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Synthesis and biological evaluation of triazepane derivatives as DPP-IV inhibitors

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ABSTRACT

A series of triazepane derivatives such as (*R*)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-ones (**7**, **13a**–**p**) and (*R*)-3-amino-1-(1,2,5-triazepan-5-yl)-4-(2,4,5-trifluorophenyl)butan-1-ones (**17a**–**e**) was synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DPP-IV) enzyme. Compounds with the acid moiety were found to be potent inhibitors of DPP-IV without inhibiting CYP 3A4. Among them, compound **13p** ((*R*)-4-[1-acetyl-2-{3-amino-4-(2,4,5-trifluorophenyl)butanoyl-1,2,5-triazepan-5-carbonyl}benzoic acid]) showed a good *in vitro* activity without inhibiting CYP.

1. Introduction

Diabetes mellitus (type 2) is a non-insulin dependent disease caused by chronic hyperglycemia, and considered under multiple metabolic disorders. It is commonly observed in patients with inadequate insulin secretion, insulin resistance, hepatic glucose overproduction or glucose intolerance [1]. Incretin, a glucagon-like peptide hormone (GLP-1) [2,3] is released from L cells of the small intestine in response to the digestion of food, and plays an important role in insulin secretion. Increased activity of incretin will lead to sustained insulin secretion, which regulates the glucose level in the body. It also controls gastric emptying, induction of satiety as well as regeneration and differentiation of islet β-cells [4,5]. Dipeptidyl peptidase IV (DPP-IV), a serine protease present in many tissues and body fluids, existing either in the membrane bound or soluble enzyme form, degrades GLP-1 (GLP-1[7–36]amide) into inactive GLP[9–36]amide [6–8] at the *N-terminus* position. Inhibition of DPP-IV increases the concentration of GLP-1 and, as a result, increases insulin secretion [9–11], which can ameliorate hyperglycemia in type 2 diabetes.

It is known that fluorine or trifluoromethyl [12,13] group at an appropriate position in the molecule alters the chemical and biological properties of the molecule. The fluorine substitution is extensively practiced in the design of potential molecules and therefore there is a growing demand for the synthesis of fluorinated molecules. Recently, several studies have emerged on the use of fluorinated molecules [14–20] as DPP-IV inhibitors, some of which are in advanced stage of development. Among them, MK-0431 (Sitagliptin, launched in the market by Merck) is a representative fluorinated DPP-IV inhibitor and its 2,4,5-trifluorophenyl moiety occupy the active pocket of DPP-IV [14].

We have been working on finding new molecules which can selectively inhibit DPP-IV without inhibiting cytochrome P450 (CYP) enzymes which play a major role in metabolizing drug molecules. Many lead candidate molecules in pharmaceutical development fail due to inhibition of one or more isozyme forms of CYP enzymes. CYP 3A4, is one of the most important cytochrome P450s present in human liver [21] has ability to metabolize >50% of administered therapeutic agents. It accounts for the large number of documented drug–drug interactions associated with CYP 3A4 inhibition [22–24].

Our continued efforts [25–27] towards finding a potential dipeptidyl peptidase IV (DPP-IV) inhibitor, we designed triazepane derivatives having (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl moiety which was conceived from MK-0431. Thus, we wish to report here the synthesis of triazepane derivatives and biological evaluation for their ability to inhibit dipeptidyl peptidase IV (DPP-IV) without inhibiting CYP. MK-0431 was used as a reference. The basic skeletons are outlined below.

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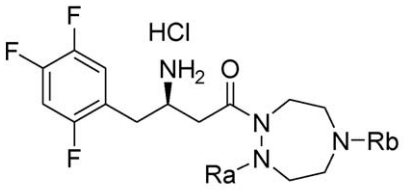
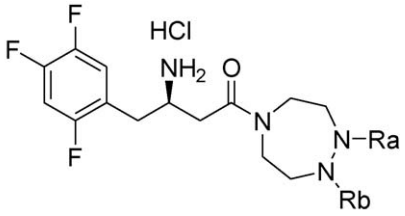
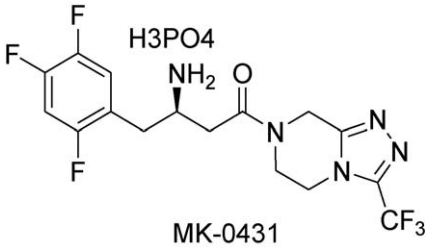


7, 13a-p



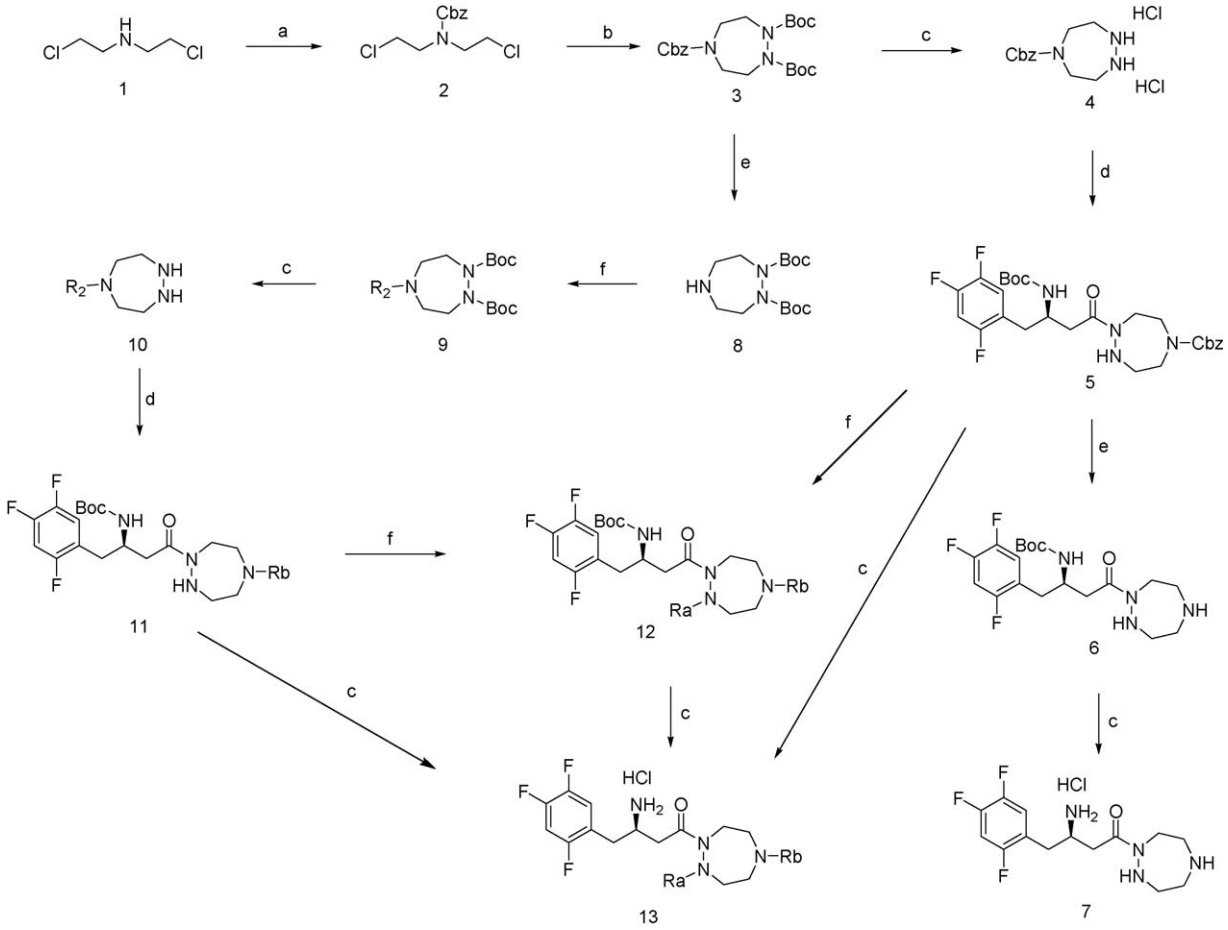
17a-e

(*R*)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**7**, **13a**–**p**) and (*R*)-3-amino-1-(1,2,5-triazepan-5-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**17a**–**e**) derivatives.

