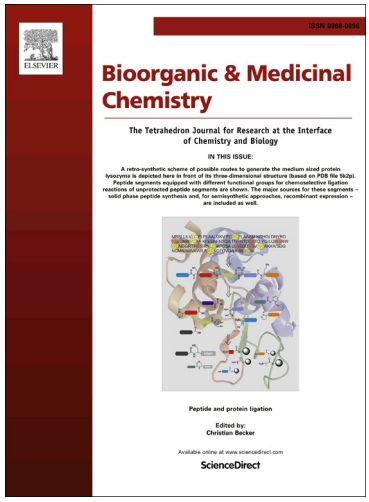
Accepted Manuscript



Design and Synthesis of a Novel Series of Orally active, Selective Somatostatin Receptor 2 Agonists for the Treatment of Type 2 diabetes

Yoshihiro Banno, Shigekazu Sasaki, Makoto Kamata, Jun Kunitomo, Yasufumi Miyamoto, Hidenori Abe, Naohiro Taya, Satoru Oi, Masanori Watanabe, Tomoko Urushibara, Masatoshi Hazama, Shin-ichi Niwa, Saku Miyamoto, Akira Horinouchi, Ken-ichi Kuroshima, Nobuyuki Amano, Shin-ichi Matsumoto, Shinichiro Matsunaga

PII: S0968-0896(17)31260-9
DOI: https://doi.org/10.1016/j.bmc.2017.09.031
Reference: BMC 13991

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 26 June 2017
Revised Date: 19 September 2017
Accepted Date: 19 September 2017

Please cite this article as: Banno, Y., Sasaki, S., Kamata, M., Kunitomo, J., Miyamoto, Y., Abe, H., Taya, N., Oi, S., Watanabe, M., Urushibara, T., Hazama, M., Niwa, S-i., Miyamoto, S., Horinouchi, A., Kuroshima, K-i., Amano, N., Matsumoto, S-i., Matsunaga, S., Design and Synthesis of a Novel Series of Orally active, Selective Somatostatin Receptor 2 Agonists for the Treatment of Type 2 diabetes, *Bioorganic & Medicinal Chemistry* (2017), doi: https://doi.org/10.1016/j.bmc.2017.09.031

Design and Synthesis of a Novel Series of Orally active, Selective Somatostatin Receptor 2 Agonists for the Treatment of Type 2 diabetes

Yoshihiro Banno, Shigekazu Sasaki, Makoto Kamata, Jun Kunitomo, Yasufumi Miyamoto, Hidenori Abe, Naohiro Taya, Satoru Oi, Masanori Watanabe, Tomoko Urushibara, Masatoshi Hazama, Shin-ichi Niwa, Saku Miyamoto, Akira Horinouchi, Ken-ichi Kuroshima, Nobuyuki Amano, Shin-ichi Matsumoto and Shinichiro Matsunaga.*

Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 26-1, Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

Abstract

The discovery of a novel series of β-methyltryptophan (β-MeTrp) derivatives as selective and orally active non-peptide somatostatin receptor 2 (SSTR2) agonists for the treatment of Type 2 diabetes is described. In our previous research, Compound **A**, β-MeTrp derivative with highly potent and selective SSTR2 agonistic activity IC_{50} (SSTR2/SSTR5) = 0.3/>100 (nM)), was identified as a drug candidate for treatment of Type 2 diabetes which lowers significantly plasma glucose level in Wistar fatty rats in its oral administrations. However, as serious increase in AUC and phospholipidosis (PLsis) were observed in its toxicological studies in rats, follow-up compounds were searched to avoid risk of PLsis with reference to their in vitro PLsis potentials evaluated on the basis of accumulation of phospholipids in HepG2 cells exposed to the compounds.

It has been found that introduction of a carbonyl group onto the piperidine and piperazine or aniline moiety of compounds **A** and **B** reduced markedly the in vitro PLsis potentials. And further modification of the compounds and their evaluation led to a discovery of compounds **3k** with lower in vitro PLsis potentials exhibiting lowering effect of hypoglycemia-induced glucagon secretion in SD rats (ED_{50} = 1.1 mg/kg) and glucose excursion in meal tolerance test in Wistar fatty diabetic rats (MED = 3.0 mg/kg) in oral administrations.

Compound **3k** was selected as a new drug candidate of selective and orally active non-peptide SSTR2 agonists for treatment of Type 2 diabetes with low in vivo PLsis potential.

KEYWORDS: non-peptide somatostatin receptor 2 agonist; β-methyltryptophan derivatives; phospholipidosis; Type 2 diabetes

*Corresponding author. Tel:+81-466-32-1200. Fax: +81-466-29-4459

e-mail: yoshihiro.banno@takeda.com

1. Introduction

It is studied well that excessive secretion of glucagon in Type 2 diabetics causes fasting and postprandial hyperglycemia through acceleration of glycogenolysis and gluconeogenesis in liver in patients. [1]

Somatostatin is a well-known regulatory hormone in endocrine and exocrine systems which suppresses secretion of glucagon, insulin, gastrin, and growth hormone depending on its binding to 5-subtypes of G-protein coupled receptors (SSTR1–5).[2,3] Among them SSTR2 has been revealed to affect significantly suppression of secretion of glucagon, and a SSTR2 agonist is anticipated to lower plasma glucose levels in hyperglycemia through inhibition of glycogenolysis and gluconeogenesis induced by glucagon and accordingly would be a novel class of therapeutic drug for type 2 diabetes without side effect of increase in body weight caused by PPARγ agonist.[4]

The research group in Merck reported selective SSTR2 agonists L-054,522, L-779,967 [5] bearing β-methyltryptophan (β-MeTrp) structure and also we developed β-MeTrp derivative **A**[6,7] as an orally active SSTR2 agonist exhibiting full agonistic activity and high selectivity over SSTR5 (IC_{50} (SSTR2/5) = 0.2/>100 (nM)). In compound **A**, (*N*,*N*-dimethylaminomethyl)aniline may mimic the amino group of Lys9 and 4-phenyl-1-piperidinecarboxamide moiety would serve as a surrogate for Phe7 in the active site of SST-14.

We found that compound **A** lowers significantly plasma glucagon levels with single dosing and decrease glucose excursion after meal challenge in Wister fatty rats. However, repeated dose toxicity study of the candidate compound revealed that it induces serious phospholipidosis (PLsis) in rats and thus following drug candidates without induction of PLsis had to be searched.

To avoid the potential risk of PLsis probably due to the cationic amphiphilic drugs (CAD) structure of β-MeTrp derivatives bearing an amino functionality essential for SSTR2 agonists activity, compounds **1**–**3** with a hydrophilic side chain were designed and synthesized for evaluation because displacement of *N*,*N*-dimethylaminomethyl group of compounds **A** and **B**[7] with weakly basic or non-basic functionalities resulted in significant decrease in activity.

In the present paper, we describe syntheses of β-MeTrp derivatives, their SAR and in vitro PLsis potentials evaluated on the basis of accumulation of phospholipids in HepG2 cells exposed to the compounds to find out a new orally active and selective non-peptide SSTR2 agonist as a therapeutic drug for Type 2 diabetes.

[Figure 1]

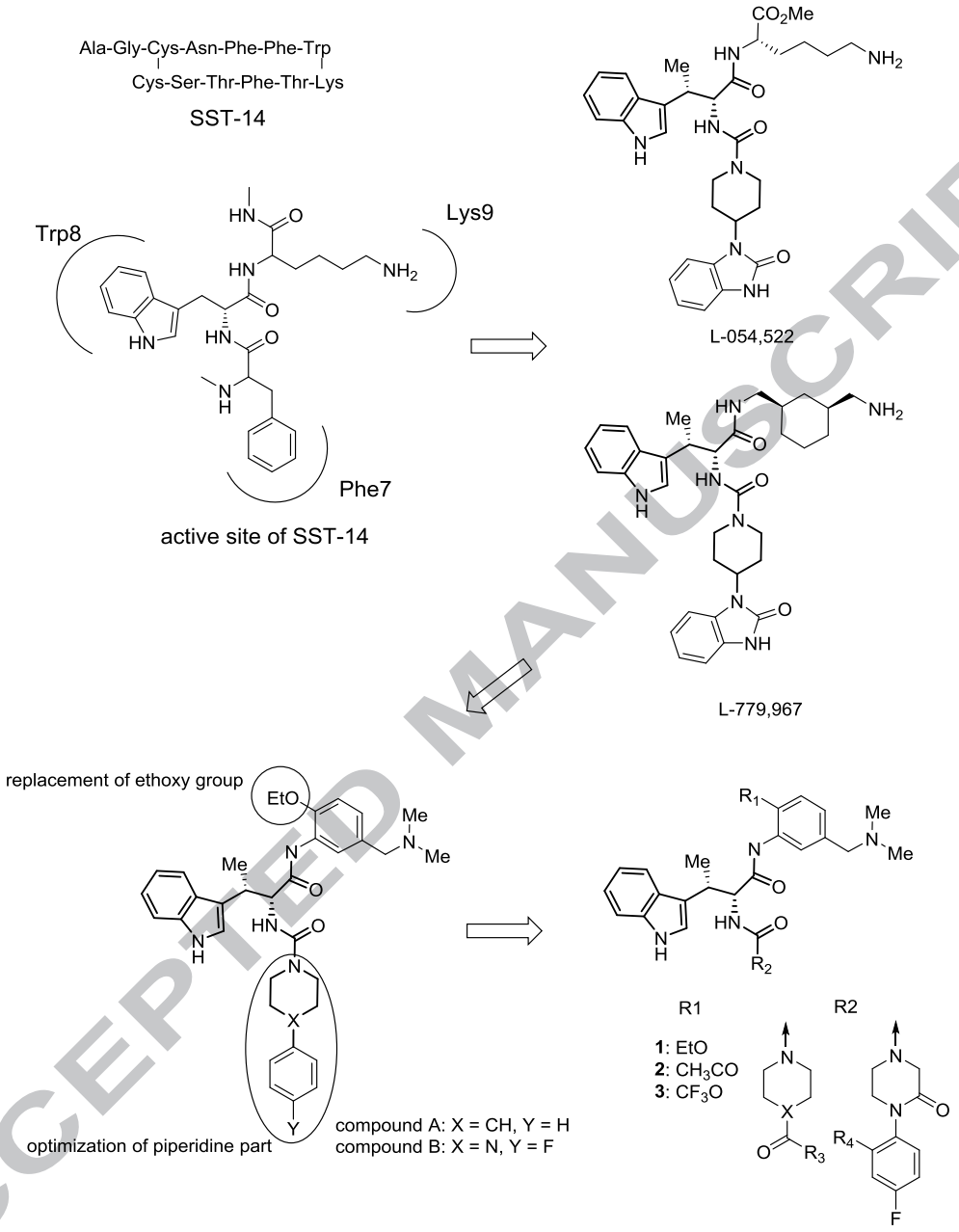


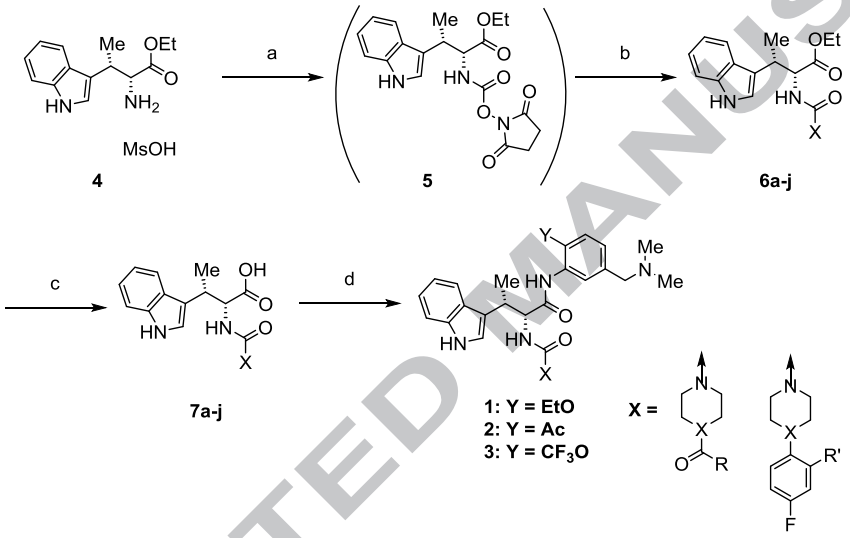
Figure 1. Drug design of Selective and orally active non-peptide SSTR agonist

2. Chemistry

Generally, compounds **1**–**3** were synthesized from **4** via urea formation with potential surrogates **8** for Phe7 followed by a peptide coupling with (*N*,*N*-dimethylaminomethyl)anilines **9** mimicking Lys9 (Scheme 1): The *N*-ureido-β-MeTrp intermediates **6** was prepared from **4** and **8** using *N*,*N*′-disuccinimidyl carbonate (DSC).[6] Hydrolysis of **6** followed by peptide coupling of **7** with **9** afforded the desired compounds without racemization.

[Scheme 1]

Scheme 1. General synthetic method of compounds 1–3.

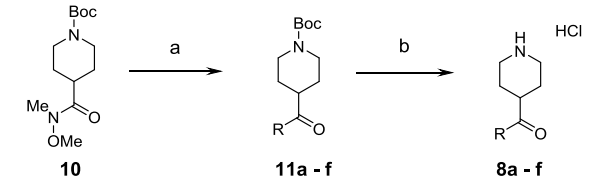


Reagents and conditions: (a) DSC, $^{i}Pr_2NEt$, MeCN; (b) piperidine derivatives or piperazine derivatives (**8a**–**j**), $^{i}Pr_2NEt$, MeCN; (c) aq. NaOH, MeOH; (d) **9a**–**c**, WSC, HOBt, $^{i}Pr_2NEt$, THF, MeCN.

Compounds **8a**–**f** were synthesized by the reactions of **10** with Grignard reagents or lithiated reagents followed by the removal of the Boc group of **10a**–**f** by treatment with HCl (Scheme 2). Alternatively, compound **8g** was prepared by the reaction of **12** with 2-furyl lithium followed by oxidation of **13** and removal of the Boc group of **14** by treatment with HCl (Scheme 3).

[Scheme 2]

Scheme 2. Syntheses of compounds 8a–f.

