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Potent and selective proline derived dipeptidyl peptidase IV inhibitors

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Abstract—In-house screening of the Merck sample collection identified proline derived homophenylalanine **3** as a DPP-IV inhibitor with modest potency (DPP-IV IC_{50} = 1.9 μM). Optimization of **3** led to compound **37**, which is among the most potent and selective DPP-IV inhibitors discovered to date.

Inhibition of dipeptidyl peptidase IV (DPP-IV) has recently emerged as a promising new approach for the treatment of type 2 diabetes mellitus.[1] DPP-IV is the enzyme responsible for inactivation of the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These two hormones are secreted in response to nutrient ingestion, and each enhances the glucose-dependent secretion of insulin. Furthermore, GLP-1 has been shown in mammals to stimulate insulin biosynthesis, inhibit glucagon secretion, slow gastric emptying, reduce appetite, and stimulate the regeneration and differentiation of islet β-cells.[1,2] More recently, the GLP-1 receptor has also been implicated in learning and neuroprotection.[3]

Due to rapid processing of GLP-1 and GIP by DPP-IV, the half-lives of the active peptides in blood are extremely short. Inhibition of DPP-IV in humans has been shown to increase circulating GLP-1 and GIP levels, and leads to decreased blood glucose levels, hemoglobin A_{1c} levels, and glucagon levels.[1,4] DPP-IV inhibitors offer a number of potential advantages over existing diabetes therapies including a lowered risk of hypoglycemia, the potential for weight loss, and the potential for the regeneration and differentiation of pancreatic β-cells.[1,2,4]

DPP-IV is a dipeptidase that selectively binds substrates with proline at the P1 position. Consequently, many known DPP-IV inhibitors possess substituted pyrrolidines or thiazolidines linked to α-amino acid derivatives (Fig. 1).[5] Screening of the Merck sample collection identified proline derivative **3** as a moderately active DPP-IV inhibitor (IC_{50} = 1.9 μM).[6] This inhibitor is unique in

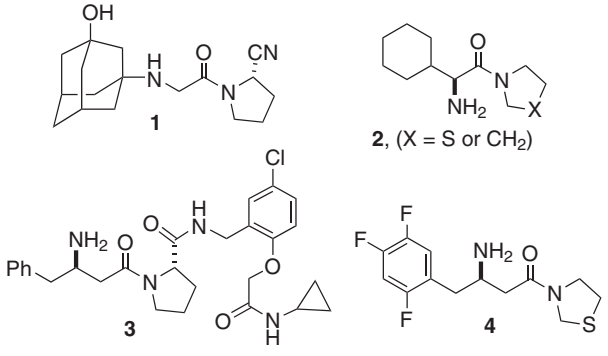

Figure 1. Some published DPP-IV inhibitors.

Keywords: Dipeptidyl peptidase IV; Selective; Proline amide; Thiazolidine; Type 2 diabetes.
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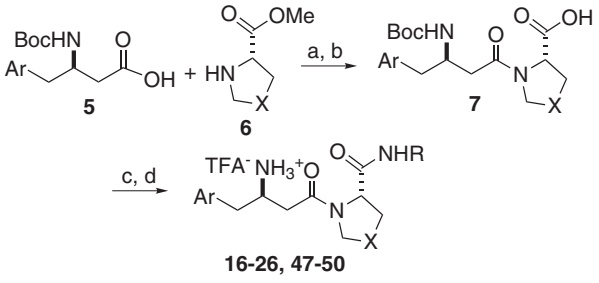
that it possesses a β-amino amide moiety rather than an α-amino amide linked to the proline nitrogen. A recent report from these laboratories describes initial optimization of the homophenylalanine moiety in the context of thiazolidine amides such as **4**.[6a] This report describes further optimization of screening hit **3** and the discovery of one of the most potent and selective proline derived DPP-IV inhibitors to date.[7]

Inhibitors were initially synthesized from acid **7**, which was prepared by coupling acids **5** with L-proline methyl ester **6** (or *R*-thiazolidine carboxylic acid methyl ester, X = S) followed by ester hydrolysis (Scheme 1). Coupling of acid **7** with various amines followed by deprotection afforded the final compounds **16**–**26** and **47**–**50**.[8] α-Amino amides **13**–**15** and **51** were synthesized the same way using *N*-Boc-*S*-cyclohexylglycine instead of **5**.

