Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of novel *N*,*N*-3-phenyl-3-benzylaminopropionanilides as potent inhibitors of cholesteryl ester transfer protein in vivo

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ARTICLE INFO

Article history:
Received 3 February 2016
Revised 1 March 2016
Accepted 2 March 2016
Available online xxxx

Keywords:
CETP inhibitor
N,*N*-3-Phenyl-3-benzylaminopropionanilide derivatives
Microsomal stability
Dose-dependent

ABSTRACT

Epidemiological studies have identified that the risk of cardiovascular events increases due to the decreased levels of high density lipoprotein-cholesterol and the elevated levels of low density lipoprotein-cholesterol. Herein, we report a novel series of *N*,*N*-3-phenyl-3-benzylaminopropionanilide derivatives, which were identified as potent cholesteryl ester transfer protein (CETP) inhibitor. The initial lead compound **L10** (IC_{50} 8.06 μM) was found by pharmacophore-based virtual screening (Dong-Mei Zhao et al., *Chin. Chem. Lett.* **2014**, *25*, 299). After systematic structure variation and biological testing against CETP, two different series were identified as scaffolds for potent CETP inhibitors. One is *N*,*N*-3-phenyl-3-benzylaminopropanamide derivatives, which were investigated in our previous paper (*Bioorg. Med. Chem.* **2015**, doi: http://dx.doi.org/10.1016/j.bmc.2015.12.010). The most potent compound **HL16** in that series has the IC_{50} of 0.69 μM. The other series is *N*,*N*-3-phenyl-3-benzylaminopropionanilide derivatives, which was investigated in current study. Further optimization of the structure–activity relationship (SAR) resulted in **H16** (IC_{50} 0.15 μM), which was discovered as a potent CETP inhibitor in vitro by BODIPY-CE fluorescence assay. In addition, the results of pharmacodynamics studies showed that **H16** exhibited both favorable HDL-C enhancement and LDL-C reduction in vivo by hamster. It also has an excellent stability in rat liver microsomal.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in both developed and developing countries.[1–3] Clinical studies have established an inverse relationship between serum levels of high density-cholesterol (HDL-C) and the incidence of cardiovascular events. Epidemiological studies have identified that for every 1.0 mg/dL increase in HDL-C, there is an approximately reduction of 2–3% in CVD risk.[4–6] Niacin, can robustly elevate HDL-C levels in current available treatments, but lacks of compliance.[7] The statins are used to reduce low density-lipoprotein (LDL-C) levels in patients at risk for CVD in clinical treatment. However, many patients on statins treatment remain at high risk to develop CVD events.[8] Thus, there is a demand for specific HDL-C raising therapies that confer better efficacy with less side effects.

Plasma cholesteryl ester transfer protein (CETP) is the key modulator facilitating the transfer cardioprotective HDL-C into very low density-lipoprotein (VLDL-C) and LDL-C.[9] CETP, secreted mainly from the liver, is a carrier glycoprotein circulating in plasma.[10] CETP has been demonstrated to participate in the movement of transferring cholesterol from peripheral tissue to the liver for catabolism, a process known as reverse cholesterol transport (CRT).[10] There are several suggestions that CETP plays a key proatherogenic role.[10] Inhibition of CETP activities is considered to be one of the most effective mechanisms to elevate the level of HDL-C.[11,12] Four small molecule inhibitors have entered into phase III of clinical trials: (1) torcetrapib, (2) anacetrapib, (3) evacetrapib and (4) dalcetrapib shown in Scheme 1. However, torcetrapib was prematurely terminated due to more mortality in the torcetrapib/atorvastatin group than the atorvastatin group.[13–15] Dalcetrapib showed modest potency, whose clinical trial III was also terminated due to the failure to show a clinically meaningful reduction in cardiovascular (CV) events.[16,17] It was shown that anacetrapib and evacetrapib led to elevated HDL-C and lowered LDL-C while avoiding torcetrapib's side effects.[18–21] TA-8995, a new CETP inhibitor, has successfully completed phase IIb trial in 2014. The clinical II trial data showed that TA-8995 reduced LDL-C levels by 45.3%, whereas increased HDL-C levels by up to 179.1%, and without serious adverse events or signs of liver toxic effects.[22,23] It remains to be seen whether these drug candidates will prevail eventually.

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http://dx.doi.org/10.1016/j.bmc.2016.03.002
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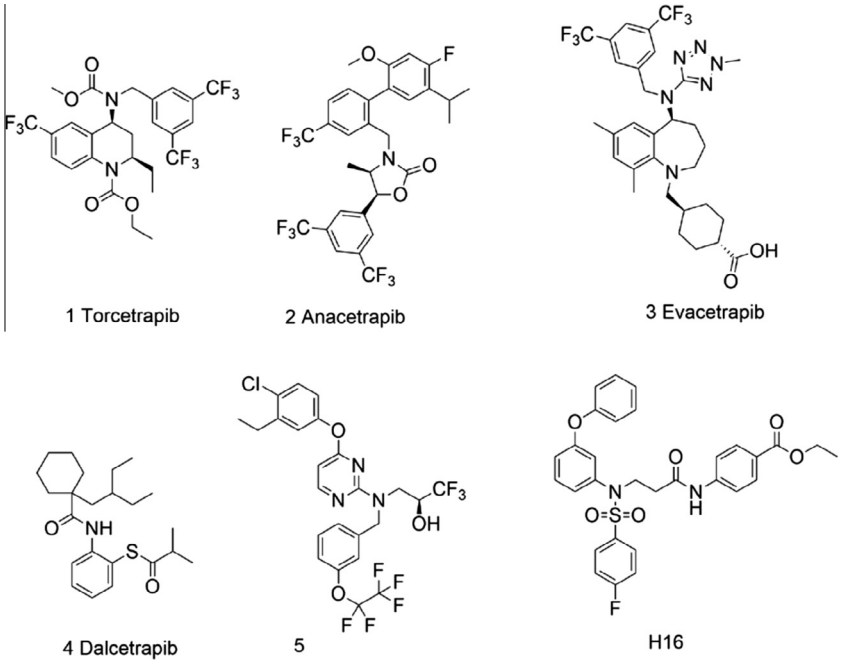


Scheme 1. Structure of CETP inhibitors.

Despite the setbacks from the terminations of torcetrapib and dalcetrapib, great progress has been made in illuminating the inhibition mechanism of CETP. A co-crystal structure of CETP with torcetrapib was published in 2012.[13] It was reported that the holo-CETP structure revealed an exceptionally long hydrophobic tunnel made of the N-terminal and the C-terminal pocket, connected by a narrow neck. Lipids can transfer through this hydrophobic tunnel between HDL and LDL, but small molecule inhibitors within the N-terminal pocket occupy the narrow neck and successfully block lipids transfer.[13] In our previous study, a pharmacophore-based virtual screening campaign was carried out and a lead compound **L10** was identified to show modest inhibitory activity against CETP.[24] In this study, using **L10** as a hit compound, we aimed to identify better lead compounds with more potency and novel structure as small-molecule CETP inhibitor as potential candidates for atherosclerosis.