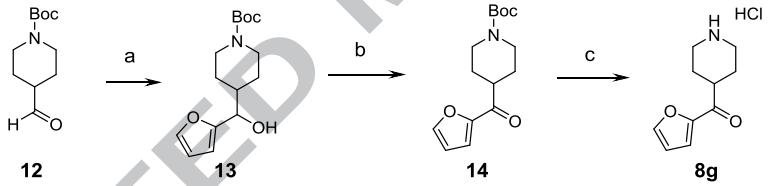


10 11a - f 8a - f

compound 11, 8	R	compound 11, 8	R
a		d	
b		e	
c		f	

Reagents and conditions: (a) RMgX (X = Cl or Br) or RLi, THF, -78 ºC; (b) 4 N HCl-AcOEt, rt. [Scheme 3]

Scheme 3. Synthesis of compound 8g



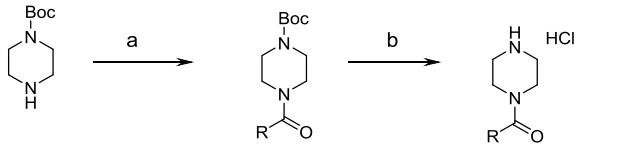
12 13 14 8g

Reagents and conditions: (a) nBuLi, furan, THF, -78 ºC; (b) $PySO_3$, Et_3N, DMSO, CH_2Cl_2, rt; (c) 4 N HCl-AcOEt, rt.

Compounds **8h** and **8i** were synthesized by acylation of **15** followed by the removal of the Boc group of **16a** and **16b** by treatment with HCl (Scheme 4).

[Scheme 4]

Scheme 4. Syntheses of compounds 8h and 8i



15

16a (R= cyclobutyl)
16b (R= tert-Bu)

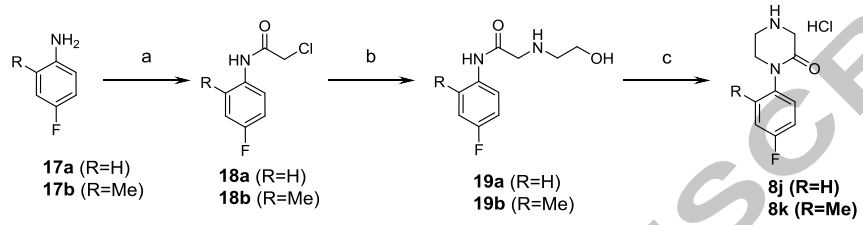
8h (R= cyclobutyl)
8i (R= tert-Bu)

Reagents and conditions: (a) acyl chloride, Et_3N, THF, rt; (b) 4 N HCl-AcOEt, rt.

1-Phenylpiperazine-2-one[7] derivatives **8j** and **8k** were synthesized by Mitsunobu reaction of **19a** and **19b** (Scheme 5).

[Scheme 5]

Scheme 5. Syntheses of compounds 8i and 8j

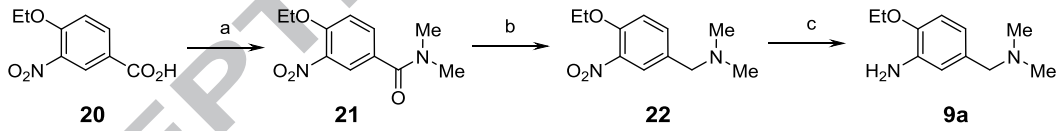


Reagents and conditions: (a) K_2CO_3, chloroacetyl chloride, AcOEt, rt; (b) ethanolamine, AcOEt, rt; (c) nBu_3P, DIAD, AcOEt, rt; (d) 10% HCl-MeOH, rt.

(*N*,*N*-Dimethylaminomethyl)anilines **9a**–**c** were synthesized from corresponding 3-nitro-*N*,*N*-dimethylbenzamide derivatives **21**, **26**, and **32** respectively by the reduction of the nitro and benzamide groups (Schemes 6–8).

[Scheme 6]

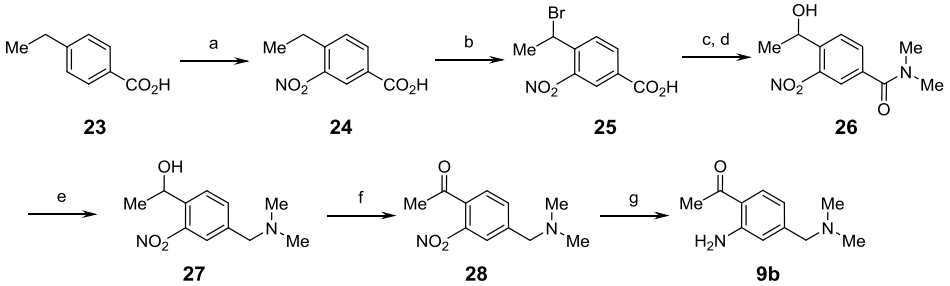
Scheme 6. Synthesis of compound 9a



Reagents and conditions: (a) dimethylamine hydrochloride, WSC, HOBt, MeCN, rt; (b) BH_3·THF, THF, reflux; c) Fe, $CaCl_2$, EtOH-H_2O, reflux.

[Scheme 7]

Scheme 7. Synthesis of compound 9b

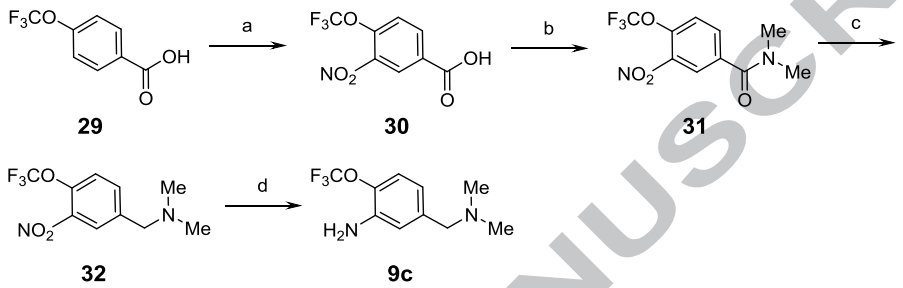


Reagents and conditions: (a) fuming HNO_3, -10 ºC; (b) NBS, AIBN, CCl_4, reflux; (c) Ag_2CO_3,

acetone, H_2O, 0 ºC - rt; (d) 2 N dimethylamine in THF, WSC, HOBt, THF, MeCN, rt; (e) (i) BH_3·THF, THF, reflux, (ii) MeOH, reflux; (f) TPAP, NMO, MS4A, THF, rt; (g) hydrazine monohydrate, Pd/C, EtOH, rt.

[Scheme 8]

Scheme 8. Synthesis of compound 9c

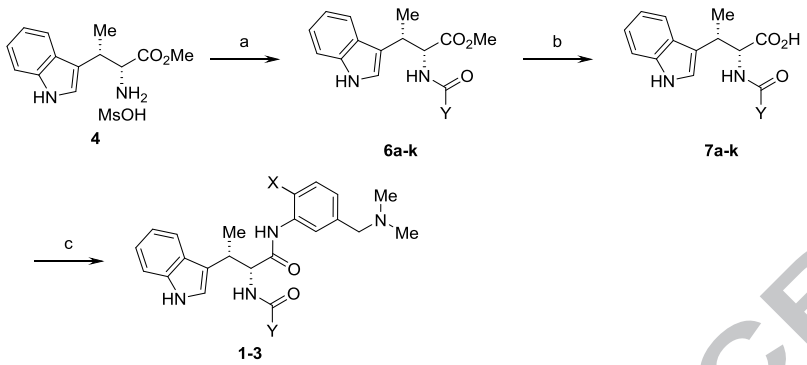


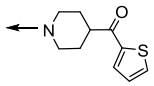
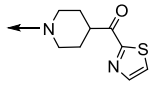
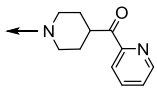
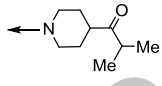
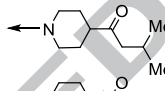
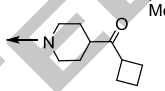
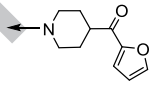
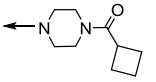
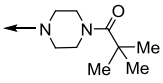
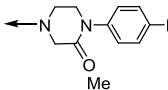
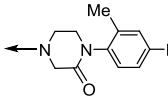
Reagents and conditions: (a) fuming HNO_3, conc. H_2SO_4, -10 ºC; (b) 2 M dimethylamine in THF, WSC, HOBt, THF, MeCN, rt; (c) (i) BH_3·THF, THF, reflux, (ii) MeOH, reflux; (d) hydrazine monohydrate, Pd/C, EtOH, rt.

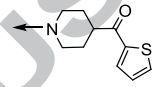
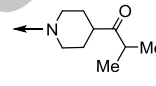
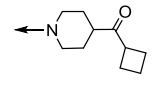
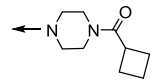
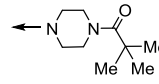
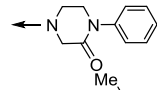
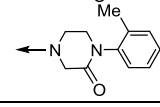
Finally, the N-ureido-β-MeTrp intermediates **6a**–**k** were prepared from **4** and **8a**–**k** using *N*,*N*-disccinimidyl carbonate (DSC)[6] and following acid hydrolysis of **6** and then peptide coupling of **7a**–**j** with **9a**–**c** (Table 1).

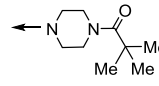
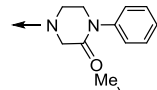
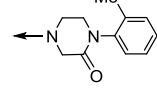
[Table 1]

Table 1. Syntheses of compounds 1-3



compound	X	Y
1a	EtO	
1b	EtO	
1c	EtO	
1d	EtO	
1e	EtO	
1f	EtO	
1g	EtO	
1h	EtO	
1i	EtO	
1j	EtO	
1k	EtO	

compound	X	Y
2a	CH_3CO	
2d	CH_3CO	
2f	CH_3CO	
2h	CH_3CO	
2i	CH_3CO	
2j	CH_3CO	
2k	CH_3CO	

compound	X	Y
3i	CF_3O	
3j	CF_3O	
3k	CF_3O	

Reagents and conditions: (a) DSC, $^{i}Pr_2NEt$, MeCN; (b) piperidine derivatives or piperazine derivatives (**8a**–**j**), $^{i}Pr_2NEt$, MeCN; (c) aq. NaOH, MeOH; (d) **9a**–**c**, WSC, HOBt, $^{i}Pr_2NEt$, THF, MeCN.

3. Results and Discussion

Compounds **1a**–**1g**, where the phenyl group on the C(4) position of the piperidine ring of compound **A** is displaced with acyl group, were designed and synthesized as analogues of compound **A** with a hydrophilic side chain and in vitro binding affinities for SSTR2 were investigated using compounds **A** and **B** as reference compounds (Table 2).

Miyamoto[8] reported in vitro PLsis potentials of the compounds could be appropriate to detect the appearance of PLsis as a side effect in pre-clinical toxicity studies in rats which are estimated on the basis of accumulation of phospholipids bearing a fluorophore by measuring fluorescent intensity in HepG2 cells exposed to the compounds. Thus, in vitro PLsis potentials of the compounds were also investigated according to the above method using amiodarone as a reference compound (Table 2).

[Table 2]

Table 2. Binding affinity of compounds 1a–g bearing a 4-acylpiperidine for SSTR2 and their in vitro PLsis potentials

compound No.	human SSTR2 binding IC_{50} (nM)	LogD	In vitro PLsis potential (% of amiodarone)
1a	0.58	1.89	98
1b	2.0	1.35	74
1c	2.6	1.57	81
1d	1.1	1.50	63
1e	0.89	2.01	76
1f	0.6	1.55	75
1g	2.3	1.14	54
Compound **A**	0.2	3.0	111
Compound **B**	0.2	2.22	99
amiodarone			100

As a Table 2 shows, displacement of the phenyl group of compound **A** with 2-thienoyl group (**1a**) led to a slightly decrease in the binding affinity for SSTR2 compared to compound **A**. Compounds substituted with a 2-thiazoyl, 2-furoyl, and 2-pyridine-2-carbonyl group (**1b**–**d**) did not show so potent activity as compound **A** in spite of exhibiting lower in vitro PLsis potentials than amiodarone. Introduction of 2-methylpropanoyl, 3-methylbutanoyl, and cyclobutanoyl group on the piperidine (**1e**–**g**) brought about recovery of about subnanomolar IC_{50} values of binding affinity for SSTR2 with significant reduction of in vitro PLsis potentials.

These results suggest that modification of piperidine ring moiety of compound **A** would give rise to new candidate drugs without induction of PLsis which prompted us modification of piperazine ring moiety of compound **B** with as potent binding affinity for SSTR2 as compound A and lower log D value than compound **A**. Thus, we designed and synthesized compounds **1h**–**k** as analogues of compound **B** with a hydrophilic side chain and in vitro binding affinities for SSTR2 and in vitro PLsis potentials were investigated (Table 3).

Although compounds **1h** and **1i** bearing a 4-acylpiperazine showed weak binding affinity, significant reduction of in vitro PLsis potentials have been observed. Compounds **1j** and **1k** bearing a 4-phenyl-3-oxopiperazine have been found to exhibit as potent binding affinity for SSTR2 as compounds **A** and **B** and lower in vitro potential of PLsis than compounds **A** and **B**. However, Compounds **1j** and **1k** resulted in insufficient lowering effect on plasma glucagon levels with single dosing in SD rats due to unfavorable pharmacokinetic profiles.

[Table 3]

Table 3. Binding affinity for SSTR2 and their in vitro PLsis potentials of compounds 1h–k modified at piperazine ring moiety of Compound B

Compound No.	SSTR2 binding IC_{50} (nM)	Log D	In vitro PLsis potential (% of amiodarone)
1h	2.1	0.73	32
1i	2.3	1.09	38
1j	0.2	1.65	36
1k	0.2	1.86	47
Compound **A**	0.2	3.0	111
Compound **B**	0.2	2.22	99
amiodarone			100

To find out orally active compounds without induction of PLsis modification of (*N*,*N*-dimethylaminomethyl)anilines moiety of compound **1** was carried out next. Thus compounds **2** and **3** where the ethoxy group of **1** was displaced with an acetyl and a trifluoromethoxy group were designed and synthesized to be evaluated (Table 4).

