

SCORE Placeholder Sheet for IFW Content

Application Number: 18354282

Document Date: 07/18/2023

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	YANG
				Art Unit	
Examiner Name					
Sheet	1	of	4	Attorney Docket Number	JHU-36631.303

U.S. PATENTS					
Exami ner Initials*	Cite No. ¹	Document Number	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² <i>(if known)</i>			
		9346814	2016-05-24	JANSEN et al.	

U.S. PUBLISHED PATENT APPLICATIONS					
Exami ner Initials*	Cite No. ³	Document Number	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ⁴ <i>(if known)</i>			
		20080280856	2008-11-13	COHEN et al.	
		20140357650	2014-12-04	JANSEN et al.	

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

FOREIGN PATENT DOCUMENTS						
Exami ner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translati on ⁸
		Country Code ⁵ Number ⁶ Kind Code ⁷ <i>(if known)</i>				
		WO 2013/107820	2013-07-25	UNIVERSITEIT ANTWERPEN et al.		
		WO 2014/001538	2014-01-03	GE HEALTHCARE LTD		
		WO 2015/114166	2015-08-06	PHILOCHEM AG		
		WO 2016/149188	2016-09-22	THE JOHNS HOPKINS UNIVERSITY		

Examiner Signature		Date Considered	
-----------------------	--	--------------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	
				Art Unit	
				Examiner Name	
Sheet	2	of	4	Attorney Docket Number	JHU-36631.303

NONPATENT LITERATURE DOCUMENTS				
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation ⁶	
		ALLINEN et al., Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004 Jul;6(1):17-32.		
		BAE et al., Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. Br J Haematol. 2008 Sep;142(5):827-30.		
		CHEN et al., Advance of molecular imaging technology and targeted imaging agent in imaging and therapy. Biomed Res Int. 2014; 2014 : 819324. PMCID: PMC3943245.		
		COENEN et al., Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. Nuclear medicine and biology. 2010; 37:727-740.		
		CHO et al., Biodistribution, tumor detection, and radiation dosimetry of 18F-DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2012; 53:1883-1891.		
		DVORAKOVA et al., Inhibitor-Decorated Polymer Conjugates Targeting Fibroblast Activation Protein. J Med Chem. 2017;60:8385-8393.		
		EDOSADA et al., Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly(2)-Pro(1)-cleaving specificity. FEBS Lett. 2006 Mar 6;580(6):1581-6.		
		FISCHER et al., Radioimmunotherapy of fibroblast activation protein positive tumors by rapidly internalizing antibodies. Clin Cancer Res. 2012;18:6208-6218.		
		FRANCO et al., Cancer associated fibroblasts in cancer pathogenesis. Semin Cell Dev Biol. 2010 Feb;21(1):33-9.		
		GARIN-CHESA et al., Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc Natl Acad Sci U S A. 1990 Sep;87(18):7235-9. PMCID: PMC54718.		
		JANSEN et al., Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Scaffold. ACS Med Chem Lett. 2013 Mar 18;4(5):491-6.		
		JANSEN et al., Extended structure-activity relationship and pharmacokinetic investigation of (4-quinolinoyl)glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP). J Med Chem. 2014 Apr 10;57(7):3053-74.		
		KELLY, Fibroblast activation protein-alpha and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. Drug Resist Updat. 2005 Feb-Apr;8(1-2):51-8.		

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	
				Art Unit	
				Examiner Name	
Sheet	3	of	4	Attorney Docket Number	
JHU-36631.303					

NONPATENT LITERATURE DOCUMENTS			
		KRAMAN et al., Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science. 2010 Nov 5;330(6005):827-30.	
		LAVERMAN et al., Immuno-PET and Immuno-SPECT of Rheumatoid Arthritis with Radiolabeled Anti-Fibroblast Activation Protein Antibody Correlates with Severity of Arthritis. J Nucl Med. 2015 May;56(5):778-83.	
		LI et al., Activatable Near-Infrared Fluorescent Probe for In Vivo Imaging of Fibroblast Activation Protein-alpha. Bioconjugate Chem. 2012 Jul. 19;23:1704-11	
		LO et al., Photodynamic molecular beacon triggered by fibroblast activation protein on cancer-associated fibroblasts for diagnosis and treatment of epithelial cancers. J Med Chem. 2009 Jan 22;52(2):358-68.	
		POPLAWSKI et al., Identification of selective and potent inhibitors of fibroblast activation protein and prolyl oligopeptidase. J Med Chem. 2013 May 9;56(9):3467-77.	
		REILLY et al., Advancing Novel Molecular Imaging Agents from Preclinical Studies to First-in-Humans Phase I Clinical Trials in Academia-A Roadmap for Overcoming Perceived Barriers. Bioconjugate chemistry. 2015; 26:625-632.	
		RETTIG et al., Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin Cancer Res. 1993 Jul 15;53(14):3327-35.	
		RYABTSOVA et al., Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. Bioorg Med Chem Lett. 2012 May 15;22(10):3412-7.	
		SCANLAN et al., Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proc Natl Acad Sci U S A. 1994 Jun 7;91(12):5657-61.	
		SCOTT et al., A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res. 2003 May;9(5):1639-47.	
		TSAI et al., Substituted 4-carboxymethylpyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. J Med Chem. 2010 Sep 23;53(18):6572-83.	
		TUXHORN et al., Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res. 2002 Sep;8(9):2912-23.	
		WELT et al., Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. J Clin Oncol. 1994 Jun;12(6):1193-203.	
		YOUN et al., In vivo noninvasive small animal molecular imaging. Osong Public Health Res Perspect. 2012; 3 :48-59.	

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				<i>Application Number</i>	
				<i>Filing Date</i>	
				<i>First Named Inventor</i> YANG	
				<i>Art Unit</i>	
				<i>Examiner Name</i>	
Sheet	4	of	4	<i>Attorney Docket Number</i>	JHU-36631.303

NONPATENT LITERATURE DOCUMENTS			
		YU et al., The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J. 2010 Mar;277(5):1126-44.	
		International Search Report and Written Opinion for PCT/US2018/057086, mailed May 10, 2019, 12 pages	
		Extended EP Search Report for EP 18871298.8, mailed May 20, 2021, 7 pages	

Examiner Signature		Date Considered	
-----------------------	--	--------------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				<i>Application Number</i>	
				<i>Filing Date</i>	
				<i>First Named Inventor</i> YANG	
				<i>Art Unit</i>	
				<i>Examiner Name</i>	
Sheet	5	of	4	<i>Attorney Docket Number</i>	JHU-36631.303

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐

See attached certification statement.

☐

The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

☒

A certification statement is not submitted herewith.

☐

Documents not provided herewith have been previously cited in parent U.S. patent application number _____ filed on _____. In compliance with 37 C.F.R. § 1.98(d), Applicants have not included copies of these documents.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jeffrey W. Childers/	Date (YYYY-MM-DD)	2023-07-18
Printed Name	Jeffrey W. Childers	Registration Number	58126

PTO Notes regarding this form:

¹ Applicant's unique citation designation number (optional).

² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.

³ Applicant's unique citation designation number (optional).

⁴ See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.

⁵ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).

⁶ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.

⁷ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.

⁸ Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		
<p>The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.</p> <p>This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.</p>			

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Xing		Yang			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	Baltimore	State/Province	MD	Country of Residenceⁱ	US	
Mailing Address of Inventor:						
Address 1		c/o The Johns Hopkins University				
Address 2		3400 North Charles Street				
City	Baltimore	State/Province	MD			
Postal Code	21218	Countryⁱ	US			
Inventor 2					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Sridhar		Nimmagadda			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	Baltimore	State/Province	MD	Country of Residenceⁱ	US	
Mailing Address of Inventor:						
Address 1		c/o The Johns Hopkins University				
Address 2		3400 North Charles Street				
City	Baltimore	State/Province	MD			
Postal Code	21218	Countryⁱ	US			
Inventor 3					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Steven		Rowe			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						

Application Data Sheet 37 CFR 1.76		Attorney Docket Number		JHU-36631.303	
		Application Number			
Title of Invention		IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)			

City	Parkville	State/Province	MA	Country of Residence ⁱ	US
------	-----------	----------------	----	-----------------------------------	----

Mailing Address of Inventor:

Address 1		c/o The Johns Hopkins University			
Address 2		3400 North Charles Street			
City	Baltimore	State/Province	MD		
Postal Code	21218	Country ⁱ	US		
Inventor 4					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Stephanie		Slania		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Baltimore	State/Province	MD	Country of Residence ⁱ	US

Mailing Address of Inventor:

Address 1		c/o The Johns Hopkins University			
Address 2		3400 North Charles Street			
City	Baltimore	State/Province	MD		
Postal Code	21218	Country ⁱ	US		
Inventor 5					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Martin	G.	Pomper		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Baltimore	State/Province	MD	Country of Residence ⁱ	US

Mailing Address of Inventor:

Address 1		c/o The Johns Hopkins University			
Address 2		3400 North Charles Street			
City	Baltimore	State/Province	MD		
Postal Code	21218	Country ⁱ	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					
Add					

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		
Customer Number	101943		
Email Address	docketing@casimirjones.com	Add Email	Remove Email

Application Information:

Title of the Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		
Attorney Docket Number	JHU-36631.303	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	9	Suggested Figure for Publication (if any)	
Filing By Reference:			
Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").			
For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).			
Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.			
Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	101943		

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	16758182	2020-04-22
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
16758182	a 371 of international	PCT/US2018/057086	2018-10-23
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2018/057086	Claims benefit of provisional	62575607	2017-10-23
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Remove
			Access Code ⁱ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

☐	<p>This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.</p> <p>NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.</p>
--------------------------	---

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant 1			
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
<input type="button" value="Clear"/>			
☒ Assignee		☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor
☐ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor: <input type="text"/>			
If the Applicant is an Organization check here. ☒			
Organization Name	The Johns Hopkins University		
Mailing Address Information For Applicant:			
Address 1	3400 North Charles Street		
Address 2			
City	Baltimore	State/Province	MD
Country	US	Postal Code	21218
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button.			

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		State/Province	
Country ⁱ		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Jeffrey W. Childers/		Date (YYYY-MM-DD)	2023-07-18
First Name	Jeffrey W.	Last Name	Childers	Registration Number
				58126

Additional Signature may be generated within this form by selecting the Add button.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU-36631.303
		Application Number	
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

- 1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
- 3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
16/758,182	22-Apr-2020

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

- ☒ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above:
- OR
- ☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

- ☒ The address associated with the above-mentioned Customer Number

OR

- ☐ The address associated with Customer Number:

OR

- ☐ Firm or Individual Name

Address

City

State

Zip

Country

Telephone

Email

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

THE JOHNS HOPKINS UNIVERSITY

- ☐ Inventor or Joint Inventor (title not required below)
- ☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)
- ☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)
- ☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature

Date (Optional)

Name

Steven Kousouris

Title

Executive Director, Johns Hopkins Technology Ventures, The Johns Hopkins University

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

- ☒ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Attorney Docket No. JHU-36631.303

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: YANG, et al.

Confirmation No.: TBD

Serial No.: TBD

Art Unit: TBD

Filed: July 18, 2023

Examiner: TBD

**FOR: IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING
FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir or Madam:

Please amend the application as indicated on the following pages.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 4 of this paper.

IN THE CLAIMS:

The present listing of claims replaces all previous versions of the claims.

1.-27. (Canceled)

28. (New) A compound of Formula (I):

B-L-A (I) wherein:

A is a targeting moiety for FAP-a, wherein A has the structure of:

Wherein:

R1x and R2x are each independently selected from the group consisting of H, OH, halogen, C1-6alk-yl, -O-C1-6alkyl, and -S-C1-6alkyl;

R3x is selected from the group consisting of H, -CN, -B(OH)₂-C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R4x is H;

R5x, R6x and R7x are each independently selected from the group consisting of H, -OH, oxo, halogen, -C1-6alkyl, -O-C1-6alkyl, -S-C1-6alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂

and -Ar₂; each of C1-6alkyl being optionally substituted with from 1 to 3 substituents selected from -OH and halogen;

R_{8x}, R_{9x} and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C1-6alkyl, -O-C1-6alkyl, -S-C1-6alkyl, and -Ar₃;

R_{10x}, R_{11x}, R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C1-6alkyl, -O-C1-6alkyl, and -S-C1-6alkyl; Ar₁, Ar₂ and

Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C1-6alkyl, -O-C1-6alkyl, and -S-C1-6alkyl;

Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1

or 2 heteroatoms selected from O, N and S; Het2 being optionally substituted with from 1 to 3 substituents selected from -NR¹³xR¹⁴x, -C1-6alkyl, -O-C1-6alkyl, and -S-C1-6alkyl;

v is 0, 1, 2, or 3; and

represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3

heteroatoms selected from O, N and S;

B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and

L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

REMARKS

Upon entry of this amendment claims 28 is pending. Claims 1-27 are canceled without prejudice. Applicant reserves the right to pursue the canceled subject matter in one or more continuation and/or divisional applications. Claim 28 is newly added. Support for the newly added claim can be found in the specification and claims as originally filed. No new matter is added.

CONCLUSION

Should there be any minor issues outstanding in this matter, the Examiner is respectfully requested to telephone the undersigned attorney at 608-662-1277. Early passage of the subject application to issue is earnestly solicited.

Respectfully Submitted,

CASIMIR JONES, S.C.

Date: July 18, 2023

/JEFFREY W. CHILDERS/
Jeffrey W. Childers, Ph.D.
Registration No. 58126
Customer No. 101943

**DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN
APPLICATION DATA SHEET (37 CFR 1.76)**

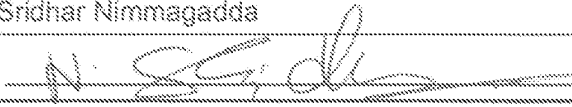
Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-alpha (FAP-alpha)
<p>As the below named inventor, I hereby declare that:</p> <p>This declaration is directed to: ☐ The attached application, or ☒ United States application or PCT international application number <u>16/758,182</u> filed on <u>22-Apr-2020</u></p> <p>The above-identified application was made or authorized to be made by me.</p> <p>I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.</p> <p>I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.</p> <p>WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p> <p>LEGAL NAME OF INVENTOR</p> <p>Inventor: <u>Xing Yang</u> Date (Optional): <u>06/24/2020</u></p> <p>Signature: <u>Xing Yang</u></p> <p>Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.</p>	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to be (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

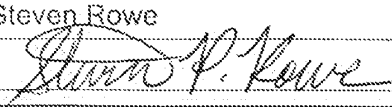
**DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN
APPLICATION DATA SHEET (37 CFR 1.76)**

Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-alpha (FAP-alpha)
As the below named inventor, I hereby declare that:	
This declaration is directed to: ☐ The attached application, or	
☒ United States application or PCT international application number <u>16/758,182</u>	
filed on <u>22-Apr-2020</u>	
The above-identified application was made or authorized to be made by me.	
I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.	
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.	
WARNING:	
Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(e) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.	
LEGAL NAME OF INVENTOR	
Inventor: <u>Sridhar Nimmagadda</u> Date (Optional): _____	
Signature: <u></u>	
Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.65. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1480, Alexandria, VA 22313-1480. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1480, Alexandria, VA 22313-1480.

If you need assistance in completing the form, call 1-800-PTO-0199 and select option 2.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-alpha (FAP-alpha)		
As the below named inventor, I hereby declare that:			
This declaration is directed to: ☐ The attached application, or			
☒ United States application or PCT international application number <u>16/758,182</u>			
filed on <u>22-Apr-2020</u>			
The above-identified application was made or authorized to be made by me.			
I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.			
I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.			
WARNING:			
Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.			
LEGAL NAME OF INVENTOR			
Inventor:	Steven Rowe		Date (Optional): <u>9-25-2020</u>
Signature:			
Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.			

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by this public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing this form, call 1-800-PTO-9199 and select option 2.

**DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN
APPLICATION DATA SHEET (37 CFR 1.76)**

Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-alpha (FAP-alpha)
<p>As the below named inventor, I hereby declare that:</p> <p>This declaration is directed to: ☐ The attached application, or ☒ United States application or PCT international application number <u>16/758,182</u> filed on <u>22-Apr-2020</u></p> <p>The above-identified application was made or authorized to be made by me.</p> <p>I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.</p> <p>I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.</p> <p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p>	
<p>LEGAL NAME OF INVENTOR</p> <p>Inventor: <u>Stephanie Slania</u> Date (Optional): <u>June 24th, 2020</u></p> <p>Signature: <u>Stephanie Slania</u></p>	
<p>Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.</p>	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

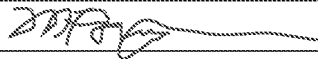
Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-alpha (FAP-alpha)
<p>As the below named inventor, I hereby declare that:</p> <p>This declaration is directed to: ☐ The attached application, or ☒ United States application or PCT international application number <u>16/758,182</u> filed on <u>22-Apr-2020</u>.</p> <p>The above-identified application was made or authorized to be made by me.</p> <p>I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.</p> <p>I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.</p> <p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p>	
LEGAL NAME OF INVENTOR	
Inventor: <u>Martin G. Pomper</u> Date (Optional): <u>June 23, 2020</u>	
Signature: 	
<p>Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.</p>	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

**CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION
UNDER 37 CFR 1.102(e) (Page 1 of 1)**

First Named Inventor:	Xing YANG	Nonprovisional Application Number (if known):	
Title of Invention:	IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently \$0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

- i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.
- ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, or the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

- i. A request for continued examination has been filed with, or prior to, this form.
- ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
- iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
- iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
- v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature <u>/Jeffrey W. Childers/</u>	Date <u>2023-07-18</u>
Name (Print/Typed) <u>Jeffrey W. Childers</u>	Practitioner Registration Number <u>58126</u>
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*	
☒ *Total of <u>1</u> forms are submitted.	

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: ECOMM.AUTH/ECOMM.WTDW

Doc Description: Internet Communications Authorized/Internet Communications Authorization Withdrawn

PTO/SB/439 (11-15)

AUTHORIZATION FOR INTERNET COMMUNICATIONS IN A PATENT APPLICATION OR REQUEST TO WITHDRAW AUTHORIZATION FOR INTERNET COMMUNICATIONS	Application No.	
	Filing Date	07/18/2023
	First Named Inventor	YANG
	Art Unit	
	Examiner Name	
	Practitioner Docket No.	JHU-36631.303

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I. To authorize permission for Internet Communications.

☒ Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via video conferencing, instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03)

II. To withdraw authorization for Internet Communications.

☐ The authorization given on _____, to the USPTO to communicate with the undersigned and any practitioner in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via Internet communications such as video conferencing, instant messaging, or electronic mail is hereby withdrawn. I understand that the withdrawal is effective when approved rather than when received.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 58126.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Jeffrey W. Childers/
Signature

2023-07-18
Date

Jeffrey W. Childers
Typed or printed name

608-662-1277
Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Juristic entities must be represented by a patent practitioner (see 37 CFR 1.31, which is applicable to any paper filed on or after September 16, 2012, that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below*.

☒ * Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-
ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application 16/758,182, filed April 22, 2020, which is a U.S. §371 National Entry Application of PCT/US2018/057086, filed October 23, 2018, which claims the benefit of U.S. Provisional Application No. 62/575,607, filed October 23, 2017, each of which is incorporated herein by reference in its entirety.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA197470 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Fibroblast-activation protein- α (FAP- α) expression has been detected on the surface of fibroblasts in the stroma surrounding >90% of the epithelial cancers examined, including malignant breast, colorectal, skin, prostate and pancreatic cancers. (Garin-Chesa, et al., 1990; Rettig, et al., 1993; Tuxhorn, et al., 2002; Scanlan, et al., 1994). It is a characteristic marker for carcinoma-associated-fibroblast (CAF), which plays a critical role in promoting angiogenesis, proliferation, invasion, and inhibition of tumor cell death. (Allinen, et al., 2004; Franco, et al., 2010). In healthy adult tissues, FAP- α expression is only limited to areas of tissue remodeling or wound healing. (Scanlan, et al., 1994; Yu, et al., 2010; Bae, et al., 2008; Kraman, et al., 2010). In addition, FAP- α -positive cells are observed during embryogenesis in areas of chronic inflammation, arthritis, and fibrosis, as well as in soft tissue and bone sarcomas. (Scanlan, et al., 1994; Yu, et al., 2010). These characteristics make FAP- α a potential imaging and radiotherapeutic target for cancer and inflammation diseases.

Because FAP- α is expressed in tumor stroma, anti-FAP antibodies have been investigated for radioimmunotargeting of malignancies, including murine F19, sibrotuzumab (a humanized version of the F19 antibody), ESC11, ESC14, and others. (Welt, et al., 1994; Scott, et al., 2003; Fischer, et al., 2012). Antibodies also demonstrated the feasibility of imaging inflammation, such as rheumatoid arthritis.

(Laverman, et al., 2015). The use of antibodies as molecular imaging agents, however, suffers from pharmacokinetic limitations, including slow blood and non-target tissue clearance (normally 2–5 days or longer) and non-specific organ uptake. Low molecular weight (LMW) agents demonstrate faster pharmacokinetics and a higher specific signal within clinically convenient times after administration. They also can be synthesized in radiolabeled form more easily and may offer a shorter path to regulatory approval. (Coenen, et al., 2010; Coenen, et al., 2012; Reilly, et al., 2015). To date, however, no LMW ligand has been reported with ideal properties for nuclear imaging of FAP- α .

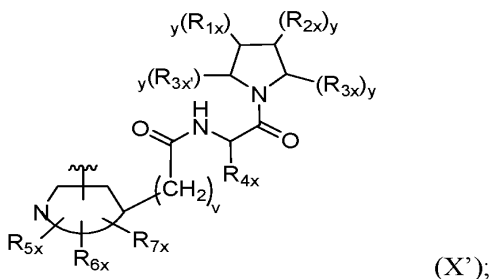
SUMMARY

In some aspects, the presently disclosed subject matter provides a compound of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

In particular aspects, A is an FAP- α targeting moiety having the structure of:



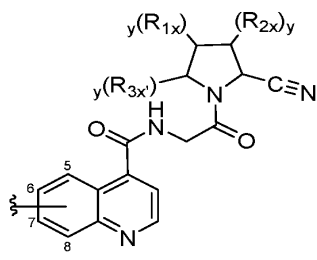
wherein each y is independently an integer selected from the group consisting of 0, 1, and 2; R_{1x}, R_{2x}, and R_{3x}, are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl; R_{4x} is H; R_{5x}, R_{6x}, and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents

selected from -OH and halogen; R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃; R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar₁, Ar₂ and Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; v is 0, 1, 2, or 3; and



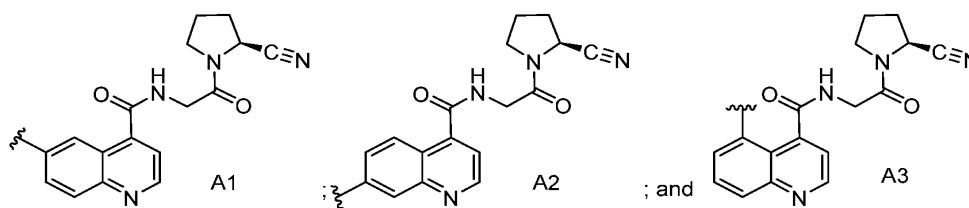
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In more particular aspects, A is an FAP- α targeting moiety having the structure of:



wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In yet more particular aspects, A is selected from the group consisting of:



In other aspects, the presently disclosed subject matter provides a pharmaceutical composition comprising a compound of formula (I).

In some aspects, the presently disclosed subject matter provides a method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

In other aspects, the presently disclosed subject matter provides a method for inhibiting fibroblast-activation protein- α (FAP- α), the method comprising administering to a subject in need thereof an effective amount of a compound of formula (I).

In yet other aspects, the presently disclosed subject matter provides a method for treating a fibroblast-activation protein- α (FAP- α)-related disease or disorder, the method comprising administering to a subject in need of treatment thereof an effective amount of a compound of formula (I), wherein the compound of formula (I) comprises a radiolabeled functional group suitable for radiotherapy.

In certain aspects, the (FAP- α)-related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG. 1A, FIG. 1B, and FIG. 1C show the synthetic pathway and structures of representative FAP-targeted agents, **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 1A shows the multi-step synthesis of the ligand precursor, tert-butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate. After each step, the reaction mixture was loaded onto a 25-g C18 cartridge and purified with a MeCN/water/TFA gradient. Identity of intermediate products was confirmed with ¹H NMR. FIG. 1B shows the full structure of optical imaging agent, **XY-FAP-01**. **XY-FAP-01** was produced with a one step reaction between the precursor and IRDye800CW-NHS. The major product was obtained at a yield of 85% after purification with HPLC. FIG. 1C shows the full structure of the SPECT imaging agent, [¹¹¹In]-**XY-FAP-02**. First, the precursor was functionalized with DOTA via a one step reaction between the precursor and DOTA-GA(t-Bu)₄-NHS. Unlabeled product was purified via HPLC to produce XY-FAP-02. Subsequent radiolabeling with ¹¹¹In and HPLC purification resulted in the radiolabeled product, [¹¹¹In]-**XY-FAP-02**;

FIG. 2 shows the inhibitory activity of **XY-FAP-01** on human recombinant FAP. The inhibitory activity of **XY-FAP-01** was determined using a fluorogenic FAP assay kit. Enzymatic activity of human recombinant FAP on a native substrate was inhibited in a concentration dependent fashion by **XY-FAP-01**. Semi-log inhibitory curves of **XY-FAP-01** activity were generated and the determined Ki value of **XY-FAP-01** was 1.26 nM;

FIG. 3A, FIG. 3B, and FIG. 3C show the assessment of the *in vitro* binding ability and specificity of **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 3A shows the concentration dependent uptake of **XY-FAP-01** in various cell lines. Cells incubated with various concentrations (range: 50 nM to 0.78 nM) of **XY-FAP-01** were imaged with the LI-COR Pearl Impulse Imager to assess uptake of agent in various FAP-positive and FAP-negative cell lines (left). Dose-response curves of **XY-FAP-01**

uptake in FAP-positive cell lines (NCIH2228, U87, and SKMEL24) and FAP-negative cell lines (PC3, NCIH226, and HCT116) were generated (right). FIG. 3B shows the inhibition of **XY-FAP-01** uptake in FAP-positive cell-lines. Cells incubated with 25-nM **XY-FAP-01** were incubated with various concentrations of either a DPPIV and FAP inhibitor, Talabostat, or a DPPIV-only inhibitor, Sitagliptin. Uptake of **XY-FAP-01** was measured and semi-log inhibitor-response curves were generated for both Talabostat and Sitagliptin. FIG. 3C shows the uptake of [¹¹¹In]-**XY-FAP-02** in FAP-positive U87 and FAP-negative PC3 cell lines. Cells were incubated with 1 μ Ci [¹¹¹In]-**XY-FAP-02** and were washed with cold PBS. Radioactivity of the cell pellets was measured and normalized to the incubated dose;

FIG. 4 is a table showing the *ex vivo* tissue biodistribution of [¹¹¹In]-**XY-FAP-01** in tumor bearing mice. At 5 min, 0.5 h, 2 h, 6 h, and 12 h after injection of 10 μ Ci [¹¹¹In]-**XY-FAP-01**, NOD/SKID mice bearing U87 and PC3 tumor xenografts were sacrificed and tissues were collected for biodistribution analysis. Additionally, mice co-injected with unlabeled **XY-FAP-02** and 10 μ Ci [¹¹¹In]-**XY-FAP-01** were sacrificed at 6 h post-injection to study the effect of blocking on uptake of the radiolabeled compound. Data presented as mean $\pm$ standard deviation. ^aStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 5 min, 0.5 h, 2 h, and 6 h post injection ($p < 0.0001$). No significant difference between the two groups were seen in the blocking study at 6 h. ^bStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 12 h post injection ($p = 0.0006$). ^cStudent's t test comparing %ID/g between PC3 tumor and U87 tumors at 6 h post injection showed significant difference between %ID/g tumors in the blocking study at 6 h versus the normal biodistribution results at 6 h ($p < 0.0001$);

FIG. 5A and FIG. 5B show the time-activity relationship of the *ex vivo* biodistribution of [¹¹¹In]-**XY-FAP-02**. FIG. 5A shows tissue time activity curves (TACs) of [¹¹¹In]-**XY-FAP-02** activity in U87 tumor, PC3 tumor, and blood. FIG. 5B shows the ratios of %ID/g between U87 tumor and PC3 tumor, blood, and muscle (mm) versus time;

FIG. 6 shows serial NIRF-imaging of **XY-FAP-01** in tumor bearing mice. NOD/SKID mice bearing FAP-positive U87 (yellow circle) and FAP-negative PC3 (red circle) tumor xenografts were injected with 10 nmol of **XY-FAP-01** via the tail

vein followed by serial NIRF-imaging on the LI-COR Pearl Impulse Imager. Representative images at 0.5 h, 1 h, 2.5 h, and 4 h after injection are shown;

FIG. 7 shows SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks; and

FIG. 8 show three-dimensional SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Figures, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

I. IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN- α (FAP- α)

FAP- α is a type II integral membrane serine protease of the prolyl oligopeptidase family, which are distinguished by their ability to cleave the Pro-AA peptide bond (where AA represents any amino acid). It has been shown to play a role in cancer by modifying bioactive signaling peptides through this enzymatic activity (Kelly, et al., 2005; Edosada, et al., 2006). FAP- α expression has been detected on the surface of fibroblasts in the stroma surrounding greater than 90% of the epithelial cancers, including, but not limited to, malignant breast, colorectal, skin, prostate,

pancreatic cancers, and the like, and inflammation diseases, including, but not limited to, arthritis, fibrosis, and the like, with nearly no expression in healthy tissues.

Accordingly, imaging and radiotherapeutic agents specifically targeting FAP- α is of clinical importance.

FAP- α exists as a homodimer to carry out its enzymatic function. Inhibitors selectively targeting FAP- α has been reported (Lo, et al., 2009; Tsai, et al., 2010; Ryabtsova, et al., 2012; Poplawski, et al., 2013; Jansen, et al., 2013; Jansen, et al., 2014). The presently disclosed subject matter provides, in part, a FAP- α selective targeting moiety that can be modified with an optical dye, a radiometal chelation complex, and other radiolabeled prosthetic groups, thus providing a platform for the imaging and radiotherapy targeting FAP- α .

Radionuclide molecular imaging, including positron emission tomography (PET), is the most mature molecular imaging technique without tissue penetration limitations. Due to its advantages of high sensitivity and quantifiability, radionuclide molecular imaging plays an important role in clinical and preclinical research (Youn, et al., 2012; Chen, et al., 2014). Many radionuclides, primarily β - and alpha emitters, have been investigated for targeted radioimmunotherapy and include both radiohalogens and radiometals (see Table 1 for representative therapeutic radionuclides).

Table 1. Representative Therapeutic Radionuclides	
β -particle emitters	^{90}Y , ^{131}I , ^{177}Lu , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb
α -particle emitters	^{225}Ac , ^{213}Bi , ^{212}Bi , ^{211}At , ^{212}Pb
Auger electron emitters	^{125}I , ^{123}I , ^{67}Ga , ^{111}In

The highly potent and specific binding moiety targeting FAP- α enables its use in nuclear imaging and radiotherapy. The presently disclosed subject matter provides the first synthesis of nuclear imaging and radiotherapy agents based on this dual-targeting moiety to FAP- α .

Accordingly, in some embodiments, the presently disclosed subject matter provides potent and selective low-molecular-weight (LMW) ligands of FAP- α , i.e., an FAP- α selective inhibitor, conjugated with a targeting moiety feasible for modification with optical dyes and radiolabeling groups, including metal chelators

and metal complexes, which enable *in vivo* optical imaging, nuclear imaging (optical, PET and SPECT), and radiotherapy targeting FAP- α . Importantly, the presently disclosed compounds can be modified, e.g., conjugated with, labeling groups without significantly losing their potency. The presently disclosed approach allows for the convenient labeling of the FAP- α ligand with optical dyes and PET or SPECT isotopes, including, but not limited to, ^{68}Ga , ^{64}Cu , ^{18}F , ^{86}Y , ^{90}Y , ^{89}Zr , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{125}I , ^{124}I , for FAP- α related imaging applications. Further, the presently disclosed approach allows for the radiolabeling of the FAP- α ligand with radiotherapeutic isotopes, including but not limited to, ^{90}Y , ^{177}Lu , ^{125}I , ^{131}I , ^{211}At , ^{111}In , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb , ^{225}Ac , ^{213}Bi , ^{212}Bi , ^{212}Pb , and ^{67}Ga , for FAP- α related radio-therapy.

In a particular embodiment, an optical agent conjugated with IRDye-800CW (**XY-FAP-01**) was synthesized and showed selective uptake *in vitro* on a FAP- α + U87 cell line and *in vivo* on a FAP- α + U87 tumor and clearly detected the tumor. In another particular embodiment, an ^{111}In labeled ligand (**XY-FAP-02-[^{111}In]**) was successfully obtained in high yield and purity from its precursor with a metal chelator. The *in vivo* study showed clear tumor radiotracer uptake in mice bearing FAP- α -positive U87 tumors with minimum non-specific organ uptake, which allows the specific imaging of FAP- α expressing tumors. The presently disclosed FAP- α targeting moiety can be adapted for use with optical dyes and radioisotopes known in the art for imaging and therapeutic applications targeting FAP- α .

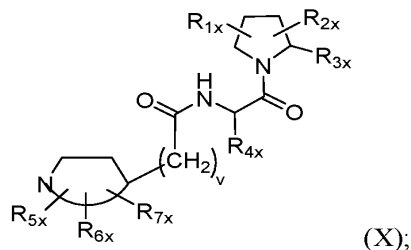
More particularly, in some embodiments, the presently disclosed subject matter provides a compound of the general structure of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

Representative targeting moieties for FAP- α are disclosed in U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014; U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016; and International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013, each of which are incorporate by reference in their entirety.

More particularly, U.S. Patent No. 9,346,814 to Jansen et al., discloses FAP- α inhibitors of formula (X), or a stereoisomer, tautomer, racemate, salt, hydrate, or solvate thereof, which are suitable for use with the presently disclosed subject matter:



wherein:

R_{1x} and R_{2x} are each independently selected from the group consisting of H, OH, halogen, C_{1-6} alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

R_{3x} is selected from the group consisting of H, $-CN$, $-B(OH)_2$, $-C(O)alkyl$, $-C(O)aryl$, $-C=C-C(O)aryl$, $-C=C-S(O)_2aryl$, $-CO_2H$, $-SO_3H$, $-SO_2NH_2$, $-PO_3H_2$, and 5-tetrazolyl;

R_{4x} is H;

R_{5x} , R_{6x} , and R_{7x} are each independently selected from the group consisting of H, $-OH$, oxo, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, $-NR_{8x}R_{9x}$, $-OR_{12x}$, $-Het_2$ and $-Ar_2$; each of C_{1-6} alkyl being optionally substituted with from 1 to 3 substituents selected from $-OH$ and halogen;

R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, $-OH$, halo, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, and $-Ar_3$;

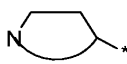
R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, $-OH$, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl; Ar_1 , Ar_2 and Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from $-NR_{10x}R_{11x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

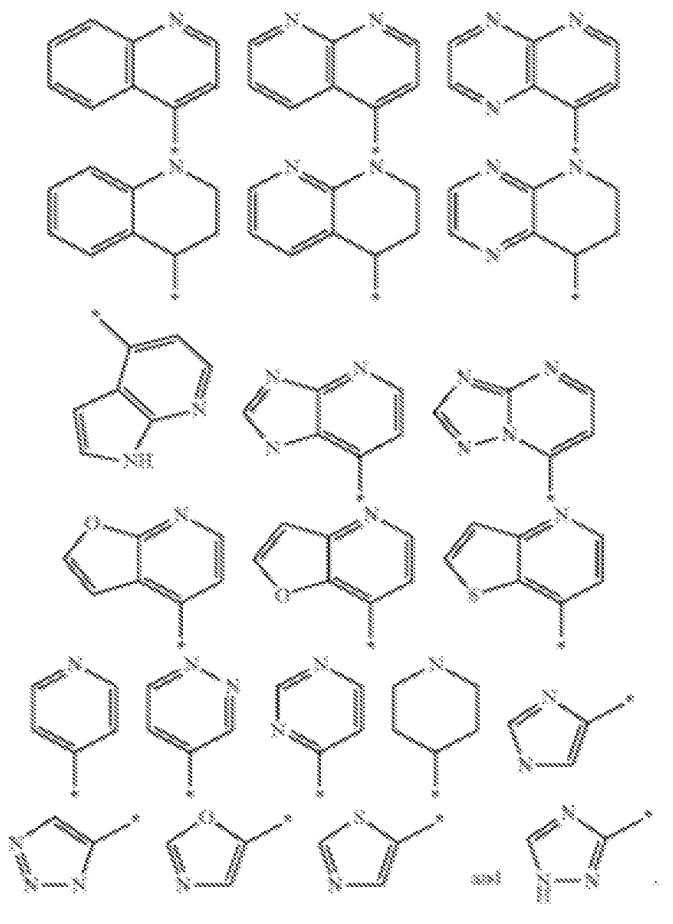
Het_2 is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het_2 being optionally substituted with from 1 to 3 substituents selected from $-NR_{13x}R_{14x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

v is 0, 1, 2, or 3; and



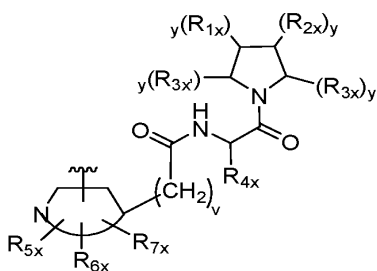
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S.

In particular embodiments,  is selected from the group consisting of:



wherein * indicates the point of attachment of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle to $-(CH_2)_n-$.

Accordingly, in some embodiments, A is an FAP- α targeting moiety having the structure of:



(X');

wherein each y is independently an integer selected from the group consisting of 0, 1, and 2;

R_{1x} , R_{2x} , and R_{3x} , are each independently selected from the group consisting of H, OH, halogen, C_{1-6} alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

R_{3x} is selected from the group consisting of H, $-CN$, $-B(OH)_2$, $-C(O)alkyl$, $-C(O)aryl$, $-C=C-C(O)aryl$, $-C=C-S(O)_2aryl$, $-CO_2H$, $-SO_3H$, $-SO_2NH_2$, $-PO_3H_2$, and 5-tetrazolyl;

R_{4x} is H;

R_{5x} , R_{6x} , and R_{7x} are each independently selected from the group consisting of H, $-OH$, oxo, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, $-NR_{8x}R_{9x}$, $-OR_{12x}$, $-Het_2$ and $-Ar_2$; each of C_{1-6} alkyl being optionally substituted with from 1 to 3 substituents selected from $-OH$ and halogen;

R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, $-OH$, halo, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, and $-Ar_3$;

R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, $-OH$, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl; Ar_1 , Ar_2 and Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from $-NR_{10x}R_{11x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

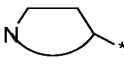
Het_2 is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het_2 being optionally substituted with from 1 to 3 substituents selected from $-NR_{13x}R_{14x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

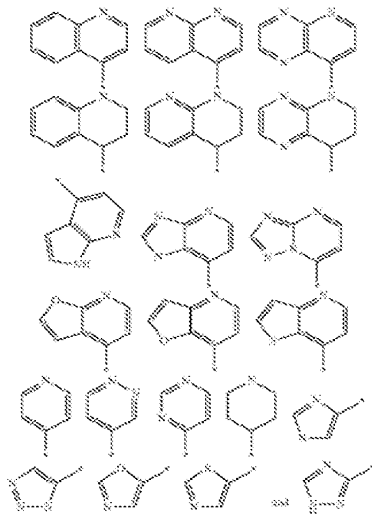
v is 0, 1, 2, or 3; and



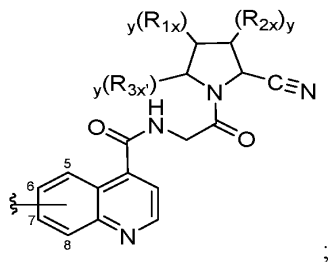
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S;

wherein  indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments,  is selected from the group consisting of:

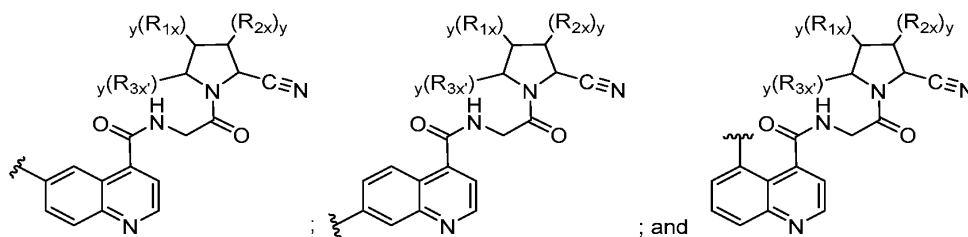


In some embodiments, A is an FAP- α targeting moiety having the structure of:

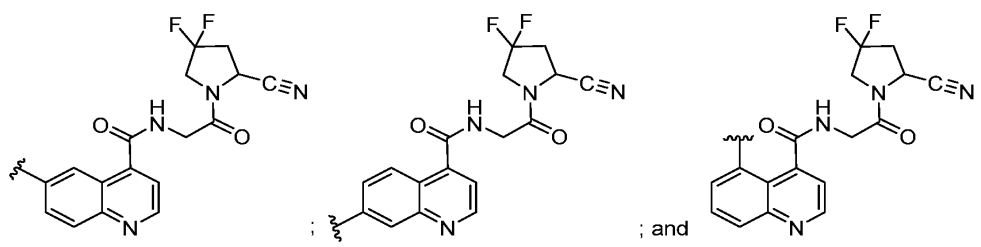


wherein y , R_{1x} , R_{2x} and $R_{3x'}$ are defined as hereinabove; $\frac{1}{2}$ — indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments, A is selected from the group consisting of:

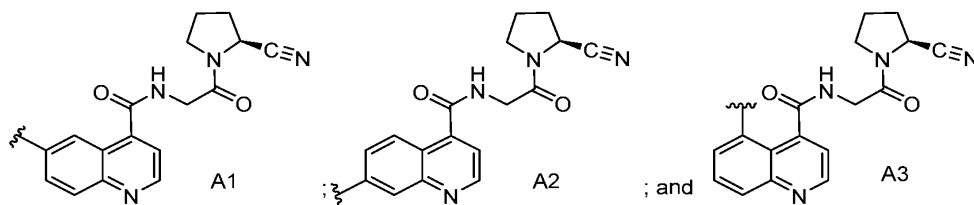


In more particular embodiments, A is selected from the group consisting of:

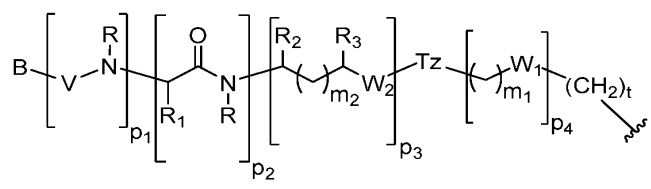


and stereoisomers thereof.

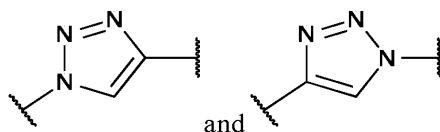
In yet more particular embodiments, A is selected from the group consisting of:



In some embodiments, the combination of L and B can be represented by:

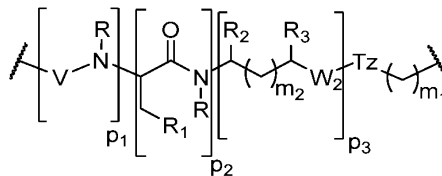


wherein the subunits associated with elements p_1 , p_2 , p_3 and p_4 may be in any order; t is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; p_1 , p_3 , and p_4 are each independently 0 or 1; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'_2-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_q-$ and $-(CH_2-CH_2-O)_q-$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R or R' is independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, and $-OR_4$, wherein R_4 is selected from the group consisting of H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, and substituted heterocycloalkyl, wherein q is defined as immediately hereinabove; Tz is a triazole group that can be present or absent and, if present, is selected from the group consisting of



; each R_1 is independently H, C_1-C_6 alkyl, C_3-C_{12} aryl, $-(CH_2)_q-C_3-C_{12}$ aryl, $-C_4-C_{16}$ alkylaryl, or $-(CH_2)_q-C_4-C_{16}$ alkylaryl; R_2 and R_3 are each independently H and $-CO_2R_5$, wherein R_5 is selected from the group consisting of H, C_1-C_6 alkyl, C_3-C_{12} aryl, and C_4-C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_5 , then the other is H; V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$; B is any optical or radiolabeled functional group suitable for optical, PET, or SPECT imaging or radiotherapy; and stereoisomers and pharmaceutically acceptable salts thereof.

In some embodiments, L has the following general structure:



wherein p_1 , p_2 , p_3 , m_1 , m_2 , q , t , Tz , W_2 , R , R_1 , R_2 , R_3 , and V are defined as hereinabove.

In some embodiments, L is selected from the group consisting of $-L_1-$, $-L_2-L_3-$, and $-L_1-L_2-L_3-$, wherein:

L_1 is $-\text{NR}-(\text{CH}_2)_q-[\text{O}-\text{CH}_2-\text{CH}_2-\text{O}]_q-\text{C}(=\text{O})-$;

L_2 is $-\text{NR}-(\text{CH}_2)_q-\text{C}(\text{COOR}_5)-\text{NR}-$; and

L_3 is $-(\text{O})\text{C}-(\text{CH}_2)_q-\text{C}(=\text{O})-$;

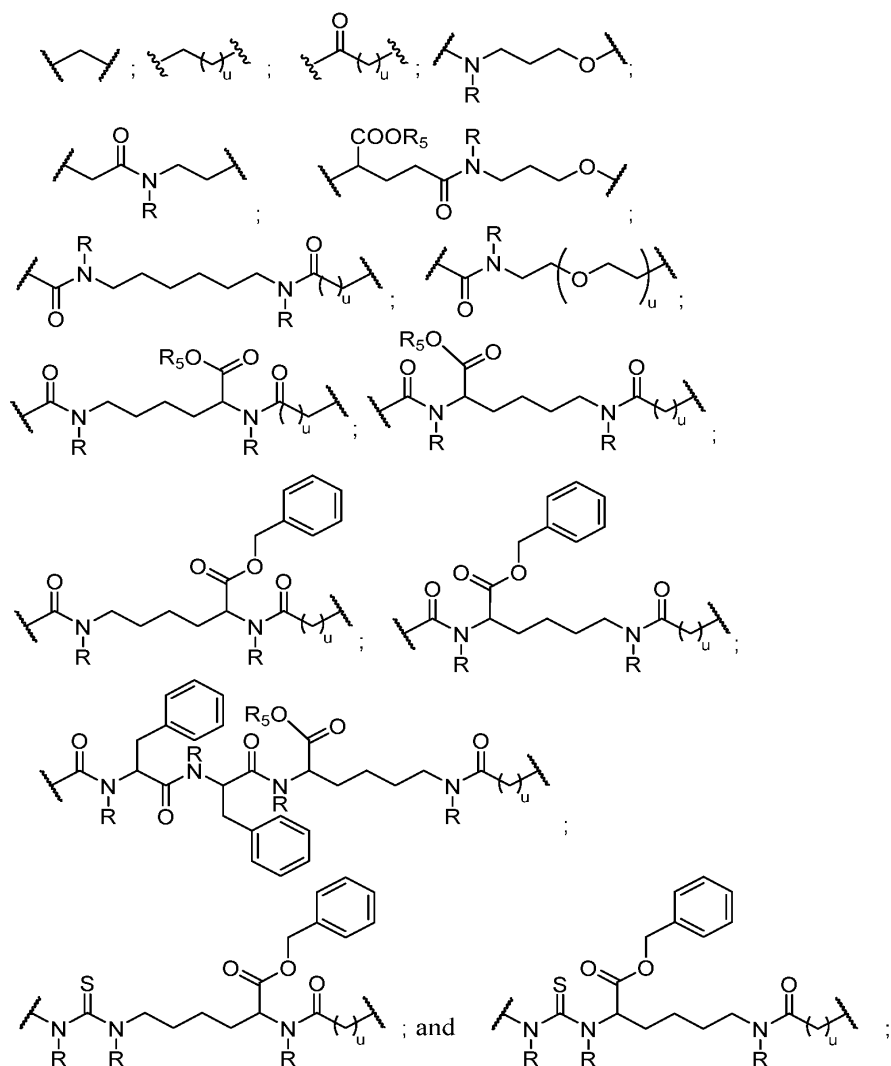
wherein each q is independently an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove.

In particular embodiments, L is:

$-(\text{CR}_6\text{H})_q-(\text{CH}_2)_q-\text{C}(=\text{O})-\text{NR}-(\text{CH}_2)_q-\text{O}-$ or $-\text{NR}-(\text{CH}_2)_q-\text{O}-$;

wherein each q and R is defined hereinabove; and R_6 is H or $-\text{COOR}_5$.

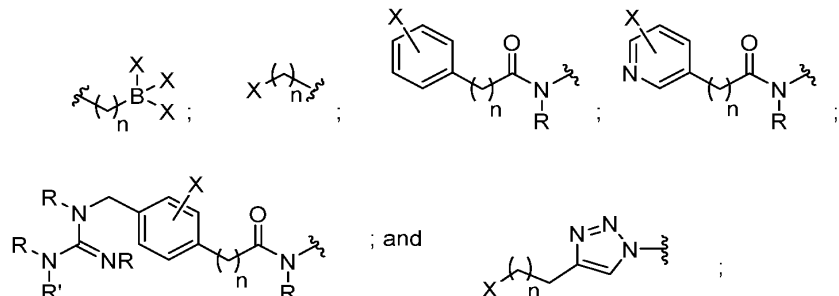
In yet more particular embodiments, L is selected from the group consisting of:



wherein u is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8; and R and R₅ are as defined hereinabove.

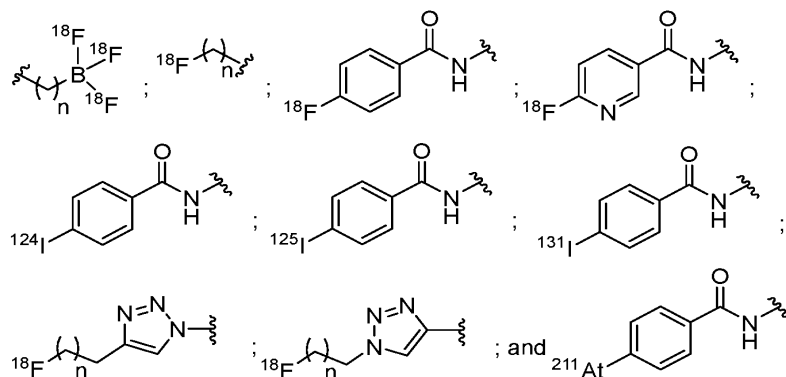
Suitable linkers are disclosed in U.S. Patent Application Publication No. US2011/0064657 A1, for "Labeled Inhibitors of Prostate Specific Membrane Antigen (PSMA), Biological Evaluation, and Use as Imaging Agents," published March 17, 2011, to Pomper et al., and U.S. Patent Application Publication No. US2012/0009121 A1, for "PSMA-Targeting Compounds and Uses Thereof," published January 12, 2012, to Pomper et al, each of which is incorporated by reference in its entirety.

In some embodiments, B is a radiolabeled prosthetic group comprising a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At . Representative radiolabeled prosthetic groups include, but are not limited to:

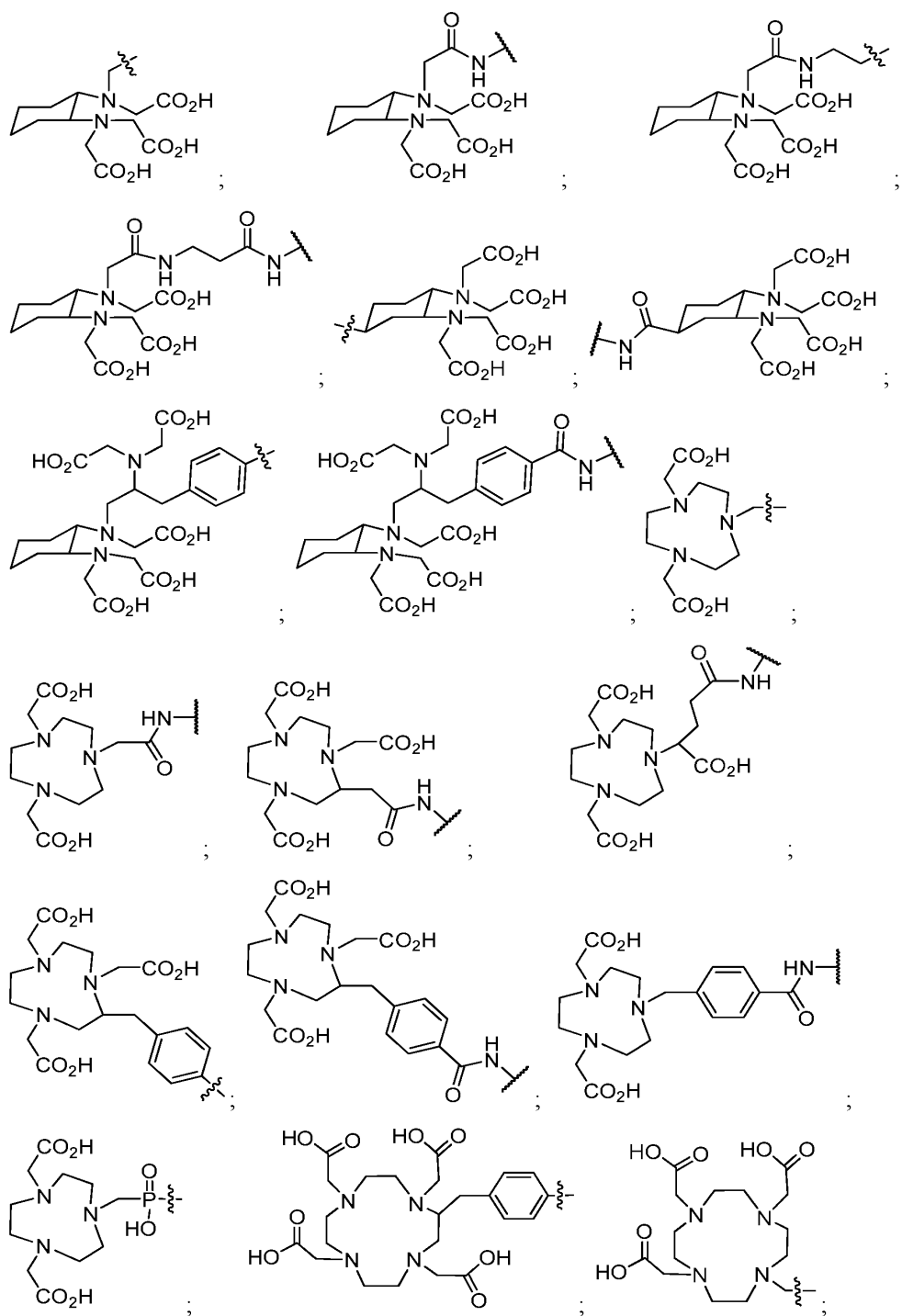


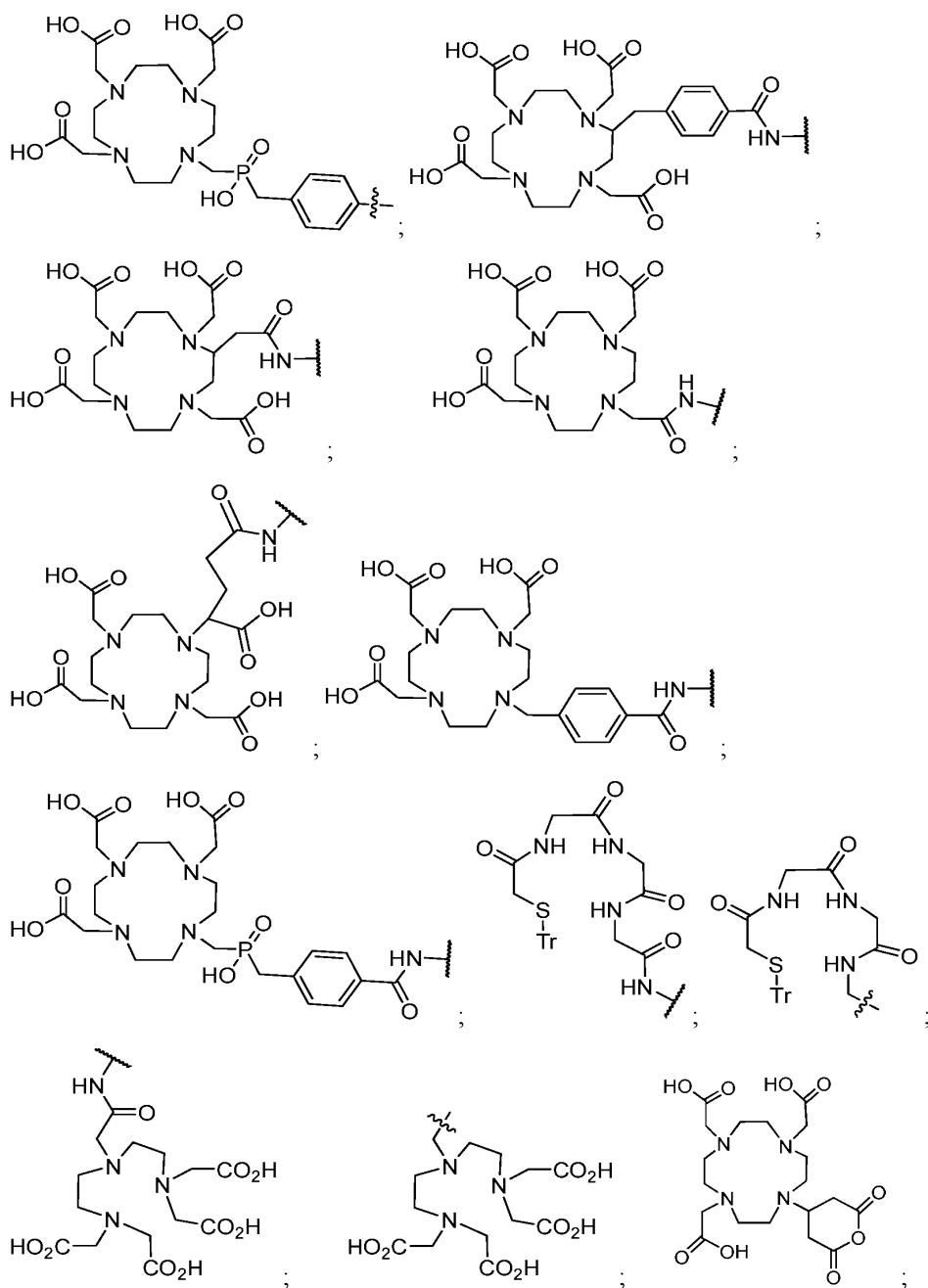
wherein each X is independently a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At ; each R and R' is defined hereinabove; and each n is independently an integer selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

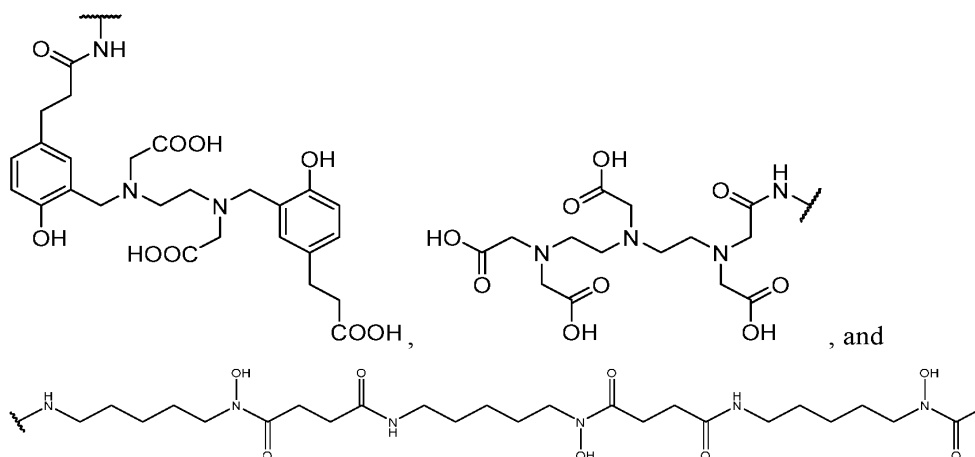
In more particular embodiments, the radiolabeled prosthetic group is selected from the group consisting of:



In other embodiments, B comprises a chelating agent. Representative chelating agents include, but are not limited to:

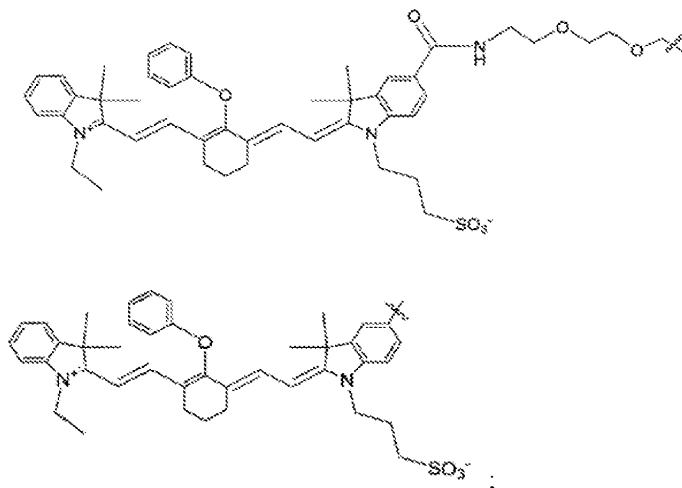


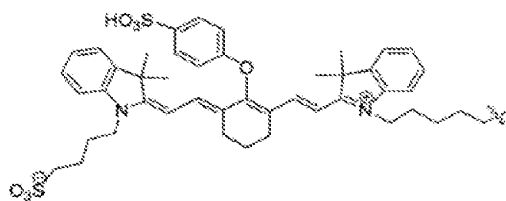
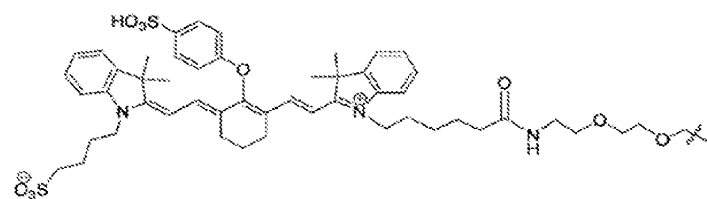
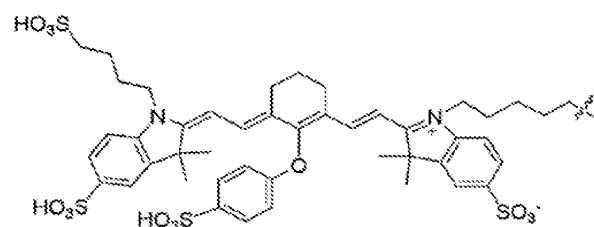
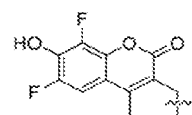
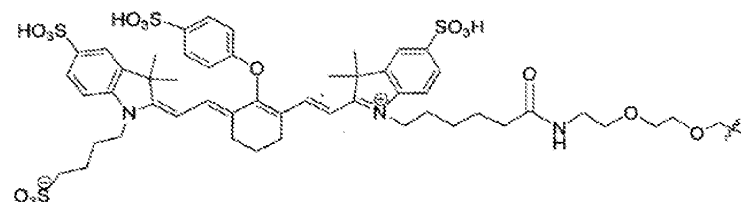
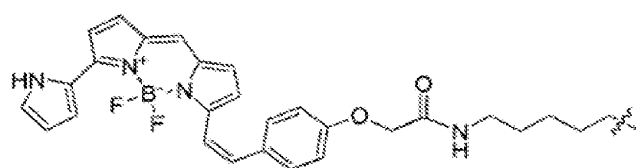
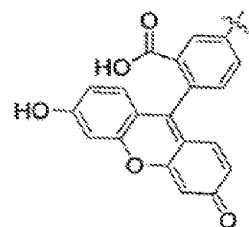
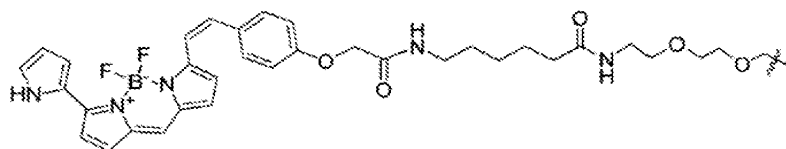


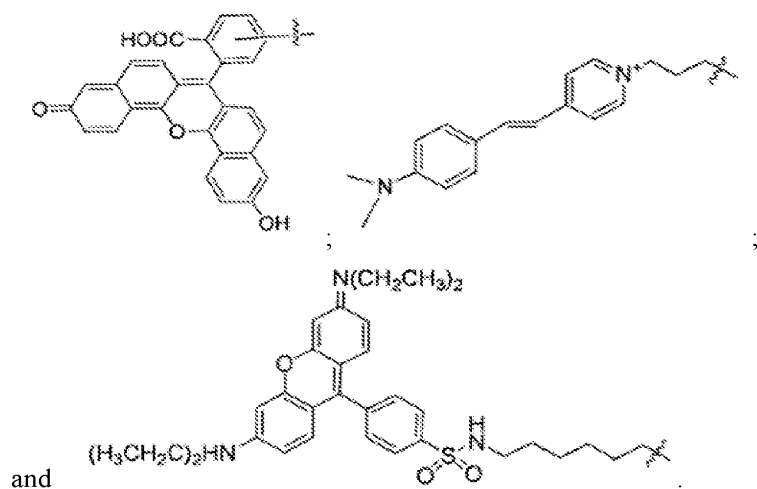


In some embodiments, B comprises an optical dye, e.g., in particular embodiments, a fluorescent dye. In some embodiments, the fluorescent dye moiety comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.

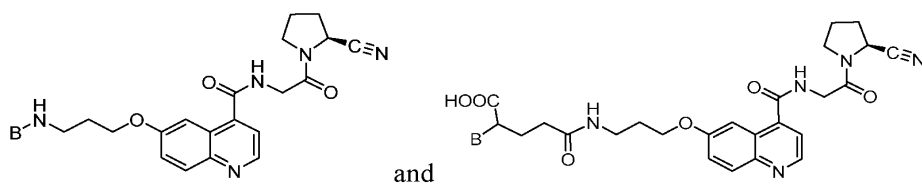
Representative optical dyes include, but are not limited to:



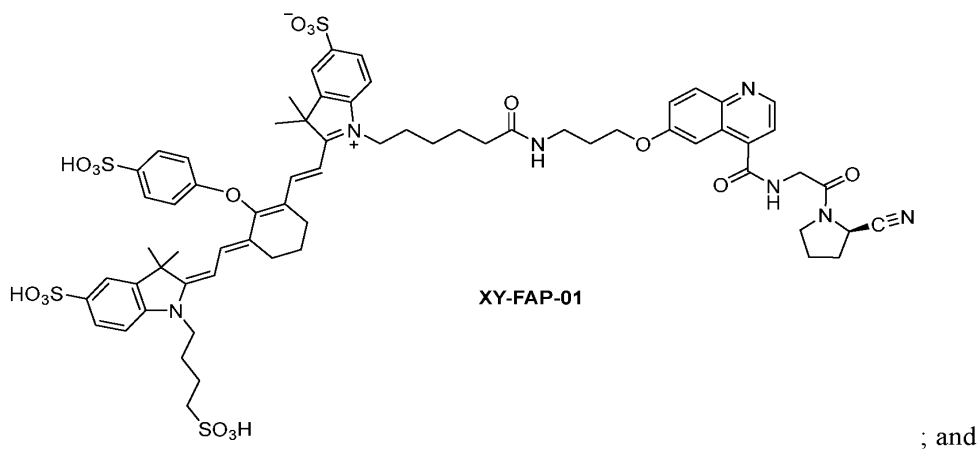


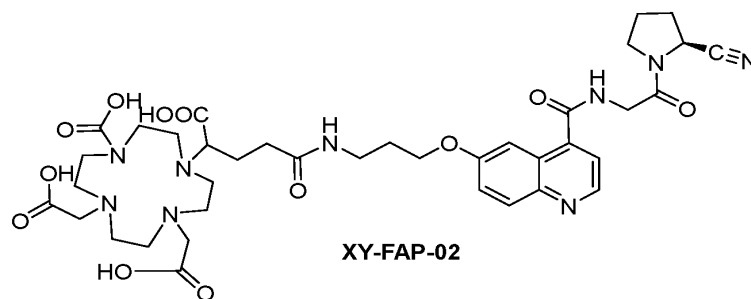


In some embodiments, the presently disclosed subject matter provides a compound selected from the group consisting of:



In particular embodiments, the compound is selected from the group consisting of:





B. Pharmaceutical Compositions and Administration

In another aspect, the present disclosure provides a pharmaceutical comprising a compound of formula (I) in admixture with a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. One of skill in the art will recognize that the pharmaceutical compositions include the pharmaceutically acceptable salts or hydrates of the compounds described above.

Pharmaceutically acceptable salts are generally well known to those of ordinary skill in the art and include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituent moieties found on the compounds described herein. When compounds of the present disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent or by ion exchange, whereby one basic counterion (base) in an ionic complex is substituted for another. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt.

When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent or by ion exchange, whereby one acidic counterion (acid) in an ionic complex is substituted for another. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-

toluenesulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

Accordingly, pharmaceutically acceptable salts suitable for use with the presently disclosed subject matter include, by way of example but not limitation, acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinolate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teclate. Other pharmaceutically acceptable salts may be found in, for example, Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

In therapeutic and/or diagnostic applications, the compounds of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained-slow release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000). Suitable routes may include oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

For injection, the agents of the disclosure may be formulated and diluted in

aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable inert carriers to formulate the compounds herein disclosed for the practice of the disclosure into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (e.g., patient) to be treated.

For nasal or inhalation delivery, the agents of the disclosure also may be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances, such as saline; preservatives, such as benzyl alcohol; absorption promoters; and fluorocarbons.

Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Generally, the compounds according to the disclosure are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from 0.01 to 1000 mg, from 0.5 to 100 mg, from 1 to 50 mg per day, and from 5 to 40 mg per day are examples of dosages that may be used. A non-limiting dosage is 10 to 30 mg per day. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, the bioavailability of the compound(s), the adsorption, distribution, metabolism, and excretion (ADME) toxicity of the compound(s), and the preference and experience of the attending physician.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and

auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

C. Methods of Imaging using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof

In some embodiments, presently disclosed subject matter provides a method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group

suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for imaging one or more cells, organs, or tissues, the method comprising exposing cells or administering to a subject an effective amount of a compound of formula (I) with an optical or radioisotopic label suitable for imaging. In some embodiments, the one or more organs or tissues include prostate tissue, kidney tissue, brain tissue, vascular tissue, or tumor tissue.

The imaging methods of the invention are suitable for imaging any physiological process or feature in which FAP- α is involved, for example, identifying areas of tissues or targets which exhibit or express high concentrations of FAP- α . Physiological processes in which FAP- α is involved include, but are not limited to: (a) proliferation diseases (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c) endocrinological disorders (including but not limited to disorders of glucose metabolism).

In certain embodiments, the radiolabeled compound is stable in vivo.

In certain embodiments, the radiolabeled compound is detected by positron emission tomography (PET) or single photon emission computed tomography (SPECT).

In certain embodiments, the optical reporting moiety is detected by fluorescence, such as fluorescence microscopy.

In certain embodiments, the presently disclosed compounds are excreted from tissues of the body quickly to prevent prolonged exposure to the radiation of the radiolabeled compound administered to the subject. Typically, the presently disclosed compounds are eliminated from the body in less than about 24 hours. More typically, the presently disclosed compounds are eliminated from the body in less than about 16 hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes. Exemplary compounds are eliminated in between about 60 minutes and about 120 minutes. In certain embodiments, the presently disclosed compounds are stable in vivo such that substantially all, e.g., more than about 50%, 60%, 70%, 80%, or 90% of the injected compound is not metabolized by the body prior to excretion.

Additionally, for in vitro applications, such as in vitro diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use, such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

Other embodiments provide kits comprising a compound of formula (I). In certain embodiments, the kit provides packaged pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of formula (I). In certain embodiments the packaged pharmaceutical composition will comprise the reaction precursors necessary to generate the compound of formula (I) upon combination with a radiolabeled precursor. Other packaged pharmaceutical compositions further comprise indicia comprising at least one of: instructions for preparing compounds of formula (I) from supplied precursors, instructions for using the composition to image cells or tissues expressing FAP- α .

In certain embodiments, a kit containing from about 1 to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier, is provided. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound of formula (I) in solution or in lyophilized form, and these components of the kit may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form. Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

In certain embodiments, a kit provides a non-radiolabeled precursor to be combined with a radiolabeled reagent on-site.

Imaging agents may be used in accordance with the presently disclosed methods by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with FAP- α . The spatial distribution may be measured using any means suitable for the particular label, for example, a gamma camera, a PET apparatus, a

SPECT apparatus, and the like. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions or fluorescence. A particularly useful imaging approach employs more than one imaging agent to perform simultaneous studies.

In general, a detectably effective amount of the imaging agent of the invention is administered to a subject. A “detectably effective amount” of the imaging agent is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent may be administered in more than one injection. The detectably effective amount of the imaging agent of the invention can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective amounts of the imaging agent also can vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art. The amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the amount of imaging agent to administer to each individual patient and the duration of the imaging study.

D. Methods of Treating a FAP- α Related Disease or Disorder using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof

In other embodiments, the presently disclosed compounds of formula (I) can be used to treat a subject afflicted with one or more FAP- α related diseases or disorders including, but not limited to: (a) proliferation (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c) endocrinological disorders (including but not limited to disorders of glucose metabolism).

Accordingly, in some embodiments, the one or more FAP- α related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and

connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

In general, the “effective amount” of an active agent or drug delivery device refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the makeup of the pharmaceutical composition, the target tissue, and the like.

In other embodiments, the method can be practiced *in vitro* or *ex vivo* by introducing, and preferably mixing, the compound and cell(s) or tumor(s) in a controlled environment, such as a culture dish or tube. The method can be practiced *in vivo*, in which case contacting means exposing the target in a subject to at least one compound of the presently disclosed subject matter, such as administering the compound to a subject via any suitable route. According to the presently disclosed subject matter, contacting may comprise introducing, exposing, and the like, the compound at a site distant to the cells to be contacted, and allowing the bodily functions of the subject, or natural (e.g., diffusion) or man-induced (e.g., swirling) movements of fluids to result in contact of the compound and the target.

The subject treated by the presently disclosed methods in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term “subject.” Accordingly, a “subject” can include a human subject for medical purposes, such as for the treatment of an existing condition or disease or the prophylactic treatment for preventing the onset of a condition or disease, or an animal (non-human) subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like. An animal may be a transgenic animal. In some

embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a condition or disease. Thus, the terms “subject” and “patient” are used interchangeably herein. In some embodiments, the subject is human. In other embodiments, the subject is non-human.

As used herein, the term “treating” can include reversing, alleviating, inhibiting the progression of, preventing or reducing the likelihood of the disease, or condition to which such term applies, or one or more symptoms or manifestations of such disease or condition.

“Preventing” refers to causing a disease, condition, or symptom or manifestation of such, or worsening of the severity of such, not to occur. Accordingly, the presently disclosed compounds can be administered prophylactically to prevent or reduce the incidence or recurrence of the disease, or condition.

II. DEFINITIONS

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs.

While the following terms in relation to compounds of formula (I) are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. These definitions are intended to supplement and illustrate, not preclude, the definitions that would be apparent to one of ordinary skill in the art upon review of the present disclosure.

The terms substituted, whether preceded by the term “optionally” or not, and substituent, as used herein, refer to the ability, as appreciated by one skilled in this art, to change one functional group for another functional group on a molecule, provided that the valency of all atoms is maintained. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. The substituents also may be further substituted (e.g., an aryl group substituent may

have another substituent off it, such as another aryl group, which is further substituted at one or more positions).

Where substituent groups or linking groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $\text{-CH}_2\text{O-}$ is equivalent to $\text{-OCH}_2\text{-}$; -C(=O)O- is equivalent to -OC(=O)- ; -OC(=O)NR- is equivalent to -NRC(=O)O- , and the like.

When the term “independently selected” is used, the substituents being referred to (e.g., R groups, such as groups R_1 , R_2 , and the like, or variables, such as “m” and “n”), can be identical or different. For example, both R_1 and R_2 can be substituted alkyls, or R_1 can be hydrogen and R_2 can be a substituted alkyl, and the like.

The terms “a,” “an,” or “a(n),” when used in reference to a group of substituents herein, mean at least one. For example, where a compound is substituted with “an” alkyl or aryl, the compound is optionally substituted with at least one alkyl and/or at least one aryl. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

A named “R” or group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative “R” groups as set forth above are defined below.

Descriptions of compounds of the present disclosure are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

Unless otherwise explicitly defined, a “substituent group,” as used herein, includes a functional group selected from one or more of the following moieties, which are defined herein:

The term hydrocarbon, as used herein, refers to any chemical group comprising hydrogen and carbon. The hydrocarbon may be substituted or unsubstituted. As would be known to one skilled in this art, all valencies must be satisfied in making any substitutions. The hydrocarbon may be unsaturated, saturated, branched, unbranched, cyclic, polycyclic, or heterocyclic. Illustrative hydrocarbons are further defined herein below and include, for example, methyl, ethyl, *n*-propyl, isopropyl, cyclopropyl, allyl, vinyl, *n*-butyl, *tert*-butyl, ethynyl, cyclohexyl, and the like.

The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched chain, acyclic or cyclic hydrocarbon group, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent groups, having the number of carbon atoms designated (i.e., C₁-C₁₀ means one to ten carbons, including 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 carbons). In particular embodiments, the term “alkyl” refers to C₁₋₂₀ inclusive, including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 carbons, linear (i.e., “straight-chain”), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (i.e., alkenyl and alkynyl) hydrocarbon radicals derived from a hydrocarbon moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom.

Representative saturated hydrocarbon groups include, but are not limited to, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *sec*-pentyl, isopentyl, neopentyl, *n*-hexyl, *sec*-hexyl, *n*-heptyl, *n*-octyl, *n*-decyl, *n*-undecyl, dodecyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, and homologs and isomers thereof.

“Branched” refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. “Lower alkyl” refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C₁₋₈ alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. “Higher alkyl” refers to an alkyl group having about 10 to about 20 carbon atoms, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, “alkyl” refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, “alkyl” refers, in particular, to C₁₋₈ branched-chain alkyls.

Alkyl groups can optionally be substituted (a “substituted alkyl”) with one or more alkyl group substituents, which can be the same or different. The term “alkyl group substituent” includes but is not limited to alkyl, substituted alkyl, halo, alkylamino, arylamino, acyl, hydroxyl, aryloxy, alkoxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as “alkylaminoalkyl”), or aryl.

Thus, as used herein, the term “substituted alkyl” includes alkyl groups, as defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

The term “heteroalkyl,” by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon group, or combinations thereof, consisting of at least one carbon atoms and at least one heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen, phosphorus, and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{N}(\text{CH}_3)-\text{CH}_3$, $-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})-\text{CH}_3$, $-\text{CH}_2-\text{CH}_2-\text{S}(\text{O})_2-\text{CH}_3$, $-\text{CH}=\text{CH}-\text{O}-\text{CH}_3$, $-\text{Si}(\text{CH}_3)_3$, $-\text{CH}_2-\text{CH}=\text{N}-\text{OCH}_3$, $-\text{CH}=\text{CH}-\text{N}(\text{CH}_3)-\text{CH}_3$, $\text{O}-\text{CH}_3$, $-\text{O}-\text{CH}_2-\text{CH}_3$, and $-\text{CN}$. Up to two or three heteroatoms may be consecutive, such as, for example, $-\text{CH}_2-\text{NH}-\text{OCH}_3$ and $-\text{CH}_2-\text{O}-\text{Si}(\text{CH}_3)_3$.

As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as $-\text{C}(\text{O})\text{NR}'$, $-\text{NR}'\text{R}$, $-\text{OR}'$, $-\text{SR}$, $-\text{S}(\text{O})\text{R}$, and/or $-\text{S}(\text{O}_2)\text{R}'$. Where “heteroalkyl” is recited, followed by recitations of specific heteroalkyl groups, such as $-\text{NR}'\text{R}$ or the like, it will be understood that the terms heteroalkyl and $-\text{NR}'\text{R}$ are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term “heteroalkyl” should not be interpreted herein as excluding specific

heteroalkyl groups, such as -NR'R'' or the like.

“Cyclic” and “cycloalkyl” refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, unsubstituted alkyl, substituted alkyl, aryl, or substituted aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl, and fused ring systems, such as dihydro- and tetrahydronaphthalene, and the like.

The term “cycloalkylalkyl,” as used herein, refers to a cycloalkyl group as defined hereinabove, which is attached to the parent molecular moiety through an alkyl group, also as defined above. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

The terms “cycloheteroalkyl” or “heterocycloalkyl” refer to a non-aromatic ring system, unsaturated or partially unsaturated ring system, such as a 3- to 10-member substituted or unsubstituted cycloalkyl ring system, including one or more heteroatoms, which can be the same or different, and are selected from the group consisting of nitrogen (N), oxygen (O), sulfur (S), phosphorus (P), and silicon (Si), and optionally can include one or more double bonds.

The cycloheteroalkyl ring can be optionally fused to or otherwise attached to other cycloheteroalkyl rings and/or non-aromatic hydrocarbon rings. Heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or a polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5-membered ring has

0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds, and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative cycloheteroalkyl ring systems include, but are not limited to pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolynyl, pyrazolidinyl, pyrazolynyl, piperidyl, piperazinyl, indolynyl, quinuclidinyl, morpholynyl, thiomorpholynyl, thiadiazinyl, tetrahydrofuranyl, and the like.

The terms “cycloalkyl” and “heterocycloalkyl”, by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl”, respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholynyl, 3-morpholynyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The terms “cycloalkylene” and “heterocycloalkylene” refer to the divalent derivatives of cycloalkyl and heterocycloalkyl, respectively.

An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. Alkyl groups which are limited to hydrocarbon groups are termed “homoalkyl.”

More particularly, the term “alkenyl” as used herein refers to a monovalent group derived from a C₁₋₂₀ inclusive straight or branched hydrocarbon moiety having at least one carbon-carbon double bond by the removal of a single hydrogen molecule. Alkenyl groups include, for example, ethenyl (i.e., vinyl), propenyl, butenyl, 1-methyl-2-buten-1-yl, pentenyl, hexenyl, octenyl, allenyl, and butadienyl.

The term “cycloalkenyl” as used herein refers to a cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

The term “alkynyl” as used herein refers to a monovalent group derived from a straight or branched C₁₋₂₀ hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon triple bond. Examples of “alkynyl” include ethynyl, 2-propynyl (propargyl), 1-propynyl, pentynyl, hexynyl, and heptynyl groups, and the like.

The term “alkylene” by itself or a part of another substituent refers to a straight or branched bivalent aliphatic hydrocarbon group derived from an alkyl group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more “alkyl group substituents.” There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as “alkylaminoalkyl”), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene (–CH₂–); ethylene (–CH₂–CH₂–); propylene (–(CH₂)₃–); cyclohexylene (–C₆H₁₀–); –CH=CH–CH=CH–; –CH=CH–CH₂–; –CH₂CH₂CH₂CH₂–, –CH₂CH=CHCH₂–, –CH₂CsCCH₂–, –CH₂CH₂CH(CH₂CH₂CH₃)CH₂–, –(CH₂)_q–N(R)–(CH₂)_r–, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxy (–O–CH₂–O–); and ethylenedioxy (–O–(CH₂)₂–O–). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being some embodiments of the present disclosure. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

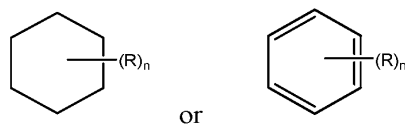
The term “heteroalkylene” by itself or as part of another substituent means a divalent group derived from heteroalkyl, as exemplified, but not limited by, –CH₂–CH₂–S–CH₂–CH₂– and –CH₂–S–CH₂–CH₂–NH–CH₂–. For heteroalkylene groups, heteroatoms also can occupy either or both of the chain termini (e.g., alkyleneoxo, alkylenedioxo, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula –C(O)OR’– represents both –C(O)OR’– and –R’OC(O)–.

The term “aryl” means, unless otherwise stated, an aromatic hydrocarbon substituent that can be a single ring or multiple rings (such as from 1 to 3 rings), which are fused together or linked covalently. The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms (in each separate ring in the case of multiple rings) selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalyl, 5-quinoxalyl, 3-quinolyl, and 6-quinolyl. Substituents for each of above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. The terms “arylene” and “heteroarylene” refer to the divalent forms of aryl and heteroaryl, respectively.

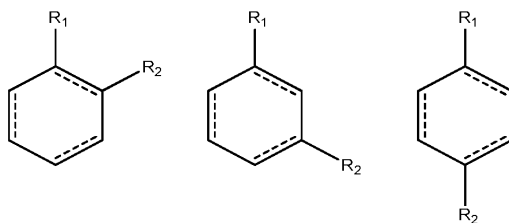
For brevity, the term “aryl” when used in combination with other terms (e.g., aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the terms “arylalkyl” and “heteroarylalkyl” are meant to include those groups in which an aryl or heteroaryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl, furylmethyl, and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like). However, the term “haloaryl,” as used herein is meant to cover only aryls substituted with one or more halogens.

Where a heteroalkyl, heterocycloalkyl, or heteroaryl includes a specific number of members (e.g. “3 to 7 membered”), the term “member” refers to a carbon or heteroatom.

Further, a structure represented generally by the formula:



as used herein refers to a ring structure, for example, but not limited to a 3-carbon, a 4-carbon, a 5-carbon, a 6-carbon, a 7-carbon, and the like, aliphatic and/or aromatic cyclic compound, including a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure, comprising a substituent R group, wherein the R group can be present or absent, and when present, one or more R groups can each be substituted on one or more available carbon atoms of the ring structure. The presence or absence of the R group and number of R groups is determined by the value of the variable “n,” which is an integer generally having a value ranging from 0 to the number of carbon atoms on the ring available for substitution. Each R group, if more than one, is substituted on an available carbon of the ring structure rather than on another R group. For example, the structure above where n is 0 to 2 would comprise compound groups including, but not limited to:



and the like.

A dashed line representing a bond in a cyclic ring structure indicates that the bond can be either present or absent in the ring. That is, a dashed line representing a bond in a cyclic ring structure indicates that the ring structure is selected from the group consisting of a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure.

The symbol () denotes the point of attachment of a moiety to the remainder of the molecule.

When a named atom of an aromatic ring or a heterocyclic aromatic ring is defined as being “absent,” the named atom is replaced by a direct bond.

Each of above terms (e.g., “alkyl,” “heteroalkyl,” “cycloalkyl,” and “heterocycloalkyl,” “aryl,” “heteroaryl,” “phosphonate,” and “sulfonate” as well as their divalent derivatives) are meant to include both substituted and unsubstituted forms of the indicated group. Optional substituents for each type of group are provided below.

Substituents for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl monovalent

and divalent derivative groups (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R'', -NR''C(O)OR', -NR-C(NR'R'')=NR'', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such groups. R', R'', R''' and R'''' each may independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. As used herein, an "alkoxy" group is an alkyl attached to the remainder of the molecule through a divalent oxygen. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7- membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1- pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

Similar to the substituents described for alkyl groups above, exemplary substituents for aryl and heteroaryl groups (as well as their divalent derivatives) are varied and are selected from, for example: halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R'', -NR''C(O)OR', -NR-C(NR'R'')=NR'', -NR-C(NR'R'')=NR'' -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxo, and fluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on aromatic ring system; and where R', R'', R''' and R'''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or

unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present.

Two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 4.

One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -(CRR')_s-X'-(C''R'')_d-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'' and R''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent and has the general formula RC(=O)-, wherein R is an alkyl, alkenyl, alkynyl, aryl, carbocyclic, heterocyclic, or aromatic heterocyclic group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as a 2-(furan-2-yl)acetyl- and a 2-phenylacetyl group. Specific examples of acyl groups include acetyl and benzoyl. Acyl groups also are intended to include amides, -RC(=O)NR', esters, -RC(=O)OR', ketones, -RC(=O)R', and aldehydes, -RC(=O)H.

The terms "alkoxyl" or "alkoxy" are used interchangeably herein and refer to a saturated (i.e., alkyl-O-) or unsaturated (i.e., alkenyl-O- and alkynyl-O-) group attached to the parent molecular moiety through an oxygen atom, wherein the terms "alkyl," "alkenyl," and "alkynyl" are as previously described and can include C₁₋₂₀ inclusive, linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, *n*-butoxyl, *sec*-butoxyl, *tert*-butoxyl, and *n*-pentoxy, neopentoxy, *n*-hexoxyl, and the like.

The term “alkoxyalkyl” as used herein refers to an alkyl-O-alkyl ether, for example, a methoxyethyl or an ethoxymethyl group.

“Aryloxy” refers to an aryl-O- group wherein the aryl group is as previously described, including a substituted aryl. The term “aryloxy” as used herein can refer to phenyloxy or hexyloxy, and alkyl, substituted alkyl, halo, or alkoxy substituted phenyloxy or hexyloxy.

“Aralkyl” refers to an aryl-alkyl-group wherein aryl and alkyl are as previously described and includes substituted aryl and substituted alkyl. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

“Aralkyloxy” refers to an aralkyl-O- group wherein the aralkyl group is as previously described. An exemplary aralkyloxy group is benzyloxy, i.e., $C_6H_5-CH_2-O-$. An aralkyloxy group can optionally be substituted.

“Alkoxycarbonyl” refers to an alkyl-O-C(=O)- group. Exemplary alkoxycarbonyl groups include methoxycarbonyl, ethoxycarbonyl, butyloxycarbonyl, and *tert*-butyloxycarbonyl.

“Aryloxycarbonyl” refers to an aryl-O-C(=O)- group. Exemplary aryloxycarbonyl groups include phenoxy- and naphthoxy-carbonyl.

“Aralkoxycarbonyl” refers to an aralkyl-O-C(=O)- group. An exemplary aralkoxycarbonyl group is benzyloxycarbonyl.

“Carbamoyl” refers to an amide group of the formula $-C(=O)NH_2$. “Alkylcarbamoyl” refers to a $R'RN-C(=O)-$ group wherein one of R and R' is hydrogen and the other of R and R' is alkyl and/or substituted alkyl as previously described. “Dialkylcarbamoyl” refers to a $R'RN-C(=O)-$ group wherein each of R and R' is independently alkyl and/or substituted alkyl as previously described.

The term carbonyldioxy, as used herein, refers to a carbonate group of the formula $-O-C(=O)-OR$.

“Acyloxy” refers to an acyl-O- group wherein acyl is as previously described.

The term “amino” refers to the $-NH_2$ group and also refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms “acylamino” and “alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

An “aminoalkyl” as used herein refers to an amino group covalently bound to an alkylene linker. More particularly, the terms alkylamino, dialkylamino, and

trialkylamino as used herein refer to one, two, or three, respectively, alkyl groups, as previously defined, attached to the parent molecular moiety through a nitrogen atom. The term alkylamino refers to a group having the structure -NHR' wherein R' is an alkyl group, as previously defined; whereas the term dialkylamino refers to a group having the structure -NR'R'' , wherein R' and R'' are each independently selected from the group consisting of alkyl groups. The term trialkylamino refers to a group having the structure -NR'R''R''' , wherein R' , R'' , and R''' are each independently selected from the group consisting of alkyl groups. Additionally, R' , R'' , and/or R''' taken together may optionally be $\text{-(CH}_2\text{)}_k\text{-}$ where k is an integer from 2 to 6. Examples include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, isopropylamino, piperidino, trimethylamino, and propylamino.

The amino group is -NR'R'' , wherein R' and R'' are typically selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

The terms alkylthioether and thioalkoxyl refer to a saturated (i.e., alkyl-S-) or unsaturated (i.e., alkenyl-S- and alkynyl-S-) group attached to the parent molecular moiety through a sulfur atom. Examples of thioalkoxyl moieties include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, *n*-butylthio, and the like.

“Acylamino” refers to an acyl-NH- group wherein acyl is as previously described. “Aroylamino” refers to an aroyl-NH- group wherein aroyl is as previously described.

The term “carbonyl” refers to the -C(=O)- group, and can include an aldehyde group represented by the general formula R-C(=O)H .

The term “carboxyl” refers to the -COOH group. Such groups also are referred to herein as a “carboxylic acid” moiety.

The terms “halo,” “halide,” or “halogen” as used herein refer to fluoro, chloro, bromo, and iodo groups. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo($\text{C}_1\text{-C}_4$)alkyl” is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term “hydroxyl” refers to the -OH group.

The term “hydroxyalkyl” refers to an alkyl group substituted with an –OH group.

The term “mercapto” refers to the –SH group.

The term “oxo” as used herein means an oxygen atom that is double bonded to a carbon atom or to another element.

The term “nitro” refers to the –NO₂ group.

The term “thio” refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term “sulfate” refers to the –SO₄ group.

The term thiohydroxyl or thiol, as used herein, refers to a group of the formula –SH.

More particularly, the term “sulfide” refers to compound having a group of the formula –SR.

The term “sulfone” refers to compound having a sulfonyl group –S(O₂)R.

The term “sulfoxide” refers to a compound having a sulfinyl group –S(O)R.

The term ureido refers to a urea group of the formula –NH—CO—NH₂.

Throughout the specification and claims, a given chemical formula or name shall encompass all tautomers, congeners, and optical- and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

Certain compounds of the present disclosure may possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as D- or L- for amino acids, and individual isomers are encompassed within the scope of the present disclosure. The compounds of the present disclosure do not include those which are known in art to be too unstable to synthesize and/or isolate. The present disclosure is meant to include compounds in racemic, scalemic, and optically pure forms. Optically active (R)- and (S)-, or D- and L-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each

asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the disclosure.

It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure. The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

As used herein the term "monomer" refers to a molecule that can undergo polymerization, thereby contributing constitutional units to the essential structure of a macromolecule or polymer.

A "polymer" is a molecule of high relative molecule mass, the structure of which essentially comprises the multiple repetition of unit derived from molecules of low relative molecular mass, i.e., a monomer.

A "dendrimer" is highly branched, star-shaped macromolecules with nanometer-scale dimensions.

As used herein, an "oligomer" includes a few monomer units, for example, in contrast to a polymer that potentially can comprise an unlimited number of monomers. Dimers, trimers, and tetramers are non-limiting examples of oligomers.

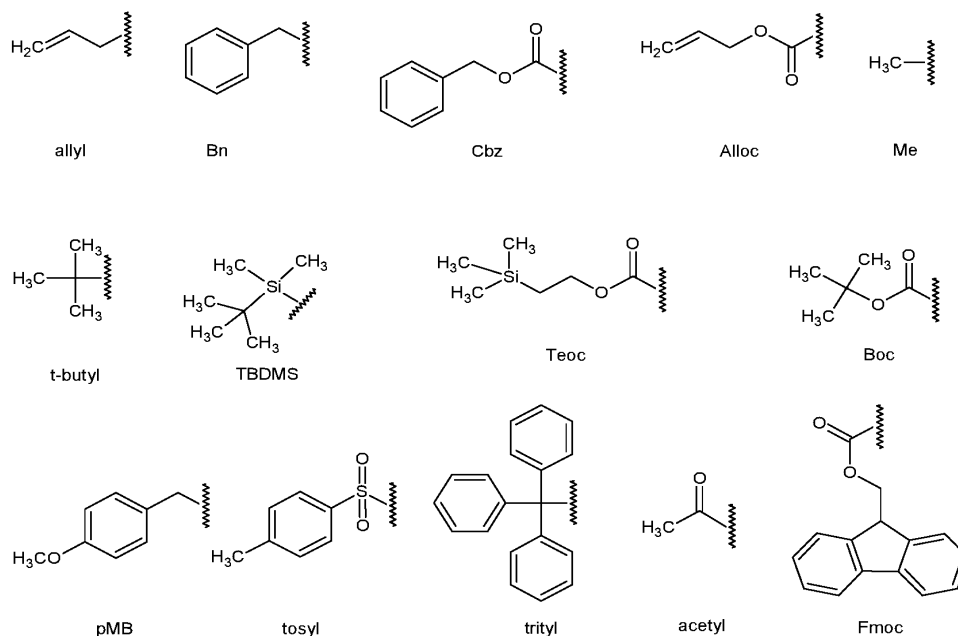
The term "protecting group" refers to chemical moieties that block some or all reactive moieties of a compound and prevent such moieties from participating in chemical reactions until the protective group is removed, for example, those moieties listed and described in T. W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, 3rd ed. John Wiley & Sons (1999). It may be advantageous, where different protecting groups are employed, that each (different) protective group be removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions allow differential removal of such protecting groups. For example, protective groups can be removed by acid, base, and hydrogenolysis. Groups such as trityl, dimethoxytrityl, acetal and tert-butyldimethylsilyl are acid labile and may be used to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by hydrogenolysis, and Fmoc groups, which are base labile. Carboxylic acid and hydroxy reactive moieties may be blocked with base labile groups such as, without limitation, methyl, ethyl, and acetyl in the presence of amines blocked with acid labile

groups such as tert-butyl carbamate or with carbamates that are both acid and base stable but hydrolytically removable.

Carboxylic acid and hydroxy reactive moieties may also be blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids may be blocked with base labile groups such as Fmoc. Carboxylic acid reactive moieties may be blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups may be blocked with fluoride labile silyl carbamates.

Allyl blocking groups are useful in the presence of acid- and base- protecting groups since the former are stable and can be subsequently removed by metal or pi-acid catalysts. For example, an allyl-protected carboxylic acid can be deprotected with a palladium(O)- catalyzed reaction in the presence of acid labile t-butyl carbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate may be attached. As long as the residue is attached to the resin, that functional group is blocked and cannot react. Once released from the resin, the functional group is available to react.

Typical blocking/protecting groups include, but are not limited to the following moieties:



Following long-standing patent law convention, the terms “a,” “an,” and “the” refer to “one or more” when used in this application, including the claims. Thus, for example, reference to “a subject” includes a plurality of subjects, unless the context clearly is to the contrary (e.g., a plurality of subjects), and so forth.

Throughout this specification and the claims, the terms “comprise,” “comprises,” and “comprising” are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about” even though the term “about” may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term “about,” when referring to a value can be meant to encompass variations of, in some embodiments, $\pm 100\%$ in some embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, the term “about” when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as

well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range.

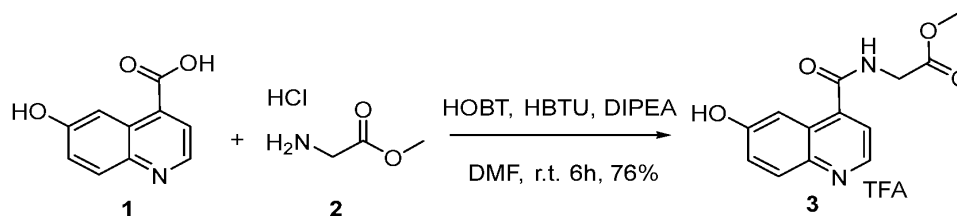
EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLE 1

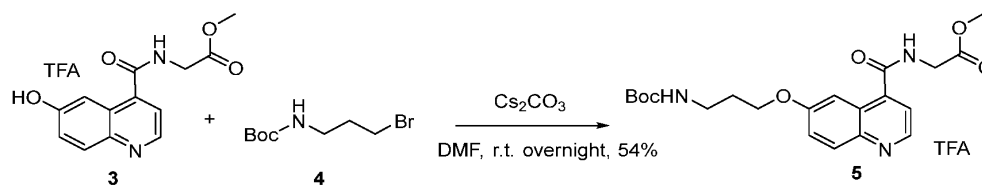
Experimental Procedures

1.1 Synthesis of XY-FAP-01.

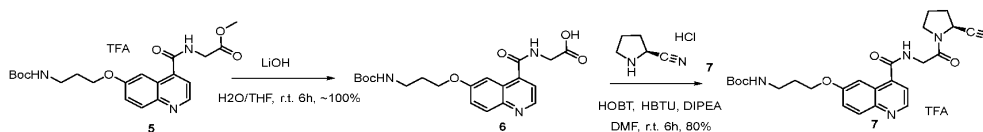


Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**): 6-Hydroxyquinoline-4-carboxylic acid (**1**) 210 mg (1.1 mmol), methyl glycinate HCl salt (**2**) 143 mg (1.1 mmol), HBTU 420 mg (1.1 mmol) and HOBt 170 mg (1.1 mmol) were dissolved in 12 mL dry DMF. To the solution, 0.77 mL of DIPEA (4.4 mmol) was added. The reaction was stirred at room temperature for 6 h. After the solvent was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and the product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 290 mg of product **3** was obtained as a yellow powder with a yield of 76%. ¹H-NMR (400 MHz, CD₃OD): δ 8.69 (s, 1H), 7.94 (d, J = 7.92 Hz, 1H), 7.57–7.51 (m, 3H), 7.42–7.37 (m, 1H), 4.21 (s, 2H), 3.81 (s, 3H). ¹³C-NMR (100 MHz, CD₃OD): δ

172.4, 160.9, 145.1, 143.7, 129.7, 129.4, 128.3, 121.8, 119.6, 112.4, 109.1, 56.8, 44.8.
MS: calculated for $[C_{13}H_{13}N_2O_4]^+$, 261.3 $[M + H]^+$; found 261.1.

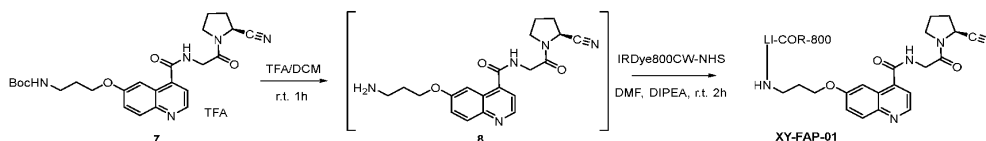


Methyl (6-(3-((tert-butoxycarbonyl)amino)propoxy)quinoline-4-carbonyl)glycinate (**5**): Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**) 360 mg (1.0 mmol), tert-butyl (3-bromopropyl)carbamate (**4**) 500 mg (2.1 mmol) were dissolved in 20 mL DMF. Cs_2CO_3 1 g (3.0 mmol) was added to the solution and the reaction was stirred at room temperature overnight. After filtration, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 270 mg of product **5** was obtained with a yield of 54%. ^1H -NMR (400 MHz, $CDCl_3$): δ 8.68–8.37 (m, 2H), 8.02 (d, $J = 9.1$ Hz, 1H), 7.80 (s, 1H), 7.72–7.64 (m, 1H), 7.40 (d, $J = 9.1$ Hz, 1H), 4.94 (br s, 1H), 4.41–4.31 (m, 2H), 4.27–4.18 (m, 2H), 3.85 (s, 3H), 3.44–3.30 (m, 2H), 2.13–2.00 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 167.2, 158.4, 144.7, 142.3, 128.4, 126.1, 124.7, 119.1, 103.7, 79.5, 60.4, 52.5, 41.4, 37.7, 29.3, 28.4. MS: calculated for $[C_{21}H_{28}N_3O_6]^+$, 418.5 $[M + H]^+$; found 418.3.



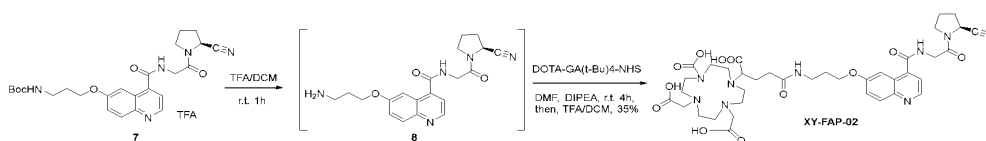
tert-Butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate (**7**): Compound **5** 110 mg (0.21 mmol) and LiOH 30 mg (1.2 mmol) was stirred in 4 mL of H_2O/THF (1/1) for 6 hours. After most of the THF was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and eluted with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1) to remove the salts. The product **6** obtained was mixed with (S)-pyrrolidine-2-carbonitrile 53 mg (0.4 mmol), HOBT 68 mg (0.4 mmol), HBTU 152 mg (0.4 mmol) and DIPEA 0.56 mL (1.6 mmol) in dry 10 mL DMF. After 6 hours, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was

purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 99 mg of **7** was obtained with a yield of 80%. ^1H NMR (400 MHz, CDCl_3): δ 8.73 (s, 1H), 7.95 (d, J = 10.2 Hz, 1H), 7.68 (br s, 1H), 7.63–7.56 (m, 1H), 7.56–7.48 (m, 1H), 7.38–7.29 (m, 1H), 5.27 (br s, 1H), 4.84–4.72 (m, 1H), 4.46–4.35 (m, 1H), 4.33–4.20 (m, 1H), 4.17–4.09 (m, 2H), 3.78–3.64 (m, 1H), 3.59–3.46 (m, 1H), 3.36 (s, 2H), 2.38–2.17 (m, 4H), 1.42 (s, 9H), 1.35–1.27 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3): δ 167.6, 167.5, 157.9, 156.2, 146.3, 130.2, 125.7, 123.7, 119.3, 118.0, 103.3, 79.0, 65.9, 46.8, 45.7, 42.2, 37.6, 29.8, 29.3, 28.4, 25.1. MS: calculated for $[\text{C}_{25}\text{H}_{32}\text{N}_5\text{O}_5]^+$, 482.6 $[\text{M} + \text{H}]^+$; found 482.3.



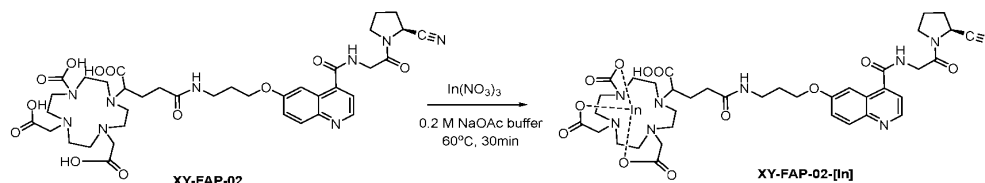
XY-FAP-01. Compound **7** (1 mg, 1.7 μmol) was treated with a 1 mL solution of TFA/methylene chloride (1/1) for 2 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMSO. To the solution, LICOR800CW-NHS ester 0.5 mg (0.43 μmol) and Et_3N 10 μL were added. After 1 h at room temperature, the solvent was removed and the product was purified by HPLC. 0.5 mg product was obtained with a yield of 85%. HPLC condition: column Phenomenex, Luna 10 x 250 mm, 10 μ . Gradient 10/90/0.1 MeCN/ H_2O /TFA to 80/20/0.1 MeCN/ H_2O /TFA within 15 min at a flow of 3 mL/min. The product was eluted at 10.1 min. MS: Calculated for $[\text{C}_{66}\text{H}_{76}\text{N}_7\text{O}_{17}\text{S}_4]^+$, 1366.4 $[\text{M} + \text{H}]^+$; found 1366.8.

1.2 Synthesis of XY-FAP-02



2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**XY-FAP-02**): Compound **7** (15 mg, 31.3 μmol) was treated with a 1-mL solution of TFA/methylene chloride (1/1) for 1 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMF. To the solution, DIPEA (27 μL , 156.5 μmol) was added, followed by dropwise addition of a solution of DOTA-GA(t-Bu) $_4$ -NHS (25 mg, 31.3 μL) in 0.5

mL of DMF. The reaction mixture was stirred for 4 h at ambient temperature and then concentrated under vacuum. The t-Bu-protected intermediate was deprotected in situ without further purification using a 1 mL mixture of TFA, H₂O and triethylsilane (TES) (95:2.5:2.5). Reaction mixture was then concentrated and purified by semipreparative HPLC, to afford the product as a white solid (8.5 mg, 33% yield). MS: calculated for [C₃₉H₅₄N₉O₁₂]⁺, 840.9 [M + H]⁺; found 840.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 µm, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02** eluted at 11.8 min.



XY-FAP-02-[In]. ^{113/115}Indium(III) 2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**XY-FAP-02-[In]**): To a solution of 2 mg (2.4 µmol) of **XY-FAP-02** in 1 mL of 0.2M AcONa, a solution of 1.4 mg (4.6 µmol) of In(NO₃)₃ in 0.5 mL water is added and warmed in a 60 °C bath for 30 min. After cooling to ambient temperature, the mixture was purified by semipreparative HPLC. The product was obtained as a white solid (1.8 mg, 79% yield). MS: calculated for [C₃₉H₅₁N₉O₁₂In]⁺, 951.7 [M + H]⁺; found 952.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 µm, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02-[In]** eluted at 14.0 min.

1.3 Radiolabeling Methods. Briefly, 20 mg **XY-FAP-02** solution in 20 mL of 0.2 M NaOAc was added to 10 mL 4.6 mCi ¹¹¹InCl₃ solution (Nordion, Ottawa, Canada) and adjusted to a final pH of 5.5-6. The mixture was heated in a water bath at 70 °C for 30 min and, after the reaction completed, was diluted with 200 mL of water for HPLC purification. The solution was purified using a Phenomenex 5 µm C₁₈ Luna 4.6 x 250 mm² column (Torrance, CA) with a flow rate of 0.6 mL/min with water (0.1% TFA) (A) and MeCN (0.1% TFA) (B) as the eluting solvents. An isocratic solution of 88% A and 12% B was utilized for purification, resulting in the labeled compound, ¹¹¹In-**XY-FAP-02**, eluting first at 18.6 min followed by the unlabeled

starting material at 23.5 min. 3.2 mCi of labeled compound was obtained as pure product with a yield of 69%. Another reaction with the identical condition was performed with 74% yield. The collected radioactivity was diluted with 20 mL of water and loaded onto activated Sep-Pak (WAT020515, Waters, Milford, MA). After the Sep-Pak was washed with 10 mL of water, ¹¹¹In-XY-FAP-02 was eluted with 1.5 mL of ethanol. The ethanol was evaporated under a gentle stream of N₂ (to a total volume of < 50 µL). The resulting solution was formulated in saline for the imaging and biodistribution studies.

1.4 FAP Inhibition Assay. The inhibitory activity of XY-FAP-01 was determined using a fluorogenic FAP Assay Kit (BPS Bioscience, San Diego, CA). Briefly, XY-FAP-01, DPP substrate, and human recombinant FAP were loaded into a 96 well plate to initiate the enzyme reaction. The reaction was left for 10 minutes at room temperature before fluorescence was measured with a VICTOR3 V multilabel plate reader (PerkinElmer Inc., Waltham, MA). Data was normalized and semi-log inhibition curves were generated in order to determine the IC₅₀ value (concentration of XY-FAP-01 where the enzyme activity is 50% inhibited) for XY-FAP-01 and subsequent enzyme inhibition constant (K_i) using the Cheng-Prusoff conversion. Generation of semi-log inhibition curves and IC₅₀ values were done using GraphPad Prism (San Diego, CA).

1.5 Cell lines. Six human cancer cell lines were used to assess binding to FAP: glioblastoma (U-87-MG), melanoma (SK-MEL-24), prostate (PC-3), non-small cell lung cancer (NCI-H2228), colorectal carcinoma (HCT 116), and lung squamous cell carcinoma (NCI-H226). From the literature, U-87-MG, SK-MEL-24, and NCI-H2228 cell lines were identified as having high levels of FAP expression [FAP-positive (+)] whereas PC-3, NCI-H226, and HCT 116 cells expressed very low levels of FAP [FAP-negative(-)]. These expression profiles were further confirmed via flow cytometry with an APC-conjugated anti-FAP antibody (R&D Systems, Minneapolis, MN) and quantitative real-time PCR. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA).

U-87-MG cells were maintained in MEM medium (Corning Cellgro, Manassas, VA), containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO) and 1% penicillin-streptomycin (Corning Cellgro, Manassas, VA), supplemented with sodium bicarbonate (Corning), sodium pyruvate (Gibco, Gaithersburg, MD), and MEM non-essential amino acids (Gibco). SK-MEL-24 cells

were maintained in MEM medium, containing 15% FBS and 1% penicillin-streptomycin, supplemented with sodium bicarbonate, sodium pyruvate, and MEM non-essential amino acids. PC-3 cells were grown in Ham's F-12K medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. NCI-H2228, NCI-H226, and HCT 116 cells were cultured in RPMI 1640 medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. All cell cultures were maintained at 37 °C and 5% carbon dioxide (CO₂) in a humidified incubator.

1.6 Cellular Uptake Studies. All cellular uptake and specific binding studies were performed in triplicate to ensure reproducibility. Cells were detached using 0.05% trypsin (Corning), resuspended in 1 million cell aliquots in binding buffer, and incubated with various concentrations (range, 50 nM to 0.78 nM) of **XY-FAP-01** for 1 hour at 37 °C and 5% CO₂. To assess the specific uptake of **XY-FAP-02**, cells were preblocked with a FAP and DPP-IV specific inhibitor (Val-boroPro, MilliporeSigma, Burlington, MA) or a DPP-IV specific inhibitor (Sitagliptin, Santa Cruz Biotechnology, Inc., Dallas, TX) at various concentrations (range, 10⁻¹⁰ M to 10⁻⁴ M) prior to incubation with 25 nM **XY-FAP-02** solution in binding buffer for 1 hour at 37 °C and 5% CO₂. Cellular uptake was terminated by washing cells with ice cold PBS (1x) three times. Cells were resuspended in binding buffer and transferred to a 96-well plate for imaging. Images were acquired on the LI-COR Pearl Impulse Imager (Lincoln, NE) using an excitation wavelength of 785 nm and detection of the emission wavelength at 800 nm. Images were analyzed using the LI-COR Pearl Impulse Software (Version 2.0) and fluorescence intensity was corrected for background signal and normalized to well area.

Cellular Uptake of ¹¹¹In-**XY-FAP-02** was also assessed in cells. Cell aliquots (1 million) were incubated with 1 µCi ¹¹¹In-**XY-FAP-02** in saline for 30 minutes at 37 °C and 5% CO₂. Cells were washed three times with cold PBS (1x) and activity of the cell pellets was measured with the 1282 CompuGamma CS gamma well counter (Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The percent uptake of the administered activity was calculated by comparison with samples of a standard dose.

1.7 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF images were acquired on the LI-COR Pearl Impulse Imager using an excitation wavelength of 785 nm and a detection wavelength of 800 nm. Mice utilized for imaging studies were anesthetized with 3% isoflurane (v/v) and maintained at 1.5% isoflurane for the

imaging procedure. NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 nmol of **XY-FAP-01** via tail vein injection and images were acquired at 30 min, 1 h, 2 h, 2.5 h, and 4 h after injection of tracer. Data were displayed and analyzed using the LI-COR Pearl Impulse Software (Version 2.0).

1.8 Small-Animal SPECT-CT Imaging. SPECT-CT studies were performed on NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts. For imaging studies, mice were anesthetized with 3% isoflurane prior to being placed on the scanner bed and kept warm with an external light source. Isoflurane levels were decreased to 1.5% for the rest of the imaging procedure. After mice were injected with 300 μCi $^{111}\text{In-XY-FAP-02}$ in 200 μL saline, SPECT-CT imaging was carried out using a CT-equipped Gamma Medica-Ideas SPECT scanner (Northridge, CA) at the indicated timepoints (30 min, 2 h, 6 h, and 24 h) post radiotracer injection. A CT scan was performed at the end of each SPECT scan for anatomical co-registration. Obtained data sets were reconstructed using the provided Gamma Medica-Ideas software and final data visualization and image generation were prepared using Amira® software (FEI, Hillsboro, OR).

1.9 Ex-vivo Biodistribution. NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 μCi $^{111}\text{In-XY-FAP-02}$ in 200 μL saline via the tail vein. At 5 min, 30 min, 2 h, 6 h, and 12 hr post injection, mice (n=4) were sacrificed by CO_2 asphyxiation and blood was immediately collected by cardiac puncture. Additionally, the heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, small intestine, large intestine, bladder, muscle, femur, FAP+ U-87-MG xenograft, and FAP- PC-3 xenograft were collected for biodistribution analysis. Each tissue was weighed and radioactivity was measuring using a 2480 Wizard² automated gamma counter (PerkinElmer, Waltham, MA). Radioactivity measurements were corrected for decay and compared with samples of a standard dilution of the initial dose to calculated percent injected dose per gram (%ID/g).

For blocking studies, mice (n=5 per group) were co-injected with unlabeled **XY-FAP-02** (50 μg per mouse) and 10 μCi $^{111}\text{In-XY-FAP-02}$ in 200 μL saline. Mice (n=5) injected with 10 μCi $^{111}\text{In-XY-FAP-02}$ in 200 μL saline served as a control. At 6 h post injection, mice were sacrificed, tissues were collected, and radioactivity was measured with the gamma well counter.

1.10 Data Analysis. Data are expressed at mean $\pm$ standard deviation (SD). Prism software (GraphPAD, San Diego, CA) was used for analysis and statistical

significance was calculated using a two-tailed Student's t test. A P-value <0.05 was considered significant.

1.11 Xenograft Tumor Model. 6-week old female NOD/SCID mice were subcutaneously injected in the upper left and right flanks with 1 million U87(FAP+) cells and PC3 cells (FAP-) in RPMI 1640 media supplemented with 1% FBS. Mice were monitored for tumor size and used for optical or SPECT/CT imaging when the size of tumor reached around 100 mm³.

EXAMPLE 2

Representative Results

2.1 FAP Inhibitory Assay. **XY-FAP-01** demonstrated high binding affinity to human recombinant FAP. The enzyme inhibitory constant (K_i) for the compound was determined to be 1.26 nM.

2.2 Cellular Uptake Studies. FAP-positive cell lines showed concentration dependent uptake of **XY-FAP-01** whereas FAP-negative cell lines showed no significant binding of **XY-FAP-01** at all concentrations (see, e.g., FIG. 3A). Saturated binding of **XY-FAP-01** was observed at concentration of 25 nM, which was subsequently used as the base concentration for all binding inhibition studies. When preblocked with a FAP and DPP-IV specific inhibitor, **XY-FAP-01** binding was significantly inhibited in FAP-positive cells (FIG. 3B). Interestingly, this phenomenon was not observed in FAP-positive cell lines preblocked with a DPP-IV specific inhibitor. These results further justify the specificity of **XY-FAP-01** for FAP over DPP-IV, since blocking of DPP-IV did not result in a change of binding ability of **XY-FAP-01**.

Similar specificity was observed with the radioactive analog, ¹¹¹In-**XY-FAP-02**. FAP positive cell line, U-87-MG, demonstrated over 30% uptake of administered radioactive dose after incubation whereas the FAP negative cell line, PC-3, had uptake of 0.01% of administered dose (FIG. 3C). Taken together, these results support the specificity of **XY-FAP-01** and ¹¹¹In-**XY-FAP-02** in the engagement of FAP *in vitro*.

2.3 Ex-vivo Biodistribution. *Ex-vivo* biodistribution of ¹¹¹In-**XY-FAP-02** results correlated with the observed imaging results (FIG. 4). Initially, the blood pool activity is very high, with over 10% %ID/g at 30 minutes post injection. With clearance of the compound, we see the blood pool activity drop significantly after 2

hours of distribution and remained less than 5% %ID/g from 2 hours post injection (FIG. 5A). High activity was also observed in pancreas, small intestines, and bladder until 2 hours post injection. Positive tumor uptake peaked at 30 minutes post injection and remained between 13-11% %ID/g up to 6 hours. Washout of tumor was observed at 12 hours post injection, with %ID/g dropping to below 5%. The PC-3, FAP negative xenograft had less than 3.5% %ID/g for all timepoints.

Co-injection of cold compound with ¹¹¹In-XY-FAP-02 resulted in significant blocking of tracer uptake in U-87 xenografts, with %ID/g dropping from 11.20% without blocking versus 0.27% with blocking ($p < 0.0001$). Additionally, blocking with cold compound resulted in %ID/g of all tissues dropping significantly, with most values being less than 0.1%. This decrease in uptake is most likely due to the blocking of non-specific binding of tracer to non-target tissues and the blocking of specific binding of FAP in U-87 xenografts.

2.4 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF imaging of XY-FAP-01 demonstrated specific uptake of tracer in the U-87-MG xenograft as early as 30 minutes post injection (FIG. 6). After one hour of distribution, tracer clearance via the bladder was observed with retained tracer uptake in the FAP positive xenograft. Tracer uptake was retained in the positive xenograft after four hours of distribution. In contrast, no significant uptake of tracer was observed in the FAP negative tumor at all imaging time points.

REFERENCES

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references (e.g., websites, databases, etc.) mentioned in the specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an

incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

- Allinen M, Beroukhi R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell*. 2004 Jul;6(1):17-32.
- Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon CJ, Koh GY, Kim J, Kim H. Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. *Br J Haematol*. 2008 Sep;142(5):827-30.
- Chen ZY, Wang YX, Lin Y, Zhang JS, Yang F, Zhou QL, Liao YY. Advance of molecular imaging technology and targeted imaging agent in imaging and therapy. *Biomed Res Int*. 2014; 2014 : 819324. PMCID: PMC3943245.
- Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740.
- Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740. Biodistribution, tumor detection, and radiation dosimetry of 18F-DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2012; 53:1883–1891.
- Edosada CY, Quan C, Tran T, Pham V, Wiesmann C, Fairbrother W, Wolf BB. Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly(2)-Pro(1)-cleaving specificity. *FEBS Lett*. 2006 Mar 6;580(6):1581-6.
- Fischer E, Chaitanya K, Wuest T, et al. Radioimmunotherapy of fibroblast activation protein positive tumors by rapidly internalizing antibodies. *Clin Cancer Res*. 2012;18:6208–6218.
- Franco OE, Shaw AK, Strand DW, Hayward SW. Cancer associated fibroblasts in cancer pathogenesis. *Semin Cell Dev Biol*. 2010 Feb;21(1):33-9.

- Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc Natl Acad Sci U S A*. 1990 Sep;87(18):7235-9. PMID: PMC54718.
- Jansen K, Heirbaut L, Cheng JD, Joossens J, Ryabtsova O, Cos P, Maes L, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Scaffold. *ACS Med Chem Lett*. 2013 Mar 18;4(5):491-6.
- Jansen K, Heirbaut L, Verkerk R, Cheng JD, Joossens J, Cos P, Maes L, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Extended structure-activity relationship and pharmacokinetic investigation of (4-quinolinoyl)glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP). *J Med Chem*. 2014 Apr 10;57(7):3053-74.
- Kelly T. Fibroblast activation protein- α and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. *Drug Resist Updat*. 2005 Feb-Apr;8(1-2):51-8.
- Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein- α . *Science*. 2010 Nov 5;330(6005):827-30.
- Laverman P, van der Geest T, Terry SY, Gerrits D, Walgreen B, Helsen MM, Nayak TK, Freimoser-Grundschober A, Waldhauer I, Hosse RJ, Moessner E, Umana P, Klein C, Oyen WJ, Koenders MI, Boerman OC. Immuno-PET and Immuno-SPECT of Rheumatoid Arthritis with Radiolabeled Anti-Fibroblast Activation Protein Antibody Correlates with Severity of Arthritis. *J Nucl Med*. 2015 May;56(5):778-83.
- Lo PC, Chen J, Stefflova K, Warren MS, Navab R, Bandarchi B, Mullins S, Tsao M, Cheng JD, Zheng G. Photodynamic molecular beacon triggered by fibroblast activation protein on cancer-associated fibroblasts for diagnosis and treatment of epithelial cancers. *J Med Chem*. 2009 Jan 22;52(2):358-68.
- Poplawski SE, Lai JH, Li Y, Jin Z, Liu Y, Wu W, Wu Y, Zhou Y, Sudmeier JL, Sanford DG, Bachovchin WW. Identification of selective and potent inhibitors of fibroblast activation protein and prolyl oligopeptidase. *J Med Chem*. 2013 May 9;56(9):3467-77.
- Reilly RM, Lam K, Chan C, Levine M. Advancing Novel Molecular Imaging Agents

- from Preclinical Studies to First-in-Humans Phase I Clinical Trials in Academia-A Roadmap for Overcoming Perceived Barriers. *Bioconjugate chemistry*. 2015; 26:625–632.
- Rettig WJ, Garin-Chesa P, Healey JH, Su SL, Ozer HL, Schwab M, Albino AP, Old LJ. Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin *Cancer Res*. 1993 Jul 15;53(14):3327-35.
- Ryabtsova O, Jansen K, Van Goethem S, Joossens J, Cheng JD, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. *Bioorg Med Chem Lett*. 2012 May 15;22(10):3412-7.
- Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, Rettig WJ. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A*. 1994 Jun 7;91(12):5657-61.
- Scott AM, Wiseman G, Welt S, Adjei A, Lee FT, Hopkins W, Divgi CR, Hanson LH, Mitchell P, Gansen DN, Larson SM, Ingle JN, Hoffman EW, Tanswell P, Ritter G, Cohen LS, Bette P, Arvay L, Amelsberg A, Vlock D, Rettig WJ, Old LJ. A Phase I dose-escalation study of sibtrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin Cancer Res*. 2003 May;9(5):1639-47.
- Tsai TY, Yeh TK, Chen X, Hsu T, Jao YC, Huang CH, Song JS, Huang YC, Chien CH, Chiu JH, Yen SC, Tang HK, Chao YS, Jiaang WT. Substituted 4-carboxymethylpyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. *J Med Chem*. 2010 Sep 23;53(18):6572-83.
- Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res*. 2002 Sep;8(9):2912-23.
- Welt S, Divgi CR, Scott AM, Garin-Chesa P, Finn RD, Graham M, Carswell EA, Cohen A, Larson SM, Old LJ, et al. Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. *J Clin Oncol*. 1994 Jun;12(6):1193-203.

Youn H., Hong K. In vivo noninvasive small animal molecular imaging. *Osong Public Health Res Perspect.* 2012; 3 :48-59. PMID: PMC3738683.

Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, McCaughan GW, Gorrell MD. The dipeptidyl peptidase IV family in cancer and cell biology. *FEBS J.* 2010 Mar;277(5):1126-44.

U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014.

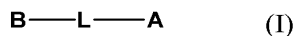
U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016.

International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013.

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED:

1. A compound of Formula (I):



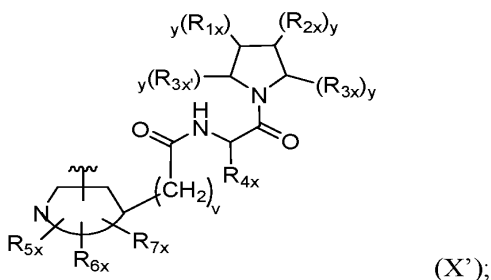
wherein:

A is a targeting moiety for FAP- α ;

B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and

L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

2. The compound of claim 1, wherein A is an FAP- α targeting moiety having the structure of:



wherein each y is independently an integer selected from the group consisting of 0, 1, and 2;

R_{1x}, R_{2x}, and R_{3x}, are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R_{4x} is H;

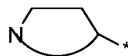
R_{5x}, R_{6x}, and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents selected from -OH and halogen;

R_{8x}, R_{9x}, and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃;

R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar_1 , Ar_2 and Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

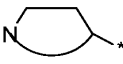
v is 0, 1, 2, or 3; and

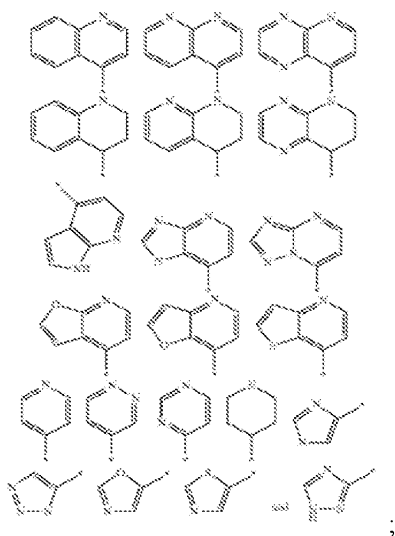


represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S;

wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof;

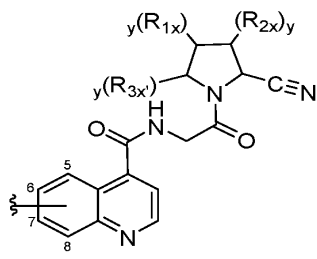
and stereoisomers and pharmaceutically acceptable salts thereof.

3. The compound of claim 2, wherein  is selected from the group consisting of:



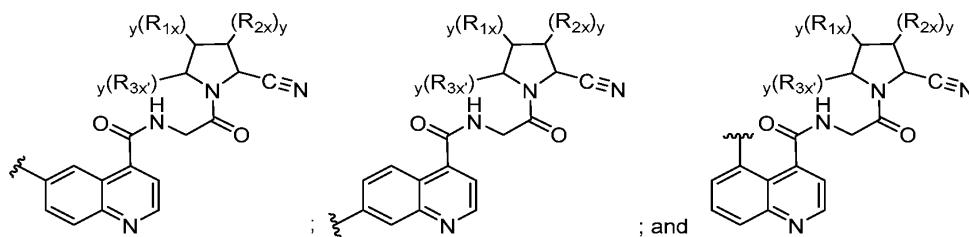
wherein * indicates the point of attachment of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle to $-(CH_2)_v-$.

4. The compound of claim 2, wherein A is an FAP- α targeting moiety having the structure of:

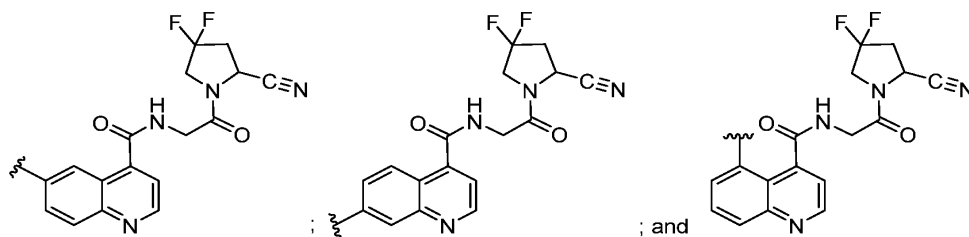


wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

5. The compound of claim 4, wherein A is selected from the group consisting of:

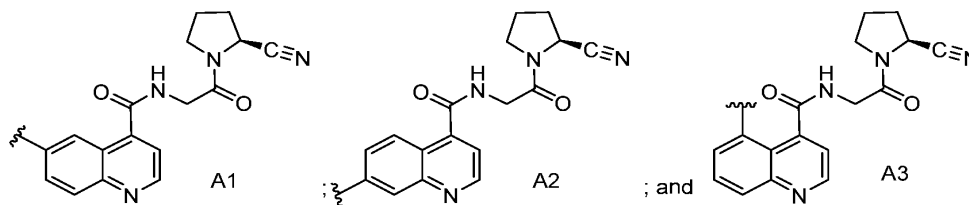


6. The compound of claim 5, wherein A is selected from the group consisting of:

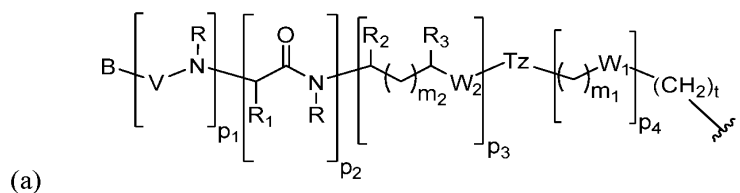


and stereoisomers thereof.

7. The compound of claim 5, wherein A is selected from the group consisting of:



8. The compound of any of claims 1-7, wherein L and B are selected from the group consisting of (a), (b), (c), or (d):



wherein:

p_1 , p_2 , p_3 and p_4 may be in any order;

t is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8;

p₁, p₃, and p₄ are each independently 0 or 1;

p₂ is an integer selected from the group consisting of 0, 1, 2, and 3, and when p₂ is 2 or 3, each R₁ is the same or different;

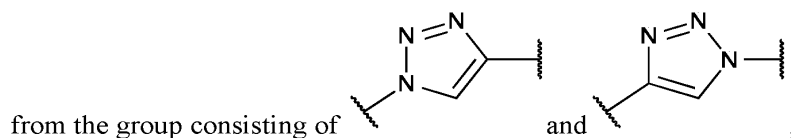
m₁ and m₂ are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6;

W₁ is selected from the group consisting of a bond, -S-, -C(=O)-NR-, and -NR-C(=O)-;

W₂ is selected from the group consisting of a bond, -S-, -CH₂-C(=O)-NR-, -C(O)-, -NRC(O)-, -NR'C(O)NR-, -NRC(S)NR'₂-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, -NR-C(O)-, -C(O)O-, -(O-CH₂-CH₂)_q- and -(CH₂-CH₂-O)_q, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8;

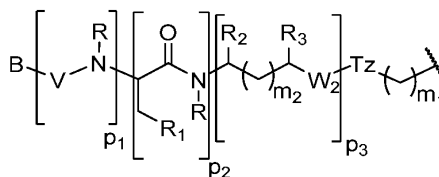
each R or R' is independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, and -OR₄, wherein R₄ is selected from the group consisting of H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, and substituted heterocycloalkyl, wherein q is defined as immediately hereinabove;

Tz is a triazole group that can be present or absent and, if present, is selected



each R₁ is independently H, C₁-C₆ alkyl, C₃-C₁₂ aryl, -(CH₂)_q-C₃-C₁₂ aryl, -C₄-C₁₆ alkylaryl, or -(CH₂)_q-C₄-C₁₆ alkylaryl; R₂ and R₃ are each independently H and -CO₂R₅, wherein R₅ is selected from the group consisting of H, C₁-C₆ alkyl, C₃-C₁₂ aryl, and C₄-C₁₆ alkylaryl, wherein when one of R₂ or R₃ is CO₂R₅, then the other is H;

V is selected from the group consisting of -C(O)-, -C(S)-, -NRC(O)-, -NRC(S)-, and -OC(O)-;



(b)

wherein p_1 , p_2 , p_3 , m_1 , m_2 , Tz , W_2 , R , R_1 , R_2 , R_3 , and V are defined as hereinabove;

(c) $-L_1-$, $-L_2-L_3-$, or $-L_1-L_2-L_3-$, wherein:

L_1 is $-NR-(CH_2)_q-[O-CH_2-CH_2-O]_q-(CH_2)_q-C(=O)-$;

L_2 is $-NR-(CH_2)_q-C(COOR_5)-NR-$; and

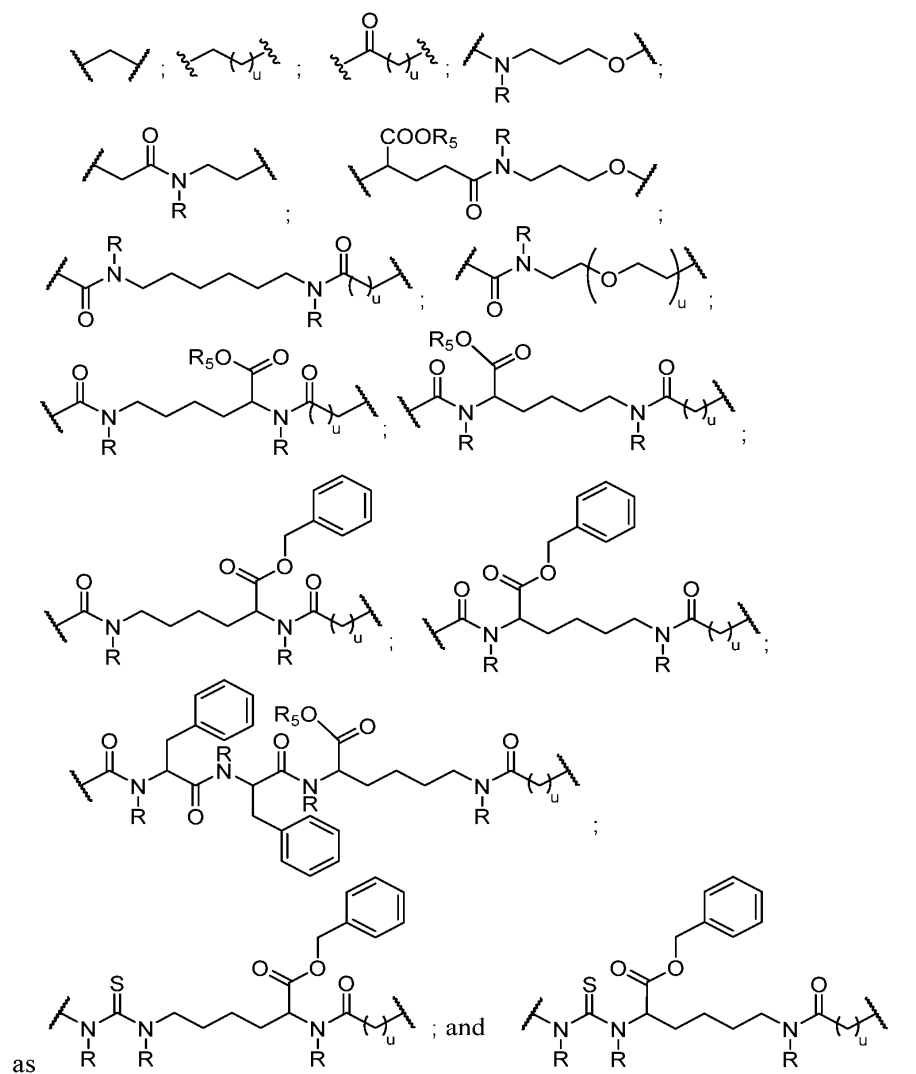
L_3 is $-(O=C)-(CH_2)_q-C(=O)-$;

wherein each q is independently an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove;

(d) $B-(CR_6H)_q-(CH_2)_q-C(=O)-NR-(CH_2)_q-O-$ or $B-NR-(CH_2)_q-O-$; wherein each q and R is defined hereinabove; and R_6 is H or $-COOR_5$; and

B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and stereoisomers and pharmaceutically acceptable salts thereof.

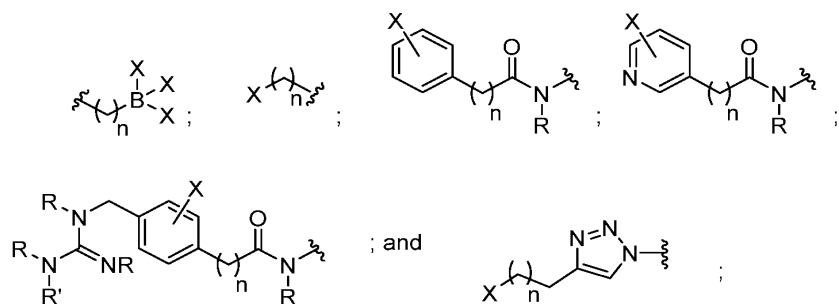
9. The compound of any of claims 1-8, wherein L is selected from the group consisting of:



wherein u is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8; and R and R₅ are as defined hereinabove.

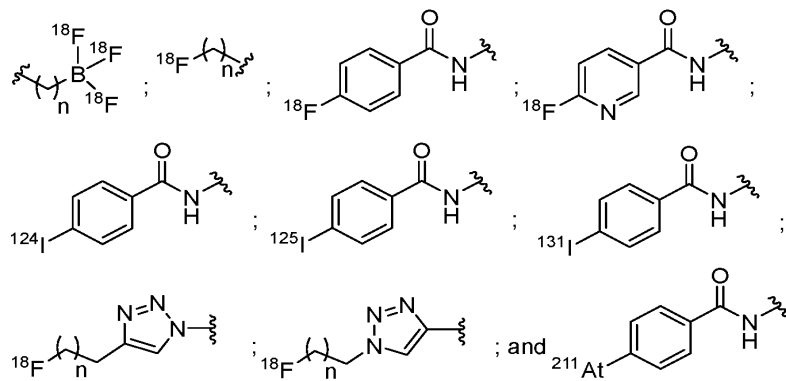
10. The compound of any of claims 1-9, wherein B is a radiolabeled prosthetic group comprising a radioisotope selected from the group consisting of ¹⁸F, ¹²⁴I, ¹²⁵I, ¹³¹I, and ²¹¹At.

11. The compound of claim 10, wherein the radiolabeled prosthetic group is selected from the group consisting of:



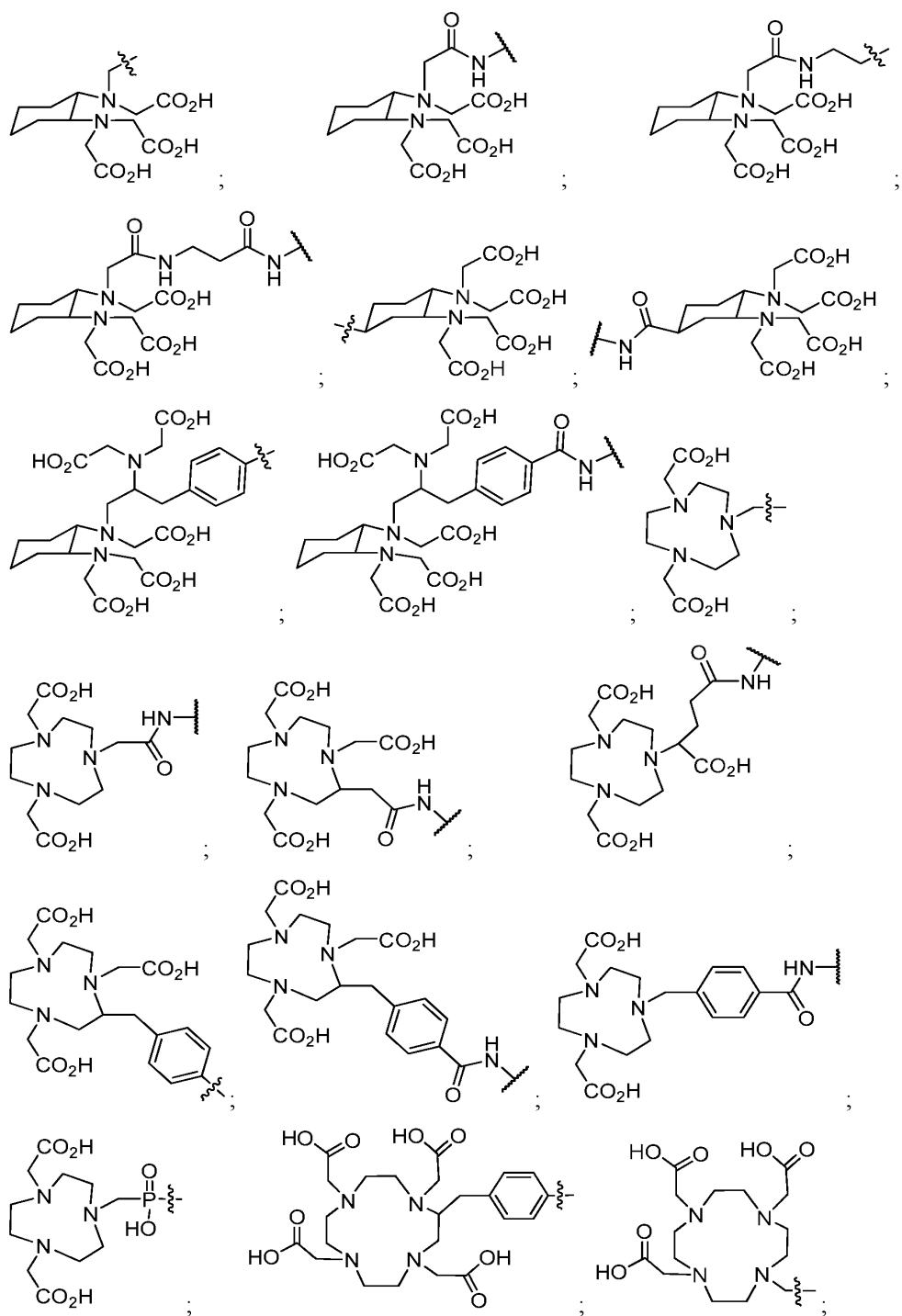
wherein each X is independently a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At ; each R and R' is defined hereinabove; and each n is independently an integer selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

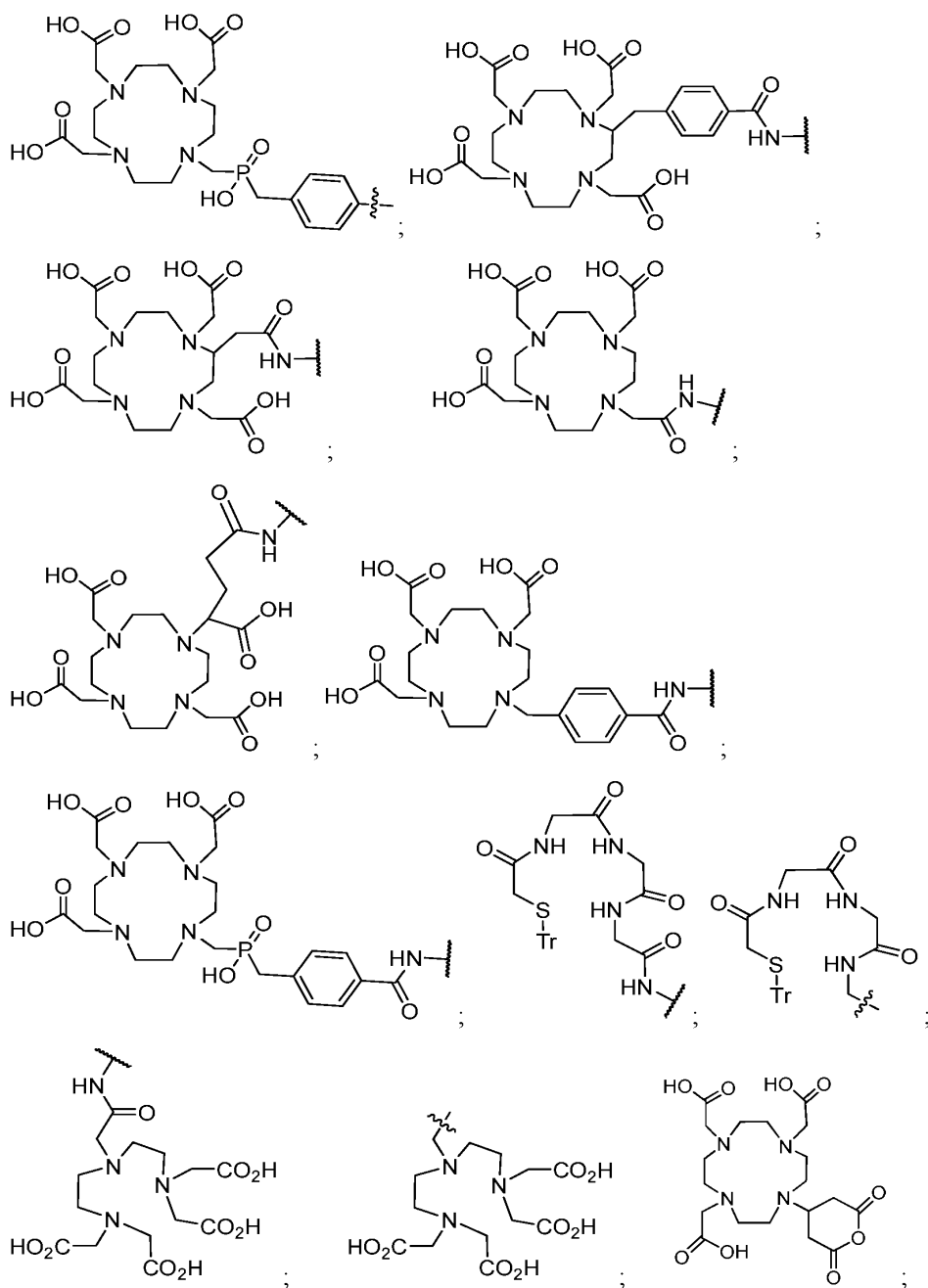
12. The compound of claim 11, wherein the radiolabeled prosthetic group is selected from the group consisting of:

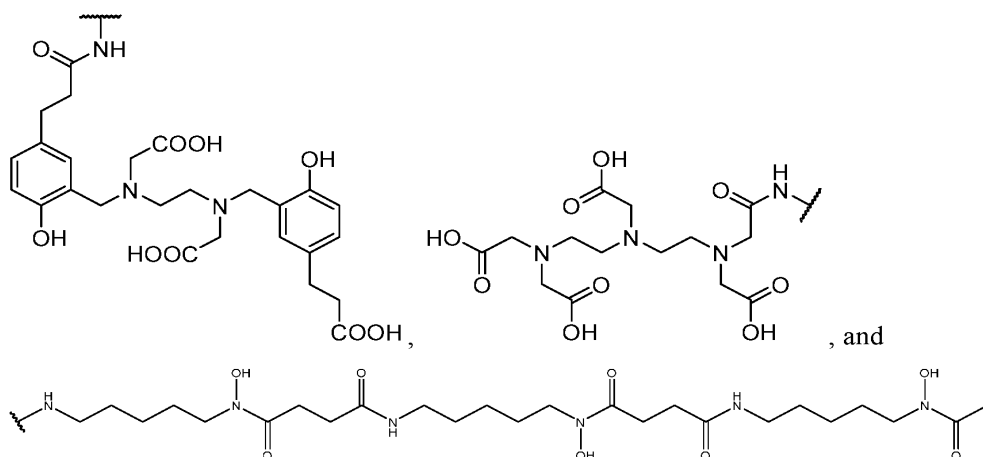


13. The compound of any of claims 1-9, wherein B comprises a chelating agent.

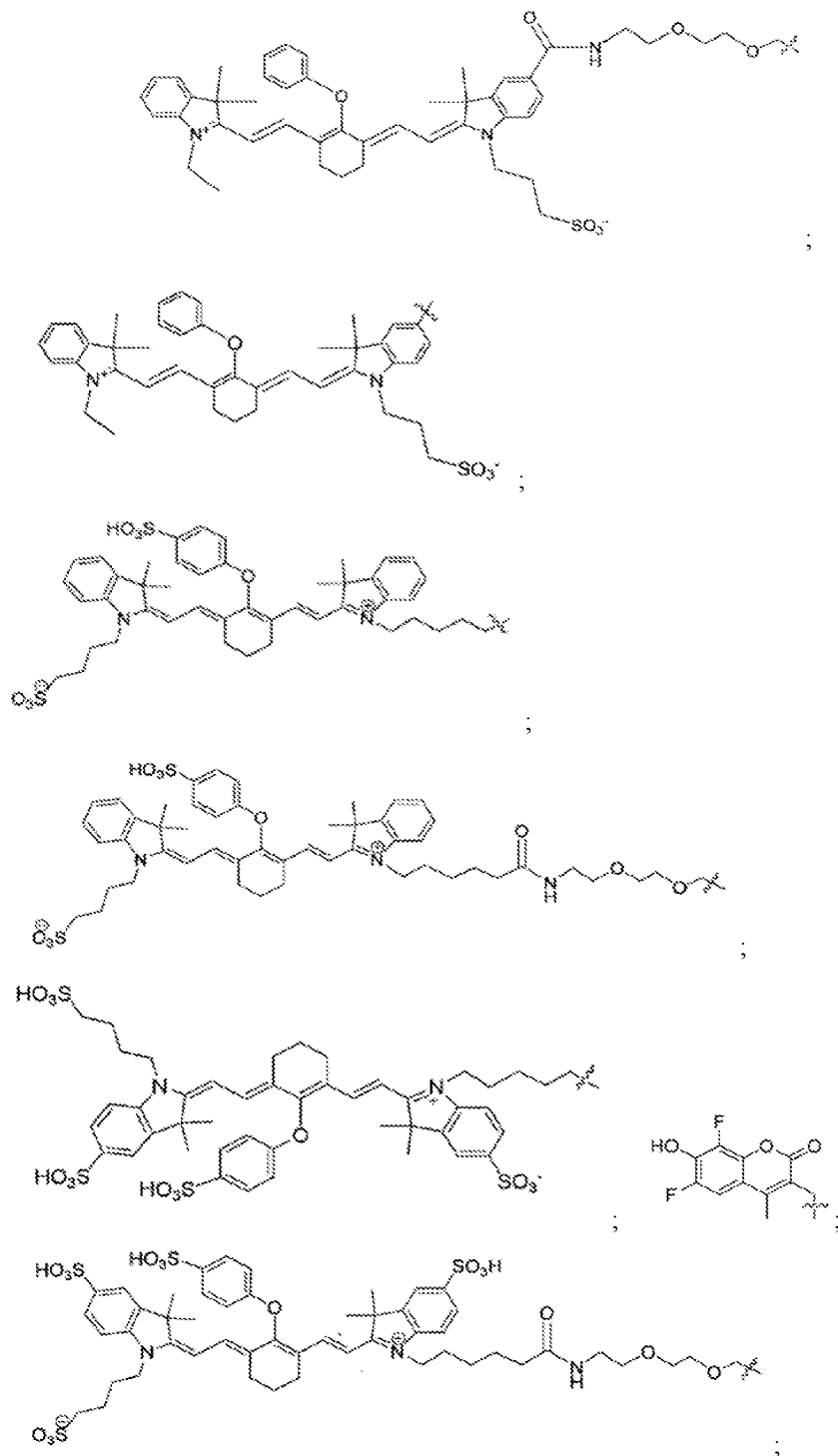
14. The compound of claim 13, wherein the chelating agent is selected from the group consisting of:

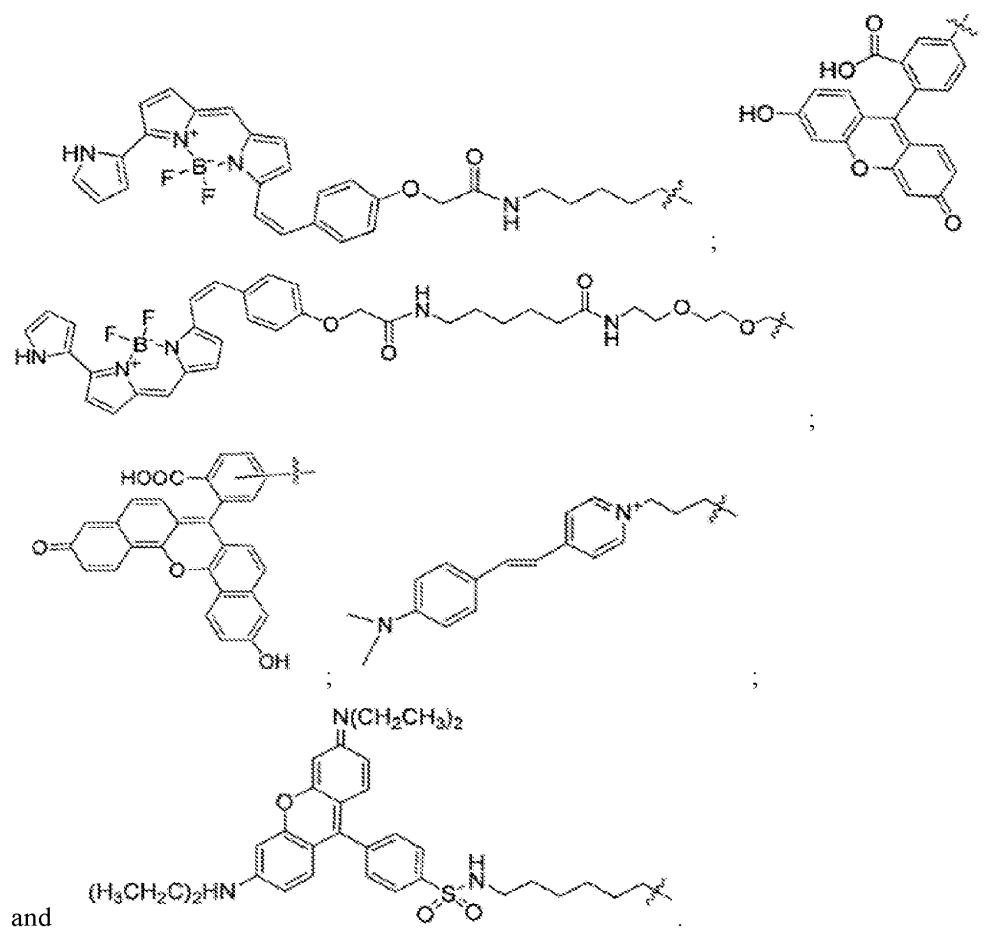




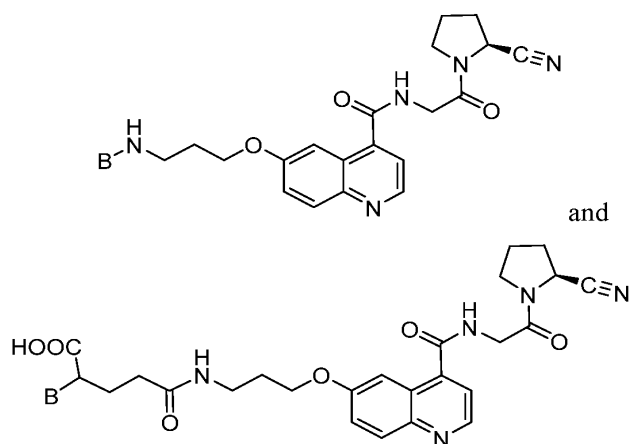


15. The compound of any of claims 1-9, wherein B comprises an optical dye.
16. The compound of claim 15, wherein the optical dye comprises a fluorescent dye.
17. The compound of claim 16, wherein the fluorescent dye is selected from the group consisting of carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, DyLight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.
18. The compound of claim 15, wherein the optical dye is selected from the group consisting of:

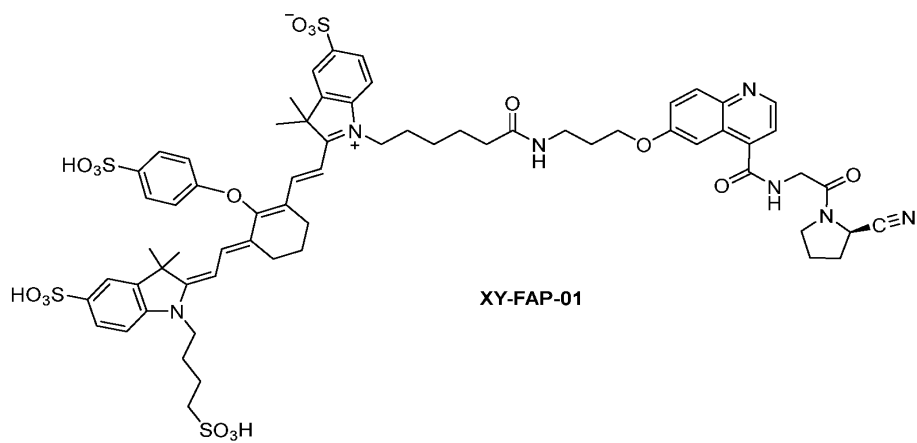




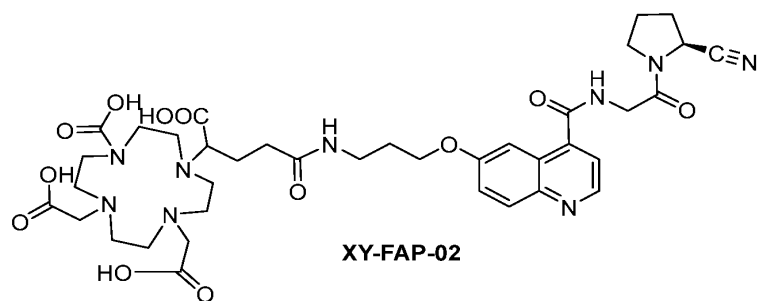
19. The compound of claims 1-9, wherein the compound is selected from the group consisting of:



20. The compound of claim 19, wherein the compound is selected from the group consisting of:



; and



21. A pharmaceutical composition comprising the compound of any of claims 1-20.

22. The composition of claim 21, further comprising one or more of pharmaceutically acceptable carriers, diluents, excipients, or adjuvants.

23. A method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound according to any of claims 1-20 or a pharmaceutical composition of claim 21 to a subject, wherein the compound of formula (I) comprises an optical or radiolabeled functional group suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

24. A method for inhibiting fibroblast-activation protein- α (FAP- α), the method comprising administering to a subject in need thereof an effective amount of a compound according to any of claims 1-20 or a pharmaceutical composition of claim 21.

25. A method for treating a fibroblast-activation protein- α (FAP- α)-related disease or disorder, the method comprising administering to a subject in need of treatment thereof an effective amount of a compound according to any of claims 1-20 or a pharmaceutical composition of claim 21, wherein the compound of formula (I) comprises a radiolabeled functional group suitable for radiotherapy.

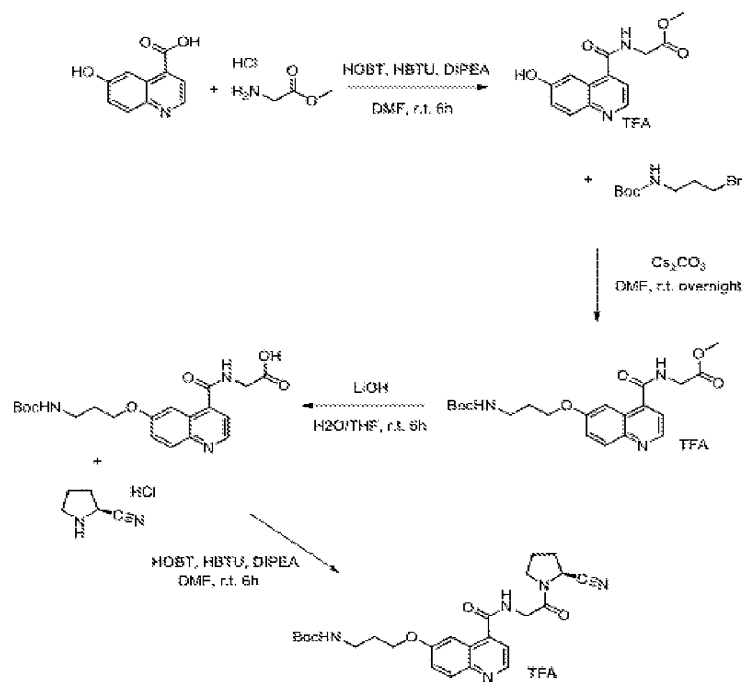
26. The method of claim 25, wherein (FAP- α)-related disease or disorder is selected from the group consisting of a proliferative disease; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

27. The method of claim 26, wherein the proliferative disease is selected from the group consisting of breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma.

ABSTRACT

Imaging and radiotherapeutics agents targeting fibroblast-activation protein- α (FAP- α) and their use in imaging and treating FAP- α related diseases and disorders are disclosed.

5

**Fig. 1A**

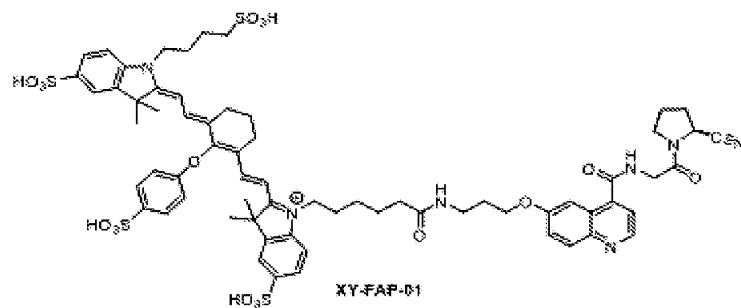


Fig. 1B

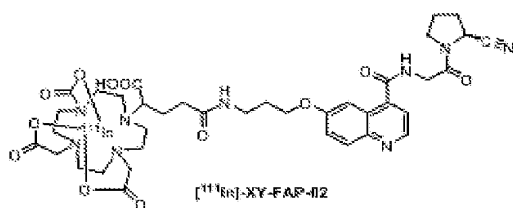


Fig. 1C

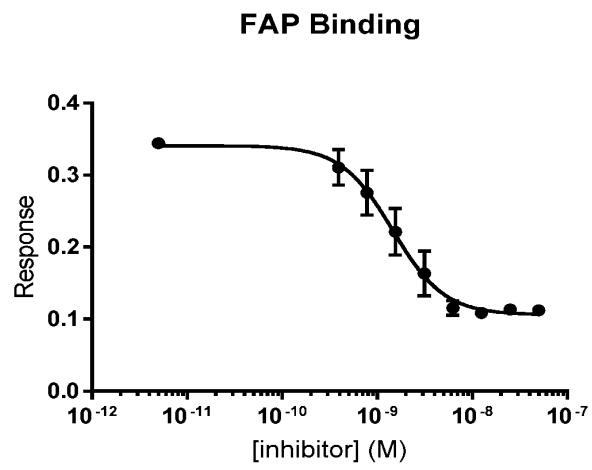


Fig. 2

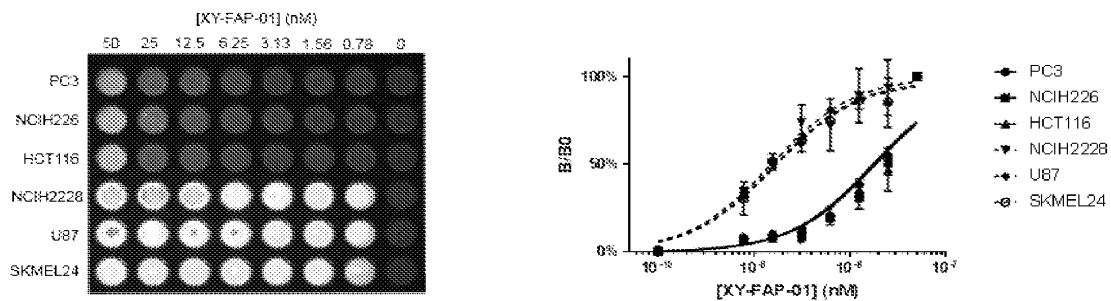


Fig. 3A

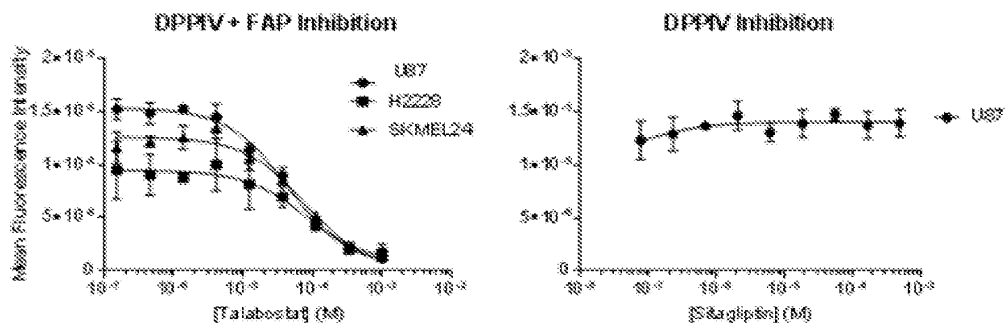


Fig. 3B

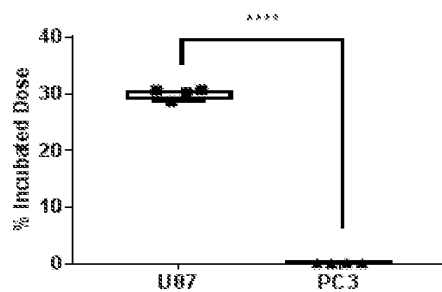
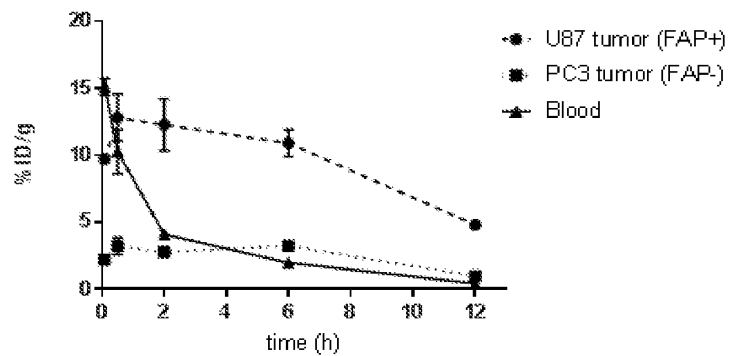
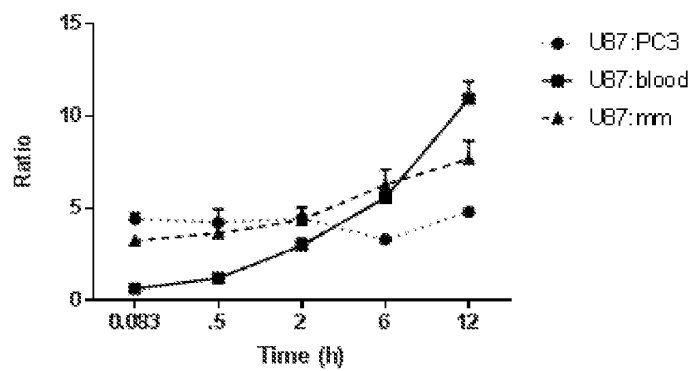


Fig. 3C

	5 min (n= 3)	0.5 h (n= 4)	2 h (n= 3)	6 hr (n= 4)	6 h - blocking (n=4)	12 h (n= 4)
blood	15.13 ± 0.62	10.64 ± 1.54	4.10 ± 0.39	2.01 ± 0.18	0.02 ± 0.001	0.42 ± 0.04
heart	6.68 ± 0.99	4.98 ± 0.71	2.61 ± 0.09	1.07 ± 0.09	0.02 ± 0.002	0.46 ± 0.04
lungs	6.99 ± 1.37	5.80 ± 0.89	2.59 ± 0.32	1.36 ± 0.13	0.04 ± 0.005	0.39 ± 0.03
liver	6.32 ± 0.77	4.90 ± 0.59	2.58 ± 0.09	2.51 ± 0.09	0.33 ± 0.058	1.66 ± 0.22
stomach	3.28 ± 0.46	2.62 ± 0.20	1.89 ± 0.14	1.19 ± 0.15	0.06 ± 0.032	0.34 ± 0.05
pancreas	14.54 ± 1.66	12.14 ± 2.85	8.11 ± 0.34	3.28 ± 0.27	0.03 ± 0.005	1.15 ± 0.52
spleen	2.93 ± 0.25	2.48 ± 0.47	1.77 ± 0.27	1.54 ± 0.19	0.06 ± 0.008	1.10 ± 0.13
fat	0.74 ± 0.09	0.73 ± 0.13	0.61 ± 0.19	0.45 ± 0.16	0.02 ± 0.017	0.15 ± 0.08
kidney	4.60 ± 0.57	3.56 ± 0.18	1.95 ± 0.06	1.79 ± 0.21	1.16 ± 0.158	0.73 ± 0.05
sm. int.	8.80 ± 1.97	8.41 ± 1.35	3.64 ± 0.45	1.66 ± 0.22	0.09 ± 0.052	0.76 ± 0.14
lrg. int.	4.67 ± 0.42	4.57 ± 0.57	2.98 ± 0.27	1.97 ± 0.47	0.36 ± 0.342	0.55 ± 0.04
bladder	2.96 ± 0.95	13.60 ± 8.80	8.94 ± 6.48	3.41 ± 0.84	1.04 ± 0.486	2.25 ± 0.57
muscle	3.00 ± 0.22	3.53 ± 0.16	2.80 ± 0.07	1.79 ± 0.15	0.02 ± 0.005	0.60 ± 0.03
femur	4.76 ± 0.12	5.83 ± 0.72	4.95 ± 0.60	3.91 ± 0.70	0.08 ± 0.021	1.38 ± 0.20
U87	9.71 ± 0.24 ^a	12.89 ± 1.45 ^a	12.28 ± 1.95 ^a	11.20 ± 1.03 ^a	0.27 ± 0.019 ^a	4.57 ± 0.54 ^a
PC3	2.20 ± 0.20	3.10 ± 0.57	2.75 ± 0.11	3.40 ± 0.34	0.11 ± 0.024 ^a	0.95 ± 0.06
U87:PC3	4.43 ± 0.31	4.23 ± 0.72	4.46 ± 0.62	3.30 ± 0.14	2.61 ± 0.440	4.82 ± 0.28
U87: blood	0.64 ± 0.04	1.22 ± 0.07	3.00 ± 0.38	5.58 ± 0.36	18.07 ± 1.197	10.94 ± 0.93
U87 mm	3.25 ± 0.26	3.66 ± 0.44	4.36 ± 0.66	6.29 ± 0.82	11.81 ± 2.086	7.68 ± 0.97

Fig. 4

**Fig. 5A****Fig. 5B**

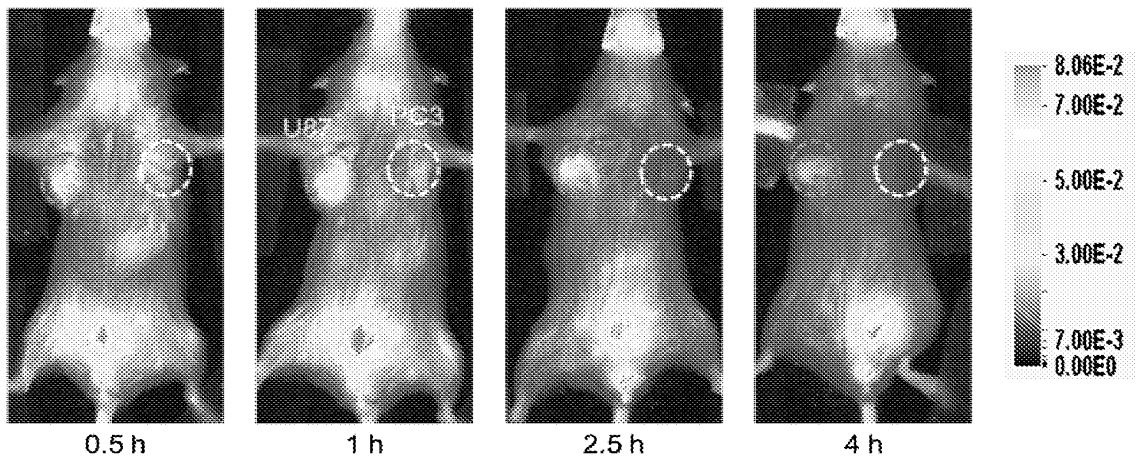


Fig. 6

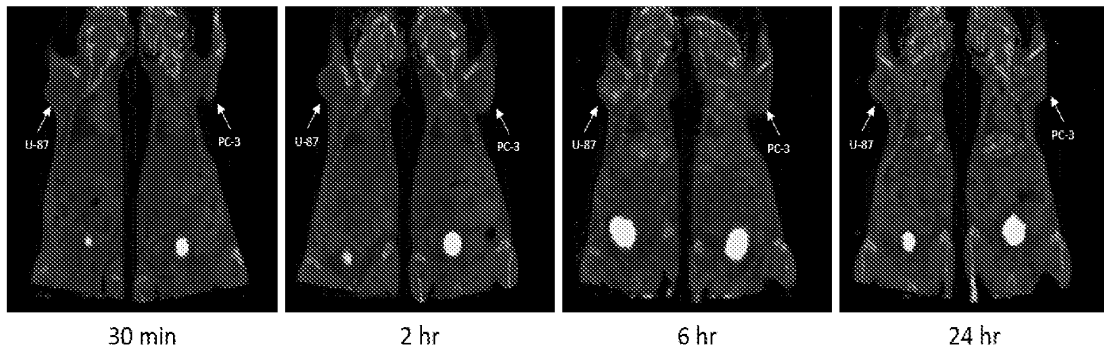


Fig. 7

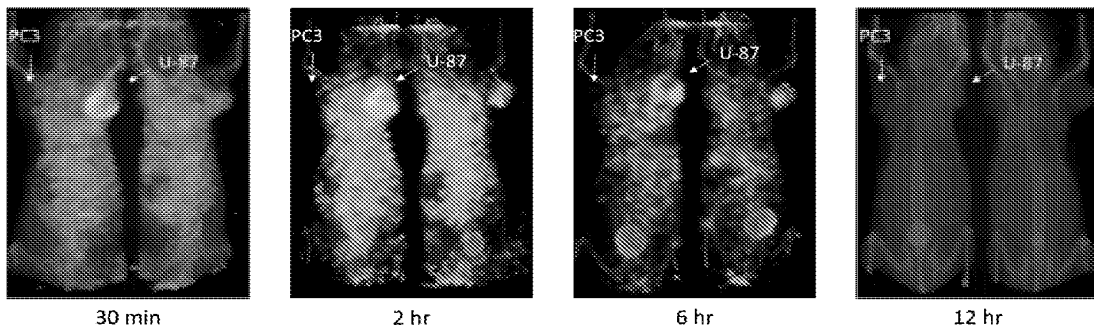


Fig. 8



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/354,282

RECEIPT DATE / TIME
07/18/2023 03:48:57 PM ET

ATTORNEY DOCKET #
J HU-36631.303

Title of Invention

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION
PROTEIN-ALPHA (FAP-ALPHA)

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 7536

FILED BY Stephanie Filandrinos

PATENT CENTER # 62461174

FILING DATE -

CUSTOMER # 101943

FIRST NAMED INVENTOR Xing Yang

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Jeffrey Childers

Documents

TOTAL DOCUMENTS: 13

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2023-07-18_36631.303_PA.pdf	4	-	146 KB
2023-07-18_36631.303_PA-A.PE.pdf (1-1)	1	Preliminary Amendment	128 KB
2023-07-18_36631.303_PA-CLM.pdf (2-3)	2	Claims	90 KB
2023-07-18_36631.303_PA-REM.pdf (4-4)	1	Applicant Arguments/Remarks Made in an Amendment	88 KB
2023-07-18_36631.303_Track1.pdf	2	Track One Request	109 KB

2023-07-18_36631.303_IDS.pdf	6	Information Disclosure Statement (IDS) Form (SB08)	338 KB
------------------------------	---	--	--------

Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems. The attached file contains comments or annotations. Comments are not recommended and may cause processing problems with the document.

2023-07-18_36631.303_ADS.pdf	10	Application Data Sheet	260 KB
------------------------------	----	------------------------	--------

Warning: This is not a USPTO supplied ADS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.

2023-07-18_36631.303_EXEC_POA.pdf	1	Power of Attorney	842 KB
-----------------------------------	---	-------------------	--------

2023-07-18_36631.303_Application.pdf	77	-	1541 KB
--------------------------------------	----	---	---------

2023-07-18_36631.303_Application-SPEC.pdf	(1-61)	61	Specification	1035 KB
---	--------	----	---------------	---------

2023-07-18_36631.303_Application-CLM.pdf	(62-76)	15	Claims	597 KB
--	---------	----	--------	--------

2023-07-18_36631.303_Application-ABST.pdf	(77-77)	1	Abstract	122 KB
---	---------	---	----------	--------

2023-07-18_36631.303_FIGURES.pdf	9	Drawings-only black and white line drawings	2952 KB
----------------------------------	---	---	---------

2023-07-18_36631.303_ExecDecls_All.pdf	7	Oath or Declaration filed	3575 KB
--	---	---------------------------	---------

2023-07-
18_36631.303_439.pdf

2

Internet Communications
Authorization

76 KB

Digest

DOCUMENT

MESSAGE DIGEST(SHA-512)

2023-07-18_36631.303_PA.pdf	589F98142A13035D0BDA4E51DD1BA0806109335269F43EC877 89DC11158B9DF42B932FB5BC70C6AE0C4D1DB4A8C569CEC2 EEA4BF0C76673D6918662DA2A1B2D6
2023-07-18_36631.303_PA- A.PE.pdf	C87350B484F36946BC1F00E2BA0D3DEEC72E5E563868A5B43 A31C28530D4B1A79B7E6115D0CC79BFB8797AA362F13056A0 FA6E0763142E736AB4F1B7FB3B6046
2023-07-18_36631.303_PA- CLM.pdf	E37326019EEF8242C9E73CB3D93D417A9C67B30215B711FE7 90692478FE039862CF4DE499306AB4D7E8F3C5E5F656929910 90A8776511D9ACADE587B26301BE6
2023-07-18_36631.303_PA- REM.pdf	9790B3D28A854D4CC329BA5D5F1232FE263C55C0F257904549 CB9CA5C20E2016500D99A27179001B34A14258DD5F99D5937 F58392EE75AC1C8DECD3667D473A8
2023-07- 18_36631.303_Track1.pdf	1CFA770E503CD3319E0BDF93021043D8A608F7511383802DB2 46BF27CA3BA83B342E64A192375CEE7F17733F27E13EB134E 3A0D7F93BD673ABCAB9D2B8124C40
2023-07-18_36631.303_IDS.pdf	B9E3D9C15AA1556E42B8AF24BDEFCCBE3DF0B46CB0703C 649B3FB0BF76018C61C1F068281308CA27B6EF326B8A7740D2 78D20CE7B90D4E40A5BD527BBC409E7
2023-07- 18_36631.303_ADS.pdf	8CA01476B26682A1346894C56533F8D114F0FD42E5FE626D00 98243154CA28E4A6695CC17E9B4878D3DB35A004ABDA4BA5 DF171FC782A1CD1157B33BDFB1B6D5
2023-07- 18_36631.303_EXEC_POA.pdf	64099E8B5B3623CDBC0D19368EFF9BB05C127C22F1473147C 0CE0990CFD37787BA6DBEACE2EE4C94B44F97EE9C26F48AA A3071A3A956F26924039FFBA17C730E

2023-07-18_36631.303_Application.pdf	7D41E713DCCE64390A7F8BBB95901E6F7D3B03827BD843D255C9FDAAF59EBC9BDBC21C3AF573C65E923B80997FA2B1C57BE3497EECAD2A732828A32A3EED221F
2023-07-18_36631.303_Application-SPEC.pdf	ED282DE61FB526452378B17C5A82BE68BCBDA86163471834F1FFFBEE721DB418AD86E4A64151AD8178405A7EC185E6366FCE2EDF4D8AA37409C3202CF07E3A94
2023-07-18_36631.303_Application-CLM.pdf	9E358B6DCD250D432C58DB1D5E3B0CF669DFC61A905756FAD1C0F46A33258BF4D4FC0B6EF6AEA6F57C69CF00C64F48E0F6F65C21AD5E5FE7181DF1B915519C62
2023-07-18_36631.303_Application-ABST.pdf	EF17C9C6F7FD9FE9684C08807F1239436EAE98F670B19C97B59BB6C45C1B519054FB3ACF56801D7D6F83AC0E8D0A8396F7CBBDD9D9C2EF745A8F903BDC CDBAD1
2023-07-18_36631.303_FIGURES.pdf	4673E55683B95B65D0CAFD7A640CC90E4C63CCDD3CB4175B6F8FBF27EFF865EB31968B6A493D0E23A3635FA28D2356BD72773CDA84A705093997187DB0E24EDC
2023-07-18_36631.303_ExecDecIs_All.pdf	71F5B35CDBE683B4AAF18EE60711C44AB1FFEE464AE7D04A153526EF437860995F7B8629133EF2EBF0DFFDD3E69FE18034EA1CCE79E6F8D92C2FFE263DB9401E
2023-07-18_36631.303_439.pdf	CA8B15D710940A714443BE075B4F028BF64C37A3BA85CCEDC24FFDED11CA3C434FE125E46FBE5529DB4DF76860744F7487D4A93F5369B4936F0704FF55CD1E22

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an

international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #
18/354,282

RECEIPT DATE / TIME
07/18/2023 03:48:57 PM ET

ATTORNEY DOCKET #
J HU-36631.303

Title of Invention

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION
PROTEIN-ALPHA (FAP-ALPHA)

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 7536

FILED BY Stephanie Filandrinis

PATENT CENTER # 62461174

AUTHORIZED BY Jeffrey Childers

CUSTOMER # 101943

FILING DATE -

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Xing Yang

Payment Information

PAYMENT METHOD
CARD / 0638

PAYMENT TRANSACTION ID
E20237HF49225607

PAYMENT AUTHORIZED BY
Stephanie Filandrinis

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
2830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	56.00	1	56.00
2817	REQUEST FOR PRIORITIZED EXAMINATION	1680.00	1	1680.00
2111	UTILITY PATENT APPL. SEARCH FEE	280.00	1	280.00
4011	BASIC FILING FEE- UTILITY	64.00	1	64.00
2311	EXAMINATION OF ORIGINAL PATENT APPLICATION	320.00	1	320.00

TOTAL AMOUNT:	\$2,400.00
--------------------------	-------------------

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

SCORE Placeholder Sheet for IFW Content

Application Number: 18354282

Document Date: 07/24/2023

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: YANG, et al.

Confirmation No.: 7536

Serial No.: 18/354,282

Art Unit: TBD

Filed: July 18, 2023

Examiner: TBD

**FOR: IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING
FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir or Madam:

Please amend the application as indicated on the following pages.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Drawings begin on page 3 of this paper.

Amendments to the Claims begin on page 4 of this paper.

Remarks begin on page 6 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the originally filed specification with the substitute specification filed herewith.

Attorney Docket No. JHU-36631.303

AMENDMENTS TO THE DRAWINGS:

Please replace the originally filed figures with the replacement figures filed herewith.



UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
18/354,282

RECEIPT DATE / TIME
07/24/2023 02:23:01 PM ET

ATTORNEY DOCKET #
JHU-36631.303

Title of Invention

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION
PROTEIN-ALPHA (FAP-ALPHA)

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 7536

FILED BY Stephanie Filandrinis

PATENT CENTER # 62491443

FILING DATE -

CUSTOMER # 101943

FIRST NAMED INVENTOR Xing Yang

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Jeffrey Childers

Documents

TOTAL DOCUMENTS: 8

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
2023-07-24_36631.303_PA2.pdf		6	-	194 KB
2023-07-24_36631.303_PA2-SA...pdf	(1-1)	1	Supplemental Response or Supplemental Amendment	131 KB
2023-07-24_36631.303_PA2-SPEC.pdf	(2-2)	1	Specification	88 KB
2023-07-24_36631.303_PA2-DRW.pdf	(3-3)	1	Drawings-only black and white line drawings	88 KB

2023-07-24_36631.303_PA2-CLM.pdf	(4-5)	2	Claims	132 KB
2023-07-24_36631.303_PA2-REM.pdf	(6-6)	1	Applicant Arguments/Remarks Made in an Amendment	132 KB
2023-07-24_36631.303_SubSpec-marked.pdf		62	Specification	1123 KB
2023-07-24_36631.303_SubSpec-clean.pdf		61	Specification	1083 KB
2023-07-24_36631.303_ReplcementFigures.pdf		9	Drawings-only black and white line drawings	3020 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
2023-07-24_36631.303_PA2.pdf	F39DF42D8DB3AF2782DDD73663E9CDDE163E90E4BA63D1449A73D8EFE5F89BD76735A8C100D5EA658794A10193B010E108EFEFB0091C3B4044D62010CDA6A24B
2023-07-24_36631.303_PA2-SA...pdf	10FB294B23D744BC60F9831AC47A001EAEADFB8B4963B5FFCFF379E47E6C44F42AA1159EF3B28664E32E519C4D051C79A4871DC6368DCFEC3525E8E1AF1E1062
2023-07-24_36631.303_PA2-SPEC.pdf	DCF1D119222C82BE271A6609AABE7B9F7B66F5D58F840B13ED5DB584B4205C001EED36D96C79D4A27405B064D45C0685BDEA3688FC094C361541D4746A61675B
2023-07-24_36631.303_PA2-DRW.pdf	47BAABA42FC99C3AF8EB12C7FB0A3A7C5A86B8C6B1D79961795029404F99C30C49135BDC6608A06EF4E434DA766FB6A34

	C4AC33AA9A26E8950ACF36E880A7C5D
2023-07-24_36631.303_PA2-CLM.pdf	B357F341D488E935AFDF5D6A4521DB655EF39F414FA50A4AA F822D083DB98530192BAD8279248C4A95B97A0EA5B1BF55DA E8902BDB16799E27206F82AFF127A6
2023-07-24_36631.303_PA2-REM.pdf	FAFD657DF82965DE22682CA1402DB963566CF22409FD8DF8C 53A87790108A1B10A540E923F77F09387AE7F0D41AE99E32BA E136AFA9EB20FAF0D0A531E2BD7EA
2023-07-24_36631.303_SubSpec-marked.pdf	60FB1A3ABF7C34BD3E82821118D3A2EC89538474EA6697CAF 9D34917EA372F17B6ED1BBC49CE0E536B4F94C3AA7E87644A C68AA47068A9D26AC9FB858A87BDBB
2023-07-24_36631.303_SubSpec-clean.pdf	A25B955AE4838D0E364AF869FC429612CD956EE38B2B0BC20 BE4E42F211994B136CCDF47AAF07CFD7C206D88CD60A1D15 7FEECCCA40B88D9698D1844903DA416
2023-07-24_36631.303_ReplcementFigures.pdf	ED37576B13EBCB8D0AE3BE72252E5E23645B4C696C299D04E 8556FA0EA84847CB5616806B97D5FC8A8A2DA930E625A631A F7FDDEF6DBE9BBA755F4C9BCB4759

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

AMENDMENTS TO THE CLAIMS:

The present listing of claims replaces all previous versions of the claims.

1.-27. (Canceled)

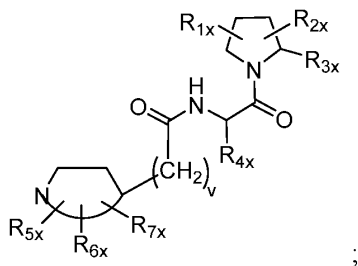
28. (Canceled)

29. (New) A compound of Formula (I):

B-L-A (I)

wherein:

A is a targeting moiety for FAP- α , wherein A has the structure of:



wherein:

R_{1x} and R_{2x} are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂-C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R_{4x} is H;

R_{5x}, R_{6x} and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents selected from -OH and halogen;

R_{8x}, R_{9x} and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃;

R_{10x}, R_{11x}, R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar₁, Ar₂ and Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from

O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

v is 0, 1, 2, or 3; and



represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S;

B is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A; or

a stereoisomer, tautomer, racemate, salt, hydrate, or solvate thereof.

REMARKS

Upon entry of this amendment claim 29 is pending. Claims 1-27 remain canceled without prejudice. Claim 28 has been canceled. Applicant reserves the right to pursue the canceled subject matter in one or more continuation and/or divisional applications. Claim 29 is newly added. Support for new claim 29 can be found in provisional patent application serial no. 62/575,607, filed October 23, 2017, on page 6, line 13, through page 8, line 3, as originally filed. No new matter is added.

Applicants have amended the specification and drawings. The specification and drawings have been amended to correct an obvious error in the chemical structure of the compound XY-FAP-02. Support for amendments to the specification and FIG. 1C can be found on page 50, lines 23-29, of the application as originally filed, which discloses that the compound XY-FAP-02 has the chemical name of 2,2',2''-(10-(1-Carboxy-4-((3-((4-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid and is formed by reaction of compound **8** with DOTA-GA(t-Bu)₄-NHS. No new matter is added.

CONCLUSION

Should there be any minor issues outstanding in this matter, the Examiner is respectfully requested to telephone the undersigned attorney at 608-662-1277. Early passage of the subject application to issue is earnestly solicited.

Respectfully Submitted,

CASIMIR JONES, S.C.

Date: July 24, 2023

/JEFFREY W. CHILDERS/
Jeffrey W. Childers, Ph.D.
Registration No. 58126
Customer No. 101943

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application is a continuation of U.S. Patent Application 16/758,182, filed April 22, 2020, which is a U.S. §371 National Entry Application of PCT/US2018/057086, filed October 23, 2018, which claims the benefit of U.S. Provisional Application No. 62/575,607, filed October 23, 2017, each of which is incorporated herein by reference in its entirety.

10

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

 This invention was made with government support under CA197470 awarded by the National Institutes of Health. The government has certain rights in the invention.

15

BACKGROUND

 Fibroblast-activation protein- α (FAP- α) expression has been detected on the surface of fibroblasts in the stroma surrounding >90% of the epithelial cancers examined, including malignant breast, colorectal, skin, prostate and pancreatic
20 cancers. (Garin-Chesa, et al., 1990; Rettig, et al., 1993; Tuxhorn, et al., 2002; Scanlan, et al., 1994). It is a characteristic marker for carcinoma-associated-fibroblast (CAF), which plays a critical role in promoting angiogenesis, proliferation, invasion, and inhibition of tumor cell death. (Allinen, et al., 2004; Franco, et al., 2010). In healthy adult tissues, FAP- α expression is only limited to areas of tissue remodeling
25 or wound healing. (Scanlan, et al., 1994; Yu, et al., 2010; Bae, et al., 2008; Kraman, et al., 2010). In addition, FAP- α -positive cells are observed during embryogenesis in areas of chronic inflammation, arthritis, and fibrosis, as well as in soft tissue and bone sarcomas. (Scanlan, et al., 1994; Yu, et al., 2010). These characteristics make FAP- α a potential imaging and radiotherapeutic target for cancer and inflammation diseases.

30 Because FAP- α is expressed in tumor stroma, anti-FAP antibodies have been investigated for radioimmunotargeting of malignancies, including murine F19, sibrotuzumab (a humanized version of the F19 antibody), ESC11, ESC14, and others. (Welt, et al., 1994; Scott, et al., 2003; Fischer, et al., 2012). Antibodies also demonstrated the feasibility of imaging inflammation, such as rheumatoid arthritis.

(Laverman, et al., 2015). The use of antibodies as molecular imaging agents, however, suffers from pharmacokinetic limitations, including slow blood and non-target tissue clearance (normally 2–5 days or longer) and non-specific organ uptake. Low molecular weight (LMW) agents demonstrate faster pharmacokinetics and a higher specific signal within clinically convenient times after administration. They also can be synthesized in radiolabeled form more easily and may offer a shorter path to regulatory approval. (Coenen, et al., 2010; Coenen, et al., 2012; Reilly, et al., 2015). To date, however, no LMW ligand has been reported with ideal properties for nuclear imaging of FAP- α .

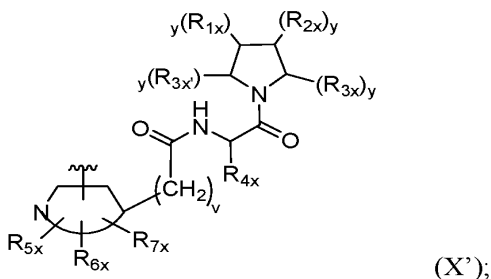
SUMMARY

In some aspects, the presently disclosed subject matter provides a compound of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

In particular aspects, A is an FAP- α targeting moiety having the structure of:



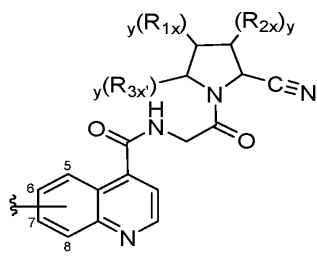
wherein each y is independently an integer selected from the group consisting of 0, 1, and 2; R_{1x}, R_{2x}, and R_{3x}, are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl; R_{4x} is H; R_{5x}, R_{6x}, and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents

selected from -OH and halogen; R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃; R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar₁, Ar₂ and Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; v is 0, 1, 2, or 3; and



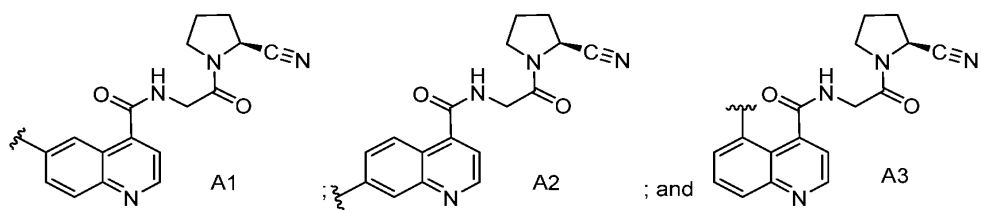
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In more particular aspects, A is an FAP- α targeting moiety having the structure of:



wherein  indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In yet more particular aspects, A is selected from the group consisting of:



In other aspects, the presently disclosed subject matter provides a pharmaceutical composition comprising a compound of formula (I).

In some aspects, the presently disclosed subject matter provides a method for
 5 imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

10 In other aspects, the presently disclosed subject matter provides a method for inhibiting fibroblast-activation protein- α (FAP- α), the method comprising administering to a subject in need thereof an effective amount of a compound of formula (I).

15 In yet other aspects, the presently disclosed subject matter provides a method for treating a fibroblast-activation protein- α (FAP- α)-related disease or disorder, the method comprising administering to a subject in need of treatment thereof an effective amount of a compound of formula (I), wherein the compound of formula (I) comprises a radiolabeled functional group suitable for radiotherapy.

20 In certain aspects, the (FAP- α)-related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders
 25 involving endocrinological dysfunction; and blood clotting disorders.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described
 30 herein below.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

5 Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG. 1A, FIG. 1B, and FIG. 1C show the synthetic pathway and structures of representative FAP-targeted agents, **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 1A shows the multi-step synthesis of the ligand precursor, tert-butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate. After each step, the reaction mixture was loaded onto a 25-g C18 cartridge and purified with a MeCN/water/TFA gradient. Identity of intermediate products was confirmed with ¹H NMR. FIG. 1B shows the full structure of optical imaging agent, **XY-FAP-01**. **XY-FAP-01** was produced with a one step reaction between the precursor and IRDye800CW-NHS. The major product was obtained at a yield of 85% after purification with HPLC. FIG. 1C shows the full structure of the SPECT imaging agent, [¹¹¹In]-**XY-FAP-02**. First, the precursor was functionalized with DOTA via a one step reaction between the precursor and DOTA-GA(t-Bu)₄-NHS. Unlabeled product was purified via HPLC to produce XY-FAP-02. Subsequent radiolabeling with ¹¹¹In and HPLC purification resulted in the radiolabeled product, [¹¹¹In]-**XY-FAP-02**;

FIG. 2 shows the inhibitory activity of **XY-FAP-01** on human recombinant FAP. The inhibitory activity of **XY-FAP-01** was determined using a fluorogenic FAP assay kit. Enzymatic activity of human recombinant FAP on a native substrate was inhibited in a concentration dependent fashion by **XY-FAP-01**. Semi-log inhibitory curves of **XY-FAP-01** activity were generated and the determined Ki value of **XY-FAP-01** was 1.26 nM;

FIG. 3A, FIG. 3B, and FIG. 3C show the assessment of the *in vitro* binding ability and specificity of **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 3A shows the concentration dependent uptake of **XY-FAP-01** in various cell lines. Cells incubated with various concentrations (range: 50 nM to 0.78 nM) of **XY-FAP-01** were imaged with the LI-COR Pearl Impulse Imager to assess uptake of agent in various FAP-positive and FAP-negative cell lines (left). Dose-response curves of **XY-FAP-01**

uptake in FAP-positive cell lines (NCIH2228, U87, and SKMEL24) and FAP-negative cell lines (PC3, NCIH226, and HCT116) were generated (right). FIG. 3B shows the inhibition of **XY-FAP-01** uptake in FAP-positive cell-lines. Cells incubated with 25-nM **XY-FAP-01** were incubated with various concentrations of either a DPPIV and FAP inhibitor, Talabostat, or a DPPIV-only inhibitor, Sitagliptin. Uptake of **XY-FAP-01** was measured and semi-log inhibitor-response curves were generated for both Talabostat and Sitagliptin. FIG. 3C shows the uptake of [¹¹¹In]-**XY-FAP-02** in FAP-positive U87 and FAP-negative PC3 cell lines. Cells were incubated with 1 μ Ci [¹¹¹In]-**XY-FAP-02** and were washed with cold PBS.

Radioactivity of the cell pellets was measured and normalized to the incubated dose; FIG. 4 is a table showing the *ex vivo* tissue biodistribution of [¹¹¹In]-**XY-FAP-01** in tumor bearing mice. At 5 min, 0.5 h, 2 h, 6 h, and 12 h after injection of 10 μ Ci [¹¹¹In]-**XY-FAP-01**, NOD/SKID mice bearing U87 and PC3 tumor xenografts were sacrificed and tissues were collected for biodistribution analysis. Additionally, mice co-injected with unlabeled **XY-FAP-02** and 10 μ Ci [¹¹¹In]-**XY-FAP-01** were sacrificed at 6 h post-injection to study the effect of blocking on uptake of the radiolabeled compound. Data presented as mean $\pm$ standard deviation. ^aStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 5 min, 0.5 h, 2 h, and 6 h post injection (p<0.0001). No significant difference between the two groups were seen in the blocking study at 6 h. ^bStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 12 h post injection (p=0.0006). ^cStudent's t test comparing %ID/g between PC3 tumor and U87 tumors at 6 h post injection showed significant difference between %ID/g tumors in the blocking study at 6 h versus the normal biodistribution results at 6 h (p<0.0001);

FIG. 5A and FIG. 5B show the time-activity relationship of the *ex vivo* biodistribution of [¹¹¹In]-**XY-FAP-02**. FIG. 5A shows tissue time activity curves (TACs) of [¹¹¹In]-**XY-FAP-02** activity in U87 tumor, PC3 tumor, and blood. FIG. 5B shows the ratios of %ID/g between U87 tumor and PC3 tumor, blood, and muscle (mm) versus time;

FIG. 6 shows serial NIRF-imaging of **XY-FAP-01** in tumor bearing mice. NOD/SKID mice bearing FAP-positive U87 (yellow circle) and FAP-negative PC3 (red circle) tumor xenografts were injected with 10 nmol of **XY-FAP-01** via the tail

vein followed by serial NIRF-imaging on the LI-COR Pearl Impulse Imager. Representative images at 0.5 h, 1 h, 2.5 h, and 4 h after injection are shown;

FIG. 7 shows SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks; and

FIG. 8 show three-dimensional SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Figures, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

I. IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN- α (FAP- α)

FAP- α is a type II integral membrane serine protease of the prolyl oligopeptidase family, which are distinguished by their ability to cleave the Pro-AA peptide bond (where AA represents any amino acid). It has been shown to play a role in cancer by modifying bioactive signaling peptides through this enzymatic activity (Kelly, et al., 2005; Edosada, et al., 2006). FAP- α expression has been detected on the surface of fibroblasts in the stroma surrounding greater than 90% of the epithelial cancers, including, but not limited to, malignant breast, colorectal, skin, prostate,

pancreatic cancers, and the like, and inflammation diseases, including, but not limited to, arthritis, fibrosis, and the like, with nearly no expression in healthy tissues. Accordingly, imaging and radiotherapeutic agents specifically targeting FAP- α is of clinical importance.

5 FAP- α exists as a homodimer to carry out its enzymatic function. Inhibitors selectively targeting FAP- α has been reported (Lo, et al., 2009; Tsai, et al., 2010; Ryabtsova, et al., 2012; Poplawski, et al., 2013; Jansen, et al., 2013; Jansen, et al., 2014). The presently disclosed subject matter provides, in part, a FAP- α selective targeting moiety that can be modified with an optical dye, a radiometal chelation
10 complex, and other radiolabeled prosthetic groups, thus providing a platform for the imaging and radiotherapy targeting FAP- α .

Radionuclide molecular imaging, including positron emission tomography (PET), is the most mature molecular imaging technique without tissue penetration limitations. Due to its advantages of high sensitivity and quantifiability, radionuclide
15 molecular imaging plays an important role in clinical and preclinical research (Youn, et al., 2012; Chen, et al., 2014). Many radionuclides, primarily β - and alpha emitters, have been investigated for targeted radioimmunotherapy and include both radiohalogens and radiometals (see Table 1 for representative therapeutic radionuclides).

20

Table 1. Representative Therapeutic Radionuclides	
β -particle emitters	^{90}Y , ^{131}I , ^{177}Lu , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb
α -particle emitters	^{225}Ac , ^{213}Bi , ^{212}Bi , ^{211}At , ^{212}Pb
Auger electron emitters	^{125}I , ^{123}I , ^{67}Ga , ^{111}In

The highly potent and specific binding moiety targeting FAP- α enables its use in nuclear imaging and radiotherapy. The presently disclosed subject matter provides the first synthesis of nuclear imaging and radiotherapy agents based on this
25 dual-targeting moiety to FAP- α .

Accordingly, in some embodiments, the presently disclosed subject matter provides potent and selective low-molecular-weight (LMW) ligands of FAP- α , i.e., an FAP- α selective inhibitor, conjugated with a targeting moiety feasible for modification with optical dyes and radiolabeling groups, including metal chelators

and metal complexes, which enable *in vivo* optical imaging, nuclear imaging (optical, PET and SPECT), and radiotherapy targeting FAP- α . Importantly, the presently disclosed compounds can be modified, e.g., conjugated with, labeling groups without significantly losing their potency. The presently disclosed approach allows for the convenient labeling of the FAP- α ligand with optical dyes and PET or SPECT isotopes, including, but not limited to, ^{68}Ga , ^{64}Cu , ^{18}F , ^{86}Y , ^{90}Y , ^{89}Zr , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{125}I , ^{124}I , for FAP- α related imaging applications. Further, the presently disclosed approach allows for the radiolabeling of the FAP- α ligand with radiotherapeutic isotopes, including but not limited to, ^{90}Y , ^{177}Lu , ^{125}I , ^{131}I , ^{211}At , ^{111}In , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb , ^{225}Ac , ^{213}Bi , ^{212}Bi , ^{212}Pb , and ^{67}Ga , for FAP- α related radio-therapy.

In a particular embodiment, an optical agent conjugated with IRDye-800CW (**XY-FAP-01**) was synthesized and showed selective uptake *in vitro* on a FAP- α + U87 cell line and *in vivo* on a FAP- α + U87 tumor and clearly detected the tumor. In another particular embodiment, an ^{111}In labeled ligand (**XY-FAP-02- ^{111}In**) was successfully obtained in high yield and purity from its precursor with a metal chelator. The *in vivo* study showed clear tumor radiotracer uptake in mice bearing FAP- α -positive U87 tumors with minimum non-specific organ uptake, which allows the specific imaging of FAP- α expressing tumors. The presently disclosed FAP- α targeting moiety can be adapted for use with optical dyes and radioisotopes known in the art for imaging and therapeutic applications targeting FAP- α .

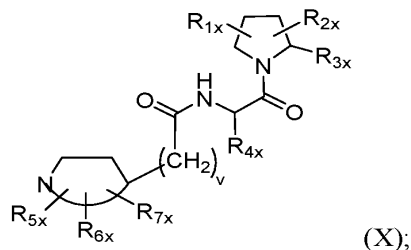
More particularly, in some embodiments, the presently disclosed subject matter provides a compound of the general structure of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

Representative targeting moieties for FAP- α are disclosed in U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014; U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016; and International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013, each of which are incorporate by reference in their entirety.

More particularly, U.S. Patent No. 9,346,814 to Jansen et al., discloses FAP- α inhibitors of formula (X), or a stereoisomer, tautomer, racemate, salt, hydrate, or solvate thereof, which are suitable for use with the presently disclosed subject matter:



5 wherein:

R_{1x} and R_{2x} are each independently selected from the group consisting of H, OH, halogen, C_{1-6} alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

R_{3x} is selected from the group consisting of H, $-CN$, $-B(OH)_2$, $-C(O)alkyl$, $-C(O)aryl$, $-C=C-C(O)aryl$, $-C=C-S(O)_2aryl$, $-CO_2H$, $-SO_3H$, $-SO_2NH_2$, $-PO_3H_2$, and
10 5-tetrazolyl;

R_{4x} is H;

R_{5x} , R_{6x} , and R_{7x} are each independently selected from the group consisting of H, $-OH$, oxo, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, $-NR_{8x}R_{9x}$, $-OR_{12x}$, $-Het_2$ and $-Ar_2$; each of C_{1-6} alkyl being optionally substituted with from 1 to 3 substituents
15 selected from $-OH$ and halogen;

R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, $-OH$, halo, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, and $-Ar_3$;

R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, $-OH$, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl; Ar_1 , Ar_2 and
20 Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from $-NR_{10x}R_{11x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

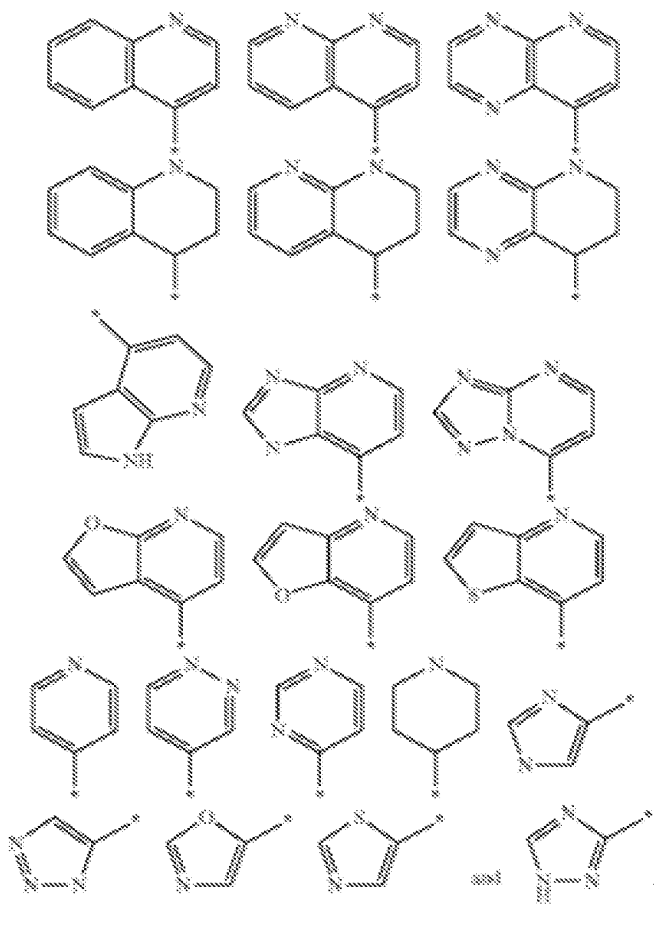
Het_2 is a 5- or 6-membered non-aromatic monocycle optionally comprising 1
25 or 2 heteroatoms selected from O, N and S; Het_2 being optionally substituted with from 1 to 3 substituents selected from $-NR_{13x}R_{14x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

v is 0, 1, 2, or 3; and



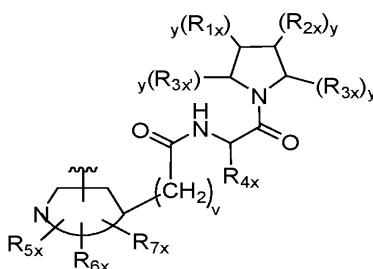
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S.

5 In particular embodiments,  is selected from the group consisting of:



wherein * indicates the point of attachment of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle to $-(CH_2)_x-$.

10 Accordingly, in some embodiments, A is an FAP- α targeting moiety having the structure of:



(X');

wherein each y is independently an integer selected from the group consisting of 0, 1, and 2;

R_{1x}, R_{2x}, and R_{3x}, are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl-, -C=C-S(O)₂aryl-, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R_{4x} is H;

R_{5x}, R_{6x}, and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents selected from -OH and halogen;

R_{8x}, R_{9x}, and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃;

R_{10x}, R_{11x}, R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar₁, Ar₂ and Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

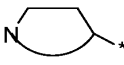
Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl;

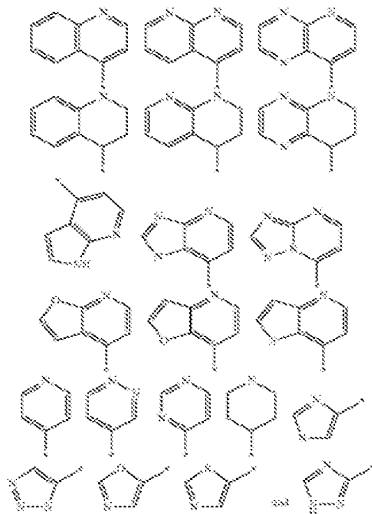
v is 0, 1, 2, or 3; and



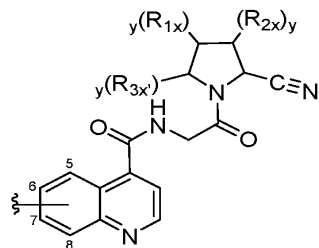
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S;

wherein  indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments,  is selected from the group consisting of:

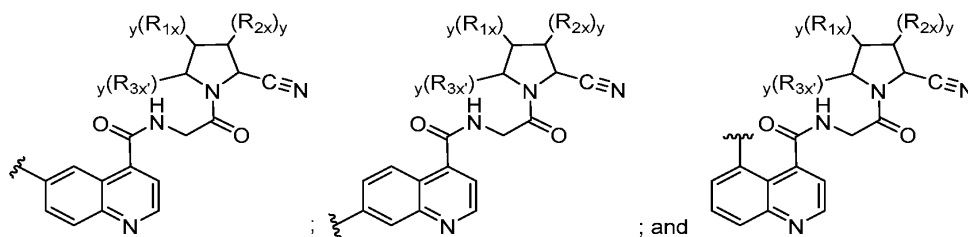


In some embodiments, A is an FAP- α targeting moiety having the structure of:

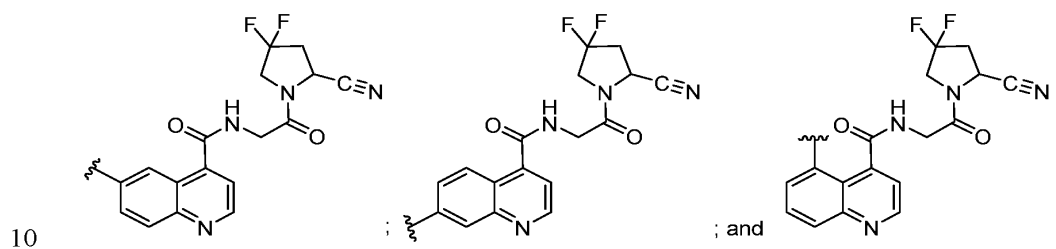


wherein y , R_{1x} , R_{2x} and $R_{3x'}$ are defined as hereinabove; $\frac{1}{2}$ indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be
 5 through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments, A is selected from the group consisting of:

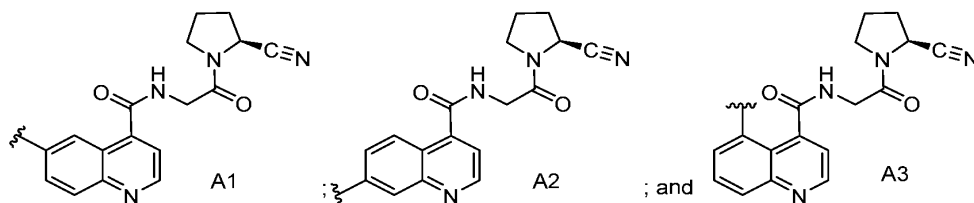


In more particular embodiments, A is selected from the group consisting of:

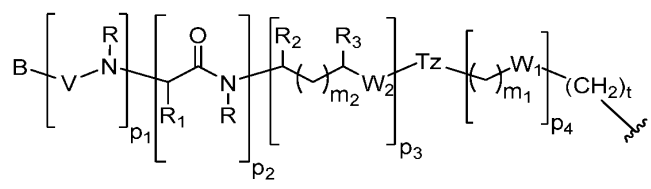


and stereoisomers thereof.

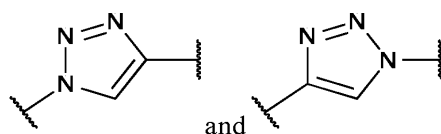
In yet more particular embodiments, A is selected from the group consisting of:



In some embodiments, the combination of L and B can be represented by:

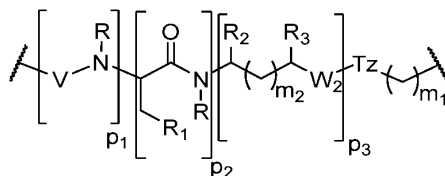


wherein the subunits associated with elements p_1 , p_2 , p_3 and p_4 may be in any order; t is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; p_1 , p_3 , and p_4 are each independently 0 or 1; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'_2-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_q-$ and $-(CH_2-CH_2-O)_q-$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R or R' is independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, and $-OR_4$, wherein R_4 is selected from the group consisting of H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, and substituted heterocycloalkyl, wherein q is defined as immediately hereinabove; Tz is a triazole group that can be present or absent and, if present, is selected from the group consisting of



; each R_1 is independently H, C_1 - C_6 alkyl, C_3 - C_{12} aryl, $-(CH_2)_q$ - C_3 - C_{12} aryl, $-C_4$ - C_{16} alkylaryl, or $-(CH_2)_q$ - C_4 - C_{16} alkylaryl; R_2 and R_3 are each independently H and $-CO_2R_5$, wherein R_5 is selected from the group consisting of H, C_1 - C_6 alkyl, C_3 - C_{12} aryl, and C_4 - C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_5 , then the other is H; V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$; B is any optical or radiolabeled functional group suitable for optical, PET, or SPECT imaging or radiotherapy; and stereoisomers and pharmaceutically acceptable salts thereof.

In some embodiments, L has the following general structure:



wherein p_1 , p_2 , p_3 , m_1 , m_2 , q , t , Tz , W_2 , R , R_1 , R_2 , R_3 , and V are defined as hereinabove.

In some embodiments, L is selected from the group consisting of $-L_1-$, $-L_2-L_3-$, and $-L_1-L_2-L_3-$, wherein:

5 L_1 is $-\text{NR}-(\text{CH}_2)_q-[\text{O}-\text{CH}_2-\text{CH}_2-\text{O}]_q-\text{C}(=\text{O})-$;

L_2 is $-\text{NR}-(\text{CH}_2)_q-\text{C}(\text{COOR}_5)-\text{NR}-$; and

L_3 is $-(\text{O})\text{C}-(\text{CH}_2)_q-\text{C}(=\text{O})-$;

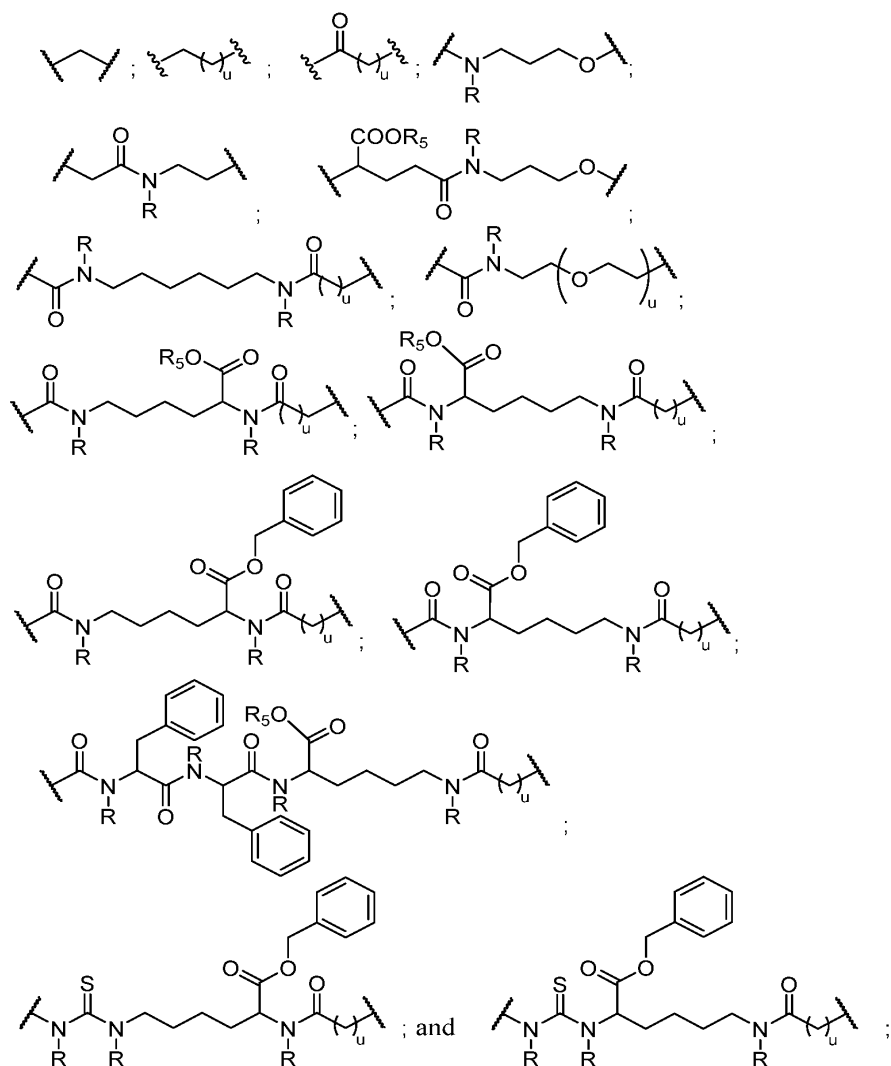
wherein each q is independently an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove.

10 In particular embodiments, L is:

$-(\text{CR}_6\text{H})_q-(\text{CH}_2)_q-\text{C}(=\text{O})-\text{NR}-(\text{CH}_2)_q-\text{O}-$ or $-\text{NR}-(\text{CH}_2)_q-\text{O}-$;

wherein each q and R is defined hereinabove; and R_6 is H or $-\text{COOR}_5$.

In yet more particular embodiments, L is selected from the group consisting of:

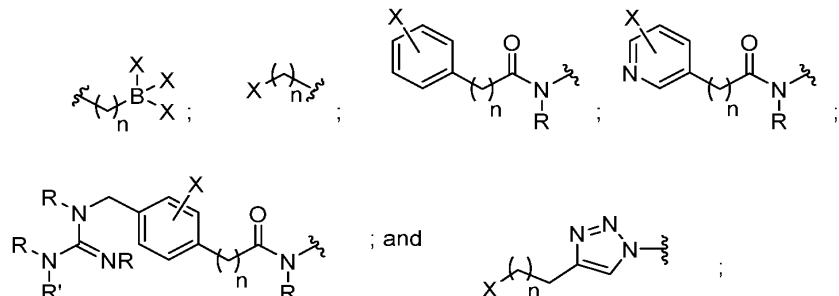


wherein u is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove.

Suitable linkers are disclosed in U.S. Patent Application Publication No.

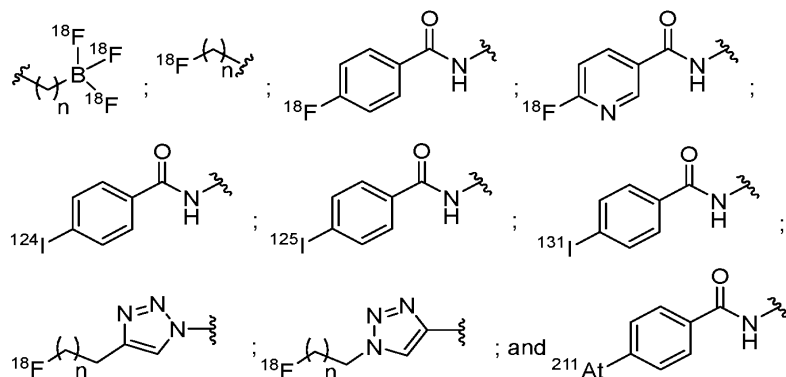
- 5 US2011/0064657 A1, for "Labeled Inhibitors of Prostate Specific Membrane Antigen (PSMA), Biological Evaluation, and Use as Imaging Agents," published March 17, 2011, to Pomper et al., and U.S. Patent Application Publication No. US2012/0009121 A1, for "PSMA-Targeting Compounds and Uses Thereof," published January 12, 2012, to Pomper et al, each of which is incorporated by reference in its entirety.

In some embodiments, B is a radiolabeled prosthetic group comprising a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At . Representative radiolabeled prosthetic groups include, but are not limited to:

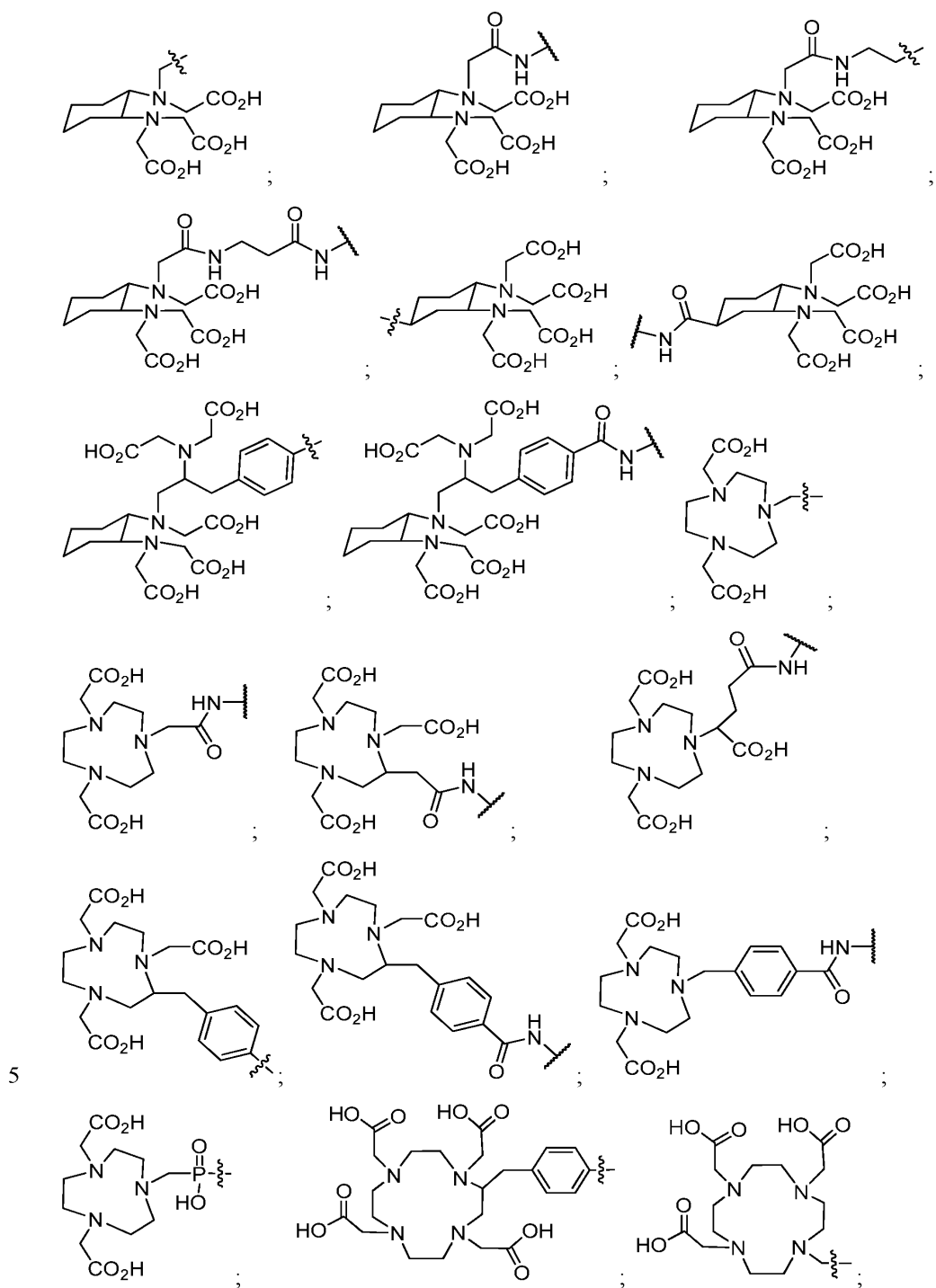


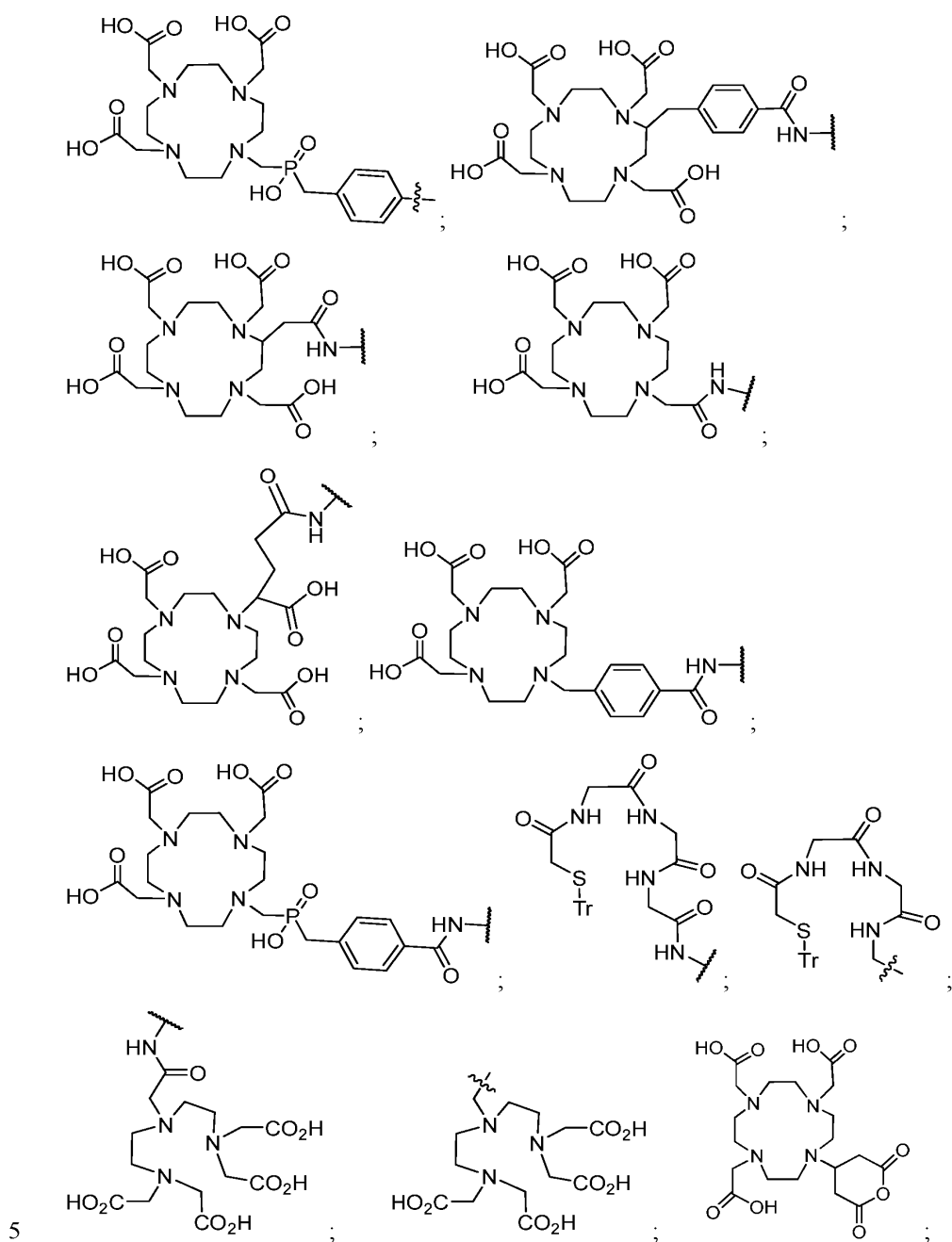
- 5 wherein each X is independently a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At ; each R and R' is defined hereinabove; and each n is independently an integer selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

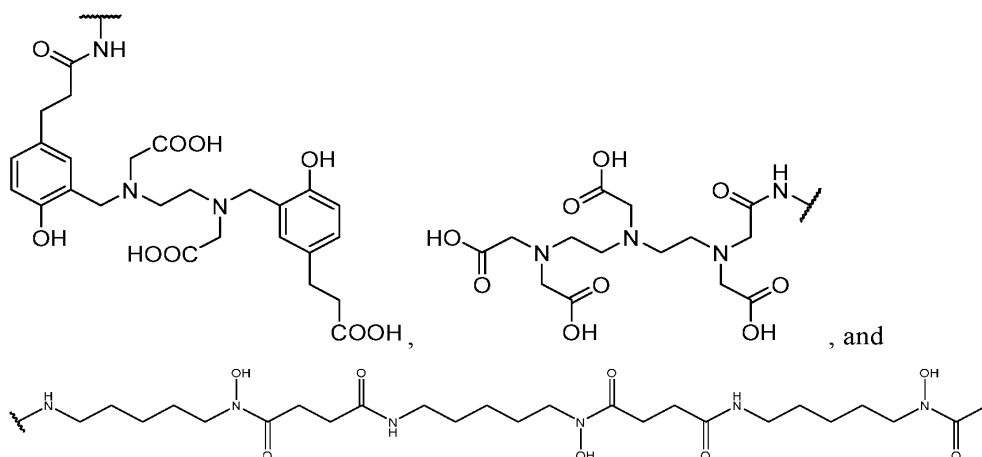
- 10 In more particular embodiments, the radiolabeled prosthetic group is selected from the group consisting of:



In other embodiments, B comprises a chelating agent. Representative chelating agents include, but are not limited to:

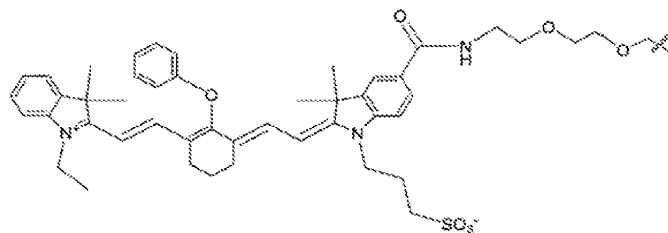
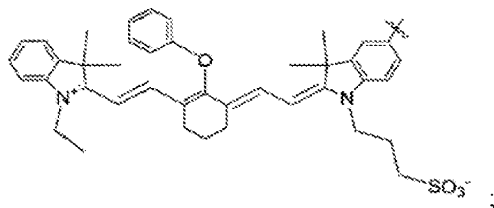




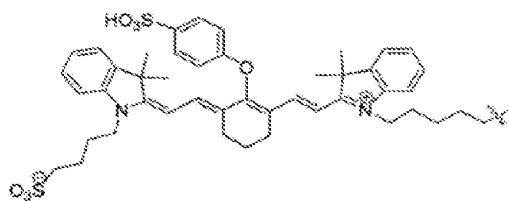


In some embodiments, B comprises an optical dye, e.g., in particular
 5 embodiments, a fluorescent dye. In some embodiments, the fluorescent dye moiety
 comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and
 merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-
 dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680,
 VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750,
 10 AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647,
 HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye
 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.

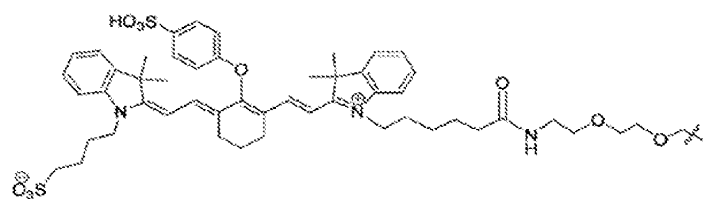
Representative optical dyes include, but are not limited to:



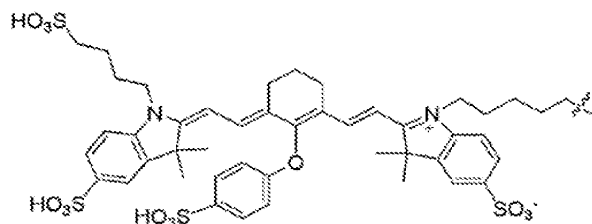
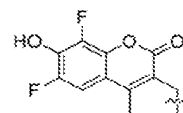
15



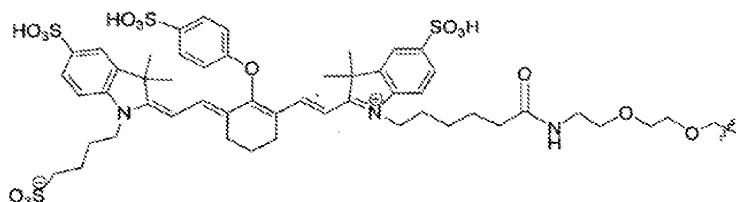
;



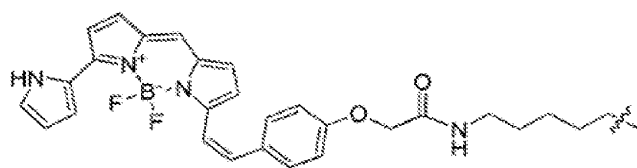
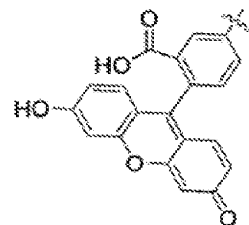
;



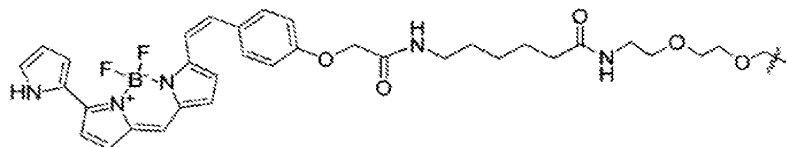
;



;

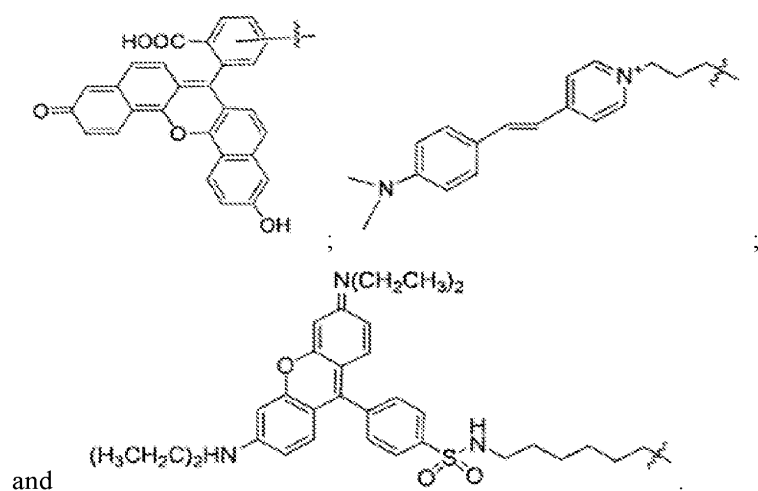


;

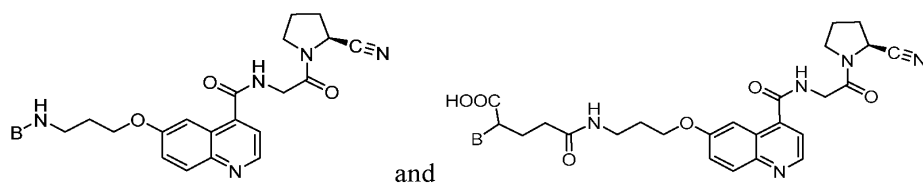


;

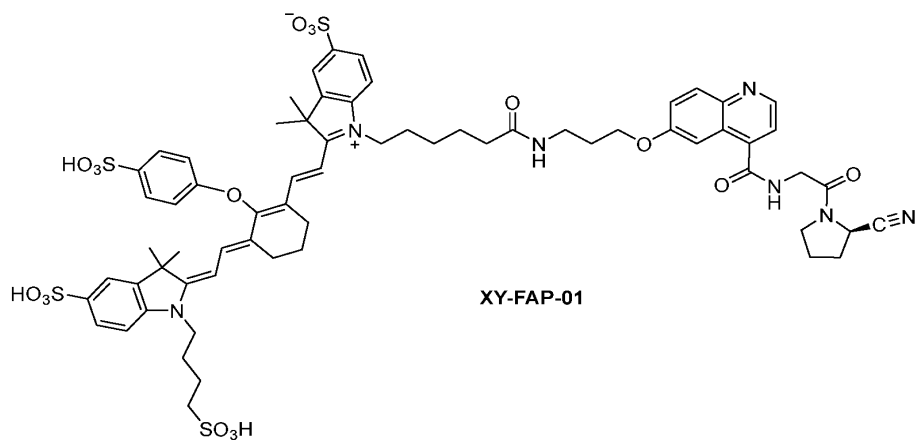
5



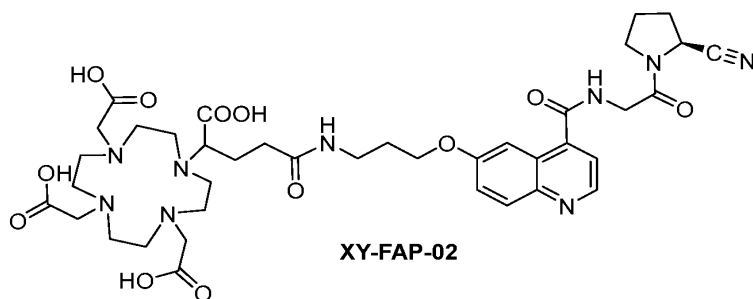
In some embodiments, the presently disclosed subject matter provides a compound selected from the group consisting of:



In particular embodiments, the compound is selected from the group consisting of:



; and



B. Pharmaceutical Compositions and Administration

In another aspect, the present disclosure provides a pharmaceutical comprising
 5 a compound of formula (I) in admixture with a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. One of skill in the art will recognize that the pharmaceutical compositions include the pharmaceutically acceptable salts or hydrates of the compounds described above.

Pharmaceutically acceptable salts are generally well known to those of
 10 ordinary skill in the art and include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituent moieties found on the compounds described herein. When compounds of the present disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the
 15 desired base, either neat or in a suitable inert solvent or by ion exchange, whereby one basic counterion (base) in an ionic complex is substituted for another. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt.

When compounds of the present disclosure contain relatively basic
 20 functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent or by ion exchange, whereby one acidic counterion (acid) in an ionic complex is substituted for another. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric,
 25 hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic,

benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-toluenesulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

Accordingly, pharmaceutically acceptable salts suitable for use with the presently disclosed subject matter include, by way of example but not limitation, acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinolate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

In therapeutic and/or diagnostic applications, the compounds of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained-slow release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000). Suitable routes may include oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra -sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

For injection, the agents of the disclosure may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the
5 formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable inert carriers to formulate the compounds herein disclosed for the practice of the disclosure into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in
10 particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and
15 the like, for oral ingestion by a subject (e.g., patient) to be treated.

For nasal or inhalation delivery, the agents of the disclosure also may be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances, such as saline; preservatives, such as benzyl alcohol; absorption
20 promoters; and fluorocarbons.

Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure
25 provided herein. Generally, the compounds according to the disclosure are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from 0.01 to 1000 mg, from 0.5 to 100 mg, from 1 to 50 mg per day, and from 5 to 40 mg per day are examples of dosages that may be used. A non-limiting dosage is 10 to 30 mg per day. The exact dosage will depend upon the route of administration, the
30 form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, the bioavailability of the compound(s), the adsorption, distribution, metabolism, and excretion (ADME) toxicity of the compound(s), and the preference and experience of the attending physician.

In addition to the active ingredients, these pharmaceutical compositions may

contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

5 Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for
10 example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked
15 polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

15 Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or
20 to characterize different combinations of active compound doses.

 Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants
25 such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

30 C. *Methods of Imaging using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof*

 In some embodiments, presently disclosed subject matter provides a method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group

suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for imaging one or more cells, organs, or tissues, the method comprising exposing cells or administering to a subject an effective amount of a compound of formula (I) with an optical or radioisotopic label suitable for imaging. In some embodiments, the one or more organs or tissues include prostate tissue, kidney tissue, brain tissue, vascular tissue, or tumor tissue.

The imaging methods of the invention are suitable for imaging any physiological process or feature in which FAP- α is involved, for example, identifying areas of tissues or targets which exhibit or express high concentrations of FAP- α . Physiological processes in which FAP- α is involved include, but are not limited to: (a) proliferation diseases (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c) endocrinological disorders (including but not limited to disorders of glucose metabolism).

In certain embodiments, the radiolabeled compound is stable in vivo.

In certain embodiments, the radiolabeled compound is detected by positron emission tomography (PET) or single photon emission computed tomography (SPECT).

In certain embodiments, the optical reporting moiety is detected by fluorescence, such as fluorescence microscopy.

In certain embodiments, the presently disclosed compounds are excreted from tissues of the body quickly to prevent prolonged exposure to the radiation of the radiolabeled compound administered to the subject. Typically, the presently disclosed compounds are eliminated from the body in less than about 24 hours. More typically, the presently disclosed compounds are eliminated from the body in less than about 16 hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes. Exemplary compounds are eliminated in between about 60 minutes and about 120 minutes. In certain embodiments, the presently disclosed compounds are stable in vivo such that substantially all, e.g., more than about 50%, 60%, 70%, 80%, or 90% of the injected compound is not metabolized by the body prior to excretion.

Additionally, for in vitro applications, such as in vitro diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use, such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

Other embodiments provide kits comprising a compound of formula (I). In certain embodiments, the kit provides packaged pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of formula (I). In certain embodiments the packaged pharmaceutical composition will comprise the reaction precursors necessary to generate the compound of formula (I) upon combination with a radiolabeled precursor. Other packaged pharmaceutical compositions further comprise indicia comprising at least one of: instructions for preparing compounds of formula (I) from supplied precursors, instructions for using the composition to image cells or tissues expressing FAP- α .

In certain embodiments, a kit containing from about 1 to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier, is provided. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound of formula (I) in solution or in lyophilized form, and these components of the kit may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form. Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

In certain embodiments, a kit provides a non-radiolabeled precursor to be combined with a radiolabeled reagent on-site.

Imaging agents may be used in accordance with the presently disclosed methods by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with FAP- α . The spatial distribution may be measured using any means suitable for the particular label, for example, a gamma camera, a PET apparatus, a

SPECT apparatus, and the like. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions or fluorescence. A particularly useful imaging approach employs more than one imaging agent to perform simultaneous studies.

5 In general, a detectably effective amount of the imaging agent of the invention is administered to a subject. A “detectably effective amount” of the imaging agent is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent may be administered in more than one injection. The detectably effective amount of the
10 imaging agent of the invention can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective amounts of the imaging agent also can vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art. The
15 amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the amount of imaging agent
20 to administer to each individual patient and the duration of the imaging study.

D. Methods of Treating a FAP- α Related Disease or Disorder using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof

 In other embodiments, the presently disclosed compounds of formula (I) can be used to treat a subject afflicted with one or more FAP- α related diseases or
25 disorders including, but not limited to: (a) proliferation (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c)
 endocrinological disorders (including but not limited to disorders of glucose
30 metabolism).

 Accordingly, in some embodiments, the one or more FAP- α related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and

connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

5 In general, the “effective amount” of an active agent or drug delivery device refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the makeup of the pharmaceutical composition, the target
10 tissue, and the like.

 In other embodiments, the method can be practiced *in vitro* or *ex vivo* by introducing, and preferably mixing, the compound and cell(s) or tumor(s) in a controlled environment, such as a culture dish or tube. The method can be practiced *in vivo*, in which case contacting means exposing the target in a subject to at least
15 one compound of the presently disclosed subject matter, such as administering the compound to a subject via any suitable route. According to the presently disclosed subject matter, contacting may comprise introducing, exposing, and the like, the compound at a site distant to the cells to be contacted, and allowing the bodily functions of the subject, or natural (e.g., diffusion) or man-induced (e.g., swirling)
20 movements of fluids to result in contact of the compound and the target.

 The subject treated by the presently disclosed methods in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term “subject.” Accordingly, a “subject” can
25 include a human subject for medical purposes, such as for the treatment of an existing condition or disease or the prophylactic treatment for preventing the onset of a condition or disease, or an animal (non-human) subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, and the like;
30 bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like. An animal may be a transgenic animal. In some

embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a condition or disease. Thus, the terms “subject” and “patient” are used interchangeably herein. In some
5 embodiments, the subject is human. In other embodiments, the subject is non-human.

As used herein, the term “treating” can include reversing, alleviating, inhibiting the progression of, preventing or reducing the likelihood of the disease, or condition to which such term applies, or one or more symptoms or manifestations of
10 such disease or condition.

“Preventing” refers to causing a disease, condition, or symptom or manifestation of such, or worsening of the severity of such, not to occur. Accordingly, the presently disclosed compounds can be administered prophylactically to prevent or reduce the incidence or recurrence of the disease, or condition.
15

II. DEFINITIONS

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly
20 understood by one of ordinary skill in the art to which this presently described subject matter belongs.

While the following terms in relation to compounds of formula (I) are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. These
25 definitions are intended to supplement and illustrate, not preclude, the definitions that would be apparent to one of ordinary skill in the art upon review of the present disclosure.

The terms substituted, whether preceded by the term “optionally” or not, and substituent, as used herein, refer to the ability, as appreciated by one skilled in this art,
30 to change one functional group for another functional group on a molecule, provided that the valency of all atoms is maintained. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. The substituents also may be further substituted (e.g., an aryl group substituent may

have another substituent off it, such as another aryl group, which is further substituted at one or more positions).

Where substituent groups or linking groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., -CH₂O- is equivalent to -OCH₂-; -C(=O)O- is equivalent to -OC(=O)-; -OC(=O)NR- is equivalent to -NRC(=O)O-, and the like.

When the term “independently selected” is used, the substituents being referred to (e.g., R groups, such as groups R₁, R₂, and the like, or variables, such as “m” and “n”), can be identical or different. For example, both R₁ and R₂ can be substituted alkyls, or R₁ can be hydrogen and R₂ can be a substituted alkyl, and the like.

The terms “a,” “an,” or “a(n),” when used in reference to a group of substituents herein, mean at least one. For example, where a compound is substituted with “an” alkyl or aryl, the compound is optionally substituted with at least one alkyl and/or at least one aryl. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

A named “R” or group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative “R” groups as set forth above are defined below.

Descriptions of compounds of the present disclosure are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

Unless otherwise explicitly defined, a “substituent group,” as used herein, includes a functional group selected from one or more of the following moieties, which are defined herein:

The term hydrocarbon, as used herein, refers to any chemical group comprising hydrogen and carbon. The hydrocarbon may be substituted or unsubstituted. As would be known to one skilled in this art, all valencies must be satisfied in making any substitutions. The hydrocarbon may be unsaturated, saturated, branched, unbranched, cyclic, polycyclic, or heterocyclic. Illustrative hydrocarbons are further defined herein below and include, for example, methyl, ethyl, *n*-propyl, isopropyl, cyclopropyl, allyl, vinyl, *n*-butyl, *tert*-butyl, ethynyl, cyclohexyl, and the like.

The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched chain, acyclic or cyclic hydrocarbon group, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent groups, having the number of carbon atoms designated (i.e., C₁-C₁₀ means one to ten carbons, including 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 carbons). In particular embodiments, the term “alkyl” refers to C₁₋₂₀ inclusive, including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 carbons, linear (i.e., “straight-chain”), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (i.e., alkenyl and alkynyl) hydrocarbon radicals derived from a hydrocarbon moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom.

Representative saturated hydrocarbon groups include, but are not limited to, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *sec*-pentyl, isopentyl, neopentyl, *n*-hexyl, *sec*-hexyl, *n*-heptyl, *n*-octyl, *n*-decyl, *n*-undecyl, dodecyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, and homologs and isomers thereof.

“Branched” refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. “Lower alkyl” refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C₁₋₈ alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. “Higher alkyl” refers to an alkyl group having about 10 to about 20 carbon atoms, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, “alkyl” refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, “alkyl” refers, in particular, to C₁₋₈ branched-chain alkyls.

Alkyl groups can optionally be substituted (a “substituted alkyl”) with one or more alkyl group substituents, which can be the same or different. The term “alkyl group substituent” includes but is not limited to alkyl, substituted alkyl, halo, alkylamino, arylamino, acyl, hydroxyl, aryloxy, alkoxy, alkylthio, arylthio, 5 aralkyloxy, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as “alkylaminoalkyl”), or aryl.

Thus, as used herein, the term “substituted alkyl” includes alkyl groups, as 10 defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

The term “heteroalkyl,” by itself or in combination with another term, means, 15 unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon group, or combinations thereof, consisting of at least one carbon atoms and at least one heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen, phosphorus, and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P and S 20 and Si may be placed at any interior position of the heteroalkyl group or at the position at which alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -Si(CH₃)₃, -CH₂-CH=N-OCH₃, 25 -CH=CH-N(CH₃)-CH₃, O-CH₃, -O-CH₂-CH₃, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃.

As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as 30 -C(O)NR’, -NR’R”, -OR’, -SR, -S(O)R, and/or -S(O)₂R’. Where “heteroalkyl” is recited, followed by recitations of specific heteroalkyl groups, such as -NR’R” or the like, it will be understood that the terms heteroalkyl and -NR’R” are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term “heteroalkyl” should not be interpreted herein as excluding specific

heteroalkyl groups, such as -NR'R'' or the like.

“Cyclic” and “cycloalkyl” refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, unsubstituted alkyl, substituted alkyl, aryl, or substituted aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl, and fused ring systems, such as dihydro- and tetrahydronaphthalene, and the like.

The term “cycloalkylalkyl,” as used herein, refers to a cycloalkyl group as defined hereinabove, which is attached to the parent molecular moiety through an alkyl group, also as defined above. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

The terms “cycloheteroalkyl” or “heterocycloalkyl” refer to a non-aromatic ring system, unsaturated or partially unsaturated ring system, such as a 3- to 10-member substituted or unsubstituted cycloalkyl ring system, including one or more heteroatoms, which can be the same or different, and are selected from the group consisting of nitrogen (N), oxygen (O), sulfur (S), phosphorus (P), and silicon (Si), and optionally can include one or more double bonds.

The cycloheteroalkyl ring can be optionally fused to or otherwise attached to other cycloheteroalkyl rings and/or non-aromatic hydrocarbon rings. Heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or a polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5-membered ring has

0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds, and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring.

5 Representative cycloheteroalkyl ring systems include, but are not limited to pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, quinuclidinyl, morpholinyl, thiomorpholinyl, thiadiazinanyl, tetrahydrofuranyl, and the like.

The terms “cycloalkyl” and “heterocycloalkyl”, by themselves or in
10 combination with other terms, represent, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl”, respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like.
15 Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The terms “cycloalkylene” and “heterocycloalkylene” refer to the divalent derivatives of
20 cycloalkyl and heterocycloalkyl, respectively.

An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. Alkyl
25 groups which are limited to hydrocarbon groups are termed “homoalkyl.”

More particularly, the term “alkenyl” as used herein refers to a monovalent group derived from a C₁₋₂₀ inclusive straight or branched hydrocarbon moiety having at least one carbon-carbon double bond by the removal of a single hydrogen molecule. Alkenyl groups include, for example, ethenyl (i.e., vinyl), propenyl, butenyl, 1-
30 methyl-2-buten-1-yl, pentenyl, hexenyl, octenyl, allenyl, and butadienyl.

The term “cycloalkenyl” as used herein refers to a cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

The term “alkynyl” as used herein refers to a monovalent group derived from a straight or branched C₁₋₂₀ hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon triple bond. Examples of “alkynyl” include ethynyl, 2-propynyl (propargyl), 1-propynyl, pentynyl, hexynyl, and heptynyl groups, and the like.

The term “alkylene” by itself or a part of another substituent refers to a straight or branched bivalent aliphatic hydrocarbon group derived from an alkyl group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more “alkyl group substituents.” There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as “alkylaminoalkyl”), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene (–CH₂–); ethylene (–CH₂–CH₂–); propylene (–(CH₂)₃–); cyclohexylene (–C₆H₁₀–); –CH=CH–CH=CH–; –CH=CH–CH₂–; –CH₂CH₂CH₂CH₂–, –CH₂CH=CHCH₂–, –CH₂CsCCH₂–, –CH₂CH₂CH(CH₂CH₂CH₃)CH₂–, –(CH₂)_q–N(R)–(CH₂)_r–, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxy (–O–CH₂–O–); and ethylenedioxy (–O–(CH₂)₂–O–). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being some embodiments of the present disclosure. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

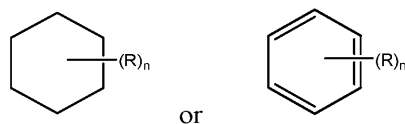
The term “heteroalkylene” by itself or as part of another substituent means a divalent group derived from heteroalkyl, as exemplified, but not limited by, –CH₂–CH₂–S–CH₂–CH₂– and –CH₂–S–CH₂–CH₂–NH–CH₂–. For heteroalkylene groups, heteroatoms also can occupy either or both of the chain termini (e.g., alkyleneoxo, alkylenedioxo, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula –C(O)OR’– represents both –C(O)OR’– and –R’OC(O)–.

The term “aryl” means, unless otherwise stated, an aromatic hydrocarbon substituent that can be a single ring or multiple rings (such as from 1 to 3 rings), which are fused together or linked covalently. The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms (in each separate ring in the case of multiple rings) selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalyl, 5-quinoxalyl, 3-quinolyl, and 6-quinolyl. Substituents for each of above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. The terms “arylene” and “heteroarylene” refer to the divalent forms of aryl and heteroaryl, respectively.

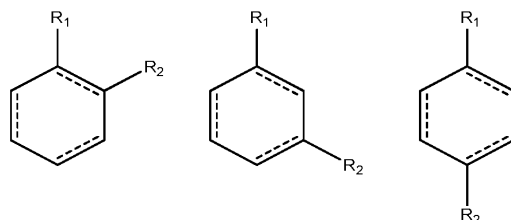
For brevity, the term “aryl” when used in combination with other terms (e.g., aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the terms “arylalkyl” and “heteroarylalkyl” are meant to include those groups in which an aryl or heteroaryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl, furylmethyl, and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like). However, the term “haloaryl,” as used herein is meant to cover only aryls substituted with one or more halogens.

Where a heteroalkyl, heterocycloalkyl, or heteroaryl includes a specific number of members (e.g. “3 to 7 membered”), the term “member” refers to a carbon or heteroatom.

Further, a structure represented generally by the formula:



as used herein refers to a ring structure, for example, but not limited to a 3-carbon, a 4-carbon, a 5-carbon, a 6-carbon, a 7-carbon, and the like, aliphatic and/or aromatic cyclic compound, including a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure, comprising a substituent R group, wherein the R group can be present or absent, and when present, one or more R groups can each be substituted on one or more available carbon atoms of the ring structure. The presence or absence of the R group and number of R groups is determined by the value of the variable “n,” which is an integer generally having a value ranging from 0 to the number of carbon atoms on the ring available for substitution. Each R group, if more than one, is substituted on an available carbon of the ring structure rather than on another R group. For example, the structure above where n is 0 to 2 would comprise compound groups including, but not limited to:



and the like.

A dashed line representing a bond in a cyclic ring structure indicates that the bond can be either present or absent in the ring. That is, a dashed line representing a bond in a cyclic ring structure indicates that the ring structure is selected from the group consisting of a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure.

The symbol () denotes the point of attachment of a moiety to the remainder of the molecule.

When a named atom of an aromatic ring or a heterocyclic aromatic ring is defined as being “absent,” the named atom is replaced by a direct bond.

Each of above terms (e.g. , “alkyl,” “heteroalkyl,” “cycloalkyl, and “heterocycloalkyl”, “aryl,” “heteroaryl,” “phosphonate,” and “sulfonate” as well as their divalent derivatives) are meant to include both substituted and unsubstituted forms of the indicated group. Optional substituents for each type of group are provided below.

Substituents for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl monovalent

and divalent derivative groups (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such groups. R', R'', R''' and R'''' each may independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. As used herein, an "alkoxy" group is an alkyl attached to the remainder of the molecule through a divalent oxygen. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7- membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1- pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

Similar to the substituents described for alkyl groups above, exemplary substituents for aryl and heteroaryl groups (as well as their divalent derivatives) are varied and are selected from, for example: halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR''', -NR-C(NR'R'')=NR''' -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxo, and fluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on aromatic ring system; and where R', R'', R''' and R'''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or

unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present.

5 Two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and
10 B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 4.

One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula
15 -(CRR')_s-X'-(C''R'')_d-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'' and R''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

20 As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent and has the general formula RC(=O)-, wherein R is an alkyl, alkenyl, alkynyl, aryl, carbocyclic, heterocyclic, or aromatic heterocyclic group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as a 2-(furan-2-yl)acetyl)- and a 2-phenylacetyl group. Specific examples of acyl groups include acetyl and benzoyl.
25 Acyl groups also are intended to include amides, -RC(=O)NR', esters, -RC(=O)OR', ketones, -RC(=O)R', and aldehydes, -RC(=O)H.

The terms "alkoxyl" or "alkoxy" are used interchangeably herein and refer to a saturated (i.e., alkyl-O-) or unsaturated (i.e., alkenyl-O- and alkynyl-O-) group
30 attached to the parent molecular moiety through an oxygen atom, wherein the terms "alkyl," "alkenyl," and "alkynyl" are as previously described and can include C₁₋₂₀ inclusive, linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, *n*-butoxyl, *sec*-butoxyl, *tert*-butoxyl, and *n*-pentoxy, neopentoxy, *n*-hexoxyl, and the like.

The term “alkoxyalkyl” as used herein refers to an alkyl-O-alkyl ether, for example, a methoxyethyl or an ethoxymethyl group.

“Aryloxy” refers to an aryl-O- group wherein the aryl group is as previously described, including a substituted aryl. The term “aryloxy” as used herein can refer to phenyloxy or hexyloxy, and alkyl, substituted alkyl, halo, or alkoxy substituted phenyloxy or hexyloxy.

“Aralkyl” refers to an aryl-alkyl-group wherein aryl and alkyl are as previously described and includes substituted aryl and substituted alkyl. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

“Aralkyloxy” refers to an aralkyl-O- group wherein the aralkyl group is as previously described. An exemplary aralkyloxy group is benzyloxy, i.e., $C_6H_5-CH_2-O-$. An aralkyloxy group can optionally be substituted.

“Alkoxy carbonyl” refers to an alkyl-O-C(=O)- group. Exemplary alkoxy carbonyl groups include methoxy carbonyl, ethoxy carbonyl, butyloxy carbonyl, and *tert*-butyloxy carbonyl.

“Aryloxy carbonyl” refers to an aryl-O-C(=O)- group. Exemplary aryloxy carbonyl groups include phenoxy- and naphthoxy-carbonyl.

“Aralkoxy carbonyl” refers to an aralkyl-O-C(=O)- group. An exemplary aralkoxy carbonyl group is benzyloxy carbonyl.

“Carbamoyl” refers to an amide group of the formula $-C(=O)NH_2$.

“Alkyl carbamoyl” refers to a $R'RN-C(=O)-$ group wherein one of R and R' is hydrogen and the other of R and R' is alkyl and/or substituted alkyl as previously described. “Dialkyl carbamoyl” refers to a $R'RN-C(=O)-$ group wherein each of R and R' is independently alkyl and/or substituted alkyl as previously described.

The term carbonyldioxy, as used herein, refers to a carbonate group of the formula $-O-C(=O)-OR$.

“Acyloxy” refers to an acyl-O- group wherein acyl is as previously described.

The term “amino” refers to the $-NH_2$ group and also refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms “acylamino” and “alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

An “aminoalkyl” as used herein refers to an amino group covalently bound to an alkylene linker. More particularly, the terms alkylamino, dialkylamino, and

trialkylamino as used herein refer to one, two, or three, respectively, alkyl groups, as previously defined, attached to the parent molecular moiety through a nitrogen atom. The term alkylamino refers to a group having the structure -NHR' wherein R' is an alkyl group, as previously defined; whereas the term dialkylamino refers to a group
5 having the structure -NR'R'' , wherein R' and R'' are each independently selected from the group consisting of alkyl groups. The term trialkylamino refers to a group having the structure -NR'R''R''' , wherein R' , R'' , and R''' are each independently selected from the group consisting of alkyl groups. Additionally, R' , R'' , and/or R''' taken together may optionally be $\text{-(CH}_2\text{)}_k\text{-}$ where k is an integer from 2 to 6.
10 Examples include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, isopropylamino, piperidino, trimethylamino, and propylamino.

The amino group is -NR'R'' , wherein R' and R'' are typically selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl,
15 substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

The terms alkylthioether and thioalkoxyl refer to a saturated (i.e., alkyl-S-) or unsaturated (i.e., alkenyl-S- and alkynyl-S-) group attached to the parent molecular moiety through a sulfur atom. Examples of thioalkoxyl moieties include, but are not
20 limited to, methylthio, ethylthio, propylthio, isopropylthio, *n*-butylthio, and the like.

“Acylamino” refers to an acyl-NH- group wherein acyl is as previously described. “Aroylamino” refers to an aroyl-NH- group wherein aroyl is as previously described.

The term “carbonyl” refers to the -C(=O)- group, and can include an aldehyde
25 group represented by the general formula R-C(=O)H .

The term “carboxyl” refers to the -COOH group. Such groups also are referred to herein as a “carboxylic acid” moiety.

The terms “halo,” “halide,” or “halogen” as used herein refer to fluoro, chloro, bromo, and iodo groups. Additionally, terms such as “haloalkyl,” are meant to
30 include monohaloalkyl and polyhaloalkyl. For example, the term “halo($\text{C}_1\text{-C}_4$)alkyl” is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term “hydroxyl” refers to the -OH group.

The term “hydroxyalkyl” refers to an alkyl group substituted with an –OH group.

The term “mercapto” refers to the –SH group.

The term “oxo” as used herein means an oxygen atom that is double bonded to
5 a carbon atom or to another element.

The term “nitro” refers to the –NO₂ group.

The term “thio” refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term “sulfate” refers to the –SO₄ group.

10 The term thiohydroxyl or thiol, as used herein, refers to a group of the formula –SH.

More particularly, the term “sulfide” refers to compound having a group of the formula –SR.

The term “sulfone” refers to compound having a sulfonyl group –S(O₂)R.

15 The term “sulfoxide” refers to a compound having a sulfinyl group –S(O)R

The term ureido refers to a urea group of the formula –NH—CO—NH₂.

Throughout the specification and claims, a given chemical formula or name shall encompass all tautomers, congeners, and optical- and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

20 Certain compounds of the present disclosure may possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as D- or L- for amino acids, and individual isomers are encompassed within the scope of the present
25 disclosure. The compounds of the present disclosure do not include those which are known in art to be too unstable to synthesize and/or isolate. The present disclosure is meant to include compounds in racemic, scalemic, and optically pure forms.

Optically active (R)- and (S)-, or D- and L-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the
30 compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each

asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the disclosure.

5 It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure. The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

10 As used herein the term "monomer" refers to a molecule that can undergo polymerization, thereby contributing constitutional units to the essential structure of a macromolecule or polymer.

A "polymer" is a molecule of high relative molecule mass, the structure of which essentially comprises the multiple repetition of unit derived from molecules of low relative molecular mass, i.e., a monomer.

15 A "dendrimer" is highly branched, star-shaped macromolecules with nanometer-scale dimensions.

As used herein, an "oligomer" includes a few monomer units, for example, in contrast to a polymer that potentially can comprise an unlimited number of monomers. Dimers, trimers, and tetramers are non-limiting examples of oligomers.

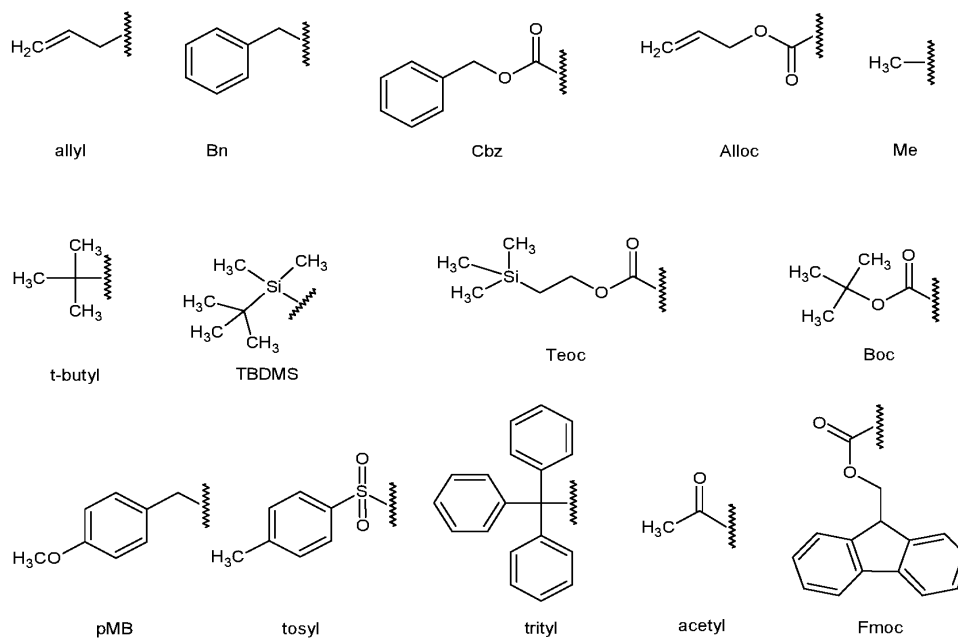
20 The term "protecting group" refers to chemical moieties that block some or all reactive moieties of a compound and prevent such moieties from participating in chemical reactions until the protective group is removed, for example, those moieties listed and described in T. W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, 3rd ed. John Wiley & Sons (1999). It may be advantageous, where
25 different protecting groups are employed, that each (different) protective group be removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions allow differential removal of such protecting groups. For example, protective groups can be removed by acid, base, and hydrogenolysis. Groups such as trityl, dimethoxytrityl, acetal and tert-butyldimethylsilyl are acid
30 labile and may be used to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by hydrogenolysis, and Fmoc groups, which are base labile. Carboxylic acid and hydroxy reactive moieties may be blocked with base labile groups such as, without limitation, methyl, ethyl, and acetyl in the presence of amines blocked with acid labile

groups such as tert-butyl carbamate or with carbamates that are both acid and base stable but hydrolytically removable.

Carboxylic acid and hydroxy reactive moieties may also be blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids may be blocked with base labile groups such as Fmoc. Carboxylic acid reactive moieties may be blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups may be blocked with fluoride labile silyl carbamates.

Allyl blocking groups are useful in the presence of acid- and base- protecting groups since the former are stable and can be subsequently removed by metal or pi-acid catalysts. For example, an allyl-protected carboxylic acid can be deprotected with a palladium(O)- catalyzed reaction in the presence of acid labile t-butyl carbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate may be attached. As long as the residue is attached to the resin, that functional group is blocked and cannot react. Once released from the resin, the functional group is available to react.

Typical blocking/protecting groups include, but are not limited to the following moieties:



Following long-standing patent law convention, the terms “a,” “an,” and “the” refer to “one or more” when used in this application, including the claims. Thus, for example, reference to “a subject” includes a plurality of subjects, unless the context
5 clearly is to the contrary (e.g., a plurality of subjects), and so forth.

Throughout this specification and the claims, the terms “comprise,” “comprises,” and “comprising” are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the
10 exclusion of other like items that can be substituted or added to the listed items.

For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in
15 all instances by the term “about” even though the term “about” may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off,
20 measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term “about,” when referring to a value can be meant to encompass variations of, in some embodiments, $\pm 100\%$ in some
embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in
25 some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, the term “about” when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all
30 numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as

well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range.

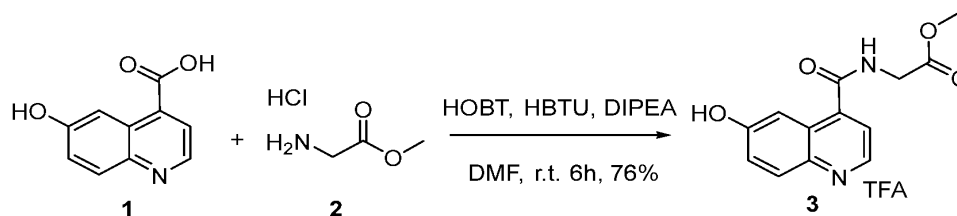
EXAMPLES

5 The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can
10 be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLE 1

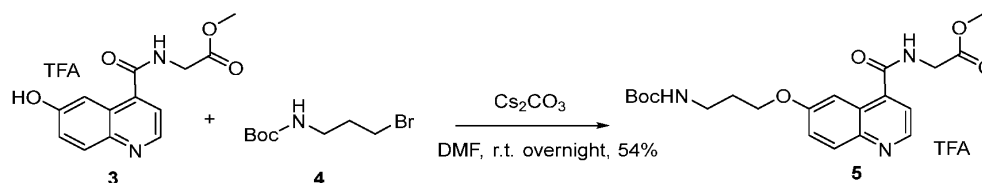
Experimental Procedures

1.1 Synthesis of XY-FAP-01.

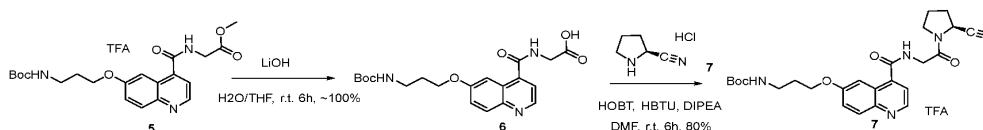


20 Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**): 6-Hydroxyquinoline-4-carboxylic acid (**1**) 210 mg (1.1 mmol), methyl glycinate HCl salt (**2**) 143 mg (1.1 mmol), HBTU 420 mg (1.1 mmol) and HOBt 170 mg (1.1 mmol) were dissolved in 12 mL dry DMF. To the solution, 0.77 mL of DIPEA (4.4 mmol) was added. The reaction was stirred at room temperature for 6 h. After the solvent was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and the
25 product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 290 mg of product **3** was obtained as a yellow powder with a yield of 76%. ¹H-NMR (400 MHz, CD₃OD): δ 8.69 (s, 1H), 7.94 (d, J = 7.92 Hz, 1H), 7.57–7.51 (m, 3H), 7.42–7.37 (m, 1H), 4.21 (s, 2H), 3.81 (s, 3H). ¹³C-NMR (100 MHz, CD₃OD): δ

172.4, 160.9, 145.1, 143.7, 129.7, 129.4, 128.3, 121.8, 119.6, 112.4, 109.1, 56.8, 44.8.
MS: calculated for $[C_{13}H_{13}N_2O_4]^+$, 261.3 $[M + H]^+$; found 261.1.

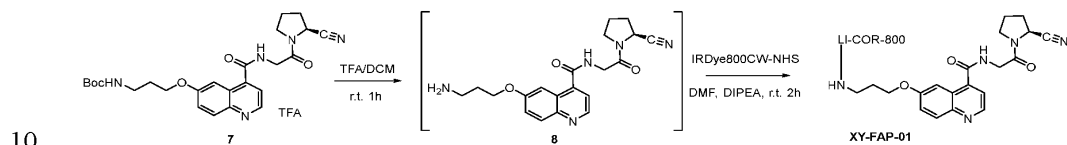


Methyl (6-(3-((tert-butoxycarbonyl)amino)propoxy)quinoline-4-carbonyl)glycinate (**5**): Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**) 360 mg (1.0 mmol), tert-butyl (3-bromopropyl)carbamate (**4**) 500 mg (2.1 mmol) were dissolved in 20 mL DMF. CS_2CO_3 1 g (3.0 mmol) was added to the solution and the reaction was stirred at room temperature overnight. After filtration, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 270 mg of product **5** was obtained with a yield of 54%. ^1H -NMR (400 MHz, $CDCl_3$): δ 8.68–8.37 (m, 2H), 8.02 (d, $J = 9.1$ Hz, 1H), 7.80 (s, 1H), 7.72–7.64 (m, 1H), 7.40 (d, $J = 9.1$ Hz, 1H), 4.94 (br s, 1H), 4.41–4.31 (m, 2H), 4.27–4.18 (m, 2H), 3.85 (s, 3H), 3.44–3.30 (m, 2H), 2.13–2.00 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 167.2, 158.4, 144.7, 142.3, 128.4, 126.1, 124.7, 119.1, 103.7, 79.5, 60.4, 52.5, 41.4, 37.7, 29.3, 28.4. MS: calculated for $[C_{21}H_{28}N_3O_6]^+$, 418.5 $[M + H]^+$; found 418.3.



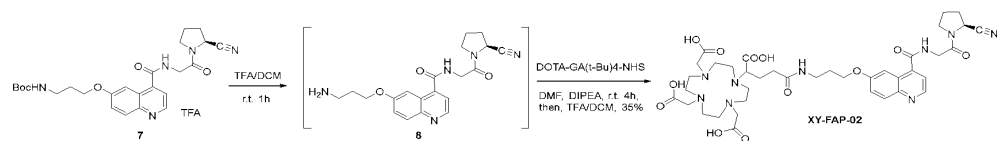
tert-Butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate (**7**): Compound **5** 110 mg (0.21 mmol) and LiOH 30 mg (1.2 mmol) was stirred in 4 mL of H_2O/THF (1/1) for 6 hours. After most of the THF was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and eluted with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1) to remove the salts. The product **6** obtained was mixed with (S)-pyrrolidine-2-carbonitrile 53 mg (0.4 mmol), HOBT 68 mg (0.4 mmol), HBTU 152 mg (0.4 mmol) and DIPEA 0.56 mL (1.6 mmol) in dry 10 mL DMF. After 6 hours, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was

purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 99 mg of **7** was obtained with a yield of 80%. ¹H NMR (400 MHz, CDCl₃): δ 8.73 (s, 1H), 7.95 (d, J = 10.2 Hz, 1H), 7.68 (br s, 1H), 7.63–7.56 (m, 1H), 7.56–7.48 (m, 1H), 7.38–7.29 (m, 1H), 5.27 (br s, 1H), 4.84–4.72 (m, 1H), 4.46–4.35 (m, 1H), 4.33–4.20 (m, 1H), 4.17–4.09 (m, 2H), 3.78–3.64 (m, 1H), 3.59–3.46 (m, 1H), 3.36 (s, 2H), 2.38–2.17 (m, 4H), 1.42 (s, 9H), 1.35–1.27 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 167.6, 167.5, 157.9, 156.2, 146.3, 130.2, 125.7, 123.7, 119.3, 118.0, 103.3, 79.0, 65.9, 46.8, 45.7, 42.2, 37.6, 29.8, 29.3, 28.4, 25.1. MS: calculated for [C₂₅H₃₂N₅O₅]⁺, 482.6 [M + H]⁺; found 482.3.



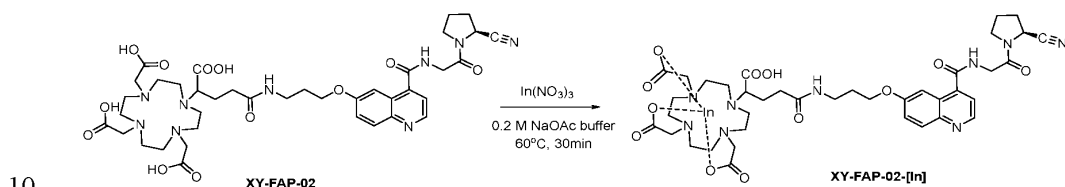
XY-FAP-01. Compound **7** (1 mg, 1.7 μmol) was treated with a 1 mL solution of TFA/methylene chloride (1/1) for 2 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMSO. To the solution, LICOR800CW-NHS ester 0.5 mg (0.43 μmol) and Et₃N 10 μL were added. After 1 h at room temperature, the solvent was removed and the product was purified by HPLC. 0.5 mg product was obtained with a yield of 85%. HPLC condition: column Phenomenex, Luna 10 x 250 mm, 10 μ. Gradient 10/90/0.1 MeCN/H₂O/TFA to 80/20/0.1 MeCN/H₂O/TFA within 15 min at a flow of 3 mL/min. The product was eluted at 10.1 min. MS: Calculated for [C₆₆H₇₆N₇O₁₇S₄]⁺, 1366.4[M+H]⁺; found 1366.8.

1.2 Synthesis of XY-FAP-02



2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**XY-FAP-02**): Compound **7** (15 mg, 31.3 μmol) was treated with a 1-mL solution of TFA/methylene chloride (1/1) for 1 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMF. To the solution, DIPEA (27 μL, 156.5 μmol) was added, followed by dropwise addition of a solution of DOTA-GA(t-Bu)₄-NHS (25 mg, 31.3 μL) in 0.5

mL of DMF. The reaction mixture was stirred for 4 h at ambient temperature and then concentrated under vacuum. The t-Bu-protected intermediate was deprotected in situ without further purification using a 1 mL mixture of TFA, H₂O and triethylsilane (TES) (95:2.5:2.5). Reaction mixture was then concentrated and purified by semipreparative HPLC, to afford the product as a white solid (8.5 mg, 33% yield). MS: calculated for [C₃₉H₅₄N₉O₁₂]⁺, 840.9 [M + H]⁺; found 840.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 μm, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02** eluted at 11.8 min.



XY-FAP-02-[In]. ^{113/115}Indium(III) 2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**XY-FAP-02-[In]**):

To a solution of 2 mg (2.4 μmol) of **XY-FAP-02** in 1 mL of 0.2M AcONa, a solution of 1.4 mg (4.6 μmol) of In(NO₃)₃ in 0.5 mL water is added and warmed in a 60 °C bath for 30 min. After cooling to ambient temperature, the mixture was purified by semipreparative HPLC. The product was obtained as a white solid (1.8 mg, 79% yield). MS: calculated for [C₃₉H₅₁N₉O₁₂In]⁺, 951.7 [M + H]⁺; found 952.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 μm, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02-[In]** eluted at 14.0 min.

1.3 Radiolabeling Methods. Briefly, 20 mg **XY-FAP-02** solution in 20 mL of 0.2 M NaOAc was added to 10 mL 4.6 mCi ¹¹¹InCl₃ solution (Nordion, Ottawa, Canada) and adjusted to a final pH of 5.5-6. The mixture was heated in a water bath at 70 °C for 30 min and, after the reaction completed, was diluted with 200 mL of water for HPLC purification. The solution was purified using a Phenomenex 5 μm C₁₈ Luna 4.6 x 250 mm² column (Torrance, CA) with a flow rate of 0.6 mL/min with water (0.1% TFA) (A) and MeCN (0.1% TFA) (B) as the eluting solvents. An isocratic solution of 88% A and 12% B was utilized for purification, resulting in the labeled compound, ¹¹¹In-**XY-FAP-02**, eluting first at 18.6 min followed by the unlabeled

starting material at 23.5 min. 3.2 mCi of labeled compound was obtained as pure product with a yield of 69%. Another reaction with the identical condition was performed with 74% yield. The collected radioactivity was diluted with 20 mL of water and loaded onto activated Sep-Pak (WAT020515, Waters, Milford, MA). After the Sep-Pak was washed with 10 mL of water, ¹¹¹In-XY-FAP-02 was eluted with 1.5 mL of ethanol. The ethanol was evaporated under a gentle stream of N₂ (to a total volume of < 50 µL). The resulting solution was formulated in saline for the imaging and biodistribution studies.

1.4 FAP Inhibition Assay. The inhibitory activity of XY-FAP-01 was determined using a fluorogenic FAP Assay Kit (BPS Bioscience, San Diego, CA). Briefly, XY-FAP-01, DPP substrate, and human recombinant FAP were loaded into a 96 well plate to initiate the enzyme reaction. The reaction was left for 10 minutes at room temperature before fluorescence was measured with a VICTOR3 V multilabel plate reader (PerkinElmer Inc., Waltham, MA). Data was normalized and semi-log inhibition curves were generated in order to determine the IC₅₀ value (concentration of XY-FAP-01 where the enzyme activity is 50% inhibited) for XY-FAP-01 and subsequent enzyme inhibition constant (K_i) using the Cheng-Prusoff conversion. Generation of semi-log inhibition curves and IC₅₀ values were done using GraphPad Prism (San Diego, CA).

1.5 Cell lines. Six human cancer cell lines were used to assess binding to FAP: glioblastoma (U-87-MG), melanoma (SK-MEL-24), prostate (PC-3), non-small cell lung cancer (NCI-H2228), colorectal carcinoma (HCT 116), and lung squamous cell carcinoma (NCI-H226). From the literature, U-87-MG, SK-MEL-24, and NCI-H2228 cell lines were identified as having high levels of FAP expression [FAP-positive (+)] whereas PC-3, NCI-H226, and HCT 116 cells expressed very low levels of FAP [FAP-negative(-)]. These expression profiles were further confirmed via flow cytometry with an APC-conjugated anti-FAP antibody (R&D Systems, Minneapolis, MN) and quantitative real-time PCR. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA).

U-87-MG cells were maintained in MEM medium (Corning Cellgro, Manassas, VA), containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO) and 1% penicillin-streptomycin (Corning Cellgro, Manassas, VA), supplemented with sodium bicarbonate (Corning), sodium pyruvate (Gibco, Gaithersburg, MD), and MEM non-essential amino acids (Gibco). SK-MEL-24 cells

were maintained in MEM medium, containing 15% FBS and 1% penicillin-streptomycin, supplemented with sodium bicarbonate, sodium pyruvate, and MEM non-essential amino acids. PC-3 cells were grown in Ham's F-12K medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. NCI-H2228,
5 NCI-H226, and HCT 116 cells were cultured in RPMI 1640 medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. All cell cultures were maintained at 37 °C and 5% carbon dioxide (CO₂) in a humidified incubator.

1.6 Cellular Uptake Studies. All cellular uptake and specific binding studies
10 were performed in triplicate to ensure reproducibility. Cells were detached using 0.05% trypsin (Corning), resuspended in 1 million cell aliquots in binding buffer, and incubated with various concentrations (range, 50 nM to 0.78 nM) of **XY-FAP-01** for 1 hour at 37 °C and 5% CO₂. To assess the specific uptake of **XY-FAP-02**, cells were preblocked with a FAP and DPP-IV specific inhibitor (Val-boroPro, MilliporeSigma,
15 Burlington, MA) or a DPP-IV specific inhibitor (Sitagliptin, Santa Cruz Biotechnology, Inc., Dallas, TX) at various concentrations (range, 10⁻¹⁰ M to 10⁻⁴ M) prior to incubation with 25 nM **XY-FAP-02** solution in binding buffer for 1 hour at 37 °C and 5% CO₂. Cellular uptake was terminated by washing cells with ice cold PBS (1x) three times. Cells were resuspended in binding buffer and transferred to a
20 96-well plate for imaging. Images were acquired on the LI-COR Pearl Impulse Imager (Lincoln, NE) using an excitation wavelength of 785 nm and detection of the emission wavelength at 800 nm. Images were analyzed using the LI-COR Pearl Impulse Software (Version 2.0) and fluorescence intensity was corrected for background signal and normalized to well area.

25 Cellular Uptake of ¹¹¹In-**XY-FAP-02** was also assessed in cells. Cell aliquots (1 million) were incubated with 1 µCi ¹¹¹In-**XY-FAP-02** in saline for 30 minutes at 37 °C and 5% CO₂. Cells were washed three times with cold PBS (1x) and activity of the cell pellets was measured with the 1282 CompuGamma CS gamma well counter (Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The percent uptake of the
30 administered activity was calculated by comparison with samples of a standard dose.

1.7 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF images were acquired on the LI-COR Pearl Impulse Imager using an excitation wavelength of 785 nm and a detection wavelength of 800 nm. Mice utilized for imaging studies were anesthetized with 3% isoflurane (v/v) and maintained at 1.5% isoflurane for the

imaging procedure. NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 nmol of **XY-FAP-01** via tail vein injection and images were acquired at 30 min, 1 h, 2 h, 2.5 h, and 4 h after injection of tracer. Data were displayed and analyzed using the LI-COR Pearl Impulse Software (Version 2.0).

5 1.8 *Small-Animal SPECT-CT Imaging.* SPECT-CT studies were performed on NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts. For imaging studies, mice were anesthetized with 3% isoflurane prior to being placed on the scanner bed and kept warm with an external light source. Isoflurane levels were decreased to 1.5% for the rest of the imaging procedure. After mice were injected
10 with 300 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline, SPECT-CT imaging was carried out using a CT-equipped Gamma Medica-Ideas SPECT scanner (Northridge, CA) at the indicated timepoints (30 min, 2 h, 6 h, and 24 h) post radiotracer injection. A CT scan was performed at the end of each SPECT scan for anatomical co-registration. Obtained data sets were reconstructed using the provided Gamma Medica-Ideas
15 software and final data visualization and image generation were prepared using Amira® software (FEI, Hillsboro, OR).

 1.9 *Ex-vivo Biodistribution.* NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline via the tail vein. At 5 min, 30 min, 2 h, 6 h, and 12 hr post injection, mice (n=4)
20 were sacrificed by CO₂ asphyxiation and blood was immediately collected by cardiac puncture. Additionally, the heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, small intestine, large intestine, bladder, muscle, femur, FAP+ U-87-MG xenograft, and FAP- PC-3 xenograft were collected for biodistribution analysis. Each tissue was weighed and radioactivity was measuring using a 2480 Wizard² automated gamma
25 counter (PerkinElmer, Waltham, MA). Radioactivity measurements were corrected for decay and compared with samples of a standard dilution of the initial dose to calculated percent injected dose per gram (%ID/g).

 For blocking studies, mice (n=5 per group) were co-injected with unlabeled **XY-FAP-02** (50 μg per mouse) and 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline. Mice
30 (n=5) injected with 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline served as a control. At 6 h post injection, mice were sacrificed, tissues were collected, and radioactivity was measured with the gamma well counter.

 1.10 *Data Analysis.* Data are expressed at mean $\pm$ standard deviation (SD). Prism software (GraphPAD, San Diego, CA) was used for analysis and statistical

significance was calculated using a two-tailed Student's t test. A P-value <0.05 was considered significant.

1.11 *Xenograft Tumor Model.* 6-week old female NOD/SCID mice were subcutaneously injected in the upper left and right flanks with 1 million U87(FAP+) cells and PC3 cells (FAP-) in RPMI 1640 media supplemented with 1% FBS. Mice were monitored for tumor size and used for optical or SPECT/CT imaging when the size of tumor reached around 100 mm³.

EXAMPLE 2

Representative Results

2.1 *FAP Inhibitory Assay.* **XY-FAP-01** demonstrated high binding affinity to human recombinant FAP. The enzyme inhibitory constant (K_i) for the compound was determined to be 1.26 nM.

2.2 *Cellular Uptake Studies.* FAP-positive cell lines showed concentration dependent uptake of **XY-FAP-01** whereas FAP-negative cell lines showed no significant binding of **XY-FAP-01** at all concentrations (see, e.g., FIG. 3A). Saturated binding of **XY-FAP-01** was observed at concentration of 25 nM, which was subsequently used as the base concentration for all binding inhibition studies. When preblocked with a FAP and DPP-IV specific inhibitor, **XY-FAP-01** binding was significantly inhibited in FAP-positive cells (FIG. 3B). Interestingly, this phenomenon was not observed in FAP-positive cell lines preblocked with a DPP-IV specific inhibitor. These results further justify the specificity of **XY-FAP-01** for FAP over DPP-IV, since blocking of DPP-IV did not result in a change of binding ability of **XY-FAP-01**.

Similar specificity was observed with the radioactive analog, ¹¹¹In-**XY-FAP-02**. FAP positive cell line, U-87-MG, demonstrated over 30% uptake of administered radioactive dose after incubation whereas the FAP negative cell line, PC-3, had uptake of 0.01% of administered dose (FIG. 3C). Taken together, these results support the specificity of **XY-FAP-01** and ¹¹¹In-**XY-FAP-02** in the engagement of FAP *in vitro*.

2.3 *Ex-vivo Biodistribution.* *Ex-vivo* biodistribution of ¹¹¹In-**XY-FAP-02** results correlated with the observed imaging results (FIG. 4). Initially, the blood pool activity is very high, with over 10% %ID/g at 30 minutes post injection. With clearance of the compound, we see the blood pool activity drop significantly after 2

hours of distribution and remained less than 5% %ID/g from 2 hours post injection (FIG. 5A). High activity was also observed in pancreas, small intestines, and bladder until 2 hours post injection. Positive tumor uptake peaked at 30 minutes post injection and remained between 13-11% %ID/g up to 6 hours. Washout of tumor was observed at 12 hours post injection, with %ID/g dropping to below 5%. The PC-3, FAP negative xenograft had less than 3.5% %ID/g for all timepoints.

Co-injection of cold compound with ¹¹¹In-XY-FAP-02 resulted in significant blocking of tracer uptake in U-87 xenografts, with %ID/g dropping from 11.20% without blocking versus 0.27% with blocking (p < 0.0001). Additionally, blocking with cold compound resulted in %ID/g of all tissues dropping significantly, with most values being less than 0.1%. This decrease in uptake is most likely due to the blocking of non-specific binding of tracer to non-target tissues and the blocking of specific binding of FAP in U-87 xenografts.

2.4 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF imaging of XY-FAP-01 demonstrated specific uptake of tracer in the U-87-MG xenograft as early as 30 minutes post injection (FIG. 6). After one hour of distribution, tracer clearance via the bladder was observed with retained tracer uptake in the FAP positive xenograft. Tracer uptake was retained in the positive xenograft after four hours of distribution. In contrast, no significant uptake of tracer was observed in the FAP negative tumor at all imaging time points.

REFERENCES

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references (e.g., websites, databases, etc.) mentioned in the specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an

incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

- 5 Allinen M, Beroukhi R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell*. 2004 Jul;6(1):17-32.
- Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon CJ, Koh GY, Kim J, Kim H.
- 10 Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. *Br J Haematol*. 2008 Sep;142(5):827-30.
- Chen ZY, Wang YX, Lin Y, Zhang JS, Yang F, Zhou QL, Liao YY. Advance of molecular imaging technology and targeted imaging agent in imaging and therapy. *Biomed Res Int*. 2014; 2014 : 819324. PMID: PMC3943245.
- 15 Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740.
- Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN
- 20 Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740. Biodistribution, tumor detection, and radiation dosimetry of 18F-DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2012; 53:1883–1891.
- 25 Edosada CY, Quan C, Tran T, Pham V, Wiesmann C, Fairbrother W, Wolf BB. Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly(2)-Pro(1)-cleaving specificity. *FEBS Lett*. 2006 Mar 6;580(6):1581-6.
- 30 Fischer E, Chaitanya K, Wuest T, et al. Radioimmunotherapy of fibroblast activation protein positive tumors by rapidly internalizing antibodies. *Clin Cancer Res*. 2012;18:6208–6218.
- Franco OE, Shaw AK, Strand DW, Hayward SW. Cancer associated fibroblasts in cancer pathogenesis. *Semin Cell Dev Biol*. 2010 Feb;21(1):33-9.

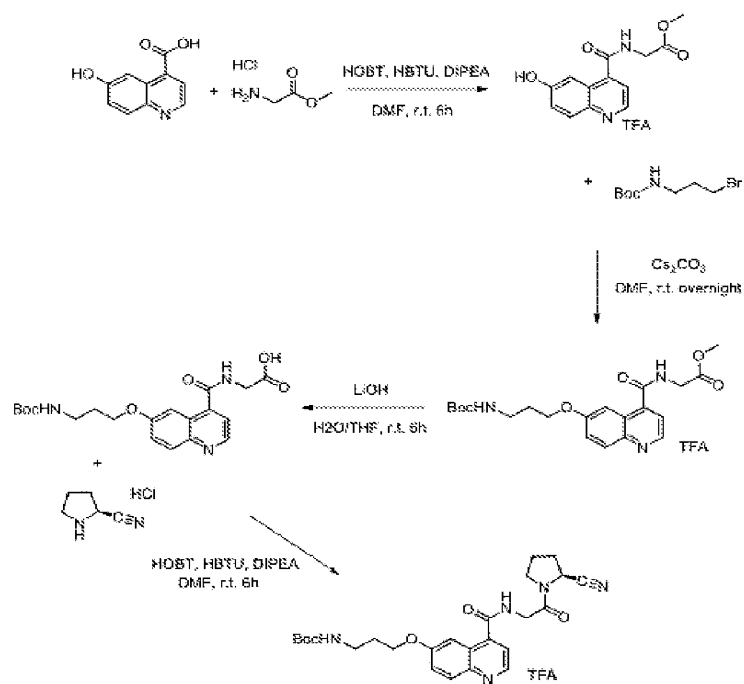
- Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc Natl Acad Sci U S A*. 1990 Sep;87(18):7235-9. PMID: PMC54718.
- Jansen K, Heirbaut L, Cheng JD, Joossens J, Ryabtsova O, Cos P, Maes L, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinyloxy)-glycyl-2-cyanopyrrolidine Scaffold. *ACS Med Chem Lett*. 2013 Mar 18;4(5):491-6.
- Jansen K, Heirbaut L, Verkerk R, Cheng JD, Joossens J, Cos P, Maes L, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Extended structure-activity relationship and pharmacokinetic investigation of (4-quinolinyloxy)glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP). *J Med Chem*. 2014 Apr 10;57(7):3053-74.
- Kelly T. Fibroblast activation protein- α and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. *Drug Resist Updat*. 2005 Feb-Apr;8(1-2):51-8.
- Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein- α . *Science*. 2010 Nov 5;330(6005):827-30.
- Laverman P, van der Geest T, Terry SY, Gerrits D, Walgreen B, Helsen MM, Nayak TK, Freimoser-Grundschober A, Waldhauer I, Hosse RJ, Moessner E, Umana P, Klein C, Oyen WJ, Koenders MI, Boerman OC. Immuno-PET and Immuno-SPECT of Rheumatoid Arthritis with Radiolabeled Anti-Fibroblast Activation Protein Antibody Correlates with Severity of Arthritis. *J Nucl Med*. 2015 May;56(5):778-83.
- Lo PC, Chen J, Stefflova K, Warren MS, Navab R, Bandarchi B, Mullins S, Tsao M, Cheng JD, Zheng G. Photodynamic molecular beacon triggered by fibroblast activation protein on cancer-associated fibroblasts for diagnosis and treatment of epithelial cancers. *J Med Chem*. 2009 Jan 22;52(2):358-68.
- Poplawski SE, Lai JH, Li Y, Jin Z, Liu Y, Wu W, Wu Y, Zhou Y, Sudmeier JL, Sanford DG, Bachovchin WW. Identification of selective and potent inhibitors of fibroblast activation protein and prolyl oligopeptidase. *J Med Chem*. 2013 May 9;56(9):3467-77.
- Reilly RM, Lam K, Chan C, Levine M. Advancing Novel Molecular Imaging Agents

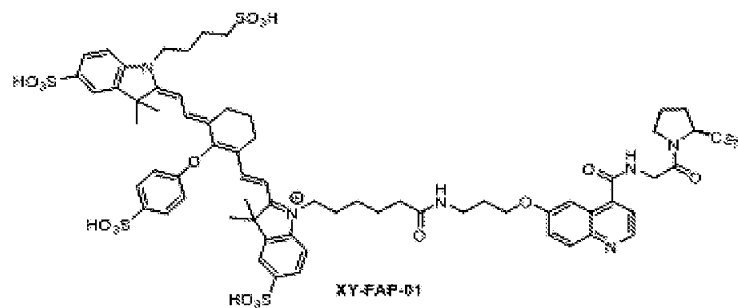
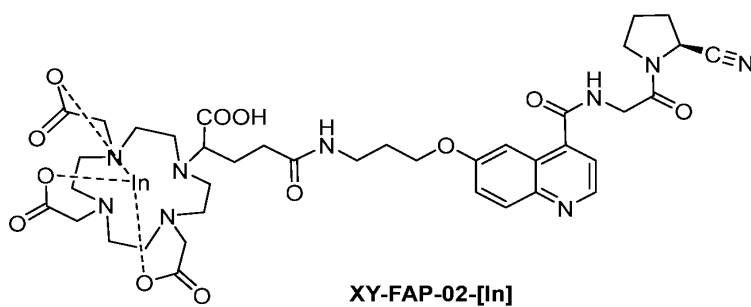
- from Preclinical Studies to First-in-Humans Phase I Clinical Trials in Academia-A Roadmap for Overcoming Perceived Barriers. *Bioconjugate chemistry*. 2015; 26:625–632.
- 5 Rettig WJ, Garin-Chesa P, Healey JH, Su SL, Ozer HL, Schwab M, Albino AP, Old LJ. Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin *Cancer Res*. 1993 Jul 15;53(14):3327-35.
- 10 Ryabtsova O, Jansen K, Van Goethem S, Joossens J, Cheng JD, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. *Bioorg Med Chem Lett*. 2012 May 15;22(10):3412-7.
- 15 Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, Rettig WJ. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A*. 1994 Jun 7;91(12):5657-61.
- 20 Scott AM, Wiseman G, Welt S, Adjei A, Lee FT, Hopkins W, Divgi CR, Hanson LH, Mitchell P, Gansen DN, Larson SM, Ingle JN, Hoffman EW, Tanswell P, Ritter G, Cohen LS, Bette P, Arvay L, Amelsberg A, Vlock D, Rettig WJ, Old LJ. A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin Cancer Res*. 2003 May;9(5):1639-47.
- 25 Tsai TY, Yeh TK, Chen X, Hsu T, Jao YC, Huang CH, Song JS, Huang YC, Chien CH, Chiu JH, Yen SC, Tang HK, Chao YS, Jiaang WT. Substituted 4-carboxymethylpyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. *J Med Chem*. 2010 Sep 23;53(18):6572-83.
- Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res*. 2002 Sep;8(9):2912-23.
- 30 Welt S, Divgi CR, Scott AM, Garin-Chesa P, Finn RD, Graham M, Carswell EA, Cohen A, Larson SM, Old LJ, et al. Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. *J Clin Oncol*. 1994 Jun;12(6):1193-203.

- Youn H., Hong K. In vivo noninvasive small animal molecular imaging. *Osong Public Health Res Perspect.* 2012; 3 :48-59. PMID: PMC3738683.
- Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, McCaughan GW, Gorrell MD. The dipeptidyl peptidase IV family in cancer and cell
5 biology. *FEBS J.* 2010 Mar;277(5):1126-44.
- U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014.
- U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016.
- 10 International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013.

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be
15 understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

1/9

**Fig. 1A**

**Fig. 1B****Fig. 1C**

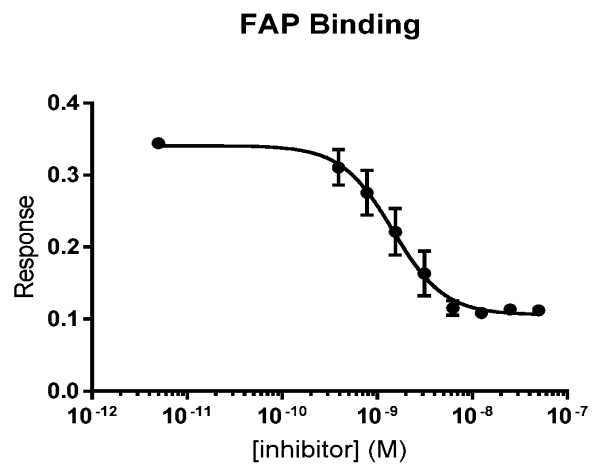
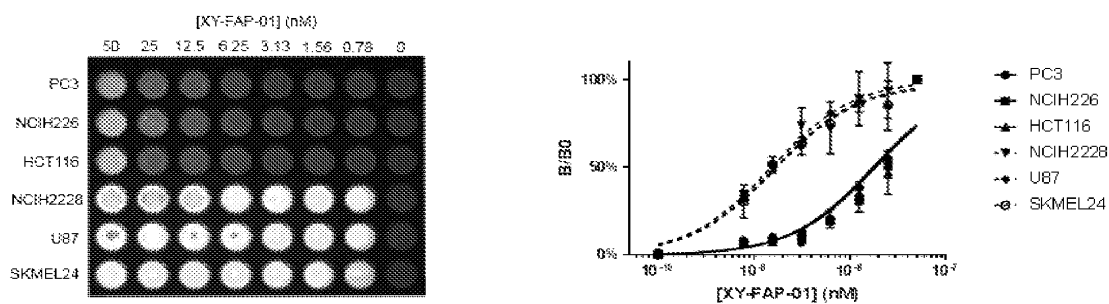
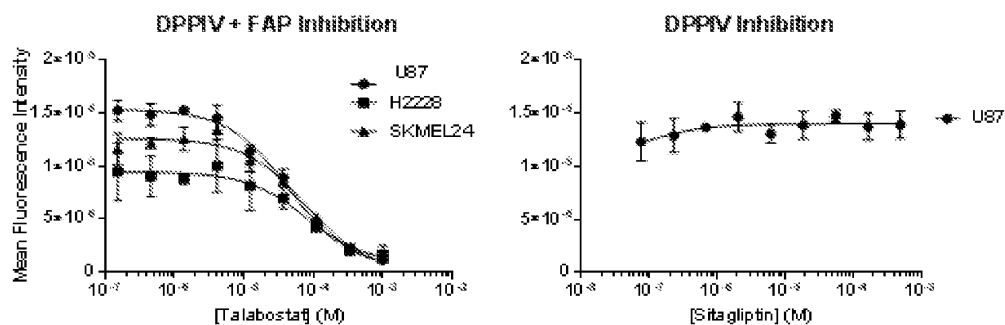
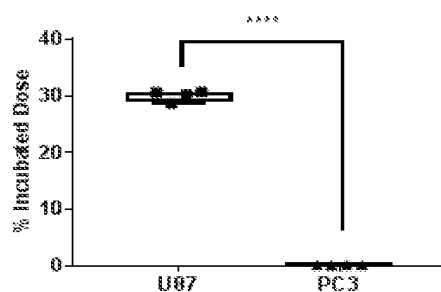


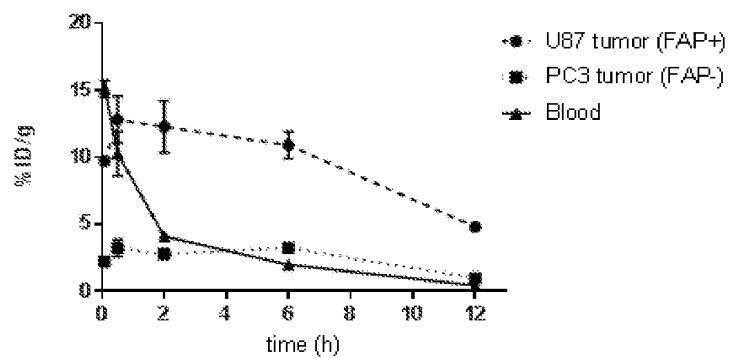
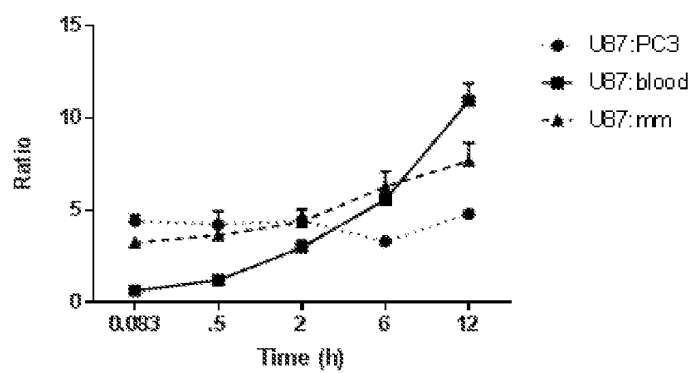
Fig. 2

**Fig. 3A****Fig. 3B****Fig. 3C**

	5 min (n= 3)	0.5 h (n= 4)	2 h (n= 3)	6 hr (n= 4)	6 h - blocking (n=4)	12 h (n= 4)
blood	15.13 ± 0.62	10.64 ± 1.54	4.10 ± 0.39	2.01 ± 0.18	0.02 ± 0.001	0.42 ± 0.04
heart	6.68 ± 0.99	4.98 ± 0.71	2.61 ± 0.09	1.07 ± 0.09	0.02 ± 0.002	0.46 ± 0.04
lungs	6.99 ± 1.37	5.60 ± 0.89	2.59 ± 0.32	1.36 ± 0.13	0.04 ± 0.005	0.39 ± 0.03
liver	6.32 ± 0.77	4.90 ± 0.59	2.58 ± 0.09	2.51 ± 0.09	0.33 ± 0.058	1.66 ± 0.22
stomach	3.26 ± 0.46	2.62 ± 0.20	1.69 ± 0.14	1.19 ± 0.15	0.06 ± 0.032	0.34 ± 0.05
pancreas	14.54 ± 1.66	12.14 ± 2.85	8.11 ± 0.34	3.28 ± 0.27	0.03 ± 0.005	1.15 ± 0.52
spleen	2.93 ± 0.25	2.48 ± 0.47	1.77 ± 0.27	1.54 ± 0.19	0.06 ± 0.008	1.10 ± 0.13
fat	0.74 ± 0.09	0.73 ± 0.13	0.61 ± 0.19	0.45 ± 0.16	0.02 ± 0.017	0.15 ± 0.08
kidney	4.60 ± 0.57	3.56 ± 0.18	1.95 ± 0.06	1.79 ± 0.21	1.16 ± 0.158	0.73 ± 0.05
sm. int.	8.80 ± 1.97	8.41 ± 1.35	3.64 ± 0.45	1.66 ± 0.22	0.09 ± 0.052	0.76 ± 0.14
lrg. int.	4.67 ± 0.42	4.57 ± 0.57	2.98 ± 0.27	1.97 ± 0.47	0.36 ± 0.342	0.55 ± 0.04
bladder	2.96 ± 0.95	13.60 ± 8.80	8.94 ± 6.48	3.41 ± 0.84	1.04 ± 0.466	2.25 ± 0.57
muscle	3.00 ± 0.22	3.53 ± 0.16	2.80 ± 0.07	1.79 ± 0.15	0.02 ± 0.005	0.60 ± 0.03
femur	4.76 ± 0.12	5.83 ± 0.72	4.95 ± 0.60	3.91 ± 0.70	0.08 ± 0.021	1.38 ± 0.20
U87	9.71 ± 0.24 ^a	12.89 ± 1.45 ^a	12.28 ± 1.95 ^a	11.20 ± 1.03 ^a	0.27 ± 0.019 ^a	4.57 ± 0.54 ^a
PC3	2.20 ± 0.20	3.10 ± 0.57	2.75 ± 0.11	3.40 ± 0.34	0.11 ± 0.024 ^a	0.95 ± 0.06
U87:PC3	4.43 ± 0.31	4.23 ± 0.72	4.46 ± 0.62	3.30 ± 0.14	2.61 ± 0.440	4.82 ± 0.28
U87:blood	0.64 ± 0.04	1.22 ± 0.07	3.00 ± 0.38	5.58 ± 0.36	18.07 ± 1.197	10.94 ± 0.93
U87:mm	3.25 ± 0.26	3.66 ± 0.44	4.36 ± 0.66	6.29 ± 0.82	11.81 ± 2.086	7.68 ± 0.97

Fig. 4

6/9

**Fig. 5A****Fig. 5B**

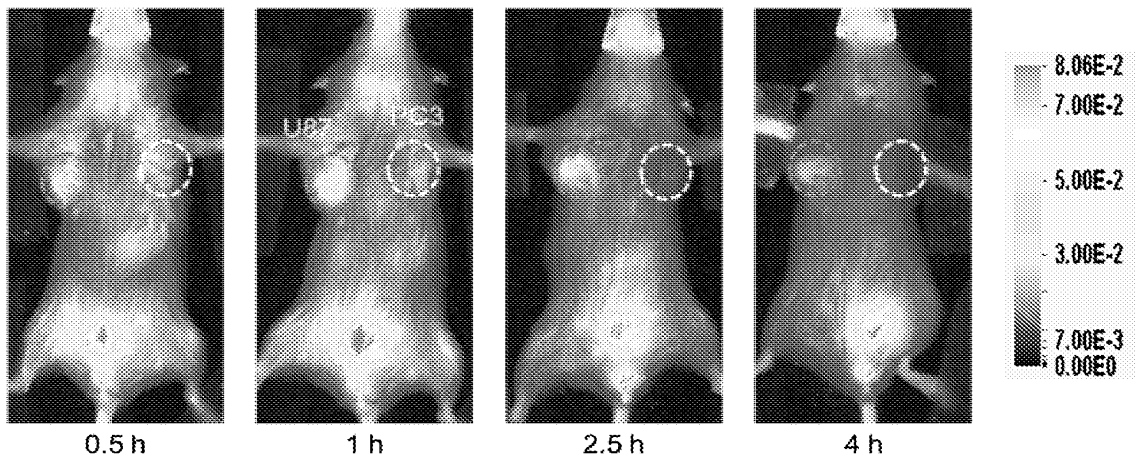
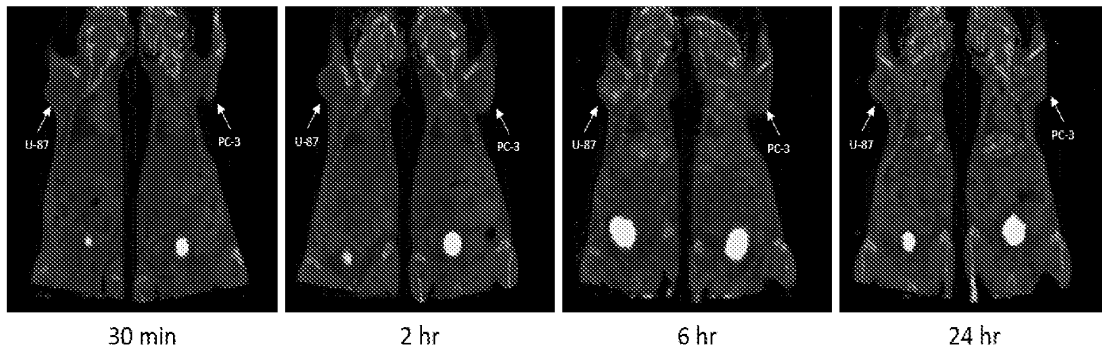


Fig. 6

8/9

***Fig. 7***

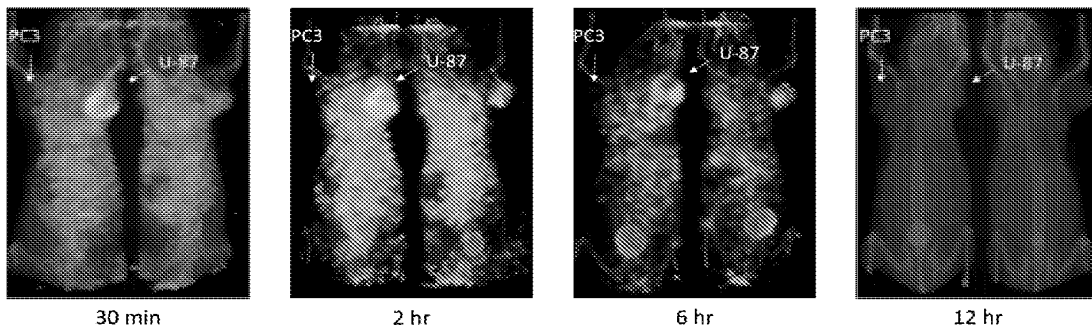


Fig. 8

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN-ALPHA (FAP-ALPHA)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application 16/758,182, filed April 22, 2020, which is a U.S. §371 National Entry Application of PCT/US2018/057086, filed October 23, 2018, which claims the benefit of U.S. Provisional Application No. 62/575,607, filed October 23, 2017, each of which is incorporated herein by reference in its entirety.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA197470 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Fibroblast-activation protein- α (FAP- α) expression has been detected on the surface of fibroblasts in the stroma surrounding >90% of the epithelial cancers examined, including malignant breast, colorectal, skin, prostate and pancreatic cancers. (Garin-Chesa, et al., 1990; Rettig, et al., 1993; Tuxhorn, et al., 2002; Scanlan, et al., 1994). It is a characteristic marker for carcinoma-associated-fibroblast (CAF), which plays a critical role in promoting angiogenesis, proliferation, invasion, and inhibition of tumor cell death. (Allinen, et al., 2004; Franco, et al., 2010). In healthy adult tissues, FAP- α expression is only limited to areas of tissue remodeling or wound healing. (Scanlan, et al., 1994; Yu, et al., 2010; Bae, et al., 2008; Kraman, et al., 2010). In addition, FAP- α -positive cells are observed during embryogenesis in areas of chronic inflammation, arthritis, and fibrosis, as well as in soft tissue and bone sarcomas. (Scanlan, et al., 1994; Yu, et al., 2010). These characteristics make FAP- α a potential imaging and radiotherapeutic target for cancer and inflammation diseases.

