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Discovery of potent and selective phenylalanine based dipeptidyl peptidase IV inhibitors

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Abstract—*anti*-Substituted β-methylphenylalanine derived amides have been shown to be potent DPP-IV inhibitors exhibiting excellent selectivity over both DPP8 and DPP9. These are among the most potent compounds reported to date lacking an electrophilic trap. The most potent compound among these is 5-oxo-1,2,4-oxadiazole **44**, which is a 3 nM DPP-IV inhibitor.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that is released from the gut during meals and serves as an enhancer of glucose stimulated insulin release from pancreatic β-cells. Chronic infusion of GLP-1 to patients with type 2 diabetes resulted in significant decreases in both blood glucose and hemoglobin A_{1c} levels;[1] however, GLP-1 is rapidly degraded in plasma by the serine protease dipeptidyl peptidase IV (DPP-IV). Inhibition of DPP-IV increases the levels of endogenous intact circulating GLP-1. Consequently, inhibition of DPP-IV is rapidly emerging as a novel therapeutic approach to the treatment of type 2 diabetes.[2]

Earlier reports from our laboratories described 4-amino cyclohexylglycine and cyclopentylglycine analogs as potent DPP-IV inhibitors lacking an electrophile.[3] Among the best compounds discovered from that series was 2,4-difluorobenzenesulfonamide **1** (Fig. 1). Compound **1** was found to have good pharmacokinetic properties and produced significant activity in an oral glucose tolerance test in lean mice. However, further development of **1** was halted because it was subsequently found to exhibit significant activity at both DPP8 (IC_{50} = 993 nM) and DPP9 (IC_{50} = 2720 nM). Inhibition of these enzymes has been correlated with toxicity in animals.[4] Despite extensive SAR studies in this original α-aminoacid series, little progress was made toward improving the selectivity against DPP8 and DPP9.

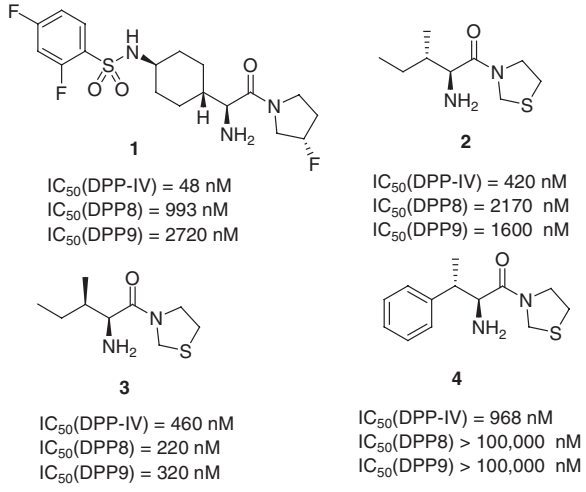

Figure 1. Lead DPP-IV inhibitors.

The selectivity profile of thiazolidides **2** and **3** looked very intriguing (Fig. 1). Although both compounds exhibited similar potency toward DPP-IV, the *threo*-isomer **2**[5] is 10-fold more selective toward DPP8 and 5-fold

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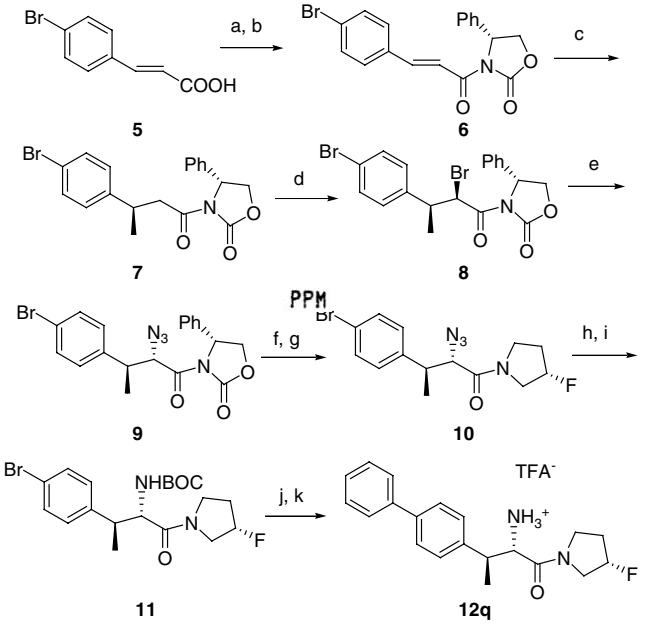
more selective toward DPP9 than allo-isomer **3**. Apparently, stereochemistry at the β-position in the α-amino acid series has some effect on the selectivity over both DPP8 and DPP9. We envisioned that we might be able to improve the selectivity over DPP8 and DPP9 by incorporating the '*threo*' bias into the α-amino acid series. To test this idea, we prepared β-methylphenylalanine thiazolidide **4**. Although the potency of compound **4** (DPP-IV, $IC_{50} = 968$ nM) was reduced relative to compound **2**, it did exhibit improved selectivity over both DPP8 and DPP9. Based on our previous results in the α-amino series, we knew that P_2-site could be optimized to improve potency. The work described here summarizes our initial efforts at optimizing the potency and selectivity of this novel series of β-methylphenylalanine based DPP-IV inhibitors.

