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Discovery of 3-aminopiperidines as potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitors

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Abstract—Substituted 3-aminopiperidines **3** were evaluated as DPP-4 inhibitors. The inhibitors showed good DPP-4 potency with superb selectivity over other peptidases (QPP, DPP8, and DPP9). Selected DPP-4 inhibitors were further evaluated for their hERG potassium channel, calcium channel, Cyp2D6, and pharmacokinetic profiles.

Type 2 diabetes mellitus is a growing health problem, with an estimated 170 million people worldwide who suffer from the disease.[1] Upon ingestion of food, the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released and regulate insulin secretion in a glucose-dependent fashion.[2] Infusion of GLP-1 was shown to stimulate insulin biosynthesis, inhibit glucagon secretion, slow gastric emptying, reduce appetite, and stimulate regeneration and differentiation of islet β-cells. Unfortunately, due to rapid (<2 min) inactivation of GLP-1 by the serine protease dipeptidyl peptidase IV (DPP-4), the reduction of blood glucose and hemoglobin A_{1c} levels can only be achieved by constant infusion of GLP-1.[2b] Inhibition of DPP-4 leads to an increased level of endogenous GLP-1 and GIP, allowing their therapeutic benefits to be realized.[2,4] Furthermore, DPP-4 inhibitors are a clinically proven, novel therapeutic approach to the treatment of type 2 diabetes.[3]

Initial work from our laboratories focused on the development of DPP-4 inhibitors lacking an electrophilic nitrile functionality. These efforts culminated in the discovery of sitagliptin **1** (DPP-4 IC_{50} 18 nM),[5] the first DPP-4 inhibitor approved by the FDA for the treatment of type 2 diabetes (Fig. 1). In a continued search for structurally diverse inhibitors,[6] DPP-4 inhibitor **2** (DPP-4 IC_{50} 21 nM) was designed using the sitagliptin X-ray crystallographic structure along with molecular modeling.[7] Replacement of the central cyclohexylamine in **2** with a 3-aminopiperidine led to the discovery of 3-aminopiperidines **3** as potent, selective, and orally bioavailable DPP-4 inhibitors.[8]

The synthesis of 3-aminopiperidine-based DPP-4 inhibitors commenced with a Horner–Wadsworth–Emmons reaction of aldehyde **4** (Scheme 1). [9] Subsequent Michael

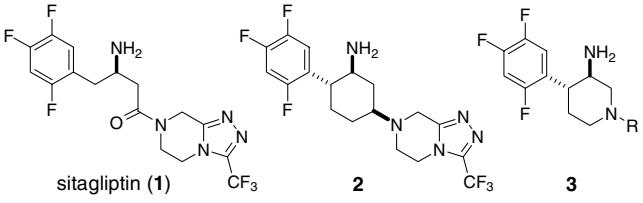

Figure 1. Select DPP-4 inhibitors.

Keywords: Dipeptidyl peptidase IV; DPP-4; DPP8; DPP9; QPP; Aminopiperidine.
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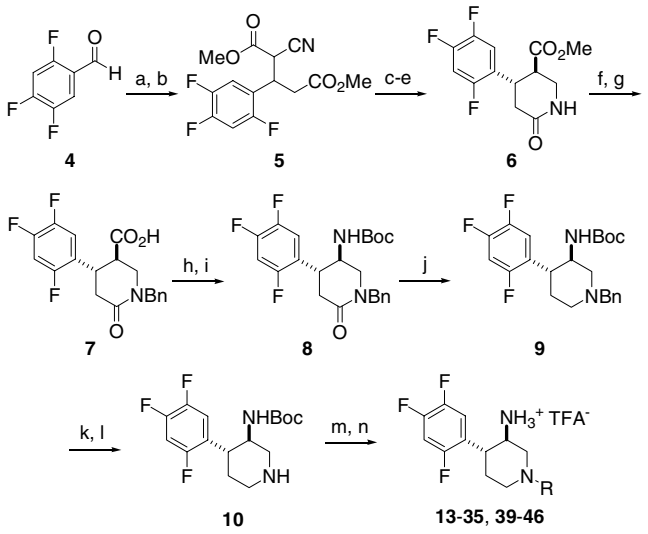
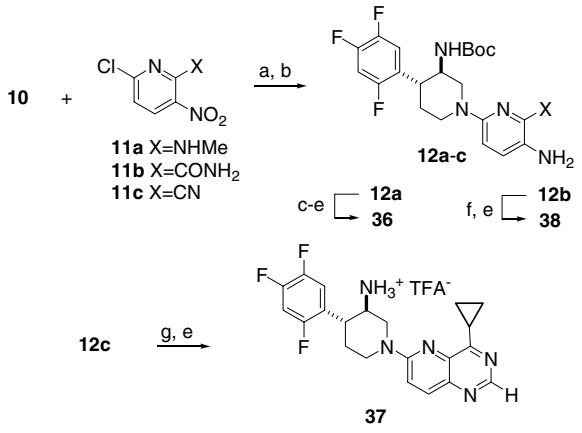


