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Article type : Research Article

Discovery of thiophene-containing biaryl amide derivatives as novel glucagon receptor antagonists

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13184

Abstract: A novel series of thiophene-containing biaryl amide glucagon receptor (GCGR) antagonists were designed and synthesized. Two compounds of this series, **14f** and **14h**, exhibited good GCGR binding (IC_{50} = 6.1 μM and 4.4 μM, respectively) and cAMP functional activities (IC_{50} = 4.4 μM and 14.4 μM, respectively). The possible binding modes of compounds **14f** and **14h** with GCGR were explored by molecular simulation.

Key words: glucagon receptor antagonist, biary amide, molecular simulation

1 INTRODUCTION

Diabetes is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin action, insulin secretion, or both, and is affecting approximately 415 million people around the globe in 2015. [1] Type 2 diabetes mellitus (T2DM) is the most prevalent form and accounts for 90 to 95% of all diabetic patients. [2] Despite the existence of several classes of drugs for treating T2DM, such as dipeptidyl peptidase-4 (DPP-4) inhibitors, [3] sodium-glucose cotransporter-2 (SGLT-2) inhibitors, [4] and glucagon-like peptide 1 (GLP-1) receptor agonists, [5] there remains a need to develop new therapies with improved safety and efficacy.

Glucagon, secreted by the α-cells in the pancreas, is a 29-amino acids peptide hormone that counter-regulates the actions of insulin. [6-7] Glucagon binds to the glucagon receptor (GCGR) and triggers a signal transduction cascade to stimulate the hepatic glucose production *via* glycogenolysis and gluconeogenesis. [8] In 2013, Siu *et al.* [9] reported the crystal structure of the seven transmembrane (7TM) helical domain of human GCGR, and indicated that the glucagon-binding site is in the 7TM domain. It was shown that impairment of insulin release and development of insulin resistance are often accompanied by absolute or relative increases of glucagon in fasting and postprandial states of T2DM, and this situation leads to hyperglycemia. [10] Thus, it is postulated that GCGR antagonists may be useful in mitigating glucagon-induced hyperglycemia, such as peptidic GCGR antagonists, [11]

antibodies, [12] antisense oligonucleotides, [13] and small-molecule antagonists [14] were demonstrated to significantly decrease blood glucose levels. To data, a number of effective small-molecule GCGR antagonists have been reported (Figure 1). The first active compound, (+)-Bay 27-9955, [15] was discovered in 2001 followed by several clinical compounds such as MK-0893 [16] in phase 2 and LGD-6972 in phase 1. Recently, Jazayeril *et al*. [17] disclosed the structure of human GCGR in complex with the antagonist MK-0893 (PDB ID: 5EE7). MK-0893 was found to bind to an allosteric site outside the 7TM helical bundle.

In this paper, we chose compound **1** as the lead compound, which is one of the possible structure of LGD-6972 according to the patent information filed by Ligand pharmaceuticals, [18-20] and adopted bioisosteric strategy to design and synthesize a series of novel thiophene-containing biaryl amide derivatives. We also described GCGR binding affinities and cAMP functional activities of these compounds. Combined structure-activity relationship (SAR) and molecular simulation studies were performed to validate their pharmacological properties.

FIGURE 1. Structures of several glucagon receptor antagonists.

Thiophene is commonly used as a building block in drugs and has proven to be an attractive bioisostere to improve potency and selectivity (Fig. 2). [21] Duloxetine [22] is an anti-depressant, which functions in the central nervous system as a selective serotonin and norepinephrine reuptake inhibitor. Eprosartan, [23] a selective angiotensin II receptor blocker, is used for the treatment of essential hypertension. Rivaroxaban [24] is an oral anti-coagulant indicated for prevention and treatment thrombo-embolic disorders.

FIGURE 2. Drugs that contain the thiophene ring.

Moreover, the structural characteristics of the thiophene-containing drugs exert an important impact on the metabolic pathways. The main metabolites of thiophene are thiophene S-oxides and thiophene epoxides, which may result in toxicity. [25] There is no

thiophene reactive metabolite in some drugs following special modification. For example, the main metabolic pathways of Rivaroxaban are cleavage of the amide bond and oxidative degradation of the morpholine group (Figure 3), [26] while its structure is characteristic of 2-CONH and 5-Cl at the thiophene ring. Therefore, we designed a novel series of 2,5-disubstituted thiophene and investigated their antagonistic effects *via* different substitutions at R_1 and R_2 (Figure 4).

FIGURE 3. Main metabolic pathways of Rivaroxaban.

FIGURE 4. Molecular design of novel glucagon receptor (GCGR) antagonists.

2 EXPERIMENTAL SECTION

2.1 Material and methods

All commercially available compounds and solvents were used without further purification. All products were characterized by their NMR and MS spectra. ^{1}H spectra and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD, and DMSO-d_6 with 400 MHz and 500 MHz instruments. Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were measured with a mass spectrometer. The purity of all compounds determined by Agilent-1100 HPLC with binary pump, photodiode array detector (DAD) and C18 column (150×4.6 mm, 5 μm) was over 95%. Compounds **14a**, **14c-14d**, **14f**, **14h-14j**, **14l-14m**, and **14o** were analyzed using MeOH/H_2O = 70/30 (v/v) at 1 mL/min, and the peak areas were calculated at 254 nm. Compounds **14b**, **14e**, **14g**, **14k**, and **14n** were analyzed using MeOH/H_2O = 80/20 (v/v) at 1 mL/min and the peak areas were calculated at 254 nm.

2.2 General procedure for synthesis of compounds 14a-14o

2.2.1 Methyl 5-methylthiophene-2-carboxylate (3)

To a solution of 5-methylthiophene-2-carboxylic acid **2** (1.0 g, 7.0 mmol) in MeOH (50 mL) under nitrogen, $SOCl_2$ (1.5 mL, 21.0 mmol) was added dropwise at 0 °C. the resulting mixture was refluxed at 80 ^oC for 3.5 h. After removal of the solvent, the crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (10:1) to afford methyl 5-methylthiophene-2-carboxylate **3** as a yellow oil (1.0 g, 90.9%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 3.7 Hz, 1H), 6.77–6.73 (m, 1H), 3.84 (s, 3H), 2.53–2.49 (m, 3H). MS (ESI, *m/z*): 157.1 $[M+H]^+$.

2.2.2 Methyl 5-(bromomethyl)thiophene-2-carboxylate (4)

Methyl 5-methylthiophene-2-carboxylate **3** (5.0 g, 32.0 mmol), *N*-bromosuccinimide (6.3 g, 35.4 mmol), and benzoylperoxide (0.78 g, 0.32 mmol) were dissolved in $CHCl_3$. The mixture was heated at 65 ^oC for 7 h and then cooled to room temperature. The resulting crude product was filtered and the filtrate was extracted with EtOAc, washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with petroleum ether/EtOAc (10:1)to yield 6.2 g (82.3%) of methyl 5-(bromomethyl)thiophene-2-carboxylate **4** as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 3.8 Hz, 1H), 7.03 (dd, *J* = 3.8, 0.6 Hz, 1H), 4.62 (s, 2H), 3.83 (dd, *J* = 6.2, 0.6 Hz, 3H).

2.2.3 *tert*-Butyl 2-(4-bromophenyl)acetate (6)

To a solution of 2-(4-bromophenyl)acetic acid **5** (10.0 g, 46.5 mmol) in *t*-BuOH (50 mL) under nitrogen, *t*-butyl pyrocarbonate (20.3 g, 93.1 mmol) and DMAP (1.7 g, 13.9 mmol) were added. The mixture was stirred at room temperature for 24 h. After removal of the solvent, the crude product was purified by flash chromatography eluting with petroleum

ether/EtOAc (10:1) to afford *tert*-butyl 2-(4-bromophenyl)acetate **6** as a yellow oil (9.4 g, 74.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.3 Hz, 2H), 7.14 (d, *J* = 8.3 Hz, 2H), 3.47 (s, 2H), 1.43 (s, 9H). MS (ESI, *m/z*): 279.1 $[M+H]^+$.

