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Dipeptidyl peptidase IV inhibitors derived from β-aminoacylpiperidines bearing a fused thiazole, oxazole, isoxazole, or pyrazole

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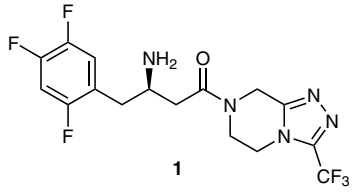
Abstract—A series of β-aminoacylpiperidines bearing various fused five-membered heterocyclic rings was synthesized as dipeptidyl peptidase IV inhibitors. Potent and relatively selective inhibition could be obtained, depending on choice of heterocycle, regioisomerism, and substitution. In particular, one analog (**74**, DPP-IV IC_{50} = 26 nM) exhibited good oral bioavailability and acceptable half-life in the rat, albeit with rather high clearance.

In recent years, one of the most active areas of diabetes research has centered on glucagon-like peptide 1 (GLP-1), an incretin hormone that stimulates glucose-dependent insulin biosynthesis and secretion, inhibits glucagon secretion, and promotes proliferation of pancreatic β cells.[1] Although GLP-1-based approaches are attractive for treatment of type 2 diabetes, therapeutic use of GLP-1 itself is limited by lack of oral activity and rapid degradation by dipeptidyl peptidase IV (DPP-IV).[2] Long-acting peptide GLP-1 analogs are in clinical development, but these still require injection.[2] DPP-IV is a widely expressed serine peptidase, specific for cleavage of an Xaa-Pro or Xaa-Ala N-terminal dipeptide from its natural substrates.[3] Orally active DPP-IV inhibitors have the potential to improve glucose tolerance by sustaining the action of endogenous GLP-1.[2,4] On the basis of animal studies and initial clinical trials, it appears that DPP-IV inhibitors are capable of significantly lowering fasting and postprandial glucose concentration, as well as reducing HbA_{1c} levels, with good tolerability and minimal risk of hypoglycemia.[4,5]

Novel β-aminoacyl derivatives of triazolo- or imidazolo-fused piperazines such as MK-0431 (**1**) have recently been reported from these laboratories as potent, selective, orally bioavailable inhibitors of DPP-IV.[6] We now describe the synthesis and biological evaluation of analogous β-aminoacylpiperidines **2** bearing various fused five-membered rings (thiazoles, oxazoles, isoxazoles, and pyrazoles).



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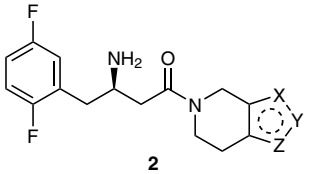