Scheme 1. Synthesis of aminopiperidine DPP-4 inhibitors **13**–**35**, **39**–**46**. Reagents and conditions: (a) $(EtO)_2POCH_2CO_2Et$, DBU, THF; (b) $NCCH_2CO_2Me$, NaOMe, MeOH, Δ; (c) H_2 (50 psi), PtO_2, HCl, MeOH; (d) K_2CO_3, tol/MeOH (1:1), Δ; (e) Me_3SiCHN_2, Et_2O/MeOH (1:1), 0 °C → rt; (f) BnBr, NaHMDS, THF/DMF (5:1), −78 °C → rt; (g) LiOH (1 N), THF/MeOH (3:1), 60 °C; (h) DPPA, TEA, tol; BnOH, Δ; (i) H_2 (45 psi), $Pd(OH)_2$, Boc_2O, MeOH; (j) BH_3, THF, Δ; HCl Δ; Boc_2O, EtOAc; (k) Chiral HPLC (ChiralCel AD column, 5% IPA/heptane); (l) H_2, $Pd(OH)_2$, MeOH; (m) RX, TEA or DIEA, tol/DMF (5:1), Δ; (n) TFA, DCM.

addition, using the sodium anion of methyl cyanoacetate, provided diester **5**. Reduction of the nitrile, cyclization, and ester regeneration yielded δ-lactam **6**, with >5:1 *trans*/*cis* selectivity.[10] Protection of the δ-lactam nitrogen as its benzyl amide, followed by saponification, afforded acid **7**. With **7** in hand, we were poised to carry out a Curtius rearrangement.[11] In the event, treatment of **7** with diphenyl phosphoryl azide (DPPA) and triethylamine (TEA), followed by trapping of the resulting isocyanate with benzyl alcohol (BnOH), and protecting group exchange, provided lactam **8**. Borane reduction of the lactam carbonyl and reprotection of the primary amine afforded piperidine **9**. The enantiomers of racemic piperidine **9** were separated using chiral HPLC to afford (2*R*,3*R*)-**9**. Hydrogenolysis of the secondary benzyl amine provided key intermediate **10**. Analogs **13**–**35** and **39**–**46** were synthesized by refluxing piperidine **10** with the appropriate halo-heterocycle,[12] followed by *tert*-butylcarbonyl (Boc) removal using trifluoroacetic acid (TFA).

Additional analogs were synthesized using intermediates **12a**–**c** (Scheme 2). Intermediates **12a**–**c** were prepared by thermal reaction of chlorides **11a**–**c**[12] with piperidine **10** followed by nitro reduction. Analog **36** was prepared by HOBt/EDC coupling of **12a** and cyclopropyl acid, followed by cyclization with acetic acid and Boc removal. Treatment of **12b** with trimethyl orthoformate and subsequent deprotection yielded analog **38**. Nitrile **12c** was converted to analog **37** via cyclopropyl Grignard addition to the nitrile, in situ trapping of the resulting imine, and deprotection.

Compounds **13**–**46** were evaluated for in vitro inhibition of human DPP-4.[13] Each inhibitor was also tested

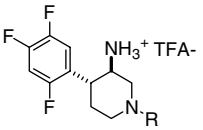


Scheme 2. Synthesis of DPP-4 inhibitors **36**–**38**. Reagents and conditions: (a) DIEA, tol, Δ; (b) Ra–Ni, THF/MeOH (10:1) 40 °C or $NiCl_2$, $NaBH_4$, MeOH/THF (3:1), 0 °C; (c) *c*-$PrCO_2H$, EDC, HOBt, DIEA, DCM; (d) AcOH, Δ; (e) TFA, DCM; (f) $(MeO)_3CMe$, tol, Δ; (g) *c*-PrMgX, THF, rt; MeOCHO, 50 °C.

against DASH family members,[14] including DPP8,[15] DPP9,[16] FAP,[17] PEP, and other proline specific enzymes with DPP-4-like activity,[18] including QPP (DPP-II).[14,19] Selectivity over DPP8 and DPP9 is of particular concern because inhibition of these enzymes is associated with toxicity in preclinical species and the relevance of this toxicity to humans is not yet known.[20] Inhibition data for DPP-4, QPP, DPP8, and DPP9 are presented in subsequent tables (FAP and PEP activity was generally weak >35 μM).