2.2.4 Methyl 5-[2-(4-bromophenyl)-3-(*tert*-butoxy)-3-oxopropyl]thiophene-2-carboxylate (7)

A solution of *tert*-butyl 2-(4-bromophenyl)acetate **6** (9.4 g, 34.7 mmol) in anhydrous THF was cooled to -78 ^{o}C under nitrogen atmosphere. To this mixture, a solution of *n*-BuLi in hexane (28.9 mL, 2.4 M) was added dropwise at -78 ^{o}C, and the resulting mixture was stirred for 1 h. A solution of methyl 5-(bromomethyl)thiophene-2-carboxylate **4** (8.2 g, 34.7 mmol) in anhydrous THF was subsequently added dropwise and the mixture was stirred for 2.5 h. The reaction was quenched with saturated ammonium chloride aqueous solution, followed by extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash chromatography eluting with petroleum ether/EtOAc (10:1) to yield 6.2 g (42.0%) of methyl 5-[2-(4-bromophenyl)-3-(*tert*-butoxy)-3-oxopropyl]thiophene-2-carboxylate **7** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 3.7 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.17–7.11 (m, 2H), 6.70 (dd, *J* = 7.1, 3.8 Hz, 1H), 3.80 (s, 3H), 3.72 (dd, *J* = 15.3, 7.0 Hz, 1H), 3.52 (dd, *J* = 14.7, 8.4 Hz, 1H), 3.12 (dd, *J* = 14.8, 7.1 Hz, 1H), 1.33 (s, 9H). MS (ESI, *m/z*): 423.0 $[M-H]^-$.

2.2.5 Methyl 5-[2-(4-bromophenyl)-3-chloro-3-oxopropyl]thiophene-2-carboxylate (8)

A solution of the compound **7** (900 mg, 2.1 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (5 mL) at room temperature for 3 h. The mixture was concentrated under reduced pressure to give a brown oil. The oil was dissolved in anhydrous CH_2Cl_2, and then to the solution was added oxalychloride (481.3 mg, 3.8 mmol) dropwise. The resulting mixture was stirred at ambient tempreature for 14 h. After removal of the solvent under reduced pressure, the resulting crude product was used in next reaction without further purification.

2.2.6 4'-Chloro-2'-methyl-biphenyl-4-amine (11a)

The protocol reported in ref. 19 was modified as follows: A mixture of 4-iodo-aniline **9** (1.0 g, 4.6 mmol), 2-methyl-4-chlorophenyl-boronic acid **10** (1.2 g, 5.9 mmol), $PdCl_2(P(o\text{-tolyl})_3)_2$ (0.47 mg, 0.59 mmol), and Na_2CO_3 (2.4 g, 22.6 mmol) in DME/EtOH/H_2O (16/8/4 mL) was heated at 125 ^oC for 2 h. The reaction mixture was cooled to room temperature, filtered and washed with EtOAc. The solution was cancentrated under reduced pressure. The resulting mixture was extracted with EtOAc. the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (10:1) to give 0.9 g (90.5%) of 4'-chloro-2'-methyl-biphenyl-4-amine **11a** as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (d, J = 2.3 Hz, 1H), 7.22 (dd, J = 8.2, 2.3 Hz, 1H), 7.13 (d, J = 8.2 Hz, 1H), 7.01 – 6.94 (m, 2H), 6.65 – 6.59 (m, 2H), 5.16 (s, 2H), 2.23 (s, 3H). MS (ESI, *m/z*): 218.1 $[M+H]^+$.

2.2.7 4'-Chloro-3'-methyl-biphenyl-4-amine (11b)

Compound **11b** was prepared according to the procedure for the preparation of compound **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (m, 1H), 7.36 (d, J = 1.7 Hz, 3H), 7.34 (s, 1H), 6.65 – 6.62 (m, 2H), 5.27 (s, 2H), 2.35 (s, 3H). MS (ESI, *m/z*): 218.0 $[M+H]^+$.

2.2.8 2',4',6'-trimethyl-biphenyl-4-amine (11c)

Compound **11c** was prepared according to the procedure for the preparation of compound **11a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (s, 2H), 6.76 – 6.69 (m, 2H), 6.63 – 6.58 (m, 2H), 5.02 (s, 2H), 2.23 (s, 3H), 1.94 (s, 6H). MS (ESI, *m/z*): 212.1 $[M+H]^+$.

2.2.9 Methyl 5-{2-(4-bromophenyl)-3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-3-oxopropyl}thiophene-2-carboxylate (12a)

A solution of the compound **8** (720 mg, 1.9 mmol) in CH_2Cl_2 was treated with 4'-chloro-2'-methyl-biphenyl-4-amine **11** (404 mg, 1.9 mmol) and *N*,*N*-diispropylethylamine (2.9 mL, 17.8 mmol), ssuccessively. The resulting mixture was then stirred for 14 h at room temperature. After removal of the solvent, the crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (4:1) to afford methyl 5-{2-(4-bromophenyl)-3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-3-oxopropyl}thiophene-2-carboxylate **12a** as a yellow solid (970 mg, 91.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.61 (h, *J* = 4.2, 3.7 Hz, 3H), 7.59 – 7.50 (m, 2H), 7.44 – 7.39 (m, 2H), 7.36 (d, *J* = 2.2 Hz, 1H), 7.29 – 7.22 (m, 3H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.98 (t, *J* = 4.1 Hz, 1H), 4.06 (t, *J* = 7.6 Hz, 1H), 3.76 (s, 3H), 3.65 (dd, *J* = 14.6, 9.0 Hz, 1H), 3.29 (dd, *J* = 14.5, 6.1 Hz, 1H), 2.19 (s, 3H). MS (ESI, *m/z*): 568.0 $[M+H]^+$.

2.2.10 Methyl 5-{2-(4-bromophenyl)-3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-3-oxopropyl}thiophene-2-carboxylate (12b)

Compound **12b** was prepared according to the procedure for the preparation of compound **12a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.66 – 7.59 (m, 6H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.46 – 7.38 (m, 4H), 6.98 (d, *J* = 3.8 Hz, 1H), 4.06 (dd, *J* = 8.7, 6.6 Hz, 1H), 3.75 (s, 3H), 3.65 (dd, *J* = 14.7, 8.7 Hz, 1H), 3.29 (dd, *J* = 14.7, 6.5 Hz, 1H), 2.37 (s, 3H). MS (ESI, *m/z*): 569.7 $[M+H]^+$.

2.2.11 Methyl 5-{2-(4-bromophenyl)-3-[(2',4',6'-trimethyl-biphenyl-4-yl)amino]-3-oxopropyl}thiophene-2-carboxylate (12c)

Compound **12c** was prepared according to the procedure for the preparation of compound **12a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.63 – 7.61 (m, 2H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.57 – 7.54 (m, 2H), 7.45 – 7.41 (m, 2H), 7.02 – 6.98 (m, 3H), 6.88 (s, 2H), 4.07 (dd, *J* = 8.8, 6.5 Hz, 1H), 3.76 (s, 3H), 3.66 (dd, *J* = 14.7, 8.8 Hz, 1H), 3.29 (dd, *J* = 14.7, 6.4 Hz, 1H), 2.23 (s, 3H), 1.89 (s, 6H). MS (ESI, *m/z*): 561.8 $[M-H]^-$.

