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Substituted piperazines as novel dipeptidyl peptidase IV inhibitors

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Abstract—Incorporation of a fluorophenyl β-amino amide moiety into piperazine screening lead **2** has resulted in the discovery of a structurally novel series of potent and selective DP-IV inhibitors. Simplification of the molecule and incorporation of multiple fluorine atoms on the phenyl ring has provided low molecular weight analogs such as compound **32**, which is a 19 nM DP-IV inhibitor with >4000-fold selectivity over QPP.

The preceding paper describes the discovery of a novel series of (*R*)-β-homophenylalanine-based dipeptidyl peptidase IV (DP-IV) inhibitors exemplified by parent compound **1** (Fig. 1).[1] In the course of our investigation of the SAR of a structurally distinct screening lead **2**, we were interested to learn the impact of a similar substitution. Thus, we have found that a marked boost in DP-IV inhibitory potency could be achieved by the incorporation of a (*R*)-β-amino amide moiety into the left-hand side of the molecule to give compounds such as **3**. Both enantiomers of the analogous α-amino amide phenylalanine derivatives were found to be >2000-fold less potent. Removal of the carbonyl group in either the (*R*)-α- or (*R*)-β-amino amides also resulted in a significant loss of intrinsic activity[2] (data not shown). Due to the clear preference for β-amino amide substitution, efforts focused on this series. Further refinement has revealed a number of structural preferences and these will be discussed herein.[3]

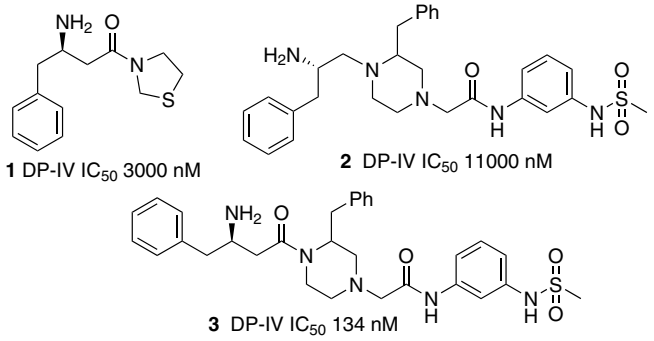

Figure 1. Initial leads.

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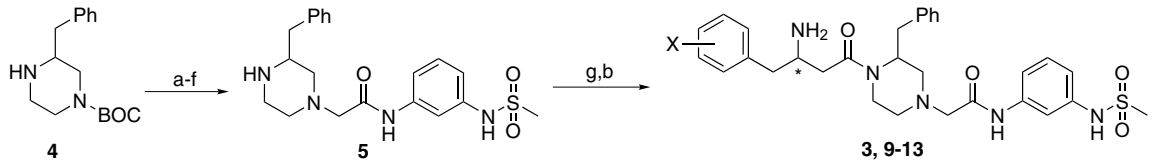
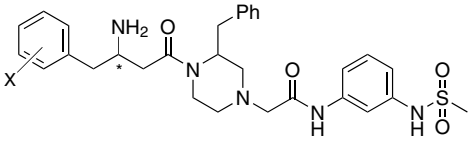


Scheme 1. Synthesis of compounds **3**, **9**–**13**. Reagents: (a) benzyl chloroformate, Et_3N, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) α-Cl-3-NO_2-acetanilide, DIEA, DMF; (d) RaNi, NH_2NH_2, MeOH, $ClCH_2CH_2Cl$; (e) MsCl, pyridine, CH_2Cl_2; (f) H_2, $Pd(OH)_2$, MeOH; (g) (*R*)- or (*S*)-$ArCH_2CH(NHBoc)CH_2CO_2H$, EDC, DMAP, CH_2Cl_2.

