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ORIGINAL ARTICLE

Synthesis and biological evaluation of novel SIPI-7623 derivatives as farnesoid X receptor (FXR) antagonists

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Abstract

Most of reported steroidal FXR antagonists are restricted due to low potency. We described the design and synthesis of novel nonsteroidal scaffold **SIPI-7623** derivatives as FXR antagonists. The most potent compound **A-11** (IC_{50} = 7.8 ± 1.1 μM) showed better activity compared to **SIPI-7623** (IC_{50} = 40.8 ± 1.7 μM) and guggulsterone (IC_{50} = 45.9 ± 1.1 μM). Docking of **A-11** in FXR's ligand-binding domain was also studied.

Keywords FXR antagonists · **SIPI-7623** · Guggulsterone · Structure activity relationship · Molecular docking

Abbreviations

FXR	Farnesoid X receptor
LBD	Ligand-binding domain
GS	Guggulsterone
TC	Total cholesterol
TG	Triacylglycerol
LDL-C	Low-density lipoprotein cholesterol

Introduction

Farnesoid X receptor (FXR), a ligand-activated transcription factor, is highly expressed in liver, intestine, kidney and adrenal glands [1]. It contains a ligand-binding domain (LBD) in the C-terminal, a DNA-binding domain in the N-terminal and a connection hinge region. After binding to diverse ligands, the LBD controls the release of corepressor proteins and the subsequent recruitment of coactivator proteins [2].

Intensive research on finding FXR antagonists has yielded some low potency steroidal compounds, such as guggulsterone (**GS**) [3], sulfated polyhydroxysterols, 16-dehydro-pregnenolone (Fig. 1). The first reported FXR antagonist **GS** displayed high affinity to progesterone receptor, androgen receptor and corticoid receptor [4, 5]. Newly discovered **12u** and **FXR-99** were identified as nonsteroidal FXR antagonists with IC_{50} values of 8.96 and 3.4 μM, respectively, which are useful tools for the study on FXR antagonists [6–9] (Fig. 1).

Our group found in a previous study that nonsteroidal compound **SIPI-7623** was able to decrease total cholesterol (TC) and triacylglycerol (TG) levels, and increase low-density lipoprotein cholesterol (LDL-C) level in hypercholesterolemic rats. We found that **SIPI-7623** is an FXR antagonist with an IC_{50} of 57.7 ± 1.9 μM via TR-FRET screening. In a dual luciferase assay, **SIPI-7623** exhibited FXR selectivity toward PPARα (IC_{50} = 44.7 ± 0.8 μM for FXR, IC_{50} > 100 μM for PPARα, EC_{50} > 100 μM for FXR, EC_{50} > 100 μM for PPARα. Its derivatives with

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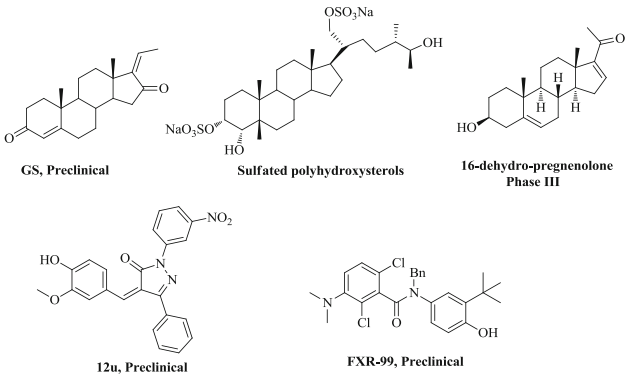


Fig. 1 Example of reported FXR antagonists

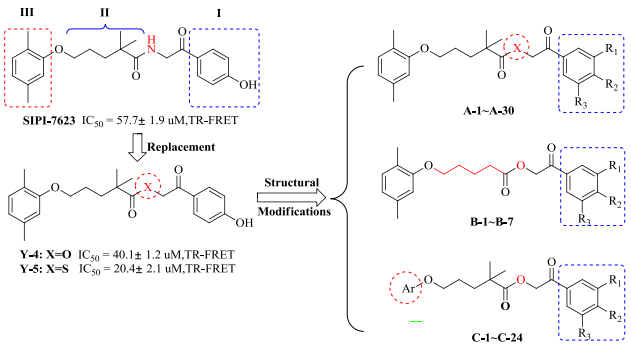


Fig. 2 Design of novel **SIPI-7623** derivatives

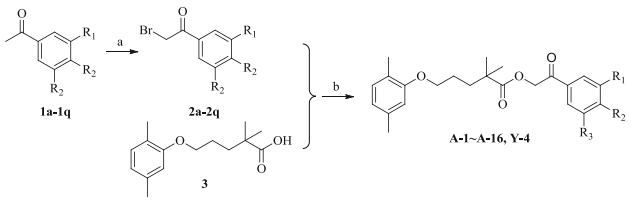
ester linker (**Y-4**, IC_{50} = 40.1 ± 1.2 μM) and thioester linker (**Y-5**, IC_{50} = 20.4 ± 2.1 μM) slightly increased FXR antagonistic activity (Fig. 2). In the course of our search for more potent FXR antagonists, we took **SIPI-7623** as a lead, focused on the substituents on the benzene ring at region **I**, the methyl group truncation on region **II** and various substituents on region **III** (Fig. 2). Thus, 61 compounds were designed, synthesized and evaluated for their activity toward FXR in vitro.

