Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5806–5811

4-Arylcyclohexylalanine analogs as potent, selective, and orally active inhibitors of dipeptidyl peptidase IV

David E. Kaelin,[a,*] Abigail L. Smenton,[a] George J. Eiermann,[b] Huaibing He,[a] Barbara Leiting,[c] Kathryn A. Lyons,[a] Reshma A. Patel,[c] Sangita B. Patel,[a] Alexsandr Petrov,[b] Giovanna Scapin,[a] Joseph K. Wu,[c] Nancy A. Thornberry,[c] Ann E. Weber[a] and Joseph L. Duffy[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Pharmacology, Merck Research Laboratories, Rahway, NJ 07065, USA*
[c]*Department of Metabolic Disorders, Merck Research Laboratories, Rahway, NJ 07065, USA*

Received 26 June 2007; revised 22 August 2007; accepted 23 August 2007
Available online 26 August 2007

Abstract—A novel series of 4-arylcyclohexylalanine DPP-4 inhibitors was synthesized and tested for inhibitory activity as well as selectivity over the related proline-specific enzymes DPP-8 and DPP-9. Optimization of this series led to **28** (DPP-4 IC_{50} = 4.8 nM), which showed an excellent pharmacokinetic profile across several preclinical species. Evaluation of **28** in an oral glucose tolerance test demonstrated that this compound effectively reduced glucose excursion in lean mice.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that has been shown to be responsible for glucose-stimulated insulin secretion following nutrient ingestion.[1] GLP-1 is rapidly inactivated, via proteolytic cleavage, by the serine protease dipeptidyl peptidase IV (DPP-4). Inhibition of DPP-4 has been shown to lead to an increase in circulating levels of endogenous GLP-1, and this in turn causes a reduction in blood glucose levels. As a result, DPP-4 inhibition has emerged as an effective method for the treatment of type 2 diabetes.[2]

Recently, JANUVIA™ (sitagliptin phosphate), a potent, selective, and orally active DPP-4 inhibitor, was approved by the FDA for the treatment of diabetes.[3] Continuing work in these laboratories has led to the discovery of a number of potent and structurally diverse DPP-4 inhibitors (Fig. 1).[4] One class of phenylalanine derivatives, typified by **1** and **2**, were shown to be potent against DPP-4 and selective over the related proline-specific peptidases DPP-8 and DPP-9 (Table 1).[4a,b] This selectivity was regarded as an absolute requirement for all structural classes in our program because of the apparent link between inhibition of DPP-8/DPP-9 and toxicity in preclinical species.[5]

Two potential issues were identified within the phenylalanine class of compounds to which **1** and **2** belong. The compounds generally exhibited ion channel binding (hERG) and a high serum potency shift (>10-fold increase in apparent IC_{50}). We believed that both of these properties were arising from the highly lipophilic biaryl moiety, so an effort was undertaken to replace

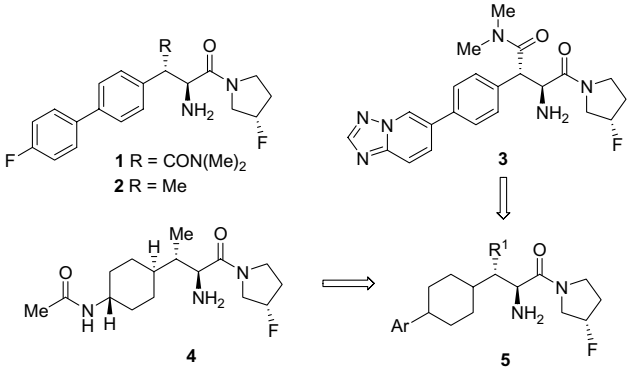


Figure 1. Structures of DPP-4 inhibitors.

