ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Scaffold

Koen Jansen,[†] Leen Heirbaut,[†] Jonathan D. Cheng,[‡] Jurgen Joossens,[†] Oxana Ryabtsova,[†] Paul Cos,[§] Louis Maes,[§] Anne-Marie Lambeir,[∥] Ingrid De Meester,[∥] Koen Augustyns,[†] and Pieter Van der Veken*,[†]

[†]Medicinal Chemistry (UAMC), Department of Pharmaceutical Sciences, University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium

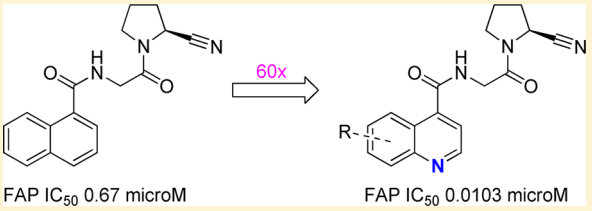
[‡]Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, Pennsylvania 19111-2497, United States

[§]Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Departments of Pharmaceutical and Biomedical Sciences, University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium

[∥]Medical Biochemistry, Department of Pharmaceutical Sciences, University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium

Supporting Information

ABSTRACT: Fibroblast activation protein (FAP) is a serine protease that is generally accepted to play an important role in tumor growth and other diseases involving tissue remodeling. Currently there are no FAP inhibitors with reported selectivity toward both the closely related dipeptidyl peptidases (DPPs) and prolyl oligopeptidase (PREP). We present the discovery of a new class of FAP inhibitors with a *N*-(4-quinolinoyl)-Gly-(2-cyanopyrrolidine) scaffold. We have explored the effects of substituting the quinoline ring and varying the position of its sp^2 hybridized nitrogen atom. The most promising inhibitors combined low nanomolar FAP inhibition and high selectivity indices (>10^3) with respect to both the DPPs and PREP. Preliminary experiments on a representative inhibitor demonstrate that plasma stability, kinetic solubility, and log *D* of this class of compounds can be expected to be satisfactory.



KEYWORDS: *Fibroblast activation protein (FAP), dipeptidyl peptidase IV (DPPIV), prolyl oligopeptidase (PREP), seprase*

Fibroblast activation protein (FAP, FAP-α, seprase) is a type II transmembrane serine protease, belonging to the prolyl oligopeptidase family. This family comprises serine proteases that cleave peptides preferentially after proline residues. Other important members of this family that are expressed in the human proteome are prolyl oligopeptidase (PREP) and the dipeptidyl peptidases (DPPs): DPPIV, DPPII, and DPP8/9.[1]

FAP expression is seen on activated stromal fibroblasts and pericytes of 90% of common human epithelial tumors examined.[2,3] It has been established that FAP expression promotes tumorigenesis in mouse models and that FAP inhibition can attenuate tumor growth.[4,5] FAP is also highly expressed in lesions characterized by activated stromal tissue such as present in cirrhosis, fibrotic diseases, osteoarthritis and rheumatoid arthritis, keloidosis, and in healing wounds.[6−11]

FAP has been demonstrated to possess both dipeptidyl peptidase and endopeptidase activity, catalyzed by the same active site. Several studies have identified in vitro substrates for FAP. Peptides found to be substrates of FAP's dipeptidyl peptidase activity include Neuropeptide Y, B-type natriuretic peptide, substance P, and peptide YY. Analogously, α2-antiplasmin, type I collagen, and gelatin were found to behave as in vitro substrates of the endopeptidase activity of FAP.[12−14] Nonetheless, the relevance of these findings under in vivo conditions has to be confirmed.