Results and discussion

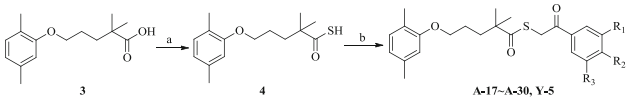
Synthesis

The synthetic route of compounds **A-1**–**A-16** (Table 1) and **Y-4** is outlined in Scheme 1. Substituted acetophenones **1a**–**1q** were converted to **2a**–**2q** with $CuBr_2$ [10, 11] and then reacted with compound **3** in the presence of K_2CO_3 to obtain **A-1**–**A-16** and **Y-4** [11, 12]. A suitable crystal of **A-4** was obtained by slowly evaporating a mixture of ethyl acetate and petroleum ether solution at ambient temperature and characterized by single-crystal X-ray diffraction.

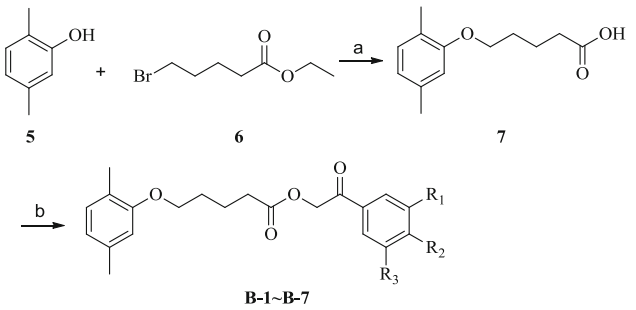
Compounds **A-17**–**A-30** (Table 1) and **Y-5** were synthesized as shown in Scheme 2. In the presence of *N,N′*-carbonyldiimidazole (CDI) in an H_2S atmosphere, compound **3** was converted to compound **4** [13], then reacted with compounds **2a**–**2n** and **2q** using a route identical to that in Scheme 2 to get compounds **A-17**–**A-30** and **Y-5**.



Scheme 1 Reagents and conditions: **a** $CuBr_2$, EtOAc/$CHCl_3$ (1:1); **b** K_2CO_3, THF



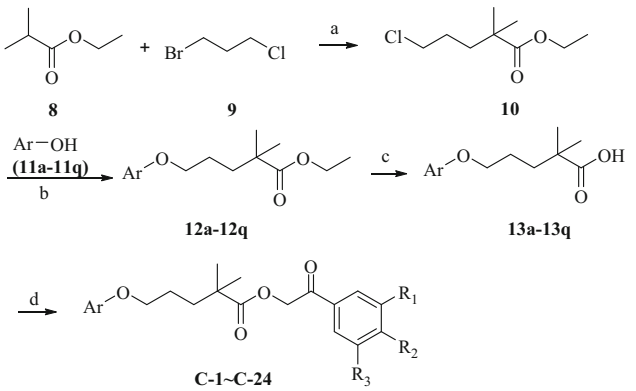
Scheme 2 Reagents and conditions: **a** H_2S, CDI, DMF; **b** compound **2a–2n** and **2q**, K_2CO_3, THF



Scheme 3 Reagents and conditions: **a** (1) K_2CO_3, DMF; (2) NaOH, EtOH; **b** compound **2j–2o** and **2q**, K_2CO_3, THF

The synthetic route of compounds **B-1**–**B-7** (Table 2) is shown in Scheme 3. 2,5-Dimethylphenol (**5**) and ethyl 5-bromopentanoate (**6**), underwent Williamson ether synthesis conditions, and then were hydrolyzed with NaOH to afford compound **7** [14–16]. After that, compounds **2j**–**2o** and **2q** were reacted with compound **7** in the presence of K_2CO_3 to generate **B-1**–**B-7**.

Compounds **C-1**–**C-24** (Table 3) were synthesized as shown in Scheme 4. Treatment of ethyl isobutyrate (**8**) with 1-bromo-3-chloropropane (**9**) afforded ethyl 5-chloro-2,2-dimethylpentanoate (**10**) [17, 18], then compound **10** reacted with appropriate phenol derivatives **11a**–**11q** to furnish compounds **12a**–**12q** which in turn were hydrolyzed with sodium hydroxide in ethanol to afford the desire compounds **13a**–**13q**. Finally, compounds **13a**–**13q** reacted with **2n** or **2q** to obtain compounds **C-1**–**C-24**.



Scheme 4 Reagents and conditions: **a** DMPU, LDA, THF; **b** K_2CO_3, DMF, TBAB; **c** NaOH, EtOH; **d** compound **2n** or **2q**, K_2CO_3, THF

Biological evaluation

In our initial study, we concluded that **SIPI-7623** derivatives with an ester and thioester linker were superior to derivatives with an amide linker in terms of antagonistic activity to FXR. We first investigated the effect on FXR antagonistic activity of different substituted groups on benzene ring at region **I** (Table 1). Derivatives without a hydrophilic hydroxyl group exhibited no activity against FXR. Interestingly, most of hydrophilic hydroxyl substituted derivatives, especially the FXR antagonistic activity of 3,5-dibromo-4-hydroxy derivatives (**A-11**, **A-27**), 3,5-dihydroxy derivatives (**A-14**, **A-30**) and 4-hydroxy-3-nitro derivative (**A-29**), are three to five fold over that in **GS** and **SIPI-7623**, with IC_{50} values of 7.8 ± 1.1, 10.8 ± 1.2, 14.8 ± 0.9, 11.1 ± 1.3 and 10.3 ± 1.2 μM, respectively. From the above results, we concluded that the phenolic group in region **I** is a key element for FXR antagonistic activity in the **SIPI-7623** derivatives.

Docking of compound **A-11** into the FXR-LBD revealed that the phenolic group formed a hydrogen bond with Arg331 (Fig. 3). This is similar to the binding mode of **SIPI-7623**. From the above SAR and docking results, we propose that the hydrophilic hydroxyl on region **I** is essential for FXR antagonistic affinity.

