US010398791B2

(12) **United States Patent**
Eder et al.

(10) **Patent No.: US 10,398,791 B2**
(45) **Date of Patent: Sep. 3, 2019**

(54) **LABELED INHIBITORS OF PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA), THEIR USE AS IMAGING AGENTS AND PHARMACEUTICAL AGENTS FOR THE TREATMENT OF PROSTATE CANCER**

(71) Applicants: **DEUTSCHES KREBSFORSCHUNGSZENTRUM**, Heidelberg (DE); **RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG**, Heidelberg (DE)

(72) Inventors: **Matthias Eder**, Mannheim (DE); **Klaus Kopka**, Dossenheim (DE); **Martin Schäfer**, Neckarsteinach (DE); **Ulrike Bauder-Wüst**, Schriesheim (DE); **Uwe Haberkorn**, Schwetzingen (DE); **Michael Eisenhut**, Heidelberg (DE); **Walter Mier**, Bensheim (DE); **Martina Benesova**, Heidelberg (DE)

(73) Assignees: **DEUTSCHES KREBSFORSCHUNGSZENTRUM**, Heidelberg (DE); **RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG**, Heidelberg (DE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **16/114,988**

(22) Filed: **Aug. 28, 2018**

(65) **Prior Publication Data**

US 2019/0060491 A1 Feb. 28, 2019

Related U.S. Application Data

(63) Continuation of application No. 15/131,118, filed on Apr. 18, 2016, now abandoned, which is a continuation-in-part of application No. PCT/EP2014/002808, filed on Oct. 17, 2014.

(30) **Foreign Application Priority Data**

Oct. 18, 2013 (EP) 13004991
Jul. 3, 2014 (EP) 14175612

(51) **Int. Cl.**

A61K 51/04	(2006.01)
C07B 59/00	(2006.01)
C07D 295/145	(2006.01)
C07D 257/02	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 51/0482*** (2013.01); ***C07B 59/002*** (2013.01); ***C07D 257/02*** (2013.01); ***C07D 295/145*** (2013.01)

(58) **Field of Classification Search**

CPC A61K 51/04; C07B 59/00; C07D 257/02

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,691,024	A	9/1987	Kunikatsu et al.
4,713,249	A	12/1987	Schröder
5,103,018	A	4/1992	Motomichi et al.
5,266,333	A	11/1993	Cady et al.
5,418,982	A	5/1995	Kishi
5,627,165	A	5/1997	Glazier
5,795,877	A	8/1998	Jackson et al.
5,863,536	A	1/1999	Jackson et al.
5,866,679	A	2/1999	DeFeo-Jones et al.
5,902,817	A	5/1999	Jackson et al.
5,948,750	A	9/1999	Garsky et al.
5,962,237	A	10/1999	Ts'o et al.
5,962,521	A	10/1999	Jackson et al.
5,968,915	A	10/1999	Jackson et al.
5,998,362	A	12/1999	Feng et al.
6,054,444	A	4/2000	Jackson et al.
6,068,830	A	5/2000	Diamandis et al.
6,127,333	A	10/2000	Brady et al.
6,174,858	B1	1/2001	Brady et al.
6,177,404	B1	1/2001	DeFeo-Jones et al.
6,232,287	B1	5/2001	Ruoslahti et al.
6,265,540	B1	7/2001	Isaacs et al.
6,342,491	B1	1/2002	Dey et al.
6,355,611	B1	3/2002	Karki et al.
6,368,598	B1	4/2002	D'Amico et al.
6,391,305	B1	5/2002	Feng et al.
6,428,785	B1	8/2002	Gocken
6,479,470	B1	11/2002	Kozikowski et al.
6,504,014	B1	1/2003	Isaacs et al.
6,511,676	B1	1/2003	Boulikas
6,518,033	B1	2/2003	Gromeier et al.
6,528,499	B1	3/2003	Kozikowski et al.
6,548,260	B1	4/2003	Tewari
6,596,755	B2	7/2003	Burman et al.
6,602,274	B1	8/2003	Chen
6,613,793	B2	9/2003	Burman et al.
6,692,724	B1	2/2004	Yang et al.
6,875,886	B2	4/2005	Frangioni
6,946,133	B1	9/2005	Schlom et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2606138	10/2005
CN	101863924	10/2010

(Continued)

OTHER PUBLICATIONS

Office Action for copending Eurasian Patent Application No. 201690495/28, dated Jul. 14, 2017.

(Continued)

Primary Examiner — Michael G. Hartley
Assistant Examiner — Jagadishwar R Samala
(74) *Attorney, Agent, or Firm* — The Marbury Law Group, PLLC

(57) **ABSTRACT**

The present invention generally relates to the field of radiopharmaceuticals and their use in nuclear medicine as tracers, imaging agents and for the treatment of various disease states of prostate cancer. Thus, the present invention concerns compounds that are represented by the general Formulae (Ia) or (Ib).

30 Claims, 20 Drawing Sheets

Patent Owner Exhibit 2079
IPR2025-01582

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,005,429	B2	2/2006	Dey et al.
7,008,765	B1	3/2006	Bussemakers et al.
7,041,786	B2	5/2006	Shailubhai et al.
7,045,605	B2	5/2006	Bander et al.
7,052,703	B1	5/2006	Pastan et al.
7,060,284	B1	6/2006	Kaumaya
7,128,893	B2	10/2006	Leamon et al.
7,129,254	B2	10/2006	Berger et al.
7,147,837	B2	12/2006	Lauffer et al.
7,153,841	B2	12/2006	Roncucci et al.
7,160,885	B2	1/2007	Currie et al.
7,166,691	B2	1/2007	Koochekpour et al.
7,192,586	B2	3/2007	Bander
7,232,805	B2	6/2007	Weinshenker et al.
7,238,785	B2	7/2007	Govindan et al.
7,282,567	B2	10/2007	Goldenberg et al.
7,361,338	B2	4/2008	Jakobovits et al.
7,381,745	B2	6/2008	Kozikowski et al.
7,399,460	B2	7/2008	Wedeking et al.
7,408,079	B2	8/2008	Pomper et al.
7,468,354	B2	12/2008	Isaacs
7,485,299	B2	2/2009	Afar et al.
7,514,078	B2	4/2009	Bander et al.
7,534,580	B2	5/2009	Reeves et al.
7,547,773	B2	6/2009	Schlom et al.
7,585,491	B2	9/2009	Govindan
7,601,332	B2	10/2009	Vlahov et al.
7,635,682	B2	12/2009	Denmeade et al.
7,638,122	B2	12/2009	Yu et al.
7,638,525	B2	12/2009	Jiang et al.
7,659,395	B2	2/2010	Pajouhesh et al.
7,662,795	B2	2/2010	Rodriquez et al.
7,691,962	B2	4/2010	Boyd et al.
7,696,185	B2	4/2010	Berkman
7,713,944	B2	5/2010	Kinberger et al.
7,740,847	B2	6/2010	Allan et al.
7,767,202	B2	8/2010	Pardoll et al.
7,767,803	B2	8/2010	Diener et al.
7,794,929	B2	9/2010	Baylin et al.
7,862,798	B2	1/2011	Leamon et al.
7,872,235	B2	1/2011	Rousso et al.
7,875,586	B2	1/2011	Kovbasnjuk et al.
7,879,981	B2	2/2011	Obata
7,910,594	B2	3/2011	Vlahov et al.
7,990,533	B2	8/2011	Maier et al.
8,000,773	B2	8/2011	Rousso et al.
8,013,991	B2	9/2011	Maier et al.
8,088,387	B2	1/2012	Steeves et al.
8,101,369	B2	1/2012	Nam et al.
8,101,713	B2	1/2012	Cuello et al.
8,105,568	B2	1/2012	Vlahov et al.
8,211,402	B2	3/2012	Babich et al.
8,153,595	B2	4/2012	Chen
8,194,660	B2	6/2012	Birze et al.
8,211,401	B2	7/2012	Babich et al.
8,211,473	B2	7/2012	Troiano et al.
8,211,635	B2	7/2012	Barton
8,227,634	B2	7/2012	Pomper et al.
8,236,330	B2	8/2012	Zale et al.
8,246,968	B2	8/2012	Zale et al.
8,258,111	B2	9/2012	Shen et al.
8,273,363	B2	9/2012	Zale et al.
8,313,728	B2	11/2012	Leamon et al.
8,388,977	B2	3/2013	Low et al.
8,394,922	B2	3/2013	Cheng et al.
8,404,817	B2	3/2013	Sherman et al.
8,414,864	B2	4/2013	Cappelletti et al.
8,414,898	B2	4/2013	Afar et al.
8,445,851	B2	5/2013	Rousso et al.
8,450,290	B2	5/2013	Worm et al.
8,465,725	B2	6/2013	Babich et al.
8,487,128	B2	7/2013	Babich et al.
8,487,129	B2	7/2013	Babich et al.
8,491,896	B2	7/2013	Goldenberg et al.
8,507,434	B2	8/2013	Popel et al.
8,557,772	B2	10/2013	Popel et al.
8,565,945	B2	10/2013	Babich et al.
8,603,499	B2	12/2013	Zale et al.
8,603,500	B2	12/2013	Zale et al.
8,603,501	B2	12/2013	Zale et al.
8,606,349	B2	12/2013	Rousso et al.
8,644,910	B2	2/2014	Rousso et al.
8,685,416	B2	4/2014	Klinman et al.
8,685,891	B2	4/2014	Muraca
8,703,918	B2	4/2014	Colombatti et al.
8,709,483	B2	4/2014	Farokhzad et al.
8,772,226	B2	6/2014	Denmeade et al.
8,772,459	B2	6/2014	Ho et al.
8,778,305	B2	7/2014	Pomper et al.
8,802,153	B2	8/2014	Cheng et al.
8,816,095	B2	8/2014	Brown et al.
8,834,842	B2	9/2014	Leamon et al.
8,840,865	B2	9/2014	Babich et al.
8,852,630	B2	10/2014	Spiegel et al.
8,858,509	B2	10/2014	Spiegel et al.
8,865,126	B2	10/2014	Leamon et al.
8,877,970	B2	11/2014	Zimmerman et al.
8,901,294	B2	12/2014	Kim et al.
8,907,058	B2	12/2014	Low et al.
8,916,167	B2	12/2014	Low et al.
8,921,378	B2	12/2014	Toermakaengas et al.
8,926,944	B2 *	1/2015	Babich C07D 255/02 424/1.65
8,926,945	B2	1/2015	Port et al.
8,940,871	B2	1/2015	Wu et al.
8,946,388	B2	2/2015	Sahin et al.
8,962,799	B2	2/2015	Babich et al.
8,987,319	B2	3/2015	Miller
9,006,415	B2	4/2015	Ren et al.
9,029,340	B2	5/2015	Lupold et al.
9,044,468	B2	6/2015	Pomper et al.
9,056,841	B2	6/2015	Pomper et al.
9,074,000	B2	7/2015	Scheinberg et al.
9,120,837	B2	9/2015	Babich et al.
9,123,725	B2	9/2015	Cho et al.
9,180,203	B2	11/2015	Cui et al.
9,180,214	B1	11/2015	Miao
9,193,763	B2	11/2015	Low et al.
9,216,218	B2	12/2015	Sahin et al.
9,226,981	B2	1/2016	Pomper et al.
9,242,012	B2	1/2016	Ma et al.
9,255,262	B2	2/2016	Wong et al.
9,278,067	B2	3/2016	Boulikas
9,295,727	B2	3/2016	Zale et al.
9,309,193	B2	4/2016	Babich et al.
9,346,846	B1	5/2016	Herzon et al.
9,371,360	B2	6/2016	Pomper et al.
9,387,344	B2	7/2016	Sgouros et al.
9,422,234	B2	8/2016	Chandran et al.
9,429,575	B2	8/2016	Ban et al.
9,433,594	B2	9/2016	Babich et al.
9,447,121	B2	9/2016	Babich et al.
9,498,546	B2	11/2016	Pomper et al.
9,556,167	B2	1/2017	Spiegel et al.
9,567,402	B2	2/2017	Liu
9,580,467	B2	2/2017	Cheng et al.
9,580,474	B2	2/2017	Viscidi et al.
9,585,957	B2	3/2017	Powell et al.
9,617,602	B2	4/2017	Joseph et al.
9,636,413	B2	5/2017	Vlahov et al.
9,687,572	B2	6/2017	Babich et al.
9,717,484	B2	8/2017	Kalloo et al.
9,745,380	B2	8/2017	Govindan et al.
9,757,084	B2	9/2017	Sgouros et al.
9,764,039	B2	9/2017	Thanos et al.
9,770,467	B2	9/2017	Dubensky, Jr. et al.
9,770,517	B2	9/2017	Govindan et al.
9,808,516	B2	11/2017	Brockstedt et al.
9,814,759	B2	11/2017	Wong et al.
9,861,444	B2	1/2018	Kalloo et al.
9,889,199	B2	2/2018	Basilion
9,932,411	B2	4/2018	Terrett et al.
9,951,049	B2	4/2018	Kamal et al.
9,951,324	B2	4/2018	Low et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

9,956,305 B2 5/2018 Babich et al.
9,988,407 B2 6/2018 Slusher et al.
10,010,624 B2 7/2018 Howard et al.
10,011,632 B2 7/2018 Wang et al.
10,016,519 B2 7/2018 Kopka et al.
10,029,023 B2 7/2018 Pomper et al.
10,064,957 B2 9/2018 Govindan et al.
2003/0049203 A1 3/2003 Elmaleh et al.
2003/0220241 A1 11/2003 DeFeo-Jones et al.
2003/0232760 A1 12/2003 Garsky et al.
2004/0001846 A1 1/2004 Israeli et al.
2004/0002478 A1 1/2004 Kozikowski et al.
2004/0002587 A1 1/2004 Watkins et al.
2004/0018203 A1 1/2004 Pastan et al.
2004/0029778 A1 2/2004 Isaacs
2004/0033195 A1 2/2004 Leamon et al.
2004/0052727 A1 3/2004 Dalton et al.
2004/0054190 A1 3/2004 Pomper et al.
2004/0058857 A1 3/2004 Yao
2004/0110723 A1 6/2004 Frangioni
2004/0146516 A1 7/2004 Roben et al.
2004/0213791 A1 10/2004 Bander et al.
2004/0229845 A1 11/2004 Frangioni
2004/0242582 A1 12/2004 Green et al.
2005/0002942 A1 1/2005 Vlahov et al.
2005/0069889 A1 3/2005 Nihei et al.
2005/0107325 A1 5/2005 Manoharan et al.
2005/0119166 A1 6/2005 Brady et al.
2005/0158780 A1 7/2005 Lupold et al.
2005/0234247 A1 10/2005 Klar et al.
2005/0239138 A1 10/2005 Hess et al.
2005/0239739 A1 10/2005 Matulic-Adamic
2005/0245486 A1 11/2005 Frangioni
2005/0255042 A1 11/2005 Lam et al.
2006/0024317 A1 2/2006 Boyd et al.
2006/0045883 A1 3/2006 Molldrem et al.
2006/0051380 A1 3/2006 Schulick et al.
2006/0052312 A1 3/2006 Erhardt et al.
2006/0062793 A1 3/2006 Webb et al.
2006/0105975 A1 5/2006 Pendergrast et al.
2006/0106047 A1 5/2006 Jiang et al.
2006/0140871 A1 6/2006 Sillerud
2006/0148718 A1 7/2006 Brady et al.
2006/0148741 A1 7/2006 Barrett et al.
2006/0155021 A1 7/2006 Lenges et al.
2006/0155146 A1 7/2006 Lenges et al.
2007/0010014 A1 1/2007 Wood et al.
2007/0020327 A1 1/2007 Fikes et al.
2007/0031326 A1 2/2007 Shirvan et al.
2007/0031438 A1 2/2007 Junghans
2007/0117153 A1 5/2007 Bieniarz et al.
2007/0128670 A1 6/2007 Klatzmann et al.
2007/0134332 A1 6/2007 Turnell et al.
2007/0141052 A1 6/2007 Watkins et al.
2007/0142296 A1 6/2007 McBride et al.
2007/0148662 A1 6/2007 Israeli et al.
2007/0160617 A1 7/2007 Ma et al.
2007/0172422 A1 7/2007 Glazier
2007/0179100 A1 8/2007 Manoharan
2007/0190029 A1 8/2007 Pardoll et al.
2007/0212337 A1 9/2007 Bedi et al.
2007/0219165 A1 9/2007 Berkman et al.
2007/0225213 A1 9/2007 Kosak
2007/0244055 A1 10/2007 Brady et al.
2007/0254316 A1 11/2007 Rodriguez et al.
2007/0254317 A1 11/2007 Busseret-Michel et al.
2008/0008649 A1 1/2008 Cappelletti et al.
2008/0008719 A1 1/2008 Bowdish et al.
2008/0089869 A1 4/2008 Denmeade et al.
2008/0089892 A1 4/2008 Allan et al.
2008/0114153 A1 5/2008 Steeves et al.
2008/0175789 A1 7/2008 Frangioni
2008/0176821 A1 7/2008 Kozikowski et al.
2008/0193381 A1 8/2008 Babich et al.
2008/0214436 A1 9/2008 Yu et al.
2008/0248052 A1 10/2008 Vlahov et al.
2008/0269105 A1 10/2008 Taft et al.
2008/0311037 A1 12/2008 Heston et al.
2009/0117042 A1 5/2009 Pomper et al.
2009/0123467 A1 5/2009 Bedi et al.
2009/0180951 A1 7/2009 Zimmeran et al.
2009/0214636 A1 8/2009 Low et al.
2009/0247614 A1 10/2009 Manoharan et al.
2009/0258002 A1 10/2009 Barrett et al.
2009/0274625 A1 11/2009 Denmeade et al.
2010/0047170 A1 2/2010 Denmeade et al.
2010/0048490 A1 2/2010 Vlahov et al.
2010/0092496 A1 4/2010 Boyd et al.
2010/0178246 A1 7/2010 Babich et al.
2010/0183509 A1 7/2010 Babich et al.
2010/0183517 A1 7/2010 Berkman
2010/0209343 A1 8/2010 Bander et al.
2010/0240701 A1 9/2010 Vlahov et al.
2010/0260677 A1 10/2010 Bhatia et al.
2010/0324008 A1 12/2010 Low et al.
2011/0008253 A1 1/2011 Babich et al.
2011/0027180 A1 2/2011 Magnani
2011/0027274 A1 2/2011 Cheng et al.
2011/0064657 A1 3/2011 Pomper et al.
2011/0124948 A1 5/2011 Yokell
2011/0142760 A1 6/2011 Pomper et al.
2011/0172254 A1 7/2011 Leamon et al.
2011/0176998 A1 7/2011 Pomper et al.
2011/0200677 A1 8/2011 Chandran et al.
2011/0288152 A1 11/2011 Low et al.
2011/0305768 A1 12/2011 Mao et al.
2012/0009121 A1 1/2012 Pomper et al.
2012/0276162 A1 11/2012 Zale et al.
2012/0322741 A1 12/2012 Low et al.
2013/0034494 A1 2/2013 Babich et al.
2013/0302409 A1 11/2013 Fuchs et al.
2013/0336888 A1 12/2013 Babich et al.
2014/0107316 A1 4/2014 Vlahov et al.
2014/0113322 A1 4/2014 Cui et al.
2014/0140925 A1 5/2014 Leamon et al.
2014/0154702 A1 6/2014 Parker et al.
2014/0187501 A1 7/2014 Bilodeau et al.
2014/0314864 A1 10/2014 Cheng et al.
2015/0023875 A1 1/2015 Farokhzad et al.
2015/0079001 A1 3/2015 Pomper et al.
2015/0104387 A1 4/2015 Pomper et al.
2015/0110715 A1 4/2015 Eder et al.
2015/0110716 A1 4/2015 Armor
2015/0246144 A1 9/2015 Pomper et al.
2015/0297735 A1 10/2015 Vlahov et al.
2015/0315196 A1 11/2015 Howard
2015/0366968 A1 12/2015 Basilion et al.
2016/0067341 A1 3/2016 Low et al.
2016/0074526 A1 3/2016 Bilodeau et al.
2016/0114060 A1 4/2016 Pomper et al.
2016/0151508 A1 6/2016 Low et al.
2016/0220694 A1 8/2016 Vlahov et al.
2016/0235865 A1 8/2016 Pomper et al.
2016/0287731 A1 10/2016 Vlahov et al.
2016/0361376 A1 12/2016 Vlahov et al.
2016/0361432 A1 12/2016 Vlahov et al.
2016/0361433 A1 12/2016 Vlahov et al.
2017/0081298 A1 3/2017 Ray et al.
2017/0151356 A1 6/2017 Govindan et al.
2017/0218464 A1 8/2017 Pomper et al.
2017/0224709 A1 8/2017 Slusher et al.
2017/0266316 A1 9/2017 Govindan et al.
2017/0275673 A1 9/2017 Luo et al.
2017/0281789 A1 10/2017 Basilion et al.
2017/0281791 A1 10/2017 Govindan et al.
2017/0333576 A1 11/2017 Pomper et al.
2018/0008668 A1 1/2018 Isaacs et al.
2018/0051039 A1 2/2018 Pomper et al.
2018/0064831 A1 3/2018 Basilion et al.
2018/0111895 A1 4/2018 Babich et al.
2018/0118847 A1 5/2018 Bander
2018/0133296 A1 5/2018 Barrett et al.
2018/0148480 A1 5/2018 Isaacs et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2018/0185511	A1	7/2018	Woodworth et al.
2018/0207299	A1	7/2018	Babich et al.
2018/0258134	A1	9/2018	Wang et al.

FOREIGN PATENT DOCUMENTS

CN	101863924	A	10/2010
DE	202014008232		3/2015
EP	0116208		8/1984
EP	1177200		6/2005
EP	1472541		9/2009
EP	2187965		5/2010
EP	2318366		5/2011
EP	2136788		10/2011
EP	2373621	A1	10/2011
EP	2373622		10/2011
EP	2389361		11/2011
EP	2408755		1/2012
EP	2644192		10/2013
EP	2644594		10/2013
EP	2648766	A1	10/2013
EP	2436376		7/2014
EP	2759535		7/2014
EP	2240171		8/2014
EP	2170075		12/2014
EP	2823826		1/2015
EP	2097111		7/2015
EP	2921482		9/2015
EP	2938364	A1	11/2015
EP	2942065		11/2015
EP	2958596	A1	12/2015
EP	2993171	A1	3/2016
EP	2706057		4/2016
EP	3038996	A1	7/2016
JP	2002506204		2/2002
JP	2004536034		12/2004
JP	2005274569		10/2005
JP	2006501149		1/2006
JP	2006514961	A	5/2006
JP	2006518712		8/2006
JP	2007521803		8/2007
JP	2009519209	A	5/2009
JP	2010515732	A	5/2010
JP	2010518112	A	5/2010
JP	2010532754		10/2010
JP	2012511023		5/2012
WO	8801622		3/1988
WO	9107418		4/1991
WO	95/33766		12/1995
WO	9945374		9/1999
WO	2000/066091	A1	11/2000
WO	2000064911		11/2000
WO	2002043773		2/2002
WO	2002062398		8/2002
WO	2002098885		12/2002
WO	2003060523		7/2003
WO	2003092742		11/2003
WO	2003097647		11/2003
WO	2004010957		2/2004
WO	2004069159		8/2004
WO	2004069285	A1	8/2004
WO	2005082023		9/2005
WO	2006012527		2/2006
WO	2006093991		9/2006
WO	2006096754		9/2006
WO	2006136564		12/2006
WO	2007006041	A2	1/2007
WO	2007022494		2/2007
WO	2007106869		9/2007
WO	2008057437		5/2008
WO	2008058192		5/2008
WO	2008088648	A2	7/2008
WO	2008098112	A2	8/2008
WO	2008101231	A2	8/2008
WO	2008121949		10/2008
WO	2009002529		12/2008
WO	2009026177		2/2009
WO	2009082606		2/2009
WO	2009070302		6/2009
WO	2009089383	A2	7/2009
WO	2009002529		12/2009
WO	2009002993		12/2009
WO	2010014933		2/2010
WO	2010/065899	A2	6/2010
WO	2010/065906	A2	6/2010
WO	2010065899		6/2010
WO	2010065902		6/2010
WO	2010108125		9/2010
WO	2011/014821	A1	2/2011
WO	2010108125		3/2011
WO	2011106639		9/2011
WO	2012078534		6/2012
WO	2012166923		12/2012
WO	2013022797		2/2013
WO	2013028664		2/2013
WO	2013/130776	A1	9/2013
WO	2014062697		4/2014
WO	2014078484		5/2014
WO	2014106208		7/2014
WO	2014127365		8/2014
WO	2014134543		9/2014
WO	2015053318		4/2015
WO	2015057250		4/2015
WO	2015171792		11/2015
WO	2016030329		3/2016
WO	2016040179		3/2016

OTHER PUBLICATIONS

Scher, B. et al., "Value of 11C-choline PET and PET/CT in patients with suspected prostate cancer," Eur. J. Nucl. Med. Mol. Imaging., 2007, 34, 45-53.

Tasch, J. et al., "A Unique Folate Hydrolase, Prostage-Specific Membrane Antingen (PSMA): A Target for Immunotherapy?" Crit. Rev. Immunol., 2001, 21, 249-261.

Rosenthal, S.A. et al., "Utility of Capromab Pendetide (ProstaScint) Imaging in the Management of Prostate Cancer," Tech Urol, 2001, 7, 27-37.

Wiberg et al. "A comparison of some properties of C=O and C=S bonds," ARKIVOC, 2011, (v) 45-56.

Beheshti, M., Langsteger, W., and Fogelman, I. (2009) Prostate cancer: role of SPECT and PET in imaging bone metastases. Semin Nucl Med 39, 396-407.

Ananias, H. J., van den Heuvel, M. C., Helfrich, W., and de Jong, I. J. (2009) Expression of the gastrin-releasing peptide receptor, the prostate stem cell antigen and the prostate-specific membrane antigen in lymph node and bone metastases of prostate cancer. Prostate 69, 1101-8.

Vavere, A. L., Kridel, S. J., Wheeler, F. B., and Lewis, J. S. (2008) 1-11C-acetate as a PET radiopharmaceutical for imaging fatty acid synthase expression in prostate cancer. J Nucl Med 49, 327-34.

Tang, Q. L., and Yao, M. Y. (2008) [Updated application of prostate-specific membrane antigen to the diagnosis and treatment of prostate cancer]. Zhonghua Nan Ke Xue 14, 79-82.

Soloviev, D., Fini, A., Chierichetti, F., Al-Nahhas, A., and Rubello, D. (2008) PET imaging with 11C-acetate in prostate cancer: a biochemical, radiochemical and clinical perspective. Eur J Nucl Med Mol Imaging 35, 942-9.

Scher, B., and Seitz, M. (2008) PET/CT imaging of recurrent prostate cancer. Eur J Nucl Med Mol Imaging 35, 5-8.

Rinnab, L., Mottaghy, F. M., Simon, J., Volkmer, B. G., de Petriconi, R., Hautmann, R. E., Wittbrodt, M., Egghart, G., Moeller, P., Blumstein, N., Reske, S., and Kuefer, R. (2008) [11C]Choline PET/CT for targeted salvage lymph node dissection in patients with biochemical recurrence after primary curative therapy for prostate cancer. Preliminary results of a prospective study. Urol Int 81, 191-7.

Reske, S. N., Blumstein, N. M., and Glatting, G. (2008) [11C]choline PET/CT imaging in occult local relapse of prostate cancer after radical prostatectomy. Eur J Nucl Med Mol Imaging 35, 9-17.

(56) **References Cited**

OTHER PUBLICATIONS

Reske S.N. (2008) [11C]choling uptake with PET/CT for the initial diagnosis of prostate cancer. relation to PSA levels, tumour stage and anti-androgenic therapy. Eur J Nucl Med Mol Imaging 35, 1740-1.

Mease, R. C., Dusich, C. L., Foss, C. A., Ravert, H. T., Dannals, R. F., Seidel, J., Prideaux, A., Fox, J. J., Sgouros, G., Kozikowski, A. P., and Pomper, M. G. (2008) N-[N-[(S)-1,3-Dicarboxypropyl]carbamoyl]-4-[18F]fluorobenzyl-L-cysteine, [18F]DCFBC: a new imaging probe for prostate cancer. Clin Cancer Res 14, 3036-43.

Luboldt, W., Kufer, R., Blumstein, N., Toussaint, T. L., Kluge, A., Seemann, M. D., and Luboldt, H. J. (2008) Prostate carcinoma: diffusion-weighted imaging as potential alternative to conventional MR and 11C-choline PET/CT for detection of bone metastases. Radiology 249, 1017-25.

Liu, T., Toriyabe, Y., Kazak, M., and Berkman, C. E. (2008) Pseudoirreversible inhibition of prostate-specific membrane antigen by phosphoramidate peptidomimetics. Biochemistry 47, 12658-60.

Li, X., Liu, Q. Wang, M., Jin, X., Yao, S., Liu, S., and Li, J. (2008) C-11 choline PET/CT imaging for differentiating malignant from benign prostate lesions. Clin Nucl Med 33, 671-6.

Igerc, I., Kohlfurst, S., Gallowitsch, H. J., Matschnig, S., Kresnik, E., Gomez-Segovia, I., and Lind, P. (2008) The value of 18F-choline PET/CT in patients with elevated PSA-level and negative prostate needle biopsy for localisation of prostate cancer. Eur J Nucl Med Mol Imaging 35, 976-83.

Husarik, D. B., Miralbell, R., Dubs, M., John, H., Giger, O. T., Gelet, A., Cservenyak, T., and Hany, T. F. (2008) Evaluation of [(18)F]-choline PET/CT for staging and restaging of prostate cancer. Eur J Nucl Med Mol Imaging 35, 253-63.

Hospers, G. A., Helmond, F. A., de Vries, E. G., Dierckx, R. A., and de Vries, E. F. (2008) PET imaging of steroid receptor expression in breast and prostate cancer Curr Pharm Des 14, 3020-32.

Colabufo, N. A., Abate, C., Contino, M., Inglese, C., Niso, M., Berardi, F., and Perrone, R. (2008) PB183, a sigma receptor ligand, as a potential PET probe for the imaging of prostate adenocarcinoma. Bioorg Med Chem Lett 18, 1990-3.

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., and Pomper, M. G. (2008) Radiohalogenated prostate-specific membrane antigen (PSMA)-based urea as imaging agents for prostate cancer. J Med Chem 51, 7933-43.

Chandran, S. S., Banerjee, S. R., Mease, R. C., Pomper, M. G., and Denmeade, S. R. (2008) Characterization of a targeted nanoparticle functionalized with a urea-based inhibitor of prostate-specific membrane antigen (PSMA). Cancer Biol Ther 7, 974-82.

Barinka, C., Byun, Y., Dusich, C. L., Banerjee, S. R., Chen, Y., Castanares, M., Kozikowski, A. P., Mease, R. C., Pomper, M. G., and Lubkowski, J. (2008) Interactions between human glutamate carboxypeptidase II and urea-based inhibitors: structural characterization. J Med Chem 51, 7737-43.

Banerjee, S. R., Foss, C. A., Castanares, M., Mease, R. C., Byun, Y., Fox, J. J., Hilton, J., Lupold, S. E., Kozikowski, A. P., and Pomper, M. G. (2008) Synthesis and evaluation of technetium-99m- and rhenium-labeled inhibitors of the prostate-specific membrane antigen (PSMA). J Med Chem 51, 4504-17.

Wu, L. Y., Anderson, M. O., Toriyabe, Y., Maung, J., Campbell, T. Y., Tajon, C., Kazak, M., Moser, J., and Berkman, C. E. (2007) The molecular pruning of a phosporamidate peptidomimetic inhibitor of prostate-specific membrane antigen. Bioorg Med Chem 15, 7434-43.