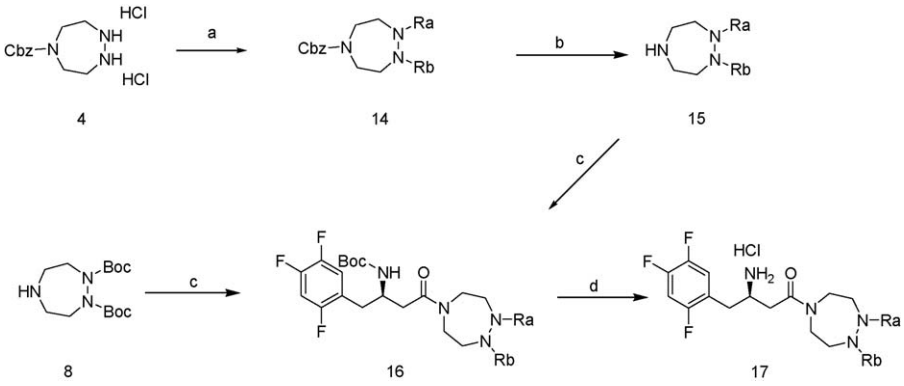


MK-0431

(*R*)-3-amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazine-7(8H)-yl-4-(2,4,5-trifluorophenyl)butan-1-one (**MK-0431**) [14].

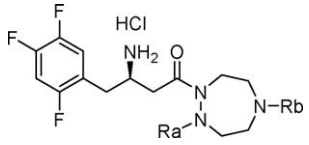


Scheme 1. Synthesis of (*R*)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**7**,**13a**–**p**) derivatives
Reagents and conditions: (a) CbzCl, Na_2CO_3, acetone/H_2O, 0 °C to room temperature; (b) BocNHNHBoc, 50% NaOH, Et_4NBr, toluene, reflux, 12 h; (c) 4 M HCl, ethyl acetate, 12 h; (d) (*R*)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid, EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (e) 10% Pd/C, H_2 balloon, MeOH, room temperature; (f) electrophile (acid or acyl halide), EDCI, Et_3N, CH_2Cl_2, room temperature.



Scheme 2. Synthesis of (*R*)-3-amino-1-(1,2,5-triazepan-5-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**17a–e**)derivatives.
Reagents and conditions: (a) Electrophile (acid halide); (b) 10% Pd/C, H_2 balloon, MeOH, room temperature, 12 h; (c) (R)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid, EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (d) 4 M HCl, ethyl acetate, room temperature, 12 h.

Table 1
In vitro DPP-IV inhibitory activity of (*R*)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**7**, **13a–l**) derivatives.



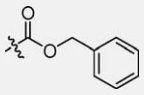
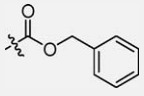
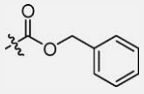
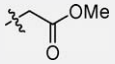
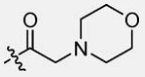
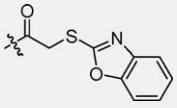
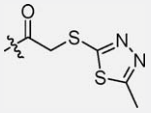
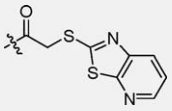
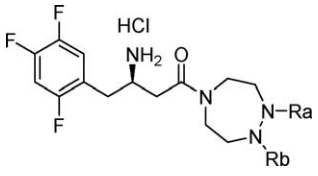
Compound	R_a	R_b	IC_{50} (nM)[a]
7	H	H	9800
13a	H		859
13b			578
13c			1200
13d	H		2500
13e	H		2900
13f	H		681
13g	H		609

Table 1 (*Continued*)

Compound	R_a	R_b	IC_{50} (nM)[a]
13h			1800
13i			700
13j			401
13k			591
13l			151
MK-0431			101

[a] The IC_{50} values were determined by a direct regression curve analysis.

Table 2
In vitro DPP-IV inhibitory activity of (*R*)-3-amino-1-(1,2,5-triazepan-5-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**17a–e**) derivatives.



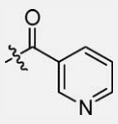
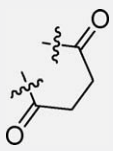
Compound	R_a	R_b	IC_{50} (nM)[a]
17a	H	H	4500
17b		H	3300
17c			1600
17d			660

Table 2 (*Continued*)

Compound	R_a	R_b	IC_{50} (nM)[a]
17e			853
MK-0431			101

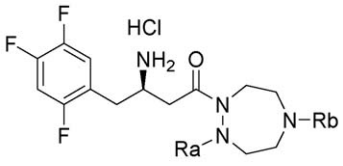
[a] The IC_{50} values were determined from a direct regression curve analysis.

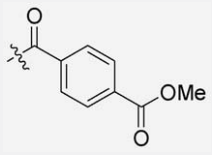
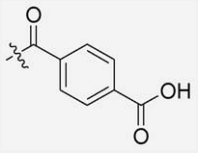
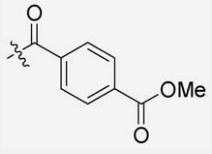
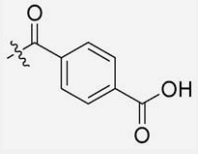
2. Results and discussion

2.1. Synthesis

The triazepane derivatives **7** and **13** were synthesized according to Scheme 1. Bis(2-chloroethyl)amine **1** was initially protected with benzyl chloroformate (CbzCl) in the presence of a base, and coupled with di-*tert*-butyl hydrazine-1,2-dicarboxylate to yield cyclic triazepane **3**. Two strategies were adopted for selective deprotection of compound **3**. One strategy was *Boc* deprotection using 4 M HCl and another was *Cbz* deprotection using 10% palladium on carbon under hydrogen atmosphere to obtain compounds **4** and **8**, respectively. Compound **4** was further coupled with (*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-(2,4,5-trifluorophenyl)butanoic acid in the presence of EDCI to give compound **5**. Compound **5** was then reduced using 10% palladium on carbon under hydrogen atmosphere to yield compound **6**, followed by *Boc* deprotection with 4 M HCl to obtain compound **7**. Also, compound **5** was deprotected or further derivatized by acylating reagents to give compound **13**. Alternatively, compound **8** was reacted with various electrophiles, then *Boc* deprotection, followed by reaction with (*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-(2,4,5-trifluorophenyl)butanoic acid to give compound **11** through compounds **9** and **10**. Compound **11** was further converted to final compounds **13** via compound **12** or directly.

Table 3
In vitro DPP-IV and CYP 3A4 inhibitory activity of (*R*)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (**13m**–**p**)derivatives.