Then, we kept the phenolic group in region **I** and the methyl groups in region **II** were removed to generate

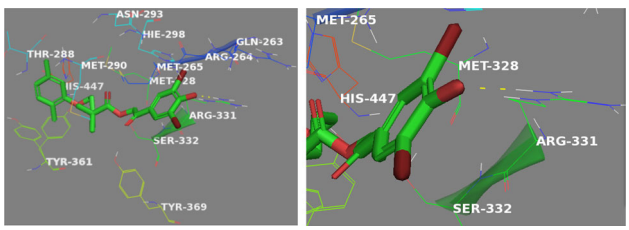


Fig. 3 Molecular docking of **A-11**

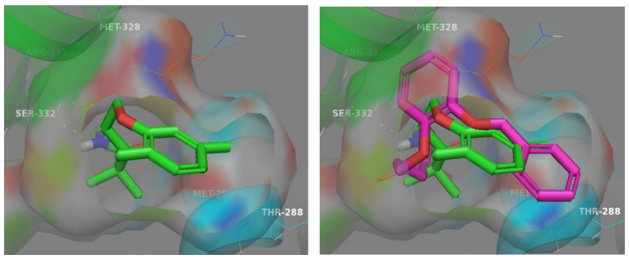


Fig. 4 SIPI-7623 (green) and **C-17** (pink) in FXR-LBD

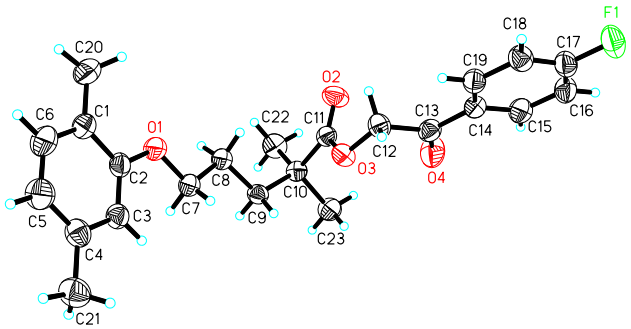


Fig. 5 Molecular solid structure projection of **A-4**

compounds **B1**–**B7** (Table 2). Compounds **B-4** and **B-7** were inactive, **B-3** and **B-5** almost totally lost activity, **B-1** (IC_{50} = 63.5 ± 3.4 μM) and **B-6** (IC_{50} = 27.5 ± 1.3 μM) displayed weak antagonistic activity compared to **GS** (IC_{50} = 47.8 ± 0.8 μM) and **SIPI-7623** (IC_{50} = 7.8 ± 1.1 μM), respectively. Only compound **B-2** (IC_{50} = 29.0 ± 2.4 μM) exhibited an equivalent antagonistic activity compared to **A-15** (IC_{50} = 22.1 ± 2.1 μM). This indicated that the truncation of methyl group results in a slightly loss of FXR antagonistic potency.

Furthermore, we investigated the effect of various substituents in region **III**, while keeping the hydrophilic hydroxyl group in region **I** and methyl group in region **II**. All of the designed derivatives demonstrated antagonistic potency toward FXR, except for **C-18** (Table 3). Unfortunately, the antagonistic potency of the designed compounds did not improve.

Docking potent analog **C-17** into FXR-LBD indicates that highly lipophilic and sterically substituents in region **III** are more favorable than the 2,5-dimethylphenoxy group in **SIPI-7623** (Fig. 4).

Conclusion

SIPI-7623 is an FXR antagonist with weak potency. SAR studies of its derivatives revealed that the core structure is a promising scaffold for the development of novel FXR antagonists. Region **I** with phenolic group is essential for

antagonistic activity, compound with methyl substituted in region **II** was more potent than compound with no substituted in region **II**, and region **III** was the key element. Compound containing 3,5-dibromo-4-hydroxy in region **I**, methyl group in region **II** and 2,5-dimethylphenoxy group in region **III**, which is **A-11**, was the most potent antagonist with an IC_{50} value of 7.8 ± 1.1 μM (Table 1).

Experimental

Chemistry

All solvents and reagents were commercially available and used without further purification. Melting points were determined on WRS-21 melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on an INOVA 400 (400 MHz) or INOVA 300 (300 MHz) spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are reported in parts per million relative to TMS, and coupling constants (J) are given in Hertz (Hz). Abbreviations for NMR signals are as follows: *s*, single; *d*, doublet; *t*, triplet; *dd*, doublet of doublets; *m*, multiplet. Electron-spray ionization mass spectra (ESI–MS) in positive mode were recorded on a HP5989A mass spectrometer. The purity of all novel compounds was checked by TLC and ^{1}H NMR. All reactions were monitored by TLC on pre-coated Silica Gel F254 plates (purchased from Yantai Jiangyou Silicone Development Co., Ltd., China) with detection by UV.

General procedure for the synthesis of compounds 2a−2q

To a stirred solution of acetophenone **1a** (5 mmol) in chloroform (150 mL) was added ethyl acetate (150 mL), $CuBr_2$ (10 mmol), and the resulting mixture was stirred at 80 °C for 6 h.

The reaction mixture was filtered, water (150 mL) was added and the mixture was extracted with dichloromethane (60 mL × 3). The combined organic layer was washed with brine and dried over MgSO4, filtered, evaporated and purified by flash column chromatography to get compound **2a**. Compounds **2b**–**2q** were prepared in a similar manner to the synthesis of compound **2a** (75–90% yield) and were used without further purification.

General procedure for the synthesis of compounds A-1–A-16, Y-4

A stirred solution of compound **2a** (10.0 mmol) and compound **3** (10.0 mmol) in THF (30 mL) was added K_2CO_3 (10.0 mmol). The resultant mixture was stirred at room temperature for 12 h. After the reaction was completed, the solution was concentrated. The oil was washed with dilute HCl and ethyl acetate, dried over $MgSO_4$, filtered, concentrated, and the residue was purified by chromatography to afford compound **A-1**. Compounds **A-2**–**A-16**, **Y-4** were prepared in a similar manner to the synthesis of compound **A-1**.