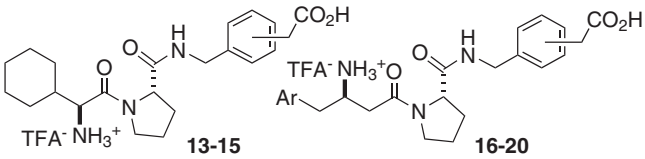
The synthesis of a series of α-substituted phenoxyacetic acids began with the coupling of phenol **8** with α-bromoesters **9** followed by *N*-deprotection (Scheme 2). In some cases, the racemic Boc-protected intermediates were resolved using chiral chromatography to give optically pure compounds. Amines **10** were then coupled to *N*-Boc-proline and then deprotected to give **11**, which in turn, were coupled with **5**. Ester hydrolysis followed by deprotection then afforded analogs **27**–**46**.

Initial SAR surrounding screening hit **3** revealed that a phenoxyacetic acid derivative was more potent against DPP-IV than the corresponding cyclopropyl amide or methyl ester (data not shown). In order to investigate



Scheme 1. Synthesis of various proline amides. Reagents: (a) EDC, HOBt, DIEA, DCM; (b) $LiOH_{aq}$, THF, MeOH; (c) EDC, HOBt, DIEA, RNH_2, DCM; (d) TFA, DCM.

Table 1. Inhibitory properties of selected phenylacetic acid derived DPP-IV inhibitors

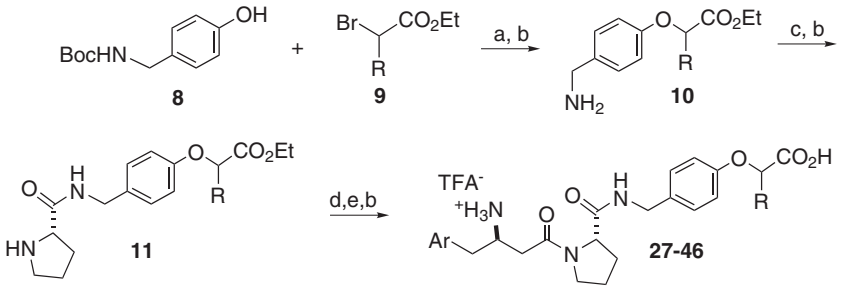


Compd	*ortho*/*meta*/*para*-Phenylacetic acid	Ar =	DPP-IV IC_{50} (nM)
13	*ortho*	—	3600
14	*meta*	—	3300
15	*para*	—	4200
16	*ortho*	Ph	1200
17	*meta*	Ph	1200
18	*para*	Ph	510
19	*para*	3,4-F_2Ph	75
20	*para*	2-FPh	54

this trend further, different positional isomers of phenylacetic acids were screened (Table 1, **16**–**18**).[9] Another potency enhancement was observed with *para*-substituted acid **18** compared to the *ortho*- and *meta*-isomers **16** and **17**. Since the corresponding *S*-cyclohexylglycine series **13**–**15** shows reduced potency against DPP-IV compared to **16**–**18**, the α-amino amide series was abandoned in favor of the *R*-homophenylalanines.

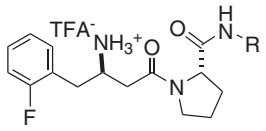
Previous work from these laboratories indicated that introduction of fluorine atoms on the pendant phenyl ring of homophenylalanines improves potency against DPP-IV.[6] This trend was also observed in the proline amide series (Table 1) with 3,4-difluoro derivative **19** (IC_{50} = 75 nM) and 2-fluoro derivative **20** (IC_{50} = 54 nM). The latter compound was nearly 10 times more potent against DPP-IV than the parent phenyl analog **18**, and we hoped to further improve DPP-IV potency by investigating different right-hand side amides while maintaining the promising 2-fluorophenyl moiety.

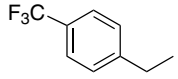
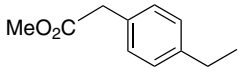
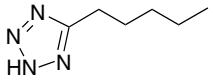
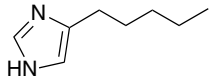
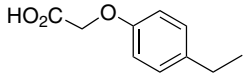
A series of nonacid bearing proline amides was next investigated (Table 2). Acids and acid bioisosteres such as **24** and **26** displayed superior potency against DPP-IV compared to nonacids. Particularly illustrative are tetrazole **24** (IC_{50} = 39 nM) and imidazole **25** (IC_{50} = 1640 nM), which are similarly tethered to the proline amide. The acid preference is also evident by



Scheme 2. Synthesis of phenoxyacetic acids. Reagents: (a) K_2CO_3, MeOH; (b) TFA, DCM; (c) Boc-*S*-Pro, EDC, HOBt, DIEA, DCM; (d) **5**, EDC, HOBt, DIEA, DCM; (e) $LiOH_{aq}$, THF, MeOH.