The β-methylphenylalanine-based DPP-IV inhibitors were prepared by EDC-mediated coupling of α-amino acids with 3-(*S*)-fluoropyrrolidine. 3-(*S*)-Fluoropyrrolidine was chosen because it improved both the selectivity and pharmacokinetic profile of these inhibitors as reported before.[3b] A representative example of the synthesis of these inhibitors is shown in Scheme 1.[6] Activation of the carboxylic acid **5** with trimethylacetyl chloride followed by addition of lithiated oxazolidinone gave acyloxazolidinone **6**. The methylcopper reagent, generated in situ by the treatment of methyl magnesium bromide and CuBr–dimethyl sulfide complex, was added to the acyloxazolidinone **6** to provide the methylated product **7** in more than 90% diastereoisomeric excess (de). The diastereoselective bromination of the dibutylboryl enolate derived from **7** with N-bromosuccinimide (NBS) followed by azide replacement with tetramethylguanidium azide (TMGA) afforded the desired compound **9** in excellent selectivity and isolated yield.

Removal of auxiliary of **9** with hydrogen peroxide mediated hydrolysis was followed by coupling with 3-(*S*)-fluoropyrrolidine, azide reduction and protection of the primary amine to give the corresponding aryl bromide **11**. Subsequent coupling of the aryl bromide **11** with aryl boronic acid via Suzuki coupling followed by deprotection of the *tert*-butylcarbamate then gave the desired compound **12q**.

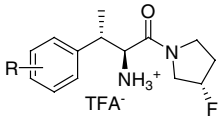
Inhibitors were tested for their selectivity profiles against a variety of DPP-IV homolog and proline-specific enzymes including quiescent cell proline dipeptidase (QPP/DPP-II), prolyl endopeptidase (PEP), amino peptidase P (APP), prolidase, DPP8, and DPP9.[7]

With the goal of finding analogs of 3-(*S*)-fluoropyrrolidide **4a** with increased potency,initially we looked at substitution on the phenyl ring in **4a**. Table 1 summarizes the DPP-IV inhibitory properties of these α-amino-acid pyrrolidides. Substitution at the 2- or 3-position on the phenyl ring had little effect on or was detrimental to activity.



Scheme 1. Reagents and conditions: (a) Et_3N, *t*-BuCOCl, THF; (b) *n*-BuLi, 4-(*R*)-4-phenyloxazolidinone, −78 °C to rt; (c) MeMgBr, CuBr–Me_2S, THF/Me_2S (1/1), −60 °C to rt ; (d) $(n\text{-Bu})_2$BOTf, DIEA, −78 °C, 1 h, then NBS, −78 °C to rt; (e) TMGA, CH_3CN, rt; (f) LiOH, H_2O_2, THF/H_2O; (g) EDC, HOBT, DIEA, (*S*)-3-fluoropyrrolidine, DMF; (h) PPh_3, THF/H_2O, 60 °C; (i) BOC_2O, $NaHCO_3$, 1,4-dioxane; (j) $PhB(OH)_2$, toluene, EtOH, 2 N aq Na_2CO_3, $Pd(dppf)_2Cl_2$, 90 °C; (k) TFA/CH_2Cl_2, 1 h.