Keyword: DPP-4 inhibitors.
* Corresponding author. Tel.: +1 732 594 2266; fax: +1 732 594 9545; e-mail: david_kaelin@merck.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.08.049

Table 1. Potency and selectivity over DPP-8 and DPP-9, selectivity over hERG, and rat PK parameters

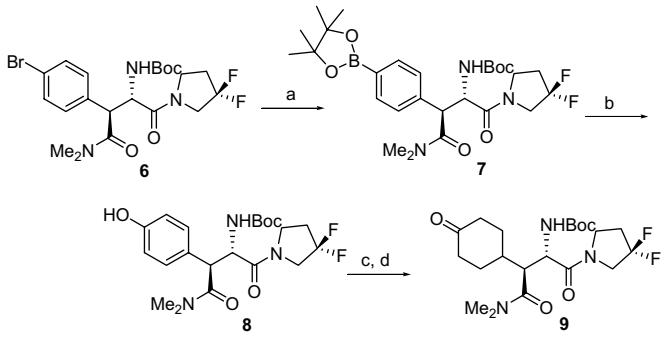
Compound	IC_{50} (μM)				Rat PK (1/2 mpk iv/po)		
	DPP-4	DPP-8	DPP-9	hERG	AUC_n (μM h/mpk)	Clp (mL/min/kg)	*F* (%)
1	0.012	>100	69	4.6	6.2	4.8	67
2	0.064	88	86	1.1	4.8	8.6	85
3	0.004	>100	>100	86	2.4	7.0	43
4	0.016	25	>100	>100	0.68	42	56

the 4-fluorophenyl group present in **1** and **2** with more polar substituents. This led to the discovery of heterocyclic derivatives like **3**, which had an improved hERG profile.[4c] Shortly thereafter, it was discovered that the phenyl ring in the phenylalanine series could be replaced with a cyclohexyl ring to give compounds like **4** that had good intrinsic potency, high selectivity against DPP-8 and DPP-9, and low ion channel binding.[6] Unfortunately, compound **4** showed a lower than anticipated effect in a murine OGTT (oral glucose tolerance test), and this was ultimately attributed to low oral exposure (as evidenced by AUC_n) in rodents. Based on these discoveries, efforts were initiated to combine the structural features present in **3** and **4** to give hybrid structures like **5** in the hopes of generating potent and selective inhibitors with improved pharmacokinetic profiles.

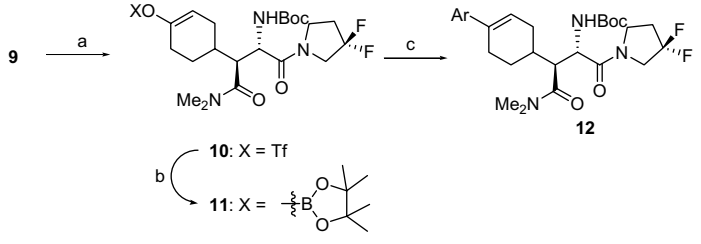
The preparation of a representative hybrid scaffold is described in Schemes 1–4. The synthesis commenced with a palladium-catalyzed coupling reaction between bis(pinacolato)diboron and the aryl bromide **6** to give **7** as was previously described.[4c] The boronate ester in **7** was then cleaved oxidatively to furnish phenol **8**. Hydrogenation of **8** in the presence of rhodium on alumina gave a mixture of diasteromeric alcohols that was subsequently oxidized to give the cyclohexanone **9** (Scheme 1).

One of two approaches was then used to install the 4-substituent. In the first approach (Scheme 2), the cyclohexanone **9** was first converted to its corresponding enol triflate **10** by treatment with 2 equivalents of LiHMDS followed by $PhNTf_2$. The enol triflate was converted to vinyl boronate **11** by employing conditions similar to those used in the preparation of **7**. Palladium-catalyzed coupling of **11** with an appropriate aryl or heteroaryl halide afforded cyclohexenes of the general structure **12**.