Our initial SAR breakthrough was the discovery that 2-pyridyl substitution played a significant role in DPP-4 potency and selectivity (Table 1).[21] 2-Pyridyl substituted analog **14** had an almost fourfold increase in potency over phenyl ring analog **13**, and greater than twofold increase in potency over 3- and 4-pyridyl analogs **15** and **16**, respectively. The pyridyl substitution also improved

Table 1. Monocyclic substitution effects on selected DPP-4 inhibitors



Compound	R	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
13	Ph	0.210	5.5	33	39
14	2-pyr	0.058	50	34	38
15	3-pyr	0.144	82	54	36
16	4-pyr	0.125	46	43	35
17	3-CF_3-2-pyr	0.111	5.3	>100	21
18	4-CF_3-2-pyr	0.067	28	7.6	13
19	5-CF_3-2-pyr	0.463	77	>100	>100
20	6-CF_3-2-pyr	0.650	57	>100	>100
21	4-CN-2-pyr	0.014	40	5.8	9.6
22	2,4-Pyrimidine	0.037	>100	60	67
23	2,6-Pyrimidine	0.090	54	33	58
24	2,5-Pyrazine	0.082	33	76	40
25	2,3-Pyridazine	0.063	>100	40	44

selectivity over QPP while maintaining good selectivity over DPP8 and DPP9, regardless of position. Substituents at position 4 maintained potency but at the cost of selectivity over the other enzymes (**18** and **21**), and a trifluoromethyl substituent at other positions decreased DPP-4 potency (**17**, **19**, and **20**). Analogs **22**–**25** contained a second nitrogen in the ring and in general maintained selectivity and potency, with pyrimidine **22** (DPP-4 IC_{50} 37 nM; QPP, DPP8, DPP9 $IC_{50} > 60$ μM) showing the best overall profile in the monocyclic series.

We next turned our attention to bicyclic inhibitors containing a 2-pyridyl derived moiety (Table 2). The first two inhibitors synthesized in this series were analogs **26** and **27**. Inhibitor **26** maintained the potency observed in the monocyclic series (DPP-4 IC_{50} 54 nM), but suffered degradation in selectivity (QPP, DPP8, DPP9 IC_{50} 10–21 μM), while inhibitor **27** significantly lost activity against DPP-4 (IC_{50} 3.7 μM).

The 6,6-biaryl series (**28**–**32** and **37**) showed good potency (DPP-4 IC_{50} 7–130 nM) and variable selectivity, with analog **32** showing the best profile in the series. 6,5-Bicyclic compounds **33**–**36** possessed similar potency and selectivity as the 6,6 series with inhibitor **34** showing a superb profile (DPP-4 IC_{50} 5 nM; QPP, DPP8, DPP9 $IC_{50} > 100$ μM).

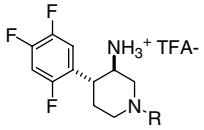
Concurrent with our investigation of DPP-4 inhibitors outlined in Table 2, we examined the profiles of bicyclic lactams **38**–**41** (Table 3). Gratifyingly, the bicyclic lactams were potent (DPP-4 IC_{50} 1.2–8 nM) and in general had good to excellent selectivity (QPP, DPP8, DPP9 $IC_{50} \geqslant 19$ μM).

Similar to the bicyclic lactam series, the triazolo-pyridazine series showed good DPP-4 potency and selectivity (Table 4). Potency increased 3- to 5-fold by the addition of alkyl substituents at the 3 position (**43**–**45**), with respect to compound **42**. With the exception of **46**, selectivity over the other peptidase screened was superb (QPP, DPP8, DPP9 $IC_{50} \geqslant 74$ μM) in the series.

Representative analogs that possessed superior potency and selectivity profiles were evaluated for hERG potassium channel,[22] rabbit L-type calcium channel,[23] and Cyp2D6 activity[24] (Table 5), and pharmacokinetic properties (Table 6). Ion channel and Cyp2D6 potencies were variable for the selected DPP-4 inhibitors (hERG IC_{50} 8.1–100 μM, Ca IC_{50} 1.0–100 μM, Cyp2D6 IC_{50} 0.1–55.4 μM) with **35** and **39** showing the cleanest overall profile.

