Bioorganic & Medicinal Chemistry Letters 21 (2011) 1880–1886

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of [(1*R*)-1-amino-2-(2,5-difluorophenyl)ethyl] cyclohexanes and 4-[(1*R*)-1-amino-2-(2,5-difluorophenyl)ethyl]piperidines as DPP-4 inhibitors

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ARTICLE INFO

Article history:
Received 21 October 2010
Revised 13 December 2010
Accepted 15 December 2010
Available online 15 January 2011

ABSTRACT

A series of 4-amino cyclohexanes and 4-substituted piperidines were prepared and evaluated for inhibition of DPP-4. Analog **20q** displayed both good DPP-4 potency and selectivity against other proteases, while derivative **20k** displayed long half life and modest oral bioavailability in rat. The most potent analog, 3-(5-aminocarbonylpyridyl piperidine **53j**, displayed excellent DPP-4 activity with good selectivity versus other proline enzymes.

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The incretin hormones glucagon-like peptide-1(GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are secreted from the gastrointestinal tract into the circulation in response to nutrient ingestion. These hormones are known to play fundamentally important roles in glucose homeostasis. They stimulate insulin biosynthesis and secretion by a glucose dependent mechanism. Additionally, GLP-1, suppresses glucagon secretion, decelerates gastric emptying, increases β-cell mass, maintains β-cell function, and reduces food intake.[1] However, both hormones are rapidly degraded ($t_{1/2}$ ~1–1.5 min) in vivo by a serine peptidase, dipeptidyl peptidase IV (DPP-4), which cleaves a dipeptide residue from the N-terminus of polypeptides. Inhibition of DPP-4 can indirectly increase circulating levels of biologically active GLP-1 and GIP.[2] Indeed, in the past decade, inhibition of DPP-4 with small molecules has become a novel therapeutic approach for the treatment of type 2 diabetes.[3,4]

Sitagliptin **1** (JANUVIA™) is a potent and selective DPP-4 inhibitor in the β-amino acid series. It is the first DPP-4 inhibitor approved by the FDA for the treatment of type 2 diabetes.[5,6] In our continuing effort to identify structurally diverse DPP-4 inhibitors, a series of substituted 4-amino cyclohexylglycine analogs in the α-amino acid series were explored, resulting in the identification of sulfonamide **2** and amide **3** as potent DPP-4 inhibitors.[7] While these analogs showed good potency against the DPP-4 enzyme, they suffered from poor selectivity against the DPP-8 and DPP-9 isoforms. In order to improve the selectivity against these off-target enzymes, our efforts were focused on the synthesis and evaluation of α/β hybrid DPP-4 inhibitors **4** (see Fig. 1). These hybrids combine elements of sitagliptin **1** with compounds **2** and **3**, leading to the discovery of potent and selective cyclohexyl substituted DPP-4 inhibitors. In addition, several piperidine analogs **5** were also prepared to determine the effect of nitrogen location on DPP-4 potency.

The synthesis of sulfonamide, amide and acyclic amine derivatives are shown in Scheme 1. Hydrogenation of ethyl 4-hydroxylphenylacetate (**6**) followed by treatment with benzyl trichloroacetimidate under acidic conditions[9] gave chromatographically separable *cis/trans* isomers **7** and **8** in a ratio of 2:1. Saponification of the *cis* ester **7** yielded acid **9**. The carboxylic acid group was activated as the mixed anhydride using pivaloyl chloride and reacted with (*R*)-5-benzyloxazolidinone to produce the desired acyl derivative **10**.[10a] Stereoselective alkylation of the

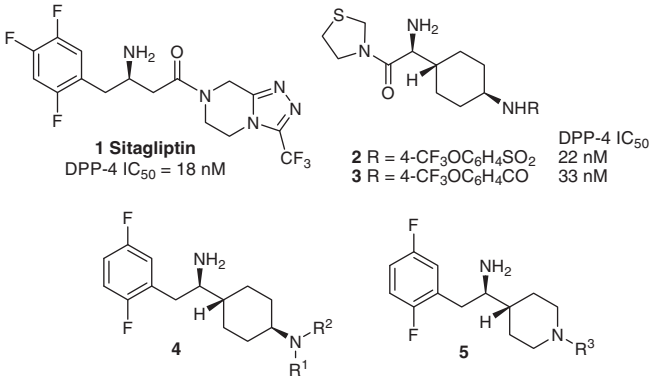

Figure 1. Rational design of DPP-4 inhibitors.