Table 2. Inhibitory properties of selected DPP-IV inhibitors

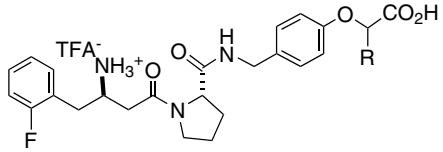


Compd	R	DPP-IV IC_{50} (nM)
21	Bn	539
22		721
23		170
24		39
25		1640
26		118

comparing methyl ester **23** to acid **20**, where a 3-fold potency boost was observed for the acid. On the other hand, the tether length of the acid appeared to be only marginally important for DPP-IV potency, with a 2-fold potency drop observed in going from phenylacetic acid **20** to phenoxyacetic acid **26**. Since an acid binding pocket in the enzyme might be responsible for the increased affinities of the acids, we sought to further improve potency against DPP-IV by optimizing the tether properties of the phenoxyacetic acid series.

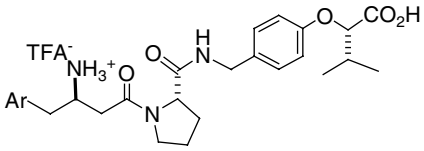
Substantial improvements in potency accompanied increasing steric bulk α to the acid in a series of substituted phenoxyacetic acids (**27**–**31**, Table 3). Diastereomerically pure *R*- and *S*-isomers of the α-methyl and α-isopropyl analogs were screened and the *S*-configurations proved optimal.[10] Indeed, *S*-isopropyl derivative **35** is 65-fold more potent than the unsubstituted acid **26**. We hoped to further improve potency by combining noncommercially available homophenylalanines **5**[11] with the optimized α-*S*-isopropyl substituent of **35**.

Table 3. Effect of α-substitution on right-hand side phenoxyacetic acids



Compd	R =	Stereochemistry α to acid	DPP-IV IC_{50} (nM)
27	Me	*R*/*S*	18
28	Et	*R*/*S*	7.1
29	*n*-Pr	*R*/*S*	10
30	*i*-Pr	*R*/*S*	3.7
31	*n*-Bu	*R*/*S*	5.1
32	Me	*R*	82
33	Me	*S*	12
34	*i*-Pr	*R*	13
35	*i*-Pr	*S*	1.8

Table 4. Inhibitory properties of selected DPP-IV inhibitors

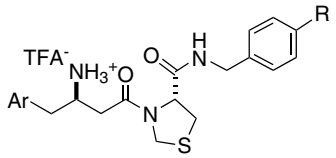


Compd	Ar =	DPP-IV IC_{50} (nM)
36	Ph	18
37	**2,5-F_2Ph**	**0.48**
38	2,4,5-F_3Ph	1.6
39	F_5Ph	8.2
40	4-CF_3–2-FPh	0.83
41	4-CF_3–2,5-F_2Ph	9.5
42	5-F–2-MePh	33
43	2-Cl–4-FPh	1.7
44	5-Cl–2-FPh	1.6
45	5-CF_3–2-FPh	12
46	3-CF_3OPh	295

As expected based on previously reported results,[6] incorporation of a 2,5-difluoro substitution pattern into the homophenylalanine moiety resulted in another boost in potency against DPP-IV (Table 4). Analog **37** is among the most potent proline derivatives yet reported, exhibiting sub-nanomolar potency against DPP-IV (IC_{50} = 0.48 nM). In contrast to past observations,[6] addition of a third fluoride at the *para*-position of **37** resulted in a potency drop against DPP-IV (**38**, IC_{50} = 1.6 nM). Not surprisingly, this aryl group was particularly sensitive to different substitution patterns. Small substituent changes led to analogs with relatively large drops in DPP-IV activity, such as the 5-fluoro-2-methyl analog **42** (IC_{50} = 33 nM). Nevertheless, many of the compounds still display excellent potency against DPP-IV.

