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Discovery of new binding elements in DPP-4 inhibition and their applications in novel DPP-4 inhibitor design

Gui-Bai Liang [a,*], Xiaoxia Qian [a], Tesfaye Biftu [a], Suresh Singh [a], Ying-Duo Gao [a], Giovanna Scapin [b], Sangita Patel [b], Barbara Leiting [c], Reshma Patel [c], Joseph Wu [c], Xiaoping Zhang [c], Nancy A. Thornberry [c], Ann E. Weber [a]

[a] Merck Research Laboratories, Department of Medicinal Chemistry, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[b] Merck Research Laboratories, Global Structural Biology, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[c] Merck Research Laboratories, Department of Metabolic Disorders, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA

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ABSTRACT

Probing with tool molecules, and by modeling and X-ray crystallography the binding modes of two structurally distinct series of DPP-4 inhibitors led to the discovery of a rare aromatic fluorine H-bond and the spatial requirement for better biaryl binding in the DPP-4 enzyme active site. These newly found binding elements were successfully incorporated into novel DPP-4 inhibitors.

Incretin hormones, primarily glucagon-like peptide 1 (GLP-1)[1,2] and glucose dependent insulinotropic polypeptide (GIP),[3] are known to stimulate glucose dependent insulin biosynthesis and secretion.[4] Inhibition of dipeptidyl peptidase IV (DPP-4) has been shown to prolong the beneficial effects of these incretin hormones by stabilizing the intact (active) form of the hormones.[5] Human clinical studies of several small molecule DPP-4 inhibitors, such as sitagliptin,[6] have shown improvement in glycemic control and improvement in β-cell function in patients with type 2 diabetes.[7] At an early stage of the DPP-4 project, which ultimately led to the discovery of sitagliptin (**16**), two structurally distinctive lead series had been studied (Fig. 1).[8,9] The glycine-based DPP-4 inhibitors (α-series **1**) were developed from compounds described in earlier publications in the field,[10] whereas the β-alanine-based DPP-4 inhibitors (β-series **2**) were developed from a high throughput screening lead.[9] Structure-activity relationship studies suggested that the pyrrolidine amide moieties of series **1** and **2** did not occupy the same pocket in the enzyme active site.

For α-series **1**, only small substituents (e.g., fluorine, nitrile) were tolerated on the pyrrolidine ring,[8b] whereas in β-series **2**, not only could the pyrrolidine amide be substituted with large groups at either the 2 and 3 positions leading to increased potency,[9b] but also the pyrrolidine itself could be replaced by piperazine and other cyclic amines.[6] In addition, in series **1** the cyclohexyl ring could be substituted at the 4-position with a variety of aryl sulfonamides providing compounds with enhanced potency;[8a] however, only small substituents such as fluorine, chlorine, and methyl group are tolerated on the benzyl group in series **2**.[9a] Before the X-ray structure of DPP-4 was published in 2003,[11] these distinct SARs provided an opportunity for us to probe the binding modes of these inhibitors in the DPP-4 enzyme active site, leading to the design of novel inhibitors.

We speculated that the fluoropyrrolidine amide moiety in the α-series and fluorobenzyl group of the β-series occupied the same hydrophobic pocket in the DPP-4 active site. To test this hypothesis, we turned our attention to aspartic acid-based analogs which embedded both a glycine unit and a β-alanine unit (Fig. 2). Starting from *N*-Boc protected aspartate **3**, these compounds were readily made through a reaction sequence of amide coupling, ester hydrolysis, second amide coupling, and finally deprotection (Scheme 1). However, when these compounds (**6a–c**)[12] were compared to their β-series analogs (**2a–c**),[9a] significantly reduced activities were observed (Table 1). All reported compounds were evaluated in vitro for their inhibition of DPP-4 activity[13] and selectivity against DPP-8 and DPP-9. Although disputed recently,[14] inhibition of DPP-8 and DPP-9 is very likely associated with toxicity in preclinical species.[15] DPP-8 and DPP-9 activities are similar, thus only DPP-8 data are reported. All new compounds were inactive

* Corresponding author. Tel.: +1 732 594 3543; fax: +1 732 594 3007.
E-mail address: gui-bai_liang@merck.com (G.-B. Liang).

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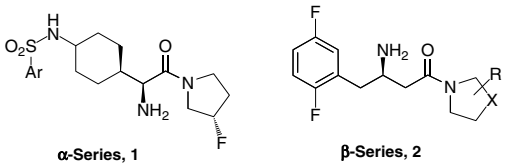


Figure 1.