2.2.12 Methyl 5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13c)

Compound **12a** (200 mg, 0.35 mmol) was dissolved in DME, followed with 4-methoxyphenyl-boronic acid (134 mg, 0.88 mmol), $PdCl_2(P(o\text{-tolyl})_3)_2$ (82.9 mg, 0.11 mmol) and DIPEA. The mixture was heated at 85 ^{o}C for 2 h. After cooling to room temperature, the mixture was filtered through a pad of celite which was rinsed three times with EtOAc and the combined organic layer was concentrated *in vacuo*. The resulting crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (4:1) to yield 135 mg (64.4%) of 5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate **13c** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.57 (d, *J* = 3.7 Hz, 1H), 7.55–7.49 (m, 6H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.16 (d, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 3.7 Hz, 1H), 3.89 (d, *J* = 3.7 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.76 (d, *J* = 11.7 Hz, 1H), 3.32 (td, *J* = 11.1, 7.3 Hz, 1H), 2.36 (s, 3H). MS (ESI, *m/z*): 594.1 $[M-H]^-$.

2.2.13 Methyl

5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-fluoro-biphenyl-4-yl)-3-oxopropyl }thiophene-2-carboxylate (13d)

Compound **13d** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.72 – 7.66 (m, 2H), 7.66 – 7.60 (m, 5H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 2.3 Hz, 1H), 7.30 – 7.22 (m, 5H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 3.8 Hz, 1H), 4.12 (dd, *J* = 9.3, 5.9 Hz, 1H), 3.76 (s, 3H), 3.74 – 3.65 (m, 1H), 3.31 (d, *J* = 5.9 Hz, 1H), 2.19 (s, 3H).MS (ESI, *m/z*): 583.8 $[M-H]^-$.

2.2.14 Methyl

5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-trifluoromethyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13e)

Compound **13e** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.66 – 7.59 (m, 5H), 7.36 (d, *J* = 2.3 Hz, 1H), 7.26 (t, *J* = 5.5 Hz, 3H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 3.8 Hz, 1H), 4.17 – 4.12 (m, 1H), 3.76 (s, 3H), 3.75 – 3.69 (m, 1H), 3.33 (s, 1H), 2.20 (s, 3H). MS (ESI, *m/z*): 633.8 $[M-H]^-$.

2.2.15 Methyl

5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-trifluoromethoxy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13f)

Compound **13f** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.77 – 7.71 (m, 2H), 7.70 – 7.57 (m, 5H), 7.35 (d, *J* = 2.2 Hz, 1H), 7.32 – 7.21 (m, 3H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 4.0 Hz, 1H), 4.19 – 4.12 (m, 1H), 4.02 (q, *J* = 7.0 Hz, 1H), 3.76 (s, 3H), 3.71 (d, *J* = 9.1 Hz, 1H), 2.19 (s, 3H). MS (ESI, *m/z*): 649.8 $[M-H]^-$.

2.2.16 Methyl 5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methy-biphenyl-4-yl)-3-oxopropyl }thiophene-2-carboxylate (13g)

Compound **13g** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.63 (m, 5H), 7.57 – 7.51 (m, 4H), 7.35 (d, *J* = 2.3 Hz, 1H), 7.25 (m, 5H), 7.16 (d, *J* = 8.3 Hz, 1H), 7.03 (d, *J* = 3.9 Hz, 1H), 4.11 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.76 (s, 3H), 3.74 – 3.68 (m, 1H), 3.30 (d, *J* = 5.7 Hz, 1H), 2.32 (s, 3H), 2.19 (s, 3H). MS (ESI, *m/z*): 579.8 $[M-H]^-$.

2.2.17 Methyl 5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-tert-butyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13h)

Compound **13h** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (d, *J* = 3.0 Hz, 1H), 7.67 – 7.61 (m, 5H), 7.56 (m, 4H), 7.48 – 7.44 (m, 2H), 7.36 (d, *J* = 2.5 Hz, 1H), 7.29 – 7.22 (m, 3H), 7.16 (dd, *J* = 8.3, 3.2 Hz, 1H), 7.04 (d, *J* = 3.6 Hz, 1H), 4.13 – 4.09 (m, 1H), 3.76 (d, *J* = 3.0 Hz, 3H), 3.74 – 3.66 (m, 1H), 3.32 – 3.26 (m, 1H), 2.20 (s, 3H), 1.30 (d, *J* = 3.1 Hz, 9H). MS (ESI, *m/z*): 621.7 $[M-H]^-$.

2.2.18 Methyl 5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(4'-tert-butyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13i)

Compound **13i** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 3H), 7.62 (d, *J* = 2.9 Hz, 4H), 7.59 – 7.52 (m, 5H), 7.45 (d, *J* = 8.8 Hz, 4H), 7.02 (d, *J* = 3.8 Hz, 1H), 4.11 (dd, *J* = 9.0, 6.1 Hz, 1H), 3.75 (s, 3H), 3.69 (d, *J* = 9.1 Hz, 1H), 3.30 (s, 1H), 2.37 (s, 3H), 1.29 (s, 9H). MS (ESI, *m/z*): 621.8 $[M-H]^-$.

2.2.19 Methyl 5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13j)

Compound **13j** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.64 – 7.56 (m, 8H), 7.51 (m, 2H), 7.48 – 7.40 (m, 2H), 7.04 – 6.98 (m, 3H), 4.09 (dt, *J* = 8.9, 4.3 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 2H), 3.70 (dd, *J* = 14.8, 9.1 Hz, 1H), 3.30 (d, *J* = 6.1 Hz, 1H), 2.37 (s, 3H). MS (ESI, *m/z*): 597.0$[M+H]^+$.

2.2.20 Methyl 5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(4'-chloro-2'-methyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13k)

Compound **13k** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 7.71 – 7.66 (m, 2H), 7.65 – 7.58 (m, 4H), 7.56 – 7.52 (m, 2H), 7.47 – 7.41 (m, 2H), 7.37 (d, *J* = 2.2 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.29 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 3.9 Hz, 1H), 4.11 (dd, *J* = 9.4, 5.6 Hz, 1H), 3.76 (d, *J* = 1.4 Hz, 3H), 3.71 (d, *J* = 9.2 Hz, 1H), 3.30 (d, *J* = 5.6 Hz, 1H), 2.37 (s, 3H), 2.21 (s, 3H). MS (ESI, *m/z*): 616.1$[M+H]^+$.

2.2.21 Methyl 5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(2',4',6'-trimethyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13l)

Compound **13l** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.65 – 7.58 (m, 4H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.48 – 7.40 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 7.00 (d, *J* = 3.8 Hz, 1H), 6.90 (s, 2H), 4.07 (dd, *J* = 8.8, 6.3 Hz, 1H), 3.75 (s, 3H), 3.73 – 3.62 (m, 1H), 3.34 – 3.27 (m, 1H), 2.37 (s, 3H), 2.24 (s, 3H), 1.90 (s, 6H). MS (ESI, *m/z*): 609.2$[M+H]^+$.

2.2.22 Methyl 5-{3-[(2',4',6'-trimethyl-biphenyl-4-yl)amino]-2-(4'-tert-butyl-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13m)

Compound **13m** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.65 – 7.58 (m, 4H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.48 – 7.40 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 7.00 (d, *J* = 3.8 Hz, 1H), 6.90 (s, 2H), 4.07 (dd, *J* = 8.8, 6.3 Hz, 1H), 3.75 (s, 3H), 3.73 – 3.62 (m, 1H), 3.34 – 3.27 (m, 1H), 2.37 (s, 3H), 2.24 (s, 3H), 1.90 (s, 6H). MS (ESI, *m/z*): 616.0 $[M+H]^+$.

2.2.23 Methyl 5-{3-[(2',4',6'-trimethyl-biphenyl-4-yl)amino]-2-(4'-methy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13n)

Compound **13n** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.65 – 7.63 (m, 2H), 7.63 – 7.59 (m, 3H), 7.55 (d, *J* = 3.0 Hz, 2H), 7.53 (d, *J* = 2.7 Hz, 2H), 7.27 – 7.21 (m, 2H), 7.03 (d, *J* = 3.8 Hz, 1H), 7.02 – 6.97 (m, 2H), 6.88 (s, 2H), 4.11 (dd, *J* = 9.1, 6.0 Hz, 1H), 3.76 (s, 3H), 3.75 – 3.67 (m, 1H), 3.32 (d, *J* = 8.8 Hz, 1H), 2.32 (s, 3H), 2.23 (s, 3H), 1.89 (s, 6H). MS (ESI, *m/z*): 574.0 $[M+H]^+$.