Because FAP- α is expressed in tumor stroma, anti-FAP antibodies have been investigated for radioimmunotargeting of malignancies, including murine F19, sibrotuzumab (a humanized version of the F19 antibody), ESC11, ESC14, and others. (Welt, et al., 1994; Scott, et al., 2003; Fischer, et al., 2012). Antibodies also demonstrated the feasibility of imaging inflammation, such as rheumatoid arthritis.

(Laverman, et al., 2015). The use of antibodies as molecular imaging agents, however, suffers from pharmacokinetic limitations, including slow blood and non-target tissue clearance (normally 2–5 days or longer) and non-specific organ uptake. Low molecular weight (LMW) agents demonstrate faster pharmacokinetics and a higher specific signal within clinically convenient times after administration. They also can be synthesized in radiolabeled form more easily and may offer a shorter path to regulatory approval. (Coenen, et al., 2010; Coenen, et al., 2012; Reilly, et al., 2015). To date, however, no LMW ligand has been reported with ideal properties for nuclear imaging of FAP- α .

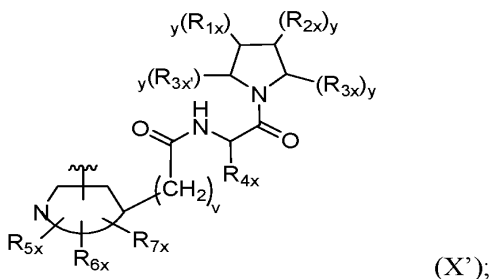
SUMMARY

In some aspects, the presently disclosed subject matter provides a compound of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

In particular aspects, A is an FAP- α targeting moiety having the structure of:



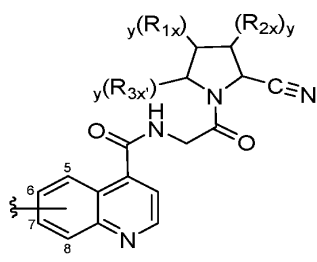
wherein each y is independently an integer selected from the group consisting of 0, 1, and 2; R_{1x}, R_{2x}, and R_{3x}, are each independently selected from the group consisting of H, OH, halogen, C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; R_{3x} is selected from the group consisting of H, -CN, -B(OH)₂, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-S(O)₂aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl; R_{4x} is H; R_{5x}, R_{6x}, and R_{7x} are each independently selected from the group consisting of H, -OH, oxo, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, -NR_{8x}R_{9x}, -OR_{12x}, -Het₂ and -Ar₂; each of C₁₋₆alkyl being optionally substituted with from 1 to 3 substituents

selected from -OH and halogen; R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, -OH, halo, -C₁₋₆alkyl, -O-C₁₋₆alkyl, -S-C₁₋₆alkyl, and -Ar₃; R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, -OH, halogen, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Ar₁, Ar₂ and Ar₃ are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar₁, Ar₂ and Ar₃ being optionally and independently substituted with from 1 to 3 substituents selected from -NR_{10x}R_{11x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; Het₂ is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het₂ being optionally substituted with from 1 to 3 substituents selected from -NR_{13x}R_{14x}, -C₁₋₆alkyl, -O-C₁₋₆alkyl, and -S-C₁₋₆alkyl; v is 0, 1, 2, or 3; and



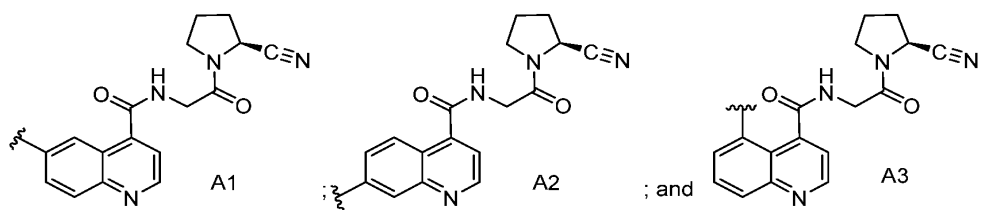
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; wherein ---^* indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In more particular aspects, A is an FAP- α targeting moiety having the structure of:



wherein ---^* indicates a point of attachment of the FAP- α binding ligand to the linker, L, or the reporter moiety, B, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In yet more particular aspects, A is selected from the group consisting of:



In other aspects, the presently disclosed subject matter provides a pharmaceutical composition comprising a compound of formula (I).

In some aspects, the presently disclosed subject matter provides a method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

In other aspects, the presently disclosed subject matter provides a method for inhibiting fibroblast-activation protein- α (FAP- α), the method comprising administering to a subject in need thereof an effective amount of a compound of formula (I).

In yet other aspects, the presently disclosed subject matter provides a method for treating a fibroblast-activation protein- α (FAP- α)-related disease or disorder, the method comprising administering to a subject in need of treatment thereof an effective amount of a compound of formula (I), wherein the compound of formula (I) comprises a radiolabeled functional group suitable for radiotherapy.

In certain aspects, the (FAP- α)-related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG. 1A, FIG. 1B, and FIG. 1C show the synthetic pathway and structures of representative FAP-targeted agents, **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 1A shows the multi-step synthesis of the ligand precursor, tert-butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate. After each step, the reaction mixture was loaded onto a 25-g C18 cartridge and purified with a MeCN/water/TFA gradient. Identity of intermediate products was confirmed with ¹H NMR. FIG. 1B shows the full structure of optical imaging agent, **XY-FAP-01**. **XY-FAP-01** was produced with a one step reaction between the precursor and IRDye800CW-NHS. The major product was obtained at a yield of 85% after purification with HPLC. FIG. 1C shows the full structure of the SPECT imaging agent, [¹¹¹In]-**XY-FAP-02**. First, the precursor was functionalized with DOTA via a one step reaction between the precursor and DOTA-GA(t-Bu)₄-NHS. Unlabeled product was purified via HPLC to produce XY-FAP-02. Subsequent radiolabeling with ¹¹¹In and HPLC purification resulted in the radiolabeled product, [¹¹¹In]-**XY-FAP-02**;

FIG. 2 shows the inhibitory activity of **XY-FAP-01** on human recombinant FAP. The inhibitory activity of **XY-FAP-01** was determined using a fluorogenic FAP assay kit. Enzymatic activity of human recombinant FAP on a native substrate was inhibited in a concentration dependent fashion by **XY-FAP-01**. Semi-log inhibitory curves of **XY-FAP-01** activity were generated and the determined Ki value of **XY-FAP-01** was 1.26 nM;

FIG. 3A, FIG. 3B, and FIG. 3C show the assessment of the *in vitro* binding ability and specificity of **XY-FAP-01** and [¹¹¹In]-**XY-FAP-02**. FIG. 3A shows the concentration dependent uptake of **XY-FAP-01** in various cell lines. Cells incubated with various concentrations (range: 50 nM to 0.78 nM) of **XY-FAP-01** were imaged with the LI-COR Pearl Impulse Imager to assess uptake of agent in various FAP-positive and FAP-negative cell lines (left). Dose-response curves of **XY-FAP-01**

uptake in FAP-positive cell lines (NCIH2228, U87, and SKMEL24) and FAP-negative cell lines (PC3, NCIH226, and HCT116) were generated (right). FIG. 3B shows the inhibition of **XY-FAP-01** uptake in FAP-positive cell-lines. Cells incubated with 25-nM **XY-FAP-01** were incubated with various concentrations of either a DPPIV and FAP inhibitor, Talabostat, or a DPPIV-only inhibitor, Sitagliptin. Uptake of **XY-FAP-01** was measured and semi-log inhibitor-response curves were generated for both Talabostat and Sitagliptin. FIG. 3C shows the uptake of [¹¹¹In]-**XY-FAP-02** in FAP-positive U87 and FAP-negative PC3 cell lines. Cells were incubated with 1 μ Ci [¹¹¹In]-**XY-FAP-02** and were washed with cold PBS. Radioactivity of the cell pellets was measured and normalized to the incubated dose;

FIG. 4 is a table showing the *ex vivo* tissue biodistribution of [¹¹¹In]-**XY-FAP-01** in tumor bearing mice. At 5 min, 0.5 h, 2 h, 6 h, and 12 h after injection of 10 μ Ci [¹¹¹In]-**XY-FAP-01**, NOD/SKID mice bearing U87 and PC3 tumor xenografts were sacrificed and tissues were collected for biodistribution analysis. Additionally, mice co-injected with unlabeled **XY-FAP-02** and 10 μ Ci [¹¹¹In]-**XY-FAP-01** were sacrificed at 6 h post-injection to study the effect of blocking on uptake of the radiolabeled compound. Data presented as mean $\pm$ standard deviation. ^aStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 5 min, 0.5 h, 2 h, and 6 h post injection ($p < 0.0001$). No significant difference between the two groups were seen in the blocking study at 6 h. ^bStudent's t test comparison of mean %ID/g of PC3 tumor versus U87 tumor demonstrated significant difference between the two groups at 12 h post injection ($p = 0.0006$). ^cStudent's t test comparing %ID/g between PC3 tumor and U87 tumors at 6 h post injection showed significant difference between %ID/g tumors in the blocking study at 6 h versus the normal biodistribution results at 6 h ($p < 0.0001$);

FIG. 5A and FIG. 5B show the time-activity relationship of the *ex vivo* biodistribution of [¹¹¹In]-**XY-FAP-02**. FIG. 5A shows tissue time activity curves (TACs) of [¹¹¹In]-**XY-FAP-02** activity in U87 tumor, PC3 tumor, and blood. FIG. 5B shows the ratios of %ID/g between U87 tumor and PC3 tumor, blood, and muscle (mm) versus time;

FIG. 6 shows serial NIRF-imaging of **XY-FAP-01** in tumor bearing mice. NOD/SKID mice bearing FAP-positive U87 (yellow circle) and FAP-negative PC3 (red circle) tumor xenografts were injected with 10 nmol of **XY-FAP-01** via the tail

vein followed by serial NIRF-imaging on the LI-COR Pearl Impulse Imager. Representative images at 0.5 h, 1 h, 2.5 h, and 4 h after injection are shown;

FIG. 7 shows SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks; and

FIG. 8 show three-dimensional SPECT-CT images of [¹¹¹In]-XY-FAP-02 at 30 min, 2 h, 6 h, and 24 h after injection in NOD/SKID female mice bearing U87 and PC3 tumor xenografts in the upper flanks.

DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Figures, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

I. IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION PROTEIN- α (FAP- α)

FAP- α is a type II integral membrane serine protease of the prolyl oligopeptidase family, which are distinguished by their ability to cleave the Pro-AA peptide bond (where AA represents any amino acid). It has been shown to play a role in cancer by modifying bioactive signaling peptides through this enzymatic activity (Kelly, et al., 2005; Edosada, et al., 2006). FAP- α expression has been detected on the surface of fibroblasts in the stroma surrounding greater than 90% of the epithelial cancers, including, but not limited to, malignant breast, colorectal, skin, prostate,

pancreatic cancers, and the like, and inflammation diseases, including, but not limited to, arthritis, fibrosis, and the like, with nearly no expression in healthy tissues. Accordingly, imaging and radiotherapeutic agents specifically targeting FAP- α is of clinical importance.

FAP- α exists as a homodimer to carry out its enzymatic function. Inhibitors selectively targeting FAP- α has been reported (Lo, et al., 2009; Tsai, et al., 2010; Ryabtsova, et al., 2012; Poplawski, et al., 2013; Jansen, et al., 2013; Jansen, et al., 2014). The presently disclosed subject matter provides, in part, a FAP- α selective targeting moiety that can be modified with an optical dye, a radiometal chelation complex, and other radiolabeled prosthetic groups, thus providing a platform for the imaging and radiotherapy targeting FAP- α .

Radionuclide molecular imaging, including positron emission tomography (PET), is the most mature molecular imaging technique without tissue penetration limitations. Due to its advantages of high sensitivity and quantifiability, radionuclide molecular imaging plays an important role in clinical and preclinical research (Youn, et al., 2012; Chen, et al., 2014). Many radionuclides, primarily β - and alpha emitters, have been investigated for targeted radioimmunotherapy and include both radiohalogens and radiometals (see Table 1 for representative therapeutic radionuclides).

Table 1. Representative Therapeutic Radionuclides	
β -particle emitters	^{90}Y , ^{131}I , ^{177}Lu , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb
α -particle emitters	^{225}Ac , ^{213}Bi , ^{212}Bi , ^{211}At , ^{212}Pb
Auger electron emitters	^{125}I , ^{123}I , ^{67}Ga , ^{111}In

The highly potent and specific binding moiety targeting FAP- α enables its use in nuclear imaging and radiotherapy. The presently disclosed subject matter provides the first synthesis of nuclear imaging and radiotherapy agents based on this dual-targeting moiety to FAP- α .

Accordingly, in some embodiments, the presently disclosed subject matter provides potent and selective low-molecular-weight (LMW) ligands of FAP- α , i.e., an FAP- α selective inhibitor, conjugated with a targeting moiety feasible for modification with optical dyes and radiolabeling groups, including metal chelators

and metal complexes, which enable *in vivo* optical imaging, nuclear imaging (optical, PET and SPECT), and radiotherapy targeting FAP- α . Importantly, the presently disclosed compounds can be modified, e.g., conjugated with, labeling groups without significantly losing their potency. The presently disclosed approach allows for the convenient labeling of the FAP- α ligand with optical dyes and PET or SPECT isotopes, including, but not limited to, ^{68}Ga , ^{64}Cu , ^{18}F , ^{86}Y , ^{90}Y , ^{89}Zr , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{125}I , ^{124}I , for FAP- α related imaging applications. Further, the presently disclosed approach allows for the radiolabeling of the FAP- α ligand with radiotherapeutic isotopes, including but not limited to, ^{90}Y , ^{177}Lu , ^{125}I , ^{131}I , ^{211}At , ^{111}In , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb , ^{225}Ac , ^{213}Bi , ^{212}Bi , ^{212}Pb , and ^{67}Ga , for FAP- α related radio-therapy.

In a particular embodiment, an optical agent conjugated with IRDye-800CW (**XY-FAP-01**) was synthesized and showed selective uptake *in vitro* on a FAP- α + U87 cell line and *in vivo* on a FAP- α + U87 tumor and clearly detected the tumor. In another particular embodiment, an ^{111}In labeled ligand (**XY-FAP-02- ^{111}In**) was successfully obtained in high yield and purity from its precursor with a metal chelator. The *in vivo* study showed clear tumor radiotracer uptake in mice bearing FAP- α -positive U87 tumors with minimum non-specific organ uptake, which allows the specific imaging of FAP- α expressing tumors. The presently disclosed FAP- α targeting moiety can be adapted for use with optical dyes and radioisotopes known in the art for imaging and therapeutic applications targeting FAP- α .

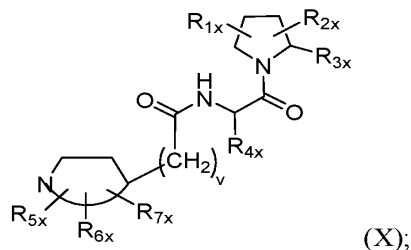
More particularly, in some embodiments, the presently disclosed subject matter provides a compound of the general structure of Formula (I):



wherein: A is a targeting moiety for FAP- α ; B is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A.

Representative targeting moieties for FAP- α are disclosed in U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014; U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016; and International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013, each of which are incorporate by reference in their entirety.

More particularly, U.S. Patent No. 9,346,814 to Jansen et al., discloses FAP- α inhibitors of formula (X), or a stereoisomer, tautomer, racemate, salt, hydrate, or solvate thereof, which are suitable for use with the presently disclosed subject matter:



wherein:

R_{1x} and R_{2x} are each independently selected from the group consisting of H, OH, halogen, C_{1-6} alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

R_{3x} is selected from the group consisting of H, $-CN$, $-B(OH)_2$, $-C(O)alkyl$, $-C(O)aryl$, $-C=C-C(O)aryl$, $-C=C-S(O)_2aryl$, $-CO_2H$, $-SO_3H$, $-SO_2NH_2$, $-PO_3H_2$, and 5-tetrazolyl;

R_{4x} is H;

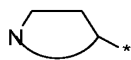
R_{5x} , R_{6x} , and R_{7x} are each independently selected from the group consisting of H, $-OH$, oxo, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, $-NR_{8x}R_{9x}$, $-OR_{12x}$, $-Het_2$ and $-Ar_2$; each of C_{1-6} alkyl being optionally substituted with from 1 to 3 substituents selected from $-OH$ and halogen;

R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, $-OH$, halo, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, and $-Ar_3$;

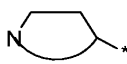
R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, $-OH$, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl; Ar_1 , Ar_2 and Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from $-NR_{10x}R_{11x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

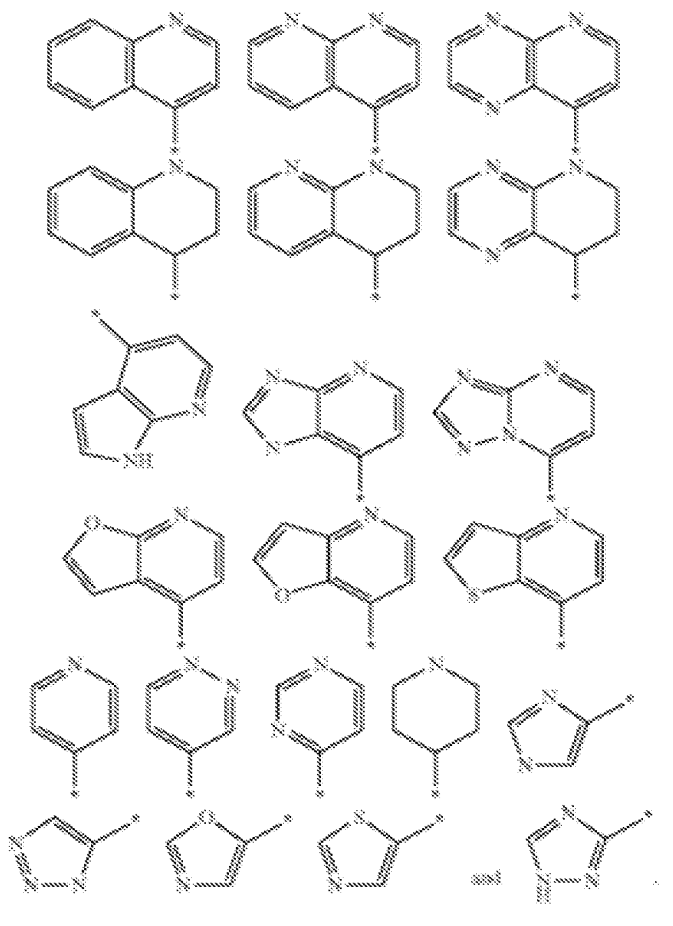
Het_2 is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het_2 being optionally substituted with from 1 to 3 substituents selected from $-NR_{13x}R_{14x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

v is 0, 1, 2, or 3; and



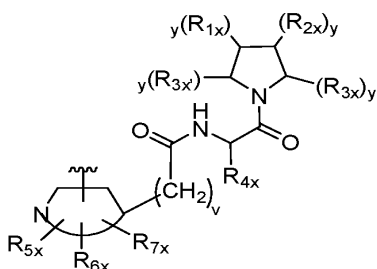
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S.

In particular embodiments,  is selected from the group consisting of:



wherein * indicates the point of attachment of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle to $-(CH_2)_x-$.

Accordingly, in some embodiments, A is an FAP- α targeting moiety having the structure of:



(X');

wherein each y is independently an integer selected from the group consisting of 0, 1, and 2;

R_{1x} , R_{2x} , and R_{3x} , are each independently selected from the group consisting of H, OH, halogen, C_{1-6} alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

R_{3x} is selected from the group consisting of H, $-CN$, $-B(OH)_2$, $-C(O)alkyl$, $-C(O)aryl$, $-C=C-C(O)aryl$, $-C=C-S(O)_2aryl$, $-CO_2H$, $-SO_3H$, $-SO_2NH_2$, $-PO_3H_2$, and 5-tetrazolyl;

R_{4x} is H;

R_{5x} , R_{6x} , and R_{7x} are each independently selected from the group consisting of H, $-OH$, oxo, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, $-NR_{8x}R_{9x}$, $-OR_{12x}$, $-Het_2$ and $-Ar_2$; each of C_{1-6} alkyl being optionally substituted with from 1 to 3 substituents selected from $-OH$ and halogen;

R_{8x} , R_{9x} , and R_{12x} are each independently selected from the group consisting of H, $-OH$, halo, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, $-S-C_{1-6}$ alkyl, and $-Ar_3$;

R_{10x} , R_{11x} , R_{13x} and R_{14x} are each independently selected from the group consisting of H, $-OH$, halogen, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl; Ar_1 , Ar_2 and Ar_3 are each independently a 5- or 6-membered aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; each of Ar_1 , Ar_2 and Ar_3 being optionally and independently substituted with from 1 to 3 substituents selected from $-NR_{10x}R_{11x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

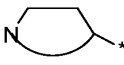
Het_2 is a 5- or 6-membered non-aromatic monocycle optionally comprising 1 or 2 heteroatoms selected from O, N and S; Het_2 being optionally substituted with from 1 to 3 substituents selected from $-NR_{13x}R_{14x}$, $-C_{1-6}$ alkyl, $-O-C_{1-6}$ alkyl, and $-S-C_{1-6}$ alkyl;

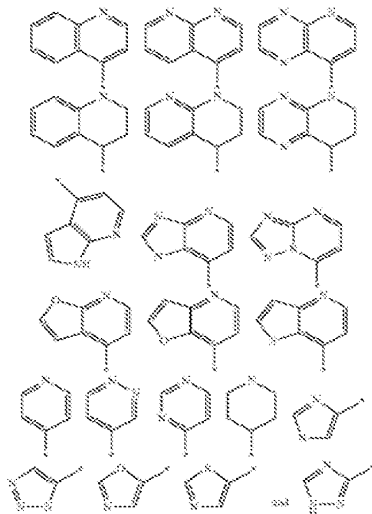
v is 0, 1, 2, or 3; and



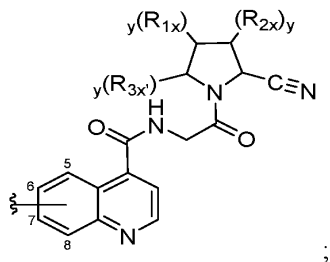
represents a 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S;

wherein  indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be through any of the carbon atoms of the 5 to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments,  is selected from the group consisting of:

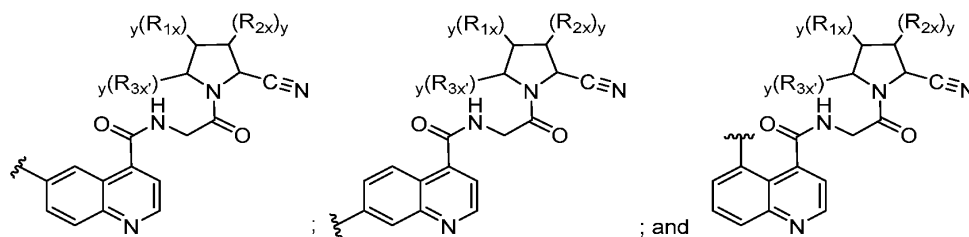


In some embodiments, A is an FAP- α targeting moiety having the structure of:

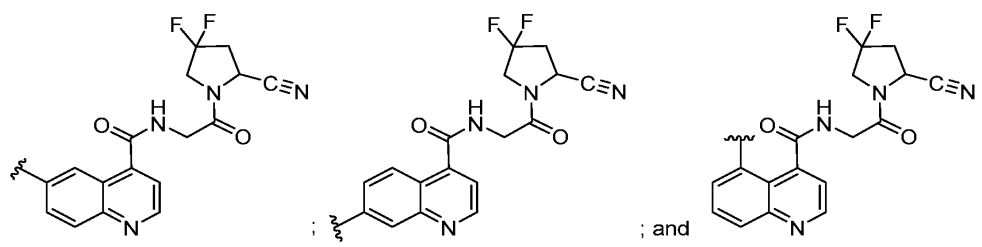


wherein y , R_{1x} , R_{2x} and $R_{3x'}$ are defined as hereinabove; $\frac{1}{2}$ — indicates a point of attachment of the FAP- α binding ligand to a linker, e.g., L, or a reporter moiety, such as an optical or radiolabeled functional group suitable for optical imaging, PET imaging, SPECT imaging or radiotherapy, wherein the point of attachment can be through any of carbon atoms 5, 6, 7, or 8 of the quinolinyl ring thereof; and stereoisomers and pharmaceutically acceptable salts thereof.

In particular embodiments, A is selected from the group consisting of:

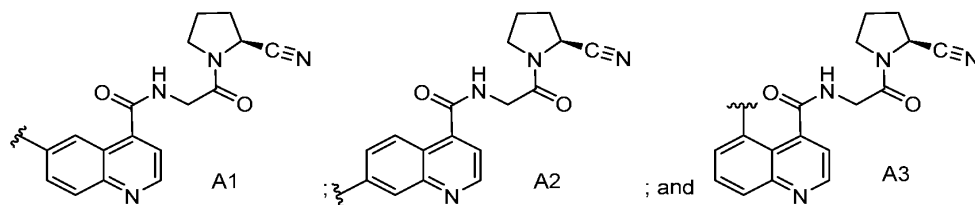


In more particular embodiments, A is selected from the group consisting of:

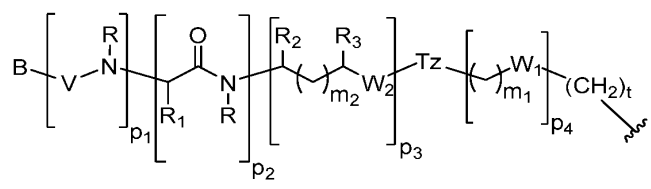


and stereoisomers thereof.

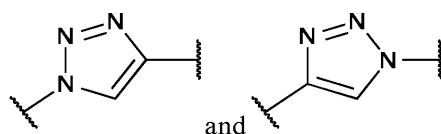
In yet more particular embodiments, A is selected from the group consisting of:



In some embodiments, the combination of L and B can be represented by:

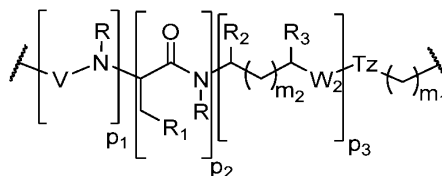


wherein the subunits associated with elements p_1 , p_2 , p_3 and p_4 may be in any order; t is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; p_1 , p_3 , and p_4 are each independently 0 or 1; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'_2-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_q-$ and $-(CH_2-CH_2-O)_q-$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R or R' is independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, and $-OR_4$, wherein R_4 is selected from the group consisting of H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, and substituted heterocycloalkyl, wherein q is defined as immediately hereinabove; Tz is a triazole group that can be present or absent and, if present, is selected from the group consisting of



; each R_1 is independently H, C_1-C_6 alkyl, C_3-C_{12} aryl, $-(CH_2)_q-C_3-C_{12}$ aryl, $-C_4-C_{16}$ alkylaryl, or $-(CH_2)_q-C_4-C_{16}$ alkylaryl; R_2 and R_3 are each independently H and $-CO_2R_5$, wherein R_5 is selected from the group consisting of H, C_1-C_6 alkyl, C_3-C_{12} aryl, and C_4-C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_5 , then the other is H; V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$; B is any optical or radiolabeled functional group suitable for optical, PET, or SPECT imaging or radiotherapy; and stereoisomers and pharmaceutically acceptable salts thereof.

In some embodiments, L has the following general structure:



wherein p_1 , p_2 , p_3 , m_1 , m_2 , q , t , Tz , W_2 , R , R_1 , R_2 , R_3 , and V are defined as hereinabove.

In some embodiments, L is selected from the group consisting of $-L_1-$, $-L_2-L_3-$, and $-L_1-L_2-L_3-$, wherein:

L_1 is $-\text{NR}-(\text{CH}_2)_q-[\text{O}-\text{CH}_2-\text{CH}_2-\text{O}]_q-\text{C}(=\text{O})-$;

L_2 is $-\text{NR}-(\text{CH}_2)_q-\text{C}(\text{COOR}_5)-\text{NR}-$; and

L_3 is $-(\text{O})\text{C}-(\text{CH}_2)_q-\text{C}(=\text{O})-$;

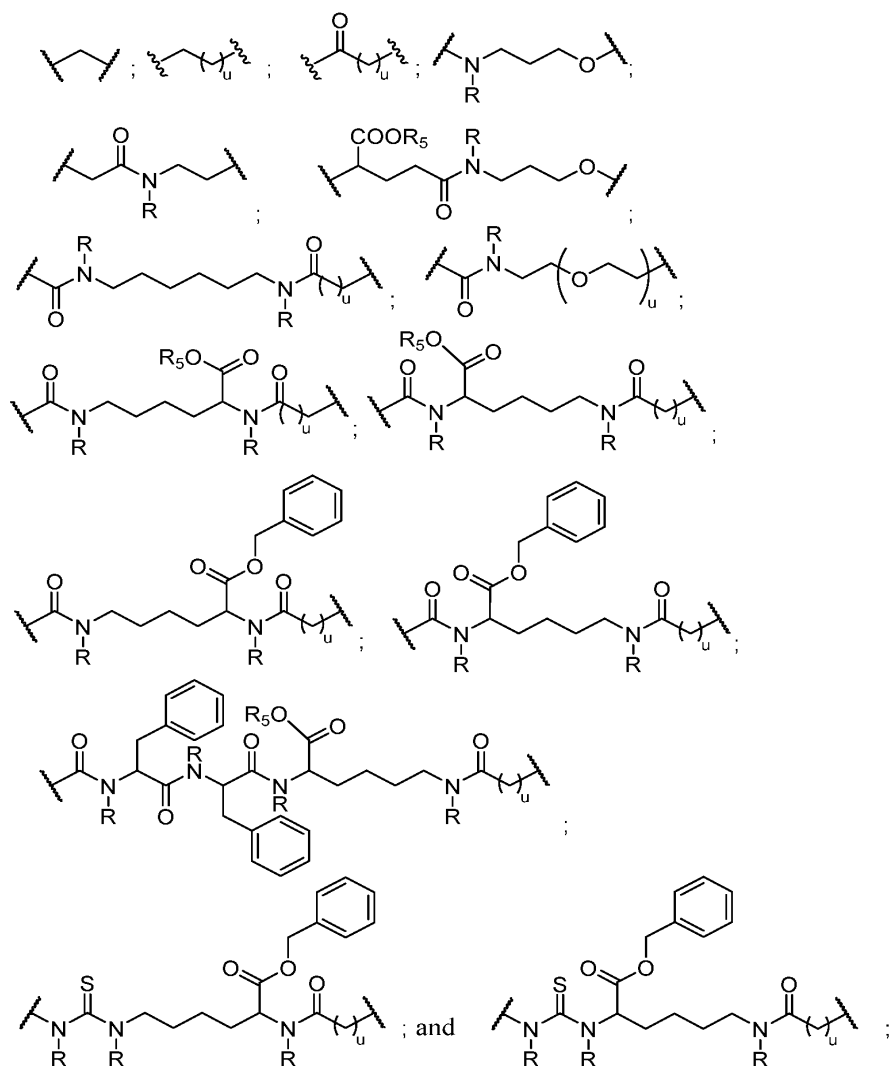
wherein each q is independently an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove.

In particular embodiments, L is:

$-(\text{CR}_6\text{H})_q-(\text{CH}_2)_q-\text{C}(=\text{O})-\text{NR}-(\text{CH}_2)_q-\text{O}-$ or $-\text{NR}-(\text{CH}_2)_q-\text{O}-$;

wherein each q and R is defined hereinabove; and R_6 is H or $-\text{COOR}_5$.

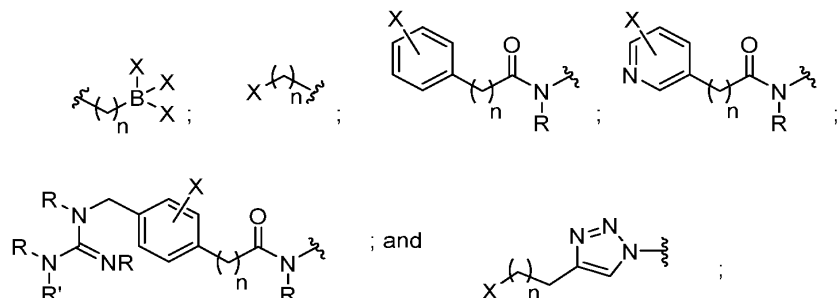
In yet more particular embodiments, L is selected from the group consisting of:



wherein u is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8; and R and R_5 are as defined hereinabove.

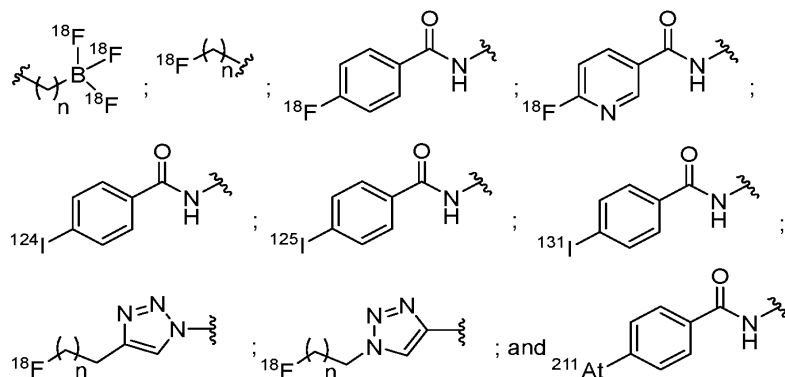
Suitable linkers are disclosed in U.S. Patent Application Publication No. US2011/0064657 A1, for "Labeled Inhibitors of Prostate Specific Membrane Antigen (PSMA), Biological Evaluation, and Use as Imaging Agents," published March 17, 2011, to Pomper et al., and U.S. Patent Application Publication No. US2012/0009121 A1, for "PSMA-Targeting Compounds and Uses Thereof," published January 12, 2012, to Pomper et al, each of which is incorporated by reference in its entirety.

In some embodiments, B is a radiolabeled prosthetic group comprising a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At . Representative radiolabeled prosthetic groups include, but are not limited to:

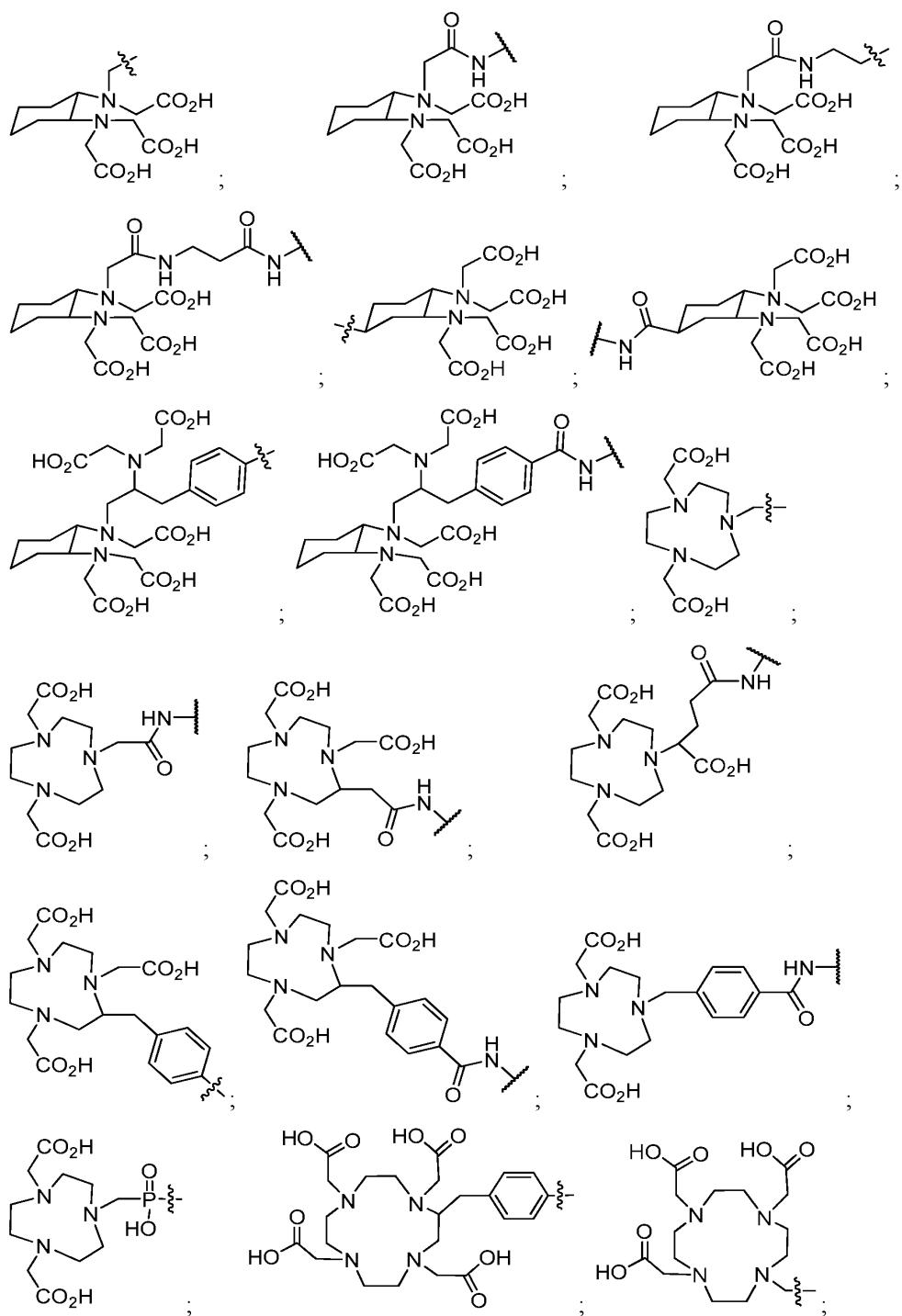


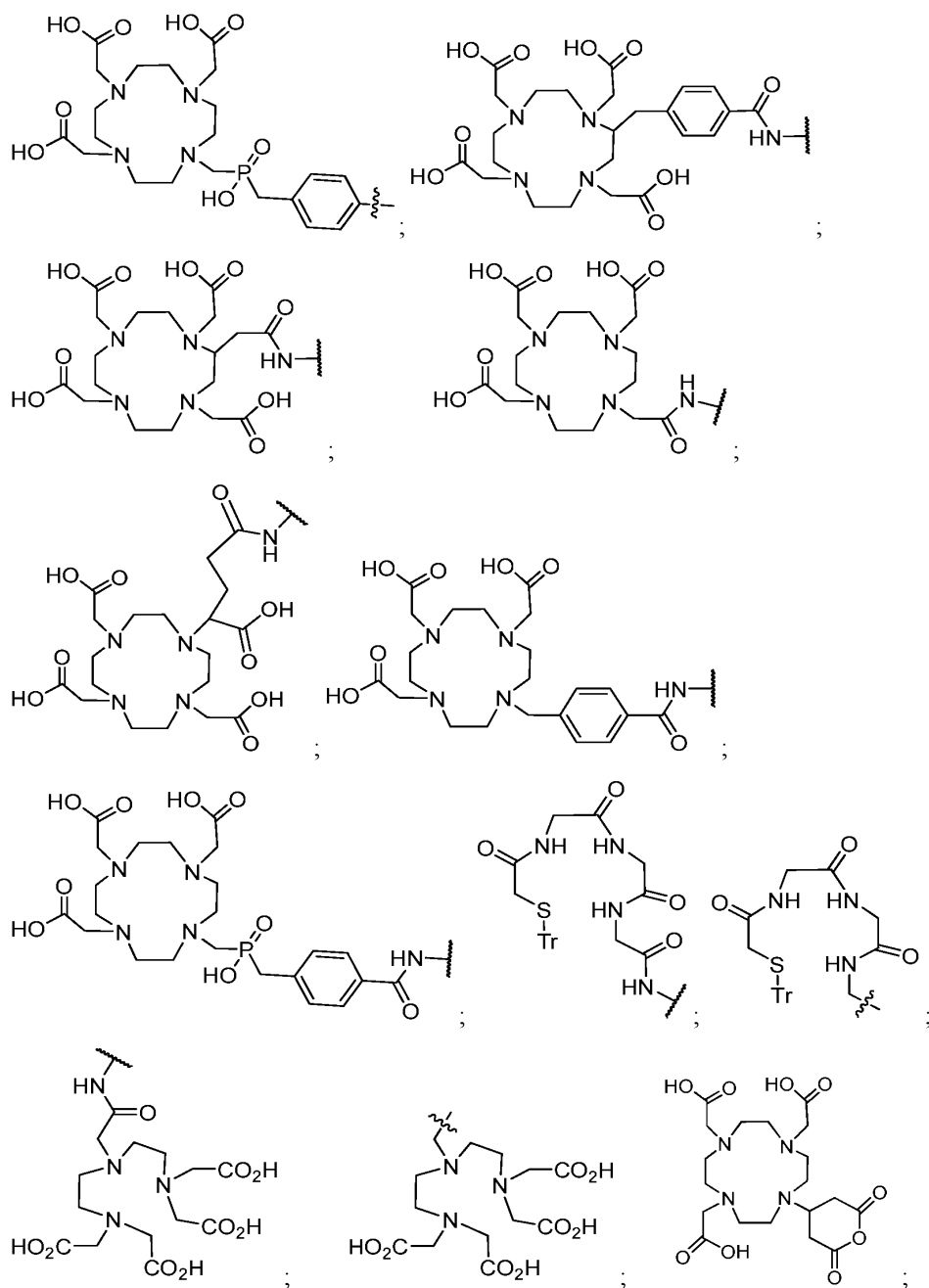
wherein each X is independently a radioisotope selected from the group consisting of ^{18}F , ^{124}I , ^{125}I , ^{131}I , and ^{211}At ; each R and R' is defined hereinabove; and each n is independently an integer selected from the group consisting of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

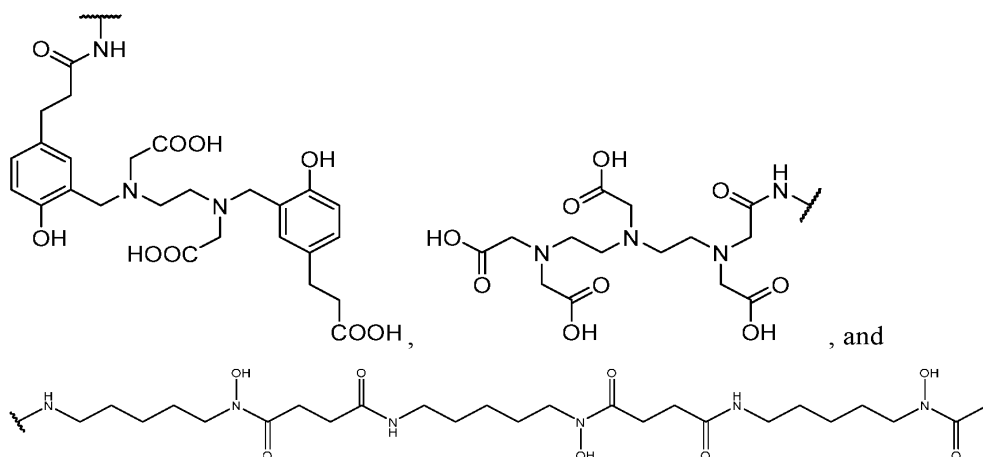
In more particular embodiments, the radiolabeled prosthetic group is selected from the group consisting of:



In other embodiments, B comprises a chelating agent. Representative chelating agents include, but are not limited to:

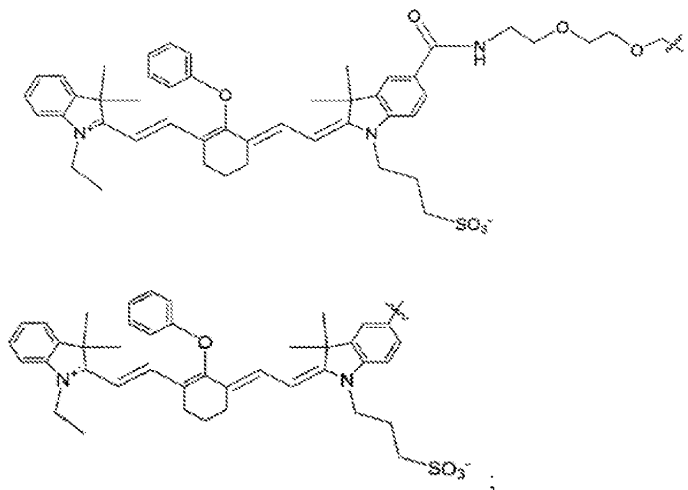


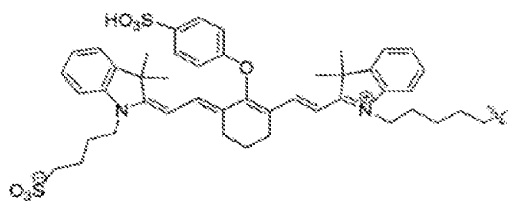




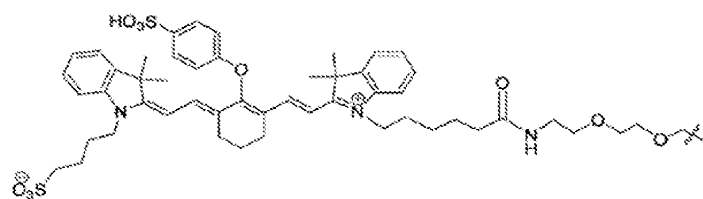
In some embodiments, B comprises an optical dye, e.g., in particular embodiments, a fluorescent dye. In some embodiments, the fluorescent dye moiety comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.

Representative optical dyes include, but are not limited to:

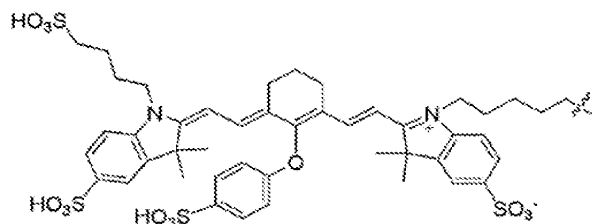
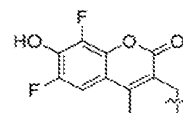




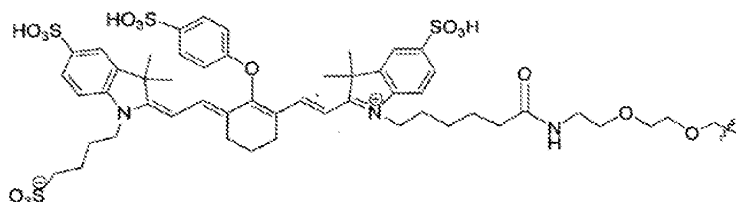
;



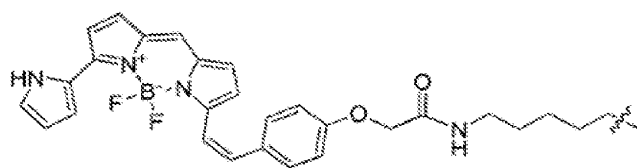
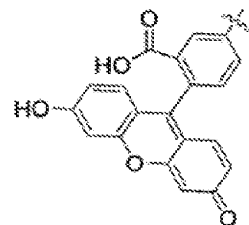
;



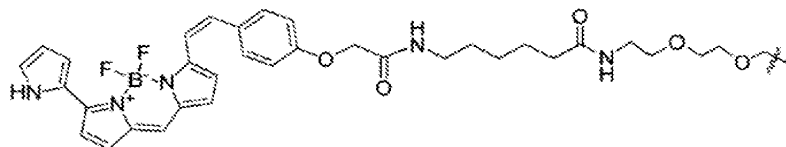
;



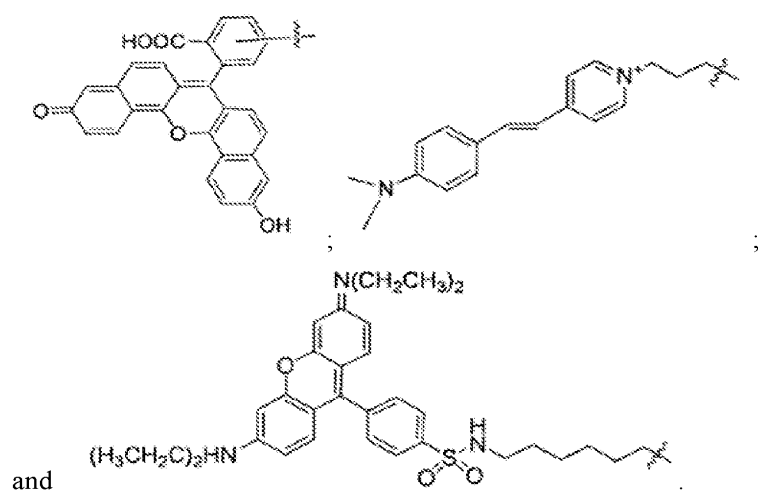
;



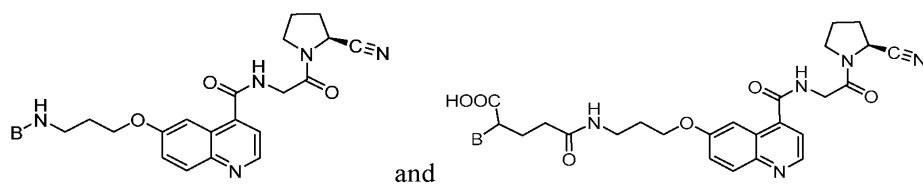
;



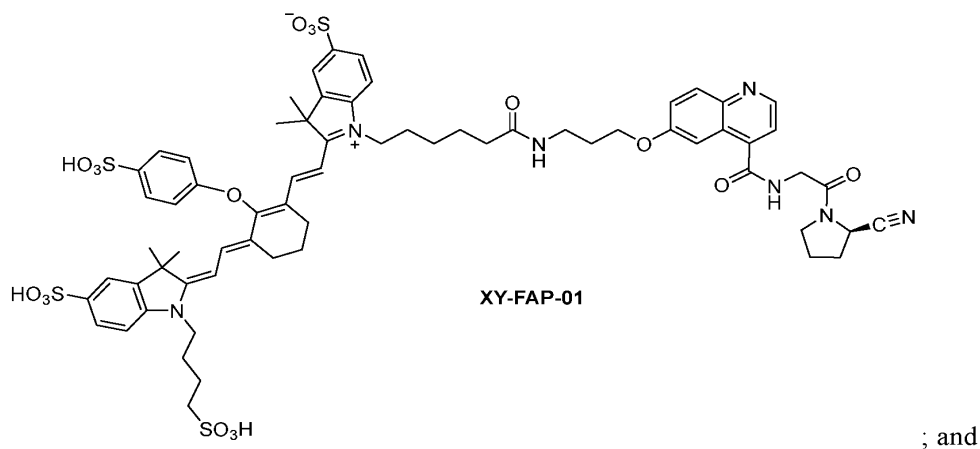
;

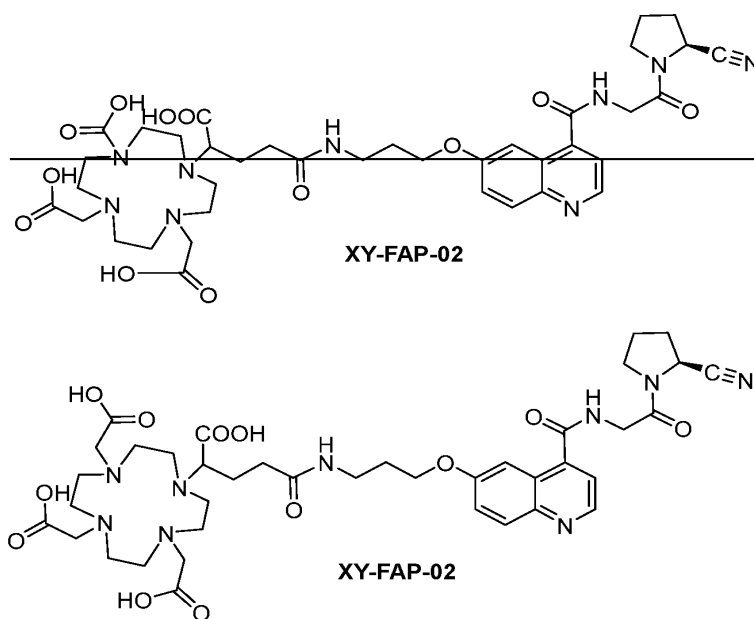


In some embodiments, the presently disclosed subject matter provides a compound selected from the group consisting of:



In particular embodiments, the compound is selected from the group consisting of:





B. *Pharmaceutical Compositions and Administration*

In another aspect, the present disclosure provides a pharmaceutical comprising a compound of formula (I) in admixture with a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant. One of skill in the art will recognize that the pharmaceutical compositions include the pharmaceutically acceptable salts or hydrates of the compounds described above.

Pharmaceutically acceptable salts are generally well known to those of ordinary skill in the art and include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituent moieties found on the compounds described herein. When compounds of the present disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent or by ion exchange, whereby one basic counterion (base) in an ionic complex is substituted for another. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt.

When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent or by ion exchange, whereby one acidic counterion (acid) in an

ionic complex is substituted for another. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-toluenesulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

Accordingly, pharmaceutically acceptable salts suitable for use with the presently disclosed subject matter include, by way of example but not limitation, acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

In therapeutic and/or diagnostic applications, the compounds of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained-slow release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington: The Science and Practice of Pharmacy (20th ed.)

Lippincott, Williams & Wilkins (2000). Suitable routes may include oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

For injection, the agents of the disclosure may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable inert carriers to formulate the compounds herein disclosed for the practice of the disclosure into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (e.g., patient) to be treated.

For nasal or inhalation delivery, the agents of the disclosure also may be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances, such as saline; preservatives, such as benzyl alcohol; absorption promoters; and fluorocarbons.

Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Generally, the compounds according to the disclosure are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from 0.01 to 1000 mg, from 0.5 to 100 mg, from 1 to 50 mg per day, and from 5 to 40 mg per day are examples of dosages that may be used. A non-limiting dosage is 10 to

30 mg per day. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, the bioavailability of the compound(s), the adsorption, distribution, metabolism, and excretion (ADME) toxicity of the compound(s), and the preference and experience of the attending physician.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

C. Methods of Imaging using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof

In some embodiments, presently disclosed subject matter provides a method for imaging a disease or disorder associated with fibroblast-activation protein- α (FAP- α), the method comprising administering a compound of formula (I), wherein the compound of formula (I) comprises an optical or radiolabeled functional group suitable for optical imaging, PET imaging, or SPECT imaging; and obtaining an image.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for imaging one or more cells, organs, or tissues, the method comprising exposing cells or administering to a subject an effective amount of a compound of formula (I) with an optical or radioisotopic label suitable for imaging. In some embodiments, the one or more organs or tissues include prostate tissue, kidney tissue, brain tissue, vascular tissue, or tumor tissue.

The imaging methods of the invention are suitable for imaging any physiological process or feature in which FAP- α is involved, for example, identifying areas of tissues or targets which exhibit or express high concentrations of FAP- α . Physiological processes in which FAP- α is involved include, but are not limited to: (a) proliferation diseases (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c) endocrinological disorders (including but not limited to disorders of glucose metabolism).

In certain embodiments, the radiolabeled compound is stable in vivo.

In certain embodiments, the radiolabeled compound is detected by positron emission tomography (PET) or single photon emission computed tomography (SPECT).

In certain embodiments, the optical reporting moiety is detected by fluorescence, such as fluorescence microscopy.

In certain embodiments, the presently disclosed compounds are excreted from tissues of the body quickly to prevent prolonged exposure to the radiation of the radiolabeled compound administered to the subject. Typically, the presently disclosed compounds are eliminated from the body in less than about 24 hours. More typically, the presently disclosed compounds are eliminated from the body in less than about 16

hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes. Exemplary compounds are eliminated in between about 60 minutes and about 120 minutes. In certain embodiments, the presently disclosed compounds are stable in vivo such that substantially all, e.g., more than about 50%, 60%, 70%, 80%, or 90% of the injected compound is not metabolized by the body prior to excretion.

Additionally, for in vitro applications, such as in vitro diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use, such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

Other embodiments provide kits comprising a compound of formula (I). In certain embodiments, the kit provides packaged pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of formula (I). In certain embodiments the packaged pharmaceutical composition will comprise the reaction precursors necessary to generate the compound of formula (I) upon combination with a radiolabeled precursor. Other packaged pharmaceutical compositions further comprise indicia comprising at least one of: instructions for preparing compounds of formula (I) from supplied precursors, instructions for using the composition to image cells or tissues expressing FAP- α .

In certain embodiments, a kit containing from about 1 to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier, is provided. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound of formula (I) in solution or in lyophilized form, and these components of the kit may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form. Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

In certain embodiments, a kit provides a non-radiolabeled precursor to be combined with a radiolabeled reagent on-site.

Imaging agents may be used in accordance with the presently disclosed methods by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with FAP- α . The spatial distribution may be measured using any means suitable for the particular label, for example, a gamma camera, a PET apparatus, a SPECT apparatus, and the like. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions or fluorescence. A particularly useful imaging approach employs more than one imaging agent to perform simultaneous studies.

In general, a detectably effective amount of the imaging agent of the invention is administered to a subject. A “detectably effective amount” of the imaging agent is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent may be administered in more than one injection. The detectably effective amount of the imaging agent of the invention can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective amounts of the imaging agent also can vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art. The amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the amount of imaging agent to administer to each individual patient and the duration of the imaging study.

D. Methods of Treating a FAP- α Related Disease or Disorder using the Compounds of Formula (I), or Pharmaceutical Compositions Thereof

In other embodiments, the presently disclosed compounds of formula (I) can be used to treat a subject afflicted with one or more FAP- α related diseases or disorders including, but not limited to: (a) proliferation (including but not limited to cancer); (b) tissue remodeling and/or chronic inflammation (including but not limited to fibrotic disease, wound healing, keloid formation, osteoarthritis, rheumatoid arthritis and related disorders involving cartilage degradation); and (c) endocrinological disorders (including but not limited to disorders of glucose

metabolism).

Accordingly, in some embodiments, the one or more FAP- α related disease or disorder is selected from the group consisting of a proliferative disease, including, but not limited to, breast cancer, colorectal cancer, ovarian cancer, prostate cancer, pancreatic cancer, kidney cancer, lung cancer, melanoma, fibrosarcoma, bone and connective tissue sarcomas, renal cell carcinoma, giant cell carcinoma, squamous cell carcinoma, and adenocarcinoma; diseases characterized by tissue remodeling and/or chronic inflammation; disorders involving endocrinological dysfunction; and blood clotting disorders.

In general, the “effective amount” of an active agent or drug delivery device refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the makeup of the pharmaceutical composition, the target tissue, and the like.

In other embodiments, the method can be practiced *in vitro* or *ex vivo* by introducing, and preferably mixing, the compound and cell(s) or tumor(s) in a controlled environment, such as a culture dish or tube. The method can be practiced *in vivo*, in which case contacting means exposing the target in a subject to at least one compound of the presently disclosed subject matter, such as administering the compound to a subject via any suitable route. According to the presently disclosed subject matter, contacting may comprise introducing, exposing, and the like, the compound at a site distant to the cells to be contacted, and allowing the bodily functions of the subject, or natural (e.g., diffusion) or man-induced (e.g., swirling) movements of fluids to result in contact of the compound and the target.

The subject treated by the presently disclosed methods in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term “subject.” Accordingly, a “subject” can include a human subject for medical purposes, such as for the treatment of an existing condition or disease or the prophylactic treatment for preventing the onset of a condition or disease, or an animal (non-human) subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, and the like;

bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like. An animal may be a transgenic animal. In some embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a condition or disease. Thus, the terms “subject” and “patient” are used interchangeably herein. In some embodiments, the subject is human. In other embodiments, the subject is non-human.

As used herein, the term “treating” can include reversing, alleviating, inhibiting the progression of, preventing or reducing the likelihood of the disease, or condition to which such term applies, or one or more symptoms or manifestations of such disease or condition.

“Preventing” refers to causing a disease, condition, or symptom or manifestation of such, or worsening of the severity of such, not to occur. Accordingly, the presently disclosed compounds can be administered prophylactically to prevent or reduce the incidence or recurrence of the disease, or condition.

II. DEFINITIONS

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs.

While the following terms in relation to compounds of formula (I) are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. These definitions are intended to supplement and illustrate, not preclude, the definitions that would be apparent to one of ordinary skill in the art upon review of the present disclosure.

The terms substituted, whether preceded by the term “optionally” or not, and substituent, as used herein, refer to the ability, as appreciated by one skilled in this art,

to change one functional group for another functional group on a molecule, provided that the valency of all atoms is maintained. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. The substituents also may be further substituted (e.g., an aryl group substituent may have another substituent off it, such as another aryl group, which is further substituted at one or more positions).

Where substituent groups or linking groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-\text{CH}_2\text{O}-$ is equivalent to $-\text{OCH}_2-$; $-\text{C}(=\text{O})\text{O}-$ is equivalent to $-\text{OC}(=\text{O})-$; $-\text{OC}(=\text{O})\text{NR}-$ is equivalent to $-\text{NRC}(=\text{O})\text{O}-$, and the like.

When the term “independently selected” is used, the substituents being referred to (e.g., R groups, such as groups R_1 , R_2 , and the like, or variables, such as “m” and “n”), can be identical or different. For example, both R_1 and R_2 can be substituted alkyls, or R_1 can be hydrogen and R_2 can be a substituted alkyl, and the like.

The terms “a,” “an,” or “a(n),” when used in reference to a group of substituents herein, mean at least one. For example, where a compound is substituted with “an” alkyl or aryl, the compound is optionally substituted with at least one alkyl and/or at least one aryl. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

A named “R” or group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative “R” groups as set forth above are defined below.

Descriptions of compounds of the present disclosure are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and

several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

Unless otherwise explicitly defined, a “substituent group,” as used herein, includes a functional group selected from one or more of the following moieties, which are defined herein:

The term hydrocarbon, as used herein, refers to any chemical group comprising hydrogen and carbon. The hydrocarbon may be substituted or unsubstituted. As would be known to one skilled in this art, all valencies must be satisfied in making any substitutions. The hydrocarbon may be unsaturated, saturated, branched, unbranched, cyclic, polycyclic, or heterocyclic. Illustrative hydrocarbons are further defined herein below and include, for example, methyl, ethyl, *n*-propyl, isopropyl, cyclopropyl, allyl, vinyl, *n*-butyl, *tert*-butyl, ethynyl, cyclohexyl, and the like.

The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched chain, acyclic or cyclic hydrocarbon group, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent groups, having the number of carbon atoms designated (i.e., C₁-C₁₀ means one to ten carbons, including 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 carbons). In particular embodiments, the term “alkyl” refers to C₁₋₂₀ inclusive, including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 carbons, linear (i.e., “straight-chain”), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (i.e., alkenyl and alkynyl) hydrocarbon radicals derived from a hydrocarbon moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom.

Representative saturated hydrocarbon groups include, but are not limited to, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *sec*-pentyl, isopentyl, neopentyl, *n*-hexyl, *sec*-hexyl, *n*-heptyl, *n*-octyl, *n*-decyl, *n*-undecyl, dodecyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, and homologs and isomers thereof.

“Branched” refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. “Lower alkyl” refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C₁₋₈ alkyl), e.g., 1, 2, 3, 4, 5, 6, 7,

or 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about 20 carbon atoms, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, "alkyl" refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, "alkyl" refers, in particular, to C₁₋₈ branched-chain alkyls.

Alkyl groups can optionally be substituted (a "substituted alkyl") with one or more alkyl group substituents, which can be the same or different. The term "alkyl group substituent" includes but is not limited to alkyl, substituted alkyl, halo, alkylamino, arylamino, acyl, hydroxyl, aryloxy, alkoxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as "alkylaminoalkyl"), or aryl.

Thus, as used herein, the term "substituted alkyl" includes alkyl groups, as defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon group, or combinations thereof, consisting of at least one carbon atoms and at least one heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen, phosphorus, and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -Si(CH₃)₃, -CH₂-CH=N-OCH₃, -CH=CH-N(CH₃)-CH₃, O-CH₃, -O-CH₂-CH₃, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃.

As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as -C(O)NR', -NR'R'', -OR', -SR, -S(O)R, and/or -S(O₂)R'. Where "heteroalkyl" is

recited, followed by recitations of specific heteroalkyl groups, such as -NR'R or the like, it will be understood that the terms heteroalkyl and -NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R" or the like.

"Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, unsubstituted alkyl, substituted alkyl, aryl, or substituted aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl, and fused ring systems, such as dihydro- and tetrahydronaphthalene, and the like.

The term "cycloalkylalkyl," as used herein, refers to a cycloalkyl group as defined hereinabove, which is attached to the parent molecular moiety through an alkyl group, also as defined above. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

The terms "cycloheteroalkyl" or "heterocycloalkyl" refer to a non-aromatic ring system, unsaturated or partially unsaturated ring system, such as a 3- to 10-member substituted or unsubstituted cycloalkyl ring system, including one or more heteroatoms, which can be the same or different, and are selected from the group consisting of nitrogen (N), oxygen (O), sulfur (S), phosphorus (P), and silicon (Si), and optionally can include one or more double bonds.

The cycloheteroalkyl ring can be optionally fused to or otherwise attached to other cycloheteroalkyl rings and/or non-aromatic hydrocarbon rings. Heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or a polycyclic group wherein at least one ring atom is a heteroatom

selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5-membered ring has 0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds, and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative cycloheteroalkyl ring systems include, but are not limited to pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, quinuclidinyl, morpholinyl, thiomorpholinyl, thiadiazinanyl, tetrahydrofuranyl, and the like.

The terms “cycloalkyl” and “heterocycloalkyl”, by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl”, respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The terms “cycloalkylene” and “heterocycloalkylene” refer to the divalent derivatives of cycloalkyl and heterocycloalkyl, respectively.

An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. Alkyl groups which are limited to hydrocarbon groups are termed “homoalkyl.”

More particularly, the term “alkenyl” as used herein refers to a monovalent group derived from a C₁₋₂₀ inclusive straight or branched hydrocarbon moiety having at least one carbon-carbon double bond by the removal of a single hydrogen molecule. Alkenyl groups include, for example, ethenyl (i.e., vinyl), propenyl, butenyl, 1-methyl-2-buten-1-yl, pentenyl, hexenyl, octenyl, allenyl, and butadienyl.

The term “cycloalkenyl” as used herein refers to a cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, 1,3-cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

The term “alkynyl” as used herein refers to a monovalent group derived from a straight or branched C₁₋₂₀ hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon triple bond. Examples of “alkynyl” include ethynyl, 2-propynyl (propargyl), 1-propynyl, pentynyl, hexynyl, and heptynyl groups, and the like.

The term “alkylene” by itself or a part of another substituent refers to a straight or branched bivalent aliphatic hydrocarbon group derived from an alkyl group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more “alkyl group substituents.” There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as “alkylaminoalkyl”), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene (–CH₂–); ethylene (–CH₂–CH₂–); propylene (–(CH₂)₃–); cyclohexylene (–C₆H₁₀–); –CH=CH–CH=CH–; –CH=CH–CH₂–; –CH₂CH₂CH₂CH₂–; –CH₂CH=CHCH₂–; –CH₂CsCCH₂–; –CH₂CH₂CH(CH₂CH₂CH₃)CH₂–; –(CH₂)_q–N(R)–(CH₂)_r–, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxy (–O–CH₂–O–); and ethylenedioxy (–O–(CH₂)₂–O–). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being some embodiments of the present disclosure. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

The term “heteroalkylene” by itself or as part of another substituent means a divalent group derived from heteroalkyl, as exemplified, but not limited by, –CH₂–CH₂–S–CH₂–CH₂– and –CH₂–S–CH₂–CH₂–NH–CH₂–. For heteroalkylene groups, heteroatoms also can occupy either or both of the chain termini (e.g., alkyleneoxo,

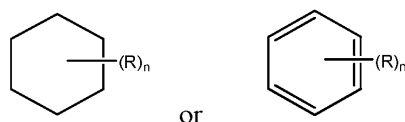
alkylenedioxo, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)OR'$ represents both $-C(O)OR'$ and $-R'OC(O)-$.

The term “aryl” means, unless otherwise stated, an aromatic hydrocarbon substituent that can be a single ring or multiple rings (such as from 1 to 3 rings), which are fused together or linked covalently. The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms (in each separate ring in the case of multiple rings) selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. The terms “arylene” and “heteroarylene” refer to the divalent forms of aryl and heteroaryl, respectively.

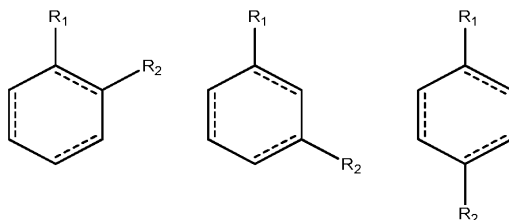
For brevity, the term “aryl” when used in combination with other terms (e.g., aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the terms “arylalkyl” and “heteroarylalkyl” are meant to include those groups in which an aryl or heteroaryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl, furylmethyl, and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like). However, the term “haloaryl,” as used herein is meant to cover only aryls substituted with one or more halogens.

Where a heteroalkyl, heterocycloalkyl, or heteroaryl includes a specific number of members (e.g. “3 to 7 membered”), the term “member” refers to a carbon or heteroatom.

Further, a structure represented generally by the formula:



as used herein refers to a ring structure, for example, but not limited to a 3-carbon, a 4-carbon, a 5-carbon, a 6-carbon, a 7-carbon, and the like, aliphatic and/or aromatic cyclic compound, including a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure, comprising a substituent R group, wherein the R group can be present or absent, and when present, one or more R groups can each be substituted on one or more available carbon atoms of the ring structure. The presence or absence of the R group and number of R groups is determined by the value of the variable “n,” which is an integer generally having a value ranging from 0 to the number of carbon atoms on the ring available for substitution. Each R group, if more than one, is substituted on an available carbon of the ring structure rather than on another R group. For example, the structure above where n is 0 to 2 would comprise compound groups including, but not limited to:



and the like.

A dashed line representing a bond in a cyclic ring structure indicates that the bond can be either present or absent in the ring. That is, a dashed line representing a bond in a cyclic ring structure indicates that the ring structure is selected from the group consisting of a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure.

The symbol () denotes the point of attachment of a moiety to the remainder of the molecule.

When a named atom of an aromatic ring or a heterocyclic aromatic ring is defined as being “absent,” the named atom is replaced by a direct bond.

Each of above terms (e.g. , “alkyl,” “heteroalkyl,” “cycloalkyl, and “heterocycloalkyl”, “aryl,” “heteroaryl,” “phosphonate,” and “sulfonate” as well as

their divalent derivatives) are meant to include both substituted and unsubstituted forms of the indicated group. Optional substituents for each type of group are provided below.

Substituents for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl monovalent and divalent derivative groups (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such groups. R', R'', R''' and R'''' each may independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. As used herein, an "alkoxy" group is an alkyl attached to the remainder of the molecule through a divalent oxygen. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7- membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1- pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

Similar to the substituents described for alkyl groups above, exemplary substituents for aryl and heteroaryl groups (as well as their divalent derivatives) are varied and are selected from, for example: halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxo, and fluoro(C₁-C₄)alkyl, in a number ranging

from zero to the total number of open valences on aromatic ring system; and where R', R'', R''' and R'''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present.

Two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 4.

One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -(CRR')_s-X'-(C''R''')_d-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'' and R''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent and has the general formula RC(=O)-, wherein R is an alkyl, alkenyl, alkynyl, aryl, carbocyclic, heterocyclic, or aromatic heterocyclic group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as a 2-(furan-2-yl)acetyl- and a 2-phenylacetyl group. Specific examples of acyl groups include acetyl and benzoyl. Acyl groups also are intended to include amides, -RC(=O)NR', esters, -RC(=O)OR', ketones, -RC(=O)R', and aldehydes, -RC(=O)H.

The terms "alkoxyl" or "alkoxy" are used interchangeably herein and refer to a saturated (i.e., alkyl-O-) or unsaturated (i.e., alkenyl-O- and alkynyl-O-) group attached to the parent molecular moiety through an oxygen atom, wherein the terms

“alkyl,” “alkenyl,” and “alkynyl” are as previously described and can include C₁₋₂₀ inclusive, linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, *n*-butoxyl, *sec*-butoxyl, *tert*-butoxyl, and *n*-pentoxyl, neopentoxyl, *n*-hexoxyl, and the like.

The term “alkoxyalkyl” as used herein refers to an alkyl-O-alkyl ether, for example, a methoxyethyl or an ethoxymethyl group.

“Aryloxyl” refers to an aryl-O- group wherein the aryl group is as previously described, including a substituted aryl. The term “aryloxyl” as used herein can refer to phenyloxyl or hexyloxyl, and alkyl, substituted alkyl, halo, or alkoxy substituted phenyloxyl or hexyloxyl.

“Aralkyl” refers to an aryl-alkyl-group wherein aryl and alkyl are as previously described and includes substituted aryl and substituted alkyl. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

“Aralkyloxyl” refers to an aralkyl-O- group wherein the aralkyl group is as previously described. An exemplary aralkyloxyl group is benzyloxyl, i.e., C₆H₅-CH₂-O-. An aralkyloxyl group can optionally be substituted.

“Alkoxy carbonyl” refers to an alkyl-O-C(=O)- group. Exemplary alkoxy carbonyl groups include methoxy carbonyl, ethoxy carbonyl, butyloxy carbonyl, and *tert*-butyloxy carbonyl.

“Aryloxy carbonyl” refers to an aryl-O-C(=O)- group. Exemplary aryloxy carbonyl groups include phenoxy- and naphthoxy-carbonyl.

“Aralkoxy carbonyl” refers to an aralkyl-O-C(=O)- group. An exemplary aralkoxy carbonyl group is benzyloxy carbonyl.

“Carbamoyl” refers to an amide group of the formula -C(=O)NH₂. “Alkylcarbamoyl” refers to a R’RN-C(=O)- group wherein one of R and R’ is hydrogen and the other of R and R’ is alkyl and/or substituted alkyl as previously described. “Dialkylcarbamoyl” refers to a R’RN-C(=O)- group wherein each of R and R’ is independently alkyl and/or substituted alkyl as previously described.

The term carbonyldioxy, as used herein, refers to a carbonate group of the formula -O-C(=O)-OR.

“Acyloxyl” refers to an acyl-O- group wherein acyl is as previously described.

The term “amino” refers to the -NH₂ group and also refers to a nitrogen containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms

“acylamino” and “alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

An “aminoalkyl” as used herein refers to an amino group covalently bound to an alkylene linker. More particularly, the terms alkylamino, dialkylamino, and trialkylamino as used herein refer to one, two, or three, respectively, alkyl groups, as previously defined, attached to the parent molecular moiety through a nitrogen atom. The term alkylamino refers to a group having the structure -NHR' wherein R' is an alkyl group, as previously defined; whereas the term dialkylamino refers to a group having the structure -NR'R'' , wherein R' and R'' are each independently selected from the group consisting of alkyl groups. The term trialkylamino refers to a group having the structure -NR'R''R''' , wherein R' , R'' , and R''' are each independently selected from the group consisting of alkyl groups. Additionally, R' , R'' , and/or R''' taken together may optionally be $\text{-(CH}_2\text{)}_k\text{-}$ where k is an integer from 2 to 6. Examples include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, isopropylamino, piperidino, trimethylamino, and propylamino.

The amino group is -NR'R'' , wherein R' and R'' are typically selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

The terms alkylthioether and thioalkoxyl refer to a saturated (i.e., alkyl-S-) or unsaturated (i.e., alkenyl-S- and alkynyl-S-) group attached to the parent molecular moiety through a sulfur atom. Examples of thioalkoxyl moieties include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, *n*-butylthio, and the like.

“Acylamino” refers to an acyl-NH- group wherein acyl is as previously described. “Aroylamino” refers to an aroyl-NH- group wherein aroyl is as previously described.

The term “carbonyl” refers to the -C(=O)- group, and can include an aldehyde group represented by the general formula R-C(=O)H .

The term “carboxyl” refers to the -COOH group. Such groups also are referred to herein as a “carboxylic acid” moiety.

The terms “halo,” “halide,” or “halogen” as used herein refer to fluoro, chloro, bromo, and iodo groups. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo($\text{C}_1\text{-C}_4$)alkyl”

is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term “hydroxyl” refers to the –OH group.

The term “hydroxyalkyl” refers to an alkyl group substituted with an –OH group.

The term “mercapto” refers to the –SH group.

The term “oxo” as used herein means an oxygen atom that is double bonded to a carbon atom or to another element.

The term “nitro” refers to the –NO₂ group.

The term “thio” refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term “sulfate” refers to the –SO₄ group.

The term thiohydroxyl or thiol, as used herein, refers to a group of the formula –SH.

More particularly, the term “sulfide” refers to compound having a group of the formula –SR.

The term “sulfone” refers to compound having a sulfonyl group –S(O₂)R.

The term “sulfoxide” refers to a compound having a sulfinyl group –S(O)R

The term ureido refers to a urea group of the formula –NH—CO—NH₂.

Throughout the specification and claims, a given chemical formula or name shall encompass all tautomers, congeners, and optical- and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

Certain compounds of the present disclosure may possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as D- or L- for amino acids, and individual isomers are encompassed within the scope of the present disclosure. The compounds of the present disclosure do not include those which are known in art to be too unstable to synthesize and/or isolate. The present disclosure is meant to include compounds in racemic, scalemic, and optically pure forms. Optically active (R)- and (S)-, or D- and L-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include

both *E* and *Z* geometric isomers.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the disclosure.

It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure. The term “tautomer,” as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

As used herein the term “monomer” refers to a molecule that can undergo polymerization, thereby contributing constitutional units to the essential structure of a macromolecule or polymer.

A “polymer” is a molecule of high relative molecule mass, the structure of which essentially comprises the multiple repetition of unit derived from molecules of low relative molecular mass, i.e., a monomer.

A “dendrimer” is highly branched, star-shaped macromolecules with nanometer-scale dimensions.

As used herein, an “oligomer” includes a few monomer units, for example, in contrast to a polymer that potentially can comprise an unlimited number of monomers. Dimers, trimers, and tetramers are non-limiting examples of oligomers.

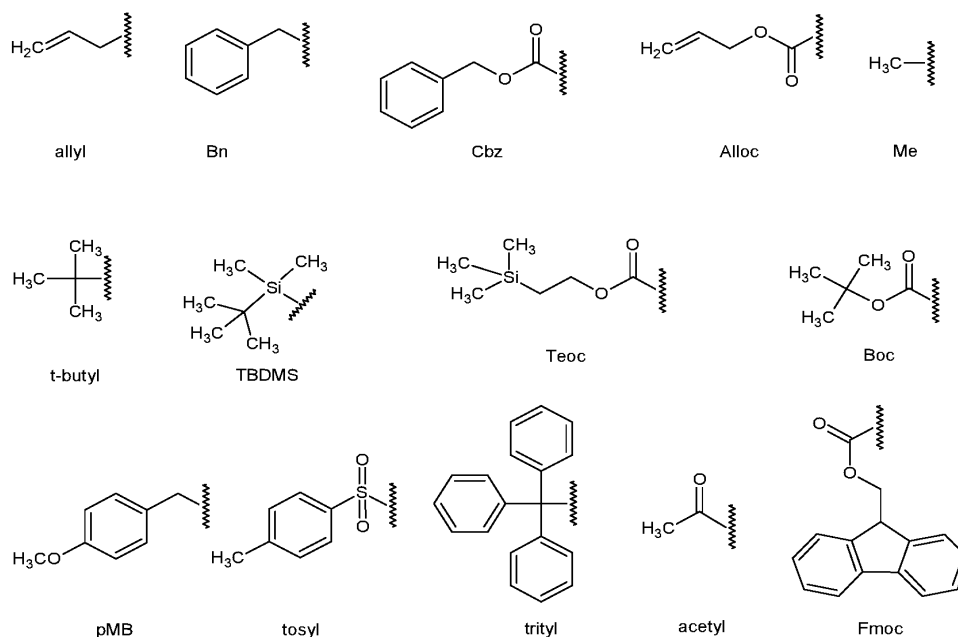
The term “protecting group” refers to chemical moieties that block some or all reactive moieties of a compound and prevent such moieties from participating in chemical reactions until the protective group is removed, for example, those moieties listed and described in T. W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, 3rd ed. John Wiley & Sons (1999). It may be advantageous, where different protecting groups are employed, that each (different) protective group be removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions allow differential removal of such protecting groups. For example, protective groups can be removed by acid, base, and hydrogenolysis. Groups such as trityl, dimethoxytrityl, acetal and tert-butyldimethylsilyl are acid labile and may be used to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by

hydrogenolysis, and Fmoc groups, which are base labile. Carboxylic acid and hydroxy reactive moieties may be blocked with base labile groups such as, without limitation, methyl, ethyl, and acetyl in the presence of amines blocked with acid labile groups such as tert-butyl carbamate or with carbamates that are both acid and base stable but hydrolytically removable.

Carboxylic acid and hydroxy reactive moieties may also be blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids may be blocked with base labile groups such as Fmoc. Carboxylic acid reactive moieties may be blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups may be blocked with fluoride labile silyl carbamates.

Allyl blocking groups are useful in the presence of acid- and base- protecting groups since the former are stable and can be subsequently removed by metal or pi-acid catalysts. For example, an allyl-blocked carboxylic acid can be deprotected with a palladium(O)- catalyzed reaction in the presence of acid labile t-butyl carbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate may be attached. As long as the residue is attached to the resin, that functional group is blocked and cannot react. Once released from the resin, the functional group is available to react.

Typical blocking/protecting groups include, but are not limited to the following moieties:



Following long-standing patent law convention, the terms “a,” “an,” and “the” refer to “one or more” when used in this application, including the claims. Thus, for example, reference to “a subject” includes a plurality of subjects, unless the context clearly is to the contrary (e.g., a plurality of subjects), and so forth.

Throughout this specification and the claims, the terms “comprise,” “comprises,” and “comprising” are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about” even though the term “about” may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off,

measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term “about,” when referring to a value can be meant to encompass variations of, in some embodiments, $\pm 100\%$ in some embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, the term “about” when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range.

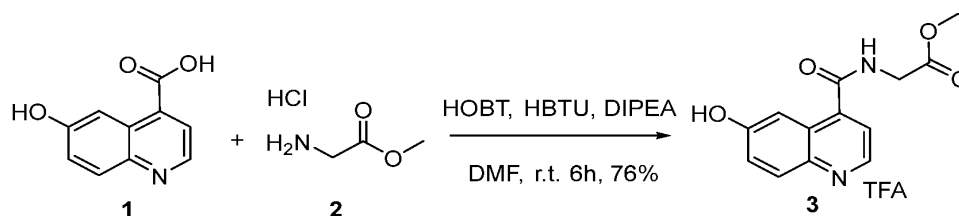
EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

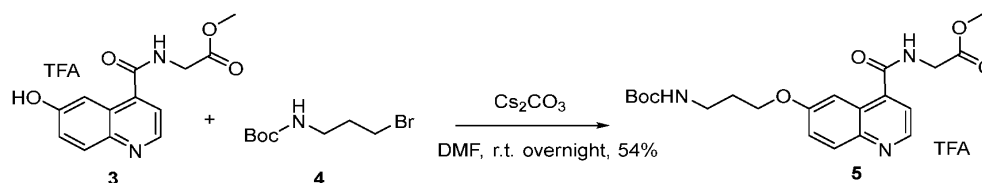
EXAMPLE 1

Experimental Procedures

1.1 Synthesis of XY-FAP-01.

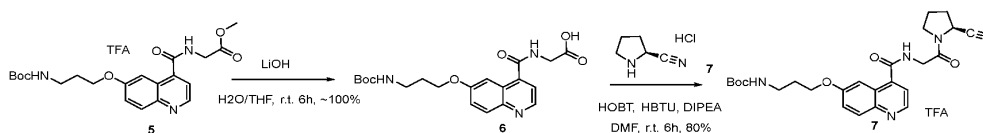


Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**): 6-Hydroxyquinoline-4-carboxylic acid (**1**) 210 mg (1.1 mmol), methyl glycinate HCl salt (**2**) 143 mg (1.1 mmol), HBTU 420 mg (1.1 mmol) and HOBT 170 mg (1.1 mmol) were dissolved in 12 mL dry DMF. To the solution, 0.77 mL of DIPEA (4.4 mmol) was added. The reaction was stirred at room temperature for 6 h. After the solvent was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and the product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 290 mg of product **3** was obtained as a yellow powder with a yield of 76%. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 8.69 (s, 1H), 7.94 (d, $J = 7.92$ Hz, 1H), 7.57–7.51 (m, 3H), 7.42–7.37 (m, 1H), 4.21 (s, 2H), 3.81 (s, 3H). $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 172.4, 160.9, 145.1, 143.7, 129.7, 129.4, 128.3, 121.8, 119.6, 112.4, 109.1, 56.8, 44.8. MS: calculated for $[\text{C}_{13}\text{H}_{13}\text{N}_2\text{O}_4]^+$, 261.3 $[\text{M} + \text{H}]^+$; found 261.1.

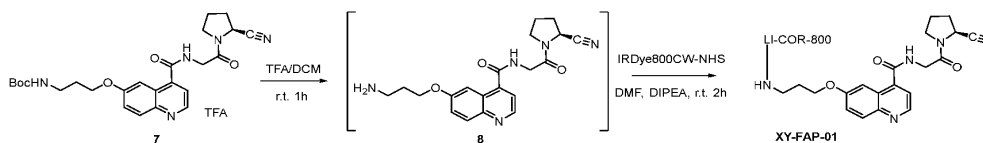


Methyl (6-(3-((tert-butoxycarbonyl)amino)propoxy)quinoline-4-carbonyl)glycinate (**5**): Methyl (6-hydroxyquinoline-4-carbonyl)glycinate (**3**) 360 mg (1.0 mmol), tert-butyl (3-bromopropyl)carbamate (**4**) 500 mg (2.1 mmol) were dissolved in 20 mL DMF. Cs_2CO_3 1 g (3.0 mmol) was added to the solution and the reaction was stirred at room temperature overnight. After filtration, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 270 mg of product **5** was obtained with a yield of 54%. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.68–8.37 (m, 2H), 8.02 (d, $J = 9.1$ Hz, 1H), 7.80 (s, 1H), 7.72–7.64 (m, 1H), 7.40 (d, $J = 9.1$ Hz, 1H), 4.94 (br s, 1H), 4.41–4.31 (m, 2H), 4.27–4.18 (m, 2H), 3.85 (s, 3H), 3.44–3.30 (m, 2H), 2.13–2.00 (m, 2H), 1.43 (s, 9H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 170.1, 167.2, 158.4, 144.7, 142.3,

128.4, 126.1, 124.7, 119.1, 103.7, 79.5, 60.4, 52.5, 41.4, 37.7, 29.3, 28.4. MS: calculated for $[C_{21}H_{28}N_3O_6]^+$, 418.5 $[M + H]^+$; found 418.3.



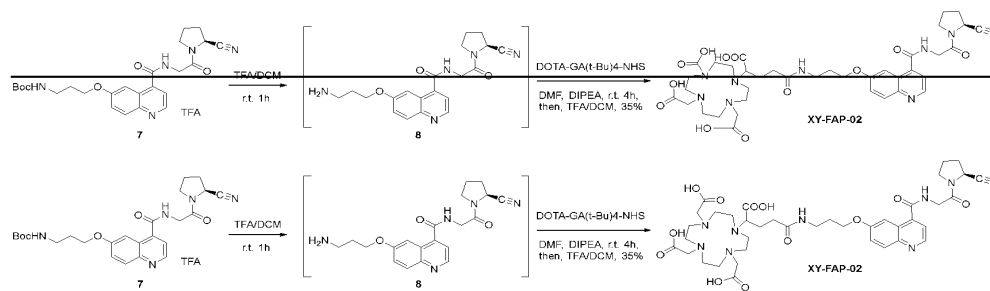
tert-Butyl(S)-(3-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl)carbamate (7): Compound **5** 110 mg (0.21 mmol) and LiOH 30 mg (1.2 mmol) was stirred in 4 mL of H₂O/THF (1/1) for 6 hours. After most of the THF was removed under vacuum, the mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada) and eluted with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1) to remove the salts. The product **6** obtained was mixed with (S)-pyrrolidine-2-carbonitrile 53 mg (0.4 mmol), HOBT 68 mg (0.4 mmol), HBTU 152 mg (0.4 mmol) and DIPEA 0.56 mL (1.6 mmol) in dry 10 mL DMF. After 6 hours, the solvent was removed under vacuum and the remaining mixture was loaded onto a 25 g C18 cartridge (Silicycle, Canada). The product was purified with a MeCN/water/TFA gradient (0/100/0.1 to 90/10/0.1). 99 mg of **7** was obtained with a yield of 80%. ¹H NMR (400 MHz, CDCl₃): δ 8.73 (s, 1H), 7.95 (d, J = 10.2 Hz, 1H), 7.68 (br s, 1H), 7.63–7.56 (m, 1H), 7.56–7.48 (m, 1H), 7.38–7.29 (m, 1H), 5.27 (br s, 1H), 4.84–4.72 (m, 1H), 4.46–4.35 (m, 1H), 4.33–4.20 (m, 1H), 4.17–4.09 (m, 2H), 3.78–3.64 (m, 1H), 3.59–3.46 (m, 1H), 3.36 (s, 2H), 2.38–2.17 (m, 4H), 1.42 (s, 9H), 1.35–1.27 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 167.6, 167.5, 157.9, 156.2, 146.3, 130.2, 125.7, 123.7, 119.3, 118.0, 103.3, 79.0, 65.9, 46.8, 45.7, 42.2, 37.6, 29.8, 29.3, 28.4, 25.1. MS: calculated for $[C_{25}H_{32}N_5O_5]^+$, 482.6 $[M + H]^+$; found 482.3.



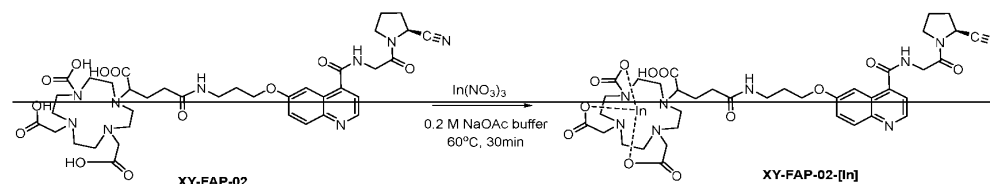
XY-FAP-01. Compound **7** (1 mg, 1.7 μmol) was treated with a 1 mL solution of TFA/methylene chloride (1/1) for 2 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMSO. To the solution, LICOR800CW-NHS ester 0.5 mg (0.43 μmol) and Et₃N 10 μL were added. After 1 h at room temperature, the solvent was removed and the product was purified by HPLC. 0.5 mg product was obtained with a yield of 85%. HPLC condition: column

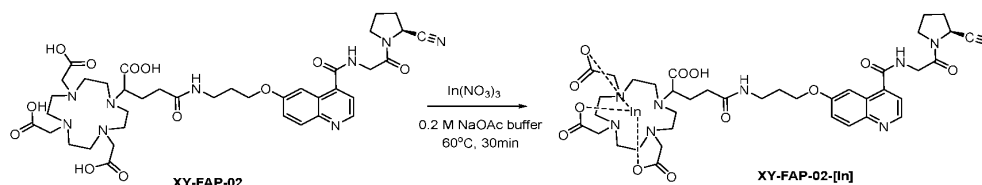
Phenomenex, Luna 10 x 250 mm, 10 μ . Gradient 10/90/0.1 MeCN/H₂O/TFA to 80/20/0.1 MeCN/H₂O/TFA within 15 min at a flow of 3 mL/min. The product was eluted at 10.1 min. MS: Calculated for [C₆₆H₇₆N₇O₁₇S₄]⁺, 1366.4[M+H]⁺; found 1366.8.

1.2 Synthesis of XY-FAP-02



2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**XY-FAP-02**): Compound 7 (15 mg, 31.3 μ mol) was treated with a 1-mL solution of TFA/methylene chloride (1/1) for 1 h. The solvent was removed under vacuum, and the remaining material re-dissolved in 0.5 mL of DMF. To the solution, DIPEA (27 μ L, 156.5 μ mol) was added, followed by dropwise addition of a solution of DOTA-GA(t-Bu)₄-NHS (25 mg, 31.3 μ L) in 0.5 mL of DMF. The reaction mixture was stirred for 4 h at ambient temperature and then concentrated under vacuum. The t-Bu-protected intermediate was deprotected in situ without further purification using a 1 mL mixture of TFA, H₂O and triethylsilane (TES) (95:2.5:2.5). Reaction mixture was then concentrated and purified by semipreparative HPLC, to afford the product as a white solid (8.5 mg, 33% yield). MS: calculated for [C₃₉H₅₄N₉O₁₂]⁺, 840.9 [M + H]⁺; found 840.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 μ m, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02** eluted at 11.8 min.





XY-FAP-02-[In]. $^{113/115}$ Indium(III) 2,2',2''-(10-(1-Carboxy-4-((3-((4-((2-((S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)oxy)propyl) amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**XY-FAP-02-[In]**): To a solution of 2 mg (2.4 μ mol) of **XY-FAP-02** in 1 mL of 0.2M AcONa, a solution of 1.4 mg (4.6 μ mol) of $\text{In}(\text{NO}_3)_3$ in 0.5 mL water is added and warmed in a 60 °C bath for 30 min. After cooling to ambient temperature, the mixture was purified by semipreparative HPLC. The product was obtained as a white solid (1.8 mg, 79% yield). MS: calculated for $[\text{C}_{39}\text{H}_{51}\text{N}_9\text{O}_{12}\text{In}]^+$, 951.7 $[\text{M} + \text{H}]^+$; found 952.5. HPLC (10 mm x 250 mm Phenomenex Luna C18 column, 10 μ m, mobile phase 95/5/0.1% to 75/25/0.1% water/acetonitrile/TFA over 20 min, flow 5 mL/min) **XY-FAP-02-[In]** eluted at 14.0 min.

1.3 Radiolabeling Methods. Briefly, 20 mg **XY-FAP-02** solution in 20 mL of 0.2 M NaOAc was added to 10 mL 4.6 mCi $^{111}\text{InCl}_3$ solution (Nordion, Ottawa, Canada) and adjusted to a final pH of 5.5-6. The mixture was heated in a water bath at 70 °C for 30 min and, after the reaction completed, was diluted with 200 mL of water for HPLC purification. The solution was purified using a Phenomenex 5 μ m C₁₈ Luna 4.6 x 250 mm² column (Torrance, CA) with a flow rate of 0.6 mL/min with water (0.1% TFA) (A) and MeCN (0.1% TFA) (B) as the eluting solvents. An isocratic solution of 88% A and 12% B was utilized for purification, resulting in the labeled compound, $^{111}\text{In-XY-FAP-02}$, eluting first at 18.6 min followed by the unlabeled starting material at 23.5 min. 3.2 mCi of labeled compound was obtained as pure product with a yield of 69%. Another reaction with the identical condition was performed with 74% yield. The collected radioactivity was diluted with 20 mL of water and loaded onto activated Sep-Pak (WAT020515, Waters, Milford, MA). After the Sep-Pak was washed with 10 mL of water, $^{111}\text{In-XY-FAP-02}$ was eluted with 1.5 mL of ethanol. The ethanol was evaporated under a gentle stream of N_2 (to a total volume of < 50 μ L). The resulting solution was formulated in saline for the imaging and biodistribution studies.

1.4 FAP Inhibition Assay. The inhibitory activity of **XY-FAP-01** was determined using a fluorogenic FAP Assay Kit (BPS Bioscience, San Diego, CA). Briefly, **XY-FAP-01**, DPP substrate, and human recombinant FAP were loaded into a 96 well plate to initiate the enzyme reaction. The reaction was left for 10 minutes at room temperature before fluorescence was measured with a VICTOR3 V multilabel plate reader (PerkinElmer Inc., Waltham, MA). Data was normalized and semi-log inhibition curves were generated in order to determine the IC₅₀ value (concentration of **XY-FAP-01** where the enzyme activity is 50% inhibited) for **XY-FAP-01** and subsequent enzyme inhibition constant (K_i) using the Cheng-Prusoff conversion. Generation of semi-log inhibition curves and IC₅₀ values were done using GraphPad Prism (San Diego, CA).

1.5 Cell lines. Six human cancer cell lines were used to assess binding to FAP: glioblastoma (U-87-MG), melanoma (SK-MEL-24), prostate (PC-3), non-small cell lung cancer (NCI-H2228), colorectal carcinoma (HCT 116), and lung squamous cell carcinoma (NCI-H226). From the literature, U-87-MG, SK-MEL-24, and NCI-H2228 cell lines were identified as having high levels of FAP expression [FAP-positive (+)] whereas PC-3, NCI-H226, and HCT 116 cells expressed very low levels of FAP [FAP-negative(-)]. These expression profiles were further confirmed via flow cytometry with an APC-conjugated anti-FAP antibody (R&D Systems, Minneapolis, MN) and quantitative real-time PCR. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA).

U-87-MG cells were maintained in MEM medium (Corning Cellgro, Manassas, VA), containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO) and 1% penicillin-streptomycin (Corning Cellgro, Manassas, VA), supplemented with sodium bicarbonate (Corning), sodium pyruvate (Gibco, Gaithersburg, MD), and MEM non-essential amino acids (Gibco). SK-MEL-24 cells were maintained in MEM medium, containing 15% FBS and 1% penicillin-streptomycin, supplemented with sodium bicarbonate, sodium pyruvate, and MEM non-essential amino acids. PC-3 cells were grown in Ham's F-12K medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. NCI-H2228, NCI-H226, and HCT 116 cells were cultured in RPMI 1640 medium (Corning Cellgro) supplemented with 10% FBS and 1% penicillin-streptomycin. All cell cultures were maintained at 37 °C and 5% carbon dioxide (CO₂) in a humidified incubator.

1.6 Cellular Uptake Studies. All cellular uptake and specific binding studies were performed in triplicate to ensure reproducibility. Cells were detached using 0.05% trypsin (Corning), resuspended in 1 million cell aliquots in binding buffer, and incubated with various concentrations (range, 50 nM to 0.78 nM) of **XY-FAP-01** for 1 hour at 37 °C and 5% CO₂. To assess the specific uptake of **XY-FAP-02**, cells were preblocked with a FAP and DPP-IV specific inhibitor (Val-boroPro, MilliporeSigma, Burlington, MA) or a DPP-IV specific inhibitor (Sitagliptin, Santa Cruz Biotechnology, Inc., Dallas, TX) at various concentrations (range, 10⁻¹⁰ M to 10⁻⁴ M) prior to incubation with 25 nM **XY-FAP-02** solution in binding buffer for 1 hour at 37 °C and 5% CO₂. Cellular uptake was terminated by washing cells with ice cold PBS (1x) three times. Cells were resuspended in binding buffer and transferred to a 96-well plate for imaging. Images were acquired on the LI-COR Pearl Impulse Imager (Lincoln, NE) using an excitation wavelength of 785 nm and detection of the emission wavelength at 800 nm. Images were analyzed using the LI-COR Pearl Impulse Software (Version 2.0) and fluorescence intensity was corrected for background signal and normalized to well area.

Cellular Uptake of ¹¹¹In-**XY-FAP-02** was also assessed in cells. Cell aliquots (1 million) were incubated with 1 µCi ¹¹¹In-**XY-FAP-02** in saline for 30 minutes at 37 °C and 5% CO₂. Cells were washed three times with cold PBS (1x) and activity of the cell pellets was measured with the 1282 CompuGamma CS gamma well counter (Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The percent uptake of the administered activity was calculated by comparison with samples of a standard dose.

1.7 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF images were acquired on the LI-COR Pearl Impulse Imager using an excitation wavelength of 785 nm and a detection wavelength of 800 nm. Mice utilized for imaging studies were anesthetized with 3% isoflurane (v/v) and maintained at 1.5% isoflurane for the imaging procedure. NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 nmol of **XY-FAP-01** via tail vein injection and images were acquired at 30 min, 1 h, 2 h, 2.5 h, and 4 h after injection of tracer. Data were displayed and analyzed using the LI-COR Pearl Impulse Software (Version 2.0).

1.8 Small-Animal SPECT-CT Imaging. SPECT-CT studies were performed on NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts. For imaging studies, mice were anesthetized with 3% isoflurane prior to being placed on the scanner bed and kept warm with an external light source. Isoflurane levels were

decreased to 1.5% for the rest of the imaging procedure. After mice were injected with 300 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline, SPECT-CT imaging was carried out using a CT-equipped Gamma Medica-Ideas SPECT scanner (Northridge, CA) at the indicated timepoints (30 min, 2 h, 6 h, and 24 h) post radiotracer injection. A CT scan was performed at the end of each SPECT scan for anatomical co-registration. Obtained data sets were reconstructed using the provided Gamma Medica-Ideas software and final data visualization and image generation were prepared using Amira® software (FEI, Hillsboro, OR).

1.9 Ex-vivo Biodistribution. NOD/SKID mice bearing FAP+ U-87-MG and FAP- PC-3 tumor xenografts were injected with 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline via the tail vein. At 5 min, 30 min, 2 h, 6 h, and 12 hr post injection, mice (n=4) were sacrificed by CO₂ asphyxiation and blood was immediately collected by cardiac puncture. Additionally, the heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, small intestine, large intestine, bladder, muscle, femur, FAP+ U-87-MG xenograft, and FAP- PC-3 xenograft were collected for biodistribution analysis. Each tissue was weighed and radioactivity was measuring using a 2480 Wizard² automated gamma counter (PerkinElmer, Waltham, MA). Radioactivity measurements were corrected for decay and compared with samples of a standard dilution of the initial dose to calculated percent injected dose per gram (%ID/g).

For blocking studies, mice (n=5 per group) were co-injected with unlabeled **XY-FAP-02** (50 μg per mouse) and 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline. Mice (n=5) injected with 10 μCi **¹¹¹In-XY-FAP-02** in 200 μL saline served as a control. At 6 h post injection, mice were sacrificed, tissues were collected, and radioactivity was measured with the gamma well counter.

1.10 Data Analysis. Data are expressed at mean $\pm$ standard deviation (SD). Prism software (GraphPAD, San Diego, CA) was used for analysis and statistical significance was calculated using a two-tailed Student's t test. A P-value <0.05 was considered significant.

1.11 Xenograft Tumor Model. 6-week old female NOD/SCID mice were subcutaneously injected in the upper left and right flanks with 1 million U87(FAP+) cells and PC3 cells (FAP-) in RPMI 1640 media supplemented with 1% FBS. Mice were monitored for tumor size and used for optical or SPECT/CT imaging when the size of tumor reached around 100 mm³.

EXAMPLE 2

Representative Results

2.1 FAP Inhibitory Assay. **XY-FAP-01** demonstrated high binding affinity to human recombinant FAP. The enzyme inhibitory constant (K_i) for the compound was determined to be 1.26 nM.

2.2 Cellular Uptake Studies. FAP-positive cell lines showed concentration dependent uptake of **XY-FAP-01** whereas FAP-negative cell lines showed no significant binding of **XY-FAP-01** at all concentrations (see, e.g., FIG. 3A). Saturated binding of **XY-FAP-01** was observed at concentration of 25 nM, which was subsequently used as the base concentration for all binding inhibition studies. When preblocked with a FAP and DPP-IV specific inhibitor, **XY-FAP-01** binding was significantly inhibited in FAP-positive cells (FIG. 3B). Interestingly, this phenomenon was not observed in FAP-positive cell lines preblocked with a DPP-IV specific inhibitor. These results further justify the specificity of **XY-FAP-01** for FAP over DPPIV, since blocking of DPPIV did not result in a change of binding ability of **XY-FAP-01**.

Similar specificity was observed with the radioactive analog, **¹¹¹In-XY-FAP-02**. FAP positive cell line, U-87-MG, demonstrated over 30% uptake of administered radioactive dose after incubation whereas the FAP negative cell line, PC-3, had uptake of 0.01% of administered dose (FIG. 3C). Taken together, these results support the specificity of **XY-FAP-01** and **¹¹¹In-XY-FAP-02** in the engagement of FAP *in vitro*.

2.3 Ex-vivo Biodistribution. *Ex-vivo* biodistribution of **¹¹¹In-XY-FAP-02** results correlated with the observed imaging results (FIG. 4). Initially, the blood pool activity is very high, with over 10% %ID/g at 30 minutes post injection. With clearance of the compound, we see the blood pool activity drop significantly after 2 hours of distribution and remained less than 5% %ID/g from 2 hours post injection (FIG. 5A). High activity was also observed in pancreas, small intestines, and bladder until 2 hours post injection. Positive tumor uptake peaked at 30 minutes post injection and remained between 13-11% %ID/g up to 6 hours. Washout of tumor was observed at 12 hours post injection, with %ID/g dropping to below 5%. The PC-3, FAP negative xenograft had less than 3.5% %ID/g for all timepoints.

Co-injection of cold compound with **¹¹¹In-XY-FAP-02** resulted in significant blocking of tracer uptake in U-87 xenografts, with %ID/g dropping from 11.20%

without blocking versus 0.27% with blocking ($p < 0.0001$). Additionally, blocking with cold compound resulted in %ID/g of all tissues dropping significantly, with most values being less than 0.1%. This decrease in uptake is most likely due to the blocking of non-specific binding of tracer to non-target tissues and the blocking of specific binding of FAP in U-87 xenografts.

2.4 Small-Animal Near Infrared Fluorescence (NIRF) Imaging. NIRF imaging of **XY-FAP-01** demonstrated specific uptake of tracer in the U-87-MG xenograft as early as 30 minutes post injection (FIG. 6). After one hour of distribution, tracer clearance via the bladder was observed with retained tracer uptake in the FAP positive xenograft. Tracer uptake was retained in the positive xenograft after four hours of distribution. In contrast, no significant uptake of tracer was observed in the FAP negative tumor at all imaging time points.

REFERENCES

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references (e.g., websites, databases, etc.) mentioned in the specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

Allinen M, Beroukhi R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell*. 2004 Jul;6(1):17-32.

- Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon CJ, Koh GY, Kim J, Kim H.
Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. *Br J Haematol*. 2008 Sep;142(5):827-30.
- Chen ZY, Wang YX, Lin Y, Zhang JS, Yang F, Zhou QL, Liao YY. Advance of molecular imaging technology and targeted imaging agent in imaging and therapy. *Biomed Res Int*. 2014; 2014 : 819324. PMID: PMC3943245.
- Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740.
- Coenen HH, Elsinga PH, Iwata R, Kilbourn MR, Pillai MR, Rajan MG, Wagner HN Jr, Zaknun JJ. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nuclear medicine and biology*. 2010; 37:727–740. Biodistribution, tumor detection, and radiation dosimetry of 18F-DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2012; 53:1883–1891.
- Edosada CY, Quan C, Tran T, Pham V, Wiesmann C, Fairbrother W, Wolf BB.
Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly(2)-Pro(1)-cleaving specificity. *FEBS Lett*. 2006 Mar 6;580(6):1581-6.
- Fischer E, Chaitanya K, Wuest T, et al. Radioimmunotherapy of fibroblast activation protein positive tumors by rapidly internalizing antibodies. *Clin Cancer Res*. 2012;18:6208–6218.
- Franco OE, Shaw AK, Strand DW, Hayward SW. Cancer associated fibroblasts in cancer pathogenesis. *Semin Cell Dev Biol*. 2010 Feb;21(1):33-9.
- Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc Natl Acad Sci U S A*. 1990 Sep;87(18):7235-9. PMID: PMC54718.
- Jansen K, Heirbaut L, Cheng JD, Joossens J, Ryabtsova O, Cos P, Maes L, Lambeir AM, De Meester I, Augustyns K, Van der Veken P. Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Scaffold. *ACS Med Chem Lett*. 2013 Mar 18;4(5):491-6.
- Jansen K, Heirbaut L, Verkerk R, Cheng JD, Joossens J, Cos P, Maes L, Lambeir

- AM, De Meester I, Augustyns K, Van der Veken P. Extended structure-activity relationship and pharmacokinetic investigation of (4-quinolinoyl)glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP). *J Med Chem*. 2014 Apr 10;57(7):3053-74.
- Kelly T. Fibroblast activation protein- α and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. *Drug Resist Updat*. 2005 Feb-Apr;8(1-2):51-8.
- Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein- α . *Science*. 2010 Nov 5;330(6005):827-30.
- Laverman P, van der Geest T, Terry SY, Gerrits D, Walgreen B, Helsen MM, Nayak TK, Freimoser-Grundschober A, Waldhauer I, Hosse RJ, Moessner E, Umana P, Klein C, Oyen WJ, Koenders MI, Boerman OC. Immuno-PET and Immuno-SPECT of Rheumatoid Arthritis with Radiolabeled Anti-Fibroblast Activation Protein Antibody Correlates with Severity of Arthritis. *J Nucl Med*. 2015 May;56(5):778-83.
- Lo PC, Chen J, Stefflova K, Warren MS, Navab R, Bandarchi B, Mullins S, Tsao M, Cheng JD, Zheng G. Photodynamic molecular beacon triggered by fibroblast activation protein on cancer-associated fibroblasts for diagnosis and treatment of epithelial cancers. *J Med Chem*. 2009 Jan 22;52(2):358-68.
- Poplawski SE, Lai JH, Li Y, Jin Z, Liu Y, Wu W, Wu Y, Zhou Y, Sudmeier JL, Sanford DG, Bachovchin WW. Identification of selective and potent inhibitors of fibroblast activation protein and prolyl oligopeptidase. *J Med Chem*. 2013 May 9;56(9):3467-77.
- Reilly RM, Lam K, Chan C, Levine M. Advancing Novel Molecular Imaging Agents from Preclinical Studies to First-in-Humans Phase I Clinical Trials in Academia-A Roadmap for Overcoming Perceived Barriers. *Bioconjugate chemistry*. 2015; 26:625-632.
- Rettig WJ, Garin-Chesa P, Healey JH, Su SL, Ozer HL, Schwab M, Albino AP, Old LJ. Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin. *Cancer Res*. 1993 Jul 15;53(14):3327-35.
- Ryabtsova O, Jansen K, Van Goethem S, Joossens J, Cheng JD, Lambeir AM, De

- Meester I, Augustyns K, Van der Veken P. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. *Bioorg Med Chem Lett*. 2012 May 15;22(10):3412-7.
- Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, Rettig WJ. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A*. 1994 Jun 7;91(12):5657-61.
- Scott AM, Wiseman G, Welt S, Adjei A, Lee FT, Hopkins W, Divgi CR, Hanson LH, Mitchell P, Gansen DN, Larson SM, Ingle JN, Hoffman EW, Tanswell P, Ritter G, Cohen LS, Bette P, Arvay L, Amelsberg A, Vlock D, Rettig WJ, Old LJ. A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin Cancer Res*. 2003 May;9(5):1639-47.
- Tsai TY, Yeh TK, Chen X, Hsu T, Jao YC, Huang CH, Song JS, Huang YC, Chien CH, Chiu JH, Yen SC, Tang HK, Chao YS, Jiaang WT. Substituted 4-carboxymethylpyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. *J Med Chem*. 2010 Sep 23;53(18):6572-83.
- Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res*. 2002 Sep;8(9):2912-23.
- Welt S, Divgi CR, Scott AM, Garin-Chesa P, Finn RD, Graham M, Carswell EA, Cohen A, Larson SM, Old LJ, et al. Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. *J Clin Oncol*. 1994 Jun;12(6):1193-203.
- Youn H., Hong K. In vivo noninvasive small animal molecular imaging. *Osong Public Health Res Perspect*. 2012; 3 :48-59. PMID: PMC3738683.
- Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Church WB, McCaughan GW, Gorrell MD. The dipeptidyl peptidase IV family in cancer and cell biology. *FEBS J*. 2010 Mar;277(5):1126-44.
- U.S. Patent Application Publication No. US2014/0357650 for Novel FAP Inhibitors to Jansen et al., published Dec. 4, 2014.

U.S. Patent No. 9,346,814 for Novel FAP Inhibitors to Jansen et al., issued May 24, 2016.

International PCT Patent Publication No. WO 2013/107820 for Novel FAP Inhibitors to Jansen et al., published July 25, 2013.

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO.	TOT CLAIMS	IND CLAIMS
18/354,282	07/18/2023		664	JHU-36631.303	1	1

CONFIRMATION NO. 7536

FILING RECEIPT



0000000060226569

101943

The Johns Hopkins University
c/o Casimir Jones
2275 Deming Way
Suite 310
Middleton, WI 53562

Date Mailed: 08/08/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Xing Yang, Baltimore, MD;
Sridhar Nimmagadda, Baltimore, MD;
Steven Rowe, Parkville, MA;
Stephanie Slania, Baltimore, MD;
Martin G. Pomper, Baltimore, MD;

Applicant(s)

The Johns Hopkins University, Baltimore, MD;

Power of Attorney: The patent practitioners associated with Customer Number 101943

Domestic Priority data as claimed by applicant

This application is a CON of 16/758,182 04/22/2020
which is a 371 of PCT/US18/57086 10/23/2018
which claims benefit of 62/575,607 10/23/2017

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 08/04/2023

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/354,282**

Projected Publication Date: 11/16/2023

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

IMAGING AND RADIOTHERAPEUTICS AGENTS TARGETING FIBROBLAST-ACTIVATION
PROTEIN-ALPHA (FAP-ALPHA)

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative,

page 2 of 4

this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit <http://www.SelectUSA.gov> or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 18/354,282		
APPLICATION AS FILED - PART I								
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY		
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)	
BASIC FEE <small>(37 CFR 1.16(a), (b), or (c))</small>	N/A	N/A	N/A	64		N/A		
SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>	N/A	N/A	N/A	280		N/A		
EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A	320		N/A		
TOTAL CLAIMS <small>(37 CFR 1.16(i))</small>	1	minus 20 = *	0	x = 0	OR			
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	1	minus 3 = *		x = 0				
APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0				
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0				
				TOTAL		TOTAL		
* If the difference in column 1 is less than zero, enter "0" in column 2.				664				
APPLICATION AS AMENDED - PART II								
AMENDMENT A	(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	OR OTHER THAN SMALL ENTITY
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE(\$)	ADDITIONAL FEE(\$)
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x =	OR	x =
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x =	OR	x =
	Application Size Fee (37 CFR 1.16(s))							
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
					TOTAL ADD'L FEE		TOTAL ADD'L FEE	
AMENDMENT B	(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	OR OTHER THAN SMALL ENTITY
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE(\$)	ADDITIONAL FEE(\$)
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	x =	OR	x =
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	x =	OR	x =
	Application Size Fee (37 CFR 1.16(s))							
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
					TOTAL ADD'L FEE		TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.								

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	18/354,282
				Filing Date	18-Jul-2023
				First Named Inventor	Yang
				Art Unit	
				Examiner Name	
Sheet	1	of	3	Attorney Docket Number	JHU-36631.303

U.S. PATENTS					
Examiner Initials*	Cite No. ¹	Document Number Number-Kind Code ² (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

U.S. PUBLISHED PATENT APPLICATIONS					
Examiner Initials*	Cite No. ³	Document Number Number-Kind Code ⁴ (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1.	2021/0038749	2021-02-11	HABERKORN et al.	

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ⁵ Number ⁶ Kind Code ⁷ (if known)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation ⁸
	1.	EP 18199641.4	2018-10-10	UNIV HEIDELBERG		
	2.	JP2021-512949A	2021-05-20	UNIV HEIDELBERG		X
	3.	WO 2010/014933	2010-02-04	THE JOHNS HOPKINS UNIVERSITY		
	4.	WO 2010/108125	2010-09-23	THE JOHNS HOPKINS UNIVERSITY		
	5.	WO 2016/065142	2016-04-28	THE JOHNS HOPKINS UNIVERSITY		
	6.	WO 2019/154886	2019-08-15	UNIV HEIDELBERG		

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				<i>Application Number</i>	18/354,282
				<i>Filing Date</i>	18-Jul-2023
				<i>First Named Inventor</i>	Yang
				<i>Art Unit</i>	
				<i>Examiner Name</i>	
Sheet	2	of	3	<i>Attorney Docket Number</i>	JHU-36631.303

FOREIGN PATENT DOCUMENTS						

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation ⁶
	1.	Third Party Preissuance Submission filed in USSN 16/758, 182, filed February 23, 2022, 6 pages.	
	2.	Third Party Observations Japanese Patent Application no. 2020-523010. Dated 24 Oct 2022. 7 pages.	
	3.	Third Party Observations for application number EP20180871298. Dated 04/03/2022. 7 pages.	
	4.	BERNHARD et al., DOTAGA-anhydride: a valuable building block for the preparation of DOTA-like chelating agents. Chemistry. 2012 Jun 18;18(25):7834-41.	
	5.	JAMOUS et al., Synthesis of peptide radiopharmaceuticals for the therapy and diagnosis of tumor diseases. Molecules. 2013 Mar 14;18(3):3379-409.	

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				<i>Application Number</i>	18/354,282
				<i>Filing Date</i>	18-Jul-2023
				<i>First Named Inventor</i>	Yang
				<i>Art Unit</i>	
				<i>Examiner Name</i>	
Sheet	3	of	3	<i>Attorney Docket Number</i>	JHU-36631.303

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

- ☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).
- OR**
- ☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).
- ☐ See attached certification statement.
- ☐ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.
- ☒ A certification statement is not submitted herewith.
- ☐ Documents not provided herewith have been previously cited in parent U.S. patent application number _____ filed on _____. In compliance with 37 C.F.R. § 1.98(d), Applicants have not included copies of these documents.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jeffrey W. Childers/	Date (YYYY-MM-DD)	2023-08-08
Name/Print	Jeffrey W. Childers	Registration Number	58,126

PTO Notes regarding this form:

¹ Applicant's unique citation designation number (optional).

² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.

³ Applicant's unique citation designation number (optional).

⁴ See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04.

⁵ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3).

⁶ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.

⁷ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.

⁸ Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

**DOCUMENT MADE AVAILABLE UNDER THE
PATENT COOPERATION TREATY (PCT)**

International application number:	PCT/EP2019/052952
International filing date:	06 February 2019 (06.02.2019)
Document type:	Certified copy of priority document
Document details:	Country/Office: EP
	Number: 18199641.4
	Filing date: 10 October 2018 (10.10.2018)
Date of receipt at the International Bureau:	21 February 2019 (21.02.2019)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

Bescheinigung

Die angehefteten
Unterlagen stimmen mit der
als ursprünglich eingereicht
geltenden Fassung der auf
dem nächsten Blatt
bezeichneten europäischen
Patentanmeldung überein.

Certificate

The attached documents are
exact copies of the text in
which the European patent
application described on the
following page is deemed to
have been filed.

Attestation

Les documents joints à la
présente attestation sont
conformes au texte,
considéré comme
initialement déposé, de la
demande de brevet
européen qui est spécifiée à
la page suivante.

Patentanmeldung Nr.

Patent application No.

Demande de brevet n°

18199641.4 / EP18199641

The organisation code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP18199641.

Der Präsident des Europäischen Patentamts;
Im Auftrag
For the President of the European Patent Office
Le Président de l'Office européen des brevets
p.o.



V. Joseph

Anmeldung Nr:
Application no.: **18199641.4**
Demande no :

Anmeldetag:
Date of filing: **10.10.18**
Date de dépôt :

Anmelder / Applicant(s) / Demandeur(s):

Universität Heidelberg
Grabengasse 1
69117 Heidelberg/DE

Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:

(Falls die Bezeichnung der Erfindung nicht angegeben ist, oder falls die Anmeldung in einer Nicht-Amtssprache des EPA eingereicht wurde, siehe Beschreibung bezüglich ursprünglicher Bezeichnung.

If no title is shown, or if the application has been filed in a non-EPO language, please refer to the description for the original title.

Si aucun titre n'est indiqué, ou si la demande a été déposée dans une langue autre qu'une langue officielle de l'OEB, se référer à la description pour le titre original.)

FAP Inhibitor

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)

Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignés lors du dépôt:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL
PT RO RS SE SI SK SM TR

FAP Inhibitor

The present invention relates to a compound, a pharmaceutical composition comprising or consisting of said compound, a kit comprising or consisting of said compound or pharmaceutical composition and use of the compound or pharmaceutical composition in the
5 diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP).

Background of the Invention

Tumor growth and spread are not only determined by the cancer cells, but also by the non-malignant constituents of the malignant lesion, which are subsumed under the term stroma.
10 The stroma may represent over 90% of the tumor mass in tumors with desmoplastic reaction such as breast, colon and pancreatic carcinoma. Especially a subpopulation of fibroblasts called cancer-associated fibroblasts (CAFs) is known to be involved in tumor growth, migration and progression. Therefore, these cells represent an attractive target for diagnosis and anti-tumor therapy.

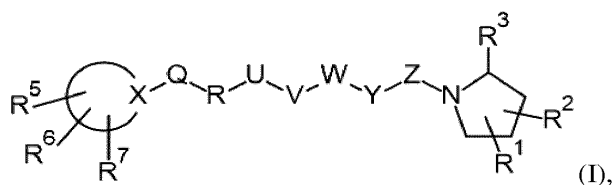
15 A distinguishing feature of CAFs is the expression of seprase or fibroblast activation protein α (FAP- α), a type II membrane bound glycoprotein belonging to the dipeptidyl peptidase 4 (DPP4) family. FAP- α has both dipeptidyl peptidase and endopeptidase activity. The endopeptidase activity distinguishes FAP- α from the other members of the DPP4 family. Identified substrates for the endopeptidase activity so far are denatured Type I collagen, α 1-
20 antitrypsin and several neuropeptides. FAP- α has a role in normal developmental processes during embryogenesis and in tissue modelling. It is not or only at insignificant levels expressed on adult normal tissues. However, high expression occurs in wound healing, arthritis, atherosclerotic plaques, fibrosis and in more than 90% of epithelial carcinomas.

The appearance of FAP- α in CAFs in many epithelial tumors and the fact that
25 overexpression is associated with a worse prognosis in cancer patients led to the hypothesis that FAP- α activity is involved in cancer development as well as in cancer cell migration and spread. Therefore, the targeting of this enzyme for imaging and endoradiotherapy can be considered as a promising strategy for the detection and treatment of malignant tumors. The present inventors developed a small molecule based on a FAP- α specific inhibitor and were able to show specific
30 uptake, rapid internalization and successful imaging of tumors in animal models as well as in tumor patients. A comparison with the commonly used radiotracer ^{18}F -fluorodeoxyglucose (^{18}F -FDG) revealed a clear superiority of the new FAP- α ligand in patients with locally advanced lung adenocarcinoma. Thus, the present invention provides inter alia: (i) detection of

smaller primary tumors and, thus the possibility of earlier diagnosis, (ii) the detection of smaller metastasis and, thus a better assessment of tumor stage, (iii) precise intra-operative guidance facilitating complete surgical removal of tumor tissue, (iv) better differentiation between inflammation and tumor tissue, (v) more precise staging of patients with tumors, (vi) better follow up of tumor lesions after antitumor therapy, (vii) the opportunity to use the molecules as theranostic agents for diagnosis and therapy. Furthermore, the molecules can be used for the diagnosis and treatment of non-malignant diseases such as chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorders.

Summary of the Invention

In a first aspect, the present invention provides a compound of Formula (I)



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other;

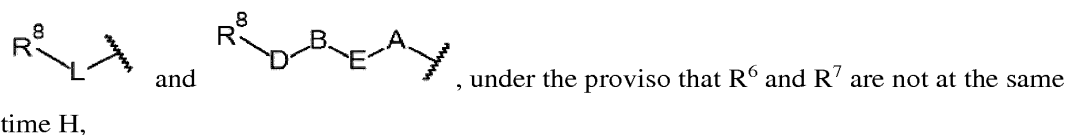
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl-, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,



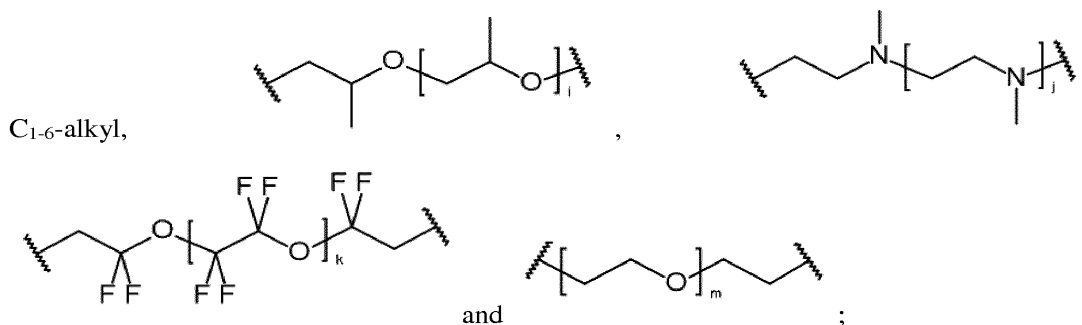
wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

- 5 A is selected from the group consisting of NR^4 , O, S, and CH_2 ;

E is selected from the group consisting of



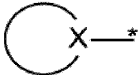
wherein i is 1, 2, or 3;

- 10 wherein j is 1, 2, or 3;

wherein k is 1, 2, or 3;

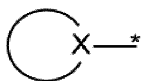
wherein m is 1, 2, or 3;

- A and E together form a group selected from a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, wherein A and E can be mono-, bi- and multicyclic, preferably monocyclic. Each A and E being optionally substituted by 1 to 4 residues from the group consisting of -H, - $\text{C}_{1-6}\text{-alkyl}$, - $\text{O}-\text{C}_{1-6}\text{-alkyl}$, - $\text{S}-\text{C}_{1-6}\text{-alkyl}$, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and - $\text{C}_{1-6}\text{-aralkyl}$, each of said - $\text{C}_{1-6}\text{-alkyl}$ being optionally substituted with from 1 to 3 substituents
- 15

selected from -OH, oxo, halo; and optionally connected to A, B, D, E or .

- B is selected from the group consisting of S, NR^4 , $\text{NR}^4\text{-O}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$ and the N-containing heterocycle is substituted with 1 to 3 substituents selected from the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$; and;
- 20

- 25 R^8 is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;



is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

5 or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof.

In a second aspect, the present invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect, and, optionally, a pharmaceutically acceptable carrier and/or excipient.

10 In a third aspect, the present invention relates to the compound of the first aspect or the pharmaceutical composition of the second aspect for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject.

In a fourth aspect, the present invention relates to a kit comprising or consisting of the compound of the first aspect or the pharmaceutical composition of the second aspect and
15 instructions for the diagnosis or treatment of a disease.

List of Figures

In the following, the content of the figures comprised in this specification is described. In this context please also refer to the detailed description of the invention above and/or below.

20 **Figure 1: In vitro characterization of ¹²⁵I-FAPI-01 and ¹⁷⁷Lu-FAPI-02.**

A. Binding of radiolabeled FAPI-01 and FAPI-02 to different human cancer cell lines as well as cell lines transfected with human FAP- α (HT-1080-FAP), murine FAP- α (HEK-muFAP) and human CD26 (HEK-CD26) after 60 min of incubation. **B.** Internalization of radiolabeled FAPI-01 and FAPI-02 into HT-1080-FAP cells after incubation for 10 min to 24 h. The internalized proportion is shown in grey and black, respectively; the extracellularly bound fraction is indicated by the white bars. **C.** Competitive binding of radiolabeled FAPI-01 and FAPI-02 to HT-1080-FAP cells after adding increasing concentrations of unlabeled FAPI-01 and Lu-FAPI-02. **D.** Internalization of FAPI-02 into FAP- α positive and negative cell lines. Blue: DAPI; green: FAPI-02-Atto488. **E+F.** Efflux kinetics of FAPI-01 and FAPI-02 after 1 h
25 incubation of HT-1080-FAP cells with radiolabeled compounds followed by incubation with compound-free medium for 1 to 24 h. All values are given as percentage of total applied dose normalized to 1 million cells (%ID/1 mio cells).
30

Figure 2: Binding specificity and relative internalization rates of FAPI derivatives.

A-C. Binding and internalization rates of FAPI-03 to FAPI-15 in relation to FAPI-02 (defined as 100%). Internalization rates after 1, 4 and 24 hrs of incubation are depicted in grey; the extracellular bound fraction is represented by the white bars. **D.** Binding of selected FAPI derivatives to HEK cells expressing murine FAP- α and human CD26 after 60 min of incubation. *Right side:* Ratio of muFAP to CD26 binding. **E.** Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound.

Figure 3: Imaging of FAPI-02 and -04 in mice bearing human FAP-positive (HT-1080-FAP) and negative (Capan-2, SK-LMS-1) tumor xenografts.

A+C, E+G. Small animal PET imaging was performed after intravenous administration of 4 nmol ^{68}Ga -FAPI-02 and -04 (10 MBq resp.) at indicated times. The radiotracer gets rapidly enriched within the tumor (indicated by the red arrow) while not accumulating in non-cancerous tissue. Furthermore, a rapid elimination via the kidneys and bladder is seen. **B+D, F+H.** Quantification of the PET images demonstrates a solid clearance of ^{68}Ga -FAPI-02 and -04 from the cardiovascular system and a constant uptake into the tumor.

Figure 4: Blocking experiments for analysis of binding specificity in vivo

A+D. Blocking of ^{68}Ga -FAPI-02 and -04 tumor accumulation by co-administration of 30 nmol unlabeled compound in HT-1080-FAP tumor bearing mice. **B+C, E+F.** Time-activity curves of ^{68}Ga -FAPI-02 and -04 in selected organs after intravenous administration with and without unlabeled compound as a competitor.

Figure 5: Organ distribution of ^{177}Lu -FAPI-02 and -04 in HT-1080-FAP tumor bearing nude mice

A-C. Biodistribution of ^{177}Lu -FAPI-02 and -04 was measured ex vivo at indicated times after intravenous administration of 1 MBq to mice bearing human FAP-positive HT-1080 tumor xenografts; n=3 for each time point. The values stated are expressed as percentage of injected dose per gram of tissue (% ID/g). The radiotracers are shown to accumulate within the FAP-expressing tumor, showing the highest enrichment after 1 h for FAPI-02 (4.5 %ID/g) and 2 h for FAPI-04 (5.4 %ID/g). **D-F.** Tumor-to-normal tissue ratios of ^{177}Lu -FAPI-02 and -04 1, 4 and 24 hrs after intravenous administration.

Figures 6-9: PET/CT imaging of FAPI-02 in cancer patients

6A-C. Maximum intensity projections (MIP) of PET/CT scans in a patient suffering from metastasized breast cancer. **D.** Maximum tissue uptake of ^{68}Ga -FAPI-02 10 min, 1 h and 3 h after intravenous administration to a patient with metastasized breast cancer.

7. MIP of PET/CT scans in patients with pancreatic cancer, non-small cell lung cancer (NSCLC) and esophageal and rectum carcinoma 1 h after administration of ^{68}Ga -FAPI-02.

8. MIP of PET/CT scans in patients with nasopharynx and larynx carcinoma 1 h after administration of ^{68}Ga -FAPI-02.

- 5 **9A+B.** Whole-body PET/CT imaging (MIP) 1 h after administration of ^{18}F -FDG and ^{68}Ga -FAPI-02 to a patient with locally advanced lung adenocarcinoma. **C+D.** Transaxial view of lung adenocarcinoma patient 1 h after administration of ^{18}F -FDG and ^{68}Ga -FAPI-02. FAPI-02 is selectively accumulated in FAP- α expressing tissue and shows significantly higher uptake in the malignant lesions compared to ^{18}F -FDG.

10 **Figures 10-16: PET/CT imaging of FAPI-04 in cancer patients**

10 Maximum intensity projections (MIP) of PET/CT scans in a patient suffering from metastasized breast cancer 10 min, 1 and 3 hrs after administration of ^{68}Ga -FAPI-04.

- 11** MIP of PET/CT scans in patients with sigma carcinoma, hypopharynx carcinoma, neuroendocrine tumors, cholangio, ovarian and small intestine carcinoma 1 h after administration of ^{68}Ga -FAPI-04.

12 MIP of PET/CT scans in a patient with lung cancer 1 h after administration of ^{68}Ga -FAPI-04.

13 MIP of PET/CT scans in a patient with oncogenic rachitis 1 h after administration of ^{68}Ga -FAPI-04.

- 20 **14** Comparative imaging of one patient with metastasized prostate cancer. MIP of PET/CT scans 1 h after application of radiolabeled DOTATOC, PSMA and FAPI-04.

15 Maximum intensity projection (MIP) and time-activity curves of a dynamic ^{68}Ga -FAPI-04 PET/CT scan in a pancreatic cancer patient.

- 16** Relative binding rates of Lu-177 labeled FAPI derivatives compared to FAPI-04 (set to 100%) after incubation for 1, 4 and 24 h on FAP-expressing HT-1080 cells; n=3.

Figure 17: Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3).

- Figure 18:** Binding of FAPI derivatives to HEK cells expressing murine FAP and human CD26 after 60 min of incubation, n=3. Values are expressed as percentage of applied dose (%ID) per 1 mio cells.

Figure 19: Biodistribution of selected FAPI derivatives in HT-1080-FAP xenotransplants 1, 4 and 24 h after intravenous administration of the radiotracers, n=3. Values are expressed as percentage of injected dose per gram of tissue (%ID/g).

Figure 20: Tumor-to-blood ratio of selected FAPI derivatives in HT-1080-FAP xenotransplants 1, 4 and 24 h after intravenous administration of the radiotracers, n=3.

Figure 21: PET imaging of Ga-68 labeled FAPI-21 and FAPI-46 in HT-1080-FAP tumor bearing mice; n=1.

5 **Figure 22:** Maximum standardized uptake values (SUV) of selected FAPI derivatives in HT-1080-FAP tumor bearing mice; n=1.

Figure 23: Maximum (SUV max, Fig. 23 A) and mean (SUV mean, Fig. 23 B) standardized uptake values of Ga-68 labeled FAPI-02 and FAPI-04 in cancer patients; n=25.

10 **Figure 24:** Intra-individual comparison of 6 patients with 6 different tumor entities undergoing FDG-PET and FAPI-PET imaging within <9 days.

Figure 25: PET/CT imaging of Ga-68 labeled FAPI-04 in patients with peritonitis carcinomatosa (A), myocarditis (B) and hip joint arthrosis (C) 1 h p.i.

Figure 26: PET/CT imaging of Ga-68 labeled FAPI-21 in cancer patients 1 h p.i.

15 **Figure 27:** PET/CT imaging of Ga-68 labeled FAPI-46 1 h p.i. and intratherapeutical imaging of Sm-153 labeled FAPI-46 30 min p.i. in cancer patients.

Figure 28: Intratherapeutical imaging of Sm-153 labeled FAPI-46 up to 20 h p.i.

Figure 29: A. Maximum intensity projection (MIP) 1 h after intravenous administration of ^{68}Ga -FAPI-46 to a patient with metastasized colorectal carcinoma. B. Imaging of Bremsstrahlung 2 h after therapeutic treatment with ^{90}Y -FAPI-46 of the same patient.

20 **Figure 30:** PET/CT imaging of Ga-68 labeled FAPI-46 1 h p.i. in lung cancer patients with idiopathic lung fibrosis. A, B. Maximum tracer uptake into tumor tissue is significantly higher than into non-exacerbated fibrotic lesions. C. Maximum tracer uptake into tumor tissue is slightly lower than into exacerbated fibrotic tissue.

25 **Figure 31:** A. Binding of Tc-99m labeled FAPI-19 to HT-1080-FAP cells, n=3. B. Competitive binding of Tc-99m labeled FAPI-19 to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3). C. Scintigraphy of Tc-99m labeled FAPI-19 in HT-1080-FAP xenotransplants, n=1.

Figure 32: A. Binding of Tc-99m labeled FAPI-34 to HT-1080-FAP cells, n=3. B. Scintigraphy of Tc-99m labeled FAPI-34 in HT-1080-FAP xenotransplants, n=1.

30 **Figure 33:** Scintigraphy of Tc-99m labeled FAPI-34 in one patient with metastasized pancreas carcinoma.

Figure 34: A. Binding of Pb-203 labeled FAPI derivatives to HT-1080-FAP cells, n=3. B. Efflux kinetics of Pb-203 labeled FAPI derivatives after incubation of HT-1080-FAP cells with radiolabeled compound for 60 min and consequent incubation with nonradioactive

medium for 1 to 24 hours, n=3. C. Competitive binding of Pb-203 labeled FAPIs to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3).

Figure 35: Scintigraphy of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenotransplants, n=1.

Figure 36: Biodistribution of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenotransplants 1, 4, 6 and 24 h after intravenous administration of the radiotracers, n=3. Values are expressed as percentage of injected dose per gram of tissue (%ID/g).

Figure 37: A. Binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells, n=3. B. Competitive binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3). C. Efflux kinetics of Cu-64 labeled FAPI-42 and FAPI-52 after incubation of HT-1080-FAP cells with radiolabeled compound for 60 min and consequent incubation with nonradioactive medium for 1 to 24 hours, n=3.

Figure 38: PET imaging of Cu-64 labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor bearing mice; n=1.

Figure 39: PET imaging of AlF-18 labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor bearing mice; n=1.

Figure 40: a. Small animal PET imaging of ^{68}Ga -labeled FAPI-02 in U87MG tumor bearing nude mice up to 140 min after intravenous administration of the radiotracer. The tumor is indicated by the red arrow. b. Biodistribution of ^{177}Lu -labeled FAPI-02 and FAPI-04 in U87MG tumor bearing nude mice 1, 4 and 24 h after intravenous administration of the radiotracers; n=3.

Figure 41: Tumor-to-organ ratios of ^{177}Lu -labeled FAPI-02 and -04 in U87MG tumor bearing mice 1, 4 and 24 h after intravenous administration.

Figure 42: Maximum intensity projection (MIP) of PET/CT scans in a glioblastoma patient 10 min, 1 and 3 h after administration of ^{68}Ga -FAPI-02.

Figure 43: Exemplary images (contrast enhanced T1 weighted MRI, FAPI-PET and fused images of both modalities) of IDH wt glioblastomas, IDH-mutant gliomas WHO grade II and IDH-mutant glioblastomas.

Figure 44: Absolute SUVmax values of all 18 gliomas.

Figure 45: Statistical Analysis of SUVmax/BG values. Boxplots of SUVmax/BG values and corresponding ROC curves in GBM versus non-GBM (a, b), IDH-mutant versus IDH wildtype gliomas (c, d) and gliomas grade II versus gliomas grade III/IV (e, f).

Detailed Descriptions of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose
5 of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary
10 of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Klbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout this specification and the claims, which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but
15 not the exclusion of any other integer or step or group of integers or steps. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being optional, preferred or advantageous may be combined with any other feature or features indicated as being optional, preferred or advantageous.

Several documents are cited throughout the text of this specification. Each of the
20 documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Some of the documents
25 cited herein are characterized as being "*incorporated by reference*". In the event of a conflict between the definitions or teachings of such incorporated references and definitions or teachings recited in the present specification, the text of the present specification takes precedence.

In the following, the elements of the present invention will be described. These elements
30 are listed with specific embodiments; however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments

with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

5 Definitions

In the following, some definitions of terms frequently used in this specification are provided. These terms will, in each instance of its use, in the remainder of the specification have the respectively defined meaning and preferred meanings.

As used in this specification and the appended claims, the singular forms "a", "an", and
10 "the" include plural referents, unless the content clearly dictates otherwise.

In the following definitions of the terms: alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkenyl and alkynyl are provided. These terms will in each instance of its use in the remainder of the specification have the respectively defined meaning and preferred meanings.

15 The term "alkyl" refers to a saturated straight or branched carbon chain. Preferably, the chain comprises from 1 to 10 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 e.g. methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *tert*-butyl, pentyl, hexyl, heptyl, or octyl. Alkyl groups are optionally substituted.

The term "heteroalkyl" refers to a saturated straight or branched carbon chain.
20 Preferably, the chain comprises from 1 to 9 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 e.g. methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, pentyl, hexyl, heptyl, octyl, which is interrupted one or more times, e.g. 1, 2, 3, 4, 5, with the same or different heteroatoms. Preferably the heteroatoms are selected from O, S, and N, e.g. -O-CH₃, -S-CH₃, -CH₂-O-CH₃, -CH₂-O-C₂H₅, -CH₂-S-CH₃, -CH₂-S-C₂H₅, -C₂H₄-O-CH₃, -C₂H₄-O-C₂H₅, -C₂H₄-S-CH₃, -
25 C₂H₄-S-C₂H₅ etc. Heteroalkyl groups are optionally substituted.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively, with preferably 3, 4, 5, 6, 7, 8, 9 or 10 atoms forming a ring, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl etc. The terms "cycloalkyl" and
30 "heterocycloalkyl" are also meant to include bicyclic, tricyclic and polycyclic versions thereof. The term "heterocycloalkyl" preferably refers to a saturated ring having five of which at least one member is a N, O or S atom and which optionally contains one additional O or one additional N; a saturated ring having six members of which at least one member is a N, O or S atom and which optionally contains one additional O or one additional N or two additional N

atoms; or a saturated bicyclic ring having nine or ten members of which at least one member is a N, O or S atom and which optionally contains one, two or three additional N atoms. “Cycloalkyl” and “heterocycloalkyl” groups are optionally substituted. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, spiro[3,3]heptyl, spiro[3,4]octyl, spiro[4,3]octyl, spiro[3,5]nonyl, spiro[5,3]nonyl, spiro[3,6]decyl, spiro[6,3]decyl, spiro[4,5]decyl, spiro[5,4]decyl, bicyclo[2.2.1]heptyl, bicyclo[2.2.2]octyl, adamantyl, and the like. Examples of heterocycloalkyl include 1-(1,2,5,6-tetrahydropyridyl), 1-piperidiny, 2-piperidiny, 3-piperidiny, 4-morpholinyl, 3-morpholinyl, 1,8 diazo-spiro-[4,5] decyl, 1,7 diazo-spiro-[4,5] decyl, 1,6 diazo-spiro-[4,5] decyl, 2,8 diazo-spiro[4,5] decyl, 2,7 diazo-spiro[4,5] decyl, 2,6 diazo-spiro[4,5] decyl, 1,8 diazo-spiro-[5,4] decyl, 1,7 diazo-spiro-[5,4] decyl, 2,8 diazo-spiro-[5,4] decyl, 2,7 diazo-spiro[5,4] decyl, 3,8 diazo-spiro[5,4] decyl, 3,7 diazo-spiro[5,4] decyl, 1-azo-7,11-dioxo-spiro[5,5] undecyl, 1,4-diazabicyclo[2.2.2]oct-2-yl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

The term “aryl” preferably refers to an aromatic monocyclic ring containing 6 carbon atoms, an aromatic bicyclic ring system containing 10 carbon atoms or an aromatic tricyclic ring system containing 14 carbon atoms. Examples are phenyl, naphthyl or anthracenyl. The aryl group is optionally substituted.

The term “aralkyl” refers to an alkyl moiety, which is substituted by aryl, wherein alkyl and aryl have the meaning as outlined above. An example is the benzyl radical. Preferably, in this context the alkyl chain comprises from 1 to 8 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, or 8, e.g. methyl, ethyl methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butenyl, *tert*-butyl, pentyl, hexyl, pentyl, octyl. The aralkyl group is optionally substituted at the alkyl and/or aryl part of the group.

The term “heteroaryl” preferably refers to a five or six-membered aromatic monocyclic ring wherein at least one of the carbon atoms are replaced by 1, 2, 3, or 4 (for the five membered ring) or 1, 2, 3, 4, or 5 (for the six membered ring) of the same or different heteroatoms, preferably selected from O, N and S; an aromatic bicyclic ring system wherein 1, 2, 3, 4, 5, or 6 carbon atoms of the 8, 9, 10, 11 or 12 carbon atoms have been replaced with the same or different heteroatoms, preferably selected from O, N and S; or an aromatic tricyclic ring system wherein 1, 2, 3, 4, 5, or 6 carbon atoms of the 13, 14, 15, or 16 carbon atoms have been replaced with the same or different heteroatoms, preferably selected from O, N and S. Examples are

oxazolyl, isoxazolyl, 1,2,5-oxadiazolyl, 1,2,3-oxadiazolyl, pyrrolyl, imidazolyl, pyrazolyl, 1,2,3-triazolyl, thiazolyl, isothiazolyl, 1,2,3-thiadiazolyl, 1,2,5-thiadiazolyl, pyridinyl, pyrimidinyl, pyrazinyl, 1,2,3-triazinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, 1-benzofuranyl, 2-benzofuranyl, indoyl, isoindoyl, benzothiophenyl, 2-benzothiophenyl, 1H-indazolyl, benzimidazolyl, benzoxazolyl, indoxazinyl, 2,1-benzosoxazolyl, benzothiazolyl, 1,2-benzisothiazolyl, 2,1-benzisothiazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinoxalinyl, quinazolinyl, quinolinyl, 1,2,3-benzotriazinyl, or 1,2,4-benzotriazinyl.

The term “heteroaralkyl” refers to an alkyl moiety, which is substituted by heteroaryl, wherein alkyl and heteroaryl have the meaning as outlined above. An example is the 2-alkylpyridinyl, 3-alkylpyridinyl, or 2-methylpyridinyl. Preferably, in this context the alkyl chain comprises from 1 to 8 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, or 8, e.g. methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butenyl, *tert*-butyl, pentyl, hexyl, heptyl, octyl. The heteroaralkyl group is optionally substituted at the alkyl and/or heteroaryl part of the group.

The terms “alkenyl” and “cycloalkenyl” refer to olefinic unsaturated carbon atoms containing chains or rings with one or more double bonds. Examples are propenyl and cyclohexenyl. Preferably, the alkenyl chain comprises from 2 to 8 carbon atoms, i.e. 2, 3, 4, 5, 6, 7, or 8, e.g. ethenyl, 1-propenyl, 2-propenyl, *iso*-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, *iso*-butenyl, *sec*-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, hexenyl, pentenyl, octenyl. Preferably the cycloalkenyl ring comprises from 3 to 8 carbon atoms, i.e. 3, 4, 5, 6, 7, or 8, e.g. 1-cyclopropenyl, 2-cyclopropenyl, 1-cyclobutenyl, 2-cyclobutenyl, 1-cyclopentenyl, 2-cyclopentenyl, 3-cyclopentenyl, cyclohexenyl, cyclopentenyl, cyclooctenyl.

The term “alkynyl” refers to unsaturated carbon atoms containing chains or rings with one or more triple bonds. An example is the propargyl radical. Preferably, the alkynyl chain comprises from 2 to 8 carbon atoms, i.e. 2, 3, 4, 5, 6, 7, or 8, e.g. ethynyl, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 3-butyne, 1-pentyne, 2-pentyne, 3-pentyne, 4-pentyne, hexynyl, pentynyl, octynyl.

In one embodiment, carbon atoms or hydrogen atoms in alkyl, heteroalkyl, cycloalkyl, aryl, aralkyl, alkenyl, cycloalkenyl, alkynyl radicals may be substituted independently from each other with one or more elements selected from the group consisting of O, S, N or with groups containing one or more elements selected from the group consisting of O, S, N.

Embodiments include alkoxy, cycloalkoxy, aryloxy, aralkoxy, alkenyloxy, cycloalkenyloxy, alkynyloxy, alkylthio, cycloalkylthio, arylthio, aralkylthio, alkenylthio, cycloalkenylthio, alkynylthio, alkylamino, cycloalkylamino, arylamino, aralkylamino, alkenylamino, cycloalkenylamino, alkynylamino radicals.

Other embodiments include hydroxyalkyl, hydroxycycloalkyl, hydroxyaryl, hydroxyaralkyl, hydroxyalkenyl, hydroxycycloalkenyl, hydroxyalanyl, mercaptoalkyl, mercaptocycloalkyl, mercaptoaryl, mercaptoaralkyl, mercaptoalkenyl, mercaptocycloalkenyl, mercaptoalkynyl, aminoalkyl, aminocycloalkyl, aminoaryl, aminoaralkyl, aminoalkenyl, aminocycloalkenyl, aminoalkynyl radicals.

In another embodiment, hydrogen atoms in alkyl, heteroalkyl, cycloalkyl, aryl, aralkyl, alkenyl, cycloalkenyl, alkynyl radicals may be substituted independently from each other with one or more halogen atoms. One radical is the trifluoromethyl radical.

If two or more radicals or two or more residues can be selected independently from each other, then the term “independently” means that the radicals or the residues may be the same or may be different.

As used herein a wording defining the limits of a range of length such as, e. g., “from 1 to 6” means any integer from 1 to 6, i. e. 1, 2, 3, 4, 5 and 6. In other words, any range defined by two integers explicitly mentioned is meant to comprise and disclose any integer defining said limits and any integer comprised in said range.

The term “halo” as used herein refers to a halogen residue selected from the group consisting of F, Br, I and Cl. Preferably, the halogen is F.

The term “linker” as used herein refers to any chemically suitable linker. Preferably, linker are not or only slowly cleaved under physiological conditions. Thus, it is preferred that the linker does not comprise recognition sequences for proteases or recognition structures for other degrading enzymes. Since it is preferred that the compounds of the invention are administered systemically to allow broad access to all compartments of the body and subsequently enrichment of the compounds of the invention wherever in the body the tumor is located, it is preferred that the linker is chosen in such that it is not or only slowly cleaved in blood. The cleavage is considered slowly, if less than 50% of the linkers are cleaved 2 h after administration of the compound to a human patient. Suitable linkers, for example, comprises or consists of optionally substituted alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, sulfonyl, amines, ethers, thioethers phosphines, phosphoramidates, carboxamides, esters, imidoesters, amidines, thioesters, sulfonamides, 3-thiopyrrolidine-2,5-dione, carbamates, ureas, guanidines, thioureas, disulfides, oximes, hydrazines, hydrazides, hydrazones, diaza bonds, triazoles, triazolines, tetrazines, platinum complexes and amino acids, or combinations thereof. Preferably, the linker comprises or consists of 1,4-piperazine, 1,3-propane and a phenolic ether or combinations thereof.

The expression "optionally substituted" refers to a group in which one, two, three or more hydrogen atoms may have been replaced independently of each other by the respective substituents.

As used herein, the term "amino acid" refers to any organic acid containing one or more amino substituents, e.g. α -, β - or γ -amino, derivatives of aliphatic carboxylic acids. In the polypeptide notation used herein, e.g. Xaa5, i.e. Xaa1Xaa2Xaa3Xaa4Xaa5, wherein Xaa1 to Xaa5 are each and independently selected from amino acids as defined, the left hand direction is the amino terminal direction and the right hand direction is the carboxy terminal direction, in accordance with standard usage and convention.

The term "conventional amino acid" refers to the twenty naturally occurring amino acids, and encompasses all stereomeric isoforms, i.e. D,L-, D- and L-amino acids thereof. These conventional amino acids can herein also be referred to by their conventional three- letter or one-letter abbreviations and their abbreviations follow conventional usage (see, for example, Immunology—A Synthesis, 2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)).

The term "non-conventional amino acid" refers to unnatural amino acids or chemical amino acid analogues, e.g. α,α -disubstituted amino acids, N-alkyl amino acids, homo-amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), and ortho-, meta- or para-aminobenzoic acid. Non-conventional amino acids also include compounds which have an amine and carboxyl functional group separated in a 1,3 or larger substitution pattern, such as β -alanine, γ -amino butyric acid, Freidinger lactam, the bicyclic dipeptide (BTD), amino-methyl benzoic acid and others well known in the art. Statine-like isosteres, hydroxyethylene isosteres, reduced amide bond isosteres, thioamide isosteres, urea isosteres, carbamate isosteres, thioether isosteres, vinyl isosteres and other amide bond isosteres known to the art may also be used. The use of analogues or non-conventional amino acids may improve the stability and biological half-life of the added peptide since they are more resistant to breakdown under physiological conditions. The person skilled in the art will be aware of similar types of substitution which may be made. A non-limiting list of non-conventional amino acids which may be used as suitable building blocks for a peptide and their standard abbreviations (in brackets) is as follows: α -aminobutyric acid (Abu), L-N-methylalanine (Nmala), α -amino- α -methylbutyrate (Mgab), L-N-methylarginine (Nmarg), aminocyclopropane (Cpro), L-N-methylasparagine (Nmasn), carboxylate L-N-methylaspartic acid (Nmasp), aninoisobutyric acid (Aib), L-N-methylcysteine (Nmcys), aminonorbornyl (Norb), L-N-methylglutamine (Nmgl), carboxylate L-N-methylglutamic acid (Nmglu),

cyclohexylalanine (Chexa), L-N-methylhistidine (Nmhis), cyclopentylalanine (Cpen), L-N-methylisoleucine (Nmile), L-N-methylleucine (Nmleu), L-N-methyllysine (Nmlys), L-N-methylmethionine (Nmmt), L-N-methylnorleucine (Nmnl), L-N-methylnorvaline (Nmna), L-N-methylornithine (Nmorn), L-N-methylphenylalanine (Nmphe), L-N-methylproline (Nmpro), L-N-methylserine (Nmser), L-N-methylthreonine (Nmthr), L-N-methyltryptophan (Nmtrp), D-ornithine (Dorn), L-N-methyltyrosine (Nmtyr), L-N-methylvaline (Nmval), L-N-methylethylglycine (Nmetg), L-N-methyl-t-butylglycine (Nmtbug), L-norleucine (Nle), L-norvaline (Nva), α -methyl-aminoisobutyrate (Maib), α -methyl- γ -aminobutyrate (Mgab), D- α -methylalanine (Dmala), α -methylcyclohexylalanine (Mchexa), D- α -methylarginine (Dmarg), α -methylcyclopentylalanine (Mcp), D- α -methylasparagine (Dmasn), α -methyl- α -naphthylalanine (Manap), D- α -methylaspartate (Dmasp), α -methylpenicillamine (Mpen), D- α -methylcysteine (Dmcys), N-(4-aminobutyl)glycine (Nglu), D- α -methylglutamine (Dmgln), N-(2-aminoethyl)glycine (Naeg), D- α -methylhistidine (Dmhis), N-(3-aminopropyl)glycine (Norn), D- α -methylisoleucine (Dmleu), N-amino- α -methylbutyrate (Nmaabu), D- α -methylleucine (Dmleu), α -naphthylalanine (Anap), D- α -methyllysine (Dmlys), N-benzylglycine (Nphe), D- α -methylmethionine (Dmmt), N-(2-carbamylethyl)glycine (Ngln), D- α -methylornithine (Dmorn), N-(carbamylmethyl)glycine (Nasn), D- α -methylphenylalanine (Dmphe), N-(2-carboxyethyl)glycine (Nglu), D- α -methylproline (Dmpro), N-(carboxymethyl)glycine (Nasp), D- α -methylserine (Dmser), N-cyclobutylglycine (Ncbu), D- α -methylthreonine (Dmthr), N-cycloheptylglycine (Nchep), D- α -methyltryptophan (Dmtrp), N-cyclohexylglycine (Nchex), D- α -methyltyrosine (Dmty), N-cyclodecylglycine (Ncdec), D- α -methylvaline (Dmval), N-cyclododecylglycine (Ncdod), D-N-methylalanine (Dnmala), N-cyclooctylglycine (Ncoct), D-N-methylarginine (Dnmarg), N-cyclopropylglycine (Ncpro), D-N-methylasparagine (Dnmasn), N-cycloundecylglycine (Ncund), D-N-methylaspartate (Dnmasp), N-(2,2-diphenylethyl)glycine (Nbhm), D-N-methylcysteine (Dnmcys), N-(3,3-diphenylpropyl)glycine (Nbhe), D-N-methylglutamine (Dnmglu), N-(3-guanidinopropyl)glycine (Narg), D-N-methylglutamate (Dnmglu), N-(1-hydroxyethyl)glycine (Ntbx), D-N-methylhistidine (Dnmhis), N-(hydroxyethyl)glycine (Nser), D-N-methylisoleucine (Dnmile), N-(imidazolethyl)glycine (Nhis), D-N-methylleucine (Dnmleu), N-(3-indolylethyl)glycine (Nhtrp), D-N-methyllysine (Dnnilys), N-methyl- γ -aminobutyrate (Nmgabu), N-methylcyclohexylalanine (Nmchexa), D-N-methylmethionine (Dnmmt), D-N-methylornithine (Dnmorn), N-methylcyclopentylalanine (Nmcp), N-methylglycine (Naia), D-N-methylphenylalanine (Dnmphe), N-methylaminoisobutyrate (Nmaib), D-N-methylproline (Dnmpro), N-(1-methylpropyl)glycine

(Nile), D-N-methylserine (Dnmser), N-(2-methylpropyl)glycine (Nleu), D-N-methylthreonine (Dnmthr), D-N-methyltryptophan (Dnmtrp), N-(1-methylethyl)glycine (Nval), D-N-methyltyrosine (Dnmtyr), N-methyl-L-naphthylalanine (Nmanap), D-N-methylvaline (Dnmval), N-methylpenicillamine (Nmpen), γ -aminobutyric acid (Gabu), N-(p-hydroxyphenyl)glycine (Nhtyr), L-/D-butylglycine (Tbug), N-(thiomethyl)glycine (Ncys), L-ethylglycine (Etg), penicillamine (Pen), L-homophenylalanine (Hphe), L- α -methylalanine (Mala), L- α -methylarginine (Marg), L- α -methylasparagine (Masn), L- α -methylaspartate (Masp), L- α -methyl-L-butylglycine (Mtbug), L- α -methylcysteine (Mcys), L-methylethylglycine (Metg), L- α -methylglutamine (MgIn), L- α -methylglutamate (MgIu), L- α -methylhistidine (Mhis), L- α -methylhomophenylalanine (Mhphe), L- α -methylisoleucine (Mile), N-(2-methylthioethyl)glycine (Nmet), L- α -methylleucine (Mleu), L- α -methyllysine (Mlys), L- α -methylmethionine (Mmet), L- α -methylnorleucine (Mnle), L- α -methylnorvaline (Mnva), L- α -methylornithine (Morn), L- α -methylphenylalanine (Mphe), L- α -methylproline (Mpro), L- α -methylserine (Mser), L- α -methylthreonine (Mthr), L- α -methyltryptophan (Mtrp), L- α -methyltyrosine (Mtyr), L- α -methylvaline (Mval), L-N-methylhomophenylalanine (Nmhphe), N-(N-(2,2-diphenylethyl)carbamylmethyl)glycine (Nnbhm), N-(N-(3,3-diphenylpropyl)carbamylmethyl)glycine (Nnbhe), 1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane (Nmbc), L-O-methyl serine (Omser), L-O-methyl homoserine (Omhser).

The term “N-containing aromatic or non-aromatic mono or bicyclic heterocycle” as used herein refers to a cyclic saturated or unsaturated hydrocarbon compound which contains at least one nitrogen atom as constituent of the cyclic chain.

The term “radioactive moiety” as used herein refers to a molecular assembly which carries a radioactive nuclide. The nuclide is bound either by covalent or coordinate bonds which remain stable under physiological conditions. Examples are [^{131}I]-3-iodobenzoic acid or ^{68}Ga -DOTA.

A “fluorescent isotope” as used herein emits electromagnetic radiation after excitation by electromagnetic radiation of a shorter wavelength.

A “radioisotope” as used herein is a radioactive isotope of an element (included by the term “radionuclide”) emitting α -, β -, and/or γ -radioation.

The term “radioactive drug” is used in the context of the present invention to refer to a biologic active compound which is modified by a radioisotope. Especially intercalating substances can be used to deliver the radioactivity to direct proximity of DNA (e.g. a ^{131}I -carrying derivative of Hoechst-33258).

The term “chelating agent” or “chelate” are used interchangeably in the context of the present invention and refer to a molecule, often an organic one, and often a Lewis base, having two or more unshared electron pairs available for donation to a metal ion. The metal ion is usually coordinated by two or more electron pairs to the chelating agent. The terms, “bidentate chelating agent”, “tridentate chelating agent, and “tetradentate chelating agent” refer to chelating agents having, respectively, two, three, and four electron pairs readily available for simultaneous donation to a metal ion coordinated by the chelating agent. Usually, the electron pairs of a chelating agent forms coordinate bonds with a single metal ion; however, in certain examples, a chelating agent may form coordinate bonds with more than one metal ion, with a variety of binding modes being possible.

The term “fluorescent dye” is used in the context of the present invention to refer to a compound that emits visible or infrared light after excitation by electromagnetic radiation of a shorter and suitable wavelength. It is understood by the skilled person, that each fluorescent dye has a predetermined excitation wavelength.

The term “contrast agent” is used in the context of the present invention to refer to a compound which increases the contrast of structures or fluids in medical imaging. The enhancement is achieved by absorbing electromagnetic radiation or altering electromagnetic fields.

The term “paramagnetic” as used herein refers to paramagnetism induced by unpaired electrons in a medium. A paramagnetic substance induces a magnetic field if an external magnetic field is applied. Unlike diamagnetism the direction of the induced field is the same as the external field and unlike ferromagnetism the field is not maintained in absence of an external field.

The term “nanoparticle” as used herein refers to particles preferably of spheric shape, with diameters of sizes between 1 and 100 nanometers. Depending on the composition, nanoparticles can possess magnetical, optical or physico-chemical qualities that can be assessed. Additionally surface modification is achievable for many types of nanoparticles.

The term “pharmaceutically acceptable salt” refers to a salt of the compound of the present invention. Suitable pharmaceutically acceptable salts of the compound of the present invention include acid addition salts which may, for example, be formed by mixing a solution of choline or derivative thereof with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compound of the invention carries an acidic moiety, suitable pharmaceutically acceptable salts

thereof may include alkali metal salts (e.g., sodium or potassium salts); alkaline earth metal salts (e.g., calcium or magnesium salts); and salts formed with suitable organic ligands (e.g., ammonium, quaternary ammonium and amine cations formed using counteranions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate).

5 Illustrative examples of pharmaceutically acceptable salts include but are not limited to: acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like (see, for example, Berge, S. M., et al, "Pharmaceutical Salts", *Journal of*
10 *Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of
25 the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily
30 undergo chemical changes under physiological conditions to provide a compound of formula (I). A prodrug is an active or inactive compound that is modified chemically through *in vivo* physiological action, such as hydrolysis, metabolism and the like, into a compound of this invention following administration of the prodrug to a patient. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in

an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme. The suitability and techniques involved in making and using prodrugs are well known by those skilled in the art. For a general discussion of prodrugs involving esters see Svensson and Tunek
5 Drug Metabolism Reviews 16.5 (1988) and Bundgaard Design of Prodrugs, Elsevier (1985). Examples of a masked carboxylate anion include a variety of esters, such as alkyl (for example, methyl, ethyl), cycloalkyl (for example, cyclohexyl), aralkyl (for example, benzyl, p-methoxybenzyl), and alkylcarbonyloxyalkyl (for example, pivaloyloxymethyl). Amines have been masked as arylcarbonyloxymethyl substituted derivatives which are cleaved by esterases
10 in vivo releasing the free drug and formaldehyde (Bundgaard J. Med. Chem. 2503 (1989)). Also, drugs containing an acidic NH group, such as imidazole, imide, indole and the like, have been masked with N-acyloxymethyl groups (Bundgaard Design of Prodrugs, Elsevier (1985)). Hydroxyl groups have been masked as esters and ethers. EP 0 039 051 (Sloan and Little, Apr. 11, 1981) discloses Mannich-base hydroxamic acid prodrugs, their preparation and use.

15 Compounds according to the invention can be synthesized according to one or more of the following methods. It should be noted that the general procedures are shown as it relates to preparation of compounds having unspecified stereochemistry. However, such procedures are generally applicable to those compounds of a specific stereochemistry, e.g., where the stereochemistry about a group is (S) or (R). In addition, the compounds having one
20 stereochemistry (e.g., (R)) can often be utilized to produce those having opposite stereochemistry (i.e., (S)) using well-known methods, for example, by inversion.

Certain compounds of the present invention can exist in unsolvated forms as well as in solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention.

25 Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual
30 isomers are all intended to be encompassed within the scope of the present invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present

invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

The term "pharmaceutical composition" as used in the present application refers to a substance and/or a combination of substances being used for the identification, prevention or treatment of a tissue status or disease. The pharmaceutical composition is formulated to be suitable for administration to a patient in order to prevent and/or treat disease. Further a pharmaceutical composition refers to the combination of an active agent with a carrier, inert or active, making the composition suitable for therapeutic use. Pharmaceutical compositions can be formulated for oral, parenteral, topical, inhalative, rectal, sublingual, transdermal, subcutaneous or vaginal application routes according to their chemical and physical properties. Pharmaceutical compositions comprise solid, semisolid, liquid, transdermal therapeutic systems (TTS). Solid compositions are selected from the group consisting of tablets, coated tablets, powder, granulate, pellets, capsules, effervescent tablets or transdermal therapeutic systems. Also comprised are liquid compositions, selected from the group consisting of solutions, syrups, infusions, extracts, solutions for intravenous application, solutions for infusion or solutions of the carrier systems of the present invention. Semisolid compositions that can be used in the context of the invention comprise emulsion, suspension, creams, lotions, gels, globules, buccal tablets and suppositories.

"Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

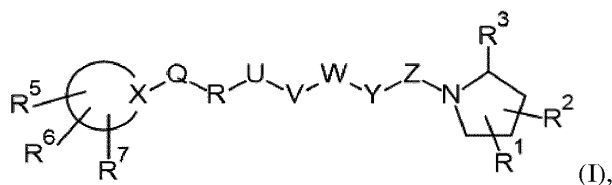
The term "carrier", as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic agent is administered. Such pharmaceutical carriers can be sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term “fibroblast activation protein (FAP)” as used herein is also known under the term “seprase”. Both terms can be used interchangeably herein. Fibroblast activation protein is a homodimeric integral protein with dipeptidyl peptidase IV (DPP-IV)-like fold, featuring an alpha/beta-hydrolase domain and an eight-bladed beta-propeller domain.

Embodiments

In the following different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

In a first aspect, the present invention provides a compound of Formula (I)



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of

Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other; preferably out of the six four groups are present of which two are C=O, one is CH₂ and one is NH; more preferably four groups are present of which two are C=O, one is CH₂ and one is NH; most preferably, V, W, Y and Z are present of which V and Z are C=O and W and Y are independently selected from CH₂ and NH;

R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl-, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aryl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,

R^8-L and $R^8-D-B-E-A$, under the proviso that R^6 and R^7 are not at the same time H, preferably R^6 is attached to the 7- or 8-quinolyl position and R^7 is attached to the 5- or 6-quinolyl position; more preferably R^6 is attached to the 7-quinolyl position and R^7 is attached to the 6-quinolyl position,

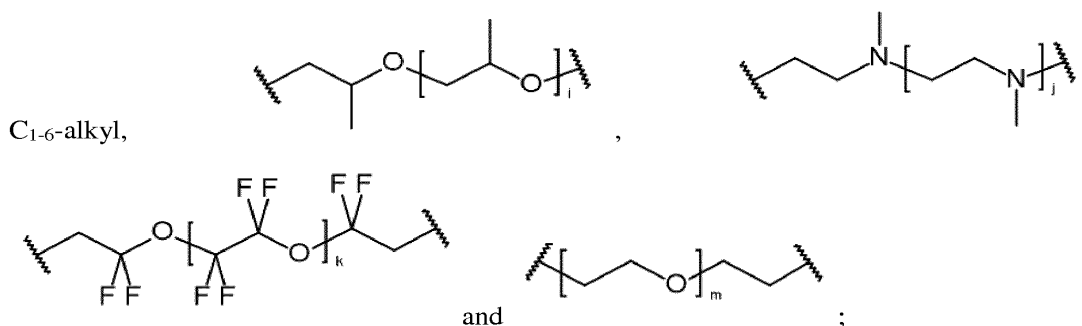
5 wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR^4 , O, S, and CH_2 ;

10 E is selected from the group consisting of



wherein i is 1, 2, or 3;

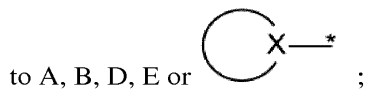
wherein j is 1, 2, or 3;

15 wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

more preferably, E is C_{1-6} -alkyl, most preferably, E is C3 or C4 alkyl;

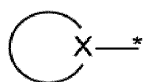
A and E together form a group selected from: a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, wherein A and E can be mono-, bi- and multicyclic, preferably monocyclic. Each A and E being optionally substituted with 1 to 4 substituents selected from -H, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-S-C_{1-6}$ -alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and $-C_{1-6}$ -aralkyl, each of said $-C_{1-6}$ -alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo; and optionally connected



25 B is selected from the group consisting of S, NR^4 , NR^4-O , NR^4-C_{1-6} -alkyl, NR^4-C_{1-6} -alkyl- NR^4 , and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle,

preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$; and

- 5 R^8 is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;

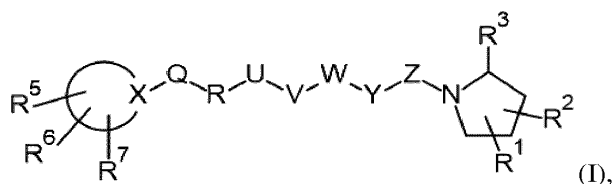


is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

- 10 or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof. Preferably, $\text{C}_{1-6}\text{-alkyl}$ is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

- In a preferred embodiment, A and E together form a group selected from the group consisting of a C_3 , C_4 , C_5 , C_6 , C_7 and C_8 monocyclic, preferably C_5 or C_6 monocyclic, or C_7 , C_8 , C_9 , C_{10} , C_{11} or C_{12} bicyclic, preferably C_7 , C_8 , C_9 and C_{10} bicyclic heterocycloalkyl, comprising 1, 2, 3, or 4, preferably 1 or 2 heteroatoms independently selected from the group consisting of N, O and S, preferably N and O, most preferably 1 or 2 N.

- 20 In a preferred embodiment of the first aspect of the present invention a compound of Formula (I) is provided:



wherein

- Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;
- 25 Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH_2 , NR^4 , C=O , C=S , C=NR^4 , HCR^4 and R^4CR^4 , with the proviso that two Os are not directly adjacent to each other; preferably out of the six four groups are present of which two are C=O , one is CH_2 and one is NH; more preferably four groups are present of which two are C=O , one is CH_2 and one is NH; most preferably, V, W, Y and Z are present of which V and Z are C=O and W and
- 30 Y are independently selected from CH_2 and NH;

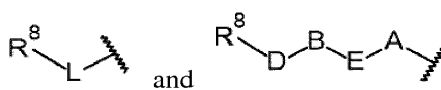
R^1 and R^2 are independently selected from the group consisting of -H, -OH, halo, C_{1-6} -alkyl, -O- C_{1-6} -alkyl, S- C_{1-6} -alkyl;

R^3 is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl-, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

- 5 R^4 is selected from the group consisting of -H, - C_{1-6} -alkyl, -O- C_{1-6} -alkyl, -S- C_{1-6} -alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and - C_{1-6} -aryl, each of said - C_{1-6} -alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R^5 is selected from the group consisting of -H, halo and C_{1-6} -alkyl;

- 10 R^6 , and R^7 are independently selected from the group consisting of -H,

 , under the proviso that R^6 and R^7 are not at the same time H, preferably R^6 is attached to the 7- or 8-quinolyl position and R^7 is attached to the 5- or 6-quinolyl position; more preferably R^6 is attached to the 7-quinolyl position and R^7 is attached to the 6-quinolyl position,

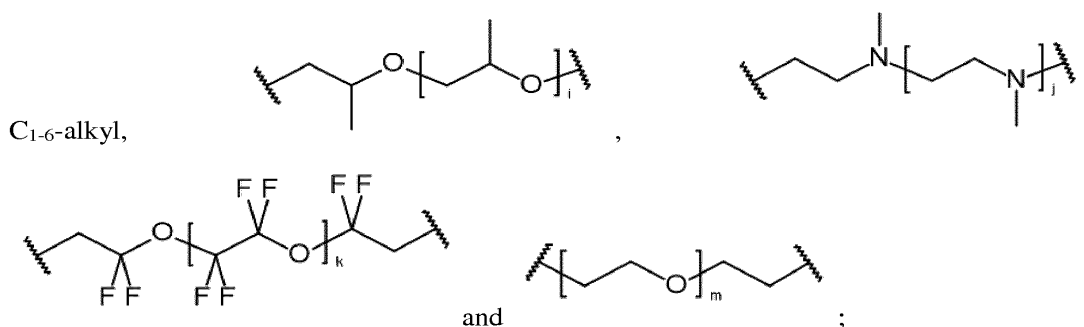
- 15 wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR⁴, O, S, and CH₂;

- 20 E is selected from the group consisting of



wherein i is 1, 2, or 3;

wherein j is 1, 2, or 3;

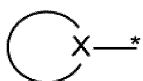
- 25 wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

more preferably, E is C_{1-6} -alkyl, most preferably, E is C3 or C4 alkyl;

B is selected from the group consisting of S, NR^4 , $\text{NR}^4\text{-O}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$; and

R^8 is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;



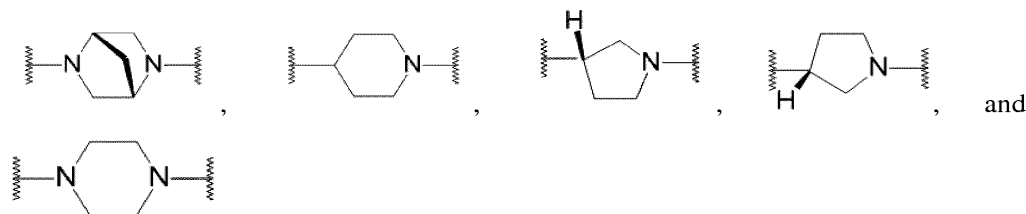
is a 1-naphthyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof. Preferably, $\text{C}_{1-6}\text{-alkyl}$ is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In another preferred embodiment of the first aspect of the present invention A and E together form a group consisting of a C_3 , C_4 , C_5 , C_6 , C_7 and C_8 monocyclic, preferably C_5 or C_6 monocyclic, or C_7 , C_8 , C_9 , C_{10} , C_{11} or C_{12} bicyclic, preferably C_7 , C_8 , C_9 and C_{10} bicyclic heterocycloalkyl, preferably comprising 1, 2, 3, or 4, more preferably 1 or 2 heteroatoms independently selected from the group consisting of N, O and S, preferably N and O, most preferably 1 or 2 N. Preferred monocyclic heterocycloalkyls are selected from the group consisting of pyrrolidinyl, piperidinyl, imidazolidinyl, 1,2-diazacyclohexanyl, 1,3-diazacyclohexanyl, piperazinyl, 1-oxo-2-azacyclohexanyl, 1-oxo-3-azacyclohexanyl, or morpholinyl, preferably piperidinyl, piperazinyl, and pyrrolidinyl. Preferred bicyclic heterocycloalkyls are selected from the group consisting of bicyclo[2.2.1] 2,5-diazaheptanyl, 3,6-diazabicyclo[3.2.1]octanyl, 3,6-diazabicyclo[3.2.2]nonyl, octahydropyrrolo[2,3-b]pyrrolyl, octahydropyrrolo[3,2-b]pyrrolyl, octahydropyrrolo[3,4-b]pyrrolyl, octahydropyrrolo[3,4-c]pyrrolyl, 9-methyl-3,7,9-triazabicyclo[3.3.1]nonanyl.

The bond between the heterocycle formed by A and E and B on one hand and/or R^6 or R^7 on the other is preferably through the heteroatom, preferably through N.

Particularly, preferred examples of the heterocycle formed by A and E are selected from the group consisting of



In a preferred embodiment of the first aspect of the present invention,

Q, R, U are CH₂ and are individually present or absent; preferably, Q and R are absent;

5 V is CH₂, C=O, C=S or C=NR⁴; preferably, V is C=O;

W is NR⁴; preferably, W is NH;

Y is HCR⁴; preferably, Y is CH₂; and

Z is C=O, C=S or C=NR⁴, preferably, Z is C=O.

In a further preferred embodiment of the first aspect of the present invention,

10 Q, R, U are absent;

V is CH₂;

W is NH;

Y is CH₂; and

Z is C=O.

15 In a further preferred embodiment of the first aspect of the present invention,

R¹ and R² are independently selected from the group consisting of -H and halo; preferably, R¹ and R² are halo; more preferably, R¹ and R² are F;

R³ is selected from the group consisting of -H, -CN, and -B(OH)₂; preferably, R³ is -CN or -B(OH)₂; more preferably, R³ is -CN;

20 R⁴ is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

25 Q, R, U are absent;

V is CH₂;

W is NH;

Y is CH₂;

Z is C=O;

30 R¹ and R² are independently selected from the group consisting of -H and halo; preferably, R¹ and R² are halo; more preferably, R¹ and R² are F;

R^3 is selected from the group consisting of -H, -CN, and -B(OH)₂; preferably, R^3 is -CN or -B(OH)₂; more preferably, R^3 is -CN;

R^4 is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is CH₂;

10 W is CH₂;

Y is NH;

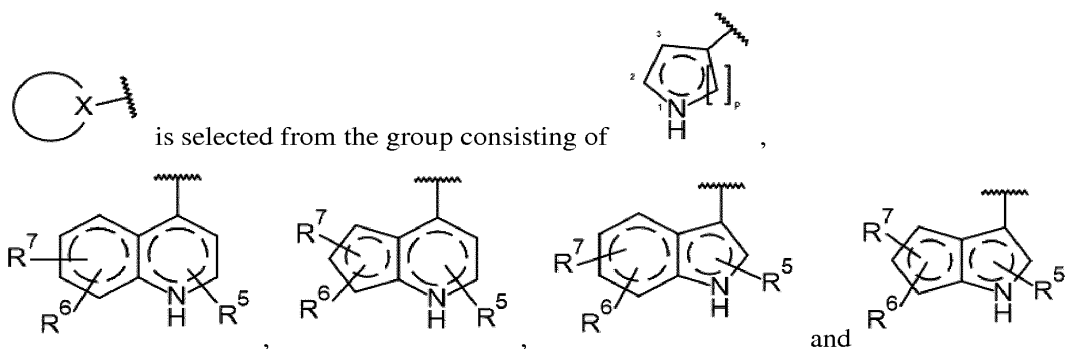
Z is C=O;

R^1 and R^2 are independently selected from the group consisting of -H and halo; preferably, R^1 and R^2 are halo; more preferably, R^1 and R^2 are F;

15 R^3 is selected from the group consisting of -H, -CN, and -B(OH)₂; preferably, R^3 is -CN or -B(OH)₂; more preferably, R^3 is -CN;

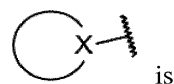
R^4 is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

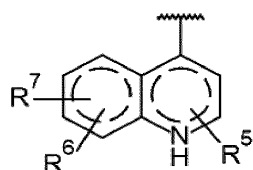
In a further preferred embodiment of the first aspect of the present invention,



optionally further comprising 1 or 2 heteroatoms selected from O, N, and S.

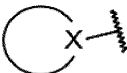
25 In a further preferred embodiment of the first aspect of the present invention,

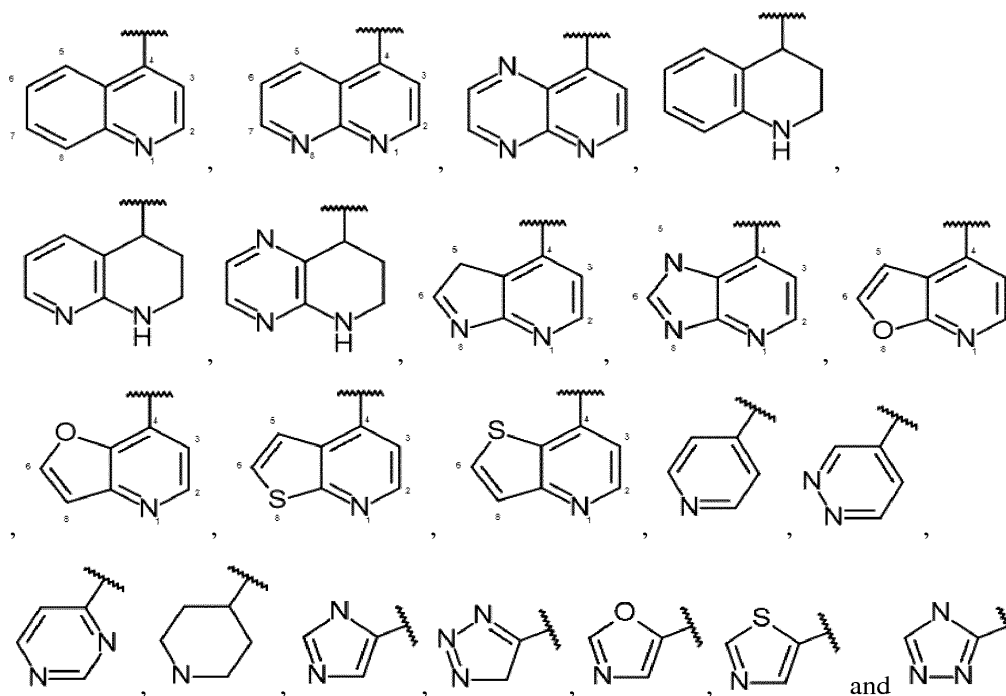




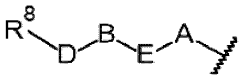
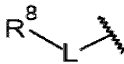
, optionally further comprising 1 or 2 heteroatoms selected from O, N, and S.

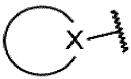
In a further preferred embodiment of the first aspect of the present invention,

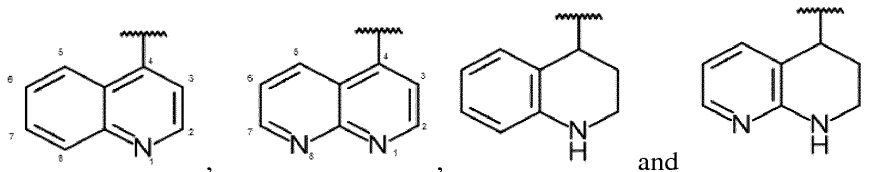
5  is selected from the group consisting of

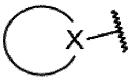
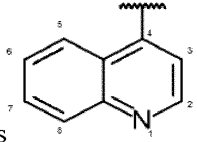


10 . R⁶, and R⁷ are independently selected from the group consisting of -H,

, and , under the proviso that R⁶ and R⁷ are not at the same time H and preferably R⁶ and R⁷ are attached on positions 5, 6 or 7.

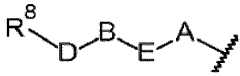
In a preferred embodiment,  is selected from the group consisting of



In another preferred embodiment  is .

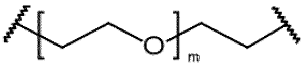
In a further preferred embodiment of the first aspect of the present invention,

- 5 R^5 and R^6 are H;

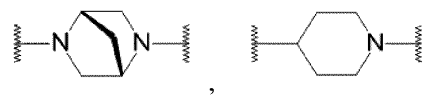
R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein

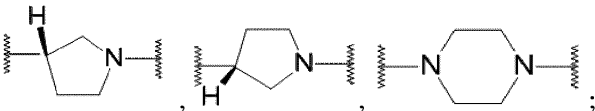
D is absent;

A is O, S, CH_2 , NH, NCH_3 ;

- 10 E is C_{1-6} -alkyl or , wherein m is 1, 2, or 3; Preferably, C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C_{1-6} -alkyl, most preferably, E is C3 or C4 alkyl; or

A and E together form a group selected from:

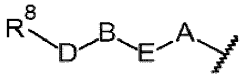


- 15 

B is NR^4 - C_{1-6} -alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C_{1-6} -alkyl,

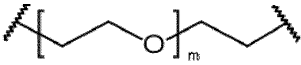
aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention, R⁵ and R⁶ are H;

- 5 R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

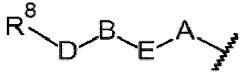
D is absent;

A is O;

- 
- 10 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

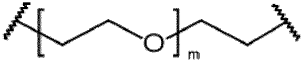
- B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing
- 15 heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention, R⁵ and R⁶ are H;

- 20 R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

A is S;

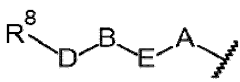
- 
- 25 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing

heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

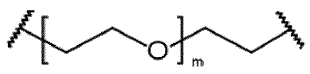
In a further preferred embodiment of the first aspect of the present invention,

- 5 R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

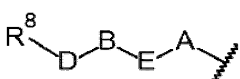
A is CH₂;

- 10 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

- B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

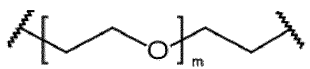
In a further preferred embodiment of the first aspect of the present invention,

- 20 R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

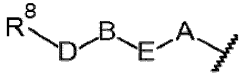
A is NH;

- 25 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and

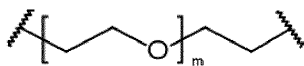
S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

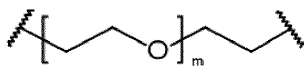
- 5 In a further preferred embodiment of the first aspect of the present invention,
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is an amino acid, preferably carrying a charged side chain;

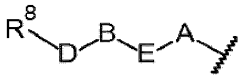
- 10 A is O;



E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

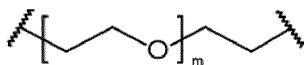
- B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or
15 bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

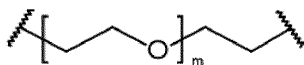
- 20 In a further preferred embodiment of the first aspect of the present invention,
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is an amino acid, preferably carrying a charged side chain;

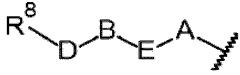
- 25 A is S;

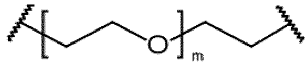


E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

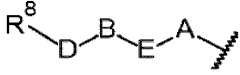
B is $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$ or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$. Preferably, $\text{C}_{1-6}\text{-alkyl}$ is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

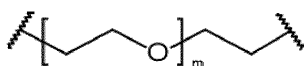
In a further preferred embodiment of the first aspect of the present invention, R^5 and R^6 are H;

R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein D is an amino acid, preferably carrying a charged side chain; A is CH_2 ;

E is $\text{C}_{1-6}\text{-alkyl}$ or , wherein m is 1, 2, or 3; Preferably, $\text{C}_{1-6}\text{-alkyl}$ is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is $\text{C}_{1-6}\text{-alkyl}$, most preferably, E is C3 or C4 alkyl; B is $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$ or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$. Preferably, $\text{C}_{1-6}\text{-alkyl}$ is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention, R^5 and R^6 are H;

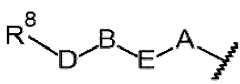
R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein D is an amino acid, preferably carrying a charged side chain; A is NH ;



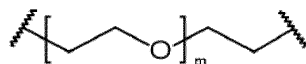
E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

- 5 B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

- 10 In a further preferred embodiment of the first aspect of the present invention, R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein D is absent;

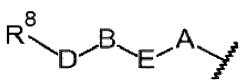
- 15 A is O;



E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, E is C₁₋₆-alkyl and C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

- 20 B is a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 nitrogen atoms.

In a further preferred embodiment of the first aspect of the present invention, R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein D is absent;

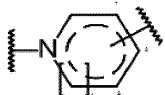
- 25

A is O;

E is C3 or C4 alkyl; more preferably, E is propyl or butyl;

B is a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 nitrogen atoms.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



, wherein

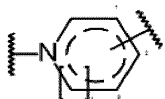
the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen;



is attached to position 1, 2, or 3, preferably to position 2;

l is 1 or 2.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



, wherein

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen;

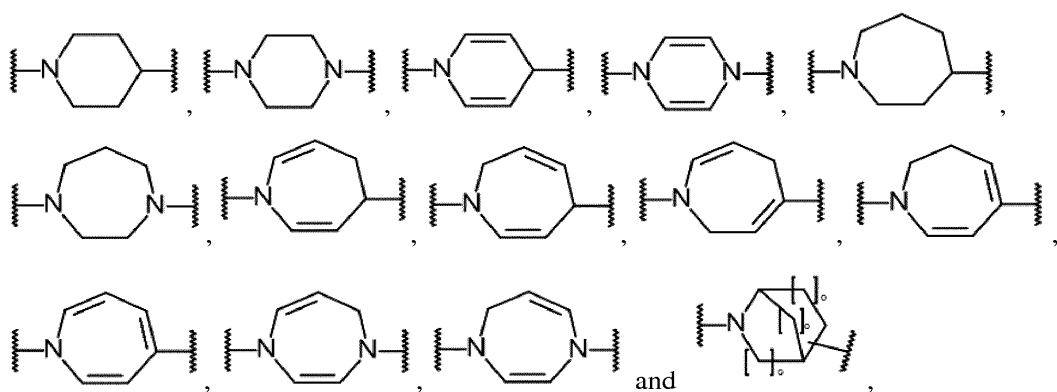


is attached to position 1, 2, or 3, preferably to position 2;

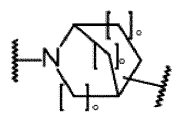
l is 1 or 2;

wherein the N-containing heterocycle is substituted with a C₁₋₆-alkyl.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle comprised in B is selected from the group consisting of:



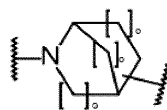
wherein the N-containing heterocycle is substituted with a C₁₋₆-alkyl



- 5 wherein if the N-containing heterocycle comprised in B is the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen, optionally comprises one or more (e.g. amino acid derived) side chains;

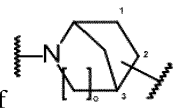
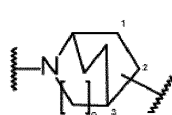
— is attached to position 1, 2, or 3, preferably to position 2;

- 10 o is 1 or 2;

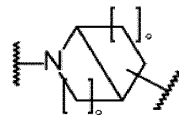


preferably, if the N-containing heterocycle comprised in B is , the N-

containing heterocycle comprised in B is selected from the group consisting of

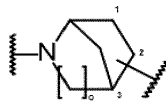


and



; more preferably, if the N-containing heterocycle

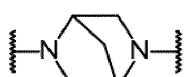
comprised in B is



, the N-containing heterocycle comprised in B is

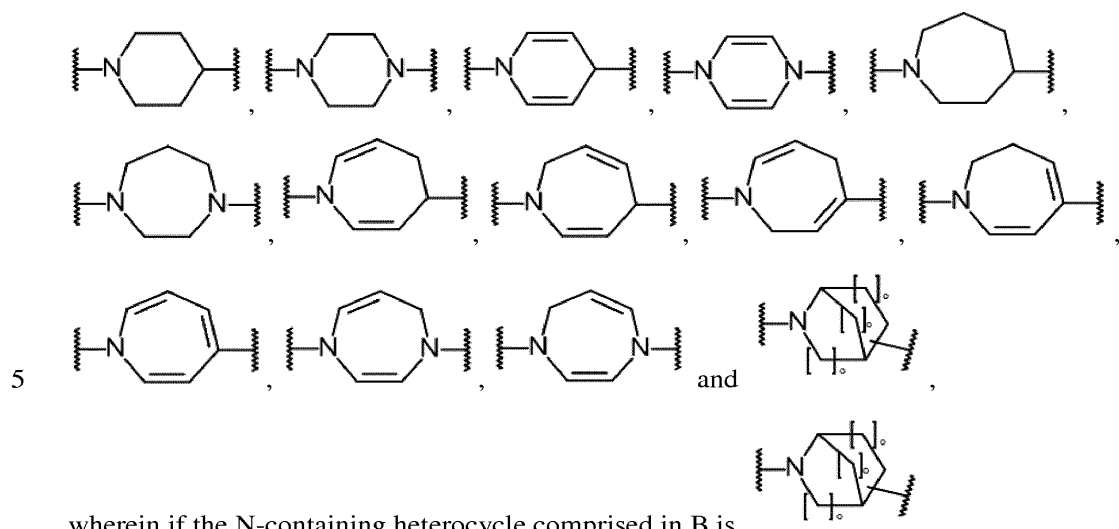


or



- 15

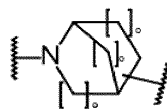
In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:



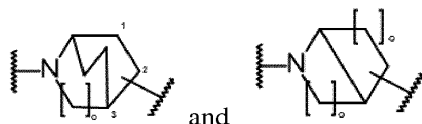
the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S,
optionally further comprises 1 nitrogen, optionally comprises one or more (e.g. amino acid
derived) side chains;

10 is attached to position 1, 2, or 3, preferably to position 2;
o is 1 or 2;

preferably, if the N-containing heterocycle comprised in B is

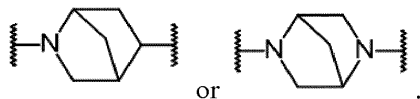


containing heterocycle comprised in B is selected from the group consisting of

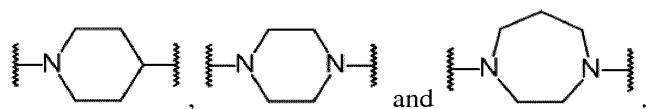


and ; more preferably, if the N-containing heterocycle

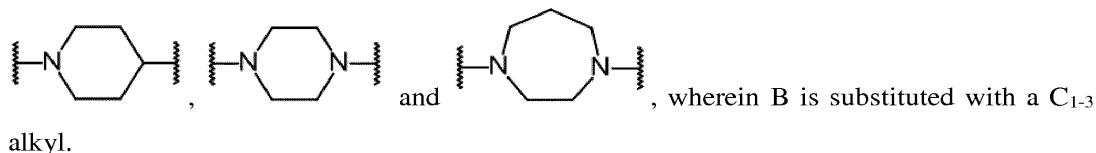
15 comprised in B is



In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:

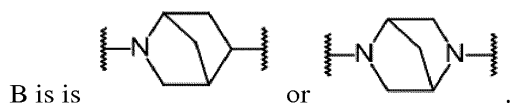


In a further preferred embodiment of the first aspect of the present invention,
5 the N-containing heterocycle comprised in B is selected from the group consisting of:



In a further preferred embodiment of the first aspect of the present invention,
R⁵ and R⁶ are H;

10 R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein
D is absent;
A is O;
E is propyl or butyl;



15 B is is or .
In a further preferred embodiment of the first aspect of the present invention,
Q, R, U are absent;
V is C=O;
W is NH;
Y is CH₂;
20 Z is C=O;

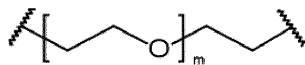
R¹ and R² are independently selected from the group consisting of -H and halo; preferably, R¹
and R² are independently selected from the group consisting of -H and F; more preferably, R¹
and R² are the same and are selected from the group consisting of -H and F;

R³ is -CN;

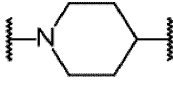
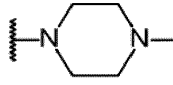
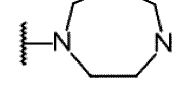
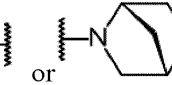
25 R⁵ and R⁶ are H;

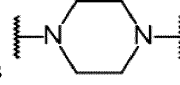
R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein
D is absent;

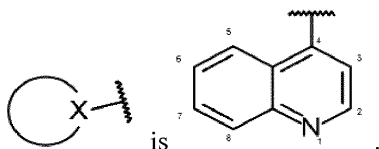
A is O;



E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; preferably, E is C₁₋₆-alkyl; preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NH-C₁₋₆-alkyl, , , , or ; preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl,

butyl, sec-butyl, tert-butyl, pentyl and hexyl; preferably, B is ; and



In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is C=O;

W is NH;

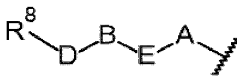
Y is CH₂;

Z is C=O;

R¹ and R² are the same and are selected from the group consisting of -H and F;

R³ is -CN;

R⁵ and R⁶ are H;

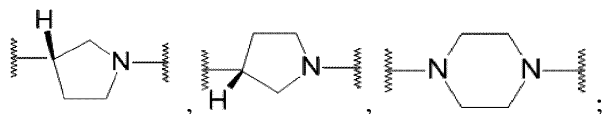
R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

A is O, S, CH₂, NH, NCH₃;

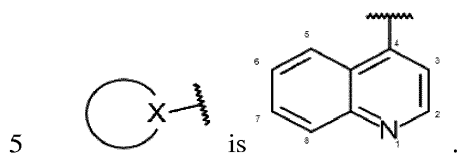
E is methyl, ethyl, propyl or butyl;

A and E together form a group selected from:



B is , optionally B is

substituted with a C₁₋₃ alkyl; preferably, B is ; and



In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is C=O;

10 W is NH;

Y is CH₂;

Z is C=O;

R¹ and R² are the same and are selected from the group consisting of -H and F;

R³ is -CN;

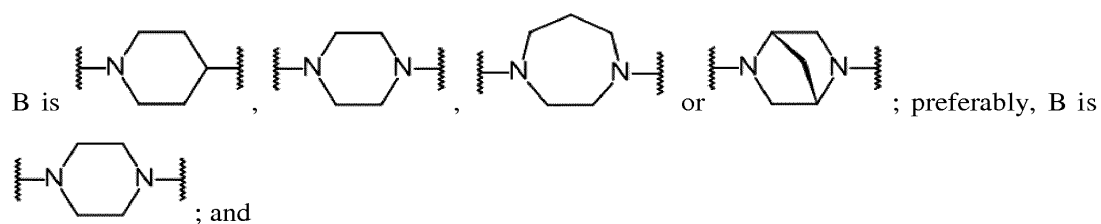
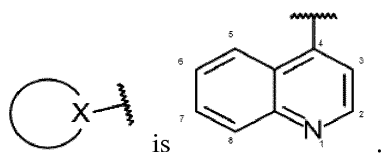
15 R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

A is O;

E is methyl, ethyl, propyl or butyl;



In a further preferred embodiment of the first aspect of the present invention,

5 Q, R, U are absent;

V is C=O;

W is NH;

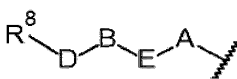
Y is CH₂;

Z is C=O;

10 R¹ and R² are the same and are selected from the group consisting of -H and F;

R³ is -CN;

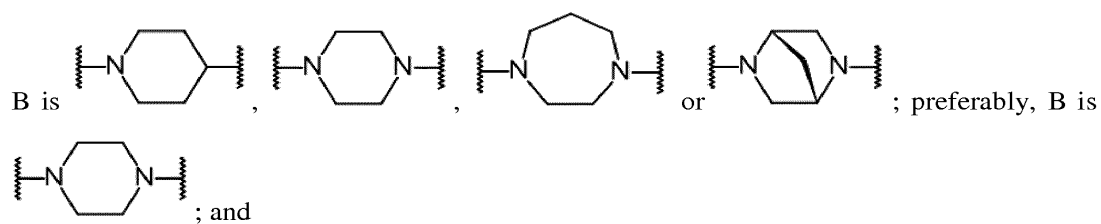
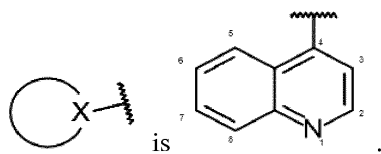
R⁵ and R⁶ are H;

R⁷ is , R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

15 A is O;

E is methyl, ethyl, propyl or butyl;



In a further preferred embodiment of the first aspect of the present invention, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

5 In a further preferred embodiment of the first aspect of the present invention, C₁₋₃-alkyl is selected from the group consisting of methyl, ethyl, propyl and i-propyl.

In a further preferred embodiment of the first aspect of the present invention, C₁₋₆-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl, phenyl-propyl, and phenyl-butyl.

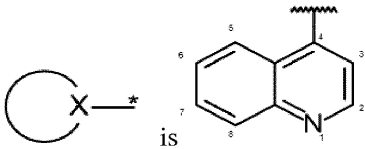
10 In a preferred embodiment of the first aspect of the present invention, the compound of the first aspect of the invention is selected from the compounds of table 1. More preferably, the compound of the first aspect of the invention is selected from the compounds of table 2. More preferably, the compound of the first aspect of the invention is selected from the group consisting of FAPI-02 and FAPI-04.

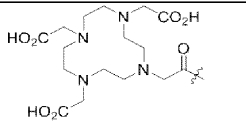
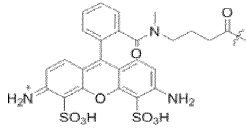
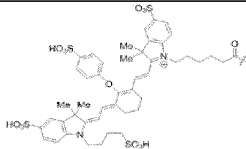
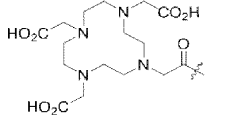
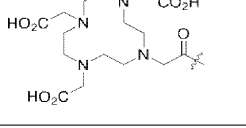
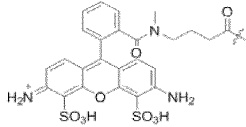
15 In a preferred embodiment of the first aspect of the present invention, the compound of the first aspect of the invention is selected from the compounds of table 1 and/or table 3. More preferably, the compound of the first aspect of the invention is selected from the compounds of table 2 and/or table 4. More preferably, the compound of the first aspect of the invention is selected from the group consisting of FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71, FAPI-72 and FAPI-73.

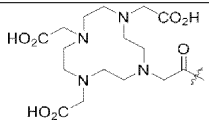
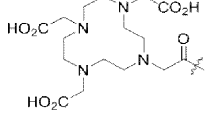
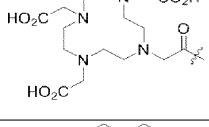
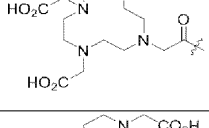
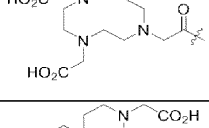
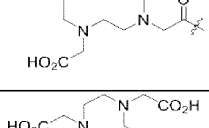
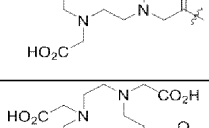
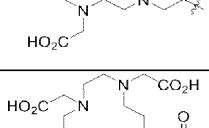
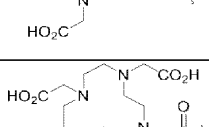
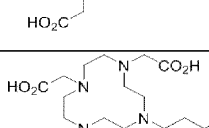
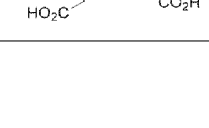
20

Table 1: Preferred compounds of the first aspect of the invention.

§ fluorescent compounds; \$ ^{99m}Tc -chelators; * Pb-chelators; R^1 and R^2 are located at the 4-

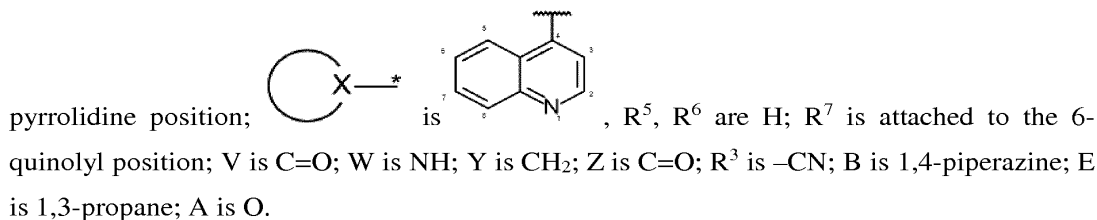
- pyrrolidine position; Q, R, U are absent;  is R^5 is H; R^6 is attached to the 7-quinolyl position; R^7 is attached to the 6-quinolyl position; V is C=O; W is NH; Y is CH_2 ; Z is C=O; R^3 is $-\text{CN}$; A is O (except FAPI-01: A is absent, R^7 is attached to the 5-quinolyl position).

name	R^1/R^2	R^6	R^7	R^8	D	B	E
FAPI-01	H/H	-	+	I	-	-	-
FAPI-02	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-02 atto 488§	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-02 dy800 CW§	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-03	H/H	-	+		-		$n\text{-C}_4\text{H}_8$
FAPI-04	F/F	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-04 atto 488§	F/F	-	+		-		$n\text{-C}_3\text{H}_6$

FAPI-05	F/F	-	+		-		<i>n</i> -C ₄ H ₈
FAPI-06	H/H	-	+		-	NH	<i>n</i> -C ₃ H ₆
FAPI-07	F/F	-	+		-	NH	<i>n</i> -C ₃ H ₆
FAPI-08	H/H	+	-		-		<i>n</i> -C ₃ H ₆
FAPI-09	F/F	+	-		-		<i>n</i> -C ₃ H ₆
FAPI-11	F/F	-	+		-		CH ₂
FAPI-12	F/F	-	+		-		<i>n</i> -C ₂ H ₄
FAPI-13	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-14	F/F	-	+		-		<i>n</i> -C ₄ H ₈
FAPI-15	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄
FAPI-16	F/F	-	+		-		<i>n</i> -C ₃ H ₆

FAPI-17	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-18	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-19^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-20	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-21	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-22	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-23	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-24	F/F	-	+		-		(C ₂ H ₄ O) 3C ₂ H ₄
FAPI-25	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-26	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄
FAPI-27^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆

FAPI-28^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-29^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-30	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-31	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-32*	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-33^s	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄
FAPI-34^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-35	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-36	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-37	F/F	-	+				
FAPI-38	F/F	-	+		D-Arg		

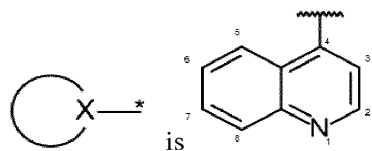


name	Purpose	R ¹ /R ²	R ⁸
⁶⁸ Ga-FAPI-02	PET	H/H	
⁶⁸ Ga-FAPI-04	PET	F/F	
Gd-FAPI-04	MRI (contrast agent)	F/F	
⁹⁰ Y-FAPI-04	radiotherapy (β^-)	F/F	
^{99m} Tc-FAPI-29	SPECT	F/F	
²⁰³ Pb-FAPI-32	SPECT	F/F	
¹⁸ F-FAPI-n.a.	PET	F/F	

Table 3: Further preferred compounds of the first aspect of the invention.

§ fluorescent compounds; \$ ^{99m}Tc-chelators; * precursors for ¹⁸F-labeling; Q, R, U are absent;

R¹ and R² are fluorine atoms located at the 4-pyrrolidine position;



5 is C=O; W is NH; Y is CH₂; Z is C=O; R³ is -CN.
 ,R⁵ and R⁷ are H; R⁶ is attached to the 6-quinolyl position; V

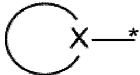
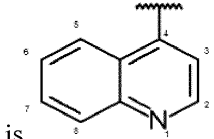
name	R ⁸	D	B	E	A
FAPI-39		-		<i>n</i> -C ₃ H ₆	CH ₂
FAPI-40		-		<i>n</i> -C ₃ H ₆	S
FAPI-41		-		<i>n</i> -C ₃ H ₆	NH
FAPI-42		-		<i>n</i> -C ₃ H ₆	O
FAPI-43[§]		-		<i>n</i> -C ₃ H ₆	O
FAPI-44[§]		-		<i>n</i> -C ₃ H ₆	O
FAPI-45[§]		-		<i>n</i> -C ₃ H ₆	O

name	R ⁸	D	B	E	A
FAPI-46		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-47		-		<i>n</i> -C ₃ H ₆	O
FAPI-48		-		<i>n</i> -C ₃ H ₆	O
FAPI-49		-		<i>n</i> -C ₃ H ₆	O
FAPI-50		-		<i>n</i> -C ₃ H ₆	O
FAPI-51		-		<i>n</i> -C ₃ H ₆	O
FAPI-52		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-53		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-54		-			
FAPI-55		-		<i>n</i> -C ₃ H ₆	NMe

name	R ⁸	D	B	E	A
FAPI-56		-			
FAPI-57		-			
FAPI-58		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-59		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-60^s		-		<i>n</i> -C ₃ H ₆	O
FAPI-61^s		-		<i>n</i> -C ₃ H ₆	O
FAPI-62^s		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-63		-			
FAPI-64		-			
FAPI-65		-			
FAPI-66		-			

name	R ⁸	D	B	E	A
FAPI-67		-			
FAPI-68		-			
FAPI-69^s		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-70^s		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-71^s		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-72[*]		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-73[*]		-		<i>n</i> -C ₃ H ₆	O

Table 4: Compounds of special interest. Q, R, U, D are absent; R¹ and R² are fluorine atoms

located at the 4-pyrrolidine position;  is , R⁵, R⁶ are H; R⁷ is attached to the 6-quinolyl position; V is C=O; W is NH; Y is CH₂; Z is C=O; R³ is –CN; B is 1,4-piperazine; E is 1,3-propane; A is O.

5

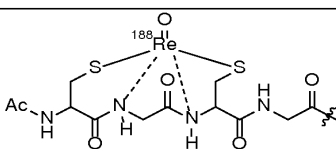
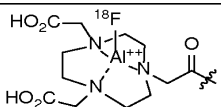
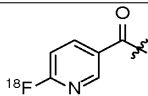
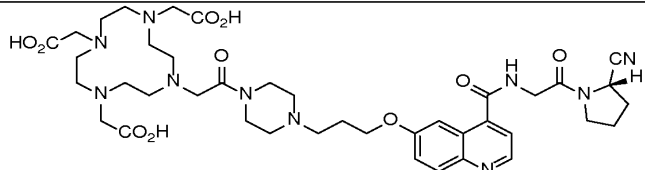
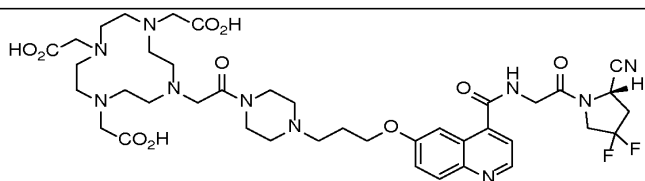
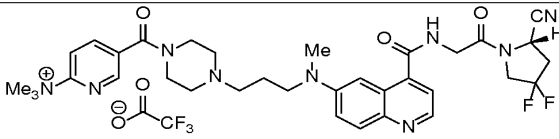
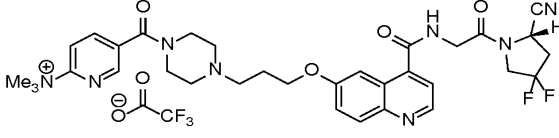
name	Purpose	R ⁸
¹⁸⁸ Re-FAPI-60	radiotherapy (β [−])	
Al ¹⁸ F-FAPI-42	PET	
¹⁸ F-FAPI-73	PET	

Table 5: Preferred precursors for radiolabelling with ¹⁸F-18; ⁶⁴Cu-64; ⁶⁸Ga-68; ^{99m}Tc-99m, Re-188; ⁹⁰Y-90, Sm-153, Lu-177.

FAPI-02 ^{€,*}	
FAPI-04 ^{€,*}	

FAPI- 34 [£]	
FAPI- 42 ^{§,§,€}	
FAPI- 46 ^{€,*}	
FAPI- 52 ^{§,§,€}	
FAPI- 69 [£]	
FAPI- 70 [£]	
FAPI- 71 [£]	

FAPI-72 ^s	
FAPI-73 ^s	

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a radioactive moiety, wherein the radioactive moiety is a fluorescent isotope, a radioisotope, a radioactive drug or combinations thereof. Preferably, the radioactive moiety is selected from the group consisting of alpha radiation emitting isotopes, beta radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting isotopes, fluorescence emitting isotopes, such as ¹¹C, ¹⁸F, ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁸⁶Re, ¹⁸⁸Re, ¹³⁹La, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁸⁸Y, ⁹⁰Y, ¹⁴⁹Pm, ¹⁶⁵Dy, ¹⁶⁹Er, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi, ²¹³Bi, ⁷²As, ⁷²Se, ⁹⁷Ru, ¹⁰⁹Pd, ¹⁰⁵Rh, ^{101m}Rh, ¹¹⁹Sb, ¹²⁸Ba, ¹²³I, ¹²⁴I, ¹³¹I, ¹⁹⁷Hg, ²¹¹At, ¹⁵¹Eu, ¹⁵³Eu, ¹⁶⁹Eu, ²⁰¹Tl, ²⁰³Pb, ²¹²Pb, ⁶⁴Cu, ⁶⁷Cu, ¹⁸⁸Re, ¹⁸⁶Re, ¹⁹⁸Au, ²²⁵Ac, ²²⁷Th and ¹⁹⁹Ag. Preferably ¹⁸F, ⁶⁴Cu, ⁶⁸Ga, ⁹⁰Y, ^{99m}Tc, ¹⁵³Sm, ¹⁷⁷Lu, ¹⁸⁸Re.

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a fluorescent dye select from the group consisting of the following classes of fluorescent dyes: Xanthenes, Acridines, Oxazines, Cynines, Styryl dyes, Coumarines, Porphines, Metal-Ligand-Complexes, Fluorescent proteins, Nanocrystals, Perylenes, Boron-dipyrromethenes and Phtalocyanines as well as conjugates and combinations of these classes of dyes.

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a chelating agent which forms a complex with divalent or trivalent metal cations. Preferably, the chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane-N,N',N,N'-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N''-pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC).

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a contrast agent which comprises or consists of a paramagnetic agent, preferably, wherein the paramagnetic agent comprises or consists of paramagnetic nanoparticles.

In a second aspect, the present invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect, and, optionally, a pharmaceutically acceptable carrier and/or excipient.

5 In a third aspect, the present invention relates to the compound of the first aspect or the pharmaceutical composition of the second aspect for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject. Preferably, the disease characterized by overexpression of fibroblast activation protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorder.

10 Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is cancer, the cancer is selected from the group consisting of breast cancer, pancreatic cancer, small intestine cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer, hepatocellular carcinoma, esophageal cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocellular carcinoma, clear cell renal carcinoma, neuroendocrine tumor, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus carcinoma, desmoid tumors, glioma, astrocytoma, cervix carcinoma and prostate cancer. Preferably, the cancer is glioma, breast cancer, colon cancer, lung cancer, head and neck cancer, liver cancer or pancreatic cancer. More preferably, the cancer is glioma.

20 Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is chronic inflammation, the chronic inflammation is selected from the group consisting of rheumatoid arthritis, osteoarthritis and Crohn's disease. Preferably, the chronic inflammation is rheumatoid arthritis.

25 Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is fibrosis, the fibrosis is selected from the group consisting of pulmonary fibrosis, such as idiopathic pulmonary fibrosis and liver cirrhosis.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is tissue remodeling, the tissue remodeling occurs after myocardial infarction.

30 Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is a keloid disorder, the keloid disorder is selected from the group consisting of scar formation, keloid tumors and keloid scar.

In a fourth aspect, the present invention relates to a kit comprising or consisting of the compound of the first aspect or the pharmaceutical composition of the second aspect and

instructions for the diagnosis or treatment of a disease. Preferably, the disease is a disease as specified above.

Examples

5 Example 1: Compound synthesis and radiochemistry

Based on a FAP- α specific inhibitor (Jansen et al., *ACS Med Chem Lett*, 2013) two radiotracers were synthesized. Radioiodine labeled FAPI-01 was obtained via an organotin stannylated precursor, which was prepared through palladium catalyzed bromine/tin exchange. FAPI-02 is a precursor for the chelation of radio metals which was synthesized in five steps. By application
10 of the same or slightly modified procedures additional compounds were prepared. The structures of these compounds are listed in table 1 and 2. Radioiodinations of the stannylated precursor were performed with peracetic acid. For chelation with Lu-177 and Ga-68 the pH of the reaction mixture was adjusted with sodium acetate and heated to 95 °C for 10 min. Stability in human serum was analyzed by precipitation and radio-HPLC analysis of the supernatant.

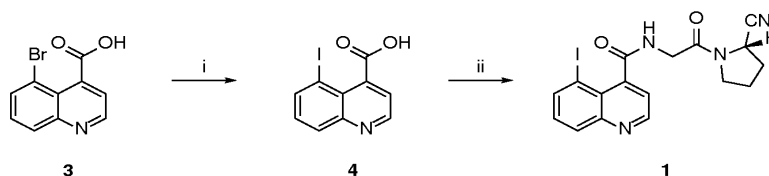
15

Reagents

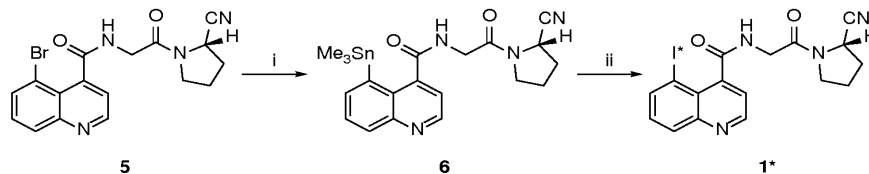
All solvents and non-radioactive reagents were obtained in reagent grade from ABCR (Karlsruhe, Germany), Sigma-Aldrich (München, Germany), Acros Organics (Geel, Belgium) or VWR (Bruchsal, Germany) and were used without further purification. Atto 488 NHS-ester
20 was obtained from AttoTec (Siegen, Germany). 2,2',2''-(10-(2-(4-nitrophenyl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-triyl)triacetic acid (DOTA-PNP) was synthesized following the protocol of Mier et al. (Mier et al., *Bioconjug Chem*, 2005). The intermediates 6-methoxyquinoline-4-carboxylic acid (**7**), 5-bromoquinoline-4-carboxylic acid (**3**) and (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate were
25 synthesized following the protocols of Jansen et al. (Jansen et al., *ACS Med Chem Lett*, 2013). The substance (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-bromoquinoline carboxamide was synthesized by a modified HBTU amidation protocol.

Compound synthesis

30 Scheme 1 depicts the initial synthesis of FAPI-01 which was achieved by performing a Br/Li-exchange with *n*-butyllithium at 5-bromoquinoline-4-carboxylic acid (**3**) and quenching with elemental iodine to obtain iodoquinoline **4**. This compound was coupled to the Gly-Pro-CN fragment by HBTU/HOBt-activation to provide non-radioactive reference material of FAPI-01 (**1**).

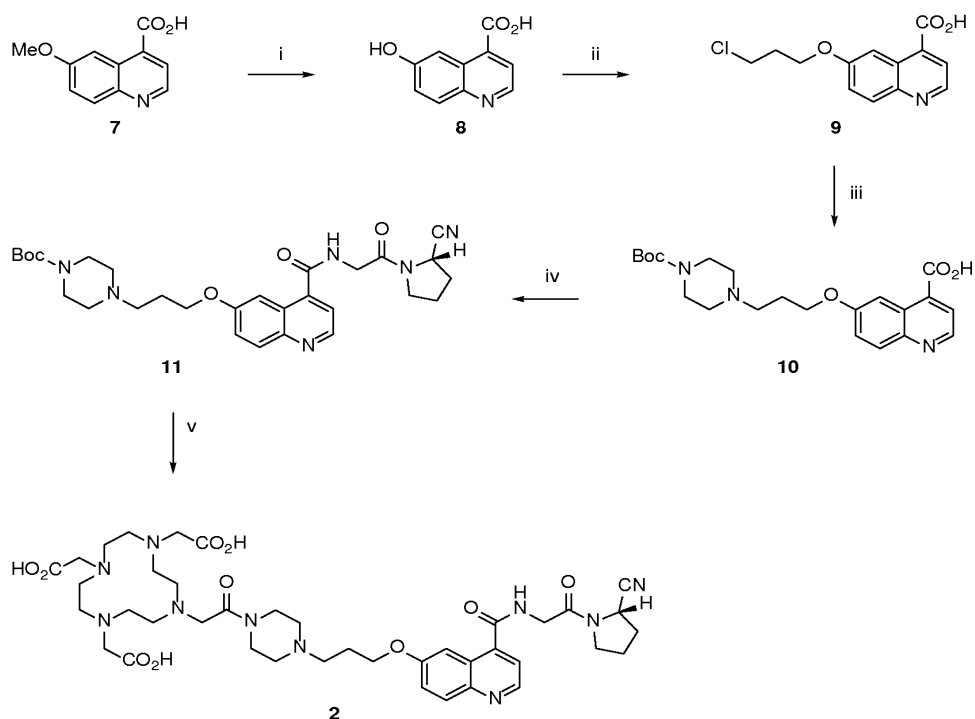


Scheme 1. Synthesis of non-radioactive FAPI-01. i) *n*BuLi, then I₂, THF; ii) HBTU/HOBt, DIPEA, H-Gly-Pro-CN, DMF. For the synthesis of radioactive FAPI-01 (**1***), the stannylated precursor **6** was obtained by palladium-catalyzed stannylation of inhibitor **5** in dioxane at 80°C (Scheme 2).



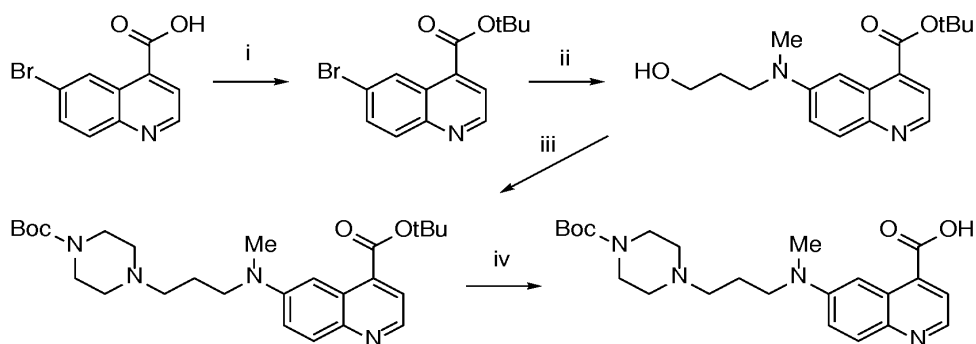
Scheme 2. Synthesis of radioactive FAPI-1 via the stannylated precursor **4**. i) (Me₃Sn)₂; (PPh₃)₂PdCl₂; dioxane 80 °C; ii) I-125 or I-131; AcOOH; 1 M HCl; MeOH.

To enable radiolabeling by incorporation of radiometals, the chelator DOTA was chemically linked to the basic scaffold of the FAP-inhibitor. As shown by Jansen et al. (Jansen et al., *ACS Med Chem Lett*, 2013), modifications at the 6-position of the quinoline-4-carboxylic acid are well tolerated without impairing target affinity and specificity. Therefore, a bifunctional linker was attached to the hydroxyl group of **8** via an ether linkage, leading way to the synthesis shown in Scheme 3. Ready available 1-bromo-3-chloropropane was chosen to create a spacer, which is unharmed during the saponification of the simultaneously formed ester bond at the end of the one-pot-process. Compound **9** was converted to the *N*-Boc protected quinolinecarboxylic acid **10** which was further coupled to H-Gly-Pro-CN by HBTU. Due to the high hygroscopicity of the free amine, compound **11** was directly converted to FAPI-02 (**2**) after the Boc-removal, solvent exchange and neutralization of excess *p*-toluenesulfonic acid.

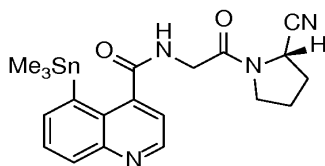


Scheme 3. Chemical synthesis of FAPI-02. i) aq. HBr 48%, 130 °C; ii) 1-bromo-3-chloropropane, Cs₂CO₃, DMF then 6 M NaOH; iii) 1-Boc-piperazine, KI, DMF; iv) HBTU/HOBt, DIPEA, H-Gly-Pro-CN, DMF; v) TosOH, MeCN, then DOTA-PNP, DIPEA, DMF.

- 5 In case of compounds incorporating group A ≠ O, the quinoline-4-carboxylic acid intermediates were synthesized by a different reaction scheme. The key step of this approach is a palladium catalyzed coupling reaction (e.g. Buchwald-Hartwig cross-coupling), which requires additional protection before and deprotection of the carboxylic acid function after the cross-coupling reaction (scheme 4).



Scheme 4. Synthesis of the building block 6-(3-(4-Boc-piperazin-1-yl)propyl)-1-(methylamino)quinoline-4-carboxylic acid for the synthesis of FAPI-46. i) DCC, tBuOH, CuCl; ii) 3-methylamino-1-propanol, Cs₂CO₃, Pd₂(dba)₃, BINAP; iii) MsCl, NEt₃, DCM then 1-Boc-piperazine, KI, DMF; iv) TFA then Boc₂O, NEt₃, DMF.



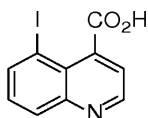
(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-trimethylstannylquinoline carboxamide (**6**)

3.88 mg (10.0 μ mol) (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-bromoquinoline carboxamide, 20 μ L (32 mg; 96 μ mol) hexamethylditin and 0.75 mg (1.07 μ mol)

- 5 bis(triphenylphosphine)palladium(II) dichloride in 1 mL dry dioxane are stirred at 80 °C overnight under an inert atmosphere. Volatiles are removed and the residue is taken up in 2 mL 50% acetonitrile/water and filtered through a C18-light cartridge before HPLC-purification. 2.78 mg (5.90 μ mol; 59%) of the product are obtained after freeze drying.

LC-MS R_t 14.77 min, m/z 473.0786 [$M(^{120}\text{Sn})+H$] $^+$

10

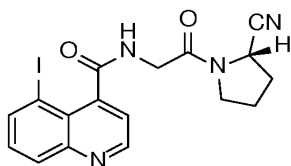


5-iodoquinoline-4-carboxylic acid (**4**)

5.42 mg (136 μ mol) of sodium hydride suspension (60% in mineral oil) are added to an solution of 30.27 mg (120 μ mol) 5-bromoquinoline-4-carboxylic acid (**3**) in 3 mL dry THF under Ar at

- 15 0°C. The ice bath is removed and the reaction mixture is cooled to -78 °C before 100 μ L (160 μ mol) *n*BuLi (1.6 M in hexanes) are added dropwise. After 15 min 64.71 mg (254 μ mol) iodine in 2 mL THF are added dropwise and the reaction is stirred for 30 min at -78 °C before allowed to reach room temperature. After 1 h the reaction is quenched by addition of 1 mL 0.5 M NaHCO_3 and ca. 30 mg (170 μ mol) sodium dithionite to remove excessive iodine. After the
- 20 removal of THF under reduced pressure the mixture is acidified to pH 2 and extracted three times with ethyl acetate (25 mL). The combined organic phases are evaporated to dryness and purified by HPLC. 18.14 mg (60.7 μ mol; 45%) of the title compound are obtained after freeze drying.

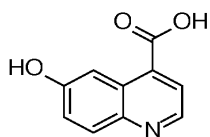
- 25 **^1H NMR** (500 MHz, DMSO-d_6) 13.95 (br, 0.3H), 8.93 (s, 1H), 8.34 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.60 (s, 1H), 7.52 (t, J = 7.9 Hz, 1H); **^{13}C NMR** (125 MHz, DMSO-d_6) 168.8, 150.3, 148.8, 141.3, 130.6, 121.0, 109.5; **LC-MS** R_t 8.65 min, m/z 299.9383 [$M+H$] $^+$



(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-trimethylstannylquinoline-4-carboxamide (**1**; FAPI-01)

9.07 mg (23.9 μ mol) HBTU in 50 μ L DMF are added to a solution of 6.21 mg (20.8 μ mol) 5-iodoquinoline-4-carboxylic acid, 7.45 mg (55.2 μ mol) HOBt and 10 μ L DIPEA in 50 μ L DMF. After 15 min (29.9 μ mol) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate in 50 μ L DMF are added. The reaction is quenched with 850 μ L water and purified by HPLC. Freeze drying provides 6.86 mg (15.8 μ mol; 76%) of the product.

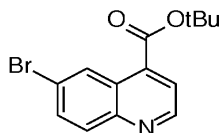
¹H NMR (600 MHz, DMSO-*d*₆) 9.06, 8.97, 8.33, 8.13, 7.56, 7.51, 4.81, 4.34, 4.06, 3.74, 3.56, 2.21, 2.17, 2.09, 2.05; **¹³C NMR** (150 MHz, DMSO-*d*₆) 167.1, 150.2, 148.8, 145.3, 141.5, 130.7, 125.3, 121.9, 119.3, 92.0, 46.3, 45.4, 42.1, 29.5, 24.9; **LC-MS** *R*_t 11.95 min, *m/z* 435.0102 [M+H]⁺



15 6-Hydroxyquinoline-4-carboxylic acid (**8**)

105 mg (477 μ mol) of raw 6-methoxyquinoline-4-carboxylic acid (**7**) are dissolved in 3 mL of 48% hydrobromic acid in water. The solution is heated to 130 °C for 4 h. The solution is brought to a slightly basic pH with 6 M NaOH after reaching room temperature. 79.2 mg (419 μ mol; 88%) of the product are obtained after by HPLC-purification and lyophilization.

¹H NMR (500 MHz, DMSO-*d*₆) 13.65 (br, 0.6H) 10.24 (s, 1H), 8.78 (d, *J* = 4.4 Hz, 1H), 8.06 (d, *J* = 2.6 Hz, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.84 (d, *J* = 4.4 Hz, 1H), 7.37 (dd, *J* = 9.1, 2.6 Hz, 1H), **¹³C NMR** (125 MHz, DMSO-*d*₆) 167.7, 156.9, 146.5, 144.1, 133.4, 131.2, 126.2, 122.3, 122.6, 106.5; **LC-MS** *R*_t 6.66 min, *m/z* 190.0415 [M+H]⁺

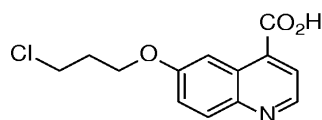


25

tert-butyl 6-bromoquinoline-4-carboxylate

98.3 mg (390 μ mol) 6-bromoquinoline-4-carboxylic acid (raw) were suspended in 5 mL tetrahydrofuran and 25.0 μ L (18.3 mg; 181 μ mol) triethylamine and added to *O*-*tert*-butyl-*N,N'*-dicyclohexylisourea (prepared the day before from neat 426 mg (2.07 mmol) dicyclohexylcarbodiimide, 173 mg (2.33 mmol) *tert*-butanol and 10.2 mg (103 μ mol) copper(I)chloride). The mixture was heated to 50 $^{\circ}$ C over night. The mixture was filtered, solvents evaporated and the product isolated by HPLC. 49.7 mg (161 μ mol; 41%) of the title compound were obtained after freeze drying.

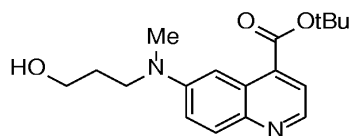
LC-MS R_t 20.40 min, m/z 251.9642 $[M-tBu]^+$



10 6-(3-chloro-1-propoxy)quinoline-4-carboxylic acid (**9**)

42.4 μ L (67.4 mg; 430 μ mol) 1-bromo-1-chloropropane are added to a suspension of 23.2 mg (123 μ mol) 6-hydroxyquinoline-4-carboxylic acid (**8**) and 190 mg (1.38 μ mol) potassium carbonate in 250 μ L DMF and heated to 60 $^{\circ}$ C over night. The reaction mixture is cooled to room temperature, diluted with 500 μ L water and 500 μ L acetonitrile before 100 μ L 6 M NaOH are added. The reaction mixture is directly purified via HPLC (5-40%) after the complete ester hydrolysis is accomplished. 26.45 mg (99.4 μ mol; 81%) of the product are obtained after lyophilization.

^1H NMR (500 MHz, DMSO- d_6) 13.75 (br, 0.4H), 8.88 (d, J = 4.4 Hz, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 4.4 Hz, 1H), 7.52 (dd, J = 9.2, 2.0 Hz, 1H), 4.24 (t, J = 5.95 Hz, 2H), 3.85 (t, J = 6.5 Hz, 2H), 2.27 (m, 2H); **^{13}C NMR** (125 MHz, DMSO- d_6) 167.6, 157.5, 147.6, 144.8, 134.0, 131.2, 125.9, 122.7, 122.2, 104.5, 64.7, 41.9, 31.6; **LC-MS** R_t 11.46 min, m/z 266.0461 $[M+H]^+$

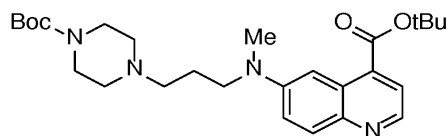


25 *tert*-butyl 6-(3-hydroxypropylmethylamino)quinoline-4-carboxylate

204.6 mg (664 μ mol) *tert*-butyl 6-bromoquinoline-4-carboxylate, 34.10 mg (54.7 μ mol) BINAP, 21.51 mg (23.5 μ mol) $Pd_2(dba)_3$ and 480.3 mg (1.47 mmol) cesium carbonate were dissolved in 6 mL toluene and 128.0 μ L (118 mg; 1.32 mmol) *N*-methyl-1,3-propanolamine were added. The mixture was stirred at 90 $^{\circ}$ C over night before solvents were removed, the

residue suspended in water/acetonitrile 1:1 and filtered before HPLC-purification. 172.7 mg (547 μ mol; 82%) of the title compound were obtained after freeze drying.

LC-MS R_t 13.41 min, m/z 261.1213 $[M-tBu+H]^+$



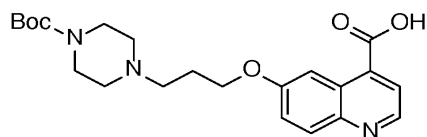
5

tert-butyl 6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methylamino)quinoline-4-carboxylate (62.8 mg (199 μ mol) *tert*-butyl 6-(3-hydroxypropylmethylamino)quinoline-4-carboxylate were dissolved in 5 mL dichloromethane and 90.0 μ L (66.6 mg; 659 μ mol) triethylamine. 20.0 μ L (29.6 mg; 258 μ mol) methanesulfonyl chloride were added at 0 $^{\circ}$ C and the mixture reacted for 60 min. 194.6 mg (1.05 mmol) 1-Boc-piperazine were added before volatiles were removed. 500 μ L dimethylformamide and 47.4 mg (286 μ mol) potassium iodide were added to the residue. The mixture was shaken at 60 $^{\circ}$ C for 120 minutes before the product was isolated by HPLC. 81.05 mg (167 μ mol; 84%) of the title compound were obtained after freeze drying.

10

LC-MS R_t 13.99 min, m/z 485.3086 $[M+H]^+$

15



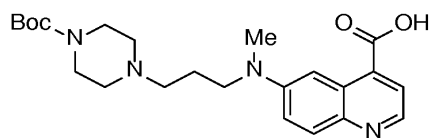
6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxylic acid (**10**)

15.13 mg (56.9 μ mol) of 6-(3-chloro-1-propoxy)quinoline-4-carboxylic acid (**9**), 55.43 mg (298 μ mol) *N*-*tert*-butoxycarbonylpiperazine and 51.05 mg (30.8 μ mol) potassium iodide are dissolved in 250 μ L DMF. The reaction is shaken at 60 $^{\circ}$ C over night. The resulting suspension is diluted with 750 μ L water before the product is purified by HPLC. After freeze drying 28.73 mg (54.3 μ mol; 95%) of the product are obtained as the corresponding TFA-salt.

20

^1H NMR (500 MHz, D_2O) 8.93 (d, J = 5.5 Hz, 1H), 8.17 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 5.5 Hz, 1H), 7.79 (dd, J = 9.3, 2.5 Hz, 1H), 7.65 (d, J = 2.5 Hz, 1H), 4.36 (t, J = 5.6 Hz, 2H), 4.27 (d, J = 13.55 Hz, 2H), 3.67 (d, J = 11.95 Hz), 3.47 (t, J = 15.5 Hz, 2 H), 3.27 (t, J = 12.7 Hz), 3.12 (td, J = 12.2, 2.65 Hz), 2.37 (m2 H), 1.47 (s, 9H); **^{13}C NMR** (125 MHz, D_2O) 155.5, 153.5, 149.0, 141.4, 134.4, 127.9, 126.6, 122.3, 118.4, 110.0, 105.1, 82.8, 65.5, 54.3, 51.5, 48.6, 40.7, 29.6, 27.4; **LC-MS** R_t 10.62 min, m/z 416.1997 $[M+H]^+$

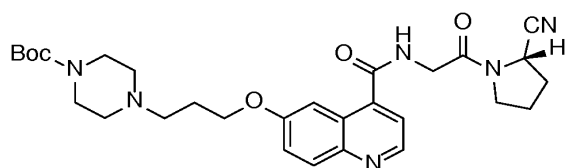
25



6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxylic acid

100.12 mg (206 μmol) *tert*-butyl 6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxylate were treated with 900 μL trifluoroacetic acid, 25 μL triisopropylsilane, 25 μL water and 50 μL trifluoromethanesulfonic acid for 60 min. The deprotected compound was precipitated with diethyl ether, dried and reacted with 60.83 mg (279 μmol) di-*tert*-butyldicarbonate and 50.0 μL (36.5 mg; 361 μmol) triethylamine in 1 mL dimethylformamide for another 60 min. 55.42 mg (129 μmol ; 65% over 2 steps) were obtained after HPLC-purification and freeze-drying.

10 **LC-MS** R_t 10.52 min, m/z 429.2463 $[\text{M}+\text{H}]^+$



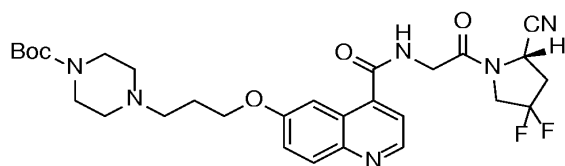
(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide (**11**)

15 9.43 mg (24.9 μmol) HBTU in 50 μL DMF are added to a solution of 10.56 mg (19.9 μmol) 6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxylic acid (**10**), 5.38 mg (39.8 μmol) HOBT and 10 μL DIPEA in 50 μL DMF. After 15 min (29.9 μmol) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate in 50 μL DMF are added. The reaction is quenched with 850 μL water and purified by HPLC. Freeze drying provides

20 12.88 mg (19.4 μmol ; 97%) of the title compound.

^1H NMR (500 MHz, DMSO- d_6) 9.04 (d, J = 5.5 Hz, 1H), 8.24 (d, J = 9.6 Hz, 1H), 8.10 (d, J = 5.5 Hz, 1H), 7.89 (d, J = 2.3 Hz, 1H), 7.85 (dd, J = 9.6, 2.3 Hz, 1H), 4.84 (t, J = 6 Hz, 1H), 4.46–4.36 (m, 4H), 4.26 (d, J = 12.0 Hz, 2H), 3.83 (m, 1H), 3.67 (m, 3H), 3.47 (t, J = 7.7 Hz, 2H), 3.27 (br, 2H), 3.11 (t, J = 11.5 Hz), 2.37 (m, 4H), 2.22 (m, 2H), 1.46 (s, 9H); **^{13}C NMR** (125 MHz, DMSO- d_6) 168.6, 168.0, 159.4, 155.5, 147.7, 141.8, 135.1, 128.2, 127.5, 123.1, 120.0, 119.1, 104.7, 82.9, 66.0, 54.3, 51.5, 47.0, 46.3, 42.3, 29.4, 27.4, 24.7, 23.1; **LC-MS** R_t 11.81 min, m/z 551.2736 $[\text{M}+\text{H}]^+$

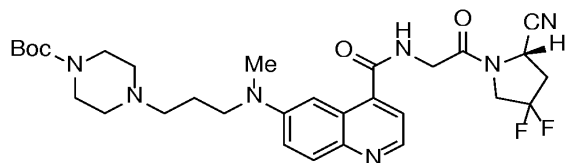
25



(*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonyl)piperazin-1-yl)-1-propoxyquinoline-4-carboxamide

13.2 mg (22.4 μ mol; 75%) were obtained following the previous protocol.

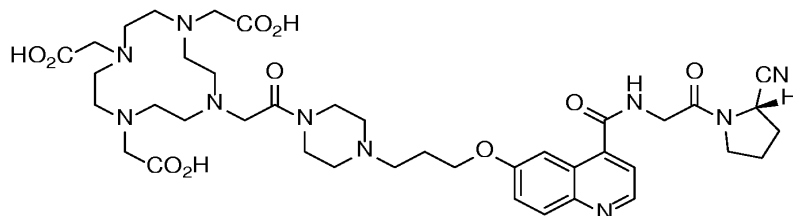
5 **LC-MS** R_t 11.84 min, m/z 605.2610 $[M+H]^+$



N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methylamino)quinoline-4-carboxamide

10 1.17 mg (1.95 μ mol; 92%) were obtained following the previous protocol.

LC-MS R_t 12.66 min, m/z 600.3057 $[M+H]^+$



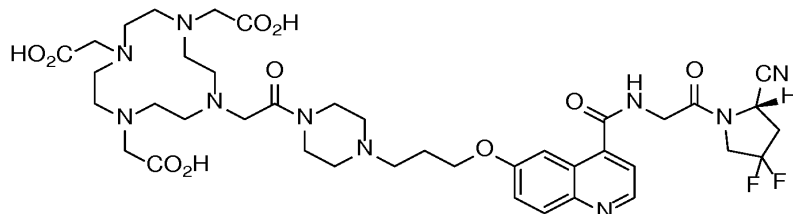
FAPI-02 (**2**)

15 4.85 mg (8.80 μ mol) (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonyl-piperazin-1-yl)-1-propoxy)quinoline-4-carboxamide (**11**) are dissolved in 1 mL acetonitrile and 4.2 mg (22.0 μ mol) 4-methylbenzenesulfonic acid monohydrate are added. The reaction is shaken at 45 $^{\circ}$ C over night, before volatiles are removed under reduced pressure. The residue is taken up in 190 μ L dimethylformamide and 10 μ L (7.3 mg; 72 μ mol) triethylamine before 6.77 mg (12.9 μ mol) of DOTA-*p*-nitrophenol ester are added. The reaction mixture is diluted with 1 mL water and purified by HPLC after shaking for two hours. 5.04 mg (6.02 μ mol; 68%) are obtained after freeze drying.

^1H NMR (600 MHz, D_2O) 9.02, 8.23, 8.07, 7.87, 7.83, 4.85, 4.45, 4.41, 4.40, 4.39, 3.83, 3.67, 3.50, 3.49, 2.40, 2.38, 2.36, 2.26, 2.22, 2.16; **^{13}C NMR** (150 MHz, D_2O) 167.9, 159.1, 147.2,

141.8, 135.4, 127.9, 127.2, 119.8, 119.0, 104.5, 65.8, 54.1, 46.8, 46.1, 42.1, 29.2, 24.5, 23.0:

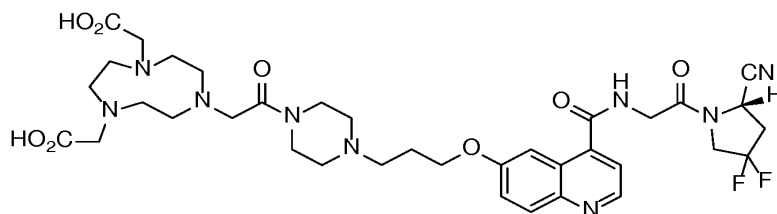
LC-MS R_t 8.37 min, m/z 837.3872 $[M+H]^+$



5 FAPI-04

3.97 mg (4.55 μ mol; 57%) were obtained following the previous protocol.

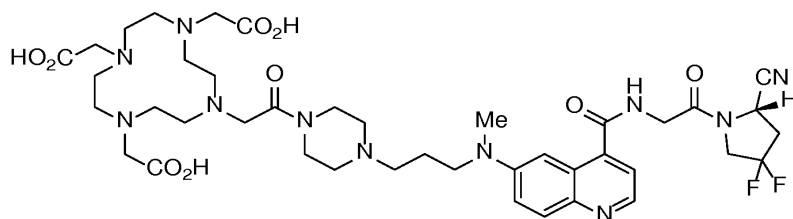
LC-MS R_t 8.80 min, m/z 873.3664 $[M+H]^+$



10 FAPI-42

1.91 mg (2.47 μ mol; 88%) were obtained following the previous protocol.

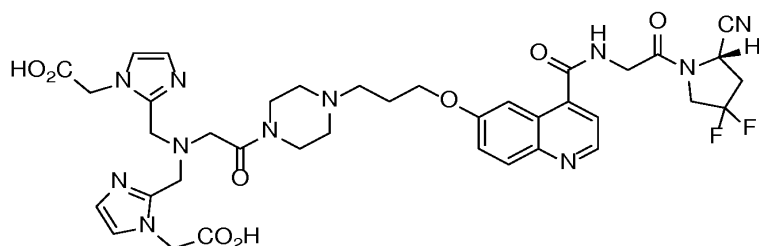
LC-MS R_t 9.37 min, m/z 386.6807 $[M+2H]^{2+}$



15 FAPI-46

39.21 mg (44.3 μ mol; 85%) were obtained following the previous protocol.

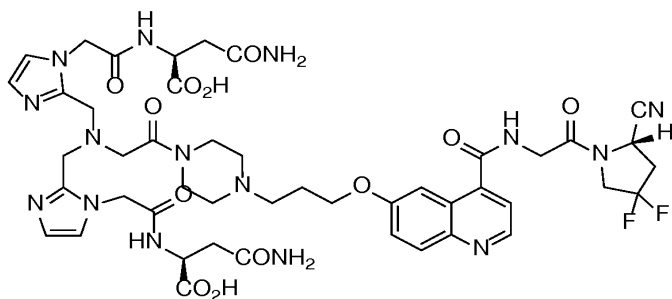
LC-MS R_t 9.03 min, m/z 443.7196 $[M+2H]^{2+}$



FAPI-19

1.09 mg (1.86 μ mol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were Boc-deprotected by the method applied for FAPI-02 and reacted with 2.74 mg (5.91 μ mol) bis((1-(2-(*tert*-butoxy)-2-oxoethyl)-1H-imidazol-2-yl)methyl)glycine, which were preactivated with 2.13 mg (5.62 μ mol) HBTU and 2.50 μ L (1.85 mg; 14.3 μ mol) DIPEA. After HPLC purification and solvent removal the residue was treated with 200 μ L of 2.5% trifluoromethanesulfonic acid in acetonitrile/trifluoroacetic acid 1:1. After precipitation with diethyl ether and HPLC purification 1.06 mg (1.29 μ mol; 70%) of the title compound were obtained.

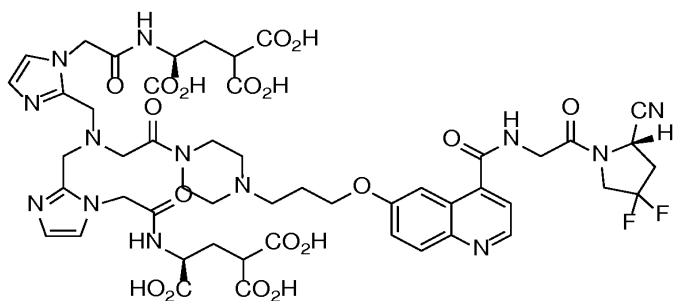
LC-MS R_t 8.91 min, m/z 820.2933 $[M+H]^+$



FAPI-28

1.00 μ L (0.74 mg; 5.73 μ mol) DIPEA was added to a solution of 0.95 mg (1.16 μ mol) FAPI-19, 0.42 mg (3.14 μ mol) HOBt and 1.10 mg (2.89 μ mol) HBTU in 50 μ L DMF. After 10 min 2.30 mg (5.34 μ mol) H-Asn(Trt)-OtBu were added and reacted for 120 min. The *tert*-butyl protecting groups were removed by 2.5% TFOH in TFA/acetonitrile 8:2. After HPLC-purification and freeze-drying, 0.79 mg (0.75 μ mol; 65%) of the title compound were obtained.

LC-MS R_t 9.23 min, m/z 524.7100 $[M+2H]^{2+}$

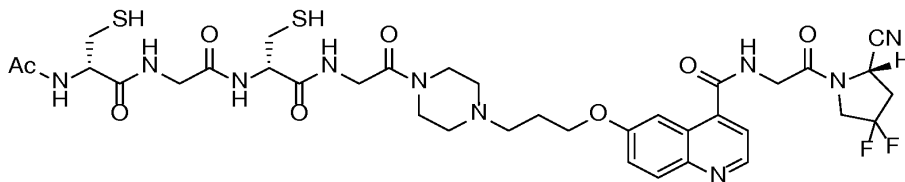


FAPI-34

1.01 mg (0.87 μmol ; 52%) were obtained following the previous protocol.

LC-MS R_t 8.87 min, m/z 583.6988 $[\text{M}+2\text{H}]^{2+}$

5

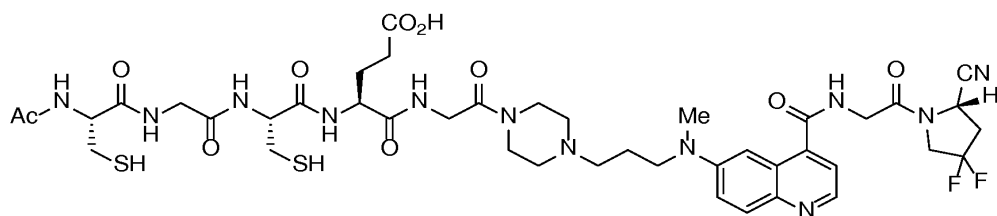


FAPI-60

3.91 mg (6.66 μmol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were deprotected for 30 min by 50 μL acetonitrile and 100 μL trifluoroacetic acid. After evaporation of the solvents and washing with diethyl ether a 10 min preincubated mixture of 8.02 mg (9.27 μmol) acetyl-Cys(Trt)-Gly-Cys(Trt)-Gly-OH, 4.31 mg (31.9 μmol) HOBt and 4.47 mg (11.8 μmol) HBTU in 150 μL dimethylformamide and 2.50 μL (1.85 mg; 14.3 μmol) DIPEA was added to the residue and reacted for 120 min. 4.66 mg (3.49 μmol ; 52%) of the *S*-trityl protected title compound were obtained after HPLC-purification and freeze-drying.

3.36 mg (2.52 μmol) of the trityl protected compound were dissolved in 50 μL acetonitrile. 3 μL Triethylsilane were and 100 μL trifluoroacetic acid were added and reacted for 30 min. 2.01 mg (2.36 μmol ; 94%; 49% over two steps) of the title compound were obtained after HPLC purification and freeze-drying.

LC-MS R_t 10.26 min, m/z 871.2703 $[\text{M}+\text{Na}]^+$

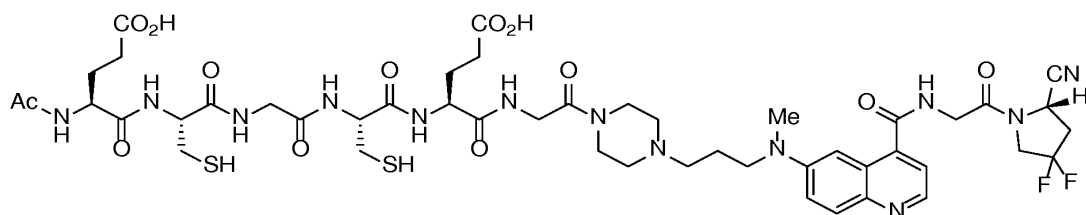


FAPI-69

0.59 mg (0.60 μmol ; 39%) were obtained following the previous protocol.

LC-MS R_t 10.25 min, m/z 991.3490 $[\text{M}+\text{H}]^+$

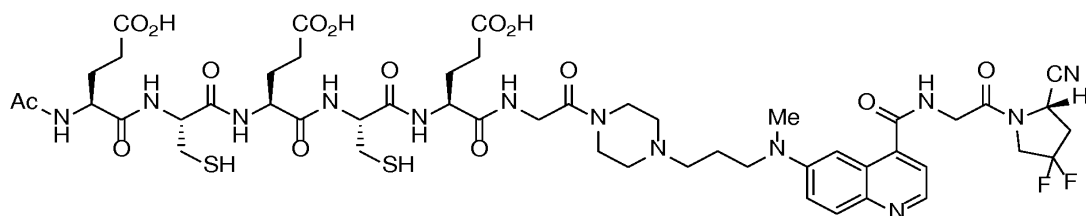
5



FAPI-70

0.61 mg (0.54 μmol ; 33%) were obtained following the previous protocol.

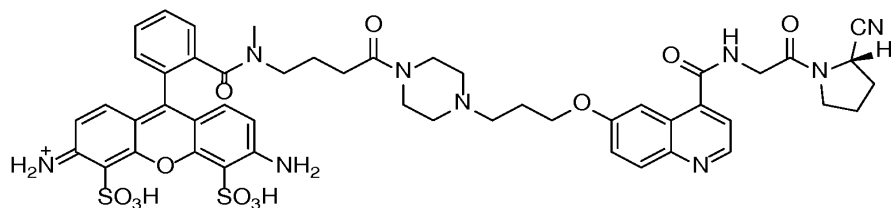
10 **LC-MS** R_t 10.14 min, m/z 1120.3884 $[\text{M}+\text{H}]^+$



FAPI-71

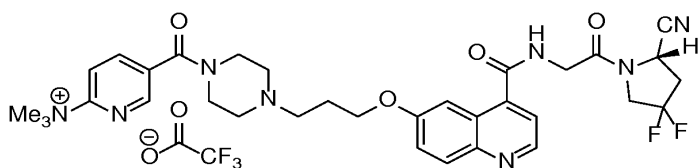
0.79 mg (0.66 μmol ; 34%) were obtained following the previous protocol.

15 **LC-MS** R_t 10.17 min, m/z 596.7075 $[\text{M}+2\text{H}]^{2+}$

Atto488-FAPI-02 (**14**)

0.66 mg (1.20 μmol) of **11** are treated with 1.33 mg (6.96 μmol) 4-methylbenzenesulfonic acid monohydrate in 250 μL acetonitrile at 45 $^{\circ}\text{C}$ for 4 hours. After removal of the solvent the residue is dissolved in 95 μL dimethylformamide and 5 μL (3.65 mg; 36.1 μmol) triethylamine. 0.54 mg (0.55 μmol) Atto 488 NHS-ester in 25 μL DMSO were added. After 60 minutes 0.49 mg (0.43 μmol ; 78%) of the title compound were isolated by HPLC and freeze drying.

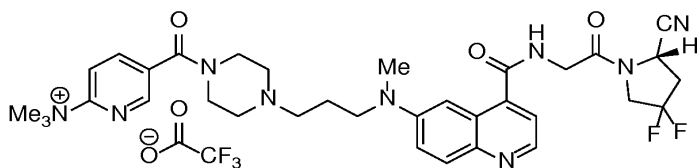
LC-MS R_t 10.19 min, m/z 1022.2706 $[\text{M}]^+$



FAPI-73

10.95 mg (18.7 μmol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were deprotected for 30 min by 100 μL acetonitrile and 200 μL trifluoroacetic acid. After evaporation of the solvents and washing with diethyl ether 15.02 mg (9.27 μmol) *N,N,N*-trimethyl-5-((2,3,5,6-tetrafluorophenoxy)-carbonyl)pyridine-2-aminium chloride was added and the mixture dissolved in 200 μL dimethylformamide and 10.0 μL (7.30 mg; 72.3 μmol) triethylamine. After 120 min the mixture was purified by HPLC and 11.24 mg (14.7 μmol ; 79%) of the title compound were obtained freeze-drying.

LC-MS R_t 9.37 min, m/z 649.2892 $[\text{M}-\text{CF}_3\text{CO}_2]^+$



FAPI-72

9.80 mg (12.6 μmol ; 70%) were obtained following the previous protocol.

LC-MS R_t 9.28 min, m/z 662.3237 $[\text{M}-\text{CF}_3\text{CO}_2]^+$

25 Compound analysis

Reverse-phase high-performance liquid chromatography (RP-HPLC) was conducted using linear gradients of acetonitrile in water (0-100% acetonitrile in 5 min; 0.1% TFA; flowrate 2 mL/min) on a Chromolith Performance RP-18e column (100 $\times$ 3 mm; Merck KGaA Darmstadt,

Germany). UV-absorbance was detected at 214 nm. An additional γ -detector was used for the HPLC-analysis of radioactive compounds. HPLC-MS characterization was performed on an ESI mass spectrometer (Exactive, Thermo Fisher Scientific, Waltham, MA, USA) connected to an Agilent 1200 HPLC system with a Hypersil Gold C18 1.9 μ m column (200 $\times$ 2.1 mm; 0-100% acetonitrile in 20 min; flowrate 200 μ L/min). Analytical Radio-HPLC was performed using a Chromolith Performance RP-18e column (100 $\times$ 3mm; Merck; 0-30% acetonitrile in 10 min; flowrate 2 mL/min). HPLC-purifications were performed on a LaPrep P110-System (Knauer, Berlin, Germany) and a Reprosil Pur 120 column (C18-aq 5 μ m 250 $\times$ 25mm; Dr. Maisch, Ammerbuch-Entringen, Germany). The water/acetonitrile-gradient (15 or 25 min; 0.1% TFA; flowrate 20 mL/min) was modified for the individual products.

Radiochemistry

Radioiodine (I-125) was purchased from Hartmann Analytik (Göttingen, Germany); radioactive lutetium (Lu-177) was obtained from ITG (München, Germany); radioactive gallium (Ga-68) was eluted from a Ge-68/Ga-68 generator purchased from Themba Labs (Somerset West, South Africa). Tc-99m was eluted from a Mo-99/Tc-99m generator (Curium Pharma, Berlin, Germany). Cu-64 was provided by UKT Tübingen (Tübingen, Germany). Sm-153 was provided by DSD Pharma (Purkersdorf, Austria). Pb-203 was provided by Lantheus (N. Billerica MA, USA). F-18-FDG and F-18-fluoride were provided by the ZAG Zyklotron AG (Eggenstein, Germany). CRS Kit for tricarbonyl was obtained from Paul Scherrer Institut (Villingen-PSI, Switzerland).

For iodinations 10 μ L of the organotin precursor of FAPI-01 (1 μ mol/mL in ethanol) were diluted with 10 μ L of 1 M HCl and 10 μ L water before 1-20 MBq iodine-125 in 0.05 M NaOH were added. The reaction was started by addition of 5 μ L of a fresh 1.9% solution of peracetic acid in glacial acetic acid. After 60 s 15 μ L of 1 M NaOH were added and the reaction was quenched by addition of 5 μ L of 5% ascorbic acid in water before HPLC purification. The obtained solution was directly used for *in vitro* experiments or evaporated to dryness under reduced pressure and taken up in 0.9% NaCl (Braun, Melsungen, Germany) in case of animal studies.

Cu-64, Lu-177 and Pb-203 labeling of DOTA-compounds was performed by addition of 5 MBq of the radionuclide to 100 μ L of a 10 μ M solution of the individual precursor in 0.1 M NaOAc (pH 5) and incubation at 95 °C for 10 min. The solution is directly used for *in vitro* experiments or diluted with 0.9% NaCl (Braun, Melsungen, Germany) in case of biodistribution studies. For

imaging studies in mice (scintigraphy, PET) the radiotracer was worked up by solid phase extraction (sep-pak light C18, Waters).

Tc(I) labeling was preceded by addition of 1 mL of the Tc-99m-pertechnetate in 0.9% saline to a CRS Kit and incubation for 20 min. After cooling to room temperature a mixture of 25.0 μ L of the precursor (1 mM in water), 150 μ L phosphate buffer (0.4 M, pH 7.4) and 240 μ L hydrochloric acid (1.0 M) was added and the final mixture adjusted to pH 5 if necessary. The reaction was performed at 95 °C for 20 minutes and worked up by solid phase extraction (sep-pak light C18, Waters). For in vivo experiments and animal studies the labeling was performed with one fifth of the reagents and 200 μ L of the CRS Kit solution after Tc(VII) reduction.

Tc(V) labeling was preceded by incubation of 30 μ L SnCl₂-solution containing 200 mM glucoheptonate with 200 μ L Tc-99m-pertechnetate in 0.9% saline for 10 min at room temperature. 5.00 μ L of the precursor (1 mM in water) and 3.75 μ L sodium hydroxide solution (0.1 M in water) were added and the final mixture was reacted at 95 °C for 20 min. For imaging studies in mice (scintigraphy) the radiotracer was worked up by solid phase extraction (sep-pak light C18, Waters).

Labeling with Ga-68 for animal studies was performed by incubating 255 μ L generator eluate (0.6 M HCl; approx. 230 MBq) with a mixture of 1 nmol DOTA-precursor, 1 μ L of 20% ascorbic acid in water and 72 μ L NaOAc (2.5 M) at 95 °C for 10 min. Remaining free radioactivity was removed by dilution with 2 mL water, solid phase extraction (sep-pak light C18, Waters), washing with 2 mL water and elution of the product with 1 mL water/ethanol 1:1. The obtained solution was evaporated to dryness under reduced pressure and the residue taken up in 0.9% NaCl (Braun).

For the formation of AIF-NOTA complexes F-18 fluoride was trapped on a waters Sep-Pak QMA plus light cartridge (46 mg sorbent; preconditioned with 0.5 M NaOAc, pH 3.9), washed with water and eluted with 500 μ L 0.1 M NaOAc (pH 3.9). For animal studies 150 μ L of the eluate were preincubated with 2 μ L of an AlCl₃ solution (10 mM in water) and 50 μ L DMSO. After 5 min the mixture was added to 40 nmol NOTA-precursor (10 μ L of a 4 mM solution in water) and 1 μ L of 20% ascorbic acid in water. The solution was reacted at 95 °C for 15 min. The product was isolated by HPLC (0-20% acetonitrile in 10 min), freed from solvents and taken up in 0.9% saline before injection.

For the formation of 6-fluoronicotinamides F-18 fluoride was trapped on a waters Sep-Pak QMA plus light cartridge (46 mg sorbent; preconditioned with 0.5 M KHCO₃), washed with water, dried and eluted with a mixture of 7.50 mg (19.9 μ mol) cryptofix 222, 1.99 mg (1.99 μ mol) KHCO₃ in 450 μ L acetonitrile and 50 μ L water. After removal of the solvent the residue

was dried by azeotropic distillation with 3×1 mL acetonitrile. The residue was taken up in 100 μ L *tert*-butanol/acetonitril 1:1 and added to 1 mg (ca. 1.3 μ mol) of a trimethylpyridin-2-aminium precursor. The solution was reacted at 75 °C for 10 min. The product was isolated by HPLC (0-30% acetonitrile in 10 min), freed from solvents and taken up in 0.9% saline before injection.

For determination of the stability in human serum the radiolabeled compounds (approx. 2.5 MBq for I-125 or 15 MBq for Lu-177) were purified (HPLC or solid phase extraction) and freed from solvent. The residues were taken up in 250 μ L human serum (Sigma-Aldrich) and incubated at 37 °C. Samples were precipitated with 30 μ L acetonitrile and analyzed by HPLC (0-30% acetonitrile in 10 min).

Example 2: In vitro characterization of FAPI derivatives

In vitro binding studies were performed using the human tumor cell lines BxPC3, Capan-2, MCF-7 (purchased from Sigma Aldrich Chemie GmbH) and SK-LMS-1 (purchased from ATCC) as well as stably transfected FAP-cell lines HT-1080-FAP, HEK-muFAP and the CD26 expressing cell line HEK-CD26 (obtained from Stefan Bauer, NCT Heidelberg). All cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum at 37°C/5% carbon dioxide. For fluorescence internalization experiments, cells were seeded on coverslips and stained with FAPI-02-Atto488 and DAPI for cell nucleus staining. Images were acquired on a laser scanning confocal microscope using a 63x oil immersion objective. Radioligand binding studies were performed using HT-1080-FAP cells. The radiolabeled compound was added to the cell culture and incubated for different time intervals ranging from 10 min to 24 h. Competition experiments were performed by simultaneous exposure to unlabeled (10^{-5} M to 10^{-9} M) and radiolabeled compound for 60 min. For efflux experiments, radioactive medium was removed after incubation for 60 min and replaced by non-radioactive medium for time intervals ranging from 1 to 24 h. For internalization experiments, surface bound activity was removed by incubating the cells with 1 M glycine-HCl buffer for 10 min. The radioactivity was measured using a γ -counter, normalized to 1 mio cells and calculated as percentage of applied dose (%ID).

Cell staining and microscopy

For internalization experiments HT-1080-FAP and HEK muFAP cells were seeded on uncoated coverslips in a 24-well plate and cultivated in culture medium containing 10% fetal calf serum to a final confluence of approx. 80-90%. The medium was removed and cells were washed with

0.5 mL PBS pH 7.4 for 2 times. FAPI-02-Atto488 (20 μ M in DMEM) was added to the cells and incubated for 2 hrs at 37°C. Cells were washed with 0.5 mL PBS pH 7.4 for 3 times and fixed with paraformaldehyde (2% in PBS) for 15 min. The overgrown coverslips were placed on microscope slides using mounting medium containing DAPI for cell nucleus staining (Fluoroshield, Sigma-Aldrich). Images were acquired on a laser scanning confocal microscope (Zeiss LSM 700; Zeiss, Oberkochen, Germany) using the Zeiss Plan-Apochromat 63x/1.4 Oil DIC III immersion objective at xy pixel settings of 0.099 x 0.099 μ m and 1 Airy unit pinhole size for each fluorophore used (488 nm for FAPI-02-Atto488, 405 nm for DAPI). The pictures were processed consistently using the ZEN 2008 software and ImageJ.

Radioligand binding studies

For radioligand binding studies, cells were seeded in 6-well plates and cultivated for 48 h to a final confluence of approx. 80-90% (1.2 - 2 mio cells/well). The medium was replaced by 1 mL fresh medium without fetal calf serum. The radiolabeled compound was added to the cell culture and incubated for different time intervals ranging from 10 min to 24 h. Competition experiments were performed by simultaneous exposure to unlabeled (10^{-5} M to 10^{-9} M) and radiolabeled compound for 60 min. For efflux experiments, radioactive medium was removed after incubation for 60 min and replaced by non-radioactive medium for time intervals ranging from 1 to 24 h. In all experiments, the cells were washed with 1 mL phosphate-buffered saline pH 7.4 for 2 times and subsequently lysed with 1.4 mL lysis buffer (0.3 M NaOH, 0.2% SDS). Radioactivity was determined in a γ -counter (Cobra II, Packard), normalized to 1 mio cells and calculated as percentage of the applied dose (%ID). Each experiment was performed 3 times, and 3 repetitions per independent experiment were acquired.

For internalization experiments the cells were incubated with the radiolabeled compound for 60 min at 37 °C and 4 °C. Cellular uptake was terminated by removing medium from the cells and washing 2 times with 1 mL PBS. Subsequently, cells were incubated with 1 mL of glycine-HCl (1 M in PBS, pH 2.2) for 10 min at room temperature to remove the surface bound activity. The cells were washed with 2 mL of ice-cold PBS and lysed with 1.4 mL of lysis buffer to determine the internalized fraction. For the cells incubated at 4 °C, all washing and elution steps were carried out using ice-cold buffers. The radioactivity was measured using a γ -counter, normalized to 1 mio cells and calculated as percentage of applied dose (%ID).

FAPI-01 selectively targets human and murine FAP- α .

In order to analyze the binding properties of FAPI-01 to its target protein, radioligand binding assays were performed using different cancer cell lines and cell lines transfected with human and murine FAP as well as the closely related membrane protein CD26, also known as DPPIV.

- 5 Both murine FAP and CD26 show a high homology to human FAP- α (muFAP: 90 % identity and 94 % similarity on amino acid level; CD26: 52 % identity and 71 % similarity with high structural resemblance) (Kelly T., *Drug Resist Updat*, 2005).

- As shown in Figure 1A, FAPI-01 shows no significant binding to FAP-negative cancer cell lines while targeting human and murine FAP- α expressing cells with high affinity (IC₅₀ human FAP- α = 39.4 nM). Additionally, no substantial binding to CD26 expressing cells was observed (0.05 $\pm$ 0.01 %), proving that FAPI-01 is selectively targeting FAP- α . This is of particular importance as CD26 is highly expressed in a variety of normal tissues including the kidneys, the liver and the small intestine. To avoid a high background signal due to unspecific CD26-binding, high selectivity of the ligand to FAP- α is of great advantage, resulting in optimal image quality.
- 10
15

FAPI-01 rapidly internalizes in FAP-positive cells but shows time-dependent efflux and robust deiodination.

- Cell-based internalization assays demonstrate a rapid uptake of FAPI-01 into the cells (Figure 1B). After 10 min of incubation, 95 % of the total bound fraction is located intracellularly (total 19.70 $\pm$ 0.28 %). In the course of 4 h, only a marginal decrease in activity is observed (total 17.00 $\pm$ 0.40 %, of which internalized 94 %).
- 20

- Iodine-labeled compounds often show a time-dependent enzymatic deiodination. This was also observed for FAPI-01 resulting in low intracellular radioactivity of this compound after longer incubation times (3.25 $\pm$ 0.29 % after 24 h). Deiodination can be minimized by reduction of deiodinase activity after lowering the temperature to 4°C, resulting in an increased radioactivity of 26.66 $\pm$ 1.59 % after 24 h.
- 25

FAPI-02 shows enhanced binding and uptake to human FAP- α as compared to FAPI-01.

- To avoid rapid loss of activity of FAPI-01 due to enzymatic deiodination, the non-halogen derivative FAPI-02 was designed in which the FAP-binding moiety is chemically linked to the chelator DOTA. In addition to the resulting enhanced stability, this modification offers the possibility to easily incorporate either diagnostic or therapeutic radionuclides, allowing FAPI-02 to be used as a theranostic compound. Similar to its iodized analogue, FAPI-02 specifically
- 30

binds to human and murine FAP- α (IC_{50} human FAP- α = 21 nM) expressing cells without addressing CD26 (%ID = 0.13 ± 0.01 %; Figure 1A). FAPI-02 internalizes rapidly into FAP- α expressing cells (20.15 ± 1.74 %ID after 60 min, of which 96 % internalized; Figure 1B), showing more stable and higher uptake rates in the course of time. Compared to the binding of FAPI-01 after 10 min of incubation, only 5 % of the activity remains after 24 h. In contrast, 34 % of the initial radioactivity of FAPI-02 is detected after 24 h of incubation. Efflux experiments demonstrate that FAPI-02 gets eliminated significantly slower than FAPI-01, showing retention of 12 % of the originally accumulated radioactivity after 24 h (FAPI-01 1.1 % ID after 24 h; Figure 1E).

Robust internalization of FAPI-02 into human and murine FAP- α expressing cells was confirmed by fluorescence laser scanning microscopy. To this end, HT-1080-FAP and HEK-muFAP cells were stained with a fluorescently labeled FAPI-02 derivative (FAPI-02-Atto488) for 1 to 2 hrs. As shown in Figure 1D, the compound gets completely internalized and accumulates in the inner of FAP- α expressing cells whereas no uptake is detectable in FAP- α negative HEK-CD26 cells.

Design of FAPI derivatives with enhanced binding properties and pharmacokinetics

Further variants of FAPI-02 were designed to increase tumor retention time, aiming for the development of a theranostic FAP-targeting agent. The variants FAPI-03 to FAPI-15 have been characterized with respect to target binding, internalization rate and target specificity. The results are shown in Figure 2.

Example 3: PET imaging and biodistribution analysis in mice

All experiments were performed in accordance with the German animal protection laws and complied with European Commission regulations for the care and use of laboratory animals. The mice were anaesthetized using isoflurane inhalation.

For *in vivo* experiments, 8 week old BALB/c *nu/nu* mice (Charles River) were subcutaneously inoculated into the right trunk with 5×10^6 with HT-1080-FAP, Capan-2 or SK-LMS-1 cells, respectively. When the size of the tumor reached approximately 1 cm³, the radiolabeled compound was injected via the tail vein (~ 10 MBq for small-animal PET imaging; ~ 1 MBq for organ distribution). PET imaging was performed up to 140 min after intravenous injection of 1 MBq of Ga-68 labeled compound per mouse using the Inveon PET small-animal PET scanner (Siemens). Images were reconstructed iteratively using the 3D-OSEM+MAP method and were converted to standardized uptake value (SUV) images. Quantification was done using

a ROI technique and expressed as SUV mean. For organ distribution of Lu-177 labeled compound (approx. 10 MBq per mouse), the animals ($n = 3$ for each time point) were sacrificed after indicated time points (from 30 min to 24 h). The distributed radioactivity was measured in all dissected organs and in blood using a γ -counter (Cobra Autogamma, Packard). The values are expressed as percentage of injected dose per gram of tissue (%ID/g).

For pharmacokinetic modeling the transport constant K_1 and the rate constants k_2 – k_4 were calculated using a two-tissue compartment model implemented in the PMOD software [4], taking into account the vascular fraction (v_B), which is associated with the volume of blood exchanging with tissue in a VOI. The rate constants that describe the compartmental fluxes include k_1 (binding to the receptor), k_2 (detachment) as well as k_3 (internalization) and k_4 (efflux) in the tumor tissue. In this model the fractional volume of distribution ($DV = K_1/k_2$) is the proportion of the region of interest in which ^{15}O -labelled water is distributed.

FAPI variants accumulate in human FAP-expressing xenografts as well as in xenografts without FAP expression by recruitment and activation of mouse fibroblasts.

Tumor accumulation of FAPI-02 and -04 was assessed by small-animal PET imaging of mice bearing xenografts from both human FAP-positive and negative tumor cells. In both cases the radiotracer gets rapidly enriched within the tumor and is maintained for at least 140 min (Figure 3A, C, E, G). At the same time, FAPI-02 and -04 show negligibly low unspecific binding and get quickly eliminated from the blood predominantly via the kidneys and bladder resulting in a low background and beneficial tumor-to-organ ratios. Simultaneous administration of unlabeled compound as competitor resulted in a complete absence of radioactivity in the tumor, demonstrating the specificity of the radiotracer to its target protein (Figure 4). Interestingly, a high tumor uptake of FAPI-02 was observed in mice bearing FAP- α positive (HT-1080-FAP) as well as FAP- α negative (Capan-2) tumor cell lines due to recruitment and activation of activated mouse fibroblasts. Pharmacokinetic characteristics of the radiotracer, calculated from PET data using a two-tissue compartment model according to Burger et al., *Nucl Med*, 1997, are given in Table 6.

Pharmacokinetic analysis of FAPI-02

	Unit	Capan-2 - comp.	HT-1080-FAP - comp.	HT-1080-FAP + comp.
vB	l/l	0.08	0.04	0.04
k1	ml/ccm/min	0.08	0.07	0.10
k2	l/min	0.16	0.13	0.32
k3	l/min	0.08	0.10	0.04
k4	l/min	0.05	0.02	0.07
Vs	ml/ccm	0.93	2.31	0.18
Vt	ml/ccm	1.44	0.87	0.48
Flux	ml/ccm/min	0.03	0.03	0.01
Chi²	----	0.10	0.11	0.26

Table 6. Pharmacokinetic characteristics of ⁶⁸Ga-FAPI-02, calculated from dynamic PET data using a two-tissue compartment model according to Burger et al., Nucl Med, 1997. vB: vascular fraction, associated with the volume of blood exchanging with tissue in a VOI (volume of interest); k1-k4: calculated rate constants; Vs: ratio of specific binding concentration to total parent at equilibrium; Vt: total distribution volume.

These observations were confirmed using ¹⁷⁷Lu-FAPI-02 and -04 in a biodistribution study, proving rapid tumor accumulation in both human FAP- α positive and negative tumors with very low activity in all the other organs (quantified uptake values see Table 7), resulting in beneficial tumor-to-organ ratios (Figure 5D-F). Similar results were obtained for ¹⁷⁷Lu-FAPI-04 in HT-1080-FAP tumor bearing mice. Compared to FAPI-02, FAPI-04 shows a higher tumor uptake especially after 24 h (Figure 5C). A calculation of the area under curve (AUC) is shown in Table 8.

	FAP1-02 (Capan-2)	FAP1-02 (HT-1080-FAP)	FAP1-04 (HT-1080-FAP)
Blood	0.83 ± 0.127	1.20 ± 0.178	1.70 ± 0.206
Brain	0.05 ± 0.010	0.06 ± 0.006	0.08 ± 0.010
Heart	0.37 ± 0.031	0.56 ± 0.085	0.80 ± 0.089
Intestines	0.30 ± 0.064	0.37 ± 0.046	0.66 ± 0.196
Kidneys	1.45 ± 0.106	1.60 ± 0.075	2.28 ± 0.477
Liver	0.36 ± 0.015	0.45 ± 0.074	0.73 ± 0.118
Lungs	0.72 ± 0.021	1.02 ± 0.152	1.50 ± 0.151
Muscle	0.94 ± 0.168	1.17 ± 0.332	0.92 ± 0.020
Spleen	0.25 ± 0.015	0.38 ± 0.051	0.48 ± 0.072
Tumor	3.82 ± 0.390	4.51 ± 0.816	4.89 ± 0.817

Table 7. Quantification of biodistribution data 1 h after intravenous administration of Lu-177 labeled FAPI-02 and -04 to tumor bearing Balb/c nude mice; $n=3$; values reported as mean %ID/g ± SD.

%ID/g	1 h	4 h	24 h	AUC
FAP1-02	4.5 ± 0.82	4.0 ± 0.56	1.12 ± 0.13	64.0
FAP1-04	4.9 ± 0.82	5.4 ± 1.51	3.0 ± 0.23	99.4
FAP1-05	6.0 ± 0.90	5.8 ± 0.60	2.8 ± 0.40	103.3
FAP1-10	3.2 ± 0.72	2.9 ± 0.12	1.1 ± 0.04	49.3
FAP1-13	6.3 ± 0.57	8.7 ± 0.77	4.8 ± 1.71	157.5
FAP1-15	3.4 ± 1.13	4.6 ± 0.32	1.1 ± 0.25	68.0

5

Table 8. Tumor uptake of selected FAPI derivatives in HT-1080-FAP tumor bearing nude mice, $n=3$. Values are reported as mean ID/g ± SD).

Example 4: Clinical PET/CT studies.

- 10 Diagnostic imaging of more than 100 patients was performed under the conditions of the updated declaration of Helsinki, § 37 (Unproven interventions in clinical practice) and in accordance to the German Pharmaceuticals Law §13 (2b) for medical reasons using either ^{68}Ga -FAPI-02 or -04, which was applied intravenously (20 nmol, 122-336 MBq), 10 min, 1 and 3
- 15 half-life of ^{68}Ga and the variable elution efficiencies obtained during the lifetime of the $^{68}\text{Ge}/^{68}\text{Ga}$ generator. FDG imaging of one patient was conducted 1 h after intravenous injection of 358 MBq ^{18}F -FDG. The PET/CT scans were performed with a Biograph mCT Flow™

PET/CT-Scanner (Siemens Medical Solution) using the following parameters: slice thickness of 5 mm, increment of 3-4 mm, soft-tissue reconstruction kernel, care dose. Immediately after CT scanning, a whole-body PET was acquired in 3D (matrix 200x200) in FlowMotion™ with 0.7 cm/min. The emission data were corrected for random, scatter and decay. Reconstruction was conducted with an ordered subset expectation maximisation (OSEM) algorithm with 2 iterations/21 subsets and Gauss-filtered to a transaxial resolution of 5 mm at full-width half-maximum (FWHM). Attenuation correction was performed using the low-dose non-enhanced CT data. The quantitative assessment of standardized uptake values (SUV) was done using a region of interest technique.

FAPI-02 and -04 rapidly accumulate in breast, pancreatic, lung, HNO, small intestine and ovarian cancer metastases in humans.

Diagnostic PET/CT scans were performed 1 h after intravenous administration of ^{68}Ga -FAPI-02 and -04 in patients with metastasized breast, lung, pancreatic, HNO, small intestine and ovarian cancer. In all patients a robust accumulation of the tracer was observed in the primary tumor as well as in lymph node and bone metastases with maximum SUV values of 48.0. In contrast, tracer uptake into normal tissue was very low (Figures 6-14). The radioactivity was cleared rapidly from the blood stream and excreted predominantly via the kidneys, resulting in high contrast images. Comparative imaging in one patient with locally advanced lung adenocarcinoma revealed an obvious advantage of FAPI-02 compared to the commonly used PET tracer ^{18}F -FDG. As shown in Figure 9, FAPI-02 shows a higher uptake with lower background activity leading to a higher contrast with better visibility of metastatic lesions. In contrast to FDG, which is highly accumulating in cells with high glucose consumption e.g. the brain, FAPI-02 selectively targets tissues where FAP- α is expressed. Comparative imaging in one patient with prostate cancer revealed an obvious advantage of FAPI-04 compared to the commonly used PET tracers ^{68}Ga -DOTATOC and ^{68}Ga -PSMA, allowing the detection of smaller tumor lesions with reduced tracer accumulation in the kidneys (Figure 14).

Discussion

The reliable diagnosis of primary tumors, metastatic lesions and affected lymph nodes is of utmost importance to allow for effective and adequate therapy planning including tumor staging and choice of treatment. For this purpose, imaging techniques represent indispensable tools for the assessment of many cancer types. Due to its high diagnostic accuracy and the possibility to evaluate both anatomic and physiologic details, combined PET/CT is the method

of choice for modern tumor diagnostics. In contrast to non-invasive imaging techniques such as MRT or CT alone, combined PET/CT, however, requires the use of radiotracers with a high affinity to target structures with enhanced expression in tumors as compared to normal tissues. An ideal tracer should specifically bind to its target protein to ensure reliable differentiation of cancerous and healthy tissue as well as low background signals resulting in high-contrast images. Affinity and specificity become even more important if the radiotracer represents a theranostic compound, i.e. offers the possibility to be loaded with either diagnostic or therapeutic nuclides, which facilitates and improves targeted and personalized treatment. Regarding a potential application of the tracer for therapeutical purposes, high target specificity assures reduced side-effects, which is especially important for the protection of radiation sensitive tissue such as bone marrow, reproductive and digestive organs.

With that in mind, the inventors developed a theranostic tracer targeting cancer-associated fibroblasts which form a major component of the tumor stroma. They are known to play a critical role in tumor growth, migration and progression and are genetically more stable than cancer cells, therefore, being less susceptible to the development of therapy resistance. In contrast to normal fibroblasts, CAFs express particular proteins which can be used as tumor-specific markers. Among these is the membrane protein FAP- α which is broadly expressed in the microenvironment of a variety of tumors and thus enables targeting of different tumor entities including pancreas, breast and lung cancer, which account for a large part of the entirety of solid tumors.

Based on a small molecule enzyme inhibitor with high affinity to its target protein, we developed the radiotracers FAPI-01 to FAPI-73 by focused chemical modification. All compounds show specific binding to human and murine FAP- α with a rapid and almost complete internalization without addressing the closely related protein CD26/DPP4. Since iodinated molecules undergo an enzymatic deiodination with efflux of free iodine, longer incubation times result in a low intracellular radioactivity. On this account, FAPI-02 and subsequent compounds were designed with the FAP-binding moiety being chemically linked to the chelator DOTA. This results in a set of theranostic compounds with favorable pharmacokinetic and biochemical properties, of which FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71, FAPI-72 and FAPI-73 represent the most favored ligands. FAPI-02 and FAPI-04 both get eliminated significantly slower than FAPI-01, with retention of 12 % (FAPI-02) and 49 % (FAPI-04) of the originally accumulated radioactivity after 24 h (FAPI-01 1.1 %) with the other favored compounds having an even stronger binding (figure 16). They rapidly internalize into FAP- α expressing cells and show

high tumor uptake rates in both tumor bearing mice and patients with metastasized epithelial carcinomas. In contrast, there is no accumulation in normal tissue and rapid clearance from the blood system, resulting in high-contrast images. The robust internalization into both human and murine FAP- α expressing cells was confirmed by confocal microscopy using fluorescence-labeled FAPI-02. In contrast to the first generation FAP-antibody F19, which has high affinity to its target protein without being internalized, FAPI-02 shows complete intracellular uptake after 1 h of incubation. The mechanism of internalization after FAP binding has been studied by Fischer et al. using FAP antibody fragments (Fabs) and a DyLight 549 anti-mouse antibody in SK-Mel-187 cells. Incubation at 37 °C led to internalization of the FAP-antibody complexes. As with our small molecule, the internalization process occurred rapidly with an almost complete internalization. Colocalization of the Fabs with a marker for early endosomes was observed after 20 minutes and with a marker for late endosomes and lysosomes after 40 minutes. Fab-mediated FAP- α internalization was suppressed by an inhibitor for dynamin dependent endocytosis, indicating that endocytosis occurs by a dynamin-dependent mechanism.

FAPI-02 and -04 get quickly eliminated from the organism by renal clearance without being retained in the renal parenchyma. In contrast to ^{18}F -FDG, which is highly accumulating in cells with high glucose consumption including inflammatory tissue or the brain, FAPI-02 gets selectively enriched in tissues where its target protein is expressed. This opens new perspectives for the detection of malign lesions in these regions. Additionally, FAP- α was also shown to be expressed by rheumatoid myofibroblast-like synoviocytes in patients with rheumatoid arthritis and osteoarthritis, atherosclerosis, fibrosis as well as in ischemic heart tissue after myocardial infarction. These observations suggest the application of FAPI-02 and -04 as imaging tracers for further indications.

The limiting factor for the detection of tumor lesions is the degree of FAP- α expression within the tumor. This largely depends on the number of activated fibroblasts, i.e. the percentage of stromal content, and/or the number of FAP- α molecules per fibroblast which may be determined by the microenvironment. Since tumor growth exceeding a size of 1 to 2 mm essentially requires the formation of a supporting stroma, visualization of small lesions in the range of 3-5 mm should be possible using FAPI-PET/CT.

As with any other targeted approach, the FAPI derivatives only achieve optimal results in tissues with sufficiently high FAP- α expression which is known to be rather heterogeneous in different cancer types and patients. Besides breast, colon and pancreatic cancer, which are

excellent candidates for FAPI imaging, further analyses have to explore whether other tumor entities such as lung cancer, head and neck cancer, ovarian cancer or hepatomas represent favorable targets.

Also, FAP- α expression was demonstrated in wound healing and fibrotic tissue, which should be kept in mind when interpreting radiological findings. These facts emphasize the necessity to properly evaluate which patients are likely to benefit from a potential FAPI therapy. Given the ability to use either diagnostic or therapeutic nuclides, FAPI-02 and -04 allow simple stratification of the appropriate patient cohort. Either way, it is already clear that both FAPI tracers represent ideal candidates for the development of a targeted radiopharmaceutical. Due to their high target affinity, rapid tumor internalization and fast body clearance, they are already ideally suitable for tumor imaging.

Example 5: FAPI characterization *in vitro* and *in vivo*

Experimental procedures and clinical evaluation

All *in vitro* and *in vivo* experiments as well as the clinical evaluation of the FAPI derivatives have been performed as described above and according to Loktev *et al.*¹ and Lindner *et al.*² A preliminary dosimetry estimate for FAPI-02 and FAPI-04 was based on two patients examined at 0.2 h, 1 h and 3 h post tracer injection using the QDOSE dosimetry software suit. Further PET/CT scans of tumor patients were acquired 1 h after injection of either FAPI-02 (n=25) or FAPI-04 (n=25); for 6 patients an intra-individual related FDG-scan (also acquired 1 h p.i.) was available. For the normal tissue of 16 organs, a 2 cm Spheric-VOI was placed in the parenchyma, for tumor lesions a threshold segmented VOI was used to quantify SUVmean/max³.

In vitro characterization of DOTA-FAPI derivatives

To assess target binding and internalization rate of the DOTA-FAPI derivatives as compared to FAPI-04, Lu-177-labeled compounds were incubated with FAP-expressing HT-1080 cells for 1, 4 and 24 h, respectively (figure 16). The membrane-bound fraction was removed by acidic elution using glycine-HCl pH 2.2 followed by alkaline cell lysis to determine the internalized fraction. As shown in Figure 16, all derivatives demonstrate higher cell binding as compared to FAPI-04 with binding values up to 500 % of the lead compound after 1 h of incubation (up to 750 % after 4 h).

To evaluate target affinity and specificity, competitive binding assays were performed using increasing concentrations of unlabeled compound as competitor of Lu-177-labeled compound

(Figure 17; respective IC₅₀ values listed in Table 9). Specificity of binding was also confirmed in a radioligand binding assay using murine FAP- and CD26-expressing HEK cells (Figure 18).

Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
FAPI-04	6.5	FAPI-39	11.3
FAPI-05	17.2	FAPI-40	12.7
FAPI-10	19.9	FAPI-41	8.3
FAPI-13	4.5	FAPI-46	13.5
FAPI-15	9.1	FAPI-47	42.0
FAPI-20	7.2	FAPI-48	26.3

Table 9. IC₅₀ values of selected FAPI derivatives as determined by competitive binding assays

Organ distribution of DOTA-FAPI derivatives in tumor-bearing mice

- 5 For analysis of pharmacokinetic profile as well as tumor uptake *in vivo*, Lu-labeled DOTA-FAPI derivatives were administered i.v. to HT-1080-FAP tumor-bearing mice. Organ distribution of the radiolabeled compounds was determined *ex vivo* in the blood, healthy tissues and the tumor. As shown in Figure 19, most of the compounds demonstrate higher tumor uptake rates as compared to FAPI-02 and FAPI-04, notably 24 h after administration. Due to increased
- 10 lipophilicity, some of the radiotracers show higher blood activities as well as an increased retention in the kidneys. Determination of tumor-blood-ratios yet reveals a clear advantage of the compounds FAPI-21 and FAPI-46 which demonstrate significantly higher ratios than FAPI-04 at all times examined (Figure 20).

Small-animal imaging of DOTA-FAPI derivatives in tumor-bearing mice

- Based on these findings, small animal PET-imaging was performed using Ga-68-labeled DOTA-FAPI derivatives up to 140 min after i.v. administration of the radiotracers in HT-1080-FAP tumor-bearing mice. The beneficial tumor-blood ratios of FAPI-21 and FAPI-46 result in high contrast images, enabling excellent visualization of the FAP-positive tumors (Figure 21).
- 20 A quantitative analysis of the tracer accumulation in the tumor, the kidneys, the liver and muscle tissue (given as SUV max values) reveals slightly lower muscular, renal and hepatic activities of FAPI-46 as compared to FAPI-21 (Figure 22).

Biodistribution and dosimetry estimate of FAPI-02 and FAPI-04 compared to FDG in cancer patients

Very similar to literature values for F-18-FDG, Ga-68-DOTATATE or Ga-68-PSMA-11, an exam with 200 MBq Ga-68-FAPI-02 and -04 corresponds to an equivalent dose of approx. 3-4 mSv. After a fast clearance via the kidneys, the normal organs show a low tracer uptake with only minimal changes between 10 min and 3 h p.i. In FAPI-02, the tumor uptake from 1 h to 3 h p.i. decreases by 75 %, whereas the tumor retention is slightly prolonged with FAPI-04 (50 % washout). At 1 h p.i. both FAPI-tracers perform equally (Figure 23). In comparison to FDG, tumor uptake is almost equal (average SUV max FDG 7.41; SUV max FAPI-02 7.37; n.s.); the background uptake in brain (11.01 vs 0.32), liver (2.77 vs 1.69) and oral/pharyngeal mucosa (4.88 vs 2.57) is significantly lower with FAPI-02; other organs were not relevantly different between FDG and FAPI-02 (Figure 24). For detailed information and results see Giesel *et al.* ³ which is incorporated by reference herewith.

PET imaging of FAPI-04 in patients with various cancers as well as non-cancerous malignancies

In addition to a rapid uptake of Ga-68-labeled FAPI-04 in different cancers including breast, pancreas, ovarian and HNO tumors, tracer accumulation was also demonstrated in peritonitis carcinomatosa (Figure 25A) as well as several inflammatory malignancies such as myocarditis (Figure 25B) and arthrosis (Figure 25C). These results indicate a potential application of Ga-68-labeled FAPIs for the detection of non-cancerous malignancies which are characterized by a chronical inflammation process involving recruitment of activated fibroblasts.

PET imaging of FAPI-21 and FAPI-46 in patients with various cancers

As shown in Figure 26, robust accumulation of Ga-68-labeled FAPI-21 was observed in different cancers including ovarian, rectal and mucoepidermoid carcinoma. Similar tumor uptake was shown for Ga-68-labeled FAPI-46 which rapidly accumulated in cholangiocellular and colorectal carcinoma, lung cancer as well as solitary fibrous sarcoma (Figure 27). Following PET/CT examination using Ga-68 labeled FAPI-46, a first therapeutical approach using the Sm-153 labeled radiotracer was taken in two cancer patients. As shown in Figure 28, robust tumor accumulation of the tracer is detectable up to 20 h after administration. FAPI-46-PET/CT imaging of three lung cancer patients with idiopathic pulmonary fibrosis revealed a clear difference of tracer accumulation in the cancerous vs. fibrotic lesions. As

shown in Figure 30, tumor uptake of Ga-68 labeled FAPI-46 was significantly higher in two patients (A, B) but slightly lower in one patient (C), as compared to the activity measured in the fibrotic tissue. The patient shown in Figure C suffered from an exacerbated lung fibrosis as compared to the two non-exacerbated cases. Therefore, the tracer is possibly useful for the differentiation of fibrosis patients with bad prognosis from patients with a good prognosis.

FAPI derivatives for radiolabeling with alternative radionuclides, e.g. Tc-99m, Pb-203, Cu-64 and F18

To enable the use of alternative radionuclides, a series of FAPI derivatives have been designed and characterized with respect to target affinity, specificity and pharmacokinetics. In some of these compounds, the original chelator DOTA has been replaced by different chelating moieties, which are ideally suitable for the incorporation of Tc-99m (FAPI-19, -27, -28, -29, -33, -34, -43, -44, -45, -60, -61, -62). FAP affinity *in vitro* and biodistribution in HT-1080-FAP xenografted mice are shown exemplarily for FAPI-19 and FAPI-34. Both compounds demonstrate a robust binding to human FAP *in vitro* (IC₅₀ FAPI-19: 6.4 nM). In contrast to FAPI-19, which shows insufficient tumor uptake *in vivo* as well as a rapid accumulation in the liver due to a shift of renal towards hepatic elimination, FAPI-34 is continuously enriched within the tumor and demonstrates significantly less hepatic uptake (Figures 31, 32). A first diagnostic application of Tc-99m labeled FAPI-34 in a pancreas cancer patient with liver metastases shows a stable tumor accumulation of the tracer up to 4 h after administration. In addition, the overall background activity is comparably low, resulting in high-contrast images (Figure 33). This offers a widespread application for diagnosis by scintigraphy and therapy after labeling with Re-188.

Pb-203 radiolabeled FAPI derivatives (FAPI-04, -32, -46 and FAPI-04tcmc) show comparable cell binding to HT-1080-FAP cells with FAPI-32 and FAPI-04tcmc reaching the highest binding values after 60 min of incubation (26.93 ± 0.846 and 21.62 ± 0.61 %ID/1 mio cells, Figure 34A). While FAPI-32 is rapidly eliminated from the tumor cells upon initial binding ($t_{1/2} = 2$ h), FAPI-04tcmc demonstrates considerably slower cell efflux ($t_{1/2} = 7$ h) but also the lowest FAP affinity as shown by the competition assay (IC₅₀ = 5.7 μ M, Figure 34C). On this account, FAPI-04 and FAPI-46, characterized by optimal half-lives and IC₅₀-values, were selected for further analysis *in vivo*. As shown in Figure 35, both compounds get continuously enriched within the tumor while showing only negligibly low binding to healthy tissue. The scintigraphic findings are confirmed in a biodistribution study, where both radiotracers demonstrate a robust tumor uptake, overall low organ activities as well as rapid renal excretion (Figure 36).

To enable radioactive labeling using Cu-64, the NOTA-derivatives FAPI-42 and FAPI-52 have been developed and characterized with respect to target affinity, specificity and pharmacokinetics. As shown in Figure 37, both tracers show a robust binding to HT-1080-FAP cells up to 24 h of incubation with similar IC₅₀-values in the lower nanomolar range (Figure 37A, B). Yet, FAPI-42 gets eliminated significantly slower than FAPI-52, resulting in a calculated *in vitro*-half-life of 12 h (Figure 37C). These results are confirmed by small animal imaging of HT-1080-FAP xenografted mice. As shown in Figure 38, both compounds demonstrate a robust tumor uptake as well as a rapid clearance from the blood stream *in vivo*. Notably, renal excretion of FAPI-42 occurs significantly faster as compared to FAPI-52, while its tumor activity remains slightly higher in the course of 2 to 24 h after administration.

The NOTA-derivatives FAPI-42 and FAPI-52 have been deployed for the formation of aluminum fluoride complexes to allow imaging with F-18. As shown in Figure 39, both compounds demonstrate a rapid tumor uptake in small animal imaging of HT-1080-FAP xenografted mice. Although both compounds are mainly excreted by the renal pathway, a biliary elimination is also observed. While the renal excretion is faster for FAPI-52 the higher tumor accumulation, longer tumor retention and the lower proportion of the biliary pathway are in favor of FAPI-42.

References

- 1 Loktev, A. *et al.* A new method for tumor imaging by targeting cancer associated fibroblasts. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, doi:10.2967/jnumed.118.210435 (2018).
- 2 Lindner, T. *et al.* Development of quinoline based theranostic ligands for the targeting of fibroblast activation protein. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, doi:10.2967/jnumed.118.210443 (2018).
- 3 Giesel, F. *et al.* FAPI-PET/CT: biodistribution and preliminary dosimetry estimate of two DOTA-containing FAP-targeting agents in patients with various cancers. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, doi:10.2967/jnumed.118.215913 (2018).

Example 6: FAPI characterization *in vitro* and *in vivo*

Preclinical data

With the objective of selectively targeting FAP-positive brain tumors, initial experiments were performed in tumor bearing mice using the human glioblastoma xenograft model U87MG. Tumor accumulation and organ distribution of radiolabeled FAPI-02 and -04 were analyzed by small animal PET imaging as well as in a biodistribution study. As shown in Fig. 40 and 41,

both FAPI-02 and -04 demonstrate rapid tumor uptake and negligibly low activities in healthy organs and the blood.

Clinical data

- 5 According to the WHO classification of 2016, gliomas are subdivided in IDH-wildtype gliomas WHO grade I-IV and IDH-mutant gliomas WHO grade II-IV. The most frequent WHO grade IV gliomas are glioblastomas.

Clinical PET-imaging was performed in 18 glioma patients (5 IDH mutant gliomas, 13 IDH-wildtype glioblastomas; see Table 10). As shown in Fig. 42-44, IDH-wildtype glioblastomas and grade III/IV, but not grade II IDH-mutant gliomas showed elevated tracer uptake. In
10 glioblastomas, spots with increased uptake in projection on contrast enhancing areas were observed.

Conclusion

- 15 Increased tracer uptake in IDH-wildtype glioblastomas and high-grade IDH-mutant astrocytomas, but not in diffuse astrocytomas may allow non-invasive distinction between low-grade IDH-mutant and high-grade gliomas and be useful for follow-up studies. The heterogeneous tracer uptake in glioblastomas may be helpful for biopsy planning.

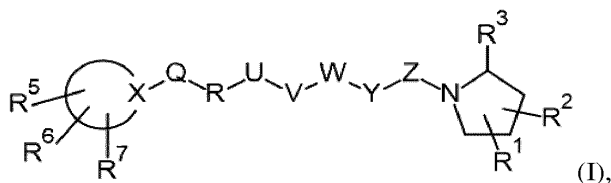
20

Table 10: Patient characteristics

Patient Number	Age (years)	IDH status	Diagnosis	WHO Grade	Localization	Biopsy Surgery	Pretreatment
1	57	wildtype	Glioblastoma	IV	parietal right	Biopsy	Radio-chemotherapy
2	65	wildtype	Glioblastoma	IV	bifrontal	Biopsy	none
3	66	wildtype	Glioblastoma	IV	parieto-temporal left	Biopsy	none
4	64	wildtype	Glioblastoma	IV	temporal right	Resection	Radio-chemotherapy
5	58	wildtype	Glioblastoma	IV	Splenium	Biopsy	none
6	48	wildtype	Glioblastoma	IV	fronto-temporo-parietal left	Resection	Radio-chemotherapy
7	20	wildtype	Glioblastoma	IV	Thalamus / temporal right	Biopsy	none
8	54	wildtype	Glioblastoma	IV	temporal left	Resection	none
9	56	wildtype	Glioblastoma	IV	basal ganglia	Partial Resection	none
10	61	wildtype	Glioblastoma	IV	temporal right	Resection	none
11	86	wildtype	Glioblastoma	IV	Corona radiata left	Biopsy	Chemotherapy
12	68	wildtype	Glioblastoma	IV	Corpus callosum	Biopsy	none
13	71	wildtype	Glioblastoma	IV	temporal left	Biopsy	none
14	29	IDH1 R132H Mutation	Glioblastoma	IV	temporo-parietal right	Resection	none
15	47	IDH1 R132H Mutation	Anaplastic astrocytoma	III	frontal right	Resection	none
16	47	IDH1 R132H Mutation	Diffuse astrocytoma	II	parietal left	Biopsy	none
17	47	IDH1 R132C Mutation	Diffuse astrocytoma	II	frontal right	Resection	none
18	43	IDH1 R132H Mutation	Diffuse astrocytoma	II	fronto-temporal left	Biopsy	Chemotherapy

Claims

1. A compound of Formula (I)



5 wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other;

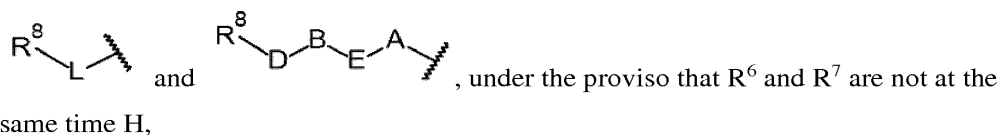
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

15 R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

20 R⁶, and R⁷ are independently selected from the group consisting of -H,



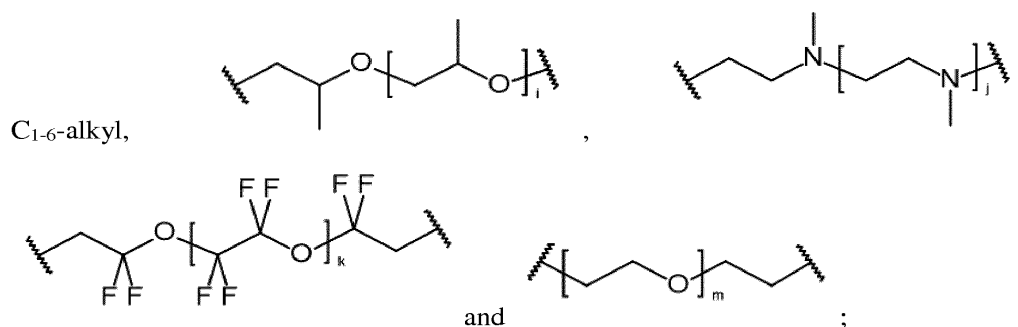
wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR⁴, O, S, and CH₂;

E is selected from the group consisting of



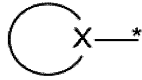
wherein i is 1, 2, or 3;

wherein j is 1, 2, or 3;

5 wherein k is 1, 2, or 3;

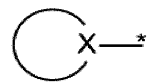
wherein m is 1, 2, or 3;

A and E together form a group selected from: a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, wherein A and E can be mono-, bi- and multicyclic, preferably monocyclic; each A and E being optionally substituted with 1 to 4 substituents selected from -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected

from -OH, oxo, halo; and optionally connected to A, B, D, E or  ;

15 B is selected from the group consisting of S, NR⁴, NR⁴-O, NR⁴-C₁₋₆-alkyl, NR⁴-C₁₋₆-alkyl-NR⁴, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein NR⁴-C₁₋₆-alkyl-NR⁴ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl; and;

20 R⁸ is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;



25 is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof.

2. The compound of claim 1, wherein

(i) Q, R, U are CH_2 and are individually present or absent;

V is CH_2 , $\text{C}=\text{O}$, $\text{C}=\text{S}$ or $\text{C}=\text{NR}^4$;

W is NR^4 ;

Y is HCR^4 ; and

Z is $\text{C}=\text{O}$, $\text{C}=\text{S}$ or $\text{C}=\text{NR}^4$; and/or

(ii) Q and R are absent;

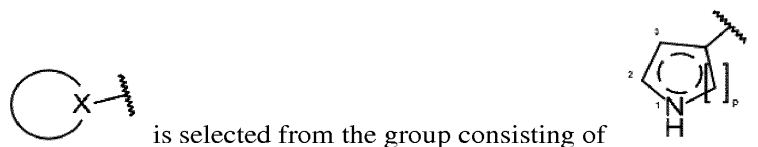
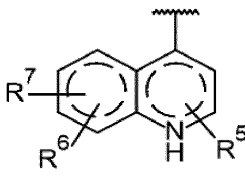
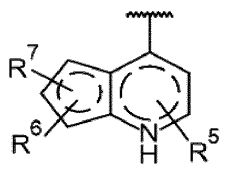
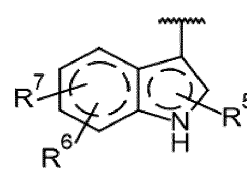
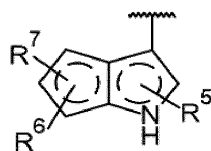
U is CH_2 and is present or absent;

R^1 and R^2 are independently selected from the group consisting of -H and halo;

R^3 is selected from the group consisting of -H, -CN, and $-\text{B}(\text{OH})_2$;

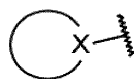
R^4 is selected from the group consisting of -H and $-\text{C}_{1-6}$ -alkyl, wherein the $-\text{C}_{1-6}$ -alkyl is optionally substituted with from 1 to 3 substituents selected from -OH.

3. The compound of claim 1 or 2, wherein

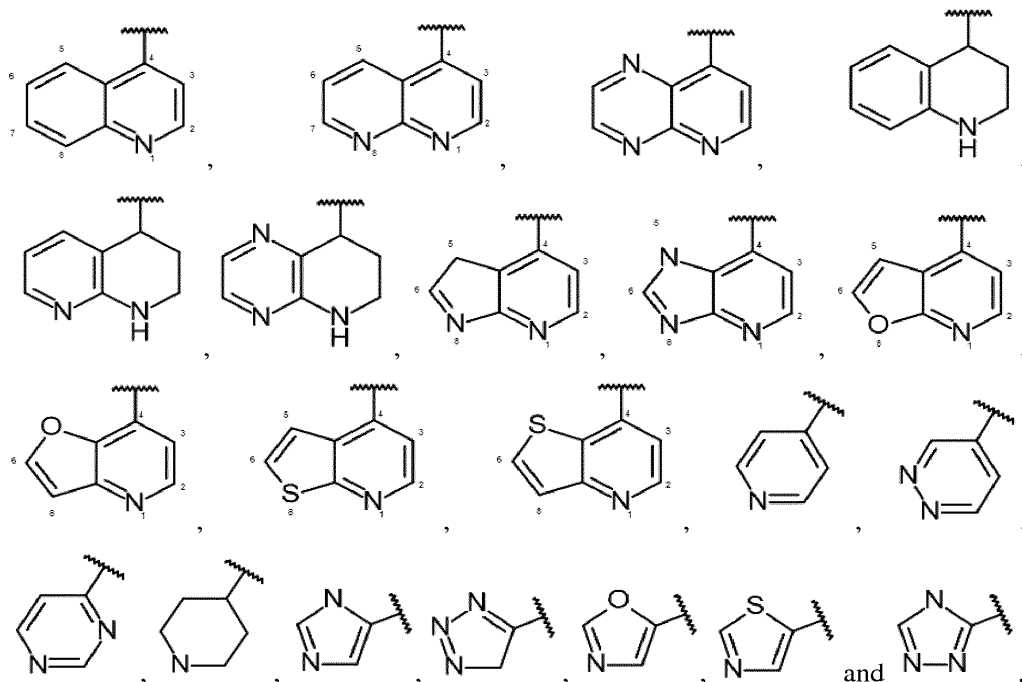


and S.

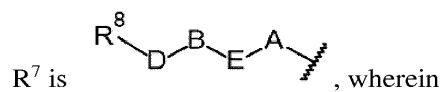
4. The compound of any of the preceding claims, wherein



is selected from the group consisting of

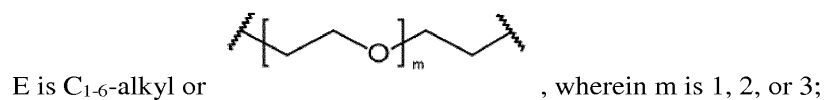
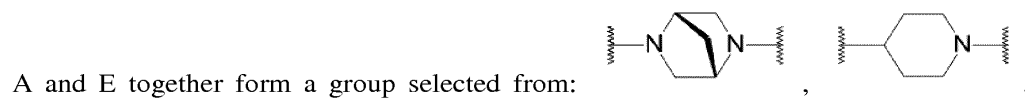
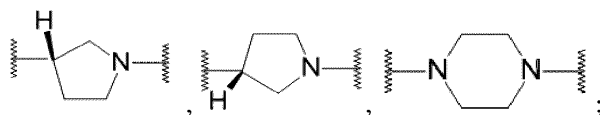


5. The compound of any of the preceding claims, wherein
 R^5 and R^6 are H;



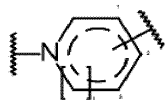
D is absent;

A is O, S, CH_2 , NH, NCH_3 ;



B is $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$ or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$.