Vees, 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 position 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, 1415-20.

Testa, C., Schiavina, R., Lodi, R., Salizzoni, E., Corti, B., Farsad, M., Kurhanewicz, J., Manferrari, F., Brunocilla, E., Tonon, C., Monetti, N., Castellucci, P., Fanti, S., Coe, M., Grigioni, W. F., Martorana, G., Canini, R., and Barbiroli, B. (2007) Prostate cancer: sextant localization with MR imaging, MR spectroscopy, and 11C-choline PET/CT. Radiology 244, 797-806.

Scattoni, V., Picchio, M., Suardi, N., Messa, C., Freschi, M., Roscigno, M., Da Pozzo, L., Bocciardi, A., Rigatti, P., and Fazio, F. (2007) Detection of lymph-node metastass with integrated [11C]choline PET/CT in patients with PSA failure after radical retropubic prostatectomy: results confirmed by open pelvic-retroperitoneal lymphadenectomy. Eur Urol 52, 423-9.

Sacha, P., Zamecnik, J., Barinka, C., Hlouchova, K., Vicha, A., Mlcochova, P., Hilgert, I., Eckschlager, T., and Konvalinka, J. (2007) Expression of glutamate carboxypeptidase II in human brain. Neuroscience 144, 1361-72.

Reske, S. N. (2007) [Nuclear imaging of prostate cancer: current status]. Der Urologe. 46, 1485-99.

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, 420-8.

Perner, S., Hofer, M. D., Kim, R., Shah, R. B., Li, H., Moller, P., Hautmann, R. E., Gschwend, J. E., Kuefer, R., and Rubin, M. A. (2007) Prostate-specific membrane antigen expression as a predictor of prostate cancer progression. Hum Pathol 38, 696-701.

Oehr, P., and Bouchelouche, K. (2007) Imaging of prostate cancer. Cuff Opin Oncol 19, 259-64.

Morris, M. J., and Scher, H. I. (2007) (11)C-acetate PET imaging in prostate cancer. Eur J Nucl Med Mol Imaging 34, 181-4.

Mlcochova, P., Plechanovova, A., Barinka, C., Mahadevan, D., Saldanha, J. W., Rulisek, L., and Konvalinka, J. (2007) Mapping of the active site of glutamate carboxypeptidase II by site-directed mutagenesis. FEBS J 274, 4731-41.

Mhawech-Fauceglia, P., Zhang, S., Terracciano, L., Sauter, G., Chadhuri, A., Herrmann, F. R., and Penetrante, R. (2007) Prostate-specific membrane antigen (PSMA) protein expression in normal and neoplastic tissues and its sensitivity and specificity in prostate adenocarcinoma: an immunohistochemical study using mutiple tumour tissue microarray technique. Histopathology 50, 472-83.

Lambert, L. A., and Mitchell, S. L. (2007) Molecular evolution of the transferrin receptor/glutamate carboxypeptidase II family. Journal of molecular evolution 64, 113-28.

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. Journal of neurochemistry 101, 682-96.

Goodman, O. B., Jr., Barwe, S. P., Ritter, B., McPherson, P. S., Vasko, A. J., Keen, J. H., Nanus, D. M., Bander, N. H., and Rajasekaran, A. K. (2007) Interaction of prostate specific membrane antigen with clathrin and the adaptor protein complex-2. Int J Oncol 31, 1199-203.

Fall, K., Garmo, H., Andren, O., Bill-Axelson, A., Adolfsson, J., Adami, H. O., Johansson, J. E., and Holmberg, L. (2007) Prostate-specific antigen levels as a predictor of lethal prostate cancer. J Natl Cancer Inst 99, 526-32.

Barinka, C., Starkova, J., Konvalinka, J., and Lubkowski, J. (2007) A high-resolution structure of ligand-free human glutamate carboxypeptidase II. Acta Crystallogr Sect F Struct Biol Cryst Commun 63, 150-3.

Barinka, C., Rovenska, M., Mlcochova, P., Hlouchova, K., Plechanovova, A., Majer, P., Tsukamoto, T., Slusher, B. S., Konvalinka, J., and Lubkowski, J. (2007) Structural insight into the pharmacophore pocket of human glutamate carboxypeptidase II. J Med Chem 50, 3267-73.

Anderson, M. O., Wu, L. Y., Santiago, N. M., Moser, J. M., Rowley, J. A., Bolstad, E. S., and Berkman, C. E. (2007) Substrate specificity of prostate-specific membrane antigen. Bioorg Med Chem 15, 6678-86.

(56) **References Cited**

OTHER PUBLICATIONS

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, 1249-54.

Reske, S. N., Blumstein, N. M., and Glatting, G. (2006) [Advancement of PET and PET/CT in prostate carcinoma]. Der Urologe. Ausg. A 45, 707-10, 712-4.

Reske, S. N., Blumstein, N. M., and Glatting, G. (2006) [PET and PET/CT in relapsing prostate carcinoma]. Der Urologe. Ausg. A 45, 1240, 1242-4, 1246-8, 1250.

Oyama, N., Kaneda, T., Nakai, M., Shioyama, R., Matsuta, Y., Tanase, K., Aoki, Y., Miwa, Y., Akino, H., Yokoyama, O., Okazawa, H., Fujibayashi, Y., and Yonekura, Y. (2006) [PET imaging in prostate cancer]. Hinyokika Kiyo 52, 503-5.

Mesters, J. R., Barinka, C., Li, W., Tsukamoto, T., Majer, P., Slusher, B. S., Konvalinka, J., and Hilgenfeld, R. (2006) Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. EMBO J 25, 1375-84.

Kinoshita, Y., Kuratsukuri, K., Landas, S., Imaida, K., Rovito, P. M., Jr., Wang, C. Y., and Haas, G. P. (2006) Expression of prostate-specific membrane antigen in normal and malignant human tissues. World J Surg 30, 628-36.

Elsasser-Beile, U., Wolf, P., Gierschner, D., Buhler, P., Schultze-Seemann, W., and Wetterauer, U. (2006) A new generation of monoclonal and recombinant antibodies against cell-adherent prostate specific membrane antigen for diagnostic and therapeutic targeting of prostate cancer. Prostate 66, 1359-70.

Cunha, A. C., Weigle, B., Kiessling, A., Bachmann, M., and Rieber, E. P. (2006) Tissue-specificity of prostate specific antigens: comparative analysis of transcript levels in prostate and non-prostatic tissues. Cancer Lett 236, 229-38.

Cimitan, M., Bortolus, R., Morassut, S., Canzonieri, V., Garbeglio, A., Baresic, T., Borsatti, E., Drigo, A., and Trovo, M. G. (2006) [18F]fluorocholine PET/CT imaging for the detection of recurrent prostate cancer at PSA relapse: experience in 100 consecutive patients. Eur J Nucl Med Mol Imaging 33, 1387-98.

Aggarwal, S., Singh, P., Topaloglu, O., Isaacs, J. T., and Denmeade, S. R. (2006) A dimeric peptide that binds selectively to prostate-specific membrane antigen and inhibits its enzymatic activity. Cancer Res 66, 9171-7.

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, 1015-26.

Yamaguchi, T., Lee, J., Uemura, H., Sasaki, T., Takahashi, N., Oka, T., Shizukuishi, K., Endou, H., Kubota, Y., and Inoue, T. (2005) Prostate cancer: a comparative study of 11C-choline PET and MR imaging combined with proton MR spectroscopy. Eur J Nucl Med Mol Imaging 32, 742-8.

Jadvar, H., Xiankui, L., Shahinian, A., Park, R., Tohme, M., Pinski, J., and Conti, P. S. (2005) Glucose metabolism of human prostate cancer mouse xenografts. Mol Imaging 4, 91-7.

Humblet, V., Lapidus, R., Williams, L. R., Tsukamoto, T., Rojas, C., Majer, P., Hin, B., Ohnishi, S., De Grand, A. M., Zaheer, A., Renze, J. T., Nakayama, A., Slusher, B. S., and Frangioni, J. V. (2005) High-affinity near-infrared fluorescent small-molecule contrast agents for in vivo imaging of prostate-specific membrane antigen. Mol Imaging 4, 448-62.

Gregor, P. D., Wolchok, J. D., Turaga, V., Latouche, J. B., Sadelain, M., Bacich, D., Heston, W. D., Houghton, A. N., and Scher, H. I. (2005) Induction of autoantibodies to syngeneic prostate-specific membrane antigen by xenogeneic vaccination. Int J Cancer 116, 415-21.

Foss, C. A., Mease, R. C., Fan, H., Wang, Y., Ravert, H. T., Dannals, R. F., Olszewski, R. T., Heston, W. D., Kozikowski, A. P., and Pomper, M. G. (2005) Radiolabeled small-molecule ligands for prostate-specific membrane antigen: in vivo imaging in experimental models of prostate cancer. Clin Cancer Res 11, 4022-8.

Vallabhajosula, S., Smith-Jones, P. M., Navarro, V., Goldsmith, S. J., and Bander, N. H. (2004) Radioimmunotherapy of prostate cancer in human xenografts using monoclonal antibodies specific to prostate specific membrane antigen (PSMA): studies in nude mice. Prostate 58, 145-55.

Scheffel, U., and Pomper, M. G. (2004) PET imaging of GRP receptor expression in prostate cancer. J Nucl Med 45, 1277-8.

O'Keefe, D. S., Bacich, D. J., and Heston, W. D. (2004) Comparative analysis of prostate-specific membrane antigen (PSMA) versus a prostate-specific membrane antigen-like gene. Prostate 58, 200-10.

Milowsky, M. I., Nanus, D. M., Kostakoglu, L., Vallabhajosula, S., Goldsmith, S. J., and Bander, N. H. (2004) Phase I trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. J Clin Oncol 22, 2522-31.

Matthies, A., Ezziddin, S., Ulrich, E. M., Palmedo, H., Biersack, H. J., Bender, H., and Guhlke, S. (2004) Imaging of prostate cancer metastases with 18F-fluoroacetate using PET/CT. Eur J Nucl Med Mol Imaging 31, 797.

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, 1729-38.

Henry, M. D., Wen, S., Silva, M. D., Chandra, S., Milton, M., and Worland, P. J. (2004) A prostate-specific membrane antigen-targeted monoclonal antibody-chemotherapeutic conjugate designed for the treatment of prostate cancer. Cancer Res 64, 7995-8001.

Ghosh, A., and Heston, W. D. (2004) Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. J Cell Biochem 91, 528-39.

Chopra, A. (2010) 68Ga-Labeled 2-{3-[5-(7-{1-benzyloxycarbonyl-5-[2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododec-1-l)acetylamino]pentylcarbamoyly}heptanoylamino)-1-carboxypentyl]ureido}pentanedioic acid. Molecular Imaging and Contrast Agent Database (MICAD) [database online]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Chopra, A. (2010) 68Ga-Labeled 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-tetraazacyclododec-1-l)acetylamino]propionylamino}p ropionylamino)pentylcarbamoyl] heptanoylamino}pentyl)ureido]pentanedioic acid. Molecular Imaging and Contrast Agent Database (MICAD) [database online]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

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. Journal of neurochemistry 89, 627-35.

Tang, H., Brown, M., Ye, Y., Huang, G., Zhang, Y., Wang, Y., Zhai, H., Chen, X., Shen, T. Y., and Tenniswood, M. (2003) Prostate targeting ligands based on N-acetylated alpha-linked acidic dipeptidase. Biochem Biophys Res Commun 307, 8-14.

Schulke, N., Varlamova, O. A., Donovan, G. P., Ma, D., Gardner, J. P., Morrissey, D. M., Arrigale, R. R., Zhan, C., Chodera, A. J., Surowitz, K. G., Maddon, P. J., Heston, W. D., and Olson, W. C. (2003) The homodimer of prostate-specific membrane antigen is a functional target for cancer therapy. Proc Natl Acad Sci U S A 100, 12590-5.

Rajasekaran, S. A., Anilkumar, G., Oshima, E., Bowie, J. U., Liu, H., Heston, W., Bander, N. H., and Rajasekaran, A. K. (2003) A novel cytoplasmic tail MXXXL motif mediates the internalization of prostate-specific membrane antigen. Mol Biol Cell 14, 4835-45.

Oyama, N., Miller, T. R., Dehdashti, F., Siegel, B. A., Fischer, K. C., Michalski, J. M., Kibel, A. S., Andriole, G. L., Picus, J., and Welch, M. J. (2003) 11C-acetate PET imaging of prostate cancer: detection of recurrent disease at PSA elapse. J Nucl Med 44, 549-55.

(56) **References Cited**

OTHER PUBLICATIONS

Meighan, M. A., Dickerson, M. T., Glinskii, O., Glinsky, V. V., Wright, G. L., Jr., and Deutscher, S. L. (2003) Recombinant glutamate carboxypeptidase II (prostate specific membrane antigen—PSMA)—cellular localization and bioactivity analyses. J Protein Chem 22, 317-26.

Hain, S. F., and Maisey, M. N. (2003) Positron emission tomography for urological tumours. BJU Int 92, 159-64.

Dimitrakopoulou-Strauss, A., and Strauss, L. G. (2003) PET imaging of prostate cancer with 11C-acetate. J Nucl Med 44, 556-8.

Shvarts, O., Han, K. R., Seltzer, M., Pantuck, A. J., and Belldegrun, A. S. (2002) Positron emission tomography in urologic oncology. Cancer Control 9, 335-42.

Rong, S. B., Zhang, J., Neale, J. H., Wroblewski, J. T., Wang, S., and Kozikowski, A. P. (2002) Molecular modeling of the interactions of glutamate carboxypeptidase II with its potent NAAG-based inhibitors. J Med Chem 45, 4140-52.

Pomper, M. G., Musachio, J. L., Zhang, J., Scheffel, U., Zhou, Y., Hilton, J., Maini, A., Dannals, R. F., Wong, D. F., and Kozikowski, A. P. (2002) 11C-MCG: synthesis, uptake selectivity, and primate PET of a probe for glutamate carboxypeptidase II (NAALADase). Mol Imaging 1, 96-101.

Oyama, N., Akino, H., Kanamaru, H., Suzuki, Y., Muramoto, S., Yonekura, Y., Sadato, N., Yamamoto, K., and Okada, K. (2002) 11C-acetate PET imaging of prostate cancer. J Nucl Med 43, 181-6.

Kotzerke, J., Gschwend, J. E., and Neumaier, B. (2002) PET for prostate cancer imaging: still a quandary or the ultimate solution? J Nucl Med 43, 200-2.

Hara, T., Kosaka, N., and Kishi, H. (2002) Development of (18)F-fluoroethylcholine for cancer imaging with PET: synthesis, biochemistry, and prostate cancer imaging. J Nucl Med 43, 187-99.

Hara, T. (2002) 11C-choline and 2-deoxy-2-[18F]fluoro-D-glucose in tumor imaging with positron emission tomography. Mol Imaging Biol 4, 267-73.

Chang, S. S., and Heston, W. D. (2002) The clinical role of prostate-specific membrane antigen (PSMA). Urol Oncol 7, 7-12.

DeGrado, T. R., Coleman, R. E., Wang, S., Baldwin, S. W., Orr, M. D., Robertson, C. N., Polascik, T. J., and Price, D. T. (2001) Synthesis and evaluation of 18F-labeled choline as an oncologic tracer for positron emission tomography: initial findings in prostate cancer. Cancer Res 61, 110-7.

DeGrado, T. R., Baldwin, S. W., Wang, S., Orr, M. D., Liao, R. P., Friedman, H. S., Reiman, R., Price, D. T., and Coleman, R. E. (2001) Synthesis and evaluation of (18)F-labeled choline analogs as oncologic PET tracers. J Nucl Med 42, 1805-14.

Bacich, D. J., Pinto, J. T., Tong, W. P., and Heston, W. D. W. (2001) Cloning, expression, genomic localization, and enzymatic activities of the mouse homolog of prostate-specific membrane antigen/NAALADase/folate hydrolase. Mammalian Genome 12, 117-123.

Slusher, B. S., Vornov, J. J., Thomas, A. G., Hum, P. D., Harukuni, I., Bhardwaj, A., Traystman, R. J., Robinson, M. B., Britton, P., Lu, X. C., Tortella, F. C., Wozniak, K. M., Yudkoff, M., Potter, B. M., and Jackson, P. F. (1999) Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. Nat Med 5, 1396-402.

Dumas, F., Gala, J. L., Berteau, P., Brasseur, F., Eschwege, P., Paradis, V., Lacour, B., Philippe, M., and Loric, S. (1999) Molecular expression of PSMA mRNA and protein in primary renal tumors. Int J Cancer 80, 799-803.

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, 3192-8.

Sweat, S. D., Pacelli, A., Murphy, G. P., and Bostwick, D. G. (1998) Prostate-specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases. Urology 52, 637-40.

Murphy, G. P., Elgamal, A. A., Su, S. L., Bostwick, D. G., and Holmes, E. H. (1998) Current evaluation of the tissue localization and diagnostic utility of prostate specific membrane antigen. Cancer 83, 2259-69.

Liu H., Rajasekaran, A. K., Moy, P., Xia, Y., Kim, S., Navarro, V., Rahmati, R., and Bander, N. H. (1998) Constitutive and antibody-induced internalization of prostate-specific membrane antigen. Cancer Res 58, 4055-60.

Kahn, D., Williams, R. D., Manyak, M. J., Haseman, M. K., Seldin, D. W., Libertino, J. A., and Maguire, R. T. (1998) 111Indium-capromab pendetide in the evaluation of patients with residual or recurrent prostate cancer after radical prostatectomy. The ProstaScint Study Group. J Urol 159, 2041-6; discussion 2046-7.

Hara, T., Kosaka, N., and Kishi, H. (1998) PET imaging of prostate cancer using carbon-11-choline. J Nucl Med 39, 990-5.

Bostwick, D. G., Pacelli, A., Blute, M., Roche, P., and Murphy, G. P. (1998) Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. Cancer 82, 2256-61.

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, 81-5.

Fair, W. R., Israeli, R. S., and Heston, W. D. (1997) Prostate-specific membrane antigen. Prostate 32, 140-8.

Jackson P.F., Cole D.C., Slusher B.S., Stetz S.L., Ross L.E., Donzanti B.A., and D.A., T. (1996) Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase N-acetylated alpha-linked acidic dipeptidase.

Wright, G. L., Jr., Haley, C., Beckett, M. L., and Schellhammer, P. F. (1995) Expression of prostate-specific membrane antigen in normal, benign, and malignant prostate tissues. Urol Oncol 1, 18-28.

Leek, J., Lench, N., Maraj, B., Bailey, A., Carr, I. M., Andersen, S., Cross, J., Whelan, P., MacLennan, K. A., Meredith, D. M., and et al. (1995) Prostate-specific membrane antigen: evidence for the existence of a second related human gene. Br J Cancer 72, 583-8.

First Office Action in counterpart Canadian Application No. 2924360, dated Jan. 11, 2018 (4 pages).

Second Office Action in counterpart Chinese Application No. 201480056250.5, dated Aug. 15, 2017 (4 pages).

Second Office Action in counterpart Eurasian Application No. 201690495/28, dated Feb. 10, 2017 (5 pages).

First Office Action in counterpart Korean Application No. 1020167012314, dated Mar. 6, 2017 (3 pages).

Second Office Action in counterpart Korean Application No. 1020167012314, dated Oct. 14, 2017 (3 pages).

Second Office Action for counterpart Georgian Application No. 14132/01, dated Mar. 2018 (2 pages).

First Office Action for counterpart Georgian Application No. 14132/01, dated Feb. 2017 (2 pages).

First Examination Report for counterpart Chilean Application No. 00883-2016, dated Apr. 12, 2018.

Third Office Action for counterpart Korean Application No. 10-2016-7012314, dated May 2, 2018 (2 pages).

Silvola J. M.U. et al., "Al18F-NOTA-Folate Accumulates in Atherosclerotic Plaques and Can be Detected by PER/CT", Poster presented Nov. 7, 2015 in Orlando, FL at the 2015 American Heart Association ReSuscitation Science Symposium (http://newsroom.heart.org/events/scientific-sessions-2015-newsroom-2942760).(3 pages).

Silvola J. M.U. et al., "Al18F-NOTA-Folate Accumulates in Atherosclerotic Plaques and Can be Detected by PET/CT", Published reference of poster, Nov. 10, 2015, at http://circ.ahajournals.org/content/132/Suppl_3/A18873?cited-by=yes&legid=circulationaha;132/Suppl_3/A18873; Circulation, 2015; 132:A18873. (2 pages).

Chinese Patent Application No. 201480071256.X, by Endocyte, Inc. et al.: Office Action dated Apr. 20, 2017; English translation (9 pages).

Eurasian Patent Application No. 201690862/28, by Endocyte, Inc. et al.: Office Action, dated May 22, 2017; English translation (2 pages).

European Patent Application No. EP 14861854, by Endocyte, Inc. et al: Partial Supplementary Search Report with Opinion; dated May 19, 2017 (15 pages).

(56) **References Cited**

OTHER PUBLICATIONS

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, 217-29.

Hwang, D. R., Mathias, C. J., Welch, M. J., McGuire, A. H., and Kadmon, D. (1990) Imaging prostate derived tumors with PET and N-(3[18F]fluoropropyl)putrescine. Int J Rad Appl Instrum B 17, 525-32.

Hwang, D. R., Lang, L. X., Mathias, C. J., Kadmon, D., and Welch, M. J. (1989) N-3[18]fluoropropylputrescine as potential PET imaging agent for prostate and prostate derived tumors. J Nucl Med 30, 1205-10.

Office Action for counterpart Chinese Application No. 201610184873.1, dated Jul. 24, 2018.

Shelley James, "Urea Based Rhenium Tricarbonyl Dipeptide Compounds as Potential Radiopharmaceuticals for PSMA Imaging"; Poster: INOR 258; The 229th ACS National Meeting, San Diego, CA, Mar. 13-17, 2005.

PCT International Search Report and Written Opinion for PCT/US2011/026238, dated Apr. 27, 2011.

PCT International Search Report and Written Opinion for PCT/US2013/070007, dated Mar. 5, 2014.

PCT International Search Report for PCT/US2008/073375 dated Oct. 26, 2008.

PCT International Search Report for PCT/US2016/012653 dated Mar. 11, 2016.

PCT International Search Report/Written Opinion for PCT/US2009/061067, completed May 28, 2010.

PCT International Search Report/Written Opinion for PCT/US2009/061049, completed Mar. 15, 2010.

PCT Search Report & Written Opinion issued in App. No. PCT/US2014/065467.

Silverman, "The Organic Chemistry of Drug Design and Drug Action," Elsevier Academic Press (2nd Ed. 2003).

Office Action for counterpart Israel Patent Application No. 245113, dated Jan. 10, 2019 (with English Translation).

Office Action for counterpart Georgian Patent Application No. AP 2014 014132, dated Jan. 11, 2019 (with English Translation).

Second Examination Report for counterpart Chilean Patent Application No. 00883-2016, dated Jan. 16, 2019 (with English Translation).

M. De Santis et al., "Role of Chemotherapy in Castration-Resistant Prostate Cancer." Der Urologe 1:39-43, 2012.

A. Heidenreich, "Immunotherapy for Metastic Prostate Cancer—Do we really need this?" Der Urologe 1(1):32-38, 2012.

T.H. Kuru et al., "MRI Navigated Stereotactic Prostate Biopsy." Der Urologe 1(1):50-56, 2012.

F. Moltzahn et al., "Bone Metastasis in Prostate Cancer." Der Urologe 1(1):20-26, 2012.

A. Omlin et al., "Inhibitors of Androgen and Estrogen Biosynthesis in Castration-Resistant Prostate Cancer." Der Urologe 1(1):8-14, 2012.

S.N. Reske et al., "Advancement of PET and PET/CT in Prostate Carcinoma." Der Urologe 45(6):707-714, 2006.

S.N. Reske, "Nuclear Imaging of Prostate Cancer." Urologe 46(11):1485-1499, 2007.

S.N. Reske et al., "PET and PET/CT in Relapsing Prostate Carcinoma," Der Urologe 45(10):1240-1250-1499, 2006.

M. Spahn et al., "How Should Hormone Therapy for Castration-Resistant Cancer be Continued?" Der Urologe 51 (1):15-19, 2012.

G. Thalmann, "Advanced Prostate Cancer Where are we going?" Der Urologe 51(1):7, 2012.

L. Weissbach, "Which Components Should 'Living Guidelines' Contain?" Der Urologe 51(1):57-59, 2012.

S. Preusser et al., "Castration-Resistant Prostate Cancer." Der Urologe 51(1):27-31, 2012.

Y-q. Wu et al., "A Mild Deprotection Procedure for Tert-butyl Esters and Tert-butyl Ethers Using ZnBr2 in Methylene Chloride." Tetrahedron Letters 41, 2847-2849, 2000.

J. Meienhofer et al., "Solid-Phase Systhesis with Attachment of Peptide to Resin Through an Amino Acid Side Chain: [8-Lysine]-Vasopressin," Proc. Nat. Acad. Sci., USA, vol. 68, No. 5, pp. 1006-1009, May 1971.

C. Foss et al., Synthesis and Validation of a Novel Small-Molecule Fluorescent Probe for PSMA Expression in Human Tumor Neovasculature, Abstract ID: 362, Molecular Imaging, vol. 4, No. 3, p. 321, Jul. 2005.

Harada, N., Kimura, H., Ono, M., and Saji, H. (2013) Preparation of asymmetric urea derivatives that target prostate-specific membrane antigen for SPECT imaging. J Med Chem 56, 7890-901.

Frigerio, B., Fracasso, G., Luison, E., Cingarlini, S., Mortarino, M., Coliva, A., Seregni, E., Bombardieri, E., Zuccolotto, G., Rosato, A., Colombatti, M., Canevari, S., and Figini, M. (2013) A single-chain fragment against prostate specific membrane antigen as a tool to build theranostic reagents for prostate cancer. Eur J Cancer 49, 2223-32.

Franc, B. L., Cho, S. Y., Rosenthal, S. A., Cui, Y., Tsui, B., Vandewalker, K. M., Holz, A. L., Poonamallee, U., Pomper, M. G., and James, R. B. (2013) Detection and localization of carcinoma within the prostate using high resolution transrectal gamma imaging (TRGI) of monoclonal antibody directed at prostate specific membrane antigen (PSMA)—Proof of concept and initial imaging results. European Journal of Radiology 82, 1877-84.

Eder, M., Eisenhut, M., Babich, J., and Haberkorn, U. (2013) PSMA as a target for radiolabelled small molecules. Eur J Nucl Med Mol Imaging 40, 819-23.

Divyya, S., Naushad, S. M., Murthy, P. V., Reddy Ch, R., and Kutala, V. K. (2013) GCPII modulates oxidative stress and prostate cancer susceptibility through changes in methylation of RASSF1, BNIP3, GSTP1 and Ec-SOD. Molecular Biology Reports 40, 5541-50.

Ceci, F., Castellucci, P., Mamede, M., Schiavina, R., Rubello, D., Fuccio, C., Ambrosini, V., Boschi, S., Martorana, G., and Fanti, S. (2013) (11)C-Choline PET/CT in patients with hormone-resistant prostate cancer showing biochemical relapse after radical prostatectomy. Eur J Nucl Med Mol Imaging 40, 149-55.

Benesova, M., Schafer, M., Bauder-Wüst, U., Mier, W., Haberkorn, U., Eisenhut, M., Kopka, K., and Eder, M. (2013) Linker Modifications of DOTA-conjugated Inhibitors of the Prostate-Specific Membrane Antigen (PSMA). Eur J Nucl Med Mol Imaging 40 (Suppl 2), S193.

Ben Jemaa, A., Sallami, S., Ceraline, J., and Oueslati, R. (2013) A novel regulation of PSMA and PSA expression by Q640X AR in 22Rv1 and LNCaP prostate cancer cells. Cell biology international 37, 464-70.

Ben Jemaa, A., Bouraoui, Y., Sallami, S., Nouira, Y., and Oueslati, R. (2013) A comparison of the biological features of prostate cancer with (PSA+, PSMA+) profile according to RKIP. BioMed Research International 2013, 409179.

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., and Babich, J. W. (2013) First-in-Man Evaluation of 2 High-Affinity PSMA-Avid Small Molecules for Imaging Prostate Cancer. J Nucl Med 54, 380-7.

Antunes, A. A., Reis, S. T., Leite, K. R., Real, D. M., Sousa-Canavez, J. M., Camara-Lopes, L. H., Dall'Oglio, M. F., and Srougi, M. (2013) PGC and PSMA in prostate cancer diagnosis: tissue analysis from biopsy samples. International Braz J Urol : official journal of the Brazilian Society of Urology 39, 649-56.

Afshar-Oromieh, A., Matcher, A., Eder, M., Eisenhut, M., Linhart, H. G., Hadaschik, B. A., Holland-Letz, T., Giesel, F. L., Kratochwil, C., Haufe, S., Haberkorn, U., and Zechmann, C. M. (2013) PET imaging with a [(68)Ga]gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. Eur J Nucl Med Mol Imaging 40, 486-95.

Afshar-Oromieh, A., Haberkorn, U., Hadaschik, B., Habl, G., Eder, M., Eisenhut, M., Schlemmer, H. P., and Roethke, M. C. (2013) PET/MRI with a 68Ga-PSMA ligand for the detection of prostate cancer. Eur J Nucl Med Mol Imaging 40, 1629-30.

(56) **References Cited**

OTHER PUBLICATIONS

Abstracts of the Annual Congress of the European Association of Nuclear Medicine. Oct. 19-23, 2013. Lyon, France. Eur J Nucl Med Mol Imaging 40 Suppl 2, S1-S477.

Zhang, Y., Guo, Z., Du, T., Chen, J., Wang, W., Xu, K., Lin, T., and Huang, H. (2012) Prostate specific membrane antigen (PSMA): A novel modulator of p38 for proliferation, migration, and survival in prostate cancer cells. Prostate 73, 835-41.

Weissbach, L. (2012) [Which components should living guidelines contain?]. Der Urologe. 51, 57-9.

Thalmann, G. (2012) [Advanced prostate cancer: where are we going?]. Der Urologe 51, 7.

Taylor, R. M., Sevems, V., Brown, D. C., Bisoffi, M., and Sillerud, L. O. (2012) Prostate cancer targeting motifs: expression of alphanu beta3, neurotensin receptor 1, prostate specific membrane antigen, and prostate stem cell antigen in human prostate cancer cell lines and xenografts. Prostate 72, 523-32.

Spahn, M., and Krebs, M. (2012) [How should hormone therapy for castration-resistant prostate cancer be continued?]. Der Urologe. 51, 15-9.

Schafer, M., Bauder-Wust, U., Leotta, K., Zoller, F., Mier, W., Haberkorn, U., Eisenhut, M., and Eder, M. (2012) A dimerized urea-based inhibitor of the prostate-specific membrane antigen for 68Ga-PET imaging of prostate cancer. EJNMMI Res 2, 23.

Preusser, S., Putora, P. M., Plasswilm, L., and Schmid, H. P. (2012) [Castration-resistant prostate cancer: surgical and radio-oncological therapeutic options]. Der Urologe. 51, 27-31.

Poulsen, M. H., Bouchelouche, K., Hoilund-Carlsen, P. F., Petersen, H., Gerke, O., Steftansen, S. I., Marcussen, N., Svolgaard, N., Vach, W., Geertsen, U., and Walter, S. (2012) [18F]fluoromethylcholine (FCH) positron emission tomography/computed tomography (PET/CT) for lymph node staging of prostate cancer: a prospective study of 210 patients. BJU Intl 110, 1666-71.