Compound	R_a	R_b	IC_{50} (nM)[a]	CYP 3A4 activity at 10 μM (%)
13m	H		439	NT
13n	H		216	92.4
13o			213	NT
13p			98	87.2
MK-0431			101	

[a] The IC_{50} values were determined from a direct regression curve analysis.

Table 4
Selectivity of compounds **13n** and **p** toward DPP-IV-related enzymes.

Compound	DPP-IV IC_{50} (nM)[a]	DPP-2 (nM)	DPP-8 (nM)
13n	216	37,153	24,547
13p	98	77,624	29,512

[a] The IC_{50} values were determined from a direct regression curve analysis.

Similarly, triazepane derivatives **17** were synthesized as shown in Scheme 2. Thus, compound **4** was acylated with various acylating reagents to afford compound **14**, and on *Cbz* deprotection gave compound **15** followed by coupling with (*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-(2,4,5-trifluorophenyl)butanoic acid resulted compound **16**. Alternatively, compound **16a** was prepared starting from compound **8** through coupling with (*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-(2,4,5-trifluorophenyl)butanoic acid followed by *Boc* deprotection yielded compound **17**.

2.2. In vitro DPP-IV inhibitory activity

The 1-[3-amino-4-(2,4,5-trifluorophenyl)butanoyl]triazepane derivatives (**7** and **13a–l**) were evaluated *in vitro* for their DPP-IV inhibition and the results are summarized in Table 1. MK0431 was used as a reference compound. The basic compound (**7**, $R_a = R_b = H$) showed weak inhibitory activity with an IC_{50} value of 9.8 μM. Introduction of the benzyloxycarbonyl group at the R_b position resulted in improved potency (**13a**, $R_a = H$, $R_b = Cbz$, $IC_{50} = 859$ nM). Furthermore, acetyl derivative at the R_a position (**13b**, $R_a = CH_3CO$, $R_b = Cbz$) showed better inhibitory activity with an IC_{50} value of 578 nM. Whereas the benzoyl substituent (**13c**, $R_a = C_6H_5CO$, $R_b = Cbz$) reduced the activity. The 5-pyrazine, 5-methyl acetate and 2-acetyl-5-methanesulfonyl derivatives showed micromolar range potencies (**13d**, **e**, **h**). However, the 5-trifluoroacetyl, 5-methanesulfonyl and 2,5-disubstituted derivatives showed better potencies (**13f**, **g**, **i–l**) with the IC_{50} values in the 151–700 nM range. Among all of the derivatives, compound **13l** (R_a = 2-(thiazolo[5,4-b]pyridine-2-ylthio)acetyl, $R_b = CH_3SO_2$) demonstrated the highest *in vitro* activity, with an IC_{50} value of 151 nM.

The 5-[3-amino-4-(2,4,5-trifluorophenyl)butanoyl]triazepane derivatives (**17a–e**) also were evaluated *in vitro*, and the results are summarized in Table 2. The basic triazepane derivative **17a** ($R_a = R_b = H$) showed a weak inhibitory activity with an IC_{50} of 4.5 μM and the introduction of substituents for R_a and R_b (**17b–e**) resulted an improved activity with the IC_{50} values in the 853–3300 nM range.

Based on the inhibitory activity data of the above two series of compounds, compound **13** derivatives were found to be promising, and therefore we further derivatized to find more potent compounds. Thus, a new set of triazepane derivatives was synthesized, evaluated for their inhibitory activity and CYP inhibition. Compounds with an acid moiety were found to exhibit good DPP-IV inhibitory activity without inhibiting CYP as shown in Table 3. The compound **13p** showed promising *in vitro* DPP-IV inhibitory activity with an IC_{50} value of 98 nM without CYP 3A4 inhibition at 10 μM concentration. We also examined the selectivity toward DPP-IV-related enzymes such as DPP-2 and DPP-8 (Table 4). Compound **13p** displayed at least 300-fold better selectivity toward DPP-IV related enzymes.

3. Conclusion

A series of triazepane derivatives (**7**, **13a–p** and **17a–e**) was synthesized, and evaluated their ability to inhibit the dipeptidyl peptidase IV (DPP-IV). Compound **13p** showed a good *in vitro* activity, selectivity without CYP inhibition. Further studies are underway to optimize this compound class for use in the treatment of diabetes.

4. Experimental

4.1. General

All reported yields are isolated yields after column chromatography or crystallization. The ^1H NMR spectra were recorded on FT-NMR Varian GEMINI-200FT or Bruker AVANCE-300 and TMS as an internal reference. ATR-FTIR spectra were recorded on Travel IR (Sens IR Technology) spectrophotometer. Low-resolution mass spectra were obtained on the Liquid Chromatography-Mass Spectrometer (LC-MS, Waters). High-resolution mass spectra were obtained on the Autospec magnetic sector mass spectrometer (Micromass, Manchester, UK).

4.1.1. Preparation of benzyl bis(2-chloroethyl)carbamate (2)

To a solution of *bis*-(2-chloroethyl)amine HCl **1** (4.5 g, 25.2 mmol) in acetone/H_2O (100 mL, 1:1), were added Na_2CO_3 (8.9 g, 84.0 mmol) and benzyl chloroformate (4.7 mL, 33.6 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature. Acetone was removed under vacuum, and aqueous residue was extracted with ethyl acetate. Organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 1/9) to give compound **2** (5.3 g, 61%, colorless oil) ^1H NMR ($CDCl_3$, 300 MHz): δ 7.36–7.35 (m, 5H), 5.15 (s, 2H), 3.69–3.65 (m, 4H), 3.61–3.55 (m, 4H); LC-MS: *m/z* 276 (MH^+).

4.1.2. Preparation of 5-benzyl-1,2-di-tert-butyl-1,2,5-triazepane-1,2,5-tricarboxylate (3)

To a solution of benzyl *bis*-(2-chloroethyl)carbamate **2** (4.2 g, 18.1 mmol) and di-*t*-butyl hydrazodiformate (5.0 g, 18.1 mmol) in toluene (36 mL), were added 50% NaOH solution (18 mL) and tetraethyl ammonium bromide (570 mg, 2.7 mmol). The mixture was stirred for 13 h under reflux condition, cooled to room temperature, and then extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 1/1) to give compound **3** (4.0 g, 51%, colorless oil). ^1H NMR ($CDCl_3$, 200 MHz): δ 7.36–7.34 (m, 5H), 5.14 (s, 2H), 4.21–4.02 (m, 2H), 3.98–3.65 (m, 1H), 3.52–3.04 (m, 5H), 1.49 (s, 18H); LC-MS: *m/z* 436 (MH^+); IR 2975, 2932, 1692, 1721.

4.1.3. Preparation of benzyl-1,2,5-triazepane-5-carboxylate·2HCl (4)

To a solution of 5-benzyl-1,2-di-*tert*-butyl-1,2,5-triazepane-1,2,5-tricarboxylate **3** (1.3 g, 2.99 mmol) in ethyl acetate (5 mL), was added 4M-HCl/1,4-dioxane (5 mL). The reaction mixture was stirred for 16 h at room temperature, and then the solvents were evaporated. The residue was crystallized with ether to give compound **4** (400 mg, 43%, white solid). m.p. 91–94 °C; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.37 (m, 5H), 5.09 (s, 2H), 4.20–3.00 (m, 8H); LC-MS: *m/z* 236 (MH^+); IR 2974, 2931, 1691.