6. The compound of any of the preceding claims, wherein
- (i) the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



, wherein

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen;

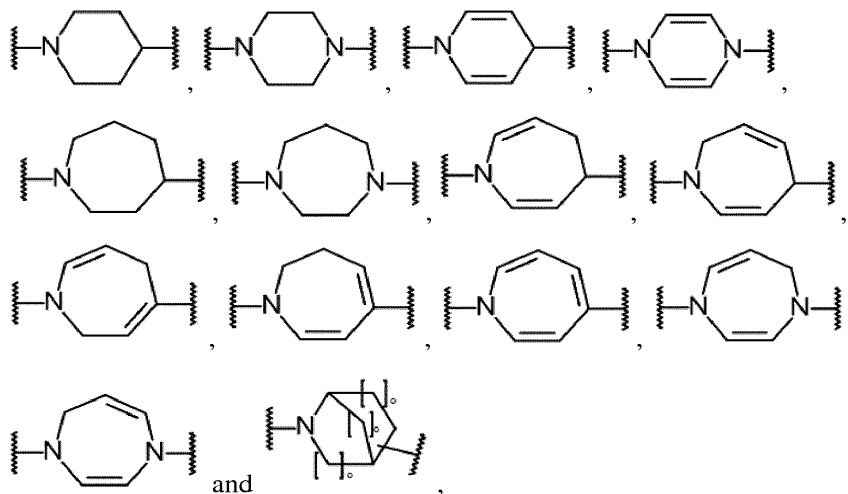


is attached to position 1, 2, or 3, preferably to position 2;

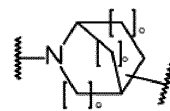
l is 1 or 2;

optionally wherein the N-containing heterocycle is substituted with a $\text{C}_{1-6}\text{-alkyl}$; and/or

- (ii) the N-containing heterocycle comprised in B is selected from the group consisting of:

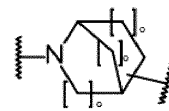


optionally wherein the N-containing heterocycle is substituted with a $\text{C}_{1-6}\text{-alkyl}$;

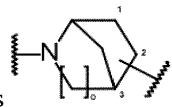
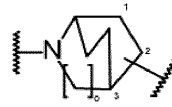


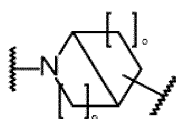
wherein if the N-containing heterocycle comprised in B is
the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O,
N and S, optionally further comprises 1 nitrogen, optionally compromises one or
more (e.g. amino acid derived) side chains;

5  is attached to position 1, 2, or 3, preferably to position 2;
o is 1 or 2,

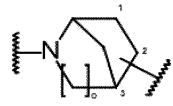


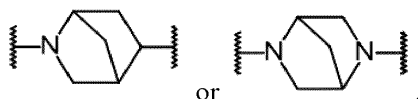
preferably, if the N-containing heterocycle comprised in B is , the

N-containing heterocycle comprised in B is ,  or



; more preferably, if the N-containing heterocycle comprised in

10 B is , the N-containing heterocycle comprised in B is



or .

7. The compound of any of the preceding claims, wherein

Q, R, U are absent;

15 V is C=O;

W is NH;

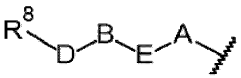
Y is CH₂;

Z is C=O;

R¹ and R² are independently selected from the group consisting of -H and halo;

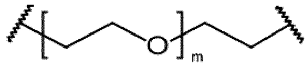
20 R³ is -CN;

R⁵ and R⁶ are H;

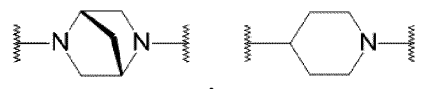
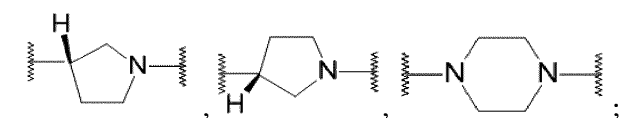
R^8
 R^7 is , wherein

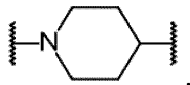
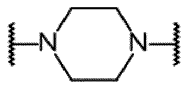
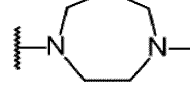
D is absent;

A is O, S, CH₂, NH, NCH₃;

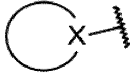
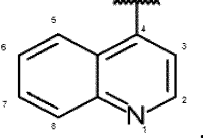
E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; or

5 A and E together form a group selected from:



B is NH-C₁₋₆-alkyl, , ,  or

, optionally B is substituted with a C₁₋₃ alkyl; and

10  is .

8. The compound of any of the preceding claims, wherein C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl, and/or

15 wherein C₁₋₆-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl, phenyl-propyl, and phenyl-butyl.

9. The compound of any of the preceding claims, wherein R⁸ is a radioactive moiety, wherein the radioactive moiety is a fluorescent isotope, a radioisotope, a radioactive drug or combinations thereof, preferably wherein the radioactive moiety is selected from the group consisting of alpha radiation emitting isotopes, beta radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting

20

isotopes, fluorescence emitting isotopes, such as ^{11}C , ^{18}F , ^{51}Cr , ^{67}Ga , ^{68}Ga , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{186}Re , ^{188}Re , ^{139}La , ^{140}La , ^{175}Yb , ^{153}Sm , ^{166}Ho , ^{88}Y , ^{90}Y , ^{149}Pm , ^{165}Dy , ^{169}Er , ^{177}Lu , ^{47}Sc , ^{142}Pr , ^{159}Gd , ^{212}Bi , ^{213}Bi , ^{72}As , ^{72}Se , ^{97}Ru , ^{109}Pd , ^{105}Rh , $^{101\text{m}}\text{Rh}$, ^{119}Sb , ^{128}Ba , ^{123}I , ^{124}I , ^{131}I , ^{197}Hg , ^{211}At , ^{151}Eu , ^{153}Eu , ^{169}Eu , ^{201}Tl , ^{203}Pb , ^{212}Pb , ^{64}Cu , ^{67}Cu , ^{188}Re , ^{186}Re , ^{198}Au , ^{225}Ac , ^{227}Th and ^{199}Ag , preferably ^{18}F , ^{64}Cu , ^{68}Ga , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{153}Sm , ^{177}Lu , ^{188}Re .

10. The compound of any of claims 1 to 8, wherein R^8 is a fluorescent dye select from the group consisting of the following classes of fluorescent dyes: Xanthenes, Acridines, Oxazines, Cynines, Styryl dyes, Coumarines, Porphines, Metal-Ligand-Complexes, Fluorescent proteins, Nanocrystals, Perylenes, Boron-dipyrromethenes and Phtalocyanines as well as conjugates and combinations of these classes of dyes.
11. The compound of any of claims 1 to 8, wherein R^8 is a chelating agent which forms a complex with divalent or trivalent metal cations, preferably wherein the chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane- $\text{N},\text{N}',\text{N},\text{N}'$ -tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine- $\text{N},\text{N},\text{N}',\text{N}',\text{N}''$ -pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC).
12. The compound of any of claims 1 to 8, wherein R^8 is a contrast agent which comprises or consists of a paramagnetic agent, preferably, wherein the paramagnetic agent comprises or consists of paramagnetic nanoparticles.
13. Pharmaceutical composition comprising or consisting of at least one compound according to any of claims 1 to 12; and, optionally, a pharmaceutically acceptable carrier and/or excipient.
14. The compound of any of claims 1 to 12 or the pharmaceutical composition of claim 13 for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject, preferably wherein the disease characterized by overexpression of fibroblast activation protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorder, preferably wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, small intestine cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer,

- 5 hepatocellular carcinoma, esophageal cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocellular carcinoma, clear cell renal carcinoma, neuroendocrine tumor, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus carcinoma, desmoid tumors, glioma, astrocytoma, cervix carcinoma and prostate cancer.
15. A kit comprising or consisting of the compound of any of claims 1 to 12 or the pharmaceutical composition of claim 13 and instructions for the diagnosis or treatment of a disease.

Summary

The present invention relates to a compound, a pharmaceutical composition comprising or consisting of said compound, a kit comprising or consisting of said compound or pharmaceutical composition and use of the compound or pharmaceutical composition in the
5 diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP).

Figure 1

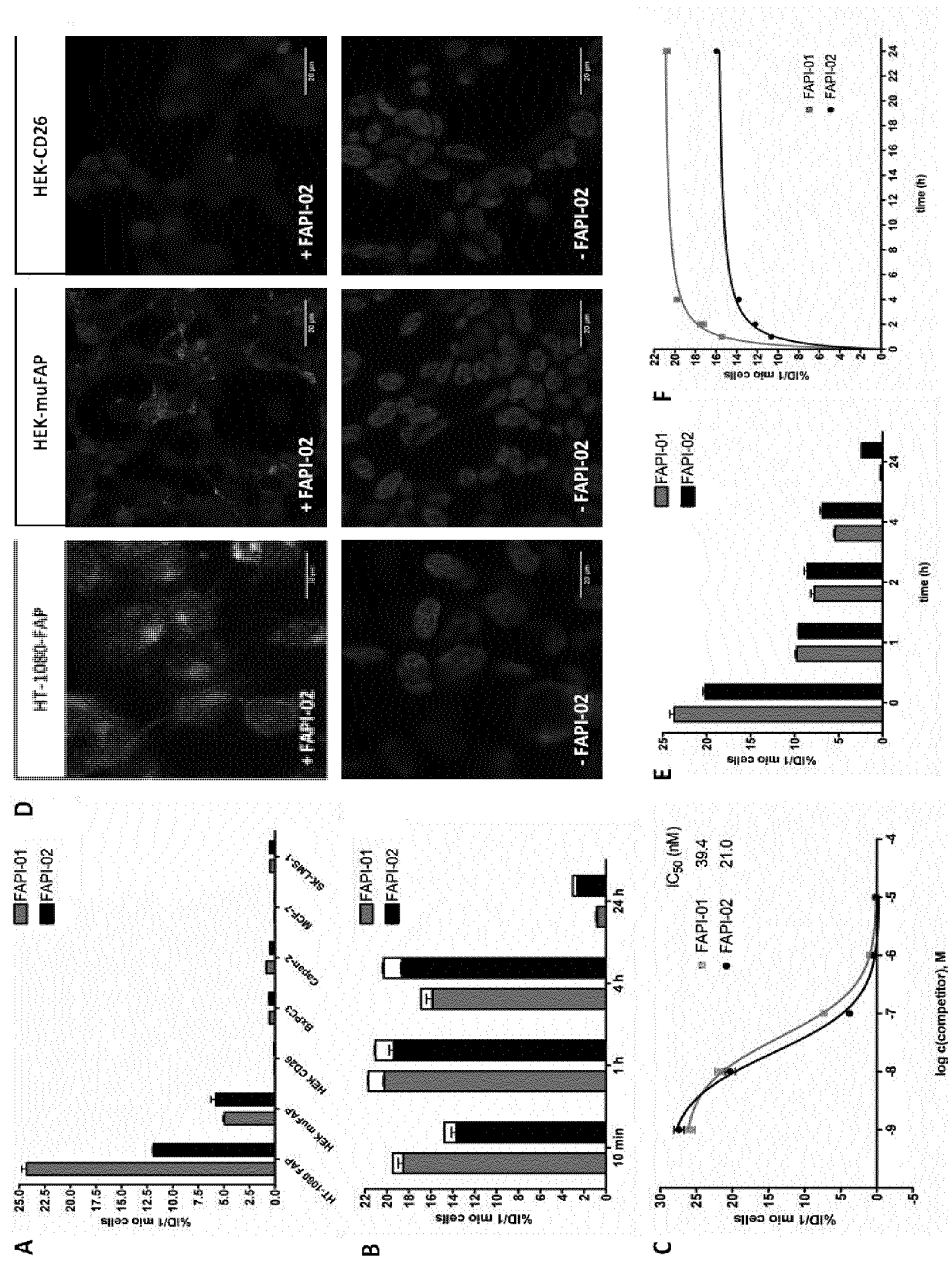


Figure 2

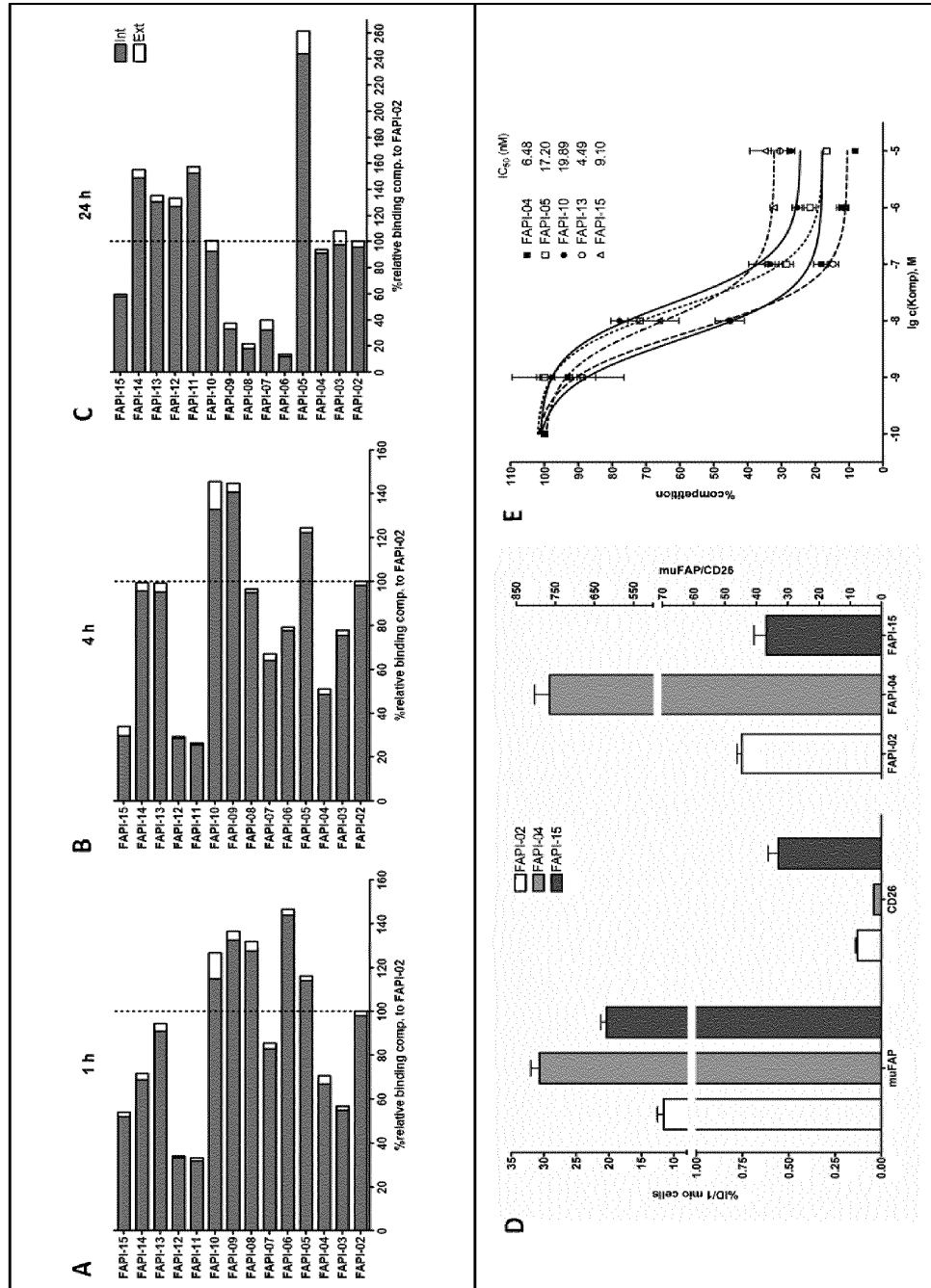


Figure 3

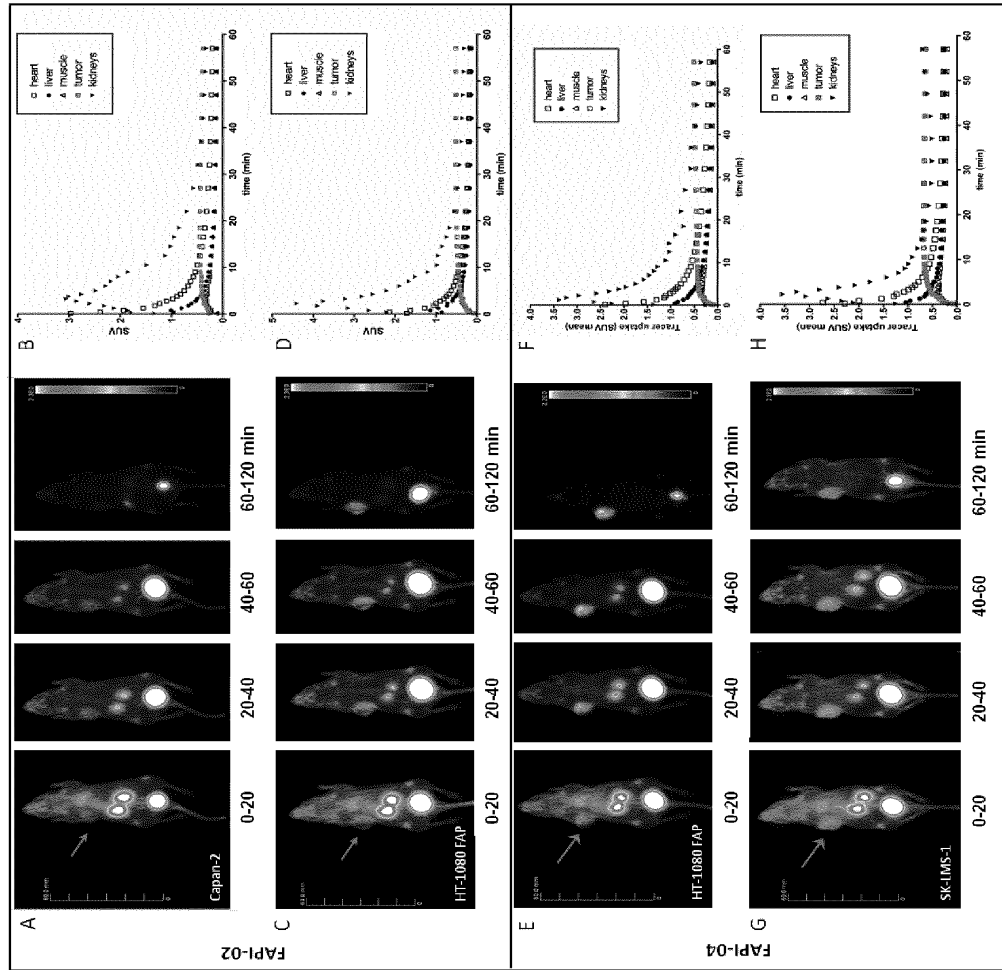


Figure 4

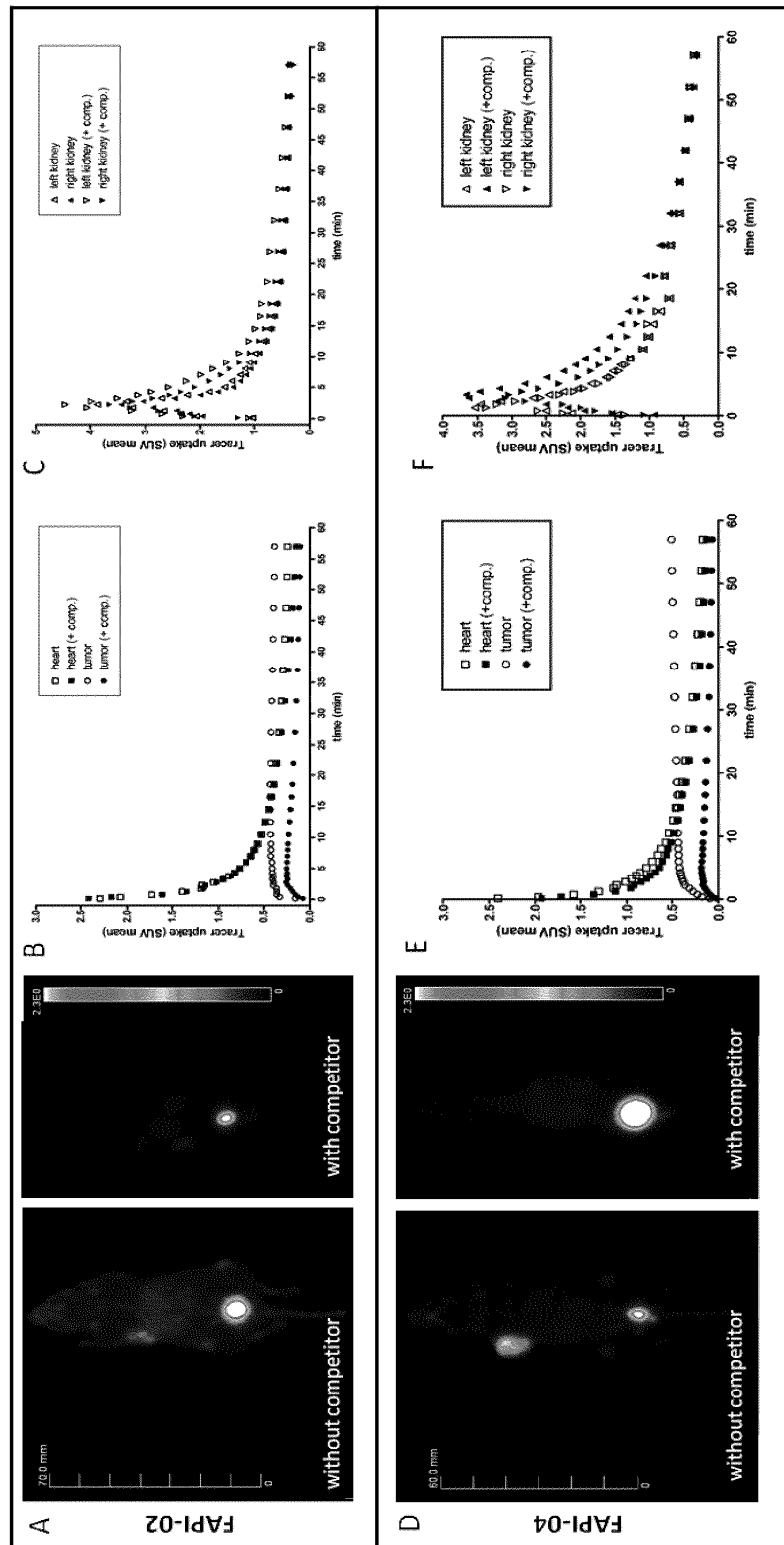


Figure 5

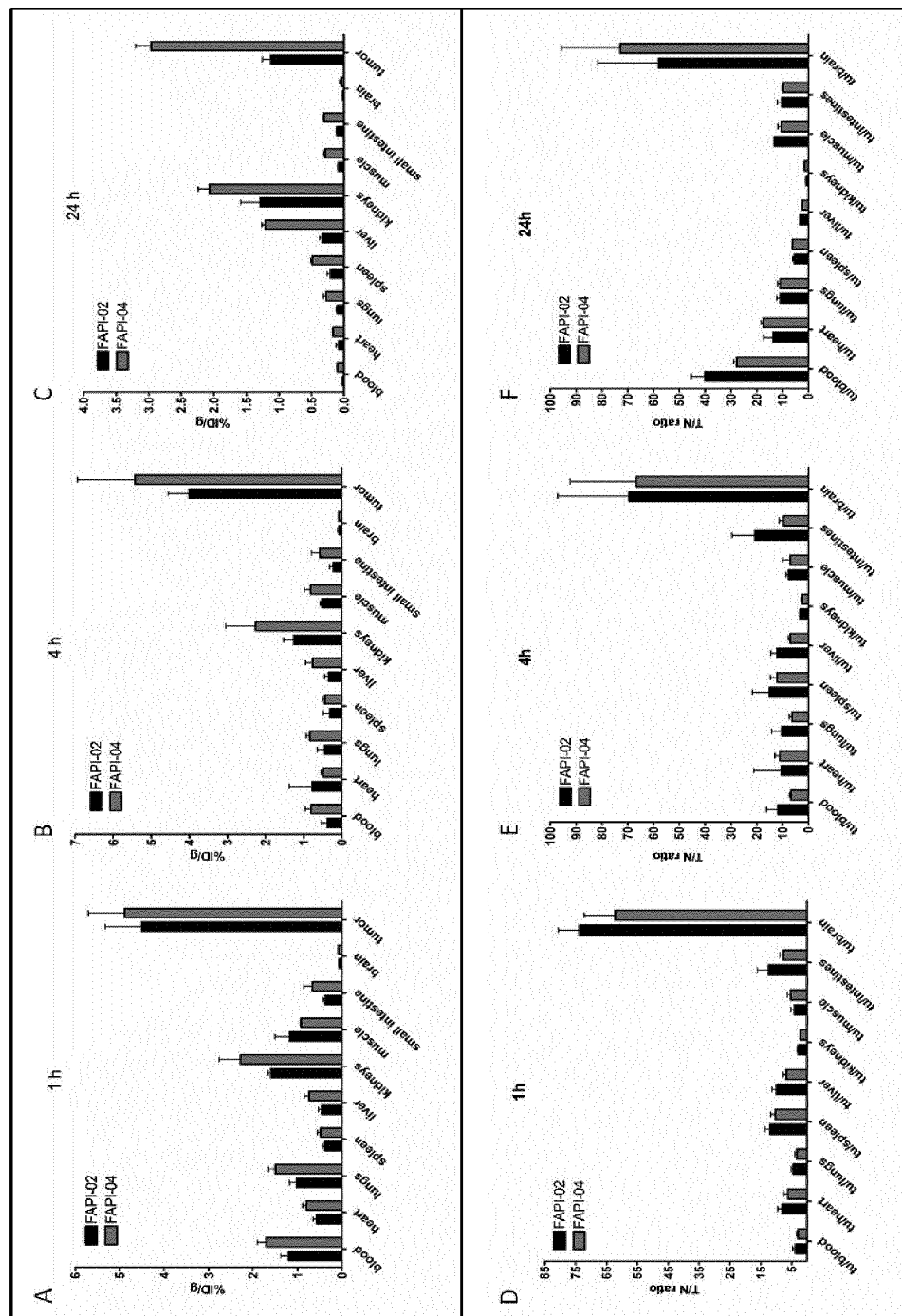


Figure 6

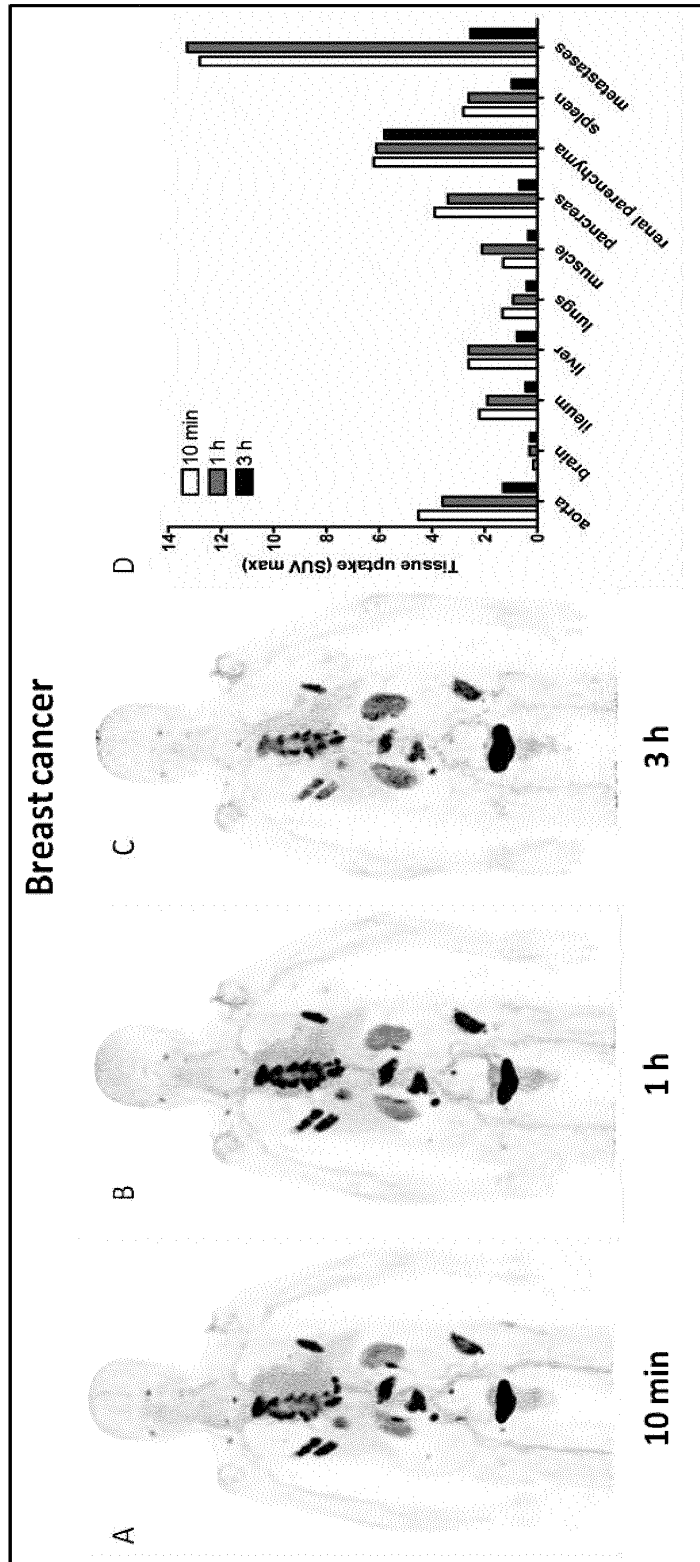


Figure 7

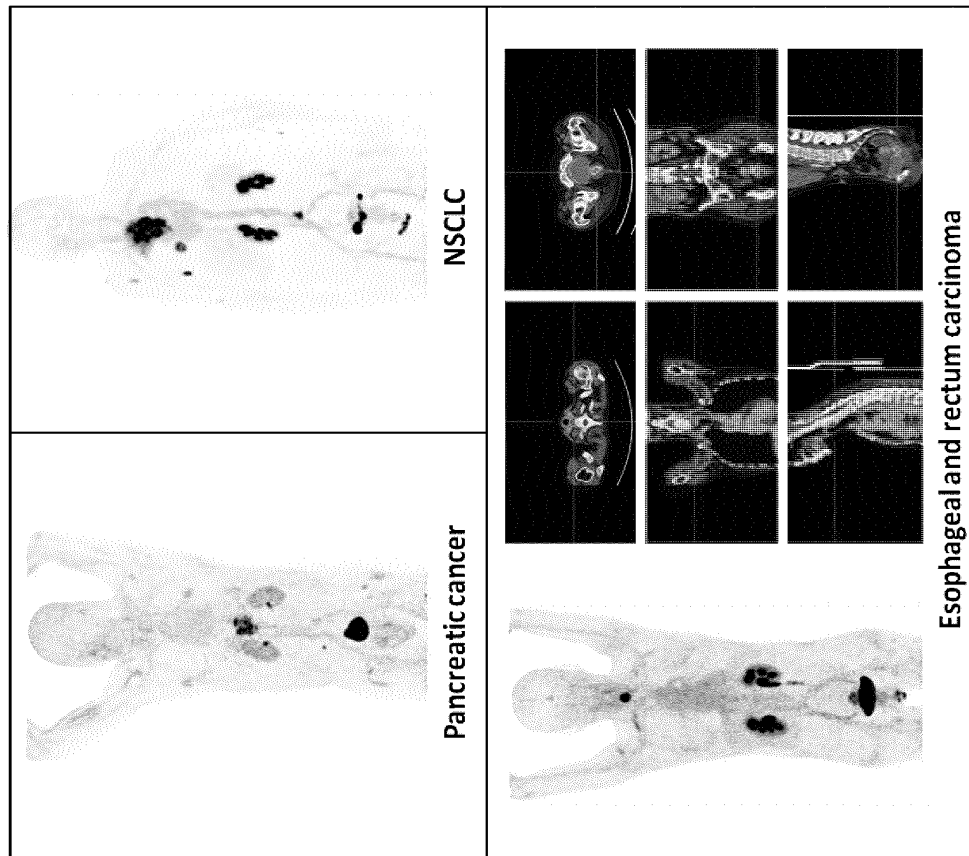


Figure 8

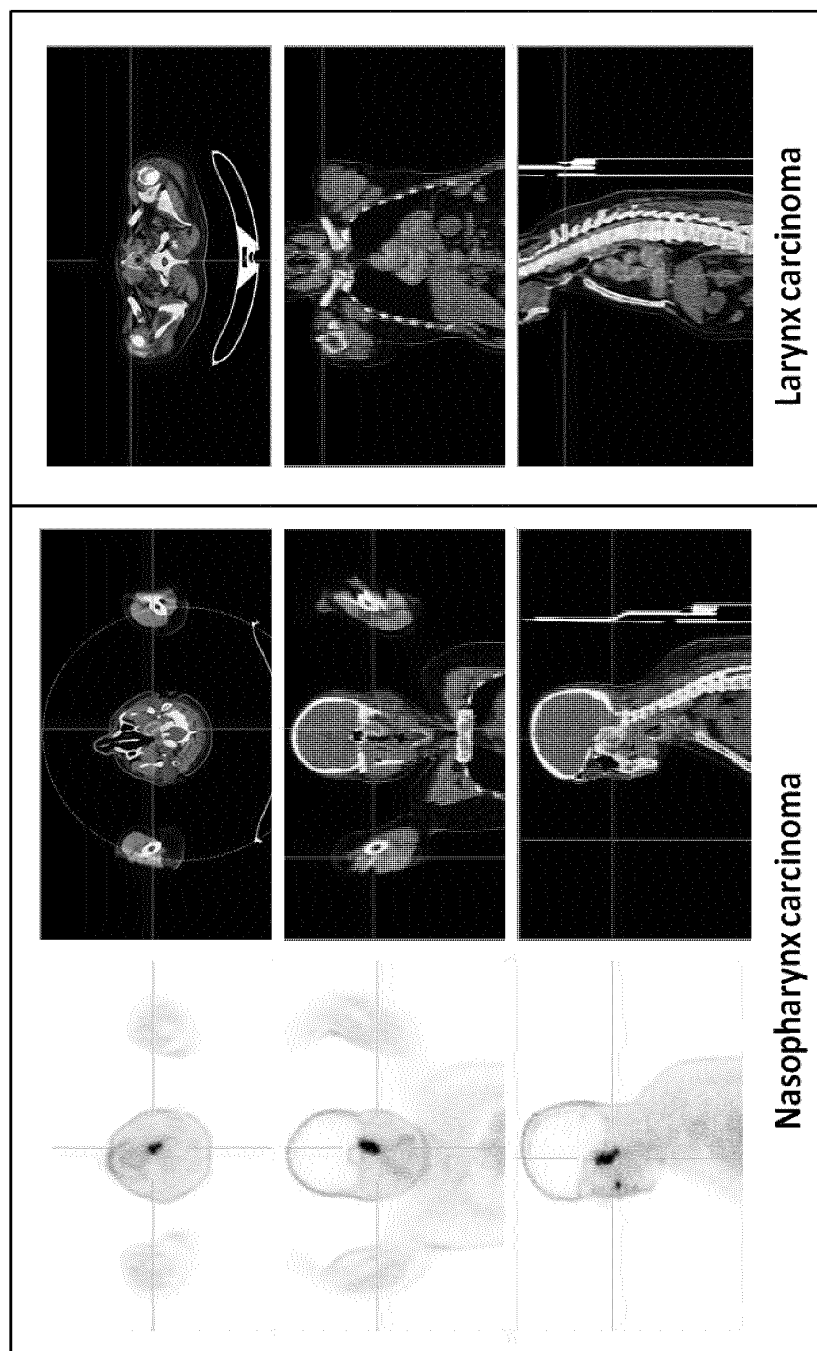


Figure 9

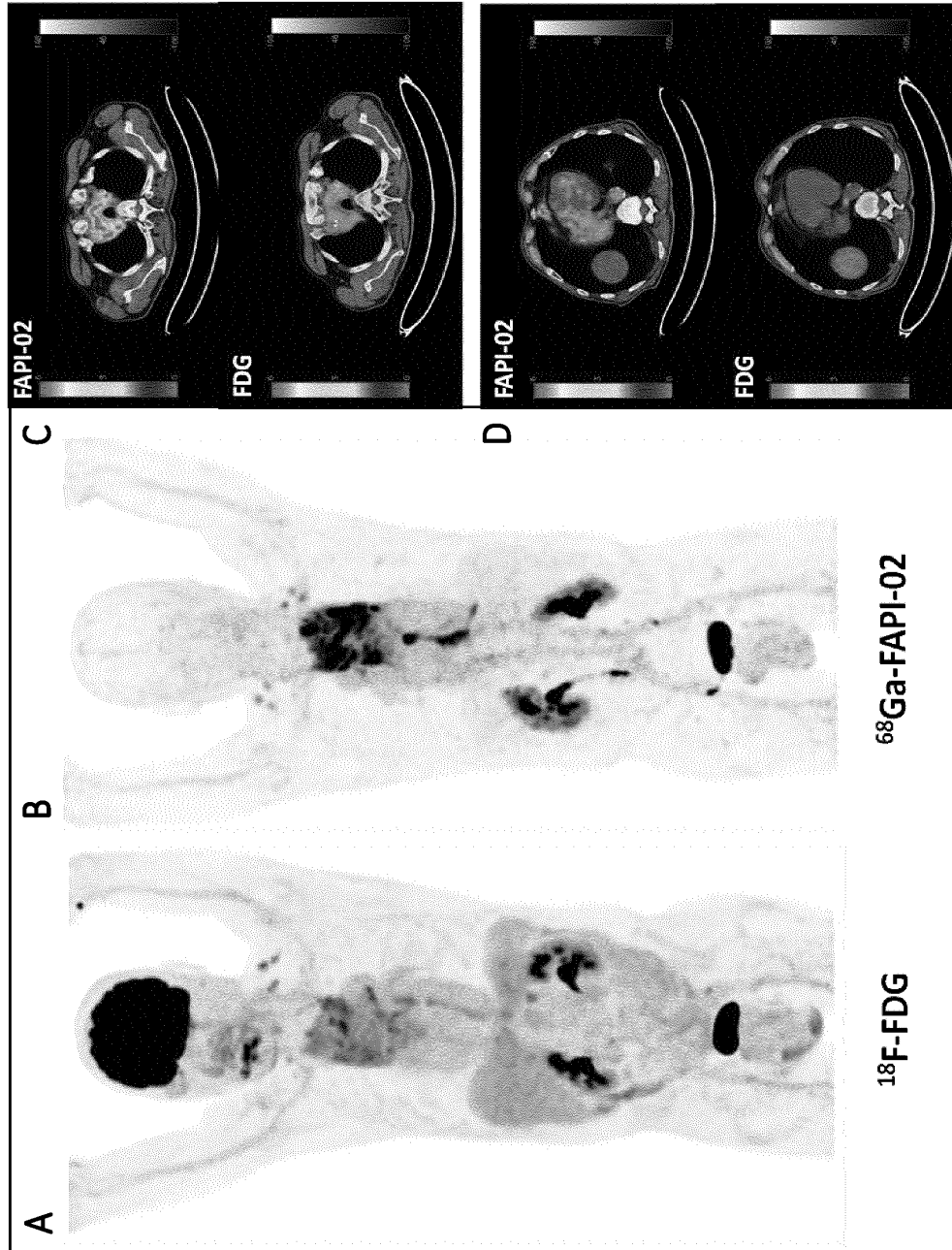


Figure 10

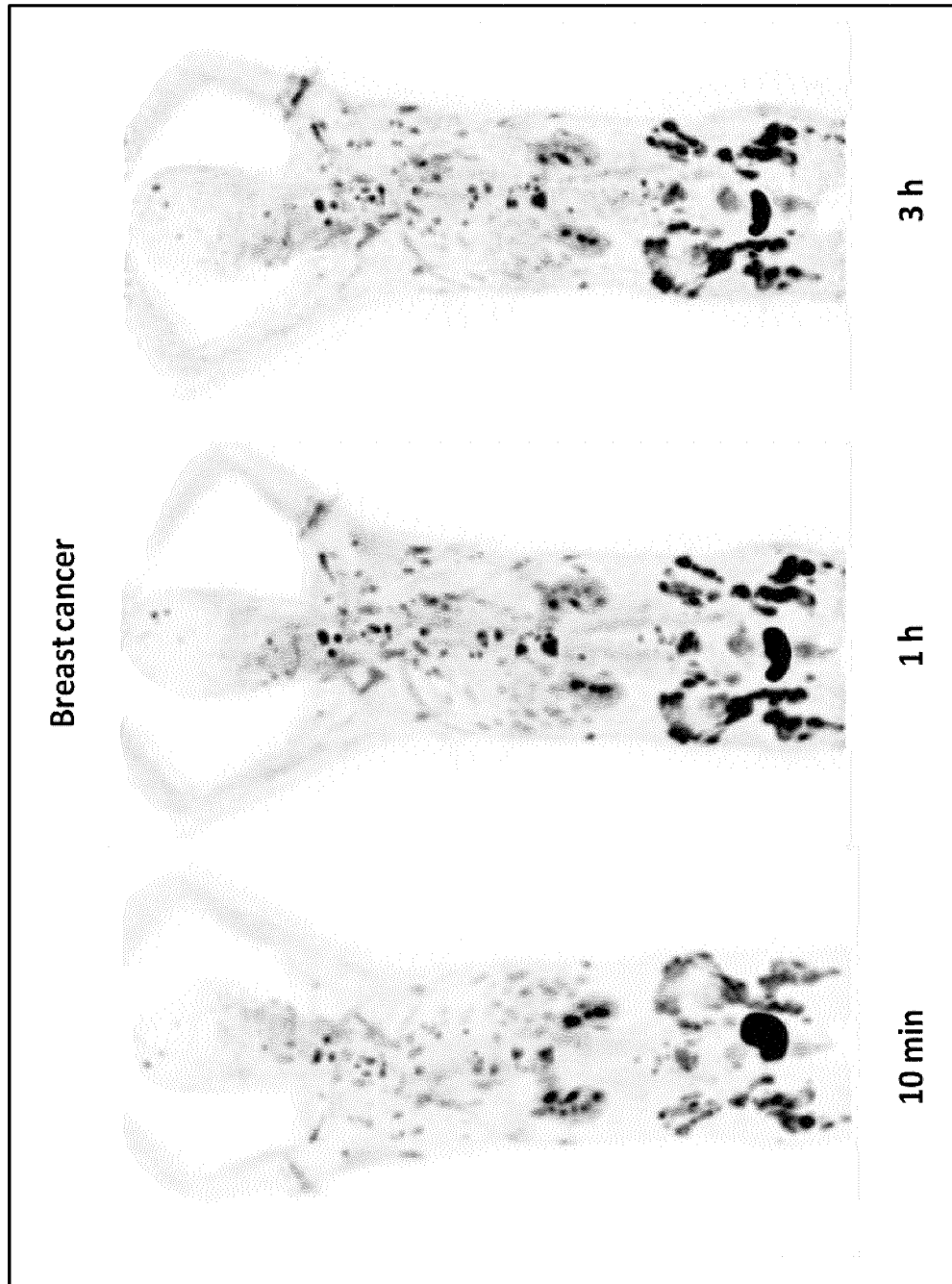


Figure 11

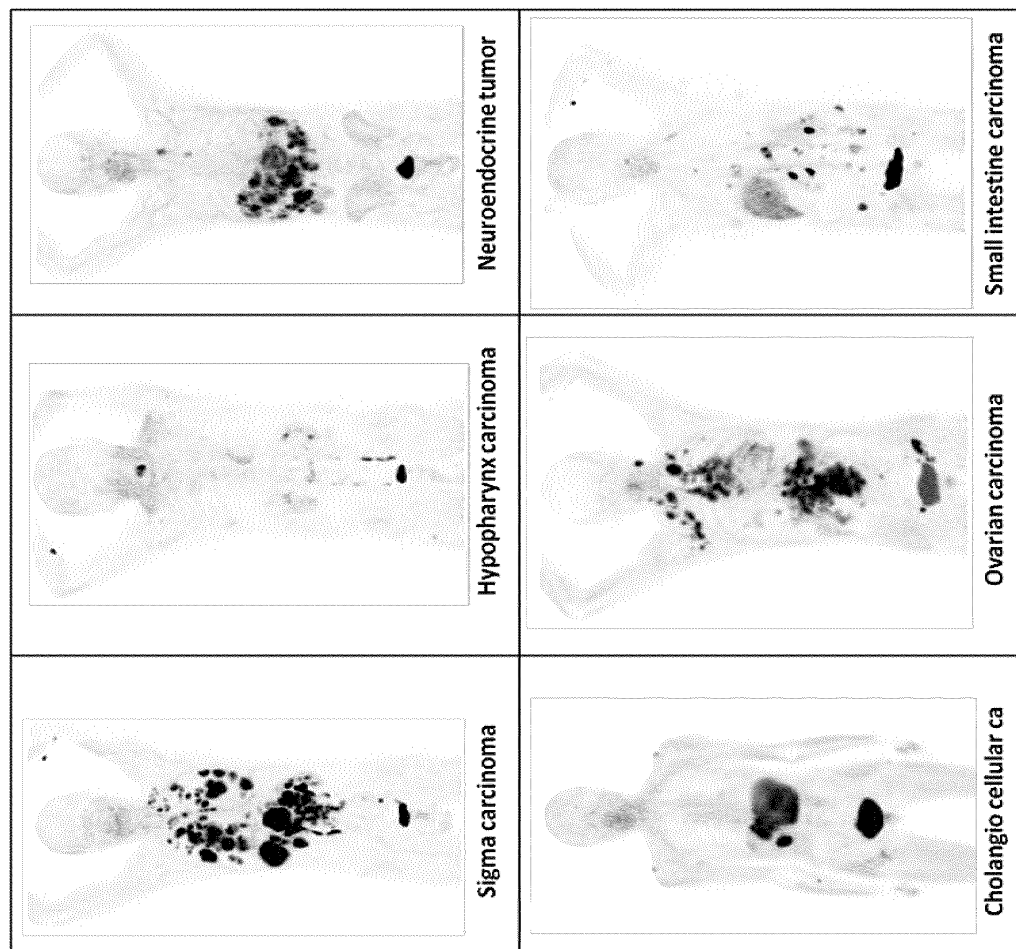


Figure 12

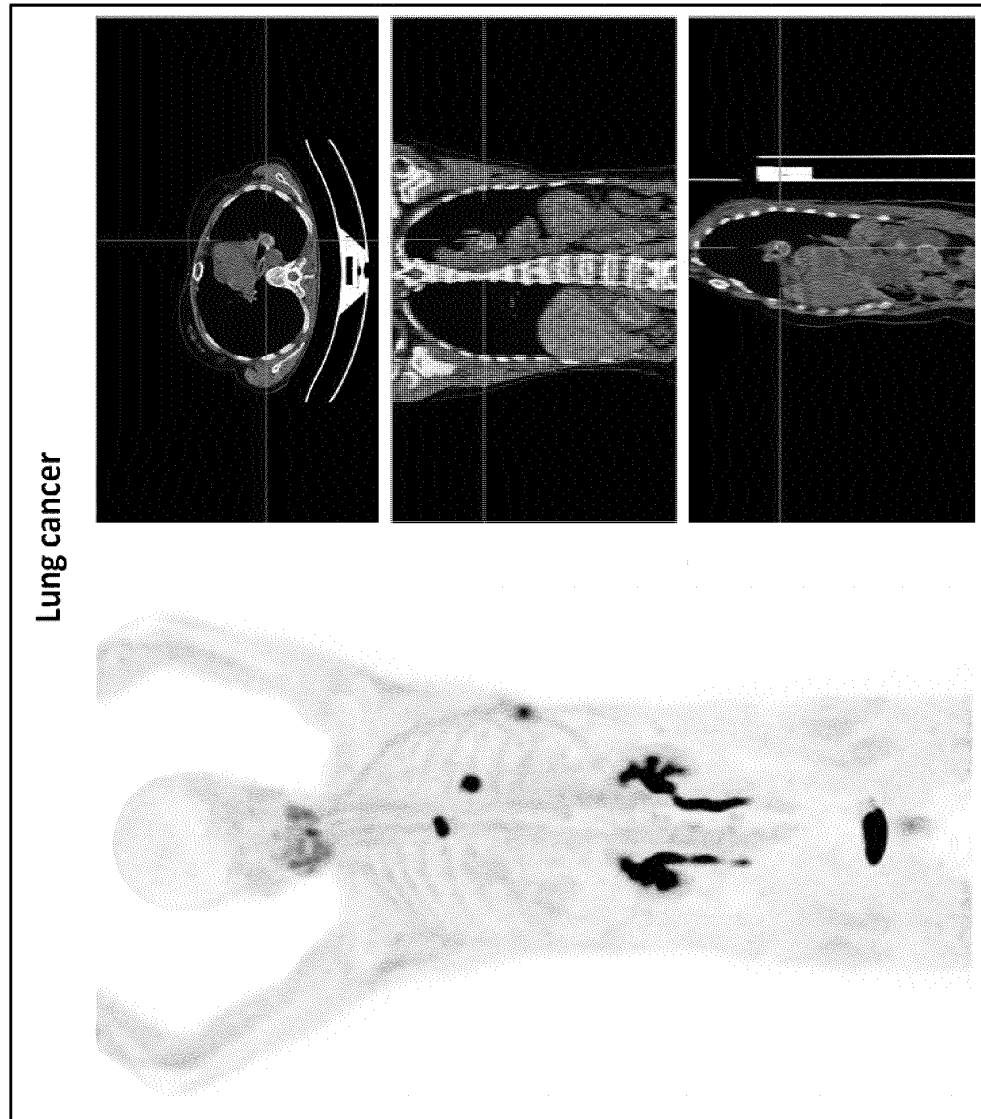


Figure 13

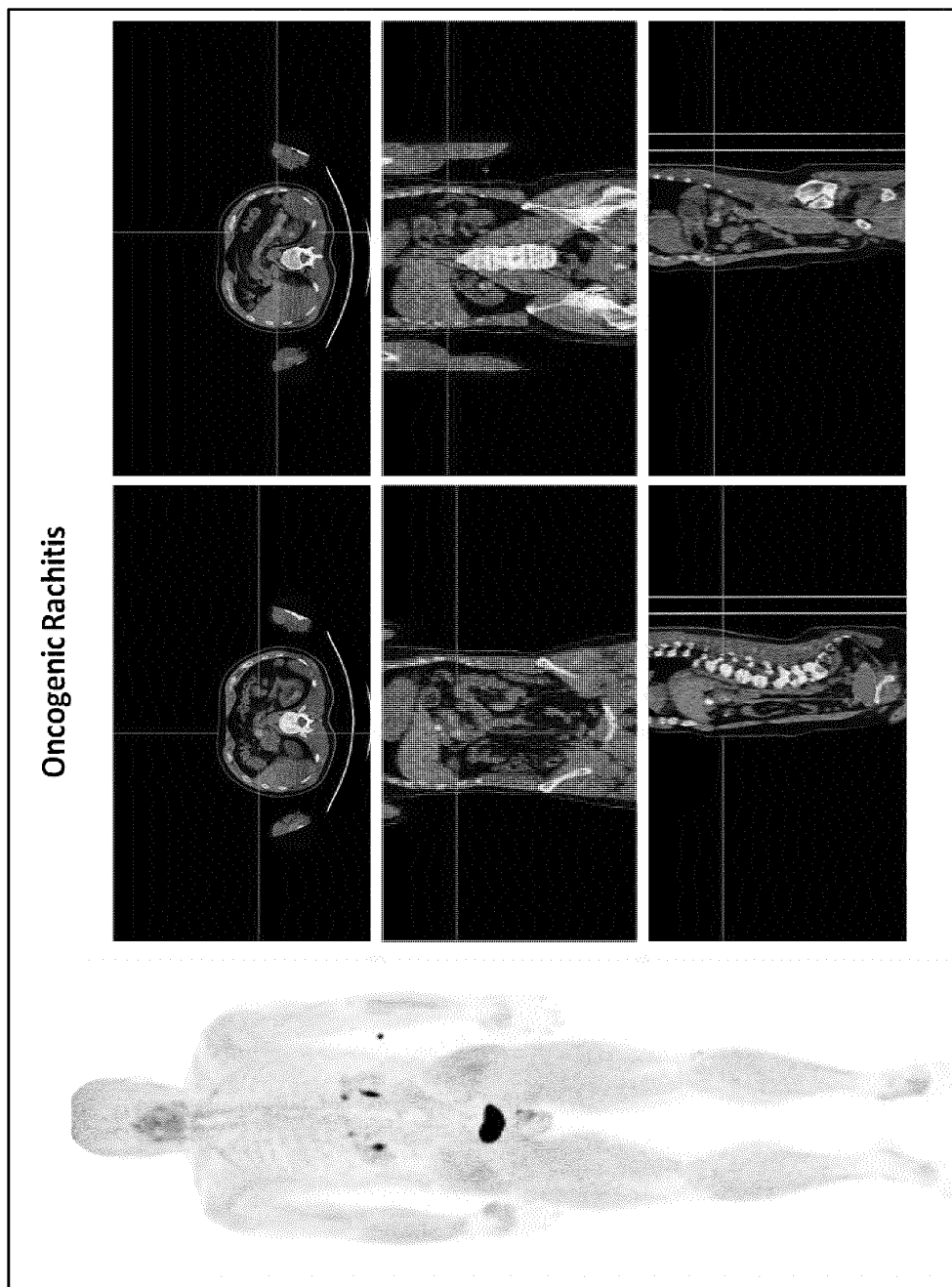
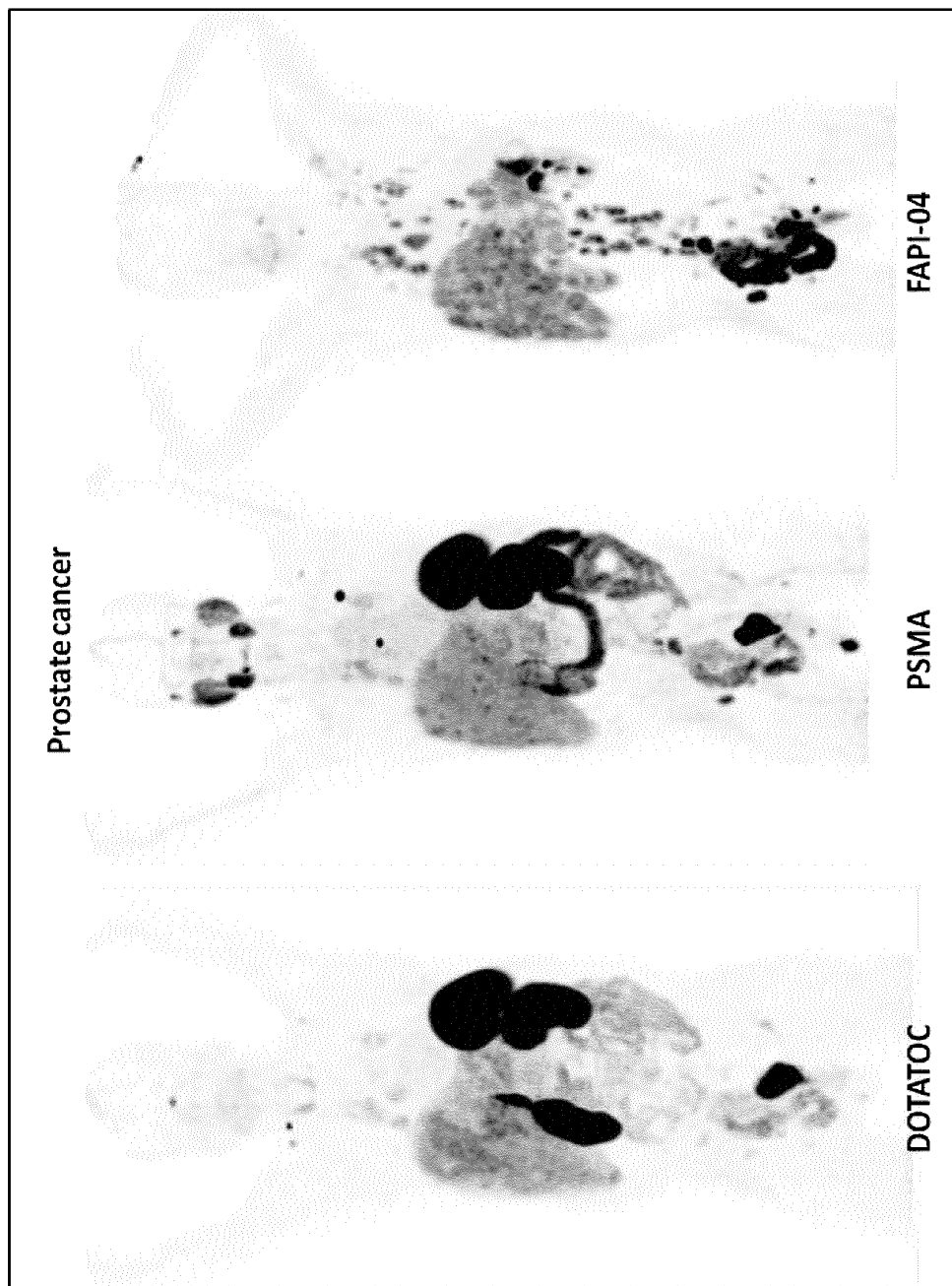


Figure 14



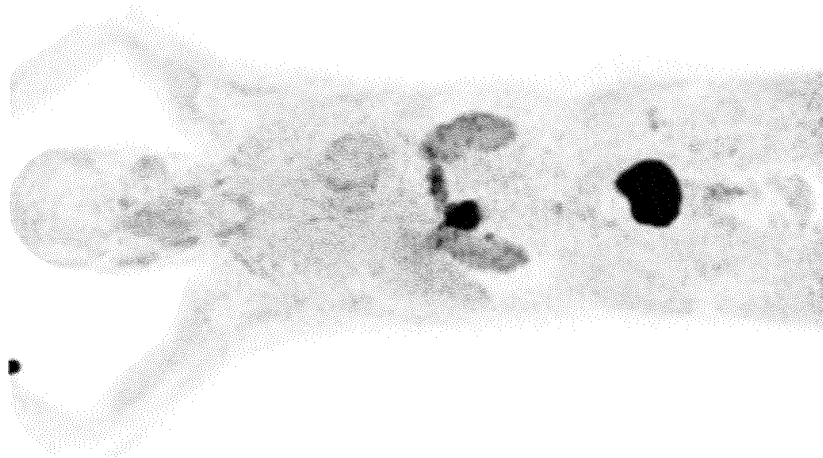
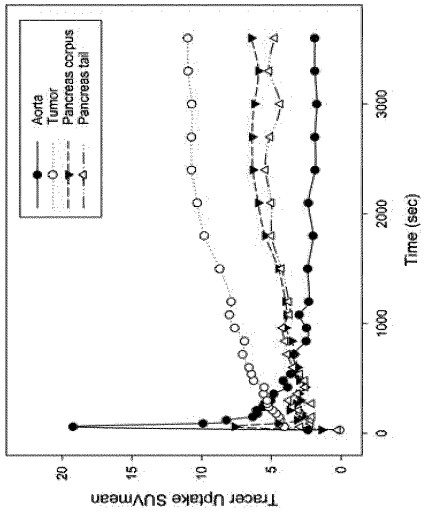
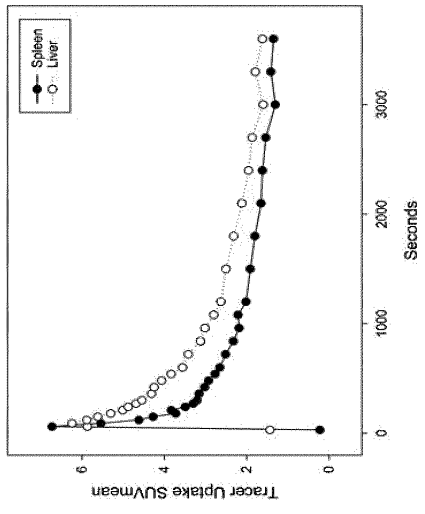
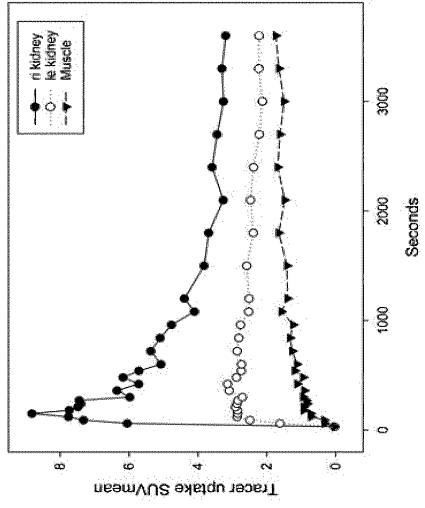


Figure 15

Figure 16

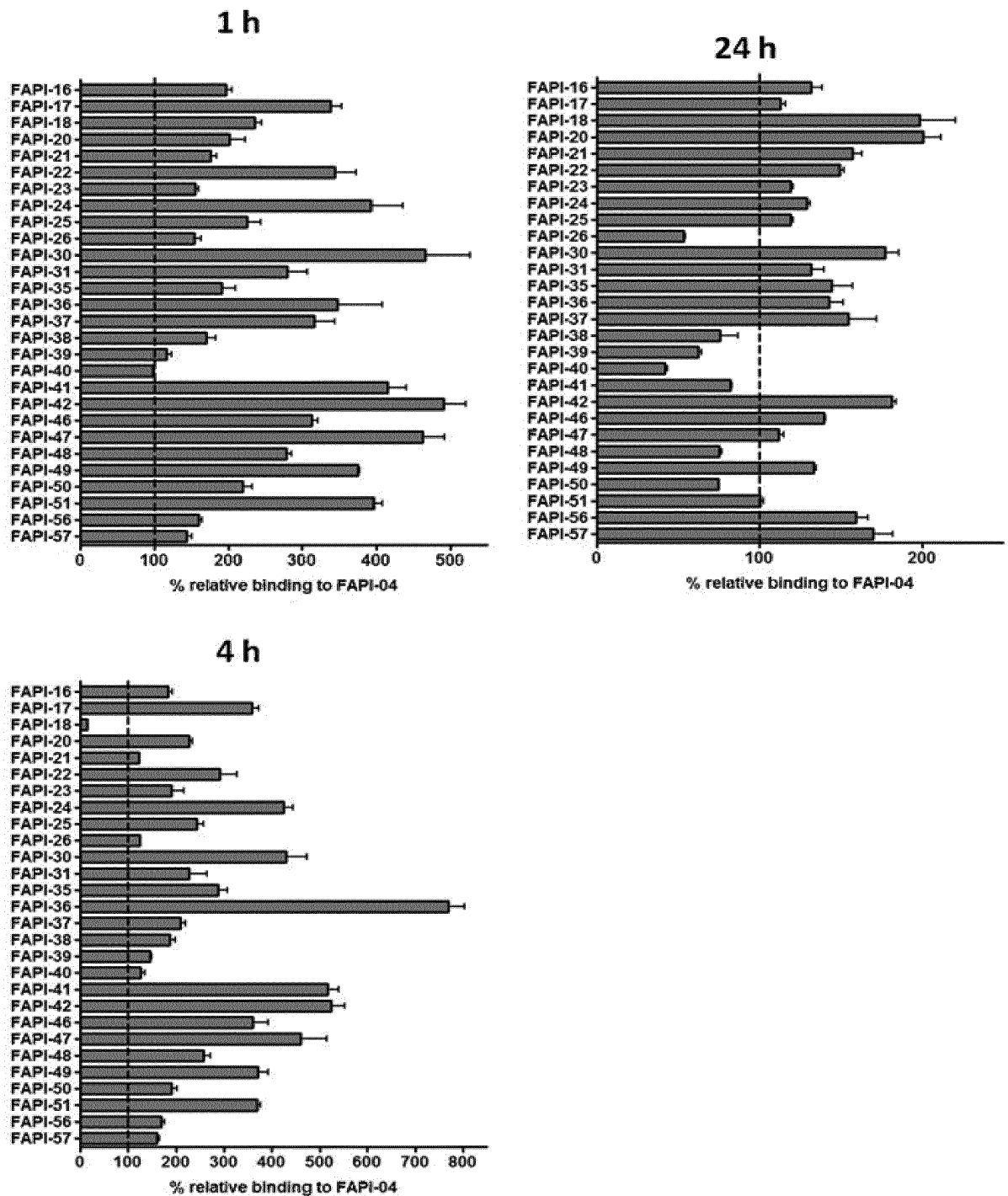


Figure 17

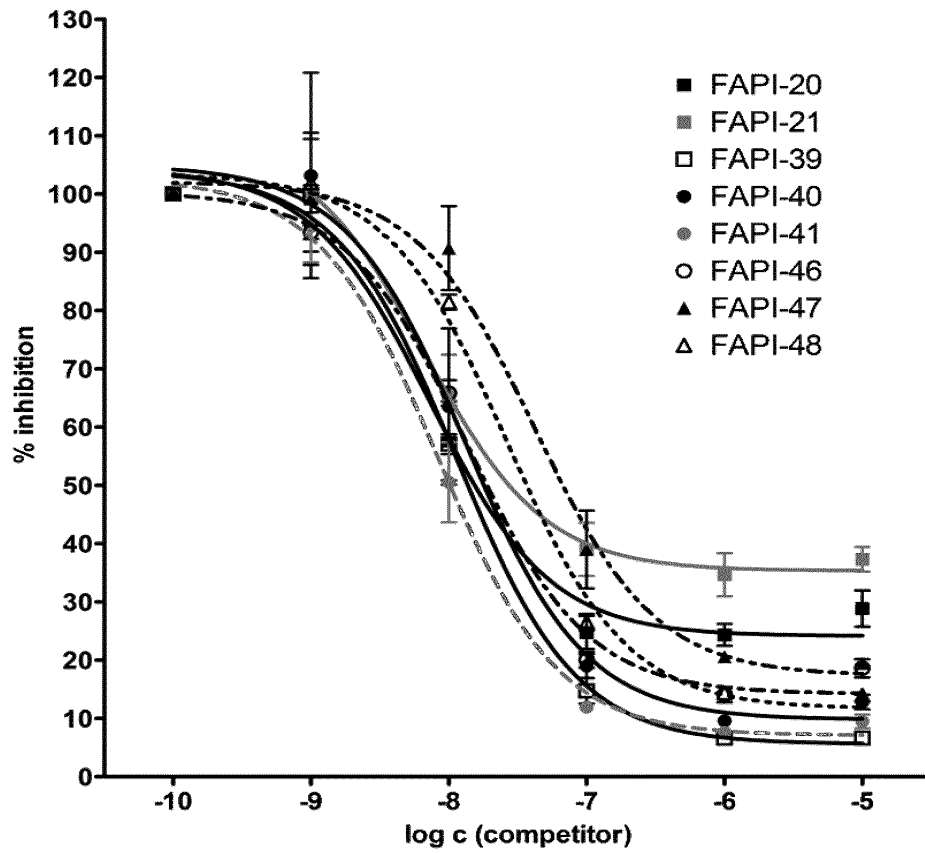


Figure 18

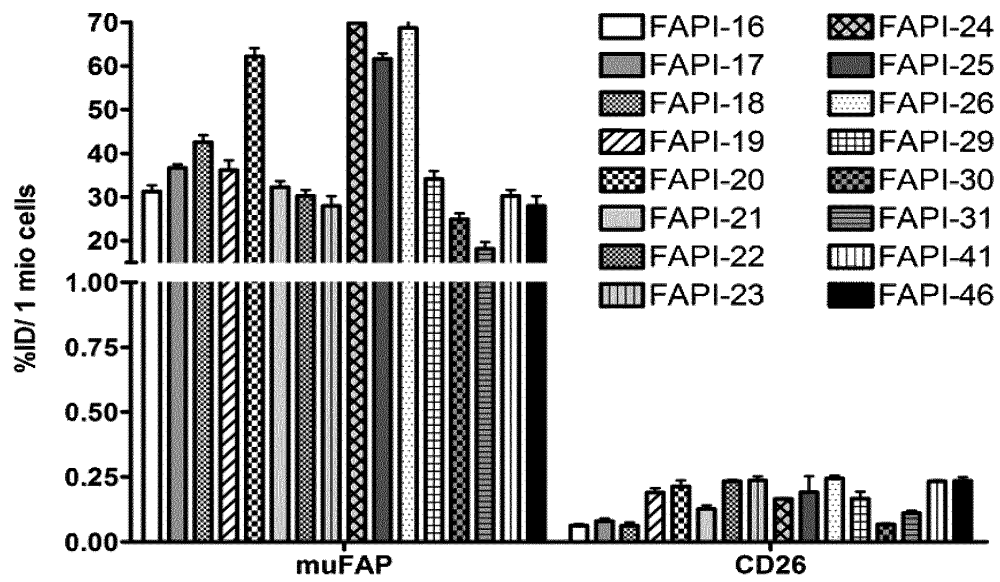


Figure 19

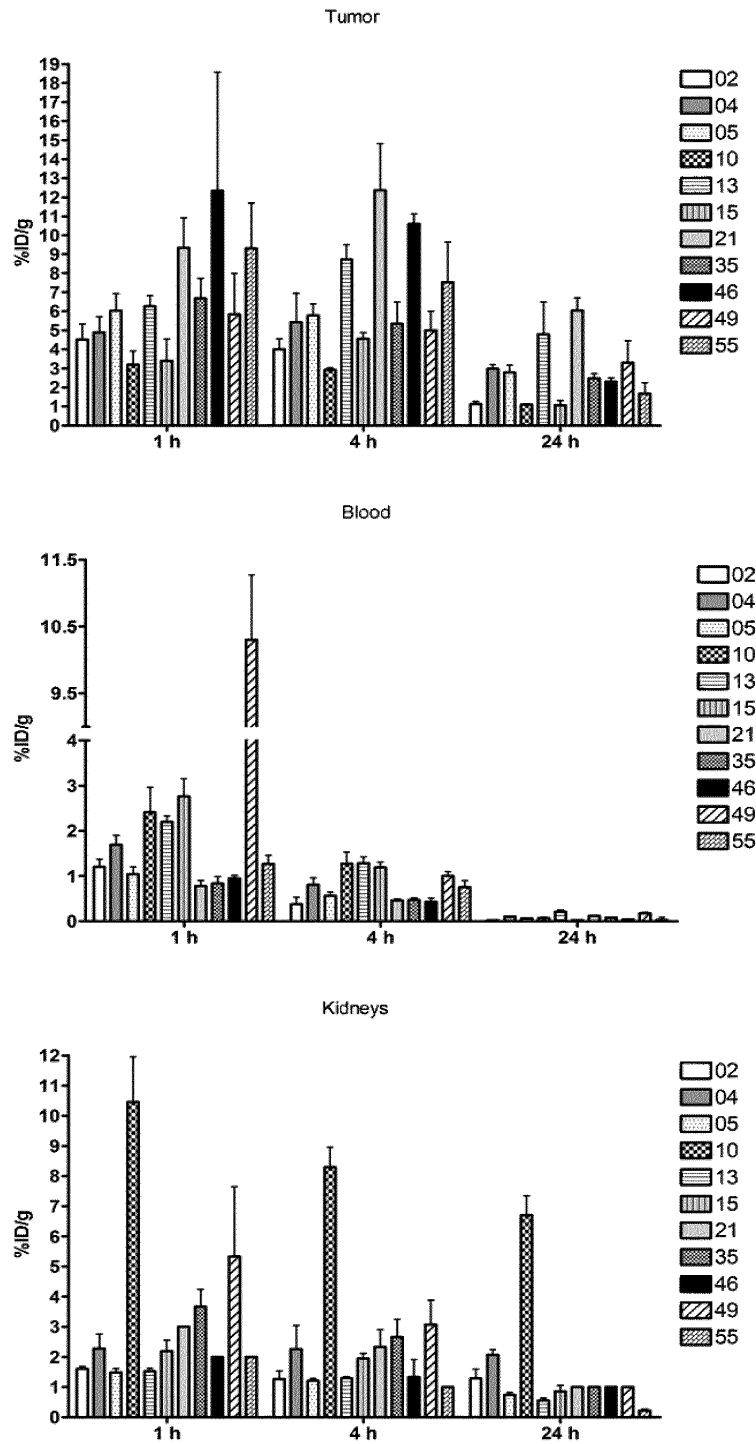
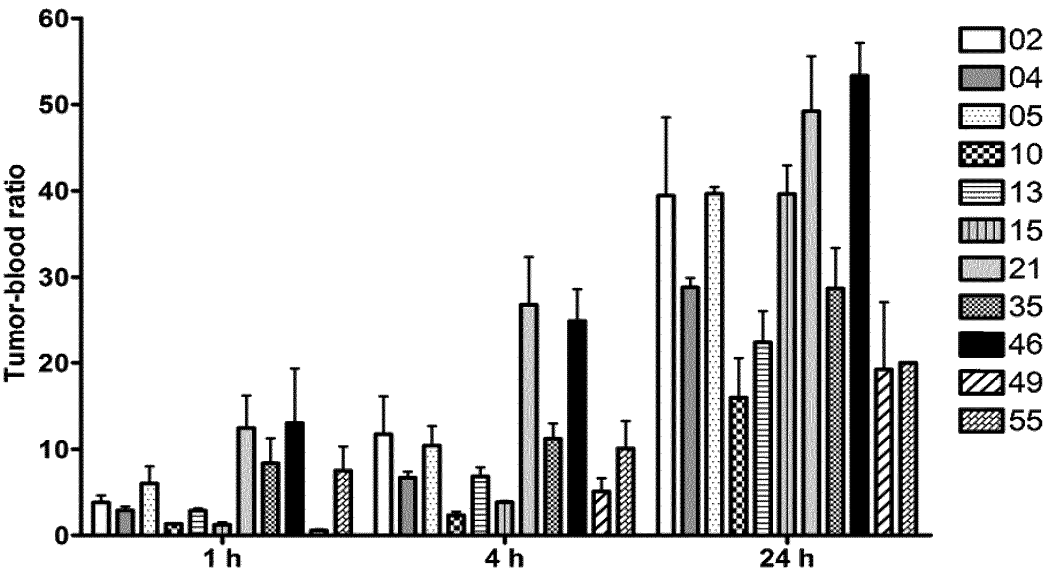


Figure 20



5

Figure 21

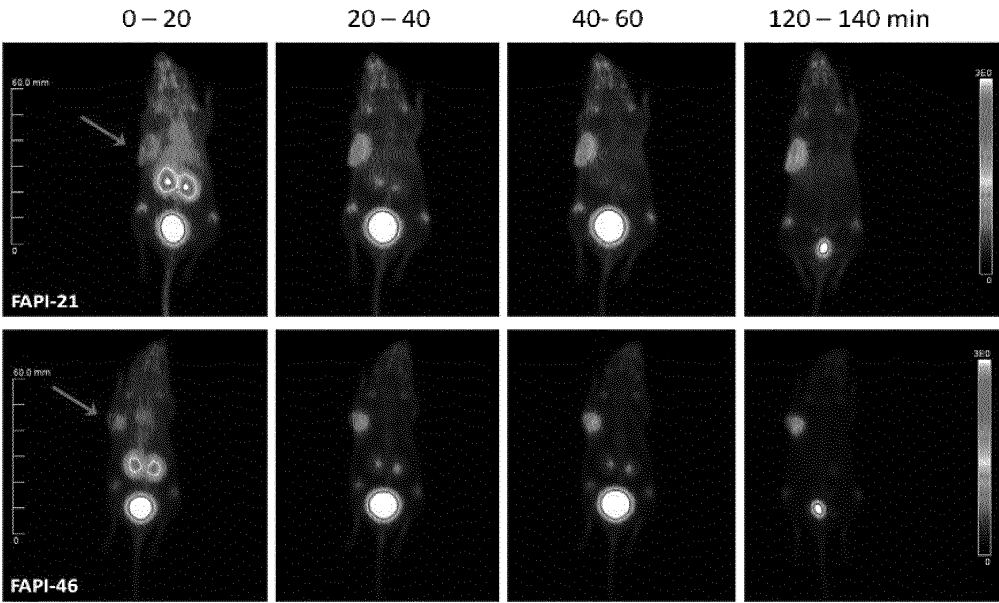


Figure 22

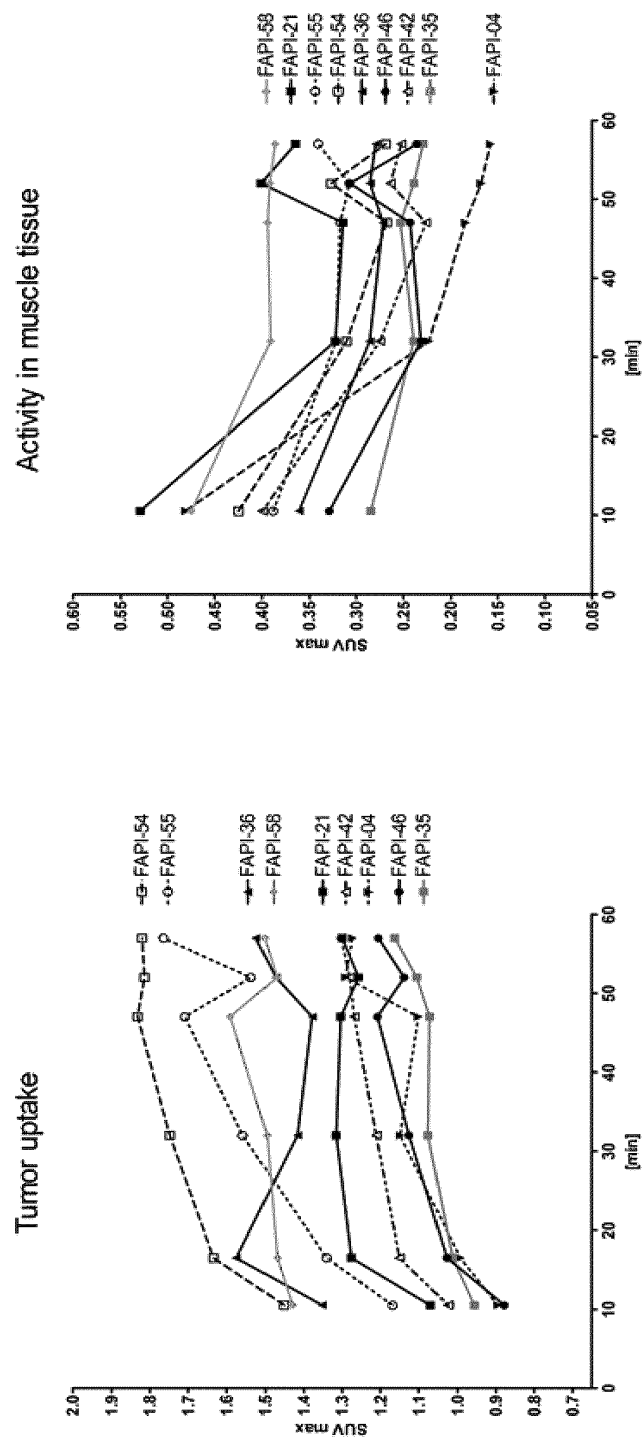


Figure 22 continued

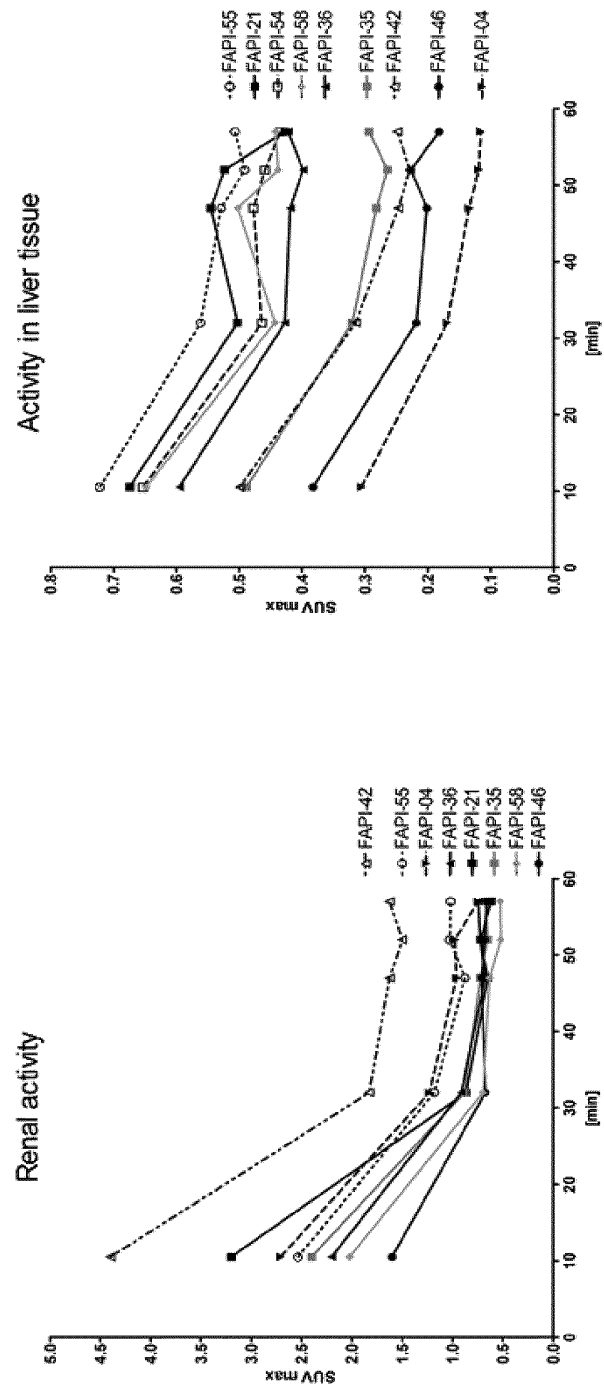


Figure 23

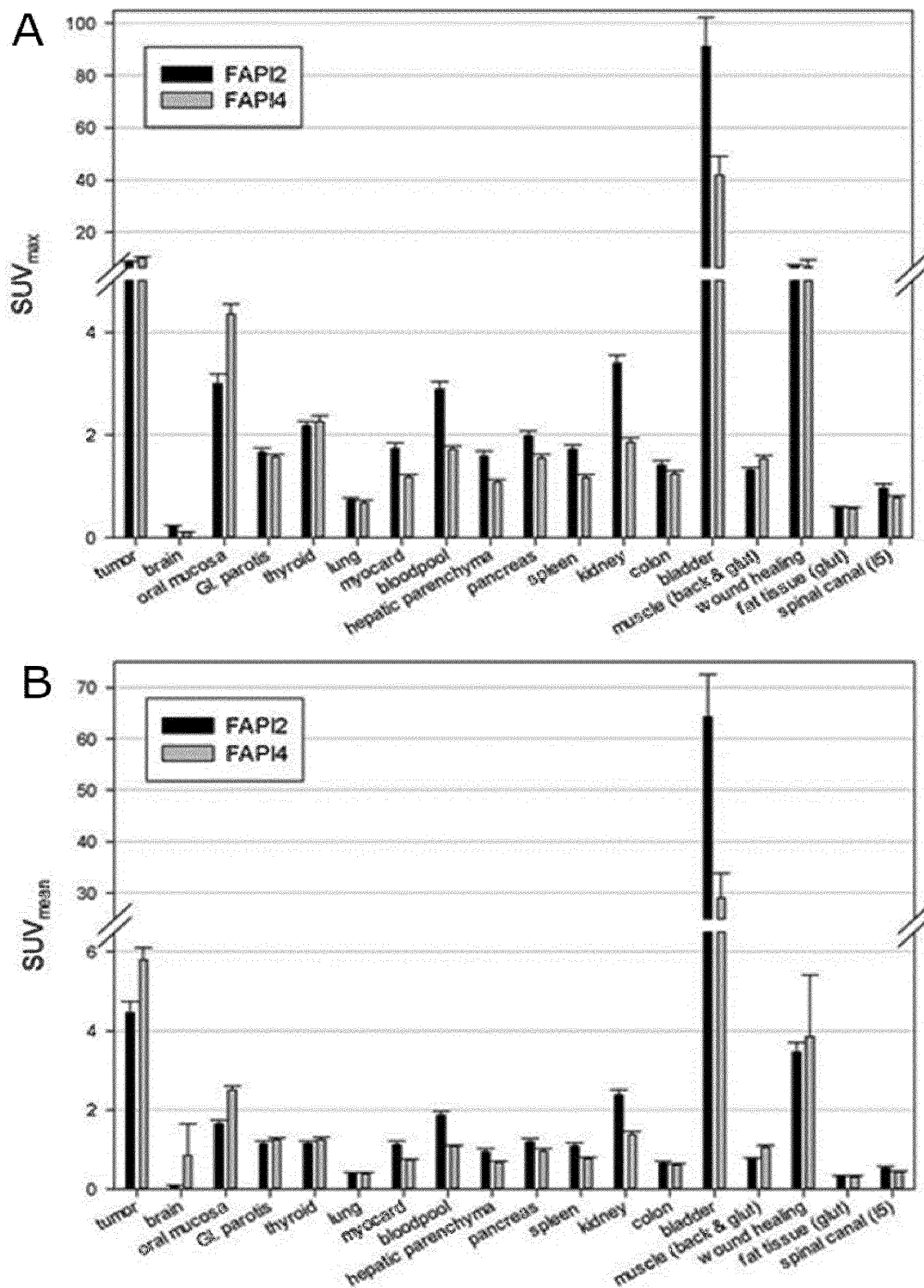


Figure 24

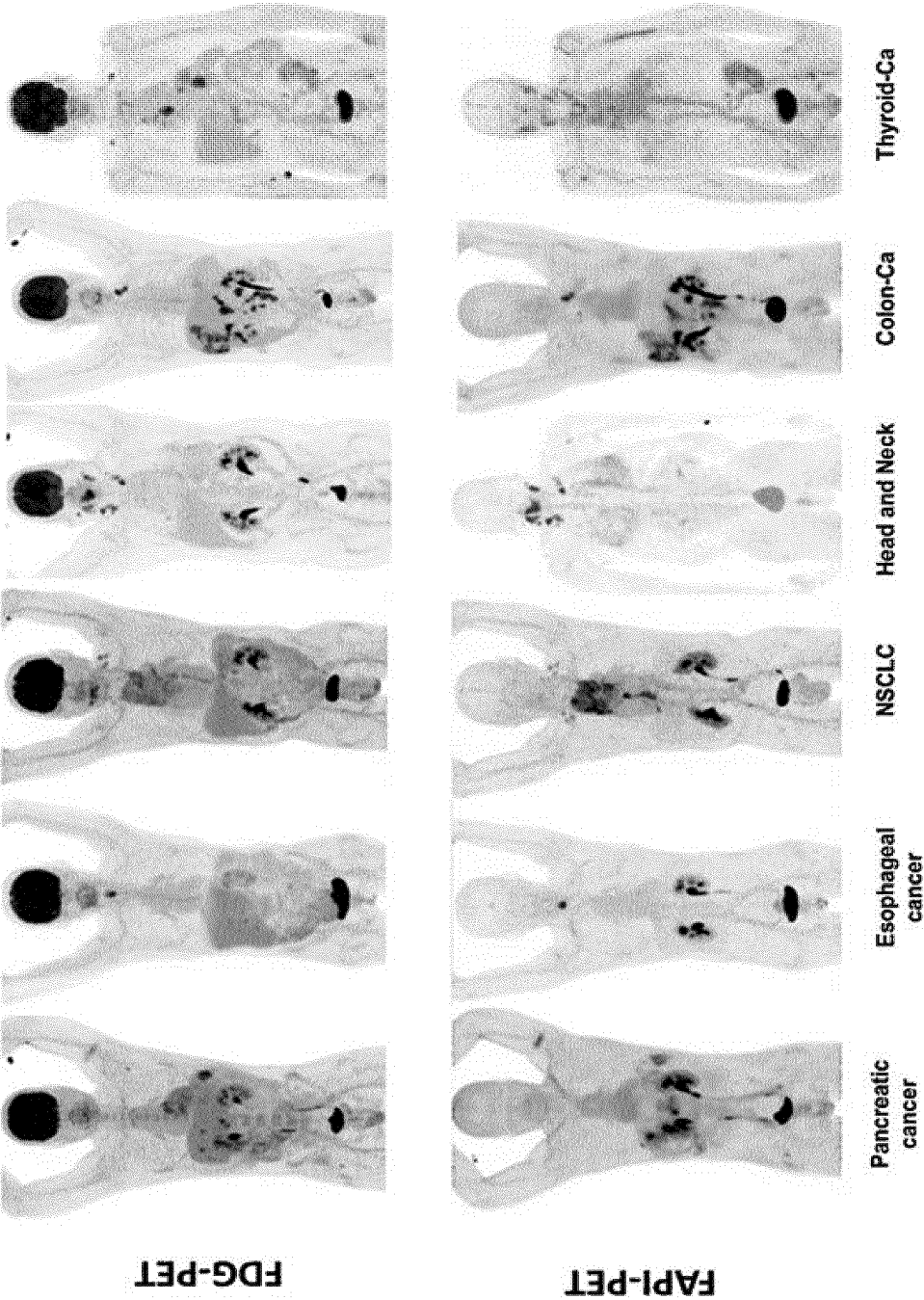


Figure 24 continued

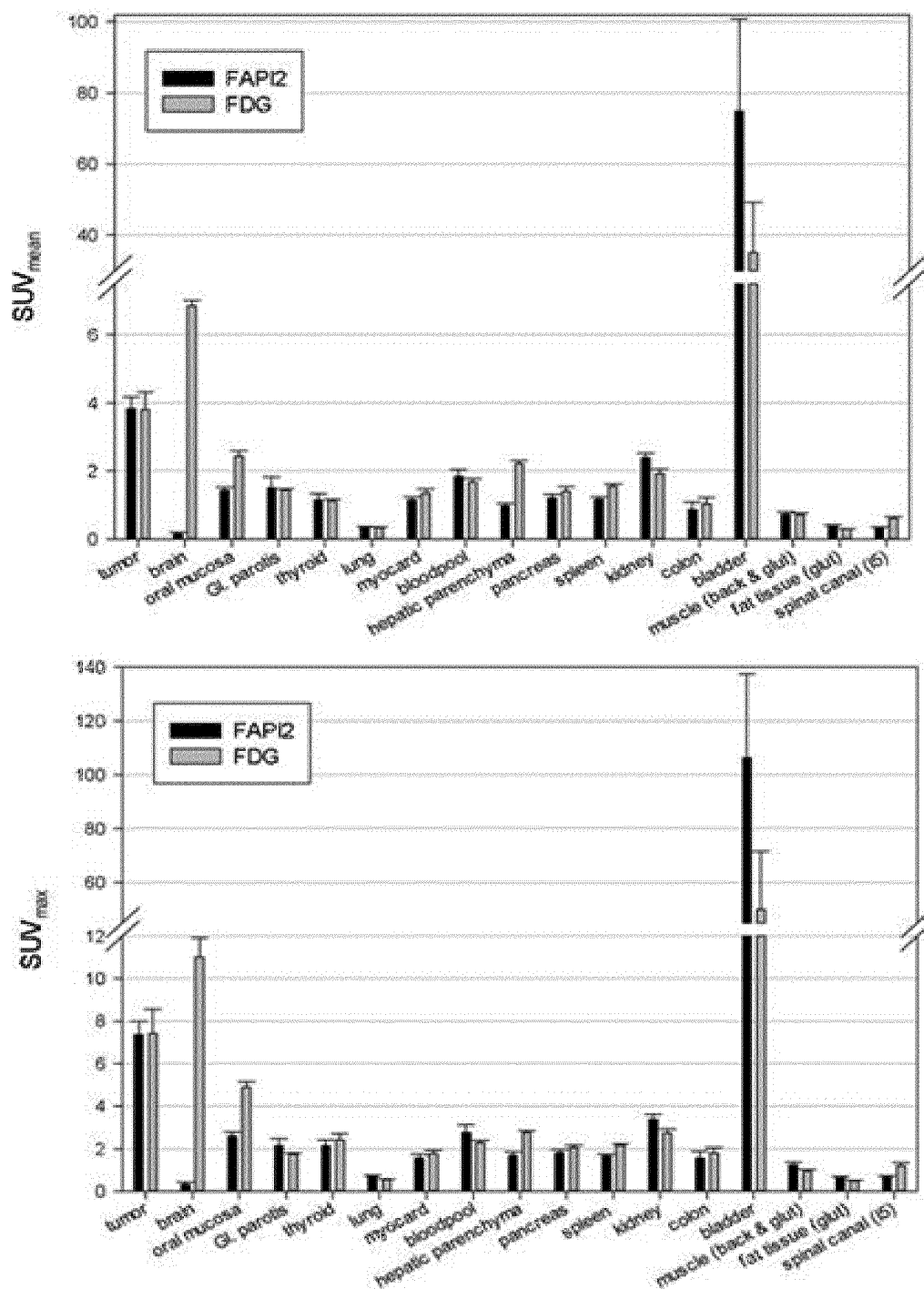


Figure 25

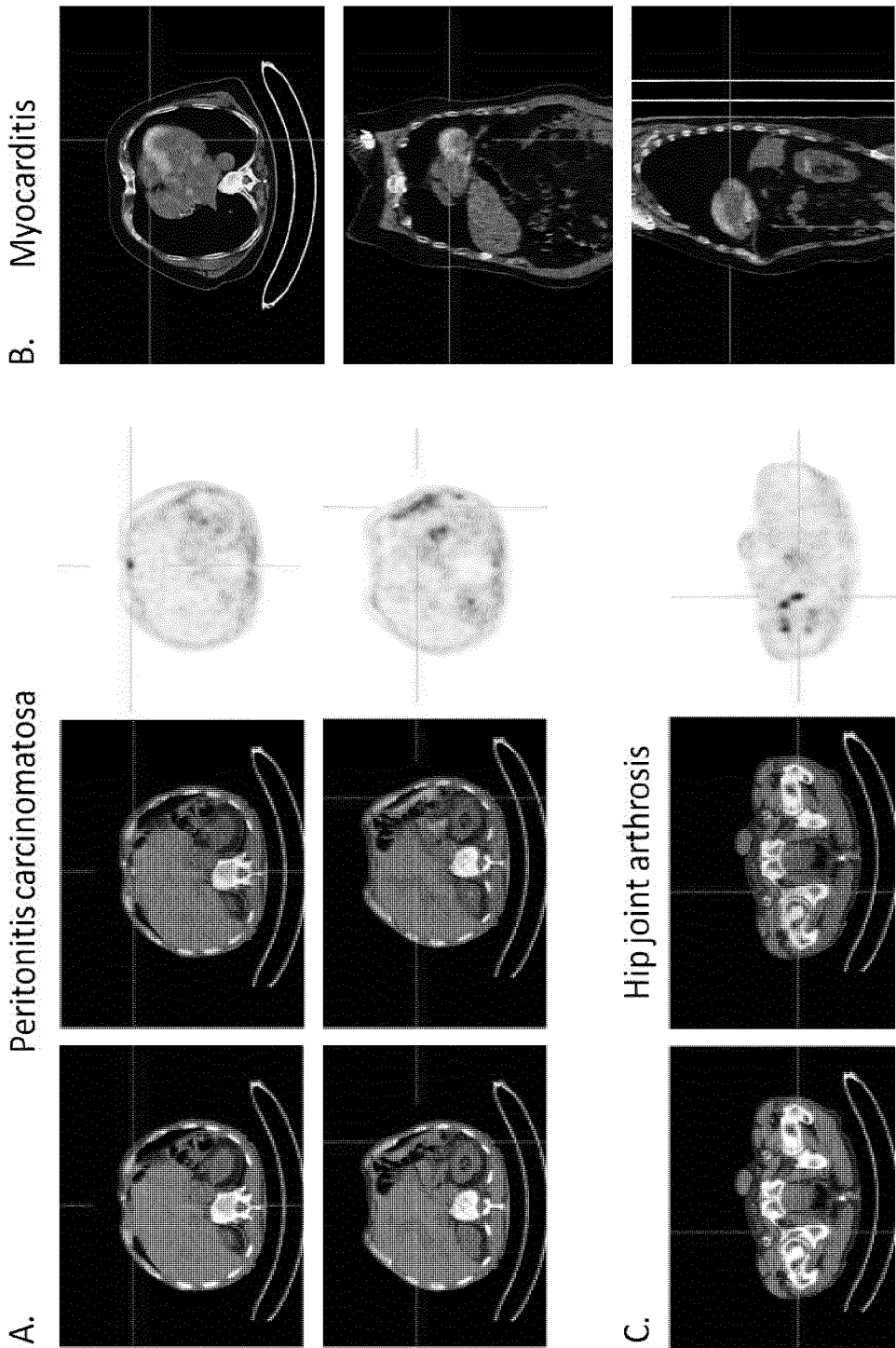
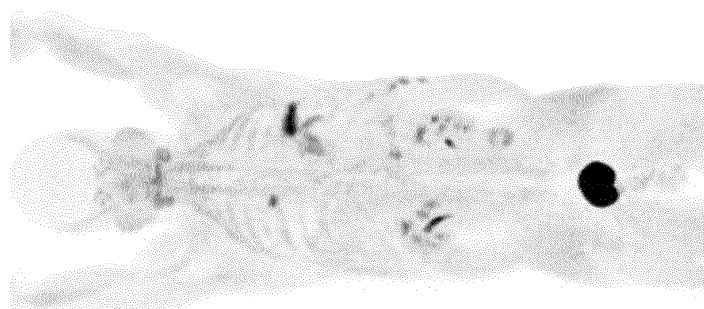
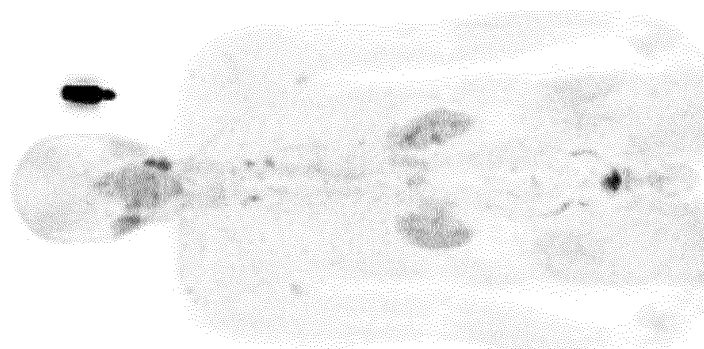


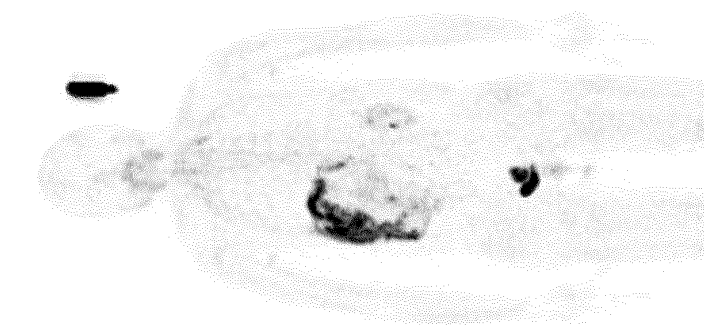
Figure 26



Rectal ca.



Mucoepidermoid ca.



Ovarial ca.

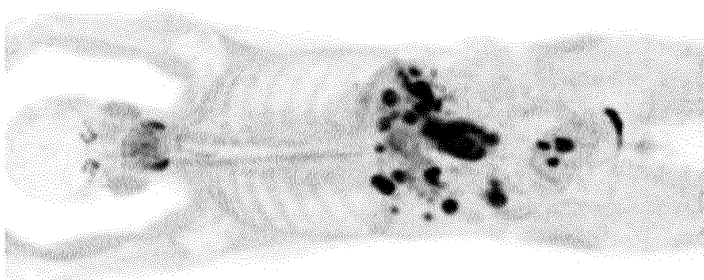


Figure 27

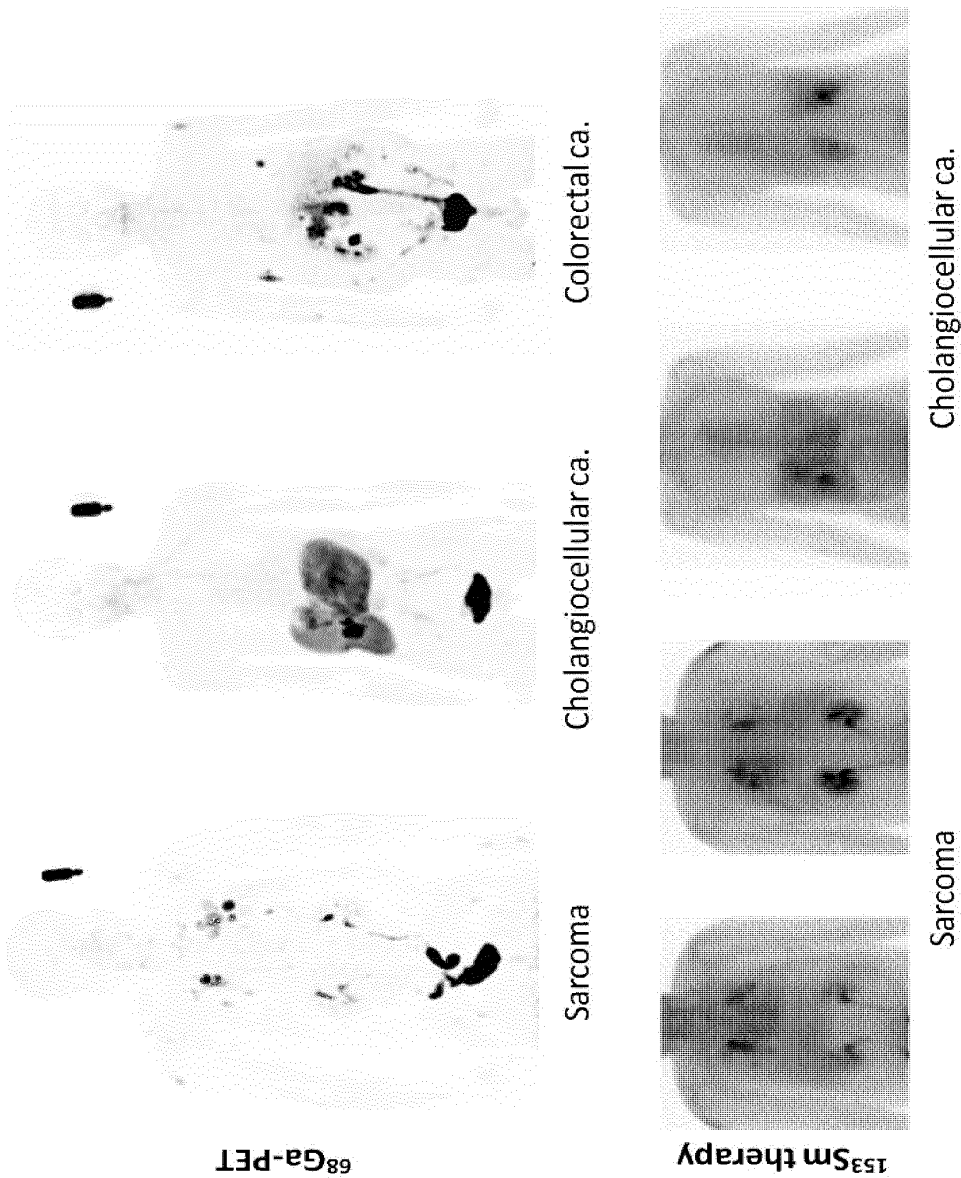


Figure 28

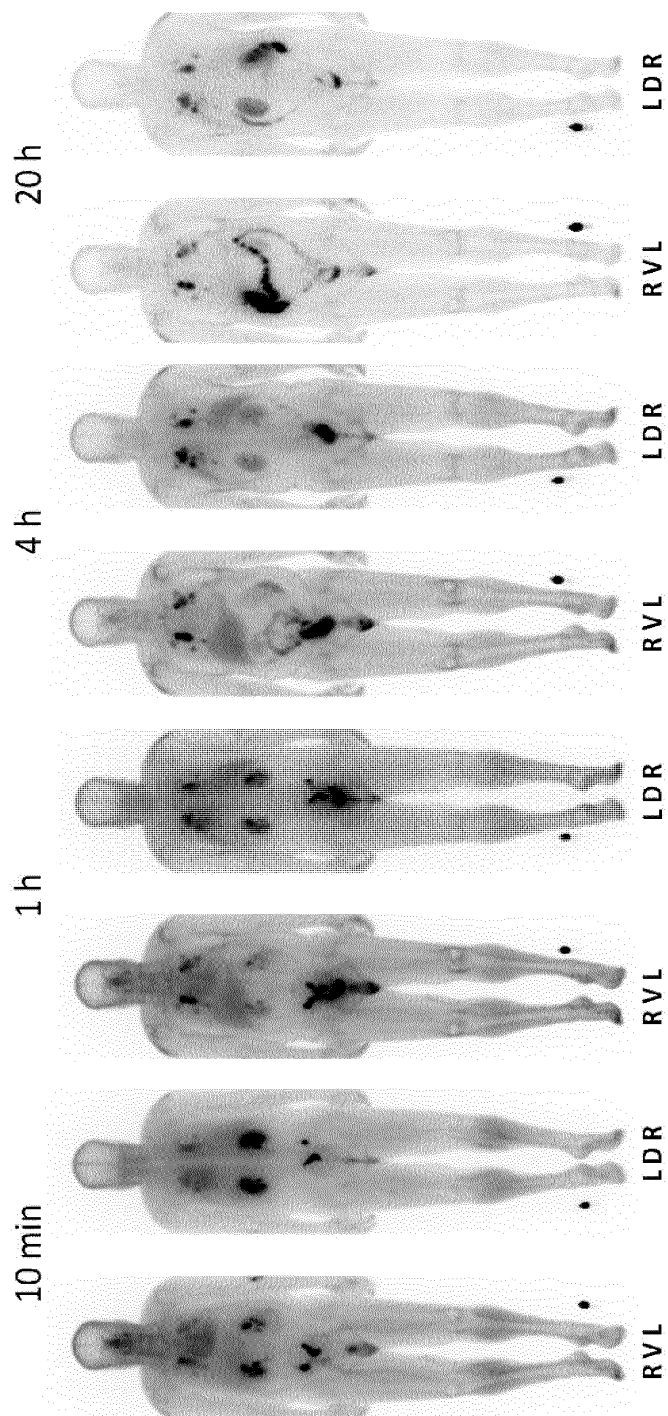


Figure 29

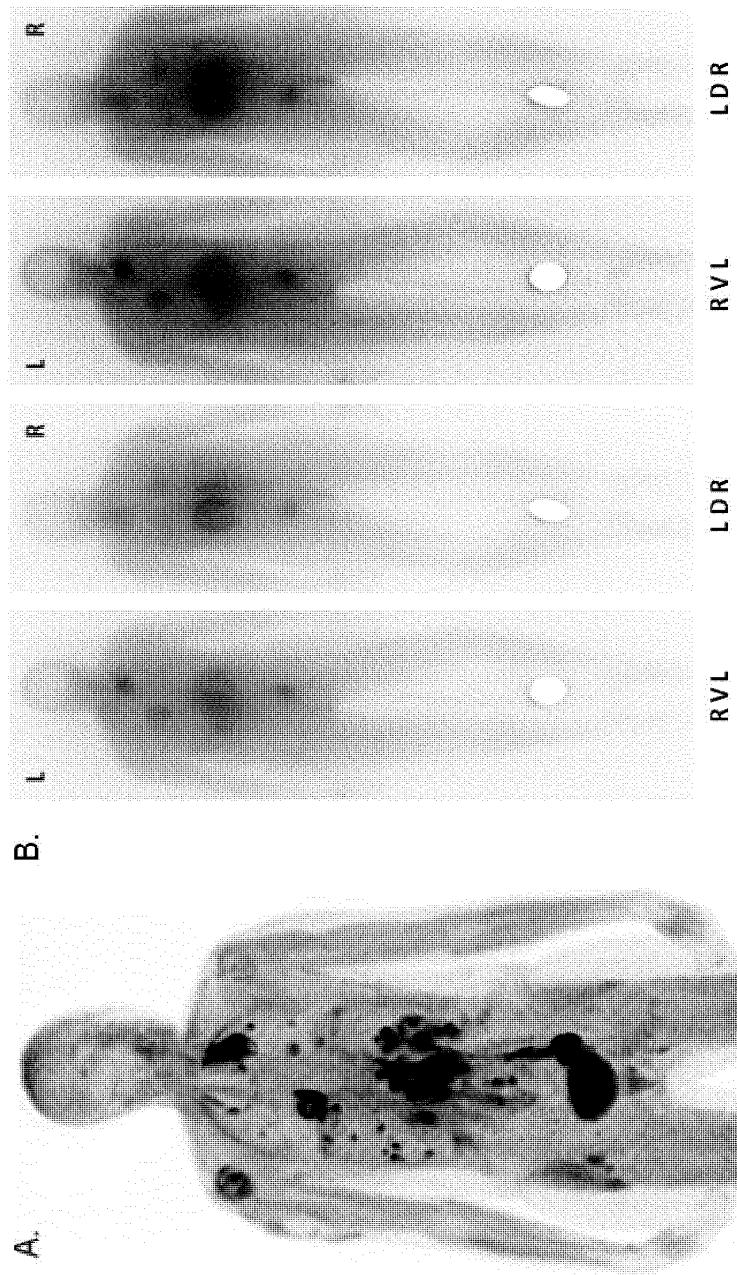




Figure 30

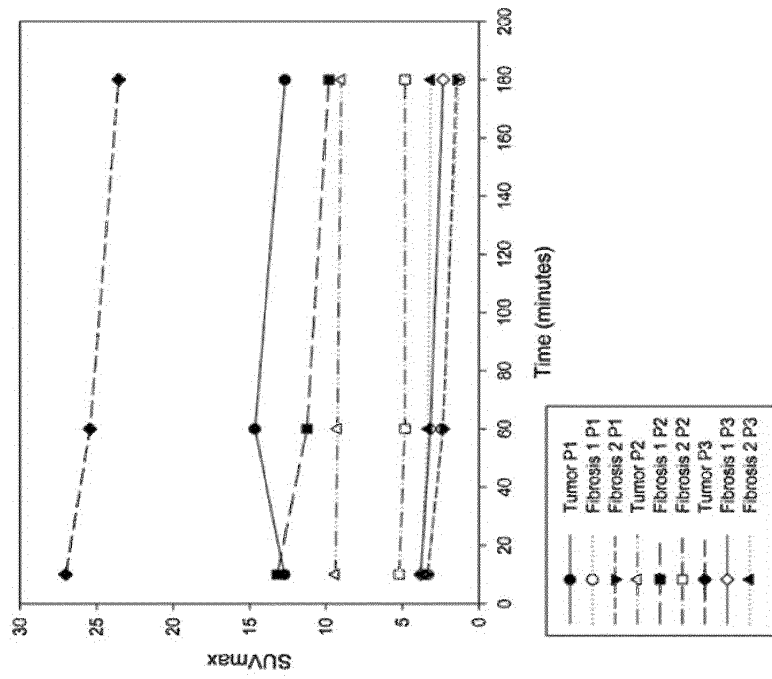
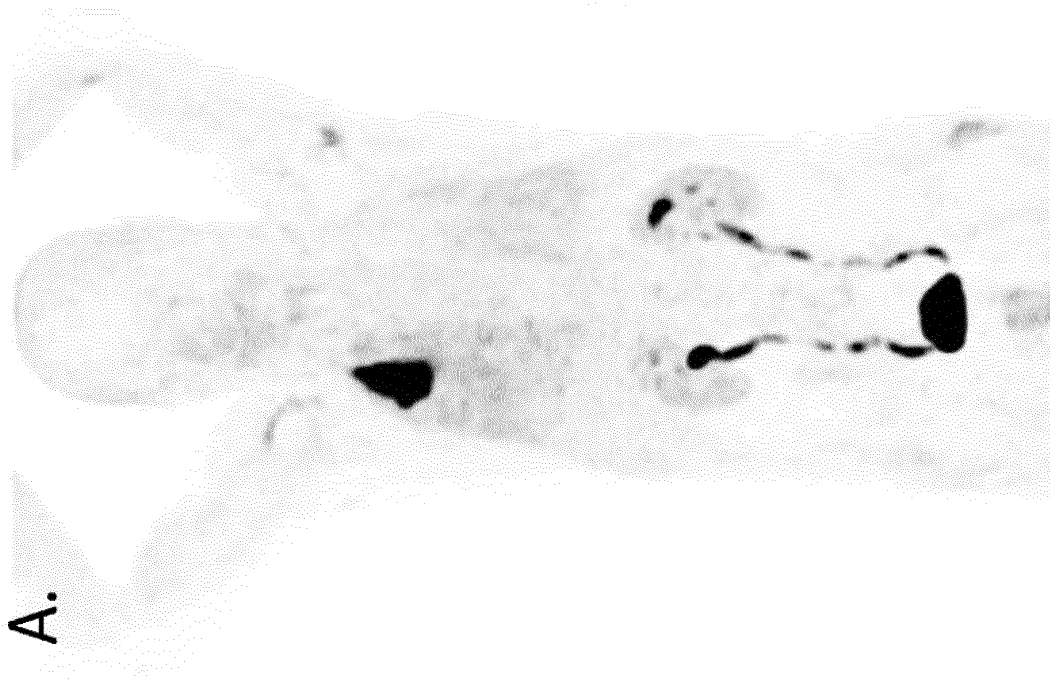
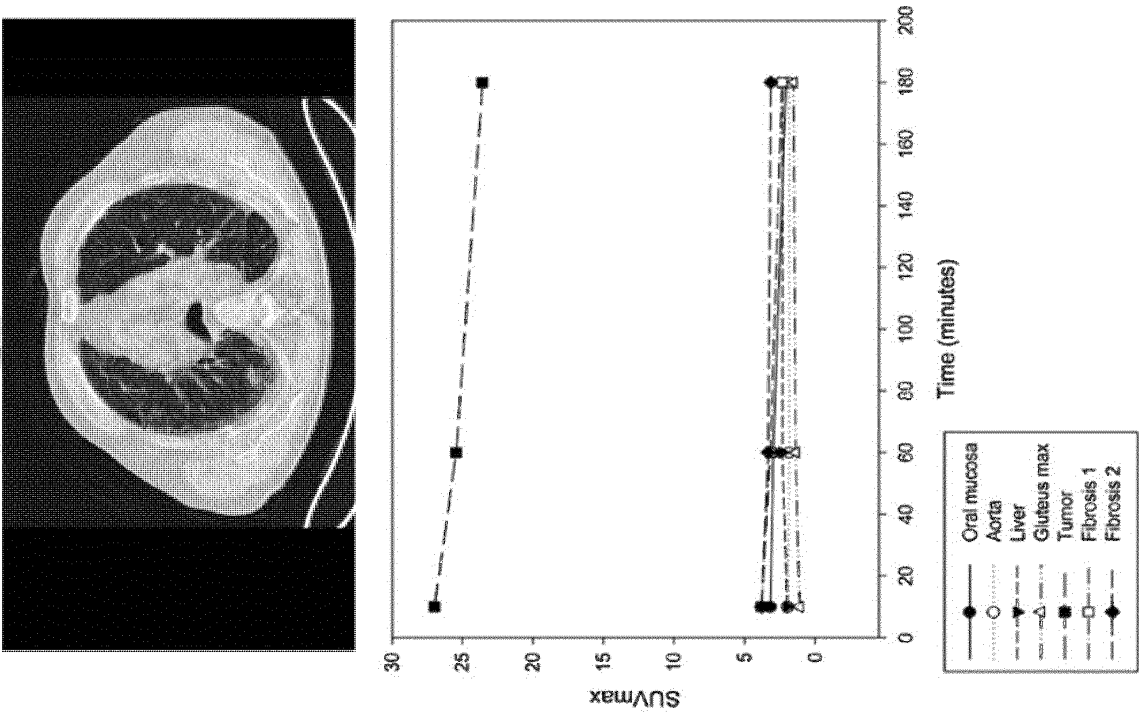
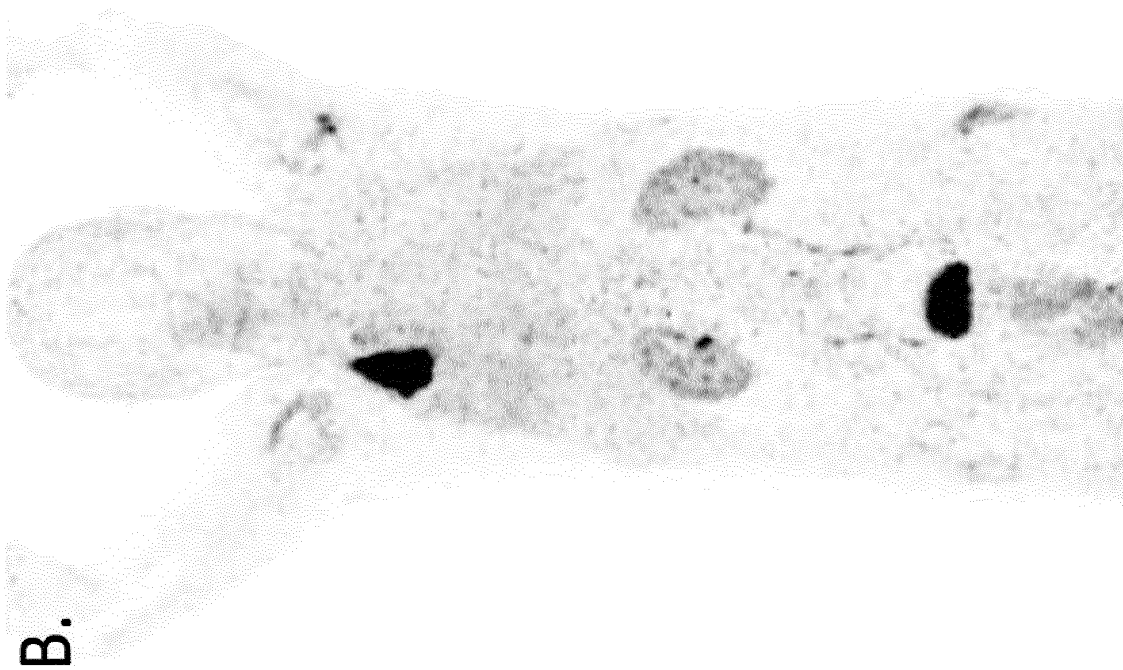
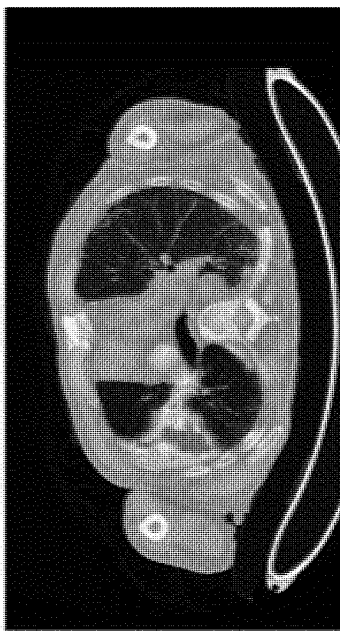
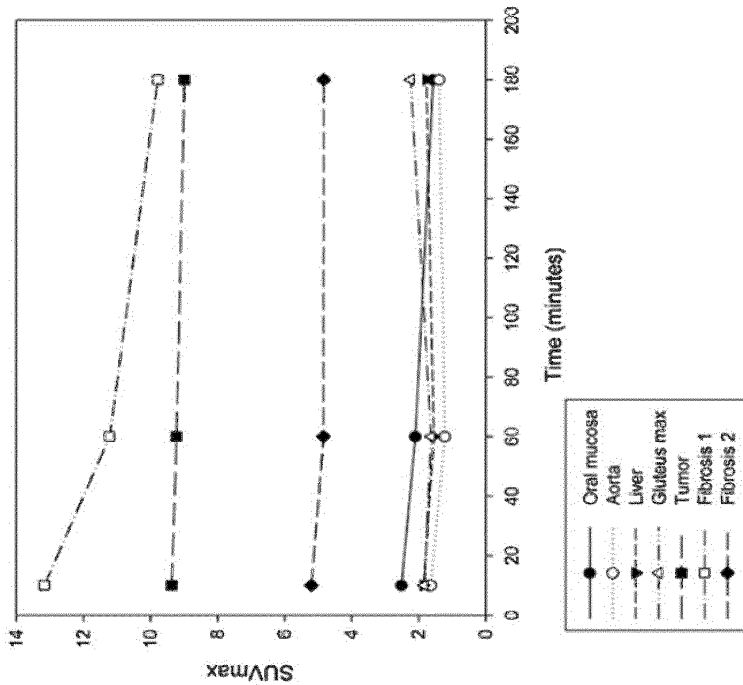
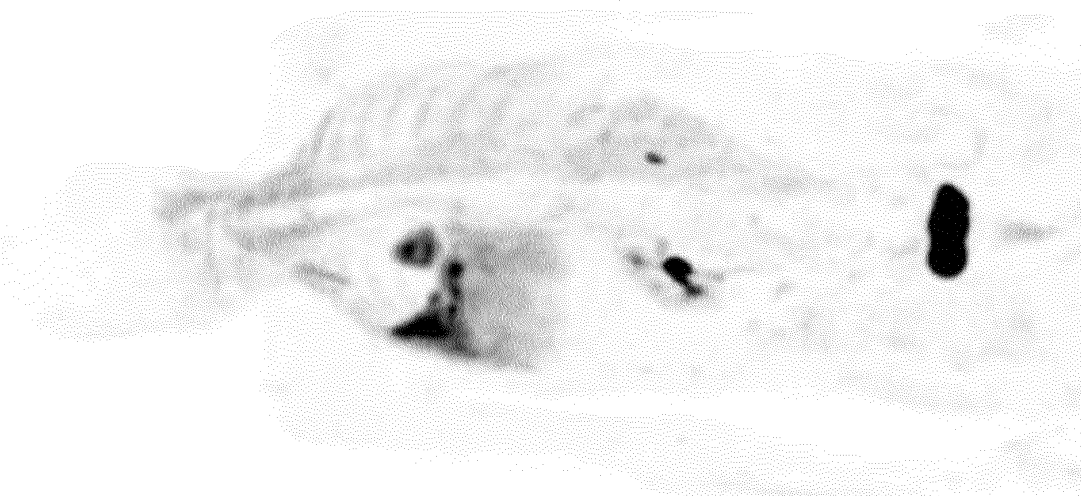


Figure 30 continued





C.

Figure 31

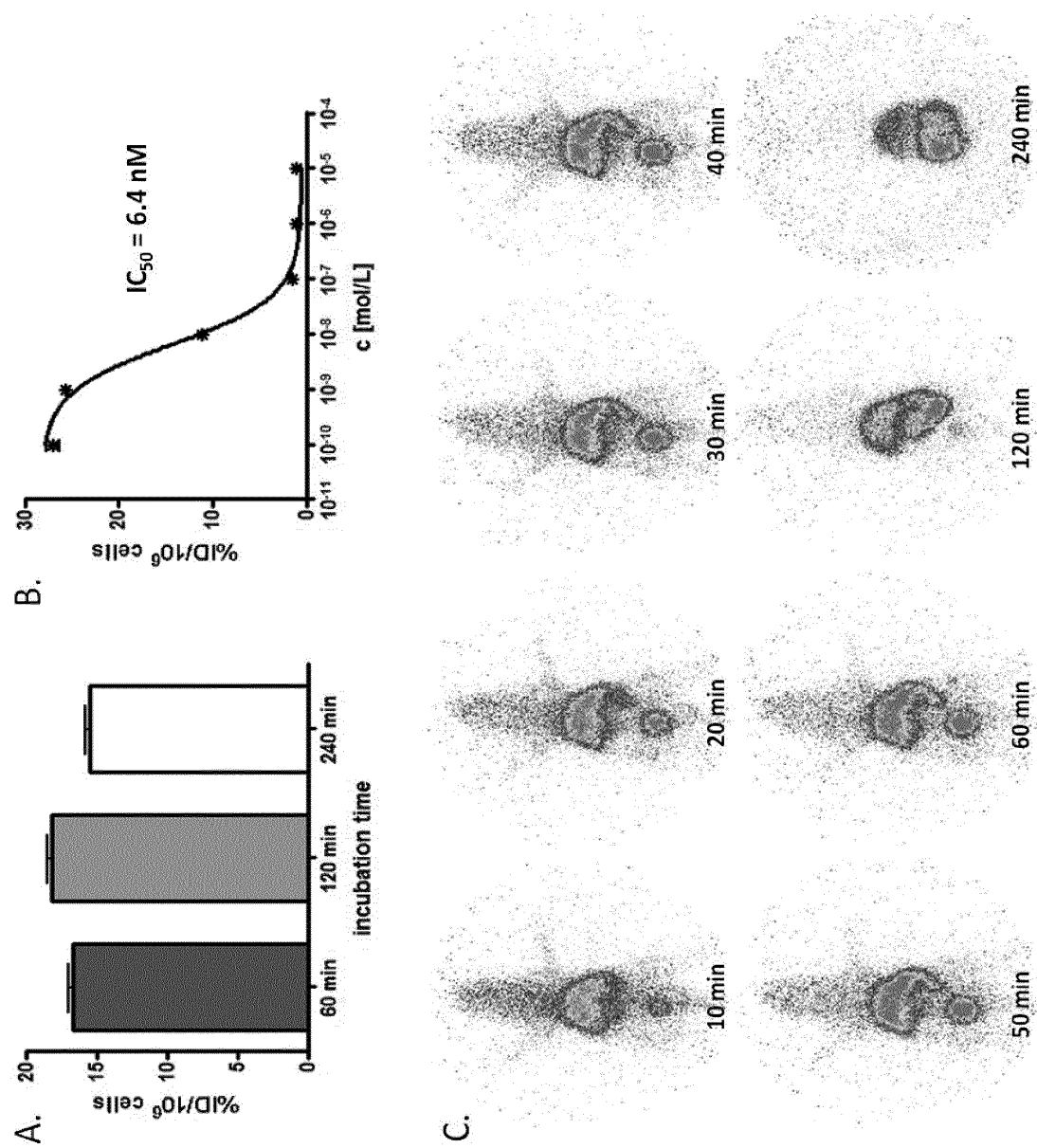


Figure 32

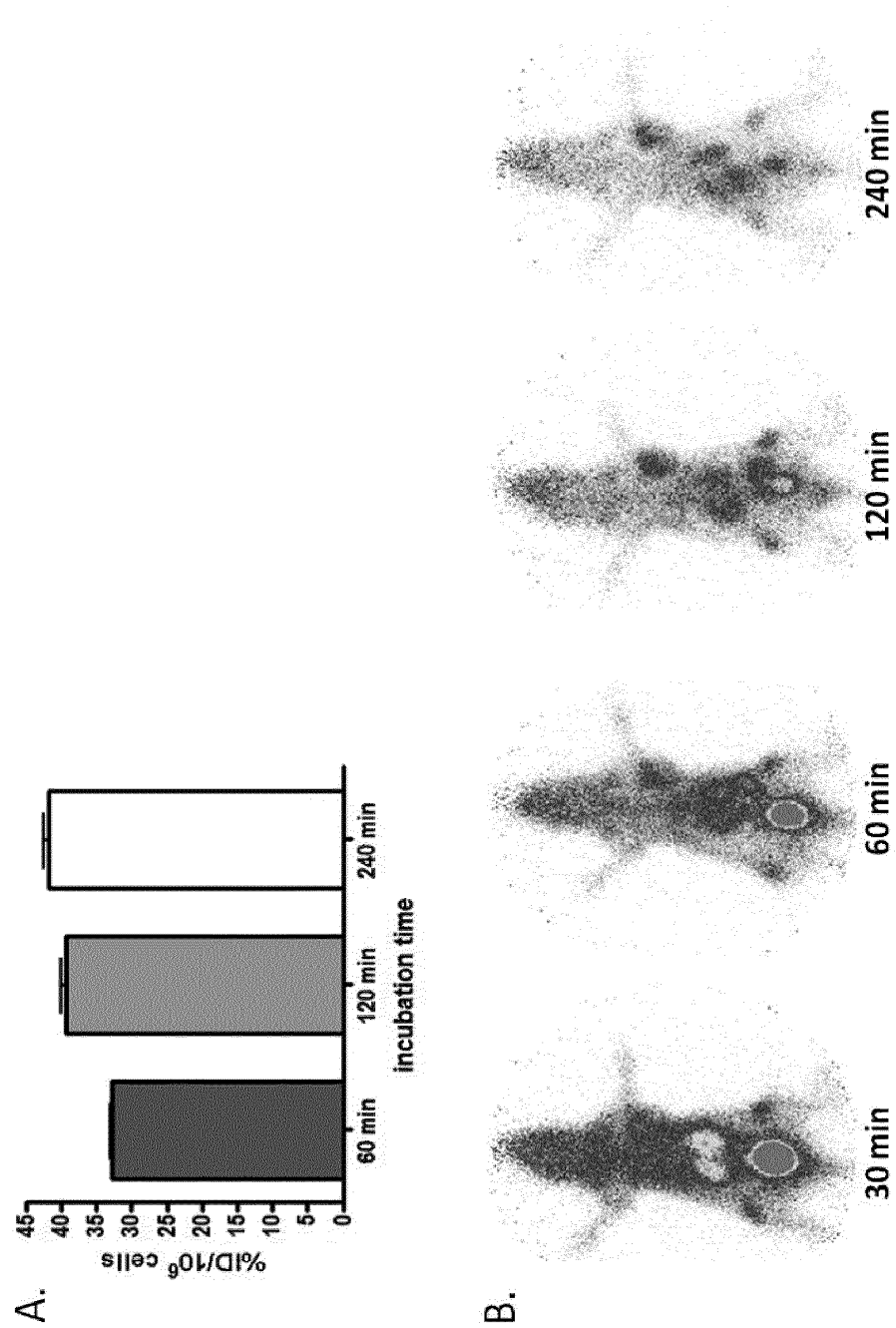


Figure 33

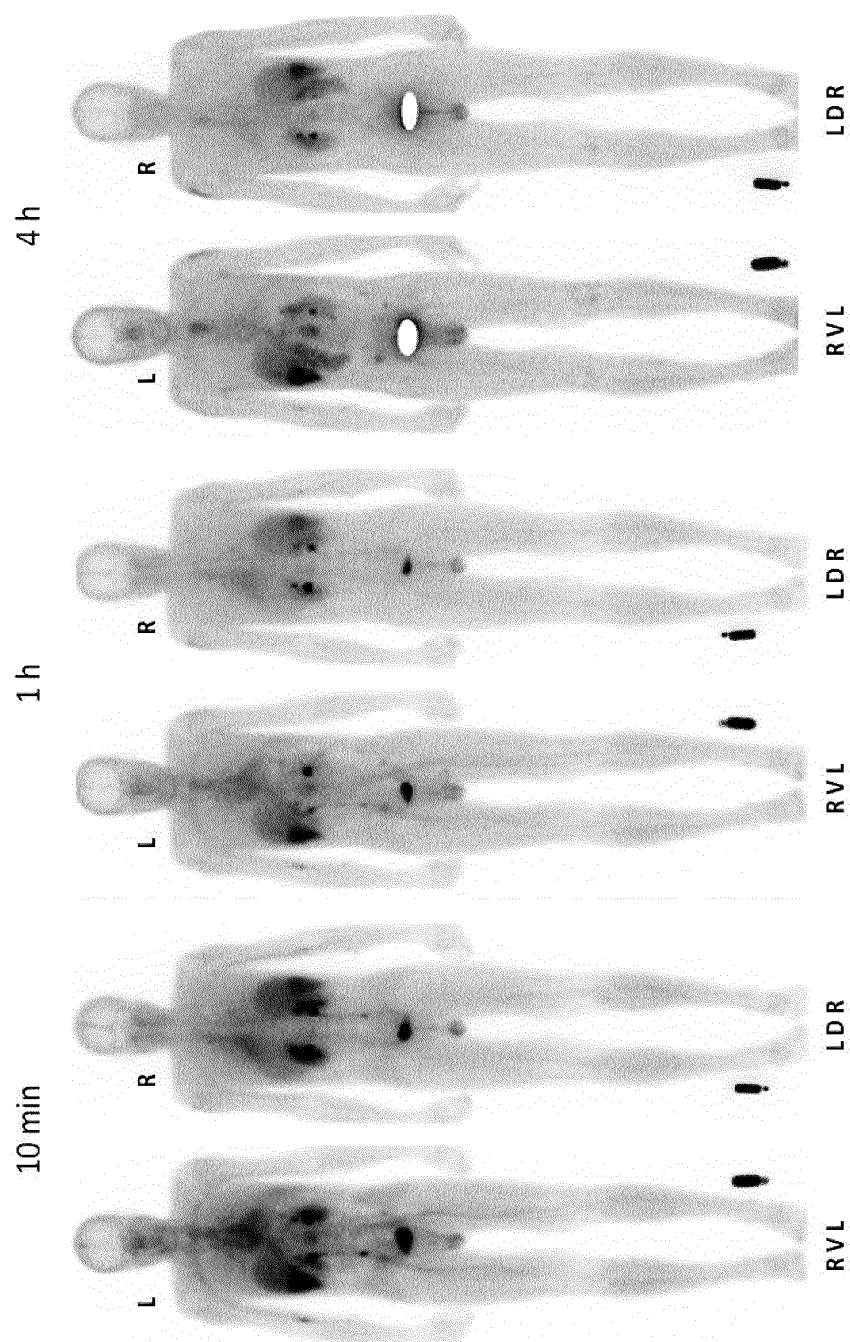


Figure 34

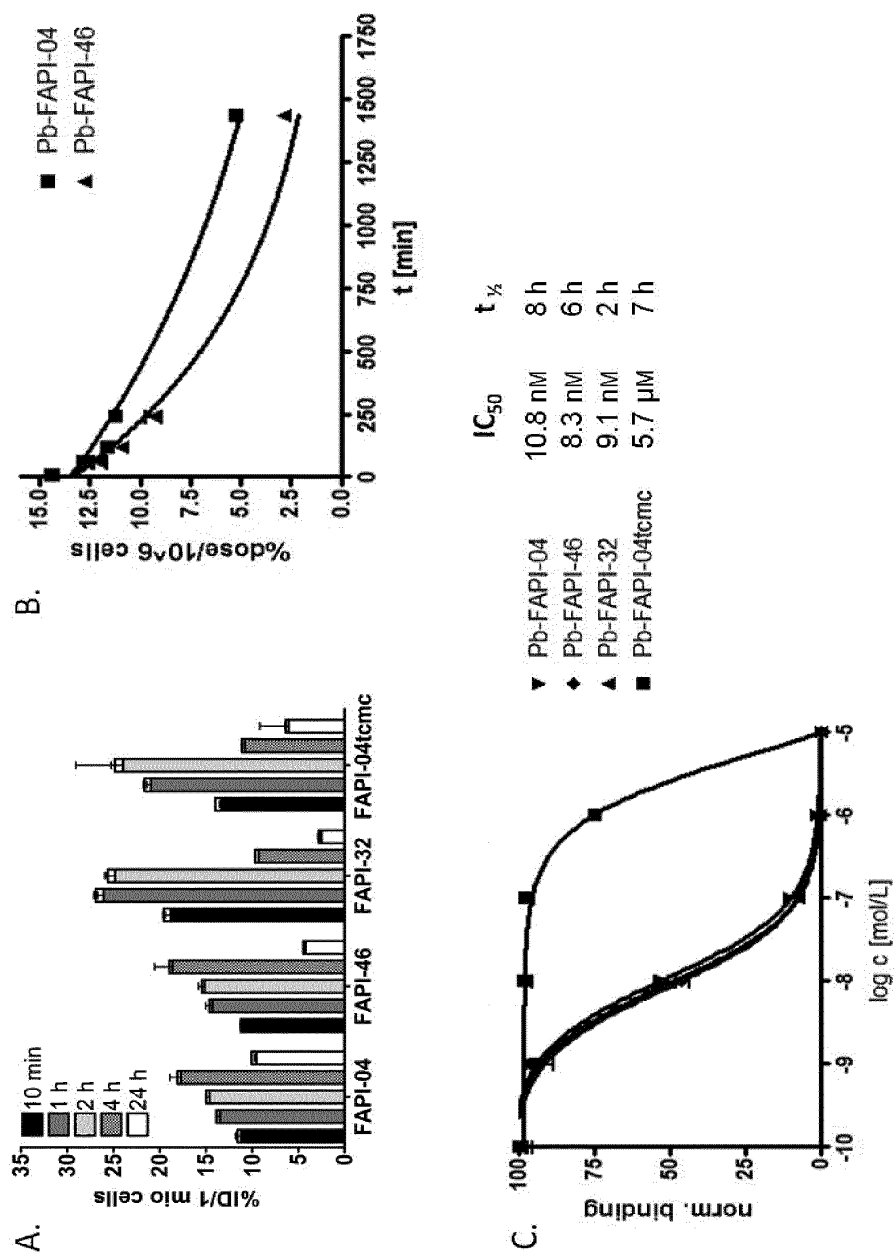


Figure 35

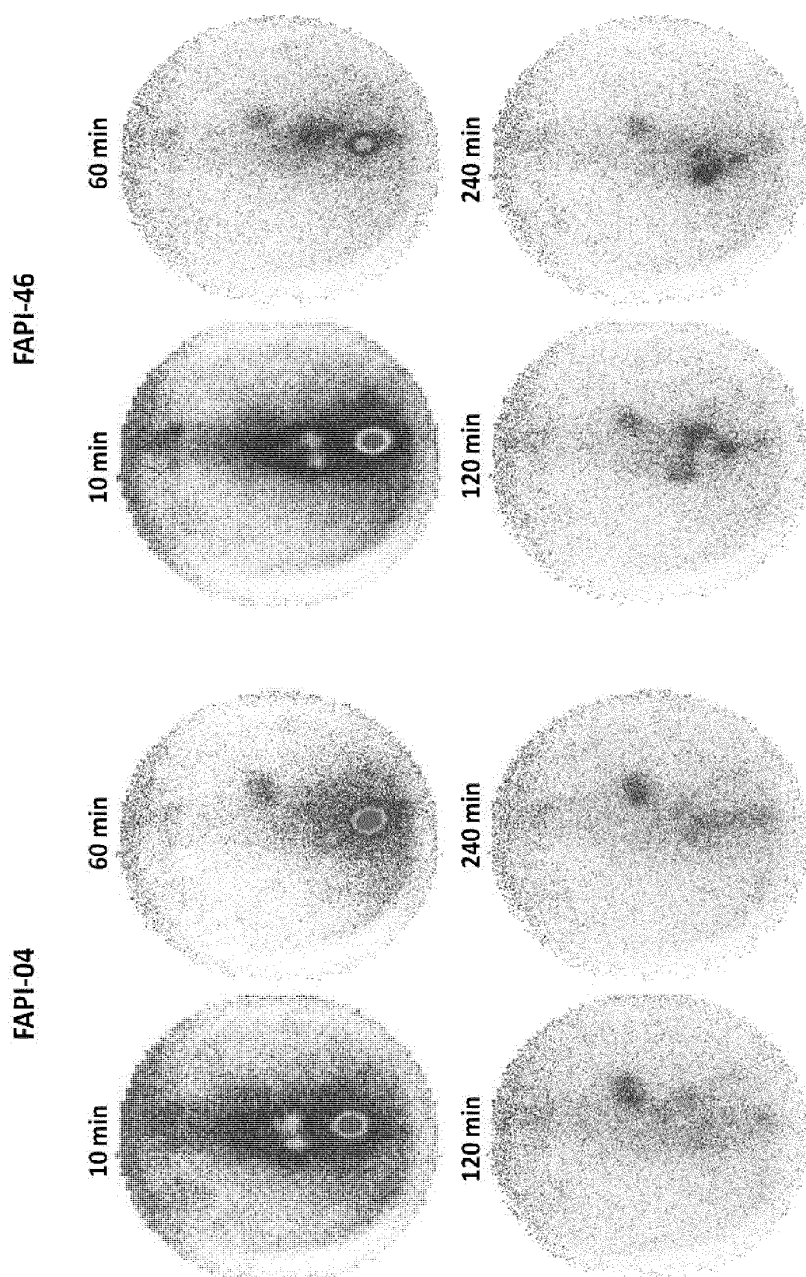


Figure 36

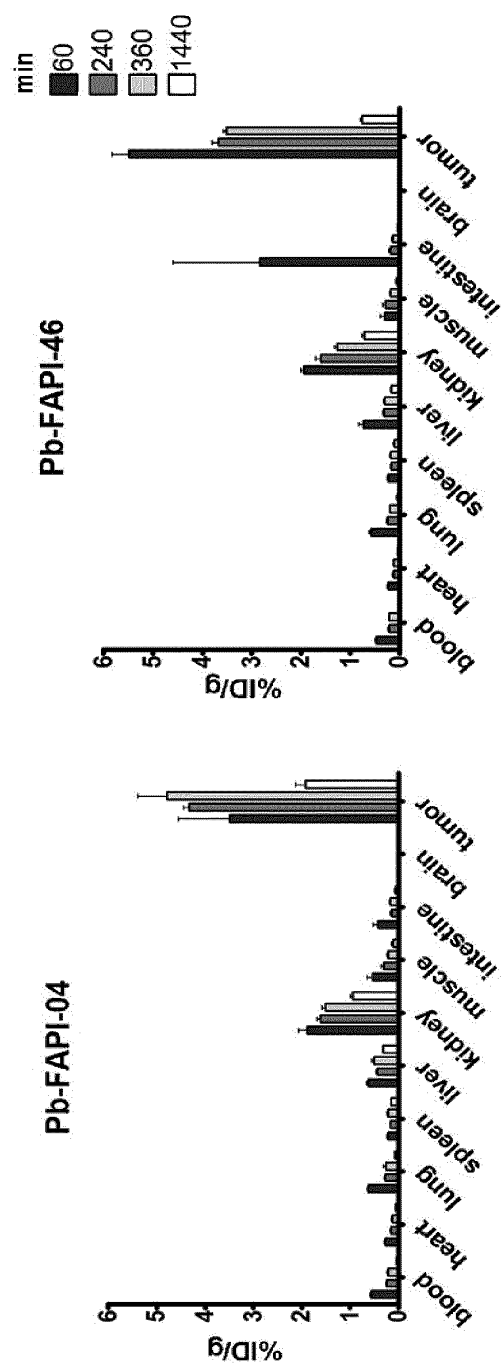


Figure 37

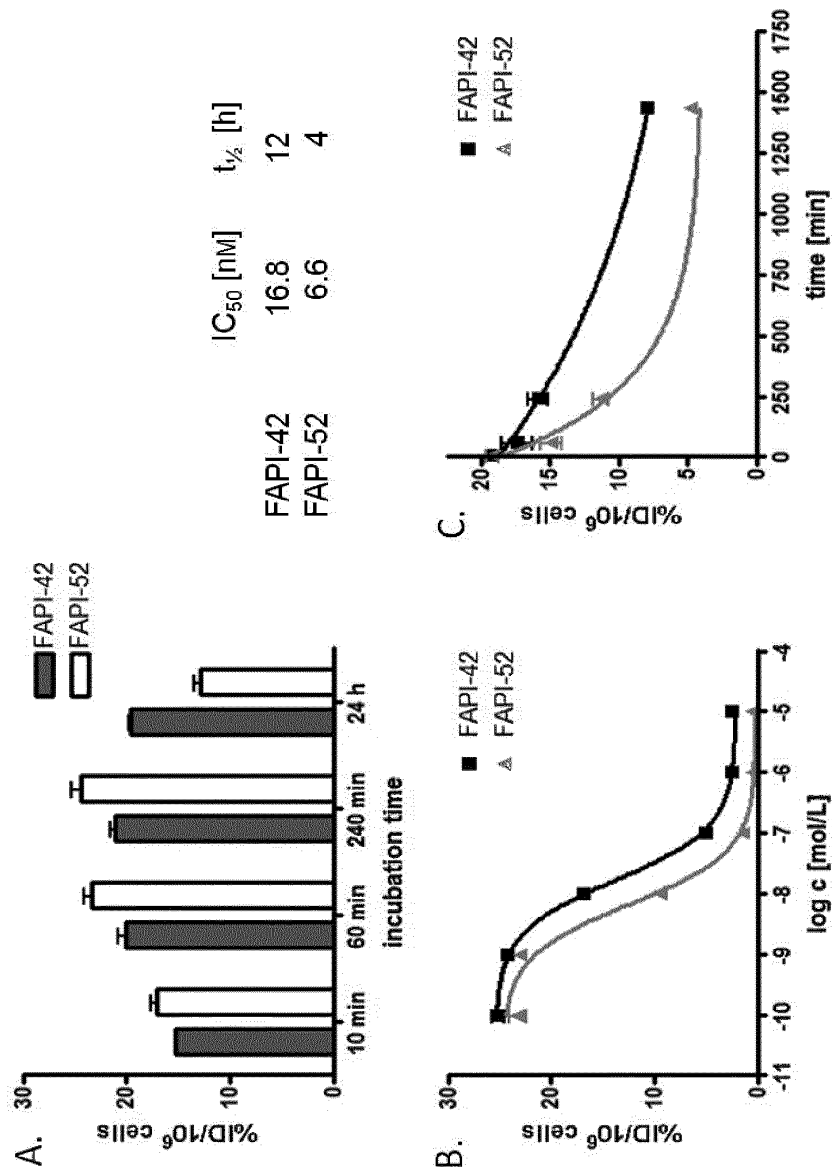


Figure 38

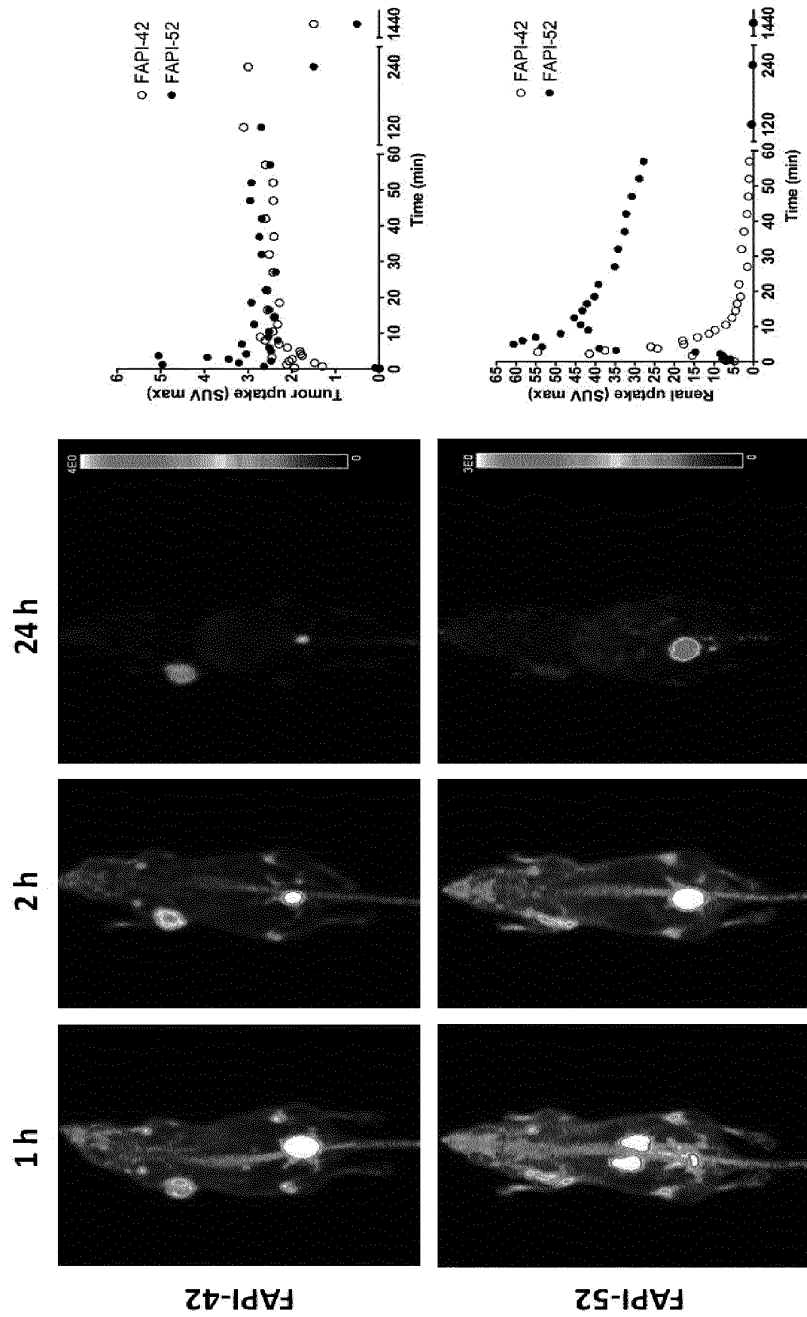


Figure 39

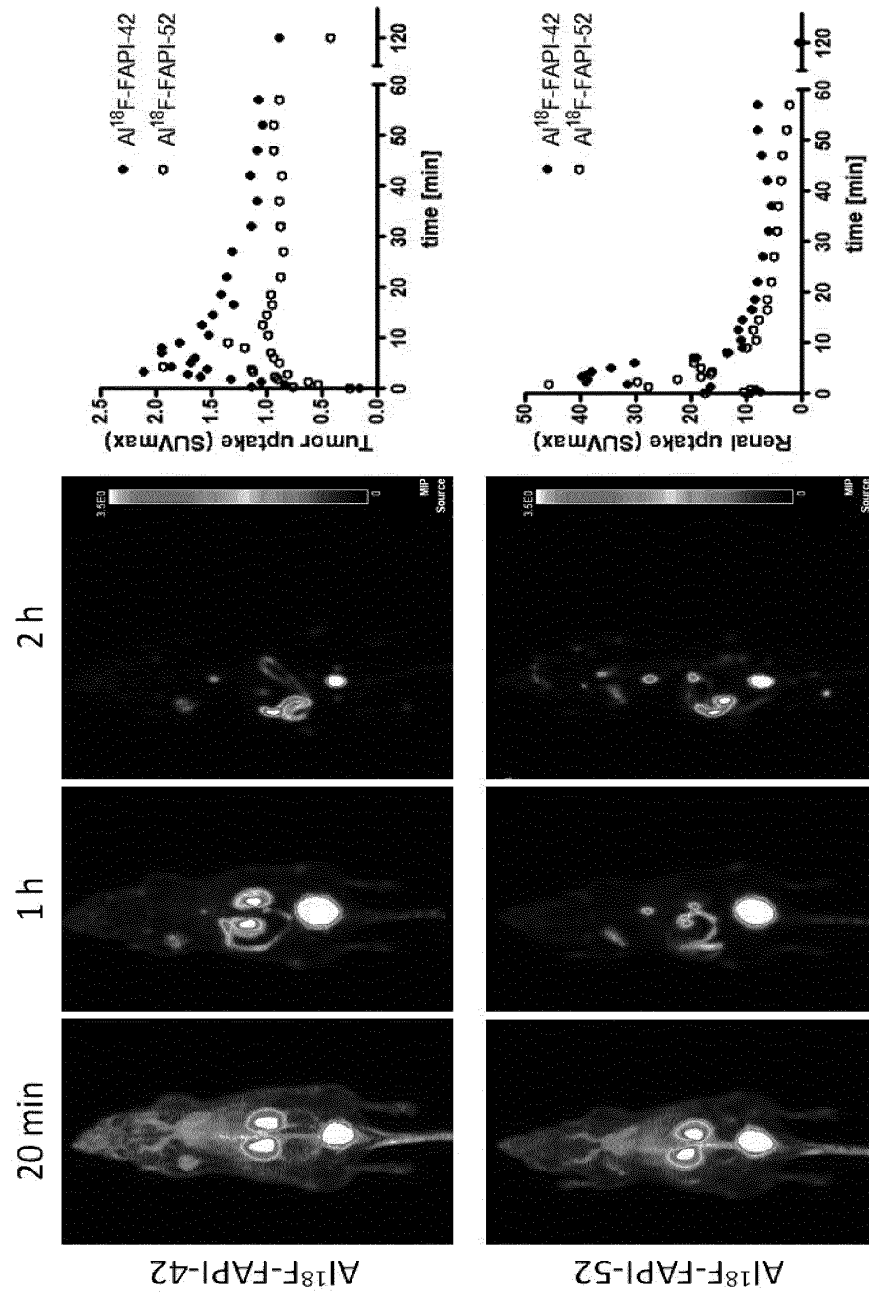


Figure 40

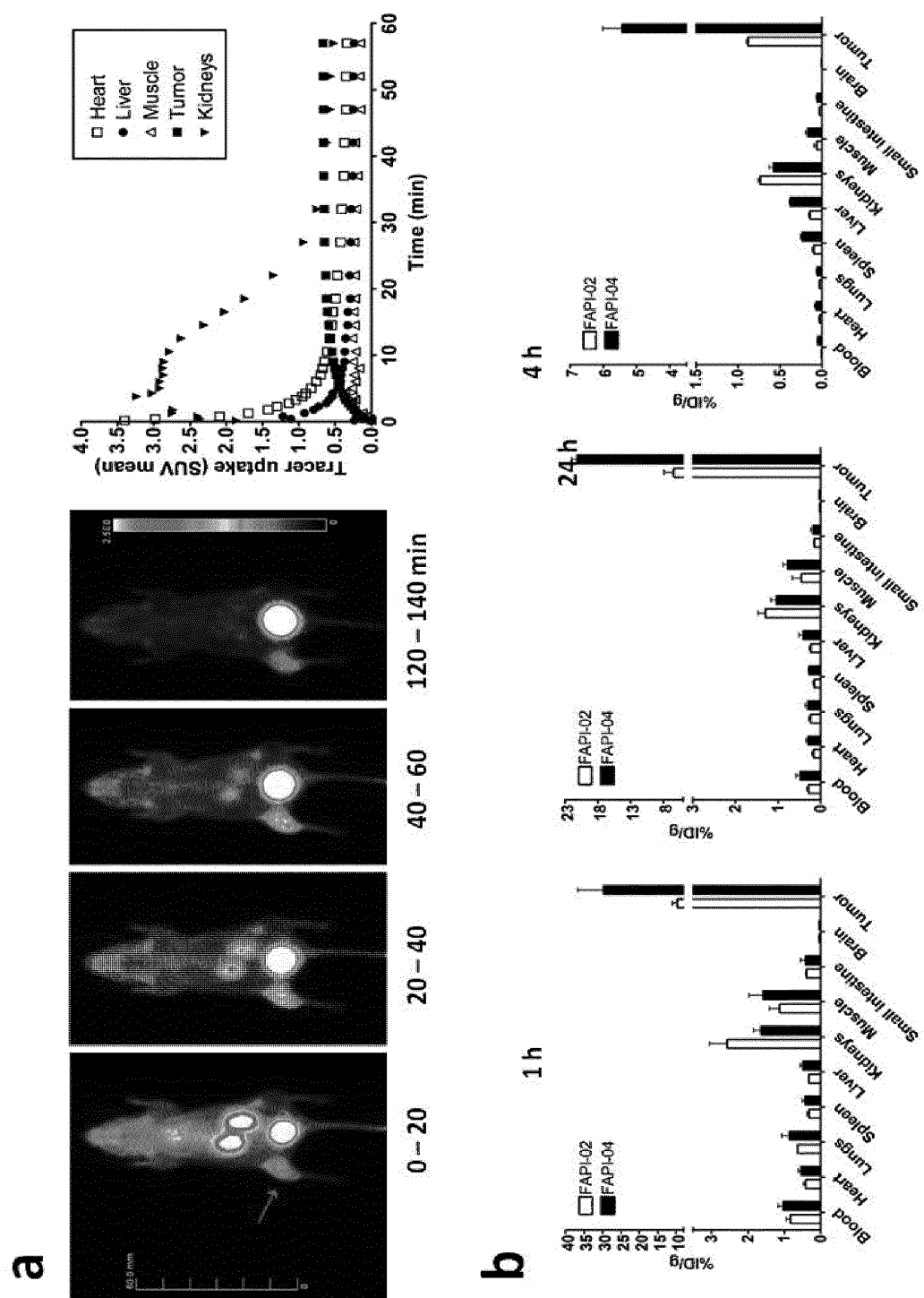


Figure 41

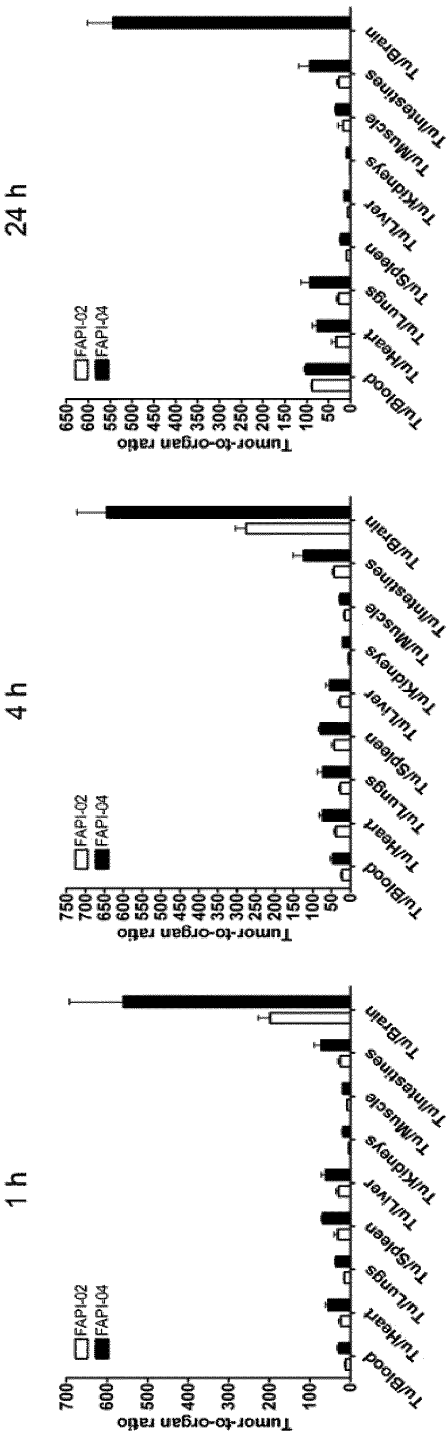


Figure 42

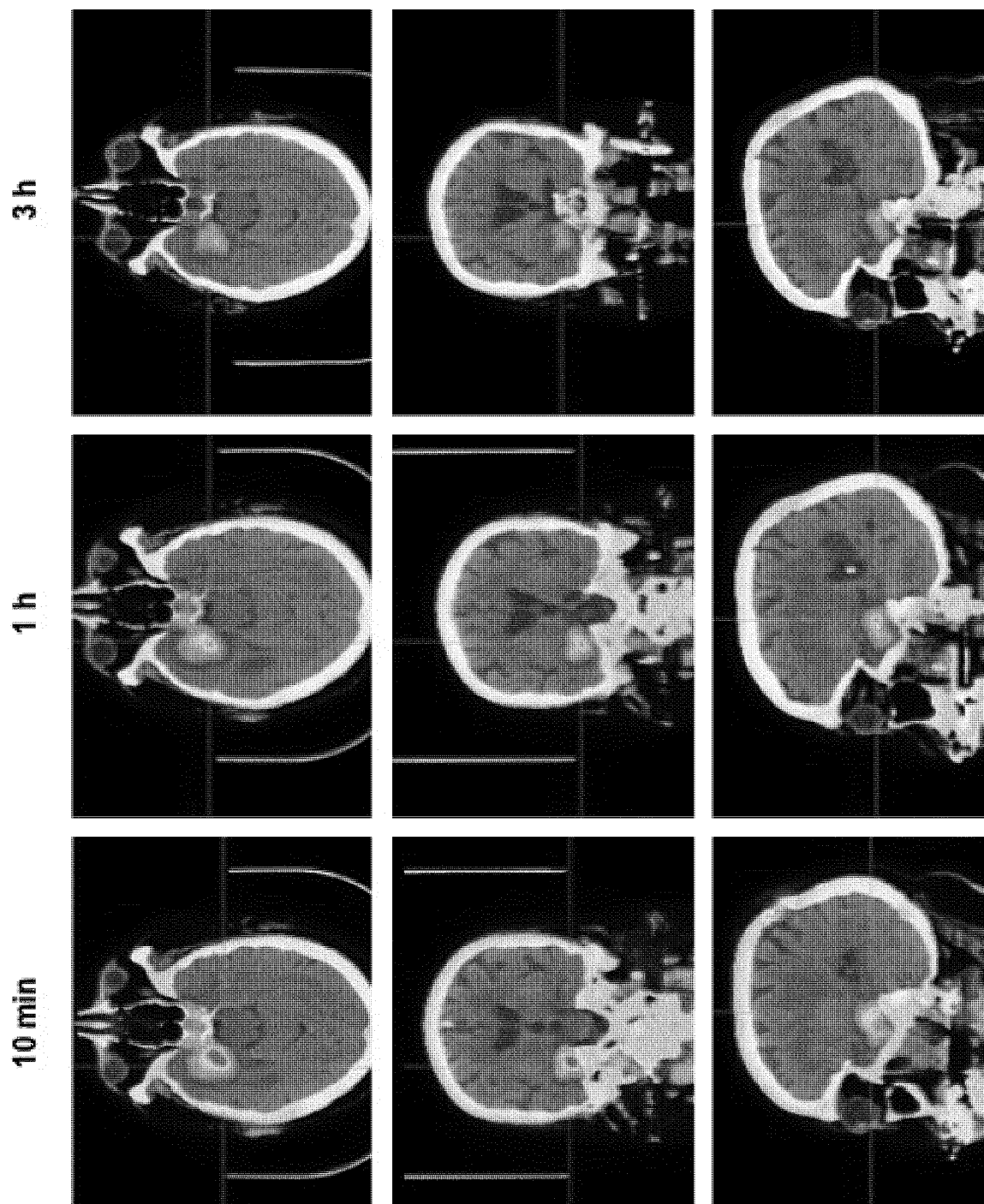


Figure 43

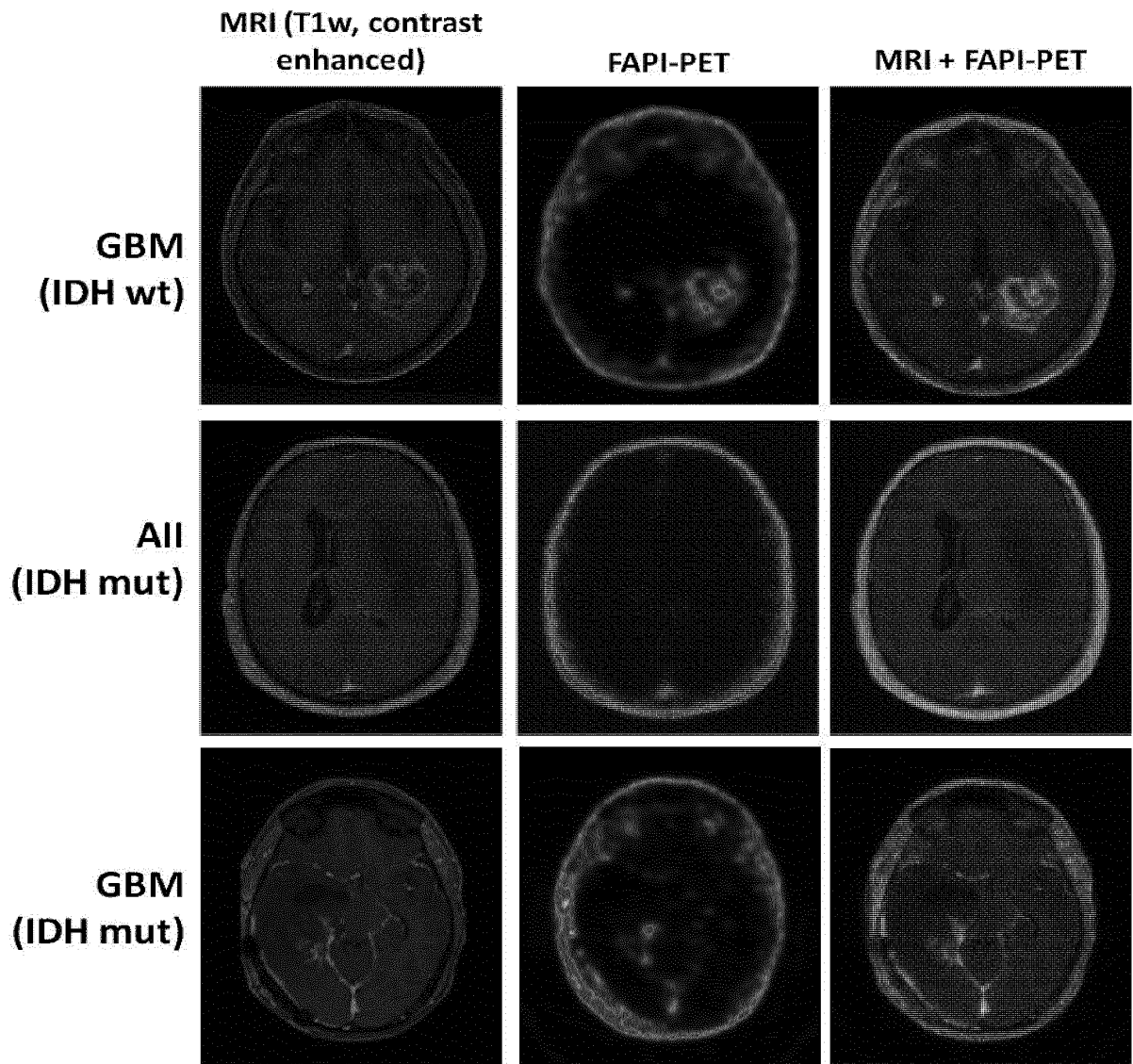


Figure 44

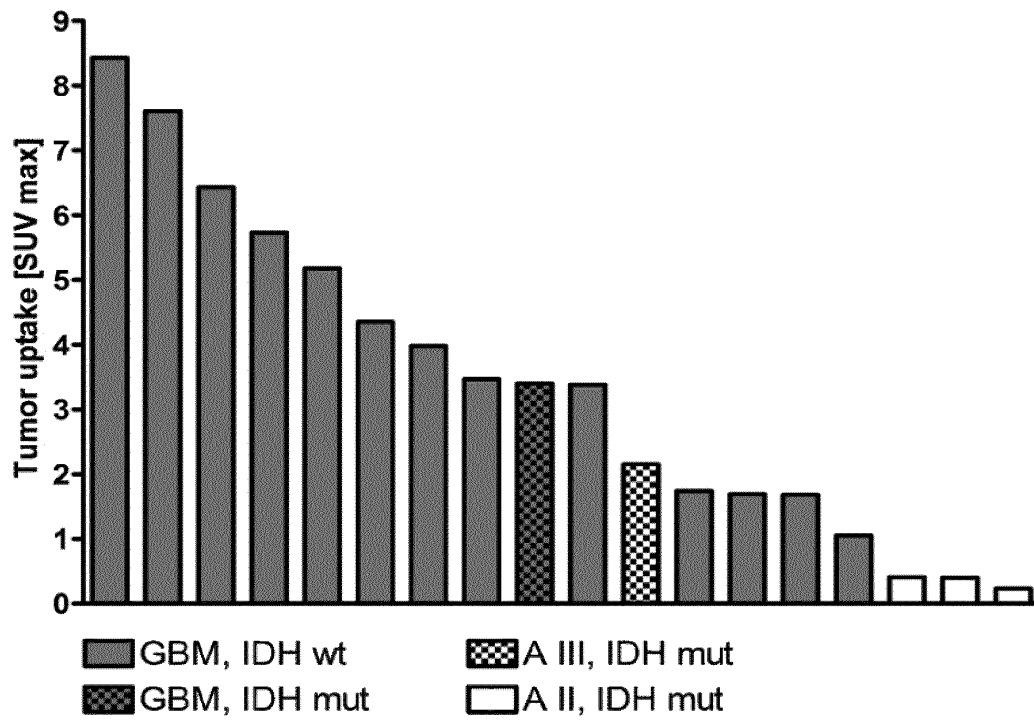
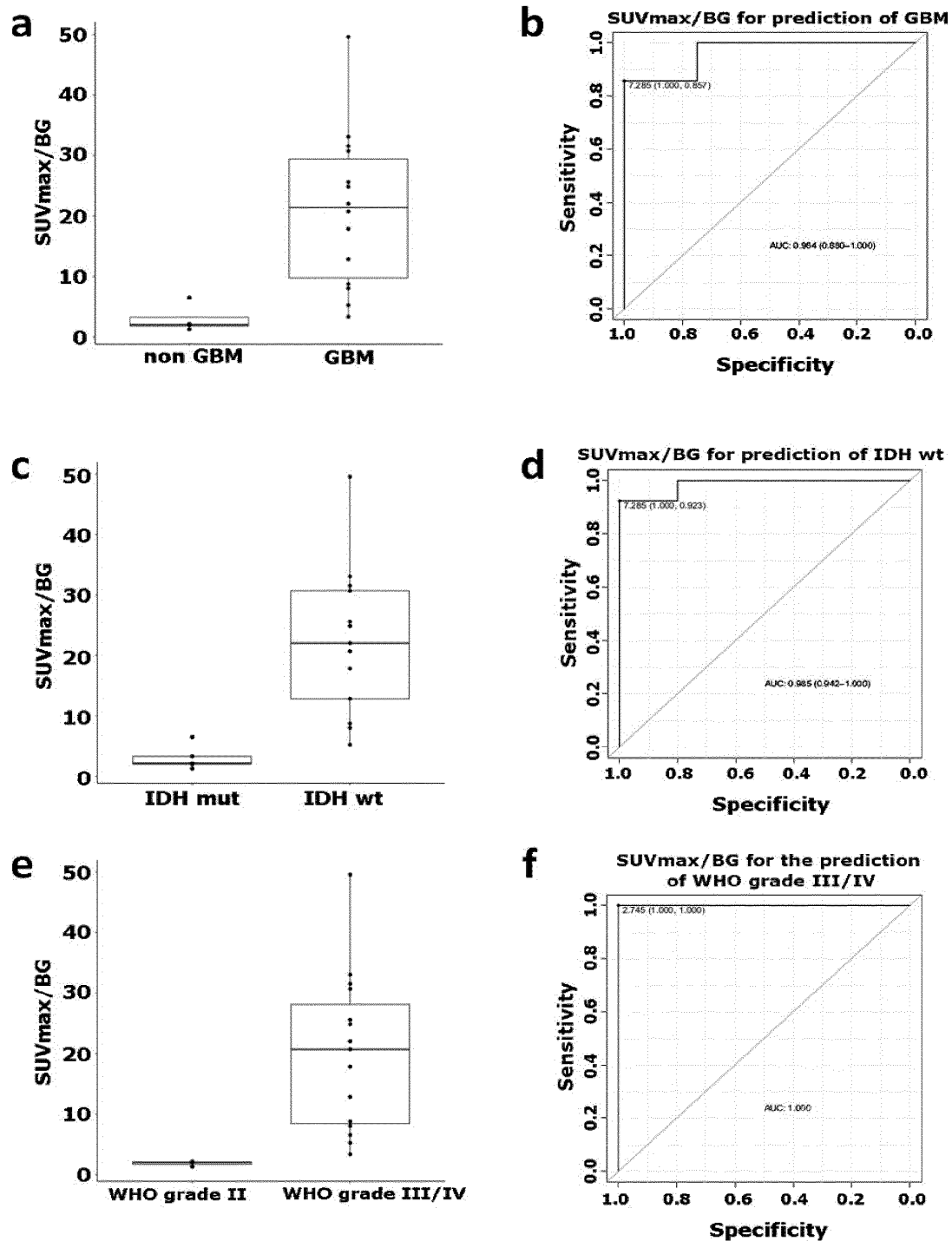


Figure 45



Data originating from sources other than the EPO may not be accurate, complete, or up to date.

The wording below is an initial machine translation of the original publication. To generate a version using the latest translation technology, go to the original language text and use Patent Translate.

FAP inhibitor

The present invention is a compound, a pharmaceutical composition comprising or comprising the compound, the compound or the pharmaceutical composition in the diagnosis or treatment of a disease characterized by overexpression of a fibroblast-activating protein (FAP). With respect to the use of kits comprising or consisting of, and the compounds or pharmaceutical compositions.

Tumor growth and spread are determined not only by cancer cells, but also by non-malignant components in malignant lesions, which fall into the stromal term. The stroma may account for 90% or more of the tumor mass in tumors with a fibrogenic response, such as breast cancer, colon cancer, and pancreatic cancer. In particular, a subpopulation of fibroblasts called cancer-related fibroblasts (CAFs) is known to be involved in tumor growth, metastasis and progression. Therefore, these cells are attractive targets for diagnostic and antitumor therapy.

A distinguishing feature of CAF is the expression of septase or fibroblast activating protein α (FAP- α), a type II membrane-bound glycoprotein belonging to the dipeptidyl peptidase 4 (DPP4) family. FAP- α has both dipeptidyl peptidase activity and endopeptidase activity. Endopeptidase activity distinguishes FAP- α from other members of the DPP4 family. Substrate of endopeptidase activity identified so far are denatured type I collagen, α 1-antitrypsin and some neuropeptides. FAP- α plays a role in normal developmental processes in embryogenesis and tissue modeling. It is not expressed in normal adult tissues or is expressed at very low levels. However, it is highly expressed in wound healing, arthritis, atherosclerotic plaques, fibrosis and over 90% epithelial cancers.

Due to the appearance of FAP- α in the CAF of many epithelial tumors and the fact that overexpression is associated with worse prognosis in cancer patients, FAP- α activity is also involved in the development of cancer and the metastasis and spread of cancer cells. It is hypothesized that it is. Therefore, targeting this enzyme for imaging and internal radiotherapy can be considered a promising strategy for the detection and treatment of malignant tumors. We have developed small molecules based on FAP- α -specific inhibitors for specific uptake, rapid internalization and tumor imaging in animal models and tumor patients. I was able to show success. This new FAP- α ligand clearly predominates in patients with locally advanced lung adenocarcinoma by comparison with the commonly used radiotracer 18F-fluorodeoxyglucose (18F-FDG). It became clear. Therefore, the present invention is, among other things, (i) detection of smaller primary tumors, thereby possible early diagnosis, (ii) detection of smaller metastases, thereby better diagnosis of tumor staging, (iii) tumors. Accurate intraoperative guidance to facilitate complete surgical resection of tissue, (iv) better differentiation of inflammation from tumor tissue, (v) more accurate staging of tumor patients, (vi) tumor lesions after antitumor therapy Provides a better follow-up, (vii) opportunity to use the molecules of the invention as a seranostic agent (for diagnostic tests that employ a particular treatment) for diagnosis and treatment. The molecules of the invention can also be used in the diagnosis and treatment of non-malignant diseases such as chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorders.

In a first aspect, the present invention describes the formula (I): [in the formula, Q, R, U, V, W, Y and Z are in Q, R, U, V, W, Y and Z. Independently present or absent, provided that at least three are present; Q, R, U, V, W, Y and Z are independently O, CH₂, NR₄, C = O, C. Selected from the group consisting of = S, C = NR₄, HCR₄ and R₄CR₄, but the two O's are not directly adjacent to each other; R₁ and R₂ are independently -H, -OH, halo, C₁-6-alkyl, - Selected from the group consisting of OC₁-6-alkyl, SC₁-6-alkyl; R₃ is -H, -CN, -B (OH) ₂, -C (O) -alkyl, -C (O).) -Aryl, -C = C-C (O) -aryl, -C = CS (O) ₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂ and 5-tetrazolyl. R₄ is selected from the group consisting of -H, -C₁-6-alkyl, -O-C₁-6-alkyl, -S-C₁-6-alkyl, aryl and -C₁-6-alkyl, each -C₁. -6-alkyl may be substituted with 1-3 substituents selected from -OH, oxo, halo and may be attached to Q, R, U, V, W, Y or Z. Well; R₅ is selected from the group consisting of -H, halo and C₁-6-alkyl;

R₆ and R₇ are independently selected from the group consisting of -H, where R₆ and R₇ are not H at the same time; where L is a linker; D, A, E and B exist independently. Or absent, preferably at least A, E and B are

present, where if present: D is a linker; A is selected from the group consisting of NR₄, O, S and CH₂. E is selected from the group consisting of C1-6-alkyl; where i is 1, 2 or 3; where j is 1, 2 or 3; where k is 1, 2 or 3; where m is 1, 2 or 3;

A and E together form a group selected from cycloalkyl, heterocycloalkyl, aryl and heteroaryl, where A and E are monocyclic, bicyclic and polycyclic. May be monocyclic; each A and E is -H, -C1-6-alkyl, -O-C1-6-alkyl, -S-C1-6-alkyl, alkenyl, heteroalkenyl, Cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C1-6-aralkyl, may be substituted with 1 to 4 residues selected from the group, each -C1-6-alkyl may be -OH. , Can be substituted with 1-3 substituents selected from oxo and halo, and may be attached to A, B, D, E or; B is S, NR₄, NR₄-O, Selected from the group consisting of NR₄-C1-6-alkyl, NR₄-C1-6-alkyl-NR₄ and 5--10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycles. The heterocycle preferably further comprises one or two heteroatoms selected from O, N and S, preferably further comprising one or two nitrogen atoms, preferably NR₄-C1-6-alkyl. -NR₄ and the N-containing heterocycle are substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl; and R₈ is radioactive. Selected from the group consisting of moieties, chelating agents, fluorescent dyes, contrasting agents and combinations thereof; are 1-naphthyl group moieties or 5-10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic. It is a heterocycle, in which there are two ring atoms between the N atom and the X; and the heterocycle is one, two or three selected from O, N and S. Heteroatoms may further be included; and X is a C atom], or pharmaceutically acceptable homovariates thereof, racemics, hydrates, solvates or salts. To do.

In a second aspect, the invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect and a pharmaceutically acceptable carrier and / or additive.

In a third aspect, the invention is a compound of the first aspect or for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast-activating protein (FAP) in an animal or human subject. The present invention relates to the pharmaceutical composition of the second aspect.

In a fourth aspect, the invention relates to a kit comprising or consisting of a compound of the first aspect or a pharmaceutical composition of the second aspect and instructions for diagnosing a disease.

Hereinafter, the contents of the figures included in the present specification will be described. In this context, see above and / or the following detailed description of the invention.

In vitro characterization of 125I-FAPI-01 and 177Lu-FAPI-02 A. Transfected with various human cancer cell lines of radiolabeled FAPI-01 and FAPI-02 and human FAP- α after 60 minutes of incubation. Binding to cell lines (HT-1080-FAP), mouse FAP- α -transfected cell lines (HEK-muFAP) and human CD26-transfected cell lines (HEK-CD26). B. Internalization of radiolabeled FAPI-01 and FAPI-02 into HT-1080-FAP cells after incubation for 10 minutes to 24 hours. Internalized percentages are shown in gray and black, respectively; extracellular binding fractions are shown in white bars. C. Competitive binding of radiolabeled FAPI-01 and FAPI-02 to HT-1080-FAP cells after addition of increased concentrations of unlabeled FAPI-01 and Lu-FAPI-02. Internalization of D. FAPI-02 into FAP- α positive and FAP- α negative cell lines. Blue: DAPI; Green: FAPI-02-Atto488. Outflow kinetics of FAPI-01 and FAPI-02 after incubating E + F. HT-1080-FAP cells with a radiolabeled compound for 1 hour and then incubating in a compound-free medium for 1 to 24 hours. All values are shown as a percentage of the total dose normalized to 1 million cells (% ID / 1 mio cells).

Binding specificity and relative internalization rate of FAPI derivatives. Binding rate and internalization rate of FAPI-03 to FAPI-15 with respect to AC FAPI-02 (defined as 100%). Internalization rates after incubation for 1 hour, 4 hours and 24 hours are shown in gray; extracellular binding fractions are shown in white bars. D. Binding of selected FAPI derivatives to HEK cells expressing mouse FAP- α and human CD26 after 60 minutes of incubation. Right: Binding rate of muFAP and CD26. E. Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after addition of an increased concentration of unlabeled compound.

Imaging of FAPI-02 and -04 in human FAP positive (HT-1080-FAP) and negative (Capan-2, SK-LMS-1) tumor xenograft-supported mice. A + C, E + G. Small animal PET imaging was performed at the time indicated after intravenous administration of 68Ga-FAPI-02 and -04 (10 MBq, respectively) 4 nmol. Radiotracers do not accumulate in non-cancerous tissue, but are rapidly concentrated within the tumor (indicated by the red arrow). In addition, rapid excretion through the kidneys and bladder is seen. Quantification of B + D, F + H. PET images demonstrates solid clearance of 68Ga-FAPI-02 and -04 from the cardiovascular system and constant uptake into tumors.

Blocking experiments to analyze binding specificity in vivo Blocking tumor accumulation of ⁶⁸Ga-FAPI-02 and -04 by co-administering 30 nmol of A + D. Unlabeled compound to HT-1080-FAP tumor-bearing mice. B + C, E + F. Time activity curves of ⁶⁸Ga-FAPI-02 and -04 in selected organs after intravenous administration with and without unlabeled compounds as competing substances.

Organ distribution of ¹⁷⁷Lu-FAPI-02 and -04 in HT-1080-FAP tumor-bearing nude mice The in vivo distribution of AC.¹⁷⁷Lu-FAPI-02 and -04 is 1MBq for human FAP-positive HT-1080 tumor xenografts. After intravenous administration to transplant-bearing mice, measured with Exvivo at the indicated time; n = 3 at each time point. The values listed are shown as a percentage (% ID / g) of dose per gram of tissue. It shows that the radiotracer accumulates in FAP-expressing tumors, with maximum enrichment of FAPI-02 (4.5% ID / g) after 1 hour and FAPI-04 (5.4% ID / g) after 2 hours.) Indicates. Tumor-to-normal tissue ratio after 1 hour, 4 hours and 24 hours of intravenous administration of D-F.¹⁷⁷ Lu-FAPI-02 and -04.

PET / CT Imaging of FAPI-02 in Cancer Patients 6A-C. Maximum Intensity Prediction (MIP) of PET / CT scans of patients suffering from metastatic breast cancer. Maximum tissue uptake 10 minutes, 1 hour, and 3 hours after intravenous administration of D.⁶⁸Ga-FAPI-02 to patients with metastatic breast cancer.

PET / CT imaging of FAPI-02 in cancer patients MIP of PET / CT scan 1 hour after administration of ⁶⁸Ga-FAPI-02 in patients with pancreatic cancer, non-small cell lung cancer (NSCLC), esophageal cancer, and rectal cancer.

PET / CT imaging of FAPI-02 in cancer patients PET / CT scan MIP 1 hour after administration of ⁶⁸Ga-FAPI-02 in patients with nasopharyngeal and laryngeal cancer.

PET / CT Imaging of FAPI-02 in Cancer Patients Systemic PET / CT Imaging (MIP) 1 hour after administration of 9A + B.¹⁸F-FDG and ⁶⁸Ga-FAPI-02 to patients with locally advanced lung adenocarcinoma. Transaxial photographs of C + D. Lung adenocarcinoma patients 1 hour after administration of ¹⁸F-FDG and ⁶⁸Ga-FAPI-02. FAPI-02 is selectively accumulated in FAP- α expressing tissues and shows significantly higher uptake into malignant lesions compared to ¹⁸F-FDG.

PET / CT imaging of FAPI-04 in cancer patients 10. Maximum projection (MIP) of PET / CT scans 10 minutes, 1 hour, and 3 hours after administration of ⁶⁸Ga-FAPI-04 in patients with metastatic breast cancer.

MIP of PET / CT scan 1 hour after administration of ⁶⁸Ga-FAPI-04 in patients with sigma cancer, hypopharyngeal cancer, neuroendocrine tumor, cholangiocarcinoma, ovarian cancer and small intestine cancer.

MIP of PET / CT scan 1 hour after administration of ⁶⁸Ga-FAPI-04 in lung cancer patients.

MIP of PET / CT scan 1 hour after administration of ⁶⁸Ga-FAPI-04 in patients with carcinogenic rickets.

Comparative imaging of one patient with metastatic prostate cancer. MIP of PET / CT scan 1 hour after application of radiolabeled DOTATATOC, PSMA and FAPI-04.

Maximum intensity projection (MIP) and temporal radioactivity curves of dynamic ⁶⁸Ga-FAPI-04 PET / CT scans in patients with pancreatic cancer.

Relative binding rate of Lu-¹⁷⁷-labeled FAPI derivative after incubation on FAP-expressing HT-1080 cells for 1, 4 and 24 hours relative to FAPI-04 (set to 100%); n = 3.

Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after addition of increased concentrations of unlabeled compound (10-10-10-5M, 60 minute incubation, n = 3).

Binding of FAPI derivatives to HEK cells expressing mouse FAP and human CD26 after 60 minutes of incubation, n = 3. Values are shown as a percentage of applied amount (% ID) per mio cell.

In vivo distribution of selected FAPI derivatives in HT-1080-FAP xenografts 1 hour, 4 hours and 24 hours after intravenous radiotracking, n = 3. Values are shown as a percentage of tissue dose per gram (% ID / g).

Tumor-to-blood ratio of selected FAPI derivatives in HT-1080-FAP xenografts 1 hour, 4 hours and 24 hours after intravenous radiotracking, n = 3.

PET imaging of Ga-68 labeled FAPI-21 and FAPI-46 in HT-1080-FAP tumor-carrying mice; n = 1.

Maximum standardized uptake value (SUV) of selected FAPI derivatives in HT-1080-FAP tumor-carrying mice; n = 1.

Maximum (SUV_{max}, FIG. 23A) and mean (SUV_{mean}, FIG. 23B) standardized uptake values of Ga-68 labeled FAPI-02 and FAPI-04 in cancer patients; n = 25.

Intra-individual comparison of 6 patients with 6 different tumor entities who underwent FDG-PET and FAPI-PET imaging within 9 days.

Ga-68 labeled FAPI-04 PET / CT imaging in patients with cancerous peritonitis (A), myocarditis (B) and hip joint arthrosis 1 hour after injection (1 h p.i.).

PET / CT imaging of Ga-68 labeled FAPI-21 in cancer patients 1 hour after injection (1 h p.i.)

PET / CT imaging of Ga-68-labeled FAPI-46 1 hour after injection (1 h p.i.) and Sm-153 labeled FAPI-46 therapeutic imaging 30 minutes after injection (30 min p.i.) in cancer patients.

Therapeutic imaging of Sm-153 labeled FAPI-46 up to 20 hours after injection (20 h p.i.)

A. Maximum projection (MIP) 1 hour after intravenous administration of ⁶⁸Ga-FAPI-46 to patients with metastatic colorectal cancer. Bremsstrahlung imaging 2 hours after treatment of the same patient with B.⁹⁰Y-FAPI-46

PET / CT imaging of Ga-68 labeled FAPI-46 1 hour after injection (1 h p.i.) in a lung cancer patient with idiopathic pulmonary fibrosis. A, B. Tracker maximal uptake into tumor tissue is significantly higher than tracker maximal uptake into non-malignant fibrous lesions. C. Maximum tracker uptake into tumor tissue is slightly lower than maximum tracker uptake into exacerbated fibrotic tissue.

Binding of A. Tc-99m labeled FAPI-19 to HT-1080-FAP cells, n = 3. B. Binding of Tc-99m labeled FAPI-19 to HT-1080-FAP cells after addition of unlabeled compound with increased concentration (10⁻¹⁰-10⁻⁵M, 60 min incubation, n = 3) .. Scintigraphy of Tc-99m labeled FAPI-19 in C.HT-1080-FAP xenotransplantation, n = 1.

Binding of A. Tc-99m labeled FAPI-34 to HT-1080-FAP cells, n = 3. B. Scintigraphy of Tc-99m labeled FAPI-34 in HT-1080-FAP xenotransplantation, n = 1.

Scintigraphy of Tc-99m labeled FAPI-34 in a patient with metastatic pancreatic cancer.

Binding of A. Pb-203 labeled FAPI derivative to HT-1080-FAP cells, n = 3. B. HT-1080-FAP cells were incubated with the radiolabeled compound for 60 minutes, followed by incubation in non-radioactive medium for 1 hour followed by outflow kinetics of the Pb-203 labeled FAPI derivative, n = 3. C. Competitive binding of Pb-203 labeled FAPI to HT-1080-FAP cells after addition of increased concentration of unlabeled compound (10⁻¹⁰-10⁻⁵M, 60 min incubation, n = 3).

Scintigraphy of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenotransplantation, n = 1.

In vivo distribution of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenografts 1 hour, 4 hours, 6 hours and 24 hours after intravenous administration of radiofollowers n = 3. Values are shown as a percentage of the amount injected per gram of tissue (% ID / g).

A. Binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells, n = 3. B. Competitive binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells after addition of increased concentration of unlabeled compound (10⁻¹⁰-10⁻⁵M, 60 min incubation) , N = 3). Outflow kinetics of Cu-64 labeled FAPI-42 and FAPI-52 after incubating C.HT-1080-FAP cells with a radiolabeled compound for 60 minutes and subsequently incubating in a non-radioactive medium for 1-24 hours, n = 3 ..

PET imaging of Cu-64 labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor-carrying mice; n = 1.

PET imaging of AIF-18-labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor-carrying mice; n = 1.

Small animal PET imaging of ⁶⁸Ga-labeled FAPI-02 in U87MG tumor-carrying nude mice up to 140 minutes after intravenous administration of the radiotracker. Tumors are indicated by red arrows. b. Biodistribution of ¹⁷⁷Lu-labeled FAPI-02 and FAPI-04 in U87MG tumor-bearing nude mice 1 hour, 4 hours and 24 hours after intravenous administration of the radiotracker; n = 3.

Tumor-to-organ ratio of ¹⁷⁷Lu-labeled FAPI-02 and -04 in U87MG tumor-bearing mice 1 hour, 4 hours and 24 hours after intravenous administration.

Maximum projection (MIP) of PET / CT scans in patients with glioblastoma 10 minutes, 1 hour and 3 hours after administration of ⁶⁸Ga-FAPI-02.

Illustrative images of IDHwt glioma, IDH mutant glioblastoma WHO grade II and IDH mutant glioblastoma (contrast-enhanced T1-weighted MRI, FAPI-PET and fusion images of both modalities).

Absolute SUVmax for all 18 gliomas.

Statistical analysis of SUVmax / BG values. Box of SUVmax / BG values in GBM vs. non-GBM (a, b), IDH mutant vs IDH wild-type glioma (c, d) and glioma grade II vs. glioma grade III / IV (e, f) Box plot and corresponding ROC curve.

Dose-dependent inhibition of enzymatic FAP activity by FAPI-04 and tarabostat. In contrast to tarabostat, a potent DPP4 inhibitor with marginal FAP activity, FAPI-04 demonstrates robust dose-dependent FAP inhibition.

Reuptake of ¹⁷⁷Lu-labeled FAPI-04 and FAPI-46 in HT-1080-FAP cells. After incubating the cells with a radiotracker for 60 minutes at 37 ° C., remove the compounds, add non-radioactive medium containing (+ Comp.) And without (-Comp.) Unlabeled compounds and incubate for 10 minutes to 6 hours. .. Already within the first 10 minutes of incubation, new uptake of the unlabeled FAPI derivative occurred, replacing part of the radiolabeled fraction, resulting in radioactivity compared to pure medium without competitors. The value drops significantly. After 6 hours of incubation, almost complete replacement of the radiolabeled FAPI has occurred. These findings indicate continuous reuptake of intact FAP molecules that returned to the plasma membrane during initial internalization, allowing new binding and internalization of FAP ligands.

Organ distribution of ¹⁷⁷Lu-FAPI-04 after single and multiple injections in HT-1080-FAP tumor-carrying nude mice. Two doses of equal doses of ¹⁷⁷Lu-FAPI-04 at 4-hour intervals resulted in an increase in overall organ activity, including tumors, measured 8 and 24 hours after the first injection. On the other hand, it is clarified that there is no change in the overall organ activity after 3 doses (higher initial dose and lower subsequent dose).

Binding of F-18-FAPI derivatives to human FAP-expressing HT1080 cells after 10-minute, 30-minute, 60-minute and 90-minute incubations, n = 3. Values are shown as a percentage (% ID) of the amount applied per 1 mio cell.

PET imaging of AIF-18-labeled FAPI-74 and FAPI-52 in HT-1080-FAP tumor-carrying mice; n = 1.

In vivo distribution of FAPI-75 in HT-1080-FAP xenografts 1 hour, 4 hours and 24 hours after intravenous radiotracking, n = 3. Values are shown as a percentage (% ID / g) of dose per gram of tissue.

PET Imaging of Patients with Non-Small Cell Lung Cancer: Robust Accumulation of F18-labeled FAPI-74 in Multiple Metastases.

Time radioactivity curves of the cardiac region (SUVmean) of FAPI-04 and -46 as an illustration of fast blood pool clearance.

FAPI-02 and FAPI-04 at different imaging time points (10 minutes, 1 hour, 3 hours after injection) in two patients with metastatic breast cancer. Rapid tumor targeting and rapid blood clearance are followed by a long plateau phase with no changes associated with image contrast (above). Compared to FAPI-02, ligand FAPI-04 is characterized by long-term tumor retention time (bottom).

The effective dose of FAPI-02 was $1.80\text{E-}02$ mSv / MBq calculated by OLINDA ($1.82\text{E-}02$ for IDAC1 / ICRP60 and $1.79\text{E-}02$ for IDAC2 / ICRP103). The effective dose of FAPI-04PET / CT was $1.64\text{E-}02$ mSv / MBq calculated by OLINDA ($1.66\text{E-}02$ for IDAC1 / ICRP60 and $1.35\text{E-}02$ for IDAC2 / ICRP103). By omitting the delayed scan 3 hours after injection in the clinical setting, the routine activity of the FAPI test can be reduced to 68 Ga of 200 MBq, and the radiation dose of such FAPI-PET / CT scan is continuously 3-4 mSv. ..

A) ^{68}Ga -FAPI-04 1 hour after injection into different tumor entities in PETCT. The highest mean SUVmax (12) was found in sarcoma cancer, esophageal cancer, breast cancer, intrahepatic cell cancer and lung cancer. The lowest FAPI uptake (mean SUVmax (6) was observed in renal cell carcinoma, differentiated thyroid cancer, adenoid cystic carcinoma, gastric cancer and pheochromocytoma. The average SUVmax of hepatocellular carcinoma, colorectal cancer, head and neck cancer, ovarian cancer, and pancreatic cancer was intermediate (SUV6 (x (12). High inter-individual variability was observed in all tumor entities. Due to low background radioactivity (SUV2), the tumor-to-background ratio is 2-fold in the intermediate uptake group and 4-fold in the high-intensity uptake group. B) Primary tumor entities showed similar SUV uptake compared to tumor entities using FAPI-04.

Illustrative PET images of different tumor entities used in the quantification shown in FIGS. 56AB.

Detailed Description of the Invention Prior to discussing the invention in detail below, it is understood that the invention is not limited to the particular method protocols and reagents described herein as they may vary. Will. Also, the terms used herein are for purposes of describing only certain embodiments and are intended to limit the scope of the invention, which is limited solely by the appended claims. It is understood that it is not. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as those skilled in the art generally understand.

Preferably, the terms used herein are "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, HGW, Nagel, B. and Klbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout the specification and subsequent claims, unless otherwise required in the context, variations such as the terms "comprise" and "comprises" and "comprising" are the integers or steps or integers or steps described. It will be understood that it means to include a group of integers or processes or does not mean to exclude a group of integers or processes. The following passages define various aspects of the invention in more detail. Each aspect defined in this way may be combined with other aspects (aspects) or aspects (aspects) unless explicitly stated on the contrary. In particular, any feature that has been shown to be optional, favorable, or advantageous is another feature that has been shown to be optional, favorable, or advantageous. Alternatively, it may be combined with features.

Several documents are cited throughout the body of this specification. Each document cited herein, including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc., is hereby by reference in its entirety, whether above or below. Incorporated in. Nothing herein is to be construed as acknowledging that the present invention does not have the right to preempt such disclosure on the grounds of prior inventions. Some of the documents cited herein are "incorporated by reference". In the event of any conflict between the definitions or teachings of such incorporated literature and the definitions or teachings cited herein, the text of this specification shall prevail.

Hereinafter, the elements of the present invention will be described. Although these elements are listed in a particular embodiment, it should be understood that they may be combined in any way and in any number to create further embodiments. The various described examples and preferred embodiments should not be construed to limit the invention to the expressly described embodiments only. It should be understood that the specification supports and embraces embodiments that expressly describe embodiments in combination with all disclosed and / or preferred elements. In addition, any permutation and combination of any of the elements described herein should be considered disclosed by the description of the present application unless the context indicates otherwise.

Definitions Below are some definitions of terms commonly used herein. These terms have the meanings defined and preferred in the rest of the specification, respectively, in each embodiment of their use.

As used herein and in the appended claims, the singular forms "a," "an," and "the" referent to multiple referents unless the content clearly dictates otherwise. Including.

The following terms provide definitions for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkenyl and alkynyl. These terms have the meanings defined and preferred in each of the examples used in the rest of this specification.

The term "alkyl" means a saturated linear or branched carbon chain. Preferably, the chain contains 1 to 10 or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms (eg, methyl). , Ethylmethyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, pentyl or octyl). The alkyl group may be substituted.

The term "heteroalkyl" means a saturated linear or branched carbon chain. Preferably, the chain contains 1 to 9, ie 1, 2, 3, 4, 5, 6, 7, 8 or 9 carbon atoms (eg, methyl, ethyl, etc.). Propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl, octyl), these once or more, eg once, twice, three times, depending on the same or different heteroatoms. It is interrupted 4 or 5 times. Preferably, the hetero atom is selected from O, S and N, eg-O-CH₃, -S-CH₃, -CH₂-O-CH₃, -CH₂-O-C₂H₅, -CH₂-S-CH₃, -CH₂-S-C₂H₅, -C₂H₄-O-CH₃, -C₂H₄-O-C₂H₅, -C₂H₄-S-CH₃, -C₂H₄-S-C₂H₅ and the like. The heteroalkyl group may be substituted.

Unless otherwise stated, the terms "cycloalkyl" and "heterocycloalkyl", in their own right or in combination with other terms, indicate cyclic versions of "alkyl" and "heteroalkyl", respectively, preferably three. 4, 5, 6, 7, 8, 9 or 10 atoms form a ring, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like. The terms "cycloalkyl" and "heterocycloalkyl" are also meant to include bicyclic, tricyclic and polycyclic versions thereof. The term "heterocycloalkyl" is preferably a 5-membered saturated ring (at least one member is an N, O or S atom and may contain one additional O or one additional N); a 6-membered saturated ring (At least one member is an N, O or S atom and may contain one additional O or one additional N or two additional N atoms; or a 9- or 10-membered saturated bicyclic ring (at least 1 member). Is an N, O or S atom, which may contain one additional N atom, two additional N atoms or three additional N atoms). The "cycloalkyl" and "heterocycloalkyl" groups may be substituted. Furthermore, in the case of heterocycloalkyl, the heteroatom can occupy the position where the heterocycle is attached to the rest of the molecule. Examples of cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, spiro [3,3] heptyl, spiro [3,4] octyl, spiro [4,3].] Octyl, Spiro [3,5] Nonyl, Spiro [5,3] Nonyl, Spiro [3,6] Decyl, Spiro [6,3] Decyl, Spiro [4,5] Decyl, Spiro [5,4] Decyl , Bicyclo [2.2.1] heptyl, bicyclo [2.2.2] octyl, adamantyl and the like. Examples of heterocycloalkyls are 1- (1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, 1,8 diazo-spiro- [4,5] decyl, 1,7 diazo-spiro- [4,5] decyl, 1,6 diazo-spiro- [4,5] decyl, 2,8 diazo-spiro [4,5] decyl, 2, 7 diazo-spiro [4,5] decyl, 2,6 diazo-spiro [4,5] decyl, 1,8 diazo-spiro- [5,4] decyl, 1,7 diazo-spiro- [5,4] Decyl, 2,8 diazo-spiro- [5,4] decyl, 2,7 diazo-spiro [5,4] decyl, 3,8 diazo-spiro [5,4] decyl, 3,7 diazo-spiro [5] , 4] decyl, 1-azo-7,11-dioxo-spiro [5,5] undecyl, 1,4-diazabicyclo [2.2.2] octa-2-yl, tetrahydrofuran-2-yl, tetrahydrofuran-3 -yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl and the like.

The term "aryl" is preferably an aromatic monocyclic ring containing 6 carbon atoms or an aromatic bicyclic ring system containing 10 carbon atoms or an aromatic containing 14 carbon atoms. It means a tricyclic ring system. Examples include phenyl, naphthyl or anthracenyl. Aryl groups may be substituted.

The term "aralkyl" means an alkyl moiety substituted with aryl, where alkyl and aryl have the meanings outlined above. An example is the benzyl group. Preferably, in this context, the alkyl chain comprises 1-8, i.e., 1, 2, 3, 4, 5, 6, 6, 7 or 8 carbon atoms (eg, methyl, ethyl). Methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butenyl, tert-butyl, pentyl, hexyl, pentyl, octyl). The aralkyl group may be substituted with an alkyl and / or aryl portion of the group.

The term "heteroaryl" is preferably the same or different 1, 2, 3 or 4 (5-membered ring) in which at least one of the carbon atoms is preferably selected from O, N and S. (In the case of) or a 5- or 6-membered aromatic monocyclic ring substituted with 1, 2, 3, 4 or 5 (in the case of a 6-membered ring) heteroatom; 8, 9 One, two, three, four, five or six carbon atoms out of ten, eleven or twelve carbon atoms are preferably the same or selected from O, N and S. Aromatic bicyclic ring system substituted with different heteroatoms; or 1, 2, 3, 4, 5, or 6 of 13, 14, 15 or 16 carbon atoms It means an aromatic tricyclic ring system in which the carbon atoms are replaced by the same or different heteroatoms, preferably selected from O, N and S. Examples include oxazolyl, isooxazolyl, 1,2,5-oxadiazolyl, 1,2,3-oxadiazolyl, pyrrolyl, imidazolyl, pyrazolyl, 1,2,3-triazolyl, thiazolyl, isothiazolyl, 1,2,3, -thiadiazolyl. 1,2,5-thiadiazolyl, pyridinyl, pyrimidinyl, pyrazinyl, 1,2,3-triazinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, 1-benzofuranyl, 2-benzofuranyl, indoyl), Isoindoyl, benzothiophenyl, 2-benzothiophenyl, 1H-indazolyl, benzoimidazolyl, benzoxazolyl, indoxazinyl, 2,1-benzoxazolyl, benzothiazolyl,

1,2 -Benzoisothiazolyl, 2,1-benzoisothiazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinoxalinyl, quinazolinyl, quinolinyl, 1,2,3-benzotriazinyl or 1,2,4-benzotriazinyl There is Nil.

The term "heteroalkyl" means an alkyl moiety substituted with a heteroaryl, where alkyl and heteroaryl have the meanings outlined above. Examples include 2-alkylpyridinyl, 3-alkylpyridinyl or 2-methylpyridinyl. Preferably, in this context, the alkyl chain comprises 1-8, i.e., 1, 2, 3, 4, 5, 6, 7 or 8 carbon atoms (eg, methyl, ethyl). Methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butenyl, tert-butyl, pentyl, hexyl, pentyl, octyl). The heteroalkyl group may be substituted with the alkyl and / or heteroaryl of the group.

"Alkene" and "cycloalkenyl" mean an olefin unsaturated carbon atom containing a chain or ring having one or more double bonds. Examples are propenyl and cyclohexenyl. Preferably, the alkenyl chain contains 2 to 8 carbon atoms, i.e. 2, 3, 4, 5, 6, 7 or 8 carbon atoms (eg, ethenyl, 1-propenyl, 2-propenyl, Iso-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, iso-butenyl, sec-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, hexenyl, pentenyl, octenyl). Preferably, the cycloalkenyl ring contains 3 to 8, ie 3, 4, 5, 6, 7 or 8 carbon atoms (eg 1-cyclopropenyl, 2-cyclopropenyl, 1-). Cyclobutenyl, 2-cyclobutenyl, 1-cyclopentenyl, 2-cyclopentenyl, 3-cyclopentenyl, cyclohexenyl, cyclopentenyl, cyclooctenyl).

The term "alkynyl" means an unsaturated carbon atom containing a chain or ring with one or more triple bonds. An example is the propargyl group. Preferably, the alkynyl chain contains 2 to 8, ie 2, 3, 4, 5, 6, 7 or 8 carbon atoms (eg, ethynyl, 1-propynyl, 2-propynyl, etc.). 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, hexynyl, pentylnyl, octynyl).

In one embodiment, the carbon or hydrogen atoms in the alkyl, heteroalkyl, cycloalkyl, aryl, aralkyl, alkenyl, cycloalkenyl, alkynyl groups are independently selected from the group consisting of O, S and N. It may be substituted with one or more elements, or with a group containing one or more elements selected from the group consisting of O, S and N.

Embodiments include alkoxy, cycloalkoxy, aryloxy, aralkylo, alkenyloxy, cycloalkenyloxy, alkynyloxy, alkylthio, cycloalkylthio, arylthio, aralkylthio, alkenylthio, cycloalkenylthio, alkynylthio, alkylamino, cycloalkyl. There are amino, arylamino, aralkylamino, alkoxyamino, cycloalkoxyamino, and alkynylamino groups.

Other embodiments include hydroxyalkyl, hydroxycycloalkyl, hydroxyaryl, hydroxyaralkyl, hydroxyalkenyl, hydroxycycloalkenyl, hydroxyalynyl, mercaptoalkyl, mercaptocycloalkyl, mercaptoaryl, mercaptoaralkyl, mercaptoalkenyl, mercaptocycloalkenyl. , Mercaptoalkynyl, aminoalkyl, aminocycloalkyl, aminoaryl, aminoaralkyl, aminoalkenyl, aminocycloalkenyl, aminoalkynyl groups.

In another embodiment, the hydrogen atoms in the alkyl group, heteroalkyl group, cycloalkyl group, aryl group, aralkyl group, alkenyl group, cycloalkenyl group, alkynyl group are independent of each other and have one or more halogens. Can be replaced by atoms. One group is a trifluoromethyl group.

When two or more groups or two or more residues can be selected independently of each other, the term "independently" may be different, with the groups or residues being the same. It means that it may be.

As used herein, a phrase defining a range limitation of length, for example "1-6", is an integer from 1 to 6, ie 1, 2, 3, 4, ... Means 5 and 6. In other words, any range defined by the two explicitly stated integers shall include and disclose any integer defining the above limitation and any integer contained within the range. Means.

As used herein, the term "halo" means a halogen residue selected from the group consisting of F, Br, I and Cl. Preferably, the halogen is F.

As used herein, the term "linker" means any chemically suitable linker. Preferably, the linker is not cleaved under physiological conditions or is cleaved only slowly. Thus, preferably, the linker does not contain a recognition sequence for proteases or a recognition structure for other degrading enzymes. It is preferred that the compounds of the invention be administered systemically, allowing widespread access to all compartments of the body, followed by enrichment of the compounds of the invention no matter where the tumor is located in the body. Therefore, the linker is preferably selected in such a way that it is not cleaved in the blood or is cleaved only slowly. Cleavage is considered slow if less than 50% of the linker is cleaved 2 hours after administration of the compound to a human patient. Suitable linkers include, for example, alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkenyl, heteroalkenyl, cycloalkenyl,

cycloheteroalkenyl, alkynyl, sulfonyl, amine, which may be substituted. , Ethers, thioethers, phosphines, phosphoramidates, carboxamides, esters, imide esters, amidins, thioesters, sulfonamides, 3-thiopyrrolidine-2,5-dione, carbamates , Ureas, guanidines, thioureas, disulfides, oximes, hydrazines, hydrazides, hydrazones, diaza conjugates, triazoles, triazolines, tetrazines, platinum complexes and amino acids or combinations thereof. Includes or consists of them. Preferably, the linker comprises or consists of 1,4-piperazine, 1,3-propane and phenolic ethers or combinations thereof.

The expression "may be substituted" means a group in which one, two, three or more hydrogen atoms may be substituted by their respective substituents independently of each other.

As used herein, the term "amino acid" is a derivative of any organic acid, aliphatic carboxylic acid, including one or more amino substituents such as α -, β - or γ -amino. Means. In the polypeptide notation used herein, eg, Xaa1 Xaa2Xaa3Xaa4Xaa5, Xaa1 to Xaa5 are each selected from independently defined amino acids, with the left-hand direction being the amino-terminal direction and the right-hand direction being the carboxy-terminal direction. And follow standard usage and conventions.

The term "conventional amino acid" means 20 naturally occurring amino acids and includes all of their stereoisomers, namely D, L-, D- and L-amino acids. These conventional amino acids can be referred to herein by conventional three-letter or one-letter abbreviations, which follow conventional usage (eg, Immunology-A Synthesis, 2nd Edition, ES). See Golub and DR Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)).

The term "non-conventional amino acid" means an unnatural amino acid or a chemical amino acid analog, such as α , α -disubstituted amino acid, N-alkyl amino acid, homo amino acid, dehydroamino acid, aromatic amino acid (phenylalanine, tyrosine and tryptophan). Other than, and means orthoaminobenzoic acid, metaamino benzoic acid or paraaminobenzoic acid. Non-conventional amino acids include compounds in which amines and carboxyl functional groups are separated by 1,3 or more substitution patterns, such as β -alanine, γ -aminobutyric acid, Freidinger lactam, bicycles. Also included are formula dipeptides (BTDs), amino-methylbenzoic acid and other compounds commonly known in the art. In addition, statin-like isostars, hydroxyethylene isostars, reduced amide bond isosteres, thioamide isostars, urea isostars, carbamate isostars, thioether isostars, vinyl isostars and other amide bonds commonly known in the art. Isostar can also be used. The use of analogs or non-conventional amino acids can improve the stability and biological half-life of the added peptide by increasing degradation resistance under physiological conditions. Those skilled in the art will recognize that similar types of substitutions can be made. A non-limiting list of non-conventional amino acids that can be used as suitable components of peptides and their standard abbreviations (in parentheses) are: α -aminobutyric acid (Abu), L- N-methylalanine (Nmala), α -amino- α -methylbutyrate (Mgab), L-N-methylarginine (Nmarg), aminocyclopropane (Cpro), L-N-methylasparagin (Nmasn), carboxylate L-N-methylasparanoic acid (Nmasp), aminoisobutyric acid (Aib), L-N-methylcysteine (Nmcys), aminonorbornyl (Norb), L-N-methylglutamine (Nmgl), carboxylate L-N-Methylglutamic acid (Nmglu), cyclohexylalanine (Chexa), L-N-methylhistidine (Nmhis), cyclopentylalanine (Cpen), L-N-methylisoleucine (Nmile), L-N-methylleucine (Nmleu), L- N-methyllysine (Nmlys), L-N-methylmethionine (Nmmt), L-N-methylnorleucine (Nmnl), L-N-methylnorvaline (Nmna), L-N-methylornithine (Nmorn), L-N-methylphenylalanine (Nmphe), L-N-methylproline (Nmpro), L-N-methylserine (Nmser), L-N-methylthreonine (Nmthr), L-N-methyltryptophane (Nmtrp), D -Ornithine (Dorn), L-N-Methyltyrosine (Nmtyr), L-N-Methylvalin (Nmval), L-N-Methylethylglycine (Nmetg), L-N-Methyl-t-Butylglycine (Nmbug), L-norleucine (Nle), L-norvaline (Nva), α -methyl-aminoisobutyrate (Maib), α -methyl- γ -aminobutyrate (Mgab), D- α -methylalanine (Dmala), α -Methylcyclohexylalanine (Mchexa), D- α -methylarginine (Dmarg), α -methylsilcopentylalanine (α -methylcopentylalanine, Mcp), D- α -methylasparagin (Dmasn), α -methyl- α -naptyl Alanine (Manap), D- α -methylaspartate (Dmasp), α -methylpeniciramine (Mpen), D- α -methylcysteine (Dmcys), N- (4-aminobutyl) glycine (Nglu), D- α -methylglutamine (Dmgln), N- (2-aminoethyl) glycine (Naeg), D- α -methylhistidine (Dmhis), N- (3-Aminopropyl) Glycine (Norn), D- α -Methylisoleucine (Dmile), N-Amino- α -Methylbutyrate (Nmaabu), D- α -Methylleucine (Dmleu), α -Naptylalanine (Anap) , D- α -methyllysine (Dmlys), N-benzylglycine (Nphe), D- α -methylmethionine (Dmmet), N- (2-carbamylethyl) glycine (Ngln), D- α -methylornithine (Ngln) Dmorn), N- (carbamylmethyl) glycine (Nasn), D- α -methylphenylalanine (Dmphe), N- (2-carboxyethyl) glycine (Nglu), D- α -methylproline (Dpro), N- (Carboxymethyl) Glycine (Nasp), D- α -Methylserine (Dmser), N-Cyclobutylglycine (Ncbut), D- α -Methylthreonine (Dmthr), N-Cycloheptylglycine (Nchep), D- α -Methyltryptophan (Dmtrp), N-cyclohexylglycine (Nchex), D- α -methyltyrosine (Dmty), N-cyclodecylglycine (Ncd), D- α -methylvaline (Dmval), N-cyclododecylglycine (Ncdod) , DN-methylalanine (Dnmala), N-cyclooctylglycine (Ncoct), DN-methylarginine (Dnmarg), N-cyclopropylglycine (Ncpro), DN-methylasparagin (Dnmasn), N -Cycloundecylglycine (Ncund), DN-methylaspartate (Dnmasp), N- (2,2-diphenylethyl) glycine (Nbhm), DN-methylcysteine (Dnmcys), N- (3, 3-Diphenylpropyl) Glycine (Nbhe), DN-Methylglutamine (Dnmgl), N- (3-

guanidinopropyl) Glycine (Narg), DN-Methylglutamate (Dnmglu), N- (1-Hydroxy) Ethyl) Glycine (Ntbx), DN-Methylhistidine (Dnmhis), N- (Hydroxyethyl) Glycine (Nser), DN-Methylisoleucine (Dnmile), N- (Imidazoli) Ruethyl) glycine (Nhis), DN-methylleucine (Dnmleu), N- (3-indrillethyl) glycine (Nhtp), DN-methyllysine (Dnnylis), N-methyl- γ -aminobutyrate (Nmgaba), N-methylcyclohexylalanine (Nmchexa), DN-methylmethionine (Dnmmt), DN-methylornithin (Dnmorn), N-methylcyclopentylalanine (Nmcpn), N-methylglycine (Nala), DN-Methylphenylalanine (Dnmphe), N-Methylaminoisobutyrate (Nmaib), DN-Methylproline (Dnmpro), N- (1-Methylpropyl) Glycin (Nile), DN-Methylserine (Nile) Dnmser), N- (2-methylpropyl) glycine (Nleu), DN-methylthreonine (Dnmthr), DN-methyltryptophan (Dnmtrp), N- (1-methylethyl) glycine (Nval), D -N-methyltyrosine (Dnmtyr), N-methyla-naptylalanine (Nmanap), DN-methylvalin (Dnmval), N-methylpenicillamine (Nmpen), γ -aminobutyric acid (Gabu), N- (p) -Hydroxyphenyl) glycine (Nhtyr), L-/-butyl glycine (Tbug), N- (thiomethyl) glycine (Ncys), L-ethylglycine (Etg), penicillamine (Pen), L-homophenylalanine (Hphe), L- α -methylalanine (Mala), L- α -methylarginine (Marg), L- α -methylasparagin (Masn), L- α -methylaspartate (Masp), L- α -methyl-t-butyl Glycin (Mtbug), L- α -Methylcysteine (Mcys), L-Methylethylglycine (Meg), L- α -Methylglutamine (MgIn), L- α -Methylglutamate (Mglu), L- α -methyl Histidine (Mhis), L- α -Methylhophenylalanine (Mhphe), L- α -Methylisoleucine (Mile), N- (2-Methylthioethyl) glycine (Nmet), L- α -Methylleusin (Mleu), L -A-Methyllysine (Mlys), L- α -methylmethionine (Mmet), L- α -methylnorleucine (Mnle), L- α -methylnorvaline (Mnva), L- α -methylornithine (Mmet) Morn), L- α -methylphenylalanine (Mphe), L- α -methylproline (Mpro), L- α -methylserine (Mser), L- α -methylthreonine (Mthr), L- α -methyltryptophane (Mtrp)), L- α -methyltyrosine (Mtyr), L- α -methylvalin (Mval), L-N-methylhomophenylalanine (Nmphe), N- (N- (2,2-diphenylethyl) carbamilmethyl) glycine (Nnbhm), N- (N- (3,3-diphenyl-propyl) carbamilmethyl) glycine (Nnbhe), 1-carboxy-1- (2,2-diphenyl-ethylamino) cyclopropane (Nmbc), L -O-Methylserine (Omser), LO-Methylhomoserin (Omher).

As used herein, the term "N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycle" is a cyclic saturated or unsaturated hydrocarbon containing at least one nitrogen atom as a component of a cyclic chain. It means a hydrogen compound.

As used herein, the term "radioactive moiety" means a molecular assembly having a radionuclide. Nuclides are bound by either covalent or coordinate bonds that remain stable under physiological conditions. Examples include [¹³¹I] -3-iodobenzoic acid or ⁶⁸Ga-DOTA.

As used herein, the "fluorescent isotope" emits electromagnetic radiation after being excited by shorter wavelength electromagnetic radiation.

As used herein, "radioisotope" is a radioisotope of an element that emits α -, β - and / or γ -radiation (included by the term "radionuclide").

The term "radioactive agent" is used in the context of the present invention to refer to a bioactive compound modified by a radioisotope. In particular, intercalating materials can be used to deliver radioactivity in the immediate vicinity of DNA (eg, ¹³¹I-supported derivatives of Hoechst-33258).

The term "chelating agent" or "chelating" is used interchangeably in the context of the present invention and is often a molecule with two or more unshared electron pairs available to donate to a metal ion. , Organic matter, and often means Lewis base. Metal ions are usually coordinated to a chelating agent with two or more electron pairs. The terms "two-ligand chelating agent", "tri-ligand chelating agent" and "four-ligand chelating agent" are readily available to simultaneously donate to metal ions coordinated by the chelating agent, respectively. It means a chelating agent having two, three and four possible electron pairs. Normally, an electron pair of a chelating agent forms a coordination bond with a single metal ion, but in a specific example, the chelating agent may form a coordination bond with more than one metal ion. Combination style is possible.

As used in the context of the present invention, the term "fluorescent dye" is used to refer to a compound that emits visible or infrared light after being excited by shorter, more suitable wavelengths of electromagnetic radiation. . It is understood by those skilled in the art that each fluorescent dye has a predetermined excitation wavelength.

The term "contrast agent" is used in the context of the present invention to refer to a compound that increases the contrast of a structure or fluid in medical imaging. Reinforcement is achieved by absorbing electromagnetic radiation or changing the electromagnetic field.

As used herein, the term "paramagnetic" means paramagnetism induced by unpaired electrons in a medium. Paramagnetic materials induce a magnetic field when an external magnetic field is applied. Unlike antimagnetism, the direction of the induced magnetic field is the same as the external magnetic field, and unlike ferromagnetic, the magnetic field is not maintained in the absence of the external magnetic field.

As used herein, the term "nanoparticle" preferably means a spherical particle having a diameter between 1 and 100 nanometers. Depending on the composition, the nanoparticles can have evaluable magnetic, optical or physicochemical qualities. In addition, surface modifications of many types of nanoparticles are achievable.

The term "pharmaceutically acceptable salt" means a salt of a compound of the invention. Suitable pharmaceutically acceptable salts of the invention include, for example, solutions of choline or derivatives thereof, such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or Includes acid addition salts that can be formed by mixing with a solution of a pharmaceutically acceptable acid such as phosphoric acid. In addition, if the compounds of the invention have acidic moieties, suitable pharmaceutically acceptable salts thereof include alkali metal salts (eg, sodium or potassium salts); alkaline earth metal salts (eg, calcium salts or magnesium). Salts); and salts formed with suitable organic ligands (eg, with counter anions such as halides, hydroxides, carboxylates, sulfates, phosphates, nitrates, alkyl sulfonates and aryl sulfonates). Includes ammonium, quaternary ammonium and amine cations formed). Examples of pharmaceutically acceptable salts include, but are not limited to: acetate, adipate, alginate, ascorbate, asparaglate, benzenesulfonate, benzoate, bicarbonate, Bisulfate, heavy tartrate, borate, bromide, butyrate, calcium edetate, camphorate, camsylate, camsylate, carbonate, chloride, citrate, Crablanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecyl sulfate, edetate, edicylate, straight (estolate), esylate, ethanesulfonate, formate, fumaric acid, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, Hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonic acid, hydroxynaphthoate, iodine Acids, Isothiocyanate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, Methyl sulfate, mucate, 2-naphthalene sulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (Embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate / diphosphate, picrate, pivalate, Polygalacturonate, propionate, salicylate, stearate, sulfate, basic acetate, succinate, tannate, tartrate, theocrate, tosylate, Triethiodide, undecanoate, valerate, etc. (eg Berge, SM, et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain particular compounds of the invention include both basic and acidic functional groups that allow the compound to be converted to either a base or an acid addition salt.

The neutral form of the compound may be regenerated by contacting the salt with a base or acid and isolating the parent compound by conventional methods. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but other salts are equivalent to the parent form of the compound for the purposes of the present invention. Is.

In addition to the salt form, the present invention provides compounds in the form of prodrugs. The prodrugs of the compounds described herein are compounds that are readily chemically modified under physiological conditions to provide the compounds of formula (I). A prodrug is an active or inactive compound that is chemically modified to a compound of the invention by in vivo physiological actions such as hydrolysis, metabolism, etc. after administration of the prodrug to a patient. In addition, prodrugs can be converted to compounds of the invention by chemical or biochemical methods in an Exvivo environment. For example, a prodrug can be slowly converted to a compound of the invention when placed in a transdermal patch reservoir with the appropriate enzyme. The suitability and technology involved in the manufacture and use of prodrugs are well known to those of skill in the art. For a general discussion of prodrugs involved in esters, see Svensson and Tunek Drug Metabolism Reviews 16.5 (1988) and Bundgaard Design of Prodrugs, Elsevier (1985). Examples of masked carboxylate anions are alkyl (eg methyl, ethyl), cycloalkyl (eg cyclohexyl), aralkyl (eg benzyl, p-methoxybenzyl) and alkylcarbonyloxymethyl (eg pivaloyloxymethyl).) And other various esters. The amine is masked as an arylcarbonyloxymethyl substituted derivative and is cleaved by esterase in vivo to release free drugs and formaldehyde (Bunggaard J. Med. Chem. 2503 (1989)). In addition, drugs containing acidic NH groups such as imidazole, imide, and indole are masked with N-acyloxymethyl groups (Bundgaard Design of Prodrugs, Elsevier (1985)). Hydroxy groups are masked as esters and ethers. EP 0 039 051 (Sloan and Little, Apr. 11, 1981) discloses the Mannich basic hydroxamic acid prodrug, its preparation and use.

The compound according to the present invention can be synthesized by one or more of the following methods. It should be noted that the general procedure has been shown in connection with the preparation of compounds with unspecified stereochemistry. However, such procedures are generally applicable when the stereochemistry

for a particular stereochemical compound, eg, a group, is (S) or (R). Further, a compound having one stereochemistry (eg, (R)) is utilized to produce a compound having the opposite stereochemistry (ie, (S)) by a well-known method, eg, inversion. Often can be done.

The particular compound of the invention can exist in a solvated form, including a non-solvated form as well as a hydrated form. In general, the solvated form is equivalent to the non-solvated form and is intended to be included within the scope of the present invention. The particular compound of the invention may be present in multiple crystalline or amorphous forms. In general, all physical forms are equivalent to the uses considered by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the invention have asymmetric carbon atoms (optical centers) or double bonds; racemates, diastereomers, geometric isomers and individual isomers are all within the scope of the invention. Intended to be included.

The compounds of the present invention may also contain an unnatural proportion of atomic isotopes in one or more atoms that make up such compounds. For example, the compound can be radiolabeled with a radioisotope such as tritium (3H), iodine-125 (125I) or carbon-14 (14C). All isotopic variations of the compounds of the invention, whether radioactive or not, are intended to be included within the scope of the invention.

As used in this application, the term "pharmaceutical composition" means a substance and / or combination of substances used for the identification, prevention or treatment of a tissue condition or disease. The pharmaceutical composition is formulated to be suitable for administration to a patient to prevent and / or treat a disease. In addition, the pharmaceutical composition means a combination with an activator, an inert or active carrier, making the composition suitable for therapeutic use. Pharmaceutical compositions may be formulated for oral, parenteral, topical, inhalative, rectal, sublingual, transdermal, subcutaneous or vaginal application routes, depending on their chemical and physical properties. it can. Pharmaceutical compositions include solid, semi-solid, liquid, transdermal therapeutic systems (TTSs). The solid composition is selected from the group consisting of tablets, coated tablets, powders, granules, pellets, capsules, effervescent tablets or transdermal therapeutic systems. Also included are liquid compositions selected from the group consisting of solutions, syrups, infusions, extracts, solutions for intravenous application, solutions for infusions or carrier system solutions of the invention. Semi-solid compositions that can be used in the context of the present invention include emulsions, suspensions, creams, lotions, gels, globules, oral tablets and suppositories.

"Pharmaceutically acceptable" is listed in the United States Pharmacopeia or other generally accepted pharmacopoeia for use in animals, especially humans, approved by federal or state regulators. It means that it has been done.

As used herein, the term "carrier" means a diluent, adjuvant, excipient or vehicle administered with a therapeutic agent. Such pharmaceutical carriers include sterile liquids such as saline solutions in water and petroleum-derived oils, animal-derived oils, plant-derived oils or synthetic-derived oils such as peanut oil, soybean oil, mineral oil, sesame oil and the like. It may be an oil containing. For intravenous administration of the pharmaceutical composition, saline is the preferred carrier. Physiological saline, aqueous dextrose and glycerol solutions can also be used as liquid carriers, especially as injection solutions. Suitable pharmaceutical additives include starch, glucose, lactose, sucrose, gelatin, malt, rice, wheat flour, milk powder (chalk), silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, Includes propylene, glycol, water, ethanol and the like. If desired, the composition may also contain small amounts of wetting or emulsifying agents or pH buffering agents. Examples of suitable drug carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term "fibroblast-activating protein (FAP)" as used herein is also known by the term "septase". Both terms can be used interchangeably herein. The fibroblast-activating protein is a homodimer integrated protein with a dipeptidyl peptidase IV (DPPIV) -like fold and is characterized by an α / β hydrolase domain and an 8-blade β -propeller domain.

Embodiments In the following, various aspects of the present invention will be defined in more detail. Each aspect defined in this way may be combined with other aspects unless explicitly indicated on the contrary. In particular, any feature that is shown to be favorable or advantageous may be combined with other features that are shown to be favorable or advantageous.

In a first aspect, the present invention describes the formula (I): [in the formula, Q, R, U, V, W, Y and Z are in Q, R, U, V, W, Y and Z. Independently present or absent, provided that at least three are present; Q, R, U, V, W, Y and Z are independently O, CH₂, NR₄, C = O, Selected from the group consisting of C = S, C = NR₄, HCR₄ and R₄CR₄, but the two O's are not directly adjacent to each other; preferably 4 out of 6 groups are present, where 2 One is C = O, one is CH₂, and one is NH; more preferably, there are four groups, where two are C = O and one is NH. CH₂, and one is NH; most preferably V, W, Y and Z are present, where V and Z are C = O and W and Y are independently CH₂ and Selected from NH; R₁ and R₂ are independently selected from the group consisting of -H, -OH, halo, C1-6-alkyl, -O-C1-6-alkyl and S-C1-6-alkyl;

R₃ is -H, -CN, -B (OH) ₂, -C (O) -alkyl, -C (O) -aryl, -C = C-C (O) -aryl, -C = CS (O) Selected from the group consisting of 2-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂ and 5-tetrazolyl; R₄ is -H, -C1-6-alkyl, -O-C1-6-alkyl, -S-C1-6-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C1-6-aralkyl, each -C1-6-alkyl selected from the group consisting of -OH, , Oxo, halo, may be substituted with 1-3 substituents, and may be attached to Q, R, U, V, W, Y or Z; R₅ is -H, Selected from the group consisting of halos and C1-6-alkyls; R₆ and R₇ are independently selected from the group consisting of -H, but R₆ and R₇ are not H at the same time; preferably R₆ is 7- or 8 -Binds to the quinolyl position, R₇ binds to the 5- or 6-quinolyl position; more preferably R₆ binds to the 7-quinolyl position, R₇ binds to the 6-quinolyl position; where , L is a linker; D, A, E and B are present or absent independently, preferably at least A, E and B are present, where D is. Linker;

A is selected from the group consisting of NR₄, O, S and CH₂; E is selected from the group consisting of C1-6-alkyl; where i is 1, 2 or 3; where j is 1, 2 or 3; where k is 1, 2 or 3; where m is 1, 2 or 3; more preferably E is C1-6-alkyl, most preferably. Preferably, E is a C₃ or C₄ alkyl; A and E together form a group selected from cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, where the heterocycloalkyl is formed. A and E may be monocyclic, bicyclic and polycyclic, preferably monocyclic; each A and E is -H, -C1-6-alkyl, -O-C1. Substituted by 1-4 substituents selected from -6-alkyl, -S-C1-6-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C1-6-aralkyl. Each -C1-6-alkyl may be substituted with 1-3 substituents selected from -OH, oxo and halo; and may also be attached to A, B, D, E or. Good to be;

B is S, NR₄, NR₄-O, NR₄-C1-6-alkyl, NR₄-C1-6-alkyl-NR₄ and 5- to 10-member N-containing aromatic or non-aromatic monocyclic or bicyclic. Selected from the group consisting of formula heterocycles, the heterocycle preferably further comprises one or two heteroatoms selected from O, N and S, preferably further comprising one or two nitrogen atoms. Preferably, the NR₄-C1-6-alkyl-NR₄ and the N-containing heterocycle are by 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. Substituted; and R₈ is selected from the group consisting of radioactive moieties, chelating agents, fluorescent dyes, contrasting agents and combinations thereof; are 1-naphthyl group moieties or 5-10-membered N-containing aromatics. Alternatively, it is a non-aromatic monocyclic or bicyclic heteroatom, wherein the heteroatom has two ring atoms between N and X; and the heteroatom is O, N and It may further contain one, two or three heteroatoms selected from S; and X is the C atom], or a pharmaceutically acceptable homozygous, racemic, water. A Japanese product, a Japanese product, or a salt product is provided. In a preferred embodiment, A and E together form a group selected from C₃, C₄, C₅, C₆, C₇ and C₈ monocyclic heterocycloalkyl, preferably C₅ or C₆ monocyclic, or. A group selected from C₇, C₈, C₉, C₁₀C₁₁ or C₁₂ bicyclic heterocycloalkyl is formed, preferably a group selected from C₇, C₈, C₉ and C₁₀ bicyclic heterocycloalkyl. The heterocycloalkyl contains 1, 2, 3 or 4, preferably 1 or 2 heteroatoms independently selected from the group consisting of N, O and S, preferably N and O, most preferably. Contains one or two N's.

In a preferred embodiment of the first aspect of the present invention, the formula (I): [In the formula, Q, R, U, V, W, Y and Z are Q, R, U, V, W, Y and Z. Independently present or absent, provided that at least 3 of them are present; Q, R, U, V, W, Y and Z are independently O, CH₂, NR₄, C. Selected from the group consisting of = O, C = S, C = NR₄, HCR₄ and R₄CR₄, but the two O's are not directly adjacent to each other; preferably 4 out of 6 groups are present, where Two are C = O, one is CH₂, and one is NH; more preferably, there are four groups, where two are C = O and one. Is CH₂, and one is NH; most preferably V, W, Y and Z are present, where V and Z are C = O and W and Y are CH₂ independently. And NH; R₁ and R₂ are independently selected from the group consisting of -H, -OH, halo, C1-6-alkyl, -O-C1-6-alkyl and S-C1-6-alkyl. ;

R₃ is -H, -CN, -B (OH) ₂, -C (O) -alkyl, -C (O) -aryl, -C = C-C (O) -aryl, -C = CS (O) Selected from the group consisting of 2-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂ and 5-tetrazolyl; R₄ is -H, -C1-6-alkyl, -O-C1-6-alkyl, -S-C1-6-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C1-6-aralkyl, each -C1-6-alkyl selected from the group consisting of -OH, , Oxo, halo, may be substituted with 1-3 substituents, and may be attached to Q, R, U, V, W, Y or Z; R₅ is -H, Selected from the group consisting of halo and C1-6-alkyl;

R6 and R7 are independently selected from the group consisting of -H, except that R6 and R7 are not H at the same time, preferably R6 is 7- or it binds to the 8-quinolyl position, R7 binds to the 5- or 6-quinolyl position; more preferably, R6 binds to the 7-quinolyl position, R7 binds to the 6-quinolyl position; here. And L is a linker; D, A, E and B are present or absent independently, preferably at least A, E and B are present and where are present: D. Is a linker;

A is selected from the group consisting of NR₄, O, S and CH₂; E is selected from the group consisting of C1-6-alkyl; where i is 1, 2 or 3; where j is 1, 2 or 3; where k is 1, 2 or 3; where m is 1, 2 or 3; more preferably E is C1-6-alkyl, most preferably. Preferably E is C3 or C4 alkyl;

B is S, NR₄, NR₄-O, NR₄-C1-6-alkyl, NR₄-C1-6-alkyl-NR₄ and 5- to 10-member N-containing aromatic or non-aromatic monocyclic or bicyclic. Selected from the group consisting of formula heterocycles, the heterocycle preferably further comprises one or two heteroatoms selected from O, N and S, preferably further comprising one or two nitrogen atoms. Preferably, the NR₄-C1-6-alkyl-NR₄ and the N-containing heterocycle are by 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-alkyl. Substituted; and R8 is selected from the group consisting of radioactive moieties, chelating agents, fluorescent dyes, contrasting agents and combinations thereof; are 1-naphthyl group moieties or 5-10-membered N-containing aromatics. Or a non-aromatic monocyclic or bicyclic heterocycle, the heteroatom having two ring atoms between the N atom and X; and the heterocycle from O, N and S. It may further contain one, two or three heteroatoms of choice; and X is the C atom], or a pharmaceutically acceptable homozygous, racemic, hydrate. , Solventates or salts. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, A and E together form a group consisting of C3, C4, C5, C6, C7 and C8 monocyclic heterocycloalkyl, preferably C5. Alternatively, a group consisting of a C6 monocyclic or C7, C8, C9, C10, C11 or C12 bicyclic heterocycloalkyl, preferably a group consisting of C7, C8, C9 and C10 bicyclic heterocycloalkyl. The heterocycloalkyl is formed, wherein the heterocycloalkyl contains 1, 2, 3 or 4, preferably 1 or 2 heteroatoms independently selected from N, O and S, preferably N and O, most of the time. It preferably contains one or two N's. Preferred monocyclic heterocycloalkyls are pyrrolidinyl, piperidinyl, imidazolidinyl, 1,2-diazacyclohexanyl, 1,3-diazacyclohexanyl, piperazinyl, 1-oxo-2-azacyclohexanyl, 1- It is selected from the group consisting of oxo-3-azacyclohexanyl or morpholinyl, preferably piperidinyl, piperazinyl and pyrrolidinyl. Preferred bicyclic heterocycloalkyls are bicyclo [2.2.1] 2,5-diazaheptanyl, 3,6-diazabicyclo [3.2.1] octanyl, 3,6-diazabicyclo [3.2.2] nonyl. , Octahydropyrrolo [2,3-b] pyrrolyl, octahydropyrrolo [3,2-b] pyrrolyl, octahydropyrrolo [3,4-b] pyrrolyl, octahydropyrrolo [3,4-c] pyrrolyl, 9 -Methyl-3,7,9-Triazabicyclo [3.3.1] Selected from the group consisting of nonanyl.

Bonds between heterocycles formed by A and E and B on the one hand and / or R6 or R7 on the other are preferably formed via heteroatoms, preferably N.

In particular, preferred examples of the heterocycle formed by A and E are selected from.

In a preferred embodiment of the first aspect of the invention, Q, R and U are CH₂ and either exist independently or are absent; preferably Q and R are absent; V is , CH₂, C = O, C = S or C = NR₄; preferably V is C = O; W is NR₄; preferably W is NH; Y is HCR₄; preferably Y is CH₂; and Z is C = O, C = S or C = NR₄, preferably Z is C = O.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is CH₂; W is NH; Y is CH₂; and Z is C = O. is there.

In a further preferred embodiment of the first aspect of the invention, R1 and R2 are independently selected from the group consisting of -H and halo; preferably R1 and R2 are halos; more preferably R1 and R2 is F; R3 is selected from the group consisting of -H, -CN and -B (OH) 2; preferably R3 is -CN or -B (OH) 2; more preferably R3. Is -CN; R4 is selected from the group consisting of -H and -C1-6-alkyl, where -C1-6-alkyl is selected by 1-3 substituents selected from -OH. It may be replaced. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is CH₂; W is NH; Y is CH₂; Z is C = O. R1 and R2 are independently selected from the group consisting of -H and halo; preferably R1 and R2 are halos; more preferably R1 and R2 are F; R3 is -H,- Selected from the group consisting of CN and -B (OH) 2; preferably R3 is -CN or -B (OH) 2; more preferably R3 is -CN; R4 is -H and- Selected

from the group consisting of C1-6-alkyl, where -C1-6-alkyl may be substituted with 1-3 substituents selected from -OH. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is CH₂; W is CH₂; Y is NH; Z is C = O. R1 and R2 are independently selected from the group consisting of -H and halo; preferably R1 and R2 are halos; more preferably R1 and R2 are F; R3 is -H,-. Selected from the group consisting of CN and -B (OH) 2; preferably R3 is -CN or -B (OH) 2; more preferably R3 is -CN; R4 is -H and- Selected from the group consisting of C1-6-alkyl, where -C1-6-alkyl may be substituted with 1-3 substituents selected from -OH. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, is selected from the group consisting of, which may comprise one or two heteroatoms selected from O, N and S.

In a further preferred embodiment of the first aspect of the invention, is, which may include one or two heteroatoms selected from O, N and S.

In a further preferred embodiment of the first aspect of the present invention, is selected from the group consisting of. R6 and R7 are independently selected from the group consisting of -H, but R6 and R7 are not H at the same time; preferably R6 and R7 bind to the 5, 6 or 7 position.

In a preferred embodiment, is selected from the group consisting of. In another preferred embodiment, is.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the 5- or 6-quinolyl position; more preferably R7. Is attached to the position of 6-quinolyl, where D is absent; A is O, S, CH₂, NH or NCH₃; E is C1-6-alkyl or, where m is 1, 2 or 3; preferably the C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; Preferably E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; or A and E together form a group selected from; B is NR₄-. It is a C1-6-alkyl-NR₄ or 5--10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycle, the heterocycle being preferably selected from O, N and S. It further comprises one or two heteroatoms, preferably further comprising one or two nitrogen atoms, wherein the N-containing heterocycle is preferably C1-6-alkyl, aryl, C1-6-aromatic. It is substituted with 1-3 substituents selected from the group consisting of. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the 5- or 6-quinolyl position; more preferably. R7 binds to the 6-quinolyl position, where D is absent; A is O; E is C1-6-alkyl or, where m is 1, 2 or 3. Preferably, the C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably E is C1-6- Alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl-NR₄ or, preferably an additional hetero selected from one or two O, N and S. It is a 5- to 10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycle containing an atom, preferably an additional 1 or 2 nitrogen atoms, preferably here. The N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the 5- or 6-quinolyl position; more preferably R7. Is attached to the position of 6-quinolyl, where D is absent; A is S; E is C1-6-alkyl or, where m is 1, 2 or 3; Preferably, the C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C1-6-. Alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl-NR₄ or 5--10-membered N-containing aromatic or non-aromatic monocyclic or dicyclic. It is a cyclic heterocycle, the heterocycle preferably further comprising one or two heteroatoms selected from O, N and S, preferably further comprising one or two nitrogen atoms, preferably here. , The N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the 5- or 6-quinolyl position; more preferably R7. Is attached to the position of 6-quinolyl, where D is

absent; A is CH₂; E is C1-6-alkyl or, where m is 1, 2 or 3; Preferably, the C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl or 5--10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic. It is a heterocycle, the heterocycle preferably further comprising one or two heteroatoms selected from O, N and S, preferably further comprising one or two nitrogen atoms, preferably said The N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R₅ and R₆ are H; R₇ is, preferably R₇ binds to the 5- or 6-quinolyl position; more preferably R₇. Is attached to the position of 6-quinolyl, where D is absent; A is NH; E is C1-6-alkyl or, where m is 1, 2 or 3. Preferably, the C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl or 5--10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic. The heterocycle is of the formula heterocycle, which further comprises one or two heteroatoms preferably selected from O, N and S, preferably further comprising one or two nitrogen atoms, preferably comprising one or two nitrogen atoms. The N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R₅ and R₆ are H; R₇ is, preferably R₇ binds to the 5- or 6-quinolyl position; more preferably R₇. Is attached at the position of 6-quinolyl, where D is an amino acid, preferably an amino acid having a charged side chain; A is O; E is C1-6-alkyl or, where , M is 1, 2 or 3; preferably C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl. More preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl or 5--10-membered N-containing aromatics. Alternatively, it may be a non-aromatic monocyclic or bicyclic heterocycle, which further comprises one or two heteroatoms selected from O, N and S, preferably one or two nitrogens. It further comprises an atom, preferably the N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. .. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R₅ and R₆ are H; R₇ is, preferably R₇ binds to the 5- or 6-quinolyl position; more preferably R₇. Is attached at the position of 6-quinolyl, where D is an amino acid, preferably an amino acid having a charged side chain; A is S; E is C1-6-alkyl or, where , M is 1, 2 or 3; preferably C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl. More preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl or 5--10-membered N-containing aromatics. Alternatively, it may be a non-aromatic monocyclic or bicyclic heterocycle, the heterocycle preferably further comprising one or two heteroatoms selected from O, N and S, preferably one or two nitrogens. It further comprises an atom, preferably the N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. .. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R₅ and R₆ are H; R₇ is, preferably R₇ binds to the 5- or 6-quinolyl position; more preferably R₇. Is attached at the position of 6-quinolyl, where D is an amino acid, preferably an amino acid having a charged side chain; A is CH₂; E is C1-6-alkyl or, where , M is 1, 2 or 3; preferably C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl. More preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR₄-C1-6-alkyl or 5--10-membered N-containing aromatics. Alternatively, it may be a non-aromatic monocyclic or bicyclic heterocycle, which further comprises one or two heteroatoms selected from O, N and S, preferably one or two nitrogens. It further comprises an atom, preferably the N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-aralkyl. .. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R₅ and R₆ are H; R₇ is, preferably R₇ binds to the 5- or 6-quinolyl position; more preferably R₇. Is attached at the position of 6-quinolyl, where D is an amino acid, preferably an amino acid having a charged side chain; A is NH; E is C1-6-alkyl or, where , M is

1, 2 or 3; preferably C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl. More preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is NR4-C1-6-alkyl or 5--10-membered N-containing aromatics. Alternatively, it may be a non-aromatic monocyclic or bicyclic heterocycle, which further comprises one or two heteroatoms selected from O, N and S, preferably one or two nitrogens. It further comprises an atom, preferably the N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C1-6-alkyl, aryl, C1-6-alkyl. .. Preferably, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is swelling, preferably R7 binds to the 5- or 6-quinolyl position; more preferably R7. Is attached to the position of 6-quinolyl, where D is absent; A is O; E is C1-6-alkyl or, where m is 1, 2 or 3. Preferably, E is a C1-6-alkyl, which C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl. More preferably, E is C1-6-alkyl, most preferably E is C3 or C4 alkyl; B is a 5- to 10-membered N-containing aromatic or non-aromatic monocyclic. Alternatively, it is a bicyclic heterocycle, which preferably further contains one or two nitrogen atoms.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the 5- or 6-quinolyl position; more preferably R7. Is attached at the position of 6-quinolyl, where D is absent; A is O; E is C3 or C4 alkyl; more preferably E is propyl or butyl; B is. It is a 5- to 10-membered N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycle, which preferably further comprises one or two nitrogen atoms.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle contained in B is the formula: [in the formula, the heterocycle is one or two heterocycles selected from O, N and S. It may further contain an atom and may contain one nitrogen; is attached at the 1, 2 or 3 position, preferably at the 2nd position; l is 1 or 2]. It is a group or non-aromatic monocyclic heterocycle.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle contained in B is the formula: [in the formula, the heterocycle is one or two heterocycles selected from O, N and S. It may further contain an atom and may further contain one nitrogen; is bonded to the 1, 2 or 3 position, preferably the 2 position; where the N-containing heterocycle is C1-. Substituted by 6-alkyl] is an aromatic or non-aromatic monocyclic heterocycle.

In a further preferred embodiment of the first aspect of the invention, the N-containing heterocycle contained in B is selected from the group consisting of, where the N-containing heterocycle is substituted with C1-6-alkyl. Here, when the N-containing heterocycle contained in B is, the heterocycle may further contain one or two heteroatoms selected from O, N and S, one. It may further contain nitrogen and may also contain one or more side chains (eg, derived from amino acids); it binds to the 1, 2 or 3 position, preferably the 2nd position; o Is 1 or 2;

Preferably, when the N-containing heterocycle contained in B is, the N-containing heterocycle contained in B is selected from the group consisting; more preferably, the N-containing heterocycle contained in B is. In some cases, the N-containing heterocycle contained in B is.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle contained in B is selected from the group consisting of, where the N-containing heterocycle contained in B, if any. The heterocycle may further contain one or two heteroatoms selected from O, N and S, further one nitrogen, and one or more (eg, derived from amino acids). May include side chains;

Is attached to the 1, 2 or 3 position, preferably to the 2 position; o is 1 or 2; preferably is contained in B if the N-containing heterocycle contained in B is. The N-containing heterocycle is selected from the group consisting of; more preferably, if the N-containing heterocycle contained in B is, then the N-containing heterocycle contained in B is.

In a further preferred embodiment of the first aspect of the present invention, the N-containing heterocycle contained in B is selected from the group consisting of.

In a further preferred embodiment of the first aspect of the invention, the N-containing heterocycle contained in B is selected from the group consisting of, where B is substituted with C1-3 alkyl.

In a further preferred embodiment of the first aspect of the invention, R5 and R6 are H; R7 is, preferably R7 binds to the position of 6-quinolyl, where D is absent. A is O; E is propyl or butyl; B is.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is C = O; W is NH; Y is CH₂; Z is C = O. R1 and R2 are independently selected from the group consisting of -H and halo; preferably R1 and R2 are independently selected from the group consisting of -H and F; more preferably R1 and R2 is identical and selected from the group consisting of -H and F; R3 is -CN; R5 and R6 are H; R7 is, preferably R7 binds to the 6-quinolyl position. , Where D is absent;

A is O; E is C1-6-alkyl or, where m is 1, 2 or 3; preferably E is C1-6-alkyl; preferably C1-6. -Alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C1-6-alkyl, most preferably. E is C3 or C4 alkyl; B is NH-C1-6-alkyl, preferably C1-6-alkyl is methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, Selected from the group consisting of tert-butyl, pentyl and hexyl; preferably B is; and is.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is C = O; W is NH; Y is CH₂; Z is C = O. R1 and R2 are identical and selected from the group consisting of -H and F; R3 is -CN; R5 and R6 are H; R7 is, preferably R7 is 6-quinolyl. Where D is absent; A is O, S, CH₂, NH or NCH₃; E is methyl, ethyl, propyl or butyl;

A and E together form the group of choice; B is and B may be substituted with C1-3 alkyl; preferably B is; and is.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is C = O; W is NH; Y is CH₂; Z is C = O. R1 and R2 are identical and selected from the group consisting of -H and F; R3 is -CN; R5 and R6 are H; R7 is, preferably R7 is 6-quinolyl. Bonded to the position of, where D is absent; A is O; E is methyl, ethyl, propyl or butyl; B is; preferably B is; and , Is.

In a further preferred embodiment of the first aspect of the invention, Q, R and U are absent; V is C = O; W is NH; Y is CH₂; Z is C = O. R1 and R2 are identical and selected from the group consisting of -H and F; R3 is CN; R5 and R6 are H; R7 is and R7 binds to the 6-quinolyl position. And here, D is absent; A is O; E is methyl, ethyl, propyl or butyl; B is out; preferably B is; and is. ..

In a first further preferred embodiment of the invention, C1-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In the first further preferred embodiment of the invention, the C1-3-alkyl is selected from the group consisting of methyl, ethyl, propyl and i-propyl.

In the first further preferred embodiment of the present invention, C1-6-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl, phenyl-propyl and phenyl-butyl.

In a further preferred embodiment of the first aspect of the invention, the compound of the first aspect of the invention is selected from the compounds of Table 1. More preferably, the compound of the first aspect of the present invention is selected from the compounds in Table 2. More preferably, the compound of the first aspect of the invention is selected from the group consisting of FAPI-02 and FAPI-04.

In a preferred embodiment of the first aspect of the invention, the compound of the first aspect of the invention is selected from the compounds of Table 1 and / or Table 3. More preferably, the first embodiment compound of the present invention is selected from the compounds in Table 2 and / or Table 4. More preferably, the compounds of the first aspect of the invention are FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71. , FAPI-72 and FAPI-73.

Table 1: Preferred compounds of the first aspect of the invention § Fluorescent compounds; § 99mTc-chelating agent; * Pb-chelating agent; R1 and R2 are at 4-pyrrolidine positions; Q, R and U are absent Yes; is, R5 is H; R6 binds to the 7-quinolyl position; R7 binds to the 6-quinolyl position; "-" indicates that R6 or R7 is H; A "+" indicates that R6 or R7 is; V is C = O; W is NH; Y is CH₂; Z is C = O; R3 is -CN; A is O (excluding FAPI-01, where A is absent and R7 binds to the 5-quinoline position).

Table 2: Particularly interesting compounds. Q, R, U and D are absent; R1 and R2 are at the 4-pyrrolidine position; are, R5, R6 are H; R7 is bound at the 6-quinolidine position; V is C = O; W is NH; Y is CH2; Z is C = O; R3 is -CN; B is 1,4-piperazine; E is 1,3-propane. And A is O.

Table 3: Further preferred compounds of the first aspect of the invention. § Fluorescent compound; § ^{99m}Tc-chelating agent; * ¹⁸F-labeled precursor; Q, R and U are absent; R1 and R2 are at 4-pyrrolidine positions; R7 binds to the 6-quinolyl position; V is C = O; W is NH; Y is CH2; Z is C = O; R3 is -CN. is there.

Table 4: Particularly interesting compounds. Q, R, U and D are absent; R1 and R2 are fluorine atoms at the 4-pyrrolidine position; are; R5, R6 are H; R7 is at the 6-quinolidine position. Combined; V is C = O; W is NH; Y is CH2; Z is C = O; R3 is -CN; B is 1,4-piperazine; E is 1,3-Propane; A is O.

In a further preferred embodiment of the first aspect of the invention, R8 is a radioactive moiety that is a fluorescent isotope, a radioisotope, a radiopharmaceutical or a combination thereof. Preferably, the radioactive moiety is an α radiation isotope, a β radiation isotope, a γ radiation isotope, an Auger electron emission isotope, an X-ray emission isotope, a fluorescent emission isotope such as ¹¹C, ¹⁸F, ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁸⁶Re, ¹⁸⁸Re, ¹³⁹La, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁸⁸Y, ⁹⁰Y, ¹⁴⁹Pm, ¹⁶⁵Dy, ¹⁶⁹Er, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi, ²¹³Bi, ⁷²As, ⁷²Se, ¹⁰⁹Pd, ¹⁰⁵Rh, ¹⁰¹mRh, ¹¹⁹Sb, ¹²⁸Ba, ¹²³I, ¹²⁴I, ¹³¹I, ¹⁹⁷Hg, ²¹¹At,

151Eu, 153Eu, 169Eu, 201Tl, 203Pb, 212Pb, 64Cu, 67Cu, 188Re, 186Re, 198Au, 227Th and 199Ag. Selected from the group. Preferably, it is selected from the group consisting of 18F, 64Cu, 68Ga, 90Y, 99mTc, 153Sm, 177Lu, 188Re and the like.

In a further preferred embodiment of the first aspect of the present invention, R8 is a fluorescent dye of the following types: xanthene, acrydin, oxazine, cyanine, styryl dye, coumarin, porphyrin, metal-ligand complex, fluorescent protein, nano. Fluorophores selected from the group consisting of crystals, perylenes, boron-dipyrromethenes and phthalocyanines, as well as composites and combinations of these types of dyes.

In a more preferred embodiment of the first aspect of the invention, R8 is a chelating agent that forms a complex with a divalent or trivalent metal cation. Preferably, the chelating agent is 1,4,7,10-tetraazacyclododecane-N, N', N"-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triaza-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N, N, N', N"-pentaacetic acid (DTPA), bis It is selected from the group consisting of (carboxymethylimidazole) glycine and 6-feedradinopyridine 3-carboxylic acid (HYNIC).

In a first further preferred embodiment of the present invention, R8 is a contrast agent comprising or consisting of a paramagnetic agent, preferably where the paramagnetic agent comprises paramagnetic nanoparticles. , Or consists of it.

In the first further preferred embodiment of the present invention, R8 is any of Tables 1-5.

In a second aspect, the invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect and a pharmaceutically acceptable carrier and / or additive.

In a third aspect, the invention is a compound of the first aspect or for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast-activating protein (FAP) in an animal or human subject. The present invention relates to the pharmaceutical composition of the second aspect. Preferably, the disease characterized by overexpression of fibroblast-activating protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorders.

Preferably, if the disease characterized by overexpression of fibroblast-activating protein (FAP) is cancer, the cancer is breast cancer, pancreatic cancer, small bowel cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovary. Cancer, hepatocellular carcinoma, esophageal carcinoma, hypopharyngeal carcinoma, nasopharyngeal carcinoma, laryngeal carcinoma, myeloma cells, bladder cancer, bile duct cell carcinoma, clear renal cell carcinoma, neuroendocrine tumor, tumor-induced osteomalacia, sarcoma, CUP It is selected from the group consisting of (unknown early stage cancer), thoracic adenocarcinoma, tendonoma, glioma, stellate cell tumor, cervical cancer and prostate cancer. Preferably, the cancer is glioma, breast cancer, colon cancer, lung cancer, head and neck cancer, liver cancer or pancreatic cancer. More preferably, the cancer is a glioma.

Preferably, if the disease characterized by overexpression of fibroblast-activating protein (FAP) is chronic inflammation, the chronic inflammation is selected from the group consisting of rheumatoid arthritis, osteoarthritis and Kron's disease. To. Preferably, the chronic inflammation is rheumatoid arthritis.

Preferably, when the disease characterized by overexpression of fibroblast-activating protein (FAP) is fibrosis, the fibrosis consists of a group consisting of pulmonary fibrosis such as idiopathic pulmonary fibrosis and liver cirrhosis. Be selected.

Preferably, if the disease characterized by overexpression of fibroblast-activating protein (FAP) is tissue remodeling, the tissue remodeling occurs after myocardial infarction.

Preferably, if the disease characterized by overexpression of fibroblast-activating protein (FAP) is a keloid disorder, the keloid disorder is selected from the group consisting of scar formation, keloid tumors and keloid scars.

In a fourth aspect, the invention relates to a kit comprising or consisting of a compound of the first aspect or a pharmaceutical composition of the second aspect and instructions for diagnosing or treating a disease. Preferably, the disease is the particular disease.

Example 1: Compound Synthesis and Radiochemistry Two radiotracers (radiotracers) were synthesized based on FAP- α specific inhibitors (Jansen et al., ACS Med Chem Lett, 2013). Radioiodine-labeled FAPI-01 was obtained via a palladium-catalyzed bromine / tin exchange-prepared organic tin stannylation precursor. FAPI-02 is a precursor for radioactive metal chelates synthesized in 5 steps. Additional compounds were prepared by applying the same or slightly modified procedures. The structures of these compounds are listed in Tables 1 and 2. Radioiodination of the stannylation precursor was performed using peracetic acid. For chelation with Lu-177 and Ga-68, the pH of the reaction mixture was adjusted with sodium acetate and heated to 95 ° C. for 10 minutes. Stability in human serum was analyzed by radio-HPLC analysis of precipitates and supernatants.

Reagents All solvents and non-radioactive reagents are obtained in reagent grade from ABCR (Karlsruhe, Germany), Sigma-Aldrich (Muenchen, Germany), Acros Organics (Geel, Belgium) or VWR (Bruchsal, Germany) without further purification. Used in. Atto 488 NHS-ester was obtained from AttoTech (Siegen, Germany). 2,2', 2''-(10- (2- (4-nitrophenyl) oxy) -2-oxoethyl) -1,4,7,10-tetraazacyclododecane-1,4,7 triyl triyl Acetic acid (DOTA-PNP) was synthesized by the protocol of Mier et al. (Mier et al., Bioconjug Chem, 2005). Intermediates 6-methoxyquinoline-4-carboxylic acid (7), 5-bromoquinoline-4-carboxylic acid (3) and (S) -1- (2-aminoacetyl) pyrrolidine-2-carbonitrile 4- Methyl benzenesulfonate was synthesized by the protocol of Jansen et al. (Jansen et al., ACS Med Chem Lett, 2013). The substance (S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -5-bromoquinoline carboxamide was synthesized by a modified HBTU amidation protocol.

The compound synthesis scheme 1 is FAPI-01 in which Br / Li exchange is performed with n-butyllithium in 5-bromoquinolinyl-4-carboxylic acid (3) and quenching is performed with elemental iodine to obtain iodoquinoline 4. Shows the initial synthesis of. This compound was coupled to the Gly-Pro-CN fragment by HBTU / HOBt-activation to give FAPI-01 non-radioactive control material (1). Scheme 1. Synthesis of non-radioactive FAPI-01. i) nBuLi, then I₂, THF; ii) HBTU / HOBt, DIPEA, H-Gly-Pro-CN, DMF.

In the synthesis of radioactive FAPI-01 (1 *), palladium-catalyzed stannylation of inhibitor 5 in dioxane at 80 ° C. gave stannylation precursor 6 (Scheme 2). Scheme 2. Synthesis of radioactive FAPI-1 via stannylation precursor 4. i) (Me₃Sn) 2; (PPh₃) 2PdCl₂; dioxane 80 ° C.; ii) I-125 or I-131; AcOOH; 1M HCl; MeOH.

The chelating agent DOTA was chemically attached to the basic backbone of the FAP inhibitor to allow radiolabeling by uptake of radioactive metals. As shown by Jansen et al. (Jansen et al. ACS Med Chem Lett, 2013), modification of the quinoline-4-carboxylic acid at the 6-position is highly resistant without compromising target affinity and specificity. Therefore, a bifunctional linker is thus attached to the hydroxyl group of 8 via an ether bond, which is the main method of synthesis shown in Scheme 3. The readily available 1-bromo-3-chloropropane was selected to create an intact spacer during the saponification of the ester bonds formed simultaneously at the end of the one-pot process. Compound 9 was converted to N-Boc-protected quinolinecarboxylic acid 10 which was further bound to H-Gly-Pro-CN by HBTU. Due to the high hygroscopicity of free amines, compound 11 was directly converted to FAPI-02 (2) after Boc removal, solvent exchange and neutralization of excess p-toluenesulfonic acid. Scheme 3. Chemical synthesis of FAPI-02. i) Appropriate amount HBr 48%, 130 ° C; ii) 1-bromo-3-chloropropane, Cs₂CO₃, DMF then 6M NaOH; iii) 1-Boc-piperazine, KI, DMF; iv) HBTU / HOBt, DIPEA, H- Gly-Pro-CN, DMF; v) TosOH, MeCN, then DOTA-PNP, DIPEA, DMF.

For compounds incorporating group A $\neq$ O, quinoline-4-carboxylic acid intermediates were synthesized by a different reaction scheme. A key step in this approach is a palladium-catalyzed coupling reaction (eg, Buchwald-Hartwig cross-coupling), which requires additional protection prior to the cross-coupling reaction and cross-coupling. Deprotection of carboxylic acid function is required after the coupling reaction (Scheme 4). Scheme 4. Synthesis of 6- (3- (4-Boc-piperazine-1-yl) propyl-1- (methyl) amino) quinoline-4-carboxylic acid, which is a component for the synthesis of FAPI-46. i) DCC, tBuOH, CuCl; ii) 3-methylamino-1-propanol, Cs₂CO₃, Pd₂ (dba)₃, BINAP; iii) MsCl, NEt₃, DCM and then 1-Boc-piperazine, KI, DMF; iv) TFA Next, Boc₂O, NEt₃, DMF.

(S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -5-trimethylstannylquinoline carboxamide (6) (S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -5-bromoquinoline carboxamide 3.88 mg (10.0 μ mol), hexamethylnitrit 20 μ L (32 mg; 96 μ mol) and bis (triphenylphosphine) palladium (II) dichloride 0.75 mg (1.07 μ mol) is stirred in 1 mL of dry dioxane overnight at 80 ° C. under an inert atmosphere. Volatile substances are removed, the residue is taken up in 50% acetonitrile / 2 mL of water, filtered through a C18 light cartridge and then purified by HPLC. After lyophilization, 2.78 mg (5.90 μ mol; 59%) of product is obtained. LC-MS Rt 14.77 min, m / z 473.0786 [M (120Sn) + H] +

5-Iodoquinoline-4-carboxylic acid (4) Sodium hydride suspension (60% in mineral oil) 5.42 mg (136 μ mol) at 0 ° C. under Ar, 5-bromoquinoline-4-carboxylic acid (3)) Add to the solution in 3 mL of 30.27 mg (120 μ mol) dry THF. After removing the ice bath and cooling the reaction mixture to -78 ° C., 100 μ L (160 μ mol) of nBuLi (1.6 M in hexane) is added dropwise. After 15 minutes, 64.71 mg (254 μ mol) of iodine in 2 mL of THF is added dropwise and the reaction is stirred at -78 ° C. for 30 minutes before reaching room temperature. After 1 hour, 31 mL of 0.5 M NaHCO₃ and about 30 mg (170 μ mol) of sodium dithionite are added to stop the reaction by removing excess iodine. After removing THF under reduced pressure, the mixture is acidified to pH 2 and extracted 3 times with ethyl acetate (25 mL). The organic phases are combined, evaporated to dryness and purified by HPLC. After lyophilization, 18.14 mg (60.7 μ mol; 45%) of the title compound is obtained. ¹H NMR (500 MHz, DMSO-d₆) 13.95 (br, 0.3H), 8.93 (s, 1H), 8.34 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.60 (s, 1H), 7.52 (t, J = 7.9 Hz, 1H); ¹³C NMR (125 MHz, DMSO-d₆) 168.8, 150.3, 148.8, 141.3, 130.6, 121.0, 109.5; LC-MS Rt 8.65 min, m / z 299.9383 [M + H] +

(S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -5-trimethylstannylquinoline carboxamide (1; FAPI-01) HBTU 9.07 mg (23.9 μ mol) in 50 μ L of DMF is added to a solution of 6.21 mg (20.8 μ mol) of 5-iodoquinoline-4-carboxylic acid, 7.45 mg (55.2 μ mol) of HOBt and 10 μ L of DIPEA in 50 μ L of DMF. After 15 minutes, methyl (S) -1- (2-aminoacetyl) pyrrolidine-2-carbonitrile 4-benzenesulfonate (29.9 μ mol) in 50 μ L of DMF is added. The reaction is stopped with 850 μ L of water and purified by HPLC. Lyophilize to give 6.86 mg (15.8 μ mol; 76%) of product. ¹H NMR (600 MHz, DMSO-d₆) 9.06, 8.97, 8.33, 8.13, 7.56, 7.51, 4.81, 4.34, 4.06, 3.74, 3.56, 2.21, 2.17, 2.09, 2.05; ¹³C NMR (150 MHz, DMSO-d₆) 167.1, 150.2, 148.8, 145.3, 141.5, 130.7, 125.3, 121.9, 119.3, 92.0, 46.3, 45.4, 42.1, 29.5, 24.9; LC-MS Rt 11.95 min, m / z 435.0102 [M + H] +

6-Hydroxyquinoline-4-carboxylic acid (8) Raw 6-methoxyquinoline-4-carboxylic acid (7) 105 mg (477 μ mol) is dissolved in 3 mL of a 48% aqueous hydrobromic acid solution. The solution is heated to 130 ° C. for 4 hours. The solution is brought to room temperature and then brought to a weakly basic pH with 6M NaOH. After HPLC-purification and lyophilization, 79.2 mg (419 μ mol; 88%) of product is obtained. ¹H NMR (500 MHz, DMSO-d₆) 13.65 (br, 0.6H) 10.24 (s, 1H), 8.78 (d, J = 4.4 Hz, 1H), 8.06 (d, J = 2.6 Hz, 1H), 7.95 (d, J = 9.1 Hz, 1H), 7.84 (d, J = 4.4 Hz, 1H), 7.37 (dd, J = 9.1, 2.6 Hz, 1H), ¹³C NMR (125 MHz, DMSO-d₆) 167.7, 156.9, 146.5, 144.1, 133.4, 131.2, 126.2, 122.3, 122.6, 106.5; LC-MS Rt 6.66 min, m / z 190.0415 [M + H] +

6-Bromoquinoline-4-carboxylic acid tert-butyl 6-bromoquinoline-4-carboxylic acid (raw) 98.3 mg (390 μ mol) was suspended in 5 mL of tetrahydrofuran and 25.0 μ L (18.3 mg; 181 μ mol) of triethylamine. O-tert-butyl-N, N'-dicyclohexylurea (the day before, pure dicyclohexylcarbodiimide 426 mg (2.07 mmol), pure tert-butanol 173 mg (2.33 mmol), pure copper (I) chloride 10.2 mg (103 μ mol)) To). The mixture was heated to 50 ° C. overnight. The mixture was filtered, the solvent was evaporated and the product was isolated by HPLC. After lyophilization, 49.7 mg (161 μ mol; 41%) of the title compound was obtained. LC-MS Rt 20.40 min, m / z 251.9642 [M-tBu] +

6- (3-Chloro-1-propoxy) quinoline-4-carboxylic acid (9) 1-bromo-1-chloropropane 42.4 μ L (67.4 mg; 430 μ mol), 6-hydroxyquinoline-4-carboxylic acid in 250 μ L of DMF Add to a suspension of acid (8) 23.2 mg (123 μ mol) and potassium carbonate 190 mg (1.38 μ mol) and heat to 60 ° C. overnight. The reaction mixture is cooled to room temperature, diluted with 500 μ L of water and 500 μ L of acetonitrile, and then 100 μ L of 6M NaOH is added. The reaction mixture is purified directly by HPLC (5-40%) after complete ester hydrolysis has been achieved. After lyophilization, 26.45 mg (99.4 μ mol; 81%) of the product is obtained. ¹H NMR (500 MHz, DMSO-d₆) 13.75 (br, 0.4H), 8.88 (d, J = 4.4 Hz, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 9.2 Hz) , 1H), 7.94 (d, J = 4.4 Hz, 1H), 7.52 (dd, J = 9.2, 2.0 Hz, 1H), 4.24 (t, J = 5.95 Hz, 2H), 3.85 (t, J = 6.5 Hz) , 2H), 2.27 (m, 2H); ¹³C NMR (125 MHz, DMSO-d₆) 167.6, 157.5, 147.6, 144.8, 134.0, 131.2, 125.9, 122.7, 122.2, 104.5, 64.7, 41.9, 31.6; LC-MS Rt 11.46 min, m / z 266.0461 [M + H] +

6- (3-Hydroxypropylmethylamino) quinoline-4-carboxylic acid tert-butyl 6-bromoquinoline-4-carboxylic acid tert-butyl 204.6 mg (664 μ mol), BINAP 34.10 mg (54.7 μ mol), Pd₂ (dba)₃ 321.51 mg (23.5 μ mol) and cesium carbonate 480.3 mg (1.47 mmol) were dissolved in 6 mL of toluene, and 128.0 μ L (118 mg; 1.32 mmol) of N-methyl-1,3-propanolamine was added. did. The mixture was stirred at 90 ° C. overnight, the solvent was removed, the residue was suspended in 1: 1 water / acetonitrile, filtered and purified by HPLC. After lyophilization, 172.7 mg (547 μ mol; 82%) of the title compound was obtained. LC-MS Rt 13.41 min, m / z 261.1213 [M-tBu + H] +

6- (3- (4-Boc-piperazine-1-yl) propyl-1- (methyl) amino) quinoline-4-carboxylic acid tert-butyl 6- (3-hydroxypropylmethylamino) quinoline-4-carboxylic acid 62.8 mg (199 μ mol) of tert-butyl was dissolved in 5 mL of dichloromethane and 90.0 μ L (66.6 mg; 659 μ mol) of triethylamine. 20.0 μ L (29.6 mg; 258 μ mol) of

metern sulfonyl chloride was added at 0 ° C. and the mixture was reacted for 60 minutes. After adding 194.6 mg (1.05 mmol) of 1-Boc-piperazine, the volatiles were removed. 500 µL of dimethylformamide and 47.4 mg (286 µmol) of potassium iodide (potassium iodide) were added to the residue. The mixture was shaken at 60 ° C. for 120 minutes and then the product was isolated by HPLC. After lyophilization, 81.05 mg (167 µmol; 84%) of the title compound was obtained. LC-MS Rt 13.99 min, m / z 485.3086 [M + H] +

6- (3- (4-tert-butoxycarbonylpiperazin-1-yl) -1-propoxy) Quinoline-4-carboxylic acid (10) 6- (3-Chloro-1-propoxy) Quinoline-4-carboxylic acid (9) Dissolve 15.13 mg (56.9 µmol), 55.43 mg (298 µmol) of N-tert-butoxycarbonylpiperazine and 51.05 mg (30.8 µmol) of potassium iodide in 250 µL of DMF. The mixture is shaken at 60 ° C. overnight. The resulting suspension is diluted with 750 µL of water and the product is purified by HPLC. After lyophilization, the product is obtained as the corresponding TFA-salt in 28.73 mg (54.3 µmol; 95%). ¹H NMR (500 MHz, D₂O) 8.93 (d, J = 5.5 Hz, 1H), 8.17 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 5.5 Hz, 1H), 7.79 (dd, J = 9.3, 2.5 Hz, 1H), 7.65 (d, J = 2.5 Hz, 1H), 4.36 (t, J = 5.6 Hz, 2H), 4.27 (d, J = 13.55 Hz, 2H), 3.67 (d, J = 11.95 Hz), 3.47 (t, J = 15.5 Hz, 2 H), 3.27 (t, J = 12.7 Hz), 3.12 (td, J = 12.2, 2.65 Hz), 2.37 (m 2H), 1.47 (s, 9H); ¹³C NMR (125 MHz, D₂O) 155.5, 153.5, 149.0, 141.4, 134.4, 127.9, 126.6, 122.3, 118.4, 110.0, 105.1, 82.8, 65.5, 54.3, 51.5, 48.6, 40.7, 29.6, 27.4; LC-MS Rt 10.62 min, m / z 416.1997 [M + H] +

6- (3- (4-Boc-piperazin-1-yl) propyl-1- (methyl) amino) quinoline-4-carboxylic acid 6- (3- (4-Boc-piperazin-1-yl) propyl-1) 100.12 mg (206 µmol) of tert-butyl - (methyl) amino) quinoline-4-carboxylic acid was treated with 900 µL of trifluoroacetic acid, 25 µL of triisopropylsilane, 25 µL of water and 50 µL of trifluoromethanesulfonic acid for 60 minutes. The deprotected compound is precipitated with diethyl ether, dried and reacted with 60.83 mg (279 µmol) of di-tert-butyl dicarbonate and 50.0 µL (36.5 mg; 361 µmol) of triethylamine in 1 mL of dimethylformamide for an additional 60 minutes. I let you. After HPLC-purification and lyophilization, 55.42 mg (129 µmol; 65% over 2 steps) was obtained. LC-MS Rt 10.52 min, m / z 429.2463 [M + H] +

(S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonylpiperazine-1-yl) -1-propoxy) quinoline-4-carboxamide (11) HBTU 9.43 mg (24.9 µmol) in 50 µL of DMF, 6- (3- (4-tert-butoxycarbonylpiperazin-1-yl) -1-propoxy) quinoline-4-carboxylic acid in 50 µL of DMF Acid (10) is added to a solution of 10.56 mg (19.9 µmol), HOBt 5.38 mg (39.8 µmol) and 10 µL of DIPEA. After 15 minutes, methyl (S) -1- (2-aminoacetyl) pyrrolidine-2-carbonitrile 4-benzenesulfonate (29.9 µmol) in 50 µL of DMF is added. The reaction is stopped with 850 µL of water and purified by HPLC. Lyophilize to give 12.88 mg (19.4 µmol; 97%) of the title compound. ¹H NMR (500 MHz, DMSO-d₆) 9.04 (d, J = 5.5 Hz, 1H), 8.24 (d, J = 9.6 Hz, 1H), 8.10 (d, J = 5.5 Hz, 1H), 7.89 (d, J = 2.3 Hz, 1H), 7.85 (dd, J = 9.6, 2.3 Hz, 1H), 4.84 (t, J = 6 Hz, 1 H), 4.46-4.36 (m, 4H), 4.26 (d, J = 12.0 Hz, 2H), 3.83 (m, 1H), 3.67 (m, 3H), 3.47 (t, J = 7.7 Hz, 2H), 3.27 (br, 2H), 3.11 (t, J = 11.5 Hz), 2.37 (m, 4H), 2.22 (m, 2H), 1.46 (s, 9H); ¹³C NMR (125 MHz, DMSO-d₆) 168.6, 168.0, 159.4, 155.5, 147.7, 141.8, 135.1, 128.2, 127.5, 123.1, 120.0, 119.1, 104.7, 82.9, 66.0, 54.3, 51.5, 47.0, 46.3, 42.3, 29.4, 27.4, 24.7, 23.1; LC-MS Rt 11.81 min, m / z 551.2736 [M + H] +

(S) -N- (2- (2-cyano-4,4-difluoropyrrolidin-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonyl-piperazine-1-yl) -1-propoxy) quinoline-4-carboxamide was performed to give 13.2 mg (22.4 µmol; 75%). LC-MS Rt 11.84 min, m / z 605.2610 [M + H] +

N- (2- (2-Cyano-4,4-difluoropyrrolidine-1-yl) -2-oxoethyl) -6- (3- (4-Boc-piperazine-1-yl) propyl-1- (methyl) amino) quinoline-4-carboxamide was performed to give 1.17 mg (1.95 µmol; 92%). LC-MS Rt 12.66 min, m / z 600.3057 [M + H] +

FAP1-02 (2) (S) -N- (2- (2-cyanopyrrolidine-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonyl-piperazine-1-yl) -1-Propoxy) Quinoline-4-carboxamid (11) 4.85 mg (8.80 mmol) is dissolved in 1 mL of acetonitrile, and 4.2 mg (22.0 µmol) of 4-methylbenzenesulfonic acid monohydrate is added thereto. .. The reaction is shaken overnight at 45 ° C. and then the volatiles are removed under reduced pressure. After incorporating the residue into 190 µL of dimethylformamide and 10 µL of triethylamine (7.3 mg; 72 µmol), 6.77 mg (12.9 mmol) of DOTA-p-nitrophenol ester is added. The reaction mixture is diluted with 1 mL of water, shaken for 2 hours and then purified by HPLC. After lyophilization, 5.04 mg (6.02 µmol; 68%) is obtained. ¹H NMR (600 MHz, D₂O) 9.02, 8.23, 8.07, 7.87, 7.83, 4.85, 4.45, 4.41, 4.40, 4.39, 3.83, 3.67, 3.50, 3.49, 2.40, 2.38, 2.36, 2.26, 2.22, 2.16; ¹³C NMR (150 MHz, D₂O) 167.9, 159.1, 147.2, 141.8, 135.4, 127.9, 127.2, 119.8, 119.0, 104.5, 65.8, 54.1, 46.8, 46.1, 42.1, 29.2, 24.5, 23.0; LC-MS Rt 8.37 min, m / z 837.3872 [M + H] +

The protocol before FAP1-04 was performed to obtain 3.97 mg (4.55 µmol; 57%). LC-MS Rt 8.80 min, m / z 873.3664 [M + H] +

The protocol prior to FAPI-42 was performed to give 1.91 mg (2.47 μ mol; 88%). LC-MS Rt 9.37 min, m / z 386.6807 [M + 2H] 2+

The protocol prior to FAPI-46 was performed to give 39.21 mg (44.3 μ mol; 85%). LC-MS Rt 9.03 min, m / z 443.7196 [M + 2H] 2+

FAPI-19 (S) -N- (2- (2-cyano-4,4-difluoropyrrolidin-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonylpiperazin-1-yl) -1-propoxy) quinoline-4-carboxamide 1.09 mg (1.86 μ mol) was pre-activated with 2.13 mg (5.62 μ mol) HBTU and 2.50 μ L (1.85 mg; 14.3 μ mol) DIPEA to FAPI- Boc-protected by the method applied to 02, bis ((1- (2- (tert-butoxy) -2-oxoethyl) -1H-imidazol-2-yl) methyl) glycine 2.74 mg (5.91 μ mol) Reacted with. After HPLC purification and solvent removal, the residue was treated with 200 μ L of 2.5% trifluoromethanesulfonic acid in 1: 1 acetonitrile / trifluoroacetic acid. After precipitation with diethyl ether and HPLC purification, 1.06 mg (1.29 μ mol; 70%) of the title compound was obtained. LC-MS Rt 8.91 min, m / z 820.2933 [M + H] +

A solution of 1.00 μ L (0.74 mg; 5.73 μ mol) of FAPI-28DIPEA, 0.95 mg (1.16 μ mol) of FAPI-19, 0.42 mg (3.14 μ mol) of HOBt and 1.10 mg (2.89 μ mol) of HBTU in 50 μ L of DMF. Was added to. After 10 minutes, 2.30 mg (5.34 μ mol) of H-Asn (Trt) -OtBu was added and reacted for 120 minutes. The tert-butyl protecting group was removed with 2.5% TFOH in 8: 2 TFA / acetonitrile. After HPLC-purification and lyophilization, 0.79 mg (0.75 μ mol; 65%) of the title compound was obtained. LC-MS Rt 9.23 min, m / z 524.7100 [M + 2H] 2+

The protocol prior to FAPI-34 was performed to give 1.01 mg (0.87 μ mol; 52%). LC-MS Rt 8.87 min, m / z 583.6988 [M + 2H] 2+

FAPI-60 (S) -N- (2- (2-cyano-4,4-difluoropyrrolidin-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonylpiperazin-1-yl) -1-propoxy) quinoline-4-carboxamide 3.91 mg (6.66 μ mol) was deprotected with 50 μ L of acetonitrile and 100 μ L of trifluoroacetic acid for 30 minutes. Acetyl-Cys (Trt) -Gly-Cys (Trt)-in 150 μ L of dimethylformamide and 2.50 μ L (1.85 mg; 14.3 μ mol) of DIPEA, after evaporating the solvent and washing with diethyl ether and pre-incubating for 10 minutes. A mixture of Gly-OH 8.02 mg (9.27 μ mol), HOBt 4.31 mg (31.9 μ mol) and HBTU 4.47 mg (11.8 μ mol) was added to the residue and reacted for 120 minutes. After HPLC-purification and lyophilization, 4.66 mg (3.49 μ mol; 52%) of the title compound S-trityl protected was obtained. 3.36 mg (2.52 μ mol) of the trityl protective compound was dissolved in 50 μ L of acetonitrile. 3 μ L of triethylsilane and 100 μ L of trifluoroacetic acid were added and reacted for 30 minutes. After HPLC purification and lyophilization, 2.01 mg of the title compound (2.36 μ mol; 94%; 49% over two steps) was obtained. LC-MS Rt 10.26 min, m / z 871.2703 [M + Na] +

The protocol prior to FAPI-69 was performed to give 0.59 mg (0.60 μ mol; 39%). LC-MS Rt 10.25 min, m / z 991.3490 [M + H] +

The protocol prior to FAPI-70 was performed to give 0.61 mg (0.54 μ mol; 33%). LC-MS Rt 10.14 min, m / z 1120.3884 [M + H] +

The protocol prior to FAPI-71 was performed to give 0.79 mg (0.66 μ mol; 34%). LC-MS Rt 10.17 min, m / z 596.7075 [M + 2H] 2+

Atto488-FAPI-02 (14) compound 11 0.66 mg (1.20 μ mol) was treated at 45 ° C. with 1.33 mg (6.96 μ mol) of 4-methylbenzenesulfonic acid monohydrate in 250 μ L of acetonitrile for 4 hours. .. After removing the solvent, the residue is dissolved in 95 μ L of dimethylformamide and 5 μ L of triethylamine (3.65 mg; 36.1 μ mol). Atto 488 NHS-ester 0.54 mg (0.55 μ mol) in 25 μ L of DMSO was added. After 60 minutes, 0.49 mg (0.43 μ mol; 78%) of the title compound was isolated by HPLC and lyophilized. LC-MS Rt 10.19 min, m / z 1022.2706 [M] +

FAPI-73 (S) -N- (2- (2-cyano-4,4-difluoropyrrolidine-1-yl) -2-oxoethyl) -6- (3- (4-tert-butoxycarbonylpiperazin-1-yl) -1-propoxy) quinoline-4-carboxamide 10.95 mg (18.7 μ mol) was deprotected with 100 μ L of acetonitrile and 200 μ L of trifluoroacetic acid for 30 minutes. After evaporating the solvent and washing with diethyl ether, N, N, N-trimethyl-5-((2,3,5,6-tetrafluorophenoxy) -carbonyl) pyridin-2-aminium chloride 15.02 mg (9.27 μ mol) was added and the mixture was dissolved in 200 μ L of dimethylformamide and 10.0 μ L of triethylamine (7.30 mg; 72.3 μ mol). After 120 minutes, the mixture was purified by HPLC and lyophilized to give 11.24 mg (14.7 μ mol; 79%) of the title compound. LC-MS Rt 9.37 min, m / z 649.2892 [M-CF₃CO₂] +

The protocol prior to FAPI-72 was performed to give 9.80 mg (12.6 μ mol; 70%). LC-MS Rt 9.28 min, m / z 662.3237 [M-CF₃CO₂] +

General conjugation of side chain protected Fmoc-amino acids (S) -N- (2- (2-cyano-4,4-difluoropyrrolidin-1-yl) -2-oxoethyl) -6- (3- (4- (4- (γ, γ-di-tert-butyl) -L-carboxy-glutamylpiperazin-1-yl) -1-propoxy) Kinolin-4-carboxamide (S) -N- (2- (2-cyano-4,4) -Difluoropyrrolidine-1-yl) -2-oxoethyl) -6- (3- (1-tert-butoxycarbonyl-piperidine-4-yl) -1-propoxy) quinoline-4-carboxamide 14.04 mg (23.9 μ mol)) Was dissolved in 50 μ L of acetonitrile and 100 μ L of trifluoroacetic acid. After 10 minutes, the volatiles were removed and the residue was washed with diethyl ether. Fmoc-L-Gla (tBu) 2-OH 14.95 mg (28.4 μ mol), HOBt 7.74 mg (57.4 μ mol), HBTU 13.46 mg (35.5 μ mol) and DIPEA 20.0 μ L (14.8 mg) in 200 μ L of dimethylformamide; A solution of 115 μ mol) was added to the dried residue. After 60 minutes, 50.0 μ L (50.4 mg; 578 μ mol) of morpholine was added and the product was isolated by HPLC after 30 minutes. After lyophilization, 15.95 mg (20.7 μ mol; 86%) of the title compound was obtained. LC-MS Rt 12.85 min, m / z 772.3643 [M + H] +

FAPI-75 (S) -N- (2- (2-cyano-4,4-difluoropyrrolidine-1-yl) -2-oxoethyl) -6- (3- (4- (γ, γ-di-tert) -Butyl) -L-carboxyglutamylpiperazin-1-yl) -1-propoxy) Quinoline-4-carboxamide 3.37 mg (4.37 μ mol) and NOTA-p-nitrophenol 4.52 mg (10.7 μ mol) in dimethylformamide It was dissolved in 100 μ L and 10.0 μ L of triethylamine (7.30 mg; 72.3 μ mol). After HPLC-purification and lyophilization, the intermediate compound was deprotected by incubating in a solution of 50 μ L of acetonitrile, 100 μ L of trifluoroacetic acid, 2.5 μ L of triisopropylsilane and 2.5 μ L of water for 60 minutes. After HPLC-purification and lyophilization, 2.62 mg (2.77 μ mol; 63%) was obtained. LC-MS Rt 9.38 min, m / z 945.3668 [M + H] +

FAPI-77-Precursor A general active ester modification protocol was performed to give 3.23 mg (3.06 μ mol; 73%). Note: After SPE, the tert-butyl protecting group was removed after solvent evaporation by radioactive fluorination, HPLC-purification and 3 minute treatment with pure TFA at 95 ° C. LC-MS Rt 16.02 min, m / z 1219.5858 [M + H] +

2- (2- (4,7,10-Tris (2- (tert-butoxy) -2-oxoethyl) -1,4,7,10-tetraazacyclododecane-1-yl) acetoxy) Tris-tBu acetate -DOTA 28.99 mg (50.6 μ mol), cesium carbonate 90.65 (278 μ mol) and 2-bromoacetate benzyl 10.28 μ L (15.0 mg; 65.5 μ mol) were suspended in 300 μ L of dimethylformamide and shaken for 2 hours. The product was isolated by HPLC, lyophilized and dissolved in 25 mL of 10% acetic acid in methanol. 50 mg of 10% Pd / C and hydrogen (atmospheric pressure) were added. After 2 hours, the solvent was removed and the title compound was isolated by HPLC. After lyophilization, 25.19 mg (39.9 μ mol; 79%) of the title compound was obtained. LC-MS Rt 14.14 min, m / z 631.4784 [M + H] +

tBu-FAPI-79 (S) -N- (2- (2-cyano-4,4-difluoropyrrolidin-1-yl) -2-oxoethyl) -6- (3- (1-tert-butoxycarbonyl-piperidine) -4-yl) -1-propoxy) quinoline-4-carboxamide (2.00 mg (3.41 μ mol)) was dissolved in 50 μ L of acetonitrile and 100 μ L of trifluoroacetic acid. After 10 minutes, the volatiles were removed and the residue was washed with diethyl ether. 2- (2- (4,7,10-Tris (2- (tert-butoxy) -2-oxoethyl) -1,4,7,10-tetraazacyclododecane-1-yl) acetic acid) Acetic acid 4.20 mg (6.60 μ mol) and 3.35 mg (8.84 μ mol) of HBTU were dissolved in 100 μ L of dimethylformamide, 10.0 μ L of DIPEA (7.40 mg; 57.4 μ mol) was added to the dried residue and allowed to react for 60 minutes. After HPLC purification and lyophilization, 2.26 mg (2.06 μ mol; 60%) of the title compound was obtained. LC-MS Rt 12.98 min, m / z 1099.7481 [M + H] +

FAPI-79tBu-FAPI-79 2.26 mg (2.06 μ mol) was dissolved in 25 μ L of acetonitrile and 100 μ L of trifluoroacetic acid and shaken at 35 ° C. for 30 minutes. After evaporating the solvent, the product was isolated by HPLC. After lyophilization, 1.58 mg (1.70 μ mol; 82%) of the title compound was obtained. LC-MS Rt 8.84 min, m / z 466.2737 [M + 2H] 2+

Analysis of Compounds Reversed Phase High Performance Liquid Chromatography (RP-HPLC) is a linear gradient of an aqueous acetonitrile solution on a Chromolith Performance RP-18e column (100 x 3 mm; Merck KGaA Darmstadt, Germany) (0-100% acetonitrile per 5 minutes). , 0.1% TFA, flow rate 2 mL / min). UV-absorbance was detected at 214 nm. An additional γ-detector was used for HPLC analysis of the radioactive compounds. Characterization of HPLC-MS was performed on an ESI mass spectrometer connected to an Agilent 1200 HPLC system equipped with a Hypersil Gold C18 1.9 μ m column (200 x 2.1 mm, 0-100% acetonitrile per 20 minutes, flow rate 200 μ L / min). (Exacte, Thermo Fisher Scientific, Waltham, MA, USA) was used. Radio-HPLC for analysis was performed using a Chromolith Performance RP-18e column (100 x 3 mm; Merck; 0-30% acetonitrile per 10 minutes; flow rate 2 mL / min). HPLC-purification was performed on a LaPrep P110-system (Knauer, Berlin, Germany) and a Reprosil Pur 120 column (C18-aq 5 μ m 250 x 25 mm;

Dr. Maisch, Ammerbuch-Entringen, Germany). The water / acetonitrile-gradient (15 or 25 min; 0.1% TFA; flow rate 20 mL / min) was varied for individual products.

Radiochemical Radioiodine (I-125) was purchased from Hartmann Analytik (Goettingen, Germany); Radioactive lutetium (Lu-177) was obtained from ITG (Muenchen, Germany); Radiogallium (Ga-68) was obtained from Themba Labs. Elution was performed from a Ge-68 / Ga-68 generator purchased from (Somerset West, South Africa). Tc-99m was eluted from a Mo-99 / Tc-99m generator (Curium Pharma, Berlin, Germany). Cu-64 was provided by UKT Tuebingen (Tuebingen, Germany). Sm-153 was provided by DSD Pharma (Purkersdorf, Austria). Pb-203 was provided by Lantheus (N. Billerica MA, USA). F-18-FDG and F-18-fluoride were provided by ZAG Zyklotron AG (Eggenstein, Germany). The CRS kit for tricarbonyl was obtained from Paul Scherrer Institut (Villingen-PSI, Switzerland).

In iodination, 10 µL of the organotin precursor of FAPI-01 (1 µmol / mL in ethanol) was diluted with 10 µL of 1 M hydrochloric acid and 10 µL of water, and then 1-20 MBq of iodine-125 in 0.05 M NaOH was added. The reaction was initiated by adding 5 µL of a fresh 1.9% peracetic acid solution in glacial acetic acid. After 60 seconds, 15 µL of 1 M NaOH was added, and the reaction was stopped by adding 5 µL of an aqueous solution of 5% ascorbic acid, and then HPLC purification was performed. The resulting solution can be used directly for in vitro experiments or, in the case of animal studies, evaporated to dryness under reduced pressure and incorporated into 0.9% NaCl (Braun, Melsungen, Germany) for use.

Cu-64, Lu-177 and Pb-203 labels for DOTA-compounds add 5 MBq of radionuclide to 100 µL of a 10 µM solution of individual precursors in 0.1 M NaOAc (pH 5) and incubate at 95 ° C. for 10 minutes. This solution is used directly for in vitro experiments or diluted with 0.9% NaCl (Braun, Melsungen, Germany) for biodistribution studies. In mouse imaging studies (scintigraphy, PET), radiotracers were post-treated with solid-phase extraction (sep-pak light C18, Waters).

Prior to Tc (I) labeling, 1 mL of Tc-99m-pertechnetium acid salt in 0.9% saline was added to the CRS kit and incubated for 20 minutes. After cooling to room temperature, a mixture of 25.0 µL of precursor (1 mM in water), 150 µL of phosphate buffer (0.4 M, pH 7.4) and 240 µL of hydrochloric acid (1.0 M) is added and the final mixture is added as needed. The pH was adjusted to 5. This reaction was carried out at 95 ° C. for 20 minutes and post-treated by solid phase extraction (sep-pak light C18, Waters). In in vivo experiments and animal studies, after Tc (VII) reduction, labeling was performed with 1/5 of the reagent and 200 µL of CRS kit solution.

Prior to Tc (V) labeling, 30 µL of SnCl₂ solution containing 200 mM glucoheptonate was incubated with 200 µL of Tc-99 m-pertechnetium acid in 0.9% saline for 10 minutes at room temperature. 5.00 µL of precursor (1 mM in water) and 3.75 µL of sodium hydroxide solution (0.1 M in water) were added and the final mixture was reacted at 95 ° C. for 20 minutes. In a mouse imaging study (scintigraphy), the radiotracer was post-treated with solid-phase extraction (sep-pak light C18, Waters).

Labeling with Ga-68 for animal studies is 250 µL of generator eluate (0.6 M HCl, about 230 MBq) at 95 ° C., 1 nmol of DOTA-precursor, 1 µL of 20% ascorbic acid aqueous solution and 72 µL of NaOAc (2.5 M). This was done by incubating with the mixture of. The remaining free radioactivity was removed by dilution with 2 mL of water, solid phase extraction (sep-pak light C18, Waters), washing with 2 mL of water, and elution with 1: 1 water / ethanol 1 mL. The resulting solution was evaporated to dryness under reduced pressure and the residue was incorporated into 0.9% NaCl (Brown).

In the formation of the AIF-NOTA complex, F-18 fluoride was trapped in a water Sep-Pak QMA plus light cartridge (adsorbent 46 mg, pretreated with 0.5 M NaOAc, pH 3.9), washed with water and washed with water. It was eluted with 500 µL of 1M NaOAc (pH 3.9). For animal studies, 150 µL of eluate was pre-incubated with 2 µL of AlCl₃ solution (10 mM in water) and 50 µL of DMSO. After 5 minutes, the mixture was added to 40 nmol of NOTA-precursor (10 µL of 4 mM aqueous solution) and 1 µL of 20% ascorbic acid aqueous solution. The solution was reacted at 95 ° C. for 15 minutes. The product was isolated by HPLC (0-20% acetonitrile per 10 minutes), solvent removed, incorporated into 0.9% saline and then injected.

In the formation of 6-fluoronicotinamide, F-18 fluoride was trapped in a Waters Sep-Pak QMA plus light cartridge (adsorbent 46 mg; pretreated with 0.5 M KHCO₃), washed with water, dried and 450 µL of acetonitrile. And eluted with a mixture of cryptofix 222 7.50 mg (19.9 µmol) and KHCO 31.99 mg (1.99 µmol) in 50 µL of water. After removing the solvent, the residue was dried by azeotropic distillation with 3 x 1 mL of acetonitrile. This residue was incorporated into 100 µL of 1: 1 tert-butanol / acetonitrile and added to 1 mg

(about 1.3 μmol) of the trimethylpyridine-2-aminoium precursor. The solution was reacted at 75 ° C. for 10 minutes. The product was isolated by HPLC (0-30% acetonitrile per 10 minutes), solvent removed, incorporated into 0.9% saline and then injected.

Instead, 6-fluoronicotinamide traps F-18 fluoride in a Waters Sep-Pak QMA plus light cartridge (46 mg adsorbent; pretreated with 0.5 M KHCO_3), washed with acetonitrile, dried and dried. Synthesized by elution with 0.5 mg (approximately 0.4-0.6 μmol) of (protected) FAPI-precursor in 0.5 mL of methanol. The solvent was removed under reduced pressure and the residue was incorporated into 100 μL of 1: 4 acetonitrile / tert-butanol. After 20 minutes at 70 ° C., the reaction mixture was diluted with water and the protected intermediate was post-treated with solid phase extraction (sep-pak light C18, Waters). The solvent was removed and 200 μL of trifluoroacetic acid was added to the residue. The mixture is heated to 95 ° C. for 3 minutes, dried under reduced pressure, diluted with water and then the product is isolated by HPLC, which in the case of compounds lacking protecting groups, directly with the diluted reaction mixture. It was conducted. For animal studies, the product was desolvated, incorporated into 0.9% saline and then injected (uncorrected radiochemical yield of about 25%).

In measuring stability in human serum, radioactively labeled compounds (about 2.5 MBq for I-125, about 15 MBq for Lu-177) were purified (HPLC or solid phase extraction) to remove the solvent. The residue was incorporated into 250 μL of human serum (Sigma-Aldrich) and incubated at 37 ° C. Samples were precipitated with 30 μL of acetonitrile and analyzed by HPLC (0-30% acetonitrile per 10 minutes).

Example 2: In vitro characterization of FAPI derivatives In vitro binding studies include human tumor cell lines BxPC3, Capan-2, MCF-7 (purchased from Sigma Aldrich Chemie GmbH) and SK-LMS-1 (purchased from ATCC) and stable. FAP-cell line HT-1080-FAP, HEK-muFAP and CD26 expressing cell line HEK-CD26 (obtained from Stefan Bauer, NCT Heidelberg) transfected into. All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum under 37 ° C./5% carbon dioxide. In the fluorescence internalization experiment, cells were seeded on a cover glass, stained with FAPI-02-Atto488 and DAPI, and subjected to cell nucleus staining. Images were acquired with a laser scanning confocal microscope using a 63x oil immersion objective lens. Radioligand binding studies were performed using HT-1080-FAP cells. Radiolabeled compounds were added to cell cultures and incubated at different time intervals ranging from 10 minutes to 24 hours. Competitive experiments were performed by simultaneous exposure to unlabeled compounds (10-5M-10-9M) and radiolabeled compounds for 60 minutes. In the spill experiment, after incubating for 60 minutes, the radioactive medium was removed and replaced with non-radioactive medium at time intervals ranging from 1 to 24 hours. In internalization experiments, surface binding activity was removed by incubating cells in 1M glycine-HCl buffer for 10 minutes. Radioactivity was measured using a γ counter, normalized to 1 mio cells and calculated as a percentage of applicable dose (% ID).

In cell staining and microscopic internalization experiments, HT-1080-FAP cells and HEK muFAP cells were seeded on uncoated coverslips of 24-well plates and cultured in 10% fetal bovine serum-containing medium for final. The concentration was about 80-90%. The medium was removed and washed twice with 0.5 mL of PBS at pH 7.4. FAPI-02-Atto488 (20 μM in DMEM) was added to the cells and incubated at 37 ° C. for 2 hours. Cells were washed 3 times with 0.5 mL of PBS at pH 7.4 and fixed with paraformaldehyde (2% in PBS) for 15 minutes. Overgrown coverslips were placed on glass slides using DAPI-containing mounting medium for cell nucleus staining (Fluoroshield, Sigma-Aldrich). The image shows a Zeiss Plan-Apochromat 63x / 1.4 oil DIC III immersion objective (63x /) for each fluorescent dye molecule used (488 for FAPI-02-Atto488, 405 nm for DAPI). Obtained on a laser scanning confocal microscope (Zeiss LSM 700; Zeiss, Oberkochen, Germany) with an xy pixel setting of 0.099x0.099 μm and a pinhole size of 1 Airy using 1.4 Oil DIC III immersion objective. Photos were consistently processed using Z ZEN 2008 software and ImageJ.

Radioligand Binding Test In the radioligand binding test, cells were seeded on a 6-well plate and cultured for 48 hours to a final approximately 80-90% confluence (1.2-2 mio cells / well). The medium was replaced with 1 mL of fresh medium without fetal bovine serum. Radiolabeled compounds were added to cell cultures and incubated at different time intervals ranging from 10 minutes to 24 hours. Competitive experiments were performed by simultaneous exposure to unlabeled (10-5M-10-9M) and radiolabeled compounds for 60 minutes. In the spill experiment, after incubating for 60 minutes, the radioactive medium was removed and replaced with non-radioactive medium at time intervals ranging from 1 to 24 hours. In all experiments, cells were washed twice with 1 mL of phosphate buffered saline at pH 7.4, followed by lysis of 1.4 ml of lysis buffer (0.3 M NaOH, 0.2% SDS). Radioactivity was measured using a counter (Cobra II, Packard), normalized to 1 mio cells and calculated as a percentage of applicable dose (% ID). Each experiment was performed 3 times and repeated 3 times for each independent experiment.

In internalization experiments, cells were incubated with radiolabeled compounds for 60 minutes at 37 ° C and 4 ° C. Cell uptake was terminated by removing the medium from the cells and washing twice with 1 mL of PBS. The cells were then incubated with 1 mL of glycine-HCl (1M in PBS, pH 2.2) for 10 minutes at room temperature to remove surface binding activity. The cells were washed with 2 mL of ice-cold PBS, lysed with 1.4 mL of lysis buffer, and the internalized fraction (internalized fraction) was measured. For cells incubated at 4 ° C., all wash and elution steps were performed using ice-cooled buffer. Radioactivity was measured using a γ counter, normalized to 1 mio cells and calculated as a percentage of applicable dose (% ID).

FAPI-01 selectively targets human and mouse FAP- α . To analyze the binding properties of FAPI-01 to its target protein, a radioligand binding assay was performed on different cancer cell lines and on CD26, a closely related membrane protein also known as human and mouse FAP and DPPIV. The transfection was performed using a cell line. Both mouse FAP and CD26 showed high homology with human FAP- α (muFAP: 90% identity and 94% similarity at the amino acid level; CD26: 52% identity and 71%. Structurally high similarity) (Kelly T., Drug Resist Updat, 2005).

As shown in FIG. 1A, FAPI-01 did not significantly bind to FAP-negative cancer cell lines and targeted human and mouse FAP- α expressing cells with high affinity (IC50 human FAP- α = 39.4 nM). In addition, no substantial binding to CD26-expressing cells was observed ($0.05 \pm 0.01\%$), demonstrating that FAPI-01 selectively targets FAP- α . This is particularly important because CD26 is highly expressed in various normal tissues, including the kidney, liver and small intestine. In order to avoid high background signals due to non-specific CD26 binding, the high selectivity of the ligand for FAP- α is a great advantage, resulting in optimal image quality.

FAPI-01 rapidly internalized into FAP-positive cells, but showed time-dependent outflow and robust deiodination. Cell-based internalization assays show rapid uptake of FAPI-01 into cells (Fig. 1B). After 10 minutes of incubation, 95% of the fully bound fraction is located intracellularly ($19.70 \pm 0.28\%$ total). Even after 4 hours, a limited decrease in activity was observed ($17.00 \pm 0.40\%$ total, 94% of which was internalized).

Iodine-labeled compounds often exhibit time-dependent enzymatic deiodination. This was also observed in FAPI-01, resulting in a decrease in intracellular radioactivity of this compound after a longer incubation time ($3.25 \pm 0.29\%$ after 24 hours). Deiodination can be minimized by reducing the activity of the deiodination enzyme after lowering the temperature to 4 ° C, resulting in $26.66 \pm 1.59\%$ radioactivity after 24 hours. increased.

FAPI-02 shows enhanced binding and uptake to human FAP- α as compared to FAPI-01. In order to avoid the rapid loss of FAPI-01 activity due to enzymatic deiodination, a non-halogen derivative FAPI-02 was designed in which the FAP binding moiety was chemically bound to the chelating agent DOTA. This modification, in addition to the resulting enhanced stability, provided the possibility of easily incorporating either a diagnostic or therapeutic radionuclide, allowing FAPI-02 to be used as a non-flammable compound. FAPI-02, similar to this iodinated analog, specifically binds to human and mouse FAP- (IC50 human FAP- α = 21 nM) -expressing cells without addressing CD26 (% ID = 0). $.13 \pm 0.01\%$; FIG. 1A). FAPI-02 was rapidly internalized in FAP- α expressing cells ($20.15 \pm 1.74\%$ ID after 60 minutes, 96% of which was internalized; FIG. 1B), which became more stable over time. It shows a higher uptake rate. Only 5% activity remains after 24 hours compared to binding after 10 minutes incubation of FAPI-01. On the other hand, the initial radioactivity of FAPI-02 was detected at 34% after 24 hours of incubation. Spill experiments demonstrated that FAPI-02 was removed significantly later than FAPI-01, indicating that after 24 hours it retained 12% of the originally accumulated radioactivity. (FAPI-01 retains 1.1% ID after 24 hours; FIG. 1E).

Robust internalization of FAPI-02 into human and mouse FAP- α expressing cells was confirmed by fluorescence laser scanning microscopy. For this purpose, HT-1080-FAP and HEK-muFAP cells were stained with a fluorescently labeled FAPI-02 derivative (FAPI-02-Atto488) for 1-2 hours. As shown in FIG. 1D, the compound is completely internalized and accumulated inside FAP- α expressing cells, but no uptake has been detected in FAP- α negative HEK-CD26 cells.

Designing FAPI Derivatives with Improved Binding Properties and Pharmacokinetics A further variant of FAPI-02 was designed to increase tumor retention time with the aim of developing seranostic FAP-targeting agents. .. Variants FAPI-03 to FAPI-15 were characterized with respect to target binding, internalization rate and target specificity. The result is shown in FIG.

Example 3: PET Imaging and Biodistribution Analysis of Mice All experiments were performed in accordance with the German Animal Protection Act and were compliant with European Commission regulations for the care and use of laboratory animals. Mice were anesthetized by inhaling isoflurane.

In an in vivo experiment, 8-week-old BALB / c nu / nu mice (Charles River) were subcutaneously inoculated into the right stem at 5×10^6 , respectively, with HT-1080-FAP, Capan-2 or SK-LMS-1 cells. When the tumor size reached about 1 cm³, the radiolabeled compound was injected via the tail vein (about 10 MBq for small animal PET imaging, about 1 MBq for organ distribution). PET imaging was performed up to 140 minutes after intravenous injection of approximately 1 MBq of Ga-68 labeled compound per mouse using an Inveon PET small animal PET scanner (Siemens). Images were iteratively reconstructed using the 3D-OSEM + MAP method and converted to standardized capture value (SUV) images. Quantified using ROI technology and shown as SUVmen. In the organ distribution of Lu-177 labeled compound (about 10 MBq per mouse), animals (n = 3 at each time point) were sacrificed after the indicated time points (30 minutes to 24 hours). Distributed radioactivity was measured in all dissected organs and blood using a γ -counter (Cobra Autogamma, Packard). Values are shown as a percentage of the injected dose per gram of tissue (% ID / g).

In pharmacokinetic modeling, transport constants K1 and rate constants k2-k4 are implemented in PMOD software [4], taking into account the vascular fraction (vB) associated with the volume of blood exchanging with tissues within the VOI. 2-Tissue compartment model was used for calculation. The rate constants that describe compartmental flux include k1 (binding to receptors), k2 (exfoliation) and k3 (internalization) and k4 (spill) within the tumor tissue. In this model, the fractional volume of the distribution ($DV = K1 / k2$) is the proportion of the region of interest in which the 150-labeled water is distributed.

FAP α variants are also accumulated in human FAP-expressing xenografts and non-FAP-expressing xenografts by recruiting and activating mouse fibroblasts. Tumor accumulation of FAP α -02 and -04 was assessed by small animal PET imaging of mice with xenografts from both human FAP positive and negative tumor cells. In both cases, the radiotracer was rapidly concentrated in the tumor and maintained for at least 140 minutes (FIGS. 3A, C, E, G). At the same time, FAP α -02 and -04 show negligibly low non-specific binding, which is rapidly removed from the blood, mainly via the kidneys and bladder, resulting in low background. And results in a beneficial tumor-to-organ ratio. Simultaneous administration of unlabeled compounds as competitors completely eliminated the radioactivity of the tumor, demonstrating the specificity of the radiotracer for the target protein (Fig. 4). Interestingly, high FAP α -02 due to recruitment and activation of activated mouse fibroblasts in mice bearing FAP α positive (HT-1080-FAP) and FAP α negative (Capan-2) tumor cell lines. Tumor uptake was observed. Table 6 shows the pharmacokinetic properties of the radiotracer calculated from PET data using the 2-tissue compartment model by Burger et al., Nucl Med, 1997.

Table 6. Pharmacokinetic properties of 68Ga-FAP α -02 calculated from PET data using a 2-tissue compartment model by Burger et al., Nucl Med, 1997. vB: Vascular fraction associated with the volume of blood exchanging with tissue within the VOI (volume of interest); k1-k4: calculated rate constant; Vs: ratio of specific binding concentration to total parent in equilibrium; Vt: Total volume of distribution

These observations were confirmed in biodistribution studies using 177Lu-FAP α -02 and -04 and showed very low activity in all other organs, as well as in both human FAP α positive and negative tumors. It demonstrated rapid tumor accumulation (see Table 7 for quantified uptake values), resulting in a beneficial tumor-to-organ ratio (Fig. 5DF). Similar results were obtained with 177Lu-FAP α -04 in mice bearing HT-1080-FAP tumors. Compared to FAP α -02, FAP α -04 shows higher tumor uptake, especially after 24 hours (Fig. 5C). The calculation of the area under the curve (AUC) is shown in Table 8.

Table 7. Quantification of biodistribution data 1 hour after intravenous administration of Lu-177 labeled FAP α -02 and -04 to tumor-bearing Balb / c nude mice; n = 3; reported values as average. % ID / g $\pm$ SD

Table 8. Tumor uptake of selected FAPI derivatives in nude mice bearing HT-1080-FAP tumors, n = 3. Values are reported as average (ID / g $\pm$ SD).

Example 4: Clinical PET / CT study Imaging of more than 100 patients is subject to renewal of the Declaration of Helsinki §37 (unproven intervention in clinical practice) in accordance with German Pharmaceutical Law §13 (2b). For medical reasons, either 68Ga-FAPI-02 or -04 was used, followed by intravenous follower administration (20 nmol, 122-336MBq) 10 minutes, 1 hour, and 3 hours later. The variation in the activity of the injected radiotracker is due to the short half-life of 68Ga and the variable elution efficiency obtained during the lifetime of the 68Ge / 68Ga generator. One patient was injected with 18F-FDG intravenously at 358 MBq and FDG imaging was performed 1 hour later. PET / CT scans were performed with a Biograph mCT FlowTM PET / CT-Scanner (Siemens Medical Solution) using the following parameters: slice thickness 5 mm, increments 3-4 mm, soft tissue reconstruction kernel, care dose. Immediately after the CT scan, whole body PET was obtained with FlowMotionTM 3D (matrix 200x200) at 0.7 cm / min. Emission data were corrected for random, scatter, and decay. The reconstruction was performed with a 2-repetition / 21 subset of ordered subset expected value maximum (OSEM) algorithm and Gaussian filtered to a horizontal axis resolution of 5 mm full width at half maximum (FWHM). Attenuation correction was performed using low-dose unemphasized CT data. Quantitative evaluation of standardized uptake values (SUVs) was performed using region of interest technology.

FAPI-02 and -04 rapidly accumulate in human metastatic breast cancer, pancreatic cancer, lung cancer, HNO cancer, small intestine cancer and ovarian cancer. Diagnostic PET / CT scans were performed on patients with metastatic breast cancer, lung cancer, pancreatic cancer, HNO cancer, small intestine cancer, and ovarian cancer 1 hour after intravenous administration of 68Ga-FAPI-02 and -04. Robust accumulation of followers was observed in primary tumors as well as in lymph nodes and bone in all patients, with a maximum SUV value of 48.0 metastases. In contrast, follower uptake into normal tissue was very low (Fig. 6-14). Radioactivity was rapidly removed from the bloodstream and most was excreted via the kidneys, resulting in high contrast images. Comparative imaging in one patient with locally advanced lung adenocarcinoma revealed a clear advantage of FAPI-02 over the commonly used PET tracker 18F-FDG. As shown in FIG. 9, FAPI-02 shows high uptake with lower background activity, which leads to better contrast of metastatic lesions with better visibility. FAPI-02 selectively targets tissues expressing FAP- α , unlike FDG, which accumulates in large amounts in cells that consume a large amount of glucose, such as the brain. Comparative image processing in a single prostate cancer patient reveals a clear advantage of FAPI-04 over the commonly used PET trackers 68Ga-DOTACTOC and 68Ga-PSMA, which goes to the kidney. Allowed detection of smaller tumor lesions with reduced follower accumulation (Fig. 14).

Discussion Reliable diagnosis of primary tumors, metastatic lesions and developed lymph nodes is paramount to enabling effective and suitable therapies that are planned to include tumor staging and treatment choices. It is a thing. To this end, image processing techniques are an essential tool for the diagnosis of many types of cancer. The PET / CT combination is the latest choice for tumor diagnosis because of its high diagnostic accuracy and the potential to evaluate both anatomical and physiological details. However, PET / CT combinations have a high affinity for target structures whose expression in tumors is enhanced compared to normal tissues, as opposed to non-invasive imaging techniques such as MRT and CT alone. Requires the use of a radioactive tracker with sex. The ideal tracker needs to specifically bind to its target protein to ensure a reliable distinction between cancerous and healthy tissue as well as a low background signal that results in a high contrast image. Affinity and peculiarity when the radiotracker exhibits a theranostic compound, i.e., which provides the possibility of carrying either a diagnostic or therapeutic nuclide that facilitates and improves targeting and personalization procedures. Gender becomes even more important. For the potential application of followers for therapeutic purposes, high target specificity guarantees reduction of side effects, which is especially important for the protection of radiosensitive tissues such as bone marrow, genitals and digestive organs.

With that in mind, the inventors have developed a theranostic chase that targets cancer-related fibroblasts, which form the major components of the tumor stroma. They are known to play important roles in tumor growth, metastasis and progression, and are more genetically stable than cancer cells, making them less susceptible to the development of therapy resistance. CAF expresses certain proteins that can be used as tumor-specific markers, as opposed to normal fibroblasts. These include the membrane protein FAP- α , which is widely expressed in the microenvironment of various tumors, including pancreatic cancer, breast cancer, and lung cancer, which make up the majority of all solid tumors. Allows targeting of various tumor entities.