2.2.24 Methyl 5-{3-[(2',4',6'-trimethyl-biphenyl-4-yl)amino]-2-(4'-methoxy-biphenyl-4-yl)-3-oxopropyl}thiophene-2-carboxylate (13o)

Compound **13o** was prepared according to the procedure for the preparation of compound **13c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.65 – 7.61 (m, 4H), 7.60 – 7.58 (m, 3H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.04 – 7.01 (m, 3H), 7.00 (d, *J* = 1.1 Hz, 2H), 6.88 (s, 2H), 4.10 (dd, *J* = 9.2, 6.1 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.74 – 3.68 (m, 1H), 3.34 – 3.28 (m, 1H), 2.23 (s, 3H), 1.89 (s, 6H). MS (ESI, *m/z*): 590.0 $[M+H]^+$.

2.2.25

2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14c)

A solution of compound **13c** in 1,4-dioxane/MeOH (1:1) was treated with NaOH *aq.* (2 *N*). Then the mixture was heated at 60 ^{o}C for 2 h. After removal of the solvent, the residue was acidified with 1 *N* HCl, and the resulting mixture was extracted with EtOAc, washed withbrine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product (2-thiophenecarboxylic acid) was used as a yellow solid which was used in next reaction without further purification. This crude 2-thiophenecarboxylic acid was dissolved in DMF, followed with addition of taurine (28.4 mg, 0.23 mmol), EDCI (65.2 mg, 0.34 mmol), HOBt (46.0 mg, 0.34 mmol) and DIPEA (0.2 mL). The reaction mixture was stirred at ambient temperature for overnight. After the reaction was processed completely, EtOA cwas added, and the organic layer was washed twice with1 *N* HCl and brine, successively. After removal of the solvent, the residue was purified by flash chromatography eluting with DCM/MeOH (15:1) to afford 2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-bipheny-4-yl)-3-oxopropyl}thiophene-2-carboxamido)ethanesulfonic acid **14c** as a white solid (100 mg, 64.1%). mp 160-162^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.40 (t, *J* = 5.4 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.62–7.56 (m, 4H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 3.7 Hz, 1H), 7.35 (d, *J* = 2.0 Hz, 1H), 7.29–7.21 (m, 3H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 3.7 Hz, 1H), 4.13 (t, *J* = 5.3 Hz, 1H), 3.78 (s, 3H), 3.65 (dt, *J* = 20.7, 10.5 Hz, 1H), 3.44 (dd, *J* = 14.1, 6.1 Hz, 2H), 3.27–3.20 (m, 1H), 2.67–2.60 (m, 2H), 2.20 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 161.9, 160.1, 148.1, 140.9, 139.9, 139.4, 139.1, 139.0, 138.5, 136.0, 133.3, 132.7, 132.4, 131.0, 130.5, 129.4, 128.9, 128.7, 127.7, 127.4, 127.0, 120.0, 115.5, 56.3, 54.6, 51.5, 37.1, 34.2, 21.2. LRMS (ESI, *m/z*): 687.1 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{32}ClN_2O_6S_2^-$, 687.1396 $[M-H]^-$; found 687.1406, purity: 98.8%.

2.2.26 2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-3-oxo-2-phenylpropyl} thiophene-2-carboxamido)ethanesulfonic acid (14a)

Compound **14a** was prepared according to the procedure for the preparation of compound **14c**. mp 151-153^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.39 (t, *J* = 5.2 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 3.5 Hz, 1H), 7.37–7.31 (m, 3H), 7.29–7.21 (m, 4H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.88 (d, *J* = 3.6 Hz, 1H), 4.10–4.01 (m, 1H), 3.63 (dd, *J* = 14.6, 9.3 Hz, 1H), 3.44 (dd, *J* = 13.5, 6.4 Hz, 2H), 3.21 (dd, *J* = 14.9, 5.9 Hz, 1H), 2.70–2.58 (m, 2H), 2.20 (s, 3H). LRMS (ESI, *m/z*): 581.1 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{29}H_{26}ClN_2O_5S_2^-$, 581.0977 $[M-H]^-$; found 581.0976, purity: 98.3%.

2.2.27 2-(5-{2-(4-bromophenyl)-3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-3-oxopropyl}thiophene-2-carboxamido)ethanesulfonic acid (14b)

Compound **14b** was prepared according to the procedure for the preparation of compound **14c**. mp 135-137^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (d, *J* = 20.2 Hz, 1H), 8.40 (s, 1H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.44–7.38 (m, 3H), 7.36 (d, *J* = 1.8 Hz, 1H), 7.29–7.21 (m, 3H), 7.17 (d, *J* = 8.2 Hz, 1H), 6.87 (d, *J* = 3.5 Hz, 1H), 4.07 (t, *J* = 7.5 Hz, 1H), 3.60 (dd, *J* = 14.6, 8.8 Hz, 1H), 3.45 (dd, *J* = 13.6, 6.4 Hz, 2H), 3.22 (dd, *J* = 14.7, 6.4 Hz, 1H), 2.65 (t, *J* = 7.3 Hz, 2H), 2.20 (s, 3H). LRMS (ESI, *m/z*): 659.0 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{29}H_{25}BrClN_2O_5S_2^-$, 659.0082 $[M-H]^-$; found 659.0089, purity: 95.3%.

2.2.28 2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-fluoro-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14d)

Compound **14d** was prepared according to the procedure for the preparation of compound **14c**. mp 151-153^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.41 (t, *J* = 5.4 Hz, 1H), 7.73–7.60 (m, 6H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.42 (d, *J* = 3.6 Hz, 1H), 7.35 (s, 1H), 7.31–7.21

(m, 5H), 7.17 (d, *J* = 8.2 Hz, 1H), 6.92 (d, *J* = 3.6 Hz, 1H), 4.14–4.09 (m, 1H), 3.66 (dd, *J* = 14.6, 9.2 Hz, 1H), 3.44 (dd, *J* = 13.7, 6.3 Hz, 2H), 3.26 (dd, *J* = 14.9, 6.0 Hz, 1H), 2.69–2.59 (m, 2H), 2.20 (s, 3H). LRMS (ESI, m/z): 674.9 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{35}H_{29}ClFN_2O_5S_2^-$, 675.1196 $[M-H]^-$; found 675.1199, purity: 99.5%.

2.2.29

2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-trifluoro-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14e)

Compound **14e** was prepared according to the procedure for the preparation of compound **14c**. mp 148-150^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.38 (t, *J* = 5.5 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.63 (dd, *J* = 22.3, 8.5 Hz, 4H), 7.38 (dd, *J* = 19.7, 2.8 Hz, 2H), 7.31–7.23 (m, 3H), 7.18 (d, *J* = 8.2 Hz, 1H), 6.91 (d, *J* = 3.7 Hz, 1H), 4.15–4.09 (m, 1H), 3.68 (dd, *J* = 14.6, 9.1 Hz, 1H), 3.44 (dd, *J* = 13.4, 6.5 Hz, 2H), 3.29 (dd, *J* = 14.7, 5.9 Hz, 1H), 2.65–2.58 (m, 2H), 2.20 (s, 3H). LRMS (ESI, *m/z*): 725.0 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{29}ClF_3N_2O_5S_2^-$, 725.1164 $[M-H]^-$; found 725.1166, purity: 97.3%.

