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Discovery of potent, selective, and orally bioavailable oxadiazole-based dipeptidyl peptidase IV inhibitors

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Abstract—A novel series of oxadiazole based amides have been shown to be potent DPP-4 inhibitors. The optimized compound **43** exhibited excellent selectivity over a variety of DPP-4 homologs.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that are released from the gut during meals and serve as enhancers of glucose-dependent insulin release from pancreatic β cells.[1] Chronic infusion of GLP-1 to patients with type 2 diabetes resulted in significant decreases in both blood glucose and hemoglobin A_{1c} levels;[2] however, GLP-1 as well as GIP is rapidly degraded in plasma by the serine protease dipeptidyl peptidase IV (DPP-4). Inhibition of DPP-4 increases the levels of endogenous intact circulating GLP-1 and GIP. Consequently, the development of DPP-4 inhibitors is rapidly emerging as a novel therapeutic approach to the treatment of type 2 diabetes.[3]

Earlier reports from our laboratories described a series of (*R*)-β-homophenylalanine-based dipeptidyl peptidase-4 inhibitors lacking an electrophile.[4] Our efforts in this area culminated in the discovery of sitagliptin,[5] which has been accepted for standard review by the U.S. Food and Drug Administration (FDA). In an effort to discover a structurally diverse back-up compound, we designed and executed the initial SAR studies of a novel series of *anti* substituted biaryl β-phenylalanine-based DPP-4 inhibitors.[6] Further optimization of this series led to the discovery of compound **2b** (Fig. 1), a potent, orally active DPP-4 inhibitor with excellent selectivity, oral bioavailability in preclinical species, and in vivo efficacy in animal models.[7]

During the course of our investigation, we became interested in replacing the central phenyl group in biphenyl lead **1a** with a heterocycle. The work described here summarizes our initial efforts at optimizing the potency, selectivity, and oral bioavailability of the resulting novel series of oxadiazole-based DPP-4 inhibitors. Quite surprisingly, the potent diastereoisomer in this series has a *syn* relationship between the β-methyl and the primary amine as revealed by the X-ray crystal structure of **20**.

Inhibitors were prepared from the protected L-aspartic acid derivative **4**, and a representative example of the synthesis of these inhibitors is shown in Scheme 1. The route began with an EDC-mediated coupling of acid **4** with pyrrolidine to provide amide **5**. The addition of potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene) to a solution of **5** in THF at −78 °C followed by quenching with methyl iodide produced **6** as the major component of a 5:1 mixture of diastereoisomers. Sapon-

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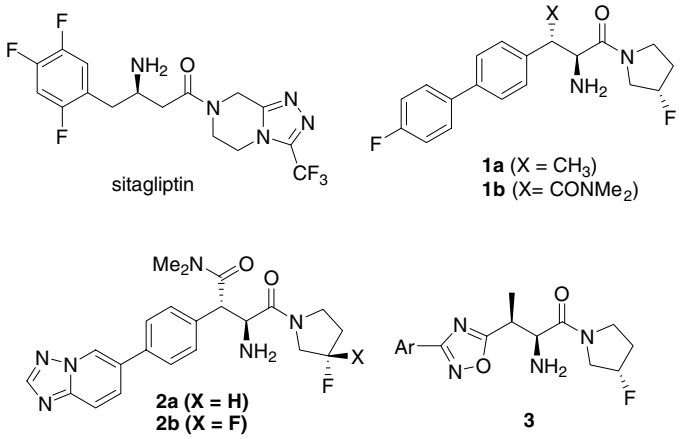
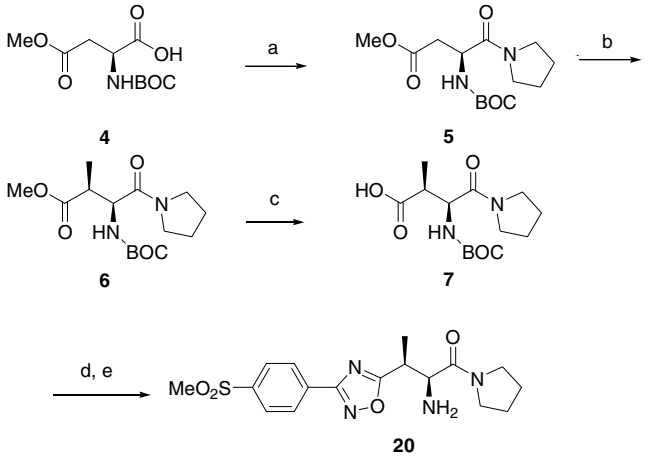


Figure 1. Lead DPP-4 inhibitors.