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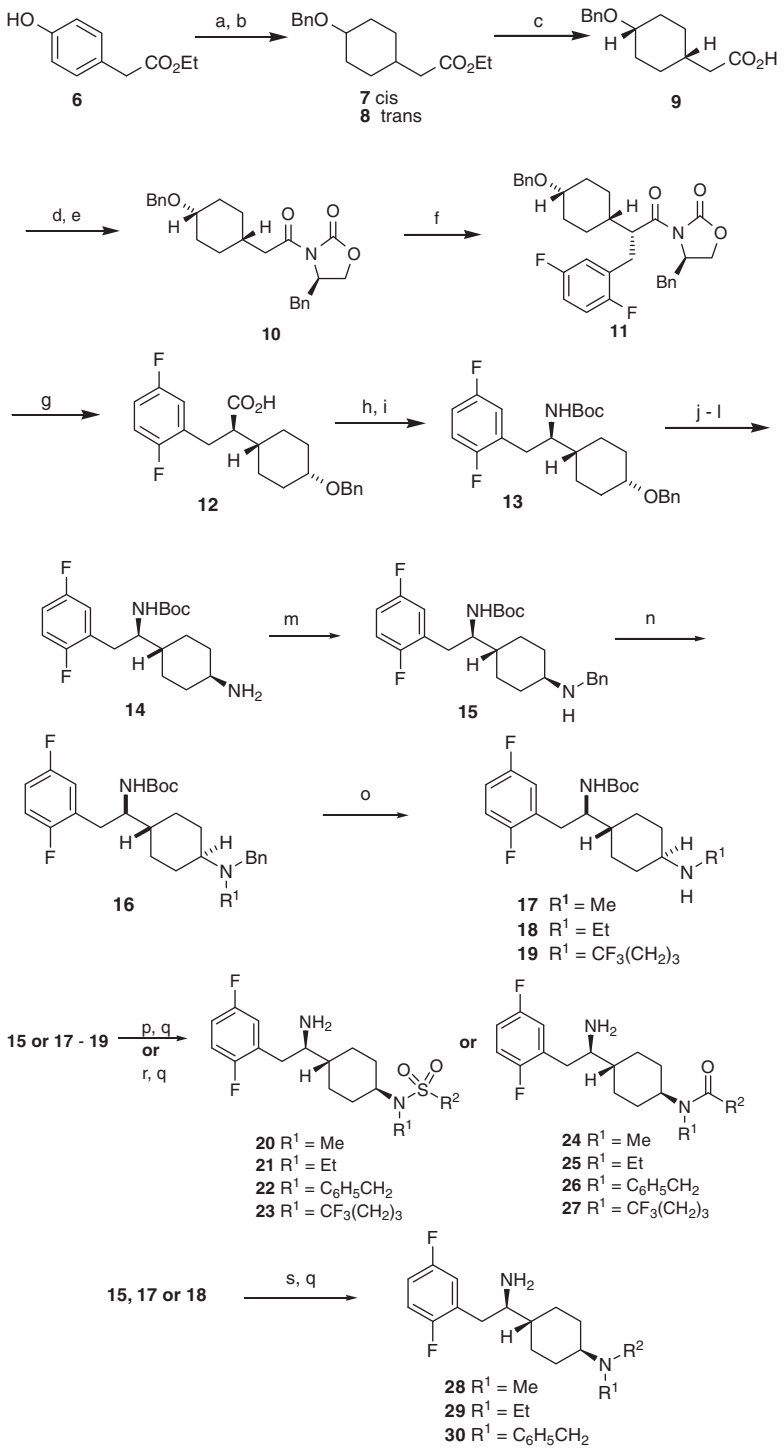
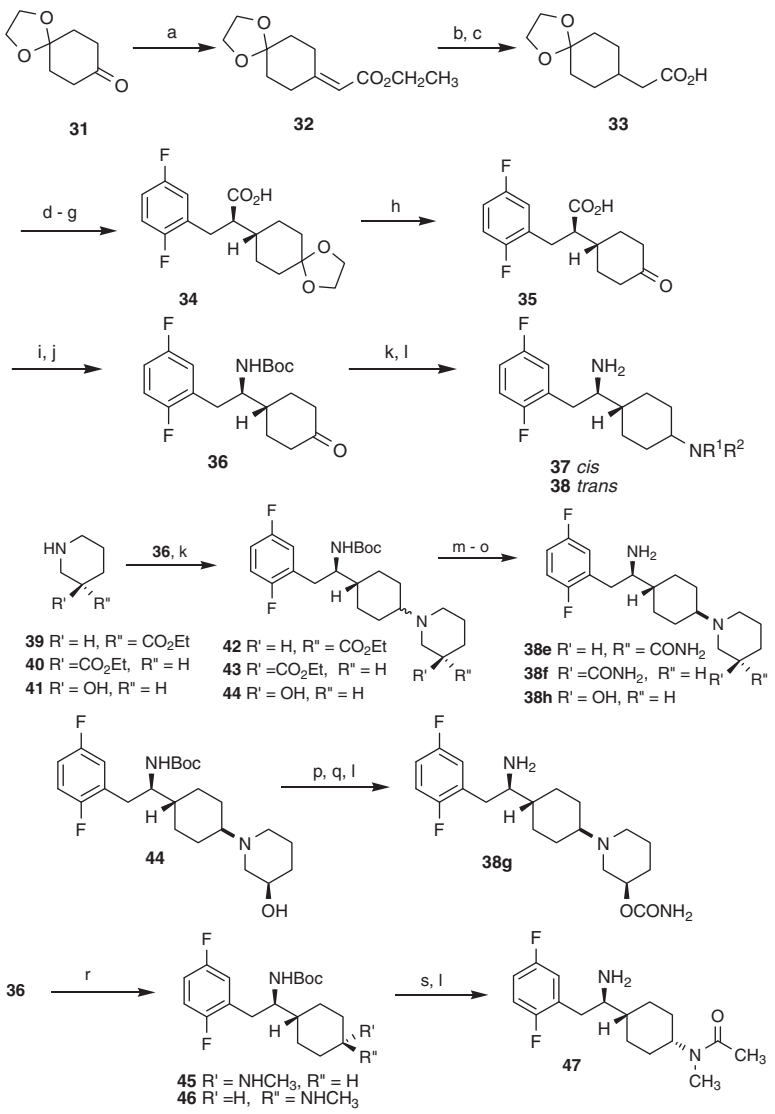
0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.12.060