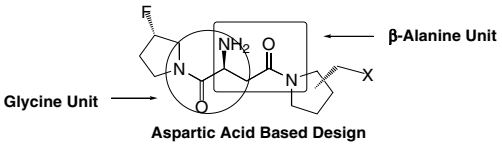
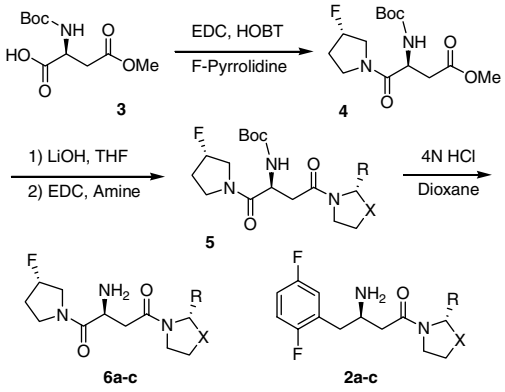


Figure 2.

(IC_{50} > 50 μM) against fibroblast activation protein and quiescent cell proline dipeptidase (QPP, also known as DPP-7).[16]

Although the simple replacement of the fluorobenzyl group with a fluoropyrrolidine amide moiety was not successful, continued SAR studies in the α-series had led to a group of oxadiazole containing compounds (**7a**–**c**),[17] and a completely different approach (amide bioisostere replacement) in the β-series had led to a group of very similar oxadiazole containing compounds (**12a**–**c**). The synthesis of the latter is outlined in Scheme 2, starting from the known β-lactam intermediate **8**.[18] Methylation yielded mostly the trans isomer **9**, which was converted to acid **10** following a route previously reported.[19] Acid **10** was then treated with corresponding amidoximes in the presence of EDC to yield oxadiazole **11**, which were then deprotected to give **12**. The similarity between their structures, as well as their DPP-4 inhibitory activities (summarized in Table 2), strongly suggest that the fluoropyrrolidine amide moiety of the α-series and the fluorobenzyl group of the β-series occupy the same pocket in the enzyme active site.[19]

To seek supporting evidence for this working hypothesis, computer modeling was carried out after the publication of the DPP-4 X-ray structure.[11] In the original structure of the enzyme bound to L-valine pyrrolidine amide **13**, two key interactions were identified: a salt bridge between the amino group of the valine amide and Glu205 and/or Glu206 and a hydrogen bond between the valine carbonyl oxygen and Arg125. When β-alanine-based DPP-4 inhibitors were docked in a similar orientation (A), many highly potent inhibitors with large substituents on the amide ring, e.g. compound **14**,[20] could not be accommodated (Fig. 3). This result prompted a computer search for new orientations that would fit a large substituent on the amide ring into the active site. The

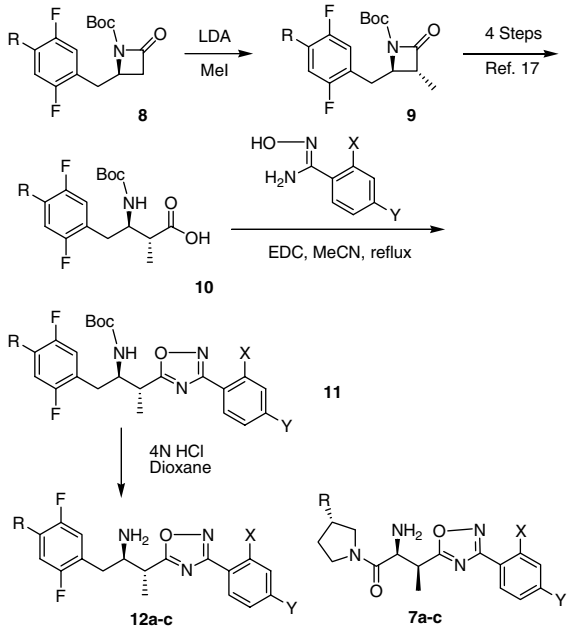


Scheme 1.

Table 1
Activities of aspartic acid-based DPP-4 inhibitors **6** and their β-series analogs **2**

Compound	X, R	DPP-4 IC_{50}[a] (nM)	DPP-8 IC_{50}[a] (nM)
6a	X=S; R=H	9800	17,000
2a		270	2100
6b	X=CH_2; R=CH_2OH	9200	18,000
2b		200	27,000
6c		8800	47,000
2c		55	>100,000

[a] Values reported are the mean of a minimum of two experiments with a standard deviation <25% of the mean, and the same for data in other tables.