Currently no inhibitors with low nanomolar FAP affinity and selectivity toward both PREP and the DPPs have been reported. Most research effort in the domain of FAP-inhibitor discovery to date has been centered around pyrrolidine-2-boronic acid derivatives.[15,16] These compounds in general also display significant affinity for one or several DPPs.[15,16] The most representative of this class, Val-*boro*Pro (Talabostat, PT-100) **1** has reached phase II clinical trials (Table 1). It was evaluated as a therapeutic drug for, among others, metastatic kidney cancer, pancreatic adenocarcinoma, nonsmall cell lung cancer, and chronic lymphocytary leukemia (Figure 1). While talabostat in several of these trials was able to induce clinical response, safety concerns potentially related to the drug's lack of selectivity led to its withdrawal from further development.[17,18]

More recently, we and the group of Jiaang have focused on 2-cyanopyrrolidine derivatives for FAP inhibitor discovery.[19,20] The 2-cyanopyrrolidine fragment has been successfully ex-

Received: November 20, 2012
Accepted: March 18, 2013
Published: March 18, 2013

ACS Publications © 2013 American Chemical Society

Petitioner GE Healthcare – Ex. 1007, p. 491

Table 1. IC_{50}s for Reference Compounds 1 and 2

Nr	IC_{50} (μM)				
	FAP	DPP IV	DPP9	DPP II	PREP
1	0.07 ± 0.01	0.022 ± 0.001	ND[a]	0.086 ± 0.007	0.98 ± 0.06
2	0.37 ± 0.002	0.0020 ± 0.0002	>100	>100	>100

[a]Not determined.

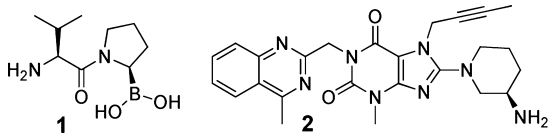


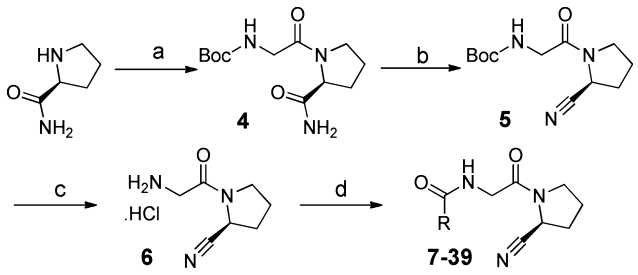
Figure 1. Structure of reference inhibitors used in this study: Val-*boro*Pro (**1**) and linagliptin (**2**).

plored before for discovery of DPP inhibitors, exemplified, for example, by the marketed DPP IV inhibitors vildagliptin and saxagliptin.[21−24] The work presented here deals with a group of 2-cyanopyrrolidines that represent the first examples of inhibitors combining low nanomolar FAP affinity and significant selectivity with respect to both PREP and the DPPs. Reference inhibitors used in this study are **1** and the FDA-approved, clinically used DPP IV inhibitor linagliptin (Tradjenta) **2** (Table 1).[25] The latter compound is structurally distinct from **1** but has also been described to possess significant FAP affinity.

Previous results pointed out that the *N*-acyl-glycyl-(2-cyanopyrrolidine) scaffold has significant potential to deliver FAP inhibitors with good selectivity toward PREP and the dipeptidyl peptidases.[19] The most potent compound identified (*N*-(1-naphthoyl)-Gly-(2-cyanopyrrolidine), **3**) displayed high nanomolar FAP-affinity.[19,20] For further optimization of this compound's potency, we proposed further exploration of the P3 area, containing the naphtyl group. A docking study furthermore indicated that the 1-naphthoyl residue could be involved in a cation−π interaction with the guanidine side chain of FAP's Arg123. A set of analogues potentially capable of corroborating this hypothesis and providing additional SAR information in the parts of chemical space surrounding the 1-naphthoyl group were prepared.