Although compounds **2d**, **2h**, **2i**, **3i** and **3j** showed lower in vitro PLsis potentials, their binding affinities for SSTR2 were decreased significantly. Compounds **2j**, **2k**, and **3k** have been found to exhibit potent binding affinities for SSTR2 with lower in vitro PLsis potentials. Among them log D values of compounds **2j** and **2k** are similar to those of **1j** and **1k** with unfavorable pharmacokinetic profiles suggesting insufficient oral activity. On the other hand it is interesting to note that compounds log D values of **3k**, regardless of its lower in vitro PLsis potentials, is similar to those of

compounds **A** and **B** showing pharmacological effects in oral administration. Furthermore, compound **3k** has been found to show excellent selectivity over other SSTR subtypes (Table 5). From these investigations and considerations, compound **3k** was selected for further evaluation in vivo.

[Table 4]

[Table 5]

Table 4. Binding affinity for SSTR2 and their in vitro PLsis potentials of compounds 2 and 3 modified at (*N,N*-dimethylaminomethyl)anilines moiety of compound 1

Compound No.	SSTR2 binding IC_{50} (nM)	Log D	In vitro PLsis potential (% of amiodarone)
2a	0.68	1.84	91
2d	2.0	1.44	58
2f	0.69	1.50	84
2h	1.80	1.04	46
2i	0.98	0.68	36
2j	0.31	1.59	37
2k	0.24	1.81	30
3i	2.8	2.18	48
3j	0.53	2.73	50
3k	0.27	2.95	58
Compound **A**	0.2	3.0	111
Compound **B**	0.2	2.22	99
amidarone			100

Table 5: Binding affinity of compound 3k for SSTR subtypes

compound	Binding affinity (nM)				
	hSSTR1	hSSTR2	hSSTR3	hSSTR4	hSSTR5
3k	5100	0.17	360	100	1700

Expectedly, compound **3k** has been found to show moderate pharmacokinetic profiles in SD rats (Figure 3) and lower hypoglycemia-induced glucagon secretion in dose dependent manner in SD rats (ED50 = 1.1 mg/kg) (Figure 4). Furthermore, oral administration of **3k** decreased glucose excursion

in meal tolerance test in Wistar fatty diabetic rats, a type 2 diabetic model and its minimum effective dose was 3.0 mg/kg (Figure 5).

Accordingly, compound **3k** was selected as a new drug candidate of selective and orally active non-peptide SSTR2 agonists for treatment of type 2 diabetes with low in vivo PLsis potential.

[Figure 3]

[Figure 4]

[Figure 5]

Figure 3. Pharmcokinetic parameters of 3k in fasted male SD rats (8-week-old, n = 3).

Pharmacokinetics of 3k in SD rat

i.v. at 0.3 mg/kg	3k	p.o. at 1 mg/kg	3k
C_{5min}	193.6 ng/mL	Cmax	10.3 ng/mL
AUCiv	118.9 ng·h/mL	Tmax	0.83 h
MRTiv	2.57 h	AUC po	22.9 ng·h/mL
VDss	6631mL/kg	MRT po	5.69 h
CLtotal	2607 mL/h/kg	BA	5.8%

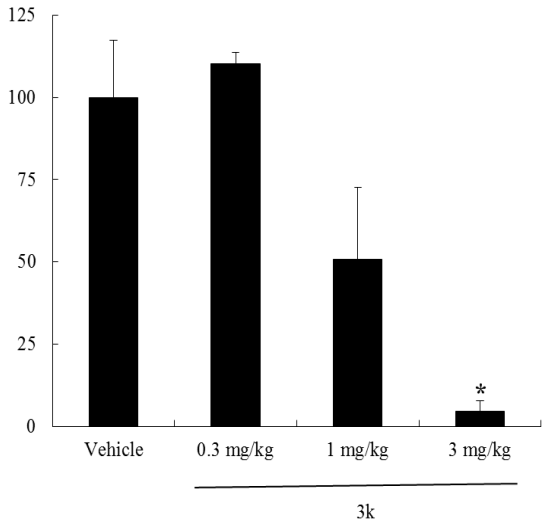


Figure 4. Suppression of glucagon secretion in normal rats after single oral administration of 3k.

The effect of **3k** on hypoglycemia-induced glucagon secretion in SD rats.

After an hour of drug adminisration insulin (2 IU/kg) was subcutaneously injected.

Blood was taken from tail vein after 30 minutes of insulin injection and plasma glucagon levels were measured by radioimmunoassay.

Data are presented as means ± SD (n = 6).

*P < 0.025 versus vehicle by one-tailed Williams' test.

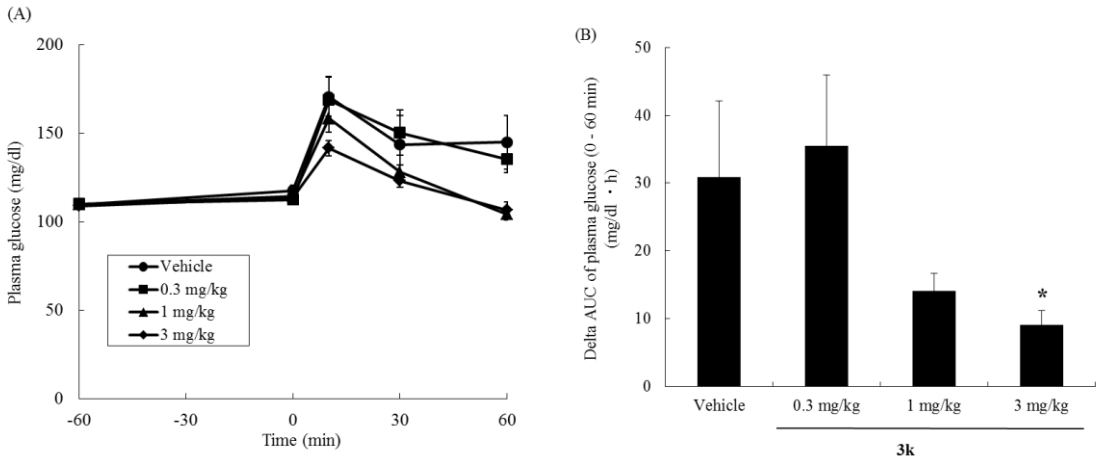


Figure 5. Acute effect of 3k on glucose excursion during meal tolerance test in type 2 diabetic rats

Female Wistar Fatty rats were fasted overnight and were given vehicle or **3k** orally. (A) shows time dependent changes in plasma glucose levels after liquid meal challenge (20 kcal/kg). (B) represents area under the curve (AUC) of delta plasma glucose shown in (A).

Data are presented as means ± SD (n = 6).

*P < 0.025 versus vehicle by one-tailed Williams' test.

4. Conclusion

We have discovered a new β-MeTrp derivative **3k** as a drug candidate of selective and orally active non-peptide somatostatin receptor 2 (SSTR2) agonists for treatment of Type 2 diabetes without induction of PLsis.

Modification of compounds **A** and **B**, exhibiting serious increase in AUC and phospholipidosis (PLsis) in their toxicological studies in rat, at piperidine and piperazine or aniline moiety was carried out to avoid risk of PLsis with reference to their in vitro PLsis potentials evaluated on the basis of accumulation of phospholipids in HepG2 cells exposed to the compounds. It has been found that introduction of a carbonyl group onto the piperidine and piperazine or aniline moiety of compounds **A** and **B** reduced markedly the in vitro PLsis potentials. And further modification of the compounds and their evaluation led to a discovery of compounds **3k** with lower in vitro PLsis potentials exhibiting lowering effect of hypoglycemia-induced glucagon secretion in SD rats (ED_{50} = 1.1 mg/kg) and glucose excursion in meal tolerance test in Wistar fatty diabetic rats (MED = 3.0 mg/kg) in oral administrations.

These results suggest strongly selective SSTR2 agonist would be a novel class of therapeutic drug for type 2 diabetes.

5. Experimental section

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE (300 MHz for ^{1}H NMR) and Bruker AVANCE II^{+}600 (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR). Chemical shifts for ^{1}H NMR are given in parts per million (ppm) downfield from tetramethysilane (δ) as the internal standard in deuterated solvent and coupling constants (*J*) are in Hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, brs = broad singlet), and coupling constants. All solvents and reagents were obtained from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was performed on Merck TLC silica gel plates 60F$_{254}$ or Fuji Silysia Chromatorex TLC plates NH. Column chromatography was performed on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, Merck). LC-MS analysis was performed on a Waters, Agilent, or Shimadzu Liquid Chromatography-Mass Spectrometer System, operating in APCI (+ or −) or ESI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing water/acetonitrile, 0.1% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase. The purities of compounds submitted for biological evaluation were determined by LC-MS analysis (detection at 220 nm). Yields are not optimized.

***tert*-Butyl 4-(2-thienylcarbonyl)piperidine-1-carboxylate (11a).** To a solution of thiophene (1.50 mL, 18.7 mmol) in THF (30 mL) was added dropwise ^{n}BuLi in hexane (1.6 M, 9.7 mL) at -30 ^{o}C and the resulting mixture was stirred at same temperature for 30 min. To the mixture was added a solution of **9** (5.0 g, 18.7 mmol) in THF (20 mL) at-78 ^{o}C, and the resulting mixture was stirred at room temperature overnight. The mixture was quenched with 1 N HCl (100 mL) at 0 ^{o}C, and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **10a** (1.18 g, 64%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.47 (9H, s), 1.70–1.96 (5H, m), 2.76–3.01 (2H, m), 3.14–3.35 (1H, m), 3.94–4.41 (2H, m), 7.15 (1H, dd, *J* = 3.9, 5.0 Hz), 7.66 (1H, dd, *J* = 1.0, 5.0 Hz), 7.74 (1H, dd, *J* = 1.1, 3.8 Hz).

Compound **11b-f** were prepared in a manner similar to that described for **11a**.

***tert*-Butyl 4-(1,3-thiazol-2-ylcarbonyl)piperidine-1-carboxylate (11b).** ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.47 (9 H, s), 1.60–1.81 (2H, m), 1.97 (2H, d, *J* = 12.3 Hz), 2.91 (2H, t, *J* = 11.9 Hz), 3.67–3.84 (1H, m), 4.18 (2H, d, *J* = 10.9 Hz), 7.69 (1H, d, *J* = 3.2 Hz), 8.02 (1H, d, *J* = 3.0 Hz).

***tert*-Butyl 4-(pyridin-2-ylcarbonyl)piperidine-1-carboxylate (11c).** ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.47 (9H, s), 1.60–1.75 (2H, m), 1.90 (2 H, d, *J* = 12.6 Hz), 2.93 (2H, t, *J* = 12.5 Hz), 3.94–4.10

(1H, m), 4.16 (2 H, s), 7.43–7.53 (1H, m), 7.80–7.90 (1H, m), 8.04 (1H, d, *J* = 7.9 Hz), 8.65–8.72 (1H, m).

***tert*-Butyl 4-isobutyrylpiperidine-1-carboxylate (11d).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.09 (6H, d, *J* = 6.8 Hz), 1.45 (9H, s), 1.49–1.86 (5H, m), 2.57–2.70 (1H, m), 2.70–2.86 (2H, m), 4.03–4.22 (2H, m).

***tert*-Butyl 4-(3-methylbutanoyl)piperidine-1-carboxylate (11e).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (6H, d, *J* = 6.6 Hz), 1.45 (9H, s), 1.47–1.60 (2H, m), 1.76 (2H, s), 2.08–2.34 (3H, m), 2.36–2.50 (1H, m), 2.76 (1H, t, *J* = 11.8 Hz), 4.06–4.18 (2H, m).

***tert*-Butyl 4-(cyclobutylcarbonyl)piperidine-1-carboxylate (11f).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.48–1.60 (2H, m), 1.70–2.36 (8H, m), 2.40–2.52 (1H, m), 2.77 (2H, t, *J* = 12.3 Hz), 3.30–3.50 (1H, m), 3.95 - 4.27 (2H, m).

***tert*-Butyl 4-[2-furyl(hydroxy)methyl]piperidine-1-carboxylate (13).** To a solution of furan (1.64 mL, 22.5 mmol) in THF (30 mL) was added dropwise ^{n}BuLi in hexane (1.6 M, 9.7 mL) at -78 ^{o}C and the resulting mixture was stirred at same temperature for 30 min then at 0 ^{o}C for 1 h. To the mixture was added a solution of **12** (2.40 g, 11.1 mmol) in THF (20 mL) at -78 ^{o}C, and the resulting mixture was stirred at -78 ^{o}C for 30 min then at 0 ^{o}C for 30 min. The mixture was quenched with H_2O (50 mL) at 0 ^{o}C, and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting precipitate was collected by filtration, washed with IPE to give **13** (2.11 g, 67%) as a coloerless crystal. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.07–1.40 (3H, m), 1.44 (9H, s), 1.85–2.01 (2H, m), 2.04 (1H, d, *J* = 4.9 Hz), 2.52–2.82 (2H, m), 3.94–4.26 (2H, m), 4.40 (1H, dd, *J* = 5.1, 7.4 Hz), 6.24 (1H, d, *J* = 3.2 Hz), 6.33 (1H, dd, *J* = 1.9, 3.2 Hz), 7.38 (1H, d, *J* = 1.7 Hz).

***tert*-Butyl 4-(2-furoyl)piperidine-1-carboxylate (14).** To a solution of **13** (2.11 g, 7.5 mmol) in CH_2Cl_2 (30 mL) was added a solution of $PySO_3$ (6.0 g, 37.7 mmol) and Et_3N (5.3 mL) in CH_2Cl_2 (30 mL) at 0 ^{o}C, and the resulting mixture was stirred at room temperature for 21 h. The mixture was quenched with water, and extracted with Et_2O. The organic layer was washed with 1N HCl, water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **14** (200 mg, 9.5%)as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 1.65–1.98 (4H, m), 2.74–2.98 (2H, m), 3.14–3.34 (1H, m), 4.16 (2H, s), 6.55 (1H, dd, *J* = 1.9, 3.6 Hz), 7.22 (1H, dd, *J* = 0.8, 3.6 Hz), 7.59 (1H, d, *J* = 1.1 Hz).