Scheme 2.

Table 2
Activities of oxadiazole containing DPP-4 inhibitors **7** and their β-series analogs **12**

Compound	R, X, Y	DPP-4 IC_{50} (nM)	DPP-8 IC_{50} (nM)
7a	R=H; X=H, Y=OCF_3	1800	>100,000
12a		4400	>100,000
7b	R=F; X=Cl; Y=Cl	615	>100,000
12b		600	>100,000
7c	R=F; X=Cl, Y=SO_2Me	40	>100,000
12c		46	>100,000

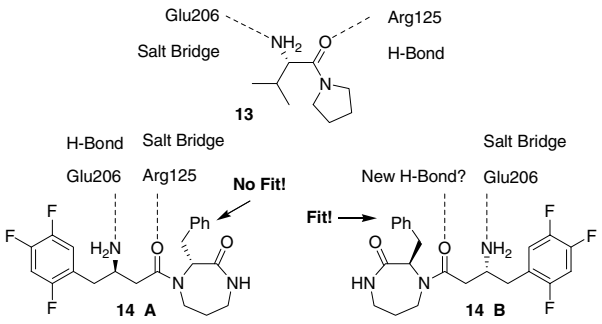


Figure 3.

search resulted in a new orientation (B) consistent with our working hypothesis that the fluoropyrrolidine amide moiety of the α-

series and fluorobenzyl group of the β-series occupy the same hydrophobic pocket in DPP-4 active site, while the free amino group keeps the same salt bridge with Glu205 and/or Glu206. However, a hydrogen bond to the carbonyl oxygen was not well defined, and no conclusions were drawn on detailed alignment and interactions until X-ray structures of the β-series of inhibitors bound DPP-4 became available.

The overlay of X-ray structures of compound **15**[21] (α-series) and sitagliptin[6] (**16**, β-series) bound to the active site of DPP-4 is shown in Figure 4. As it was proposed, the fluoropyrrolidine amide moiety of the α-series and the fluorobenzyl group of the β-series do occupy the same pocket in the enzyme active site, even though sitagliptin **16** lacks the sterically demanding benzyl substitution. In this new orientation, the carbonyl oxygen of **16** forms a hydrogen bond to Tyr547 through a bridging water molecule in the active site.[6] In the complex with compound **15**, the water molecule was displaced by the dimethyl amide moiety which forms a hydrogen bond directly with Tyr547 (in green), which was re-oriented to have a better alignment for hydrogen bonding.[21]

An unexpected discovery was that the fluorine (Fig. 4, X) at the 2 position of the benzyl group in **16** occupied the same space as the pyrrolidine amide oxygen (Fig. 4, X) in **15** and formed a hydrogen bond to Asn710. The distance between the fluorine and the NH nitrogen is measured at 3.2 Å, in the range of a typical hydrogen bond. Although rarely observed, aromatic carbon-bound fluorine is known to participate in significant hydrogen bonds and can also interact with charged molecules.[22] This newly discovered binding element became a focal point in our continued search for structurally diverse new leads. We envisaged that a carbonyl oxygen could be used to replace the fluorine, form the same hydrogen bond, and therefore, retain the binding energy. Thus, valerolactam-based DPP-4 inhibitors **20** were designed and synthesized (Scheme 3).

Using cesium carbonate as a base, alkylation of pyridone went exclusively on nitrogen to yield **17**. Michael addition was carried out in neat α-methylbenzylamine. Diastereomers of **18** were not easily separated, so the mixture was carried on, and the two diasteromers of the *t*-Boc protected final product were separated by HPLC using a Chiral-Cell OJ column. The absolute configuration of **20a** was confirmed by X-ray crystal structure, and others were assigned analogously, based on their DPP-4 inhibitory activities.

