Discovery of Potent and Selective Dipeptidyl Peptidase IV Inhibitors Derived from β-Aminoamides Bearing Subsituted Triazolopiperazines†

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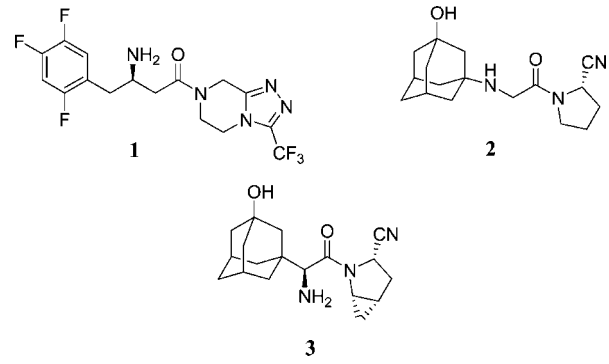
A series of β-aminoamides bearing triazolopiperazines have been discovered as potent, selective, and orally active dipeptidyl peptidase IV (DPP-4) inhibitors by extensive structure–activity relationship (SAR) studies around the triazolopiperazine moiety. Among these, compound **34b** with excellent in vitro potency (IC_{50} = 4.3 nM) against DPP-4, high selectivity over other enzymes, and good pharmacokinetic profiles exhibited pronounced in vivo efficacy in an oral glucose tolerance test (OGTT) in lean mice. On the basis of these properties, compound **34b** has been profiled in detail. Further refinement of the triazolopiperazines resulted in the discovery of a series of extremely potent compounds with subnanomolar activity against DPP-4 (**42b**−**49b**), that is, 4-fluorobenzyl-substituted compound **46b**, which is notable for its superior potency (IC_{50} = 0.18 nM). X-ray crystal structure determination of compounds **34b** and **46b** in complex with DPP-4 enzyme revealed that (*R*)-stereochemistry at the 8-position of triazolopiperazines is strongly preferred over (*S*) with respect to DPP-4 inhibition.

Introduction

Type 2 diabetes is a chronic disease characterized by elevated plasma glucose in the presence of high endogenous insulin levels, causing serious vascular complications, significant morbidity, and mortality. This metabolic disorder is a growing public health problem, affecting approximately 150 million people worldwide, and the prevalence of type 2 diabetes is expected to reach 220 million by 2010.[1] Although current type 2 diabetes therapies that increase the concentration of circulating insulin have proven therapeutically beneficial, these often show undesirable side effects such as hypoglycemia and weight gain.[2] Accordingly, there is a significant unmet medical need. Recently, inhibition of dipeptidyl peptidase IV (DPP-4[a]), a serine protease, has emerged as a new potential approach for the treatment of type 2 diabetes.[3] 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).[4,5] This hormone is released in the gut in response to ingestion of food. GLP-1, in turn, stimulates insulin biosynthesis and secretion, while inhibiting the release of glucagon.[5] Because GLP-1 regulates insulin in a strictly glucose-dependent manner, GLP-1 therapy may pose little or no risk of hypoglycemia. Other beneficial effects of GLP-1 therapy include slowing gastric emptying[6] and reduction of appetite.[7] Furthermore, intriguing data suggesting a potential role in restoration of β-cell function in rodents indicate that this mechanism might actually slow or even reverse disease progression.[8] 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.[9] Consequently, inhibition of DPP-4 would increase the half-life of GLP-1 and prolong the beneficial effects of this incretin hormone. Sitagliptin, (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (**1**),[10] a DPP-4 inhibitor recently approved by the U.S. Food and Drug Administration (FDA), is a potent, selective, and orally active antidiabetic agent that has the potential to provide a new treatment option for patients with type 2 diabetes. Several other DPP-4 inhibitors are currently being evaluated in late stage clinical trials, including compounds **2** (LAF-237)[11a] and **3** (BMS-477118;[11b]

Chart 1



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† This paper is dedicated to two outstanding Merck scientists, the late Drs. Michael H. Fisher and Barbara Leiting, who made important contributions to the discovery of sitagliptin at Merck.

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[a] Abbreviations: DPP-4, dipeptidyl peptidase 4; SAR, structure−activity relationship; OGTT, oral glucose tolerance test; GLP-1, glucacon-like peptide 1; DPP-8, dipeptidyl peptidase 8; DPP-9, dipeptidyl peptidase 9; FAP, seprase or fibroblast activation protein; QPP, quiescent cell proline dipeptidase; DPP-II, dipeptidyl peptidase II; DPP-7, dipeptidyl peptidase 7; F, oral bioavailability; PD, pharmacodynamic, DIO, diet-induced obesity; AUC, area under the curve, Cl_p, clearance.

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Chart 2

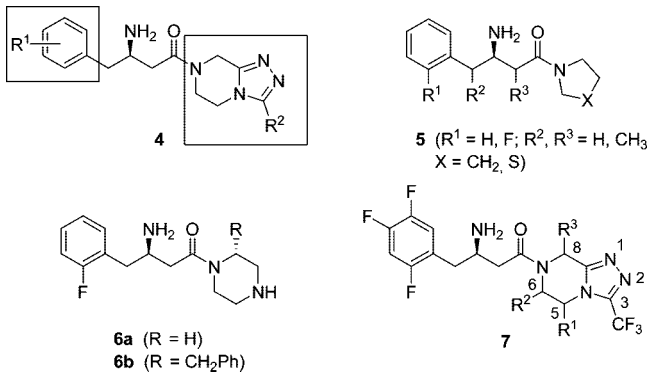
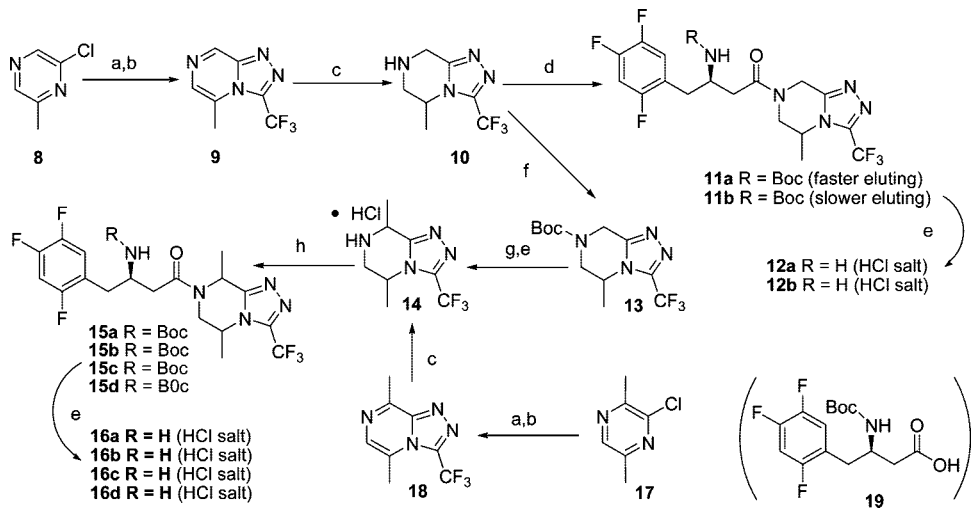


Chart 1). A recent report[12] has highlighted the detailed structure–activity relationships (SARs) of compound **1** by using a variety of substituents R^1 and R^2 on the left phenyl and the right triazolopiperazine, respectively, of the structure **4** (Chart 2), which ultimately led to the discovery of compound **1**. Subsequently, we became interested in the modifications of the β-aminoamide backbone for the further optimization of the β-aminoamide leads. Substitution of alkyl around the β-aminoamide backbone was found to be detrimental to potency. Alkyl substitution along with other modifications such as lengthening, shortening, or tethering were already proven to be ineffective in the corresponding thiazolidine[13a] and the piperazine series[13b] (**5** and **6**; >10-fold less active, data not shown). Because the SAR trends of these series are generally in line with those of the triazolopiperazine series, as previously reported from these laboratories,[13a,b] similar SAR studies were, therefore, not of interest for the triazolopiperazine series (**7**). Importantly, a significant increase in potency (>20-fold) was previously observed with the incorporation of a benzyl substituent into the piperazine moiety as exemplified by **6b** (**6b**, DPP-4 IC_{50} = 139 nM; **6a**, DPP-4 IC_{50} = 3700 nM).[13b] Previous SAR trends suggested that the incorporation of a benzyl might likewise increase the DPP-4 potency in the triazolopiperazine series. Thus, efforts focused on alkyl substitution around the triazolopiperazine moiety (5-, 6-, and 8-positions in **7**, Chart 2) to provide a series of extremely potent DPP-4 inhibitors beyond sitagliptin. Herein, we describe the synthesis, SARs, and biological properties of a series of close analogues of compound **1**.

Chemistry. The β-aminoacid derived DPP-4 inhibitors in this report were synthesized by standard peptide coupling of β-aminoacids with fused heterocycles as previously reported[10a,b] except that 1-hydroxy-7-azabenzotriazole (HOAT) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) were employed in place of 1-hydroxybenzotriazole (HOBT) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) for the coupling reactions of β-aminoacids and heterocycles with α-substituents. Initial synthetic efforts focused on the synthesis of triazolopiperazines bearing a methyl substituent or dimethyl substituents at the 5, 6, and 8 positions around the triazolopiperazine moiety. Two different approaches to the triazolopyrazine ring system are described in Schemes 1–3. The first approach started with commercially available pyrazines, for example, 2-chloro-6-methylpyrazine **8** (Scheme 1), which was converted to the corresponding hydrazinopyrazine intermediate. The treatment of the hydrazinopyrazine with trifluoroacetic anhydride (TFAA) followed by polyphosphoric acid (PPA) gave 6-methyl-substituted triazolopyrazine **9**, using conditions similar to those reported earlier (Scheme 1).[10a,b] Subsequent catalytic hydrogenation of fused heterocycle **9** proceeded smoothly to give the target triazolopiperazine intermediate **10**. Coupling of triazolopiperazine **10** with the β-aminoacid **19** typically gave a mixture of two diastereomers, **11a** and **11b**, in a 1:1 ratio, as determined by analytical chiral HPLC. These *N*-Boc-protected isomers were easily separable by HPLC, using a preparative chiral OD column. Upon removal of the *N*-Boc-group of **11a** and **11b**, the desired compounds **12a** and **12b**, respectively, were obtained. For the introduction of an additional methyl substituent at the 8-position, heterocycle **10** was transformed into the *N*-Boc-protected triazolopiperazine **13**, which was then alkylated with iodomethane via an *N*-Boc-assisted alkylation strategy[14] and subsequently deprotected to give the desired 5,8-dimethyl triazolopiperazine **14**. Coupling of triazolopiperazine **14** with the β-aminoacid **19** gave a mixture of four diastereomers (**15a**−**d**) in a ∼1:1:1:1 ratio, as determined by analytical HPLC. These isomers were also easily separable by HPLC, using a preparative chiralCel OD column. Alternatively, two of the above four diasteromers, that is, **15b** and **15c**, could be obtained using 3-chloro-2,5-dimethylpyrazine **17** as starting material. Catalytic hydrogenation of dimethyl-substituted triazolopyrazine **18** followed by coupling with acid **19** gave rise to only two isomers, which were identical with **15b** and **15c**, as confirmed by chiral HPLC analysis. These isomers were tentatively assigned as *cis*-dimethyl diastereomers, with 5*S*,8*S* and 5*R*,8*R* stereochemistry, respectively, arising from the anticipated Pd-mediated *cis*-reduction of the pyrazine. In a second approach, oxadiazole intermediate **20**, the preparation of which was reported earlier,[15] was treated with a variety of substituted ethylenediamines (**21**, **24**, and **27**) to give a mixture of regioisomers with known stereochemistry (Scheme 2). For example, treatment of oxadiazole **20** with (*S*)-(−)-1,2-diaminopropane **21** in refluxing methanol followed by *N*-Boc-protection gave a mixture of two regioisomers, **22a** and **22b** with (*S*)-stereochemistry in ∼2.4:1 ratio, resulting from the preferred displacement of chlorine in oxadiazole **20** by the less-hindered nitrogen of the diamine as reported earlier.[15] These regioisomers (**22a** and **22b**) were easily separable by flash chromatography. Subsequent deprotection of **22a** followed by coupling with the β-aminoacid **19** provided the desired regioisomer **12a**. Oxadiazole **20** was likewise treated with (*R*)-(+)-1,2-diaminopropane **24** and converted to the corresponding regioisomers **25a** and **25b** with (*R*)-stereochemistry. Coupling products obtained from both major isomers **22a** and **25a** turned out to be identical with compound **11a** and **11b**, respectively, as indicated by chiral HPLC. Compound **28** with 5,5-*gem*-dimethyl groups was prepared in an exclusive manner by treatment of oxadiazole **20** with 1,2-diamino-methylpropane **27**.