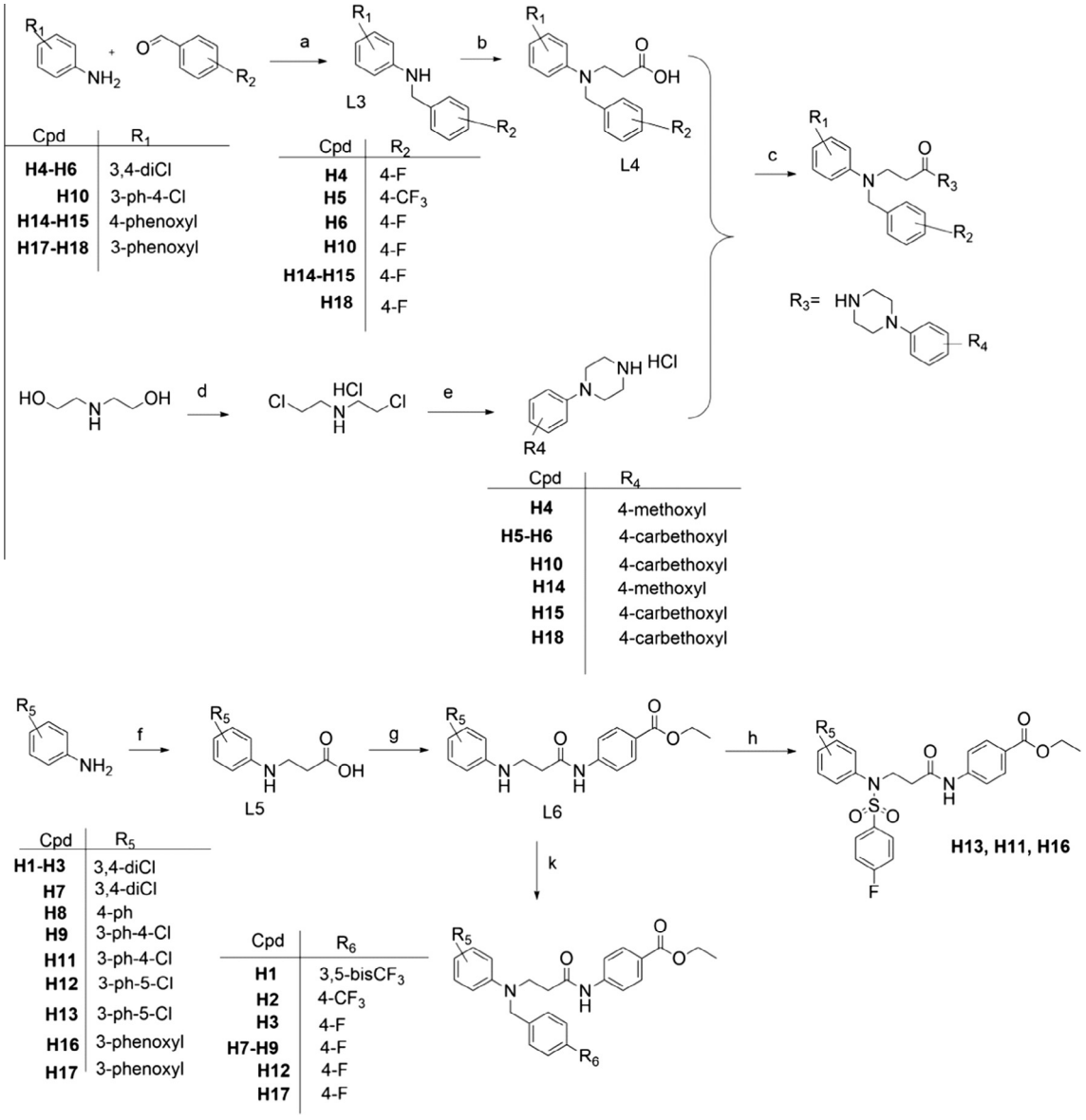
Following the structure–activity relationship (SAR) of compound **L10**, we focused on *N*,*N*-3-phenyl-3-benzylaminopropanamides derivatives. In our earlier work, we have demonstrated that *N*,*N*-3-phenyl-3-benzylaminopropanamides derivatives exhibited CETP inhibition activities.[24] This discovery have been briefly reported in an earlier paper (Dong-mei Zhao et al. doi: http://dx.doi.org/10.1016/j.bmc.2015.12.010).[24] Different from most of reported inhibitors which require a central core ring for activity, the propionamide is a flexible scaffold as an unusually simple class of CETP inhibitors. This novel class thereby represents a flexible family exhibiting inhibitory activity against CETP. We hypothesized that propionamide acted as a link to connect potential pharmacophore. Thus the formation of modified amide terminus, benzyl and aniline moiety (Scheme 2) are important to improve CETP inhibition. Here we report our further efforts to optimize the activity of propionamide series through the modification or replacement of the amide terminus (B moiety), benzyl (C moiety) and aniline moiety (A moiety). As a result, the introduction of 3-phenoxyl into A moiety and 4-fluobenzenesulfonyl into C moiety leads to discovery of **H16** (IC_{50} 0.15 μM), which was manifested as a potent CETP inhibitor in vitro by BODIPY-CE fluorescence assay.

Scheme 2. General structure of the target compounds **L10** derivatives.

2. Results and discussion

2.1. Chemistry

Since the propionamide moiety gives an important contribution to the potency of CETP inhibition, we tried to explore the chemical space based on this core moiety. Chemical diversity around the propionamide scaffold was generated from a highly versatile and straightforward synthesized intermediate that in one final step could be converted in parallel to many desired compounds. Compounds **H1**–**H18** were prepared according to Scheme 3. Overall, they were prepared in 4 or 5 steps. The key intermediate **L3** was prepared by standard reductive amination or alkylation sequences with commercially corresponding aniline and substituted benzaldehyde. The Secondary amine **L3** was treated with acrylic acid through michael reaction at 50 °C for 6 h to generate the key link **L4**. In this reaction, we found the yield of product from the solvent of acrylic acid was higher than some literature reported acetonitrile or toluene as solvent. The next step **L4** was treated with HOBT, EDCI and DIEA in DMF at 0 °C for 2 h, followed by various substituent aliphatic amine or arylpiperazines by condensation reaction to obtain the desired target compounds. In another synthetic route the first step was synthesizing the linker **L5** of the scaffold by commercially corresponding aniline with acrylic acid through michael reaction at 50 °C. The next step **L5** was treated with HOBT, EDCI and DIEA in DMF at 0 °C for 2 h, followed by ethyl



Scheme 3. Synthetic routes. Reagents and conditions: (a) AcOH, Pd/C, H_2; (b) 10% HCl, acrylic acid, 50 °C; (c) substituted amines, HOBt, EDCI, DIEA, RT; (d) DCM, reflux; (e) *n*-BuOH, K_2CO_3, reflux; (f) 10% HCl, acrylic acid, 50 °C; (g) ethyl 4-aminobenzoate, HOBt, EDCI, DIEA, RT; (h) DCM, azabenzene, 4-fluobenzenesulfonyl chloride; (k) DMF, K_2CO_3, substituent benzyl bromide, 90 °C.