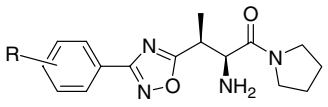
Scheme 1. Reagents and conditions: (a) EDC, HOBT, DIEA, pyrrolidine, DMF; (b) KHMDS, MeI, −78 °C; (c) LiOH, THF, H_2O; (d) CDI, 4-methanesulfonylbenzamidoxime, rt, 1 h, then 110 °C, 12 h; (e) TFA/CH_2Cl_2, 1 h.

ification of **6** with an aqueous solution of lithium hydroxide thus provided carboxylic acid **7**. Activation of acid **7** with 1,1-carbonyl diimidazole followed by addition of the substituted benzamidoxime and heating at 110 °C in DMF provided the desired product after Boc deprotection.[8]

Inhibitors were tested for their selectivity profiles against a variety of DPP-4 homologs and proline-specific enzymes including quiescent cell proline dipeptidase (QPP/DPP-II), prolyl endopeptidase (PEP), amino peptidase P (APP), prolidase, DPP8, and DPP9.[9] Since significant off-target activity was only observed with QPP (<10,000 nM), QPP data are presented for comparison.[10] Safety studies using a dual DPP8/DPP9 selective inhibitor suggest that inhibition of DPP8 and/or DPP9 may be associated with toxicity in preclinical species.[11] While the relevance of these findings to human toxicity is unknown, DPP8 and DPP9 binding data for these compounds are included for comparison.

The effect of substitution on the phenyl group was first examined. Table 1 summarizes the DPP-4 inhibitory

Table 1. Inhibitory properties of selected DPP-4 inhibitors



Compound	R	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
8	H	19.7	5.7	>100	>100
9	2-CHF_2	1.82	1.0	>100	>100
10	2-OCF_3	12.4	2.5	>100	>100
11	2-F	4.79	4.5	>100	>100
12	2-CF_3	4.82	1.8	>100	>100
13	2-Cl	0.99	2.2	>100	>100
14	3-CF_3	15.6	4.3	>100	>100
15	4-Cl	3.82	3.8	>100	>100
16	4-OCF_3	1.79	5.2	>100	>100
17	4-CF_3	1.61	6.0	>100	>100
18	4-SO_2CF_3	0.33	24.4	>100	>100
19	4-SO_2NH_2	0.19	26.8	>100	>100
20	4-SO_2Me	0.122	15.0	>100	>100
21	4-$NHSO_2Me$	0.52	5.4	>100	>100
22	3-Cl, 5-Cl	7.36	2.5	>100	>100
23	2-F, 4-F	4.59	8.1	>100	>100
24	2-Cl, 4-Cl	0.20	1.4	>100	>100
25	2-Cl, 4-Br	0.16	1.3	>100	>100
26	2-Cl, 4-F	0.83	3.0	>100	>100
27	2-Cl 4-$NHSO_2Me$	0.008	1.4	>100	>100
28	2-Cl, 4-SO_2Me	0.017	6.4	>100	>100
29	2-F, 4-SO_2Me	0.043	14.1	>100	>100

properties of these α-amino acid pyrrolidides. Introduction of a fluorine at the 2-position increased potency by 4-fold (**11**), while a 20-fold increase in potency was achieved when a chlorine was introduced at the 2-position (**13**). Substitution at the 3-position has little effect on potency. Introduction of a polar group at the 4-position improved potency dramatically. A 160-fold increase in potency was achieved when a methanesulfonyl group was incorporated at the 4-position (**20**). We also observed substantial additive effects of substitution at both the 2- and 4-position on the phenyl ring. A 2500-fold increase in potency relative to the unsubstituted phenyl group was achieved when a chlorine was introduced at the 2-position and a methanesulfonamide group was

introduced at the 4-position (**27**). Incorporating a methanesulfonyl group at the 4-position and a fluorine at the 2-position (**29**) improved not only the potency but also the selectivity (>320-fold window over inhibition of QPP).