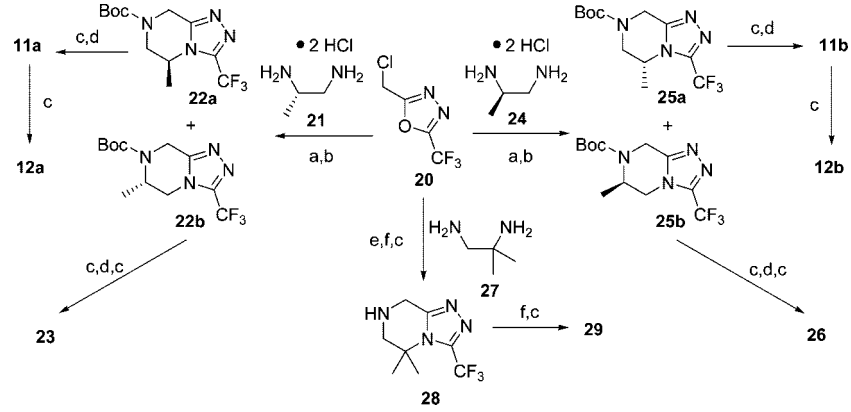
Alkylation at the 8-position of the triazolopiperazine moiety was conveniently achieved by using an *N*-Boc-assisted alkylation strategy (Scheme 3). Deprotonation of the *N*-Boc compound **31**, prepared by Boc protection of the previously reported triazolopiperazine **30**,[10] with *n*-butyllithium followed by the treatment with iodomethane afforded the desired 8-methyl-substituted compound **32**. Deprotection of the *N*-Boc group of heterocycle **32** followed by coupling with β-aminoacid **19** provided a mixture of **33a** and **33b**, which were easily separable by chiral HPLC using a preparative chiralCel OD column as in the previous cases. Absolute stereochemistry of the methyl group at the 8-position of the

Scheme 1[a]



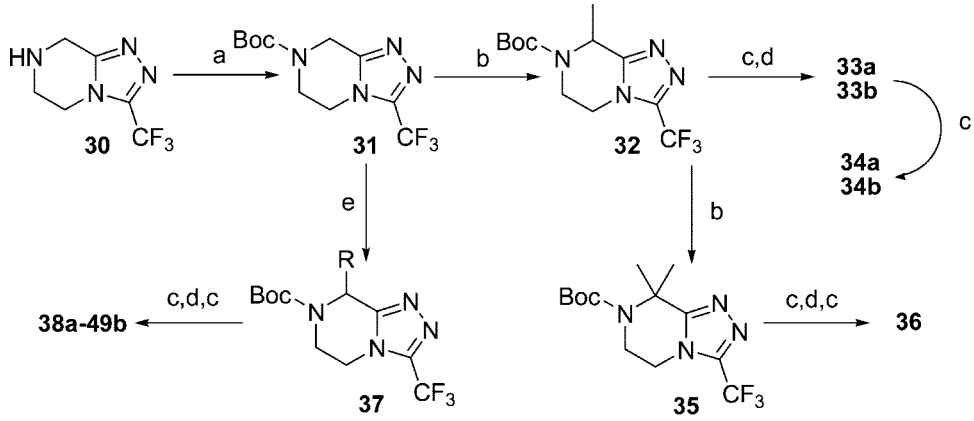
[a] Reagents: (a) NH_2-$NH_2 \cdot H_2O$, ~100 °C, 30 min.; (b) (i) $(CF_3CO)_2O$, 0 °C to room temperature, (ii) PPA, 120 °C, 18 h; (c) H_2, 10% Pd/C, EtOH/THF; (d) **19**, EDC, DMF, then chiral separation of **11a** and **11b** on ChiralCel OD column; (e) satd HCl/MeOH; (f) $(Boc)_2O$, CH_2Cl_2; (g) toluene, TMEDA, *n*-BuLi, −78 °C, 10 min, then MeI; (h) **19**, HOAT, HATU, DIPEA, DMF, rt, 18 h.

Scheme 2[a]



[a] Reagents: (a) DIPEA, MeOH, reflux; (b) $(Boc)_2O$, CH_2Cl_2, then separation of isomers by flash chromatography; (c) satd HCl/MeOH; (d) **19**, HOAT, HATU, DIPEA, DMF, rt, 18 h; (e) (i) DIPEA, MeOH, rt, 2 h, (ii) superphosphoric acid, 110 °C, 18 h; (f) **19**, EDC, DMF, rt, 18 h.

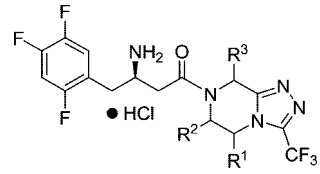
Scheme 3[a]



[a] Reagents: (a) $(Boc)_2O$, CH_2Cl_2; (b) toluene, TMEDA, *n*-BuLi, −78 °C, 10 min, then MeI; (c) satd HCl/MeOH; (d) **19**, HOAT, HATU, DIPEA, DMF, rt, 18 h, then chiral separation of **33a** and **33b** on chiralCel OD column; (e) toluene, TMEDA, *n*-BuLi, −78 °C, 10 min, then R′CH_2-Br or R′CHO.

triazolopiperazine moiety in compound **34b** was definitively assigned by X-ray crystal structure determination to be (*R*). Repeated methylation with intermediate **32** provided 8,8-*gem*-dimethyl-substituted compound **35**. Similarly, a variety of alkyl- or benzyl-substituted compounds in Table 2 were prepared by the *N*-Boc-assisted alkylation strategy using intermediate **31**, except that ethyl analogues **38a** and **38b** were prepared using a procedure similar to that described for the preparation of **12a** and **12b**. Trapping of an anion intermediate from **31** with 4-fluorobenzaldehyde provided a mixture of diasteromers, which were converted to hydroxy compounds (**47a**−**d**). Absolute stereochemistry of the 4-fluo-

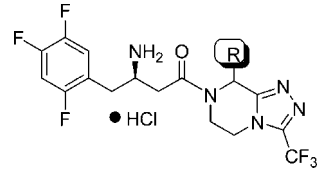
Table 1. Effects of Methyl Substituents around Triazolopiperazines on Inhibitory Properties[a]



cmpd	R^1	R^2	R^3	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)	DPP-9 IC_{50} (nM)
1	H	H	H	18	>100000	48000	>100000
12a	(*S*)-CH_3	H	H	23	>100000	23000	23000
12b	(*R*)-CH_3	H	H	14	>100000	33000	53000
23	H	(*S*)-CH_3	H	91	74000	>100000	53000
26	H	(*R*)-CH_3	H	42	>100000	75000	>100000
34a	H	H	(*S*)-CH_3	88	>100000	>100000	>100000
34b	H	H	(*R*)-CH_3	4.3	>100000	17000	>100000
29	*di*-CH_3	H	H	92	77000	66000	54000
36	H	H	*di*-CH_3	175	23000	6000	20000
16a	CH_3	H	CH_3	100	>100000	>100000	>100000
16b	CH_3	H	CH_3	209	>100000	>100000	>100000
16c	CH_3	H	CH_3	12	>100000	70000	52000
16d	CH_3	H	CH_3	11	>100000	44000	62000

[a] The IC_{50} results are an average of three independent titrations, unless otherwise noted, having calculated standard errors below 15%.

Table 2. Inhibitory Properties of Alkyl-Substituted Triazolopiperazine Analogues[a]



cmpd	R	DPP-4 IC_{50}(nM)	QPP IC_{50}(nM)	DPP-8 IC_{50}(nM)	DPP-9 IC_{50}(nM)
38a	-Et	113	>100000	>100000	>100000
38b	-Et	5.0	47000	8000	>100000
39a	-CH_2CF_3	123	30000	>100000	>100000
39b	-CH_2CF_3	5.7	60000	1600	26000
40a	-$CH_2CH{=}CH_2$	1.5	33000	3000	41000
40b	-$CH_2CH{=}CH_2$	32	62000	72000	>100000
41a	-$CH_2CON(CH_3)_2$	377	>100000	>100000	>100000
41b	-$CH_2CON(CH_3)_2$	2.8	>100000	30000	>100000
42a	-CH_2Ph	140	87000	>100000	>100000
42b	-CH_2Ph	0.66	52000	622	24000
43a	-CH_2(4-methoxyphenyl)	320	>100000	>100000	>100000
43b	-CH_2(4-methoxyphenyl)	0.43	57000	367	18000
44a	-CH_2 (2-trifluoromethylphenyl)	438	83000	>100000	>100000
44b	-CH_2 (2-trifluoromethylphenyl)	0.31	41000	8000	>100000
45a	-CH_2(2-fluorophenyl)	131	76000	>100000	>100000
45b	-CH_2(2-fluorophenyl)	0.46	47000	1103	39000
46a	-CH_2(4-fluorophenyl)	116	39000	>100000	>100000
46b	-CH_2(4-fluorophenyl)	0.18	33000	332	20000
47a	-CH(OH)(4-fluorophenyl)	430	48000	>100000	>100000
47b	-CH(OH)(4-fluorophenyl)	0.32	36000	326	89000
47c	-CH(OH)(4-fluorophenyl)	90	>100000	40000	>100000
47d	-CH(OH)(4-fluorophenyl)	0.50	41000	628	>100000
48a	-CH_2 (3,5-*bis*-trifluoromethylphenyl)	587	>100000	>100000	>100000
48b	-CH_2 (3,5-*bis*-trifluoromethylphenyl)	6.3	>100000	>100000	>100000
49a	-CH_2(2-pyridyl)	132	>100000	>100000	>100000
49b	-CH_2(2-pyridyl)	0.40	>100000	5000	>100000

[a] When two diasteromers with unknown stereochemistry at the 8-position were obtained, they were designated with letters "a" and "b" in the order of elution. Four diasteromers (**47a**−**d**) with additional unknown stereochemistry of OH substituent were designated with letters "a−d" in the order of elution. Based on the X-ray structure determination of **46b**, slower eluting isomers were tentatively assigned as (*R*)-isomers. The IC_{50} results are an average of three independent titrations, unless otherwise noted, having calculated standard errors below 15%.

robenzyl group at the 8-position of the triazolopiperazine in compound **46b** was again assigned by X-ray structure of **46b** in complex with DPP-4 to be (*R*).

Results and Discussion

Compounds **12a**−**49b** were evaluated in vitro for their inhibition of DPP-4.[16] The inhibitors were also tested against DPP-4 homologues in the DPP-4 gene family, including DPP-8,[17] DPP-9,[18] fibroblast activation protein (FAP, also called seprase),[19] and other proline specific enzymes with DPP-4 like activity, including quiescent cell proline dipeptidase (QPP, also known as DPP-II and DPP-7),[20] amino peptidase P, and prolidase. None of the compounds in this report showed any significant inhibition against these other enzymes (IC_{50}s >10000

nM). Because significant QPP off-target activity was often observed for the β-aminoacid derived DPP-4 inhibitors reported from these laboratories earlier,[13] QPP data are presented for comparison. Safety studies using a DPP8/9 selective inhibitor suggest that inhibition of DPP8 and DPP9 is associated with profound toxicity in rats and dogs.[21] Thus, selectivity profiles against DPP-8 and DPP-9 were also obtained for safety reasons.

In vitro inhibitory activities for the selected triazolopiperazine-based DPP-4 inhibitors are listed in Table 1. Pronounced SARs were evident in this series. 5-Methyl analogues (**12a** and **12b**) were similar in potency to compound **1** (DPP-4 $IC_{50} = 18$ nM). A slight preference for (*R*)- stereochemistry over (*S*) at the 5-position was observed (**12a** vs **12b**). A stereochemical preference was more evident in 6-methyl analogues (**23** vs **26**) and even more so in 8-methyl analogues (**34a** vs **34b**). Notably, compound **34b** with (*R*)-methyl at the 8-position, which was 20-fold more potent than its corresponding (*S*)-isomer, **34a**, showed a 4-fold increase in potency over the parent compound **1**. Introduction of *gem*-dimethyl groups at either the 5- or the 8-position resulted in a significant decrease in the DPP-4 potency (**29** and **36**). Interestingly, two of the four 5,8-dimethyl analogues (**16c** and **16d**) were slightly more potent than compound **1**.

