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Discovery of potent, selective, and orally bioavailable pyridone-based dipeptidyl peptidase-4 inhibitors

Jinyou Xu,[a,*] Lan Wei,[a] Robert Mathvink,[a] Scott D. Edmondson,[a] Anthony Mastracchio,[a] George J. Eiermann,[c] Huaibing He,[a] Joseph F. Leone,[a] Barbara Leiting,[b] Kathryn A. Lyons,[a] Frank Marsilio,[b] Reshma A. Patel,[b] Aleksandr Petrov,[c] Joseph K. Wu,[b] Nancy A. Thornberry[b] and Ann E. Weber[a]

[a]*Department of Medicinal Chemistry, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Metabolic Disorders, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Pharmacology, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*

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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. The optimized compound exhibited good pharmacokinetic profiles in three preclinical species.

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-stimulated 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 4 (DPP-4). Inhibition of DPP-4 increases the levels of endogenous intact circulating GLP-1 and GIP. Consequently, inhibition of DPP-4 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 that area culminated in the discovery of sitagliptin, which is currently in the phase III clinical trials.[5] In an effort to discover a structurally diverse back-up compound to sitagliptin, we designed and executed the initial SAR studies of a novel series of *anti*-substituted biaryl

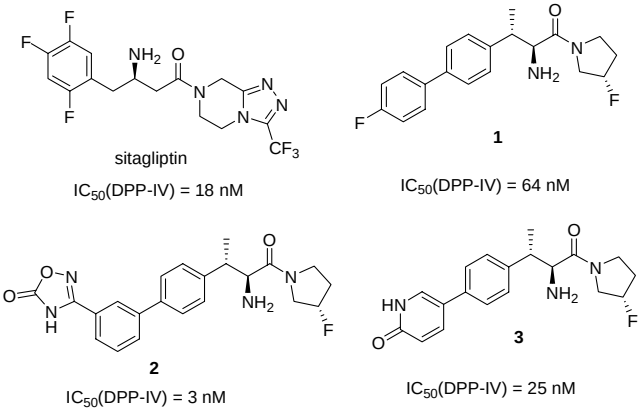

Figure 1. Lead DPP-4 inhibitors.

β-methylphenylalanine-based dipeptidyl peptidase-4 inhibitors.[6] While lipophilic compounds in this series such as **1** (Fig. 1) exhibited good oral bioavailability in rats, they usually had poor selectivity over off-target enzymes such as quiescent cell proline dipeptidase (QPP) and hERG.[7] Incorporation of an acidic heterocycle at the 3′-position on the pendant phenyl group in **1** increased potency against DPP-4 as well as selectivity over QPP and hERG. However, incorporation of the polar functionality was detrimental to pharmacokinetic prop-

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* Corresponding author. Tel.: +1 732 594 2217; e-mail: Jinyou_ Xu@merck.com

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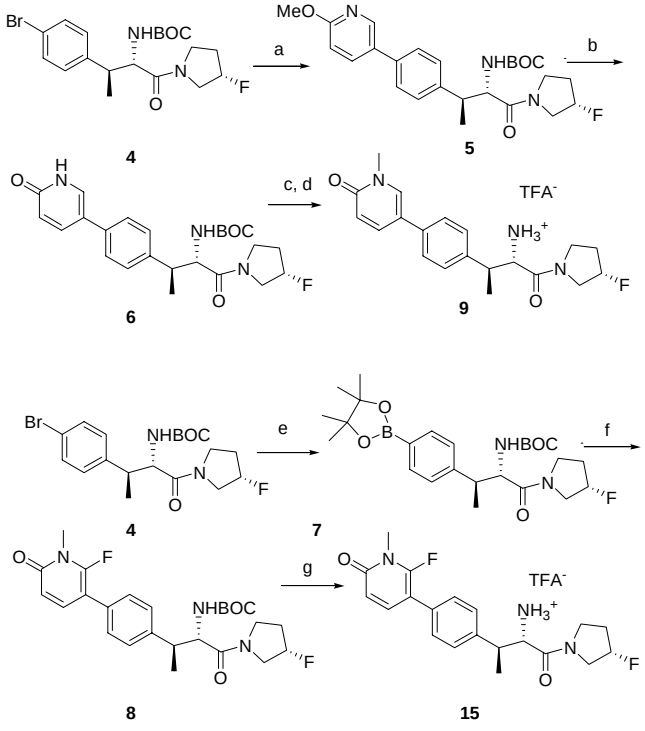
erties as evidenced by the extremely poor oral bioavailability of 5-oxo-1,2,4-oxadiazole **2**.[6] These preliminary results suggest that there is a fine balance among lipophilicity, selectivity, and oral bioavailability.