2-Oxo-2-phenylethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-1)** Yield 85.2%; white solid, mp: 58.6–61.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.96 (d, J = 8.4 Hz, 2H), 7.69 (t, J = 7.4 Hz, 1H), 7.56 (t, J = 7.7 Hz, 2H), 6.98 (d, J = 7.5 Hz, 1H), 6.73 (s, 1H), 6.63 (d, J = 7.5 Hz, 1H), 5.48 (s, 2H), 3.93 (t, J = 5.5 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.90–1.63 (m, 4H), 1.24 (s, 6H); ESI–MS *m/z*: 367 $[M - H]^-$.

2-Oxo-2-(p-tolyl)ethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-2)** Yield 83.7%; white solid, mp: 57.7–59.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.86 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 6.98 (d, J = 7.5 Hz, 1H), 6.72 (s, 1H), 6.62 (d, J = 7.5 Hz, 1H), 5.43 (s, 2H), 3.93 (t, J = 5.4 Hz, 2H), 2.38 (s, 3H), 2.25 (s, 3H), 2.10 (s, 3H), 1.88 -1.59 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 381 $[M - H]^-$.

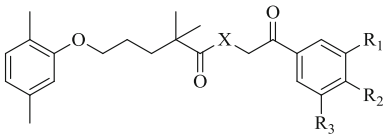
2-(4-Methoxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-3)** Yield 84.2%; white solid, mp: 55.0–57.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.94 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 7.5 Hz, 1H), 6.72 (s, 1H), 6.62 (d, J = 7.5 Hz, 1H), 5.41 (s, 2H), 3.92 (t, J = 7.7 Hz, 2H), 3.85 (s, 3H), 2.25 (s, 3H), 2.10 (s, 3H), 1.87–1.59 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 397 $[M - H]^-$, 399 $[M + H]^+$.

2-(4-Fluorophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-4) (Table 4, Fig. 5)** Yield 80.8%; white solid, mp: 50 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.05 (dd, J = 8.8, 5.5 Hz, 2H), 7.39 (t, J = 8.9 Hz, 2H), 6.98 (d, J = 7.5 Hz, 1H), 6.72 (s, 1H), 6.62 (d, J = 7.4 Hz, 1H), 5.46 (s, 2H), 3.93 (t, J = 5.3 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.80-1.70 (m, 4H), 1.24 (s, 6H); ESI–MS *m/z*: 385 $[M - H]^-$.

2-(4-Chlorophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-5)** Yield 89.2%; white solid, mp: 50.4–51.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.97 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 7.4 Hz, 1H), 6.72 (s, 1H), 6.62 (d, J = 7.6 Hz, 1H), 5.46 (s, 2H), 3.93 (t, J = 5.1 Hz, 2H), 2.24 (s, 3H), 2.09 (s, 3H), 1.83–1.55 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 401 $[M - H]^-$.

2-(4-Bromophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate **(A-6)** Yield 81.5%; white solid, mp: 52.4–53.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.89 (d,

Table 1 Structures of **A-1**–**A-30** and screening results



Compd	*X*	R_1	R_2	R_3	Activation ratio at 10 μM[a]	Inhibition ration at 50 μM[a]	IC_{50}[b] (μM)
A-1	O	H	H	H	0.2%	11.5%	ND[d]
A-2	O	H	CH_3	H	0.3%	13.8%	ND
A-3	O	H	OCH_3	H	0.8%	11.8%	ND
A-4	O	H	F	H	− 0.3%	10.8%	ND
A-5	O	H	Cl	H	0.6%	8.8%	ND
A-6	O	H	Br	H	0.8%	11.1%	ND
A-7	O	H	I	H	8.9%	17.2%	ND
A-8	O	H	CF_3	H	0.6%	11.5%	ND
A-9	O	H	NO_2	H	0.0%	11.8%	ND
A-10	O	CH_3	OH	CH_3	1.4%	37.4%	ND
A-11	O	Br	OH	Br	3.1%	88.1%	7.8 ± 1.1
A-12	O	F	OH	H	4.1%	66.6%	33.0 ± 1.2
A-13	O	NO_2	OH	H	16.7%	17.0%	ND
A-14	O	OH	H	OH	2.1%	80.1%	14.8 ± 0.9
A-15	O	H	H	OH	− 0.7%	78.2%	22.1 ± 2.1
A-16	O	H	COOH	H	4.7%	30.0%	ND
A-17	S	H	H	H	0.3%	14.8%	ND
A-18	S	H	CH_3	H	0.3%	19.2%	ND
A-19	S	H	OCH_3	H	0.0%	17.2%	ND
A-20	S	H	F	H	0.6%	12.1%	ND
A-21	S	H	Cl	H	0.6%	9.1%	ND
A-22	S	H	Br	H	2.8%	10.1%	ND
A-23	S	H	I	H	4.7%	13.8%	ND
A-24	S	H	CF_3	H	0.3%	7.1%	ND
A-25	S	H	NO_2	H	0.6%	14.5%	ND
A-26	S	CH_3	OH	CH_3	1.1%	35.7%	ND
A-27	S	Br	OH	Br	− 0.3%	92.2%	10.8 ± 1.2
A-28	S	F	OH	H	3.0%	68.3%	30.5 ± 0.9
A-29	S	NO_2	OH	H	3.3%	82.8%	10.3 ± 1.2
A-30	S	OH	H	OH	− 0.7%	80.9%	11.1 ± 1.3
Y-4	O	H	OH	H	ND	ND	42.5 ± 2.3
Y-5	S	H	OH	H	ND	ND	23.8 ± 1.3
GS[c]							45.9 ± 1.6
SIPI-7623[c]							40.8 ± 1.7