Piperidin-4-yl(2-thienyl)methanone hydrochloride (8a). To a solution of *tert*-butyl 4-(2-thienylcarbonyl)piperidine-1-carboxylate **11a** (600 mg, 2 mmol) in AcOEt (5 mL) was added 4 N HCl in AcOEt (5 mL), and the resulting mixture was stirred at room temperature for 1 h. The resulting precipitate was collected by filtration, washed with IPE to give **8a** (433 mg, 92%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.71–2.05 (4H, m), 2.90–3.11 (2H, m), 3.30 (2H, d, J = 12.8 Hz), 3.57–3.74 (1H, m), 7.29 (1H, dd, J = 3.8, 4.9 Hz), 8.06 (1H, dd, J = 0.9, 4.9 Hz), 8.10 (1H, dd, J = 1.1, 3.8 Hz), 8.90 (1H, s), 9.24 (1H, s).

Compounds **8b-g** were prepared in a similar manner to that described for **8a**.

Piperidin-4-yl(1,3-thiazol-2-yl)methanone hydrochloride (8b). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.75–1.98 (2H, m), 2.10 (2H, dd, J = 2.4, 13.9 Hz), 2.94–3.15 (2H, m), 3.74–3.94 (1H, m), 4.39 (2H, s), 8.19 (1H, d, J = 3.0 Hz), 8.28 (1H, d, J = 3.0 Hz), 9.06 (1H, s), 9.25 (1H, s).

Piperidin-4-yl(pyridin-2-yl)methanone hydrochloride (8c). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.69–1.89 (2H, m), 1.95–2.08 (2H, m), 2.95–3.14 (2H, m), 3.26–3.34 (2H, m), 3.97–4.16 (1H, m), 7.47 (1H, s), 7.66–7.76 (1H, m), 7.95–8.13 (2H, m), 8.71–8.80 (1H, m), 8.96 (1H, s), 9.15 (1H, s).

2-Methyl-1-piperidin-4-ylpropan-1-one hydrochloride (8d). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.00 (6H, d, J = 7.0 Hz), 1.50–1.74 (2H, m), 1.88 (2H, dd, J = 2.0, 13.7 Hz), 2.74–3.00 (4H, m), 3.16–3.29 (2H, m), 8.98 (2H, s).

3-Methyl-1-piperidin-4-ylbutan-1-one hydrochloride (8e). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.85 (6H, d, J = 6.8 Hz), 1.52–1.71 (2H, m), 1.84–2.11 (3H, m), 2.38 (2H, d, J = 7.0 Hz), 2.58–2.74 (1H, m), 2.76–2.99 (2H, m), 3.14–3.30 (2H, m), 8.83 (1H, s), 9.08 (1H, s).

Cyclobutyl(piperidin-4-yl)methanone hydrochloride (8f). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.53–1.80 (3H, m), 1.81–2.29 (7H, m), 2.64–2.76 (1H, m), 2.86 (2H, td, J = 2.6, 12.4 Hz), 3.13–3.28 (2H, m), 3.41–3.61 (1H, m), 9.04 (2H, s).

2-Furyl(piperidin-4-yl)methanone hydrochloride (8g). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.66–2.03 (4H, m), 2.86–3.11 (2H, m), 3.31 (2H, d, J = 13.0 Hz), 3.39–3.57 (1H, m), 6.76 (1H, dd, J = 1.6, 3.5 Hz), 7.60 (1H, d, J = 3.6 Hz), 8.05 (1H, d, J = 1.3 Hz), 8.76 (1H, s), 9.07 (1H, s).

***tert*-Butyl 4-(cyclobutylcarbonyl)piperazine-1-carboxylate (16a).** To a solution of **15** (4.47 g, 24 mmol) THF (50 mL) was added Et_3N (4.2 mL) and cyclobutanecarbonyl chloride (2.37 g, 20 mmol),

and the resulting mixture was stirred at room temperature for 2 h. The mixture was poured into water, and extracted with AcOEt. The organic layer was washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from hexane to give **16a** (4.45 g, 83 %) as a colorless crystal. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 1.79–2.05 (2H, m), 2.08–2.24 (2H, m), 2.26–2.46 (2H, m), 3.18–3.34 (3H, m), 3.35–3.47 (4H, m), 3.51–3.65 (2H, m).

Compound **16b** was prepared in a manner similar to that described for **16a**.
***tert*-Butyl 4-(2,2-dimethylpropanoyl)piperazine-1-carboxylate (16b).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (9H, s), 1.47 (9H, s), 3.37–3.46 (4H, m), 3.57–3.65 (4H, m).

1-(Cyclobutylcarbonyl)piperazine hydrochloride (8h). A mixture of **16a** (4.29 g, 16 mmol) and 4 N HCl in AcOEt (10 mL) was stirred at room temperature for 6 h. After evaporation of the solvent, the resulting precipitate was collected by filtration, washed with AcOEt to give **8h** (2.71 g, 83 %) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.66–1.82 (1H, m), 1.83–2.00 (1H, m), 2.01–2.25 (4H, m), 3.03 (3H, s), 3.29–3.44 (1H, m), 3.50–3.58 (3H, m), 3.61–3.71 (2H, m), 9.28 (1H, s), 9.66 (1H, s).

Compound **8i** was prepared in a manner similar to that described for **8h**.
1-(2,2-Dimethylpropanoyl)piperazine hydrochloride (8i). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19 (9H, s), 2.98–3.10 (4H, m), 3.68–3.82 (4H, m), 9.40 (1H, s 9.70 (1H, s).

2-Chloro-*N*-(4-fluorophenyl)acetamide (18a). To a mixture of 4-fluoroaniline **17a** (9.47 mL, 100 mmol), AcOiPr (100 mL) and 2 M $KHCO_3$ aq. (100 ml) was stirred at 0 ^{o}C was added dropwise chloroacetyl chloride (9.56 mL, 120 mmol), and the resulting mixture was stirred at room temperature for 30 min. The mixture was poured into waster and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from AcOEt-hexane to give **18a** (17.9 g, 95 %) as a colorless prism. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.20 (2H, s), 7.02–7.10 (2H, m), 7.48–7.55 (2H, m), 8.21 (1H, brs).

Compound **18b** was prepared in a manner similar to that described for **18a**.
2-Chloro-*N*-(4-fluoro-2-methylphenyl)acetamide (18b). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.29 (3H, s), 4.24 (2H, s), 6.90–6.96 (2H, m), 7.72–7.77 (1H, m), 8.12 (1H, brs).

***N*-(4-Fluorophenyl)-2-[(2-hydroxyethyl)amino]acetamide (19a).** A mixture of 2-chloro-*N*-(4-fluorophenyl)acetamide **18a** (9.38 g, 50.0 mmol), 2-aminoethanol (12.1 mL, 200 mmol) and AcOiPr (50 mL) was stirred at 60 ^{o}C for 1.5 h. After being cooled, the mixture was

poured into water and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from AcOEt to give **19a** (8.55 g, 81 %) as a colorless prism. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.41 (1H, brs), 2.61 (2H, t, J = 5.4 Hz), 3.28 (2H, s), 3.46 (2H, q, J = 5.4 Hz), 4.63 (1H, t, J = 5.4 Hz), 7.10–7.18 (2H, m), 7.61–7.69 (2H, m), 9.94 (1H, brs).

Compound **19b** was prepared in a manner similar to that described for **19a**. ***N*-(4-Fluoro-2-methylphenyl)-2-[(2-hydroxyethyl)amino]acetamide (19b).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.92 (2H, brs), 2.27 (3H, s), 2.85–2.88 (2H, m), 3.45 (2H, s), 3.75–3.78 (2H, m), 6.87–6.94 (2H, m), 7.90–7.95 (1H, m), 9.24 (1H, brs).

1-(4-Fluorophenyl)piperazin-2-one hydrochloride (8j). To a solution of **19a** (4.14 g, 20.0 mmol) and PBu_3 (5.98 mL, 24 mmol) in THF (100 mL) was added dropwise Di-*tert*-butyl Azodicarboxylate (5.08 g, 25.0 mmol) in toluene, and the resulting mixture was stirred at room temperature for 1h. The mixture was added 10% HCl in MeOH (15 mL), and concentrated *in vacuo*. The residue was crystallized from EtOH to give **8j** (2.02 g, 44%) as a colorless prism. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50–3.53 (2H, m), 3.84–3.87 (4H, m), 7.24–7.32 (2H, m), 7.33–7.40 (2H, m), 9.81 (2H, s).

Compound **8k** was prepared in a manner similar to that described for **8j**. **1-(4-Fluoro-2-methylphenyl)piperazin-2-one hydrochloride (8k).** ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.17 (3H, s), 3.47–3.60 (2H, m), 3.62–3.70 (1H, m), 3.75–3.84 (2H, m), 3.92 (1H, d, J = 16.6 Hz), 7.08–7.20 (2H, m), 7.25 (1H, dd, J = 5.7, 8.7 Hz), 9.85 (2H, brs).

4-Ethyl-3-nitrobenzoic acid (24). 4-Ethylbenzoic acid **23** (50 g, 0.33 mol) was added slowly to HNO_3 (fuming) (300 mL) under -10 ^{o}C, and the resulting mixture was stirred at -15 ^{o}C for 1 h. The mixture was added ice-water, and the resulting precipitate was collected by filtration, washed with water to give **24** (62.4 g, 96%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, t, J = 7.5 Hz), 3.00 (2H, q, J = 7.5 Hz), 5.07 (1H, s), 7.52 (1H, d, J = 8.1 Hz), 8.24 (1H, dd, J = 1.8, 8.0 Hz), 8.59 (1H, d, J = 1.9 Hz).

4-(1-Bromoethyl)-3-nitrobenzoic acid (25). To a suspension of **24** (61 g, 0.31 mol)in CCl_4 (900 mL) was added AIBN (2.32 g) and NBS (52.7 g), and the resulting mixture was refluxed for 2 h. The precipitate was removed by filtration, washed with CCl_4, the filtrate was concentrated *in vacuo* to give **25** (86.0 g, quant.) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (3H, d, J = 6.8 Hz), 5.80 (1H, q, J = 6.8 Hz), 8.03 (1H, d, J = 8.3 Hz), 8.33 (1H, dd, J = 1.7, 8.3 Hz), 8.55 (1H, d, J

= 1.7 Hz), 9.02 (1H, s).

4-(1-Hydroxyethyl)-*N*,*N*-dimethyl-3-nitrobenzamide (26). To a solution of **25** (86.0 g, 0.31 mol) in acetone (400 mL) and water (400 mL) was added $AgCO_3$ (117 g, 0.69 mol) at 0 °C, and the resulting mixture was stirred room temperature overnight. c.HCl was added to the mixture, and the resulting precipitate was removed by filtration, the filtrate was concentrated *in vacuo*. The residue was poured into water and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo* to give yellow oil. This oil was dissolved in THF (300 mL) and MeCN (300 mL), and WSC (57.5 g, 0.30 mol), HOBt (46.0 g, 0.34 mol), 2M dimethylamine in THF (150 mL) at 0 °C, and the resulting mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was poured into water and extracted with AcOEt. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **26** (41.7 g, 61%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.53 (3H, d, J = 6.4 Hz), 3.00 (3H, s), 3.12 (4H, s), 5.33–5.47 (1H, m), 7.63 (1H, dd, J = 1.6, 8.0 Hz), 7.88 (1H, d, J = 8.1 Hz), 7.91 (1H, d, J = 1.5 Hz).

1-{4-[(Dimethylamino)methyl]-2-nitrophenyl}ethanol (27). To a solution of **26** (41.7 g, 0.17 mol) in THF (200 mL) was added dropwise 1.0 M BH_3-THF complex in THF (700 mL), and the resulting mixture was refluxed for 3 h. After being cooled, MeOH (500 mL) was added to the mixture, and the resulting mixture was refluxed overnight. After evaporation of the solvent, the residue was pruified by column chromatography to give **26** (35.8 g, 91%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.57 (3H, d, J = 6.4 Hz), 2.24 (6H, s), 3.46 (2H, s), 7.60 (1H, dd, J = 1.6, 8.0 Hz), 7.78 (1H, d, J = 8.1 Hz), 7.85 (1H, d, J = 1.5 Hz).

1-{4-[(Dimethylamino)methyl]-2-nitrophenyl}ethanone (28). To a solution of **27** (101 mg, 0.45 mmol) in CH_2Cl_2 (5 mL) was added MS4A (100 mg), NMO (92 mg, 0.68 mmol) and TPAP (16 mg, 0.05 mmol) at room temperature, and the resulting mixture was stirred at room temperature for 30 min. Insoluble material was removed by filtration, washed with AcOEt, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **28** (82.8 mg, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.27 (6H, s), 2.55 (3H, s), 3.52 (2H, s), 7.40 (1H, d, J = 7.9 Hz), 7.67 (1H, dd, J = 7.7, 1.3 Hz), 8.04 (1H, s).

1-{2-Amino-4-[(dimethylamino)methyl]phenyl}ethanone (9b). To a solution of **28** (103.5 mg, 0.47 mmol) in EtOH (2 mL) was added 10% Pd/C (20 mg), then hydrazine monohydrate (0.068 mL) was added to the mixture, and the resulting mixture was stirred at room temperature overnight. After removal of the Pd/C, the residue was concentrated *in vacuo*. The residue was pruified by column

chromatography to give **9b** (74.6 mg, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.25 (6H, s), 2.56 (3H, s), 3.33 (2H, s), 6.26 (2H, s), 6.56–6.67 (1H, m), 7.65 (1H, d, J = 8.1 Hz).

Compound **9a** was prepared in a manner similar to that described for **9b**.

4-Ethoxy-N,N-dimethyl-3-nitrobenzamide (21). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (3H, t, J = 7.1 Hz), 3.08 (6H, s), 4.23 (2H, q, J = 7.0 Hz), 7.10 (1H, d, J = 8.8 Hz), 7.65 (1H, dd, J = 2.2, 8.8 Hz) , 7.93 (1H, d, J = 2.2 Hz).

(4-Ethoxy-3-nitrobenzyl)dimethylamine (22). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (3H, t, J = 6.7 Hz), 2.23 (6H, s) , 3.38 (2H, s), 4.17 (2H, q, J = 7.0 Hz), 7.01 (1H, d, J = 8.6 Hz), 7.45 (1H, dd, J = 2.2, 8.6 Hz), 7.74 (1H, d, J = 2.2 Hz)

(4-Ethoxy-3-aminobenzyl)dimethylamine (9a). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (3H, t, J = 7.0 Hz), 2.21 (6H, s) , 3.28 (2H, s), 3.77 (2H, s), 4.05 (2H, q, J = 7.0 Hz) , 6.60 (1H, dd, J = 1.9, 8.1 Hz) , 6.71 (1H, d, J = 8.1 Hz) , 6.70 (1H, d, J = 1.9 Hz).