Scheme 1. Synthesis of sulfonamides **20**–**23**, amides **24**–**27**, and amines **28**–**30**. Reagents and conditions: (a) H_2, 5% Rh/Al_2O_3, 1300 psi, 40 °C; (b) $CCl_3C(=NH)OBn$; TfOH, cyclohexane, CH_2Cl_2; (c) KOH, EtOH, H_2O (*cis* isomer **7**); (d) pivaloyl chloride, Et_3N, Et_2O; (e) (*R*)-benzyloxazolidinone, BuLi, THF, −78 °C; (f) 2,5-$F_2C_6H_3CH_2Br$, $NaN(TMS)_2$, THF, −78 °C; (g) LiOH, H_2O_2, THF, H_2O, rt; (h) DPPA, DIPEA, $PhCH_3$, 110 °C; (i) *t*-BuOH, TMSCl, CH_2Cl_2, rt; (j) H_2, 20% $Pd(OH)_2/C$, EtOAc; (k) $Zn(N_3)_2{\cdot}2C_5H_5N$, Ph_3P, DEAD, imidazole, CH_2Cl_2, rt; (l) H_2, 10%Pd/C, EtOAc; (m) PhCHO, $NaBH_3CN$, MeOH, rt; (n) R^1CHO, $NaBH_3CN$, CH_3OH, rt; (o) H_2, 10% Pd/C, HOAc; EtOH; (p) R^2SO_2Cl, Et_3N, CH_2Cl_2, rt; (q) HCl, MeOH, rt; (r) R^2COCl, Et_3N, CH_2Cl_2 or Ac_2O, 4-DMAP, CH_2Cl_2 (R^2 = Me) or R^2CO_2H, EDC, HOBt, DMF rt; (s) R^2CHO, $NaBH_3CN$, HOAc, MeOH, rt.

sodium enolate of **10** with 2,5-difluorobenzyl bromide gave intermediate **11** in good yield.[10] Removal of the chiral auxiliary using lithium hydroperoxide generated the acid **12**.[10] Subsequent Curtius rearrangement of **12** by treatment with diphenylphosphoryl azide and trapping the isocyanate intermediate with *tert*-butanol provided the *N*-Boc amino compound **13**. Through hydrogenolysis, Mitsunobu reaction with zinc azide and catalytic reduction, the benzyl ether **13** was successfully converted to the amine **14**. Reductive amination of **14** with benzaldehyde gave the benzylamine intermediate **15**. The methyl, ethyl and trifluorobutylamines **17**–**19** were obtained in excellent yield via reductive amination of **15** with the corresponding aldehydes followed by removal of benzyl group under hydrogenolysis conditions. Treatment of **15** and **17**–**19** with a variety of commercially available sulfonyl chlorides afforded sulfonamide derivatives **20**–**23**. The amide analogs **24**–**27** were synthesized by reaction of **15**, **17**–**19** with acid chlorides,



Scheme 2. Synthesis of pyrrolidine, piperidine, piperazine and acetamide substituted **37**, **38** and **47**. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, rt; (b) H_2, 10%Pd/C, EtOH; (c) KOH, EtOH, H_2O, rt; (d) pivaloyl chloride, Et_3N, Et_2O, rt; (e) (*R*)-benzyloxazolidinone, BuLi, THF, −78 °C; (f) 2,5-$F_2C_6H_3CH_2Br$, $NaN(TMS)_2$, THF, −78 °C; (g) LiOH, H_2O_2, THF, H_2O, rt; (h) HCl, THF, H_2O, rt; (i) DPPA, DIPEA, $PhCH_3$, 110 °C; (j) *t*-BuOH, TMSCl, CH_2Cl_2, rt; (k) NHR^1R^2, $NaBH(OAc)_3$, HOAc, $ClCH_2CH_2Cl$, rt; (l) HCl, MeOH, rt; (m) NaOH, EtOH, H_2O, rt (*trans* isomer); (n) NH_3, EDC, HOBt, DMF, rt; (o) TFA, CH_2Cl_2, rt; (p) $Cl_3C(O)NCO$, rt; (q) NH_3, MeOH, rt; (r) $CH_3NH_2{\cdot}HCl$, Et_3N, $NaBH_3CN$, HOAc, MeOH, rt; (s) Ac_2O, Et_3N, CH_2Cl_2, rt (*cis* isomer **45**).

anhydrides or EDC activation of the carboxylic acids. Preparation of N,N-disubstituted amine derivatives **28**–**30** was accomplished by reductive amination of amines **15**, **17** or **18** with the corresponding aldehydes.