A general pathway for the synthesis of the target compounds **3**−**39** is displayed in Scheme 1. Commercially available prolinamide was coupled to *N*-Boc-glycine giving *N*-Boc-glycine-prolinamide **4** and subsequently dehydrated with trifluoroacetic anhydride to yield the corresponding nitrile **5**. The nitrile was deprotected in acetonitrile to give hydrochloric acid salt **6**, which was then coupled with a carboxylic acid or acyl chloride to give final compounds.

Scheme 1. Synthesis of Target Compounds 3-39[a]



[a]Reagents and conditions: (a) Boc-glycine, HATU, DIPEA, DMF-DCM, rt, 71%; (b) trifluoroacetic anhydride, pyridine, THF, 0 °C, 92%; (c) (i) TFA, MeCN, 0 °C, 24h; (ii) HCl, diethylether, 84%; (d) HATU, DIPEA, R-COOH or 1-chloro-*N*,*N*,2-trimethylprop-1-en-1-amine, RCOOH, TEA or RCOCl, TEA, 20−76%.

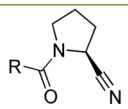
All compounds synthesized in this study were evaluated as inhibitors of FAP, DPP IV, DPP9, DPP II, and PREP using a chromogenic substrate assay.[19] It is worthwhile to stipulate that DPP9 potencies reported can reasonably be expected to also be indicative for inhibitor affinities toward the highly homologous DPP8.[26,27]

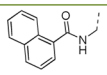
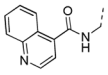
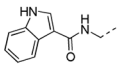
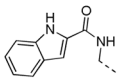
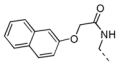
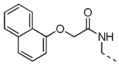
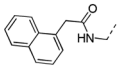
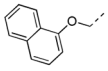
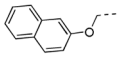
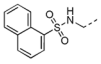
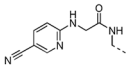
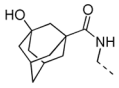
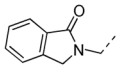
The results summarized in Table 2 show that the *N*-(4-quinolinoyl) substituted compound **7** has about 60 times more FAP-affinity than the initial *N*-(1-naphthoyl) based 'hit' **3**. Its potency as an inhibitor of FAP also clearly stands out among the other compounds in Table 2. The presence of other azaheteroaromatic substituents, as, for example, in the *N*-(indolyl-3-acyl) containing **8** and its regio-isomeric congener **9**, leads to a drastic drop in FAP-affinity. The same affinity trend was observed for compounds **10**−**12**: in these molecules, the electron density in the naphthyl ring can reasonably be expected to be higher than in compound **3**. Directly inspired by the docking study, these molecules were designed to gain affinity from a stronger cation−π interaction with FAP's Arg123. Further deviation from 'hit' structure **3** was investigated with compounds **13** and **14** in which the acylated glycine amine function was changed for an oxygen atom. This, however, was also found to be detrimental for FAP potency. Likewise and in line with results obtained earlier, introduction of a sulfonamide function replacing the P3 acyl group in **15** decreases potency very significantly. The ((5-cyanopyridin-2-yl)amino)acetamide **16** and 3-hydroxyadamantane-1-carboxamide **17** are nonbasic analogues of the *N*-substituted glycyl-(2-cyanopyrroldine) DPPIV inhibitors NVP-DPP 728 and vildagliptin, respectively.[24] By introducing an amide group instead of the parent structures' basic P2-amine function that is critical for DPP IV interaction, we hoped to decrease DPP IV potency, while retaining affinity for the phylogenetically very closely related FAP. Only moderate inhibition of the latter was observed however. Finally, an analogue of compound **18** with a P1-pyrrolidine-2-boronate residue instead of a 2-cyanopyrrolidine group has been described as a low nanomolar FAP inhibitor.[28] Nitrile **18**, however, loses almost all FAP inhibition when compared to its boronate analogue.