Pinto, F., Totaro, A., Palermo, G., Calarco, A., Sacco, E., D'Addessi, A., Racioppi, M., Valentini, A., Gui, B., and Bassi, P. (2012) Imaging in prostate cancer staging: present role and future perspectives. Urol Int 88, 125-36.

Pavlicek, J., Ptacek, J., and Barinka, C. (2012) Glutamate carboxypeptidase II: an overview of structural studies and their importance for structure-based drug design and deciphering the reaction mechanism of the enzyme. Curr Med Chem 19, 1300-9.

Omlin, A., and Gillessen, S. (2012) [Inhibitors of androgen and estrogen biosynthesis in castration-resistant prostate cancer]. Der Urologe. 51, 8-14.

Nedrow-Byers, J. R., Jabbes, M., Jewett, C., Ganguly, T., He, H., Liu, T., Benny, P., Bryan, J. N., and Berkman, C. E. (2012) A phosphoramidate-based prostate-specific membrane antigen-targeted SPECT agent. Prostate 72, 904-12.

Moltzahn, F., and Thalmann, G. N. (2012) [Bone metastasis in prostate cancer]. Der Urologe. 51, 20-6.

Meinhardt, W., van der Poel, H. G., Valdes Olmos, R. A., Bex, A., Brouwer, O. R., and Horenblas, S. (2012) Laparoscopic sentinel lymph node biopsy for prostate cancer: the relevance of locations outside the extended dissection area. Prostate Cancer 2012, 751753.

Malik, N., Zlatopolskiy, B., Machulla, H.-J., Reske, S. N., and Solbach, C. (2012) One pot radiofluorination of a new Potential PSMA ligand [Al18F]NOTA-DUPA-Pep. Journal of Labelled Compounds and Radiopharmaceuticals 55, 320-325.

Lutje, S., Boerman, O. C., van Rij, C. M., Sedelaar, M., Helfrich, W., Oyen, W. J., and Mulders, P. F. (2012) Prospects in radionuclide imaging of prostate cancer. Prostate 72, 1262-72.

Liu, T., Wu, L. Y., Fulton, M. D., Johnson, J. M., and Berkman, C. E. (2012) Prolonged androgen deprivation leads to downregulation of androgen receptor and prostate-specific membrane antigen in prostate cancer cells. Int J Oncol 41, 2087-92.

Liu, T., Nedrow-Byers, J. R., Hopkins, M. R., Wu, L. Y., Lee, J., Reilly, P. T., and Berkman, C. E. (2012) Targeting prostate cancer cells with a multivalent PSMA inhibitor-guided streptavidin conjugate. Bioorg Med Chem Lett 22, 3931-4.

Lees K., Durve, M., and Parker, C. (2012) Active surveillance in prostate cancer: patient selection and triggers for intervention. Curr Opin Urol 22, 210-5.

Kuru, T. H., Tulea, C., Simpfendorfer, T., Popeneciu, V., Roethke, M., Hadaschik, B. A., and Hohenfellner, M. (2012) [MRI navigated stereotactic prostate biopsy: fusion of MRI and real-time transrectal ultrasound images for perineal prostate biopsies]. Der Urologe. 51, 50-6.

Kosuri, S., Akhtar, N. H., Smith, M., Osborne, J. R., and Tagawa, S. T. (2012) Review of salvage therapy for biochemically recurrent prostate cancer: the role of imaging and rationale for systemic salvage targeted anti-prostate-specific membrane antigen radioimmunotherapy. Advances in Urology 2012, 921674.

Klotz, L. (2012) Cancer overdiagnosis and overtreatment. Curr Opin Urol 22, 203-9.

Jadvar, H. (2012) Molecular imaging of prostate cancer: PET radiotracers. AJR Am J Roentgenol 199, 278-91.

Hlouchova, K., Navratil, V., Tykvart, J., Sacha, P., and Konvalinka, J. (2012) GCPII variants, paralogs and orthologs. Curr Med Chem 19, 1316-22.

Heidenreich, A. (2012) [Immunotherapy for metastatic prostate cancer: do we really need this?]. Der Urologe. 51, 32-8.

Grant, C. L., Caromile, L. A., Ho, V., Durrani, K., Rahman, M. M., Claffey, K. P., Fong, G. H., and Shapiro, L. H. (2012) Prostate specific membrane antigen (PSMA) regulates angiogenesis independently of VEGF during ocular neovascularization. PLoS One 7, e4 1285.

Graham, K., Lesche, R., Gromov, A. V., Bohnke, N., Schafer, M., Hassfeld, J., Dinkelborg, L., and Kettschau, G. (2012) Radiofluorinated derivatives of 2-(phosphonomethyl)pentanedioic acid as inhibitors of prostate specific membrane antigen (PSMA) for the imaging of prostate cancer. J Med Chem 55, 9510-20.

Foss, C. A., Mease, R. C., Cho, S. Y., Kim, H. J., and Pomper, M. G. (2012) GCPII imaging and cancer. Curr Med Chem 19, 1346-59.

Fortuin, A. S., Deserno, W. M., Meijer, H. J., Jager, G. J., Takahashi, S., Debats, O. A., Reske, S. N., Schick, C., Krause, B. J., van Oort, I., Witjes, A. J., Hoogeveen, Y. L., van Lin, E. N., and Barentsz, J. O. (2012) Value of PET/CT and MR lymphography in treatment of prostate cancer patients with lymph node metastases. Int J Radiat Oncol Biol Phys 84(3), 712-8.

Eder, M., Schafer, M., Bauder-Wust, U., Hull, W. E., Wangler, C., Mier, W., Haberkorn, U., and Eisenhut, M. (2012) (68)Ga-Complex Lipophilicity and the Targeting Property of a Urea-Based PSMA Inhibitor for PET Imaging. Bioconjugate Chem 23, 688-97.

De Santis, M., and Bachner, M. (2012) [Role of chemotherapy in castration-resistant prostate cancer: are there new approaches?]. Der Urologe. 51, 39-43.

Chen, Z., Penet, M. F., Nimmagadda, S., Li, C., Banerjee, S. R., Winnard, P. T., Jr., Artemov, D., Glunde, K., Pomper, M. G., and Bhujwalla, Z. M. (2012) PSMA-targeted theranostic nanoplex for prostate cancer therapy. ACS Nano 6, 7752-62.

Chen, Y., Pullambhatla, M., Banerjee, S. R., Byun, Y., Stathis, M., Rojas, C., Slusher, B. S., Mease, R. C., and Pomper, M. G. (2012) Synthesis and biological evaluation of low molecular weight fluorescent imaging agents for the prostate-specific membrane antigen. Bioconjugate Chem 23, 2377-85.

Chen, J., Zhao, Y., Li, X., Sun, P., Wang, M., Wang, R., and Jin, X. (2012) Imaging primary prostate cancer with 11C-Choline PET/CT: relation to tumour stage, Gleason score and biomarkers of biologic aggressiveness. Radiology and Oncology 46(3), 179-88.

Baiz, D., Pinder, T. A., Hassan, S., Karpova, Y., Salsbury, F., Welker, M. E., and Kulik, G. (2012) Synthesis and characterization of a novel prostate cancer-targeted phosphatidylinositol-3-kinase inhibitor prodrug. J Med Chem 55, 8038-46.

Afshar-Oromieh, A., Haberkorn, U., Eder, M., Eisenhut, M., and Zechmann, C. M. (2012) [68Ga]Gallium-labelled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: comparison with 18F-FECH. Eur J Nucl Med Mol Imaging 39, 1085-6.

C. Dusich et al., General Approach for the Preparation of Fluorescent PSMA/GCPII Inhibitors, Abstract ID: 470 Poster board space: 29, Molecular Imaging, vol. 5, No. 3, pp. 322-323, Jul. 2006.

(56) **References Cited**

OTHER PUBLICATIONS

Banerjee, S. et al., "Sequential SPECT and Optical Imaging of Experimental Models of Prostate Cancer with a Dual Modality Inhibitor of the Prostate-Specific Membrane Antigen," Angewandte Chemie International Edition, 2011, 50, 9167-9170.

Banerjee, S.R. et al. "Synthesis and Evaluation of Technetium-99m- and Rhenium-Labeled Inhibitors of the Prostate-Specific Membrane Antigen (PSMA)," J. Med. Chem. Aug. 14, 2008; 51(15): 4504-4517.

Bennett, V.J.,"Analysis of fluorescently labeled substance P analogs: binding, imaging and receptor activation," BMC chemical Biology, 2001, 1:1. doi: 10.1186/1472-6769-1-1.

Chen, Ying, et al., "Radiohalogenated Prostate-Specific Membrane Antigen (PSMA)-Based Ureas as Imaging Agents for Prostate Cancer," J. Med. Chem., 2008, 51 (24), pp. 7933-7943.

Cole et al., "Cancer theranostics: the rise of targeted magnetic nanoparticles," Trends in Biotechnology, 2011, 29, 323-332.

Davis, Mindy I., et al., "Crystal Structure of Prostate-Specific Membrane Antigen, A Tumor Marker and Peptidase", Apr. 26, 2005, PNAS, vol. 102, No. 17, pp. 5981-5986.

Definition of ligand, Random House Kernerman Webster's College IMMM Dictionary, downloaded on Jan. 25, 2014 from http://www.thefreed ictionary. com/ligand, 1 page.

Eder et al., "68Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging," Bioconjugate Chemistry, 2012; 23:688-697.

Farokhzad, et al., "Nanoparticle-Aptamer Bioconjugates: A New Approach for Targeting Prostate Cancer Cells," Cancer Research 64, pp. 7668-7672 (2004).

Foss, Catherine A., et al. "Radiolabeled small-molecule ligands for prostate-specific membrane antigen: in vivo imaging in experimental models of prostate cancer." Clinical cancer research 11.11 (2005): 4022-4028.

Gomez-Hens et al., "Long wavelength fluorophores: new trends in their analytical use," Trends in Analytical Chemistry, 2004; 23: 127-136.

Henne, et al., "Synthesis and activity of a folate peptide camptothecin prodrug," ScienceDirect, Bioorganic & Medical Chemistry Letters 16 (2006) 5350-5355.

Hiller, Shawn M., et al., "Preclinical Evaluation of Novel Glutamate-Urea-Lysine Analogues That Target Prostate-Specific Membrane Antigen as Molecular Imaging Pharmaceuticals for Prostate Cancer," Cancer Res. Sep. 1, 2009; 69 (17):6932-40.

Jackson, Paul F., et al., "Design of NAALADase Inhibitors: A Novel Neuroprotective Strategy", 2001, Current Medicinal Chemistry, vol. 8, No. 8, pp. 949-957.

Jayaprakash, Sarva, et al. "Design and synthesis of a PSMA inhibitor-doxorubicin conjugate for targeted prostate cancer therapy." ChemMedChem 1.3 (2006): 299-302.

Kaur, G. et al., "Biological evaluation of tubulysin A: a potential anticancer and antiangiogenic natural product," Biochem. J., 2006, 396, 235-242.

Kozikowski, Alan P., et al., "Design of Remarkably Simple, Yet Potent Urea-Based Inhibitors of Glutamate Carbozypeptidase II (NAALADase)"2, 001, Journal of Medicinal Chemistry, vol., 44, No. 3, pp. 298-301.

Kozikowski, Alan P., et al., "Synthesis of Urea-Based Inhibitors as Active Site Probes of Glutamate Carboxypeptodase II: Efficacy as Analgesic Agents", 2004, Journal of Medicinal Chemistry, vol., 47, No. 7, pp. 1729-1738.

Kularatne et al., "Prostate-specific membrane antigen targeted imaging and therapy of prostate cancer using a PSMA inhibitor as a homing ligand," Molecular Pharmaceutics, 6(3): 780-789 (2009).

Kularatne, S., "Synthesis and Biological Analysis of Prostate-Specific Membrane Antigen-Targeted Anticancer Prodrugs," J. Med. Chem, 2010, 53(21) , 7767-7777.

Larock, "Comprehensive Organic Transformations, a guide to functional group preparations," VCH Publishers, Inc. New York (1989).

Lu, G. et al., "Synthesis and SAR of 99mTc/Re-labeled small molecule prostate specific membrane antigen inhibitors with novel polar chelates," Bioorganic and Medicinal Chemistry Letters, 2013, 23, 1557-1563.

Lupold, et al., "Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen." Cancer Res. 2002; 62:4029-4033.

Majer, Pavel., et al., "Synthesis and Biological Evaluation of Thiol-Based Inhibitors of Glutamate Carboxypeptodase II: Discovery of an Orally Active GCP II Inhibitor", 2003, Journal of Medicinal Chemistry, vol. 46, No. 10, pp. 1989-1996.

Maresca, K. P., "A Series of Halogenated Heterodimeric Inhibitors of Prostate Specific Membrane Antigen (PSMA) as Radiolabeled Probes for Targeting Prostate Cancer," J. Med. Chem., 2009, 52 (2), pp. 347-357.

Maresca, K., et al., "Molecular targeting of prostate cancer with small molecule inhibitors of prostate specific membrane antigen (PSMA)," J. Nucl. Med. 2007, 48 (Supplement 2):25P.

Martin, et al., Helv. Chim. Acta, 78, 486-504 (1995) and Abstract.

McNamara et al, Cell type specific delivery of siRNAs with aptamer-siRNA chimeras, Nature Biotechnolgy, 2006; 24: 1005-1015.

Melby, et at., Cancer Research 53(8), pp. 1755-1760 (1993).

Mesters, et al., et al., "Structure of Glutamate Carboxypeptidase II, a Drug Target in Neuronal Damage and Prostate Cancer" 2006, The EMBO Journal, vol. 25, No. 6, pp. 1375-1384.

Olsnes, S., et al., Immunology Today, 10, pp. 291-295 (1989).

Paranjpe, et al., "Tumor-targeted bioconjugate based delivery of camptothecin: design, synthesis and in vitro evaluation," ScienceDirect Journal of Controlled Release 100 (2004) 275-292.

Pathalk et al., Enzymic protecting group techniques in organic synthesis, Stereosel. Biocatal. 775-797 (2000).

Peltier et al., "The Total Synthesis ofTubulysin D," J. Am. Chem. Soc. 128:16018-19 (2006).

Pubchem, Compound summary for: CID 58099954, Aug. 19, 2012.

Radioisotopes in Medicine, from http://www.word-nuclear.org/information-library/non-power-nuclear applications/radioisotopes-research/radioisotopes-in-medicine.aspx, Dec. 28, 2016, pp. 1-20.

Ranasinghe, M. G., et al., "Facile Synthesis of Unsymmetrical Thiolsulfonates via Sulfonylation of Mercaptans", 1988, Synthetic Communications, vol. 18, No. 3, pp. 227-232.

Reddy et al., "PS MA-specific anti-tumor activity of the targeted-tubulysin conjugate, EC 1169," American Association for Cancer Research Annual Meeting (Apr. 8, 2013) Poster.

Reddy et al., "PS MA-specific anti-tumor activity of the targeted-tubulysin conjugate, EC 1169," American Association for Cancer Research Annual Meeting (Apr. 8, 2013) Presentation Abstract.

Roy, et al., "DUPA Conjugation of a Cytotoxic Indenoisoquinoline Topoisomerase I Inhibitor for Selective Prostate Cancer Cell Targeting," J. Med. Chem. 58 (2015) 3094-3103.

Theodora E. Greene & Peter G.M. Wuts, "Protective Groups ion Organic Synthesis," 2d edition, John Wiley & Sons, Inc. New York (1991).

Truffert, et al., Tetrahedron, 52:3005 (1996).

Vlahov, et al., "Design and regioselective synthesis of a new generation of targeted chemotherapeutics. Part 1: EC145, a folic acid conjugate of desacetylvinblastine monohydrazide," ScienceDirect, Bioorganic & Medical Chemistry Letters 16 (2006) 5093-5096.

Wang et al., "Prostate-Specific Membrane Antigen Targeted Tubulysin Conjugates for Cancer Therapy," 246th ACS National Meeting and Exposition (Sep. 8, 2013) Poster.

Yang, et al., "Characterization of the pH of Folate Receptor-Containing Endosomes and the Rate of Hydrolysis of Internalized Acid-Labile Folate-Drug Conjugates," JPET 321: 462-468, 2007.

Benesova, M. et al., "Linker Modifications of DOTA-conjugated Inhibitors of the Prostate-Specific Membrane Antigen (PSMA)," poster, presented at the European Association of Nuclear Medicine Conference on Oct. 21, 2013.

Benesova, M. et al., "Linker Modifications of DOTA-conjugated Inhibitors of the Prostate-Specific Membrane Antigen (PSMA)," abstract, Eur. J. Nucl. Med. Mal. Imaging, available Oct. 16, 2013, 40, Suppl. 2, S193.

(56) **References Cited**

OTHER PUBLICATIONS

Rinnab, L. et al., "Evaluation of [11C]-choline positron-emission/computed tomography in patients with increasing prostate-specific antigen levels after primary treatment for prostate cancer," BJU Int, 2007, 100, 786,793.
Reske, S.N. et al., "Imaging Prostate Cancer with 11C-Choline PET/CT," J. Nucl. Med., 2006, 47, 1249-1254.
Zophel, K. et al., "Is 11C-choline the most appropriate tracer for KP prostate cancer?" Eur J Nucl Med Mo/ Imaging, 2004, 31, 756-759.
Extended European Search Report for European Application No. 18175078, dated Sep. 14, 2018.
Office Action for counterpart Indonesia Patent Application No. P00201603202, dated Jan. 28, 2019 (with English Translation).
Examination Report for counterpart Australian Patent Application No. 2018200419, dated Oct. 24, 2018.
Office Action for counterpart Japanese Patent Application No. 2017-210775, dated Nov. 6, 2018, with English Translation.
Supplementary Partial European Search Report for counterpart European Application No. 14861854.9, dated May 19, 2017.
Zophel et al., "Is 11C-choline the most appropriate tracer for prostate cancer? Against." Eur J Nucl Med Mol Imaging, 2004, 31: 756-759.
First Examination Report for counterpart Saudi Arabian Application No. 516370842, with English translation.
Vees, H., et al., "18F-choline and/or 11 C-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, 2007, 99, 1415-1420.
Larson, S. M., et al., "Tumor Localization of 16β-18F-Fluoro-5a-Dihydrotestosterone Versus 18F-FDG in Patients with Progressive, Metastatic Prostate Cancer," J Nucl Med, 2004, 45, 366-373.
Schuster, D.M., et al., "Initial Experience with the Radiotracer Anti-1-Am ino-3-18F-Fluorocyclobutane-1-Carboxylic Acid with PET/CT in Prostate Carcinoma," J. Nucl. Med., 2007, 48, 56-63.
Tehrani, O.S., et al., "Tumor Imaging Using 1-(2'-deoxy-2'-18F-Fluoro-β-D-Arabinofuranosyl) Thymine and PET," J. Nucl. Med., 2007, 48, 1436-1441.
Mease RC., et al., "N-[N-[(S)-1, 3-Dicarboxypropyl]Carbam oyl]-4-[18F[F luorobenzyl-L-Cysteine, [18F]DCFBC: A New Imaging Probe for Prostate Cancer," Clin. Cancer Res., 2008, 14, 3036-3043.
Zhou, J., et al., "Naag Peptidase Inhibitors and Their Potential for Diagnosis and Therapy," Nat Rev Drug Discovery, 2005, 4, 1015-1026.
Schulke, N., et al., "The homodimer of prostate-specific membrane antigen is a functional target for cancer therapy," Proc. Natl. Acad. Sci., USA, 2003, 100, 12590-12595.
Nan, F., et al., "Dual Function Glutamate-Related Ligands: Discovery of a Novel, Potent Inhibitor of Glutamate Carboxypeptidase II Possessing mGluR3 Agonist Activity," J. Med. Chem., 2000, 43, 772-774.
Lange, P.H., "ProstaScint scan for staging prostate cancer," Urology, 2001, 57, 402-406.
Haseman, M.K., et al., "Capromab Pendetide Imaging of Prostate Cancer," Cancer Bi other Radiopharm, 2000, 15, 131-140.
Mier W., et al., "Conjugation of DOTA Using Isolated Phenolic Active Esters: The Labeling and Biodistribution of Albumin as Blood Pool Marker," Bioconjugate Chem., 2005, 16: 237-240.
Schafer et al., "A dimerized urea-based inhibitor of the prostate-specific membrane antigen for 68Ga-PET imaging of prostate cancer," EJNMMI Research, 2012, 2, 23, 11 pages.
Humblet, V. et al., "An HPLC/mass spectrometry platform for the development of multimodality contrast agents and targeted therapeutics: prostate-specific membrane antigen small molecule derivatives," Contrast Med. Mal. Imaging, 2006, 1, 196-211.
Pomper, M.G., et al., "11C-MCG: synthesis, uptake selectivity, and primate PET of a probe for glutamate carboxypeptidase II (NAALADase)," Mal Imaging, 2002, 1, 96-101.
Japanese Office Action dated Mar. 23, 2017, which issued during prosecution of Japanese Application No. P016-524427.
Humblet, et al., "An HPLC/mass spectrometry platform for the development of multimodality contrast agents and targeted therapeutics: prostate-specific membrane antigen small molecule derivatives" Contrast Media & Molecular Imaging, Jul. 2006 1:196-211.
Eurasian Office Action dated Feb. 10, 2017, which issued during prosecution of Eurasian Application No. 201690495/28.
Liu M., et al., "Synthesis and Biological Evaluation of Diethylenetriamine Pentaacetic acid-Polyethylene Glycol Folate: A new Folate-Derived, 99mTc-Based Radiopharmaceutical," Bioconjugate Chem., 2005 vol. 16, p. 1126-1132.
Muller C., et al. "Synthesis and in Vitro/in Vivo Evaluation of Novel 99mTc(Co)3-Folates," Bioconjugate Chem., 2006 vol. 17, p. 797-806.
Viola-Villegas N., et al. "Targeting Gallium to Cancer Cells through the Folate Receptor," Drug Target Insights, 2008 vol. 3, p. 13-25.
Viola-Villegas N., et al. "Targeting the Folate Receptor (FR): Imaging and Cytotoxicity of Rel Conjugates in FR-Overexpressing Cancer Cells," ChemMedChem, 2008 vol. 3, p. 1387-1394.
Zhou J., "In vivo evaluation of medical device-associated inflammation using macrophage-specific position emission tomography (PET) imaging," Bioorganic and Medicinal Chemistry Letters, 2013 vol. 23, p. 2044-2047.
Kularatne SA., et al. "Comparative Analysis of Folate Derived PET Imaging Agents with [18F]-2-Fluoro-2-deoxy-D-glucose Using Rodent Inflammatory Paw Model," Molecular Pharmaceutics, 2013 vol. 10, p. 3103-3111.
Minner, S., Wittmer, C., Graefen, M., Salomon, G., Steuber, T., Haese, A., Huland, H., Bokemeyer, C., Yekebas, E., Dierlamm, J., Balabanov, S., Kilic, E., Wilczak, W., Simon, R., Sauter, G., and Schlomm, T. (2011) High level PSMA expression is associated with early PSA recurrence in surgically treated prostate cancer. Prostate 71, 281-8.
Malik, N., Machulla, H. J., Solbach, C., Winter, G., Reske, S. N., and Zlatopolskiy, B. (2011) Radiosynthesis of a new PSMA targeting ligand ([18F]FPy-DUPA-Pep). Appl Radiat Isot 69, 1014-8.
Lord, M., Ratib, O., and Vallee, J. P. (2011) (1)(8)F-Fluorocholine integrated PET/MRI for the initial staging of prostate cancer. Eur J Nud Med Mol Imaging 38, 2288.
Ho, C. L., Liu, I. H., Wu, Y. H., Chen, L. C., Chen, C. L, Lee, W. C., Chuang, C. H., Lee, T. W., Lin, W. J., Shen, L. H., and Chang, C. H. (2011) Molecular imaging, pharmacokinetics, and dosimetry of In-AMBA in human prostate tumor-bearing mice. J Biomed Biotechnol 2011, 101497.
Hillier, S. M., Kern, A. M., Maresca, K. P., Marquis, J. C., Eckelman, W. C., Joyal, J. L., and Babich, J. W. (2011) 123I-MIP-1072, a small-molecule inhibitor of prostate-specific membrane antigen, is effective at monitoring tumor response to taxane therapy. J Nucl Med 52, 1087-93.
Fortmuller, K., Alt, K., Gierschner, D., Wolf, P., Baum, V., Freudenberg, N., Wetterauer, U., Elsasser-Beile, U., and Buhler, P. (2011) Effective targeting of prostate cancer by lymphocytes redirected by a PSMA x CD3 bispecific single-chain diabody. Prostate 71, 588-96.
Evans, M. J., Smith-Jones, P. M., Wongvipat, J., Navarro, V., Kim, S., Bander, N. H., Larson, S. M., and Sawyers, C. L. (2011) Noninvasive measurement of androgen receptor signaling with a positron-emitting radiopharmaceutical that targets prostate-specific membrane antigen. Proc Natl Acad Sci U S A 108, 9578-82.
Emonds, K. M., Swinnen, J. V., van Weerden, W. M., Vanderhoydonc, F., Nuyts, J., Mortelmans, L., and Mottaghy, F. M. (2011) Do androgens control the uptake of 18F-FDG, 11C-choline and 11C-acetate in human prostate cancer cell lines? Eur J Nucl Med Mol Imaging 38, 1842-53.
Dahl, M., Bouchelouche, P., Kramer-Marek, G., Capala, J., Nordling, J., and Bouchelouche, K. (2011) Sarcosine induces increase in HER2/neu expression in androgen-dependent prostate cancer cells. Molecular Biology Reports 38, 4237-43.
Chuu, C. P., Kokontis, J. M., Hiipakka, R. A., Fukuchi, J., Lin, H. P., Lin, C. Y., Huo, C., Su, L. C., and Liao, S. (2011) Androgen suppresses proliferation of castration-resistant LNCaP 104-R2 prostate cancer cells through androgen receptor, Skp2, and c-Myc. Cancer science 102, 2022-8.

(56) **References Cited**

OTHER PUBLICATIONS

Chen, Y., Pullambhatla, M., Byun, Y., Foss, C. A., Nimmagadda, S., Senthamizhchelvan, S., Sgouros, G., Mease, R. C., and Pomper, M. G. (2011) 2-(3-{1-Carboxy-5-[(6-[18F]fluoro-pyridine-3-carbonyl)-amino]-pentyl}-urei do)-pentanedioic acid, [18F]DCFPyL, a PSMA-based PET Imaging Agent for Prostate Cancer. Clin Cancer Res 17(24), 7645-53.

Bouchelouche, K., Tagawa, S. T., Goldsmith, S. J., Turkbey, B., Capala, J., and Choyke, P. (2011) PET/CT Imaging and Radioimmunotherapy of Prostate Cancer. Semin Nucl Med 41, 29-44.

Banerjee, S. R., Pullambhatla, M., Sheilal, H., Lisok, A., Mease, R. C., and Pomper, M. G. (2011) A Modular Strategy to Prepare Multivalent Inhibitors of Prostate-Specific Membrane Antigen (PSMA). Oncotarget 2(12), 1244-53.

Zhang, A. X., Murelli, R. P., Barinka, C., Michel, J., Cocleaza, A., Jorgensen, W. L., Lubkowski, J., and Spiegel, D. A. (2010) A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules. J Am Chem Soc 132, 12711-6.

Wang, H., Byun, Y., Barinka, C., Pullambhatla, M., Bhang, H. E., Fox, J. J., Lubkowski, J., Mease, R. C., and Pomper, M. G. (2010) Bioisosterism of urea-based GCPII inhibitors: Synthesis and structure-activity relationship studies. Bioorg Med Chem Lett 20, 392-7.

Rioja, J., Rodriguez-Fraile, M., Lima-Favaretto, R., Rincon-Mayans, A., Penuelas-Sanchez, I., Zudaire-Bergera, J. J., and Parra, R. O. (2010) Role of positron emission tomography in urological oncology. BJU Int 106, 1578-93.

Poulsen, M. H., Bouchelouche, K., Gerke, O., Petersen, H., Svolgaard, B., Marcussen, N., Svolgaard, N., Ogren, M., Vach, W., Hoilund-Carlsen, P. F., Geertsen, U., and Walter, S. (2010) [18F]-fluorocholine positron-emission/computed tomography for lymph node staging of patients with prostate cancer: preliminary results of a prospective study. BJU Int 106, 639-44.

Mertens, K., Sleets, D., Lambert, B., Acou, M., De Vos, F., and Goethals, I. (2010) PET with (18)F-labelled choline-based tracers for tumour imaging: a review of the literature. Eur J Nucl Med Mol Imaging 37, 2188-93.

Maresca, K., Hillier, S., Lu, G., Marquis, J., Zimmerman, G., Eckelman, W., Joyal, J., and Babich, J. (2010) Influence of functionalized chelators on affinity and pharmacokinetics of 99mTc(CO)3-labeled small molecules targeting prostate specific membrane antigen (PSMA). J Nucl Med 51, 250.

Liu, T., Wu, L. Y., Hopkins, M. R., Choi, J. K., and Berkman, C. E. (2010) A targeted low molecular weight near-infrared fluorescent probe for prostate cancer. Bioorg Med Chem Lett 20, 7124-6.

Kularatne, S. A., Venkatesh, C., Santhapuram, H. K., Wang, K., Vaitilingam, B., Henne, W. A., and Low, P. S. (2010) Synthesis and biological analysis of prostate-specific membrane antigen-targeted anticancer prodrugs. J Med Chem 53, 7767-77.

Jambor, I., Borra, R., Kemppainen, J., Lepomaki, V., Parkkola, R., Dean, K., Alanen, K., Arponen, E., Nurmi, M., Aronen, H. J., and Minn, H. (2010) Functional imaging of localized prostate cancer aggressiveness using 11C-acetate PET/CT and 1H-MR spectroscopy. J Nucl Med 51, 1676-83.

Hong, H., Zhang, Y., Sun, J., and Cai, W. (2010) Positron emission tomography imaging of prostate cancer. Amino Acids 39, 11-27.

Holland, J. P., Divilov, V., Bander, N. H., Smith-Jones, P. M., Larson, S. M., and Lewis, J. S. (2010) 89Zr-DFO-J591 for immunoPET of prostate-specific membrane expression in vivo. J Nucl Med 51, 1293-300.

Giovacchini, G., Picchio, M., Coradeschi, E., Bettinardi, V., Gianolli, L., Scattoni, V., Cozzaini, C., Di Muzio, N., Rigatti, P., Fazio, F., and Messa, C. (2010) Predictive factors of [(11)C]choline PET/CT in patients with biochemical failure after radical prostatectomy. Eur J Nucl Med Mol Imaging 37, 301-9.

Bouchelouche, K., Turkbey, B., Choyke, P., and Capala, J. (2010) Imaging prostate cancer: an update on positron emission tomography and magnetic resonance imaging. Current urology reports 11, 180-90.

Bouchelouche, K., Choyke, P. L., and Capala, J. (2010) Prostate specific membrane antigen—a target for imaging and therapy with radionuclides. Discov Med 9, 55-61.

Bouchelouche, K., and Capala, J. (2010) 'Image and treat': an individualized approach to urological tumors. Curr Opin Oncol 22, 274-80.

Banerjee, S. R., Pullambhatla, M., Byun, Y., Nimmagadda, S., Green, G., Fox, J. J., Horti, A., Mease, R. C., and Pomper, M. G. (2010) 68Ga-labeled inhibitors of prostate-specific membrane antigen (PSMA) for imaging prostate cancer. J Med Chem 53, 5333-41.