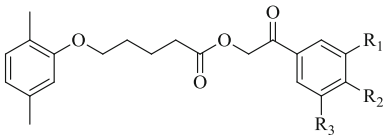
[a]Results are given as the mean of two independent experiments

[b]Results are given as the mean ± SD of three independent experiments

[c]Used as a positive control

[d]Not determined

Table 2 Structures of **B-1**–**B-7** and screening results



Compd	R_1	R_2	R_3	Activation ratio at 10 μM[a] (%)	Inhibition ration at 50 μM[a] (%)	IC_{50}^{b} (μM)
B-1	H	OH	H	− 0.3	50.9	63.5 ± 3.4
B-2	H	H	OH	− 1.1	78.6	29.0 ± 2.4
B-3	OH	H	OH	5.3	45.5	ND[d]
B-4	F	OH	H	1.1	10.9	ND
B-5	CH_3	OH	CH_3	0.2	44.6	ND
B-6	Br	OH	Br	1.2	76.4	27.5 ± 1.3
B-7	NO_2	OH	H	0.8	12.7	ND
GS[c]						47.8 ± 0.8
SIPI-7623[c]						37.7 ± 2.1

[a]Results are given as the mean of two independent experiments

[b]Results are given as the mean ± SD of three independent experiments

[c]Used as a positive control

[d]Not determined

J = 8.5 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.72 (s, 1H), 6.63 (d, *J* = 7.5 Hz, 1H), 5.46 (s, 2H), 3.93 (t, *J* = 4.9 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.75 (t, *J* = 10.0 Hz, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 445 $[M - H]^-$.

***2-(4-Iodophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-7)** Yield 75.1%; white solid, mp: 77.2–77.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.96 (d, *J* = 8.2 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.72 (s, 1H), 6.62 (d, *J* = 7.3 Hz, 1H), 5.44 (s, 2H), 3.92 (s, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.73 (s, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 493 $[M - H]^-$.

***2-oxo-2-(4-(Trifluoromethyl)phenyl)ethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-8)** Yield 80.2%; white solid, mp: 53.5–54.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.14 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 7.4 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.4 Hz, 1H), 5.51 (s, 2H), 3.92 (d, *J* = 4.8 Hz, 2H), 2.24 (s, 3H), 2.10 (s, 3H), 1.74 (s, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 435 $[M - H]^-$.

***2-(4-Nitrophenyl)-2-oxoethyl5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-9)** Yield 78.1%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.37 (d, *J* = 8.7 Hz, 2H), 8.19 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.72 (s, 1H), 6.62 (d, *J* = 7.4 Hz, 1H), 5.54 (s, 2H), 3.95–3.90 (m, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.74 (s, 4H), 1.24 (s, 6H); ESI–MS *m/z*: 412 $[M - H]^-$.

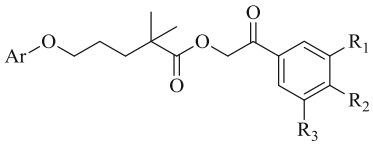
***2-(4-Hydroxy-3,5-dimethylphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-10)** Yield 61.3%; white solid, mp: 99.3–102.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.31 (s, 1H), 7.59 (s, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.72 (s, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 5.34 (s, 2H), 3.93 (t, *J* = 5.6 Hz, 2H), 2.25 (s, 3H), 2.21 (s, 6H), 2.10 (s, 3H), 1.81–1.66 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 411 $[M - H]^-$, 413 $[M + H]^+$,435 $[M + Na]^+$.

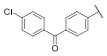
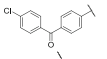
***2-(3,5-Dibromo-4-hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-11)** Yield 64.1%; white solid, mp: 83.2–86.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (s, 2H), 7.02 (d, *J* = 7.4 Hz, 1H), 6.68 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 5.19 (s, 2H), 3.98 (t, *J* = 5.2 Hz, 2H), 2.33 (s, 3H), 2.20 (s, 3H), 1.98–1.76 (m, 4H), 1.33 (s, 6H); ESI–MS *m/z*: 541 $[M - H]^-$.

***2-(3-Fluoro-4-hydroxyphenyl)-2-oxoethyl-5-(2,5-imethylphenoxy)-2,2-dimethylpentanoate* (A-12)** Yield 65.2%; white solid, mp: 87.1–88.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.04 (dd, *J* = 15.4, 7.1 Hz, 2H), 6.68 (d, *J* = 7.6 Hz, 1H), 6.66 (s, 1H), 5.24 (s, 2H), 3.99 (t, *J* = 5.5 Hz, 2H), 2.33 (s, 3H), 2.21 (s, 3H), 1.91–1.79 (m, 4H), 1.35 (s, 6H); ESI–MS *m/z*: 401 $[M - H]^-$.

***2-(4-Hydroxy-3-nitrophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-13)** Yield 57.5%; white solid, mp: 50.2–52.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.19 (s, 1H), 8.44 (d, *J* = 2.2 Hz, 1H), 8.09 (d,