4-aminobenzoate by condensation reaction to obtain the key intermediate **L6**. These intermediates were used to in the next step to furnish the desired target compounds. The amide terminus was synthesized by two steps. Thionyl chloride and 2,2′-azanediylbis (ethan-1-ol) were used as starting materials to give bis(2-chloroethyl)amine hydrochloride by chlorination, and then the corresponding aryl piperazines were obtained by cyclization.

2.2. Results and discussion

2.2.1. In vitro activity and structure–activity relationships

In an attempt to evaluate the ability of various *N*,*N*-3-phenyl-3-benzylaminopropionanilide derivatives to inhibit CETP, all these new synthetic compounds and reference compound anacetrapib were initially assayed for their inhibitory effects against CETP by BODIPY-CE fluorescence assay with CETP RP Activity Assay Kit (Catalog # RB-RPAK; Roar). The results of compound assay are shown in Table 1. Most of the target compounds exhibited moderate CETP inhibition activity, while only one compound showed unexpected activity with $IC_{50} > 50$ μM, indicating that the introduction of propionamide scaffold was able to fit the

Table 1
Structure–activity relationship (SAR) of *N*,*N*-3-phenyl-3-benzylaminopropionanilides derivatives **H1**–**H18**

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
H1	5.4	**H10**	2.9
H2	9.5	**H11**	1.3
H3	2.0	**H12**	3.4
H4	4.9	**H13**	2.3
H5	18.2	**H14**	>50
H6	12.4	**H15**	6.7
H7	9.1	**H16**	0.15
H8	16.7	**H17**	0.24
H9	2.7	**H18**	0.69
Anacetrapib[a]			0.06

[a] The positive control.

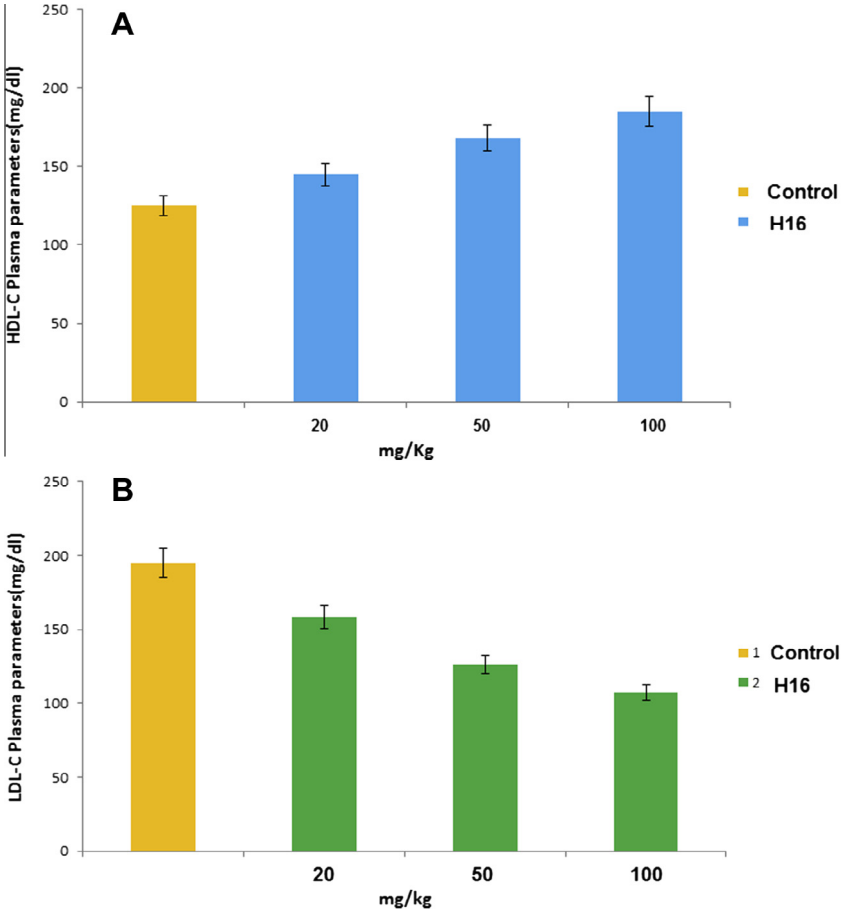


Figure 1. In vivo dose-dependent analysis of hamster treated with **H16** for 4 weeks. (A) HDL-C plasma parameters (mg/dl); (B) LDL-C plasma parameters (mg/dl) (ig). Control: high-fat diet-fed group untreated with **H16**; **H16**: high-fat diet-fed hamster treated with **H16** (20, 50, 100 mg/kg/d). $n = 6$.

moiety into the large pocket of CETP. As summarized below, several key structural changes provided critical insights into the general SAR of this series.

Initial modifications focused on substituted benzyl moiety. The results showed that 4-fluorobenzyl was the most important than 3,5-bis(trifluoromethyl)benzyl and 4-(trifluoromethyl)benzyl to improve CETP inhibition activity. However, while 4-fluorobenzenesulfonyl was introduced to further explore the requirements for activity. Compounds **H11** (IC_{50} 1.3 μM) and **H16** (IC_{50} 0.15 μM) displayed marked improvement in potency compared to **H9** (IC_{50} 2.7 μM) and **H17** (IC_{50} 0.24 μM) respectively. For the modifications of the substituted amide terminus, in order to investigate hydrophilicity and adjust lipophilicity, ethyl 4-(piperazin-1-yl) benzoate, ethyl 4-aminobenzoate and 1-(4-methoxyphenyl)-piperazine were employed. The activity of compounds **H3**, **H4** and **H6** showed that ethyl 4-aminobenzoate seems to be optimal for CETP inhibition. Compound **H3** (IC_{50} 2.0 μM) with ethyl 4-aminobenzoate through hydrolysis reaction changed to 4-aminobenzoic acid was also investigated. But the effectiveness of compound **H7** (IC_{50} 9.1 μM) was diminished. For that reason ethyl 4-aminobenzoate was chosen for further developed structures. Results from the modification of the aniline moiety indicated that increased aniline space volume with 4-chloro-3-biphenyl, 5-chloro-3-biphenyl or 4-biphenyl the potency showed nearly no improvement. However, introduction of flexible 3-phenoxyaniline moiety resulted in 10-fold improvement over 3,4-dichloroaniline (**H17** exhibit IC_{50} 0.24 μM while **H3** IC_{50} 2.0 μM). Compound **H18** (IC_{50} 0.69 μM) with 3-phenoxyaniline moiety was 10-fold potent than compound **H15** (IC_{50} 6.7 μM) with 4-phenoxyaniline moiety. Therefore, it was concluded that 3-phenoxyaniline provides an important contribution to the potency.