A potency enhancing effect of a substituent at the 8-position of triazolopiperazines was again observed with a variety of alkyl- or benzyl-substituted compounds, as shown in Table 2. The unequivocal stereochemical assignment can be made only to the pair of **46a** and **46b** based on the X-ray structure determination of **46b**. When two diasteromers with unknown stereochemistry at the 8-position were obtained, they were designated with letters "a" and "b" in the order of elution. Four diasteromers (**47a**−**d**) with additional unknown stereochemistry of OH substituent were designated with letters "a−d" in the order of elution. One carbon elongated analogues with an ethyl or trifluoroethyl substituent (**38b** and **39b**) showed similar potency to compound **34b**, with increased DPP-8 activity (2-fold and 10-fold, respectively). The markedly increased potency against both DPP-4 and DPP-8 was evident in allyl analogue **40a** (1.5 nM and 3000 nM, respectively). It was notable that an amide substituent restored the DPP-4 selectivity over DPP-8 (**41b**; DPP-4 $IC_{50} = 2.8$ nM; DPP-8 $IC_{50} = 30000$ nM). Incorporation of a benzyl, substituted benzyl, or a pyridyl group into the 8-position of triazolopiperazine moiety (**42**−**49**) resulted in a dramatic improvement of DPP-4 potency over compound **1**, although submicromolar DPP-8 activities were often observed. In general, slower eluting isomers (**41b**−**49b**), tentatively assigned as (*R*)-isomers, with superior potency against DPP-4 were >200-fold more potent than their corresponding faster eluting isomers (**41a**−**49a**), except for compound **40**. The paramount effect of the substituent on the inhibition of DPP-4 was demonstrated in compound **46b** with a (*R*)-4-fluorobenzyl substituent, which was ~600- and 100-fold more potent than its corresponding (*S*)-isomer, **46a**, and compound **1**, respectively. Compound **46b** is the most potent, noncovalent DPP-4 inhibitor reported to date from these laboratories. While the DPP-8 activity (332 nM) was unacceptable, DPP-4 selectivity over DPP-8 is still >1000-fold (0.18 nM vs 332 nM).

Because inhibition of DPP-8 and DPP-9 is reported to be associated with significant toxicity, this issue had to be addressed by modifications on these extremely potent DPP-4 inhibitors. The effect of a hydrophilic group, for example, the -OH group, on the DPP-4 selectivity over DPP-8 was very small (<2-fold), as observed with compounds **47b** and **47d** compared to that of **46b**. Surprisingly, DPP-8 activity was significantly reduced in both **44b** and **48b** by employing 2-CF_3-phenyl and 3,5-bis-CF_3-phenyl group, respectively ($IC_{50} = 8000$ nM and >100000 nM, respectively), while maintaining superior DPP-4 potency ($IC_{50} = 0.31$ nM and 6.3 nM, respectively). A pyridyl heterocycle was also highly effective in lowering DPP-8 potency (5000 nM), and compound **49b** showed significantly increased DPP-4 selectivity over DPP-8.

Table 3. Pharmacokinetic Profiles of Selected DPP-4 Inhibitors[a]

cmpd	species	Cl_p (mL/min/kg)	$t_{1/2}$ (h)	PO AUC_{norm} (μM·h/mpk)	POC_{max} (μM)	*F* (%)
1	rat	60	1.7	0.52	0.33	76
12b	rat	154	1.9	0.090	0.058	35
34b	rat	54	2.4	0.422	0.133	57
	dog	7.8	8.8	4.591	0.756	91
	monkey	29	6.0	1.176	0.410	85
41b	rat	87	2.7	0.034	0.004	9
16c	rat	85	2.1	0.206	0.092	45
45b	rat	74	1.4	0.116	0.064	26
46b	rat	29	1.5	0.929	0.360	76
47b	rat	63	2.6	0.038	0.020	8
48b	rat	50	4.1	0.103	0.042	19
49b	rat	94	1.9	0.032	0.016	9

[a] The reported data are an average generated after 1 mg/kg iv and 2 mg/kg po doses in $n = 2$ animals/dose, except for orally dosed rats where $n = 3$. Dose solution was 1.0 mg/mL in ethanol/water (5:95, v/v) for both routes of administration.

Compounds with superior potency along with high DPP-4 selectivity were selected and further evaluated in pharmacokinetic studies (Table 3). While compound **12b** is as potent as compound **1** against DPP-4, it showed decreased oral bioavailability ($F = 35\%$) with very high clearance (154 mL/min/kg) and poor systemic exposure after oral dosing. Unlike compound **12b**, compound **34b** exhibited good oral bioavailability in rats ($F = 57\%$). In addition, compound **34b** showed excellent oral bioavailability in dogs and monkeys (91% and 85%, respectively). Clearance is relatively high in rats and monkeys (54 and 29 mL/min/kg, respectively), but lower in dogs (7.8 mL/min/kg) as in the case of compound **1**. It was notable that compound **34b** showed similar pharmacokinetic profiles with significantly increased half-lives in dogs and monkeys (8.8 and 6.0 h, respectively) compared to that of compound **1** (4.9 and 3.7 h, respectively). While amide-substituted compound **41b** showed poor oral bioavailability ($F = 9\%$), compound **16c**, incorporating methyl groups at both the 5- and 8-positions of the triazolopiperazine moiety, showed fair oral bioavailability in rats ($F = 45\%$) with higher clearance (85 mL/min/kg) and lower oral exposure compared to that of compound **34b**. Compounds **45b** with 2-fluorobenzyl and **46b** with 4-fluorobenzyl exhibited a pronounced difference in oral bioavailability in the rat (26% and 76%, respectively). Notably, oral bioavailability of compound **46b** in Zucker fa/fa rats was excellent ($F = 100\%$) but poor in mice ($F = 6\%$). Hydroxy compound **47b** exhibited poor oral bioavailability in rats ($F = 8\%$) with high clearance (63 mL/min/kg) and poor oral exposure. Both compounds with high DPP-4 selectively over DPP8, **48b** with bis-CF_3 and **49b** with a pyridyl group, showed poor oral bioavailability in the rat, 19 and 9%, respectively.

Compound **34b** showed high selectivity (>1000-fold) for DPP-4 over the other proline specific peptidases. Further profiling in an extensive panel of receptor and ion channel binding and enzyme inhibition assays showed no significant activity (data not shown).

X-ray crystal structure determination for two potent compounds, **34b** and **46b** (Figures 1 and 2, respectively), in complex with DPP-4 enzyme revealed that the absolute stereochemistry

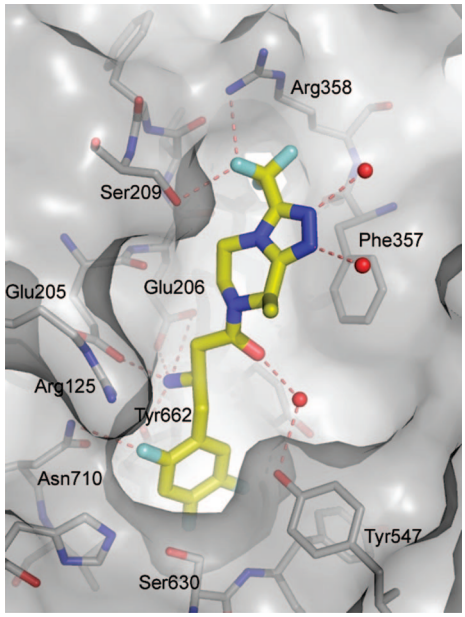


Figure 1. Compound **34b** bound to DPP-4.

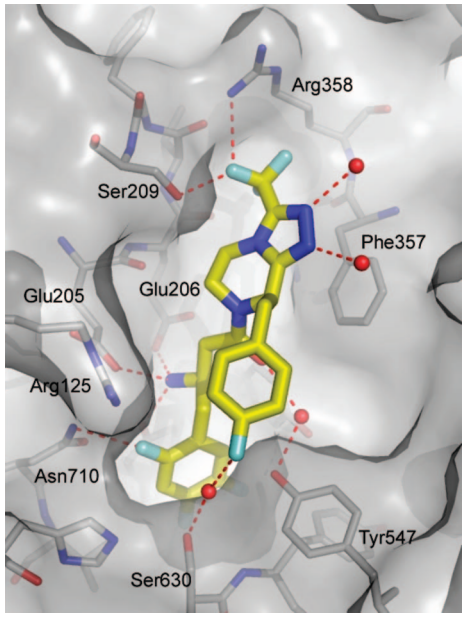


Figure 2. Compound **46b** bound to DPP-4.

at the 8-position of the triazolopiperazine in the more potent diastereomer is (*R*). As in the case of compound **1**, the 2,4,5-trifluorophenyl moiety fully occupies the S1 hydrophobic pocket, and the (*R*)-β-amino group forms four hydrogen bonding interactions with the side chains of a tyrosine (Tyr662) and two glutamate residues (Glu205 and Glu206). A water molecule bridges the carboxylic oxygen and the hydroxyl of Tyr547. Several other water-mediated interactions are also present between the nitrogen atoms of the triazolopiperazine and protein atoms. The triazolopiperazine is stacked against the side chain of Phe357. The trifluoromethyl substituent interacts with the side chains of Arg358 and Ser209. In compound **34b**, the (*R*)-methyl group at the 8-position of the triazolopiperazine points toward a relatively open area of the DPP-4 binding site and provides further surface complementarity to the side chain of Phe357, thus, contributing to the about 4-fold enhancement of potency observed for **34b** in comparison to compound **1** (Table 1). The structure also explains the decrease in potency observed with compound **34a** having the opposite stereochemistry at the methyl center (IC_{50} = 88 nM), because the (*S*)-methyl group would be within clashing distance from Phe357. In compound **46b**, the (*R*)-4-fluorobenzyl-group extends toward the same open area and interacts with the pocket formed by the side chains of Tyr547 and Arg125 (Figure 2), suggesting that relatively large groups are well tolerated at this position, as exemplified by compound **48b** with a bis-3,5-CF_3-benzyl group. The superior DPP-4 potency of compound **46b** (IC_{50} = 0.18 nM) may be attributed to the additional water molecule-bridged hydrogen bonding interaction between 4-fluorophenyl and Ser630.

Based on its excellent in vitro potency, selectivity, and pharmacokinetic profile, compound **34b** was chosen for the assessment of its ability to improve glucose tolerance in lean mice.[10a] Results on an oral glucose tolerance test (OGTT) and corresponding pharmacodynamic (PD) studies in lean mice are shown in Figure 3. Administration of single oral doses significantly reduced the dextrose-induced blood glucose excursion in a dose-dependent manner from 0.1 mg/kg (34% reduction) to 1.0 mg/kg (55% reduction) when administered 60 min before an oral dextrose challenge (5 g/kg; Figure 3a). In a separate OGTT experiment, the PD profile of compound **34b** was determined. Plasma DPP-4 inhibition, compound concentration, and active GLP-1 levels were measured 20 min after dextrose challenge (Figure 3). Maximal efficacy was observed at 1 mg/kg, corresponding to a plasma concentration of approximately 190 nM and >80% inhibition of plasma DPP-4 activity.[22] The inhibition of DPP-4, achieved with compound **34b** at 1 mg/kg, was comparable to the inhibition observed with compound **1** at 3.0 mg/kg. Maximal efficacy at a 1 mg/kg dose resulted in a 3-fold increase in active GLP-1, analogous to what is observed upon glucose challenge in DPP-4-deficient mice[23] and observed with compound **1** at 3 mg/kg. These results were in agreement with the improved DPP-4 activity of **34b** compared to compound **1** in mice (IC_{50} = 11 nM vs 69 nM). Acute lowering of blood glucose was also demonstrated in diet-induced obese (DIO) mice, which are hyperglycemic and hyperinsulinemic and show impaired glucose tolerance in response to a dextrose challenge consistent with the insulin resistance observed in type 2 diabetes mellitus (Figure 4). Near normalization of the glucose excursion relative to lean controls was seen following a 3 mg/kg oral dose of compound **34b**.