Radio tracers of FAPI-01 to FAPI-73 were developed by intensively performing chemical modification based on a small molecule enzyme inhibitor having a high affinity for this target protein. All compounds exhibit specific binding of rapid and near-complete internalization to FAP- α in humans and mice without addressing the closely related proteins CD26 / DPP4. Since the iodinated molecule undergoes enzymatic deiodination with the outflow of free iodine, the intracellular radioactivity decreases as the incubation time increases. For this reason, FAPI-02 and subsequent compounds were designed so that the FAP binding moiety was chemically bound to the chelating agent DOTA. The result is a series of theranostic compounds with favorable pharmacokinetic and biochemical properties, of which FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71, FAPI-72 and FAPI-73 are the most preferred ligands. Both FAPI-02 and FAPI-04 were removed significantly later than FAPI-01, and after 24 hours 12% (FAPI-02) and 49% (FAPI-04) of the original accumulated radioactivity were retained (FAPI-04). FAPI-01 is 1.1%), and other preferred compounds have even stronger binding strength (Fig. 16). They rapidly become endogenous to FAP- α -expressing cells and show high tumor uptake rates in both tumor-bearing mice and patients with metastatic epithelial carcinoma. In contrast, normal tissue has no accumulation and clearance from the blood system is fast, resulting in high contrast images. Robust internalization into both human and mouse FAP- α expressing cells was confirmed by confocal microscopy using fluorescently labeled FAPI-02. In contrast to the first generation FAP antibody F19, which is not internalized and has a high affinity for its target protein, FAPI-02 shows complete intracellular uptake after 1 hour of incubation. The mechanism of internalization after FAP binding has been studied by Fisher et al. Using FAP antibody fragments (Fabs) and DyLight 549 anti-mouse antibody in SK-Mel-187 cells. Incubation at 37 ° C. resulted in internalization of the FAP-antibody double complex. Like our small molecule, the internalization process took place rapidly with near complete internalization. Coexistence of early endosome markers and Fab was observed after 20 minutes, and coexistence of late endosome and lysosomal markers and Fab was observed after 40 minutes. Fab-mediated FAP- α internalization is suppressed by inhibitors of dynamin-dependent endocytosis, indicating that endocytosis is caused by a dynamin-dependent mechanism.

FAPI-02 and -04 are not retained in the renal parenchyma and are quickly removed from the organism by renal clearance. Unlike 18F-FDG, which accumulates heavily in glucose-consuming cells, including inflamed tissues or brain, FAPI-02 is selectively concentrated in tissues expressing the target protein. This opens up new perspectives for detecting malignant lesions in these areas. In addition, FAP- α is also expressed by rheumatoid myofibroblast-like synovial cells in patients with rheumatoid arthritis and osteoarthritis, atherosclerosis, fibrosis and ischemic heart tissue after myocardial infarction. Was also shown. These observations suggest the application of FAPI-02 and -04 as imaging trackers for further indications.

A limiting factor for the detection of tumor lesions is the degree of FAP- α expression within the tumor. This largely depends on the number of activated fibroblasts, i.e. the interstitial content, and / or the number of FAP- α molecules per fibroblast that can be determined by the microenvironment. Since the growth of tumors larger than 1-2 mm basically requires the formation of supporting stroma, it is possible to visualize small lesions in the range of 3-5 mm using FAPI-PET / CT. ..

Like other targeting approaches, FAPI derivatives achieve optimal results only in tissues with sufficiently high FAP- α expression, which is known to be fairly heterogeneous in different cancer types and patients. In addition to breast cancer, colon cancer, and pancreatic cancer, which are good candidates for FAPI imaging, it is necessary to investigate whether other tumor entities such as lung cancer, head and neck cancer, ovarian cancer, and liver cancer are preferred targets.

Expression of FAP- α was also demonstrated in wound healing and fibrotic tissue, which should be noted when interpreting radiological findings. These facts underscore the need to adequately assess which patients are likely to benefit from potential FAPI therapy. Given the ability to use either diagnostic or therapeutic nuclides, FAPI-02 and -04 allow easy stratification of the appropriate patient cohort. In any case, it is already clear that both FAPI trackers are ideal candidates for the development of targeted radiopharmaceuticals. Due to their high target affinity, rapid tumor internalization and rapid systemic clearance, they are already ideally suitable for tumor imaging.

Example 5: characterization and clinical evaluation of FAPI in vitro and in vivo All in vitro and in vivo experiments, as well as clinical evaluation of FAPI derivatives, were performed according to Loctev et al. 1 and Lindner et al. 2 as described above. It was. Preliminary dose estimates for FAPI-02 and FAPI-04 were examined 0.2 hours, 1 hour, and 3 hours after injection of the follower using the QDOSE dose estimation software suit. Based on the patient. Further PET / CT scans for tumor patients were taken 1 hour after injection of either FAPI-02 (n = 25) or FAPI-04 (n = 25); for 6 patients, an intra-individually relevant FDG. A scan (also taken one hour

later) was available. For normal tissues of 16 organs, 2 cm of Physical-VOI was placed in parenchyma, and for tumor lesions, SUVmean / max3 was quantified using threshold split VOI.

Incubation characterization of DOTA-FAPI derivatives To evaluate the target binding and internalization rates of DOTA-FAPI derivatives in comparison to FAPI-04, Lu-177-labeled compounds were used with FAP-expressing HT-1080 cells for 1 hour and 4 hours respectively. Incubated for hours and 24 hours (Fig. 16). The membrane-bound fraction was removed by acidic elution with glycine-HCl of pH 2.2, followed by lysis of alkaline cells, and the internalized fraction (internalized fraction) was measured. As shown in FIG. 16, all derivatives have higher cell binding compared to FAPI-04 so that the binding value is 500% of the maximum lead compound after 1 hour of incubation (up to 750% after 4 hours). Shown.

Competitive binding assays were performed with increased concentrations of unlabeled compounds as competitors to Lu-177 labeled compounds to assess target affinity and specificity (FIG. 17; each listed in Table 9). IC50 value).

Table 9. IC50 values of selected FAPI derivatives measured by competitive binding assay

Lu-labeled DOTA-FAPI derivatives were intravenously administered to HT-1080-FAP tumor-bearing mice to analyze the organ distribution pharmacokinetic profile of DOTA-FAPI derivatives in tumor-bearing mice and tumor uptake in vivo. Organ distribution of radiolabeled compounds was measured with Exvivo in blood, healthy tissues and tumors. As shown in FIG. 19, most compounds show higher tumor uptake rates, especially 24 hours after dosing, as compared to FAPI-02 and FAPI-04. Due to increased lipophilicity, some radiotracers show higher blood activity as well as increased retention in the kidney. Tumor-blood ratio measurements reveal a clear advantage of the compounds FAPI-21 and FAPI-46, which always show significantly higher ratios than FAPI-04 in experiments (Fig. 20).

Small animal imaging of DOTA-FAPI derivatives in tumor-bearing mice Based on these findings, 140 minutes after intravenous administration of radiotracers to HT-1080-FAP tumor-bearing mice using Ga-68-labeled DOTA-FAPI derivatives. Small animal PET imaging was performed up to. The beneficial tumor-blood ratios of FAPI-21 and FAPI-46 provide high contrast images and allow for excellent visualization of FAP positive tumors (FIG. 21). Quantitative analysis of follower accumulation in tumor, kidney, liver and muscle tissue (given as SUVmax value) shows that FAPI-46 has slightly lower muscle, kidney and liver activity compared to FAPI-21. (Fig. 22).

In vivo distribution and dosimetric estimates of FAPI-02 and FAPI-04 compared to FDG in cancer patients Similar to the literature values of F-18-FDG, Ga-68-DOTATE, or Ga-68-PSMA-11, In the 200 MBq Ga-68-FAPI-02 and -04 experiments, it corresponds to an equivalent dose of about 3-4 mSv. After rapid clearance via the kidney between 10 minutes and 3 hours after injection, normal organs show low follower uptake with minimal change. In FAPI-02, tumor uptake 1 to 3 hours after injection was reduced by 75%, while in FAPI-04 (50% flush), tumor retention was slightly prolonged. Tumor uptake of both FAPI-trackers is equally skillful 1 hour after injection (Fig. 23). Tumor uptake is approximately equal compared to FDG (mean SUVmaxFDG 7.41; SUVmaxFAPI-02 7.37; n.s.); brain (11.01 vs 0.32), liver (2.01 vs. 0.32) on FAPI-02. Background uptake in 77 vs 1.69) and oral / pharyngeal mucosa (4.88 vs 2.57) is significantly lower; there is no relevant difference between FDG and FAPI-02 in other organs (FIG. 24). See Giesel et al.3, which is incorporated herein by reference, for more information and results.

PET Imaging of FAPI-04 in Patients with Various Cancers and Non-Cancer Malignancies In addition to rapid uptake of Ga-68 labeled FAPI-04 in various cancers including breast cancer, pancreatic cancer, ovarian cancer, HNO tumor, cancerous Tracker accumulation was also shown in some inflammatory malignancies such as peritonitis (Fig. 25A) and myocarditis (Fig. 25B) and arthritis (Fig. 25C). These results indicate the potential application of Ga-68 labeled FAPs to the detection of non-cancerous malignancies characterized by a process of chronic inflammation involved in the recruitment of activated fibroblasts.

PET Imaging of FAPI-21 and FAPI-46 in Various Cancer Patients Robust Accumulation of Ga-68 Labeled FAPI-21 in Various Cancers, Including Ovarian Cancer, Rectal Cancer and Squamous Cell Carcinoma, as shown in FIG. 26. Was observed. Ga-68-labeled FAPI-46, which rapidly accumulated in bile duct cell

carcinoma and colorectal cancer, lung cancer, and solitary fibrous sarcoma, showed similar tumor uptake (Fig. 27). Following PET / CT examination with Ga-68 labeled FAPI-46, the first therapeutic approach with Sm-153 labeled radiotracer was performed on two cancer patients. As shown in FIG. 28, the follower's robust tumor accumulation is detectable up to 20 hours after administration. FAPI-46-PET / CT imaging of three patients with idiopathic pulmonary fibrosis lung cancer showed a clear difference in tracker accumulation between cancerous and fibrotic lesions. As shown in FIG. 30, tumor uptake of Ga-68 labeled FAPI-46 was significantly higher in 2 patients (A, B) compared to activity measured in fibrous tissue, but in 1 patient. It was slightly lower in the patient (C) of. The patient shown in FIG. C suffered from exacerbated pulmonary fibrosis as compared to two non-exacerbated cases. Therefore, this tracker can be useful in differentiating patients with poor prognosis from those with good prognosis.

FAPI Derivatives for Radiolabeling with Alternative Radionuclides such as Tc-99m, Pb-203, Cu-64 and F18 A series of FAPI derivatives have been designed to enable the use of alternative radionuclides and have target affinity, specificity and pharmacokinetics. In some of these compounds, the original chelating agent DOTA was Tc-99m (FAPI-19, -27, -28, -29, -33, -34, -43, -44, -45, -60, It has been replaced by different chelated moieties ideally suitable for uptake of -61, -62). For FAPI-19 and FAPI-34, in vitro FAP affinity and biological distribution in HT-1080-FAP xenograft mice are illustrated. Both compounds show robust binding to human FAP in vitro (IC50FAPI-19: 6.4 nM). FAPI-34 continues within the tumor, in contrast to FAPI-19, which shows inadequate tumor uptake in vivo and rapid accumulation in the liver upon excretion from the kidney towards the liver. It is enriched and shows significantly less liver uptake (FIGS. 31 and 32). The first diagnostic application of Tc-99m labeled FAPI-34 in patients with pancreatic cancer with liver metastases showed stable tumor accumulation of followers up to 4 hours post-dose. Also, since the overall background radioactivity is relatively low, a high contrast image can be obtained (FIG. 33). This provides a wide range of applications for diagnosis by scintigraphy and therapy after labeling with Re-188.

Pb-203 radiolabeled FAPI derivatives (FAPI-04, -32, -46 and FAPI-04tcmc) show equivalent cell binding to HT-1080-FAP cells, FAPI-32 and after 60 minutes of incubation. FAPI-04tcmc has reached the highest binding value (26.93 ± 0.846 and $21.62 \pm 0.61\%$ ID / 1mio cells, FIG. 34A). Not only does FAPI-32 exhibit a fairly slow cell outflow ($t_{1/2} = 7$ hours), whereas FAPI-32 is rapidly eliminated from tumor cells during initial binding ($t_{1/2} = 2$ hours). It also shows the lowest FAP affinity, as shown by the competitive assay (IC50 = 5.7 μ M, FIG. 34C). For this reason, FAPI-04 and FAPI-46, which feature optimal half-life and IC50 values, were selected for further analysis in vivo. As shown in FIG. 35, both compounds show negligibly low binding to healthy tissue, but are continuously concentrated within the tumor. Scintigraphic findings have been confirmed in biodistribution studies, where both radiotracers show robust tumor uptake, overall low organ activity, and rapid renal excretion (Fig. 36).

NOTA derivatives FAPI-42 and FAPI-52 have been developed and characterized for target affinity, specificity and pharmacokinetics to enable radiolabeling with Cu-64. As shown in FIG. 37, both trackers show robust binding to HT-1080-FAP cells up to 24 hours of incubation at similar IC50 values in the lower nanomolar range (FIGS. 37A, B). .. Nevertheless, FAPI-42 was eliminated significantly slower than FAPI-52, with a calculated in vitro half-life of 12 hours (Fig. 37C). These results are confirmed by small animal imaging of HT-1080-FAP xenograft mice. As shown in FIG. 38, both compounds exhibit robust tumor uptake as well as rapid clearance from in vivo bloodstream. In particular, renal excretion of FAPI-42 is significantly faster compared to FAPI-52, but its tumor activity remains slightly higher between 2 and 24 hours after administration.

The NOTA derivatives FAPI-42 and FAPI-52 were arranged for the formation of an aluminum fluoride complex that allows imaging on the F-18. As shown in FIG. 39, both compounds show rapid tumor uptake in small animal imaging of HT-1080-FAP xenograft mice. Both compounds are excreted primarily by the renal pathway, but bile excretion has also been observed. Although renal excretion is faster with FAPI-52, FAPI-42 has the advantage of higher tumor accumulation, longer tumor residence time, and lower proportion of biliary pathways.

Reference 1 Loktev, A. et al. A new method for tumor imaging by targeting cancer associated fibroblasts. Journal of nuclear medicine: official publication, Society of Nuclear Medicine, doi: 10.2967 / jnumed.118.210435 (2018) .2 Lindner, T. et al. Development of quinoline based theranostic ligands for the targeting of fibroblast activation protein. Journal of nuclear medicine: official publication, Society of Nuclear Medicine, doi: 10.2967 / jnumed.118.210443 (2018). 3 Giesel, F. et al. FAPI-PET / CT: biodistribution and preliminary dosimetry estimate of two DOTA-containing FAP-targeting agents in patients with various cancers. Journal of nuclear medicine: official publication, Society of Nuclear Medicine, doi: 10.2967 / jnumed.118.215913 (2018).

Example 6: In vitro and in vivo FAPI characterization Preclinical data Initial experiments were performed on tumor-bearing mice using the human glioblastoma xenograft model U87MG with the aim of selectively targeting FAP-positive brain tumors. Tumor accumulation and organ distribution of radiolabeled FAPI-02 and -04 were analyzed by small animal PET imaging and biodistribution studies. As shown in FIGS. 40 and 41, both FAPI-02 and -04 show rapid tumor uptake and negligibly low activity in healthy organs and blood.

Clinical Data According to the 2016 WHO classification, gliomas are subdivided into IDH wild-type gliomas WHO grades I-IV and IDH mutant gliomas WHO grades II-IV. The most common WHO grade IV glioma is glioblastoma. Clinical PET imaging was performed on 18 patients with glioma (5 with IDH mutant glioma, 13 with IDH wild-type glioma; see Table 10). Tracker uptake was shown in grade III / IV IDH mutant gliomas excluding IDH wild-type glioblastoma and grade II, as shown in FIGS. 42-44. In glioblastoma, spots with increased uptake were observed in projection to the contrast-enhancing region.

CONCLUSIONS: Low-grade IDH mutations and high-grade gliomas have increased follower uptake in IDH wild-type gliomas and high-grade IDH mutant astrocytomas, but not in diffuse dehydrogenomas. It enables non-invasive distinction and may be useful for follow-up studies. Heterogeneous follower uptake in glioblastoma can be useful for biopsy planning.

Example 7: In vitro and in vivo characterization of FAPI Reuptake experiments In the reuptake experiments, ¹⁷⁷Lu-labeled FAPI-04 and -46 (5 MBq / nmol in DMEM) were added to HT-1080-FAP cells at 4 ° C. and respectively. Incubated at 37 ° C. for 60 minutes. Radioactive medium was removed and cells were washed twice with phosphate buffered saline (PBS) at pH 7.4. Subsequently, non-radioactive medium containing or not containing unlabeled FAPI (1 μM) was added at time intervals ranging from 10 minutes to 6 hours. The cells were washed twice with PBS pH 7.4. To remove surface binding activity, cells were incubated with glycine-HCl (1M in PBS, pH 2.2) for 10 minutes at room temperature. After washing twice with ice-cold PBS, cells were lysed with 1.4 mL of lysis buffer (0.3 M NaOH, 0.2% SDS) and the internalized fraction (internalized fraction) was measured. .. For cells incubated at 4 ° C., all wash and elution steps were performed using ice-cooled buffer. Radioactivity was measured using a γ counter (Packard Cobra II), normalized to 1 mio cells and calculated as a percentage of applicable dose (% AD; see FIG. 47).

Enzyme Inhibition Assay To measure the potential inhibitory effect of FAPI-04 on enzyme FAP activity, an enzyme inhibition assay was performed using recombinant human FAP protein (1 pmol / well) in a 48-well plate. After incubating FAPI-04 or tarabostat (0 to 1000 nM / well) with human FAP for 30 minutes at 37 ° C, the fluorescent FAP substrate Z-GP-AMC was added to bring the final concentration to 0 to 200 μM / well at 37 ° C. Incubated for 60 minutes. The enzymatic activity of FAP was determined by measuring the fluorescence intensity of the reaction product AMC at 360/460 nm using a SpectraMax M2 plate reader (Molecular Devices, San Jose, USA) (see Figure 46).

Multiple administration of FAPI-04 to mice bearing HT-1080-FAP tumors In an in vivo distribution experiment, 8-week-old BALB / c nu / nu mice (Challless River) were subjected to 5 mio HT-1080-FAP cells, respectively. The right trunk was subcutaneously inoculated. When the tumor size reached about 1 cm³, the radiolabeled compound was injected via the tail vein. The first group of animals received a single dose of ¹⁷⁷Lu-FAPI-04 (2 MBq per animal), while the second group received two doses of 1 MBq each, with a second dose 4 hours after the first injection. went. The third group was administered three times in total, and the first administration was 1 MBq per mouse, and then 0.5 MBq was administered 2 hours later, and an additional 0.5 MBq was administered 4 hours later. Animals (n = 3 at each time point) were sacrificed 8 and 24 hours after the first injection. The distributed radioactivity was measured using a γ counter (Cobra Autogamma, Packard) in all dissected organs and blood. Values are shown as a percentage (% ID / g) of dose per gram of tissue (see Figure 48).

Example 8: In vitro and in vivo characterization of FAPI Experimental operation and clinical evaluation All in vitro and in vivo experiments and clinical evaluation of FAPI derivatives were performed according to Loktev et al. 1 and Lindner et al. 2 as described in the first document. It was.

Results In vitro characterization of F-18-FAPI derivatives All experiments were performed in a manner similar to FAPI-42 (AIF-18 labeled) or FAPI-72 (F-18 nicotinamide labeled).

Table 11: EC50 values of selected FAPI derivatives measured by competitive binding assay

Measurement of Blood Pool Clearance In order to estimate the clearance rate of compounds, the half-life was calculated from the SUVmean value of the heart (0.375-60 minutes) as a representation of the blood pool by the estimated two-phase exponential decay. All selected compounds cleared very quickly with a half-life of less than 10 minutes. The calculated Plateau value was higher for Ga-68 labeled FAPI-13, -21, -36 and AIF-18 labeled FAPI-74, which is theoretically non-specific. Corresponds to higher fraction compounds that have not been cleared due to binding or by remaining in the circulation (Table 12). As an example of fast clearance, the temporal radioactivity curves of FAPI-04 and -46 between 0 and 15 minutes are shown in FIG.

Table 12. Blood pool half-life and virtual plateau values of selected FAPI derivatives calculated from SUVmean values by estimated two-phase exponential decay. For clarity, only the rate-determining half-life values are listed.

Small animal imaging of F-18-FAPI derivatives in tumor-bearing mice Based on these findings, small animal PET-imaging performed F-18-labeled NOTA up to 140 minutes after administration of the radiotracker to HT-1080-FAP tumor-bearing mice. -And F-18-nicotinamide-labeled FAPI derivatives were used. The F-18-nicotinamide derivatives FAPI-72, -73 and -77 showed unwanted accumulation and bile excretion in the liver, while FAPI-78 was excreted from the kidney but not tumor uptake. In the case of the AIF-18 labeled NOTA derivatives FAPI-74 and -75, high target specificity and fast clearance were observed, resulting in high contrast images that allowed excellent visualization of FAP positive tumors. (Fig. 50).

In the organ distribution pharmacokinetic profile of F-18-FAPI derivatives in tumor-bearing mice and in vivo tumor uptake analysis, AIF-18-labeled FAPI-75 was intravenously administered to HT-1080-FAP tumor-bearing mice. Organ distribution of radiolabeled compounds was measured with Exvivo in blood, healthy tissues and tumors. As shown in FIG. 51, this compound showed high tumor uptake, but higher accumulation in healthy tissues was observed compared to Ga-68 labeled DOTA derivatives and performance in PET imaging was comparable.

Items The following items indicate preferred embodiments of the present invention. 1. Equation (I): [In the equation, Q, R, U, V, W, Y and Z are conditions in which at least three of Q, R, U, V, W, Y and Z are present. Independently present or absent; Q, R, U, V, W, Y and Z are independently O, CH₂, NR₄, C = O, C = S, C = NR₄, HCR₄. And R₄CR₄ selected from the group, but the two O's are not directly adjacent to each other; R₁ and R₂ are independently -H, -OH, halo, C₁-6-alkyl, -O-C₁-6-alkyl, Selected from the group consisting of S-C₁-6-alkyl; R₃ is -H, -CN, -B (OH) ₂, -C (O) -alkyl, -C (O) -aryl, -C = C Selected from the group consisting of -C (O) -aryl, -C = C-S (O) ₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂ and 5-tetrazolyl; R₄ is -H,- From the group consisting of C₁-6-alkyl, -O-C₁-6-alkyl, -S-C₁-6-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C₁-6-aralkyl Selected, each -C₁-6-alkyl may be substituted with 1-3 substituents selected from -OH, oxo and halo, and Q, R, U, V, W, Y or Z. May be combined with;

R₅ is selected from the group consisting of -H, halo and C₁-6-alkyl; R₆ and R₇ are independently selected from the group consisting of -H, but R₆ and R₇ are not H at the same time; , L is a linker; D, A, E and B are present or absent independently, preferably at least A, E and B are present, where D is. A linker is selected from the group consisting of NR₄, O, S and CH₂; E is selected from the group consisting of C₁-6-alkyl; where i is 1, 2 or 3; Where j is 1, 2 or 3; where k is 1, 2 or 3; where m is 1, 2 or 3;

A and E together form a group selected from cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, where A and E are monocyclic, bicyclic. And may be polycyclic, preferably monocyclic; each A and E is -H, -C₁-6-alkyl, -O-C₁-6-alkyl, -S-C₁-6- It may be substituted with 1 to 4 substituents selected from alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl and -C₁-6-aralkyl, each -C₁-6-alkyl, -OH, oxo and halo, may be substituted with 1-3 substituents; and may be attached to A, B, D, E or; B is S, NR₄, NR₄. Select from the group consisting of —O, NR₄-C₁-6-alkyl, NR₄-C₁-6-alkyl-NR₄ and 5--10-

membered N-containing aromatic or non-aromatic monocyclic or bicyclic heterocycles. The heterocycle preferably further comprises 1 or 2 heteroatoms selected from O, N and S, preferably further comprising 1 or 2 nitrogen atoms, preferably NR₄-C₁-. The 6-alkyl-NR₄ and its N-containing heterocycle are substituted with 1-3 substituents selected from the group consisting of C₁-6-alkyl, aryl, C₁-6-aralkyl; and R₈. Is selected from the group consisting of radioactive moieties, chelating agents, fluorescent dyes, contrasting agents and combinations thereof; is a 1-naphthyl moiety or a 5-10-membered N-containing aromatic or non-aromatic monocyclic or two. It is a cyclic heterocycle, in which there are two ring atoms between the N atom and the X; and the heterocycle is selected from O, N and S 1, 2 or 3 It may further contain an additional hetero atom; and X is a C atom], or a pharmaceutically acceptable homovariant, racemic, hydrate, solvate or salt thereof.

2. (i) Q, R and U are CH₂ and either exist independently or are absent; V is CH₂, C = O, C = S or C = NR₄; W is NR₄ Is; Y is HCR₄; and Z is C = O, C = S or C = NR₄; and / or (ii) Q and R are absent; U is CH₂ and is there? , Or absent; R₁ and R₂ are independently selected from the group consisting of -H and halo; R₃ is selected from the group consisting of -H, -CN and -B (OH) 2; R₄ is , -H and -C₁-6-alkyl, wherein -C₁-6-alkyl may be substituted with 1-3 substituents selected from -OH, item 1. Compound.

3. The compound according to claim 1 or 2, wherein is selected from the group consisting of, which may further comprise one or two heteroatoms selected from O, N and S.

4. The compound according to any one of items 1 to 3 selected from the group consisting of.

5. R₅ and R₆ are H; R₇ is, where D is absent; A is O; E is C₁-6-alkyl or, where m is 1, 2 or 3; A and E together form a group selected from; B is an NR₄-C₁-6-alkyl or 5--10-member N-containing aromatic or non-aromatic. A group monocyclic or bicyclic heterocycle, the heterocycle preferably further comprising one or two heteroatoms selected from O, N and S, preferably one or two nitrogen atoms. Further included, preferably the N-containing heterocycle is substituted with 1-3 substituents selected from the group consisting of C₁-6-alkyl, aryl and C₁-6-aralkyl, items 1-. The compound according to any one of 4.

6. (i) The N-containing heterocycle contained in B may further contain one or two heteroatoms selected from O, N and S in the formula: [In the formula, one heteroatom. Nitrogen may further be included; is attached at the 1, 2 or 3 position, preferably at the 2 position; l is 1 or 2], which is an aromatic or non-aromatic monocyclic hetero. It is a ring; and / or the N-containing heterocycle contained in (ii) B is selected from the group consisting of, where the N-containing heterocycle is substituted with C₁-6-alkyl; where , B contains an N-containing heteroatom, which may further comprise one or two heteroatoms selected from O, N and S, further comprising one nitrogen. It may also contain one or more side chains (eg, derived from amino acids); it binds to the 1, 2 or 3 position, preferably the 2nd position; o is 1 or 2 If the N-containing heterocycle contained in B is, then the N-containing heterocycle contained in B is; more preferably, the N-containing heterocycle contained in B is. The compound according to any one of items 1 to 5, wherein the N-containing heterocycle contained in the B is.

7. Q, R and U are absent; V is C = O; W is NH; Y is CH₂; Z is C = O; R₁ and R₂ are independently- Selected from the group consisting of H and halo; R₃ is -CN; R₅ and R₆ are H; R₇ is, where D is absent; A is O, S, CH₂, NH or NCH₃; E is C₁-6-alkyl or where m is 1, 2 or 3; or A and E together form the group of choice; B is. The compound according to any one of items 1-6, which is NH-C₁-6-alkyl, where B may be substituted with C₁-3 alkyl; and is.

8. C₁-6-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl, and / or C₁-6-aralkyl is benzyl, phenyl. The compound according to any one of items 1 to 7, selected from the group consisting of -ethyl, phenyl-propyl and phenyl-butyl.

9. R₈ is a radioactive moiety which is a fluorescent isotope, a radioisotope, a radiopharmaceutical or a combination thereof, preferably an α radiation isotope, a β radiation isotope, a γ radiation isotope, an Auger electron. Emission isotopes, X-ray emission isotopes, fluorescent emission isotopes such as ¹⁸F, ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁸⁶Re, ¹⁸⁸Re, ¹³⁹La, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁸⁸Y, ⁹⁰Y, ¹⁴⁹Pm, ¹⁶⁵Dy , ¹⁶⁹Er, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi, ²¹³Bi, ⁷²As, ⁷²Se, ⁹⁷Ru, ¹⁰⁹Pd, ¹⁰⁵Rh, ¹⁰¹mRh, ¹¹⁹Sb, ¹²⁸Ba, ¹²³I, ¹²⁴I, ¹³¹I, ¹⁹⁷Hg, ²¹¹At, ¹⁵¹Eu, ¹⁵³Eu, ¹⁶⁹Eu, ²⁰¹T , ²¹²Pb, ⁶⁴Cu, ⁶⁷Cu, ¹⁸⁸Re, ¹⁸⁶Re, ¹⁹⁸Au, ²²⁵Ac, ²²⁷Th and ¹⁹⁹Ag. The compound according to any one of items 1 to 8, which is a radioactive moiety selected from the group.

10. R₈ is a group consisting of the following types of fluorescent dyes: xanthene, acrydin, oxazine, cyanine, styryl dye, coumarin, porphyrin, metal-ligand complex, fluorescent protein, nanocrystals, perylene, boron-

dipyrrromethene and phthalocyanine. The compound according to any one of items 1 to 8, which is a fluorescent dye selected from the above, and a composite and combination of these types of dyes.

11. R8 is a chelating agent in the form of a complex having a divalent or trivalent metal cation, preferably 1,4,7,10-tetraazacyclododecane-N, N', N, N'-. Tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N A chelate selected from the group consisting of, N, N', N', N''-pentaacetic acid (DTPA), bis- (carboxymethylimidazole) glycine and 6-hydrazinopyridine 3-carboxylic acid (HYNIC). The compound according to any one of items 1 to 8, which is an agent.

12. R8 is a contrast agent containing or consisting of a paramagnetic agent, preferably, wherein the paramagnetic agent contains or consists of paramagnetic nanoparticles, any of items 1-8. The compound according to one item.

13. A pharmaceutical composition comprising or consisting of a compound according to at least one item 1-12, and optionally a pharmaceutically acceptable carrier and / or additive.

14. The compound or item according to any one of items 1-12 for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast-activating protein (FAP) in an animal or human subject. 13. The pharmaceutical composition according to 13, preferably in which the disease characterized by overexpression of the fibroblast-activating protein (FAP) is cancer, chronic inflammation, atherosclerosis, fibrosis, histology. Selected from the group consisting of modeling and keroid disorders, preferably the cancers are breast cancer, pancreatic cancer, small bowel cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer, hepatocellular carcinoma, esophageal cancer, hypopharyngeal cancer, Nasopharyngeal cancer, laryngeal cancer, myeloma cells, bladder cancer, bile duct cell cancer, clear renal cell cancer, neuroendocrine tumor, tumor-induced bone softening, sarcoma, CUP (unknown early stage cancer), thoracic adenocarcinoma, tendonoma A compound or pharmaceutical composition selected from the group consisting of, glioma, stellate cell tumor, cervical cancer and prostate cancer.

15. A kit comprising or comprising the compound according to any one of items 1 to 12 or the pharmaceutical composition according to item 13 and instructions for diagnosing a disease.

(19) 日本国特許庁 (JP)

(12) 公表特許公報 (A)

(11) 特許出願公表番号

特表2021-512949

(P2021-512949A)

(43) 公表日 令和3年5月20日 (2021.5.20)

(51) Int. Cl.	F I	テーマコード (参考)
C07D 401/14 (2006.01)	C07D 401/14	C S P 4 C 0 6 3
A61P 17/02 (2006.01)	A61P 17/02	4 C 0 8 4
A61P 37/06 (2006.01)	A61P 37/06	4 C 0 8 5
A61P 9/10 (2006.01)	A61P 9/10	4 C 0 8 6
A61P 29/00 (2006.01)	A61P 29/00	4 H 0 4 5
審査請求 未請求 予備審査請求 未請求 (全 134 頁) 最終頁に続く		

(21) 出願番号 特願2020-563822 (P2020-563822)
 (86) (22) 出願日 平成31年2月6日 (2019.2.6)
 (85) 翻訳文提出日 令和2年10月5日 (2020.10.5)
 (86) 国際出願番号 PCT/EP2019/052952
 (87) 国際公開番号 W02019/154886
 (87) 国際公開日 令和1年8月15日 (2019.8.15)
 (31) 優先権主張番号 18155420.5
 (32) 優先日 平成30年2月6日 (2018.2.6)
 (33) 優先権主張国・地域又は機関
 欧州特許庁 (EP)
 (31) 優先権主張番号 18199641.4
 (32) 優先日 平成30年10月10日 (2018.10.10)
 (33) 優先権主張国・地域又は機関
 欧州特許庁 (EP)

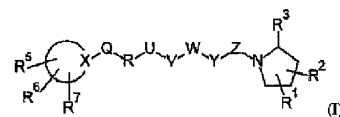
(71) 出願人 509223977
 ウニベルジテート ハイデルベルク
 ドイツ連邦共和国 69117 ハイデル
 ベルク, グラベンガッセ 1
 (74) 代理人 100145403
 弁理士 山尾 憲人
 (74) 代理人 100150500
 弁理士 森本 靖
 (74) 代理人 100176474
 弁理士 秋山 信彦
 (72) 発明者 ウーヴェ・ハーベルコルン
 ドイツ68723シュヴェツィンゲン、ケ
 ーニッヒシェッカー82番

最終頁に続く

(54) 【発明の名称】 F A P阻害物質

(57) 【要約】

本発明は、線維芽細胞活性化タンパク質 (F A P) の過剰発現を特徴とする疾患の診断または処置における、化合物、該化合物を含むか、または該化合物からなる医薬組成物、該化合物または医薬組成物を含むか、またはそれらからなるキット、および該化合物または医薬組成物の使用に関する。

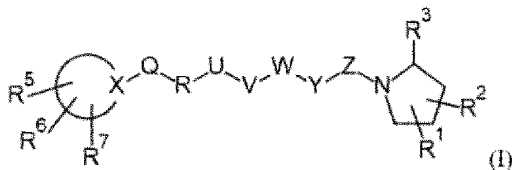


【特許請求の範囲】

【請求項 1】

式 (I) :

【化 1】



10

〔式中、Q、R、U、V、W、YおよびZは、Q、R、U、V、W、YおよびZの中の少なくとも三つが存在する条件で、独立して存在するか、または不存在であり；

Q、R、U、V、W、YおよびZは、独立してO、CH₂、NR⁴、C=O、C=S、C=NR⁴、HCR⁴およびR⁴CR⁴からなる群から選択され、ただし二つのOは互いに直接隣接しない；

R¹およびR²は、独立して-H、-OH、ハロ、C₁-₆-アルキル、-O-C₁-₆-アルキル、S-C₁-₆-アルキルからなる群から選択され；

R³は、-H、-CN、-B(OH)₂、-C(O)-アルキル、-C(O)-アリール、-C=C-C(O)-アリール、-C=C-S(O)₂-アリール、-CO₂H、-SO₃H、-SO₂NH₂、-PO₃H₂および5-テトラゾリルからなる群から選択され；

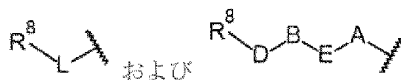
20

R⁴は、-H、-C₁-₆-アルキル、-O-C₁-₆-アルキル、-S-C₁-₆-アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび-C₁-₆-アラルキルからなる群から選択され、各-C₁-₆-アルキルは、-OH、オキソおよびハロから選択される1~3個の置換基によって置換されていてよく、またQ、R、U、V、W、YまたはZに結合されていてよく；

R⁵は、-H、ハロおよびC₁-₆-アルキルからなる群から選択され；

R⁶およびR⁷は、独立して-H、

【化 2】



30

からなる群から選択され、ただしR⁶およびR⁷は同時にHではない；

ここで、Lはリンカーであり；

D、A、EおよびBは独立して存在するか、または不存在であり、好ましくは、少なくともA、EおよびBは存在し、ここで、存在する場合；

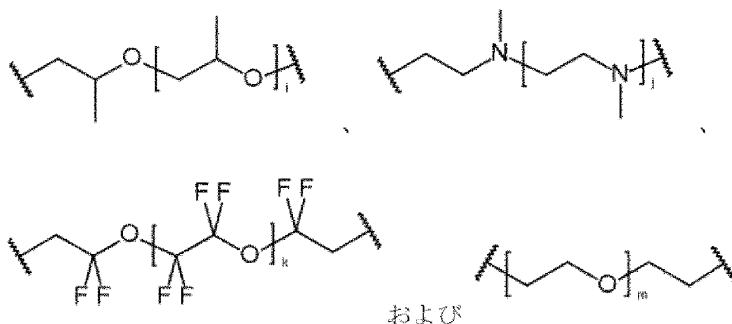
Dはリンカーであり；

Aは、NR⁴、O、SおよびCH₂からなる群から選択され；

Eは、C₁-₆-アルキル、

40

【化 3】



10

からなる群から選択され；

ここで、 i は 1、2 または 3 であり；

ここで、 j は 1、2 または 3 であり；

ここで、 k は 1、2 または 3 であり；

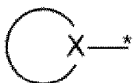
ここで、 m は 1、2 または 3 であり；

B は、 S 、 NR^4 、 NR^4-O 、 NR^4-C_{1-6} -アルキル、 NR^4-C_{1-6} -アルキル- NR^4 および 5～10-員 N -含有芳香族または非芳香族単環式もしくは二環式ヘテロ環からなる群から選択され、該ヘテロ環は好ましくは O 、 N および S から選択される 1 または 2 個のヘテロ原子をさらに含み、好ましくは 1 または 2 個の窒素原子をさらに含み、ここに好ましくは、 NR^4-C_{1-6} -アルキル- NR^4 および該 N -含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アラルキルからなる群から選択される 1～3 個の置換基によって置換されており；そして、

20

R^8 は、放射性部分、キレート剤、蛍光色素、造影剤およびそれらの組み合わせからなる群から選択され；

【化 4】



30

は、1-ナフチル部分または、5～10-員 N -含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、ここに該ヘテロ環は N 原子と X との間に 2 個の環原子が存在し；また、該ヘテロ環は O 、 N および S から選択される 1、2 または 3 個のヘテロ原子をさらに含んでいてよく；そして、 X は C 原子である]

で示される化合物、またはその薬学的に許容できる互変異性体、ラセミ体、水和物、溶媒和物もしくは塩。

【請求項 2】

(i) Q 、 R および U が CH_2 であり、独立して存在するか、または不存在であり；

40

V が、 CH_2 、 $C=O$ 、 $C=S$ または $C=NR^4$ であり；

W が NR^4 であり；

Y が HCR^4 であり；そして

Z が $C=O$ 、 $C=S$ または $C=NR^4$ であり；および/または

(ii) Q および R が不存在であり；

U が CH_2 であり、存在するか、または不存在であり；

R^1 および R^2 が、独立して $-H$ およびハロからなる群から選択され；

R^3 が、 $-H$ 、 $-CN$ および $-B(OH)_2$ からなる群から選択され；

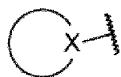
R^4 が、 $-H$ および C_{1-6} -アルキルからなる群から選択され、ここに C_{1-6} -アルキルは $-OH$ から選択される 1～3 個の置換基によって置換されていてよい、請求

50

項 1 に記載の化合物。

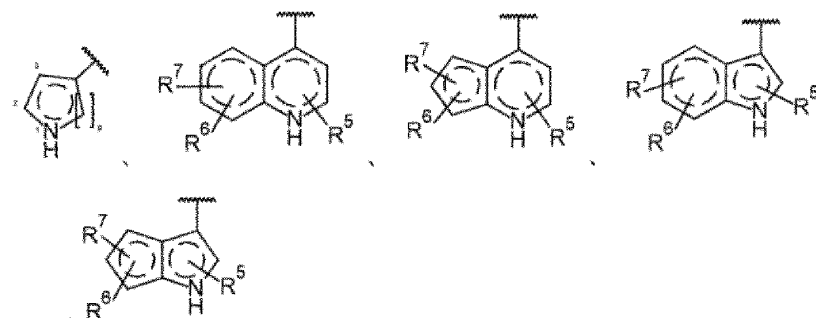
【請求項 3】

【化 5】



が、

【化 6】

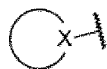


および

からなる群から選択され、これらは、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでよい、請求項1または2に記載の化合物。

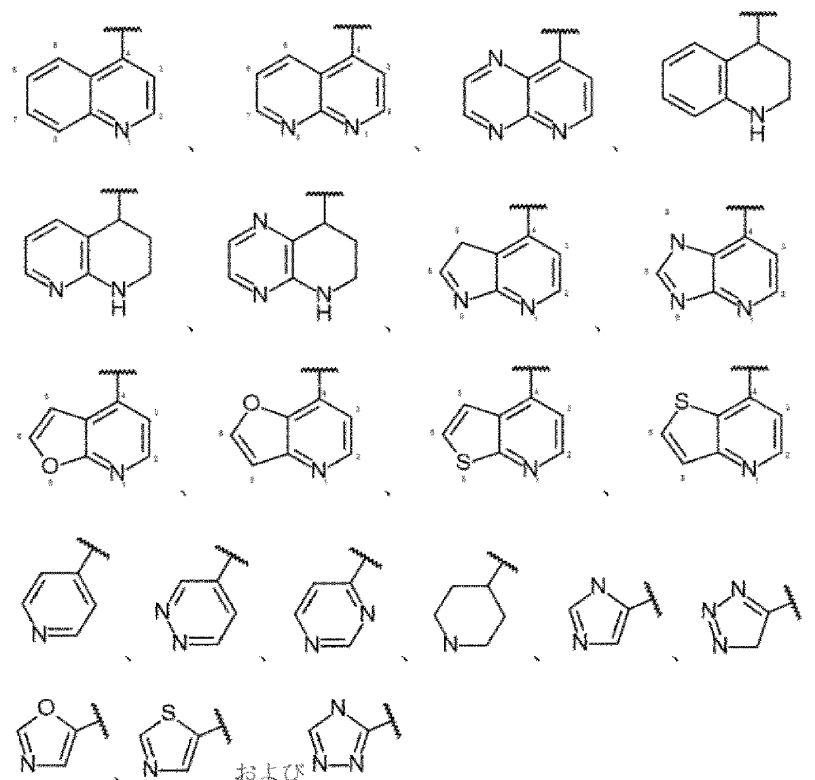
【請求項 4】

【化 7】



が、

【化 8】



10

20

からなる群から選択される、請求項 1 ～ 3 のいずれか一項に記載の化合物。

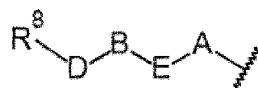
【請求項 5】

R^5 および R^6 が H であり；

R^7 が、

30

【化 9】



であり、ここで、

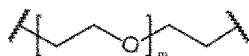
D は不存在であり；

A は O であり；

E は、 C_{1-6} - アルキルまたは

【化 10】

40



であり、ここで、 m が 1、2 または 3 であり；

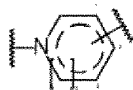
B が、 NR^4-C_{1-6} - アルキルまたは 5 - ~ 10 - 員 N - 含有芳香族または非芳香族単環式もしくはまたは二環式ヘテロ環であり、該ヘテロ環は好ましくは O、N および S から選択される 1 または 2 個のヘテロ原子をさらに含み、好ましくは 1 または 2 個の窒素原子をさらに含み、ここに好ましくは、N - 含有ヘテロ環は C_{1-6} - アルキル、アリールおよび C_{1-6} - アラルキルからなる群から選択される 1 ~ 3 個の置換基によって置換されている、請求項 1 ~ 4 のいずれか一項に記載の化合物。

50

【請求項 6】

(i) B に含まれる N-含有ヘテロ環が、式：

【化 1 1】



〔式中、ヘテロ環は O、N および S から選択される 1 または 2 個のヘテロ原子をさらに含んでいてよく、さらに 1 個の窒素を含んでいてよく；

【化 1 2】



10

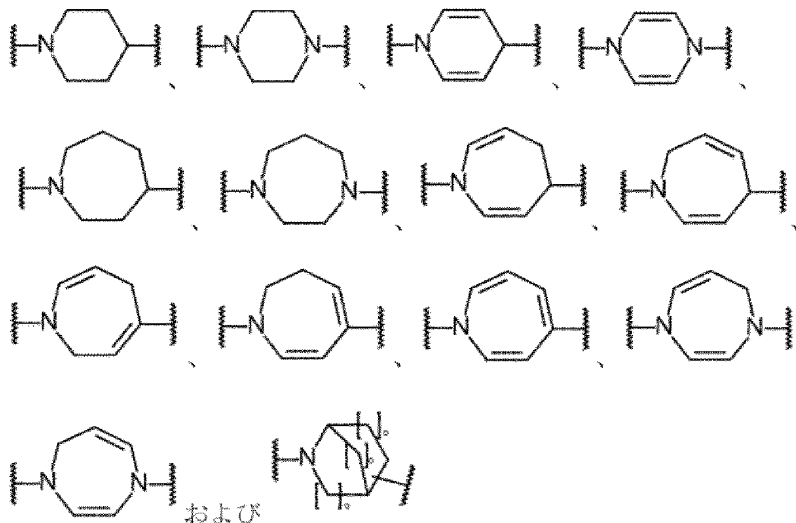
は、1、2 または 3 位に結合し、好ましくは 2 位に結合し；

l は 1 または 2 である〕

で示される芳香族または非芳香族単環式ヘテロ環であり；および／または

(ii) B に含まれる N-含有ヘテロ環が、

【化 1 3】



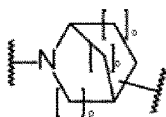
20

30

からなる群から選択され、

ここで、B に含まれる N-含有ヘテロ環が

【化 1 4】



40

である場合、該ヘテロ環は、O、N および S から選択される 1 または 2 個のヘテロ原子をさらに含んでいてよく、さらに 1 個の窒素を含んでいてよく、また、一つまたはそれ以上の (例えばアミノ酸由来) 側鎖を含んでいてよく；

【化 1 5】

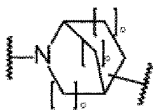


は、1、2または3位に結合し、好ましくは2位に結合し；

o は1または2であり、

好ましくは、Bに含まれるN-含有ヘテロ環が

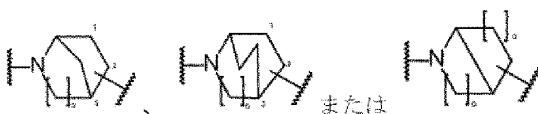
【化 1 6】



10

である場合、該Bに含まれるN-含有ヘテロ環は

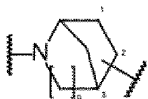
【化 1 7】



20

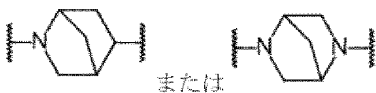
であり；より好ましくは、Bに含まれるN-含有ヘテロ環が

【化 1 8】



である場合、該Bに含まれるN-含有ヘテロ環は

【化 1 9】



30

である、請求項1～5のいずれか一項に記載の化合物。

【請求項 7】

Q、RおよびUが不存在であり；

VがC=Oであり；

WがNHであり；

YがCH₂であり；

ZがC=Oであり；

R¹ および R² が、独立して-Hおよびハロからなる群から選択され；

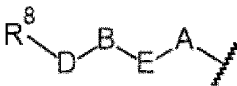
R³ が-CNであり；

R⁵ および R⁶ がHであり；

R⁷ が

40

【化 2 0】



50

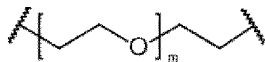
であり、ここで、

Dは不存在であり；

AはOであり；

Eは、 $C_1 - 6$ - アルキルまたは

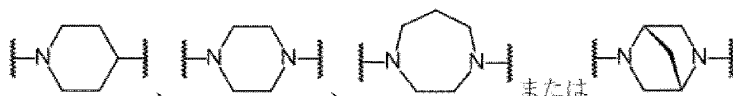
【化 2 1】



であり、ここで、mは1、2または3であり；

Bは、 $NH - C_1 - 6$ - アルキル、

【化 2 2】



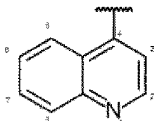
であり；そして

【化 2 3】



は

【化 2 4】



である、請求項 1 ～ 6 のいずれか一項に記載の化合物。

【請求項 8】

$C_1 - 6$ - アルキルが、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され、および／または

$C_1 - 6$ - アラルキルが、ベンジル、フェニル-エチル、フェニル-プロピルおよびフェニル-ブチルからなる群から選択される、請求項 1 ～ 7 のいずれか一項に記載の化合物。

【請求項 9】

R^8 が、蛍光同位体、放射性同位体、放射性薬剤またはそれらの組み合わせである放射性部分であって、好ましくは、 α 放射線放出同位体、 β 放射線放出同位体、 γ 放射線放出同位体、オージェ電子放出同位体、X-ray放出同位体、蛍光放出同位体、例えば ^{18}F 、 ^{51}Cr 、 ^{67}Ga 、 ^{68}Ga 、 ^{111}In 、 ^{99m}Tc 、 ^{186}Re 、 ^{188}Re 、 ^{139}La 、 ^{140}La 、 ^{175}Yb 、 ^{153}Sm 、 ^{166}Ho 、 ^{88}Y 、 ^{90}Y 、 ^{49}Pm 、 ^{165}Dy 、 ^{169}Er 、 ^{177}Lu 、 ^{47}Sc 、 ^{142}Pr 、 ^{159}Gd 、 ^{212}Bi 、 ^{213}Bi 、 ^{72}As 、 ^{72}Se 、 ^{97}Ru 、 ^{109}Pd 、 ^{105}Rh 、 ^{101m}Rh 、 ^{119}Sb 、 ^{128}Ba 、 ^{123}I 、 ^{124}I 、 ^{131}I 、 ^{197}Hg 、 ^{211}At 、 ^{151}Eu 、 ^{153}Eu 、 ^{169}Eu 、 ^{201}Tl 、 ^{203}Pb 、 ^{212}Pb 、 ^{64}Cu 、 ^{67}Cu 、 ^{188}Re 、 ^{186}Re 、 ^{198}Au 、 ^{225}Ac 、 ^{227}Th

および¹⁹⁹Agからなる群から選択される放射性部分である、請求項1～8のいずれか一項に記載の化合物。

【請求項10】

R⁸が、以下の種類の蛍光色素：キサンテン、アクリジン、オキサジン、シアニン、ステリル色素、クマリン、ポルフィン、金属-配位子錯体、蛍光タンパク質、ナノ結晶、ペリレン、ホウ素-ジピロメテンおよびフタロシアニンからなる群から選択される蛍光色素、ならびにこれらの種類の色素の複合物および組み合わせである、請求項1～8のいずれか一項に記載の化合物。

【請求項11】

R⁸が、2価または3価金属カチオンを有する錯体の形態のキレート剤であって、好ましくは、1,4,7,10-テトラアザシクロドデカン-N,N',N,N'-テトラ酢酸(DOTA)、エチレンジアミンテトラ酢酸(EDTA)、1,4,7-トリアザシクロノナン-1,4,7-トリ酢酸(NOTA)、トリエチレントラミン(TETA)、イミノニ酢酸、ジエチレントリアミン-N,N,N',N',N''-ペンタ酢酸(DTPA)、ビス-(カルボキシメチルイミダゾール)グリシンおよび6-ヒドラジノピリジン3-カルボン酸(HYNIC)からなる群から選択されるキレート剤である、請求項1～8のいずれか一項に記載の化合物。

【請求項12】

R⁸が、常磁性剤を含むか、またはそれからなる造影剤であって、好ましくは、ここで、常磁性剤が、常磁性ナノ粒子を含むか、またはそれからなる、請求項1～8のいずれか一項に記載の化合物。

【請求項13】

少なくとも一つの請求項1～12による化合物、および場合により製薬的に許容できる担体および/または添加物を含むか、またはそれらからなる医薬組成物。

【請求項14】

動物またはヒトの対象における線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患の診断または処置に使用するための、請求項1～12のいずれか一項に記載の化合物または請求項13に記載の医薬組成物であって、好ましくは、該線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患が、癌、慢性炎症、アテローム性動脈硬化症、線維症、組織リモデリングおよびケロイド障害からなる群から選択され、好ましくは、癌が乳癌、膵臓癌、小腸癌、結腸癌、直腸癌、肺癌、頭頸部癌、卵巣癌、肝細胞癌、食道癌、下咽頭癌、上咽頭癌、喉頭癌、骨髄腫細胞、膀胱癌、胆管細胞癌、腎明細胞癌、神経内分泌腫瘍、腫瘍誘発性骨軟化症、肉腫、CUP(未知の初期癌)、胸腺癌、類腫瘍、神経膠腫、星状細胞腫、頸癌および前立腺癌からなる群から選択される、化合物または医薬組成物。

【請求項15】

請求項1～12のいずれか一項に記載の化合物もしくは請求項13に記載の医薬組成物および疾患の診断のためのインストラクションを含むか、またはそれらからなるキット。

【発明の詳細な説明】

【技術分野】

【0001】

本発明は、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患の診断または処置における、化合物、該化合物を含むか、または該化合物からなる医薬組成物、該化合物または医薬組成物を含むか、またはそれらからなるキット、および該化合物または医薬組成物の使用に関する。

【背景技術】

【0002】

腫瘍の増殖や拡散は、癌細胞だけで決定されるのではなく、間質なる用語に分類される、悪性病変における非悪性成分によっても決定される。間質は、乳癌、結腸癌、膵臓癌などの、線維形成反応を伴う腫瘍では、腫瘍量の90%以上を占めることがある。特に、癌関

連線維芽細胞(CAF)と称される線維芽細胞の亜集団は、腫瘍の増殖、転移および進行に関与していることが知られている。したがって、これらの細胞は、診断および抗腫瘍療法のための魅力的な標的である。

【0003】

CAFの際立った特徴は、ジペプチジルペプチダーゼ4(DPP4)ファミリーに属するII型膜結合糖タンパク質であるセプラーゼまたは線維芽細胞活性化タンパク質 α (FAP- α)の発現である。FAP- α は、ジペプチジルペプチダーゼ活性とエンドペプチダーゼ活性の両方を有する。エンドペプチダーゼ活性は、FAP- α をDPP4ファミリーの他のメンバーと区別する。これまでに同定されたエンドペプチダーゼ活性の基質は、変性I型コラーゲン、 α 1-アンチトリプシンおよびいくつかの神経ペプチドである。FAP- α は、胚発生期間や組織モデリングにおける正常な発生過程に役割を果たしている。これは、成人の正常組織では発現していないか、または極めて少ないレベルでしか発現していない。しかしながら、創傷治癒、関節炎、アテローム硬化性プラーク、線維症および90%を超える上皮癌では、高く発現している。

【0004】

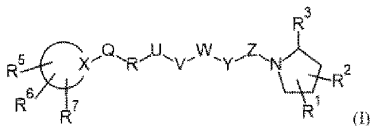
多くの上皮腫瘍のCAFにFAP- α が出現し、過剰発現が癌患者の予後悪化と関連している事実から、FAP- α 活性は癌の発生、および癌細胞の転移や拡散にも関与しているという仮説が立てられている。したがって、この酵素を画像化や内部放射線療法の標的にすることは、悪性腫瘍の検出および処置のための有望な戦略と考えることができる。本発明者らは、FAP- α 特異的な阻害物質に基づいた低分子を開発し、動物モデルおよび腫瘍患者において、特異的な取り込み、迅速なインターナリゼーション(内在化)および腫瘍の画像化の成功を示すことができた。一般的に使用されている放射性追跡子(トレーサー)である ^{18}F -フルオロデオキシグルコース(^{18}F -FDG)との比較により、局所進行性肺腺癌患者においてこの新しいFAP- α リガンドが明白に優位であることが明らかになった。したがって、本発明はとりわけ、(i)より小さな原発性腫瘍の検出、それによる早期診断の可能性、(ii)より小さな転移の検出、それによる腫瘍の病期のより良い診断、(iii)腫瘍組織の完全な外科切除を容易にする正確な術中指導、(iv)炎症と腫瘍組織のより良い鑑別、(v)腫瘍患者のより正確な病期分類、(vi)抗腫瘍療法後の腫瘍病変のより良い経過観察、(vii)診断および治療のためのセラノスティック(特定の治療を採用する診断テストに関する)剤として本発明の分子を使用する機会、を提供する。本発明の分子はさらに、慢性炎症、アテローム性動脈硬化症、線維症、組織リモデリングおよびケロイド障害などの非悪性疾患の診断および処置にも使用できる。

【発明の概要】

【0005】

第一の態様において、本発明は、式(I)：

【化1】



〔式中、Q、R、U、V、W、YおよびZは、Q、R、U、V、W、YおよびZの中の少なくとも三つが存在する条件で、独立して存在するか、または不存在であり；

Q、R、U、V、W、YおよびZは、独立してO、 CH_2 、 NR^4 、 $\text{C}=\text{O}$ 、 $\text{C}=\text{S}$ 、 $\text{C}=\text{NR}^4$ 、 HCR^4 および R^4CR^4 からなる群から選択され、ただし二つのOは互いに直接隣接しない；

R^1 および R^2 は、独立して $-\text{H}$ 、 $-\text{OH}$ 、ハロ、 C_{1-6} -アルキル、 $-\text{O}-\text{C}_{1-6}$ -アルキル、 $\text{S}-\text{C}_{1-6}$ -アルキルからなる群から選択され；

R^3 は、 $-\text{H}$ 、 $-\text{CN}$ 、 $-\text{B}(\text{OH})_2$ 、 $-\text{C}(\text{O})$ -アルキル、 $-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{S}(\text{O})_2$ -アリール、 $-\text{CO}_2\text{H}$ 、 $-\text{SO}_3\text{H}$ 、

$-\text{SO}_2\text{NH}_2$ 、 $-\text{PO}_3\text{H}_2$ および 5-テトラゾリルからなる群から選択され；

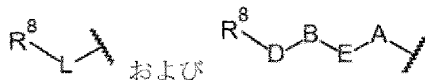
R^4 は、 $-\text{H}$ 、 $-\text{C}_{1-6}$ -アルキル、 $-\text{O}-\text{C}_{1-6}$ -アルキル、 $-\text{S}-\text{C}_{1-6}$ -アルキル、アリールおよび $-\text{C}_{1-6}$ -アラルキルからなる群から選択され、各 $-\text{C}_{1-6}$ -アルキルは、 $-\text{OH}$ 、オキソ、ハロから選択される 1～3 個の置換基によって置換されていてよく、また、Q、R、U、V、W、Y または Z に結合されていてよく；

R^5 は、 $-\text{H}$ 、ハロおよび C_{1-6} -アルキルからなる群から選択され；

【0006】

R^6 および R^7 は、独立して $-\text{H}$ 、

【化2】



10

からなる群から選択され、ただし R^6 および R^7 は同時に H ではない；

ここで、L はリンカーであり；

D、A、E および B は独立して存在するか、または不存在であり、好ましくは、少なくとも A、E および B は存在し、ここで、存在する場合；

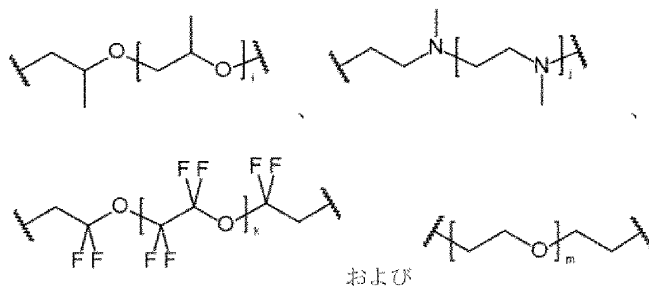
D はリンカーであり；

A は、 NR^4 、O、S および CH_2 からなる群から選択され；

E は、 C_{1-6} -アルキル、

20

【化3】



30

からなる群から選択され；

ここで、i は 1、2 または 3 であり；

ここで、j は 1、2 または 3 であり；

ここで、k は 1、2 または 3 であり；

ここで、m は 1、2 または 3 であり；

【0007】

A および E は一緒になって、シクロアルキル、ヘテロシクロアルキル、アリールおよびヘテロアリールから選択される基を形成し、ここで、A および E は、単環式、二環式および多環式であってもよく、好ましくは単環式であり；各 A および E は、 $-\text{H}$ 、 $-\text{C}_{1-6}$ -アルキル、 $-\text{O}-\text{C}_{1-6}$ -アルキル、 $-\text{S}-\text{C}_{1-6}$ -アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび $-\text{C}_{1-6}$ -アラルキルからなる群から選択される 1～4 個の残基によって置換されていてよく、各 $-\text{C}_{1-6}$ -アルキルは、 $-\text{OH}$ 、オキソおよびハロから選択される 1～3 個の置換基によって置換されていてよく、また、A、B、D、E または

40

【化4】



に結合されていてよく；

50

Bは、S、NR⁴、NR⁴-O、NR⁴-C₁₋₆-アルキル、NR⁴-C₁₋₆-アルキル-NR⁴および5～10員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環からなる群から選択され、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、NR⁴-C₁₋₆-アルキル-NR⁴および該N-含有ヘテロ環は、C₁₋₆-アルキル、アリール、C₁₋₆-アラルキルからなる群から選択される1～3個の置換基によって置換されており；そして、

R⁸は、放射性部分、キレート剤、蛍光色素、造影剤およびそれらの組み合わせからなる群から選択され；

【化5】



は、1-ナフチル基部分または5～10員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、ここに該ヘテロ環はN原子とXとの間に2個の環原子が存在し；また、該ヘテロ環はO、NおよびSから選択される1、2または3個のヘテロ原子をさらに含んでいてよく；そして、XはC原子である]

で示される化合物、またはその薬学的に許容できる互変異性体、ラセミ体、水和物、溶媒和物もしくは塩、を提供する。

【0008】

第2の態様において、本発明は、少なくとも一つの第1の態様の化合物、および製薬的に許容できる担体および／または添加物を含むか、またはそれらからなる医薬組成物に関する。

【0009】

第3の態様において、本発明は、動物またはヒトの対象における線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患の診断または処置に使用するための、第1の態様の化合物または第2の態様の医薬組成物に関する。

【0010】

第4の態様において、本発明は、第1の態様の化合物もしくは第2の態様の医薬組成物および疾患の診断のためのインストラクションを含むか、またはそれらからなるキットに関する。

【図面の簡単な説明】

【0011】

以下では、本明細書に含まれる図の内容について説明する。なお、この文脈においては、上記および／または以下の本発明の詳細な説明を参照すること。

【0012】

【図1】¹²⁵I-FAPI-01および¹⁷⁷Lu-FAPI-02のインビトロ特性評価 A. 60分間のインキュベーション後の、放射性標識FAPI-01およびFAPI-02のさまざまなヒト癌細胞株ならびにヒトFAP-αでトランスフェクトされた細胞株(HT-1080-FAP)、マウスのFAP-αでトランスフェクトされた細胞株(HEK-muFAP)およびヒトCD26でトランスフェクトされた細胞株(HEK-CD26)への結合。B. 10分～24時間のインキュベーション後の放射性標識FAPI-01およびFAPI-02のHT-1080-FAP細胞への内在化。内在化された割合はそれぞれ灰色と黒色で示し；細胞外結合画分は白色バーで示している。C. 濃度を増加させた非標識FAPI-01およびLu-FAPI-02を添加した後の放射性標識FAPI-01およびFAPI-02のHT-1080-FAP細胞への競合結合。D. FAPI-02のFAP-α陽性細胞株およびFAP-α陰性細胞株への内在化。青：DAPI；緑：FAPI-02-Att488。E+F. HT-1080-FAP細胞を放射性標識化合物と1時間インキュベートした後、化合物を含まない培地で1～24時間インキュベートした後のFAPI-01およびFAPI-02の流出動態。すべての値は、10

10

20

30

40

50

0 万細胞に正規化した総適用量のパーセンテージ(% I D / 1 m i o 細胞)で示している。

【0013】

【図2】F A P I 誘導体の結合特異性および相対的な内在化率。A - C. F A P I - 0 2 (100%と定義)に関してのF A P I - 0 3 ~ F A P I - 1 5 の結合率及び内在化率。1 時間、4 時間および24 時間のインキュベーション後の内在化率を灰色で示し; 細胞外結合画分を白色バーで示している。D. 60 分間のインキュベーション後のマウスのF A P - α およびヒトCD26を発現するHEK細胞への選択されたF A P I 誘導体の結合。右側: m u F A P とCD26の結合率。E. 濃度を増加させた非標識化合物を添加した後の選択されたF A P I 誘導体のHT-1080-FAP細胞への競合結合。

【0014】

【図3】ヒトFAP陽性(HT-1080-FAP)および陰性(Capan-2、SK-LMS-1)腫瘍異種移植担持マウスにおけるF A P I - 0 2 および-04のイメージング。A + C、E + G. 小動物PETイメージングは、 ^{68}Ga -F A P I - 0 2 および-04(それぞれ10MBq)4nmolを静脈内投与後に示された時刻で行った。放射性追跡子は非癌性組織には蓄積されないが、腫瘍内で急速に濃縮される(赤い矢印で示される)。さらに、腎臓および膀胱を介した急速な排出が見られる。B + D、F + H. PET画像の定量化は、心血管系からの ^{68}Ga -F A P I - 0 2 および-04の固体クリアランスならびに腫瘍への一定の取り込みを証明している。

【0015】

【図4】インビボでの結合特異性を分析するためのブロッキング実験 A + D. 非標識化合物30nmolをHT-1080-FAP腫瘍担持マウスに共同投与することによる ^{68}Ga -F A P I - 0 2 および-04の腫瘍蓄積のブロッキング。B + C、E + F. 競合物質として非標識化合物を使用する場合と使用しない場合の静脈内投与後の選択された臓器における ^{68}Ga -F A P I - 0 2 および-04の時間放射活性曲線(time activity curve)。

【0016】

【図5】HT-1080-FAP腫瘍担持ヌードマウスにおける ^{177}Lu -F A P I - 0 2 および-04の臓器分布 A - C. ^{177}Lu -F A P I - 0 2 および-04の生体内分布は、1MBqをヒトFAP陽性のHT-1080腫瘍異種移植担持マウスに静脈内投与した後、指示された時刻でエキスピドで測定し; 各時点でn = 3。記載された値は、組織1グラムあたりの投与量に対するパーセンテージ(% I D / g)として示している。放射性追跡子がFAP発現腫瘍内に蓄積することを示し、最大の濃縮は1時間後にF A P I - 0 2 (4.5% I D / g)で、2時間後にF A P I - 0 4 (5.4% I D / g)で示している。D-F. ^{177}Lu -F A P I - 0 2 および-04の静脈内投与1時間後、4時間後および24時間後の腫瘍対正常組織の比。

【0017】

【図6】癌患者におけるF A P I - 0 2 のPET/CTイメージング6A - C. 転移性乳癌に罹患している患者のPET/CTスキャンの最大強度予測(MIP)。D. ^{68}Ga -F A P I - 0 2 の転移性乳癌患者への静脈内投与10分後、1時間後、3時間後の最大組織取り込み。

【0018】

【図7】癌患者におけるF A P I - 0 2 のPET/CTイメージング膵臓癌、非小細胞肺癌(NSCLC)、食道癌、直腸癌患者における ^{68}Ga -F A P I - 0 2 投与1時間後のPET/CTスキャンのMIP。

【0019】

【図8】癌患者におけるF A P I - 0 2 のPET/CTイメージング鼻咽頭および喉頭癌患者における ^{68}Ga -F A P I - 0 2 投与1時間後のPET/CTスキャンのMIP。

【0020】

【図9】癌患者におけるF A P I - 0 2 のPET/CTイメージング9A + B. ^{18}F -FDGおよび ^{68}Ga -F A P I - 0 2 を局所進行性肺腺癌患者に投与して1時間後の全

10

20

30

40

50

身PET/CTイメージング(MIP)。C+D.肺腺癌患者の ^{18}F -FDGおよび ^{68}Ga -FAPI-02投与1時間後の経軸写真。FAPI-02はFAP- α 発現組織に選択的に蓄積され、 ^{18}F -FDGと比較して悪性病変への有意に高い取り込みを示している。

【0021】

【図10-16】癌患者におけるFAPI-04のPET/CTイメージング 10. 転移性乳癌患者の ^{68}Ga -FAPI-04投与10分後、1時間後、3時間後のPET/CTスキャンの最大値投影(MIP)。

【0022】

【図11】シグマ癌、下咽頭癌、神経内分泌腫瘍、胆管癌、卵巣癌および小腸癌患者における ^{68}Ga -FAPI-04投与1時間後のPET/CTスキャンのMIP。 10

【0023】

【図12】肺癌患者における ^{68}Ga -FAPI-04投与1時間後のPET/CTスキャンのMIP。

【0024】

【図13】発癌性くる病患者における ^{68}Ga -FAPI-04投与1時間後のPET/CTスキャンのMIP。

【0025】

【図14】転移性前立腺癌患者1名の比較画像診断。放射性標識DOTATATOC、PSMAおよびFAPI-04適用1時間後のPET/CTスキャンのMIP。 20

【0026】

【図15】膵臓癌患者における動的な ^{68}Ga -FAPI-04のPET/CTスキャンの最大強度投影(MIP)および時間放射活性曲線。

【0027】

【図16】FAP発現HT-1080細胞上で1、4および24時間インキュベーションした後のLu-177標識FAPI誘導体のFAPI-04(100%に設定)と比較した相対的な結合率; $n=3$ 。

【0028】

【図17】濃度を増加させた非標識化合物($10^{-10} \sim 10^{-5} \text{ M}$ 、60分間インキュベーション、 $n=3$)を添加した後の選択されたFAPI誘導体のHT-1080-FAP細胞への競合結合。 30

【0029】

【図18】60分間のインキュベーション後のFAPI誘導体のマウスFAPおよびヒトCD26を発現するHEK細胞への結合、 $n=3$ 。値は、1mio細胞あたりの適用量(%ID)に対するパーセンテージとして示している。

【0030】

【図19】放射性追跡子静脈内投与1時間後、4時間後24時間後のHT-1080-FAP異種移植における選択されたFAPI誘導体の生体内分布、 $n=3$ 。値は、組織の1グラムあたりの投与量に対するパーセンテージとして示している(%ID/g)。

【0031】

【図20】放射性追跡子静脈内投与1時間後、4時間後および24時間後のHT-1080-FAP異種移植における選択されたFAPI誘導体の腫瘍対血液の比、 $n=3$ 。 40

【0032】

【図21】HT-1080-FAP腫瘍担持マウスにおけるGa-68標識FAPI-21およびFAPI-46のPETイメージング; $n=1$ 。

【0033】

【図22】HT-1080-FAP腫瘍担持マウスにおける選択されたFAPI誘導体の最大標準化取り込み値(SUV); $n=1$ 。

【0034】

【図23】癌患者におけるGa-68標識FAPI-02およびFAPI-04の最大(50

SUVmax、図23A)および平均(SUVmean、図23B)標準化取り込み値； $n = 25$ 。

【0035】

【図24】9日以内にFDG-PETおよびFAP I-PETイメージングを受けた6種類の異なる腫瘍実体を有する患者6人の交差比較(Intra-individual comparison)。

【0036】

【図25】注射1時間後(1h p.i.)の癌性腹膜炎(A)、心筋炎(B)および股関節症(hip joint arthrosis)患者におけるGa-68標識FAP I-04 PET/CTイメージング。

【0037】

【図26】注射1時間後(1h p.i.)の癌患者におけるGa-68標識FAP I-21のPET/CTイメージング

【0038】

【図27】癌患者における注射1時間後(1h p.i.)のGa-68標識FAP I-46のPET/CTイメージングおよび注射30分後(30min p.i.)のSm-153標識FAP I-46治療用イメージング(intratherapeutic imaging)

【0039】

【図28】注射20時間後(20h p.i.)までのSm-153標識FAP I-46の治療用イメージング

【0040】

【図29】A. 転移性結腸直腸癌患者への ^{68}Ga -FAP I-46静脈内投与1時間後の最大値投影(MIP)。B. ^{90}Y -FAP I-46で同一患者を治療処置して2時間後の制動放射イメージング

【0041】

【図30】特発性肺線維症を有する肺癌患者における注射1時間後(1h p.i.)のGa-68標識FAP I-46のPET/CTイメージング。A、B. 腫瘍組織への追跡子最大取り込みは、非増悪性線維性病変への追跡子最大取り込みより有意に高い。C. 腫瘍組織への追跡子最大取り込みは、増悪線維性組織への追跡子最大取り込みより若干低い。

【0042】

【図31】A. Tc-99m標識FAP I-19のHT-1080-FAP細胞への結合、 $n = 3$ 。B. 濃度を増加させた非標識化合物を添加した後のTc-99m標識FAP I-19のHT-1080-FAP細胞への結合($10^{-10} \sim 10^{-5} \text{ M}$ 、60分間のインキュベーション、 $n = 3$)。C. HT-1080-FAP異種移植におけるTc-99m標識FAP I-19のシンチグラフィー、 $n = 1$ 。

【0043】

【図32】A. Tc-99m標識FAP I-34のHT-1080-FAP細胞への結合、 $n = 3$ 。B. HT-1080-FAP異種移植におけるTc-99m標識FAP I-34のシンチグラフィー、 $n = 1$ 。

【0044】

【図33】一人の転移性膵臓癌患者におけるTc-99m標識FAP I-34のシンチグラフィー。

【0045】

【図34】A. Pb-203標識FAP I誘導体のHT-1080-FAP細胞への結合、 $n = 3$ 。B. HT-1080-FAP細胞を放射性標識化合物と60分間インキュベートし、非放射性培地で1～時間続いてインキュベートした後のPb-203標識FAP I誘導体の流出動態、 $n = 3$ 。C. 濃度を増加させた非標識化合物を添加した後のPb-203標識FAP IのHT-1080-FAP細胞への競合結合($10^{-10} \sim 10^{-5} \text{ M}$ 、60分間のインキュベーション、 $n = 3$)。

【0046】

【図35】HT-1080-FAP異種移植におけるPb-203標識FAP I-04お

10

20

30

40

50

よびFAP I-46のシンチグラフィー、 $n=1$ 。

【0047】

【図36】放射性追跡子静脈内投与1時間後、4時間後、6時間後および24時間後のHT-1080-FAP異種移植におけるPb-203標識FAP I-04およびFAP I-46の生体内分布 $n=3$ 。値は、組織1グラム当たりの注入された量のパーセンテージ($\%ID/g$)として示されている。

【0048】

【図37】A. Cu-64標識FAP I-42およびFAP I-52のHT-1080-FAP細胞への結合、 $n=3$ 。B. 濃度を増加させた非標識化合物を添加した後のCu-64標識FAP I-42およびFAP I-52のHT-1080-FAP細胞への競合結合($10^{-10} \sim 10^{-5} M$ 、60分間のインキュベーション、 $n=3$)。C. HT-1080-FAP細胞を放射性標識化合物と60分間インキュベートし、非放射性培地で1~24時間続いてインキュベートした後のCu-64標識FAP I-42およびFAP I-52の流出動態、 $n=3$ 。

【0049】

【図38】HT-1080-FAP腫瘍担持マウスにおけるCu-64標識FAP I-42およびFAP I-52のPETイメージング； $n=1$ 。

【0050】

【図39】HT-1080-FAP腫瘍担持マウスにおけるAlF-18標識FAP I-42およびFAP I-52のPETイメージング； $n=1$ 。

【0051】

【図40】a. 放射性追跡子静脈内投与後140分までのU87MG腫瘍担持ヌードマウスにおける68Ga標識FAP I-02の小動物PETイメージング。腫瘍は赤い矢印で示している。b. 放射性追跡子静脈内投与1時間後、4時間後および24時間後のU87MG腫瘍担持ヌードマウスにおける ^{177}Lu -標識FAP I-02およびFAP I-04の生体内分布； $n=3$ 。

【0052】

【図41】静脈内投与1時間後、4時間後および24時間後のU87MG腫瘍担持マウスにおける ^{177}Lu -標識FAP I-02および-04の腫瘍対臓器の比。

【0053】

【図42】68Ga-FAP I-02投与10分後、1時間後および3時間後の神経膠芽腫患者におけるPET/CTスキャンの最大値投影(MIP)。

【0054】

【図43】IDHwt神経膠芽腫、IDH変異神経膠腫WHOグレードIIおよびIDH変異神経膠芽腫の例示的な画像(コントラスト強化T1強調MRI、FAP I-PETおよび両モダリティの融合画像)。

【0055】

【図44】18種類の神経膠腫すべての絶対SUVmax値。

【0056】

【図45】SUVmax/BG値の統計分析。GBM対非GBM(a、b)、IDH変異型vs IDH野生型神経膠腫(c、d)および神経膠腫グレードII対神経膠腫グレードII I/IV(e、f)におけるSUVmax/BG値の箱ひげ図および対応するROC曲線。

【0057】

【図46】FAP I-04およびタラボスタットによる酵素的FAP活性の用量依存性阻害。限界的なFAP活性を有する強力なDPP4阻害物質であるタラボスタットとは対照的に、FAP I-04はロバストな用量依存性FAP阻害を証明している。

【0058】

【図47】HT-1080-FAP細胞における ^{177}Lu -標識FAP I-04およびFAP I-46の再取り込み。37℃で細胞を放射性追跡子と60分間インキュベートした後、化合物を除去し、非標識化合物を含む(+Comp.)および含まない(-Comp.)

非放射性培地を加え、10分～6時間インキュベートする。インキュベーションの最初の10分以内にすでに、非標識FAP I誘導体の新たな取り込みが発生し、放射性標識画分の一部が置換され、その結果、競合相手のない純粋な培地と比較して、放射性値が優位に低下する。6時間のインキュベーション後、放射性標識FAP Iのほぼ完全な置換が発生している。これらの所見は、最初の内在化時に細胞膜に戻ったインタクトなFAP分子の継続的な再取り込みを示し、FAPリガンドの新たな結合および内在化を可能にする。

【0059】

【図48】HT-1080-FAP腫瘍担持ヌードマウスにおける単回および多回注射後の ^{177}Lu -FAP I-04の臓器分布。等量の ^{177}Lu -FAP I-04を4時間間隔で2回投与することにより、最初の注射8時間後と24時間後に測定された、腫瘍を含む全体的な臓器活性の増加をもたらした。これに対して、3回投与(初期投与量を高くし、その後の投与量を低くした)では、全体的な臓器活性に変化がないことを明らかにする。

10

【0060】

【図49】10分、30分、60分および90分のインキュベーション後のF-18-FAP I誘導体のヒトFAP発現HT1080細胞への結合、 $n=3$ 。値は、1mio細胞あたりの適用された量のパーセンテージ(%ID)として示されている。

【0061】

【図50】HT-1080-FAP腫瘍担持マウスにおけるAIF-18標識FAP I-74およびFAP I-52のPETイメージング； $n=1$ 。

20

【0062】

【図51】放射性追跡子静脈内投与1時間後、4時間後および24時間後のHT-1080-FAP異種移植におけるFAP I-75の生体内分布、 $n=3$ 。値は、組織1グラムあたりの投与量に対するパーセンテージ(%ID/g)として示されている。

【0063】

【図52】非小細胞性肺癌患者のPETイメージング：多重転移におけるF18-標識FAP I-74のロバストな蓄積。

【0064】

【図53】高速血液プールクリアランスの図示としてのFAP I-04および-46の心臓領域(SUVmean)の時間放射性曲線。

30

【0065】

【図54】転移性乳癌患者2人における異なるイメージング時点(注射10分後、1時間後、3時間後)でのFAP I-02とFAP I-04。急速な腫瘍標的化および迅速な血液クリアランスの後に、画像コントラストに関連する変化がない長いプラトー期が続く(上)。FAP I-02と比較して、リガンドFAP I-04は長期腫瘍保持時間を特徴とする(下)。

【0066】

【図55】FAP I-02の実効線量は、OLINDAで計算して $1.80\text{E}-02\text{mSv}/\text{MBq}$ であった(IDAC1/ICRP60では $1.82\text{E}-02$ 、IDAC2/ICRP103では $1.79\text{E}-02$)。FAP I-04PET/CTの実効線量は、OLINDAで計算して $1.64\text{E}-02\text{mSv}/\text{MBq}$ であった(IDAC1/ICRP60では $1.66\text{E}-02$ 、IDAC2/ICRP103では $1.35\text{E}-02$)。臨床現場で注射3時間後での遅延スキャンを省略すれば、FAP I検査のルーチン活動は 200MBq の ^{68}Ga に減少でき、このようなFAP I-PET/CTスキャンの放射線量は連続して $3-4\text{mSv}$ となる。

40

【0067】

【図56】A)PETCTにおける異なる腫瘍実体への注射1時間後の ^{68}Ga -FAP I-04。最も高い平均SUVmax(>12)は、肉腫癌、食道癌、乳房癌、胆管細胞癌および肺癌で見つかった。最も低いFAP I取り込み(平均SUVmax<6)は、腎細胞癌、分化型甲状腺癌、腺様嚢胞癌、胃癌および褐色細胞腫で観察された。肝細胞癌、結

50

腸直腸癌、頭頸部癌、卵巣癌、膵臓癌の平均 SUV_{max} は中間 ($SUV_6 < x < 12$) だった。すべての腫瘍実体内で、高い個体間変動が観察された。低バックグラウンド放射活性 (SUV_2) のため、腫瘍対バックグラウンドの比は、中間取り込み群では > 2 倍、高強度取り込み群では > 4 倍である。B) 原発性腫瘍実体では、FAP I-04 を使用した腫瘍実体と比較して、類似の SUV 取り込みを示した。

【0068】

【図57】図56のA-Bに示される定量化に使用された、異なる腫瘍実体の例示的なPET画像。

【0069】

本発明の詳細な説明

本発明を以下に詳細に説明する前に、本発明は、本明細書に記載される特定の方法プロトコールおよび試薬が変化し得るので、これらに限定されないことが理解されるであろう。また、本明細書で使用される用語は、特定の実施態様のみを説明する目的のためのものであり、添付の特許請求の範囲のみによって限定される本発明の範囲を限定することを意図していないことが理解される。別に定義しない限り、本明細書で使用されるすべての技術的および科学的用語は、当業者が一般的に理解するのと同じ意味を有する。

【0070】

好ましくは、本明細書で使用される用語は、「A multilingual glossary of biotechnological terms: (IUPAC Recommendations)」, Leuenberger, H.G.W, Nagel, B. and Klibi, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland)に記載されているように定義する。

【0071】

本明細書およびそれに続く特許請求の範囲全体を通して、文脈上別段の要求がない限り、用語「comprise」および「comprises」および「comprising」のような変動は、記載される整数または工程または整数もしくは工程のグループを包含することを意味し、他の整数または工程または整数もしくは工程のグループを除外することを意味しないと理解されるであろう。以下のパッセージでは、本発明のさまざまな態様をより詳細に定義する。このように定義される各態様は、反対に明確に示されない限り、他の態様 (aspect) または態様 (aspects) と組み合わせてもよい。特に、任意であること、好ましいこと、または有利であることが示されているいずれかの特徴は、任意であること、好ましいこと、または有利であることが示されている他の特徴 (feature) または特徴 (features) と組み合わせてもよい。

【0072】

本明細書の本文全体にわたっていくつかの文書が引用されている。本明細書に引用される各文書 (すべての特許、特許出願、科学出版物、製造業者の仕様書、インストラクションなどを含む) は、上記または下記にかかわらず、その全体を参照することにより、ここに組み込まれる。本明細書のいかなる記述も、本発明が先行発明を理由に、そのような開示を先取りする権利を有していないことを認めるものと解釈されるものではない。本明細書に引用される文書の一部は、「参照により組み込まれている」ことを特徴とする。このような組み込まれる文献の定義または教示と、本明細書で引用される定義または教示との間に矛盾がある場合には、本明細書の本文が優先される。

【0073】

以下では、本発明の要素について説明する。これらの要素は、特定の実施態様で列挙されているが、さらなる実施態様を作成するために、いずれかの方法でおよびいずれかの数で組み合わせてもよいことが理解されるべきである。さまざまに記載される実施例および好ましい実施態様は、本発明を明示的に記載される実施態様のみに限定するように解釈されるべきではない。本明細書は、明示的に記載される実施態様を、あらゆる開示されるおよび/または好ましい要素と組み合わせる実施態様を支持し、包含すると理解されるべきである。さらに、本願に記載されるすべての要素のいずれかの順列および組み合わせは、文脈がそうでないことを示さない限り、本願の説明によって開示されているとみなされる

10

20

30

40

50

べきである。

【0074】

定義

以下では、本明細書でよく使用される用語の定義をいくつか示す。これらの用語は、その使用の各実例において、本明細書の残りの部分において、それぞれ定義される意味および好ましい意味を有する。

【0075】

本明細書および添付の特許請求の範囲で使用されるように、単数形の「a」、「an」および「the」は、内容が明らかに他のことを指示しない限り、複数の指示対象を含む。

【0076】

以下に用語：アルキル、ヘテロアルキル、シクロアルキル、ヘテロシクロアルキル、アリール、アラルキル、ヘテロアリール、ヘテロアラルキル、アルケニルおよびアルキニルの定義を提供する。これらの用語は、本明細書の残りの部分で使用される各実例において、それぞれ定義される意味および好ましい意味を有する。

【0077】

用語「アルキル」は、飽和の直鎖または分岐炭素鎖を意味する。好ましくは、該鎖は、1～10個、すなわち1個、2個、3個、4個、5個、6個、7個、8個、9個または10個の炭素原子を含む(例えばメチル、エチルメチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、tert-ブチル、ペンチル、ヘキシル、ペンチルまたはオクチル)。アルキル基は置換されていてよい。

【0078】

用語「ヘテロアルキル」は、飽和の直鎖または分岐炭素鎖を意味する。好ましくは、該鎖は、1～9個、すなわち1個、2個、3個、4個、5個、6個、7個、8個または9個の炭素原子を含む(例えばメチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec-ブチル、tert-ブチル、ペンチル、ヘキシル、ペンチル、オクチル)、これらは、同一または異なるヘテロ原子によって1回またはそれ以上、例えば1回、2回、3回、4回または5回中断される。好ましくは、ヘテロ原子は、O、SおよびNから選択され、例えば-O-CH₃、-S-CH₃、-CH₂-O-CH₃、-CH₂-O-C₂H₅、-CH₂-S-CH₃、-CH₂-S-C₂H₅、-C₂H₄-O-CH₃、-C₂H₄-O-C₂H₅、-C₂H₄-S-CH₃、-C₂H₄-S-C₂H₅などである。ヘテロアルキル基は置換されていてよい。

【0079】

用語「シクロアルキル」および「ヘテロシクロアルキル」は、別段の記載がない限り、それ自体または他の用語と組み合わせて、「アルキル」および「ヘテロアルキル」の環状バージョンをそれぞれ示し、好ましくは、3個、4個、5個、6個、7個、8個、9個または10個の原子が環、例えばシクロプロピル、シクロブチル、シクロペンチル、シクロヘキシル、シクロヘプチル、シクロオクチルなどを形成する。用語「シクロアルキル」および「ヘテロシクロアルキル」はまた、それらの二環式、三環式および多環式バージョンを含むことを意味する。用語「ヘテロシクロアルキル」は、好ましくは、5員飽和環(少なくとも1員がN、OまたはS原子であり、1つのさらなるOまたは1つのさらなるNを含んでいてよい)；6員飽和環(少なくとも1員がN、OまたはS原子であり、1つのさらなるOまたは1つのさらなるNまたは2つのさらなるN原子を含んでいてよい)；または9員または10員飽和二環式環(少なくとも1員がN、OまたはS原子であり、1つのさらなるN原子、2つのさらなるNまたは3つのさらなるN原子を含んでいてよい)を意味する。「シクロアルキル」および「ヘテロシクロアルキル」基は、置換されていてよい。さらに、ヘテロシクロアルキルの場合、ヘテロ原子は、分子の残りの部分にヘテロ環が結合している位置を占めることができる。シクロアルキルの例としては、シクロプロピル、シクロブチル、シクロペンチル、シクロヘキシル、1-シクロヘキセニル、3-シクロヘキセニル、シクロヘプチル、スピロ[3,3]ヘプチル、スピロ[3,4]オクチル、スピ

10

20

30

40

50

ロ [4, 3] オクチル、スピロ [3, 5] ノニル、スピロ [5, 3] ノニル、スピロ [3, 6] デシル、スピロ [6, 3] デシル、スピロ [4, 5] デシル、スピロ [5, 4] デシル、ビシクロ [2, 2, 1] ヘプチル、ビシクロ [2, 2, 2] オクチル、アダマンチルなどがある。ヘテロシクロアルキルの例としては、1-(1, 2, 5, 6-テトラヒドロピリジル)、1-ピペリジニル、2-ピペリジニル、3-ピペリジニル、4-モルホリニル、3-モルホリニル、1, 8 ジアゾースピロ [4, 5] デシル、1, 7 ジアゾースピロ [4, 5] デシル、1, 6 ジアゾースピロ [4, 5] デシル、2, 8 ジアゾースピロ [4, 5] デシル、2, 7 ジアゾースピロ [4, 5] デシル、2, 6 ジアゾースピロ [4, 5] デシル、1, 8 ジアゾースピロ [5, 4] デシル、1, 7 ジアゾースピロ [5, 4] デシル、2, 8 ジアゾースピロ [5, 4] デシル、2, 7 ジアゾースピロ [5, 4] デシル、3, 8 ジアゾースピロ [5, 4] デシル、3, 7 ジアゾースピロ [5, 4] デシル、1-アゾ-7, 11-ジオキソースピロ [5, 5] ウンデシル、1, 4-ジアザビシクロ [2, 2, 2] オクタ-2-イル、テトラヒドロフラン-2-イル、テトラヒドロフラン-3-イル、テトラヒドロチエン-2-イル、テトラヒドロチエン-3-イル、1-ピペラジニル、2-ピペラジニルなどがある。

【0080】

用語「アリール」とは、好ましくは6個の炭素原子を含有する芳香族単環式環、10個の炭素原子を含有する芳香族二環式環系または14個の炭素原子を含有する芳香族三環式環系を意味する。例としては、フェニル、ナフチルまたはアントラセニルがある。アリール基は置換されていてよい。

【0081】

用語「アラルキル」とは、アリールによって置換されているアルキル部分を意味し、ここで、アルキルおよびアリールは、上記で概説された意味を有する。例としてはベンジル基がある。好ましくは、この文脈において、アルキル鎖は、1~8個、すなわち1個、2個、3個、4個、5個、6個、7個または8個の炭素原子を含む(例えばメチル、エチル、メチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec-ブチル、tert-ブチル、ペンチル、ヘキシル、ペンチル、オクチル)。アラルキル基は、基のアルキルおよび/またはアリール部分で置換されていてよい。

【0082】

用語「ヘテロアリール」とは、好ましくは、炭素原子の中の少なくとも1個が、好ましくはO、NおよびSから選択される同一または異なる1個、2個、3個または4個(5員環の場合)または1個、2個、3個、4個または5個(6員環の場合)のヘテロ原子によって置換されている5または6員芳香族単環式環; 8個、9個、10個、11個または12個の炭素原子の中の1個、2個、3個、4個、5個または6個の炭素原子が、好ましくはO、NおよびSから選択される同一または異なるヘテロ原子によって置換されている芳香族二環式環系; または13個、14個、15個または16個の炭素原子の中の1個、2個、3個、4個、5個または6個の炭素原子が、好ましくはO、NおよびSから選択される同一または異なるヘテロ原子によって置換されている芳香族三環式環系を意味する。例としては、オキサゾリル、イソオキサゾリル、1, 2, 5-オキサジアゾリル、1, 2, 3-オキサジアゾリル、ピロリル、イミダゾリル、ピラゾリル、1, 2, 3-トリアゾリル、チアゾリル、イソチアゾリル、1, 2, 3-チアジアゾリル、1, 2, 5-チアジアゾリル、ピリジニル、ピリミジニル、ピラジニル、1, 2, 3-トリアジニル、1, 2, 4-トリアジニル、1, 3, 5-トリアジニル、1-ベンゾフラニル、2-ベンゾフラニル、インドイル(indoyl)、イソインドイル(isoindoyl)、ベンゾチオフェニル、2-ベンゾチオフェニル、1H-インダゾリル、ベンゾイミダゾリル、ベンゾオキサゾリル、インドオキサジニル(indoxazinyll)、2, 1-ベンゾオキサゾリル、ベンゾチアゾリル、1, 2-ベンゾイソチアゾリル、2, 1-ベンゾイソチアゾリル、ベンゾトリアゾリル、キノリニル、イソキノリニル、キノキサリニル、キナゾリニル、キノリニル、1, 2, 3-ベンゾトリアジニルまたは1, 2, 4-ベンゾトリアジニルがある。

【0083】

用語「ヘテロアラルキル」とは、ヘテロアリールによって置換されているアルキル部分
を意味し、ここで、アルキルおよびヘテロアリールは、上記で概説された意味を有する。
例としては、2-アルキルピリジニル、3-アルキルピリジニルまたは2-メチルピリジ
ニルがある。好ましくは、この文脈において、アルキル鎖は、1～8個、すなわち1個、
2個、3個、4個、5個、6個、7個または8個の炭素原子を含む(例えばメチル、エチ
ルメチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec-ブテ
ニル、tert-ブチル、ペンチル、ヘキシル、ペンチル、オクチル)。ヘテロアラルキル
基は、基のアルキルおよび／またはヘテロアリールで置換されていてよい。

【0084】

「アルケニル」および「シクロアルケニル」とは、一つまたはそれ以上の二重結合を有
する鎖または環を含有するオレフィン不飽和炭素原子を意味する。例としては、プロペ
ニルおよびシクロヘキセニルがある。好ましくは、アルケニル鎖は、2～8個、すなわち2
個、3個、4個、5個、6個、7個または8個の炭素原子を含む(例えばエテニル、1-
プロペニル、2-プロペニル、イソプロペニル、1-ブテニル、2-ブテニル、3-ブ
テニル、イソブテニル、sec-ブテニル、1-ペンテニル、2-ペンテニル、3-ペ
ンテニル、4-ペンテニル、ヘキセニル、ペンテニル、オクテニル)。好ましくは、シク
ロアルケニル環は、3～8個、すなわち3個、4個、5個、6個、7個または8個の炭素
原子を含む(例えば1-シクロプロペニル、2-シクロプロペニル、1-シクロブテニル
、2-シクロブテニル、1-シクロペンテニル、2-シクロペンテニル、3-シクロペン
テニル、シクロヘキセニル、シクロペンテニル、シクロオクテニル)。

【0085】

用語「アルキニル」とは、一つまたはそれ以上の三重結合を有する鎖または環を含有す
る不飽和炭素原子を意味する。例としてはプロパルギル基がある。好ましくは、アルキ
ニル鎖は、2～8個、すなわち2個、3個、4個、5個、6個、7個または8個の炭素原子
を含む(例えばエチニル、1-プロピニル、2-プロピニル、1-ブチニル、2-ブチ
ニル、3-ブチニル、1-ペンチニル、2-ペンチニル、3-ペンチニル、4-ペンチニル
、ヘキシニル、ペンチニル、オクチニル)。

【0086】

一実施態様において、アルキル、ヘテロアルキル、シクロアルキル、アリール、アラ
ルキル、アルケニル、シクロアルケニル、アルキニル基中の炭素原子または水素原子は、互
いに独立して、O、SおよびNからなる群から選択される一つまたはそれ以上の元素によ
って置換されていてよく、またはO、SおよびNからなる群から選択される一つまたはそ
れ以上の元素を含む基によって置換されていてよい。

【0087】

実施態様としては、アルコキシ、シクロアルコキシ、アリールオキシ、アラ
ルコキシ、アルケニルオキシ、シクロアルケニルオキシ、アルキニルオキシ、アルキルチ
オ、シクロアルキルチオ、アリールチオ、アラ
ルキルチオ、アルケニルチオ、シクロアルケニルチオ
、アルキニルチオ、アルキルアミノ、シクロアルキルアミノ、アリールアミノ、アラ
ルキルアミノ、アルケニルアミノ、シクロアルケニルアミノ、アルキニルアミノ基がある。

【0088】

他の実施態様としては、ヒドロキシアルキル、ヒドロキシシクロアルキル、ヒドロキシ
アリール、ヒドロキシアラルキル、ヒドロキシアルケニル、ヒドロキシシクロアルケニ
ル、ヒドロキシアリニル、メルカプトアルキル、メルカプトシクロアルキル、メルカプト
アリール、メルカプトアラルキル、メルカプトアルケニル、メルカプトシクロアルケニ
ル、メルカプトアルキニル、アミノアルキル、アミノシクロアルキル、アミノアリー
ル、アミノアラルキル、アミノアルケニル、アミノシクロアルケニル、アミノアルキ
ニル基がある。

【0089】

別の実施態様において、アルキル基、ヘテロアルキル基、シクロアルキル基、アリー
ル基、アラルキル基、アルケニル基、シクロアルケニル基、アルキニル基中の水素原子は、

互いに独立して、1つまたはそれ以上のハロゲン原子によって置換され得る。一つの基としては、トリフルオロメチル基がある。

【0090】

2つもしくは2つ以上の基または2つもしくは2つ以上の残基が互いに独立して選択され得る場合、用語「独立して」とは、基または残基が同一であってもよく、異なっているもよいことを意味する。

【0091】

本明細書で使用されるように、例えば「1～6」のような長さの範囲の限定を定義する語句は、1から6までのいずれかの整数、すなわち1、2、3、4、5および6を意味する。言い換えれば、明示的に記載された2つの整数によって定義されるいずれかの範囲は、上記の限定を定義するいずれかの整数と、前記範囲に含まれるいずれかの整数とを含み、開示することを意味する。

【0092】

本明細書で使用される用語「ハロ」とは、F、Br、IおよびClからなる群から選択されるハロゲン残基を意味する。好ましくは、ハロゲンはFである。

【0093】

本明細書で使用される用語「リンカー」とはいずれかの化学的に適当なリンカーを意味する。好ましくは、リンカーは、生理学的条件下では切断されないか、またはゆっくりとしか切断されない。このように、好ましくは、リンカーは、プロテアーゼに対する認識配列や他の分解酵素に対する認識構造を含まない。本発明の化合物は、全身的に投与され、体内のすべての区画への広範なアクセスを可能にし、続いて、体内のどこに腫瘍が位置していても本発明の化合物が濃縮されることが好ましいため、リンカーは、血中では切断されないか、またはゆっくりとしか切断されないような形で選択されることが好ましい。化合物をヒト患者に投与してから2時間後にリンカーの50%未満が切断される場合、切断はゆっくりと見なされる。適当なリンカーは、例えば置換されていてよいアルキル、ヘテロアルキル、シクロアルキル、シクロヘテロアルキル、アリール、ヘテロアリール、アラキル、ヘテロアラキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、スルホニル、アミン類、エーテル類、チオエーテル類、ホスフィーン類、ホスホルアミデート類、カルボキサミド類、エステル類、イミドエステル類、アミジン類、チオエステル類、スルホンアミド類、3-チオピロリジン-2,5-ジオン、カルバメート類、尿素類、グアニジン類、チオ尿素類、ジスルフィド類、オキシム類、ヒドラジン類、ヒドラジド類、ヒドラゾン類、ジアザ結合類、トリアゾール類、トリアゾリン類、テトラジン類、白金錯体類およびアミノ酸類またはそれらの組み合わせを含むか、またはそれらからなる。好ましくは、該リンカーは、1,4-ピペラジン、1,3-プロパンおよびフェノール系エーテルまたはそれらの組み合わせを含むか、またはそれらからなる。

【0094】

表現「置換されていてよい」とは、1個、2個、3個またはそれ以上の水素原子が、互いに独立してそれぞれの置換基によって置換されていてよい群を意味する。

【0095】

本明細書で使用されるように、用語「アミノ酸」は、1つまたはそれ以上のアミノ置換基、例えば α -、 β -または γ -アミノを含むいずれかの有機酸、脂肪族カルボン酸の誘導体を意味する。本明細書で使用されるポリペプチド表記、例えばXaa5、すなわちXaa1Xaa2Xaa3Xaa4Xaa5において、Xaa1～Xaa5は、それぞれ独立して定義されるアミノ酸から選択され、左手方向はアミノ末端方向であり、右手方向はカルボキシ末端方向であり、標準的な使用法および慣例に従ったものである。

【0096】

用語「従来のアミノ酸」とは、20種類の天然に存在するアミノ酸を意味し、それらの立体異性体すべて、すなわちD、L-、D-およびL-アミノ酸を包含する。これらの従来のアミノ酸は、本明細書において、従来の3文字または1文字の略語で言及されること

ができ、それらの略語は、従来の使用法に従う(例えば Immunology—A Synthesis, 2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)を参照)。

【0097】

用語「非従来のなアミノ酸」とは、非天然アミノ酸または化学的アミノ酸類似体、例えば α, α -二置換アミノ酸、N-アルキルアミノ酸、ホモアミノ酸、デヒドロアミノ酸、芳香族アミノ酸(フェニルアラニン、チロシンおよびトリプトファン以外の)、およびオルトアミノ安息香酸、メタアミノ安息香酸またはパラアミノ安息香酸を意味する。非従来のなアミノ酸には、アミンとカルボキシル官能基が1, 3またはそれ以上の置換パターンで分かれている化合物、例えば、 β -アラニン、 γ -アミノ酪酸、フライディンガーラクタム(Freidinger Lactam)、二環式ジペプチド(BTD)、アミノ-メチル安息香酸および当技術分野で一般的に知られている他の化合物も含まれる。また、スタチン様イソスター、ヒドロキシエチレンイソスター、還元アミド結合イソスター、チオアミドイソスター、尿素イソスター、カルバメートイソスター、チオエーテルイソスター、ビニルイソスターおよび当技術分野で一般的に知られている他のアミド結合イソスターも使用することができる。類似体または非従来型のアミノ酸を使用することにより、生理的条件下での分解耐性が高まるため、添加されたペプチドの安定性および生物学的半減期を改善し得る。当業者は、同様のタイプの置換が行われ得ることを認識するであろう。ペプチドの適当な構成要素として使用し得る非従来のなアミノ酸の非限定的なリストおよびそれらの標準的な略語(括弧内)は、以下の通りである： α -アミノ酪酸(Abu)、L-N-メチルアラニン(Nma la)、 α -アミノ- α -メチルブチラート(Mgab u)、L-N-メチルアルギニン(Nma rg)、アミノシクロプロパン(Cpro)、L-N-メチルアスパラギン(Nmas n)、カルボキシレートL-N-メチルアスパラギン酸(Nmas p)、アミノイソ酪酸(Aib)、L-N-メチルシステイン(Nmcys)、アミノノルボルニル(Norb)、L-N-メチルグルタミン(Nmgl n)、カルボキシレート L-N-メチルグルタミン酸(Nmgl u)、シクロヘキシルアラニン(Chexa)、L-N-メチルヒスチジン(Nmhis)、シクロペンチルアラニン(Cpen)、L-N-メチルイソロイシン(Nm ile)、L-N-メチルロイシン(Nm leu)、L-N-メチルリシン(Nm lys)、L-N-メチルメチオニン(Nm met)、L-N-メチルノルロイシン(Nm nle)、L-N-メチルノルバリン(Nm nva)、L-N-メチルオルニチン(Nm orn)、L-N-メチルフェニルアラニン(Nm phe)、L-N-メチルプロリン(Nm pro)、L-N-メチルセリン(Nm ser)、L-N-メチルスレオニン(Nm thr)、L-N-メチルトリプトファン(Nm trp)、D-オルニチン(Dorn)、L-N-メチルチロシン(Nm tyr)、L-N-メチルバリン(Nm val)、L-N-メチルエチルグリシン(Nm etg)、L-N-メチル- ϵ -ブチルグリシン(Nm tbug)、L-ノルロイシン(N ile)、L-ノルバリン(N va)、 α -メチル-アミノイソブチラート(Maib)、 α -メチル- γ -アミノブチラート(Mgab u)、D- α -メチルアラニン(Dma la)、 α -メチルシクロヘキシルアラニン(Mchexa)、D- α -メチルアルギニン(Dma rg)、 α -メチルシルコペンチルアラニン(α -methy lcy l copenty l a l a n i n e、Mcp en)、D- α -メチルアスパラギン(Dmas n)、 α -メチル- α -ナブチルアラニン(Manap)、D- α -メチルアスパルタート(Dmas p)、 α -メチルベニシラミン(Mpen)、D- α -メチルシステイン(Dmcys)、N-(4-アミノブチル)グリシン(Ng I u)、D- α -メチルグルタミン(Dmgl n)、N-(2-アミノエチル)グリシン(Naeg)、D- α -メチルヒスチジン(Dmhis)、N-(3-アミノプロピル)グリシン(Norn)、D- α -メチルイソロイシン(Dm ile)、N-アミノ- α -メチルブチラート(Nmaabu)、D- α -メチルロイシン(Dm leu)、 α -ナブチルアラニン(Anap)、D- α -メチルリシン(Dm lys)、N-ベンジルグリシン(Nphe)、D- α -メチルメチオニン(Dm met)、N-(2-カルバミルエチル)グリシン(Ng I n)、D- α -メチルオルニチン(Dm orn)、N-(カルバミルメチル)グリシン(Nasn)、D- α -メチルフェニルアラニン(Dmphe)、N-(2-カルボキシエチル)グリシン

(N g I u)、D- α -メチルプロリン(D m p r o)、N-(カルボキシメチル)グリシン(N a s p)、D- α -メチルセリン(D m s e r)、N-シクロブチルグリシン(N c b u t)、D- α -メチルスレオニン(D m t h r)、N-シクロヘプチルグリシン(N c h e p)、D- α -メチルトリプトファン(D m t r p)、N-シクロヘキシルグリシン(N c h e x)、D- α -メチルチロシン(D m t y)、N-シクロデシルグリシン(N c d e c)、D- α -メチルバリン(D m v a l)、N-シクロドデシルグリシン(N c d o d)、D-N-メチルアラニン(D n m a l a)、N-シクロオクチルグリシン(N c o c t)、D-N-メチルアルギニン(D n m a r g)、N-シクロプロピルグリシン(N c p r o)、D-N-メチルアスパラギン(D n m a s n)、N-シクロウンデシルグリシン(N c u n d)、D-N-メチルアスパルタート(D n m a s p)、N-(2,2-ジフェニルエチル)グリシン(N b h m)、D-N-メチルシステイン(D n m c y s)、N-(3,3-ジフェニルプロピル)グリシン(N b h e)、D-N-メチルグルタミン(D n m g l n)、N-(3-グアニジノプロピル)グリシン(N a r g)、D-N-メチルグルタマート(D n m g l u)、N-(1-ヒドロキシエチル)グリシン(N t b x)、D-N-メチルヒスチジン(D n m h i s)、N-(ヒドロキシエチル)グリシン(N s e r)、D-N-メチルイソロイシン(D n m i l e)、N-(イミダゾリルエチル)グリシン(N h i s)、D-N-メチルロイシン(D n m l e u)、N-(3-インドリルエチル)グリシン(N h t r p)、D-N-メチルリシン(D n n i l y s)、N-メチル- γ -アミノブチラート(N m g a b u)、N-メチルシクロヘキシルアラニン(N m c h e x a)、D-N-メチルメチオニン(D n m m e t)、D-N-メチルオルニチン(D n m o r n)、N-メチルシクロペンチルアラニン(N m c p e n)、N-メチルグリシン(N a l a)、D-N-メチルフェニルアラニン(D n m p h e)、N-メチルアミノイソブチラート(N m a i b)、D-N-メチルプロリン(D n m p r o)、N-(1-メチルプロピル)グリシン(N i l e)、D-N-メチルセリン(D n m s e r)、N-(2-メチルプロピル)グリシン(N l e u)、D-N-メチルスレオニン(D n m t h r)、D-N-メチルトリプトファン(D n m t r p)、N-(1-メチルエチル)グリシン(N v a l)、D-N-メチルチロシン(D n m t y r)、N-メチル- α -ナプチルアラニン(N m a n a p)、D-N-メチルバリン(D n m v a l)、N-メチルペニシラミン(N m p e n)、 γ -アミノ酪酸(G a b u)、N-(p-ヒドロキシフェニル)グリシン(N h t y r)、L- α -ナプチルグリシン(T b u g)、N-(チオメチル)グリシン(N c y s)、L-エチルグリシン(E t g)、ペニシラミン(P e n)、L-ホモフェニルアラニン(H p h e)、L- α -メチルアラニン(M a l a)、L- α -メチルアルギニン(M a r g)、L- α -メチルアスパラギン(M a s n)、L- α -メチルアスパルタート(M a s p)、L- α -メチル- γ -ナプチルグリシン(M t b u g)、L- α -メチルシステイン(M c y s)、L-メチルエチルグリシン(M e t g)、L- α -メチルグルタミン(M g l n)、L- α -メチルグルタマート(M g l u)、L- α -メチルヒスチジン(M h i s)、L- α -メチルホモフェニルアラニン(M h p h e)、L- α -メチルイソロイシン(M i l e)、N-(2-メチルチオエチル)グリシン(N m e t)、L- α -メチルロイシン(M l e u)、L- α -メチルリシン(M l y s)、L- α -メチルメチオニン(M m e t)、L- α -メチルノルロイシン(M n l e)、L- α -メチルノルバリン(M n v a)、L- α -メチルオルニチン(M o r n)、L- α -メチルフェニルアラニン(M p h e)、L- α -メチルプロリン(M p r o)、L- α -メチルセリン(M s e r)、L- α -メチルスレオニン(M t h r)、L- α -メチルトリプトファン(M t r p)、L- α -メチルチロシン(M t y r)、L- α -メチルバリン(M v a l)、L-N-メチルホモフェニルアラニン(N m h p h e)、N-(N-(2,2-ジフェニルエチル)カルバミルメチル)グリシン(N n b h m)、N-(N-(3,3-ジフェニルプロピル)カルバミルメチル)グリシン(N n b h e)、1-カルボキシ-1-(2,2-ジフェニル-エチルアミノ)シクロプロパン(N m b c)、L-O-メチルセリン(O m s e r)、L-O-メチルホモセリン(O m h s e r)。

【0098】

本明細書で使用される用語「N含有芳香族または非芳香族単環式または二環式ヘテロ環」とは、環状鎖の構成要素として少なくとも1つの窒素原子を含有する環状飽和または不

10

20

30

40

50

飽和炭化水素化合物を意味する。

【0099】

本明細書で使用される用語「放射性部分」とは、放射性核種を持つ分子集合体を意味する。核種は、生理学的条件下で安定的に維持される共有結合または配位結合のどちらかによって結合される。例としては、 $[^{131}\text{I}]$ -3-ヨード安息香酸または ^68Ga -DOTAがある。

【0100】

本明細書で使用される「蛍光同位体」は、より短い波長の電磁放射線によって励起された後に電磁放射線を放出する。

【0101】

本明細書で使用される「放射性同位体」とは、 α -、 β -および/または γ -放射線を放出する元素(用語「放射性核種」によって含まれる)の放射性同位体である。

【0102】

用語「放射性薬剤」は、本発明の文脈では、放射性同位体によって修飾される生物学的活性化合物を指すために使用される。特にインターカレート物質は、放射活性をDNAの直接の近くに送達するために使用することができる(例えばHoechst-33258の ^{131}I -担持誘導体)。

【0103】

用語「キレート剤」または「キレート」とは、本発明の文脈で互換的に使用され、金属イオンに供与するために利用可能な2つまたはそれ以上の非共有電子対を有する分子、多くの場合、有機物、および多くの場合、ルイス塩基を意味する。金属イオンは、通常、キレート剤に2つまたはそれ以上の電子対で配位している。用語「二配位子キレート剤」、「三配位子キレート剤」および「四配位子キレート剤」とは、それぞれ、キレート剤によって配位された金属イオンに同時に供与するために容易に利用可能な二つ、三つおよび四つの電子対を有するキレート剤を意味する。通常、キレート剤の電子対は、単一の金属イオンと配位結合を形成するが、特定の例では、キレート剤は、一つを超える金属イオンと配位結合を形成してもよく、さまざまな結合様式が可能である。

【0104】

本発明の文脈で使用される用語「蛍光色素」は、より短くて適当な波長の電磁放射線によって励起された後に可視光または赤外光を放出する化合物を指すために使用される。。各蛍光色素が所定の励起波長を有することは、当業者には理解されている。

【0105】

用語「造影剤」は、本発明の文脈において、医用イメージングにおいて構造または流体のコントラストを増加させる化合物を指すために使用される。強化は、電磁放射線を吸収するか、または電磁場を変更することによって達成される。

【0106】

本明細書で使用される用語「常磁性の」とは、媒体中の不対電子によって誘導される常磁性を意味する。常磁性物質は、外部磁場が印加されると磁場を誘導する。反磁性とは異なり、誘導磁場の方向は外部磁場と同一であり、強磁性とは異なり、磁場は外部磁場がない場合は維持されない。

【0107】

本明細書で使用される用語「ナノ粒子」とは、好ましくは、1~100ナノメートルの間の大きさの直径を有する球形の粒子を意味する。組成に応じて、ナノ粒子は、評価可能な磁氣的、光学的または物理化学的な品質を有することができる。さらに、多くの種類のナノ粒子の表面修飾が達成可能である。

【0108】

用語「薬学的に許容できる塩」とは、本発明の化合物の塩を意味する。本発明の化合物適切な薬学的に許容できる塩には、例えば、コリンまたはその誘導体の溶液を、塩酸、硫酸、フマル酸、マレイン酸、コハク酸、酢酸、安息香酸、クエン酸、酒石酸、炭酸またはリン酸などの薬学的に許容できる酸の溶液と混合することによって形成され得る酸付加塩

が含まれる。さらに、本発明の化合物が酸性部分を有する場合、その適切な薬学的に許容できる塩には、アルカリ金属塩類(例えば、ナトリウム塩またはカリウム塩);アルカリ土類金属塩類(例えば、カルシウム塩またはマグネシウム塩類);および適切な有機リガンドで形成される塩(例えば、ハロゲン化物、水酸化物、カルボン酸塩、硫酸塩、リン酸塩、硝酸塩、スルホン酸アルキルおよびスルホン酸アリールなどの対アニオンを用いて形成されるアンモニウム、第四級アンモニウムおよびアミンカチオン)が含まれる。薬学的に許容できる塩の用例として以下の塩があるがこれらに限定されない:酢酸塩、アジピン酸塩、アルギン酸塩、アスコルビン酸塩、アスパラギン酸塩、ベンゼンスルホン酸塩、安息香酸塩、重炭酸塩、重硫酸塩、重酒石酸塩、ホウ酸塩、臭化物、酪酸塩、エデト酸カルシウム、樟脳酸塩(camphorate)、樟脳スルホン酸塩、カンシル酸塩(camsylate)、炭酸塩、塩化物、クエン酸塩、クラブラン酸塩、シクロペンタンプロピオン酸塩、ジグルコン酸塩、二塩酸塩、硫酸ドデシル、エデト酸塩、エジシル酸塩、ストレート(estolate)、エシレート(esylate)、エタンスルホン酸塩、ギ酸塩、フマル酸塩、グルセプト酸塩(glucoseptate)、グルコヘプトン酸塩(glucoseheptonate)、グルコン酸塩、グルタミン酸塩、グリセロリン酸塩、グリコリルアルサルニル酸塩(glycolylarsanilate)、ヘミ硫酸塩(hemisulfate)、ヘプタン酸塩(heptanoate)、ヘキサン酸塩(hexanoate)、レゾルシン酸ヘキシル(hexylresorcinate)、ヒドラバミン、臭化水素酸塩、塩酸塩、ヨウ化水素酸塩(hydroiodide)、2-ヒドロキシエタンスルホン酸、ヒドロキシナフトエ酸塩、ヨウ化物、イソチオシアネート(isothiocyanate)、乳酸塩、ラクトビオン酸塩、ラウリン酸塩、ラウリル硫酸塩、リンゴ酸塩、マレイン酸塩、マロン酸塩、マンデル酸塩、メシル酸塩(mesylate)、メタンスルホン酸塩、硫酸メチル、ムチン酸塩(mucate)、2-ナフタレンスルホン酸塩、ナプシル酸塩(napsylate)、ニコチン酸塩、硝酸塩、N-メチルグルカミンアンモニウム塩、オレイン酸塩、シュウ酸塩、パモ酸塩(エンボン酸塩)、パルミチン酸塩、パントテン酸塩、ペクチン酸塩(pectinate)、過硫酸塩、3-フェニルプロピオン酸塩、リン酸塩/ニリン酸塩、ピクリン酸塩、ピバリン酸塩(pivalate)、ポリガラクトン酸塩(polygalacturonate)、プロピオン酸塩、サリチル酸塩、ステアリン酸塩、硫酸塩、塩基性酢酸塩、コハク酸塩、タンニン酸塩、酒石酸塩、テオクル酸塩、トシル酸塩(tosylate)、トリエチオダイド、ウンデカン酸塩、吉草酸塩など(例えばBerge, S. M., et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19を参照)。本発明の特定の特定の化合物は、化合物が塩基または酸付加塩のいずれかに変換されることを可能にする塩基性および酸性の両方の官能基を含む。

【0109】

化合物の中性形態は、塩を塩基または酸に接触させ、親化合物を従来の方法で単離することにより再生してもよい。化合物の親形態は、極性溶媒への溶解性のような特定の物理的性質において様々な塩の形態とは異なるが、それ以外の塩は、本発明の目的のために化合物の親形態と同等である。

【0110】

塩の形態に加えて、本発明は、プロドラッグの形態の化合物を提供する。本明細書に記載の化合物のプロドラッグは、生理学的条件下で容易に化学変化を受けて式(I)の化合物を提供する化合物である。プロドラッグは、プロドラッグを患者に投与した後に、加水分解、代謝などのインビボ生理学的作用によって本発明の化合物に化学的に修飾される活性または不活性の化合物である。さらに、プロドラッグは、エクスピボ環境下で化学的または生化学的方法によって本発明の化合物に変換することができる。例えば、プロドラッグは、適切な酵素で経皮パッチリザーバーに配置すると、ゆっくりと本発明の化合物に変換され得る。プロドラッグの製造および使用に関する適合性および技術は、当業者によく知られている。エステルに関するプロドラッグの一般的な議論については、Svensson and Tunek Drug Metabolism Reviews 16.5 (1988) and Bundgaard Design of Prodrugs, Elsevier (1985)を参照すること。マスクされたカルボン酸塩陰イオンの例としては、アルキル(例えばメチル、エチル)、シクロアルキル(例えばシクロヘキシル)、アラキル(例えばベンジル、p-メトキシベンジル)およびアルキルカルボニルオキシアルキル(例えば

ビパロイルオキシメチル)などのさまざまなエステルがある。アミンは、アリールカルボニルオキシメチル置換誘導体としてマスクされており、インビボでエステラーゼによって切断され、遊離薬物およびホルムアルデヒドを放出する (Bungaard J. Med. Chem. 2503 (1989))。また、イミダゾール、イミド、インドールなどの酸性NH基を含有する薬剤は、N-アシルオキシメチル基でマスクされている (Bundgaard Design of Prodrugs, Elsevier (1985))。ヒドロキシル基は、エステルおよびエーテルとしてマスクされている。EP 0 039 051 (Sloan and Little, Apr. 11, 1981)には、マンニッヒ塩基性ヒドロキサム酸プロドラッグ、その調製および使用が開示されている。

【0111】

本発明による化合物は、以下の方法のうちの1種またはそれ以上の方法によって合成することができる。一般的な手順は、不特定の立体化学を有する化合物の調製に関連して示されていることに留意すべきである。しかしながら、そのような手順は、一般に、特定の立体化学の化合物、例えば、基に関する立体化学が(S)または(R)である場合に適用可能である。さらに、一方の立体化学(例えば、(R))を有する化合物は、よく知られている方法、例えば、反転によって、反対の立体化学(すなわち、(S))を有する化合物を製造するために利用され得ることが多い。

【0112】

本発明の特定の化合物は、非溶媒和形態、ならびに水和物形態を含む溶媒和形態で存在することができる。一般に、溶媒和形態は、非溶媒和形態と同等であり、本発明の範囲内に包含されることが意図されている。本発明の特定の化合物は、複数の結晶または非晶質形態で存在してもよい。一般に、すべての物理的形態は、現在の発明により考慮された用途に同等であり、現在の発明の範囲内にあるように意図されている。

【0113】

本発明の特定の化合物は、不斉炭素原子(光学中心)または二重結合を有し;ラセミ体、ジアステレオマー、幾何学的異性体および個々の異性体は、すべて本発明の範囲内に包含されると意図されている。

【0114】

本発明の化合物はまた、そのような化合物を構成する1つまたはそれ以上の原子に不自然な割合の原子同位体を含み得る。例えば、化合物は、例えばトリチウム(^3H)、ヨウ素-125(^{125}I)または炭素-14(^{14}C)のような放射性同位体で放射性標識され得る。本発明の化合物のすべての同位体変動は、放射性であるか否かにかかわらず、本発明の範囲内に包含されると意図されている。

【0115】

本出願で使用される用語「医薬組成物」とは、組織の状態または疾患の同定、予防または処置のために使用される物質および/または物質の組み合わせを意味する。医薬組成物は、疾患を予防および/または処置するために患者に投与するのに適しているように製剤化する。さらに、医薬組成物は、活性剤、不活性または活性の担体との組み合わせを意味し、組成物を治療的使用に適したものにする。医薬組成物は、その化学的および物理的特性に応じて、経口、非経口、局所、吸入(inhalative)、直腸、舌下、経皮、皮下または腔への適用経路用に製剤化することができる。医薬組成物は、固体、半固体、液体、経皮吸収治療システム(TTS)を含む。固体組成物は、錠剤、コーティング錠、粉末、粒状、ベレット、カプセル、発泡性錠剤または経皮吸収治療システムからなる群から選択される。また、溶液、シロップ、輸液、抽出物、静脈内適用のための溶液、輸液のための溶液または本発明の担体システム溶液からなる群から選択される液体組成物も含まれる。本発明の文脈で使用することができる半固形組成物には、エマルジョン、懸濁液、クリーム、ローション、ゲル、グロビュール、口腔内錠剤および座薬が含まれる。

【0116】

「薬学的に許容できる」とは、連邦政府または州政府の規制当局によって承認されたか、または動物、特に人間に使用するための米国薬局方またはその他の一般的に認められている薬局方にリストされていることを意味する。

【0117】

本明細書で使用されるように、用語「担体」とは、治療剤とともに投与される希釈剤、アジュバント、賦形剤またはビヒクルを意味する。このような医薬担体は、無菌液体、例えば生理食塩水水溶液 (saline solutions in water) および、石油由来油、動物由来油、植物由来油または合成由来油、例えばピーナッツ油、ダイズ油、鉱油、ゴマ油などを含む油類であってもよい。医薬組成物を静脈内投与する場合、生理食塩水が好ましい担体である。また、生理食塩水、水性デキストロースおよびグリセロール溶液も液体担体として、特に注射用の溶液として採用することができる。適切な医薬品添加剤には、デンプン、グルコース、ラクトース、スクロース、ゼラチン、モルト、米、小麦粉、粉乳 (c h a l k)、シリカゲル、ステアリン酸ナトリウム、モノステアリン酸グリセロール、タルク、塩化ナトリウム、乾燥スキムミルク、グリセロール、プロピレン、グリコール、水、エタノールなどが含まれる。所望であれば、組成物はまた、少量の湿潤剤または乳化剤またはpH緩衝剤を含み得る。好適な薬剤担体の例は、E. W. Martinによる"Remington's Pharmaceutical Sciences"に記載されている。

10

【0118】

本明細書で使用される用語「線維芽細胞活性化タンパク質 (FAP)」とは、「セプラゼ」という用語でも知られている。両方の用語は、本明細書において互換的に使用することができる。線維芽細胞活性化タンパク質は、ジペプチジルペプチダーゼIV (DPPIV) 様フォールドを有するホモ二量体統合タンパク質であり、 α/β ヒドロラーゼドメインおよび8ブレード β プロペラドメインを特徴とする。

20

【0119】

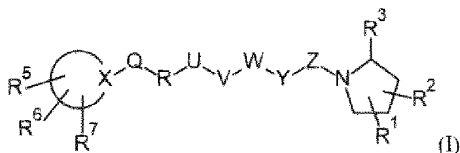
実施態様

以下では、本発明のさまざまな態様をより詳細に定義する。このように定義される各態様は、反対に明確に示されない限り、他の態様と組み合わせてもよい。特に、好ましいまたは有利であると示されるいずれかの特徴は、好ましいまたは有利であると示される他の特徴と組み合わせてもよい。

【0120】

第1の態様において、本発明は、式(I)：

【化6】



30

〔式中、Q、R、U、V、W、YおよびZは、Q、R、U、V、W、YおよびZの中の少なくとも3個が存在する条件で、独立して存在するか、または不存在であり；

Q、R、U、V、W、YおよびZは、独立してO、CH₂、NR⁴、C=O、C=S、C=NR⁴、HCR⁴ およびR⁴CR⁴ からなる群から選択され、ただし二つのOは互いに直接隣接していない；好ましくは、6個の基のうちの4個が存在し、ここで、2個はC=Oであり、1個はCH₂であり、そして1個はNHであり；より好ましくは、4個の基が存在し、ここで、2個はC=Oであり、1個はCH₂であり、そして1個はNHであり；最も好ましくは、V、W、YおよびZが存在し、ここで、VおよびZはC=Oであり、WおよびYは、独立してCH₂ およびNHから選択され；

40

R¹ およびR² は、独立して-H、-OH、ハロ、C₁₋₆-アルキル、-O-C₁₋₆-アルキルおよびS-C₁₋₆-アルキルからなる群から選択され；

【0121】

R³ は、-H、-CN、-B(OH)₂、-C(O)-アルキル、-C(O)-アリール、-C=C-C(O)-アリール、-C=C-S(O)₂-アリール、-CO₂H、-SO₃H、-SO₂NH₂、-PO₃H₂ および5-テトラゾリルからなる群から選択され；

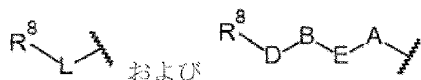
50

R^4 は、 $-H$ 、 $-C_{1-6}$ -アルキル、 $-O-C_{1-6}$ -アルキル、 $-S-C_{1-6}$ -アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび $-C_{1-6}$ -アラルキルからなる群から選択され、各 $-C_{1-6}$ -アルキルは、 $-OH$ 、オキソ、ハロから選択される1~3個の置換基によって置換されていてよく、また、Q、R、U、V、W、YまたはZに結合されていてよく；

R^5 は、 $-H$ 、ハロおよび C_{1-6} -アルキルからなる群から選択され；

R^6 および R^7 は、独立して $-H$ 、

【化7】



10

からなる群から選択され、ただし R^6 および R^7 が同時にHでない；好ましくは、 R^6 は7-または8-キノリルの位置に結合し、 R^7 は5-または6-キノリルの位置に結合し；より好ましくは、 R^6 は7-キノリルの位置に結合し、 R^7 は6-キノリルの位置に結合し；

ここで、Lはリンカーであり；

D、A、EおよびBは独立して存在するか、または不存在であり、好ましくは、少なくともA、EおよびBが存在し、ここで、存在する場合；

Dはリンカーであり；

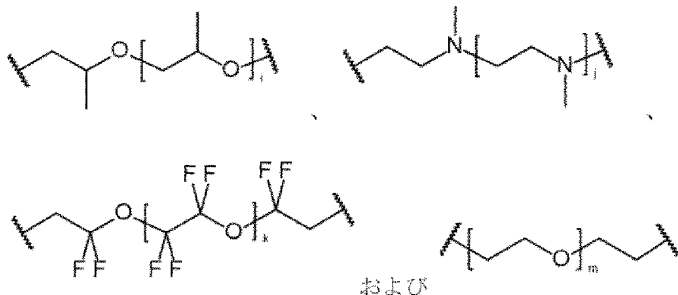
20

【0122】

Aは、 NR^4 、O、Sおよび CH_2 からなる群から選択され；

Eは、 C_{1-6} -アルキル、

【化8】



30

からなる群から選択され；

ここで、iは1、2または3であり；

ここで、jは1、2または3であり；

ここで、kは1、2または3であり；

ここで、mは1、2または3であり；

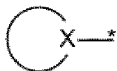
40

より好ましくは、Eは C_{1-6} -アルキルであり、最も好ましくは、EはC3またはC4アルキルであり；

AおよびEは一緒になって、シクロアルキル、ヘテロシクロアルキル、アリールおよびヘテロアリールから選択される基、好ましくはヘテロシクロアルキルを形成し、ここで、AおよびEは、単環式、二環式および多環式であってもよく、好ましくは単環式であり；各AおよびEは、 $-H$ 、 $-C_{1-6}$ -アルキル、 $-O-C_{1-6}$ -アルキル、 $-S-C_{1-6}$ -アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび $-C_{1-6}$ -アラルキルから選択される1~4個の置換基によって置換されていてよく、各 $-C_{1-6}$ -アルキルは、 $-OH$ 、オキソおよびハロから選択される1~3個の置換基によって置換されていてよく；また、A、B、D、

50

E または
【化 9】



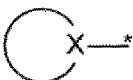
に結合されていてよく；

【0123】

B は、S、NR⁴、NR⁴-O、NR⁴-C₁₋₆-アルキル、NR⁴-C₁₋₆-アルキル-NR⁴ および 5～10-員 N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環からなる群から選択され、該ヘテロ環は好ましくは O、N および S から選択される 1 または 2 個のヘテロ原子をさらに含み、好ましくは 1 または 2 個の窒素原子をさらに含み、ここに好ましくは、NR⁴-C₁₋₆-アルキル-NR⁴ および該 N-含有ヘテロ環は、C₁₋₆-アルキル、アリール、C₁₋₆-アラールキルからなる群から選択される 1～3 個の置換基によって置換されており；そして、

R⁸ は、放射性部分、キレート剤、蛍光色素、造影剤およびそれらの組み合わせからなる群から選択され；

【化 10】



は、1-ナフチル基部分または 5～10-員 N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、ここに該ヘテロ環は N 原子と X との間に 2 個の環原子が存在し；また、該ヘテロ環は、O、N および S から選択される 1、2 または 3 個のヘテロ原子をさらに含んでいてよく；そして、X は C 原子である]

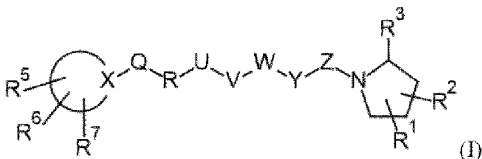
で示される化合物、または薬学的に許容できる互変異性体、ラセミ体、水和物、溶媒和物もしくは塩を提供する。

好ましい実施態様において、A および E は一緒になって、C₃、C₄、C₅、C₆、C₇ および C₈ 単環式ヘテロシクロアルキルから選択される基を形成し、好ましくは C₅ または C₆ 単環式、または C₇、C₈、C₉、C₁₀、C₁₁ もしくは C₁₂ 二環式ヘテロシクロアルキルから選択される基を形成し、好ましくは C₇、C₈、C₉ および C₁₀ 二環式ヘテロシクロアルキルから選択される基を形成し、ここに該ヘテロシクロアルキルは独立して N、O および S、好ましくは N および O からなる群から選択される 1、2、3 または 4 個、好ましくは 1 または 2 個のヘテロ原子を含み、最も好ましくは 1 または 2 個の N を含む。

【0124】

本発明の第 1 の態様の好ましい実施態様において、式 (I)：

【化 11】



〔式中、Q、R、U、V、W、Y および Z は、Q、R、U、V、W、Y および Z の中の少なくとも 3 個が存在する条件で、独立して存在するか、または不存在であり；

Q、R、U、V、W、Y および Z は、独立して O、CH₂、NR⁴、C=O、C=S、C=NR⁴、HCR⁴ および R⁴CR⁴ からなる群から選択され、ただし二つの O が互いに直接隣接しない；好ましくは、6 個の基のうちの 4 個が存在し、ここで、2 個は C=O

であり、1個は CH_2 であり、そして1個は NH であり；より好ましくは、4個の基が存在し、ここで、2個は $\text{C}=\text{O}$ であり、1個は CH_2 であり、そして1個は NH であり；最も好ましくは、 V 、 W 、 Y および Z が存在し、ここで、 V および Z は $\text{C}=\text{O}$ であり、 W および Y は、独立して CH_2 および NH から選択され；

R^1 および R^2 は、独立して $-\text{H}$ 、 $-\text{OH}$ 、ハロ、 $\text{C}_1 - 6$ - アルキル、 $-\text{O}-\text{C}_1 - 6$ - アルキルおよび $\text{S}-\text{C}_1 - 6$ - アルキルからなる群から選択され；

【0125】

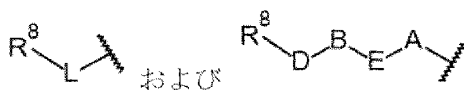
R^3 は、 $-\text{H}$ 、 $-\text{CN}$ 、 $-\text{B}(\text{OH})_2$ 、 $-\text{C}(\text{O})$ -アルキル、 $-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{S}(\text{O})_2$ -アリール、 $-\text{CO}_2\text{H}$ 、 $-\text{SO}_3\text{H}$ 、 $-\text{SO}_2\text{NH}_2$ 、 $-\text{PO}_3\text{H}_2$ および 5-テトラゾリルからなる群から選択され；

R^4 は、 $-\text{H}$ 、 $-\text{C}_1 - 6$ - アルキル、 $-\text{O}-\text{C}_1 - 6$ - アルキル、 $-\text{S}-\text{C}_1 - 6$ - アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび $-\text{C}_1 - 6$ - アラルキルからなる群から選択され、各 $-\text{C}_1 - 6$ - アルキルは、 $-\text{OH}$ 、オキソ、ハロから選択される1~3個の置換基によって置換されていてよく、また、 Q 、 R 、 U 、 V 、 W 、 Y または Z に結合されていてよく；

R^5 は、 $-\text{H}$ 、ハロおよび $\text{C}_1 - 6$ - アルキルからなる群から選択され；

R^6 および R^7 は、独立して $-\text{H}$ 、

【化12】



からなる群から選択され、ただし R^6 および R^7 は同時に H ではない、好ましくは、 R^6 は7-または8-キノリルの位置に結合し、 R^7 は5-または6-キノリルの位置に結合し；より好ましくは、 R^6 は7-キノリルの位置に結合し、 R^7 は6-キノリルの位置に結合し；

ここで、 L はリンカーであり；

D 、 A 、 E および B は独立して存在するか、または不存在であり、好ましくは、少なくとも A 、 E および B は存在し、ここで、存在する場合；

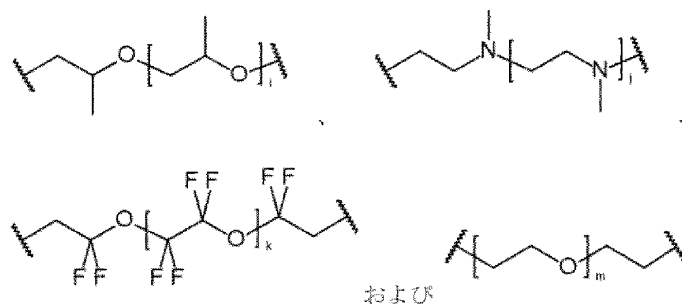
D はリンカーであり；

【0126】

A は、 NR^4 、 O 、 S および CH_2 からなる群から選択され；

E は、 $\text{C}_1 - 6$ - アルキル、

【化13】



からなる群から選択され；

ここで、 i は1、2または3であり；

ここで、 j は1、2または3であり；

ここで、 k は1、2または3であり；

ここで、 m は1、2または3であり；

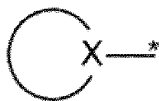
より好ましくは、Eは C_{1-6} -アルキルであり、最も好ましくは、EはC3またはC4アルキルであり；

【0127】

Bは、S、 NR^4 、 NR^4-O 、 NR^4-C_{1-6} -アルキル、 NR^4-C_{1-6} -アルキル- NR^4 および5～10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環からなる群から選択され、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、 NR^4-C_{1-6} -アルキル- NR^4 および該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アラールキルからなる群から選択される1～3個の置換基によって置換されており；そして、

R^8 は、放射性部分、キレート剤、蛍光色素、造影剤およびそれらの組み合わせからなる群から選択され；

【化14】



は、1-ナフチル基部分または5～10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環はN原子とXとの間に2個の環原子が存在し；また、該ヘテロ環は、O、NおよびSから選択される1、2または3個のヘテロ原子をさらに含んでいてよく；そして、XはC原子である]

で示される化合物、または薬学的に許容できる互変異性体、ラセミ体、水和物、溶媒和物もしくは塩を提供する。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0128】

本発明の第1の態様のさらなる好ましい実施態様において、AおよびEは一緒になって、 C_3 、 C_4 、 C_5 、 C_6 、 C_7 および C_8 単環式ヘテロシクロアルキルからなる基を形成し、好ましくは C_5 もしくは C_6 単環式、または C_7 、 C_8 、 C_9 、 C_{10} 、 C_{11} もしくは C_{12} 二環式ヘテロシクロアルキルからなる基を形成し、好ましくは C_7 、 C_8 、 C_9 および C_{10} 二環式ヘテロシクロアルキルからなる基を形成し、ここに該ヘテロシクロアルキルは独立してN、OおよびS、好ましくはNおよびOから選択される1、2、3または4個、好ましくは1または2個のヘテロ原子を含み、最も好ましくは1または2個のNを含む。好ましい単環式ヘテロシクロアルキルは、ピロリジニル、ピペリジニル、イミダゾリジニル、1,2-ジアザシクロヘキサニル、1,3-ジアザシクロヘキサニル、ピペラジニル、1-オキソ-2-アザシクロヘキサニル、1-オキソ-3-アザシクロヘキサニルまたはモルホリニル、好ましくは、ピペリジニル、ピペラジニルおよびピロリジニルからなる群から選択される。好ましい二環式ヘテロシクロアルキルは、ビシクロ[2.2.1] 2,5-ジアザヘプタニル、3,6-ジアザビシクロ[3.2.1] オクタニル、3,6-ジアザビシクロ[3.2.2] ノニル、オクタヒドロピロロ[2,3-b] ピロリル、オクタヒドロピロロ[3,2-b] ピロリル、オクタヒドロピロロ[3,4-b] ピロリル、オクタヒドロピロロ[3,4-c] ピロリル、9-メチル-3,7,9-トリアザビシクロ[3.3.1] ノナニルからなる群から選択される。

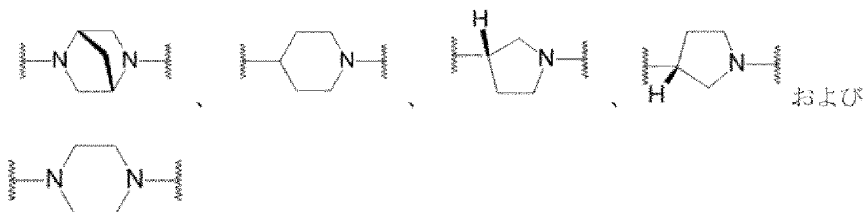
【0129】

一方ではAおよびEおよびB、ならびに／または他方では R^6 もしくは R^7 によって形成されるヘテロ環の間の結合は、好ましくはヘテロ原子、好ましくはNを介して形成される。

【0130】

特に、AおよびEによって形成されるヘテロ環の好ましい例としては、

【化 1 5】



から選択される。

10

【0131】

本発明の第1の態様の好ましい実施態様において、

Q、RおよびUは CH_2 であり、独立して存在するか、または不存在であり；好ましくは、QおよびRは不存在であり；

Vは、 CH_2 、 $\text{C}=\text{O}$ 、 $\text{C}=\text{S}$ または $\text{C}=\text{NR}^4$ であり；好ましくは、Vは $\text{C}=\text{O}$ であり；

Wは NR^4 であり；好ましくは、Wは NH であり；

Yは HCR^4 であり；好ましくは、Yは CH_2 であり；そして

Zは $\text{C}=\text{O}$ 、 $\text{C}=\text{S}$ または $\text{C}=\text{NR}^4$ であり、好ましくは、Zは $\text{C}=\text{O}$ である。

【0132】

20

本発明の第1の態様のさらなる好ましい実施態様において、

Q、RおよびUは不存在であり；

Vは CH_2 であり；

Wは NH であり；

Yは CH_2 であり；そして

Zは $\text{C}=\text{O}$ である。

【0133】

本発明の第1の態様のさらなる好ましい実施態様において、

R^1 および R^2 は、独立して $-\text{H}$ およびハロからなる群から選択され；好ましくは、 R^1 および R^2 は F であり；

R^3 は、 $-\text{H}$ 、 $-\text{CN}$ および $-\text{B}(\text{OH})_2$ からなる群から選択され；好ましくは、 R^3 は $-\text{CN}$ または $-\text{B}(\text{OH})_2$ であり；より好ましくは、 R^3 は $-\text{CN}$ であり；

R^4 は、 $-\text{H}$ および $-\text{C}_{1-6}$ -アルキルからなる群から選択され、ここで、 $-\text{C}_{1-6}$ -アルキルは、 $-\text{OH}$ から選択される1~3個の置換基によって置換されていてよい。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

30

【0134】

本発明の第1の態様のさらなる好ましい実施態様において、

Q、RおよびUは不存在であり；

Vは CH_2 であり；

Wは NH であり；

Yは CH_2 であり；

Zは $\text{C}=\text{O}$ であり；

R^1 および R^2 は、独立して $-\text{H}$ およびハロからなる群から選択され；好ましくは、 R^1 および R^2 は F であり；

R^3 は、 $-\text{H}$ 、 $-\text{CN}$ および $-\text{B}(\text{OH})_2$ からなる群から選択され；好ましくは、 R^3 は $-\text{CN}$ または $-\text{B}(\text{OH})_2$ であり；より好ましくは、 R^3 は $-\text{CN}$ であり；

R^4 は、 $-\text{H}$ および $-\text{C}_{1-6}$ -アルキルからなる群から選択され、ここで、 $-\text{C}_{1-6}$ -アルキルは、 $-\text{OH}$ から選択される1~3個の置換基によって置換されていてよい。

40

50

好ましくは、 $C_1 - 6$ -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0135】

本発明の第1の態様のさらなる好ましい実施様態において、

Q、RおよびUは不存在であり；

Vは CH_2 であり；

Wは CH_2 であり；

YはNHであり；

Zは $C=O$ であり；

R^1 および R^2 は、独立して-Hおよびハロからなる群から選択され；好ましくは、 R^1 および R^2 はハロであり；より好ましくは、 R^1 および R^2 はFであり；

R^3 は、-H、-CNおよび $-B(OH)_2$ からなる群から選択され；好ましくは、 R^3 は-CNまたは $-B(OH)_2$ であり；より好ましくは、 R^3 は-CNであり；

R^4 は、-Hおよび $C_1 - 6$ -アルキルからなる群から選択され、ここで、 $C_1 - 6$ -アルキルは、-OHから選択される1～3個の置換基によって置換されていてよい。好ましくは、 $C_1 - 6$ -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0136】

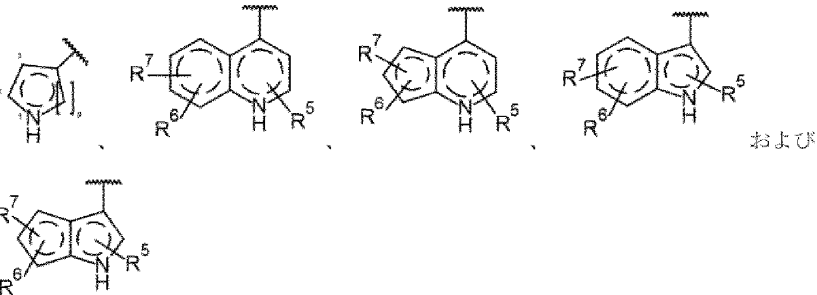
本発明の第1態様のさらなる好ましい実施様態において、

【化16】



は、

【化17】



からなる群から選択される、これらは、O、NおよびSから選択される1または2個のヘテロ原子を含んでいてよい。

【0137】

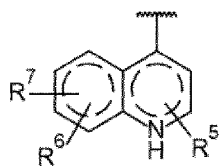
本発明の第1態様のさらなる好ましい実施態様において、

【化18】



は、

【化 1 9】

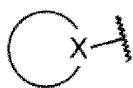


であり、これは、O、NおよびSから選択される1または2個のヘテロ原子を含んでいてよい。

【0138】

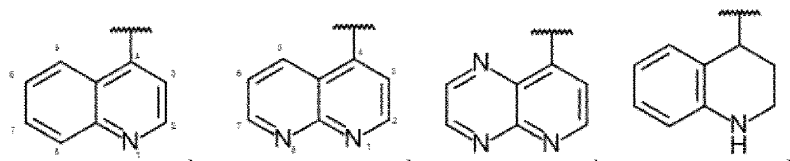
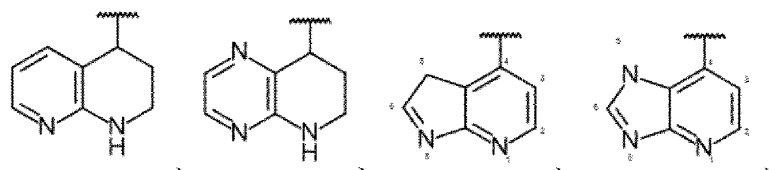
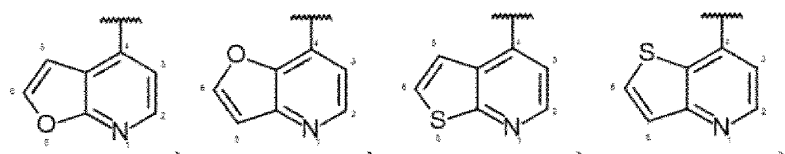
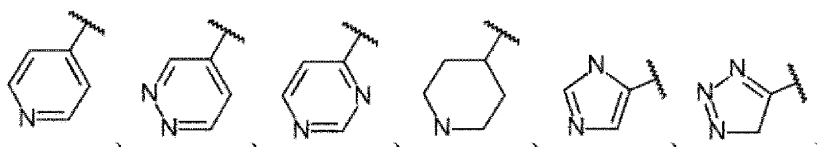
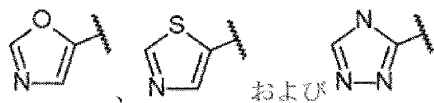
本発明の第1態様のさらなる好ましい実施態様において、

【化 2 0】



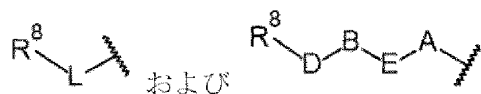
は、

【化 2 1】



および

からなる群から選択される。 R^6 および R^7 は、独立して -H、
【化 2 2】



からなる群から選択され、ただし R^6 および R^7 が同時に H ではない；好ましくは、 R^6
および R^7 は、5、6 または 7 位に結合する。

【0139】

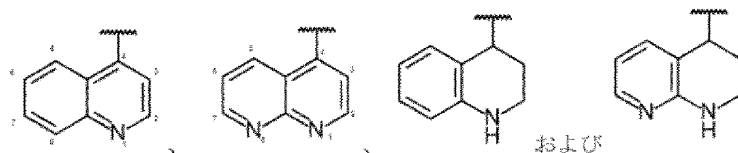
好ましい実施態様において、

【化 2 3】



は、

【化 2 4】



からなる群から選択される。

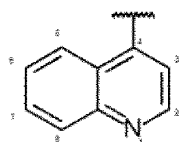
他の好ましい実施態様において、

【化 2 5】



は、

【化 2 6】



である。

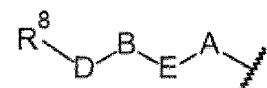
【0140】

本発明の第 1 の態様のさらなる好ましい実施様態において、

R^5 および R^6 は H であり；

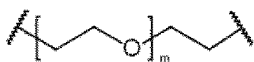
R^7 は

【化 2 7】



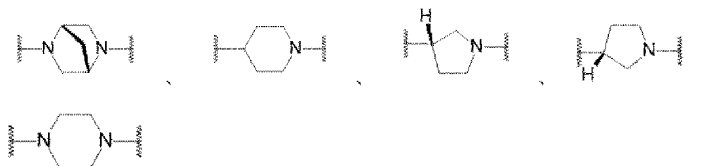
であり、好ましくは、 R^7 は、5-または 6-キノリルの位置に結合し；より好ましくは

、 R^7 は6-キノリルの位置に結合し、ここで、
 Dは不存在であり；
 Aは、O、S、 CH_2 、NHまたは NCH_3 であり；
 Eは $C_1 - 6$ -アルキルまたは
 【化28】



であり、ここで、mは1、2または3であり；好ましくは、 $C_1 - 6$ -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、Eは $C_1 - 6$ -アルキルであり、最も好ましくは、EはC3またはC4アルキルであり；または
 AおよびEは一緒になって、

【化29】



から選択される基を形成し；

Bは、 $NR^4 - C_1 - 6$ -アルキル- NR^4 または5-~10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 $C_1 - 6$ -アルキル、アリール、 $C_1 - 6$ -アラルキルからなる群から選択される1~3個の置換基によって置換されている。好ましくは、 $C_1 - 6$ -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択される。

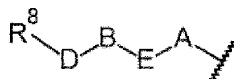
【0141】

本発明の第1の態様のさらなる好ましい実施様態において、

R^5 および R^6 はHであり；

R^7 は、

【化30】



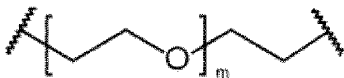
であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

Dは不存在であり；

AはOであり；

Eは $C_1 - 6$ -アルキルまたは

【化 3 1】



であり、ここで、 m は1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は C_{1-6} -アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

10

B は、 NR^4-C_{1-6} -アルキル- NR^4 または、好ましくはさらに1または2個のO、NおよびSから選択されるヘテロ原子を含み、好ましくはさらに1または2個の窒素原子を含む5～10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、好ましくは、ここで、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アラールキルからなる群から選択される1～3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0142】

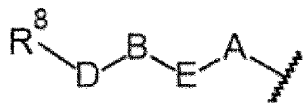
本発明の第1の態様のさらなる好ましい実施態様において、

20

R^5 および R^6 はHであり；

R^7 は

【化 3 2】



であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで

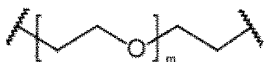
30

D は不存在であり；

A はSであり；

E は、 C_{1-6} -アルキルまたは

【化 3 3】



であり、ここで、 m は1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は C_{1-6} -アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

40

B は、 NR^4-C_{1-6} -アルキル- NR^4 または5～10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アラールキルからなる群から選択される1～3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

50

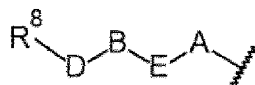
【0143】

本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 はHであり；

R^7 は

【化34】



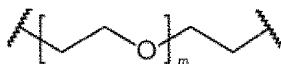
であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

Dは不存在であり；

Aは CH_2 であり；

Eは C_{1-6} -アルキルまたは

【化35】



であり、ここで、mは1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、Eは C_{1-6} -アルキルであり、最も好ましくは、EはC3またはC4アルキルであり；

Bは、 NR^4-C_{1-6} -アルキルまたは5-~10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アララルキルからなる群から選択される1~3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択される。

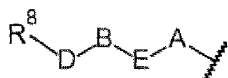
【0144】

本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 はHであり；

R^7 は

【化36】



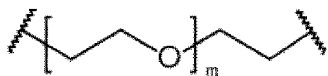
であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

Dは不存在であり；

AはNHであり；

Eは、 C_{1-6} -アルキルまたは

【化 37】



であり、ここで、 m は1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は C_{1-6} -アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

10

B は、 NR^4-C_{1-6} -アルキルまたは5-~10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アララルキルからなる群から選択される1~3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0145】

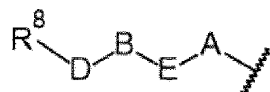
本発明の第1の態様のさらなる好ましい実施態様において、

20

R^5 および R^6 はHであり；

R^7 は

【化 38】



であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

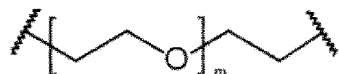
30

D はアミノ酸であり、好ましくは荷電側鎖を有するアミノ酸であり；

A はOであり；

E は、 C_{1-6} -アルキルまたは

【化 39】



であり、ここで、 m は1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は C_{1-6} -アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

40

B は、 NR^4-C_{1-6} -アルキルまたは5-~10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アララルキルからなる群から選択される1~3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選

50

択される。

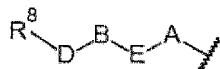
【0146】

本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 はHであり；

R^7 は

【化40】



10

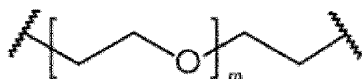
であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

Dはアミノ酸であり、好ましくは荷電側鎖を有するアミノ酸であり；

AはSであり；

Eは、 C_{1-6} -アルキルまたは

【化41】



20

であり、ここで、mは1、2または3であり；好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、Eは C_{1-6} -アルキルであり、最も好ましくは、EはC3またはC4アルキルであり；

Bは、 NR^4-C_{1-6} -アルキルまたは5-~10-員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アララルキルからなる群から選択される1~3個の置換基によって置換されている。好ましくは、 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択される。

30

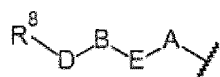
【0147】

本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 はHであり；

R^7 は

【化42】



40

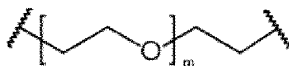
であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し；より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

Dはアミノ酸であり、好ましくは荷電側鎖を有するアミノ酸であり；

Aは CH_2 であり；

Eは、 C_{1-6} -アルキルまたは

【化 4 3】



であり、ここで、 m は1、2または3であり；好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、*i* - プロピル、ブチル、*sec* - ブチル、*tert* - ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は $C_1 - 6$ - アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

B は、 $NR^4 - C_1 - 6$ - アルキルまたは5 - ~ 10 - 員N - 含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N - 含有ヘテロ環は、 $C_1 - 6$ - アルキル、アリール、 $C_1 - 6$ - アラルキルからなる群から選択される1 ~ 3個の置換基によって置換されている。好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、*i* - プロピル、ブチル、*sec* - ブチル、*tert* - ブチル、ペンチルおよびヘキシルからなる群から選択される。

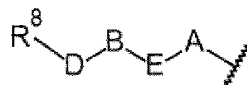
【0148】

本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 はHであり；

R^7 は

【化 4 4】



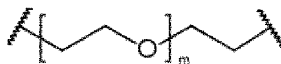
であり、好ましくは、 R^7 は、5 - または6 - キノリルの位置に結合し；より好ましくは、 R^7 は6 - キノリルの位置に結合し、ここで、

D はアミノ酸であり、好ましくは荷電側鎖を有するアミノ酸であり；

A はNHであり；

E は、 $C_1 - 6$ - アルキルまたは

【化 4 5】



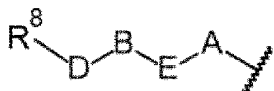
であり、ここで、 m は1、2または3であり；好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、*i* - プロピル、ブチル、*sec* - ブチル、*tert* - ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、 E は $C_1 - 6$ - アルキルであり、最も好ましくは、 E はC3またはC4アルキルであり；

B は、 $NR^4 - C_1 - 6$ - アルキルまたは5 - ~ 10 - 員N - 含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、該N - 含有ヘテロ環は、 $C_1 - 6$ - アルキル、アリール、 $C_1 - 6$ - アラルキルからなる群から選択される1 ~ 3個の置換基によって置換されている。好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、*i* - プロピル、ブチル、*sec* - ブチル、*tert* - ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0149】

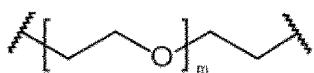
本発明の第1の態様のさらなる好ましい実施態様において、

R^5 および R^6 は H であり ;
 R^7 は
 【化 46】



であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し ; より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

D は不存在であり ;
 A は O であり ;
 E は、 C_{1-6} -アルキルまたは
 【化 47】



であり、ここで、m は 1、2 または 3 であり ; 好ましくは、E は C_{1-6} -アルキルであり、該 C_{1-6} -アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択され ; より好ましくは、E は C_{1-6} -アルキルであり、最も好ましくは、E は C3 または C4 アルキルであり ;

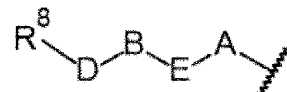
B は、5-~10-員 N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくは 1 または 2 個の窒素原子をさらに含む。

【0150】

本発明の第 1 の態様のさらなる好ましい実施態様において、

R^5 および R^6 は H であり ;
 R^7 は

【化 48】



であり、好ましくは、 R^7 は、5-または6-キノリルの位置に結合し ; より好ましくは、 R^7 は6-キノリルの位置に結合し、ここで、

D は不存在であり ;
 A は O であり ;
 E は C3 または C4 アルキルであり ; より好ましくは、E はプロピルまたはブチルであり ;

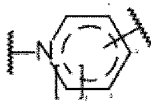
B は、5-~10-員 N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、該ヘテロ環は好ましくは 1 または 2 個の窒素原子をさらに含む。

【0151】

本発明の第 1 の態様のさらなる好ましい実施態様において、

B に含まれる N-含有ヘテロ環は、式 :

【化 4 9】



〔式中、ヘテロ環は、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、1個の窒素を含んでいてよく；

【化 5 0】



10

は、1、2または3位に結合し、好ましくは2位に結合し；

1は1または2である〕

で示される芳香族または非芳香族単環式ヘテロ環である。

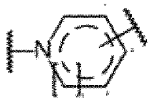
【0152】

本発明の第1の態様のさらなる好ましい実施態様において、

Bに含まれるN-含有ヘテロ環は、式：

【化 5 1】

20



〔式中、ヘテロ環は、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、1個の窒素をさらに含んでいてよく；

【化 5 2】



30

は、1、2または3位に結合し、好ましくは2位に結合し；

ここで、N-含有ヘテロ環はC₁ - 6 - アルキルによって置換されている〕

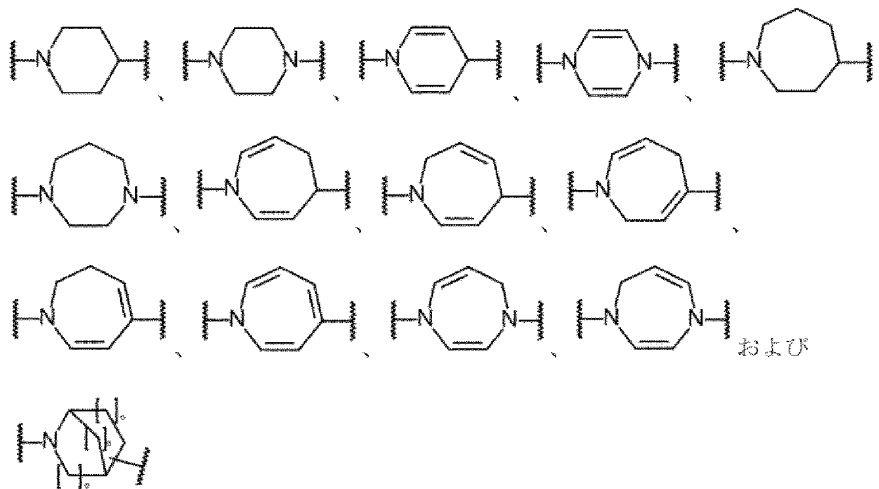
で示される芳香族または非芳香族単環式ヘテロ環である。

【0153】

本発明の第1の態様のさらなる好ましい実施態様において、

Bに含まれるN-含有ヘテロ環は、

【化 5 3】



10

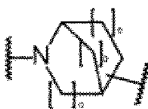
からなる群から選択され、

ここで、N-含有ヘテロ環はC₁ - 6 - アルキルによって置換されており、

ここで、Bに含まれるN-含有ヘテロ環は

20

【化 5 4】



である場合、該ヘテロ環は、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、1個の窒素をさらに含んでよく、また、一つまたはそれ以上の(例えばアミノ酸由来)側鎖を含んでいてよく、

【化 5 5】

30



は、1、2または3位に結合し、好ましくは、2位に結合し；

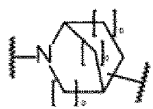
oは1または2であり；

【0154】

好ましくは、Bに含まれるN-含有ヘテロ環が

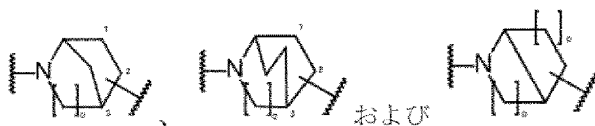
【化 5 6】

40



である場合、Bに含まれるN-含有ヘテロ環は、

【化 5 7】



からなる群から選択され；より好ましくは、Bに含まれるN-含有ヘテロ環が

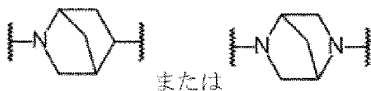
【化 5 8】



10

である場合、Bに含まれるN-含有ヘテロ環は

【化 5 9】



20

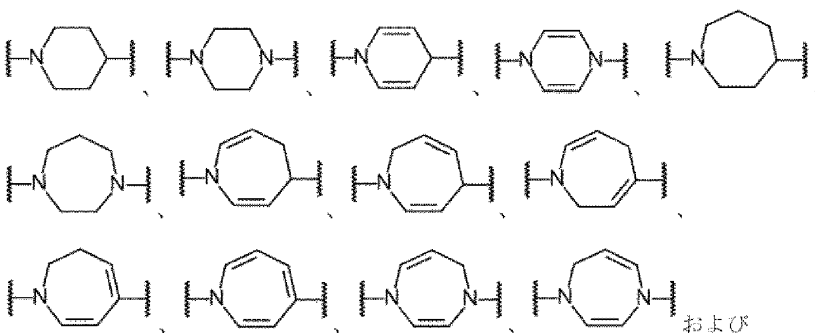
である。

【0155】

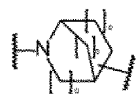
本発明の第1の態様のさらなる好ましい実施態様において、

Bに含まれるN-含有ヘテロ環は、

【化 6 0】



30

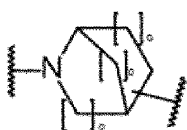


40

からなる群から選択され、

ここで、Bに含まれるN-含有ヘテロ環が

【化 6 1】



50

である場合、該ヘテロ環は、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、1個の窒素をさらに含んでよく、また、一つまたはそれ以上の(例えばアミノ酸由来)側鎖を含んでいてよく；

【0156】

【化62】



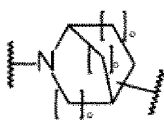
10

は、1、2または3位に結合し、好ましくは、2位に結合し；

oは1または2であり；

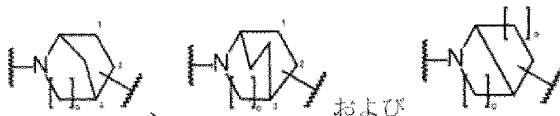
好ましくは、Bに含まれるN-含有ヘテロ環が

【化63】



である場合、Bに含まれるN-含有ヘテロ環は、

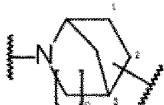
【化64】



20

からなる群から選択され；より好ましくは、Bに含まれるN-含有ヘテロ環が

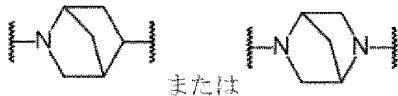
【化65】



30

である場合、Bに含まれるN-含有ヘテロ環は

【化66】



である。

【0157】

本発明の第1の態様のさらなる好ましい実施態様において、

Bに含まれるN-含有ヘテロ環は、

【化67】



からなる群から選択される。

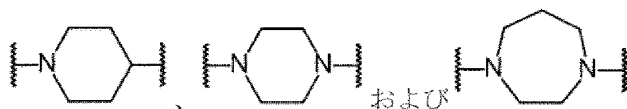
【0158】

40

50

本発明の第 1 の態様のさらなる好ましい実施態様において、
B に含まれる N-含有ヘテロ環は、

【化 68】



からなる群から選択され、ここで、B は C₁ - ₃ アルキルによって置換されている。

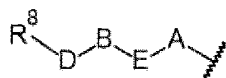
【0159】

本発明の第 1 の態様のさらなる好ましい実施態様において、

R⁵ および R⁶ は H であり；

R⁷ は

【化 69】



であり、好ましくは、R⁷ は 6-キノリルの位置に結合し、ここで、

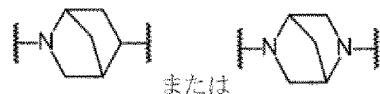
D は不存在であり；

A は O であり；

E はプロピルまたはブチルであり；

B は

【化 70】



である。

【0160】

本発明の第 1 の態様のさらなる好ましい実施態様において、

Q、R および U は不存在であり；

V は C=O であり；

W は NH であり；

Y は CH₂ であり；

Z は C=O であり；

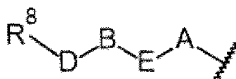
R¹ および R² は、独立して -H およびハロからなる群から選択され；好ましくは、R¹ および R² は、独立して -H および F からなる群から選択され；より好ましくは、R¹ および R² は同一であり、-H および F からなる群から選択され；

R³ は -CN であり；

R⁵ および R⁶ は H であり；

R⁷ は

【化 71】



であり、好ましくは、R⁷ は 6-キノリルの位置に結合し、ここで、

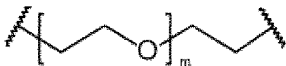
D は不存在であり；

【 0 1 6 1 】

A は O であり；

E は、 $C_1 - 6$ - アルキルまたは

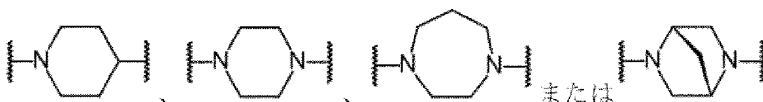
【 化 7 2 】



であり、ここで、m は 1、2 または 3 であり；好ましくは、E は $C_1 - 6$ - アルキルであり；好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、i - プロピル、ブチル、sec - ブチル、tert - ブチル、ペンチルおよびヘキシルからなる群から選択され；より好ましくは、E は $C_1 - 6$ - アルキルであり、最も好ましくは、E は C 3 または C 4 アルキルであり；

B は $NH - C_1 - 6$ - アルキル、

【 化 7 3 】



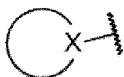
であり；好ましくは、 $C_1 - 6$ - アルキルは、メチル、エチル、プロピル、i - プロピル、ブチル、sec - ブチル、tert - ブチル、ペンチルおよびヘキシルからなる群から選択され；好ましくは、B は

【 化 7 4 】



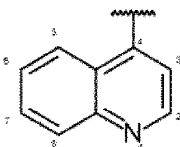
であり；そして

【 化 7 5 】



は

【 化 7 6 】



である。

【 0 1 6 2 】

本発明の第 1 の態様のさらなる好ましい実施態様において、

Q、R および U は不存在であり；

V は $C=O$ であり；W は NH であり；Y は CH_2 であり；Z は $C=O$ であり；

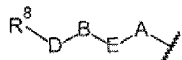
R^1 および R^2 は同一であり、 $-H$ および F からなる群から選択され；

R^3 は $-CN$ であり；

R^5 および R^6 は H であり；

R^7 は

【化 77】



であり、好ましくは、 R^7 は 6-キノリルの位置に結合し、ここで、

D は不存在であり；

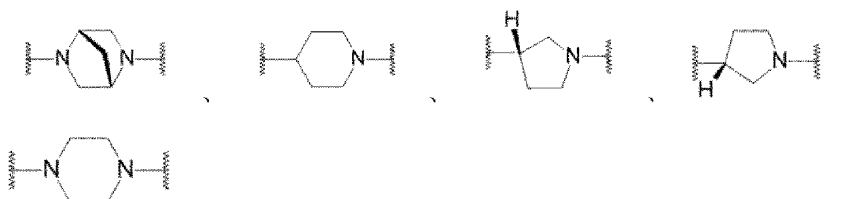
A は O 、 S 、 CH_2 、 NH または NCH_3 であり；

E は、メチル、エチル、プロピルまたはブチルであり；

【0163】

A および E は一緒になって

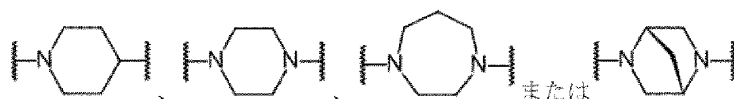
【化 78】



から選択される基を形成し；

B は

【化 79】



であり、B は、 C_{1-3} アルキルによって置換されていてよく；好ましくは、B は

【化 80】



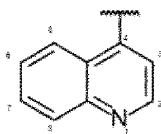
であり；そして

【化 81】



は

【化 82】



10

20

30

40

50

である。

【0164】

本発明の第1の態様のさらなる好ましい実施態様において、

Q、RおよびUは不存在であり；

VはC=Oであり；

WはNHであり；

YはCH₂であり；

ZはC=Oであり；

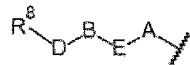
R¹ および R² は同一であり、-HおよびFからなる群から選択され；

R³ は-CNであり；

R⁵ および R⁶ はHであり；

R⁷ は

【化83】



であり、好ましくは、R⁷ は6-キノリルの位置に結合し、ここで、

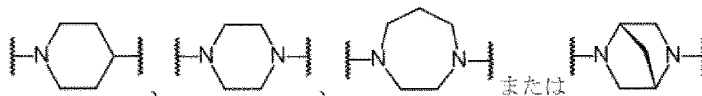
Dは不存在であり；

AはOであり；

Eは、メチル、エチル、プロピルまたはブチルであり；

Bは、

【化84】



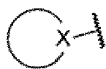
であり、好ましくは、Bは

【化85】



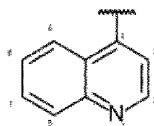
であり；そして、

【化86】



は

【化87】



である。

【0165】

本発明の第1の態様のさらなる好ましい実施態様において、

Q、RおよびUは不存在であり；

10

20

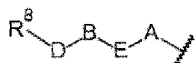
30

40

50

VはC=Oであり；
 WはNHであり；
 YはCH₂であり；
 ZはC=Oであり；
 R¹およびR²は同一であり、-HおよびFからなる群から選択され；
 R³はCNであり；
 R⁵およびR⁶はHであり；
 R⁷は

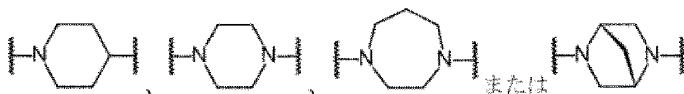
【化88】



10

であり、R⁷は6-キノリルの位置に結合し、ここで、
 Dは不存在であり；
 AはOであり；
 Eは、メチル、エチル、プロピルまたはブチルであり；
 Bは、

【化89】



20

であり；好ましくは、Bは

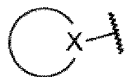
【化90】



であり；そして、

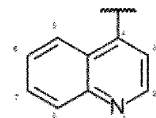
30

【化91】



は

【化92】



40

である。

【0166】

本発明の第1のさらなる好ましい実施態様において、C₁-₆-アルキルは、メチル、エチル、プロピル、i-プロピル、ブチル、sec-ブチル、tert-ブチル、ペンチルおよびヘキシルからなる群から選択される。

【0167】

本発明の第1のさらなる好ましい実施態様において、C₁-₃-アルキルは、メチル、エチル、プロピルおよびi-プロピルからなる群から選択される。

50

【0168】

本発明の第1のさらなる好ましい実施態様において、 C_{1-6} -アラルキルは、ベンジル、フェニル-エチル、フェニル-プロピルおよびフェニル-ブチルからなる群から選択される。

【0169】

本発明の第1の態様のさらなる好ましい実施態様において、本発明の第1の態様の化合物は表1の化合物から選択される。より好ましくは、本発明の第1の態様の化合物は表2の化合物から選択される。より好ましくは、本発明の第1の態様の化合物は、F A P I - 02およびF A P I - 04からなる群から選択される。

【0170】

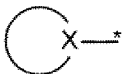
本発明の第1の態様の好ましい実施態様において、本発明の第1の態様の化合物は、表1および/または表3の化合物から選択される。より好ましくは、本発明の第1の態様の化合物は表2および/または表4の化合物から選択される。より好ましくは、本発明の第1の態様の化合物は、F A P I - 02、F A P I - 04、F A P I - 46、F A P I - 34、F A P I - 42、F A P I - 52、F A P I - 69、F A P I - 70、F A P I - 71、F A P I - 72およびF A P I - 73からなる群から選択される。

【0171】

表1：本発明の第1の態様の好ましい化合物

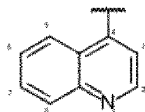
S 蛍光化合物；\$^{99m}Tc-キレート剤；* Pb-キレート剤；R¹およびR²は4-ピロリジンの位置にあり；Q、RおよびUは不存在であり；

【化93】



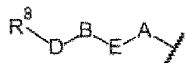
は

【化94】



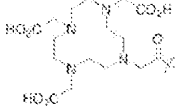
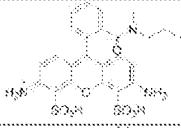
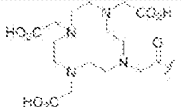
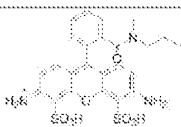
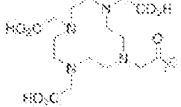
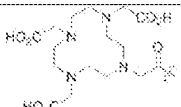
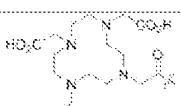
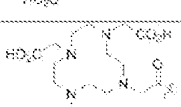
であり、R⁵はHであり；R⁶は7-キノリルの位置に結合し；R⁷は6-キノリルの位置に結合し；「-」は、R⁶またはR⁷がHであることを示し；「+」は、R⁶またはR⁷が

【化95】



であることを示し；VはC=Oであり；WはNHであり；YはCH₂であり；ZはC=Oであり；R³は-CNであり；AはOである(Aが存在せず、R⁷が5-キノリルの位置に結合するF A P I - 01除外)。

【表 1 - 1】

名称	R ¹ /R ²	R ⁶	R ⁷	R ⁸	D	B	E
FAP1-01	H/H	-	+	I	-	-	-
FAP1-02	H/H	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-02 atto 488 [®]	H/H	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-02 dy800 CW [®]	H/H	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-03	H/H	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-04	F/F	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-04 atto 488 [®]	F/F	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-05	F/F	-	+		-		<i>n</i> -C ₈ H ₁₇
FAP1-06	H/H	-	+		-	NH	<i>n</i> -C ₈ H ₁₇
FAP1-07	F/F	-	+		-	NH	<i>n</i> -C ₈ H ₁₇
FAP1-08	H/H	+	-		-		<i>n</i> -C ₈ H ₁₇

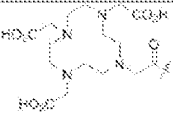
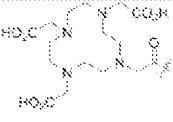
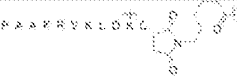
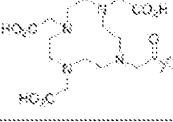
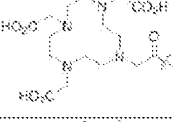
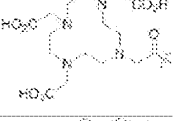
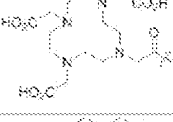
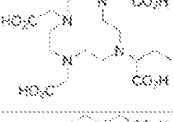
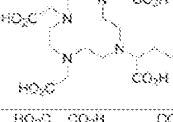
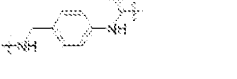
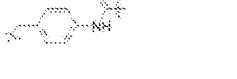
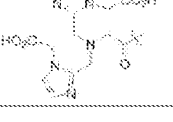
10

20

30

40

【表 1 - 2】

FAP1-09	F/F	+	-		-		<i>n</i> -C ₈ H ₁₆
FAP1-10	F/F	-	+				<i>n</i> -C ₈ H ₁₆
FAP1-11	F/F	-	+		-		CH ₂
FAP1-12	F/F	-	+		-		<i>n</i> -C ₈ H ₁₆
FAP1-13	F/F	-	+		-		<i>n</i> -C ₈ H ₁₆
FAP1-14	F/F	-	+		-		<i>n</i> -C ₈ H ₁₆
FAP1-15	F/F	-	+		-		(C ₂ H ₄ O) C ₂ H ₄
FAP1-16	F/F	-	+		-		<i>n</i> -C ₈ H ₁₆
FAP1-17	F/F	-	+				<i>n</i> -C ₈ H ₁₆
FAP1-18	F/F	-	+				<i>n</i> -C ₈ H ₁₆
FAP1-19 [†]	F/F	-	+		-		<i>n</i> -C ₈ H ₁₆

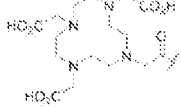
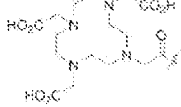
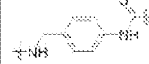
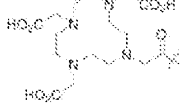
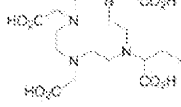
10

20

30

40

【表 1 - 3】

FAP1-20	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-21	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-22	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-23	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-24	F/F	-	+		-		(C ₂ H ₅ O) C ₂ H ₄
FAP1-25	F/F	-	+				<i>n</i> -C ₈ H ₁₈
FAP1-26	F/F	-	+		-		(C ₂ H ₅ O) C ₂ H ₄
FAP1-27 ³	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-28 ³	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-29 ³	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAP1-30	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈

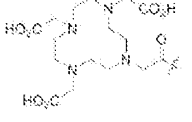
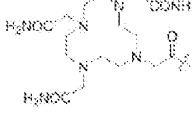
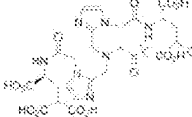
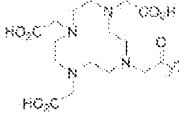
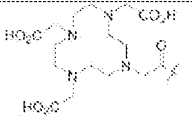
10

20

30

40

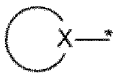
【表 1 - 4】

FAPI- 31	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAPI- 32*	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAPI- 33 ³	F/F	-	+		-		(C ₂ H ₅ O) C ₂ H ₄
FAPI- 34 ⁸	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAPI- 35	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAPI- 36	F/F	-	+		-		<i>n</i> -C ₈ H ₁₈
FAPI- 37	F/F	-	+				<i>n</i> -C ₈ H ₁₈
FAPI- 38	F/F	-	+		D-Aix		<i>n</i> -C ₈ H ₁₈

【 0 1 7 2】

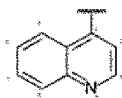
表 2 : 特に興味深い化合物。Q、R、U および D は不存在であり ; R¹ および R² は 4 -
ピロリジンの位置にあり ;

【化 9 6】



は

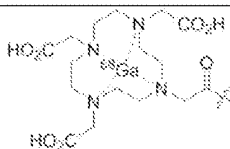
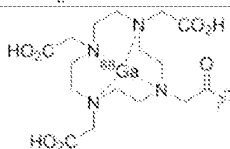
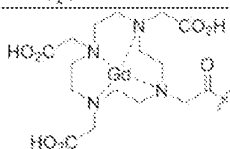
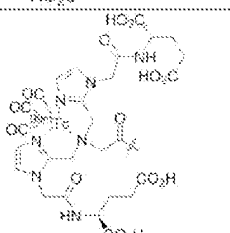
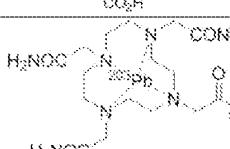
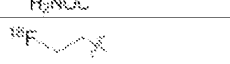
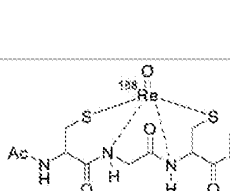
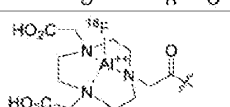
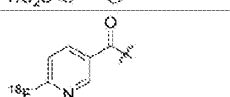
【化 9 7】



であり、R⁵、R⁶ は H であり ; R⁷ は 6 -キノリルの位置に結合し ; V は C = O であり
 ; W は NH であり ; Y は CH₂ であり ; Z は C = O であり ; R⁸ は -CN であり ; B は 1

, 4-ピペラジンであり; Eは1,3-プロパンであり; AはOである。

【表2】

名称	目的	R ¹ /R ²	R ³
⁶⁷ Ga-FAPI-02	PET	H/H	
⁶⁸ Ga-FAPI-04	PET	F/F	
Gd-FAPI-04	MRI (造影剤)	F/F	
⁹⁰ Y-FAPI-04	放射線療法 (β ⁻)	F/F	
^{99m} Tc-FAPI-29	SPECT	F/F	
²⁰³ Pb-FAPI-32	SPECT	F/F	
¹⁸ F-FAPI-n. a.	PET	F/F	
¹⁸⁸ Re-FAPI-60	放射線療法 (β ⁻)	F/F	
Al ¹⁸ F-FAPI-42	PET	F/F	
¹⁸ F-FAPI-72	PET	F/F	

10

20

30

40

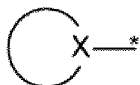
50

【0173】

表3：本発明の第1の態様のさらなる好ましい化合物。

S 蛍光化合物； ^{99m}Tc -キレート剤； ^{18}F -標識の前駆体；Q、RおよびUは
不存在であり； R^1 および R^2 は4-ピロリジンの位置にあり；

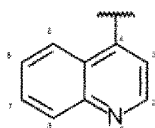
【化98】



は

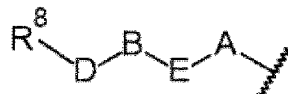
10

【化99】



であり、 R^5 および R^6 はHであり； R^7 は6-キノリルの位置に結合し、

【化100】



20

であり；Vは $\text{C}=\text{O}$ であり；Wは NH であり；Yは CH_2 であり；Zは $\text{C}=\text{O}$ であり； R^3 は $-\text{CN}$ である。

【表 3 - 1】

名称	R ¹ /R ²	R ³	D	B	E	A
FAP1-39	F/F		-		<i>n</i> -C ₃ H ₇	CH ₃
FAP1-40	F/F		-		<i>n</i> -C ₃ H ₇	S
FAP1-41	F/F		-		<i>n</i> -C ₃ H ₇	NH
FAP1-42	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-43 ³	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-44 ³	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-45 ¹	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-46	F/F		-		<i>n</i> -C ₃ H ₇	NH ₂
FAP1-47	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-48	F/F		-		<i>n</i> -C ₃ H ₇	O
FAP1-49	F/F		-		<i>n</i> -C ₃ H ₇	O

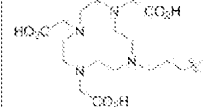
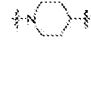
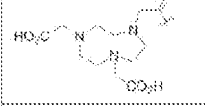
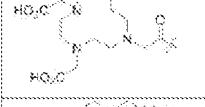
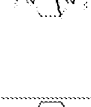
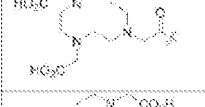
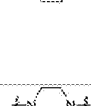
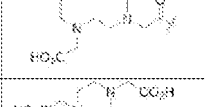
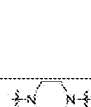
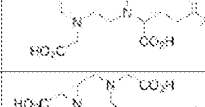
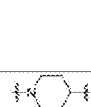
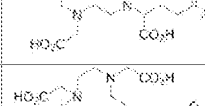
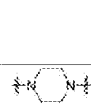
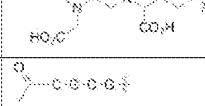
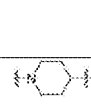
10

20

30

40

【表 3 - 2】

FAP1-50	F/P		~		<i>n</i> -C ₅ H ₁₁	0
FAP1-51	F/P		~		<i>n</i> -C ₅ H ₁₁	0
FAP1-52	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-53	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-54	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-55	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-56	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-57	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-58	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-59	F/P		~		<i>n</i> -C ₅ H ₁₁	NMe
FAP1-60 ¹	F/P		~		<i>n</i> -C ₅ H ₁₁	0

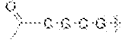
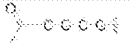
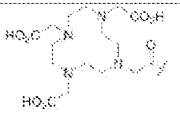
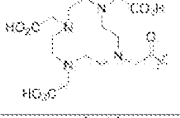
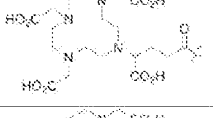
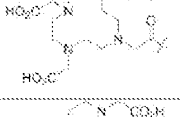
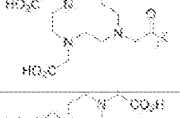
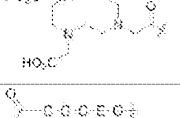
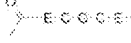
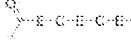
10

20

30

40

【表 3 - 3】

FAP1-61 ¹	F/F		-		<i>p</i> -C ₆ H ₆	0
FAP1-62 ¹	F/F		-		<i>p</i> -C ₆ H ₆	NMe
FAP1-63	F/F		-			10
FAP1-64	F/F		-			20
FAP1-65	F/F		-			30
FAP1-66	F/F		-			40
FAP1-67	F/F		-			
FAP1-68	F/F		-			
FAP1-69 ¹	F/F		-		<i>p</i> -C ₆ H ₆	NMe
FAP1-70 ¹	F/F		-		<i>p</i> -C ₆ H ₆	NMe
FAP1-71 ¹	F/F		-		<i>p</i> -C ₆ H ₆	NMe

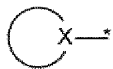
【表 3 - 4】

FAP1-72*	F/F		-		<i>p</i> -C ₆ H ₅	NMe
FAP1-73*	F/F		-		<i>p</i> -C ₆ H ₅	0
FAP1-74	H/H		-		<i>p</i> -C ₆ H ₅	0
FAP1-75	F/F				<i>p</i> -C ₆ H ₅	0
FAP1-76	F/F		-		<i>p</i> -C ₆ H ₅	0
FAP1-77	F/F				<i>p</i> -C ₆ H ₅	0
保護された FAP1-77	F/F				<i>p</i> -C ₆ H ₅	0
FAP1-78	F/F				<i>p</i> -C ₆ H ₅	0
保護された FAP1-78	F/F				<i>p</i> -C ₆ H ₅	0
FAP1-79	F/F				<i>p</i> -C ₆ H ₅	0
FAP1-80	F/F				<i>p</i> -C ₆ H ₅	NMe

【0174】

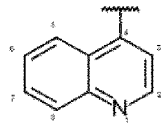
表 4 : 特に興味深い化合物。Q、R、U および D は不存在であり ; R¹ および R² は、4-ピロリジンの位置にあるフッ素原子であり ;

【化 1 0 1】



は

【化 1 0 2】



10

であり； R^5 、 R^6 はHであり； R^7 は6-キノリルの位置に結合し；VはC=Oであり；WはNHであり；YはCH₂であり；ZはC=Oであり； R^8 は-CNであり；Bは1,4-ピペラジンであり；Eは1,3-プロパンであり；AはOである。

【表 4】

名称	目的	R^9
^{188}Re -FAP1-60	放射線療法 (β^-)	
$\text{Al } ^{18}\text{F}$ -FAP1-42	PET	
^{18}F -FAP1-73	PET	

20

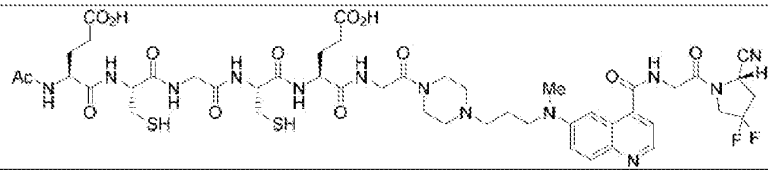
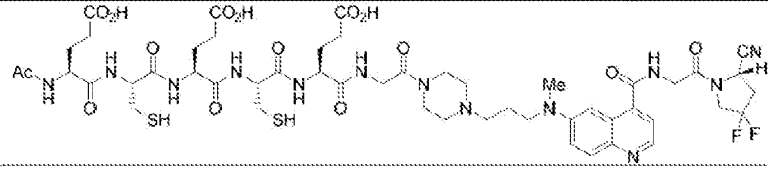
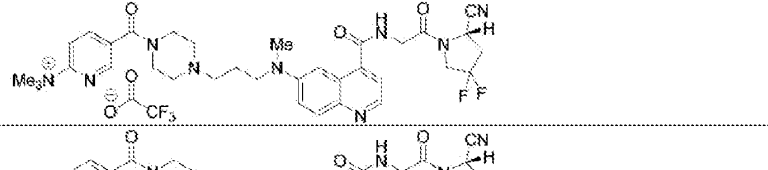
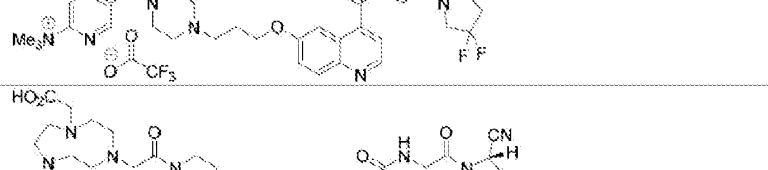
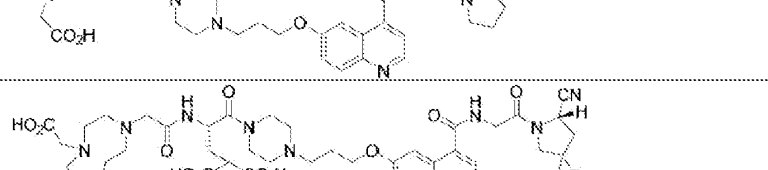
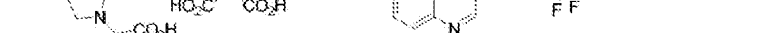
30

【 0 1 7 5】

表5: ${}^8\text{F}-18$; ${}^8\text{Cu}-64$; ${}^6\text{Ga}-68$; ${}^8\text{Tc}-99\text{m}$; $\text{Re}-188$; ${}^8\text{Y}-90$, $9\text{m}-153$, $\text{Lu}-177$ での放射標識のための好ましい前駆体。

FAP1- 02 ^{a,c}	
FAP1- 04 ^{b,c}	
FAP1- 34 ^c	
FAP1- 42 ^{d,e,f,g}	
FAP1- 46 ^{e,f}	
FAP1- 52 ^{d,e,f,g}	
FAP1- 69 ^f	

【表 5-2】

FAP1-70 ^A	
FAP1-71 ^A	
FAP1-72 ^B	
FAP1-73 ^B	
FAP1-74 ^{B,3,4}	
FAP1-75 ^{B,3,4}	

【0176】

本発明の第1の態様のさらなる好ましい実施態様において、R^Bは、蛍光同位体、放射性同位体、放射性薬剤またはそれらの組み合わせである放射性部分である。好ましくは、該放射性部分は、 α 放射線放出同位体、 β 放射線放出同位体、 γ 放射線放出同位体、オージェ電子放出同位体、X-ray放出同位体、蛍光放出同位体、例えば¹¹C、¹⁸F、⁵¹Cr、⁶⁷Ga、⁶⁸Ga、¹¹¹In、^{99m}Tc、¹⁸⁶Re、¹⁸⁸Re、¹³⁹La、¹⁴⁰La、¹⁷⁵Yb、¹⁵³Sm、¹⁶⁶Ho、⁸⁸Y、⁹⁰Y、¹⁴⁹Pm、¹⁶⁵Dy、¹⁶⁹Er、¹⁷⁷Lu、⁴⁷Sc、¹⁴²Pr、¹⁵⁹Gd、²¹²Bi、²¹³Bi、⁷²As、⁷²Se、⁹⁷Ru、¹⁰⁹Pd、¹⁰⁵Rh、^{101m}Rh、¹¹⁹Sb、¹²⁸Ba、¹²³I、¹²⁴I、¹³¹I、¹⁹⁷Hg、²¹¹At、¹⁵¹Eu、¹⁵³Eu、¹⁶⁹Eu、²⁰¹Tl、²⁰³Pb、²¹²Pb、⁶⁴Cu、⁶⁷Cu、¹⁸⁸Re、¹⁸⁶Re、¹⁹⁸Au、²²⁵Ac、²²⁷Thおよび¹⁹⁹Agからなる群から選択される。好ましくは、¹⁸F、⁶⁴Cu、⁶⁸Ga、⁹⁰Y、^{99m}Tc、¹⁵³Sm、¹⁷⁷Lu、¹⁸⁸Reなどのからなる群から選択される。

【0177】

本発明の第1の態様のさらなる好ましい実施態様において、R^Bは、以下の種類の蛍光色素：キサンテン、アクリジン、オキサジン、シアニン、スチリル色素、クマリン、ポリフィーン、金属-配位子錯体、蛍光タンパク質、ナノ結晶、ペリレン、ホウ素-ジピロメ

テンおよびフタロシアニンからなる群から選択される蛍光色素、ならびにこれらの種類の色素の複合物および組み合わせである。

【0178】

本発明の第1の様態のさらなる好ましい実施様態において、 R^8 は、2価または3価の金属カチオンと錯体を形成するキレート剤である。好ましくは、キレート剤は、1,4,7,10-テトラアザシクロドデカン-N,N',N,N'-テトラ酢酸(DOTA)、エチレンジアミンテトラ酢酸(EDTA)、1,4,7-トリアザシクロノナン-1,4,7-トリ酢酸(NOTA)、トリエチレントラミン(TEETA)、イミノニ酢酸、ジエチレントリアミン-N,N,N',N',N''-ペンタ酢酸(DTPA)、ビス-(カルボキシメチルイミダゾール)グリシンおよび6-フィードラジノピリジン3-カルボン酸(HYNIC)からなる群から選択される。

10

【0179】

本発明の第1のさらなる好ましい実施態様において、 R^8 は、常磁性剤を含むか、またはそこからなる造影剤であって、好ましくは、ここで、常磁性剤が、常磁性ナノ粒子を含むか、またはそれからなる。

【0180】

本発明の第1のさらなる好ましい実施態様において、 R^8 は、表1~5のいずれかの R^8 である。

【0181】

第2の態様において、本発明は、少なくとも一つの第1の態様の化合物、および製薬的に許容できる担体および/または添加物を含むか、またはそれらからなる医薬組成物に関する。

20

【0182】

第3の態様において、本発明は、動物またはヒトの対象における線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患の診断または処置に使用するための、第1の態様の化合物または第2の態様の医薬組成物に関する。好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患は、癌、慢性炎症、アテローム性動脈硬化症、線維症、組織リモデリングおよびケロイド障害からなる群から選択される。

【0183】

好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴する疾患が癌である場合、該癌は、乳癌、膵臓癌、小腸癌、結腸癌、直腸癌、肺癌、頭頸部癌、卵巣癌、肝細胞癌、食道癌、下咽頭癌、上咽頭癌、喉頭癌、骨髄腫細胞、膀胱癌、胆管細胞癌、腎明細胞癌、神経内分泌腫瘍、腫瘍誘発性骨軟化症、肉腫、CUP(未知の初期癌)、胸腺癌、類腫瘍、神経膠腫、星細胞腫、頸癌および前立腺癌からなる群から選択される。好ましくは、該癌は、神経膠腫、乳癌、結腸癌、肺癌、頭頸部癌、肝臓癌または膵臓癌である。より好ましくは、該癌は神経膠腫である。

30

【0184】

好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患が慢性炎症である場合、該慢性炎症は、リウマチ性関節炎、骨関節症およびクローン病からなる群から選択される。好ましくは、該慢性炎症はリウマチ性関節炎である。

40

【0185】

好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患が線維症である場合、該線維症は、特発性肺線維症などの肺線維症および肝臓硬変からなる群から選択される。

【0186】

好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患が組織リモデリングである場合、該組織リモデリングは、心筋梗塞の後に発生する。

【0187】

好ましくは、線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患がケロイド障害である場合、該ケロイド障害は、瘢痕形成、ケロイド腫瘍およびケロイド瘢痕か

50

らなる群から選択される。

【0188】

第4の態様において、本発明は、第1の態様の化合物もしくは第2の態様の医薬組成物および疾患の診断または処置のためのインストラクションを含むか、またはそれらからなるキットに関する。好ましくは、該疾患は前記特定の疾患である。

【実施例】

【0189】

実施例1：化合物の合成および放射化学

FAP- α 特異的な阻害物質 (Jansen et al., ACS Med Chem Lett, 2013) に基づき、二つの放射性追跡子 (放射性トレーサー) を合成した。放射性ヨウ素標識 FAPI-01 は、パラジウム触媒による臭素/スズ交換により調製した有機スズスタンニル化前駆体を介して得た。FAPI-02 は、5段階で合成した放射性金属キレートのための前駆体である。同じ操作または若干変更した操作を適用することにより、追加の化合物を調製した。これらの化合物の構造を表1および表2に列挙する。スタンニル化前駆体の放射性ヨウ素化は、過酢酸を用いて行った。Lu-177 および Ga-68 とのキレート化のために、反応混合物の pH を酢酸ナトリウムで調節し、95℃に10分間加熱した。ヒト血清における安定性は、沈殿および上清のラジオ-HPLC 分析によって分析した。

【0190】

試薬

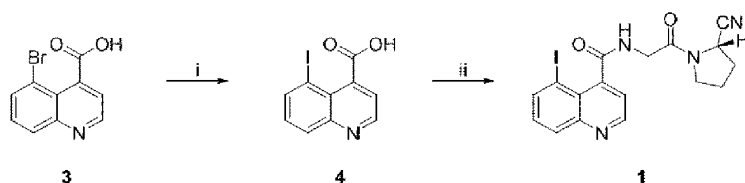
全ての溶媒および非放射性試薬は、ABC R (Karlruhe, Germany)、Sigma-Aldrich (Muenchen, Germany)、Acros Organics (Geel, Belgium) または VWR (Bruchsal, Germany) から試薬グレードで入手し、さらなる精製なしで使用した。Attom 488 NHS-エステルは Attomtec (Siegen, Germany) から入手した。2,2',2''-(10-(2-(4-ニトロフェニル)オキシ)-2-オキソエチル)-1,4,7,10-テトラアザシクロドデカン-1,4,7トリイル)トリ酢酸 (DOTA-PNP) は、Mierらのプロトコールによって合成した (Mier et al., Bioconj Chem, 2005)。中間体である6-メトキシキノリン-4-カルボン酸(7)、5-ブロモキノリン-4-カルボン酸(3)および(S)-1-(2-アミノアセチル)ピロリジン-2-カルボニトリル4-ベンゼンスルホン酸メチルは、Jansenらのプロトコールによって合成した。(Jansen et al., ACS Med Chem Lett, 2013)。物質(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキソエチル)-5-ブロモキノリンカルボキサミドは修飾HBTUアミド化プロトコールによって合成した。

【0191】

化合物の合成

スキーム1は、5-ブロモキノリル-4-カルボン酸(3)において、n-ブチルリチウムを用いてBr/Li交換を行い、元素状ヨウ素でクエンチングしてヨードキノリン4を得るFAPI-01の初期合成を示している。この化合物をHBTU/HOBt-活性化によりGly-Pro-CNフラグメントにカップリングさせ、FAPI-01の非放射性的対照物質(1)を得た。

【化103】

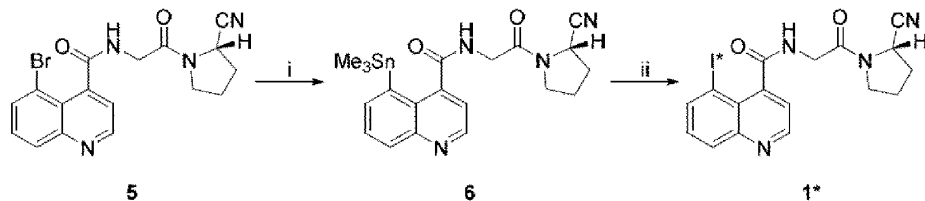


スキーム1. 非放射性 FAPI-01 の合成。i) nBuLi、次に I₂、THF; ii) HBTU/HOBt、DIPEA、H-Gly-Pro-CN、DMF。

【0192】

放射性FAP I-01(1*)の合成において、80℃、ジオキサン中、阻害物質5のパラジウム触媒スタンニル化によって、スタンニル化前駆体6を得た(スキーム2)。

【化104】



10

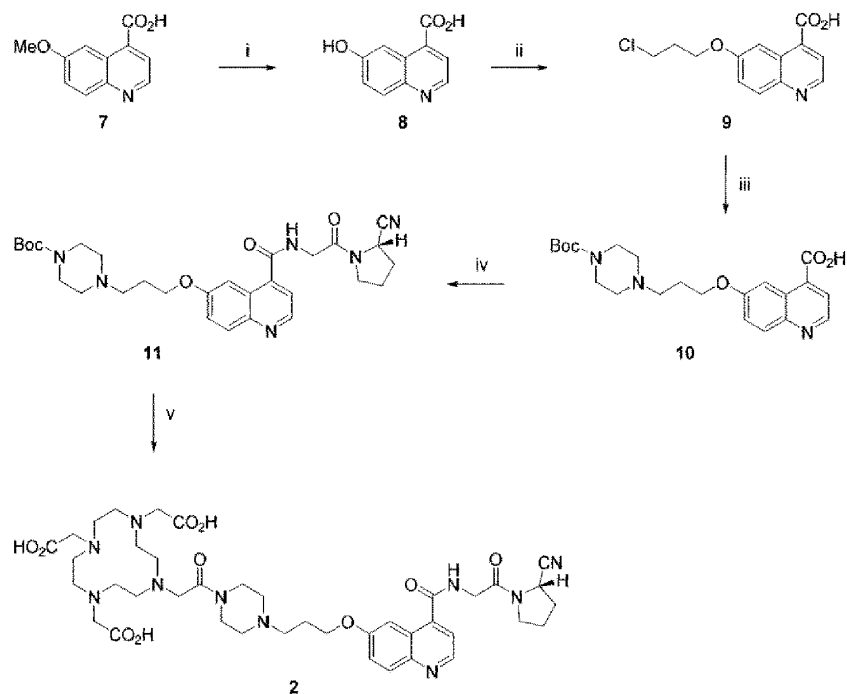
スキーム2.スタンニル化前駆体4を介した放射性FAP I-1の合成。i) $(\text{Me}_3\text{Sn})_2$; $(\text{PPh}_3)_2\text{PdCl}_2$; ジオキサン 80℃; ii) I-125またはI-131; AcOOH ; 1M HCl ; MeOH 。

【0193】

放射性金属の取り込みによる放射性標識を可能にするために、キレート剤であるDOTAをFAP阻害物質の基本的な骨格に化学的に結合させた。Jansenら(Jansen et al. ACS Med Chem Lett, 2013)によって示されたように、キノリン-4-カルボン酸の6-位置での修飾は、標的親和性および特異性を損なうことなく耐性が高い。したがって、したがって、二官能性リンカーを、エーテル結合を介して8のヒドロキシル基に結合させ、これはスキーム3に示される合成の主な方法になっている。すぐに入手可能な1-ブロモ-3-クロロプロパンを選択し、ワンポットプロセスの最後に同時に形成されたエステル結合の鹼化の間に無傷であるスペーサーを作った。化合物9をN-Boc保護キノリネカルボン酸10に変換し、これをさらにHBTUによりH-Gly-Pro-CNに結合させた。遊離アミンは吸湿性が高いため、化合物11はBoc除去、溶媒交換および過剰なp-トルエンシルホン酸の中和後に、直接FAP I-02(2)に変換された。

20

【化105】

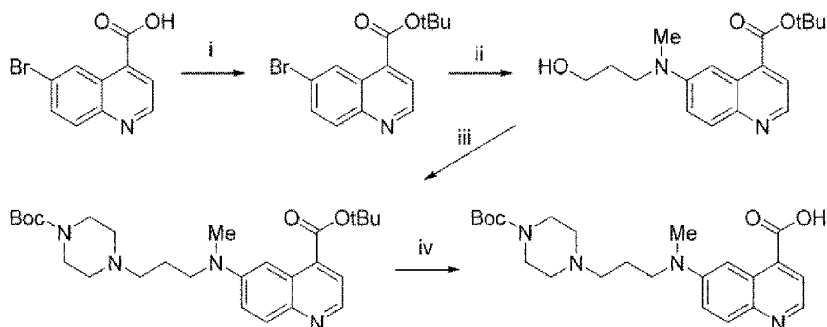


スキーム3. FAPI-02の化学合成。i)適量HBr、48%、130℃；ii)1-ブromo-3-クロロプロパン、Cs₂CO₃、DMF、次に6M NaOH；iii)1-Boc-ピペラジン、KI、DMF；iv)HBTU/HOBt、DIPEA、H-Gly-Pro-CN、DMF；v)TosOH、MeCN、次にDOTA-PPN、DIPEA、DMF。

【0194】

基A≠Oを組み込んでいる化合物の場合は、キノリン-4-カルボン酸中間体を別の反応スキームによって合成した。このアプローチの鍵となる工程は、パラジウム触媒によるカップリング反応(例えば、ブッフバルト-ハートウィッグクロスカップリング)であり、これは、クロスカップリング反応の前に追加の保護を必要とし、クロスカップリング反応の後にカルボン酸機能の脱保護を必要とする(スキーム4)。

【化106】

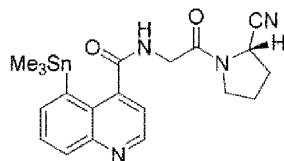


スキーム4. FAPI-46の合成のための構成要素である6-(3-(4-Boc-ピペラジン-1-イル)プロピル-1-(メチル)アミノ)キノリン-4-カルボン酸の合成。i

) DCC, *t*BuOH, CuCl; ii) 3-メチルアミノ-1-プロパノール、Cs₂CO₃、Pd₂(dba)₃、BINAP; iii) MsCl、NEt₃、DCM 次に1-Boc-ピペラジン、KI、DMF; iv) TFA 次にBoc₂O、NEt₃、DMF。

【0195】

【化107】



10

(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキシエチル)-5-トリメチルスタニルキノリンカルボキサミド(6)

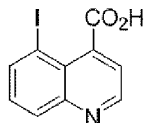
(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキシエチル)-5-ブロモキノリンカルボキサミド3.88mg(10.0μmol)、ヘキサメチルニズ20μL(32mg; 96μmol)およびビス(トリフェニルホスフィン)パラジウム(II)ジクロライド0.75mg(1.07μmol)を乾燥ジオキサン1mL中で、不活性雰囲気下、80℃で一晩攪拌する。揮発性物質を除去し、残留物を50%アセトニトリル/水2mLに取り入れ、C18ライトカートリッジで濾過した後、HPLC精製する。凍結乾燥後、生成物2.78mg(5.90μmol; 59%)を得る。

20

LC-MS R_t 14.77 min, m/z 473.0786 [M(¹²⁰Sn)+H]⁺

【0196】

【化108】



30

5-ヨードキノール-4-カルボン酸(4)

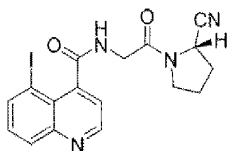
水素化ナトリウム懸濁液(鉱油中60%)5.42mg(136μmol)を、0℃、Arの下、5-ブロモキノリン-4-カルボン酸(3)30.27mg(120μmol)の乾燥THF3mL中の溶液に添加する。氷浴を除去し、反応混合物を-78℃に冷却した後、nBuLi(ヘキサン中1.6m)100μL(160μmol)を滴下する。15分後、THF2mL中のヨウ素64.71mg(254μmol)を滴下し、反応物を-78℃で30分間攪拌した後、室温に到達させる。1時間後、0.5M NaHCO₃1mLと亜ジチオン酸ナトリウム約30mg(170μmol)を加え、過剰なヨウ素を除去することによって反応を停止させる。THFを減圧下で除去した後、混合物をpH2に酸性化し、酢酸エチル(25mL)で3回抽出する。有機相を一緒にし、蒸発乾固し、HPLCで精製する。凍結乾燥後、表題の化合物18.14mg(60.7μmol; 45%)を得る。

40

¹H NMR (500 MHz, DMSO-d₆) 13.95 (br, 0.3H), 8.93 (s, 1H), 8.34 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.60 (s, 1H), 7.52 (t, J = 7.9 Hz, 1H); ¹³C NMR (125 MHz, DMSO-d₆) 168.8, 150.3, 148.8, 141.3, 130.6, 121.0, 109.5; LC-MS R_t 8.65 min, m/z 299.9383 [M+H]⁺

【0197】

【化109】

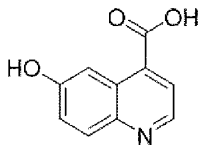


(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキソエチル)-5-トリメチルスタンニルキノリンカルボキサミド(1; FAPI-01)

DMF 50 μ L 中の HBTU 9.07 mg (23.9 μ mol) を、DMF 50 μ L 中の 5-ヨードキノリン-4-カルボン酸 6.21 mg (20.8 μ mol)、HOBt 7.45 mg (55.2 μ mol) および DIPEA 10 μ L の溶液に添加する。15 分後、DMF 50 μ L 中の (S)-1-(2-アミノアセチル)ピロリジン-2-カルボニトリル 4-ベンゼンスルホン酸メチル (29.9 μ mol) を添加する。反応を水 850 μ L で停止させ、HPLC で精製する。凍結乾燥し、生成物 6.86 mg (15.8 μ mol; 76%) を得る。¹H NMR (600 MHz, DMSO-d₆) 9.06, 8.97, 8.33, 8.13, 7.56, 7.51, 4.81, 4.34, 4.06, 3.74, 3.56, 2.21, 2.17, 2.09, 2.05; ¹³C NMR (150 MHz, DMSO-d₆) 167.1, 150.2, 148.8, 145.3, 141.5, 130.7, 125.3, 121.9, 119.3, 92.0, 46.3, 45.4, 42.1, 29.5, 24.9; LC-MS R_t 11.95 min, m/z 435.0102 [M+H]⁺

【0198】

【化110】



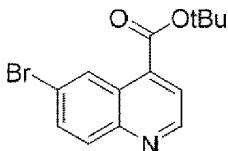
6-ヒドロキシキノリン-4-カルボン酸(8)

生 6-メトキシキノリン-4-カルボン酸(7) 105 mg (477 μ mol) を、48% 臭化水素酸水溶液 3 mL に溶解する。この溶液を 130 °C に 4 時間加熱する。この溶液を、室温に到達させた後、6 M NaOH で弱塩基性 pH にする。HPLC-精製および凍結乾燥後、生成物 79.2 mg (419 μ mol; 88%) を得る。

¹H NMR (500 MHz, DMSO-d₆) 13.65 (br, 0.6H), 10.24 (s, 1H), 8.78 (d, J = 4.4 Hz, 1H), 8.06 (d, J = 2.6 Hz, 1H), 7.95 (d, J = 9.1 Hz, 1H), 7.84 (d, J = 4.4 Hz, 1H), 7.37 (dd, J = 9.1, 2.6 Hz, 1H), ¹³C NMR (125 MHz, DMSO-d₆) 167.7, 156.9, 146.5, 144.1, 133.4, 131.2, 126.2, 122.3, 122.6, 106.5; LC-MS R_t 6.66 min, m/z 190.0415 [M+H]⁺

【0199】

【化111】



6-ブロモキノリン-4-カルボン酸 tert-ブチル

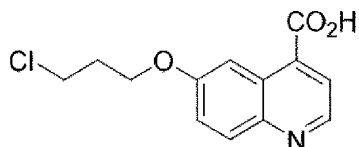
6-ブロモキノリン-4-カルボン酸(生) 98.3 mg (390 μ mol) をテトラヒドロフラン 5 mL および トリエチルアミン 25.0 μ L (18.3 mg; 181 μ mol) に懸濁させ、O-tert-ブチル-N,N'-ジシクロヘキシル尿素(前日、純ジシクロヘキシルカルボジミド 426 mg (2.07 mmol)、純 tert-ブタノール 173 mg (2.33

mmol)、純銅(I)塩化物10.2mg(103μmol)から調製)に添加する。この混合物を一晚50℃に加熱した。この混合物を濾過し、溶媒を蒸発させ、生成物をHPLCで単離させた。凍結乾燥後、表題の化合物49.7mg(161μmol; 41%)を得た。

LC-MS R_t 20.40 min, m/z 251.9642 $[M-tBu]^+$

【0200】

【化112】



10

6-(3-クロロ-1-プロポキシ)キノリン-4-カルボン酸(9)

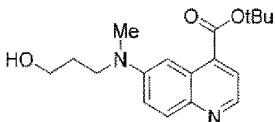
1-ブロモ-1-クロロプロパン42.4μL(67.4mg; 430μmol)を、DMF250μL中の6-ヒドロキシキノリン-4-カルボン酸(8)23.2mg(123μmol)および炭酸カリウム190mg(1.38μmol)の懸濁液に添加し、一晚、60℃に加熱する。この反応混合物を室温に冷却し、水500μLおよびアセトニトリル500μLで希釈した後、6M NaOH100μLを添加する。この反応混合物を、完全なエステル加水分解が達成した後、HPLC(5~40%)によって直接精製する。凍結乾燥後、生成物26.45mg(99.4μmol; 81%)を得る。

20

^1H NMR (500 MHz, DMSO- d_6) 13.75 (br, 0.4H), 8.88 (d, J = 4.4 Hz, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 4.4 Hz, 1H), 7.52 (dd, J = 9.2, 2.0 Hz, 1H), 4.24 (t, J = 5.95 Hz, 2H), 3.85 (t, J = 6.5 Hz, 2H), 2.27 (m, 2H); ^{13}C NMR (125 MHz, DMSO- d_6) 167.6, 157.5, 147.6, 144.8, 134.0, 131.2, 125.9, 122.7, 122.2, 104.5, 64.7, 41.9, 31.6; LC-MS R_t 11.46 min, m/z 266.0461 $[M+H]^+$

【0201】

【化113】



30

6-(3-ヒドロキシプロピルメチルアミノ)キノリン-4-カルボン酸tert-ブチル

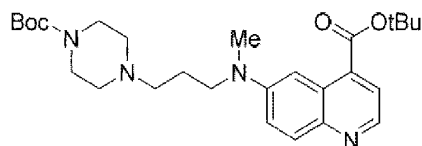
6-ブロモキノリン-4-カルボン酸tert-ブチル204.6mg(664μmol)、BINAP34.10mg(54.7μmol)、Pd₂(dba)₃21.51mg(23.5μmol)および炭酸セシウム480.3mg(1.47mmol)をトルエン6mLに溶解し、N-メチル-1,3-プロパノールアミン128.0μL(118mg; 1.32mmol)を添加した。この混合物を90℃で一晩攪拌した後、溶媒を除去し、残留物を1:1の水/アセトニトリルに懸濁させ、濾過した後、HPLCによって精製した。凍結乾燥後、表題の化合物172.7mg(547μmol; 82%)を得た。

40

LC-MS R_t 13.41 min, m/z 261.1213 $[M-tBu+H]^+$

【0202】

【化 1 1 4】



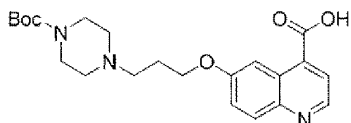
6-(3-(4-Boc-ピペラジン-1-イル)プロピル-1-(メチル)アミノ)キノリン-4-カルボン酸 tert-ブチル

6-(3-ヒドロキシプロピルメチルアミノ)キノリン-4-カルボン酸 tert-ブチル 62.8 mg (199 μmol) をジクロロメータン 5 mL およびトリエチルアミン 90.0 μL (66.6 mg; 659 μmol) に溶解した。メータースルホンクロライド 20.0 μL (29.6 mg; 258 μmol) を 0℃ で添加し、この混合物を 60 分間反応させた。1-Boc-ピペラジン 194.6 mg (1.05 mmol) を添加した後、揮発性物質を除去した。ジメチルホルムアミド 500 μL およびヨウ化カリウム (potassium ヨウ化物) 47.4 mg (286 μmol) を残留物に加えた。この混合物を 60℃ で 120 分間振盪した後、HPLC で生成物を単離させた。凍結乾燥後、表題の化合物 81.05 mg (167 μmol; 84%) を得た。

LC-MS R_t 13.99 min, m/z 485.3086 $[M+H]^+$

【0203】

【化 1 1 5】



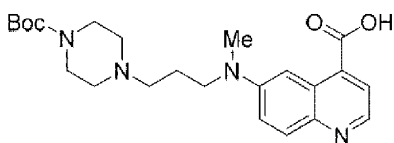
6-(3-(4-tert-ブトキシカルボニルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボン酸 (10)

6-(3-クロロ-1-プロポキシ)キノリン-4-カルボン酸 (9) 15.13 mg (56.9 μmol)、N-tert-ブトキシカルボニルピペラジン 55.43 mg (298 μmol) およびヨウ化カリウム 51.05 mg (30.8 μmol) を DMF 250 μL に溶解する。この混合物を 60℃ で一晩振盪する。得られた懸濁液を水 750 μL で希釈した後、生成物を HPLC で精製する。凍結乾燥後、生成物を対応する TFA-塩として 28.73 mg (54.3 μmol; 95%) 得る。

^1H NMR (500 MHz, D_2O) 8.93 (d, J = 5.5 Hz, 1H), 8.17 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 5.5 Hz, 1H), 7.79 (dd, J = 9.3, 2.5 Hz, 1H), 7.65 (d, J = 2.5 Hz, 1H), 4.36 (t, J = 5.6 Hz, 2H), 4.27 (d, J = 13.55 Hz, 2H), 3.67 (d, J = 11.95 Hz), 3.47 (t, J = 15.5 Hz, 2H), 3.27 (t, J = 12.7 Hz), 3.12 (td, J = 12.2, 2.65 Hz), 2.37 (m2 H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, D_2O) 155.5, 153.5, 149.0, 141.4, 134.4, 127.9, 126.6, 122.3, 118.4, 110.0, 105.1, 82.8, 65.5, 54.3, 51.5, 48.6, 40.7, 29.6, 27.4; LC-MS R_t 10.62 min, m/z 416.1997 $[M+H]^+$

【0204】

【化 1 1 6】



10

20

30

40

50

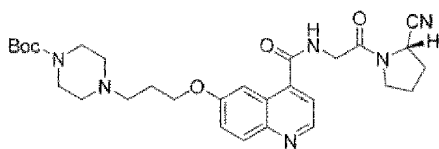
6-(3-(4-Boc-ピペラジン-1-イル)プロピル-1-(メチル)アミノ)キノリン-4-カルボン酸

6-(3-(4-Boc-ピペラジン-1-イル)プロピル-1-(メチル)アミノ)キノリン-4-カルボン酸 *tert*-ブチル 100.12 mg (206 μ mol) を、トリフルオロ酢酸 900 μ L、トリイソプロピルシラン 25 μ L、水 25 μ L およびトリフルオロメタンスルホン酸 50 μ L で 60 分間処理した。脱保護された化合物をジエチルエーテルで沈殿させ、乾燥させ、ジメチルホルムアミド 1 mL 中の二炭酸ジ-*tert*-ブチル 60.83 mg (279 μ mol) およびトリエチルアミン 50.0 μ L (36.5 mg; 361 μ mol) とさらに 60 分間反応させた。HPLC-精製および凍結乾燥後、55.42 mg (129 μ mol; 65% 2 工程をかけた) を得た。

LC-MS R_t 10.52 min, m/z 429.2463 [M+H]⁺

【0205】

【化117】



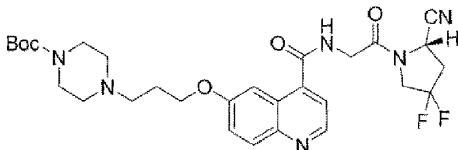
(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキソエチル)-6-(3-(4-*tert*-ブトキシカルボニルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボキサミド(11)

DMF 50 μ L 中の HBTU 9.43 mg (24.9 μ mol) を、DMF 50 μ L 中の 6-(3-(4-*tert*-ブトキシカルボニルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボン酸(10) 10.56 mg (19.9 μ mol)、HOBt 5.38 mg (39.8 μ mol) および DIPEA 10 μ L の溶液に添加する。15 分後、DMF 50 μ L 中の (S)-1-(2-アミノアセチル)ピロリジン-2-カルボニトリル 4-ベンゼンスルホン酸メチル(29.9 μ mol) を添加する。この反応を水 850 μ L で停止させ、HPLC で精製する。凍結乾燥させ、表題の化合物 12.88 mg (19.4 μ mol; 97%) を得る。

¹H NMR (500 MHz, DMSO-d₆) 9.04 (d, J = 5.5 Hz, 1H), 8.24 (d, J = 9.6 Hz, 1H), 8.10 (d, J = 5.5 Hz, 1H), 7.89 (d, J = 2.3 Hz, 1H), 7.85 (dd, J = 9.6, 2.3 Hz, 1H), 4.84 (t, J = 6 Hz, 1H), 4.46-4.36 (m, 4H), 4.26 (d, J = 12.0 Hz, 2H), 3.83 (m, 1H), 3.67 (m, 3H), 3.47 (t, J = 7.7 Hz, 2H), 3.27 (br, 2H), 3.11 (t, J = 11.5 Hz), 2.37 (m, 4H), 2.22 (m, 2H), 1.46 (s, 9H); ¹³C NMR (125 MHz, DMSO-d₆) 168.6, 168.0, 159.4, 155.5, 147.7, 141.8, 135.1, 128.2, 127.5, 123.1, 120.0, 119.1, 104.7, 82.9, 66.0, 54.3, 51.5, 47.0, 46.3, 42.3, 29.4, 27.4, 24.7, 23.1; LC-MS R_t 11.81 min, m/z 551.2736 [M+H]⁺

【0206】

【化118】



(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキソエチル)-6-(3-(4-*tert*-ブトキシカルボニルピペラジン-1-イル)-1-ブ

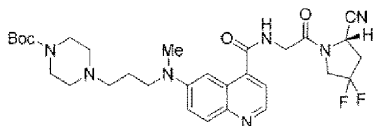
ロポキシ)キノリン-4-カルボキサミド

前のプロトコールを行い、13.2 mg (22.4 μ mol; 75%)を得た。

LC-MS R_t 11.84 min, m/z 605.2610 [M+H]⁺

【0207】

【化119】



10

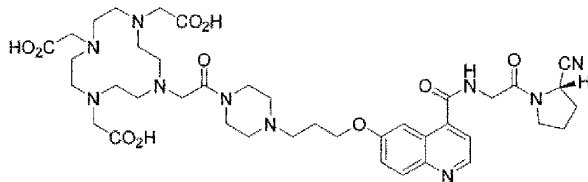
N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキソエチル)-6-(3-(4-Boc-ピペラジン-1-イル)プロピル-1-(メチル)アミノ)キノリン-4-カルボキサミド

前のプロトコールを行い、1.17 mg (1.95 μ mol; 92%)を得た。

LC-MS R_t 12.66 min, m/z 600.3057 [M+H]⁺

【0208】

【化120】



20

F A P I - 0 2 (2)

(S)-N-(2-(2-シアノピロリジン-1-イル)-2-オキソエチル)-6-(3-(4-tert-ブトキシカルボニル-ピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボキサミド(11) 4.85 mg (8.80 μ mol)をアセトニトリル1 mLに溶解し、それに4-メチルベンゼンスルホン酸-水和物 4.2 mg (22.0 μ mol)を添加する。この反応物を45℃で一晩振盪した後、減圧下で揮発性物質を除去する。残留物をジメチルホルムアミド190 μ Lおよびトリエチルアミン10 μ L(7.3 mg; 72 μ mol)に取り入れた後、DOTA-p-ニトロフェノールエステル6.77 mg(12.9 μ mol)を添加する。この反応混合物を水1 mLで希釈し、2時間振盪した後、HPLCで精製する。凍結乾燥後、5.04 mg(6.02 μ mol; 68%)を得る。

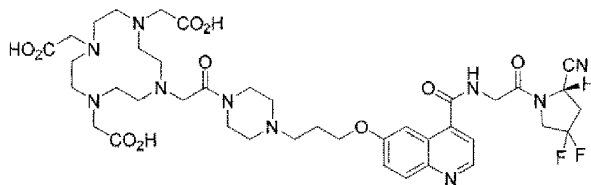
30

¹H NMR (600 MHz, D₂O) 9.02, 8.23, 8.07, 7.87, 7.83, 4.85, 4.45, 4.41, 4.40, 4.39, 3.83, 3.67, 3.50, 3.49, 2.40, 2.38, 2.36, 2.26, 2.22, 2.16; ¹³C NMR (150 MHz, D₂O) 167.9, 159.1, 147.2, 141.8, 135.4, 127.9, 127.2, 119.8, 119.0, 104.5, 65.8, 54.1, 46.8, 46.1, 42.1, 29.2, 24.5, 23.0; LC-MS R_t 8.37 min, m/z 837.3872 [M+H]⁺

40

【0209】

【化 1 2 1】



F A P I - 0 4

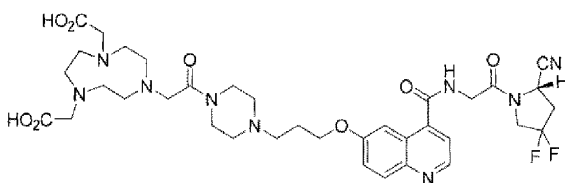
10

前のプロトコルを行い、3.97 mg (4.55 μ mol; 57%)を得た。

LC-MS R_t 8.80 min, m/z 873.3664 $[M+H]^+$

【 0 2 1 0 】

【化 1 2 2】



20

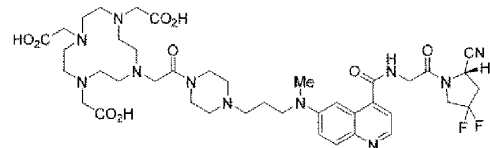
F A P I - 4 2

前のプロトコルを行い、1.91 mg (2.47 μ mol; 88%)を得た。

LC-MS R_t 9.37 min, m/z 386.6807 $[M+2H]^{2+}$

【 0 2 1 1 】

【化 1 2 3】



30

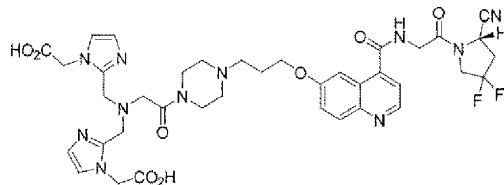
F A P I - 4 6

前のプロトコルを行い、39.21 mg (44.3 μ mol; 85%)を得た。

LC-MS R_t 9.03 min, m/z 443.7196 $[M+2H]^{2+}$

【 0 2 1 2 】

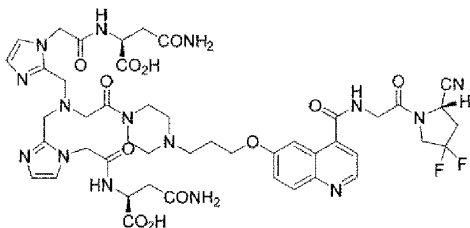
【化 1 2 4】



LC-MS R_t 8.91 min, m/z 820.2933 [M+H]⁺

【化 1 2 5】

10

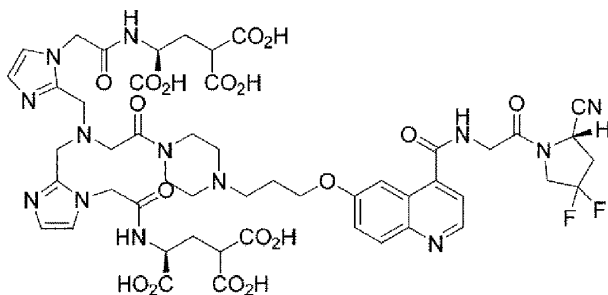


20

LC-MS R_t 9.23 min, m/z 524.7100 [M+2H]²⁺

【化 1 2 6】

30



40

LC-MS R_t 8.87 min, m/z 583.6988 [M+2H]²⁺

【 0 2 1 5 】

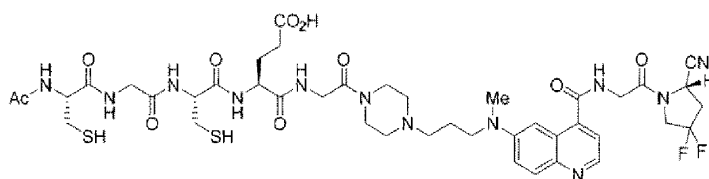
CC(=O)N[C@@H](CS)C(=O)NC(=O)N[C@@H](CS)C(=O)N1CCN(CC1)CCCOc2ccc3ncn(c3cc2)C(=O)N[C@@H]4C[C@H](F)C(F)[C@H]4C#N

10

20

【 0 2 1 6 】

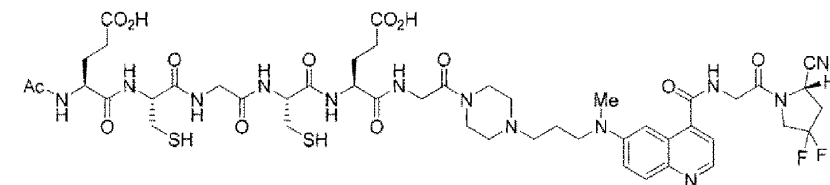
【化 1 2 8】



30

【 0 2 1 7 】

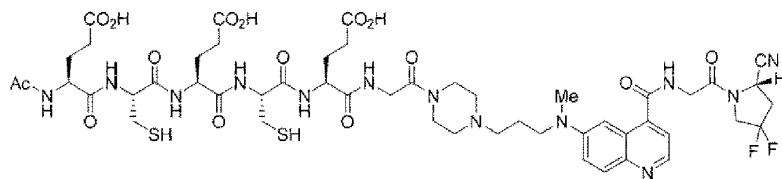
【化 1 2 9】



40

【 0 2 1 8 】

【化130】



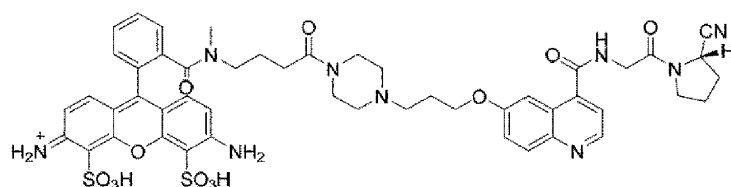
F A P I - 7 1

前のプロトコールを行い、0.79 mg (0.66 μmol; 34%)を得た。

LC-MS R_t 10.17 min, m/z 596.7075 [$M+2H$] $^{2+}$

【0219】

【化131】



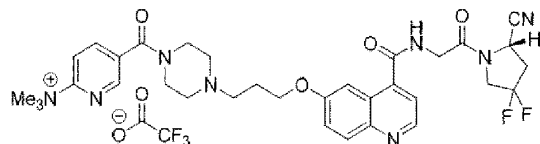
A t t o 4 8 8 - F A P I - 0 2 (1 4)

化合物11 0.66 mg (1.20 μmol)を、45℃でアセトニトリル250 μL中の4-メチルベンゼンスルホン酸一水和物1.33 mg (6.96 μmol)で4時間処理した。溶媒を除去した後、残留物をジメチルホルムアミド95 μLおよびトリエチルアミン5 μL (3.65 mg; 36.1 μmol)に溶解する。DMSO 25 μL中のA t t o 488 NHS-エステル0.54 mg (0.55 μmol)を添加した。60分後、HPLCで表題の化合物0.49 mg (0.43 μmol; 78%)を単離させ、凍結乾燥させた。

LC-MS R_t 10.19 min, m/z 1022.2706 [M] $^{+}$

【0220】

【化132】



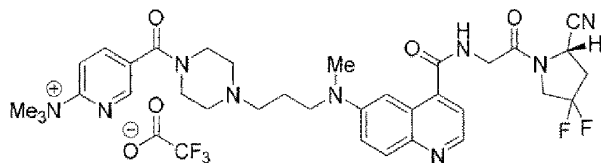
F A P I - 7 3

(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキソエチル)-6-(3-(4-tert-ブトキシカルボニルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボキサミド10.95 mg (18.7 μmol)を、アセトニトリル100 μLおよびトリフルオロ酢酸200 μLで30分間脱保護した。溶媒を蒸発させ、ジエチルエーテルで洗浄した後、N,N,N-トリメチル-5-((2,3,5,6-テトラフルオロフェノキシ)-カルボニル)ピリジン-2-アミニウムクロライド15.02 mg (9.27 μmol)を添加し、この混合物をジメチルホルムアミド200 μLおよびトリエチルアミン10.0 μL (7.30 mg; 72.3 μmol)に溶解した。120分後、混合物をHPLCで精製し、凍結乾燥させ、表題の化合物11.24 mg (14.7 μmol; 79%)を得た。

LC-MS R_t 9.37 min, m/z 649.2892 [$M-CF_3CO_2$] $^{+}$

【0221】

【化 1 3 3】



F A P I - 7 2

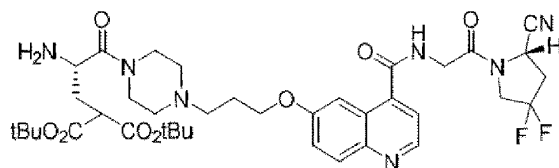
前のプロトコールを行い、9.80 mg (12.6 μmol; 70%)を得た。

LC-MS R_t 9.28 min, m/z 662.3237 $[M-CF_3CO_2]^+$

【0 2 2 2】

側鎖保護 Fmoc-アミノ酸の一般的な接合

【化 1 3 4】



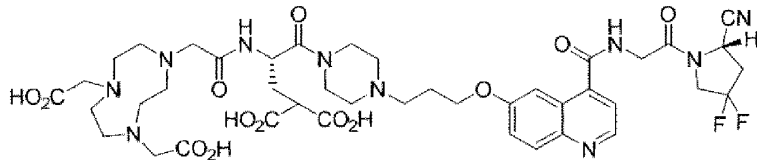
(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキシエチル)-6-(3-(4-(γ,γ-ジ-tert-ブチル)-L-カルボキシグルタミルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボキサミド

(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキシエチル)-6-(3-(1-tert-ブトキシカルボニル-ピペリジン-4-イル)-1-プロポキシ)キノリン-4-カルボキサミド 14.04 mg (23.9 μmol) を、アセトニトリル 50 μL およびトリフルオロ酢酸 100 μL に溶解した。10分後、揮発性物質を除去し、残留物をジエチルエーテルで洗浄した。ジメチルホルムアミド 200 μL 中の Fmoc-L-Gla(tBu)₂-OH 14.95 mg (28.4 μmol)、HOBt 7.74 mg (57.4 μmol)、HBTU 13.46 mg (35.5 μmol) および DIPEA 20.0 μL (14.8 mg; 115 μmol) の溶液を乾燥された残留物に添加した。60分後、モルホリン 50.0 μL (50.4 mg; 578 μmol) を添加し、30分後 HPLC で生成物を単離させた。凍結乾燥後、表題の化合物 15.95 mg (20.7 μmol; 86%) を得た。

LC-MS R_t 12.85 min, m/z 772.3643 $[M+H]^+$

【0 2 2 3】

【化 1 3 5】



F A P I - 7 5

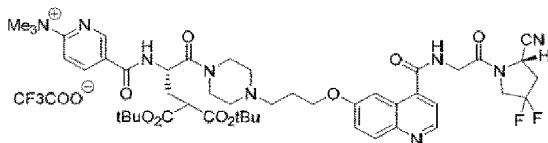
(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキシエチル)-6-(3-(4-(γ,γ-ジ-tert-ブチル)-L-カルボキシグルタミルピペラジン-1-イル)-1-プロポキシ)キノリン-4-カルボキサミド 3.37 mg (4.37 μmol) および NOTA-p-ニトロフェノール 4.52 mg (10.7 μmol) をジメチルホルムアミド 100 μL およびトリエチルアミン 10.0 μL (7.30 mg; 7

2.3 μmol)に溶解した。HPLC-精製および凍結乾燥後、中間体化合物を、アセトニトリル50 μL 、トリフルオロ酢酸100 μL 、トリイソプロピルシラン2.5 μL および水2.5 μL の溶液中で60分間インキュベートすることによって脱保護した。HPLC-精製および凍結乾燥後、2.62 mg (2.77 μmol ; 63%)を得た。

LC-MS R_t 9.38 min, m/z 945.3668 $[\text{M}+\text{H}]^+$

【0224】

【化136】



10

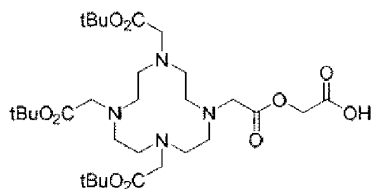
F A P I - 77 - 前駆体

一般的な活性エステル修飾プロトコールを行い、3.23 mg (3.06 μmol ; 73%)を得た。注：SPEを行った後、放射性フッ素化、HPLC-精製および95℃下純TFAでの3分間処理による溶媒蒸発後、tert-ブチル保護基を除去した。

LC-MS R_t 16.02 min, m/z 1219.5858 $[\text{M}+\text{H}]^+$

【0225】

【化137】



20

2-(2-(4,7,10-トリス(2-(tert-ブトキシ)-2-オキソエチル)-1,4,7,10-テトラアザシクロデカン-1-イル)アセトキシ)酢酸

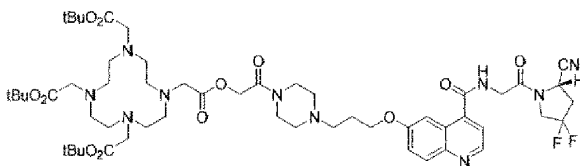
30

トリス- $t\text{Bu}$ -DOTA 28.99 mg (50.6 μmol)、炭酸セシウム90.65 (278 μmol)および2-ブロモ酢酸ベンジル10.28 μL (15.0 mg; 65.5 μmol)をジメチルホルムアミド300 μL に懸濁させ、2時間振盪した。生成物をHPLCで単離させ、凍結乾燥し、メーターノール中の10%酢酸25 mLに溶解した。10%Pd/C50 mgおよび水素(雰囲気圧)を添加した。2時間後、溶媒を除去し、HPLCで表題の化合物を単離させた。凍結乾燥後、表題の化合物25.19 mg (39.9 μmol ; 79%)を得た。

LC-MS R_t 14.14 min, m/z 631.4784 $[\text{M}+\text{H}]^+$

【0226】

【化138】



40

t B u - F A P I - 79

(S)-N-(2-(2-シアノ-4,4-ジフルオロピロリジン-1-イル)-2-オキソエチル)-6-(3-(1-tert-ブトキシカルボニル-ピペリジン-4-イル)-1-

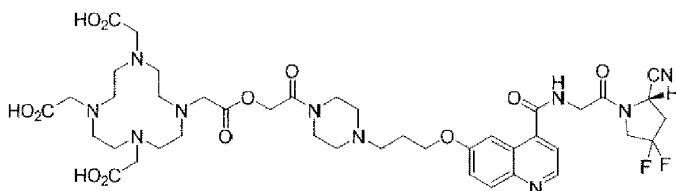
50

プロポキシ)キノリン-4-カルボキサミド 2.00 mg (3.41 μmol) を、アセトニトリル 50 μL およびトリフルオロ酢酸 100 μL に溶解した。10 分後、揮発性物質を除去し、残留物をジエチルエーテルで洗浄した。2-(2-(4,7,10-トリス(2-(tert-ブトキシ)-2-オキソエチル)-1,4,7,10-テトラアザシクロデカン-1-イル)アセトキシ)酢酸 4.20 mg (6.60 μmol) および HBTU 3.35 mg (8.84 μmol) をジメチルホルムアミド 100 μL に溶解し、DIPEA 10.0 μL (7.40 mg; 57.4 μmol) を乾燥された残留物に添加し、60 分間反応させた。HPLC 精製および凍結乾燥後、表題の化合物 2.26 mg (2.06 μmol ; 60%) を得た。

LC-MS R_t 12.98 min, m/z 1099.7481 $[\text{M}+\text{H}]^+$

【0227】

【化139】



FAP I-79

tBu-FAP I-79 2.26 mg (2.06 μmol) をアセトニトリル 25 μL およびトリフルオロ酢酸 100 μL に溶解し、35 $^{\circ}\text{C}$ で30分間振盪した。溶媒を蒸発させた後、生成物をHPLCで単離させた。凍結乾燥後、表題の化合物 1.58 mg (1.70 μmol ; 82%) を得た。

LC-MS R_t 8.84 min, m/z 466.2737 $[\text{M}+2\text{H}]^{2+}$

【0228】

化合物の分析

逆相高速液体クロマトグラフィー(RP-HPLC)は、Chromolith Performance RP-18eカラム(100 $\times$ 3 mm; Merck KGaA Darmstadt, Germany)でのアセトニトリル水溶液の直線勾配(5分あたりアセトニトリル0-100%、0.1% TFA、流量2 mL/分)を用いて行った。UV-吸光度は214 nmで検出した。放射性化合物のHPLC分析には、付加的な γ -検出器を使用した。HPLC-MSの特性評価は、Hypersil Gold C18 1.9 μm カラム(200 $\times$ 2.1 mm、20分あたりアセトニトリル0-100%、流量200 μL /分)を備えたAgilent 1200 HPLCシステムに接続したESI質量分析計(Exactive, Thermo Fisher Scientific, Waltham, MA, USA)を用いて行った。分析用ラジオ-HPLCは、Chromolith Performance RP-18eカラム(100 $\times$ 3 mm; Merck; 10分あたりアセトニトリル0-30%、流量2 mL/分)を用いて行った。HPLC-精製は、LaPrep P110-system(Knauer, Berlin, Germany)およびReprosil Pur 120カラム(C18-aq 5 μm 250 $\times$ 25mm; Dr. Maisch, Ammerbuch-Entringen, Germany)で行った。水/アセトニトリル-勾配(15または25分; 0.1% TFA; 流量20 mL/分)は個々の生成物に対して変更した。

【0229】

放射化学

放射性ヨウ素(I-125)は、Hartmann Analytik(Goettingen, Germany)から購入し; 放射性ルテチウム(Lu-177)はITG(Muenchen, Germany)から得; 放射性ガリウム(Ga-68)は、Themba Labs(Somerset West, South Africa)から購入したGa-68/Ga-68ジェネレータから溶出させた。Tc-99mは、Mo-99/Tc-99mジェネレータ(Curium Pharma, Berlin, Germany)から溶出させた。Cu-64は、UKT Tuebingen(Tuebingen, Germany)により提供された。Sm-153は、DSD Pharma(Purkersdorf,

Austria)により提供された。Pb-203は、Lantheus(N. Billerica MA, USA)により提供された。F-18-FDGおよびF-18-フッ化物は、ZAG Zyklotron AG(Eggenstein, Germany)により提供された。トリカルボニルのためのCRSキットは、Paul Scherrer Institut(Villingen-PSI, Switzerland)から得た。

【0230】

ヨウ素化において、FAP1-01の有機スズ前駆体(エタノール中1μmol/mL)10μLを1M 塩酸10μLおよび水10μLで希釈した後、0.05M NaOH中のヨウ素-125を1-20MBq添加した。氷酢酸中の新鮮な1.9%過酢酸溶液を5μL添加することにより反応を開始させた。60秒後、1M NaOHを15μL添加し、5%アスコルビン酸の水溶液を5μL添加することによって反応を停止させた後、HPLC精製を行った。得られた溶液をインビトロ実験に直接使用するか、または動物研究の場合には減圧下で蒸発乾固させ、0.9%NaCl(Braun, Melsungen, Germany)に取り入れて使用する。

【0231】

DOTA-化合物のCu-64、Lu-177およびPb-203標識は、放射性核種5MBqを0.1M NaOAc(pH5)中の個々の前駆体の10μM溶液100μLに添加し、95℃で10分間インキュベートすることによって行った、この溶液は、インビトロ実験に直接使用するか、または生体内分布研究の場合には0.9%NaCl(Braun, Melsungen, Germany)で希釈して使用する。マウスのイメージング研究(シンチグラフィ、PET)において、放射性追跡子を固相抽出(sep-pak light C18, Waters)で後処理した。

【0232】

Tc(I)標識の前に、0.9%食塩水中のTc-99m-過テクネチウム酸塩1mLをCRSキットに添加し、20分間インキュベートした。室温に冷めた後、前駆体(水中1mM)25.0μL、リン酸緩衝液(0.4M、pH7.4)150μLおよび塩酸(1.0M)240μLの混合物を添加し、最終混合物を必要に応じてpH5に調整した。この反応は95℃で20分間行い、固相抽出(sep-pak light C18, Waters)で後処理した。インビボ実験や動物研究において、Tc(VII)還元後、試薬の5分の1とCRSキット溶液200μLを用いて標識を行った。

【0233】

Tc(V)標識の前に、200mM グルコヘプトン酸塩を含有するSnCl2溶液30μLを0.9%生理食塩水中のTc-99m-過テクネチウム酸塩200μLで、室温で10分間インキュベートした。前駆体(水中1mM)5.00μLおよび水酸化ナトリウム溶液(水中0.1M)3.75μLを添加し、最終混合物を95℃で20分間反応させた。マウスのイメージング研究(シンチグラフィ)において、放射性追跡子を固相抽出(sep-pak light C18, Waters)で後処理した。

【0234】

動物研究のためのGa-68での標識は、ジェネレーター溶出液(0.6M HCl、約230MBq)255μLを95℃でDOTA-前駆体1nmol、20%アスコルビン酸水溶液1μLおよびNaOAc(2.5M)72μLの混合物で10分間インキュベートすることによって行った。残った遊離放射性は、水2mLでの希釈、固相抽出(sep-pak light C18, Waters)、水2mLでの洗浄、1:1水/エタノール1mLでの溶出によって除去した。得られた溶液を減圧下で蒸発乾固させ、残留物を0.9%NaCl(Braun)に取り取り入れた。

【0235】

AlF-NO₂A錯体の形成において、F-18フッ化物を水Sep-Pak QMA plus lightカートリッジ(吸着剤46mg、0.5M NaOAcで前処理、pH3.9)にトラップし、水で洗浄し、0.1M NaOAc(pH3.9)500μLで溶出させた。動物研究において、溶離液150μLをAlCl₃溶液(水中10mM)2μLおよびDMSO50μLでブレインキュベートした。5分後、この混合物をNO₂A-前駆体(4mM 水溶液10μL)40nmolおよび20%アスコルビン酸の水溶液1μLに添加した。この溶液

を95℃で15分間反応させた。生成物をHPLC(10分あたりアセトニトリル0-20%)で単離させ、溶媒を除去し、0.9%食塩水に取り入れた後注射した。

【0236】

6-フルオロニコチンアミドの形成において、F-18フッ化物をWaters Sep-Pak QMA plus lightカートリッジ(吸着剤46mg; 0.5M K₂HCO₃で前処理)にトラップし、水で洗浄し、乾燥させ、アセトニトリル450μLおよび水50μL中のcryptofix 222 7.50mg(19.9μmol)、KHCO₃ 1.99mg(1.99μmol)の混合物で溶出させた。溶媒を除去した後、残留物をアセトニトリル3×1mLで共沸蒸留によって乾燥させた。この残留物を1:1tert-ブタノール/アセトニトリル100μLに取り入れ、トリメチルピリジーン-2-アミニウム前駆体1mg(約1.3μmol)に添加した。この溶液を75℃で10分間反応させた。生成物をHPLC(10分あたりアセトニトリル0-30%)で単離させ、溶媒を除去し、0.9%食塩水に取り入れた後、注射した。

【0237】

その代わりに、6-フルオロニコチンアミドは、F-18フッ化物をWaters Sep-Pak QMA plus lightカートリッジ(吸着剤46mg; 0.5M K₂HCO₃で前処理)にトラップし、アセトニトリルで洗浄し、乾燥させ、メーターノール0.5mL中の(保護)FAPI-前駆体0.5mg(約0.4-0.6μmol)で溶出させることによって合成した。減圧下で溶媒を除去し、残留物を1:4アセトニトリル/tert-ブタノール100μLに取り入れた。70℃で20分後、反応混合物を水で希釈し、保護された中間体を固相抽出(sep-pak light C18, Waters)で後処理した。溶媒を除去し、トリフルオロ酢酸200μLを残留物に加えた。この混合物を95℃に3分間加熱し、減圧下で乾燥させ、水で希釈した後、生成物をHPLCで単離させ、これは、保護基を欠く化合物の場合、希釈された反応混合物で直接行われた。動物研究の場合は、生成物を溶媒を除去し、0.9%生理食塩水に取り入れた後注射した(無補正の放射化学的収量約25%)。

【0238】

ヒト血清中の安定性の測定において、放射性標識化合物(I-125では約2.5MBq、Lu-177では約15MBq)を精製(HPLCまたは固相抽出)し、溶媒を除去した。残留物をヒト血清(Sigma-Aldrich)250μLに取り入れ、37℃でインキュベートした。試料をアセトニトリル30μLで沈殿させ、HPLC(10分あたりアセトニトリル0-30%)で分析した。

【0239】

実施例2: FAPI誘導体のインビトロ特性評価

インビトロ結合研究は、ヒト腫瘍細胞株BxPC3、Capan-2、MCF-7(Sigma Aldrich Chemie GmbHから購入)およびSK-LMS-1(ATCCから購入)ならびに安定的にトランスフェクトされたFAPI-細胞株HT-1080-FAPI、HEK-muFAPIおよびCD26発現細胞株HEK-CD26(Stefan Bauer、NCT Heidelbergから得た)を用いて行った。全ての細胞を37℃/5%二酸化炭素下、10%ウシ胎児血清含有ダルベッコ変法イーグル培地(DMEM)中で培養した。蛍光内在化実験において、細胞をカバーガラス上に播種し、FAPI-02-Att-488およびDAPIで染色して、細胞核染色を行った。画像は、63倍油浸対物レンズを用いたレーザー走査型共焦点顕微鏡で取得した。放射性リガンド結合研究はHT-1080-FAPI細胞を用いて行った。放射性標識化合物を細胞培養物に加え、10分~24時間の範囲の異なる時間間隔でインキュベートした。競合実験は、非標識化合物(10⁻⁵M~10⁻⁹M)および放射性標識化合物に同時に60分間曝露させることによって行った。流出実験において、60分間インキュベートした後に放射性培地を除去し、1~24時間の範囲の時間間隔で非放射性培地に置き換えた。内在化実験において、細胞を1Mグリシン-HCl緩衝液で10分間インキュベートすることによって、表面結合活性を除去した。放射活性は、γカウンタを用いて測定し、1mio細胞に正規化し、適用線量のパーセンテージ(%ID)として計算した。

【0240】

細胞染色および顕微鏡観察

内在化実験において、HT-1080-FAP細胞およびHEK-muFAP細胞を24-ウェルプレートのコーティングされていないカバースリップ上に播種し、10%ウシ胎児血清含有培地で培養して、最終的に約80-90%のコンフルエンスにした。培地を除去し、pH7.4のPBS0.5mLで2回洗浄した。FAPI-02-Atto488(DMEM中20μM)を細胞に添加し、37℃で2時間インキュベートした。細胞をpH7.4のPBS0.5mLで3回洗浄し、パラホルムアルデヒド(PBS中2%)で15分間固定した。過成長したカバーガラスを、細胞核染色のためのDAPI含有マウンティング培地を用いてスライドガラス上に配置した(Fluoroshield, Sigma-Aldrich)。画像は、使用された各蛍光色素分子(FAPI-02-Atto488に対して488、DAPIに対して405nm)に対して、Zeiss Plan-Apochromat 63x/1.4油DIC III浸液対物レンズ(63x/1.4 Oil DIC III immersion objective)を用いて、0.099x0.099μmのxy画素設定および1Airy単位のピンホールサイズでレーザー走査型共焦点顕微鏡(Zeiss LSM 700; Zeiss, Oberkochen, Germany)上で取得した。写真は、ZEN 2008 softwareおよびImageJを用いて一貫して処理した。

10

【0241】

放射性リガンド結合試験

放射性リガンド結合試験において、細胞を6-ウェルプレートに播種し、48時間培養して、最終的に約80-90%のコンフルエンス(1.2-2mio細胞/ウェル)にした。培地を、ウシ胎児血清を含まない新鮮な培地1mLに置き換えた。放射性標識化合物を細胞培養物に加え、10分~24時間の範囲の異なる時間間隔でインキュベートした。競合実験は、非標識(10^{-5} M~ 10^{-9} M)および放射性標識化合物に60分間同時に暴露させることによって行った。流出実験において、60分間インキュベートした後に放射性培地を除去し、1~24時間の範囲の時間間隔で非放射性培地に置き換えた。全ての実験において、細胞をpH7.4のリン酸緩衝食塩水1mLで2回洗浄し、続いて溶解緩衝液(0.3M NaOH、0.2%のSDS)1.4mL溶解した。放射活性は、カウンター(Cobra II, Packard)を用いて測定し、1mio細胞に正規化し、適用線量のパーセンテージ(%ID)として計算した。各実験を3回行い、独立実験ごとに3回繰り返した。

20

【0242】

内在化実験において、細胞を37℃および4℃下で、放射性標識化合物とともに60分間インキュベートした。細胞の取り込みは、培地を細胞から除去し、PBS 1mLで2回洗浄することによって終了させた。続いて、細胞を室温でグリシン-HCl(PBS中1M、pH2.2)1mLで10分間インキュベートし、表面結合活性を除去した。細胞を、氷冷のPBS2mLで洗浄し、溶解緩衝液1.4mLで溶解し、内在化画分(内在化された画分)を測定した。4℃でインキュベートする細胞において、全ての洗浄および溶出工程は氷冷の緩衝液を用いて行った。放射活性は、γカウンターを用いて測定し、1mio細胞に正規化し、適用線量のパーセンテージ(%ID)として計算した。

30

【0243】

FAP-01は、ヒトおよびマウスのFAP-αを選択的に標的とする。

40

FAP-01のその標的タンパク質への結合性質を分析するために、放射性リガンド結合アッセイを、異なる癌細胞株ならびに、ヒトおよびマウスのFAPならびにDPP-IVとしても知られる密接に関連する膜タンパク質であるCD26でトランスフェクトされた細胞株、を用いて行った。マウスのFAPとCD26は両方とも、ヒトのFAP-αと高い相同性を示した(muFAP: アミノ酸レベルでの90%の同一性と94%の類似性; CD26: 52%の同一性と71%の構造的に高い類似性)(Kelly T., Drug Resist Updat, 2005)。

【0244】

図1Aに示されるように、FAP-01は、FAP-陰性癌細胞株には有意に結合せず、ヒトおよびマウスのFAP-α発現細胞を高親和性で標的とした(IC₅₀ ヒトのF

50

$AP-\alpha = 39.4 \text{ nM}$)。また、CD26発現細胞への実質的な結合は観察されなかった($0.05 \pm 0.01\%$)ことから、FAP I-01がFAP- α を選択的に標的としていることが証明された。これは、CD26が腎臓、肝臓、小腸を含む様々な正常組織で高度に発現していることから、特に重要である。非特異的なCD26結合による高いバックグラウンドシグナルを避けるためには、FAP- α に対するリガンドの高い選択性が大きな利点であり、結果的に最適な画像品質をもたらす。

【0245】

FAP I-01は、FAP陽性細胞に急速に内在化したが、時間依存性の流出およびロバストな脱ヨウ素化を示している。

細胞ベースの内在化アッセイは、FAP I-01の細胞への迅速な取り込みを示している(図1B)。10分間のインキュベーション後、全結合画分の95%が細胞内に位置している(合計 $19.70 \pm 0.28\%$)。4時間の経過でも、活性の低下は限定的でしか観察されなかった(合計 $17.00 \pm 0.40\%$ 、そのうち94%が内在化された)。

【0246】

ヨウ素-標識化合物は、しばしば時間依存性の酵素脱ヨウ素化を示す。これは、FAP I-01においても観察され、結果的により長いインキュベーション時間後にこの化合物の細胞内放射活性が低下した(24時間後に $3.25 \pm 0.29\%$)。脱ヨウ素化は、温度を4℃に下げた後、脱ヨウ素酵素の活性を低下させることによって最低限に抑えることができ、結果的に、24時間後に $26.66 \pm 1.59\%$ の放射活性が増加した。

【0247】

FAP I-02は、FAP I-01と比べて、ヒトFAP- α への結合および取り込みが強化されていることを示している。

酵素脱ヨウ素化によるFAP I-01の活性の急速な損失を避けるために、FAP結合部分をキレート剤DOTAに化学結合させたノンハロゲン誘導体FAP I-02を設計した。この修飾は、結果的に強化された安定性に加え、診断または治療用放射性核種のいずれを容易に繰り込む可能性を提供し、FAP I-02の不燃性化合物としての使用を可能にした。FAP I-02は、このヨウ素化アナログと類似して、CD26をアドレスすることなく、ヒトおよびマウスのFAP- (IC_{50}) ヒトのFAP- $\alpha = 21 \text{ nM}$)発現細胞に特異的に結合する($\%ID = 0.13 \pm 0.01\%$; 図1A)。FAP I-02は、FAP- α 発現細胞に急速に内在化し(60分後 $20.15 \pm 1.74\% ID$ 、そのうち96%が内在化した; 図1B)、これは時間の経過とともに、より安定した、より高い取り込み率を示している。FAP I-01の10分間のインキュベーション後の結合と比べて、24時間後には5%の活性しか残っていない。一方、FAP I-02の初期放射活性は、24時間のインキュベーション後に34%が検出された。流出実験では、FAP I-02がFAP I-01より有意的に遅く除去されることを実証し、これは24時間後には最初に蓄積されていた放射活性の12%を保持していることを示している(FAP I-01は24時間後に1.1%のIDを保持している; 図1E)。

【0248】

蛍光レーザー走査顕微鏡観察によって、ヒトおよびマウスのFAP- α 発現細胞へのFAP I-02のロバストな内在化を確認した。この目的のために、HT-1080-FAPおよびHEK-muFAP細胞を、蛍光標識FAP I-02誘導体(FAP I-02-Atto488)で1~2時間染色した。図1Dに示されるように、化合物はFAP- α 発現細胞の内部に完全に内在化され、蓄積されるが、FAP- α 陰性のHEK-CD26細胞では取り込みが検出されていない。

【0249】

結合性質と薬物動態が向上されたFAP I誘導体の設計

FAP I-02のさらなるバリエーション(変異体)を、セラノスティックなFAP-標的化剤の開発を目指して、腫瘍の保持時間を増加させるように設計した。バリエーションFAP I-03~FAP I-15を、標的結合、内在化率および標的特異性に関して特徴付けた。その結果を図2に示す。

【0250】

実施例3：マウスのPETイメージングおよび生体内分布分析

すべての実験は、ドイツ動物保護法に従って行われ、実験動物のケアと使用のための欧州委員会の規制に準拠していた。マウスはイソフルラン吸入により麻酔をかけた。

【0251】

インビボ実験において、8週齢のBALB/c nu/nuマウス(Charles River)を、HT-1080-FAP、Capan-2またはSK-LMS-1細胞をそれぞれ 5×10^6 で右幹に皮下接種した。腫瘍の大きさが約 1 cm^3 に達したとき、放射性標識化合物を、尾静脈を介して注射した(小動物PETイメージングでは約 10 MBq 、臓器分布では約 1 MBq)。PETイメージングは、マウス1匹あたり約 1 MBq のGa-68標識化合物を静脈内注射した後、Inveon PET小動物PETスキャナー(Siemens)を用いて、140分後まで行った。画像は3D-OSEM+MAP法を用いて反復的に再構成され、標準化取り込み値(SUV)画像に転換させた。ROI技術を用いて定量化を行い、 SUV_{mean} として示した。Lu-177標識化合物(マウス1匹あたり約 10 MBq)の臓器分布において、指示された時間点(30分~24時間)の後に動物(各時間点の $n=3$)を犠牲にした。分布された放射活性は、 γ -カウンター(Cobra Autogamma, Packard)を用いて、解剖されたすべての臓器および血液で測定した。値は、組織1グラム当たりの注射された用量のパーセンテージ($\% \text{ ID/g}$)として示した。

【0252】

薬物動態学的モデル化において、輸送定数 K_1 および速度定数 k_2-k_4 は、VOI内の組織と交換する血液の体積に関連する血管画分(v_B)を考慮して、PMODソフトウェア[4]に実装された2-組織コンパートメントモデルを用いて計算した。コンパートメントフラックス(コンパートメント flux)を説明する速度定数は、腫瘍組織内の k_1 (受容体への結合)、 k_2 (剥離)ならびに k_3 (内在化)および k_4 (流出)を含む。このモデルでは、分布の分画体積($DV = K_1 / k_2$)は、 ^{15}O -標識水が分布している関心領域の割合である。

【0253】

FAP I バリエーションは、マウス線維芽細胞の動員および活性化により、ヒトFAP発現異種移植片およびFAP発現していない異種移植片においても蓄積される。

FAP I-02および-04の腫瘍蓄積は、ヒトFAP陽性腫瘍細胞および陰性腫瘍細胞の両方からの異種移植片を有するマウスの小動物PETイメージングによって評価された。どちらの場合も、放射性追跡子は腫瘍内で急速に濃縮され、少なくとも140分間維持された(図3A、C、E、G)。同時に、FAP I-02および-04は、無視してよい程度に低い非特異的な結合、主に腎臓および膀胱を介して血液から迅速に除去されることを示しており、その結果、低バックグラウンドおよび有益な腫瘍対臓器の比をもたらす。非標識化合物を競合相手として同時に投与すると、腫瘍に放射活性が完全になくなり、これは放射性追跡子の標的タンパク質に対する特異性を実証している(図4)。興味深いことに、FAP- α 陽性(HT-1080-FAP)およびFAP- α 陰性(Capan-2)腫瘍細胞株を有するマウスで活性化マウス線維芽細胞の動員と活性化による、FAP I-02の高い腫瘍取り込みが観察された。Burger et al., Nucl Med, 1997による2-組織コンパートメントモデルを用いてPETデータから計算した放射性追跡子の薬物動態特性を表6に示す。

【表 6】

FAPI-02の薬物動態分析				
ユニット		Capan-2	HT-1080-FAP	HT-1080-FAP
		- comp.	- comp.	+ comp.
vB	l/l	0.08	0.04	0.04
k1	ml/ccm/分	0.08	0.07	0.10
k2	1/分	0.16	0.13	0.32
k3	1/分	0.08	0.10	0.04
k4	1/分	0.05	0.03	0.07
Vs	ml/ccm	0.93	2.31	0.18
Vt	ml/ccm	1.44	0.87	0.38
Flux	ml/ccm/分	0.03	0.03	0.01
Chi ²	----	0.10	0.11	0.26

表 6. Burger et al., Nucl Med, 1997による2-組織コンパートメントモデルを用いてPETデータから計算した⁶⁸Ga-FAPI-02の薬物動態特性。vB:VOI(関心体積)内の組織と交換する血液の体積に関連する血管画分; k1-k4:計算した速度定数; Vs:平衡状態での総ペARENTに対する特定の結合濃度の比; Vt:総分布体積【0254】

これらの観察では、生体内分布研究で¹⁷⁷Lu-FAPI-02および-04を用いて確認され、他のすべての臓器で非常に低い活性を示すとともに、ヒトFAP-α陽性および陰性腫瘍の両方への急速な腫瘍蓄積を証明し(定量化取り込み値は表7を参照)、結果的に有益な腫瘍対臓器の比が得られた(図5D-F)。HT-1080-FAP腫瘍を有するマウスにおける¹⁷⁷Lu-FAPI-04で類似の結果が得られた。FAPI-02と比較して、FAPI-04は、特に24時間後に高い腫瘍取り込みを示している(図5C)。曲線下面積(AUC)の計算を表8に示す。

【表 7】

	FAPI-02 (Capan-2)	FAPI-02 (HT-1080-FAP)	FAPI-04 (HT-1080-FAP)
血液	0.83 ± 0.127	1.20 ± 0.178	1.70 ± 0.206
脳	0.05 ± 0.010	0.06 ± 0.006	0.08 ± 0.010
心臓	0.37 ± 0.031	0.56 ± 0.085	0.80 ± 0.089
腸	0.30 ± 0.064	0.37 ± 0.046	0.66 ± 0.196
腎臓	1.45 ± 0.106	1.60 ± 0.075	2.28 ± 0.477
肝臓	0.36 ± 0.015	0.45 ± 0.074	0.73 ± 0.118
肺	0.72 ± 0.021	1.02 ± 0.152	1.50 ± 0.151
筋肉	0.94 ± 0.168	1.17 ± 0.332	0.92 ± 0.020
脾臓	0.25 ± 0.015	0.38 ± 0.051	0.48 ± 0.072
腫瘍	3.82 ± 0.390	4.51 ± 0.816	4.89 ± 0.817

表 7. Lu-177 標識 FAPI-02 および -04 を、腫瘍を有する Balb/c ノードマウスへの静脈内投与 1 時間後の生体内分布データの定量化; $n = 3$; 平均として報告された値 $\%ID/g \pm SD$

【表 8】

%ID/g	1時間	4時間	24時間	AUC
FAPI-02	4.5 $\pm$ 0.82	4.0 $\pm$ 0.56	1.12 $\pm$ 0.13	64.0
FAPI-04	4.9 $\pm$ 0.82	5.4 $\pm$ 1.51	3.0 $\pm$ 0.23	99.4
FAPI-05	6.0 $\pm$ 0.90	5.8 $\pm$ 0.60	2.8 $\pm$ 0.40	103.3
FAPI-10	3.2 $\pm$ 0.72	2.9 $\pm$ 0.12	1.1 $\pm$ 0.04	49.3
FAPI-13	6.3 $\pm$ 0.57	8.7 $\pm$ 0.77	4.8 $\pm$ 1.71	157.5
FAPI-15	3.4 $\pm$ 1.13	4.6 $\pm$ 0.32	1.1 $\pm$ 0.25	68.0

10

表 8. HT-1080-FAP 腫瘍を有するノードマウスにおける選択された FAPI 誘導体の腫瘍取り込み、 $n = 3$ 。値は平均として報告される ($ID/g \pm SD$)。

【0255】

実施例 4: 臨床 PET/CT 研究

100 人を超える患者の画像診断は、ヘルシンキ宣言 § 37 (臨床現場での実証されていない介入) の更新の条件下で、ドイツ医薬品法 § 13 (2b) に従って、医療上の理由から ^{68}Ga -FAPI-02 または -04 のいずれかを用いて行い、追跡子静脈内投与 ($20 nmol$ 、 $122-336 MBq$) 10 分後、1 時間後、3 時間後に適用した。注射された放射性追跡子の活性の変動は、 ^{68}Ga の短い半減期と、 ^{68}Ge / ^{68}Ga ジェネレーターの寿命中に得られた変動する溶出効率によるものである。1 人の患者を対象に、 ^{18}F -FDG を $358 MBq$ 静脈内注射して 1 時間後に FDG イメージングを行った。PET/CT スキャンは、Biograph mCT FlowTM PET/CT-Scanner (Siemens Medical Solution) で、以下のパラメータを用いて行った: スライス厚 $5 mm$ 、増分 $3 \sim 4 mm$ 、軟部組織再建カーネル、ケア線量。CT スキャンの直後に、全身 PET を FlowMotionTM の 3D (マトリックス 200×200) で $0.7 cm$ /分で取得した。放出データを、ランダム、散乱、減衰について補正した。再構成は、2 反復/21 サブセットの順序部分集合期待値最大 (OSEM) アルゴリズムで実行し、半値全幅 (FWHM) で $5 mm$ の横軸解像度にガウスフィルター処理した。低線量非強調 CT データを用いて減衰補正を行った。関心領域技術を用いて標準化取り込み値 (SUV) の定量的評価を行った。

20

【0256】

FAPI-02 および -04 は、ヒトの転移性乳癌、膵臓癌、肺癌、HNO 癌、小腸癌および卵巣癌に急速に蓄積される。

転移性乳癌、肺癌、膵臓癌、HNO 癌、小腸癌、卵巣癌患者に ^{68}Ga -FAPI-02 および -04 静脈内投与 1 時間後に診断 PET/CT スキャンを行った。すべての患者において、原発性腫瘍ならびにリンパ節および骨において追跡子のロバストな蓄積が観察され、転移の最大 SUV 値は 48.0 であった。対照的に、正常組織への追跡子の取り込みは非常に低かった (図 6-14)。放射活性は血流から急速に除去され、大部分は腎臓を経由して排泄され、結果的に高コントラスト画像が得られた。1 人の局所進行性肺腺癌患者における比較画像診断により、一般的に使用されている PET 追跡子 ^{18}F -FDG と比較して、FAPI-02 の明らかな優位性が明らかになった。図 9 に示されるように、FAPI-02 は、より低いバックグラウンド放射活性 (background activity) とともに高い取り込みを示し、これは転移性病変のより良い可視性のより高いコントラストにつながる。FAPI-02 は、脳などのブドウ糖消費量の多い細胞に多く蓄積される FDG とは異なり、FAPI- α が発現している組織を選択的に標的としている。1 人の前立腺癌患者における比較画像処理により、一般的に使用されている PET 追跡子 ^{68}Ga -DOT

30

40

50

A T O C および ^{68}Ga -P S M A と比較して、F A P I - 0 4 の明らかな利点が明らかになり、これは腎臓への追跡子蓄積が減少されたより小さな腫瘍病変の検出を可能にした(図 1 4)。

【 0 2 5 7 】

考察

原発性腫瘍、転移性病変および発症したリンパ節の信頼できる診断は、腫瘍病期分類および処置の選択を含むものを計画している効果的かつ適した療法を可能にするのに最も重要なものである。この目的のために、画像処理技術は多くの種類の癌の診断のために不可欠な手段である。P E T / C T の組み合わせは、その高い診断精度ならびに解剖学および生理学的詳細の両方を評価する可能性のため、最新の腫瘍診断のための選択法である。しかしながら、P E T / C T の組み合わせは、M R T や C T 単独のような非侵襲性画像処理技術とは対照的に、腫瘍内での発現が正常組織と比較して増強された標的構造に対して高い親和性を有する放射性追跡子の使用を必要とする。理想的な追跡子は、癌性組織と健康的な組織の信頼できる区別ならびに高コントラスト画像をもたらす低バックグラウンドシグナルを確保するように、その標的タンパク質に特異的に結合する必要がある。放射性追跡子がセラノスティックな化合物を示す場合、すなわち、標的化および個別化処置を容易にしかつ改善する診断用核種または治療用核種のいずれかを搭載する可能性を提供する場合、親和性および特異性はさらに重要となる。治療的な目的のための追跡子の潜在的応用については、高い標的特異性は副作用の軽減を保証し、これは、骨髄、生殖器および消化器などの放射線感受性組織の保護に特に重要である。

【 0 2 5 8 】

そのことを念頭に置いて、発明者らは、腫瘍間質の主要成分を形成する癌関連線維芽細胞を標的とするセラノスティックな追跡子を開発した。それらは、腫瘍の増殖、転移および進行に重要な役割を果たすことが知られており、癌細胞よりも遺伝的に安定しているため、療法抵抗性の発生の影響を受けにくくなっている。C A F は、正常な線維芽細胞とは対照的に、腫瘍特異的マーカーとして使用できる特定のタンパク質を発現している。これらの中には、様々な腫瘍の微小環境に広く発現している膜タンパク質 F A P - α が含まれており、これによって、固形腫瘍全体の大部分を占めている膵臓癌、乳癌、肺癌を含むさまざまな腫瘍の実体の標的化を可能にする。

【 0 2 5 9 】

この標的タンパク質に対する親和性の高い小分子酵素阻害物質を基に、化学修飾を集中的に行うことにより、F A P I - 0 1 ~ F A P I - 7 3 の放射性追跡子を開発した。すべての化合物は、密接に関連するタンパク質 C D 2 6 / D P P 4 をアドレスすることなく、ヒトおよびマウスの F A P - α への迅速かつほぼ完全な内在化の特異的な結合を示している。ヨウ素化分子は遊離ヨウ素の流出とともに酵素的な脱ヨウ素化を受けるため、インキュベーション時間が長くなると細胞内放射活性が低くなる。このため、F A P I - 0 2 および後続の化合物は、F A P 結合部分がキレート剤 D O T A に化学的に結合されるように設計した。この結果、好ましい薬物動態および生化学的な性質を有する一連のセラノスティックな化合物が得られ、そのうち、F A P I - 0 2、F A P I - 0 4、F A P I - 4 6、F A P I - 3 4、F A P I - 4 2、F A P I - 5 2、F A P I - 6 9、F A P I - 7 0、F A P I - 7 1、F A P I - 7 2 および F A P I - 7 3 が、最も好ましいリガンドである。F A P I - 0 2 および F A P I - 0 4 の両方は、F A P I - 0 1 より有意に遅く除去され、24 時間後には元の蓄積放射活性の 12 % (F A P I - 0 2) と 49 % (F A P I - 0 4) が保持され (F A P I - 0 1 は 1.1 %)、他の好ましい化合物はさらなる強い結合力を有している(図 1 6)。それらは F A P - α 発現細胞に急速に内在化し、腫瘍担持マウスおよび転移性上皮癌患者の両方で高い腫瘍取り込み率を示している。対照的に、正常組織には蓄積がなく、血液系からのクリアランスが早いいため、高コントラスト画像が得られる。ヒトおよびマウスの F A P - α 発現細胞の両方へのロバストな内在化は、蛍光標識 F A P I - 0 2 を用いて共焦点顕微鏡によって確認された。内在化されずにその標的タンパク質に対して高い親和性を有する第 1 世代 F A P 抗体 F 1 9 とは対照的に、F A P I - 0 2

は、1時間のインキュベーション後に完全な細胞内取り込みを示している。FAP結合後の内在化のメカニズムは、SK-Mel-187細胞におけるFAP抗体断片(Fabs)およびDyLight 549抗マウス抗体を用いた、Fischerらによって研究されている。37℃でのインキュベーションにより、FAP-抗体複合体の内在化が生じた。我々の小分子と同様に、内在化プロセスは、ほぼ完全な内在化を伴って急速に起こった。20分後に初期エンドソームのマーカ-とFabの共存が、40分後に後期エンドソームおよびリソソームのマーカ-とFabの共存が観察された。Fab介在FAP- α 内在化は、ダイナミン依存性エンドサイトーシスの阻害物質によって抑制されており、これはエンドサイトーシスがダイナミン依存性メカニズムによって発生することを示している。

【0260】

FAPI-02および-04は、腎実質に保持されずに腎クリアランスによって生物からすぐに除去される。炎症組織または脳を含むブドウ糖消費量の多い細胞に多く蓄積する¹⁸F-FDGとは異なり、FAPI-02は標的タンパク質が発現している組織に選択的に濃縮される。これにより、これらの領域の悪性病変を検出するための新しい展望が開かれる。さらに、FAP- α はまた、リウマチ性関節炎および骨関節症、アテローム性動脈硬化症、線維症患者ならびに心筋梗塞後の虚血性心臓組織において、リウマチ筋線維芽細胞様滑膜細胞によって発現されることも示された。これらの観察は、さらなる適応症のためのイメージング追跡子としてのFAPI-02と-04の適用を示唆している。

【0261】

腫瘍病変の検出のための制限因子は、腫瘍内のFAP- α 発現の程度である。これは、活性化線維芽細胞の数、すなわち間質含有量、および/または微小環境によって決定される線維芽細胞あたりのFAP- α 分子の数におおに左右される。1~2mmを超えるサイズの腫瘍の増殖は、基本的に支持間質の形成を必要とするため、FAPI-PET/CTを用いて3~5mmの範囲の小さな病変を可視化することが可能である。

【0262】

他の標的化アプローチと同様に、FAPI誘導体は、異なる癌型や患者においてかなり不均一であると知られている十分に高いFAP- α 発現を伴う組織でのみ最適な結果を達成する。FAPIイメージングの優れた候補である乳癌、結腸癌、膵臓癌の他に、肺癌、頭頸部癌、卵巣癌、肝腫などの他の腫瘍実体が好ましいターゲットであるかどうかを探究する必要がある。

【0263】

また、FAP- α の発現は、創傷治癒および線維性組織で実証されたが、これは放射線所見を解釈する際に留意すべきである。これらの事実は、どの患者が潜在的なFAPI療法の利益を受ける可能性が高いかを適切に評価する必要性を強調している。診断用または治療用核種のいずれかを使用する能力を考えると、FAPI-02および-04は、適切な患者コホートの簡単な層別化を可能にする。いずれにしても、FAPI追跡子は両方とも標的化放射性医薬品の開発に理想的な候補であることはすでに明らかである。その高い標的親和性、迅速な腫瘍内在化および迅速な全身クリアランスにより、それらはすでに腫瘍イメージングに理想的に適している。

【0264】

実施例5：インビトロおよびインビボでのFAPIの特性評価

実験方法と臨床的評価

FAPI誘導体の臨床評価と同様に、すべてのインビトロおよびインビボ実験は、上記のように、Loktevら¹およびLindnerら²に従って行った。FAPI-02およびFAPI-04の予備的な線量推定値は、QDOSE線量推定ソフトウェアツールを用いて、追跡子を注射して0.2時間後、1時間後および3時間後で検査された2人の患者に基づいている。腫瘍患者に対するさらなるPET/CTスキャンは、FAPI-02(n=25)またはFAPI-04(n=25)のいずれかの注射1時間後に取得された；6人の患者については、個人内関連FDGスキャン(同じく1時間後に取得された)が利用可能であった。16の臓器の正常組織については、2cmのSpheric-VOIを柔

組織に配置し、腫瘍病変については閾値分割VOIを用いて SUV_{mean}/max^3 を定量化した。

【0265】

DOTA-FAPI誘導体のインビトロ特性評価

DOTA-FAPI誘導体の標的結合および内在化率をFAPI-04と比較して評価するために、Lu-177標識化合物をFAP発現HT-1080細胞とそれぞれ1時間、4時間および24時間インキュベートした(図16)。膜結合画分は、pH2.2のグリシン-HClを用いた酸性溶出によって除去し、その後アルカリ細胞溶解を行い、内在化画分(内在化された画分)を測定した。図16に示されるように、すべての誘導体は、FAPI-04と比べて、結合値が1時間のインキュベーション後に最大リード化合物の50% (4時間後に最大750%)となるように高い細胞結合を示している。

【0266】

標的親和性および特異性を評価するために、Lu-177標識化合物の競合相手として非標識化合物の濃度を増加させて、競合結合アッセイを行った(図17;表9に記載されているそれぞれの IC_{50} 値)。

【表9】

化合物	IC_{50} (nM)	化合物	IC_{50} (nM)
FAPI-04	6.5	FAPI-39	11.3
FAPI-05	17.2	FAPI-40	12.7
FAPI-10	19.9	FAPI-41	8.3
FAPI-13	4.5	FAPI-46	13.5
FAPI-15	9.1	FAPI-47	42.0
FAPI-20	7.2	FAPI-48	26.3

表9.競合結合アッセイによって測定された、選択されたFAPI誘導体の IC_{50} 値

【0267】

腫瘍担持マウスにおけるDOTA-FAPI誘導体の臓器分布

薬物動態プロファイルならびにインビボでの腫瘍取り込みを分析するために、Lu-標識DOTA-FAPI誘導体をHT-1080-FAP腫瘍担持マウスに静脈内投与した。放射性標識化合物の臓器分布は、血液、健康な組織、腫瘍においてエクスピボで測定した。図19に示されるように、ほとんどの化合物は、FAPI-02およびFAPI-04と比較して、特に投与24時間後により高い腫瘍取り込み率を示している。親油性の増加により、一部の放射性追跡子はより高い血液活性ならびに腎臓での保持の増加を示している。腫瘍-血液比の測定は、実験においていつもFAPI-04より有意に高い比率を示している化合物FAPI-21およびFAPI-46の明確な利点を明らかにする(図20)。

【0268】

腫瘍担持マウスにおけるDOTA-FAPI誘導体の小動物イメージング

これらの所見に基づき、Ga-68標識DOTA-FAPI誘導体を用いて放射性追跡子をHT-1080-FAP腫瘍担持マウスに静脈内投与した後、140分後まで小動物PETイメージングを行った。FAPI-21およびFAPI-46の有益な腫瘍-血液比は、高コントラスト画像をもたらし、FAP陽性腫瘍の優れた可視化を可能にする(図21)。腫瘍、腎臓、肝臓および筋肉組織における追跡子蓄積の定量分析(SUV_{max} 値として与えられる)では、FAPI-21と比較してFAPI-46の筋肉、腎臓および肝臓活性がわずかに低いことを示している(図22)。

【0269】

癌患者におけるFDGと比較したFAPI-02およびFAPI-04の生体内分布および線量測定推定値

F-18-FDG、Ga-68-DOTATEまたはGa-68-PSMA-11の文献値と類似して、200MBqのGa-68-FAPI-02および-04の実験では、約3-4mSvの等価線量に相当する。注射10分後～3時間後の間に、腎臓を經由して迅速にクリアランスされた後、正常な臓器では、最小限度の変化しかない低い追跡子取り込みを示している。FAPI-02において、注射1時間後～3時間後の腫瘍取り込みは75%減少されるが、FAPI-04(50%洗い流し)では腫瘍の保持がわずかに延長されている。注射1時間後、両方のFAPI-追跡子の腫瘍の取り込みは等しく技能している(図23)。FDGと比較して、腫瘍の取り込みはほぼ等しく(平均 SUV_{max} FDG 7.41; SUV_{max} FAPI-02 7.37; n.s.); FAPI-02での脳(11.01 vs 0.32)、肝臓(2.77 vs 1.69)および口腔/咽頭粘膜(4.88 vs 2.57)におけるバックグラウンド取り込みは有意に低く; 他の臓器ではFDGとFAPI-02の間に関連性のある違いはない(図24)。詳細な情報および結果については、参照により本文中に組み込まれているGieselら³を参照すること。

【0270】

さまざまな癌ならびに非癌性悪性腫瘍患者におけるFAPI-04のPETイメージング

乳癌、膵臓癌、卵巣癌、HNO腫瘍を含むさまざまな癌におけるGa-68標識FAPI-04の迅速な取り込みに加えて、癌性腹膜炎(図25A)ならびに心筋炎(図25B)や関節症(図25C)のようないくつかの炎症性悪性腫瘍においても追跡子蓄積が示された。これらの結果は、活性化線維芽細胞の動員に關与する慢性炎症のプロセスを特徴とする非癌性悪性腫瘍の検出へのGa-68標識FAPIの潜在的応用を示している。

【0271】

各種癌患者におけるFAPI-21とFAPI-46のPETイメージング

図26に示されるように、卵巣癌、直腸癌および粘液性類表皮癌を含むさまざまな癌においてGa-68標識FAPI-21のロバストな蓄積が観察された。胆管細胞性癌および結腸直腸癌、肺癌ならびに単発性線維性肉腫に急速に蓄積したGa-68標識FAPI-46では類似の腫瘍取り込みが示された(図27)。Ga-68標識FAPI-46を用いたPET/CT検査に続いて、Sm-153標識放射性追跡子を用いた最初の治療的アプローチを2人の癌患者で行った。図28に示されるように、追跡子のロバストな腫瘍蓄積は、投与後20時間まで検出可能である。

特発性肺線維症肺癌患者3人のFAPI-46-PET/CTイメージングでは、癌性病変と線維性病変での追跡子蓄積に明確な違いがあることが示された。図30に示されるように、Ga-68標識FAPI-46の腫瘍取り込みは、線維組織で測定された活性と比較して、2人の患者(A、B)では有意に高かったが、1人の患者(C)ではわずかに低かった。図Cに示される患者は、2つの非増悪例と比較して、増悪肺線維症を患っていた。したがって、この追跡子は、予後の悪い線維症患者と予後の良い患者を鑑別するのに有用であり得る。

【0272】

代替放射性核種、例えばTc-99m、Pb-203、Cu-64およびF18での放射性標識のためのFAPI誘導体

代替放射性核種の使用を可能にするために、一連のFAPI誘導体が設計され、標的親和性、特異性及び薬物動態に関して特徴づけられている。これらの化合物のいくつかにおいて、元のキレート剤DOTAは、Tc-99m(FAPI-19、-27、-28、-29、-33、-34、-43、-44、-45、-60、-61、-62)の取り込みに理想的に適した異なるキレート化部分によって置き換えられている。FAPI-19およびFAPI-34について、HT-1080-FAPI異種移植マウスにおけるインビトロでのFAPI親和性及び生物分布が例示されている。両方の化合物は、インビトロでのヒトFAPIへのロバストな結合を示している(IC_{50} FAPI-19: 6.4 nM)。インビボでの腫瘍取り込みが不十分であり、ならびに腎臓から肝臓の方への排出により肝臓に急速に蓄積することを示しているFAPI-19とは対照的に、FAPI-34は、腫瘍内に継続的に濃縮され、が有意に少ない肝臓取り込みを示している(図31、32)。肝

臓転移を伴う膵臓癌患者における $Tc-99m$ 標識 $FAP I-34$ の最初の診断的適用では、投与後4時間まで追跡子の安定した腫瘍蓄積を示している。また、全体的なバックグラウンド放射活性が比較的低いため、高コントラスト画像が得られる(図33)。これにより、 $Re-188$ で標識した後、シンチグラフィーおよび療法による診断のための広範な応用を提供している。

【0273】

$Pb-203$ 放射性標識 $FAP I$ 誘導体($FAP I-04$ 、 -32 、 -46 および $FAP I-04 tcm c$)は、 $HT-1080-FAP$ 細胞への同等の細胞結合を示しており、60分間のインキュベーション後に $FAP I-32$ および $FAP I-04 tcm c$ が最も高い結合値に達している(26.93 ± 0.846 および $21.62 \pm 0.61\% ID/1 m i o$ 細胞、図34A)。 $FAP I-32$ が最初の結合($t 1/2=2$ 時間)時に腫瘍細胞から急速に排除されるのに対し、 $FAP I-04 tcm c$ は、かなり遅い細胞流出($t 1/2=7$ 時間)を示すだけでなく、競合アッセイ($IC_{50}=5.7 \mu M$ 、図34C)によって示されるように、最も低い FAP 親和性も示している。この理由により、インビボでのさらなる分析のために、最適な半減期および IC_{50} 値を特徴とする $FAP I-04$ および $FAP I-46$ を選択した。図35に示されるように、両方の化合物は、健康な組織への結合が無視してよい程度に低いことを示しているが、腫瘍内で継続的に濃縮されている。シンチグラフィーによる所見は生体内分布研究で確認されており、ここで、両方の放射性追跡子はロバストな腫瘍取り込み、全体的に低い臓器活性ならびに急速な腎臓排泄を示している(図36)。

【0274】

$Cu-64$ を用いた放射性標識を可能にするために、 $NOTA$ 誘導体 $FAP I-42$ および $FAP I-52$ が開発し、標的親和性、特異性および薬物動態に関して特徴付けている。図37に示されるように、両方の追跡子は、より低いナノモル範囲において類似の IC_{50} 値でのインキュベーション24時間まで $HT-1080-FAP$ 細胞へのロバストな結合を示している(図37A、B)。それでも、 $FAP I-42$ は $FAP I-52$ より有意にゆっくり排除され、計算されたインビトロ半減期は12時間になっている(図37C)。これらの結果は、 $HT-1080-FAP$ 異種移植マウスの小動物イメージングによって確認される。図38に示されるように、両方の化合物は、ロバストな腫瘍取り込みならびにインビボ血流からの迅速なクリアランスを示している。特に、 $FAP I-42$ の腎臓排泄は、 $FAP I-52$ と比較して有意に速いが、その腫瘍活性は、投与後2~24時間の間にわずかに高いままである。

【0275】

$NOTA$ 誘導体 $FAP I-42$ および $FAP I-52$ は、 $F-18$ でのイメージングを可能にするフッ化アルミニウム錯体の形成のために配置された。図39に示されるように、両方の化合物は、 $HT-1080-FAP$ 異種移植マウスの小動物イメージングにおいて、迅速な腫瘍取り込みを示している。両方の化合物は主に腎臓経路によって排出されるが、胆汁排出も観察されている。腎臓排泄は $FAP I-52$ の方が速いが、腫瘍蓄積がより多く、腫瘍の滞留時間がより長く、胆道経路の割合がより低いことは、 $FAP I-42$ が有利である。

【0276】

参考文献

- 1 Loktev, A. et al. A new method for tumor imaging by targeting cancer associated fibroblasts. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, doi: 10.2967/jnumed.118.210435 (2018).
- 2 Lindner, T. et al. Development of quinoline based theranostic ligands for the targeting of fibroblast activation protein. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, doi: 10.2967/jnumed.118.210443 (2018).
- 3 Giesel, F. et al. FAPI-PET/CT: biodistribution and preliminary dosimetry estim

ate of two DOTA-containing FAP-targeting agents in patients with various cancers. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, doi: 10.2967/jnumed.118.215913 (2018).

【0277】

実施例6：インビトロおよびインビボでのFAP I 特性評価

前臨床データ

FAP陽性脳腫瘍を選択的に標的化することを目的として、ヒト神経膠芽腫異種移植モデルU87MGを用いて、腫瘍担持マウスで初期実験を行った。放射性標識FAP I-02および-04の腫瘍蓄積および臓器分布は、小動物PETイメージングならびに生体内分布研究で分析した。図40および41に示されるように、FAP I-02および-04の両方は、迅速な腫瘍取り込みを示し、健康な臓器および血液中では、無視してよい程度に低い活性を示している。

10

【0278】

臨床データ

2016年のWHO分類によると、神経膠腫はIDH野生型神経膠腫WHOグレードI~IVとIDH変異神経膠腫WHOグレードII~IVに細分化されている。最も頻度の高いWHOグレードIV神経膠腫は神経膠芽腫である。

神経膠腫患者18人(IDH変異神経膠腫5人、IDH野生型神経膠芽腫13人；表10参照)で臨床PETイメージングを行った。図42-44に示されるように、IDH野生型神経膠芽腫およびグレードIIを除いたグレードIII/IVのIDH変異体神経膠腫で追跡子取り込みを示した。神経膠芽腫において、コントラスト向上領域への投影において取り込みが増加したスポットが観察された。

20

【0279】

結論

IDH野生型神経膠芽腫および高悪性度IDH変異星細胞腫における追跡子取り込みは増加するが、びまん性星細胞腫では増加しないことから、低悪性度IDH変異と高悪性度神経膠腫の非侵襲性区別を可能にし、追跡研究に有用であり得る。神経膠芽腫における不均一な追跡子取り込みは、生検計画に役に立ち得る。

【表 10】

患者 番号	年齢	IDH状態	診断	WHO グレード	局在性	生検 手術	前処置
1	57	野生型	神経膠芽腫	IV	右頭頂(parietal right)	生検	放射線化学療法
2	65	野生型	神経膠芽腫	IV	両側前頭葉(bifrontal)	生検	なし
3	66	野生型	神経膠芽腫	IV	左前頭側頭(parieto-temporal left)	生検	なし
4	64	野生型	神経膠芽腫	IV	右側頭(temporal right)	切除	放射線化学療法
5	58	野生型	神経膠芽腫	IV	膨大部(Splenium)	生検	なし
6	48	野生型	神経膠芽腫	IV	左前側頭頂部(fronto-temporo-parietal left)	切除	放射線化学療法
7	20	野生型	神経膠芽腫	IV	視床/右側頭(Thalamus / temporal right)	生検	なし
8	54	野生型	神経膠芽腫	IV	左側頭(temporal left)	切除	なし
9	56	野生型	神経膠芽腫	IV	大脳基底核(basal ganglia)	部分的な切除	なし
10	61	野生型	神経膠芽腫	IV	右側頭(temporal right)	切除	なし
11	86	野生型	神経膠芽腫	IV	左放射冠(Corona radiata left)	生検	化学療法
12	68	野生型	神経膠芽腫	IV	脳梁(Corpus callosum)	生検	なし
13	71	野生型	神経膠芽腫	IV	左側頭(temporal left)	生検	なし
14	29	IDH1 R132H 変異	神経膠芽腫	IV	右側頭頂部(temporo-parietal right)	切除	なし
15	47	IDH1 R132H 変異	退形成性星細胞腫(anaplastic astrocytoma)	III	右前頭(frontal right)	切除	なし
16	47	IDH1 R132H 変異	びまん性星細胞腫	II	左頭頂(parietal left)	生検	なし
17	47	IDH1 R132C 変異	びまん性星細胞腫	II	右前頭	切除	なし
18	43	IDH1 R132H 変異	びまん性星細胞腫	II	左前側頭(fronto-temporal left)	生検	化学療法

【 0 2 8 0 】

実施例 7 : FAPI のインビトロおよびインビボ特性評価

再取り込み実験

再取り込み実験において、 ^3H -ラベル FAPI-04 および 46 (DMEM 中 $5\text{ MBq}/\text{nmol}$) を HT-1080-FAP 細胞に添加し、それぞれ 4°C および 37°C で 60 分間インキュベートした。放射性培地を除去し、細胞を pH 7.4 のリン酸緩衝食塩水 (PBS) で 2 回洗浄した。続いて、10 分～6 時間の範囲の時間間隔で、非ラベル FAPI ($1\text{ }\mu\text{M}$) を含む、または含まない非放射性培地を加えた。この細胞を pH 7.4 の

PBSで2回洗浄した。表面結合活性を除去するために、細胞を室温でグリシン-HCl (PBS中1M、pH2.2)で10分間インキュベートした。氷冷のPBSで2回洗浄した後、細胞を溶解緩衝液(0.3M NaOH、0.2% SDS)1.4mLで溶解し、内在化画分(内在化された画分)を測定した。4℃でインキュベートする細胞について、すべての洗浄および溶出工程は、氷冷の緩衝液を用いて行った。放射活性は、 γ カウンター(Packard Cobra II)を用いて測定し、1mio細胞に正規化し、適用線量のパーセンテージとして計算した(%AD; 図47を参照)。

【0281】

酵素阻害アッセイ

FAP I-04の酵素FAP活性に対する潜在的な阻害効果を測定するために、48ウェルプレート中で組換えヒトFAPタンパク質(1pmol/ウェル)を用いて酵素阻害アッセイを行った。37℃でFAP I-04またはタラボスタット(0~1000nM/ウェル)をヒトFAPと30分間インキュベートした後、蛍光性FAP基質Z-GP-AMCを添加して最終濃度を0~200 μ M/ウェルにし、37℃で60分間インキュベートした。FAPの酵素活性は、SpectraMax M2プレートリーダー(Molecular Devices, San Jose, USA)を用いて、360/460nmで反応生成物AMCの蛍光強度を測定することにより決定した(図46を参照)。

【0282】

HT-1080-FAP腫瘍を有するマウスへのFAP I-04の多重投与

生体内分布実験において、8週齢のBALB/c nu/nuマウス(Challies River)を、それぞれ5mioのHT-1080-FAP細胞を右体幹に皮下接種した。腫瘍のサイズが約1cm³に達したら、放射性標識化合物を尾静脈を介して注射した。動物の第1群には¹⁷⁷Lu-FAP I-04(動物あたり2MBq)を一回投与し、一方、第2群には1MBqずつ2回投与し、1回目の注射の4時間後に二回目の投与を行った。第3群には合計3回の投与を行い、最初の投与はマウス1匹あたり1MBq、その後2時間後に0.5MBq、4時間後に追加で0.5MBqを投与した。動物(各時点でn=3)を、最初の注射の8時間後と24時間後に犠牲にした。分布された放射活性は、 γ カウンター(Cobra Autogamma, Packard)を用いて、解剖したすべての臓器および血液中の放射活性を測定した。値は、組織1グラムあたりの投与量に対するパーセンテージ(%ID/g)として示す(図48を参照)。

【0283】

実施例8: FAP Iのインビトロおよびインビボ特性評価

実験操作および臨床評価

FAP I誘導体のすべてのインビトロおよびインビボ実験ならびに臨床評価は、最初の文献に記載されているように、Loktevら¹およびLindnerら²に従って行った。

【0284】

結果

F-18-FAP I誘導体のインビトロ特性評価

すべての実験は、FAP I-42(AIF-18標識)またはFAP I-72(F-18ニコチンアミド標識)と類似する方法で実施した。

【表11】

化合物	IC ₅₀ (nM)	化合物	IC ₅₀ (nM)
FAP I-72	2.4	FAP I-74	9.2
FAP I-73	5.4	FAP I-75	2.9

表11: 競合結合アッセイによって測定された、選択されたFAP I誘導体のEC₅₀値

【0285】

血液プールのクリアランスの測定

化合物のクリアランス率を推定するために、推定された二相指数関数的減衰によって血

液プールの表現として心臓の SUV_{mean} 値 ($0.375 - 60$ 分) から半減期を計算した。選択されたすべての化合物は、 10 分未満の半減期で非常に速くクリアされた。計算されたプラトー値 (Plateau value) は、Ga-68 標識 FAPI-13、-21、-36 および AIF-18 標識 FAPI-74 の方がより高かったが、これは理論的に、非特異的な結合のために、または循環中に残っていることによってクリアされていないより高い画分の化合物に相当する (表 12)。速いクリアランスの例として、 $0 \sim 15$ 分間の間の FAPI-04 および -46 の時間放射活性曲線を図 53 に示す。

【表 12】

化合物	血液プールクリアランス ($T_{1/2}$ [分])	プラトー値 ($SUV_{平均}$)
FAPI-04	7.1	0.21
FAPI-13	8.5	0.27
FAPI-21	8.1	0.31
FAPI-36	5.0	0.58
FAPI-46	6.3	0.19
FAPI-74	2.4	0.32

表 12. 推定された 2 相指数関数的減衰によって SUV_{mean} 値から計算された、選択された FAPI 誘導体の血液プール半減期および仮想プラトー値。明確にするために、律速半減期の値のみを列挙する。

【0286】

腫瘍担持マウスにおける F-18-FAPI 誘導体の小動物イメージング

これらの所見に基づき、小動物 PET-イメージングは、放射性追跡子を H-1080-FAP 腫瘍担持マウスに投与後 140 分までの F-18-標識 NOTA-および F-18-ニコチンアミド-標識 FAPI 誘導体を用いて行った。F-18-ニコチンアミド誘導体である FAPI-72、-73 および -77 は肝臓への好ましくない蓄積と胆汁排泄を示し、一方 FAPI-78 は腎臓から排出されたが、腫瘍取り込みは示していない。AIF-18 標識 NOTA 誘導体である FAPI-74 および -75 の場合には、高い標的の特異性および速いクリアランスが観察され、その結果、FAP 陽性腫瘍の優れた可視化を可能にする高コントラスト画像が得られた (図 50)。

【0287】

腫瘍担持マウスにおける F-18-FAPI 誘導体の臓器分布

薬物動態プロファイルならびにインビボでの腫瘍取り込みの分析において、AIF-18 標識 FAPI-75 を H-1080-FAP 腫瘍担持マウスに静脈内投与した。放射性標識化合物の臓器分布は、血液、健康な組織および腫瘍におけるエクスピボで測定した。図 51 に示されるように、この化合物は高い腫瘍取り込みを示すが、Ga-68 標識 DOTA 誘導体と比較して、健康な組織でのより高い蓄積が観察され、PET イメージングにおける性能は同等だった。

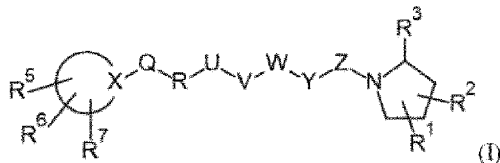
【0288】

項目

以下の項目は、本発明の好ましい実施態様を示す。

1. 式 (I):

【化 140】



[式中、Q、R、U、V、W、Y および Z は、Q、R、U、V、W、Y および Z の中の少

なくとも三つが存在する条件で、独立して存在するか、または不存在であり；

Q、R、U、V、W、YおよびZは、独立してO、 CH_2 、 NR^4 、 $\text{C}=\text{O}$ 、 $\text{C}=\text{S}$ 、 $\text{C}=\text{NR}^4$ 、 HCR^4 および R^4CR^4 からなる群から選択され、ただし二つのOは互いに直接隣接しない；

R^1 および R^2 は、独立して $-\text{H}$ 、 $-\text{OH}$ 、ハロ、 C_1-6 -アルキル、 $-\text{O}-\text{C}_1-6$ -アルキル、 $\text{S}-\text{C}_1-6$ -アルキルからなる群から選択され；

R^3 は、 $-\text{H}$ 、 $-\text{CN}$ 、 $-\text{B}(\text{OH})_2$ 、 $-\text{C}(\text{O})$ -アルキル、 $-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{C}(\text{O})$ -アリール、 $-\text{C}=\text{C}-\text{S}(\text{O})_2$ -アリール、 $-\text{CO}_2\text{H}$ 、 $-\text{SO}_3\text{H}$ 、 $-\text{SO}_2\text{NH}_2$ 、 $-\text{PO}_3\text{H}_2$ および5-テトラゾリルからなる群から選択され；

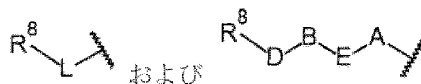
R^4 は、 $-\text{H}$ 、 $-\text{C}_1-6$ -アルキル、 $-\text{O}-\text{C}_1-6$ -アルキル、 $-\text{S}-\text{C}_1-6$ -アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアルケニル、アルキニル、アリールおよび $-\text{C}_1-6$ -アラキルからなる群から選択され、各 $-\text{C}_1-6$ -アルキルは、 $-\text{OH}$ 、オキソおよびハロから選択される1~3個の置換基によって置換されていてよく、またQ、R、U、V、W、YまたはZに結合されていてよく；

【0289】

R^5 は、 $-\text{H}$ 、ハロおよび C_1-6 -アルキルからなる群から選択され；

R^6 および R^7 は、独立して $-\text{H}$ 、

【化141】



からなる群から選択され、ただし R^6 および R^7 は同時にHではない；

ここで、Lはリンカーであり；

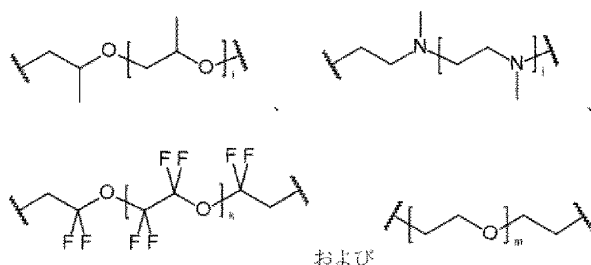
D、A、EおよびBは独立して存在するか、または不存在であり、好ましくは、少なくともA、EおよびBは存在し、ここで、存在する場合；

Dはリンカーであり；

Aは、 NR^4 、O、Sおよび CH_2 からなる群から選択され；

Eは、 C_1-6 -アルキル、

【化142】



からなる群から選択され；

ここで、iは1、2または3であり；

ここで、jは1、2または3であり；

ここで、kは1、2または3であり；

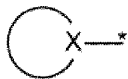
ここで、mは1、2または3であり；

【0290】

AおよびEは一緒になって、シクロアルキル、ヘテロシクロアルキル、アリールおよびヘテロアリールから選択される基、好ましくはヘテロシクロアルキルを形成し、ここで、AおよびEは、単環式、二環式および多環式であってもよく、好ましくは単環式であり；各AおよびEは、 $-\text{H}$ 、 $-\text{C}_1-6$ -アルキル、 $-\text{O}-\text{C}_1-6$ -アルキル、 $-\text{S}-\text{C}_1-6$ -アルキル、アルケニル、ヘテロアルケニル、シクロアルケニル、シクロヘテロアル

ケニル、アルキニル、アリールおよび $-C_{1-6}$ -アラルキルから選択される1~4個の置換基によって置換されていてよく、各 $-C_{1-6}$ -アルキルは、 $-OH$ 、オキソおよびハロから選択される1~3個の置換基によって置換されていてよく；また、A、B、D、Eまたは

【化143】



に結合されていてよく；

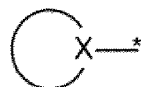
10

Bは、S、 NR^4 、 NR^4-O 、 NR^4-C_{1-6} -アルキル、 NR^4-C_{1-6} -アルキル- NR^4 および5~10員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環からなる群から選択され、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、 NR^4-C_{1-6} -アルキル- NR^4 および該N-含有ヘテロ環は、 C_{1-6} -アルキル、アリール、 C_{1-6} -アラルキルからなる群から選択される1~3個の置換基によって置換されており；そして、

R^B は、放射性部分、キレート剤、蛍光色素、造影剤およびそれらの組み合わせからなる群から選択され；

【化144】

20



は、1-ナフチル部分または5~10員N-含有芳香族または非芳香族単環式もしくは二環式ヘテロ環であり、ここに該ヘテロ環はN原子とXとの間に2個の環原子が存在し；また、該ヘテロ環はO、NおよびSから選択される1、2または3個のヘテロ原子をさらに含んでいてよく；そして、XはC原子である]

で示される化合物、またはその薬学的に許容できる互変異性体、ラセミ体、水和物、溶媒和物もしくは塩。

30

【0291】

2.(i)Q、RおよびUが CH_2 であり、独立して存在するか、または不存在であり；

Vが、 CH_2 、 $C=O$ 、 $C=S$ または $C=NR^4$ であり；

Wが NR^4 であり；

Yが HCR^4 であり；そして

Zが $C=O$ 、 $C=S$ または $C=NR^4$ であり；および/または

(ii)QおよびRが不存在であり；

Uが CH_2 であり、存在するか、または不存在であり；

R^1 および R^2 が、独立して $-H$ およびハロからなる群から選択され；

40

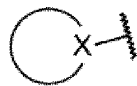
R^3 が、 $-H$ 、 $-CN$ および $-B(OH)_2$ からなる群から選択され；

R^4 が、 $-H$ および $-C_{1-6}$ -アルキルからなる群から選択され、ここに $-C_{1-6}$ -アルキルは $-OH$ から選択される1~3個の置換基によって置換されていてよい、項目1に記載の化合物。

【0292】

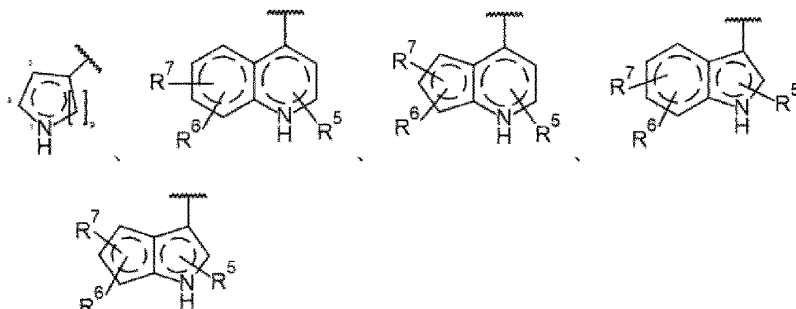
3.

【化 1 4 5】



が、

【化 1 4 6】



10

および

からなる群から選択され、これらは、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよい、請求項1または2に記載の化合物。

20

【0 2 9 3】

4.

