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Triazolopiperazine-amides as dipeptidyl peptidase IV inhibitors: Close analogs of JANUVIA™ (sitagliptin phosphate)

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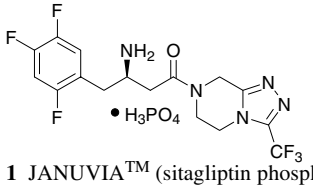
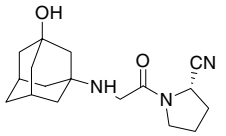
Abstract—A series of β-aminoamides bearing triazolopiperazines has been prepared and evaluated as potent, selective, orally active dipeptidyl peptidase IV (DPP-4) inhibitors. Efforts at optimization of the β-aminoamide series, which ultimately led to the discovery of JANUVIA™ (sitagliptin phosphate, compound **1**), are described.

The discovery of a biological function of dipeptidyl peptidase IV (DPP-4), a serine protease, has spurred recent intense research efforts directed toward the development of DPP-4 inhibitors as new potential anti-diabetic agents.[1] DPP-4 inhibitors function as indirect stimulators of insulin secretion and this effect is believed to be mediated primarily by enhancing the action of the incretin hormone glucagon-like peptide 1 (GLP-1).[2] This hormone is released in the gut in response to food intake. GLP-1, in turn, stimulates the pancreas to synthesize and secrete insulin, while inhibiting the release of glucagon. GLP-1 regulates insulin in a strictly glucose-dependent manner, thus posing little or no risk of hypoglycemia. Other beneficial effects of GLP-1 therapy include the slowing of gastric emptying[3] and reduction of appetite.[4] Furthermore, recent data suggesting a potential role for GLP-1 in restoration of β-cell function in rodents indicate that this mechanism might even slow or reverse disease progression.[5] However, GLP-1 is rapidly degraded in vivo through the action of DPP-4, which cleaves a dipeptide from the N-terminus to give the inactive GLP-1[9–36]amide.[6] Thus, inhibition of DPP-4 would increase the half-life of GLP-1 and prolong the beneficial effects of this incretin hormone. Compound **1**, JANUVIA™ (sitagliptin phosphate), a potent, selective, and orally active DPP-4 inhibitor, has recently been approved by the U.S. Food and Drug Administration (FDA) for the treatment of type 2 diabetes (Fig. 1). Several other DPP-4 inhibitors are currently being evaluated in late stage human clinical trials, including vildagliptin (**2**) and saxagliptin (**3**).[7]

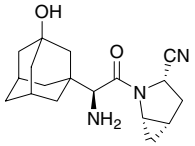
Extensive structure–activity relationship (SAR) studies around the left side phenyl ring and the right side triazolopiperazine moiety in the β-aminoamide series



1 JANUVIA™ (sitagliptin phosphate)



2 Vildagliptin (LAF-237)



3 Saxagliptin (BMS477118)

Figure 1. DPP-4 inhibitors.

Keywords: Close analogs of Januvia; Sitagliptin; Type 2 diabetes; Triazolopiperazine-amides; Dipeptidyl peptidase IV inhibitors; DPP-4 inhibitor; GLP-1; DPP-8; DPP-9.
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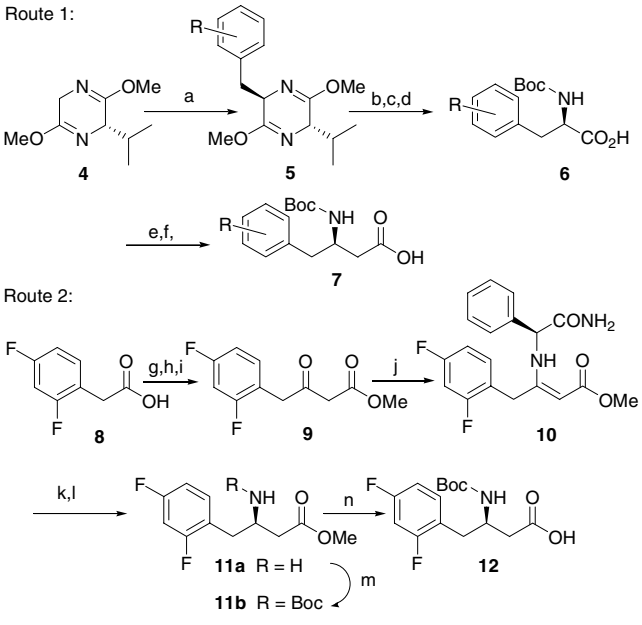
provided various close analogs of sitagliptin (**1**).[8] While the discovery of sitagliptin (**1**) was previously reported,[9] herein, our initial efforts at optimization of β-aminoamide series are described in detail.