2.2.30

2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-trifluoromethoxy-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14f)

Compound **14f** was prepared according to the procedure for the preparation of compound **14c**. mp 161-163^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.42 (t, *J* = 5.5 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.67 (dd, *J* = 8.5, 1.2 Hz, 4H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.47–7.41 (m, 3H), 7.35 (d, *J* = 1.9 Hz, 1H), 7.30–7.21 (m, 3H), 7.17 (d, *J* = 8.2 Hz, 1H), 6.92 (d, *J* = 3.7 Hz, 1H), 4.15 (dd, *J* = 11.3, 6.0 Hz, 1H), 3.67 (dd, *J* = 14.8, 9.1 Hz, 1H), 3.44 (dd, *J* = 14.2, 6.1 Hz, 2H), 3.26 (dd, *J* = 14.7, 5.9 Hz, 1H), 2.68–2.60 (m, 2H), 2.20 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.6, 161.9, 149.0, 148.0, 140.9, 140.3, 140.3, 139.3, 139.2, 138.8,

138.5, 136.1, 132.7, 132.4, 131.0, 130.5, 129.7, 129.6, 128.7, 128.2, 127.8, 127.0, 122.6, 120.1, 54.6, 51.5, 37.1, 34.2, 21.2. LRMS (ESI, *m/z*): 741.0 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{29}ClF_3N_2O_6S_2^-$, 741.1113 $[M-H]^-$; found 741.1117, purity: 99.8%.

2.2.31

2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-methyl-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14g)

Compound **14g** was prepared according to the procedure for the preparation of compound **14c**. mp 150-152^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.39 (t, *J* = 5.3 Hz, 1H), 7.63 (dd, *J* = 10.5, 8.6 Hz, 4H), 7.54 (dd, *J* = 7.8, 3.6 Hz, 4H), 7.41 (d, *J* = 3.6 Hz, 1H), 7.35 (s, 1H), 7.31–7.20 (m, 5H), 7.17 (d, *J* = 8.2 Hz, 1H), 6.91 (d, *J* = 3.5 Hz, 1H), 4.19–3.99 (m, 1H), 3.67 (dd, *J* = 14.5, 9.1 Hz, 1H), 3.46 (dd, *J* = 13.6, 6.3 Hz, 2H), 3.25 (dd, *J* = 14.6, 5.6 Hz, 1H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.33 (s, 3H), 2.20 (s, 3H). LRMS (ESI, *m/z*): 671.0 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{32}ClN_2O_5S_2^-$, 671.1147 $[M-H]^-$; found 671.1458, purity: 98.2%.

2.2.32

2-(5-{3-[(4'-chloro-2'-methyl-biphenyl-4-yl)amino]-2-(4'-*t*-butyl-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14h)

Compound **14h** was prepared according to the procedure for the preparation of compound **14c**. mp 160-162^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.39 (d, *J* = 5.2 Hz, 1H), 7.60 (ddd, *J* = 19.3, 12.6, 8.4 Hz, 8H), 7.50–7.30 (m, 4H), 7.26 (dd, *J* = 12.8, 9.6 Hz, 3H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 3.5 Hz, 1H), 4.11 (d, *J* = 6.2 Hz, 1H), 3.67 (dd, *J* = 14.3, 9.2 Hz, 1H), 3.44 (dd, *J* = 13.7, 6.5 Hz, 3H), 3.25 (dd, *J* = 14.8, 5.6 Hz, 1H), 2.64 (t, *J* = 7.3 Hz, 2H), 2.20 (s, 3H), 1.30 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.7, 161.8, 151.0, 148.1, 140.2, 139.9, 139.5, 139.4, 139.2, 138.1, 137.6, 134.8, 132.3, 132.1, 131.2, 130.8, 129.4, 129.3, 128.6, 127.8, 127.8, 127.5, 126.9, 119.9, 54.8, 51.5, 37.2, 35.4, 32.3,

21.4. LRMS (ESI, *m/z*): 713.2 $[M-H]^-$. HRMS (ESI, *m/z*) calcd for $C_{39}H_{38}ClN_2O_5S_2^-$, 713.1916 $[M-H]^-$; found 713.1915, purity: 99.6%.

2.2.33

2-(5-{2-(4'-*t*-butyl-bipheny-4-yl)-3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14i)

Compound **14i** was prepared according to the procedure for the preparation of compound **14c**. mp 139-141^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.39 (t, *J* = 5.3 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.64–7.51 (m, 8H), 7.49–7.37 (m, 5H), 6.90 (d, *J* = 3.6 Hz, 1H), 4.14–4.06 (m, 1H), 3.66 (dd, *J* = 14.7, 9.0 Hz, 1H), 3.43 (dd, *J* = 13.7, 6.1 Hz, 2H), 3.26 (dd, *J* = 14.6, 5.9 Hz, 1H), 2.62 (t, *J* = 7.3 Hz, 2H), 2.35 (d, *J* = 13.8 Hz, 3H), 1.30 (s, 9H). LRMS (ESI, *m/z*): 713.2 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{39}H_{38}ClN_2O_5S_2^-$, 713.1916 $[M-H]^-$; found 713.1928, purity: 96.9%.

2.2.34

2-(5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(4'-methoxy-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14j)

Compound **14j** was prepared according to the procedure for the preparation of compound **14c**. mp 158-160^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.37 (t, *J* = 5.4 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.64–7.55 (m, 7H), 7.54–7.37 (m, 5H), 7.00 (d, *J* = 8.9 Hz, 2H), 6.89 (d, *J* = 3.7 Hz, 1H), 4.12–4.08 (m, 1H), 3.78 (s, 3H), 3.65 (dd, *J* = 14.7, 8.9 Hz, 1H), 3.43 (dd, *J* = 13.7, 6.4 Hz, 2H), 3.25 (dd, *J* = 14.6, 6.0 Hz, 1H), 2.65–2.58 (m, 2H), 2.37 (s, 3H). LRMS (ESI, *m/z*): 687.1 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{32}ClN_2O_6S_2^-$, 687.1396 $[M-H]^-$; found 687.1401, purity: 97.6%.

2.2.35

2-(5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(4'-chloro-2'-methyl-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14k)

Compound **14k** was prepared according to the procedure for the preparation of compound **14c**. mp 166-169^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.37 (t, *J* = 5.2 Hz, 1H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.62 (dd, *J* = 15.0, 10.0 Hz, 4H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.49–7.43 (m, 2H), 7.43–7.39 (m, 1H), 7.37 (d, *J* = 1.7 Hz, 1H), 7.35–7.29 (m, 3H), 7.28 (d, *J* = 1.9 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 6.90 (d, *J* = 3.6 Hz, 1H), 4.12 (dd, *J* = 9.1, 3.4 Hz, 1H), 3.68 (dd, *J* = 14.5, 9.4 Hz, 1H), 3.45 (dd, *J* = 13.5, 6.3 Hz, 2H), 3.25 (dd, *J* = 14.6, 5.6 Hz, 1H), 2.65 (t, *J* = 7.3 Hz, 2H), 2.37 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.1, 162.5, 147.4, 139.5, 139.5, 138.7, 137.6, 137.4, 136.8, 136.0, 135.5, 135.2, 132.3, 132.1, 130.3, 129.1, 128.6, 128.4, 128.3, 128.0, 127.0, 126.3, 126.1, 125.0, 124.7, 119.7, 54.2, 49.5, 35.0, 33.3, 18.7, 18.3. LRMS (ESI, *m/z*): 705.1 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{36}H_{31}Cl_2N_2O_5S_2^-$, 705.1057 $[M-H]^-$; found 705.1065, purity: 95.8%.

