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Discovery of potent and selective orally bioavailable β-substituted phenylalanine derived dipeptidyl peptidase IV inhibitors

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Abstract—*anti*-Substituted biaryl β-methylphenylalanine derived amides have been shown to be potent DPP-IV inhibitors that suffer from suboptimal selectivity and pharmacokinetics. This letter describes the substitution of the β-methyl substituent with β-polar substituents, culminating in the discovery of a β-dimethylamide substituted phenylalanine derivative with an excellent potency, selectivity, and pharmacokinetic profile.

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 incretin hormones 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. Consequently, inhibition of DPP-IV in humans has been shown to increase circulating GLP-1 and GIP levels, which 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.

Previous work from these laboratories describes the conversion of a moderately potent and poorly selective dipeptidyl peptidase IV (DPP-IV) inhibitor (**1**) to potent and selective β-methylphenylalanine derived DPP-IV inhibitors with the general structure **2** (Fig. 1).[5] The conversion of the ethyl group of **1** to a biaryl group (**2**) increased potency against DPP-IV as well as selectivity over DPP8 and DPP9. Furthermore, the incorporation of polar aryl groups into **2** further increased DPP-IV potency and provided additional selectivity over off-target enzymes (e.g., quiescent peptidyl peptidase—QPP) and hERG.[6] Unfortunately, the latter benefits were achieved at the expense of inferior rat pharmacokinetic profiles. Previous work in this series demonstrated that more sterically demanding groups could be substituted for the β-methyl group of **2** without sacrificing potency at

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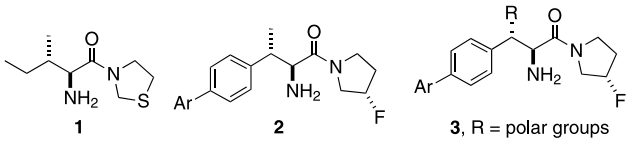
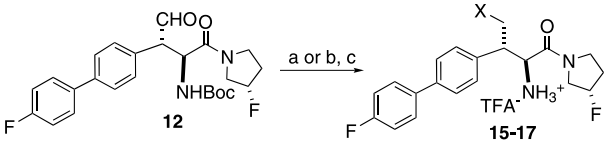


Figure 1. Classic DPP-IV inhibitor isoleucyl thiazolidide (**1**) and phenylalanine derived DPP-IV inhibitors.



Scheme 2. Synthesis of DPP-IV inhibitors **15**–**17**. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) $NaCNBH_3$, $(ClCH_2)_2$, AcOH, R_2NH; (c) TFA, DCM.

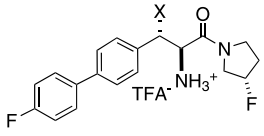
DPP-IV (data not shown). We hoped to introduce more polar substituents at the β-position (**3**) in order to improve the potency and selectivity profile of this promising new series of DPP-IV inhibitors.

Inhibitors were synthesized using an effective combination of a chiral CBS reduction with a Kazmaier–Claisen rearrangement (Scheme 1). The route begins with a Horner–Emmons reaction of aldehyde **4** to give the corresponding methyl styrenone. A chiral reduction of this enone with the (*R*)-2-methyl-CBS-oxazaborolidine reagent (*R*-CBS) then afforded allylic alcohol **5** in excellent yield and enantioselectivity (typically 80–90% ee).[7] The optical activity could be further enriched (>98% ee) by recrystallization from cyclohexane. Next, the allylic alcohol was coupled with Boc-glycine to give **6**. Exposure of **6** to Kazmaier's enolate-Claisen rearrangement conditions[8] followed by esterification of the resulting acid with trimethylsilyl diazomethane then afforded *N*-Boc amino ester **7** in excellent yield and stereoselectivity. Hydrolysis of the ester was followed by coupling with (*S*)-3-fluoropyrrolidine[9] to give the corresponding amide. Subsequent coupling of the aryl bromide with aryl boronic acids via Suzuki coupling then gave **8**. Ozonolysis of **8** followed by oxidation of the resulting aldehyde yielded acid **10**, which could be deprotected to afford analog **18**.[10] Alternatively, **10** could be coupled with amines then deprotected to afford amides **20**–**22** and **24**–**53**. The primary amide (**11**, *R*, *R*′ = H) was dehydrated to the nitrile, which was then converted to the corresponding tetrazole and deprotected to afford **23**.