3-Nitro-4-(trifluoromethoxy)benzoic acid (30). To a solution of 4-trifluoromethylbenzoic acid **29** (1.50 g, 7.3 mmol) in H_2SO_4 (8 mL) was added HNO_3 (4 mL) at 0 ^{o}C, and the resulting mixture was stirred at room temperature for 1.5 h. The mixture was poured into ice-water, and the resulting precipitate was collected by filtration, washed with H_2O to give **30** (1.87 g, 96%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.56–7.63 (1H, m), 8.39 (1H, dd, J = 2.3, 8.7 Hz), 8.70 (1H, d, J = 2.3 Hz).

***N,N*-Dimethyl-3-nitro-4-(trifluoromethoxy)benzamide (31).** A mixture of **29** (4.03 g, 16 mmol), dimethylamine hydrochloride (1.96 g, 24 mmol), DIEA (4.25 mL), WSC (5.75 g, 30 mmol), HOBt (3.98 g, 29.5 mmol) and MeCN (50 mL) was stirred at room temperature for 12 h. The mixture was poured into waster, and the resulting precipitate was collected by filtration, washed with H_2O and n-hexane to give **31** (3.66 g, 80%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.09 (6H, d, J = 31.7 Hz), 7.43–7.59 (1H, m), 7.74 (1H, dd, J = 2.1, 8.5 Hz), 8.06 (1H, d, J = 2.1 Hz).

***N,N*-Dimethyl-1-[3-nitro-4-(trifluoromethoxy)phenyl]methanamine (32).** A mixture of **31** (3.66 g, 13 mmol) and 1.0 M BH_3-THF complex in THF (55 mL) was refluxed for 6 h. After being cooled, H_2O was added to the mixture, and concentrated *in vacuo*. The residue was dissolved in MeOH (10 mL), 6 N HCl was added to the solution, and refluxed for 16 h. After being cooled, the mixture was quenched with 2 N NaOH, and extracted with AcOEt and THF. The organic layer was concentrated *in vacuo*. The residue was pruified by column chromatography to give **32** (2.90 g, 84%) as a yellow

oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.26 (6H, s), 3.48 (2H, s), 7.39 (1H, dd, J = 1.3, 8.5 Hz), 7.62 (1H, dd, J = 2.2, 8.4 Hz), 7.96 (1H, d, J = 2.1 Hz).

5-[(Dimethylamino)methyl]-2-(trifluoromethoxy)aniline (9c). To a solution of **31** (17.8 g, 67 mmol) in EtOH (200 mL) was added 10% Pd/C (2 g), then hydrazine monohydrate (9.8 mL) was added to the mixture, and the resulting mixture was stirred at room temperature overnight. After removal of the Pd/C, the residue was concentrated *in vacuo*. The residue was pruified by column chromatography to give **9c** (13.6 g, 86%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.23 (6H, s), 3.31 (2H, s), 3.83 (2H, s), 6.65 (1H, dd, J = 1.9, 8.3 Hz), 6.79 (1H, d, J = 1.9 Hz), 7.06 (1H, dd, J = 1.5, 8.3 Hz).

(2*R*,3*S*)-3-(1*H*-Indol-3-yl)-2-({[4-(2-thienylcarbonyl)piperidin-1-yl]carbonyl}amino)butanoic acid (7a). To a suspension of ethyl (2*R*,3*S*)-2-amino-3-(1*H*-indol-3-yl)butanoate methanesulfonate **4** (606 mg, 1.8 mmol) and CDI (476 mg, 2.9 mmol) in MeCN (10 mL) was added dropwise DIEPA (0.67 mL) and stirred at 0 ^{o}C for 30 min. **8a** (410 mg, 1.8 mmol) and DIPEA (0.35 mL) was added to the mixture, and stirred ar room temperature overnight. The mixture was added sat. NaHCO3 aq., and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **6a** as a amorphous solid. The obtained material was disolved in EtOH (5mL) and was added dropwise 4 N NaOH (1.4 mL). The resulting mixture was stirred room temperature overnight. After evaporation of the solvent, the residue was acidified with 1N HCl and the resulting precipitate was collected by filtration, washed with H_2O to give **7a** (630 mg, 81%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.33 (3H, d, J = 7.2 Hz), 1.38–1.53 (2H, m), 1.74 (2H, d, J = 10.6 Hz), 2.77–2.90 (2H, m), 3.44–3.60 (2H, m), 3.97–4.11 (2H, m), 4.38–4.51 (1H, m), 6.35 (1H, d, J = 8.5 Hz), 6.93–7.08 (2H, m), 7.14 (1H, d, J=2.3 Hz), 7.24–7.35 (2H, m), 7.54 (1H, d, J = 7.7 Hz), 8.00–8.08 (2H, m), 10.82 (1H, d, J = 1.7 Hz).

Compound **7b-k** was prepared in a manner similar to that described for **7a**.

(2*R*,3*S*)-3-(1*H*-Indol-3-yl)-2-({[4-(1,3-thiazol-2-ylcarbonyl)piperidin-1-yl]carbonyl}amino)butanoic acid (7b). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.28–1.37 (3H, m), 1.39–1.52 (2H, m), 1.87 (2H, d, J = 12.6 Hz), 2.77–2.90 (2H, m), 3.47–3.62 (1H, m), 3.73 (1H, ddd, J = 3.8, 7.8, 11.4 Hz), 4.05 (2H, t, J = 13.9 Hz), 4.39–4.51 (1H, m), 6.37 (1H, d, J = 8.7 Hz), 6.93–7.08 (2H, m), 7.14 (1H, d, J = 2.3 Hz), 7.32 (1H, d, J = 8.1 Hz), 7.53 (1H, d, J = 7.9 Hz), 8.20 (2H, dd, J = 3.0, 19.0 Hz), 10.82 (1H, d, J = 1.7 Hz).

(2*R*,3*S*)-3-(1*H*-Indol-3-yl)-2-({[4-(pyridin-2-ylcarbonyl)piperidin-1-yl]carbonyl}amino)

butanoic acid (7c). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.30–1.36 (3H, m), 1.37–1.56 (3H, m), 1.78 (2H, d, J = 11.5 Hz), 2.74–2.94 (2H, m), 3.24–3.70 (7H, m), 3.89–4.17 (3H, m), 4.44 (1H, t, J = 7.9 Hz), 6.36 (1H, d, J = 8.5 Hz), 6.93–7.10 (3H, m), 7.15 (1H, d, J = 2.3 Hz), 7.32 (1H, d, J = 7.9 Hz), 7.53 (1H, d, J = 7.9 Hz), 7.63–7.73 (1H, m), 7.90–8.11 (2H, m), 8.76 (1H, d, J = 4.5 Hz), 10.84 (1H, s).

(2*R*,3*S*)-3-(1*H*-Indol-3-yl)-2-{[(4-isobutyrylpiperidin-1-yl)carbonyl]amino} butanoic acid (7d). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.99 (6H, d, J = 7.0 Hz), 1.11–1.28 (2H, m), 1.32 (3H, d, J = 7.0 Hz), 1.67 (2H, d, J=11.5 Hz), 2.60–2.90 (3H, m), 3.47–3.63 (1H, m), 3.97 (2H, t, J=14.0 Hz), 6.30 (1H, d, J=8.5 Hz), 6.88–7.08 (2H, m), 7.13 (1H, d, J=2.5 Hz), 7.31 (1H, d, J=8.1 Hz), 7.53 (1H, d, J=7.7 Hz), 10.82 (1H, d, J=1.5 Hz), 12.22 (1H, s).

(2*R*,3*S*)-3-(1*H*-Indol-3-yl)-2-({[4-(3-methylbutanoyl)piperidin-1-yl]carbonyl}amino) butanoic acid (7e). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.85 (6H, d, J = 6.6Hz), 1.11–1.30 (2H, m), 1.32 (3H, d, J = 7.2 Hz), 1.70 (2H, d, J = 11.3 Hz), 1.89–2.13 (1H, m), 2.28–2.42 (2H, m), 2.59–2.79 (2H, m), 3.46–3.63 (1H, m), 3.96 (2H, t, J=13.9 Hz), 4.32–4.50 (1H, m), 6.30 (1H, d, J = 8.7 Hz), 6.90–7.08 (2H, m), 7.13 (1H, d, J = 2.3 Hz), 7.31 (1H, d, J = 7.9 Hz), 7.52 (1H, d, J = 7.9 Hz), 10.82 (1H, d, J = 1.3 Hz), 12.22 (1H, s).

(2*R*,3*S*)-2-({[4-(Cyclobutylcarbonyl)piperidin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl) butanoic acid (7f). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.09–1.27 (2H, m), 1.32 (3H, d, J = 7.2 Hz), 1.60–1.80 (3H, m), 1.83–1.99 (1H, m), 1.99–2.17 (4H, m), 2.52–2.62 (1H, m), 2.62–2.83 (2H, m), 3.39–3.62 (2H, m), 3.94 (2H, t, J = 13.9 Hz), 4.42 (1H, t, J = 7.9 Hz), 6.30 (1H, d, J = 8.5 Hz), 6.88–7.09 (2H, m), 7.13 (1H, d, J = 2.1Hz), 7.31 (1H, d, J = 7.9 Hz), 7.52 (1H, d, J = 7.7 Hz), 10.82 (1H, s), 12.19 (1H, s).

(2*R*,3*S*)-2-({[4-(2-Furoyl)piperidin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)butanoic acid (7g). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.33 (3H, d, J = 7.2 Hz), 1.36–1.54 (2H, m), 1.71 (2H, d, J = 10.7 Hz), 2.69–2.96 (2H, m), 3.49–3.61 (1H, m), 4.04 (2H, t, J = 13.9 Hz), 4.36–4.51 (1H, m), 6.37 (1H, d, J = 8.7 Hz), 6.73 (1H, dd, J = 1.8, 3.7 Hz), 6.91–7.09 (2H, m), 7.14 (1H, d, J = 2.3 Hz), 7.32 (1H, d, J = 7.9 Hz), 7.48–7.63 (2H, m), 8.01 (1H, d, J = 1.1 Hz), 10.83 (1H, d, J = 1.9 Hz), 12.21 (1H, s).

(2*R*,3*S*)-2-({[4-(Cyclobutylcarbonyl)piperazin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)butanoic acid (7h). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.32 (3H, d, J = 7.2 Hz), 1.64–1.80 (1H, m), 1.82–1.97 (1H, m), 1.99–2.24 (3H, m), 3.17–3.42 (10H, m), 3.48–3.63 (1H, m), 4.39–4.50 (1H, m), 6.46 (1H, d, J = 8.7 Hz), 6.92–6.99 (1H, m), 7.00–7.08 (1H, m), 7.13 (1H, d, J = 2.3 Hz), 7.31 (1H, d, J = 8.1 Hz),

7.52 (1H, d, *J* = 7.5 Hz), 10.81 (1H, s), 12.19 (1H, s)..

(2*R*,3*S*)-2-({[4-(2,2-Dimethylpropanoyl)piperazin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)butanoic acid (7i). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19 (9H, s), 1.33 (3H, d, *J* = 7.2 Hz), 3.19–3.67 (10H, m), 4.38–4.49 (1H, m), 6.42 (1H, d, *J* = 8.5 Hz), 6.91–7.09 (1H, m), 7.14 (1H, d, *J* = 2.3 Hz), 7.32 (1H, d, *J* = 7.9 Hz), 7.53 (1H, d, *J* = 7.7 Hz), 10.81 (1H, d, *J* = 1.7 Hz).

(2*R*,3*S*)-2-({[4-(4-Fluorophenyl)-3-oxopiperazin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)butanoic acid (7j). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.35 (3H, d, *J* = 7.2 Hz), 3.54–3.72 (5H, m), 4.03 (1H, d, *J* = 17.5 Hz), 4.18 (1H, d, *J* = 17.5 Hz), 4.48 (1H, dd, *J* = 7.4, 8.2 Hz), 6.63 (1H, d, *J* = 8.7 Hz), 6.94–7.00 (1H, m), 7.03–7.08 (1H, m), 7.17 (1H, d, *J* = 2.3 Hz), 7.19–7.27 (2H, m), 7.31–7.36 (3H, m), 7.54 (1H, d, *J* = 7.7 Hz), 10.83 (1H, d, *J* = 1.7 Hz), 12.28 (1H, brs).

(2*R*,3*S*)-2-({[4-(4-Fluoro-2-methylphenyl)-3-oxopiperazin-1-yl]carbonyl}amino)-3-(1*H*-indol-3-yl)butanoic acid (7k). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.35 (3H, d, *J* = 7.2 Hz), 2.05 (3H, d, *J* = 20.0 Hz), 3.35–3.84 (5H, m), 4.01 (1H, dd, *J* = 8.6, 17.6 Hz), 4.18 (1H, d, *J* = 17.6 Hz), 4.50 (1H, t, *J* = 7.5 Hz), 6.67 (1H, d, *J* = 8.7 Hz), 6.95–7.00 (1H, m), 7.03–7.25 (5H, m), 7.29–7.34 (1H, m), 7.54 (1H, d, *J* = 7.7 Hz), 10.82 (1H, s), 12.29 (1H, brs).

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl)propyl]-4-(2-thienylcarbonyl)piperidine-1-carboxamide (1a).** To a mixture of **7a** (150 mg, 0.34 mmol), **9a** (78 mg, 0.4 mmol) , WSC (72 mg, 0.38 mmol) and HOBt (58 mg, 0.43 mmol) in MeCN (2 mL) and THF(2 mL) was added DIEA (0.15 mL) at room temperature, and the resulting mixture was sitrred at room temperature overnight. The mixture was added sat. NaHCO3 aq., and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was pruified by column chromatography to give **1a** (123.5 mg, 59%) as a amorphous solid. ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.20–1.31 (4H, m), 1.53–1.59 (3H, m), 1.71–1.81 (2H, m), 1.82–1.89 (3H, m), 2.18–2.25 (6H, m), 2.82–3.04 (1H, m), 3.19-3.29 (1H, m), 3.32–3.36 (2H, m), 3.65 (1H, quin, *J* = 7.2 Hz), 3.81–3.96 (3H, m), 4.02–4.13 (1H, m), 4.82–4.91 (1H, m), 5.32 (1H, d, *J* = 7.7 Hz), 6.65–6.73 (1H, m), 6.90–6.98 (1H, m), 7.05–7.18 (4H, m), 7.29–7.33 (1H, m), 7.63–7.69 (1H, m), 7.71–7.76 (1H, s), 7.96 (1H, s), 8.15 (1H, brs), 8.18 (1H, d, *J* = 2.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.1, 14.7, 17.7, 28.3, 28.5, 34.8, 43.4, 43.5, 45.0, 45.1, 60.2, 63.8, 64.2, 110.7, 111.3, 116.9, 119.3, 119.6, 120.7, 121.9, 122.2, 124.4, 126.6, 126.9, 128.2, 130.7, 131.8, 134.0, 136.4, 143.2, 146.5, 157.0, 170.0, 194.6. LC–MS calcd for $C34H41N5O4S^+$ (*m/e*), 616, obsd 616 (M+1).