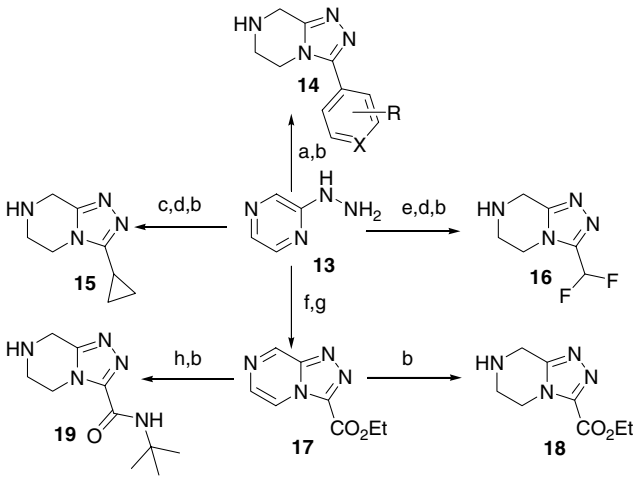
The β-amino acid derived DPP-4 inhibitors in this report were prepared by standard peptide coupling of β-amino acids (**7** and **12**, Scheme 1) with fused heterocycles (**14**–**19**, Scheme 2) as reported earlier.[9] Two different approaches to non-commercially available β-amino acids are described in Scheme 1.

First, using Scholkopf's *bis*-lactam strategy,[10] the starting dihydropyrazine **4** was converted to α-amino acid **6** with the requisite (*R*)-stereochemistry following the procedure similar to those previously reported (Route 1, Scheme 1).[8a,9] Subsequent one-carbon extension gave rise to the desired β-amino acid **7** for the synthesis of DPP-4 inhibitors. In a second approach (Route 2, Scheme 1), 2,4-difluorophenylacetic acid was converted to β-ketoester **9** in three steps according to the known procedure.[11] Treatment of β-ketoester **9** with (*S*)-phenylglycine amide followed by asymmetric hydrogenation provided compound **11a**. Enantiomeric excess (ee) of methyl ester **11b** was determined to be 98.5% using chiral HPLC (ChiralPak AD column). Boc-protection of **11a** followed by hydrolysis afforded β-aminoacid **12**.

Several approaches for the variation of the substituents on triazolopiperazines are described in Scheme 2. First, direct condensation of hydrazinopyrazine **13** with corresponding fluorobenzoic acid or isonicotinic acid in polyphosphoric acid (PPA) followed by catalytic hydrogenation afforded aryl-substituted triazolopiperazine **14**.



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, BnBr, 75–80%; (b) i—1 N HCl, rt, 16 h, ii—MeOH; (c) $(Boc)_2O$, Et_3N, CH_2Cl_2; (d) LiOH, 1:1 THF/H_2O, 62–70% (3 steps); (e) Et_3N, *iso*-butyl chloroformate, −30 °C, CH_2N_2; (f) silver benzoate, 1,4-dioxane/H_2O (5:1), sonication, 81–86% (2 steps); (g) $(COCl)_2$, cat. DMF, CH_2Cl_2; (h) 2,4,6-collidine, Meldrum's acid, 45% (2 steps); (i) MeOH, reflux, 4 h, 81%; (j) (*S*)-phenylglycine amide, MeOH, AcOH, 40 °C, 2 h, 87%; (k) 90 psi H_2, THF/MeOH = 5:1, PtO_2, 42%; (l) MeOH/AcOH/H_2O, Pearlman's catalyst; (m) $(Boc)_2O$, Et_3N, CH_2Cl_2, 59% (2 steps); (n) 1 N aqueous LiOH, THF, 87%.