Table 3 Structures of **C-1–C-27** and screening results



Compd	R_1	R_2	R_3	Ar	Activation ratio at 10 μM[a]	Inhibition ration at 50 μM[a]	IC_{50}[b] (μM)
C-1	H	OH	H		-0.9%	60.5%	30.2±2.1
C-2	OH	H	OH		-1.5%	79.1%	18.5±1.2
C-3	OH	H	OH		-1.5%	51.8%	55.1±0.6
C-4	OH	H	OH		-0.75	74.9%	18.7±1.1
C-5	OH	H	OH		2.7%	78.2%	16.5±0.8
C-6	OH	H	OH		-0.9%	70.85	27.5±0.6
C-7	OH	H	OH		0.8%	64.6%	30.2±2.1
C-8	OH	H	OH		-0.9%	80.2%	19.5±2.0
C-9	OH	H	OH		-0.8%	67.3%	25.0±1.8
C-10	OH	H	OH		-0.7%	76.4%	16.9±1.2
C-11	OH	H	OH		0.4%	67.3%	29.8±1.4
C-12	OH	H	OH		0.9%	80.6%	16.6±1.6
C-13	OH	H	OH		-1.3%	70.4%	19.6±2.2
C-14	OH	H	OH		0.3%	70.9%	17.0±1.4
C-15	OH	H	OH		-0.2%	69.1%	23.7±2.3
C-16	OH	H	OH		-1.3%	51.8%	54.5±2.2
C-17	OH	H	OH		-1.2%	78.2%	15.9±0.8
C-18	OH	H	OH		1.0%	21.8%	>100
C-19	H	OH	H		0.2%	59.5%	40.6±0.9
C-20	H	OH	H		-1.1%	64.4%	38.3±1.7
C-21	H	OH	H		0.4%	66.6%	38.3±1.2
C-22	H	OH	H		0.6%	56.3%	55.1±3.2
C-23	H	OH	H		2.1%	74.3%	20.3±2.1
C-24	H	OH	H		0.1%	52.1%	59.4±1.2
GS[c]							43.8±1.6
SIPI-7623[c]							42.0±2.3

[a]Results are given as the mean of two independent experiments

[b]Results are given as the mean ± SD of three independent experiments

[c]Used as a positive control

Table 4 Crystal data and structure refinement for **A-4**

Identification code	**A-4**
Empirical formula	C_{23} H_{27} F O_4
Formula weight	386.45
Temperature	296(2) K
Wavelength	1.54178 A
Crystal system, space group	Monoclinic, C 2/*c*
Unit cell dimensions	*a* = 31.1731(4) A alpha = 90°
Volume	*b* = 12.7247(2) A beta = 97.7680(10)°
Z, Calculated density	*c* = 10.92390(10) A gamma = 90°
	4293.40(10) A^3
	8, 1.196 Mg/m^3
Absorption coefficient	0.708 mm^{-1}
F(000)	1648
Crystal size	0.19 × 0.15 × 0.12 mm
Theta range for data collection	3.76°–67.38°
Limiting indices	$-36 \leq h \leq 36, -15 \leq k \leq 14, -12 \leq l \leq 2$
Reflections collected/unique	14602/3731 [*R*(int) = 0.0202]
Completeness to theta = 67.38	96.8%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7529 and 0.6774
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3731/0/253
Goodness-of-fit on F^2	1.057
Final *R* indices [$I > 2$ sigma(I)]	R_1 = 0.0448, wR_2 = 0.1263
R indices (all data)	R_1 = 0.0487, wR_2 = 0.1308
Largest diff. peak and hole	0.195 and − 0.194 e A^{-3}

J = 8.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 5.43 (s, 2H), 3.92 (t, *J* = 5.1 Hz, 2H), 2.24 (s, 3H), 2.09 (s, 3H), 1.81–1.66 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 428 $[M - H]^-$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-14)** Yield 65.7%; white solid, mp: 116.1–117.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.78 (d, *J* = 1.8 Hz, 2H), 6.72 (s, 1H), 6.63 (d, *J* = 7.4 Hz, 1H), 6.52 (s, 1H), 5.33 (s, 2H), 3.93 (t, *J* = 5.3 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.79–1.65 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 399 $[M - H]^-$, 423 $[M + Na]^+$.

***2-(3-Hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (A-15)** Yield 52.6%; white solid, mp: < 50 °C; ^{1}H NMR (300 MHz, DMSO) δ: 9.88 (s, 1H), 7.45–7.27 (m, 3H), 7.07 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 7.3 Hz, 1H), 6.71 (d, *J* = 6.7 Hz, 1H), 6.62 (d, *J* = 7.4 Hz, 1H), 5.39 (s, 2H), 3.95–3.89 (m, 2H), 2.24 (s, 3H), 2.10 (s, 3H), 1.74 (s, 2H), 1.63 (s, 2H), 1.23 (s, 3H), 1.12 (s, 3H); ESI–MS *m/z*: 383 $[M - H]^-$.

***4-(2-((5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoyl)oxy)acetyl)benzoic acid* (A-16)** Yield 32.4%; white solid, mp: 104.9–107.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.08 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.73 (s, 1H), 6.63 (d, *J* = 7.4 Hz, 1H), 5.50 (s, 2H), 3.93 (d, *J* = 4.2 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.75 (s, 4H), 1.24 (s, 6H); ESI–MS *m/z*: 411 $[M - H]^-$.

***2-(4-Hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoate* (Y-4)** Yield 83%; white solid, mp: 105.6–108.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80–7.82 (m, 2H), 6.98–7.00 (d, *J* = 7.6 Hz, 1H), 6.85–6.87 (m, 2H), 6.63–6.64 (m, 2H), 5.6 (s, 1H), 5.23 (s, 2H), 3.94–3.97 (m, 2H), 2.30 (s, 3H), 2.18 (s, 3H), 1.81–1.85 (m, 4H), 1.32 (s, 6H); ESI–MS *m/z*: 383$[M - H]^-$.

General procedure for the synthesis of compound 4

CDI (10.0 mmol) was dissolved in DMF (20 mL), and this solution was added a solution of the carboxylic acid **3** (10.0 mmol) in DMF (2 mL). Subsequently, the reaction mixture was stirred at room temperature for 1 h. Then, H_2S gas was bubbled gently through the reaction mixture for 2 h. 0.5 M sulfuric acid (40 mL) was added, and the mixture was extracted with ethyl acetate (25 mL × 3). The organic layer was dried with $MgSO_4$, filtrated, concentrated and purified by chromatography to yield compound **4**. Yield 75.4%, waxy oil, ESI–MS *m/z*: 265 $[M - H]^-$.