【化 1 4 7】



が

10

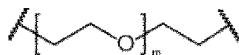


30

5. R^5 および R^6 が H であり ;
 R^7 が、

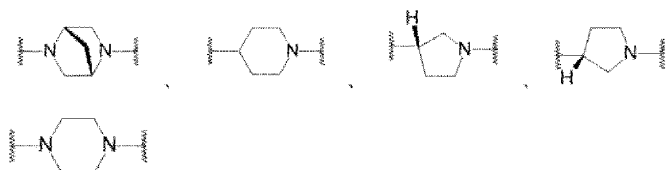
$$R^8-D-B-E-A-\text{[wavy line]}$$

40



ITM Exhibit 1004, Page 566 of 1652

【化 1 5 1】



から選択される基を形成し；

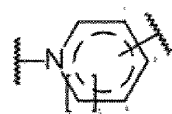
Bが、 $\text{NR}^4 - \text{C}_{1-6}$ -アルキルまたは5-~10-員N-含有芳香族または非芳香族単環式もしくはまたは二環式ヘテロ環であり、該ヘテロ環は好ましくはO、NおよびSから選択される1または2個のヘテロ原子をさらに含み、好ましくは1または2個の窒素原子をさらに含み、ここに好ましくは、N-含有ヘテロ環は C_{1-6} -アルキル、アリー

10

【0 2 9 5】

6. (i) Bに含まれるN-含有ヘテロ環が、式：

【化 1 5 2】



20

〔式中、ヘテロ環はO、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、1個の窒素をさらに含んでいてよく；

【化 1 5 3】



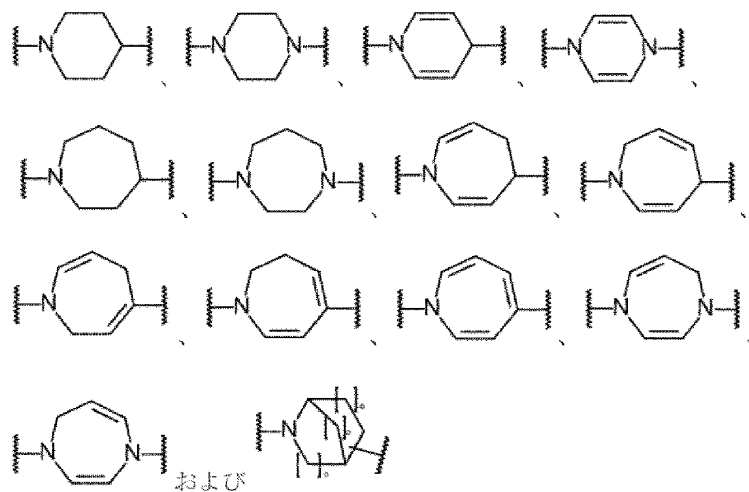
30

は、1、2または3位に結合し、好ましくは2位に結合し；

lは1または2である]

で示される芳香族または非芳香族単環式ヘテロ環であり；および／または (i i) Bに含まれるN-含有ヘテロ環が、

【化 1 5 4】



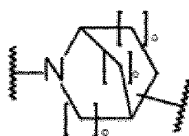
10

からなる群から選択され、ここで、N-含有ヘテロ環はC₁ - 6 - アルキルによって置換されており；

20

ここで、Bに含まれるN-含有ヘテロ環が

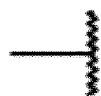
【化 1 5 5】



である場合、該ヘテロ環は、O、NおよびSから選択される1または2個のヘテロ原子をさらに含んでいてよく、さらに1個の窒素を含んでいてよく、また、一つまたはそれ以上の(例えばアミノ酸由来)側鎖を含んでいてよく；

30

【化 1 5 6】



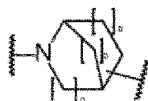
は、1、2または3位に結合し、好ましくは2位に結合し；

oは1または2であり、

好ましくは、Bに含まれるN-含有ヘテロ環が

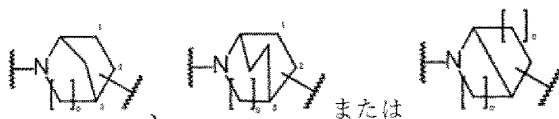
40

【化 1 5 7】



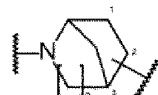
である場合、該Bに含まれるN-含有ヘテロ環は

【化 1 5 8】



であり；より好ましくは、Bに含まれるN-含有ヘテロ環が

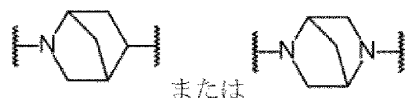
【化 1 5 9】



10

である場合、該Bに含まれるN-含有ヘテロ環は

【化 1 6 0】



である、項目 1 ～ 5 のいずれか一項に記載の化合物。

【0 2 9 6】

20

7. Q、RおよびUが不存在であり；

VがC=Oであり；

WがNHであり；

YがCH₂であり；

ZがC=Oであり；

R¹ および R² が、独立して-Hおよびハロからなる群から選択され；

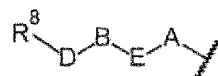
R³ が-CNであり；

R⁵ および R⁶ がHであり；

R⁷ が

【化 1 6 1】

30



であり、ここで、

Dは不存在であり；

AはO、S、CH₂、NHまたはNCH₃であり；

Eは、C₁ - 6 - アルキルまたは

【化 1 6 2】

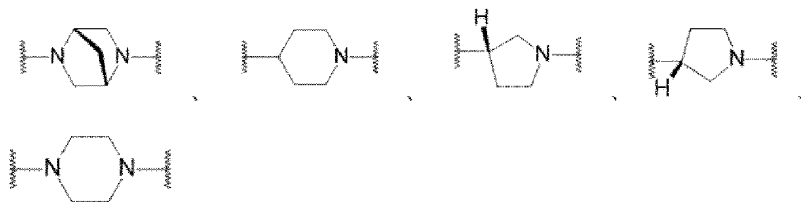
40



であり、ここで、mは1、2または3であり；または

AおよびEは一緒になって

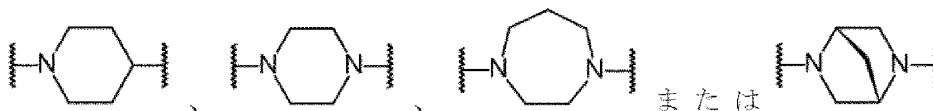
【化 1 6 3】



から選択される基を形成し；

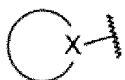
B は、 $\text{NH}-\text{C}_{1-6}$ -アルキル、

【化 1 6 4】



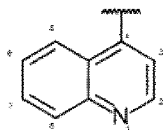
であり、B は C_{1-3} アルキルによって置換されていてよく；そして

【化 1 6 5】



は

【化 1 6 6】



である、項目 1 ～ 6 のいずれか一項に記載の化合物。

【0 2 9 7】

8. C_{1-6} -アルキルが、メチル、エチル、プロピル、*i*-プロピル、ブチル、*sec*-ブチル、*tert*-ブチル、ペンチルおよびヘキシルからなる群から選択され、および／または

C_{1-6} -アラルキルが、ベンジル、フェニル-エチル、フェニル-プロピルおよびフェニル-ブチルからなる群から選択される、項目 1 ～ 7 のいずれか一項に記載の化合物。

【0 2 9 8】

9. R^8 が、蛍光同位体、放射性同位体、放射性薬剤またはそれらの組み合わせである放射性部分であって、好ましくは、 α 放射線放出同位体、 β 放射線放出同位体、 γ 放射線放出同位体、オージェ電子放出同位体、X-ray 放出同位体、蛍光放出同位体、例えば $^1\text{8 F}$ 、 ^{51}Cr 、 ^{67}Ga 、 ^{68}Ga 、 ^{111}In 、 $^{99\text{m}}\text{Tc}$ 、 ^{186}Re 、 ^{188}Re 、 ^{139}La 、 ^{140}La 、 ^{175}Yb 、 ^{153}Sm 、 ^{166}Ho 、 ^{88}Y 、 ^{90}Y 、 ^{149}Pm 、 ^{165}Dy 、 ^{169}Er 、 ^{177}Lu 、 ^{47}Sc 、 ^{142}Pr 、 ^{159}Gd 、 ^{212}Bi 、 ^{213}Bi 、 ^{72}As 、 ^{72}Se 、 ^{97}Ru 、 ^{109}Pd 、 ^{105}Rh 、 $^{101\text{m}}\text{Rh}$ 、 ^{119}Sb 、 ^{128}Ba 、 ^{123}I 、 ^{124}I 、 ^{131}I 、 ^{197}Hg 、 ^{211}At 、 ^{151}Eu 、 ^{153}Eu 、 ^{169}Eu 、 ^{201}Tl 、 ^{203}Pb 、 ^{212}Pb 、 ^{64}Cu 、 ^{67}Cu 、 ^{188}Re 、 ^{186}Re 、 ^{198}Au 、 ^{225}Ac 、 ^{227}Th および ^{199}Ag からなる群から選択される放射性部分である、項目 1 ～ 8 のいずれか

一項に記載の化合物。

【0299】

10. R^B が、以下の種類の蛍光色素：キサンテン、アクリジン、オキサジン、シアニン、スチリル色素、クマリン、ポルフィン、金属-配位子錯体、蛍光タンパク質、ナノ結晶、ペリレン、ホウ素-ジピロメテンおよびフタロシアニンからなる群から選択される蛍光色素、ならびにこれらの種類の色素の複合物および組み合わせである、項目1～8のいずれか一項に記載の化合物。

【0300】

11. R^B が、2価または3価金属カチオンを有する錯体の形態のキレート剤であって、好ましくは、1,4,7,10-テトラアザシクロドデカン-N,N',N,N'-テトラ酢酸(DOTA)、エチレンジアミンテトラ酢酸(EDTA)、1,4,7-トリアザシクロノナン-1,4,7-トリ酢酸(NOTA)、トリエチレントトラミン(TETA)、イミノニ酢酸、ジエチレントリアミン-N,N,N',N',N''-ペンタ酢酸(DTPA)、ビス-(カルボキシメチルイミダゾール)グリシンおよび6-ヒドラジノピリジン3-カルボン酸(HYNIC)からなる群から選択されるキレート剤である、項目1～8のいずれか一項に記載の化合物。

【0301】

12. R^B が、常磁性剤を含むか、またはそれからなる造影剤であって、好ましくは、ここで、常磁性剤が、常磁性ナノ粒子を含むか、またはそれからなる、項目1～8のいずれか一項に記載の化合物。

【0302】

13. 少なくとも一つの項目1～12による化合物、および場合により製薬的に許容できる担体および/または添加物を含むか、またはそれらからなる医薬組成物。

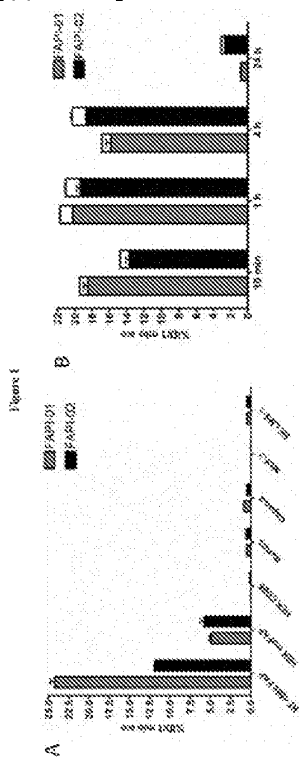
【0303】

14. 動物またはヒトの対象における線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患の診断または処置に使用するための、項目1～12のいずれか一項に記載の化合物または項目13に記載の医薬組成物であって、好ましくは、該線維芽細胞活性化タンパク質(FAP)の過剰発現を特徴とする疾患が、癌、慢性炎症、アテローム性動脈硬化症、線維症、組織リモデリングおよびケロイド障害からなる群から選択され、好ましくは、癌が乳癌、膵臓癌、小腸癌、結腸癌、直腸癌、肺癌、頭頸部癌、卵巣癌、肝細胞癌、食道癌、下咽頭癌、上咽頭癌、喉頭癌、骨髄腫細胞、膀胱癌、胆管細胞癌、腎明細胞癌、神経内分泌腫瘍、腫瘍誘発性骨軟化症、肉腫、CUP(未知の初期癌)、胸腺癌、類腺腫、神経膠腫、星状細胞腫、頸癌および前立腺癌からなる群から選択される、化合物または医薬組成物。

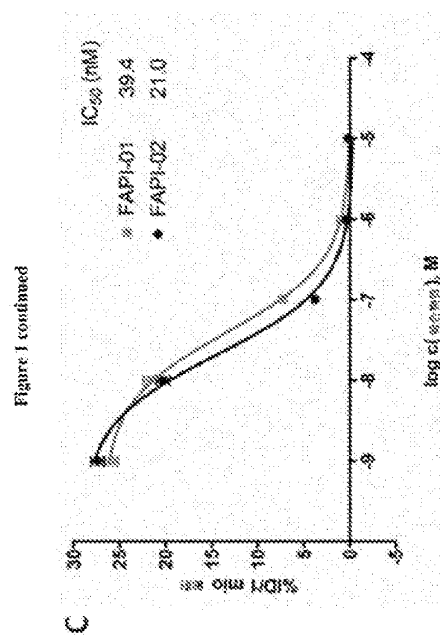
【0304】

15. 項目1～12のいずれか一項に記載の化合物もしくは項目13に記載の医薬組成物および疾患の診断のためのインストラクションを含むか、またはそれらからなるキット。

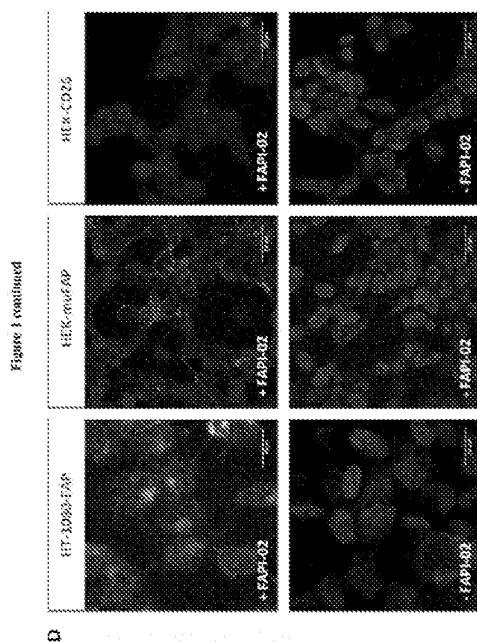
【圖 1 - 1】



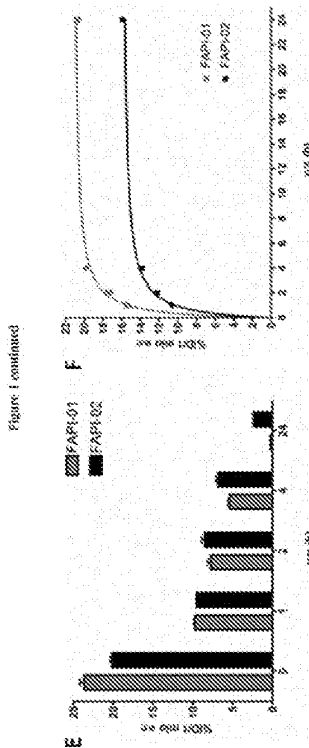
【圖 1 - 2】



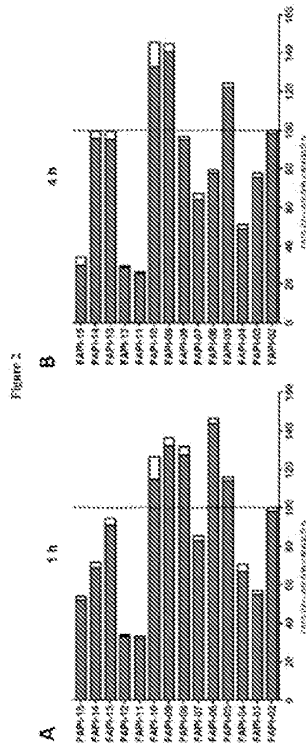
【圖 1 - 3】



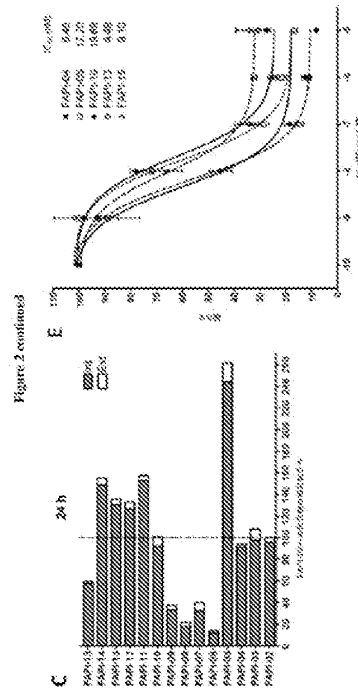
【圖 1 - 4】



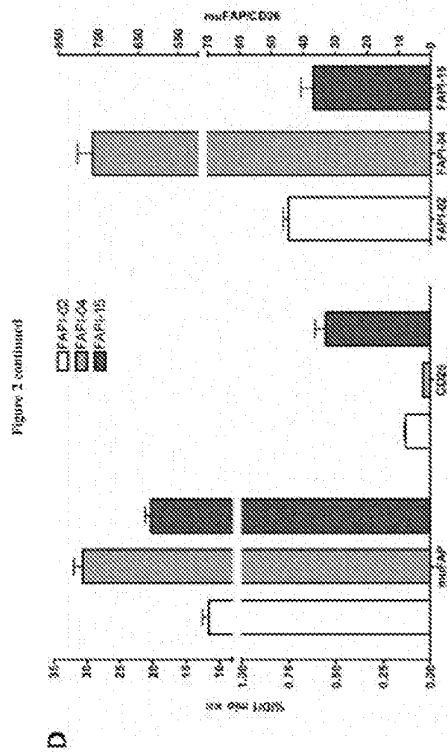
【図 2 - 1】



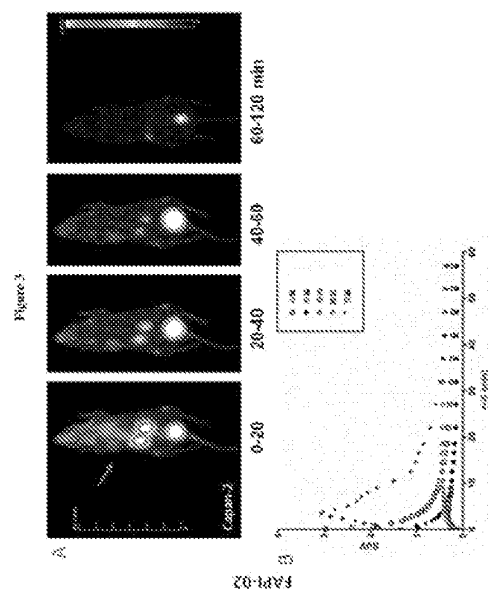
【図 2 - 2】



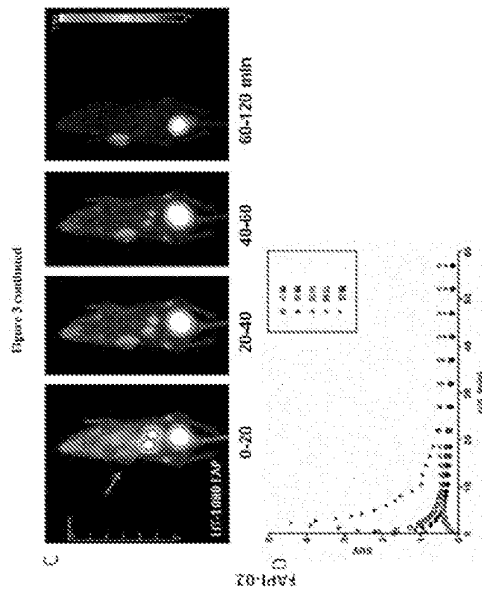
【図 2 - 3】



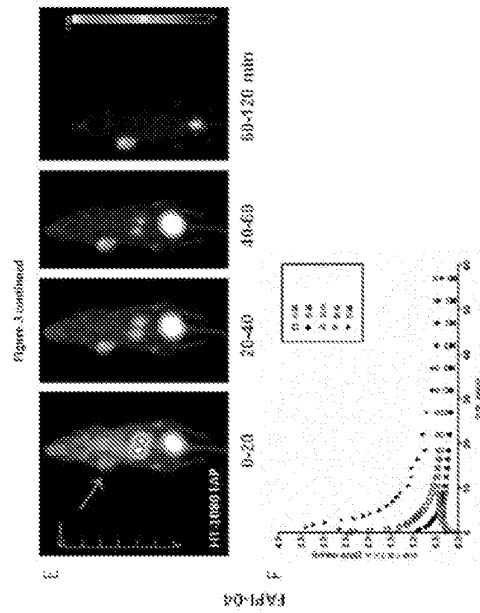
【図 3 - 1】



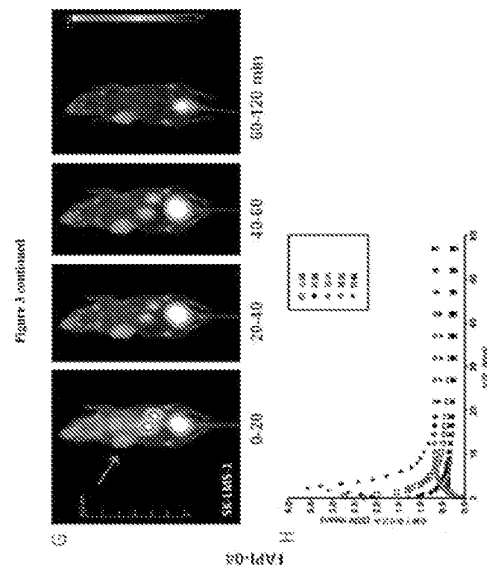
【 図 3 - 2 】



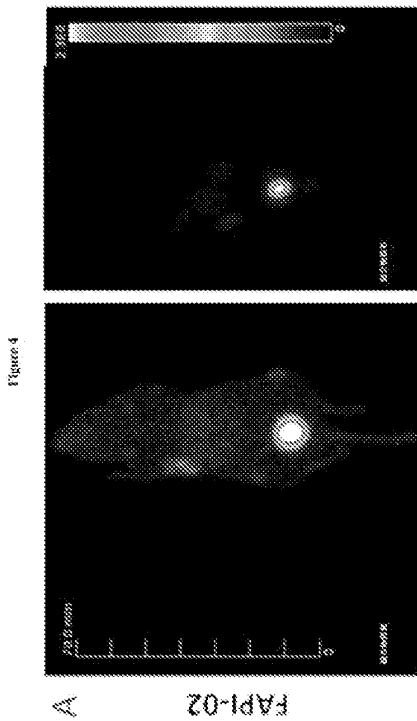
【 図 3 - 3 】



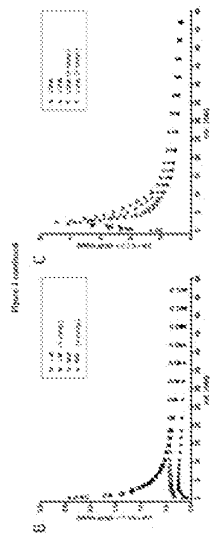
【 図 3 - 4 】



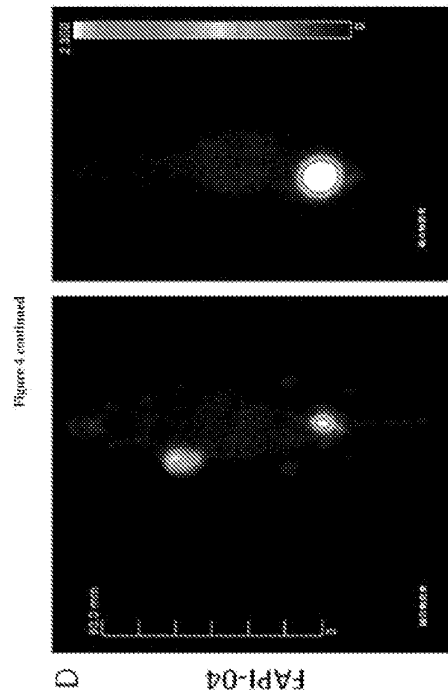
【 図 4 - 1 】



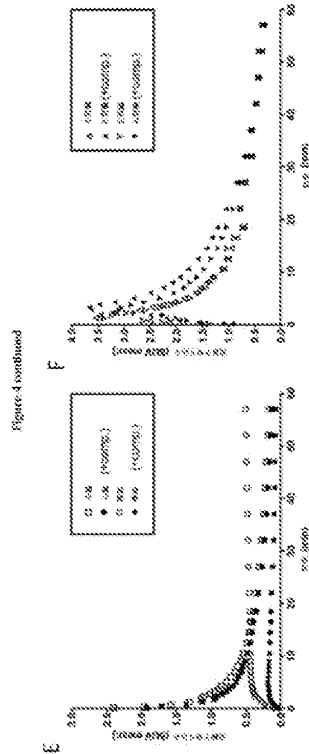
【 図 4 - 2 】



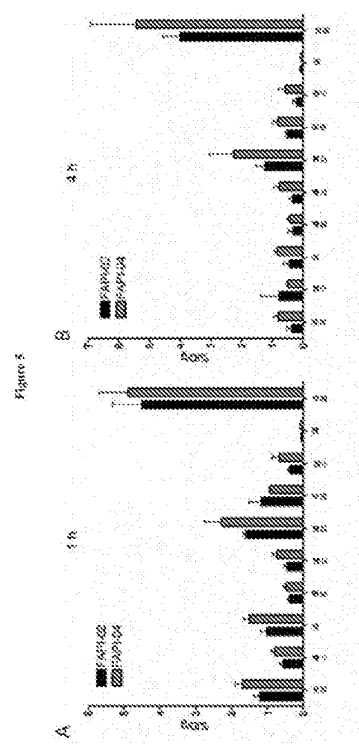
【 図 4 - 3 】



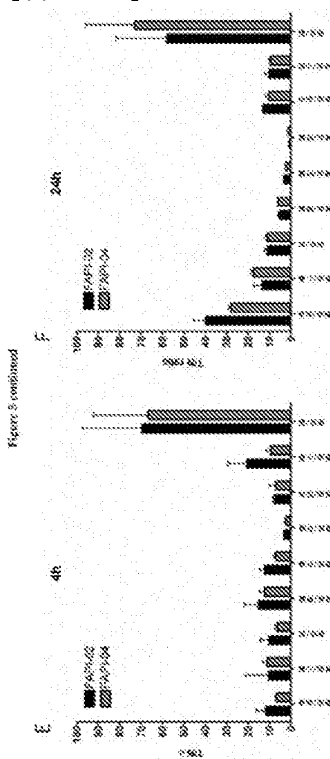
【 図 4 - 4 】



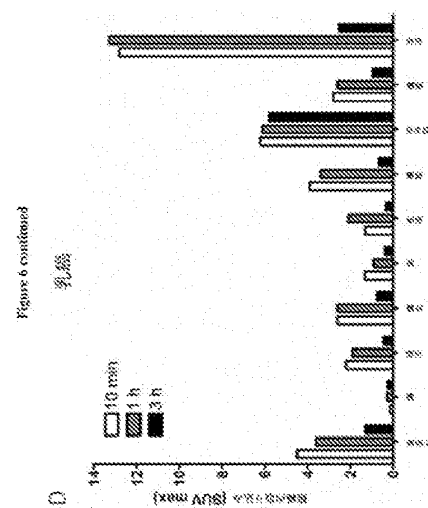
【 図 5 - 1 】



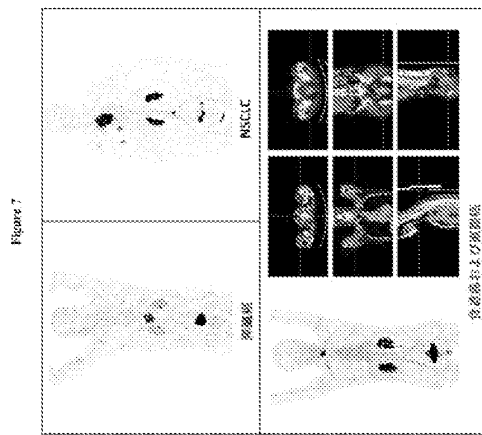
【圖 5-3】



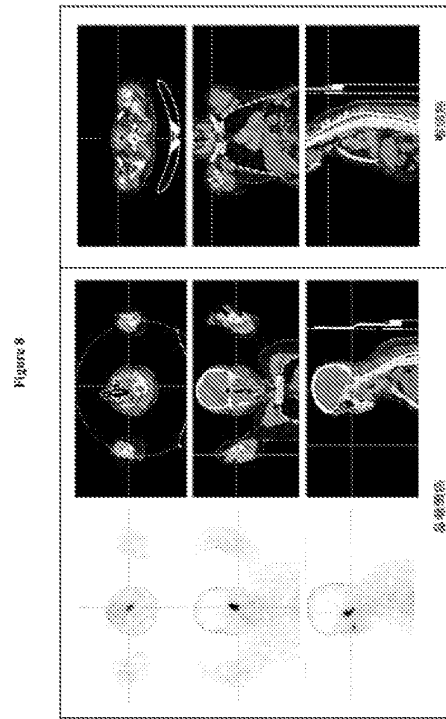
【圖 6-2】



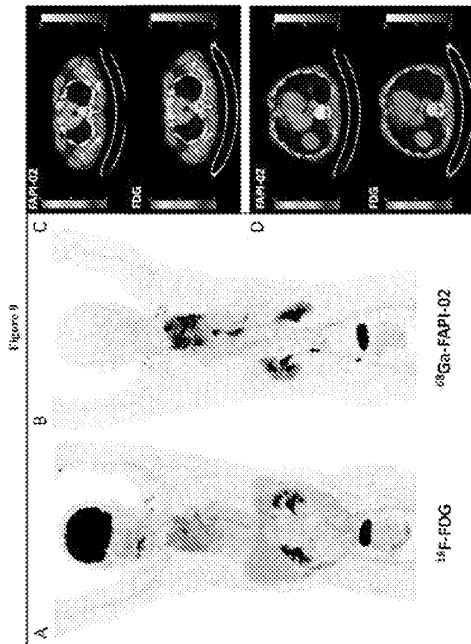
【 図 7 】



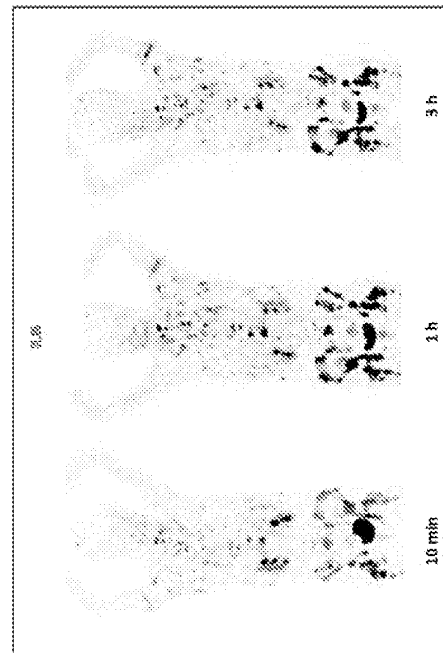
【 図 8 】



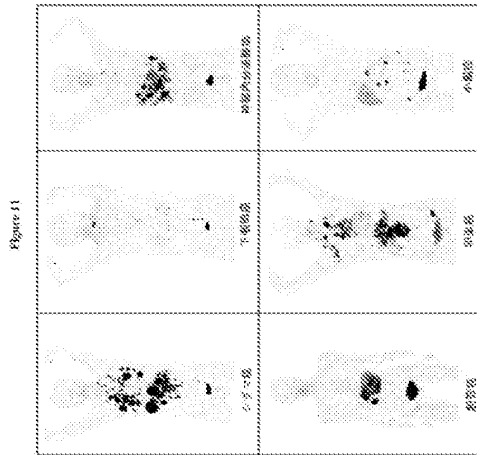
【 図 9 】



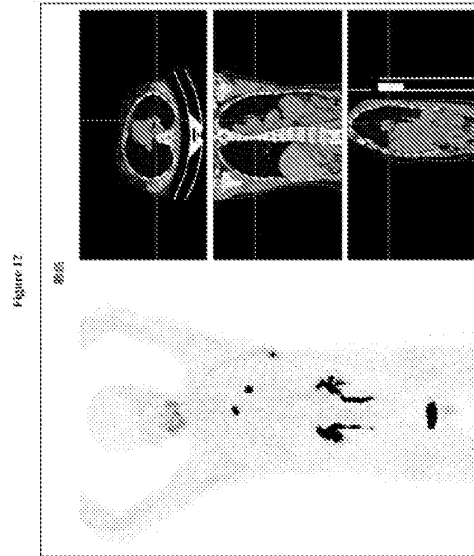
【 図 10 】



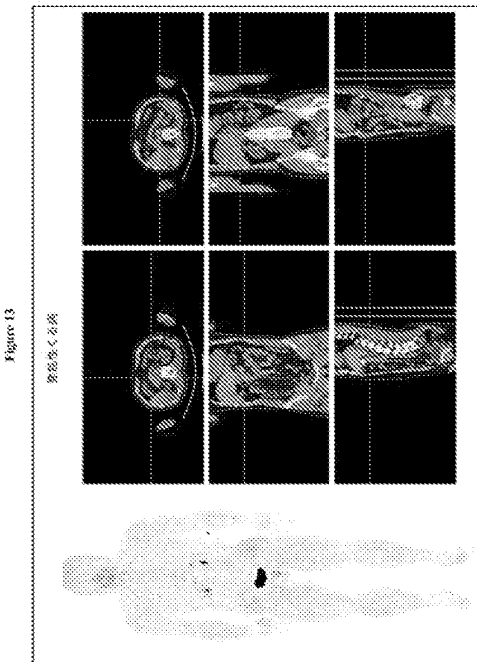
【図 1 1】



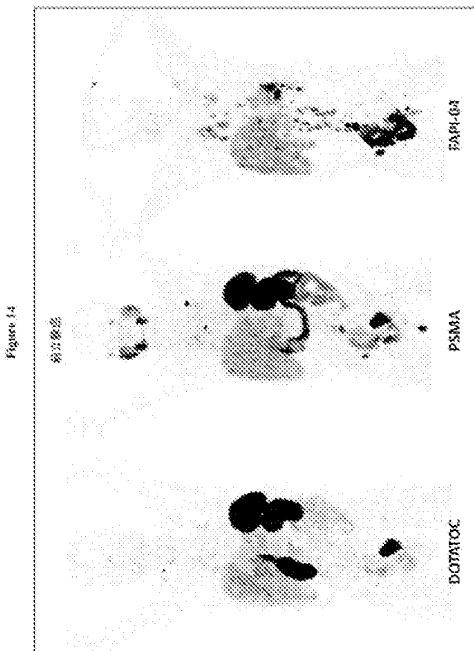
【図 1 2】



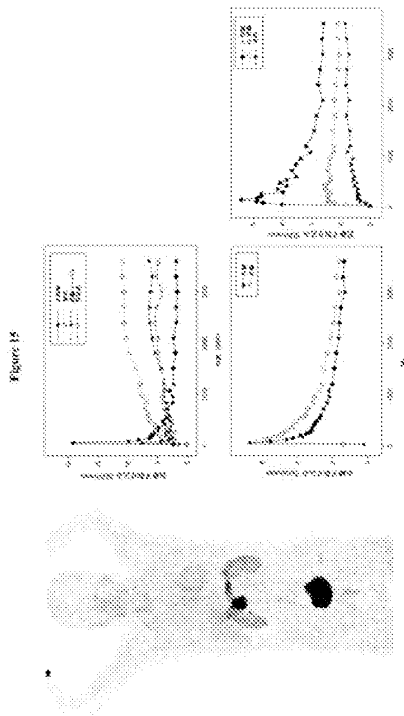
【図 1 3】



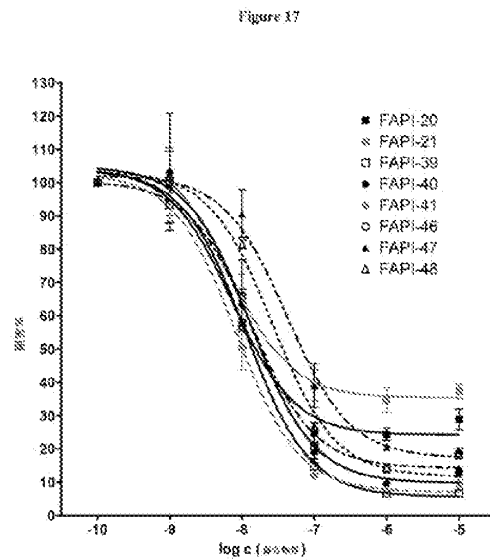
【図 1 4】



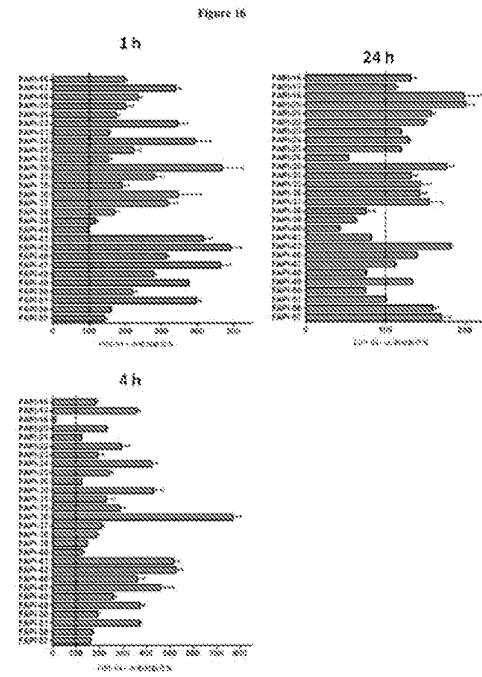
【図 15】



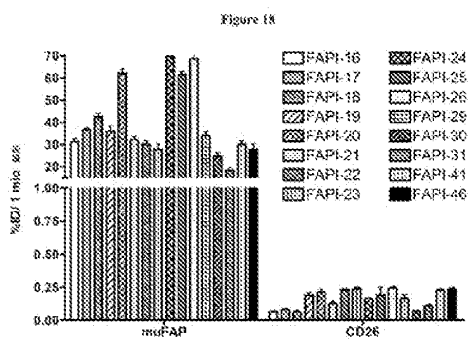
【図 17】



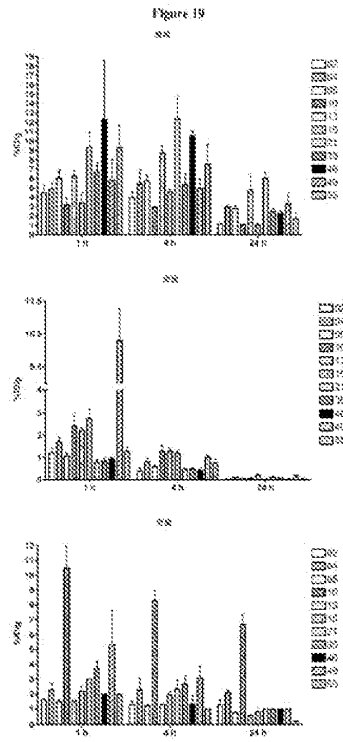
【図 16】



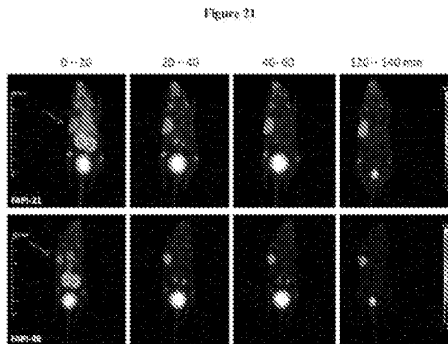
【図 18】



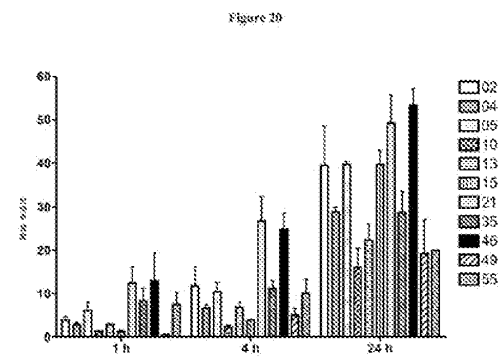
【図 19】



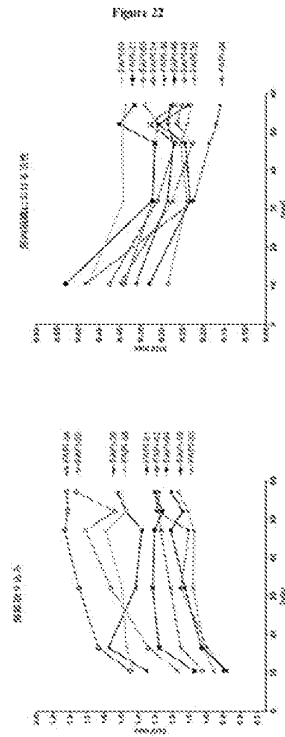
【図 21】



【図 20】

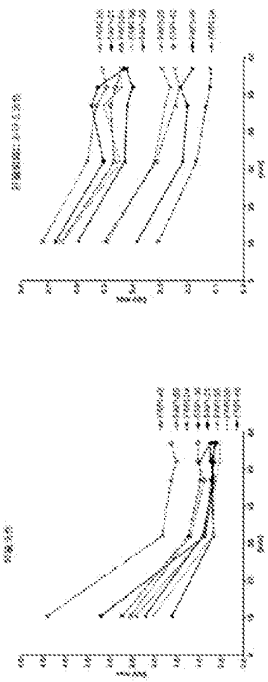


【図 22-1】



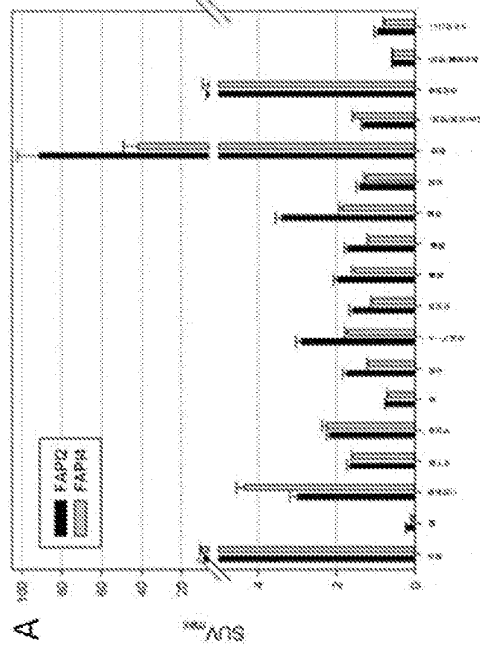
【図 2 2 - 2】

Figure 22 continued



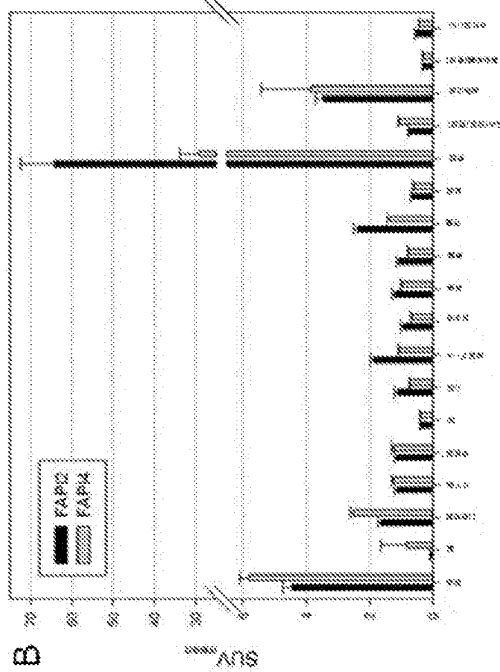
【図 2 3 - 1】

Figure 23



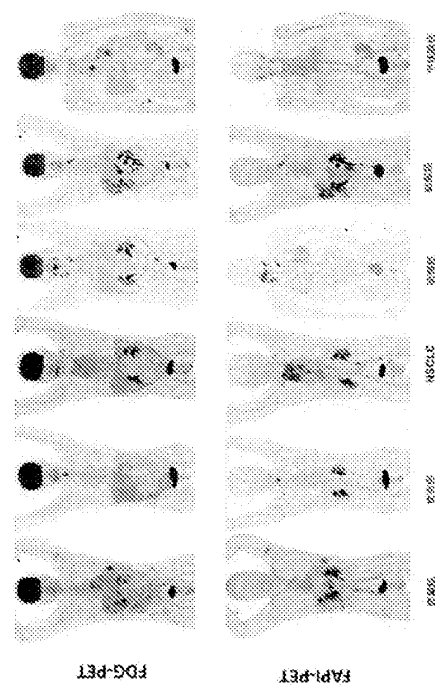
【図 2 3 - 2】

Figure 23 continued



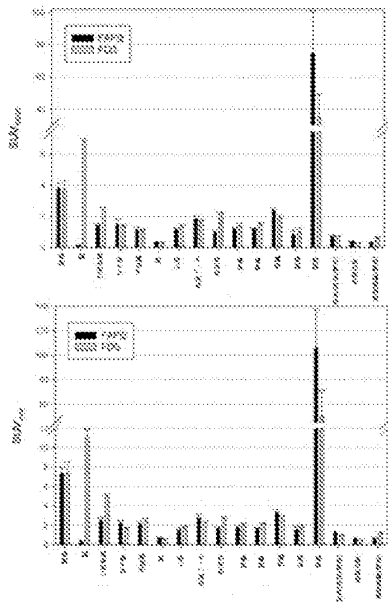
【図 2 4 - 1】

Figure 24



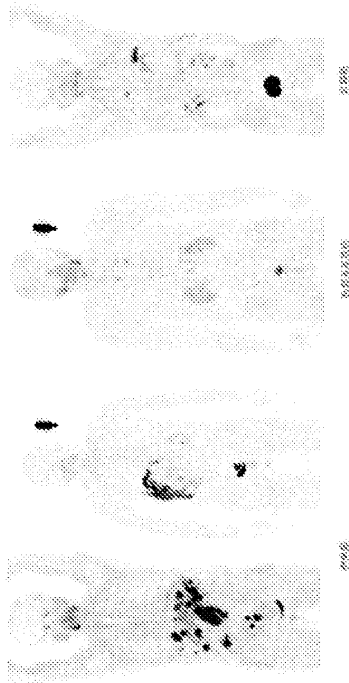
【圖 24-2】

Figure 24 continued



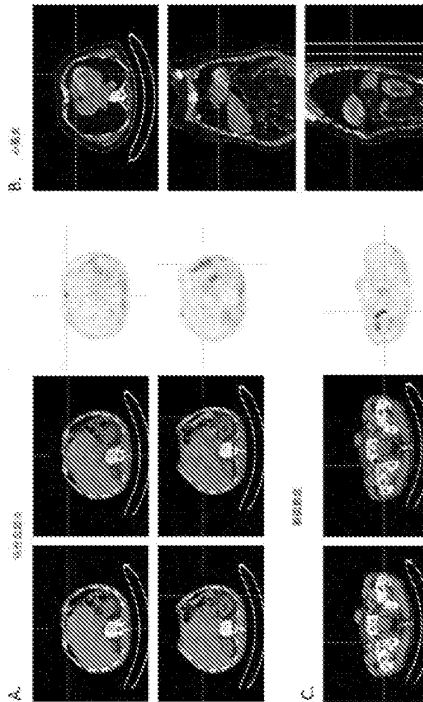
【图 26】

Figure 36



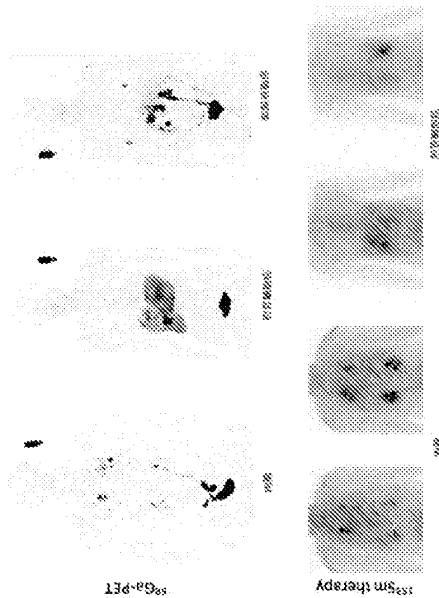
【 図 2 5 】

Figure 28



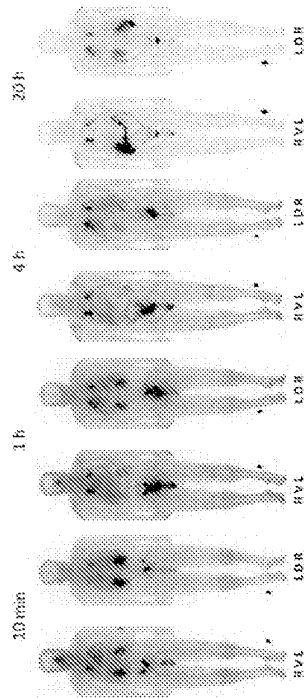
【图 27】

Figure 27



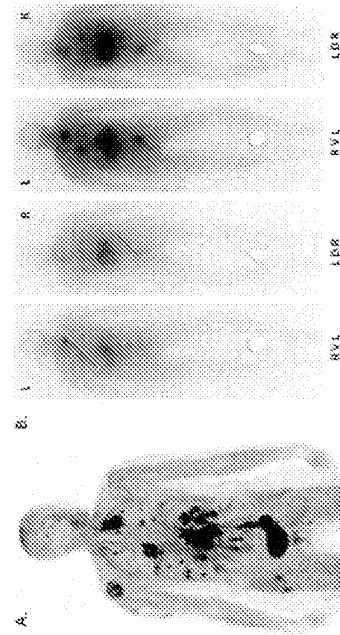
【図 28】

Figure 28



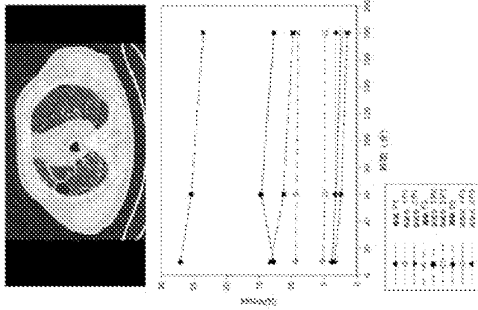
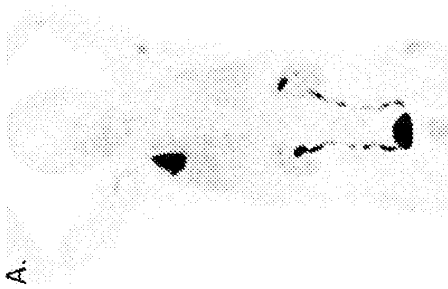
【図 29】

Figure 29



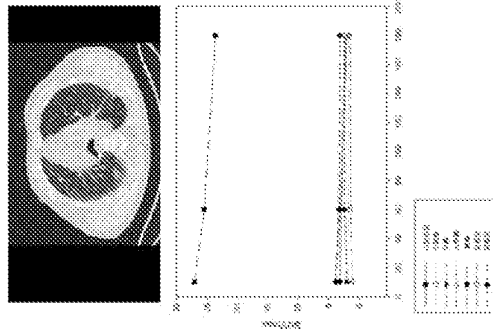
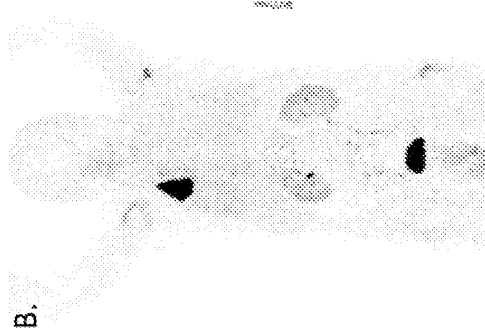
【図 30 - 1】

Figure 30

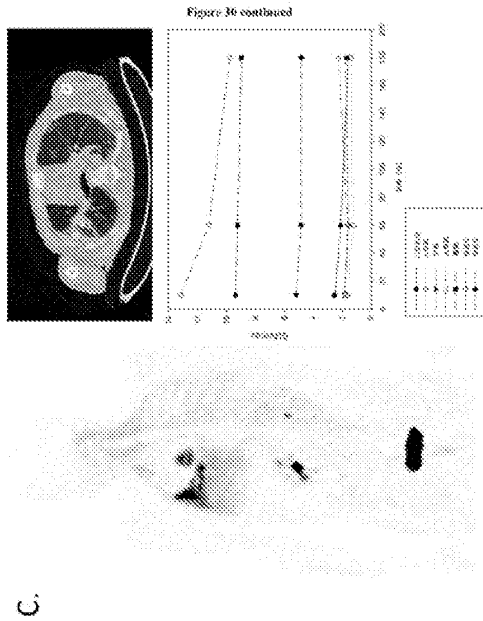


【図 30 - 2】

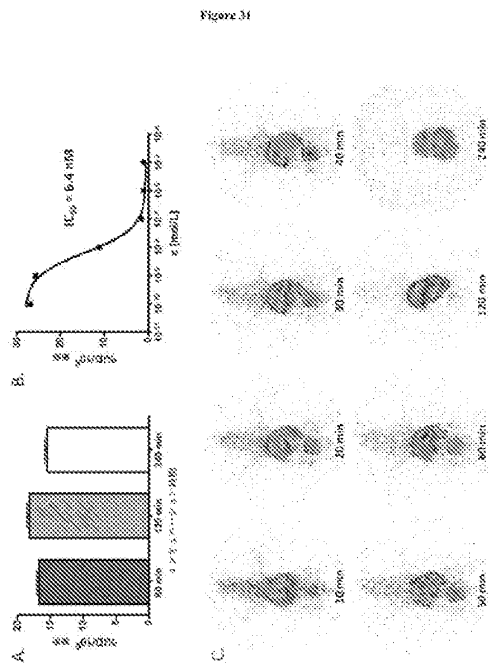
Figure 30 continued



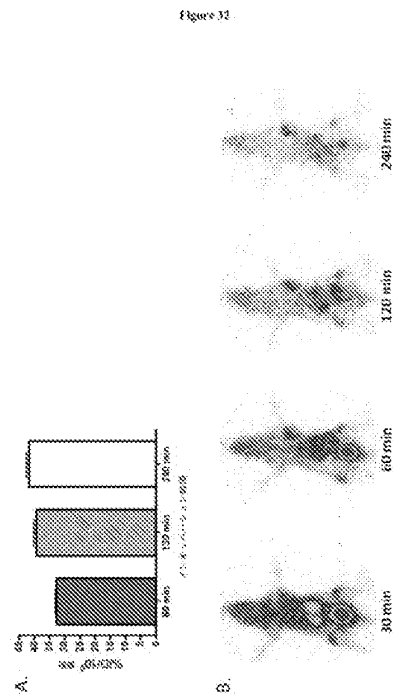
【図 30-3】



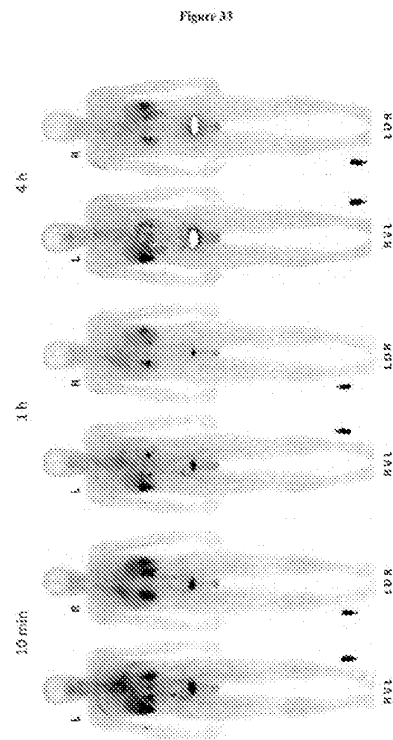
【図 31】



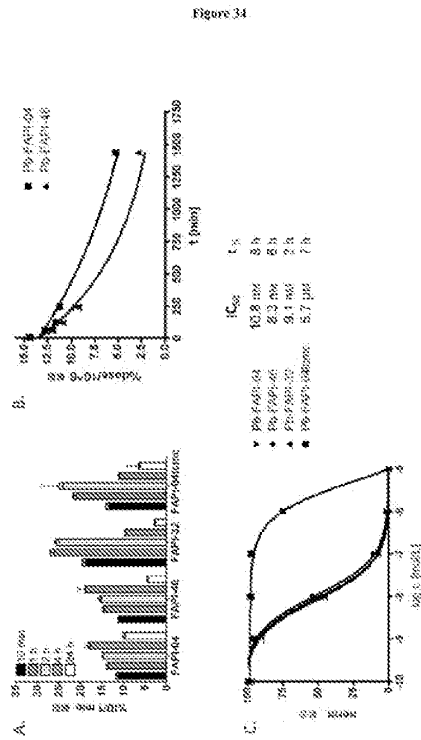
【図 32】



【図 33】

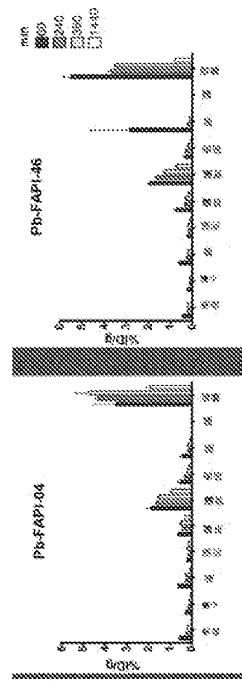


【図 3 4】



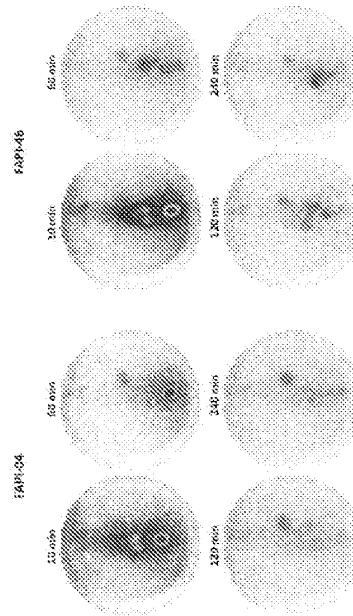
【図 3 6】

Figure 36



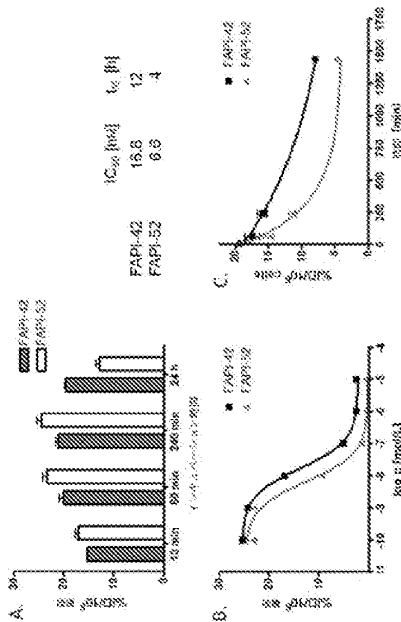
【図 3 5】

Figure 35

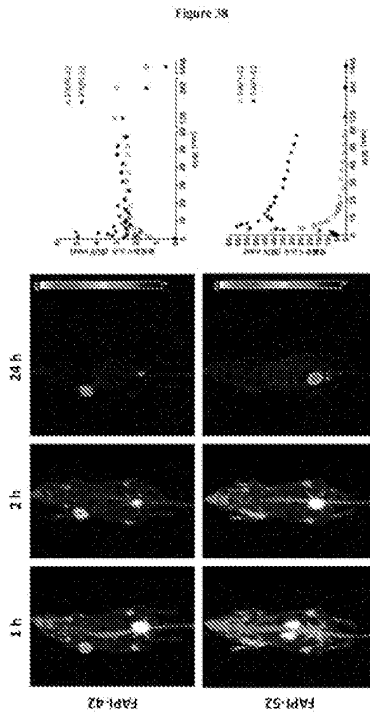


【図 3 7】

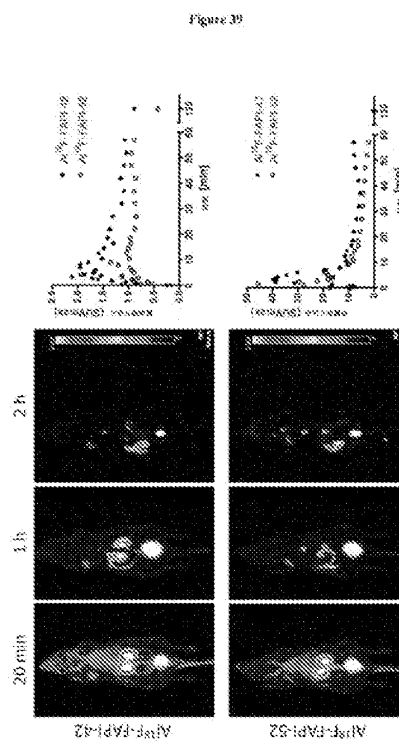
Figure 37



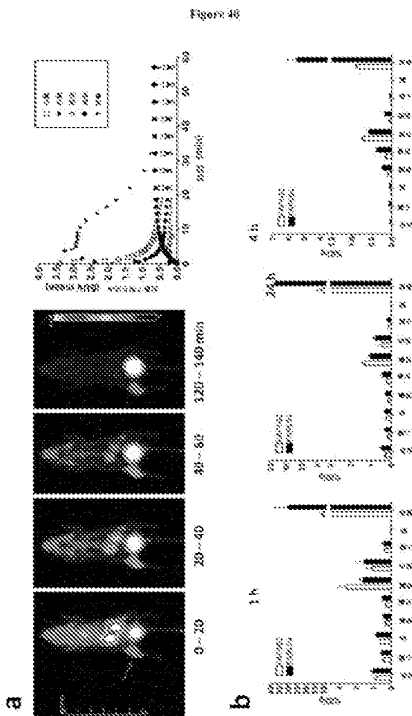
【図 38】



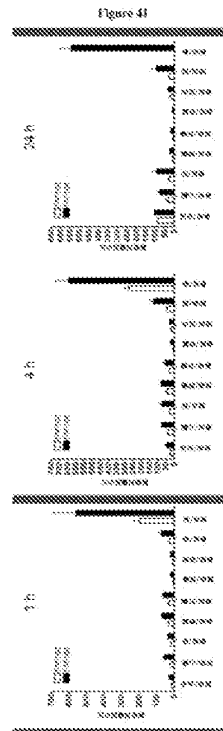
【図 39】



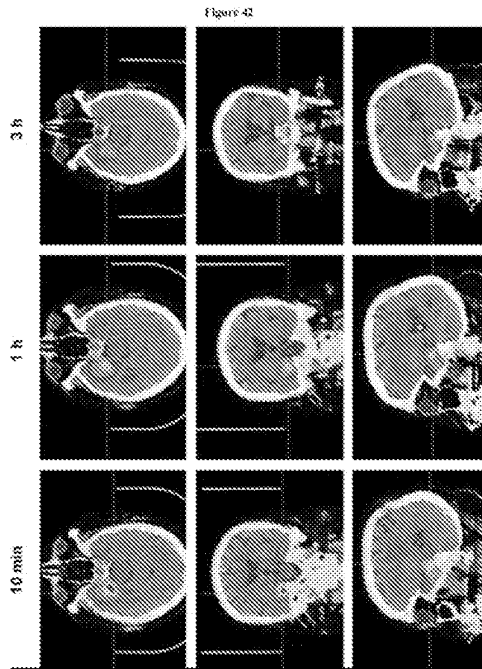
【図 40】



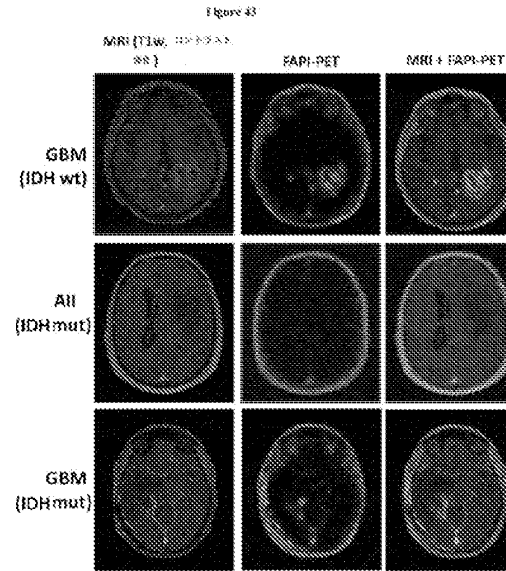
【図 41】



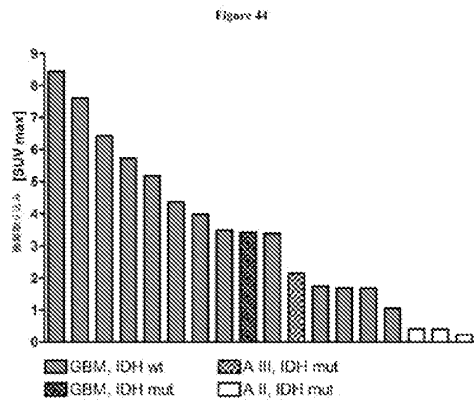
【図 4 2】



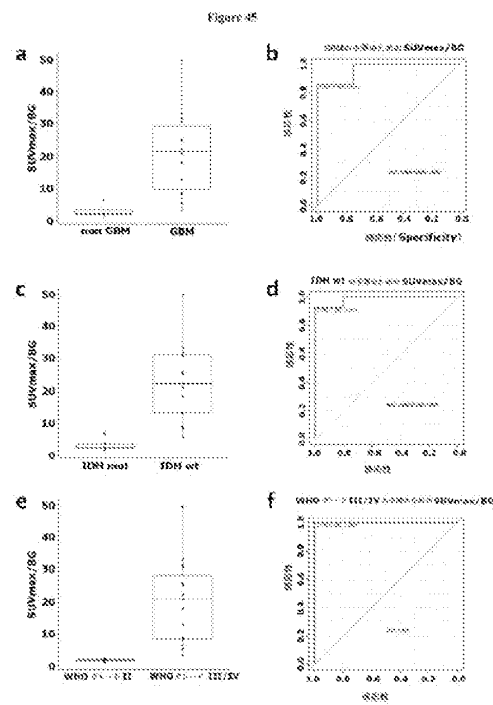
【図 4 3】



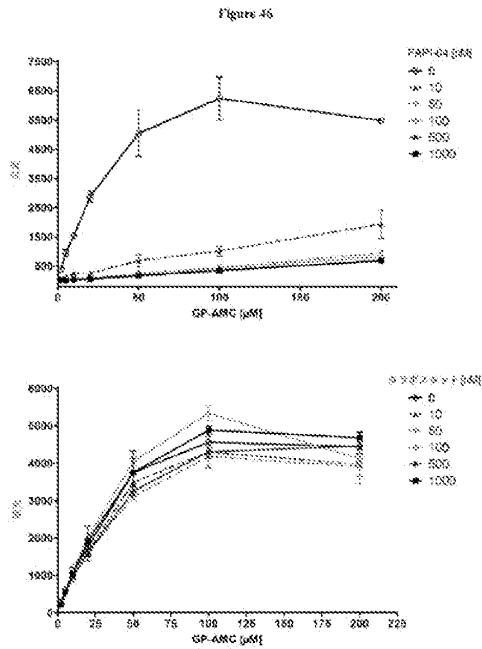
【図 4 4】



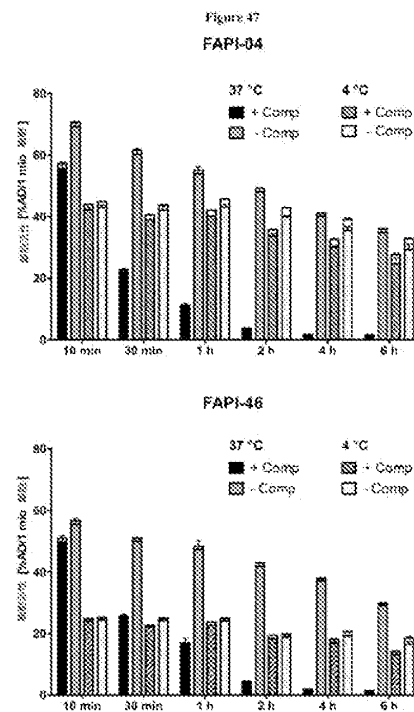
【図 4 5】



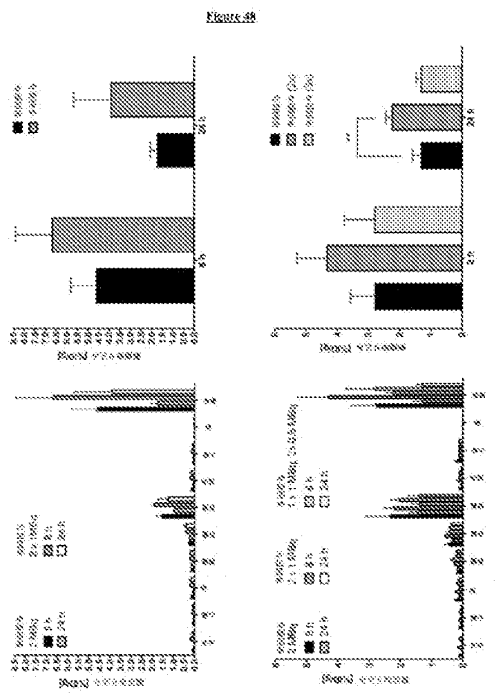
【図 46】



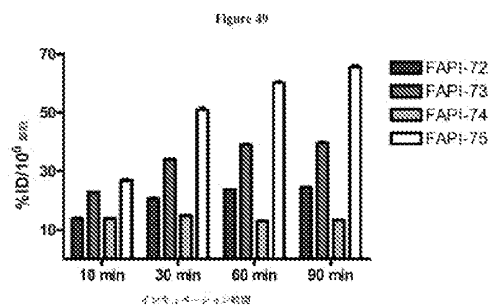
【図 47】



【図 48】

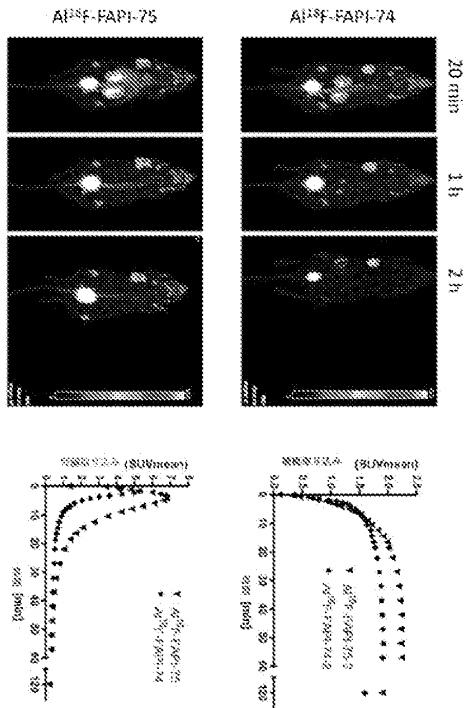


【図 49】



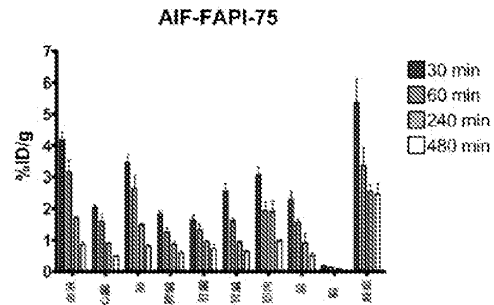
【図 50】

Figure S9



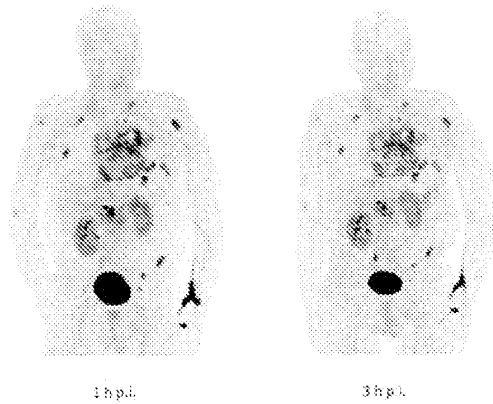
【 図 5 1 】

Figure S1



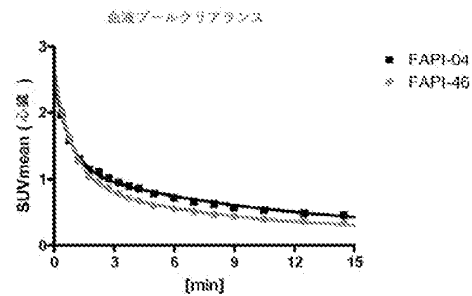
【図 5 2】

Figure S2



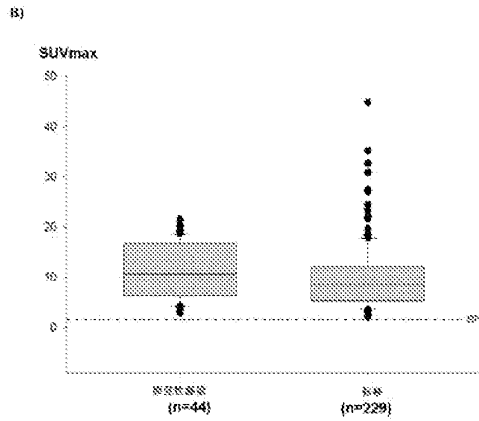
【圖 5 3】

Figure S3



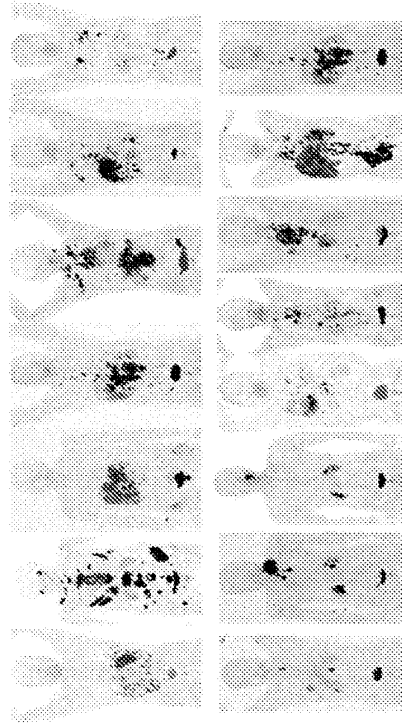
【図 5 6 - 2】

Figure 56 continued



【図 5 7】

Figure 57



【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/052952

A. CLASSIFICATION OF SUBJECT MATTER		
INV. C07D401/12	C07D401/14	C07D405/14 A61K31/4709 A61P35/00
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPO		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C07D A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/107820 A1 (UNIV ANTWERPEN [BE]; FOX CHASE CANCER CT [US]) 25 July 2013 (2013-07-25) page 709, line 21 - page 88, line 15; claims	1-15
Y	WO 2013/033396 A2 (TUFTS COLLEGE [US]; BACHOVCHIN WILLIAM W [US]; LAI HUNG-SEN [US]; POPL) 7 March 2013 (2013-03-07) claims; examples	1-15
Y	WO 2017/165473 A1 (UNIV JOHNS HOPKINS [US]) 28 September 2017 (2017-09-28) claims; examples	1-15
	----- -/-	
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
15 March 2019		29/03/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Gavriliu, Daniela

Form PCT/ISA/210 (second sheet) (April 2005)

page 1 of 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/052952

G(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AL JAMMAZ ER AL.: "Rapid and efficient synthesis of [18F]fluoronicotinamides, [18F]fluoroisonicotinamides and [18F]fluorobenzamides as potential pet radiopharmaceuticals for melanoma imaging", JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, vol. 54, no. 6, 2011, pages 312-317, XP002782854, DOI: 10.1002/jlcr.1869 the whole document	1-15
Y	WO 2011/158189 A1 (CENTRE NAT RECH SCIENT [FR]; UNIV PARIS DESCARTES [FR]; DALKO PETER [F] 22 December 2011 (2011-12-22) the whole document	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/052952

Box No. II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-6, 8-15(all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)

International Application No. PCT/ EP2019/ 052952

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-6, 8-15(all partially)

The present claim 1 relates to an extremely large number of possible compounds. Support and disclosure within the meaning of Articles 6 and 5 PCT are to be found, however, for only a very small proportion of the compounds claimed, namely for compounds as claimed in claim 7. Claim 1 can in no way be considered to be a reasonable generalisation of the actual examples since it include numerous possibilities which cannot be considered as equivalents, homologues or analogues of the examples (see the the present examples which contain all of them a quinoliyn-4-y] moiety as embodiment for the ring X, the substituents Q, R, U are always absent, R1/R2 are H or F, V=CO, W=NH, Y=CH2, Z=CO). Consequently, the search was carried out for those parts of the application which do appear to be clear (concise and supported by the examples), namely for the compounds as defined in Claims 7.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/052952

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013107820 A1	25-07-2013	EP 2804859 A1	26-11-2014
		US 2014357650 A1	04-12-2014
		WO 2013107820 A1	25-07-2013
WO 2013033396 A2	07-03-2013	AU 2012301810 A1	10-04-2014
		AU 2017204121 A1	06-07-2017
		CA 2846852 A1	07-03-2013
		CN 103945856 A	23-07-2014
		CN 108383893 A	10-08-2018
		EP 2753334 A2	16-07-2014
		JP 6207509 B2	04-10-2017
		JP 2014527070 A	09-10-2014
		JP 2018016629 A	01-02-2018
		US 2014255300 A1	11-09-2014
		US 2016346401 A1	01-12-2016
		US 2019054181 A1	21-02-2019
		WO 2013033396 A2	07-03-2013
WO 2017165473 A1	28-09-2017	AU 2017238181 A1	18-10-2018
		BR 112018069507 A2	29-01-2019
		CA 3018709 A1	28-09-2017
		CN 109311827 A	05-02-2019
		EP 3433238 A1	30-01-2019
		KR 20180134918 A	19-12-2018
		WO 2017165473 A1	28-09-2017
WO 2011158189 A1	22-12-2011	CA 2801807 A1	22-12-2011
		EP 2397466 A1	21-12-2011
		EP 2582672 A1	24-04-2013
		US 2013190284 A1	25-07-2013
		WO 2011158189 A1	22-12-2011

Form PCT/ISA/210 (patent family annex) (April 2005)

フロントページの続き

(51) Int. Cl.		F I		テーマコード (参考)
A 6 1 P	35/00	(2006. 01)	A 6 1 P	35/00
A 6 1 K	31/496	(2006. 01)	A 6 1 K	31/496
A 6 1 K	38/07	(2006. 01)	A 6 1 K	38/07
A 6 1 K	38/08	(2019. 01)	A 6 1 K	38/08
C 0 7 D	405/14	(2006. 01)	C 0 7 D	405/14
A 6 1 K	33/34	(2006. 01)	A 6 1 K	33/34
A 6 1 K	33/244	(2019. 01)	A 6 1 K	33/244
A 6 1 K	33/241	(2019. 01)	A 6 1 K	33/241
A 6 1 K	33/24	(2019. 01)	A 6 1 K	33/24
A 6 1 K	49/00	(2006. 01)	A 6 1 K	49/00
A 6 1 K	51/04	(2006. 01)	A 6 1 K	51/04 2 0 0
C 0 7 K	5/10	(2006. 01)	A 6 1 K	51/04 1 0 0
			C 0 7 K	5/10

(31) 優先権主張番号 18155419.7

(32) 優先日 平成30年2月6日(2018.2.6)

(33) 優先権主張国・地域又は機関
欧州特許庁(EP)

(81) 指定国・地域 AP(BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), EA(AM, AZ, BY, KG, KZ, RU, TJ, TM), EP(AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OA(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG), AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT

(72) 発明者 アナスタシア・ロクテフ

ドイツ 6 9 1 2 4 ハイデルベルク、アルベルト・フリッツ・シュトラッセ 3 7 アー番

(72) 発明者 トーマス・リントナー

ドイツ 6 8 7 2 3 シュヴェツィンゲン、エアフルター・シュトラッセ 1 0 番

(72) 発明者 ヴァルター・ミアー

ドイツ 6 4 6 2 5 ベンスハイム、ブリュックヴェーク 2 1 番

(72) 発明者 フレデリク・ギーゼル

ドイツ 6 9 1 1 5 ハイデルベルク、アルテ・エッペルハイマー・シュトラッセ 2 4 / 1 番

(72) 発明者 クレメンス・クラトホヴィル

ドイツ 6 9 4 9 3 ヒルシュベルク・アー・デー・ペー、ガルテンシュトラッセ 9 番

F ターム(参考) 4C063 AA03 AA05 BB07 CC25 CC45 CC47 CC79 DD14 EE01

4C084 AA02 AA07 AA12 BA01 BA16 BA23 CA59 NA14 ZA361 ZA362

ZA891 ZA892 ZB081 ZB082 ZB111 ZB112 ZB261 ZB262

4C085 HH03 HH11 KB07 KB09 KB12 KB18 KB20 KB55 LL01 LL18

4C086 AA01 AA02 AA03 BC58 GA07 GA08 GA12 GA16 HA01 HA05

HA06 HA09 HA13 MA01 MA04 NA14 ZA36 ZA89 ZB08 ZB11
ZB26

4H045 AA10 AA30 BA13 BA70 BA71 EA28 FA30 FA40 GA21

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 February 2010 (04.02.2010)

PCT

(10) International Publication Number
WO 2010/014933 A2

(51) International Patent Classification:

C07D 211/34 (2006.01) *A61P 9/10* (2006.01)
A61K 31/33 (2006.01) *A61P 9/00* (2006.01)
A61K 31/44 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:

PCT/US2009/052456

(22) International Filing Date:

31 July 2009 (31.07.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/085,462 1 August 2008 (01.08.2008)
61/111,791 6 November 2008 (06.11.2008)

US

US

(71) Applicant (for all designated States except US): **THE JOHNS HOPKINS UNIVERSITY** [US/US]; 3400 North Charles Street, Baltimore, Maryland 21218 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **POMPER, Martin, Gilbert** [US/US]; 101 Churchwardens Road, Baltimore, MD 21212 (US). **MEASE, Ronnie, Charles** [US/US]; 12320 Cannonball Road, Fairfax, VA 22030 (US). **CHEN, Ying** [CN/US]; 14 Breezy Tree Court, Apartment 1, Timonium, MD 21093 (US).

(74) Agent: **HADDAWAY, Keith G.**; VENABLE LLP, P.O. Box 34385, Washington, District of Columbia 20043-9998 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

[Continued on next page]

(54) Title: PSMA-BINDING AGENTS AND USES THEREOF

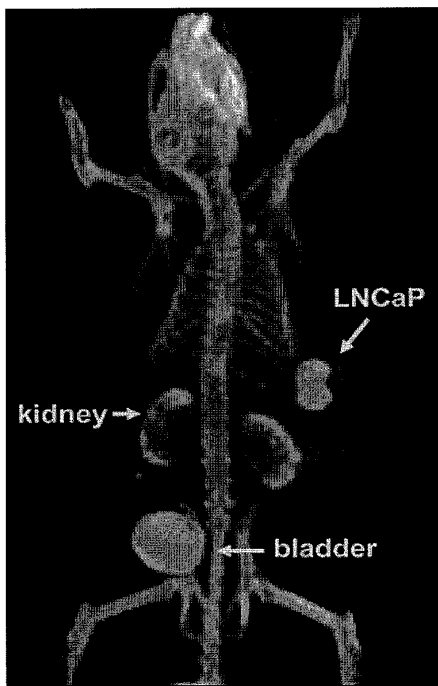


FIG 5

(57) Abstract: Prostate-specific membrane antigen (PSMA) binding compounds having radioisotope substituents are described, as well as chemical precursors thereof. Compounds include pyridine containing compounds, compounds having phenylhydrazine structures, and acylated lysine compounds. The compounds allow ready incorporation of radionuclides for single photon emission computed tomography (SPECT) and positron emission tomography (PET) for imaging, for example, prostate cancer cells and angiogenesis.

WO 2010/014933 A2



AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))

PSMA-BINDING AGENTS AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATION**

5 **[0001]** This application claims priority to U.S. Provisional Application Nos. 61/085,462
filed August 1, 2008, and 61/111,791 file November 6, 2008, the entire contents of which are
hereby incorporated by reference. This invention was made using U. S. Government support
under NIH grant NIH U24 92871, NIH R21 CA114111, NIH CA111982, and DOD PC050825.
The government has certain rights in this invention.

10

BACKGROUNDField of the Invention

[0002] The present invention relates generally to radioisotope labeled prostate specific
membrane antigen (PSMA) binding compounds, chemical precursors of radioisotope labeled
15 PSMA binding compounds and imaging methods using the radioisotope labeled compounds.

Background

[0003] Prostate cancer (PCa) is the second leading cause of cancer-related death in men
(1). Only one half of tumors due to PCa are clinically localized at diagnosis and one half of
20 those represent extracapsular spread. Localization of that spread as well as determination of the
total body burden of PCa have important implications for therapy, particularly as new
combination and focal therapies become available. Also critically needed are targeted agents
that can provide a readout on the biology of the tumor, with the ability to predict which tumors
will lie dormant and which will develop into aggressive, metastatic disease. The current clinical
25 standard for localizing cancer – including PCa – is shifting from the anatomic techniques such as
computed tomography (CT) and magnetic resonance (MR) imaging to more physiologically
relevant methods that employ molecular imaging, such as MR spectroscopy, single photon
emission computed tomography (SPECT) and positron emission tomography (PET) (2). Such
newer methods that utilize molecular imaging may provide the biological readout necessary for
30 understanding tumor physiology, enabling more accurate prognosis and therapeutic monitoring.
Molecular imaging may provide a way to not only detect tumors *in vivo*, but also to provide
information regarding the biology of the lesion, if a mechanism-specific agent is used. For
example, [¹⁸F]FDHT can be used to study the androgen receptor status of tumors (3).

[0004] Unlike many other cancers, PCa is particularly difficult to detect using existing molecular imaging tracers. There are several reasons for this, including the relatively slow growth and metabolic rate of PCa compared to other malignancies as well as the small size of the organ and proximity to the urinary bladder, into which most radiopharmaceuticals are eventually excreted.

[0005] Because of the relatively low metabolism of PCa, PET with [¹⁸F]fluorodeoxyglucose (FDG-PET) has proved ineffectual for diagnostic imaging of this disease. Other promising, experimental radiopharmaceuticals for imaging PCa are emerging, including those of the choline series (4)(5)(6), radiolabeled acetates (7), anti-1-amino-3-
10 [¹⁸F]fluorocyclobutyl-1-carboxylic acid (anti[¹⁸F]F-FACBC) (8)(9), 1-(2-deoxy-2-[¹⁸F]fluoro-L-arabinofuranosyl)-5-methyluracil ([¹⁸F]FMAU) (10) and [¹⁸F]fluorodihydrotestosterone ([¹⁸F]FDHT) (3). Each has its benefits and detriments, with no single agent ideal, i.e., easy to synthesize, little metabolism and demonstrating tumor-specific uptake, in all PCa phenotypes.

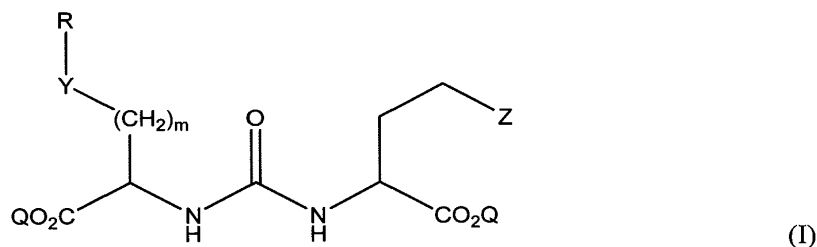
[0006] Overexpressed on most solid tumor neovasculature (11) as well as in prostate
15 cancer, the prostate-specific membrane antigen (PSMA) is becoming an attractive target for cancer imaging and therapy (12)(13). PSMA-based agents can report on the presence of this marker, which is increasingly recognized as an important prognostic determinate in PCa (14). It is also the target for a variety of new PCa therapies (15). ProstaScint™ is an ¹¹¹In-labeled monoclonal antibody against PSMA that is clinically available for imaging PCa. ProstaScint™
20 and radiolabeled variations of this antibody are fraught with long circulation times and poor target to nontarget tissue contrast, limiting the utility of these agents (16)(17)(18).

SUMMARY OF THE INVENTION

[0007] The present invention satisfies the long standing and unmet need for new tissue-
25 specific compounds for imaging prostate cancer and angiogenesis. The present invention, in particular, provides imaging agents which differ from the prior art in modifications which were not previously known or suggested. Furthermore, the invention provides imaging agents that offer better contrast between target tissues and non-target tissues.

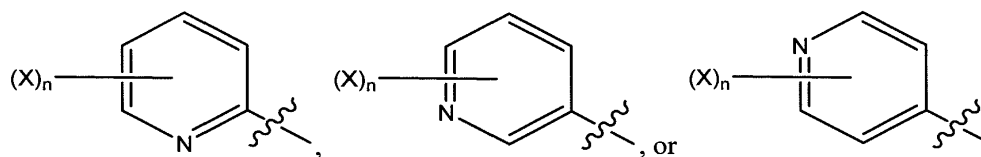
[0008] The invention relates to compounds having the structure (I) shown below.

30



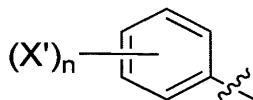
wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group.

- 5 **[0009]** In some embodiments of Formula I, m is 0, 1, 2, 3, 4, 5, or 6; R is a pyridine ring with the structure



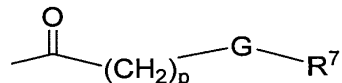
- wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃,
 10 Si(R²)₃, Hg(R²)₂, B(OH)₂, -NHNH₂, -NHN=CHR³, -NHNH-CH₂R³; n is 1, 2, 3, 4, or 5; Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted; R² is C₁-C₆ alkyl; and R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl
 15 each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²)₂, or B(OH)₂.

- [0010]** In some embodiments of Formula I, m is 0, 1, 2, 3, 4, 5, or 6; Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p,
 20 NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted; R is



wherein X' is selected from the group consisting of NHNH_2 , -NHN=CHR^3 , and $\text{-NHNH-CH}_2\text{R}^3$; wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$; and n is 1, 2, 3, 4, or 5.

[0011] In other embodiments of Formula I, m is 4, Y is NR' , and R is



wherein G is O, NR' or a covalent bond; R' is H or $\text{C}_1\text{-C}_6$ alkyl; p is 1, 2, 3, or 4, and R^7 is selected from the group consisting of NH_2 , N=CHR^3 , $\text{NH-CH}_2\text{R}^3$, wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$.

[0012] Some compounds of the present invention interact with the prostate-specific membrane antigen (PSMA). As a result, when the compounds comprise a radioisotope, they may be suitable as imaging agents, diagnostic agents, and/or therapeutic agents.

[0013] In many cases, the radioisotope used in the compound is short-lived. Therefore, radioisotopically labeled compounds are prepared immediately or shortly before use, or only in sufficient quantity for administration. For this reason, the invention also includes precursors to radioisotopically labeled compounds, which may be chemically converted into the radioisotopically labeled compounds of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **Figure 1** shows an overlay of the best poses for **3**, **6** and **8** with the crystal ligand, i.e., **3** as it is co-crystallized with PSMA, in the presence of water molecule in the active site of PSMA (PDB ID: 3D7H). Dark spheres (zinc ions), light sphere (chloride ion).

[0015] **Figure 2** shows an overlay of the best poses (**3**, **6** and **8**) with the crystal ligand (**3**) in the absence of water molecule in the active site of PSMA. Dark sphere (zinc ions), light sphere (chloride ion).

[0016] **Figure 3** shows $[\text{}^{125}\text{I}]\text{3}$ SPECT-CT in PCa tumor models (4 h postinjection).

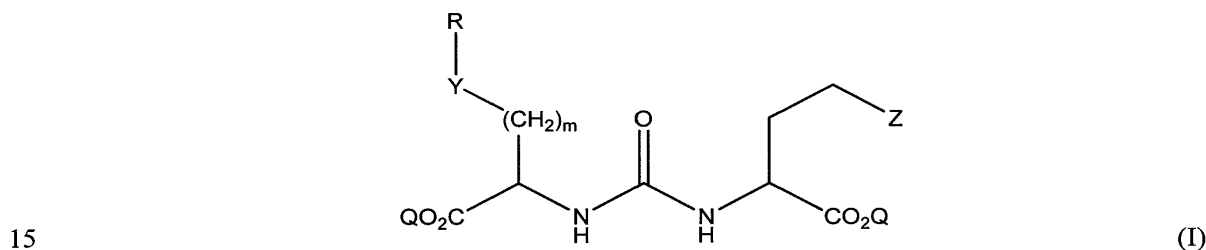
Note uptake within the PSMA+ PIP tumor only. Uptake within kidneys is due in large measure to the specific binding of $[\text{}^{125}\text{I}]\text{3}$ to renal cortex.

[0017] **Figure 4** shows [^{18}F]6 PET coregistered to CT in PCa tumor models (~100 min postinjection). Note uptake within the PSMA+ PIP tumor only. Uptake within kidneys is due in large measure to the specific binding of [^{125}I]3 to renal cortex. There is more intense tumor uptake and less liver seen with this agent than with [^{125}I]3.

5 [0018] **Figure 5** shows [^{125}I]8 SPECT-CT in PSMA+ LNCaP tumors (4 h postinjection). Note intense uptake within tumor. A similar result was obtained for PSMA+ PIP but not PSMA-flu tumors (data not shown). There is less renal and liver uptake with this agent than the halobenzoylated analogues, [^{125}I]3 and [^{18}F]6, respectively.

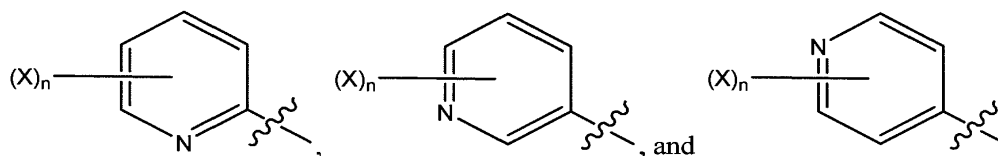
10 DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0019] Embodiments of the invention include compounds according to formula I, shown below:



wherein Z is tetrazole or CO₂Q, and each Q is independently selected from hydrogen or a protecting group.

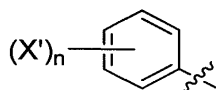
20 [0020] In exemplary embodiments (A), m is 0, 1, 2, 3, 4, 5, or 6, R is a pyridine ring selected from the group consisting of



wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²)₂, B(OH)₂, -NHNH₂, -NHN=CHR³, -NHNH-CH₂R³; n is 1, 2, 3, 4, or 5; Y is O,
 25 S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent

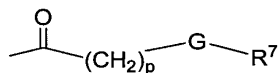
bond; p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted; R² is C₁-C₆ alkyl; and R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃,
 5 Sn(R²)₃, Si(R²)₃, Hg(R²)₂, or B(OH)₂; or a pharmaceutically acceptable salt thereof.

[0021] In other embodiments (B), m is 0, 1, 2, 3, 4, 5, or 6; Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; p is 1, 2, or 3; R' is H or C₁-C₆ alkyl; R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted;
 10 R is



wherein X' is selected from the group consisting of NHNH₂, -NHN=CHR³, and -NHNH-CH₂R³; wherein R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of
 15 bromine, or a radioisotope of astatine; NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²)₂, or B(OH)₂; R² is C₁-C₆ alkyl; n is 1, 2, 3, 4, or 5; or a pharmaceutically acceptable salt thereof.

[0022] In yet other embodiments (C), m is 4; Y is NR'; and R is



wherein G is O, NR' or a covalent bond; R' is H or C₁-C₆ alkyl; p is 1, 2, 3, or 4, and R⁷ is
 20 selected from the group consisting of NH₂, N=CHR³, NH-CH₂R³, wherein R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine; NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²)₂, or B(OH)₂; R² is C₁-C₆ alkyl; or a pharmaceutically acceptable salt thereof.

[0023] The compounds herein described may have one or more asymmetric centers or planes. Compounds of the present invention containing an asymmetrically substituted atom may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms (racemates), by asymmetric synthesis, or by synthesis from optically active starting materials. Resolution of the racemates
 30 can be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column.

Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Cis and trans geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral
5 (enantiomeric and diastereomeric), and racemic forms, as well as all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

[0024] The compounds herein described may have one or more charged atoms. For example, the compounds may be zwitterionic, but may be neutral overall. Other embodiments
10 may have one or more charged groups, depending on the pH and other factors. In these embodiments, the compound may be associated with a suitable counter-ion. It is well known in the art how to prepare salts or exchange counter-ions. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting
15 free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Counter-ions may be changed, for example, by ion-exchange techniques such as ion-exchange chromatography. All zwitterions, salts and counter-ions are intended, unless the counter-ion or salt is specifically indicated. In certain embodiments, the salt or counter-ion may be
20 pharmaceutically acceptable, for administration to a subject. Pharmaceutically acceptable salts are discussed later.

[0025] When any variable occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with (X)_n, where n is 1, 2,
25 3, 4, or 5, then said group may optionally be substituted with up to five X groups and each occurrence is selected independently from the definition of X. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

[0026] As indicated above, various substituents of the various formulae are "substituted" or "may be substituted." The term "substituted," as used herein, means that any one or more
30 hydrogens on the designated atom or group is replaced with a substituent, provided that the designated atom's normal valence is not exceeded, and that the substitution results in a stable compound. When a substituent is oxo (keto, i.e., =O), then 2 hydrogens on an atom are replaced.

The present invention is intended to include all isotopes (including radioisotopes) of atoms occurring in the present compounds. When the compounds are substituted, they may be so substituted at one or more available positions, typically 1, 2, 3 or 4 positions, by one or more suitable groups such as those disclosed herein. Suitable groups that may be present on a "substituted" group include e.g., halogen; cyano; hydroxyl; nitro; azido; amino; alkanoyl (such as a C₁-C₆ alkanoyl group such as acyl or the like); carboxamido; alkyl groups (including cycloalkyl groups, having 1 to about 8 carbon atoms, for example 1, 2, 3, 4, 5, or 6 carbon atoms); alkenyl and alkynyl groups (including groups having one or more unsaturated linkages and from 2 to about 8, such as 2, 3, 4, 5 or 6, carbon atoms); alkoxy groups having one or more oxygen linkages and from 1 to about 8, for example 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those having one or more thioether linkages and from 1 to about 8 carbon atoms, for example 1, 2, 3, 4, 5 or 6 carbon atoms; alkylsulfinyl groups including those having one or more sulfinyl linkages and from 1 to about 8 carbon atoms, such as 1, 2, 3, 4, 5, or 6 carbon atoms; alkylsulfonyl groups including those having one or more sulfonyl linkages and from 1 to about 8 carbon atoms, such as 1, 2, 3, 4, 5, or 6 carbon atoms; aminoalkyl groups including groups having one or more N atoms and from 1 to about 8, for example 1, 2, 3, 4, 5 or 6, carbon atoms; carbocyclic aryl having 4, 5, 6 or more carbons and one or more rings, (e.g., phenyl, biphenyl, naphthyl, or the like, each ring either substituted or unsubstituted aromatic); arylalkyl having 1 to 3 separate or fused rings and from 6 to about 18 ring carbon atoms, (e.g. benzyl); arylalkoxy having 1 to 3 separate or fused rings and from 6 to about 18 ring carbon atoms (e.g. O-benzyl); or a saturated, unsaturated, or aromatic heterocyclic group having 1 to 3 separate or fused rings with 3 to about 8 members per ring and one or more N, O or S atoms, (e.g. coumarinyl, quinolinyl, isoquinolinyl, quinazolinyl, pyridyl, pyrazinyl, pyrimidyl, furanyl, pyrrolyl, thienyl, thiazolyl, triazinyl, oxazolyl, isoxazolyl, imidazolyl, indolyl, benzofuranyl, benzothiazolyl, tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholinyl, piperazinyl, and pyrrolidinyl). Such heterocyclic groups may be further substituted, e.g. with hydroxy, alkyl, alkoxy, halogen and amino.

[0027] As used herein, "alkyl" is intended to include branched, straight-chain, and cyclic saturated aliphatic hydrocarbon groups. Examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, and *sec*-pentyl. In certain embodiments, alkyl groups are C₁-C₆ alkyl groups or C₁-C₄ alkyl groups. Particular alkyl groups are methyl, ethyl, propyl, butyl, and 3-pentyl. The term "C₁-C₆ alkyl" as used herein means straight-chain, branched, or cyclic C₁-C₆ hydrocarbons which are completely saturated

and hybrids thereof such as (cycloalkyl)alkyl. Examples of C₁-C₆ alkyl substituents include methyl (Me), ethyl (Et), propyl (including *n*-propyl (n-Pr, ⁿPr), *iso*-propyl (i-Pr, ⁱPr), and cyclopropyl (c-Pr, ^cPr)), butyl (including *n*-butyl (n-Bu, ⁿBu), *iso*-butyl (i-Bu, ⁱBu), *sec*-butyl (s-Bu, ^sBu), *tert*-butyl (t-Bu, ^tBu), or cyclobutyl (c-Bu, ^cBu)), and so forth. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. Cycloalkyl groups typically will have 3 to about 8 ring members. In the term "(cycloalkyl)alkyl", cycloalkyl, and alkyl are as defined above, and the point of attachment is on the alkyl group. This term encompasses, but is not limited to, cyclopropylmethyl, cyclopentylmethyl, and cyclohexylmethyl.

10 **[0028]** As used herein, "alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration comprising one or more unsaturated carbon-carbon bonds, which may occur in any stable point along the chain, such as ethenyl and propenyl. Alkenyl groups typically will have 2 to about 8 carbon atoms, more typically 2 to about 6 carbon atoms.

15 **[0029]** As used herein, "alkynyl" is intended to include hydrocarbon chains of either a straight or branched configuration comprising one or more carbon-carbon triple bonds, which may occur in any stable point along the chain, such as ethynyl and propynyl. Alkynyl groups typically will have 2 to about 8 carbon atoms, more typically 2 to about 6 carbon atoms.

[0030] As used herein, "haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen atoms. Examples of haloalkyl include, but are not limited to, mono-, di-, or tri-fluoromethyl, mono-, di-, or tri-chloromethyl, mono-, di-, tri-, tetra-, or penta-fluoroethyl, and mono-, di-, tri-, tetra-, or penta-chloroethyl, etc. Typical haloalkyl groups will have 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

25 **[0031]** As used herein, "alkoxy" represents an alkyl group as defined above attached through an oxygen bridge. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, *n*-propoxy, *i*-propoxy, *n*-butoxy, 2-butoxy, *t*-butoxy, *n*-pentoxy, 2-pentoxy, 3-pentoxy, isopentoxy, neopentoxy, *n*-hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy. Alkoxy groups typically have 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

[0032] As used herein, "haloalkoxy" represents a haloalkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge. Haloalkoxy groups will have 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

30 **[0033]** As used herein, "alkylthio" includes those groups having one or more thioether linkages and typically from 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

[0034] As used herein, the term "alkylsulfinyl" includes those groups having one or more sulfoxide (SO) linkage groups and typically from 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

[0035] As used herein, the term "alkylsulfonyl" includes those groups having one or more sulfonyl (SO₂) linkage groups and typically from 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

[0036] As used herein, the term "alkylamino" includes those groups having one or more primary, secondary and/or tertiary amine groups and typically from 1 to about 8 carbon atoms, more typically 1 to about 6 carbon atoms.

[0037] As used herein, "Halo" or "halogen" refers to fluoro, chloro, bromo, or iodo; and "counter-ion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, sulfate, and the like.

[0038] As used herein, "carbocyclic group" is intended to mean any stable 3- to 7-membered monocyclic or bicyclic or 7- to 13-membered bicyclic or tricyclic group, any of which may be saturated, partially unsaturated, or aromatic. In addition to those exemplified elsewhere herein, examples of such carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctanyl, [4.3.0]bicyclononanyl, [4.4.0]bicyclodecanyl, [2.2.2]bicyclooctanyl, fluorenyl, phenyl, naphthyl, indanyl, and tetrahydronaphthyl.

[0039] As used herein, the term "aryl" includes groups that contain 1 to 3 separate or fused rings and from 6 to about 18 ring atoms, without hetero atoms as ring members. Examples of aryl groups include but are not limited to phenyl, and naphthyl, including 1-naphthyl and 2-naphthyl.

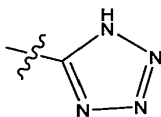
[0040] As used herein, "heterocyclic group" is intended to include saturated, partially unsaturated, or unsaturated (aromatic) groups having 1 to 3 (possibly fused) rings with 3 to about 8 members per ring at least one ring containing an atom selected from N, O or S. The nitrogen and sulfur heteroatoms may optionally be oxidized. The term or "heterocycloalkyl" is used to refer to saturated heterocyclic groups.

[0041] A heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. A nitrogen in the heterocycle may optionally be quaternized.

[0042] As used herein, the term "heteroaryl" is intended to include any stable 5- to 7-membered monocyclic or 10- to 14-membered bicyclic heterocyclic aromatic ring system which comprises carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O and S. In exemplary embodiments, the total number of S and O atoms in the aromatic heterocycle is not more than 2, and typically not more than 1.

[0043] Examples of heteroaryl include, but are not limited to, those exemplified elsewhere herein and further include acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolynyl, carbazolyl, NH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H.6HA, 5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolynyl, imidazolyl, 1H-indazolyl, indolenyl, indolynyl, indolizynyl, indolyl, 3H-indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolynyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolynyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizynyl, quinoxalynyl, quinuclidinyl, tetrahydrofuran, tetrahydroisoquinolinyl, tetrahydroquinolinyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl. Exemplary heteroaryl groups include, but are not limited to, pyridinyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrrolidinyl, morpholinyl, piperidinyl, piperazinyl, and imidazolyl.

[0044] In certain embodiments, Z is tetrazole or CO₂Q. When Z is tetrazole, the tetrazole ring is attached through the carbon atom, as shown below.



[0045] In certain embodiments, Q is a protecting group. As used herein, a "protecting group" is a chemical substituent which can be selectively removed by readily available reagents

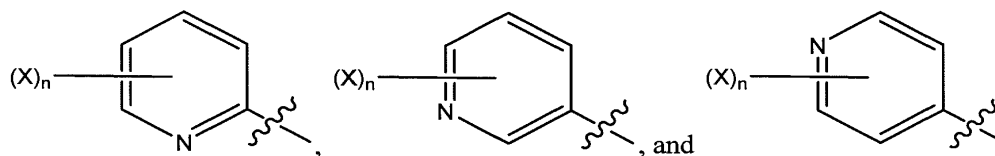
which do not attack the regenerated functional group or other functional groups in the molecule. Suitable protecting groups may be found, for example in Wutz et al. ("Greene's Protective Groups in Organic Synthesis, Fourth Edition," Wiley-Interscience, 2007). Protecting groups for protection of the carboxyl group, as described by Wutz et al. (pages 533-643), are used in certain
 5 embodiments. In some embodiments, the protecting group is removable by treatment with acid. Specific examples of protecting groups include but are not limited to, benzyl, p-methoxybenzyl (PMB), tertiary butyl (^tBu), methoxymethyl (MOM), methoxyethoxymethyl (MEM), methylthiomethyl (MTM), tetrahydropyranyl (THP), tetrahydrofuranyl (THF), benzyloxymethyl (BOM), trimethylsilyl (TMS), triethylsilyl (TES), t-butyldimethylsilyl (TBDMS), and
 10 triphenylmethyl (trityl, Tr).

[0046] In some embodiments, R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted. In certain embodiments, R⁸ describes the sidechain of a natural or synthetic α -amino acid. Specific examples of R⁸ include hydrogen, methyl (CH₃), isopropyl (CH(CH₃)₂), 2,2-dimethylethyl (CH₂CH(CH₃)₂), 2-methylpropyl (CH(CH₃)CH₂CH₃), phenyl, 4-
 15 hydroxyphenyl, hydroxymethyl (CH₂OH), carboxymethyl (CH₂CO₂H), thiomethyl (CH₂SH), imidazolylmethyl, indolylmethyl, and so forth.

[0047] Certain embodiments include compounds according to formula I where Z is CO₂Q. In other embodiments, Q is hydrogen. In some specific embodiments, Z is CO₂Q and Q is hydrogen.

[0048] Certain embodiments include compounds according to formula I, where m is 1, 2, 3, or 4.

[0049] Other embodiments include compounds according to formula I wherein m is 0, 1, 2, 3, 4, 5, or 6; R is a pyridine ring selected from the group consisting of

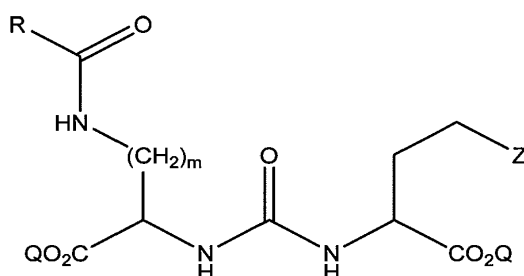


25 wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), B(OH)₂, -NHNH₂, -NHN=CHR³, -NHNH-CH₂R³. In certain embodiments, n is 1. Each Q is independently selected from hydrogen or a protecting group; Z is tetrazole or CO₂Q; Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR',
 30 NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O),

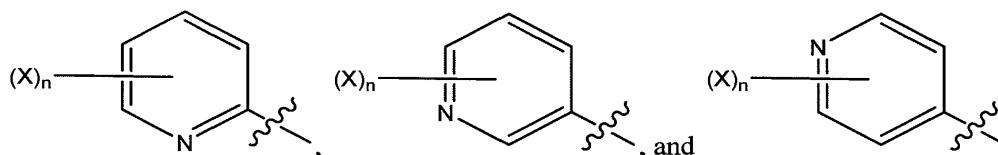
NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is alkyl, aryl or heteroaryl, each of which may be substituted; R² is C₁-C₆ alkyl; and R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, a radioisotope of astatine; NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), or B(OH)₂. In certain

embodiments, R³ is aryl, substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine.

[0050] Other embodiments include compounds having the structure



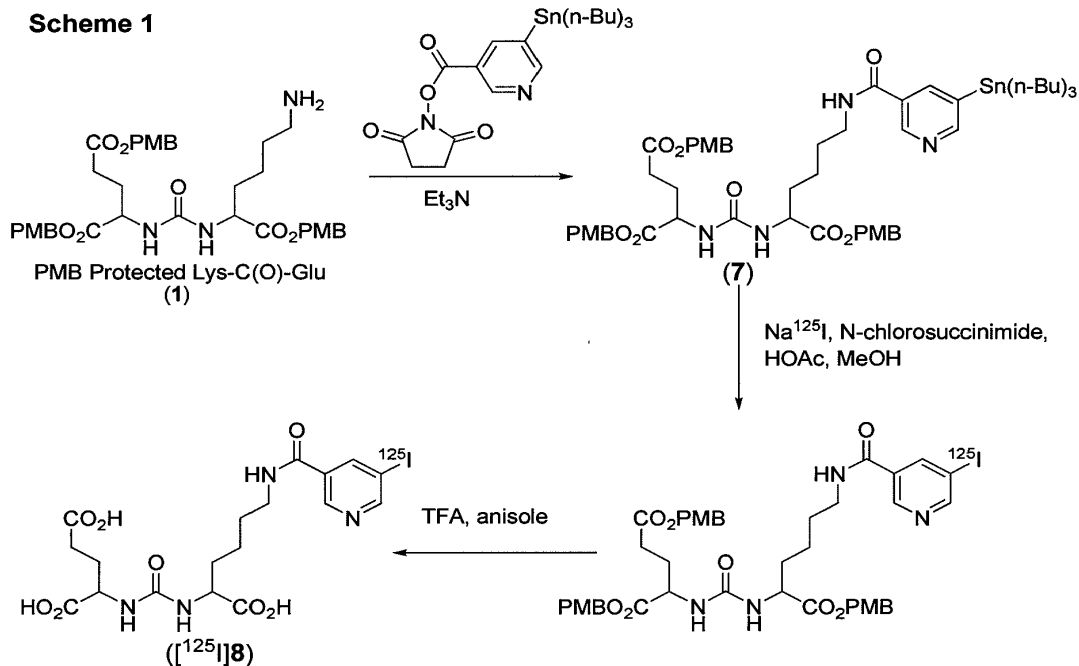
wherein m is not 0. R is a pyridine ring selected from the group consisting of



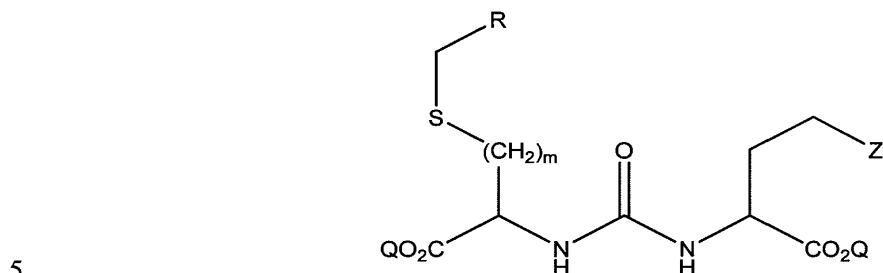
wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), B(OH)₂, -NHNH₂, -NHN=CHR³, or -NHNH-CH₂R³. R² is C₁-C₆ alkyl; and R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, or a radioisotope of astatine; NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), or B(OH)₂. In certain

embodiments, n is 1. Other specific embodiments include compounds where X is fluorine, iodine, or a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine. In certain embodiments, R³ is aryl, substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine. Specific embodiments include compounds having the structure shown above, where Z is CO₂Q, Q is hydrogen, and m is 4.

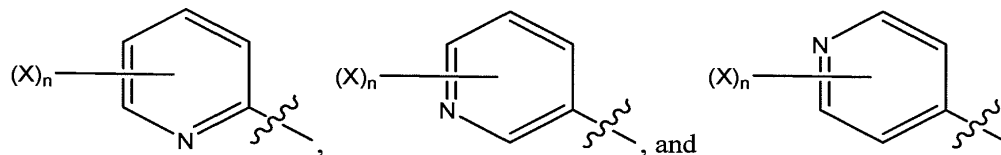
[0051] Compounds according to this embodiment can be prepared, for example, from p-methoxybenzyl (PMB) protected precursor Lys-C(O)-Glu according to Scheme 1 shown below.



[0052] Other embodiments include compounds having the structure



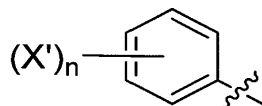
wherein m is not 0. R is a pyridine ring selected from the group consisting of



wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, $\text{B}(\text{OH})_2$, $-\text{NHNH}_2$, $-\text{NHN}=\text{CHR}^3$, $-\text{NHNH}-\text{CH}_2\text{R}^3$. R^2 is C_1 - C_6 alkyl; and R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a

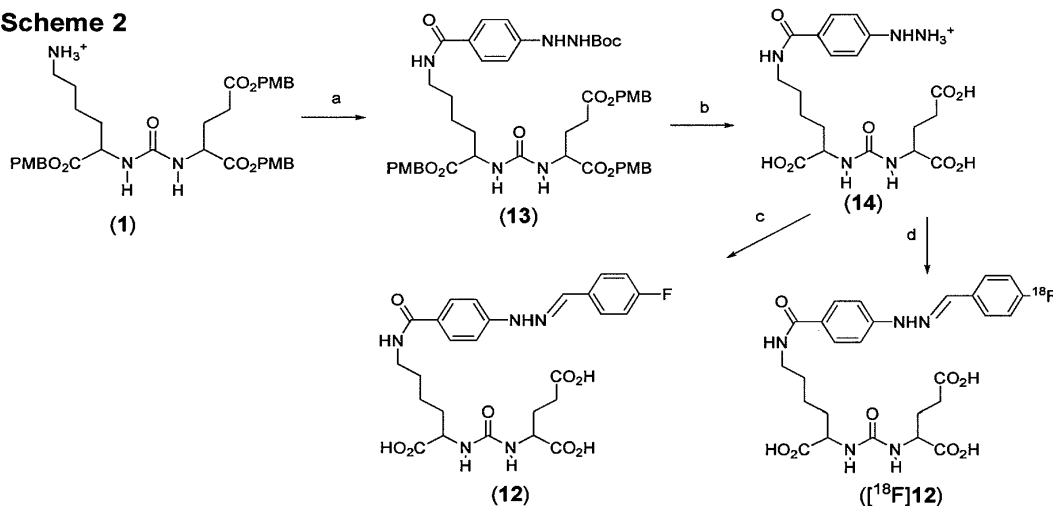
radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, or a radioisotope of astatine; NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$. In certain embodiments, n is 1. Other specific embodiments include compounds where X is fluorine, iodine, or a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine. In certain embodiments, R^3 is aryl, substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine. Specific embodiments include compounds having the structure shown above, where Z is CO_2Q , Q is hydrogen, and m is 1, 2, or 3.

[0053] Other embodiments include compounds according to formula I wherein R is the structure below



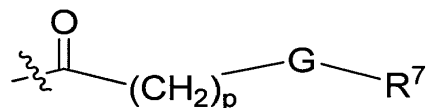
wherein X' is selected from the group consisting of $-\text{NHNH}_2$, $-\text{NHN}=\text{CHR}^3$, $-\text{NHNH}-\text{CH}_2\text{R}^3$. In such embodiments, R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine; NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, $\text{B}(\text{OH})_2$. R^2 is C_1 - C_6 alkyl; and R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, or a radioisotope of astatine; NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$. In certain embodiments, R^3 is aryl, substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine. Specific embodiments include compounds where n is 1.

[0054] Compounds according to this embodiment can be prepared, for example, from hydrazine substituted phenyl precursors, followed by derivatization with an alkyl, alkenyl, alkynyl, aryl, or heteroaryl reagent, each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, or a radioisotope of astatine NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, and $\text{B}(\text{OH})_2$ as illustrated in Scheme 2 below.

Scheme 2

a. succinimidyl 4-[2-(*tert*-butoxycarbonyl)hydrazino]benzoate, triethylamine, DMF, CH_2Cl_2 ; b. TFA, CH_2Cl_2 ; c. 4-fluorobenzaldehyde, 50 mM KH_2PO_4 , CH_3CN ; d. 4-[^{18}F]fluorobenzaldehyde, 50 mM KH_2PO_4 .

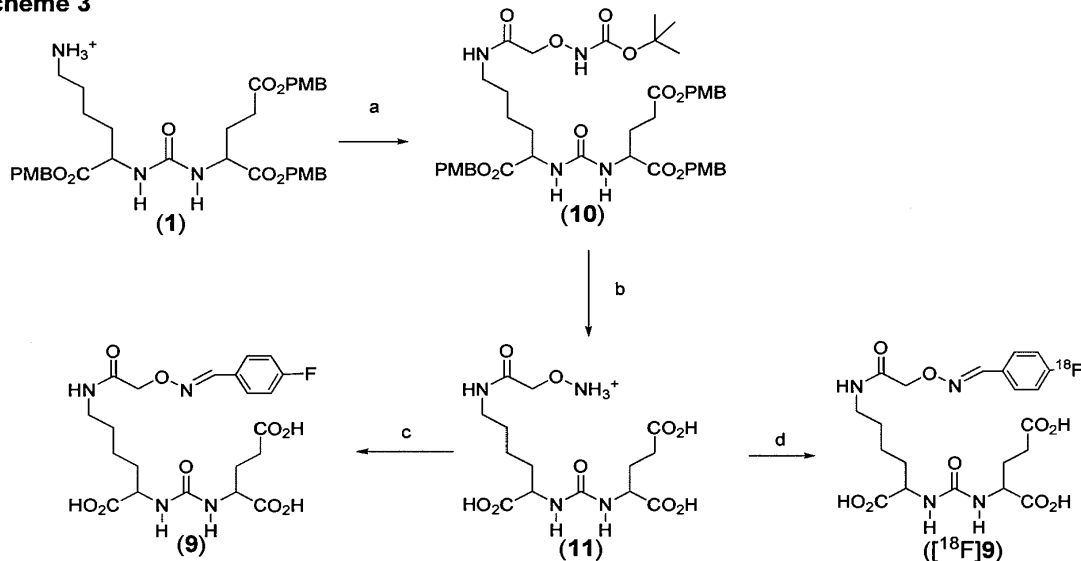
[0055] Other embodiments include compounds according to formula I wherein m is 4, Y is NR' , and R is



wherein G is O, NR' or a covalent bond, R' is H or $\text{C}_1\text{--C}_6$ alkyl, and p is 1, 2, 3, or 4. R^7 can be selected from NH_2 , N=CHR^3 , and $\text{NH-CH}_2\text{R}^3$, wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl, each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$, where R^2 is $\text{C}_1\text{--C}_6$ alkyl. In certain embodiments, R^3 is aryl, substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine. In certain embodiments, G is O or NR' .

[0056] Compounds according to this embodiment can be prepared, for example, by acylation of PMB protected Lys-C(O)-Glu with an acylating agent bearing a free or protected amine, followed by deprotection of the amine, if necessary, and derivatization with an alkyl, alkenyl, alkynyl, aryl, or heteroaryl reagent, each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine,

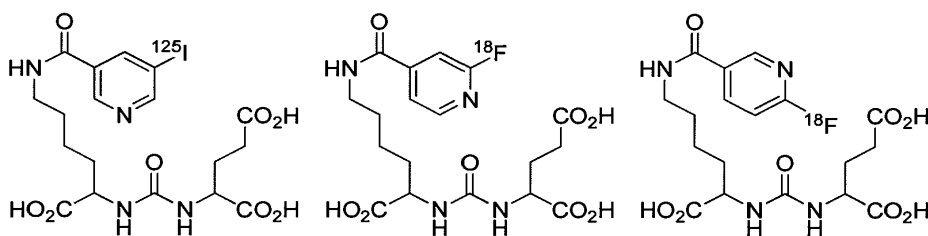
or a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²)₂, or B(OH)₂, as illustrated by Scheme 3 shown below.

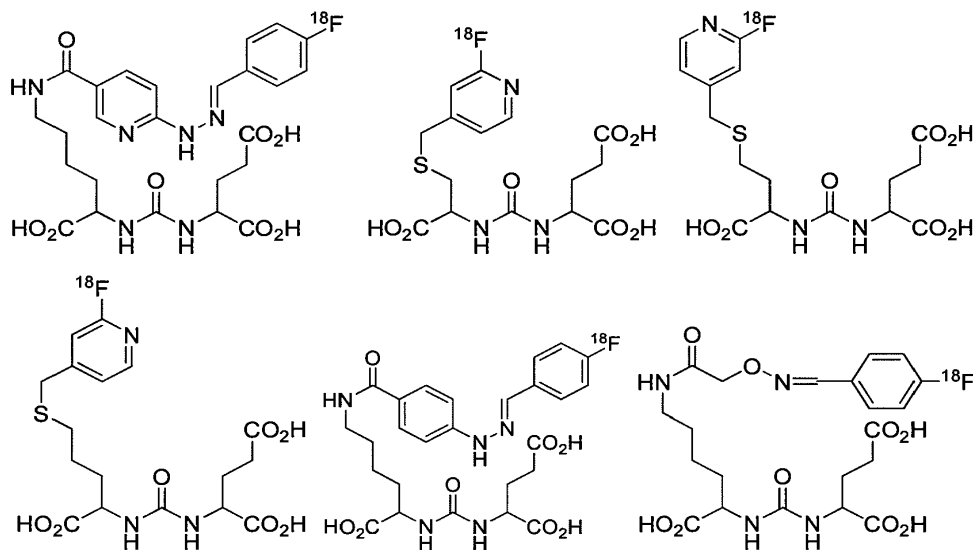
Scheme 3

- 5 a. *N*-tert-butyloxycarbonyl-*O*-(carboxymethyl)hydroxyamine hydroxysuccinimidyl ester, triethylamine, CH₂Cl₂; b. TFA, anisole; c. *p*-fluorobenzaldehyde, triethylamine, methanol; d. *p*-[¹⁸F]fluorobenzaldehyde, triethylamine, methanol.

- [0057] Other embodiments include compounds according to any of the embodiments discussed herein, which comprise a radioisotope. Specific exemplary radioisotopes include ¹⁸F, ¹²³I, ¹²⁴I, ¹²⁵I, ¹²⁶I, ¹³¹I, ⁷⁵Br, ⁷⁶Br, ⁷⁷Br, ⁸⁰Br, ^{80m}Br, ⁸²Br, ⁸³Br and ²¹¹At. Radioisotope containing compounds of any embodiment of the present invention can be prepared with sufficient radiolabel to be used in imaging applications. In other words, the compounds can be prepared with radioisotope concentrations greater than natural abundance, when a particular radioisotope occurs naturally.

[0058] Specific examples of compounds according to the previous embodiments include the structures shown below



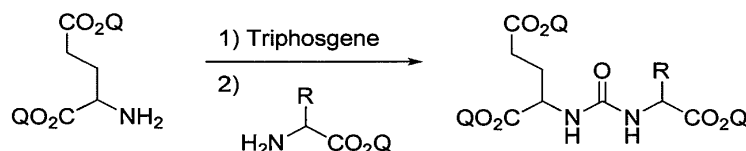


[0059] Other embodiments include pharmaceutically acceptable salts of the compounds described in the previous embodiments.

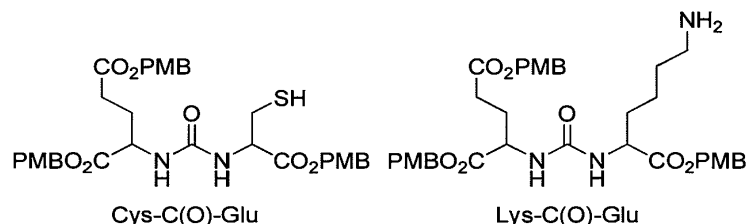
5 **[0060]** Compounds according to the invention, in particular various radiolabeled compounds, may be used for diagnostic, imaging, or therapeutic purposes. For example, some compounds, e.g. those labeled with ^{125}I and ^{123}I , are designed for SPECT imaging, while some compounds, e.g. those labeled with ^{18}F and ^{124}I , are designed for PET imaging, and some radioisotopically labeled compounds may be used therapeutically. In general, the suitability of a particular radioisotope for a particular purpose is well understood in the art. Other exemplary
10 embodiments are compounds used as precursors for radiolabeled compounds, in which a substituent may be directly exchanged for a radioisotope in one or more steps. Unless described otherwise, the terms "converted," "derivatized," "exchanged," or "reacted" are intended to encompass one or more steps. Examples of substituents that may be exchanged for
15 radioisotopes include halogens, NO_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, and $\text{B}(\text{OH})_2$. Other compounds are precursors which may be chemically reacted with a radioisotopically labeled reagent to produce a stable radioisotopically labeled compound. Compounds bearing substituents such as halogen, $-\text{NH}_2$, $-\text{NHNH}_2$, $\text{Sn}(\text{R}^2)_3$, and $\text{B}(\text{OH})_2$, for example, may be converted into radioisotopically labeled compounds by chemical reactions known to those in the
20 art.

[0061] Compounds of the present invention may be made by methods known in the art. For example, the asymmetrical ureas used as precursors may be produced by the general scheme shown below, where R is the sidechain of a natural or synthetic amino acid, which bears a group

that can be further derivatized. Specific examples of amino acids include lysine, cysteine, homocysteine, serine, threonine, tyrosine, phenylalanine and substituted phenylalanine. Substituted phenylalanine has the structure of phenylalanine where the phenyl sidechain is substituted by, for example, nitro, amino or halogen.

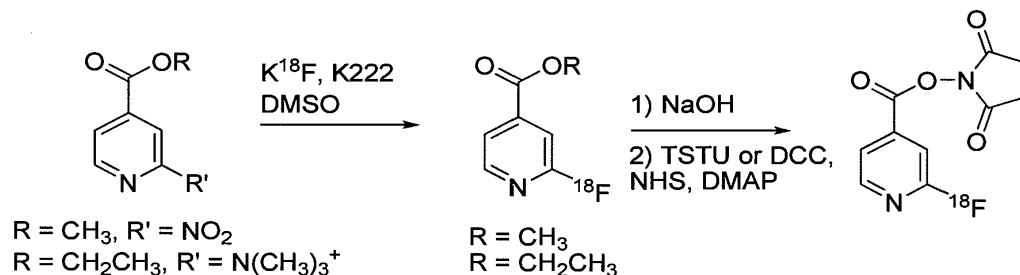


[0062] Protected urea precursors Cys-C(O)-Glu, and Lys-C(O)-Glu (shown below), where Q is p-methoxybenzyl (PMB) are used to synthesize exemplary compounds. Preparation of precursor Cys-C(O)-Glu is described, for example by Kozikowski et al. (29), while the preparation of Lys-C(O)-Glu is described, for example, by Banerjee et al. (19).

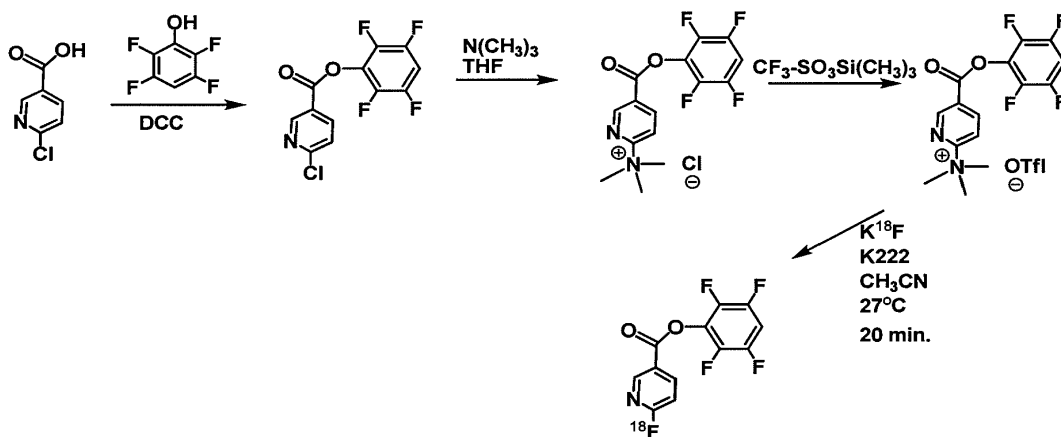


[0063] Compounds of the present invention may be prepared, for example, by reaction of a protected urea precursor with a reagent substituted with a radioisotope or other substituent which may be converted or derivatized into a radioisotope containing compound. A protected urea precursor, such as those described above may be reacted, for example with an activated benzoate or pyridine carboxylate. The synthesis of both the halobenzoate and pyridine carboxylate radionuclide-bearing precursors have been described (20)(21)(22)(23)(25)(37)(38).

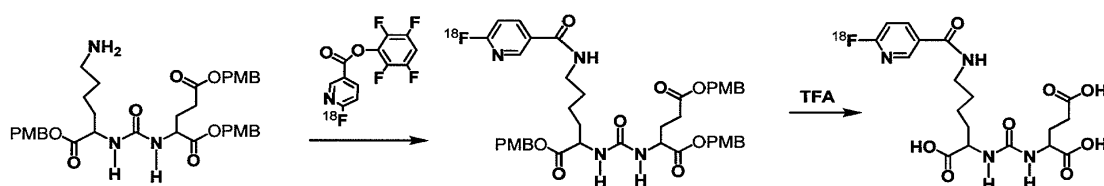
[0064] Pyridinecarboxylate ^{18}F precursors, such as n-hydroxysuccinimide-activated pyridine carboxylates, can be prepared, for example, by the scheme shown below.



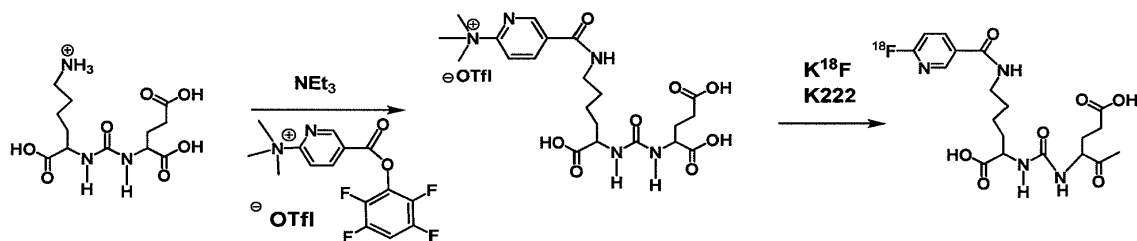
[0065] Other ^{18}F pyridine precursors may be prepared by methods described by Olberg et al. (J. Labeled Compd. Radiopharm, vol. 52:Supplement 1, p. S160, 2009), shown below.



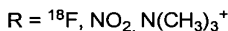
[0066] An ^{18}F pyridinecarboxylate precursor may be used to prepare compounds according to the present invention, for example, according to the scheme shown below.



[0067] Similarly, a precursor may be prepared, which can then be converted into an ^{18}F -substituted compound. For example, compounds may be prepared according to the scheme below.



[0068] Other compounds may be produced from a suitable protected precursor, such as those discussed above, by reaction with bromomethyl pyridine compounds bearing radioisotope substituents, or substituents which may be converted to radioisotopes or derivatized with radioisotope containing compounds. For example, the scheme below shows preparation of an exemplary compound from PMB protected precursor Cys-C(O)-Glu.

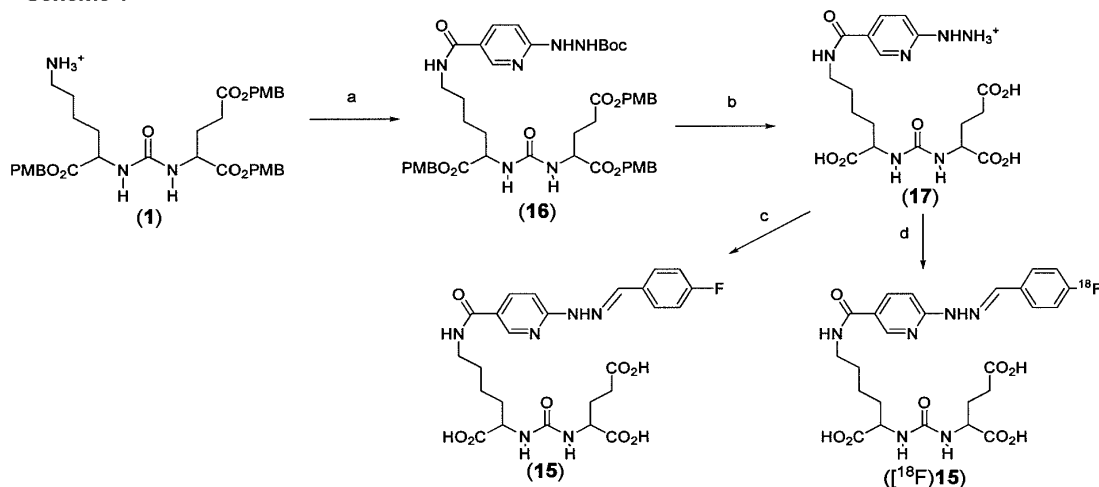


[0069] Bromomethyl pyridine compounds, such as ^{18}F , NO_2 or $\text{N}(\text{CH}_3)_3^+$ substituted bromomethyl pyridine, suitable for preparation of compounds according to the present invention can be prepared, for example, according to the scheme shown below.



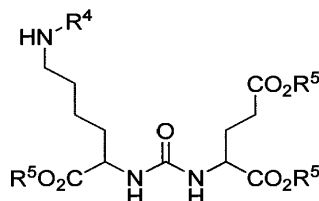
15

Scheme 4

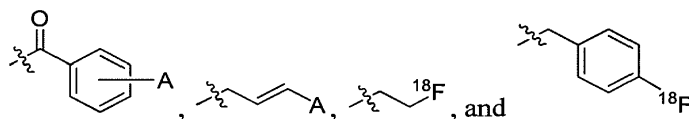


a. succinimidyl 6-(*N*-*tert*-butoxycarbonyl-hydrazino)-nicotinate, triethylamine, CH_2Cl_2 ;
 b. TFA, CH_2Cl_2 ; c. 4-fluorobenzaldehyde, 50 mM KH_2PO_4 , CH_3CN ; d. 4-
 $[^{18}\text{F}]$ fluorobenzaldehyde, 50 mM KH_2PO_4 .

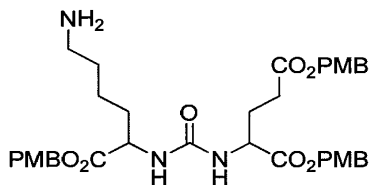
5 [0071] Other embodiments include compounds having the formula



wherein R^5 is hydrogen or *p*-methoxybenzyl; R^4 is selected from the group consisting of hydrogen,

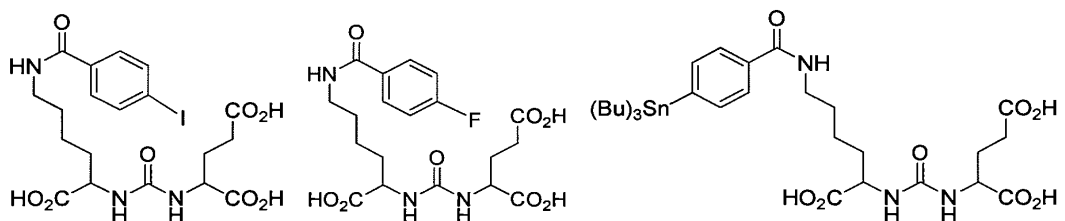


10 wherein A is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, a radioisotope of astatine, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, or HgCl . Further embodiments include compounds according to the structure above, which comprise a radioisotope. One specific embodiment includes the compound having the formula shown below, also known as PMB protected Lys-C(O)-Glu.

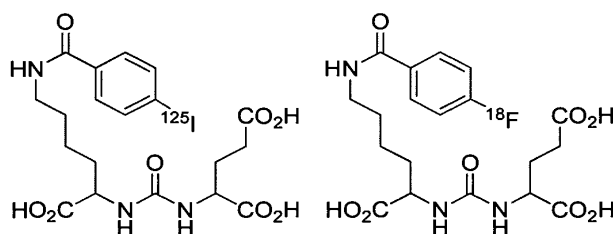


15

wherein PMB is p-methoxybenzyl. Another specific embodiment includes the compound 2-[3-(5-amino-1-carboxy-pentyl)-ureido]-pentanedioic acid, also known as Lys-C(O)-Glu. Other exemplary embodiments include compounds shown below.



5 Exemplary radioisotope containing compounds include the compounds shown below.



[0072] Other embodiments of the inventions include methods of imaging one or more cells, organs or tissues comprising exposing cells to or administering to a subject an effective amount of a compound with an isotopic label suitable for imaging. In some embodiments, the one or more organs or tissues include prostate tissue, kidney tissue, brain tissue, vascular tissue or tumor tissue.

[0073] In another embodiment, the imaging method is suitable for imaging studies of PSMA inhibitors, for example, by studying competitive binding of non-radiolabeled inhibitors. In still another embodiment, the imaging method is suitable for imaging of cancer, tumor or neoplasm. In a further embodiment, the cancer is selected from eye or ocular cancer, rectal cancer, colon cancer, cervical cancer, prostate cancer, breast cancer and bladder cancer, oral cancer, benign and malignant tumors, stomach cancer, liver cancer, pancreatic cancer, lung cancer, corpus uteri, ovary cancer, prostate cancer, testicular cancer, renal cancer, brain cancer (e.g., gliomas), throat cancer, skin melanoma, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's Sarcoma, Kaposi's Sarcoma, basal cell carcinoma and squamous cell carcinoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, angiosarcoma, hemangioendothelioma, Wilms Tumor, neuroblastoma, mouth/pharynx cancer, esophageal cancer, larynx cancer, lymphoma, neurofibromatosis, tuberous sclerosis, hemangiomas, and lymphangiogenesis.

[0074] The imaging methods of the invention are suitable for imaging any physiological process or feature in which PSMA is involved. Typically, imaging methods are suitable for identification of areas of tissues or targets which express high concentrations of PSMA. Typical applications include imaging glutamateric neurotransmission, presynaptic glutamatergic neurotransmission, malignant tumors or cancers that express PSMA, prostate cancer (including metastasized prostate cancer), and angiogenesis. Essentially all solid tumors express PSMA in the neovasculature. Therefore, methods of the present invention can be used to image nearly all solid tumors including lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal and stomach. Also, certain benign lesions and tissues including endometrium, schwannoma and Barrett's esophagus can be imaged according to the present invention.

[0075] The methods of imaging angiogenesis provided by the present invention are suitable for use in imaging a variety of diseases and disorders in which angiogenesis takes place. Illustrative, non-limiting, examples include tumors, collagen vascular disease, cancer, stroke, vascular malformations, retinopathy. Methods of imaging angiogenesis provided by the present invention are also suitable for use in diagnosis and observation of normal tissue development.

[0076] PSMA is frequently expressed in endothelial cells of capillary vessels in peritumoral and endotumoral areas of various malignancies such that compounds of the invention and methods of imaging using same are suitable for imaging such malignancies.

[0077] In certain embodiments, the radiolabeled compound is stable *in vivo*.

[0078] In certain embodiments, the radiolabeled compound is detected by positron emission tomography (PET) or single photon emission computed tomography (SPECT).

[0079] In one embodiment, the invention provides a method wherein the subject is a human, rat, mouse, cat, dog, horse, sheep, cow, monkey, avian, or amphibian. In another embodiment, the cell is *in vivo* or *in vitro*. Typical subjects to which compounds of the invention may be administered will be mammals, particularly primates, especially humans. For veterinary applications, a wide variety of subjects will be suitable, e. g. livestock such as cattle, sheep, goats, cows, swine and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, a wide variety of mammals will be suitable subjects including rodents (e.g. mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like. Additionally, for *in vitro* applications, such as *in vitro* diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use such as mammalian,

particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

[0080] In certain methods of the invention the compounds of the invention are excreted from tissues of the body quickly to prevent prolonged exposure to the radiation of the radiolabeled compound administered to the patient. Typically compounds of the invention are eliminated from the body in less than about 24 hours. More typically, compounds of the invention are eliminated from the body in less than about 16 hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes. Exemplary compounds are eliminated in between about 60 minutes and about 120 minutes.

[0081] In some embodiments, the compounds bind strongly to the PSMA protein, for instance by incorporating structural features which reside in an accessory binding site. For example, in compound 3, the 4-iodobenzoyl group resides in a hydrophobic pocket accessory to the S1 binding site (39).

[0082] In certain embodiments, compounds of the invention are stable *in vivo* such that substantially all, e.g., more than about 50%, 60%, 70%, 80%, or 90% of the injected compound is not metabolized by the body prior to excretion. Typical subjects to which compounds of the invention may be administered will be mammals, particularly primates, especially humans. For veterinary applications, a wide variety of subjects will be suitable, e.g. livestock such as cattle, sheep, goats, cows, swine and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, a wide variety of mammals will be suitable subjects including rodents (e.g. mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like. Additionally, for *in vitro* applications, such as *in vitro* diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

[0083] Other embodiments of the invention provide methods of treating tumors comprising administering to a subject a therapeutically effective amount of a compound according to the present invention comprising a therapeutically effective radioisotope. In certain embodiments, the tumor cells may express PSMA, such as prostate tumor cells or metastasized prostate tumor cells. In other embodiments, a tumor may be treated by targeting adjacent or nearby cells which express PSMA. For example, vascular cells undergoing angiogenesis associated with a tumor may be targeted. Essentially all solid tumors express PSMA in the

neovasculature. Therefore, methods of the present invention can be used to treat nearly all solid tumors including lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal and stomach. Also, certain benign lesions and tissues including endometrium, schwannoma and Barrett's esophagus can be treated according to the present invention. Examples of therapeutically effective radioisotopes include ^{131}I and ^{211}At .

[0084] Other embodiments provide kits comprising a compound according to the invention. In certain embodiments, the kit provides packaged pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of the invention. In certain embodiments the packaged pharmaceutical composition will comprise the reaction precursors necessary to generate the compound of the invention upon combination with a radiolabeled precursor. Other packaged pharmaceutical compositions provided by the present invention further comprise indicia comprising at least one of: instructions for preparing compounds according to the invention from supplied precursors, instructions for using the composition to image cells or tissues expressing PSMA, or instructions for using the composition to image glutamatergic neurotransmission in a patient suffering from a stress-related disorder, or instructions for using the composition to image prostate cancer.

[0085] In certain embodiments, a kit according to the invention contains from about 1 to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound of the invention in solution or in lyophilized form, and these components of the kit of the invention may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form. Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

[0086] In certain embodiments, a kit provides a non-radiolabeled precursor to be combined with a radiolabeled reagent on-site. Examples of radioactive reagents include $\text{Na}[^{125}\text{I}]$, $\text{Na}[^{131}\text{I}]$, $\text{Na}[^{123}\text{I}]$, $\text{Na}[^{124}\text{I}]$, $\text{K}[^{18}\text{F}]$, $\text{Na}[^{76}\text{Br}]$, $\text{Na}[^{75}\text{Br}]$, $\text{Na}[^{211}\text{At}]$. Other radiolabeled reagents include activated radiolabeled benzoyl compounds, radiolabeled pyridine carboxylates,

radiolabeled bromomethyl pyridine compounds, and radiolabeled aldehydes discussed previously.

[0087] Imaging agents of the invention may be used in accordance with the methods of the invention by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with PSMA. The spatial distribution may be measured using any means suitable for the particular label, for example, a gamma camera, a PET apparatus, a SPECT apparatus, and the like. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions. A particularly useful imaging approach employs more than one imaging agent to perform simultaneous studies.

[0088] In general, a detectably effective amount of the imaging agent of the invention is administered to a subject. In accordance with the invention, "a detectably effective amount" of the imaging agent of the invention is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent of the invention may be administered in more than one injection. The detectably effective amount of the imaging agent of the invention can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective amounts of the imaging agent of the invention can also vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art. The amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the amount of imaging agent to administer to each individual patient and the duration of the imaging study.

[0089] A "pharmaceutically acceptable carrier" refers to a biocompatible solution, having due regard to sterility, pH, isotonicity, stability, and the like and can include any and all solvents, diluents (including sterile saline, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other aqueous buffer solutions), dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, and the like. The pharmaceutically acceptable carrier may also contain

stabilizers, preservatives, antioxidants, or other additives, which are well known to one of skill in the art, or other vehicle as known in the art.

[0090] As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making non-toxic acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, conventional non-toxic acid salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, mesylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, HOOC-(CH₂)_n-COOH where n is 0-4, and the like. The pharmaceutically acceptable salts of the present invention can be synthesized from a parent compound that contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used, where practicable. Lists of additional suitable salts may be found, e.g., in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985).

[0091] The contents of all cited references (including literature references, issued patents, published patent applications) as cited throughout this application are hereby expressly incorporated by reference. The invention and the manner and process of making and using it, are described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same.

[0092] It is to be understood that the foregoing describes exemplary embodiments of the present invention and that modifications may be made therein without departing from the spirit or scope of the present invention as set forth in the appended claims.

EXAMPLES

[0093] The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The practice of the present invention will employ, unless otherwise indicated, conventional techniques, which are within the skill of the art. Such techniques are explained fully in the literature.

Synthesis

[0094] **General Procedures.** All reagents and solvents were purchased from either Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA). The tosylate salt of PMB-protected Lys-C(O)-Glu (compound 1) was prepared according to a reported procedure (19). ¹H NMR spectra were obtained on a Varian Mercury 400 MHz or a Bruker Avance 400 MHz Spectrometer. ESI mass spectra were obtained on an API 150EX™ or a Bruker Esquire 3000 plus system. High-resolution mass spectra (HRMS) were performed on a JEOL JMS-AX505HA mass spectrometer in the Mass Spectrometry Facility at the University of Notre Dame. HPLC purification of reference compounds was performed on a Waters 625 LC system with a Waters 490E multiwavelength UV/Vis detector (Milford, MA).

[0095] [¹²⁵I]NaI was purchased from MP Biomedicals (Costa Mesa, CA). [¹⁸F]Fluoride was produced by 18 MeV proton bombardment of a high pressure [¹⁸O]H₂O target using a General Electric PETtrace biomedical cyclotron (Milwaukee, WI). Solid-phase extraction cartridges (C₁₈ plus, Sep-Pak) were purchased from Waters Associates. Radioactivity was measured in a Capintec CRC-10R dose calibrator (Ramsey, NJ). The specific radioactivity was calculated as the radioactivity eluting at the retention time of product during the semi-preparative HPLC purification divided by the mass corresponding to the area under the curve of the UV absorption.

EXAMPLE 1

[0096] **2-{3-[5-(4-Iodo-benzoylamino)-1-(4-methoxy-benzyloxycarbonyl)-pentyl]-ureido}-pentanedioic acid bis-(4-methoxy-benzyl) ester (2).** To a solution of 1 (0.126 g, 0.148 mmol) in CH₂Cl₂ (4 mL) was added triethylamine (0.1 mL, 0.712 mmol), followed by *N*-hydroxysuccinimidyl-4-iodobenzoate (24) (0.073 g, 0.212 mmol). After stirring for 2 h at room temperature, the solvent was evaporated on a rotary evaporator. The crude material was purified on a silica column using methanol/methylene chloride (5:95) to afford 0.127g (94%) of 2. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.17-7.26 (m, 6H),

6.77-6.86 (m, 7H), 5.37-5.46 (m, 2H), 4.93-5.09 (m, 6H), 4.32-4.40 (m, 2H), 3.76-3.77 (m, 9H), 3.30-3.33 (m, 2H), 2.30-2.36 (m, 2H), 2.07-2.12 (m, 1H), 1.84-1.92 (m, 1H), 1.70-1.79 (m, 1H), 1.49-1.57 (m, 3H), 1.25-1.33 (m, 2H). ESI-Mass calcd for $C_{43}H_{48}IN_3O_{11}Na$ $[M + Na]^+$ 932.2, found 932.7.

5

EXAMPLE 2

[0097] 2-{3-[1-Carboxy-5-(4-iodo-benzoylamino)-pentyl]-ureido}-pentanedioic acid

(3). A solution of 3% anisole in TFA (15 mL) was added to **2** (0.117 g, 0.129 mmol) at 0°C.

The mixture was stirred at room temperature for 30 min then concentrated on a rotary

10 evaporator. The crude material was purified by HPLC (Econosil C18 10 μ , 250 x 10 mm, H₂O/CH₃CN/TFA (70/30/0.1), 4 mL/min, **3** eluting at 11 min) to afford 0.040 g (57%) of **3**. ¹H NMR (400 MHz, D₂O:CD₃CN = 1:1 (v/v)) δ 7.79 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 4.08-4.16 (m, 2H), 3.26 (m, 2H), 2.35 (m, 2H), 2.00-2.03 (m, 1H), 1.72-1.84 (m, 2H), 1.52-1.62 (m, 3H), 1.34-1.36 (m, 2H). ESI-Mass calcd for $C_{19}H_{24}IN_3O_8Na$ $[M + Na]^+$ 572.1, found 572.0.
15 FAB-HRMS calcd for $C_{19}H_{25}IN_3O_8$ $[M + H]^+$ 550.0686, found 550.0648.

EXAMPLE 3

[0098] 2-{3-[1-(4-Methoxy-benzyloxycarbonyl)-5-(4-tributylstannanyl-

benzoylamino)-pentyl]-ureido}-pentanedioic acid bis-(4-methoxy-benzyl) ester (4). To a

20 solution of **1** (0.120 g, 0.148 mmol) in CH₂Cl₂ (6 mL) was added triethylamine (0.1 ml, 0.712 mmol), followed by *N*-hydroxysuccinimidyl-4-tributylstannylbenzoate (**24**) (0.075 g, 0.147 mmol). After stirring for 2 h at room temperature, the reaction mixture was condensed on a rotary evaporator. The crude material was purified on a silica column using methanol/methylene chloride (5:95) to afford 0.130 g (86%) of **4**. ¹H NMR (400 MHz, CDCl₃) δ 7.68 (d, J = 8.4 Hz,
25 2H), 7.49 (d, J = 7.6 Hz, 2H), 7.18-7.24 (m, 6H), 6.80-6.85 (m, 6H), 6.47 (m, 1H), 5.44-5.47 (m, 2H), 4.95-5.09 (m, 6H), 4.41-4.45 (m, 2H), 3.76-3.77 (m, 9H), 3.32-3.38 (m, 2H), 2.35-2.37 (m, 2H), 2.08-2.16 (m, 1H), 1.90-1.94 (m, 1H), 1.70-1.79 (m, 1H), 1.45-1.64 (m, 9H), 1.24-1.30 (m, 8H), 1.01-1.06 (m, 6H), 0.85-0.87 (m, 9H). ESI-Mass calcd for $C_{55}H_{75}N_3O_{11}SnNa$ $[M + Na]^+$ 1096.4, found 1096.7.

30

EXAMPLE 4

[0099] 2-{3-[5-(4-Fluoro-benzoylamino)-1-(4-methoxy-benzyloxycarbonyl)-pentyl]-ureido}-pentanedioic acid bis-(4-methoxy-benzyl) ester (5). To a solution of **1** (0.120 g,

0.164 mmol) in CH₂Cl₂ (4 mL) was added triethylamine (0.1 mL, 0.712 mmol), followed by *N*-hydroxysuccinimidyl-4-fluorobenzoate (22) (0.043 g, 0.181 mmol). After stirring for 2 h at room temperature, the solvent was evaporated on a rotary evaporator. The crude material was purified by on a silica column using methanol/methylene chloride (5:95) to afford 0.120 g (91%) of **5**. ¹H NMR (400 MHz, CDCl₃) δ 7.78 (m, 2H), 7.16-7.24 (m, 6H), 7.01 (m, 2H), 6.80-6.85 (m, 7H), 5.51-5.64 (m, 2H), 4.93-5.09 (m, 6H), 4.34-4.40 (m, 2H), 3.75-3.77 (m, 9H), 3.28-3.34 (m, 2H), 2.26-2.38 (m, 2H), 2.04-2.15 (m, 1H), 1.82-1.91 (m, 1H), 1.68-1.74 (m, 1H), 1.44-1.57 (m, 3H), 1.25-1.33 (m, 2H). ESI-Mass calcd for C₄₃H₄₈FN₃O₁₁Na [M + Na]⁺ 824.3, found 824.7.

10

EXAMPLE 5

[0100] 2-{3-[1-Carboxy-5-(4-fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid (6).

A solution of 3% anisole in TFA (15 mL) was added to **5** (0.081 g, 0.1 mmol) at 0°C. The mixture was stirred at room temperature for 20 min then concentrated on a rotary

15

evaporator. The crude material was purified by HPLC (Econosil C18 10μm, 250 x 10 mm, H₂O/CH₃CN/TFA (75/25/0.1), 4 mL/min, with purified **6** eluting at about 9 min) to afford 0.035 g (79%) of **6**. ¹H NMR (400 MHz, D₂O) δ 7.66-7.69 (m, 2H), 7.11-7.16 (m, 2H), 4.12-4.19 (m, 2H), 3.28-3.31 (m, 2H), 2.39-2.43 (m, 2H), 2.07-2.09 (m, 1H), 1.79-1.90 (m, 2H), 1.55-1.69 (m, 3H), 1.39-1.40 (m, 2H). ESI-Mass calcd for C₁₉H₂₄FN₃O₈Na [M + Na]⁺ 464.1, found 464.4.

20

FAB-HRMS calcd for C₁₉H₂₅FN₃O₈ [M + H]⁺ 442.1626, found 442.1646.

EXAMPLE 6

[0101] 2-(3-{1-(4-methoxy-benzyloxycarbonyl)-5-[(5-tributylstannanyl-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid bis-(4-methoxy-benzyl) ester (7).

25

To a solution of **1** (0.120 g, 0.148 mmol) in CH₂Cl₂ (2 mL) was added triethylamine (0.1 mL, 0.712 mmol), followed by *N*-hydroxysuccinimidyl-5-(tri-*n*-butylstannyl)-3-pyridinecarboxylate (25) (0.075 g, 0.147 mmol). After stirring for 30 min at room temperature, the crude material was purified on a silica column using methanol/methylene chloride (5:95) to afford 0.115 g (76%) of **7**. ¹H NMR (400 MHz, CDCl₃) δ 8.85 (s, 1H), 8.65 (s, 1H), 8.19 (s, 1H), 7.19-7.24 (m, 6H), 6.81-6.85 (m, 6H), 6.65 (m, 1H), 5.32-5.35 (m, 1H), 5.22-5.25 (m, 1H), 4.96-5.10 (m, 6H), 4.40-4.47 (m, 2H), 3.70-3.77 (m, 9H), 3.34 (m, 2H), 2.35-2.39 (m, 2H), 2.10-2.15 (m, 1H), 1.90-1.94 (m, 1H), 1.72-1.79 (m, 1H), 1.46-1.59 (m, 9H), 1.27-1.36 (m, 8H), 1.02-1.25 (m, 6H), 0.84-0.87 (m, 9H). ESI-Mass calcd for C₅₄H₇₅IN₄O₁₁Sn [M + H]⁺ 1075.4, found 1075.5.

30

EXAMPLE 7

[0102] **2-(3-{1-carboxy-5-[(5-iodo-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid (8).** To a solution of **7** (0.025 g, 0.023 mmol) in 2 mL methanol was added
5 0.020 mL acetic acid and sodium iodide (0.017 g, 0.113 mmol), followed by *N*-chlorosuccinimide (0.025 g, 0.187 mmol). After 20 min at room temperature, the solvent was removed under a stream of nitrogen. A solution of TFA in CH₂Cl₂ (1:1, 2 mL) was then added to the residue. After 1 h at room temperature, **8** (0.008 g, 62%) was isolated by HPLC (Econosphere C18 10μ, 250 x 10 mm, H₂O/CH₃CN/TFA (85/15/0.1), 4 mL/min, product peak
10 eluting at 10 min). ¹H NMR (400 MHz, D₂O) δ 9.00-9.15 (m, 3H), 4.18-4.24 (m, 2H), 3.40-3.41 (m, 2H), 2.45-2.49 (m, 2H), 2.12-2.13 (m, 1H), 1.85-1.97 (m, 2H), 1.64-1.73 (m, 3H), 1.44 (m, 2H). ESI-Mass calcd for C₁₈H₂₄IN₄O₈ [M + H]⁺ 551.1, found 551.0. FAB-HRMS calcd for C₁₈H₂₄IN₄O₈ [M + H]⁺ 551.0639, found 551.0607.

15 EXAMPLE 8

[0103] **2-{3-[1-carboxy-5-(4-[¹²⁵I]iodo-benzoylamino)-pentyl]-ureido}-pentanedioic acid ([¹²⁵I]**3**).** [¹²⁵I]**3** was prepared *via* iododestannylation of the corresponding tri-*n*-butylstannyl precursor **4** followed by deprotection. To a solution of **4** (1 mg, 0.932 μmol) in 0.1 mL methanol was added 0.001 mL acetic acid and [¹²⁵I]NaI, followed by *N*-chlorosuccinimide
20 (0.25 mg, 0.187 μmol) in 0.025 mL of methanol. After stirring at room temperature for 20 min, the solvent was removed under a stream of N₂. A solution of 3% anisole in TFA (0.1 mL) was then added to the residue. After 5 min at room temperature, [¹²⁵I]**3** was isolated by HPLC (Econosil C18 10μ, 250 x 4.6 mm, H₂O/CH₃CN/TFA (72/28/0.1), 1 mL/min). Reverse phase radio-HPLC purification of [¹²⁵I]**3** was performed using a Waters 510 pump, Waters 490E
25 variable wavelength UV/Vis detector at 254 nm and a Bioscan Flow Count PMT radioactivity detector (Washington, DC). The yields of this reaction were 65-80% (three separate determinations). Specific radioactivity was always > 700 Ci/mmol (25.9 GBq/μmol).

EXAMPLE 9

30 [0104] ***N*-Hydroxysuccinimidyl-4-[¹⁸F]fluorobenzoate [¹⁸F]SFB.** *N*-hydroxysuccinimidyl-4-[¹⁸F]iodobenzoate ([¹⁸F]SFB) was prepared by a literature procedure (23) and purified by HPLC (Econosphere C18 10μ, 250 x 10 mm, H₂O/CH₃CN/TFA (75/25/0.1), 5 mL/min, product peak eluting at 19 min).

[0105] **2-{3-[1-carboxy-5-(4-[¹⁸F]fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid ([¹⁸F]6).** In a vial containing 2 mg of 1 and 0.002 mL of Et₃N was added [¹⁸F]SFB in CH₂Cl₂. The reaction mixture was heated at 45°C for 20 min, and then the solvent was removed under a stream of nitrogen. 0.1 mL of 3% anisole/TFA was then added and the reaction mixture was heated at 45°C for 5 min. The final product ([¹⁸F]6) was obtained after HPLC purification (Econosphere C18 10μ., 250 x 10 mm, H₂O/CH₃CN/TFA [80/20/0.1], 4 mL/min). The decay-corrected yields of [¹⁸F]6 were 30-35%, based on starting [¹⁸F]fluoride (three separate determinations). The mean synthesis time was 180 min from the time of addition of [¹⁸F]fluoride. Starting from 40 mCi [¹⁸F]fluoride, the specific radioactivity of [¹⁸F]6 was found to be 250-300 Ci/mmol (9.1 - 11.1 GBq/μmol).

EXAMPLE 10

[0106] **2-(3-{1-carboxy-5-[(5-[¹²⁵I]iodo-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid ([¹²⁵I]8).** [¹²⁵I]8 was prepared using iododestannylation of the corresponding tri-*n*-butylstannyl precursor 7 followed by deprotection. To a solution of 7 (0.05 mg, 0.047 μmol) in 0.05 mL of methanol was added 0.002 mL acetic acid, [¹²⁵I]NaI, followed by *N*-chlorosuccinimide (0.1 mg, 0.749 μmol) in 0.010 mL of methanol. After 20 min at room temperature, the solvent was removed under a stream of nitrogen. A solution of 3% anisole in TFA (0.1 mL) was then added to the residue. After 5 min at room temperature, [¹²⁵I]8 was isolated by HPLC (Econosil C18 10μ., 250 x 4.6 mm, H₂O/CH₃CN/TFA [85/15/0.1], 1 mL/min). Reverse phase radio-HPLC purification of [¹²⁵I]8 was performed using a Waters 510 pump, Waters 490E variable wavelength UV/Vis detector at 354 nm and a Bioscan Flow Count PMT radioactivity detector (Washington DC). The yields of this reaction were 59-75% (three separate determinations). Specific radioactivity was > 2,000 Ci/mmol (74.0 GBq/μmol) in each case.

EXAMPLE 11

[0107] **Synthesis of 2-(3-{1-carboxy-5-[2-(4-fluoro-benzylideneaminoxy)-acetyl-amino]-pentyl}-ureido)-pentanedioic acid (9).** To a solution of compound 1 (0.062 g, 0.073 mmol) in CH₂Cl₂ (2 mL) was added triethylamine (0.045 mL, 0.320 mmol), followed by *N*-*tert*-butyloxycarbonyl-O-(carboxymethyl)hydroxyamine hydroxysuccinimidyl ester (0.025 g, 0.087 mmol, *Bioconjugate Chemistry* 1993, 4, 515-20). After stirring for 30 min at room temperature, the solvent was evaporated. The crude material was purified by a silica column

using methanol/methylene chloride (5:95) to afford 0.055 g (89%) of compound **10**. ¹H NMR (400 MHz, CDCl₃) δ 7.98 (bs, 1H), 7.91 (s, 1H), 7.25-7.27 (m, 6H), 6.85-6.88 (m, 6H), 5.56-5.63 (m, 2H), 5.01-5.11 (m, 6H), 4.47-4.53 (m, 1H), 4.27-4.38 (m, 3H), 3.79 (m, 9H), 3.30-3.38 (m, 1H), 3.15-3.21 (m, 1H), 2.36-2.41 (m, 2H), 2.10-2.15 (m, 1H), 1.89-1.95 (m, 1H), 1.74-1.81 (m, 1H), 1.23-1.61 (m, 14H). ESI-Mass calcd for C₄₃H₅₇N₄O₁₄ [M + H]⁺ 853.4, found 853.5.

[0108] A solution of 3% anisole in TFA (1 mL) was added to compound **10** (0.031 g, 0.036 mmol). The mixture was stirred at room temperature for 5 min, then concentrated on a rotary evaporator. The crude material was purified by HPLC (Econosil C18 10μ, 250 × 10 mm, H₂O/CH₃CN/TFA (90/10/0.1), 4 mL/min) to afford 0.009 g (49%) of compound **11**. ¹H NMR (400 MHz, D₂O) δ 4.68 (s, 2H), 4.28-4.35 (m, 2H), 3.34 (m, 2H), 2.58 (m, 2H), 2.24 (m, 1H), 1.78-2.13 (m, 3H), 1.62 (m, 2H), 1.49 (m, 2H). ESI-Mass calcd for C₁₄H₂₅N₄O₉ [M]⁺ 393.2, found 393.3.

[0109] To a solution of compound **11** (0.005 g, 0.010 mmol) in methanol (0.3 mL) was added triethylamine (0.0075 ml, 0.05 mmol), followed by 4-fluorobenzaldehyde (0.0017 ml, 0.016 mmol). After 30 min at room temperature, the reaction mixture was purified by HPLC (Econosil C18 10μ, 250 × 10 mm, H₂O/CH₃CN/TFA (75/25/0.1) and compound **9** (0.002 g, 41%) was obtained. ¹H NMR (400 MHz, D₂O : CD₃CN = 1:1) δ 8.26 (s, 1H), 7.56-7.80 (m, 2H), 7.10-7.14 (m, 2H), 4.53 (s, 2H), 4.13-4.17 (m, 1H), 3.96-4.00 (m, 1H), 3.16 (m, 2H), 2.37 (m, 2H), 2.10-2.16 (m, 1H), 1.80-1.88 (m, 2H), 1.65 (m, 1H), 1.54 (m, 1H), 1.42 (m, 1H), 1.28 (m, 1H). ESI-Mass calcd for C₂₁H₂₇F₄O₉Na [M + Na]⁺ 521.2, found 521.3. FAB-HRMS calcd for C₂₁H₂₈FN₄O₉ [M + H]⁺ 499.1840, found 499.1869.

EXAMPLE 12

[0110] **Synthesis of 2-(3-{1-carboxy-5-[2-(4-[¹⁸F]fluoro-benzylideneaminoxy)-acetylaminol]-pentyl}-ureido)-pentanedioic acid ([¹⁸F]**9**).** 4-[¹⁸F]fluorobenzaldehyde was synthesized by using literature procedure (*Nuclear Medicine and Biology* 19 (1992) 275–281) and purified by HPLC (H₂O/CH₃CN/TFA 70/30/0.1). The HPLC eluent of 4-[¹⁸F]fluorobenzaldehyde was diluted with H₂O, passed through a C18 Sep Pak, eluted with 2 mL of methanol. To a solution of compound **11** (1 mg) in methanol (0.05 mL) was added [¹⁸F]4-fluorobenzaldehyde in 2 mL methanol. After stirred 30 min at room temperature, the reaction mixture was purified by HPLC to give compound [¹⁸F]**9**. The radiochemical yield (decay corrected and based on starting [¹⁸F]fluoride, n = 2) was 6-9%. The specific activity of final compound was found to be 350-1300 mCi/μmol.

EXAMPLE 13

- [0111] **Synthesis of 2-[3-(1-carboxy-5-{4-[N'-(4-fluoro-benzylidene)-hydrazino]-benzoylamino}-pentyl)-ureido]-pentanedioic acid (12).** To a solution of compound **1** (0.030 g, 0.035 mmol) in CH₂Cl₂ (2 mL) was added triethylamine (0.020 mL, 0.142 mmol), followed by succinimidyl 4-[2-(*tert*-butoxycarbonyl)hydrazino benzoate (0.020 g, 0.057 mmol, *Bioconjugate Chem.* **1991**, 2, 333-336) in DMF (0.2 mL). After stirred for 1 hour at room temperature, the solvent was evaporated. The crude material was purified by a silica column using methanol/methylene chloride (5:95) to afford 0.025 g (78%) of compound **13**. ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, *J* = 8.0 Hz, 2H), 7.19-7.23 (m, 6H), 6.81-6.85 (m, 6H), 6.68 (d, *J* = 8.8 Hz, 2H), 6.61 (s, 2H), 6.15 (bs, 1H) 5.68 (m, 2H), 4.95-5.07 (m, 6H), 4.34-4.45 (m, 2H), 3.74 (m, 9H), 3.25 (m, 2H), 2.31 (m, 2H), 2.10 (m, 1H), 1.84 (m, 1H), 1.19-1.74 (m, 14H). ESI-Mass calcd for C₄₈H₅₉N₅O₁₃Na [M + Na]⁺ 936.4, found 935.9.
- [0112] A solution of TFA/CH₂Cl₂ 1:1 (2 mL) was added to compound **13** (0.025 g, 0.027 mmol). The mixture was stirred at room temperature for 1 hour, then concentrated on a rotary evaporator. The crude material was purified by HPLC (Econosphere C18 10μ, 250 × 10 mm, H₂O/CH₃CN/TFA (92/8/0.1), 4 mL/min) to afford 0.010 g (64%) of compound **14**. ¹H NMR (400 MHz, D₂O) δ 7.72(d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 4.15-4.23 (m, 2H), 3.35 (m, 2H), 2.46(m, 2H), 2.10-2.17 (m, 1H), 1.80-1.95 (m, 2H), 1.59-1.74 (m, 3H), 1.45 (m, 2H). ESI-Mass calcd for C₁₉H₂₈N₅O₈ [M + H]⁺ 454.2, found 453.9.
- [0113] To a solution of compound **14** (0.004 g, 0.009 mmol) in water (0.030 mL) was added 0.1 mL of 50 mM KH₂PO₄, followed by 4-fluorobenzaldehyde (0.0011 g, 0.009 mmol) in 0.05 mL acetonitrile. The reaction mixture was heated at 90°C for 10 min, then purified by HPLC (Econosphere C18 10μ, 250 × 10 mm, H₂O/CH₃CN/TFA (65/35/0.1) and compound **12** (0.003 g, 76%) was obtained. ¹H NMR (400 MHz, D₂O:CD₃CN = 3:2) δ 7.82 (s, 1H), 7.64 (m, 4H), 7.11 (m, 4H), 4.14 (m, 2H), 3.24 (m, 2H), 2.01 (m, 2H), 1.94 (m, 1H), 1.80 (m, 2H), 1.52-1.63 (m, 3H), 1.35 (m, 2H). ESI-Mass calcd for C₂₆H₃₁FN₅O₈ [M + H]⁺ 560.2, found 560.1.

EXAMPLE 14

- [0114] **Synthesis of 2-[3-(1-carboxy-5-{4-[N'-(4-[¹⁸F]fluoro-benzylidene)-hydrazino]-benzoylamino}-pentyl)-ureido]-pentanedioic acid (¹⁸F)12).** 4-[¹⁸F]fluorobenzaldehyde was synthesized by using a known procedure (Nuclear Medicine and Biology 33 (2006) 677-683). To the crude DMSO solution of 4-[¹⁸F]fluorobenzaldehyde was added 1-2 mg of compound **14**,

0.2 mL of 50 mM KH_2PO_4 . The vial was closed and heated at 90°C for 15 min. The reaction mixture was then purified by HPLC (Ecomosphere C18 10μ , 250×10 mm, $\text{H}_2\text{O}/\text{CH}_3\text{CN}/\text{TFA}$ [70/30/0.1], 4 mL/min). The radiochemical yield (decay corrected and based on starting [^{18}F] fluoride, $n = 2$) was 30-55%. The compound **12** decomposed soon after been made. The specific activity of final compound was not determined.

EXAMPLE 15

[0115] Synthesis of 2-{3-[1-carboxy-5-({6-[N'-(4-fluoro-benzylidene)-hydrazino]-pyridine-3-carbonyl}-amino)-pentyl]-ureido}-pentanedioic acid (15). To a solution of

compound **1** (0.040 g, 0.047 mmol) in CH_2Cl_2 (2 mL) was added triethylamine (0.020 mL, 0.14 mmol), followed by succinimidyl 6-(N'-tert-butoxycarbonyl-hydrazino)-nicotinate (0.020 g, 0.057 mmol, *Bioconjugate Chem.* **1991**, 2, 333-336). After stirred for 1 hour at room temperature, the solvent was evaporated. The crude material was purified by a silica column using methanol/methylene chloride (10:90) to afford 0.032 g (74%) of compound **16**. ^1H NMR (400 MHz, CDCl_3) δ 8.54 (s, 1H), 7.90 (m, 1H), 7.17-7.23 (m, 6H), 6.90-7.05 (m, 3H), 6.79-6.84 (m, 6H), 6.55 (d, $J = 8.8$ Hz, 2H), 5.79 (m, 2H), 4.94-5.07 (m, 6H), 4.38-4.45 (m, 2H), 3.74 (m, 9H), 3.26 (m, 2H), 2.33 (m, 2H), 2.07 (m, 1H), 1.85 (m, 1H), 1.68 (m, 1H), 1.18-1.55 (m, 13H). ESI-Mass calcd for $\text{C}_{47}\text{H}_{59}\text{N}_6\text{O}_{13}$ $[\text{M} + \text{H}]^+$ 915.4, found 914.9.

[0116] A solution of TFA/ CH_2Cl_2 1:1 (2 mL) was added to compound **16 (0.032 g, 0.035 mmol). The mixture was stirred at room temperature for 1 hour, then concentrated on a rotary evaporator. The crude material was purified by HPLC (Econosphere C18 10μ , 250×10 mm, $\text{H}_2\text{O}/\text{CH}_3\text{CN}/\text{TFA}$ (92/8/0.1), 4 mL/min) to afford 0.009 g (45%) of compound **17**. ^1H NMR (400 MHz, D_2O) δ 8.07-8.40 (m, 2H), 7.00-7.13 (m, 1H), 4.18-4.24 (m, 2H), 3.38 (m, 2H), 2.48 (m, 2H), 2.14 (m, 1H), 1.86-1.98 (m, 2H), 1.62-1.65 (m, 3H), 1.44 (m, 2H). ESI-Mass calcd for $\text{C}_{18}\text{H}_{27}\text{N}_6\text{O}_8$ $[\text{M} + \text{H}]^+$ 455.2, found 455.0.**

[0117] To a solution of compound **17 (0.005 g, 0.0011 mmol) in water (0.030 mL) was added 50 mM KH_2PO_4 0.1 mL, followed by 4-fluorobenzaldehyde (0.002 g, 0.0016 mmol) in 0.05 mL acetonitrile. The reaction mixture was heated at 90°C for 10 min, then purified by HPLC (Econosphere C18 10μ , 250×10 mm, $\text{H}_2\text{O}/\text{CH}_3\text{CN}/\text{TFA}$ (80/20/0.1) and compound **15** (0.002 g, 41%) was obtained. ^1H NMR (400 MHz, D_2O) δ 8.38 (m, 1H), 8.22 (m, 2H), 7.83 (m, 2H), 7.20 (m, 3H), 4.26 (m, 2H), 3.41 (m, 2H), 2.52 (m, 2H), 2.11 (m, 1H), 1.92 (m, 2H), 1.73 (m, 3H), 1.47 (m, 2H). ESI-Mass calcd for $\text{C}_{25}\text{H}_{30}\text{FN}_6\text{O}_8$ $[\text{M} + \text{H}]^+$ 561.2, found 560.9.**

EXAMPLE 16

[0118] **Synthesis of 2-{3-[1-carboxy-5-({6-[N'-(4-[¹⁸F]fluoro-benzylidene)-hydrazino]-pyridine-3-carbonyl)-amino)-pentyl]-ureido}-pentanedioic acid ([¹⁸F]15)**

[0119] 4-[¹⁸F]fluorobenzaldehyde was synthesized by using a known procedure (Nuclear Medicine and Biology 33 (2006) 677–683). To the crude DMSO solution of 4-[¹⁸F]fluorobenzaldehyde was added 1–2 mg of compound **16**, 0.2 mL of 50 mM KH₂PO₄. The vial was closed and heated at 90°C for 15 min. The reaction mixture was then purified by HPLC (Ecomosphere C18 10μ, 250 × 10 mm, H₂O/CH₃CN/TFA [80/20/0.1], 4 mL/min). The radiochemical yield (decay corrected and based on starting [¹⁸F]fluoride, n = 1) was 49%.

EXAMPLE 17

In Vitro Binding.

[0120] **NAALADase Assay.** NAAG hydrolysis was performed essentially as described previously (26)(27). Briefly, LNCaP cell extracts were prepared by sonication in NAALADase buffer (50 mM Tris [pH 7.4] and 0.5% Triton X-100). Cell lysates were incubated with or without inhibitor at 37°C for 10 min. Following the incubation the radiolabeled substrate *N*-acetyl-L-aspartyl-L-(3,4-³H)glutamate (NEN Life Science Products, Boston, MA) was added to a final concentration of 30 nM at 37°C for 10–15 min. The reaction was stopped by the addition of an equal volume of ice-cold 100 mM sodium phosphate and 2 mM EDTA. Products were partitioned by AG 1-X8 formate resin (Bio-Rad Laboratories, Hercules, CA) anion exchange chromatography, eluted with 1 M sodium formate, and quantified by liquid scintillation counting. Inhibition curves were determined using semi-log plots and IC₅₀ values determined at the concentration at which enzyme activity was inhibited by 50%. Assays were performed in triplicate with the entire inhibition study being repeated at least once to confirm affinity and mode of inhibition. Data were collected during the linear phase of hydrolysis (i.e., < 20% cleavage of total substrate). Enzyme inhibitory constants (K_i values) were generated using the Cheng-Prusoff conversion (28). Data analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California).

[0121] PSMA activity was also determined using a fluorescence-based assay according to a previously reported procedure (29). Briefly, lysates of LNCaP cell extracts were incubated with inhibitor in the presence of 4 μM NAAG. The amount of reduced glutamate was measured by incubating with a working solution of the Amplex Red glutamic acid kit (Molecular Probes Inc., Eugene, OR, USA). The fluorescence was determined by reading with a VICTOR3V

multilabel plate reader (Perkin Elmer Inc., Waltham, MA, USA) with excitation at 490 nm and emission at 642 nm.

[0122] The NAALADase inhibition assay was undertaken to determine the K_i value for **3**, **6** and **8** (26). The concentration of each compound was varied from 0.01 nM to 1000 nM against a fixed amount of [^3H]NAAG (30 nM). The NAALADase (PSMA) was prepared from LNCaP cell lysates. The percentage of the enzymatic cleavage product, [^3H]glutamate, produced was measured by scintillation counting and was plotted against the logarithmic concentration of the compound under study. Linear regression of the resulting data were solved for 50% [^3H]glutamate production (50% inhibition) and resulted in K_i values of 0.010 nM for **3**, 0.256 nM for **6** and 0.351 nM for **8** (Table 1). That result is in keeping with other compounds of this class (30). When these compounds were evaluated through a fluorescence-based inhibition assay as a second check on affinity, K_i values of **3**, **6**, and **8** were 0.010, 0.194, and 0.557 nM, respectively.

15 **Table 1.** PSMA *in vitro* Inhibitory Activities and Calculated ClogD values

compd	K_i [nM] ^a	SD ^b	K_i [nM] ^c	SD ^b	ClogD
3	0.010	0.003	0.010	0.004	-5.16
6	0.256	0.038	0.194	0.134	-5.64
8	0.351	0.257	0.557	0.265	-5.88

^aobtained from the NAALADase (radiometric) assay. ^b95% confidence interval. ^cobtained from a fluorescence-based assay.

EXAMPLE 18 - Modeling of Inhibitors in the Active Site of PSMA

20 [0123] **Protein Preparation.** The 3-D coordinates of GCPII for docking studies were prepared as GCPII crystal structures in complex with 2-PMPA (PDB ID: 2PVW) or compound **3** (PDB ID:3D7H) through a clean-up process implemented in Discovery Studio 2.0 (DS 2.0), which can correct for structural disorder, fix bond order and connectivity of amino acid residues. The CHARMM forcefield that was applied to the protein and the binding site for docking studies was obtained through an automated method by using the option of finding sites from receptor cavities. Two zinc ions and one chloride ion in the active site were typed as Zn^{2+} and Cl^- , with formal charges of +2 and -1, respectively.

25 [0124] **Docking Studies with CDOCKER.** Docking studies of compounds **3**, **6** and **8** were performed with two conformers of 2PVW using the CDOCKER module implemented in

DS 2.0 by modifying the default settings (Top hits: 20, random conformations: 20, random conformation dynamics steps: 1000, random conformations dynamics target temperature: 1000, orientation to refine: 20, maximum bad orientations: 800, orientation vdW energy threshold: 300, simulation heating steps: 2000, heating target temperature: 700, cooling steps: 5000, cooling target temperature: 300, Grid extension: 8, ligand partial charge: CHARMM). The best pose of each ligand with high CDOCKER energy was used for generating the overlay structures (Figure 1 and 2) with crystal ligand **3** (shown in lighter color) from GCPII complex (PDB ID: 3D7H). Seven water molecules within 3Å from crystal ligand **3** were included for docking studies with 3D7H.

10 **[0125]** The PSMA crystal structure complexed with **3**, which was deposited in the protein data bank (PDB ID: 3D7H) was resolved. Details of PSMA co-crystallized with **3** as well as with other urea-based PSMA inhibitors such as DCIT, DCMC and DCFBC, are described by Barinka et al. (39). As predicted from modeling studies, only the binding conformation of the arginine patch region was found in the PSMA complex with **3**. To elucidate
15 potential binding modes of the other two compounds (**6** and **8**), docking studies using the 3-D coordinates of 3D7H in the presence or in the absence of water molecules in the active site were carried out. The best poses of **3**, **6** and **8** from the docking studies using the CDOCKER module are shown in Figure 1, overlaid with crystal ligand, i.e., the compound as it is co-crystallized with PSMA, of **3**. As shown in Figure 1, all of the radionuclide-bearing moieties (4-iodophenyl,
20 4-fluorophenyl, and 5-iodo-3-pyridyl) were located within the arginine patch of the S1 binding site. 4-Iodophenyl and 4-fluorophenyl groups protruded deeply within the subpocket compared to the 5-iodo-3-pyridyl moiety. CDOCKER scores of the three poses are ordered **3** (80.63) > **6** (72.39) > **8** (69.78). Aromatic rings of **3**, **6**, and **8** provide π - π interactions with the guanidine functions of Arg 463 and Arg 534, leading to stabilization of the ligand within the subpocket. In
25 the case of **8**, the nitrogen of the pyridine ring enables electrostatic interaction with the carboxylate of Asp 465 and hydrogen bonding with one water molecule. Docking results of PSMA without water molecules in the active site showed that the radionuclide-bearing moieties of **6** and **8** were outside of the subpocket and projected into the tunnel region (Figure 2) while the 4-iodophenyl group of **3** was projected into the subpocket. Based on *in vitro* PSMA
30 inhibitory activities and molecular modeling studies, it appears that ligand interaction with the subpocket of the S1 binding site contributes more to binding affinity than interaction with the tunnel region.

[0126] Not surprisingly, **3**, **6** and **8** assume similar conformations within the PSMA active site. The radionuclide-bearing moiety resides within the arginine patch region of the S1' binding site in each case, however, that of **8** is not as deep within the pocket (Figure 1). Compound **3** has been co-crystallized with PSMA, and the binding conformation for that compound shows productive π - π stacking with Arg 463 and Arg 534 of the enzyme, whereas the pyridine moiety of **8** is unable to form a similarly productive π - π interaction. However, unlike **3** or **6**, **8** is able to interact with Asp 465 (*via* the pyridine nitrogen), with Asp 453 (*via* the carbonyl oxygen) and a water molecule because the carbonyl group of the radionuclide-bearing moiety points toward the S1 subpocket. Those additional interactions likely offset the less productive π - π bonding geometry of **8**, providing a high-affinity interaction and an imaging agent that gives clear delineation of tumor (Figure 5). While **6** adopts a very similar conformation within the active site as **3**, it binds with significantly lower affinity, likely due to additional interactions of the iodine within the positively-charged arginine patch for **3**. In all, the affinities of **3**, **6** and **8** (Table 1) track with predications based on molecular modeling.

Biodistribution and Imaging

[0127] **Cell Lines and Mouse Models:** PC-3 PIP (PSMA⁺) and PC-3 flu (PSMA⁻) cell lines were obtained from Dr. Warren Heston (Cleveland Clinic) and were maintained as previously described (19). All cells were grown to 80 - 90% confluence before trypsinization and formulation in Hank's Balanced Salt Solution (HBSS, Sigma, St. Louis, MO) for implantation into mice.

[0128] All animal studies were carried out in full compliance with institutional guidelines related to the conduct of animal experiments. Male severe-combined immunodeficient (SCID) mice (Charles River Laboratories, Wilmington, MA) were implanted subcutaneously with 1 - 5 x 10⁶ cells forward of each shoulder. PC-3 PIP cells were implanted behind the left shoulder and PC-3 flu cells were implanted behind the right shoulder. Mice were imaged or used in biodistribution assays when the tumor xenografts reached 3 - 5 mm in diameter.

***Ex Vivo* Biodistribution and Imaging**

EXAMPLE 19

[0129] **Compound [¹²⁵I]**3**.** Xenograft-bearing SCID mice were injected via the tail vein with 74 Bq (2 μ Ci) of [¹²⁵I]**3**. Four mice each were sacrificed by cervical dislocation at 30, 60,

120, 300 min, 12, 24 and 48 hours p.i. The heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, muscle, small and large intestines, urinary bladder and PIP and flu tumors were quickly removed. A 0.1 mL sample of blood was also collected. The organs were weighed and the tissue radioactivity measured with an automated gamma counter (1282 Compugamma CS, Pharmacia/LKB Nuclear, Inc, Gaithersburg, MD). The percent-injected dose per gram of tissue (% ID/g) was calculated by comparison with samples of a standard dilution of the initial dose. All measurements were corrected for decay.

[0130] Table 2 outlines the *ex vivo* rodent tissue distribution results of [¹²⁵I]3. The blood, kidney, urinary bladder, spleen and PSMA⁺ PC-3 PIP tumor display high uptake at the initial, 30 min postinjection (p.i.) time point. By 60 min p.i., the urinary bladder displays the highest uptake while the uptake in PSMA⁺ PC-3 PIP tumor also achieves its highest absolute value. The kidney achieves its maximal uptake at 24 h p.i. The values noted in the kidney are largely due to specific binding rather than renal clearance, due to the expression of high amounts of PSMA in the proximal renal tubule (31)(32). Urinary bladder uptake represents excretion at all time points, i.e., there was no specific binding to bladder wall. Tumor uptake demonstrates a high degree of specificity represented by the PIP:flu ratio of 10:1 at 60 min and rising to 140:1 at 48 h. Radiopharmaceutical uptake within tumor relative to other organs also increases with time. Site-specific blockade studies were not performed as they were perceived to be redundant in light of the use of engineered (PC-3 PIP and PC-3 flu) tumors to determine binding specificity.

Table 2. *Ex vivo* biodistribution of [¹²⁵I]3 in tumor-bearing mice.^{a,b}

Organ	30 min	60 min	2 h	5 h	12 h	24 h	48 h
blood	0.9 ± 0.8 (10)	0.6 ± 0.2 (22)	0.3 ± 0.1 (36)	0.4 ± 0.2 (31)	0.1 ± 0.03 (125)	ND	ND
heart	2.7 ± 0.9 (3)	1.9 ± 0.3 (7)	1.4 ± 0.7 (8)	0.9 ± 0.2 (14)	0.5 ± 0.2 (25)	0.5 ± 0.2 (28)	0.1 ± 0.1 (100)
lung	5.8 ± 2.4 (1.5)	4.5 ± 0.5 (3)	3.6 ± 2.8 (3)	3.5 ± 0.6 (3.5)	2.6 ± 0.8 (5)	1.1 ± 0.8 (12)	0.5 ± 0.04 (22)
liver	7.7 ± 3.1 (1)	7.5 ± 0.8 (2)	5.9 ± 3.5 (2)	4.5 ± 1.0 (3)	1.4 ± 0.2 (9)	1.4 ± 0.6 (9)	0.7 ± 0.1 (16)
stomach	1.5 ± 0.9 (6)	1.5 ± 0.3 (9)	1.6 ± 1.5 (8)	0.8 ± 0.03 (15)	0.3 ± 0.06 (39)	0.7 ± 0.6 (18)	0.4 ± 0.2 (25)
pancreas	2.0 ± 0.3 (4)	2.3 ± 0.3 (6)	2.0 ± 0.7 (6)	1.6 ± 0.5 (8)	0.6 ± 0.2 (21)	1.2 ± 0.7 (11)	1.1 ± 0.6 (10)
spleen	83 ± 8 (0.1)	141 ± 14 (0.1)	104 ± 43 (0.1)	119 ± 9 (0.1)	69 ± 39 (0.2)	39 ± 6 (0.3)	22 ± 8.6 (0.5)
fat	4.5 ± 1.1 (2)	5.6 ± 1.0 (2)	6.2 ± 0.8 (2)	6.8 ± 1.3 (2)	3.8 ± 0.8 (3)	1.6 ± 1.9 (8)	2.8 ± 0.7 (4)

Organ	30 min	60 min	2 h	5 h	12 h	24 h	48 h
kidney	119 ± 15 (0.1)	121 ± 17 (0.1)	111 ± 34 (0.1)	132 ± 12 (0.1)	169 ± 29 (0.1)	234 ± 140 (0.1)	101 ± 30 (0.1)
muscle	2.7 ± 2.4 (3)	0.8 ± 0.2 (17)	0.6 ± 0.2 (21)	0.4 ± 0.1 (31)	1.0 ± 0.1 (12.5)	0.4 ± 0.1 (33)	0.25 ± 0.03 (44)
small int.	4.9 ± 1.9 (2)	3.8 ± 0.4 (3.5)	1.5 ± 0.3 (8)	1.5 ± 0.2 (8)	1.0 ± 0.4 (12.5)	0.25 ± 0.1 (54)	0.1 ± 0.1 (110)
large int.	1.4 ± 0.6 (6)	1.0 ± 0.2 (13.5)	0.6 ± 0.1 (21)	0.7 ± 0.6 (17)	0.6 ± 0.2 (21)	1.6 ± 1.9 (9)	0.15 ± 0.1 (73)
bladder	5.2 ± 1.7 (2)	6.1 ± 0.8 (2)	4.0 ± 2.6 (3)	3.0 ± 1.7 (4)	0.8 ± 0.3 (16)	0.2 ± 0.2 (64)	0.3 ± 0.04 (37)
PC-3 PIP	8.8 ± 4.7	13.5 ± 2.1	11.8 ± 5.6	12.4 ± 6.4	12.5 ± 4.8	13.4 ± 5.1	11.0 ± 0.2
PC-3 flu	1.8 ± 1.0	1.2 ± 0.3	0.7 ± 0.3	0.6 ± 0.05	0.3 ± 0.1	0.1 ± 0.06	0.08 ± 0.06
PIP:flu	5	11	18	19	48	131	140

a. Values are in % ID/g ± SD; ND = not determined; n = 4 except at 48 h where n = 3. Int. = intestine.

b. Pip tumor:organ ratios are in parentheses

5 EXAMPLE 20

[0131] Compound [¹⁸F]6. *Ex vivo* biodistribution proceeded as for [¹²⁵I]3 with the following exceptions: Mice were injected with 3.7 MBq (100 [μCi] of [¹⁸F]6 and uptake times were 30, 60, 120 and 300 minutes p.i.

[0132] Table 3 illustrates tissue uptake of [¹⁸F]6. This radiopharmaceutical also displayed rapid, specific uptake within PSMA⁺ PIP tumors (8.58 ± 3.09% ID/g at 30 min. p.i.) and kidney (72.05 ± 3.19% ID/g at 30 min. p.i.). Uptake in and washout from nonspecific tissues was low and fast, respectively. Only the liver and spleen display uptake values that rival those seen in PIP tumor. The spleen exhibits the highest uptake of any nonspecific tissue (12.67 ± 0.36% ID/g at 30 min. p.i.), perhaps due to the presence of GCPIII, a close homolog of GCPII/PSMA (33). It is speculated that [¹⁸F]6 may bind to GCPIII as well as to GCPII, as several other PSMA ligands have been shown to do so (34).

Table 3. *Ex vivo* biodistribution of [¹⁸F]6 in tumor-bearing mice.^{a,b}

Organ	30 min	60 min	2 h	5 h
blood	2.5 ± 1.7 (3)	0.7 ± 0.5 (9)	0.4 ± 0.2 (9)	0.03 ± 0.00 (117)
heart	0.8 ± 0.1 (11)	0.2 ± 0.02 (35)	0.15 ± 0.05 (25)	0.03 ± 0.01 (117)
lung	1.7 ± 0.3 (5)	0.5 ± 0.1 (13)	1.0 ± 0.9 (4)	0.1 ± 0.1 (35)
liver	8.7 ± 1.8 (1)	5.8 ± 0.6 (1)	11.7 ± 7.0 (0.3)	1.0 ± 0.6 (3.5)
stomach	0.8 ± 0.1	0.25 ± 0.1	0.3 ± 0.1	0.04 ± 0.02

Organ	30 min	60 min	2 h	5 h
	(11)	(26)	(13)	(88)
pancreas	0.8 ± 0.1 (11)	0.3 ± 0.1 (21)	0.15 ± 0.03 (25)	0.05 ± 0.03 (70)
spleen	12.7 ± 0.4 (0.7)	7.2 ± 1.6 (1)	4.4 ± 1.2 (1)	1.0 ± 0.6 (3.5)
kidney	72 ± 3 (0.1)	48 ± 5 (0.1)	29 ± 12 (0.1)	14 ± 9 (0.3)
muscle	2.4 ± 3.1 (4)	0.5 ± 0.7 (13)	0.2 ± 0.1 (19)	0.1 ± 0.1 (35)
small int.	1.7 ± 0.7 (5)	0.6 ± 0.2 (11)	0.3 ± 0.2 (12)	0.1 ± 0.04 (35)
large int.	1.2 ± 0.8 (7)	0.5 ± 0.1 (13)	0.2 ± 0.1 (19)	0.6 ± 0.6 (6)
bladder	6.8 ± 3.5 (1)	17 ± 21 (0.4)	11 ± 8 (0.3)	5.2 ± 8.9 (0.7)
PC-3 PIP	8.6 ± 3.1	6.4 ± 0.9	3.7 ± 1.2	3.5 ± 2.3
PC-3 flu	0.8 ± 0.3	0.3 ± 0.05	0.25 ± 0.1	0.1 ± 0.1
PIP:flu	11	21	15	35

a. Values are in % ID/g ± SD, n = 4, int. = intestine.

b. Pip tumor:organ ratios are in parentheses.

EXAMPLE 21

5 **[0133]** **Compound [¹²⁵I]8.** PC-3 PIP and PC-3 flu xenograft-bearing SCID mice were injected via the tail vein with 74 KBq (2 µCi) of [¹²⁵I]8. Four mice each were sacrificed by cervical dislocation at 30, 60, 240 min., 8 and 24 hours p.i. The heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, muscle, small and large intestines, urinary bladder and PIP and flu tumors were quickly removed. A 0.1 mL sample of blood was also collected. All the organs
10 were weighed and the tissue radioactivity measured with an automated gamma counter (1282 Compugamma CS, Pharmacia/LKB Nuclear, Inc, Gaithersburg, MD). The %ID/g was calculated by comparison with samples of a standard dilution of the initial dose. All measurements were corrected for decay.

[0134] Table 4 outlines the *ex vivo* rodent tissue distribution results of [¹²⁵I]8. The liver, spleen, kidney and PSMA⁺ PC-3 PIP tumor displayed high uptake at the initial, 30 min p.i. time point. By 60 min p.i., the kidney displayed the highest uptake while that in PSMA⁺ PC-3 PIP tumor remained steady, showing a similar value to that at 30 min. By 24 h, there was complete clearance of radioactivity from the individual, nontarget organs. The values noted in the kidney are largely due to specific binding rather than renal clearance, as for the other
15 radiopharmaceuticals discussed above. Urinary bladder uptake represented excretion at all time points, i.e., there was no specific binding to bladder wall, while tumor uptake demonstrated a
20

high degree of specificity represented by the PIP:flu uptake ratio of 18:1 at 30 min, rising to 48:1 at 24 h. As for [^{125} I]**3**, the radiopharmaceutical uptake within tumor relative to other organs increases with time.

5 **Table 4.** *Ex vivo* biodistribution of [^{125} I]**8** in tumor-bearing mice.^{a,b}

Organ	30 min	60 min	4 h	8 h	24 h
blood	2.5 ± 1.4 (6)	1.1 ± 0.5 (11)	0.25 ± 0.2 (17)	0.06 ± 0.01 (23)	0.00 ± 0.00
heart	0.9 ± 0.2 (16)	0.4 ± 0.3 (30)	0.06 ± 0.02 (20)	0.02 ± 0.01 (70)	0.00 ± 0.00
lung	2.7 ± 3.2 (5)	2.3 ± 1.0 (5)	0.3 ± 0.1 (14)	0.1 ± 0.05 (12)	0.01 ± 0.00 (9)
liver	8.2 ± 1.2 (2)	9.7 ± 1.2 (1)	4.8 ± 0.9 (1)	1.6 ± 0.4 (1)	0.04 ± 0.03 (2)
stomach	0.8 ± 0.3 (18)	0.5 ± 0.3 (23)	0.1 ± 0.05 (42)	0.06 ± 0.02 (23)	0.01 ± 0.01 (9)
pancreas	0.9 ± 0.2 (15)	0.8 ± 0.3 (16)	0.3 ± 0.3 (14)	0.03 ± 0.00 (47)	0.00 ± 0.00
spleen	26 ± 12 (0.5)	13.0 ± 6.8 (1)	1.25 ± 0.4 (3)	0.5 ± 0.2 (3)	0.03 ± 0.02 (3)
fat	3.5 ± 0.2 (4)	1.4 ± 0.5 (8)	0.04 ± 0.1 (105)	0.2 ± 0.2 (7)	0.00 ± 0.01
kidney	160 ± 27 (0.1)	205 ± 46 (0.06)	71 ± 27 (0.06)	24 ± 10 (0.06)	0.97 ± 0.94 (0.1)
muscle	1.9 ± 2.4 (7)	0.9 ± 0.7 (13)	0.2 ± 0.3 (21)	0.1 ± 0.1 (14)	0.00 ± 0.00
small int.	0.8 ± 0.2 (18)	1.1 ± 1.3 (11)	0.2 ± 0.05 (21)	0.1 ± 0.02 (14)	0.01 ± 0.01 (9)
Large int.	0.9 ± 0.4 (16)	0.6 ± 0.3 (21)	0.2 ± 0.1 (21)	0.1 ± 0.1 (14)	0.00 ± 0.00
bladder	1.9 ± 0.4 (8)	4.8 ± 4.9 (2.5)	7.0 ± 3.6 (0.6)	2.8 ± 2.1 (0.5)	0.05 ± 0.01 (2)
PC-3 PIP	14.2 ± 9.5	12.1 ± 4.9	4.2 ± 1.5	1.4 ± 0.4	0.09 ± 0.04
PC-3 flu	0.8 ± 0.1	0.6 ± 0.25	0.1 ± 0.02	0.03 ± 0.01	0.00 ± 0.00
PIP:flu	18	20	42	47	

a. Values are in % ID/g ± SD; n = 4; int. = intestine.

b. Pip tumor:organ ratios are in parentheses.

10 **[0135]** Regarding *ex vivo* biodistribution, **3** demonstrates only about twice the tumor uptake at 1 h p.i. as **6**, while its affinity is about twenty-five times higher. The target to nontarget (PIP:flu) ratio for **6**, however, is higher than for **3**, reflecting lower nonspecific binding. Those target to nontarget ratios rise to approximately 140 at 48 hours p.i. for **3** and to 31 at 5 hours p.i. for **6**. Compound **8** differs from **3** in that the aromatic ring is a pyridine and the iodine is substituted at the three-position. At one hour p.i. **8** demonstrated a similarly high

tumor uptake (12.05 ± 4.92 %ID/g) compared to **3**, but had a much higher target to nontarget (PIP:flu) ratio at that time (22), which rose to 48.3 at 24 h p.i. Interestingly, the affinity of **8** is the lowest among the three compounds tested (Table 1), however, it provides the highest target to nontarget ratio at 1 h p.i. As demonstrated in previous work with ^{99m}Tc -labeled compounds of this series, it is again shown that there is not a clear relationship between affinity and *in vivo* tumor uptake selectivity. Notably, all of these affinities are quite high and the tumors are clearly delineated (Figures 3-5).

In Vivo Biodistribution and Imaging

EXAMPLE 22

[0136] Compound [^{125}I]3**.** A single SCID mouse implanted with both a PC-3 PIP and a PC-3 flu xenograft was injected intravenously with 37 MBq (1 mCi) of [^{125}I]**3** in saline. At 4 and 6 h p.i. the mouse was anesthetized with isoflurane and maintained under 1% isoflurane in oxygen. The mouse was positioned on the X-SPECT (Gamma Medica, Northridge, CA) gantry and was scanned using two low energy, high-resolution pinhole collimators (Gamma Medica) rotating through 360° in 6° increments for 45 seconds per increment. All gamma images were reconstructed using Lunagem software (Gamma Medica, Northridge, CA). Immediately following SPECT acquisition, the mice were then scanned by CT (X-SPECT) over a 4.6 cm field-of-view using a 600 μA , 50 kV beam. The SPECT and CT data were then coregistered using the supplier's software (Gamma Medica, Northridge, CA) and displayed using AMIDE (<http://amide.sourceforge.net/>). Data were reconstructed using the Ordered Subsets-Expectation Maximization (OS-EM) algorithm.

[0137] Figure 3 shows a SPECT-CT image of radiopharmaceutical uptake at 4 h p.i. Note the intense uptake in the PC-3 PIP and absence of uptake in the PC-3 flu tumor. Thyroid uptake of the radiotracer indicates the presence of free [^{125}I]iodide due to deiodination by dehalogenases (35)(36). The amount of free [^{125}I]iodide, however, is small in comparison to the amount of PC-3 PIP tumor uptake (thyroid:muscle = 2; PIP tumor:thyroid = 12.5). The small amount of radiopharmaceutical uptake seen in the liver, with no concurrent gastrointestinal uptake, is likely due to the hydrophilic nature of [^{125}I]**3** (ClogD = -5.16 at pH 7.4).

EXAMPLE 23

[0138] Compound [^{18}F]6**.** *In vivo* PET-CT: A SCID mouse bearing subcutaneous PC-3 PIP and PC-3 flu xenografts was anesthetized using 3% isoflurane in oxygen for induction and

1.5% isoflurane in oxygen at 0.8 L/min flow for maintenance and positioned prone on the gantry of a GE eXplore Vista small animal PET scanner (GE Healthcare, Milwaukee, WI). The mouse was injected intravenously with 7.4 MBq (200 μ Ci) of [^{18} F]6 followed by image acquisition using the following protocol: The images were acquired as a pseudodynamic scan, i.e., a sequence of successive whole-body images acquired in three bed positions for a total of 90 min. The dwell time at each position was 5 minutes, such that a given bed position (or mouse organ) was revisited every 15 min. An energy window of 250 - 700 keV was used. Images were reconstructed using the FORE/2D-OSEM method (2 iterations, 16 subsets) and included corrections for radioactive decay, scanner dead time and scattered radiation.

- 10 [0139] **Figure 4** shows the averaged results of the dynamic scan from 94-120 min p.i. The uptake pattern for [^{18} F]6 is very similar to that seen for [^{125} I]3: easily observed within PIP tumor, none within flu tumor, high renal uptake and a modest degree of liver uptake. That result was expected due to similar ClogD values for [^{18} F]6 (-5.64 at pH 7.4) and [^{125} I]3. As for [^{125} I]3, the urinary bladder is visualized due to the continually accumulating presence of radioactive
- 15 urine, however, specific binding to bladder wall was not demonstrated.

EXAMPLE 24

- [0140] **Compound [^{125} I]8.** A single SCID mouse implanted with a LNCaP xenograft was injected intravenously with 37 MBq (1 mCi) of [^{125} I]8 in saline. At 4 h p.i., the mouse was
- 20 anesthetized with isoflurane and maintained under 1% isoflurane in oxygen. The mouse was positioned on the X-SPECT (Gamma Medica, Northridge, CA) gantry and was scanned using two low energy, high-resolution pinhole collimators (Gamma Medica) rotating through 360° in 6° increments for 45 seconds per increment. All gamma images were reconstructed using Lunagem software (Gamma Medica, Northridge, CA). Immediately following SPECT
- 25 acquisition, the mice were then scanned by CT (X-SPECT) over a 4.6 cm field-of-view using a 600 μ A, 50 kV beam. The SPECT and CT data were then coregistered using the supplier's software (Gamma Medica, Northridge, CA) and displayed using AMIDE (<http://amide.sourceforge.net/>). Data were reconstructed using the Ordered Subsets-Expectation Maximization (OS-EM) algorithm.
- 30 [0141] **Figure 5** shows a SPECT-CT image of radiopharmaceutical uptake at 4 h p.i. Tumor uptake and retention was high, with slow washout, while the washout of [^{125} I]8 from nontarget tissue was rapid.

Comparative Data for Target/nontarget ratios for phenyl versus pyridine analogs.

[0142] Target/non-target ratios are given for compound **3** (4-iodobenzoyl derivative) at 5 hours post injection and compound **8** (3-iodo-5-carboxyl-pyridyl derivative) at 4 hour post-injection. The target/non-target ratios are shown in Table 5 below.

5 **Table 5**

Tumor (T)/organ	Cmpd. 3 (5 hours post injection)	Cmpd. 8 (4 hours post injection)
T/blood	31	17
T/heart	14	20
T/Lung	3.5	14
T/liver	3	1
T/stomach	15	42
T/Pancreas	8	14
T/Spleen	0.1	3
T/fat	2	105
T/kidney	0.1	0.1
T/muscle	31	21
T/small Intest	8	21
T/large Intest	17	21

[0143] It appears that the improved target/non-target ratios for **8** versus **3** is due to the faster non-target clearance of **8** even though the blood clearance of each are comparable and **3** has higher tumor retention especially at later time points. Compound **3** is more lipophilic than **8** and has higher uptake in fat. The retention of **3** in fat could be providing a slow release of **3** for uptake in tumor and normal organs.

[0144] The high and prolonged tumor and kidney (PSMA rich in mice) uptake of **3** is due to this compounds tight binding to PSMA. The 4-iodophenyl moiety resides in a hydrophobic pocket accessory to the S1 binding site and provides additional hydrophobic-hydrophobic interactions (39). Pyridine analogs are more polar than **3** so they should have reduced hydrophobic-hydrophobic interactions in this binding site.

[0145] Compound **6** has even better target/non-target ratios than compound **8**. Therefore the background clearance of the more polar pyridine analog of **6** should give even better tumor-non-target ratios.

20

REFERENCES

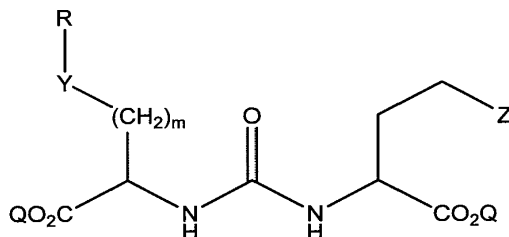
1. Jemal, A., Murray, T., Samuels, A., Ghafoor, A., Ward, E., and Thun, M. J. (2003) Cancer statistics, 2003. *CA Cancer J Clin* **53**, 5-26.
2. Geus-Oei, L. F., and Oyen, W. J. (2008) Predictive and prognostic value of FDG-PET. *Cancer Imaging* **8**, 70-80
3. Larson, S. M., Morris, M., Gunther, I., Beattie, B., Humm, J. L., Akhurst, T. A., Finn, R. D., Erdi, Y., Pentlow, K., Dyke, J., Squire, O., Bornmann, W., McCarthy, T., Welch, M., and Scher, H. (2004) Tumor localization of 16beta-18F-fluoro-5alpha-dihydrotestosterone versus 18F-FDG in patients with progressive, metastatic prostate cancer. *J Nucl Med* **45**, 366-373
4. Scher, B., Seitz, M., Albinger, W., Tiling, R., Scherr, M., Becker, H. C., Souvatzoglou, M., Gildehaus, F. J., Wester, H. J., and Dresel, S. (2007) Value of 11C-choline PET and PET/CT in patients with suspected prostate cancer. *Eur J Nucl Med Mol Imaging* **34**, 45-53
5. Reske, S. N., Blumstein, N. M., Neumaier, B., Gottfried, H. W., Finsterbusch, F., Kocot, D., Moller, P., Glatting, G., and Perner, S. (2006) Imaging prostate cancer with 11C-choline PET/CT. *J Nucl Med* **47**, 1249-1254
6. Veas, H., Buchegger, F., Albrecht, S., Khan, H., Husarik, D., Zaidi, H., Soloviev, D., Hany, T. F., and Miralbell, R. (2007) 18F-choline and/or 11C-acetate positron emission tomography: detection of residual or progressive subclinical disease at very low prostate-specific antigen values (<1 ng/mL) after radical prostatectomy. *BJU Int* **99**, 1415-1420
7. Ponde, D. E., Dence, C. S., Oyama, N., Kim, J., Tai, Y. C., Laforest, R., Siegel, B. A., and Welch, M. J. (2007) 18F-fluoroacetate: a potential acetate analog for prostate tumor imaging--*in vivo* evaluation of 18F-fluoroacetate versus 11C-acetate. *J Nucl Med* **48**, 420-428
8. Schuster, D. M., Votaw, J. R., Nieh, P. T., Yu, W., Nye, J. A., Master, V., Bowman, F. D., Issa, M. M., and Goodman, M. M. (2007) Initial experience with the radiotracer anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid with PET/CT in prostate carcinoma. *J Nucl Med* **48**, 56-63
9. Oka, S., Hattori, R., Kurosaki, F., Toyama, M., Williams, L. A., Yu, W., Votaw, J. R., Yoshida, Y., Goodman, M. M., and Ito, O. (2007) A preliminary study of anti-1-amino-3-18F-fluorocyclobutyl-1-carboxylic acid for the detection of prostate cancer. *J Nucl Med* **48**, 46-55
10. Tehrani, O. S., Muzik, O., Heilbrun, L. K., Douglas, K. A., Lawhorn-Crews, J. M., Sun, H., Mangner, T. J., and Shields, A. F. (2007) Tumor imaging using 1-(2'-deoxy-2'-18F-fluoro-beta-D-arabinofuranosyl)thymine and PET. *J Nucl Med* **48**, 1436-1441
11. Chang, S. S., Reuter, V. E., Heston, W. D., Bander, N. H., Grauer, L. S., and Gaudin, P. B. (1999) Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res* **59**, 3192-3198.
12. Zhou, J., Neale, J. H., Pomper, M. G., and Kozikowski, A. P. (2005) NAAG peptidase inhibitors and their potential for diagnosis and therapy. *Nat Rev Drug Discov* **4**, 1015-1026
13. Chang, S. S. (2004) Overview of prostate-specific membrane antigen. *Rev Urol* **6 Suppl** **10**, S13-18
14. Murphy, G. P., Kenny, G. M., Ragde, H., Wolfert, R. L., Boynton, A. L., Holmes, E. H., Misrock, S. L., Bartsch, G., Klocker, H., Pointner, J., Reissigl, A., McLeod, D. G., Douglas, T., Morgan, T., and Gilbaugh, J., Jr. (1998) Measurement of serum prostate-

- specific membrane antigen, a new prognostic marker for prostate cancer. *Urology* **51**, 89-97.
15. Galsky, M. D., Eisenberger, M., Moore-Cooper, S., Kelly, W. K., Slovin, S. F., DeLaCruz, A., Lee, Y., Webb, I. J., and Scher, H. I. (2008) Phase I trial of the prostate-specific membrane antigen-directed immunoconjugate MLN2704 in patients with progressive metastatic castration-resistant prostate cancer. *J Clin Oncol* **26**, 2147-2154
 16. Lange, P. H. (2001) PROSTASCINT scan for staging prostate cancer. *Urology* **57**, 402-406.
 17. Haseman, M. K., Rosenthal, S. A., and Polascik, T. J. (2000) Capromab Pendetide imaging of prostate cancer. *Cancer Biother Radiopharm* **15**, 131-140.
 18. Rosenthal, S. A., Haseman, M. K., and Polascik, T. J. (2001) Utility of capromab pendetide (ProstaScint) imaging in the management of prostate cancer. *Tech Urol* **7**, 27-37.
 19. Banerjee, S. R., Foss, C. A., Mease, R. C., Fox, J., Kozikowski, A. P., and Pomper, M. G. (2008) Synthesis and evaluation of ^{99m}Tc/Re labeled PSMA inhibitors. *J Med Chem* **51**, 4504-4517.
 20. Vaidyanathan, G., and Zalutsky, M. R. (1994) Improved synthesis of N-succinimidyl 4-[¹⁸F]fluorobenzoate and its application to the labeling of a monoclonal antibody fragment. *Bioconjug Chem* **5**, 352-356
 21. Vaidyanathan, G., and Zalutsky, M. R. (2006) Synthesis of N-succinimidyl 4-[¹⁸F]fluorobenzoate, an agent for labeling proteins and peptides with ¹⁸F. *Nature protocols* **1**, 1655-1661
 22. Vaidyanathan, G., and Zalutsky, M. R. (1992) Labeling proteins with fluorine-18 using N-succinimidyl-4-[¹⁸F]fluorobenzoate. *Int J Rad Appl Instrum Part B* **19**, 275-281
 23. Chen, X., Park, R., Shahinian, A. H., Tohme, M., Khankaldyian, V., Bozorgzadeh, M. H., Bading, J. R., Moats, R., Laug, W. E., and Conti, P. S. (2004) ¹⁸F-labeled RGD peptide: initial evaluation for imaging brain tumor angiogenesis. *Nucl Med Biol* **31**, 179-189
 24. Dekker, B., Keen, H., Shaw, D., Disley, L., Hastings, D., Hadfield, J., Reader, A., Allan, D., Julyan, P., Watson, A., and Zweit, J. (2005) Functional comparison of annexin V analogues labeled indirectly and directly with iodine-124. *Nucl Med Biol* **32**, 403-413
 25. Garg, S., Garg, P. K., and Zalutsky, M. R. (1991) N-succinimidyl 5-(trialkylstannyl)-3-pyridinecarboxylates: a new class of reagents for protein radioiodination. *Bioconjug Chem* **2**, 50-56
 26. Lupold, S. E., Hicke, B. J., Lin, Y., and Coffey, D. S. (2002) Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. *Cancer Res* **62**, 4029-4033
 27. Robinson, M. B., Blakely, R. D., Couto, R., and Coyle, J. T. (1987) Hydrolysis of the brain dipeptide N-acetyl-L-aspartyl-L-glutamate. Identification and characterization of a novel N-acetylated alpha-linked acidic dipeptidase activity from rat brain. *J Biol Chem* **262**, 14498-14506
 28. Cheng, H. C. (2001) determination of KB or Ki from IC50. A closer look at the Cheng-Prusoff equation, the Schild plot and related power equations. *J. Pharmacol. Toxicol. Methods* **46**, 61-71
 29. Kozikowski, A. P., Nan, F., Conti, P., Zhang, J., Ramadan, E., Bzdega, T., Wroblewska, B., Neale, J. H., Pshenichkin, S., and Wroblewski, J. T. (2001) Design of remarkably simple, yet potent urea-based inhibitors of glutamate carboxypeptidase II (NAALADase). *J Med Chem* **44**, 298-301.
 30. Kozikowski, A. P., Zhang, J., Nan, F., Petukhov, P. A., Grajkowska, E., Wroblewski, J. T., Yamamoto, T., Bzdega, T., Wroblewska, B., and Neale, J. H. (2004) Synthesis of

- urea-based inhibitors as active site probes of glutamate carboxypeptidase II: efficacy as analgesic agents. *J Med Chem* **47**, 1729-1738
31. Slusher, B. S., Tsai, G., Yoo, G., and Coyle, J. T. (1992) Immunocytochemical localization of the N-acetyl-aspartyl-glutamate (NAAG) hydrolyzing enzyme N-acetylated alpha-linked acidic dipeptidase (NAALADase). *J Comp Neurol* **315**, 217-229
 32. Silver, D. A., Pellicer, I., Fair, W. R., Heston, W. D., and Cordon-Cardo, C. (1997) Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res* **3**, 81-85
 33. Bzdega, T., Crowe, S. L., Ramadan, E. R., Sciarretta, K. H., Olszewski, R. T., Ojeifo, O. A., Rafalski, V. A., Wroblewska, B., and Neale, J. H. (2004) The cloning and characterization of a second brain enzyme with NAAG peptidase activity. *J Neurochem* **89**, 627-635
 34. Hlouchova, K., Barinka, C., Klusak, V., Sacha, P., Mlcochova, P., Majer, P., Rulisek, L., and Konvalinka, J. (2007) Biochemical characterization of human glutamate carboxypeptidase III. *J Neurochem* **101**, 682-696
 35. Bakker, W. H., Krenning, E. P., Breeman, W. A., Koper, J. W., Kooij, P. P., Reubi, J. C., Klijn, J. G., Visser, T. J., Docter, R., and Lamberts, S. W. (1990) Receptor scintigraphy with a radioiodinated somatostatin analogue: radiolabeling, purification, biologic activity, and *in vivo* application in animals. *J Nucl Med* **31**, 1501-1509
 36. Bakker, W. H., Krenning, E. P., Breeman, W. A., Kooij, P. P., Reubi, J. C., Koper, J. W., de Jong, M., Lameris, J. S., Visser, T. J., and Lamberts, S. W. (1991) *In vivo* use of a radioiodinated somatostatin analogue: dynamics, metabolism, and binding to somatostatin receptor-positive tumors in man. *J Nucl Med* **32**, 1184-1189.
 37. Garg, S., Garg, P.K., Zhao, X-G., Friedman, H.S., Bigner, D.D., and Zalutsky, M.R., Radioiodination of a monoclonal antibody using N-succinimidyl 5-iodo-3-pyridinecarboxylate Nucl. Med. Biol. 20: 835-842 (1993);
 38. Ghirmai, S., Mume, E., Tolmachev, V., and Sjoberg, S., Synthesis and radioiodination of some daunorubicin and doxorubicin derivatives Carbohydrate Research 340 15-24 (2005).
 39. Barinka, C., Byun, Y., Dusich, C.L., Banerjee, S.R., Chen, Y., Castanares, M., Kozikowski, A.P., Mease, R.C., Pomper, Martin G., and Lubkowski, J., Interactions between human glutamate carboxypeptidase II and urea-based inhibitors: Structural Characterizations. *J. Med. Chem.* 51: 7737-7743 (2008).

CLAIMS

1. A compound having the structure



wherein

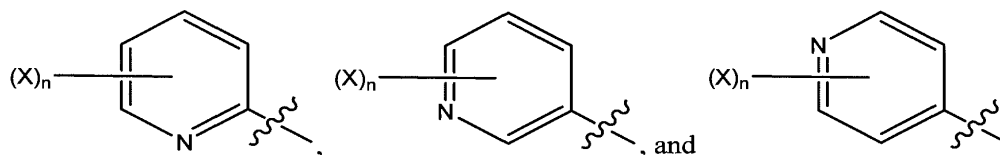
Z is tetrazole or CO₂Q;

each Q is independently selected from hydrogen or a protecting group; and

wherein

(A) m is 0, 1, 2, 3, 4, 5, or 6;

R is a pyridine ring selected from the group consisting of



wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), B(OH)₂, -NHNH₂, -NHN=CHR³, -NHNH-CH₂R³;

n is 1, 2, 3, 4, or 5;

Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is hydrogen, alkyl, aryl or heteroaryl, each of which may be substituted;

R² is C₁-C₆ alkyl; and

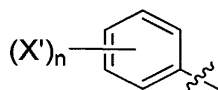
R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of

bromine, or a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$;
OR

(B) m is 0, 1, 2, 3, 4, 5, or 6;

Y is O, S, $\text{N}(\text{R}')$, $\text{C}(\text{O})$, $\text{NR}'\text{C}(\text{O})$, $\text{C}(\text{O})\text{N}(\text{R}')$, $\text{OC}(\text{O})$, $\text{C}(\text{O})\text{O}$, $\text{NR}'\text{C}(\text{O})\text{NR}'$, $\text{NR}'\text{C}(\text{S})\text{NR}'$, $\text{NR}'\text{S}(\text{O})_2$, $\text{S}(\text{CH}_2)_p$, $\text{NR}'(\text{CH}_2)_p$, $\text{O}(\text{CH}_2)_p$, $\text{OC}(\text{O})\text{CHR}^8\text{NHC}(\text{O})$, $\text{NHC}(\text{O})\text{CHR}^8\text{NHC}(\text{O})$, or a covalent bond; wherein p is 1, 2, or 3, R' is H or C_1 - C_6 alkyl, and R^8 is hydrogen alkyl, aryl or heteroaryl, each of which may be substituted;

R is



wherein

X' is selected from the group consisting of NHNH_2 , $-\text{NHN}=\text{CHR}^3$, and $-\text{NHNH}-\text{CH}_2\text{R}^3$; wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine; NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, or $\text{B}(\text{OH})_2$;

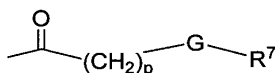
R^2 is C_1 - C_6 alkyl;

n is 1, 2, 3, 4, or 5; OR

(C) m is 4,

Y is NR' , and

R is



wherein

G is O, NR' or a covalent bond;

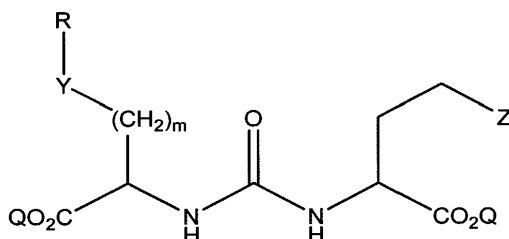
R' is H or C_1 - C_6 alkyl;

p is 1, 2, 3, or 4, and

R^7 is selected from the group consisting of NH_2 , $\text{N}=\text{CHR}^3$, $\text{NH}-\text{CH}_2\text{R}^3$, wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine, NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, $\text{B}(\text{OH})_2$; and R^2 is C_1 - C_6 alkyl.

2. A compound according to claim 1, wherein Z is CO_2Q .

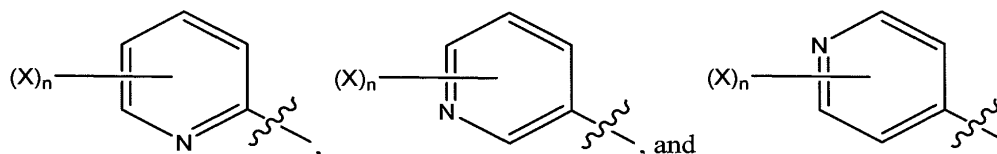
3. A compound according to claim 1 or 2, wherein Q is hydrogen.
4. A compound according to any one of claims 1-3, where m is 1, 2, 3, or 4.
5. A compound according to any one of claims 1-3, having the structure



wherein

m is 0, 1, 2, 3, 4, 5, or 6;

R is a pyridine ring selected from the group consisting of



wherein X is fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, a radioisotope of astatine, NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), B(OH)₂, -NHNH₂, -NHN=CHR³, and -NHNH-CH₂R³;

each Q is independently selected from hydrogen or a protecting group;

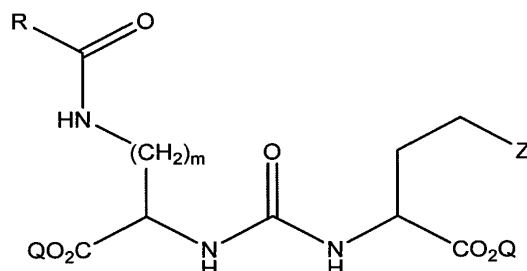
Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is hydrogen, alkyl, aryl or heteroaryl, each of which may be substituted;

Z is tetrazole or CO₂Q;

R² is C₁-C₆ alkyl; and

R³ is alkyl, alkenyl, alkynyl, aryl, or heteroaryl, each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine, bromine, a radioisotope of bromine, or a radioisotope of astatine; NO₂, NH₂, N⁺(R²)₃, Sn(R²)₃, Si(R²)₃, Hg(R²), or B(OH)₂.

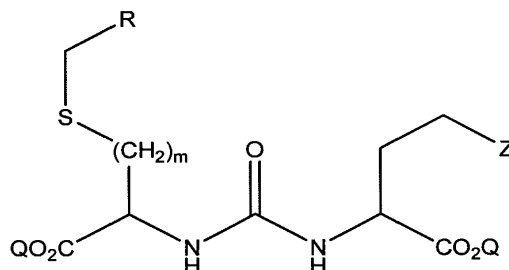
6. A compound according to claim 5, having the structure



wherein m is not 0.

7. A compound according to claim 6, where Z is CO₂Q, Q is hydrogen, and m is 4.

8. A compound according to claim 5, having the structure



wherein m is not 0.

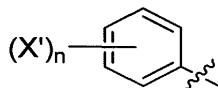
9. A compound according to claim 8, where Z is CO₂Q, Q is hydrogen, and m is 1, 2, or 3.

10. A compound according to any one of claims 1-4, wherein

m is 0, 1, 2, 3, 4, 5, or 6;

Y is O, S, N(R'), C(O), NR'C(O), C(O)N(R'), OC(O), C(O)O, NR'C(O)NR', NR'C(S)NR', NR'S(O)₂, S(CH₂)_p, NR'(CH₂)_p, O(CH₂)_p, OC(O)CHR⁸NHC(O), NHC(O)CHR⁸NHC(O), or a covalent bond; wherein p is 1, 2, or 3, R' is H or C₁-C₆ alkyl, and R⁸ is hydrogen, alkyl, aryl or heteroaryl, each of which may be substituted;

R is



wherein

X' is selected from the group consisting of NHNH_2 , $-\text{NHN}=\text{CHR}^3$, $-\text{NHNH}-\text{CH}_2\text{R}^3$; wherein R^3 is alkyl, alkenyl, alkynyl, aryl, or heteroaryl, each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine; NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, and $\text{B}(\text{OH})_2$, where R^2 is $\text{C}_1\text{-C}_6$ alkyl;

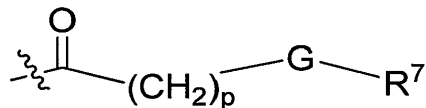
n is 1, 2, 3, 4, or 5.

11. A compound according to any one of claims 1-10, wherein n is 1.

12. A compound according to any one of claims 1-11, wherein X or X' is fluorine, iodine, or a radioisotope of fluorine or iodine, bromine, a radioisotope of bromine, or a radioisotope of astatine.

13. A compound according to any one of claims 1-11, wherein X or X' is fluorine, iodine, or a radioisotope of fluorine or iodine.

14. A compound according to any one of claims 1-4, wherein m is 4, Y is NR' , and R is



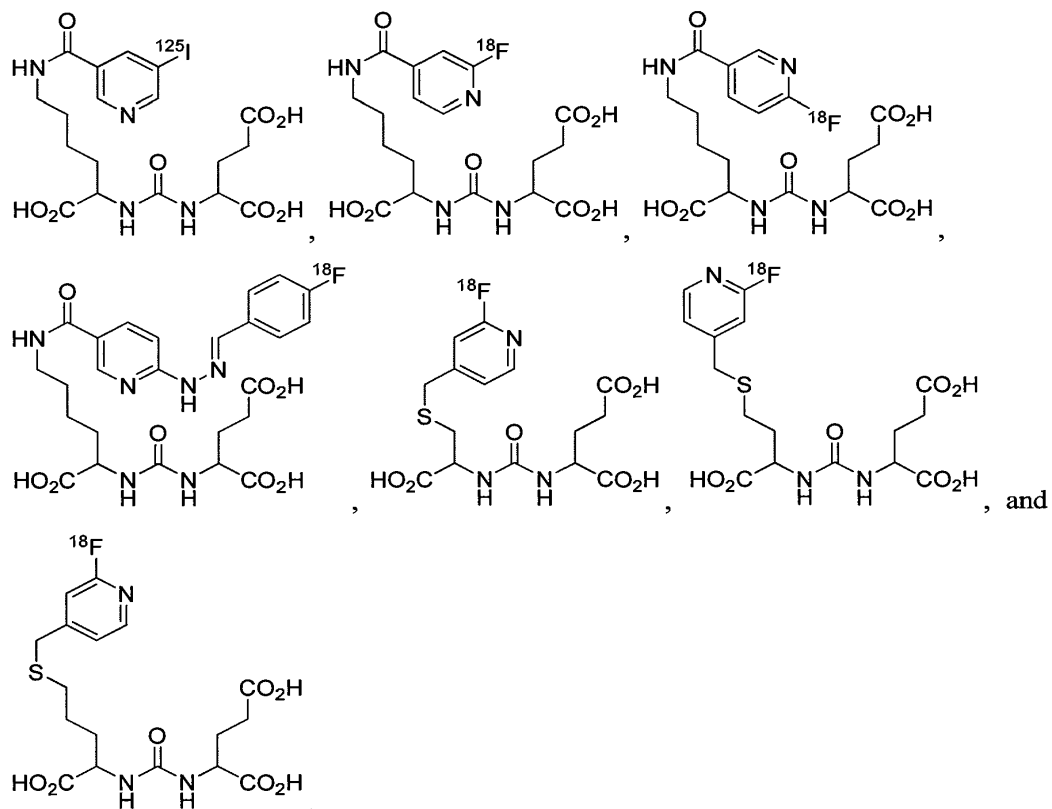
wherein G is O, NR' or a covalent bond,

p is 1, 2, 3, or 4, and

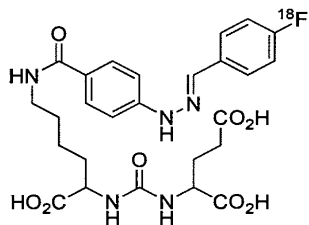
R^7 is selected from the group consisting of NH_2 , $\text{N}=\text{CHR}^3$, $\text{NH}-\text{CH}_2\text{R}^3$, wherein R^3 is alkyl, alkenyl, alkynyl, aryl, heteroaryl each of which is substituted by fluorine, iodine, a radioisotope of fluorine, a radioisotope of iodine, chlorine bromine, a radioisotope of bromine, or a radioisotope of astatine NO_2 , NH_2 , $\text{N}^+(\text{R}^2)_3$, $\text{Sn}(\text{R}^2)_3$, $\text{Si}(\text{R}^2)_3$, $\text{Hg}(\text{R}^2)$, and $\text{B}(\text{OH})_2$, wherein R^2 is $\text{C}_1\text{-C}_6$ alkyl.

15. A compound according to claim 14, wherein G is O or NR' .

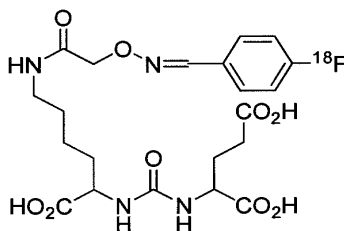
16. A compound according to any one of claims 1-15, wherein R comprises a radioisotope.
17. A compound according to claim 16, wherein the radioisotope is selected from the group consisting of ^{18}F , ^{123}I , ^{124}I , ^{125}I , ^{126}I , ^{131}I , ^{75}Br , ^{76}Br , ^{77}Br , ^{80}Br , $^{80\text{m}}\text{Br}$, ^{82}Br , ^{83}Br and ^{211}At .
18. A compound according to claim 1 selected from the group consisting of



19. A compound according to claim 1, having the structure



20. A compound according to claim 1, having the structure



21. A method of imaging one or more cells, organs or tissues comprising exposing the cell to or administering to a organism an effective amount of a compound according to any one of claims 16-20.
22. The method according to claim 21, wherein the one or more organs or tissues includes prostate tissue, kidney tissue, brain tissue vascular tissue or tumor tissue.
23. A method of treating a tumor comprising administering a therapeutically effective amount of a compound according to any one of claims 1-17 comprising a therapeutically effective radioisotope.
24. A kit comprising a compound according to any one of claims 1-20.

1/5

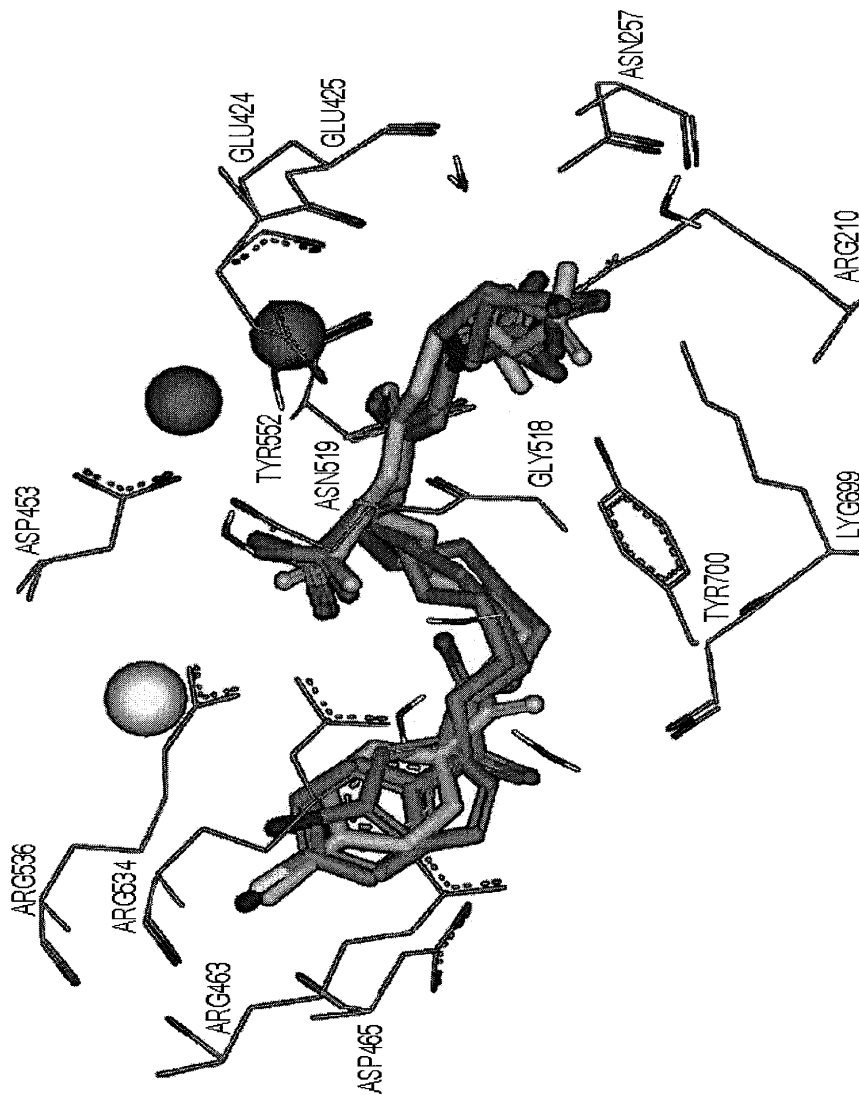


FIG 1

2/5

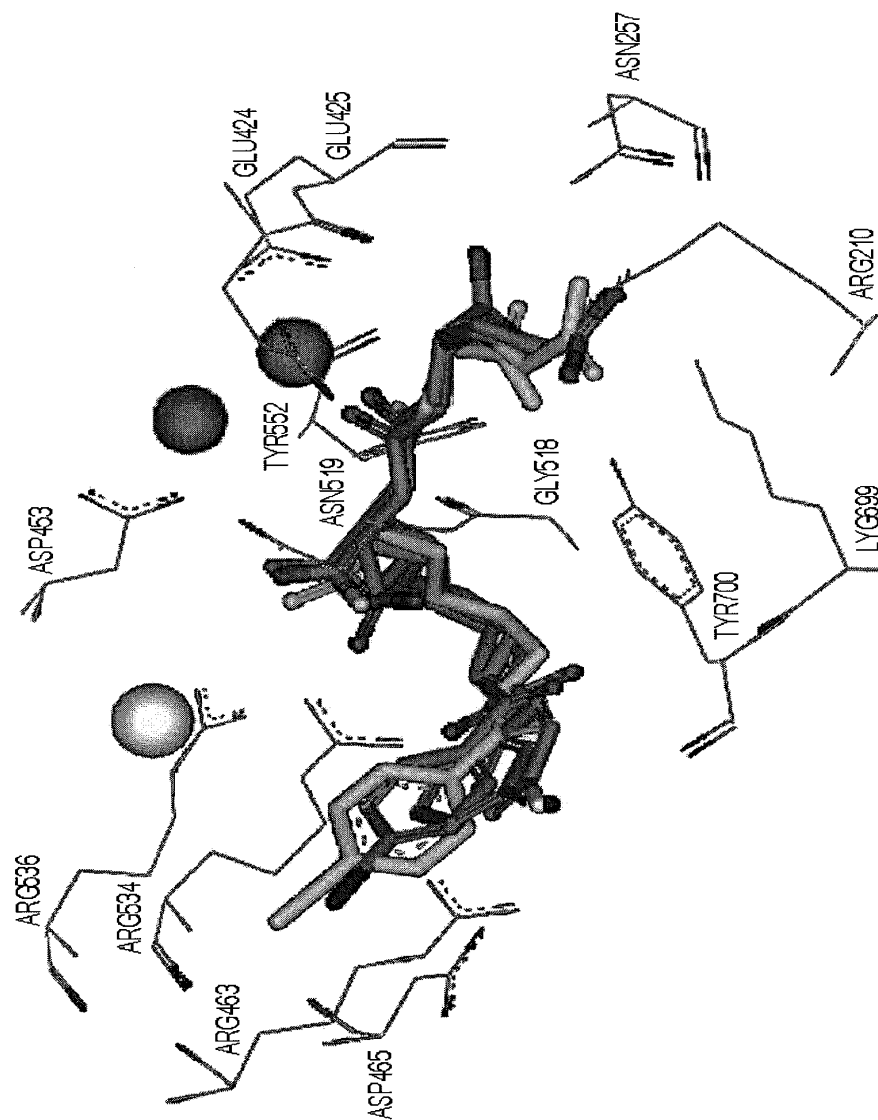


FIG 2

3/5

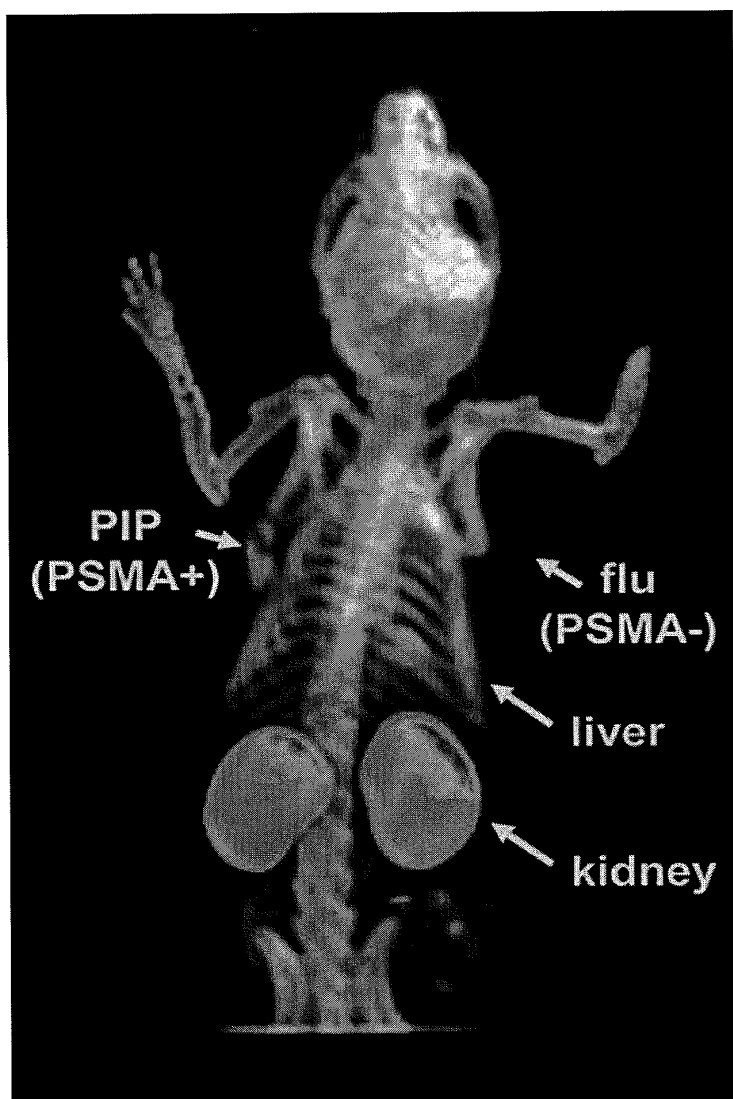


FIG 3

4/5

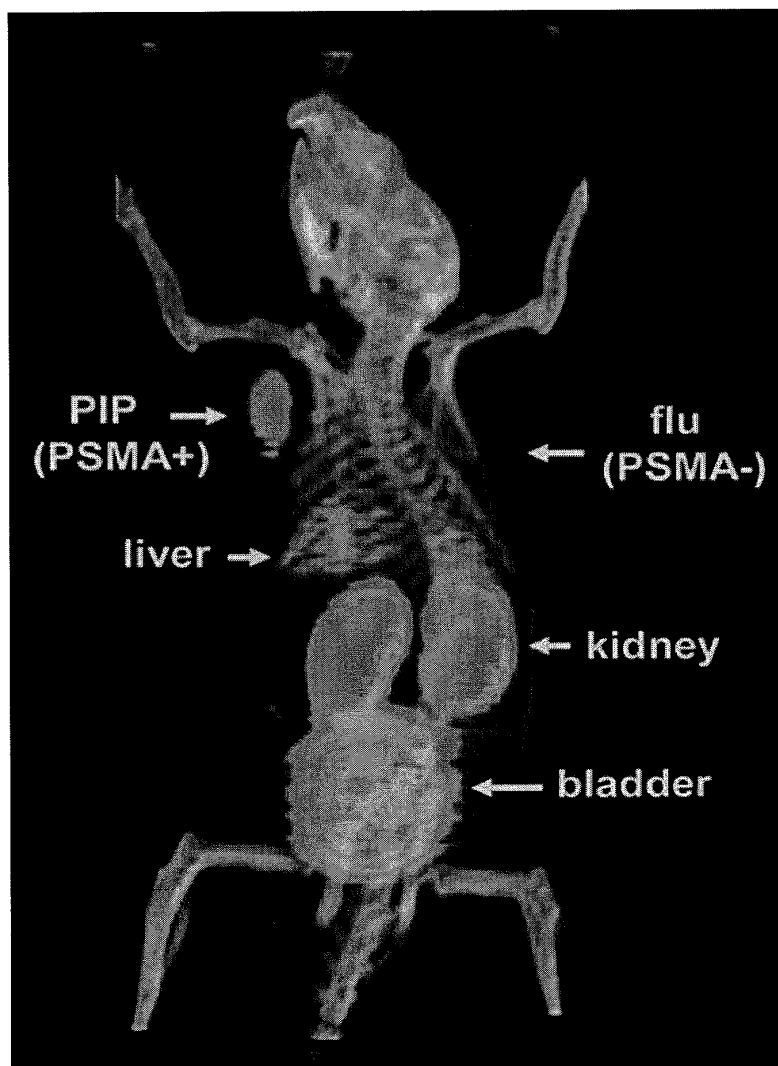


FIG 4

5/5

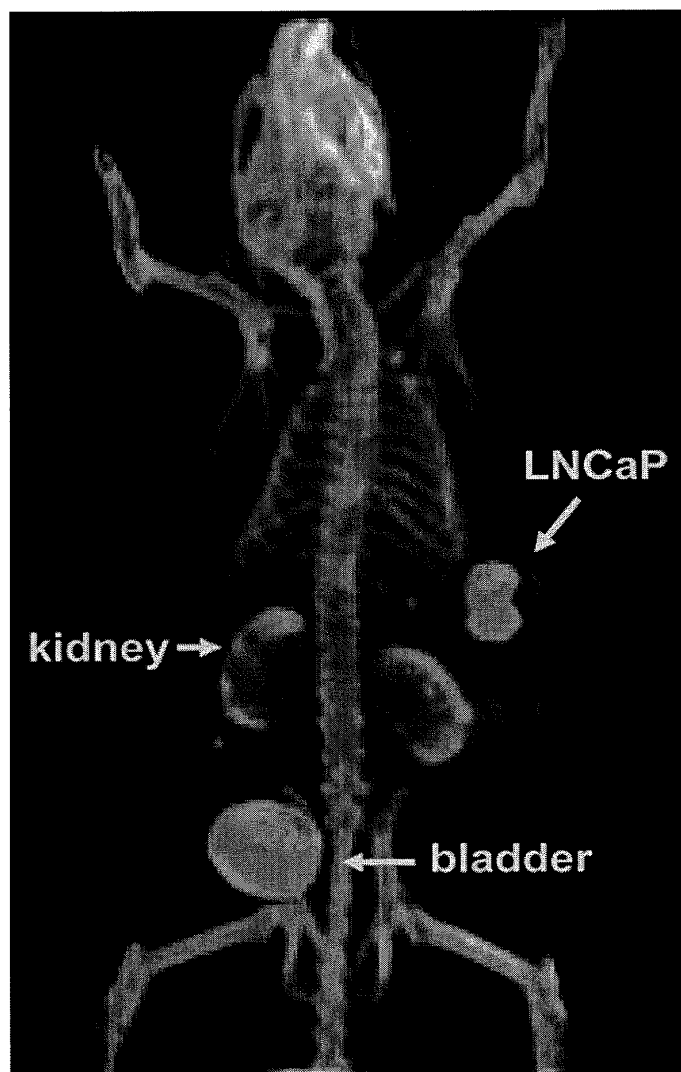


FIG 5

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 September 2010 (23.09.2010)

(10) International Publication Number
WO 2010/108125 A2

PCT

(51) International Patent Classification:
C07D 257/00 (2006.01) *A61K 49/00* (2006.01)
A61K 31/41 (2006.01) *A61P 35/00* (2006.01)

CHEN, Ying [CN/US]; 14 Breezy Tree Court, Apartment
I, Timonium, Maryland 21093 (US).

(21) International Application Number:
PCT/US2010/028020

(74) Agent: **HADDAWAY, Keith G.**; VENABLE LLP, P.O.
Box 34385, Washington, DC20043-9998 (US).

(22) International Filing Date:
19 March 2010 (19.03.2010)

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/161,485 19 March 2009 (19.03.2009) US
61/161,484 19 March 2009 (19.03.2009) US
61/248,067 2 October 2009 (02.10.2009) US
61/248,934 6 October 2009 (06.10.2009) US

(71) Applicant (for all designated States except US): **THE
JOHNS HOPKINS UNIVERSITY** [US/US]; 3400
North Charles Street, Baltimore, Maryland 21218 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **POMPER, Martin
G.** [US/US]; 101 Churchwarden's Road, Baltimore,
Maryland 21212 (US). **MEASE, Ronnie C.** [US/US];
12320 Cannonball Road, Fairfax, Virginia 22030 (US).
RAY, Sangeeta [IN/US]; 3358 North Chatham Road,
Apartment L, Ellicott City, Maryland 21042 (US).

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,
TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

[Continued on next page]

(54) Title: PSMA-TARGETING COMPOUNDS AND USES THEREOF

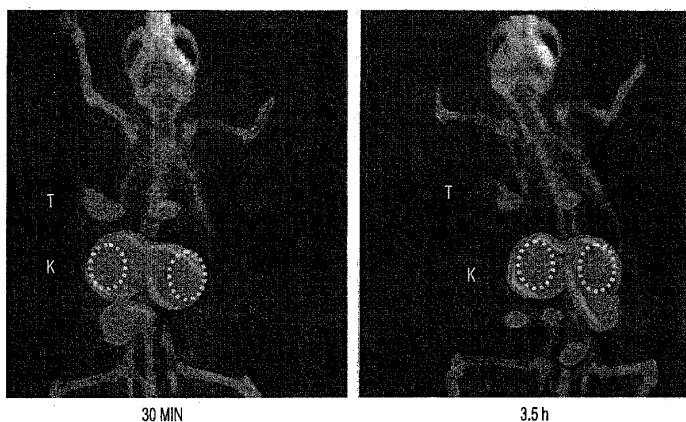


FIG. 1

(57) Abstract: Prostate-specific membrane antigen (PSMA) targeting compounds are described. Uses of the compounds for imaging, therapy, cell sorting, and tumor mapping are also described.

WO 2010/108125 A2



-
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*
- Published:**
- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

PSMA-TARGETING COMPOUNDS AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This application claims priority to U.S. Provisional Application Nos. 61/161,484 filed March 19, 2009, 61/161,485, filed March 19, 2009, 61/248,067, filed October 2, 2009, and 61/248,934, filed October 6, 2009. The entire content of each Provisional Application is hereby incorporated by reference in their entirety. This invention was made using U. S. Government support under NIH grant NIH U24 CA92871. The government has certain rights in this invention.

BACKGROUNDField of the Invention

[0002] The present invention relates to prostate specific membrane antigen (PSMA) binding compounds, chemical precursors of PSMA binding compounds and imaging methods of using the compounds.

Background

[0003] Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in men in the United States (*Cancer Facts & Figures*; American Cancer Society: Atlanta, GA, 2009). In 2009, it is estimated that 192,000 men will be diagnosed with prostate cancer and 27,000 men will die of the disease. Only one half of tumors due to PCa are clinically localized at diagnosis and one half of those represent extracapsular spread. Localization of that spread as well as determination of the total body burden of PCa have important implications for therapy, particularly as new combination and focal therapies become available.

[0004] The prostate-specific membrane antigen (PSMA), while expressed in prostate tumor epithelium, has a curious property in that it is expressed in the neovasculature of many solid tumors but not in that of prostate cancer (Chang et al., *Cancer Res.*, vol. 59, pp. 3192-3198, 1999; Chang et al., *Clin. Cancer Res.*, vol. 5, pp. 2674-2681, 1999; Gong et al., *Cancer Metastasis Rev.*, vol. 18, pp. 483-490, 1999; Chang et al., *Mol. Urol.*, vol. 3, pp. 313-320, 1999; Baccala et al., *Urology*, vol. 70, pp. 385-390, 2007; Chang et al., *Urology*, vol. 57, pp. 801-805,

2001Milowsky et al., *J. Clin. Oncol.*, vol. 25, pp. 540-547, 2007). Because of that property, an ^{111}In -labeled monoclonal antibody to an extracellular epitope of PSMA, ^{111}In -J591, was capable of identifying renal, bladder, lung, breast, colorectal and pancreatic tumors in a Phase I clinical imaging study (Milowsky et al., *J. Clin. Oncol.*, vol. 25, pp. 540-547, 2007). That study validated ^{111}In -J591 as a vascular targeting agent in human subjects. Since then other reports have further studied PSMA expression in certain tumor types. Baccala et al. noted that clear cell renal cell carcinoma expresses significantly more PSMA in its neovasculature than does the papillary variety (Baccala et al., *Urology*, vol. 70, pp. 385-390, 2007). Furthermore, angiomyolipoma, a benign renal lesion, did not express PSMA. As an enzyme with an extracellular active site, PSMA represents an excellent target for imaging and therapy directed toward solid tumor neovasculature in addition to prostate cancer itself. PSMA-based agents can report on the presence of this marker, which is increasingly recognized as an important prognostic determinate in PCa (Murphy et al., *Urology*, vol. 51, pp. 89-97, 1998). It is also the target for a variety of new PCa therapies (Galsky et al., *J Clin Oncol*, vol. 26, pp. 2147-2154, 2008).

[0005] ProstaScint™ is an ^{111}In -labeled monoclonal antibody against PSMA that is clinically available for imaging PCa. Radioimmunotherapy based on ProstaScint™ and radiolabeled variations of this antibody are fraught with similar difficulties to the use of radiolabeled antibodies for imaging, including prolonged circulation times, poor target to nontarget tissue contrast, unpredictable biological effects and the occasional need for pre-targeting strategies, limiting the utility of these agents (Lange, P. H., *Urology*, vol. 57, pp. 402-406, 2001; Haseman et al., *Cancer Biother Radiopharm*, vol. 15, pp. 131-140, 2000; Rosenthal et al., *Tech Urol*, vol. 7, pp. 27-37, 2001). Furthermore, antibodies may have less access to tumor than low molecular weight agents, which can be manipulated pharmacologically.

[0006] The development of low molecular weight radiotherapeutic agents is much different from developing radiopharmaceuticals for imaging in that longer tumor residence times can often be important for the former.

[0007] Complete detection and eradication of primary tumor and metastatic foci are required to effect a cure in patients with cancer; however, current preoperative assessment often misses small metastatic deposits. More sensitive imaging techniques than computed tomography, magnetic resonance imaging and even positron emission tomography (PET), which can be used easily in the operating suite, are required. An old technique, recently revisited

because of improved optics and fluorescent dye chemistry, is intraoperative photodiagnosis (PDD) (Toda, *Keio J. Med.*, vol. 57, pp. 155-161, 2008). Fluorescein dyes have been used intraoperatively to identify brain tumors and verify the clarity of tumor margins since 1948 (Toda, *Keio J. Med.*, vol. 57, pp. 155-161, 2008). A recent report describes its utility in identifying brain metastases (Okuda et al., *Minim. Invasive Neurosurg.*, vol. 50, pp. 382-384, 2007). A long history of the use of 5-aminolevulinic acid (5-ALA) for brain tumor resection is also evident, and its use has been associated with improvement in progression-free survival (Stummer et al., *Lancet Oncol.*, vol. 7, pp. 392-401, 2006). PDD can be performed easily during surgery due to the lack of a need for complex imaging equipment. All that is needed is a light-emitting diode to excite the fluorophore, which can be administered systemically or “painted” on the tissue directly. More recent incarnations of PDD have used quantum dots (Arndt-Jovin et al., *IEEE Trans Nanobioscience*, 2009), and more advanced dyes, such as indocyanine green (ICG) (Gotoh et al., *J. Surg. Oncol.*, 2009), which emit in the near-infrared (NIR) region of the spectrum, enabling reasonable tissue penetration of emitted (and detected) light. Applications have included nontargeted approaches, such as preoperative evaluation of the vascular integrity of surgical flaps or identification of nodules of hepatocellular carcinoma (Matsui et al., *Plast. Reconstr. Surg.*, vol. 123, pp. 125e-127e, 2009). Targeted approaches are also emerging, such as use of a fluorophore-conjugated anti-CEA antibody to identify colon or pancreatic cancer (Kaushal et al., *J. Gastrointest. Surg.*, vol. 12, pp. 1938-1950, 2008), or the use of NIR activatable probes that emit light only when cleaved by a tumor-associated protease (Sheth et al., *Gynecol. Oncol.*, vol. 112, pp. 616-622, 2009).

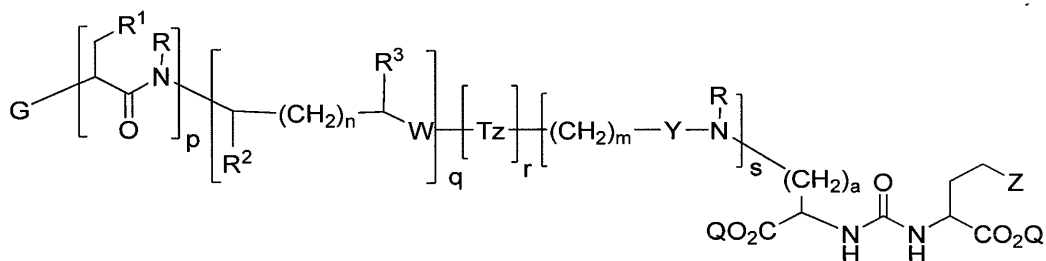
[0008] Recently, the application of ^{68}Ga -labeled peptides has attracted considerable interest for cancer imaging because of the physical characteristics of Ga-68 (Reubi et al., *J Nucl Med*, vol. 49, pp. 1735-1738, 2008). Ga-68 is available from an in-house $^{68}\text{Ge}/^{68}\text{Ga}$ generator (^{68}Ge , $t_{1/2} = 270.8$ day), which renders it independent of an onsite cyclotron. Therefore, ^{68}Ga -based PET agents possess significant commercial potential and serve as a convenient alternative to cyclotron-based isotopes for positron emission tomography (PET), such as ^{18}F or ^{124}I . ^{68}Ga has a high positron-emitting fraction (89% of its total decay). The maximum positron energy of ^{68}Ga (max. energy = 1.92 MeV, mean = 0.89 MeV) is higher than that of ^{18}F (max = 0.63 MeV, mean = 0.25 MeV). However, a study of spatial resolution using Monte Carlo analysis revealed that under the assumption of 3 mm spatial resolution for most PET detectors, the full-width-at-half-maximum (FWHM) of ^{18}F and ^{68}Ga are indistinguishable in soft tissue (3.01 mm vs. 3.09

mm) (Sanchez-Crespo et al., *Eur J Nucl Med Mol Imaging*, vol. 31, pp. 44-51, 2004). That finding implies that with the standard spatial resolution of 5 to 7 mm for current clinical scanners, image quality using ^{68}Ga -based radiotracers will likely be indistinguishable from that of ^{18}F -based agents, stimulating interest in the development of ^{68}Ga -labeled compounds for medical imaging (Sanchez-Crespo et al., *Eur J Nucl Med Mol Imaging*, vol. 31, pp. 44-51, 2004; Khan et al., *Eur J Surg Oncol*, vol. 35, pp. 561-567, 2009; Fani et al., *Contrast Media Mol Imaging*, vol. 3, pp. 67-77, 2008). With a physical half-life of 68 min, ^{68}Ga is also matched nicely to the pharmacokinetics of many peptides used for imaging. Few ^{68}Ga -labeled, mechanism-based radiotracers for prostate cancer have been reported previously, and none for PSMA. Furthermore, ^{68}Ga is introduced to biomolecules through macrocyclic chelators, which allows possible kit formulation and wide availability of the corresponding imaging agents.

SUMMARY OF THE INVENTION

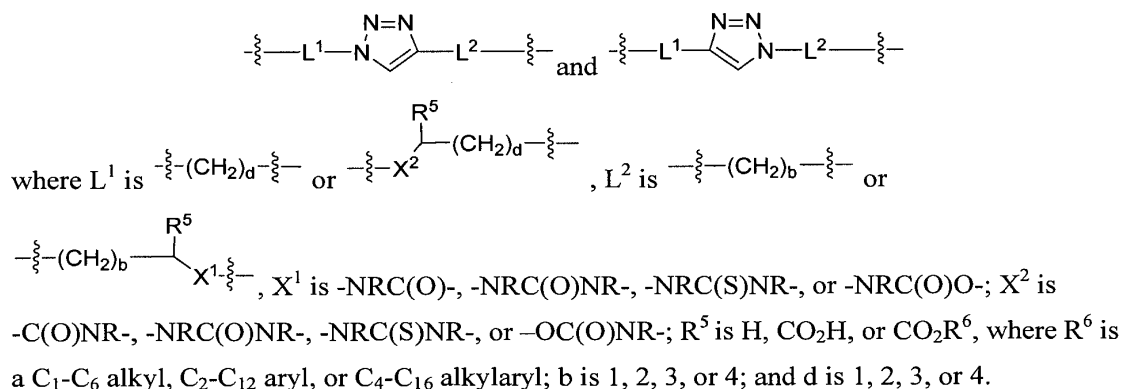
[0009] The present invention satisfies the long standing and unmet need for new imaging and therapeutic compounds for targeting prostate cancer and cancer angiogenesis. The present invention, in particular, provides therapeutic compounds and imaging agents which differ from the prior art in modifications which were not previously known or suggested. Furthermore, the invention provides imaging agents that offer better contrast between target tissues and non-target tissues. The invention also provides compounds with greater cellular retention and low molecular weight.

[0010] Embodiments of the invention include compounds having the structure



[0011] wherein the subunits associated with elements p, q, r, and s may be in any order. Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4, and R is each independently H or $\text{C}_1\text{-C}_4$ alkyl.

[0012] Variable r is 0 or 1. Tz is a triazole group selected from the group consisting of

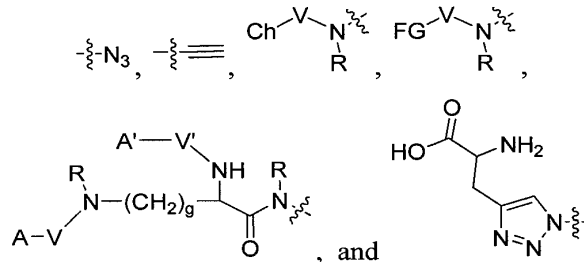


[0013] Variable q is 0 or 1. W is -NRC(O)-, -NRC(O)NR-, -NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-; R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein if one of R² and R³ is CO₂H or CO₂R², then the other is H; n is 1, 2, 3, 4, 5 or 6.

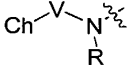
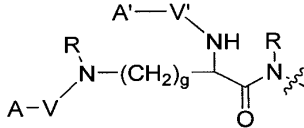
[0014] Variable s is 0 or 1. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O); and m is 1, 2, 3, 4, 5, or 6.

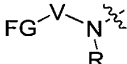
[0015] Variable p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl.

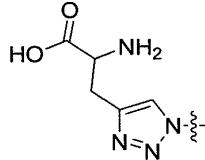
[0016] G is a moiety selected from the group consisting of

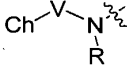


where Ch is a metal chelating moiety, optionally including a chelated metal; FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum; one of A and A' is Ch and the other is FG; V and V' are independently -C(O)-, -NRC(O)-, -NRC(S)-, or -OC(O)-; and g is 1, 2, 3, 4, 5, or 6. The following conditions also apply:

- 1) when G is , or  and r is 0, then q and s are both 1;

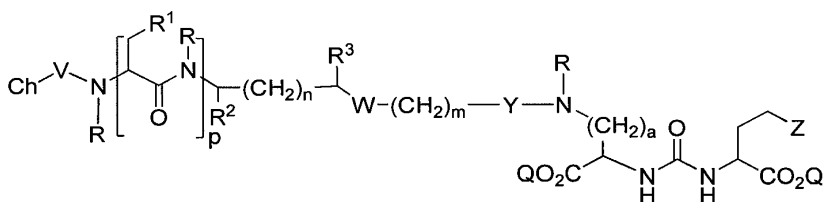
- 2) when G is  and r is 0, then q and s are both 0 or both 1;

- 3) when G is  then p is 0 and R² is H, and the structure optionally includes a chelated metal ion.

- 4) when G is  and r is 0, then if p is 0, then one of R² and R³ is CO₂R², and the other is H; and

- 5) when g is  or , then r is 0.

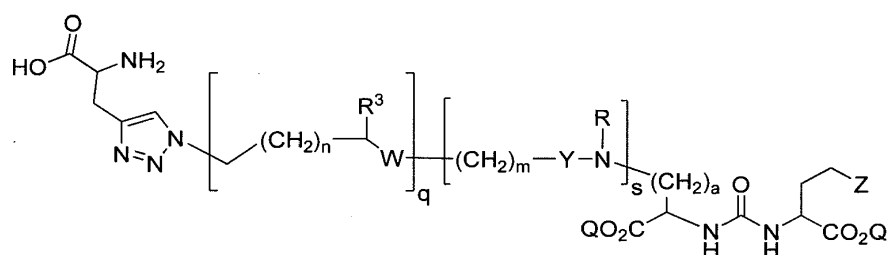
[0017] Embodiments include compounds having the structure



wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4, and R is each independently H or C₁-C₄ alkyl. Ch is a metal chelating moiety optionally including a chelated metal. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O). V is -C(O)-, -NRC(O)-, -NRC(S)-, or -OC(O)-. In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; and p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl,

wherein when one of R^2 and R^3 is CO_2H or CO_2R^2 , the other is H, and when p is 0, one of R^2 and R^3 is CO_2R^4 , and the other is H.

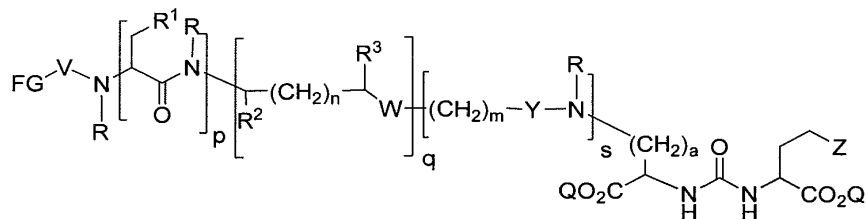
[0018] Some embodiments further include a chelated metal. In some embodiments, the chelated metal is Tc, In, Ga, Y, Lu, Re, Cu, Ac, Bi, Pb, Sm, Sc, Co, Ho, Gd, Eu, Tb, or Dy. In some embodiments, the chelated metal is an isotope, for example. In some embodiments, the isotope is Tc-94m, Tc-99m, In-111, Ga-67, Ga-68, Y-86, Y-90, Lu-177, Re-186, Re-188, Cu-64, Cu-67, Co-55, Co-57, Sc-47, Ac-225, Bi-213, Bi-212, Pb-212, Sm-153, Ho-166, or Dy-166. Embodiments include compounds having the structure



optionally including a chelated metal ion. Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. R is each independently H or C_1 - C_4 alkyl. W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$. Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})-$;

[0019] In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; q is 0 or 1; and s is 0 or 1. R^3 is H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl. Some embodiments further include a chelated metal ion. In some embodiments, the metal ion is Tc, Re, Cu, or Ga. In some embodiments, the metal ion is Tc-99m, Re-186, Re-188, Cu-64, or Ga-68. In some embodiments, the metal ion is Tc-99m.

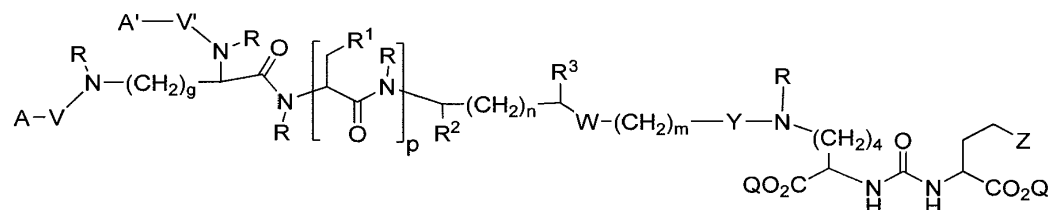
[0020] Embodiments include compounds having the structure



where p , q , and s are in the order drawn, and q and s are either both 0 or both 1. Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or

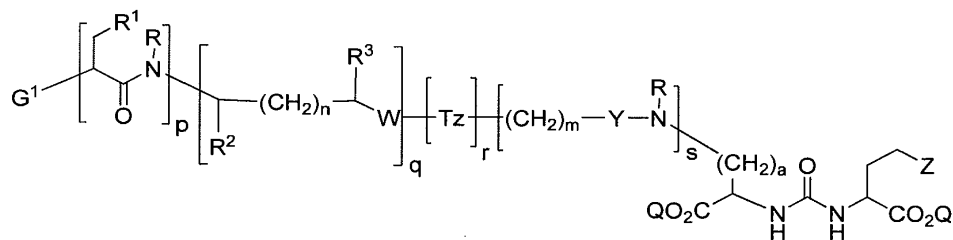
4. FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum. R is each independently H or C₁-C₄ alkyl. V is -C(O)- or -NRC(O)- or -NRC(S)-. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O). In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or CO₂R², where R² is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R² and R³ is CO₂H or CO₂R², the other is H. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum.

[0021] Embodiments include compounds having the structure

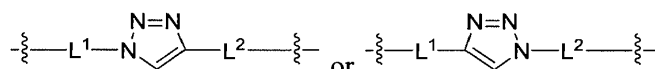


wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. One of A and A' is Ch and the other is FG, where FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum and Ch is metal chelating moiety optionally including a chelated metal. R is each independently H or C₁-C₄ alkyl. V or V' are independently -C(O)-, -NRC(O)-, or -NRC(S)-. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O). In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; and g is 1, 2, 3, 4, 5, or 6; p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R² and R³ is CO₂H or CO₂R², the other is H. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum. Some embodiments further include a chelated metal.

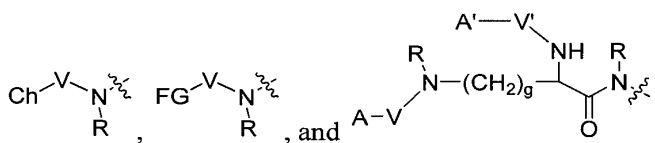
[0022] Embodiments include compounds having the structure



wherein subunits associated with p, q, r, and s may be in any order. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. R is each independently H or C₁-C₄ alkyl. In this exemplary embodiment r is 1. Tz is a triazole group having the structure



where L¹ is $-\text{---}(\text{CH}_2)_d\text{---}$ or $-\text{---}\text{X}^2\text{---}$; L² is $-\text{---}(\text{CH}_2)_b\text{---}$ or $-\text{---}\text{X}^1\text{---}$; X¹ is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, or -NRC(O)O-; X² is -C(O)NR-, -NRC(O)NR-, NRC(S)NR-, or -OC(O)NR-; R⁵ is H, CO₂H, or CO₂R⁶, where R⁶ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl; b is 1, 2, 3, or 4; and d is 1, 2, 3, or 4. In exemplary embodiments q is 0 or 1, W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-; n is 1, 2, 3, 4, 5 or 6; and R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein if one of R² and R³ is CO₂H or CO₂R², then the other is H. In exemplary embodiments s is 0 or 1; Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O); and m is 1, 2, 3, 4, 5, or 6. In exemplary embodiments p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different; and R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. G¹ is a moiety selected from the group consisting of



where Ch is a metal chelating moiety, optionally including a chelated metal; FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum; one of A and A' is Ch and the other is FG; V and V' are each independently $-C(O)-$, $-NRC(O)-$, $-NRC(S)-$, or $-OC(O)-$; and g is 1, 2, 3, 4, 5, or 6. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum. Some embodiments include a chelated metal.

[0023] Embodiments of the invention include methods of imaging one or more cells, organs or tissues by exposing the cell to or administering to a organism an effective amount of a compound discussed above, where the compound includes a fluorescent dye moiety, or a metal isotope suitable for imaging.

[0024] Embodiments of the invention include methods of treating a tumor comprising administering a therapeutically effective amount of a compound discussed above, where the compound includes a therapeutically effective radioisotope.

[0025] Embodiments of the invention include methods for sorting cells by exposing the cells to a compound discussed above, where the compound includes a fluorescent dye moiety, followed by separating cells which bind the compound from cells which do not bind the compound.

[0026] Embodiments of the invention include methods of intraoperative tumor mapping comprising administering an effective amount of a compound discussed above to a subject, where the compound includes a fluorescent dye moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **Figure 1** shows SPECT-CT images of a PSMA+ LNCaP tumor-bearing mouse injected intravenously with exemplary compound $[^{99m}\text{Tc}]SRV32$.

[0028] **Figure 2.** GE eXplore VISTA pseudodynamic PET image (co-registered with the corresponding CT image) of a PSMA+ LNCaP tumor-bearing mouse injected intravenously with 0.2 mCi (7.4 MBq) of exemplary compound $[^{68}\text{Ga}]SRV27$.

[0029] **Figure 3.** GE eXplore VISTA PET image (co-registered with the corresponding CT image) of a PSMA+ PIP and PSMA- flu tumor-bearing mouse injected intravenously with 0.2 mCi (7.4 MBq) of exemplary compound $[^{68}\text{Ga}]SRV100$.

[0030] **Figure 4** shows a synthetic scheme for exemplary compound SRV100 and $[^{111}\text{In}]SRV100$.

- [0031] **Figure 5** shows SPECT-CT images of a PSMA+ PC-3 PIP tumor-bearing mouse injected intravenously with exemplary compound [^{111}In]SRV27.
- [0032] **Figure 6** shows SPECT-CT images of a PSMA+ PC-3 PIP tumor-bearing mouse injected intravenously with exemplary compound [^{111}In]SRV100.
- [0033] **Figure 7** shows SPECT-CT images of a PSMA+ PC-3 PIP tumor-bearing mouse injected intravenously with exemplary dual modality compound [^{111}In]SRV73.
- [0034] **Figure 8** shows the absorbance and emission spectra, and quantum yield of exemplary compound YC-27.
- [0035] **Figure 9** shows the fluorescence decay of exemplary compound YC-27.
- [0036] **Figure 10** shows an IC_{50} curve of compound YC-27 using a fluorescence-based NAALADase assay
- [0037] **Figure 11** shows *in vivo* imaging of a NOD/SCID mouse (mouse #1), bearing PC3-PIP (forward left flank) and PC3-flu (forward right flank) tumors. Mouse #1 received 10 nmol of YC-27 and dorsal (animal prone) and ventral (animal supine) views were obtained. Dorsal and ventral views at 40 min p.i. (A, B, respectively); 18.5 h (C, D); 23 h (E, F); 42.5 h (G, H); 68 h (I, J). Dorsal view of pre-injection image (K). Dorsal and ventral views 70.5 h p.i. (L, M). Images after midline laparotomy (N) and individually harvested organs (O) on a Petri dish at 70.5 h p.i.. Images were scaled to the same maximum (arbitrary units).
- [0038] **Figure 12** shows *in vivo* imaging of a NOD/SCID mouse (mouse #2) (left panel), bearing PC3-PIP (forward left flank) and PC3-flu (forward right flank) tumors. Mouse #2 received 1 nmol of YC-27 and dorsal (animal prone) and ventral (animal supine) views were obtained. Dorsal and ventral views of the pre-injection image (A, B, respectively); 10 min p.i. (C, D); 20.5 h (E, F); 24 h (G, H). Images after midline laparotomy (I) and individually harvested organs (J) on a Petri dish at 24 h p.i.. *Right Panels:* Mouse #3 in same orientation as mouse #2. Mouse #3 received 1 nmol of YC-27 co-injected with 1 μmol of DCIBzL, which served as a blocking agent to test binding specificity. Images were scaled to the same maximum (arbitrary units).
- [0039] **Figure 13** shows SPECT-CT images of a PSMA+ LNCaP tumor-bearing mouse injected intravenously with exemplary compound [$^{99\text{m}}\text{Tc}$]SRVI34B.
- [0040] **Figure 14** shows SPECT-CT images of a PSMA+ PC3-PIP tumor-bearing mouse injected intravenously with exemplary compound [$^{99\text{m}}\text{Tc}$]SRVI34B.

[0041] **Figure 15** shows SPECT-CT images of a PSMA+ PC3-PIP (forward left flank) and PSMA- PC3-flu (forward right flank) tumor-bearing mouse injected intravenously with exemplary compound [^{99m}Tc]SRVI34A.

[0042] **Figure 16** shows SPECT-CT images of a PSMA+ PC3-PIP (forward left flank) and PSMA- PC3-flu (forward right flank) tumor-bearing mouse injected intravenously with exemplary compound [^{99m}Tc]SRVI34B.

[0043] **Figure 17** shows PC3-PIP and PC3-flu cells treated with fluorescent compound YC-VIII-36 (green, top left) and DAPI (blue), and PC3-PIP and PC3-flu cells treated with both YC-VIII-36 and PSMA inhibitor, PMPA.

[0044] **Figure 18** shows PC3-PIP cells treated with DAPI (blue) and varying concentrations of YC-VIII-36 (green).

[0045] **Figure 19** shows time dependent internalization of YC-VIII-36 into PC3-PIP cells treated with YC-VIII-36 (green) and DAPI (blue).

[0046] **Figure 20** shows titration and detection of varying amounts of YC-VIII-36 injected subcutaneously into a nude mouse. (IVIS spectrum with 10 second exposure followed by spectral unmixing)

[0047] **Figure 21** shows fluorescence images of a PSMA+ PC3-PIP and PSMA- PC3-flu tumor-bearing mouse injected intravenously with exemplary compound YC-VIII-36.

[0048] **Figure 22** shows fluorescence images of a PSMA+ PC3-PIP and PSMA- PC3-flu tumor-bearing mouse injected intravenously with exemplary compound YC-VIII-36 180 minutes after injection (top) and biodistribution of exemplary compound YC-VIII-36 180 minutes after injection (bottom).

[0049] **Figure 23** shows FACS analysis showing the percent subpopulation of PSMA positive cells in PC3-flu, PC3-PIP, and LNCaP cells.

[0050] **Figure 24** shows cell sorting results for PC3-PIP cells treated with exemplary compound YC-VIII-36, including initial percentage (top center), and after 3 passages of sorting (bottom).

[0051] **Figure 25** shows the number of spiked PIP-pos cells into 10 million of PC3-flu detectable by 100 nM compound YC-VIII-36 in flow cytometry (BD LSR-II). Gate P1 is total number of single cells counted; gate P2 at higher intensity is the number of Pip-pos cells detected and gate P3 at lower intensity.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0052] Some embodiments of the current invention are discussed in detail below. In describing embodiments, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. A person skilled in the relevant art will recognize that other equivalent components can be employed and other methods developed without departing from the broad concepts of the current invention. All references cited anywhere in this specification are incorporated by reference as if each had been individually incorporated.

[0053] Where a range of values is provided in the present application, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The end values of any range are included in the range.

Definitions

[0054] The following terms below generally have the meaning that would be readily understood by persons skilled in the art. The definitions are provided herein for clarity. Where a definition excludes an art-recognized meaning, the term should be taken to have the meaning set forth below. Where the art-recognized meaning and the meaning below differ but are not exclusive, the intended meaning is clear by the context in which it is used.

[0055] As used herein, "agent" is a non-peptide, small molecule compound.

[0056] By "cell substrate" is meant the cellular or acellular material (e.g., extracellular matrix, polypeptides, peptides, or other molecular components) that is in contact with the cell.

[0057] By "control" is meant a standard or reference condition.

[0058] By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, organ or subject.

[0059] By "effective amount" is meant a quantity sufficient to produce a measurable difference, when compared with a control. For example, an amount sufficient to produce a measurable image, when the compound is used for imaging, or an amount sufficient to ameliorate the symptoms of a disease, when the compound is used for therapy. The effective amount of an active therapeutic agent for the treatment of a disease or injury varies depending

upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending clinician will decide the appropriate amount and dosage regimen.

[0060] By "modifies" is meant alters. An agent that modifies a cell, substrate, or cellular environment produces a biochemical alteration in a component (e.g., polypeptide, nucleotide, or molecular component) of the cell, substrate, or cellular environment.

[0061] As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

[0062] By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

[0063] By "therapeutic delivery device" is meant any device that provides for the release of a therapeutic agent. Exemplary therapeutic delivery devices include tablets and pills, described below, as well as syringes, osmotic pumps, indwelling catheters, delayed-release and sustained-release biomaterials.

[0064] As used herein, the terms "treat," "treating," "treatment," "therapeutic" and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

[0065] The compounds herein described may have one or more asymmetric centers or planes. Compounds of the present invention containing an asymmetrically substituted atom may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms (racemates), by asymmetric synthesis, or by synthesis from optically active starting materials. Resolution of the racemates can be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Cis and trans geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral (enantiomeric and diastereomeric), and racemic forms, as well as all geometric isomeric forms

of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

[0066] The compounds herein described may have one or more charged atoms. For example, the compounds may be zwitterionic, but may be neutral overall. Other embodiments may have one or more charged groups, depending on the pH and other factors. In these embodiments, the compound may be associated with a suitable counter-ion. It is well known in the art how to prepare salts or exchange counter-ions. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Counter-ions may be changed, for example, by ion-exchange techniques such as ion-exchange chromatography. All zwitterions, salts and counter-ions are intended, unless the counter-ion or salt is specifically indicated. In certain embodiments, the salt or counter-ion may be pharmaceutically acceptable, for administration to a subject. Pharmaceutically acceptable salts are discussed later.

[0067] As used herein, a "protecting group" is a chemical substituent which can be selectively removed by readily available reagents which do not attack the regenerated functional group or other functional groups in the molecule. Suitable protecting groups are known in the art and continue to be developed. Suitable protecting groups may be found, for example in Wutz et al. ("Greene's Protective Groups in Organic Synthesis, Fourth Edition," Wiley-Interscience, 2007). Protecting groups for protection of the carboxyl group, as described by Wutz et al. (pages 533-643), are used in certain embodiments. In some embodiments, the protecting group is removable by treatment with acid. Specific examples of protecting groups include but are not limited to, benzyl, p-methoxybenzyl (PMB), tertiary butyl (^tBu), methoxymethyl (MOM), methoxyethoxymethyl (MEM), methylthiomethyl (MTM), tetrahydropyranyl (THP), tetrahydrofuranyl (THF), benzyloxymethyl (BOM), trimethylsilyl (TMS), triethylsilyl (TES), t-butyl dimethylsilyl (TBDMS), and triphenylmethyl (trityl, Tr). Persons skilled in the art will recognize appropriate situations in which protecting groups are required and will be able to select an appropriate protecting group for use in a particular circumstance.

[0068] As used herein, "alkyl" is intended to include branched, straight-chain, and cyclic saturated aliphatic hydrocarbon groups. Examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, and *sec*-pentyl. In certain embodiments, alkyl groups are C₁-C₆ alkyl groups or C₁-C₄ alkyl groups. Particular alkyl groups are methyl, ethyl, propyl, butyl, and 3-pentyl. The term "C₁-C₆ alkyl" as used herein means straight-chain, branched, or cyclic C₁-C₆ hydrocarbons which are completely saturated and hybrids thereof such as (cycloalkyl)alkyl. Examples of C₁-C₆ alkyl substituents include methyl (Me), ethyl (Et), propyl (including *n*-propyl (n-Pr, ⁿPr), *iso*-propyl (i-Pr, ⁱPr), and cyclopropyl (c-Pr, ^cPr)), butyl (including *n*-butyl (n-Bu, ⁿBu), *iso*-butyl (i-Bu, ⁱBu), *sec*-butyl (s-Bu, ^sBu), *tert*-butyl (t-Bu, ^tBu), or cyclobutyl (c-Bu, ^cBu)), and so forth. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. Cycloalkyl groups typically will have 3 to about 8 ring members. In the term "(cycloalkyl)alkyl", cycloalkyl, and alkyl are as defined above, and the point of attachment is on the alkyl group. This term encompasses, but is not limited to, cyclopropylmethyl, cyclopentylmethyl, and cyclohexylmethyl. The alkyl group may be substituted or unsubstituted. Substituents are not counted towards the total number of atoms in the alkyl group, so long as the total atoms in the substituent(s) are not larger than the alkyl group.

[0069] As used herein, the term "aryl" includes aromatic groups that contain 1 to 3 separate or fused rings and from 2 to about 12 carbon atoms, and up to 3 heteroatoms as ring members. Examples of heteroatoms include nitrogen, oxygen or sulfur atoms. The aryl group may have 0, 1, 2 or 3 heteroatoms as ring members. Examples of aryl groups include but are not limited to phenyl, biphenyl and naphthyl, including 1-naphthyl and 2-naphthyl. Examples of aryl groups having heteroatoms include quinolinyl, isoquinolinyl, quinazolinyl, pyridyl, pyrazinyl, pyrimidyl, furanyl, pyrrolyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, triazinyl, oxazolyl, isoxazolyl, imidazolyl, indolyl, benzofuranyl, and benzothiazolyl, among others. The aryl group may be substituted or unsubstituted. Substituents are not counted towards the total number of atoms in the aryl group, so long as the total atoms in the substituent(s) are not larger than the aryl group.

[0070] As used herein, the term "alkylaryl" includes alkyl groups, as defined above, substituted by aryl groups, as defined above. The aryl group may be connected at any point on the alkyl group. The term C₄-C₁₆ alkylaryl includes alkylaryl groups having a total of 4 to 16 carbon atoms, counting the carbon atoms on the alkyl group and aryl group together. Examples

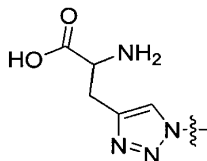
of alkylaryl groups include but are not limited to benzyl (phenylmethyl), phenylethyl, and naphthylmethyl. The alkylaryl group may be substituted or unsubstituted. Substituents are not counted towards the total number of atoms in the alkylaryl group, so long as the total atoms in the substituent(s) are not larger than the alkylaryl group.

[0071] The term "substituted," as used herein, means that any one or more hydrogens on the designated atom or group is replaced with a substituent, provided that the designated atom's normal valence is not exceeded, and that the substitution results in a stable compound. When a substituent is oxo (keto, i.e., =O), then 2 hydrogens on an atom are replaced. The present invention is intended to include all isotopes (including radioisotopes) of atoms occurring in the present compounds. When the compounds are substituted, they may be so substituted at one or more available positions, typically 1, 2, 3 or 4 positions, by one or more suitable groups such as those disclosed herein. Suitable groups that may be present on a "substituted" group include e.g., halogen; cyano; hydroxyl; nitro; azido; amino; alkanoyl (such as a C₁-C₆ alkanoyl group such as acyl or the like); carboxamido; alkyl groups (including cycloalkyl groups, having 1 to about 8 carbon atoms, for example 1, 2, 3, 4, 5, or 6 carbon atoms); alkenyl and alkynyl groups (including groups having one or more unsaturated linkages and from 2 to about 8, such as 2, 3, 4, 5 or 6, carbon atoms); alkoxy groups having one or more oxygen linkages and from 1 to about 8, for example 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those having one or more thioether linkages and from 1 to about 8 carbon atoms, for example 1, 2, 3, 4, 5 or 6 carbon atoms; alkylsulfinyl groups including those having one or more sulfinyl linkages and from 1 to about 8 carbon atoms, such as 1, 2, 3, 4, 5, or 6 carbon atoms; alkylsulfonyl groups including those having one or more sulfonyl linkages and from 1 to about 8 carbon atoms, such as 1, 2, 3, 4, 5, or 6 carbon atoms; aminoalkyl groups including groups having one or more N atoms and from 1 to about 8, for example 1, 2, 3, 4, 5 or 6, carbon atoms; carbocyclic aryl having 4, 5, 6 or more carbons and one or more rings, (e.g., phenyl, biphenyl, naphthyl, or the like, each ring either substituted or unsubstituted aromatic); arylalkyl having 1 to 3 separate or fused rings and from 6 to about 18 ring carbon atoms, (e.g. benzyl); arylalkoxy having 1 to 3 separate or fused rings and from 6 to about 18 ring carbon atoms (e.g. O-benzyl); or a saturated, unsaturated, or aromatic heterocyclic group having 1 to 3 separate or fused rings with 3 to about 8 members per ring and one or more N, O or S atoms, (e.g. coumarinyl, quinolinyl, isoquinolinyl, quinazolinyl, pyridyl, pyrazinyl, pyrimidyl, furanyl, pyrrolyl, thienyl, thiazolyl, triazinyl, oxazolyl, isoxazolyl, imidazolyl, indolyl, benzofuranyl,

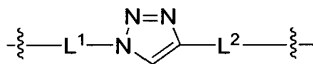
benzothiazolyl, tetrahydrofuranyl, tetrahydropyranyl, piperidiny, morpholinyl, piperazinyl, and pyrrolidinyl). Such heterocyclic groups may be further substituted, e.g. with hydroxy, alkyl, alkoxy, halogen and amino.

[0072] As used herein, where an internal substituent is flanked by bonds (for example -NRC(O)-) the order of the atoms is fixed, the orientation of the group may not be reversed, and is inserted into a structure in the orientation presented. In other words -NRC(O)- is not the same as -C(O)NR-. As used herein the term C(O) (for example -NRC(O)-) is used to indicate a carbonyl (C=O) group, where the oxygen is bonded to the carbon by a double bond.

[0073] A substituent bearing a broken bond, such as the example shown below, means that the substituent is directly bonded to the molecule at the indicated position. No additional methylene (CH₂) groups are implied.



[0074] Substituents bearing two broken bonds, such as the example shown below, means that the orientation of the atoms is as-indicated, left to right and should be inserted into a molecule in the orientation shown. No additional methylene (CH₂) groups are implied unless specifically indicated.

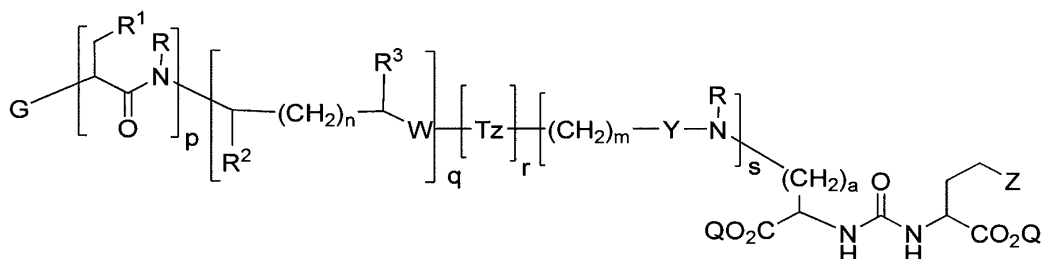


Embodiments

[0075] As described herein, all embodiments or subcombinations may be used in combination with all other embodiments or subcombinations, unless mutually exclusive.

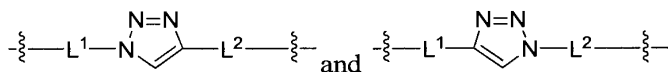
[0076] In some of the following embodiments, Z is CO₂Q. In some of the following embodiments, Q is H. In some of the following embodiments, m is 4, 5, or 6. In some of the following embodiments, m is 6. In some of the following embodiments, n is 2, 3, or 4. In some of the following embodiments, n is 3. In some of the following embodiments, a is 3 or 4. In some of the following embodiments, a is 4. In some of the following embodiments, Y is -C(O)-. In some of the following embodiments, W is -NHC(O)-.

[0077] Embodiments of the invention include compounds having the structure



wherein the subunits associated with elements p, q, r, and s may be in any order. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4, and R is each independently H or C₁-C₄ alkyl.

[0078] Variable r is 0 or 1. Tz is a triazole group selected from the group consisting of



where L¹ is $\text{---}(\text{CH}_2)_d\text{---}$ or $\text{---}X^2\text{---}$, L² is $\text{---}(\text{CH}_2)_b\text{---}$ or

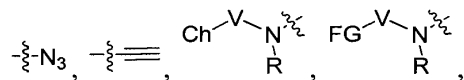
$\text{---}(\text{CH}_2)_b\text{---}X^1\text{---}$, X¹ is -NRC(O)-, -NRC(O)NR-, -NRC(S)NR-, or -NRC(O)O-; X² is -C(O)NR-, -NRC(O)NR-, -NRC(S)NR-, or -OC(O)NR-; R⁵ is H, CO₂H, or CO₂R⁶, where R⁶ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl; b is 1, 2, 3, or 4; and d is 1, 2, 3, or 4.

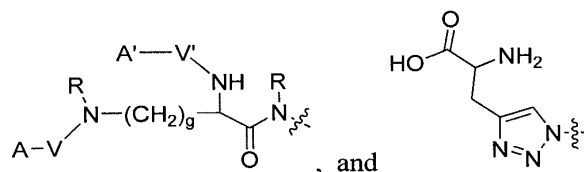
[0079] Variable q is 0 or 1. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-; R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein if one of R² and R³ is CO₂H or CO₂R², then the other is H; n is 1, 2, 3, 4, 5 or 6.

[0080] Variable s is 0 or 1. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-; and m is 1, 2, 3, 4, 5, or 6.

[0081] Variable p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl.

[0082] G is a moiety selected from the group consisting of



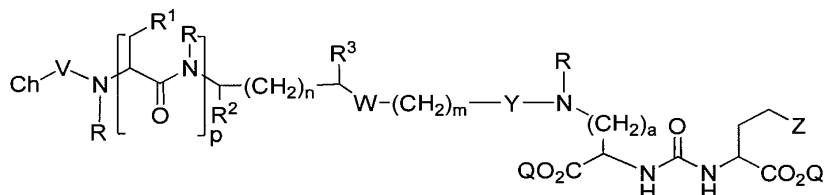


where Ch is a metal chelating moiety, optionally including a chelated metal; FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum; one of A and A' is Ch and the other is FG; V and V' are independently $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, or $-\text{OC}(\text{O})-$; and g is 1, 2, 3, 4, 5, or 6. The following conditions also apply:

- 1) when G is , or and r is 0, then q and s are both 1;
- 2) when G is and r is 0, then q and s are both 0 or both 1;
- 3) when G is then p is 0 and R^2 is H, and the structure optionally includes a chelated metal ion;
- 4) when G is and r is 0, then if p is 0, then one of R^2 and R^3 is CO_2R^2 , and the other is H; and
- 5) when g is $-\text{N}_3$ or $-\text{C}\equiv\text{N}$, then r is 0.

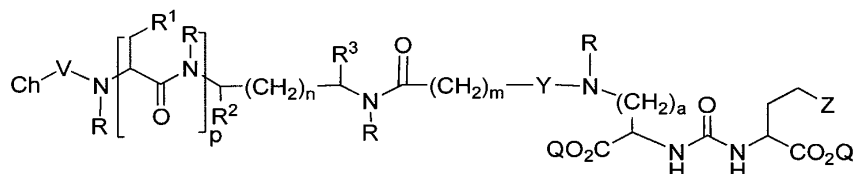
[0083] In some embodiments, Z is CO_2Q . In some embodiments, Q is H. In some embodiments, m is 4, 5, or 6. In some embodiments, m is 6. In some embodiments, n is 2, 3, or 4. In some embodiments, n is 3. In some embodiments, a is 4. In some embodiments, subunits associated with elements p, q and s are in the order drawn and r may be in any location, including between one of p, q, or s. In some embodiments r is 0.

[0084] Embodiments include compounds having the structure

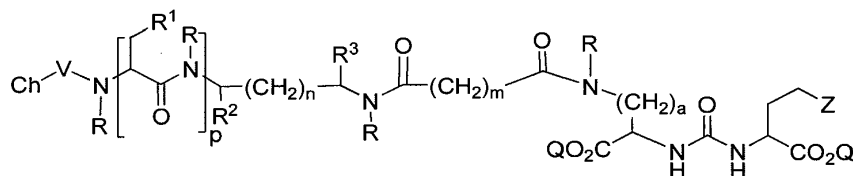


wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4, and R is each independently H or C₁-C₄ alkyl. Ch is a metal chelating moiety optionally including a chelated metal. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O). V is -C(O)-, -NRC(O)-, -NRC(S)-, or -OC(O)-. In exemplary embodiment m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; and p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R² and R³ is CO₂H or CO₂R⁴, the other is H, and when p is 0, one of R² and R³ is CO₂R⁴, and the other is H.

[0085] In some embodiments, the compound has the structure shown below.



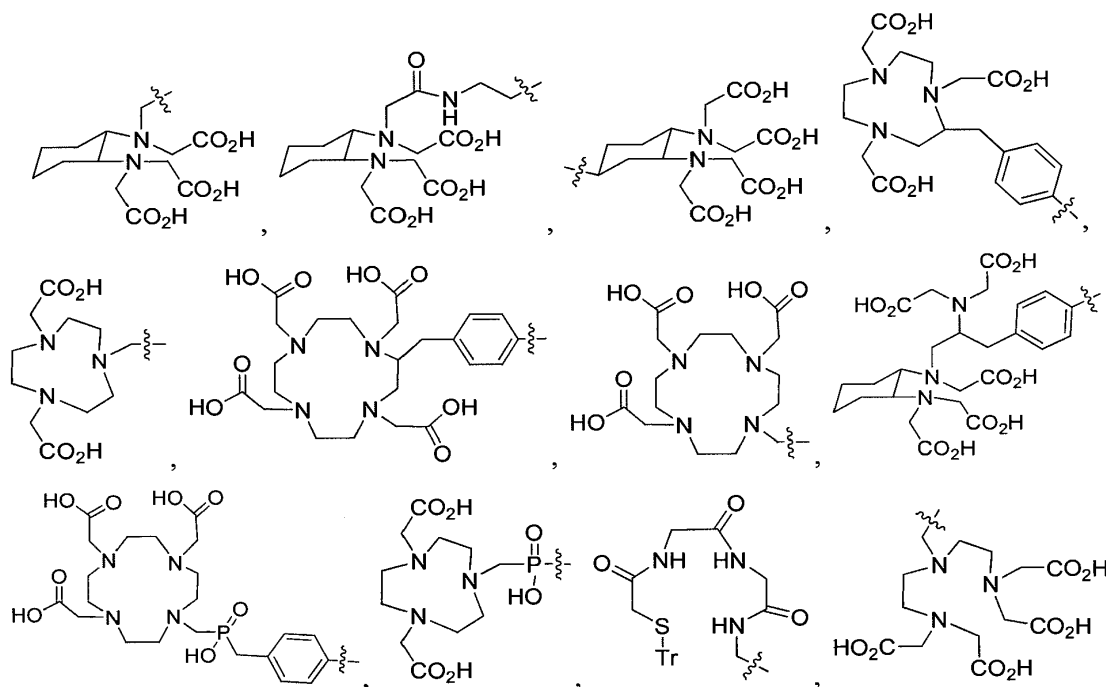
[0086] In some embodiments, the compound has the structure shown below.

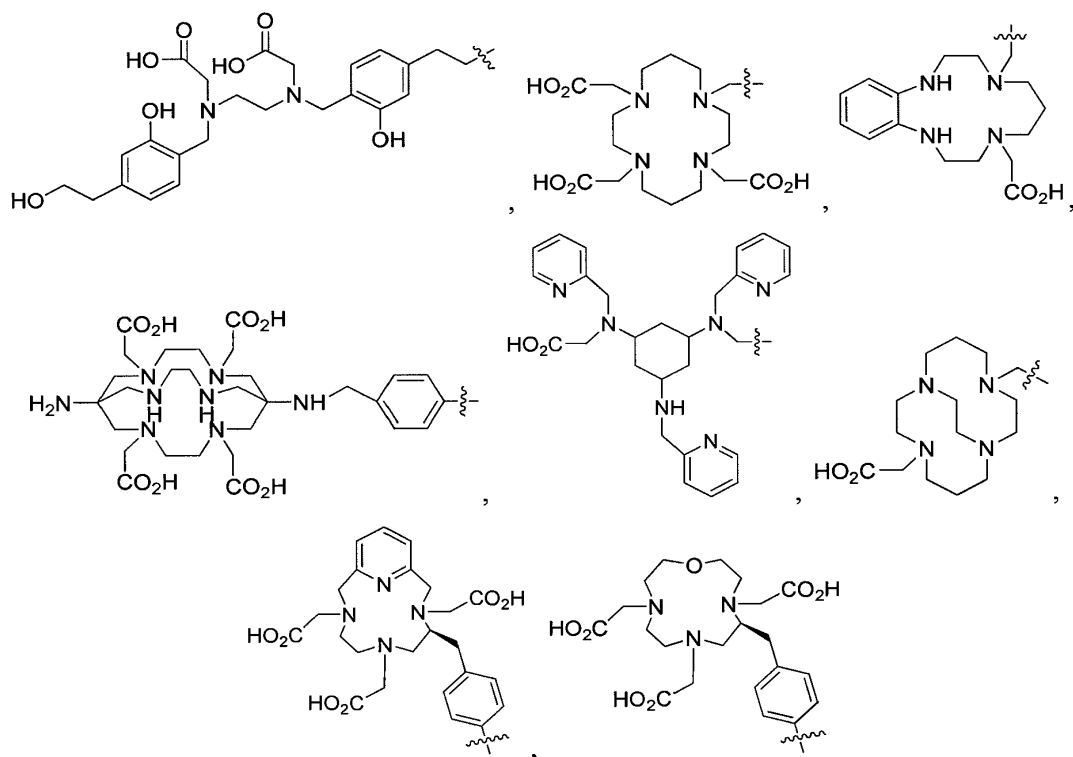


[0087] In some embodiments, p is 1, 2 or 3. When p is 2 or 3, each R¹ may be the same or different. When two R¹ groups are different, the two may be in any order. In some embodiments, p is 2. In some embodiments, p is 2, and both R¹ are the same. In some embodiments, R¹ is C₂-C₁₂ aryl. In some embodiments R¹ is phenyl. In some embodiments, R³ is CO₂H and R² is H. In some embodiments, R² is CO₂H and R³ is H. In some embodiments, R² and R³ are both H.

[0088] In some embodiments, p is 0. In some embodiments where p is 0, R^2 is CO_2R^4 , and R^3 is H. In some embodiments where p is 0, R^3 is CO_2R^4 , and R^2 is H. In some embodiments R^4 is $\text{C}_2\text{-C}_{12}$ aryl, or $\text{C}_4\text{-C}_{16}$ alkylaryl. In some embodiments R^4 is benzyl.

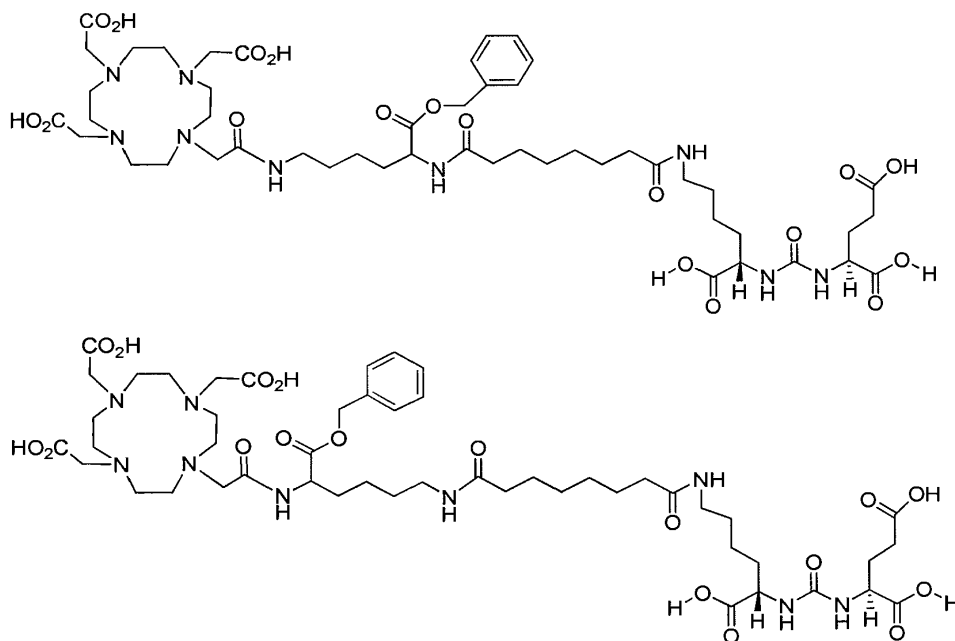
[0089] Ch is a metal chelating moiety optionally including a chelated metal. A metal chelating moiety is a chemical moiety that non-covalently binds a metal atom, usually with a plurality of non-covalent interactions. Ch includes any additional atoms or linkers necessary to attach the metal chelating moiety to the rest of the compound. For instance linking groups having alkyl, aryl, combination of alkyl and aryl, or alkyl and aryl groups having heteroatoms may be present in the chelating moiety. Numerous metal chelating moieties are known in the art. Any acceptable chelator can be used with the present invention as long as compatible and capable of chelating a desired metal. Examples of metal chelating moieties (Ch) include, but are not limited to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and Diethylenetriaminepentaacetic acid (DTPA). In some embodiments, Ch has a structure shown below.

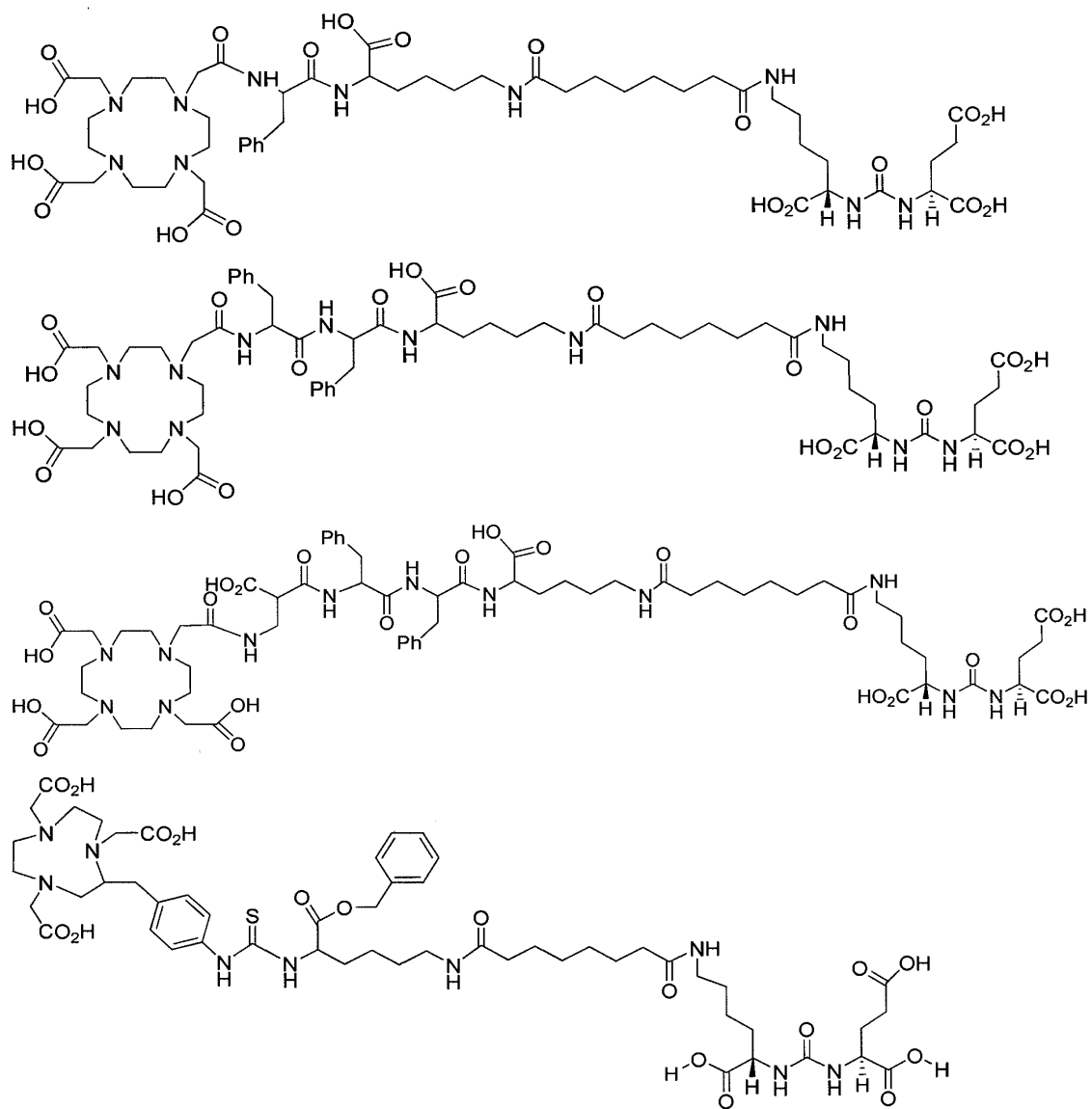


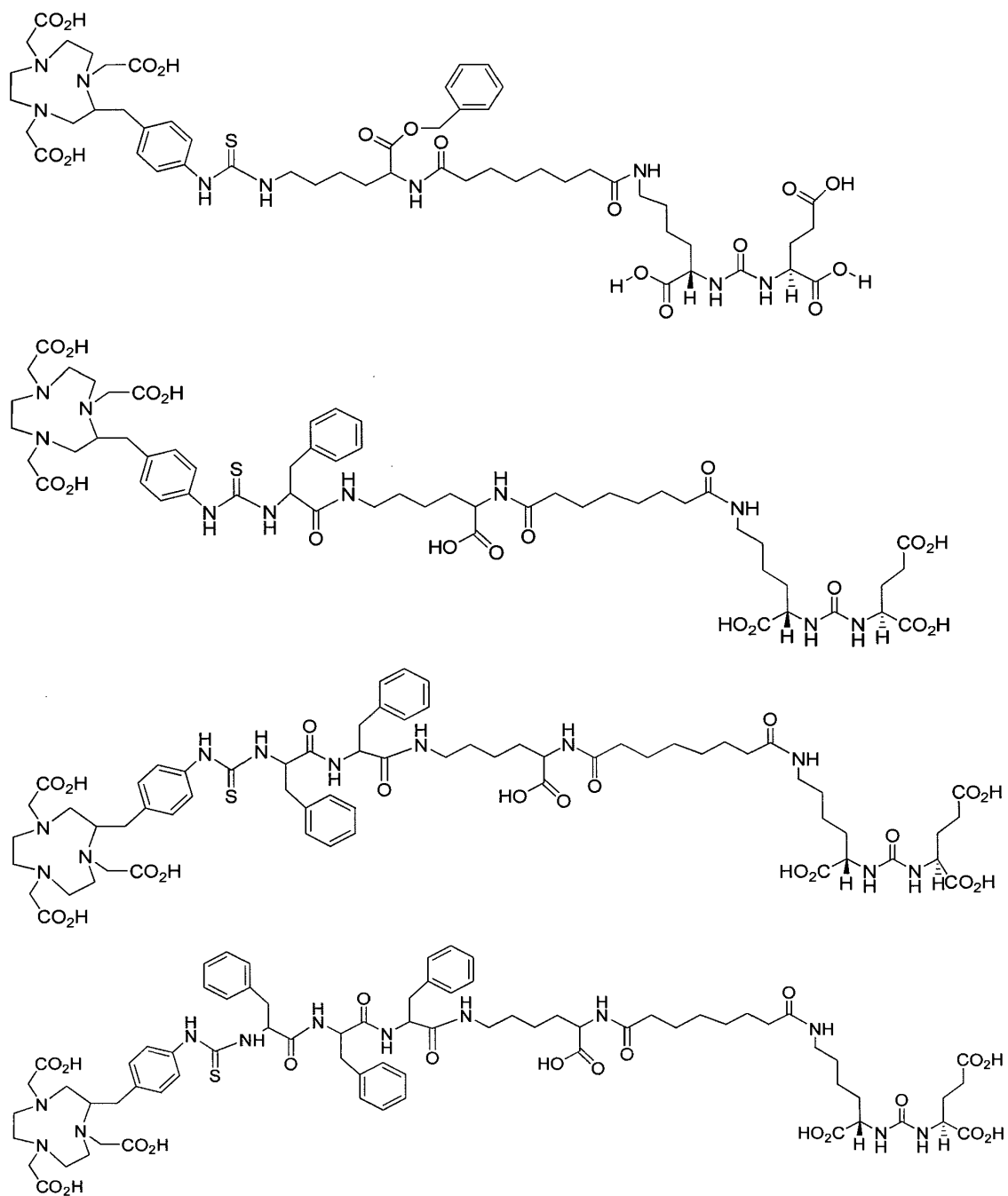


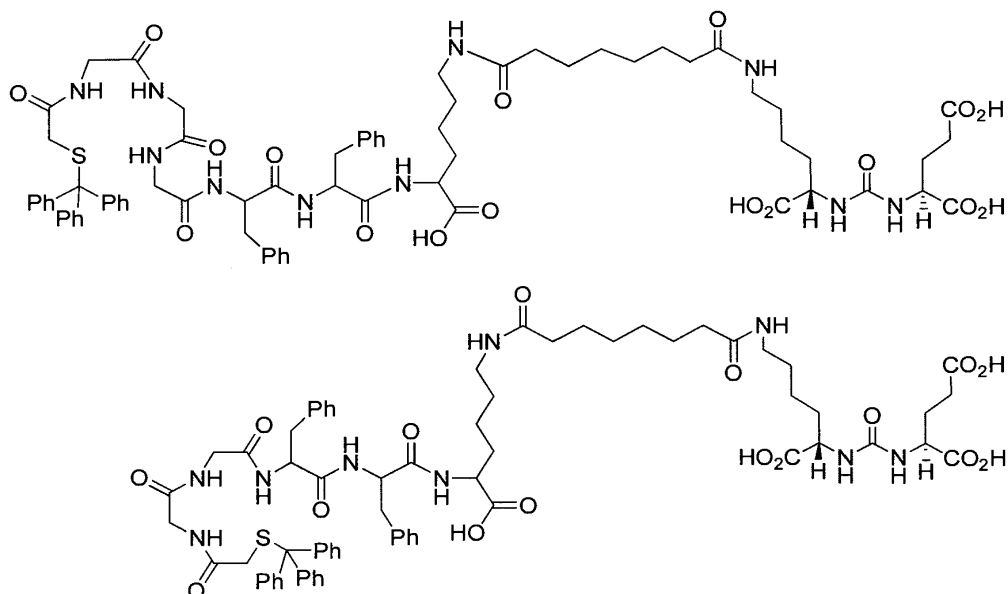
[0090]

Examples of specific compounds include the compounds shown below.



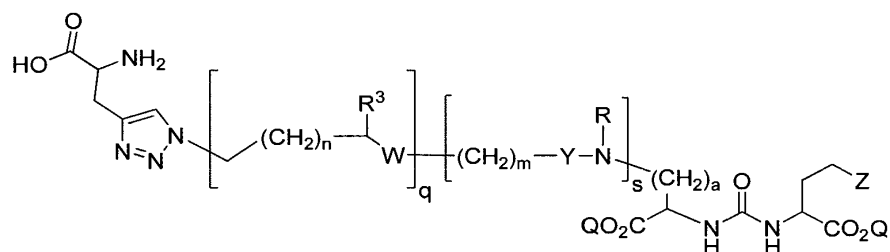






[0091] In some embodiments, the compound further includes a chelated metal. In some embodiments, the chelated metal is Tc, In, Ga, Y, Lu, Re, Cu, Ac, Bi, Pb, Sm, Sc, Co, Ho, Gd, Eu, Tb, or Dy. In some embodiments, the chelated metal is Tc, Ga, In, Cu, Y, Ac, Lu, Re, or Bi. In some embodiments the metal is an isotope, for example a radioactive isotope. In some embodiments, the isotope is Tc-99m, In-111, Ga-67, Ga-68, Y-86, Y-90, Lu-177, Re-186, Re-188, Cu-64, Cu-67, Co-55, Co-57, Sc-47, Ac-225, Bi-213, Bi-212, Pb-212, Sm-153, Ho-166, or Dy-166. In some embodiments, the isotope is Tc-99m, In-111, Ga-67, Ga-68, Y-90, Lu-177, Re-186, Re-188, Cu-67, Ac-225, Bi-213, or Bi-212.

[0092] Embodiments include compounds having the structure

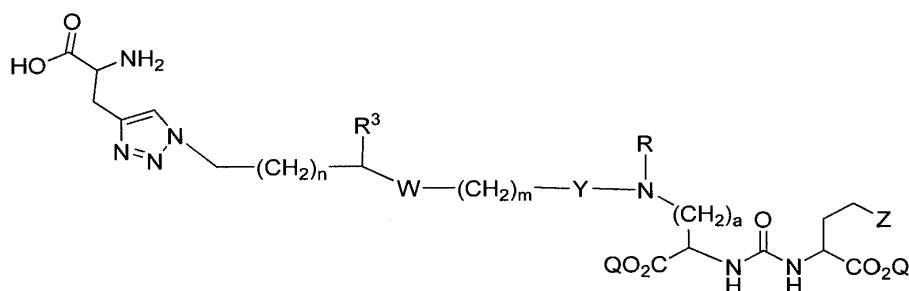


optionally including a chelated metal ion. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. R is each independently H or C₁-C₄ alkyl. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-,

-OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-. In some embodiments, subunits associated with q and s may be in the order shown or the reverse thereof.

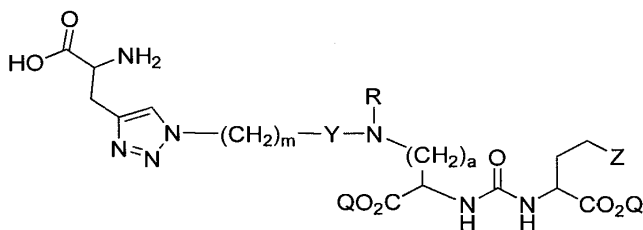
[0093] In exemplary embodiment m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; q is 0 or 1; and s is 0 or 1. R³ is H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. Some embodiments further include a chelated metal ion. In some embodiments, the metal ion is Tc, Re, Ga, or Cu. In some embodiments, the metal ion is Tc-99m, Re-186, Re-188, Cu-64, or Ga-68. In some embodiments, the metal ion is Tc-99m, Re-186 or Re-188.

[0094] In some embodiments, the compound has the structure



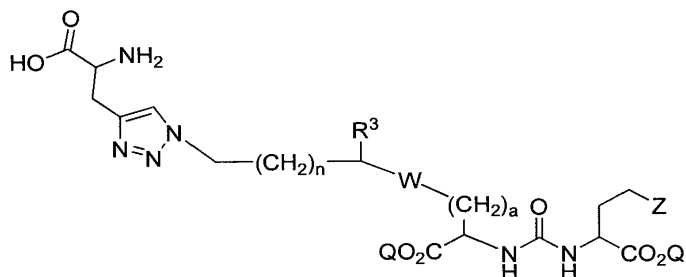
optionally including a chelated metal ion. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group and a is 1, 2, 3, or 4. R is each independently H or C₁-C₄ alkyl. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-. In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; and n is 1, 2, 3, 4, 5 or 6. R³ is H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl.

[0095] In some embodiments, the compound has the structure



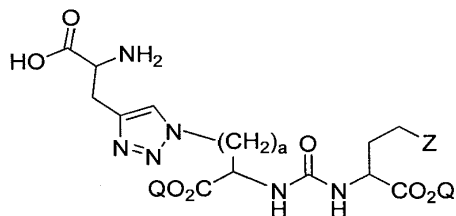
where a is 1, 2, 3, or 4. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-, and In exemplary embodiment m is 1, 2, 3, 4, 5, or 6.

[0096] In some embodiments, the compound has the structure



where a is 1, 2, 3, or 4. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-, and n is 1, 2, 3, 4, 5 or 6. R^3 is H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl.

[0097] In some embodiments, the compound has the structure



where a is 1, 2, 3, or 4.

[0098] In some embodiments, Y is -C(O)-.

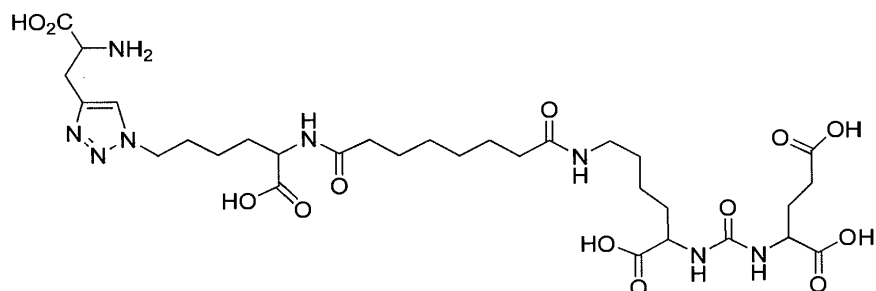
[0099] In some embodiments, W is -NHC(O)-.

[00100] In some embodiments, m is 4, 5, or 6. In some embodiments, m is 6.

[00101] In some embodiments, n is 2, 3, or 4. In some embodiments, n is 3.

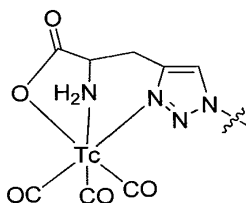
[00102] In some embodiments, R^3 is CO₂H. In some embodiments, R^3 is H. In some embodiments, R^3 is CO₂R⁴.