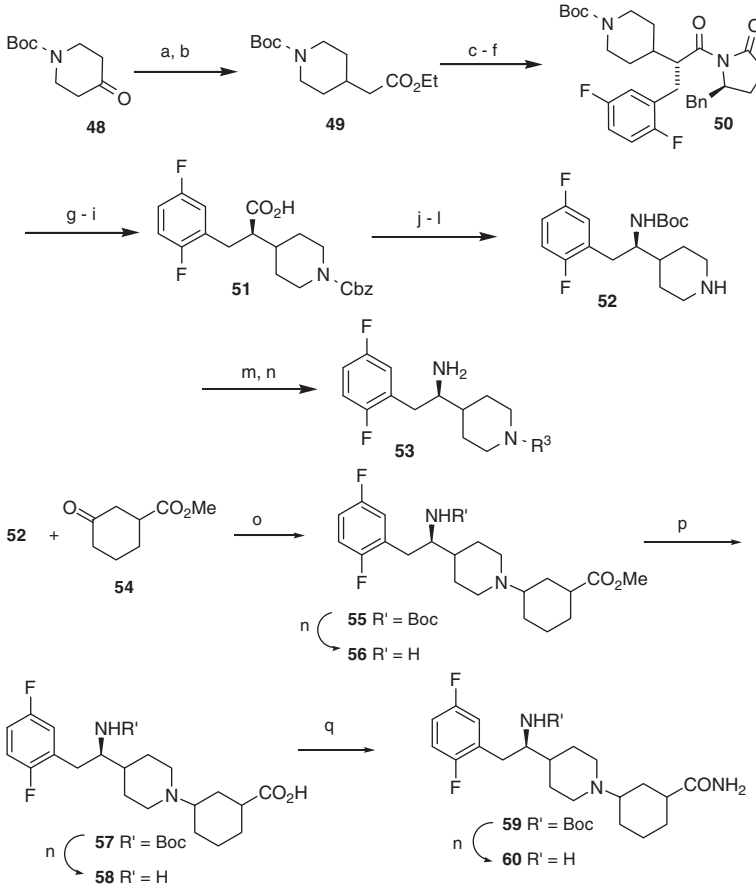
The preparations of pyrrolidine, piperidine and piperazine analogs are outlined in Scheme 2. Condensation of commercially available 1,4-cyclohexanedione mono-ethylene ketal (**31**) with triethyl phosphonoacetate proceeded smoothly to give the unsaturated ester **32**. The saturated acid **33** was obtained by hydrogenation and saponification of **32**. Utilizing the same synthetic sequence as described for the preparation of **12** from the acid **9**, the 2,5-difluorobenzyl moiety was installed in the α-position of carboxylic acid **33**, yielding compound **34**. Hydrolysis of the acetal group afforded the ketone intermediate **35**. The carboxylic acid of **35** was converted into the *N*-Boc protected amine using the same synthetic protocol described for the conversion of **12** to **13**, affording the key ketone compound **36**. Reductive amination with a variety of secondary amines followed by deprotection, yielded the desired compounds **37** (*cis*) and **38** (*trans*). The nipecotamide derivatives **38e** and **38f**, with known configurations, were prepared from ethyl (*S*)-nipecotate (**39**) or ethyl (*R*)-nipecotate (**40**). Reductive amination of ketone **36** was carried out using ethyl (*S*)-nipecotate (**39**) to give separable *cis* and *trans* isomers **42**. Saponification of the ester group of the *trans* isomer followed by EDC-mediated reaction with ammonia produced nipecotamide compound **38e** after removal of the Boc group. A similar sequence beginning with ethyl (*R*)-nipecotate (**40**) and ketone **36** gave the (*R*) diastereomer **38f**. The same treatment of 3(*R*)-hydroxylpiperidine hydrochloride (**41**) with the ketone **36** generated the 3(*R*)-hydroxylpiperidine derivative **38h**. Alternatively, the *trans*-piperidin-3(*R*)-ol intermediate **44** was reacted with trichloroacetyl isocyanate, followed by treatment with methanolic ammonia to remove the trichloroacetyl group. Subsequent deprotection then produced the carbamate analog **38g**.