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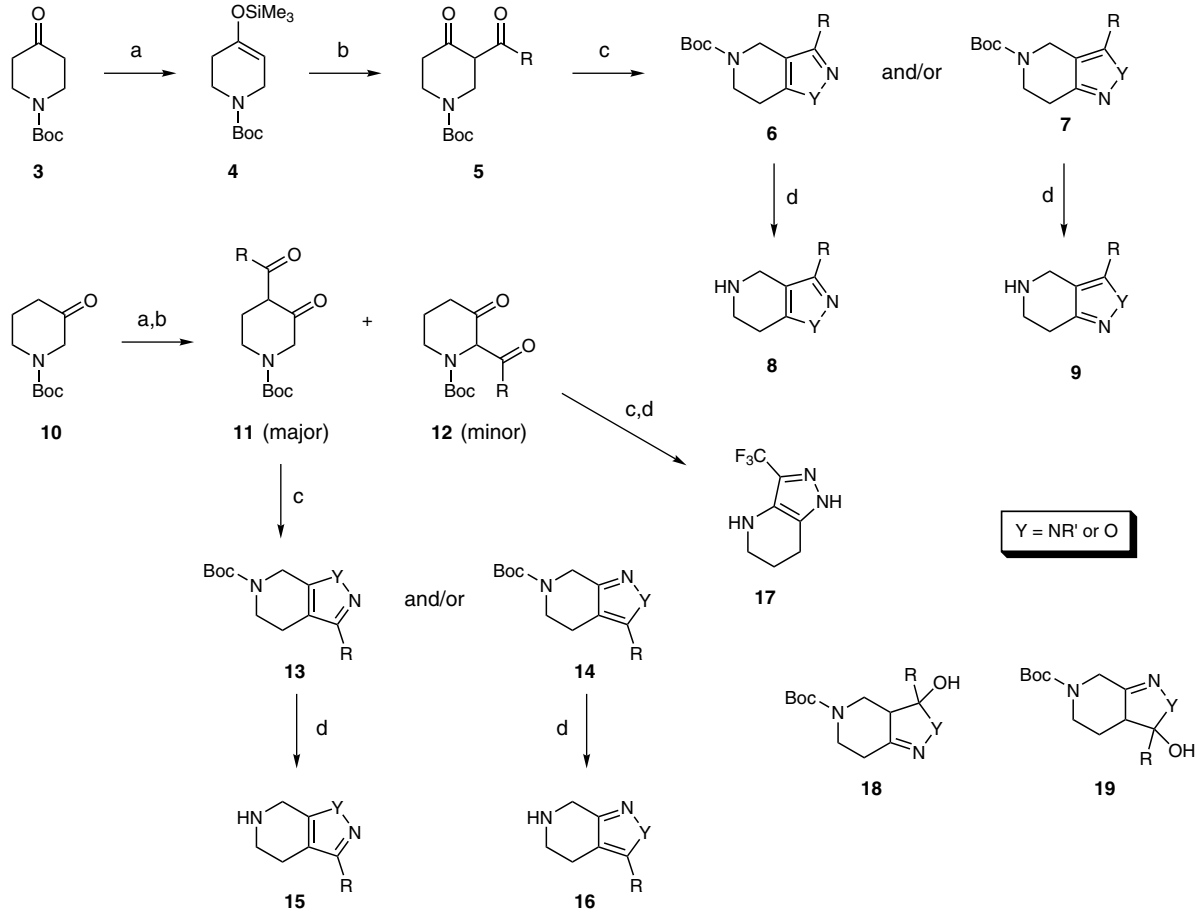
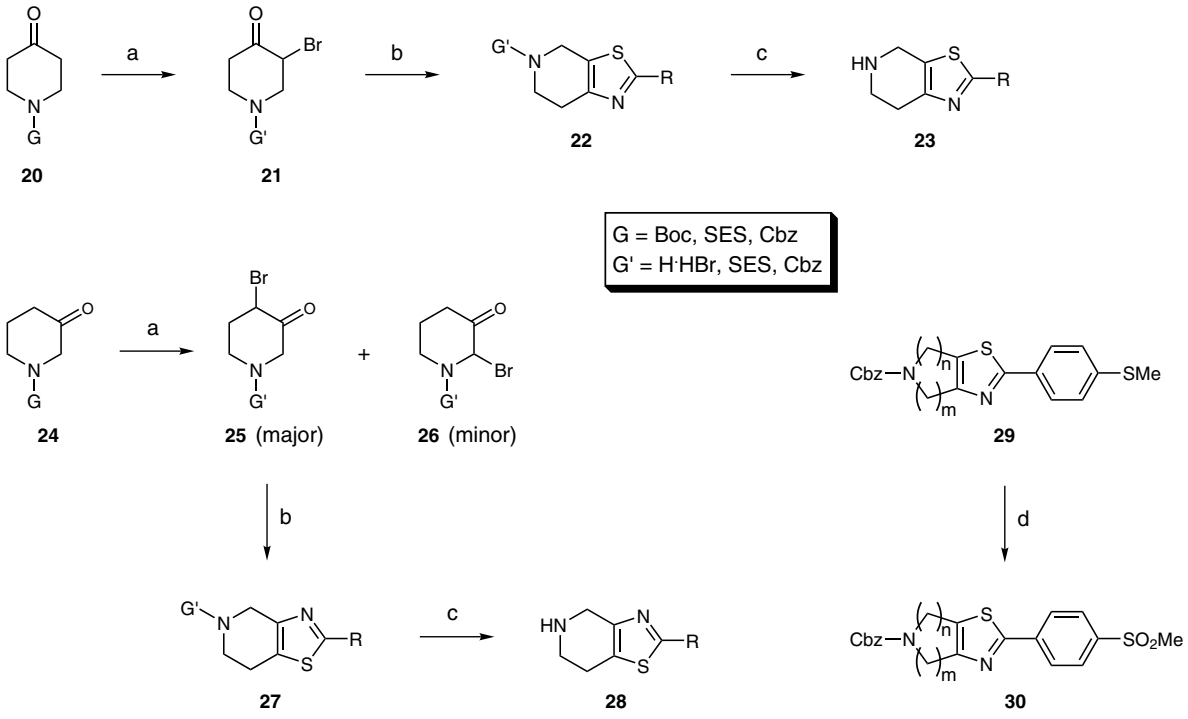
Using conditions similar to those reported for *N*-benzyl analogs,[7] 1-Boc-piperidin-4-one (**3**) was converted to its silyl enol ether **4**[8] and acylated to give the diketone **5** (Scheme 1). Condensation of **5** with hydrazines or hydroxylamine typically gave a mixture of pyrazolopiperidine or isoxazolopiperidine regioisomers **6** and **7**, which were deprotected to give, respectively, the amines **8** and **9**. Similarly, the corresponding piperidin-3-one **10** was transformed to the major diketone **11**, which was separated from the minor isomer **12**. As above, **11** could be converted to bicyclic products **13** and **14**, which were deprotected to give, respectively, amines **15** and **16**. The minor diketone isomer **12** (R = CF_3) afforded another pyrazolopiperidine regioisomer **17**. It should be noted that, in some cases (especially where R is perfluoroalkyl), condensation of the diketones with hydrazines or hydroxylamine led to hydrated adducts such as **18** and **19** as major products. Although relatively resistant to dehydration at this stage, if these hydroxy intermediates were carried forward, they could be successfully dehydrated during the EDC- or HATU-mediated coupling with the protected β-amino acid (see Scheme 4). Isomers **6** and **7** (R = CF_3, Y = NMe) were assigned by NOE experiments. Other pairs of pyrazolopiperidine regioisomers were assigned by analogy on the basis of NMR and TLC behavior. Isoxazolopyrimidine regioisomers **15** and **16** (R = CF_3, Y = O; only **16** obtained pure) were definitively assigned by ^{15}N, ^{19}F, and ^{13}C NMR experiments after synthesis from hydroxylamine–^{15}N hydrochloride.