2.2.2. In vitro rat liver microsomal stability

Microsomal incubation is an effective enzyme system for metabolism studies. In human P450 metabolism stability was important for drug discovery. Based on its in vitro properties, in order to examine compound **H16** metabolism stability, rat liver microsomal was employed. We chose LC–MS/MS approach to detect **H16** metabolism stability. The compound **H16** was eluted out LC–MS/MS at 17.1 min after quantitative assessment 36% was oxidized or reduced. The result showed that it has an excellent stability in rat liver microsomal.

2.2.3. In vivo dose-dependent study in hamsters

Based on the result of in vitro CETP inhibitory assay, potent inhibitor **H16** was selected for the in vivo assay. Male Golden Syrian hamsters (8 weeks old, weight 120–130 g) were randomly divided into control and treated groups ($n = 6$). Hamsters were placed on high cholesterol and lipid diet for 5 weeks, and dyslipidemia model was induced. After oral administration of **H16** (formulation ethanol/cremophor/saline = 5:5:90) at dose of 20, 50 and 100 mg/kg/day for four weeks, the blood samples were drawn from orbit and the serum lipid levels were measured. The weights of hamsters were also observed during the dosing process and no hamster died. The results indicated that LDL-C was reduced by 19.3%, 36.2%, 45.8% and HDL-C was increased by 16.2%, 34.7%,

48.2%. The results were presented in Figure 1, confirming that **H16** had significant lipid regulating potency, and also exhibits dose-dependency in vivo.

3. Conclusion

In the present study, we report a novel CETP inhibitor and biological evaluation in vitro by BODIPY-CE fluorescence assay. Most of the synthetic compounds showed moderate inhibition activity, fortunately, several representatives of *N*,*N*-3-phenyl-3-benzylaminopropionanilide derivatives were identified to exhibit remarkable potent CETP inhibition. After limited further structure–activity relationship study, compounds substituted with 4-fluorobenzenesulfonyl and 3-phenoxyaniline were found to have potent CETP inhibition. **H16** exhibits an IC_{50} of 0.15 μM in vitro and dose-dependency for lipid regulating activity in vivo by hamster. In addition, **H16** also has an excellent stability in rat liver microsomal. In summary, these results demonstrate that the newly synthetic compounds have the potential to be developed as leads and novel scaffold. It is highly expected that through further structural modifications, the novel scaffold will produce promising CETP inhibition agents.

4. Experimental

4.1. Chemistry

All solvents were purchased from Aladdin (Shanghai China) were used without further purification, all the chemicals were purchased from commercial sources with purity >98%, Melting points (mp) were determined in open capillaries on a Buchi 353 melting point apparatus (Buchi Labortechnik, Flawil, Switzerland) and were uncorrected. The solvents used for reactions that moisture sensitive were distilled and performed under argon atmosphere. The purity and homogeneity of the compounds by chromatography either on a glass column using silica gel (100–200 mesh) assessed by TLC and HPLC chromatography. Mass spectra were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). NMR spectra were recorded at 400 MHz for ^{1}H and 400 MHz for ^{13}C on a Bruker spectrometer with TMS as an internal standard, $CDCl_3$, or DMSO-d_6 as solvent, and coupling constants (*J*) were in hertz (Hz), and the signals were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet.

4.1.1. 3,4-Dichloro-*N*-(4-fluorobenzyl)aniline

To a 250 mL three-necked round bottom flask 3,4-dichloroaniline (5.2 g, 32.1 mmol), 4-fluorobenzaldehyde (4.8 g, 38.5 mmol) and acetic acid (0.5 mL) were dissolved in ethanol 30.0 mL. The solution was heated to 40 °C for 2 h and then Pd/C (0.3 g) was added, with hydrogen reaction 5 h. The reaction mixture was filtered, washed with ethanol, the filtrate was then concentrated in vacuum to give target product (7.5 g, 84.7% yield) as a yellow solid. ESI-MS (*m*/*z*): 270.1[M+H]$^+$.

4.1.2. 3-((3,4-Dichlorophenyl)(4-fluorobenzyl)amino)propanoic acid

In a solution of 3,4-dichloro-*N*-(4-fluorobenzyl)aniline (7.5 g, 27.8 mmol), dissolved acrylic acid (20.0 mL) and 10% hydrochloric acid (0.5 mL) and then the mixture was heated at 50 °C for 6 h, and then cooled to room temperature. The reaction mixture was poured into ice water. The precipitate was filtered, washed with water, and then purified by column chromatography (silica gel) to give target product (7.9 g, 83.1% yield) as a white solid. ESI-MS (*m*/*z*): 242.1[M+H]$^+$.

4.1.3. 4-Substituted-phenylpiperazine (L6)

In a solution of 2,2′-azanediylbis(ethan-1-ol) (11.0 g, 104.7 mmol), dissolved in dry DCM (100.0 mL), thionyl chloride (37.4 g, 314.0 mmol) was added in a slow stream at below 5 °C with ice bath, and then the mixture was heated at reflux for 4.5 h, and then cooled to room temperature. The precipitate was filtered, washed with ethanol, dried in infrared lamp and obtained as a white solid bis(2-chloroethyl)aminehydrochloride, mp: 212–216 °C.

A mixture of bis(2-chloroethyl)aminehydrochloride (7.3 g, 41.0 mmol), K_2CO_3 (5.7 g, 41.3 mmol), dissolved in 1-butanol, the substituted aniline (41.0 mmol) was added. The solution was heated at reflux for 8 h and then cooled to room temperature. The precipitate was filtered, washed with water, dried in infrared lamp and obtained as a white solid used directly in the next step without further purification.

4.1.4. 3-(3,4-Dichlorophenylamino)propanoic acid

A mixture of 3,4-dichloroaniline (4.6 g, 28.4 mmol), acrylic acid (15.0 mL) and 10% hydrochloric acid (0.5 mL) was added to a 250 mL three-necked round bottom flask. The solution was heated to 40 °C for 2 h and then cooled to room temperature.[23] The reaction mixture was poured into ice water. The precipitate was filtered, washed with water, and then purified by column chromatography (silica gel) to give target product (3.5 g, 72.1% yield) as a yellow solid. Mp: 49.5–51.2 °C.

4.1.5. 3-(3,4-Dichlorophenylamino)propanamides

In a solution of 3-((3,4-dichlorophenyl)(4-fluorobenzyl)amino) propanoic acid (0.7 g, 2.0 mmol) dissolved in dry DMF (10.0 mL) 1-hydroxybenzotrizole (HOBt, 0.3 g, 2.0 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 0.4 g, 2.0 mmol) was added. The mixture was stirred at room temperature for 1.5 h, and then the DIEA (0.6 g, 4.0 mmol) and corresponding amine (2.0 mmol) were added. The reaction mixture was stirred at room temperature for 2 h and poured into ice water. The precipitate was filtered, washed with water, dried in infrared lamp and obtained as a white solid, and the resulting residue was purified by column chromatography (10% EtOAc/petroleum ether, silica) or recrystallization (petroleum ether/ethyl acetate) to provide the title compounds as a white to yellow solid.