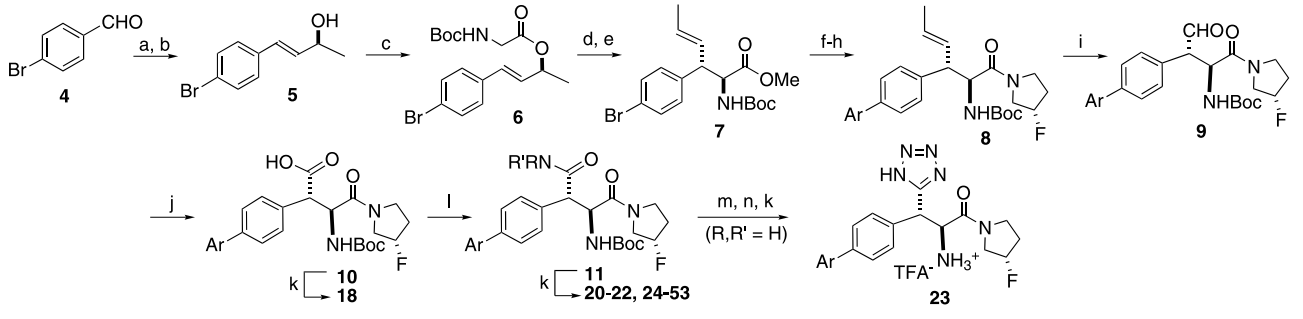
Additional analogs were synthesized from aldehyde **12** by reduction to the corresponding alcohol followed by deprotection to give **15** (X = OH, Scheme 2). Alternatively, reductive amination of **12** followed by deprotection afforded diamines **16** and **17**.

Clearly, the β-substituent of biaryl phenylalanines plays a significant role in DPP-IV potency and selectivity (Table 1). Unsubstituted biaryl phenylalanine **13**, for example, was more than an order of magnitude less potent against DPP-IV than the β-methyl analog **14**.[5] Potency could be further improved by changing the β-methyl substituent from analog **14** to a carboxylic acid moiety. Acid **18** (DPP-IV IC_{50} = 6.6 nM) was 10 times more potent against DPP-IV and showed a superior overall selectivity profile (Table 1).[11] Similarly, dimethylamide

Table 1. Effects of polar substituents at the β-position of biaryl phenylalanines



Compd	X =	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
13	H	0.98	>100	>100	>100
14	Me	0.064	2.7	87	86
15	CH_2OH	0.39	15	>100	>100
16	CH_2NMe_2	1.6	2.5	16	>100
17	$CH_2N(CH_2)_4$	3.5	0.7	49	>100
18	CO_2H	0.0066	>100	>100	>100
19	CO_2Me	0.22	21	>100	>100
20	$CONH_2$	0.11	>100	>100	>100
21	CONHMe	0.037	>100	41	>100
22	$CONMe_2$	0.012	45	>100	69
23	Tetrazole	0.192	>100	>100	>100



Scheme 1. Enantioselective synthesis of *anti*-substituted β-polar biaryl phenylalanine derived DPP-IV inhibitors. Reagents and conditions: (a) $(EtO)_2POCH_2COCH_3$, DBU, THF; (b) *R*-CBS, Catecholborane, tol, −78 → −30 °C; (c) EDC, HOBt, Boc-Gly, DIEA, DCM; (d) LHMDS, $ZnCl_2$, THF, −78 °C; (e) $TMSCHN_2$, Et_2O, MeOH; (f) LiOH, H_2O, THF, MeOH; (g) EDC, (*S*)-3-fluoropyrrolidine, HOBt, DIEA, DCM; (h) $ArB(OH)_2$, tol, EtOH, 2 N Na_2CO_3, $Pd(dppf)_2Cl_2$, Δ; (i) O_3, MeOH, DCM then Me_2S; (j) $NaClO_2$, H_2O, *t*-BuOH, isobutylene; (k) TFA, DCM; (l) EDC, HOBt, RR'NH, DIEA, DCM; (m) cyanuric chloride, DMF; (n) NaN_3, tol, Et_3N–HCl, Δ.

22 (DPP-IV IC_{50} = 12 nM) showed improved potency and overall selectivity compared to **14**. With the exception of these two analogs and monomethylamide **21**, the remainder of the β-polar groups gave analogs with reduced potency and/or selectivity against DPP-IV. Consequently, an investigation of various amide groups at the β-position was initiated.