N-Protected piperidin-4-ones **20** were α-brominated to provide **21**[9] (Scheme 2). Although the Boc group was lost, the SES [2-(trimethylsilyl)ethylsulfonyl][10] and Cbz protecting groups were retained. Condensation of **21** with thioamides[11] afforded thiazolopiperidines **22**, which were deprotected to give the amines **23**. Similarly, piperidin-3-ones **24** were brominated to give **25** along with a minor amount of the isomer **26**. As above, **25** was converted to thiazolopiperidine **27** and then deprotected to provide amine **28**. In both series, a Cbz-protected thiazolopiperidine **29** bearing a 4-(methylthio)phenyl substituent could be oxidized to the corresponding sulfone **30**. Because, we were unable to prepare oxazolopiperidines and some thiazolo derivatives by the pathway in Scheme 2, an alternative route was developed (Scheme 3). Azido alcohol **31**[12] was reduced to the amine **32**,[12b] which was acylated to give **33**. Following oxidation of the alcohol, the resulting ketone **34** was cyclized to a thiazolopiperidine with Lawesson's reagent or to an oxazolopiperidine with Burgess reagent. Subsequent deprotection afforded amines **23** and **35**, respectively.



Scheme 1. Reagents and conditions: (a) (1) LiHMDS, THF, −78°C; (2) TMSCl, −78°C; (b) (1) MeLi, THF, −10°C; (2) RCOCl or $(RCO)_2O$, −78°C; (c) $RNHNH_2$, EtOH or 2-methoxyethanol, Δ; or $HCl{\cdot}NH_2OH$, AcOH, Δ; or $HCl{\cdot}NH_2OH$, aq NaOH, EtOH, Δ; (d) anhydrous 4 M HCl/dioxane.

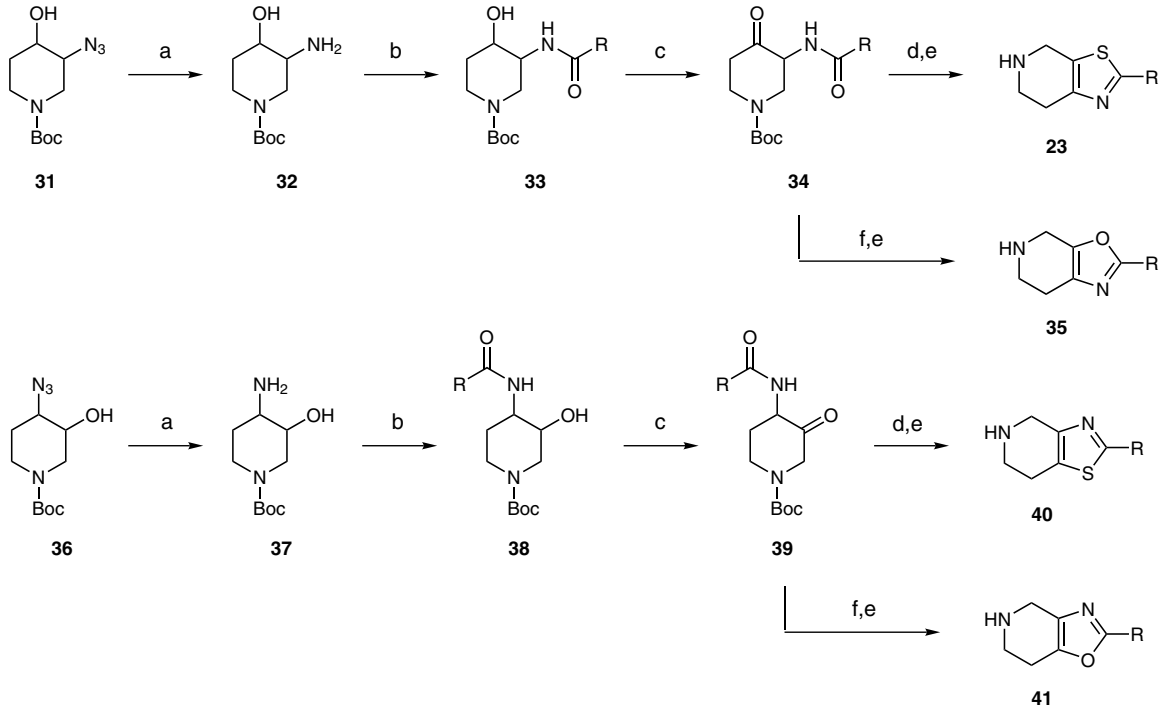


Scheme 2. Reagents and conditions: (a) Br_2, $CHCl_3$, 5–20 °C; (b) $RCSNH_2$, DMF, 100 °C; (c) For Boc: anhydrous 4 M HCl/dioxane; for SES: CsF, DMF, 95 °C; for Cbz: anhydrous 30% HBr/AcOH; (d) MCPBA, DMF.

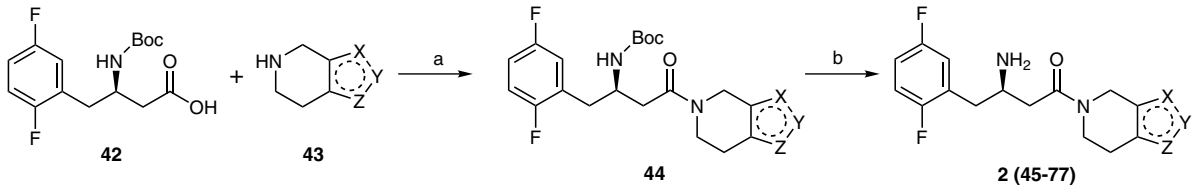
A similar sequence from the azido alcohol **36**[12] led to the isomeric bicyclic products **40** and **41**.