Compounds **1b-k, 2a,d,f,h-k, 3i-k** was prepared in a manner similar to that described for **1a**.

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(1,3-thiazol-2-ylcarbonyl)piperidine-1-carboxamide (1b).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.20–1.57 (3H, m), 1.52–1.60 (3H, m), 1.66–1.78 (3H, m), 1.93–2.03 (2H, m), 2.21 (6H, s), 2.87–2.96 (1H, m), 2.97–3.06 (1H, m), 3.29–3.35 (2H, m), 3.60–3.68 (1H, m), 3.70–3.97 (4H, m), 4.83–4.92 (1H, m), 5.31 (1H, d, *J* = 7.7 Hz), 6.65–6.73 (1H, m), 6.92 (1H, dd, *J* = 2.0, 8.3 Hz), 7.06 (1H, d, *J* = 2.2 Hz), 7.07–7.17 (2H, m), 7.28–7.33 (1H, m), 7.65–7.70 (1H, m), 7.74 (1H, d, *J* = 8.1 Hz), 7.96 (1H, s), 7.98–8.03 (1H, m), 8.12 (1H, brs), 8.16–8.22 (1H, m). ^{13}C NMR ($CDCl_3$) *δ*: 14.7, 17.7, 27.6, 27.8, 34.8, 43.4, 43.6, 43.9, 45.2 (2C), 60.1, 63.9, 64.2, 110.7, 111.3, 117.0, 119.3, 119.6, 120.7, 121.9, 122.2, 124.3, 126.6, 126.7, 126.9, 131.2, 136.4, 144.8, 146.4, 156.9, 166.3, 169.9, 194.9. LC–MS calcd for $C33H40N6O4S^+$ (*m/e*), 617, obsd 617 (M+1).

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(pyridin-2-ylcarbonyl)piperidine-1-carboxamide (1c).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.18–1.28 (3H, m), 1.52–1.59 (1H, m), 1.61–1.71 (3H, m), 1.77 (3H, brs), 1.86–1.98 (2H, m), 2.16–2.25 (6H, m), 2.89–2.97 (1H, m), 2.99–3.07 (1H, m), 3.28–3.35 (2H, m), 3.60–3.69 (1H, m), 3.80–3.96 (3H, m), 3.98–4.10 (2H, m), 4.88 (1H, t, *J* = 7.3 Hz), 5.25–5.35 (1H, m), 6.90–6.95 (1H, m), 7.04–7.11 (2H, m), 7.12–7.16 (1H, m), 7.31 (1H, d, *J* = 8.1 Hz), 7.48 (1H, ddd, *J* = 1.3, 4.8, 7.5 Hz), 7.70–7.77 (1H, m), 7.79–7.88 (1H, m), 7.98 (1H, s), 8.00–8.06 (1H, m), 8.13 (1H, brs), 8.16–8.21 (1H, m). ^{13}C NMR ($CDCl_3$) *δ*: 14.7, 17.7, 22.9, 27.7, 27.9, 34.8, 42.0, 43.6, 43.7, 45.2, 60.1, 63.9, 64.2,110.7, 111.3, 117.1, 119.3,119.6, 120.7, 121.9, 122.2, 122.6, 124.3, 126.7, 126.9, 127.2, 131.1, 136.4, 137.1, 146.4, 148.9, 152.5, 157.0, 170.0, 202.9. LC–MS calcd for $C35H42N6O4^+$ (*m/e*), 611, obsd 611 (M+1).

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-isobutyrylpiperidine-1-carboxamide (1d).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.05–1.12 (6H, m), 1.20–1.25 (3H, m), 1.48–1.60 (5H, m), 1.70–1.77 (2H, m), 2.17–2.24 (6H, m), 2.64 (1H, tt, J = 11.2, 3.7 Hz), 2.72–2.82 (2H, m), 2.87 (1H, ddd, J = 13.2, 12.1, 2.9 Hz), 3.29–3.36 (2H, m), 3.63 (1H, quin, J = 7.2 Hz), 3.79–3.94 (3H, m), 4.00 (1H, d, J = 13.2 Hz), 4.86 (1H, t, J = 7.3 Hz), 5.22–5.32 (1H, m), 6.66–6.71 (1H, m), 6.89–6.95 (1H, m), 7.03–7.10 (2H, m), 7.11–7.17 (1H, m), 7.28–7.32 (1H, m), 7.72 (1H, d, J = 7.7 Hz), 7.93 (1H, s), 8.14 (1H, brs), 8.18 (1H, d, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$) *δ*: 14.7, 17.7, 18.4, 18.5, 27.5, 27.6, 34.8, 38.8, 43.5, 43.6, 45.2 (2C), 46.6, 60.1, 63.9, 64.2, 110.7, 111.3, 116.9,119.3, 119.6, 120.7, 121.9, 122.1, 124.3, 126.6, 126.9, 131.1, 136.4, 146.4, 157.0, 169.9, 215.7. LC–MS calcd for $C33H45N5O4^+$ (*m/e*), 576, obsd 576 (M+1).

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(3-methylbutanoyl)piperidine-1-carboxamide (1e).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*:

0.86–0.96 (6H, m), 1.18–1.25 (3H, m), 1.43–1.60 (5H, m), 1.79 (2H, brs), 2.11–2.24 (7H, m), 2.27–2.35 (2H, m), 2.42 (1H, tt, J = 11.3, 3.8 Hz), 2.71–2.81 (1H, m), 2.82–2.92 (1H, m), 3.26–3.37 (2H, m), 3.63 (1H, quin, J = 7.2 Hz), 3.79–3.94 (3H, m), 3.99 (1H, d, J = 13.2 Hz), 4.86 (1H, t, J = 7.5 Hz), 5.22–5.34 (1H, m), 6.64–6.74 (1H, m), 6.92 (1H, dd, J = 8.3, 2.0 Hz), 7.01–7.10 (2H, m), 7.12–7.17 (1H, m), 7.30 (1H, d, J = 8.1 Hz), 7.72 (1H, d, J = 7.7 Hz), 7.93 (1H, s), 8.12 (1H, brs), 8.18 (1H, d, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.7, 22.6 (2C), 24.3, 27.1, 27.3, 34.8, 43.5, 43.6, 45.2 (2C), 48.7, 49.6, 60.1, 63.9, 64.2, 110.7, 111.3, 116.9, 119.3, 119.6, 120.6, 121.9, 122.2, 124.3, 126.6, 126.9, 131.2, 136.4, 146.3, 157.0, 169.9, 211.7. LC–MS calcd for $C34H47N5O4^+$ (*m/e*), 590, obsd 590 (M+1).

4-(Cyclobutylcarbonyl)-*N*-[(1*R*,2*S*)-1-[({5-[(dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl)propyl]piperidine-1-carboxamide (1f). ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.17–1.26 (3H, m), 1.44–1.60 (5H, m), 1.69–1.89 (3H, m), 1.94–2.02 (1H, m), 2.07–2.16 (2H, m), 2.17–2.28 (8H, m), 2.46 (1H, tt, J = 11.1, 3.7 Hz), 2.72–2.81 (1H, m), 2.81–2.91 (1H, m), 3.29–3.35 (2H, m), 3.35–3.43 (1H, m), 3.63 (1H, quin, J = 7.2 Hz), 3.78–3.93 (3H, m), 3.97 (1H, d, J = 13.2 Hz), 4.79–4.92 (1H, m), 5.28 (1H, d, J = 7.7 Hz), 6.69 (1H, d, J = 8.4 Hz), 6.93 (1H, dd, J = 8.1, 1.8 Hz), 7.01–7.10 (2H, m), 7.14 (1H, t, J = 7.3 Hz), 7.30 (1H, d, J = 8.1 Hz), 7.72 (1H, d, J = 8.1 Hz), 7.93 (1H, s), 8.13 (1H, brs), 8.18 (1H, d, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.7, 17.9, 24.6, 24.6, 27.3, 27.4, 34.8, 43.5, 43.6, 43.7, 45.2 (2C), 46.4, 60.1, 63.9, 64.2, 110.7, 111.3, 117.0, 119.3, 119.6, 120.7, 121.9, 122.2, 124.3, 126.6, 126.9, 131.1, 136.4, 146.4, 157.0, 169.9, 212.6. LC–MS calcd for $C34H45N5O4^+$ (*m/e*), 588, obsd 588 (M+1).

***N*-[(1*R*,2*S*)-1-[({5-[(Dimethylamino)methyl]-2-ethoxyphenyl}amino)carbonyl]-2-(1*H*-indol-3-yl)propyl]-4-(2-furoyl)piperidine-1-carboxamide (1g).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.19–1.28 (3H, m), 1.56 (3H, d, J = 7.0 Hz), 1.67–1.78 (2H, m), 1.80–1.90 (2H, m), 2.21 (6H, s), 2.83–2.92 (1H, m), 2.94–3.04 (1H, m), 3.23 (1H, tt, J = 11.1, 3.8 Hz), 3.30–3.37 (2H, m), 3.65 (1H, quin, J = 7.2 Hz), 3.81–3.96 (3H, m), 4.07 (1H, d, J = 13.2 Hz), 4.88 (1H, t, J = 7.3 Hz), 5.30 (1H, d, J = 7.7 Hz), 6.55 (1H, dd, J = 3.5, 1.7 Hz), 6.70 (1H, d, J = 8.1 Hz), 6.93 (1H, dd, J = 8.1, 1.8 Hz), 7.03–7.11 (2H, m), 7.12–7.17 (1H, m), 7.20–7.24 (1H, m), 7.31 (1H, d, J = 8.1 Hz), 7.59 (1H, dd, J = 1.8, 0.7 Hz), 7.74 (1H, d, J = 7.7 Hz), 7.95 (1H, s), 8.06 (1H, brs), 8.18 (1H, d, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.7, 27.6, 27.8, 34.9, 43.4, 43.6, 44.0, 45.3 (2C), 60.1, 63.9, 64.2, 110.7, 111.3, 112.4, 117.0, 117.4, 119.3, 119.7, 120.7, 121.9, 122.2, 124.3, 126.7, 126.9, 131.2, 136.4, 146.3, 146.4, 152.1, 156.9, 169.9, 190.8. LC–MS calcd for $C34H41N5O5^+$ (*m/e*), 600, obsd 600 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-ethoxyphenyl]amino}carbonyl)-2-(1*H*-indol-3-yl)propyl]-4-(cyclobutylcarbonyl)piperazine-1-carboxamide (1h).** ^{1}H NMR (600MHz, $CDCl_3$) δ:

1.19–1.26 (3H, m), 1.56 (3H, d, J = 7.3 Hz), 1.83–1.92 (1H, m), 1.97 (1H, dquin, J = 11.0, 9.0 Hz), 2.11–2.18 (2H, m), 2.20–2.38 (8H, m), 3.18–3.35 (9H, m), 3.36–3.44 (1H, m), 3.48–3.56 (1H, m), 3.57–3.64 (2H, m), 3.79–3.97 (2H, m), 4.79–4.89 (1H, m), 5.28–5.41 (1H, m), 6.69 (1H, d, J = 8.4 Hz), 6.90–6.94 (1H, m), 7.01–7.09 (2H, m), 7.12–7.17 (1H, m), 7.29–7.33 (1H, m), 7.70 (1H, d, J = 7.7 Hz), 7.82–7.90 (1H, m), 8.16 (2H, d, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.8, 18.0, 22.9, 25.1, 34.8, 37.2, 41.2, 43.7, 43.8, 44.6, 45.2 (2C), 60.2, 63.9, 64.2, 110.6, 111.3, 116.6, 119.2, 119.6, 120.7, 122.0, 122.2, 124.5, 126.6, 126.8, 131.2, 136.4, 146.4, 157.0, 169.7, 173.4. LC–MS calcd for $C33H44N6O4^+$ (*m/e*), 589, obsd 589 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-ethoxyphenyl]amino}carbonyl)-2-(1*H*-indol-3-yl) propyl]-4-(2,2-dimethylpropanoyl)piperazine-1-carboxamide (1i).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.23 (3H, t, J = 7.0 Hz), 1.26–1.30 (9H, m), 1.52–1.60 (3H, m), 2.16–2.22 (6H, m), 3.26–3.33 (4H, m), 3.35–3.41 (2H, m), 3.53–3.67 (5H, m), 3.80–3.94 (2H, m), 4.80–4.89 (1H, m), 5.33 (1H, d, J = 7.7 Hz), 6.66–6.73 (1H, m), 6.89–6.95 (1H, m), 7.03–7.09 (2H, m), 7.12–7.18 (1H, m), 7.28–7.34 (1H, m), 7.71 (1H, d, J = 8.1 Hz), 7.86 (1H, s), 8.11–8.19 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.8, 28.4 (3C), 34.9, 38.7, 43.8 (2C), 44.7 (2C), 45.2 (2C), 60.1, 63.9, 64.2, 110.7, 111.3, 116.7, 119.2, 119.6, 120.6, 122.0, 122.2, 124.4, 126.6, 126.8, 131.2, 136.4, 146.3, 157.1, 169.7, 176.6. LC–MS calcd for $C33H46N6O4^+$ (*m/e*), 591, obsd 591 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-ethoxyphenyl]amino}carbonyl)-2-(1*H*-indol-3-yl) propyl]-4-(4-fluorophenyl)-3-oxopiperazine-1-carboxamide (1j).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.24–1.32 (3H, m), 1.57 (3H, d, J = 7.3 Hz), 2.14–2.26 (6H, m), 3.30–3.35 (2H, m), 3.60–3.73 (4H, m), 3.73–3.81 (1H, m), 3.81–3.97 (2H, m), 4.05–4.13 (1H, m), 4.16–4.24 (1H, m), 4.78–4.91 (1H, m), 5.36 (1H, d, J = 7.7 Hz), 6.70 (1H, d, J = 8.1 Hz), 6.87–6.96 (1H, m), 7.03–7.12 (4H, m), 7.15 (1H, t, J = 7.5 Hz), 7.20–7.25 (2H, m), 7.31 (1H, d, J = 8.1 Hz), 7.67–7.76 (1H, m), 7.84–7.93 (1H, m), 8.11-8.25 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.6, 34.7, 40.8, 45.2 (2C), 48.1, 49.7, 60.2, 63.9, 64.2, 110.6, 111.4, 116.2, 116.3, 116.6, 119.1, 119.6,120.7, 122.0, 122.3, 124.5, 126.5, 126.8, 127.3, 127.4, 131.24, 136.5, 137.5, 146.4, 156.2,160.4, 165.0, 169.6. LC–MS calcd for $C34H39N6O4F^+$ (*m/e*), 615, obsd 615 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-ethoxyphenyl]amino}carbonyl)-2-(1*H*-indol-3-yl) propyl]-4-(4-fluoro-2-methylphenyl)-3-oxopiperazine-1-carboxamide (1k).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.22–1.28 (3H, m), 1.57 (3H, d, J = 7.3 Hz), 2.17–2.24 (9H, m), 3.32 (2H, s), 3.43–3.52 (1H, m), 3.57–3.75 (3H, m), 3.78-–3.95 (3H, m), 4.06–4.13 (1H, m), 4.16–4.24 (1H, m), 4.86 (1H, t, J = 7.5 Hz), 5.38 (1H, d, J = 6.6 Hz), 6.70 (1H, d, J = 8.4 Hz), 6.92–7.01 (3H, m), 7.04–7.11 (3H, m), 7.15 (1H, t, J = 7.5 Hz), 7.29–7.34 (1H, m), 7.69–7.75 (1H, m), 7.89 (1H, d, J = 4.8 Hz), 8.17 (2H, d,