Conclusions

A series of β-aminoamides bearing triazolopiperazines have been prepared and evaluated as potent, selective, and orally active DPP-4 inhibitors. It was demonstrated that β-aminoacids in conjunction with triazolopiperazines with the appropriate substitution provide extremely potent DPP-4 inhibitors showing high selectivity over other related enzymes, good pharmacokinetic profiles, and high in vivo efficacy in an OGTT in lean mice. It was also demonstrated that (*R*)-stereochemistry at the 8-position of the triazolopiperazine moiety is strongly preferred over (*S*), with respect to DPP-4 inhibition, as confirmed by X-ray crystal structure determination for the potent compounds **34b** and **46b** in complex with DPP-4 enzyme. Fine tuning of relative inhibitory properties against DPP-4 and DPP-8 by variations of substituents (R^1, R^2, and R^3) around the triazolopiperazine moiety provided a series of potent DPP-4 inhibitors suitable for further studies. Among three substituents, R^3, with (*R*)-stereochemistry at the 8-position, was of critical importance for the superior potency and more effective than R^1 at the 5-position. R^2 at the 6-position was least effective. Compound **34b** (IC_{50} = 4.3 nM), showing a 4-fold increase in DPP-4 activity over

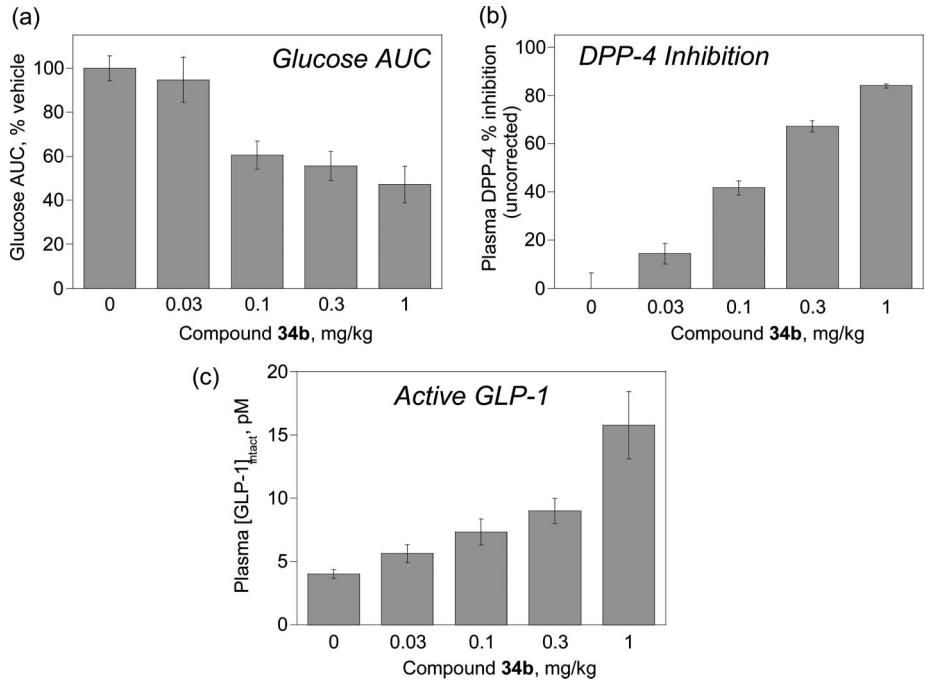


Figure 3. Effects of compound **34b** on (a) glucose AUC, (b) DPP-4 inhibition, and (c) GLP-1 levels after an oral glucose tolerance test in lean C57BL/6N male mice. Compound **34b** was administered 60 min prior to an oral dextrose challenge (5 g/kg). Plasma samples were collected for analysis 20 min postdextrose administration. Data are represented as mean ± SEM ($n = 7$ group).

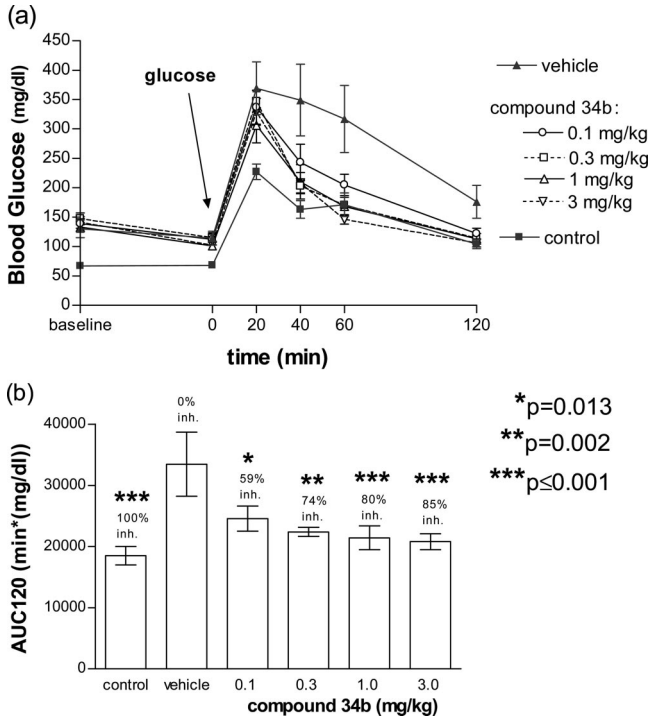


Figure 4. (a) Effect of compound **34b** on glucose levels after an oral glucose tolerance test in diet-induced obese (DIO) C57BL/6N mice. Compound **34b** or water (vehicle) was administered 60 min prior to an oral dextrose challenge (5 g/kg). Control animals received water only. (b) The glucose AUC was determined from 0 to 120 min. Percent inhibition values for each treatment were generated from the AUC data normalized to the dextrose-challenged lean controls. Data are represented as mean ± SEM ($n = 7$).

compound **1**, exhibited pronounced in vivo efficacy in the lean mice OGTT. With excellent DPP-4 potency, high selectivity, good pharmacokinetic profile, and in vivo efficacy in hand, we further profiled compound **34b**. Unfortunately, in studies in anesthetized dogs to assess cardiovascular activity, compound **34b** showed unacceptable dose-dependent prolongation of QRS and QTc intervals in the ECG, which precluded further development of compound **34b**.

Experimental Section

General. All commercial chemicals and solvents are reagent grade and were used without further purification, unless otherwise specified. ^{1}H NMR spectra were recorded on a Varian InNova 500 MHz instrument in $CDCl_3$ or CD_3OD solutions. Low-resolution mass spectra (MS) were determined on a Micromass Platform Liquid Chromatography–Mass Spectrometer (LC-MS), using a Waters Xterrra MSC18 3.5 μm, 50 × 3.0 mm column with a binary solvent system, where solvent A = water, 0.05% trifluoroacetic acid (by volume) and solvent B = acetonitrile, 0.05% trifluoroacetic acid (by volume). The LC method used a flow rate = 1.0 mL/min, with the following gradient: $t = 0$ min, 90% solvent A; $t = 3.75$ min, 2.0% solvent A; $t = 4.75$ min, 2% solvent A; $t = 4.76$ min, 90% solvent A; $t = 5.5$ min, 90% solvent A. High-resolution mass spectra were acquired from a Micromass Q-TOF quadrupole-time-of-flight mass spectrometer. All MS experiments were performed using electrospray ionization (EI) in positive ion mode. Leucine enkephalin was applied as a lock-mass reference for accurate mass analysis. The above-described LC-MS HPLC method serves as a gross analysis of purity over a broad range, and all final compounds show a single peak (>95% purity) using this analytical method. Purity of key target compounds was determined using Waters SunFireC18 4.6 × 50 mm column 5 μ, with a binary solvent system, where solvent A = water, 0.1% trifluoroacetic acid (by volume) and solvent B = acetonitrile, 0.1% trifluoroacetic acid (by volume). The LC method used a flow rate = 4.0 mL/min, with the following gradient: $t = 0$ min, 90% solvent A; $t = 4$ min, 100% solvent B; λ 214 nm. The final purity using these methods is noted with the analytical data for each compound. The *N*-Boc-protected compounds as a mixture of diastereomers were separated by HPLC using

ChiralCel OD, ChiralPak AD, or ChiralCel OJ column and 5∼15% ethanol/hexane or 5∼10% isopropanol/heptane as solvents.

5-Methyl-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine (9). To 2-chloro-6-methylpyrazine (2.925 g, 22.75 mmol) was added hydrazine hydrate (15 mL) at rt. The flask was immersed in a preheated oil bath (∼52 °C) and then heated up to 100 °C over 30 min (cloudy heterogeneous mixture became a clear, bright yellow solution). After being cooled to rt, the flask was kept in a refrigerator for 1 h. The white needle-shaped solid was filtered, washed with cold hydrazine hydrate, and dried to give the desired 2-hydrazino-6-methylpyrazine (1.460 g). The filtrate was kept in a refrigerator for 2 h and 0.869 g of a second crop of crystals was collected (82% yield): ^{1}H NMR (500 MHz, CD_3OD) δ 9.16 (s, 1H), 8.46 (s, 1H), 2.54 (s, 3H). MS *m*/*z* 124.9 $(M + H)^+$. To the above intermediate (2.320 g, 18.71 mmol) was added 50 mL of trifluoroacetic anhydride dropwise at 0 °C (highly exothermic!). After being stirred at rt for 1 h, the reaction mixture was concentrated to give a viscous material. To the above material was added ∼50 mL of polyphosphoric acid (PPA) and the reaction was stirred at 120 °C for 18 h. The hot PPA solution was added to ice and neutralized by the addition of ammonium hydroxide (highly exothermic!). The aqueous solution was extracted with ethyl acetate (3×), washed with brine, and dried over anhydrous $MgSO_4$. Concentration followed by flash chromatography (silica gel, 1:1 hexanes/ethyl acetate, then 100% ethyl acetate) afforded the title compound as a solid (1.421 g, 38% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (s, 1H), 7.94 (s, 1H), 2.83 (s, 3H). MS *m*/*z* 203.0 $(M + H)^+$.

5-Methyl-3-(trifluoromethyl)-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyrazine (10). 5-Methyl-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*] pyrazine **9** (720 mg, 3.56 mmol) was hydrogenated under a hydrogen atmosphere (balloon) with 10% Pd/C (400 mg) as a catalyst in an ethanol/THF mixture (10 mL/5 mL) at rt for 18 h. The reaction mixture was filtered through celite and concentrated. Purification by flash chromatography (silica gel, 100% EtOAc, then 10% MeOH/CH_2Cl_2) gave 729 mg of the title compound as a colorless viscous oil (99% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 4.59 (m, 1H), 4.28 (d, 1H, J = 16.9 Hz), 4.10 (d, 1H, J = 16.9 Hz), 3.24 (dd, 1H, J = 4.4, 14.0 Hz), 3.10 (dd, 1H, J = 2.5, 13.9 Hz), 1.55 (d, 3H, J = 6.7 Hz). MS *m*/*z* 207.0 $(M + H)^+$.

***tert*-Butyl[(1*R*)-3-[(5*S*)-5-methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate (11a) and *tert*-Butyl[(1*R*)-3-[(5*R*)-5-methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate (11b). Method A.** To a solution of **10** (281 mg, 1.364 mmol) and β-aminoacid **19** (454 mg, 1.364 mmol) in DMF (2.5 mL) was added EDC (313.8 mg, 1.639 mmol). After being stirred at rt for 18 h, DMF was evaporated to give a viscous residue, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Concentration gave a 1:1 mixture of compound **11a** and compound **11b** (481.5 mg). Separation of a 100 mg portion of the mixture using chiral HPLC (chiral OD column) afforded compound **11a** (34.7 mg) and compound **11b** (39.5 mg) as solid (50% yield). **11a** (faster eluting): ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.00∼7.15 (m), 6.90∼6.95 (m), 5.68 (d, J = 18.1 Hz), 5.30∼5.40 (m), 5.24 (d, J = 16.9 Hz), 4.84 (d, J = 14.2 Hz), 4.76 (d, J = 16.7 Hz), 4.70∼4.72 (m), 4.49 (d, J = 18.3), 4.00∼4.25 (m), 3.69 (d, J = 14.9 Hz), 3.25 (d, J = 12.1), 2.80∼3.05 (m), 2.60∼2.75 (m), 1.55 (d, J = 6.2), 1.50 (d, J = 6.4 Hz), 1.41 (s), 1.38 (s). MS *m*/*z* 522.1 $(M + H)^+$. **11b** (slower eluting): ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.05∼7.15 (m), 6.90∼6.95 (m), 5.65 (d, J = 18.3 Hz), 5.35 (br d, J = 6.7 Hz), 5.17 (d, J = 17.2), 4.65∼4.86 (m), 4.47 (d, J = 18.3 Hz), 4.10∼4.40 (m), 4.01 (d, J = 13.5 Hz), 3.54 (d, J = 13.9 Hz), 3.27∼3.30 (m), 2.60∼3.10 (m), 1.45∼1.60 (m), 1.40 (s), 1.38 (s). MS *m*/*z* 522.1 $(M + H)^+$.