In terms of selectivity, all inhibitors in Table 2 display very limited to no affinity for the DPPs. As mentioned earlier, this can be rationalized by the absence of a basic P2 amine function known to be engaged in salt bridging with two acidic glutamate residues in the active center of the DPPs. The corresponding Glu203 and Glu204 in FAP have a different orientation to accommodate endopeptidase substrates, and thus also allow acylated P2-amines to enter FAP's active center.[15] As reported earlier, selectivity of *N*-acylated inhibitors with respect to the endopeptidase PREP is far less evident. Nonetheless, a comparison of initial 'hit' **3** ($SI_{[FAP/PREP]}$ = 5.3) and our new

Petitioner GE Healthcare – Ex. 1007, p. 492

Table 2. Enzymatic Evaluation Data for Compounds 3−18



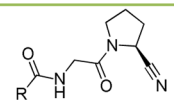
Nr	R	IC50 (μM) FAP	DPP IV	DPP 9	DPP II	PREP
3		0.67 ± 0.04	>100	>100	>100	3.3 ± 0.2
7		0.0103 ± 0.0004[a,b]	>100	>100	>100	0.86 ± 0.07
8		3.6 ± 0.2	>100	>25	>100	13.2 ± 0.4
9		15.4 ± 0.4	>100	>100	>100	>100
10		14.0 ± 0.7	>100	>100	>100	7.7 ± 0.5
11		9.4 ± 0.5	>100	>100	>100	5.6 ± 0.5
12		8.5 ± 0.3	>100	>100	>100	0.60 ± 0.06
13		>12.5	>100	>100	>100	33 ± 2
14		>12.5	>100	>100	>100	>50
15		17.6 ± 0.5	>100	>100	>100	8.3 ± 0.5
16		11.8 ± 0.4	>100	>100	>100	>100
17		2.1 ± 0.1	>100	>10	>100	2.4 ±0.3
18		42 ± 2	>100	>100	>100	39 ± 3

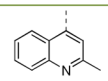
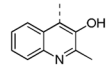
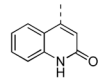
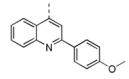
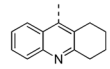
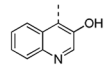
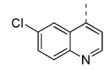
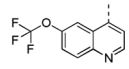
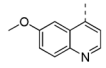
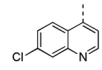
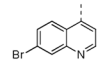
[a]FAP-affinity (K_i) was determined to be 3.0 ± 0.4 nM. [b]The mean result of three separate measurements.

'lead' 7 ($SI_{[FAP/PREP]}$ = 85) indicates that selectively optimizing for FAP affinity is possible.

Next, we turned our attention to the effect of substituting the *N*-(4-quinolinoyl) ring in 7 (Table 3). Overall, none of the evaluated substituents was able to improve FAP potency significantly, with substituents in the 2- and 3-position of the quinoline ring as in compounds **19**−**24**, having a clearly negative effect on this parameter. Identifying the factors that cause this effect is not evident from these six analogues, but both steric (**19**, **22**, and **23**) and electronic (**20**, **21**, and **24**, with an increased electron density in the pyridine ring compared to 7) factors seem to be contributive. Remarkably, PREP-affinity is affected in a more intricate manner by these substitution types: the negative effect of 3-hydroxylation in **20** and **24** significantly contrasts with the influence of a 2-hydroxyl functionality in **21**. Introducing a chlorine or bromine