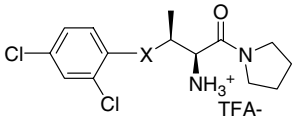
The effect of the central heterocycle on the potency and selectivity was next briefly explored. Table 2 summarizes the DPP-4 inhibitory properties of these α-amino acid pyrrolidides. Among the heterocycles we prepared, the 1,2,4-oxadiazole analog **24** gave the best overall potency and selectivity profile. Given also the ease of synthesis of 1,2,4-oxadiazoles, further optimization of potency, selectivity, and pharmacokinetic profiles was thus continued with the 1,2,4-oxadiazole series.

The effect of modification of the pyrrolidine amide was next explored (Table 3). In general, consistent with what we observed in the related α-amino acid series, the 3,3-difluoropyrrolidine analog **36** displayed markedly lower selectivity for inhibition of DPP-4 over QPP. The (*S*)- and (*R*)-3-fluoropyrrolidides **34** and **35** exhibited a moderate decrease in DPP-4 potency relative to pyrrolidide **27**. Since better pharmacokinetic profiles were observed with analogs which incorporated (*S*)-3-fluoropyrrolidine, further optimization was continued with compounds derived from (*S*)-3-fluoropyrrolidine.[12]

The effect of changing the β-substituent in this series was also explored (Table 4) In general, potency could be further improved by increasing the lipophilicity at the β-position as evidenced by compounds **38** and **39**. Since analogs incorporating cyclopropylmethyl group at the β-position exhibited much better improved pharmacokinetic properties relative to analogs with isobutyl group, the cyclopropylmethyl group was incorporated into other derivatives. The optimized compound, **43**, exhibited excellent selectivity over a variety of DPP-4 homologs.

Representative analogs were selected for in vivo evaluation of pharmacokinetic properties in rats and possible ion channel activity as a measure of potential off-target activity (Table 5). The latter is illustrated here with data for binding to the hERG potassium channel.[13] In general, these compounds exhibited excellent selectivity with regard to ion channel binding. However, with few exceptions, these compounds exhibited a relatively short half-life and moderate clearance in rats. The optimized com-

Table 2. Effect of changing the heterocycle



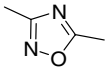
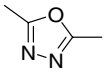
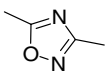
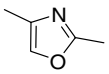
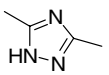
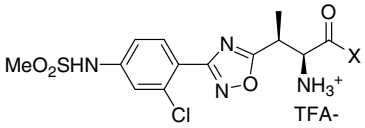
Compound	X	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
24		0.203	1.4	>100	>100
30		0.56	0.85	>100	>100
31		1.21	4.2	>100	>100
32		1.83	1.05	>100	>100
33		3.05	5.41	>100	>100

Table 3. Effect of changing the right-hand side amide



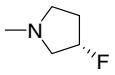
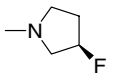
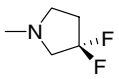
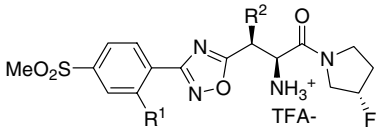
Compound	X	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
27		0.008	1.4	>100	>100
34		0.019	2.57	>100	>100
35		0.019	1.7	>100	>100
36		0.019	0.16	>100	>100