For *trans*-*N*-methyl acetamide analog **24a**, the corresponding *cis* isomer **47** was prepared from the ketone **36**. Reductive amination with methylamine hydrochloride salt yielded a mixture of the *cis* isomer **45** and the *trans* isomer **46** in a 2:3 ratio. After separation of the isomers, acetylation of the *cis* amine **45** was followed by removal of the Boc group to yield the *cis* acetamide **47**.

In addition to exploring substituent effects in the cyclohexyl series, we were also interested in examining substitution of a piperidine series. The chemistry to access those compounds is summarized in Scheme 3. The ester intermediate **49** was obtained from condensation of commercially available 1-Boc-4-piperidinone (**48**) with triethyl phosphonoacetate followed by catalytic hydrogenation. Utilizing the same synthetic sequence to prepare acyl derivative **11** from ester **7** depicted in Scheme 1, the intermediate **49** was successfully transformed into compound **50**. After exchanging the Boc protecting group for a Cbz group, the chiral auxiliary was removed, giving *N*-Cbz piperidine derivative **51**. Curtius rearrangement followed by reaction with *tert*-butanol yielded the key intermediate **52** upon hydrogenolysis. Sulfonylation, acylation or N-arylation then produced a series of N-substituted piperidine analogs **53**. Installation of a 3-substituted cyclohexane group on the piperidine nitrogen was accomplished via a different route. Methyl 3-oxo-1-cyclohexanecarboxylate **54**, prepared from the corresponding acid with trimethylsilyl diazomethane, was reductively aminated with piperidine **52** under standard conditions, producing the chromatographically separable diastereomeric products **55**. Saponification followed by EDC-mediated amination generated the acid and amide derivatives, **57** and **59**. Treatment of the ester, acid and amide intermediates with hydrogen chloride in methanol then afforded analogs **56**, **58** and **60**.

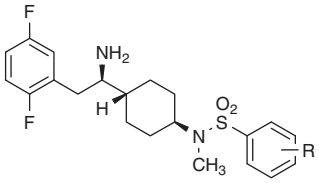
The cyclohexyl and piperidine analogs were assessed for DPP-4 inhibitory activity and selectivity profiles against other proline specific enzymes including quiescent cell proline dipeptidase (QPP/DPP-II),[11] dipeptidyl-peptidase 8 (DPP-8),[12] dipeptidyl-peptidase 9 (DPP-9),[13] and fibroblast activation protein (FAP).[14] Selectivity against DPP-8 and DPP-9 is of particular importance because preclinical studies have suggested that inhibition of the enzymes DPP-8 and DPP-9 may cause toxicity in animal models. However, the relevance of this adverse effect in humans has not been unequivocally demonstrated to date.[8] In this report, all compounds showed weak affinity to FAP (>35 μM), and only DPP-4, QPP, DPP-8 and DPP-9 data are listed.

A series of sulfonamide derivatives was evaluated for inhibition of the DPP-4 enzyme in vitro and these results are reported in Table 1 and Table 2 along with the selectivity against the QPP, DPP-8 and DPP-9 enzymes. Table 1 describes the inhibitory properties of *N*-methyl benzenesulfonamides **20** possessing different substituents on the phenyl ring. The substituents varied from small groups such as fluoro, cyano, and amino to the relatively bulky isoxazole and thiadiazole ring systems. Relative to the unsubstituted benzenesulfonamide **20a** (IC_{50} = 0.14 μM), analogs with those substituents at the 2-, 3- or 4-position of the phenyl ring displayed comparable DPP-4 activity except for 4-trifluoromethoxy derivative **20f**, which was about three-fold less potent in DPP-4 inhibition. In addition to the benzensulfonamides, our research also focused on the alkyl sulfonamides and heterocyclic sulfonamides described in Table 2. In the *N*-methyl sulfonamide series, **20l**–**20q**, the 2,5-dimethylisoxazole derivative **20q** was found to be the most potent DPP-4 inhibitor with excellent selectivity against the off-target enzymes. Variation of the R1 group of the



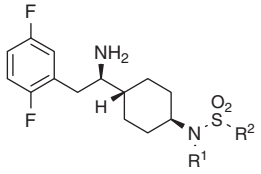
Scheme 3. Synthesis of piperidines **53**, **56**, **58** and **60**. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, rt; (b) H_2, 10%Pd/C, EtOH; (c) KOH, EtOH, H_2O, rt; (d) pivaloyl chloride, Et_3N, Et_2O, rt; (e) (*R*)-benzyloxazolidinone, *n*-BuLi, THF, −78 °C; (f) 2,5-$F_2C_6H_3CH_2Br$, $NaN(TMS)_2$, THF, −78 °C; (g) HCl, MeOH, rt; (h) CbzCl, Et_3N, CH_2Cl_2, rt; (i) LiOH, H_2O_2, THF, H_2O, rt; (j) DPPA, DIPEA, $PhCH_3$, 100 °C; (k) *t*-BuOH, TMSCl, CH_2Cl_2, rt; (l) H_2, 10%Pd/C, EtOH; (m) CH_3SO_2Cl, or CH_3COCl, Et_3N, CH_2Cl_2 or R^3X, $Pd_2(dba)_3$, (*o*-biphenyl)PCy_2, Cs_2CO_3, toluene, 70–100 °C, overnight; (n) HCl, MeOH, rt; (o) decaborane, MeOH, rt; (p) LiOH, THF, H_2O, MeOH, 75 °C, 3 h; (q) NH_3 in dioxane, HOBt, EDC, DMF, rt.