Alt, K., Wiehr, S., Ehrlichmann, W., Reischl, G., Wolf, P., Pichler, B. J., Elsasser-Beile, U., and Buhler, P. (2010) High-resolution animal PET imaging of prostate cancer xenografts with three different 64Cu-labeled antibodies against native cell-adherent PSMA. Prostate 70, 1413-21.

Zaheer, A., Cho, S. Y., and Pomper, M. G. (2009) New agents and techniques for imaging prostate cancer. J Nucl Med 50, 1387-90.

Rinnab, L., Simon, J., Hautmann, R. E., Cronauer, M. V., Hohl, K., Buck, A. K., Reske, S. N., and Mottaghy, F. M. (2009) [(11)C]choline PET/CT in prostate cancer patients with biochemical recurrence after radical prostatectomy. World J Urol 27, 619-25.

Pillarsetty, N., Punzalan, B., and Larson, S. M. (2009) 2-18F-Fluoropropionic acid as a PET imaging agent for prostate cancer. J Nucl Med 50, 1709-14.

Maresca, K. P., Hillier, S. M., Femia, F. J., Keith, D., Barone, C., Joyal, J. L., Zimmerman, C. N., Kozikowski, A. P., Barrett, J. A., Eckelman, W. C., and Babich, J. W. (2009) A series of halogenated heterodimeric inhibitors of prostate specific membrane antigen (PSMA) as radiolabeled probes for targeting prostate cancer. J Med Chem 52, 347-57.

Mannweiler, S., Amersdorfer, P., Trajanoski, S., Terrett, J. A., King, D., and Mehes, G. (2009) Heterogeneity of prostate-specific membrane antigen (PSMA) expression in prostate carcinoma with distant metastasis. Pathology oncology research : POR 15, 167-72.

Lapi, S. E., Wahnishe, H., Pham, D., Wu, L. Y., Nedrow-Byers, J. R., Liu, T., Vejdani, K., VanBrocklin, H. F., Berkman, C. E., and Jones, E. F. (2009) Assessment of an 18F-labeled phosphoramidate peptidomimetic as a new prostate-specific membrane antigen-targeted imaging agent for prostate cancer. J Nucl Med 50, 2042-8.

Kwee, S. A., Coel, M. N., Ly, B. H., and Lim, J. (2009) (18)F-Choline PET/CT imaging of RECIST measurable lesions in hormone refractory prostate cancer. Ann Nucl Med 23, 541-8.

Kularatne, S. A., Zhou, Z., Yang, J., Post, C. B., and Low, P. S. (2009) Design, synthesis, and preclinical evaluation of prostate-specific membrane antigen targeted (99m)Tc-radioimaging agents. Mol Pharm 6, 790-800.

Kularatne, S. A., Wang, K., Santhapuram, H. K., and Low, P. S. (2009) Prostate-specific membrane antigen targeted imaging and therapy of prostate cancer using a PSMA inhibitor as a homing ligand. Mol Pharm 6, 780-9.

Klusak, V., Barinka, C., Plechanovova, A., Mlcochova, P., Konvalinka, J., Rulisek, L., and Lubkowski, J. (2009) Reaction mechanism of glutamate carboxypeptidase II revealed by mutagenesis, X-ray crystallography, and mmputational methods. Biochemistry 48, 4126-38.

Jadvar, H. (2009) Molecular imaging of prostate cancer with 18F-fluorodeoxyglucose PET. Nat Rev Urol 6, 317-23.

Humblet, V., Misra, P., Bhushan, K. R., Nasr, K., Ko, Y. S., Tsukamoto, T., Pannier, N., Frangioni, J. V., and Maison, W. (2009) Multivalent scaffolds for affinity maturation of small molecule cell surface binders and their application to prostate tumor targeting. J Med Chem 52, 544-50.

Hlouchova, K., Barinka, C., Konvalinka, J., and Lubkowski, J. (2009) Structural insight into the evolutionary and pharmacologic homology of glutamate carboxypeptidases II and III. FEBS J 276, 4448-62.

Hillier, S. M., Maresca, K. P., Femia, F. J., Marquis, J. C., Foss, C. A., Nguyen, N., Zimmerman, C. N., Barrett, J. A., Eckelman, W. C., Pomper, M. G., Joyal, J. L., and Babich, J. W. (2009) Preclinical evaluation of novel glutamate-urea-lysine analogues that target prostate-specific membrane antigen as molecular imaging pharmaceuticals for prostate cancer. Cancer Res 69, 6932-40.

(56) **References Cited**

OTHER PUBLICATIONS

Haffner, M. C., Kronberger, I. E., Ross, J. S., Sheehan, C. E., Zitt, M., Muhlmann, G., Ofner, D., Zelger, B., Ensinger, C., Yang, X. J., Geley, S., Margreiter, R., and Bander, N. H. (2009) Prostate-specific membrane antigen expression in the neovasculature of gastric and colorectal cancers. Hum Pathol 40, 1754-61.

Elsasser-Beile, U., Reischl, G., Wiehr, S., Buhler, P., Wolf, P., Alt, K., Shively, J., Judenhofer, M. S., Machulla, H. J., and Pichler, B. J. (2009) PET imaging of prostate cancer xenografts with a highly specific antibody against the prostate-specific membrane antigen. J Nucl Med 50, 606-11.

Elsasser-Beile, U., Buhler, P., and Wolf, P. (2009) Targeted therapies for prostate cancer against the prostate specific membrane antigen. Curr Drug Targets 10, 118-25.

Chen, Y., Dhara, S., Banerjee, S. R., Byun, Y., Pullambhatla, M., Mease, R. C., and Pomper, M. G. (2009) A low molecular weight PSMA-based fluorescent imaging agent for cancer. Biochem Biophys Res Commun 390, 624-9.

Bouchelouche, K., Capala, J., and Oehr, P. (2009) Positron emission tomography/computed tomography and radioimmunotherapy of prostate cancer. Curr Opin Oncol 21, 469-74.

Belloli, S., Jachetti, E., Moresco, R. M., Picchio, M., Lecchi, M., Valtorta, S., Freschi, M., Michelini, R. H., Bellone, M., and Fazio, F. (2009) Characterization of preclinical models of prostate cancer using PET-based molecular imaging. Eur J Nucl Med Mol Imaging 36, 1245-55.

Zechmann, C. M., Afshar-Oromieh, A., Armor, T., Stubbs, J. B., Mier, W., Hadaschik, B., Joyal, J., Kopka, K., Debus, J., Babich, J. W., and Haberkorn, U. (2014) Radiation dosimetry and first therapy results with a I/ I-labeled small molecule (MIP-1095) targeting PSMA for prostate cancer therapy. Eur J Nucl Med Mol Imaging 41, 1280-92.

Huang, S. S., Wang, X., Zhang, Y., Doke, A., DiFilippo, F. P., and Heston, W. D. (2014) Improving the biodistribution of PSMA-targeting tracers with a highly negatively charged linker. Prostate 74, 702-13.

Wiehr, S., Buhler, P., Gierschner, D., Wolf, P., Rolle, A. M., Kesenheimer, C., Pichler, B. J., and Elsasser-Beile, U. (2014) Pharmacokinetics and PET imaging properties of two recombinant anti-PSMA antibody fragments in comparison to their parental antibody. Prostate 74. 743-55.

Weineisen, M., Simecek, J., Schottelius, M., Schwaiger, M., and Wester, H. J. (2014) Synthesis and preclinical evaluation of DOTAGA-conjugated PSMA ligands for functional imaging and endoradiotherapy of prostate cancer. EJNMMI Res 4, 63.

Wang, X., Huang, S. S., Heston, W. D., Guo, H., Wang, B. C., and Basilion, J. P. (2014) Development of targeted near-infrared imaging agents for prostate cancer. Mol Cancer Ther 13(11), 2595-606.

Uprimny, C., Kroiss, A., Nilica, B., Buxbaum, S., Decristoforo, C., Horninger, W., and Virgolini, I. J. (2014) Ga-PSMA ligand PET versus F-NaF PET: evaluation of response to Ra therapy in a prostate cancer patient. Eur J Nucl Med Mol Imaging 42, 362-63.

Hillier, S. M., Maresca, K. P., Lu, G., Merkin, R. D., Marquis, J. C., Zimmerman, C. N., Eckelman, W. C., Joyal, J. L., and Babich, J. W. (2013) 99mTc-labeled small-molecule inhibitors of prostate-specific membrane antigen for molecular imaging of prostate cancer. J Nucl Med 54, 1369-76.

Jadvar, H. (2013) Imaging evaluation of prostate cancer with (18)F-fluorodeoxyglucose PET/CT: utility and limitations. Eur J Nucl Med Mol Imaging 40 (Suppl 1), S5-S10.

Jemaa, A. B., Bouraoui, Y., Sallami, S., Banasr, A., Nouira, Y., Horchani, A., and Oueslati, R. (2013) Cellular distribution and heterogeneity of Psa and Psma expression in normal, hyperplasia and human prostate cancer. La tunisie Medicale 91, 458-463.

Kasperzyk, J. L., Finn, S. P., Flavin, R., Fiorentino, M., Lis, R., Hendrickson, W. K., Clinton, S. K., Sesso, H. D., Giovannucci, E. L., Stampfer, M. J., Loda, M., and Mucci, L. A. (2013) Prostate-specific membrane antigen protein expression in tumor tissue and risk of lethal prostate cancer. Cancer Epidemiol Biomarkers Prev 22, 2354-63.

Kasten, B. B., Liu, T., Nedrow-Byers, J. R., Benny, P. D., and Berkman, C. E. (2013) Targeting prostate cancer cells with PSMA inhibitor-guided gold nanoparticles. Bioorg Med Chem Lett 23, 565-8.

Tykvart, J., Schimer, J., Barinkova, J., Pachl, P., Postova-Slavetinska, L., Majer, P., Konvalinka, J., and Sacha, P. (2014) Rational design of urea-based glutamate carboxypeptidase II (GCPII) inhibitors as versatile tools for specific drug targeting and delivery. Bioorg Med Chem 22, 4099-108.

Ristau, B. T., O'Keefe, D. S., and Bacich, D. J. (2014) The prostate-specific membrane antigen: Lessons and current clinical implications from 20 years of research. Urol Oncol 32, 272-279.

Ray Banerjee, S., Pullambhatla, M., Foss, C. A., Nimmagadda, S., Ferdani, R., Anderson, C. J., Mease, R. C., and Pomper, M. G. (2014) 64Cu-Labeled Inhibitors of Prostate-Specific Membrane Antigen for PET Imaging of Prostate Cancer. J Med Chem 57, 2657-69.

Rais, R., Rojas, C., Wozniak, K., Wu, Y., Zhao, M., Tsukamoto, T., Rudek, M. A., and Slusher, B. S. (2014) Bioanalytical method for evaluating the pharmacokinetics of the GCP-II inhibitor 2-phosphonomethyl pentanedioic acid (2-PMPA). Journal of pharmaceutical and biomedical analysis 88, 162-9.

Pavlicek, J., Ptacek, J., Cerny, J., Byun, Y., Skuttetyova, L., Pomper, M. G., Lubkowski, J., and Barinka, C. (2014) Structural characterization of P1'-diversified urea-based inhibitors of glutamate carboxypeptidase II. Bioorg Med Chem Lett 24, 2340-5.

Lutje, S., Rijpkema, M., Franssen, G. M., Fracasso, G., Helfrich, W., Eek, A., Oyen, W. J., Colombatti, M., and Boerman, O. C. (2014) Dual-Modality Image-Guided Surgery of Prostate Cancer with a Radiolabeled Fluorescent Anti-PSMA Monoclonal Antibody. J Nucl Med 55, 995-1001.

Liu, T., Mendes, D. E., and Berkman, C. E. (2014) Functional prostate-specific membrane antigen is enriched in exosomes from prostate cancer cells. Int J Oncol 44, 918-22.

Krohn, T., Verburg, F. A., Pufe, T., Neuhuber, W., Vogg, A., Heinzel, A., Mottaghy, F. M., and Behrendt, F. F. (2014) [Ga]PSMA-HBED uptake mimicking lymph node metastasis in coeliac ganglia: an important pitfall in clinical practice. Eur J Nucl Med Mol Imaging 42, 210-14.

Kovar, J. L., Cheung, L. L., Simpson, M. A., and Olive, D. M. (2014) Pharmacokinetic and Biodistribution Assessment of a Near Infrared-Labeled PSMA-Specific Small Molecule in Tumor-Bearing Mice. Prostate cancer 2014, 104248.

Huang, B., Otis, J., Joice, M., Kotlyar, A., and Thomas, T. P. (2014) PSMA-Targeted Stably Linked "Dendrimer-Glutamate Urea-Methotrexate" as a Prostate Cancer Therapeutic. Biomacromolecules 15, 915-23.

Haberkom, U., Mier, W., Dimitrakopoulou-Strauss, A., Eder, M., Kopka, K., and Altmann, A. (2014) Mechanistic and high-throughput approaches for the design of molecular imaging probes and targeted therapeutics. Clinical and Translational Imaging 2, 33-41.

El-Zaria, M. E., Genady, A. R., Janzen, N., Petlura, C. I., Beckford Vera, D. R., and Valliant, J. F. (2014) Preparation and evaluation of carborane-derived inhibitors of prostate specific membrane antigen (PSMA). Dalton Trans 43, 4950-61.

Eiber, M., Nekolla, S. G., Maurer, T., Weirich, G., Wester, H. J., and Schwaiger, M. (2014) Ga-PSMA PET/MR with multimodality image analysis for primary prostate cancer. Abdom Imaging 40, 1769-71.

Eder, M., Schafer, M., Bauder-Wüst, U., Haberkorn, U., Eisenhut, M., and Kopka, K. (2014) Preclinical evaluation of a bispecific low-molecular heterodimer targeting both PSMA and GRPR for improved PET imaging and therapy of prostate cancer. The Prostate 74, 659-68.

Eder, M., Neels, O., Muller, M., Bauder-Wust, U., Remde, Y., Schafer, M., Hennrich, U., Eisenhut, M., Afshar-Oromieh, A., Haberkorn, U., and Kopka, K. (2014) Novel Preclinical and Radiopharmaceutical Aspects of [68Ga]Ga-PSMA-HBED-CC: A New PET Tracer for Imaging of Prostate Cancer. Pharmaceuticals 7, 779-96.

(56) **References Cited**

OTHER PUBLICATIONS

Afshar-Oromieh, A., Zechmann, C. M., Matcher, A., Eder, M., Eisenhut, M., Linhart, H. G., Holland-Letz, T., Hadaschik, B. A., Giesel, F. L., Debus, J., and Haberkorn, U. (2014) Comparison of PET imaging with a Ga-labelled PSMA ligand and F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. Eur J Nud Med Mol Imaging 41, 11-20.

Afshar-Oromieh, A., Haberkorn, U., Schlemmer, H. P., Fenchel, M., Eder, M., Eisenhut, M., Hadaschik, B. A., Kopp-Schneider, A., and Rothke, M. (2014) Comparison of PET/CT and PET/MRI hybrid systems using a 68Ga-labelled PSMA ligand for the diagnosis of recurrent prostate cancer: initial experience. Eur J Nucl Med Mol Imaging 41, 887-97.

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., and Haberkorn, U. (2014) The diagnostic value of PET/CT imaging with the Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. Eur J Nucl Med Mol Imaging 42, 197-209.

Whitaker, H. C., Shiong, L. L., Kay, J. D., Gronberg, H., Warren, A. Y., Seipel, A., Wiklund, F., Thomas, B., Wiklund, P., Miller, J. L., Menon, S., Ramos-Montoya, A., Vowler, S. L., Massie, C., Egevad, L., and Neal, D. E. (2013) N-acetyl-L-aspartyl-L-glutamate peptidase-like 2 is overexpressed in cancer and promotes a pro-migratory and pro-metastatic phenotype. Oncogene 33, 5274-87.

Simone, C. B., 2nd and Hahn, S. M. (2013) What's in a Label? Radioimmunotherapy for metastatic prostate cancer. Clin Cancer Res 19, 4908-10.

Rybalov, M., Breeuwsma, A. J., Leliveld, A. M., Pruim, J., Dierckx, R. A., and de Jong, I. J. (2013) Impact of total PSA, PSA doubling time and PSA velocity on detection rates of (11)C-Choline positron emission tomography in recurrent prostate cancer. World J Urol 31, 319-23.

Rothke, M. C., Afshar-Oromieh, A., and Schlemmer, H. P. (2013) [Potential of PET/MRI for diagnosis of prostate cancer]. Radiologe 53, 676-81.

Roethke, M. C., Kuru, T. H., Afshar-Oromieh, A., Schlemmer, H. P., Hadaschik, B. A., and Fenchel, M. (2013) Hybrid positron emission tomography-magnetic resonance imaging with gallium 68 prostate-specific membrane antigen tracer: a next step for imaging of recurrent prostate cancer-preliminary results. Eur Urol 64, 862-4.

Kim, D., Kim, S. K., Valencia, C. A., and Liu, R. (2013) Tribody: robust self-assembled trimeric targeting ligands with high stability and significantly improved target-binding strength. Biochemistry 52, 7283-94.

Ray Banerjee, S., Pullambhatla, M., Foss, C. A., Falk, A., Byun, Y., Nimmagadda, S., Mease, R. C., and Pomper, M. G. (2013) Effect of Chelators on the Pharmacokinetics of Tc-Labeled Imaging Agents for the Prostate-Specific Membrane Antigen (PSMA). J Med Chem 56, 6108-21.

Parker, S. A., Diaz, I. L., Anderson, K. A., and Batt, C. A. (2013) Design, production, and characterization of a single-chain variable fragment (ScFv) derived from the prostate specific membrane antigen (PSMA) monoclonal antibody J591. Protein Expr Purif 89, 136-145.

Osborne, J. R., Green, D. A., Spratt, D. E., Lyashchenko, S., Fareedy, S. B., Robinson, B. D., Beattie, B. J., Jain, M., Lewis, J. S., Christos, P., Larson, S. M., Bander, N. H., and Scherr, D. S. (2014) A Prospective Pilot Study of Zr-J591/Prostate Specific Membrane Antigen Positron Emission Tomography in Men with Localized Prostate Cancer Undergoing Radical Prostatectomy. J Urol 191, 1439-46.

Osborne, J. R., Akhtar, N. H., Vallabhajosula, S., Anand, A., Deh, K., and Tagawa, S. T. (2013) Prostate-specific membrane antigen-based imaging. Urol Oncol 31, 144-54.

Nedrow-Byers, J. R., Moore, A. L., Ganguly, T., Hopkins, M. R., Fulton, M. D., Benny, P. D., and Berkman, C. E. (2013) PSMA-targeted SPECT agents: mode of binding effect on in vitro performance. Prostate 73, 355-62.

Mease RC, Foss CA, and MG, P. (2013) PET imaging in prostate cancer: focus on prostate-specific membrane antigen. Curr Top Med Chem 13, 951-962.

Lu, G., Maresca, K. P., Hillier, S. M., Zimmerman, C. N., Eckelman, W. C., Joyal, J. L., and Babich, J. W. (2013) Synthesis and SAR of (9)(9)mTc/Re-labeled small molecule prostate specific membrane antigen inhibitors with novel polar chelates. Bioorg Med Chem Lett 23, 1557-63.

Lesche, R., Kettschau, G., Gromov, A. V., Bohnke, N., Bodcowski, S., Monning, U., Hegele-Hartung, C., Dohr, O., Dinkelborg, L. M., and Graham, K. (2014) Preclinical evaluation of BAY 1075553, a novel F-labelled inhibitor of prostate-specific membrane antigen for PET imaging of prostate cancer. Eur J Nucl Med Mol Imaging 41, 89-101.

Office Action for co-pending Canadian Application No. 2924360, dated Sep. 11, 2018.

Office Action for counterpart Eurasian Patent Application No. 201690495, dated Dec. 20, 2018 (with English translation).

* cited by examiner

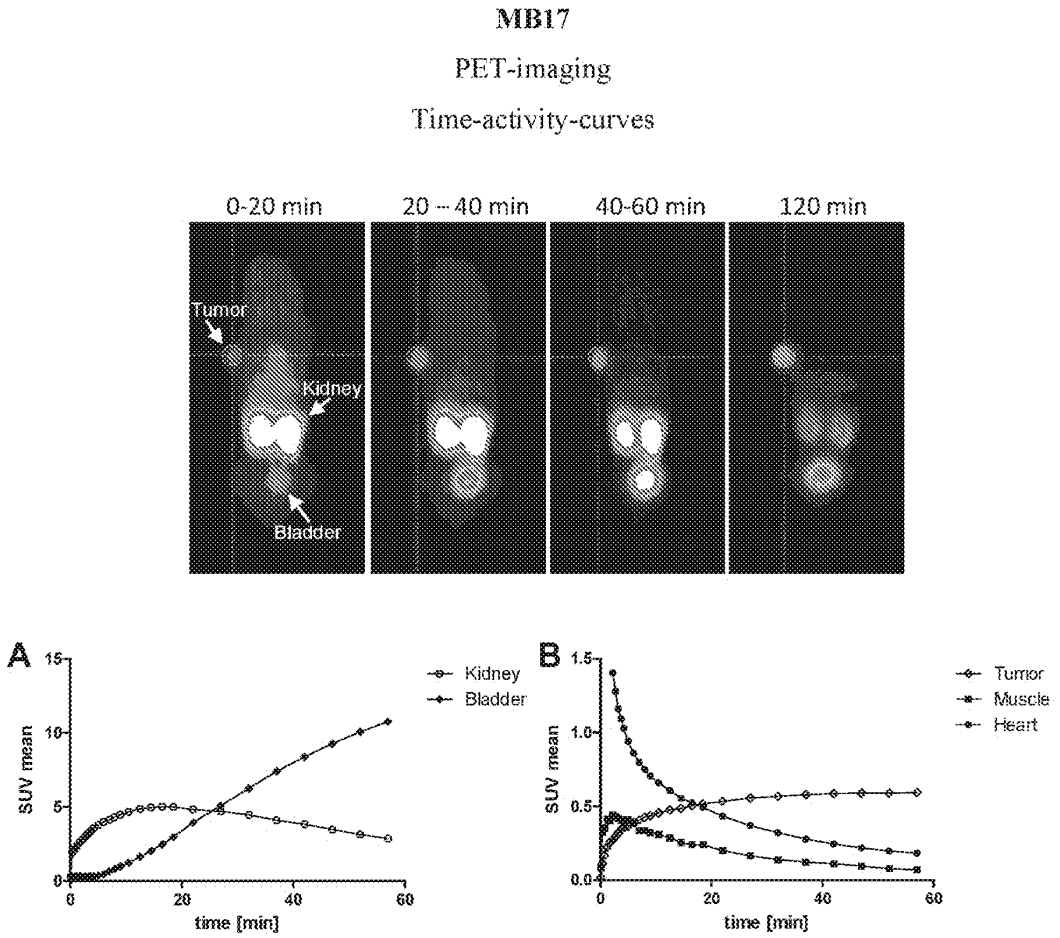

MB17
PET-imaging
Time-activity-curves
0-20 min
20 – 40 min
40-60 min
120 min
Tumor
Kidney
Bladder
A
SUV mean
time [min]
Kidney
Bladder
B
SUV mean
time [min]
Tumor
Muscle
Heart

FIG. 1

Organ distribution at 1 h post injection

	MB17			MB17 blocked		
	%ID/g	SD	n	%ID/g	SD	n
Blood	1.08	0.25	3	1.24	0.92	3
Heart	0.53	0.15	3	0.49	0.27	3
Lung	1.41	0.41	3	1.10	0.53	3
Spleen	2.13	0.16	3	0.53	0.36	3
Liver	1.17	0.10	3	0.73	0.07	3
Kidney	113.34	24.45	3	2.38	1.40	3
Muscle	0.50	0.07	3	0.24	0.09	3
Intestine	0.46	0.13	3	0.35	0.23	3
Brain	0.12	0.01	3	0.07	0.01	3
LNCaP Tumor	8.47	4.09	3	0.98	0.32	3

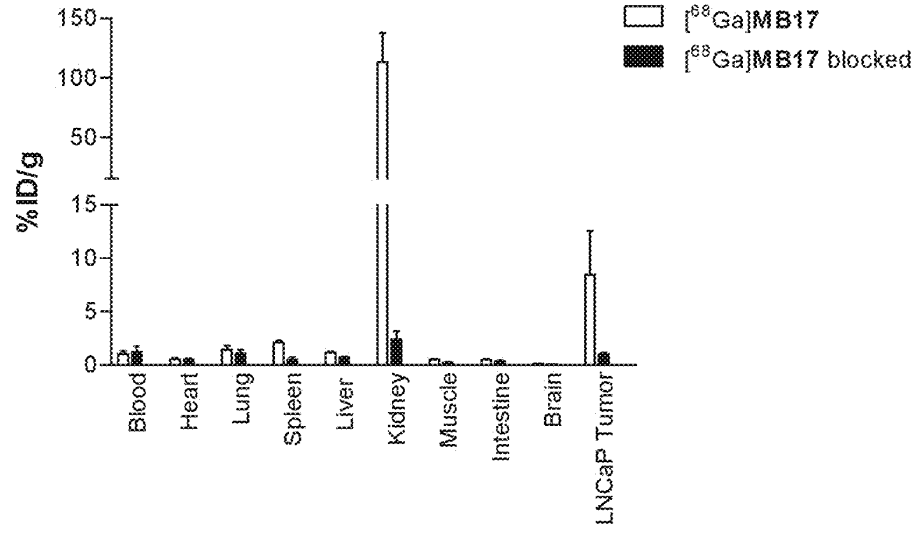


FIG. 2

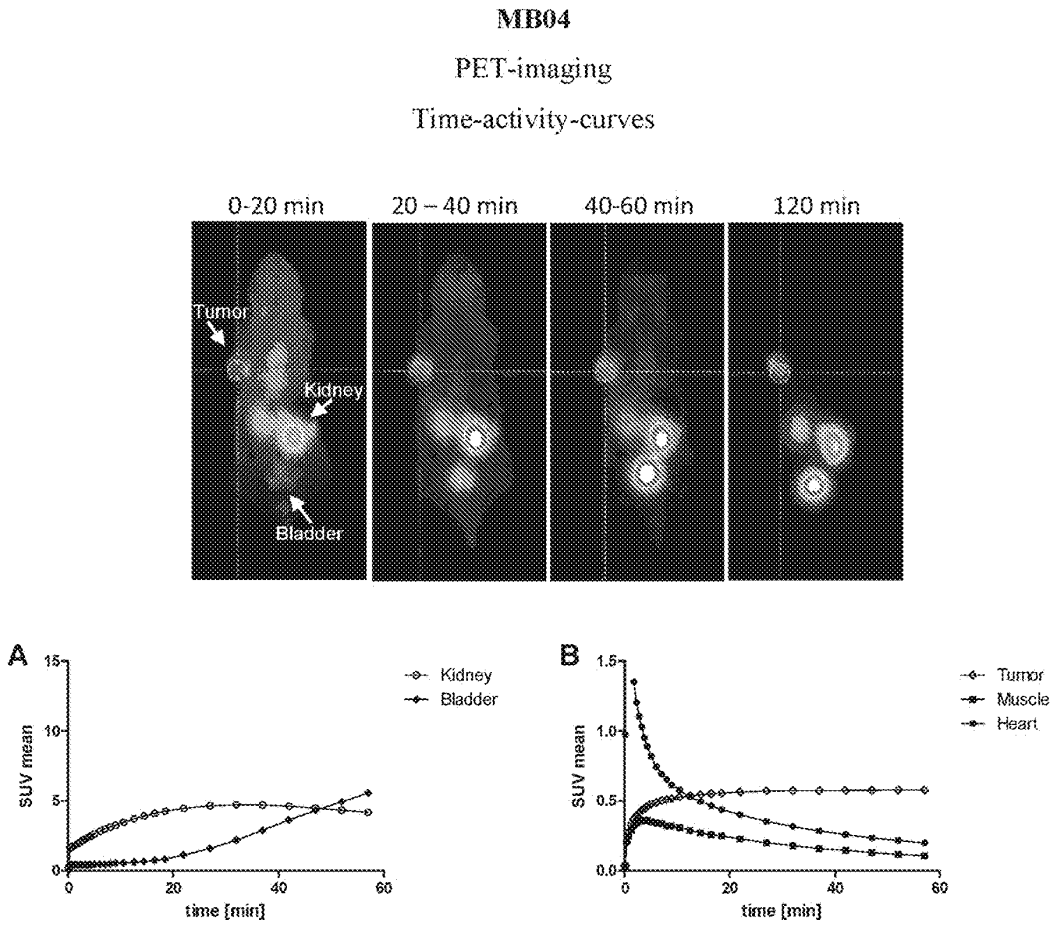
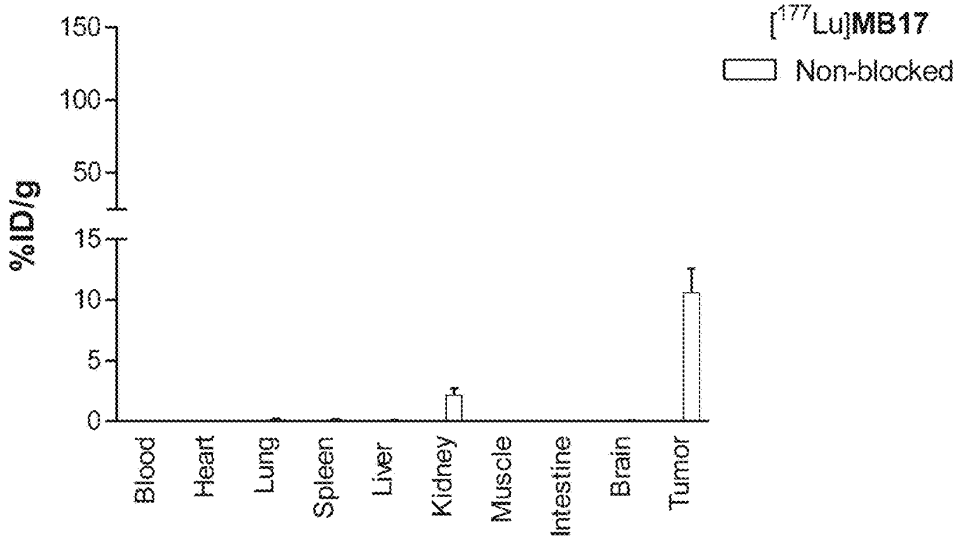

MB04
PET-imaging
Time-activity-curves
0-20 min
20 – 40 min
40-60 min
120 min
Tumor
Kidney
Bladder
A
SUV mean
time [min]
Kidney
Bladder
B
SUV mean
time [min]
Tumor
Muscle
Heart

FIG. 3



24 h	%ID/g	SD	n
Blood	0,01	0,00	5
Heart	0,02	0,01	5
Lung	0,11	0,13	5
Spleen	0,13	0,05	5
Liver	0,08	0,03	5
Kidney	2,13	1,36	5
Muscle	0,02	0,01	5
Intestine	0,02	0,01	5
Brain	0,05	0,03	5
LNCaP Tumor	10,58	4,50	5