Table 6 depicts the rat pharmacokinetic properties. Compounds **32**, **35**, and **39** suffered from rapid clearance

Table 2. Bicyclic substitution effects on selected DPP-4 inhibitors



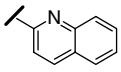
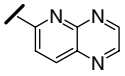
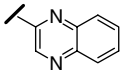
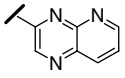
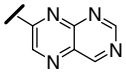
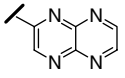
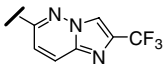
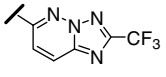
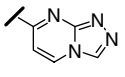
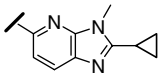
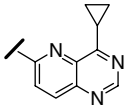
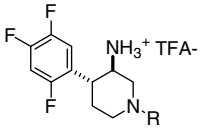
Compound	R	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
26		0.054	21	10	13
27		3.7	32	>100	>100
28		0.007	35	25	50
29		0.012	27	10	37
30		0.13	58	66	>100
31		0.043	87	>100	>100
32		0.013	93	54	>100
33		0.022	64	49	75
34		0.005	>100	>100	>100
35		0.026	>100	30	43
36		0.002	41	>100	57
37		0.008	9.9	28	32

Table 3. Bicyclic lactams as potent and selective DPP-4 inhibitors



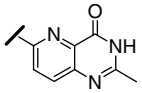
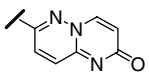
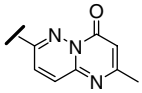
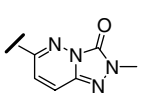
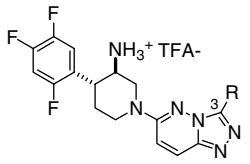
Compound	R	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
38		0.007	46	80	>100
39		0.0012	>100	>100	19
40		0.008	>100	>100	>100
41		0.005	57	>100	>100

Table 4. Substituted triazolo-pyridazines as selective DPP-4 inhibitors



Compound	R	IC_{50} (μM)			
		DPP-4	QPP	DPP8	DPP9
42	H	0.031	>100	>100	>100
43	Me	0.006	74	>100	>100
44	Et	0.006	90	>100	91
45	CF_3	0.009	96	>100	90
46	4-F–Ph	0.016	15	40	>100

Table 5. Ion channel and Cyp2D6 activity of selected DPP-4 inhibitors

Compound	hERG IC_{50} (μM)	Ca IC_{50} (μM)	Cyp2D6 IC_{50} (μM)
22	26.1	26.9	0.95
32	9.9	4.1	11.6
34	8.1	1.0	0.1
35	100	100	20.6
39	100	84.6	55.4
40	33.2	18.5	16.2
43	20.3	2.0	8.8

Table 6. Pharmacokinetic properties of selected DPP-4 inhibitors in the rat (1/2 mpk iv/po)

Compound	Clp (mL/min/kg)	$t_{1/2}$ (h)	F (%)	po AUC_{norm} (μM h kg/mg)
32	62	1.4	45	0.34
35	54	1.5	19	0.17
39	35	1.6	12	0.15
40	18	1.4	74	1.73
43	12	4.7	100	4.3

(35–62 mL/min/kg), short half-life (1.4–1.6 h), low oral bioavailability (12–45%), and low normalized oral AUC (0.15–0.34 μM h kg/mg). Compound **40** showed an improved pharmacokinetic profile, but had a short half-life (1.4 h). DPP-4 inhibitor **43** demonstrated the best overall rat pharmacokinetic profile with low clearance (12 mL/min/kg), good half-life (4.7 h), high oral bioavailability (100%), and high normalized oral AUC (4.3 μM h kg/mg).

In summary, we have discovered a novel series of 3-aminopiperidines that are potent and selective DPP-4 inhibitors. The 2-pyridyl substitution proved to be a key discovery in increasing the potency and selectivity of this structural class. Further investigation afforded bicyclic inhibitors that have increased DPP-4 potency ($IC_{50} < 10$ nM) and selectivity ($IC_{50} > 50$ μM) over other DASH proteins. While compounds **35** and **39** demonstrated low activity at ion channels and Cyp2D6, they suffered from poor pharmacokinetic properties. On the other hand, inhibitor **43** demonstrated excellent pharmacokinetics but poor Ca channel selectivity (2.0 μM). Current efforts are focused on improving potassium channel, calcium channel, and Cyp2D6 selectivity, while maintaining DPP-4 potency, selectivity over other DASH proteins, and excellent pharmacokinetics. These and other discoveries will be reported in due course.

Acknowledgments

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