Method B. For **11a**, to a solution of **22a** (50.0 mg, 0.163 mmol) in MeOH (1 mL) was added saturated methanolic HCl solution (1 mL) at 0 °C. After being stirred at room temperature for 1 h, the solution was concentrated to give a deprotected product as a white solid (39.0 mg, 99% yield): LC/MS *m*/*e* 206.9 $(M + H)^+$. To the above solid (36 mg, 0.148 mmol) and β-aminoacid **19** (49.3 mg, 0.148 mmol) in DMF (1.5 mL) were added DIPEA (31 μL, 0.178 mmol), HOAT (24.2 mg, 0.178 mmol), and HATU (67.7 mg, 0.178 mmol) sequentially at rt. After being stirred at rt for 18 h, DMF was evaporated to give a viscous residue, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Concentration followed by purification by preparative TLC (100% EtOAc) afforded only compound **11a** (45 mg, 53% yield) as a foamy solid, which was identical to the compound **11a** separated from the mixture of diastereomers as judged by ^{1}H NMR, LC/MS, and HPLC. For **11b**, starting with **25a**, the same procedures were followed as in the above synthesis of **11a**.

(2*R*)-4-[(5*S*)-5-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (12a). To a solution of *N*-Boc-protected compound **11a** (29.5 mg, 0.057 mmol) in MeOH (1 mL) was added saturated methanolic HCl solution (1 mL) at 0 °C. After being stirred at rt for 1 h, the solution was concentrated to give the title compound (26.1 mg, 100% yield) as a white foamy solid. 100% purity by HPLC (t_r = 1.51 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.45 (m), 7.20∼7.30 (m), 5.52 (d, J = 16.4 Hz), 5.26 (d, J = 16.3 Hz), 4.75∼4.90 (m), 4.72 (d, J = 14.2 Hz), 4.55 (d, J = 17.9), 4.10∼4.20 (m), 3.85∼3.96 (m), 3.81 (br d), 3.12 (d, J = 6.4 Hz), 2.92∼2.97 (m), 1.46 (d, J = 5.9 Hz); MS *m*/*z* 422.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{17}H_{17}F_6N_5O$ $(M + H)^+$ *m*/*e*, 422.1415; found, 422.1427.

(2*R*)-4-[(5*R*)-5-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine (12b). Following the same procedure for the synthesis of **12a**, the title compound was prepared as a white foamy solid. 100% purity by HPLC (t_r = 1.52 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.50 (m), 7.20∼7.30 (m), 5.56 (d, J = 17.8 Hz), 5.27 (d, J = 16.5 Hz), 4.80∼4.90 (m), 4.75 (d, J = 13.3 Hz), 4.51 (d, J = 17.8), 4.14 (br d), 3.90∼4.00 (m), 3.78 (br d), 2.80∼3.20 (m), 1.54 (d, J = 5.3 Hz), 1.43 (d, J = 6.2 Hz); MS *m*/*z* 422.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{17}H_{17}F_6N_5O$ $(M + H)^+$ *m*/*e*, 422.1415; found, 422.1411.

***tert*-Butyl-5-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (13).** To a solution of **10** (1.031 g, 5.00 mmol) in CH_2Cl_2 (10 mL) was added $(Boc)_2O$ (1.091 g, 5.00 mmol) at rt. The solution was stirred at room temperature for 2 h. Concentration followed by flash chromatography (H/E = 1:1) gave the title compound (1.355

g, 89% yield) as a white, foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.25∼5.50 (m, 1H), 4.20∼4.65 (m, 3H), 3.25∼3.50 (m, 1H), 1.54 (s, 9H), 1.52 (d, 3H, J = 6.6 Hz); MS m/z 307.0 $(M + H)^+$.

5,8-Dimethyl-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]-triazolo[4,3-*a*]pyrazine (14). Method A. To a solution of **13** (1.009 g, 3.30 mmol) in toluene (14 mL) was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (547 μL, 3.63 mmol) followed by *n*-BuLi (1.60 M in hexanes, 2.50 mL, 3.99 mmol) at −78 °C. The brown colored solution was stirred at −78 °C for 10 min. After the addition of neat iodomethane (226 μL, 3.63 mmol) at −78 °C, the solution was stirred at −78 °C for an additional 10 min and then warmed to rt. After being stirred at rt for 2 h, the reaction was quenched with aqueous NH_4Cl. The aqueous layer was extracted with EtOAc (3×), washed with brine, and dried over anhydrous $MgSO_4$. Concentration followed by separation by preparative TLC (H/E = 1:1) afforded the methylated product as a solid (52.0 mg, 5% yield): MS m/z 321.0 (M + H). Following the same procedures as in the synthesis of **12a**, the above methylated product (51.2 mg, 0.128 mmol) was converted to the title compound as a foamy solid (45.5 mg, 80% yield). ^{1}H NMR (500 MHz, CD_3OD, mixture) δ 4.95∼5.10 (m, 1H), 4.02 (dd, 1H, J = 5.3, 13.8 Hz), 3.84 (d, 1H, J = 3.2 Hz), 3.53∼3.58 (m, 1H), 1.94 (d, 1.5H, J = 6.8 Hz), 1.87 (d, 1.5 H, J = 6.8 Hz), 1.68∼1.71 (m, 3H); MS m/z 220.9 $(M + H)^+$.

Method B. Starting with **17**, the same procedures were followed as in the synthesis of **10**.

***tert*-Butyl[(1*R*)-3-[5,8-dimethyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate (15a−d). Method A.** To a solution of **14** (35.0 mg, 0.136 mmol) and a β-aminoacid **19** (45.4 mg, 0.136 mmol) in DMF (1.0 mL) were added DIPEA (28.4 mL, 0.163 mmol), HOAT (22.2 mg, 0.163 mmol), and HATU (62 mg, 0.163 mmol) sequentially at rt. After being stirred at rt for 18 h, DMF was evaporated to give a viscous residue, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Concentration followed by purification by preparative TLC gave a mixture of four diastereomers (43 mg) as a solid. Separation of the mixture using chiral HPLC (chiral OD column) afforded compound **15a** (first eluting: 4.1 mg), compound **15b** (second eluting: 3.6 mg), **15c** (third eluting: 3.8 mg), and compound **15b** (fourth eluting: 3.5 mg; 21% yield). For **15a**, ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.05∼7.15 (m), 6.85∼6.95 (m), 6.06 (q), 5.30∼5.55 (m), 4.90 (d, J=14.0 Hz), 4.60∼4.70 (m), 4.00∼4.20 (m), 3.69 (dd, J = 2.7, 14.4 Hz), 3.22 (dd, J = 3.2, 14.2 Hz), 2.90∼3.10 (m), 2.80∼2.90 (br d), 2.56∼2.65 (m), 1.71 (d, J = 6.6 Hz), 1.66 (d, J = 6.9 Hz), 1.50 (d, J = 6.4 Hz), 1.45 (d, J = 6.4 Hz), 1.41 (s), 1.36 (s); MS m/z 558.0 (M + Na); for **15b**, ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.05∼7.16 (m), 6.82–6.98 (m), 5.92–6.12 (m), 5.23–5.48 (m), 4.84–5.04 (m), 4.35–4.56 (m), 4.16 (q, J=7.3), 3.37–3.58 (m), 2.97 (d, J = 7.8 Hz), 2.80 (dd, J = 3.2,12.8 Hz), 2.62 (dd, J = 6.0, 16.0 Hz), 1.70 (d, J = 16.3 Hz), 1.66 (d, J = 6.2 Hz), 1.41 (s), 1.38 (s); MS m/z 536.3 $(M + H)^+$; for **15c**, ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.91 (q, J = 8.7 Hz), 6.91 (q, J = 9.6 Hz), 5.96–6.12 (m), 5.29–5.49 (m), 4.88–5.05 (m), 4.35–4.46 (m), 4.15 (q, J = 7.1 Hz), 3.34–3.55 (m), 2.93–3.04 (m), 2.64–2.80 (m), 1.62–1.78 (m), 1.40 (s); MS m/z 536.3 $(M + H)^+$; for **15d**, ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.05∼7.15 (m), 6.85∼6.95 (m), 6.06 (q), 5.30∼5.45 (m), 4.90 (d, J = 14.1 Hz), 4.60∼4.70 (m), 4.10∼4.25 (m), 3.92 (d, J = 14.4 Hz), 3.70 (dd, J = 3.0, 14.6 Hz), 3.24 (dd, J = 3.2, 14.2 Hz), 2.90∼3.10 (m), 2.70∼2.85 (m), 2.65 (dd, J = 5.0, 15.8 Hz), 1.69 (d, J = 6.9 Hz), 1.64 (d, J = 6.8 Hz), 1.49 (d, J = 6.4 Hz), 1.45 (d, J = 6.4 Hz), 1.41 (s), 1.40 (s); MS m/z 558.1 (M + Na).

Method B for the Synthesis of 15b and 15c. Starting with **14** derived from **17**, the same procedures were followed as in the synthesis of **15a**−**d**. Only two isomers obtained using Method B for the synthesis of compound **14** were identical with the compounds **15b** and **15c**, respectively, as judged by ^{1}H NMR, LC/MS, and HPLC.

(2*R*)-4-[5,8-Dimethyl-3-(trifluoromethyl)-5,6-dihydro-[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (16a−d). Compounds **16a**−**d** were prepared following the same procedure for the synthesis of **12a**−**b**. For **16a**, 100% purity by HPLC (t_r = 1.60 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.42 (m), 7.20∼7.30 (m), 5.89 (q, J = 6.9 Hz), 5.52 (q, J = 6.6 Hz), 4.05 (d, J = 15.1 Hz), 3.85∼3.95 (m), 3.66 (dd, J = 3.0, 14.6 Hz), 3.40 (dd, J = 3.5, 14.2 Hz), 3.05∼3.16 (m), 3.03 (dd, J = 7.1, 17.4 Hz), 2.93 (dd, J = 4.8, 17.6 Hz), 2.86 (d, J = 6.7 Hz), 1.65 (d, J = 6.9 Hz), 1.61 (d, J = 6.9 Hz), 1.41 (m); MS m/z 436.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{18}H_{19}F_6N_5O$ $(M + H)^+$ m/e, 436.1572; found, 436.1585. For **16b**, 100% purity by HPLC (t_r = 1.58 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.38 (dd, J = 8.6, 17.3 Hz), 7.20 (dd, J = 10.1, 16.9 Hz), 5.81–5.89 (m), 5.39–5.56 (m), 4.51–4.62 (m), 4.43–4.50 (m), 4.18 (d, J = 14.1 Hz), 3.89 (t, J = 5.5 Hz), 3.50–3.72 (m), 3.31–3.32 (m), 3.09 (d, J = 7.1 Hz), 2.80–2.91 (m), 1.50–1.76 (m); MS m/z 436.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{18}H_{19}F_6N_5O$ $(M + H)^+$ m/e, 436.1572; found, 436.1576. For **16c**, 100% purity by HPLC (t_r = 1.56 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.34–7.46 (m), 7.22 (dd, J=9.9, 16.5 Hz), 5.86–5.96 (m), 5.40–5.48 (m), 4.60–4.70 (m), 4.34–4.46 (m), 4.21 (d, J=14.4 Hz), 3.88–3.96 (m), 3.52–3.65 (m), 3.04–3.16 (m), 2.94–3.02 (m), 1.55–1.73 (m); MS m/z 436.3 $(M + H)^+$; HRMS (ES^+) calcd for $C_{18}H_{19}F_6N_5O$ $(M + H)^+$ m/e, 436.1572; found, 436.1585. For **16d**, 100% purity by HPLC (t_r = 1.60 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) 7.30∼7.45 (m), 7.20∼7.30 (m), 5.76 (q, J = 6.9 Hz), 5.50 (q, J = 6.8 Hz), 4.75∼4.80 (m), 4.05 (d, J = 15.2 Hz), 3.90∼4.00 (m), 3.81 (dd, J = 2.2, 14.6 Hz), 3.42 (dd, J = 3.2, 14.4 Hz), 3.05∼3.20 (m), 2.99 (dd, J = 3.2, 17.6 Hz), 2.70∼2.85 (m), 1.68 (d, J = 6.9 Hz), 1.57 (d, J = 6.9 Hz), 1.50 (d, J = 6.6 Hz), 1.37 (d, J = 6.4 Hz); MS m/z 436.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{18}H_{19}F_6N_5O$ $(M + H)^+$ m/e, 436.1572; found, 436.1577.