FIG. 4

MB-02

PET-imaging

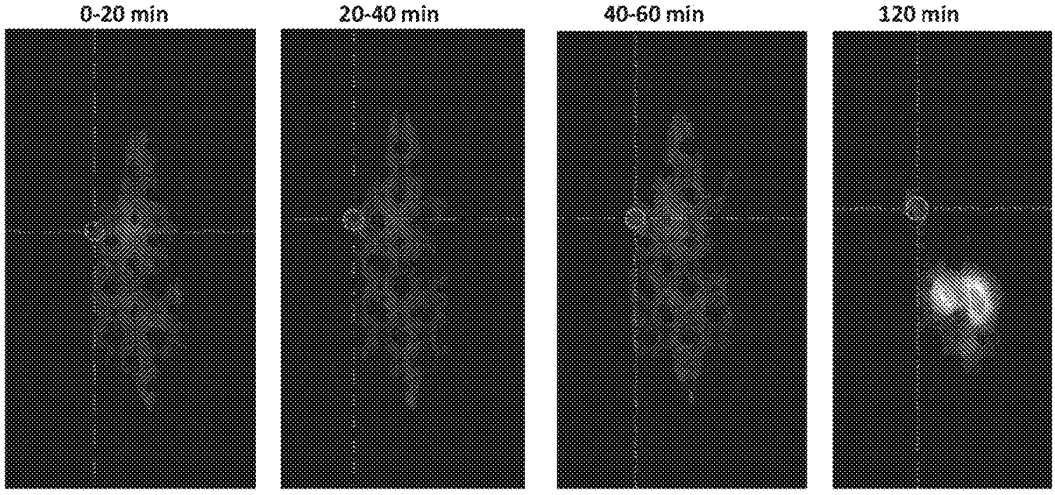


FIG. 5

MB-03

PET-imaging

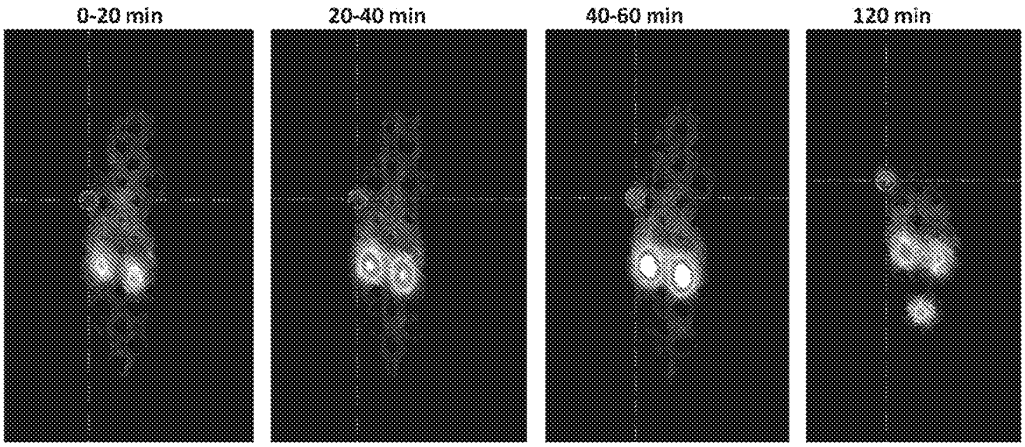


FIG. 6

MB-10

PET-imaging

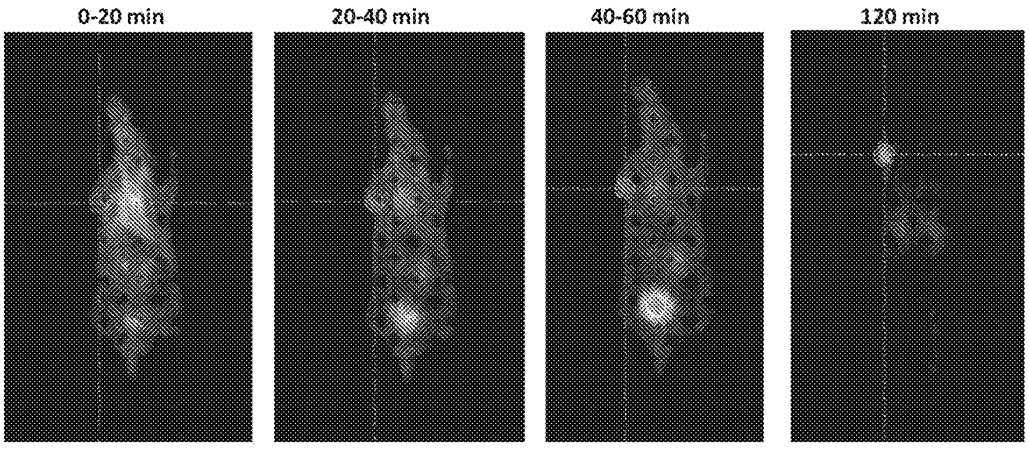


FIG. 7

MB-17.D

PET-imaging

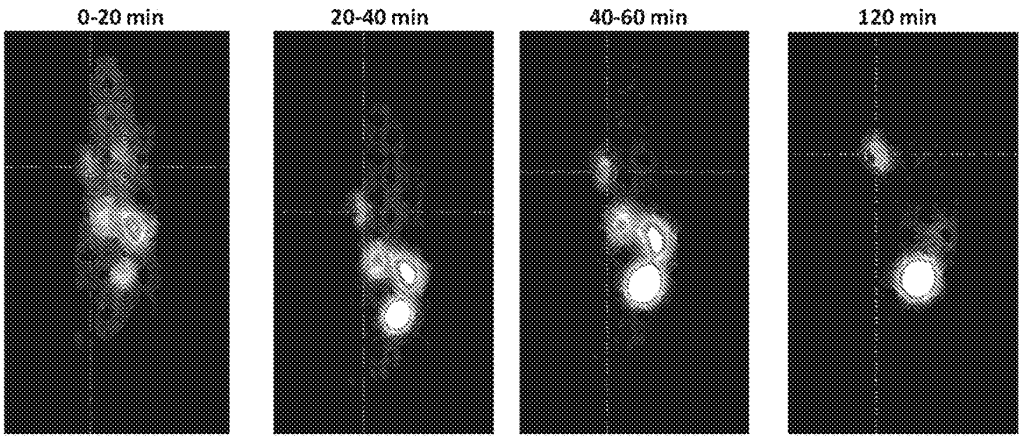


FIG. 8

MB-22

PET-imaging

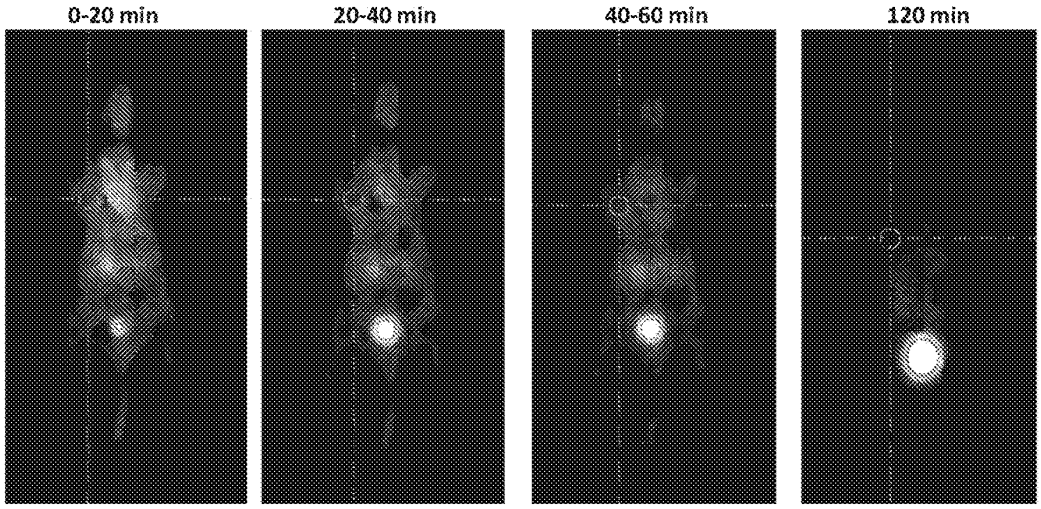


FIG. 9

MB-24

PET-imaging

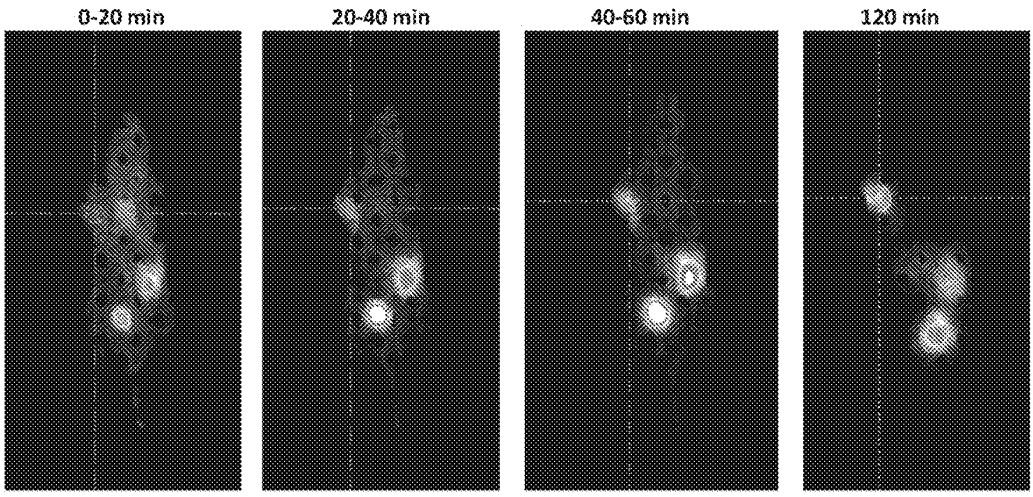


FIG. 10

MB-25

PET-imaging

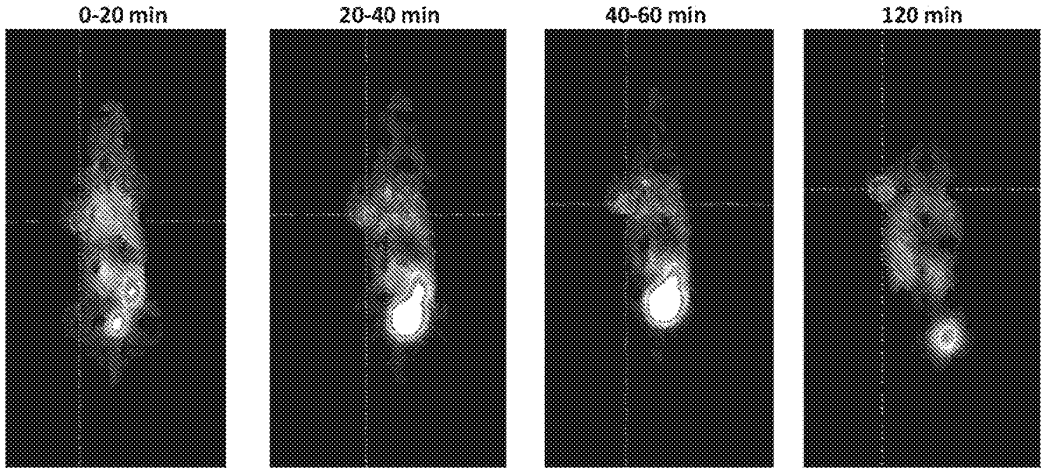


FIG. 11

MB-31

PET-imaging

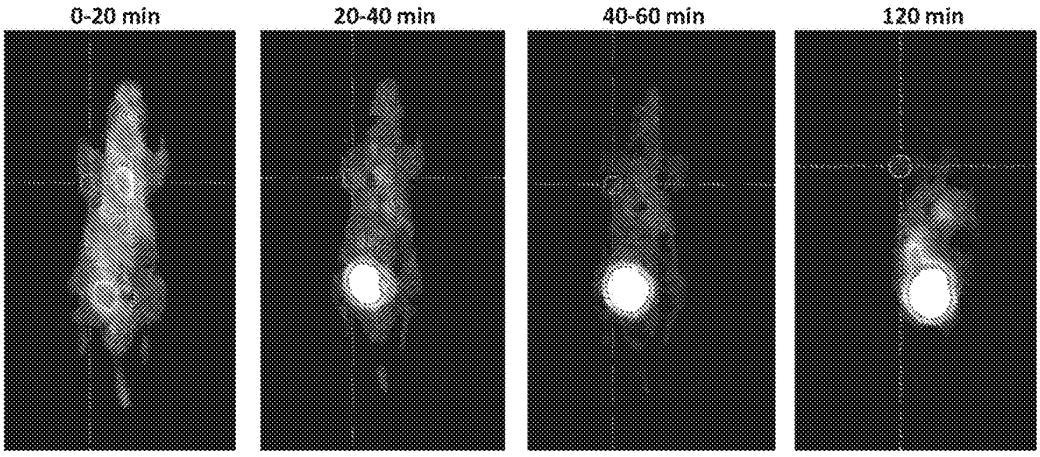


FIG. 12

MB-33

PET-imaging

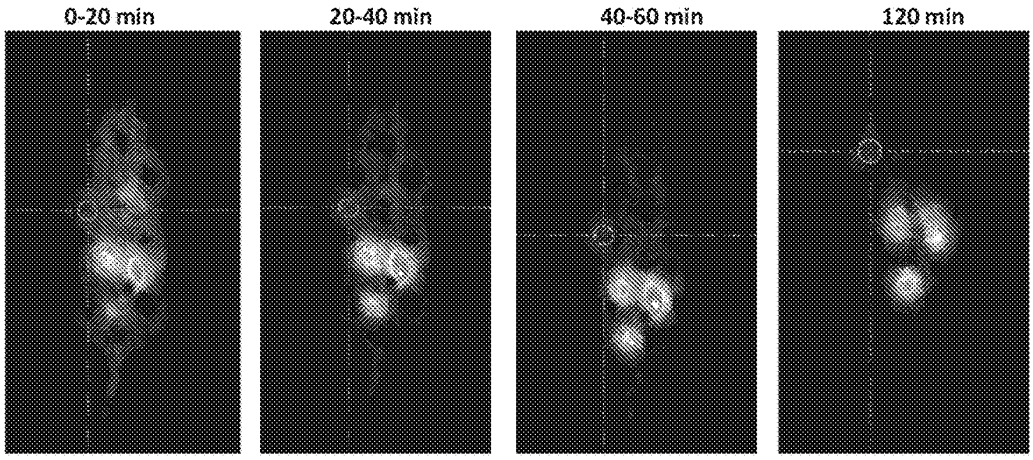


FIG. 13

MB-35

PET-imaging

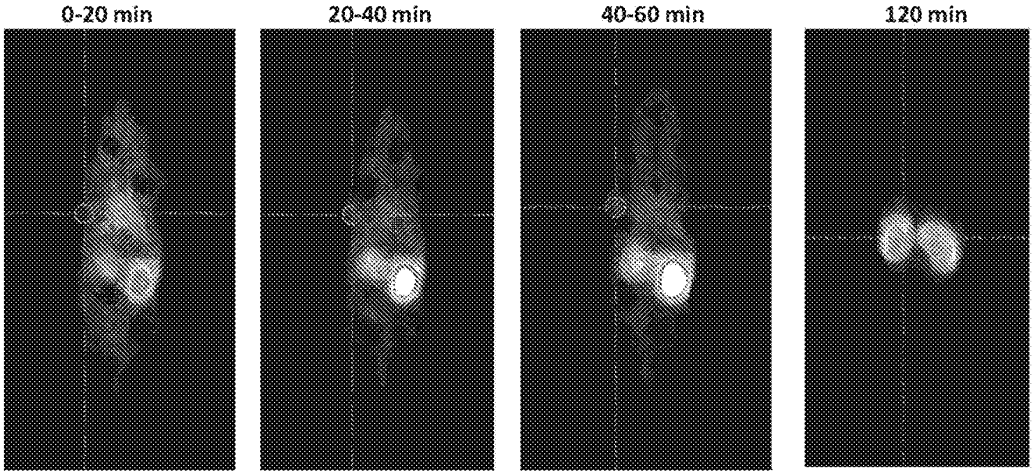


FIG. 14

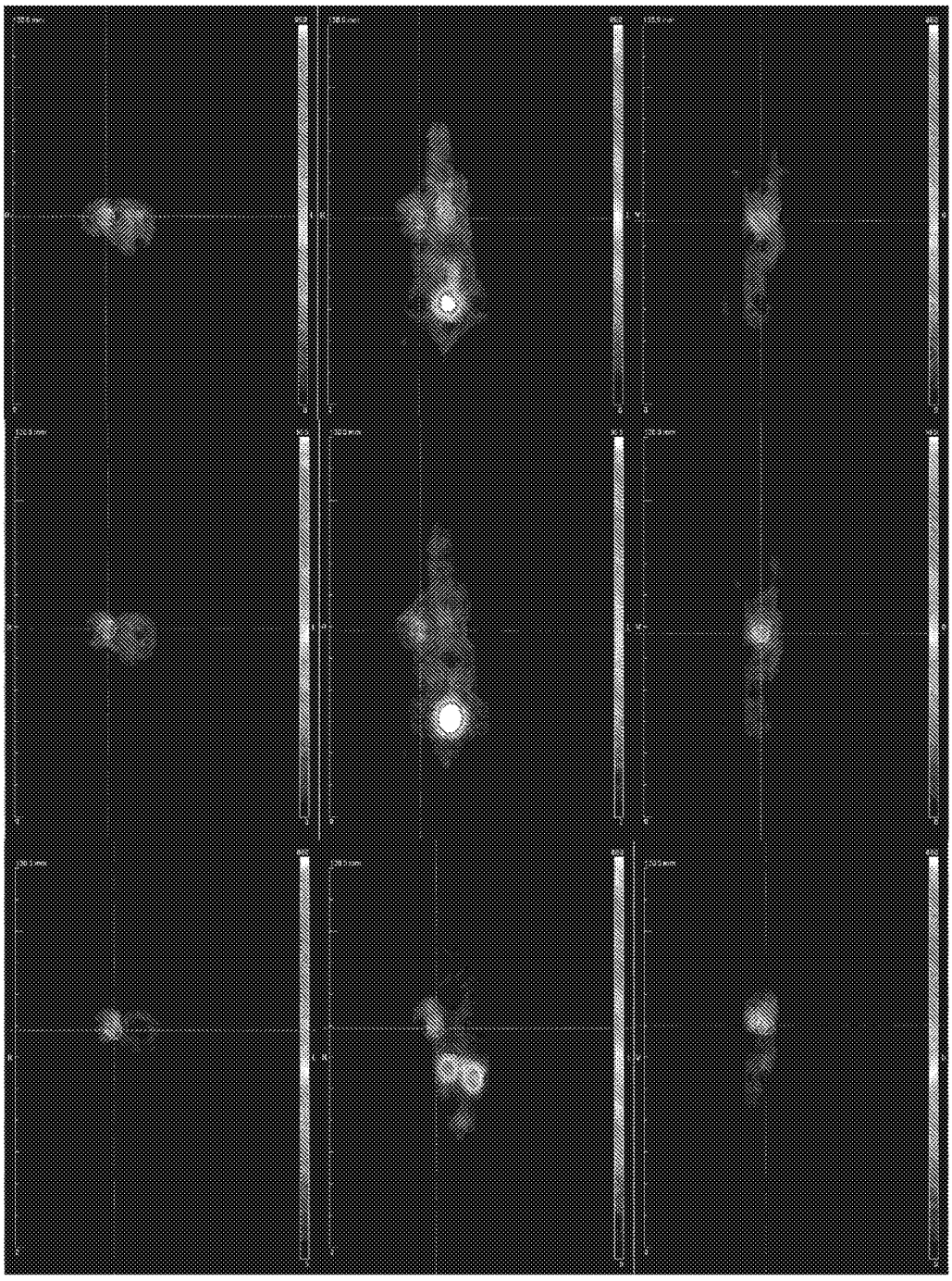


FIG. 15

MB-17 vs MB-17.D

PET-imaging after 2 hours post injection, comparison of whole-body scan

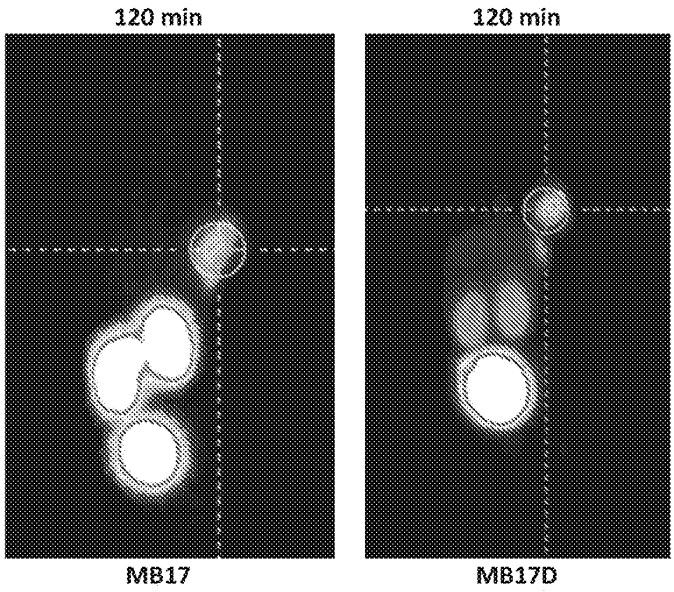


FIG. 16

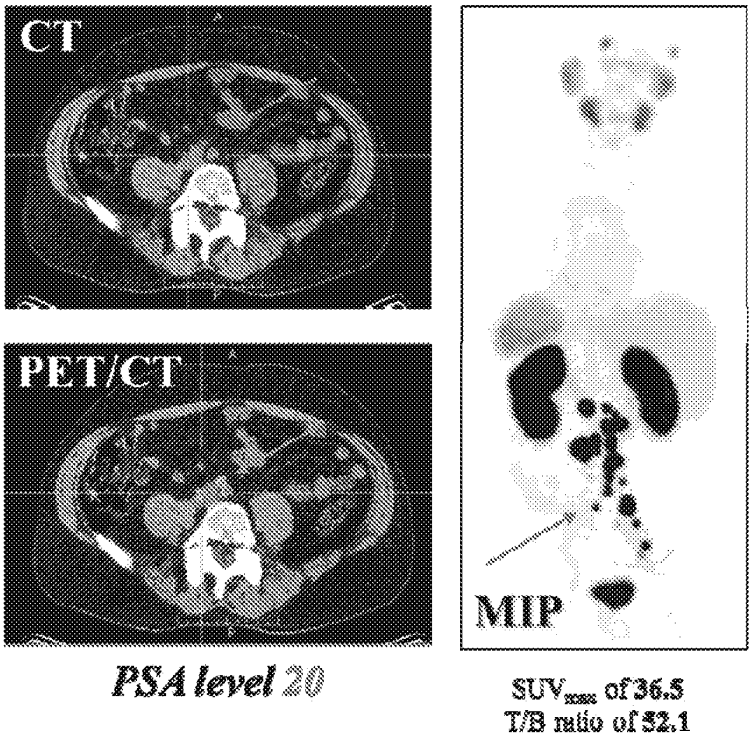


FIG. 17A

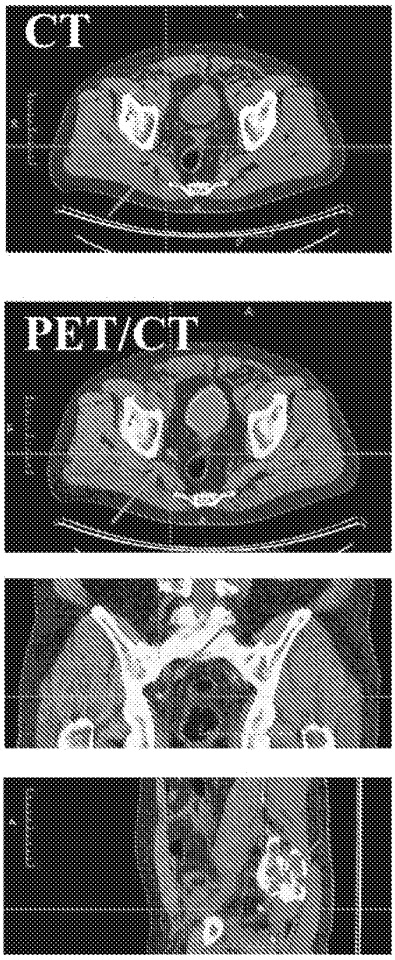


FIG. 17B

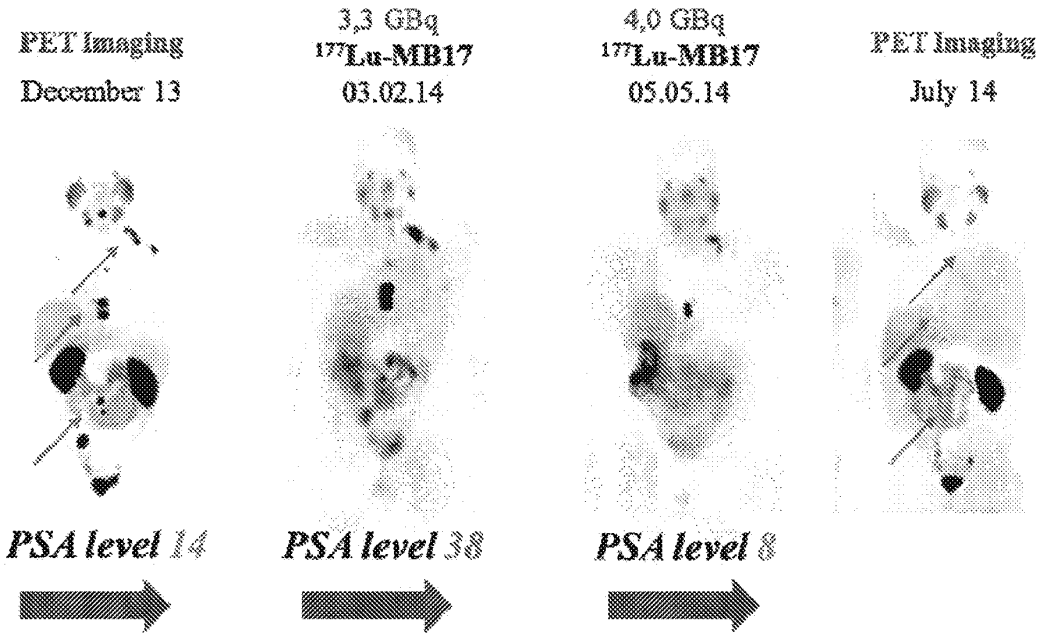

PET Imaging
December 13
3,3 GBq
177Lu-MB17
03.02.14
4,0 GBq
177Lu-MB17
05.05.14
PET Imaging
July 14
PSA level 14
PSA level 38
PSA level 8

FIG. 18A

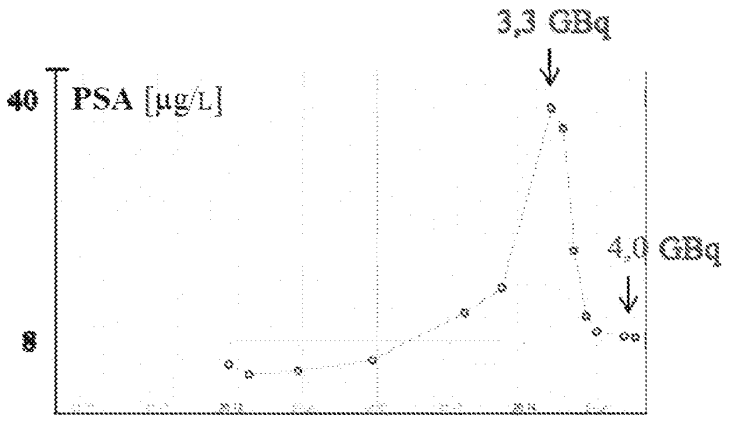


FIG. 18B

LABELED INHIBITORS OF PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA), THEIR USE AS IMAGING AGENTS AND PHARMACEUTICAL AGENTS FOR THE TREATMENT OF PROSTATE CANCER

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

This application is a continuation of U.S. patent application Ser. No. 15/131,118 filed Apr. 18, 2016, which is a continuation-in-part application of international patent application Serial No. PCT/EP2014/002808 filed Oct. 17, 2014, which published as PCT Publication No. WO 2015/055318 on Apr. 23, 2015, which claims benefit of European patent application Serial Nos. 13004991.9 filed Oct. 18, 2013 and 14175612.2 filed Jul. 3, 2014.

The foregoing applications, and all documents cited therein are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The present invention generally relates to the field of radiopharmaceuticals and their use in nuclear medicine as tracers, imaging agents and for the treatment of various disease states of prostate cancer.

BACKGROUND OF THE INVENTION

Prostate cancer (PCa) is the leading cancer in the US and European population. At least 1-2 million men in the western hemisphere suffer from prostate cancer and it is estimated that the disease will strike one in six men between the ages of 55 and 85. There are more than 300,000 new cases of prostate cancer diagnosed each year in USA. The mortality from the disease is second only to lung cancer. Currently anatomic methods, such as computed tomography (CT), magnetic resonance (MR) imaging and ultrasound, predominate for clinical imaging of prostate cancer. An estimated $ 2 billion is currently spent worldwide on surgical, radiation, drug therapy and minimally invasive treatments. However, there is presently no effective therapy for relapsing, metastatic, androgen-independent prostate cancer.

A variety of experimental low molecular weight PCa imaging agents are currently being pursued clinically, including radiolabeled choline analogs [^{18}F]fluorodihydrotestosterone ([^{18}F]FDHT), anti-1-amino-3-[^{18}F]fluorocyclobutyl-1-carboxylic acid (anti[18F]F-FACBC, [^{11}C]acetate and 1-(2-deoxy-2-[^{18}F]flouro-L-arabinofuranosyl)-5-methyluracil (–[^{18}F]FMAU) (Scher, B.; et al. *Eur J Nucl Med Mol Imaging* 2007, 34, 45-53; Rinnab, L.; et al. *BJU Int* 2007, 100, 786,793; Reske, S. N.; et al. J Nucl Med 2006, 47, 1249-1254; Zophel, K.; Kotzerke, *J. Eur J Nucl Med Mol Imaging* 2004, 31, 756-759; Vees, H.; et al. *BJU Int* 2007, 99, 1415-1420; Larson, S. M.; et al. *J Nucl Med* 2004, 45, 366-373; Schuster, D. M.; et al. *J Nucl Med* 2007, 48, 56-63; Tehrani, O. S.; et al. *J Nucl Med* 2007, 48, 1436-1441). Each operates by a different mechanism and has certain advantages, e.g., low urinary excretion for [^{11}C] choline, and disadvantages, such as the short physical half-life of positron-emitting radionuclides.

It is well known that tumors may express unique proteins associated with their malignant phenotype or may over-express normal constituent proteins in greater number than normal cells. The expression of distinct proteins on the surface of tumor cells offers the opportunity to diagnose and characterize disease by probing the phenotypic identity and biochemical composition and activity of the tumor. Radioactive molecules that selectively bind to specific tumor cell surface proteins provide an attractive route for imaging and treating tumors under non-invasive conditions. A promising new series of low molecular weight imaging agents targets the prostate-specific membrane antigen (PSMA) (Mease R. C. et al. Clin Cancer Res. 2008, 14, 3036-3043; Foss, C. A.; et al. *Clin Cancer Res* 2005, 11, 4022-4028; Pomper, M. G.; et al. Mol Imaging 2002, 1, 96-101; Zhou, J.; etr al. *Nat Rev Drug Discov* 2005, 4, 1015-1026; WO 2013/022797).

PSMA is a trans-membrane, 750 amino acid type II glycoprotein that has abundant and restricted expression on the surface of PCa, particularly in androgen-independent, advanced and metastatic disease (Schulke, N.; et al. Proc Natl Acad Sci USA 2003, 100, 12590-12595). The latter is important since almost all PCa become androgen independent over the time. PSMA possesses the criteria of a promising target for therapy, i.e., abundant and restricted (to prostate) expression at all stages of the disease, presentation at the cell surface but not shed into the circulation and association with enzymatic or signaling activity (Schulke, N.; et al. Proc. Natl. Acad. Sci. USA 2003, 100, 12590-12595). The PSMA gene is located on the short arm of chromosome 11 and functions both as a folate hydrolase and neuropeptidase. It has neuropeptidase function that is equivalent to glutamate carboxypeptidase II (GCPII), which is referred to as the "brain PSMA", and may modulate glutamatergic transmission by cleaving N-acetylaspartylglutamate (NAAG) to N-acetylaspartate (NAA) and glutamate (Nan, F.; et al. J Med Chem 2000, 43, 772-774). There are up to 10^6 PSMA molecules per cancer cell, further suggesting it as an ideal target for imaging and therapy with radionuclide-based techniques (Tasch, J.; et al. *Crit Rev Immunol* 2001, 21, 249-261).