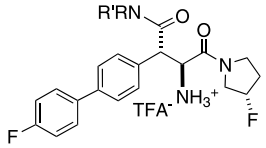
With the exception of aminotetetrazole **28**, tertiary amides at the β-position were more potent against DPP-IV than secondary amides (Table 2). Tetrazole **28** was equipotent to **22**, but suffered from a drop in selectivity against DPP8. Pyrrolidide amide **32** proved to be the most potent of the tertiary amides (DPP-IV IC_{50} = 9.2 nM), but this analog also showed reduced selectivity against all of the counterscreens. Attention was next focused on replacing the fluoropyrrolidide moiety, which is an effective P_1-site proline mimic for DPP-IV.[9,12]

In general, only small changes were tolerated at the P_1-site (Table 3). While four- and five-membered rings each showed good activity against DPP-IV, a substantial drop in potency was observed with six-membered rings (DPP-IV IC_{50} >500 nM for **44** and **45**). Pyrrolidide **38**, thiazolidide **41**, and cyanopyrrolidide **43** had comparable or increased potency relative to **22**, but each of these analogs exhibited increased inhibition of DPP8 and DPP9. Especially noteworthy was the very potent cyanopyrrolidide **43** (DPP-IV IC_{50} = 3.3 nM), which shows significant activity at both DPP8 (IC_{50} = 2.1 μM) and DPP9 (IC_{50} = 2.2 μM). Since activity in these homologs has been correlated with toxicity in animals,[13] further pursuit of this analog was halted. Also noteworthy was fluoroazetidide **36**, which was similar to **22** with respect to DPP-IV potency and selectivity.

Because the introduction of polar biaryl groups afforded increased potency and selectivity in the β-methyl series,[5] we decided to incorporate some of the improved aryl groups into the β-dimethylamide scaffold (Table 4). As expected, polar aryl groups afforded compounds with comparable or improved selectivity profiles. Most noteworthy among these analogs were tetrazole **50**, methylsulfone **51**, and 4-pyridyl analog **53** (IC_{50}'s = 2.2, 21, and 20 nM, respectively). Each of these analogs shows acceptable selectivity profiles in the counterscreens.

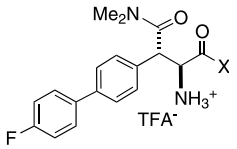
Inhibitors that possessed superior potency and selectivity profiles were evaluated for their rat pharmacokinetic

Table 2. Effects of amide groups at the β-position of biaryl phenylalanines



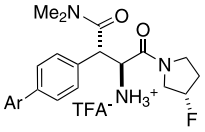
Compd	–NRR′ =	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
24	–NHEt	0.11	90	>100	>100
25	–NH*i*-Pr	0.099	76	26	>100
26	–NH*c*-Pr	0.071	55	26	>100
27	$-NH(CH_2)_2OH$	0.094	>100	32	>100
28	–NHtetrazole	0.011	>100	19	>100
29	–NMeEt	0.020	43	18	>100
30	$-NEt_2$	0.059	48	52	>100
31	$-N(CH_2)_3$	0.044	5.3	47	>100
32	$-N(CH_2)_4$	0.0092	5.6	15	62
33	–NMe(OMe)	0.045	>100	27	>100
34	$-NHNMe_2$	0.067	>100	22	>100

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



Compd	X =	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
35	$-N(CH_2)_3$	0.10	>100	>100	>100
36		0.017	58	>100	>100
37		0.029	9.4	>100	>100
38	$-N(CH_2)_4$	0.0075	26	22	66
39		0.030	94	37	>100
40		0.027	7.1	8.0	46
41		0.0091	8.5	13	26
42		0.230	75	>100	>100
43		0.0033	40	2.1	2.6
44	$-N(CH_2)_5$	0.55	38	>100	>100
45		0.52	77	>100	>100

Table 4. Influence of various biaryl groups



Compd	Ar =	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
46	2,4-F_2Ph	0.013	15	40	>100
47	3,4-F_2Ph	0.021	44	14	81
48	2,5-F_2Ph	0.056	11	6.9	>100
49	3-CNPh	0.043	57	0.46	57
50	3-(Tetrazole)Ph	0.0022	29	>100	>100
51	3-($MeSO_2$)Ph	0.021	81	>100	>100
52	3-pyr	0.019	57	12	>100
53	4-pyr	0.020	89	>100	100

Table 5. 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)
18	0.25	3.1	16	76
22	4.8	3.5	67	4.6
28	55	0.20	<1	41
32	18	1.7	21	1.5
38	16	2.0	100	1.2
50	18	0.74	<1	>100

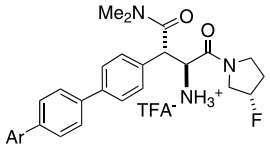
properties (Table 5). Activity at hERG was also measured as an indicator of general off-target activity.[6,14] Although **22** showed moderate activity against hERG, this compound displayed excellent oral bioavailability (67%) and half-life (3.5 h) in the rat. More potent compounds such as **18**, **28**, and **50** all possess low bioavailabilities compared to **22**. Analogs **32** and **38** are roughly equipotent to **22** and display acceptable pharmacokinetic profiles, but each of these compounds was inferior in terms of selectivity against DPP8, DPP9, and hERG. Consequently, **22** was chosen for further evaluation (Table 6). We were pleased to find that this compound displayed excellent oral bioavailabilities and half-lives in dogs and rhesus monkeys (Table 7).