4.1.6. Ethyl 4-(3-((3,5-bis(trifluoromethyl)benzyl)(3,4-dichlorophenyl)amino)propanamido)benzoate (H1)

Light white solid. Yield: 78%; mp: 108.4–109.2 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.64 (s, 1H), 7.48 (s, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.06 (d, *J* = 9.0 Hz, 1H), 6.64 (d, *J* = 2.9 Hz, 1H), 6.34 (dd, *J* = 9.0, 3.0 Hz, 1H), 4.57 (s, 2H), 4.24 (d, *J* = 7.1 Hz, 2H), 3.78 (s, 2H), 2.65 (s, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.29 (s), 166.30 (s), 146.44 (s), 141.83 (s), 140.79 (s), 133.21 (s), 132.12 (s), 131.90 (s), 130.86 (s), 130.64 (s), 126.35 (s), 125.88 (s), 123.97 (s), 122.16 (s), 121.31 (s), 120.65 (s), 118.79 (s), 113.92 (s), 112.26 (s), 77.20 (s), 76.99 (s), 76.78 (s), 61.00 (s), 54.54 (s), 47.44 (s), 34.93 (s), 26.82 (s), 14.17 (s). HRMS calcd for $C_{27}H_{22}Cl_2F_6N_2O_3Na$, [M+Na]$^+$, 629.0809; found 629.0809.

4.1.7. Ethyl 4-(3-((3,4-dichlorophenyl)(4-(trifluoromethyl) benzyl)amino)propanamido)benzoate (H2)

White solid. Yield: 76%; mp: 121.2–122.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.7 Hz, 2H), 7.55 (dd, *J* = 8.2, 3.2 Hz, 4H), 7.40 (s, 1H), 7.22 (s, 2H), 6.79 (d, *J* = 2.9 Hz, 1H), 6.54–6.51 (m, 1H), 4.65 (s, 2H), 4.40–4.35 (m, 2H), 3.89 (t, *J* = 6.4 Hz, 2H), 2.72 (d, *J* = 6.4 Hz, 2H), 1.41 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.59 (s), 164.54 (s), 161.41 (s), 142.15 (s), 136.97 (s), 128.46 (s), 126.04 (t, *J* = 5.3 Hz), 121.90 (s), 120.99 (d, *J* = 3.8 Hz), 115.46

(s), 114.10 (s), 109.11 (s), 108.32 (s), 107.55 (s), 72.62 (s), 72.31 (s), 71.99 (s), 50.05 (s), 42.80 (s), 34.00 (s), 29.76 (s), 25.19–24.74 (m), 24.18 (s), 9.58 (s). HRMS calcd for $C_{26}H_{23}Cl_2F_3N_2O_3Na$, $[M+Na]^+$, 561.0935; found 561.0936.

4.1.8. Ethyl 4-(3-((3,4-dichlorophenyl)(4-fluorobenzyl)amino) propanamido)benzoate (H3)

White solid. Yield: 78%; mp: 104.2–104.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 9.0 Hz, 1H), 7.22 (dd, J = 8.6, 5.6 Hz, 2H), 7.12 (t, J = 8.8 Hz, 2H), 6.89 (d, J = 2.9 Hz, 1H), 6.68 (dd, J = 9.1, 3.0 Hz, 1H), 4.59 (s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 3.76 (d, J = 6.8 Hz, 2H), 2.70 (t, J = 6.8 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.02 (s), 141.51 (s), 133.24 (s), 130.83 (s), 128.15 (d, J = 7.9 Hz), 118.81 (s), 115.80 (s), 115.59 (s), 114.37 (s), 112.74 (s), 77.27 (d, J = 11.6 Hz), 77.01 (s), 76.69 (s), 60.94 (s), 54.63 (s), 47.35 (s), 35.30 (s), 14.33 (s). HRMS calcd for $C_{25}H_{23}Cl_2FN_2O_3Na$, $[M+Na]^+$, 511.0967; found 511.0968.

4.1.9. 3-((3,4-Dichlorophenyl)(4-fluorobenzyl)amino)-1-(4-(4-methoxyphenyl)piperazin-1-yl)propan-1-one (H4)

White solid. Yield: 80%; mp: 115.5–116.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 9.0 Hz, 1H), 7.15 (dd, J = 8.5, 5.4 Hz, 2H), 7.01 (t, J = 8.6 Hz, 2H), 6.87 (t, J = 8.8 Hz, 4H), 6.77 (d, J = 3.0 Hz, 1H), 6.53 (dd, J = 9.0, 3.0 Hz, 1H), 4.54 (s, 2H), 3.82 (d, J = 6.7 Hz, 2H), 3.79 (s, 5H), 3.55 (s, 2H), 3.02 (dd, J = 11.2, 4.9 Hz, 4H), 2.66 (t, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.33 (s), 147.27 (s), 133.21 (d, J = 10.0 Hz), 130.76 (s), 128.00 (d, J = 8.0 Hz), 119.59 (s), 119.13 (s), 115.77 (s), 115.56 (s), 114.62 (s), 113.66 (s), 112.04 (s), 77.28 (d, J = 11.5 Hz), 77.02 (s), 76.70 (s), 55.57 (s), 54.05 (s), 47.32 (s), 30.24 (s). HRMS calcd for $C_{27}H_{28}Cl_2FN_3O_2Na$, $[M+Na]^+$, 538.1440; found 538.1440.

4.1.10. Ethyl 4-(4-(3-((3,4-dichlorophenyl)(4-(trifluoromethyl) benzyl)amino)propanoyl)piperazin-1-yl)benzoate (H5)

White solid. Yield: 73%; mp: 121.2–122.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (s, 1H), 7.38 (dd, J = 13.9, 7.7 Hz, 2H), 7.34–7.28 (m, 5H), 7.23 (d, J = 7.8 Hz, 1H), 6.86 (d, J = 2.6 Hz, 1H), 6.64 (dd, J = 9.1, 2.8 Hz, 1H), 4.65 (s, 2H), 4.29 (d, J = 7.1 Hz, 2H), 3.62–3.54 (m, 4H), 3.14 (d, J = 4.3 Hz, 4H), 2.71 (t, J = 6.9 Hz, 2H), 1.31 (d, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.27 (s), 166.40 (s), 153.41 (s), 146.91 (s), 141.95 (s), 131.16 (s), 130.78 (s), 126.58 (s), 125.68 (d, J = 3.6 Hz), 121.10 (s), 114.04 (s), 113.47 (s), 111.87 (s), 77.17 (s), 76.96 (s), 76.75 (s), 60.43 (s), 54.39 (s), 47.57 (d, J = 26.5 Hz), 47.32 (s), 47.28–47.17 (m), 44.91 (s), 41.12 (s), 30.27 (s), 14.34 (s). HRMS calcd for $C_{30}H_{30}Cl_2F_3N_3O_3Na$, $[M+Na]^+$, 630.1514; found 630.1514.