[00103] Examples of compounds include those having the structure shown below

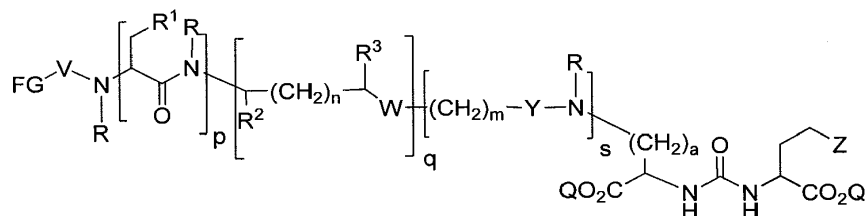


[00104] In some embodiments, the compound further includes a chelated metal ion. In some embodiments, the metal ion is Tc, Re, Cu, or Ga. In some embodiments, the metal ion is Tc-99m, Re-186, Re-188, Cu-64, or Ga-68. In some embodiments, the metal ion is Tc-99m.

[00105] The metal ion chelates to the triazole amino acid portion of the molecule to form a structure shown below using Tc as an example.



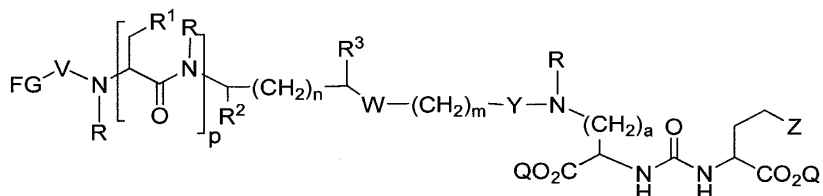
[00106] Embodiments include compounds having the structure



where p, q, and s are in the order drawn, and q and s are either both 0 or both 1. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum. R is each independently H or C₁-C₄ alkyl. V is -C(O)- or -NRC(O)- or -NRC(S)-. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O). In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or

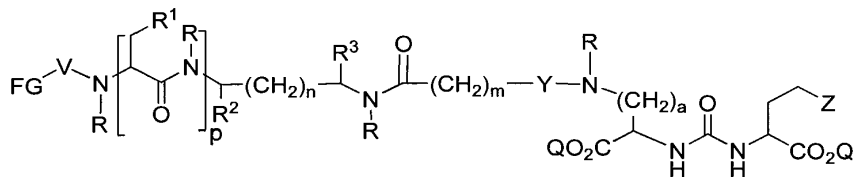
CO_2R^2 , where R^2 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein when one of R^2 and R^3 is CO_2H or CO_2R^2 , the other is H. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum.

[00107] Some embodiments have the structure shown below.

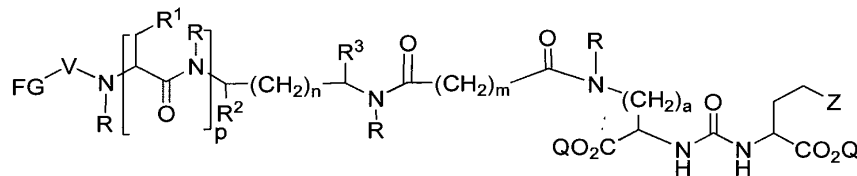


wherein Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3, or 4. FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum. R is each independently H or C_1 - C_4 alkyl. V is $-\text{C}(\text{O})-$ or $-\text{NRC}(\text{O})-$ or $-\text{NRC}(\text{S})-$. W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$. Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})-$. In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different. R^1 is H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl. R^2 and R^3 are independently H, CO_2H , or CO_2R^2 , where R^2 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein when one of R^2 and R^3 is CO_2H or CO_2R^2 , the other is H. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum.

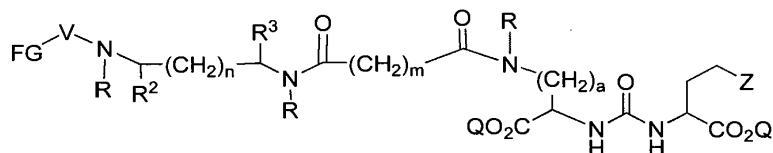
[00108] In some embodiments, the compound has the structure shown below.



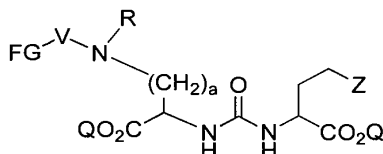
[00109] In some embodiments, the compound has the structure shown below.



[00110] In some embodiments, the compound has the structure shown below.



[00111] In some embodiments, the compound has the structure shown below.



[00112] In some embodiments, p is 1, 2 or 3. In some embodiments, p is 2. In some embodiments R¹ is C₂-C₁₂ aryl. In some embodiments, R¹ is phenyl.

[00113] In some embodiments, p is 0.

[001114] In some embodiments, R³ is CO₂H and R² is H. In some embodiments, R² is CO₂H and R³ is H. In some embodiments, R² is CO₂R⁴, and R³ is H. In some embodiments, R³ is CO₂R⁴, and R² is H. In some embodiments R⁴ is C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. In some R⁴ is benzyl. In some embodiments, R² is H, and R³ is H.

[00115] In some embodiments V is $-C(O)-$ or $-NRC(S)-$.

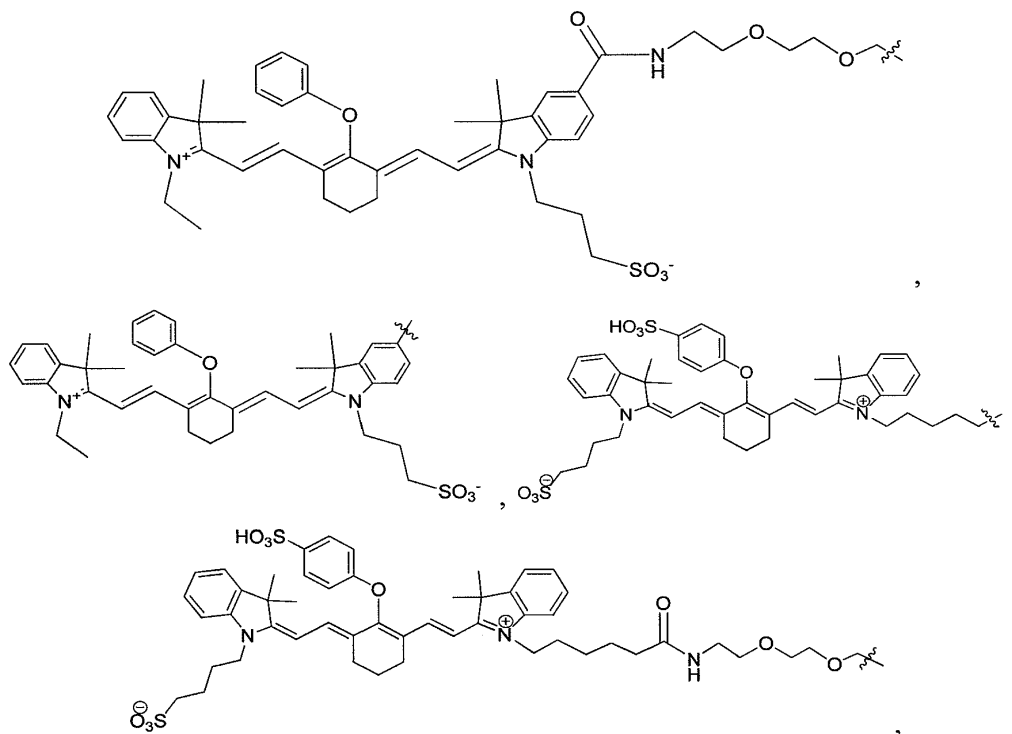
[001116] FG is a fluorescent dye moiety that emits light in the visible or near infrared spectrum. In some embodiments, FG is a fluorescent dye moiety which emits in the near infrared spectrum. FG includes any additional atoms or linkers necessary to attach the fluorescent dye moiety to the rest of the compound. For instance linking groups having alkyl, aryl, combination of alkyl and aryl, or alkyl and aryl groups having heteroatoms may be present in the chelating moiety, so long as the linker does not interfere with the fluorescence of the dye. In some embodiments, the fluorescent dye moiety includes a poly(ethyleneglycol) linker. Numerous fluorescent dye moieties are known in the art, and will be readily apparent to one of ordinary skill. Many fluorescent dyes are commercially available with activated groups used to react with protein sidechains or other compounds.

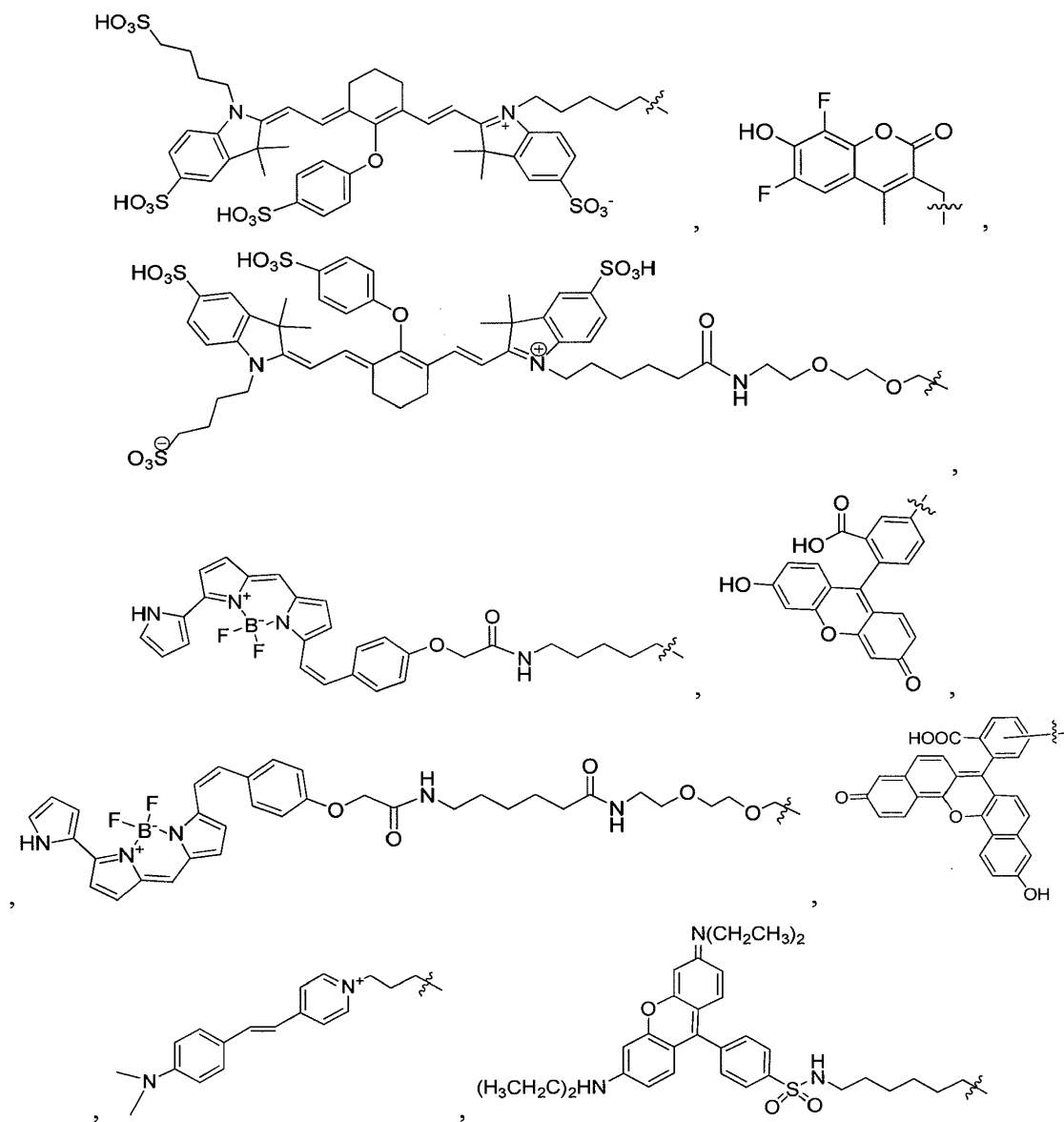
[00117] Examples of fluorescent compounds which may form all or part of the structure of FG include carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine, merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, and boron-dipyrromethane (BODIPY) compounds, to name a few.

[00118] Examples of fluorescent dye moieties include those described in WO 20089/109832, incorporated by reference herein in its entirety.

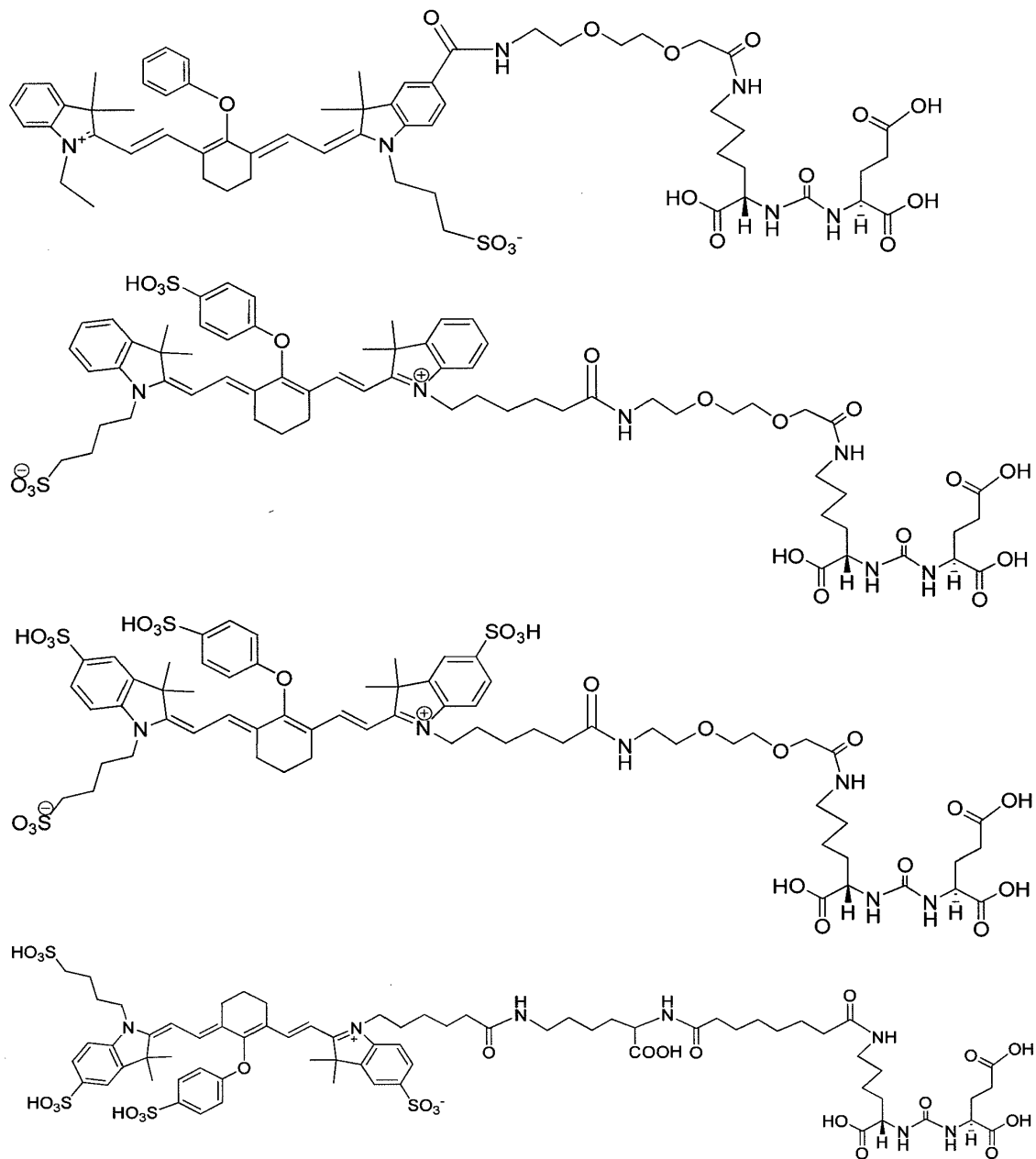
[00119] Specific dyes which emit in the near infrared spectrum include commercially available compounds Cy5, Cy5.5, and Cy7, available from GE Healthcare; VivoTag-680, VivoTag-S680, and VivoTag-S750, available from VisEn Medical; AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, and AlexaFluor790, available from Invitrogen; Dy677, Dy676, Dy682, Dy752, and Dy780, available from Dyonics; DyLight547, and DyLight647, available from Pierce; HiLyte Fluor 647, HiLyte Fluor 680, and HiLyte Fluor 750, available from AnaSpec; IRDye 800CW, IRDye 800RS, and IRDye 700DX, available from Li-Cor; and ADS780WS, ADS830WS, and ADS832WS, available from American Dye Source.

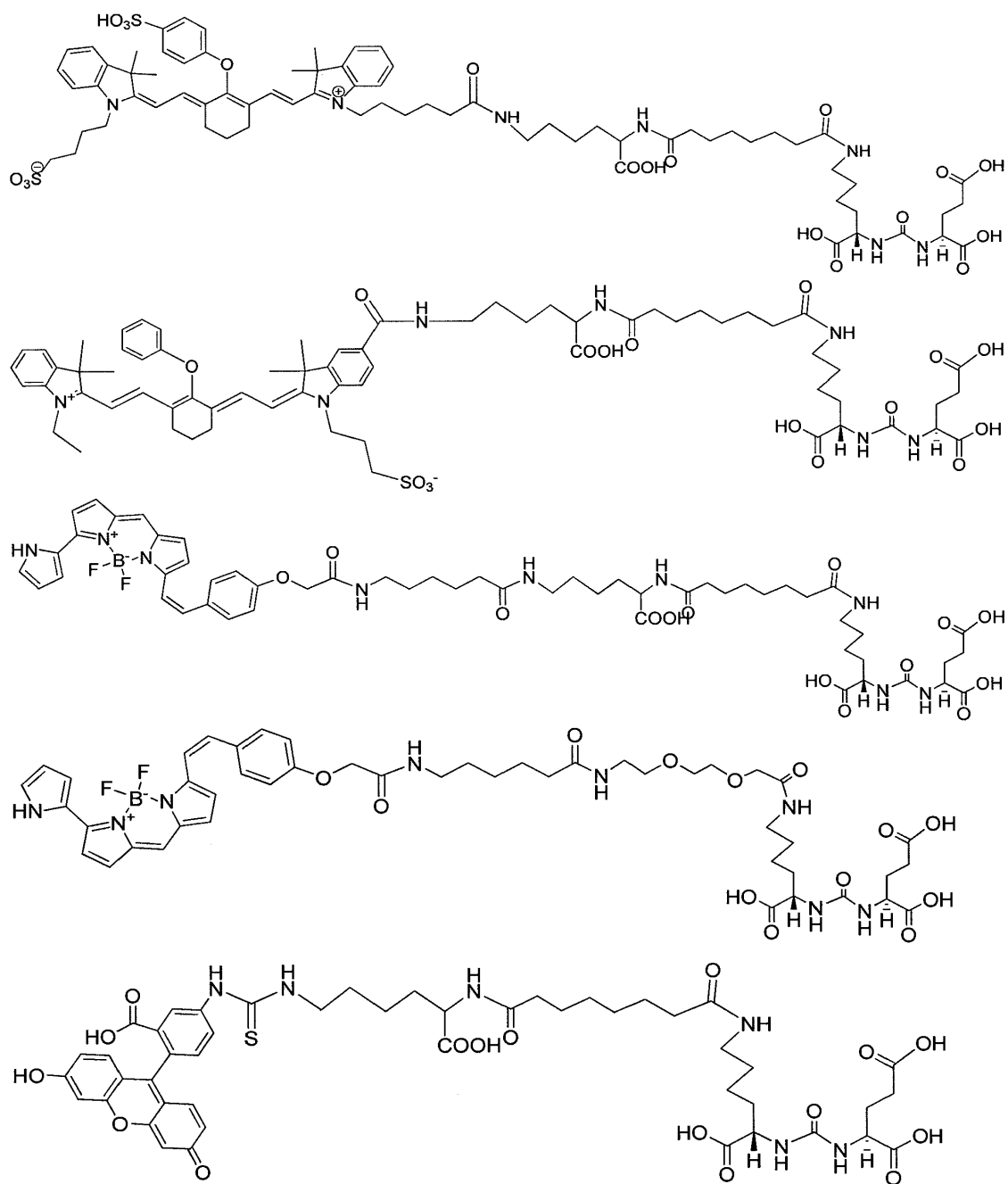
[00120] In some embodiments, FG is a structure shown below.

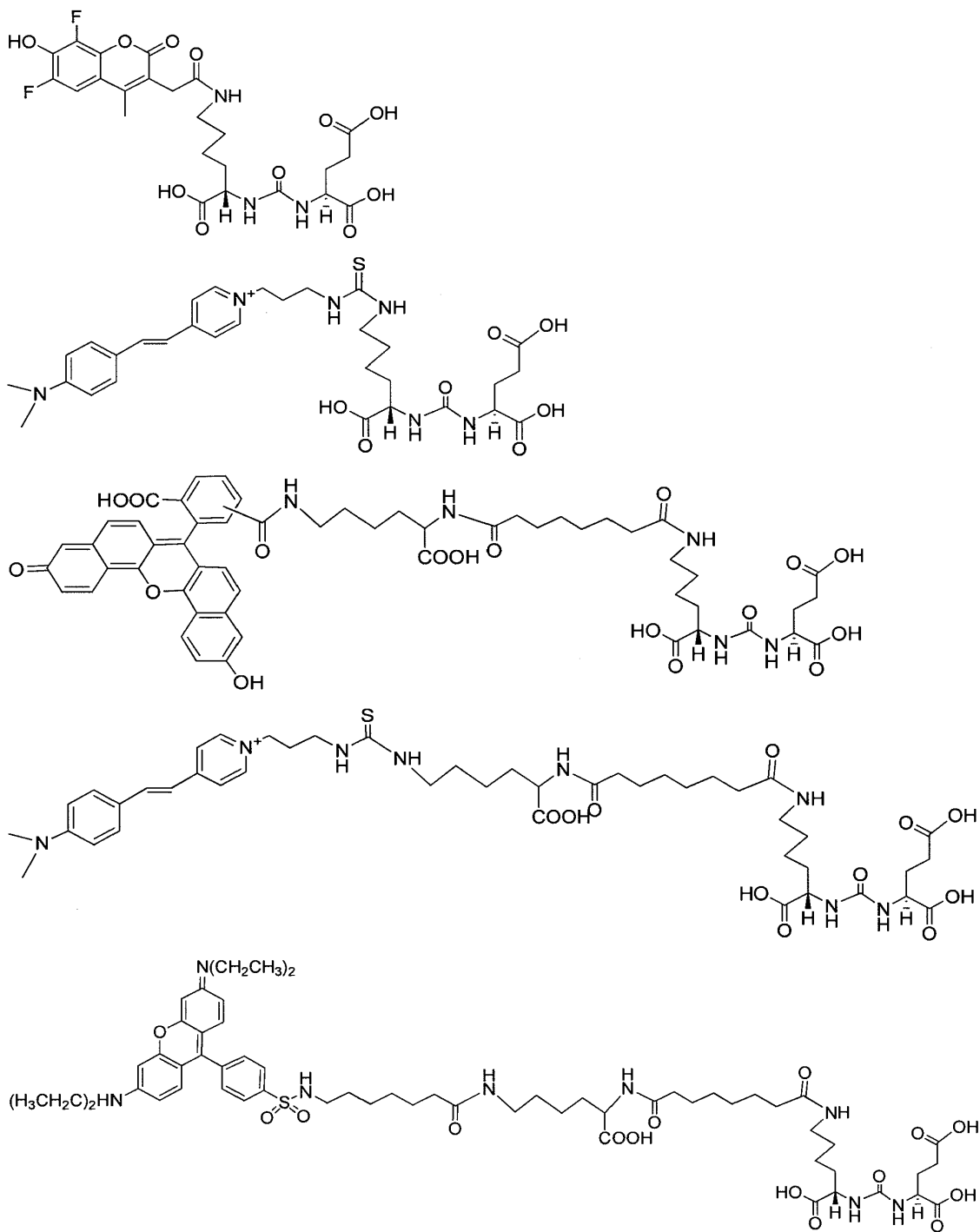




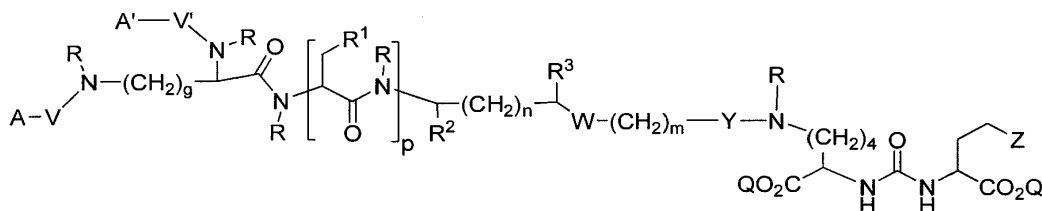
[00121] Exemplary compounds include those shown below.





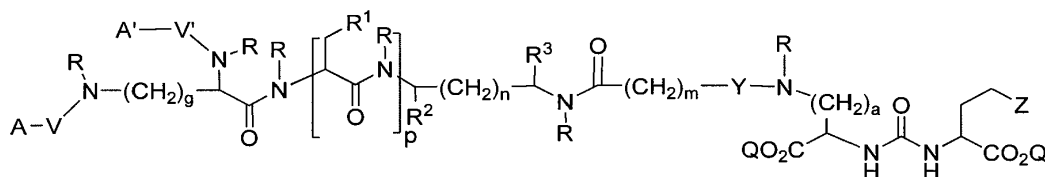


[00122] Embodiments include compounds having the structure

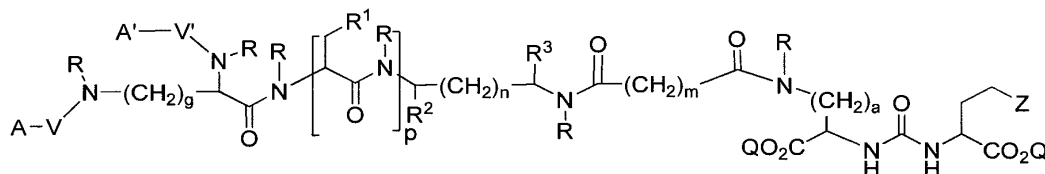


wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4. One of A and A' is Ch and the other is FG, where FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum and Ch is metal chelating moiety optionally including a chelated metal. R is each independently H or C₁-C₄ alkyl. V or V' are independently -C(O)-, -NRC(O)-, or -NRC(S)-. W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-. Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-. In exemplary embodiments m is 1, 2, 3, 4, 5, or 6; n is 1, 2, 3, 4, 5 or 6; and g is 1, 2, 3, 4, 5, or 6; p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different. R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R¹ and R² is CO₂H or CO₂R², the other is H. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum. Some embodiments further include a chelated metal.

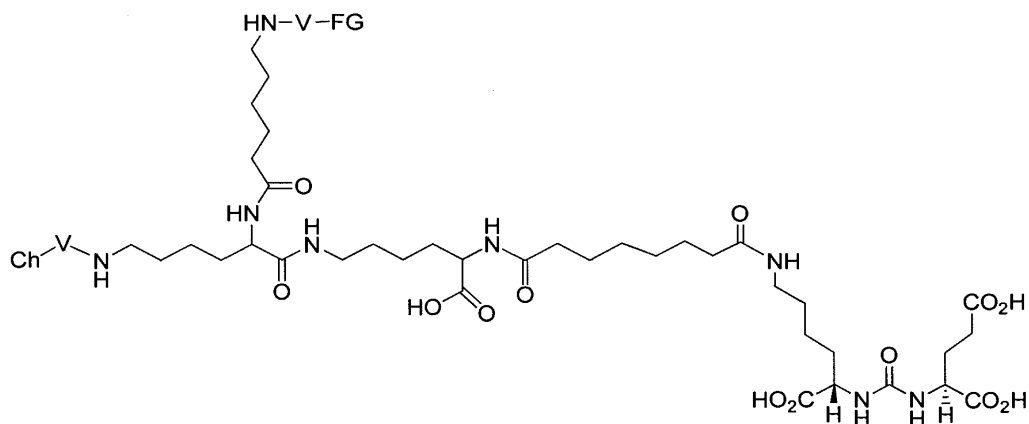
[00123] In some embodiments, the compound has the structure shown below.



[00124] In some embodiments, the compound has the structure shown below.

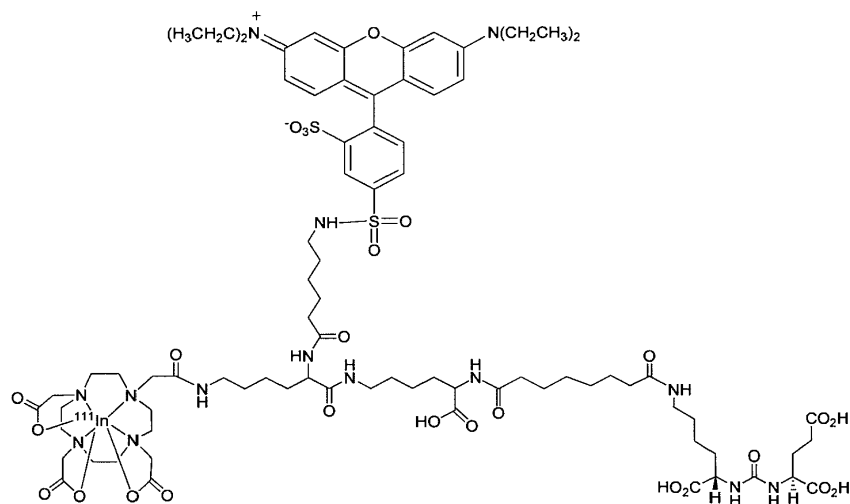


[00133] Some embodiments have the structure

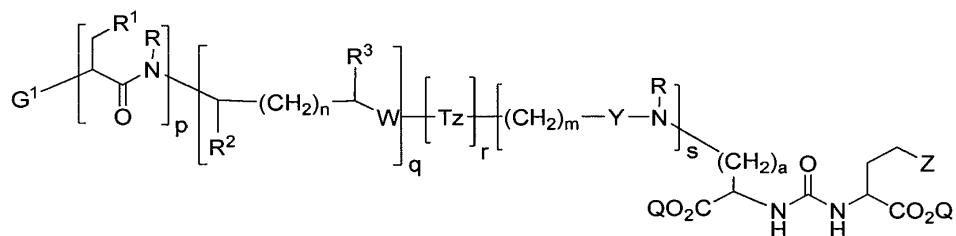


where FG and Ch are described previously, and each V is individually –C(O)– or –NRC(S)–.

[00134] Specific examples of compounds include the structures shown below.

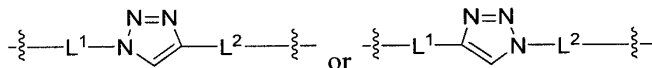


[00135] Embodiments include compounds having the structure

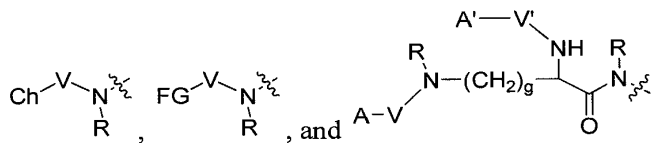


wherein the subunits associated with elements p, q, r, and s may be in any order. Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, and a is 1, 2, 3,

or 4. R is each independently H or C₁-C₄ alkyl. In an exemplary embodiment r is 1. Tz is a triazole group having the structure

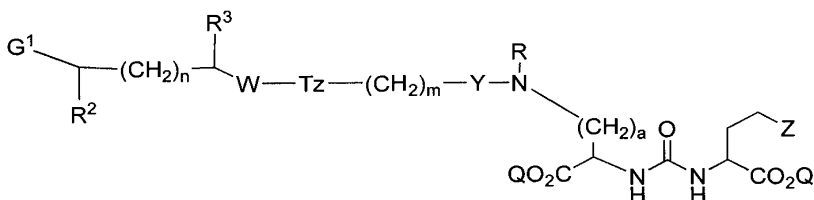


where L¹ is $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$ or $-\text{X}^2-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$; L² is $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$ or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{X}^1-$; X¹ is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, or -NRC(O)O-; X² is -C(O)NR-, -NRC(O)NR-, NRC(S)NR-, or -OC(O)NR-; R⁵ is H, CO₂H, or CO₂R⁶, where R⁶ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl; b is 1, 2, 3, or 4; and d is 1, 2, 3, or 4. In exemplary embodiments q is 0 or 1, W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-; n is 1, 2, 3, 4, 5 or 6; and R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein if one of R² and R³ is CO₂H or CO₂R², then the other is H. In exemplary embodiments s is 0 or 1; Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O); and m is 1, 2, 3, 4, 5, or 6. In exemplary embodiments p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different; and R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. G¹ is a moiety selected from the group consisting of

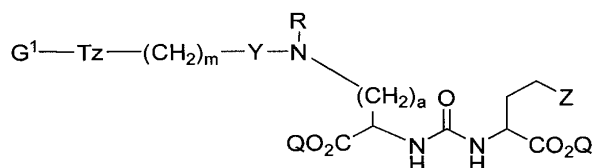


where Ch is a metal chelating moiety, optionally including a chelated metal; FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum; one of A and A' is Ch and the other is FG; V and V' are each independently -C(O)-, -NRC(O)-, -NRC(S)-, or -OC(O)-; and g is 1, 2, 3, 4, 5, or 6. In some embodiments, the fluorescent dye moiety emits in the near infrared spectrum. Some embodiments include a chelated metal. In some embodiments the subunits associated with elements p, q, and s are in the order shown. In some embodiments, p is 0.

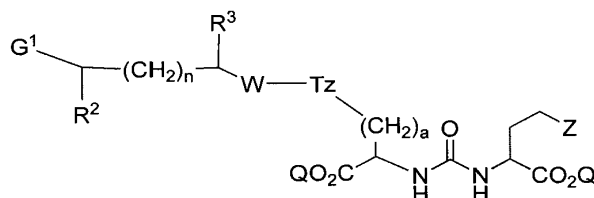
[00136] Some embodiments have the structure shown below.



[00137] Some embodiments have the structure shown below.



[00138] Some embodiments have the structure shown below.



[00139] In some embodiments Y is -C(O)-. In some embodiments, W is -NRC(O)-.

[00140] In some embodiments, p is 1, 2 or 3. In some embodiments, p is 2. In some embodiments R¹ is C₂-C₁₂ aryl. In some embodiments, R¹ is phenyl.

[00141] In some embodiments, p is 0.

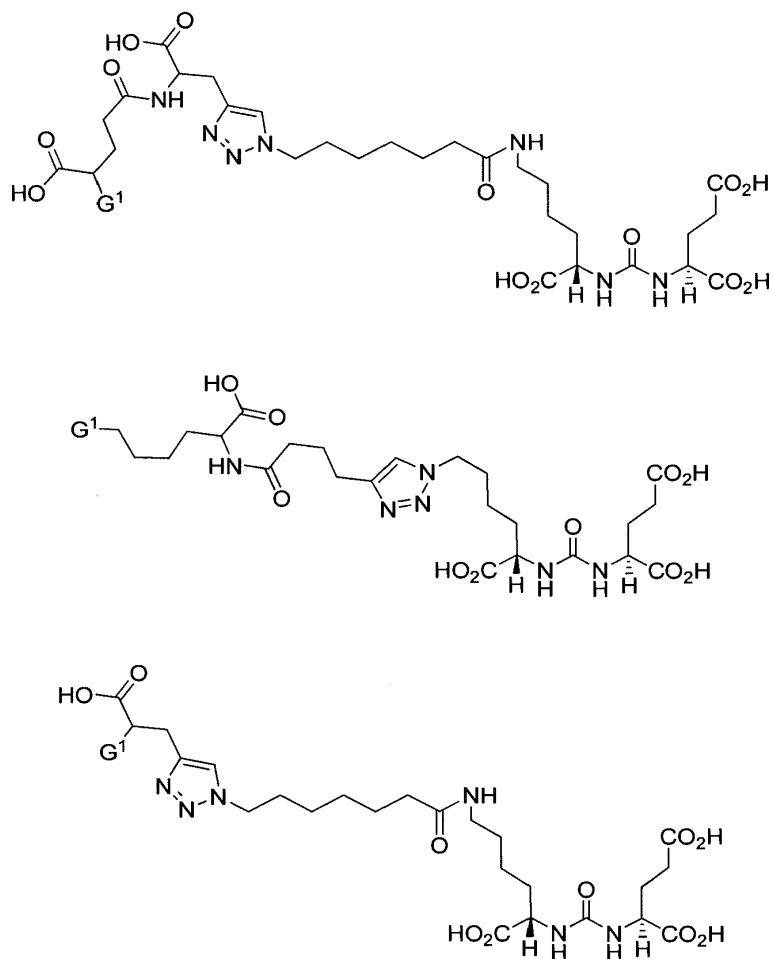
[00142] In some embodiments, R³ is CO₂H and R² is H. In some embodiments, R² is CO₂H and R³ is H. In some embodiments, R² is CO₂R⁴, and R³ is H. In some embodiments, R³ is CO₂R⁴, and R² is H. In some embodiments R⁴ is C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl. In some R⁴ is benzyl. In some embodiments, R² is H, and R³ is H.

[00143] In some embodiments, V and V' are individually -C(O)- or -NRC(S)-.

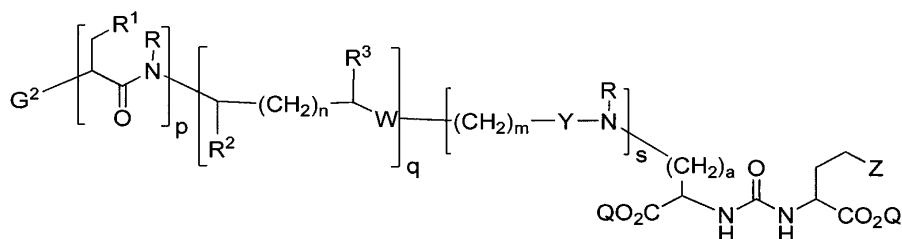
[00144] FG is a fluorescent dye moiety which emits light in the visible or near infrared spectrum. Exemplary fluorescent dye moieties are described previously and may be used in these embodiments.

[00145] Ch is a metal chelating group. Suitable metal chelating groups are described previously. In some embodiments, the compound further includes a chelated metal. The list of exemplary metals and isotopes thereof are described previously and may be used in these embodiments.

[00146] Examples of compounds include the structures shown below



[00147] Embodiments of the invention also include intermediates used to make triazole compounds according to the various embodiments of the invention. Embodiments include compounds having the structure

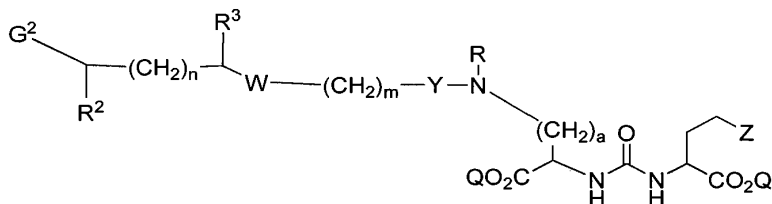


wherein the subunits associated with elements p , q , r , and s may be in any order. Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4, and R is each independently H or C_1 - C_4 alkyl. Variable q is 0 or 1. W is $-NRC(O)-$, $-NRC(O)NR-$, $NRC(S)NR-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, or $-C(O)O-$; R^2 and R^3 are independently H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein if one of R^2 and R^3 is CO_2H or CO_2R^2 , then the other is H; n is 1, 2, 3, 4, 5 or 6.

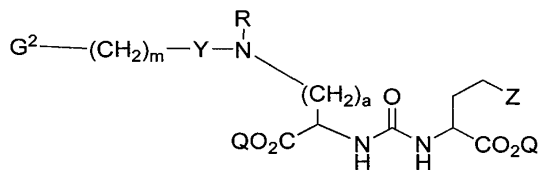
[00148] Variable s is 0 or 1. Y is $-C(O)-$, $-NRC(O)-$, $-NRC(S)-$, $-OC(O)-$; and m is 1, 2, 3, 4, 5, or 6.

[00149] Variable p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different. R^1 is H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl. G^2 is $-\frac{1}{2}N_3$ or $-\frac{1}{2}C\equiv C$.

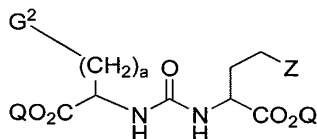
[00150] In some embodiments, the compound has the structure shown below.



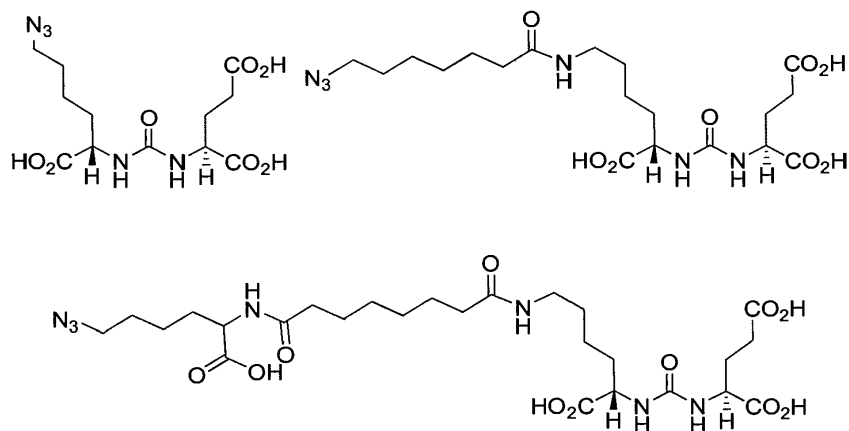
[00151] In some embodiments, the compound has the structure shown below.



[00152] In some embodiments, the compound has the structure shown below.



[00153] Examples include the compounds shown below.



[00154] Other embodiments include pharmaceutically acceptable salts of the compounds described in any of the previous embodiments. As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making non-toxic acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, conventional non-toxic acid salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, malefic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, mesylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, HOOC-(CH₂)_n-COOH where n is 0-4, and the like. The pharmaceutically acceptable salts of the present invention can be synthesized from a parent compound that contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water

or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used, where practicable. Lists of additional suitable salts may be found, e.g., in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985).

Preparation

[00155] The compounds described in the above embodiments may be made using procedures known in the art. In general, the materials used will be determined by the desired structure, and the type of linkage used.

[00156] Often, the compounds are prepared by sequentially adding components to a preformed urea, such as the lysine-urea-glutamate compounds described in Banerjee et al. (J. Med. Chem. vol. 51, pp. 4504-4517, 2008). Other urea-based compounds may also be used as building blocks.

[00157] Compounds are assembled by reactions between different components, to form linkages such as ureas (-NRC(O)NR-), thioureas (-NRC(S)NR-), amides (-C(O)NR- or -NRC(O)-), or esters (-C(O)O- or -OC(O)-). Urea linkages may be readily prepared by reaction between an amine and an isocyanate, or between an amine and an activated carbonamide (-NRC(O)-). Thioureas may be readily prepared from reaction of an amine with an isothiocyanate. Amides (-C(O)NR- or -NRC(O)-) may be readily prepared by reactions between amines and activated carboxylic acids or esters, such as an acyl halide or N-hydroxysuccinimide ester. Carboxylic acids may also be activated in situ, for example, with a coupling reagent, such as a carbodiimide, or carbonyldiimidazole (CDI). Esters may be formed by reaction between alcohols and activated carboxylic acids. Triazoles are readily prepared by reaction between an azide and an alkyne, optionally in the presence of a copper (Cu) catalyst.

[00158] Protecting groups may be used, if necessary, to protect reactive groups while the compounds are being assembled. Suitable protecting groups, and their removal, will be readily available to one of ordinary skill in the art.

[00159] In this way, the compounds may be easily prepared from individual building blocks, such as amines, carboxylic acids, and amino acids.

[00160] Often, a Ch or FB group is placed on the compound by adding a metal chelating group or fluorescent dye to the compound toward the end of a synthesis, for example by reacting a reactive amine on the compound with an activated metal chelating group or fluorescent dye. A

wide variety of metal chelating groups and fluorescent dyes are known in the art, with activated functional groups for reacting with amines. The type of metal chelating group will be determined, in part by the desired metal. Selecting a metal chelating group for a particular metal atom will be apparent to one of ordinary skill in the art. The fluorescent dye used will be determined, in part, by the desired wavelength of fluorescence, and may be readily selected by one of ordinary skill in the art.

[00161] Exemplary procedures for specific compounds are described in the Examples below. Other compounds within the scope of the claims can be prepared using readily apparent modifications of these procedures.

Uses

[00162] Compounds described above, including various radiolabeled compounds, may be used for diagnostic, imaging, or therapeutic purposes. In general, the suitability of a particular radioisotope for a particular purpose (i.e. imaging or therapeutic) is well understood in the art. Other exemplary embodiments are compounds used as precursors for radiolabeled compounds, in which a metal or radioactive isotope of a metal may be added to the compound. Some compounds according to the invention are intermediates for forming other compounds of the invention.

Imaging

[00163] Embodiments include methods of imaging one or more cells, organs or tissues comprising exposing cells to or administering to a subject an effective amount of a compound with an isotopic label suitable for imaging. In some embodiments, the one or more organs or tissues include prostate tissue, kidney tissue, brain tissue, vascular tissue or tumor tissue. The cells, organs or tissues may be imaged while within an organism, either by whole body imaging or intraoperative imaging, or may be excised from the organism for imaging.

[00164] In another embodiment, the imaging method is suitable for imaging studies of PSMA inhibitors, for example, by studying competitive binding of non-radiolabeled inhibitors. In still another embodiment, the imaging method is suitable for imaging of cancer, tumor or neoplasm. In a further embodiment, the cancer is selected from eye or ocular cancer, rectal cancer, colon cancer, cervical cancer, prostate cancer, breast cancer and bladder cancer, oral cancer, benign and malignant tumors, stomach cancer, liver cancer, pancreatic cancer, lung

cancer, corpus uteri, ovary cancer, prostate cancer, testicular cancer, renal cancer, brain cancer (e.g., gliomas), throat cancer, skin melanoma, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's Sarcoma, Kaposi's Sarcoma, basal cell carcinoma and squamous cell carcinoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, angiosarcoma, hemangioendothelioma, Wilms Tumor, neuroblastoma, mouth/pharynx cancer, esophageal cancer, larynx cancer, lymphoma, neurofibromatosis, tuberous sclerosis, hemangiomas, and lymphangiogenesis.

[00165] The imaging methods of the invention are suitable for imaging any physiological process or feature in which PSMA is involved. Typically, imaging methods are suitable for identification of areas of tissues or targets which express high concentrations of PSMA. Typical applications include imaging glutamateric neurotransmission, presynaptic glutamatergic neurotransmission, malignant tumors or cancers that express PSMA, prostate cancer (including metastasized prostate cancer), and angiogenesis. Essentially all solid tumors express PSMA in the neovasculature. Therefore, methods of the present invention can be used to image nearly all solid tumors including lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal and stomach. Also, certain benign lesions and tissues including endometrium, schwannoma and Barrett's esophagus can be imaged according to the present invention.

[00166] The methods of imaging angiogenesis are suitable for use in imaging a variety of diseases and disorders in which angiogenesis takes place. Illustrative, non-limiting, examples include tumors, collagen vascular disease, cancer, stroke, vascular malformations, and retinopathy. Methods of imaging angiogenesis are also suitable for use in diagnosis and observation of normal tissue development.

[00167] PSMA is frequently expressed in endothelial cells of capillary vessels in peritumoral and endotumoral areas of various malignancies such that compounds of the invention and methods of imaging using same are suitable for imaging such malignancies.

[00168] In certain embodiments, the radiolabeled compound is stable *in vivo*.

[00169] In certain embodiments, the radiolabeled compound is detectable by positron emission tomography (PET) or single photon emission computed tomography (SPECT).

[00170] In some embodiments, the subject is a human, rat, mouse, cat, dog, horse, sheep, cow, monkey, avian, or amphibian. In another embodiment, the cell is *in vivo* or *in vitro*. Typical subjects to which compounds of the invention may be administered will be mammals,

particularly primates, especially humans. For veterinary applications, a wide variety of subjects will be suitable, e. g. livestock such as cattle, sheep, goats, cows, swine and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, a wide variety of mammals will be suitable subjects including rodents (e.g. mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like. Additionally, for *in vitro* applications, such as *in vitro* diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications. In other *in vitro* applications, the cells or tissues are present in culture or in suspension.

[00171] Imaging agents of the invention may be used in accordance with the methods of the invention by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with PSMA. The spatial distribution may be measured using any means suitable for the particular label, for example, a gamma camera, a PET apparatus, a SPECT apparatus, a fluorescence camera and the like. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions. A particularly useful imaging approach employs more than one imaging agent, or a bimodal agent having a fluorescent dye moiety and a metal chelating group, such as those described above, to perform simultaneous studies.

[00172] In general, a detectably effective amount of the imaging agent is administered to a subject. As used herein, "a detectably effective amount" of the imaging agent is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent may be administered in more than one injection. The detectably effective amount of the imaging agent can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective amounts of the imaging agent can also vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art. The amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the

amount of imaging agent to administer to each individual patient and the duration of the imaging study.

[00173] In some embodiments, the compounds are excreted from tissues of the body quickly to prevent prolonged exposure to the radiation of the radiolabeled compound administered to the patient. Generally, the compounds are excreted from tissues of the body slowly enough to allow sufficient time for imaging or other use. Typically compounds of the invention are eliminated from the body in less than about 24 hours. More typically, compounds of the invention are eliminated from the body in less than about 16 hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes. Exemplary compounds are eliminated in between about 60 minutes and about 120 minutes.

[00174] In some embodiments of the invention, the compounds are designed to increase uptake in PSMA positive cells (i.e. tumor cells). For example, highly hydrophilic compounds may be excreted quickly. Compounds with increased hydrophobicity, such as compounds having hydrophobic linkers, may have longer circulation times, thereby providing more prolonged supply of tracer to bind to cells. According to embodiments of compounds according to the invention, hydrophobicity can be increased when, for example, p is 1 or more, or when R² or R³ is CO₂R⁴

Therapeutic Uses

[00175] Embodiments of the invention include methods of treating a tumor comprising administering a therapeutically effective amount of a compound discussed above, where the compound includes a therapeutically effective radioisotope. The development of low molecular weight radiotherapeutic agents is much different from developing radiopharmaceuticals for imaging in that longer tumor residence times may be important for the former.

[00176] In some embodiments, the tumor cells may express PSMA, such as prostate tumor cells or metastasized prostate tumor cells. In other embodiments, a tumor may be treated by targeting adjacent or nearby cells which express PSMA. For example, vascular cells undergoing angiogenesis associated with a tumor may be targeted. Essentially all solid tumors express PSMA in the neovasculature. Therefore, methods of the present invention can be used to treat nearly all solid tumors including lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal and stomach. Also, certain

benign lesions and tissues including endometrium, schwannoma and Barrett's esophagus can be treated according to the present invention. Examples of therapeutically effective radioisotopes include ^{90}Y , ^{177}Lu , ^{186}Re , ^{188}Re , ^{67}Cu , ^{225}Ac , ^{213}Bi , ^{212}Bi , ^{67}Ga , ^{111}In , ^{153}Sm , ^{212}Pb , ^{131}I and ^{211}At .

Cell Sorting

[00177] Embodiments include methods for sorting cells by exposing the cells to a compound discussed above, where the compound includes a fluorescent dye moiety, followed by separating cells which bind the compound from cells which do not bind the compound.

[00178] Fluorescent compounds described above bind to PSMA on cells that express PSMA on the cell surface. In some cases, fluorescent compound is internalized. Cells binding the fluorescent compound appear fluorescent, and may be imaged using fluorescence microscopy. Fluorescence-activated cell sorting (FACS) or flow cytometry may be used to separate PSMA positive cells from PSMA negative cells.

Intraoperative Tumor Mapping

[00179] Embodiments of the invention include methods of intraoperative tumor mapping or intraoperative photodiagnosis (PDD) by administering an effective amount of a compound discussed above to a subject, where the compound includes a fluorescent dye moiety. According to such embodiments, an effective amount of a compound is an amount sufficient to produce a detectable level of fluorescence when used for intraoperative tumor mapping or PDD. The compounds bind to, and may be internalized into, cells, particularly tumor cells, that express PSMA. The fluorescent compounds thereby define the boundaries of the tumor, allowing for accurate surgical removal. The compounds that includes a fluorescent dye moiety may also be used to visualize circulating tumor cells that express PSMA.

Pharmaceutical Compositions and Kits

[00180] The compounds discussed herein can be formulated into various compositions, for use in diagnostic, imaging or therapeutic treatment methods. The compositions (*e.g.* pharmaceutical compositions) can be assembled as a kit. Generally, a pharmaceutical composition comprises an effective amount (*e.g.*, a pharmaceutically effective amount, or detectably effective amount) of a compound described above.

[00181] A composition of the invention can be formulated as a pharmaceutical composition, which comprises a compound of the invention and pharmaceutically acceptable carrier. By a "pharmaceutically acceptable carrier" is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. For a discussion of pharmaceutically acceptable carriers and other components of pharmaceutical compositions, see, *e.g.*, Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, 1990. Some suitable pharmaceutical carriers will be evident to a skilled worker and include, *e.g.*, water (including sterile and/or deionized water), suitable buffers (such as PBS), physiological saline, cell culture medium (such as DMEM), artificial cerebral spinal fluid, or the like.

[00182] A pharmaceutical composition or kit of the invention can contain other pharmaceuticals, in addition to the compound. The other agent(s) can be administered at any suitable time during the treatment of the patient, either concurrently or sequentially.

[00183] One skilled in the art will appreciate that the particular formulation will depend, in part, upon the particular agent that is employed, and the chosen route of administration. Accordingly, there is a wide variety of suitable formulations of compositions of the present invention.

[00184] One skilled in the art will appreciate that a suitable or appropriate formulation can be selected, adapted or developed based upon the particular application at hand. Dosages for compositions of the invention can be in unit dosage form. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for animal (*e.g.* human) subjects, each unit containing a predetermined quantity of an agent of the invention, alone or in combination with other therapeutic agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle.

[00185] One skilled in the art can easily determine the appropriate dose, schedule, and method of administration for the exact formulation of the composition being used, in order to achieve the desired effective amount or effective concentration of the agent in the individual patient.

[00186] The dose of a composition of the invention, administered to an animal, particularly a human, in the context of the present invention should be sufficient to produce at least a detectable amount of a diagnostic or therapeutic response in the individual over a reasonable time frame. The dose used to achieve a desired effect will be determined by a variety of factors, including the potency of the particular agent being administered, the pharmacodynamics associated with the agent in the host, the severity of the disease state of infected individuals, other medications being administered to the subject, etc. The size of the dose also will be determined by the existence of any adverse side effects that may accompany the particular agent, or composition thereof, employed. It is generally desirable, whenever possible, to keep adverse side effects to a minimum. The dose of the biologically active material will vary; suitable amounts for each particular agent will be evident to a skilled worker.

[00187] Other embodiments provide kits including a compound according to the invention. In certain embodiments, the kit provides packaged pharmaceutical compositions having a pharmaceutically acceptable carrier and a compound of the invention. In some embodiments the packaged pharmaceutical composition will include the reaction precursors necessary to generate the compound of the invention upon combination with a radionuclide. Other packaged pharmaceutical compositions provided by the present invention further comprise indicia comprising at least one of: instructions for preparing compounds according to the invention from supplied precursors, instructions for using the composition to image cells or tissues expressing PSMA, or instructions for using the composition to image glutamatergic neurotransmission in a patient suffering from a stress-related disorder, or instructions for using the composition to image prostate cancer.

[00188] In certain embodiments, a kit according to the invention contains from about 1 mCi to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound of the invention in solution or in lyophilized form, and these components of the kit of the invention may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form.

Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

[00189] A "pharmaceutically acceptable carrier" refers to a biocompatible solution, having due regard to sterility, pH, isotonicity, stability, and the like and can include any and all solvents, diluents (including sterile saline, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other aqueous buffer solutions), dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, and the like. The pharmaceutically acceptable carrier may also contain stabilizers, preservatives, antioxidants, or other additives, which are well known to one of skill in the art, or other vehicle as known in the art.

[00190] The contents of all cited references (including literature references, issued patents, published patent applications) as cited throughout this application are hereby expressly incorporated by reference. The invention and the manner and process of making and using it, are described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same.

[00191] While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be defined by the scope of the claims that follow and that such claims be interpreted as broadly as is reasonable.

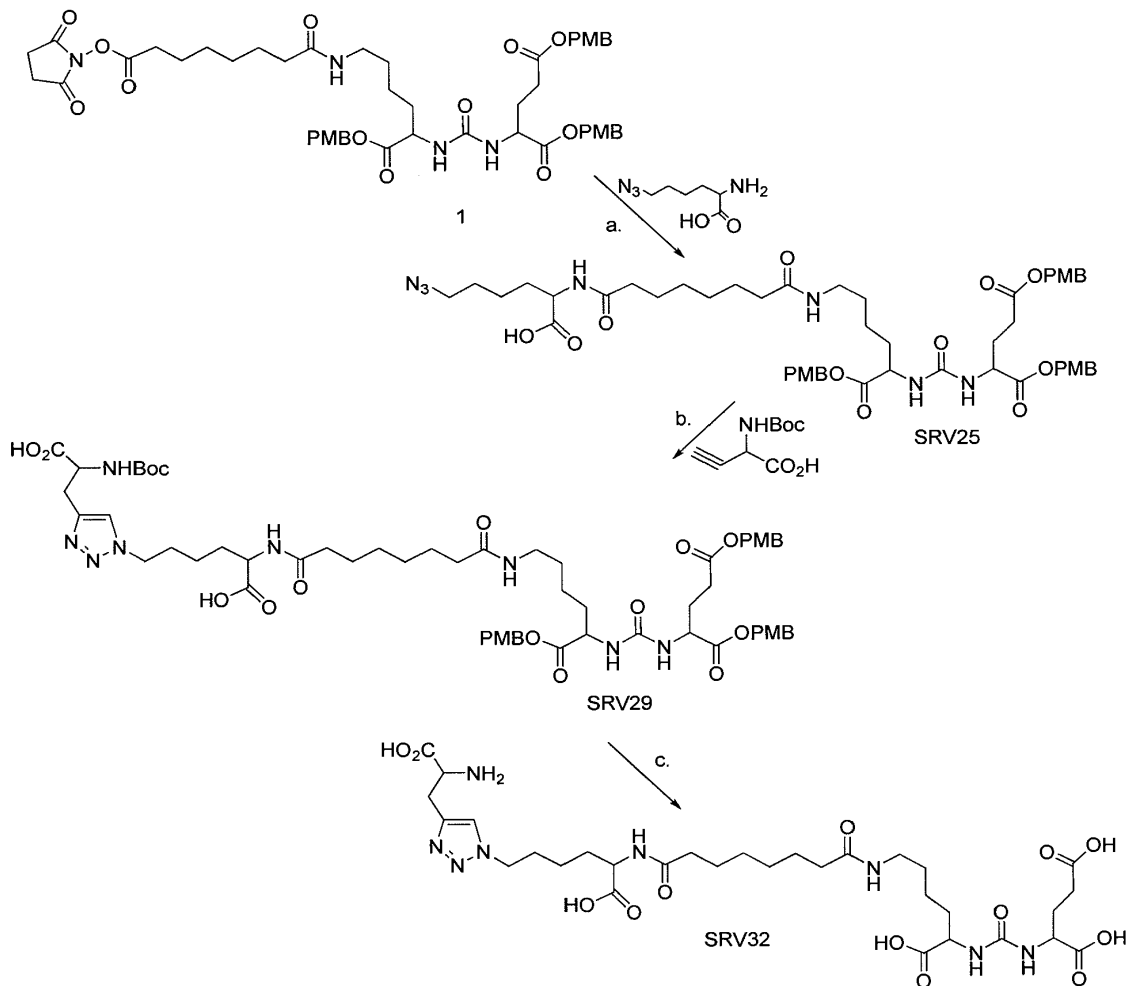
[00192] It is to be understood that the foregoing describes exemplary embodiments of the present invention and that modifications may be made therein without departing from the spirit or scope of the present invention as set forth in the appended claims.

EXAMPLES

[00193] The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The practice of the present invention will employ, unless otherwise indicated, conventional techniques, which are within the skill of the art. Such techniques are explained fully in the literature.

EXAMPLE 1

[00194] 2-{3-[5-(7-{5-[4-(2-Amino-2-carboxy-ethyl)-[1,2,3]triazol-1-yl]-1-carboxy-pentylcarbamoyl}-heptanoylamino)-1-carboxy-pentyl]-ureido}-pentanedioic acid, **compound SRV32**. The compound SRV32 was prepared in three steps following the scheme shown below.



a. TEA, DMF, 16h, rt; b. $\text{Cu}(\text{OAc})_2$, Sodium ascorbate, water/t-BuOH(1:1), rt, 18 h;
 b. $\text{Cu}(\text{OAc})_2$, Na-ascorbate, H_2O , rt, 12 h;
 c. TFA/ CH_2Cl_2 (1:1), 16 h, rt.

[00195] The compound 1 was prepared from literature method (Banerjee et al., J Med Chem, vol. 51, pp. 4504-4517, 2008). To a solution of compound 1 (100mg, 0.107 mmol in 5 ml

DMF) was added H-Lys(ϵ -azide)-OH (20 mg, 0.107 mmol) (Boc-Lys(Azide)-OH was purchased from Anaspec. The removal of Boc group was done by treating the commercial compound with 1:1 TFA:CH₂Cl₂ at room temperature for 4 hr, and the solution was stirred for 16h at rt. The solvent was removed under vacuum. The solid residue thus obtained was dissolved in 10 ml ethyl acetate and extracted with 3× 10 mL water. Organic layer was dried under vacuum to get a colorless solid as the protected azido urea compound SRV25. ESIMS: 991[M+1]⁺. To the Compound SRV25 (60 mg, 0.06 mmol in 1 ml t-BuOH), was added N(a)-Boc-L-propargylglycine (Anaspec) (14 mg, 0.012 mmol in 1 ml t-BuOH), followed by Cu(OAc)₂·H₂O (2 mg, 0.012 mmol in 1 ml water) and sodium ascorbate (4.75 mg, 0.024 mmol in 1 ml water) and the mixture stirred at room temperature for 12 h. The product was extracted into CH₂Cl₂ and washed twice with aqueous NaCl. The aqueous phases were re-extracted with CH₂Cl₂. The organic phases were combined, dried over Na₂SO₄ and evaporated. The product, compound SRV29, was purified by a silica gel pipette column eluted with solution of 90/10 CH₂Cl₂/MeOH. ESIMS: Calcd for C₆₀H₈₂N₈O₁₈ 1203.57, found 1204[M+1]⁺.

[00196] The compound SRV29 was dissolved in 2 ml 1/1 CHCl₃/TFA and stirred overnight. The solution was removed under vacuum to get a colorless solid. The solid was washed 3 times with 5 ml CH₂Cl₂ to remove impurities. The crude solid, compound SRV32 was further purified by HPLC using a 85/15 water/ acetonitrile (0.1% TFA in each) flow rate 4 ml, R_t=10.2 min. ESMS: 742.77[M+1]⁺, ¹H NMR (D₂O) δ : 7.46(m, 1H), 5.2(m, 2H) 4.35(m, 1H), 4.26(m, 1H), 4.18(m, 1H), 3.80-3.70(m, 1H), 3.18(t, *J* = 6 Hz, 2H), 2.69(m, 2H), 2.51(t, *J* = 7.6 Hz, 2H), 2.40-2.18(m, 25H).

[00197] Radiolabeling with Tc-99m was performed by the same procedure described previously (Banerjee et al., J Med Chem, vol. 51, pp. 4504-4517, 2008).

Biodistribution and Imaging

[00198] A single SCID mouse implanted with both a PC-3 PIP (PSMA+) and a PC-3 flu (PSMA-) xenograft was injected intravenously with compound ^{99m}Tc-SRV32 in saline. At 0.5 hr, 1 hr, 2 h, and 5 h p.i. the mouse was anesthetized with isoflurane and maintained under 1% isoflurane in oxygen. The mouse was positioned on the X-SPECT (Gamma Medica, Northridge, CA) gantry and was scanned using two low energy, high-resolution pinhole collimators (Gamma Medica) rotating through 360° in 6° increments for 45 seconds per increment. All gamma images were reconstructed using Lunagem software (Gamma Medica, Northridge, CA).

Immediately following SPECT acquisition, the mice were then scanned by CT (X-SPECT) over a 4.6 cm field-of-view using a 600 μ A, 50 kV beam. The SPECT and CT data were then coregistered using the supplier's software (Gamma Medica, Northridge, CA) and displayed using AMIDE (<http://amide.sourceforge.net/>). Data were reconstructed using the Ordered Subsets-Expectation Maximization (OS-EM) algorithm.

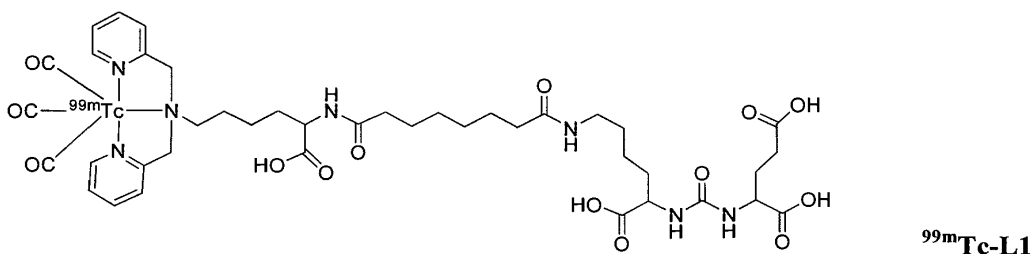
[00199] Tissue biodistribution was measured. Results are summarized in the following table. ^{99m}Tc -SRV32 exhibited high uptake ($\sim 7\%$ ID/g at 30 minutes), and good clearance from non-target tissues.

Tissue	30 min	60 min	120 min	300 min
blood	1.38 ± 0.4	0.63 ± 0.1	0.61 ± 0.3	0.19 ± 0.1
liver	14.26 ± 1.0	9.81 ± 2.0	5.65 ± 0.5	3.06 ± 0.6
stomach	0.77 ± 0.1	0.42 ± 0.09	0.29 ± 0.1	0.18 ± 0.1
spleen	26.10 ± 9.0	17.31 ± 6.6	5.80 ± 1.9	1.26 ± 0.5
kidney	139.53 ± 17.2	144.65 ± 15.1	151.23 ± 37.1	80.00 ± 8.4
muscle	0.56 ± 0.1	0.40 ± 0.2	0.16 ± 0.1	0.51 ± 0.6
small intestine	1.94 ± 1.1	0.74 ± 0.3	0.39 ± 0.2	0.26 ± 0.2
large intestine	0.61 ± 0.1	0.36 ± 0.1	0.53 ± 0.4	2.96 ± 1.3
bladder	1.07 ± 0.1	3.09 ± 2.6	5.39 ± 7.1	2.74 ± 2.1
PC-3 PIP	6.67 ± 1.6	5.32 ± 1.2	3.77 ± 0.8	2.19 ± 0.5
PC-3 flu	0.75 ± 0.2	0.45 ± 0.3	0.35 ± 0.2	0.43 ± 0.4

[00200] A single SCID mouse implanted with a PSMA+ LnCaP xenograft was injected intravenously with compound ^{99m}Tc -SRV32 in saline. At 0.5 hr, and 3.5 hr p.i. the mouse was anesthetized with isoflurane and maintained under 1% isoflurane in oxygen. The mouse was positioned on the X-SPECT (Gamma Medica, Northridge, CA) gantry and was scanned using two low energy, high-resolution pinhole collimators (Gamma Medica) rotating through 360° in 6° increments for 45 seconds per increment. All gamma images were reconstructed using Lunagem software (Gamma Medica, Northridge, CA). Immediately following SPECT acquisition, the mice were scanned by CT (X-SPECT) over a 4.6 cm field-of-view using a 600 μ A, 50 kV beam. The SPECT and CT data were then coregistered using the supplier's software (Gamma Medica, Northridge, CA) and displayed using AMIDE (<http://amide.sourceforge.net/>). Data were reconstructed using the Ordered Subsets-Expectation Maximization (OS-EM) algorithm. Images are shown in **Figure 1**.

COMPARATIVE EXAMPLE 1

[00201] Under the same conditions, tumor uptake for compound ^{99m}Tc -L1, shown below, was determined. Results are summarized in the following table. The data show that while ^{99m}Tc -L1 shows good retention, compound ^{99m}Tc -SRV32 has greater retention *in vivo* both for target tumor and nontarget tissues, and lower GI uptake than the previous ^{99m}Tc -L1 compound at initial time points.



Tissue	30 min	60 min	120 min	300 min
PC-3 PIP	7.9 ± 4	3.9 ± 0.6	2.0 ± 0.8	0.8 ± 0.5
PC-3 flu	0.3 ± 0.2	0.2 ± 0.1	0.05 ± 0.02	0.01 ± 0.01

EXAMPLE 2 – ^{68}Ga CompoundsGeneral

[00202] Solvents and chemicals obtained from commercial sources were of analytical grade or better and used without further purification. All experiments were performed in duplicate or triplicate to ensure reproducibility. Analytical thin-layer chromatography (TLC) was performed using Aldrich aluminum-backed 0.2 mm silica gel Z19, 329-1 plates and visualized by ultraviolet light (254 nm), I2 and 1% ninhydrin in EtOH. Flash chromatography was performed using silica gel purchased from Bodman (Aston PA), MP SiliTech 32-63 D 60Å. ^1H NMR spectra were recorded on either a Varian Mercury 400 MHz or on a Bruker ultrashieldTM 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield by reference to proton resonances resulting from incomplete deuteration of the NMR solvent. Low resolution ESI mass spectra were obtained on a Bruker Daltonics Esquire 3000 Plus spectrometer. Higher-resolution FAB mass spectra were obtained on a JOEL JMS-AX505HA mass spectrometer in the mass spectrometer facility at the University of Notre Dame. Optical rotation was measured on a Jasco P-1010 polarimeter. Infrared spectra were obtained on a

Bruker Tensor 27 spectrometer. High-performance liquid chromatography (HPLC) purification of new compounds was performed using a Phenomenex C₁₈ Luna 10- μ m 250 mm² column on a Waters 600E Delta LC system with a Waters 486 tunable absorbance UV/Vis detector, both controlled by Empower software.

[00203] For purification of radiolabeled [⁶⁸Ga]SRV100, a Varian Microsorb-Mv C₁₈ 250 x 4.6mm² column was used. HPLC was performed using the following isocratic conditions: For Method 1, the mobile phase was 80% solvent A (0.1% TFA in water) and 20% solvent B (0.1% TFA in CH₃CN), flow rate 4 mL/min; for Method 2, the mobile phase was 80% solvent A and 20% solvent B, flow rate 1 mL/min. Method 1 was used for purification of compounds SRV27, [^{69/71}Ga]SRV27, SRV100, [^{69/71}Ga]SRV100 and [⁶⁸Ga]SRV27.

[00204] For purification of [⁶⁸Ga]SRV100 Method 2 was used. For radiosynthetic purification, HPLC was performed on a Varian Prostar System (Palo Alto, CA), equipped with a model 490 UV absorbance detector and a Bioscan NaI scintillation detector connected to a Bioscan Flow-count system controlled by Empower software.

Radiochemistry

[00205] ⁶⁸Ga labeling protocol for compound SRV27 was done following a literature procedure (Zhernosekov et al., J Nucl Med, vol. 48, pp. 1741-1748, 2007). A detailed description is given below.

1. 13.2mCi of ⁶⁸Ga in 7 mL of 0.1N HCl were obtained from more than 1-year-old 740-MBq generator. The solution was transferred on a cation-exchange cartridge, Phenomenex Strata-X-C tubes (33 μ m strong cation exchange resin, part no. 8B-S029-TAK, 30 mg/1ml).
2. The column was eluted with 5 ml of a solution of 20/80 0.10N hydrochloric acid/acetone. The eluant remaining on the cation-exchanger was removed by passage of nitrogen. These two processes aimed to remove most of the remaining chemical and radiochemical impurities from the resin, whereas ⁶⁸Ga(III) should quantitatively remain on the column.
3. The column was filled with 150 μ L of a 2.4/97.6 0.05N HCl/acetone solution. About 2 min standing appeared to be best for complete desorption of the ⁶⁸Ga(III) from the resin into the liquid phase. An additional 250 μ L of this mixture were applied, and the purified ⁶⁸Ga(III) was obtained in 400 μ L of this eluent overall.
4. The fraction (400 μ L eluent) was used directly for the labeling of DOTA-urea compound. The processed activity was added to 500 μ L pure H₂O in a standard glass reagent

vial containing 100 μ l (92 nmol, 1 mg/mL solution) of ligand. No buffer solution was added. The reaction vial was heated at 95°C for 10 min. The complexation was monitored by injecting aliquots of 100 μ l (210 μ Ci) of the solution in HPLC. Product obtained = 160 μ Ci. Radiochemical Yield = $(160/210) \times 100 = 76.19\%$ (without decay correction). Solvent system 80/20 water/acetonitrile (0.1% TFA in each), R_t (retention time) = 25 min for the compound and R_t = 19 min for the free ligand. Product obtained = 5.92 MBq. For [^{68}Ga]SRV27, radiochemical yield: 76.2% (without decay correction). HPLC was performed by Method 1 as described in the General experimental section. R_t = 25 min for the desired product and R_t = 19 min for the free ligand. For [^{68}Ga]SRV100, radiochemical yield: 70%. HPLC was performed by Method 2 as mentioned in General experimental section. R_t = 22.5 min for the desired product and R_t = 16 min for the free ligand.

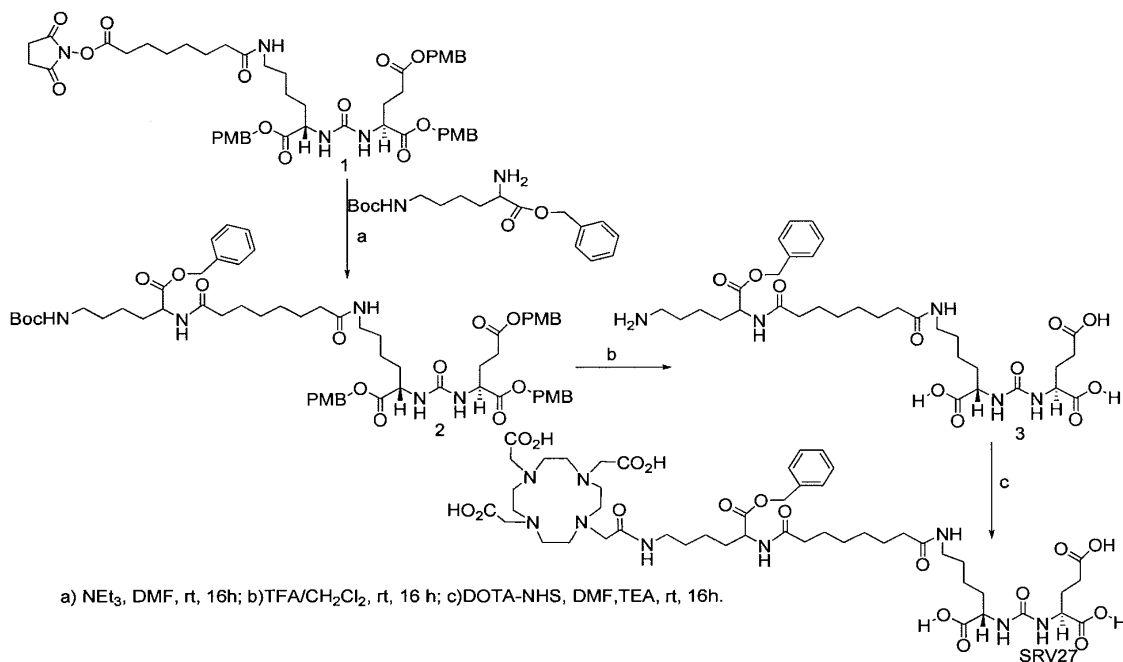
Cell Lines and Tumor Models

[00206] PC-3 PIP (PSMA+) and PC-3 flu (PSMA⁻) cell lines were obtained from Dr. Warren Heston (Cleveland Clinic) and were maintained as previously described (Mease et al., *Clin Cancer Res*, vol. 14, pp. 3036-3043, 2008). LNCaP cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and were maintained as per ATCC guidelines. All cells were grown to 80–90% confluence before trypsinization and formulation in Hank's Balanced Salt Solution (HBSS, Sigma, St. Louis, MO) for implantation into mice.

[00207] Animal studies were undertaken in compliance with institutional guidelines related to the conduct of animal experiments. For biodistribution studies of [^{68}Ga]SRV27, and [^{68}Ga]SRV100 and imaging studies of [^{68}Ga]SRV100, male SCID mice (NCI) were implanted subcutaneously with $1 - 5 \times 10^6$ PSMA+ PC-3 PIP and PSMA- PC-3 flu cells behind either shoulder. For imaging studies of [^{68}Ga]SRV27, male SCID mice (NCI) were implanted subcutaneously with 5×10^6 LNCaP cells behind the right shoulder. Mice were imaged or used in biodistribution studies when the tumor xenografts reached 3 - 5 mm in diameter.

Synthesis of SRV27

[00208] 2-{3-[5-(7-{1-Benzoyloxycarbonyl-5-[2-(4,7,10-tris-carboxymethyl-1,4,7,10tetraazacyclododec-1-yl)-acetylamino]-pentylcarbamoyl}-heptanoylamino)-1-carboxy-pentyl]-ureido}-pentanedioic acid (SRV27). Compound SRV27 was prepared in three steps according the following scheme.



[00209] Compound 1 was prepared according to a literature method (Banerjee et al., J Med Chem, vol. 51, pp. 4504-4517, 2008). To a solution of compound 1 (100 mg, 0.11 mmol in 5 mL DMF) was added H-Lys(Boc)-OBz (36 mg, 0.11 mmol) (Hamachi et al., *Chem. Eur. J.*, vol. 5, pp. 1503-1511, 1999). The solution was stirred for 16 h at ambient temperature. The solvent was removed under vacuum. The solid residue thus obtained was dissolved in 10 mL ethyl acetate and extracted with 3 × 10 mL water. The organic layer was dried under vacuum to provide a colorless solid ESIMS: 1154 [M+1]⁺. This crude compound was dissolved in 3 mL CHCl₃ followed by addition of 3 mL TFA at 0°C. The solution was allowed to stir overnight at ambient temperature. The volume of the solution was reduced under vacuum and the solid residue was washed with 3 × 5 mL CH₂Cl₂ to remove impurities. The colorless solid residue, 3, was dried under vacuum to give 80 mg of compound 3. Compound 3 was purified further by using a 2 g Sep Pak C₁₈ cartridge with a solution of 85/15 water/acetonitrile (0.1% TFA in each). ¹H NMR (D₂O, δ): 7.5 (bm, 5H), 4.27 (m, 1H), 4.12 (m, 1H), 3.99 (m, 1H), 3.04 (m, 4H), 2.38 (m, 2H), 2.3-1.0 (m, 27H). ESIMS: 694 [M+1]⁺. To a solution of DOTA-mono-NHS (54 mg, 0.11 mmol in 5 mL DMF) was added 3 (80mg, 0.08 mmol) and TEA (60 μL, 0.43 mmol) and the solution was allowed to stir for 16 h at ambient temperature. Solvent was removed under vacuum and the crude solid, **SRV27**, was purified by HPLC Method 1, retention time 19 min. Yield: ~ 40%. ESMS: 1080[M+1]⁺, HRESI⁺- MS: Calcd. for C₄₉H₇₇N₉O₁₈, 1080.5487 [M+H],

found: 1080.5459. ¹H NMR (D₂O) δ: 7.88 (m, 4H), 4.26-4.1 (m, 5H), 3.45-3.18 (m, 16H), 2.52-2.43 (m, 16H), 2.40-2.18 (m, 25H). ¹³C (CD₃CO₂D) δ: 177.5, 177.6, 175.3, 172.3, 160.6, 160.2, 159.8, 159.5, 135.5, 128.5, 128.4, 119.9, 117, 114.0, 111.3, 67.3, 55.5, 53.1, 51.0, 49.9, 30.7, 28.0, 26.4, 25.1.

[00210] 2-[3-[5-(7-{1-Benzoyloxycarbonyl-5-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-pentylcarbamoyle]-heptanoylamino)-1-carboxy-pentyl]-ureido]-pentanedioic acid Gallium (III), SRV31 ([^{69/71}Ga]-SRV27). To a solution of GaNO₃ (10 mg, 39 μmol) in deionized water was added compound SRV27 (4.2 mg, 39 μmol) in 1 mL deionized water and the resulting solution was heated in boiling water for 10 min. The solvent was evaporated to dryness and the crude residue was purified by HPLC using a 80/20 water/acetonitrile (0.1% TFA in each), flow rate 8 ml/min. Retention time for the product was at 12 min. Yield: ~35% ESMS: 1146[M+1]⁺, ¹H NMR (D₂O) δ: 7.88 (m, 4H), 4.26-4.1 (m, 5H), 3.45 (m, 8H) 3.18 (m, 8H), 2.69 (m, 8H), 2.51 (m, 8H), 2.40-2.18 (m, 25H).

SRV100

[00211] 2-[3-(1-Carboxy-5-{7-[5-carboxy-5-(3-phenyl-2-{3-phenyl-2-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-propionylamino]-propionylamino)-pentylcarbamoyle]-heptanoylamino}-pentyl)-ureido]-pentanedioic acid, (SRV100). Compound SRV100 was prepared according to the scheme shown in **Figure 4**. Fmoc-Lys(Boc)-Wang resin (100 mg, 0.43 mM) was allowed to swell with CH₂Cl₂ (3 mL) followed by DMF (3 mL). A solution of 20 % piperidine in DMF (3 x 3 mL) was added to the resin that was then shaken gently on a mechanical shaker for 30 min at ambient temperature. The resin was washed with DMF (3 x 3 mL) and CH₂Cl₂ (3 x 3 mL). Formation of free amine was assessed by the Kaiser test (Kaiser et al., *Anal Biochem*, vol. 34, pp. 595-598, 1970). After swelling the resin in DMF, a solution of Fmoc-Phe-OH (3eq), HBTU (3eq), HOBt (3eq), and DIPEA (4.0eq) in DMF was added and gently shaken for 2 h. The resin was then washed with DMF (3 x 3 mL) and CH₂Cl₂ (3 x 3 mL). The coupling efficiency was assessed by the Kaiser Test. That aforementioned sequence was repeated for two more coupling steps with Fmoc-Phe-OH and DOTA-(*t*-butyl ester)₃-CO₂H. The resulting compound was cleaved from the resin using TFA: CH₂Cl₂ (1:1) and concentrated under vacuum to produce the free amine. The concentrated product was purified by using a C₁₈ SepPak Vac 2g column. The product was eluted with a solution 70/30 water/acetonitrile (0.1% TFA in each). ESIMS: 827 [M+1]⁺. Lyophilized amine

(10 mg, 12 μ mol in 2 mL DMF) was added to **1** (prepared separately) (20 mg, 21.4 μ mol in 1 mL DMF) followed by TEA (214 μ mol, 30 μ L) and then stirred at 25°C for 16 h. After solvent removal, solid residue was treated with 3 mL TFA: CH₂Cl₂ to remove the PMB group. The residue was washed 2 $\times$ 5 mL CH₂Cl₂ to remove impurities. The colorless solid residue thus obtained was purified by a C18 SepPak Vac 2g column using an eluent of 70/30 water/acetonitrile (0.1% TFA in each) to produce **SRV100** (SR-V-100). The product was further purified using preparative RP-HPLC by Method 1, retention time 17 min. Yield: ~ 30%. ESMS *m/z*: 1284[M+H]⁺, HRESI⁺-MS: Calcd. for C₆₈H₉₀N₁₁O₂₀, 1284.6365 [M+H], found: 1284.6358. ¹H NMR (CD₃CO₂D) δ : 7.35-7.20(m, 10H), 4.86 (bm, 2H), 4.57-4.46 (4H), 4.4-2.8 (m, 14 H), 2.51 (t, 2h), 2.4-1.2 (m, 28H). ¹³C (CD₃CO₂D) δ : 176.5, 177, 177.06, 177.6, 173.6, 173.24, 161.3, 160.92, 160.53, 160.14, 159.77, 137.95, 137.06, 130.5, 129.5, 127.9, 127.71,, 120.8, 118.0, 115.1, 112.3, 56.1, 55.5, 53.5, 53.3, 40.1, 38.8, 36.832.6, 31.8, 30.7, 29.42, 27.9, 26.53.

[00212] **2-[3-(1-Carboxy-5-{7-[5-carboxy-5-(3-phenyl-2-{3-phenyl-2-[2-(4,7,10-tris-carboxymethyl-1,4,7,10tetraaza-cyclododec-1-yl)-acetylamino]-propionylamino}-propionylamino)-pentylcarbamoyl]-heptanoylamino}-pentyl)-ureido]-pentanedioic acid Gallium (III), [^{69/71}Ga]SRV100.** This compound was prepared according to the same general procedure as described for [^{69/71}Ga]SRV27. Compound [^{69/71}Ga]SRV100 was purified by Method 1, retention time 22 min. Yield: ~ 30%. ESMS *m/z*: 1351[M+H]⁺, HRESI⁺-MS: Calcd. For C₆₈H₈₆GaN₁₁NaO₂₀, 1372.5204 [M+Na]⁺, found: 1372.5199.

Compound Characterization – Lipophilicity

[00213] Partition coefficients, log_{o/w} (pH = 7.4) values were determined according to a literature procedure (Antunes et al., *Bioconjug Chem*, vol. 18, pp. 84-92, 2007). Briefly, a solution of either [⁶⁸Ga]SRV27 or [⁶⁸Ga]SRV100 was added to a presaturated solution of 1-octanol (5 mL) mixed with phosphate buffered saline (PBS) (5 mL) in a 15 mL centrifuge tube. After vigorously shaking the mixture, it was centrifuged at 3,000 rpm for 5 min. Aliquots (100 μ L) were removed from the two phases and the radioactivity was measured in a γ -counter, 1282 Compugamma CS (LKB, Wallac, Turku, Finland).

[00214] On analysis of the reaction mixture by HPLC, the retention time of the radiolabeled compound was slightly longer than the corresponding free ligand. The specific radioactivity of purified [⁶⁸Ga]SRV27 and [⁶⁸Ga]SRV100 was between 3.0 and 6.0 MBq/nmol.

The log $P_{\text{octanol/water}}$ values for [^{68}Ga]SRV27 and [^{68}Ga]SRV100 were approximately -3.9 as determined by the shake-flask method (Antunes et al., *Bioconjug Chem*, vol. 18, pp. 84-92, 2007). However, using an HPLC method, we found that the HPLC retention times for SRV100 (28 min) and [$^{69/71}\text{Ga}$]SRV100 (32 min) were longer than for SRV27 (19 min) and [$^{69/71}\text{Ga}$]SRV27 (24 min). It is evident that SRV100 and the corresponding gallium compound were more lipophilic than SRV27 and its gallium-labeled analog, which is reasonable in light of the presence of two phenylalanine residues in the long linker of SRV100, while SRV27 has only one lysine residue protected as the benzyl ester.

Cell Binding Assay

[00215] Ki values for SRV27, [$^{69,71}\text{Ga}$]SRV27, SRV100 and [$^{69,71}\text{Ga}$]SRV100 were determined using a competitive N-acetyl aspartyl glutamate (NAAG) fluorescence cell binding assay adapted from the literature (Kozikowski et al., *J Med Chem*, vol. 47, pp. 1729-1738, 2004). All compounds were found to be strong inhibitors of PSMA. Compounds SRV27 and [$^{69,71}\text{Ga}$]SRV27 had inhibitory capacities of 2.9 nM and 29 nM, respectively. For SRV100 and [$^{69,71}\text{Ga}$]SRV100, values were 1.23 nM and 0.44 nM, respectively.

Ex vivo Biodistribution

[00216] PSMA+ PC-3 PIP and PSMA- PC-3 flu xenograft-bearing SCID mice were injected *via* the tail vein with 30 μCi (1.1 MBq) of [^{68}Ga]SRV27 or [^{68}Ga]SRV100. In case each four mice were sacrificed by cervical dislocation at 30, 60, 120, 180 min p.i. For [^{68}Ga]SRV27 and at 5, 60, 120, 180 min p.i. for [^{68}Ga]SRV100. The heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, muscle, small and large intestines, urinary bladder, and PC-3 PIP and flu tumors were quickly removed. A 0.1 mL sample of blood was also collected. Each organ was weighed, and the tissue radioactivity was measured with an automated gamma counter (1282 Compugamma CS, Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The %ID/g was calculated by comparison with samples of a standard dilution of the initial dose. All measurements were corrected for decay.

[00217] Compound [^{68}Ga]SRV27 was assessed for its pharmacokinetics *ex vivo* in severe-combined immunodeficient (SCID) mice bearing both PSMA+ PC3-PIP and PSMA- PC3- flu xenografts (Chang et al., *Cancer Res*, vol. 59, pp. 3192-3198, 1999). **Table 1** shows the percent injected dose per gram (%ID/g) of radiotracer in selected organs for [^{68}Ga]SRV27.

Table 1. *Ex vivo* tissue biodistribution of [^{68}Ga]SRV27

Tissue	30 min	60 min	120 min	180 min
blood	2.20 $\pm$ 0.90	1.93 $\pm$ 0.70	0.80 $\pm$ 0.30	0.62 $\pm$ 0.34
heart	0.70 $\pm$ 0.13	0.50 $\pm$ 0.08	0.21 $\pm$ 0.08	0.20 $\pm$ 0.02
liver	0.84 $\pm$ 0.24	0.83 $\pm$ 0.10	0.42 $\pm$ 0.07	0.50 $\pm$ 0.03
stomach	0.73 $\pm$ 0.13	0.75 $\pm$ 0.32	0.24 $\pm$ 0.07	0.24 $\pm$ 0.05
spleen	4.90 $\pm$ 1.10	3.35 $\pm$ 1.20	0.43 $\pm$ 0.19	0.32 $\pm$ 0.13
kidney	97.19 $\pm$ 16.07	64.68 $\pm$ 4.10	5.35 $\pm$ 2.12	2.13 $\pm$ 0.11
muscle	0.46 $\pm$ 0.16	0.25 $\pm$ 0.07	0.08 $\pm$ 0.04	0.05 $\pm$ 0.01
small intestine	0.79 $\pm$ 0.12	0.70 $\pm$ 0.34	0.26 $\pm$ 0.11	0.34 $\pm$ 0.20
large intestine	0.77 $\pm$ 0.14	0.95 $\pm$ 0.53	0.34 $\pm$ 0.10	0.46 $\pm$ 0.10
bladder	8.96 $\pm$ 5.30	25.29 $\pm$ 8.63	2.70 $\pm$ 4.02	5.39 $\pm$ 2.98
PC-3 PIP	3.78 $\pm$ 0.90	3.32 $\pm$ 0.33	1.31 $\pm$ 0.06	1.10 $\pm$ 0.19
PC-3	flu 0.82 $\pm$ 0.20	0.67 $\pm$ 0.08	0.41 $\pm$ 0.09	0.39 $\pm$ 0.02
PIP:flu	4.61	4.93	3.24	2.77
PIP:muscle	8.30	13.13	17.40	20.37
flu:muscle	1.80	2.67	5.37	7.34

[00218] Compound [^{68}Ga]SRV27 showed clear PSMA-dependent binding in PSMA+ PC3 PIP xenografts, reaching a maximum uptake of 3.78 $\pm$ 0.90 (SEM) %ID/g at 30 min post-injection (p.i.). The blood, spleen and kidney displayed highest uptake at 30 min. By 60 min, the urinary bladder showed highest uptake, however, this uptake represents excretion at all time points. The high values noted in kidney are partially due to high expression of PSMA within proximal renal tubules (Silver et al., *Clin Cancer Res*, vol. 3, pp. 81-85, 1997; Slusher et al., *J Comp Neurol*, vol. 315, pp. 271-229, 1992). Rapid clearance from the kidneys was demonstrated, decreasing from 97.19 $\pm$ 16.07 %ID/g at 30 min to 2.31 $\pm$ 0.11%ID/g at 3 h. The radioactivity in the PSMA+ PIP tumor cleared more slowly, from its aforementioned value at 30 min to 1.08 $\pm$ 0.19 %ID/g at 3 h.

[00219] Compound [^{68}Ga]SRV100 was also investigated for its pharmacokinetic characteristics in tumor bearing mice at 5 min, 1 h, 2 h and 3 h p.i. **Table 2** shows the %ID/g of radiotracer in selected organs for [^{68}Ga]SRV100.

Table 2. *Ex vivo* tissue biodistribution of [^{68}Ga]SRV100

Tissue	5 min	60 min	120 min	180 min
blood	6.28 ± 0.08	$.41 \pm 0.05$	0.15 ± 0.07	0.13 ± 0.01
heart	2.01 ± 0.24	0.19 ± 0.07	0.05 ± 0.03	0.03 ± 0.01
lung	4.59 ± 0.68	0.74 ± 0.54	0.20 ± 0.05	0.14 ± 0.03
liver	1.57 ± 0.16	0.24 ± 0.09	0.19 ± 0.03	0.14 ± 0.02
stomach	2.38 ± 0.35	0.38 ± 0.16	0.18 ± 0.02	0.04 ± 0.02
pancreas	1.52 ± 0.19	0.25 ± 0.14	0.08 ± 0.03	0.04 ± 0.02
spleen	5.17 ± 2.22	2.43 ± 1.07	0.78 ± 0.15	0.34 ± 0.09
fat	1.03 ± 0.02	0.40 ± 0.04	0.08 ± 0.02	0.02 ± 0.01
kidney	64.75 ± 12.00	26.57 ± 10.93	12.25 ± 1.79	10.04 ± 1.22
muscle	1.58 ± 0.33	0.12 ± 0.08	0.03 ± 0.02	0.00 ± 0.01
small intestine	2.04 ± 0.25	0.23 ± 0.05	0.09 ± 0.04	0.06 ± 0.03
large intestine	2.02 ± 0.49	0.50 ± 0.70	0.12 ± 0.03	0.12 ± 0.03
bladder	5.97 ± 1.50	7.65 ± 3.34	1.41 ± 1.17	0.75 ± 0.54
PC-3 PIP	6.61 ± 0.55	2.80 ± 1.32	3.29 ± 0.77	1.80 ± 0.16
PC-3 flu	2.63 ± 0.51	0.16 ± 0.08	0.18 ± 0.03	0.12 ± 0.03
PIP:flu	2.50	17.30	18.28	15.20
Pip:muscle	4.17	23.27	122.13	436.29
flu:muscle	1.67	1.34	6.68	28.70

[00220] As for [^{68}Ga]SRV27, [^{68}Ga]SRV100 showed PSMA-dependent tumor uptake. After a peak, flow-related, uptake at 5 min p.i. of $6.61 \pm 0.55\%$, [^{68}Ga]SRV100 demonstrated a 2 h tumor uptake value of $3.29 \pm 0.77\%$, which dropped to $1.80 \pm 0.16\%$ at 3 h. Uptake in blood was high at 5 min and rapidly washed out within 1 h. Non-target organs such as kidney, spleen and lung showed high uptake at 5 min and rapidly washed out with time. With the exception of the kidneys and spleen, clearance from blood and normal organs was faster for [^{68}Ga]SRV100 than for [^{68}Ga]SRV27. Again, high kidney uptake is associated with high expression of PSMA within proximal renal tubules (Silver et al., *Clin Cancer Res*, vol. 3, pp. 81-85, 1997; Slusher et al., *J Comp Neurol*, vol. 315, pp. 271-229, 1992). Similar to [^{68}Ga]SRV27, [^{68}Ga]SRV100 demonstrated faster clearance of radioactivity from kidney than from the PSMA+ tumor. However, the rate of clearance from kidney for [^{68}Ga]SRV100 was much slower than for [^{68}Ga]SRV27, i.e., $65 \pm 12\%$ at 5 min p.i. and $10.04 \pm 1.22\%$ at 3 h.

Small Animal PET Imaging

[00221] A single SCID mouse implanted with a PSMA+ LNCaP xenograft was injected intravenously with 0.2 mCi (7.4 MBq) of [^{68}Ga]SRV27 in 200 μL 0.9% NaCl. At 0.5 h p.i., the mouse was anesthetized with 3% isoflurane in oxygen for induction and maintained under 1.5% isoflurane in oxygen at a flow rate of 0.8 L/min. The mouse was positioned in the prone position

on the gantry of a GE eXplore VISTA small animal PET scanner (GE Healthcare, Milwaukee, WI). Image acquisition was performed using the following protocol: The images were acquired as a pseudodynamic scan, i.e., a sequence of successive whole-body images were acquired in three bed positions for a total of 120 min. The dwell time at each position was 5 min, such that a given bed position (or mouse organ) was revisited every 15 min. An energy window of 250 - 700 keV was used. Images were reconstructed using the FORE/2D-OSEM method (two iterations, 16 subsets) and included correction for radioactive decay, scanner dead time, and scattered radiation. After PET imaging, the mobile mouse holder was placed on the gantry of an X-SPECT (Gamma Medica Ideas, Northridge, CA) small animal imaging device to acquire the corresponding CT. Animals were scanned over a 4.6 cm field-of-view using a 600 μ A, 50 kV beam. The PET and CT data were then co-registered using Amira 5.2.0 software (Visage Imaging Inc., Carlsbad, CA).

[00222] Imaging studies of [^{68}Ga]SRV100 and blocking studies of [^{68}Ga]SRV27 were carried out on PSMA+ PC-3 PIP and PSMA- PC-3 flu xenograft-bearing SCID mice or PSMA+ PC-3 PIP (25.9 MBq in 100 μ L NaCl) xenograft-bearing SCID mice. At 30 min, 1 h and 2 h p.i. the mice were anesthetized and whole-body images were obtained using the PET scanner as mentioned above, in two bed positions, 15 min at each position for a total of 30 min using the same energy window. Images were reconstructed and co-registered with the corresponding CT images using the same methods as described above.

[00223] **Figures 2 and 3** demonstrate the high target selectivity of [^{68}Ga]SRV27 and [^{68}Ga]SRV100 by delineating the PSMA+ tumors. Although a PSMA- control tumor was not included in **Figure 2**, a separate blocking study was performed for [^{68}Ga]SRV27, in which an animal pre-treated with 50 mg/kg of the known PSMA-binding ligand, 2-(phosphonomethyl)pentanedioic acid (2-PMPA) (Jackson et al., *J Med Chem*, vol. 39, pp. 619-622, 1996), did not demonstrate PSMA+ tumor uptake, attesting to the binding specificity of this compound. The more quantitative, *ex vivo* studies of [^{68}Ga]SRV27 and [^{68}Ga]SRV100 further supported high PSMA target specificity, demonstrating target-to-nontarget (PIP/flu) ratios of approximately 5 and 18 at 1 h and 2 h p.i., respectively. One hour and 2 h PSMA+ tumor uptake values for these compounds, $3.32 \pm 0.33\%$ and $3.29 \pm 0.77\%$, respectively, for [^{68}Ga]SRV27 and [^{68}Ga]SRV100, are comparable to other radiometallated PSMA inhibitors (Banerjee et al., *J Med Chem*, vol. 51, pp. 4504-4517, 2008). As shown in **Figures 2 and 3** those values are sufficient for clear tumor imaging. Notably, PIP tumors contain about one order of magnitude lower

PSMA than LNCaP tumors (data not shown), which are often employed to assess for binding specificity of PSMA-targeting agents. PIP/flu is the preferred comparison as both are derived from PC-3 cells, providing a more controlled study.

[00224] Intense radiotracer uptake was seen only in the kidneys and tumor for both [^{68}Ga]SRV27 (**Figure 2**) and [^{68}Ga]SRV100 (**Figure 3**). As noted above for the *ex vivo* study, the intense renal uptake was partially due to specific binding of the radiotracer to proximal renal tubules (Silver et al., *Clin Cancer Res*, vol. 3, pp. 81-85, 197; Slusher et al., *J Comp Neurol*, vol. 315, pp. 271-229, 1992) as well as to excretion of this hydrophilic compound. Apart from the kidneys, only the PSMA+ tumor demonstrated significant radiotracer uptake.

Discussion

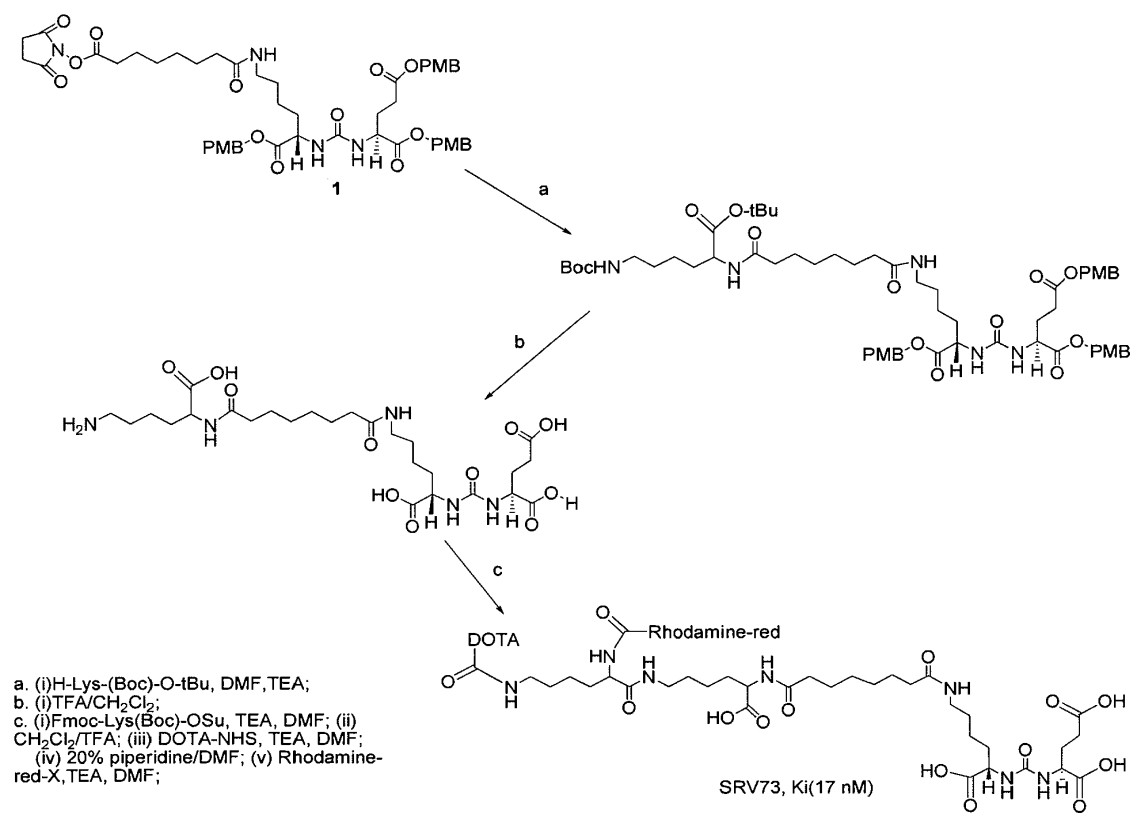
[00225] Because of its demonstrated clinical utility and the appearance of dual modality (PET/computed tomography (CT)) systems, clinical PET imaging has been accelerating worldwide and may soon become the dominant technique in nuclear medicine. PET isotopes tend to be short-lived and enable synthesis of “physiologic” radiotracers, namely, those that incorporate ^{15}O , ^{13}N or ^{11}C , enabling precise conformity to the tracer principle. Being essentially isosteric to H, ^{18}F enables nearly tracer-level studies, with important caveats, particularly for [^{18}F]fluorodeoxyglucose (FDG), which is by far the most commonly used radiopharmaceutical for PET. But, in part because FDG does not accumulate well within many tumor types, including prostate cancer, there has been a re-emergence in the development of radiometallated peptides, often employing $^{99\text{m}}\text{Tc}$, that target G-protein coupled receptors. Gallium-68 provides a link between PET and single photon emission computed tomography (SPECT) since metal chelating methodology needed for $^{99\text{m}}\text{Tc}$ can also be applied to ^{68}Ga . A further analogy is the convenience of use of a $^{68}\text{Ge}/^{68}\text{Ga}$ generator (PET), as with $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ (SPECT), to provide readily available isotope, with no need for an in-house cyclotron. Although ^{18}F -labeled, low molecular weight PSMA inhibitors have shown promise in preclinical imaging studies (Mease et al., *Clin Cancer Res*, vol. 14, pp. 3036-3043, 2008; Lapi et al., *J Nucl Med*, vol. 50, pp. 2042-2048, 2009), the availability of generator-produced ^{68}Ga and the extension to PET from our published $^{99\text{m}}\text{Tc}$ -labeled series of PSMA-binding radiometallated imaging agents (Banerjee et al., *J Med Chem*, vol. 51, pp. 4504-4517, 2008) provided the rationale for this study.

EXAMPLE 3

[00226] Compound **SRV27** and **SRV100** were prepared as described in Example 2. In-111 labeling was generally performed by treatment of **SRV27** or **SRV100** or **SRV73** with $^{111}\text{InCl}_3$, in 200 mM aqueous NaOAc ~60 °C for 30 minutes. Specifically, for **SRV27**, 60 μl of **SRV27** (2 mg/mL, sodium acetate) was combined with 100 μl sodium acetate and 3 mCi $^{111}\text{InCl}_3$ in a 1.5 ml appendorf tube and left at for 60°C for 30 min. The radiolabeled product was diluted with 800 μl water and purified by HPLC. Radiolabeling yield is 1.7 mCi (~57%) and radiochemical purity was > 99.9%

SRV73

[00227] Compound **SRV73** was prepared by the method outlined in the scheme below. Compound **SRV73** is a bimodal compound having a fluorescent dye moiety and a metal chelating moiety



Small animal PET imaging

[00228] SPECT imaging experiments for [^{111}In]SRV27, [^{111}In]SRV100 and [^{111}In]SRV73 were performed using the same general procedure described for [$^{99\text{m}}\text{Tc}$]SRV32 described in Example 1.

[00229] SPECT-CT imaging experiment of compound [^{111}In]SRV27 (**Figure 5**) illustrated clear PSMA-dependent binding in PSMA+ PC3 PIP xenografts within 1 h post injection. The high values noted in kidney are partially due to high expression of PSMA within proximal renal tubules (Silver et al., Clin Cancer Res, vol. 3, pp. 81-85, 1997; Slusher et al., J Comp Neurol, vol. 315, pp. 271-229, 1992). Rapid clearance from the kidneys was observed while the activity retained in PSMA+ tumor even after four days post injection.

[00230] SPECT-CT imaging experiment of compound [^{111}In]SRV100 (**Figure 6**) demonstrated similar clear PSMA-dependent binding in PSMA+ PC3 PIP xenografts within 2 h post injection. The high values noted in kidney are partially due to high expression of PSMA within proximal renal tubules (Silver et al., Clin Cancer Res, vol. 3, pp. 81-85, 1997; Slusher et al., J Comp Neurol, vol. 315, pp. 271-229, 1992). Rapid clearance from the kidneys was observed while the activity retained in PSMA+ tumor even after four days post injection. The longer tumor activity retention for [^{111}In]SRV27 and [^{111}In]SRV100 might be useful Y-90/Lu-177 based radiotherapeutic application.

[00231] **Figure 7** demonstrates clear tumor uptake for [^{111}In]SRV73 at 7 h post injection. This is significant since after attaching a bulky fluorescent dye, rhodamine, the compound retains its PSMA binding activity. This is an example of dual modality application for this class of compounds.

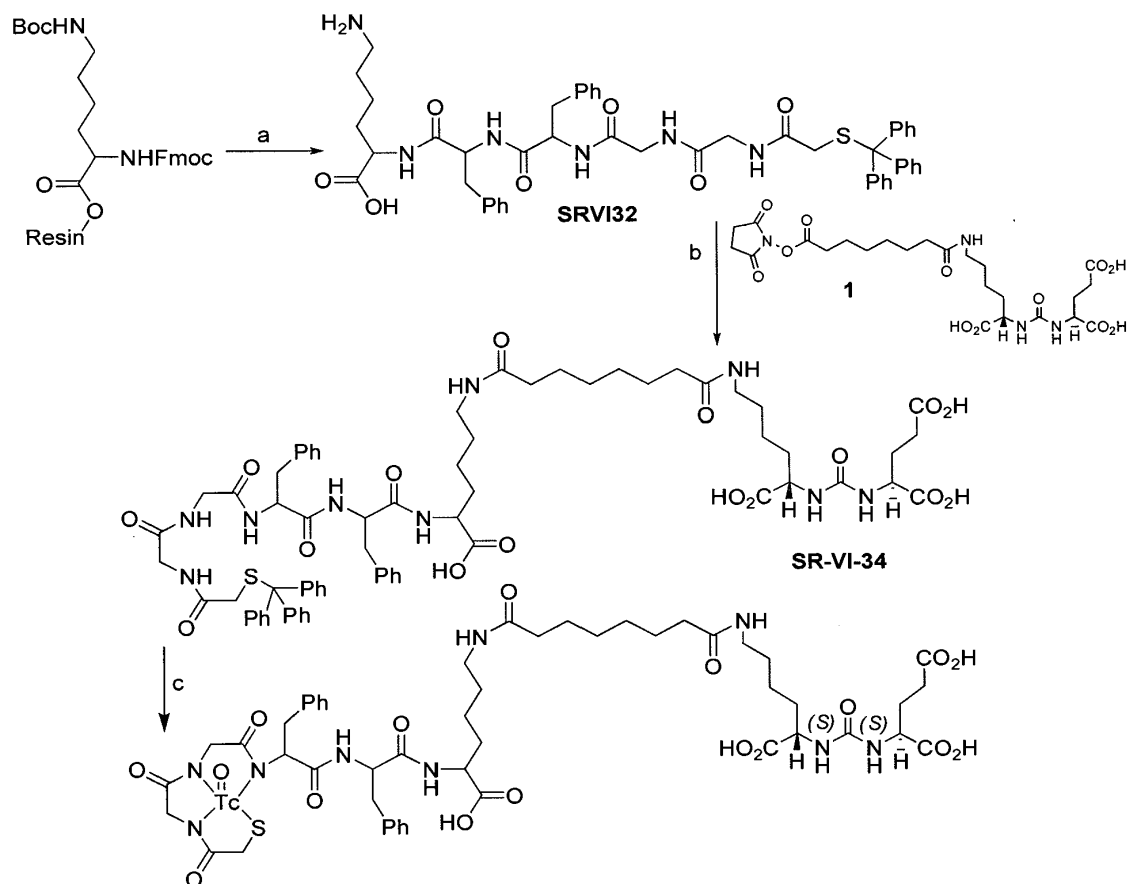
EXAMPLE 4

SRVI34

[00232] **2-{3-[1-Carboxy-5-(7-{5-carboxy-5-[3-phenyl-2-(3-phenyl-2-{2-[2-(2-tritylsulfanyl-acetylamino)-acetylamino]-acetylamino}-propionylamino)-propionylamino]-pentylcarbamoyl}-heptanoylamino)-pentyl]-ureido}-pentanedioic acid (SRVI34).** SRVI34 was prepared according to the scheme below. Lys(Boc)-Wang resin (100 mg, 0.43 mM) was allowed to swell with CH_2Cl_2 (3 mL) followed by DMF (3 mL). A solution of 20% piperidine in DMF (3 x 3 mL) was added to the resin that was then shaken gently on a mechanical shaker for 30 min at ambient temperature. The resin was washed with DMF (3 x 3 mL) and CH_2Cl_2 (3 x 3

mL). Formation of free amine was assessed by the Kaiser test. After swelling the resin in DMF, a solution of Fmoc-Phe-OH (3 eq), HBTU (3eq), HOBt (3 eq), and DIPEA (4.0 eq) in DMF was added and gently shaken for 2 h. The resin was then washed with DMF (3 x 3 mL) and CH₂Cl₂ (3 x 3 mL). The coupling efficiency was assessed by the Kaiser Test. That aforementioned sequence was repeated for four more coupling steps with Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Gly-OH and S-trityl mercaptoacetic acid. Finally the product was cleaved from the resin using TFA:CH₂Cl₂ (1:1) and concentrated under vacuum to produce the free amine (SRVI32). The concentrated product was purified by using a C₁₈ SepPak Vac 2g column. The product was eluted with a solution 70/30 water/acetonitrile (0.1% TFA in each). ESIMS: [M+1]⁺.

Lyophilized SRVI32 (10 mg, 12 μmol in 2 mL DMF) was added to the urea (compound 1 described in Example 2) (20 mg, 21.4 μmol in 1 mL DMF) followed by TEA (214 μmol, 30 μL) and then stirred at 25°C for 16 h. The residue was washed 2 x 5 mL CH₂Cl₂ to remove impurities. The colorless solid residue thus obtained was purified by a C₁₈ SepPak Vac 2g column using an eluent of 70/30 water/acetonitrile (0.1% TFA in each). The product was further purified using preparative RP-HPLC by Method 1, retention time 17 min. Yield: ~ 30%. ESMS m/z: 1328 [M+H]⁺, ¹H NMR (D₂O/CD₃CN (1:1) δ: 7.98 (m, 5H), 7.90-7.76 (m, 18H), 7.66 (m, 2H), 5.11 (m, 1H), 4.82-4.72 (m, 3H), 4.28 (m, 2H), 4.16 (m, 2H), 3.68 (m, 5H), 3.49-3.32 (m, 2H), 3.00 (m, 2H), 2.69 (m, 4H), 2.64-1.74 (m, 26H).



a. (i) 20% piperidine/DMF; (ii) Fmoc-Phe-OH, HOBT, HBTU, DIEA; (iii) 20% piperidine/DMF; (iv) Fmoc-Phe-OH, HOBT, HBTU, DIEA; (v) 20% piperidine/DMF; (vi) Fmoc-Gly-OH, HOBT, HBTU, DIEA; (vii) 20% piperidine/DMF; (viii) Fmoc-Gly-OH, HOBT, HBTU, DIEA; (ix) 20% piperidine/DMF; (x) (S-Ph₃)-CH₂CO₂H, HOBT, HBTU, DIEA; (xi) TFA/CH₂Cl₂; b. TEA, DMF; c. TcO₄⁻, SnCl₂, sodium ascorbate, Na-tartrate, NH₄OAc, pH 7.5.

[00233] Radiolabeling with Tc-99m: Radiolabeling was performed following a literature procedure (Wang et al., Nature Protocols, vol. 1, pp. 1477-1480, 2006). Briefly, 1 mg (75.3 μmol) of compound SRVI34 was dissolved in 1 ml of 0.5 M ammonium acetate buffer at pH 8. Disodium tartarate dihydrate was dissolved in the labelling buffer of 0.5 M ammonium acetate (pH 8) to a concentration of 50 mg/ml. Ascorbic acid-HCl solution was prepared by dissolving ascorbic acid in 10 mM HCl to a concentration of 1.0 mg/ml. A solution of SRVI34 (80 μl) was combined to a solution of 45 μl 0.25 M ammonium acetate, 15 μl tartarate buffer, followed by 5 μl of the freshly prepared 4 mg/ml SnCl₂ · 2H₂O solution in the ascorbate-HCl

solution. The final pH will be about 8–8.5. After vortexing, was added 20 mCi of ^{99m}Tc -pertechnetate in 200 μl saline and was heated the solution at 90–100°C for 20 min. Reaction mixture was cooled, diluted 850 μl of water and purified by HPLC using a Phenomenex C_{18} Luna $10 \times 250 \text{ mm}^2$ column on a Waters 600E Delta LC system with a Waters 486 tunable absorbance UV/Vis detector, both controlled by Empower software. HPLC solvent system, flow rate= 4 ml/min, a gradient, 0-5 min, 80/20 water/acetonitrile (0.1% TFA in each solvent), 5-25 min 40/60 water/acetonitrile (0.1% TFA in each solvent) and 25-35 min 80/20 (0.1% TFA in each solvent) was used. Two radiolabeled products were found, called as [^{99m}Tc]SRVI34A (5.52 mCi) (retention time 17.5 min) and [^{99m}Tc]SRVI34B (6 mCi) (retention time 18.9 min). SRVI34A and SRVI32B are diastereomers, syn and anti-isomers with respect to the $\text{Tc}=\text{O}$ group. Each product was neutralized with 50 μl of 1 M sodium bicarbonate and evaporated to dryness under vacuum. The obtained solid residues was dissolved in 200 μl saline and used for imaging and biodistribution studies.

Ex vivo Biodistribution

[00234] PSMA+ PC-3 PIP and PSMA- PC-3 flu xenograft-bearing SCID mice were injected *via* the tail vein with 30 μCi [^{99m}Tc]SRVI34B. Four mice were sacrificed by cervical dislocation at 30, 60, 120, and 300 min p.i. The heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, muscle, small and large intestines, urinary bladder, and PC-3 PIP and flu tumors were quickly removed. A 0.1 mL sample of blood was also collected. Each organ was weighed, and the tissue radioactivity was measured with an automated gamma counter (1282 Compugamma CS, Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The %ID/g was calculated by comparison with samples of a standard dilution of the initial dose. All measurements were corrected for decay.

[00235] Compound [^{99m}Tc]SRVI34B was assessed for its pharmacokinetics *ex vivo* in severe-combined immunodeficient (SCID) mice bearing both PSMA+ PC3-PIP and PSMA-PC3- flu xenografts (Chang et al., *Cancer Res*, vol. 59, pp. 3192-3198, 1999). **Table 3** shows the percent injected dose per gram (%ID/g) of radiotracer in selected organs for [^{99m}Tc]SRVI34B.

Table 3: Biodistribution data for [^{99m}Tc]SRVI34B (n = 4)

	30 min	60 min	120 min	300 min
Blood	1.13±1.06	0.69±0.08	0.27±0.09	0.23±0.00
heart	1.11±0.06	0.70±0.16	0.61±0.07	0.46±0.05
lung	4.08±0.31	4.84±1.26	4.02±0.73	2.79±0.74
liver	1.55±0.23	0.92±0.37	0.50±0.085	0.24±0.09
stomach	0.79±0.23	0.77±0.17	0.54±12	0.27±0.08
pancreas	1.72±0.74	1.42±0.45	1.02±0.29	0.94±0.46
spleen	56.44±16.49	64.24±13.29	58.27±18.26	24.49±3.63
fat	2.18037±0.50	2.13±0.58	1.82±0.37	0.99±0.03
kidney	62.45±1.63	96.38±22.74	104.84±19.03	116.14±2.71
muscle	1.20±0.12	0.74±0.04	1.29±1.31	0.45±0.31
small intestine	1.03±0.40	1.43±0.66	0.79±0.33	0.23±0.12
large intestine	0.61±0.03	0.63±0.38	0.35±0.12	1.30±0.08
bladder	1.28±0.25	2.07±0.96	0.87±0.33	0.51±0.00
PC-3 PIP	6.11±0.94	7.99±2.26	6.96±1.13	4.81±0.66
PC-3 flu	0.98±0.38	0.76±0.51	0.50±0.28	0.22±0.11
PIP:flu	6.28	10.56	14.05	22.18

Small Animal SPECT-CT Imaging

[00236] Imaging experiments for [^{99m}Tc]SRVI34A and [^{99m}Tc]SRVI34B were done following the same procedures as was done for [^{99m}Tc]SRV32 (Example 1).

[00237] **Figures 13, 14, 15, and 16** demonstrate the high target selectivity of [^{99m}Tc]SRVI34B by delineating the PSMA+ tumors. The compound [^{99m}Tc]SRVI34B exhibited high uptake in PSMA+ tumor and no uptake in PSMA- tumor. The tumor uptake remains high 4.88% ID/g even at 5 hr post inject (p.i.). However this compound showed very high kidney uptake 116% ID/g even at 5 hr p.i. In addition this compound showed high spleen uptake 24.5 %ID/g at 5 hr p.i.

EXAMPLE 5

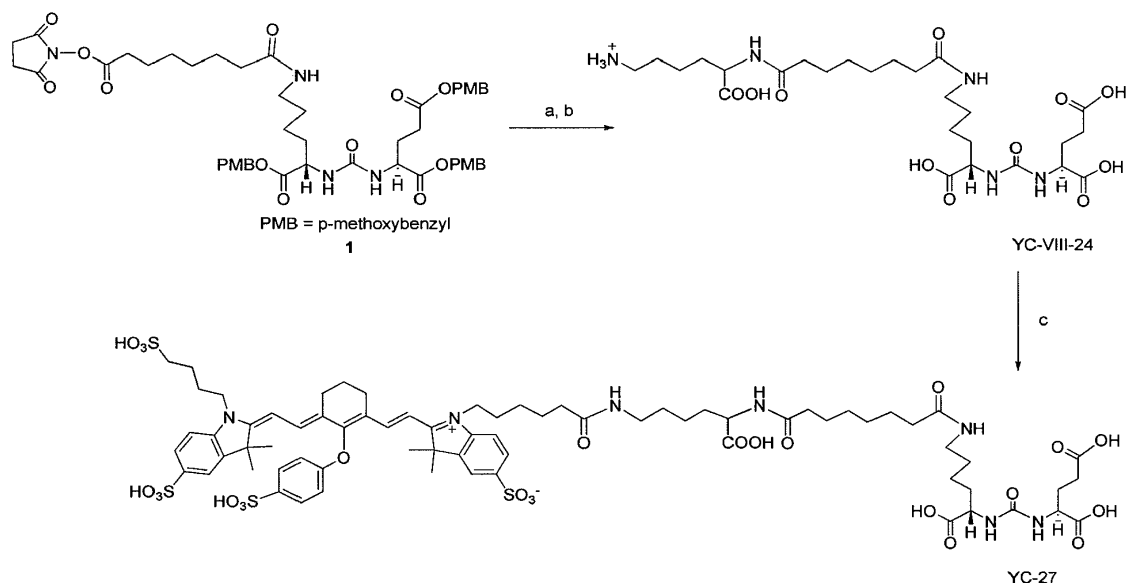
General

[00238] All reagents and solvents were purchased from either Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA). 2-{3-[5-[7-(2,5-Dioxo-pyrrolidin-1-yl)oxycarbonyl]-heptanoylamino]-1-(4-methoxy-benzoyloxycarbonyl)-pentyl]-ureido}-pentanedioic acid bis-(4-methoxy-benzyl) ester (**1**) was prepared according to (Banerjee et al., J. Med. Chem., vol. 51, pp. 4504-4517, 2008). H-Lys(Boc)-OBu-HCl was purchased from Chem-

Impex International (Wood Dale, IL). The *N*-hydroxysuccinimide (NHS) ester of IRDye 800CW was purchased from LI-COR Biosciences (Lincoln, NE). ^1H NMR spectra were obtained on a Bruker Avance 400 MHz Spectrometer. ESI mass spectra were obtained on a Bruker Esquire 3000 plus system. Purification by high-performance liquid chromatography (HPLC) was performed on a Varian Prostar System (Varian Medical Systems, Palo Alto, CA).

YC-27

[00239] Compound YC-27 was prepared according to the scheme shown below.



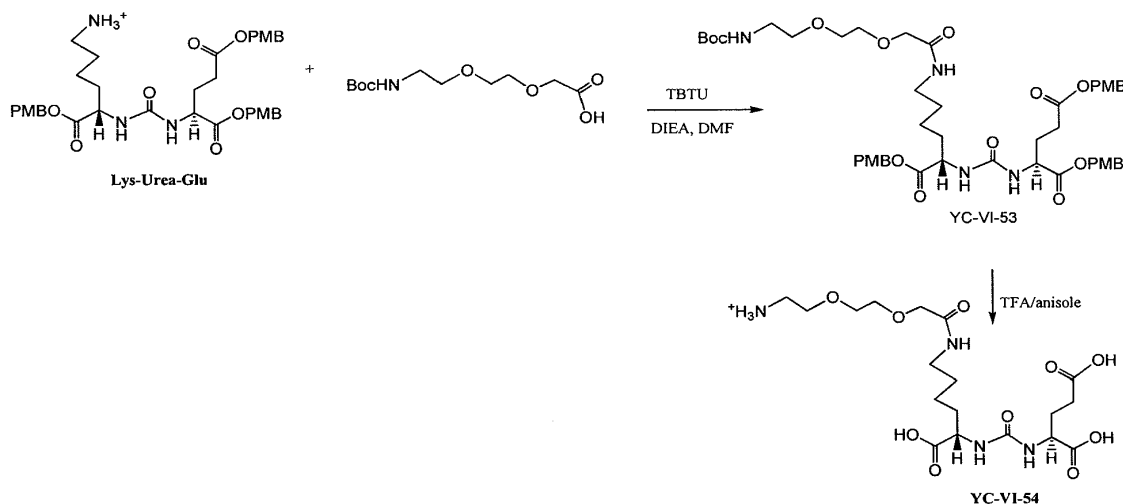
(a) H-Lys(Boc)-OBu·HCl, Et₃N, CH₂Cl₂; (b) TFA:CH₂Cl₂ = 1:1; (c) IRDye800CW-NHS, DIPEA, DMSO

[00240] **Trifluoroacetate salt of 2-(3-{5-[7-(5-amino-1-carboxy-pentylcarbamoyl)-heptanoylamino]-1-carboxy-pentyl}-ureido)-pentanedioic acid (YC-VIII-24).** To a solution of 1 (0.065 g, 0.020 mmol) in CH₂Cl₂ (2 mL) was added triethylamine (0.040 mL, 0.285 mmol), followed by H-Lys(Boc)-OBu·HCl (0.028 g, 0.083 mmol). After stirring for 2 h at room temperature, the solvent was evaporated on a rotary evaporator. A solution of TFA/CH₂Cl₂ 1:1 (2 mL) was then added to the residue and stirred for 1 h at room temperature. The crude material was purified by HPLC (column, Econosphere C18, 10μ, 250 × 10 mm; retention time, 15 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 min = 5% B, 25 min = 25% B; flow rate, 4 mL/min) to afford 0.032 g (66%) of YC-VIII-24. ^1H NMR

(400 MHz, D₂O) δ 4.24-4.28 (m, 1H), 4.17-4.20 (m, 1H), 4.08-4.12 (m, 1H), 3.08-3.12 (m, 2H), 2.88-2.92 (m, 2H), 2.41-2.44 (m, 2H), 2.19-2.21 (m, 2H), 2.05-2.16 (m, 3H), 1.57-1.93 (m, 7H), 1.21-1.50 (m, 10H), 1.21 (m, 4H). ESI-Mass calcd for C₂₆H₄₆N₅O₁₁ [M]⁺ 604.3, found 604.0.

[00241] YC-27. To a solution of **YC-VIII-24** (0.3 mg, 0.43 μ mol) in DMSO (0.1 mL) was added *N,N*-diisopropylethylamine (0.002 mL, 11.4 μ mol), followed by the NHS ester of IRDye 800CW (0.3 mg, 0.26 μ mol). After stirring for **YC-VIII-24** for 2 h at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 22 min, mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 min = 0% B, 5 min = 0% B, 45 min = 100% B; flow rate, 1 mL/min) to afford 0.3 mg (72%) of **YC-27**. ESI-Mass calcd for C₇₂H₉₇N₇O₂₅S₄ [M]⁺ 1587.5, found 794.3 [M+H]²⁺, 1587.6 [M]⁺.

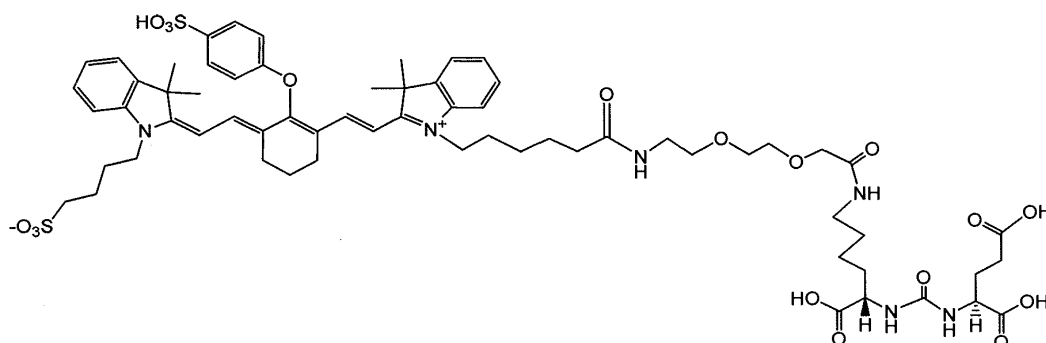
Synthesis of precursor YC-VI-54



[00242] To a solution of **Lys-Urea-Glu** (0.103 g, 0.121 mmol, Banerjee et al *J. Med. Chem.*, vol. 51, pp. 4507-4517, 2008) in DMF (2 mL) was added Boc-NH-PEG-COOH (0.060 g, 0.135 mmol) and TBTU (0.040g, 0.125 mmol), followed by *N,N'*-diisopropylethylamine (0.042 mL, 0.241 mmol). After stirring overnight at room temperature, the solvent was evaporated on a rotary evaporator. The crude material was purified by a silica column using methanol/methylene chloride (5:95) to afford 0.101 g (0.109 mmol, 90%) of **YC-VI-53**, which was dissolved in a solution of 3% anisole in TFA (1 mL). The mixture was reacted at room

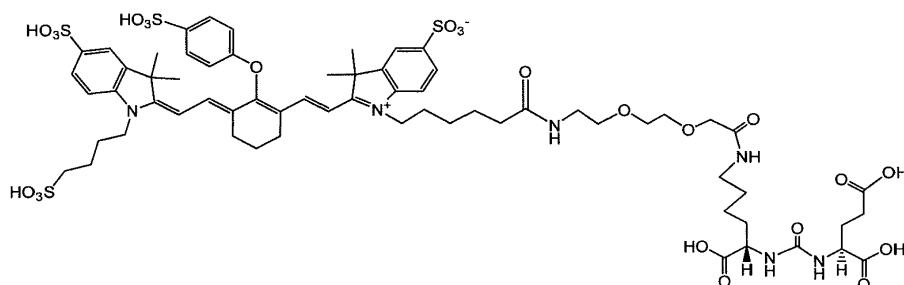
temperature for 10 min, then concentrated on a rotary evaporator. The crude material was purified by HPLC (Econosphere C18 10u, 250 x 10 mm, H₂O/CH₃CN/TFA (92/8/0.1), 4 mL/min, Compound **YC-VI-54** eluting at 11 min) to afford 0.035 g (57%) of compound **YC-VI-54**. ¹H NMR (400 MHz, D₂O) δ 4.17-4.21 (m, 1H), 4.10-4.13 (m, 1H), 4.00 (s, 2H), 3.67-3.71 (m, 6H), 3.14-3.20 (m, 4H), 2.43-2.46 (m, 2H), 2.08-2.13 (m, 1H), 1.87-1.93 (m, 1H), 1.76-1.79 (m, 1H), 1.63-1.67 (m, 1H), 1.45-1.50 (m, 2H), 1.33-1.40 (m, 2H). ESI-Mass calcd for C₁₈H₃₃N₄O₁₀ [M]⁺ 465.2, found 465.2.

YC-VIII-11



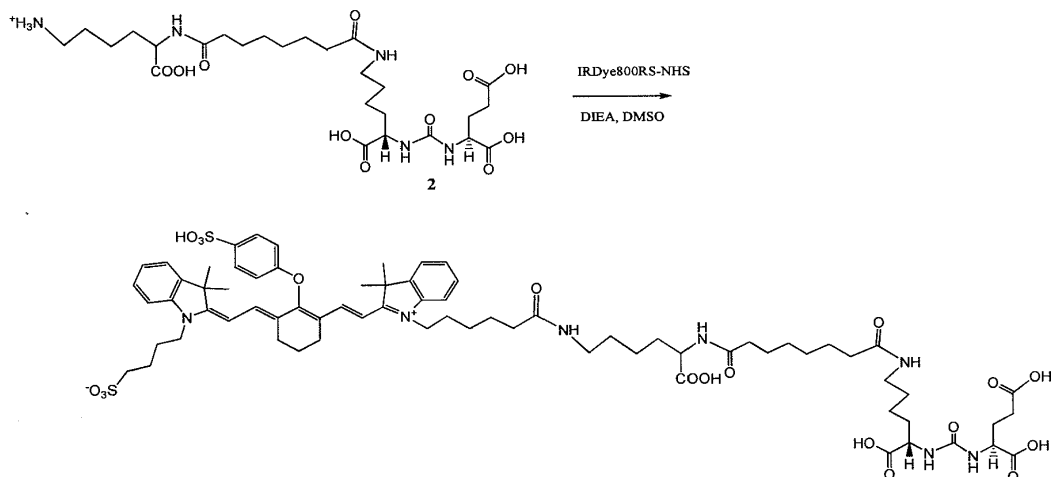
[00243] To a solution of compound **YC-VI-54** (0.3 mg, 53 μ mol) in DMSO (0.05 mL) was added *N,N*-diisopropylethylamine (0.002 mL, 11.4 μ mol), followed by NHS ester of IRDye 800RS (0.2 mg, 0.21 μ mol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 x 4.6 mm; retention time, 28 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.2 mg (75%) of compound **YC-VIII-11**. ESI-Mass calcd for C₆₄H₈₄N₆O₁₈S₂ [M]⁺ 1288.5, found 1288.9.

YC-VIII-12

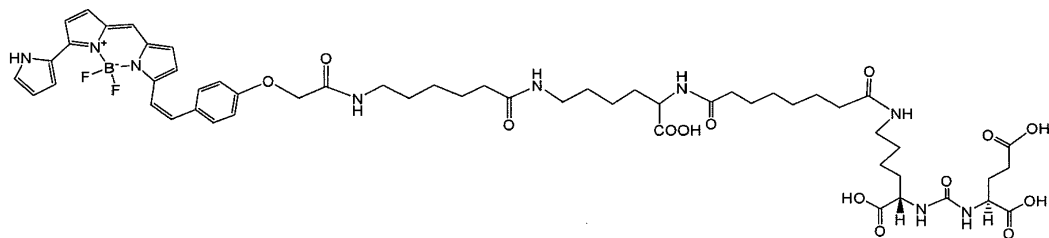


[00244] To a solution of compound **YC-VI-54** (0.3 mg, 53 μmol) in DMSO (0.05 mL) was added *N,N*-diisopropylethylamine (0.002 mL, 11.4 μmol), followed by NHS ester of IRDye800CW (0.2 mg, 0.17 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 22 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.2 mg (80%) of compound **YC-VIII-12**. ESI-Mass calcd for C₆₄H₈₄N₆O₂₄S₄ [M]⁺ 1448.4, found 1448.7.

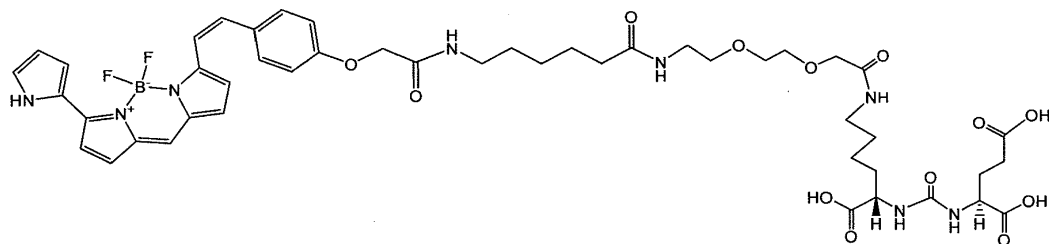
YC-VIII-28



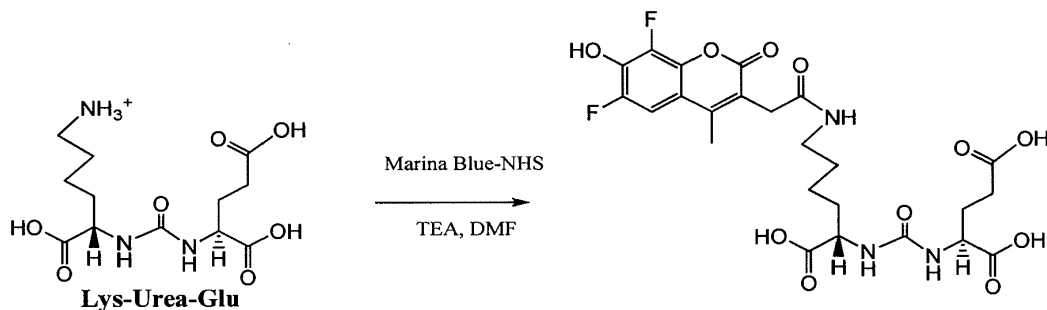
[00245] To a solution of **YC-VIII-24** (prepared as described previously for **YC-27**) (0.3 mg, 0.42 μmol) in DMSO (0.1 mL) was added *N,N*-diisopropylethylamine (0.002 mL, 11.5 μmol), followed by NHS ester of IRDye 800RS (0.3 mg, 0.31 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 27 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.3 mg (67%) of compound **YC-VIII-28**. ESI-Mass calcd for C₇₂H₉₇N₇O₁₉S₂ [M]⁺ 1427.6, found 714.4 [M+H]²⁺, 1427.8 [M]⁺.

YC-VIII-30

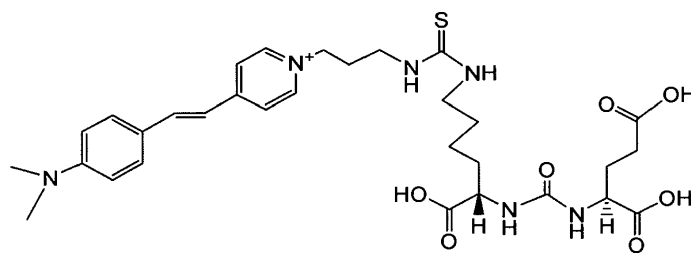
[00246] To a solution of **YC-VIII-24** (0.5 mg, 0.70 μmol) in DMSO (0.1 mL) was added *N,N*-diisopropylethylamine (0.005 mL, 28.7 μmol), followed by NHS ester of BODIPY 650/665-X (0.3 mg, 0.47 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 28 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.4 mg (75%) of compound **YC-VIII-30**. ESI-Mass calcd for C₅₅H₇₃BF₂N₉O₁₄ [M+H]⁺ 1132.5, found 1132.0.

YC-VIII-31

[00247] To a solution of **YC-VI-54** (0.5 mg, 0.70 μmol) in DMSO (0.1 mL) was added *N,N*-diisopropylethylamine (0.005 mL, 28.7 μmol), followed by NHS ester of BODIPY 650/665-X (0.3 mg, 0.47 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 29 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.4 mg (86%) of compound **YC-VIII-31**. ESI-Mass calcd for C₄₇H₅₉BF₂N₈O₁₃ [M]⁺ 992.4, found 992.9.

YC-VIII-41

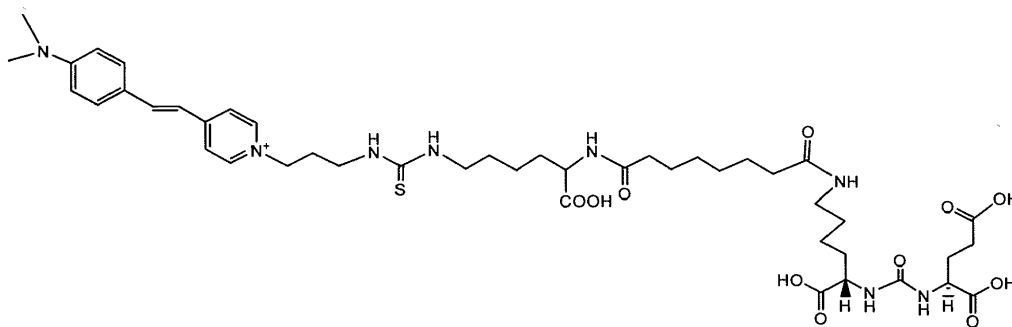
[00248] To a solution of Lys-Urea-Glu (4.0 mg, 9.6 μmol) in DMF (0.5 mL) was added triethylamine (0.01 mL, 71.7 μmol), followed by Marina Blue-NHS ester (1.8 mg, 4.9 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 10 μ , 250 $\times$ 10 mm; retention time, 14 min; mobile phase, H₂O/CH₃CN/TFA = 85/15/0.1; flow rate, 4 mL/min) to afford 2.5 mg (89%) of compound **YC-VIII-41**. ¹H NMR (400 MHz, D₂O) δ 7.40 (d, J = 11.6 Hz, 1H), 4.23-4.31 (m, 1H), 4.15-4.19 (m, 1H), 3.64 (s, 2H), 3.19-3.23 (m, 2H), 2.49-2.53 (m, 2H), 2.39 (s, 3H), 2.06-2.17 (m, 1H), 1.95-1.99 (m, 1H), 1.83-1.90 (m, 1H), 1.72-1.80 (m, 1H), 1.52-1.55 (m, 2H), 1.40-1.45 (m, 2H). ESI-Mass calcd for C₂₄H₂₈F₂N₃O₁₁ [M+H]⁺ 572.2, found 571.8.

YC-VIII-52

[00249] To a solution of Lys-Urea-Glu (4.0 mg, 9.6 μmol) in DMSO (0.5 mL) was added *N,N*-diisopropylethylamine (0.020 mL, 114.8 μmol), followed by 4-[2-(4-dimethylamino-phenyl)-vinyl]-1-(3-isothiocyanato-propyl)-pyridium (3 mg, 7.4 μmol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 10 μ , 250 $\times$ 10 mm; retention time, 13 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1%

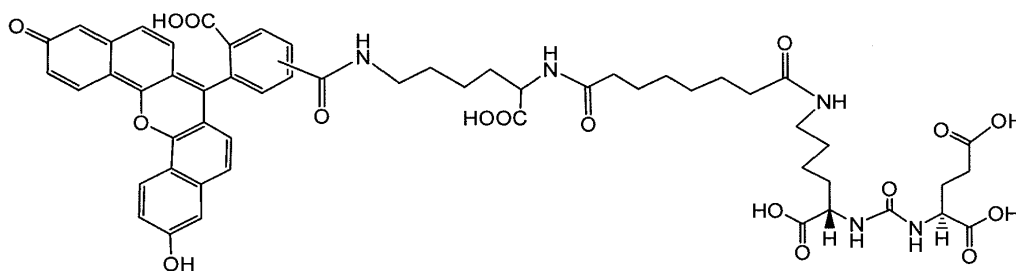
TFA in CH₃CN; gradient, 0 mins = 10% B, 20 mins = 60% B; flow rate, 4 mL/min) to afford 1.3 mg (24%) of compound **YC-VIII-52**. ESI-Mass calcd for C₃₁H₄₃N₆O₇S [M]⁺ 643.3, found 642.9.

YC-VIII-74



[00250] To a solution of **YC-VIII-24** (3.0 mg, 4.2 μ mol) in DMSO (0.5 mL) was added *N,N*-diisopropylethylamine (0.020 mL, 114.8 μ mol), followed by 4-[2-(4-dimethylamino-phenyl)-vinyl]-1-(3-isothiocyanato-propyl)-pyridium (2 mg, 4.9 μ mol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 15 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 2 mg (47%) of compound **YC-VIII-74**. ESI-Mass calcd for C₄₅H₆₇N₈O₁₁S [M]⁺ 927.5, found 927.0.

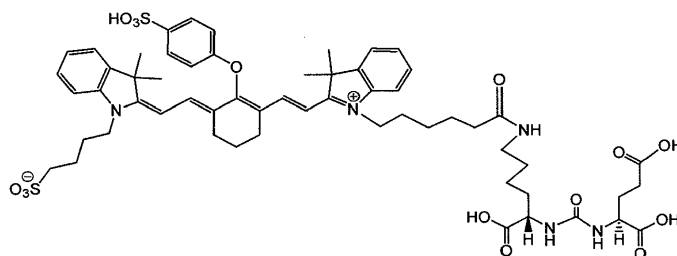
YC-VIII-63



[00251] To a solution of **YC-VIII-24** (5.0 mg, 7.0 μ mol) in DMF (1 mL) was added triethylamine (0.020 mL, 143.5 μ mol), followed by NHS ester of 5-(and-6)-carboxynaphthofluorescein (4.0 mg, 7.0 μ mol). After 1 hour at room temperature, the reaction

mixture was purified by HPLC (column, Econosphere C18 10 μ , 250 $\times$ 10 mm; retention time, minor product at 17 min, major product at 20 min); mobile phase, H₂O/CH₃CN/TFA = 70/30/0.1; flow rate, 4 mL/min) to afford 0.3 mg of minor and 2.2 mg of major product (two isomers of **YC-VIII-63**). ESI-Mass calcd for C₅₅H₅₉N₅O₁₇ [M]⁺ 1061.4, found 1061.6 (for both minor and major product).

YC-IX-92



[00252] To a solution of Lys-Urea-Glu (0.2 mg, 0.48 μ mol) in DMSO (0.05 mL) was added *N,N*-diisopropylethylamine (0.002 mL, 11.5 μ mol), followed by NHS ester of IRDye 800RS (0.2 mg, 0.21 μ mol). After 2 hour at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 23 min; mobile phase, A = 0.1% TFA in H₂O, B = 0.1% TFA in CH₃CN; gradient, 0 mins = 0% B, 5 mins = 0% B, 45 mins = 100% B; flow rate, 1 mL/min) to afford 0.2 mg (84%) of compound **YC-IX-92**. ESI-Mass calcd for C₅₈H₇₃N₅O₁₅S₂ [M]⁺ 1143.5, found 572.5 [M+H]²⁺, 1144.0 [M]⁺.

Characterization - Fluorescence

[00253] Fluorescence spectra were recorded using a Varian Cary Eclipse fluorescence spectrophotometer (Varian Medical Systems) with excitation from a Xenon arc lamp. **YC-27** was dissolved in water. All of the fluorescence measurements were performed in aqueous solution under ambient conditions. The fluorescence quantum yield of **YC-27** was measured using an aqueous solution of ICG (Φ = 0.016 (Sevick-Muraca et al., Photochem. Photobiol., vol. 66, pp. 55-64, 1997), excitation wavelength at 775 nm) as the standard (**Figure 8**). The fluorescence intensity data were collected in the spectral region 780 – 900 nm over which quantum yield was integrated. Time-resolved intensity decays were recorded using a PicoQuant Fluotime 100 time-correlated single-photon counting (TCSPC) fluorescence lifetime spectrometer (PicoQuant, Berlin, DE). The excitation was obtained using a pulsed laser diode

(PicoQuant PDL800-B) with a 20 MHz repetition rate. The fluorescence intensity decay of **YC-27** was analyzed in terms of the single-exponential decay using the PicoQuant Fluofit 4.1 software with deconvolution of the instrument response function and nonlinear least squares fitting. The goodness-of-fit was determined by the χ^2 value.

[00254] The electronic spectrum of **YC-27** exhibited an absorbance maximum at 774 nm with an extinction coefficient of $158,900 \text{ M}^{-1}$. Upon excitation, **YC-27** provided intense fluorescence with an emission maximum at 792 nm and a fluorescence lifetime of 443 psec in aqueous solution (**Figure 9**). Using an excitation wavelength of 775 nm, **YC-27** demonstrated a fluorescence quantum yield of 0.053 in aqueous solution relative to ICG, which demonstrated a quantum yield of 0.016 (**Figure 8**) (Sevick-Muraca et al., Photochem. Photobiol., vol. 66, pp. 55-64, 1997), attesting to the efficiency of this IRDye 800CW-based compound. That is significant because ICG has been used previously for intraoperative tumor mapping (K. Gotoh, T. Yamada, O. Ishikawa, H. Takahashi, H. Eguchi, M. Yano, H. Ohigashi, Y. Tomita, Y. Miyamoto, and S. Imaoka, A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. J. Surg. Oncol., 2009).

In vitro NAALADase Activity

[00255] PSMA inhibitory activity of **YC-27** was determined using a fluorescence-based assay according to a previously reported procedure (Chen et al., J. Med. Chem., vol. 51, pp. 7933-7943, 2008). Briefly, lysates of LNCaP cell extracts (25 μL) were incubated with the inhibitor (12.5 μL) in the presence of 4 μM *N*-acetylaspartylglutamate (NAAG) (12.5 μL) for 120 min. The amount of glutamate released by NAAG hydrolysis was measured by incubation with a working solution (50 μL) of the Amplex Red Glutamic Acid Kit (Molecular Probes Inc., Eugene, OR) for 60 min. Fluorescence was measured with a VICTOR³V multilabel plate reader (Perkin Elmer Inc., Waltham, MA) with excitation at 530 nm and emission at 560 nm. Inhibition curves were determined using semi-log plots, and IC_{50} values were determined at the concentration at which enzyme activity was inhibited by 50%. Assays were performed in triplicate. Enzyme inhibitory constants (K_i values) were generated using the Cheng-Prusoff conversion [19]. Data analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California).

[00256] This assay is free from the interference of IRDye 800CW because the excitation/emission maxima of IRDye 800CW are remote from those of resorufin ($\lambda_{\text{ex}} = 563 \text{ nm}$,

$\lambda_{em} = 587 \text{ nm}$), which provides the fluorescent readout in the assay. The K_i value of **YC-27** was 0.37 nM with 95% confidence intervals from 0.18 nM to 0.79 nM. Under the same experimental conditions, the K_i value of the known PSMA inhibitor ZJ-43 (Zhou et al., Nat. Rev. Drug Discov., vol. 4, pp. 1015-1026, 2005) was 2.1 nM, indicating the high inhibitory capacity of **YC-27**. The inhibition curve of **YC-27**, which is expressed with respect to the amount of glutamate released from hydrolysis of NAAG, is shown in **Figure 10**.

Biodistribution and Imaging

[00257] Cell Culture and Animal Models. Both PSMA-expressing (PSMA+ PC3-PIP) and non-expressing (PSMA- PC3-flu) prostate cancer cell lines (Chang et al, Cancer Res., vol. 59, pp. 3192-3198, 1999) were grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Invitrogen) and 1% Pen-Strep (Biofluids, Camarillo, CA). All cell cultures were maintained in 5% carbon dioxide (CO_2), at 37.0°C in a humidified incubator. Animal studies were undertaken in compliance with the regulations of the Johns Hopkins Animal Care and Use Committee. Six- to eight-week-old male, non-obese diabetic (NOD)/severe-combined immunodeficient (SCID) mice (Charles River Laboratories, Wilmington, MA) were implanted subcutaneously (s.c.) with PC3-PIP and PC3-flu cells (2×10^6 in 100 μL of Matrigel) at the forward left and right flanks, respectively. Mice were imaged or used in *ex vivo* biodistribution assays when the xenografts reached 5 to 7 mm in diameter.

[00258] *In vivo* Imaging and *Ex vivo* Biodistribution. Mouse #1 was injected with 10 nmol and mouse #2 with 1 nmol of **YC-27** in 200 μL of PBS intravenously (i.v.) *via* the lateral tail vein. Mouse #3 was injected with 1 nmol of **YC-27** and also co-injected with 1 μmol of the known PSMA inhibitor 2-{3-[1-carboxy-5-(4-iodo-benzoylamino)-pentyl]-ureido}-pentanedioic acid (DCIBzL) (Chen et al., J. Med. Chem., vol. 51, pp. 7933-7943, 2008; Barinka et al., J. Med. Chem. vol. 51, pp. 7737-7743, 2008) in 200 μL of PBS i.v. to assess for PSMA binding specificity. Images were acquired at an array of post-injection (p.i.) time points starting at 10 min p.i. using a dedicated small animal optical imaging instrument, the Pearl Imager (LI-COR Biosciences). The Pearl Imager uses diffusive lasers optimized for IRDye 800CW. The instrument employs a CCD camera with a field-of-view of 11.2 cm x 8.4 cm at the surface of the imaging bed. The scan time was less than 30 sec to complete white light, 700 nm channel and 800 nm channel image acquisition. Images are displayed using a pseudocolor output with

corresponding scale. All images were acquired at the same parameter settings and are scaled to the same maximum values. Imaging bed temperature was adjusted to 37°C. Animals received inhalational anesthesia (isoflurane) through a nose cone attached to the imaging bed. Animals were sacrificed by cervical dislocation for *ex vivo* imaging studies at the end of acquisition of the *in vivo* images. *Ex vivo* images were acquired first by midline surgical laparotomy and then again by harvesting liver, spleen, stomach, small intestine, kidneys, urinary bladder, PC3-PIP and PC3-flu tumors and displaying them individually on plastic Petri dishes. Estimates of signal output were provided by drawing three circular regions of interest within each tumor and determining the average signal (arbitrary units)/area using the manufacturer's software.

[00259] **Figure 11** (mouse #1) depicts the pharmacokinetic behavior of **YC-27** *in vivo*. In this experiment 10 nmol of **YC-27** was administered intravenously and the animal was imaged repeatedly over a three day period. Although difficult to quantify as these are planar images, one can see clearly increased uptake in the PSMA+ PC3-PIP tumor relative to the control (PSMA-negative) PC3-flu tumor at 18.5 h p.i. through 70.5 h p.i. (**Figure 11C through 11M**). Using quantitative real time polymerase chain reaction (qRT-PCR) we measured the relative amounts of PSMA mRNA expression in extracts of the tumors in mice #1-3, and confirmed that PC3-PIP tumors (left flank) expressed PSMA mRNA at levels several million times higher than PC3-flu tumors (right flank) (data not shown). Panels **11L** and **11M** show emission from the intact, living, unshaven animal, while panels **11N** and **11O** are postmortem studies with organs exposed. Note that in **11L** one can barely discern the kidneys, a known target site for PSMA (Tasch et al., Crit. Rev. Immunol., vol. 21, pp. 249-261, 2001; Pomper et al., Mol. Imaging, vol. 1, pp. 96-101, 2002; Kinoshita et al., World J. Surg., vol. 30, pp. 628-636, 2006), while the kidneys are clearly visible in **11O** when exposed. A portion of that renal light emission may be due to clearance of this relatively hydrophilic compound. The estimated target-to-nontarget ratio (PC-3 PIP vs. PC-3 flu light output) was 10 when comparing the tumors from panel M (70.5 h p.i.).

[00260] The experiment in **Figure 12** was performed with 10-fold less **YC-27** administered than in the previous experiment. Despite reducing the concentration of **YC-27**, PSMA+ PC3-PIP tumor could be seen clearly at one day p.i. (**Figure 12**, mouse #2, *Left Panels*). DCIBzL, a known, high-affinity PSMA inhibitor, was co-administered with **YC-27** as a test of binding specificity (**Figure 12**, mouse #3, *Right Panels*). Nearly all of the light emission from target tumor, as well as kidneys, was blocked, demonstrating the specificity of

this compound for PSMA *in vivo*. The estimated target-to-nontarget ratio (PC-3 PIP vs. PC-3 flu light output) was 26 when comparing the tumors from panel F (20.5 h p.i.). By administering 1 nmol to this ~ 25 g mouse, we have realized the high sensitivity of *in vivo* optical imaging, rivaling that of the radiopharmaceutical-based techniques. For example, 1 nmol converts to 1.6 μg injected. If we synthesized a similar compound labeled with ^{18}F or other radionuclide at 1,000 mCi/ μmol (37 GBq/ μmol), and administered a standard dose of 200 μCi (7.4 MBq) to a mouse, we would be injecting 0.3 μg .

[00261] Interestingly, in mouse #1, which received 10 nmol of **YC-27**, we observed a small degree of non-specific uptake at the 23 h time point, manifested as uptake within PSMA-negative PC3-flu tumors. That finding could be due to enhanced permeability and retention of **YC-27**. No non-specific uptake/retention was observed at a similar, 20.5 h, time point in mouse #2, which received a 10-fold lower dose. That finding suggests the need for further optimization of dose and timing for *in vivo* applications.

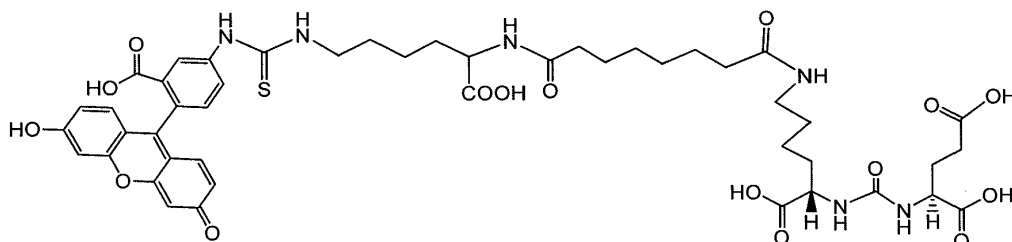
Discussion

[00262] A wide variety of low molecular weight PSMA-based imaging agents have been synthesized, including those using the urea scaffold (Banerjee et al., J. Med. Chem., vol. 51, pp. 4504-4517, 2008; Chen et al., J. Med. Chem., vol. 51, pp. 7933-7943, 2008; Zhou et al., Nat. Rev. Drug Discov., vol. 4, pp. 1015-1026, 2005; Pomper et al., Mol. Imaging, vol. 1, pp. 96-101, 2002; Foss et al., Clin. Cancer Res., vol. 11, pp. 4022-4028, 2005; Humblet et al., Mol. Imaging, vol. 4, 448-462, 2005; Misra et al., J. Nucl. Med., vol. 48, pp. 1379-1389, 2007; Mease et al., Clin. Cancer Res., vol. 14, pp. 3036-3043, 2008; Liu et al., Prostate, vol. 68, pp. 955-964, 2008; Humblet et al., J. Med. Chem., vol. 52, pp. 544-550, 2009; Kularatne et al., Mol. Pharm., vol. 6, pp. 790-800, 2009; Hillier et al., Cancer Res., vol. 69, pp. 6932-6940, 2009). Those compounds have primarily been radiopharmaceuticals, but optical agents exist. In two separate studies Humblet et al. reported the synthesis of mono- and polyvalent NIR fluorescent phosphonate derivatives for imaging PSMA, but little accumulation in PSMA-expressing tumors was evident in the former study (Humblet et al., Mol. Imaging, vol. 4, pp. 448-462, 2005) while no *in vivo* results were reported in the latter (Humblet et al., J. Med. Chem., vol. 52, pp. 544-550, 2009). Liu et al have also synthesized fluorescent phosphonate derivatives and have demonstrated their PSMA-binding specificity and intracellular localization *in vitro* (Liu et al., Prostate, vol. 68, pp. 955-964, 2008). Recently Kularatne et al. have synthesized fluorescent

(fluorescein and rhodamine) urea derivatives that demonstrate PSMA migration to endosomes (Kularatne et al., Mol. Pharm., vol. 6, pp. 790-800, 2009). We arrived at **YC-27** based on structure-activity relationships developed for PSMA-binding ureas, which were focused on improving pharmacokinetics for use *in vivo* by optimization of the linker-chelate complex (Banerjee et al., J. Med. Chem., vol. 51, pp. 4504-4517, 2008). Calculated hydrophobicity values (Ghose et al., J. Phys. Chem. A, vol. 102, pp. 3762-3772, 1998) suggest that **YC-27** should be considerably more hydrophobic (ALogD = 5.96) than radiopharmaceuticals such as [¹²⁵I]DCIBzL (ALogD = 1.19), perhaps accounting for its long tumor retention, which is desirable for an optical imaging agent intended for intraoperative use. We confirmed greater hydrophobicity of **YC-27** relative to DCIBzL through reverse-phase HPLC (data not shown)

EXAMPLE 6

Synthesis of YC-VIII-36



[00263] To a solution of **YC-VIII-24** (prepared as described in Example 5) (1.5 mg, 0.21 μ mol) in DMF (1 mL) was added triethylamine (0.005 mL, 35.9 μ mol), followed by fluorescein isothiocyanate isomer 1 (1 mg, 2.57 μ mol). After 2 hours at room temperature, the reaction mixture was purified by HPLC (column, Econosphere C18 5 μ , 150 $\times$ 4.6 mm; retention time, 15 min; mobile phase, H₂O/CH₃CN/TFA = 75/25/0.1; flow rate, 1 mL/min) to afford 1.5 mg (72%) of compound **YC-VIII-36**. ESI-Mass calcd for C₄₇H₅₇N₆O₁₆S [M+H]⁺ 993.4, found 992.8.

Cell Labeling

[00264] PSMA positive PIP cells, and PSMA negative FLU cells were treated with compound **YC-VIII-36** (40nM) and 4',6-diamidino-2-phenylindole (DAPI, blue). **Figure 17** shows fluorescence of cells expressing PSMA (green fluorescence, top left). PIP and FLU cells

were treated with both **YC-VIII-36** and PSMA inhibitor PMPA (5 μ M), showing inhibition of cellular fluorescence by PMPA (**Figure 17**, bottom).

[00265] **Figure 18** shows PC3-PIP cells treated with DAPI (blue) and varying concentrations of **YC-VIII-36** (green).

[00266] **Figure 19** shows time dependent internalization of **YC-VIII-36** into PC3-PIP cells treated with **YC-VIII-36** (green) and DAPI (blue). The time dependent internalization study was done as described (Liu et al., Prostate vol. 68, pp. 955-964, 2008) with appropriate modifications. Briefly, PC3-PIP cells were seeded as above. The cells were first pre-chilled by incubating with ice cold complete growth media and then incubated with ice cold complete growth media containing 500 nM of compound **YC-VIII-36** at 40 C for 1 hr. After 1hr of incubation the excess compound was removed by washing the wells twice with ice-cold complete growth media and then the wells were replenished with pre-warmed complete growth media. The chamber slides containing cells were incubated for 10 min, 30 min, 60 min and 180 min at 37°C in a humidified incubator.

In vivo Imaging

[00267] **Figure 20** shows titration and detection of varying amounts of **YC-VIII-36** injected subcutaneously into a nude mouse. (IVIS spectrum with 10 second exposure followed by spectral unmixing)

[00268] **Figure 21 and 22** (top) shows fluorescence images of a PSMA+ PC3-PIP and PSMA- PC3-flu tumor-bearing mouse injected intravenously with exemplary compound **YC-VIII-36**. Compound **YC-VIII-36** (150 μ g) was injected into the tail vein of a nude mouse. The excitation frequency was 465 nm with a 5 s exposure. Fluorescence emission was measured at 500, 520, 540, and 580 nm, followed by spectral unmixing.

[00269] **Figure 22** (bottom) shows the biodistribution of compound **YC-VII-36** (150 μ g) 180 minutes after injection.

FACS and Cell Sorting

[00270] *Flow cytometric analysis (FCA)*: Confluent flasks of PC3-PIP, PC3-flu and LNCap cells were trypsinized, washed with complete growth media (to neutralize trypsin) and counted. Approximately 5 million of each cell type in suspension was incubated with 1mM of compound **YC-VIII-36** for 30 min with occasional shaking at 37°C in the humidified incubator

with 5% CO₂. After incubation, the cells were washed twice with ice cold KRB buffer and fixed with 2% paraformaldehyde (ice cold). The samples were stored on ice and protected from light until the FCA was done. FCA was performed using a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA). For data acquisition, singlets were gated as the prominent cluster of cells identified from a plot of side scatter (SSC) width versus forward scatter (FSC) width to ensure that cell aggregates were excluded from analysis. 50,000 total events were counted to estimate the positively stained cells from a plot of FI-1 (X-axis) versus FI-2 (Y-axis). All data were analyzed using CellQuest version 3.3 software.

[00271] Flow sorting: PC3-PIP cells were labeled with 1mM of compound **YC-VIII-36** for 30 min at 37°C in the humidified incubator with 5% CO₂. Cells were washed twice with ice cold KRB buffer and stored on ice. Flow sorting was performed using FACS Aria system (Becton Dickinson, San Jose, CA) within 10-15 minutes after completion of last wash. Both the stained (positive) and also the unstained (negative) subpopulations were collected in sterile tubes containing 3 ml of complete growth media. Following sorting, cells were centrifuged, resuspended in warm complete growth media, transferred to tissue culture flasks and incubated at 37°C in the humidified incubator with 5% CO₂ for culture. The sorted subpopulations, “PIP-positive (PIP-pos)” and “PIP-negative (PIP-neg)” cells, were re-analyzed by FCA (as above) at passage 3 for further confirmation of their heterogeneity.

[00272] *Determination of saturation dose in flow cytometry:* Approximately 5 million cells each of PIP-pos (sorted) and PC3-flu were labeled as above with varying doses of compound #. The cells were washed twice with ice cold KRB buffer and fixed with 2% paraformaldehyde (ice cold). The samples were stored on ice and protected from light till the FCA was done. Singlets were gated as above in a plot of SSC vs. FSC to exclude the aggregates. Standard gating was used on X-axis (FI-1) for analysis of stained cells in all the doses.

[00273] PC3-flu, PC3-PIP, and LNCaP cells were treated with compound YC-VIII-36, and analyzed using fluorescence activated cell sorting (FACS) to determine the percentage of cells expressing PSMA on the cell surface. **Figure 23** shows FACS analysis showing the percent subpopulation of PSMA positive cells in PC3-flu, PC3-PIP, and LNCaP cells. As expected PC3-flu (PSMA-) cells (left) show a very small percentage, while PC3-PIP (PSMA+, center) and LNCaP (PSMA+ right) show greater percentages.

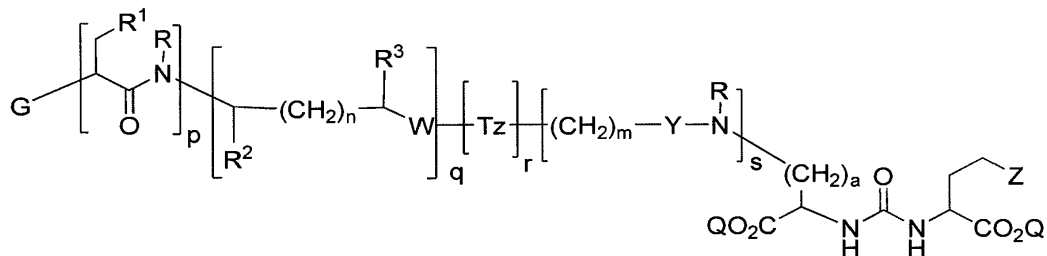
[00274] PC3-PIP (PSMA+) cells were sorted using FACS following treatment with compound YC-VIII-36. **Figure 24** shows cell sorting of PC3-PIP cells, including initial

percentage (top center), and after 3 passages of sorting (bottom). Region R2 indicates positive PSMA surface expression, as indicated by binding compound YC-VIII-36. The results show an increase in the percentage of PSMA expressing cells following three rounds of cell sorting.

[00275] *Determination of detection limit (Figure 25):* PIP-pos cells were mixed with 10 million of PC3-flu cells in triplicates in different ratios- 1 in 10^6 , 10^5 , 10^4 , 10^3 and 10^2 respectively. All the tubes containing cell suspensions in complete growth media including controls [10 million PC3-flu cells with 0% PIP-pos cells and 10 million PIP-pos cells (100%)] were incubated with 100 nM of compound # **YC-VIII-36** at 37°C in the humidified incubator with 5% CO₂ as above, with occasional stirring. The cells were washed, fixed with 2% paraformaldehyde as above and analyzed with LSRII (Becton Dickinson, San Jose, CA) for the determination of detection limit. Singlets were gated as above in a plot of SSC vs. FSC to exclude the aggregates. 1 million total events were counted to estimate the positively stained cells from plot of FI-1 (X-axis) versus FI-2 (Y-axis). Two gates, P2 at higher intensity (103 and above) and P3 at lower intensity (102-103) on X-axis (FI-1) was applied for analysis of positive cells as described in figure 4. All the data were analyzed using DIVA 6.1.3 software.

CLAIMS

1. A compound having the structure



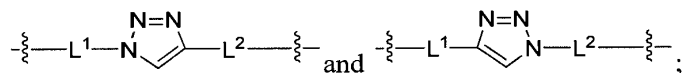
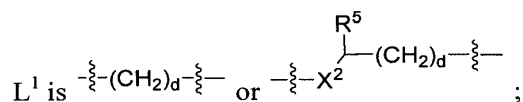
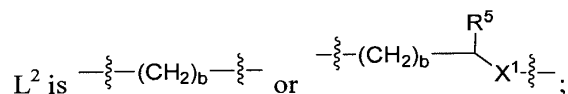
wherein the subunits associated with elements p, q, r, and s may be in any order;

Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4;

R is each independently H or C₁-C₄ alkyl;

r is 0 or 1;

Tz is a triazole group selected from the group consisting of



X¹ is -NRC(O)-, -NRC(O)NR-, -NRC(S)NR-, or -NRC(O)O-;

X² is -C(O)NR-, -NRC(O)NR-, -NRC(S)NR-, or -OC(O)NR-;

R^5 is H, CO_2H , or CO_2R^6 , where R^6 is a $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_{12}$ aryl, or $\text{C}_4\text{-C}_{16}$ alkylaryl;

b is 1, 2, 3, or 4;

d is 1, 2, 3, or 4;

q is 0 or 1;

W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$

R^2 and R^3 are independently H, CO_2H , or CO_2R^4 , where R^4 is a $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_{12}$ aryl, or $\text{C}_4\text{-C}_{16}$ alkylaryl, wherein if one of R^2 and R^3 is CO_2H or CO_2R^2 , then the other is H;

n is 1, 2, 3, 4, 5 or 6;

s is 0 or 1;

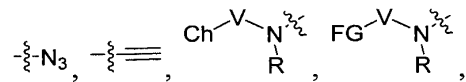
Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})-$;

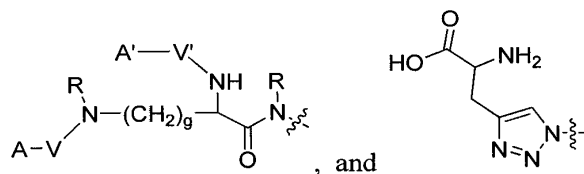
m is 1, 2, 3, 4, 5, or 6;

p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different;

R^1 is H, $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_{12}$ aryl, or $\text{C}_4\text{-C}_{16}$ alkylaryl;

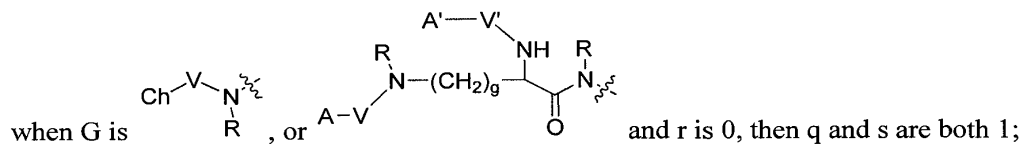
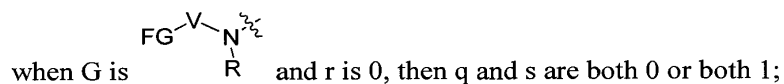
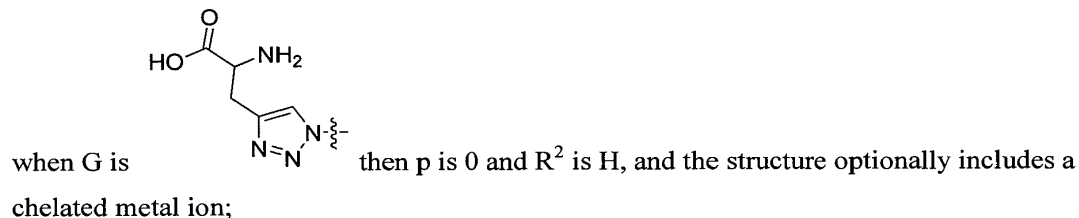
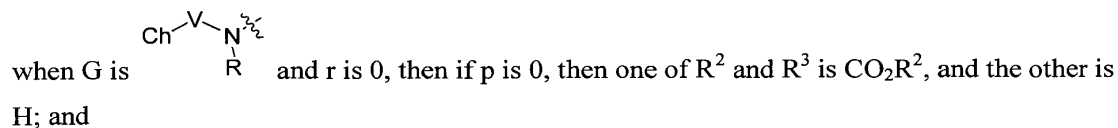
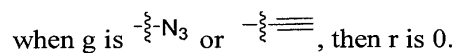
G is a moiety selected from the group consisting of



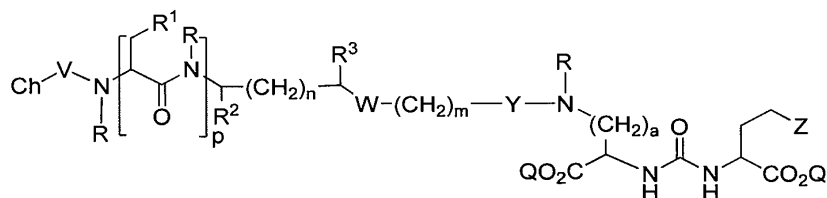


where Ch is a metal chelating moiety, optionally including a chelated metal; FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum; one of A and A' is Ch and the other is FG; V and V' are independently $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, or $-\text{OC}(\text{O})-$; and g is 1, 2, 3, 4, 5, or 6;

with the condition that:



2. A compound according to claim 1 having the structure



wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4;

R is each independently H or C₁-C₄ alkyl,

Ch is a metal chelating moiety optionally including a chelated metal;

W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-

Y is $-C(O)-$, $-NRC(O)-$, $-NRC(S)-$, $-OC(O)-$;

V is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, or $-\text{OC}(\text{O})-$;

m is 1, 2, 3, 4, 5, or 6;

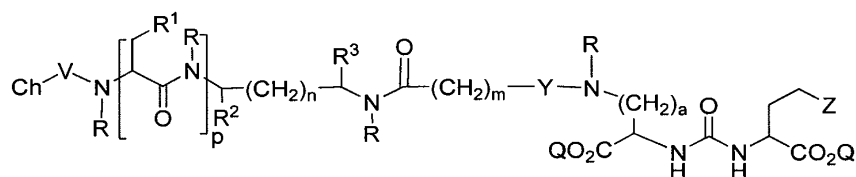
n is 1, 2, 3, 4, 5 or 6;

p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different;

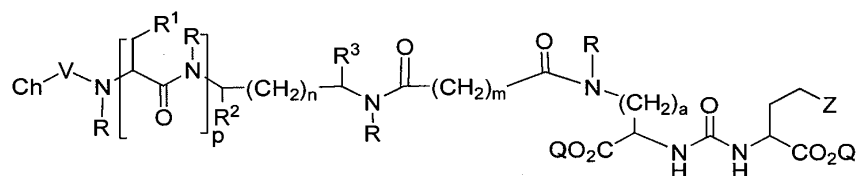
R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl

R² and R³ are independently H, CO₂H, or CO₂R⁴, where R⁴ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R² and R³ is CO₂H or CO₂R², the other is H, and when p is 0, one of R² and R³ is CO₂R⁴, and the other is H.

3. A compound according to claim 2 having the structure

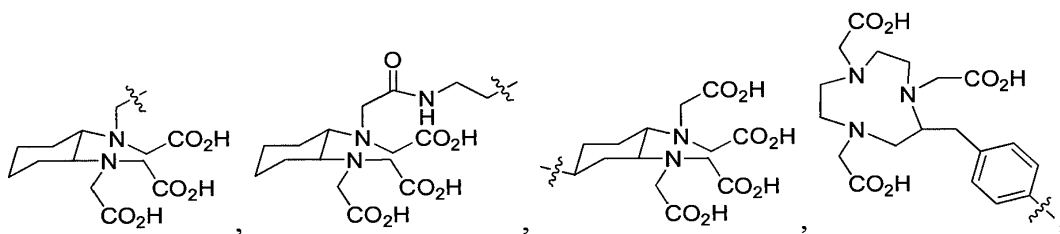


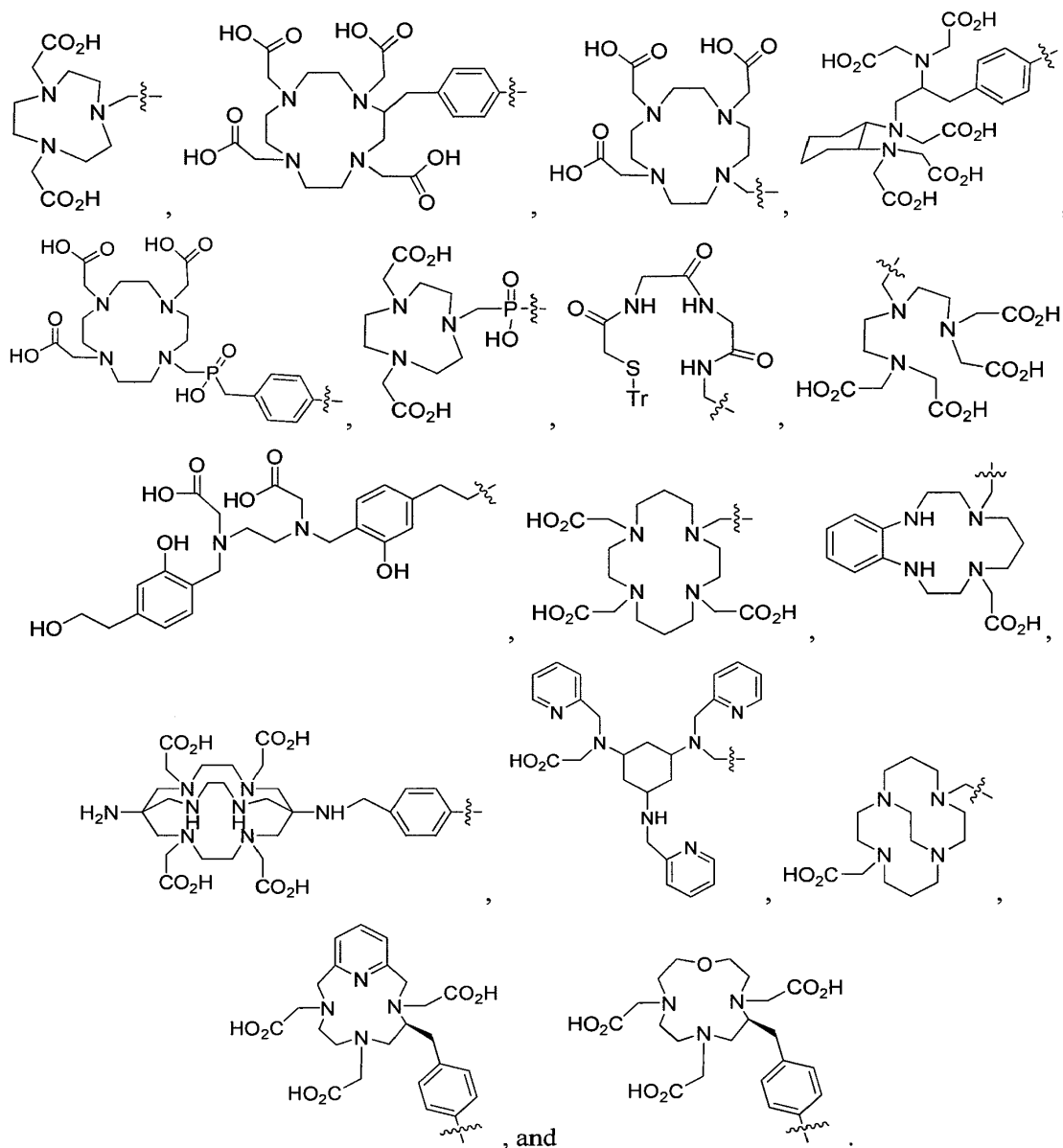
4. A compound according to claim 3 having the structure



5. A compound according to any one of claims 2-4 wherein p is 1, 2 or 3.
6. A compound according to claim 5 wherein p is 2.
7. A compound according to any one of claims 5-6, wherein R³ is CO₂H and R² is H.
8. A compound according to any one of claims 5-6 wherein R² is CO₂H and R³ is H.
9. A compound according to any one of claims 2-4 where p is 0
10. A compound according to claim 9 wherein R² is CO₂R⁴, and R³ is H.
11. A compound according to claim 9 wherein R³ is CO₂R⁴, and R² is H.
12. A compound according to any one of claims 1-11 wherein V is -C(O)- or -NRC(S)-
13. A compound according to any one of claims 1-12 wherein Z is CO₂Q.

14. A compound according to any one of claims 1-13 wherein Q is H.
15. A compound according to any one of claims 1-14 wherein m is 4, 5, or 6.
16. A compound according to claim 15 wherein m is 6.
17. A compound according to any one of claims 1-16 wherein n is 2, 3, or 4.
18. A compound according to claim 17, wherein n is 3.
19. A compound according to any one of claims 2-6, and 9-18 wherein R⁴ is C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl.
20. A compound according to claim 19, wherein R⁴ is benzyl.
21. A compound according to any one of claims 2-8, and 12-20 wherein R¹ is C₂-C₁₂ aryl.
22. A compound according to claim 21 wherein R¹ is phenyl.
23. A compound according to any one of claims 1-22, wherein Ch comprises DOTA, or DTPA.
24. A compound according to any one of claims 1-23 wherein Ch is selected from the group consisting of

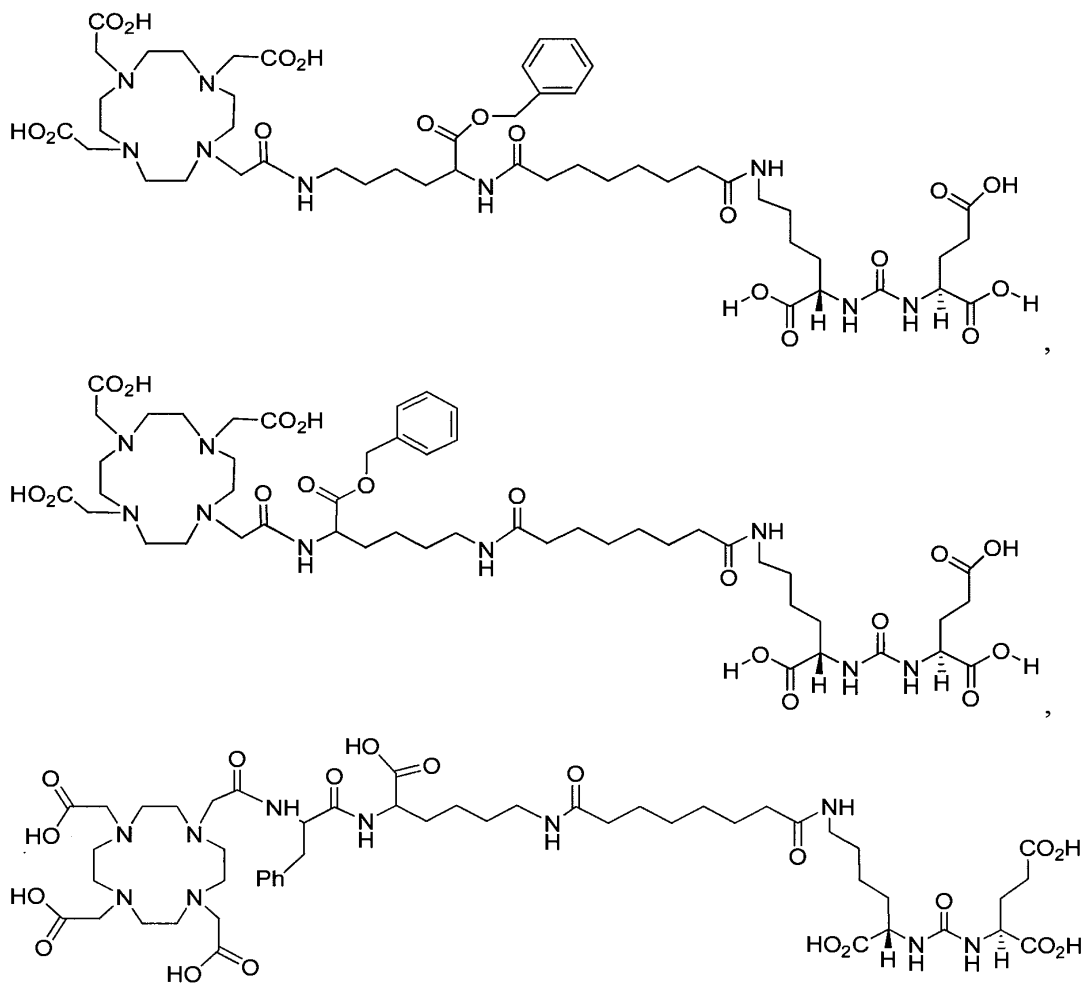


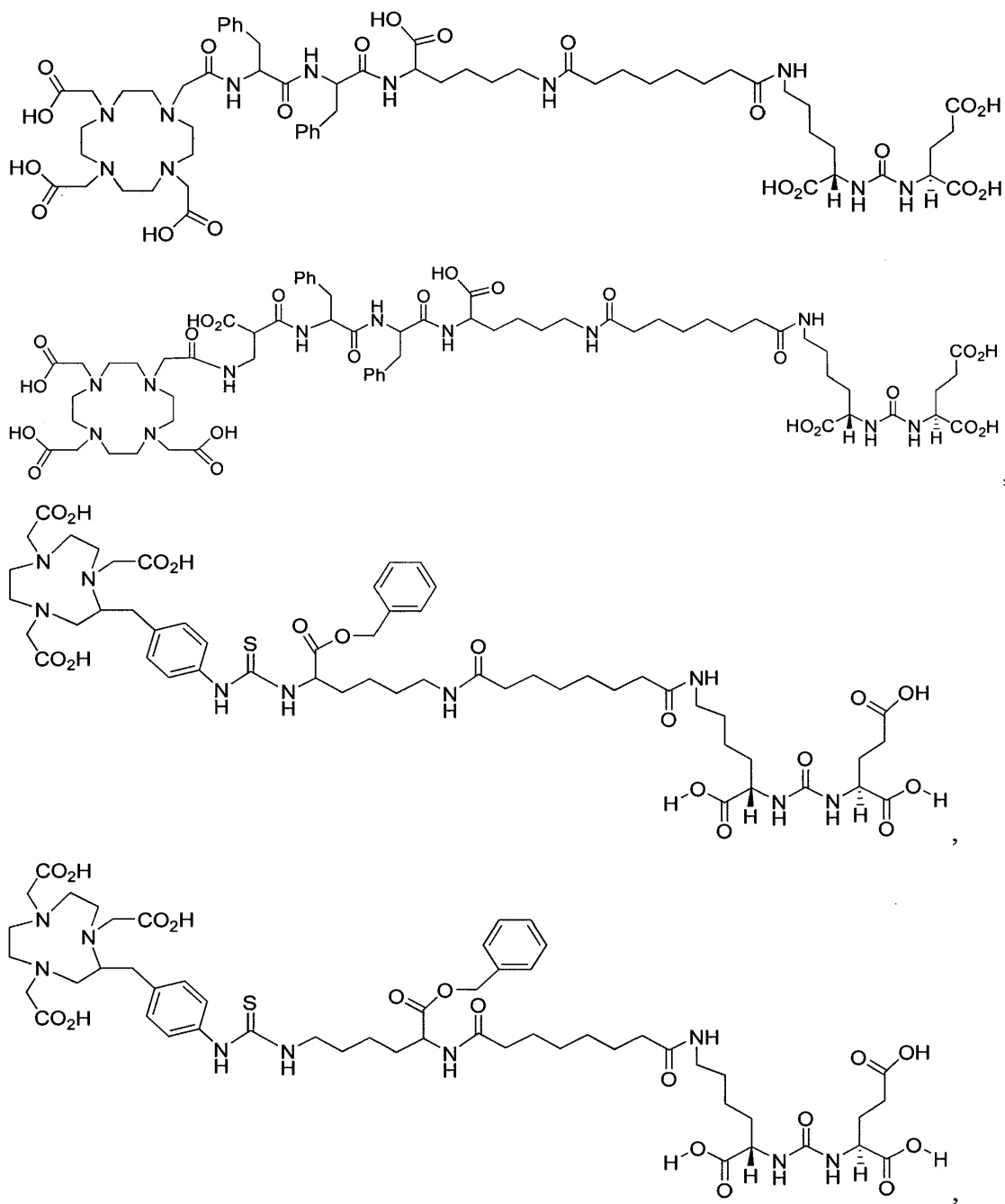


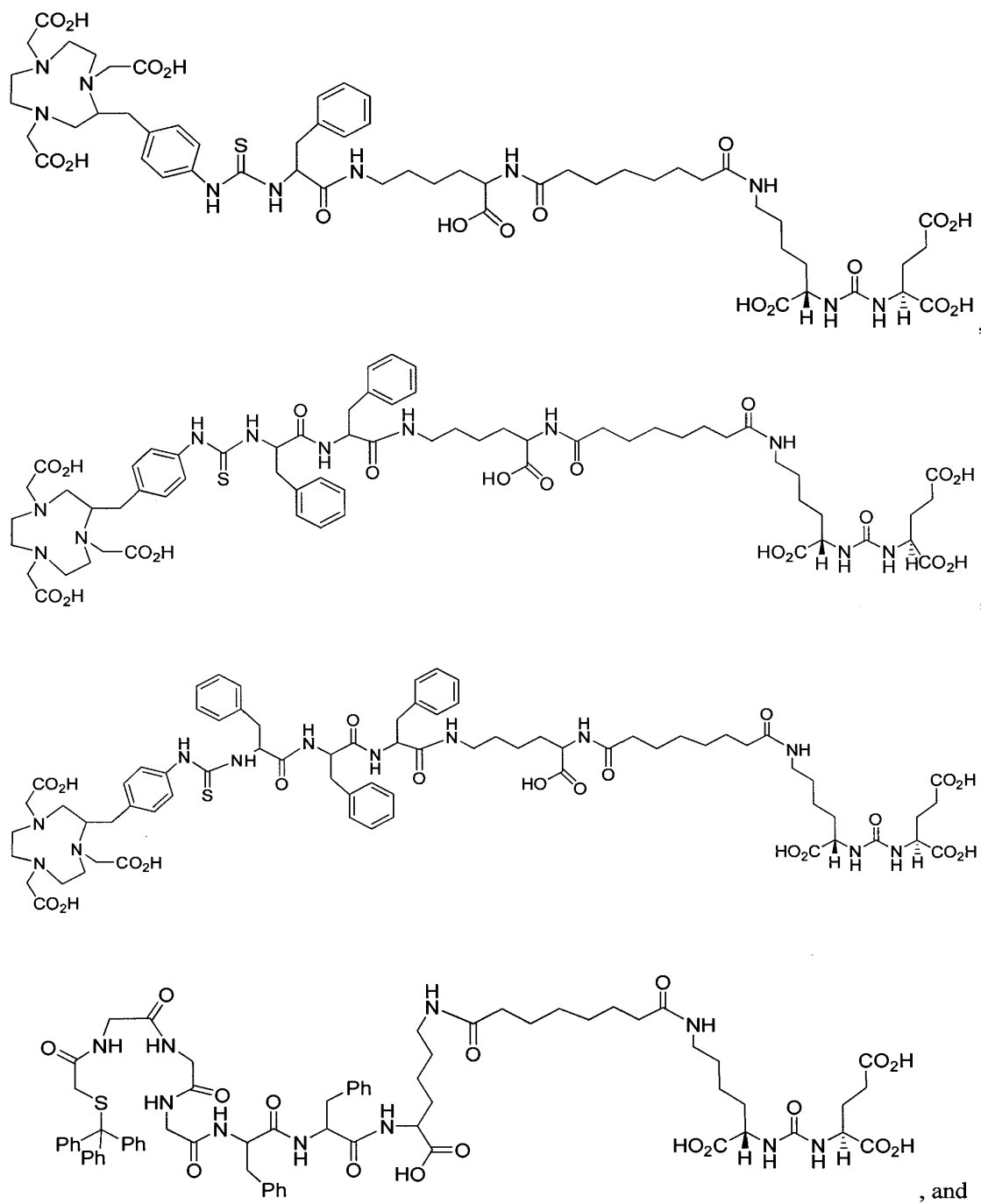
25. A compound according to any one of claims 1-23 having a chelated metal.

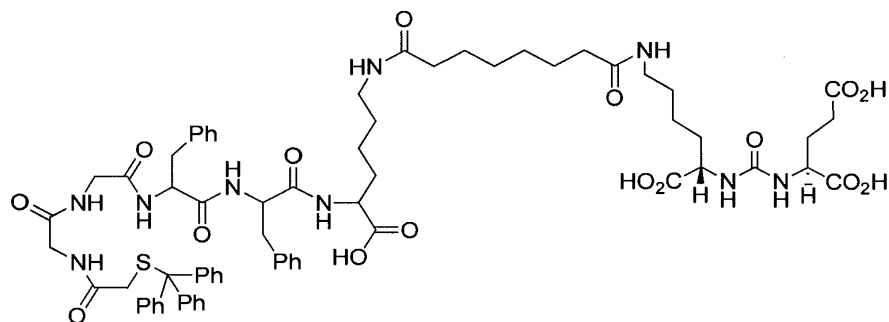
26. A compound according to claim 25 wherein the chelated metal is Tc, In, Ga, Y, Lu, Re, Cu, Ac, Bi, Pb, Sm, Sc, Co, Ho, Gd, Eu, Tb, or Dy.

27. A compound according to claim 26 wherein the metal is a radioactive isotope.
28. A compound according to claim 27 wherein the isotope is Tc-94m, Tc-99m, In-111, Ga-67, Ga-68, Y-86, Y-90, Lu-177, Re-186, Re-188, Cu-64, Cu-67, Co-55, Co-57, Sc-47, Ac-225, Bi-213, Bi-212, Pb-212, Sm-153, Ho-166, or Dy-166.
29. A compound according to claim 2 selected from the group consisting of

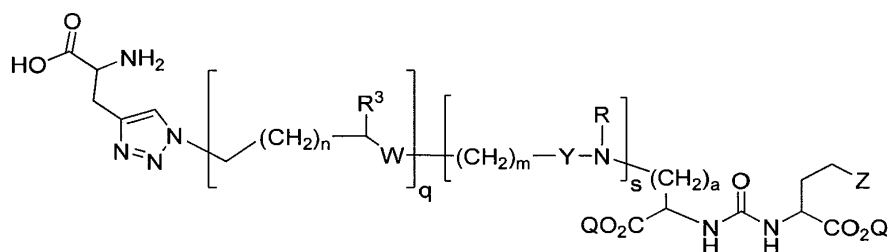








Claim 30. A compound according to claim 1 having the structure



optionally including a chelated metal ion;

wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4

R is each independently H or C₁-C₄ alkyl;

W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-

Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O);

a is 1, 2, 3, or 4;

m is 1, 2, 3, 4, 5, or 6;

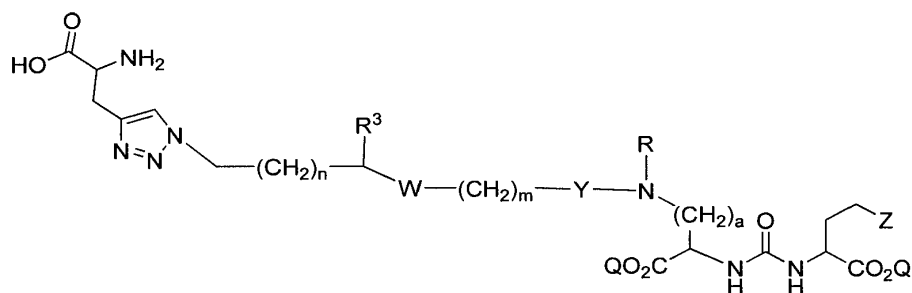
n is 1, 2, 3, 4, 5 or 6;

q is 0 or 1;

s is 0 or 1; and

R^3 is H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl.

31. A compound according to claim 30 having the structure



optionally including a chelated metal ion;

wherein Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group,

R is each independently H or C_1 - C_4 alkyl;

W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$

Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})$;

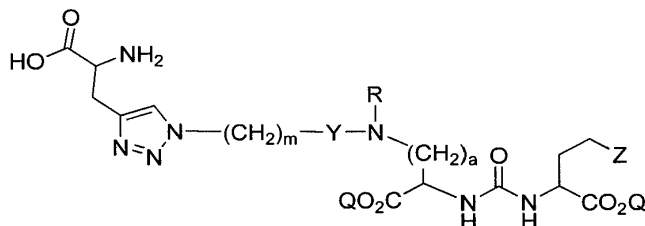
a is 1, 2, 3, or 4;

m is 1, 2, 3, 4, 5, or 6;

n is 1, 2, 3, 4, 5 or 6;

R^3 is H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl.

32. A compound according to claim 30 having the structure

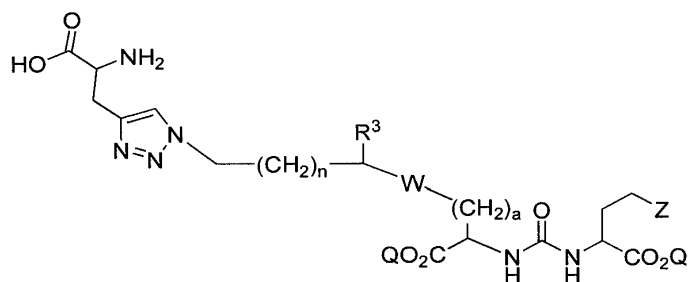


Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})$;

a is 1, 2, 3, or 4;

m is 1, 2, 3, 4, 5, or 6;

33. A compound according to claim 30 having the structure



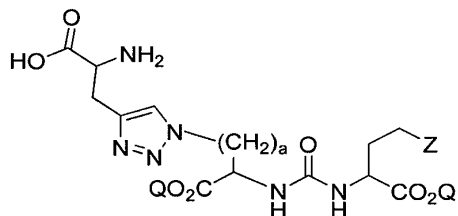
W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$

a is 1, 2, 3, or 4;

n is 1, 2, 3, 4, 5 or 6;

R^3 is H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl.

34. A compound according to claim 30 having the structure



a is 1, 2, 3, or 4;

35. A compound according to any one of claims 30-34 wherein a is 4.

36. A compound according to any one of claims 30-35 wherein Z is CO_2Q .

37. A compound according to any one of claims 30-36 wherein Q is H.

38. A compound according to any one of claims 30-32 and 35-37 wherein Y is $-\text{C}(\text{O})-$.

39. A compound according to any one of claims 30-31, 33, and 35-37, wherein W is $-\text{NHC}(\text{O})-$.

40. A compound according to any one of claims 30-32, and 35-39, wherein m is 4, 5, or 6.

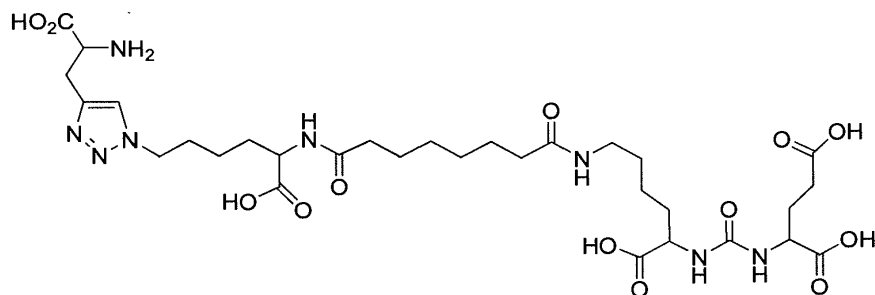
41. A compound according to claim 40 wherein m is 6.

42. A compound according to any one of claims 30-31, 33, 35-41 wherein n is 2, 3, or 4.

43. A compound according to claim 42, wherein n is 3.

44. A compound according to any one of claims 30-31, 33, 35-42, wherein R^3 is CO_2H .

45. A compound according to claim 30 having the structure



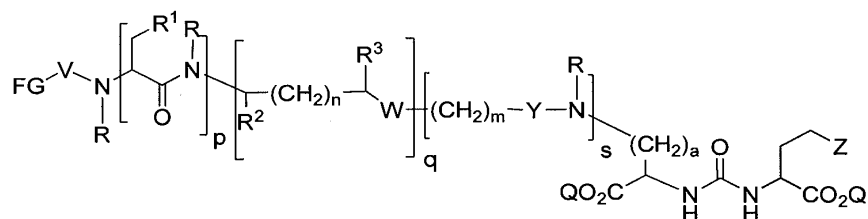
46. A compound according to any one of claims 30-45 having a chelated metal ion.

47. A compound according to claim 46 wherein the chelated metal ion is Tc, Re, Cu, or Ga.

48. A compound according to claim 47 wherein the metal ion is Tc-99m, Re-186, Re-188, Cu-64, or Ga-68.

49. A compound according to claim 48, wherein the metal ion is Tc-99m.

50. A compound according to claim 1 having the structure



where p, q, and s are in the order drawn, and q and s are either both 0 or both 1;

wherein Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4;

FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum;

R is each independently H or C₁-C₄ alkyl;

V is $-\text{C}(\text{O})-$ or $-\text{NRC}(\text{O})-$ or $-\text{NRC}(\text{S})-$;

m is 1, 2, 3, 4, 5, or 6;

n is 1, 2, 3, 4, 5 or 6;

W is $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, or $-\text{C}(\text{O})\text{O}-$

R^2 and R^3 are independently H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein if one of R^2 and R^3 is CO_2H or CO_2R^2 , then the other is H;

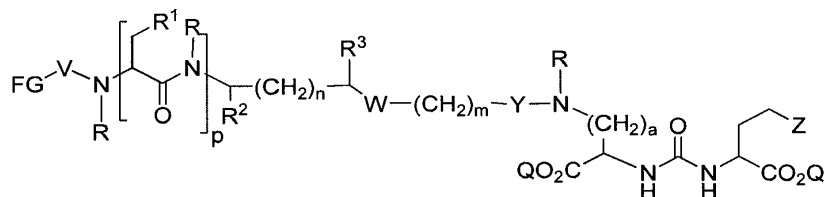
Y is $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, $-\text{OC}(\text{O})-$;

p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different;

R^1 is H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl

R^2 and R^3 are independently H, CO_2H , or CO_2R^2 , where R^2 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein when one of R^2 and R^3 is CO_2H or CO_2R^2 , the other is H.

51. A compound according to claim 50 having the structure



wherein Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4

FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum;

R is each independently H or C₁-C₄ alkyl;

V is -C(O)- or -NRC(O)- or -NRC(S)-;

W is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, -NRC(O)O-, -OC(O)NR-, -OC(O)-, -C(O)NR-, or -C(O)O-

Y is -C(O)-, -NRC(O)-, -NRC(S)-, -OC(O)-;

a is 1, 2, 3, or 4;

m is 1, 2, 3, 4, 5, or 6;

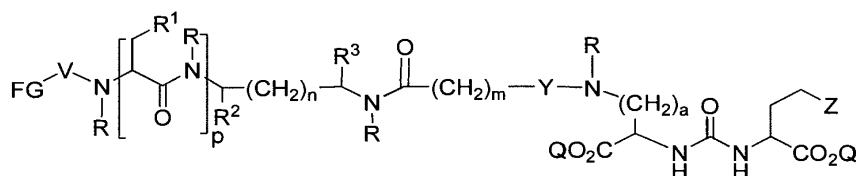
n is 1, 2, 3, 4, 5 or 6;

p is 0, 1, 2, or 3, and when p is 2 or 3, each R¹ may be the same or different;

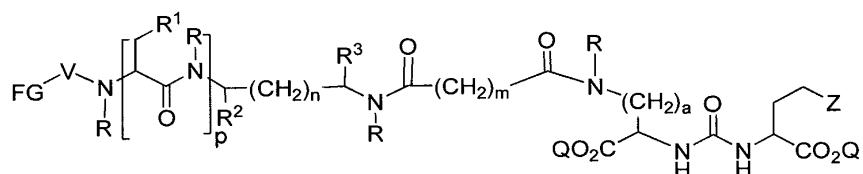
R¹ is H, C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl

R² and R³ are independently H, CO₂H, or CO₂R², where R² is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl, wherein when one of R² and R³ is CO₂H or CO₂R², the other is H.

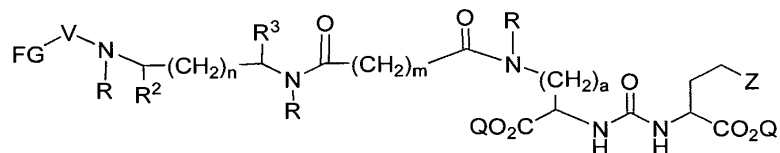
52. A compound according to claim 50 having the structure



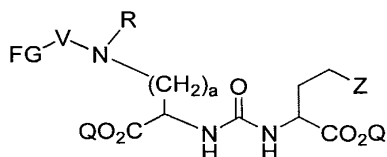
53. A compound according to claim 52 having the structure



54. A compound according to claim 53 having the structure



55. A compound according to claim 51 having the structure



56. A compound according to any one of claims 51-53 wherein p is 1, 2 or 3.

57. A compound according to any one of claims 50-53 wherein p is 2.

58. A compound according to any one of claims 50-53 wherein p is 0.

59. A compound according to any one of claims 50-58, wherein R³ is CO₂H and R² is H.

60. A compound according to any one of claims 50-58 wherein R² is CO₂H and R³ is H.

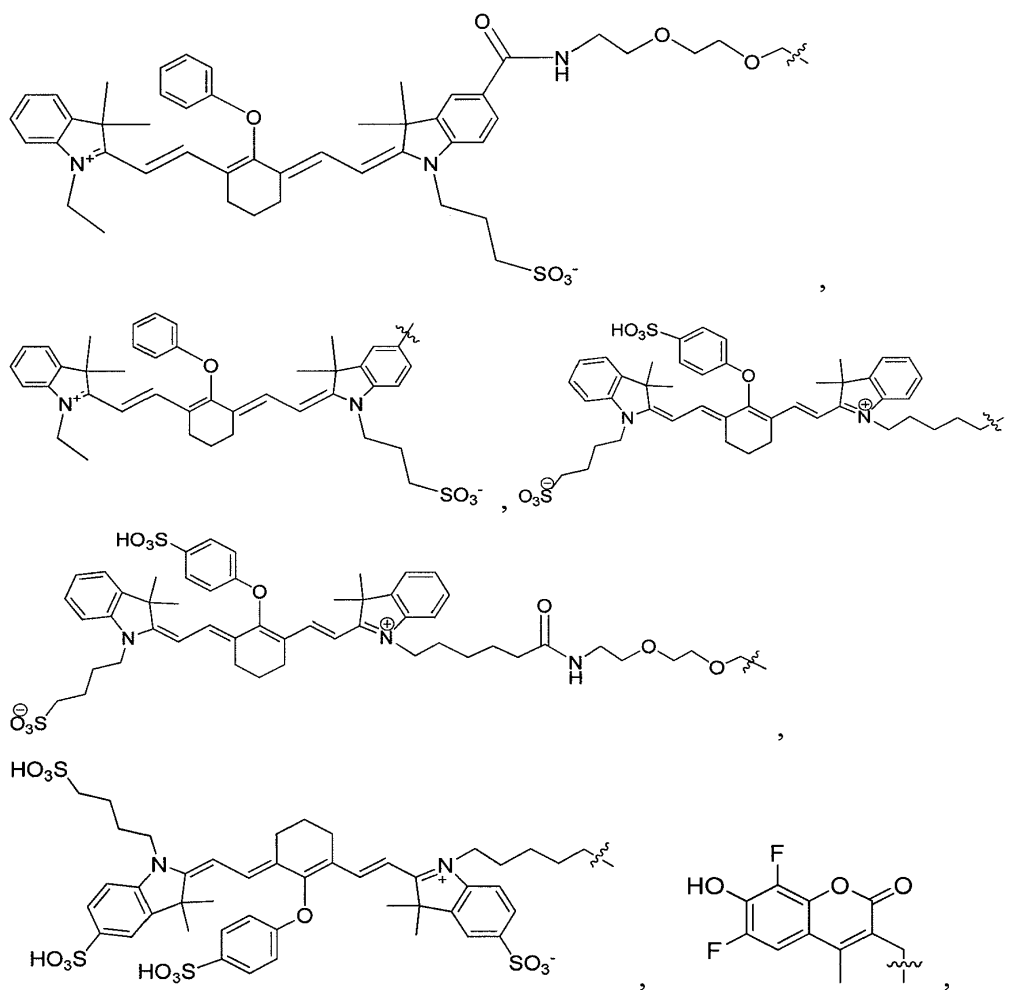
61. A compound according to any one of claims 50-58 wherein R² is CO₂R⁴, and R³ is H.

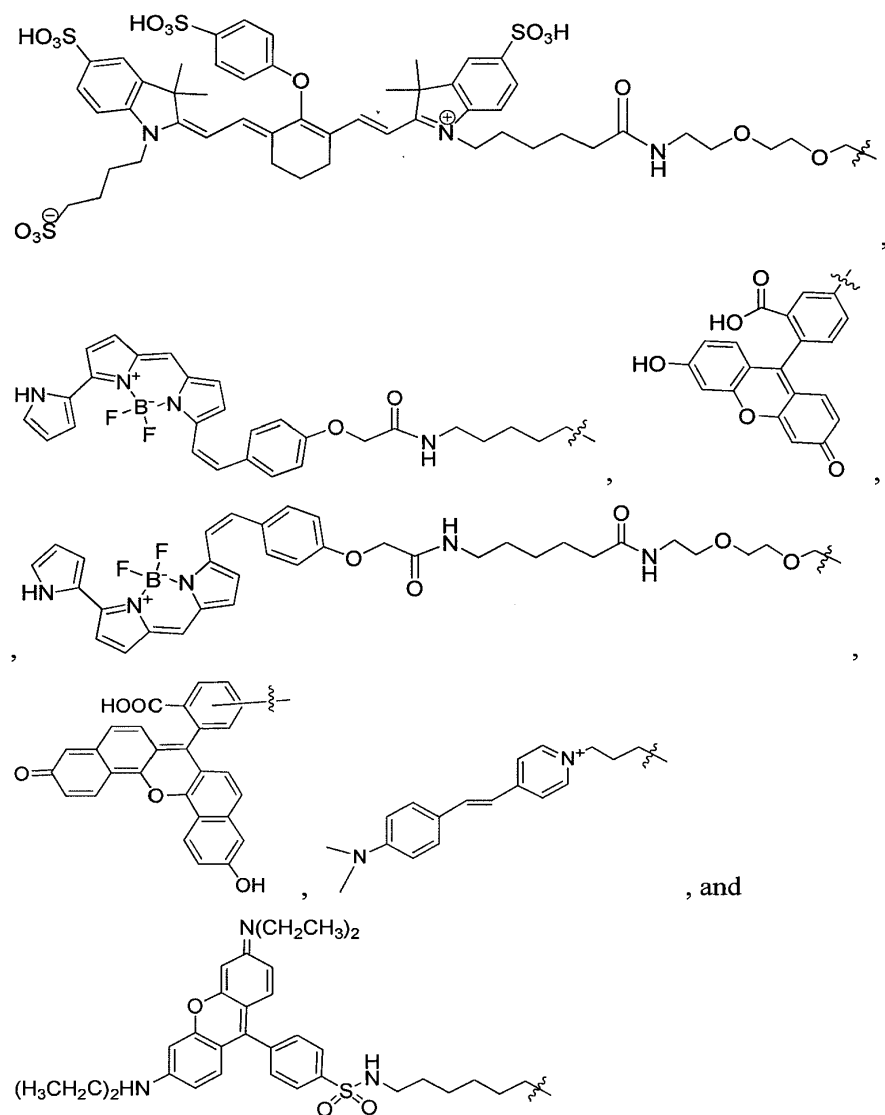
62. A compound according to any one of claims 50-58 wherein R³ is CO₂R⁴, and R² is H.

63. A compound according to any one of claims 50-58, wherein R^2 is H, and R^3 is H.
64. A compound according to any one of claims 50-63 wherein V is $-C(O)-$ or $-NRC(S)-$
65. A compound according to any one of claims 50-64 wherein Z is CO_2Q .
66. A compound according to any one of claims 50-65 wherein Q is H.
67. A compound according to any one of claims 50-66 wherein m is 4, 5, or 6.
68. A compound according to claim 67 wherein m is 6.
69. A compound according to any one of claims 50-68 wherein n is 2, 3, or 4.
70. A compound according to claim 69, wherein n is 3.
71. A compound according to any one of claims 50-58, 61-62, and 64-70 wherein R^4 is C_2-C_{12} aryl, or C_4-C_{16} alkylaryl.
72. A compound according to claim 71, wherein R^4 is benzyl.
73. A compound according to any one of claims 50-53, 56-57, and 59-72 wherein R^1 is C_2-C_{12} aryl.
74. A compound according to claim 73 wherein R^1 is phenyl.
75. A compound according to any one of claims 50-74 wherein FG is a fluorescent dye moiety which emits in the near infrared spectrum.
76. A compound according to any one of claims 50-74 wherein FG comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine,

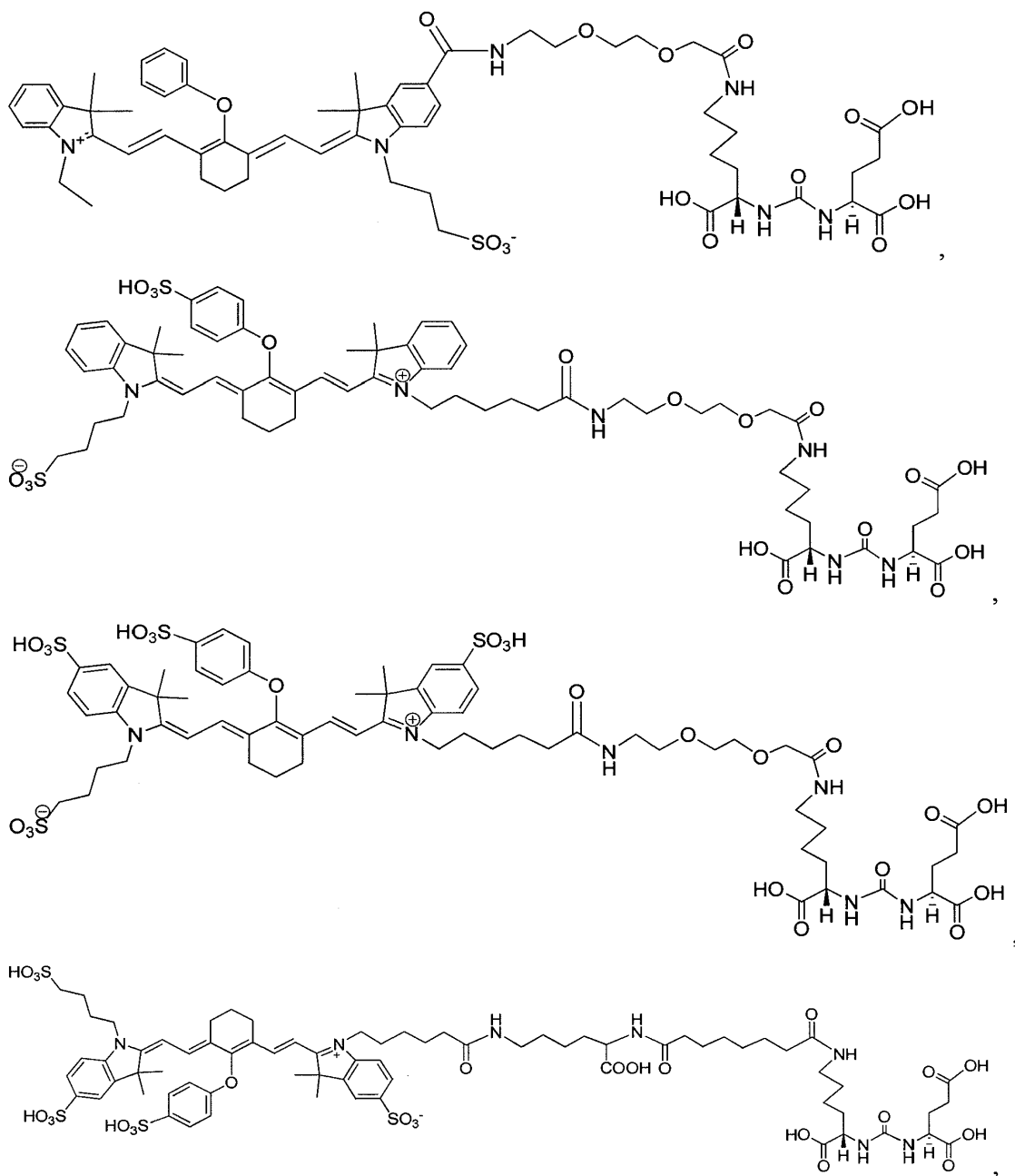
coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.

77. A compound according to any one of claims 50-74 wherein FG has the a structure selected from the group consisting of

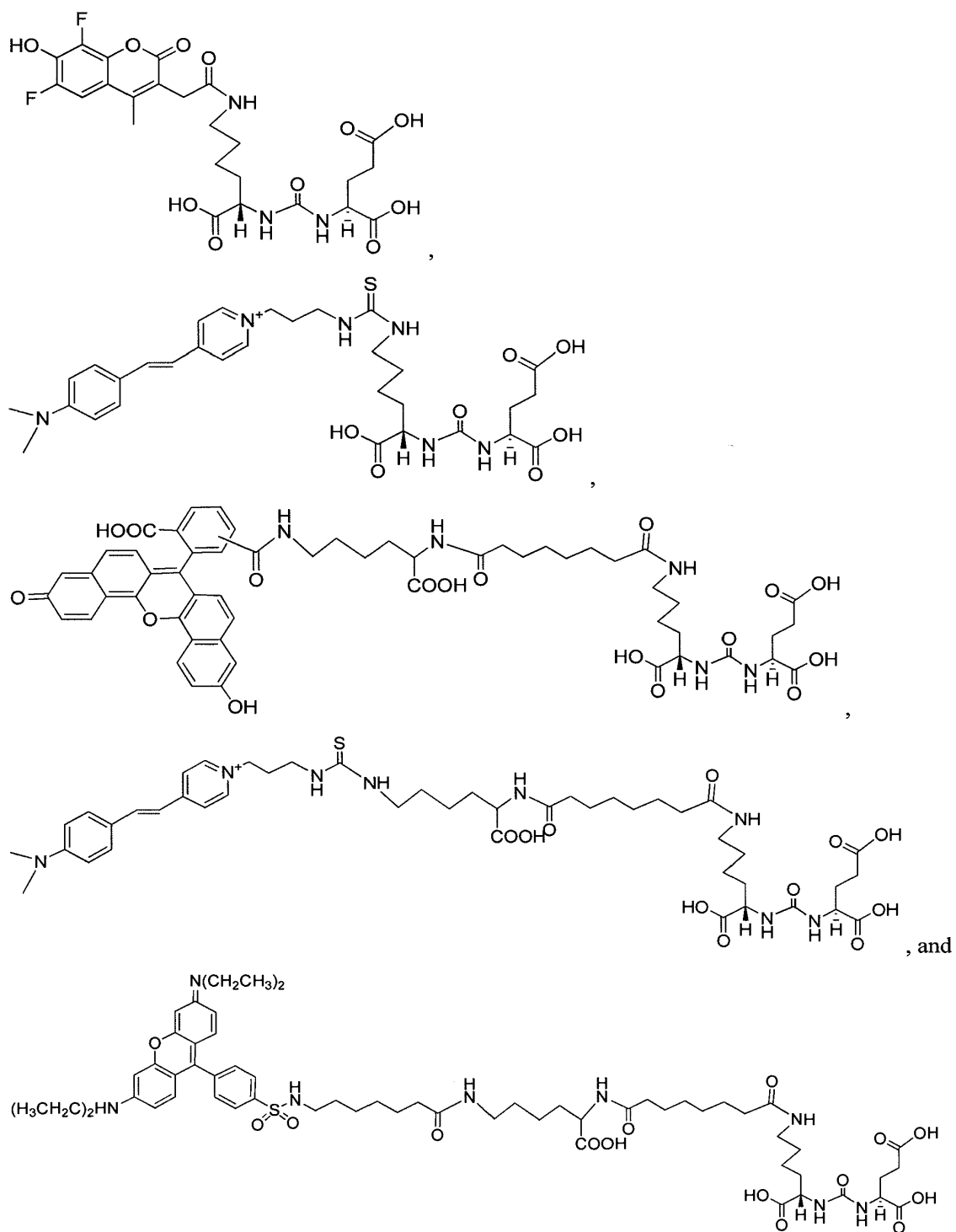




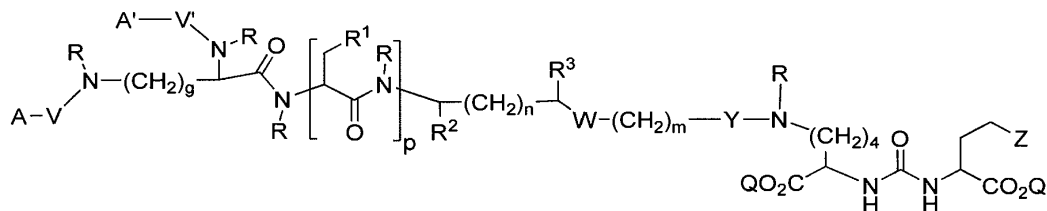
78. A compound according to claim 50 selected from the group consisting of







Claim 79. A compound according to claim 1 having the structure



wherein Z is tetrazole or CO_2Q ; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4;

one of A and A' is Ch and the other is FG, where FG is a fluorescent dye moiety which emits in the visible or near infrared spectrum and Ch is metal chelating moiety optionally including a chelated metal.

R is each independently H or C_1 - C_4 alkyl;

V or V' are independently $-C(O)-$, $-NRC(O)-$, or $-NRC(S)-$;

W is $-NRC(O)-$, $-NRC(O)NR-$, $NRC(S)NR-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, or $-C(O)O-$

Y is $-C(O)-$, $-NRC(O)-$, $-NRC(S)-$, $-OC(O)-$;

m is 1, 2, 3, 4, 5, or 6;

n is 1, 2, 3, 4, 5 or 6;

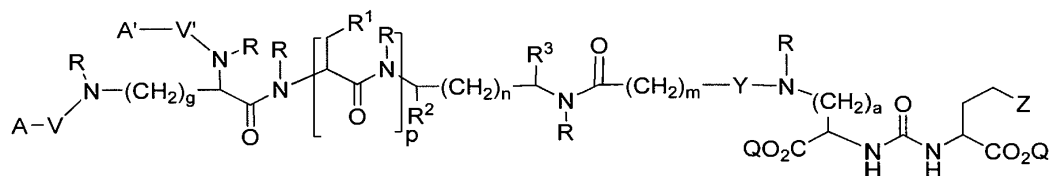
g is 1, 2, 3, 4, 5, or 6;

p is 0, 1, 2, or 3, and when p is 2 or 3, each R^1 may be the same or different;

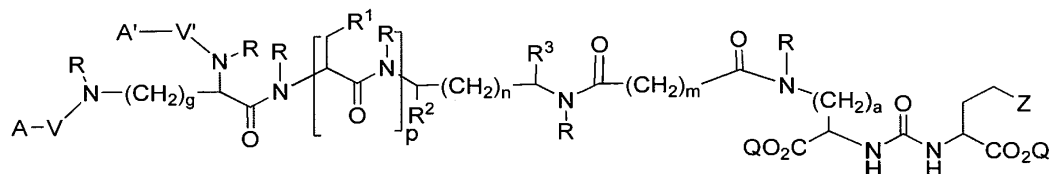
R^1 is H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl

R^2 and R^3 are independently H, CO_2H , or CO_2R^4 , where R^4 is a C_1 - C_6 alkyl, C_2 - C_{12} aryl, or C_4 - C_{16} alkylaryl, wherein when one of R^1 and R^2 is CO_2H or CO_2R^2 , the other is H.

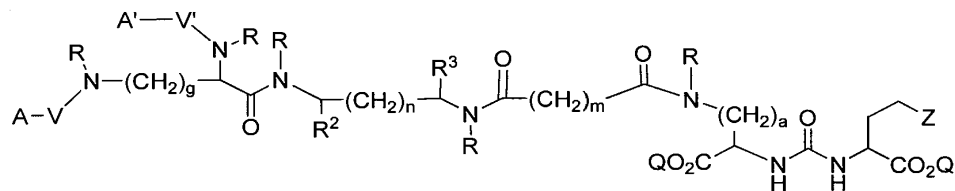
80. A compound according to claim 73 having the structure



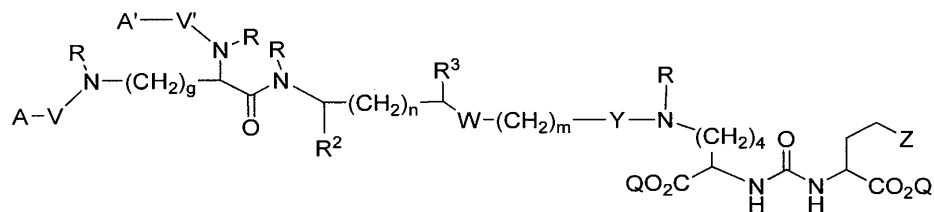
81. A compound according to claim 74 having the structure



82. A compound according to claim 75 having the structure



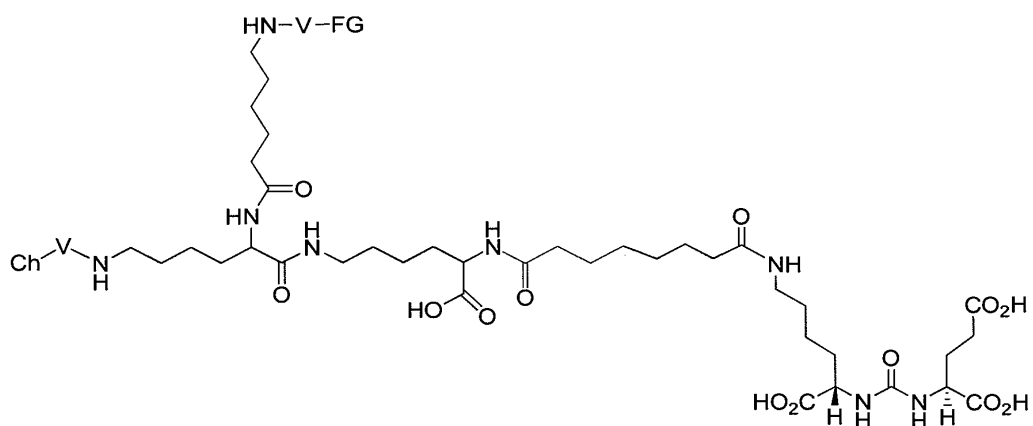
83. A compound according to claim 73 having the structure



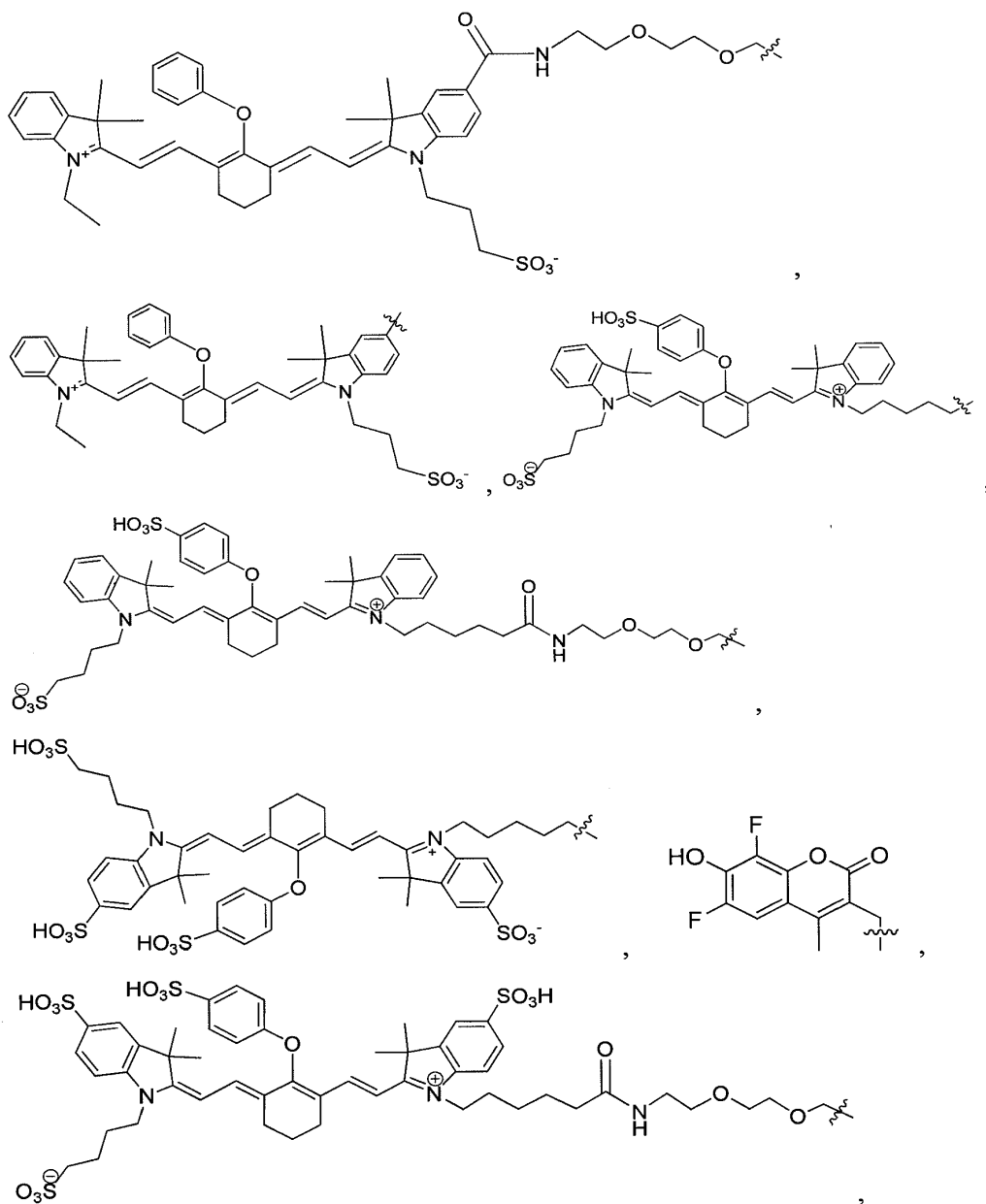
84. A compound according to any one of claims 79-81 wherein p is 1, 2 or 3.

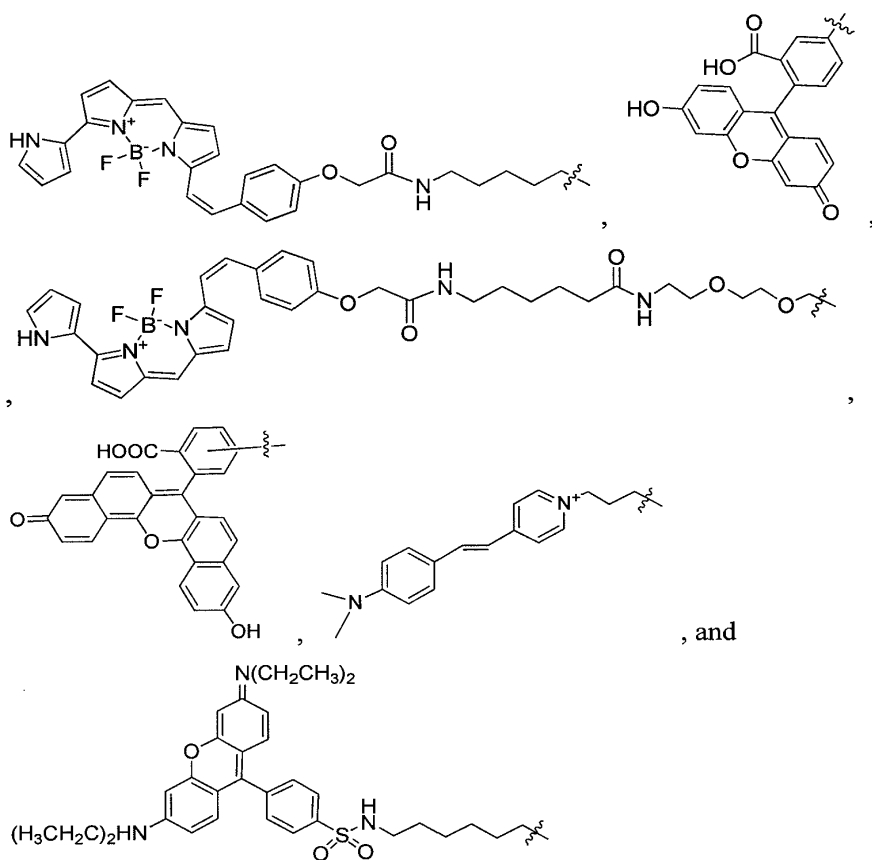
85. A compound according to claim 84 wherein p is 2.
86. A compound according to any one of claims 79-81 wherein p is 0.
87. A compound according to any one of claims 79-86, wherein R^3 is CO_2H and R^2 is H.
88. A compound according to any one of claims 79-86 wherein R^2 is CO_2H and R^3 is H.
89. A compound according to any one of claims 79-86 wherein R^2 is CO_2R^4 , and R^3 is H.
90. A compound according to any one of claims 79-86 wherein R^3 is CO_2R^4 , and R^2 is H.
91. A compound according to any one of claims 79-86 wherein R^3 is H and R^2 is H.
92. A compound according to any one of claims 79-91 wherein V and V' are individually $-C(O)-$ or $-NRC(S)-$
93. A compound according to any one of claims 79-92 wherein Z is CO_2Q .
94. A compound according to any one of claims 79-93 wherein Q is H.
95. A compound according to any one of claims 79-94 wherein m is 4, 5, or 6.
96. A compound according to claim 95 wherein m is 6.
97. A compound according to any one of claims 79-96 wherein n is 2, 3, or 4.
98. A compound according to claim 97, wherein n is 3.
99. A compound according to any one of claims 79-86, and 89-98 wherein R^4 is C_2-C_{12} aryl, or C_4-C_{16} alkylaryl.

100. A compound according to claim 99, wherein R^4 is benzyl.
101. A compound according to any one of claims 79-81, 84-85, and 87-100 wherein R^1 is C_2 - C_{12} aryl.
102. A compound according to claim 101 wherein R^1 is phenyl.
103. A compound according to claim 79 having the structure



104. A compound according to any one of claims 79-103 wherein FG is a fluorescent dye moiety which emits in the near infrared spectrum.
105. A compound according to any one of claims 79-103 wherein FG comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.
106. A compound according to any one of claims 79-103 wherein FG has a structure selected from the group consisting of



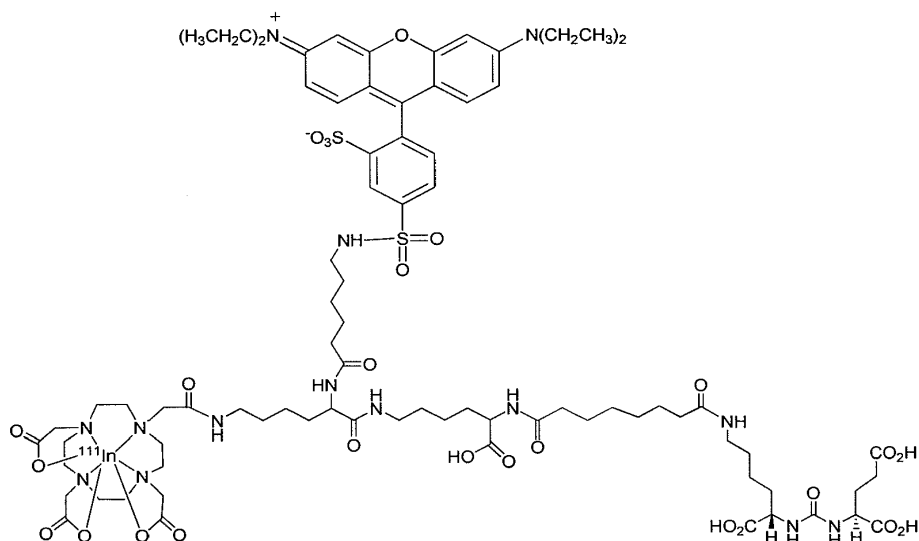


107. A compound according to any one of claims 79-106, wherein Ch comprises DOTA, or DTPA

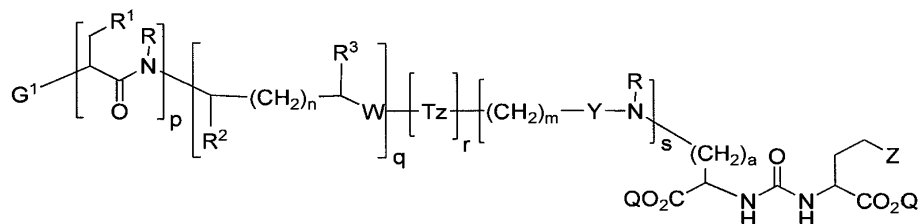
108. A compound according to any one of claims 79-106 wherein Ch is selected from the group consisting of



109. A compound according to any one of claims 79-108 having a chelated metal.
110. A compound according to claim 109 wherein the chelated metal is Tc, In, Ga, Y, Lu, Re, Cu, Ac, Bi, Pb, Sm, Sc, Co, Ho, Gd, Eu, Tb, or Dy.
111. A compound according to claim 110 wherein the metal is a radioactive isotope.
112. A compound according to claim 111 wherein the isotope is Tc-94m, Tc-99m, In-111, Ga-67, Ga-68, Y-86, Y-90, Lu-177, Re-186, Re-188, Cu-64, Cu-67, Co-55, Co-57, Sc-47, Ac-225, Bi-213, Bi-212, Pb-212, Sm-153, Ho-166, or Dy-166.
113. A compound according to claim 79 having the structure



114. A compound according to claim 1 having the structure



121

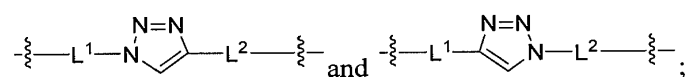
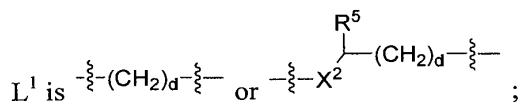
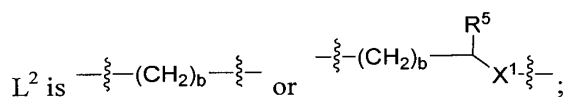
wherein the subunits associated with elements p, q, r, and s may be in any order;

Z is tetrazole or CO₂Q; each Q is independently selected from hydrogen or a protecting group, a is 1, 2, 3, or 4;

R is each independently H or C₁-C₄ alkyl,

r is 1

Tz is a triazole group selected from the group consisting of



X¹ is -NRC(O)-, -NRC(O)NR-, NRC(S)NR-, or -NRC(O)O-;

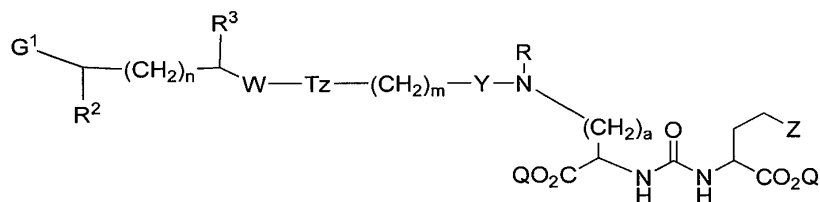
X² is -C(O)NR-, -NRC(O)NR-, NRC(S)NR-, or -OC(O)NR-;

R⁵ is H, CO₂H, or CO₂R⁶, where R⁶ is a C₁-C₆ alkyl, C₂-C₁₂ aryl, or C₄-C₁₆ alkylaryl;

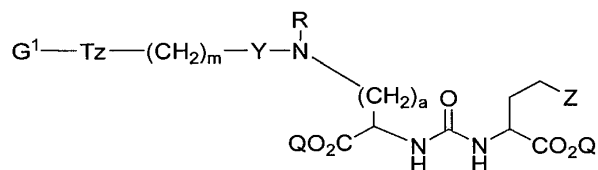
b is 1, 2, 3, or 4;

d is 1, 2, 3, or 4;

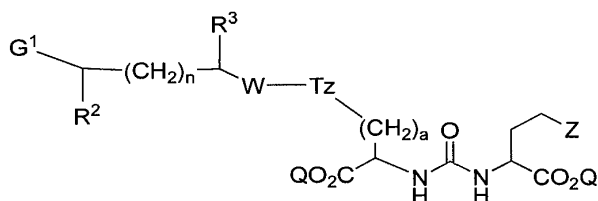
q is 0 or 1;



116. A compound according to claim 114 having the structure



117. A compound according to claim 114 having the structure



118. A compound according to any one of claims 114-116 wherein Y is $-\text{C}(\text{O})-$.

119. A compound according to any one of claims 114-115, and 117 wherein W is $-\text{NRC}(\text{O})-$.

120. A compound according to any one of claims 114, and 118-119 wherein p is 1, 2 or 3.

121. A compound according to claim 120 wherein p is 2.

122. A compound according to any one of claims 114, and 118-119 wherein p is 0.

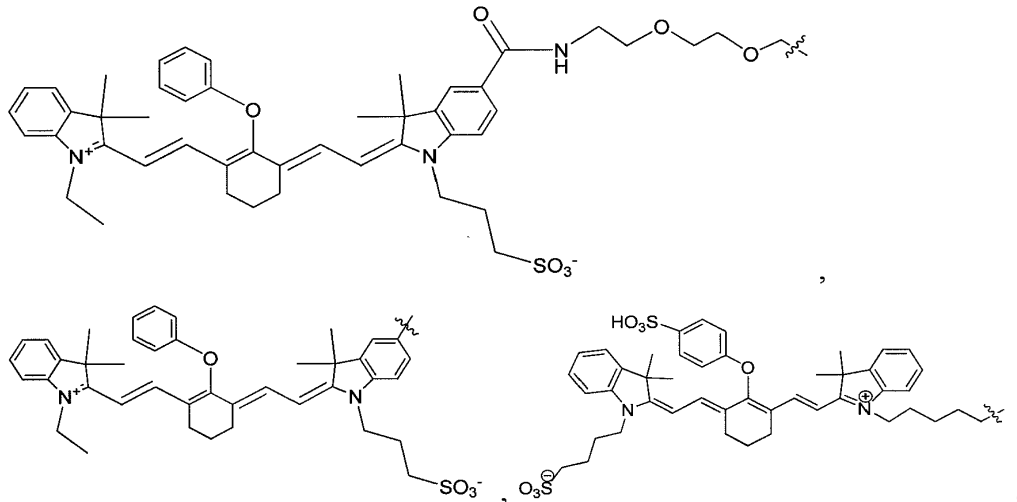
123. A compound according to any one of claims 114-115, 117-122, wherein R^3 is CO_2H and R^2 is H.
124. A compound according to any one of claims 114-115, 117-122 wherein R^2 is CO_2H and R^3 is H.
125. A compound according to any one of claims 114-115, 117-122 wherein R^2 is CO_2R^4 , and R^3 is H.
126. A compound according to any one of claims 114-115, 117-122 wherein R^3 is CO_2R^4 , and R^2 is H.
127. A compound according to any one of claims 114-115, 117-122, and 125-126 wherein R^4 is $\text{C}_2\text{-C}_{12}$ aryl, or $\text{C}_4\text{-C}_{16}$ alkylaryl.
128. A compound according to claim 127, wherein R^4 is benzyl.
129. A compound according to any one of claims 114-115, 117-122 wherein R^3 is H and R^2 is H.
130. A compound according to any one of claims 114-129 wherein V and V' are individually $-\text{C}(\text{O})-$ or $-\text{NRC}(\text{S})-$.
131. A compound according to any one of claims 114-130 wherein Z is CO_2Q .
132. A compound according to any one of claims 114-131 wherein Q is H.
133. A compound according to any one of claims 114-116, and 118-132 wherein m is 4, 5, or 6.
134. A compound according to claim 133 wherein m is 6.
135. A compound according to any one of claims 114-115, and 117-134 wherein n is 2, 3, or 4.

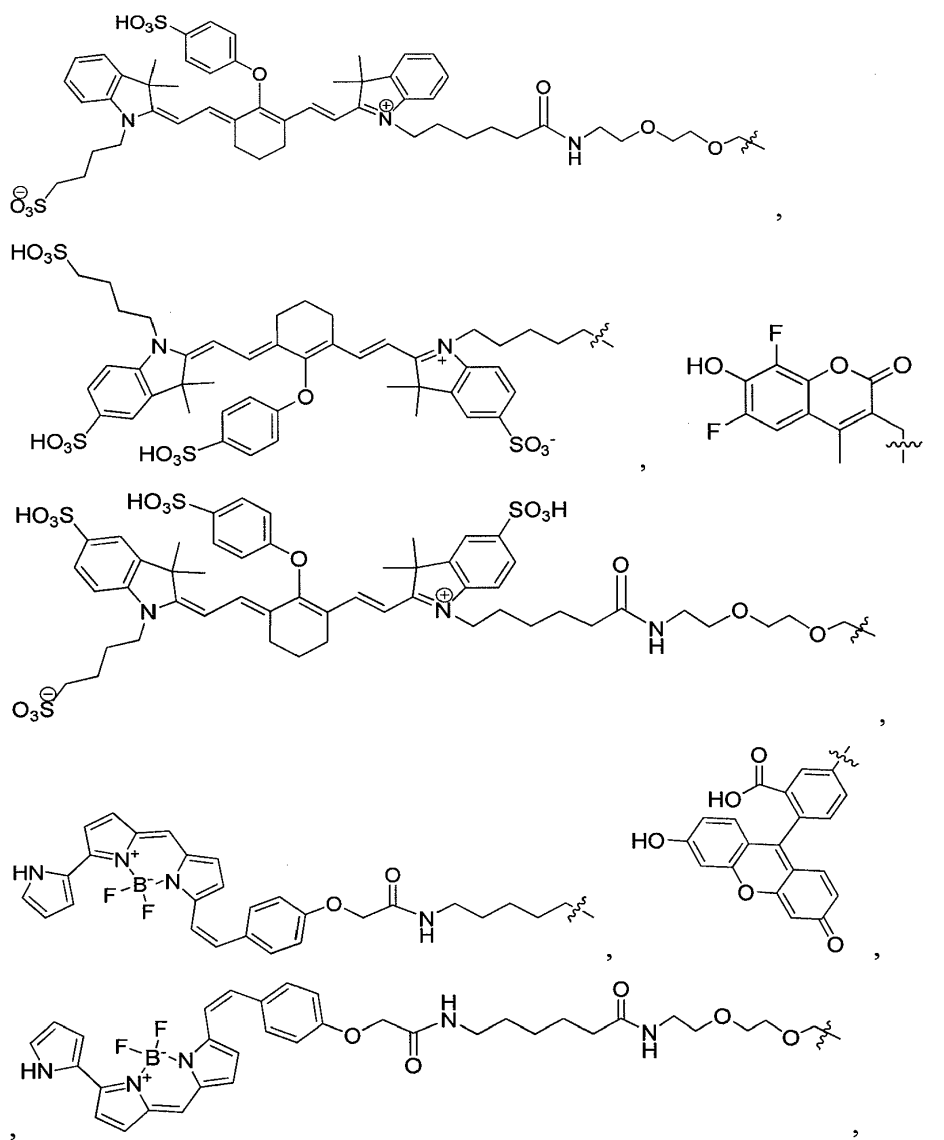
136. A compound according to claim 135, wherein n is 3.

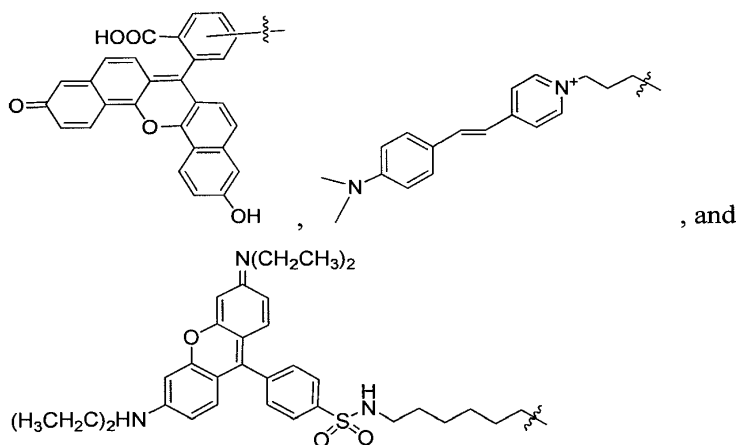
137. A compound according to any one of claims 114-136 wherein FG is a fluorescent dye moiety which emits in the near infrared spectrum.

138. A compound according to any one of claims 114-136 wherein FG comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750,, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS.

139. A compound according to any one of claims 114-136 wherein FG has a structure selected from the group consisting of

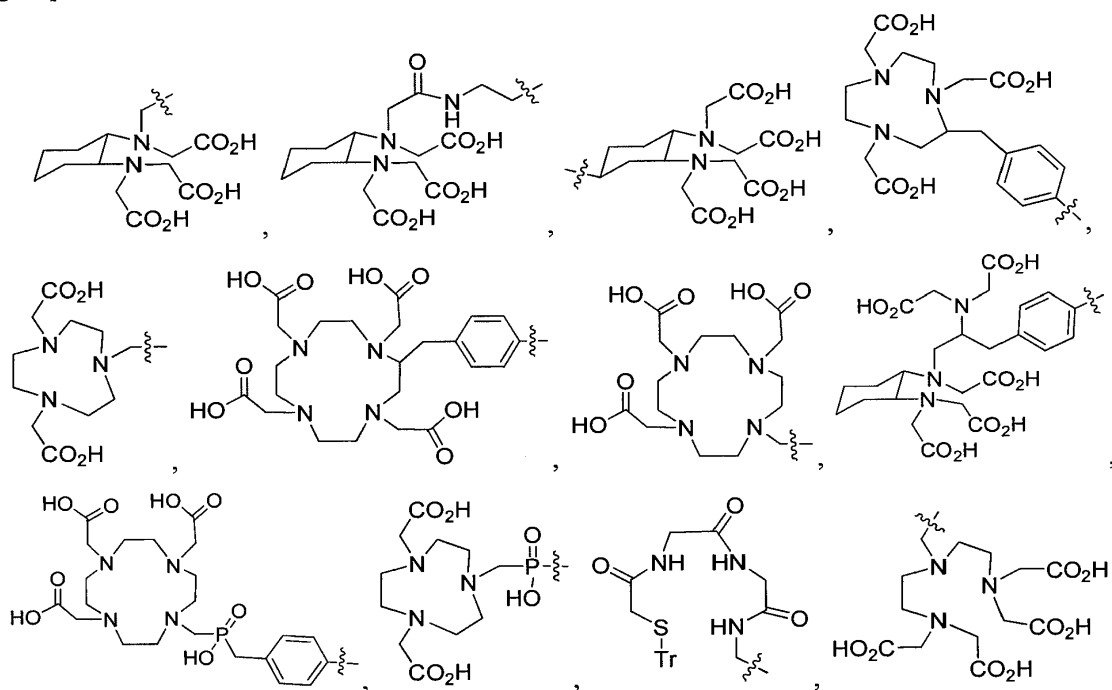


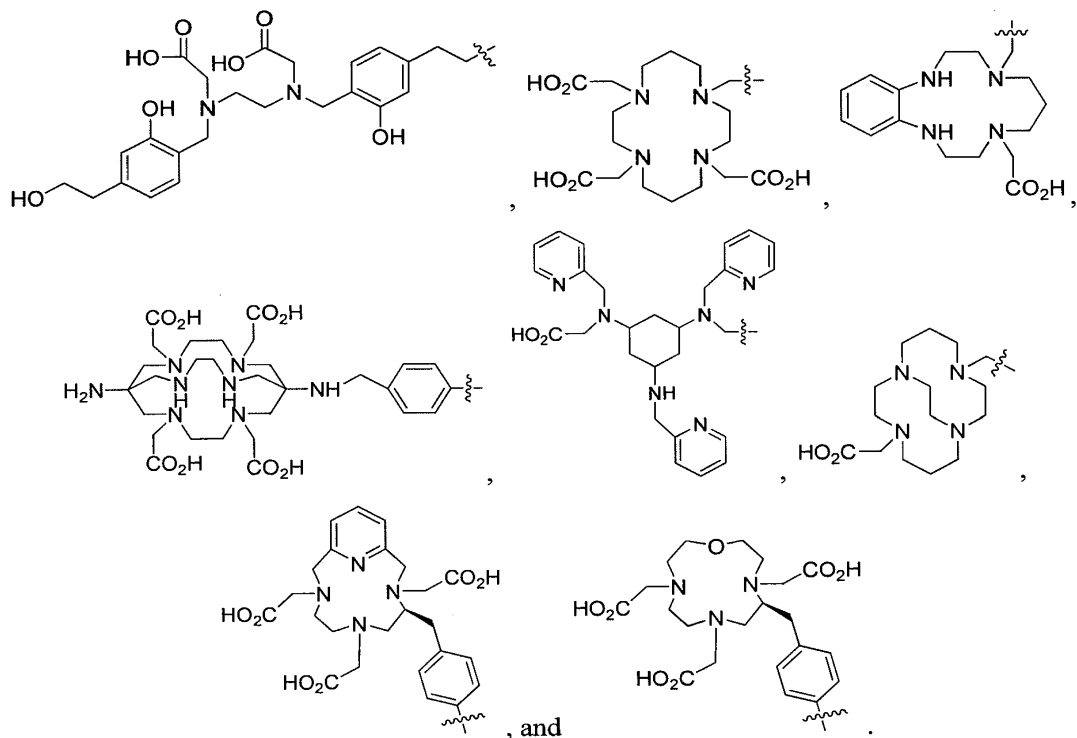




140. A compound according to any one of claims 114-139, wherein Ch comprises DOTA, or DTPA.

141. A compound according to any one of claims 114-140 wherein Ch is selected from the group consisting of





142. A compound according to any one of claims 114-141 having a chelated metal.

143. A compound according to claim 142 wherein the chelated metal is Tc, In, Ga, Y, Lu, Re, Cu, Ac, Bi, Pb, Sm, Sc, Co, Ho, Gd, Eu, Tb, or Dy.

144. A compound according to claim 143 wherein the metal is a radioactive isotope.

145. A compound according to claim 144 wherein the isotope is Tc-94m, Tc-99m, In-111, Ga-67, Ga-68, Y-86, Y-90, Lu-177, Re-186, Re-188, Cu-64, Cu-67, Co-55, Co-57, Sc-47, Ac-225, Bi-213, Bi-212, Pb-212, Sm-153, Ho-166, or Dy-166.

146. A method of imaging one or more cells, organs or tissues by exposing the cell to or administering to a organism an effective amount of a compound according to any one of claims 1-145, where the compound includes a fluorescent dye moiety, or a metal isotope suitable for imaging.

147. A method of treating a tumor comprising administering a therapeutically effective amount of a compound according to any one of claims 1-145, where the compound includes a therapeutically effective radioisotope.

148. A method for sorting cells by exposing the cells to a compound according to any one of claims 1-145, where the compound includes a fluorescent dye moiety, followed by separating cells which bind the compound from cells which do not bind the compound.

149. A method for intraoperative tumor mapping comprising administering an effective amount of a compound according to any one of claims 1-145, where the compound includes a fluorescent dye moiety.

150. A kit comprising a compound according to any one of claims 1-145.

1/27

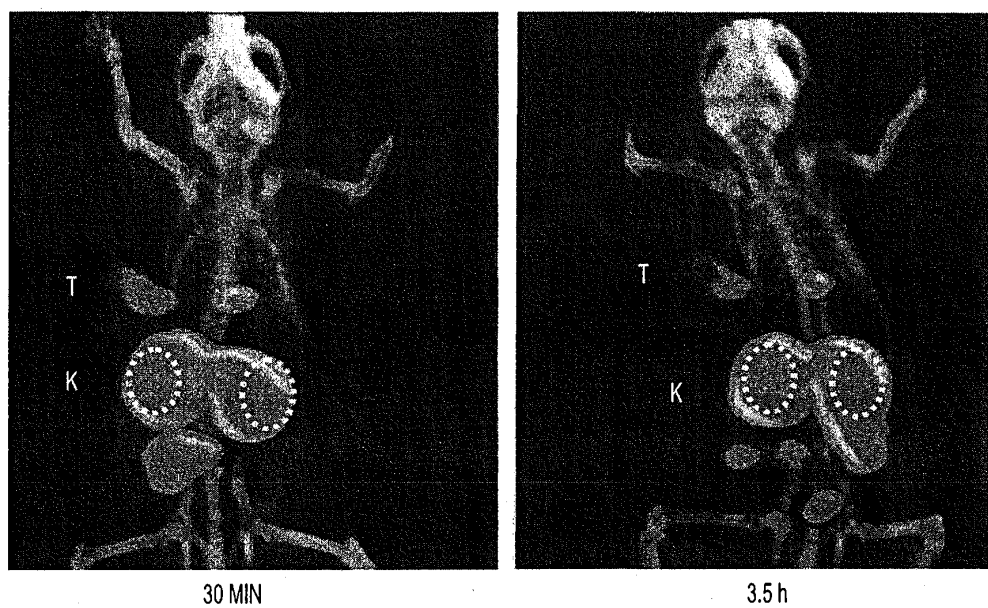


FIG. 1

SUBSTITUTE SHEET (RULE 26)

2/27

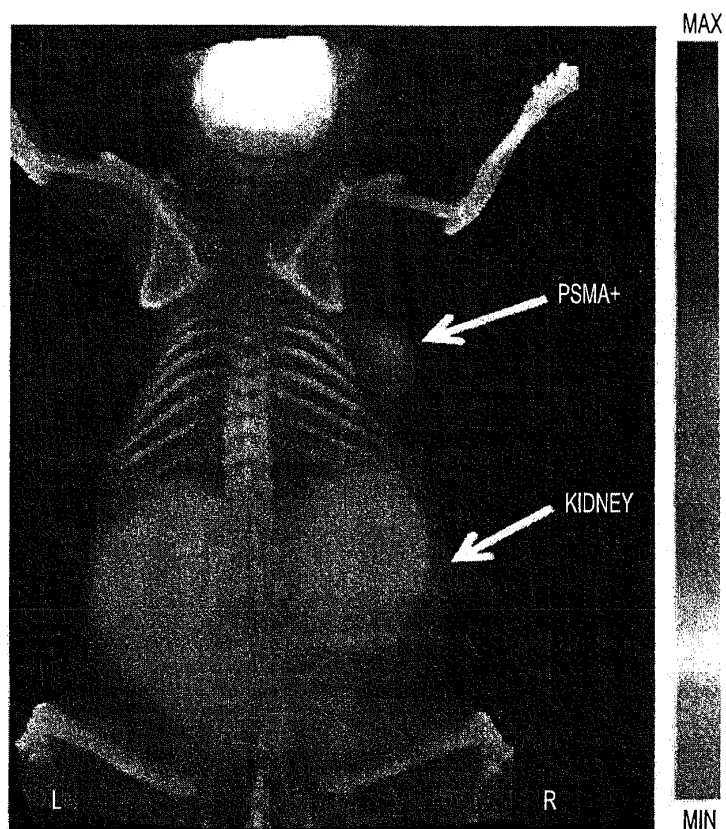


FIG. 2

SUBSTITUTE SHEET (RULE 26)

3/27

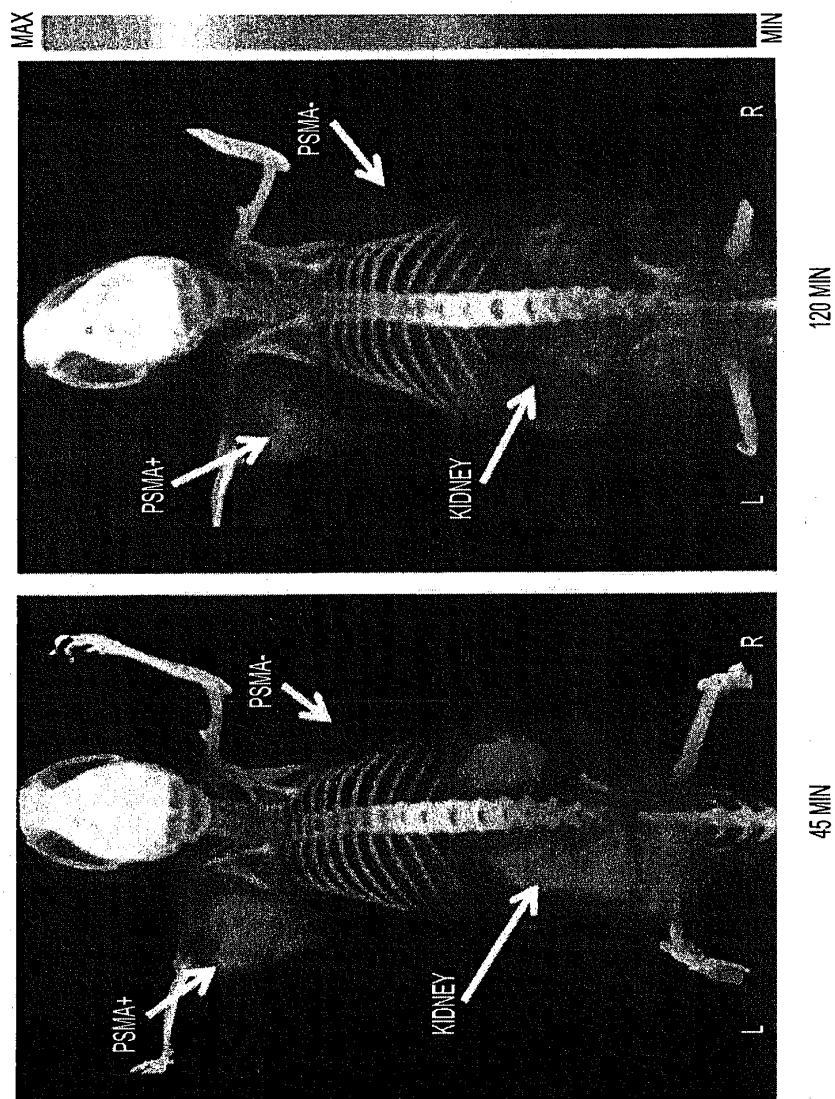
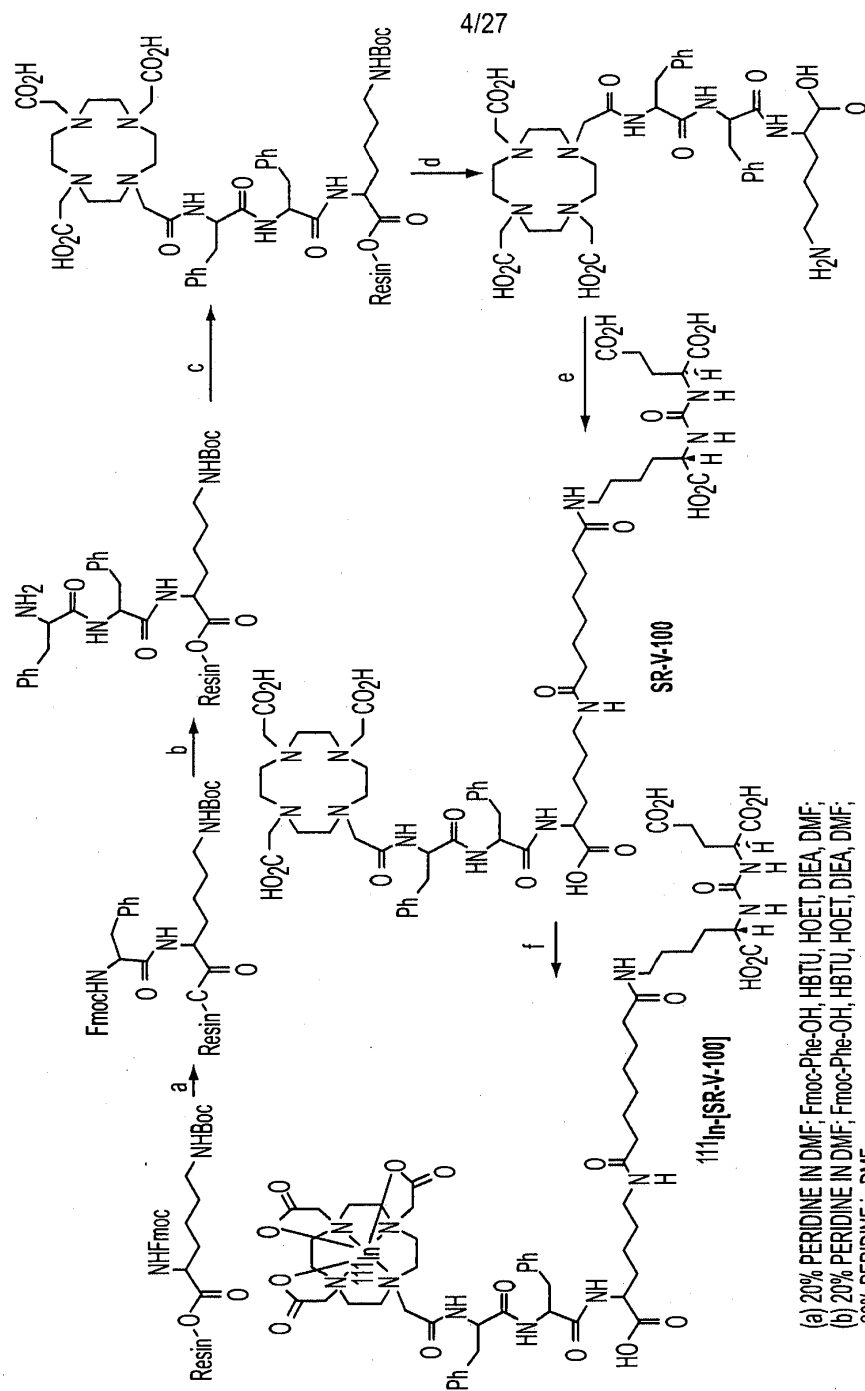


FIG. 3

SUBSTITUTE SHEET (RULE 26)



- (a) 20% PERIDINE in DMF; Fmoc-Phe-OH, HBTU, HOET, DIEA, DMF;
 (b) 20% PERIDINE in DMF; Fmoc-Phe-OH, HBTU, HOET, DIEA, DMF;
 20% PERIDINE in DMF
 (c) DOTA-*tris*(*tert*-butylester)-CO₂H, HBTU, HOBT, DIEA, DMF;
 (d) TFA, CH₂Cl₂, t, OVERNIGHT;
 (e) DSS-urea, TEA, DMF, rt, OVERNIGHT;
 (f) $^{111}\text{InCl}_3$ 200 mM NaOAc, 60°C, 30 min

FIG. 4

SUBSTITUTE SHEET (RULE 26)

5/27

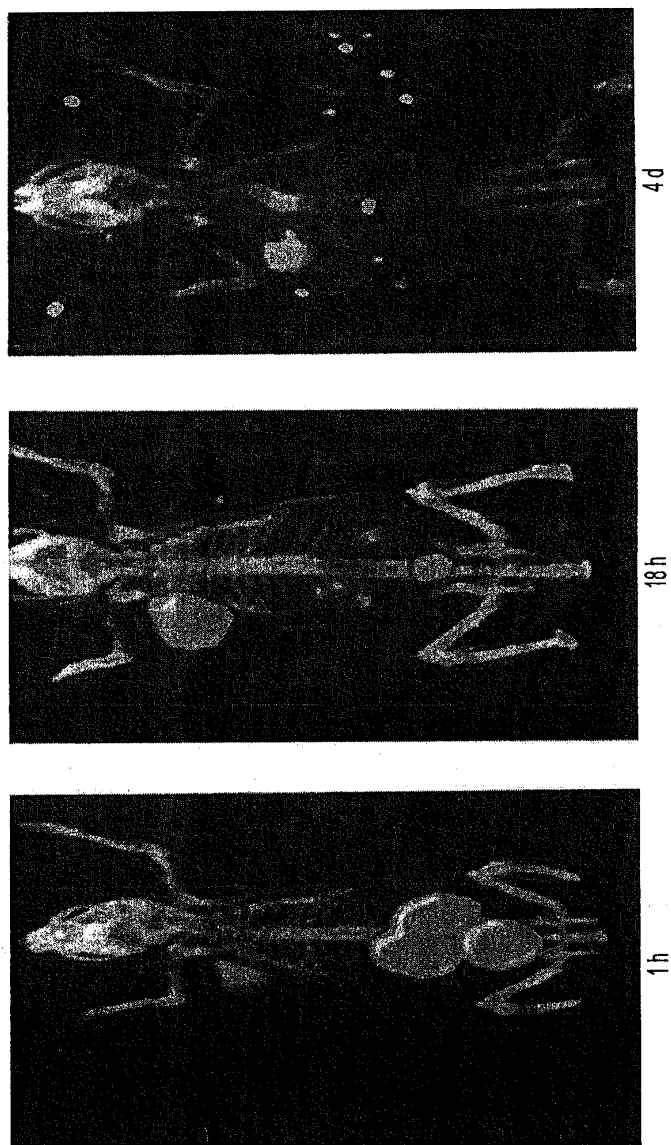


FIG. 5

SUBSTITUTE SHEET (RULE 26)

6/27

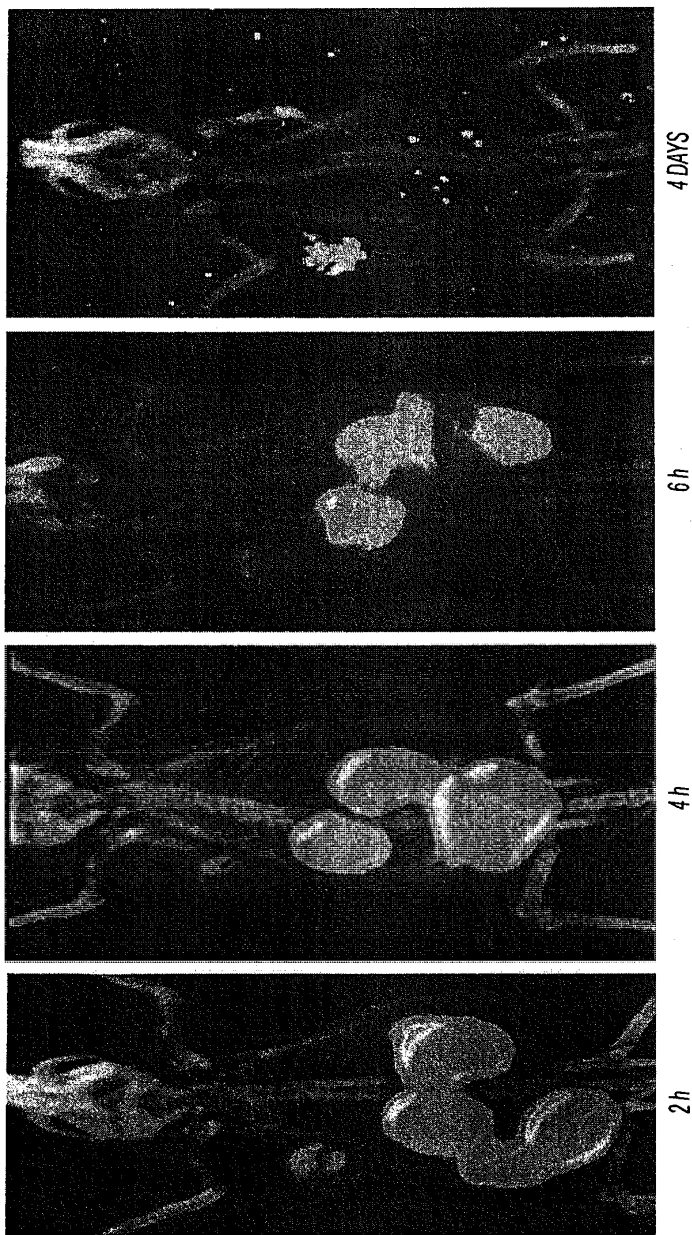


FIG. 6

SUBSTITUTE SHEET (RULE 26)

7/27

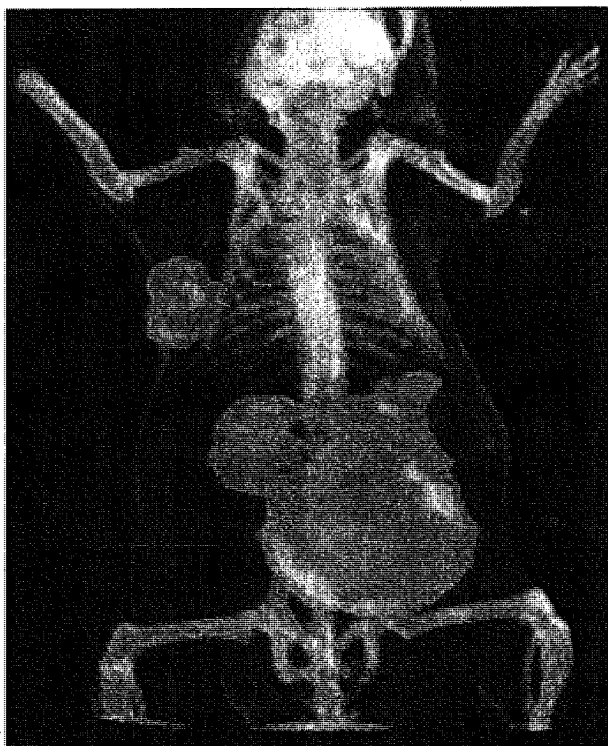


FIG. 7

SUBSTITUTE SHEET (RULE 26)

8/27

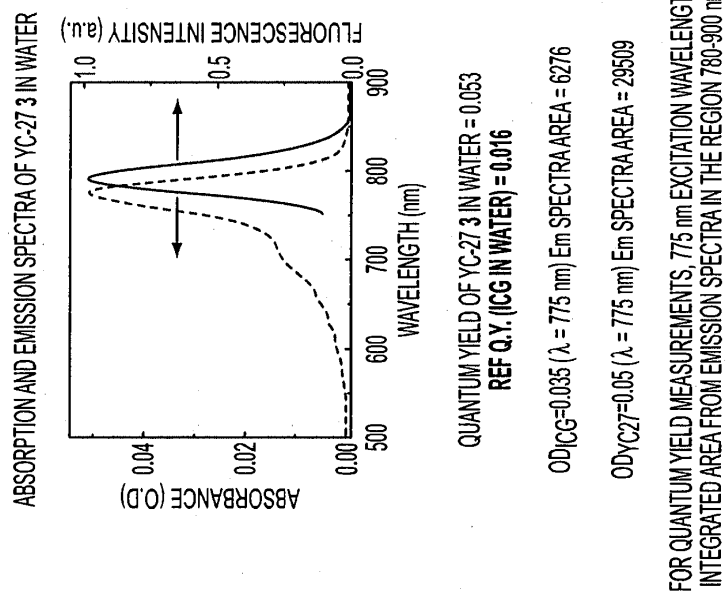


FIG. 8

SUBSTITUTE SHEET (RULE 26)

9/27

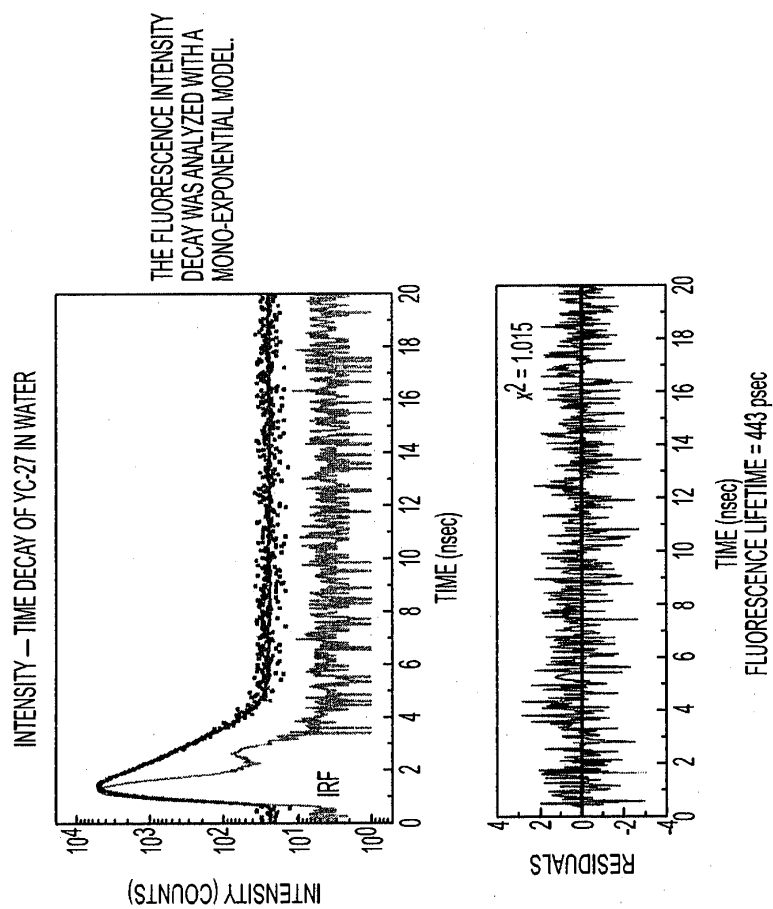


FIG. 9

SUBSTITUTE SHEET (RULE 26)

10/27

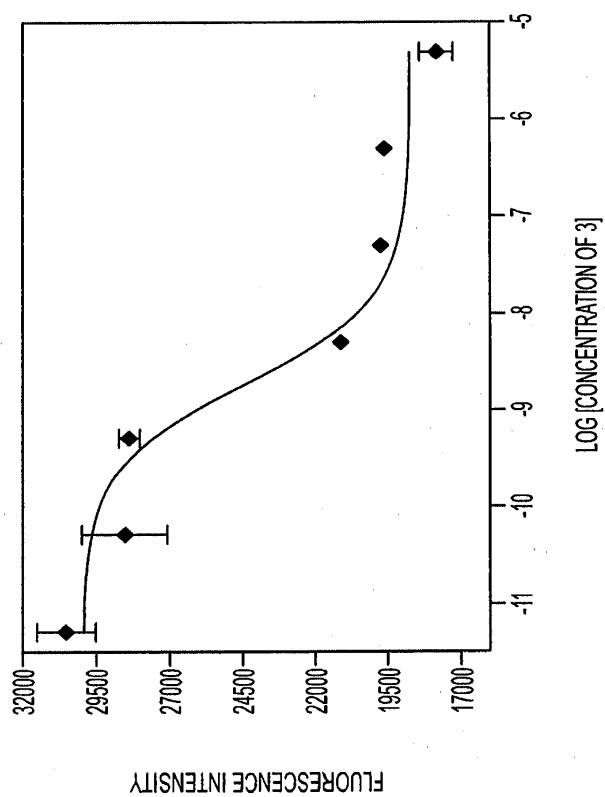
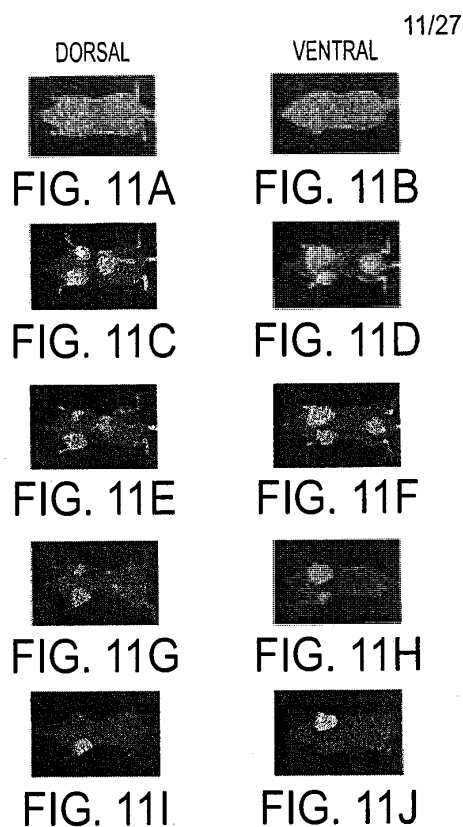
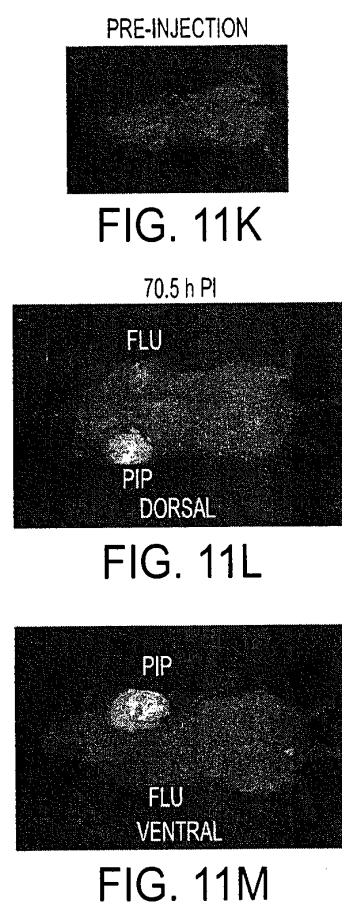
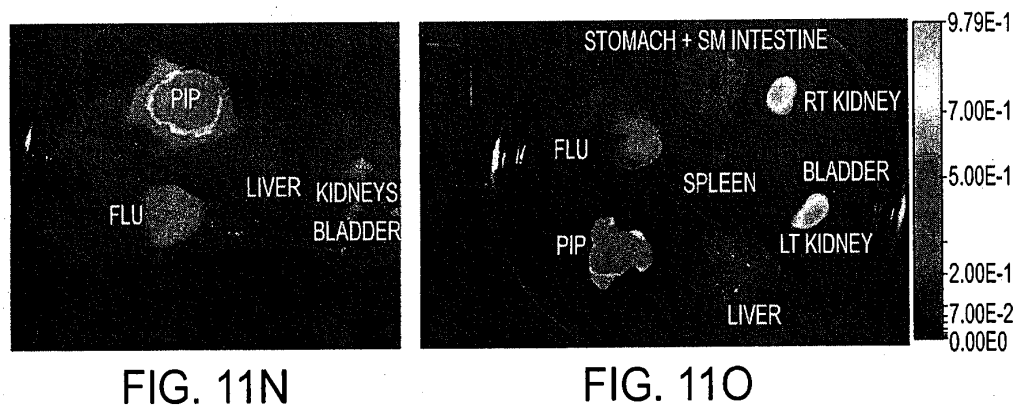


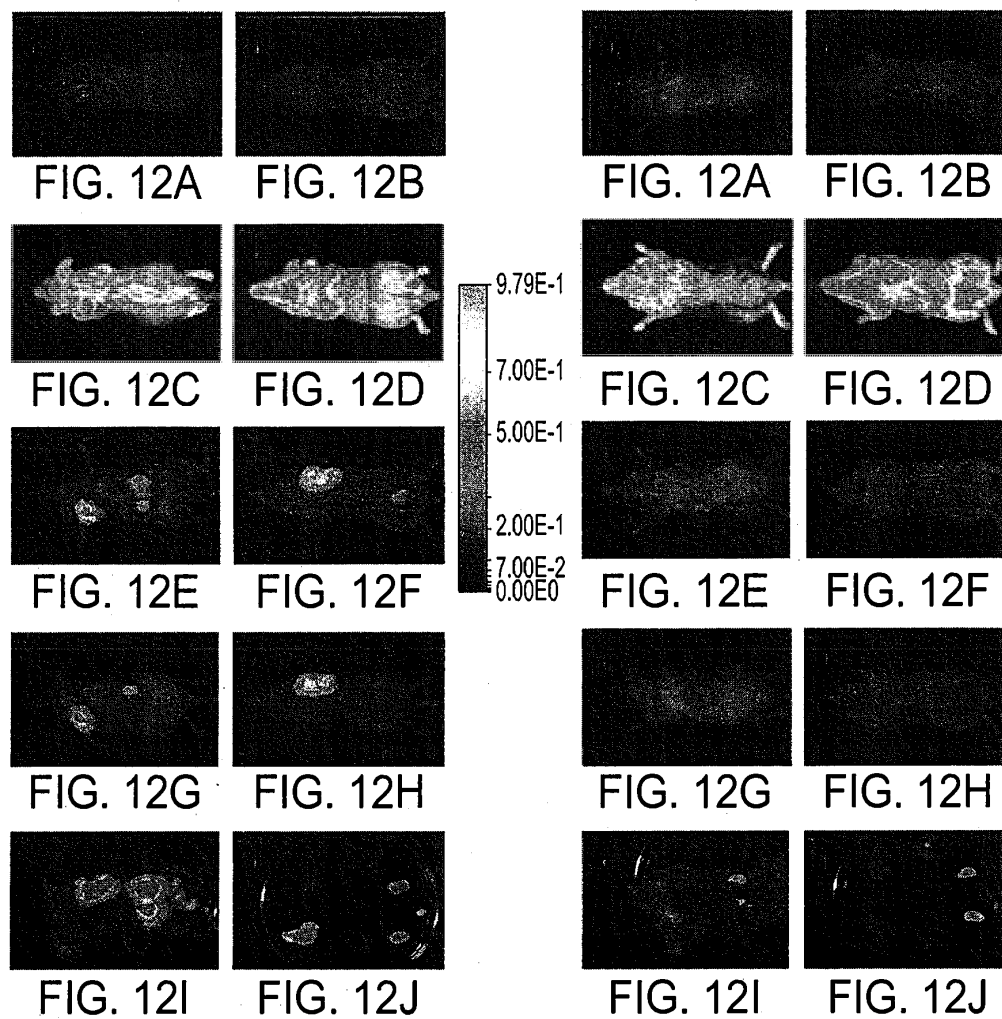
FIG. 10

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)

12/27



SUBSTITUTE SHEET (RULE 26)

13/27

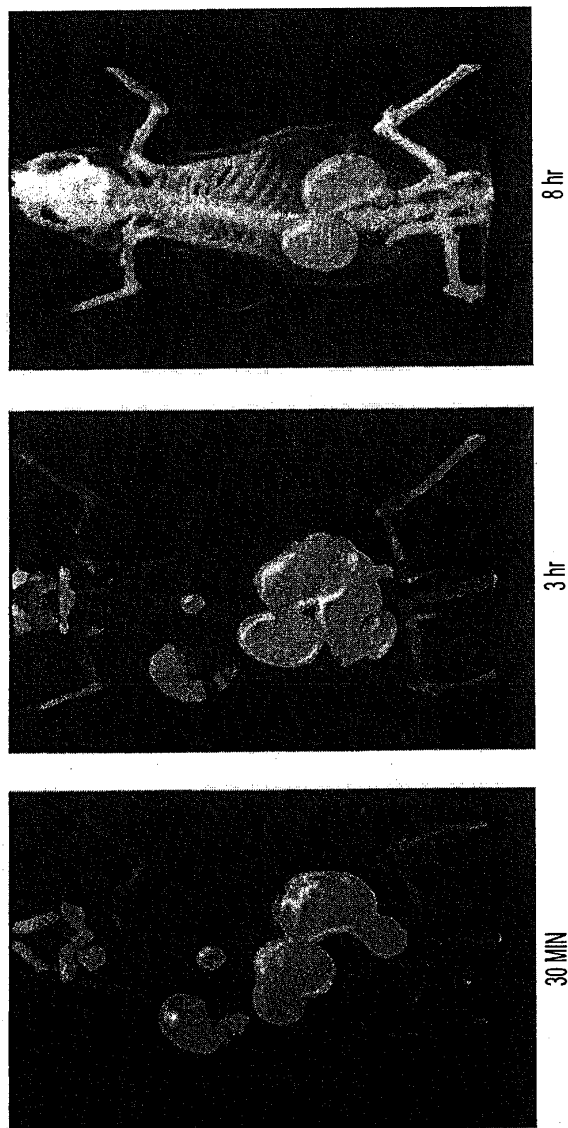


FIG. 13

SUBSTITUTE SHEET (RULE 26)

14/27

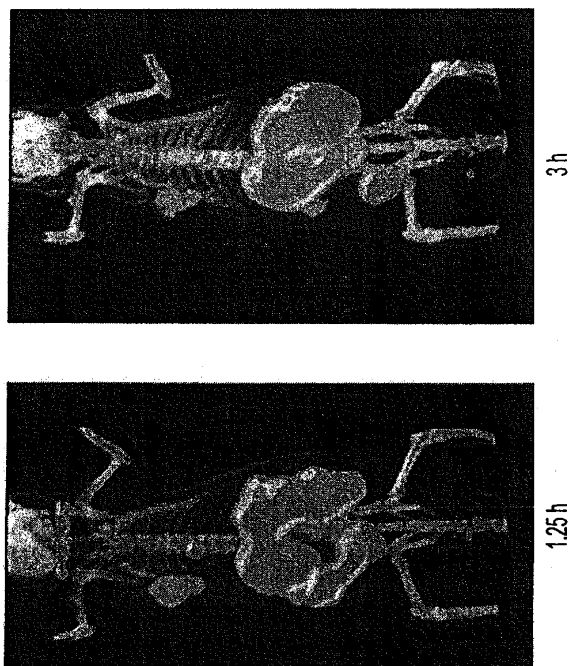


FIG. 14

SUBSTITUTE SHEET (RULE 26)

15/27

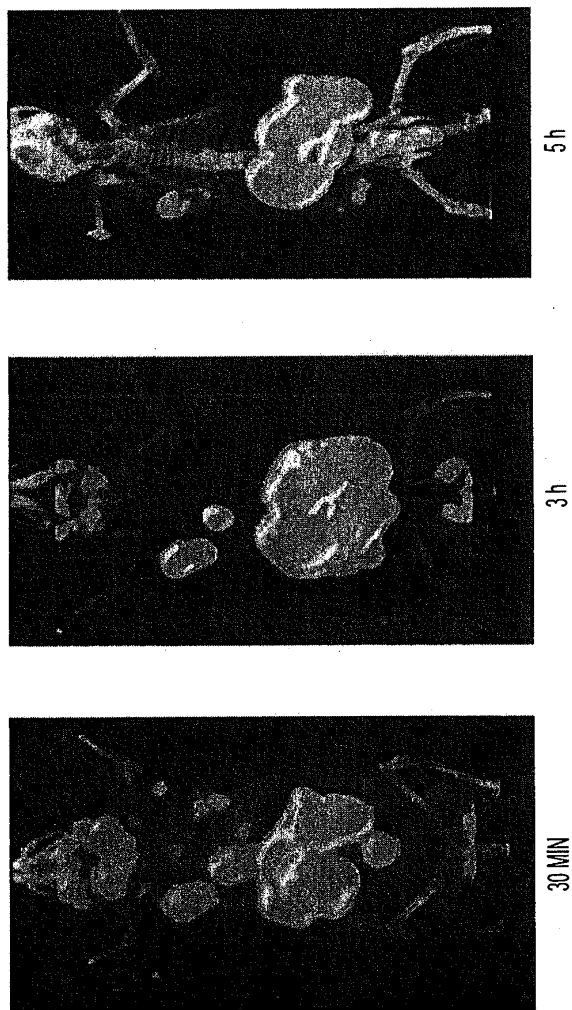


FIG. 15

SUBSTITUTE SHEET (RULE 26)

16/27

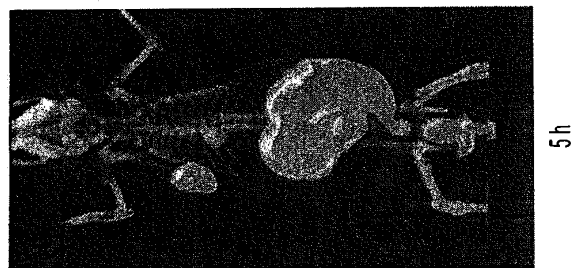
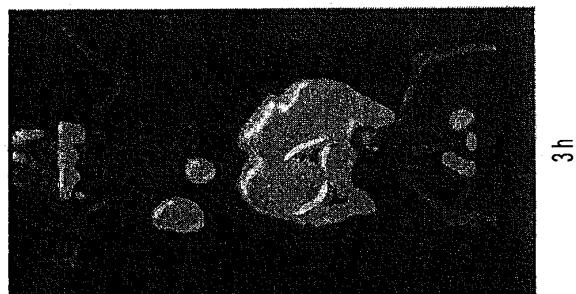
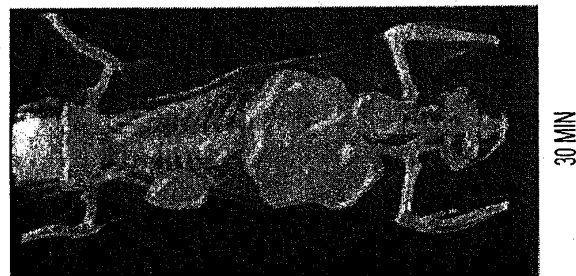


FIG. 16

SUBSTITUTE SHEET (RULE 26)

17/27

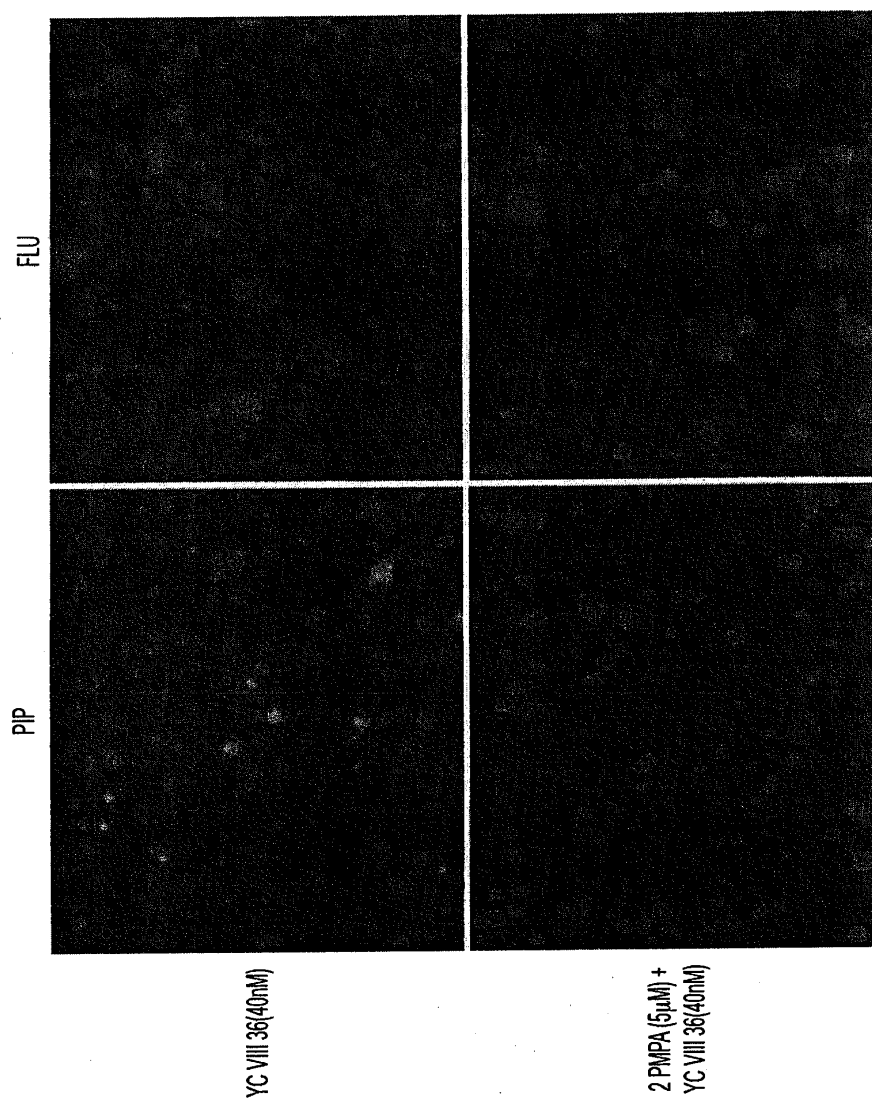


FIG. 17

SUBSTITUTE SHEET (RULE 26)

18/27

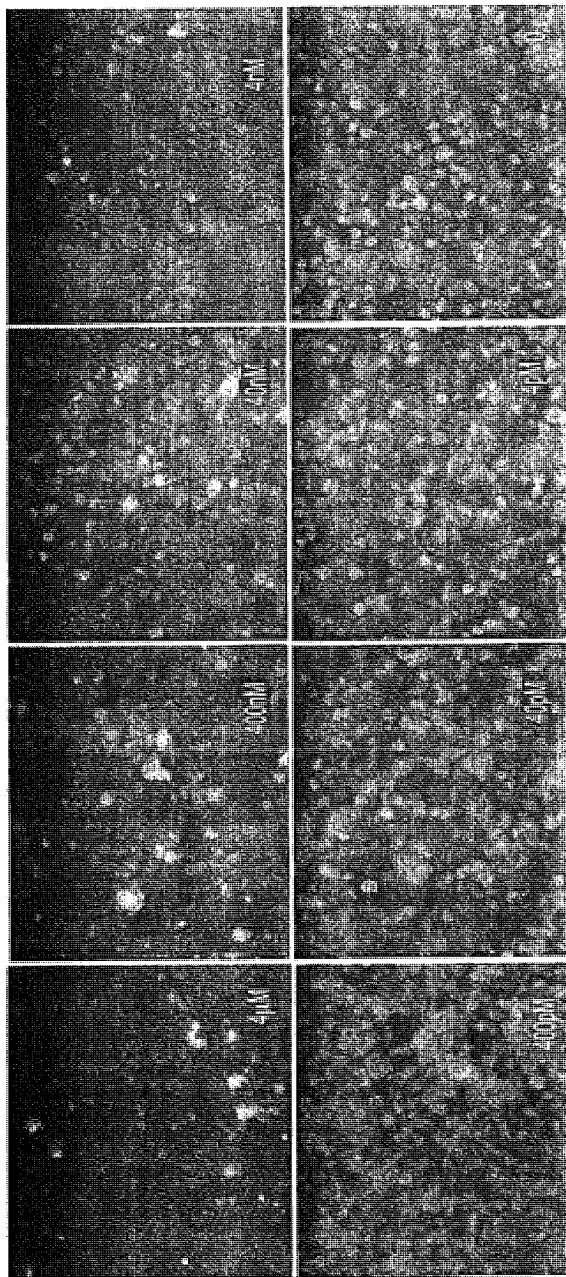


FIG. 18

SUBSTITUTE SHEET (RULE 26)

19/27

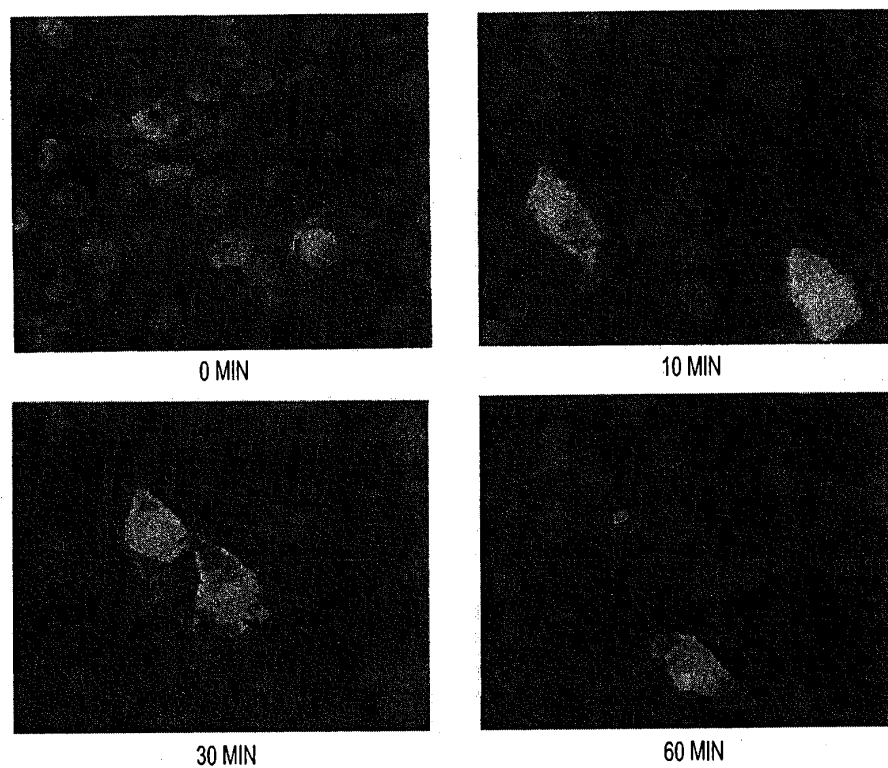


FIG. 19

SUBSTITUTE SHEET (RULE 26)

20/27

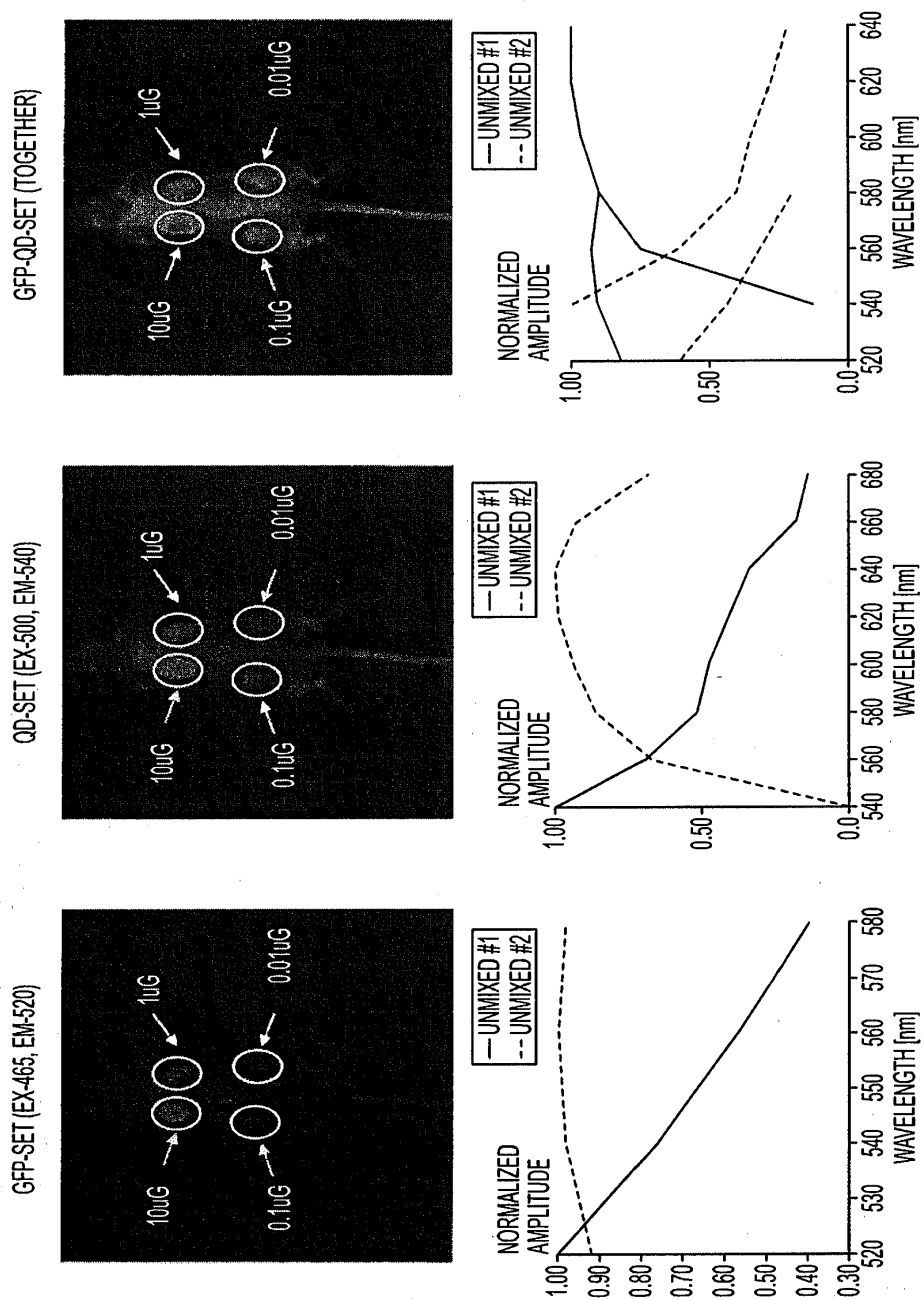


FIG. 20

SUBSTITUTE SHEET (RULE 26)

21/27

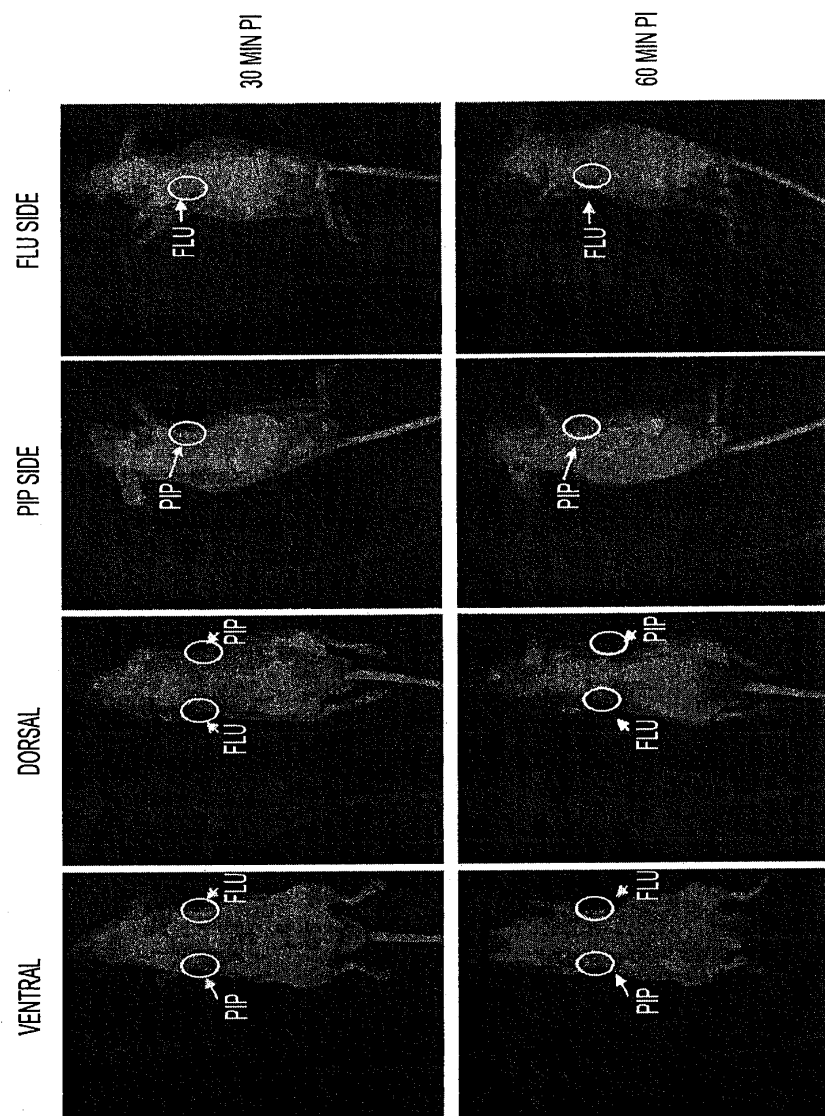


FIG. 21

SUBSTITUTE SHEET (RULE 26)

22/27

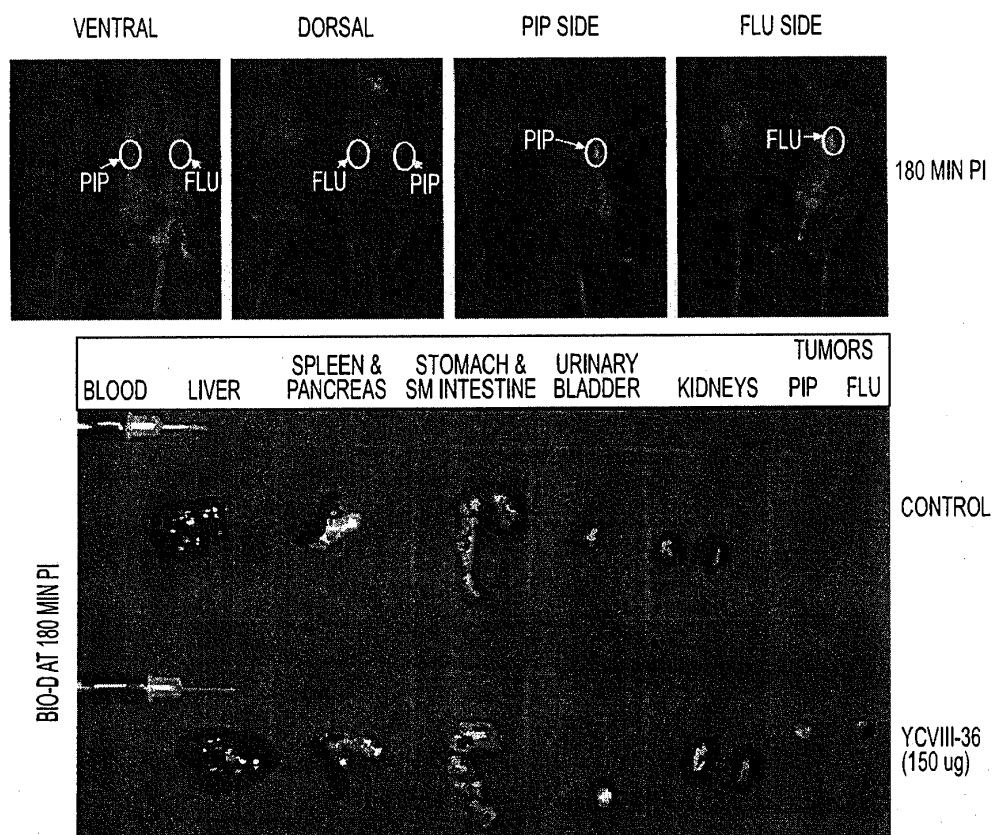
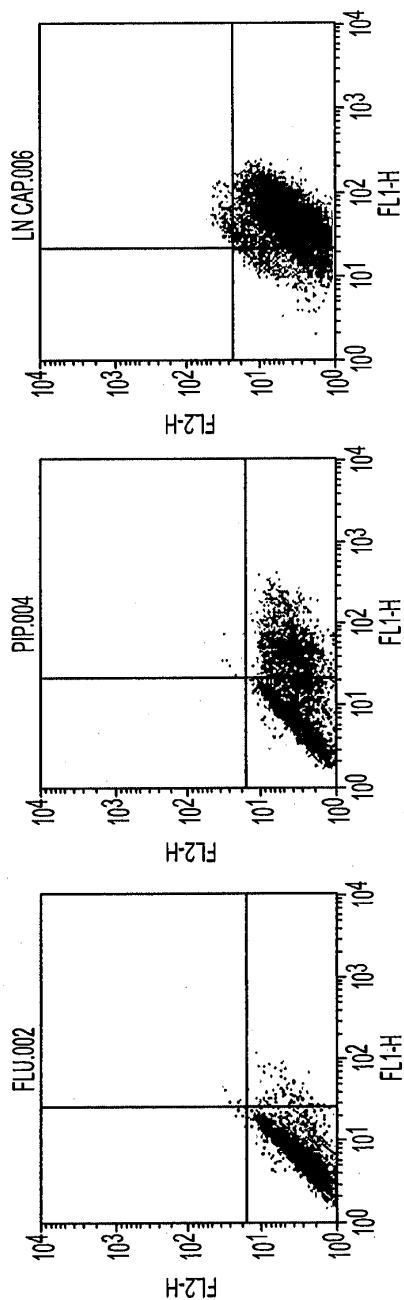


FIG. 22

23/27



FILE: FLU.002
ACQUISITION DATE: 23-JUL-09
GATE: G1
GATED EVENTS: 10805

QUAD	EVENTS	% GATED
UL	9	0.08
UR	5	0.05
LL	10718	99.19
LR	73	0.68

FILE: PIP.004
ACQUISITION DATE: 23-JUL-09
GATE: G1
GATED EVENTS: 10528

QUAD	EVENTS	% GATED
UL	2	0.02
UR	8	0.08
LL	8636	82.03
LR	1882	17.88

FILE: LN.CAP.006
ACQUISITION DATE: 23-JUL-09
GATE: G1
GATED EVENTS: 11179

QUAD	EVENTS	% GATED
UL	0	0.00
UR	137	1.23
LL	1290	11.54
LR	9752	87.23

FIG. 23

SUBSTITUTE SHEET (RULE 26)

24/27

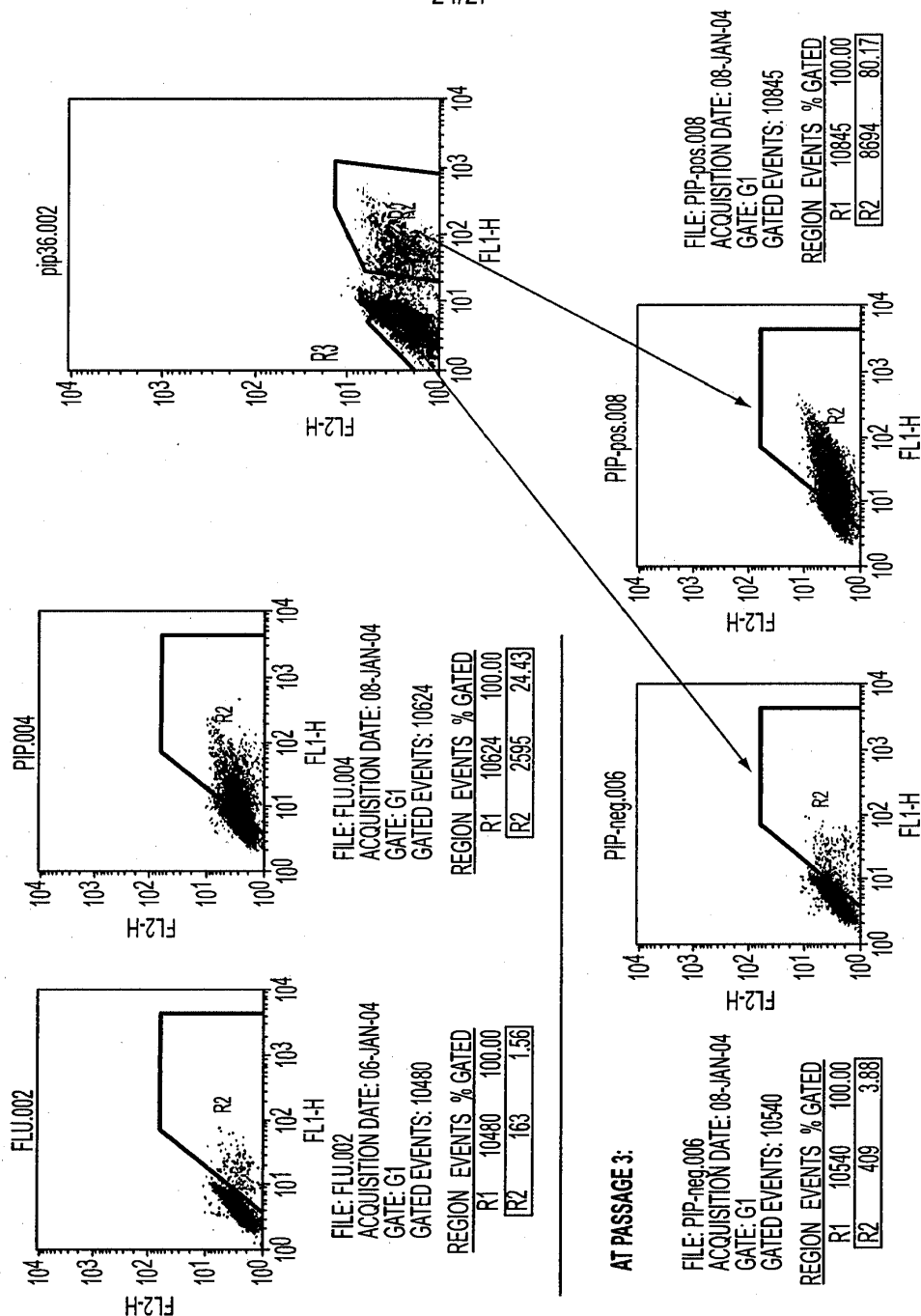


FIG. 24

25/27

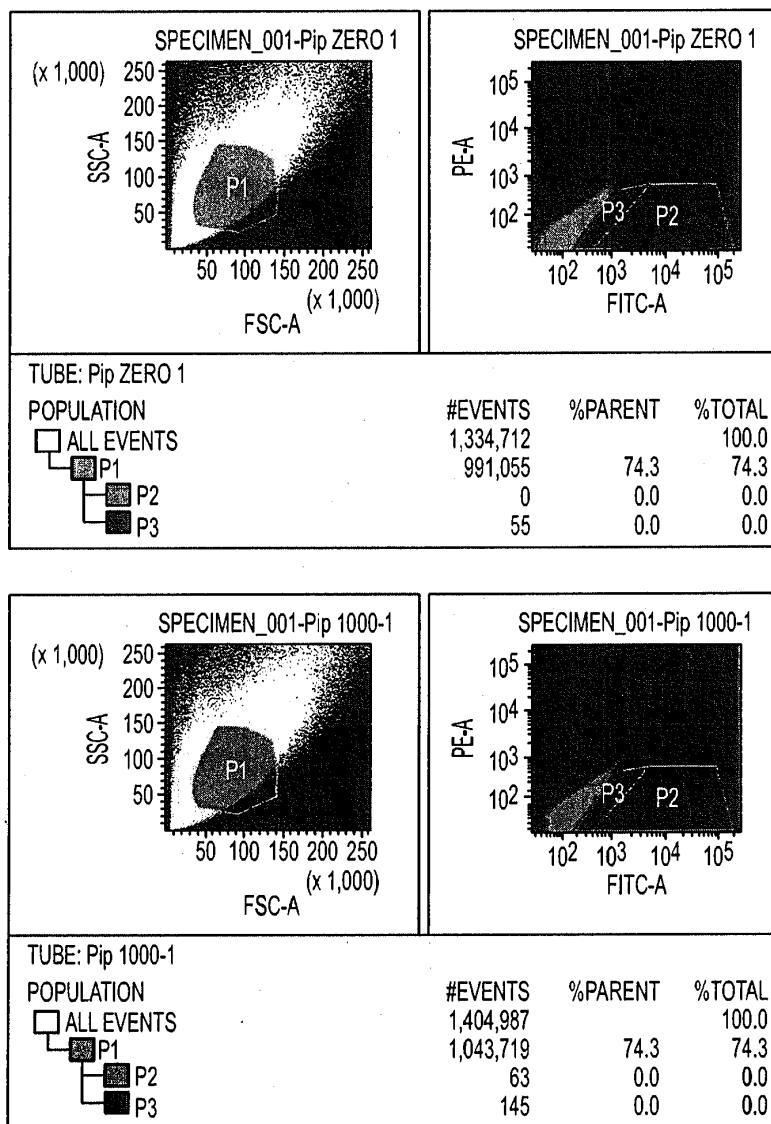
TO
FIG. 25B

FIG. 25A

SUBSTITUTE SHEET (RULE 26)

26/27

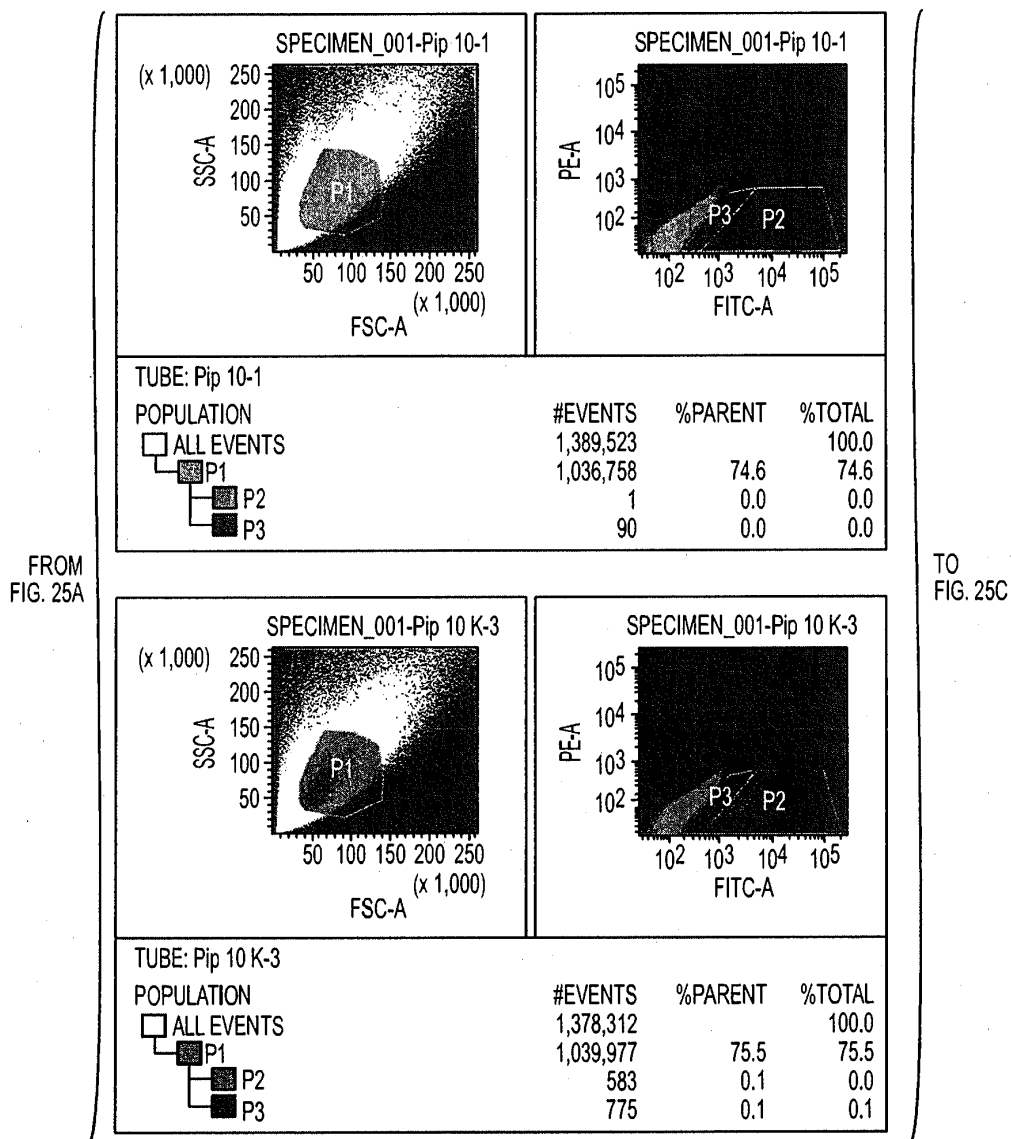


FIG. 25B

SUBSTITUTE SHEET (RULE 26)

27/27

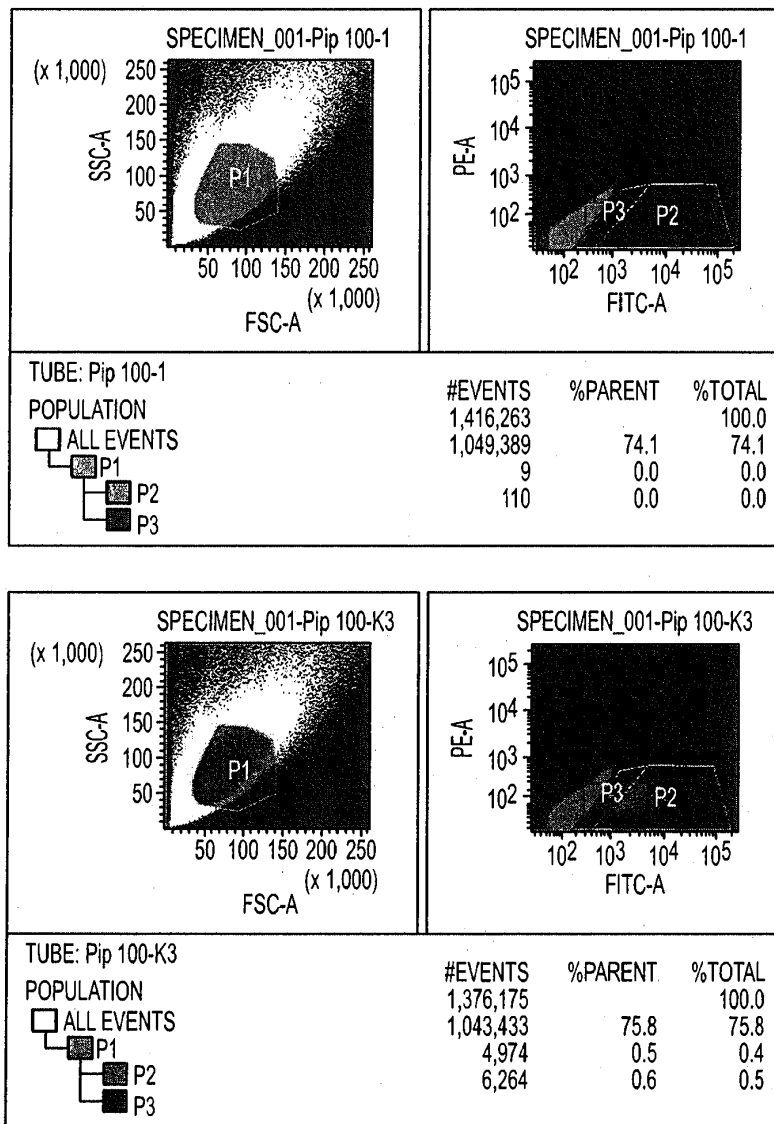
FROM
FIG. 25B

FIG. 25C

SUBSTITUTE SHEET (RULE 26)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
28 April 2016 (28.04.2016)

(10) International Publication Number
WO 2016/065142 A2

(51) International Patent Classification:
C07D 249/04 (2006.01) *A61K 51/04* (2006.01)
A61K 49/10 (2006.01) *A61K 47/48* (2006.01)
A61K 49/06 (2006.01) *A61P 35/00* (2006.01)

(74) Agent: **CHILDERS, Jeffrey W.**; Wade II, Suite 400,
5430 Wade Park Blvd., Raleigh, North Carolina 27607
(US).