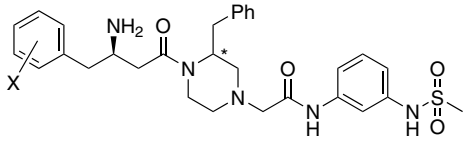
Inhibitors were initially synthesized as mixtures of diastereomers from compound **4** (Scheme 1). Protection as the benzyl carbamate (Cbz) and removal of the *tert*-butylcarbamate (Boc) group was followed by alkylation with alpha-chloro-3-nitroacetanilide. Selective reduction of the nitro group in the presence of the Cbz-protecting group, sulfonamide formation, and removal of the benzylcarbamate provided intermediate **5**. Standard 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) mediated peptide coupling of **5** with appropriately substituted optically active Boc-protected β-amino acids[1] followed by deprotection gave the final compounds[4] **3**, and **9**–**13** (structures defined in Table 1). The stereoselective synthesis of compounds **14**–**32** (structures defined in Tables 2–5) from enantiomerically pure intermediates[5] **6** is shown in Scheme 2. Peptide coupling of **6** with appropriately substituted β-amino acids, and Boc deprotection of the resultant intermediate **7** provided compound **18**. Alternatively, the benzyl group in **7** was

Table 1. Inhibitory properties of (*R*) and (*S*) β-amino amides



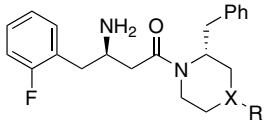
Compd	X	Amine stereochemistry	DP-IV IC_{50}, nM	QPP IC_{50}, nM
3	H	*R*	134	7700
9	3,4-DiF	*R*	44	450
10	2-F	*R*	34	13,000
11	H	*S*	65,000	19,000
12	3,4-DiF	*S*	36,000	15,000
13	2-F	*S*	19,000	26,000

Table 2. Effect of C-2 stereochemistry on DP-IV inhibitory properties



Compd	X	Benzyl group stereochemistry	DP-IV IC_{50}, nM	QPP IC_{50}, nM
14	3,4-DiF	*R*	30	640
15	2-F	*R*	14	19,000
16	3,4-DiF	*S*	1100	280
17	2-F	*S*	690	12,000

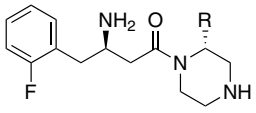
Table 3. Effect of truncation or replacement of the piperazine core on DP-IV inhibitory properties



Compd	X	R	DP-IV IC_{50}, nM	QPP IC_{50}, nM
18	N	$-CH_2Ph$	405	15,000
19	N	$-CH_2CONH_2$	114	66,000
20	N	$-CH_3$	225	>100,000
21	N	H	139	>100,000
22	O	—	108	>100,000
23[a]	CH	H	1040	>100,000

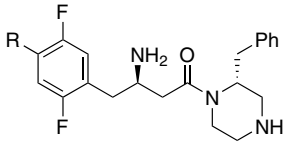
[a] Mixture of diastereomers at piperidine 2-position.

Table 4. Effect of C-2 substitution of truncated analogs on DP-IV inhibitory properties

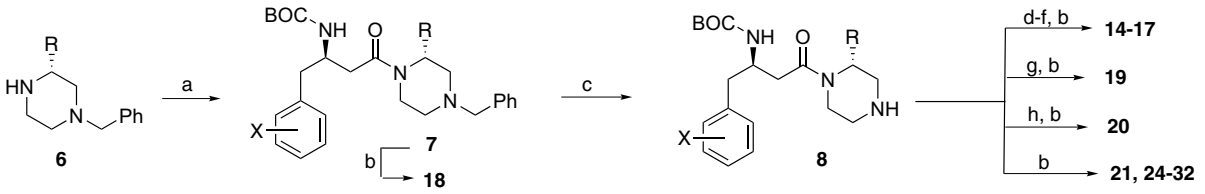


Compd	R	DP-IV IC_{50}, nM	QPP IC_{50}, nM
21	Benzyl	139	>100,000
24	H	3700	>100,000
25	Methyl	1600	>100,000
26	Isopropyl	922	50,000
27	4-Fluorophenyl	610	23,000
28	4-Thiazolylmethyl	143	>100,000
29	3-Pyridylmethyl	155	>100,000
30	4-Fluorobenzyl	166	>100,000

Table 5. Effect of incorporation of multiple fluorine atoms on DP-IV inhibitory properties