4.1.4. Preparation of (R)-benzyl-1-(3-(tert-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carboxylate (5)

To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid (333 mg, 1.00 mmol) in CH_2Cl_2 (5 mL), were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 552 mg, 1.92 mmol), TEA (1.39 mL, 10 mmol) and 5-benzyl-1,2,5-triazepane-5-carboxylate·2HCl **4** (369 mg, 1.20 mmol). The reaction mixture was stirred for 12 h at room temperature. Organic layer was extracted from H_2O/CH_2Cl_2 and washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 2/1) to obtain compound **5** (440 mg, 80%, colorless oil). ^1H NMR ($CDCl_3$, 200 MHz): δ 7.35–7.32 (m, 5H), 7.12–6.99 (m, 1H), 6.88–6.81 (m, 1H), 5.48–5.32 (br, 1H), 5.12 (s,

2H), 4.14–4.10 (m, 1H), 3.73–3.46 (m, 8H), 3.00–2.70 (m, 4H), 1.36 (s, 9H); LC-MS: *m*/*z* 551 (MH^+); IR 2905, 2845, 1691.

*4.1.5. Preparation of (R)-tert-butyl-4-oxo-4-(1,2,5-triazepan-1-yl)-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (**6**)*

To a solution of (*R*)-benzyl 1-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carboxylate **5** (35 mg, 0.064 mmol) in methanol (2 mL), was added 10%-Pd/C (7 mg, 20 mol%). The reaction mixture was stirred for 10 h in hydrogen atmosphere, filtered through celite, washed with additional methanol and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 2/1) to give compound **6** (24 mg, 90%). m.p. 76–78 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.03–6.82 (m, 1H), 6.79–6.60 (m, 1H), 5.60–5.47 (br, 1H), 4.21–3.83 (m, 2H), 3.31–3.03 (m, 3H), 2.86–2.53 (m, 4H), 2.29–2.11 (m, 4H), 1.22 (s, 9H); LC-MS: *m*/*z* 417 (MH^+).

*4.1.6. Preparation of (R)-3-amino-1-(1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**7**)*

To a solution of [(*R*)-*tert*-butyl-4-oxo-4-(1,2,5-triazepan-1-yl)-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate] **6** (18 mg, 0.043 mmol) in ethyl acetate (1 mL), was added 4 M-HCl/1,4-dioxane (1 mL) and the mixture was stirred for 16 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **7** (12 mg, 80%, white solid). m.p. 115–117 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.13 (br, 1H), 9.94, 9.08 (br, 1H), 8.00 (br, 2H), 7.37–7.34 (m, 2H), 4.61–4.35 (m, 1H), 4.17–4.14 (m, 1H), 3.94–3.89 (m, 1H), 3.76–3.14 (m, 5H), 2.79–2.76 (m, 2H), 2.60–2.53 (m, 3H); LC-MS: *m*/*z* 317 (MH^+); IR 3423, 2840, 1631.

*4.1.7. Preparation of [1,2,5]triazepane-1,2-dicarboxylic acid di-tert-butyl ester (**8**)*

To a solution of 5-benzyl 1,2-di-*tert*-butyl-1,2,5-triazepane-1,2,5-tricarboxylate **3** (1.0 g, 2.30 mmol) in methanol (5 mL), added 10% Pd/C (200 mg, 20 mol%). The reaction mixture was stirred for 10 h under hydrogen atmosphere, filtered through celite, washed with additional methanol and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 9/1) to give compound **8** (1.1 g, 79%, yellow oil). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.02–3.93 (m, 2H), 3.16–2.98 (m, 3H), 2.85–2.75 (m, 3H), 1.49 (s, 18H); LC-MS: *m*/*z* 302 (MH^+); IR 2975, 2933, 1697.

*4.2. General procedure for the synthesis of compound **13** (Example: **13m** and **n**)*

*4.2.1. Preparation of di-tert-butyl-5-(4-(methoxycarbonyl)benzoyl)-1,2,5-triazepane-1,2-dicarboxylate (**9m**)*

To a solution of 4-(methoxycarbonyl)benzoic acid (1 g, 5.55 mmol) in CH_2Cl_2, were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 2.13 g, 11.1 mmol), DMAP (340 mg, 2.77 mmol), di-*tert*-butyl-1,2,5-triazepane-1,2-dicarboxylate **8** (2 g, 6.66 mmol) and TEA (3.87 mL, 27.75 mmol). The reaction mixture was stirred for 4 h at room temperature. Organic layer was extracted from H_2O/CH_2Cl_2 and washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 1/1) to obtain compound **9m** (2.45 g, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10 (dd, J = 1.5, 8 Hz, 2H), 7.44–7.40 (m, 2H), 4.27–4.09 (m, 2H), 3.94 (s, 3H), 3.85–3.74 (m, 1H), 3.39–3.14 (m, 4H), 2.97–2.96 (m, 1H), 1.51 (s, 9H), 1.48 (s, 9H); LC-MS: *m*/*z* 464 (MH^+); IR 2977, 2936, 1697, 1721.

*4.2.2. Methyl-4-(1,2,5-triazepane-5-carbonyl)benzoate dihydrochloride (**10m**)*

To a solution of di-*tert*-butyl-5-(4-(methoxycarbonyl)benzoyl)-1,2,5-triazepane-1,2-dicarboxylate **9m** (2.3 g, 4.9 mmol) in ethyl acetate (18 mL), was added 4 M-HCl/1,4-dioxane (18 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, the residue was crystallized with ether to yield **10m** (1.32 g, 79%). ^{1}H NMR (MeOH-d_4, 300 MHz): δ 8.04–7.97 (m, 2H), 7.58–7.49 (m, 2H), 4.08–3.94 (m, 2H), 3.83 (s, 3H), 3.56–3.36 (m, 4H), 3.29–3.06 (m, 2H); LC-MS: *m*/*z* 264 (MH^+). IR 2977, 1662.

*4.2.3. (R)-Methyl-4-(1-(3-(tert-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoate (**11m**)*

To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid (320 mg, 0.96 mmol) in CH_2Cl_2, were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 552 mg, 1.92 mmol), TEA (1.34 mL, 9.6 mmol) and 4-(1,2,5-triazepane-5-carbonyl)benzoate dihydrochloride **10m** (643 mg, 1.92 mmol). The reaction mixture was stirred for 12 h at room temperature. Organic layer was extracted from H_2O/CH_2Cl_2 and washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 1/1) to obtain **11m** (250 mg, 45%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10–8.08 (m, 2H), 7.45–7.39 (m, 2H), 7.11–7.06 (m, 1H), 6.92–6.89 (m, 1H), 5.48 (br, 1H), 4.30–4.18 (m, 1H), 3.99 (s, 3H), 3.94–3.77 (m, 3H), 3.69–3.43 (m, 4H), 3.30–3.18 (m, 1H), 3.08–2.87 (m, 4H), 2.84–2.76 (m, 1H), 1.37 (s, 9H); LC-MS: *m*/*z* 579 (MH^+); IR 2846, 1660, 1630.

*4.2.4. Preparation of (R)-methyl-4-(1-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoate·HCl (**13m**)*

To a solution of (*R*)-methyl-4-(1-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoate **11m** (30 mg, 0.052 mmol) in ethyl acetate (2 mL), was added 4 M HCl/1,4-dioxane (2 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **13m** (20 mg, 76%, white solid). m.p. 158–160 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.06 (br, 3H), 7.93 (m, 2H), 7.47–7.45 (m, 4H), 3.81 (s, 3H), 3.70–3.33 (m, 7H), 3.30–3.28 (m, 2H), 3.15–2.66 (m, 5H); HRMS (free base, $C_{23}H_{25}F_3N_4O_4$): calcd., 478.1828; found, 478.1837; IR 3432, 2925, 1677, 1631.