An oral glucose tolerance test (OGTT) was used to assess the ability of **22** to improve glucose tolerance in mice. In lean animals, **22** was orally administered 1 h prior to dextrose challenge and significantly reduced blood glucose excursion in a dosage-dependent manner from 0.1 mg/kg (21% reduction) to 3.0 mg/kg (64% reduction). In the corresponding pharmacodynamic (PD) assay, compound-mediated DPP-IV inhibition and plasma compound concentrations were dosage-dependent 10 min following dextrose challenge. At the 1.0 mg/kg dosage, the plasma concentration of **22** was 940 nM and plasma DPP-IV activity was inhibited by >75%.[15] The administration of **22** at dosages from 1.0 to 3 mg/kg also significantly increased active GLP-1 plasma concentrations in this assay. The observed levels of DPP-IV inhibition in the PD assay did not, however, correspond to the expected efficacy of **22** in light of the mouse DPP-IV potency (murine IC_{50} = 50 nM).

In order to resolve this apparent disconnect between efficacy and in vitro potency, the potency of **22** against mouse and human DPP-IV was measured in the presence of increasing amounts of mouse and human serum, respectively (Table 7). Potency against mouse DPP-IV steadily dropped with increasing serum concentrations, displaying an IC_{50} = 230 nM at 50% mouse serum. This serum shift was attributed to high levels of non-covalent plasma protein binding. Protein binding was even more pronounced in human serum, with an IC_{50} = 387 nM at 50% human serum. The measured potency of **22** in mouse in the presence of 50% serum (IC_{50} = 230 nM) agrees well with the pharmacodynamic study described above. Thus, the high levels of protein binding observed with **22** explain the apparent disconnect between efficacy and plasma drug levels observed in the mouse pharmacodynamic model.

In conclusion, optimization of *anti*-substituted β-polar substituents of biarylphenylalanine derived DPP-IV inhibitors led to the discovery of the potency and selectivity enhancing dimethylamide group at the β-position. Further investigation into pyrrolidine right-hand side replacements and biaryl left-hand side substituents led to the discovery of a series of highly potent and selective phenylalanine derived DPP-IV inhibitors. Since compound **22** exhibits the best balance of potency, selectivity, and rat pharmacokinetics of this series, this compound was profiled further. Compound **22** possesses an excellent pharmacokinetic profile in three species and excellent in vivo efficacy in a lean mouse OGTT. Nevertheless, the off-target ion channel activity at hERG combined with the observed serum shift of **22** has prompted further investigation into optimization of this series of inhibitors.

Table 6. Pharmacokinetic properties of **22** in the dog and rhesus monkey (1/2 mpk iv/po)



Species	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)
Dog	1.5	6.1	90
Rhesus	2.4	4.7	56

Table 7. Potency of **22** in the presence of increasing amounts of mouse and human serum (mouse and human DPP-IV IC_{50}'s are given in nM)

Species	0% Serum	3% Serum	15% Serum	50% Serum	80% Serum
Mouse	—	50	71	230	—
Human	12	18	39	387	376

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12. SAR at the P_1 position of dipeptide α-amino amide inhibitors has been investigated with DPP-IV and QPP (DPP-II). For a lead reference, see: Senten, K.; Veken, P. V.; De Meeser, I.; Lambeir, A.-M.; Scharpe, S.; Haemers, A.; Augustyns, K. *J. Med. Chem.* **2003**, *46*, 5005.
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14. IC_{50}'s against human calcium and sodium channels were also measured, and activity at hERG is indicative of activity at these other ion channels.
15. It should be noted that the % inhibition as determined using the in vitro assay underestimates the % inhibition achieved in vivo, since compound **22** is a competitive, reversible inhibitor and the assay of plasma DPP-IV activity requires dilution of plasma (resulting in dilution of the total inhibitor) and the presence of substrate that competes with inhibitor for binding to the enzyme.