2.2.36

2-(5-{3-[(4'-chloro-3'-methyl-biphenyl-4-yl)amino]-2-(2',4',6'-trimethyl-bipheny-4-yl)-3-oxopropyl} thiophene-2-carboxamido)ethanesulfonic acid (14l)

Compound **14l** was prepared according to the procedure for the preparation of compound **14c**. mp 159-161^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.34 (t, *J* = 5.4 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.63 (dd, *J* = 13.0, 9.0 Hz, 3H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.47–7.40 (m, 2H), 7.37 (d, *J* = 3.7 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 2H), 6.90 (s, 2H), 6.86 (d, *J* = 3.7 Hz, 1H), 4.06 (dd, *J* = 9.1, 6.3 Hz, 1H), 3.67 (dd, *J* = 14.5, 9.1 Hz, 1H), 3.44 (dd, *J* = 13.7, 6.2 Hz, 2H), 3.25 (dd, *J* = 14.5, 5.7 Hz, 1H), 2.66–2.58 (m, 2H), 2.38 (s, 3H), 2.25 (s, 3H), 1.90 (s, 6H). LRMS (ESI, *m/z*): 699.2 $[M-H]^-$. HRMS (ESI, *m/z*): calcd for $C_{38}H_{36}ClN_2O_5S_2^-$, 699.1760 $[M-H]^-$; found 699.1765, purity: 98.9%.

2.2.37

2-(5-{2-(4'-*t*-butyl-bipheny-4-yl)-3-oxo-3-[(2',4',6'-trimethyl-bipheny-4-yl)amino]-propyl }thiophene-2-carboxamido)ethanesulfonic acid (14m)

Compound **14m** was prepared according to the procedure for the preparation of compound **14c**. mp 165-168^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.39 (t, *J* = 5.5 Hz, 1H), 7.63 (dd, *J* = 8.4, 4.5 Hz, 4H), 7.56 (dd, *J* = 11.7, 8.4 Hz, 4H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 3.7 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.92–6.83 (m, 3H), 4.10 (dd, *J* = 11.8, 6.6 Hz, 1H), 3.72–3.58 (m, 1H), 3.44 (dd, *J* = 14.0, 6.3 Hz, 2H), 3.25 (dd, *J* = 14.6, 5.9 Hz, 1H), 2.66–2.59 (m, 2H), 2.24 (s, 3H), 1.90 (s, 6H), 1.31 (s, 9H). LRMS (ESI, *m/z*): 707.2 [M-H]$^{-}$. HRMS (ESI, *m/z*): calcd for $C_{41}H_{43}N_2O_5S_2^{-}$, 707.2619 [M-H]$^{-}$; found 707.2627, purity: 95.2%.

2.2.38

2-(5-{2-(4'-methyl-bipheny-4-yl)-3-oxo-3-[(2',4',6'-trimethyl-bipheny-4-yl)amino]propyl }thiophene-2-carboxamido)ethanesulfonic acid (14n)

Compound **14n** was prepared according to the procedure for the preparation of compound **14c**. mp 180-182^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.38 (t, *J* = 5.4 Hz, 1H), 7.65–7.59 (m, 4H), 7.54 (dd, *J* = 8.2, 2.6 Hz, 4H), 7.40 (d, *J* = 3.7 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.94–6.86 (m, 3H), 4.08 (d, *J* = 6.2 Hz, 1H), 3.67 (dd, *J* = 14.9, 9.1 Hz, 1H), 3.44 (dd, *J* = 13.9, 6.2 Hz, 2H), 3.27–3.22 (m, 1H), 2.68–2.59 (m, 2H), 2.33 (s, 3H), 2.23 (s, 3H), 1.90 (s, 6H). LRMS (ESI, *m/z*): 665.2 [M-H]$^{-}$. HRMS (ESI, *m/z*): calcd for $C_{38}H_{37}N_2O_5S_2^{-}$, 665.2149 [M-H]$^{-}$; found 665.2155, purity: 96.3%.

2.2.39

2-(5-{2-(4'-methoxy-bipheny-4-yl)-3-oxo-3-[(2',4',6'-trimethyl-bipheny-4-yl)amino]propyl}thiophene-2-carboxamido)ethanesulfonic acid (14o)

Compound **14o** was prepared according to the procedure for the preparation of compound **14c**. mp 179-180^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.36 (s, 1H), 7.61 (dd, *J* = 15.6, 8.2 Hz, 6H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 3.7 Hz, 1H), 7.00 (dd, *J* = 8.6, 2.9 Hz, 4H), 6.92–6.84 (m, 3H), 4.07 (d, *J* = 6.9 Hz, 1H), 3.79 (s, 3H), 3.66 (dd, *J* = 14.4, 9.2 Hz, 1H), 3.44 (dd, *J* = 13.3, 6.4 Hz, 2H), 3.25 (dd, *J* = 14.7, 6.0 Hz, 1H), 2.63 (t, *J* = 7.2 Hz, 2H), 2.24 (s, 3H), 1.90 (s, 6H). LRMS (ESI, *m/z*): 681.3 [M-H]$^{-}$. HRMS (ESI, *m/z*): calcd for $C_{38}H_{37}N_2O_6S_2^-$, 681.2099 [M-H]$^{-}$; found 681.2105, purity: 95.8%.

2.3 Biological evaluation method

2.3.1 Construction of GCGR vector

The wild-type human GCGR was cloned into the EcoRI and HindIII sites of the pcDNA3.1/V5-His-TOPO vector (Invitrogen, Carlsbad, CA, USA). Sequences of receptor clones were confirmed by sequencing with ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

2.3.2 GCGR binding assay

According to the previously reported method, [9] cells were harvested 24 h after transfections with GCGR and incubated with blocking buffer (F12 supplemented with 33 mM HEPES and 0.1% bovine serum albumin (BSA), pH 7.4) for 2 h. Then the binding assay were performed at room temperature for 3 h by incubating the cells with constant concentration of [^{125}I]-glucagon (40 pM, PerkinElmer, Boston, MA, USA) and different concentrations of unlabeled peptides (glucagon, 3.57 pM-1 μM) or compounds (1.28 nM - 100 μM). After cells were washed by ice-cold PBS for three times, they were lysed and counted for radioactivity

(counts per minute, CPM) in a scintillation counter (MicroBeta Plate Counter, PerkinElmer) using a scintillation cocktail (OptiPhase SuperMix, PerkinElmer).

2.3.3 cAMP assays

Based on the manufacturer's instructions and the previously published method, [27] cAMP accumulation was measured using HTRF-cAMP dynamic kit (Cisbio International, Gif sur Yvette Cedex, France). Firstly, HEK293T cells were transfected with GCGR. Then, cells were transferred to 384-well plates at a density of 3,000 cells per well and incubated for a further 24 h at 37^{o}C. After cells were incubated for 30 min in assay buffer (DMEM, 1 mM 3-isobutyl-1-methylxanthine) with different concentration of compounds at 37^{o}C, lysis buffer containing HTRF reagents was added to stop the reactions. Then, plates were incubated for 60 min at room temperature, and time-resolved FRET signals were measured after excitation at 620 nm and 650 nm by EnVision (PerkinElmer).

2.4 Molecular docking studies

Docking modes was generated by the Glide program from Schrödinger Suite. [28] The receptor protein was obtained from the Protein Data Bank (PDB ID: 5EE7), which was reported to bind with MK-0893, and processed by removing solvents and unwanted molecules. [29] The receptor grid was sized to 15 Å in each direction and MK-0893 was selected as the reference. The low energy 3-dementional structures of all compounds were generated by OPLS2001 force field under its default parameters and were refined by LigPrep. [30] Docking studies were carried out using Glide in standard precision mode, with up to 40 conformers saved per molecule. According to assessment of the Glide score, the top scoring conformer for each compound was exported to a Maestro-formatted output file.