4.1.11. Ethyl 4-(4-(3-((3,4-dichlorophenyl)(4-fluorobenzyl) amino)propanoyl)piperazin-1-yl)benzoate (H6)

White solid. Yield: 76%; mp: 99.7–100.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.9 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.30 (s, 2H), 7.22 (d, J = 9.0 Hz, 1H), 6.87 (d, J = 9.0 Hz, 2H), 6.76 (d, J = 3.0 Hz, 1H), 6.50 (dd, J = 9.0, 3.0 Hz, 1H), 4.66 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.85 (d, J = 6.9 Hz, 2H), 3.78 (d, J = 5.3 Hz, 2H), 3.58 (d, J = 5.3 Hz, 2H), 3.32 (d, J = 4.6 Hz, 4H), 2.70 (t, J = 6.8 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.47 (s), 166.48 (s), 153.50 (s), 147.19 (s), 133.18 (s), 131.25 (s), 130.78 (s), 128.01 (d, J = 8.0 Hz), 121.20 (s), 119.68 (s), 115.78 (s), 115.57 (s), 114.12 (s), 113.68 (s), 112.07 (s), 77.34 (s), 77.03 (s), 76.71 (s), 60.50 (s), 54.12 (s), 47.68 (d, J = 17.6 Hz), 47.25 (s), 45.01 (s), 41.19 (s), 30.26 (s), 29.70 (s), 14.42 (s). HRMS calcd for $C_{29}H_{30}Cl_2FN_3O_3Na$, $[M+Na]^+$, 580.1546; found 580.1546.

4.1.12. 4-(3-((3,4-Dichlorophenyl)(4-fluorobenzyl)amino) propanamido)benzoic acid (H7)

White solid. Yield: 68%; mp: 109.6–110.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 7.87 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 9.0 Hz, 1H), 7.21 (d, J = 5.3 Hz, 2H), 7.14 (d, J = 8.8 Hz, 3H), 6.89 (d, J = 2.9 Hz, 1H), 6.68 (dd, J = 9.1, 3.0 Hz, 1H), 4.59 (s, 2H), 3.77 (t, J = 6.7 Hz, 2H), 2.69 (t, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.52 (s), 167.38 (s), 162.80 (s), 147.99 (s), 143.51 (s), 132.02 (d, J = 7.4 Hz), 130.93 (d, J = 17.9 Hz), 129.13 (s), 128.73 (d, J = 8.1 Hz), 118.81 (s), 117.62 (s), 115.78 (d, J = 21.4 Hz), 113.74 (s), 113.09 (s), 65.49 (s), 53.27 (s), 47.63 (s), 40.60 (s), 40.39 (s), 40.18 (s), 39.97 (s), 39.76 (s), 39.59 (s), 39.45 (d, J = 21.0 Hz), 34.88 (s), 30.47 (s), 19.12 (s), 14.01 (s). HRMS calcd for $C_{23}H_{19}Cl_2FN_2O_3Na$, $[M+Na]^+$, 483.0654; found 483.0653.

4.1.13. Ethyl 4-(3-([1,1′-biphenyl]-4-yl(4-fluorobenzyl)amino) propanamido)benzoate (H8)

White solid. Yield: 73%; mp: 143.2–143.8 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, J = 8.4 Hz, 1H), 7.59 (s, 1H), 7.58–7.50 (m, 6H), 7.41 (d, J = 7.7 Hz, 2H), 7.30 (s, 1H), 7.21 (s, 2H), 6.99 (t, J = 8.6 Hz, 2H), 6.83 (d, J = 49.2 Hz, 2H), 4.59 (s, 2H), 4.38 (q, J = 7.1 Hz, 2H), 3.90 (s, 2H), 2.74 (s, 2H), 1.40 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.08 (s), 141.75 (s), 130.81 (s), 129.03–127.46 (m), 127.88–127.46 (m), 126.35 (s), 118.79 (s), 115.56 (d, J = 21.5 Hz), 115.39–115.22 (m), 77.29 (d, J = 11.5 Hz), 77.03 (s), 76.72 (s), 60.91 (s), 35.41 (s), 31.94 (s), 29.72 (s), 22.71 (s), 21.27 (s), 20.50 (s), 14.36 (s). HRMS calcd for $C_{31}H_{28}FN_2O_3Na$, $[M+Na]^+$, 519.2059; found 519.2060.

4.1.14. Ethyl 4-(3-((6-chloro-[1,1′-biphenyl]-3-yl)(4-fluorobenzyl)amino)propanamido)benzoate (H9)

White solid. Yield: 82%; mp: 104.6–105.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 6.7 Hz, 2H), 7.35 (dd, J = 7.4, 5.8 Hz, 3H), 7.29–7.21 (m, 3H), 7.12 (t, J = 8.8 Hz, 2H), 6.73–6.68 (m, 1H), 6.66 (d, J = 3.0 Hz, 1H), 4.60 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 3.80 (t, J = 6.7 Hz, 2H), 2.71 (t, J = 6.7 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.74 (s), 165.78 (s), 162.76 (s), 160.35 (s), 147.10 (s), 143.83 (s), 140.51 (s), 139.98 (s), 130.67 (s), 129.62 (s), 128.98–128.51 (m), 128.51–128.39 (m), 127.95 (s), 124.60 (s), 118.89 (s), 118.53 (s), 115.81 (s), 115.60 (s), 115.23 (s), 113.45 (s), 60.87 (s), 53.43 (s), 47.68 (s), 40.61 (s), 40.40 (s), 40.19 (s), 39.98 (s), 39.77 (s), 39.57 (s), 39.36 (s), 35.11 (s), 14.68 (s). HRMS calcd for $C_{31}H_{28}ClFN_2O_3Na$, $[M+Na]^+$, 553.1670; found 553.1670.

4.1.15. Ethyl 4-(4-(3-((6-chloro-[1,1′-biphenyl]-3-yl)(4-fluorobenzyl)amino)propanoyl)piperazin-1-yl)benzoate (H10)

White solid. Yield: 67%; mp: 98.5–99.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 15.8 Hz, 4H), 7.24 (s, 3H), 7.18–7.06 (m, 3H), 6.95 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.6 Hz, 1H), 6.62 (s, 1H), 4.60 (s, 2H), 4.24 (d, J = 6.9 Hz, 2H), 3.72 (s, 2H), 3.56 (s, 4H), 3.28 (s, 4H), 2.71 (s, 2H), 1.28 (d, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.87 (s), 166.01 (s), 153.87 (s), 147.06 (s), 140.43 (s), 139.94 (s), 135.09 (s), 131.06 (s), 129.51 (s), 128.71 (d, J = 8.0 Hz), 128.42 (s), 119.07 (s), 115.71 (s), 115.57 (s), 114.94 (s), 113.80 (s), 113.13 (s), 60.28 (s), 52.99 (s), 47.42 (s), 44.66 (s), 40.18 (s), 40.04 (s), 39.90 (s), 39.77 (s), 39.63 (s), 30.34 (s), 14.68 (s). HRMS calcd for $C_{35}H_{35}ClFN_3O_3Na$, $[M+Na]^+$, 622.2248; found 622.2247.