General procedure for the synthesis of compounds A-17–A-30, Y-5

Compounds **A-17**–**A-30**, **Y-5** were prepared in a similar manner to that described in the synthesis of compounds **A-1**–**A-16** from compound **4** in place of compound **3**.

***S-(2-Oxo-2-phenylethyl)5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-17)** Yield 88.4%; oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96–7.91 (m, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.52 (d, *J* = 7.8 Hz, 2H), 7.02 (d, *J* = 7.4 Hz, 1H), 6.68 (d, *J* = 7.7 Hz, 1H), 6.66 (s, 1H), 5.32 (s, 2H), 3.99 (t, *J* = 5.7 Hz, 2H), 2.33 (s, 3H), 2.21 (s, 3H), 1.94–1.79 (m, 4H), 1.35 (s, 6H); ESI–MS *m/z*: 383 $[M - H]^-$.

***S-(2-Oxo-2-(p-tolyl)ethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-18)** Yield 84.1%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.91 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.71 (s, 1H), 6.63 (d, *J* = 11.5 Hz, 1H), 4.46 (s, 2H), 3.89 (t, *J* = 6.0 Hz, 2H), 2.38 (s, 3H), 2.25 (s, 3H), 2.09 (s, 3H), 1.75–1.63 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 397 $[M - H]^-$.

***S-(2-(4-Methoxyphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-19)** Yield 86.6%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.99 (d, *J* = 9.0 Hz, 2H), 7.06 (d, *J* = 6.9 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.70 (s, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 4.43 (s, 2H), 3.89 (t, *J* = 5.9 Hz, 2H), 3.85 (s, 3H), 2.25 (s, 3H), 2.09 (s, 3H), 1.78–1.62 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 413 $[M - H]^-$.

***S-(2-(4-Fluorophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-20)** Yield 81.9%; oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (dd, *J* = 8.8, 5.3 Hz, 2H), 7.16 (t, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.66 (d, *J* = 7.6 Hz, 1H), 6.63 (s, 1H), 5.26 (s, 2H), 3.96 (t, *J* = 5.6 Hz, 2H), 2.31 (s, 3H), 2.17 (s, 3H), 1.92–1.73 (m, 4H), 1.32 (s, 6H); ESI–MS *m/z*: 401 $[M - H]^-$.

***S-(2-(4-Chlorophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-21)** Yield 87.1%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.02 (d, *J* = 6.6 Hz, 2H), 7.62 (d, *J* = 6.6 Hz, 2H), 6.98 (d, *J* = 6.5 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.7 Hz, 1H), 4.49 (s, 2H), 3.89 (t, *J* = 5.2 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.77–1.61 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 417 $[M - H]^-$.

***S-(2-(4-Bromophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-22)** Yield 83.2%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.94 (d, *J* = 8.7 Hz, 2H), 7.76 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.70 (s, 1H), 6.62 (d, *J* = 7.4 Hz, 1H), 4.48 (s, 2H), 3.89 (t, *J* = 5.9 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.76–1.69 (m, 2H), 1.68 -1.62 (m, 2H), 1.22 (s, 6H); ESI–MS *m/z*: 463 $[M - H]^-$.

***S-(2-(4-Iodophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-23)** Yield 75.9%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.94 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 4.46 (s, 2H), 3.89 (t, *J* = 6.0 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.76–1.69 (m, 2H), 1.68–1.62 (m, 2H), 1.22 (s, 6H); ESI–MS *m/z*: 509 $[M - H]^-$.

***S-(2-Oxo-2-(4-(trifluoromethyl)phenyl)ethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-24)** Yield 76.2%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.20 (d, *J* = 8.1 Hz, 2H), 7.92 (d, *J* = 8.3 Hz, 2H), 6.97 (d, *J* = 7.4 Hz, 1H), 6.70 (s, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 4.55 (s, 2H), 3.88 (t, *J* = 5.9 Hz, 2H), 2.24 (s, 3H), 2.09 (s, 3H), 1.75–1.60 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 451 $[M - H]^-$.

***S-(2-(4-Nitrophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-25)** Yield 63.7%; oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.36 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 7.4 Hz, 1H), 6.69 (s, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 4.56 (s, 2H), 3.88 (t, *J* = 5.8 Hz, 2H), 2.24 (s, 3H), 2.08 (s, 3H), 1.75–1.61 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 428 $[M - H]^-$.

***S-(2-(4-Hydroxy-3,5-dimethylphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-26)** Yield 61.5%; white solid, mp: 104.1–104.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.28 (s, 1H), 7.64 (s, 2H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 7.3 Hz, 1H), 4.38 (s, 2H), 3.89 (t, *J* = 5.8 Hz, 2H), 2.23 (d, *J* = 10.9 Hz, 9H), 2.09 (s, 3H), 1.74–1.63 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 427 $[M - H]^-$.

***S-(2-(3,5-Dibromo-4-hydroxyphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate* (A-27)** Yield 59.2%; white solid, mp: 90.5–92.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.16 (s, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.71 (s, 1H), 6.62 (d, *J* = 11.2 Hz, 1H), 4.44 (s, 2H), 3.89 (t,

J = 5.8 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.76–1.61 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 557 $[M - H]^-$.