5,8-Dimethyl-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine (18). Starting with 3-chloro-2,5-dimethylpyrazine, the same procedures were followed as in the synthesis of **9**. For **18**, ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 3.01 (s, 3H), 2.75 (s, 3H); MS m/z 217.3 $(M + H)^+$.

***tert*-Butyl-(5*S*)-5-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (22a) and *tert*-Butyl-(6*S*)-6-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (22b).** To **20** (3.11 g, 16.8 mmol)[15] and (*S*)-(−)-1,2-diaminopropane **21** (2.47 g, 16.8 mmol) in MeOH (20 mL) was added DIPEA (8.78 mL, 50.4 mmol) at rt. The solution was stirred at rt for 4 h and then refluxed for 18 h. After concentration, the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Concen-

tration gave a viscous oil (1.05 g, 30% yield): MS *m/z* 206.9 (M + 1). To the above crude oil (1.05 g, 5.1 mmol) in CH_2Cl_2 (10 mL) was added $(Boc)_2O$ (1.20 g, 5.50 mmol) at rt. The solution was stirred at rt for 1 h. Concentration followed by flash chromatography (H/E = 4:1) gave **22a** (103 mg) and **22b** (243 mg; 22% yield). For **22a**, ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (d, 1H, J = 17.8 Hz), 4.90∼5.00 (br s, 1H), 4.48 (d, 1H, J = 18.0 Hz), 4.20 (dd, 1H, J = 4.5, 12.5 Hz), 4.07 (d, 1H, J = 12.5 Hz), 1.53 (s, 9H), 1.26 (d, 3H, J = 7.1 Hz); MS *m/z* 307.0 $(M + H)^+$. For **22b**, ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20∼5.50 (m, 1H), 4.20∼4.70 (m, 3H), 3.25∼3.50 (m, 1H), 1.54 (s, 9H), 1.52 (d, 3H, J = 6.9 Hz); MS *m/z* 307.0 $(M + H)^+$.

(2*R*)-4-[(6*S*)-6-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (23). Starting with **22b**, the same procedures were followed as in the synthesis of **12a**. Purity (100%) by HPLC (t_r = 1.50 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.45 (m), 7.20∼7.30 (m), 5.64 (d, J = 18.3 Hz), 5.30∼5.45 (br s), 5.17 (d, J = 17.0 Hz), 4.70∼4.90 (m), 4.10∼4.50 (m), 3.85∼4.00 (br s), 2.90∼3.20 (m), 2.75∼2.85 (m), 1.30 (d, J = 5.7 Hz), 1.23 (d, J = 6.4 Hz); MS *m/z* 422.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{17}H_{17}F_6N_5O$ $(M + H)^+$ *m/e*, 422.1415; found, 422.1422.

***tert*-Butyl-(5*R*)-5-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (25a) and *tert*-Butyl-(6*R*)-6-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (25b).** Starting with **20** (1.433 g, 7.75 mmol) and (*R*)-(+)-1,2-diaminopropane **24** (1.139 g, 7.75 mmol), the same procedures were followed as in the synthesis of **22a** and **22b**. For **25a**, ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20∼5.50 (m, 1H), 4.10∼4.70 (m, 3H), 3.20∼3.50 (m, 1H), 1.53 (s, 9H), 1.50 (s, 3H); MS *m/z* 307.0 $(M + H)^+$; for **25b**, ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.29 (d, 1H, J = 17.9 Hz), 4.90∼5.00 (br s, 1H), 4.45 (d, 1H, J = 17.9 Hz), 4.19 (dd, 1H, J = 4.8, 12.8 Hz), 4.07 (d, 1H, J = 12.6 Hz), 1.52 (s, 9H), 1.25 (d, 3H, J = 7.1 Hz); MS *m/z* 307.0 $(M + H).^+$

(2*R*)-4-[(6*R*)-6-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (26). Starting with **25b**, the same procedures were followed as in the synthesis of **12a**. Purity (95%) by HPLC (t_r = 1.48 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.45 (m), 7.20∼7.30 (m), 5.56 (d, J = 18.3 Hz), 5.30∼5.40 (br s), 5.15 (d, J = 16.7 Hz), 4.65∼4.90 (m), 4.45 (d, J = 18.3 Hz), 4.20∼4.40 (m), 3.90∼4.00 (br s), 3.00∼3.15 (m), 2.75∼2.95 (m), 1.31 (d, J = 6.1 Hz), 1.19 (d, J = 6.8 Hz); MS *m/z* 422.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{17}H_{17}F_6N_5O$ $(M + H)^+$ *m/e*, 422.1415; found, 422.1415.

5,5-Dimethyl-3-(trifluoromethyl)-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyrazine (28). To **20** (2.17 g, 11.73 mmol) and **27** (1.23 mL, 11.73 mmol) in MeOH (10 mL) was added DIPEA (2.04 mL, 11.73 mmol) at 0 °C. The solution was stirred at 0 °C for 30 min and at rt for 2 h. After filtering off a white solid, the filtrate was concentrated to give a viscous oil, which was dissolved in superphosphoric acid (20 mL). After being stirred at 110 °C for 18 h, the mixture was poured into ice, then the mixture was adjusted to basic pH with NH_4OH. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Concentration followed by flash chromatography (10% MeOH/CH_2Cl_2, then CH_2Cl_2/MeOH/NH_4OH = 80:15:1) gave the title compound (201 mg, 8% yield): ^{1}H NMR (500 MHz, CD_3OD) δ 4.19 (s, 2H), 3.06 (s, 2H), 1.62 (s, 6H); MS *m/z* 220.9 $(M + H)^+$.

(2*R*)-4-[5,5-Dimethyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (29). Starting with **28**, the same procedures were followed as in the synthesis of **12a**. 94.9% purity by HPLC (t_r = 1.63 min).^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.36∼7.46 (m), 7.20∼7.30 (m), 4.90∼5.15 (m), 3.80∼4.00 (m), 2.85∼3.20 (m), 1.67 (s), 1.66 (s), 1.64 (s), 1.60 (s); MS *m/z* 436.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{18}H_{19}F_6N_5O$ $(M + H)^+$ *m/e*, 436.1572; found, 436.1567.

***tert*-Butyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo-[4,3-*a*]pyrazine-7(8*H*)-carboxylate (31).** To a solution of **30** (20.11 g, 88.01 mmol)[10a] in CH_2Cl_2 (150 mL) was added $(Boc)_2O$ (19.21 g, 88.01 mmol) at rt. The solution was stirred at rt for 4 h. Concentration followed by flash chromatography (H/E = 1:1) gave the title compound (23.24 g, 90% yield) as a white foamy solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.90 (s, 2H), 4.17 (t, 2H, J = 5.5 Hz), 3.94 (t, 2H, J = 5.5 Hz), 1.51 (s, 9H); MS *m/z* 293 $(M + H)^+$.

***tert*-Butyl-8-methyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (32).** To a solution of **31** (1.026 g, 3.51 mmol) in toluene (14 mL) was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (557 μL, 3.69 mmol) followed by *n*-BuLi (1.60 M in hexanes, 2.31 mL, 3.69 mmol) at −78 °C. The brown colored solution was stirred at −78 °C for 10 min. After the addition of neat iodomethane (230 μL, 3.69 mmol) to the above solution at −78 °C, the solution was stirred at −78 °C for an additional 10 min, and then warmed to rt over 1 h. The reaction was quenched with aqueous NH_4Cl. The aqueous layer was extracted with EtOAc (3×), washed with brine, and dried over anhydrous $MgSO_4$. Concentration followed by separation by flash chromatography (H/E = 4:1, then 1:1) afforded the title compound as a foamy solid (0.752 g, 70% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.56∼5.65 (br s, 1H), 4.45∼4.60 (br s, 1H), 4.23 (dd, 1H, J = 3.2, 12.3 Hz), 4.06 (dt, 1H, J = 4.3, 12.1 Hz), 3.33 (br t, 1H, J = 11.6 Hz), 1.64 (d, 3H, J = 6.9 Hz), 1.53 (s, 9H); MS *m/z* 307.0 $(M + H)^+$.

***tert*-Butyl[(1*R*)-3-[(8*S*)-8-methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate (33a) and *tert*-Butyl[(1*R*)-3-[(8*R*)-8-methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-yl]-3-oxo-1-(2,4,5-trifluorobenzyl)propyl]carbamate (33b).** Compounds **33a** and **33b** were prepared essentially following the same procedure for the synthesis of **15a**,**b**. For **33a** (faster eluting): ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.00∼7.15 (m), 6.80∼7.00 (m), 6.12 (m), 5.20∼5.50 (m), 5.10 (d, J=13.3 Hz), 4.00∼4.35 (m), 3.69 (br t, J = 11.7 Hz), 3.21 (br t, J = 11.7 Hz), 2.90∼3.10 (m), 2.70∼2.90 (m), 2.58∼2.70 (m), 1.73 (d, J = 6.1 Hz), 1.66 (d, J = 6.4 Hz), 1.38 (s). MS *m/z* 522.1 $(M + H)^+$. For **33b** (slower eluting): ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.05∼7.15 (m), 6.90∼7.00 (m), 6.11 (m), 5.20∼5.50 (m), 5.08 (d, J = 11.4 Hz), 4.00∼4.35 (m), 3.67 (br t, J = 12.1 Hz), 3.22 (br t, J = 12.1 Hz), 2.80∼3.05 (m), 2.60∼2.80 (m), 1.70 (d, J = 6.6 Hz), 1.65 (d, J = 6.4 Hz), 1.40 (s). MS *m/z* 522.1 $(M + H)^+$.

(2*R*)-4-[(8*S*)-8-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (34a). Following the same procedure for the synthesis of **12a**, the title compound was prepared as a white foamy solid. Purity (97%) by HPLC (t_r = 1.46 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers)

δ 7.35∼7.42 (m), 7.18∼7.30 (m), 5.93 (q, J = 7.1 Hz), 5.47 (m), 4.36 (d, J = 12.1 Hz), 4.15∼4.30 (m), 4.08 (m), 3.85∼3.95 (br s), 3.70∼3.80 (m), 3.11 (d, J = 7.1 Hz), 2.80∼3.05 (m), 1.67 (d, J = 6.9 Hz), 1.61 (d, J = 6.9 Hz); MS m/z 422.1 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{17}H_{17}F_6N_5O$ (M + H)$^+$ m/e, 422.1415,; found, 422.1421.

(2*R*)-4-[(8*R*)-8-Methyl-3-(trifluoromethyl)-5,6-dihydro[[1,-2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (34b). Following the same procedure for the synthesis of **12a**, the title compound was prepared as a white foamy solid. Purity (100%) by HPLC (t_r = 1.46 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.45 (m), 7.20∼7.30 (m), 5.97 (q, J = 6.8 Hz), 5.48 (m), 4.15∼4.40 (m), 3.90∼4.05 (m), 3.70∼3.80 (m), 3.30∼3.40 (m), 2.90∼3.20 (m), 2.75∼2.82 (m), 1.70 (d, J = 6.6 Hz), 1.57 (d, J = 6.8 Hz); MS m/z 422.2 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{17}H_{17}F_6N_5O$ (M + H)$^+$ m/e, 422.1415; found, 422.1411.