4.1.16. Ethyl 4-(3-((*N*-(6-chloro-[1,1′-biphenyl]-3-yl)-4-fluorophenyl)sulfonamido)propanamido)benzoate (H11)

White solid. Yield: 78%; mp: 138.6–139.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.7 Hz, 2H), 7.74 (s, 1H), 7.70–7.66 (m, 2H),

7.57 (d, J = 8.6 Hz, 2H), 7.43 (s, 1H), 7.42–7.39 (m, 3H), 7.30 (s, 1H), 7.21 (s, 2H), 7.03 (d, J = 2.6 Hz, 1H), 6.96 (dd, J = 8.5, 2.6 Hz, 1H), 4.38 (d, J = 7.1 Hz, 2H), 3.94 (t, J = 6.8 Hz, 2H), 2.73 (t, J = 6.8 Hz, 2H), 1.40 (d, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.44 (s), 166.06 (s), 141.49 (s), 137.87 (s), 137.57 (s), 132.66 (s), 131.42 (s), 130.74 (d, J = 12.6 Hz), 130.41 (d, J = 9.4 Hz), 129.10 (s), 128.13 (dd, J = 17.1, 13.9 Hz), 118.80 (s), 116.69–116.55 (m), 116.37 (d, J = 22.5 Hz), 60.86 (s), 47.58 (s), 37.33 (s), 29.62 (s), 14.27 (s). HRMS calcd for $C_{30}H_{25}ClFN_2O_5SNa$, $[M+Na]^+$, 603.1132; found 603.1133.

4.1.17. Ethyl 4-(3-((5-chloro-[1,1′-biphenyl]-3-yl)(4-fluorobenzyl)amino)propanamido)benzoate (H12)

White solid. Yield: 75%; mp: 146.8–147.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 7.49–7.35 (m, 6H), 7.21–7.16 (m, 2H), 6.99 (dd, J = 14.8, 6.1 Hz, 3H), 6.76 (d, J = 29.4 Hz, 2H), 4.60 (s, 2H), 4.37 (d, J = 7.1 Hz, 2H), 3.91 (t, J = 6.5 Hz, 2H), 2.74 (t, J = 6.4 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.70 (s), 166.21 (s), 163.18 (s), 160.74 (s), 148.95 (s), 143.89 (s), 140.45 (s), 135.68 (s), 130.77 (s), 128.84 (s), 128.15 (d, J = 8.0 Hz), 127.87 (s), 127.09 (s), 118.87 (s), 115.63 (d, J = 21.5 Hz), 115.47–115.07 (m), 111.44 (s), 110.03 (s), 77.41 (s), 77.09 (s), 76.77 (s), 60.98 (s), 54.44 (s), 47.24 (s), 35.35 (s), 14.36 (s). HRMS calcd for $C_{31}H_{27}ClFN_2O_3Na$, $[M+Na]^+$, 553.1670; found 553.1670.

4.1.18. Ethyl 4-(3-((*N*-(5-chloro-[1,1′-biphenyl]-3-yl)-4-fluorophenyl)sulfonamido)propanamido)benzoate (H13)

White solid. Yield: 72%; mp: 143.5–144.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.7 Hz, 2H), 7.70 (dd, J = 8.8, 4.9 Hz, 3H), 7.57 (d, J = 8.6 Hz, 2H), 7.53 (t, J = 1.6 Hz, 1H), 7.45–7.36 (m, 5H), 7.22 (t, J = 8.5 Hz, 2H), 7.16 (t, J = 1.6 Hz, 1H), 6.99 (t, J = 1.8 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 3.97 (t, J = 6.9 Hz, 2H), 2.74 (t, J = 6.9 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.63 (s), 166.77 (s), 166.20 (s), 164.22 (s), 143.80 (s), 141.82 (s), 140.52 (s), 138.28 (s), 135.04 (s), 133.15 (s), 130.90–130.29 (m), 129.03 (s), 128.49 (s), 127.52–127.37 (m), 127.37–126.83 (m), 125.93 (d, J = 12.8 Hz), 118.89 (s), 116.60 (s), 116.38 (s), 77.42 (s), 77.10 (s), 76.79 (s), 60.94 (s), 53.50 (s), 47.67 (s), 37.29 (s), 29.72 (s), 14.36 (s). HRMS calcd for $C_{30}H_{25}ClFN_2O_5SNa$, $[M+Na]^+$, 603.1132; found 603.1133.

4.1.19. 3-((4-Fluorobenzyl)(4-phenoxyphenyl)amino)-1-(4-(4-methoxyphenyl)piperazin-1-yl)propan-1-one (H14)

White solid. Yield: 78%; mp: 155.4–156.2 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, J = 9.0 Hz, 2H), 7.29 (m, 2H), 7.12 (dd, J = 8.5, 5.6 Hz, 2H), 7.10 (dd, J = 16.0, 8.5 Hz, 3H), 6.96 (t, J = 7.4 Hz, 2H), 6.95 (d, J = 9.1 Hz, 2H), 6.91–6.86 (m, 2H), 6.48 (dd, J = 8.4, 2.3 Hz, 1H), 6.16 (dd, J = 7.9, 2.0 Hz, 1H), 4.53 (s, 2H), 3.83 (d, J = 7.1 Hz, 2H), 3.79 (s, 3H), 3.55 (m, 2H), 3.03 (m, 2H), 2.68 (d, J = 7.0 Hz, 4H), 2.64 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) d 169.87, 166.01, 160.66, 153.87, 147.07, 140.43, 139.94, 131.06, 129.51, 128.43, 119.06, 118.22, 115.66, 115.57, 114.94, 113.80, 113.15, 60.28, 52.99, 47.44, 46.69, 44.66, 30.35. ESI-MS (m/z):540.2[M+H]$^+$. HRMS calcd for $C_{33}H_{34}FN_3O_3Na$, $[M+Na]^+$,562.2482; found 562.2481.