3 Results and Discussion

3.1 Chemistry

The synthesis route to thiophene-containing GCGR antagonists (**14a**-**14o**) is shown in Scheme 1. It mainly consisted of three fragments. The first fragment methyl 5-(bromomethyl) thiophene-2-carboxylate **4** was prepared by esterification and bromination of commercially available compound **2**, and esterification of 4-bromophenyl acetic acid **5** afforded the second fragment **6**. Then, benzeneacetyl chloride **8** was generated by alkylation of compound **6** with compound **4** followed by hydrolysis and chloroformylation. Compound **9** experienced a classical Suzuki coupling reaction with various boric acids **10** to provide the third fragment **11**. Suzuki coupling reaction of amide **12** with R_1-$B(OH)_2$, hydrolysis, and condensation gave the target compounds **14a-14o**.

3.2 Biological evaluation

Compounds **14a-14o** were evaluated by GCGR binding and cAMP functional assays *in vitro*, using compound **1** as the positive control. As shown in Table 1, the preliminary structure and activity relationship (SAR) at the hydrophobic pocket was firstly investigated. The effects of regiochemical substitution and electronics on the phenyl ring (R_1) were explored. Compounds **14a** (R_1 = H) and **14b** (R_1 = Br) exhibited similar GCGR binding activities (IC_{50} = 38.4 μM and 39.9 μM, respectively), while 4-Br substituted derivate **14b** demonstrated more potent than **14a** in cAMP functional assay. Then, various substituents on the phenyl group were investigated. Compound **14c** containing 4-methoxyphenyl improved GCGR binding activity (IC_{50} = 6.9 μM), which was similar to the positive control compound **1** (IC_{50} = 6.5 μM). In addition, introducing electron-withdrawing group, compounds **14d-14f** displayed potent GCGR binding affinity and cAMP accumulation effects, with IC_{50} values of 22.9 μM, 16.3 μM, and 6.1 μM for GCGR binding, respectively. Among these derivatives, compound **14f** showed better GCGR binding activity and cAMP activity (IC_{50} = 6.1 μM and 4.4 μM, respectively) than compound **1**. Introducing *tert*-butylphenyl substitute on the phenyl

group, the compound **14h** displayed good GCGR binding affinity (IC_{50} = 4.4 μM) with good cAMP activity (IC_{50} = 13.4 μM). It was shown that the introduction of bulky groups might improve receptor binding affinity with GCGR. To investigate the importance of the R_2 group of these compounds, compounds **14i-14o** were designed and synthesized. Replacement of 2-methyl-4-chlorophenyl group (R_2) in compound **14h** to 3-methyl-4-chlorophenyl and 2,4,6-trimethylphenyl group, compounds **14i-14o** led to sharply reduced GCGR binding affinity and cAMP functional activity.

3.3 Molecular docking of compounds 1, 14f, and 14 h with GCGR

Figure 5 displayed the putative binding modes of compounds **1**, **14f**, and **14h** with GCGR. The binding mode of positive control compound **1** indicates that the *tert*-butylbiphenyl moiety formed hydrophobic interactions with residue Phe345, and the 4-chloro-2-methylbiphenyl moiety formed hydrophobic interactions with residues Pro356, Leu357, and Val360. In addition, 2-aminoethanesulfurous acid moiety occupied in the polar TM6-TM7 cleft, which was consisted of residues Arg346, Lys349, Ser350, and Lys405 (Figure 5A).

Overlay view of compounds **1, 14f**, and **14h** suggests that these three compounds occupied the allosteric site and with similar orientation (Figure 5B). Moreover, the polar and nonpolar moieties of compounds **14f** and **14h** extended into the polar cleft and hydrophobic pocket, respectively.

Compared with the *tert*-butylbiphenyl moiety of compound **1**, the 4-trifluoromethoxybiphenyl moiety of compound **14f** (Figure 5C) and the *t*-butylbiphenyl moiety of compound **14h** (Figure 5D) formed more hydrophobic interactions with residues Leu329 and Leu352, and the thiophene ring formed extra hydrophobic interaction with residue Leu399. Furthermore, within the polar TM6-TM7 cleft, amide group of compounds **14f** and **14h** could form a hydrogen bond with residues Ser350 and Lys349, respectively; the sulfonic acid group formed two hydrogen bonds with residues Arg346 and Lys349, which

were not observed in compound **1**. Clearly, the above distinctive interactions imply that introduction of thiophene ring could be the main reason for the improvement of GCGR binding affinity.

FIGURE 5. (A) Binding mode of compound **1**. **(B)** Superimposition of compounds **14f** (yellow), **14h** (green) and **1** (purple) with glucagon receptor (GCGR). **(C)** Binding mode of compound **14f**. **(D)** Binding mode of compound **14h**. All figures were prepared using PyMol (http://www.pymol.org).

4 CONCLUSIONS

In summary, we discovered a novel series of thiophene-containing derivatives as GCGR antagonists. SAR studies of this series of compounds focused on the two parts of phenyl rings, among them, compounds **14f** and **14h** showed better binding activities than lead compound **1** with IC_{50} values of 6.1 μM and 4.4 μM, respectively. Moreover, the binding modes of these three compounds were built to describe their interaction with GCGR.

Acknowledgments

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (21632008, 81620108027, 21672231, and 21472209), the Major Project of Chinese National Programs for Fundamental Research and Development (2015CB910304), SA-SIBS scholarship program, Shanghai Science and Technology Development Fund (15DZ2291600 and 15QA1404400) and the Thousand Talents Program in China, and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA12040201 and XDA12040202).

Figure legends

FIGURE 1. Structures of several glucagon receptor antagonists.

FIGURE 2. Drugs that contain the thiophene ring.

FIGURE 3. Main metabolic pathways of Rivaroxaban.

FIGURE 4. Molecular design of novel glucagon receptor (GCGR) antagonists.

FIGURE 1S. NMR and HPLC spectrums of compound **14a**

FIGURE 2S. NMR and HPLC spectrums of compound **14b**

FIGURE 3S. NMR and HPLC spectrums of compound **14c**

FIGURE 4S. NMR and HPLC spectrums of compound **14d**

FIGURE 5S. NMR and HPLC spectrums of compound **14e**

FIGURE 6S. NMR and HPLC spectrums of compound **14f**

FIGURE 7S. NMR and HPLC spectrums of compound **14g**

FIGURE 8S. NMR and HPLC spectrums of compound **14h**

FIGURE 9S. NMR and HPLC spectrums of compound **14i**

FIGURE 10S. NMR and HPLC spectrums of compound **14j**

FIGURE 11S. NMR and HPLC spectrums of compound **14k**

FIGURE 12S. NMR and HPLC spectrums of compound **14l**

FIGURE 13S. NMR and HPLC spectrums of compound **14m**

FIGURE 14S. NMR and HPLC spectrums of compound **14n**

FIGURE 15S. NMR and HPLC spectrums of compound **14o**

FIGURE 16S. ^{1}H NMR spectra for intermediates.