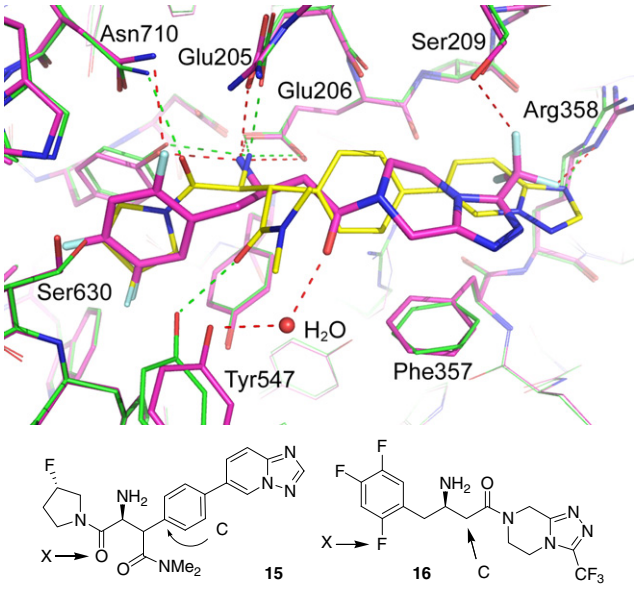
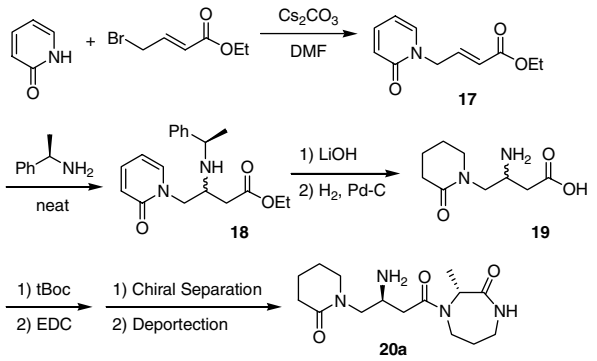
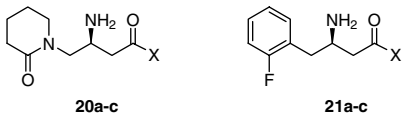


Figure 4. Overlay of X-ray structures of **15** (yellow) and **16** (magenta) bound to the active site of DPP-4.

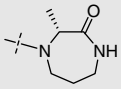
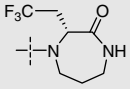
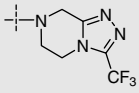


Scheme 3.

Table 3
Activities of valerolactam containing DPP-4 inhibitors **20** and their β-series analogs **21**



20a-c **21a-c**

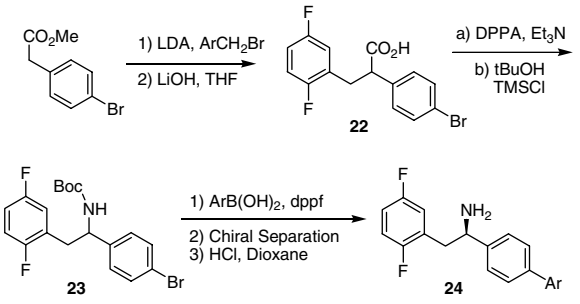
Compound	X	DPP-4 IC_{50} (nM)	DPP-8 IC_{50} (nM)
20a		20	41,000
21a		40	87,000
20b		120	15,000
21b		85	27,000
20c		120	43,000
21c		98	75,000

When these valerolactam DPP-4 inhibitors (**20**) were compared to their 2-fluorophenyl analogs **21**,[19] very similar potencies were observed (Table 3), indicating the arylfluorine to Asn710 H-bond was successfully replaced with a carbonyl to Asn710 H-bond. These new DPP-4 inhibitors are potent and selective against DPP-8. Unfortunately, preliminary rat PK studies on **20a** showed poor oral bioavailability, much lower than that of fluorophenyl analogs reported earlier.[20]

Additional analysis of the X-ray structure overlay indicated an interesting alignment of C1 of the biaryl moiety in compound **15** (Fig. 4, C) with the α-carbon of the β-alanine unit in compound **16** (Fig. 4, C), suggesting another possible hybrid structure: replacement of the amidomethylene moiety in the β-series with the biaryl unit in the α-series to provide compounds **24**.

The synthesis of this new series is shown in Scheme 4. The lithium enolate of methyl phenylacetate was alkylated with 2,5-difluorobenzylbromide, followed by ester hydrolysis. The resulting carboxylic acid **22** was subjected to Curtius rearrangement with diphenylphosphoryl azide under basic condition, and the product was captured in situ by *tert*-butanol to give the *t*-Boc protected amino aryl bromide **23**. Suzuki coupling with various aromatic boronic acids, followed by chiral separation and acid deprotection of the *t*-Boc group yielded biaryl compounds **24**. Again, the absolute configuration of **24d** was confirmed by X-ray crystal structure (Fig. 5) and the others by analogy based on DPP-4 inhibitory activities.