Table 4. Inhibitory properties of selected DPP-4 inhibitors



Compound	R^1	R^2	IC_{50}(μM)			
			DPP-4	QPP	DPP8	DPP9
37	Cl	Me	0.040	3.5	>100	>100
38	Cl	cPr-CH_2	0.013	1.7	>100	>100
39	Cl	iPr-CH_2	0.013	3.7	>100	>100
40	Cl	$(CH_2)_2OH$	0.070	44.8	>100	>100
41	H	cPr-CH_2	0.052	20.4	>100	>100
42	Me	cPr-CH_2	0.031	2.92	>100	>100
43	F	cPr-CH_2	0.019	17.6	>100	>100

Table 5. Pharmacokinetic properties of selected DPP-4 inhibitors in the rat (1 mpk iv/2 mpk po) and hERG binding

Compound	Clp (mL/min/kg)	$t_{1/2}$ (h)	*F* (%)	hERG IC_{50} (μM)
24	13.8	2.71	35	36.7
28	6.0	0.82	27	>100
41	11.2	1.8	49	>100
43	18.7	1.3	36	58.5

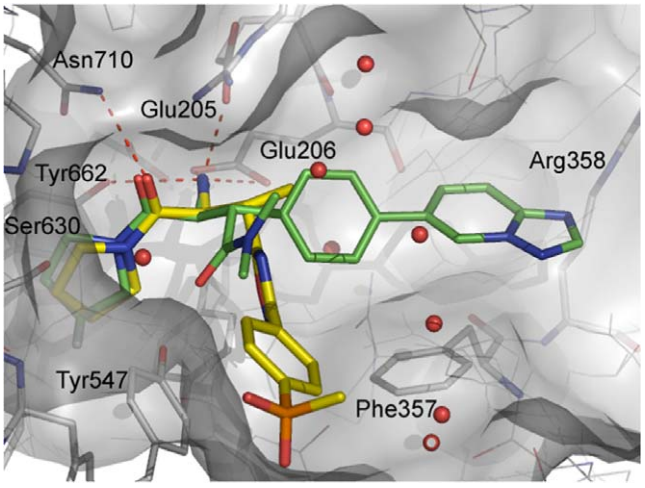


Figure 2. Compound **20** bound to DPP-4. The overlay of compound **20** (yellow) and **2a** (green, 2FJP.pdb) shows the different orientation of the two compounds. Interactions of compound **20** with DPP-4 are shown as red dotted lines. The hydrogen-bond network present between the ordered water molecules, compound **20**, and protein atoms has been omitted for clarity.

pound **43** displayed moderate oral bioavailability in rats ($F = 36\%$) and excellent oral bioavailability in dogs ($F = 95\%$). Unfortunately, **43** exhibited a short half-life in both rats ($t_{1/2} = 1.3$ h) and dogs ($t_{1/2} = 1.75$ h).

Co-crystallization of **20** with the DPP-4 enzyme indicates that the major interactions of compound **20** with DPP-4 are similar to those observed with the Val-Pro and Diprotin A substrate analogs and several other classes of inhibitors reported to date (Fig. 2).[14] The pyrrolidine moiety is located in the P-1 site, adjacent to the catalytic Ser630. The α-amino acid group forms four hydrogen-bond interactions with the side chains of Glu205, Glu206, Tyr662, and Asn710. The major difference between the binding mode of compound **20** and that of other amino-acid derived compounds such as **2** is that it does not extend across the binding site to interact with Arg358. The methylsulfonylphenyl ring stacks against the side chain of Tyr547, and the methylsulfonyl group extends toward a polar surface in an area of the binding site that has not been utilized by any previously reported compounds, although aminomethylpyrimidines have been shown to extend in the same general direction.[14h] The stereochemistry at the β-position is critical for the binding potency, since the corresponding *anti*-diastereoisomer is typically 10-fold less potent than the *syn*-diastereoisomer in this series. The difference in binding modes accounts for the switch in stereochemical preference observed in this series (*syn* over *anti*) relative to the biaryl derivatives **1** and **2**.

In summary, we have discovered a novel series of potent, selective, and orally bioavailable DPP-4 inhibitors. These are among the most potent compounds reported to date lacking an electrophilic trap. The optimized compound **43** exhibited excellent selectivity over a variety of DPP-4 homologs. However, further development of this compound was not pursued due to the short half-life observed upon oral administration in both rats and dogs.

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