The radio-immunoconjugate of the anti-PSMA monoclonal antibody (mAb) 7E11, known as the PROSTASCINT® scan, is currently being used to diagnose prostate cancer metastasis and recurrence. However, this agent tends to produce images that are challenging to interpret (Lange, P. H. PROSTASCINT scan for staging prostate cancer. *Urology* 2001, 57, 402-406; Haseman, M. K.; et al. *Cancer Biother Radiopharm* 2000, 15, 131-140; Rosenthal, S. A.; et al. *Tech Urol* 2001, 7, 27-37). More recently, monoclonal antibodies have been developed that bind to the extracellular domain of PSMA and have been radiolabeled and shown to accumulate in PSMA-positive prostate tumor models in animals. However, diagnosis and tumor detection using monoclonal antibodies has been limited by the low permeability of the monoclonal antibody in solid tumors.

The selective targeting of cancer cells with radiopharmaceuticals, either for imaging or therapeutic purposes is challenging. A variety of radionuclides are known to be useful for radio-imaging or cancer radiotherapy, including ^{111}In, ^{90}Y, ^{68}Ga, ^{177}Lu, ^{99m}Tc, ^{123}I and ^{131}I. Recently it has been shown that some compounds containing a glutamate-urea-glutamate (GUG) or a glutamate-urea-lysine (GUL) recognition element linked to a radionuclide-ligand conjugate exhibit high affinity for PSMA.

Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

New agents that will enable rapid visualization of prostate cancer and specific targeting to allow radiotherapy present are needed.

Thus, the object of the present invention is to develop ligands that interact with PSMA and carry appropriate radionuclides which provide a promising and novel targeting option for the detection, treatment and management of prostate cancer.

The solution of said object is achieved by providing the embodiments characterized in the claims.

The inventors found new compounds which are useful radiopharmaceuticals and their use in nuclear medicine as tracers, imaging agents and for the treatment of various disease states of prostate cancer.

The novel imaging agents with structural modifications in the linker region have improved tumor targeting properties and pharmacokinetics. The pharmacophore presents three carboxylic groups able to interact with the respective side chains of PSMA and an oxygen as part of zinc complexation in the active center. Besides these obligatory interactions, the inventors were able to optimize the lipophilic interactions in the linker region.

It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

FIG. **1**: PET—Imaging of MB17. Whole-body coronal microPET images of an athymic male nude mice bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB17 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected. Graph A shows the respective time-activity-curves of kidney and baldder and graph B the respective time-activity-curves of heart, muscle and tumor. The values are expressed as mean SUV (standardized uptake values).

FIG. **2**: Organ Distribution at 1 h post injection. Organ distribution at one hour post injection of 0.06 nmol of the ^{68}Ga labeled PSMA inhibitor MB17. PSMA-blocking by co-administration of 2 mg/kg body weight 2-PMPA indicates PSMA-specific uptake in the tumor and the kidneys. Data are expressed as mean % ID/g tissue ±SD (n=3).

FIG. **3**: PET—Imaging of MB4. Whole-body coronal microPET images of an athymic male nude mice bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB4 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected. Graph A shows the respective time-activity-curves of kidney and bladder and graph B the respective time-activity-curves of heart, muscle and tumor. The values are expressed as mean SUV (standardized uptake values)

FIG. **4**: Organ distribution expressed as % ID/g tissue ±SD (n=5) 24 h post injection of 0.06 nmol of the ^{177}Lu-labeled MB17. Organ distribution with ^{177}Lu shows that the high initial kidney uptake is nearly completely washed out (2.13±1.36% ID/g) after 24 hours while the tumor uptake remained high and even increased (10.58±4.50% ID/g). Other organs as liver (0.08±0.03% ID/g), lung (0.11±0.13% ID/g) and spleen (0.13±0.05% ID/g) showed very low uptake. The favourable pharmacokinetics led to extremly high tumor-to-background ratios (Tumor/Blood: 1058; Tumor/Muscle: 529) after 24 hours

FIG. **5**: PET—Imaging of MB 2. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB2 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **6**: PET—Imaging of MB 3. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB 3 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **7**: PET—Imaging of MB10. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB10 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **8**: PET—Imaging of MB17.D. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB17.D were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected. MB17D: stereoisomer of MB17(L); synthesis based on Fmoc-3(2-naphthyl)-D-alanine

FIG. **9**: PET—Imaging of MB22. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB22 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **10**: PET—Imaging of MB 24. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB 24 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **11**: PET—Imaging of MB25. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB25 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **12**: PET—Imaging of MB31. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB31 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **13**: PET—Imaging of MB33. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of [^{68}Ga]MB33 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **14**: PET—Imaging of MB35. Whole-body coronal microPET images of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and

pharmacokinetic properties of [^{68}Ga]MB35 were evaluated by dynamic microPET scans. Approximately 15 MBq/mouse were injected.

FIG. **15**: PET scan of a mouse injected with ^{68}Ga-CHX-DTPA. On the left the caudal, in the centre the dorsal and on the right the lateral view. The pictures cover the time spans of 20-40 min (top), 40-60 min (centre) and 120-140 min (bottom).

FIG. **16**: MB-17 vs MB-17.D. Whole-body coronal microPET images of athymic male nude mice bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties of the stereoisomers MB-17 and MB-17 D were directly compared at 2 hours post injection.

FIGS. **17**A-B: Human PET/CT imaging ^{68}Ga-labeled MB17. (a) First clinical experience with ^{68}Ga-labeled MB17 PET/CT demonstrates the detection of small lymph node metastases 1 hour post injection, primarily due to a high radiotracer uptake. Red arrows point to a representative lesion with a SUVmax of 36.5 and a tumor-to-background ratio of 52.1 one hour post injection. MIP=maximum intensity projection of the PET 1 h post injection. (b) The significant advantage of ^{68}Ga-labeled MB17 PET/CT is the sensitive detection of lesions even at low PSA level.

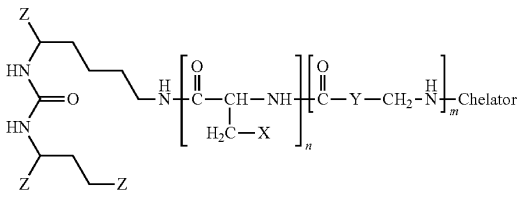
FIGS. **18**A-B: PET imaging of patient with multiple prostate cancer metastasis. (a) First scan demonstrate initial PET imaging of the patient with multiple prostate cancer metastases with blood PSA value of 14. Two months later 3.3 GBq of ^{177}Lu-labeled MB17 was applied. At this time point, the amount of PSA in blood reached a value of 38. After the first cycle, the PSA level decreased to 8. Three months after the first cycle another 4 GBq of ^{177}Lu-labeled MB17 was applied. The control PET scan was performed one month after the second cycle. The treatment has shown a significant impact on the tumor lesions and PSA value and resulted in a reduction of bone pain. (b) The graph demonstrates the significant impact on the PSA value which decreased after the first application of the therapeutic dose of ^{177}Lu-labeled MB17.

DETAILED DESCRIPTION OF THE INVENTION

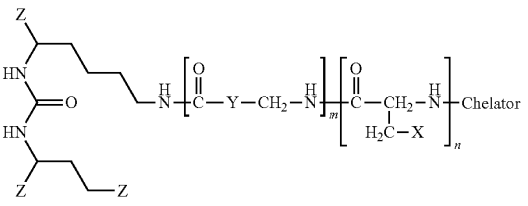
The present invention relates to radiopharmaceuticals and their use in nuclear medicine as tracers, imaging agents and for the treatment of various disease states of prostate cancer.

Thus, the present invention concerns compounds that are represented by the general Formulae (Ia) or (Ib):

Formula (Ia)



Formula (Ib)



with:

n:	0, 1
m:	1, 2, 3, 4
Z:	—CO_2H, —SO_2H, —SO_3H, —SO_4H, —PO_2H, —PO_3H, —PO_4H_2
X:	Naphthyl, Phenyl, Biphenyl, Indolyl (=2,3-benzopyrrolyl), Benzothiazolyl
Y:	Aryl, Alkylaryl, Cyclopentyl, Cyclohexyl, Cycloheptyl
Chelator radical of:	1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (=DOTA), N,N''-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N''-diacetic acid (=HBED—CC), 1,4,7-triazacyclononane-1,4,7-triacetic acid (=NOTA), 2-(4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl)pentanedioic acid (NODAGA), 2-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentanedioic acid (DOTAGA), 1,4,7-triazacyclononane phosphinic acid (TRAP), 1,4,7-triazacyclononane-1-[methyl(2-carboxyethyl)phosphinic acid]-4,7-bis[methyl(2-hydroxymethyl)phosphinic acid] (NOPO), 3,6,9,15-tetraazabicyclo[9.3.1[[.]]]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (=PCTA), N'-{5-[Acetyl(hydroxy)amino]pentyl}-N-[5-({4-[(5-aminopentyl)(hydroxy)amino]-4-oxobutanoyl}amino)pentyl]-N-hydroxysuccinamide (DFO), Diethylenetriaminepentaacetic acid (DTPA) Trans-cyclohexyl-diethylenetriaminepentaacetic acid (CHX—DTPA) 1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid (oxo-Do3A) p-isothiocyanatobenzyl-DTPA (SCN—Bz—DTPA) 1-(p-isothiocyanatobenzyl)-3-methyl-DTPA (1B3M) 2-(p-isothiocyanatobenzyl)-4-methyl-DTPA (1M3B) 1-(2)-methyl-4-isocyanatobenzyl-DTPA (MX—DTPA)

If not stated otherwise, in the present invention the "alkyl" residue (preferably: C_1 to C_{10}) can be linear or branched, unsubstituted or substituted. Preferred alkyl residues are methyl, ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, n-pentanyl, n-hexanyl. The same also applies to the corresponding cycloalkyl compounds having preferably 3 to 10 carbon atoms.

"Aryl" refers to an aromatic monocyclic or polycyclic ring system having 6 to 14 carbon atoms, preferably 6 to 10 carbon atoms. The aryl group can be substituted, where appropriate, with one or several ring substituents, like alkyl groups. Preferred aryl groups are phenyl, benzyl or naphthyl.

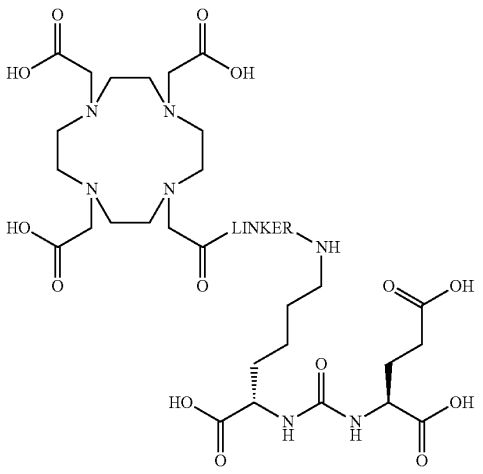
Although it is preferred that the Z-Group is $—CO_2H$ it may be easily replaced with biosteric replacements such as $—SO_2H$, $—SO_3H$, $—SO_4H$, $—PO_2H$, $—PO_3H$, $—PO_4H_2$, see e.g. "The Practice of Medicinal Chemistry" (Academic Press New York, 1996), page 203.

Within the meaning of the invention, all residues are considered combinable unless stated otherwise in the definition of the residues. All conceivable subgroupings thereof are considered to be disclosed.

In a preferred embodiment, the motif specifically binding to cell membranes of neoplastic cells is a motif specifically binding to cell membranes of cancerous cells, preferably wherein said motif may comprise a prostate-specific membrane antigen (PSMA), in particular wherein said PSMA may comprise a glutamate-urea-lysine motif according to the following formula in Scheme 1.

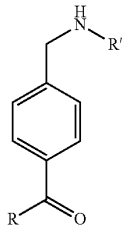
Thus, preferred molecules of the present invention consist of three principal components (Scheme 1): the hydrophilic PSMA binding motif (radical of Glu-Urea-Lys=Glu-NH—CO—NH-Lys), a variable linker and the chelator which is preferably a radical of DOTA.

Scheme 1: Structure of preferred compounds of the present invention



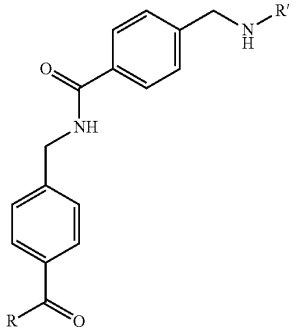
The different preferred linkers are shown below, wherein R=radical of Glu-urea-Lys and R'=radical of DOTA (as a preferred example for the chelator), as shown above.

MB2 Linker



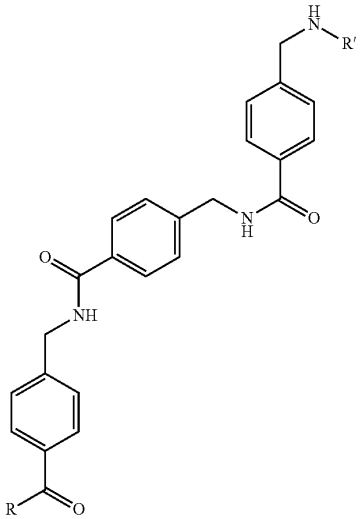
Chemical Formula: $C_{36}H_{54}N_8O_{15}$
Molecular Weight: 838.88 g/mol

MB3 Linker



Chemical Formula: $C_{44}H_{61}N_9O_{16}$
Molecular Weight: 972.03 g/mol

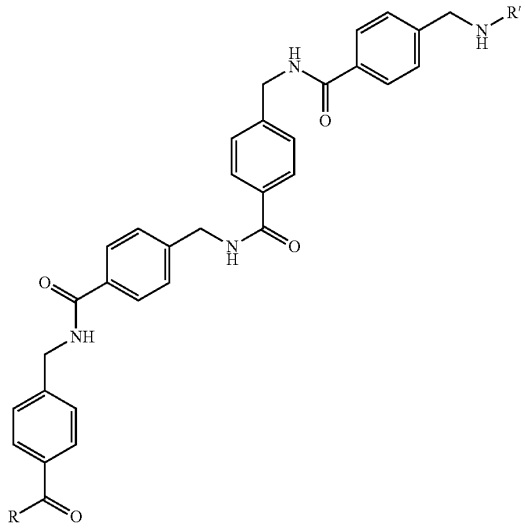
MB4 Linker



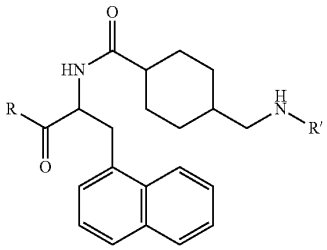
Chemical Formula: $C_{52}H_{68}N_{10}O_{17}$
Molecular Weight: 1105.18 g/mol

-continued

MB10 Linker

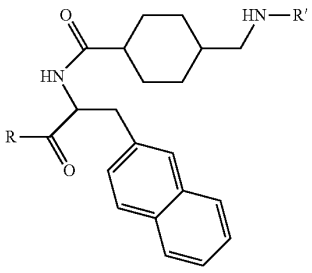


Chemical Formula: $C_{65}H_{70}N_{11}O_8$
Molecular Weight: 1238.33 g/mol



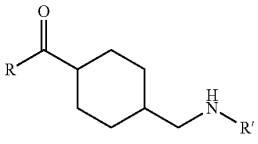
or

MB 17 Linker



Chemical Formula: $C_{49}H_{71}N_9O_{16}$
Molecular Weight: 1042.16 g/mol

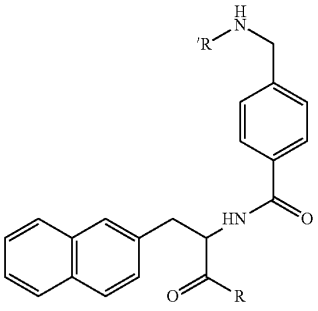
MB22 Linker



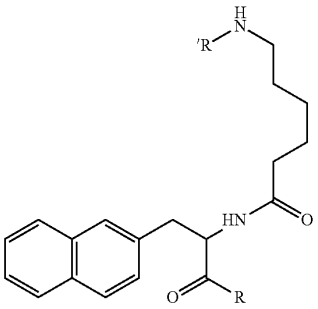
Chemical Formula: $C_{36}H_{60}N_8O_{15}$
Molecular Weight: 844.92 g/mol

-continued

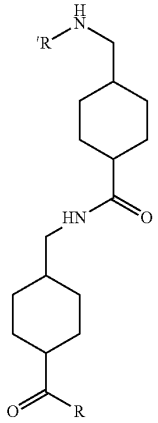
MB24



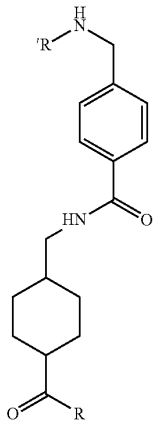
MB25



MB31

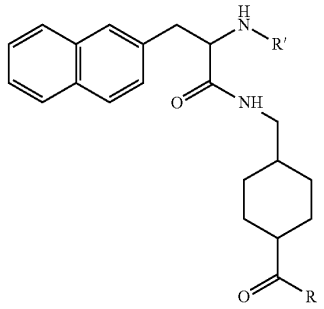


MB33

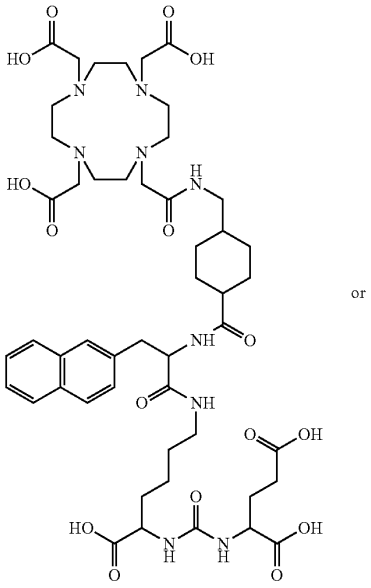


-continued

MB35

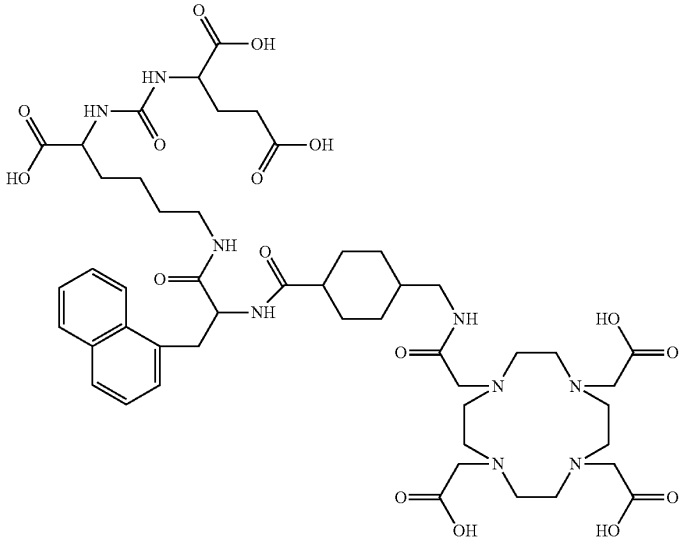


Preferred compounds of the present invention are e.g.



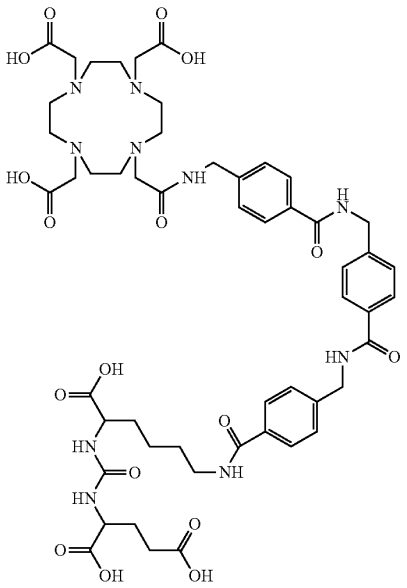
or

MB17

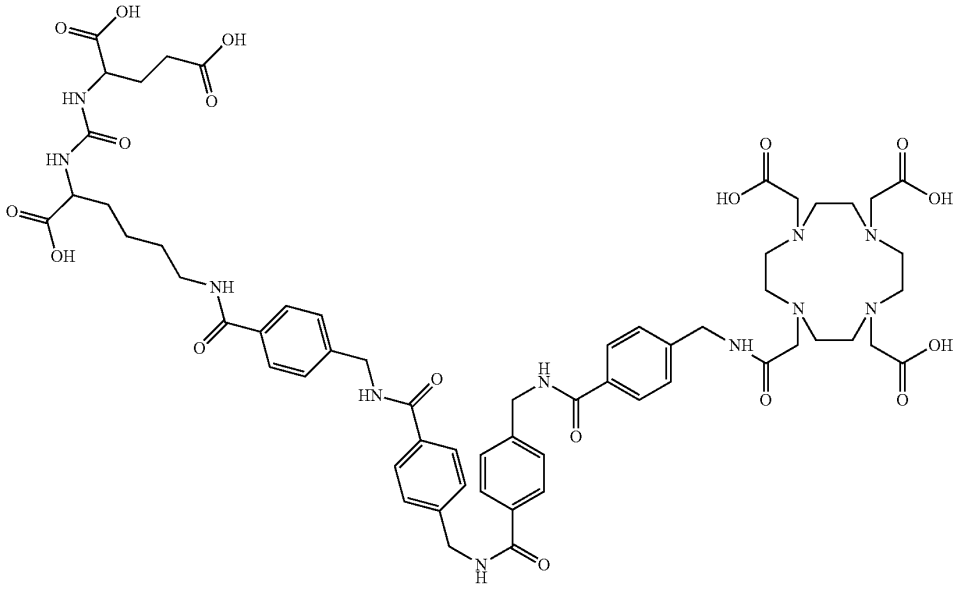


-continued

MB 4

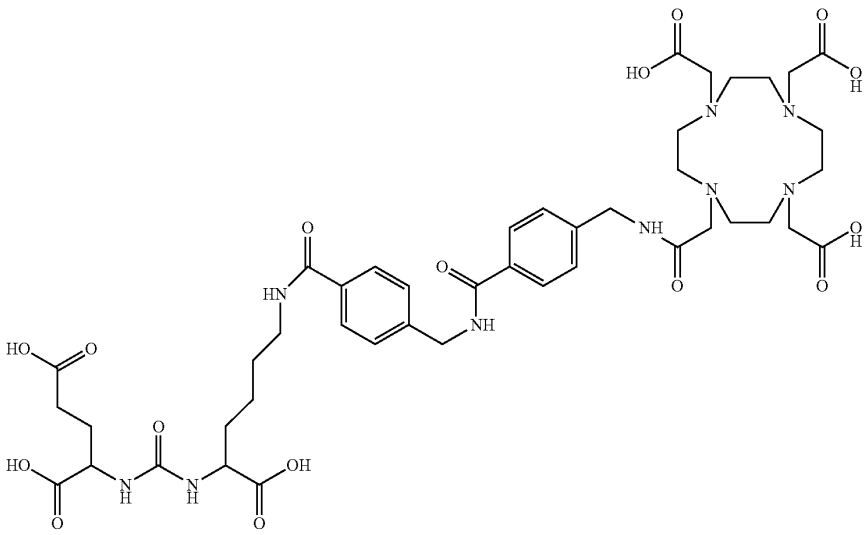


MB 10

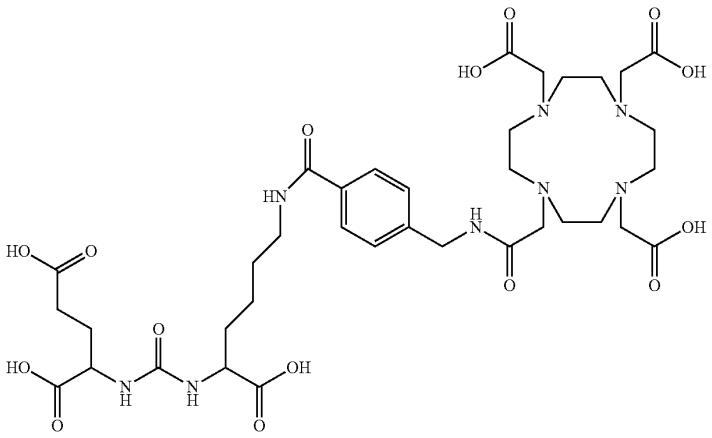


-continued

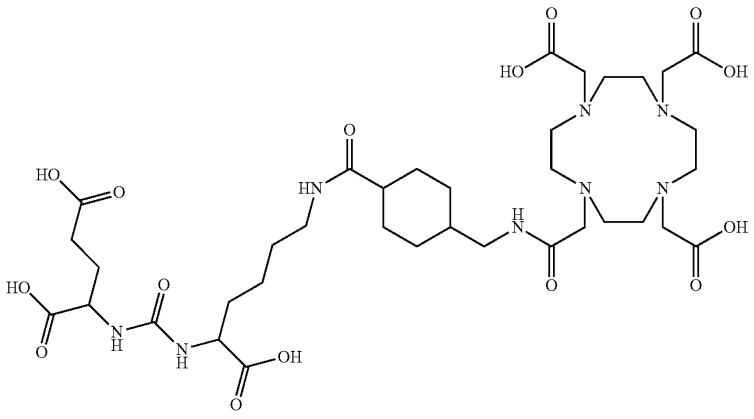
MB 3



MB 2

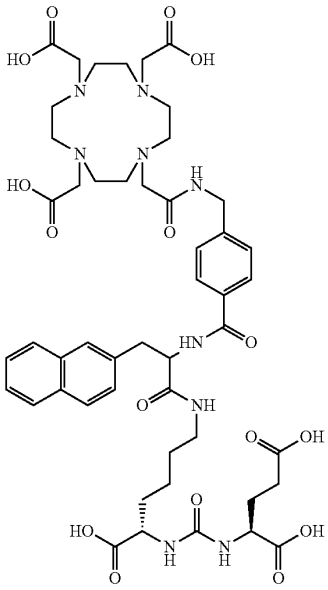


MB 22

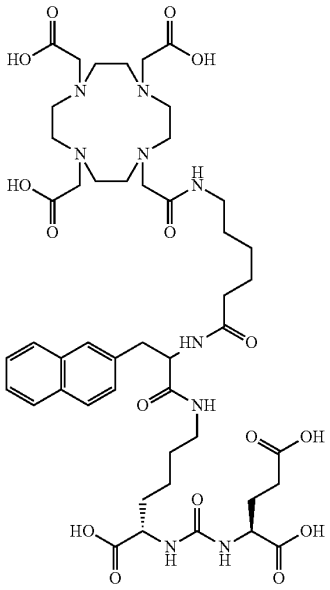


-continued

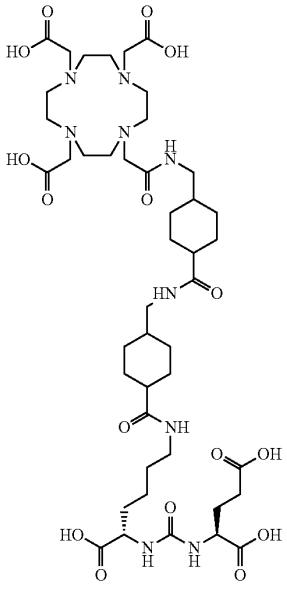
MB24



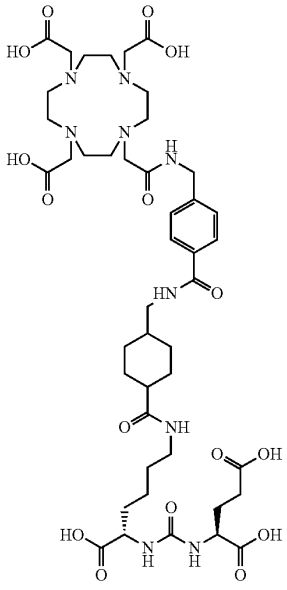
MB25



MB31

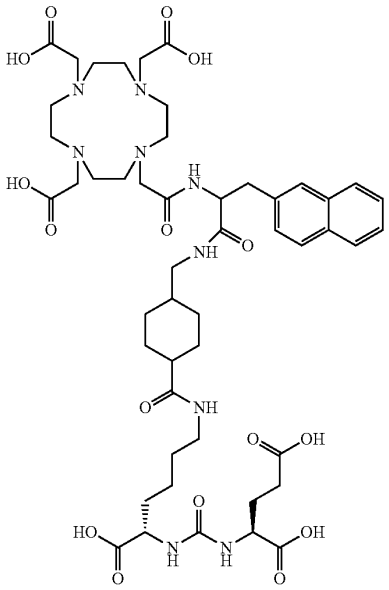


MB33



-continued

MB35



The invention also relates to pharmaceutically acceptable salts of the compounds of general formula (Ia) and/or (Ib). The invention also relates to solvates of the compounds, including the salts as well as the active metabolites thereof and, where appropriate, the tautomers thereof according to general formula (Ia) and/or (Ib) including prodrug formulations.

A "pharmaceutically acceptable salt" is a pharmaceutically acceptable, organic or inorganic acid or base salt of a compound of the invention. Representative pharmaceutically acceptable salts include, e.g., alkali metal salts, alkali earth salts, ammonium salts, water-soluble and water-insoluble salts, such as the acetate, carbonate, chloride, gluconate, glutamate, lactate, laurate, malate or tartrate.

The term "prodrug" refers to a precursor of a drug that is a compound which upon administration to a patient, must undergo chemical conversion by metabolic processes before becoming an active pharmacological agent. Illustrative prodrugs of compounds in accordance with Formula (Ia) and/or (Ib) are esters and amides, preferably alkyl esters of fatty acid esters. Prodrug formulations here may comprise all substances which are formed by simple transformation including hydrolysis, oxidation or reduction either enzymatically, metabolically or in any other way. A suitable prodrug contains e.g. a substance of general formula (Ia) and/or (Ib) bound via an enzymatically cleavable linker (e.g. carbamate, phosphate, N-glycoside or a disulfide group) to a dissolution-improving substance (e.g. tetraethylene glycol, saccharides, formic acids or glucuronic acid, etc.). Such a prodrug of a compound according to the invention can be applied to a patient, and this prodrug can be transformed into a substance of general formula (Ia) and/or (Ib) so as to obtain the desired pharmacological effect.

Some compounds of Formula (Ia) and/or (Ib) are encompassed in form of the racemates, their enantiomers and optionally in form of their diastereomers and all possible mixtures thereof.

According to the invention all chiral C-atoms shall have D- and/or L-configuration; also combinations within one compound shall be possible, i.e. some of the chiral C-atoms may be D- and others may be L-configuration.

The obtained compounds can be optionally separated by known methods (e.g. *Allinger, N. L. und Elliel E. L. in "Topics in Stereochemistry" Vol. 6, Wiley Interscience,* 1971) in their enantiomers and/or diasteromers. One possible method of enantiomeric separation is the use of chromatography.