Since thiazolidine amides are a common motif in many reported DPP-IV inhibitors, we replaced the pyrrolidine moiety with a thiazolidine in selected compounds (Table 5). In each case, a potency boost was observed with the

Table 5. Thiazolidines are more potent than their pyrrolidine counterparts



Compd	Ar =	R =	DPP-IV IC_{50} (nM)
47	Ph	–CH_2CO_2H	161
48	2-FPh	–CH_2CO_2Me	87
49	2-FPh	–CH_2CO_2H	37
50	2,5-F_2Ph	–OCH(*i*-Pr)CO_2H (*S*)	0.30

Table 6. Selectivity profiles of selected DPP-IV inhibitors

Compd	DPP-IV IC_{50} (μM)	QPP IC_{50} (μM)	DPP8 IC_{50} (μM)	DPP9 IC_{50} (μM)	FAP IC_{50} (μM)	PEP IC_{50} (μM)	APP IC_{50} (μM)	Prolidase	hERG IC_{50} (μM)
19	0.075	>100	>100	>100	>100	—	—	—	79
22	0.72	55	5.3	>100	>100	—	>100	>100	0.87
24	0.039	>100	>100	>100	>100	—	>100	>100	—
37	0.00048	>100	>100	86	21	39	>100	>100	76
39	0.0082	>100	>100	>100	48	>100	>100	>100	>100
48	0.087	59	>100	>100	>100	100	>100	>100	9.7
49	0.037	>100	>50	71	>100	—	—	—	—
51	7.2	>100	4.1	0.45	>100	>100	—	—	>90

thiazolidine derivatives compared to the corresponding proline derivative, leading to **50** (IC_{50} = 0.30 nM), one of the most potent DPP-IV inhibitors yet reported.

In order to ensure that these inhibitors are neither substrates nor irreversible inhibitors of DPP-IV, the inhibition kinetics and off-rate of potent inhibitor **37** were measured as a representative example. The resulting progress curves (Fig. 2) are indicative of a typical slow binding reversible inhibitor,[12] and the formation of steady state velocities observed with **37** indicate that this inhibitor is not a substrate for DPP-IV. Taken together, these data indicate that **37** is a reversible inhibitor of DPP-IV.

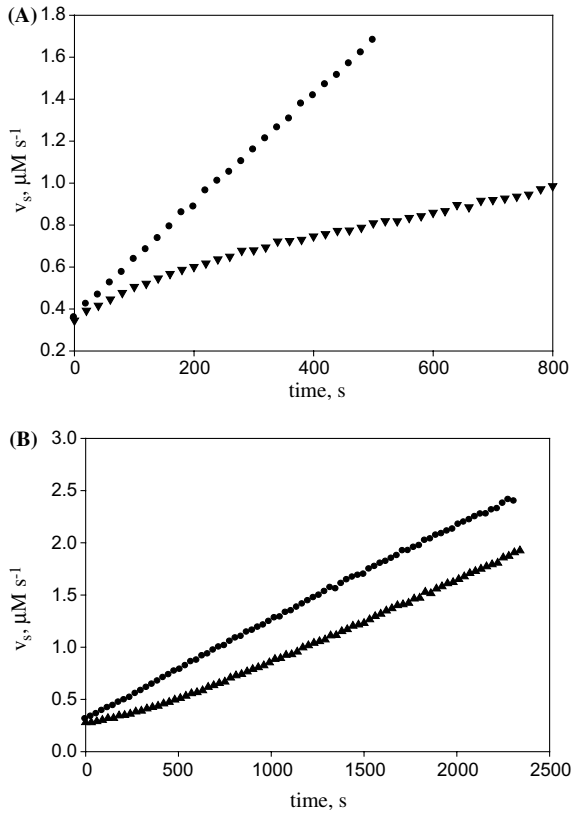


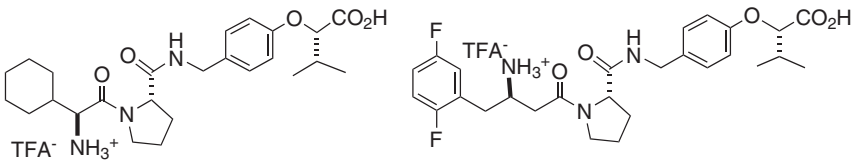
Figure 2. (A) Human recombinant DPP-IV was studied by liberation of AMC from substrate Gly-Pro-AMC in the absence (dots) and presence (triangles) of **37**. The data were fit to the integrated rate equation for slow binding inhibition by nonlinear regression analysis. Binding constant $k_{on} = 2.0 \pm 0.5 \cdot 10^6 M^{-1} s^{-1}$ was calculated from from individual data sets. (B) The dissociation rate was determined by pre-incubation of DPP-IV with **37** under the same conditions and subsequent 100-fold dilution. Eight individual data sets were fit as described above to determine $k_{off} = 1.2 \pm 0.3 \cdot 10^{-3} s^{-1}$, which corresponds to $t_{1/2}$ = 10 min.