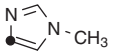
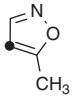
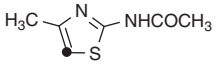
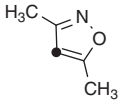
Table 1
N-Methylbenzenesulfonamide substitiution



Entry	R	DPP-IV IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitaglptin	0.027	>100	69	>100
2		0.022	1.18	0.93	1.40
20a	H	0.14	20	61	62
20b	2-CF_3	0.11	20	65	27
20c	3-CN	0.085	9.6	>69	47
20d	4-CN	0.18	23	>100	30
20e	4-NH_2	0.14	19	69	61
20f	4-CF_3O	0.40	29	>100	>100
20g	3-$CONH_2$	0.099	20	34	31
20h	4-$CONH_2$	0.084	15	65	28
20i	2,4-diF	0.15	17	55	32
20j		0.13	13	76	24
20k		0.094	8.5	>100	16

Table 2
N-Alkyl-*N*-aryl/alkylsulfonamide substitutions



Entry	R^1	R^2	DPP-4 IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitagliptin		0.018	>100	69	>100
20l	CH_3		0.28	57	71	>100
20m	CH_3		0.50	>100	>100	>100
20n	CH_3		0.17	43	>100	>100
20o	CH_3		0.10	34	62	39
20p	CH_3		0.11	35	>100	57
20q	CH_3		0.055	63	>100	>100
21a	CH_3CH_2		0.038	29	>100	64
22a	$C_6H_5CH_2$		0.67	10	>100	51
23a	$CF_3(CH_2)_3$		0.57	40	>100	>100
21b	CH_3CH_2	CH_3	0.26	68	>100	85
22b	$C_6H_5CH_2$	CH_3	1.3	11	>100	55
23b	$CF_3(CH_2)_3$	CH_3	2.4	86	>100	>100

2,5-dimethylisoxazole derivative **20q** was then examined. As seen in analogs **21a**–**23a**, sterically bulkier ($R^1 = C_6H_5CH_2$) or larger linear groups ($R1 = CF_3(CH_2)_3$) resulted in a decrease in DPP-4 potency and, in some cases, decreased selectivity towards QPP and DPP-9. *N*-Ethyl-3,5-dimethylisoxazole analog **21a** was slightly more active than the corresponding *N*-methyl analog **20q** in DPP-4 inhibition but two-fold less selective over QPP. Ethyl, benzyl and trifluorobutyl groups were also examined in methanesulfonamide series (entries **21b**–**23b**), giving analogs with somewhat decreased potency and selectivity.

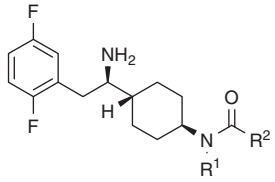
DPP-4 inhibitory properties for some representative cyclohexyl amide analogs are summarized in Table 3. *N*-Methyl-substituted alkyl/heterocylic amides **24a**–**24k** displayed modest potency and a varying degree of selectivity over QPP, DPP-8 and DPP-9 enzymes. It was observed that the *N*-methyl-3,5-dimethyl-isoxazole amide derivative **24j** was slightly less potent and selective relative to the analogous sulfonamide **20q**. In the case of acetamide, the *cis* isomer **47** was synthesized and evaluated in the DPP-4 inhibitory assay. The *cis* isomer **47** was significantly less potent and selective than the *trans* isomer **24a**.[15] Compounds **25**–**27** are analogs of the *N*-methyl acetamide **24a** where the *N*-methyl group was replaced by ethyl, benzyl or trifluorobutyl groups. Compared with the parent compound **24a**, these replacements resulted in no improvement in DPP-4 potency. Noticeably, the *N*-benzylacetamide 26 and *N*-triflurobutylacetamide 27 enhanced DPP-4 inhibitory activity by >10-fold comparing to their analogous sulfonamide derivatives **22b** and **23b**.

Analogs bearing acyclic amine side chains at the 4-position of the cyclohexane ring were also prepared and in vitro profiles are summarized in Table 4. The substitution on nitrogen included a combination of methyl, ethyl, trifluorobutyl, benzyl, 3- or 4-cyanobenzyl groups. However, these derivatives gave DPP-4 inhibitory activities in the range of 0.29–0.91 μM, with, in most cases, modest to good selectivity over off-target enzymes.