J = 2.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.7, 17.6, 17.8, 34.7, 40.9, 45.2 (2C), 48.0, 49.7, 60.2, 63.9, 64.2, 110.7, 111.4, 114.1, 114.3, 116.6, 117.8, 117.9, 119.2, 119.7, 120.7, 122.0, 122.3, 124.5, 126.5, 126.8, 131.2, 136.4, 136.5, 146.4, 156.3, 161.1, 164.7, 169.6. LC–MS calcd for $C35H41N6O4F^+$ (*m/e*), 629, obsd 629 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(2-thienylcarbonyl)piperidine-1-carboxamide (2a).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.50–1.55 (3H, m), 1.73–1.88 (4H, m), 2.27–2.36 (6H, m), 2.50–2.55 (3H, m), 2.85–3.04 (2H, m), 3.16–3.29 (1H, m), 3.44–3.57 (2H, m), 3.74–3.85 (1H, m), 3.94–4.08 (2H, m), 4.75–4.83 (1H, m), 5.14–5.24 (1H, m), 6.97–7.06 (1H, m), 7.07–7.21 (4H, m), 7.27–7.32 (1H, m), 7.61–7.68 (2H, m), 7.70–7.79 (2H, m), 8.15–8.26 (1H, m), 8.51–8.75 (1H, m), 11.90 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.9, 28.3, 28.4, 28.5, 33.9, 43.4, 43.5, 45.1 (x2), 45.2, 61.0, 63.8, 111.2, 117.1, 119.1, 119.6, 121.2, 121.3, 121.6, 122.2, 123.0, 126.9, 128.2, 131.6, 131.7, 133.9, 136.3, 140.3, 143.3, 145.7, 156.8, 171.8, 194.7, 201.6. LC–MS calcd for $C34H39N5O4S^+$ (*m/e*), 614, obsd 614 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-isobutyrylpiperidine-1-carboxamide (2d).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.08 (6H, d, J = 6.6 Hz), 1.51–1.65 (5H, m), 1.72–1.77 (2H, m), 2.26–2.32 (6H, m), 2.48–2.55 (3H, m), 2.59–2.68 (1H, m), 2.72–2.93 (3H, m), 3.40–3.55 (2H, m), 3.78 (1H, quin, J = 6.8 Hz), 3.88–4.06 (2H, m), 4.73–4.84 (1H, m), 5.10–5.20 (1H, m), 6.97–7.05 (1H, m), 7.06–7.18 (3H, m), 7.26–7.32 (1H, m), 7.61–7.68 (1H, m), 7.72–7.78 (1H, m), 8.18 (1H, brs), 8.64 (1H, d, J = 1.5 Hz), 11.88 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.9, 18.4, 18.5, 27.4, 27.5, 28.4, 33.9, 38.8, 43.5, 43.5, 45.3, 46.7 (2C), 60.9, 63.9, 111.1, 117.1, 119.2, 119.6, 121.1, 121.2, 121.6, 122.2, , 123.0, 126.9, 131.6, 136.2, 140.3, 145.8, 156.8, 171.8, 201.5, 215.9. LC–MS calcd for $C33H43N5O4^+$ (*m/e*), 574, obsd 574 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(cyclobutylcarbonyl)piperidine-1-carboxamide (2f).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.46–1.62 (5H, m), 1.74 (2H, dd, J = 13.0, 2.8 Hz), 1.81–1.87 (1H, m), 1.95–2.02 (1H, m), 2.07–2.14 (2H, m), 2.19–2.27 (2H, m), 2.29 (6H, s), 2.46 (1H, tt, J = 11.2, 3.8 Hz), 2.52 (3H, s), 2.72–2.92 (2H, m), 3.33–3.42 (1H, m), 3.44–3.55 (2H, m), 3.73–3.82 (1H, m), 3.84–3.98 (2H, m), 4.76 (1H, dd, J = 6.8, 5.7 Hz), 5.15 (1H, d, J = 6.6 Hz), 6.97–7.04 (1H, m), 7.07–7.19 (3H, m), 7.29 (1H, d, J = 8.1 Hz), 7.58–7.66 (1H, m), 7.75 (1H, d, J = 8.1 Hz), 8.19 (1H, brs), 8.64 (1H, d, J = 1.1 Hz), 11.87 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.9, 17.9, 24.6, 24.6, 27.2, 27.3, 28.4, 33.9, 43.5, 43.6 (2C), 45.3, 46.5, 48.5, 61.0, 63.8, 111.1, 117.1, 119.1, 119.6, 121.1, 121.2, 121.6 (2C), 122.2, 123.0, 126.9, 131.6, 136.2, 140.3, 156.8, 171.8, 201.5, 212.7. LC–MS calcd for $C34H43N5O4^+$ (*m/e*), 586, obsd 586 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(cyclobutylcarbonyl)piperazine-1-carboxamide (2h).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.47–1.57 (3H, m), 1.81–1.91 (1H, m), 1.92–2.01 (1H, m), 2.08–2.18 (2H, m), 2.25 (6H, s), 2.29–2.38 (2H, m), 2.51 (3H, s), 3.14–3.40 (7H, m), 3.41–3.49 (2H, m), 3.52–3.66 (2H, m), 3.75–3.84 (1H, m), 4.77 (1H, t, J = 6.1 Hz), 5.14 (1H, d, J = 7.0 Hz), 7.02 (1H, t, J = 7.5 Hz), 7.06–7.18 (3H, m), 7.31–7.33 (1H, m), 7.61–7.67 (1H, m), 7.71–7.80 (1H, m), 8.19 (1H, brs), 8.64 (1H, s), 11.90 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.9, 18.0, 25.1, 25.1, 28.5, 33.9, 37.2, 41.1, 43.5, 43.7, 44.6, 45.5 (2C), 60.9, 64.1, 111.2, 116.9, 119.1, 119.7, 120.9, 121.0, 121.6, 122.3, 122.9, 126.9, 131.5, 136.2, 140.2, 146.9, 156.8, 171.4, 173.4, 201.6. LC–MS calcd for $C33H42N6O4^+$ (*m/e*), 587, obsd 587 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(2,2-dimethylpropanoyl)piperazine-1-carboxamide (2i).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.27 (9H, s), 1.50–1.56 (3H, m), 2.25 (6H, s), 2.51 (3H, s), 3.29–3.50 (6H, m), 3.55–3.68 (4H, m), 3.75–3.84 (1H, m), 4.78 (1H, t, J = 6.1 Hz), 5.15 (1H, d, J = 7.0 Hz), 6.98–7.06 (1H, m), 7.07–7.15 (3H, m), 7.30 (1H, d, J = 8.1 Hz), 7.62–7.67 (1H, m), 7.72–7.77 (1H, m), 8.11 (1H, brs), 8.65 (1H, s), 11.91 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.9, 28.4 (3C), 28.4, 33.9, 38.7, 43.7 (2C), 44.7 (2C), 45.5 (2C), 60.8, 64.1, 111.2, 117.0, 119.1, 119.7, 120.9, 121.0, 121.6, 122.3, 122.9, 126.9, 131.5, 136.2, 140.2, 146.9, 156.9, 171.4, 176.6, 201.6. LC–MS calcd for $C33H44N6O4^+$ (*m/e*), 589, obsd 589 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(4-fluorophenyl)-3-oxopiperazine-1-carboxamide (2j).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.47–1.55 (3H, m), 2.28 (6H, s), 2.50–2.55 (3H, m), 3.42–3.50 (2H, m), 3.63–3.68 (2H, m), 3.72–3.76 (2H, m), 3.85–3.92 (1H, m), 4.02–4.10 (1H, m), 4.36 (1H, d, J = 16.9 Hz), 4.79 (1H, t, J = 5.7 Hz), 5.08 (1H, d, J = 6.2 Hz), 7.01–7.17 (7H, m), 7.22–7.25 (1H, m), 7.31 (1H, d, J = 8.1 Hz), 7.70 (1H, d, J = 7.7 Hz), 7.75–7.82 (1H, m), 8.20 (1H, brs), 8.69 (1H, d, J = 1.5 Hz), 12.00 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 16.2, 28.5, 33.6, 40.6, 45.5, 48.2, 49.7, 60.6, 64.1, 111.2, 116.1, 116.3, 116.9, 119.1, 119.8, 121.0, 121.0, 121.7, 122.4, 123.0, 126.7, 127.3, 127.4, 131.6, 136.4, 137.6, 137.7, 140.3, 156.1, 160.4, 162.1, 165.0,171.2, 201.9. LC–MS calcd for $C34H37N6O4F^+$ (*m/e*), 613, obsd 613 (M+1).

***N*-[(1*R*,2*S*)-1-[({2-Acetyl-5-[(dimethylamino)methyl]phenyl}amino)carbonyl]-2-(1*H*-indol-3-yl) propyl]-4-(4-fluoro-2-methylphenyl)-3-oxopiperazine-1-carboxamide (2k).** ^{1}H NMR (600MHz, $CDCl_3$) δ: 1.52 (3H, d, J = 7.0 Hz), 2.18 (3H, d, J = 13.6 Hz), 2.29 (6H, s), 2.54 (3H, d, J = 5.9 Hz), 3.39–3.54 (3H, m), 3.56–3.76 (2H, m), 3.76–3.94 (2H, m), 4.05 (1H, d, J = 16.9 Hz), 4.38 (1H, dd, J

= 16.7, 12.7 Hz), 4.79 (1H, t, J = 5.7 Hz), 5.03–5.16 (1H, m), 6.85–7.20 (7H, m), 7.31 (1H, d, J = 8.1 Hz), 7.70 (1H, d, J = 7.3 Hz), 7.75–7.84 (1H, m), 8.21 (1H, brs), 8.69 (1H, brs), 12.02 (1H, d, J = 10.6 Hz). ^{13}C NMR ($CDCl_3$) *δ*: 16.2d, 17.8, 28.5, 33.6, 40.6, 45.4(2C), 48.1, 49.8, 60.6, 63.9, 111.2, 114.1, 116.9, 117.8, 119.1, 119.8, 121.0, 121.1, 121.7, 122.5, 123.1, 126.6, 128.4, 131.7, 136.4, 137.6, 140.3, 146.6, 156.1, 161.1, 162.8, 164.7, 171.2, 201.9. LC–MS calcd for $C35H39N6O4F^+$ (*m/e*), 627, obsd 627 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-(trifluoromethoxy)phenyl]amino}carbonyl)-2-(1*H*-indol-3-yl)propyl]-4-(2,2-dimethylpropanoyl)piperazine-1-carboxamide (3i).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.27 (9H, s), 1.51–1.59 (3H, m), 2.22 (6H, s), 3.24–3.42 (6H, m), 3.53–3.63 (4H, m), 3.71 (1H, quin, J = 7.2 Hz), 4.74–4.84 (1H, m), 5.08–5.24 (1H, m), 7.03 (1H, dd, J = 8.4, 2.2 Hz), 7.06–7.19 (4H, m), 7.33 (1H, d, J = 8.4 Hz), 7.62–7.71 (1H, m), 8.03–8.10 (1H, m), 8.17 (1H, d, J = 1.8 Hz), 8.21 (1H, brs). ^{13}C NMR ($CDCl_3$) *δ*: 17.5, 28.3 (3C), 33.6, 38.7, 43.7, 44.6 (2C), 45.3 (2C), 60.2, 63.6, 111.5, 116.7, 118.9, 119.8, 120.0, 120.0, 121.3, 121.8, 122.3,122.5, 124.7, 126.5, 129.8, 136.4, 137.2, 138.4, 157.2, 170.2, 176.7. LC/MS (ESI) m/z 631 ($M+H^+$). LC–MS calcd for $C32H41N6O4F3^+$ (*m/e*), 631, obsd 631 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-(trifluoromethoxy)phenyl]amino}carbonyl)-2-(1*H*-indol-3-yl)propyl]-4-(4-fluorophenyl)-3-oxopiperazine-1-carboxamide (3j).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.51–1.57 (3H, m), 2.23 (6H, s), 3.31–3.40 (2H, m), 3.61–3.81 (5H, m), 4.01–4.09 (1H, m), 4.12–4.19 (1H, m), 4.80 (1H, t, J = 7.2 Hz), 5.18–5.30 (1H, m), 7.01–7.24 (9H, m), 7.33 (1H, d, J = 8.1 Hz), 7.66–7.72 (1H, m), 8.07 (1H, s), 8.16 (1H, d, J = 1.8 Hz), 8.26 (1H, brs). ^{13}C NMR ($CDCl_3$) *δ*: 17.1, 33.5, 40.7, 45.4 (2C), 48.0, 49.7, 60.3, 63.6, 111.5, 116.2, 116.3, 116.7, 118.9, 119.8, 120.0, 121.8, 122.4, 122.5, 124.8, 126.3, 127.3, 127.4, 129.7, 136.5, 137.2, 137.4d, 138.5, 156.3, 160.5, 162.1, 164.7, 170.1. LC–MS calcd for $C33H34N6O4F4^+$ (*m/e*), 655, obsd 655 (M+1).