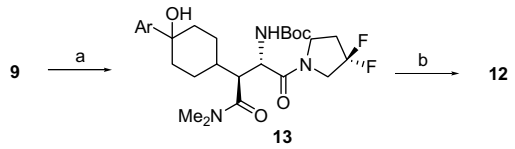
The second approach that was used to prepare the common intermediate **12** commenced with the addition of an appropriately substituted aryllithium or arylmagnesium bromide reagent to cyclohexanone **9** (Scheme 3). The resultant diastereomeric mixture of benzylic alcohols **13** was then dehydrated by the action of Burgess reagent. This method was employed in instances where the requisite organometallic reagent was commercially available (e.g., 4-fluorophenylmagnesium bromide).



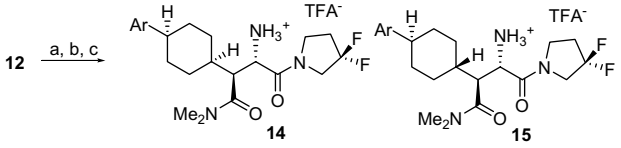
Scheme 1. (a) Bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 90 °C, 12 h, 90–98%; (b) H_2O_2 (30% aq), aq NaOH (3*N*), THF, 86–97%; (c) 5% Rh/Al_2O_3, H_2 (50 psi), MeOH, 12 h; (d) Dess-Martin periodinane, CH_2Cl_2, 56–81% (2 steps).



Scheme 2. (a) LiHMDS, THF, −78 °C, 1.5 h, then $PhNTf_2$, −78 to 5 °C, 48–93%; (b) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, dppf, KOAc, *p*-dioxane, 80 °C, 46–57%; (c) Ar–Br, $Pd(dppf)Cl_2$, K_2CO_3, DMF, 80 °C, 56–87%.



Scheme 3. (a) Ar–Li or Ar–MgBr(Cl), THF, −78 °C to rt; (b) Burgess reagent, THF, 49–79% (2 steps).



Scheme 4. (a) 10% Pd/C, H_2 (1 atm), MeOH; (b) HPLC (Chiracel OD, *i*-PrOH/hexane), 16–32% (2 steps); (c) TFA, CH_2Cl_2, 88–100%.

The double bond in **12** was reduced by hydrogenation in the presence of palladium on carbon to give a mixture of *cis*- and *trans*-cyclohexane diastereomers that, in most cases, was separated by HPLC (Scheme 4). Cleavage of the Boc-protecting group afforded the target compounds **14** and **15** as single diastereomers. An identical procedure was employed, starting with the known aryl bromide,[4a] to prepare analogs that contained a β-methyl substituent in place of the β-dimethylamide group.

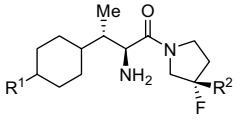
Table 2 lists the DPP-4 inhibitory potency and selectivity over the proline-specific enzymes DPP-8 and DPP-9 for several of the β-methyl-substituted inhibitors.[7a,b] It should be noted that compounds **16**–**18** were analyzed for inhibitory activity as mixtures (ca. 1:1) of *cis* and *trans* cyclohexane isomers. Compounds **16** and **17** showed high intrinsic potency against DPP-4, but the apparent potency was decreased in the presence of 50% human serum. Compound **18** on the other hand was highly potent both in the presence and absence of human serum, despite a substantial serum shift. All three inhibitors exhibited low micromolar activity against the related proline-specific peptidases DPP-8 and DPP-9. For this reason, an alternate scaffold was investigated in an attempt to mitigate the unwanted off-target activity. We opted not to separate the *cis* and *trans* isomers of **16**–**18** at this stage, but rather to use the determined activity of the mixtures as a guide to which analogs should be focused on first in the alternate scaffold design.

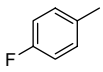
It had been shown previously that replacement of the β-methyl substituent present in the phenylcyclohexylalanine inhibitors with a polar group often resulted in an increase in selectivity over DPP-8 and DPP-9.[4b,c] Hence, this strategy was employed for the synthesis of additional inhibitors.