Table 5 lists the in vitro data for cyclohexyl core systems substituted with several cyclic amines such as the 3,3-difluoropyrrolidine, piperidine and trizazolopiperazine derivatives. Both *cis* isomer **37** and *trans* isomer **38** were prepared for the pyrrolidine (**37a** and **38a**), triazolopiperazine (**37b** and **38b**), and piperidine (**37c** and **38c**) analogs. In case of piperidine analogs, only the *trans* diastereomer (**38c** and **38d**) was more active than the *cis* diastereomer (**37c** and **37d**) in the DPP-4 inhibitory assay. Diastereomers **38e** and **38f** with known configuration at the 3-position were also prepared, of which the *R*-isomer **38f** was slightly more active than the *S*-isomer **38e** and >150-fold more selective against the proline enzymes DPP-8 and DPP-9. However, replacement of the amide group with a carbamate (**38g**) or hydroxyl group (**38h**) resulted in decreased DPP-4 potency. Interestingly, analogs **37b** and **38b** containing the sitagliptin right hand side chain, 3-trifluoromethyl-triazolopyrazine, were found to be significantly lesspotent than sitagliptin as DPP-4 inhibitors.

Table 6 shows the DPP-4 inhibitory data for the piperidine class of analogs. Small groups like the acetyl and methanesulfonyl derivatives (compounds **53a** and **53b**) showed modest activity in DPP-4 inhibition. Aryl groups, such as the unsubstituted pyridyl derivatives, **53c**–**53e**, also displayed modest DPP-4 activity with the 3-pyridyl derivative **53d** being the most potent DPP-4 inhibitor. Several substituted 3-pyridyl analogs were subsequently prepared. It was found that incorporation of cyano, methyl, fluoro, ester or amide groups on the pyridine ring generally improved DPP-4 inhibitory activity by one- to six-fold (see entries **53f**–**53j**). The primary amide analog **53j** was the most potent DPP-4 inhibitor in this series. However, these substitutions produced little or no effect in enhancing selectivity over QPP, DPP-8 and DPP-9. The 5-pyrimidine derivative **53l** showed slightly improved potency over the 3-pyridyl analog **53d** and was substantially more potent and selective a DPP-4 inhibitor than the 2-pyrimidine analog **53k**. Methoxy

Table 3
N-alkylamide substitution



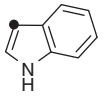
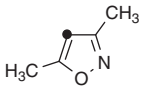
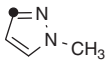
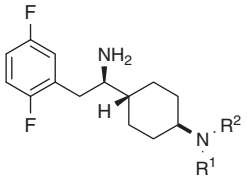
Entry	R^1	R^2	DPP-4 IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitagliptin		0.018	>100	69	>100
3			0.033	0.69	0.17	0.57
24a	CH_3	CH_3	0.12	83	>100	63
47	CH_3	CH_3	0.99	>100	5.8	12
24b	CH_3	$(CH_3)_2C$	0.13	>100	>100	53
24c	CH_3	$(CH_3)_3C$	0.11	81	50	16
24d	CH3	$NH_2CH_2CH_2$	0.73	>100	>100	76
24e	CH_3	$HOOCCH_2CH_2$	0.14	>100	>100	>100
24f	CH_3		0.14	58	>100	51
24g	CH_3		0.12	18	70	12
24h	CH_3		0.12	58	>100	81
24i	CH_3		0.11	3.6	35	32
24j	CH_3		0.14	32	58	13
24k	CH_3		0.14	21	>100	30
25	CH_3CH_2	CH_3	0.26	>100	>100	>100
26	$C_6H_4CH_2$	CH_3	0.11	17	>100	86
27	$CF_3(CH_2)_3$	CH_3	0.10	51	>100	>100

Table 4
N,N-acyclic substitution

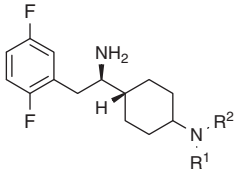


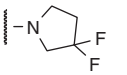
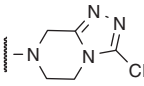
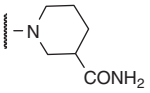
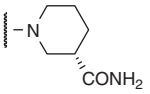
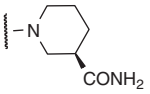
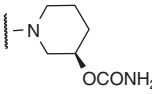
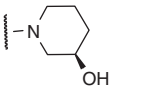
Entry	R^1	R^2	DPP-4 IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitagliptin		0.018	>100	69	>100
19	H	$CF_3(CH_2)_3$	0.91	4.8	75	45
28a	CH_3	4-$CNC_6H_5CH_2$	0.29	12	>100	10
28b	CH_3	3-$CNC_6H_5CH_2$	0.22	14	>100	15
29a	CH_3CH_2	$C_6H_5CH_2$	0.71	27	76	17
30a	$C_6H_5CH_2$	$CF_3(CH_2)_3$	0.69	23	>100	43

and hydroxy substitution on the 5-pyrimidine ring (**53m** and **53n**) were well tolerated in terms of DPP-4 inhibition.