***tert*-Butyl-8,8-dimethyl-3-(trifluoromethyl)-5,6-dihydro-1,2,4-triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (35).** Following the same procedure for the synthesis of **32**, the title compound was prepared as a white solid (47% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.16 (t, 2H, J = 4.8 Hz), 3.92 (t, 2H, J = 5.3 Hz), 1.95 (s, 6H), 1.55 (s, 9H); MS m/z 321.0 (M + H)$^+$.

(2*R*)-4-[8,8-Dimethyl-3-(trifluoromethyl)-5,6-dihydro[[1,-2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (36). Starting with **35**, the same procedures were followed as in the synthesis of **12a**. Purity (100%) by HPLC (t_r = 1.58 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.45 (m), 7.20∼7.30 (m), 4.34 (br s), 3.86 (br s), 3.05∼3.15 (m), 2.99 (dd, J = 3.9, 17.6 Hz), 2.86 (dd, J = 8.0, 17.4 Hz), 1.99 (s), 1.94 (s); MS m/z 436.1 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{18}H_{19}F_6N_5O$ (M + H)$^+$ m/e, 436.1572; found, 436.1564.

General Procedure for the Synthesis of 37. To a solution of **31** in toluene was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine followed by *n*-BuLi (1.60 M in hexanes) at −78 °C. The brown colored solution was stirred at −78 °C for 10 min. After the addition of neat bromide (R′CH_2Br) or aldehyde (R′CHO) to the above solution at −78 °C, the solution was stirred at −78 °C for an additional 10 min, and then warmed to rt over 1 h. The reaction was quenched with aqueous NH_4Cl. The aqueous layer was extracted with EtOAc (3×), washed with brine, and dried over anhydrous $MgSO_4$. After purification by flash chromatography (hexanes/ethyl acetate), the *N*-Boc-protected compounds were used for the next step.

(2*R*)-4-[8-Ethyl-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, trifluoroacetate (38a and 38b). Compounds **38a** and **38b** were prepared from 2-chloro-3-ethylpyrazine by a procedure similar to that described for the synthesis of compounds **12a** and **12b**. To a solution of the corresponding intermediate triazolopiperazine (56 mg, 0.25 mmol) in DMF (3 mL) were added β-aminoacid **19** (100 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), HOAT (51 mg, 0.37 mmol), and DIPEA (109 μL, 0.63 mmol). The resultant mixture was stirred at rt for 18 h and partitioned between ethyl acetate and saturated aqueous $NaHCO_3$ solution. The organic layer was washed with water (3×), brine (1×), and dried over anhydrous $MgSO_4$. After filtration and concentration in vacuo, the product was purified by preparative TLC (H/E = 1:1). Diastereomers were separated on a ChiralCel OJ column using 10% ethanol/hexanes as eluent. Treatment of each of the *N*-Boc-protected diastereomers with a 1:1 mixture of trifluoracetic acid and dichlormethane followed by concentration provided the corresponding trifluoroacetate salts. For **38a**, 98.2% purity by HPLC (t_r = 1.53 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.33∼7.38 (m), 7.22∼7.29 (m), 5.89 (q, J = 6.4 Hz), 5.23 (q, J = 6.4 Hz), 4.32∼4.37 (m, 1H), 4.18∼4.25 (m), 4.09 (m), 3.87∼3.91 (m), 3.75∼3.81 (m), 3.1 (d, J = 7.1 Hz), 2.82–3.07 (m), 1.95∼2.12 (m), 1.1 (t, J = 7.4 Hz). 1.05 (t, J = 7.4 Hz); MS m/z 436 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{18}H_{19}F_6N_5O$ (M + H)$^+$ m/e, 436.1572; found, 436.1583. For **38b**, 100% purity by HPLC (t_r = 1.54 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.40 (m), 7.23∼7.29 (m), 5.90 (q, J = 6.2 Hz), 5.23 (q, J = 6.2 Hz), 4.11∼4.39 (m), 3.91∼4.04 (m), 3.75∼3.81 (m), 3.01∼3.19 (m), 2.69∼2.85 (m), 1.93∼2.13 (m), 1.15 (t, J = 7.4 Hz), 1.07 (t, J = 7.4 Hz). MS m/z 436 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{18}H_{19}F_6N_5O$ (M + H)$^+$ m/e, 436.1572; found, 436.1577.

General Procedure for the Synthesis of 39a−49b. To a solution of *N*-Boc-protected compound **37** in MeOH was added saturated methanolic HCl solution at 0 °C. After being stirred at rt for 1 h, the solution was concentrated to give a white foamy solid. To the above compound and a β-aminoacid **19** in DMF were added DIPEA, HOAT, and HATU sequentially at rt. After being stirred at rt for 18 h, DMF was evaporated to give a viscous residue, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Preliminary purification of a mixture of diastereomers by either preparative TLC or flash chromatography (hexanes/ethyl acetate) was followed by further separation of diastereomers by chiral HPLC using chiral columns (ChiralCel OD, ChiralPak AD, or ChiralCel OJ column, 5∼10% ethanol/hexane or 5∼15% *iso*-propanol/heptane). Treatment of each of the *N*-Boc-protected diastereomers with a methanolic HCl solution at 0 °C followed by concentration provided the corresponding compounds (**39a**−**49b**) as HCl salts.

(2*R*)-4-[8-(2,2,2-Trifluoroethyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (39a). Purity (100%) by HPLC (t_r = 1.67 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.35∼7.40 (m), 7.15∼7.30 (m), 6.38 (dd, J = 3.9, 9.4 Hz), 5.90∼5.95 (m), 5.81 (d, J = 9.4 Hz), 4.8∼5.0 (m, overlapped with CD_3OD), 4.20∼4.40 (m), 4.10∼4.20 (m), 3.70∼3.90 (m), 3.35∼3.45 (m), 2.70∼3.15 (m); MS m/z 490.0 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{18}H_{16}F_9N_5O$ (M + H)$^+$ m/e, 490.1289; found, 490.1290.

(2*R*)-4-[8-(2,2,2-Trifluoroethyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (39b). Purity (100%) by HPLC (t_r = 1.67 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.42 (m), 7.20∼7.30 (m), 6.41 (dd, J = 3.9, 9.6 Hz), 5.80 (d, J = 8.0 Hz), 5.35 (dd, J = 3.2, 9.7 Hz), 4.8∼5.0 (m, overlapped with CD_3OD), 4.64 (dd, J = 4.4, 13.3 Hz), 4.45∼4.51 (m), 4.15∼4.40 (m), 3.70∼4.10 (m), 3.50∼3.65 (m), 3.38∼3.45 (m), 2.40∼3.30 (m), 2.61∼2.67 (m); MS m/z 490.0 (M + H)$^+$; HRMS (ES$^+$) calcd for $C_{18}H_{16}F_9N_5O$ (M + H)$^+$ m/e, 490.1289; found, 490.1295.

(2*R*)-4-[8-Allyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (40a). Purity (100%) by HPLC (t_r = 1.60 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.45 (m), 7.20∼7.30 (m), 6.04 (dd, J = 5.7, 8.4 Hz), 5.84∼6.01 (m), 5.41 (t, J = 6.9 Hz), 5.02∼5.30 (m), 4.61 (s), 4.17∼4.39 (m), 4.02 (dt, J = 4.4, 12.1 Hz), 3.86∼3.95 (m), 3.76∼3.86 (m), 3.30∼3.45 (overlapped with CD_3OD), 2.65∼3.15

(m); MS *m/z* 448.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{19}H_{19}F_6N_5O$ $(M + H)^+$ *m/e*, 448.1572; found, 448.1580.

(2*R*)-4-[8-Allyl-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (40b). Purity (100%) by HPLC (t_r = 1.60 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.45 (m), 7.20∼7.30 (m), 6.03 (dd, J = 5.5, 8.5 Hz), 5.87∼5.96 (m), 5.74∼5.82 (m), 5.41 (t, J = 6.8 Hz), 5.19 (d, J = 17.2 Hz), 5.15 (d, J = 9.8 Hz), 5.05 (d, J = 10.7 Hz), 4.60 (s), 4.30∼4.40 (m), 4.18∼4.30 (m), 4.09 (dt, J = 4.6, 12.1 Hz), 3.75∼3.92 (m), 3.37∼3.45 (m), 3.11 (d, J = 7.1 Hz), 2.98∼3.05 (m), 2.72∼2.89 (m); MS *m/z* 448.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{19}H_{19}F_6N_5O$ $(M + H)^+$ *m/e*, 448.1572; found, 448.1573.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl]-*N*,*N*-dimethylacetamide, HCl (41a). Purity (100%) by HPLC (t_r = 1.45 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.45 (m), 7.15∼7.30 (m), 6.13 (m), 5.87 (d, J = 7.7 Hz), 4.58 (s), 4.15∼4.40 (m), 3.90∼4.15 (m), 3.80∼3.90 (m), 3.59 (dd, J = 2.9, 17.4 Hz), 3.40∼3.50 (m), 2.70∼3.20 (m); MS *m/z* 493.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{20}H_{22}F_6N_6O_2$ $(M + H)^+$ *m/e*, 493.1787; found, 493.1790.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]- 3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl]-*N*,*N*-dimethylacetamide, HCl (41b). Purity (100%) by HPLC (t_r = 1.44 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.40 (m), 7.15∼7.30 (m), 6.13 (t, J = 7.3 Hz), 5.84∼5.86 (m), 4.58 (s), 4.10∼4.42 (m), 3.95∼4.05 (m), 3.80∼3.90 (m), 3.20∼3.50 (m), 2.70∼3.15 (m); MS *m/z* 493.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{20}H_{22}F_6N_6O_2$ $(M + H)^+$ *m/e*, 493.1787; found, 493.1784.

(2*R*)-4-[8-Benzyl-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (42a). Purity (100%) by HPLC (t_r = 1.72 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.12–7.30 (m), 6.21 (dd, J = 5.5, 7.6 Hz), 5.44–5.54 (m), 5.01 (dd, J=4.4, 14.2 Hz), 4.39 (dd, J = 3.6, 12.3 Hz), 4.07–4.17 (m), 3.76–3.78 (m), 3.54–3.60 (m), 3.36–3.46 (m), 2.91–2.96 (m), 2.67–2.75 (m), 2.56–2.61 (m), 2.46 (dd, J = 10.6, 17.2 Hz), 1.44 (dd, J = 3.5, 17.4 Hz); MS *m/z* 498.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{21}F_6N_5O$ $(M + H)^+$ *m/e*, 498.1729; found, 498.1708.

(2*R*)-4-[8-Benzyl-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (42b). Purity (100%) by HPLC (t_r = 1.80 min). ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.10–7.48 (m), 6.22 (dd, J = 5.5, 8.1 Hz), 5.46–5.54 (m), 5.16–5.20 (m), 4.10–4.36 (m), 3.84–4.04 (m), 3.48–3.74 (m), 2.61–3.01 (m); MS *m/z* 498.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{21}F_6N_5O$ $(M + H)^+$ *m/e*, 498.1729; found, 498.1715.

(2*R*)-4-[8-(4-Methoxybenzyl)-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (43a). Purity (98.5%) by HPLC (t_r = 1.82 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.14–7.26 (m), 7.08 (d, J = 8.3 Hz), 6.83 (d, J = 8.3 Hz), 6.14–6.18 (m), 5.42–5.50 (m), 5.01 (dd, J = 4.7, 14.2 Hz), 4.36–4.40 (m), 4.07–4.18 (m), 3.73 (d, J = 9.8 Hz), 3.51–3.58 (m), 2.94 (d, J = 7.4 Hz), 2.71–2.77 (m), 2.47–2.60 (m), 1.55 (dd, J = 2.8, 17.4 Hz); MS *m/z* 528.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{24}H_{23}F_6N_5O_2$ $(M + H)^+$ *m/e*, 528.1834; found, 528.1832.