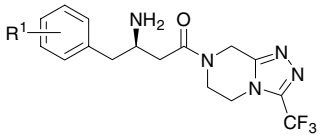
Scheme 2. Reagents and conditions: (a) fluorobenzoic acid or isonicotinic acid, PPA, 150 °C, 18 h, 8–16%; (b) H_2, 10% Pd/C, EtOH, rt, 18 h, 80–93%; (c) cyclopropanecarbonyl chloride, py, reflux, 47%; (d) PPA, 150 °C, 18 h, 57–74%; (e) $(CHF_2CO)_2O$, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h, 100%; (f) ethyl oxalyl chloride, Et_3N, CH_3CN, 27%; (g) PTSA, toluene, reflux, 6 h, 31%; (h) *t*-$BuNH_2$, 35%.

Acylation of starting hydrazinopyrazine **13** with cyclopropanecarbonyl chloride or difluoroacetic anhydride followed by PPA cyclization and hydrogenation afforded **15** and **16**, respectively. Similarly, hydrazinopyrazine **13** was acylated with ethyl oxalyl chloride and then cyclized under mild conditions to give **17**,[12] which was hydrogenated to afford **18** or converted to *tert*-butyl amide **19** by treatment with *tert*-butyl amine.

Compounds in Tables 1 and 2 were evaluated in vitro for their inhibition of DPP-4.[13] The inhibitors were also tested against DPP-4 structural homologs in the DPP-4 gene family, including DPP8,[14] DPP9,[15] fibroblast activation protein (FAP, also called seprase),[16] and other proline specific enzymes with DPP-4 like activity, including quiescent cell proline dipeptidase (QPP, also known as DPP-II),[13,17] amino peptidase P, and prolidase. Since significant QPP off-target activity was often observed for the β-amino acid derived DPP-4 inhibitors reported from these laboratories earlier,[8] QPP data are presented for comparison. Safety studies using a DPP8/9 selective inhibitor suggest that inhibition of DPP8 and/or DPP9 is associated with profound toxicity in preclinical species.[18] Although the relevance of these findings to human toxicity is unknown, treatment-related dermatological toxicity in monkeys was observed with a non-selective DPP-4, DPP8, and DPP9 inhibitor.[19] Thus, selectivity profiles against DPP8 and DPP9 were also obtained for safety reasons.

In the course of the SAR development of this triazolopiperazine series, an interesting fluorine effect on DPP-4 activity was observed. The trend was consistent with the SAR observed with a previously reported thiazolidine series.[8] In the case of mono-fluoro substitution,

Table 1. Effects of R^1 substituent on inhibitory properties of selected DPP-4 inhibitors[a]