Table 1. Inhibitory properties of selected DPP-IV inhibitors



Compd	R	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
4a	H	1.4	59	>100	>100
12a	2-F	1.1	19	>100	>100
12b	2-F, 4-Cl	0.48	5.6	>100	>100
12c	2-Cl, 4-F	4.2	46	>100	>100
12d	2-CF_3, 4-F	54	>100	>100	>100
12e	3-F	3.7	14	>100	>100
12f	3-Cl	1.1	5.7	>100	>100
12g	3,5-DiF	3.7	7.8	>100	>100
12h	3,4-DiF	1.1	11	>100	78
12i	3-Cl, 4-F	2.8	5.2	57	34
12j	4-F	0.28	23	>100	95
12k	4-Br	0.25	3.6	>100	>100
12l	4-Cl	0.17	5.9	>100	>100
12m	4-SCH_3	0.36	5.9	61	67
12n	4-CF_3	0.60	5.2	>100	>100
12o	4-SO_2Me	3.6	36	>100	>100
12p	4-OMe	0.77	14	>100	>100
12q	4-Ph	0.12	3.0	85	>100
12r	3-F, 4-Ph	2.0	48	>100	>100
12s	4-Piperidinyl	7.9	28	>100	>100
12t	4-(2-Pyrazoloyl)	1.7	1.5	63	>100
12u	4-(2-Thiazolyl)	1.5	0.72	>100	>100
12v	4-(2-Pyridyl)	0.24	4.6	>100	>100
12w	4-(3-Pyridyl)	0.11	4.8	>100	>100
12x	4-(4-Pyridyl)	0.11	3.1	>100	>100
12y	4-(3,5-Pyrimidinyl)	0.58	10.0	57	>100

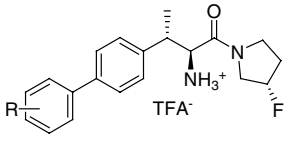
Lipophilic groups were well tolerated at the 4-position. Introduction of a fluorine at the 4-position of the phenyl ring increased potency by 5-fold (**12j**). An 8-fold increase in potency was achieved when a chlorine was introduced at the 4-position (**12l**). Incorporating a phenyl group at the 4-position of the phenyl ring increased the potency by 10-fold (**12q**). Replacement of the pendant phenyl group in **12q** with a heterocycle usually resulted in a decrease in potency. However, the potency of 3-pyridyl analog **12w** and 4-pyridyl analog **12x** was comparable to **12q**.

In order to further increase the potency and selectivity over QPP, we looked at substitution on the pendant phenyl group in the biphenyl lead **12q**. The results are summarized in Table 2. Substitution at the 2′-position on the pendant phenyl ring had little effect on the potency. Polar groups were well tolerated at the 3′-position. Incorporating a carboxylic acid group at this position (**20**) increased potency as well as selectivity (>170-fold window over inhibition of QPP). Analogs incorporating a polar group at the 4′-position were all less potent than **12q**. Introduction of a fluorine at the 4′-position of the phenyl ring increased potency by 2-fold (**25**).

Since polar groups were well tolerated at 3′-position, we decided to incorporate a heterocycle at that position to see if it could improve the potency and selectivity of this series. The results are summarized in Table 3. Neutral or basic heterocycles had little effect on potency. However, analogs incorporating an acidic heterocycle such as tetrazole or 5-oxo-1,2,4-oxadiazole exhibited improved potency and selectivity (**43** and **44**).

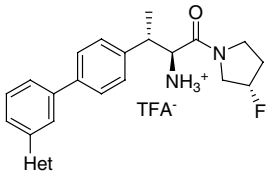
Representative analogs were selected for evaluation of pharmacokinetic properties in the rat and possible ion channel activity as a measure of general off-target activity (Table 4). The latter is illustrated here with binding to the hERG potassium channel.[8] Compound **25** exhibited excellent pharmacokinetic properties in rats (F = 85%). However, it had demonstrable ion channel binding (hERG binding K_i = 1100 nM). Analogs incorporating a polar group at the 3′-position generally exhibited enhanced selectivity over QPP and ion channel binding. However, incorporation of the polar functionality was