A representative series of the above inhibitors was assessed for their selectivity profiles against other dipeptidyl peptidase homologs and proline-specific enzymes (Table 6) including quiescent prolyl peptidase (QPP/DPP-II), dipeptidyl peptidases 8 and 9 (DPP8 and DPP9), seprase (FAP), prolidase, amino peptidase P (APP), and prolyl endopeptidase (PEP).[9,13] The same series of inhibitors was also tested against the hERG potassium channel as a general test of off-target activity.[14] For comparison purposes, cyclohexylglycine derivative **51** (Fig. 3) was also screened against this same series of enzymes. All of the homophenylalanine derived acids (**19**, **37**, **39**, and **49**) and tetrazole **24** displayed excellent selectivity against all of the counterscreens. On the other hand, the nonacids all possess increased activity against hERG, with the trifluoromethylbenzyl analog **22** being the most potent (hERG IC_{50} = 870 nM). Cyclohexylglycine analog **51** was only moderately potent against DPP-IV and inactive against hERG, but it was relatively potent against DPP8 and DPP9. The above data indicates that the acid functionality in this series is important for selectivity over hERG while the β-amino amide functionality is important for selectivity over DPP8 and DPP9. Selectivity over these homologs is important because inhibition of these enzymes has been associated with multi-organ toxicity in pre-clinical species.[15]

The rat pharmacokinetic profiles of the potent amino acids examined were uniformly poor (Table 7), although thiazolidine analog **49** showed improved oral

Table 7. Pharmacokinetic properties of selected DPP-IV inhibitors in the rat (1/2 mpk iv/po)

Compd	Clp ($mL\,min^{-1}\,kg^{-1}$)	$t_{1/2}$ (h)	Oral AUC_{norm} ($\mu M\,h\,kg\,mg^{-1}$)	F (%)
19	47	0.36	0.020	<1.0
22	78	0.78	0.183	38
24	54	0.20	—	<1.0
37	150	0.63	0.003	1.2
39	47	0.36	<0.001	<1.0
48	140	0.10	0.031	11
49	25	0.19	0.151	10



51, DPP-IV IC_{50} = 7200 nM **37**, DPP-IV IC_{50} = 0.48 nM

Figure 3. Cyclohexylglycine analog **51** and potent and selective DPP-IV inhibitor **37**.

bioavailability and exposure. In the case of methyl ester **48**, a rapid and complete conversion of the ester to acid **49** was observed. In order to determine the reason for the poor oral exposures, three representative compounds (**19**, **20**, and **37**) were administered orally to portal vein cannulated rats. Compared to a well absorbed positive control (theophylline), all three compounds were poorly absorbed ([compound] < 10 nM). Thus, the poor bioavailability of these proline derived acids can be attributed to poor oral absorption of this series of inhibitors. It appears that the acid functionality contributes to the poor absorption since **22** possesses improved oral bioavailability and exposure compared to the other analogs.

In conclusion, a series of potent and highly selective proline derived DPP-IV inhibitors has been described, culminating in the discovery of analog **37**, a 0.48 nM DPP-IV inhibitor with >50,000-fold selectivity over all other enzymes tested (Fig. 3). In contrast, cyclohexylglycine derived α-amino amide analog **51** is a poor DPP-IV inhibitor with no selectivity over DPP8 and DPP9. Consequently, it appears that the excellent selectivity profile of the above described inhibitors is derived from the β-amino amide functionality combined with the carboxylic acid moiety. Future efforts in this structure class will focus on maintaining the excellent potency and selectivity while improving the pharmacokinetic profile of these homophenylalanine derived DPP-IV inhibitors.

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8. All final compounds were characterized by ^{1}H NMR and LC–MS.
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10. In the case of the α-methyl series the stereochemistry was confirmed by synthesis of optically pure derivatives via Mitsunobu reaction of **8** with *R*- or *S*-lactic acid esters. In the case of the isopropyl substituent stereochemistry was confirmed by X-ray crystal structure of **37**.
11. (a) As described in Ref. a optically active β-amino acids **5** were prepared using Schollkopf's synthesis of α-amino acids followed by Arndt–Eistert homologation. See: Schollkopf, U. *Tetrahedron* **1983**, *39*, 2085; (b) Podlech, J.; Seebach, D. *Liebigs Ann.* **1995**, 1217.
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