4.1.20. Ethyl 4-(4-(3-((4-fluorobenzyl)(4-phenoxyphenyl)amino)-propanoyl)piperazin-1-yl)benzoate (H15)

White solid. Yield: 74%; mp: 162.2–162.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 9.0 Hz, 2H), 7.27 (dt, J = 8.2, 6.6 Hz, 4H), 7.14 (d, J = 8.8 Hz, 2H), 6.98 (dd, J = 11.2, 8.3 Hz, 3H), 6.86 (t, J = 9.3 Hz, 4H), 6.71 (d, J = 9.1 Hz, 2H), 4.55 (s, 2H), 4.23 (d, J = 7.1 Hz, 2H), 3.68 (s, 2H), 3.57 (s, 4H), 3.30 (d, J = 4.4 Hz, 4H), 2.70 (t, J = 7.0 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.94 (s), 157.10 (s), 131.07 (s), 130.15 (s), 128.64 (d, J = 8.0 Hz), 123.34 (s), 118.66 (s), 115.66 (s), 115.52 (s), 113.79 (s), 107.83 (s), 106.27 (s), 103.12 (s), 60.28 (s), 53.13 (s), 47.52 (s), 44.65 (s), 40.04 (s), 39.90 (s), 39.76 (s), 30.39 (s), 14.68 (s). HRMS calcd for $C_{35}H_{36}FN_3O_4Na$, $[M+Na]^+$, 604.2588; found 604.2587.

4.1.21. Ethyl 4-(3-((4-fluoro-*N*-(3-phenoxyphenyl)phenyl)sulfonamido)propanamido)benzoate (H16)

White solid. Yield: 72%; mp: 143.7–144.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.6 Hz, 2H), 7.78 (s, 1H), 7.71–7.38 (m, 4H), 7.34 (t, J = 7.9 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.16 (dd, J = 11.8, 5.1 Hz, 3H), 6.94 (d, J = 7.9 Hz, 2H), 6.81 (d, J = 7.5 Hz, 1H), 6.63 (s, 1H), 4.38 (q, J = 7.1 Hz, 2H), 3.91 (t, J = 6.8 Hz, 2H), 2.69 (t, J = 6.8 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.15 (s), 158.13 (s), 156.05 (s), 141.72 (s), 140.09 (s), 130.78 (s), 129.92 (s), 124.06 (s), 123.24 (s), 119.28 (s), 118.60 (s), 118.34 (s), 116.48 (s), 116.25 (s), 77.37 (s), 77.05 (s), 76.73 (s), 47.47 (s), 37.15 (s), 29.72 (s), 14.36 (s). HRMS calcd for $C_{30}H_{27}FN_2O_6SNa$, $[M+Na]^+$, 585.1472; found 585.1471.

4.1.22. Ethyl 4-(3-((4-fluorobenzyl)(3-phenoxyphenyl)amino)propanamido)benzoate (H17)

White solid. Yield: 76%; mp: 133.2–133.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.4 Hz, 3H), 7.34–7.29 (m, 2H), 7.21–7.07 (m, 4H), 7.02–6.92 (m, 4H), 6.55 (s, 1H), 6.43 (s, 2H), 4.51 (s, 2H), 4.41–4.35 (m, 2H), 3.83 (t, J = 6.5 Hz, 2H), 2.70 (s, 2H), 1.41 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.16 (s), 163.14 (s), 160.70 (s), 158.57 (s), 156.91 (s), 141.85 (s), 130.79 (s), 129.68 (s), 123.24 (s), 118.87 (d, J = 9.2 Hz), 115.60 (s), 115.39 (s), 77.38 (s), 77.06 (s), 76.74 (s), 65.64 (s), 60.94 (s), 54.83 (s), 47.50 (s), 29.73 (s), 14.36 (s). HRMS calcd for $C_{31}H_{29}FN_2O_4Na$, $[M+Na]^+$, 535.2001; found 535.2001.

4.1.23. Ethyl 4-(4-(3-((4-fluorobenzyl)(3-phenoxyphenyl)amino)propanoyl)piperazin-1-yl)benzoate (H18)

White solid. Yield: 75%; mp: 152.7–153.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.29 (d, J = 9.0 Hz, 2H), 7.28–7.26 (m, 2H), 7.13 (dd, J = 8.5, 5.6 Hz, 2H), 7.11 (dd, J = 16.0, 8.5 Hz, 3H), 7.06 (t, J = 7.4 Hz, 1H), 6.95 (d, J = 9.1 Hz, 2H), 6.91–6.86 (m, 2H), 6.47 (dd, J = 8.4, 2.3 Hz, 1H), 6.27 (t, J = 2.3 Hz, 1H), 6.17 (dd, J = 7.9, 2.0 Hz, 1H), 4.53 (s, 2H), 4.23 (d, J = 7.1 Hz, 2H), 3.67 (t, J = 7.0 Hz, 2H), 3.60–3.49 (m, 4H), 3.32 (s, 4H), 2.68 (d, J = 7.0 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) d 169.85, 166.01, 162.24, 160.64, 157.94, 157.10, 153.87, 149.67, 135.24, 131.07, 130.68, 130.15, 128.67, 128.61, 123.34, 119.06, 118.66, 115.66, 115.52, 113.79, 107.83, 106.27, 103.12, 60.28, 40.32, 40.18, 40.04, 39.90, 39.76, 39.62, 39.48, 30.39, 14.68. .ESI-MS (m/z): 582.2[M+H]$^+$. HRMS calcd for $C_{35}H_{36}FN_3O_4Na$, $[M+Na]^+$, 604.2588; found 604.2687.

4.2. Biological evaluation

4.2.1. In vitro CETP inhibitory assay

The CETP RP Activity Assay Kit (Catalog # RB-RPAK; Roar) uses a donor molecule containing a fluorescent self-quenched neutral lipid, that is, transferred to an acceptor by CETP (Catalog # R8899; Roar). CETP-mediated transfer of the fluorescence neutral lipid to the acceptor molecule results in an increase in fluorescence (ExEm = 465/535 nm). Inhibitor of CETP will inhibit the lipid transfer and therefore decrease the fluorescence intensity. The testing compounds are dissolved using 100% DMSO. It is important to make sure that each compound is dissolved totally. It is recommended to vibrate the solution on oscillator for more than 30 s then store them at nitrogen cabinet. Dilute the stocking

compounds (10 mM) with DMSO for 8 points titration (1:5 serial dilutions) in 96-well dilution plate. The assay was performed according to the instruction for the CETP inhibitor screening kit and recombinant CETP.

4.2.2. In vitro rat liver microsomal stability

Rat liver microsomes were prepared from KM rats (200–220 g) by differential centrifugation according to the method reported by our laboratory.[25] Microsomes (final concentration: 2.0 mg protein/mL) were mixed with compound **H16** at concentrations of 100 μM in 0.1 M potassium phosphate buffer (pH 7.4) to a total volume of 5.0 mL. The reactions were initiated by adding NADPH (1.0 mM). In order to investigate P450 metabolism the control groups excluded NADPH. Each incubation were performed in thrice. The mixtures were incubated at 37 °C for 60 min with gentle shaking, 10 point times were detected and then the reactions were quenched by mixing with two volumes of ice-cold acetonitrile. The resulting mixtures were vortex-mixed and centrifuged at 16,000 rpm for 10 min. The precipitated protein was washed with cold 0.05 M Tris–HCl (pH 7.4) for three times then reconstituted with 4.0 mL of water for LC–MS/MS analysis.

Acknowledgements

The work was supported by 81373324 and grants (J1103606 and J1210029) of the National Natural Science Foundation of China and the program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University.

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