Several analogs were prepared that incorporated the triazolopyridine present in **18** onto the β-dimethylamido scaffold and the data for these compounds, obtained as single diastereomers, are summarized in Table 3. Both the 3,3-difluoropyrrolidine and (*S*)-fluoropyrrolidine amides were investigated. The more active monofluoro diastereomer **20** exhibited good potency against DPP-4 and also showed significant selectivity over DPP-8 and DPP-9. When dosed in rats, compound **20** showed only moderate oral bioavailability and a short half-life.[8] Alternatively, difluoropyrrolidine amide **22** possessed similar potency and selectivity to **20** but had a substantially improved pharmacokinetic profile in rats. However, due to the relatively high clearance (Clp = 40 mL/min/kg) of **22**, the AUC_n (0.93 μM h/mpk) for this compound was lower than we desired.

Next, we prepared several additional analogs (**23**–**30**) that incorporated the dimethylamido side chain and the difluoropyrrolidine amide (Table 4). Compound **28**, which contained the regioisomeric triazolopyridine present in **3**,[4c] showed excellent potency against DPP-4 and high selectivity over DPP-8 and DPP-9. However, this compound did exhibit a larger than expected decrease in potency in the presence of 50% human serum. Finally, compound **28** was found to be inactive against

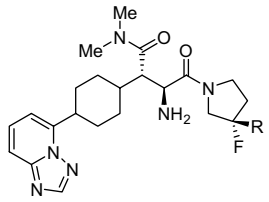
Table 2. Potency in the presence of 50% human serum (HS) and selectivity over DPP-8 and DPP-9



Compound[a]	R^1	R^2	IC_{50} (μM)			
			DPP-4 (0% HS)	DPP-4 (50% HS)	DPP-8	DPP-9
16		H	0.012	0.39	3.6	3.1
17		F	0.0046	0.15	6.4	11
18		F	0.0015	0.028	4.3	1.7

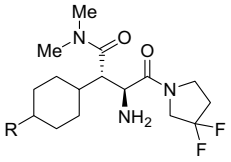
[a] All compounds are a mixture (ca. 1:1) of *cis*- and *trans*-cyclohexane diastereomers.

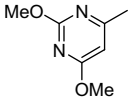
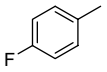
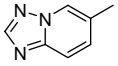
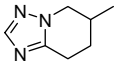
Table 3. Potency and selectivity over DPP-8/DPP-9 and selected rat PK data



Compound	R	IC_{50} (μM)				Rat PK	
		DPP-4 (0% HS)	DPP-4 (50% HS)	DPP-8	DPP-9	$t_{1/2}$ (h)	F (%)
19 (Diast A)	H	0.31	0.82	>100	3.7		
20 (Diast B)	H	0.0023	0.026	53	28	0.54	31
21 (Diast A)	F	0.26	0.67	95	2.6		
22 (Diast B)	F	0.0025	0.023	33	25	2.9	98

Table 4. Potency and selectivity over DPP-8/DPP-9



Compound	R	IC_{50} (μM)			
		DPP-4 (0% HS)	DPP-4 (50% HS)	DPP-8	DPP-9
23 (Diast A)		0.10	2.7	>100	40
24 (Diast B)		0.26	1.9	>100	>100
25 (Diast A)		0.15	1.5	>100	15
26 (Diast B)		0.011	0.29	41	21
27 (Diast A)		0.032	0.13	>100	38
28 (Diast B)		0.0048	0.13	30	38
29 (Diast A)		0.22	0.45	>100	50
30 (Diast B)		0.038	0.12	22	>100

the hERG ion channel (IC_{50} > 90 μM) so for this reason it was selected for further in vivo characterization despite the high serum shift.