Replacement of the β-methyl group in **1** with a polar substituent such as dimethylamide improved the potency, selectivity, and pharmacokinetic profile of this promising new series of DPP-4 inhibitors. However, these compounds still suffered from poor selectivity over hERG and a large serum shift.[8] During the course of our investigation, we discovered pyridone **3**, which exhibited a promising selectivity and pharmacokinetic profile. The work described here summarizes our initial efforts at optimizing the potency, selectivity, and oral bioavailability of this novel series of pyridone-based DPP-4 inhibitors. The optimized compound (**9**) exhibited good pharmacokinetic profiles in three preclinical species.

The β-methylphenylalanine-based DPP-4 inhibitors were prepared from the known aryl bromide **4** (Scheme 1).[6] The route began with a Suzuki coupling reaction of aryl bromide **4** with 4-methoxy-3-pyridinylboronic acid to give biaryl compound **5**. Demethylation of **5** with pyridine hydrochloride followed by selective N-methylation and deprotection of the *tert*-butylcarbamate thus provided pyridone **9**. Alternatively, the aryl bromide **4** could be first converted to boronate **7**, which was subsequently coupled to various aryl bromides as illustrated by the synthesis of pyridone **15**.



Scheme 1. Reagents and conditions: (a) $ArB(OH)_2$, toluene, EtOH, 2 N Na_2CO_3, $Pd(dppf)_2Cl_2$, 90 °C; (b) PyHCl, 190 °C; (c) Cs_2CO_3, MeI, DMF; (d) TFA/CH_2Cl_2, 1 h; (e) bis(pinacolato)diboron, $Pd(dppf)_2Cl_2$, KOAc, DMF, 80 °C; (f) ArBr, toluene, EtOH, 2 N Na_2CO_3, $Pd(dppf)_2Cl_2$, 90 °C; (g) TFA/CH_2Cl_2, 1 h.

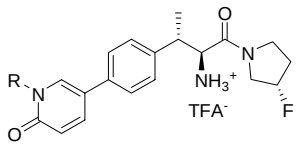
Inhibitors were tested for their selectivity profiles against a variety of DPP-4 homologs and proline-specific enzymes including quiescent cell proline dipeptidase (QPP/DPP-II), prolyl endopeptidase (PEP), amino peptidase P (APP), prolidase, DPP8, and DPP9. Since significant off-target activity was only observed with QPP (<10,000 nM), QPP data are presented for comparison.[9] 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.[10] Thus, DPP8 and DPP9 data are also included for safety reasons.

With the goal of finding analogs of pyridone **3** with increased potency and selectivity, initially we looked at the effect of substitution on the pyridone nitrogen in **3**. Table 1 summarizes the DPP-4 inhibitory properties of these α-aminoacid pyrrolidides. Unfortunately, substitution on the nitrogen was not tolerated as the potency decreased as the size of R increased. While *N*-methyl pyridone **9** was slightly less potent than the unsubstituted analog **3**, it showed improved PK properties and selectivity over ion channels (see below). Thus, further SAR studies were conducted on this lead.

To further increase the potency and selectivity over QPP, we next looked at substitution on the pendant pyridone group in lead **9**. The results are summarized in Table 2. Substitution on the heteroaryl ring usually had little effect or was detrimental to activity. Interestingly, introduction of a bulky substituent at 6-position usually improved the selectivity over QPP. Especially noteworthy was compound **22** which exhibited excellent selectivity over QPP.