Table 3. Effect of Substituents on the 4-Quinolinoyl Residue



Nr	R	IC50 (μM) FAP	DPP IV	DPP 9	DPP II	PREP
19		0.67 ± 0.02	>100	>100	>100	0.64 ± 0.04
20		5.9 ± 0.4	>100	>100	>100	53.6 ±1.3
21		1<IC50 <10	>100	>100	>100	0.35 ± 0.02
22		>10	>100	>100	>100	1 ± 0.1
23		0.77 ± 0.06	>100	>100	>100	18.5± 1.4
24		0.16 ± 0.01	>100	>100	>100	38 ± 2
25		0.0099 ± 0.0003	>100	>100	>100	16.8 ± 0.5
26		0.011 ± 0.0004[a]	>100	>100	>100	>50
27		0.014 ± 0.001	>100	>100	>100	0.84 ± 0.07
28		0.012 ± 0.001	>100	>100	>100	0.71 ± 0.05
29		0.0103 ± 0.0007	>100	>100	>100	0.61 ± 0.06
30		0.0092 ± 0.0005	>100	>100	>100	6.1 ± 0.4
31		0.0071 ± 0.0003	>100	>100	>100	1.4 ± 0.08
32		0.0062 ± 0.0004	>100	>100	>100	1.2 ± 0.1
33		0.19 ± 0.01	>100	>100	>100	0.35 ± 0.03
34		0.37 ± 0.02	>100	>100	>100	0.36 ± 0.02

[a]FAP-affinity (K_i) was determined to be 4.1 ± 0.6 nM.

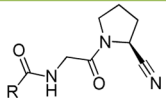
substituent at the 5-position of the quinoline ring such as in analogues **25** and **26** has limited effect on FAP potency. Most remarkably, the selectivity with respect to PREP of these inhibitors sharply increases ($SI_{[FAP/PREP]}$ > 1000), rendering

Petitioner GE Healthcare – Ex. 1007, p. 493

them the most favorable affinity/selectivity profile of all FAP-inhibitors we had identified so far. Functionalizing the quinoline ring's 6-position as in compounds **27**−**30** does not generally lead to marked differences in the affinity and selectivity profiles of the corresponding inhibitors compared to 'lead' **7**. The most notable compound in this subseries of molecules is the 6-methoxyquinoline **30**, which displays 10-fold less PREP affinity than its congeners. The 7-substituted chloroquinoline **31** and 7-bromoquinoline **32** again do not display relevantly changed FAP affinity when compared to **7**, but they do have moderately increased selectivity toward PREP. Finally, 8-chloroquinoline **33** and 8-bromoquinoline **34** were found to possess significantly reduced FAP potency, possibly due to steric effects. These modifications, however, have little effect on the PREP inhibitory potential of the corresponding molecules.

We subsequently studied the effect of varying the position of the sp^2 hybridized nitrogen atom of the quinoline ring (Table 4). We considered these investigations highly instrumental for understanding the striking difference in FAP affinity between 'hit' **3** and 'lead' **7**. If inhibitor affinity would depend significantly on the ring nitrogen's position, this could be indicative for a specific interaction with the enzyme, other than the cation−π complexation we originally hypothesized. On the basis of our docking study, the favorable contribution to binding of the latter would be less dependent on the nature of the (iso-)quinolinoyl isomer selected.

Table 4. Effect of Nitrogen Position in the (Iso-)Quinolinoyl Ring



Nr	R	IC50 (μM) FAP	DPP IV	DPP 9	DPP II	PREP
35		4.8 ± 0.2	>100	>100	>100	0.61 ± 0.07
7		0.0103 ± 0.0004	>100	>100	>100	0.86 ± 0.07
36		0.42 ± 0.04	>100	>100	>100	3.4 ± 0.2
37		0.107 ± 0.008	>100	>100	>100	2.4 ± 0.1
38		0.158 ± 0.004	>100	>100	>100	11.7 ± 0.8
39		2.17 ± 0.09	>100	>100	>100	8.2 ± 0.8

With FAP-affinities spanning almost 3 orders of magnitude, evaluation results of compounds **35**−**39** nonetheless reveal a pivotal importance of the nitrogen's position. Of all the positional isomers synthesized, the 4-quinolinoyl ring of 'lead' **7** clearly displays the best results and takes in a singular position within this series. The 4-isoquinolinoyl and 8-quinolinoyl derivatives (**35** and **39**, respectively) are characterized by very low FAP-affinity, even when compared to 'hit' **3**.