Finally, the Boc-protected β-amino acid **42**[6] was coupled with the fused piperidines **43** to furnish **44**, which yielded the target DPP-IV inhibitors **45**–**77** (corresponding to general structure **2**) upon Boc removal (Scheme 4).

As previously reported,[13] compounds were determined as inhibitors of human recombinant DPP-IV using the



Scheme 3. Reagents and conditions: (a) Ph_3P, H_2O, THF; (b) RCOCl or $(RCO)_2O$, Et_3N, CH_2Cl_2; (c) Dess–Martin periodinane, CH_2Cl_2; or $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2, −60 °C; (d) Lawesson's reagent, toluene, Δ; (e) anhydrous 4 M HCl/dioxane; (f) Burgess reagent, THF, Δ.



Scheme 4. Reagents and conditions: (a) EDC, HOBT, iPr_2NEt, CH_2Cl_2; or HATU, HOAT, iPr_2NEt, CH_2Cl_2; (b) anhydrous 4 M HCl/dioxane.

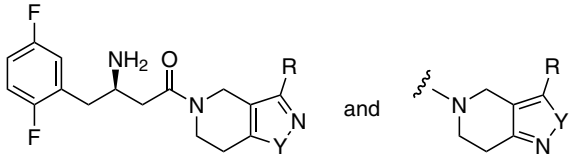
substrate Gly-Pro-AMC, which is cleaved to release the fluorescent leaving group, 7-amino-4-methylcoumarin. In addition, the compounds were counter-screened against human peptidases structurally or functionally related to DPP-IV,[14] including quiescent cell proline dipeptidase (QPP),[15] DPP8, and DPP9. Although these enzymes share some general substrate preferences with DPP-IV, their physiological function is unclear. In contrast to DPP-IV, they are localized intracellularly[14] and are not known to be involved in the metabolism of GLP-1. Inhibition of DPP8 and/or DPP9 is reported to be associated with significant toxicity.[16]

Pyrazolopiperidines with ring fusion 'A' (i.e., those derived from diketones **5**), **45**–**58**, exhibited moderate potency (generally 100–300 nM) against DPP-IV, frequently with >100-fold selectivity versus the other peptidases (Table 1). No pronounced structure–activity relationships were evident, although the combination of alkyl and bulky 4-fluorophenyl substituents at distal positions on the pyrazole ring (isomer A-1, compounds **51** and **57**) appeared unfavorable for DPP-IV inhibition, whereas the same substituents at proximal ring positions (isomer A-2, compounds **52** and **58**) were associated with increased potency at one or more of the off-target peptidases. The isoxazolopiperidine **59** was similar in activity to the corresponding pyrazolopiperidine **46**.

The ring fusion 'B' pyrazolo- and isoxazolopiperidine derivatives (those derived from diketones **11**), **60**–**64**, tended to be somewhat more potent DPP-IV inhibitors than their ring fusion 'A' counterparts (**60** vs **46**, **61** vs **47**, **64** vs **59**), although this was not accompanied by an improvement in selectivity (Table 2). Analog **65** with ring fusion 'C' (derived from diketone **12**) was 5-fold and 15-fold less potent on DPP-IV than corresponding analogs **46** (ring fusion 'A') and **60** (ring fusion 'B'), respectively.