The invention also relates to pharmaceutical preparations which contain a therapeutically effective amount of the active ingredients (compound according to the invention of formula (Ia) or (Ib) together with organic or inorganic solid or liquid, pharmaceutically acceptable carriers which are suited for the intended administration and which interact with the active ingredients without drawbacks.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, material, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of a patient without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

A "patient" includes an animal, such as a human, monkey, cow, horse, cat or dog. The animal can be a mammal such as a non-primate and a primate (e.g., monkey and human). In one embodiment, a patient is a human being.

In general, the Formula (Ia) or (Ib) compound or pharmaceutical compositions thereof, may be administered orally or via a parenteral route, usually injection or infusion.

A "parenteral administration route" means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramusclular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticluare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The dosage of the compounds according to the invention is determined by the physician on the basis of the patient-specific parameters, such as age, weight, sex, severity of the disease, etc. The dosage is preferably from 0.00001 mg/kg to 100 mg/kg body weight, preferably from 0.001 to 50 mg/kg body weight and most preferably from 0.01 to 10 mg/kg body weight.

Corresponding to the kind of administration, the medicament is suitably formulated, e.g. in the form of solutions or suspensions, simple tablets or dragees, hard or soft gelatine capsules, suppositories, ovules, preparations for injection, which are prepared according to common galenic methods.

The compounds according to the invention can be formulated, where appropriate, together with further active substances and with excipients and carriers common in pharmaceutical compositions, e.g.—depending on the preparation to be produced—talcum, gum arabic, lactose, starch, magnesium stearate, cocoa butter, aqueous and non-aqueous carriers, fatty bodies of animal or vegetable origin, paraffin derivatives, glycols (in particular polyethylene glycol), various plasticizers, dispersants or emulsifiers, pharmaceutically compatible gases (e.g. air, oxygen, carbon dioxide, etc.), preservatives.

In order to produce liquid preparations, additives, such as sodium chloride solution, ethanol, sorbitol, glycerine, olive oil, almond oil, propylene glycol or ethylene glycol, can be used.

When solutions for infusion or injection are used, they are preferably aqueous solutions or suspensions, it being possible to produce them prior to use, e.g. from lyophilized preparations which contain the active substance as such or together with a carrier, such as mannitol, lactose, glucose, albumin and the like. The ready made solutions are sterilized and, where appropriate, mixed with excipients, e.g. with preservatives, stabilizers, emulsifiers, solubilizers, buffers and/or salts for regulating the osmotic pressure. The sterilization can be obtained by sterile filtration using filters having a small pore size according to which the composition can be lyophilized, where appropriate. Small amounts of antibiotics can also be added to ensure the maintenance of sterility.

The phrases "effective amount" or "therapeutically-effective amount" as used herein means that amount of a compound, material, or composition which may comprise a compound of the invention, or other active ingredient which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment. A therapeutically effective amount with respect to a compound of the invention means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment of prevention of a disease. Used in connection with a compound of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease, or enhances the therapeutic efficacy of or synergies with another therapeutic agent.

As used herein, the terms "treating" or "treatment" is intended to encompass also diagnosis, prophylaxis, prevention, therapy and cure.

The terms "prevent", "preventing," and "prevention" refer to the prevention of the onset, recurrence, or spread of the disease in a patient resulting from the administration of a prophylactic or therapeutic agent.

Depending on whether the inventive Formula (Ia) and/or (Ib) compounds are to be used as radio-imaging agents or radio-pharmaceuticals different radionuclides are complexed to the chelator. Illustrative radionuclides include, for example, ^{89}Zr, ^{44}Sc, ^{111}In, ^{90}Y, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{177}Lu, ^{99m}Tc, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{149}Tb, ^{152}Tb, ^{155}Tb, ^{161}Tb, ^{153}Gd, ^{155}Gd, ^{157}Gd, ^{213}Bi, ^{225}Ac, ^{230}U, ^{223}Ra, ^{165}Er and Fe. According to one aspect of this invention, the radionuclide is ^{111}In, ^{90}Y, ^{68}Ga, ^{64}Cu, ^{153}Gd, ^{155}Gd, ^{213}Bi, ^{225}Ac, Fe, or ^{177}Lu.

As noted above, complexes of the compounds according Formula (Ia) or (Ib) may contain one or more radionuclides which are suitable for use as radio-imaging agents or as therapeutics for the treatment of rapidly proliferating cells, for example, PSMA expressing prostate cancer cells. According to the present invention they are called "metal complexes" or "radiopharmaceuticals".

Preferred imaging methods are positron emission tomography (PET) or single photon emission computed tomography (SPECT).

Accordingly, in one embodiment, a pharmaceutical composition is provided including a complex that includes a radionuclide and a compound of Formula (Ia) or Formula (Ib), a salt, solvate, stereoisomer, or tautomer thereof, and a pharmaceutically acceptable carrier.

According to another aspect, a pharmaceutical composition is provided, which is suitable for in vivo imaging and radiotherapy. Suitable pharmaceutical compositions may contain a radio imaging agent, or a radiotherapeutic agent that has a radionuclide either as an element, i.e. radioactive iodine, or a radioactive metal chelate complex of the compound of Formula (Ia) and/or (Ib) in an amount sufficient for imaging, together with a pharmaceutically acceptable radiological vehicle. The radiological vehicle should be suitable for injection or aspiration, such as human serum albumin; aqueous buffer solutions, e.g., tris(hydromethyl) aminomethane (and its salts), phosphate, citrate, bicarbonate, etc; sterile water physiological saline; and balanced ionic solutions containing chloride and or dicarbonate salts or normal blood plasma cautions such as calcium potassium, sodium and magnesium.

The concentration of the imaging agent or the therapeutic agent in the radiological vehicle should be sufficient to provide satisfactory imaging. For example, when using an aqueous solution, the dosage is about 1.0 to 100 millicuries. The actual dose administered to a patient for imaging or therapeutic purposes, however, is determined by the physician administering treatment. The imaging agent or therapeutic agent should be administered so as to remain in the patient for about 1 hour to 10 days, although both longer and shorter time periods are acceptable. Therefore, convenient ampoules containing 1 to 10 mL of aqueous solution may be prepared.

Imaging may be carried out in the normal manner, for example by injecting a sufficient amount of the imaging composition to provide adequate imaging and then scanning with a suitable imaging or scanning machine, such as a tomograph or gamma camera. In certain embodiments, a method of imaging a region in a patient includes the steps of: (i) administering to a patient a diagnostically effective amount of a compound complexed with a radionuclide; exposing a region of the patient to the scanning device; and (ii) obtaining an image of the region of the patient. In certain embodiments of the region imaged is the head or thorax. In other embodiments, the compounds and complexes of Formula I(a) and/or (Ib) target the PSMA protein.

Thus, in some embodiments, a method of imaging tissue such as spleen tissue, kidney tissue, or PSMA-expressing tumor tissue is provided including contacting the tissue with a complex synthesized by contacting a radionuclide and a Formula (Ia) and/or Formula (Ib) compound.

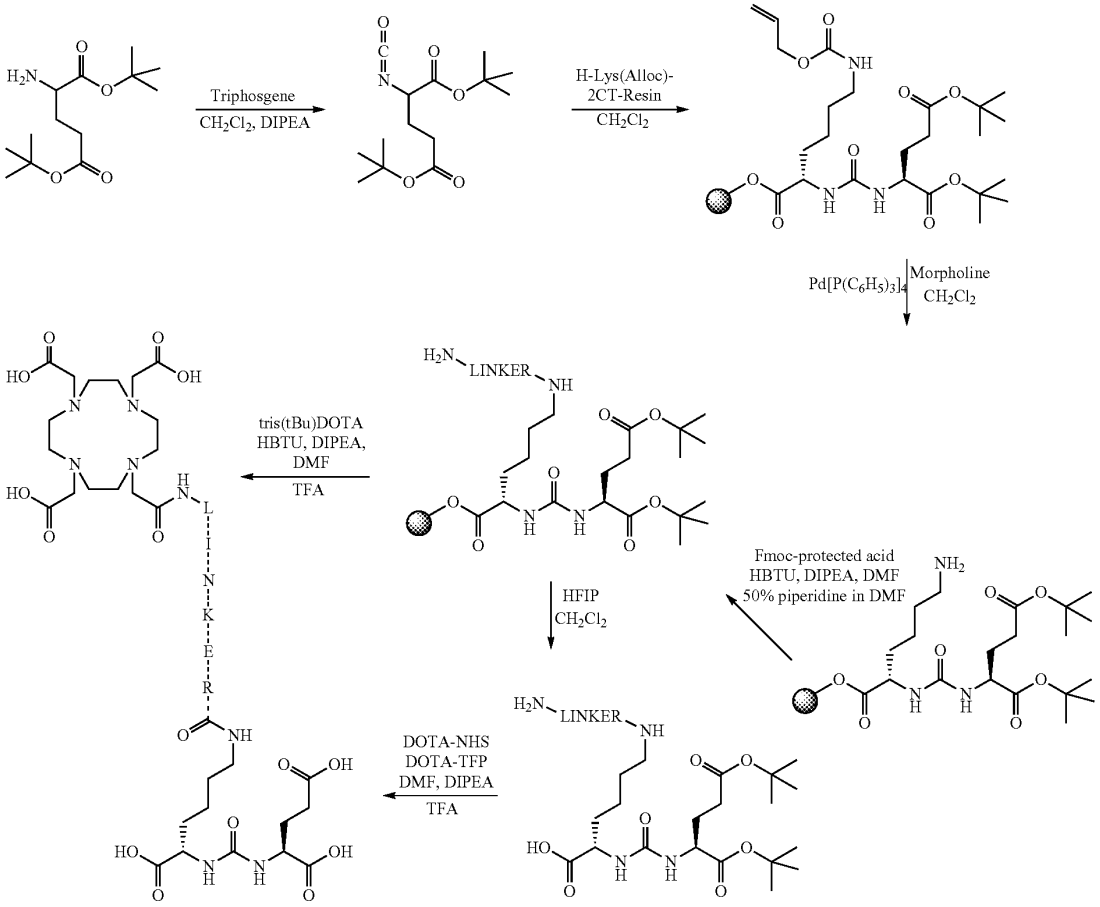
The amount of the compound of the present invention, or a formulation which may comprise a complex of a metal and

a compound according to Formula (Ia) and/or (Ib), or its salt, solvate, stereoisomer, or tautomer that is administered to a patient depends on several physiological factors that are routinely used by the physician, including the nature of imaging to be carried out, tissue to be targeted for imaging or therapy and the body weight and medical history of the patient to be imaged or treated using a radiopharmaceutical.

Accordingly in another aspect, the invention provides a method for treating a patient by administering to a patient a therapeutically effective amount of a Formula (Ia) and/or (Ib) compound complexed to a radionuclide, or a pharmaceutically acceptable salt or solvate of the complex to treat a patient suffering from a cell proliferative disease or disorder. Specifically, the cell proliferative disease or disorder to be treated or imaged using a compound, pharmaceutical composition or radiopharmaceutical in accordance with this invention is a cancer, for example, prostate cancer and/or prostate cancer metastasis in e.g. lung, liver, kidney, bones, brain, spinal cord, bladder, etc.

The synthesis of the compounds of the present invention is described in detail in the example section. An overview of the synthesis is exemplified in Scheme 2 concerning DOTA conjugated-PSMA inhibitors. However, a person skilled in the art would be able to modify the reactions e.g. by using another chelator. Thus, this scheme shall not be understood to limit the compounds of the present invention to the DOTA chelator only.

Scheme 2



The synthesized compounds are chemically characterized by RP-HPLC, MS, and/or NMR.

The novel chelator-conjugated imaging agents with structural modifications in the linker region have improved tumor targeting properties and pharmacokinetics. The pharmacophore presents three carboxylic group able to interact with the respective side chains of PSMA and an oxygen as part of zinc complexation in the active center. Besides these obligatory interactions, the inventors were able to optimize the lipophilic interactions in the linker region.

The preclinical evaluation includes in vitro assays (affinity, internalization) and in vivo experiments WET screening and organ distribution).

The compounds of the present invention are better than known reference compounds with regard to kidney clearance and enrichment in the tumor. The binding affinity of PSMA inhibitors of the present invention can be influenced by linker modifications. Two cyclic moieties and at least one aromatic moiety in the linker region of the substance seem to be preferable and resulted in the high affinity compounds MB4 and MB17. In this regard, a very promising compound is MB17.

Thus, the compounds of the present invention represent novel PSMA-targeting probes with optimal characteristics which was also confirmed by organ distribution and small animal PET imaging. The compounds of the present invention show a high PSMA-specific tumor uptake. In addition, they are characterized by an early enrichment in the bladder and also the maximum kidney uptake. With regard to therapeutic use, this gives clear clinical advantages for the compounds of the present invention compared to other PSMA-inhibitors. In the PET diagrams the compounds of the present invention, in particular MB17, show a rapid background clearance as well as a substantial reduction of the enrichment in the kidney after 2 hours while it is further accumulated and retained in the PSMA-expressing tumor. Also first in vivo treatments with MB 17 showed promising data (c.f. FIGS. **17** and **18**).

Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

The below example explains the invention in more detail but are not construed to limit the invention in any way to the exemplified embodiments only.

EXAMPLES

Example 1

Synthesis of DOTA-conjugated Inhibitors

The DOTA conjugated-PSMA inhibitors are synthesized via solid-phase peptide synthesis (c.f. Scheme 2). In a first step, the isocyanate of the glutamyl moiety was generated in situ by adding a mixture of 3 mmol of bis(tert-butyl)-L-glutamate hydrochloride and 3 mL of N-ethyldiisopropylamine (DIPEA) in 200 mL of dry CH_2Cl_2 to a solution of 1 mmol triphosgene in 10 mL of dry CH_2Cl_2 at 5° C. for 3 h. After the reaction, 0.5 mmol of a resin-immobilized (2-chloro-tritylresin) ε-allyloxycarbonyl protected lysine was added and reacted for 16 h with gentle agitation. The resin was filtered off and the allyloxy-protecting group was removed using 50 mg tetrakis-(triphenyl)palladium and 400 μL morpholine in 4 mL CH_2Cl_2 for 2 h.

The subsequent synthesis of the peptidomimetic PSMA binding motif was performed according to standard Fmoc protocol. The following coupling of the linker part was performed using 2 mmol of the corresponding Fmoc-protected acid, 3.96 mmol of HBTU and 2 mmol of N-ethyldiisopropylamine in a final volume of 4 mL DMF. After activation with 3.95 eq of HBTU and DIPEA for 2 h, 4 eq of tris(t-bu)-DOTA (Chematech) relative to the resin loading were reacted in a final volume of 3 mL DMF. The product was cleaved from the resin in a 2 mL mixture consisting of trifluoroacetic acid, triisopropylsilane, and water (95:2.5:2.5).

The chelator was also conjugated by using HBTU activated DOTA-NHS ester (CheMatech) or DOTA-TFP ester (Mier W., Hoffend J., Kramer S., Schuhmacher J., Hull W. E., Eisenhut M., Haberkorn U., *Bioconjugate Chem.* 2005, 16: 237-240).

Analysis of the synthesized molecules was performed using reversed-phase high performance liquid chromatography (RP-HPLC; Chromolith RP-18e, 100×4.6 mm; Merck, Darmstadt, Germany) with a linear A-B gradient (0% B to 100% B in 6 min) at a flow rate of 4 mL/min (analysis) or 6 mL/min (purification). Solvent A consisted of 0.1% aqueous TFA and solvent B was 0.1% TFA in CH_3CN. The HPLC system (L6200 A; Merck-Hitachi, Darmstadt, Germany) was equipped with a UV and a gamma detector (Bioscan; Washington, USA). UV absorbance was measured at 214 nm. Mass spectrometry was performed with a MALDI-MS Daltonics Microflex system (Bruker Daltonics, Bremen, Germany).

Example 2

Radiolabeling

Typically, 1.5 nmol of a synthesized compound of Example 1 (dissolved in 0.1 M HEPES buffer pH 7.5) was added in a volume of 100 μL to a mixture of 10 μL 2.1 M HEPES solution and 40 μL $[^{68}Ga]Ga^{3+}$ eluate (40 MBq). The pH of the labeling solution was adjusted to 4.5.

The radiolabeling of the compounds resulted in a radiochemical yield of >97% after 15 minutes at 95° C. and was determined by RP-HPLC and TLC. Subsequent purification was done using Sep-Pak C18 cartridges.

Example 3

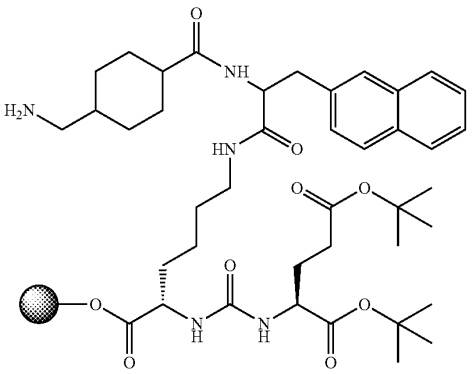
Synthesis of Compounds MB4 and MB17

The isocyanate of the glutamyl moiety was generated in situ by adding a mixture of 3 mmol of bis(tert-butyl) L-glutamate hydrochloride and 1.5 mL of N-ethyldiisopropylamine (DIPEA) in 200 mL of dry CH_2Cl_2 to a solution of 1 mmol triphosgene in 10 mL of dry CH_2Cl_2 at 0° C. over 4 h. After agitation of the reaction mixture for 1 h at 25° C., 0.5 mmol of the resin-immobilized (2-chloro-tritylresin) c-allyloxycarbonyl protected lysine in 4 mL DCM was added and reacted for 16 h with gentle agitation. The resin was filtered off and the allyloxy-protecting group was removed using 30 mg tetrakis(triphenyl)palladium(0) and 400 μL morpholine in 4 mL CH_2Cl_2 for 3 hours. The following coupling of 3 times 4-(Fmoc-aminomethyl)benzoic acid (in case of MB4) or Fmoc-3-(2-naphthyl)-L-alanine and trans-4-(Fmoc-aminomethyl)cyclohexanecarboxylic acid (in case of MB17), respectively, was performed stepwise using 2 mmol of the Fmoc-protected acid, 1.96 mmol of HBTU and 2 mmol of N-ethyldiisopropylamine in a final volume of 4 mL DMF. After activation with 3.95 eq of HBTU and DIPEA for 2 h, 4 eq of tris(t-bu)-DOTA (Chematech) relative to the resin loading were reacted for 3 h in a final volume of 3 mL DMF. The product was cleaved from the resin in a 2 mL mixture consisting of trifluoroacetic acid, triisopropylsilane, and water (95:2.5:2.5). Purification was performed using RP-HPLC and the purified product was analysed by analytical RP-HPLC and MALDI-MS.

For preparing MB-17D which is the stereoisomer of MB17(L), the synthesis was based on Fmoc-3(2-naphthyl)-D-alanine. If not stated otherwise, in the present description MB17 means the L-stereoisomer.

Example 4

Coupling to Various Chelators



The chelators (DOTA, NOTA, NODAGA, DTPA, CHX-DTPA, PCTA, Do3A) were coupled to the MB17 linker by solid phase synthesis. In general, 13 μmol of resin which was coupled with the PSMA binding motif was swollen with DCM in a syringe with a filter. After washing the resin 5× with DMF, it was incubated 2× for 5 min with 20% of piperidine in DMF to deprotect the N-terminus. Another 5× washing with DMF followed.

Between 1.5 and 4 equivalents of the chelator (depending of the chelator), $0.98 \times n_{chelator}$ HATU (if needed) and 10 equivalents of DIPEA were dissolved in 500 μl of DMF, the solution was drawn up into the syringe containing the resin and incubated overnight. Next, the resin was washed 5× each with DMF, methanol, DCM and diethyl ether and dried over vacuum.

To check the state of the reaction, test separations were used. This was achieved by washing a small amount of resin with DCM into a filter tip and adding 100 μl of separation solution containing 95% TFA, 2.5% water and 2.5% TIPS. After 30 min of incubation, the solution was pipetted into ice cold diethyl ether and centrifuged. The diethyl ether was decanted and the remaining pellet was dissolved in 35 μl of ACN:H_2O (1:1) and analysed by HPLC (0-100% ACN in water within 5 min) and LC/MS.

If the desired product was obtained, the complete peptide was separated from the resin. The dried resin was incubated with 500 μl of the separation solution (95% TFA, 2.5% H_2O, 2.5% TIPS) for 2 hours. The resulting solution was mixed with ice cold diethyl ether and centrifuged (4000 min^{-1}, 5 min). The supernatant was discarded, new diethyl ether was added and the receptacle was shaken vigorously to resuspend the pellet. Again, the solution was centrifuged (4000 min^{-1}, 5 min) and the resulting supernatant discarded. The pellet was then vacuum dried and finally resuspended in 1 ml of ACN:H_2O (1:1).

Purification was achieved by preparative HPLC, the peaks were analysed by analytic HPLC (0-100% ACN in water within 5 min) and LC/MS and those containing the product were pooled and lyophilized.

Example 5

Radiolabelling

^{177}Lu-labelling

^{177}Lu (approx. 100 MBq) was mixed with 200 μl of 0.4 M sodium acetate buffer containing Chelex (pH=5). 10 μl of a 1 mM solution of the compound in 10% DMSO in water, 2 μl of a saturated solution of ascorbic acid and 40 μl of the solution containing ^{177}Lu were mixed and heated to 95° C. for 10 min. The labelling was checked by radio-HPLC (0-100% ACN in water within 5 min, Monolith column).

^{68}Ga-labelling

For the PET scan CHX-DTPA was labelled with ^{68}Ga. 1 ml of ^{68}Ga was eluted from a ^{68}Ge/^{68}Ga generator with 0.6 M HCl. 298 μl NaOAc buffer and 1 μl of a 10 mM solution of CHX-DTPA in DMSO was added and incubated for 5 min. Afterwards the product was purified using a SOLA cartridge. Washing was done with a 0.9% NaCl solution and for elution ethanol was used. The ethanol then was vaporized and the remaining product was dissolved in 100 μl of a 0.9% NaCl solution and 10 μl of phosphate buffer.

Example 6

Determination of the IC_{50} Value

A filter plate MultiScreen$_{HTS}$-DV was incubated at room temperature with 100 μl PBS with 1% BSA per well for 30 min. After removing the PBS/BSA solution 10^5 LNCaP cells in 50 μl of Opti-MEM were applied to each well. Different concentrations of the compounds (leading to concentrations of 0, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 500, 1000 and 5000 nM in each well) in 300 μl of Opti-MEM were mixed with 3 μl of a 150 nM solution of ^{125}I-labeled MIP-1466 in Opti-MEM. 50 μl of the resulting solution were added to each well, each concentration was pipetted in quadruples. Each well now contained the radioactively labelled ligand in a concentration of 0.75 nM and the competitive, not labelled ligand in the concentration mentioned above. The plate was then incubated for 45 min at room temperature on a shaker.

After the incubation, the cells were washed 2× with 100 μl of ice cold PBS and 1× with 200 μl of ice cold PBS. Finally, the filters were collected and the remaining radioactivity was measured with a gamma counter. Each tube was measured for 5 min.

The data measured by the gamma counter were evaluated with Graphpad Prism to achieve an inhibition concentration 50 (IC_{50}) against the radioactively labelled MIP-1095.

Conjugate	IC_{50} [nM]
MB17-DOTA	0.13 ± 0.08
MB17-NOTA	0.14 ± 0.08
MB17-DTPA	0.12 ± 0.05
MB17-CHX-DTPA	0.06 ± 0.04
MB17-PCTA	0.10 ± 0.06
MB17-DO3A	0.10 ± 0.05
MB17-NODAGA	0.09 ± 0.08

Example 7

μ PET—Imaging Using CHX-DTPA-MB17

Before injection into the mouse, the solution containing the purified ^{68}Ga-CHX-DTPA-coupled PSMA inhibitor was sterile-filtered. 100 μl of this solution was taken up into a syringe and then injected into a BALB/c nude mouse LNCaP xenograft, intravenously into the tail vein. The PET scan was recorded for 140 min with a Siemens Inveon PET (FIG. **15**)

Example 8

Determination of the Competitive Binding Affinity

In order to compare the series of novel compounds the competitive binding affinity and the specific internalization

was analyzed using the PSMA expressing cell line LNCaP. To determine specific cellular uptake, cells were blocked with 2-(phosphonomethyl)-pentanedioic acid (PMPA). The inhibition potency was also investigated by the enzyme-based NAALADase assay.

Cell Culture

For binding studies and in vivo experiments LNCaP cells (metastatic lesion of human prostatic adenocarcinoma, ATCC CRL-1740) were cultured in RPMI medium supplemented with 10% fetal calf serum and Glutamax (PAA, Austria). During cell culture, cells were grown at 37° C. in an incubator with humidified air, equilibrated with 5% CO2. The cells were harvested using trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA; 0.25% trypsin, 0.02% EDTA, all from PAA, Austria) and washed with PBS.

Cell Binding and Internalization

The competitive cell binding assay and internalization experiments were performed as described previously (Eder et al. 2012). Briefly, the respective cells (10^5 per well) were incubated with the radioligand (68Ga-labeled [Glu-urea-Lys (Ahx)]2-HBED-CC (Schafer et al., 2012) in the presence of 12 different concentrations of analyte (0-5000 nM, 100 μL/well). After incubation, washing was carried out using a multiscreen vacuum manifold (Millipore, Billerica, Mass.). Cell-bound radioactivity was measured using a gamma counter (Packard Cobra II, GMI, Minnesota, USA). The 50% inhibitory concentration (IC50) was calculated by fitting the data using a nonlinear regression algorithm (GraphPad Software). Experiments were performed three times.

To determine the specific cell uptake and internalization, 10^5 cells were seeded in poly-L-lysine coated 24-well cell culture plates 24 h before incubation. After washing, the cells were incubated with 25 nM of the radiolabeled compounds for 45 min at 37° C. and at 4° C., respectively. Specific cellular uptake was determined by competitive blocking with 2-(phosphonomethyl)pentanedioic acid (500 μM final concentration, PMPA, Axxora, Loerrach, Germany). Cellular uptake was terminated by washing 4 times with 1 mL of ice-cold PBS. Cells were subsequently incubated twice with 0.5 mL glycine-HCl in PBS (50 mM, pH=2.8) for 5 min to remove the surface-bound fraction. The cells were washed with 1 mL of ice-cold PBS and lysed using 0.3 N NaOH (0.5 mL). The surface-bound and the internalized fractions were measured in a gamma counter. The cell uptake was calculated as per cent of the initially added radioactivity bound to 10^6 cells [% ID/10^6 cells].

Naaladase Assay

Recombinant human PSMA (rhPSMA, R&D systems, Wiesbaden, Germany) was diluted in assay buffer (50 mM HEPES, 0.1 M NaCl, pH 7.5) to 0.4 μg/mL. The substrate Ac-Asp-Glu (Sigma, Taufkirchen, Germany, 40 μM final concentration) was mixed with natGa labeled analyte at concentrations ranging from 0.05 nM to 1000 nM in a final volume of 125 assay buffer. The mixtures were combined with 125 μL of the rhPSMA solution (0.4 μg/mL) and incubated for one hour at 37° C. The reaction was stopped by heating at 95° C. for 5 minutes. 250 μL of a 15 mM solution of ortho-phthaldialdehyde (Sigma, Taufkirchen, Germany) was added to all vials and incubated for 10 minutes at ambient temperature. Finally, 200 μL of the reaction solutions were loaded onto a F16 Black Maxisorp Plate (Nunc, Langenselbold, Germany) and read at excitation and emission wavelengths of 330 nm and 450 nm, respectively, using a microplate reader (DTX-880, Beckman Coulter, Krefeld, Germany). The data were analyzed by a one site-total binding regression algorithm of GraphPad (GraphPad Software, California, USA).

Biodistribution

7- to 8-week-old male BALB/c nu/nu mice (Charles River Laboratories) were subcutaneously inoculated into the right trunk with 5×10^6 cells of LNCaP (in 50% Matrigel; Becton Dickinson, Heidelberg, Germany). The tumors were allowed to grow until approximately 1 cm3 in size. The radiolabeled compounds were injected into the tail vein (approx. 1 MBq per mouse; 0.06 nmol). At 1 h after injection the animals were sacrificed. Organs of interest were dissected, blotted dry, and weighed. The radioactivity was measured using a gamma counter and calculated as % ID/g.

MicroPET

For the microPET studies, 10-25 MBq of the radiolabeled compounds in a volume of 0.15 ml (~0.5 nmol) were injected via a lateral tail vein into mice bearing LNCaP tumor xenografts. The anesthetized animals (2% sevoflurane, Abbott, Wiesbaden, Germany) were placed in prone position into the Inveon small animal PET scanner (Siemens, Knoxville, Tenn., USA) to perform dynamic microPET scans and 20 min-static scans; c.f. FIG. **1**, **3**, **5-14**

TABLE A

Substance	IC_{50} [nM]	Internalization [% IA/10^6 cells]
MB2	2.75 ± 0.82	8.78 ± 3.96 for Ga-68 5.22 ± 0.67 for Lu-177
MB3	10.51 ± 6.06	3.65 ± 1.32 for Lu-177
MB4	0.74 ± 0.50	14.18 ± 0.98 for Ga-68 14.25 ± 4.61 for Lu-177
MB10	8.67 ± 1.58	6.96 ± 3.90 for Lu-177
MB17	0.13 ± 0.08	17.02 ± 4.36 for Ga-68 17.51 ± 3.99 for Lu-177
MB17.D	12.41 ± 5.10	2.60 ± 0.14 for Lu-177
MB22	52.80	1.15 ± 0.19 for Lu-177
MB24	3.33	7.26 ± 2.76 for Lu-177
MB25	6.64	3.91 ± 0.54 for Lu-177
MB31	91.80	0.53 ± 0.48 for Lu-177
MB33	59.33	1.96 ± 0.20 for Lu-177
MB35	26.18	0.97 ± 0.17 for Lu-177

The present example shows that the binding affinity of PSMA inhibitors can be influenced by linker modifications. Two cyclic moieties and at least one aromatic moiety in the linker region of the substance seem to be preferable and resulted in the high affinity compounds MB4 and MB17. These novel variants show low nanomolar affinity to LNCap cell line and were specifically internalized at 37° C. up to 48% ID/10^6 cells. Former studies showed that besides binding affinity the internalization properties of PSMA-targeting probes are highly important and high internalization rates are essential for high in vivo tumor uptake and retention. Thus, MB17 represents a novel PSMA-targeting probe with optimal characteristics which was also confirmed by organ distribution and small animal PET imaging. MB 17 shows a high PSMA-specific tumor uptake (FIG. **2**). In addition, dynamic PET imaging of MB17 (FIG. **2**) shows an early enrichment in the bladder and also the maximum kidney uptake (highest point in the time-activity-curve) is as early as 15 min after injection of the radiotracer and diminishes substantially already after 20 minutes. With regard to therapeutic use, this gives clear clinical advantages for MB17 compared to other PSMA-inhibitors. In the PET diagrams (FIG. **1**) MB17 shows a rapid background clearance as well as a substantial reduction of the enrichment in the kidney after 2 hours while it is further accumulated and retained in the PSMA-expressing tumor.

In addition, organ distribution with ^{177}Lu (FIG. **4**) showed that the high initial kidney uptake is nearly completely washed out (2.13±1.36% ID/g) after 24 hours while the tumor uptake remained high and even increased (10.58±4.50% ID/g). Other organs as liver (0.08±0.03% ID/g), lung (0.11±0.13% ID/g) and spleen (0.13±0.05% ID/g) showed very low uptake. The favourable pharmacokinetics led to extremly high tumor-to-background ratios (Tumor/Blood: 1058; Tumor/Muscle: 529) after 24 hours

Table A clearly confirms that the chemical modifications in the linker region of the molecule affect the biological properties, e.g. affinity and internalization efficacy. MB17 and MB4 show the most promising binding properties on cells.