Table 2. Inhibitory properties of selected DPP-IV inhibitors



Compd	R	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
12q	H	0.13	3.0	85	>100
13	2-F	0.10	1.1	>100	>100
14	2-Cl	0.086	0.38	>100	>100
15	2-OMe	0.11	3.8	>100	>100
16	2-CF_3	0.13	2.5	>100	>100
17	2-Me	0.15	2.5	>100	>100
18	3-CO_2Et	0.075	0.49	>100	>100
19	3-$NHSO_2$Me	0.15	0.25	>100	>100
20	3-COOH	0.056	9.2	>100	>100
21	3-SO_2Me	0.11	2.9	23	45
22	3-SO_2NH_2	0.14	3.6	>100	>100
23	3-$NHSO_2CF_3$	0.027	1.3	>100	59
24	3-$CONH_2$	0.10	0.82	39	37
25	4-F	0.064	2.7	88	86
26	4-CF_3	0.51	0.67	64	>100
27	4-OMe	0.13	0.33	74	>100
28	4-Me	0.39	1.1	77	>100
29	4-$NHSO_2$Me	0.71	1.8	>100	>100
30	4-CO_2Et	0.44	0.12	>100	>100
31	4-CO_2H	0.37	8.6	>100	>100
32	4-SO_2Me	0.42	1.2	>100	>100
33	3,5-DiF	0.24	2.0	9.8	79
34	2,5-DiF	0.15	1.3	>100	>100
35	3,4-DiF	0.06	2.0	42	48
36	2,4-DiF	0.037	2.5	>100	>100

Table 3. Inhibitory properties of selected DPP-IV inhibitors



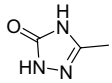
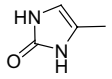
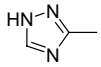
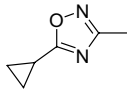
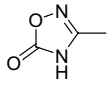
Compd	R	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
37		0.14	0.87	58	>100
38		0.069	0.96	>100	>100
39		0.095	1.1	47	56
40		0.11	0.83	17	57
41		0.098	0.35	>100	>100
42		0.14	0.096	55	>100
43		0.006	2.9	>100	52
44		0.003	1.1	>100	>100

Table 4. Pharmacokinetic properties of selected DPP-IV inhibitors in the rat (1/2 mpk iv/po) and hERG binding

Compd	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)	hERG IC_{50} (μM)
20	50.8	0.4	2.3	>100
25	8.6	2.2	85	1.1
43	132.4	0.3	3.5	59
44	23.9	1.1	4.2	28

detrimental to pharmacokinetic properties as evidenced by the extremely poor oral bioavailability of acid **20** and heterocycles **43** and **44**.

In summary, we have discovered a novel series of potent and selective DPP-IV inhibitors. These are among the most potent compounds reported to date lacking an electrophilic trap. The most potent compound among these is heterocycle **44**, which is a 3 nM DPP-IV inhibitor with good selectivity over related proline peptidases and ion channel activity. However, this compound lacks oral bioavailability in rats. More lipophilic compounds such as **25** exhibited excellent oral bioavailability in rats, but suffered from poor selectivity over QPP and ion channel activity. These preliminary results suggest that there is a fine balance among lipophilicity, selectivity, and oral bioavailability. Incorporation of a polar group at the β-position led to further optimization of these properties. These results will be described in the due course.

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7. (a) None of the compounds in this report showed any significant inhibition against APP, PEP, and prolidase (IC_{50}s > 10,000 nM). For assay conditions for DPP-IV and QPP inhibition, see: 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) All multiple determinations of the IC_{50} values were with 1.4-fold of the reported average; (c) IC_{50} determinations for hERG were carried as described in Lankas, G.; Leiting, B.; Sinha Roy, R.; Eiermann, G.; Biftu, T.; Kim, D.; Ok, H.; Weber, A. E.; Thornberry, N. A. Inhibition of DPP8/9 Results in Toxicity in Preclinical Species: Potential Importance of Selective Dipeptidyl Peptidase IV Inhibition for Treatment of Type 2 DM. Presented at the American Diabetes Association 64th Scientific Session, Orlando, FL, June, 2004, Abstract 7-OR.
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