A striking effect of regioisomerism was observed in the thiazolopiperidines and, to a lesser extent, the oxazolopiperidines. Those designated as ring fusion 'D', **66**–**72**, were only modest inhibitors of DPP-IV (Table 3). In addition, several of these, especially those bearing an aryl substituent, were poorly selective relative to other peptidases. In contrast, Table 4 shows that **73**–**77**, designated as ring fusion 'E', had marked gains in DPP-IV potency compared to their ring fusion 'D' analogs (e.g., 5-fold for **73** vs **67**, 14-fold for **74** vs **68**, 20-fold for **75** vs **69**, and 90-fold for **76** vs **71**). The 1.4 nM

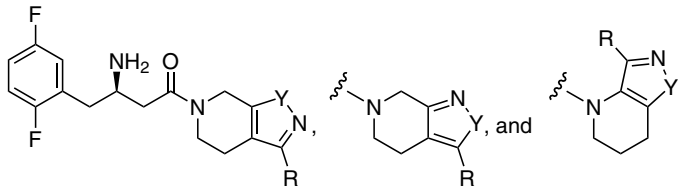
Table 1. Inhibition of DPP-IV and other peptidases by pyrazolo- and isoxazolopiperidines (ring fusion 'A')



Isomer A-1 Isomer A-2

Compd	Y	R	Isomer	IC_{50} (μM)			
				DPP-IV	QPP	DPP8	DPP9
45	NH	Me	A-1/A-2	0.25	71	24	63
46	NH	CF_3	A-1/A-2	0.11	71	21	65
47	NMe	CF_3	A-1	0.13	38	68	>100
48	NMe	CF_3	A-2	0.15	51	>100	>100
49	NCH_2CF_3	CF_3	A-1	0.18	16	71	>100
50	NCH_2CF_3	CF_3	A-2	0.15	18	>100	>100
51	NPh-4-F	Me	A-1	0.35	17	18	24
52	NPh-4-F	Me	A-2	0.16	5.5	9.4	27
53	NH	CF_2CF_3	A-1/A-2	0.16	67	72	>100
54	NMe	CF_2CF_3	A-1	0.22	40	56	>100
55	NMe	CF_2CF_3	A-2	0.30	92	>100	>100
56	NH	Ph-4-F	A-1/A-2	0.27	43	22	50
57	NMe	Ph-4-F	A-1	0.78	26	62	>100
58	NMe	Ph-4-F	A-2	0.15	4.0	58	>100
59	O	CF_3	A-2	0.13	54	>100	>100

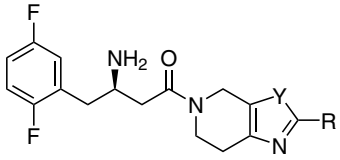
Table 2. Inhibition of DPP-IV and other peptidases by pyrazolo- and isoxazolopiperidines (ring fusion 'B' and 'C')



Isomer B-1 Isomer B-2 Isomer C

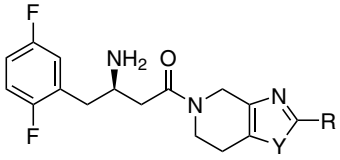
Compd	Y	R	Isomer	IC_{50} (μM)			
				DPP-IV	QPP	DPP8	DPP9
60	NH	CF_3	B-1/B-2	0.039	30	10	13
61	NMe	CF_3	B-1	0.072	67	32	26
62	NMe	CF_3	B-2	0.13	85	65	>100
63	NH	Cyclopropyl	B-1/B-2	0.16	43	11	34
64	O	CF_3	B-2	0.085	>100	5.8	36
65	NH	CF_3	C	0.59	31	>100	>100

Table 3. Inhibition of DPP-IV and other peptidases by thiazolo- and oxazolopiperidines (ring fusion 'D')



Compd	Y	R	IC_{50} (μM)			
			DPP-IV	QPP	DPP8	DPP9
66	S	Me	0.48	80	7.1	20
67	S	CF_3	0.31	63	29	41
68	S	Ph-4-F	0.36	15	4.3	7.8
69	S	Ph-4-CF_3	0.26	11	3.0	9.6
70	S	Ph-4-SMe	0.38	1.9	3.3	5.1
71	S	Ph-4-SO_2Me	0.13	35	3.2	6.5
72	O	Cyclopropyl	0.29	56	28	30