*4.2.5. Preparation of (R)-4-(1-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoic acid·HCl (**13n**)*

To a solution of (*R*)-methyl-4-(1-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoate **11m** (50 mg, 0.087 mmol) in THF (2 mL) and MeOH (2 mL), was added $LiOH·H_2O$ (10.5 mg, 0.174 mmol in 1 mL water). The reaction mixture was stirred for 12 h at room temperature, and then acidified by 1 N HCl to adjust around pH 4. The reaction mixture was diluted with brine and ethyl acetate. The organic layer was separated, dried and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 9/1) to give (*R*)-4-(1-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluoro phenyl)butanoyl)-2,5-dihydro-1H-1,2,5-triazepane-5-carbonyl)benzoic acid (45 mg, 92%,) ^{1}H NMR ($CDCl_3$, 300 M Hz): δ 8.08–8.05 (m, 2H), 7.61–7.44 (m, 2H), 7.15–7.09 (m, 1H), 6.94–6.89 (m, 1H), 5.44 (br, 1H), 4.35–4.01 (m, 2H), 4.00–3.80 (m, 3H), 3.66–3.44 (m, 4H), 3.30–3.00 (m, 2H), 2.96–2.80 (m, 4H), 1.37 (s, 9H); LC-MS: *m*/*z* 565 (MH^+).

To a solution of (*R*)-4-(1-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-2,5-dihydro-1H-1,2,5-triazepane-5-carbonyl)benzoic acid (45 mg, 0.080 mmol) in ethyl acetate (2 mL), was added 4 M-HCl/1,4-dioxane (2 mL) and the mixture was stirred for 12 h at room temperature. The solvents

were evaporated, and the residue was crystallized with ether to give compound **13n** (32 mg, 81% white solid). m.p., 179–182 °C; ^{1}H NMR (DMSO-d_6,300 MHz): δ 8.85 (br, 3H), 7.96–7.80 (m, 2H), 7.52–7.47 (m, 4H), 4.20–3.56 (m, 10H), 3.39–3.36 (m, 2H), 2.94–2.72 (m, 3H); HRMS calcd. for $C_{22}H_{23}F_3N_4O_4$: 464.1671; found, 464.1666; IR 3432, 2921, 1679, 1631.

Compound **13a** was directly prepared from compound **5** by *Boc* deprotection, whereas compounds **13b** and **c** were prepared from compound **5** via compound **12** in two steps by reaction of electrophiles like acetyl chloride and benzoyl chloride, respectively, followed by *Boc* deprotection.

*4.2.6. (R)-Benzyl-1-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carboxylate·HCl (**13a**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.96 (br, 3H), 7.55–7.52 (m, 3H), 7.50–7.35 (m, 4H), 5.07 (s, 2H), 3.70–3.65 (m, 4H), 3.56–3.42 (m, 3H), 2.94–2.93 (m, 2H), 2.83–2.77 (m, 4H); LC-MS: *m*/*z* 451 (MH^+); IR 3420, 2846, 1699, 1683, 1639.

*4.2.7. (R)-Benzyl 1-acetyl-2-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbo xylate·HCl (**13b**)*

^{1}H NMR (MeOH-d_4, 300 MHz): δ 7.54 (m, 2H), 7.36–7.35 (m, 5H), 5.09 (s, 2H), 4.26–3.90 (m, 2H), 3.71–3.35 (m, 5H), 3.20–3.10 (m, 1H), 3.05–2.95 (m, 3H), 2.65–2.64 (m, 2H), 2.00 (s, 3H); LC-MS: *m*/*z* 493 (MH^+); IR 3449, 2882, 1667, 1631.

*4.2.8. (R)-Benzyl 1-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-2-benzoyl-1,2,5-triazepane-5-carboxylate·HCl (**13c**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.15–8.03 (brs, 3H), 7.42–7.02 (m, 12H), 4.91 (s, 2H), 4.38–4.15 (m, 1H), 3.93–3.21 (m, 8H), 3.08–3.06 (m, 2H), 3.01–2.90 (m, 2H); LC-MS: *m*/*z* 555 (MH^+); IR 3447, 2941, 1667, 1631.

Compounds **13d–g** were prepared from compound **8** in sequence of steps via compounds **9–11** by introducing various groups on N-5 by known means.

*4.2.9. (R)-3-Amino-1-(5-(pyrazin-2-yl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13d**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.56 (s, 1H), 8.31 (br, 1H), 7.81 (br, 1H), 7.27–7.18 (m, 1H), 7.15–7.06 (m, 1H), 4.11–3.86 (m, 6H), 3.69–3.63 (m, 2H), 2.99–2.94 (m, 6H), 2.78–2.72 (m, 2H); LC-MS: *m*/*z* 395 (MH^+); IR 3431, 2912, 1697, 1631.

*4.2.10. (R)-Methyl-2-(1-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepan-5-yl)acetate·HCl (**13e**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.23 (brs, 3H), 7.62–7.51 (m, 2H), 4.32 (s, 2H), 3.86–3.26 (m, 11H), 3.00–2.73 (m, 6H); LC-MS: *m*/*z* 389 (MH^+); IR 3431, 2910, 1699, 1631.

*4.2.11. (R)-3-Amino-1-(5-(2,2,2-trifluoroacetyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13f**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.09 (brs, 3H), 7.54–7.49 (m, 2H), 3.78–3.35 (m, 8H), 2.98–2.92 (m, 4H), 2.81–2.79 (m, 2H); LC-MS: *m*/*z* 413 (MH^+); IR 3432, 2927, 2867, 1680, 1633.

*4.2.12. (R)-3-Amino-1-(5-(methylsulfonyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13g**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.07 (brs, 3H), 7.54–7.46 (m, 2H), 4.01 (br, 1H), 3.63–3.62 (m, 2H), 3.37–3.29 (m, 4H), 3.00–2.93 (m, 4H), 2.90 (s, 3H), 2.88–2.78 (m, 2H); LC-MS: *m*/*z* 395 (MH^+); IR 3342, 2925, 1631.

Compounds **13h–l** were prepared from compound **8** by primarily fixing methanesulfonyl group on N-5 followed by in sequence of steps via compounds **9–11**. Then, reaction of compound **11** with various acyl halides to have compound **12** followed by *Boc* deprotection to give compound **13h–l**.

*4.2.13. (R)-1-(2-Acetyl-5-(methylsulfonyl)-1,2,5-triazepan-1-yl)-3-amino-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13h**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.14 (br, 3H), 7.59–7.58 (m, 2H), 4.24–4.19 (m, 2H), 3.85–3.78 (m, 1H), 3.60–3.36 (m, 4H), 3.24–3.00 (m, 4H), 2.99 (s, 3H), 2.97–2.76 (m, 1H), 2.23–1.93 (m, 4H); LC-MS: *m*/*z* 437 (MH^+); IR 3341, 2947, 2888, 1631.

*4.2.14. (R)-3-Amino-1-(5-(methylsulfonyl)-2-(2-morpholinoacetyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13i**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.45 (br, 3H), 7.68–7.56 (m, 2H), 4.98–4.62 (m, 3H), 4.33–3.82 (m, 7H), 3.64–3.39 (m, 10H), 3.12–2.82 (m, 6H); LC-MS: *m*/*z* 522 (MH^+); IR 3393, 2932, 2880, 1679, 1634.