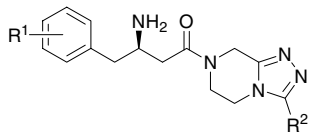
Compound	R^1	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)	DPP-9 IC_{50} (nM)
20	2-F	98	14,000	75,000	>100,000
21	3-F	135	31,000	>100,000	>100,000
22	4-F	272	>100,000	65,000	>100,000
23	3,4-Di-F	128	98,000	46,000	>100,000
24	2,4-Di-F	82	>100,000	83,000	>100,000
25	2,5-Di-F	27	>100,000	69,000	>100,000
26	2,3,5-Tri-F	805	42,000	>100,000	>100,000
27	2,3,6-Tri-F	151	>100,000	>100,000	>100,000
28	2,4,6-Tri-F	87	>100,000	>100,000	>100,000
1 (Sitagliptin)	2,4,5-Tri-F	18	>100,000	48,000	>100,000
29	2,3,4,5,6-Penta-F	1018	66,000	>100,000	>100,000
30	2-CF_3	486	>100,000	>100,000	>100,000
31	3-CF_3	366	>100,000	>100,000	>100,000
32	4-CF_3	511	>100,000	>100,000	>100,000
33	2-Cl	145	>100,000	>100,000	>100,000
34	3-Cl	59	>100,000	>100,000	>100,000
35	4-Cl	264	>100,000	26,000	72,000
36	3,4-Di-Cl	1580	>100,000	>100,000	>100,000
37	2,4-Di-Cl	23	>100,000	30,000	49,000
38	2,5-Di-Cl	180	>100,000	>100,000	>100,000
39	2-F, 5-Cl	21	>100,000	>100,000	98,000
40	2,5-Di-F, 4-Cl	76	46,000	49,000	>100,000
41	2-Cl-, 4,5-di-F	84	>100,000	>100,000	>100,000

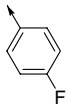
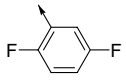
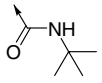
[a] Unless otherwise noted, values reported are means of a minimum of two experiments with a standard deviation <25% of the mean.

2-fluoro analog **20** was slightly more potent than 3-fluoro analog **21**, and 4-fluoro analog **22** was the least active. Sequential addition of one or two more fluorine atoms further increased the DPP-4 potency. In the case of difluoro-analogs (**23**–**25**), highest DPP-4 potency was achieved with the addition of fluorine atoms at the 2- and 5-positions (**25**, IC_{50} = 27 nM). Interestingly, reordering of three fluorine atoms on the phenyl of the most potent 2,4,5-trifluoro-compound **1** (sitagliptin) resulted in a significant decrease in the DPP-4 potency ($\geqslant$5-fold, **26**–**28**). It was notable that pentafluoro-analog **29** was the least active in the fluorine-substitution series (DPP-4 IC_{50} = 1018 nM). Trifluoromethyl substituted analogs (**30**–**32**) were uniformly less active than their corresponding fluoro-analogs (**21**–**23**). Unlike the fluorine series, the preferred positions for chlorine atoms changed in both mono- and di-substitution cases. In the case of mono-chloro substitution, the 3-position is the optimal position for DPP-4 potency (**35**; DPP-4 IC_{50} = 59 nM). Compound **34** was ca. 2-fold more potent than the corresponding 3-fluoro compound **21**. Among three dichloro-substituted compounds (**36**–**38**) the 2,4-dichloro compound **37**exhibited the highest potency (DPP-4 IC_{50} = 23 nM). Compound **37** was ca. 4-fold more potent than the corresponding 2,4-difluoro-compound **24** and was as potent as 2,5-difluoro compound **25**. Interestingly, compound **39** with mixed halides (2-F, 5-Cl) exhibited a similar activity as 2,5-difluoro-compound **25**. Displacement of a fluorine atom with a chlorine atom at either the 2- or 4-position of compound **1** (sitagliptin) resulted in a 4- to 5-fold decrease in the DPP-4 potency (**40** and **41**). In general, most of the triazolopiperazine analogs exhibited high selectivity over counterscreens.