Table 4. Inhibition of DPP-IV and other peptidases by thiazolo- and oxazolopiperidines (ring fusion 'E')



Compd	Y	R	IC_{50} (μM)			
			DPP-IV	QPP	DPP8	DPP9
73	S	CF_3	0.057	95	63	>100
74	S	Ph-4-F	0.026	11	16	43
75	S	Ph-4-CF_3	0.013	15	7.9	28
76	S	Ph-4-SO_2Me	0.0014	51	8.6	25
77	O	Cyclopropyl	0.18	>100	51	>100

IC_{50} for **76** demonstrates a remarkable activity enhancement attributable to the methanesulfonyl substituent in this series. This compound was >6000-fold selective for DPP-IV over the other peptidases. Although less potent, the trifluoromethyl thiazole derivative **73** was also notable for its very high selectivity (>1000-fold).

Selected compounds were evaluated for pharmacokinetics in male Sprague–Dawley rats (typically dosed at 1 mg/kg iv and 2 mg/kg po). Trifluoromethyl thiazole derivative **73** had moderate half-life (2.0 h) but very high clearance (250 mL/min/kg) and low oral bioavailability (16%). The 4-(methanesulfonyl)phenyl analog **76** had reduced clearance (60 mL/min/kg) but no improvement in half-life (1.5 h), and oral bioavailability was only 20%. However, the corresponding 4-fluorophenyl analog **74** displayed improved half-life (3.3 h) and oral bioavailability (55%), albeit with rather high clearance (91 mL/min/kg).

In conclusion, a series of β-aminoacyl amides derived from piperidines bearing a fused pyrazole, isoxazole, thiazole, or oxazole was prepared and evaluated as DPP-IV inhibitors. Among the pyrazolopiperidines, ring fusion 'B' tended to give more potent DPP-IV inhibition than ring fusion 'A', and ring fusion 'C' was least effective. There were no pronounced substituent effects, and the isoxazolopiperidines were similar in activity to their pyrazolo counterparts. A clear structure–activity trend was evident in the thiazolopiperidine series. Here ring fusion 'E' was strongly preferred over ring fusion 'D' with respect to DPP-IV inhibitory potency and selectivity. A lesser effect was observed for the oxazolopiperidines. Excellent DPP-IV inhibitory potency (IC_{50} = 1.4 nM for **76**) and selectivity over other peptidases (>1000-fold for **76** and **73**) could be achieved in the thiazolopiperidines with ring fusion 'E'. Compound **74** (DPP-IV IC_{50} = 26 nM; selectivity >400-fold vs other peptidases), despite rather high clearance, had good oral bioavailability (55%) and an acceptable half-life (3.3 h) in rats.

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and Dr. Judith E. Fenyk-Melody (Laboratory Animal Resources) as well as to Ms. Ida Ita (Medicinal Chemistry) for valuable assistance in the pharmacokinetics experiments.