Conflict of Interest statement

The authors declare that they have no conflict of interest

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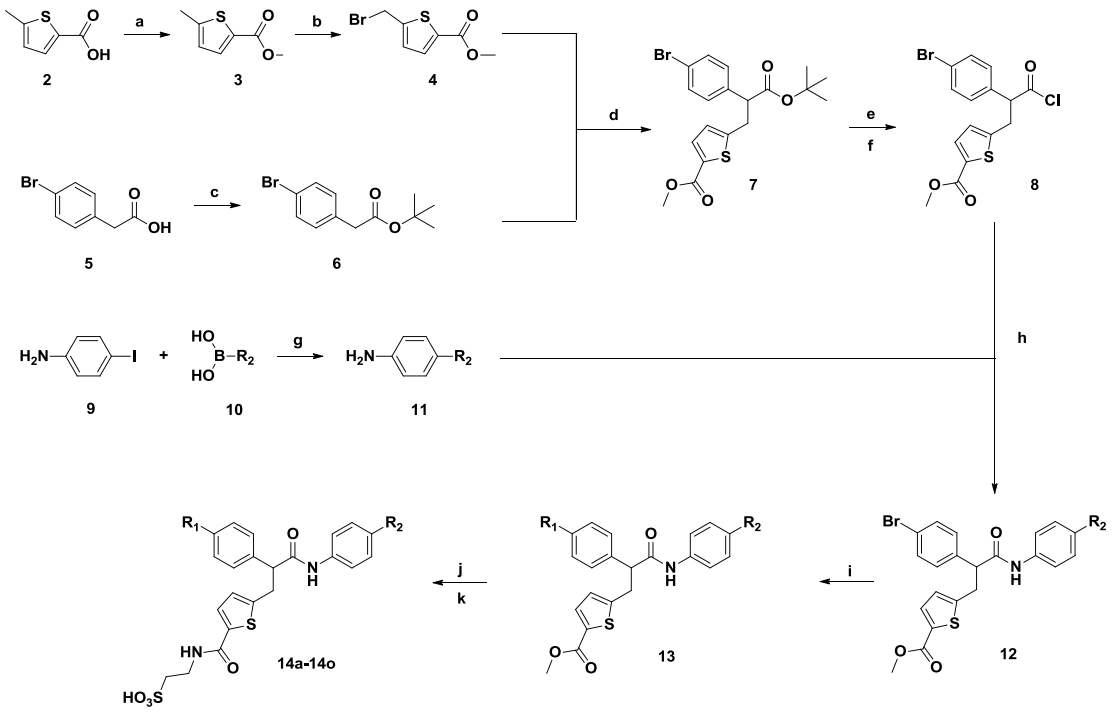
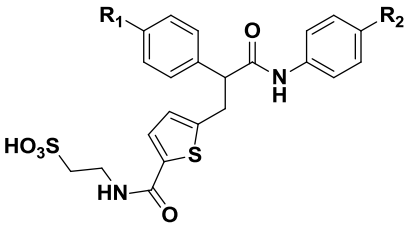
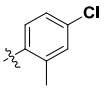
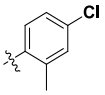
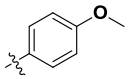
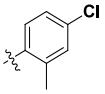
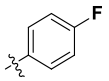
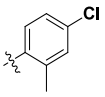
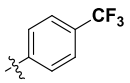
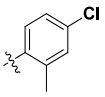
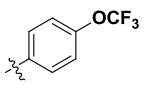
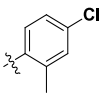
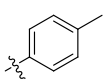
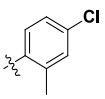
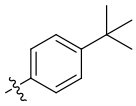
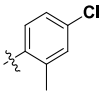
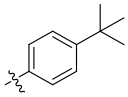
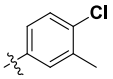
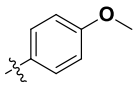
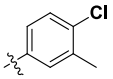
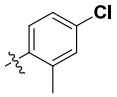
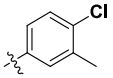
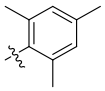
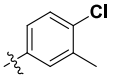
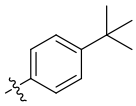
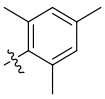
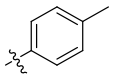
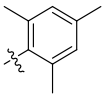
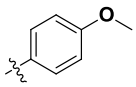
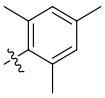


Scheme 1. Reagents and conditions: a) $SOCl_2$, MeOH, reflux; b) NBS, BPO, $CHCl_3$, reflux; c) Boc_2O, DMAP, *t*-BuOH, r.t.; d) *n*-BuLi, THF, -78 ^oC; e) TFA, DCM, r.t.; f) CO_2Cl_2, DCM, r.t.; g) $PdCl_2(P(o\text{-tolyl})_3)_2$, Na_2CO_3, DME/EtOH/H_2O, 125 ^oC; h) DIPEA, DCM, r.t.; i) R_1-$B(OH)_2$, $PdCl_2(P(o\text{-tolyl})_3)_2$, DIPEA, DME, 85$^oC$; j) NaOH, 1,4-dioxane/MeOH/H_2O, r.t.; k) Taurine, HOBt, EDCI, DIPEA, r.t..

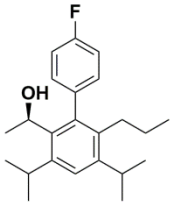
Table 1. *In vitro* glucagon receptor (GCGR) binding and cAMP functional activities of compounds **14a-14o**.



Compd.	R_1	R_2	GCGR Binding[a] IC_{50} (μM)	GCGR cAMP[a] IC_{50} (μM)
14a	**H**		38.4 ± 13.2	NA [b]
14b	**Br**		39.9 ± 8.3	25.8 ± 2.1
14c			6.9 ± 5.6	24.6 ± 3.1
14d			22.9 ± 10.9	28.0 ± 8.4
14e			16.3 ± 6.1	7.1±1.0
14f			6.1 ± 1.8	4.4 ± 0.6
14g			115.7 ± 69.1	13.5 ± 2.2

14h			4.4 ± 1.0	13.4 ± 2.3
14i			31.6 ± 25.8	13.5 ± 3.4
14j			NA [b]	24.7 ± 2.7
14k			15.0 ± 1.3	20.1 ± 0.4
14l			18.1 ± 13.7	16.5 ± 1.2
14m			213.5 ± 207.0	3.5 ± 1.2
14n			25.0 ± 10.6	9.2 ± 0.3
14o			110.3 ± 19.6	40.5 ± 17.5
1 [c]			6.5 ± 1.4	10.9 ± 2.3

[a] Activities are reported as means ± SEM (N ≥ 3). [b] No activity. [c] Possible structure in the patent of WO2010019830A1.



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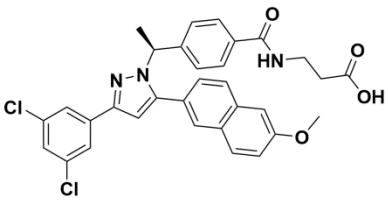

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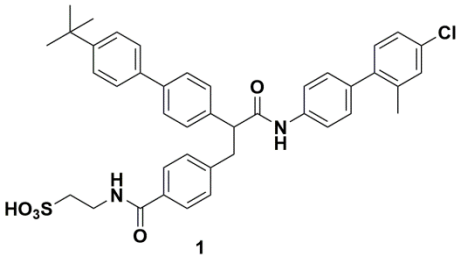

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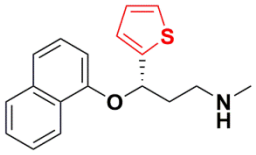
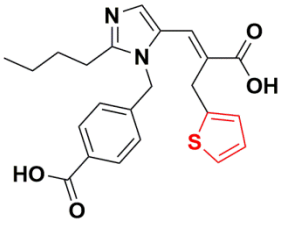
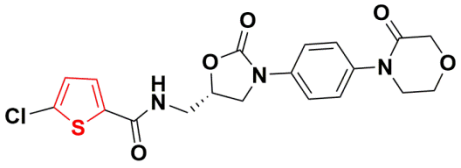
(Ligand)



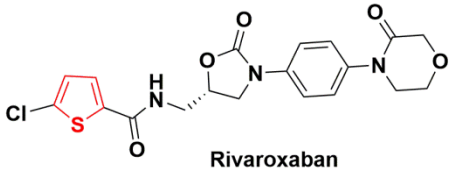
Duloxetine



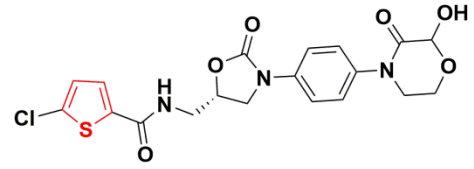
Eprosartan



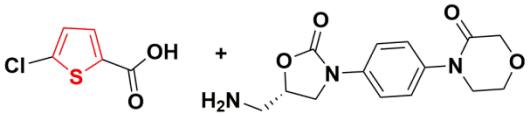
Rivaroxaban



Rivaroxaban



Oxidative degradation of the morpholine group



Hydrolysis of amide bond