In conclusion, we have identified the *N*-(4-quinolinoyl)-glycyl-(2-cyanopyrrolidine) scaffold as highly promising for discovery of FAP-inhibitors. It is the first scaffold type demonstrated to have potential for rendering compounds that combine low nanomolar FAP-affinity and a selectivity index $>10^3$ with respect to both the DPPs and PREP. Our SAR investigations so far have mainly focused on the P3 part of the scaffold but already allow to conclude that the *N*-(4-quinolinoyl) residue is a critical element in determining FAP-affinity. As such, it was found to hold a privileged position within the series of different quinolinoyl and isoquinolinoyl isomers evaluated. Furthermore, we have shown that introduction of substituents at the 5-position of the quinolinoyl ring system is a possible means of maximizing inhibitor selectivity with respect to PREP. So far, however, we have not been able to identify the eventual specific interactions between FAP and the 4-quinolinoyl ring that could underlie the observed affinities.

To this end, we are currently expanding our SAR-data set by investigating additional modification types in the P3, P2, and P1 region of these molecules. In addition, preclinical ADME and in vivo pharmacokinetic parameters of the optimal molecules will be determined. Inhibitor **7** has a kinetic solubility of >200 μM and a log *D* of 0.51 at pH 7.4. Furthermore, **7** was stable for >24 h both in PBS buffer at pH 7.4 and in rat plasma. Furthermore, **26**, which together with inhibitor **25** shares low-nanomolar FAP-affinity and a FAP/PREP selectivity index $>10^3$, has a kinetic solubility of >200 μM and a log *D* of 0.7 at pH 7.4. This molecule was stable for 24 h in PBS and human plasma and 80% stable after 24 h in mouse plasma. It displayed mouse microsomal stability of 70% over 24 h.

■ ASSOCIATED CONTENT

Supporting Information

Analytical data and experimental procedures for synthetic preparation, enzymatic evaluation, and physicochemical characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pieter.vanderveken@ua.ac.be. Tel: +32-3 265 27 08.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was financially supported by the Fund for Scientific Research Flanders/FWO-Vlaanderen (to I.D.M. and A-M.L.), the research (BOF)-fund of the University of Antwerp (to K.J., I.D.M., K.A., and P.V.d.V.), and the Hercules foundation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are indebted to Nicole Lamoen and Sophie Lyssens for excellent technical assistance.

Petitioner GE Healthcare – Ex. 1007, p. 494

■ ABBREVIATIONS

FAP, fibroblast activation protein; PREP, prolyl oligopeptidase; DPPII, dipeptidyl peptidase II; DPPIV, dipeptidyl peptidase IV; DPP9, dipeptidyl peptidase 9; HATU, 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; SI, selectivity index

■ REFERENCES

(1) Aertgeerts, K.; Levin, I.; Shi, L.; Snell, G. P.; Jennings, A.; Prasad, G. S.; Zhang, Y.; Kraus, M. L.; Salakian, S.; Sridhar, V.; Wijnands, R.; Tennant, M. G. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein alpha. *J. Biol. Chem.* **2005**, *280*, 19441−19444.

(2) Garin-Chesa, P.; Old, L. J.; Rettig, W. J. Cell-surface protein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7235−9.

(3) Scanlan, M. J.; Raj, B. K.; Calvo, B.; Garin-Chesa, P.; Sanz-Moncasi, M. P.; Healey, J. H.; Old, L. J.; Rettig, W. J. Molecular cloning of fibroblast activation protein-alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5657−5661.

(4) Santos, A. M.; Jung, J.; Aziz, N.; Kissil, J. L.; Puré, E. Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. *J. Clin. Invest.* **2009**, *119*, 3613−25.

(5) Cheng, J. D.; Valianou, M.; Canutescu, A. A.; Jaffe, E. K.; Lee, H. O.; Wang, H.; Lai, J. H.; Bachovchin, W. W.; Weiner, L. M. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. *Mol. Cancer Ther* **2005**, *4*, 351−360.