References and notes

1. (a) Kieffer, T. J.; Habener, J. F. *Endocrine Rev.* **1999**, *20*, 876; (b) Holst, J. J.; Gromada, J. *Am. J. Physiol. Endocrinol. Metab.* **2004**, *287*, E199; (c) Perry, T.; Greig, N. H. *Trends Pharmacol. Sci.* **2003**, *24*, 377.
2. (a) Holst, J. J. *Exp. Opin. Emerg. Drugs* **2004**, *9*, 155; (b) Mentlein, R. *Exp. Opin. Investig. Drugs* **2005**, *14*, 57; (c) Ahrén, B.; Schmitz, O. *Horm. Metab. Res.* **2004**, *36*, 867; (d) Holst, J. J.; Deacon, C. F. *Curr. Opin. Pharmacol.* **2004**, *4*, 589; (e) Knudsen, L. B. *J. Med. Chem.* **2004**, *47*, 4128; (f) D'Alessio, D. A.; Vahl, T. P. *Am. J. Physiol. Endocrinol. Metab.* **2004**, *286*, E882.
3. (a) Lambeir, A. M.; Durinx, C.; Scharpé, S.; De Meester, I. *Crit. Rev. Clin. Lab. Sci.* **2003**, *40*, 209; (b) Demuth, H.-U.; Heins, J.. In *Dipeptidyl Peptidase IV (CD26) in Metabolism and the Immune Response*; Fleischer, B., Ed.; Landes: Austin, 1995, pp 1–35; (c) Mentlein, R. *Int. Rev. Cytol.* **2004**, *235*, 165; (d) Boonacker, E.; Van Noorden, J. F. *Eur. J. Cell Biol.* **2003**, *82*, 53; (e) Chen, T.; Smyth, D.; Abbott, C. A. *J. Biol. Regul. Homeost. Agents* **2004**, *18*, 47.
4. (a) Weber, A. E. *J. Med. Chem.* **2004**, *47*, 4135; (b) Deacon, C. F.; Ahrén, B.; Holst, J. J. *Exp. Opin. Investig. Drugs* **2004**, *13*, 1091; (c) Drucker, D. J. *Exp. Opin. Investig. Drugs* **2003**, *12*, 87; (d) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. *Exp. Opin. Ther. Patents* **2003**, *13*, 499; (e) Wiedeman, P. E.; Trevillyan, J. M. *Curr. Opin. Investig. Drugs* **2003**, *4*, 412.
5. (a) Barlocco, D. *Curr. Opin. Investig. Drugs* **2004**, *5*, 1094; (b) McIntyre, J. A.; Castañer, J. *Drugs Future* **2004**, *29*, 887.
6. 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.
7. Boumendjel, A.; Roberts, J. C.; Hu, E.; Pallai, P. V.; Rebek, J., Jr. *J. Org. Chem.* **1996**, *61*, 4434.
8. Irie, T.; Fukushi, K.; Namba, H.; Iyo, M.; Ikota, N.; Nagatsuka, S.; Ueda, T.; Nishiura, M.; Takatoku, K; Yomoda, I. U.S. Patent 6,306,882, 2001. *Chem. Abstr.* **1999**, *130*, 81402.
9. Preparation of compound **21** (G′ = H·HBr) by a similar route: Gangjee, A.; Elzein, E.; Queener, S. F.; McGuire, J. J. *J. Med. Chem.* **1998**, *41*, 1409.
10. Weinreb, S. M.; Demko, D. M.; Lessen, T. A.; Demers, J. P. *Tetrahedron Lett.* **1986**, *27*, 2099.
11. Noncommercial thioamides were prepared by reaction of the corresponding amides with Lawesson's reagent or P_2S_5 or from the nitrile by the method of Ohkawa, S.; Naruo, K.; Miwatashi, S.; Kimura, H. PCT Int. Patent Appl. WO 01/74811, 2001. *Chem. Abstr.* **2001**, *135*, 303878.
12. (a) Caldwell, C. G. Unpublished work from Merck Research Laboratories; (b) Sheppeck, J. E.; Duan, J.; Xue, C.-B.; Wasserman, Z. PCT Int. Patent Appl. WO 02/096426, 2002. *Chem. Abstr.* **2002**, *138*, 14059.
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. (a) Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 1; (b) Chen, T.; Ajami, K.; McCaughan, G. W.; Gorrell, M. D.; Abbott, C. A. In *Dipeptidyl Peptidases in Health and Disease*; Hildebrandt, M., Klapp, B. F., Hoffman, T., Demuth, H.-U., Eds.; Kluwer Academic/Plenum: New York, 2003; pp 79–86.
15. QPP is also known as dipeptidyl peptidase VII and is very similar, if not identical, to dipeptidyl peptidase II. See Ref. 13.
16. Lankas, G.; Leiting, B.; Sinha Roy, R.; Eiermann, G.; Biftu, T.; Kim, D.; Ok, H.; Weber, A. E.; Thornberry, N. A. Abstracts of Papers, 64th Annual Scientific Sessions of the American Diabetes Association, Orlando, FL, 4–8 June 2004; Abstract 7-OR.