*4.2.15. (R)-3-Amino-1-(2-(2-(benzo[d]oxazol-2-ylthio)acetyl)-5-(methylsulfonyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13j**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20–8.10 (m, 3H), 7.80–7.30 (m, 6H), 4.50–2.80 (m, 18H); LC-MS: *m*/*z* 586 (MH^+); IR 3441, 2932, 2880, 1758, 1665.

*4.2.16. (R)-3-Amino-1-(2-(2-(5-methyl-1,3,4-thiadiazol-2-ylthio)acetyl)-5-(methylsulfonyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13k**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.11 (m, 3H), 7.6–7.50 (m, 2H), 4.55–2.66 (m, 21H); LC-MS: *m*/*z* 567 (MH^+); IR 3423, 2927, 2862, 1669.

*4.2.17. (R)-3-Amino-1-(5-(methylsulfonyl)-2-(2-(thiazolo[5,4-b]pyridin-2-ylthio)acetyl)-1,2,5-triazepan-1-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**13l**)*

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.50 (m, 1H), 8.15–7.95 (m, 3H), 7.55–7.25 (m, 3H), 4.40–2.65 (m, 18H); LC-MS: *m*/*z* 603 (MH^+); IR 3443, 2925, 1671, 1679.

*4.2.18. Preparation of (R)-methyl-4-(1-acetyl-2-(3-amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-5-carbonyl)benzoate·HCl (**13o**)*

To a solution of (*R*)-4-{1-[3-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyryl]-[1,2,5]triazepane-5-carbonyl}benzoic acid methyl ester **11m** (90 mg, 0.156 mmol) in CH_2Cl_2, were added acetyl chloride (22 μL, 0.311 mmol) and TEA (65 μL, 0.467 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature. Organic layer was extracted from H_2O/CH_2Cl_2 and washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 7/1) to obtain compound **12o** (75 mg, 78%). ^{1}H NMR ($CDCl_3$, 300 M Hz): δ 8.12–8.04 (m, 2H), 7.53–7.43 (m, 2H), 7.12–7.03 (m, 1H), 6.93–6.91 (m, 1H), 5.25 (br, 1H), 4.59–4.40 (m, 1H), 4.38–4.29 (m, 1H), 4.26–4.13 (m, 1H), 4.11 (s, 3H), 3.95–3.42 (m, 4H), 3.01–2.94 (m, 3H), 2.64–2.49 (m, 2H), 2.30–1.98 (m, 3H), 1.37 (s, 9H); LC-MS: *m*/*z* 621 (MH^+); IR 3440, 2922, 2881, 1661, 1631.

To a solution of (*R*)-4-{1-acetyl-2-[3-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyryl]-[1,2,5]triazepane-5-carbonyl}benzoic acid methyl ester **12o** (25 mg, 0.040 mmol) in ethyl acetate (1 mL), added 4 M-HCl/1,4-dioxane (1 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **13o** (18 mg, 81%, white solid). 169–171 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.08 (br, 3H), 7.95 (m, 2H), 7.50–7.47 (m, 4H), 4.21–4.09 (m, 2H), 3.81 (s, 3H), 3.67–3.57 (m, 3H), 3.50 (s, 3H), 3.40–3.00 (m, 3H), 2.95–2.61 (m, 3H), 2.07–1.76 (m, 2H); HRMS (free base, $C_{25}H_{27}F_3N_4O_5$): calcd., 520.1934; found, 520.1899; IR 3441, 2923, 2867, 1669, 1631.

*4.2.19. Preparation of (R)-4-{1-acetyl-2-[3-amino-4-(2,4,5-trifluorophenyl)butyryl]-[1,2,5]triazepine-5-carbonyl}benzoic acid·HCl (**13p**)*

To a solution of (*R*)-4-{1-acetyl-2-[3-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyryl]-[1,2,5]triazepane-5-carbonyl}benzoic acid methyl ester **12o** (50 mg, 0.081 mmol) in THF (1 mL) and MeOH (1 mL), was added $LiOH{\cdot}H_2O$ (7 mg, 0.161 mmol in 1 mL water). The reaction mixture was stirred for 12 h at room temperature, and then acidified by 2 N HCl to adjust around pH 4. The reaction mixture was diluted with brine and ethyl acetate. The organic layer was separated, dried and evaporated to give (*R*)-4-{1-acetyl-2-[3-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)-butyryl]-[1,2,5]triazepane-5-carbonyl}benzoic acid (46.5 mg, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.23–8.08 (m, 2H), 7.61–7.33 (m, 2H), 7.23–7.07 (m, 1H), 7.00–6.80 (m, 1H), 5.30 (br, 1H), 4.70–4.38 (m, 2H), 3.90–3.38 (m, 7H), 3.03–2.64 (s, 3H), 2.80–2.26 (m, 4H), 1.38 (s, 9H); LC-MS: *m*/*z* 607 (MH^+).

To a solution of (*R*)-4-{1-acetyl-2-[3-*tert*-butoxycarbonylamino-4-(2,4,5-trifluorophenyl)butyryl]-[1,2,5]triazepane-5-carbonyl}benzoic acid (36 mg, 0.059 mmol) in ethyl acetate (2 mL), was added 4 M-HCl/1,4-dioxane (2 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **13p** (30 mg, 93%, white solid). 186–188 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.00–7.98 (m, 2H), 7.54–7.51 (m, 4H), 4.20–3.80 (m, 2H), 3.74–3.63 (m, 2H), 3.57 (s, 3H), 3.41–3.30 (m, 5H), 3.17–2.78 (m, 3H), 2.08–1.84 (m, 2H); IR 3441, 2925, 2889, 1664, 1631; HRMS calcd. for $C_{24}H_{25}F_3N_4O_5$: 506.1777; found, 506.1743.

*4.3. General procedure for the synthesis of compound **17** (Example: **17d**)*

*4.3.1. Preparation of benzyl-1-acetyl-2-nicotinoyl-1,2,5-triazepane-5-carboxylate (**14d**)*

To a solution of benzyl-1,2,5-triazepane-5-carboxylate·HCl **4** (100 mg, 0.324 mmol) in CH_2Cl_2, were added acetyl chloride (0.023 mL, 0.324 mmol) and TEA (0.18 mL, 1.298 mmol) at 0 °C. The reaction mixture was stirred for 12 h at room temperature, and diluted with brine and CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 4/1) to obtain benzyl-1-acetyl-1,2,5-triazepane-5-carboxylate (45 mg, 45%). ^{1}H NMR ($CDCl_3$, 300 M Hz): δ 7.36–7.33 (m, 5H), 5.70 (br, 1H), 5.16–5.12 (m, 2H), 4.16–4.08 (m, 5H), 3.08–3.00 (m, 1H), 2.94–2.88 (m, 2H), 2.04 (s, 3H); LC-MS: *m*/*z* 278 (MH^+).

To a solution of benzyl-1-acetyl-1,2,5-triazepane-5-carboxylate (200 mg, 0.721 mmol) in CH_2Cl_2, were added nicotinoyl chloride (257 mg, 1.442 mmol) and TEA (0.3 mL, 2.164 mmol) at 0°C. The reaction mixture was stirred for 48 h at room temperature and diluted with brine and CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 9/1) to obtain compound **14d** (160 mg, 58%). ^{1}H NMR ($CDCl_3$, 300 M Hz): δ 8.73–8.55 (m, 2H), 7.87–7.84 (m, 1H), 7.53–7.31 (m, 6H), 5.22 (s, 2H), 4.70–4.20 (m, 2H), 3.84–3.39 (m, 6H), 2.19–1.79 (m, 3H); LC-MS: *m*/*z* 383 (MH^+); IR 3036, 2947, 1679,1660.