(6) Wang, X. M.; Yao, T.-W.; Nadvi, N. A.; Osborne, B.; McCaughan, G. W.; Gorrell, M. D. Fibroblast activation protein and chronic liver disease. *Front. Biosci.* **2008**, *13*, 3168−3180.

(7) Acharya, P. S.; Zukas, A.; Chandan, V.; Katzenstein, A. L.; Pure, E. Fibroblast activation protein: a serine protease expressed at the remodeling interface in idiopathic pulmonary fibrosis. *Hum. Pathol.* **2006**, *37*, 352−360.

(8) Dienus, K.; Bayat, A.; Gilmore, B. F.; Seifert, O. Increased expression of fibroblast activation protein-alpha: implications for the development of a new treatment option. *Arch. Dermatol. Res.* **2010**, *302*, 725−731.

(9) Milner, J. M.; Kevorkian, L.; Young, D. A.; Jones, D.; Wait, R.; Donell, S. T.; Barksby, E.; Patterson, A. M.; Middleton, J.; Cravatt, B. F.; Clark, I. M.; Rowan, A. D.; Cawston, T. Fibroblast activation protein alpha is expressed by chondrocytes following a pro-inflammatory stimulus and is elevated in osteoarthritis. *Arthritis Res. Ther.* **2006**, *8*, R23.

(10) Milner, M.; Patel, A.; Rowan, A. D. Emerging role of serine proteinases in tissue turnover in arthritis. *Arthritis Rheum.* **2008**, *58*, 3644−3656.

(11) Bauer, S.; Jendro, M. C.; Wadle, A.; Kleber, S.; Stenner, F.; Dinser, R.; Reich, A.; Faccin, E.; Gödde, S.; Dinges, H.; Müller-Ladner, U.; Renner, C. Fibroblast activation protein is expressed by rheumatoid myofibroblast-like synoviocytes. *Arthritis Res. Ther.* **2006**, *8*, R171.

(12) Brokopp, C. E.; Schoenauer, R.; Richards, P.; Bauer, S.; Lohmann, C.; Emmert, M. Y.; Weber, B.; Winnik, S.; Aikawa, E.; Graves, K.; Genoni, M.; Vogt, P.; Lüscher, T. F.; Renner, C.; Hoerstrup, S. P.; Matter, C. M. Fibroblast activation protein is induced by inflammation and degrades type I collagen in thin-cap fibroatheroma. *Eur. Heart J.* **2011**, *32*, 2713−2722.

(13) O'Brien, P.; O'Connor, B. F. Seprase: an overview of an important matrix serine protease. *Biochim. Biophys. Acta* **2008**, *1784*, 1130−1145.

(14) Keane, F. M.; Nadvi, N. A.; Yao, T.-W.; Gorrell, M. D.; Neuropeptide, Y. B-type natriuretic peptide, substance P and peptide YY are novel substrates of fibroblast activation protein-alpha. *FEBS J.* **2011**, *278*, 1326−1332.

(15) Wolf, B. B.; Quan, C.; Tran, T.; Wiesman, C.; Sutherlin, C. On the edge of vamidation-Cancer protease fibroblast activation protein. *Mini-Rev. Med. Chem.* **2008**, *8*, 719−727.

(16) Hu, Y.; Ma, L.; Wu, M.; Wong, M. S.; Li, B.; Corral, S.; Yu, Z.; Nomanbhoy, T.; Alemayehu, S.; Fuller, S. R.; Rosenblum, J. S.; Rozenkrants, N.; Minimo, L. C.; Ripka, W. C.; Szardenings, A. K.; Kozarich, J. W.; Shreder, K. R. Synthesis and structure−activity relationship of N-alkyl-Gly-*boro*Pro inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4239−4242.