Comparison of DPP-4 inhibition of the new biaryl compounds **24** and their α-series analogs **25**[23] is summarized in Table 4. Biaryl



Scheme 4.

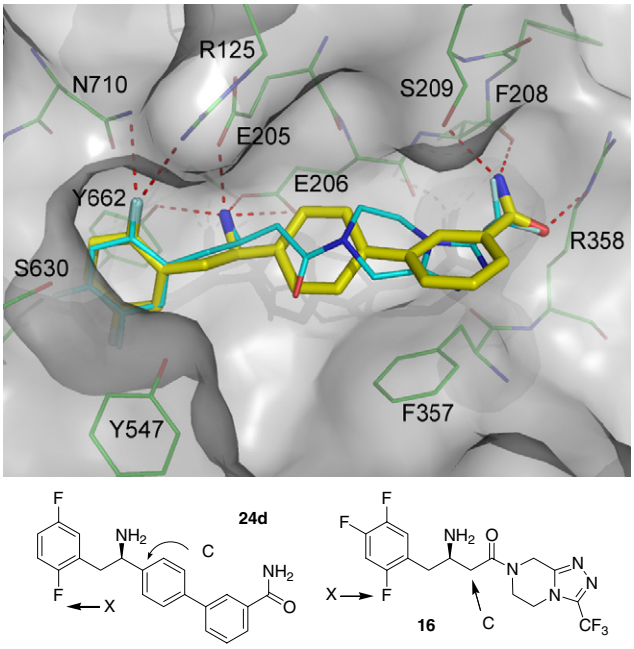
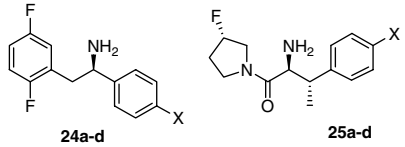
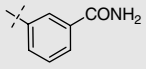


Figure 5. Overlay of X-ray structures of **24d** (yellow) and **16** (cyan) bound to the active site of DPP-4.

Table 4
Activities of biaryl containing DPP-4 inhibitors **24** and their α-series analogs **25**



Compound	–X	DPP-4 IC_{50} (nM)	DPP-8 IC_{50} (nM)
24a	–Br	1300	>100,000
25a		245	>100,000
24b		253	64,000
25b		110	>100,000
24c		107	>100,000
25c		71	>100,000
24d		32	>100,000
25d		100	39,000

inhibitor **24d**, in particular, showed good potency (DPP-4 IC_{50} = 32 nM) and selective against DPP8. More importantly, its pharmacokinetic properties[24] (Cl_p, 13 mL/min/kg; $t_{1/2}$, 3.8 h; C_{max}, 2.1 μM; F%, 130) compare favorably to the α-series analogs.

The X-ray structure of **24d**,[25] shown in Fig. 5, revealed that it overlapped with Sitaglitin **16** almost perfectly in the DPP-4 active site. Not only the aromatic fluorine is in the same position for H-bonding, but also the carboxamide group of **24d** is overlapping with the trifluoromethyl group of **16**, and engaged in favorable interactions with the side chains of residue F208, S209, and R358 (Fig. 5). This observation is consistent with our working hypothesis mentioned earlier that this new orientation of the biaryl moiety would result in better inhibitors of DPP-4.

In summary, our efforts to seek common features of two structurally distinct series of DPP-4 inhibitors led to compounds which provided insights into the binding modes of these inhibitors in the DPP-4 active site. Computer modeling based on the DPP-4 X-ray structure provided credible supporting evidence of the possible alignment of the two inhibitor classes. Finally the availability of the X-ray structures of enzyme-inhibitor complexes allowed detailed analysis of specific interactions and structural unit alignment. With newly discovered binding elements, such as the aromatic fluorine H-bond, we were able to design structurally distinct, potent and selective inhibitors of DPP-4. These studies further demonstrated the value of structural biology and computer modeling in medicinal chemistry programs.