*4.3.2. Preparation of 1-(2-nicotinoyl-1,2,5-triazepan-1-yl)ethanone (**15d**)*

To a solution of benzyl-1-acetyl-2-nicotinoyl-1,2,5-triazepane-5-carboxylate **14d** (150 mg, 0.392 mmol) in methanol (3 mL), added 10%-Pd/C (83 mg, 20 mol%). The reaction mixture was stirred for 12 h in hydrogen atmosphere, filtered through celite, washed with additional methanol and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 4/1) to give compound **15d** (81 mg, 83%). ^{1}H NMR (CD_3OD, 200 MHz): δ 8.72–8.63 (m, 1H), 8.09–7.89 (m, 1H), 7.59–7.43 (m, 1H), 4.60–4.01 (m,4H), 3.74–3.01 (m, 5H), 2.35–1.96 (m, 3H); LC-MS: *m*/*z* (relative intensity) 249 (MH^+); IR 3390, 2938, 1660, 1658.

*4.3.3. Preparation of (R)-tert-butyl-4-(1-acetyl-2-nicotinoyl-1,2,5-triazepan-5-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (**16d**)*

To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid (50 mg, 0.150 mmol) in CH_2Cl_2 (2 mL), were added 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI, 58 mg, 0.300 mmol), DMAP (1 mg, 0.008 mmol), TEA (0.006 mL, 45 mmol) and (2-nicotinoyl-1,2,5-triazepan-1-yl)ethanone **15d** (45 mg, 0.18 mmol). The reaction mixture was stirred for 12 h at room temperature and diluted with brine and CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/MeOH: 4/1) to obtain **16d** (66 mg, 78%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.78–8.67 (m, 2H), 7.80–7.76 (m, 1H), 7.42–7.32 (m, 1H), 7.18–7.00 (m, 1H), 6.95–6.88 (m, 1H), 5.32 (s, 1H), 4.66–4.12 (m, 1H), 4.08–3.72 (m, 2H), 3.65–3.04 (m, 6H), 2.97–2.90 (m, 2H), 2.65–2.57 (m, 2H), 2.27–2.12 (m, 3H), 1.39 (s, 9H); LC-MS: *m*/*z* 564 (MH^+).

*4.3.4. (R)-1-(1-Acetyl-2-nicotinoyl-1,2,5-triazepan-5-yl)-3-amino-4-(2,4,5-trifluorophenyl)butan-1-one hydrochloride·HCl (**17d**)*

To a solution of (*R*)-*tert*-butyl-4-(1-acetyl-2-nicotinoyl-1,2,5-triazepan-5-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate **16d** (30 mg, 0.054 mmol) in ethyl acetate (1 mL), was added 4 M-HCl/1,4-dioxane (1 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **17d** (25 mg, 93%, white solid). 171–173 °C; ^{1}H NMR (MeOH-d_4, 300 MHz): δ 9.08–9.00 (m, 2H), 8.88–8.51 (m, 1H), 8.11–8.04 (m, 1H), 7.30–7.20 (m, 1H), 7.15–7.13 (m, 1H), 4.69–4.39 (m, 1H), 4.30–4.02 (m, 2H), 3.82–3.63 (m, 4H), 3.10–2.99 (m, 3H), 2.81–2.60 (m, 2H), 2.01–1.86 (m, 6H); LC-MS: *m*/*z* 463 (MH^+); IR 3423, 2927, 2871, 1631.

*4.3.5. Preparation of (R)-3-amino-1-(1,2,5-triazepan-5-yl)-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**17a**)*

To a solution of (*R*)-3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoic acid (33 mg, 0.1 mmol) in CH_2Cl_2 (1 mL), were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 54 mg, 0.3 mmol), TEA (0.07 mL, 0.5 mmol) and di-*tert*-butyl-1,2,5-triazepane-1,2-dicarboxylate **8** (30 mg, 0.1 mmol). The reaction mixture was stirred for 12 h at room temperature and diluted with brine and CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane: 1/1) to obtain compound **16a** (43 mg, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05–6.97 (m, 1H), 6.94–6.88 (m, 1H), 5.64 (br, 1H), 4.22–4.07 (m, 3H), 3.90–3.80 (m, 1H), 3.76–3.39 (m, 3H), 3.27–3.39 (m, 3H), 3.27–3.05 (m, 2H), 2.93–2.90 (m, 2H), 2.58–2.53 (m, 2H), 1.46 (s, 18H), 1.37 (s, 9H); LC-MS: *m*/*z* 162 (MH^+); IR 3433, 2844, 1630.

To a solution of (*R*)-di-*tert*-butyl-5-(3-(*tert*-butoxycarbonylamino)-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-1,2-dicarboxylate **16a** (30 mg, 0.049 mmol) in ethyl acetate (1 mL), was added 4 M-HCl/1,4-dioxane (1 mL) and the mixture was stirred for 16 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give compound **17a** (18 mg, 98%, white solid). 117–119 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.19 (brs, 3H), 7.63–7.50 (m, 2H), 3.73–3.57 (m, 5H), 3.35–3.11 (m, 4H), 3.07–2.91 (m, 2H), 2.82–2.66 (m, 2H); LC-MS: *m*/*z* 317 (MH^+); IR 3423, 2930, 1631.

*4.3.6. (R)-1-(1-Acetyl-1,2,5-triazepan-5-yl)-3-amino-4-(2,4,5-trifluorophenyl)butan-1-one·HCl (**17b**)*

^{1}H NMR (MeOH-d_4, 300 MHz): δ 7.35–7.28 (m, 1H), 7.18–7.08 (m, 1H), 3.80–3.67 (m, 7H), 3.64–3.48 (m, 2H), 3.10–2.83 (m, 4H), 2.81–2.60 (m, 3H), 2.19–1.93 (m, 3H); LC-MS: *m*/*z* 359 (MH^+); IR 3415, 2923, 2853, 1692, 1633.

*4.3.7. (R)-1,1′-(5-(3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)-1,2,5-triazepane-1,2-diyl)diethanone·HCl (**17c**)*

^{1}H NMR (MeOH-d_4, 300 MHz): δ 7.28–7.18 (m, 1H), 7.16–7.10 (m, 1H), 4.35–4.29 (m, 2H), 4.10–3.90 (m, 1H), 3.74–3.71 (m, 2H), 3.56–3.43 (m, 3H), 3.20–2.95 (m, 5H), 2.87–2.50 (m, 2H), 2.17–1.87 (m, 6H); LC-MS: *m*/*z* 401 (MH^+); IR 3423, 2932, 2888, 1633.

*4.3.8. (R)-3-(3-Amino-4-(2,4,5-trifluorophenyl)butanoyl)hexahydro-1H-pyridazino[1,2-a][1,2,5]-triazepane-7,10-dione·HCl (**17e**)*

^{1}H NMR (MeOH-d_4, 300 MHz): δ 7.31–7.22 (m, 1H), 7.19–7.10 (m, 1H), 3.86–3.42 (m, 8H), 2.98–2.95 (m, 3H), 2.91–2.57 (m, 6H); LC-MS: *m*/*z* 399 (MH^+); IR 3419, 2927, 2853, 1631.