In order to improve DPP-4 potency as well as pharmacokinetic properties, the effects of substituents on the triazolopiperazine moiety were investigated. Both aryl and heteroaryl substituents were found to be as effective as alkyl or perfluoroalkyl substituents in terms of DPP-4 potency. However, these analogs (**42**–**44**) displayed unacceptable DPP8 activity (IC_{50}: 1–4 μM), whereas the effects of aryl and heteroaryl substituents on DPP9 activity were insignificant. Since inhibition of DPP8 and/or DPP9 is associated with significant toxicity in preclinical species, further development of a series of compounds with aryl or heteroaryl substituent was discontinued. While ethyl ester and *tert*-butyl amide analogs (**45**, **50**, and **51**) showed moderate DPP-4 activity, hydroxyl analog **46** exhibited a 3-fold increase in DPP-4 potency over these analogs.[20] Ethyl analog **47** was equipotent to cyclopropyl analog **48**. Deletion of one fluorine from a CF_3-group at R^2 resulted in a slight decrease in potency (**49**: DPP-4 IC_{50} = 29 nM). Representative potent compounds were selected for the evaluation in rat pharmacokinetic studies (Table 3). While both 2,4-dichloro- and 2-fluoro-, 5-chloro-analogs (**37** and **39**) are comparable to compound **1** (sitagliptin) in terms of DPP-4 potency, they showed faster clearance and disappointing oral bioavailability compared to compound **1**. Additional halo-substituted analogs **40** and **41** also exhibited decreased oral

Table 2. Effects of R^1 and R^2 substituents on inhibitory properties of selected DPP-4 inhibitors[a]



Compound	R^1	R^2	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)	DPP-9 IC_{50} (nM)
42	2,5-Di-F		60	38,000	979	>100,000
43	2,5-Di-F		30	95,000	1010	58,000
44	2,5-Di-F		65	>100,000	4200	84,000
45	2,5-Di-F		190	>100,000	37,000	78,000
46	2,5-Di-F		69	>100,000	>100,000	>100,000
47	2,4,5-Tri-F	Et	37	>100,000	39,000	>100,000
48	2,4,5-Tri-F		30	83,000	54,000	>100,000
49	2,4,5-Tri-F		29	>100,000	86,000	>100,000
50	2,4,5-Tri-F		219	>100,000	22,000	78,000
51	2,4,5-Tri-F		234	46,000	63,000	>100,000

[a] Unless otherwise noted, values reported are means of a minimum of two experiments with a standard deviation <25% of the mean.

Table 3. Pharmacokinetic properties of selected DPP-4 inhibitors in the rat (iv, 1 mg/kg; po, 2 mg/kg)

Compound	CL (mL/min/kg)	$t_{1/2}$ (h)	AUC norm ($\mu M\ h\ kg\ mg^{-1}$)	F (%)
1	60	1.7	0.523	76
37	98	2.6	0.144	36
39	99	1.7	0.180	43
40	58	2.1	0.371	54
41	78	1.7	0.238	47
47	70	1.7	0.016	2
48	68	1.8	0.016	3
49	66	1.3	0.251	39

bioavailability in the rat comparable to compound **1** (sitagliptin). Replacement of CF_3 in compound **1** with an ethyl or cyclopropyl substituent resulted in a loss of oral bioavailability (**47** and **48**: $F = 2$–3%). Deletion of one fluorine atom from the CF_3-substituent in compound **1** resulted in a significant decrease in oral exposure and oral bioavailability (**49**).

In summary, we have discovered a series of novel triazolopiperazine-based DPP-4 inhibitors, which are among the most selective DPP-4 inhibitors reported to date. Reordering of three fluorine atoms (**26**–**28**), displacement of a fluorine atom with a chlorine (**40** and **41**) in the left side phenyl ring of compound **1** (sitagliptin), and deletion of a fluorine from triazolopiperazine ring (**49**) gave rise to several close analogs of compound **1**. Among these analogs compound **40** was found to possess the best pharmacokinetic profile, however, its DPP-4 activity was suboptimal. Decreased DPP-4 potency and oral bioavailability of *des*-fluoro-analog **49** demonstrated that the CF_3 group on the triazolopip-

erazine moiety of compound **1** is optimal. Importantly, it was discovered that some substituents on the triazolopiperazine moiety often afford the unacceptable DPP8 activity (**42**–**44**). This result suggests that we need to take precautions in designing DPP-4 inhibitors even in highly selective series such as the triazolopiperazine series.

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20. For the synthesis of compound **46**, the coupling product **52** was employed as shown below.