Example 9

Clinical Data Concerning MB17

PET/CT imaging was performed using the radiotracer MB17 labeled with Ga-68 (c.f FIG. **17**)

The ^{68}Ge/^{68}Ga-generator used for radiopharmaceutical production was purchased from IDB-Holland BV (Baarle-Nassau, The Netherlands). Disposable cassette kits and chemicals including the precursor in GMP-compliant grade used for the radiosynthesis were obtained from ABX advanced biochemical compounds (Radeberg, Germany). An Ultimate 3000 HPLC system (Dionex) (acetonitrile (A), water+0.1% TFA (B); gradient: 0.5 min 95% B, 10.0 min 80% A, flowrate: 2 mL/min) equipped with a Chromolith Performance RP-18e column (100×4.6 mm, Merck) and a NaI radiodetector (Raytest) was used to determine the radiochemical purity. Residual solvents were determined using a 6850 Series gas chromatograph (Agilent Technologies). Endotoxin testing was performed with an Endosafe®-PTS device (Charles River).

2 μg of MB17 were dissolved in 1.5 M acetate buffer pH 4.5 (1 mL) and 1 M ascorbic acid (10 μL) and transferred into the reaction vessel. The ^{68}Ge/^{68}Ga-generator was eluted with 10 mL of 0.6 M HCl and the eluate diluted with 9 mL of ultrapure water. The mixture was then transferred to a cation exchange cartridge (Macherey-Nagel PS-H+, Size M) and eluted with 5 M NaCl solution (1.2 mL) into the preheated reaction vessel (100° C.). The reaction mixture was heated for 10 minutes. The crude reaction mixture was then removed from the reaction vessel and transferred to a pre-conditioned (10 mL EtOH/10 mL ultrapure water) C18 cartridge (Waters Sep-Pak light). 9 mL ultrapure water was used to rinse the reaction vessel and passed over the C18 cartridge. The C18 cartridge was washed with another 5 mL of ultrapure water. The final product was eluted from the C18 cartridge with 2 mL of EtOH/H_2O (v:v 1:1), sterile filtered (Millipore Cathivex-GV, 0.22 μm) and diluted with 10 mL of phosphate buffered saline (PBS) solution pH 7.4 (according to Eur. Ph. 8.0 (4005000)). The ^{68}Ga-MB17 complex solution was applied to patients via an intravenous bolus.

Example 10

Human Therapy with ^{177}Lu-labeled MB17

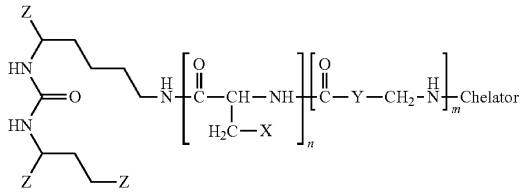
For therapy, the PSMA ligand MB17 was radiolabeled with Lu-177. $^{177}LuCl_3$ was obtained from Perkin Elmer (4 GBq, NEZ307D, 0.04 M HCl). 80 nmoles of MB17 were dissolved in 400 μL sodium acetate buffer (0.4 M, pH 5) supplemented with 5 μL of 20% ascorbic acid. The solution was transferred to the $^{177}LuCl_3$ and incubated for 10 minutes at 95° C. Finally, 2 mL 0.9% NaCl was added. For quality control, ITLC and radio-HPLC was performed.

The ^{177}Lu-labeled MB17 was applied to patients via an intravenous bolus (5 mL, slowly within 30 seconds). The intravenous application was accompanied by an infusion of 0.9% NaCl for 4.5 h starting at 0.5 h before injection. Reference is made to FIG. **18**.

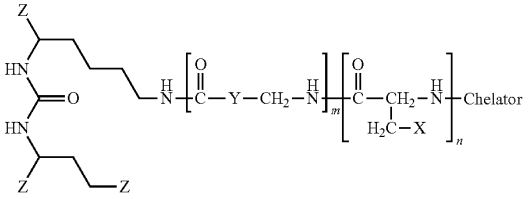
The invention is further described by the following numbered paragraphs:

1. A compound of Formula (Ia) or (Ib):

(Ia)



(Ib)



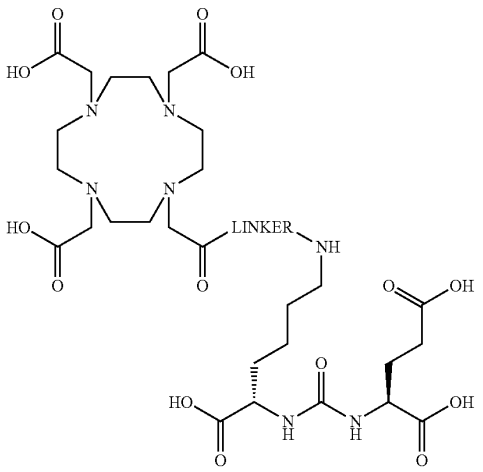
with:

n:	0, 1
m:	1, 2, 3, 4
Z:	—CO_2H, —SO_2H, —SO_3H, —SO_4H, —PO_2H, —PO_3H, —PO_4H_2
X:	Naphthyl, Phenyl, Biphenyl, Indolyl (=2,3-benzopyrrolyl), Benzothiazolyl
Y:	Aryl, Alkylaryl, Cyclopentyl, Cyclohexyl, Cycloheptyl
Chelator radical of:	1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (=DOTA), N,N''-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N''-diacetic acid (=HBED—CC), 1,4,7-triazacyclononane-1,4,7-triacetic acid (=NOTA), 2-(4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl)pentanedioic acid (NODAGA), 2-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentanedioic acid (DOTAGA), 1,4,7-triazacyclononane phosphinic acid (TRAP),

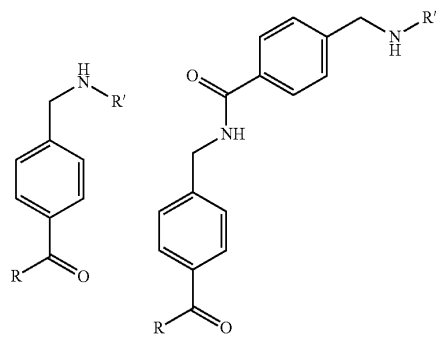
-continued

1,4,7-triazacyclononane-1-[methyl(2-carboxyethyl)phosphinic acid]-4,7-bis[methyl(2-hydroxymethyl)phosphinic acid] (NOPO),
3,6,9,15-tetraazabicyclo[9.3.1.]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (=PCTA),
N'-{5-[Acetyl(hydroxy)amino]pentyl}-N-[5-({4-[(5-aminopentyl)(hydroxy)amino]-4-oxobutanoyl}amino)pentyl]-N-hydroxysuccinamide (DFO),
Diethylenetriaminepentaacetic acid (DTPA)
Trans-cyclohexyl-diethylenetriaminepentaacetic acid (CHX—DTPA)
1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid (oxo-Do3A)
p-isothiocyanatobenzyl-DTPA (SCN—Bz—DTPA)
1-(p-isothiocyanatobenzyl)-3-methyl-DTPA (1B3M)
2-(p-isothiocyanatobenzyl)-4-methyl-DTPA (1M3B)
1-(2)-methyl-4-isocyanatobenzyl-DTPA (MX—DTPA).

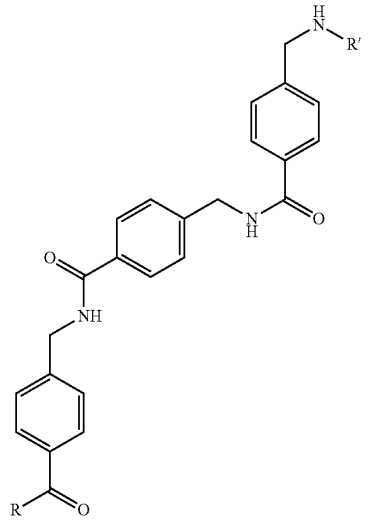
2. The compound of paragraph 1 having the structure R'-LINKER-R, wherein R'=radical of DOTA and R=radical of Glu-Urea-Lys:



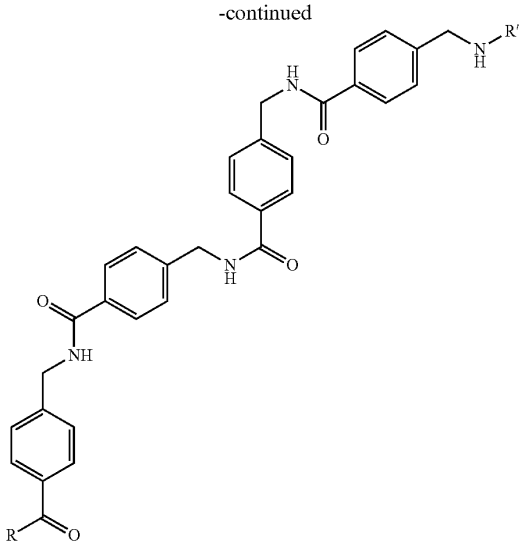
wherein the compound R'-LINKER-R is selected from:

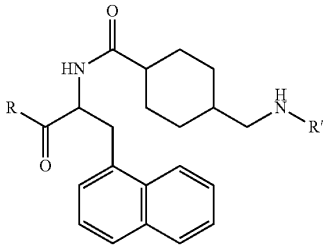


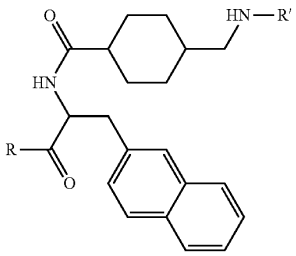
-continued

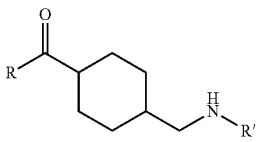


-continued

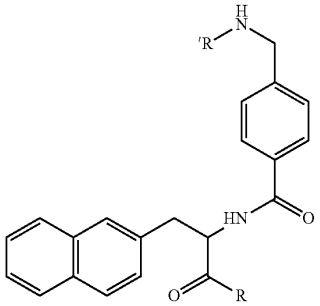


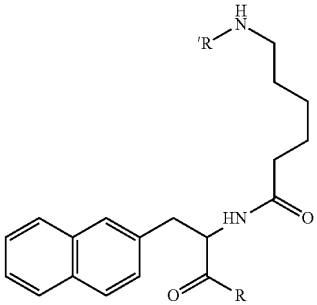


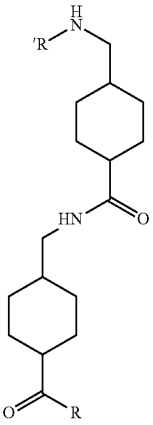


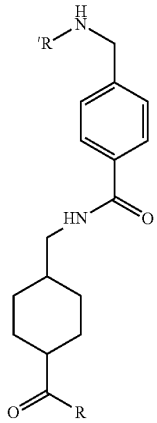


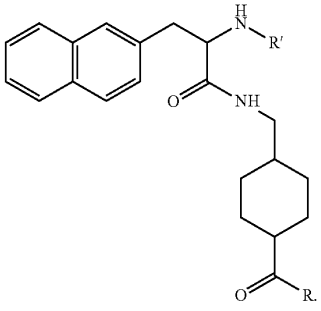
-continued





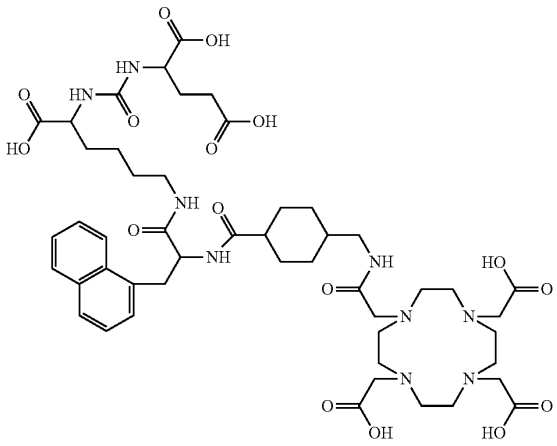




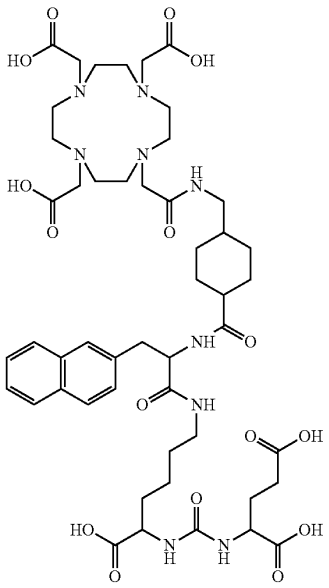


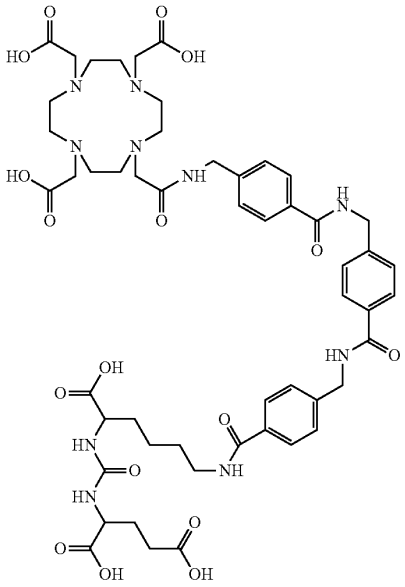
3. The compound of paragraph 1 or 2, selected from the following:

37

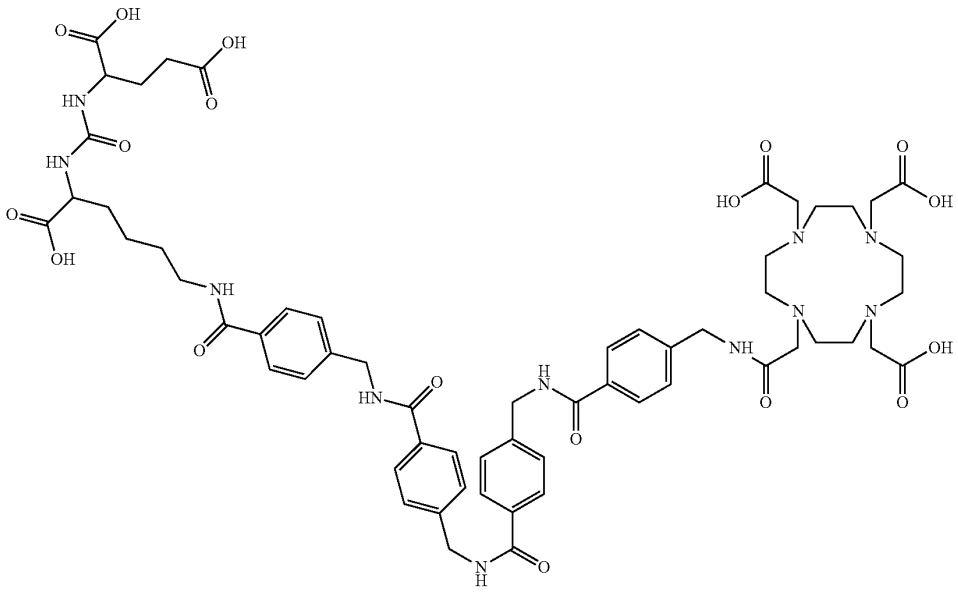


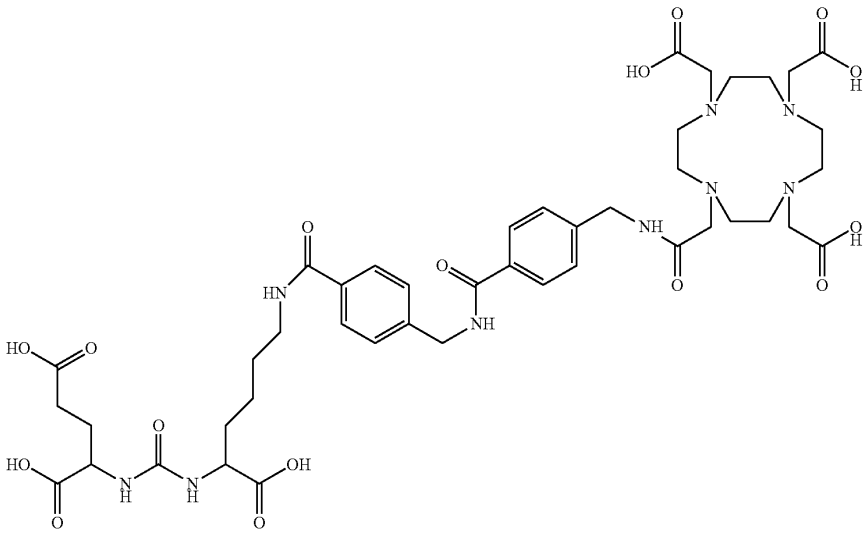
38

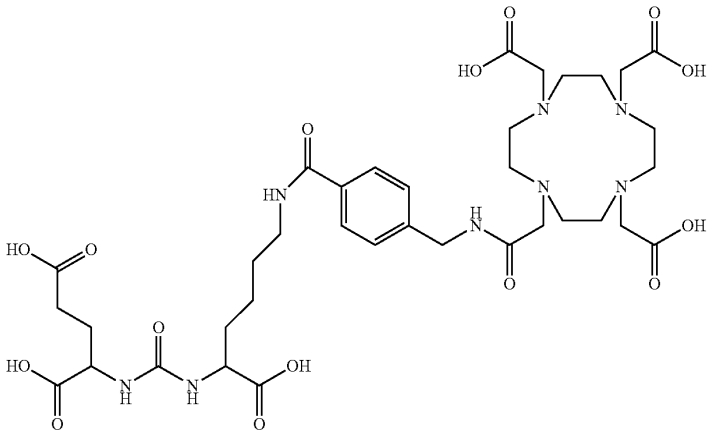




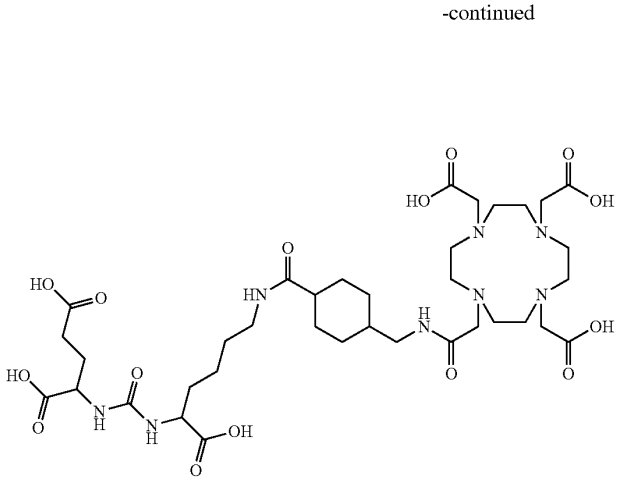
-continued

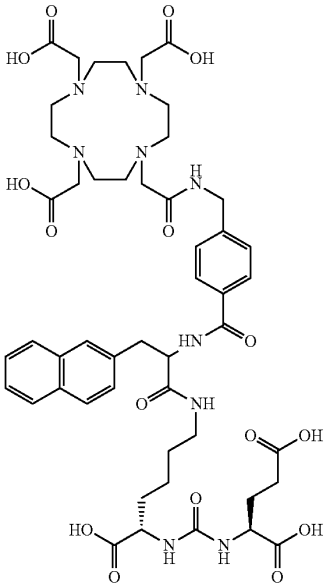


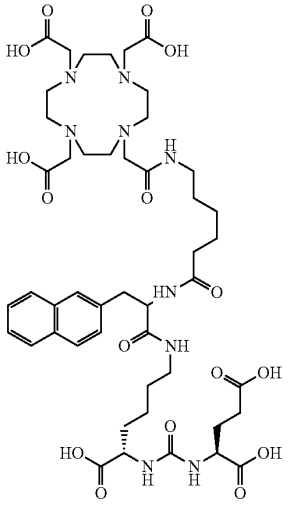


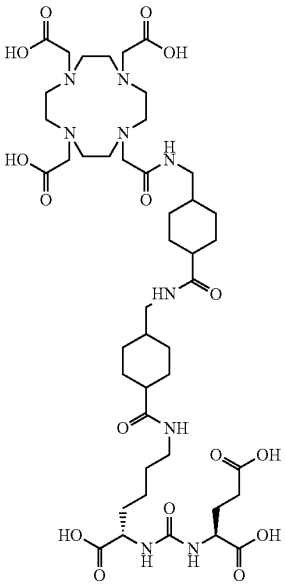


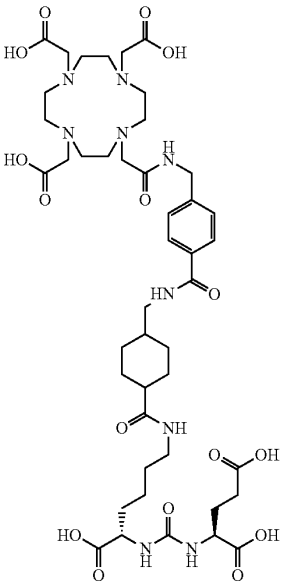
-continued



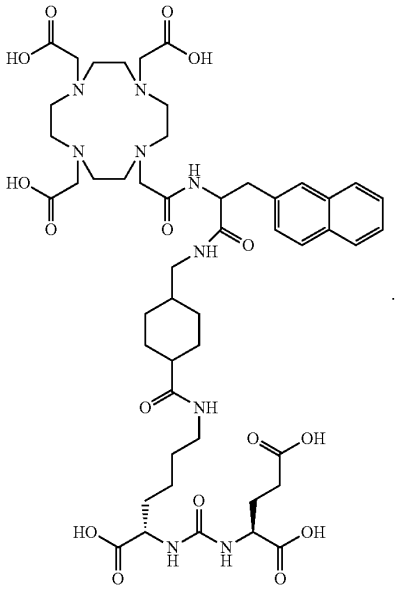








-continued



4. Use of the compound of any of paragraphs 1 to 3 for the preparation of radiolabled compounds.

5. A metal complex comprising a radionuclide and a compound of any of paragraphs 1 to 3.

6. The metal complex of paragraph 5, wherein the radionuclide is ^{111}In, ^{90}Y, ^{68}Ga, ^{177}Lu, ^{99m}Tc, ^{64}Cu, ^{153}Gd, ^{155}Gd, ^{157}Gd, ^{213}Bi, ^{225}Ac or Fe.

7. A pharmaceutical composition comprising a compound of any of paragraphs 1 to 3 or metal complex of paragraph 5 or 6, or a pharmaceutically acceptable salt, or ester thereof, and a pharmaceutically acceptable carrier.

8. Compound of any of paragraphs 1 to 3 or metal complex of paragraph 5 or 6 for use in a method of imaging in a patient.

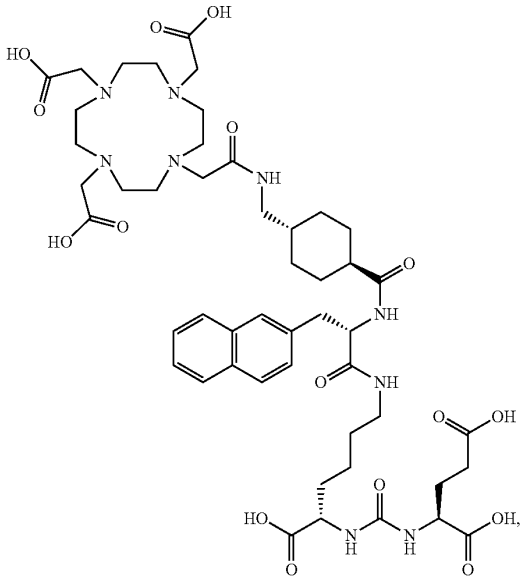
9. Compound of any of paragraphs 1 to 3 or metal complex of paragraph 5 or 6 for use in a method of diagnosing prostate cancer and/or metastasis thereof.

10. Compound of any of paragraphs 1 to 3 or metal complex of paragraph 5 or 6 for use in a method of treating prostate cancer and/or metastasis thereof.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

What is claimed is:

1. A compound of the formula:



or a salt thereof.

2. The compound or salt thereof according to claim **1**, wherein the compound or salt is lyophilized.

3. A composition comprising:

(1) the compound and/or a salt thereof according to claim **1**; and

(2) optionally a pharmaceutically acceptable carrier.

4. The composition of claim 3,

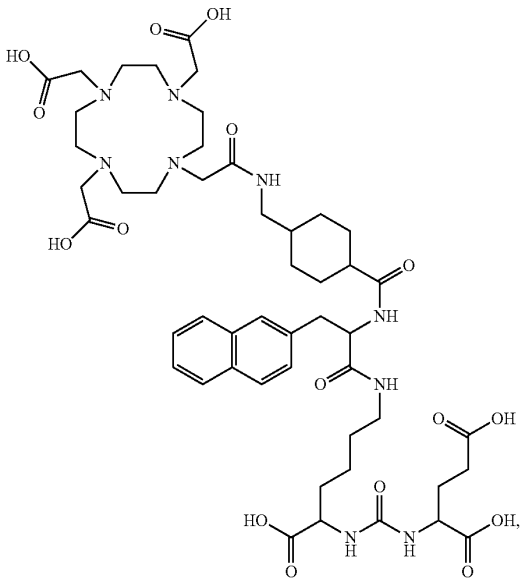
wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

5. The composition of claim 3, wherein the composition is a buffered solution.

6. The composition of claim 3, wherein the compound and/or a salt thereof, and optionally the pharmaceutically acceptable carrier, are lyophilized.

7. A compound of the formula:



or a salt thereof.

8. The compound or salt thereof according to claim 7, wherein the compound or salt is lyophilized.

9. A composition comprising:

(1) the compound and/or a salt thereof according to claim 7; and

(2) optionally a pharmaceutically acceptable carrier.

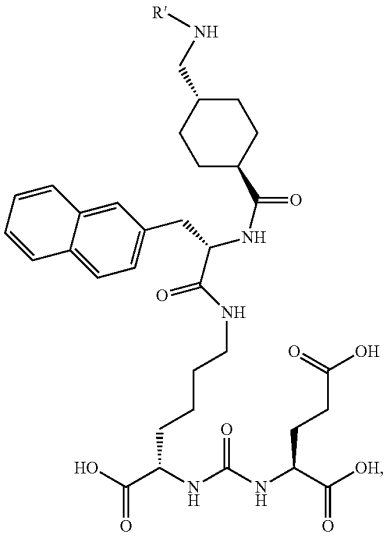
10. The composition of claim 9,

wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

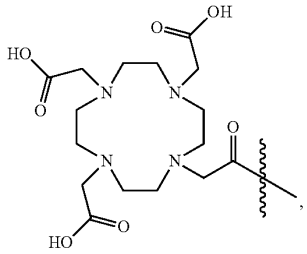
11. The composition of claim 9, wherein the composition is a buffered solution.

12. A compound of the formula:



or a salt thereof,

wherein R' is a chelator of the formula:



wherein ^{177}Lu is complexed to the chelator.

13. A composition comprising:

(1) the compound and/or a salt thereof according to claim 12; and

(2) optionally a pharmaceutically acceptable carrier.

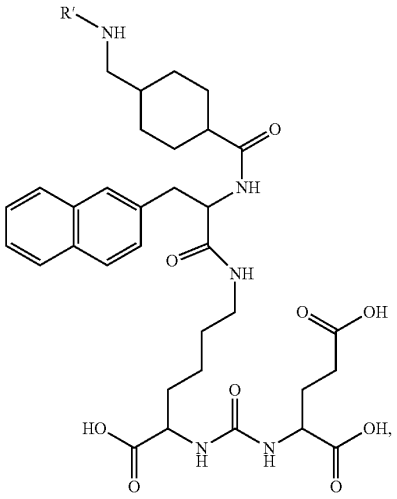
14. The composition of claim 13,

wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

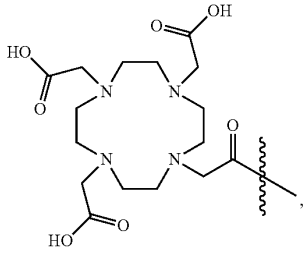
15. The composition of claim 13, wherein the composition is a buffered solution.

16. A compound of the formula:



or a salt thereof,

wherein R' is a chelator of the formula:



wherein ^{177}Lu is complexed to the chelator.

17. A composition comprising:

(1) the compound and/or a salt thereof according to claim **16**; and

(2) optionally a pharmaceutically acceptable carrier.

18. The composition of claim **17**,

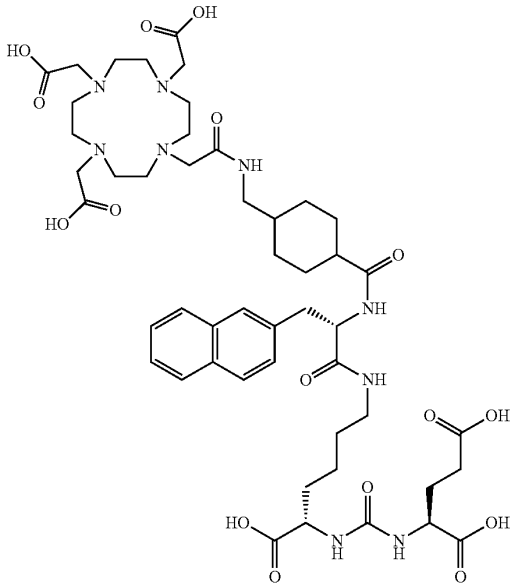
wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

19. The composition of claim **17**, wherein the composition is a buffered solution.

20. The composition of claim **9**, wherein the compound and/or a salt thereof, and optionally the pharmaceutically acceptable carrier, are lyophilized.

21. The compound of claim **7** having the formula:



or a salt thereof.

22. The compound or salt thereof according to claim **21**, wherein the compound or salt is lyophilized.

23. A composition comprising:

(1) the compound and/or a salt thereof according to claims **21**; and

(2) optionally a pharmaceutically acceptable carrier.

24. The composition of claim **23**,

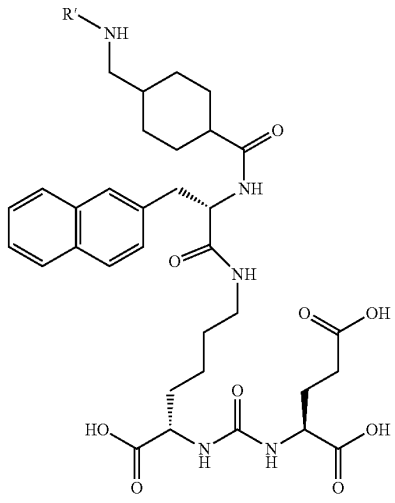
wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

25. The composition according to claim **23**, wherein the composition is a buffered solution.

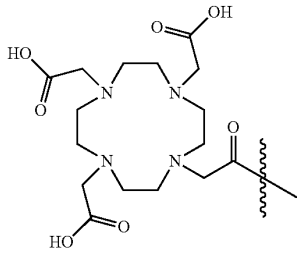
26. The composition of claim **23**, wherein the compound and/or a salt thereof, and optionally the pharmaceutically acceptable carrier, are lyophilized.

27. The compound of claim **16** having the formula:



or a salt thereof,

wherein R' is a chelator of the formula:



wherein ^{177}Lu is complexed to the chelator.

28. A composition comprising:

(1) the compound and/or a salt thereof according to claims **27**; and

(2) optionally a pharmaceutically acceptable carrier.

29. The composition of claim **28**,

wherein the composition further comprises an excipient, and

wherein the excipient is different from said pharmaceutically acceptable carrier.

30. The composition of claim **28**, wherein the composition is a buffered solution.

* * * * *