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References and notes

1. Deacon, C. F.; Hughes, T. E.; Holst, J. J. *Diabetes* **1998**, *47*, 764.
2. Pederson, R. A.; White, H. A.; Schlenzig, D.; Pauly, R. P.; McIntosh, C. H.; Demuth, H.-U. *Diabetes* **1998**, *47*, 1235.
3. Gautier, J. F.; Fetita, S.; Sobngwi, E.; Salaun-Martin, C. *Diabetes Metab.* **2005**, *31*, 233.
4. Holst, J. J.; Gromada, J. *Am. J. Physiol. Endocrinol. Metab.* **2004**, *146*, E199.
5. Ahren, B.; Schmitz, O. *Horm. Metab. Res.* **2004**, *36*, 867.
6. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, K.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R.; Wu, J.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
7. (a) Herman, G. et al *J. Clin. Endocrin. Metabol.* **2006**, *91*, 4612; (b) Raz, I. et al *Diabetologia* **2006**, *49*, 2564; (c) Aschner, P. et al *Diabetes Care* **2006**, *29*, 2632; (d) Hermansen, K.; Kipnes, M.; Luo, E.; Fanurik, D.; Khatami, J.; Stein, P. *Diabetes Obes. Metab.* **2007**, *9*, 733.
8. (a) Parmee, E. R.; He, J.; Mastracchio, A.; Edmondson, S. D.; Colwell, L.; Eiermann, G.; Feeney, W. P.; Habulihas, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Petrov, A.; DiSalvo, J.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 43; (b) Caldwell, C. G.; Chen, P.; He, J.; Parmee, E. R.; Leiting, B.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Eiermann, G. J.; Petrov, A.; He, H.; Lyons, K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265.
9. (a) Xu, J.; OK, H.; Gonzales, E. J.; Colwell, L.; Habulihas, B.; He, H.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4459; (b) Edmondson, S.; Mastracchio, A.; Beconi, M.; Colwell, L. F., Jr.; Habulihaz, B.; He, H.; Kumar, S.;

Leiting, B.; Lyons, K. A.; Mao, A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Zhu, L.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5151.
10. Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.
11. Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.* **2003**, *10*, 19.
12. All new compounds were characterized by ^{1}H NMR and LC–MS prior to submission for biological evaluation.
13. Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. *Biochem. J.* **2003**, *371*, 525.
14. Burkey, B. F.; Hoffmann, P. K.; Hassiepen, U.; Trappe, J.; Juedes, M.; Foley, J. E. *Diabetes Obes. Metab.* **2008**. doi:10.1111/j.1463-1326.2008.00860.x.
15. Lankas, G. R.; Leiting, B.; Sinha Roy, R.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C-C.; Edmondson, S.; Feeney, W. P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
16. Maes, M.-B.; Lambier, A.-M.; Gilany, K.; Senten, K.; Van der veken, P.; Leiting, B.; Augustyns, K.; Scharpe, S.; De Meester, I. *Biochem. J.* **2005**, *386*, 315.
17. Xu, J.; Lan, W.; Mathvink, R. J.; Edmondson, S.; Eiermann, G.; He, H.; Leone, J.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5373.
18. Angelaud, R.; Zhong, Y.-L.; Maligres, P.; Lee, J.; Askin, D. *J. Org. Chem.* **2005**, *70*, 1949.
19. At the time of this study, the stereochemistry of neither **7** nor **12** could be unambiguously assigned, and '*anti* configuration was assumed for both series. Later, X-ray structural analysis showed that **12c** is indeed '*anti*, but **7c** is actually '*syn*. However, X-structures also showed that the backbones of both compounds fit in the enzyme active site the same way regardless of the orientation of the chiral methyl groups.
20. Liang, G.-B.; Qian, X.; Feng, D.; Biftu, T.; Eiermann, G.; He, H.; Leiting, B.; Lyons, K.; Petrov, A.; Sinha-Roy, R.; Zhang, B.; Wu, J.; Zhang, X.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1903.
21. Edmondson, S. D.; Mastracchio, A.; Mathvink, R. J.; He, J.; Harper, B.; Park, Y.-J.; Beconi, M.; DiSalvo, J.; Eiermann, G.; He, H.; Leiting, B.; Leone, J.; Levorse, D.; Lyons, K.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Shang, J.; Sinha-Roy, R.; Smith, A.; Wu, J. K.; Xu, S.; Zhu, B.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2006**, *49*, 3614.
22. Razgulin, A. V.; Mecozzi, S. *J. Med. Chem.* **2006**, *49*, 7902.
23. Xu, J.; Lan, W.; Mathvink, R. J.; He, J.; Park, Y.-J.; He, H.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2533.
24. Pharmacokinetic parameters were obtained following an IV (1 mg/kg) or P.O. (2 mg/kg) dose (amorphous trifluoroacetic acid salt) in water. Cl_p, plasma clearance; C_{max}, peak plasma concentration after indicated oral dosing; F%, oral bioavailability.
25. The coordinates for the structure of DPP-4 in complex with **24d** were deposited with the Protein Data Bank, Accession code 3D4L.