4.4. DPP-IV in vitro activity

10 μL of Caco-2 cell lysate was suspended in Tris–HCl (pH 7.5) and 40 μM Ala-Pro-AFC (ICN Biomedicals, Inc.) was added. After treatment of compounds, the mixture was incubated for 60 min at 24 °C. AFC as a indicator of DPP-IV activity was detected at 405 nm/510 nm (Ex/Em) by Fluorometer, Synergy HT (Biotek). IC_{50} was calculated by Prism 4.0 software (GarphPad Software, Inc.).

4.5. CYP assay

The CYP 3A4 enzyme assay was carried out using fluorometric enzyme assays with Vivid CYP 3A4 assay kit (PanVera, USA, CA), in a 96-well microtiter plate following the manufacturer's instruction with some modification. Test compounds including the ketoconazole is known as CYP 3A4 inhibitor was prepared in acetonitrile to give final concentrations of 10 μM. Briefly, to each well of the microtiter plate was added NADP generating solution (1.0 mM $NADP^+$, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2{\cdot}6H_2O$, and 0.4 U/mL glucose 6-phosphate dehydrogenase in 10 mM K_3PO_4, pH 8.0) followed by the vehicle acetonitrile (control) and the test samples. The plate was covered and then incubated at 37 °C for 20 min. Enzyme reaction was initiated by the addition of enzyme/substrate (E/S) mixture (0.5 pmol CYP 3A4 enzyme and 5 μM dibenzylfluorescein, DBF). The plate was further incubated for 20 min, followed by the addition of the stop solution to terminate the enzyme activity. Background reading was measured in a similar manner except for the E/S mixture which was added after the enzyme reaction was terminated. The fluorescence of DBF metabolite fluorescein was measured on a fluorescence plate reader with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The effect of test compounds on CYP 3A4 enzyme was calculated as the percentage of the enzyme activity.

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References

[1] S. Wild, G. Roglic, A. Green, R. Sicree, H. King, Diabetes Care 27 (2004) 1047–1053.
[2] L.B. Knudsen, J. Med. Chem. 47 (2004) 4128–4134.
[3] D.J. Drucker, Endocrinology 142 (2001) 521–527.
[4] J.J. Holst, E.F. Deacon, Curr. Opin. Pharmacol. 4 (2004) 589–596.
[5] D.J. Drucker, Gastroenterology 122 (2002) 531–544.
[6] T.J. Kieffer, C.H.S. McIntosh, T.A. Pederson, Endocrinology 136 (1995) 3585–3596.
[7] C.F. Deacon, M.A. Nauck, M. Toft-Nielson, L. Pridal, B. Willms, J.J. Holst, Diabetes 44 (1995) 1126–1131.
[8] R. Mentlein, Regul. Pept. 85 (1999) 9–24.
[9] B. Ahren, J.J. Holst, H. Martensson, B. Balkan, Eur. J. Pharmacol. 404 (2000) 239–245.
[10] C.F. Deacon, T.E. Hughes, J.J. Joist, Diabetes 47 (1998) 764–769.
[11] J.A. Pospisilik, S.G. Stafford, H.-U. Demuth, R. Brownsey, W. Parkhous, D.T. Finegood, D.H. McIntosh, R.A. Pederson, Diabetes 51 (2002) 943–950.
[12] L.W. Hertel, J.S. Kroin, J.W. Misner, J.M. Tustin, J. Org. Chem. 53 (1988) 2406–2409.
[13] R. Filler, et al. (Eds.), Organo Fluorine Compounds in Medicinal Chemistry and Biomedical Applications, Studies in Organic Chemistry, vol. 48, 1993, pp. 362.
[14] D. Kim, L. Wang, M. Beconi, G.J. Eiermann, M.H. Fisher, H. He, G.J. Hickey, J.E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M.E. McCann, R.A. Patel, A. Petrov, G. Scapin, S.B. Patel, R.S. Roy, J.K. Wu, M.J. Wyvratt, B.B. Zhang, L. Zhu, N.A. Thornberry, A. Weber, J. Med. Chem. 48 (2005) 141–151.
[15] D. Kim, J.E. Kowalchick, L.L. Brockunier, E.R. Parmee, G.J. Eiermann, M.H. Fisher, H. He, B. Leiting, K. Lyons, G. Scapin, S.B. Pater, A. Petrov, K.D. Pryor, R.S. Roy, J.K. Wu, X. Zhang, M.J. Wyvratt, B.B. Zhang, L. Zhu, N.A. Thornberry, A.E. Weber, J. Med. Chem. 51 (2008) 589–602.
[16] S.D. Edmondson, L. Wei, J. Xu, J. Shang, S. Xu, J. Pang, A. Chaudhary, D.C. Dean, H. He, B. Leiting, K.A. Lyons, R.A. Patel, S.B. Patel, G. Scapin, J.K. Wu, M.G. Beconi, N.A. Thornberry, A.E. Weber, Bioorg. Med. Chem. Lett. 18 (2008) 2409–2413.
[17] N.A. Thornberry, A.E. Weber, Curr. Top. Med. Chem. 7 (2007) 557–568.
[18] I. Idris, R. Donnelly, Diab. Obesity Metab. 9 (2007) 153–165.
[19] A. Matsuyama-Yokono, A. Tahara, R. Nakano, Y. Someya, I. Nagase, M. Hayakawa, M. Shibasaki, Biochem. Pharmacol. 76 (2008) 98–107.
[20] H. Fukushima, A. Hiratate, M. Takahashi, A. Mikami, M. Saito-Hori, E. Munetomo, K. Kitano, S. Chonan, H. Saito, A. Suzuki, Y. Takaoka, K. Yamamoto, Bioorg. Med. Chem. Lett. 16 (2008) 4093–4106.
[21] F.P. Guengerich, Cytochrome P450: structure, mechanism and biochemistry, in: P.R. Ortiz de Montellano (Ed.), Biochemistry, Plenum Press, New York, 1995, pp. 473–525.
[22] J. Lilja, K. Kivisto, P. Neuvonen, Clin. Pharmacol. Ther. 68 (2000) 384–390.
[23] J.H. Lin, A.Y.H. Lu, Pharmacol. Rev. 49 (1997) 403–449.
[24] F.P. Guengerich, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 1–17.
[25] J.H. Ahn, M.S. Shin, S.H. Jung, S.K. Kang, K.R. Kim, S.D. Rhee, N.S. Kang, S.Y. Kim, S.K. Sohn, S.G. Kim, M.S. Jin, J.O. Lee, H.G. Cheon, S.S. Kim, Bioorg. Med. Chem. Lett. 17 (2007) 2622–2628.
[26] M.A. Jun, W.S. Park, S.K. Kang, K.Y. Kim, K.R. Kim, S.D. Rhee, M.A. Bae, N.S. Kang, S.K. Sohn, S.G. Kim, J.O. Lee, D.H. Lee, H.G. Cheon, S.S. Kim, J.H. Ahn, Eur. J. Med. Chem. 43 (2008) 1889–1902.
[27] J.H. Ahn, W.S. Park, M.A. Jun, M.S. Shin, S.K. Kang, K.Y. Kim, S.D. Rhee, M.A. Bae, K.R. Kim, S.G. Kim, S.Y. Kim, S.K. Sohn, N.S. Kang, J.O. Lee, D.H. Lee, H.G. Cheon, S.S. Kim, Bioorg. Med. Chem. Lett. 18 (2008) 6525–6529.