(2*R*)-4-[8-(4-Methoxybenzyl)-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (43b). Purity (100%) by HPLC (t_r = 1.82 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.21–7.36 (m), 7.19 (d, J = 9.0 Hz), 7.05 (d, J = 9.0 Hz), 6.92 (d, J = 8.2 Hz), 6.77 (d, J = 8.5 Hz), 6.16 (dd, J = 5.3, 8.0 Hz), 5.48 (t, J = 7.0 Hz), 4.20–4.36 (m), 4.10–4.18 (m), 3.74–4.04 (dt, J = 4.4, 12.2 Hz), 3.68–3.78 (m), 3.58–3.66 (m), 3.46–3.55 (m), 3.28–3.34 (m), 2.93–3.02 (m), 2.70–2.92 (m), 2.58–2.66 (m), 1.61 (dd, J = 7.7, 17.1 Hz); MS *m/z* 528.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{24}H_{23}F_6N_5O_2$ $(M + H)^+$ *m/e*, 528.1834; found, 528.1827.

(2*R*)-4-Oxo-4-[3-(trifluoromethyl)-8-[2-(trifluoromethyl)-benzyl]-5,6-dihydro [1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (44a). Purity (100%) by HPLC (t_r = 1.99 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) 7.70 (d, J = 8.0 Hz), 7.55–7.64 (m), 7.38–7.48 (m), 7.11–7.28 (m), 6.32 (dd, J = 5.3, 10.1 Hz), 5.38 (dd, J = 3.7, 10.7 Hz), 4.05 (dd, J = 3.9, 14.0 Hz), 4.20–4.43 (m), 4.10–4.14 (m), 3.93–4.09 (m), 3.21–3.72 (m), 2.67–2.79 (m), 2.58–2.63 (m), 2.43–2.49 (m); MS *m/z* 566.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{24}H_{20}F_9N_5O$ $(M + H)^+$ *m/e*, 566.1602; found, 566.1597.

(2*R*)-4-Oxo-4-[3-(trifluoromethyl)-8-[2-(trifluoromethyl)-benzyl]-5,6-dihydro [1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (44b). Purity (100%) by HPLC (t_r = 2.00 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) 7.82 (d, J = 8.0 Hz), 7.52–7.68 (m), 7.34–7.46 (m), 7.12–7.32 (m), 6.30 (dd, J = 4.8, 10.1 Hz), 5.38 (m), 4.35–4.42 (m), 4.24–4.28 (m), 4.12–4.18 (m), 3.88–4.02 (m), 3.51–3.65 (m), 2.91–2.98 (m), 2.81–2.90 (m), 2.75 (d, J = 6.2 Hz), 2.55–2.65 (m); MS *m/z* 566.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{24}H_{20}F_9N_5O$ $(M + H)^+$ *m/e*, 566.1602; found, 566.1596.

(2*R*)-4-[8-(2-Fluorobenzyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (45a). Purity (100%) by HPLC (t_r = 1.82 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.20∼7.35 (m), 7.00∼7.15 (m), 6.26 (m), 5.53 (dd, J = 3.6, 10.5), 4.98 (dd, J = 3.9, 14.4 Hz), 4.39 (dd, J = 3.2, 12.4 Hz), 4.15∼4.30 (m), 4.12 (d,t, J = 4.4, 12.3 Hz), 3.70∼3.80 (m), 3.30∼3.60 (m), 2.85∼3.00 (m), 2.81 (dd, J = 6.6, 14.2 Hz), 2.55∼2.75 (m), 1.64 (dd, J = 3.4, 17.2 Hz); MS *m/z* 515.9 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O$ $(M + H)^+$ *m/e*, 516.1635; found, 516.1616.

(2*R*)-4-[8-(2-Fluorobenzyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (45b). Purity (100%) by HPLC (t_r = 1.84 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.15∼7.40 (m), 6.95∼7.10 (m), 6.29 (dd, J=5.1, 9.4 Hz), 5.54 (m), 4.92 (dd, J = 3.9, 14.2 Hz), 4.33∼4.36 (m), 4.15∼4.30 (m), 4.01 (dt, J = 4.1, 12.3 Hz), 3.70∼3.80 (m), 3.60∼3.70 (m), 3.40∼3.50 (m), 3.30∼3.26 (m), 2.70∼3.00, 1.62 (dd, J = 8.0, 17.2 Hz); MS *m/z* 515.9 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O$ $(M + H)^+$ *m/e*, 516.1635; found, 516.1640.

(2*R*)-4-[(8*S*)-8-(4-Fluorobenzyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (46a). Purity (100%) by HPLC (t_r = 1.84 min); ^{1}H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.10∼7.30 (m), 6.95∼7.05 (m), 6.13 (t, J = 7.3 Hz), 5.49 (br m), 5.01 (dd, J = 3.9, 14.2 Hz), 4.37∼4.40 (m), 4.05∼4.30 (m), 3.79 (br m), 3.40∼3.60 (m), 3.40 (dd, J = 5.5, 8.7 Hz), 2.98 (d, J = 7.4 Hz), 2.70∼2.85 (m), 2.55∼2.70 (m),

1.64 (dd, J = 3.0, 17.4 Hz); MS m/z 515.9 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O$ $(M + H)^+$ m/e, 516.1635; found, 516.1629.

(2*R*)-4-[(8*R*)-8-(4-Fluorobenzyl)-3-(trifluoromethyl)-5,6-dihydro[[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (46b). Purity (100%) by HPLC (t_r = 1.86 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.15∼7.36 (m), 7.11 (t, J = 8.7 Hz), 6.95 (t, J = 8.7 Hz), 6.19 (dd, J = 5.5, 8.5 Hz), 5.52 (t, J = 6.6 Hz), 4.80∼4.95 m, overlapped with CD_3OD), 4.25∼4.35 (m), 4.10∼4.25 (m), 4.00 (dt, J = 4.1, 12.4 Hz), 3.55∼3.80 (m), 3.20∼3.40 (m, overlapped with CD_3OD), 2.70∼3.05 (m), 1.64 (dd, J = 8.0, 16.9 Hz); MS m/z 515.9 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O$ $(M+H)^+$ m/e, 516.1635; found, 516.1620.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl](4-fluorophenyl)methanol, HCl (47a). Purity (100%) by HPLC (t_r = 1.67 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.61 (dd, J = 5.3, 8.7 Hz), 7.51 (dd, J = 5.5, 8.5 Hz), 7.42 (dd, J = 5.3, 8.5 Hz), 7.05∼7.30 (m), 6.75 (d, J = 4.8 Hz), 6.18 (d, J = 3.9 Hz), 5.49 (d, J = 4.8 Hz), 5.45 (d, J = 3.9 Hz), 5.38 (br t, J = 6.7 Hz), 5.05 (d, J = 9.2 Hz), 4.60∼4.75 (m), 4.35∼4.50 (m), 4.05∼4.30 (m), 3.80∼3.95 (m), 3.65∼3.75 (m), 3.50∼3.55 (m), 2.80∼3.00 (m), 2.65∼2.75 (m), 2.50∼2.55 (m), 1.68 (dd, J = 3.0, 17.2 Hz); MS m/z 532.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O_2$ $(M + H)^+$ m/e, 532.1583; found, 532.1570.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl](4-fluorophenyl)methanol, HCl (47b). Purity (100%) by HPLC (t_r = 1.69 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.65 (br t), 7.40∼7.55 (m), 7.15∼7.35 (m), 6.99 (t, J = 8.2 Hz), 6.75 (s), 6.16 (d, J = 2.7 Hz), 5.55 (br s), 5.45 (d, J = 2.2 Hz), 5.39 (s), 5.00 (br d), 4.60∼4.70 (m), 4.30∼4.50 (m), 4.15∼4.30 (m), 3.50∼4.05 (m), 2.65∼3.00 (m), 1.70∼1.80; MS m/z 532.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O_2$ $(M + H)^+$ m/e, 532.1583; found, 532.1569.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl](4-fluorophenyl)methanol, HCl (47c). Purity (100%) by HPLC (t_r = 1.73 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.62 (m), 7.42 (dd, J = 5.5, 8.4 Hz), 7.34 (q, J = 8.1 Hz), 7.20∼7.30 (m), 7.09 (t, J = 8.7 Hz), 7.02 (t, J = 8.4 Hz), 6.02 (d, J = 3.7 Hz), 5.70 (s), 5.54 (d, J = 3.7 Hz), 5.35 (d, J = 4.2 Hz), 5.28 (d, J = 3.6 Hz), 5.22 (s), 4.81 (dd, J = 3.9, 14.2 Hz), 4.60∼4.70 (m), 4.44 (d, J = 13.3 Hz), 3.90∼4.30 (m), 3.60∼3.90 (m), 3.05 (d, J = 7.1 Hz), 2.70∼3.04 (m), 2.60∼2.70 (t); MS m/z 532.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O_2$ $(M + H)^+$ m/e, 532.1583; found, 532.1571.

2-[7-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(trifluoromethyl)-5,6,7,8-tetrahydro[[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl](4-fluorophenyl)methanol, HCl (47d). Purity (100%) by HPLC (t_r = 1.73 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 7.30∼7.45 (m), 7.20∼7.30 (m), 7.00∼7.10 (m), 6.01 (d, J = 3.9 Hz), 5.62 (s), 5.46 (s), 5.1 (d, J = 3.7 Hz), 4.75 (d, J = 11.5 Hz), 4.48 (d, J = 11.0 Hz), 4.20∼4.30 (m), 4.05∼4.20 (m), 3.80∼3.90 (m), 3.95∼3.10 (m), 2.89 (d, J = 5.5 Hz), 2.38 (t); MS m/z 532.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{23}H_{20}F_7N_5O_2$ $(M + H)^+$ m/e, 532.1583; found, 532.1574.

[1,2,4]Triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (48a). Purity (100%) by HPLC (t_r = 2.40 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) 7.87–7.94 (m), 7.16–7.28 (m), 6.26 (t, J = 7.5 Hz), 5.65 (br s), 4.13–4.40 (m), 3.77–3.83 (m), 3.43–3.67 (m), 2.88–3.02 (m), 2.68–2.86 (m); MS m/z 634.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{25}H_{19}F_{12}N_5O$ $(M + H)^+$ m/e, 634.1476; found, 634.1490.

(2*R*)-4-[8-[3,5-Bis(trifluoromethyl)benzyl]-3-(trifluoromethyl)-5,6-dihydro [1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (48b). Purity (100%) by HPLC (t_r = 2.23 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) 7.82–7.97 (m), 7.23–7.28 (m), 4.06–4.85 (m), 3.52–3.85 (m), 2.74–3.18 (m); MS m/z 634.0 $(M + H)^+$; HRMS (ES^+) calcd for $C_{25}H_{19}F_{12}N_5O$ $(M + H)^+$ m/e, 634.1476; found, 634.1468.

(2*R*)-4-Oxo-4-[8-(pyridin-2-ylmethyl)-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (49a). Purity (100%) by HPLC (t_r = 1.26 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 8.84 (d, J = 6.1 Hz), 8.62 (dt, J = 1.5, 7.8 Hz), 8.16 (d, J = 8 Hz), 8.04 (t, J = 7.1 Hz), 7.32–7.37 (m), 7.19–7.25 (m), 6.23 (t, J = 7.4 Hz), 4.35–4.44 (m), 4.27 (dt, J = 4.4, 12.4 Hz), 3.91–3.97 (m), 3.75–3.82 (m), 2.93–3.03 (m); MS m/z 499.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{22}H_{20}F_6N_6O$ $(M + H)^+$ m/e, 499.1681; found, 499.1677.

(2*R*)-4-Oxo-4-[8-(pyridin-2-ylmethyl)-3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, HCl (49b). Purity (100%) by HPLC (t_r = 1.26 min); ^1H NMR (500 MHz, CD_3OD, mixture of rotamers) δ 8.82 (d, J = 4.6 Hz), 8.57 (dt, J = 1.6, 8.0 Hz), 8.12 (d, J = 8 Hz), 8.02 (t, J = 7.3 Hz), 7.33–7.38 (m), 7.22–7.28 (m), 6.27 (t, J = 7.5 Hz), 4.43 (dd, J = 2.8, 12.6 Hz), 4.34 (dd, J = 3.9, 14.6 Hz), 4.27 (dt, J = 3.9, 12.1 Hz), 3.90–3.97 (m), 3.70–3.82 (m), 2.89–3.09 (m); MS m/z 499.1 $(M + H)^+$; HRMS (ES^+) calcd for $C_{22}H_{20}F_6N_6O$ $(M + H)^+$ m/e, 499.1681; found, 499.1670.

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Supporting Information Available: The X-ray crystallographic data of compounds **34b** and **46b** bound to DPP-4. This material is available free of charge via the Internet at http://pubs.acs.org.

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