***N*-[(1*R*,2*S*)-1-({[5-[(Dimethylamino)methyl]-2-(trifluoromethoxy)phenyl]amino}carbonyl)-2-(1*H*-indol-3-yl)propyl]-4-(4-fluoro-2-methylphenyl)-3-oxopiperazine-1-carboxamide (3k).** ^{1}H NMR (600MHz, $CDCl_3$) *δ*: 1.54 (3H, d, J = 7.3 Hz), 2.16–2.20 (3H, m), 2.32 (6H, d, J = 3.7 Hz), 3.42–3.55 (3H, m), 3.57–3.79 (3H, m), 3.81–3.91 (1H, m), 4.02–4.10 (1H, m), 4.14–4.24 (1H, m), 4.80 (1H, t, J = 7.2 Hz), 5.39 (1H, brs), 6.89–7.18 (8H, m), 7.33 (1H, d, J = 8.1 Hz), 7.69 (1H, d, J = 8.1 Hz), 8.14 (1H, d, J = 12.1 Hz), 8.20 (1H, s), 8.26 (1H, brs). ^{13}C NMR ($CDCl_3$) *δ*: 17.2, 17.8, 33.4, 40.8, 44.7 (2C), 48.0, 49.7d, 60.3, 63.0, 111.5, 114.2, 116.7, 117.8, 119.0, 119.8, 120.0, 121.9, 122.5, 122.8, 125.2, 126.4, 128.5, 130.0, 136.3, 136.5 (2C), 137.6, 137.7, 156.5, 161.2, 162.8, 164.5, 170.2. LC–MS calcd for $C34H36N6O4F4^+$ (*m/e*), 669, obsd 669 (M+1). HRMS (m/z): [M]+ calcd. for

$C_{34}H_{36}N_6O_4F4$, 669.2807; found 669.2783.

Somatostatin receptor binding assays .

Five types of human SSTR (SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5) cDNAs cloned by PCR based on the published sequence were inserted into the expression vector pAKKO-111. Each vector was then introduced into CHO dhfr- cells by calcium phosphate-mediated transfection. Transformed CHO cells were cultured in a selection medium (ribonucleic acid and deoxyribonucleic acid free α -MEM medium containing 10% dialysed FCS), and a single colony expressing the high level of each receptor subtype was isolated. Transformed CHO cells (1x109 cells) were dispersed using phosphate-bufferd saline (PBS), pH 7.4 containing 0.2 mM EDTA and suspended in a 10 mM sodium carbonate buffer containing 1 mM EDTA, 0.25 mM PMSF, 20 mg/mL of leupeptin, 10 mg/mL of phosphoramidon, and 1 mg/mL pepstatin. After the cells were homogenized with a polytron homogenizer, the homogenates were centrifuged at 1,000 x g for 10 min. The supernatants were ultracentrifuged twice at 100,000 x g for 60 min. The pellets were then suspended in an assay buffer (25 mM Tris-HCl, pH 7.4 containing 0.1 % BSA, 1 mM EDTA, 0.25 mM PMSF, 20 mg/mL of leupeptin, 10 mg/mL of phosphoramidon, and 1 mg/mL pepstatin) and then used as membrane fractions. Binding assays were performed in 96-well plates. Each cell membrane fraction (1 mg protein was used for SSTR1 and 0.5 mg protein for SSTR2-5) dissolved in the assay buffer was incubated with 50 pM [125I] somatostatin-14 (Tyr11) (Amersham, UK) and the test compound at various concentrations for 60 min at room temperature. Nonspecfic binding was defined as [125I] somatostatin-14 (Tyr11) binding in the presence of 1 mM SST-14 (Peptide Research Inc, Japan). The binding reaction was terminated by rapid filtration through GF/B glass filter plates pre-soaked in 0.2 % polyethylenimine and 0.02 % BSA, followed by washing three times with 300 mL of 50 mM Tris-HCl, pH 7.5. Then the plates were dried for 2 hours at 37 ^{o}C and Microscint-O scintillation fluid was added. The radioactivity retained in the filters was determined with Topcount scintillation counter (Packard, USA). The concentration of test compound causing 50% inhibit ion of the specific value (IC50 value) was derived by fitting the data into a pseudo-Hill equation: log [%SB/(100-%SB)] =n [log(C)-log(IC50)] , where %SB is a specific binding, n is a pseudo-Hill constant, and C is the concentration of the test compound.

Human SSTR2 GTPgS binding assay.

Human SSTR2 membrane fraction (4 μg protein was used for each assay) was suspended in the GTPγS assay buffer (50 mM Tris-HCl, pH 7.5 containing 0.1 % BSA, 1 mM EDTA, 10 mM $MgCl_2$, 150 mM NaCl, 10 μM GDP, 0.25 mM PMSF, 20 μg/mL of leupeptin, 10 μg/mL of phosphoramidon, and 1μg/mL pepstatin) and incubated with 0.33 nM [^{35}S] GTPγS (NEN) and test compound over a range of concentrations from 0.1 nM to 10,000 nM. The mixture was incubated for 60 min at room

temperature and then filtered onto GF/C plates. After washing 3 times with 300 μL of 50 mM Tris-HCl buffer pH 7.4 , the plates were dried for 1 hour at 37 ^{o}C. The radioactivity retained in the plates were determined with Topcount scintillation counter (Packard, USA). The efficacy of the compounds was determined from the maximum value of the SST-14 dose response curve. The concentration of the compounds that produced a half-maximum response was represented by the EC_{50}.

Pharmacokinetics of the compounds in rats.

Male Sprague-Dawley rats (8 weeks) were housed two per cage in a room with controlled lighting (12 h light, 12 h dark, lights on at 06:00 h) and temperature (21 ^{o}C - 24 ^{o}C). Food and water were available *ad libitum*. Test compound was prepared as a 2 mg/mL solution in 0.5 % methylcellulose and orally administered at a dose of 10 mg/kg. Blood samples were collected from a jugular vein under anethetization with ether at 1 h after administration. The plasma concentrations of the test compounds were determined using human SSTR2 binding assay described above.

Acknowledgment. We thank Dr. Tetsuji Kawamoto for helpful discussions.

References

1.(a) Unger, R. H.; Orci, L. The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet* **1975**, *1*, 14–16; (b) Dobbs, R. E.; Sakurai, H.; Sasaki, H; Faloona, G.; Valverde, I.; Baetens, D.; Orici, L.; Unger, R. Glucagon: role in the hyperglycemia of diabetes mellitus. *Science* **1975**, *187*, 544–547; (c) Hansen, L. H.; Larger, E.; Chaput, J.- C.; Vissuzaine, C.; Holst, J. J.; Madsen, O. D.; Yakushiji, F.; De Meyts, P.; Assan, R.; Nishimura, E. Identification of a hGluR Gene Deletion Mutation in a Patient with Hyperglucagonemia and Pseudoadenomatous Hyperplasia of Pancreatic Alpha-Cells. Presented at the 81st Annual Meeting of the Endocrine Society, San Diego, CA, June 12–15, **1999**; P3–441.

2. (a) Cordelier, P.; Esteve, J. P.; Bousquest, C.; Delesque, N.; O'Carroll, A.; Schall, A. V.; Vaysse, N.; Susini, C.; Buscail, L. Characterization of the antiproliferative signal mediated by the somatostatin receptor subtype sst5. *Proc. Natl. Acad. Sci.* **1997**, *94*, 9343–9348. (b) Rossowski, W.; Coy, D. H. Specific inhibition of rat pancreatic insulin and glucagon release by receptor-selective somatostatin analogues. *Biochem. Biophys. Res. Comm.* **1994**, *205*, 341–346. (c) Parmar, R. M.; Chan, W. W. –S.; Dashkevicz, M.; Hayes, E. C.; Rohrer, S. P.; Smith, R. G.; Schaeffer, J. M.; Blake, A. D. Nonpeptidl somatostatin agonist demonstrates that sst2 and sst5 inhibit stimulated growth hormone secretion from rat anterior pituitary cells. *Biochem. Biophys. Res. Comm.* **1999**, *263*, 276–280.

3. (a) McCann, S. M. The Discovery of Growth Hormone (GH)-Releasing Hormone and GH Release-Inhibiting Hormone. *Endocrinology* **1992**, *131*, 2042–2043. (b) Brazeau, P.; Vale, W.;

Burgus, R.; Ling, N.; Bucher, M.; Rivier, J.; Guillemin, R. Hypothalamic polypeptide that inhibits the secretion of the immunoreactive growth hormone. *Science* **1973**, *179*, 77–79. (c) Gerich, J. E.; Lovinger, R.; Grodsky, G. M. Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: evidence for a preferential effect on glucagon secretion. *Endocrinology* **1975**, *96*, 749–754. (d) Johansson, C.; Wisen, O.; Efendic, S.; Uvnas-Wallensten, K. Effects of somatostatin on gastrointestinal propagation and absorption of oral glucose in man. *Digestion* **1981**, *22*, 126–137. (e) Mandarino, L.; Stenner, D.; Blanchard, W.; Nissen, S.; Gerich, J.; Ling, N.; Brazeau, P.; Bohlen, P.; Esch, F.; Guillemen, R. Slelective effects of somatostatin-14, -25, and -28 on *in vitro* insulin and glucagons secretion. *Nature* **1981**, *291*, 76–77. (f) Vaysse, N.; Pradayrol, L.; Chayvialle, A.; Pignal, F.; Esteve, J.; Susini, J.; Descos, F.; Ribet, A. Effects of somatostatin-14 and somatostatin-28 on bombesin-stimulated release of gastrin, insulin and glucagons in the dog. *Endocrinology* **1981**, *108*, 1843–1847. (g) Krejs, G. J. Physiological role of somatostatin in the digestive tract: gastric acid secretion, intestinal absorption, and motility. *Scand. J. Gastroenterol.* **1986**, 21 (suppl 119), 47–53. (h) Reichin, S. Somatostatin (part 2). *N. Engl. J. Med.* **1983**, *309*, 1556–1563.

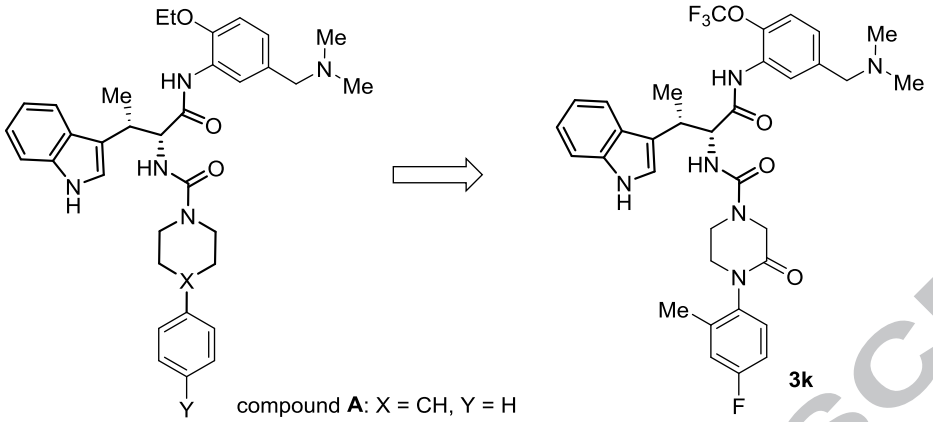
4. Hussein, Z.; Wentworth, J.M.; Nankervis, A.J.; Proietto, J.; Colman, P.G. Effectiveness and side effects of thiazolidinediones for type 2 diabetes: real-life experience from a tertiary hospital. Med. J. Aust. **2004**, 181, 536–539.

5. (a)Berk, S. C.; Rohrer, S. P., Degrado, S. J.; Birzin, E. T.; Mosley, R. T.; Hutchins, S. M.; Pasternak, A.; Schaeffer, J. M.; Underwood, D. J.; Chapman, K. T.; A Combinatorial Approach toward the Discovery of Non-Peptide, Subtype-Selective Somatostatin Receptor 29 Ligands. *J. Comb. Chem.* **1999**, *1*, 388–396. (b) Rohrer, S. P.; Birzin, E. T.; Mosley, R. T.; Berk, S. C., Hutchins, S. M.; Shen, D.; Xiong, Y., Hayes, E. C.; Parmar, R. M., Foor, F.; Mitra. S. W.; Degrado, S. J.; Shu, M.; Klopp, J. M.; Cai, S.; Blake, A.; Chan, W. W. S.; Pasternak, A.; Yang, L.; Patchett, A. A.; Smith, R. G.; Chapman, K. T.; Schaeffer, J. M. Rapid Identification of Subtype-Selective Agonists of the Somatostatin Receptor Through Combinational Chemistry. *Science* **1998**, *282*, 737–740. (c) Zhou, C.; Guo, L.; Morriello, G.; Pasternak, A.; Pan, Y.; Rohrer, S. P.; Birzin, E. T.; Huskey, S. W.; Jacks, T.; Schleim, K. D.; Cheng, K.; Schaeffer, J. M.; Patchett, A. A.; Yang, L. Nipecotic and *iso*-nipecotic amides as potent and selective somatostatin subtype-2 receptor agonists**.** *Bioorg. Med. Chem. Lett.* **2001**, *11*, 415–417.

6. Sawai Y.; Mizuno M.; Ito T; Kawakami J.; Yamano M. A practical synthesis of enantiopure b-methyltryptophan ethyl ester for a preparation of diabetes drug. Tetrahedron, **2009**, 7122–7128.

7. Abe H, Matsunaga S.; Watanabe M.; WO 2004046107 A1

8. Miyamoto S.; Matsumoto A.;, Mori I.; Horinouchi A. Relationship between in vitro phospholipidosis assay using HepG2 cells and 2-week toxicity studies in rats. *Toxicology Mechanisms and Methods*, **2009**, 19, 477–485.



compound **A**: X = CH, Y = H
compound **B**: X = N, Y = F

3k