(21) International Application Number:
PCT/US2015/056909

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(22) International Filing Date:
22 October 2015 (22.10.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/067,185 22 October 2014 (22.10.2014) US

(71) Applicant: **THE JOHNS HOPKINS UNIVERSITY**
[US/US]; 3400 North Charles Street, Baltimore, Maryland
21218 (US).

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

(72) Inventors: **POMPER, Martin G.**; 101 Churchwardens
Road, Baltimore, Maryland 21212 (US). **MEASE, Ronnie
C.**; 12320 Cannonball Road, Fairfax, Virginia 22030 (US).
RAY, Sangeeta; 9577 Joey Dr., Ellicott City, Maryland
21042 (US). **CHEN, Ying**; 1804 Reuter Rd, Lutherville-ti-
monium, Maryland 21093 (US). **YANG, Xing**; 909 W.
University Parkway, Apt. 307, Baltimore, Maryland 21210
(US).

Published:

— *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*



WO 2016/065142 A2

(54) Title: NEW SCAFFOLDS AND MULTIFUNCTIONAL INTERMEDIATES FOR IMAGING PSMA AND CANCER THER-
APY

(57) Abstract: Carbamate and beta-amino acid urea-based scaffolds that have high binding affinity to PSMA are disclosed. These scaffolds can be radiolabeled and used for imaging cells and tumors that express PSMA or for cancer radiotherapy. These com-
pounds also can comprise a fluorescent dye and be used for imaging cells and tumors that express PSMA or for photodynamic ther-
apy.

NEW SCAFFOLDS AND MULTIFUNCTIONAL INTERMEDIATES
FOR IMAGING PSMA AND CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the benefit of U.S. Provisional Application No. 62/067,185, filed, October 22, 2014, which is incorporated herein by reference in its entirety.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

- 10 This invention was made with government support under R01 CA134675 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

BACKGROUND

- 15 Prostate-specific membrane antigen (PSMA) is among the most intensively targeted biomarkers for imaging metastatic prostate cancer. PSMA is a zinc-dependent metallopeptidase that catalyzes the hydrolysis of a series of *N*-acylpolyglutamate derivatives (Mesters, 2006; Barinka, 2004; Pinto, 1996). It is expressed within certain normal tissues,(Ghosh, 2004) but transitions to abundant
20 plasma membrane expression in the epithelium of most prostate cancer and within other solid tumor neovasculature (Rajasekaran, 2005). PSMA membrane expression is associated with metastasis (Chang, 2001), castration resistance (Bander, 2005) and progression of prostate cancer (Perner, 2007).

- Several different scaffolds are available for synthesis of small-molecule
25 PSMA inhibitors, and have been reviewed (Byun, 2009; Tsukamoto, 2007; Zhou, 2005). As shown FIG. 1, those potent scaffolds share common features, namely; a) a pentanedioic acid as a glutamate mimic to fit within the S1' binding pocket of the PSMA active site; and b) a zinc-binding group to interact with the catalytic zinc atom at the PSMA active site. A substituent (R) can reside either within the S1 binding
30 pocket or within a void in the protein that extends to the surface. Scaffolds composed of phosphonates or phosphinates,(Jackson, 1996; Jackson, 2000), phosphoramidates (Maung, 2004) and ureas (Kozikowski, 2001; Kozikowski, 2004; Maung, 2004) of general structures **1-3**, as well as thiol **4** (Majer, 2003; Stoermer, 2012) and

hydroxamate **5** (Stoermer, 2003) have been reported as effective zinc binding groups for PSMA inhibition. However, the presence of a zinc binding moiety and a glutamate mimic residing in the S1' pocket are themselves not sufficient for high binding as demonstrated by gly-urea-glu compound **8** (FIG. 2A and FIG. 2B) (Wang, 2013). Of the reported PSMA binding scaffolds, urea-based inhibitors, first introduced by Kozikowski in 2001 (Kozikowski, 2001) for inhibition of glutamate carboxypeptidase II within the central nervous system, have been utilized the most for targeting PSMA due to their high binding affinity and synthetic simplicity (Kozikowski, 2001; Kozikowski, 2001; Foss, 2012; Mease, 2013; Vargas, 2015; Chen, 200; Barinka, 2008). A variety of low-molecular-weight compounds based on the various scaffolds discussed above, primarily the ureas, have been labeled with radionuclides for positron emission tomography (PET) and single photon emission computed tomography (SPECT), namely, $^{125/124}\text{I}$, $^{99\text{m}}\text{Tc}$, ^{111}In , ^{18}F , ^{11}C , ^{68}Ga , ^{64}Cu , and ^{86}Y , and have demonstrated PSMA-targeted imaging of prostate cancer in experimental models (Foss, 2012; Mease, 2013; Vargas, 2015; Banerjee, 2008; Banerjee, 2015; Banerjee, 2010; Banerjee, 2014; Chen, 2008; Chen, 2011; Eder, 2012; Foss, 2005; Hillier, 2013; Kularatne, 2009; Lapi, 2009; Maresca, 2009; Mease, 2008; Ray Banerjee, 2013; Weineisen, 2014). Several of these have been translated to phase 0-1 clinical trials, where they have enabled visualization of both primary and metastatic bone and soft-tissue lesions due to prostate cancer. (Afshar-Oromieh, 2015; Afshar-Oromieh, 2012; Afshar-Oromieh, 2013; Afshar-Oromieh, 2014; Barrett, 2013; Cho, 2012; Rowe, 2015; Szabo, 2015; Vallabhajosula, 2014). In addition, one agent [^{18}F]DCFPyL has also shown a higher sensitivity for detection of metastatic clear cell renal carcinoma compared to conventional imaging methods (S.P. Rowe, 2015).

However, clinical imaging studies also exhibited considerable uptake in non-target PSMA-expressing tissues such as the salivary glands and kidneys, bringing to light potential dose-limiting off-target effects, particularly for radiotherapeutic analogs. Additional PSMA-binding scaffolds that might preserve the positive imaging characteristics of the ureido scaffolds but clear from the non-target organs were sought. The carbamate scaffold was chosen because it would retain the overall geometry of the existing inhibitors, differing only with an O for NH substitution, which eliminates a potential hydrogen bonding donor group present in the ureas. The

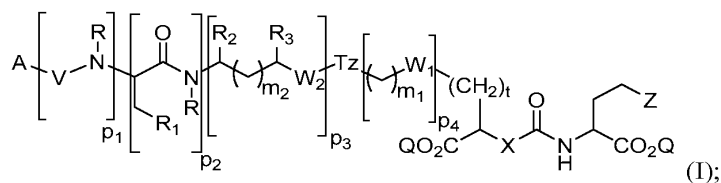
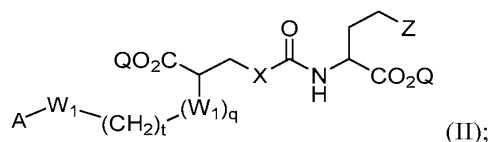
only PSMA-binding carbamate reported is gly-amino-pentanedioic acid **1** (Wang, 2013) displayed low binding affinity to PSMA, most likely due to the absence of productive binding within the S1 pocket, similar to ureido compound **8**. Herein a new class of potent PSMA inhibitors based on the carbamate scaffold to maintain
5 glutamate and S1 pocket side chain geometry and for putative binding to zinc have been reported. Carbamate scaffolds may complement the existing urea and other scaffolds upon which inhibitors, imaging and therapeutic agents targeting PSMA have been based.

Because of the favorable pharmacokinetic profile of this class of compounds,
10 i.e., low nonspecific binding, lack of metabolism in vivo and reasonable tumor residence times, it has been reasoned that urea and carbamate-based agents could also be used for molecular radiotherapy. This will be in analogy with radioimmunotherapy (RIT), which has proved remarkably successful in the treatment of lymphoma with two commercial products routinely integrated into clinical practice. However, (RIT)
15 is fraught with difficulties inherent in the use of radiolabeled antibodies for imaging, including prolonged circulation times, unpredictable biological effects and the occasional need for pre-targeting strategies. Furthermore, antibodies may have less access to tumor than low molecular weight agents, which can be manipulated pharmacologically. Therefore a need remains for low molecular weight compounds
20 with high binding affinity to PSMA for the imaging and radiotherapy of tumors.

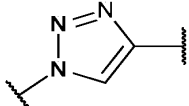
Further, for the aforementioned reasons, fluorescent-linker-carbamate based PSMA inhibitors have also been investigated. Targeted fluorescent PSMA binding compounds may find utility in fluorescence guided surgery and biopsy of PSMA positive tumors and tissues, the former providing visual confirmation of complete
25 removal of PSMA containing tissue. Moreover, carbamate conjugates of photosensitizing dyes also provide PSMA targeted photodynamic therapy agents.

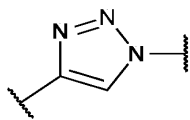
SUMMARY

In some aspects, the presently disclosed subject matter provides a compound
30 of formula (I) or formula (II):



wherein the subunits associate with elements p_1 , p_2 , p_3 and p_4 may be in any order; Z is tetrazole or CO_2Q ; Q is H or a protecting group; X is O or NH; q is an integer selected from the group consisting of 0 and 1; t is an integer selected from the group consisting of 1, 2, 3, and 4; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; p_1 , p_3 , and p_4 are each independently 0 or 1; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_q-$ and $-(CH_2-CH_2-O)_q-$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl or C_4 - C_{16} alkylaryl; Tz is a triazole group that can be present or

absent and is selected from the group consisting of  and



; each R_1 is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl or C_4 - C_{16} alkylaryl; R_2 and R_3 are each independently H and CO_2R_4 , wherein R_4 is selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_4 , then the other is H; V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$; A is selected

from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can optionally comprise one or more radioactive isotope suitable for imaging and /or radiotherapy, or a photosensitizing dye suitable for imaging and/or photodynamic therapy; and
5 stereoisomers and pharmaceutically acceptable salts thereof.

In certain aspects, the compound of formula (I) or formula (II) further comprises a radioactive isotope suitable for imaging and /or radiotherapy, or a photosensitizing dye suitable for imaging and/or photodynamic therapy.

In other aspects, the presently disclosed subject matter provides a method for
10 imaging one or more prostate-specific membrane antigen (PSMA)-expressing tumors or cells, the method comprising contacting the one or more tumors or cells with an effective amount of a compound of formula (I) and/or formula (II), including compounds of formula (Ia), (Ib), (Ic), and (IIa), and making an image, wherein the compound of formula (I) further comprises a radioactive isotope or a photosensitizing
15 dye suitable for imaging.

In yet other aspects, the presently disclosed subject matter provides a method for treating or preventing a disease or condition associate with one or more PSMA expressing tumors or cells, the method comprising administering at least one compound of formula (I) and/or formula (II), including compounds of formula (Ia),
20 (Ib), (Ic), and (IIa), to a subject in an amount effective to treat or prevent the disease or condition wherein the compound of formula (I) and/or formula (II) further comprises a radioactive isotope suitable for radiotherapy or a photosensitizing dye suitable for photodynamic therapy.

Certain aspects of the presently disclosed subject matter having been stated
25 hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

30 BRIEF DESCRIPTION OF THE FIGURES

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG.1 shows scaffolds available for synthesis of small-molecule PSMA inhibitors; those potent scaffolds share common features, namely; a) a pentanedioic acid (green) as a glutamate mimic to fit within the S1' binding pocket of the active site; and b) a zinc-binding group (blue) to interact with the catalytic zinc atom at the PSMA active site (blue); a substituent (R) can reside either within the S1 binding pocket or within a void in the protein that extends to the surface;

FIG. 2A and FIG. 2B show (FIG. 2A) urea, a carbamate, and reversed carbamate-based PSMA binding compounds; (FIG. 2B) binding affinities of urea, carbamate and reversed carbamate-based PSMA compounds;

FIG. 3A and FIG. 3B show (FIG. 3A) the lysine-carbamate scaffold used to design compounds of the presently disclosed subject matter: amino-pentanedioic acid (NPA) corresponding to a carbamate scaffold; (FIG. 3B) the general structure, high binding affinity compounds, and versatile intermediates of the presently disclosed carbamates;

FIG. 4 shows the general structure, high binding affinity compounds, and versatile intermediates of the presently disclosed beta-amino acid ureas;

FIG. 5 is a synthesis scheme for the presently disclosed carbamates;

FIG. 6 is a synthesis scheme for the presently disclosed beta-amino acid ureas;

FIG. 7A, FIG. 7B, FIG. 7C, and FIG. 7D show docking of **9 (YC-I-26)**, **12 (XY-20)**, and **13 (XY-48)** to PSMA; (FIG. 7A) urea **9 (YC-I-26)**; (FIG. 7B) Lys-NPA Carbamate **12 (XY-20)**; (FIG. 7C) Lys-OPA Carbamate **13 (XY-48)**; (FIG. 7D) overlay of **9 (YC-I-26)** (red), **12 (XY-20)** (blue) and **13 (XY-48)** (green);

FIG. 8A, FIG. 8B, FIG. 8C, and FIG. 8D show positron emission tomography-computed tomography (PET-CT) images in male NOD-SCID mice injected with the radiolabeled [¹⁸F]**XY-20** compound at (FIG. 8A) 5-15 minutes, (FIG. 8B) 15-25 minutes, (FIG. 8C) 25-35 minutes and (FIG. 8D) 50-60 minutes after injection; the mice on the right in each panel were pretreated with ZJ43 and the mice on the left in each panel were not pretreated with ZJ43. The green arrows point to the PSMA-negative PC3-flu tumor and the red arrows point to the PSMA-expressing (PC3-PIP) tumor (target); 200 microCi of radiotracer was injected;

FIG. 9 shows TLC analysis of Metabolism of [¹²⁵I]**31** ([¹²⁵I]**XY-26**) and [¹²⁵I]**32** ([¹²⁵I]**XY-57**) in PC 3 PIP (PSMA+) and PC3 flu (PSMA-) cells;

FIG. 10 shows three PSMA binding scaffolds: the lysine-glutamate urea **1**, cysteine-glutamate urea **2**, and glutamate-glutamate urea **3**;

FIG. 11 shows a cysteine-NPA carbamate and a glutamate-NPA carbamate scaffolds used to design compounds of the presently disclosed subject matter;

5 FIG. 12 shows the cysteine-glutamate urea scaffold used for PSMA binding and imaging for over 10 years starting with C-11 labeled DCMC, F-18 labeled DCFBC both for PET imaging with the latter currently in use in patients, and I-125 labeled DCIBC for SPECT imaging and or radiotherapy;

FIG. 13 shows two specific examples of IRDye800CW-linker-ureas.

10 The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

DETAILED DESCRIPTION

15 The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Examples and Figures, in which some, but not all embodiments of the presently disclosed subject matter are illustrated. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these
20 embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Examples and
25 Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

30 I. NEW SCAFFOLDS AND MULTIFUNCTIONAL INTERMEDIATES FOR IMAGING PSMA AND CANCER THERAPY

The presently disclosed subject matter provides new carbamate and beta-amino acid urea-based scaffolds that have high binding affinity to PSMA. These

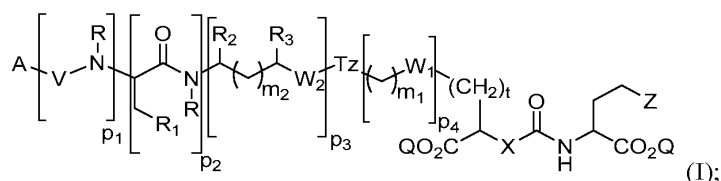
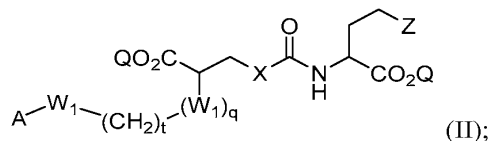
scaffolds can be radiolabeled and used to image cells and tumors that express PSMA. Some advantages to the presently disclosed methods include, but are not limited to, lower renal uptake of the presently disclosed compounds compared with existing urea-based PSMA-targeted radiotracers, as well as more rapid renal clearance as compared to compounds known in the art.

In some embodiments, the presently disclosed carbamate and beta-amino acid urea-based scaffolds complement existing urea and other scaffolds upon which inhibitors, imaging, and therapeutic agents targeting PSMA have been based. Versatile intermediates for carbamate beta-amino acid scaffolds can be functionalized in one or two steps toward PET imaging agents.

In other embodiments, the presently disclosed carbamate based scaffolds can be conjugated with near infrared dye molecules and their use in imaging PSMA and PSMA targeted photodynamic therapy agents also are provided.

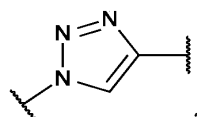
15 A. Compounds of Formula (I) or Formula (II)

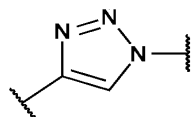
Accordingly, in some embodiments, the presently disclosed subject matter provides a compound of formula (I) or formula (II):



20 wherein the subunits associate with elements p₁, p₂, p₃ and p₄ may be in any order; Z is tetrazole or CO₂Q; Q is H or a protecting group; X is O or NH; q is an integer selected from the group consisting of 0 and 1; t is an integer selected from the group consisting of 1, 2, 3, and 4; p₂ is an integer selected from the group consisting of 0, 1, 2, and 3, and when p₂ is 2 or 3, each R₁ is the same or different; p₁, p₃, and p₄ are each independently 0 or 1; m₁ and m₂ are each an integer independently selected

- from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NRC(O)NR-$, $-NRC(S)NR-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_r-$ and $-(CH_2-CH_2-O)_r-$, wherein r is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl; Tz is a triazole group that can be present

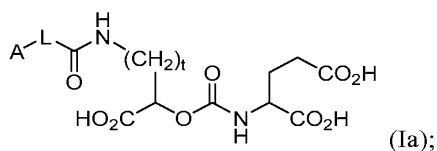
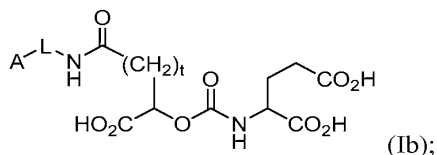
or absent and is selected from the group consisting of  and

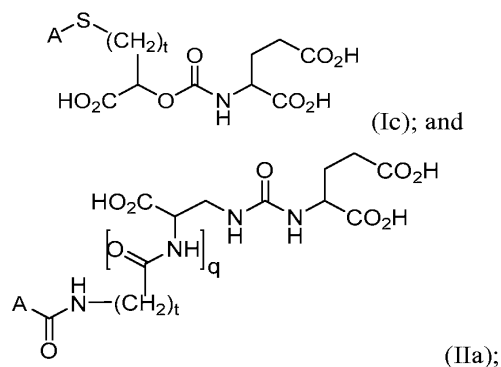


; each R_1 is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl or C_4 - C_{16}

- 10 alkylaryl; R_2 and R_3 are each independently H and CO_2R_4 , wherein R_4 is selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_4 , then the other is H; V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$; A is selected from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can optionally comprise one or more radioactive isotope suitable for imaging and /or radiotherapy, or a photosensitizing dye suitable for imaging and/or photodynamic therapy; and stereoisomers and pharmaceutically acceptable salts thereof.

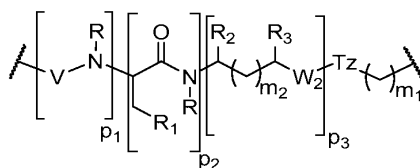
- 20 In particular embodiments, the compound of formula (I) is selected from the group consisting of:





wherein L is a linker that can be present or absent, and has the following

5 general structure:

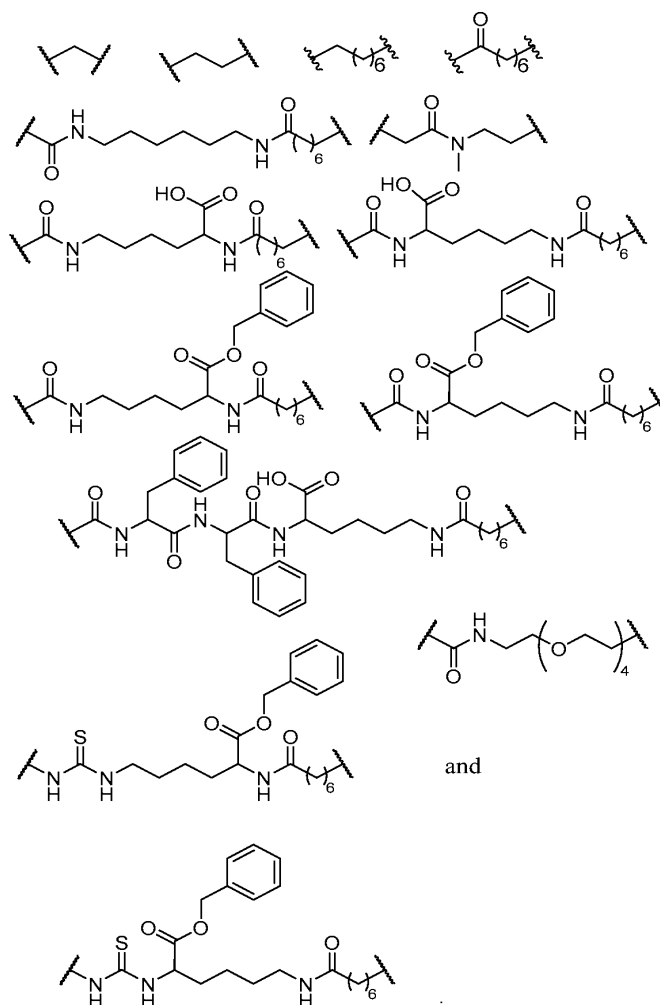


wherein p_1 , p_2 , p_3 , m_1 , m_2 , q , t , Tz , W_2 , R , R_1 , R_2 , R_3 , V and A are defined as hereinabove.

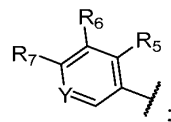
Suitable linkers are disclosed in U.S. Patent Application Publication No.

10 US2011/0064657 A1, for "Labeled Inhibitors of Prostate Specific Membrane Antigen (PSMA), Biological Evaluation, and Use as Imaging Agents," published March 17, 2011, to Pomper et al., and U.S. Patent Application Publication No. US2012/0009121 A1, for "PSMA-Targeting Compounds and Uses Thereof," published January 12, 2012, to Pomper et al, each of which is incorporated by reference in its entirety.

15 In yet more particular embodiments, L is selected from the group consisting of:



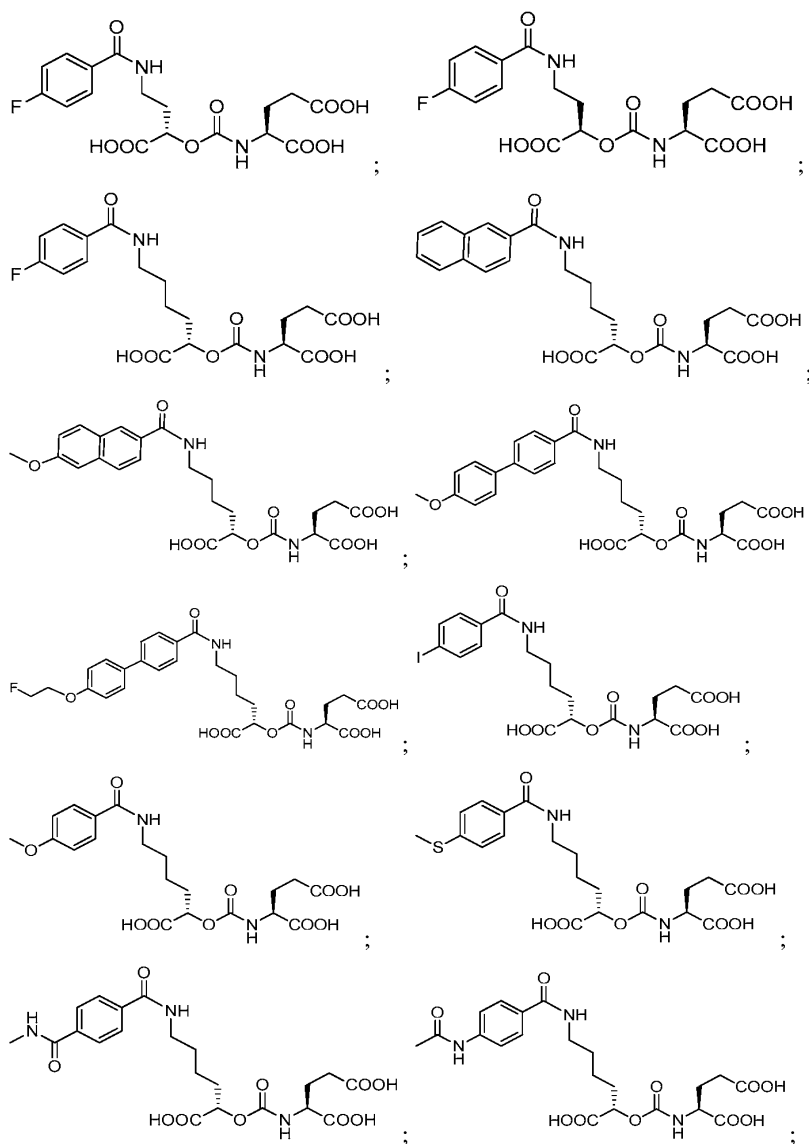
In some embodiments, A has the following general structure:

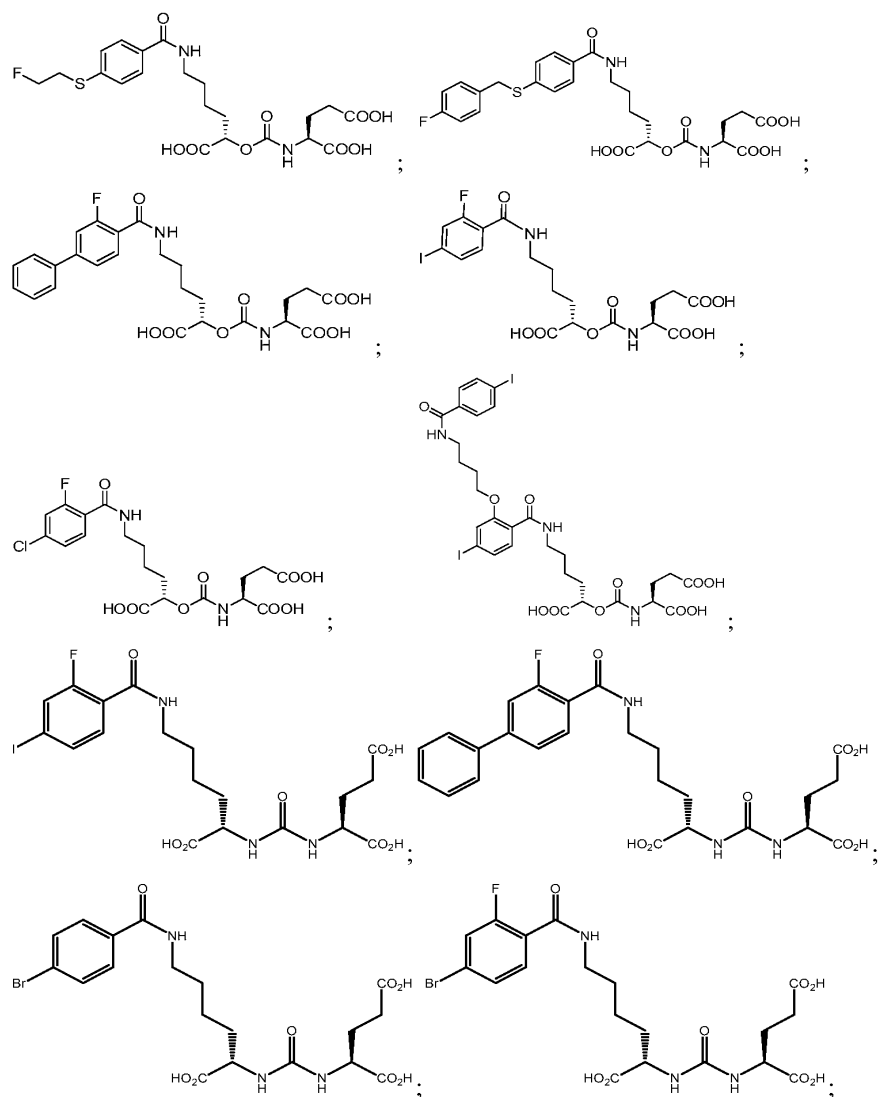


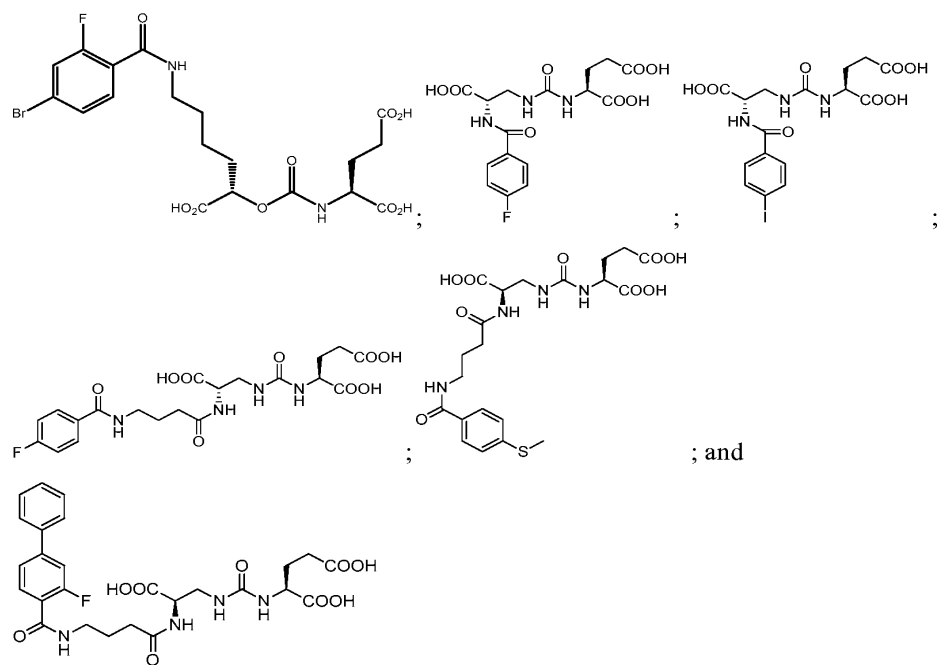
- wherein: Y is CH or N; and R₅, R₆, and R₇ are each independently selected
- 5 from the group consisting of H, halogen, alkoxy, alkylthioether, substituted and unsubstituted aryl, CH₂-NH-C(=NH)-NH₂, -NH-(C=O)-R₈, wherein R₈ is alkyl, - (C=O)-NR₉R₁₀, wherein R₉ and R₁₀ are each independently selected from the group consisting of H and alkyl, -X₁-(CH₂)_p-Ph-X₂, -X₁-(CH₂)_p-X₂, -X₁-(CH₂)_p-NH-C(=O)-

Ph-X₂, wherein each X₁ is independently O or S, each p is independently an integer selected from the group consisting of 1, 2, 3, and 4, each Ph is phenyl, and each X₂ is halogen.

In particular embodiments, the compound of formula (I) is selected from the group consisting of:

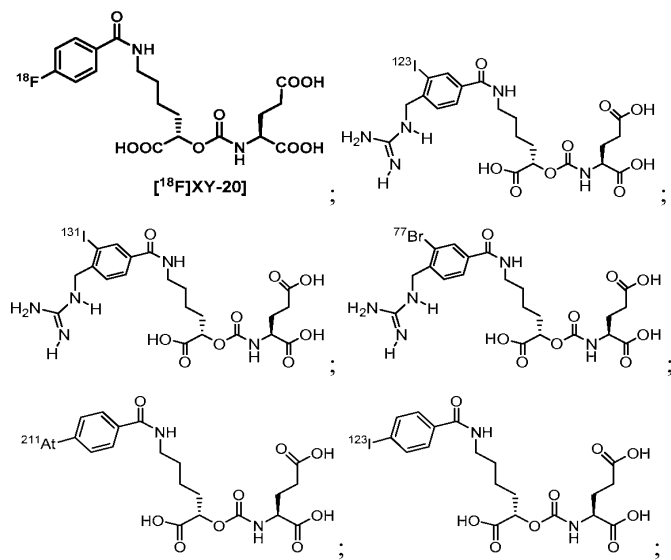




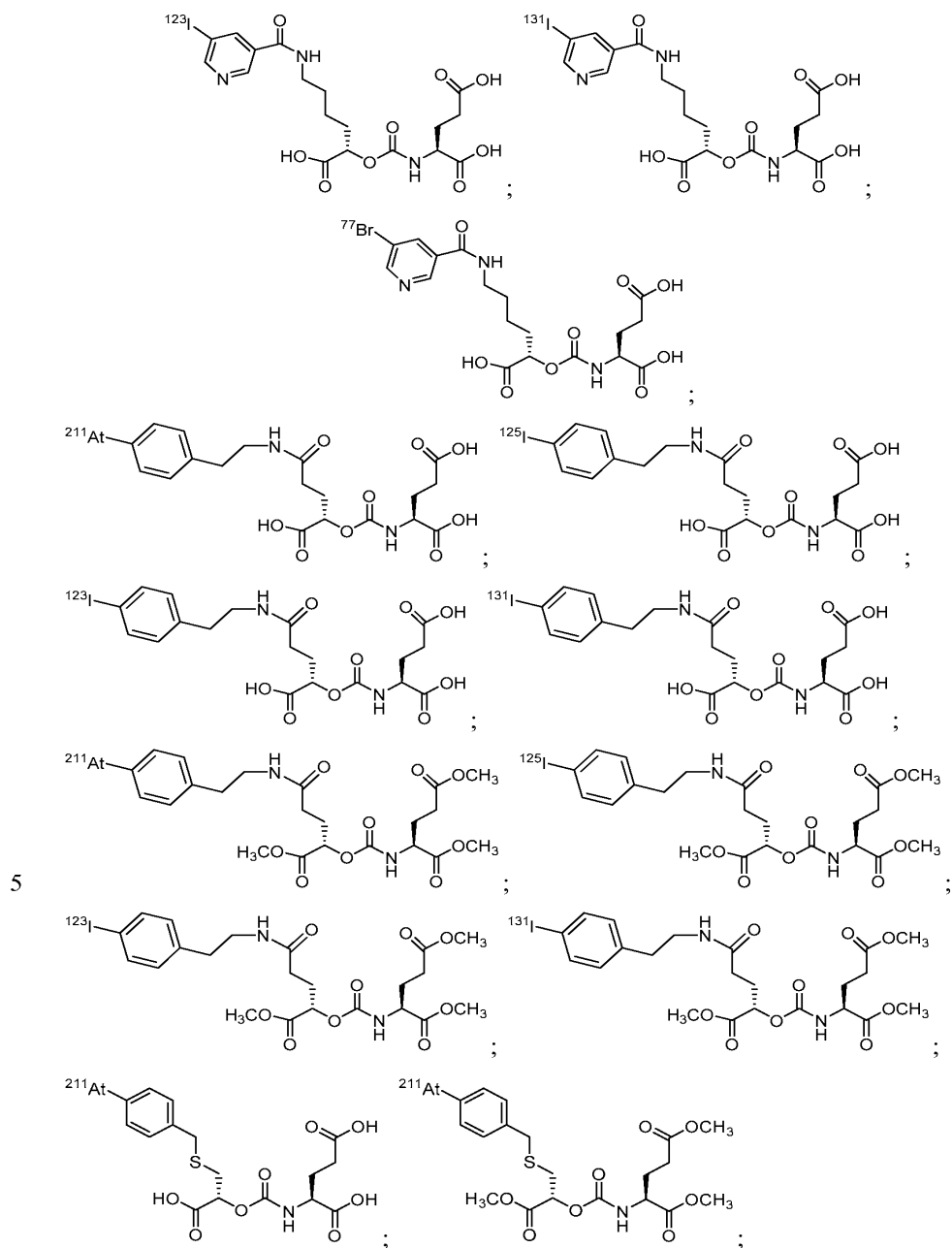


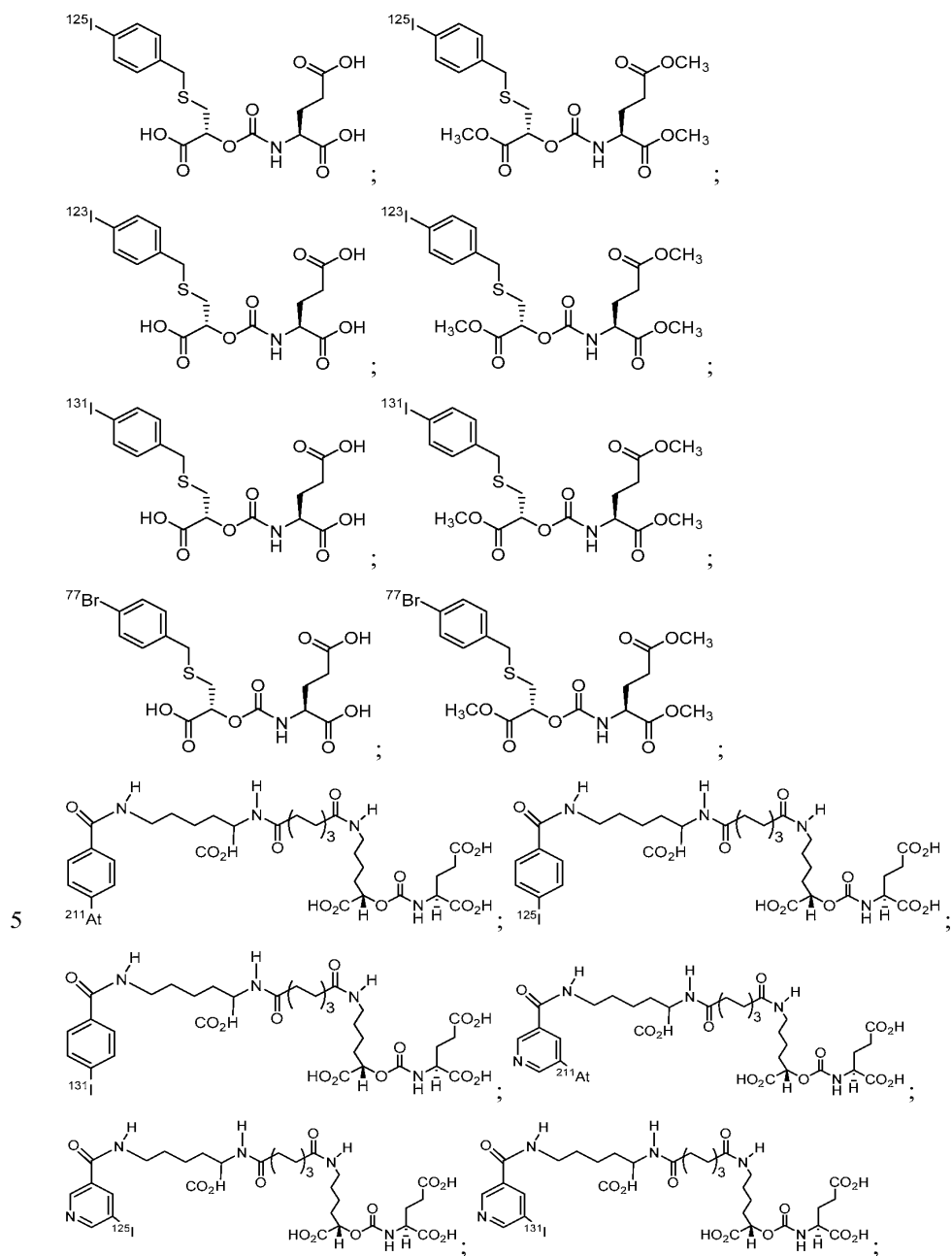
In yet more particular embodiments, the compound of formula (I) is selected

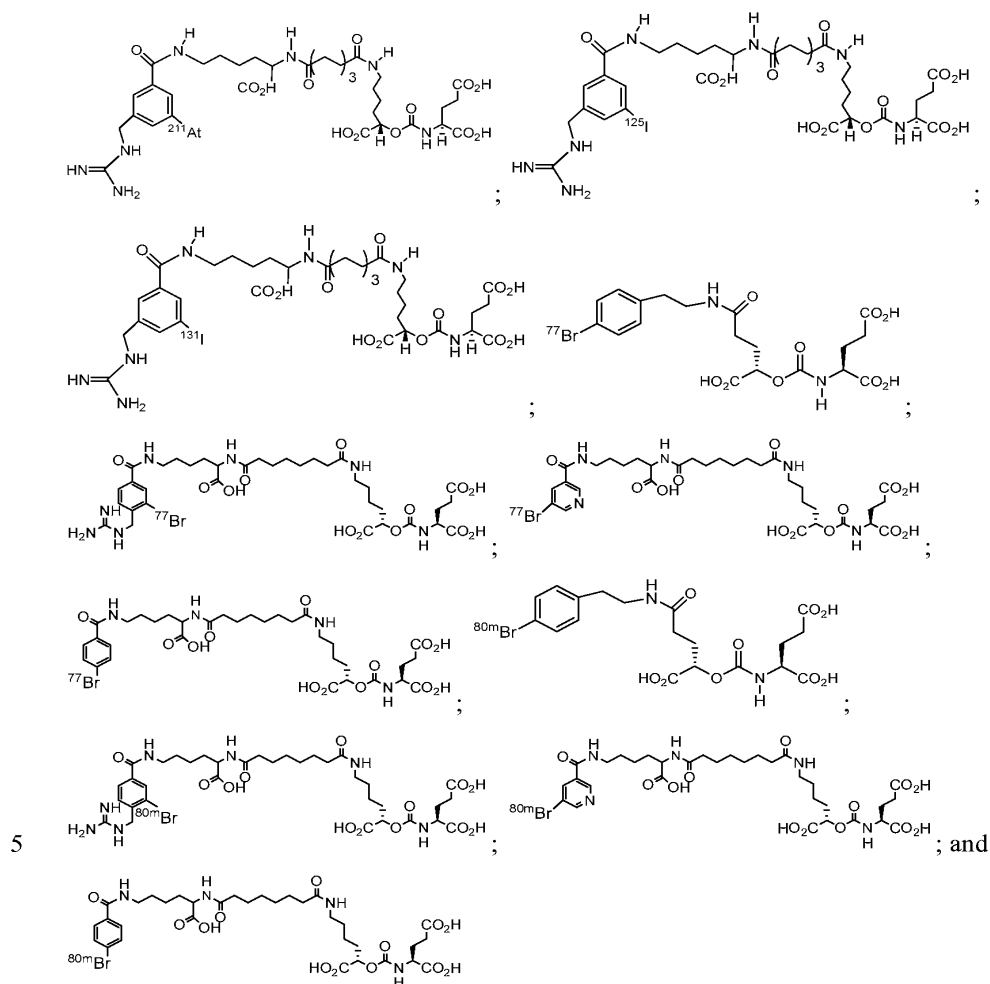
5 from the group consisting of:







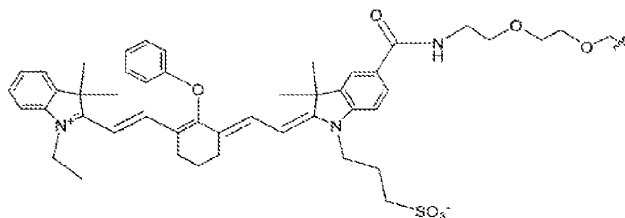




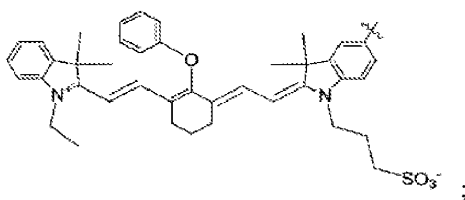
In some embodiments, the photosensitizing dye suitable for imaging and/or photodynamic therapy is a fluorescent dye moiety which emits in the visible or near infrared spectrum, wherein the fluorescent dye moiety comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye 800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS; and wherein R₁ or R₂ can optionally be a radioactive

isotope suitable for imaging or radiotherapy or optionally substituted with said radioisotope.

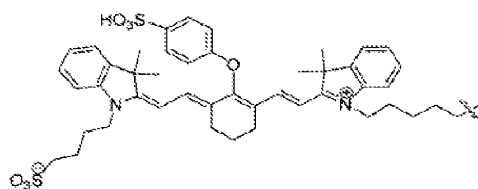
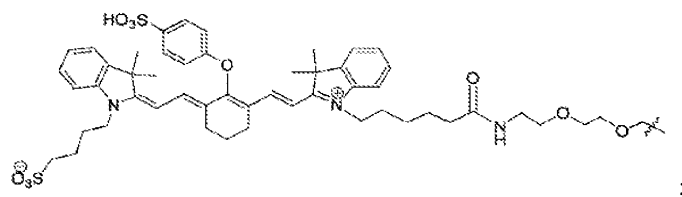
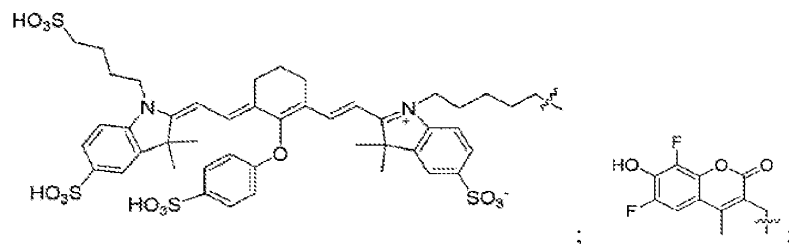
In specific embodiments, the fluorescent dye moiety is selected from the group

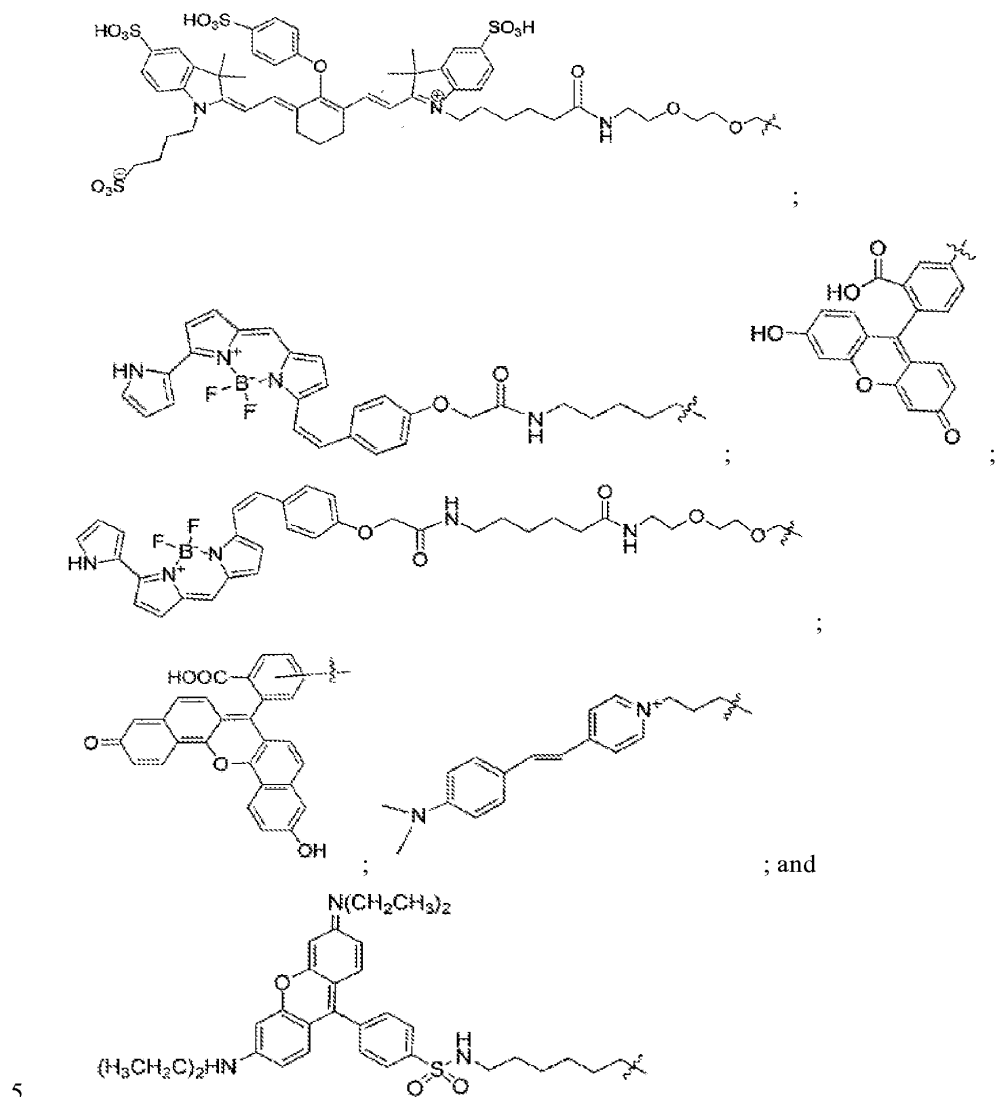


consisting of:

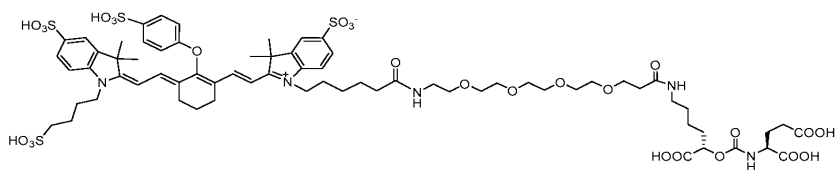


5



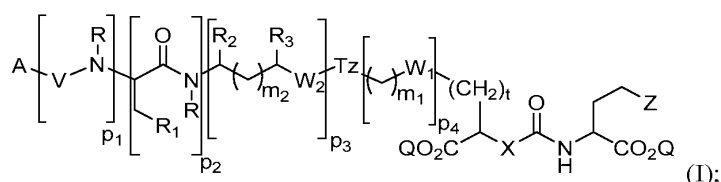


In more specific embodiments, the compound of formula (I) is

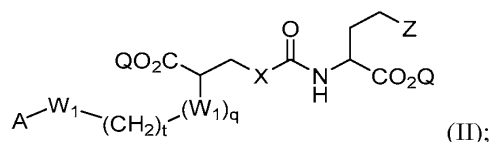


B. Methods of Using Compounds of Formula (I) or Formula (II) for Imaging a PSMA-expressing Tumor or Cell

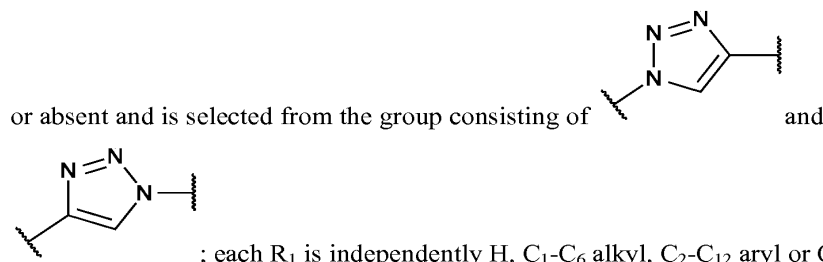
In some embodiments, the presently disclosed subject matter provides a method for imaging one or more prostate-specific membrane antigen (PSMA)-expressing tumors or cells, the method comprising contacting the one or more tumors or cells with an effective amount of a compound of formula (I and/or formula (II), including compounds of formula (Ia), (Ib), (Ic), and (IIa), and making an image, the compound of formula (I) or formula (II) comprising:



10



wherein the subunits associate with elements p_1 , p_2 , p_3 and p_4 may be in any order; Z is tetrazole or CO_2Q ; Q is H or a protecting group; X is O or NH; q is an integer selected from the group consisting of 0 and 1; t is an integer selected from the group consisting of 1, 2, 3, and 4; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; p_1 , p_3 , and p_4 are each independently 0 or 1; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$; W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'_2-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_{q-}$ and $-(CH_2-CH_2-O)_{q-}$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R is independently H, C_1-C_6 alkyl, C_2-C_{12} aryl, and C_4-C_{16} alkylaryl; Tz is a triazole group that can be present



- 5 when one of R₂ or R₃ is CO₂R₄, then the other is H; V is selected from the group consisting of -C(O)-, -C(S)-, -NRC(O)-, -NRC(S)-, and -OC(O)-; A is selected from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can optionally comprise one or more radioactive isotope suitable for imaging and /or radiotherapy, and
- 10 photosensitizing dye suitable for imaging and/or photodynamic therapy.

"Contacting" means any action which results in at least one compound comprising the imaging agent of the presently disclosed subject matter physically contacting at least one PSMA-expressing tumor or cell. Contacting can include exposing the cell(s) or tumor(s) to the compound in an amount sufficient to result in

15 contact of at least one compound with at least one cell or tumor.

By "making an image", it is meant using PET, SPECT or florescent optical imaging to form an image of a cell, tissue, tumor, part of body, and the like. The presently disclosed methods may include one or more radioactive isotopes capable of emitting radiation suitable for detection with PET or SPECT or a photosensitizing dye

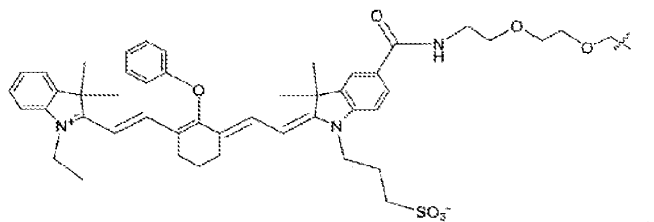
20 suitable for fluorescent optical imaging.

In some embodiments, the image is made using PET the image is made using positron emission tomography (PET) and the radiohalogen is selected from the group consisting of ¹⁸F or ¹²⁴I.

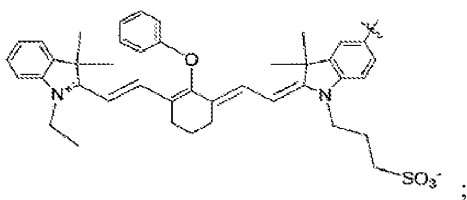
In some other embodiments, the image is made using Single-photon emission computed tomography (SPECT) and the radiohalogen is selected from the group consisting of ⁷⁷Br, ¹³¹I, ¹²⁵I, and ¹²³I.

25

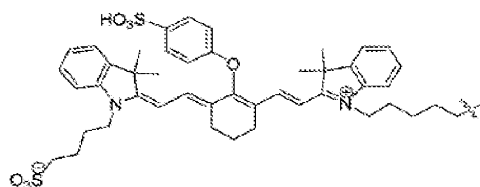
In yet some other embodiments, the image is made using florescent optical imaging and the photosensitizing dye suitable for imaging is selected from the group consisting of:



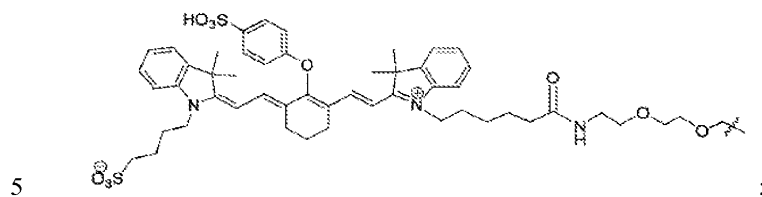
;



;

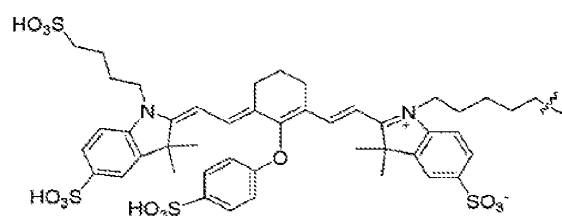


;

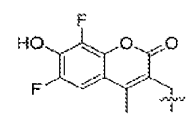


;

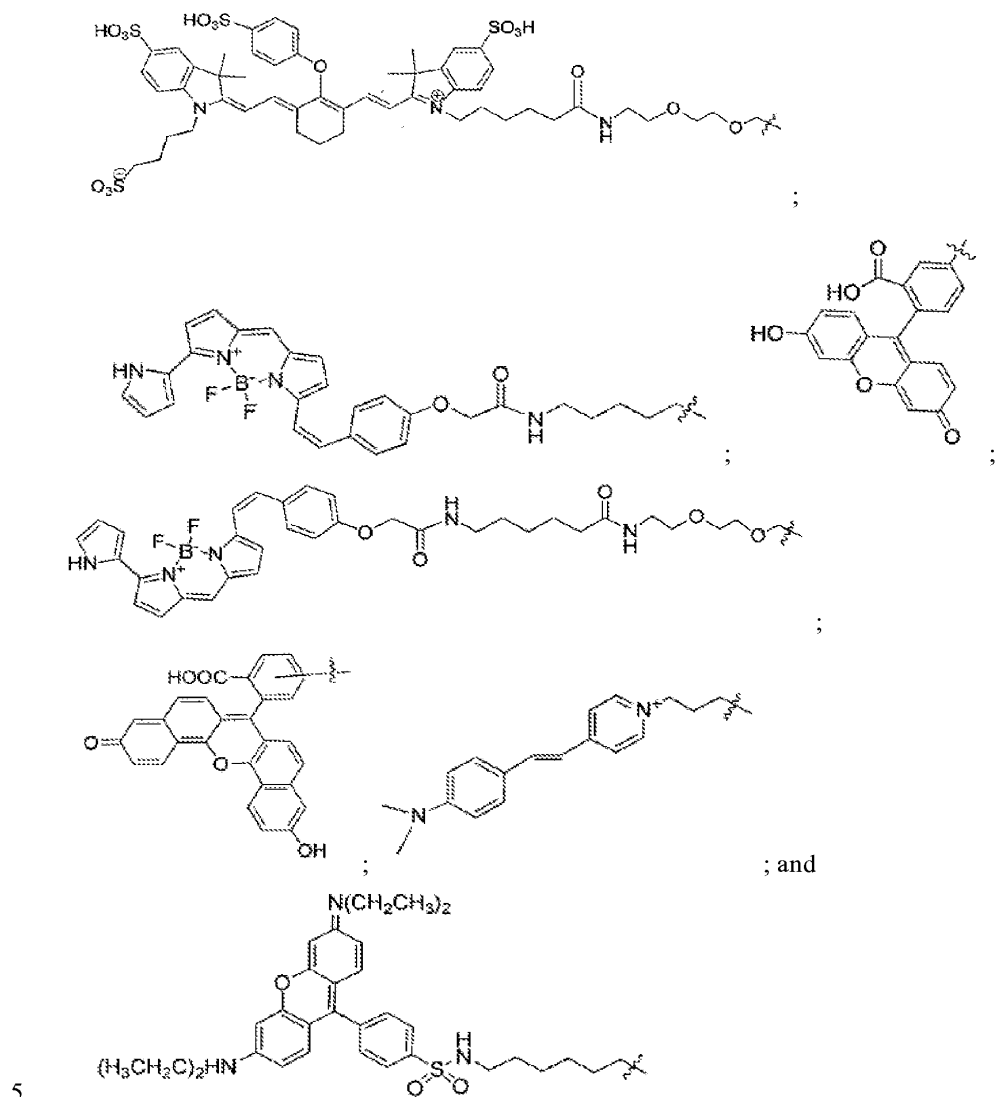
5



;



;



In certain embodiments, the one or more PSMA-expressing tumors or cells is selected from the group consisting of: a prostate tumor or cell, a metastasized prostate tumor or cell, a lung tumor or cell, a renal tumor or cell, a glioblastoma, a pancreatic tumor or cell, a bladder tumor or cell, a sarcoma, a melanoma, a breast tumor or cell, a colon tumor or cell, a germ cell, a pheochromocytoma, an esophageal tumor or cell, a stomach tumor or cell, and combinations thereof. In yet more certain

embodiments, the one or more PSMA-expressing tumors or cells is a prostate tumor or cell.

In some embodiments, the one or more PSMA-expressing tumors or cells is *in vitro*, *in vivo*, or *ex vivo*. In particular embodiments, the one or more PSMA-expressing tumors or cells is present in a subject.

In some embodiments, the tumor or cell is found in a subject. The subject treated by the presently disclosed methods in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term "subject." Accordingly, a "subject" can include a human subject for medical purposes, such as for the treatment of an existing condition or disease or the prophylactic treatment for preventing the onset of a condition or disease, or an animal (non-human) subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, monkeys, apes, and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like. An animal may be a transgenic animal. In some embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a "subject" can include a patient afflicted with or suspected of being afflicted with a condition or disease. Thus, the terms "subject" and "patient" are used interchangeably herein. In some embodiments, the subject is human. In other embodiments, the subject is non-human.

In some embodiments, a detectably effective amount of the imaging agent of the presently disclosed methods is administered to a subject. In accordance with the presently disclosed subject matter, "a detectably effective amount" of the imaging agent is defined as an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of the imaging agent may be administered in more than one injection. The detectably effective amount of the imaging agent can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual,

idiosyncratic responses of the individual, the dosimetry, and instrument and film-related factors. Optimization of such factors is well within the level of skill in the art.

In general, the “effective amount” of an active agent refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of
5 ordinary skill in this art, the effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the makeup of the pharmaceutical composition, the target tissue, and the like.

It is preferable to have the compound comprising the imaging agent to localize
10 to the tumor or cell quickly after administration so as to minimize any side effects to the subject. Accordingly, in some embodiments, the compound comprising the imaging agent substantially localizes to the tumor or cell within about 60 minutes to about 240 minutes of administration and, in some embodiments, about 60 minutes. In other embodiments, the compound comprising the imaging agent substantially
15 localizes to the tumor or cell within about 30 minutes of administration. In still other embodiments, the compound comprising the imaging agent substantially localizes to the tumor or cell within about 10 minutes of administration.

It is also preferable that the compounds of the presently disclosed subject matter are excreted from tissues of the body quickly to prevent prolonged exposure to
20 the radiation of the radiolabeled compound administered to the patient. Typically compounds of the presently disclosed subject matter are eliminated from the body in less than about 24 hours. More preferably, compounds of the presently disclosed subject matter are eliminated from the body in less than about 16 hours, 12 hours, 8 hours, 6 hours, 4 hours, 2 hours, 90 minutes, or 60 minutes.

25 In some embodiments, the presently disclosed methods comprise clearance of the compound comprising the imaging agent from the tumor or cell in the subject. At least one advantage of the presently disclosed methods is that, in some embodiments, there is more rapid clearance of the compound comprising the imaging agent from the kidneys than from the tumor of the subject.

30 In other embodiments, for example for fluorescence guided surgery and biopsy of PSMA positive tumors and tissues the compound comprising the photosensitizing dye suitable for imaging is visible at about 4 hours after injection and presents the brightest signal at about 24 hours after injection.

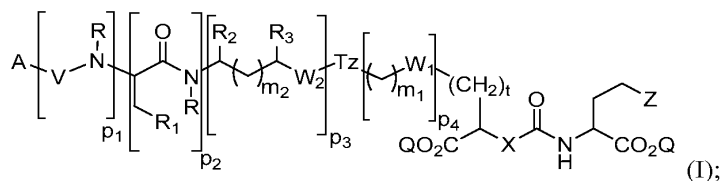
In some embodiments, the presently disclosed methods use compounds that are stable *in vivo* such that substantially all, e.g., more than about 50%, 60%, 70%, 80%, or more preferably 90% of the injected compound is not metabolized by the body prior to excretion. In other embodiments, the compound comprising the imaging agent is stable *in vivo*.

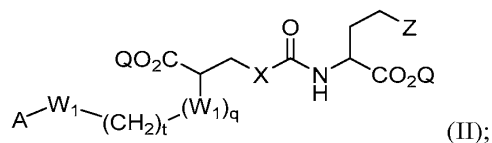
A “tumor,” as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all precancerous and cancerous cells and tissues.

In some embodiments, the tumor cells express PSMA, such as prostate tumor cells or metastasized prostate tumor cells. In other embodiments, a tumor may be treated by targeting adjacent or nearby cells which express PSMA. For example, vascular cells undergoing angiogenesis associated with a tumor may be targeted. Essentially all solid tumors express PSMA in the neovasculature. Therefore, methods of the presently disclosed subject matter can be used to image nearly all solid tumors including, but not limited to, lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal, and stomach tumors. Also, certain benign lesions and tissues including, but not limited to, endometrium, schwannoma and Barrett's esophagus, can be imaged according to the presently disclosed methods.

C. *Methods of Using Compounds of Formula (I) or Formula (II) for Treating a disease or condition associated with one or more PSMA-expressing Tumor or Cell*

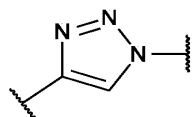
In some embodiments, the presently disclosed subject matter provides a method for treating or preventing a disease or condition associated with one or more PSMA expressing tumors or cells, the method comprising administering at least one compound of formula (I), and/or formula (II), including compounds of formula (Ia), (Ib), (Ic), and (IIa), to a subject in an amount effective to treat or prevent the disease or condition, the compound of formula (I) and/or formula (II) comprising:





wherein the subunits associate with elements p_1 , p_2 , p_3 and p_4 may be in any order; Z is tetrazole or CO_2Q ; Q is H or a protecting group; X is O or NH; q is an integer selected from the group consisting of 0 and 1; t is an integer selected from the group consisting of 1, 2, 3, and 4; p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different; p_1 , p_3 , and p_4 are each independently 0 or 1; m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6; W_1 is selected from the group consisting of a bond, $-\text{S}-$, $-\text{C}(=\text{O})-\text{NR}-$, and $-\text{NR}-\text{C}(=\text{O})-$; W_2 is selected from the group consisting of a bond, $-\text{S}-$, $-\text{CH}_2-\text{C}(=\text{O})-\text{NR}-$, $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $-\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, $-\text{NR}-\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $-(\text{O}-\text{CH}_2-\text{CH}_2)_q-$ and $-(\text{CH}_2-\text{CH}_2-\text{O})_q-$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8; each R is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl; Tz is a triazole group that can be present

or absent and is selected from the group consisting of

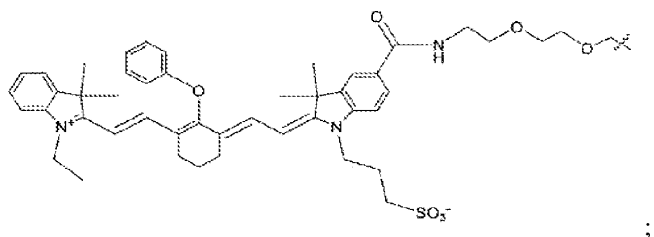
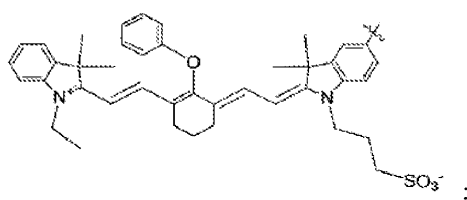
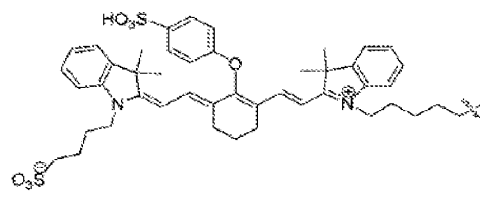
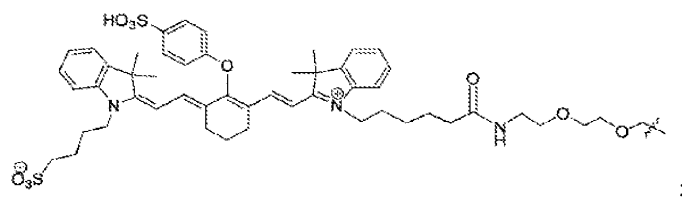
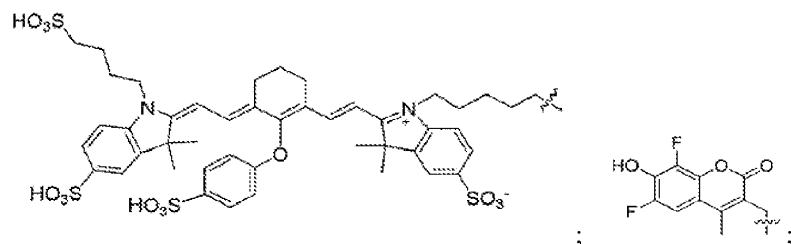


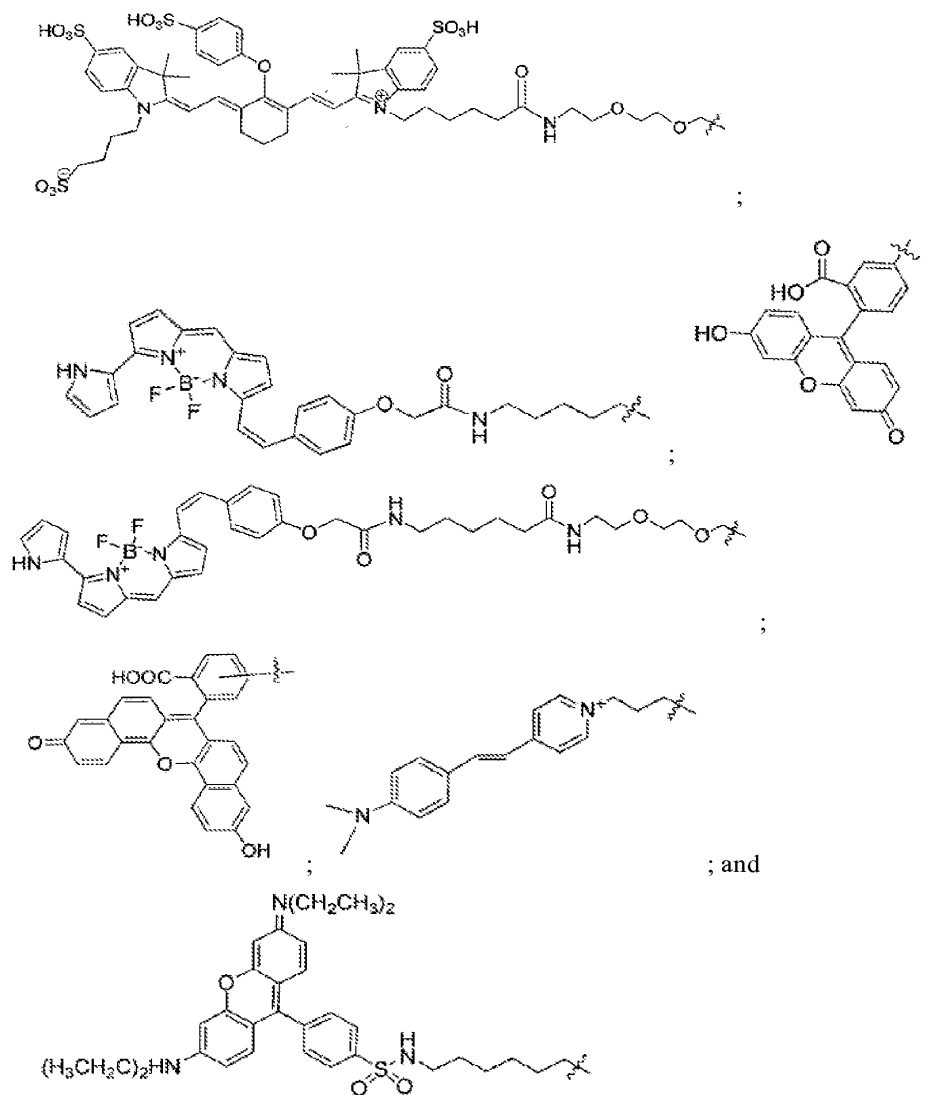
; each R_1 is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl or C_4 - C_{16}

alkylaryl; R_2 and R_3 are each independently H and CO_2R_4 , wherein R_5 is selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_4 , then the other is H; V is selected from the group consisting of $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{S})-$, and $-\text{OC}(\text{O})-$; A is selected from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can optionally comprise one or more radioactive isotope suitable for radiotherapy, and photosensitizing dye suitable for photodynamic therapy.

In further embodiments, the radiohalogen suitable for radiotherapy is selected from the group consisting of $^{80\text{m}}\text{Br}$, ^{77}Br , ^{125}I , ^{123}I , ^{131}I and ^{211}At . In yet other

embodiment, wherein the photosensitizing dye suitable for photodynamic therapy is selected from the group consisting of





In specific embodiments, the disease or condition is a prostate cancer, renal cancer, head cancer, neck cancer, head and neck cancer, lung cancer, breast cancer, prostate cancer, colorectal cancer, esophageal cancer, stomach cancer, leukemia/lymphoma, uterine cancer, skin cancer, endocrine cancer, urinary cancer, pancreatic cancer, gastrointestinal cancer, ovarian cancer, cervical cancer, adenomas, and tumor neovasculature. In more specific embodiments, the disease or condition is

prostate cancer. Accordingly, the presently disclosed compounds can be administered prophylactically to prevent or reduce the incidence or recurrence of the cancer or the tumor neovasculature.

A “cancer” in a subject refers to the presence of cells possessing characteristics typical of cancer-causing cells, for example, uncontrolled proliferation, loss of specialized functions, immortality, significant metastatic potential, significant increase in anti-apoptotic activity, rapid growth and proliferation rate, and certain characteristic morphology and cellular markers. In some circumstances, cancer cells will be in the form of a tumor; such cells may exist locally within a subject, or circulate in the blood stream as independent cells, for example, leukemic cells.

A cancer can include, but is not limited to, renal cancer, head cancer, neck cancer, head and neck cancer, lung cancer, breast cancer, prostate cancer, colorectal cancer, esophageal cancer, stomach cancer, leukemia/lymphoma, uterine cancer, skin cancer, endocrine cancer, urinary cancer, pancreatic cancer, gastrointestinal cancer, ovarian cancer, cervical cancer, and adenomas. In more specific embodiments, the disease or condition is prostate cancer. In some embodiments, a detectably effective amount of the therapeutic agent of the presently disclosed methods is administered to a subject.

D. Definitions

i. Chemical Definitions

While the following terms in relation to compounds of formula (I) are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter. These definitions are intended to supplement and illustrate, not preclude, the definitions that would be apparent to one of ordinary skill in the art upon review of the present disclosure.

The terms substituted, whether preceded by the term “optionally” or not, and substituent, as used herein, refer to the ability, as appreciated by one skilled in this art, to change one functional group for another functional group provided that the valency of all atoms is maintained. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. The substituents also may be further substituted (e.g., an aryl group substituent may have another

substituent off it, such as another aryl group, which is further substituted, for example, with fluorine at one or more positions).

Where substituent groups or linking groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-\text{CH}_2\text{O}-$ is equivalent to $-\text{OCH}_2-$; $-\text{C}(=\text{O})\text{O}-$ is equivalent to $-\text{OC}(=\text{O})-$; $-\text{OC}(=\text{O})\text{NR}-$ is equivalent to $-\text{NRC}(=\text{O})\text{O}-$, and the like.

As used herein, where an internal substituent is flanked by bonds (for example, $-\text{NRC}(\text{O})-$) the order of the atoms is fixed, the orientation of the group may not be reversed, and is inserted into a structure in the orientation presented. In other words $-\text{NRC}(\text{O})-$ is not the same as $-\text{C}(\text{O})\text{NR}-$. As used herein the term $\text{C}(\text{O})$ (for example $-\text{NRC}(\text{O})-$) is used to indicate a carbonyl ($\text{C}=\text{O}$) group, where the oxygen is bonded to the carbon by a double bond.

When the term “independently selected” is used, the substituents being referred to (e.g., R groups, such as groups R_1 , R_2 , and the like, or variables, such as “m” and “n”), can be identical or different. For example, both R_1 and R_2 can be substituted alkyls, or R_1 can be hydrogen and R_2 can be a substituted alkyl, and the like.

The terms “a,” “an,” or “a(n),” when used in reference to a group of substituents herein, mean at least one. For example, where a compound is substituted with “an” alkyl or aryl, the compound is optionally substituted with at least one alkyl and/or at least one aryl. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

A named “R” or group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative “R” groups as set forth above are defined below.

Descriptions of compounds of the present disclosure are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds

which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

The term hydrocarbon, as used herein, refers to any chemical group comprising hydrogen and carbon. The hydrocarbon may be substituted or unsubstituted. As would be known to one skilled in this art, all valencies must be satisfied in making any substitutions. The hydrocarbon may be unsaturated, saturated, branched, unbranched, cyclic, polycyclic, or heterocyclic. Illustrative hydrocarbons are further defined herein below and include, for example, methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, allyl, vinyl, n-butyl, tert-butyl, ethynyl, cyclohexyl, methoxy, diethylamino, and the like.

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched chain, acyclic or cyclic hydrocarbon group, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent groups, having the number of carbon atoms designated (i.e., C₁-C₁₀ means one to ten carbons). In particular embodiments, the term "alkyl" refers to C₁₋₂₀ inclusive, linear (i.e., "straight-chain"), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (i.e., alkenyl and alkynyl) hydrocarbon radicals derived from a hydrocarbon moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom.

Representative saturated hydrocarbon groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, sec-pentyl, iso-pentyl, neopentyl, n-hexyl, sec-hexyl, n-heptyl, n-octyl, n-decyl, n-undecyl, dodecyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, and homologs and isomers thereof.

"Branched" refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. "Lower alkyl" refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C₁₋₈ alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about

20 carbon atoms, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, "alkyl" refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, "alkyl" refers, in particular, to C₁₋₈ branched-chain alkyls.

In certain embodiments, alkyl groups are C₁-C₆ alkyl groups or C₁-C₄ alkyl groups. The term "C₁-C₆ alkyl" as used herein means straight-chain, branched, or cyclic C₁-C₆ hydrocarbons which are completely saturated and hybrids thereof, such as (cycloalkyl)alkyl. Examples of C₁-C₆ alkyl substituents include methyl (Me), ethyl (Et), propyl (including n-propyl (n-Pr, ⁿPr), iso-propyl (i-Pr, ⁱPr), and cyclopropyl (c-Pr, ⁰Pr)), butyl (including n-butyl (n-Bu, ⁿBu), iso-butyl (i-Bu, ⁱBu), sec-butyl (s-Bu, ^sBu), tert-butyl (t-Bu, ^tBu), or cyclobutyl (c-Bu, ⁰Bu)), and so forth.

Alkyl groups can optionally be substituted (a "substituted alkyl") with one or more alkyl group substituents, which can be the same or different. The term "alkyl group substituent" includes but is not limited to alkyl, substituted alkyl, halo, arylamino, acyl, hydroxyl, aryloxy, alkoxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as "alkylaminoalkyl"), or aryl.

Thus, as used herein, the term "substituted alkyl" includes alkyl groups, as defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon group, or combinations thereof, consisting of at least one carbon atoms and at least one heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen, phosphorus, and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -

CH=CH-O-CH₃, -Si(CH₃)₃, -CH₂-CH=N-OCH₃, -CH=CH-N(CH₃)-CH₃, -O-CH₃, -O-CH₂-CH₃, and -CN. Up to two or three heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃.

As described above, heteroalkyl groups, as used herein, include those groups
5 that are attached to the remainder of the molecule through a heteroatom, such as -C(O)R', -C(O)NR', -NR'R'', -OR', -SR, and/or -SO₂R'. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as -NR'R'' or the like, it will be understood that the terms heteroalkyl and -NR'R'' are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity.
10 Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as -NR'R'' or the like.

In the term "(cycloalkyl)alkyl", cycloalkyl, and alkyl are as defined above, and the point of attachment is on the alkyl group. This term encompasses, but is not limited to, cyclopropylmethyl, cyclopentylmethyl, and cyclohexylmethyl. The alkyl
15 group may be substituted or unsubstituted.

"Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined
20 herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, alkyl, substituted alkyl, aryl, or substituted aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and
25 cycloheptyl.

Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl, and fused ring systems, such as dihydro- and tetrahydronaphthalene, and the like.

The terms "cycloheteroalkyl" or "heterocycloalkyl" refer to a non-aromatic
30 ring system, unsaturated or partially unsaturated ring system, such as a 3- to 10-member substituted or unsubstituted cycloalkyl ring system, including one or more heteroatoms, which can be the same or different, and are selected from the group consisting of nitrogen (N), oxygen (O), sulfur (S), phosphorus (P), and silicon (Si),

and optionally can include one or more double bonds.

The cycloheteroalkyl ring can be optionally fused to or otherwise attached to other cycloheteroalkyl rings and/or non-aromatic hydrocarbon rings. Heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or a polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5-membered ring has 0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds, and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative cycloheteroalkyl ring systems include, but are not limited to pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, quinuclidinyl, morpholinyl, thiomorpholinyl, thiadiazinanyl, tetrahydrofuranyl, and the like.

The terms “cycloalkyl” and “heterocycloalkyl”, by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of “alkyl” and “heteroalkyl”, respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The terms “cycloalkylene” and “heterocycloalkylene” refer to the divalent derivatives of cycloalkyl and heterocycloalkyl, respectively.

The term “cycloalkylalkyl,” as used herein, refers to a cycloalkyl group as

defined hereinabove, which is attached to the parent molecular moiety through an alkyl group, also as defined above. Examples of cycloalkylalkyl groups include cyclopropylmethyl and cyclopentylethyl.

5 An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. Alkyl groups which are limited to hydrocarbon groups are termed "homoalkyl."

10 More particularly, the term "alkenyl" as used herein refers to a monovalent group derived from a C₁₋₂₀ inclusive straight or branched hydrocarbon moiety having at least one carbon-carbon double bond by the removal of a single hydrogen atom. Alkenyl groups include, for example, ethenyl (i.e., vinyl), propenyl, butenyl, 1-methyl-2-buten-1-yl, pentenyl, hexenyl, octenyl, and butadienyl.

15 The term "cycloalkenyl" as used herein refers to a cyclic hydrocarbon containing at least one carbon-carbon double bond. Examples of cycloalkenyl groups include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadiene, cyclohexenyl, 1,3-cyclohexadiene, cycloheptenyl, cycloheptatrienyl, and cyclooctenyl.

20 The term "alkynyl" as used herein refers to a monovalent group derived from a straight or branched C₁₋₂₀ hydrocarbon of a designed number of carbon atoms containing at least one carbon-carbon triple bond. Examples of "alkynyl" include ethynyl, 2-propynyl (propargyl), 1-propynyl, pentynyl, hexynyl, heptynyl, and allenyl groups, and the like.

25 The term "alkylene" by itself or a part of another substituent refers to a straight or branched bivalent aliphatic hydrocarbon group derived from an alkyl group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or
30 unsubstituted nitrogen atoms (also referred to herein as "alkylaminoalkyl"), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene (—CH₂—); ethylene (—CH₂—CH₂—); propylene (—(CH₂)₃—); cyclohexylene (—C₆H₁₀—); —CH=CH—CH=CH—; —CH=CH—CH₂—; —CH₂CH₂CH₂CH₂—,

-CH₂CH=CHCH₂-, -CH₂CsCCH₂-, -CH₂CH₂CH(CH₂CH₂CH₃)CH₂-, -(CH₂)_q-N(R)-(CH₂)_r-, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxy (-O-CH₂-O-); and ethylenedioxy (-O-(CH₂)₂-O-). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being some embodiments of the present disclosure. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

10 The term “heteroalkylene” by itself or as part of another substituent means a divalent group derived from heteroalkyl, as exemplified, but not limited by, -CH₂-CH₂-S-CH₂-CH₂- and -CH₂-S-CH₂-CH₂-NH-CH₂-. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxo, alkylenedioxo, alkyleneamino, alkylenediamino, and the like). Still further, for
15 alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula -C(O)OR’- represents both -C(O)OR’- and -R’OC(O)-.

20 The term “aryl” means, unless otherwise stated, an aromatic hydrocarbon substituent that can be a single ring or multiple rings (such as from 1 to 3 rings), which are fused together or linked covalently.

25 The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms (in each separate ring in the case of multiple rings) selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of
30 aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl,
2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, indazolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents

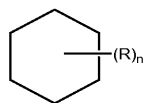
described below. The terms “arylene” and “heteroarylene” refer to the divalent forms of aryl and heteroaryl, respectively.

For brevity, the term “aryl” when used in combination with other terms (e.g., aryloxo, arylthioxo, arylalkyl) includes both aryl and heteroaryl rings as defined
5 above. Thus, the terms “arylalkyl” and “heteroarylalkyl” are meant to include those groups in which an aryl or heteroaryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl, furylmethyl, and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxyethyl, 2-pyridyloxyethyl, 3-(1-naphthyloxy)propyl, and
10 the like). The term “haloaryl,” however, as used herein, is meant to cover only aryls substituted with one or more halogens.

Where a heteroalkyl, heterocycloalkyl, or heteroaryl includes a specific number of members (e.g. “3 to 7 membered”), the term “member” refers to a carbon or heteroatom.

15 As used herein, the term “alkylaryl” includes alkyl groups, as defined above, substituted by aryl groups, as defined above. The aryl group may be connected at any point on the alkyl group. The term C₄-C₁₆ alkylaryl includes alkylaryl groups having a total of 4 to 16 carbon atoms, counting the carbon atoms on the alkyl group and aryl group together. Examples of alkylaryl groups include but are not limited to benzyl
20 (phenylmethyl), phenyl ethyl, and naphthylmethyl. The alkylaryl group may be substituted or unsubstituted. Substituents are not counted towards the total number of atoms in the alkylaryl group, so long as the total atoms in the substituent(s) are not larger than the alkylaryl group.

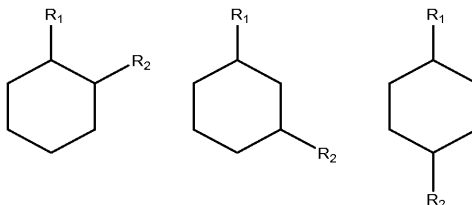
Further, a structure represented generally by the formula:



25 as used herein refers to a ring structure, for example, but not limited to a 3-carbon, a 4-carbon, a 5-carbon, a 6-carbon, a 7-carbon, and the like, aliphatic and/or aromatic cyclic compound, including a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure, comprising a substituent R group, wherein
30 the R group can be present or absent, and when present, one or more R groups can each be substituted on one or more available carbon atoms of the ring structure. The

presence or absence of the R group and number of R groups is determined by the value of the variable “n,” which is an integer generally having a value ranging from 0 to the number of carbon atoms on the ring available for substitution. Each R group, if more than one, is substituted on an available carbon of the ring structure rather than on another R group. For example, the structure above where n is 0 to 2 would

5 comprise compound groups including, but not limited to:



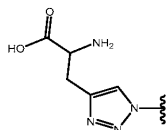
and the like.

A dashed line representing a bond in a cyclic ring structure indicates that the bond can be either present or absent in the ring. That is, a dashed line representing a bond in a cyclic ring structure indicates that the ring structure is selected from the group consisting of a saturated ring structure, a partially saturated ring structure, and an unsaturated ring structure.

10

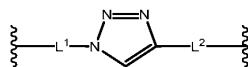
A substituent bearing a broken bond, such as the example shown below, means that the substituent is directly bonded to the molecule at the indicated position. No additional methylene (CH₂) groups are implied. The symbol () denotes the point of attachment of a moiety to the remainder of the molecule.

15



Substituents bearing two broken bonds, such as the example shown below, means that the orientation of the atoms is as-indicated, left to right and should be inserted into a molecule in the orientation shown. No additional methylene (CH₂) groups are implied unless specifically indicated.

20



When a named atom of an aromatic ring or a heterocyclic aromatic ring is defined as being “absent,” the named atom is replaced by a direct bond.

25

Each of above terms (e.g. , “alkyl,” “heteroalkyl,” “cycloalkyl, and

“heterocycloalkyl,” “aryl,” “heteroaryl,” “phosphonate,” and “sulfonate” as well as their divalent derivatives) are meant to include both substituted and unsubstituted forms of the indicated group. Optional substituents for each type of group are provided below.

- 5 Substituents for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl monovalent and divalent derivative groups (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR'', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such groups. R', R'', R''' and R'''' each may independently refer to hydrogen, substituted or unsubstituted
- 10 heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. As used herein, an “alkoxy” group is an alkyl attached to the remainder of the molecule through a divalent oxygen. When a compound of the disclosure includes
- 15 more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7- membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1- pyrrolidiny and 4-
- 20 morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term “alkyl” is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

- 25 Similar to the substituents described for alkyl groups above, exemplary substituents for aryl and heteroaryl groups (as well as their divalent derivatives) are varied and are selected from, for example: halogen, -OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -C(O)NR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)OR', -NR-C(NR'R'')=NR'', -NR-
- 30

C(NR'R'')=NR'''-S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxo, and fluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on aromatic ring system; and where R', R'', R''' and R''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the disclosure includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R''' groups when more than one of these groups is present.

Two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally form a ring of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 4.

One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -(CRR')_s-X'-(C''R''')_d-, where s and d are independently integers of from 0 to 3, and X' is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'' and R''' may be independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent and has the general formula RC(=O)-, wherein R is an alkyl, alkenyl, alkynyl, aryl, carbocyclic, heterocyclic, or aromatic heterocyclic group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as an acetylfuran and a phenacyl group. Specific examples of acyl groups include acetyl and benzoyl.

The terms "alkoxyl" or "alkoxy" are used interchangeably herein and refer to a

saturated (i.e., alkyl-O-) or unsaturated (i.e., alkenyl-O- and alkynyl-O-) group attached to the parent molecular moiety through an oxygen atom, wherein the terms “alkyl,” “alkenyl,” and “alkynyl” are as previously described and can include C₁₋₂₀ inclusive, linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, n-butoxyl, sec-butoxyl, t-butoxyl, and n-pentoxyl, neopentoxyl, n-hexoxyl, and the like.

The term “alkoxyalkyl” as used herein refers to an alkyl-O-alkyl ether, for example, a methoxyethyl or an ethoxymethyl group.

“Aryloxyl” refers to an aryl-O- group wherein the aryl group is as previously described, including a substituted aryl. The term “aryloxyl” as used herein can refer to phenyloxyl or hexyloxyl, and alkyl, substituted alkyl, halo, or alkoxy substituted phenyloxyl or hexyloxyl.

“Aralkyl” refers to an aryl-alkyl-group wherein aryl and alkyl are as previously described, and included substituted aryl and substituted alkyl. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

“Aralkyloxyl” refers to an aralkyl-O- group wherein the aralkyl group is as previously described. An exemplary aralkyloxyl group is benzyloxyl.

“Alkoxy carbonyl” refers to an alkyl-O-CO- group. Exemplary alkoxy carbonyl groups include methoxycarbonyl, ethoxycarbonyl, butyloxycarbonyl, and t-butyloxycarbonyl.

“Aryloxy carbonyl” refers to an aryl-O-CO- group. Exemplary aryloxy carbonyl groups include phenoxy- and naphthoxy-carbonyl.

“Aralkoxy carbonyl” refers to an aralkyl-O-CO- group. An exemplary aralkoxy carbonyl group is benzyloxy carbonyl.

“Carbamoyl” refers to an amide group of the formula -CONH₂.

“Alkylcarbamoyl” refers to a R’RN-CO- group wherein one of R and R’ is hydrogen and the other of R and R’ is alkyl and/or substituted alkyl as previously described.

“Dialkylcarbamoyl” refers to a R’RN-CO- group wherein each of R and R’ is independently alkyl and/or substituted alkyl as previously described.

The term carbonyldioxy, as used herein, refers to a carbonate group of the formula -O-CO-OR.

“Acyloxyl” refers to an acyl-O- group wherein acyl is as previously described.

The term “amino” refers to the -NH₂ group and also refers to a nitrogen

containing group as is known in the art derived from ammonia by the replacement of one or more hydrogen radicals by organic radicals. For example, the terms “acylamino” and “alkylamino” refer to specific N-substituted organic radicals with acyl and alkyl substituent groups respectively.

5 An “aminoalkyl” as used herein refers to an amino group covalently bound to an alkylene linker. More particularly, the terms alkylamino, dialkylamino, and trialkylamino as used herein refer to one, two, or three, respectively, alkyl groups, as previously defined, attached to the parent molecular moiety through a nitrogen atom. The term alkylamino refers to a group having the structure -NHR' wherein R' is an
10 alkyl group, as previously defined; whereas the term dialkylamino refers to a group having the structure -NR'R'' , wherein R' and R'' are each independently selected from the group consisting of alkyl groups. The term trialkylamino refers to a group having the structure -NR'R''R''' , wherein R' , R'' , and R''' are each independently selected from the group consisting of alkyl groups. Additionally, R' , R'' , and/or R'''
15 taken together may optionally be $\text{-(CH}_2\text{)}_k\text{-}$ where k is an integer from 2 to 6. Examples include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, iso-propylamino, piperidino, trimethylamino, and propylamino.

 The amino group is -NR'R'' , wherein R' and R'' are typically selected from
20 hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

 The terms alkylthioether and thioalkoxyl refer to a saturated (i.e., alkyl-S-) or unsaturated (i.e., alkenyl-S- and alkynyl-S-) group attached to the parent molecular
25 moiety through a sulfur atom. Examples of thioalkoxyl moieties include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

 “Acylamino” refers to an acyl-NH- group wherein acyl is as previously described. “Aroylamino” refers to an aroyl-NH- group wherein aroyl is as previously described.

30 The term “carbonyl” refers to the -(C=O)- group.

 The term “carboxyl” refers to the -COOH group. Such groups also are referred to herein as a “carboxylic acid” moiety.

 The terms “halo,” “halide,” or “halogen” as used herein refer to fluoro, chloro,

bromo, and iodo groups. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C₁-C₄)alkyl” is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

5 The term “hydroxyl” refers to the –OH group.

 The term “hydroxyalkyl” refers to an alkyl group substituted with an –OH group.

 The term “mercapto” refers to the –SH group.

10 The term “oxo” as used herein means an oxygen atom that is double bonded to a carbon atom or to another element.

 The term “nitro” refers to the –NO₂ group.

 The term “thio” refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

 The term “sulfate” refers to the –SO₄ group.

15 The term thiohydroxyl or thiol, as used herein, refers to a group of the formula –SH.

 The term ureido refers to a urea group of the formula –NH—CO—NH₂.

20 Unless otherwise explicitly defined, a “substituent group,” as used herein, includes a functional group selected from one or more of the following moieties, which are defined herein:

 (A) –OH, –NH₂, –SH, –CN, –CF₃, –NO₂, oxo, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

25 (B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl, substituted with at least one substituent selected from:

 (i) oxo, –OH, –NH₂, –SH, –CN, –CF₃, –NO₂, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

30 (ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl, substituted with at least one substituent selected from:

 (a) oxo, –OH, –NH₂, –SH, –CN, –CF₃, –NO₂, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, substituted with at least one substituent selected from oxo, -OH, -NH₂, -SH, -CN, -CF₃, -NO₂, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, and unsubstituted heteroaryl.

A “lower substituent” or “lower substituent group,” as used herein means a group selected from all of the substituents described hereinabove for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₈ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₅-C₇ cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 5 to 7 membered heterocycloalkyl.

A “size-limited substituent” or “size-limited substituent group,” as used herein means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C₁-C₂₀ alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C₄-C₈ cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 4 to 8 membered heterocycloalkyl.

Throughout the specification and claims, a given chemical formula or name shall encompass all tautomers, congeners, and optical- and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure. The term “tautomer,” as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the

disclosure.

Certain compounds of the present disclosure possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present disclosure. The compounds of the present disclosure do not include those which are known in art to be too unstable to synthesize and/or isolate. The present disclosure is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms (racemates), by asymmetric synthesis, or by synthesis from optically active starting materials. Resolution of the racemates can be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column. Many geometric isomers of olefins, C=N double bonds, and the like also can be present in the compounds described herein, and all such stable isomers are contemplated in the presently disclosed subject matter. Cis and trans geometric isomers of the compounds of the presently disclosed subject matter are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral (enantiomeric and diastereomeric), and racemic forms, as well as all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

The compounds herein described may have one or more charged atoms. For example, the compounds may be zwitterionic, but may be neutral overall. Other embodiments may have one or more charged groups, depending on the pH and other factors. In these embodiments, the compound may be associated with a suitable counter-ion. It is well known in the art how to prepare salts or exchange counter-ions. Generally, such salts can be prepared by reacting free acid forms of these compounds

with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two.

- 5 Counter-ions may be changed, for example, by ion-exchange techniques such as ion-exchange chromatography. All zwitterions, salts and counter-ions are intended, unless the counter-ion or salt is specifically indicated. In certain embodiments, the salt or counter-ion may be pharmaceutically acceptable, for administration to a subject. Pharmaceutically acceptable salts are discussed later.

- 10 As used herein, a "protecting group" is a chemical substituent which can be selectively removed by readily available reagents which do not attack the regenerated functional group or other functional groups in the molecule. Suitable protecting groups are known in the art and continue to be developed. Suitable protecting groups may be found, for example in Wutz et al. ("Greene's Protective Groups in Organic
15 Synthesis, Fourth Edition," Wiley-Interscience, 2007). Protecting groups for protection of the carboxyl group, as described by Wutz et al. (pages 533-643), are used in certain embodiments. In some embodiments, the protecting group is removable by treatment with acid. Specific examples of protecting groups include but are not limited to, benzyl, p-methoxybenzyl (PMB), tertiary butyl (^tBu),
20 methoxymethyl (MOM), methoxyethoxymethyl (MEM), methylthiomethyl (MTM), tetrahydropyranyl (THP), tetrahydrofuranyl (THF), benzyloxymethyl (BOM), trimethylsilyl (TMS), triethylsilyl (TES), t-butyldimethylsilyl (TBDMS), and triphenylmethyl (trityl, Tr). Persons skilled in the art will recognize appropriate situations in which protecting groups are required and will be able to select an
25 appropriate protecting group for use in a particular circumstance.

- Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon
30 by ¹³C- or ¹⁴C-enriched carbon are within the scope of this disclosure.

The compounds of the present disclosure may also contain unnatural proportions of atomic isotopes at one or more of atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive

isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present disclosure, whether radioactive or not, are encompassed within the scope of the present disclosure.

ii. *Pharmaceutical Salts*

5 The compounds of the present disclosure may exist as pharmaceutically acceptable salts. The term “pharmaceutically acceptable salts” is meant to include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituent moieties found on the compounds described herein. Pharmaceutically acceptable salts are generally well known to those of
10 ordinary skill in the art, and may include, by way of example but not limitation, acetate, benzenesulfonate, besylate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate,
15 lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrates, (e.g. (+)-tartrates, (-)-tartrates or mixtures thereof including racemic mixtures), or teoclate. These salts may be prepared by methods known to those skilled in art. Other pharmaceutically
20 acceptable salts may be found in, for example, Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams & Wilkins (2000).

Also included are base addition salts such as sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can
25 be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric,
30 hydriodic, or phosphorous acids and the like, as well as the salts derived organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like.

Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like, see, for example, Berge et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

Certain compounds of the present disclosure can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present disclosure. Certain compounds of the present disclosure may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present disclosure and are intended to be within the scope of the present disclosure.

In addition to salt forms, the present disclosure provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present disclosure. Additionally, prodrugs can be converted to the compounds of the present disclosure by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present disclosure when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

iii. Pharmaceutical Compositions

The compounds disclosed herein can be formulated into various compositions, for use in diagnostic and imaging methods. Generally, a pharmaceutical composition comprises an effective amount (e.g., a or detectably effective amount) of a compound described hereinabove.

A presently disclosed composition can be formulated as a pharmaceutical composition, which comprises a presently disclosed compound and pharmaceutically acceptable carrier. By a "pharmaceutically acceptable carrier" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a

subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. For a discussion of pharmaceutically acceptable carriers and other components of pharmaceutical compositions, see, e.g., Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, 1990. Some suitable pharmaceutical carriers will be evident to a skilled worker and include, e.g., water (including sterile and/or deionized water), suitable buffers (such as PBS), physiological saline, cell culture medium (such as DMEM), artificial cerebral spinal fluid, or the like.

One skilled in the art will appreciate that the particular formulation will depend, in part, upon the particular agent that is employed, and the chosen route of administration. Accordingly, there is a wide variety of suitable formulations of compositions of the presently disclosed subject matter.

One skilled in the art will appreciate that a suitable or appropriate formulation can be selected, adapted or developed based upon the particular application at hand. Dosages for presently disclosed compositions can be in unit dosage form. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for animal (e.g. human) subjects, each unit containing a predetermined quantity of a presently disclosed agent, alone or in combination with other therapeutic agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle.

One skilled in the art can easily determine the appropriate dose, schedule, and method of administration for the exact formulation of the composition being used, in order to achieve the desired effective amount or effective concentration of the agent in the individual patient.

The dose of a presently disclosed composition, administered to an animal, particularly a human, in the context of the presently disclosed subject matter should be sufficient to produce at least a detectable amount of a diagnostic response in the individual over a reasonable time frame. The dose used to achieve a desired effect will be determined by a variety of factors, including the potency of the particular agent being administered, the pharmacodynamics associated with the agent in the

host, the severity of the disease state of infected individuals, other medications being administered to the subject, and the like. The size of the dose also will be determined by the existence of any adverse side effects that may accompany the particular agent, or composition thereof, employed. It is generally desirable, whenever possible, to keep adverse side effects to a minimum. The dose of the biologically active material will vary; suitable amounts for each particular agent will be evident to a skilled worker.

A "pharmaceutically acceptable carrier" refers to a biocompatible solution, having due regard to sterility, p[Eta], isotonicity, stability, and the like and can include any and all solvents, diluents (including sterile saline, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other aqueous buffer solutions), dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, and the like. The pharmaceutically acceptable carrier also can contain stabilizers, preservatives, antioxidants, or other additives, which are well known to one of skill in the art, or other vehicles as known in the art.

iv. General Definitions

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation. Particular definitions are provided herein for clarity. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs.

A "cancer" in an animal refers to the presence of cells possessing characteristics typical of cancer-causing cells, for example, uncontrolled proliferation, loss of specialized functions, immortality, significant metastatic potential, significant increase in anti-apoptotic activity, rapid growth and proliferation rate, and certain characteristic morphology and cellular markers. In some circumstances, cancer cells will be in the form of a tumor; such cells may exist locally within an animal, or circulate in the blood stream as independent cells.

By "control" is meant a standard or reference condition.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, organ, organism, or subject.

The term "administering" as used herein refers to contacting a subject with a presently disclosed agent.

By "delivery device" is meant any device that provides for the release of an imaging agent. Exemplary delivery devices include tablets and pills, described below,
5 as well as syringes, osmotic pumps, indwelling catheters, delayed-release and sustained-release biomaterials.

Following long-standing patent law convention, the terms "a," "an," and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a subject" includes a plurality of subjects, unless the context
10 clearly is to the contrary (e.g., a plurality of subjects), and so forth.

Throughout this specification and the claims, the terms "comprise," "comprises," and "comprising" are used in a non-exclusive sense, except where the context requires otherwise. Likewise, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the
15 exclusion of other like items that can be substituted or added to the listed items.

For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes, dimensions, proportions, shapes, formulations, parameters, percentages, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in
20 all instances by the term "about" even though the term "about" may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off,
25 measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be obtained by the presently disclosed subject matter. For example, the term "about," when referring to a value can be meant to encompass variations of, in some embodiments, $\pm 100\%$ in some
embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in
30 some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, the term "about" when used in connection with one or more numbers

or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

EXAMPLE 1

Synthesis and Evaluation of Carbamate-Based Agents for Imaging of PSMA and Cancer Radiotherapy

Material and Methods

General Procedures. Solvents and chemicals purchased from commercial sources were of analytical grade or better and used without further purification. Tert-butyl-1,3-diisopropylisourea, L-glutamic acid di-tert-butyl ester, N^ε-Boc-lysine-tert-butyl ester hydrochloride, 1-hydroxybenzotriazole monohydrate and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Chem Impex International Inc. (Wooddale, IL). 1,1'-Carbonyldiimidazole, iodomethane, N-hydroxysuccinimide, (diacetoxyiodo)benzene, triethylsilane (Et₃SiH), diisopropylethylamine (DIEA) and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO). 4-bromo-2-nitro-benzaldehyde was purchased from Combi-Block (San Diego, CA). (S)-6-((tert-butoxycarbonyl)amino)-

2-hydroxyhexanoic acid (Makarsen, 2009) 4-iodo-2-nitro-benzaldehyde (Litosh, 2009) and (S)-dimethyl 2-hydroxypentanedioate (Winkler, 2013) were synthesized by previously reported procedures. Analytical thin-layer chromatography (TLC) was performed using Aldrich aluminum-backed 0.2 mm silica gel Z19, 329-1 plates and visualized by ultraviolet light (254 nm), I₂ and 1 % ninhydrin in EtOH. Flash chromatography was performed using silica gel (MP SiliTech 32-63 D 60Å) purchased from Bodman (Aston, PA). All in vitro PSMA binding studies were performed in triplicate. ¹H NMR spectra were recorded on a Bruker Ultrashield™ 400 MHz or 500 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield by reference to proton resonances resulting from incomplete deuteration of the NMR solvent. Low resolution ESI mass spectra were obtained on a Bruker Daltonics Esquire 3000 Plus spectrometer. High resolution mass spectra were obtained at the University of Notre Dame Mass Spectrometry & Proteomics Facility, Notre Dame, IN, using ESI either by direct infusion on a Bruker microTOF-II or by LC elution via an ultra-high pressure Dionex RSLC with C18 column coupled to a Bruker microTOF-Q II. HPLC purification was performed using a Phenomenex C18 Luna 10 × 250 mm² column on Agilent Technologies 1260 Infinity Preparative HPLC System. HPLC purification of [¹⁸F] labeled compounds were performed on a Varian Prostar System (Palo Alto, CA), equipped with a Varian ProStar 325 UV-Vis variable wavelength detector and a Bioscan Flow-count in-line Radioactivity detector, all controlled by Galaxie software. The specific radioactivity was calculated as the ratio of the radioactivity eluting at the retention time of product during the preparative HPLC purification to the mass corresponding to the area under the curve of the UV absorption.

25 *Synthesis and characterization of Carbamates.*

(S)-6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoic acid 14. (S)-6-amino-2-hydroxyhexanoic acid 200 mg (1.36 mmol) and K₂CO₃ 550 mg (4 mmol) were dissolved in 10 mL of water. Di-tert-butyl dicarbonate (300 mg, 1.36 mmol) was added to the solution. The reaction was stirred at room temperature overnight. After acidified to pH 2 by concentrated HCl, the aqueous layer was extracted by methylene chloride and the organic layer was dried with sodium sulfate. 250 mg (S)-6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoic acid was obtained, after the organic solvent was removed under vacuum. The yield was 74 %.

(S)-tert-butyl 6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoate **15**. (S)-6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoic acid **14** (250 mg, 1.00 mmol) was mixed with tert-butyl-1,3-diisopropylisourea (600 mg, 3.00 mmol) in 2 mL anhydrous methylene chloride. The reaction was sealed and kept at room temperature for 36 h.

5 135 mg (S)-tert-butyl 6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoate was obtained after flash column chromatography and the yield was 44 %. (¹H NMR (400 MHz, CDCl₃): δ 4.56(br, 1H), 4.06(dd, *J*₁ = 7.2 Hz, *J*₂ = 4.4 Hz, 1H), 3.14(m, 2H), 2.25(br, 1H), 1.83-1.75(m, 1H), 1.65-1.35(m, 23H); ¹³C NMR (125 MHz, CDCl₃): δ 174.6, 156.1, 82.5, 79.2, 70.4, 40.6, 34.0, 29.8, 28.5, 28.1, 22.0. MS: Calculated for [C₁₅H₃₀NO]⁺, 304.2118 [M+H]⁺; Found 304.2147.

(S)-1-(tert-butoxy)-6-((tert-butoxycarbonyl)amino)-1-oxohexan-2-yl 1H-imidazole-1-carboxylate **16**. (S)-tert-butyl 6-((tert-butoxycarbonyl)amino)-2-hydroxyhexanoate **15** (131 mg, 0.43 mmol) and 1,1'-carbonyldiimidazole (105 mg, 0.65 mmol) were mixed in 5 mL anhydrous methylene chloride. The reaction was

15 kept at room temperature for 1 h. 113 mg (S)-1-(tert-butoxy)-6-((tert-butoxycarbonyl)amino)-1-oxohexan-2-yl 1H-imidazole-1-carboxylate was obtained by flash column chromatography and the yield was 81 %. (¹H NMR (400 MHz, CDCl₃): δ 8.19(s, 1H), 7.50(s, 1H), 7.18(s, 1H), 5.14(m, 1H), 4.61(br, 1H), 3.21-3.16(m, 2H), 2.05-1.96(m, 2H), 1.60-1.42(m, 22H); ¹³C NMR (125 MHz, CDCl₃): δ 167.8, 156.0, 148.3, 137.2, 130.9, 117.2, 83.3, 79.3, 75.8, 40.2, 30.6, 29.7, 28.4, 28.0, 22.4. MS: Calculated for [C₁₉H₃₂N₃O₆]⁺, 398.2286 [M+H]⁺; Found 398.2309.

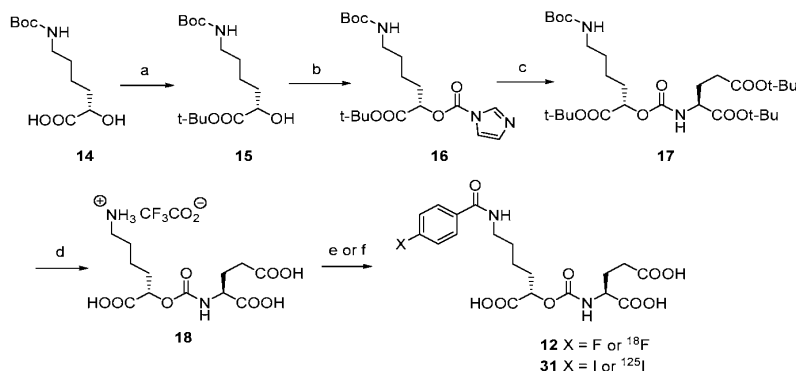
(10S,14S)-tri-tert-butyl 2,2-dimethyl-4,12-dioxo-3,11-dioxa-5,13-diazahexadecane-10,14,16-tricarboxylate **17**. (S)-1-(tert-butoxy)-6-((tert-butoxycarbonyl)amino)-1-oxohexan-2-yl 1H-imidazole-1-carboxylate **16** (139 mg, 0.35 mmol) and L-glutamic acid di-tert-butyl ester (170 mg, 0.66 mmol) were mixed neat. The reaction was kept at 45 °C for 2 days. (10S,14S)-tri-tert-butyl 2,2-dimethyl-4,12-dioxo-3,11-dioxa-5,13-diazahexadecane-10,14,16-tricarboxylate was obtained 210 mg by flash column chromatography and the yield was 100 %. ¹H NMR (400 MHz, CDCl₃): δ 5.5(br, 1H), 4.88-4.70(m, 1H), 4.59(br, 1H), 4.32-4.23(m, 1H),

25 3.16-3.08(m, 2H), 2.45-2.28(m, 2H), 2.25-2.10(m, 1H), 1.95-1.76(m, 3H), 1.60-1.42(m, 22H). ¹³C NMR (125 MHz, CDCl₃): δ 172.1, 171.1, 169.9, 156.0, 155.4, 82.4, 81.9, 80.6, 79.1, 73.1, 53.8, 40.4, 31.4, 30.9, 29.6, 28.4, 28.1, 28.0, 22.4. MS: Calculated for [C₂₉H₅₂N₂NaO₁₀]⁺, 611.3514 [M+Na]⁺; Found 611.3502.

(S)-2-((((S)-5-amino-1-carboxypentyl)oxy)carbonyl)amino) pentanedioic acid trifluoroacetic acid salt, 18: (10*S*,14*S*)-tri-tert-butyl 2,2-dimethyl-4,12-dioxo-3,11-dioxa-5,13-diazahexadecane-10,14,16-tricarboxylate (**17**) (237 mg) was treated with a 6 mL solution of TFA/methylene chloride(1/1) for 2 h. The final product was
 5 purified by HPLC giving 90 mg, yield 70 %. ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 4.80(m, 1H), 4.20-4.11(m, 1H, two isomers), 2.90(t, J = 6.8 Hz, 2H), 2.43(t, J = 6.8 Hz, 2H), 2.16-2.09(m, 1H), 1.91-1.83(m, 3H), 1.61-1.58(m, 2H), 1.43-1.37(m, 2H). ¹³C NMR (125 MHz, D₂O/CD₃CN 1:1) δ 177.1, 175.7/175.5 (two isomers), 163.1/162.8(two isomers), 157.3, 73.4, 53.4, 39.2, 36.5, 30.0, 26.2, 26.0, 21.4. MS: Calculated for [C₁₂H₂₁N₂O₈]⁺, 321.1292 [M+H]⁺; Found 321.1310. HPLC (10 × 250
 10 mm Phenomenix Luna C18 column, mobile phase 100/0/0.1 % water/acetonitrile/TFA, flow 10 mL/min). **18** eluted at 14.5 min.

(S)-2-((((S)-1-carboxy-5-(4-fluorobenzamido)pentyl)oxy)carbonyl)amino)pentanedioic acid 12 (XY-20). As outlined in scheme 1 (S)-2-
 15 (((((S)-5-amino-1-carboxypentyl)oxy)carbonyl)amino) pentanedioic acid TFA salt **18** (10 mg, 0.024 mmol) was dissolved in 1 mL DMSO and 20 μL triethylamine. To the solution, N-succinimidyl 4-fluorobenzoate (11.5 mg, 0.045 mmol) was added. The resulting solution was kept at room temperature for 2 hours. After the solvent was removed under vacuum and HPLC purification, **12 (XY-20)** (8 mg) was obtained.
 20 Yield is 75 %. HPLC (10 × 250 mm Phenomenix Luna C18 column, mobile phase 75/25/0.1% water/acetonitrile/TFA, flow 4 mL/min). **12 (XY-20)** eluted at 12 min. ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 8.11(m, 2H), 7.51(t, J = 8.8 Hz, 2H), 5.16(m, 1H), 4.52-4.45(m, 1H, two rotamers), 3.64(t, J = 7.2 Hz, 2H), 2.74(t, J = 7.2 Hz, 2H), 2.46-2.41(m, 1H), 2.22-2.12(m, 3H), 1.91-1.80(m, 2H), 1.77-1.68(m, 2H).
 25 ¹³C NMR (125 MHz, D₂O/CD₃CN 1:1): δ 176.3, 174.8, 174.2, 168.4, 166.0/164.0 (coupled with F), 156.9, 130.9, 130.2/130.1(coupled with F), 116.0/115.9(coupled with F), 73.2, 53.6, 39.9, 30.9, 30.3, 28.7, 26.7, 22.5. MS: Calculated for [C₁₉H₂₃FN₂NaO₉]⁺, 465.1280 [M+Na]⁺; Found 465.1295.

30 Scheme 1.



(a) t-butyl-1,3-diisopropylisourea, CH₂Cl₂, rt, 3d, (b) carbonyldiimidazole, CH₂Cl₂, rt, 1h, (c) L-glutamic acid di-tert-butyl ester, neat, 45°C, 2d, (d) TFA/CH₂Cl₂(1/1), rt, 2 h, (e) N-succinimidyl-4-fluorobenzoate or N-succinimidyl-4-[¹⁸F]fluorobenzoate, Et₃N, DMSO, rt, 2h; (f) N-succinimidyl-4-iodobenzoate or N-succinimidyl-4-[¹²⁵I]iodobenzoate, diisopropylethyl amine, DMSO rt 1h.

General procedure for the PSMA binding carbamates. The key intermediate **18** was treated with 1.5 eq of carboxylic acid NHS ester and 6 eq of triethylamine in DMSO for 2 h at room temperature. The final product was purified by HPLC.

NMR and MS for carbamates (structures and PSMA binding are given in Example 3).

XY-10: ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 4.81(dd, *J*₁ = 9.2 Hz, *J*₂ = 8.0 Hz, 1H), 4.21-4.11(m, 1H, two rotamers), 2.42(t, *J* = 7.6 Hz, 2H), 2.15-2.06(m, 1H), 1.94-1.82(m, 1H), 1.72-1.51(m, 3H), 0.83-0.80(m, 6H). MS: 306 (M+H⁺).

XY-16: ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 8.20(m, 2H), 7.58(m, 2H), 5.32-5.28(m, 1H), 4.65-2.52(m, 1H, two rotamers), 3.92-3.75(m, 2H), 2.82(t, *J* = 7.6 Hz, 2H), 2.58-2.28(m, 4H). MS: 415 (M+H⁺).

XY-17: ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 8.23(dd, *J*₁ = 8.8 Hz, *J*₂ = 5.6 Hz, 2H), 7.63(t, *J* = 8.8 Hz, 2H), 5.34(dd, *J*₁ = 8.8 Hz, *J*₂ = 4.0 Hz, 1H), 4.65-2.56(m, 1H, two rotamers), 3.90(t, *J* = 6.4 Hz, 2H), 2.82(t, *J* = 7.2 Hz, 2H), 2.58-2.28(m, 4H). MS: 415 (M+H⁺).

XY-21: ¹H NMR (400 MHz, D₂O/CD₃CN 1:1): δ 8.84(s, 1H), 8.55-8.32(m, 4H), 8.15-8.04(m, 2H), 5.36(m, 1H), 4.52-4.45(m, 1H, two rotamers), 3.90(t, *J* = 7.2 Hz, 2H), 2.94(t, *J* = 7.2 Hz, 2H), 2.66-2.51(m, 1H), 2.42-2.32(m, 3H), 2.19-2.10(m, 2H), 2.07-1.98(m, 2H). MS: 475 (M+H⁺).

XY-22: ^1H NMR (400 MHz, $\text{D}_2\text{O}/\text{CD}_3\text{CN}$ 1:1): δ 8.77(s, 1H), 8.41-8.29(m, 3H), 7.82(s, 1H), 7.71(d, $J = 8.8$ Hz, 1H), 5.36(m, 1H), 4.65-4.63(m, 1H, two rotamers), 4.41(s, 3H), 3.88(t, $J = 7.2$ Hz, 2H), 2.90(t, $J = 7.2$ Hz, 2H), 2.63-2.58(m, 1H), 2.40-2.32(m, 3H), 2.15-2.12(m, 2H), 2.03-2.00(m, 2H). MS: 505 ($\text{M} + \text{H}^+$).

5 XY-23: MS: 531 ($\text{M} + \text{H}^+$).

XY-24: ^1H NMR (400 MHz, $\text{D}_2\text{O}/\text{CD}_3\text{CN}$ 1:1): δ 8.27(d, $J = 8.4$ Hz, 2H), 8.15-8.09(m, 3H), 7.51(d, $J = 6.0$ Hz, 1H), 5.29-5.14(m, 3H), 4.77-4.74(m, 1H), 4.58-4.50(m, 1H), 3.80(t, $J = 7.2$ Hz, 2H), 2.86(t, $J = 7.2$ Hz, 2H), 2.45-2.41(m, 1H), 2.33-2.29(m, 3H), 2.06-2.00(m, 2H), 1.93-1.80(m, 2H). MS: 563 ($\text{M} + \text{H}^+$).

10 XY-26: MS: 551 ($\text{M} + \text{H}^+$).

XY-27: MS: 455 ($\text{M} + \text{H}^+$).

XY-28: MS: 471 ($\text{M} + \text{H}^+$).

XY-29: MS: 482 ($\text{M} + \text{H}^+$).

XY-30: MS: 482 ($\text{M} + \text{H}^+$).

15 XY-38: MS: 503 ($\text{M} + \text{H}^+$).

XY-39: MS: 565 ($\text{M} + \text{H}^+$).

XY-42: MS: 519 ($\text{M} + \text{H}^+$).

XY-43: MS: 569 ($\text{M} + \text{H}^+$).

XY-49: MS: 477 ($\text{M} + \text{H}^+$).

20 XY-50: MS: 868 ($\text{M} + \text{H}^+$).

NAALADase Assay. The PSMA inhibitory activity was determined using a modification of the fluorescence-based Amplex Red Glutamic Acid Assay (Life Technologies, Grand Island, NY)(Kozikowski, 2004). Briefly, lysates of LNCaP cell extracts (25 μL) were incubated with the inhibitor (12.5 μL) in the presence of 4 μM N-acetylaspartylglutamate (NAAG) (12.5 μL) for 120 min. The amount of the glutamate released by NAAG hydrolysis was measured by incubating with a working solution (50 μL) of the Amplex Red Glutamic Acid Kit for 60 min. Fluorescence was measured with a VICTOR3V multilabel plate reader (Perkin Elmer Inc., Waltham, MA) with excitation at 490 nm and emission at 642 nm. Inhibition curves were determined using semi-log plots and IC_{50} values were determined at the concentration at which enzyme activity was inhibited by 50 %. Enzyme inhibitory constants (K_i values) were generated using the Cheng-Prusoff conversion (Cheng, 1973). Assays were performed in triplicate. Data analysis was performed using

GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California).

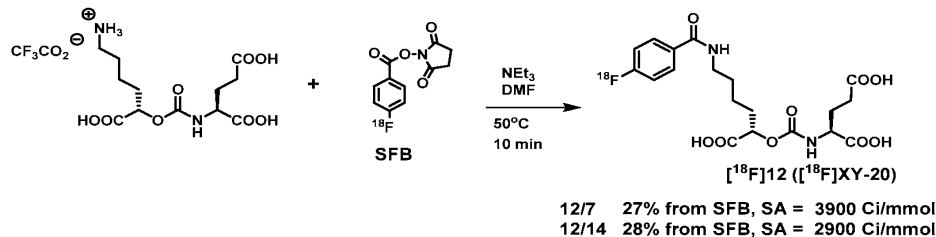
Cell Lines and Mouse Models. Sublines of the androgen-independent PC3 human prostate cancer cell line, originally derived from an advanced androgen independent bone metastasis, were used. These sublines have been modified to express high (PC3 PIP) or possess low (PC3 flu) levels of PSMA, and were generously provided by Dr. Warren Heston (Cleveland Clinic). PSMA-expressing (PC3 PIP), non-expressing (PC3 flu) PCa cell lines, were grown in RPMI 1640 medium (Corning Cellgro, Manassas, VA) containing 10 % fetal bovine serum (FBS) (Sigma-Aldrich, St.Louis, MO) and 1 % Penicillin-Streptomycin (Corning Cellgro, Manassas, VA). PC-3 PIP cells were grown under 20 µg/mL of puromycin selection in the growthmedium to maintain PSMA expression. All cell cultures were maintained in an atmosphere containing 5 % carbon dioxide (CO₂), at 37.0 °C in a humidified incubator. Animal studies were carried out in full compliance with the regulations of the Johns Hopkins Animal Care and Use Committee. Six- to eight-week-old male, non-obese diabetic (NOD)/severe-combined immunodeficient (SCID) mice (Johns Hopkins Immune Compromised Core) were implanted subcutaneously (sc) with PC3 PIP (PSMA+) and PC3 flu (PSMA-) cells (1 x 10⁶ in 100 µL of HBSS (Corning Cellgro, Manassas, VA) at the forward right and left flanks, respectively. Mice were imaged or used in ex vivo biodistribution assays when the xenografts reached 5 to 7 mm in diameter.

Radiosynthesis.

(S)-2-(((S)-1-carboxy-5-(4-[¹⁸F]fluorobenzamido)pentyl)oxy)carbonyl)amino)pentanedioic acid [¹⁸F]12 (**[¹⁸F]XY-20**). [¹⁸F]**SFB** was prepared according to a literature procedure (J. Label. Comp. Radiopharm, 2008, 51, 68-71). A methylene chloride solution of [¹⁸F]**SFB** was evaporated under a stream of argon gas. To this residue was added 3 mg of (S)-2-(((S)-5-amino-1-carboxypentyl)oxy)carbonyl)amino)-pentanedioic acid **18** in 200 µL dry DMF and 5 µL triethylamine. This was heated for 10 min at 50 °C, cooled to room temperature, acidified with trifluoroacetic acid, diluted with water, and injected onto a radio-HPLC (10 × 250 mm Phenomenix Luna C18 column, mobile phase 75/25/0.1 % water/acetonitrile/TFA, flow 4 mL/min). **12 (XY-20)** eluted at 12 min.

The product HPLC fraction was collected, neutralized with sodium bicarbonate, concentrated under vacuum, and dissolved in sterile saline for injection.

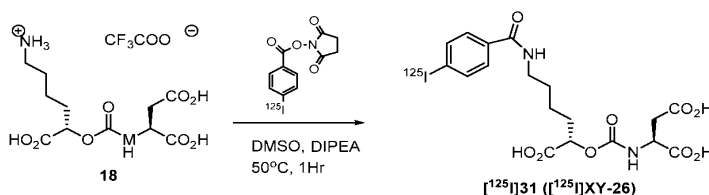
Scheme 2.



- 5 (S)-2-((((S)-1-carboxy-5-(4-
[¹²⁵I]iodobenzamido)pentyl)oxy)carbonyl)amino) pentanedioic acid [¹⁸F]31
([¹⁸F]XY-26). N-succinimidyl-4-[¹²⁵I]iodobenzoate was prepared by a modification
of the method of Dekker et al. (Dekker et al., 2005). In particular, to a solution of 0.1
mg N-succinimidyl-4-tributylstannylbenzoate (Dekker et al., 2005) in 100 µL
10 methanol was added 2 µL glacial acetic acid, 7.3 mCi of Na[¹²⁵I] (Perkin Elmer,
Billerica, MA), and 5 µL of solution of N-chlorosuccinimide in methanol (10mg N-
chlorosuccinimide in 1.5mL methanol). This was allowed to stand at room temperature
for 20 min, then diluted with 200 µL methanol and injected onto a semi-preparative-
HPLC (10 X 250 mm, 10 micron, Phenomenex Luna C18 column, 55/45/0.1
15 water/acetonitrile/trifluoroacetic acid, flow = 4 mL/min). N-succinimidyl-4-
[¹²⁵I]iodobenzoate eluted at 14 min. This was diluted with 20 mL water, loaded onto
an activated Waters C18 Sep-Pak Plus cartridge, washed with 10 mL water, dried
with a stream of nitrogen for 2 min, then eluted with 2 mL methylene chloride
through a Na₂SO₄ drying cartridge. The methylene chloride solution of N-
20 succinimidyl-4-[¹²⁵I]iodobenzoate (5.9 mCi) was stored at 0-2 °C.
The methylene chloride solution was then evaporated to dryness under a stream of
nitrogen and to this was added a solution of **18** (2mg/200µL DMSO). To this solution
is added 5µL diisopropylethylamine. Reaction is shaken and allowed to stand at room
temperature for one hour. The reaction is then acidified by the addition of 20µL TFA
25 and diluted with 1 mL water. This is injected onto a semi-preparative-HPLC (10 X
250 mm, 10 micron, Phenomenex Luna C18 column, 72/28/0.1
water/acetonitrile/trifluoroacetic acid, flow = 4 mL/min). Retention times of [¹²⁵I]**31**
was 12 min. Product fraction was diluted with 40 mL water, loaded onto an activated

Waters C18 Sep-Pak Plus cartridge, washed with 10 mL water, dried with a stream of nitrogen for 2min, then eluted with 2mL ethanol. Ethanol solution was concentrated under a stream of nitrogen until dryness and reconstituted in buffer for in-vitro assay. Starting with 2.0 mCi and 2.2 mCi of N-succinimidyl-4- ^{125} Iiodobenzoate, 1.8 and
 5 2.0mCi of ^{125} I]**31** was prepared.

Scheme 3.



10 *Imaging study.* Dynamic and whole-body PET and CT images were acquired on an eXplore VISTA small-animal PET (GE Healthcare) and an X-SPECT small SPECT/CT system (Gamma Medica Ideas), respectively. For imaging studies, anesthesia was induced with 3% and maintained under 1.5 % isoflurane (v/v) in oxygen. PET or PET-CT imaging studies were performed on NOD/SCID mice
 15 bearing PSMA+ PC-3 PIP and PSMA- PC-3 flu tumors. Following intravenous injection of ^{18} F]**12** (^{18} F]**XY-20**) PET images were acquired at 10 min, 30 min, 1 h, 2 h and 4 h p.i. as a pseudodynamic scan, i.e., a sequence of successive whole-body images were acquired in two bed positions. The dwell time at each bed position was 10 min for a total scan time of 20 minutes. An energy window of 250 – 700 keV was
 20 used. Images were reconstructed using the FORE/2D-OSEM method (one iteration, 16 subsets) and included correction for radioactive decay, scanner dead time, and scattered radiation.

Biodistribution. Mice bearing PSMA+ PC-3 PIP and PSMA- PC-3 flu xenografts Chang et al., 1999) were injected *via* the tail vein with 740 kBq (20 μ Ci)
 25 of ^{18} F]**12** (^{18} F]**XY-20**) in 200 μ L of saline. At various time points post-injection, mice were sacrificed by cervical dislocation and the blood immediately collected by cardiac puncture. The heart, lungs, liver, stomach, pancreas, spleen, fat, kidney, muscle, small and large intestines, urinary bladder, PSMA+ PC-3 PIP and PSMA- PC-3 flu tumors were collected. Each organ was weighed, and the tissue radioactivity

was measured with an automated gamma counter (1282 Compugamma CS, Pharmacia/ LKB Nuclear, Inc., Mt. Waverly, Vic. Australia). The % ID/g was calculated by comparison with samples of a dilution of a standard dose. All measurements were corrected for decay. Data are expressed as mean $\pm$ standard deviation (SD).

Molecular Modeling. All molecular modeling experiments were performed using Discovery Studio 4.0 developed by Accelrys, Inc. (San Diego, CA).

Protein and Ligand Structure Preparation. The X-ray structure of PSMA co-crystallized with the competitive inhibitor DCIBzL (PDB: 3D7H) was downloaded from the protein data bank (RCSB, <http://www.rcsb.org/pdb/home/home.do>). The water molecules were removed while the co-crystallized ligand was used as a template to sketch the compounds using the Sketch Molecules module in Discovery Studio.

In Situ Ligand Minimization. Each ligand was minimized while binding to its target protein using the in situ ligand minimization module with the following parameters: CHARMM as an input force field, minimization algorithm as smart minimizer, maximum minimization steps equal to 1000, minimization with RMS gradient equal to 0.001 Å, and minimization energy change set equal to zero. After the minimization protocol was executed, the in situ minimized ligands were stripped of their nonpolar hydrogens to simplify the overall view. The protein was depicted in the form of a light gray line ribbon. The bound ligand is depicted as a stick with atoms color-coded according to element: carbon (gray), nitrogen (blue), and oxygen (red) (FIG. 13).

In vitro stability studies. PC-3 PIP (PSMA+) and PC-3 flu (PSMA-) cells were cultured as previously described (Banerjee et al., 2014). 300,000 PIP or flu cells were seeded into three wells each of a 6 well plate using RPMI 1640 + 10 % fetal bovine serum + 1 % Penicillin-Streptomycin (Corning Cellgro, Manassas, VA) and were grown to 80 % confluency. At the time of assay, the culture medium was refreshed and 50 μ Ci (1.35 kBq) of [^{125} I]**31** ([^{125} I]**XY-26**) or [^{125} I]**32** ([^{125} I]**XY-57**) was added to both a PIP- and flu-containing well. After radiotracer addition, the plate was returned to the incubator (humidified 37 °C, 5% CO₂) for 30 minutes. The medium was then carefully removed and saved for counting in a LKB Wallac 1282 Compugamma gamma counter (Mount Waverly, Vic, Australia). The cells were

washed twice with ambient temperature PBS, pH 7.4 followed by the addition of ddH₂O to lyse the cells. Lysis took place over 30 minutes inside the incubator. The lysates were then collected and counted using the gamma counter. Equal amounts of radioactivity from the supernatant and lysates were spotted onto silica gel 60 RP-18
5 F254S glass TLC plates (EMD Millipore Corp., Billerica, MA) and the plates were developed using a mobile phase consisting of 55 % acetonitrile, 45 % water and 0.1 % trifluoroacetic acid. The TLC plate was dried and exposed to Kodak Biomax x-ray film (Fisher Scientific) prior to digitizing using the MCID Core package (Interfocus Imaging, Cambridge, UK). Standards solutions of [¹²⁵I]**31** ([¹²⁵I]**XY-26**) and [¹²⁵I]**32**
10 ([¹²⁵I]**XY-57**) had R_f values of 0.8. Intracellular and extracellular metabolites of [¹²⁵I]**31** ([¹²⁵I]**XY-26**) in PC-3 PIP cells had an R_f value of approximately 0.44.

Results

Chemistry. Among the potent urea-based PSMA binding ligands, 4-fluorobenzoyl-
15 and 4-iodobenzoyl-lys-glu urea, compounds **9** (**YC-I-26**) and **10** (**YC-I-27**) respectively, have produced some of the highest affinities reported (FIG. 2A and FIG. 2B) (Chen, 2008). Based on these ureas, NPA- carbamate **12** (**XY-20**) (Scheme 1) was the initial target compound. It has been reasoned that the 4-fluorobenzoyl side-chain would provide high affinity and specificity by utilizing the S1 binding pocket
20 and be amenable to radiolabeling with ¹⁸F. The synthesis of **12** (**XY-20**) started with *N*-Boc protected (*S*)-6-amino-2-hydroxyhexanoic acid **14** (Scheme 1). The carboxylic acid as the tert-butyl ester was first protected and then converted alcohol **15** to *N*-imidazolecarbamate **16**. Compound **16** reacted slowly with L-glutamic acid di-tert-butyl ester under neat conditions and provided a quantitative yield of **17**. Upon
25 deprotection intermediate **18** was obtained in 70 % yield as a trifluoroacetate salt. NPA-carbamate **12** (**XY-20**) was obtained by treating **18** with *N*-succinimidyl-4-fluorobenzoate in the presence of triethylamine.

X-ray co-crystal studies with bound urea-based ligands such as **10** have demonstrated a unique cation- π interaction with PSMA, with the benzoyl group of the
30 ligand fully inserted into the “arginine patch” of the PSMA S1 binding pocket and an adjacent hydrophobic subpocket that can accommodate the *para*-iodo substituent of **10** (Barinka, 2008). That additional interaction likely accounted for the increase in binding affinity of **10** compared to **9** (Chen, 2008; Barinka, 2008). To increase the

binding affinities of the iodinated carbamates **31** has been prepared by reacting *N*-succinimidyl-4-iodobenzoate with **18**, respectively (Scheme 1). For PET imaging with ^{18}F , the 4-iodo-benzoyl group would require an ^{18}F fluoro substituent in the activated ortho position.

- 5 *In-vitro PSMA binding.* The PSMA inhibitory activities of the prepared compounds were measured using a modification of the fluorescence-based Amplex Red Glutamic Acid assay (Kozikowski, 2004) (FIG. 2B). Carbamates **12 (XY-20)** and reversed carbamate **13 (XY-48)** inhibited PSMA at $K_i = 42$ nM and 9.2 nM, respectively, significantly less potent than the corresponding ureido analog **9 (XY-I-26)**, ($K_i = 0.25$ nM) (Chen, 2008). Carbamate **31 (XY-26)** and reversed carbamate **32 (XY-57)** inhibited PSMA at $K_i = 0.9$ nM and 0.04 nM, respectively. Reversed carbamates **23 (XY-52)** and **24 (XY-47)** demonstrated potent inhibition of PSMA, with $K_i = 0.11$ nM and 0.21 nM, respectively, while the corresponding ureas **26 (XY-58)** and **27 (XY-44)** inhibited PSMA with $K_i = 0.04$ and 0.02 nM, respectively.
- 15 *PSMA Inhibitor Docking Studies.* Urea **9 (XY-I-26)**, NPA-carbamate **12 (XY-20)** and OPA- reversed carbamate **13 (XY-48)** were subjected to *in situ* ligand minimization docking experiments based on the reported X-ray crystal structure of PSMA known as 3D7H.(Barinka, 2008). The results are shown in FIG. 7A, FIG. 7B, FIG. 7C, and FIG. 7D. The urea N-H in **9 (YC-I-26)** binds to the carbonyl oxygen of
- 20 G518 asymmetrically, with the Lys-N-H (1.92 Å) slightly closer to the carbonyl oxygen than Glu-N-H (2.03 Å). Lys-OPA reversed carbamate **13 (XY-48)**, with the Glu-N-H changed to O resulting in the loss of a hydrogen bond, retained nearly all of the features of **9 (YC-I-26)**, when binding to PSMA (FIG. 7C), and the Lys-N-H distance to G518 slightly increased 1.95 Å, with the carbamate shifting slightly
- 25 towards the zinc (FIG. 7C vs. FIG. 7A). This resulted in a 30-fold reduction in binding affinity compared to **9 (YC-I-26)**. NPA carbamate **12 (XY-20)**, with the Lys-N-H changed to O, altered the binding mode dramatically (FIG. 7B), with the carbamate shifting toward the zinc resulting an increase in hydrogen bond distance to G518 (2.448 Å) (FIG. 7B). This decreased the binding affinity of **12 (XY-20)** by
- 30 more than 150-fold compared to **9 (YC-I-26)**.

Radiochemistry. Carbamate ^{18}F **12** (^{18}F **XY-20**) was prepared by reacting *N*-succinimidyl-4- ^{18}F fluorobenzoate (Tang, 2008), ^{18}F SFB, with **18** respectively

(Scheme 2). ^{125}I labeled analog ^{125}I 131 (^{125}I XY-26) (Scheme 3) was prepared likewise using N-succinimidyl-4- ^{125}I iodobenzoate (Zalutsky, 1987).

Biodistribution and imaging. Whole body micro-PET CT images of [^{18}F]12 ([^{18}F]XY-20) are shown in FIG. 8A, FIG. 8B, FIG. 8C, and FIG. 8D and its biodistribution is shown in Table 1. [^{18}F]12 ([^{18}F]XY-20) exhibited a modest PC-3 PIP tumor uptake of 6.33 ± 1.55 % injected dose/g (% ID/g) at 30 min and radioactivity rapidly cleared from all organs including PC-3 PIP tumor. PET-CT imaging confirms both the rapid tumor uptake and clearance from all tissues. In the PET-CT imaging, co-injection of [^{18}F]12 ([^{18}F]XY-20) with an excess of known competing inhibitor (S)-2-(3-((S)-1-carboxy-3-methylbutyl)ureido)pentanedioic acid (ZJ-43), showed complete blockade of tumor uptake indicating that the tumor uptake is PSMA specific (FIG. 8A, FIG. 8B, FIG. 8C, and FIG. 8D).

Table 1. Biodistribution of [^{18}F]12 ([^{18}F]XY-20)

	30 min ^b	60 min ^c	90 min ^c	180 min ^c
blood	2.51 ± 1.75	1.07 ± 0.26	0.20 ± 0.14	0.08 ± 0.06
heart	1.12 ± 0.74	0.25 ± 0.08	0.11 ± 0.02	0.07 ± 0.04
lung	2.07 ± 1.51	0.85 ± 0.55	0.33 ± 0.05	0.14 ± 0.04
liver	11.0 ± 1.77	12.5 ± 3.01	11.0 ± 0.87	4.62 ± 3.80
spleen	3.76 ± 6.48	0.45 ± 0.31	0.27 ± 0.04	0.13 ± 0.08
fat	1.23 ± 1.88	0.15 ± 0.05	0.11 ± 0.06	0.04 ± 0.02
kidney	14.2 ± 8.47	6.97 ± 7.02	1.51 ± 0.22	0.50 ± 0.18
Sm.int.	1.89 ± 1.58	0.54 ± 0.19	0.27 ± 0.11	0.08 ± 0.04
Lrg int.	3.82 ± 5.87	0.62 ± 0.27	0.18 ± 0.03	0.16 ± 0.05
muscle	0.86 ± 0.81	0.27 ± 0.12	0.06 ± 0.01	0.03 ± 0.01
bone	2.05 ± 2.39	0.72 ± 0.31	0.79 ± 0.16	0.63 ± 0.14
bladder	22.4 ± 22.5	5.50 ± 3.45	2.11 ± 1.21	2.74 ± 0.48
PC-3 Pip	6.33 ± 1.55	3.52 ± 1.48	0.94 ± 0.24	0.22 ± 0.07
PC-3 flu	1.89 ± 0.78	0.72 ± 0.12	0.37 ± 0.08	0.12 0.03

^aValues expressed as percent injected dose/gram $\pm$ standard deviation;

b. N = 7; c. N = 4

In-vitro stability studies. Because NPA carbamates [^{18}F]**12** ([^{18}F]**XY-20**) and OPA reversed carbamate [^{18}F]**13** ([^{18}F]**XY-48**) (FIG. 2A) exhibited such differing pharmacokinetics (FIG. 2B), the stability of NPA carbamate [^{125}I]**31** ([^{125}I]**XY-26**) and OPA reversed carbamates [^{125}I]**32** ([^{125}I]**XY-57**) in-vitro have been tested. The radioiodinated compounds were utilized for the convenience of the long half-life of I-125. Polar radiometabolite(s) in extracellular and intracellular fluid were only seen for NPA [^{125}I]**31** ([^{125}I]**XY-26**) in PC-3 PIP cells (FIG. 9). This suggests that NPA carbamates may be subject to PSMA specific metabolism and may be the reason for their rapid clearance from PSMA expressing tumors and organs.

10 Discussion

Recent clinical PET imaging using either ^{18}F , ^{124}I or ^{68}Ga labeled urea based PSMA inhibitors have produced high quality PET images allowing the detection of both primary and metastatic lesions, some of which were undetectable by conventional imaging. Although all agents exhibited renal uptake, some agents (Ex: [^{18}F]DCFB (Cho, 2012), and [^{68}Ga]DOTA-DUPA-Pep (Reske, 2013)) showed persistent blood pool occupancy but low salivary gland uptake. On the other hand, [^{18}F]DCFPyL (Szabo, 2015), [^{124}I]-MIP-1095 (Zechmann, 2014), and [^{68}Ga]DKFZ-11 (Afshar-Oromieh, 2012; Afshar-Oromieh, 2013) showed rapid clearance from the blood but high uptake in salivary and lacrimal glands. The latter has also been observed in radiopharmaceutical therapy studies using [^{177}Lu]-DKFZ-617 (Kratochwil, 2015) and [^{131}I]-**MIP-1095** (Zechmann, 2014). Absorbed dose estimates for [^{131}I]-**MIP-1095** identified the salivary glands, lower large intestinal wall, and kidneys as dose-limiting organs (Zechmann, 2014). Therefore, new scaffolds that would preserve the positive distribution characteristics of the urea-based agents but provide better clearance from non-target organs were sought.

However, NPA carbamate [^{18}F]**12** ([^{18}F]**XY-20**) and OPA reversed carbamate [^{18}F]**13** ([^{18}F]**XY-48**) exhibited drastically different pharmacokinetics: [^{18}F]**12** ([^{18}F]**XY-20**) had modest PSMA+ PC-3 PIP tumor uptake but rapidly cleared from both PC-3 PIP tumor and normal tissues including kidneys whereas OPA reversed carbamate [^{18}F]**13** ([^{18}F]**XY-48**) had high and prolonged tumor and kidney uptake. Selective metabolism of NPA-carbamate [^{125}I]**31** ([^{125}I]**XY-26**) was observed only in PSMA+ PC-3 PIP cells in-vitro, suggesting that NPA-carbamates may be PSMA substrates. In addition, *in situ* ligand minimization docking experiments showed a

difference in the binding of **12** (**XY-20**) and **13** (**XY-48**). Although compounds **12** (**XY-20**) and **13** (**XY-48**) both have one less hydrogen bonding moiety compared to urea **9** (**XY-I-26**), compound **13** (**XY-48**) possesses a similar position with regards to the zinc molecule and G518 in the binding site. For compound **12** (**XY-20**) the hydrogen bonding distance to G518 is increased. The resulting weaker hydrogen bonding in **12** (**XY-20**) may be the reason for its lower PSMA binding affinity compared to both **9** (**XY-I-26**) and **13**(**XY-48**).

EXAMPLE 2

10 Synthesis of Beta-amino acid Urea Agents for for Imaging of PSMA and Cancer
Radiotherapy

(R)-2-amino-3-(3-((S)-1,5-di-tert-butoxy-1,5-dioxopentan-2-yl)ureido)propanoic acid. L-glutamic acid di-tert-butyl ester hydrochloride 600 mg (2 mmol) and triethylamine 1 mL were dissolved in 20 mL anhydrous methylene chloride. The solution was cooled to -78 °C. Triphosgene 200 mg (0.66 mmol) was dissolved in 3 mL anhydrous methylene chloride and added by drop. The reaction was slowly warmed up to room temperature and stirred for 30 min. (R)-3-amino-2-((tert-butoxycarbonyl)amino)propanoic acid 400 mg (2 mmol) and triethylamine 0.4 mL were added. The reaction was kept at room temperature for 24 h. The organic layer was washed with 2N HCl and dried with sodium sulfate. After the solvent was removed under vacuum, 1.0 g (R)-2-amino-3-(3-((S)-1,5-di-tert-butoxy-1,5-dioxopentan-2-yl)ureido)propanoic acid was obtained and used for the next steps without further purification. The yield was around 100%. (¹H NMR (400 MHz, CDCl₃): δ 6.17-6.00(br, 3H), 4.31(m, 2H), 3.71-3.51(m, 2H), 2.37-2.32(m, 2H), 2.11-2.06(m, 1H), 1.91-1.80(m, 1H) 1.51-1.41(m, 27H). MS: 446 (M+H⁺)

(S)-2-(3-((R)-2-amino-2-carboxyethyl)ureido)pentanedioic acid. (R)-2-amino-3-(3-((S)-1,5-di-tert-butoxy-1,5-dioxopentan-2-yl)ureido)propanoic acid 300 mg was treated with a 6 mL solution of TFA/methylene chloride(1/1) for 2 h. The final product was purified by HPLC in 150 mg, yield 60%. (¹H NMR (400 MHz, CDCl₃): δ 4.17(dd, *J*₁ = 9.6 Hz, *J*₂ = 5.6 Hz, 1H), 4.00(dd, *J*₁ = 6.0 Hz, *J*₂ = 4.0 Hz, 1H), 3.66(dd, *J*₁ = 15.2 Hz, *J*₂ = 4.0 Hz, 1H), 3.56(dd, *J*₁ = 15.2 Hz, *J*₂ = 6.0 Hz, 1H), 2.40(t, *J* = 7.2 Hz, 2H), 2.31-2.05(m, 1H), 1.92-1.83(m, 1H). MS: 278 (M+H⁺)

NMR and MS for beta-amino acid ureas (Structures and PSMA binding are given in Example 3).

XY-34: MS: 440 ($M+H^+$)

XY-36: MS: 508 ($M+H^+$)

5 **XY-35:** MS: 585 ($M+H^+$)

XY-37: MS: 585 ($M+H^+$)

XY-40: MS: 513 ($M+H^+$)

XY-46: MS: 561 ($M+H^+$).

10

EXAMPLE 3

Binding Affinity data of Representative Compounds of Formula (I) and Formula (II)

Table 3. Binding Affinity Data of Representative Compounds of Formula (I) and Formula(II)				
Entry	Compound structure	IC ₅₀ (nM)	K _i (nM)	Cmpd
1		113	22	XY-10
2		4,500	900	XY-16
3		4,900	980	XY-17
4		210	42	XY-20

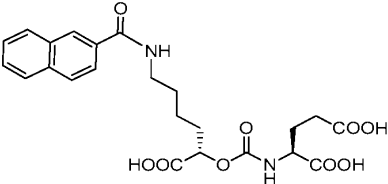
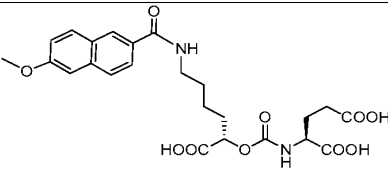
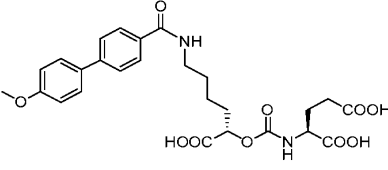
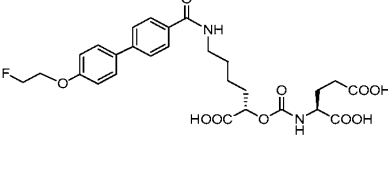
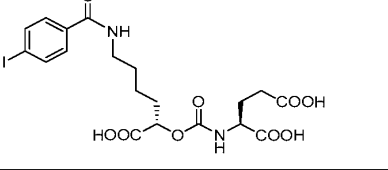
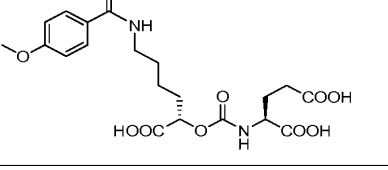
Table 3. Binding Affinity Data of Representative Compounds of Formula (I) and Formula(II)				
Entry	Compound structure	IC ₅₀ (nM)	K _i (nM)	Cmpd
5		60	12	XY-21
6		104	20	XY-22
7		30	6	XY-23
8		120	22	XY-24
9		4.3	0.9	XY-26
10		100	21	XY-27

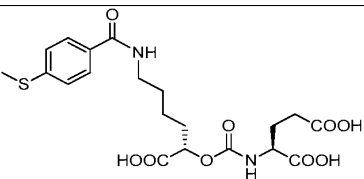
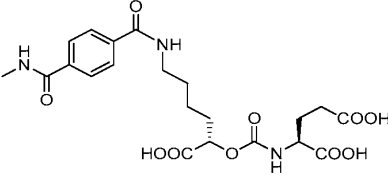
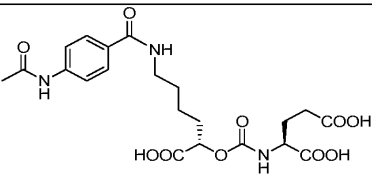
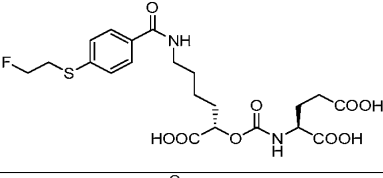
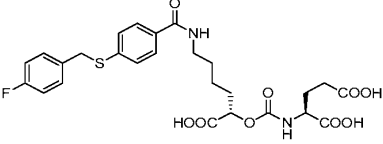
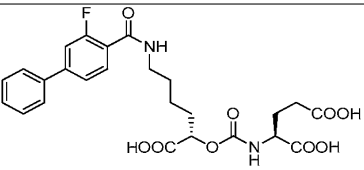
Table 3. Binding Affinity Data of Representative Compounds of Formula (I) and Formula(II)				
Entry	Compound structure	IC ₅₀ (nM)	K _i (nM)	Cmpd
11		24	4.8	XY-28
12		1,300	270	XY-30
13		660	130	XY-29
14		41	8.3	XY-38
15		46	9.3	XY-39
16		86	13	XY-42

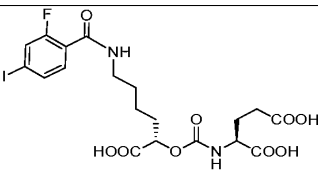
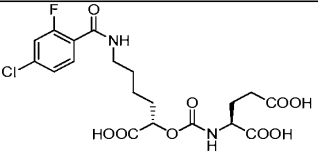
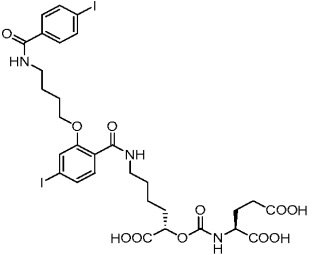
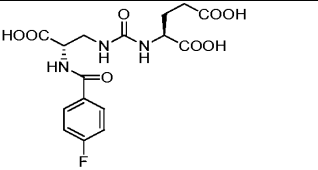
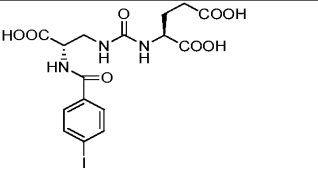
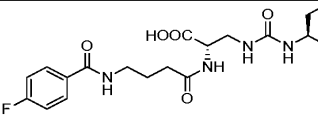
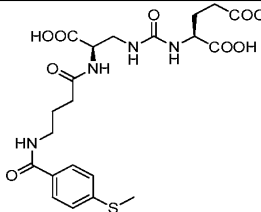
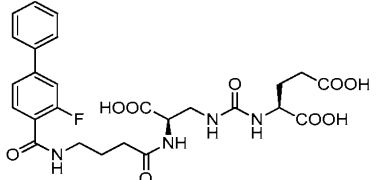
Table 3. Binding Affinity Data of Representative Compounds of Formula (I) and Formula(II)				
Entry	Compound structure	IC ₅₀ (nM)	K _i (nM)	Cmpd
17		1.6	0.32	XY-43
18		192	38	XY-49
19		3,800	770	XY-50
20		43,000	8,700	XY-34
21		12,000	2,400	XY-36
22		8,000	1,600	XY-35

Table 3. Binding Affinity Data of Representative Compounds of Formula (I) and Formula(II)				
Entry	Compound structure	IC ₅₀ (nM)	K _i (nM)	Cmpd
23		1.3	0.26	XY-40
24		2.0	0.4	XY-46

Some other additional data, including the binding affinity K_i, distribution coefficient CLogD, and PSA, of representative compounds of Formula (I) are provided in Table 4. In these exemplary, non-limiting embodiments, the most potent compounds contain a fluorine atom at R₁ and a large halogen, e.g., either Br or I, or a phenyl ring at the 4-position of the arylbenzoyl moiety, i.e., R₂ and are annotated with an "*".

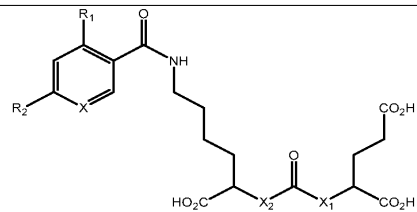
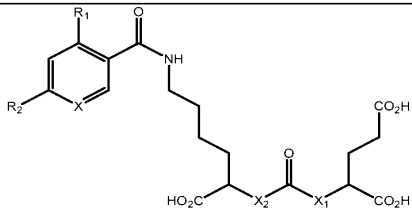
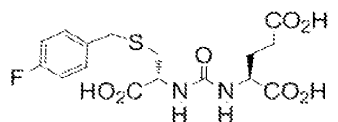
Table 4. Binding Affinity Data of Representative Compounds of Formula (I)								
								
Cmpd	X ₂	X ₁	X	R ₁	R ₂	K _i (nM)	CLogD	PSA
DCFBC [†]	---	---	---	---	---	14	-4.7	178
DCIBC ^{††}	---	---	---	---	---	0.03	---	---
YC-I-27	NH	NH	CH	H	I	0.01	-5.2	182
YC-I-26	NH	NH	CH	H	F	0.3	-5.6	182
DCFPyL ^{†††}	NH	NH	N	H	F	1.1	-6.4	195

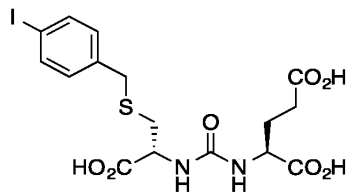
Table 4. Binding Affinity Data of Representative Compounds of Formula (I)

								
Cmpd	X ₂	X ₁	X	R ₁	R ₂	K _i (nM)	CLogD	PSA
C8 ^{††††}	---	---	---	---	---	0.35	---	---
XY-44*	NH	NH	CH	F	I	0.017	-5.5	182
XY-45*	NH	NH	CH	F	C ₆ H ₅	0.1	-5.1	182
XY-59	NH	NH	CH	H	Br	0.02	-5.4	182
XY-58*	NH	NH	CH	F	Br	0.036	-5.8	182
XY-20	O	NH	CH	H	F	42	-4.7	179
XY-26	O	NH	CH	H	I	0.9	-4.3	179
XY-28	O	NH	CH	H	SCH ₃	4.8	-4.6	205
XY-43*	O	NH	CH	F	I	0.32	-4.6	179
XY-42	O	NH	CH	F	C ₆ H ₅	13	-4.2	179
XY-49	O	NH	CH	F	Cl	38	-5.5	179
XY-56	O	NH	CH	F	Br	--	-4.9	179

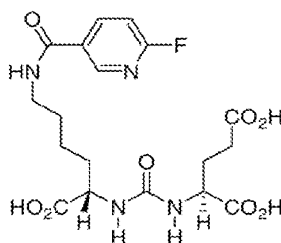
[†]DCFBC is N-[N-[(S)-1,3-Dicarboxypropyl]carbamoyl]-4-fluorobenzyl-L-cysteine:



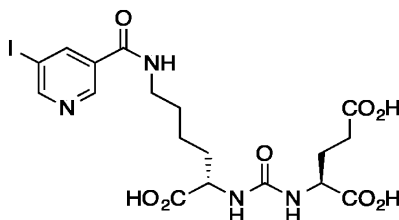
^{††}DCIBC is N-[N-[(S)-1,3-Dicarboxypropyl]carbamoyl]-4-iodobenzyl-L-cysteine



^{†††}DCFPyL is 2-(3-{1-carboxy-5-[(6-fluoro-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid:



††††C8 is



5

EXAMPLE 4

Synthesis and use of Radiohalogenated Lysine, Glutamate and Cysteine-NPA carbamate compounds for Imaging and Cancer Radiotherapy

The development of low molecular weight radiotherapeutic agents is different from developing radiopharmaceuticals for imaging in that longer tumor residence times are required for the former. Many radionuclides, primarily β - and alpha emitters, have been investigated for targeted radioimmunotherapy and include both radiohalogens and radiometals. The studies presented in the disclosed subject matter have been focused on radiohalogens, ^{125}I , ^{123}I , ^{131}I , ^{211}At , ^{77}Br (Table 5), with several specific examples of appropriate ways of introducing them into PSMA-targeting molecules. Several of these radionuclides (^{123}I , ^{131}I , ^{77}Br) also emit imaging gamma rays providing both imaging and radiotherapeutic applications.

Table 5. Therapeutic Radionuclides

β -particle emitters	^{90}Y , ^{131}I , ^{177}Lu , ^{153}Sm , ^{186}Re , ^{188}Re , ^{67}Cu , ^{212}Pb , ^{166}Ho , ^{47}Sc
α -particle emitters	^{225}Ac , ^{213}Bi , ^{212}Bi , ^{211}At , ^{212}Pb , ^{227}Th , ^{223}Ra
Auger electron emitters	^{125}I , ^{123}I , ^{67}Ga , ^{111}In , $^{80\text{m}}\text{Br}$, ^{77}Br

20

These radiohalogens are covalently bound to the targeting moiety and unlike

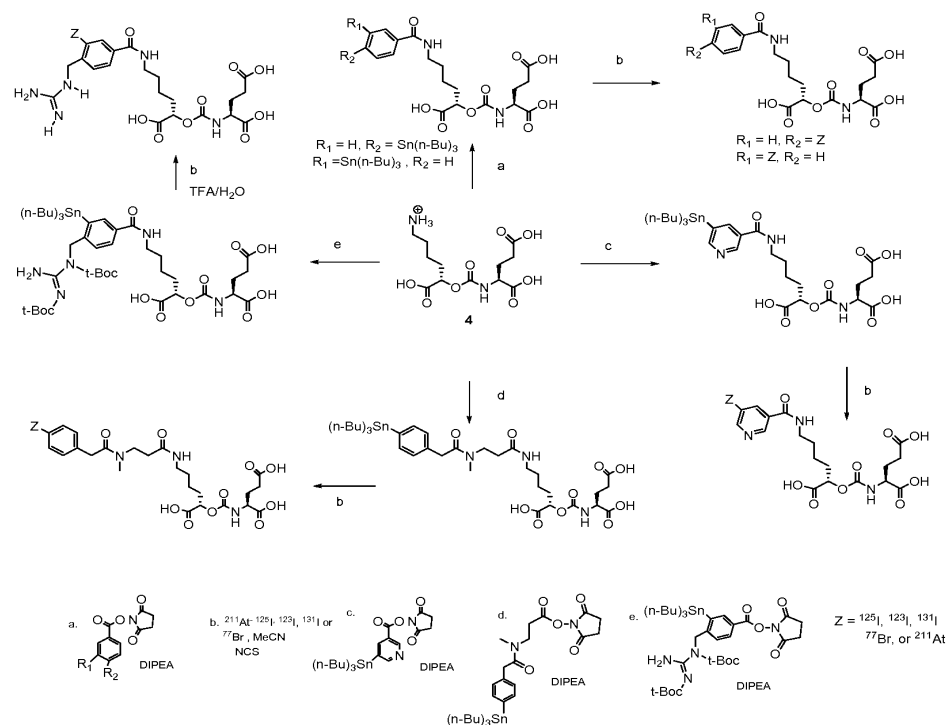
large chelated radiometals are small enough that the entire radiolabeled PSMA inhibitor can fit within the PSMA binding cavity thereby retaining the high binding affinity. The same radiolabeled prosthetic groups can be conjugated to linker-inhibitor conjugates to move the radiolabeled portion of the inhibitor to the exterior of the PSMA protein.

Radiohalogenated PSMA binding radiotherapeutics can be built upon multiple PSMA binding scaffolds. The urea based scaffolds include: the lysine-glutamate urea, cysteine-glutamate urea, and glutamate-glutamate urea (FIG. 10). PSMA inhibitors built upon novel lysine- reversed carbamate scaffold (NPA) have been disclosed in example 1 of the present application, and cysteine -NPA carbamate and glutamate—NPA carbamate scaffolds, as shown FIG.11, have been envisioned. These compounds are based on extensive structure-activity relationships – not merely of the imaging precursors or PSMA binding compounds, but on actual imaging agents already synthesized and tested in vivo – as well as on molecular modeling.

The lead compound in the lysine-glutamate ureas is **YC-I-27**. This iodinated compound has high and prolonged tumor uptake and a very high PSMA binding affinity $K_i = 0.01$ nM (Chen et al., 2008). It has also been co-crystallized with PSMA. This has shown that the bulky iodo-phenyl moiety is accommodated by a hydrophobic auxiliary sub-pocket extending beyond the normal binding pocket and the additional hydrophobic-hydrophobic interactions accounts for the high binding affinity (Barinka et al., 2008). Table 4 summarizes the halogen containing compounds that have been prepared to date and demonstrates the high binding affinity for the halogenated compounds using the new lysine-NPA reversed carbamate scaffold. This indicates the utility of the new Lysine-NPA scaffold and suggests that it can be applied to PSMA targeted radiotherapy of prostate cancer. This also suggests that cysteine-NPA scaffold as well as glutamate-NPA carbamate scaffold will also produce PSMA targeted radiotherapeutic agents.

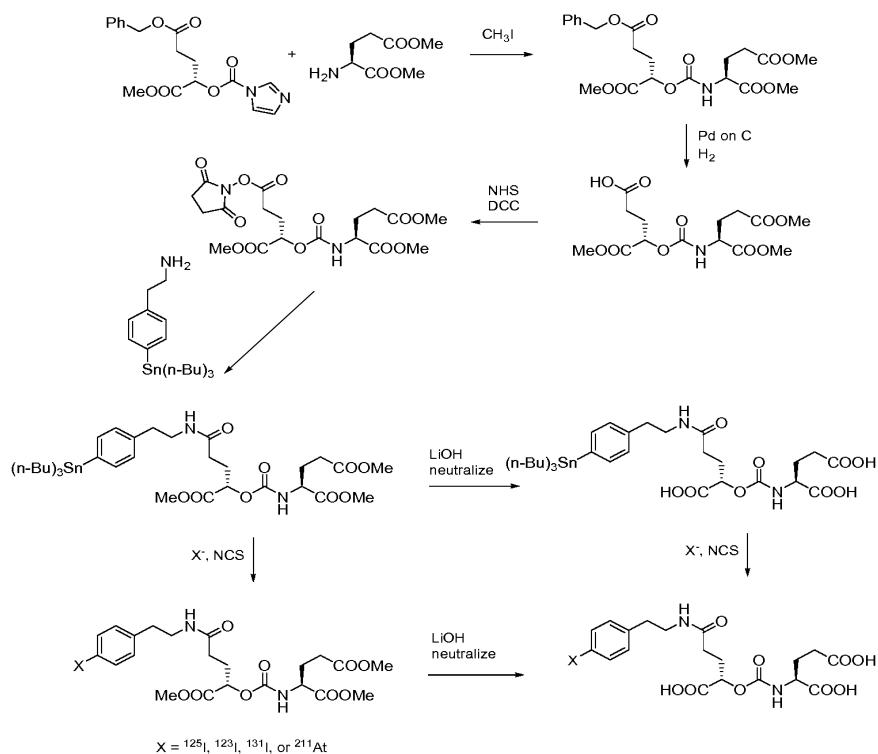
Synthesis of Radiohalogenated Lysine – NPA carbamate compounds. Parent lysine-NPA carbamate scaffold reported in example 1 of the present application, and can be utilized, as shown in Scheme 4, to prepare radiohalogenated lysine –NPA carbamates utilizing known stannane containing prosthetic groups (Garg, et al., 1991; Vaidyanathan G, and Zalutsky MR, 2007; Talanov et al., 2006).

Scheme 4.



Synthesis of Radiohalogenated Glutamate-NPA carbamate compounds.

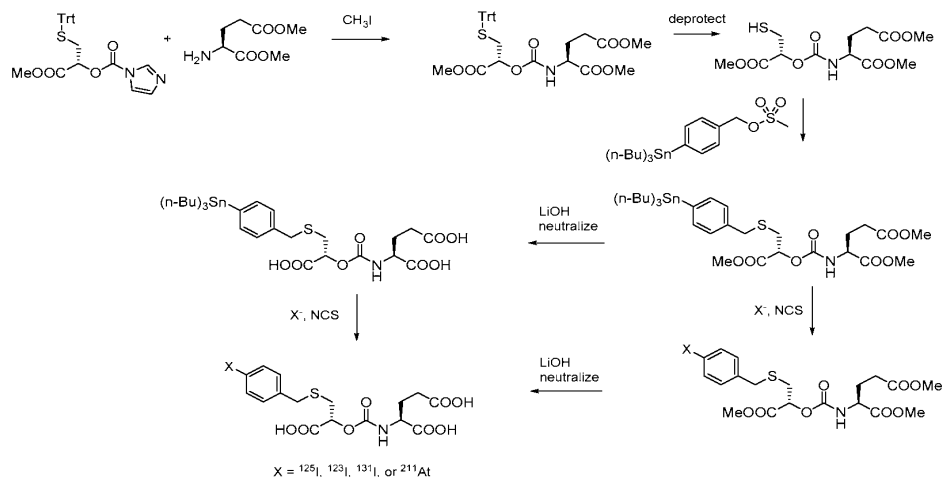
- 5 Glutamate-glutamate ureas have been used by others to conjugate bulky radiometal chelating agents, fluorescent molecule, and chemotherapeutics (Kularatne et al., 2009; International Patent Applications Nos. PCT/US2008/073375, PCT/US2009/061067, and PCT/US2011/026238). These compounds are too large to utilize the non-pharmacophore binding pocket and must utilize a void region to extend outside of the
- 10 protein. A synthetic route to an analogous radiohalogenated glutamate-NPA carbamate is shown in Scheme 5.

Scheme 5.

Synthesis of Radiohalogenated Cysteine-NPA carbamate compound. The cysteine-glutamate urea scaffold has been utilized by us for PSMA binding and imaging for over 10 years starting with C-11 labeled DCMC (Pomper et al., 2002; Foss et al., 2005), continuing with F-18 labeled DCFBC (Mease et al., 2008; Cho et al., 2012) both for PET imaging with the latter currently in use in patients, and I-125 labeled DCIBC (Dusich, 2008) for SPECT imaging and or radiotherapy (FIG. 11).

A synthetic route to analogous radiohalogenated cysteine-NPA carbamates is shown in Scheme 6.

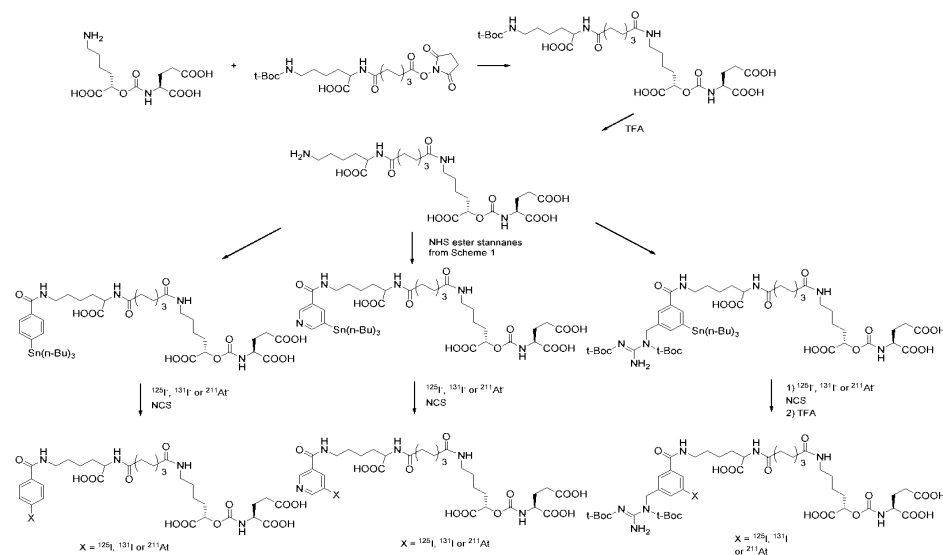
Scheme 6



Homologs of the cysteine-NPA carbamates may have even higher binding affinity because the extended alkyl chain will permit a deeper penetration of the 4-halobenzyl group into the non-pharmacophore binding pocket. These compounds can be prepared analogously using Scheme 3 starting with commercial N^α-Fmoc-S-trityl-L-homocysteine and L-5-[S-trityl]-[N-9-fluorenylmethyloxycarbonyl]-mercaptonorvaline.

Lysine glutamate ureas and glutamate-glutamate ureas linker conjugates have been used to attach bulky radiometal chelates or fluorescent molecules for PSMA specific imaging and radiotherapy, with a focus on the use of polyethylene glycol (PEG) and lysine-suberate linkers (Banerjee et al., 2008; Chen et al., 2012). The new PSMA binding scaffolds described herein can also be used to prepare radiolabeled linker conjugates. A route to the synthesis of a lysine-suberate-lysine-NPA carbamate and its use in preparing radiohalogenated linker NPA carbamates is shown in Scheme 7.

Scheme 7.

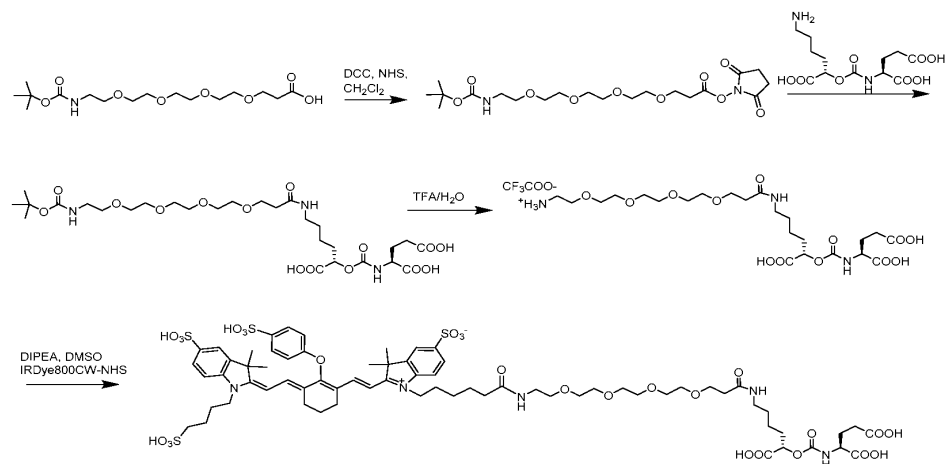


EXAMPLE 4

PSMA Binding Carbamates Conjugated to Fluorescent Molecules for PSMA Targeted Imaging Guided Surgery and Photodynamic Therapy

The preparation and use of PSMA binding ureas conjugated to fluorescent molecules via various linkers for imaging PSMA expressing tumors and tissues (Chen et al., 2009; Banerjee et al., 2011; Chen et al., 2012) and photodynamic therapy (PDT) have been previously described. Two specific examples of IR-Dye-800-CW-linker-ureas are shown in FIG.13. PSMA inhibitors built upon lysine-carbamate scaffolds (NPA) including F-18 labeled analogs have been disclosed in the present application, and the F-18 labeled NPA compounds demonstrated selective uptake in PSMA positive tumor mouse xenografts. Various dyes and linkers previously disclosed in Patent Application No. PCT/US2010/028020 for use with ureas can be attached to the NPA scaffolds to provide novel optical agents for imaging prostate cancer. A route to the synthesis is shown in scheme 8.

Scheme 8.



REFERENCES

- 5 All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references are herein incorporated by reference to the same extent as if each individual publication, patent application, patent, and other reference was
- 10 specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art. In case of a conflict between the specification and any of the incorporated references, the
- 15 specification (including any amendments thereof, which may be based on an incorporated reference), shall control. Standard art-accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein
- 20 International PCT Patent Application Publication No. PCT/US2008/073375 to Low PS, Kularatne SA. for PSMA binding ligand-linker conjugates and methods for using, published 2009/02/26 (WO 2009/026177A1);
- International PCT Patent Application Publication No. PCT/US2009/061067 to

Low PS, Kularatne SA. for PSMA binding ligand-linker conjugates and methods for using, published 2010/04/22 (WO 2010/045598 A2);

International PCT Patent Application Publication No. PCT/US2011/026238 to Low PS, Chelvam V, Kim Y. for PSMA binding ligand-linker conjugates and

5 methods for using, published 2011/09/01 (WO 2011/106639 A1);

International PCT Patent Application Publication No. PCT/US03/00680 to Pomper MG, Zhang J, Kozikowski AP, Musachio JL. for Imaging agents and methods of imaging NAALADase of PSMA, published 2003/07/24 (WO 03/60523 A1);

10 International PCT Patent Application Publication No. PCT/US2008/007947 to Pomper, M.G., Ray, S., Mease, R.C., Foss, C. for Labeled inhibitors of prostate specific membrane antigen (PSMA), biological evaluation, and use as imaging agents, published 2008/12/31 (WO 2009/002529 A2);

15 International PCT Patent Application Publication No. PCT/US2009/052456 to Pomper MG, Mease RC, Chen Y. for Preparation of glutamic acid heterodimer prostate-specific membrane antigen PSMA binding agents for therapeutic and imaging use, published 2010/02/04 (WO 2010/014933 A2);

International PCT Patent Application Publication No. PCT/US2010/028020 to Pomper, Martin G.; Mease, Ronnie C.; Ray, Sangeeta; Chen, Ying. for PSMA-targeting compounds and uses thereof, published 2010/09/23 (WO 2010/108125 A2);

20 International PCT Patent Application Publication No. PCT/US2012/067162 to Pomper, Martin G. Mease, Ronnie C., Ray, Sangeeta, Shallal, Hassan for Homomultivalent and heteromultivalent inhibitors of prostate specific membrane antigen (PSMA) and uses thereof, published 2013/06/06 (WO2013/082338 A1)

25 International PCT Patent Application Publication No. PCT/US2011/026238 to Low P.S., Chelvam V., Kim Y. for PSMA binding ligand-linker conjugates and methods for using, published 2011/09/01 (WO2011/106639);

International PCT Patent Application Publication No. PCT/US2009/061067 to Low P.S., Chelvam V., Kim Y. for PSMA binding ligand-linker conjugates and methods for using, published 2010/04/22 (WO 2010/045598 A2)

30 International PCT Patent Application Publication No. PCT/US2008/073375 to Low P.S., Chelvam V., Kim Y. for PSMA binding ligand-linker conjugates and methods for using, published 2009/02/26 (WO 2009/026177 A1);

U.S. Patent Application Publication No. US 2004/0054190 A1 to Pomper

- MG, Zhang J, Kozikowski AP, Musachio JL. for Imaging agents and methods of imaging NAALADase of PSMA, Licensed to: Molecular Insight Pharmaceuticals, Inc. published 2004/03/18 (10/340,864);
- U.S. Patent Application Publication No. US 2008/0193381 A1 to Babich JW, Zimmerman CN, Maresca KP. for Heterodimers of glutamic acid, published 5 2008/08/14 (11/936,659);
- U.S. Patent Application Publication No. US 2013/0034494 A1 to Babich JW, Zimmerman C, Joyal JL, Lu G. for Radiolabeled prostate specific membrane antigen inhibitors, published 2013/02/07 (13/566,674); Afshar-Oromieh, A.; Haberkorn, U.; Eder, M.; Eisenhut, M.; Zechmann, C. M., [68Ga]Gallium-labelled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: comparison with 18F-FECH. *European journal of nuclear medicine and molecular imaging* **2012**, 39 (6), 1085-6;
- 10 Afshar-Oromieh, A.; Malcher, A.; Eder, M.; Eisenhut, M.; Linhart, H. G.; Hadaschik, B. A.; Holland-Letz, T.; Giesel, F. L.; Kratochwil, C.; Haufe, S.; Haberkorn, U.; Zechmann, C. M., PET imaging with a [68Ga]gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. *European journal of nuclear medicine and molecular imaging* **2013**, 40 (4), 486-95;
- 15 Afshar-Oromieh, A.; Haberkorn, U.; Hadaschik, B.; Habl, G.; Eder, M.; Eisenhut, M.; Schlemmer, H. P.; Roethke, M. C., PET/MRI with a 68Ga-PSMA ligand for the detection of prostate cancer. *European journal of nuclear medicine and molecular imaging* **2013**, 40 (10), 1629-30;
- 20 Afshar-Oromieh, A.; Zechmann, C. M.; Malcher, A.; Eder, M.; Eisenhut, M.; Linhart, H. G.; Holland-Letz, T.; Hadaschik, B. A.; Giesel, F. L.; Debus, J.; Haberkorn, U., Comparison of PET imaging with a (68)Ga-labelled PSMA ligand and (18)F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *European journal of nuclear medicine and molecular imaging* **2014**, 41 (1), 11-20;
- 25 Afshar-Oromieh, A.; Avtzi, E.; Giesel, F. L.; Holland-Letz, T.; Linhart, H. G.; Eder, M.; Eisenhut, M.; Boxler, S.; Hadaschik, B. A.; Kratochwil, C.; Weichert, W.; Kopka, K.; Debus, J.; Haberkorn, U., The diagnostic value of PET/CT imaging with the (68)Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. *European journal of nuclear medicine and molecular imaging* **2015**, 42 (2), 197-209;
- 30

- Ai-Darwich, M. J., Plenevaux, A., Lemaire, C., Fiore, G.D., Christianes, L., Comar, D., Luxen, A., Enantioselective synthesis of no-carrier-added (S)-4-chloro-2-[¹⁸F]fluorophenylalanine and (S)-α-methyl-4-chloro-2-[¹⁸F]fluorophenylalanine. *J. Fluor. Chem.* **1996**, 80, 117-124;
- 5 Bander, N. H.; Milowsky, M. I.; Nanus, D. M.; Kostakoglu, L.; Vallabhajosula, S.; Goldsmith, S. J., Phase I trial of 177lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2005**, 23 (21), 4591-601;
- 10 Banerjee SR, Foss CA, Castanares M, Mease RC, Byun Y, Fox JJ, Hilton J, Lupold S, Kozikowski AP, Pomper MG. Synthesis and evaluation of technetium-99m- and rhenium-labeled inhibitors of the prostate-specific membrane antigen (PSMA). *J Med Chem* **2008**; 51:4504-4517;
- 15 Banerjee SR, Pullambhatla M, Byun Y, Nimmagadda S, Foss CA, Green G, Fox JJ, Lupold SE, Mease RC, Pomper MG. Sequential SPECT and optical imaging of experimental models of prostate cancer with a dual modality inhibitor of the prostate-specific membrane antigen. *Angew Chem Int Ed Engl* **2011**; 50:9167-70.
- Banerjee SR, Pullambhatla M, Byun Y, Nimmagadda S, Green G, Fox JJ, Horti A, Mease RC, Pomper MG. 68Ga-labeled inhibitors of prostate-specific membrane antigen (PSMA) for imaging prostate cancer. *J Med Chem* **2010**, 53:5333-5341;
- 20 Banerjee, S.R, Pullambhatla, M., Foss, C.A., Nimmagadda, S. et al. 64Cu-labeled inhibitors of prostate-specific membrane antigen for PET imaging of prostate cancer. *Journal of medicinal chemistry*, **2014**, 57(6), 2657-69;
- 25 Banerjee, S. R.; Foss, C. A.; Pullambhatla, M.; Wang, Y.; Srinivasan, S.; Hobbs, R.; Baidoo, K. E.; Brechbiel, M. W.; Nimmagadda, S.; Mease, R. C.; Sgouros, G.; Pomper, M. G., Preclinical Evaluation of 86Y-Labeled Inhibitors of Prostate-Specific Membrane Antigen for Dosimetry Estimates. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **2015**;
- 30 Barinka C, Byun Y, Dusich CL, Ray S, Chen Y, Castanares M, Kozikowski AP, Mease RC, Pomper MG, Lubkowski J. Interactions between Human Glutamate Carboxypeptidase II and Urea-based Inhibitors: Structural Characterization. *J Med Chem*, **2008**; 51:7737-7743;

- Barinka, C.; Sacha, P.; Sklenar, J.; Man, P.; Bezouska, K.; Slusher, B. S.; Konvalinka, J., Identification of the N-glycosylation sites on glutamate carboxypeptidase II necessary for proteolytic activity. *Protein science : a publication of the Protein Society* **2004**, *13* (6), 1627-35;
- 5 Barrett, J. A.; Coleman, R. E.; Goldsmith, S. J.; Vallabhajosula, S.; Petry, N. A.; Cho, S.; Armor, T.; Stubbs, J. B.; Maresca, K. P.; Stabin, M. G.; Joyal, J. L.; Eckelman, W. C.; Babich, J. W., First-in-Man Evaluation of 2 High-Affinity PSMA-Avid Small Molecules for Imaging Prostate Cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **2013**, *54* (3), 380-7;
- 10 Byun, Y., Mease, R.C., Lupold, S., Pomper, M.G., Drug Design of Zinc-Enzyme Inhibitors. Functional Structureal and Disease Applications. In *Recent Developments of Diagnostic and Therapeutic Agents Targeting Glutamate Carboxypeptidase II (GCPII)* Supuran, C. T., Winum, J.Y., Ed. John Wiley & Sons: Hoboken, **2009**; pp 881-910;
- 15 Chang, S. S.; Reuter, V. E.; Heston, W. D.; Bander, N. H.; Grauer, L. S.; Gaudin, P. B., Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res* **1999**, *59* (13), 3192-8;
- 20 Chang, S. S.; Reuter, V. E.; Heston, W. D.; Gaudin, P. B., Comparison of anti-prostate-specific membrane antigen antibodies and other immunomarkers in metastatic prostate carcinoma. *Urology* **2001**, *57* (6), 1179-83;
- Cheng, Y.; Prusoff, W. H., Relationship between the inhibition constate (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I₅₀) of an enzymatic reaction. *Biochem Pharmacol* **1973**, *22*, 3099-3108;
- 25 Chen, Y.; Foss, C. A.; Byun, Y.; Nimmagadda, S.; Pullambhatla, M.; Fox, J. J.; Castanares, M.; Lupold, S. E.; Babich, J. W.; Mease, R. C.; Pomper, M. G., Radiohalogenated Prostate-Specific Membrane Antigen (PSMA)-Based Ureas as Imaging Agents for Prostate Cancer. *Journal of medicinal chemistry* **2008**, *51* (24), 7933-7943;
- 30 Chen Y, Dhara S, Banerjee S, Byun Y, Pullambhatla M, Mease RC, Pomper MG. A low molecular weight PSMA-based fluorescent imaging agent for cancer. *Biochem. Biophys Res. Commun.* 390: 624-629 (**2009**);
- Chen, Y.; Pullambhatla, M.; Banerjee, S. R.; Byun, Y.; Stathis, M.; Rojas, C.;

- Slusher, B. S.; Mease, R. C.; Pomper, M. G., Synthesis and biological evaluation of low molecular weight fluorescent imaging agents for the prostate-specific membrane antigen. *Bioconjugate chemistry* **2012**, *23* (12), 2377-85;
- Chen, Y.; Pullambhatla, M.; Foss, C. A.; Byun, Y.; Nimmagadda, S.;
- 5 Senthamizhchelvan, S.; Sgouros, G.; Mease, R. C.; Pomper, M. G., 2-(3-{1-Carboxy-5-[(6-[¹⁸F]fluoro-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid, [¹⁸F]DCFPyL, a PSMA-based PET imaging agent for prostate cancer. *Clin Cancer Res* **2011**, *17* (24), 7645-53;
- Cho SY, Gage KL, Mease RC, et al. Biodistribution, tumor detection, and
- 10 radiation dosimetry of ¹⁸F-DCFBC, a low molecular weight inhibitor of PSMA, in patients with metastatic prostate cancer. *J. Nucl. Med.* **2012**; 53:1883-1891.
- Dekker B, Keen H, Shaw D, Disley L, Hastings D, Hadfield J, Reader A, Allan D, Julyan P, Watson A, Zweit J. Functional comparison of annexin V analogues labeled indirectly and directly with iodine-124. *Nuclear Medicine and Biology* **2005**;
- 15 32:403-413;
- Dusich CL. Imaging prostate cancer: Design, synthesis and biological evaluation of optical and radioactive prostate-specific membrane antigen (PSMA) inhibitors. PhD Thesis Johns Hopkins University 2008;
- Eder, M.; Schafer, M.; Bauder-Wust, U.; Hull, W. E.; Wangler, C.; Mier, W.;
- 20 Haberkorn, U.; Eisenhut, M., ⁶⁸Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjugate chemistry* **2012**, *23* (4), 688-97;
- Foss CA, Mease RC, Fan H, Wang Y, Ravert HT, Dannals RF, Olszewski RT, Heston WD, Kozikowski AP, Pomper MG. Radiolabeled small molecule ligands for
- 25 prostate-specific membrane antigen: in vivo imaging in experimental models of prostate cancer. *Clin Cancer Res* **2005**; 11:4022-4028;
- Foss, C. A.; Mease, R. C.; Cho, S. Y.; Kim, H. J.; Pomper, M. G., GCPII imaging and cancer. *Current medicinal chemistry* **2012**, *19* (9), 1346-59;
- Garg S, Garg PK, Zalutsky MR. *N*-succinimidyl 5-(trialkylstannyl)-3-
- 30 pyridinecarboxylates: a new class of reagents for protein radioiodination. *Bioconjugate Chem.* **1991**; 2:50-56;

- Ghosh, A.; Heston, W. D., Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. *Journal of cellular biochemistry* **2004**, 91 (3), 528-39;
- Glaser, M., Arstad, E., Luthra, S.K., Robins, E.G., Two-step radiosynthesis of
5 [18F]N-succinimidy1-4-fluorobenzoate ([¹⁸F]SFB. *J. Label. Compd. Radiopharm.* **2009**, 52, 327-330;
- Hillier SM, Maresca KP, Femia FJ, Marquis JC, Foss CA, Nguyen N, Zimmerman CN, Barrett JA, Eckelman WC, Pomper MG, Joyal JL, Babich JW. Preclinical Evaluation of Novel Glutamate-Urea-Lysine Analogues That Target
10 Prostate-Specific Membrane Antigen as Molecular Imaging Pharmaceuticals for Prostate Cancer. *Cancer Res* **2009**; 69: 6932-40;
- Hillier, S. M.; Maresca, K. P.; Lu, G.; Merkin, R. D.; Marquis, J. C.; Zimmerman, C. N.; Eckelman, W. C.; Joyal, J. L.; Babich, J. W., 99mTc-labeled small-molecule inhibitors of prostate-specific membrane antigen for molecular
15 imaging of prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **2013**, 54 (8), 1369-76;
- Jackson, P. F.; Cole, D. C.; Slusher, B. S.; Stetz, S. L.; Ross, L. E.; Donzanti, B. A.; Trainor, D. A., Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase. *Journal of medicinal
20 chemistry* **1996**, 39 (2), 619-22;
- Jackson, P. F.; Tays, K. L.; Maclin, K. M.; Ko, Y. S.; Li, W.; Vitharana, D.; Tsukamoto, T.; Stoermer, D.; Lu, X. C.; Wozniak, K.; Slusher, B. S., Design and pharmacological activity of phosphinic acid based NAALADase inhibitors. *Journal of medicinal chemistry* **2001**, 44 (24), 4170-5; Kabalka, G. W.; Mereddy, A. R., A facile
25 no-carrier-added radioiodination procedure suitable for radiolabeling kits. *Nuclear medicine and biology* **2004**, 31 (7), 935-8;
- Kratochwil, C.; Giesel, F. L.; Eder, M.; Afshar-Oromieh, A.; Benesova, M.; Mier, W.; Kopka, K.; Haberkorn, U., [Lu]Lutetium-labelled PSMA ligand-induced remission in a patient with metastatic prostate cancer. *European journal of nuclear
30 medicine and molecular imaging* **2015**;
- Kularatne SA, Zhou Z, Yang J, Post CB, Low PS. Design, synthesis, and preclinical evaluation of prostate-specific membrane antigen (PSMA)-targeted-99mTc radioimaging agents. *Molecular Pharmaceutics* **2009**; 6(3) 790-800;

- Kurth M, Pelegrin A, Rose, K, Offord RE, Pochon S, Mach J-P, Buchegger F. Site-specific conjugation of a radioiodinated phenethylamine derivative to a monoclonal antibody results in increased radioactivity localization in tumor. *J. Med. Chem.* **1993**; 36:1255-1261;
- 5 Kozikowski, A. P.; Nan, F.; Conti, P.; Zhang, J.; Ramadan, E.; Bzdega, T.; Wroblewska, B.; Neale, J. H.; Pshenichkin, S.; Wroblewski, J. T., Design of remarkably simple, yet potent urea-based inhibitors of glutamate carboxypeptidase II (NAALADase). *Journal of medicinal chemistry* **2001**, 44 (3), 298-301;
- Kozikowski, A. P.; Zhang, J.; Nan, F.; Petukhov, P. A.; Grajkowska, E.;
- 10 Wroblewski, J. T.; Yamamoto, T.; Bzdega, T.; Wroblewska, B.; Neale, J. H., Synthesis of urea-based inhibitors as active site probes of glutamate carboxypeptidase II: efficacy as analgesic agents. *Journal of medicinal chemistry* **2004**, 47 (7), 1729-38;
- Lapi, S. E.; Wahnische, H.; Pham, D.; Wu, L. Y.; Nedrow-Byers, J. R.; Liu, T.; Vejdani, K.; VanBrocklin, H. F.; Berkman, C. E.; Jones, E. F., Assessment of an 18F-
- 15 labeled phosphoramidate peptidomimetic as a new prostate-specific membrane antigen-targeted imaging agent for prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **2009**, 50 (12), 2042-8;
- Litosh, V. A. H., M.N.; Stupi, B.P.; Wu, W.; Metzker, M.L. Nucleotides and nucleosides and methods for their use in DNA sequencing. 2009;
- 20 Majer, P.; Jackson, P. F.; Delahanty, G.; Grella, B. S.; Ko, Y. S.; Li, W.; Liu, Q.; Maclin, K. M.; Polakova, J.; Shaffer, K. A.; Stoermer, D.; Vitharana, D.; Wang, E. Y.; Zakrzewski, A.; Rojas, C.; Slusher, B. S.; Wozniak, K. M.; Burak, E.; Limsakun, T.; Tsukamoto, T., Synthesis and biological evaluation of thiol-based inhibitors of glutamate carboxypeptidase II: discovery of an orally active GCP II
- 25 inhibitor. *Journal of medicinal chemistry* **2003**, 46 (10), 1989-96;
- Makarasen, A., Nishikawa, T., Isobe, M., Synthesis of four lysine-linked cereulide analogues showing ionophoric activity towards potassium cations as lead compounds for emetic toxin detection by immunoassays. *Synthesis* **2009**, 2184-2004;
- Maresca, K. P.; Hillier, S. M.; Femia, F. J.; Keith, D.; Barone, C.; Joyal, J. L.;
- 30 Zimmerman, C. N.; Kozikowski, A. P.; Barrett, J. A.; Eckelman, W. C.; Babich, J. W., A series of halogenated heterodimeric inhibitors of prostate specific membrane antigen (PSMA) as radiolabeled probes for targeting prostate cancer. *Journal of medicinal chemistry* **2009**, 52 (2), 347-57;

- Mease RC, Dusich CL, Foss CA, Ravert HT, Dannals RF, Seidel J, Prideaux A, Fox JJ, Sgouros G, Kozikowski AP, Pomper MG. Synthesis and *in vivo* evaluation of N-[N-[(S)-1,3-dicarboxypropyl]carbamoyl]-4-[18F]fluorobenzyl-L-cysteine, [18F]DCFBC: a new imaging probe for prostate cancer. *Clin Cancer Res* **2008**; 14:3036-3043;
- Mease, R. C.; Foss, C. A.; Pomper, M. G., PET imaging in prostate cancer: focus on prostate-specific membrane antigen. *Current topics in medicinal chemistry* **2013**, 13 (8), 951-62;
- Mesters, J. R.; Barinka, C.; Li, W.; Tsukamoto, T.; Majer, P.; Slusher, B. S.; Konvalinka, J.; Hilgenfeld, R., Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. *Embo J* **2006**, 25 (6), 1375-84;
- Perner, S.; Hofer, M. D.; Kim, R.; Shah, R. B.; Li, H.; Moller, P.; Hautmann, R. E.; Gschwend, J. E.; Kuefer, R.; Rubin, M. A., Prostate-specific membrane antigen expression as a predictor of prostate cancer progression. *Human pathology* **2007**, 38 (5), 696-701;
- Pinto, J. T.; Suffoletto, B. P.; Berzin, T. M.; Qiao, C. H.; Lin, S.; Tong, W. P.; May, F.; Mukherjee, B.; Heston, W. D., Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. *Clin Cancer Res* **1996**, 2 (9), 1445-51;
- Maung, J.; Mallari, J. P.; Girtsman, T. A.; Wu, L. Y.; Rowley, J. A.; Santiago, N. M.; Brunelle, A. N.; Berkman, C. E., Probing for a hydrophobic a binding register in prostate-specific membrane antigen with phenylalkylphosphonamides. *Bioorganic & medicinal chemistry* **2004**, 12 (18), 4969-79;
- Pomper MG, Musachio JL, Zhang J, Zhou Y, Scheffel U, Hilton J, Maini A, Dannals RF, Wong DF, Kozikowski AP. 11C-MCG: Synthesis, uptake selectivity and primate PET of a probe for glutamate carboxypeptidase II (NAALADase.) *Mol Imaging* **2002**; 1:96-101;
- Rajasekaran, A. K.; Anilkumar, G.; Christiansen, J. J., Is prostate-specific membrane antigen a multifunctional protein? *American journal of physiology. Cell physiology* **2005**, 288 (5), C975-81;
- Ray Banerjee, S.; Pullambhatla, M.; Foss, C. A.; Falk, A.; Byun, Y.; Nimmagadda, S.; Mease, R. C.; Pomper, M. G., Effect of chelators on the pharmacokinetics of (99m)Tc-labeled imaging agents for the prostate-specific

- membrane antigen (PSMA). *Journal of medicinal chemistry* **2013**, 56 (15), 6108-21;
- Reske, S. N.; Winter, G.; Baur, B.; Machulla, H. J.; Kull, T., Comment on Afshar-Oromieh et al.: PET imaging with a [⁶⁸Ga]gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of
- 5 tumour lesions. *European journal of nuclear medicine and molecular imaging* **2013**, 40 (6), 969-70;
- Rowe, S. P. G., K.L.; Macura, K.; Guner, G.; Faraj, S.F.; Munari, E.; Rodriquez, R.; Han, M.; Blackford, A.; Netto, G.; Lodge, M.; Mease, R.C.; Pomper, M.G., Cho, S.Y., PSMA-based low molecular weight ¹⁸F-DCFBC PET/CT for
- 10 detection and characterization of primary prostate cancer. *J. Nucl. Med.* **2015**, In Press;
- Rowe SP, Gorin MA, Hammers HJ, Javadi MS, Hawasli H, Szabo Z, Cho SY, Pomper MG, Allaf ME. Imaging of metastatic clear cell renal cell carcinoma with PSMA-target ¹⁸F-DCFPyL PET/CT;
- 15 Silver, D. A.; Pellicer, I.; Fair, W. R.; Heston, W. D.; Cordon-Cardo, C., Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res* **1997**, 3 (1), 81-5;
- Slusher, B. S.; Tsai, G.; Yoo, G.; Coyle, J. T., Immunocytochemical localization of the N-acetyl-aspartyl-glutamate (NAAG) hydrolyzing enzyme N-
- 20 acetylated alpha-linked acidic dipeptidase (NAALADase). *The Journal of comparative neurology* **1992**, 315 (2), 217-29;
- Stoermer, D.; Vitharana, D.; Hin, N.; Delahanty, G.; Duvall, B.; Ferraris, D. V.; Grella, B. S.; Hoover, R.; Rojas, C.; Shanholtz, M. K.; Smith, K. P.; Stathis, M.; Wu, Y.; Wozniak, K. M.; Slusher, B. S.; Tsukamoto, T., Design, synthesis, and
- 25 pharmacological evaluation of glutamate carboxypeptidase II (GCP II) inhibitors based on thioalkylbenzoic acid scaffolds. *Journal of medicinal chemistry* **2012**, 55 (12), 5922-32;
- Stoermer, D.; Liu, Q.; Hall, M. R.; Flanary, J. M.; Thomas, A. G.; Rojas, C.; Slusher, B. S.; Tsukamoto, T., Synthesis and biological evaluation of hydroxamate-
- 30 Based inhibitors of glutamate carboxypeptidase II. *Bioorganic & medicinal chemistry letters* **2003**, 13 (13), 2097-100;
- Szabo, Z.; Mena, E.; Rowe, S. P.; Plyku, D.; Nidal, R.; Eisenberger, M. A.; Antonarakis, E. S.; Fan, H.; Dannals, R. F.; Chen, Y.; Mease, R. C.; Vranesic, M.;

- Bhatnagar, A.; Sgouros, G.; Cho, S. Y.; Pomper, M. G., Initial Evaluation of [F]DCFPyL for Prostate-Specific Membrane Antigen (PSMA)-Targeted PET Imaging of Prostate Cancer. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging* **2015**;
- 5 Tsukamoto, T.; Wozniak, K. M.; Slusher, B. S., Progress in the discovery and development of glutamate carboxypeptidase II inhibitors. *Drug discovery today* **2007**, *12* (17-18), 767-76
- Tang, G., Zeng, W., Yu, M., Kabalka, G. Facile synthesis of N-succinimidyl-4-[¹⁸F]fluorobenzoate ([¹⁸F]SFB for protein labeling. *J Label Comp Radiopharm*,
10 **2008**, *51*, 68-71;
- Talanov, VS, Garmestani K, Regino CAS, Milenic DE, Plascjak PS, Waldmann TA, Brechbiel MW. Preparation and in vivo evaluation of a novel stabilized linker for ²¹¹At labeling of protein. *Nucl. Med. Biol.* **2006**; *33*:469-480;
- Vaidyanathan G, Zalutsky MR. Synthesis of N-succinimidyl 4-
15 guanidinomethyl-3-[¹²⁵I]iodobenzoate: a radio-iodination agent for labeling internalizing proteins and peptides. *Nat Protoc* **2007**; *2*:282-286;
- Vallabhajosula, S.; Nikolopoulou, A.; Babich, J. W.; Osborne, J. R.; Tagawa, S. T.; Lipai, I.; Solnes, L.; Maresca, K. P.; Armor, T.; Joyal, J. L.; Crummet, R.; Stubbs, J. B.; Goldsmith, S. J., ^{99m}Tc-labeled small-molecule inhibitors of prostate-specific membrane antigen: pharmacokinetics and biodistribution studies in healthy
20 subjects and patients with metastatic prostate cancer. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **2014**, *55* (11), 1791-8;
- Vargas, H. A.; Grimm, J.; O, F. D.; Sala, E.; Hricak, H., Molecular imaging of prostate cancer: translating molecular biology approaches into the clinical realm.
25 *European radiology* **2015**
- Wang, H.; Byun, Y.; Barinka, C.; Pullambhatla, M.; Bhang, H. E.; Fox, J. J.; Lubkowski, J.; Mease, R. C.; Pomper, M. G., Bioisosterism of urea-based GCPII inhibitors: Synthesis and structure-activity relationship studies. *Bioorganic & medicinal chemistry letters* **2010**, *20* (1), 392-7;
- 30 Wang, E. K., H.C.; Szardenings, A.K., Liu, C.; Walsh, J.C.; Chen, G.; Sinha, A.; Kasi, D.; Gangadharmath, U.B.; Yu, C.; Zhang, W.; Zhao, T.; Mocharla, V.P. PSMA Imaging Agents. 28.02.2013, **2013**;
- Weineisen, M. S., J.; Schottelius, M.; Schwaiger, M.; Wester, H-J., Synthesis

and preclinical evaluation of DOTAGA-conjugated PSMA ligands for functional imaging and endoradiotherapy of prostate cancer. *EJNMMI* **2014**, 4 (63);

Winkler, J. W.; Uddin, J.; Serhan, C. N.; Petasis, N. A., Stereocontrolled total synthesis of the potent anti-inflammatory and pro-resolving lipid mediator resolvin

- 5 D3 and its aspirin-triggered 17R-epimer. *Organic letters* **2013**, 15 (7), 1424-7;

Zalutsky, M. R.; Narula, A. S., A method for the radiohalogenation of proteins resulting in decreased thyroid uptake of radioiodine. *Int J Rad Appl Instrum Part A* **1987**, 38 (1051-1055);

- 10 Zechmann, C. M.; Afshar-Oromieh, A.; Armor, T.; Stubbs, J. B.; Mier, W.; Hadaschik, B.; Joyal, J.; Kopka, K.; Debus, J.; Babich, J. W.; Haberkorn, U., Radiation dosimetry and first therapy results with a (124)I/ (131)I-labeled small molecule (MIP-1095) targeting PSMA for prostate cancer therapy. *European journal of nuclear medicine and molecular imaging* **2014**, 41 (7), 1280-92;

- 15 Zhong, C.; Zhao, X.; Sarva, J.; Kozikowski, A.; Neale, J. H.; Lyeth, B. G., NAAG peptidase inhibitor reduces acute neuronal degeneration and astrocyte damage following lateral fluid percussion TBI in rats. *J Neurotrauma* **2005**, 22 (2), 266-76;

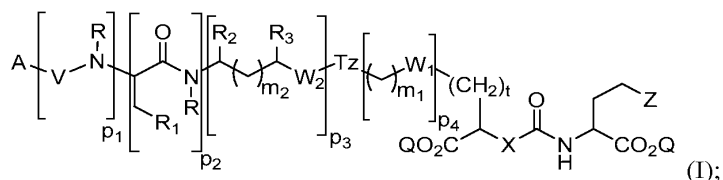
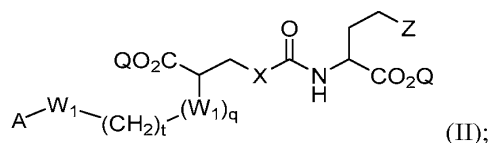
Zhou J, Neale JH, Pomper MG, Kozikowski AP. NAAG Peptidase inhibitors and their potential for diagnosis and therapy. *Nat Rev Drug Discovery* **2005**; 4:1015-1026.

20

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

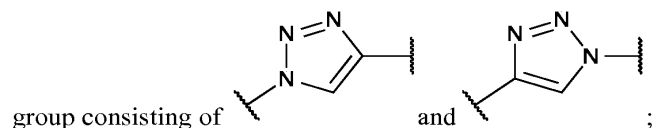
THAT WHICH IS CLAIMED:

1. A compound of formula (I) or formula (II):



- 5 wherein the subunits associate with elements p_1 , p_2 , p_3 and p_4 may be in any order;
- Z is tetrazole or CO_2Q ;
- Q is H or a protecting group;
- X is O or NH;
- 10 q is an integer selected from the group consisting of 0 and 1;
- t is an integer selected from the group consisting of 1, 2, 3, and 4;
- p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different;
- p_1 , p_3 , and p_4 are each independently 0 or 1;
- 15 m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6;
- W_1 is selected from the group consisting of a bond, $-\text{S}-$, $-\text{C}(=\text{O})-\text{NR}-$, and $-\text{NR}-\text{C}(=\text{O})-$;
- W_2 is selected from the group consisting of a bond, $-\text{S}-$, $-\text{CH}_2-\text{C}(=\text{O})-\text{NR}-$, $-\text{C}(\text{O})-$, $-\text{NRC}(\text{O})-$, $-\text{NRC}(\text{O})\text{NR}-$, $-\text{NRC}(\text{S})\text{NR}-$, $-\text{NRC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NR}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NR}-$, $-\text{NR}-\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $-(\text{O}-\text{CH}_2-\text{CH}_2)_r-$ and $-(\text{CH}_2-\text{CH}_2-\text{O})_r-$,
- 20 wherein r is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8;
- each R is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl;

Tz is a triazole group that can be present or absent and is selected from the



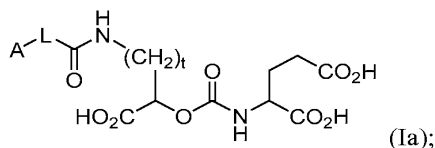
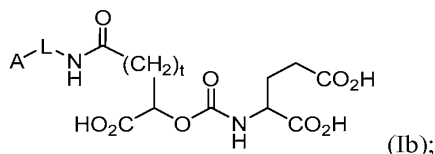
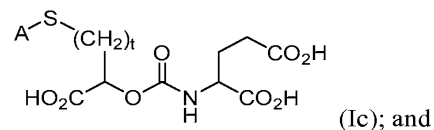
each R_1 is independently H, C_1 - C_6 alkyl, C_2 - C_{12} aryl or C_4 - C_{16} alkylaryl;

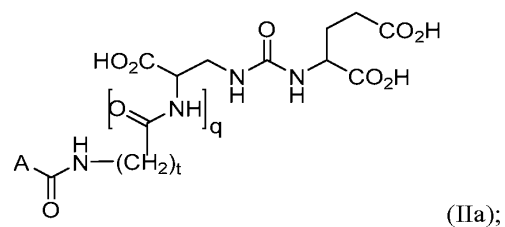
R_2 and R_3 are each independently H and CO_2R_4 , wherein R_4 is selected from
 5 the group consisting of H, C_1 - C_6 alkyl, C_2 - C_{12} aryl, and C_4 - C_{16} alkylaryl, wherein
 when one of R_2 or R_3 is CO_2R_4 , then the other is H;

V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$.

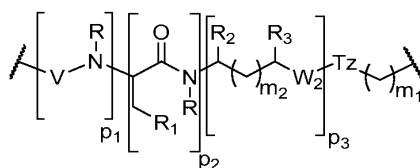
A is selected from the group consisting of naphthyl, biphenyl, substituted or
 10 unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can
 optionally comprise one or more radioactive isotope suitable for imaging and /or
 radiotherapy, or a photosensitizing dye suitable for imaging and/or photodynamic
 therapy; and stereoisomers and pharmaceutically acceptable salts thereof.

15 2. The compound of claim 1, wherein the compound of formula (I) is
 selected from the group consisting of:



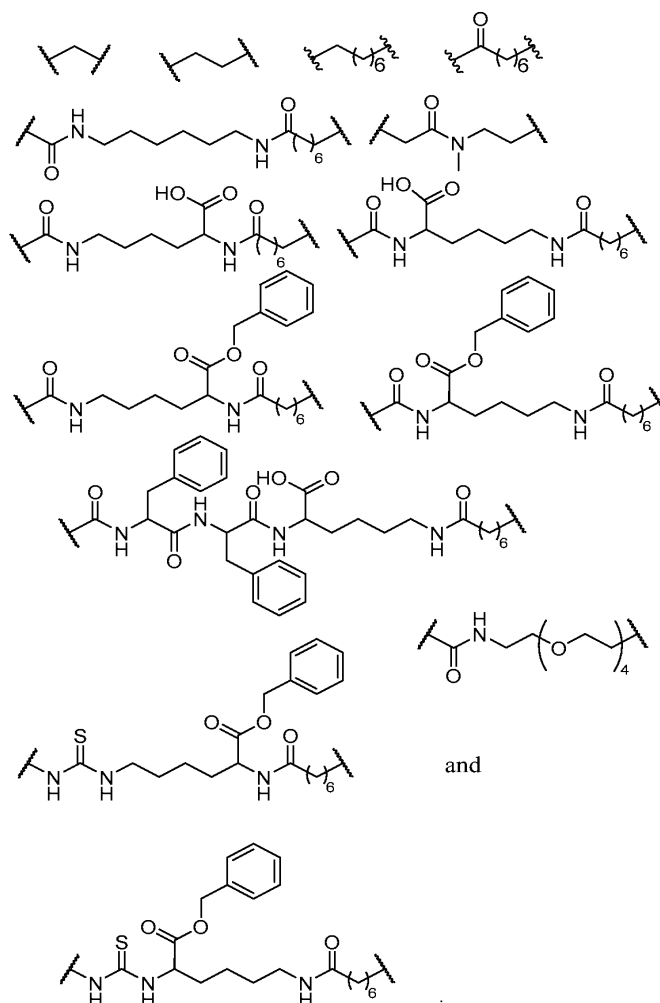


wherein L is a linker that can be present or absent, and has the following general structure:

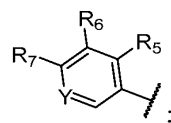


5 wherein p_1 , p_2 , p_3 , m_1 , m_2 , q , t , Tz , W_2 , R , R_1 , R_2 , R_3 , V and A are defined as hereinabove.

3. The compound of claim 1, wherein L is selected from the group consisting of:



4. The compound of claim 1, wherein A has the following general structure:



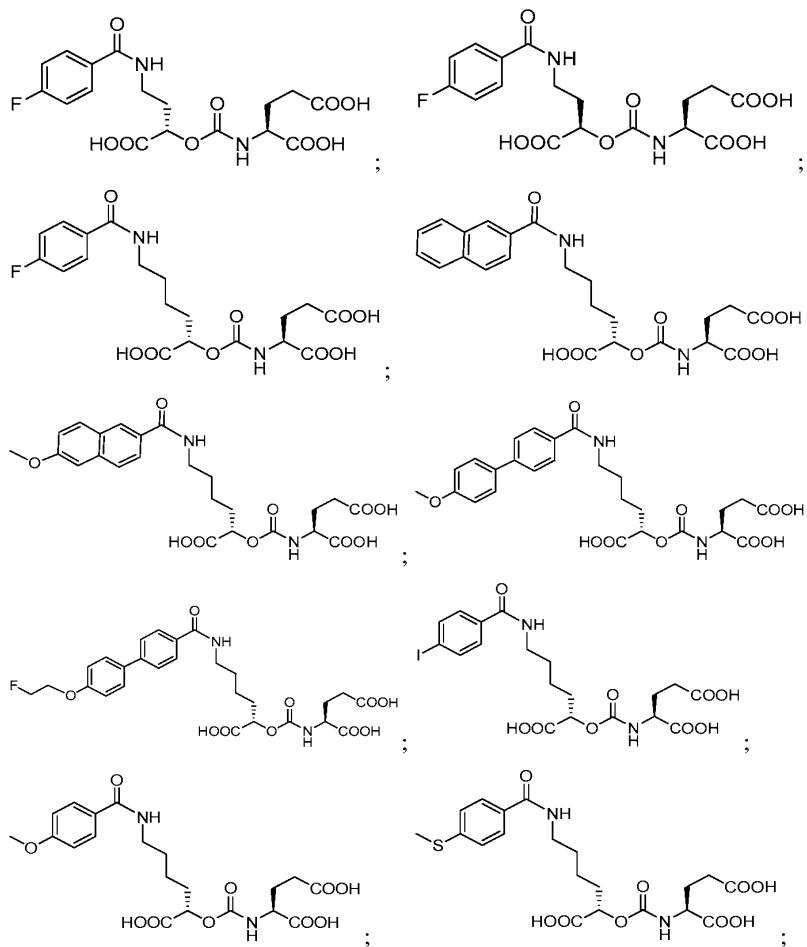
5

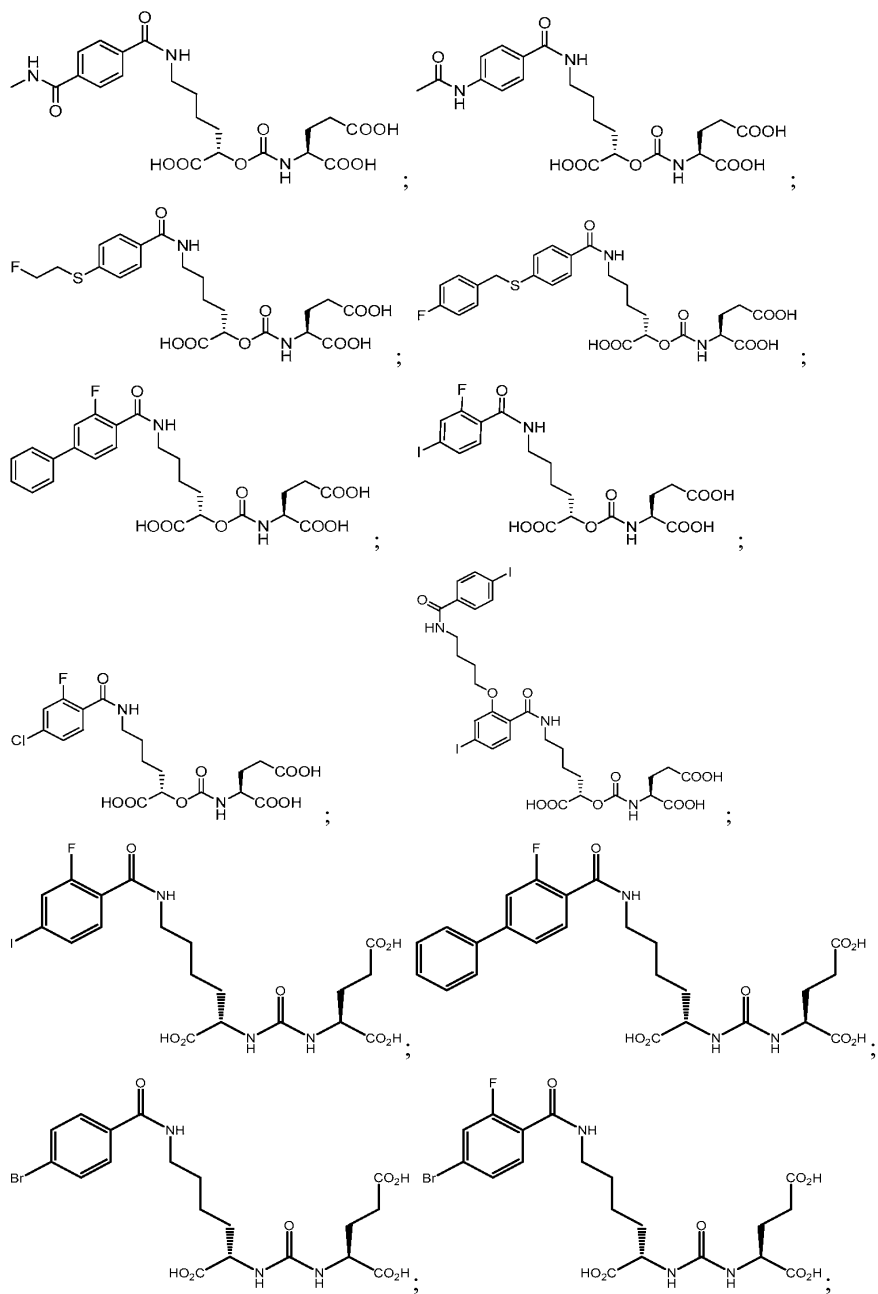
wherein:

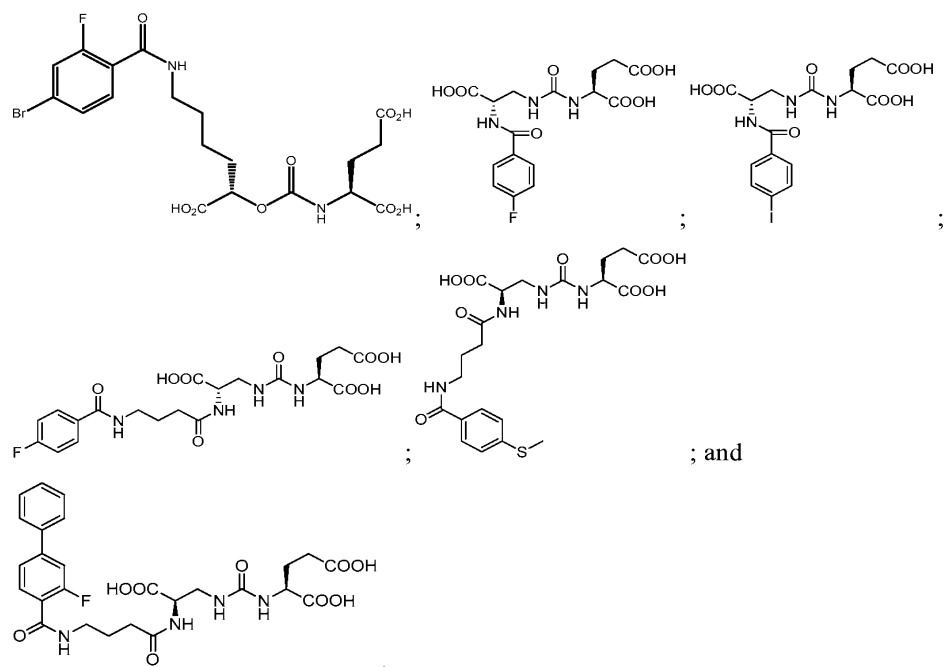
Y is CH or N;

and R₅, R₆, and R₇ are each independently selected from the group consisting of H, halogen, alkoxy, alkylthioether, substituted and unsubstituted aryl, CH₂-NH-C(=NH)-NH₂-NH-(C=O)-R₈, wherein R₈ is alkyl, -(C=O)-NR₉R₁₀, wherein R₉ and R₁₀ are each independently selected from the group consisting of H and alkyl, -X₁-(CH₂)_p-Ph-X₂, -X₁-(CH₂)_p-X₂, -X₁-(CH₂)_p-NH-C(=O)-Ph-X₂, wherein each X₁ is independently O or S, each p is independently an integer selected from the group consisting of 1, 2, 3, and 4, each Ph is phenyl, and each X₂ is halogen.

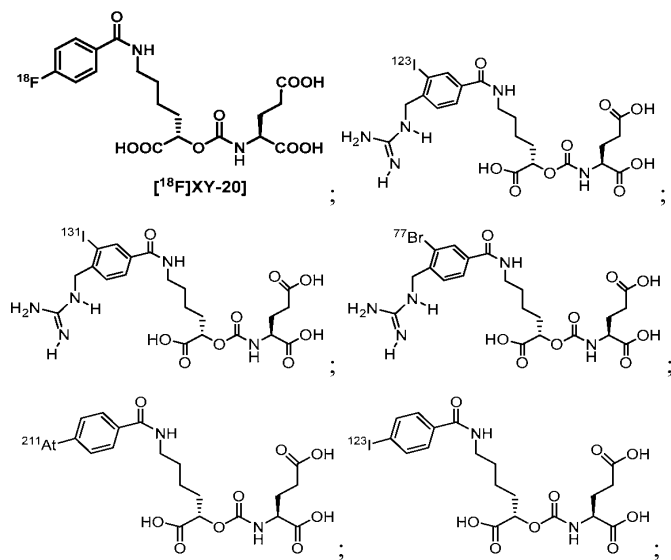
5. The compound of claim 1, wherein the compound of formula (I) is selected from the group consisting of:

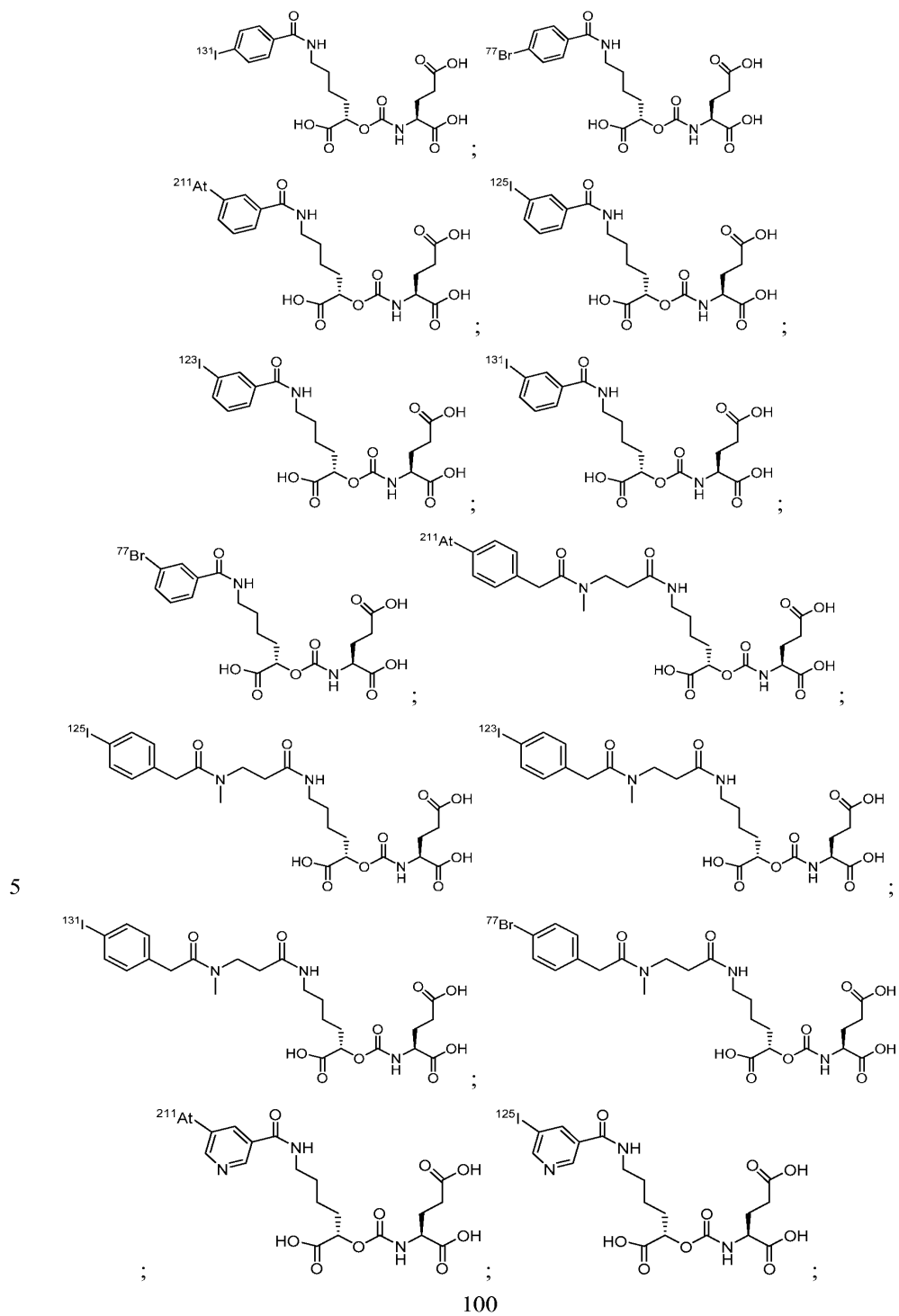


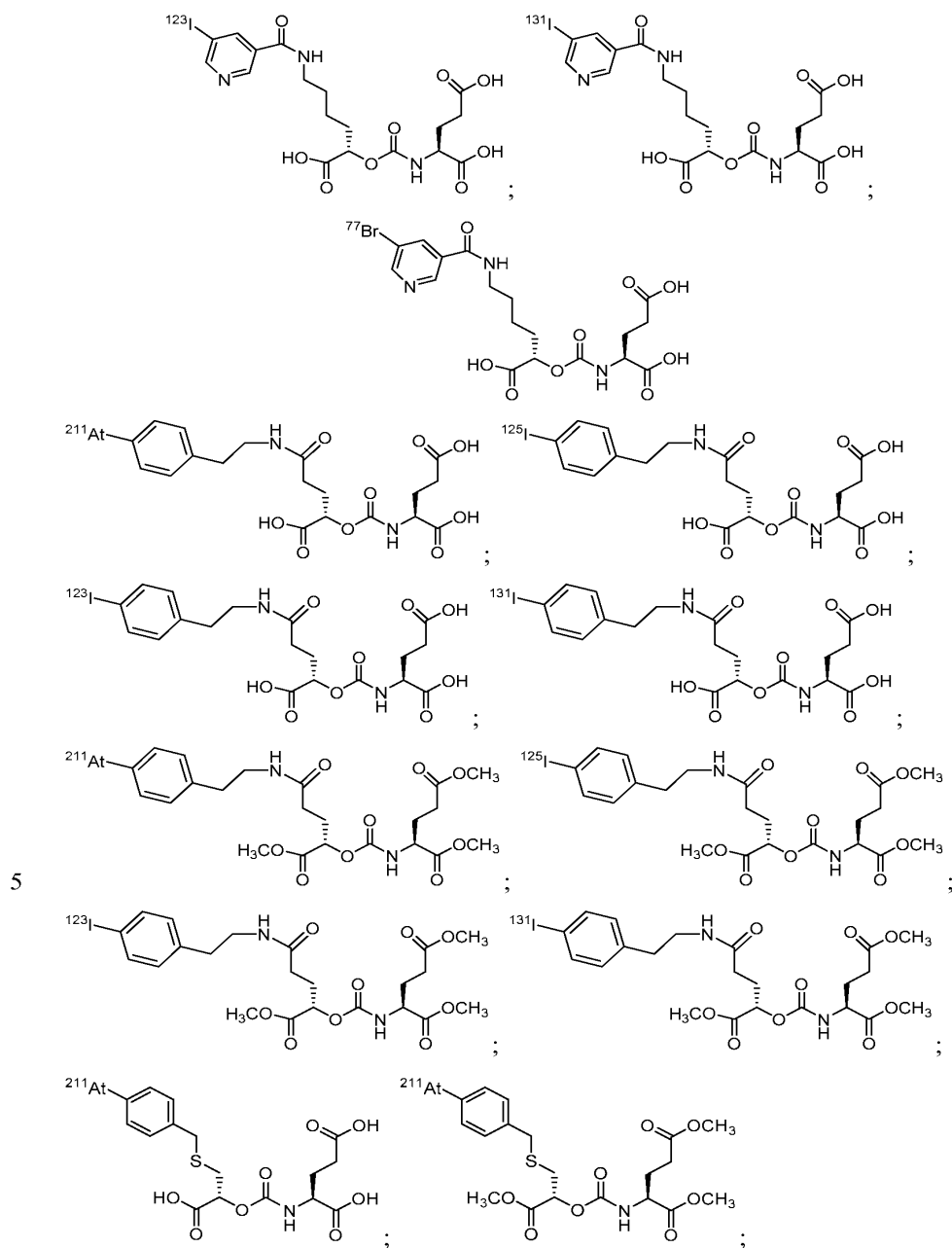


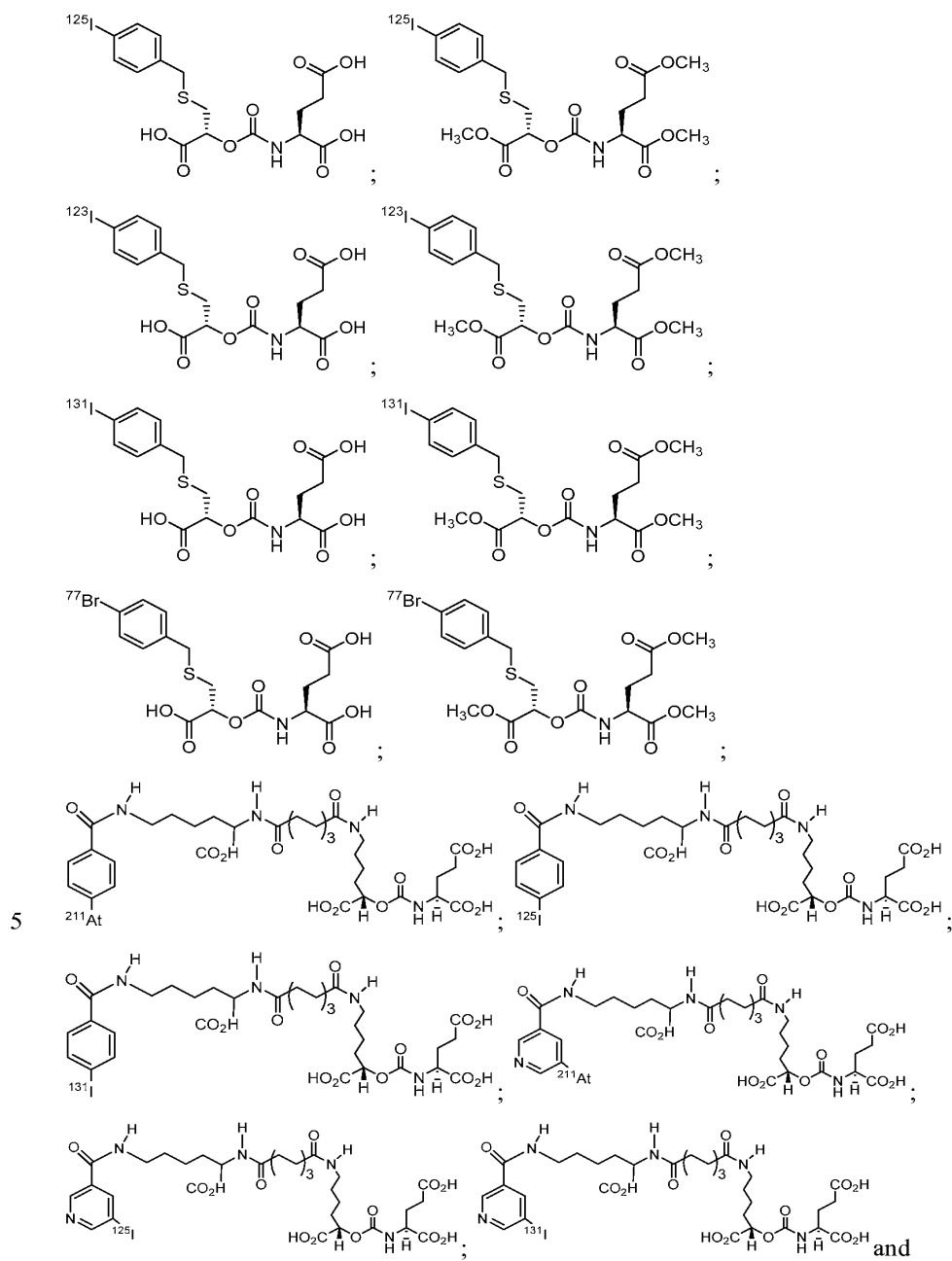


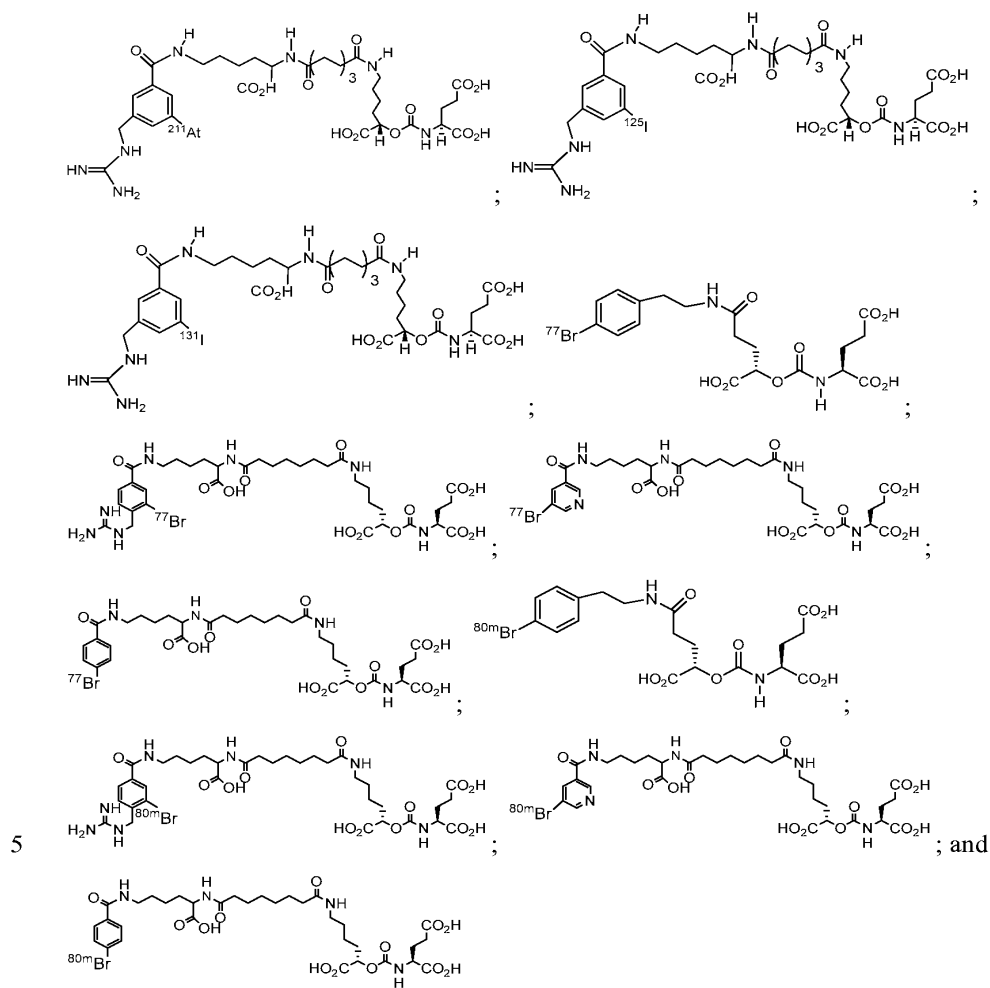
- 5 6. The compound of claim 1, wherein the compound of formula (I) is selected from the group consisting of:







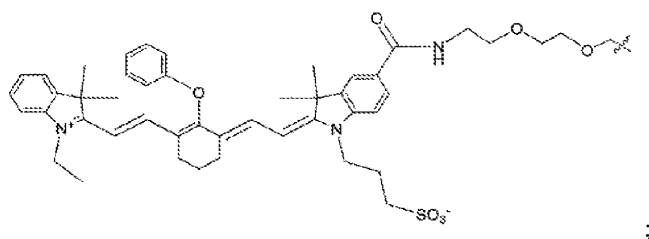
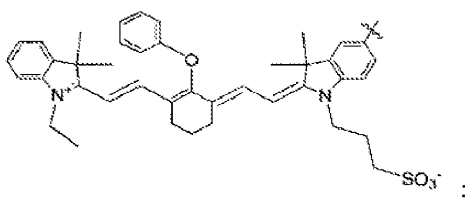
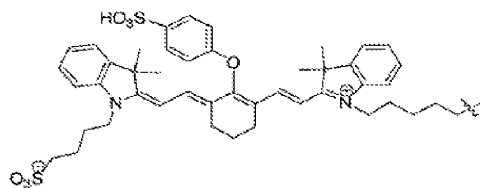
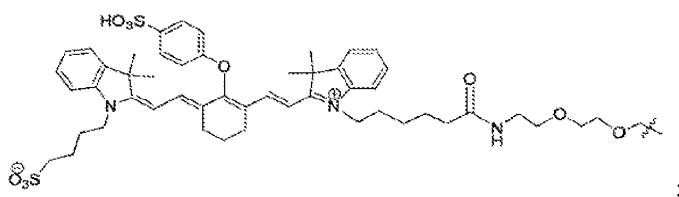




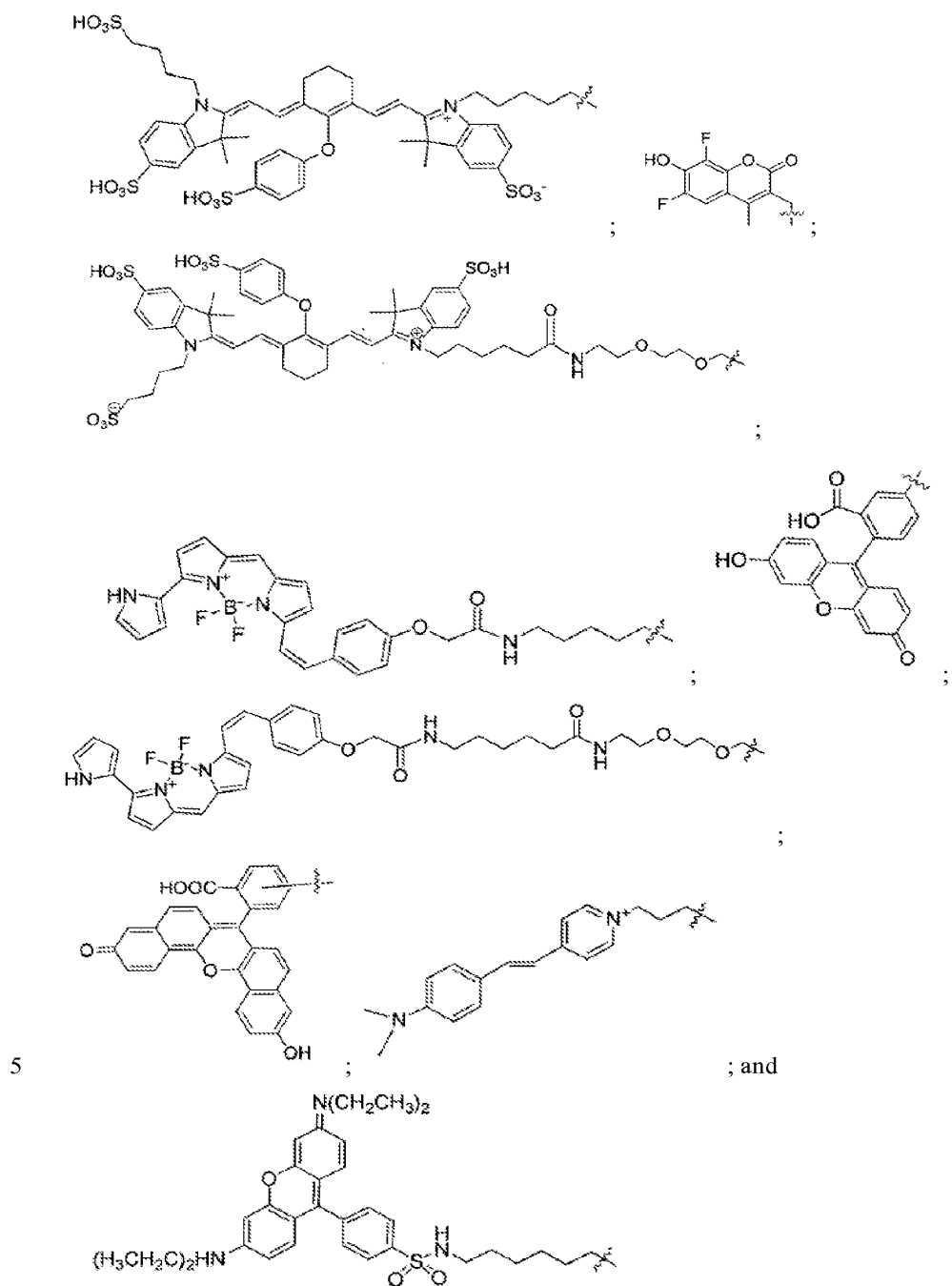
7. The compound of claim 1, wherein the photosensitizing dye suitable for imaging and/or photodynamic therapy is a fluorescent dye moiety which emits in the visible or near infrared spectrum, wherein the fluorescent dye moiety comprises carbocyanine, indocarbocyanine, oxacarbocyanine, thiocarbocyanine and merocyanine, polymethine, coumarine, rhodamine, xanthene, fluorescein, boron-dipyrromethane (BODIPY), Cy5, Cy5.5, Cy7, VivoTag-680, VivoTag-S680, VivoTag-S750, AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790, Dy677, Dy676, Dy682, Dy752, Dy780, DyLight547, Dylight647, HiLyte Fluor 647, HiLyte Fluor 680, HiLyte Fluor 750, IRDye 800CW, IRDye

800RS, IRDye 700DX, ADS780WS, ADS830WS, and ADS832WS; and wherein R₁ or R₂ can optionally be a radioactive isotope suitable for imaging or radiotherapy or optionally substituted with said radioisotope.

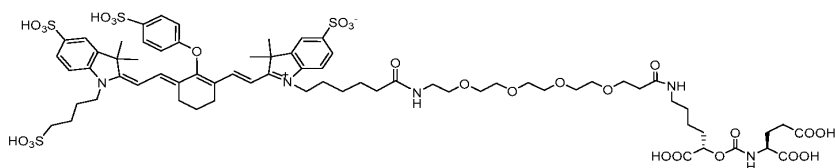
- 5 8. The compound of claim 7, wherein the fluorescent dye moiety is selected from the group consisting of:



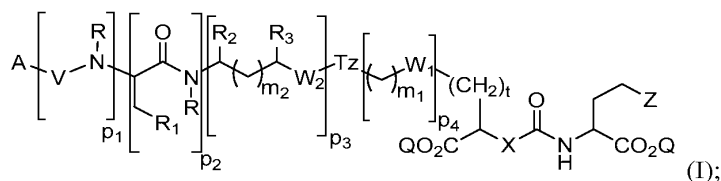
10



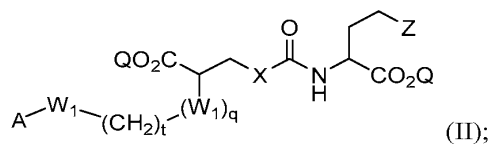
9. The compound of claim 7, wherein the compound is



10. A method for imaging one or more prostate-specific membrane antigen (PSMA) tumors or cells, the method comprising contacting the one or more tumors or cells with an effective amount of a compound of formula (I) and/or formula (II), including compounds of formula (Ia), (Ib), (Ic), and (IIa), and making an image, the compound of formula (I) or formula (II) comprising:



10



(II);

wherein the subunits associate with elements p₁, p₂, p₃ and p₄ may be in any order;

Z is tetrazole or CO₂Q;

Q is H or a protecting group;

15 X is O or NH;

q is an integer selected from the group consisting of 0 and 1;

t is an integer selected from the group consisting of 1, 2, 3, and 4;

p₂ is an integer selected from the group consisting of 0, 1, 2, and 3, and when p₂ is 2 or 3, each R₁ is the same or different;

20 p₁, p₃, and p₄ are each independently 0 or 1;

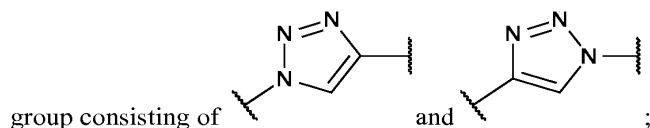
m₁ and m₂ are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6;

W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$;

W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NRC(O)NR-$, $-NRC(S)NR-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_r-$ and $-(CH_2-CH_2-O)_r-$,
 5 wherein r is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8;

each R is independently H, C_1-C_6 alkyl, C_2-C_{12} aryl, and C_4-C_{16} alkylaryl;

Tz is a triazole group that can be present or absent and is selected from the



10 each R_1 is independently H, C_1-C_6 alkyl, C_2-C_{12} aryl or C_4-C_{16} alkylaryl;

R_2 and R_3 are each independently H and CO_2R_4 , wherein R_4 is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_{12} aryl, and C_4-C_{16} alkylaryl, wherein when one of R_2 or R_3 is CO_2R_4 , then the other is H;

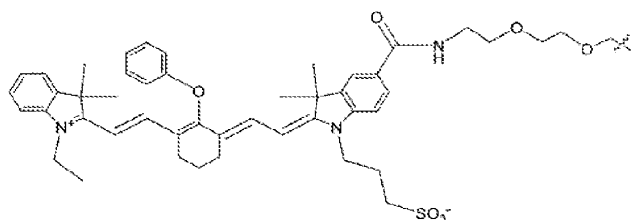
V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$.
 15

A is selected from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can optionally comprise one or more radioactive isotope suitable for imaging or a photosensitizing dye suitable for imaging; and stereoisomers and pharmaceutically
 20 acceptable salts thereof.

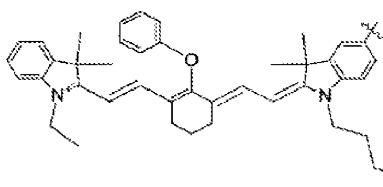
11. The method of claim 10, wherein the image is made using positron emission tomography (PET) and the radiohalogen is selected from the group consisting of ^{18}F and ^{124}I .
 25

12. The method of claim 10, wherein the image is made using Single-photon emission computed tomography (SPECT) and the radiohalogen is selected from the group consisting of ^{77}Br , ^{131}I , ^{125}I , and ^{123}I .

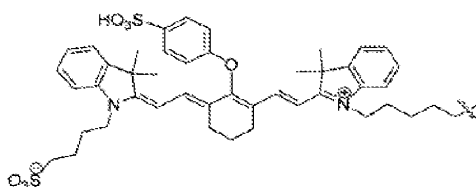
13. The method of claim 10, wherein the image is made using florescent optical imaging and the photosensitizing dye suitable for imaging is selected from the group consisting of



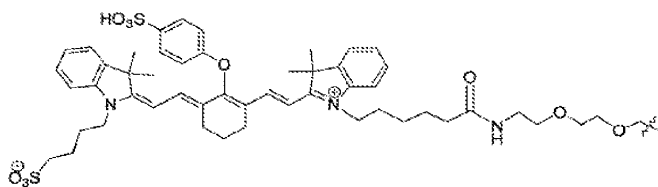
:



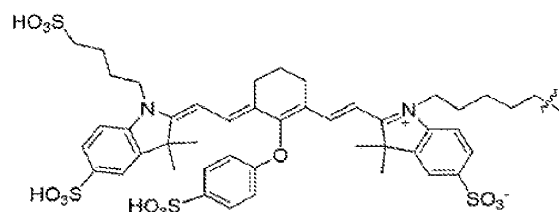
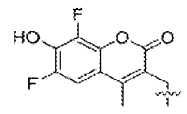
:



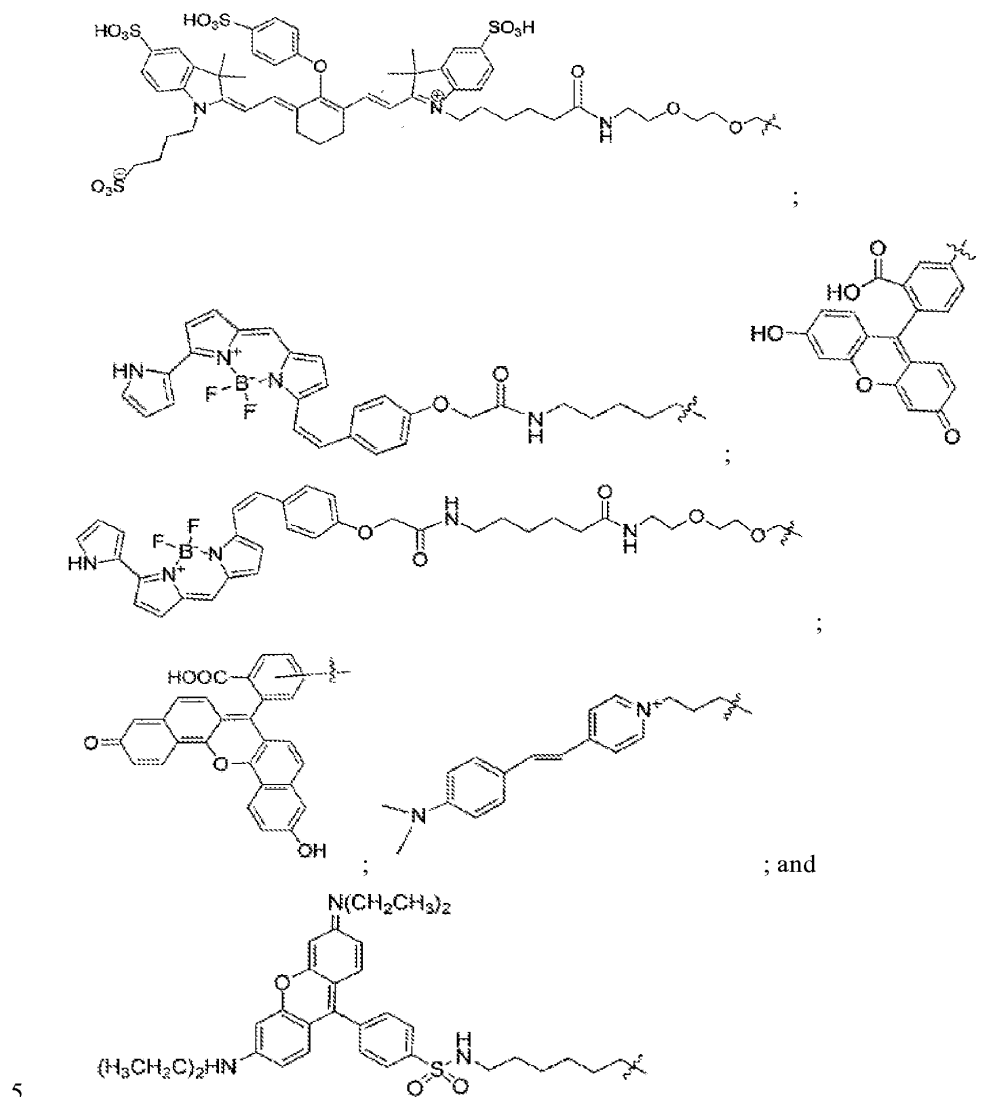
•



•



•



pheochromocytoma, an esophageal tumor or cell, a stomach tumor or cell, and combinations thereof.

15 15. The method of claim 10, wherein the one or more PSMA-expressing tumors or cells is a prostate tumor or cell.

 16. The method of claim 10, wherein the one or more PSMA-expressing tumors or cells is *in vitro*, *in vivo*, or *ex vivo*.

10 17. The method of claim 10, wherein the one or more PSMA-expressing tumors or cells is present in a subject.

 18. The method of claim 17, wherein the compound of formula (I) comprising the imaging agent substantially localizes to the tumor or cell within about 15 60 minutes of administration.

 19. The method of claim 17, wherein the compound of formula (I) comprising the radioisotope suitable for imaging substantially localizes to the tumor or cell within about 30 minutes of administration.

20

 20. The method of claim 17, wherein the compound of formula (I) comprising the radioisotope suitable for imaging substantially localizes to the tumor or cell within about 10 minutes of administration.

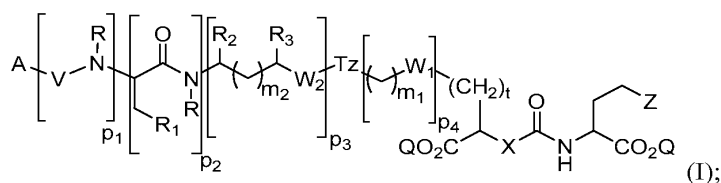
25 21. The method of claim 17, wherein the compound of formula (I) comprising the radioisotope suitable for imaging is cleared from the tumor or cell in the subject.

 22. The method of claim 21, wherein the compound of formula (I) comprising the radioisotope suitable for imaging is cleared more rapidly from a 30 subject's kidneys than from a tumor of the subject.

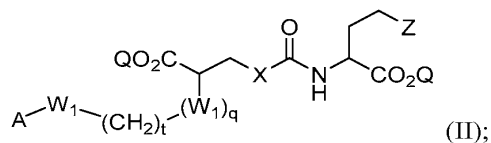
23. The method of claim 17, wherein the compound comprising the photosensitizing dye suitable for imaging is visible at about 4 hours after injection.

24. The method of claim 17, wherein the compound comprising the photosensitizing dye suitable for imaging presents the brightest signal at about 24 hours after injection.

25. A method for treating or preventing a disease or condition associated with one or more PSMA expressing tumors or cells, the method comprising administering at least one compound of formula (I) and/or formula (II), including compounds of formula (Ia), (Ib), (Ic), and (IIa), to a subject in an amount effective to treat or prevent the disease or condition, the compound of formula (I) and/or formula (II) comprising:



15



wherein the subunits associate with elements p_1 , p_2 , p_3 and p_4 may be in any order;

Z is tetrazole or CO_2Q ;

Q is H or a protecting group;

20 X is O or NH;

q is an integer selected from the group consisting of 0 and 1;

t is an integer selected from the group consisting of 1, 2, 3, and 4;

p_2 is an integer selected from the group consisting of 0, 1, 2, and 3, and when p_2 is 2 or 3, each R_1 is the same or different;

25 p_1 , p_3 , and p_4 are each independently 0 or 1;

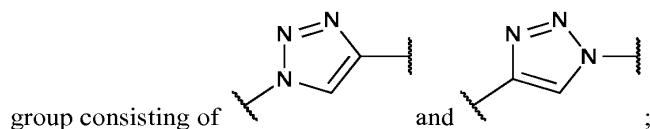
m_1 and m_2 are each an integer independently selected from the group consisting of 0, 1, 2, 3, 4, 5, and 6;

W_1 is selected from the group consisting of a bond, $-S-$, $-C(=O)-NR-$, and $-NR-C(=O)-$;

- 5 W_2 is selected from the group consisting of a bond, $-S-$, $-CH_2-C(=O)-NR-$, $-C(O)-$, $-NRC(O)-$, $-NR'C(O)NR-$, $-NRC(S)NR'_2-$, $-NRC(O)O-$, $-OC(O)NR-$, $-OC(O)-$, $-C(O)NR-$, $-NR-C(O)-$, $-C(O)O-$, $-(O-CH_2-CH_2)_r-$ and $-(CH_2-CH_2-O)_r-$, wherein r is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, and 8;

each R is independently H, C_1-C_6 alkyl, C_2-C_{12} aryl, and C_4-C_{16} alkylaryl;

- 10 Tz is a triazole group that can be present or absent and is selected from the



each R_1 is independently H, C_1-C_6 alkyl, C_2-C_{12} aryl or C_4-C_{16} alkylaryl;

R_2 and R_3 are each independently H and CO_2R_4 , wherein R_4 is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_{12} aryl, and C_4-C_{16} alkylaryl, wherein

- 15 when one of R_2 or R_3 is CO_2R_4 , then the other is H;

V is selected from the group consisting of $-C(O)-$, $-C(S)-$, $-NRC(O)-$, $-NRC(S)-$, and $-OC(O)-$.

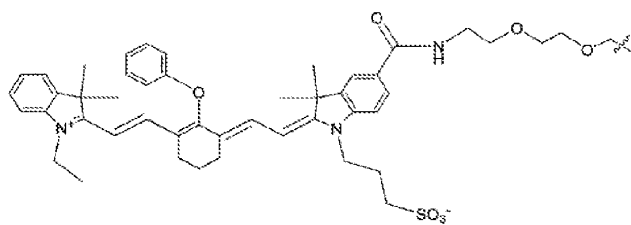
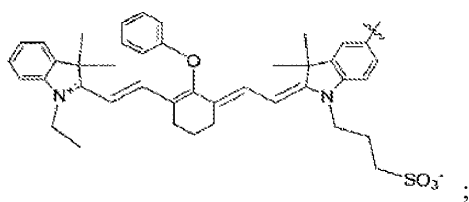
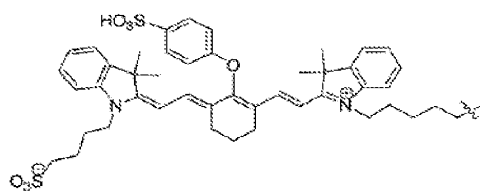
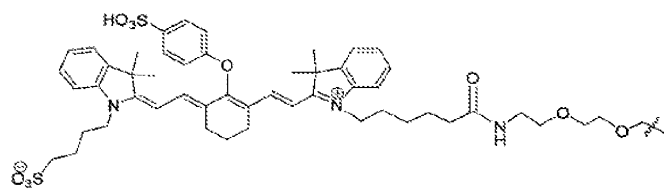
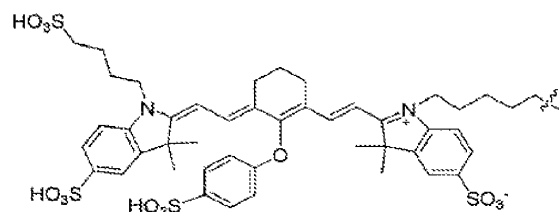
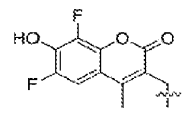
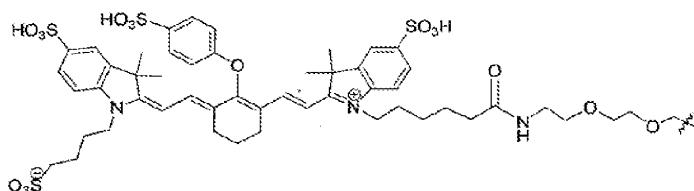
A is selected from the group consisting of naphthyl, biphenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, each of which can

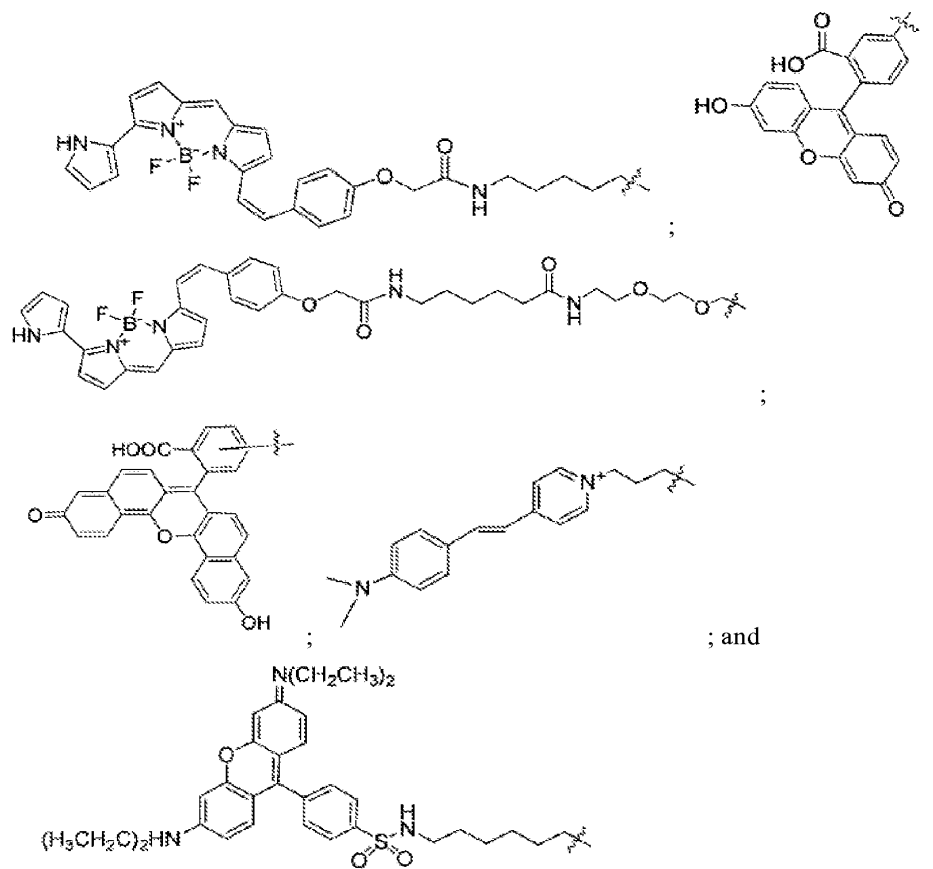
- 20 optionally comprise one or more radioactive isotope suitable for radiotherapy, or a photosensitizing dye suitable for photodynamic therapy.

26. The method of claim 25, wherein the radiohalogen suitable for radiotherapy is selected from the group consisting of ^{80m}Br , ^{77}Br , ^{125}I , ^{123}I , ^{131}I and ^{211}At .

25

27. The method of claim 25, wherein the photosensitizing dye suitable for photodynamic therapy is selected from the group consisting of





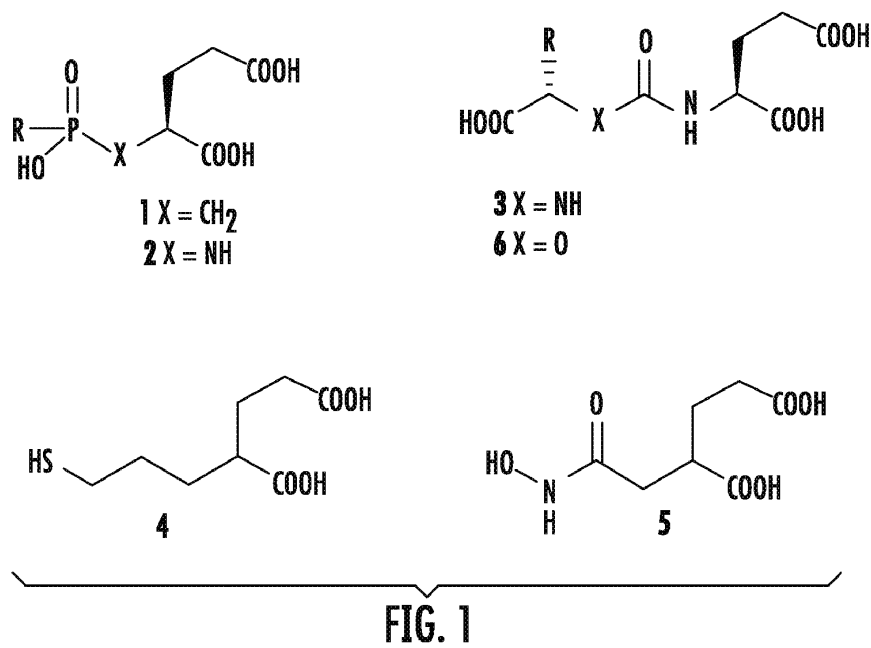
5

28. The method of claim 27, wherein the disease or condition is a prostate cancer, renal cancer, head cancer, neck cancer, head and neck cancer, lung cancer, breast cancer, prostate cancer, colorectal cancer, esophageal cancer, stomach cancer, leukemia/lymphoma, uterine cancer, skin cancer, endocrine cancer, urinary cancer, pancreatic cancer, gastrointestinal cancer, ovarian cancer, cervical cancer, adenomas, and tumor neovasculation.

29. The method of claim 28, wherein the disease or condition is prostate cancer.

15

1/13



SUBSTITUTE SHEET (RULE 26)

2/13

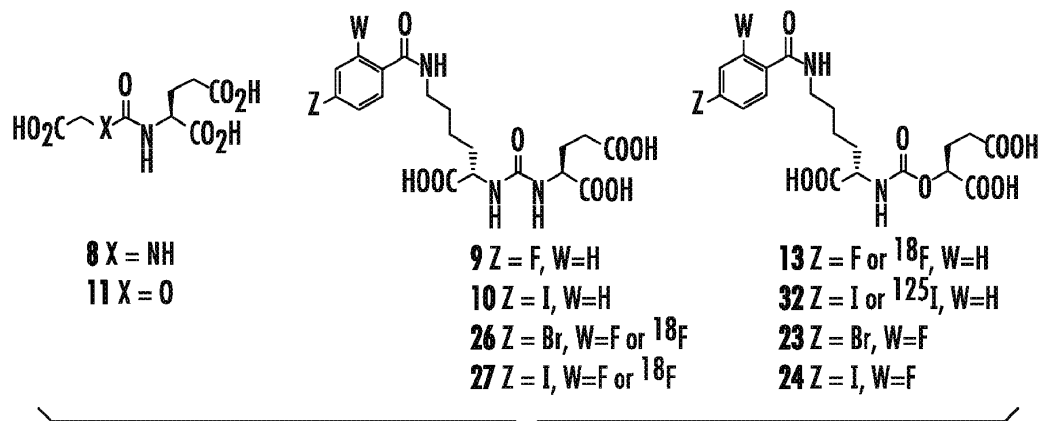


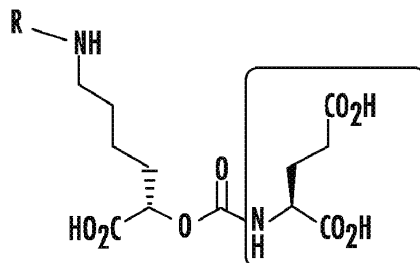
FIG. 2A

COMPOUND	K _i (nM)	COMPOUND	K _i (nM)
8 (WANG, 2013)	>1,000	23	0.11
9 (CHEN, 2008)	0.25	24	0.21
10 (CHEN, 2008)	0.01	26	0.04
11 (WANG, 2013)	>10,000	27	0.02
12	42	31	0.9
13	9.2	32	0.04

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

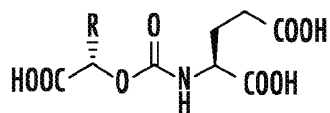
3/13



AMINO-PENTANEIOIC ACID (NPA)

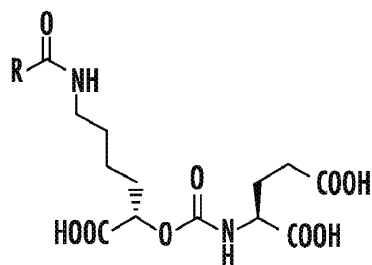
FIG. 3A

A) CARBAMATES



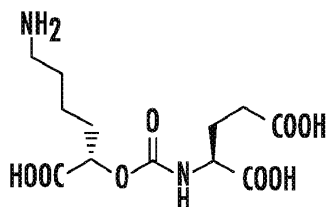
GENERAL STRUCTURE

R = ANY ALKYL, ARYL GROUP SIDECHAINS



HIGH BINDING AFFINITY STRUCTURES

R = ANY ALKYL, ARYL GROUP SIDECHAINS



VERSATILE INTERMEDIATE FOR FUNCTIONALIZATION

FIG. 3B

SUBSTITUTE SHEET (RULE 26)

4/13

B) BETA-AMINOACID UREAS

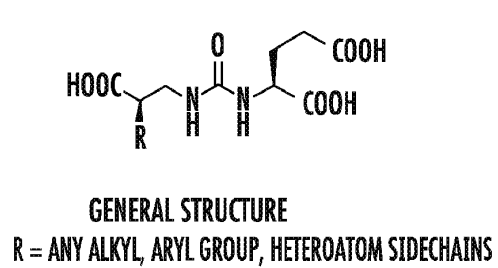
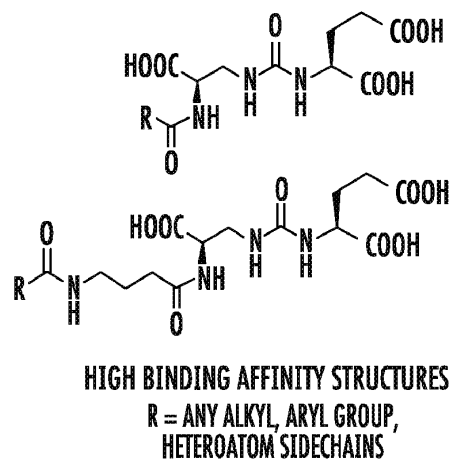
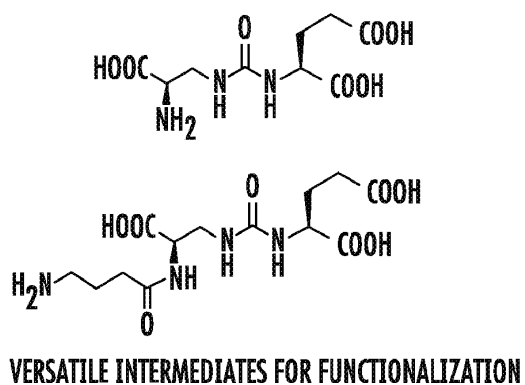
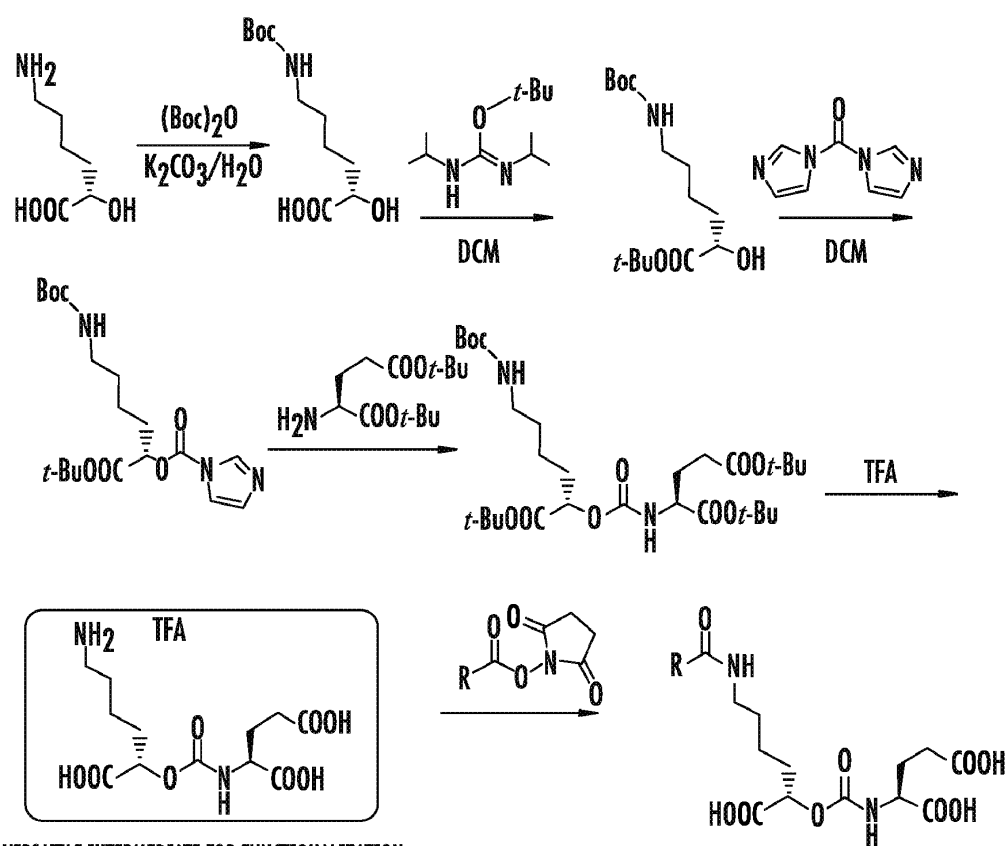


FIG. 4

SUBSTITUTE SHEET (RULE 26)

5/13



VERSATILE INTERMEDIATE FOR FUNCTIONALIZATION

FIG. 5

SUBSTITUTE SHEET (RULE 26)

6/13

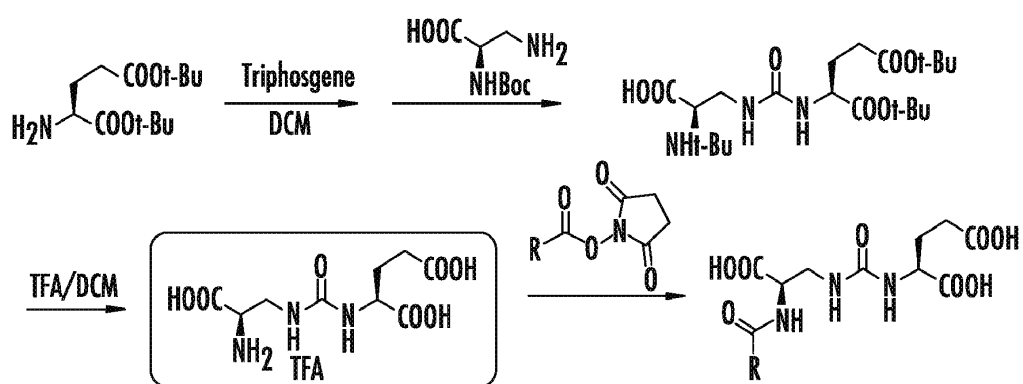


FIG. 6

SUBSTITUTE SHEET (RULE 26)

7/13

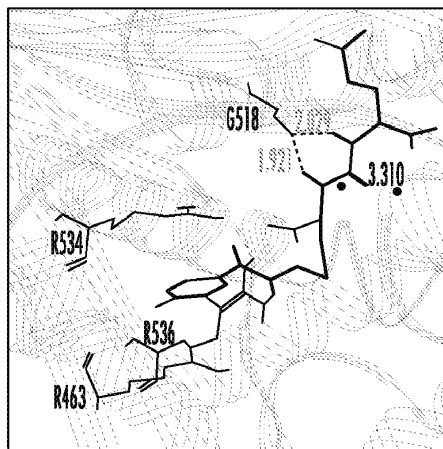


FIG. 7A

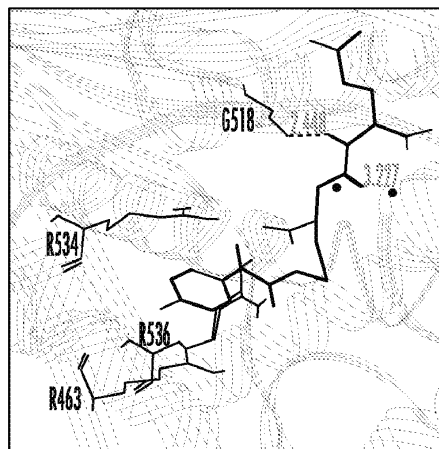


FIG. 7B

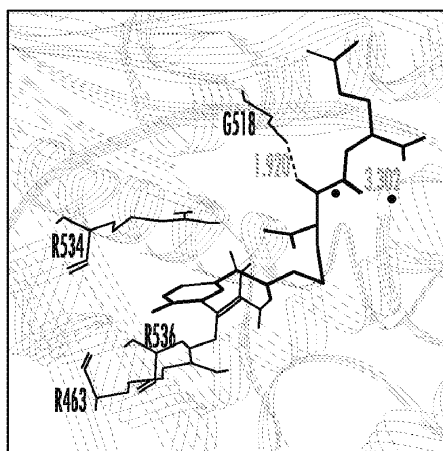


FIG. 7C

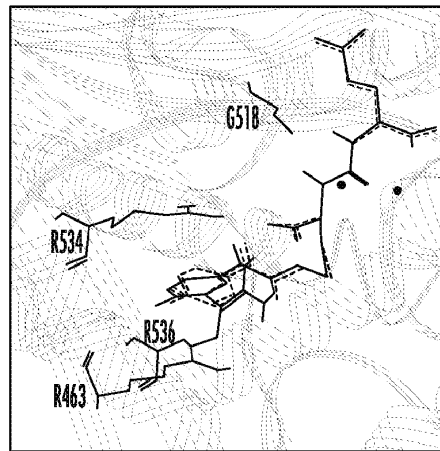


FIG. 7D

SUBSTITUTE SHEET (RULE 26)

8/13

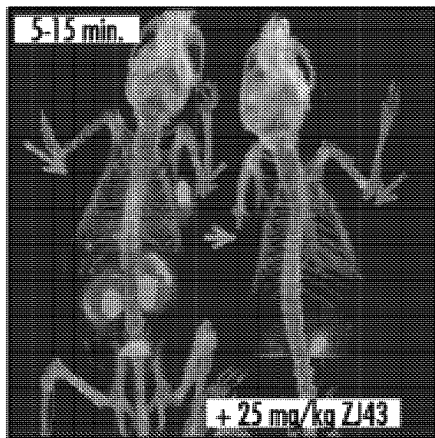


FIG. 8A

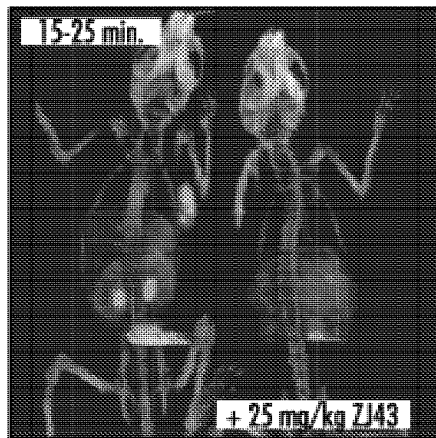


FIG. 8B

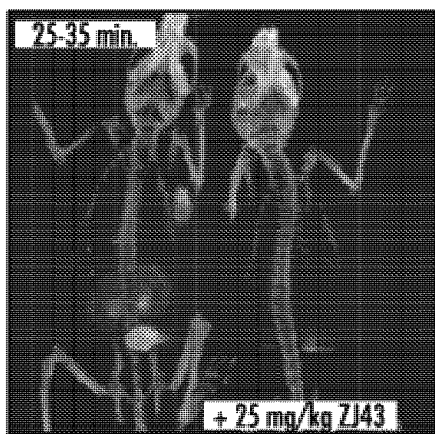


FIG. 8C

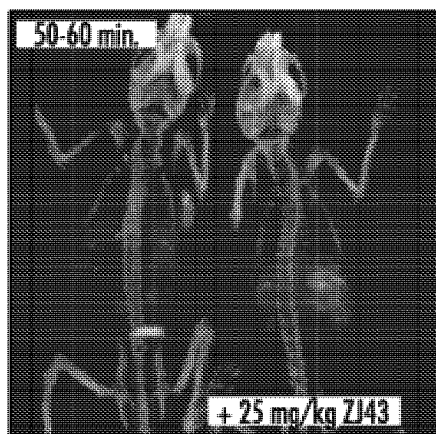


FIG. 8D



SUBSTITUTE SHEET (RULE 26)

9/13

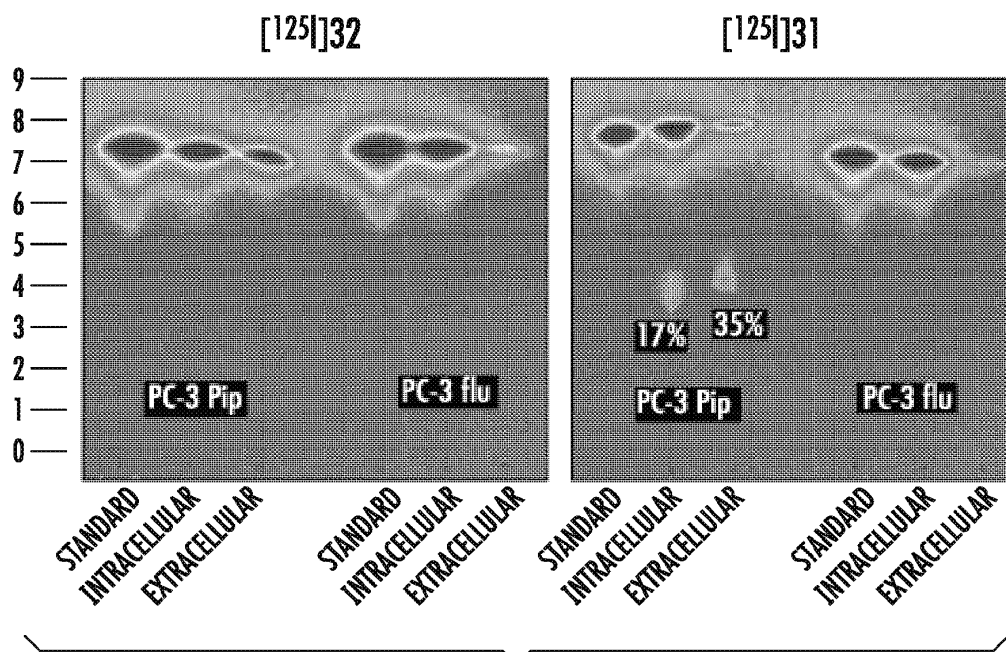
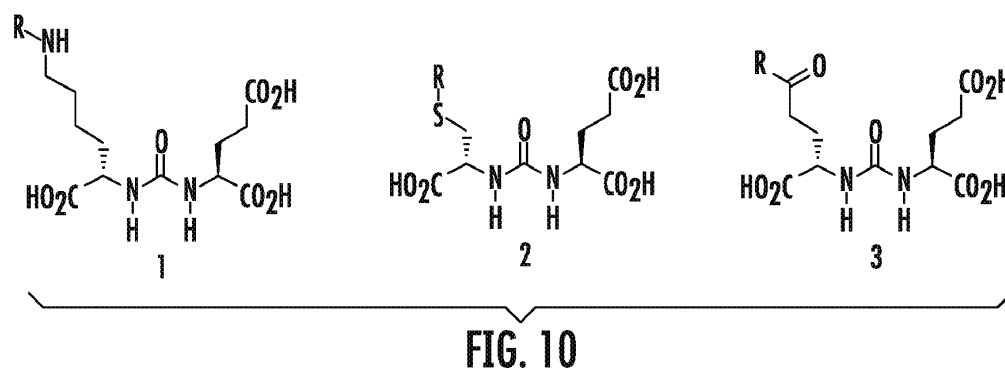


FIG. 9

SUBSTITUTE SHEET (RULE 26)

10/13



SUBSTITUTE SHEET (RULE 26)

11/13

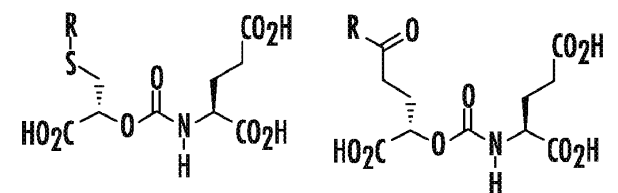


FIG. 11

SUBSTITUTE SHEET (RULE 26)

12/13

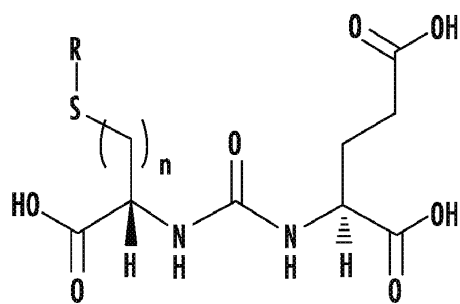
DCMC OR MCG $R = {}^{11}\text{CH}_3$, $n = 1$ DCFBC $R = -\text{CH}_2\text{C}_6\text{H}_5\text{X}$, $\text{X} = {}^{18}\text{F}$, $n = 1$ DCIBC $R = -\text{CH}_2\text{C}_6\text{H}_5\text{X}$, $\text{X} = {}^{125}\text{I}$, $n = 1$

FIG. 12

SUBSTITUTE SHEET (RULE 26)

13/13

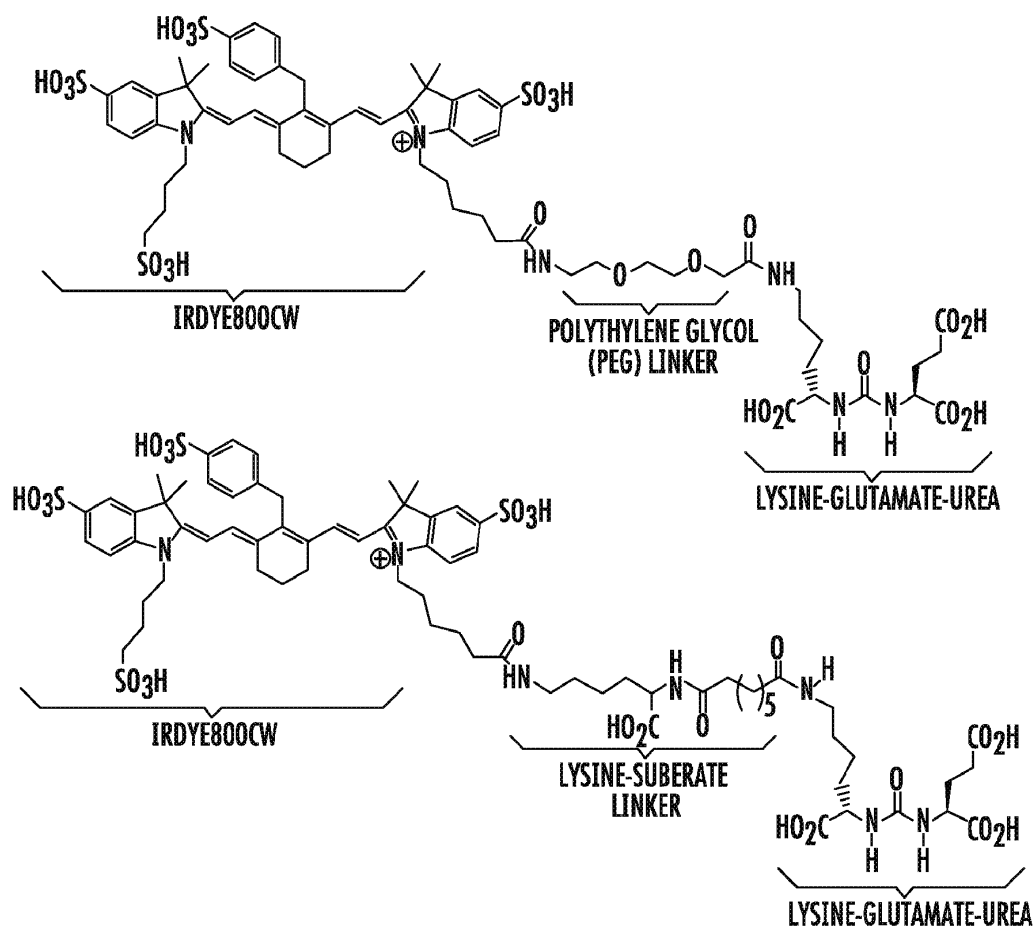


FIG. 13

SUBSTITUTE SHEET (RULE 26)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
15 August 2019 (15.08.2019)



(10) International Publication Number
WO 2019/154886 A1

(51) International Patent Classification:

C07D 401/12 (2006.01) A61K 31/4709 (2006.01)
C07D 401/14 (2006.01) A61P 35/00 (2006.01)
C07D 405/14 (2006.01)

(21) International Application Number:

PCT/EP2019/052952

(22) International Filing Date:

06 February 2019 (06.02.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

18155420.5 06 February 2018 (06.02.2018) EP
18155419.7 06 February 2018 (06.02.2018) EP
18199641.4 10 October 2018 (10.10.2018) EP

(71) Applicant: UNIVERSITÄT HEIDELBERG [DE/DE];
Grabengasse 1, 69117 Heidelberg (DE).

(72) Inventors: HABERKORN, Uwe; Königsäcker 82, 68723
Schwetzingen (DE). LOKTEV, Anastasia; Albert-Fritz-
Str. 37a, 69124 Heidelberg (DE). LINDNER, Thomas; Er-
furter Str. 10, 68723 Schwetzingen (DE). MIER, Walter;
Brückweg 21, 64625 Bensheim (DE). GIESEL, Frederik;
Alte Eppelheimer Str. 24/1, 69115 Heidelberg (DE). KRA-
TOCHWIL, Clemens; Gartenstr. 9, 69493 Hirschberg
a.d.B. (DE).

(74) Agent: ZWICKER, Jörk; Zwicker Schnappauf & Part-
ner Patentanwälte PartG mbB, Hansastr. 32, 80686 Munich
(DE).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,
KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

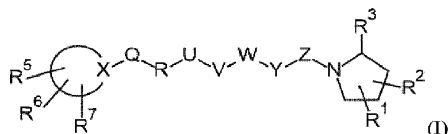
Published:

— with international search report (Art. 21(3))



WO 2019/154886 A1

(54) Title: FAP INHIBITOR



(57) Abstract: The present invention relates to a compound of formula (I), a pharmaceutical composition comprising or consisting of said compound, a kit comprising or consisting of said compound or pharmaceutical composition and use of the compound or pharmaceutical composition in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP).

FAP Inhibitor

The present invention relates to a compound, a pharmaceutical composition comprising or consisting of said compound, a kit comprising or consisting of said compound or pharmaceutical composition and use of the compound or pharmaceutical composition in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP).

Background of the Invention

Tumor growth and spread are not only determined by the cancer cells, but also by the non-malignant constituents of the malignant lesion, which are subsumed under the term stroma. The stroma may represent over 90% of the tumor mass in tumors with desmoplastic reaction such as breast, colon and pancreatic carcinoma. Especially a subpopulation of fibroblasts called cancer-associated fibroblasts (CAFs) is known to be involved in tumor growth, migration and progression. Therefore, these cells represent an attractive target for diagnosis and anti-tumor therapy.

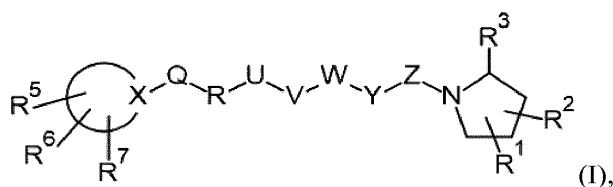
A distinguishing feature of CAFs is the expression of seprase or fibroblast activation protein α (FAP- α), a type II membrane bound glycoprotein belonging to the dipeptidyl peptidase 4 (DPP4) family. FAP- α has both dipeptidyl peptidase and endopeptidase activity. The endopeptidase activity distinguishes FAP- α from the other members of the DPP4 family. Identified substrates for the endopeptidase activity so far are denatured Type I collagen, α 1-antitrypsin and several neuropeptides. FAP- α has a role in normal developmental processes during embryogenesis and in tissue modelling. It is not or only at insignificant levels expressed on adult normal tissues. However, high expression occurs in wound healing, arthritis, atherosclerotic plaques, fibrosis and in more than 90% of epithelial carcinomas.

The appearance of FAP- α in CAFs in many epithelial tumors and the fact that overexpression is associated with a worse prognosis in cancer patients led to the hypothesis that FAP- α activity is involved in cancer development as well as in cancer cell migration and spread. Therefore, the targeting of this enzyme for imaging and endoradiotherapy can be considered as a promising strategy for the detection and treatment of malignant tumors. The present inventors developed a small molecule based on a FAP- α specific inhibitor and were able to show specific uptake, rapid internalization and successful imaging of tumors in animal models as well as in tumor patients. A comparison with the commonly used radiotracer ^{18}F -fluorodeoxyglucose (^{18}F -FDG) revealed a clear superiority of the new FAP- α ligand in patients with locally advanced lung adenocarcinoma. Thus, the present invention provides inter alia: (i) detection of

smaller primary tumors and, thus the possibility of earlier diagnosis, (ii) the detection of smaller metastasis and, thus a better assessment of tumor stage, (iii) precise intra-operative guidance facilitating complete surgical removal of tumor tissue, (iv) better differentiation between inflammation and tumor tissue, (v) more precise staging of patients with tumors, (vi) better follow up of tumor lesions after antitumor therapy, (vii) the opportunity to use the molecules as theranostic agents for diagnosis and therapy. Furthermore, the molecules can be used for the diagnosis and treatment of non-malignant diseases such as chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorders.

Summary of the Invention

In a first aspect, the present invention provides a compound of Formula (I)



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other;

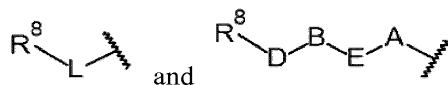
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,



under the proviso that R⁶ and R⁷ are not at the same time H,

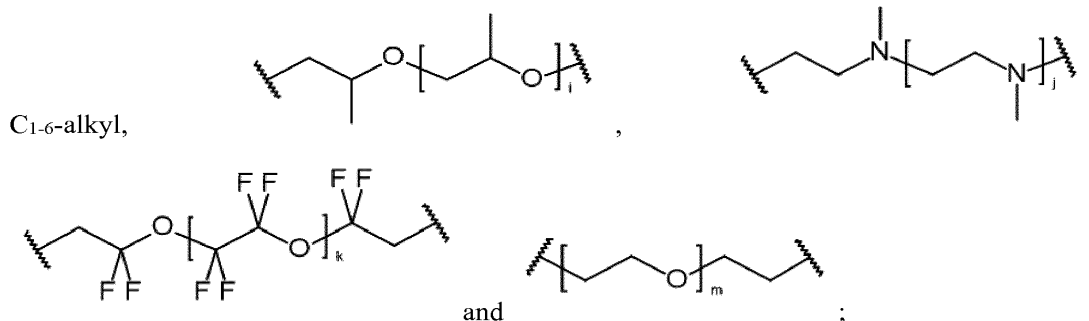
wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

- 5 A is selected from the group consisting of NR^4 , O, S, and CH_2 ;

E is selected from the group consisting of



wherein i is 1, 2, or 3;

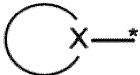
- 10 wherein j is 1, 2, or 3;

wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

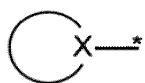
A and E together form a group selected from a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, wherein A and E can be mono-, bi- and multicyclic, preferably monocyclic. Each A and E being

- 15 optionally substituted by 1 to 4 residues from the group consisting of -H, $\text{-C}_{1-6}\text{-alkyl}$, $\text{-O-C}_{1-6}\text{-alkyl}$, $\text{-S-C}_{1-6}\text{-alkyl}$, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and $\text{-C}_{1-6}\text{-aralkyl}$, each of said $\text{-C}_{1-6}\text{-alkyl}$ being optionally substituted with from 1 to 3 substituents

selected from -OH, oxo, halo; and optionally connected to A, B, D, E or .

B is selected from the group consisting of S, NR^4 , $\text{NR}^4\text{-O}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$, $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$,
20 and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein $\text{NR}^4\text{-C}_{1-6}\text{-alkyl-NR}^4$ and the N-containing heterocycle is substituted with 1 to 3 substituents selected from the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$; and;

- 25 R^8 is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;



is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

5 or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof.

In a second aspect, the present invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect, and, optionally, a pharmaceutically acceptable carrier and/or excipient.

10 In a third aspect, the present invention relates to the compound of the first aspect or the pharmaceutical composition of the second aspect for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject.

In a fourth aspect, the present invention relates to a kit comprising or consisting of the compound of the first aspect or the pharmaceutical composition of the second aspect and
15 instructions for the diagnosis or treatment of a disease.

List of Figures

In the following, the content of the figures comprised in this specification is described. In this context please also refer to the detailed description of the invention above and/or below.

20 **Figure 1: In vitro characterization of ¹²⁵I-FAPI-01 and ¹⁷⁷Lu-FAPI-02.**

A. Binding of radiolabeled FAPI-01 and FAPI-02 to different human cancer cell lines as well as cell lines transfected with human FAP- α (HT-1080-FAP), murine FAP- α (HEK-muFAP) and human CD26 (HEK-CD26) after 60 min of incubation. **B.** Internalization of radiolabeled FAPI-01 and FAPI-02 into HT-1080-FAP cells after incubation for 10 min to 24 h. The internalized proportion is shown in grey and black, respectively; the extracellularly bound fraction is indicated by the white bars. **C.** Competitive binding of radiolabeled FAPI-01 and FAPI-02 to HT-1080-FAP cells after adding increasing concentrations of unlabeled FAPI-01 and Lu-FAPI-02. **D.** Internalization of FAPI-02 into FAP- α positive and negative cell lines. Blue: DAPI; green: FAPI-02-Atto488. **E+F.** Efflux kinetics of FAPI-01 and FAPI-02 after 1 h
25 incubation of HT-1080-FAP cells with radiolabeled compounds followed by incubation with compound-free medium for 1 to 24 h. All values are given as percentage of total applied dose normalized to 1 million cells (%ID/1 mio cells).
30

Figure 2: Binding specificity and relative internalization rates of FAPI derivatives.

A-C. Binding and internalization rates of FAPI-03 to FAPI-15 in relation to FAPI-02 (defined as 100%). Internalization rates after 1, 4 and 24 hrs of incubation are depicted in grey; the extracellular bound fraction is represented by the white bars. **D.** Binding of selected FAPI derivatives to HEK cells expressing murine FAP- α and human CD26 after 60 min of incubation. *Right side:* Ratio of muFAP to CD26 binding. **E.** Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound.

Figure 3: Imaging of FAPI-02 and -04 in mice bearing human FAP-positive (HT-1080-FAP) and negative (Capan-2, SK-LMS-1) tumor xenografts.

A+C, E+G. Small animal PET imaging was performed after intravenous administration of 4 nmol ^{68}Ga -FAPI-02 and -04 (10 MBq resp.) at indicated times. The radiotracer gets rapidly enriched within the tumor (indicated by the red arrow) while not accumulating in non-cancerous tissue. Furthermore, a rapid elimination via the kidneys and bladder is seen. **B+D, F+H.** Quantification of the PET images demonstrates a solid clearance of ^{68}Ga -FAPI-02 and -04 from the cardiovascular system and a constant uptake into the tumor.

Figure 4: Blocking experiments for analysis of binding specificity in vivo

A+D. Blocking of ^{68}Ga -FAPI-02 and -04 tumor accumulation by co-administration of 30 nmol unlabeled compound in HT-1080-FAP tumor bearing mice. **B+C, E+F.** Time-activity curves of ^{68}Ga -FAPI-02 and -04 in selected organs after intravenous administration with and without unlabeled compound as a competitor.

Figure 5: Organ distribution of ^{177}Lu -FAPI-02 and -04 in HT-1080-FAP tumor bearing nude mice

A-C. Biodistribution of ^{177}Lu -FAPI-02 and -04 was measured ex vivo at indicated times after intravenous administration of 1 MBq to mice bearing human FAP-positive HT-1080 tumor xenografts; n=3 for each time point. The values stated are expressed as percentage of injected dose per gram of tissue (% ID/g). The radiotracers are shown to accumulate within the FAP-expressing tumor, showing the highest enrichment after 1 h for FAPI-02 (4.5 %ID/g) and 2 h for FAPI-04 (5.4 %ID/g). **D-F.** Tumor-to-normal tissue ratios of ^{177}Lu -FAPI-02 and -04 1, 4 and 24 hrs after intravenous administration.

Figures 6-9: PET/CT imaging of FAPI-02 in cancer patients

6A-C. Maximum intensity projections (MIP) of PET/CT scans in a patient suffering from metastasized breast cancer. **D.** Maximum tissue uptake of ^{68}Ga -FAPI-02 10 min, 1 h and 3 h after intravenous administration to a patient with metastasized breast cancer.

7. MIP of PET/CT scans in patients with pancreatic cancer, non-small cell lung cancer (NSCLC) and esophageal and rectum carcinoma 1 h after administration of ^{68}Ga -FAPI-02.

8. MIP of PET/CT scans in patients with nasopharynx and larynx carcinoma 1 h after administration of ^{68}Ga -FAPI-02.

- 5 **9A+B.** Whole-body PET/CT imaging (MIP) 1 h after administration of ^{18}F -FDG and ^{68}Ga -FAPI-02 to a patient with locally advanced lung adenocarcinoma. **C+D.** Transaxial view of lung adenocarcinoma patient 1 h after administration of ^{18}F -FDG and ^{68}Ga -FAPI-02. FAPI-02 is selectively accumulated in FAP- α expressing tissue and shows significantly higher uptake in the malignant lesions compared to ^{18}F -FDG.

10 **Figures 10-16: PET/CT imaging of FAPI-04 in cancer patients**

10 Maximum intensity projections (MIP) of PET/CT scans in a patient suffering from metastasized breast cancer 10 min, 1 and 3 hrs after administration of ^{68}Ga -FAPI-04.

- 11** MIP of PET/CT scans in patients with sigma carcinoma, hypopharynx carcinoma, neuroendocrine tumors, cholangio, ovarian and small intestine carcinoma 1 h after administration of ^{68}Ga -FAPI-04.

12 MIP of PET/CT scans in a patient with lung cancer 1 h after administration of ^{68}Ga -FAPI-04.

13 MIP of PET/CT scans in a patient with oncogenic rachitis 1 h after administration of ^{68}Ga -FAPI-04.

- 20 **14** Comparative imaging of one patient with metastasized prostate cancer. MIP of PET/CT scans 1 h after application of radiolabeled DOTATOC, PSMA and FAPI-04.

15 Maximum intensity projection (MIP) and time-activity curves of a dynamic ^{68}Ga -FAPI-04 PET/CT scan in a pancreatic cancer patient.

- 16** Relative binding rates of Lu-177 labeled FAPI derivatives compared to FAPI-04 (set to 100%) after incubation for 1, 4 and 24 h on FAP-expressing HT-1080 cells; n=3.

Figure 17: Competitive binding of selected FAPI derivatives to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3).

- Figure 18:** Binding of FAPI derivatives to HEK cells expressing murine FAP and human CD26 after 60 min of incubation, n=3. Values are expressed as percentage of applied dose (%ID) per 1 mio cells.