Compd	R	DP-IV IC_{50}, nM	QPP IC_{50}, nM
31	H	51	65,000
32	F	19	78,000



Scheme 2. Diastereoselective synthesis of compounds **14**–**32**. Reagents: (a) (*R*)-ArCH$_2$CH(NHBoc)CH$_2$CO$_2$H, EDC, HOBt, DIEA, DMF; (b) TFA, CH$_2$Cl$_2$; (c) H$_2$, Pd(OH)$_2$, MeOH [for compound **28** ACE–Cl, ClCH$_2$CH$_2$Cl]; (d) α-Cl-3-NO$_2$-acetanilide, DIEA, DMF; (e) H$_2$, Pd(OH)$_2$, MeOH; (f) MsCl, pyridine, CH$_2$Cl$_2$; (g) 2-chloroacetamide, DIEA, DMF; (h) NaBH(OAc)$_3$, (CH$_2$O)$_n$, 4 Å mol. sieves, ClCH$_2$CH$_2$Cl.

removed by hydrogenolysis (or by treatment with 1-chloroethyl chloroformate when R = 4-thiazolylmethyl) and the resultant intermediate **8** converted to compounds **14**–**17** by elaboration of the right-hand side as discussed in Scheme 1. Alkylation of **8** with 2-chloroacetamide and Boc removal provided compound **19**. Reductive amination of **8** with paraformaldehyde followed by deprotection of the Boc group gave compound **20**. Compounds **21** and **24**–**32** were prepared by Boc deprotection of **8**. The requisite precursor of morpholine analog **22** was synthesized according to literature precedent.[6] Compound **23** was synthesized from racemic 2-benzylpiperidine.

All compounds synthesized were tested for DP-IV activity and selectivity over DP-IV homologs and other proline specific enzymes with DP-IV-like activity.[7,8] Without exception, the piperazine analogs described herein are inactive ($IC_{50's}$ >100 μM) at PEP (prolyl endopeptidase), prolidase, and APP (aminopeptidase P) and as such these data will not be presented. However, some inhibition of QPP (quiescent cell proline peptidase, also known as DPP-II or DPP7), which has been implicated to play a role in immune function, has been observed in this series. Thus inhibitory potential of test compounds at DP-IV and QPP will be reported.

The work described in the previous paper focused on the optimization of a β-homophenylalanine thiazolidide series and we hoped to utilize this information in the design of analogs in the piperazine series. A key modification was the introduction of one or more fluorine atoms on the phenyl ring. In particular, the 3,4-difluoro and 2-fluoro-substituted analogs were quite promising, thus the corresponding isomers were prepared in the piperazine series. While the (*R*) stereochemistry was shown to be essential in the thiazolidide series, we wanted to confirm this in the structurally unique piperazine series. As shown in Table 1, the same general trend for increased DP-IV inhibitory potency when going from the unsubstituted phenyl analog **3** to the 3,4-difluorophenyl analog **9** (3-fold increase) and the 2-fluorophenyl analog **10** (4-fold increase) was also observed in the piperazine series. Preference for the (*R*) stereochemistry of the β-amino amide moiety is clearly maintained. Most interestingly, there was quite a pronounced increase in selectivity (>350-fold) over QPP seen with the 2-fluorophenyl analog and, therefore, the remaining SAR studies concentrated primarily on this substitution pattern.

Having demonstrated the dramatic influence of amine stereochemistry on DP-IV inhibitory potency, we then wished to define the stereochemical preference of the substituent at the 2-position of the piperazine ring. The individual diastereomers were prepared as described in Scheme 2 and the compounds evaluated in vitro (Table 2). Once again there was a strong preference for one isomer. For the 2-fluoro analog, the (*R*,*R*) diastereomer **15** was 50-fold more potent (DP-IV IC_{50} = 14 nM) than the corresponding (*R*,*S*) isomer **17**. Importantly, compound **15** also had an excellent selectivity profile as it was >1000-fold selective over QPP.

With potent and selective DP-IV inhibitors now in hand, we wanted to evaluate the pharmacokinetic profiles of compounds in this series. The initial results were not encouraging: compound **15** had an unusually high clearance (130 mL/min/kg) and low (<1%) oral bioavailability when dosed in rats.[9] To address this issue we first began simplifying the molecule by truncation of the right-hand side at various points. Gratifyingly, the low molecular weight unsubstituted piperazine **21** and morpholine **22** retained good in vitro potency (DP-IV IC_{50} = 139 and 108 nM, respectively) and excellent selectivity (>700- and >900-fold, respectively) over QPP (Table 3). Interestingly, a significant loss in potency (DP-IV IC_{50} = 1040 nM) was observed with piperidine compound **23**.