(17) Eager, R. M.; Cunningham, C. C.; Senzer, N. N.; Stephenson, J.; Anthony, S. P.; O'Day, S. J.; Frenette, G.; Pavlick, A. C.; Jones, B.; Uprichard, M. Nemunaitis, Phase II assessment of talabostat and cisplatin in second-line stage IV melanoma J. *BMC Cancer* **2009**, *9*, 263.

(18) Narra, K.; Mullins, S. R.; Lee, H. O.; Stzremkowski-Brun, R.; Magalong, K.; Christansen, V. J.; McKee, P. A.; Egleston, B.; Cohen, S. J.; Weiner, L. M.; Meropol, N. J.; Cheng, J. D. Phase II trial of single agent Val-boroPro (talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. *Cancer Biol. Ther.* **2007**, *7*, 1691−1699.

(19) Ryabtsova, O.; Jansen, K.; Van Goethem, S.; Joossens, J.; Cheng, J. D.; Lambeir, A.-M.; De Meester, I.; Augustyns, K.; Van der Veken, P. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3412−3417.

(20) Tsai, T. Y.; Yeh, T. K.; Chen, X.; Hsu, T.; Jao, Y. C.; Huang, C. H.; Song, J. S.; Huang, Y. C.; Chien, C. H.; Chiu, Y. H.; Yen, S. C.; Tang, H. K.; Chao, Y. S.; Jiaang, W. K. Substituted 4-carboxymethylpyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. *J. Med. Chem.* **2010**, *53*, 6573−6583.

(21) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J. Med. Chem.* **2003**, *46*, 2774−2789.

(22) Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S. P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L.G. S. Synthesis of novel potent dipeptidyl peptidase IV Inhibitors with enhanced chemical stability: interplay between the N-terminal amino acid alkyl side chain and the cyclopropyl group of α-aminoacyl-L-*cis*-4,5-methanoprolinenitrile-based inhibitors. *J. Med. Chem.* **2004**, *47*, 2587−2598.

(23) Ashworth, D. A.; Atrash, B.; Baker, G. A.; Baxter, A. J.; Jenkins, P. D.; Jones, M. D.; Szelke, M. 4-Cyanothiazolidides as very potent, stable inhibitors of dipeptidyl peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.

(24) Van der Veken, P.; Haemers, A.; Augustyns, K. Prolyl peptidases related to dipeptidyl peptidase IV: Potential of specific inhibitors in drug discovery. *Curr. Top. Med. Chem.* **2007**, *7*, 621−635.

(25) Thomas, L.; Eckhardt, M.; Langkopf, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. 8-(3-(*R*)-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2007**, *50*, 6450−6453.

(26) Van Goethem, S.; Mateeussen, V.; Joossens, J.; Lambeir, A. M.; Chen, X.; De Meester, I.; Haemers, A.; Augustyns, K.; Van der Veken, P. Structure−activity relationship studies on isoindoline inhibitors of dipeptidyl peptidases 8 and 8 (DPP8, DPP9): is DPP8-selectivity attainable? *J. Med. Chem.* **2011**, *54*, 5737−5746.

(27) Dubois, V.; Van Ginneken, C.; De Cock, H.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Chen, X.; Scharpe, S.; De Meester, I. Enzyme activity and immunohistochemical localization of dipeptidyl

Petitioner GE Healthcare – Ex. 1007, p. 495

peptidase 8 and 9 in male reproductive tissues. *J. Histochem. Cytochem.* **2009**, *57*, 531−541.

(28) Tran, T.; Quan, C.; Edosada, C. Y.; Mayeda, M.; Wiesmann, C.; Sutherlin, D.; Wolf, B. B. Synthesis and structure−activity relationship of N-acyl-Gly-, N-acyl-Sar- and N-blocked-boroPro inhibitors of FAP, DPP4 and POP. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1438−1442.

Petitioner GE Healthcare – Ex. 1007, p. 496