Figure 19: Biodistribution of selected FAPI derivatives in HT-1080-FAP xenotransplants 1, 4 and 24 h after intravenous administration of the radiotracers, n=3. Values are expressed as percentage of injected dose per gram of tissue (%ID/g).

Figure 20: Tumor-to-blood ratio of selected FAPI derivatives in HT-1080-FAP xenotransplants 1, 4 and 24 h after intravenous administration of the radiotracers, n=3.

Figure 21: PET imaging of Ga-68 labeled FAPI-21 and FAPI-46 in HT-1080-FAP tumor bearing mice; n=1.

5 **Figure 22:** Maximum standardized uptake values (SUV) of selected FAPI derivatives in HT-1080-FAP tumor bearing mice; n=1.

Figure 23: Maximum (SUV max, Fig. 23 A) and mean (SUV mean, Fig. 23 B) standardized uptake values of Ga-68 labeled FAPI-02 and FAPI-04 in cancer patients; n=25.

10 **Figure 24:** Intra-individual comparison of 6 patients with 6 different tumor entities undergoing FDG-PET and FAPI-PET imaging within <9 days.

Figure 25: PET/CT imaging of Ga-68 labeled FAPI-04 in patients with peritonitis carcinomatosa (A), myocarditis (B) and hip joint arthrosis (C) 1 h p.i.

Figure 26: PET/CT imaging of Ga-68 labeled FAPI-21 in cancer patients 1 h p.i.

15 **Figure 27:** PET/CT imaging of Ga-68 labeled FAPI-46 1 h p.i. and intratherapeutical imaging of Sm-153 labeled FAPI-46 30 min p.i. in cancer patients.

Figure 28: Intratherapeutical imaging of Sm-153 labeled FAPI-46 up to 20 h p.i.

Figure 29: A. Maximum intensity projection (MIP) 1 h after intravenous administration of ^{68}Ga -FAPI-46 to a patient with metastasized colorectal carcinoma. B. Imaging of Bremsstrahlung 2 h after therapeutic treatment with ^{90}Y -FAPI-46 of the same patient.

20 **Figure 30:** PET/CT imaging of Ga-68 labeled FAPI-46 1 h p.i. in lung cancer patients with idiopathic lung fibrosis. A, B. Maximum tracer uptake into tumor tissue is significantly higher than into non-exacerbated fibrotic lesions. C. Maximum tracer uptake into tumor tissue is slightly lower than into exacerbated fibrotic tissue.

25 **Figure 31:** A. Binding of Tc-99m labeled FAPI-19 to HT-1080-FAP cells, n=3. B. Competitive binding of Tc-99m labeled FAPI-19 to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3). C. Scintigraphy of Tc-99m labeled FAPI-19 in HT-1080-FAP xenotransplants, n=1.

Figure 32: A. Binding of Tc-99m labeled FAPI-34 to HT-1080-FAP cells, n=3. B. Scintigraphy of Tc-99m labeled FAPI-34 in HT-1080-FAP xenotransplants, n=1.

30 **Figure 33:** Scintigraphy of Tc-99m labeled FAPI-34 in one patient with metastasized pancreas carcinoma.

Figure 34: A. Binding of Pb-203 labeled FAPI derivatives to HT-1080-FAP cells, n=3. B. Efflux kinetics of Pb-203 labeled FAPI derivatives after incubation of HT-1080-FAP cells with radiolabeled compound for 60 min and consequent incubation with nonradioactive

medium for 1 to 24 hours, n=3. C. Competitive binding of Pb-203 labeled FAPIs to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3).

5 **Figure 35:** Scintigraphy of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenotransplants, n=1.

Figure 36: Biodistribution of Pb-203 labeled FAPI-04 and FAPI-46 in HT-1080-FAP xenotransplants 1, 4, 6 and 24 h after intravenous administration of the radiotracers, n=3. Values are expressed as percentage of injected dose per gram of tissue (%ID/g).

10 **Figure 37:** A. Binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells, n=3. B. Competitive binding of Cu-64 labeled FAPI-42 and FAPI-52 to HT-1080-FAP cells after adding increasing concentrations of unlabeled compound (10^{-10} to 10^{-5} M, incubation for 60 min, n=3). C. Efflux kinetics of Cu-64 labeled FAPI-42 and FAPI-52 after incubation of HT-1080-FAP cells with radiolabeled compound for 60 min and consequent incubation with nonradioactive medium for 1 to 24 hours, n=3.

15 **Figure 38:** PET imaging of Cu-64 labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor bearing mice; n=1.

Figure 39: PET imaging of AIF-18 labeled FAPI-42 and FAPI-52 in HT-1080-FAP tumor bearing mice; n=1.

20 **Figure 40:** a. Small animal PET imaging of ^{68}Ga -labeled FAPI-02 in U87MG tumor bearing nude mice up to 140 min after intravenous administration of the radiotracer. The tumor is indicated by the red arrow. b. Biodistribution of ^{177}Lu -labeled FAPI-02 and FAPI-04 in U87MG tumor bearing nude mice 1, 4 and 24 h after intravenous administration of the radiotracers; n=3.

25 **Figure 41:** Tumor-to-organ ratios of ^{177}Lu -labeled FAPI-02 and -04 in U87MG tumor bearing mice 1, 4 and 24 h after intravenous administration.

Figure 42: Maximum intensity projection (MIP) of PET/CT scans in a glioblastoma patient 10 min, 1 and 3 h after administration of ^{68}Ga -FAPI-02.

30 **Figure 43:** Exemplary images (contrast enhanced T1 weighted MRI, FAPI-PET and fused images of both modalities) of IDH wt glioblastomas, IDH-mutant gliomas WHO grade II and IDH-mutant glioblastomas.

Figure 44: Absolute SUVmax values of all 18 gliomas.

Figure 45: Statistical Analysis of SUVmax/BG values. Boxplots of SUVmax/BG values and corresponding ROC curves in GBM versus non-GBM (a, b), IDH-mutant versus IDH wildtype gliomas (c, d) and gliomas grade II versus gliomas grade III/IV (e, f).

Figure 46: Dose-dependent inhibition of enzymatic FAP activity by FAPI-04 and Talabostat. In contrast to Talabostat, a potent DPP4 inhibitor with marginal FAP activity, FAPI-04 demonstrates robust, dose-dependent FAP inhibition.

Figure 47: Reuptake of ^{177}Lu -labeled FAPI-04 and FAPI-46 in HT-1080-FAP cells. Following incubation of the cells with the radiotracers for 60 min at 37°C, the compounds are removed and non-radioactive medium with (+ Comp.) and without unlabeled compound (- Comp.) added and incubated for 10 min to 6 h. Already within the first ten minutes of incubation, renewed uptake of the unlabeled FAPI derivatives occurs, displacing parts of the radiolabeled fraction, which results in significantly lower radioactivity values as compared to pure medium without competitor. After 6 h of incubation, almost complete displacement of the radiolabeled FAPIs has occurred. These findings indicate a continuous reuptake of intact FAP molecules back to the cell membrane upon initial internalization, allowing renewed binding and internalization of FAP ligands.

Figure 48: Organ distribution of ^{177}Lu -labeled FAPI-04 after single and multiple injection in HT-1080-FAP tumor bearing nude mice. Administration of two equal doses of ^{177}Lu -FAPI-04 at intervals of 4 h results in increased overall organ activities, including the tumor, measured 8 and 24 h after the first injection. In contrast, administration of three doses (higher initial dose, lower subsequent doses) reveals no change in the overall organ activities.

Figure 49: Binding of F-18-FAPI derivatives to HT1080 cells expressing human FAP after 10, 30, 60 and 90 min of incubation, n=3. Values are expressed as percentage of applied dose (%ID) per 1 mio cells.

Figure 50: PET imaging of AIF-18 labeled FAPI-74 and FAPI-52 in HT-1080-FAP tumor bearing mice; n=1.

Figure 51: Biodistribution of FAPI-75 in HT-1080-FAP xenotransplants 1, 4 and 24 h after intravenous administration of the radiotracer, n=3. Values are expressed as percentage of injected dose per gram of tissue (%ID/g).

Figure 52: PET imaging of patient with non-small cell lung cancer: Robust accumulation of F18-labeled FAPI-74 in multiple metastases

Figure 53: Time activity curves of the heart region (SUVmean) for FAPI-04 and -46 as illustration of the fast blood pool clearance.

Figure 54: FAPI-02 and FAPI-04 at the different imaging time-point (10 min, 1h and 3h p.i.) in two patients with metastasized breast cancer. Rapid tumor targeting and fast blood clearance is followed by a long plateau phase without relevant change in image contrast (top).

In comparison to FAPI-02 the ligand FAPI-04 is characterized by a prolonged tumor retention time (bottom).

Figure 55: The effective dose of FAPI-02 was 1.80E-02 mSv/MBq calculated with OLINDA (1.82E-02 with IDAC1 / ICRP60, 1.79E-02 with IDAC2 / ICRP103). The effective dose for FAPI-04 PET/CT was 1.64E-02 mSv/MBq calculated with OLINDA (1.66E-02 with IDAC1 / ICRP60, 1.35E-02 with IDAC2 / ICRP103). If the delayed scan at 3h p.i. is omitted in clinical practice, the routine activity for an FAPI-exam could be reduced to 200 MBq ^{68}Ga ; consecutively the radiation dose of such a FAPI-PET/CT scan would be 3-4 mSv.

Figure 56: A) ^{68}Ga -FAPI-04 after 1h post injection in different tumor entities in PET/CT. The highest average SUVmax (>12) were found in sarcoma, esophageal, breast, cholangiocellular carcinoma and lung cancer. The lowest FAPI uptake (average SUVmax <6) was observed in renal cell, differentiated thyroid, adenoid-cystic, gastric carcinoma and pheochromocytoma. The average SUVmax of hepatocellular carcinoma, colorectal carcinoma, head-neck-cancer, ovarian carcinoma, pancreatic carcinoma was intermediate (SUV $6 < x < 12$). Within all tumor entities a high inter-individual variation was observed. Due to low background activity (SUV 2), the tumor-to-background ratios are > 2 -fold in the intermediate and > 4 -fold in the high intensity uptake group. B) Primary tumour entities presented similar SUV-uptake compared tumour entities using FAPI-04

Figure 57: Exemplary PET images of different tumor entities that have been used for the quantifications shown in figure 56 A-B.

Detailed Descriptions of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Klbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout this specification and the claims, which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be

understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being optional, preferred or advantageous may be combined with any other feature or features indicated as being optional, preferred or advantageous.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Some of the documents cited herein are characterized as being "*incorporated by reference*". In the event of a conflict between the definitions or teachings of such incorporated references and definitions or teachings recited in the present specification, the text of the present specification takes precedence.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments; however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

25

Definitions

In the following, some definitions of terms frequently used in this specification are provided. These terms will, in each instance of its use, in the remainder of the specification have the respectively defined meaning and preferred meanings.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents, unless the content clearly dictates otherwise.

In the following definitions of the terms: alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkenyl and alkynyl are provided.

These terms will in each instance of its use in the remainder of the specification have the respectively defined meaning and preferred meanings.

The term "alkyl" refers to a saturated straight or branched carbon chain. Preferably, the chain comprises from 1 to 10 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 e.g. methyl, ethyl
5 methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *tert*-butyl, pentyl, hexyl, pentyl, or octyl. Alkyl groups are optionally substituted.

The term "heteroalkyl" refers to a saturated straight or branched carbon chain. Preferably, the chain comprises from 1 to 9 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 e.g. methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, pentyl, hexyl, pentyl, octyl,
10 which is interrupted one or more times, e.g. 1, 2, 3, 4, 5, with the same or different heteroatoms. Preferably the heteroatoms are selected from O, S, and N, e.g. -O-CH₃, -S-CH₃, -CH₂-O-CH₃, -CH₂-O-C₂H₅, -CH₂-S-CH₃, -CH₂-S-C₂H₅, -C₂H₄-O-CH₃, -C₂H₄-O-C₂H₅, -C₂H₄-S-CH₃, -C₂H₄-S-C₂H₅ etc. Heteroalkyl groups are optionally substituted.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with
15 other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively, with preferably 3, 4, 5, 6, 7, 8, 9 or 10 atoms forming a ring, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl etc. The terms "cycloalkyl" and "heterocycloalkyl" are also meant to include bicyclic, tricyclic and polycyclic versions thereof. The term "heterocycloalkyl" preferably refers to a saturated ring having five of which at least
20 one member is a N, O or S atom and which optionally contains one additional O or one additional N; a saturated ring having six members of which at least one member is a N, O or S atom and which optionally contains one additional O or one additional N or two additional N atoms; or a saturated bicyclic ring having nine or ten members of which at least one member is a N, O or S atom and which optionally contains one, two or three additional N atoms.
25 "Cycloalkyl" and "heterocycloalkyl" groups are optionally substituted. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, spiro[3,3]heptyl, spiro[3,4]octyl, spiro[4,3]octyl, spiro[3,5]nonyl, spiro[5,3]nonyl, spiro[3,6]decyl, spiro[6,3]decyl, spiro[4,5]decyl, spiro[5,4]decyl, bicyclo[2.2.1]heptyl, bicyclo[2.2.2]octyl,
30 adamantyl, and the like. Examples of heterocycloalkyl include 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, 1,8 diazo-spiro-[4,5] decyl, 1,7 diazo-spiro-[4,5] decyl, 1,6 diazo-spiro-[4,5] decyl, 2,8 diazo-spiro[4,5] decyl, 2,7 diazo-spiro[4,5] decyl, 2,6 diazo-spiro[4,5] decyl, 1,8 diazo-spiro-[5,4] decyl, 1,7 diazo-spiro-

[5,4] decyl, 2,8 diazo-spiro-[5,4] decyl, 2,7 diazo-spiro[5,4] decyl, 3,8 diazo-spiro[5,4] decyl, 3,7 diazo-spiro[5,4] decyl, 1-azo-7,11-dioxo-spiro[5,5] undecyl, 1,4-diazabicyclo[2.2.2]oct-2-yl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

5 The term “aryl” preferably refers to an aromatic monocyclic ring containing 6 carbon atoms, an aromatic bicyclic ring system containing 10 carbon atoms or an aromatic tricyclic ring system containing 14 carbon atoms. Examples are phenyl, naphthyl or anthracenyl. The aryl group is optionally substituted.

10 The term “aralkyl” refers to an alkyl moiety, which is substituted by aryl, wherein alkyl and aryl have the meaning as outlined above. An example is the benzyl radical. Preferably, in this context the alkyl chain comprises from 1 to 8 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, or 8, e.g. methyl, ethyl methyl, ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butenyl, *tert*-butyl, pentyl, hexyl, pentyl, octyl. The aralkyl group is optionally substituted at the alkyl and/or aryl part of the group.

15 The term “heteroaryl” preferably refers to a five or six-membered aromatic monocyclic ring wherein at least one of the carbon atoms are replaced by 1, 2, 3, or 4 (for the five membered ring) or 1, 2, 3, 4, or 5 (for the six membered ring) of the same or different heteroatoms, preferably selected from O, N and S; an aromatic bicyclic ring system wherein 1, 2, 3, 4, 5, or 6 carbon atoms of the 8, 9, 10, 11 or 12 carbon atoms have been replaced with the same or
20 different heteroatoms, preferably selected from O, N and S; or an aromatic tricyclic ring system wherein 1, 2, 3, 4, 5, or 6 carbon atoms of the 13, 14, 15, or 16 carbon atoms have been replaced with the same or different heteroatoms, preferably selected from O, N and S. Examples are oxazolyl, isoxazolyl, 1,2,5-oxadiazolyl, 1,2,3-oxadiazolyl, pyrrolyl, imidazolyl, pyrazolyl, 1,2,3-triazolyl, thiazolyl, isothiazolyl, 1,2,3-thiadiazolyl, 1,2,5-thiadiazolyl, pyridinyl, pyrimidinyl, pyrazinyl, 1,2,3-triazinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, 1-benzofuranyl, 2-
25 benzofuranyl, indoyl, isoindoyl, benzothiophenyl, 2-benzothiophenyl, 1H-indazolyl, benzimidazolyl, benzoxazolyl, indoxazinyl, 2,1-benzosoxazolyl, benzothiazolyl, 1,2-benzisothiazolyl, 2,1-benzisothiazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinoxalinyl, quinazolinyl, quinolinyl, 1,2,3-benzotriazinyl, or 1,2,4-benzotriazinyl.

30 The term “heteroaralkyl” refers to an alkyl moiety, which is substituted by heteroaryl, wherein alkyl and heteroaryl have the meaning as outlined above. An example is the 2-alkylpyridinyl, 3-alkylpyridinyl, or 2-methylpyridinyl. Preferably, in this context the alkyl chain comprises from 1 to 8 carbon atoms, i.e. 1, 2, 3, 4, 5, 6, 7, or 8, e.g. methyl, ethyl methyl,

ethyl, propyl, *iso*-propyl, butyl, *iso*-butyl, *sec*-butenyl, *tert*-butyl, pentyl, hexyl, pentyl, octyl. The heteroalkyl group is optionally substituted at the alkyl and/or heteroaryl part of the group.

The terms “alkenyl” and “cycloalkenyl” refer to olefinic unsaturated carbon atoms containing chains or rings with one or more double bonds. Examples are propenyl and cyclohexenyl. Preferably, the alkenyl chain comprises from 2 to 8 carbon atoms, i.e. 2, 3, 4, 5, 6, 7, or 8, e.g. ethenyl, 1-propenyl, 2-propenyl, *iso*-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, *iso*-butenyl, *sec*-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, hexenyl, pentenyl, octenyl. Preferably the cycloalkenyl ring comprises from 3 to 8 carbon atoms, i.e. 3, 4, 5, 6, 7, or 8, e.g. 1-cyclopropenyl, 2-cyclopropenyl, 1-cyclobutenyl, 2-cyclobutenyl, 1-cyclopentenyl, 2-cyclopentenyl, 3-cyclopentenyl, cyclohexenyl, cyclopentenyl, cyclooctenyl.

The term “alkynyl” refers to unsaturated carbon atoms containing chains or rings with one or more triple bonds. An example is the propargyl radical. Preferably, the alkynyl chain comprises from 2 to 8 carbon atoms, i.e. 2, 3, 4, 5, 6, 7, or 8, e.g. ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, hexynyl, pentylnyl, octynyl.

In one embodiment, carbon atoms or hydrogen atoms in alkyl, heteroalkyl, cycloalkyl, aryl, aralkyl, alkenyl, cycloalkenyl, alkynyl radicals may be substituted independently from each other with one or more elements selected from the group consisting of O, S, N or with groups containing one or more elements selected from the group consisting of O, S, N.

Embodiments include alkoxy, cycloalkoxy, aryloxy, aralkoxy, alkenyloxy, cycloalkenyloxy, alkynyloxy, alkylthio, cycloalkylthio, arylthio, aralkylthio, alkenylthio, cycloalkenylthio, alkynylthio, alkylamino, cycloalkylamino, arylamino, aralkylamino, alkenylamino, cycloalkenylamino, alkynylamino radicals.

Other embodiments include hydroxyalkyl, hydroxycycloalkyl, hydroxyaryl, hydroxyaralkyl, hydroxyalkenyl, hydroxycycloalkenyl, hydroxyalanyl, mercaptoalkyl, mercaptocycloalkyl, mercaptoaryl, mercaptoaralkyl, mercaptoalkenyl, mercaptocycloalkenyl, mercaptoalkynyl, aminoalkyl, aminocycloalkyl, aminoaryl, aminoaralkyl, aminoalkenyl, aminocycloalkenyl, aminoalkynyl radicals.

In another embodiment, hydrogen atoms in alkyl, heteroalkyl, cycloalkyl, aryl, aralkyl, alkenyl, cycloalkenyl, alkynyl radicals may be substituted independently from each other with one or more halogen atoms. One radical is the trifluoromethyl radical.

If two or more radicals or two or more residues can be selected independently from each other, then the term “independently” means that the radicals or the residues may be the same or may be different.

As used herein a wording defining the limits of a range of length such as, e. g., “from 1 to 6” means any integer from 1 to 6, i. e. 1, 2, 3, 4, 5 and 6. In other words, any range defined by two integers explicitly mentioned is meant to comprise and disclose any integer defining said limits and any integer comprised in said range.

5 The term “halo” as used herein refers to a halogen residue selected from the group consisting of F, Br, I and Cl. Preferably, the halogen is F.

 The term “linker” as used herein refers to any chemically suitable linker. Preferably, linker are not or only slowly cleaved under physiological conditions. Thus, it is preferred that the linker does not comprise recognition sequences for proteases or recognition structures for
10 other degrading enzymes. Since it is preferred that the compounds of the invention are administered systemically to allow broad access to all compartments of the body and subsequently enrichment of the compounds of the invention wherever in the body the tumor is located, it is preferred that the linker is chosen in such that it is not or only slowly cleaved in blood. The cleavage is considered slowly, if less than 50% of the linkers are cleaved 2 h after
15 administration of the compound to a human patient. Suitable linkers, for example, comprises or consists of optionally substituted alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, sulfonyl, amines, ethers, thioethers phosphines, phosphoramidates, carboxamides, esters, imidoesters, amidines, thioesters, sulfonamides, 3-thiopyrrolidine-2,5-dione, carbamates,
20 ureas, guanidines, thioureas, disulfides, oximes, hydrazines, hydrazides, hydrazones, diaza bonds, triazoles, triazolines, tetrazines, platinum complexes and amino acids, or combinations thereof. Preferably, the linker comprises or consists of 1,4-piperazine, 1,3-propane and a phenolic ether or combinations thereof.

 The expression “optionally substituted” refers to a group in which one, two, three or
25 more hydrogen atoms may have been replaced independently of each other by the respective substituents.

 As used herein, the term "amino acid" refers to any organic acid containing one or more amino substituents, e.g. α -, β - or γ -amino, derivatives of aliphatic carboxylic acids. In the polypeptide notation used herein, e.g. Xaa₁Xaa₂Xaa₃Xaa₄Xaa₅, wherein Xaa₁ to
30 Xaa₅ are each and independently selected from amino acids as defined, the left hand direction is the amino terminal direction and the right hand direction is the carboxy terminal direction, in accordance with standard usage and convention.

 The term "conventional amino acid" refers to the twenty naturally occurring amino acids, and encompasses all stereomeric isoforms, i.e. D,L-, D- and L-amino acids thereof. These

conventional amino acids can herein also be referred to by their conventional three- letter or one-letter abbreviations and their abbreviations follow conventional usage (see, for example, Immunology—A Synthesis, 2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)).

5 The term "non-conventional amino acid" refers to unnatural amino acids or chemical amino acid analogues, e.g. α,α -disubstituted amino acids, N-alkyl amino acids, homo-amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), and ortho-, meta- or para-aminobenzoic acid. Non-conventional amino acids also include compounds which have an amine and carboxyl functional group separated in a 1,3 or
10 larger substitution pattern, such as β -alanine, γ -amino butyric acid, Freidinger lactam, the bicyclic dipeptide (BTD), amino-methyl benzoic acid and others well known in the art. Statine-like isosteres, hydroxyethylene isosteres, reduced amide bond isosteres, thioamide isosteres, urea isosteres, carbamate isosteres, thioether isosteres, vinyl isosteres and other amide bond isosteres known to the art may also be used. The use of analogues or non-conventional amino
15 acids may improve the stability and biological half-life of the added peptide since they are more resistant to breakdown under physiological conditions. The person skilled in the art will be aware of similar types of substitution which may be made. A non-limiting list of non-conventional amino acids which may be used as suitable building blocks for a peptide and their standard abbreviations (in brackets) is as follows: α -aminobutyric acid (Abu), L-N-methylalanine (Nmala), α -amino- α -methylbutyrate (Mgab), L-N-methylarginine (Nmarg), aminocyclopropane (Cpro), L-N-methylasparagine (Nmasn), carboxylate L-N-methylaspartic acid (Nmasp), aninoisobutyric acid (Aib), L-N-methylcysteine (Nmcy), aminonorbornyl (Norb), L-N-methylglutamine (Nmgl), carboxylate L-N-methylglutamic acid (Nmglu), cyclohexylalanine (Chexa), L-N-methylhistidine (Nmhis), cyclopentylalanine (Cpen), L-N-methylisoleucine (Nmile), L-N-methylleucine (Nmleu), L-N-methyllysine (Nmlys), L-N-methylmethionine (Nmmet), L-N-methylnorleucine (Nmnl), L-N-methylnorvaline (Nmna), L-N-methylornithine (Nmorn), L-N-methylphenylalanine (Nmphe), L-N-methylproline (Nmpro), L-N-methylserine (Nmser), L-N-methylthreonine (Nmthr), L-N-methyltryptophan (Nmtrp), D-ornithine (Dorn), L-N-methyltyrosine (Nmtyr), L-N-methylvaline (Nmval), L-N-methylethylglycine (Nmetg), L-N-methyl-t-butylglycine (Nmtbg), L-norleucine (Nle), L-norvaline (Nva), α -methyl-aminoisobutyrate (Maib), α -methyl- γ -aminobutyrate (Mgab), D- α -methylalanine (Dmala), α -methylcyclohexylalanine (Mchexa), D- α -methylarginine (Dmarg), α -methylcyclopentylalanine (Mcpn), D- α -methylasparagine (Dmasn), α -methyl- α -naphthylalanine (Manap), D- α -methylaspartate (Dmasp), α -methylpenicillamine (Mpen), D- α -

methylcysteine (Dmcys), N-(4-aminobutyl)glycine (Nglu), D- α -methylglutamine (Dmgln), N-(2-aminoethyl)glycine (Naeg), D- α -methylhistidine (Dmhis), N-(3-aminopropyl)glycine (Norn), D- α -methylisoleucine (Dmile), N-amino- α -methylbutyrate (Nmaabu), D- α -methylleucine (Dmleu), α -naphthylalanine (Anap), D- α -methyllysine (Dmlys), N-benzylglycine
 5 (Nphe), D- α -methylmethionine (Dmmet), N-(2-carbamylethyl)glycine (Ngln), D- α -methylornithine (Dmorn), N-(carbamylmethyl)glycine (Nasn), D- α -methylphenylalanine (Dmphe), N-(2-carboxyethyl)glycine (Nglu), D- α -methylproline (Dmpro), N-(carboxymethyl)glycine (Nasp), D- α -methylserine (Dmser), N-cyclobutylglycine (Ncbut), D- α -methylthreonine (Dmthr), N-cycloheptylglycine (Nchep), D- α -methyltryptophan (Dmtrp),
 10 N-cyclohexylglycine (Nchex), D- α -methyltyrosine (Dmty), N-cyclodecylglycine (Ncdec), D- α -methylvaline (Dmval), N-cyclododecylglycine (Ncdod), D-N-methylalanine (Dnmala), N-cyclooctylglycine (Ncoct), D-N-methylarginine (Dnmarg), N-cyclopropylglycine (Ncpro), D-N-methylasparagine (Dnmasn), N-cycloundecylglycine (Ncund), D-N-methylaspartate (Dnmasp), N-(2,2-diphenylethyl)glycine (Nbhm), D-N-methylcysteine (Dnmcys), N-(3,3-diphenylpropyl)glycine (Nbhe), D-N-methylglutamine (Dnmgln), N-(3-guanidinopropyl)glycine (Narg), D-N-methylglutamate (Dnmglu), N-(1-hydroxyethyl)glycine (Ntbx), D-N-methylhistidine (Dnmhis), N-(hydroxyethyl)glycine (Nser), D-N-methylisoleucine (Dnmile), N-(imidazolethyl)glycine (Nhis), D-N-methylleucine (Dnmleu), N-(3-indolylyethyl)glycine (Nhtrp), D-N-methyllysine (Dnnilys), N-methyl- γ -aminobutyrate (Nmgabu), N-methylcyclohexylalanine (Nmchexa), D-N-methylmethionine (Dnmmet), D-N-methylornithine (Dnmorn), N-methylcyclopentylalanine (Nmcpen), N-methylglycine (NaIa), D-N-methylphenylalanine (Dnmphe), N-methylaminoisobutyrate (Nmaib), D-N-methylproline (Dnmpro), N-(1-methylpropyl)glycine (Nile), D-N-methylserine (Dnmser), N-(2-methylpropyl)glycine (Nleu), D-N-methylthreonine
 25 (Dnmthr), D-N-methyltryptophan (Dnmtrp), N-(1-methylethyl)glycine (Nval), D-N-methyltyrosine (Dnmtyr), N-methyl- α -naphthylalanine (Nmanap), D-N-methylvaline (Dnmval), N-methylpenicillamine (Nmpen), γ -aminobutyric acid (Gabu), N-(p-hydroxyphenyl)glycine (Nhtrp), L-/butylglycine (Tbug), N-(thiomethyl)glycine (Ncys), L-ethylglycine (Etg), penicillamine (Pen), L-homophenylalanine (Hphe), L- α -methylalanine (Mala), L- α -methylarginine (Marg), L- α -methylasparagine (Masn), L- α -methylaspartate (Masps), L- α -methyl-t-butylglycine (Mtbug), L- α -methylcysteine (Mcys), L-methylethylglycine (Metg), L- α -methylglutamine (Mgln), L- α -methylglutamate (Mglu), L- α -methylhistidine (Mhis), L- α -methylhomophenylalanine (Mhphe), L- α -methylisoleucine (Mile), N-(2-methylthioethyl)glycine (Nmet), L- α -methylleucine (Mleu), L- α -methyllysine (Mlys), L- α -

methylnorleucine (Mnle), L- α -methylnorvaline (Mnva), L- α -methylornithine (Morn), L- α -methylphenylalanine (Mphe), L- α -methylproline (Mpro), L- α -methylserine (Mser), L- α -methylthreonine (Mthr), L- α -methyltryptophan (Mtrp), L- α -methyltyrosine (Mtyr), L- α -methylvaline (Mval), L-N-methylhomophenylalanine (Nmhphe),
5 N-(N-(2,2-diphenylethyl)carbamylmethyl)glycine (Nnbhm), N-(N-(3,3'-diphenylpropyl)-carbamylmethyl)glycine (Nnbhe), 1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane (Nmhc), L-O-methyl serine (Omser), L-O-methyl homoserine (Omhsr).

The term "N-containing aromatic or non-aromatic mono or bicyclic heterocycle" as used herein refers to a cyclic saturated or unsaturated hydrocarbon compound which contains
10 at least one nitrogen atom as constituent of the cyclic chain.

The term "radioactive moiety" as used herein refers to a molecular assembly which carries a radioactive nuclide. The nuclide is bound either by covalent or coordinate bonds which remain stable under physiological conditions. Examples are [^{131}I]-3-iodobenzoic acid or ^{68}Ga -DOTA.

15 A "fluorescent isotope" as used herein emits electromagnetic radiation after excitation by electromagnetic radiation of a shorter wavelength.

A "radioisotope" as used herein is a radioactive isotope of an element (included by the term "radionuclide") emitting α -, β -, and/or γ -radioation.

The term "radioactive drug" is used in the context of the present invention to refer to a
20 biologic active compound which is modified by a radioisotope. Especially intercalating substances can be used to deliver the radioactivity to direct proximity of DNA (e.g. a ^{131}I -carrying derivative of Hoechst-33258).

The term "chelating agent" or "chelate" are used interchangeably in the context of the present invention and refer to a molecule, often an organic one, and often a Lewis base, having
25 two or more unshared electron pairs available for donation to a metal ion. The metal ion is usually coordinated by two or more electron pairs to the chelating agent. The terms, "bidentate chelating agent", "tridentate chelating agent, and "tetradentate chelating agent" refer to chelating agents having, respectively, two, three, and four electron pairs readily available for simultaneous donation to a metal ion coordinated by the chelating agent. Usually, the electron
30 pairs of a chelating agent forms coordinate bonds with a single metal ion; however, in certain examples, a chelating agent may form coordinate bonds with more than one metal ion, with a variety of binding modes being possible.

The term "fluorescent dye" is used in the context of the present invention to refer to a compound that emits visible or infrared light after excitation by electromagnetic radiation of a

shorter and suitable wavelength. It is understood by the skilled person, that each fluorescent dye has a predetermined excitation wavelength.

The term “contrast agent” is used in the context of the present invention to refer to a compound which increases the contrast of structures or fluids in medical imaging. The enhancement is achieved by absorbing electromagnetic radiation or altering electromagnetic fields.

The term “paramagnetic” as used herein refers to paramagnetism induced by unpaired electrons in a medium. A paramagnetic substance induces a magnetic field if an external magnetic field is applied. Unlike diamagnetism the direction of the induced field is the same as the external field and unlike ferromagnetism the field is not maintained in absence of an external field.

The term “nanoparticle” as used herein refers to particles preferably of spheric shape, with diameters of sizes between 1 and 100 nanometers. Depending on the composition, nanoparticles can possess magnetical, optical or physico-chemical qualities that can be assessed. Additionally surface modification is achievable for many types of nanoparticles.

The term "pharmaceutically acceptable salt" refers to a salt of the compound of the present invention. Suitable pharmaceutically acceptable salts of the compound of the present invention include acid addition salts which may, for example, be formed by mixing a solution of choline or derivative thereof with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compound of the invention carries an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts (e.g., sodium or potassium salts); alkaline earth metal salts (e.g., calcium or magnesium salts); and salts formed with suitable organic ligands (e.g., ammonium, quaternary ammonium and amine cations formed using counteranions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate). Illustrative examples of pharmaceutically acceptable salts include but are not limited to: acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, hydroxynaphthoate,

iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like (see, for example, Berge, S. M., et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide a compound of formula (I). A prodrug is an active or inactive compound that is modified chemically through *in vivo* physiological action, such as hydrolysis, metabolism and the like, into a compound of this invention following administration of the prodrug to a patient. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme. The suitability and techniques involved in making and using prodrugs are well known by those skilled in the art. For a general discussion of prodrugs involving esters see Svensson and Tunek *Drug Metabolism Reviews* 16.5 (1988) and Bundgaard *Design of Prodrugs*, Elsevier (1985). Examples of a masked carboxylate anion include a variety of esters, such as alkyl (for example, methyl, ethyl), cycloalkyl (for example, cyclohexyl), aralkyl (for example, benzyl, p-methoxybenzyl), and alkylcarbonyloxyalkyl (for example, pivaloyloxymethyl). Amines have been masked as arylcarbonyloxymethyl substituted derivatives which are cleaved by esterases *in vivo* releasing the free drug and formaldehyde (Bundgaard *J. Med. Chem.* 2503 (1989)). Also, drugs containing an acidic NH group, such as imidazole, imide, indole and the like, have been masked with N-acyloxymethyl groups (Bundgaard *Design of Prodrugs*, Elsevier (1985)).

Hydroxyl groups have been masked as esters and ethers. EP 0 039 051 (Sloan and Little, Apr. 11, 1981) discloses Mannich-base hydroxamic acid prodrugs, their preparation and use.

Compounds according to the invention can be synthesized according to one or more of the following methods. It should be noted that the general procedures are shown as it relates to preparation of compounds having unspecified stereochemistry. However, such procedures are generally applicable to those compounds of a specific stereochemistry, e.g., where the stereochemistry about a group is (S) or (R). In addition, the compounds having one stereochemistry (e.g., (R)) can often be utilized to produce those having opposite stereochemistry (i.e., (S)) using well-known methods, for example, by inversion.

Certain compounds of the present invention can exist in unsolvated forms as well as in solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

The term "pharmaceutical composition" as used in the present application refers to a substance and/or a combination of substances being used for the identification, prevention or treatment of a tissue status or disease. The pharmaceutical composition is formulated to be suitable for administration to a patient in order to prevent and/or treat disease. Further a pharmaceutical composition refers to the combination of an active agent with a carrier, inert or active, making the composition suitable for therapeutic use. Pharmaceutical compositions can be formulated for oral, parenteral, topical, inhalative, rectal, sublingual, transdermal, subcutaneous or vaginal application routes according to their chemical and physical properties. Pharmaceutical compositions comprise solid, semisolid, liquid, transdermal therapeutic systems (TTS). Solid compositions are selected from the group consisting of tablets, coated

tablets, powder, granulate, pellets, capsules, effervescent tablets or transdermal therapeutic systems. Also comprised are liquid compositions, selected from the group consisting of solutions, syrups, infusions, extracts, solutions for intravenous application, solutions for infusion or solutions of the carrier systems of the present invention. Semisolid compositions that can be used in the context of the invention comprise emulsion, suspension, creams, lotions, gels, globules, buccal tablets and suppositories.

“Pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

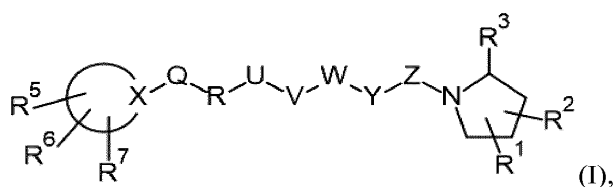
The term “carrier”, as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic agent is administered. Such pharmaceutical carriers can be sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term “fibroblast activation protein (FAP)” as used herein is also known under the term “seprase”. Both terms can be used interchangeably herein. Fibroblast activation protein is a homodimeric integral protein with dipeptidyl peptidase IV (DPPIV)-like fold, featuring an alpha/beta-hydrolase domain and an eight-bladed beta-propeller domain.

Embodiments

In the following different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

In a first aspect, the present invention provides a compound of Formula (I)



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

- 5 Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other; preferably out of the six four groups are present of which two are C=O, one is CH₂ and one is NH; more preferably four groups are present of which two are C=O, one is CH₂ and one is NH; most preferably, V, W, Y and Z are present of which V and Z are C=O and W and Y are independently selected from CH₂ and NH;

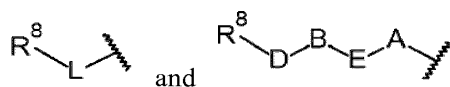
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl-, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

- 15 R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aryl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

- 20 R⁶, and R⁷ are independently selected from the group consisting of -H,



under the proviso that R⁶ and R⁷ are not at the same time H, preferably R⁶ is attached to the 7- or 8-quinolyl position and R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁶ is attached to the 7-quinolyl position and R⁷ is attached to the 6-quinolyl position,

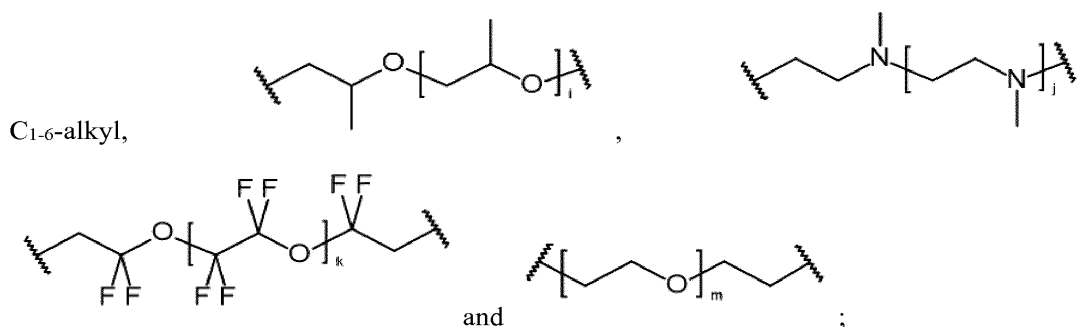
- 25 wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR⁴, O, S, and CH₂;

- 30 E is selected from the group consisting of



wherein i is 1, 2, or 3;

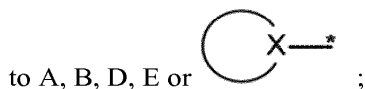
wherein j is 1, 2, or 3;

5 wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

A and E together form a group selected from: a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, wherein A and E can be mono-, bi- and multicyclic,
 10 preferably monocyclic. Each A and E being optionally substituted with 1 to 4 substituents selected from -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo; and optionally connected



15 B is selected from the group consisting of S, NR⁴, NR⁴-O, NR⁴-C₁₋₆-alkyl, NR⁴-C₁₋₆-alkyl-NR⁴, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein NR⁴-C₁₋₆-alkyl-NR⁴ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl,
 20 aryl, C₁₋₆-aralkyl; and

R⁸ is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;

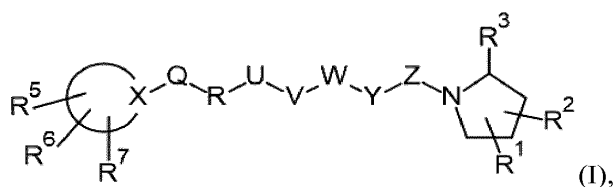
is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

25

or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a preferred embodiment, A and E together form a group selected from the group consisting of a C₃, C₄, C₅, C₆, C₇ and C₈ monocyclic, preferably C₅ or C₆ monocyclic, or C₇, C₈, C₉, C₁₀, C₁₁ or C₁₂ bicyclic, preferably C₇, C₈, C₉ and C₁₀ bicyclic heterocycloalkyl, comprising 1, 2, 3, or 4, preferably 1 or 2 heteroatoms independently selected from the group consisting of N, O and S, preferably N and O, most preferably 1 or 2 N.

In a preferred embodiment of the first aspect of the present invention a compound of Formula (I) is provided:



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other; preferably out of the six four groups are present of which two are C=O, one is CH₂ and one is NH; more preferably four groups are present of which two are C=O, one is CH₂ and one is NH; most preferably, V, W, Y and Z are present of which V and Z are C=O and W and Y are independently selected from CH₂ and NH;

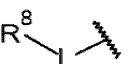
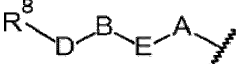
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl-, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aryl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,

R^8  and R^8 , under the proviso that R^6 and R^7 are not at the same time H, preferably R^6 is attached to the 7- or 8-quinolyl position and R^7 is attached to the 5- or 6-quinolyl position; more preferably R^6 is attached to the 7-quinolyl position and R^7 is attached to the 6-quinolyl position,

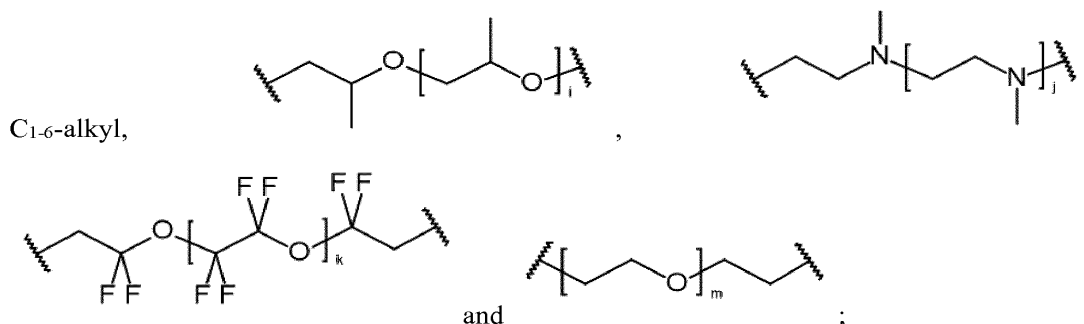
5 wherein L is a linker,

wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR^4 , O, S, and CH_2 ;

10 E is selected from the group consisting of



wherein i is 1, 2, or 3;

wherein j is 1, 2, or 3;

15 wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

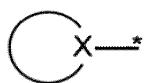
more preferably, E is C_{1-6} -alkyl, most preferably, E is C3 or C4 alkyl;

B is selected from the group consisting of S, NR^4 , NR^4 -O, NR^4 - C_{1-6} -alkyl, NR^4 - C_{1-6} -alkyl- NR^4 , and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle,

20 preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein NR^4 - C_{1-6} -alkyl- NR^4 and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C_{1-6} -alkyl, aryl, C_{1-6} -aralkyl; and

R^8 is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye,

25 a contrast agent and combinations thereof;



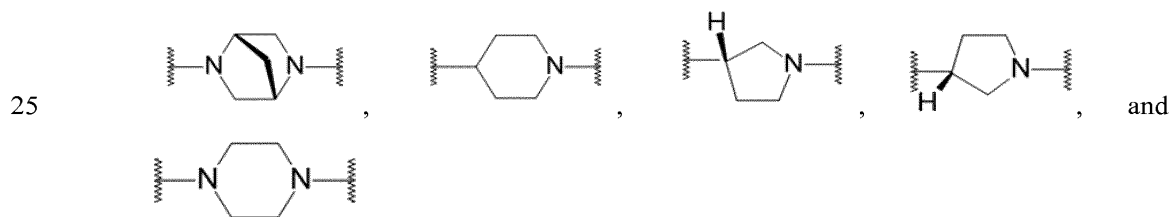
is a 1-naphthyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

- 5 or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In another preferred embodiment of the first aspect of the present invention A and E together form a group consisting of a C₃, C₄, C₅, C₆, C₇ and C₈ monocyclic, preferably C₅ or C₆ monocyclic, or C₇, C₈, C₉, C₁₀, C₁₁ or C₁₂ bicyclic, preferably C₇, C₈, C₉ and C₁₀ bicyclic heterocycloalkyl, preferably comprising 1, 2, 3, or 4, more preferably 1 or 2 heteroatoms independently selected from the group consisting of N, O and S, preferably N and O, most preferably 1 or 2 N. Preferred monocyclic heterocycloalkyls are selected from the group consisting of pyrrolidinyl, piperidinyl, imidazolidinyl, 1,2-diazacyclohexanyl, 1,3-diazacyclohexanyl, piperazinyl, 1-oxo-2-azacyclohexanyl, 1-oxo-3-azacyclohexanyl, or morpholinyl, preferably piperidinyl, piperazinyl, and pyrrolidinyl. Preferred bicyclic heterocycloalkyls are selected from the group consisting of bicyclo[2.2.1] 2,5-diazaheptanyl, 3,6-diazabicyclo[3.2.1]octanyl, 3,6-diazabicyclo[3.2.2]nonyl, octahydropyrrolo[2,3-b]pyrrolyl, octahydropyrrolo[3,2-b]pyrrolyl, octahydropyrrolo[3,4-b]pyrrolyl, octahydropyrrolo[3,4-c]pyrrolyl, 9-methyl-3,7,9-triazabicyclo[3.3.1]nonanyl.

The bond between the heterocycle formed by A and E and B on one hand and/or R⁶ or R⁷ on the other is preferably through the heteroatom, preferably through N.

Particularly, preferred examples of the heterocycle formed by A and E are selected from the group consisting of



- In a preferred embodiment of the first aspect of the present invention,
 Q, R, U are CH₂ and are individually present or absent; preferably, Q and R are absent;
 V is CH₂, C=O, C=S or C=NR⁴; preferably, V is C=O;
 30 W is NR⁴; preferably, W is NH;

Y is HCR^4 ; preferably, Y is CH_2 ; and

Z is $\text{C}=\text{O}$, $\text{C}=\text{S}$ or $\text{C}=\text{NR}^4$, preferably, Z is $\text{C}=\text{O}$.

In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

5 V is CH_2 ;

W is NH ;

Y is CH_2 ; and

Z is $\text{C}=\text{O}$.

In a further preferred embodiment of the first aspect of the present invention,

10 R^1 and R^2 are independently selected from the group consisting of -H and halo; preferably, R^1 and R^2 are halo; more preferably, R^1 and R^2 are F;

R^3 is selected from the group consisting of -H, -CN, and $-\text{B}(\text{OH})_2$; preferably, R^3 is -CN or $-\text{B}(\text{OH})_2$; more preferably, R^3 is -CN;

15 R^4 is selected from the group consisting of -H and $-\text{C}_{1-6}$ -alkyl, wherein the $-\text{C}_{1-6}$ -alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

20 V is CH_2 ;

W is NH ;

Y is CH_2 ;

Z is $\text{C}=\text{O}$;

25 R^1 and R^2 are independently selected from the group consisting of -H and halo; preferably, R^1 and R^2 are halo; more preferably, R^1 and R^2 are F;

R^3 is selected from the group consisting of -H, -CN, and $-\text{B}(\text{OH})_2$; preferably, R^3 is -CN or $-\text{B}(\text{OH})_2$; more preferably, R^3 is -CN;

30 R^4 is selected from the group consisting of -H and $-\text{C}_{1-6}$ -alkyl, wherein the $-\text{C}_{1-6}$ -alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is CH_2 ;

W is CH₂;

Y is NH;

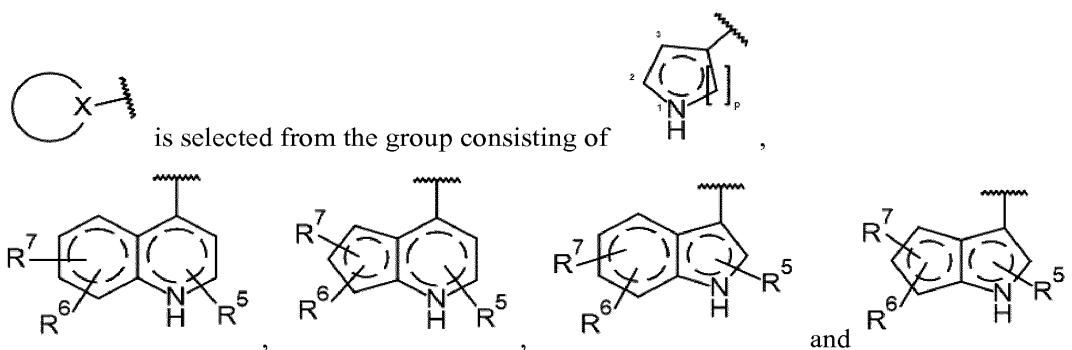
Z is C=O;

R¹ and R² are independently selected from the group consisting of -H and halo; preferably, R¹ and R² are halo; more preferably, R¹ and R² are F;

R³ is selected from the group consisting of -H, -CN, and -B(OH)₂; preferably, R³ is -CN or -B(OH)₂; more preferably, R³ is -CN;

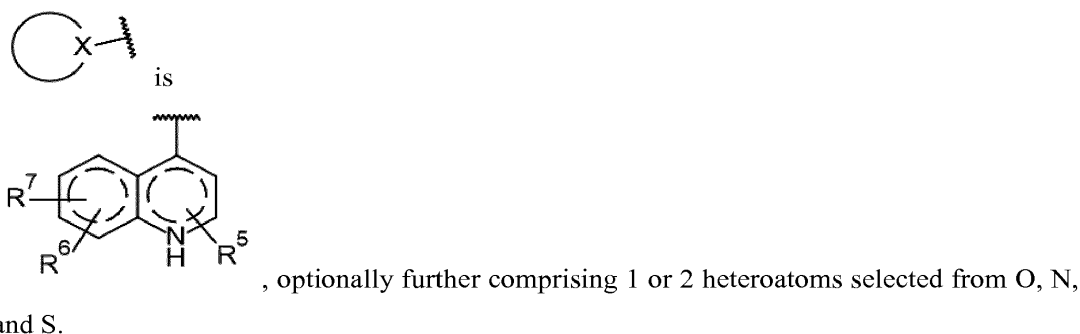
R⁴ is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,



optionally further comprising 1 or 2 heteroatoms selected from O, N, and S.

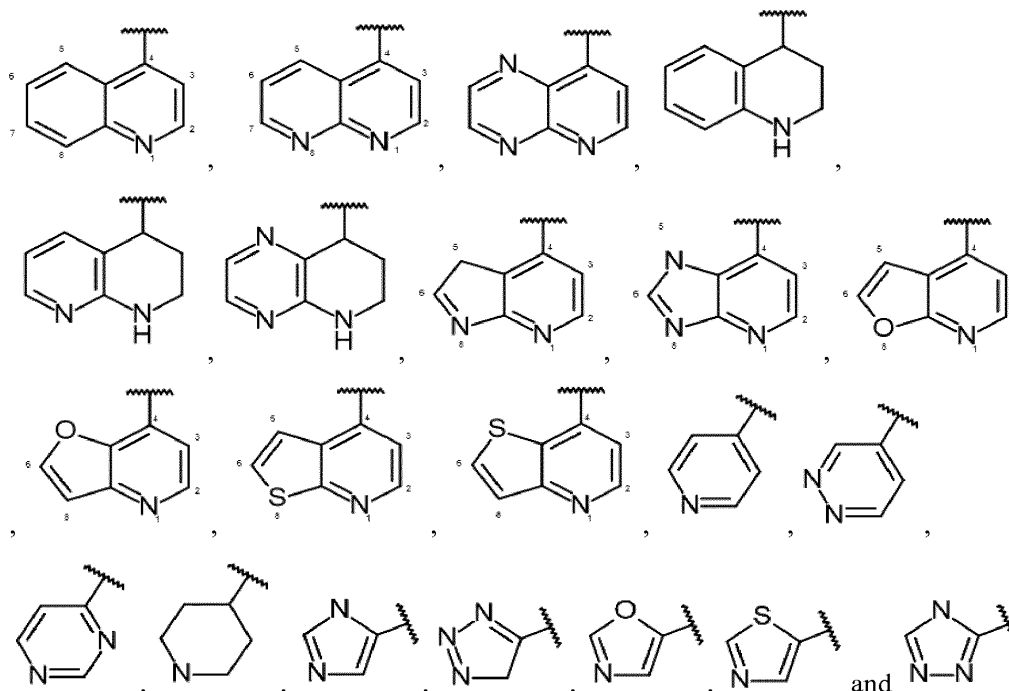
In a further preferred embodiment of the first aspect of the present invention,



In a further preferred embodiment of the first aspect of the present invention,

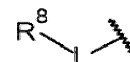


is selected from the group consisting of



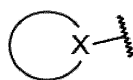
5

. R^6 , and R^7 are independently selected from the group consisting of $-H$,

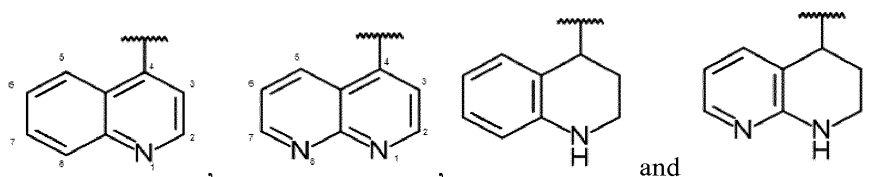


and $R^8-D-B-E-A$, under the proviso that R^6 and R^7 are not at the same time H and preferably R^6 and R^7 are attached on positions 5, 6 or 7.

10 In a preferred embodiment,

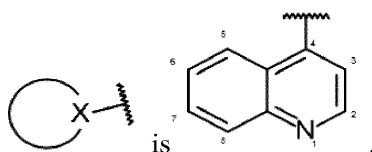


is selected from the group consisting of

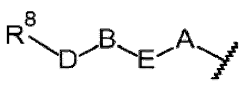


and

In another preferred embodiment

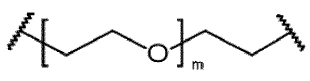


In a further preferred embodiment of the first aspect of the present invention,
 R^5 and R^6 are H;

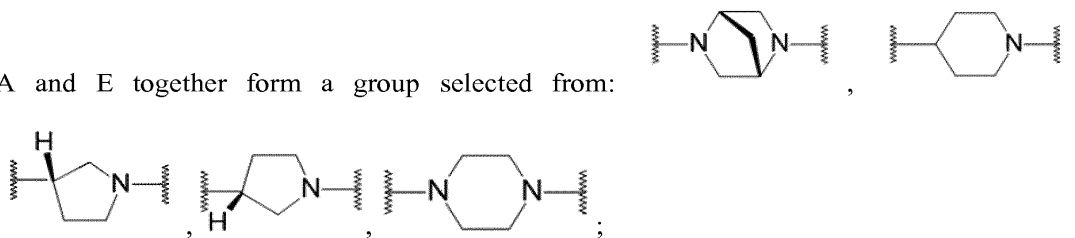
R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein

D is absent;

A is O, S, CH_2 , NH, NCH_3 ;

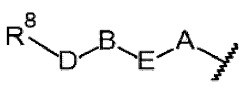
E is C_{1-6} -alkyl or , wherein m is 1, 2, or 3; Preferably, C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C_{1-6} -alkyl, most preferably, E is C3 or C4 alkyl; or

A and E together form a group selected from:

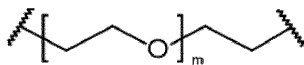


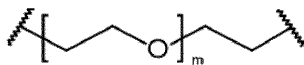
B is NR^4 - C_{1-6} -alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C_{1-6} -alkyl, aryl, C_{1-6} -aralkyl. Preferably, C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,
 R^5 and R^6 are H;

R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein D is absent;

A is O;

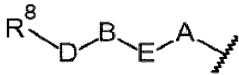


E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

- 5 B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

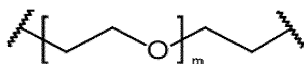
In a further preferred embodiment of the first aspect of the present invention,

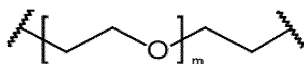
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

- 15 D is absent;

A is S;

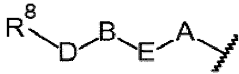


E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

- 20 B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

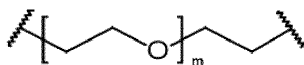
In a further preferred embodiment of the first aspect of the present invention,

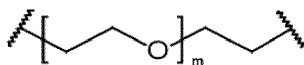
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

A is CH₂;

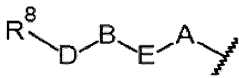


E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

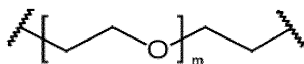
In a further preferred embodiment of the first aspect of the present invention,

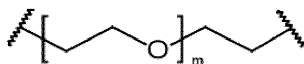
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is absent;

A is NH;

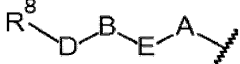


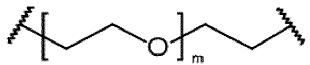
E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

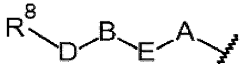
R⁵ and R⁶ are H;

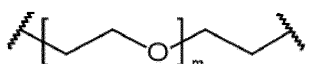
R^8
 R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein
 D is an amino acid, preferably carrying a charged side chain;
 A is O;

- 5 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;
 B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and
 10 S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention,

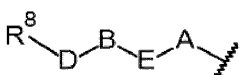
- 15 R⁵ and R⁶ are H;

R^8
 R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein
 D is an amino acid, preferably carrying a charged side chain;
 A is S;

- 20 E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;
 B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and
 25 S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

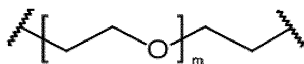
In a further preferred embodiment of the first aspect of the present invention,

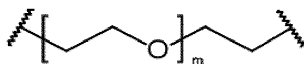
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is an amino acid, preferably carrying a charged side chain;

5 A is CH₂;

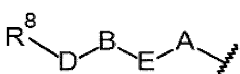


E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

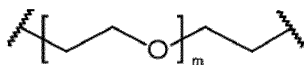
15 In a further preferred embodiment of the first aspect of the present invention,

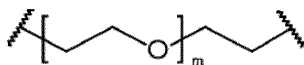
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 5- or 6-quinolyl position; more preferably R⁷ is attached to the 6-quinolyl position, wherein

D is an amino acid, preferably carrying a charged side chain;

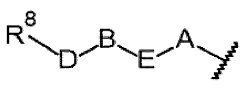
20 A is NH;



E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

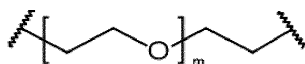
B is NR⁴-C₁₋₆-alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl. Preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

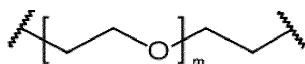
In a further preferred embodiment of the first aspect of the present invention,
 R^5 and R^6 are H;

R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein

5 D is absent;

A is O;

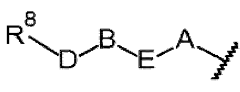


E is C_{1-6} -alkyl or , wherein m is 1, 2, or 3; Preferably, E is C_{1-6} -alkyl and C_{1-6} -alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C_{1-6} -alkyl, most preferably,

10 E is C3 or C4 alkyl;

B is a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 nitrogen atoms.

In a further preferred embodiment of the first aspect of the present invention,
 R^5 and R^6 are H;

15 R^7 is , preferably R^7 is attached to the 5- or 6-quinolyl position; more preferably R^7 is attached to the 6-quinolyl position, wherein

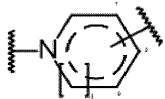
D is absent;

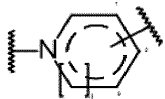
A is O;

E is C3 or C4 alkyl; more preferably, E is propyl or butyl;

20 B is a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 nitrogen atoms.

In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



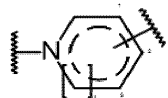
25 , wherein

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S,
optionally further comprises 1 nitrogen;

 is attached to position 1, 2, or 3, preferably to position 2;

l is 1 or 2.

In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic
heterocycle:



, wherein

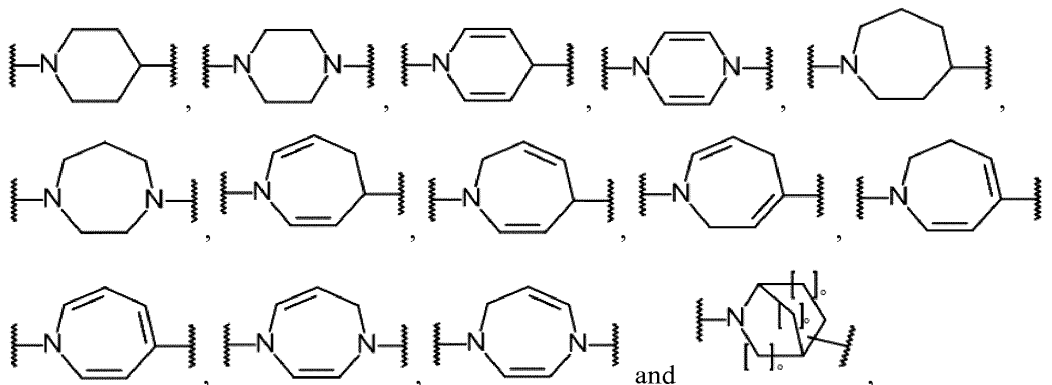
the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S,
optionally further comprises 1 nitrogen;

 is attached to position 1, 2, or 3, preferably to position 2;

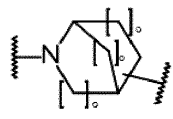
l is 1 or 2;

wherein the N-containing heterocycle is substituted with a C₁₋₆-alkyl.

In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:



wherein the N-containing heterocycle is substituted with a C₁₋₆-alkyl



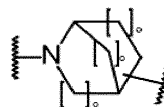
wherein if the N-containing heterocycle comprised in B is

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S,
optionally further comprises 1 nitrogen, optionally compromises one or more (e.g. amino acid
derived) side chains;

 is attached to position 1, 2, or 3, preferably to position 2;

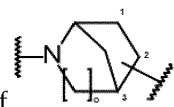
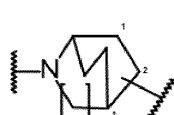
o is 1 or 2;

preferably, if the N-containing heterocycle comprised in B is

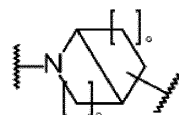


, the N-

containing heterocycle comprised in B is selected from the group consisting of

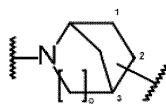


and

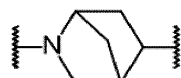


; more preferably, if the N-containing heterocycle

5 comprised in B is



, the N-containing heterocycle comprised in B is

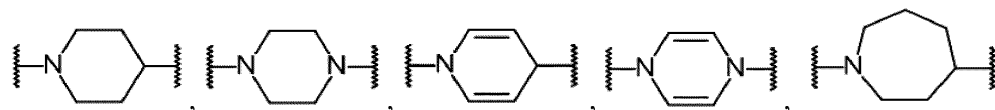
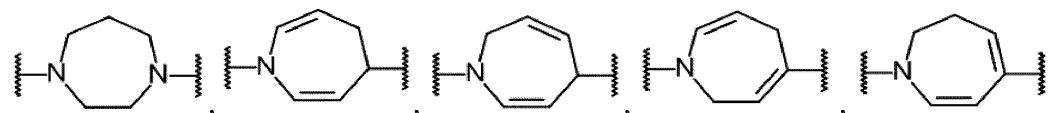
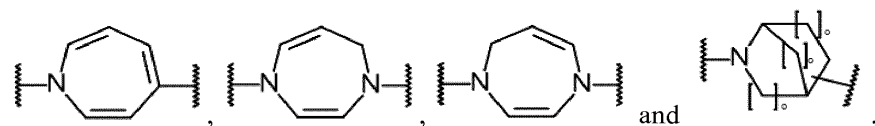


or

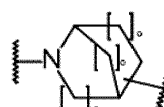


In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:

10



and

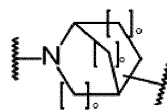


wherein if the N-containing heterocycle comprised in B is

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S,
optionally further comprises 1 nitrogen, optionally comprises one or more (e.g. amino acid
15 derived) side chains;

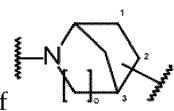
— is attached to position 1, 2, or 3, preferably to position 2;
o is 1 or 2;

preferably, if the N-containing heterocycle comprised in B is

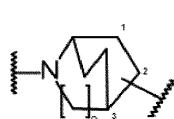


, the N-

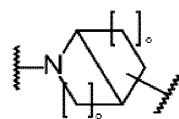
containing heterocycle comprised in B is selected from the group consisting of



5

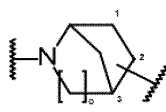


and



; more preferably, if the N-containing heterocycle

comprised in B is



, the N-containing heterocycle comprised in B is

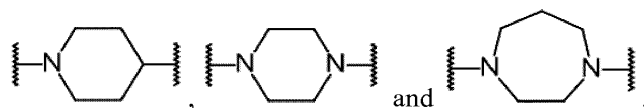


or

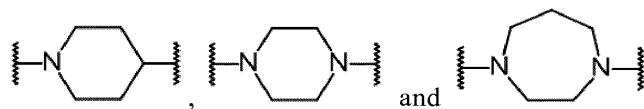


In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:

10



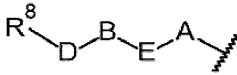
In a further preferred embodiment of the first aspect of the present invention,
the N-containing heterocycle comprised in B is selected from the group consisting of:



, wherein B is substituted with a C₁₋₃ alkyl.

15

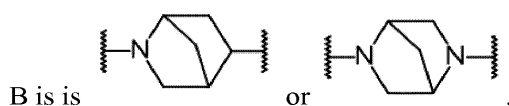
In a further preferred embodiment of the first aspect of the present invention,
R⁵ and R⁶ are H;

R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein
D is absent;

A is O;

20

E is propyl or butyl;



In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is C=O;

5 W is NH;

Y is CH₂;

Z is C=O;

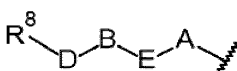
R¹ and R² are independently selected from the group consisting of -H and halo; preferably, R¹

and R² are independently selected from the group consisting of -H and F; more preferably, R¹

10 and R² are the same and are selected from the group consisting of -H and F;

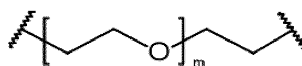
R³ is -CN;

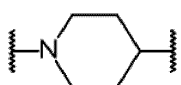
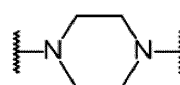
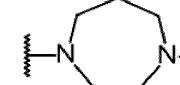
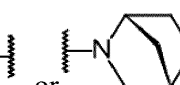
R⁵ and R⁶ are H;

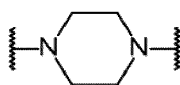
R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein

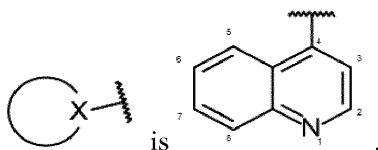
D is absent;

15 A is O;

E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; preferably, E is C₁₋₆-alkyl; preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl; more preferably, E is C₁₋₆-alkyl, most preferably, E is C3 or C4 alkyl;

20 B is NH-C₁₋₆-alkyl, , ,  or ; preferably, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl,

butyl, sec-butyl, tert-butyl, pentyl and hexyl; preferably, B is ; and



In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is C=O;

W is NH;

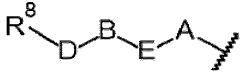
Y is CH₂;

5 Z is C=O;

R¹ and R² are the same and are selected from the group consisting of -H and F;

R³ is -CN;

R⁵ and R⁶ are H;

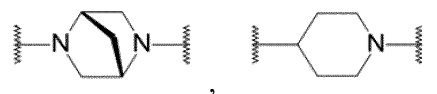
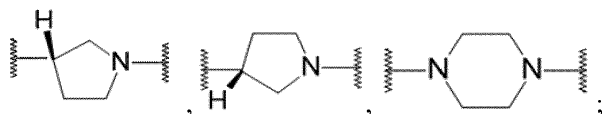
R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein

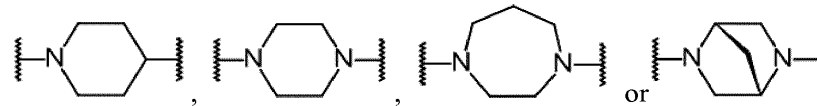
10 D is absent;

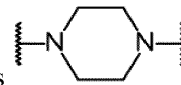
A is O, S, CH₂, NH, NCH₃;

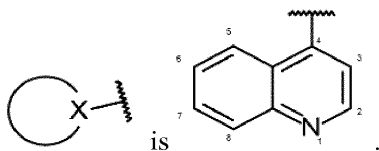
E is methyl, ethyl, propyl or butyl;

A and E together form a group selected from:



15 B is , optionally B is

substituted with a C₁₋₃ alkyl; preferably, B is ; and



In a further preferred embodiment of the first aspect of the present invention,

20 Q, R, U are absent;

V is C=O;

W is NH;

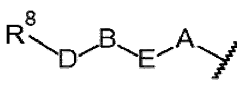
Y is CH₂;

Z is C=O;

R¹ and R² are the same and are selected from the group consisting of -H and F;

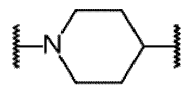
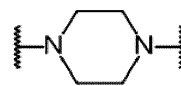
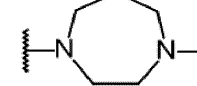
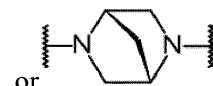
R³ is -CN;

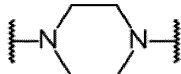
R⁵ and R⁶ are H;

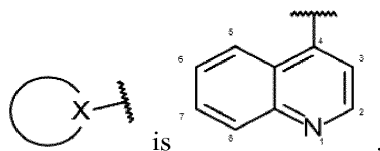
5 R⁷ is , preferably R⁷ is attached to the 6-quinolyl position, wherein D is absent;

A is O;

E is methyl, ethyl, propyl or butyl;

B is , ,  or ; preferably, B is

10 ; and



In a further preferred embodiment of the first aspect of the present invention,

Q, R, U are absent;

V is C=O;

15 W is NH;

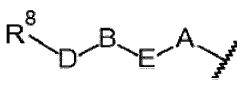
Y is CH₂;

Z is C=O;

R¹ and R² are the same and are selected from the group consisting of -H and F;

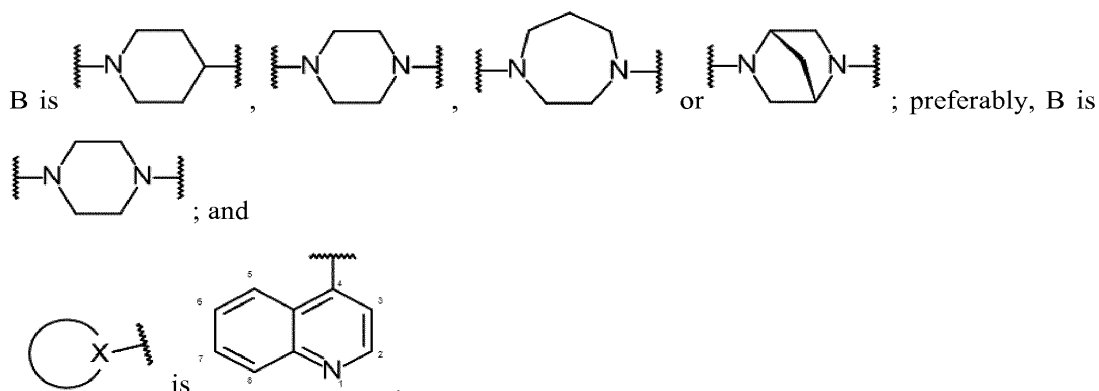
R³ is -CN;

20 R⁵ and R⁶ are H;

R⁷ is , R⁷ is attached to the 6-quinolyl position, wherein D is absent;

A is O;

E is methyl, ethyl, propyl or butyl;



5 In a further preferred embodiment of the first aspect of the present invention, C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl.

In a further preferred embodiment of the first aspect of the present invention, C₁₋₃-alkyl is selected from the group consisting of methyl, ethyl, propyl and i-propyl.

10 In a further preferred embodiment of the first aspect of the present invention, C₁₋₆-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl, phenyl-propyl, and phenyl-butyl.

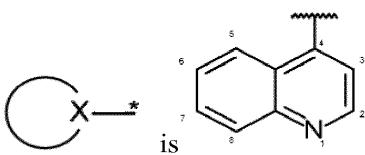
In a preferred embodiment of the first aspect of the present invention, the compound of the first aspect of the invention is selected from the compounds of table 1. More preferably, the compound of the first aspect of the invention is selected from the compounds of table 2. More preferably, the compound of the first aspect of the invention is selected from the group consisting of FAPI-02 and FAPI-04.

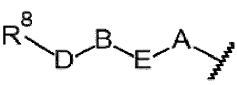
In a preferred embodiment of the first aspect of the present invention, the compound of the first aspect of the invention is selected from the compounds of table 1 and/or table 3. More preferably, the compound of the first aspect of the invention is selected from the compounds of table 2 and/or table 4. More preferably, the compound of the first aspect of the invention is selected from the group consisting of FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71, FAPI-72 and FAPI-73.

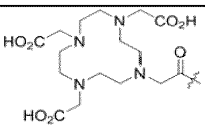
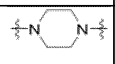
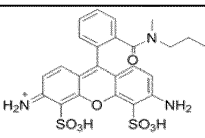
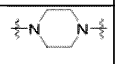
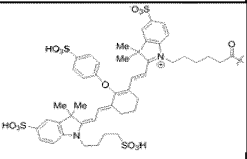
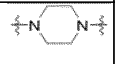
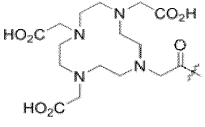
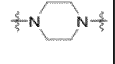
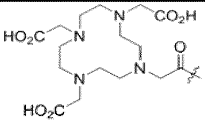
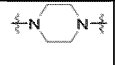
25

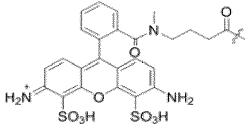
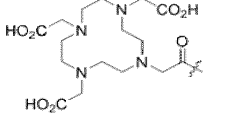
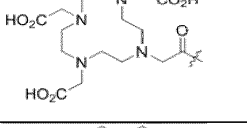
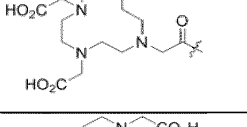
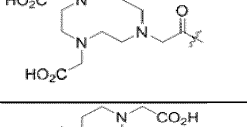
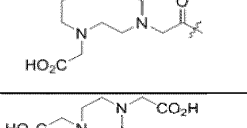
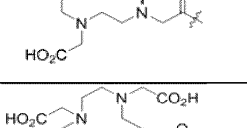
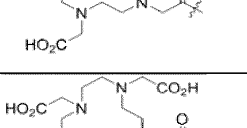
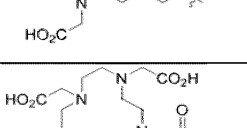
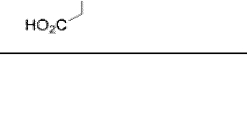
Table 1: Preferred compounds of the first aspect of the invention.

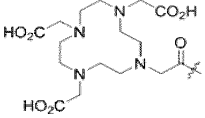
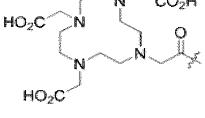
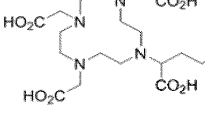
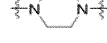
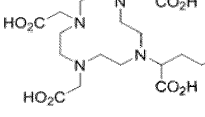
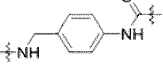
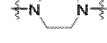
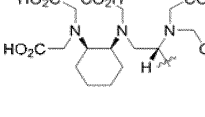
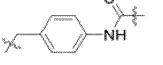
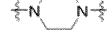
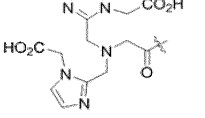
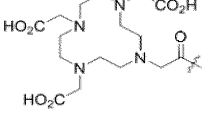
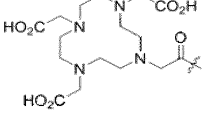
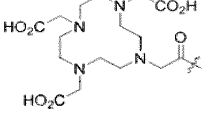
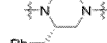
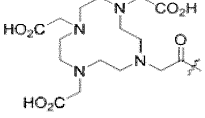
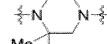
§ fluorescent compounds; \$ ^{99m}Tc -chelators; * Pb-chelators; R^1 and R^2 are located at the 4-

pyrrolidine position; Q, R, U are absent;  is R^5 is H; R^6 is attached to the 7-quinolyl position; R^7 is attached to the 6-quinolyl position; ‘-’ indicates that

- 5 R^6 or R^7 being H; ‘+’ indicates R^6 or R^7 being ; V is C=O; W is NH; Y is CH_2 ; Z is C=O; R^3 is $-\text{CN}$; A is O (except FAPI-01: A is absent, R^7 is attached to the 5-quinolyl position).

name	R^1/R^2	R^6	R^7	R^8	D	B	E
FAPI-01	H/H	-	+	I	-	-	-
FAPI-02	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-02 atto 488[§]	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-02 dy800 CW^{\$}	H/H	-	+		-		$n\text{-C}_3\text{H}_6$
FAPI-03	H/H	-	+		-		$n\text{-C}_4\text{H}_8$
FAPI-04	F/F	-	+		-		$n\text{-C}_3\text{H}_6$

name	R ¹ /R ²	R ⁶	R ⁷	R ⁸	D	B	E
FAPI-04 atto 488^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-05	F/F	-	+		-		<i>n</i> -C ₄ H ₈
FAPI-06	H/H	-	+		-	NH	<i>n</i> -C ₃ H ₆
FAPI-07	F/F	-	+		-	NH	<i>n</i> -C ₃ H ₆
FAPI-08	H/H	+	-		-		<i>n</i> -C ₃ H ₆
FAPI-09	F/F	+	-		-		<i>n</i> -C ₃ H ₆
FAPI-10	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-11	F/F	-	+		-		CH ₂
FAPI-12	F/F	-	+		-		<i>n</i> -C ₂ H ₄
FAPI-13	F/F	-	+		-		<i>n</i> -C ₃ H ₆

name	R ¹ /R ²	R ⁶	R ⁷	R ⁸	D	B	E
FAPI-14	F/F	-	+		-		<i>n</i> -C ₄ H ₈
FAPI-15	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄
FAPI-16	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-17	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-18	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-19^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-20	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-21	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-22	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-23	F/F	-	+		-		<i>n</i> -C ₃ H ₆

name	R ¹ /R ²	R ⁶	R ⁷	R ⁸	D	B	E
FAPI-24	F/F	-	+		-		(C ₂ H ₄ O) 3C ₂ H ₄
FAPI-25	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-26	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄
FAPI-27^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-28^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-29^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-30	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-31	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-32*	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-33^s	F/F	-	+		-		(C ₂ H ₄ O) 2C ₂ H ₄

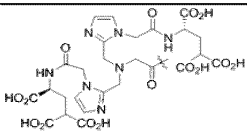
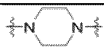
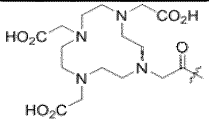
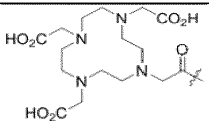
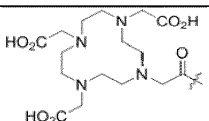
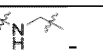
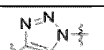
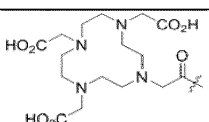
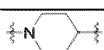
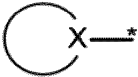
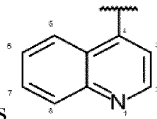
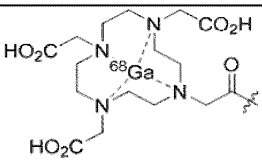
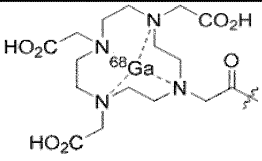
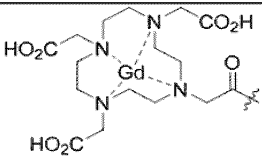
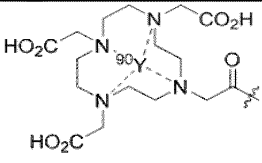
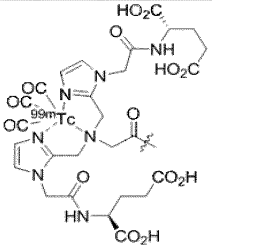
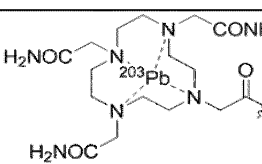
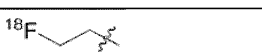
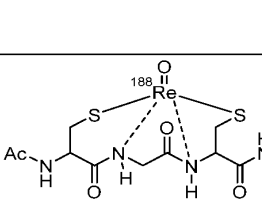
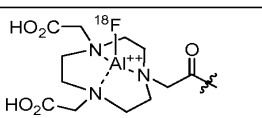
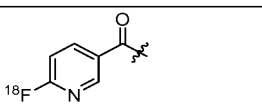
name	R ¹ /R ²	R ⁶	R ⁷	R ⁸	D	B	E
FAPI-34^s	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-35	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-36	F/F	-	+		-		<i>n</i> -C ₃ H ₆
FAPI-37	F/F	-	+				<i>n</i> -C ₃ H ₆
FAPI-38	F/F	-	+		D-Arg		<i>n</i> -C ₃ H ₆

Table 2: Compounds of special interest. Q, R, U, D are absent; R¹ and R² are located at the 4-

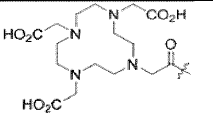
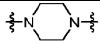
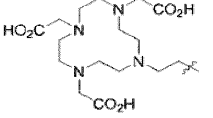
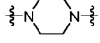
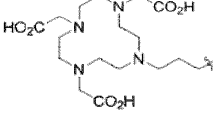
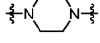
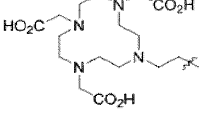
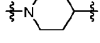
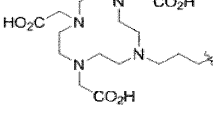
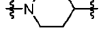
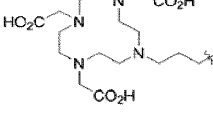
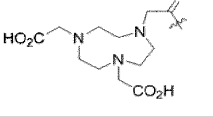
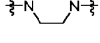
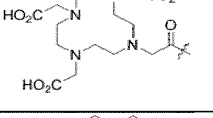
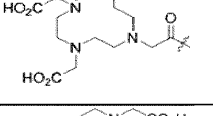
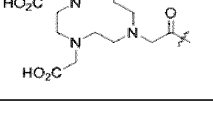
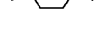
pyrrolidine position;  is , R⁵, R⁶ are H; R⁷ is attached to the 6-quinolyl position; V is C=O; W is NH; Y is CH₂; Z is C=O; R³ is -CN; B is 1,4-piperazine; E is 1,3-propane; A is O.

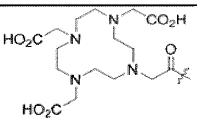
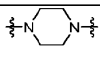
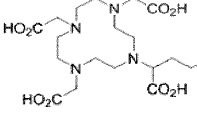
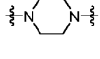
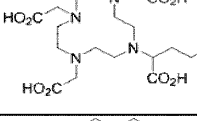
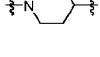
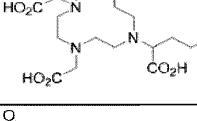
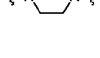
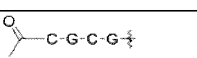
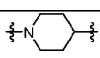
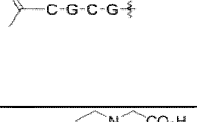
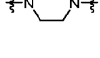
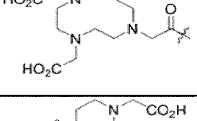
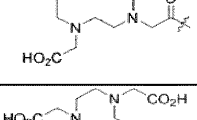
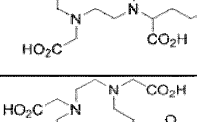
name	Purpose	R ¹ /R ²	R ⁸
⁶⁸ Ga-FAPI-02	PET	H/H	
⁶⁸ Ga-FAPI-04	PET	F/F	

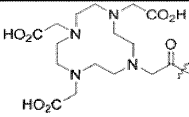
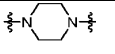
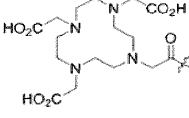
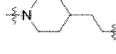
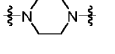
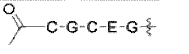
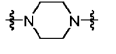
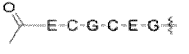
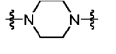
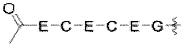
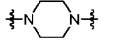
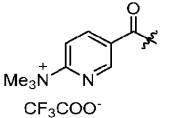
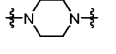
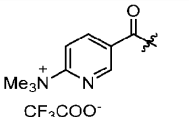
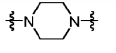
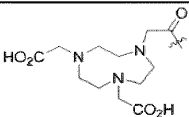
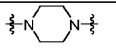
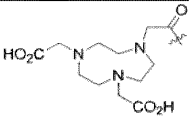
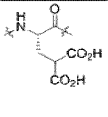
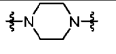
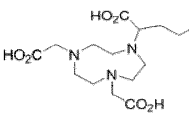
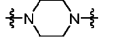
Gd-FAPI-04	MRI (contrast agent)	F/F	
⁹⁰ Y-FAPI-04	radiotherapy (β ⁻)	F/F	
^{99m} Tc-FAPI-29	SPECT	F/F	
²⁰³ Pb-FAPI-32	SPECT	F/F	
¹⁸ F-FAPI-n.a.	PET	F/F	
¹⁸⁸ Re-FAPI-60	radiotherapy (β ⁻)	F/F	
Al ¹⁸ F-FAPI-42	PET	F/F	
¹⁸ F-FAPI-72	PET	F/F	


 is R^5 and R^6 are H; R^7 is attached to the 6-quinolyl position and

$$R^8 - D - B - E - A - \text{[wavy line]}$$
ITM Exhibit 1004, Page 999 of 1652

name	R ¹ /R ²	R ⁸	D	B	E	A
FAPI-46	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-47	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-48	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-49	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-50	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-51	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-52	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-53	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-54	F/F		-			
FAPI-55	F/F		-		<i>n</i> -C ₃ H ₆	NMe

name	R ¹ /R ²	R ⁸	D	B	E	A
FAPI-56	F/F		-			
FAPI-57	F/F		-			
FAPI-58	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-59	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-60^s	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-61^s	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-62^s	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-63	F/F		-			
FAPI-64	F/F		-			
FAPI-65	F/F		-			
FAPI-66	F/F		-			

name	R ¹ /R ²	R ⁸	D	B	E	A
FAPI-67	F/F		-			
FAPI-68	F/F		-			
FAPI-69^s	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-70^s	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-71^s	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-72[*]	F/F		-		<i>n</i> -C ₃ H ₆	NMe
FAPI-73[*]	F/F		-		<i>n</i> -C ₃ H ₆	O
FAPI-74	H/H		-		<i>n</i> -C ₃ H ₆	O
FAPI-75	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-76	F/F		-		<i>n</i> -C ₃ H ₆	O

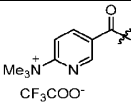
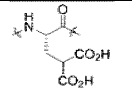
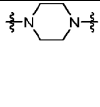
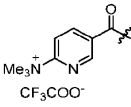
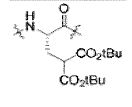
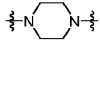
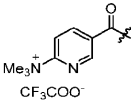
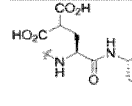
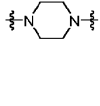
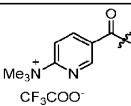
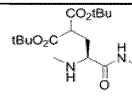
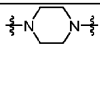
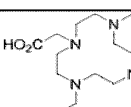
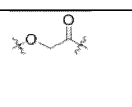
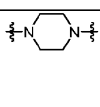
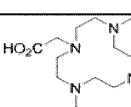
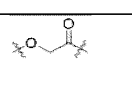
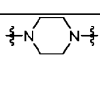
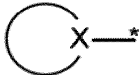
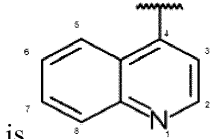
name	R ¹ /R ²	R ⁸	D	B	E	A
FAPI-77	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-77 protected	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-78	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-78 protected	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-79	F/F				<i>n</i> -C ₃ H ₆	O
FAPI-80	F/F				<i>n</i> -C ₃ H ₆	NMe

Table 4: Compounds of special interest. Q, R, U, D are absent; R¹ and R² are fluorine atoms

located at the 4-pyrrolidine position;  is , R⁵, R⁶ are H; R⁷ is attached to the 6-quinolyl position; V is C=O; W is NH; Y is CH₂; Z is C=O; R³ is -CN; B is 1,4-piperazine; E is 1,3-propane; A is O.

5

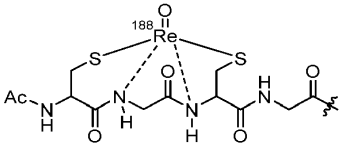
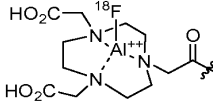
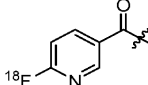
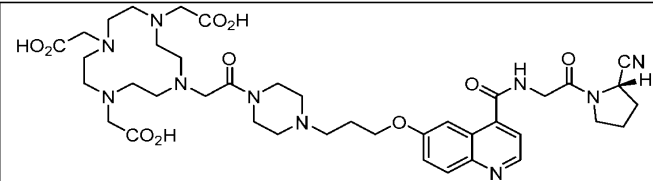
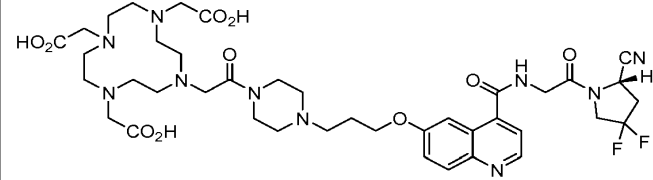
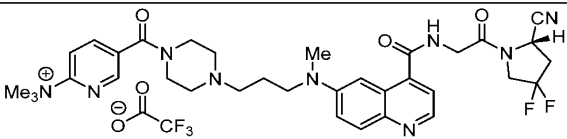
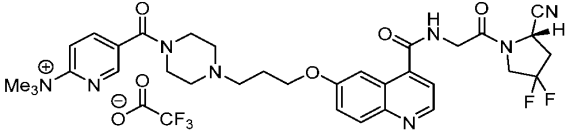
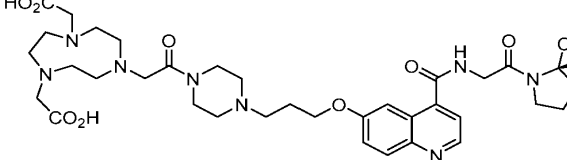
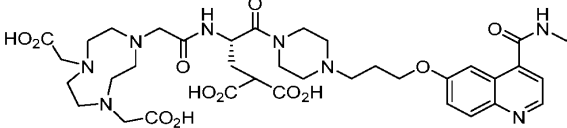
name	Purpose	R ⁸
¹⁸⁸ Re-FAPI-60	radiotherapy (β ⁻)	
Al ¹⁸ F-FAPI-42	PET	
¹⁸ F-FAPI-73	PET	

Table 5: Preferred precursors for radiolabelling with ¹⁸F-18; ⁶⁴Cu-64; ⁶⁸Ga-68; ^{99m}Tc-99m, Re-188; * Y-90, Sm-153, Lu-177.

FAPI-02 ^{e,*}	
FAPI-04 ^{e,*}	

FAP1- 34[£]	
FAP1- 42^{§,\$,€}	
FAP1- 46^{€,*}	
FAP1- 52^{§,\$,€}	
FAP1- 69[£]	
FAP1- 70[£]	
FAP1- 71[£]	

FAPI-72 [§]	
FAPI-73 [§]	
FAPI-74 ^{§,§,€}	
FAPI-75 ^{§,§,€}	

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a radioactive moiety, wherein the radioactive moiety is a fluorescent isotope, a radioisotope, a radioactive drug or combinations thereof. Preferably, the radioactive moiety is selected from the group consisting of alpha radiation emitting isotopes, beta radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting isotopes, fluorescence emitting isotopes, such as ¹¹C, ¹⁸F, ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁸⁶Re, ¹⁸⁸Re, ¹³⁹La, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁸⁸Y, ⁹⁰Y, ¹⁴⁹Pm, ¹⁶⁵Dy, ¹⁶⁹Er, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi, ²¹³Bi, ⁷²As, ⁷²Se, ⁹⁷Ru, ¹⁰⁹Pd, ¹⁰⁵Rh, ^{101m}Rh, ¹¹⁹Sb, ¹²⁸Ba, ¹²³I, ¹²⁴I, ¹³¹I, ¹⁹⁷Hg, ²¹¹At, ¹⁵¹Eu, ¹⁵³Eu, ¹⁶⁹Eu, ²⁰¹Tl, ²⁰³Pb, ²¹²Pb, ⁶⁴Cu, ⁶⁷Cu, ¹⁸⁸Re, ¹⁸⁶Re, ¹⁹⁸Au, ²²⁵Ac, ²²⁷Th and ¹⁹⁹Ag. Preferably ¹⁸F, ⁶⁴Cu, ⁶⁸Ga, ⁹⁰Y, ^{99m}Tc, ¹⁵³Sm, ¹⁷⁷Lu, ¹⁸⁸Re.

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a fluorescent dye select from the group consisting of the following classes of fluorescent dyes: Xanthenes, Acridines, Oxazines, Cynines, Styryl dyes, Coumarines, Porphines, Metal-Ligand Complexes, Fluorescent proteins, Nanocrystals, Perylenes, Boron-dipyrromethenes and Phtalocyanines as well as conjugates and combinations of these classes of dyes.

In a further preferred embodiment of the first aspect of the present invention, R⁸ is a chelating agent which forms a complex with divalent or trivalent metal cations. Preferably, the chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane-N,N',N',N'-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-

triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N''-pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC).

5 In a further preferred embodiment of the first aspect of the present invention, R⁸ is a contrast agent which comprises or consists of a paramagnetic agent, preferably, wherein the paramagnetic agent comprises or consists of paramagnetic nanoparticles.

In a further preferred embodiment of the first aspect of the invention, R⁸ is selected from any R⁸ of tables 1 to 5.

10 In a second aspect, the present invention relates to a pharmaceutical composition comprising or consisting of at least one compound of the first aspect, and, optionally, a pharmaceutically acceptable carrier and/or excipient.

In a third aspect, the present invention relates to the compound of the first aspect or the pharmaceutical composition of the second aspect for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or
15 a human subject. Preferably, the disease characterized by overexpression of fibroblast activation protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorder.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is cancer, the cancer is selected from the group consisting of breast cancer,
20 pancreatic cancer, small intestine cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer, hepatocellular carcinoma, esophageal cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocellular carcinoma, clear cell renal carcinoma, neuroendocrine tumor, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus carcinoma, desmoid tumors, glioma,
25 astrocytoma, cervix carcinoma and prostate cancer. Preferably, the cancer is glioma, breast cancer, colon cancer, lung cancer, head and neck cancer, liver cancer or pancreatic cancer. More preferably, the cancer is glioma.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is chronic inflammation, the chronic inflammation is selected from the group
30 consisting of rheumatoid arthritis, osteoarthritis and Crohn's disease. Preferably, the chronic inflammation is rheumatoid arthritis.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is fibrosis, the fibrosis is selected from the group consisting of pulmonary fibrosis, such as idiopathic pulmonary fibrosis and liver cirrhosis.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is tissue remodeling, the tissue remodeling occurs after myocardial infarction.

Preferably, if the disease characterized by overexpression of fibroblast activation protein (FAP) is a keloid disorder, the keloid disorder is selected from the group consisting of scar formation, keloid tumors and keloid scar.

In a fourth aspect, the present invention relates to a kit comprising or consisting of the compound of the first aspect or the pharmaceutical composition of the second aspect and instructions for the diagnosis or treatment of a disease. Preferably, the disease is a disease as specified above.

Examples

Example 1: Compound synthesis and radiochemistry

Based on a FAP- α specific inhibitor (Jansen et al., *ACS Med Chem Lett*, 2013) two radiotracers were synthesized. Radioiodine labeled FAPI-01 was obtained via an organotin stannylated precursor, which was prepared through palladium catalyzed bromine/tin exchange. FAPI-02 is a precursor for the chelation of radio metals which was synthesized in five steps. By application of the same or slightly modified procedures additional compounds were prepared. The structures of these compounds are listed in table 1 and 2. Radioiodinations of the stannylated precursor were performed with peracetic acid. For chelation with Lu-177 and Ga-68 the pH of the reaction mixture was adjusted with sodium acetate and heated to 95 °C for 10 min. Stability in human serum was analyzed by precipitation and radio-HPLC analysis of the supernatant.

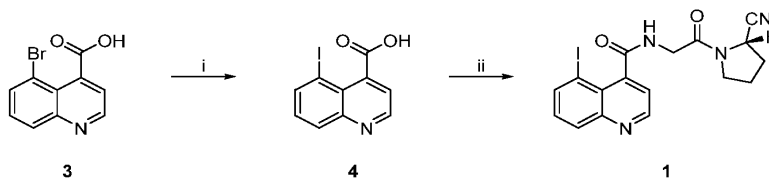
Reagents

All solvents and non-radioactive reagents were obtained in reagent grade from ABCR (Karlsruhe, Germany), Sigma-Aldrich (München, Germany), Acros Organics (Geel, Belgium) or VWR (Bruchsal, Germany) and were used without further purification. Atto 488 NHS-ester was obtained from AttoTec (Siegen, Germany). 2,2',2''-(10-(2-(4-nitrophenyl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-triyl)triacetic acid (DOTA-PNP) was synthesized following the protocol of Mier et al. (Mier et al., *Bioconjug Chem*, 2005). The intermediates 6-methoxyquinoline-4-carboxylic acid (**7**), 5-bromoquinoline-4-carboxylic acid (**3**) and (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate were synthesized following the protocols of Jansen et al. (Jansen et al., *ACS Med Chem Lett*, 2013).

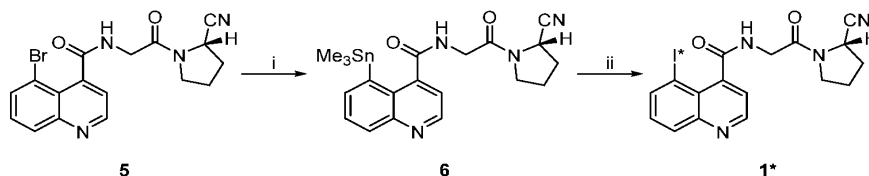
The substance (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-bromoquinoline carboxamide was synthesized by a modified HBTU amidation protocol.

Compound synthesis

- 5 Scheme 1 depicts the initial synthesis of FAPI-01 which was achieved by performing a Br/Li-exchange with *n*-butyllithium at 5-bromoquinoline-4-carboxylic acid (**3**) and quenching with elemental iodine to obtain iodoquinoline **4**. This compound was coupled to the Gly-Pro-CN fragment by HBTU/HOBt-activation to provide non-radioactive reference material of FAPI-01 (**1**).

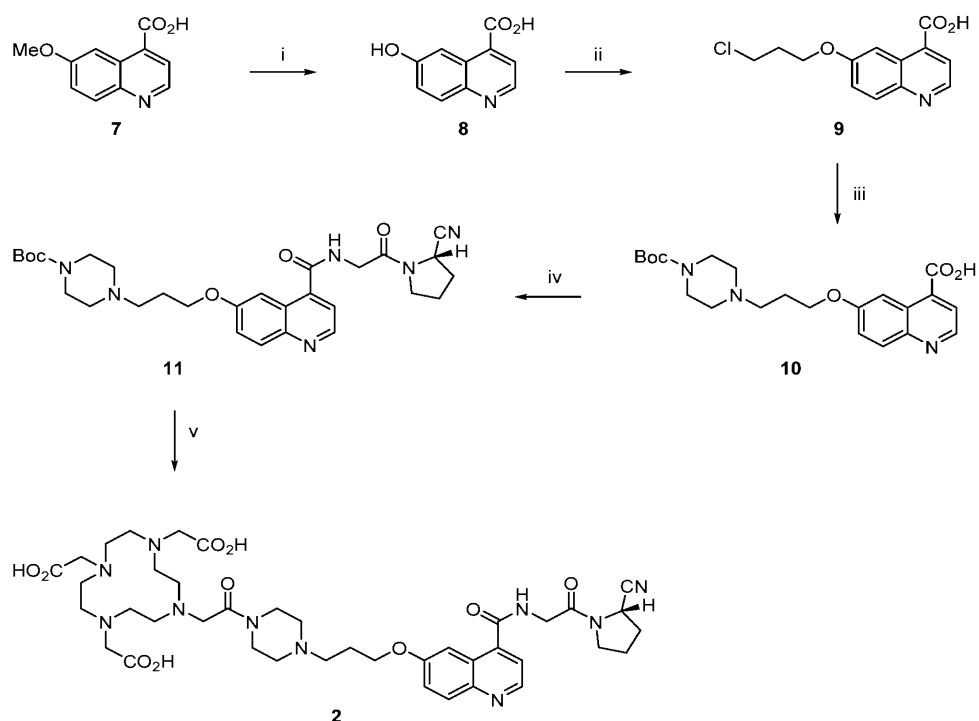


Scheme 1. Synthesis of non-radioactive FAPI-01. i) *n*BuLi, then I₂, THF; ii) HBTU/HOBt, DIPEA, H-Gly-Pro-CN, DMF. For the synthesis of radioactive FAPI-01 (**1***), the stannylated precursor **6** was obtained by palladium-catalyzed stannylation of inhibitor **5** in dioxane at 80°C (Scheme 2).



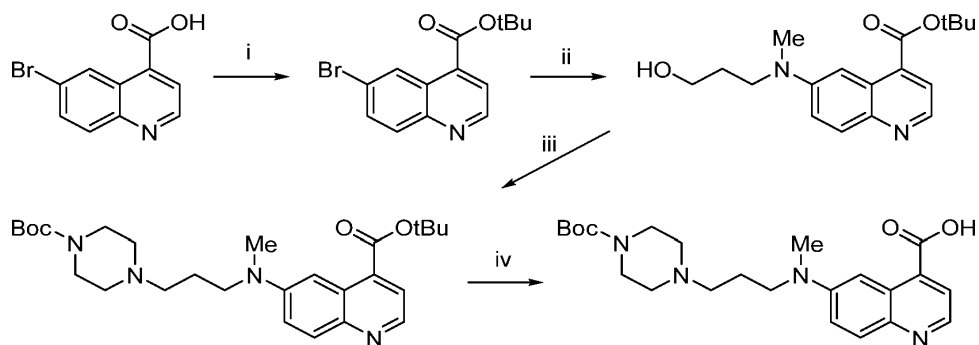
- 15 **Scheme 2.** Synthesis of radioactive FAPI-1 via the stannylated precursor **4**. i) (Me₃Sn)₂; (PPh₃)₂PdCl₂; dioxane 80 °C; ii) I-125 or I-131; AcOOH; 1 M HCl; MeOH.

To enable radiolabeling by incorporation of radiometals, the chelator DOTA was chemically linked to the basic scaffold of the FAP-inhibitor. As shown by Jansen et al. (Jansen et al., *ACS Med Chem Lett*, 2013), modifications at the 6-position of the quinoline-4-carboxylic acid are well tolerated without impairing target affinity and specificity. Therefore, a bifunctional linker was attached to the hydroxyl group of **8** via an ether linkage, leading way to the synthesis shown in Scheme 3. Ready available 1-bromo-3-chloropropane was chosen to create a spacer, which is unharmed during the saponification of the simultaneously formed ester bond at the end of the one-pot-process. Compound **9** was converted to the *N*-Boc protected quinolinecarboxylic acid **10** which was further coupled to H-Gly-Pro-CN by HBTU. Due to the high hygroscopicity of the free amine, compound **11** was directly converted to FAPI-02 (**2**) after the Boc-removal, solvent exchange and neutralization of excess *p*-toluenesulfonic acid.

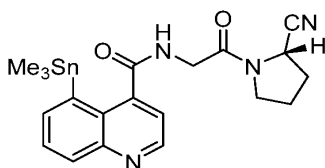


Scheme 3. Chemical synthesis of FAPI-02. i) aq. HBr 48%, 130 °C; ii) 1-bromo-3-chloropropane, Cs₂CO₃, DMF then 6 M NaOH; iii) 1-Boc-piperazine, KI, DMF; iv) HBTU/HOBt, DIPEA, H-Gly-Pro-CN, DMF; v) TosOH, MeCN, then DOTA-PNP, DIPEA, DMF.

- 5 In case of compounds incorporating group A ≠ O, the quinoline-4-carboxylic acid intermediates were synthesized by a different reaction scheme. The key step of this approach is a palladium catalyzed coupling reaction (e.g. Buchwald-Hartwig cross-coupling), which requires additional protection before and deprotection of the carboxylic acid function after the cross-coupling reaction (scheme 4).



Scheme 4. Synthesis of the building block 6-(3-(4-Boc-piperazin-1-yl)propyl)-1-(methylamino)quinoline-4-carboxylic acid for the synthesis of FAPI-46. i) DCC, tBuOH, CuCl; ii) 3-methylamino-1-propanol, Cs₂CO₃, Pd₂(dba)₃, BINAP; iii) MsCl, NEt₃, DCM then 1-Boc-piperazine, KI, DMF; iv) TFA then Boc₂O, NEt₃, DMF.



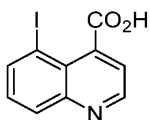
(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-(trimethylstannyl)quinoline caboxamide (**6**)

3.88 mg (10.0 μ mol) (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-bromoquinoline caboxamide, 20 μ L (32 mg; 96 μ mol) hexamethylditin and 0.75 mg (1.07 μ mol)

- 5 bis(triphenylphosphine)palladium(II) dichloride in 1 mL dry dioxane are stirred at 80 °C over night under an inert atmosphere. Volatiles are removed and the residue is taken up in 2 mL 50% acetonitrile/water and filtered through a C18-light cartridge before HPLC-purification. 2.78 mg (5.90 μ mol; 59%) of the product are obtained after freeze drying.

LC-MS R_t 14.77 min, m/z 473.0786 [$M(^{120}\text{Sn})+H$]⁺

10



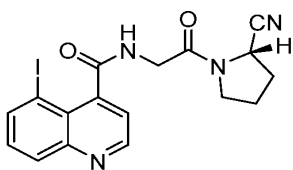
5-iodoquinoline-4-carboxylic acid (**4**)

5.42 mg (136 μ mol) of sodium hydride suspension (60% in mineral oil) are added to an solution of 30.27 mg (120 μ mol) 5-bromoquinoline-4-carboxylic acid (**3**) in 3 mL dry THF under Ar at

- 15 0°C. The ice bath is removed and the reaction mixture is cooled to -78 °C before 100 μ L (160 μ mol) *n*BuLi (1.6 M in hexanes) are added dropwise. After 15 min 64.71 mg (254 μ mol) iodine in 2 mL THF are added dropwise and the reaction is stirred for 30 min at -78 °C before allowed to reach room temperature. After 1 h the reaction is quenched by addition of 1 mL 0.5 M NaHCO₃ and ca. 30 mg (170 μ mol) sodium dithionite to remove excessive iodine. After the
- 20 removal of THF under reduced pressure the mixture is acidified to pH 2 and extracted three times with ethyl acetate (25 mL). The combined organic phases are evaporated to dryness and purified by HPLC. 18.14 mg (60.7 μ mol; 45%) of the title compound are obtained after freeze drying.

¹H NMR (500 MHz, DMSO-*d*₆) 13.95 (br, 0.3H), 8.93 (s, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.12

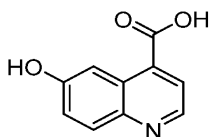
- 25 (d, *J* = 8.4 Hz, 1H), 7.60 (s, 1H), 7.52 (t, *J* = 7.9 Hz, 1H); **¹³C NMR** (125 MHz, DMSO-*d*₆) 168.8, 150.3, 148.8, 141.3, 130.6, 121.0, 109.5; **LC-MS** R_t 8.65 min, m/z 299.9383 [$M+H$]⁺



(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-5-trimethylstannylquinoline-4-carboxamide (**1**; FAPI-01)

9.07 mg (23.9 μ mol) HBTU in 50 μ L DMF are added to a solution of 6.21 mg (20.8 μ mol) 5-iodoquinoline-4-carboxylic acid, 7.45 mg (55.2 μ mol) HOBt and 10 μ L DIPEA in 50 μ L DMF. After 15 min (29.9 μ mol) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate in 50 μ L DMF are added. The reaction is quenched with 850 μ L water and purified by HPLC. Freeze drying provides 6.86 mg (15.8 μ mol; 76%) of the product.

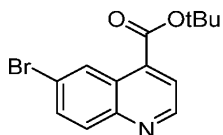
¹H NMR (600 MHz, DMSO-*d*₆) 9.06, 8.97, 8.33, 8.13, 7.56, 7.51, 4.81, 4.34, 4.06, 3.74, 3.56, 2.21, 2.17, 2.09, 2.05; **¹³C NMR** (150 MHz, DMSO-*d*₆) 167.1, 150.2, 148.8, 145.3, 141.5, 130.7, 125.3, 121.9, 119.3, 92.0, 46.3, 45.4, 42.1, 29.5, 24.9; **LC-MS** *R*_t 11.95 min, *m/z* 435.0102 [*M*+*H*]⁺



15 6-Hydroxyquinoline-4-carboxylic acid (**8**)

105 mg (477 μ mol) of raw 6-methoxyquinoline-4-carboxylic acid (**7**) are dissolved in 3 mL of 48% hydrobromic acid in water. The solution is heated to 130 °C for 4 h. The solution is brought to a slightly basic pH with 6 M NaOH after reaching room temperature. 79.2 mg (419 μ mol; 88%) of the product are obtained after by HPLC-purification and lyophilization.

20 **¹H NMR** (500 MHz, DMSO-*d*₆) 13.65 (br, 0.6H) 10.24 (s, 1H), 8.78 (d, *J* = 4.4 Hz, 1H), 8.06 (d, *J* = 2.6 Hz, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.84 (d, *J* = 4.4 Hz, 1H), 7.37 (dd, *J* = 9.1, 2.6 Hz, 1H), **¹³C NMR** (125 MHz, DMSO-*d*₆) 167.7, 156.9, 146.5, 144.1, 133.4, 131.2, 126.2, 122.3, 122.6, 106.5; **LC-MS** *R*_t 6.66 min, *m/z* 190.0415 [*M*+*H*]⁺

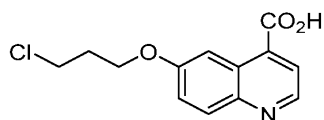


25

tert-butyl 6-bromoquinoline-4-carboxylate

98.3 mg (390 μ mol) 6-bromoquinoline-4-carboxylic acid (raw) were suspended in 5 mL tetrahydrofuran and 25.0 μ L (18.3 mg; 181 μ mol) triethylamine and added to *O*-*tert*-butyl-*N,N'*-dicyclohexylisourea (prepared the day before from neat 426 mg (2.07 mmol) dicyclohexylcarbodiimide, 173 mg (2.33 mmol) *tert*-butanol and 10.2 mg (103 μ mol) copper(I)chloride). The mixture was heated to 50 °C over night. The mixture was filtered, solvents evaporated and the product isolated by HPLC. 49.7 mg (161 μ mol; 41%) of the title compound were obtained after freeze drying.

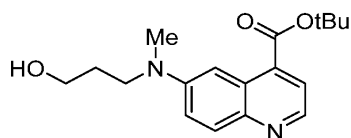
LC-MS R_t 20.40 min, m/z 251.9642 $[M-tBu]^+$



10 6-(3-chloro-1-propoxy)quinoline-4-carboxylic acid (**9**)

42.4 μ L (67.4 mg; 430 μ mol) 1-bromo-1-chloropropane are added to a suspension of 23.2 mg (123 μ mol) 6-hydroxyquinoline-4-carboxylic acid (**8**) and 190 mg (1.38 μ mol) potassium carbonate in 250 μ L DMF and heated to 60 °C over night. The reaction mixture is cooled to room temperature, diluted with 500 μ L water and 500 μ L acetonitrile before 100 μ L 6 M NaOH are added. The reaction mixture is directly purified via HPLC (5-40%) after the complete ester hydrolysis is accomplished. 26.45 mg (99.4 μ mol; 81%) of the product are obtained after lyophilization.

¹H NMR (500 MHz, DMSO-*d*₆) 13.75 (br, 0.4H), 8.88 (d, *J* = 4.4 Hz, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.94 (d, *J* = 4.4 Hz, 1H), 7.52 (dd, *J* = 9.2, 2.0 Hz, 1H), 4.24 (t, *J* = 5.95 Hz, 2H), 3.85 (t, *J* = 6.5 Hz, 2H), 2.27 (m, 2H); ¹³C NMR (125 MHz, DMSO-*d*₆) 167.6, 157.5, 147.6, 144.8, 134.0, 131.2, 125.9, 122.7, 122.2, 104.5, 64.7, 41.9, 31.6; LC-MS R_t 11.46 min, m/z 266.0461 $[M+H]^+$

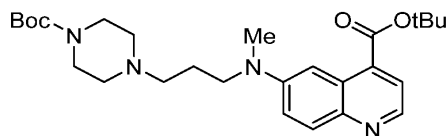


25 *tert*-butyl 6-(3-hydroxypropylmethylamino)quinoline-4-carboxylate

204.6 mg (664 μ mol) *tert*-butyl 6-bromoquinoline-4-carboxylate, 34.10 mg (54.7 μ mol) BINAP, 21.51 mg (23.5 μ mol) Pd₂(dba)₃ and 480.3 mg (1.47 mmol) cesium carbonate were dissolved in 6 mL toluene and 128.0 μ L (118 mg; 1.32 mmol) *N*-methyl-1,3-propanolamine were added. The mixture was stirred at 90 °C over night before solvents were removed, the

residue suspended in water/acetonitrile 1:1 and filtered before HPLC-purification. 172.7 mg (547 μ mol; 82%) of the title compound were obtained after freeze drying.

LC-MS R_t 13.41 min, m/z 261.1213 $[M-tBu+H]^+$



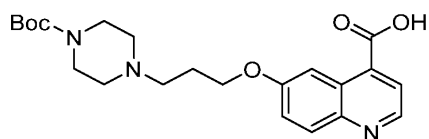
5

tert-butyl 6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxylate (62.8 mg (199 μ mol)) and *tert*-butyl 6-(3-hydroxypropylmethylamino)quinoline-4-carboxylate were dissolved in 5 mL dichloromethane and 90.0 μ L (66.6 mg; 659 μ mol) triethylamine. 20.0 μ L (29.6 mg; 258 μ mol) methanesulfonyl chloride were added at 0 $^{\circ}$ C and the mixture reacted for 60 min. 194.6 mg (1.05 mmol) 1-Boc-piperazine were added before volatiles were removed. 500 μ L dimethylformamide and 47.4 mg (286 μ mol) potassium iodide were added to the residue. The mixture was shaken at 60 $^{\circ}$ C for 120 minutes before the product was isolated by HPLC. 81.05 mg (167 μ mol; 84%) of the title compound were obtained after freeze drying.

10

LC-MS R_t 13.99 min, m/z 485.3086 $[M+H]^+$

15



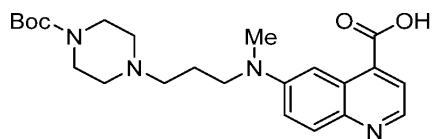
6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxylic acid (**10**)

15.13 mg (56.9 μ mol) of 6-(3-chloro-1-propoxy)quinoline-4-carboxylic acid (**9**), 55.43 mg (298 μ mol) *N*-*tert*-butoxycarbonylpiperazine and 51.05 mg (30.8 μ mol) potassium iodide are dissolved in 250 μ L DMF. The reaction is shaken at 60 $^{\circ}$ C over night. The resulting suspension is diluted with 750 μ L water before the product is purified by HPLC. After freeze drying 28.73 mg (54.3 μ mol; 95%) of the product are obtained as the corresponding TFA-salt.

20

^1H NMR (500 MHz, D_2O) 8.93 (d, J = 5.5 Hz, 1H), 8.17 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 5.5 Hz, 1H), 7.79 (dd, J = 9.3, 2.5 Hz, 1H), 7.65 (d, J = 2.5 Hz, 1H), 4.36 (t, J = 5.6 Hz, 2H), 4.27 (d, J = 13.55 Hz, 2H), 3.67 (d, J = 11.95 Hz), 3.47 (t, J = 15.5 Hz, 2 H), 3.27 (t, J = 12.7 Hz), 3.12 (td, J = 12.2, 2.65 Hz), 2.37 (m, 2 H), 1.47 (s, 9H); **^{13}C NMR** (125 MHz, D_2O) 155.5, 153.5, 149.0, 141.4, 134.4, 127.9, 126.6, 122.3, 118.4, 110.0, 105.1, 82.8, 65.5, 54.3, 51.5, 48.6, 40.7, 29.6, 27.4; **LC-MS** R_t 10.62 min, m/z 416.1997 $[M+H]^+$

25

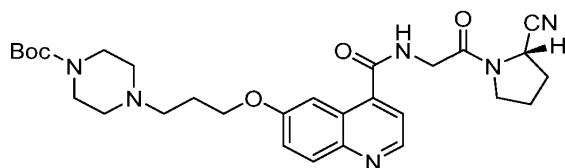


6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxylic acid

100.12 mg (206 μ mol) *tert*-butyl 6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxylate were treated with 900 μ L trifluoroacetic acid, 25 μ L

5 triisopropylsilane, 25 μ L water and 50 μ L trifluoromethanesulfonic acid for 60 min. The deprotected compound was precipitated with diethyl ether, dried and reacted with 60.83 mg (279 μ mol) di-*tert*-butyldicarbonate and 50.0 μ L (36.5 mg; 361 μ mol) triethylamine in 1 mL dimethylformamide for another 60 min. 55.42 mg (129 μ mol; 65% over 2 steps) were obtained after HPLC-purification and freeze-drying.

10 **LC-MS** R_t 10.52 min, m/z 429.2463 $[M+H]^+$



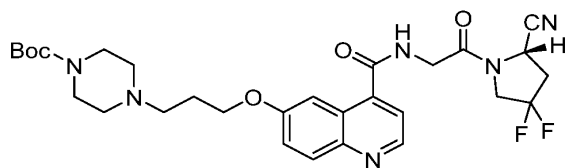
(*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide (**11**)

15 9.43 mg (24.9 μ mol) HBTU in 50 μ L DMF are added to a solution of 10.56 mg (19.9 μ mol) 6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxylic acid (**10**), 5.38 mg (39.8 μ mol) HOBT and 10 μ L DIPEA in 50 μ L DMF. After 15 min (29.9 μ mol) (*S*)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate in 50 μ L DMF are added. The reaction is quenched with 850 μ L water and purified by HPLC. Freeze drying provides

20 12.88 mg (19.4 μ mol; 97%) of the title compound.

¹H NMR (500 MHz, DMSO-*d*₆) 9.04 (d, *J* = 5.5 Hz, 1H), 8.24 (d, *J* = 9.6 Hz, 1H), 8.10 (d, *J* = 5.5 Hz, 1H), 7.89 (d, *J* = 2.3 Hz, 1H), 7.85 (dd, *J* = 9.6, 2.3 Hz, 1H), 4.84 (t, *J* = 6 Hz, 1H), 4.46–4.36 (m, 4H), 4.26 (d, *J* = 12.0 Hz, 2H), 3.83 (m, 1H), 3.67 (m, 3H), 3.47 (t, *J* = 7.7 Hz, 2H), 3.27 (br, 2H), 3.11 (t, *J* = 11.5 Hz), 2.37 (m, 4H), 2.22 (m, 2H), 1.46 (s, 9H); **¹³C NMR** (125 MHz, DMSO-*d*₆) 168.6, 168.0, 159.4, 155.5, 147.7, 141.8, 135.1, 128.2, 127.5, 123.1, 120.0, 119.1, 104.7, 82.9, 66.0, 54.3, 51.5, 47.0, 46.3, 42.3, 29.4, 27.4, 24.7, 23.1; **LC-MS** R_t 11.81 min, m/z 551.2736 $[M+H]^+$

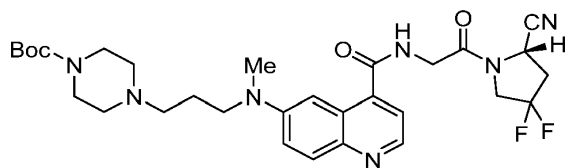
25



(*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonyl)piperazin-1-yl)-1-propoxyquinoline-4-carboxamide

13.2 mg (22.4 μ mol; 75%) were obtained following the previous protocol.

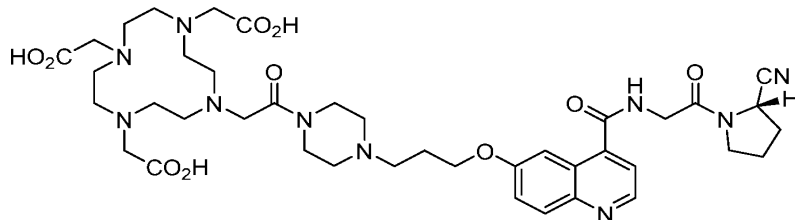
5 **LC-MS** R_t 11.84 min, m/z 605.2610 $[M+H]^+$



N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-Boc-piperazin-1-yl)propyl-1-(methyl)amino)quinoline-4-carboxamide

10 1.17 mg (1.95 μ mol; 92%) were obtained following the previous protocol.

LC-MS R_t 12.66 min, m/z 600.3057 $[M+H]^+$



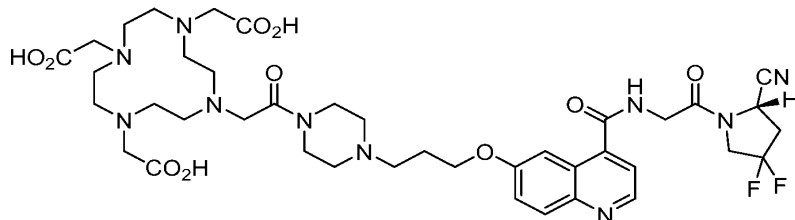
FAPI-02 (2)

15 4.85 mg (8.80 μ mol) (*S*)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonyl-piperazin-1-yl)-1-propoxy)quinoline-4-carboxamide (**11**) are dissolved in 1 mL acetonitrile and 4.2 mg (22.0 μ mol) 4-methylbenzenesulfonic acid monohydrate are added. The reaction is shaken at 45 $^{\circ}$ C over night, before volatiles are removed under reduced pressure. The residue is taken up in 190 μ L dimethylformamide and 10 μ L (7.3 mg; 72 μ mol) triethylamine before 6.77 mg (12.9 μ mol) of DOTA-*p*-nitrophenol ester are added. The reaction mixture is diluted with 1 mL water and purified by HPLC after shaking for two hours. 5.04 mg (6.02 μ mol; 68%) are obtained after freeze drying.

^1H NMR (600 MHz, D_2O) 9.02, 8.23, 8.07, 7.87, 7.83, 4.85, 4.45, 4.41, 4.40, 4.39, 3.83, 3.67, 3.50, 3.49, 2.40, 2.38, 2.36, 2.26, 2.22, 2.16; **^{13}C NMR** (150 MHz, D_2O) 167.9, 159.1, 147.2,

141.8, 135.4, 127.9, 127.2, 119.8, 119.0, 104.5, 65.8, 54.1, 46.8, 46.1, 42.1, 29.2, 24.5, 23.0:

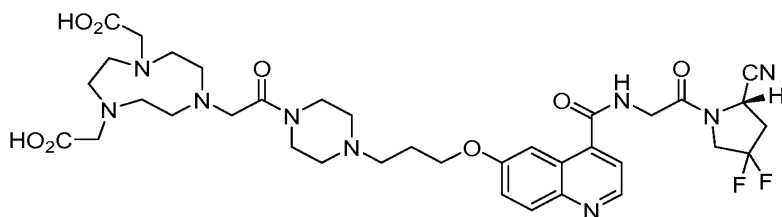
LC-MS R_t 8.37 min, m/z 837.3872 $[M+H]^+$



5 FAPI-04

3.97 mg (4.55 μ mol; 57%) were obtained following the previous protocol.

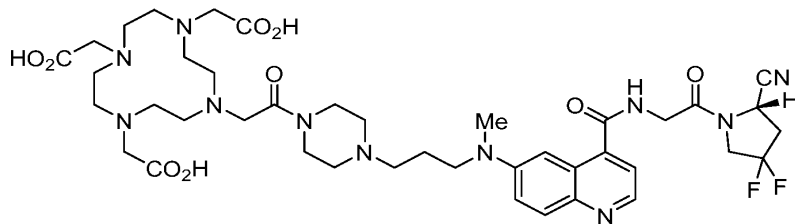
LC-MS R_t 8.80 min, m/z 873.3664 $[M+H]^+$



10 FAPI-42

1.91 mg (2.47 μ mol; 88%) were obtained following the previous protocol.

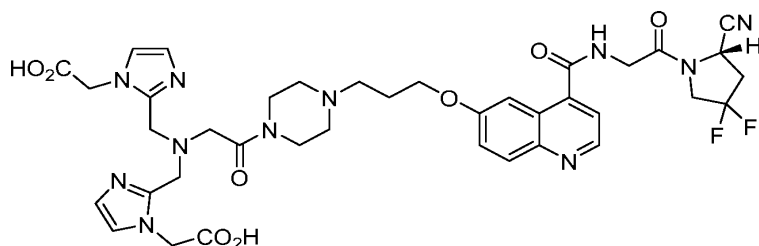
LC-MS R_t 9.37 min, m/z 386.6807 $[M+2H]^{2+}$



15 FAPI-46

39.21 mg (44.3 μ mol; 85%) were obtained following the previous protocol.

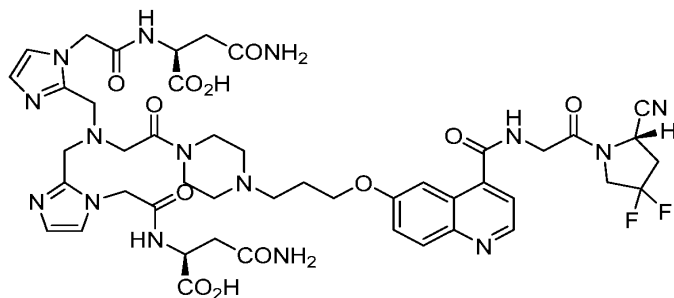
LC-MS R_t 9.03 min, m/z 443.7196 $[M+2H]^{2+}$



FAPI-19

1.09 mg (1.86 μmol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were Boc-deprotected by the method applied for FAPI-02 and reacted with 2.74 mg (5.91 μmol) bis((1-(2-(*tert*-butoxy)-2-oxoethyl)-1H-imidazol-2-yl)methyl)glycine, which were preactivated with 2.13 mg (5.62 μmol) HBTU and 2.50 μL (1.85 mg; 14.3 μmol) DIPEA. After HPLC purification and solvent removal the residue was treated with 200 μL of 2.5% trifluoromethanesulfonic acid in acetonitrile/trifluoroacetic acid 1:1. After precipitation with diethyl ether and HPLC purification 1.06 mg (1.29 μmol ; 70%) of the title compound were obtained.

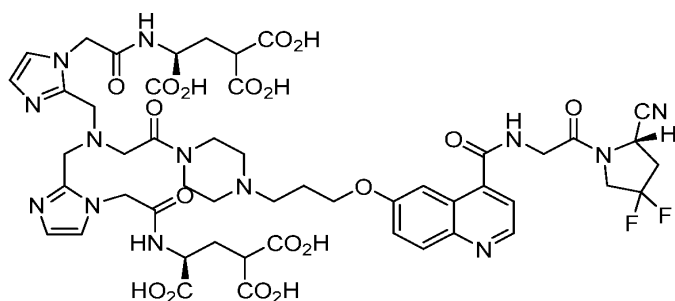
LC-MS R_t 8.91 min, m/z 820.2933 $[\text{M}+\text{H}]^+$



FAPI-28

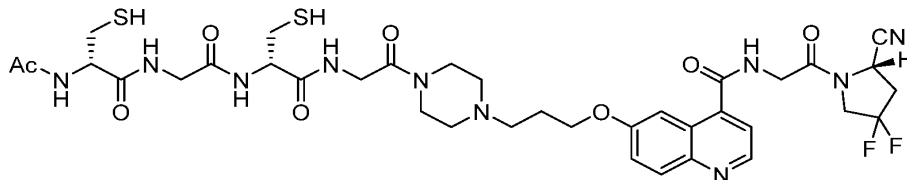
1.00 μL (0.74 mg; 5.73 μmol) DIPEA was added to a solution of 0.95 mg (1.16 μmol) FAPI-19, 0.42 mg (3.14 μmol) HOBt and 1.10 mg (2.89 μmol) HBTU in 50 μL DMF. After 10 min 2.30 mg (5.34 μmol) H-Asn(Trt)-OtBu were added and reacted for 120 min. The *tert*-butyl protecting groups were removed by 2.5% TFOH in TFA/acetonitrile 8:2. After HPLC-purification and freeze-drying, 0.79 mg (0.75 μmol ; 65%) of the title compound were obtained.

LC-MS R_t 9.23 min, m/z 524.7100 $[\text{M}+2\text{H}]^{2+}$



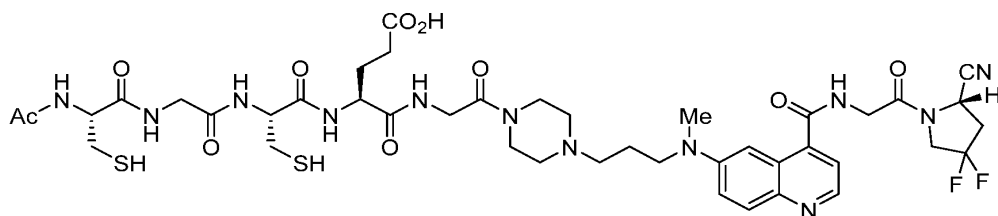
LC-MS R_t 8.87 min, m/z 583.6988 [M+2H]²⁺

5



3.36 mg (2.52 μ mol) of the trityl protected compound were dissolved in 50 μ L acetonitrile. 3 μ L Triethylsilane were and 100 μ L trifluoroacetic acid were added and reacted for 30 min. 2.01 mg (2.36 μ mol; 94%; 49% over two steps) of the title compound were obtained after HPLC purification and freeze-drying.

20 **LC-MS** R_t 10.26 min, m/z 871.2703 [M+Na]⁺

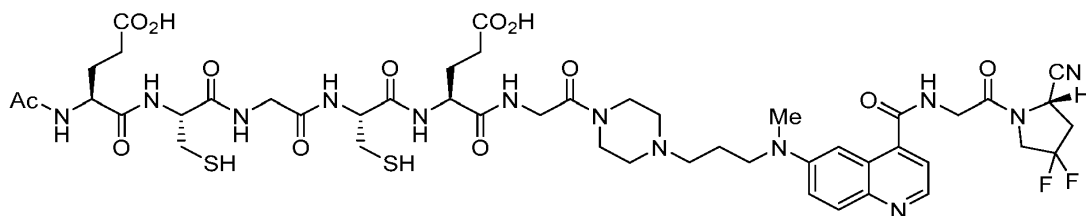


FAPI-69

0.59 mg (0.60 μmol ; 39%) were obtained following the previous protocol.

LC-MS R_t 10.25 min, m/z 991.3490 $[\text{M}+\text{H}]^+$

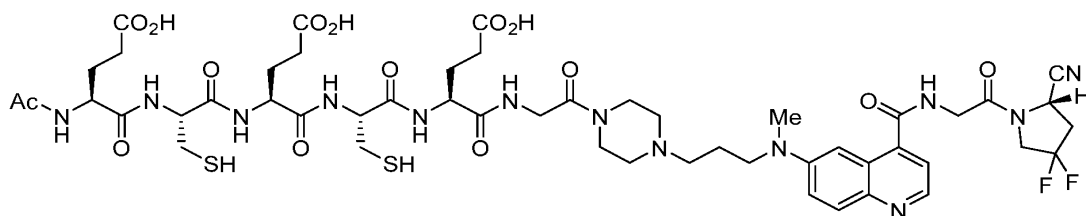
5



FAPI-70

0.61 mg (0.54 μmol ; 33%) were obtained following the previous protocol.

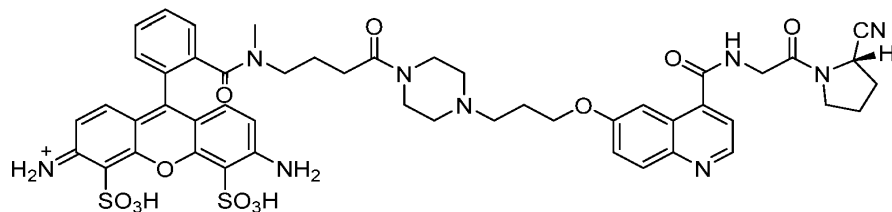
10 **LC-MS** R_t 10.14 min, m/z 1120.3884 $[\text{M}+\text{H}]^+$



FAPI-71

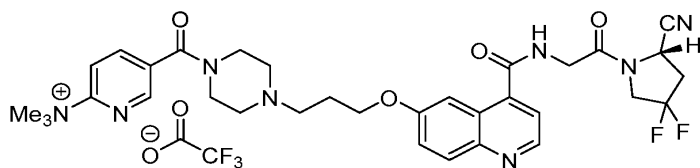
0.79 mg (0.66 μmol ; 34%) were obtained following the previous protocol.

15 **LC-MS** R_t 10.17 min, m/z 596.7075 $[\text{M}+2\text{H}]^{2+}$

Atto488-FAPI-02 (**14**)

0.66 mg (1.20 μmol) of **11** are treated with 1.33 mg (6.96 μmol) 4-methylbenzenesulfonic acid monohydrate in 250 μL acetonitrile at 45 $^{\circ}\text{C}$ for 4 hours. After removal of the solvent the residue is dissolved in 95 μL dimethylformamide and 5 μL (3.65 mg; 36.1 μmol) triethylamine. 0.54 mg (0.55 μmol) Atto 488 NHS-ester in 25 μL DMSO were added. After 60 minutes 0.49 mg (0.43 μmol ; 78%) of the title compound were isolated by HPLC and freeze drying.

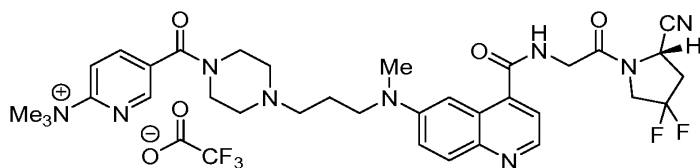
LC-MS R_t 10.19 min, m/z 1022.2706 $[\text{M}]^+$



FAPI-73

10.95 mg (18.7 μmol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-*tert*-butoxycarbonylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were deprotected for 30 min by 100 μL acetonitrile and 200 μL trifluoroacetic acid. After evaporation of the solvents and washing with diethyl ether 15.02 mg (9.27 μmol) *N,N,N*-trimethyl-5-((2,3,5,6-tetrafluorophenoxy)-carbonyl)pyridine-2-aminium chloride was added and the mixture dissolved in 200 μL dimethylformamide and 10.0 μL (7.30 mg; 72.3 μmol) triethylamine. After 120 min the mixture was purified by HPLC and 11.24 mg (14.7 μmol ; 79%) of the title compound were obtained freeze-drying.

LC-MS R_t 9.37 min, m/z 649.2892 $[\text{M}-\text{CF}_3\text{CO}_2]^+$

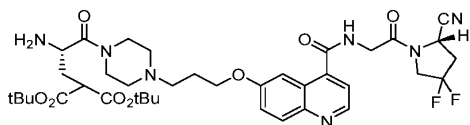


FAPI-72

9.80 mg (12.6 μmol ; 70%) were obtained following the previous protocol.

LC-MS R_t 9.28 min, m/z 662.3237 $[\text{M}-\text{CF}_3\text{CO}_2]^+$

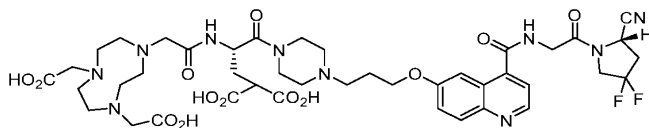
25 **General Attachment of side chain protected Fmoc-amino acids**



(*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-(γ,γ -di-*tert*-butyl)-L-carboxy-glutamylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide

14.04 mg (23.9 μ mol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-1-propoxy)quinoline-4-carboxamide were dissolved in 50 μ L acetonitrile and 100 μ L trifluoroacetic acid. After 10 min the volatiles were removed; the residue was washed with diethyl ether. A solution of 14.95 mg (28.4 μ mol) Fmoc-L-Gla(tBu)₂-OH, 7.74 mg (57.4 μ mol) HOBt, 13.46 mg (35.5 μ mol) HBTU and 20.0 μ L (14.8 mg; 115 μ mol) DIPEA in 200 μ L dimethylformamide was added to the dried residue. After 60 min 50.0 μ L (50.4 mg; 578 μ mol) morpholine were added and the product was isolated by HPLC after 30 min. 15.95 mg (20.7 μ mol; 86%) of the title compound were obtained after freeze drying.

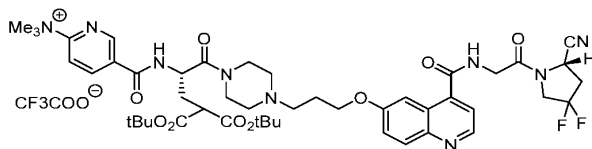
LC-MS *R*_t 12.85 min, *m/z* 772.3643 [M+H]⁺



FAP1-75

3.37 mg (4.37 μ mol) of (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(4-(γ,γ -di-*tert*-butyl)-L-carboxyglutamylpiperazin-1-yl)-1-propoxy)quinoline-4-carboxamide were and 4.52 mg (10.7 μ mol) NOTA-*p*-nitrophenol were dissolved in 100 μ L dimethylformamide and 10.0 μ L (7.30 mg; 72.3 μ mol) triethylamine. After HPLC-purification and freeze-drying the intermediate compound was deprotected by a 60 min incubation in a solution of 50 μ L acetonitrile, 100 μ L trifluoroacetic acid, 2.5 μ L triisopropylsilane and 2.5 μ L water. 2.62 mg (2.77 μ mol; 63%) were obtained after HPLC-purification and freeze-drying.

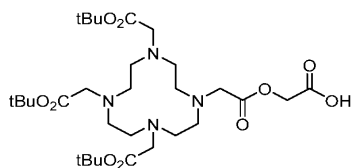
LC-MS *R*_t 9.38 min, *m/z* 945.3668 [M+H]⁺



FAP1-77-precursor

3.23 mg (3.06 μ mol; 73%) were obtained following the general active ester modification protocol. Note: The *tert*-butyl protecting groups were removed after radiofluorination, HPLC-purification and evaporation of solvents by treatment with neat TFA at 95 °C for 3 min followed by SPE work up.

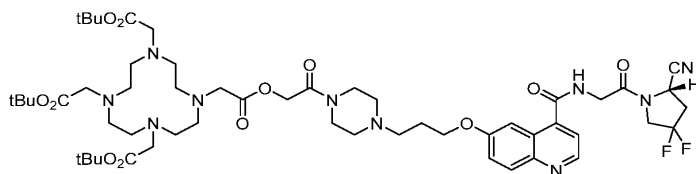
LC-MS *R*_t 16.02 min, *m/z* 1219.5858 [M+H]⁺



2-(2-(4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetoxy)acetic acid

- 5 28.99 mg (50.6 μ mol) tris-*t*Bu-DOTA, 90.65 (278 μ mol) cesium carbonate and 10.28 μ L (15.0 mg; 65.5 μ mol) benzyl 2-bromoacetate were suspended in 300 μ L dimethylformamide and shaken for 2 h. The product was isolated by HPLC, freeze dried and dissolved in 25 ml 10% acetic acid in methanol. 50 mg 10% Pd/C and hydrogen (ambient pressure) were added. After 2 hours. Solvents were removed and the title compound isolated by HPLC. After freeze drying
- 10 25.19 mg (39.9 μ mol; 79%) of the title compound were obtained.

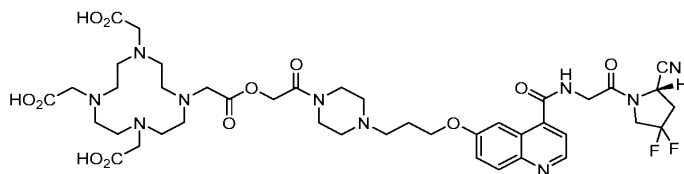
LC-MS R_t 14.14 min, m/z 631.4784 $[M+H]^+$



tBu-FAPI-79

- 15 2.00 mg (3.41 μ mol) (*S*)-*N*-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-(3-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-1-propoxy)quinoline-4-carboxamide were dissolved in 50 μ L acetonitrile and 100 μ L trifluoroacetic acid. After 10 min the volatiles were removed; the residue was washed with diethyl ether. 4.20 mg (6.60 μ mol) 2-(2-(4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetoxy)acetic acid and 3.35 mg (8.84 μ mol)
- 20 HBTU dissolved in 100 μ L dimethylformamide and 10.0 μ L (7.40 mg; 57.4 μ mol) DIPEA were added to the dried residue and reacted for 60 min. 2.26 mg (2.06 μ mol; 60%) of the title compound were obtained after HPLC purification and freeze drying.

LC-MS R_t 12.98 min, m/z 1099.7481 $[M+H]^+$



25

FAP1-79

2.26 mg (2.06 μmol) tBu-FAP1-79 were dissolved in 25 μL acetonitrile and 100 μL trifluoroacetic acid and shaken at 35 $^{\circ}\text{C}$ for 30 min. After evaporation of the solvents the product was isolated by HPLC. 1.58 mg (1.70 μmol ; 82%) of the title compound were obtained after
5 freeze drying.

LC-MS R_t 8.84 min, m/z 466.2737 $[\text{M}+2\text{H}]^{2+}$

Compound analysis

Reverse-phase high-performance liquid chromatography (RP-HPLC) was conducted using
10 linear gradients of acetonitrile in water (0-100% acetonitrile in 5 min; 0.1% TFA; flowrate 2 mL/min) on a Chromolith Performance RP-18e column (100 $\times$ 3 mm; Merck KGaA Darmstadt, Germany). UV-absorbance was detected at 214 nm. An additional γ -detector was used for the HPLC-analysis of radioactive compounds. HPLC-MS characterization was performed on an ESI mass spectrometer (Exactive, Thermo Fisher Scientific, Waltham, MA, USA) connected
15 to an Agilent 1200 HPLC system with a Hypersil Gold C18 1.9 μm column (200 $\times$ 2.1 mm; 0-100% acetonitrile in 20 min; flowrate 200 $\mu\text{L}/\text{min}$). Analytical Radio-HPLC was performed using a Chromolith Performance RP-18e column (100 $\times$ 3mm; Merck; 0-30% acetonitrile in 10 min; flowrate 2 mL/min). HPLC-purifications were performed on a LaPrep P110-System (Knauer, Berlin, Germany) and a Reprosil Pur 120 column (C18-aq 5 μm 250 $\times$ 25mm; Dr.
20 Maisch, Ammerbuch-Entringen, Germany). The water/acetonitrile-gradient (15 or 25 min; 0.1% TFA; flowrate 20 mL/min) was modified for the individual products.

Radiochemistry

Radioiodine (I-125) was purchased from Hartmann Analytik (Göttingen, Germany);
25 radioactive lutetium (Lu-177) was obtained from ITG (München, Germany); radioactive gallium (Ga-68) was eluted from a $\text{Ge-68}/\text{Ga-68}$ generator purchased from Themba Labs (Somerset West, South Africa). Tc-99m was eluted from a $\text{Mo-99}/\text{Tc-99m}$ generator (Curium Pharma, Berlin, Germany). Cu-64 was provided by UKT Tübingen (Tübingen, Germany). Sm-153 was provided by DSD Pharma (Purkersdorf, Austria). Pb-203 was provided by Lantheus
30 (N. Billerica MA, USA). F-18-FDG and F-18-fluoride were provided by the ZAG Zyklotron AG (Eggenstein, Germany). CRS Kit for tricarbonyl was obtained from Paul Scherrer Institut (Villingen-PSI, Switzerland).

For iodinations 10 μL of the organotin precursor of FAP1-01 (1 $\mu\text{mol}/\text{mL}$ in ethanol) were diluted with 10 μL of 1 M HCl and 10 μL water before 1-20 MBq iodine-125 in 0.05 M NaOH

were added. The reaction was started by addition of 5 μ L of a fresh 1.9% solution of peracetic acid in glacial acetic acid. After 60 s 15 μ L of 1 M NaOH were added and the reaction was quenched by addition of 5 μ L of 5% ascorbic acid in water before HPLC purification. The obtained solution was directly used for *in vitro* experiments or evaporated to dryness under reduced pressure and taken up in 0.9% NaCl (Braun, Melsungen, Germany) in case of animal studies.

Cu-64, Lu-177 and Pb-203 labeling of DOTA-compounds was performed by addition of 5 MBq of the radionuclide to 100 μ L of a 10 μ M solution of the individual precursor in 0.1 M NaOAc (pH 5) and incubation at 95 °C for 10 min. The solution is directly used for *in vitro* experiments or diluted with 0.9% NaCl (Braun, Melsungen, Germany) in case of biodistribution studies. For imaging studies in mice (scintigraphy, PET) the radiotracer was worked up by solid phase extraction (sep-pak light C18, Waters).

Tc(I) labeling was preceded by addition of 1 mL of the Tc-99m-pertechnetate in 0.9% saline to a CRS Kit and incubation for 20 min. After cooling to room temperature a mixture of 25.0 μ L of the precursor (1 mM in water), 150 μ L phosphate buffer (0.4 M, pH 7.4) and 240 μ L hydrochloric acid (1.0 M) was added and the final mixture adjusted to pH 5 if necessary. The reaction was performed at 95 °C for 20 minutes and worked up by solid phase extraction (sep-pak light C18, Waters). For *in vivo* experiments and animal studies the labeling was performed with one fifth of the reagents and 200 μ L of the CRS Kit solution after Tc(VII) reduction.

Tc(V) labeling was preceded by incubation of 30 μ L SnCl₂-solution containing 200 mM glucoheptonate with 200 μ L Tc-99m-pertechnetate in 0.9% saline for 10 min at room temperature. 5.00 μ L of the precursor (1 mM in water) and 3.75 μ L sodium hydroxide solution (0.1 M in water) were added and the final mixture was reacted at 95 °C for 20 min. For imaging studies in mice (scintigraphy) the radiotracer was worked up by solid phase extraction (sep-pak light C18, Waters).

Labeling with Ga-68 for animal studies was performed by incubating 255 μ L generator eluate (0.6 M HCl; approx. 230 MBq) with a mixture of 1 nmol DOTA-precursor, 1 μ L of 20% ascorbic acid in water and 72 μ L NaOAc (2.5 M) at 95 °C for 10 min. Remaining free radioactivity was removed by dilution with 2 mL water, solid phase extraction (sep-pak light C18, Waters), washing with 2 mL water and elution of the product with 1 mL water/ethanol 1:1. The obtained solution was evaporated to dryness under reduced pressure and the residue taken up in 0.9% NaCl (Braun).

For the formation of AlF-NOTA complexes F-18 fluoride was trapped on a waters Sep-Pak QMA plus light cartridge (46 mg sorbent; preconditioned with 0.5 M NaOAc, pH 3.9), washed

with water and eluted with 500 μ L 0.1 M NaOAc (pH 3.9). For animal studies 150 μ L of the eluate were preincubated with 2 μ L of an AlCl_3 solution (10 mM in water) and 50 μ L DMSO. After 5 min the mixture was added to 40 nmol NOTA-precursor (10 μ L of a 4 mM solution in water) and 1 μ L of 20% ascorbic acid in water. The solution was reacted at 95 $^{\circ}\text{C}$ for 15 min.

- 5 The product was isolated by HPLC (0-20% acetonitrile in 10 min), freed from solvents and taken up in 0.9% saline before injection.

For the formation of 6-fluoronicotinamides F-18 fluoride was trapped on a waters Sep-Pak QMA plus light cartridge (46 mg sorbent; preconditioned with 0.5 M KHCO_3), washed with water, dried and eluted with a mixture of 7.50 mg (19.9 μ mol) cryptofix 222, 1.99 mg (1.99 μ mol) KHCO_3 in 450 μ L acetonitrile and 50 μ L water. After removal of the solvent the residue was dried by azeotropic distillation with 3 $\times$ 1 mL acetonitrile. The residue was taken up in 100 μ L *tert*-butanol/acetonitrile 1:1 and added to 1 mg (ca. 1.3 μ mol) of a trimethylpyridin-2-aminium precursor. The solution was reacted at 75 $^{\circ}\text{C}$ for 10 min. The product was isolated by HPLC (0-30% acetonitrile in 10 min), freed from solvents and taken up in 0.9% saline before injection.

Alternatively 6-fluoronicotinamides were synthesized by trapping F-18 fluoride on a waters Sep-Pak QMA plus light cartridge (46 mg sorbent; preconditioned with 0.5 M KHCO_3), washed with acetonitrile, dried and eluted with 0.5 mg (ca. 0.4 – 0.6 μ mol) of the (protected) FAPI-precursor in 0.5 mL methanol. The solvent was removed *in vacuo* and the residue taken up in 100 μ L acetonitrile/*tert*-butanol 1:4. After 20 min at 70 $^{\circ}\text{C}$ the reaction mixture was diluted with water and the protected intermediates worked up by solid phase extraction (sep-pak light C18, Waters). The solvents were removed and 200 μ L of trifluoroacetic acid were added to the residue. The mixture was heated to 95 for 3 min, dried *in vacuo* and diluted with water before the product was isolated by HPLC, which was directly performed with the diluted reaction mixture in case of compounds lacking protecting groups. The products were freed from solvents and taken up in 0.9% saline before injection in case of animal studies. (Uncorrected radiochemical yield approx. 25%)

For determination of the stability in human serum the radiolabeled compounds (approx. 2.5 MBq for I-125 or 15 MBq for Lu-177) were purified (HPLC or solid phase extraction) and freed from solvent. The residues were taken up in 250 μ L human serum (Sigma-Aldrich) and incubated at 37 $^{\circ}\text{C}$. Samples were precipitated with 30 μ L acetonitrile and analyzed by HPLC (0-30% acetonitrile in 10 min).

Example 2: In vitro characterization of FAPI derivatives

In vitro binding studies were performed using the human tumor cell lines BxPC3, Capan-2, MCF-7 (purchased from Sigma Aldrich Chemie GmbH) and SK-LMS-1 (purchased from ATCC) as well as stably transfected FAP-cell lines HT-1080-FAP, HEK-muFAP and the CD26 expressing cell line HEK-CD26 (obtained from Stefan Bauer, NCT Heidelberg). All cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum at 37°C/5% carbon dioxide. For fluorescence internalization experiments, cells were seeded on coverslips and stained with FAPI-02-Atto488 and DAPI for cell nucleus staining. Images were acquired on a laser scanning confocal microscope using a 63x oil immersion objective. Radioligand binding studies were performed using HT-1080-FAP cells. The radiolabeled compound was added to the cell culture and incubated for different time intervals ranging from 10 min to 24 h. Competition experiments were performed by simultaneous exposure to unlabeled (10^{-5} M to 10^{-9} M) and radiolabeled compound for 60 min. For efflux experiments, radioactive medium was removed after incubation for 60 min and replaced by non-radioactive medium for time intervals ranging from 1 to 24 h. For internalization experiments, surface bound activity was removed by incubating the cells with 1 M glycine-HCl buffer for 10 min. The radioactivity was measured using a γ -counter, normalized to 1 mio cells and calculated as percentage of applied dose (%ID).

20 Cell staining and microscopy

For internalization experiments HT-1080-FAP and HEK muFAP cells were seeded on uncoated coverslips in a 24-well plate and cultivated in culture medium containing 10% fetal calf serum to a final confluence of approx. 80-90%. The medium was removed and cells were washed with 0.5 mL PBS pH 7.4 for 2 times. FAPI-02-Atto488 (20 μ M in DMEM) was added to the cells and incubated for 2 hrs at 37°C. Cells were washed with 0.5 mL PBS pH 7.4 for 3 times and fixed with paraformaldehyde (2% in PBS) for 15 min. The overgrown coverslips were placed on microscope slides using mounting medium containing DAPI for cell nucleus staining (Fluoroshield, Sigma-Aldrich). Images were acquired on a laser scanning confocal microscope (Zeiss LSM 700; Zeiss, Oberkochen, Germany) using the Zeiss Plan-Apochromat 63x/1.4 Oil DIC III immersion objective at xy pixel settings of 0.099 x 0.099 μ m and 1 Airy unit pinhole size for each fluorophore used (488 nm for FAPI-02-Atto488, 405 nm for DAPI). The pictures were processed consistently using the ZEN 2008 software and ImageJ.

Radioligand binding studies

For radioligand binding studies, cells were seeded in 6-well plates and cultivated for 48 h to a final confluence of approx. 80-90% (1.2 - 2 mio cells/well). The medium was replaced by 1 mL fresh medium without fetal calf serum. The radiolabeled compound was added to the cell culture and incubated for different time intervals ranging from 10 min to 24 h. Competition experiments were performed by simultaneous exposure to unlabeled (10^{-5} M to 10^{-9} M) and radiolabeled compound for 60 min. For efflux experiments, radioactive medium was removed after incubation for 60 min and replaced by non-radioactive medium for time intervals ranging from 1 to 24 h. In all experiments, the cells were washed with 1 mL phosphate-buffered saline pH 7.4 for 2 times and subsequently lysed with 1.4 mL lysis buffer (0.3 M NaOH, 0.2% SDS). Radioactivity was determined in a γ -counter (Cobra II, Packard), normalized to 1 mio cells and calculated as percentage of the applied dose (%ID). Each experiment was performed 3 times, and 3 repetitions per independent experiment were acquired.

For internalization experiments the cells were incubated with the radiolabeled compound for 60 min at 37 °C and 4 °C. Cellular uptake was terminated by removing medium from the cells and washing 2 times with 1 mL PBS. Subsequently, cells were incubated with 1 mL of glycine-HCl (1 M in PBS, pH 2.2) for 10 min at room temperature to remove the surface bound activity. The cells were washed with 2 mL of ice-cold PBS and lysed with 1.4 mL of lysis buffer to determine the internalized fraction. For the cells incubated at 4 °C, all washing and elution steps were carried out using ice-cold buffers. The radioactivity was measured using a γ -counter, normalized to 1 mio cells and calculated as percentage of applied dose (%ID).

FAP-01 selectively targets human and murine FAP- α .

In order to analyze the binding properties of FAP-01 to its target protein, radioligand binding assays were performed using different cancer cell lines and cell lines transfected with human and murine FAP as well as the closely related membrane protein CD26, also known as DPP-IV. Both murine FAP and CD26 show a high homology to human FAP- α (muFAP: 90 % identity and 94 % similarity on amino acid level; CD26: 52 % identity and 71 % similarity with high structural resemblance) (Kelly T., *Drug Resist Updat*, 2005).

As shown in Figure 1A, FAP-01 shows no significant binding to FAP-negative cancer cell lines while targeting human and murine FAP- α expressing cells with high affinity (IC_{50} human FAP- α = 39.4 nM). Additionally, no substantial binding to CD26 expressing cells was observed (0.05 ± 0.01 %), proving that FAP-01 is selectively targeting FAP- α . This is of particular importance as CD26 is highly expressed in a variety of normal tissues including the kidneys, the liver and the small intestine. To avoid a high background signal due to unspecific CD26-

binding, high selectivity of the ligand to FAP- α is of great advantage, resulting in optimal image quality.

5 **FAPI-01 rapidly internalizes in FAP-positive cells but shows time-dependent efflux and robust deiodination.**

Cell-based internalization assays demonstrate a rapid uptake of FAPI-01 into the cells (Figure 1B). After 10 min of incubation, 95 % of the total bound fraction is located intracellularly (total 19.70 ± 0.28 %). In the course of 4 h, only a marginal decrease in activity is observed (total 17.00 ± 0.40 %, of which internalized 94 %).

10 Iodine-labeled compounds often show a time-dependent enzymatic deiodination. This was also observed for FAPI-01 resulting in low intracellular radioactivity of this compound after longer incubation times (3.25 ± 0.29 % after 24 h). Deiodination can be minimized by reduction of deiodinase activity after lowering the temperature to 4°C, resulting in an increased radioactivity of 26.66 ± 1.59 % after 24 h.

15

FAPI-02 shows enhanced binding and uptake to human FAP- α as compared to FAPI-01.

To avoid rapid loss of activity of FAPI-01 due to enzymatic deiodination, the non-halogen derivative FAPI-02 was designed in which the FAP-binding moiety is chemically linked to the chelator DOTA. In addition to the resulting enhanced stability, this modification offers the possibility to easily incorporate either diagnostic or therapeutic radionuclides, allowing FAPI-02 to be used as a theranostic compound. Similar to its iodized analogue, FAPI-02 specifically binds to human and murine FAP- α (IC_{50} human FAP- α = 21 nM) expressing cells without addressing CD26 (%ID = 0.13 ± 0.01 %; Figure 1A). FAPI-02 internalizes rapidly into FAP- α expressing cells (20.15 ± 1.74 %ID after 60 min, of which 96 % internalized; Figure 1B), showing more stable and higher uptake rates in the course of time. Compared to the binding of FAPI-01 after 10 min of incubation, only 5 % of the activity remains after 24 h. In contrast, 34 % of the initial radioactivity of FAPI-02 is detected after 24 h of incubation. Efflux experiments demonstrate that FAPI-02 gets eliminated significantly slower than FAPI-01, showing retention of 12 % of the originally accumulated radioactivity after 24 h (FAPI-01 1.1 % ID after 24 h; Figure 1E).

30 Robust internalization of FAPI-02 into human and murine FAP- α expressing cells was confirmed by fluorescence laser scanning microscopy. To this end, HT-1080-FAP and HEK-muFAP cells were stained with a fluorescently labeled FAPI-02 derivative (FAPI-02-Atto488) for 1 to 2 hrs. As shown in Figure 1D, the compound gets completely internalized and

accumulates in the inner of FAP- α expressing cells whereas no uptake is detectable in FAP- α negative HEK-CD26 cells.

Design of FAPI derivatives with enhanced binding properties and pharmacokinetics

- 5 Further variants of FAPI-02 were designed to increase tumor retention time, aiming for the development of a theranostic FAP-targeting agent. The variants FAPI-03 to FAPI-15 have been characterized with respect to target binding, internalization rate and target specificity. The results are shown in Figure 2.

10 Example 3: PET imaging and biodistribution analysis in mice

All experiments were performed in accordance with the German animal protection laws and complied with European Commission regulations for the care and use of laboratory animals. The mice were anaesthetized using isoflurane inhalation.

- For *in vivo* experiments, 8 week old BALB/c *nu/nu* mice (Charles River) were subcutaneously
15 inoculated into the right trunk with 5×10^6 with HT-1080-FAP, Capan-2 or SK-LMS-1 cells, respectively. When the size of the tumor reached approximately 1 cm³, the radiolabeled compound was injected via the tail vein (~10 MBq for small-animal PET imaging; ~1 MBq for organ distribution). PET imaging was performed up to 140 min after intravenous injection of 1 MBq of Ga-68 labeled compound per mouse using the Inveon PET small-animal PET
20 scanner (Siemens). Images were reconstructed iteratively using the 3D-OSEM+MAP method and were converted to standardized uptake value (SUV) images. Quantification was done using a ROI technique and expressed as SUV mean. For organ distribution of Lu-177 labeled compound (approx. 10 MBq per mouse), the animals ($n = 3$ for each time point) were sacrificed after indicated time points (from 30 min to 24 h). The distributed radioactivity was measured
25 in all dissected organs and in blood using a γ -counter (Cobra Autogamma, Packard). The values are expressed as percentage of injected dose per gram of tissue (%ID/g).

- For pharmacokinetic modeling the transport constant K1 and the rate constants k2–k4 were calculated using a two-tissue compartment model implemented in the PMOD software [4], taking into account the vascular fraction (vB), which is associated with the volume of blood
30 exchanging with tissue in a VOI. The rate constants that describe the compartmental fluxes include k1 (binding to the receptor), k2 (detachment) as well as k3 (internalization) and k4 (efflux) in the tumor tissue. In this model the fractional volume of distribution ($DV=K1/k2$) is the proportion of the region of interest in which ¹⁵O-labelled water is distributed.

FAP- α variants accumulate in human FAP-expressing xenografts as well as in xenografts without FAP expression by recruitment and activation of mouse fibroblasts.

Tumor accumulation of FAPI-02 and -04 was assessed by small-animal PET imaging of mice bearing xenografts from both human FAP-positive and negative tumor cells. In both cases the radiotracer gets rapidly enriched within the tumor and is maintained for at least 140 min (Figure 3A, C, E, G). At the same time, FAPI-02 and -04 show negligibly low unspecific binding and get quickly eliminated from the blood predominantly via the kidneys and bladder resulting in a low background and beneficial tumor-to-organ ratios. Simultaneous administration of unlabeled compound as competitor resulted in a complete absence of radioactivity in the tumor, demonstrating the specificity of the radiotracer to its target protein (Figure 4). Interestingly, a high tumor uptake of FAPI-02 was observed in mice bearing FAP- α positive (HT-1080-FAP) as well as FAP- α negative (Capan-2) tumor cell lines due to recruitment and activation of activated mouse fibroblasts. Pharmacokinetic characteristics of the radiotracer, calculated from PET data using a two-tissue compartment model according to Burger et al., *Nucl Med*, 1997, are given in Table 6.

Pharmacokinetic analysis of FAPI-02

	Unit	Capan-2 - comp.	HT-1080-FAP - comp.	HT-1080-FAP + comp.
vB	l/l	0.08	0.04	0.04
k1	ml/ccm/min	0.08	0.07	0.10
k2	l/min	0.16	0.13	0.32
k3	l/min	0.08	0.10	0.04
k4	l/min	0.05	0.02	0.07
Vs	ml/ccm	0.93	2.31	0.18
Vt	ml/ccm	1.44	0.87	0.48
Flux	ml/ccm/min	0.03	0.03	0.01
Chi²	----	0.10	0.11	0.26

Table 6. Pharmacokinetic characteristics of ⁶⁸Ga-FAPI-02, calculated from dynamic PET data using a two-tissue compartment model according to Burger et al., *Nucl Med*, 1997. vB: vascular fraction, associated with the volume of blood exchanging with tissue in a VOI (volume of interest); k1-k4: calculated rate constants; Vs: ratio of specific binding concentration to total parent at equilibrium; Vt: total distribution volume.

These observations were confirmed using ¹⁷⁷Lu-FAPI-02 and -04 in a biodistribution study, proving rapid tumor accumulation in both human FAP- α positive and negative tumors with very low activity in all the other organs (quantified uptake values see Table 7), resulting in

beneficial tumor-to-organ ratios (Figure 5D-F). Similar results were obtained for ^{177}Lu -FAPI-04 in HT-1080-FAP tumor bearing mice. Compared to FAPI-02, FAPI-04 shows a higher tumor uptake especially after 24 h (Figure 5C). A calculation of the area under curve (AUC) is shown in Table 8.

5

	FAPI-02 (Capan-2)	FAPI-02 (HT-1080-FAP)	FAPI-04 (HT-1080-FAP)
Blood	0.83 ± 0.127	1.20 ± 0.178	1.70 ± 0.206
Brain	0.05 ± 0.010	0.06 ± 0.006	0.08 ± 0.010
Heart	0.37 ± 0.031	0.56 ± 0.085	0.80 ± 0.089
Intestines	0.30 ± 0.064	0.37 ± 0.046	0.66 ± 0.196
Kidneys	1.45 ± 0.106	1.60 ± 0.075	2.28 ± 0.477
Liver	0.36 ± 0.015	0.45 ± 0.074	0.73 ± 0.118
Lungs	0.72 ± 0.021	1.02 ± 0.152	1.50 ± 0.151
Muscle	0.94 ± 0.168	1.17 ± 0.332	0.92 ± 0.020
Spleen	0.25 ± 0.015	0.38 ± 0.051	0.48 ± 0.072
Tumor	3.82 ± 0.390	4.51 ± 0.816	4.89 ± 0.817

Table 7. Quantification of biodistribution data 1 h after intravenous administration of Lu-177 labeled FAPI-02 and -04 to tumor bearing Balb/c nude mice; $n=3$; values reported as mean %ID/g $\pm$ SD.

%ID/g	1 h	4 h	24 h	AUC
FAPI-02	4.5 ± 0.82	4.0 ± 0.56	1.12 ± 0.13	64.0
FAPI-04	4.9 ± 0.82	5.4 ± 1.51	3.0 ± 0.23	99.4
FAPI-05	6.0 ± 0.90	5.8 ± 0.60	2.8 ± 0.40	103.3
FAPI-10	3.2 ± 0.72	2.9 ± 0.12	1.1 ± 0.04	49.3
FAPI-13	6.3 ± 0.57	8.7 ± 0.77	4.8 ± 1.71	157.5
FAPI-15	3.4 ± 1.13	4.6 ± 0.32	1.1 ± 0.25	68.0

10

Table 8. Tumor uptake of selected FAPI derivatives in HT-1080-FAP tumor bearing nude mice, $n=3$. Values are reported as mean ID/g $\pm$ SD).

Example 4: Clinical PET/CT studies.

15 Diagnostic imaging of more than 100 patients was performed under the conditions of the updated declaration of Helsinki, § 37 (Unproven interventions in clinical practice) and in accordance to the German Pharmaceuticals Law §13 (2b) for medical reasons using either ^{68}Ga -

FAPI-02 or -04, which was applied intravenously (20 nmol, 122-336 MBq), 10 min, 1 and 3 hours post tracer administration. Variation of injected radiotracer activity is due to the short half-life of ^{68}Ga and the variable elution efficiencies obtained during the lifetime of the $^{68}\text{Ge}/^{68}\text{Ga}$ generator. FDG imaging of one patient was conducted 1 h after intravenous injection of 358 MBq ^{18}F -FDG. The PET/CT scans were performed with a Biograph mCT FlowTM PET/CT-Scanner (Siemens Medical Solution) using the following parameters: slice thickness of 5 mm, increment of 3-4 mm, soft-tissue reconstruction kernel, care dose. Immediately after CT scanning, a whole-body PET was acquired in 3D (matrix 200x200) in FlowMotionTM with 0.7 cm/min. The emission data were corrected for random, scatter and decay. Reconstruction was conducted with an ordered subset expectation maximisation (OSEM) algorithm with 2 iterations/21 subsets and Gauss-filtered to a transaxial resolution of 5 mm at full-width half-maximum (FWHM). Attenuation correction was performed using the low-dose non-enhanced CT data. The quantitative assessment of standardized uptake values (SUV) was done using a region of interest technique.

FAPI-02 and -04 rapidly accumulate in breast, pancreatic, lung, HNO, small intestine and ovarian cancer metastases in humans.

Diagnostic PET/CT scans were performed 1 h after intravenous administration of ^{68}Ga -FAPI-02 and -04 in patients with metastasized breast, lung, pancreatic, HNO, small intestine and ovarian cancer. In all patients a robust accumulation of the tracer was observed in the primary tumor as well as in lymph node and bone metastases with maximum SUV values of 48.0. In contrast, tracer uptake into normal tissue was very low (Figures 6-14). The radioactivity was cleared rapidly from the blood stream and excreted predominantly via the kidneys, resulting in high contrast images. Comparative imaging in one patient with locally advanced lung adenocarcinoma revealed an obvious advantage of FAPI-02 compared to the commonly used PET tracer ^{18}F -FDG. As shown in Figure 9, FAPI-02 shows a higher uptake with lower background activity leading to a higher contrast with better visibility of metastatic lesions. In contrast to FDG, which is highly accumulating in cells with high glucose consumption e.g. the brain, FAPI-02 selectively targets tissues where FAP- α is expressed. Comparative imaging in one patient with prostate cancer revealed an obvious advantage of FAPI-04 compared to the commonly used PET tracers ^{68}Ga -DOTATOC and ^{68}Ga -PSMA, allowing the detection of smaller tumor lesions with reduced tracer accumulation in the kidneys (Figure 14).

Discussion

The reliable diagnosis of primary tumors, metastatic lesions and affected lymph nodes is of utmost importance to allow for effective and adequate therapy planning including tumor staging and choice of treatment. For this purpose, imaging techniques represent indispensable tools for the assessment of many cancer types. Due to its high diagnostic accuracy and the possibility to evaluate both anatomic and physiologic details, combined PET/CT is the method of choice for modern tumor diagnostics. In contrast to non-invasive imaging techniques such as MRT or CT alone, combined PET/CT, however, requires the use of radiotracers with a high affinity to target structures with enhanced expression in tumors as compared to normal tissues.

An ideal tracer should specifically bind to its target protein to ensure reliable differentiation of cancerous and healthy tissue as well as low background signals resulting in high-contrast images. Affinity and specificity become even more important if the radiotracer represents a theranostic compound, i.e. offers the possibility to be loaded with either diagnostic or therapeutic nuclides, which facilitates and improves targeted and personalized treatment.

Regarding a potential application of the tracer for therapeutical purposes, high target specificity assures reduced side-effects, which is especially important for the protection of radiation sensitive tissue such as bone marrow, reproductive and digestive organs.

With that in mind, the inventors developed a theranostic tracer targeting cancer-associated fibroblasts which form a major component of the tumor stroma. They are known to play a critical role in tumor growth, migration and progression and are genetically more stable than cancer cells, therefore, being less susceptible to the development of therapy resistance. In contrast to normal fibroblasts, CAFs express particular proteins which can be used as tumor-specific markers. Among these is the membrane protein FAP- α which is broadly expressed in the microenvironment of a variety of tumors and thus enables targeting of different tumor entities including pancreas, breast and lung cancer, which account for a large part of the entirety of solid tumors.

Based on a small molecule enzyme inhibitor with high affinity to its target protein, we developed the radiotracers FAPI-01 to FAPI-73 by focused chemical modification. All compounds show specific binding to human and murine FAP- α with a rapid and almost complete internalization without addressing the closely related protein CD26/DPP4. Since iodinated molecules undergo an enzymatic deiodination with efflux of free iodine, longer incubation times result in a low intracellular radioactivity. On this account, FAPI-02 and subsequent compounds were designed with the FAP-binding moiety being chemically linked to the chelator DOTA. This results in a set of theranostic compounds with favorable

pharmacokinetic and biochemical properties, of which FAPI-02, FAPI-04, FAPI-46, FAPI-34, FAPI-42, FAPI-52, FAPI-69, FAPI-70, FAPI-71, FAPI-72 and FAPI-73 represent the most favored ligands. FAPI-02 and FAPI-04 both get eliminated significantly slower than FAPI-01, with retention of 12 % (FAPI-02) and 49 % (FAPI-04) of the originally accumulated radioactivity after 24 h (FAPI-01 1.1 %) with the other favored compounds having an even stronger binding (figure 16). They rapidly internalize into FAP- α expressing cells and show high tumor uptake rates in both tumor bearing mice and patients with metastasized epithelial carcinomas. In contrast, there is no accumulation in normal tissue and rapid clearance from the blood system, resulting in high-contrast images. The robust internalization into both human and murine FAP- α expressing cells was confirmed by confocal microscopy using fluorescence-labeled FAPI-02. In contrast to the first generation FAP-antibody F19, which has high affinity to its target protein without being internalized, FAPI-02 shows complete intracellular uptake after 1 h of incubation. The mechanism of internalization after FAP binding has been studied by Fischer et al. using FAP antibody fragments (Fabs) and a DyLight 549 anti-mouse antibody in SK-Mel-187 cells. Incubation at 37 °C led to internalization of the FAP-antibody complexes. As with our small molecule, the internalization process occurred rapidly with an almost complete internalization. Colocalization of the Fabs with a marker for early endosomes was observed after 20 minutes and with a marker for late endosomes and lysosomes after 40 minutes. Fab-mediated FAP- α internalization was suppressed by an inhibitor for dynamin dependent endocytosis, indicating that endocytosis occurs by a dynamin-dependent mechanism.

FAPI-02 and -04 get quickly eliminated from the organism by renal clearance without being retained in the renal parenchyma. In contrast to ^{18}F -FDG, which is highly accumulating in cells with high glucose consumption including inflammatory tissue or the brain, FAPI-02 gets selectively enriched in tissues where its target protein is expressed. This opens new perspectives for the detection of malign lesions in these regions. Additionally, FAP- α was also shown to be expressed by rheumatoid myofibroblast-like synoviocytes in patients with rheumatoid arthritis and osteoarthritis, atherosclerosis, fibrosis as well as in ischemic heart tissue after myocardial infarction. These observations suggest the application of FAPI-02 and -04 as imaging tracers for further indications.

The limiting factor for the detection of tumor lesions is the degree of FAP- α expression within the tumor. This largely depends on the number of activated fibroblasts, i.e. the percentage of stromal content, and/or the number of FAP- α molecules per fibroblast which may be determined by the microenvironment. Since tumor growth exceeding a size of 1 to 2 mm essentially

requires the formation of a supporting stroma, visualization of small lesions in the range of 3-5 mm should be possible using FAPI-PET/CT.

As with any other targeted approach, the FAPI derivatives only achieve optimal results in tissues with sufficiently high FAP- α expression which is known to be rather heterogeneous in different cancer types and patients. Besides breast, colon and pancreatic cancer, which are excellent candidates for FAPI imaging, further analyses have to explore whether other tumor entities such as lung cancer, head and neck cancer, ovarian cancer or hepatomas represent favorable targets.

Also, FAP- α expression was demonstrated in wound healing and fibrotic tissue, which should be kept in mind when interpreting radiological findings. These facts emphasize the necessity to properly evaluate which patients are likely to benefit from a potential FAPI therapy. Given the ability to use either diagnostic or therapeutic nuclides, FAPI-02 and -04 allow simple stratification of the appropriate patient cohort. Either way, it is already clear that both FAPI tracers represent ideal candidates for the development of a targeted radiopharmaceutical. Due to their high target affinity, rapid tumor internalization and fast body clearance, they are already ideally suitable for tumor imaging.

Example 5: FAPI characterization *in vitro* and *in vivo*

Experimental procedures and clinical evaluation

All *in vitro* and *in vivo* experiments as well as the clinical evaluation of the FAPI derivatives have been performed as described above and according to Loktev *et al.*¹ and Lindner *et al.*² A preliminary dosimetry estimate for FAPI-02 and FAPI-04 was based on two patients examined at 0.2 h, 1 h and 3 h post tracer injection using the QDOSE dosimetry software suit. Further PET/CT scans of tumor patients were acquired 1 h after injection of either FAPI-02 (n=25) or FAPI-04 (n=25); for 6 patients an intra-individual related FDG-scan (also acquired 1 h p.i.) was available. For the normal tissue of 16 organs, a 2 cm Spheric-VOI was placed in the parenchyma, for tumor lesions a threshold segmented VOI was used to quantify SUV_{mean}/max³.

In vitro characterization of DOTA-FAPI derivatives

To assess target binding and internalization rate of the DOTA-FAPI derivatives as compared to FAPI-04, Lu-177-labeled compounds were incubated with FAP-expressing HT-1080 cells for 1, 4 and 24 h, respectively (figure 16). The membrane-bound fraction was removed by acidic

elution using glycine-HCl pH 2.2 followed by alkaline cell lysis to determine the internalized fraction. As shown in Figure 16, all derivatives demonstrate higher cell binding as compared to FAPI-04 with binding values up to 500 % of the lead compound after 1 h of incubation (up to 750 % after 4 h).

- 5 To evaluate target affinity and specificity, competitive binding assays were performed using increasing concentrations of unlabeled compound as competitor of Lu-177-labeled compound (Figure 17; respective IC₅₀ values listed in Table 9). Specificity of binding was also confirmed in a radioligand binding assay using murine FAP- and CD26-expressing HEK cells (Figure 18).

Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
FAPI-04	6.5	FAPI-39	11.3
FAPI-05	17.2	FAPI-40	12.7
FAPI-10	19.9	FAPI-41	8.3
FAPI-13	4.5	FAPI-46	13.5
FAPI-15	9.1	FAPI-47	42.0
FAPI-20	7.2	FAPI-48	26.3

Table 9. IC₅₀ values of selected FAPI derivatives as determined by competitive binding assays

10 Organ distribution of DOTA-FAPI derivatives in tumor-bearing mice

For analysis of pharmacokinetic profile as well as tumor uptake *in vivo*, Lu-labeled DOTA-FAPI derivatives were administered i.v. to HT-1080-FAP tumor-bearing mice. Organ distribution of the radiolabeled compounds was determined *ex vivo* in the blood, healthy tissues and the tumor. As shown in Figure 19, most of the compounds demonstrate higher tumor uptake rates as compared to FAPI-02 and FAPI-04, notably 24 h after administration. Due to increased lipophilicity, some of the radiotracers show higher blood activities as well as an increased retention in the kidneys. Determination of tumor-blood-ratios yet reveals a clear advantage of the compounds FAPI-21 and FAPI-46 which demonstrate significantly higher ratios than FAPI-04 at all times examined (Figure 20).

20

Small-animal imaging of DOTA-FAPI derivatives in tumor-bearing mice

Based on these findings, small animal PET-imaging was performed using Ga-68-labeled DOTA-FAPI derivatives up to 140 min after i.v. administration of the radiotracers in HT-1080-FAP tumor-bearing mice. The beneficial tumor-blood ratios of FAPI-21 and FAPI-46 result in high contrast images, enabling excellent visualization of the FAP-positive tumors (Figure 21).

25

A quantitative analysis of the tracer accumulation in the tumor, the kidneys, the liver and muscle tissue (given as SUV max values) reveals slightly lower muscular, renal and hepatic activities of FAPI-46 as compared to FAPI-21 (Figure 22).

5 Biodistribution and dosimetry estimate of FAPI-02 and FAPI-04 compared to FDG in cancer patients

Very similar to literature values for F-18-FDG, Ga-68-DOTATATE or Ga-68-PSMA-11, an exam with 200 MBq Ga-68-FAPI-02 and -04 corresponds to an equivalent dose of approx. 3-4 mSv. After a fast clearance via the kidneys, the normal organs show a low tracer uptake with
10 only minimal changes between 10 min and 3 h p.i. In FAPI-02, the tumor uptake from 1 h to 3 h p.i. decreases by 75 %, whereas the tumor retention is slightly prolonged with FAPI-04 (50 % washout). At 1 h p.i. both FAPI-tracers perform equally (Figure 23). In comparison to FDG, tumor uptake is almost equal (average SUV max FDG 7.41; SUV max FAPI-02 7.37; n.s.); the background uptake in brain (11.01 vs 0.32), liver (2.77 vs 1.69) and oral/pharyngeal mucosa
15 (4.88 vs 2.57) is significantly lower with FAPI-02; other organs were not relevantly different between FDG and FAPI-02 (Figure 24). For detailed information and results see Giesel *et al.* ³ which is incorporated by reference herewith.

20 PET imaging of FAPI-04 in patients with various cancers as well as non-cancerous malignancies

In addition to a rapid uptake of Ga-68-labeled FAPI-04 in different cancers including breast, pancreas, ovarian and HNO tumors, tracer accumulation was also demonstrated in peritonitis carcinomatosa (Figure 25A) as well as several inflammatory malignancies such as myocarditis (Figure 25B) and arthrosis (Figure 25C). These results indicate a potential application of Ga-
25 68-labeled FAPIs for the detection of non-cancerous malignancies which are characterized by a chronic inflammation process involving recruitment of activated fibroblasts.

PET imaging of FAPI-21 and FAPI-46 in patients with various cancers

As shown in Figure 26, robust accumulation of Ga-68-labeled FAPI-21 was observed in
30 different cancers including ovarian, rectal and mucoepidermoid carcinoma. Similar tumor uptake was shown for Ga-68-labeled FAPI-46 which rapidly accumulated in cholangiocellular and colorectal carcinoma, lung cancer as well as solitary fibrous sarcoma (Figure 27). Following PET/CT examination using Ga-68 labeled FAPI-46, a first therapeutical approach

using the Sm-153 labeled radiotracer was taken in two cancer patients. As shown in Figure 28, robust tumor accumulation of the tracer is detectable up to 20 h after administration.

FAP-46-PET/CT imaging of three lung cancer patients with idiopathic pulmonary fibrosis revealed a clear difference of tracer accumulation in the cancerous vs. fibrotic lesions. As shown in Figure 30, tumor uptake of Ga-68 labeled FAPI-46 was significantly higher in two patients (A, B) but slightly lower in one patient (C), as compared to the activity measured in the fibrotic tissue. The patient shown in Figure C suffered from an exacerbated lung fibrosis as compared to the two non-exacerbated cases. Therefore, the tracer is possibly useful for the differentiation of fibrosis patients with bad prognosis from patients with a good prognosis.

FAP-46 derivatives for radiolabeling with alternative radionuclides, e.g. Tc-99m, Pb-203, Cu-64 and F18

To enable the use of alternative radionuclides, a series of FAP-46 derivatives have been designed and characterized with respect to target affinity, specificity and pharmacokinetics. In some of these compounds, the original chelator DOTA has been replaced by different chelating moieties, which are ideally suitable for the incorporation of Tc-99m (FAP-19, -27, -28, -29, -33, -34, -43, -44, -45, -60, -61, -62). FAP affinity *in vitro* and biodistribution in HT-1080-FAP xenografted mice are shown exemplarily for FAP-19 and FAP-34. Both compounds demonstrate a robust binding to human FAP *in vitro* (IC₅₀ FAP-19: 6.4 nM). In contrast to FAP-19, which shows insufficient tumor uptake *in vivo* as well as a rapid accumulation in the liver due to a shift of renal towards hepatic elimination, FAP-34 is continuously enriched within the tumor and demonstrates significantly less hepatic uptake (Figures 31, 32). A first diagnostic application of Tc-99m labeled FAP-34 in a pancreas cancer patient with liver metastases shows a stable tumor accumulation of the tracer up to 4 h after administration. In addition, the overall background activity is comparably low, resulting in high-contrast images (Figure 33). This offers a widespread application for diagnosis by scintigraphy and therapy after labeling with Re-188.

Pb-203 radiolabeled FAP-46 derivatives (FAP-04, -32, -46 and FAP-04tcmc) show comparable cell binding to HT-1080-FAP cells with FAP-32 and FAP-04tcmc reaching the highest binding values after 60 min of incubation (26.93 ± 0.846 and 21.62 ± 0.61 %ID/1 mio cells, Figure 34A). While FAP-32 is rapidly eliminated from the tumor cells upon initial binding ($t_{1/2} = 2$ h), FAP-04tcmc demonstrates considerably slower cell efflux ($t_{1/2} = 7$ h) but also the lowest FAP affinity as shown by the competition assay (IC₅₀ = 5.7 μ M, Figure 34C). On this account, FAP-04 and FAP-46, characterized by optimal half-lives and IC₅₀-values, were selected for

further analysis *in vivo*. As shown in Figure 35, both compounds get continuously enriched within the tumor while showing only negligibly low binding to healthy tissue. The scintigraphic findings are confirmed in a biodistribution study, where both radiotracers demonstrate a robust tumor uptake, overall low organ activities as well as rapid renal excretion (Figure 36).

- 5 To enable radioactive labeling using Cu-64, the NOTA-derivatives FAPI-42 and FAPI-52 have been developed and characterized with respect to target affinity, specificity and pharmacokinetics. As shown in Figure 37, both tracers show a robust binding to HT-1080-FAP cells up to 24 h of incubation with similar IC₅₀-values in the lower nanomolar range (Figure 37A, B). Yet, FAPI-42 gets eliminated significantly slower than FAPI-52, resulting in a
10 calculated *in vitro*-half-life of 12 h (Figure 37C). These results are confirmed by small animal imaging of HT-1080-FAP xenografted mice. As shown in Figure 38, both compounds demonstrate a robust tumor uptake as well as a rapid clearance from the blood stream *in vivo*. Notably, renal excretion of FAPI-42 occurs significantly faster as compared to FAPI-52, while its tumor activity remains slightly higher in the course of 2 to 24 h after administration.
- 15 The NOTA-derivatives FAPI-42 and FAPI-52 have been deployed for the formation of aluminum fluoride complexes to allow imaging with F-18. As shown in Figure 39, both compounds demonstrate a rapid tumor uptake in small animal imaging of HT-1080-FAP xenografted mice. Although both compounds are mainly excreted by the renal pathway, a biliary elimination is also observed. While the renal excretion is faster for FAPI-52 the higher
20 tumor accumulation, longer tumor retention and the lower proportion of the biliary pathway are in favor of FAPI-42.

References

- 1 Loktev, A. *et al.* A new method for tumor imaging by targeting cancer associated fibroblasts. *Journal of nuclear medicine : official publication, Society of Nuclear*
25 *Medicine*, doi:10.2967/jnumed.118.210435 (2018).
- 2 Lindner, T. *et al.* Development of quinoline based theranostic ligands for the targeting of fibroblast activation protein. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, doi:10.2967/jnumed.118.210443 (2018).
- 3 Giesel, F. *et al.* FAPI-PET/CT: biodistribution and preliminary dosimetry estimate of two DOTA-containing FAP-targeting agents in patients with various cancers. *Journal*
30 *of nuclear medicine : official publication, Society of Nuclear Medicine*, doi:10.2967/jnumed.118.215913 (2018).

Example 6: FAPI characterization *in vitro* and *in vivo*Preclinical data

With the objective of selectively targeting FAP-positive brain tumors, initial experiments were performed in tumor bearing mice using the human glioblastoma xenograft model U87MG.

- 5 Tumor accumulation and organ distribution of radiolabeled FAPI-02 and -04 were analyzed by small animal PET imaging as well as in a biodistribution study. As shown in Fig. 40 and 41, both FAPI-02 and -04 demonstrate rapid tumor uptake and negligibly low activities in healthy organs and the blood.

10 Clinical data

According to the WHO classification of 2016, gliomas are subdivided in IDH-wildtype gliomas WHO grade I-IV and IDH-mutant gliomas WHO grade II-IV. The most frequent WHO grade IV gliomas are glioblastomas.

- 15 Clinical PET-imaging was performed in 18 glioma patients (5 IDH mutant gliomas, 13 IDH-wildtype glioblastomas; see Table 10). As shown in Fig. 42-44, IDH-wildtype glioblastomas and grade III/IV, but not grade II IDH-mutant gliomas showed elevated tracer uptake. In glioblastomas, spots with increased uptake in projection on contrast enhancing areas were observed.

20 Conclusion

Increased tracer uptake in IDH-wildtype glioblastomas and high-grade IDH-mutant astrocytomas, but not in diffuse astrocytomas may allow non-invasive distinction between low-grade IDH-mutant and high-grade gliomas and be useful for follow-up studies. The heterogeneous tracer uptake in glioblastomas may be helpful for biopsy planning.

25

Table 10: Patient characteristics

Patient Number	Age (years)	IDH status	Diagnosis	WHO Grade	Localization	Biopsy Surgery	Pretreatment
1	57	wildtype	Glioblastoma	IV	parietal right	Biopsy	Radio-chemotherapy
2	65	wildtype	Glioblastoma	IV	bifrontal	Biopsy	none
3	66	wildtype	Glioblastoma	IV	parieto-temporal left	Biopsy	none
4	64	wildtype	Glioblastoma	IV	temporal right	Resection	Radio-chemotherapy
5	58	wildtype	Glioblastoma	IV	Splenium	Biopsy	none
6	48	wildtype	Glioblastoma	IV	fronto-temporo-parietal left	Resection	Radio-chemotherapy
7	20	wildtype	Glioblastoma	IV	Thalamus / temporal right	Biopsy	none
8	54	wildtype	Glioblastoma	IV	temporal left	Resection	none
9	56	wildtype	Glioblastoma	IV	basal ganglia	Partial Resection	none
10	61	wildtype	Glioblastoma	IV	temporal right	Resection	none
11	86	wildtype	Glioblastoma	IV	Corona radiata left	Biopsy	Chemotherapy
12	68	wildtype	Glioblastoma	IV	Corpus callosum	Biopsy	none
13	71	wildtype	Glioblastoma	IV	temporal left	Biopsy	none
14	29	IDH1 R132H Mutation	Glioblastoma	IV	temporo-parietal right	Resection	none
15	47	IDH1 R132H Mutation	Anaplastic astrocytoma	III	frontal right	Resection	none
16	47	IDH1 R132H Mutation	Diffuse astrocytoma	II	parietal left	Biopsy	none
17	47	IDH1 R132C Mutation	Diffuse astrocytoma	II	frontal right	Resection	none
18	43	IDH1 R132H Mutation	Diffuse astrocytoma	II	fronto-temporal left	Biopsy	Chemotherapy

Example 7: FAPI characterization *in vitro* and *in vivo*Reuptake experiments

For reuptake experiments, ^{177}Lu -labeled FAPI-04 and -46 (5 MBq/nmol in DMEM) were added to HT-1080-FAP cells and incubated for 60 min at 4 and 37°C, respectively. Radioactive medium was removed and cells were washed twice with phosphate-buffered saline (PBS) pH 7.4. Subsequently, non-radioactive medium with and without unlabeled FAPI (1 μM) was added for time intervals ranging from 10 min to 6 h. The cells were washed twice with PBS pH 7.4. To remove the surface bound activity, the cells were incubated with glycine-HCl (1 M in PBS, pH 2.2) for 10 min at room temperature. After washing twice with ice-cold PBS, the cells were lysed with 1.4 mL of lysis buffer (0.3 M NaOH, 0.2% SDS) to determine the internalized fraction. For the cells incubated at 4 °C, all washing and elution steps were carried out using ice-cold buffers. The radioactivity was measured using a γ -counter (Packard Cobra II), normalized to 1 mio cells and calculated as percentage of applied dose (%AD; see Fig. 47).

Enzyme inhibition assay

To determine potential inhibitory effects of FAPI-04 on the enzymatic FAP activity, enzyme inhibition assays were performed using recombinant human FAP protein (1 pmol/well) in a 48-well plate. After incubation of FAPI-04 or Talabostat (0 – 1000 nM/well) with human FAP for 30 min at 37 °C, the fluorogenic FAP substrate Z-GP-AMC was added to a final concentration of 0 – 200 μM /well and incubated for 60 min at 37°C. The enzymatic activity of FAP was determined by measuring the fluorescence intensity of the reaction product AMC at 360/460 nm using the SpectraMax M2 Plate Reader (Molecular Devices, San José, USA) (see Fig. 46).

Multiple administration of FAPI-04 to HT-1080-FAP tumor bearing mice

For biodistribution experiments, 8 week old BALB/c *nu/nu* mice (Charles River) were subcutaneously inoculated into the right trunk with 5 mio HT-1080-FAP cells, respectively. When the size of the tumor reached approximately 1 cm^3 , the radiolabeled compound was injected via the tail vein. The first group of animals was administered a single dose of ^{177}Lu -FAPI-04 (2 MBq per animal), whereas the second group received two doses of 1 MBq each, with the second dose given 4 h after the first injection. The third group was administered three doses in total, with an initial dose of 1 MBq per mouse, followed by 0.5 MBq 2 h and additional 0.5 MBq 4 h after the first injection. The animals ($n = 3$ for each time point) were sacrificed 8 and 24 h after the first injection. The distributed radioactivity was measured in all dissected

organs and in blood using a γ -counter (Cobra Autogamma, Packard). The values are expressed as percentage of injected dose per gram of tissue (%ID/g) (see Fig. 48).

Example 8: FAPI characterization *in vitro* and *in vivo*

5 Experimental Procedures and Clinical Evaluation

All *in vitro* and *in vivo* experiments as well as the clinical evaluation of the FAPI derivatives have been performed as described in the initial document and according to Loktev *et al.*¹ and Lindner *et al.*²

10 Results

In vitro Characterization of F-18-FAPI Derivatives

All experiments were carried out analogous to FAPI-42 (AlF-18 labeling) or FAPI-72 (F-18 nicotinamide labeling).

Compound	IC ₅₀ (nM)	Compound	IC ₅₀ (nM)
FAPI-72	2.4	FAPI-74	9.2
FAPI-73	5.4	FAPI-75	2.9

Table 11. EC₅₀ values of selected FAPI derivatives as determined by competitive binding assays

Determination of blood pool clearance

To estimate the rate of clearance of the compound, half-life times were calculated by a presumed two phase exponential decay from the SUVmean values (0.375-60 min) of the heart as representation of the blood pool. All selected compounds were cleared very fast with half-life times below 10 min. The calculated plateau values, which were higher for Ga-68 labeled FAPI-13, -21, -36 and AlF-18 labeled FAPI-74 theoretically correspond to a higher fraction of compound which is not cleared due to unspecific binding or by remaining in circulating (Table 12). As an example for the fast clearance the time activity curves for FAPI-04 and -46 between 0 and 15 min are shown in Figure 53.

Compound	Blood pool-clearance ($T_{1/2}$ [min])	Plateau value (SUVmean)
FAP1-04	7.1	0.21
FAP1-13	5.5	0.27
FAP1-21	5.1	0.31
FAP1-36	5.0	0.58
FAP1-46	5.3	0.19
FAP1-74	2.4	0.32

Table 12. Blood pool half lives and the hypothetical plateau value of selected FAP1-derivatives calculated from SUVmean-values by a presumed two phase exponential decay. For clarity only the rate determining half-life values are listed.

5 *Small-Animal Imaging of F-18-FAP1 Derivatives in Tumor-Bearing Mice*

Based on these findings, small animal PET-imaging was performed using F-18-labeled NOTA- and F-18-nicotinamide-labeled FAP1 derivatives up to 140 min after i.v. administration of the radiotracers in HT-1080-FAP tumor-bearing mice. The F-18-nicotinamide derivatives FAP1-72, -73 and -77 showed an unfavorable accumulation in the liver as well as a biliary excretion, while FAP1-78 was renally excreted but showed no tumor uptake. In case of the AlF-18 labeled
10 NOTA derivatives FAP1-74 and -75 a high target specificity and fast clearance was observed, resulting in high contrast images, which enable excellent visualization of the FAP-positive tumors (Figure 50).

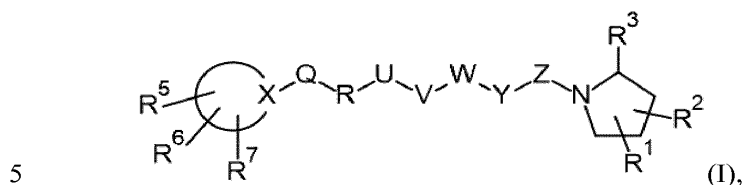
15 *Organ Distribution of F-18-FAP1 Derivatives in Tumor-Bearing Mice*

For analysis of pharmacokinetic profile as well as tumor uptake *in vivo*, AlF-18-labeled FAP1-75 was administered i.v. to HT-1080-FAP tumor-bearing mice. Organ distribution of the radiolabeled compound was determined *ex vivo* in the blood, healthy tissues and the tumor. As shown in Figure 51, the compounds demonstrates high tumor uptake, although in comparison
20 to Ga-68 labeled DOTA derivatives a higher accumulation in healthy tissue is observed, while performance in PET imaging was equal.

Items

The following items represent preferred embodiments of the present invention.

1. A compound of Formula (I)



wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

10 Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other;

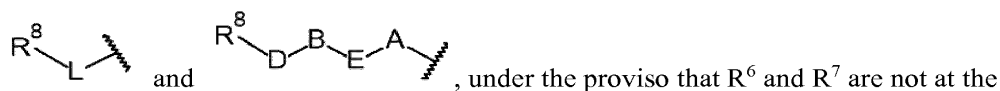
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

15 R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

20 R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,



under the proviso that R⁶ and R⁷ are not at the same time H,

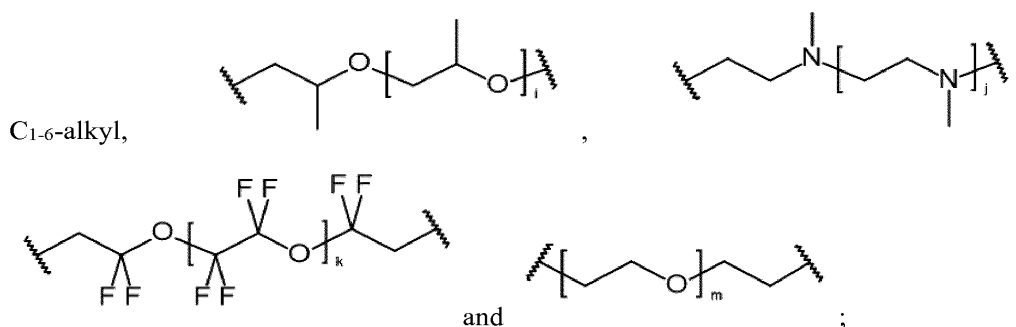
wherein L is a linker,

25 wherein D, A, E, and B are individually present or absent, preferably wherein at least A, E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR⁴, O, S, and CH₂;

E is selected from the group consisting of



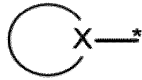
wherein i is 1, 2, or 3;

wherein j is 1, 2, or 3;

5 wherein k is 1, 2, or 3;

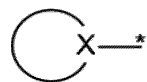
wherein m is 1, 2, or 3;

A and E together form a group selected from: a cycloalkyl, heterocycloalkyl, aryl and heteroaryl, preferably heterocycloalkyl, wherein A and E can be mono-, bi- and multicyclic, preferably monocyclic; each A and E being optionally substituted with 1 to 4 substituents selected from -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected

from -OH, oxo, halo; and optionally connected to A, B, D, E or  ;

B is selected from the group consisting of S, NR⁴, NR⁴-O, NR⁴-C₁₋₆-alkyl, NR⁴-C₁₋₆-alkyl-NR⁴, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein NR⁴-C₁₋₆-alkyl-NR⁴ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl; and;

20 R⁸ is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;



is a 1-naphtyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof.

2. The compound of item 1, wherein

(i) Q, R, U are CH₂ and are individually present or absent;

V is CH₂, C=O, C=S or C=NR⁴;

W is NR⁴;

Y is HCR⁴; and

Z is C=O, C=S or C=NR⁴; and/or

(ii) Q and R are absent;

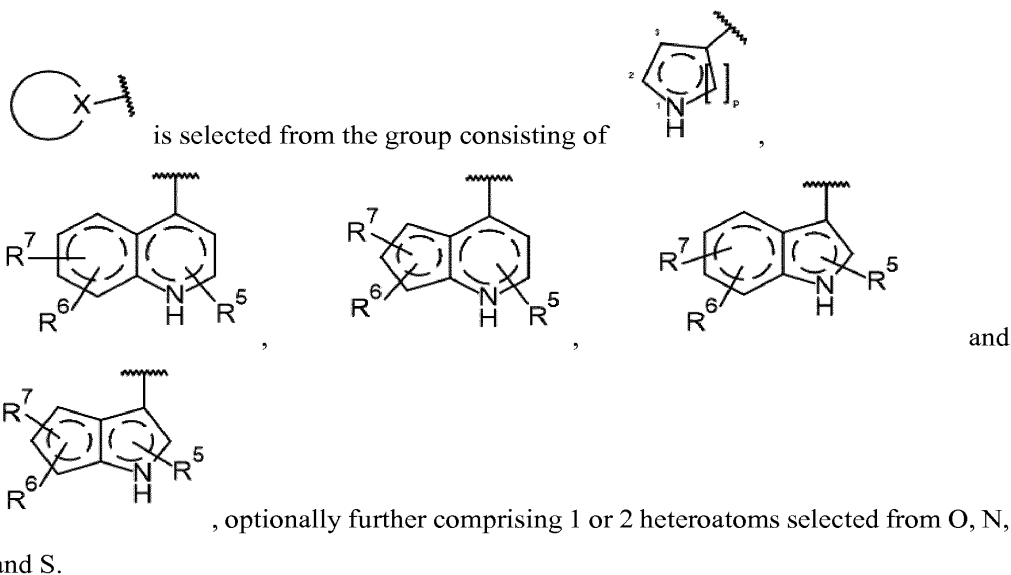
U is CH₂ and is present or absent;

R¹ and R² are independently selected from the group consisting of -H and halo;

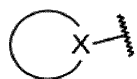
R³ is selected from the group consisting of -H, -CN, and -B(OH)₂;

R⁴ is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH.

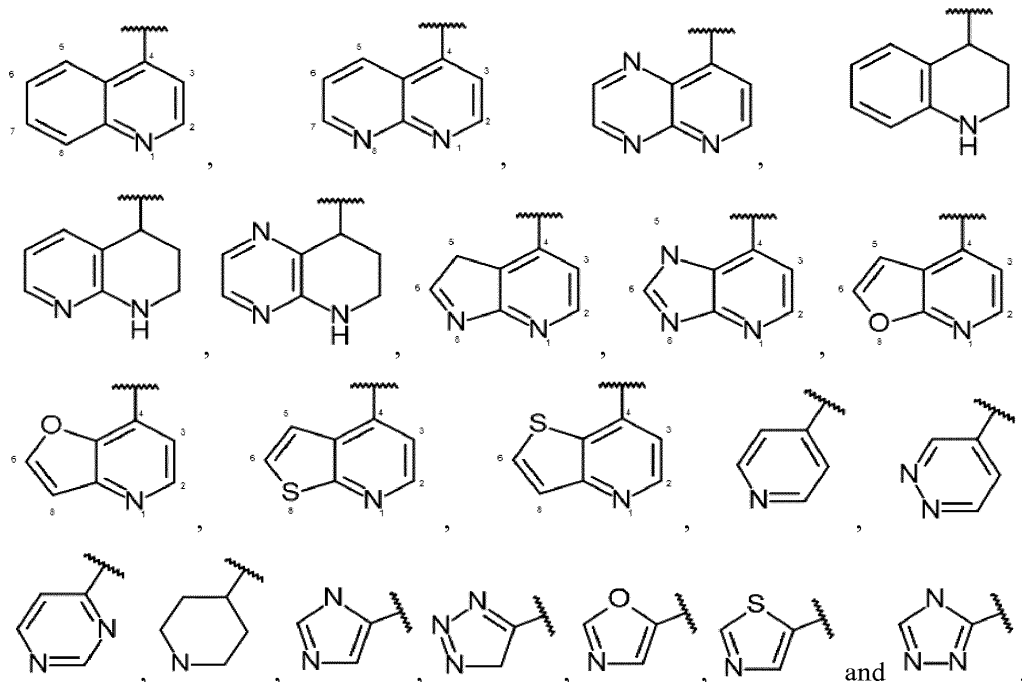
3. The compound of item 1 or 2, wherein



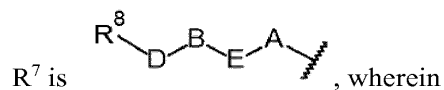
4. The compound of any of the preceding items, wherein



is selected from the group consisting of



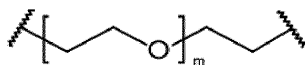
5. The compound of any of the preceding items, wherein
 R^5 and R^6 are H;



10

D is absent;

A is O, S, CH_2 , NH, NCH_3 ;

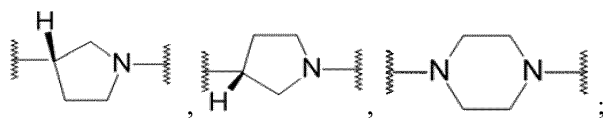


E is C_{1-6} -alkyl or

, wherein m is 1, 2, or 3;



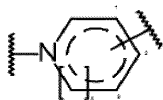
A and E together form a group selected from:



B is $\text{NR}^4\text{-C}_{1-6}\text{-alkyl}$ or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of $\text{C}_{1-6}\text{-alkyl}$, aryl, $\text{C}_{1-6}\text{-aralkyl}$.

6. The compound of any of the preceding items, wherein

(i) the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



, wherein

the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen;

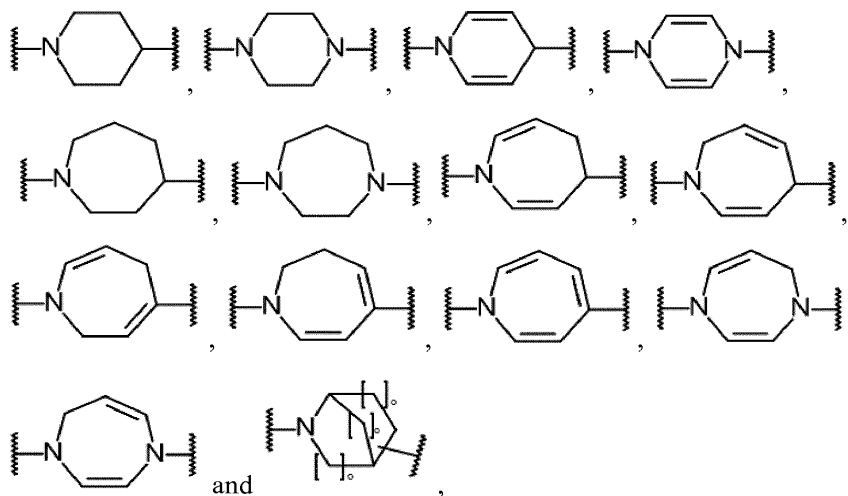


is attached to position 1, 2, or 3, preferably to position 2;

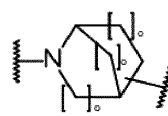
l is 1 or 2;

optionally wherein the N-containing heterocycle is substituted with a $\text{C}_{1-6}\text{-alkyl}$; and/or

(ii) the N-containing heterocycle comprised in B is selected from the group consisting of:

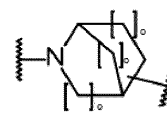


optionally wherein the N-containing heterocycle is substituted with a $\text{C}_{1-6}\text{-alkyl}$;



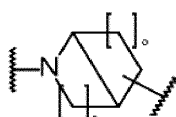
wherein if the N-containing heterocycle comprised in B is
the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O,
N and S, optionally further comprises 1 nitrogen, optionally compromises one or
more (e.g. amino acid derived) side chains;

5 is attached to position 1, 2, or 3, preferably to position 2;
o is 1 or 2,



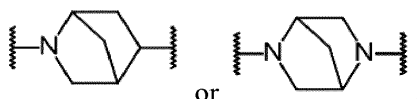
preferably, if the N-containing heterocycle comprised in B is , the

N-containing heterocycle comprised in B is , or



; more preferably, if the N-containing heterocycle comprised in

10 B is , the N-containing heterocycle comprised in B is



7. The compound of any of the preceding items, wherein

Q, R, U are absent;

15 V is C=O;

W is NH;

Y is CH₂;

Z is C=O;

R¹ and R² are independently selected from the group consisting of -H and halo;

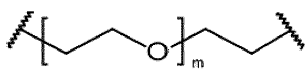
20 R³ is -CN;

R⁵ and R⁶ are H;

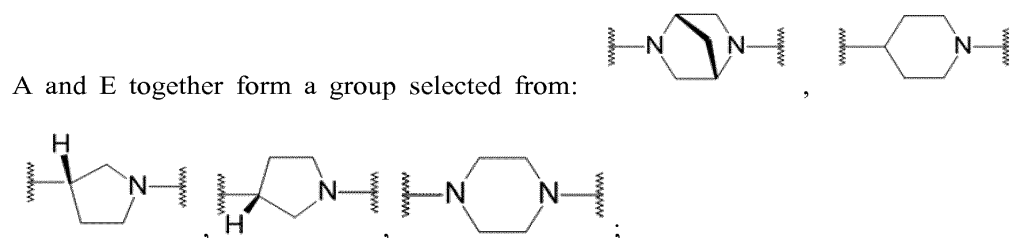
R^7 is $R^8-D-B-E-A$, wherein

D is absent;

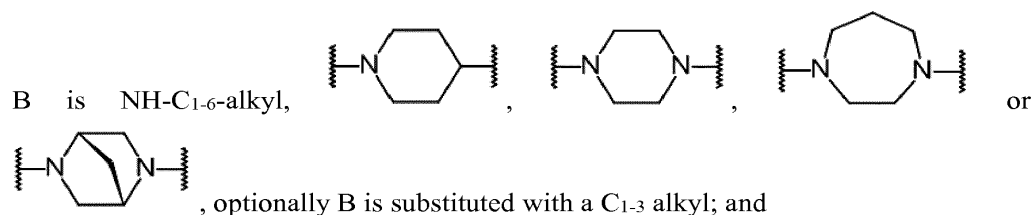
A is O, S, CH₂, NH, NCH₃;

E is C₁₋₆-alkyl or , wherein m is 1, 2, or 3; or

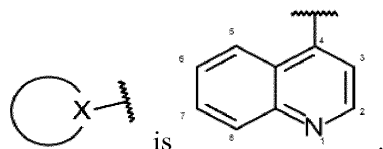
5 A and E together form a group selected from:



B is NH-C₁₋₆-alkyl,



10



8. The compound of any of the preceding items, wherein C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl, and/or

15 wherein C₁₋₆-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl, phenyl-propyl, and phenyl-butyl.

9. The compound of any of the preceding items, wherein R⁸ is a radioactive moiety, wherein the radioactive moiety is a fluorescent isotope, a radioisotope, a radioactive drug or combinations thereof, preferably wherein the radioactive moiety is selected from the group consisting of alpha radiation emitting isotopes, beta radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting

20

isotopes, fluorescence emitting isotopes, such as ^{11}C , ^{18}F , ^{51}Cr , ^{67}Ga , ^{68}Ga , ^{111}In , $^{99\text{m}}\text{Tc}$, ^{186}Re , ^{188}Re , ^{139}La , ^{140}La , ^{175}Yb , ^{153}Sm , ^{166}Ho , ^{88}Y , ^{90}Y , ^{149}Pm , ^{165}Dy , ^{169}Er , ^{177}Lu , ^{47}Sc , ^{142}Pr , ^{159}Gd , ^{212}Bi , ^{213}Bi , ^{72}As , ^{72}Se , ^{97}Ru , ^{109}Pd , ^{105}Rh , $^{101\text{m}}\text{Rh}$, ^{119}Sb , ^{128}Ba , ^{123}I , ^{124}I , ^{131}I , ^{197}Hg , ^{211}At , ^{151}Eu , ^{153}Eu , ^{169}Eu , ^{201}Tl , ^{203}Pb , ^{212}Pb , ^{64}Cu , ^{67}Cu , ^{188}Re , ^{186}Re , ^{198}Au , ^{225}Ac , ^{227}Th and ^{199}Ag , preferably ^{18}F , ^{64}Cu , ^{68}Ga , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{153}Sm , ^{177}Lu , ^{188}Re .

10. The compound of any of items 1 to 8, wherein R^8 is a fluorescent dye select from the group consisting of the following classes of fluorescent dyes: Xanthenes, Acridines, Oxazines, Cynines, Styryl dyes, Coumarines, Porphines, Metal-Ligand-Complexes, Fluorescent proteins, Nanocrystals, Perylenes, Boron-dipyrromethenes and Phtalocyanines as well as conjugates and combinations of these classes of dyes.
11. The compound of any of items 1 to 8, wherein R^8 is a chelating agent which forms a complex with divalent or trivalent metal cations, preferably wherein the chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane-N,N',N,N'-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N"-pentaacetic acid (DTPA), bis-(carboxymethylimidazole)glycine and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC).
12. The compound of any of items 1 to 8, wherein R^8 is a contrast agent which comprises or consists of a paramagnetic agent, preferably, wherein the paramagnetic agent comprises or consists of paramagnetic nanoparticles.
13. Pharmaceutical composition comprising or consisting of at least one compound according to any of items 1 to 12; and, optionally, a pharmaceutically acceptable carrier and/or excipient.
14. The compound of any of items 1 to 12 or the pharmaceutical composition of claim 13 for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject, preferably wherein the disease characterized by overexpression of fibroblast activation protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorder, preferably wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, small intestine cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer, hepatocellular carcinoma,

esophageal cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocellular carcinoma, clear cell renal carcinoma, neuroendocrine tumor, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus carcinoma, desmoid tumors, glioma, astrocytoma, cervix carcinoma and prostate cancer.

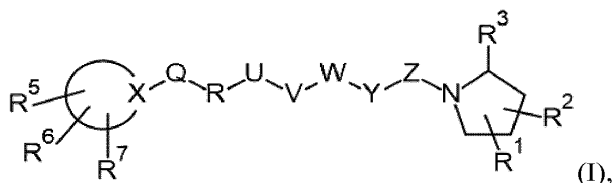
5

15. A kit comprising or consisting of the compound of any of items 1 to 12 or the pharmaceutical composition of claim 13 and instructions for the diagnosis or treatment of a disease.

10

Claims

1. A compound of Formula (I)



5 wherein

Q, R, U, V, W, Y, Z are individually present or absent under the proviso that at least three of Q, R, U, V, W, Y, Z are present;

Q, R, U, V, W, Y, Z are independently selected from the group consisting of O, CH₂, NR⁴, C=O, C=S, C=NR⁴, HCR⁴ and R⁴CR⁴, with the proviso that two Os are not directly adjacent to each other;

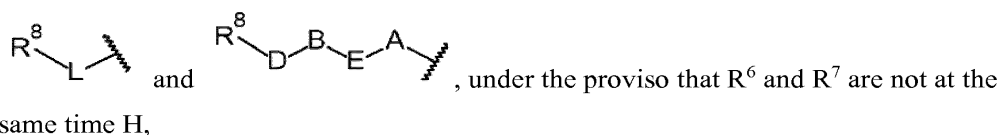
R¹ and R² are independently selected from the group consisting of -H, -OH, halo, C₁₋₆-alkyl, -O-C₁₋₆-alkyl, S-C₁₋₆-alkyl;

R³ is selected from the group consisting of -H, -CN, -B(OH)₂, -C(O)-alkyl, -C(O)-aryl, -C=C-C(O)-aryl, -C=C-S(O)₂-aryl, -CO₂H, -SO₃H, -SO₂NH₂, -PO₃H₂, and 5-tetrazolyl;

R⁴ is selected from the group consisting of -H, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -S-C₁₋₆-alkyl, alkenyl, heteroalkenyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, and -C₁₋₆-aralkyl, each of said -C₁₋₆-alkyl being optionally substituted with from 1 to 3 substituents selected from -OH, oxo, halo and optionally connected to Q, R, U, V, W, Y or Z;

R⁵ is selected from the group consisting of -H, halo and C₁₋₆-alkyl;

R⁶, and R⁷ are independently selected from the group consisting of -H,



wherein L is a linker,

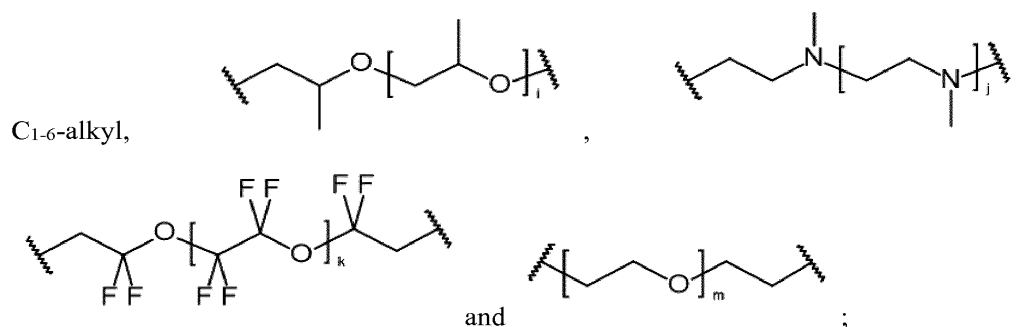
wherein D, A, E, and B are individually present or absent, preferably wherein at least A,

E, and B are present, wherein when present:

D is a linker;

A is selected from the group consisting of NR⁴, O, S, and CH₂;

E is selected from the group consisting of



wherein i is 1, 2, or 3;

wherein j is 1, 2, or 3;

5 wherein k is 1, 2, or 3;

wherein m is 1, 2, or 3;

B is selected from the group consisting of S, NR⁴, NR⁴-O, NR⁴-C₁₋₆-alkyl, NR⁴-C₁₋₆-alkyl-NR⁴, and a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein NR⁴-C₁₋₆-alkyl-NR⁴ and the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C₁₋₆-alkyl, aryl, C₁₋₆-aralkyl; and;

R⁸ is selected from the group consisting of radioactive moiety, chelating agent, fluorescent dye, a contrast agent and combinations thereof;

15 is a 1-naphthyl moiety or a 5 to 10- membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, wherein there are 2 ring atoms between the N atom and X; said heterocycle optionally further comprising 1, 2 or 3 heteroatoms selected from O, N and S; and X is a C atom;

or a pharmaceutically acceptable tautomer, racemate, hydrate, solvate, or salt thereof.

20

2. The compound of claim 1, wherein

(i) Q, R, U are CH₂ and are individually present or absent;

V is CH₂, C=O, C=S or C=NR⁴;

W is NR⁴;

25 Y is HCR⁴; and

Z is C=O, C=S or C=NR⁴; and/or

(ii) Q and R are absent;

U is CH₂ and is present or absent;

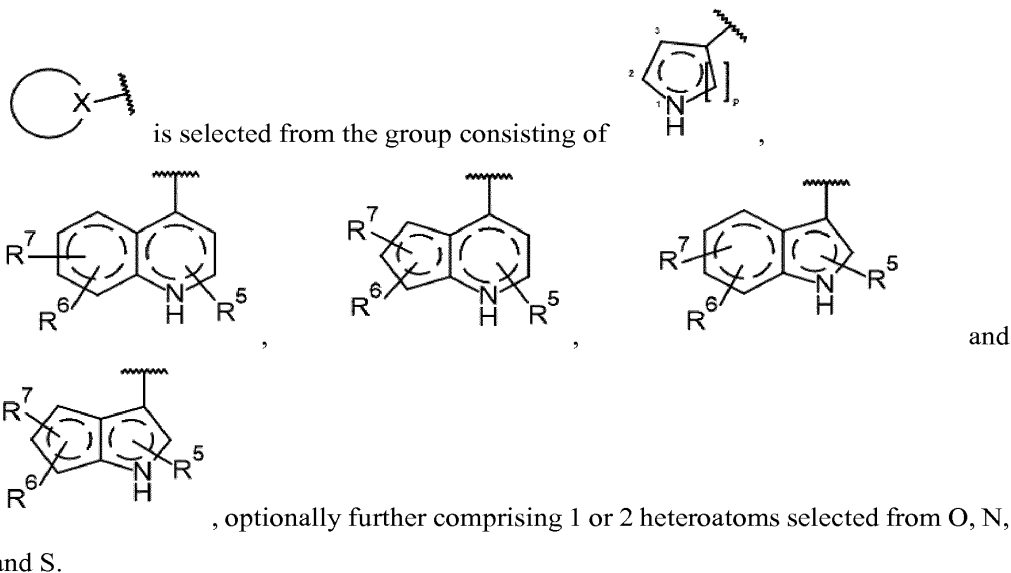
R^1 and R^2 are independently selected from the group consisting of -H and halo;

R^3 is selected from the group consisting of -H, -CN, and -B(OH)₂;

R^4 is selected from the group consisting of -H and -C₁₋₆-alkyl, wherein the -C₁₋₆-alkyl is optionally substituted with from 1 to 3 substituents selected from -OH.

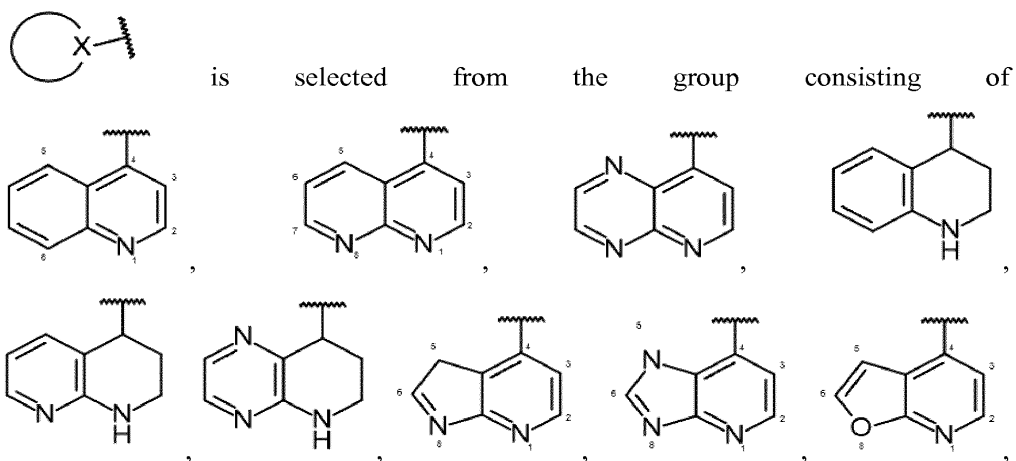
5

3. The compound of claim 1 or 2, wherein

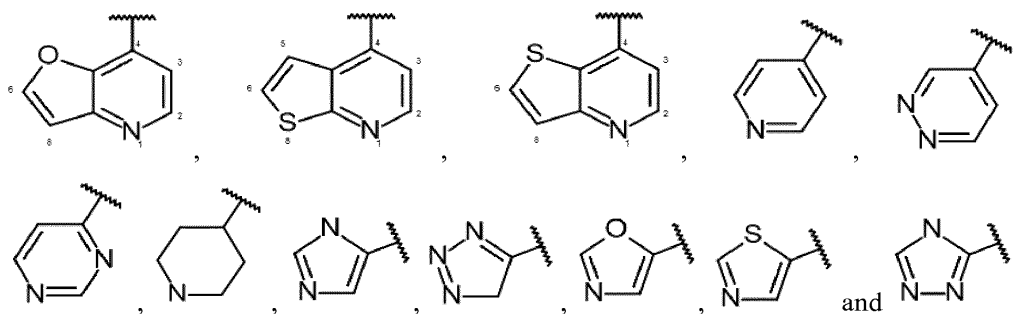


10

4. The compound of any of the preceding claims, wherein

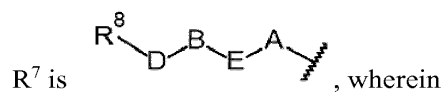


15



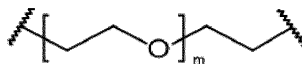
5. The compound of any of the preceding claims, wherein

5 R^5 and R^6 are H;



D is absent;

A is O;



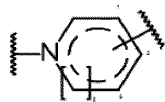
E is C_{1-6} -alkyl or , wherein m is 1, 2, or 3;

10 B is NR^4 - C_{1-6} -alkyl or a 5- to 10-membered N-containing aromatic or non-aromatic mono- or bicyclic heterocycle, preferably further comprising 1 or 2 heteroatoms selected from O, N, and S, preferably further comprising 1 or 2 nitrogen atoms, preferably wherein the N-containing heterocycle is substituted with 1 to 3 substituents selected the group consisting of C_{1-6} -alkyl, aryl, C_{1-6} -aralkyl.

15

6. The compound of any of the preceding claims, wherein

(i) the N-containing heterocycle comprised in B is an aromatic or non-aromatic monocyclic heterocycle:



, wherein

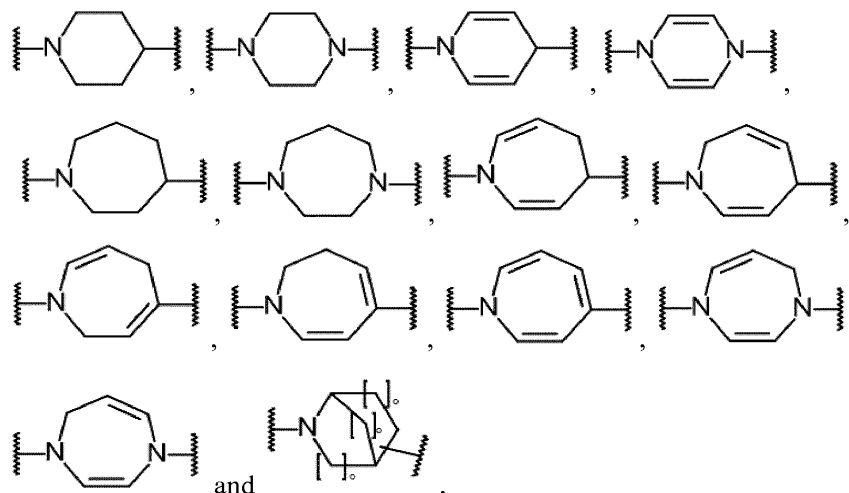
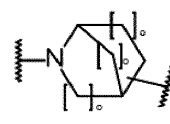
20

the heterocycle optionally further comprises 1 or 2 heteroatoms selected form O, N and S, optionally further comprises 1 nitrogen;

is attached to position 1, 2, or 3, preferably to position 2;

l is 1 or 2; and/or

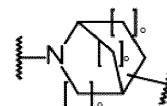
- (ii) the N-containing heterocycle comprised in B is selected from the group consisting of:



wherein if the N-containing heterocycle comprised in B is the heterocycle optionally further comprises 1 or 2 heteroatoms selected from O, N and S, optionally further comprises 1 nitrogen, optionally compromises one or more (e.g. amino acid derived) side chains;

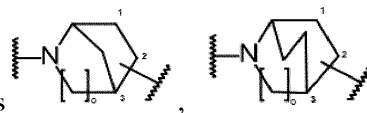
10 is attached to position 1, 2, or 3, preferably to position 2; o is 1 or 2,

preferably, if the N-containing heterocycle comprised in B is

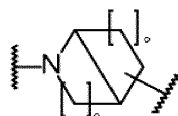


, the

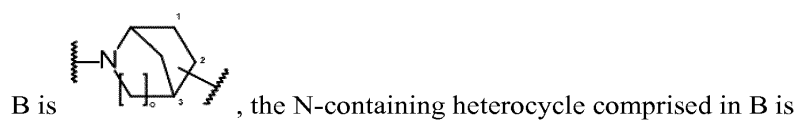
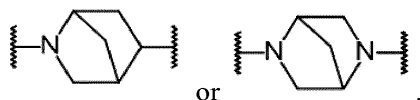
N-containing heterocycle comprised in B is



or



15 ; more preferably, if the N-containing heterocycle comprised in



7. The compound of any of the preceding claims, wherein

5 Q, R, U are absent;

V is C=O;

W is NH;

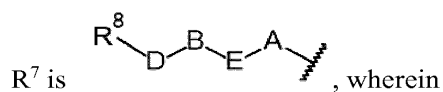
Y is CH₂;

Z is C=O;

10 R¹ and R² are independently selected from the group consisting of -H and halo;

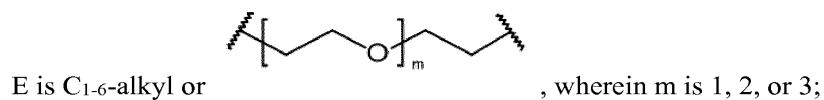
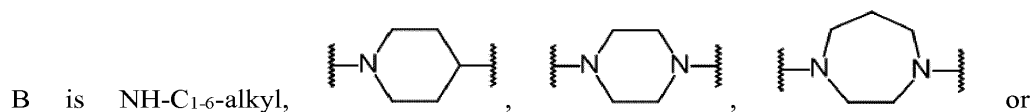
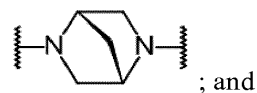
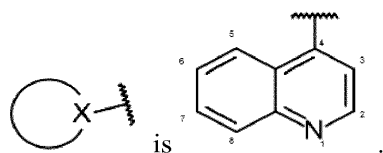
R³ is -CN;

R⁵ and R⁶ are H;



D is absent;

15 A is O;



20

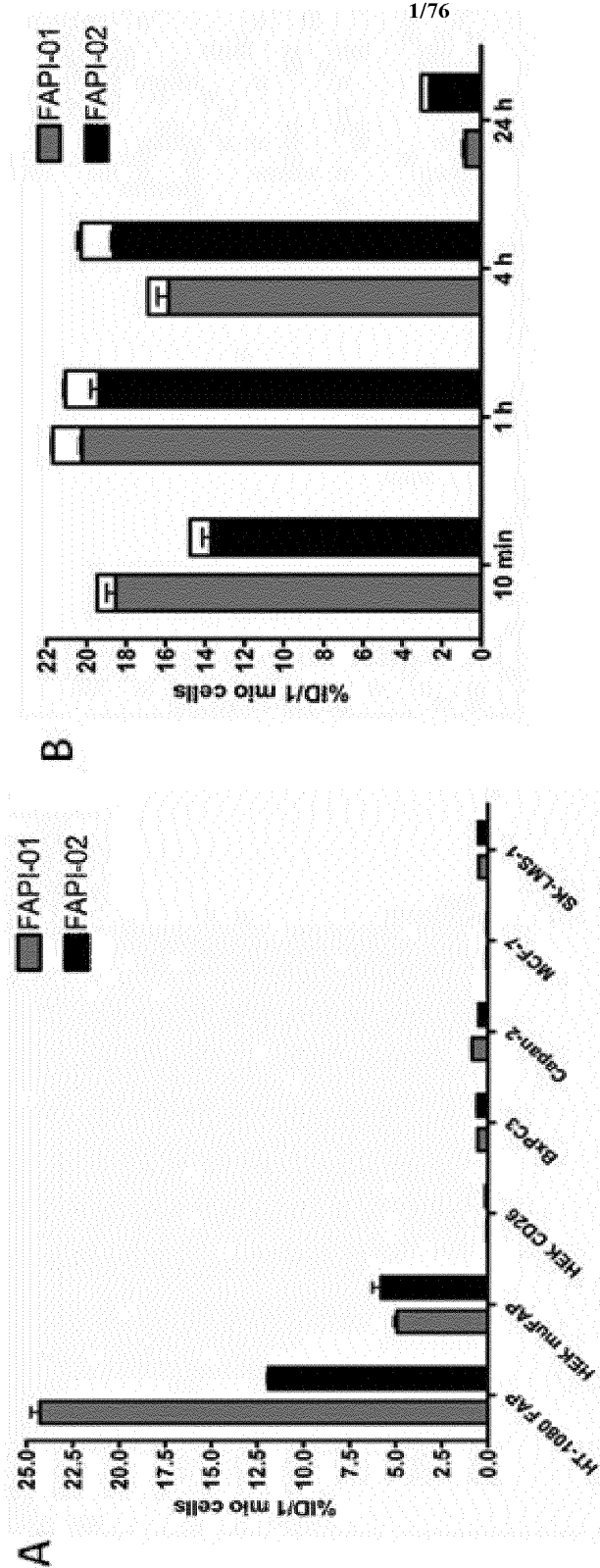
8. The compound of any of the preceding claims, wherein C₁₋₆-alkyl is selected from the group consisting of methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, tert-butyl, pentyl and hexyl, and/or
wherein C₁₋₆-aralkyl is selected from the group consisting of benzyl, phenyl-ethyl,
5 phenyl-propyl, and phenyl-butyl.
9. The compound of any of the preceding claims, wherein R⁸ is a radioactive moiety, wherein the radioactive moiety is a fluorescent isotope, a radioisotope, a radioactive drug or combinations thereof, preferably wherein the radioactive moiety is selected from the
10 group consisting of alpha radiation emitting isotopes, beta radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting isotopes, fluorescence emitting isotopes, such as ¹⁸F, ⁵¹Cr, ⁶⁷Ga, ⁶⁸Ga, ¹¹¹In, ^{99m}Tc, ¹⁸⁶Re, ¹⁸⁸Re, ¹³⁹La, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁵³Sm, ¹⁶⁶Ho, ⁸⁸Y, ⁹⁰Y, ¹⁴⁹Pm, ¹⁶⁵Dy, ¹⁶⁹Er, ¹⁷⁷Lu, ⁴⁷Sc, ¹⁴²Pr, ¹⁵⁹Gd, ²¹²Bi, ²¹³Bi, ⁷²As, ⁷²Se, ⁹⁷Ru, ¹⁰⁹Pd, ¹⁰⁵Rh, ^{101m}Rh, ¹¹⁹Sb, ¹²⁸Ba, ¹²³I, ¹²⁴I, ¹³¹I, ¹⁹⁷Hg,
15 ²¹¹At, ¹⁵¹Eu, ¹⁵³Eu, ¹⁶⁹Eu, ²⁰¹Tl, ²⁰³Pb, ²¹²Pb, ⁶⁴Cu, ⁶⁷Cu, ¹⁸⁸Re, ¹⁸⁶Re, ¹⁹⁸Au, ²²⁵Ac, ²²⁷Th and ¹⁹⁹Ag.
10. The compound of any of claims 1 to 8, wherein R⁸ is a fluorescent dye select from the group consisting of the following classes of fluorescent dyes: Xanthenes, Acridines,
20 Oxazines, Cynines, Styryl dyes, Coumarines, Porphines, Metal-Ligand-Complexes, Fluorescent proteins, Nanocrystals, Perylenes, Boron-dipyrromethenes and Phtalocyanines as well as conjugates and combinations of these classes of dyes.
11. The compound of any of claims 1 to 8, wherein R⁸ is a chelating agent which forms a complex with divalent or trivalent metal cations, preferably wherein the chelating agent is selected from the group consisting of 1,4,7,10-tetraazacyclododecane-N,N',N,N'-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), triethylenetetramine (TETA), iminodiacetic acid, diethylenetriamine-N,N,N',N',N''-pentaacetic acid (DTPA), bis-
25 (carboxymethylimidazole)glycine and 6-Hydrazinopyridine-3-carboxylic acid (HYNIC).
30
12. The compound of any of claims 1 to 8, wherein R⁸ is a contrast agent which comprises or consists of a paramagnetic agent, preferably, wherein the paramagnetic agent comprises or consists of paramagnetic nanoparticles.

35

13. Pharmaceutical composition comprising or consisting of at least one compound according to any of claims 1 to 12; and, optionally, a pharmaceutically acceptable carrier and/or excipient.
- 5 14. The compound of any of claims 1 to 12 or the pharmaceutical composition of claim 13 for use in the diagnosis or treatment of a disease characterized by overexpression of fibroblast activation protein (FAP) in an animal or a human subject, preferably wherein the disease characterized by overexpression of fibroblast activation protein (FAP) is selected from the group consisting of cancer, chronic inflammation, atherosclerosis, fibrosis, tissue remodeling and keloid disorder, preferably wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, small intestine cancer, colon cancer, rectal cancer, lung cancer, head and neck cancer, ovarian cancer, hepatocellular carcinoma, esophageal cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocellular carcinoma, clear cell renal carcinoma, neuroendocrine tumor, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus carcinoma, desmoid tumors, glioma, astrocytoma, cervix carcinoma and prostate cancer.
- 10 15. A kit comprising or consisting of the compound of any of claims 1 to 12 or the pharmaceutical composition of claim 13 and instructions for the diagnosis of a disease.
- 15 20

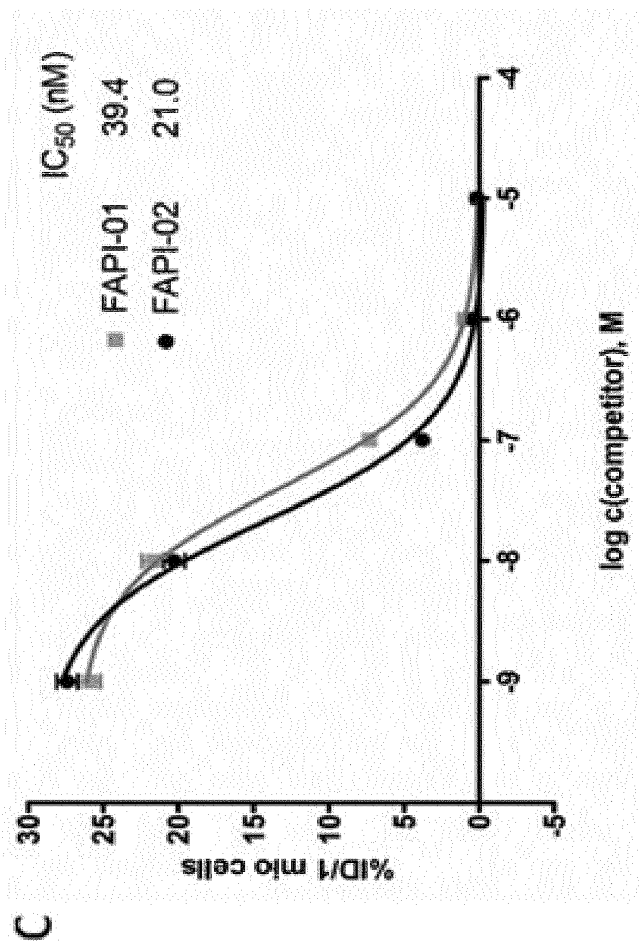
1/76

Figure 1



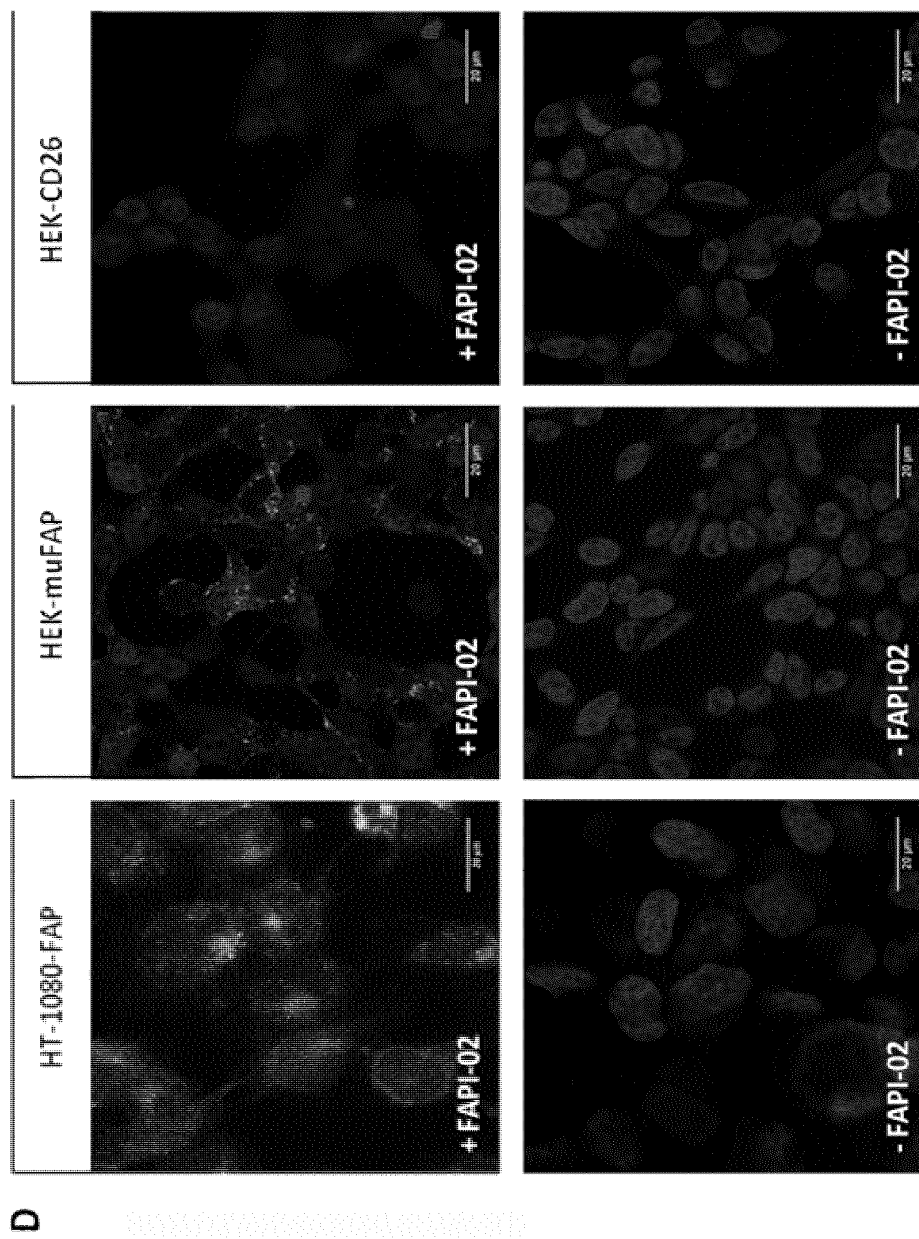
SUBSTITUTE SHEET (RULE 26)

Figure 1 continued



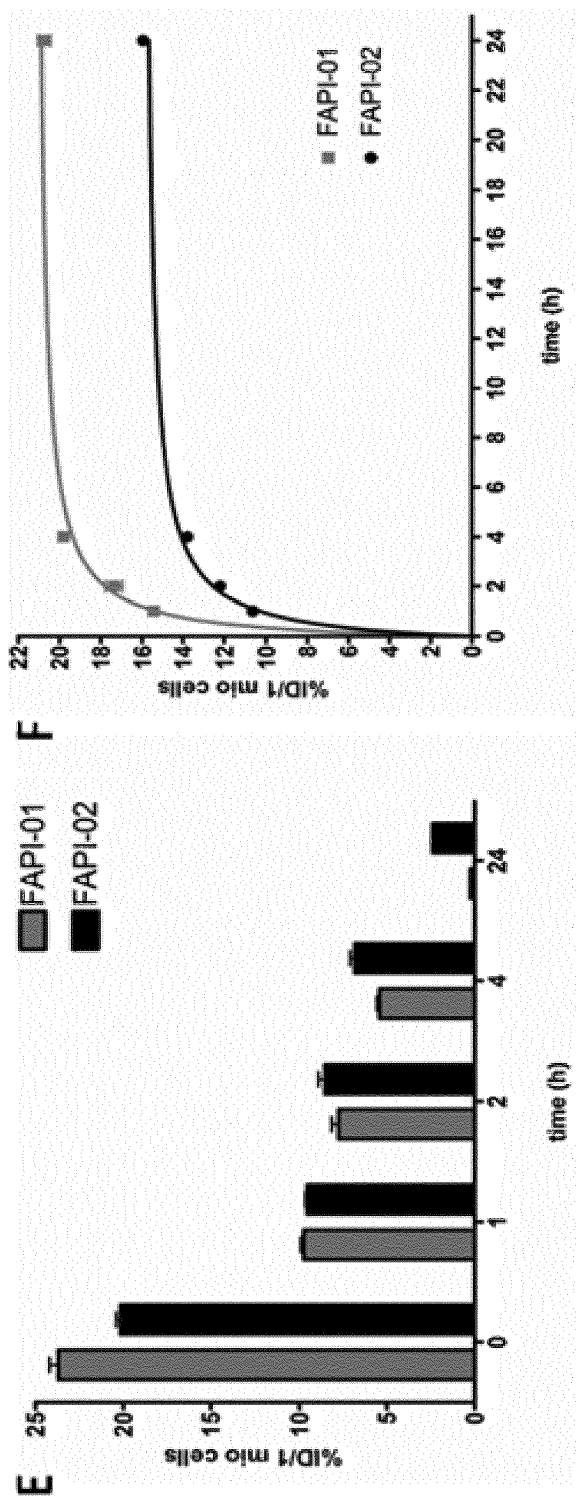
SUBSTITUTE SHEET (RULE 26)

Figure 1 continued



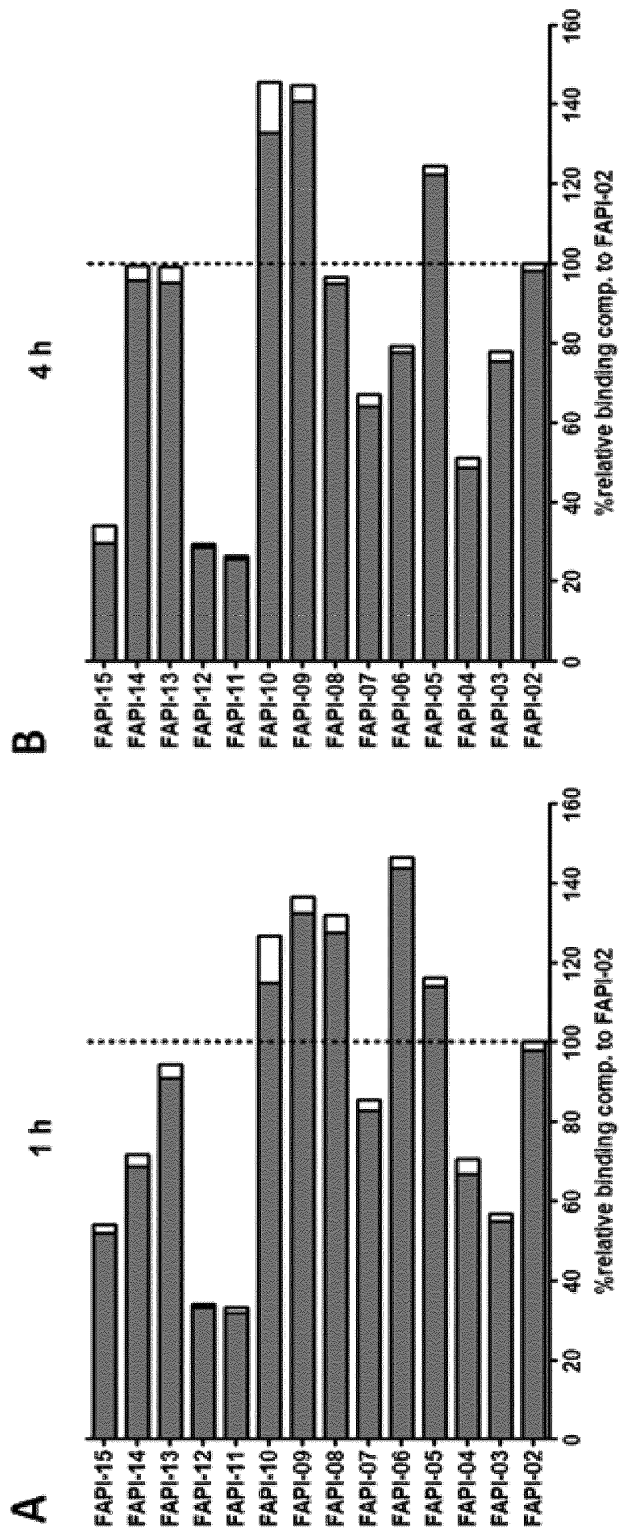
SUBSTITUTE SHEET (RULE 26)

Figure 1 continued



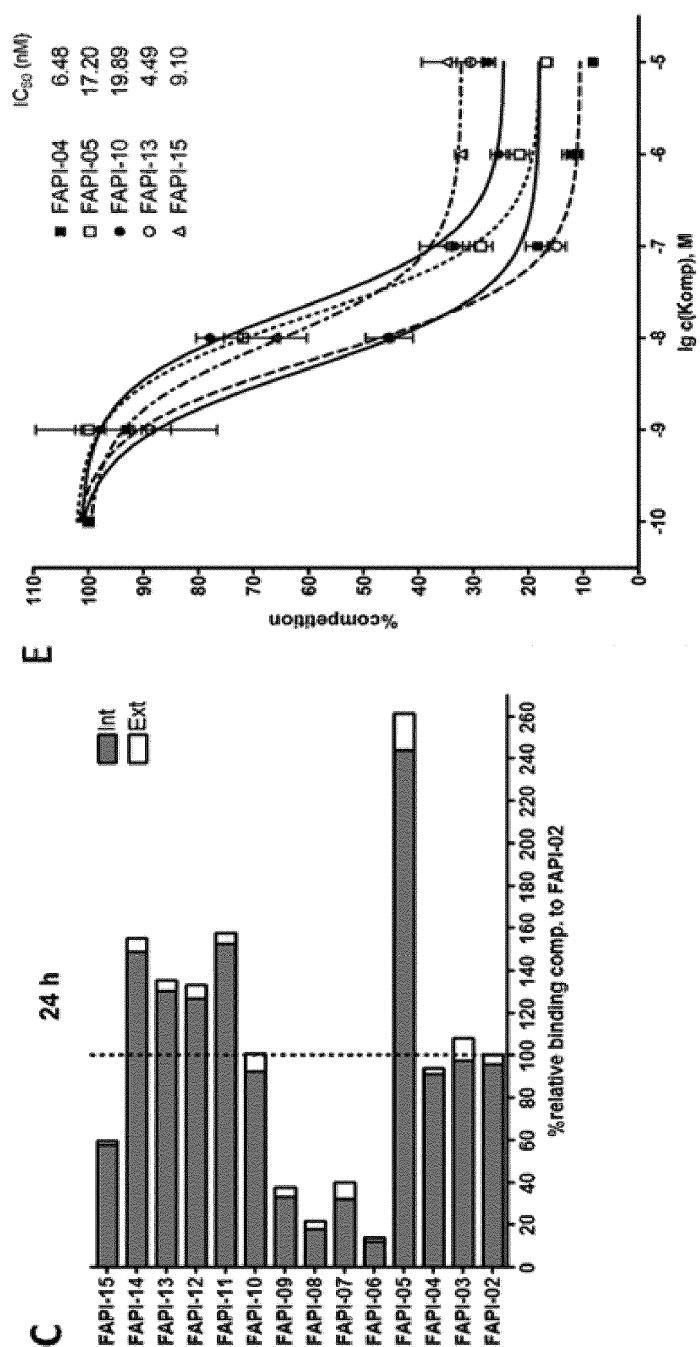
SUBSTITUTE SHEET (RULE 26)⁷

Figure 2



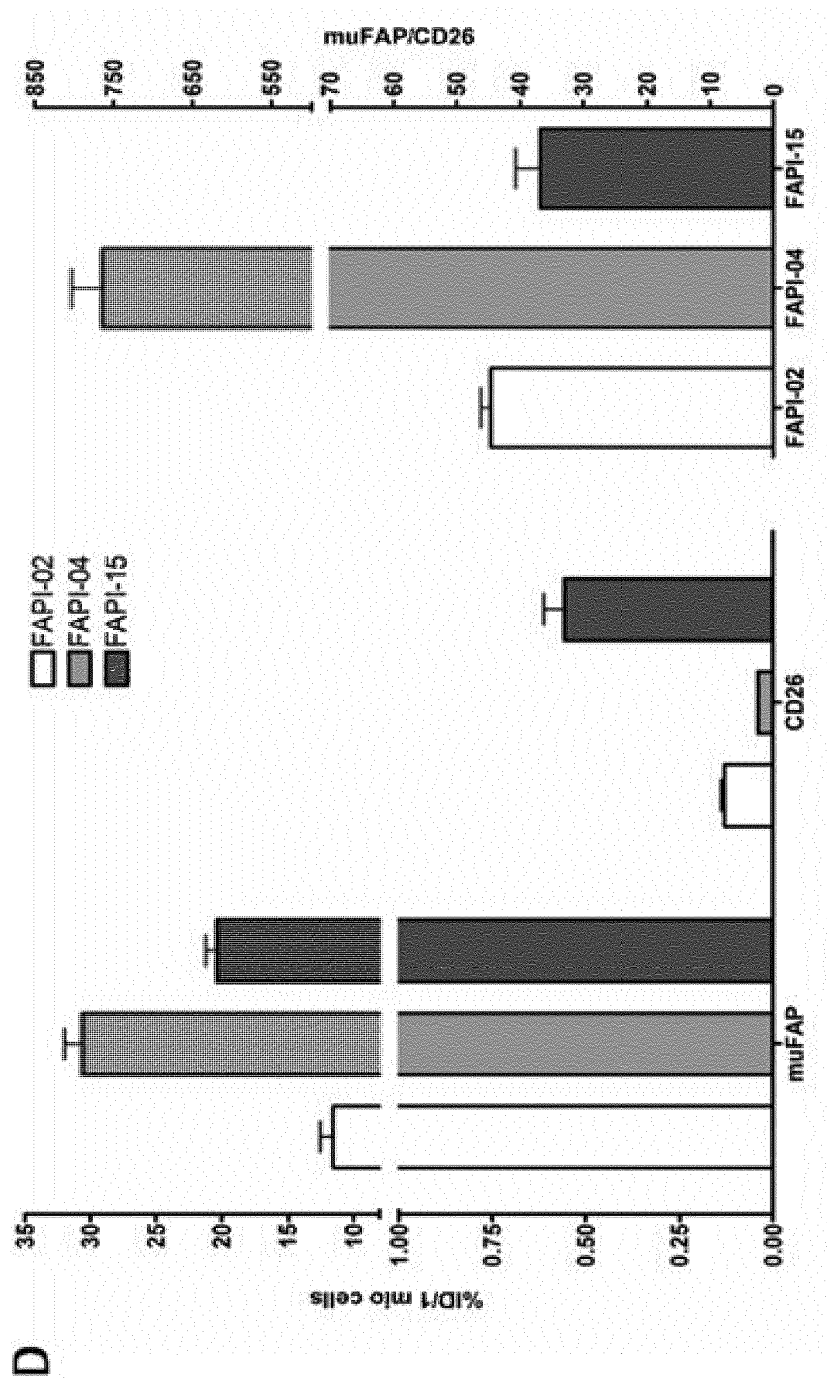
SUBSTITUTE SHEET (RULE 26)

Figure 2 continued



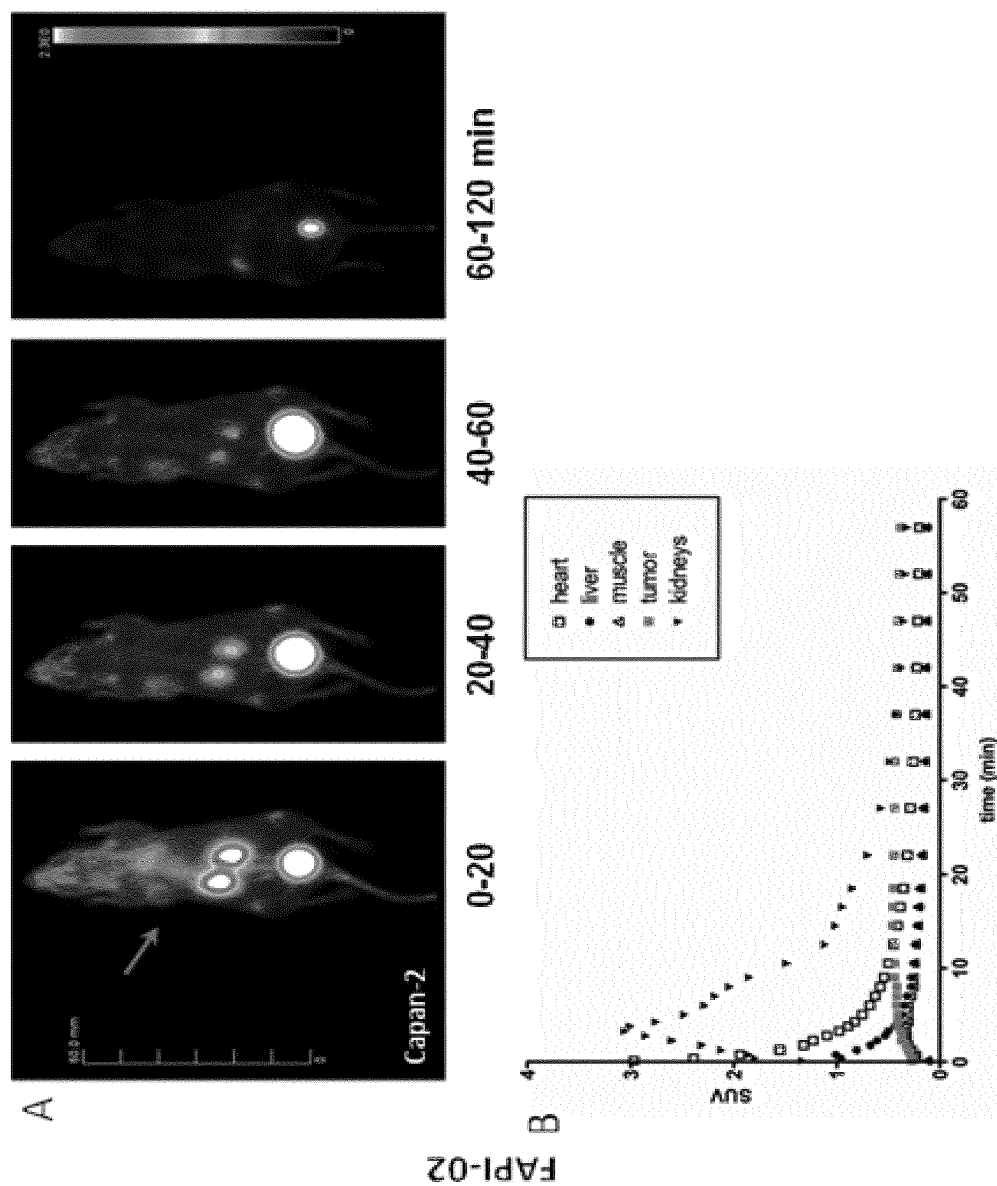
SUBSTITUTE SHEET (RULE 26)

Figure 2 continued



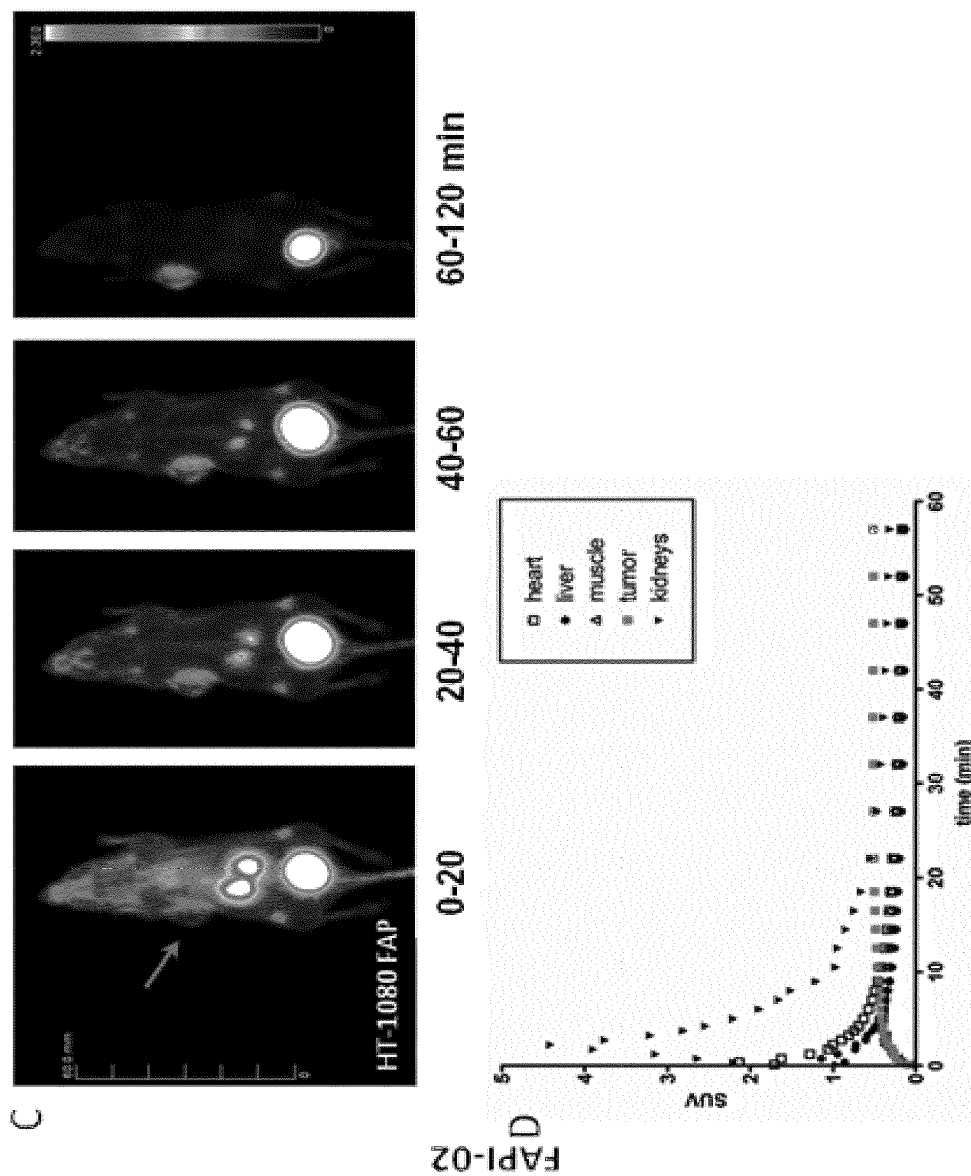
SUBSTITUTE SHEET (RULE 26)

Figure 3



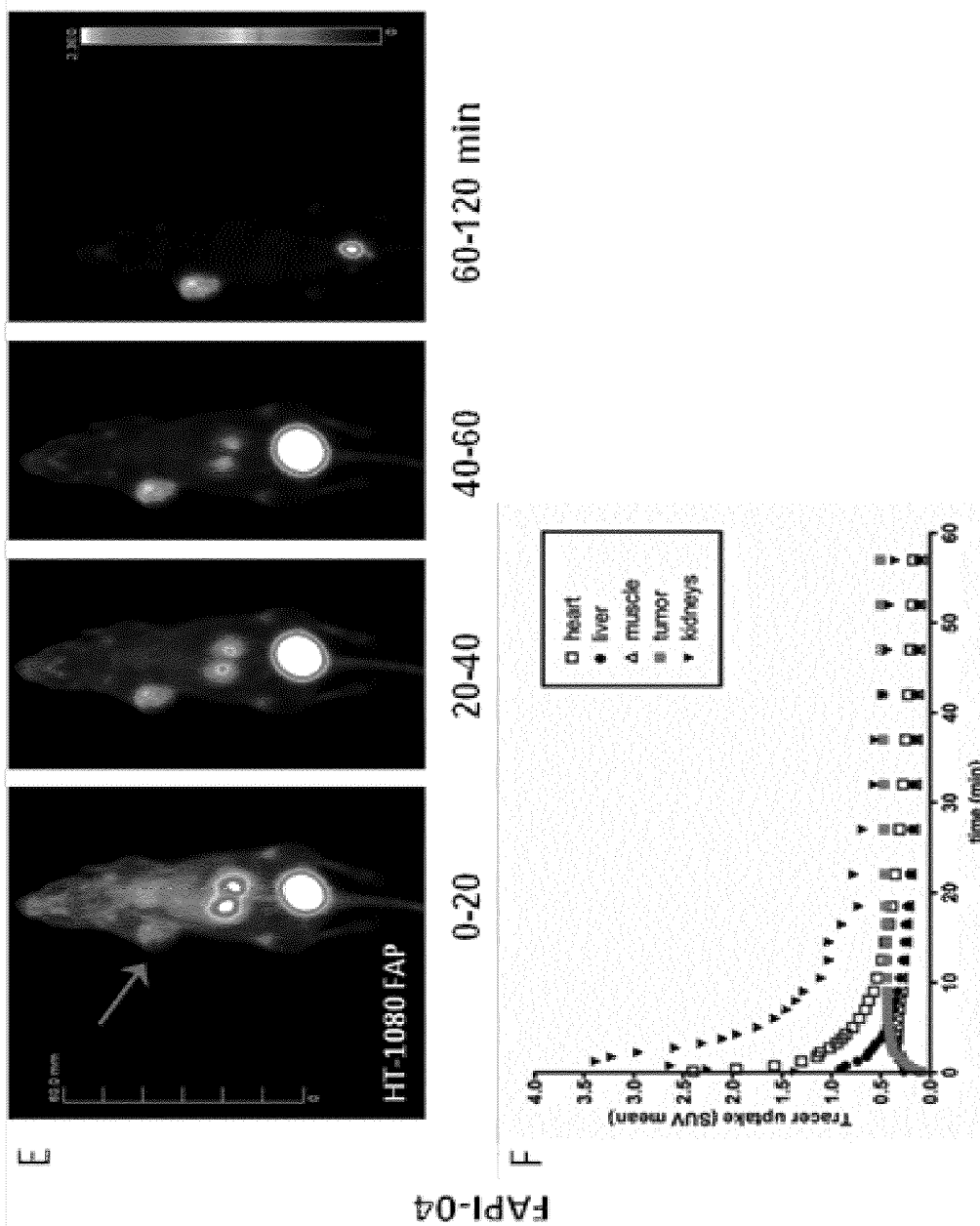
SUBSTITUTE SHEET (RULE 26)

Figure 3 continued



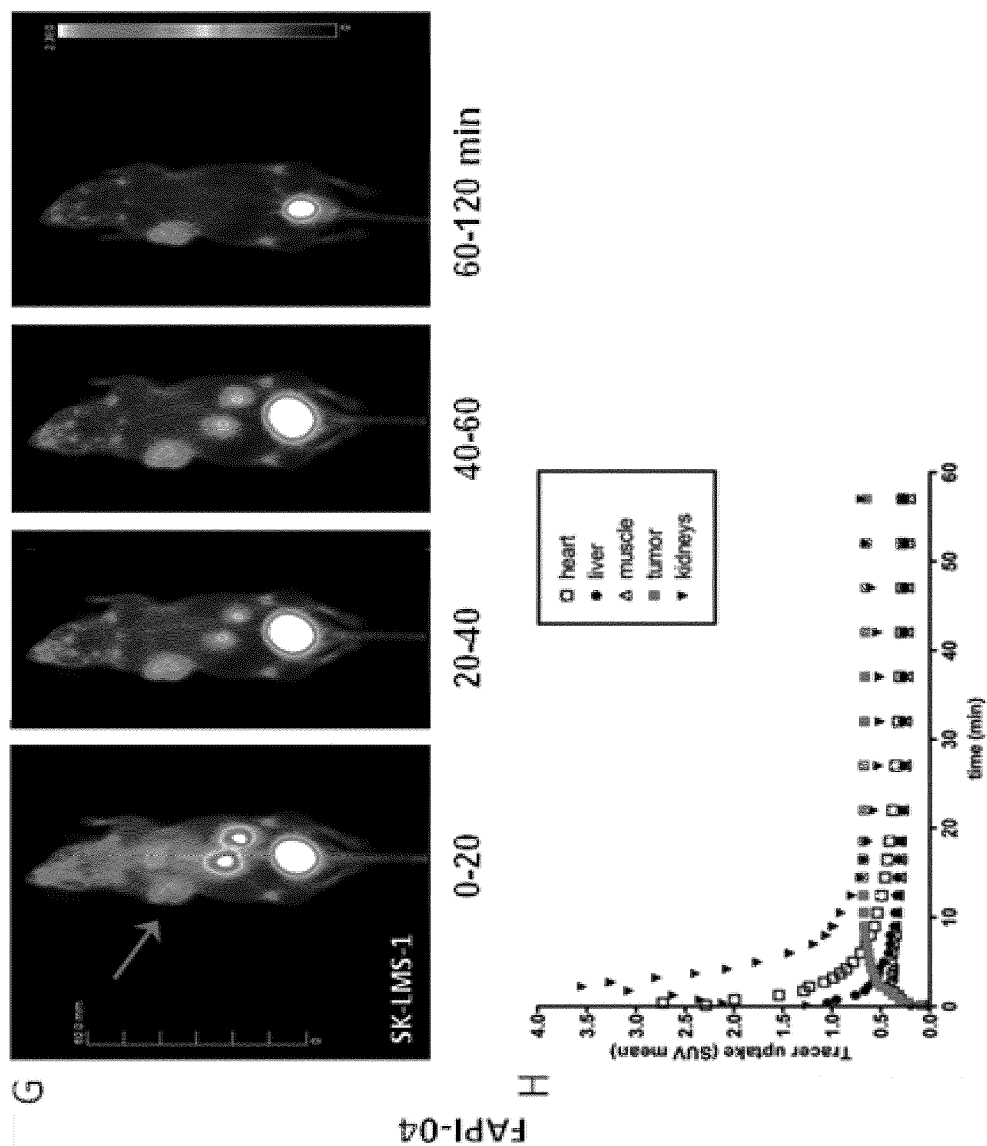
SUBSTITUTE SHEET (RULE 26)

Figure 3 continued



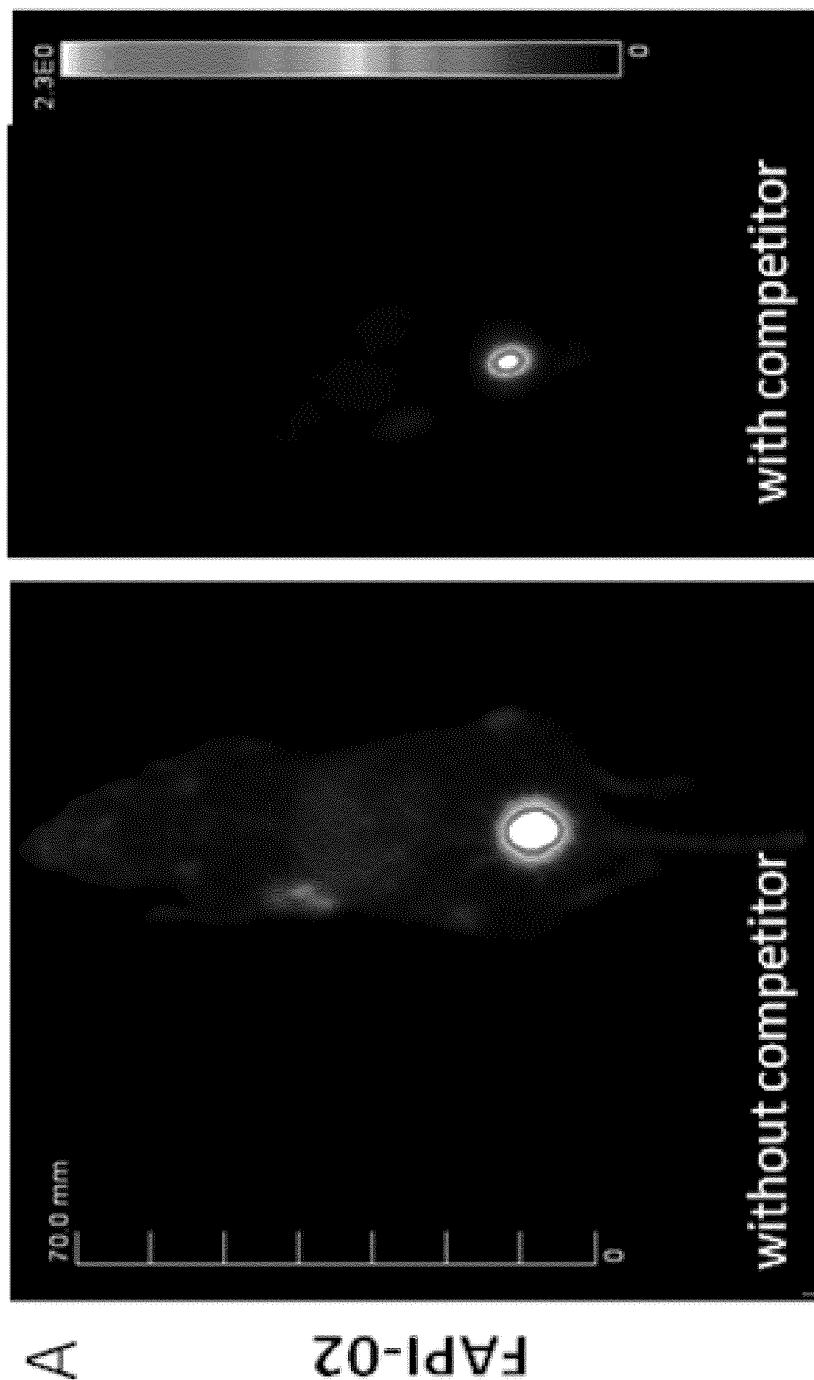
SUBSTITUTE SHEET (RULE 26)

Figure 3 continued



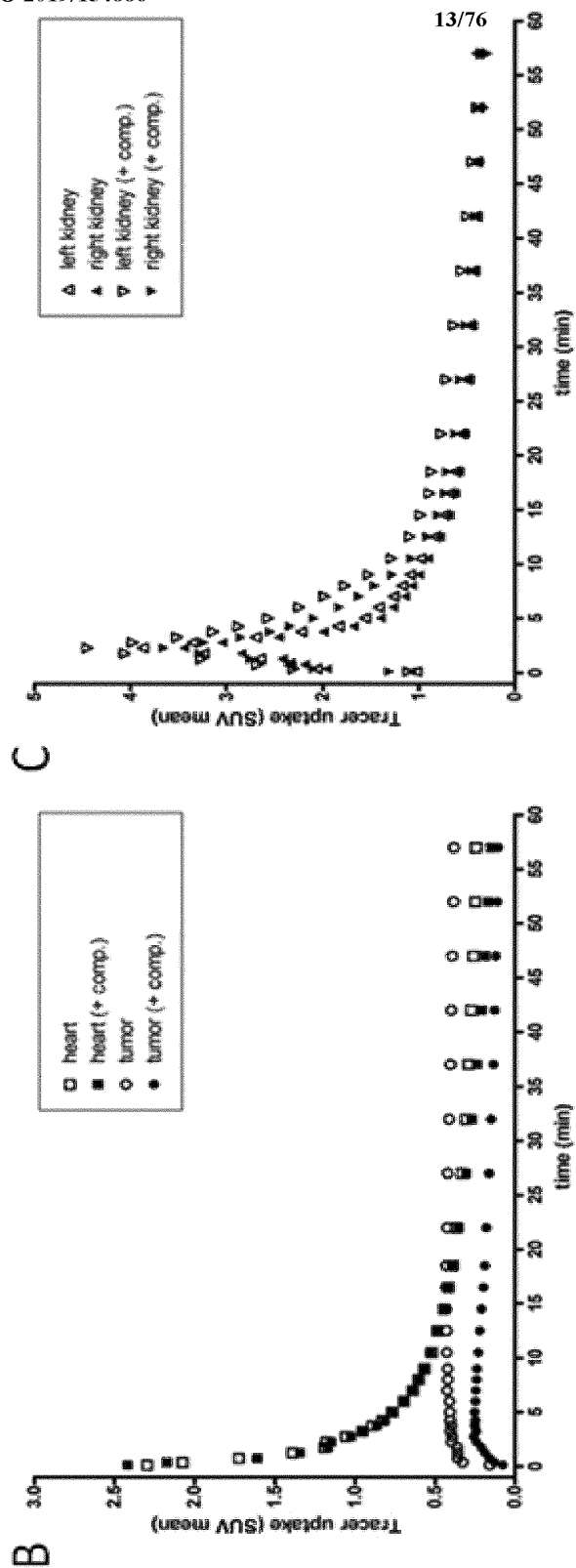
SUBSTITUTE SHEET (RULE 26)

Figure 4



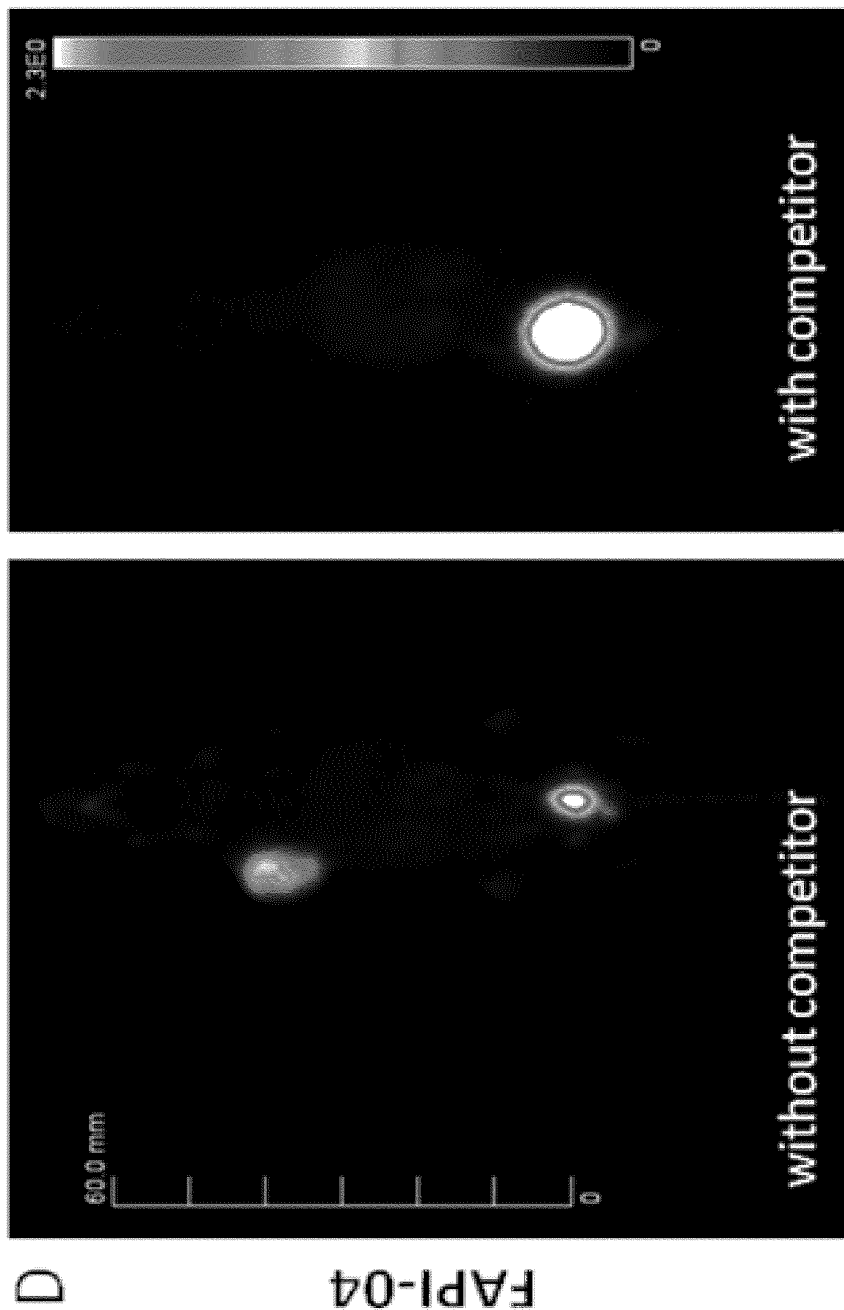
SUBSTITUTE SHEET (RULE 26)

Figure 4 continued



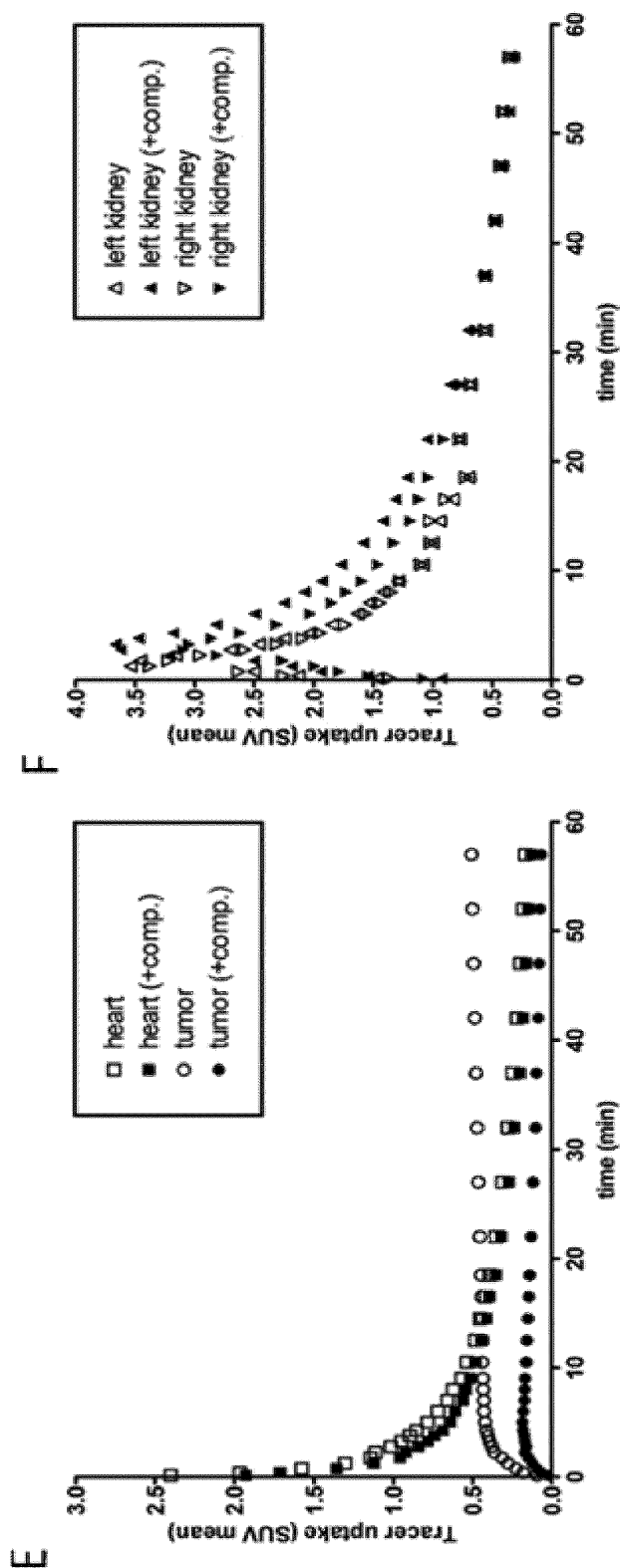
SUBSTITUTE SHEET (RULE 26)

Figure 4 continued



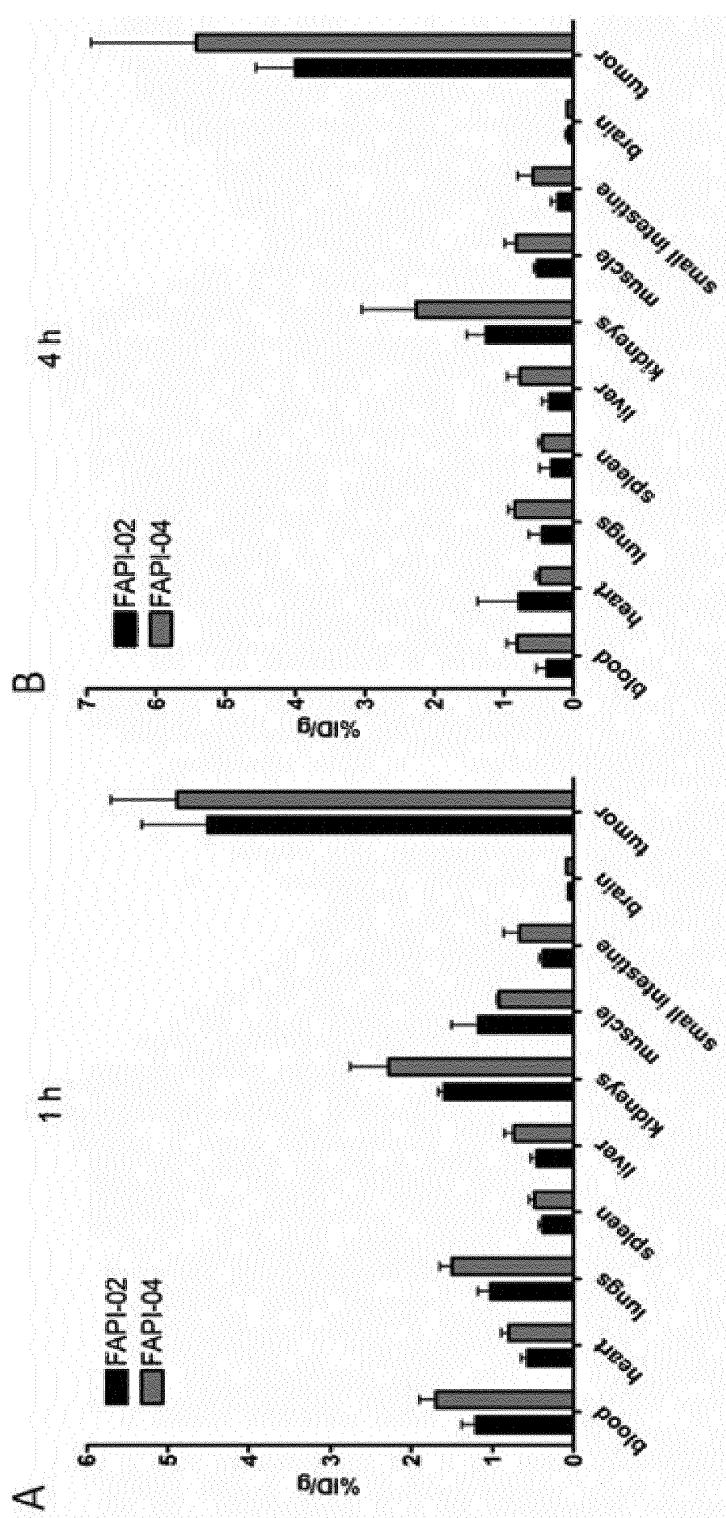
SUBSTITUTE SHEET (RULE 26)

Figure 4 continued



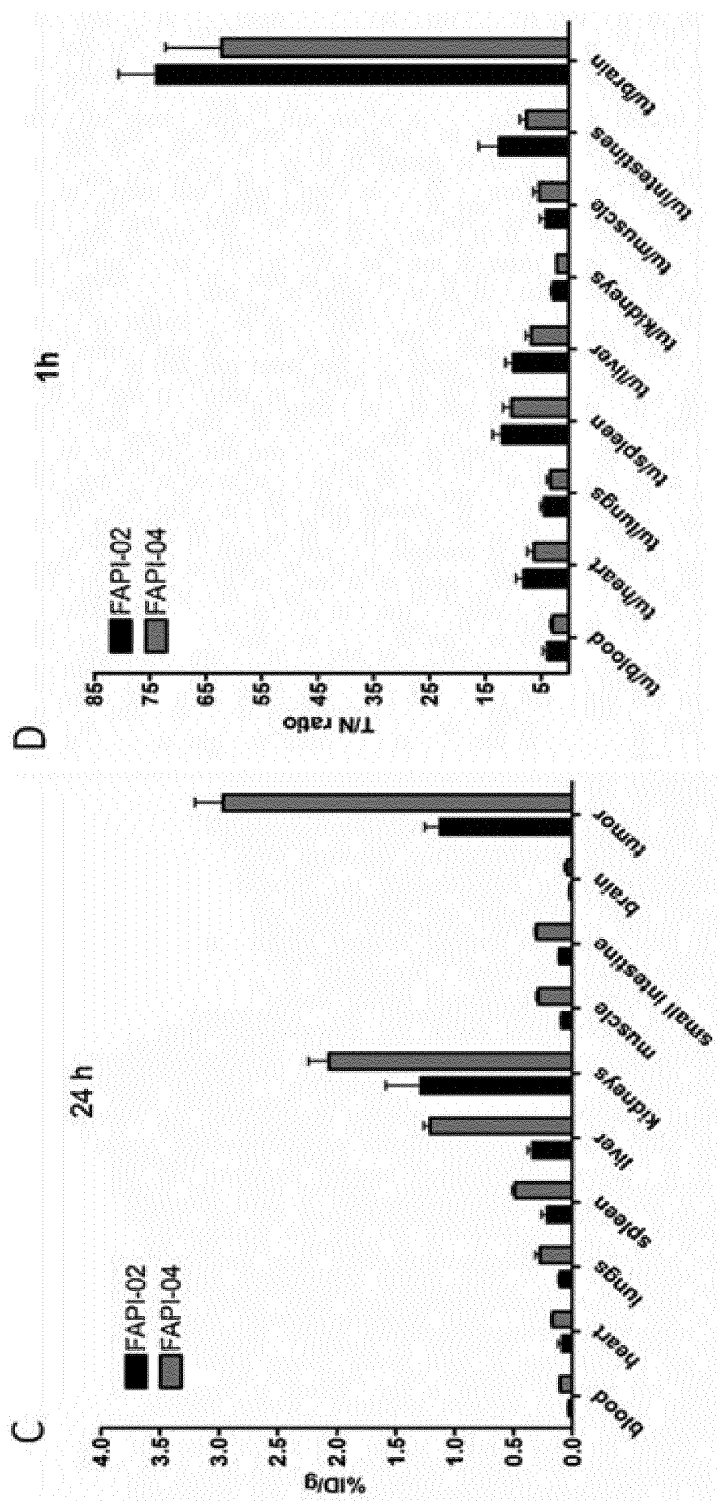
SUBSTITUTE SHEET (RULE 26)

Figure 5



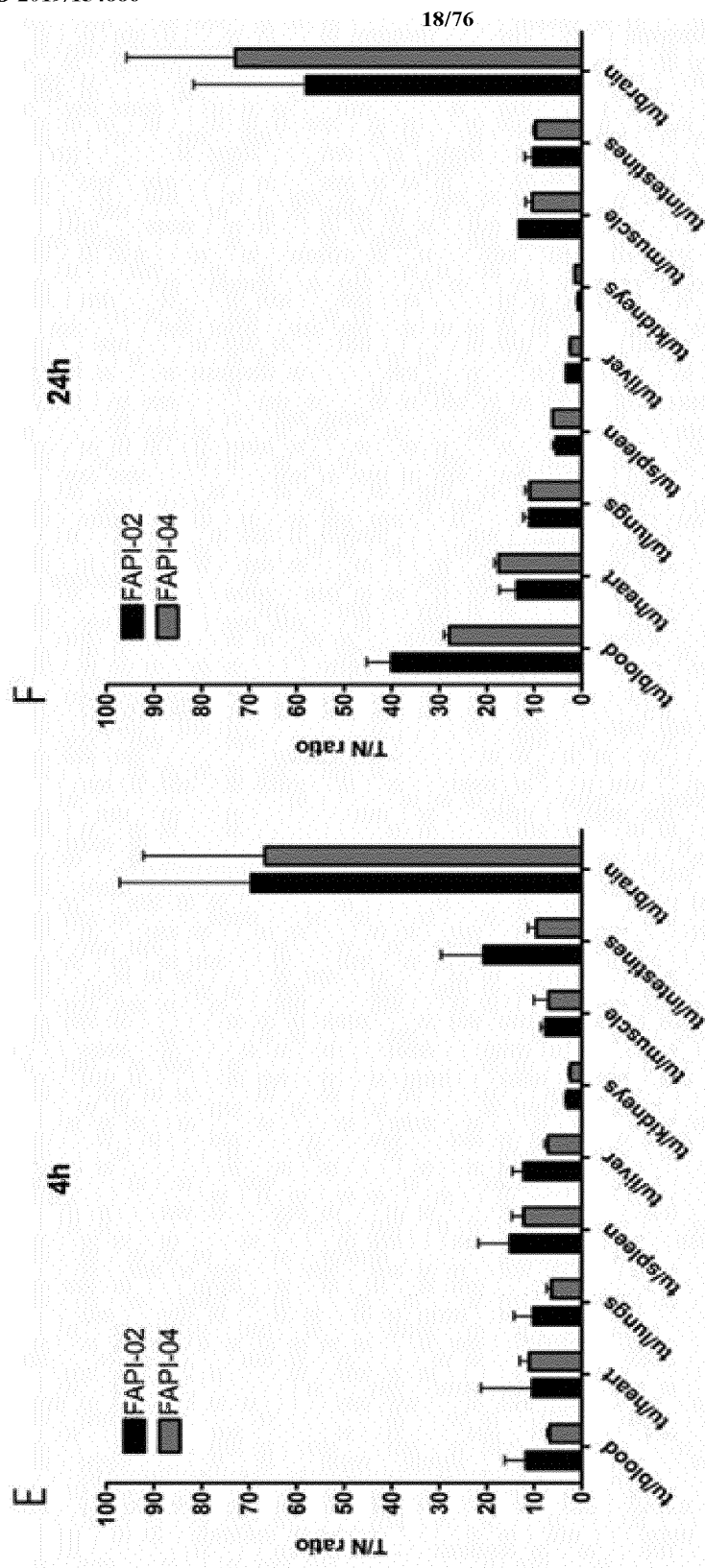
SUBSTITUTE SHEET (RULE 26)

Figure 5 continued



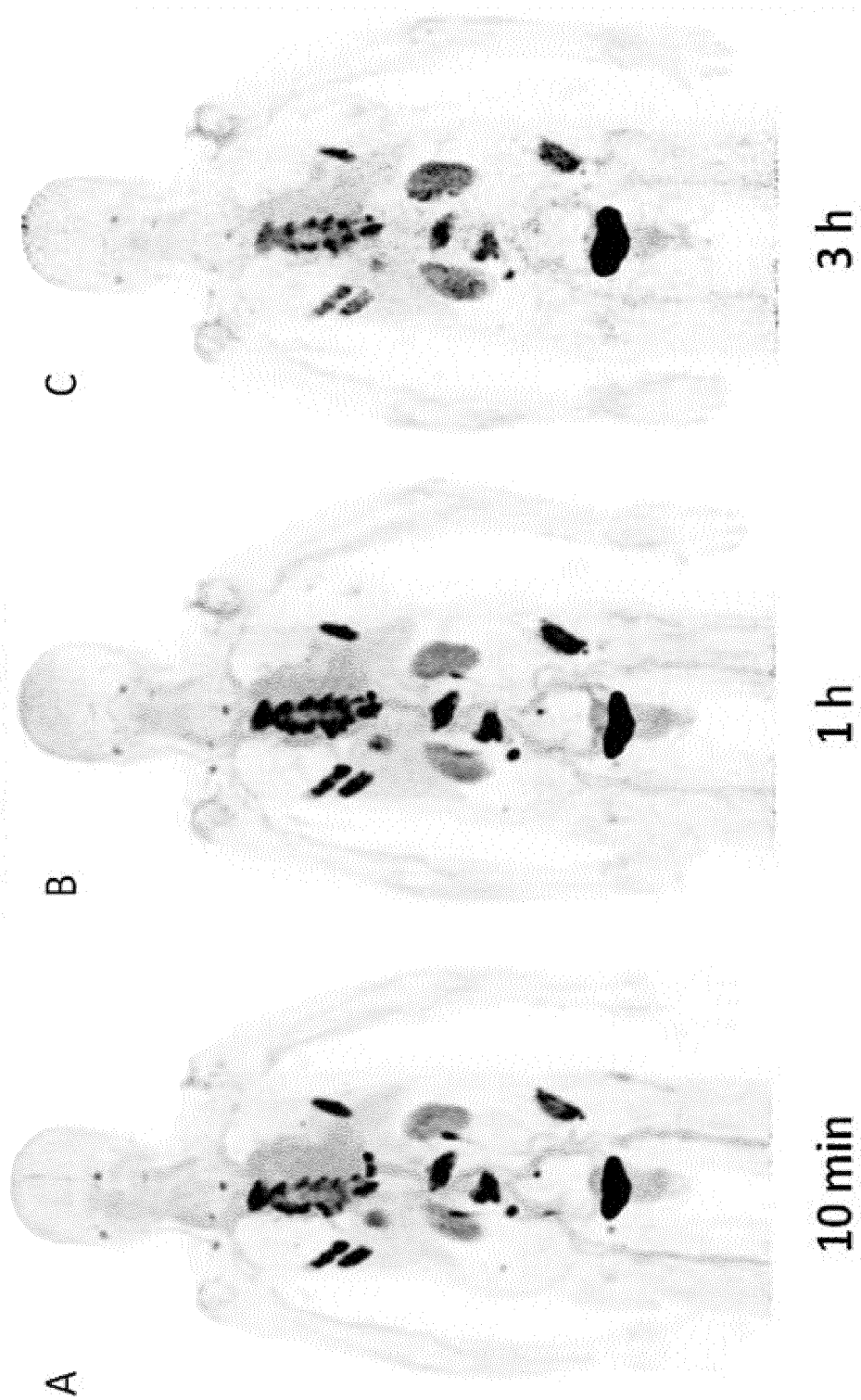
SUBSTITUTE SHEET (RULE 26)

Figure 5 continued



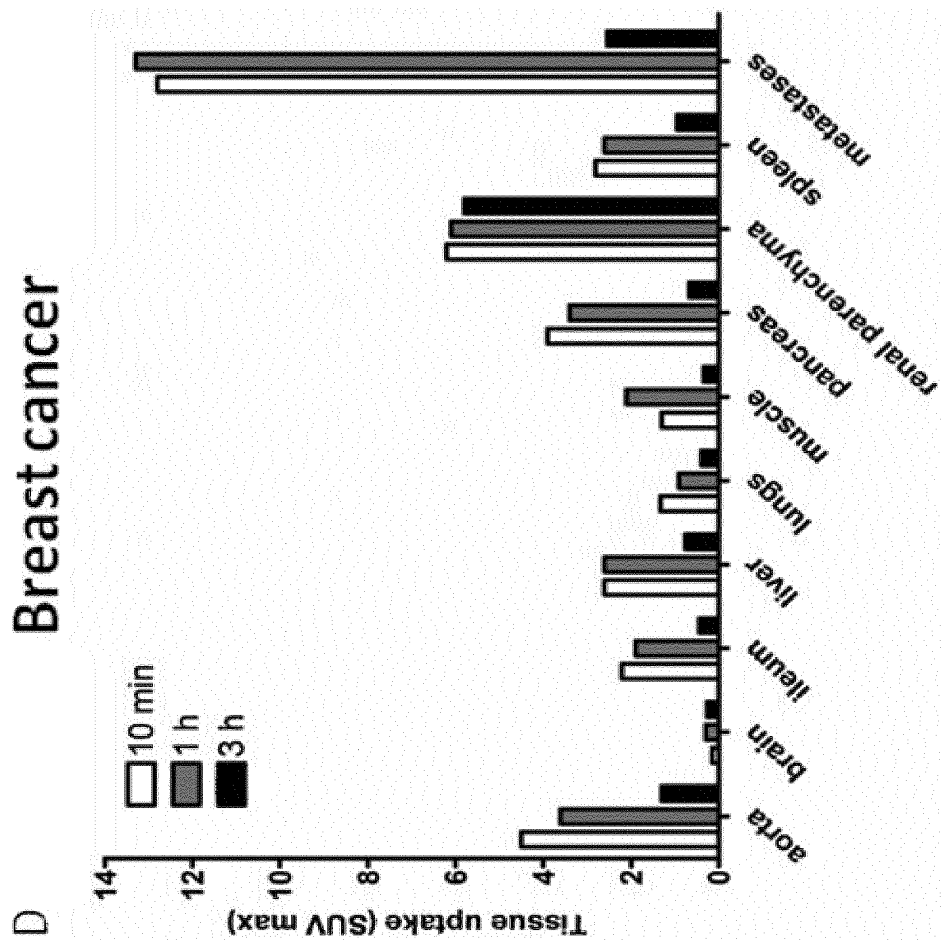
SUBSTITUTE SHEET (RULE 26)

Figure 6
Breast cancer



SUBSTITUTE SHEET (RULE 26)

Figure 6 continued



SUBSTITUTE SHEET (RULE 26)

Figure 7

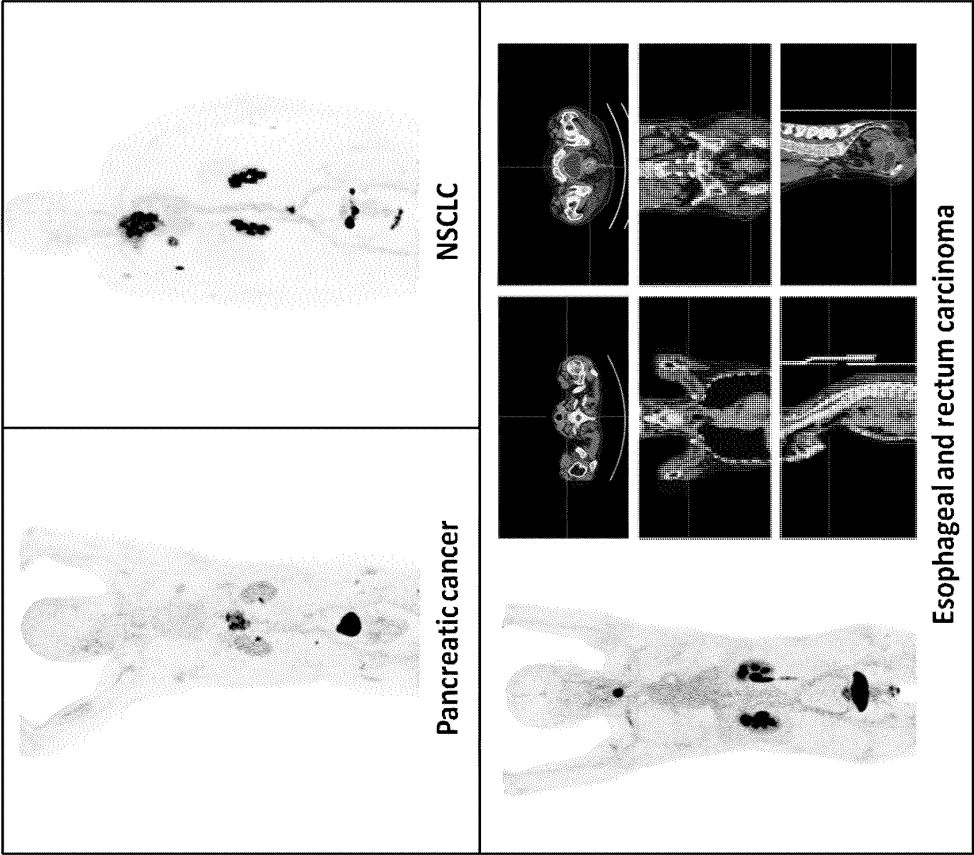
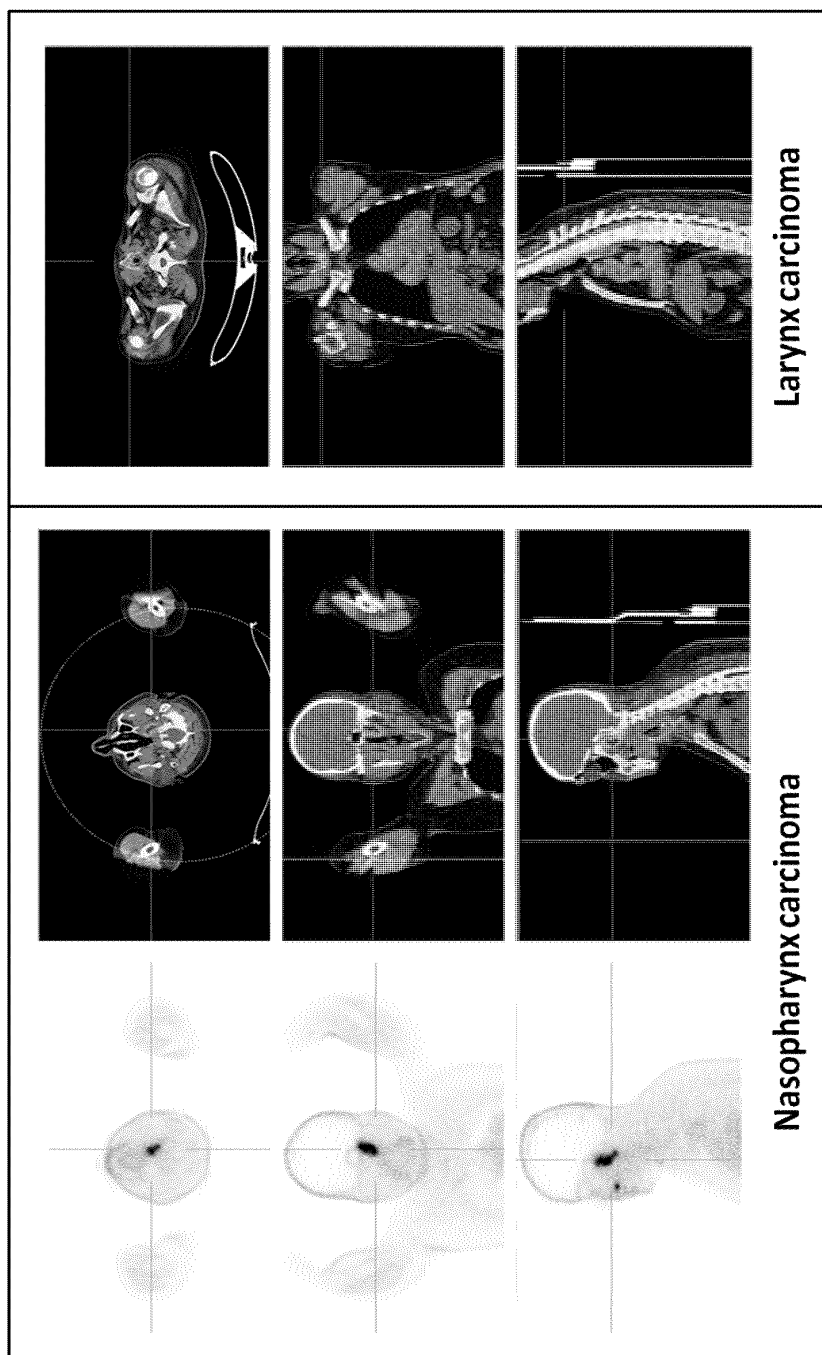


Figure 8



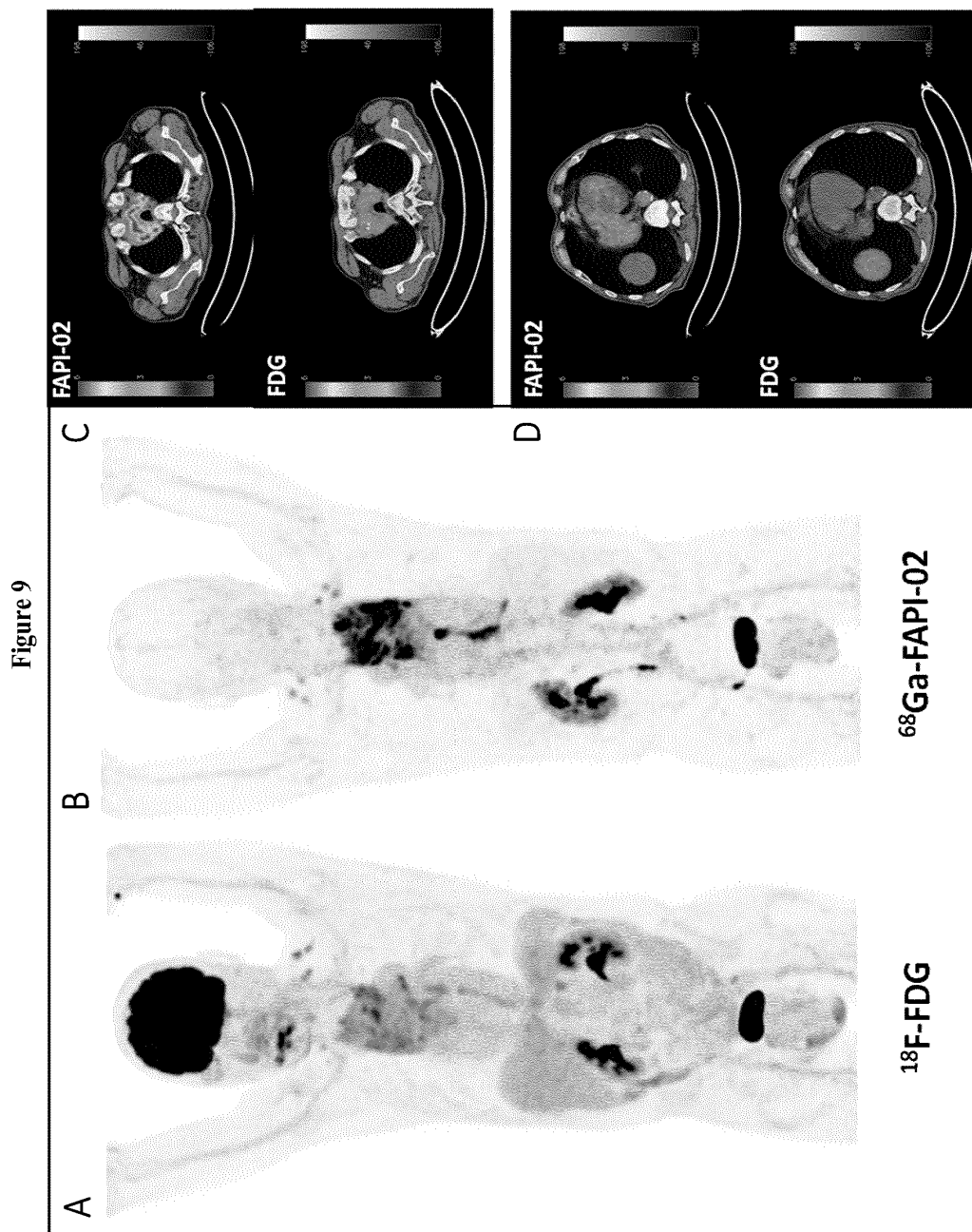


Figure 10

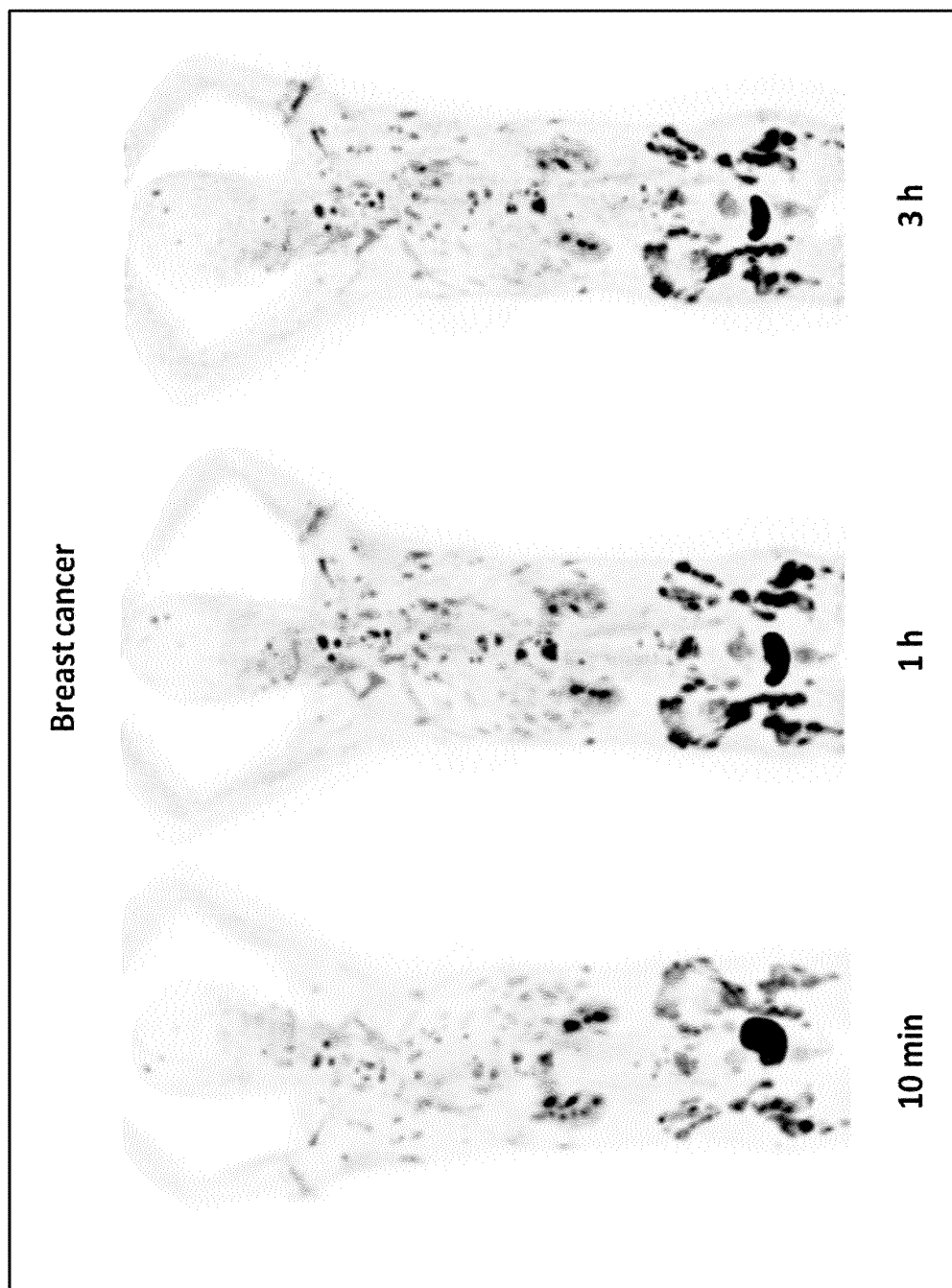


Figure 11