The pharmacokinetic profile of **28** was determined for several species (Table 5). In general, **28** exhibited excellent oral bioavailability and low clearance. The half-life across species was generally consistent. Importantly, the oral AUC_n was high in all species, so **28** was tested further in our primary pharmacodynamic assay.

A lean mouse oral glucose tolerance test (OGTT) was performed using **28** in order to determine the effect of the inhibitor on blood glucose excursion following a dextrose challenge (Fig. 2). Gratifyingly, **28**, when administered orally as a single dose 60 min prior to a dextrose challenge, reduced blood glucose levels in a dose-dependent fashion down to a minimum effective dose (MED) of 0.03 mpk which resulted in a 23% reduction in blood glucose excursion relative to vehicle control. The MED for both **1** and **4** was 0.1 mpk which resulted in a 21% and 26% reduction in blood glucose, respectively.

X-ray crystal structures of the more active diastereomers **20** and **28** bound to the active site of DPP-4 were obtained (Fig. 3).[9a,b] These structures established unambiguously the 1,4-*trans* relationship of the cyclohexyl

Table 5. PK data for **28**

Species	Selected pharmacokinetic parameters (1/2 mpk iv/po)				
	AUC_n (μM h)	Clp (mL/min/kg)	C_{max} (μM)	$t_{1/2}$ (h)	F (%)
Rat	4.4	5.3	3.3	4.1	63
Dog[a]	46	0.94	16	3.9	100
Rhesus	6.5	5.6	2.8	4.1	97

[a] po dose—0.5 mpk.

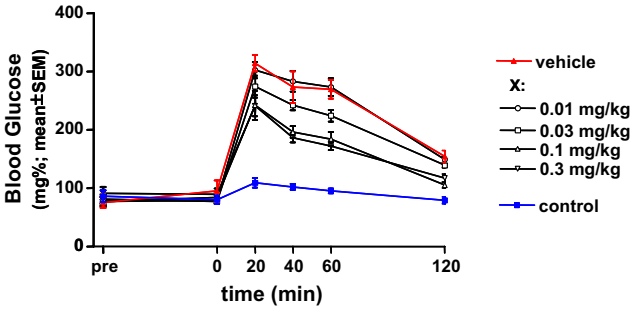


Figure 2. Effects of **28** on glucose levels after an oral glucose tolerance test in lean mice.

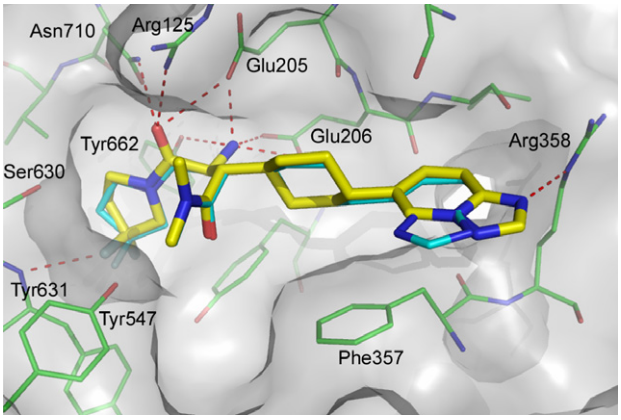


Figure 3. Inhibitors **20** (cyan) and **28** (yellow) bound to DPP-4. Interactions of the inhibitors with DPP-4 are shown as dotted red lines.

substituents in these inhibitors. The more potent diastereomer of each pair in Tables 3 and 4 is presumed to be *trans* by analogy. The interactions between **20**/**28** and DPP-4 are similar to those seen with related compounds.[4c] It is noteworthy that the heterocycle in **28** is capable of participating in a hydrogen bonding interaction with Arg358 whereas the heterocycle in **20** cannot. This interaction appears to have no impact on intrinsic potency.