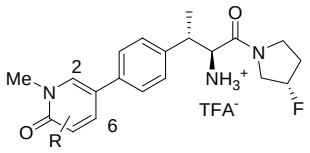
The effect of changing the right-hand side amide in this series was next explored. In general, consistent with what we observed in the related α-amino acid series, only small changes were tolerated at the P_1-site (Table 3).[8,11] While 4- and 5-membered rings were well tolerated, a substantial drop in potency against DPP-4 was observed with the 6-membered ring compound **30**.

Table 1. Effect of substitution on the pyridone nitrogen



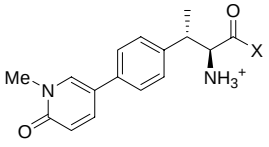
Compound	R	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
3	H	0.025	5.0	>100	>100
9	Me	0.034	8.0	>100	>100
10	Et	0.074	6.1	>100	>100
11	cPr-CH_2	0.117	3.9	>100	>100
12	CH_2CO_2Et	0.090	5.9	>100	>100
13	CF_2CO_2Et	0.092	2.5	>100	>100
14	4-FPh	0.229	2.1	>100	>100

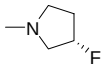
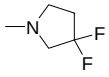
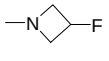
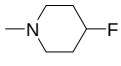
Table 2. Inhibitory properties of selected DPP-4 inhibitors



Compound	R	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
9	H	0.034	8.0	>100	>100
15	2-F	0.029	14	>100	>100
16	2-Me	0.32	6.2	>100	>100
17	2-Me, 5-Br	0.57	9.0	>100	>100
18	5-F	0.049	7.1	41.3	>100
19	5-Br	0.069	7.4	>100	>100
20	6-F	0.061	5.5	>100	>100
21	6-Me	0.052	15	>100	>100
22	6-CH_2OH	0.038	>100	>100	>100
23	6-OMe	0.067	24	>100	>100
24	5-Cl, 6-F	0.037	4.5	>100	>100
25	5-F, 6-$NHCO_2Et$	0.12	8.5	>100	59

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



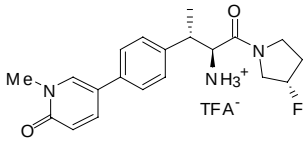
Compound	X	IC_{50} (μM)			
		DPP-IV	QPP	DPP8	DPP9
9		0.034	8.0	>100	>100
26		0.026	0.55	>100	>100
27		0.048	3.9	>100	>100
28		0.026	0.81	>100	>100
29		0.031	16	>100	>100
30		1.1	1.1	>100	>100

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.[7] Although **22** is the most selective compound in this series, it exhibited poor oral bioavailability. The most potent compound in this series, **3**, had demonstrable ion channel binding (hERG binding K_i = 8300 nM). Pyridone **9** exhibited excellent selectivity over ion channel binding and good oral bioavailability in rat. Consequently, **9** was chosen for further evaluation (Table 5). We were pleased to find that **9** showed excellent oral bioavailability in dogs and monkeys (95% and 51%, respectively). An oral glucose tolerance test (OGTT) was used to assess the ability of **9** to improve glucose tolerance in mice. In lean animals, **9** was orally administered 1 h prior to dextrose challenge and reduced blood glucose excursion in a dosage-dependent manner from 1 mg/kg (33% reduction) to 10 mg/kg (56% reduction).

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

Compound	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)	hERG IC_{50} (μM)
3	35.8	1.9	10	8.3
9	7.7	1.4	34	>100
15	11.2	1.1	61	>100
22	68.1	2.6	3	>100

Table 5. Pharmacokinetic properties of compound **9** in the dog and rhesus monkey (1/2 mpk iv/po)



Species	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)
Dog	4.7	3.8	95
Rhesus	6.7	3.9	50

In summary, we have discovered a novel series of potent and selective DPP-4 inhibitors. The optimized compound **9** exhibited good pharmacokinetic profiles in three preclinical species. In vitro and in vivo metabolism studies revealed that N-demethylation occurred, leading to the formation of the initial lead compound **3**, which had unacceptable hERG binding. Replacement of the *N*-methyl pyridone with a less metabolically labile group and improving the potency of **9** will be the focus of future work in this series. These results will be reported in due course.

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