In addition to aromatic subunits on the right hand side of the piperidine ring, several 3-substitued cyclohexyl rings were also attached to the piperidine core (entries **56**, **58** and **60**, unknown stereochemistry). In each case, one diastereomer was found to be significantly more active than the other diastereomer in DPP-4 inhibition. Only the active isomers are listed in Table 6. Analogs **56**, **58** and **60** showed good potency with modest selectivity versus off-target enzymes. Overall, the piperidine compounds **53a**–**60** did not dramatically improve the in vitro profile relative to the cyclohexane derivatives **37a**–**38h**.

Table 5
Piperidine or pyrolidine substitution

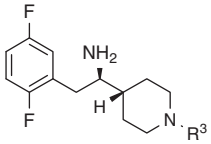


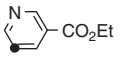
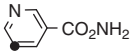
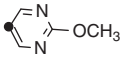
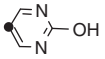
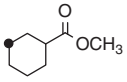
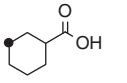
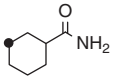
Entry	*cis* *trans*	NR^1R^2	DPP-4 IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitagliptin		0.018	>100	69	>100
37a			1.0	16	22	8.1
38a	*cis* *trans*		0.85	31	>100	>100
37b			0.28	>100	10	14
38b	*cis* *trans*		0.33	18	>100	93
37c	*cis*		1.8	17	21	12
38c	*trans*		0.33	77	56	28
37d			1.3	54	14	16
38d	*cis* *trans*		0.057	62	68	32
38e	*trans*		0.2	80	25	27
38f	*trans*		0.07	11	>100	77
38g	*trans*		0.31	72	58	36
38h	*trans*		0.27	>100	33	37

Several selected DPP-4 inhibitors were evaluated in a rat pharmacokinetic model and the results are summarized in Table 7. In general, the majority of the tested analogs suffered from high clearance rate, low exposure levels and poor oral bioavailability.

In summary, with combination of the α-amino acid derivatives **2** and **3** with the β-amino acid compound **1**, we have discovered a novel series of substituted cyclohexane and piperidine derivatives as DPP-4 inhibitors. These classes of inhibitors significantly improved selectivities against proline specific enzymes QPP, DPP-8 and DPP-9. Among the cyclohexane analogs, *N*-methyl-(2,5-dimethylisoxazol-yl)sulfonamide **20q** showed the most promising potency (IC_{50} = 55 nM) and selectivity (>46 μM vs off-target enzymes) as a DPP-4 inhibitor but suffered from poor pharmacokinetic properties. In the piperidine series, 3-(5-aminocarbonylpyridyl) derivative **53j** was found to be the most potent DPP-4 inhibitor (IC_{50} = 18 nM), with potency comparable to sitagliptin and good selectivity against other proline enzymes (IC_{50} = 18–27 μM). For those derivatives examined in rat PK studies, 4-(1,2,3-thiadiazolyl)benzensulfonamide analogs **20k** has a long half

Table 6
N-substitution piperidine derivatives



Entry	R^3	DPP-4 IC_{50} (μM)	QPP IC_{50} (μM)	DPP-8 IC_{50} (μM)	DPP-9 IC_{50} (μM)
1	Sitagliptin	0.018	>100	69	>100
53a	CH_3CO	0.45	66	>100	>100
53b	CH_3SO_2	0.13	65	95	59
53c		0.45	19	29	19
53d		0.11	37	30	16
53e		0.43	34	5.5	4.2
53f		0.077	39	46	18
53g		0.071	28	19	3.1
53h		0.11	52	32	7.2
53i		0.082	11	37	25
53j		0.018	22	18	27
53k		0.67	30	31	24
53l		0.075	35	35	16
53m		0.078	42	47	23
53n		0.072	48	26	12
56		0.054	13	39	28
58		0.051	85	72	23
60		0.021	23	58	19

life ($t_{1/2}$ = 3.5 h) and modest oral bioavailability (25% *F*), but the clearance was unacceptably high.

Table 7
Pharmacokinetic properties of selected inhibitors in rat (1/2 mpk iv/p.o)

Entry	Clp (ml/min/kg)	$t_{1/2}$ (h)	C_{max} (μM)	*F*%
20g	204	nd	0.019	13
20k	180	3.5	0.19	25
20q	116	3.0	0.032	2.2
24a	177	1.0	0.12	9.7
38f	201	2.1	0.024	24
53g	154	2.8	0.069	22
53l	171	0.92	0.019	4.5

Acknowledgments

We thank colleagues Jacqueline Hicks and Robert Wilkening for their valuable discussions and revisions regarding this manuscript. We also thank Tesfaye Biftu for insight and guidance during the manuscript preparation.

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15. At the time of this study, X-ray structural analysis was limited to complexation of analog **24a** in the DPP-4 enzyme cavity as discussed previously (Ref. 5a). Two key interactions were identified from this complex: a salt bridge between the free amino group and Glu205 and/or Glu206 and weak a hydrogen bond between the amide carbonyl oxygen and the hydroxyl of Tyr547 through a bridging water molecule in active site.