In conclusion, the hybridization of DPP-4 inhibitors **3** and **4** gave a new class of compounds that effectively incorporated the desirable properties of each. The 4-aryl and heteroarylcyclohexylalanines described are highly potent and selective over DPP-8 and DPP-9. One member of this new class, **28**, has improved selectivity over hERG compared to **1** and an improved pharmacokinetic profile relative to **4**. Compound **28** showed good efficacy in a murine OGTT experiment.

Acknowledgments

We are grateful to D. Hora, J. Fenyk-Melody, I. Capodanno, P. Cunningham, M. Donnelly, J. Hausamann, C. Nunes, X. Shen, J. Strauss, and K. Vakerich for in vivo pharmacokinetic studies.

References and notes

1. For recent GLP-1 references, see: (a) Holst, J. J. *Curr. Opin. Endocrin. Diabetes* **2005**, *12*, 56; (b) Knudsen, L. B. *J. Med. Chem.* **2004**, *47*, 4128; (c) Vahl, T. P.; D'Alessio, D. A. *Expert Opin. Invest. Drugs* **2004**, *13*, 177, and references therein.
2. For reviews on DPP-4 inhibitors, see: (a) Nielson, L. L. *Drug Discovery Today* **2005**, *10*, 703; (b) Augustyns, K.; Van der Veken, P.; Haemers, A. *Expert Opin. Ther. Patents* **2005**, *15*, 1387; (c) Mentlein, R. *Expert Opin. Invest. Drugs* **2005**, *14*, 57; (d) Weber, A. E. *J. Med. Chem.* **2004**, *47*, 4135; (e) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. *Expert Opin. Ther. Patents* **2003**, *13*, 499, and references therein.
3. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsillio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
4. (a) Xu, J.; Wei, L.; Mathvink, R.; He, J.; Park, Y.-J.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2533; (b) Edmondson, S. D.; Mastracchio, A.; Duffy, J. L.; Eiermann, G. J.; He, H.; Ita, I.; Leiting, B.; Leone, J. F.; Lyons, K. A.; Makarewicz, A. M.; Patel, R. A.; Petrov, A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3048; (c) Edmondson, S. D.; Mastracchio, A.; Mathvink, R. J.; He, J.; Harper, B.; Park, Y.-J.; Beconi, M.; Di Salvo, J.; Eiermann, G. J.; He, H.; Leiting, B.; Leone, J. F.; Levorse, D. A.; 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; (d) Parmee, E. R.; He, J.; Mastracchio, A.; Edmondson, S. D.; Colwell, L.; Eiermann, G.; Feeney, W. P.; Habulihaz, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Petrov, A.; Di Salvo, J.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 43; (e) 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. A.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265.
5. Lankas, G. R.; Leiting, B.; Sinha Roy, R.; Eiermann, G. J.; Biftu, T.; Cahn, C.-C.; Edmondson, S. D.; 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. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
6. Duffy, J. L.; Kirk, B. A.; Wang, L.; Eiermann, G. J.; He, H.; Leiting, B.; Lyons, K. A.; Patel, R. A.; Patel, S. B.; Petrov, A.; Scapin, G.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2879.
7. For assay conditions for DPP-4 inhibition, see: (a) 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. *J. Biochem.* **2003**, *371*, 525; (b) For assay conditions for DPP-8 and DPP-9, see Ref. 5.

8. Pharmacokinetic parameters were analyzed with WATSON software (version 6.4.0.04 Innaphase Corp) by noncompartmental methods using formulas described in Gibaldi, M.; Perrier, D. In *Pharmacokinetics*; Swarbrick, J., Ed., 2nd ed.; Marcel Dekker: New York, 1982; p 409.
9. X-ray crystal structures were obtained following the published protocol: (a) Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; 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.; Sinha Roy, R.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141; (b) The structures of DPP-4 bound to compound **20** and **28** have been deposited with the RCSB Protein Data Bank, Accession Nos. 2QT9 and 2QTB, respectively.