We next began a survey of replacements of the benzyl group at the 2-position of the piperazine ring, which would not have the potential metabolic liabilities of an unsubstitued phenyl ring. As shown in Table 4, removal of the benzyl group altogether, or replacement by an alkyl group resulted in a significant loss of potency. The 4-fluorophenyl compound **27**, lacking a benzylic methylene group, suffered a 4-fold loss in DP-IV inhibitory potency whereas all of the other benzylic aromatic groups were of comparable potency (DP-IV IC_{50}s of 143–166 nM) and selectivity (>600-fold over QPP) to that of the unsubstituted benzylic analog **21**.

The preceding paper[1] describes the dramatic effect on potency when additional fluorine atoms are incorporated into the fluorophenyl β-amino amide moiety of a thiazolidide series of DP-IV inhibitors. Thus, the 2,5-difluorophenyl and 2,4,5-trifluorophenyl analogs are 3- and 7-fold more potent, respectively, than the 2-fluorophenyl analog. We were also interested in incorporating these same modifications into the current piperazine

lead. When the corresponding 2,5-difluorophenyl analog **31** and 2,4,5-trifluorophenyl analog **32** were prepared in the truncated series, similar increases in potency relative to the corresponding 2-fluorophenyl analog **21** were achieved. Excellent selectivity of >1000-fold over QPP was maintained.

To determine whether we had achieved any improvement in oral bioavailability, a subset of potent and selective compounds were chosen from within these series for pharmacokinetic analysis in rats.[9] Unfortunately, compounds **21**, **22**, **29**, and **30** also showed uniformly poor pharmacokinetics typified by an exceptionally high clearance, short half-life (<2 h), and low overall oral bioavailability (<4%). With the hope of providing some guidance for future synthetic efforts within this class of compounds, we initiated in vitro metabolism studies, choosing compound **21** as a representative example. Incubation with rat liver microsomes resulted in extensive metabolism of this compound, primarily by piperazine ring oxidation.

In summary, we have discovered a series of structurally novel compounds, which are potent and selective DP-IV inhibitors. For example, compound **15** has an IC_{50} of 14 nM at DP-IV with >1000-fold selectivity over QPP. Significantly, truncation of the right-hand side of the molecule provided low molecular weight analog **32**, which maintained good in vitro potency (DP-IV IC_{50} = 19 nM) and selectivity (>4000-fold) over QPP. Having ascertained the metabolic liabilities of compounds in this series, we have modified the piperazine ring of compound **32**, resulting in major improvements in the pharmacokinetic profile. These modifications will be the subject of future communications.

References and notes

1. Xu, J.; Ok, H. O.; Gonzalez, E. J.; Colwell, L. F., Jr.; Habulihaz, B.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett*. **2004**, *14*, preceding paper. doi:10.1016/j.bmcl.2004.06.099.
2. The α- and β-amino amines were prepared by reductive amination of piperazine intermediate **5** with the requisite aldehydes. The aldehydes were available by lithium aluminum hydride reduction of the corresponding Weinreb amides.
3. This work was reported in part; Brockunier, L. L. *227th National Meeting of the ACS*, Anaheim, CA, March 2004. Abstract 100.
4. Final compounds were characterized by ^{1}H NMR, mass spectrometry and HPLC analysis.
5. Requisite enatiomerically pure piperazines **6** were prepared according to a literature procedure outlined for the synthesis of (*R*) and (*S*)-2-methylpiperazine. Kiely, J. S.; Priebe, S. R. *Org. Prep. Proced. Int.* **1990**, *22*, 761.
6. Shawe, T. T.; Koenig, G. J.; Ross, A. A. *Synth. Commun.* **1997**, *27*, 1777. Chromatographic separation provided the individual diastereomers.
7. (a) All IC_{50} determinations for DP-IV and other proline peptidases were carried out as described in: 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. (b) With the exception of compound **31**, all multiple determinations of the DP-IV IC_{50} values were within 1.6-fold of the reported average; compound **31** was within 2.8-fold of the average. All QPP values were within 1.3-fold of the reported average.
8. For references on proline specific peptidases see: (a) Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 1; (b) Cunningham, D. F.; O'Connor, B. *Biochim. Biophys. Acta* **1997**, *1343*, 160.
9. Male Sprague-Dawley rats were dosed intravenously at 1 mg/kg and orally at 2 mg/kg. Plasma drug levels were determined by LC/MS/MS.