S-(2-(3-Fluoro-4-hydroxyphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate **(A-28)** Yield 62.5%; white solid, mp: 63.8–66.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.00 (s, 1H), 7.81–7.73 (m, 2H), 7.07 (t, J = 8.8 Hz, 1H), 6.97 (d, J = 7.6 Hz, 1H), 6.70 (s, 1H), 6.62 (d, J = 7.6 Hz, 1H), 4.40 (s, 2H), 3.89 (t, J = 5.8 Hz, 2H), 2.24 (s, 3H), 2.09 (s, 3H), 1.78–1.62 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 417 $[M - H]^-$.

S-(2-(4-Hydroxy-3-nitrophenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate **(A-29)** Yield 59.4%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.14 (s, 1H), 8.50 (s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 7.3 Hz, 1H), 6.70 (s, 1H), 6.62 (d, J = 7.4 Hz, 1H), 4.47 (s, 2H), 3.89 (t, J = 5.7 Hz, 2H), 2.24 (s, 3H), 2.08 (s, 3H), 1.77–1.62 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 444 $[M - H]^-$.

S-(2-(3,5-Dihydroxyphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate **(A-30)** Yield 58.8%; white solid, mp: 124.5–124.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.68 (s, 2H), 6.98 (d, J = 7.4 Hz, 1H), 6.84 (d, 2H), 6.71 (s, 1H), 6.62 (d, J = 7.4 Hz, 1H), 6.50 (s, 1H), 4.36 (s, 2H), 3.90 (t, J = 5.8 Hz, 2H), 2.25 (s, 3H), 2.10 (s, 3H), 1.76–1.62 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 415 $[M - H]^-$.

S-(2-(4-Hydroxyphenyl)-2-oxoethyl)-5-(2,5-dimethylphenoxy)-2,2-dimethylpentanethioate **(Y-5)** Yield 67%; white solid, mp: 90.3–93.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82–7.80 (m, 2H), 7.0–6.99 (d,1H, J = 7.2 Hz), 6.88–6.86 (m, 2H), 6.67–6.63 (m, 2H), 5.68 (s, 1H), 5.24 (s, 2H), 3.97–3.94 (m, 2H), 2.30 (s, 3H), 2.18 (s, 3H), 1.86–1.83 (m, 4H), 1.32 (s, 6H); ESI–MS *m/z*: 423$[M + Na]^+$.

General procedure for the synthesis of compound 7

A solution of compound **5** (10 mmol) and K_2CO_3 (12 mmol) in DMF (25 mL) was stirred at room temperature for 0.5 h, then compound **6** (12 mmol) and tetrabutyl ammonium bromide (TBAB, 0.1 mmol) were added into the above solution. The reaction mixture was stirred overnight at room temperature; after the reaction was completed, water was added to the solution and extracted with DCM (40 mL × 3). The organic layer was dried with $MgSO_4$, filtrated and evaporated at reduced pressure; the residue and NaOH (20 mmol) were dissolved in EtOH (40 mL), and then the reaction was allowed to 80 °C and stirred for 2 h. After the reaction was completed, it was added water (150 mL), extracted with DCM (40 mL × 3), evaporated at reduced pressure, and the residue was purified by chromatography to afford compound 7. Yield 80.5%, waxy oil, ESI–MS *m/z*: 221$[M - H]^-$.

General procedure for the synthesis of compounds B-1–B-7

Compounds **B-1**–**B-7** were prepared in a similar manner to that described in the synthesis of compound **A-1**.

2-(4-Hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy) pentanoate **(B-1)** Yield 64.8%; white solid, mp: 80.6–80.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.51 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 8.8 Hz, 2H), 6.75 (s, 1H), 6.63 (d, J = 7.4 Hz, 1H), 5.38 (s, 2H), 3.97 (t, J = 5.9 Hz, 2H), 2.53–2.50 (m, 2H), 2.26 (s, 3H), 2.10 (s, 3H), 1.85–1.74 (m, 4H); ESI–MS *m/z*: 355 $[M - H]^-$.

2-(3-Hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy) pentanoate **(B-2)** Yield 59.8%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.90 (s, 1H), 7.43–7.33 (m, 2H), 7.33–7.29 (m, 1H), 7.08 (dd, J = 7.9, 2.3 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 6.75 (s, 1H), 6.63 (d, J = 7.5 Hz, 1H), 5.43 (s, 2H), 3.97 (t, J = 5.9 Hz, 2H), 2.26 (s, 3H), 2.10 (s, 3H), 1.85–1.74 (m, 4H); ESI–MS *m/z*: 355 $[M - H]^-$, 357 $[M + H]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy) pentanoate **(B-3)** Yield 64.2%; white solid, mp: 150.2–151.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 6.99 (d, J = 7.4 Hz, 1H), 6.78 (d, J = 2.0 Hz, 2H), 6.75 (s, 1H), 6.63 (d, J = 7.4 Hz, 1H), 6.51 (s, 1H), 5.35 (s, 2H), 3.97 (t, J = 5.9 Hz, 2H), 2.26 (s, 3H), 2.10 (s, 3H), 1.85–1.74 (m, 4H); ESI–MS *m/z*: 371 $[M - H]^-$.

2-(3-Fluoro-4-hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)pentanoate **(B-4)** Yield 55.8%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.80–7.66 (m, 2H), 7.09 (t, J = 9.5 Hz, 1H), 6.95 (d, J = 7.4 Hz, 1H), 6.69 (s, 1H), 6.60 (d, J = 7.4 Hz, 1H), 4.80 (s, 2H), 3.91 (t, J = 5.9 Hz, 2H), 2.28 (t, J = 7.1 Hz, 2H), 2.23 (s, 3H), 2.07 (s, 3H), 1.76–1.65 (m, 4H); ESI–MS *m/z*: 373 $[M - H]^-$.

2-(4-Hydroxy-3,5-dimethylphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)pentanoate **(B-5)** Yield 59.5%; white solid, mp: 83.5–85.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.60 (s, 2H), 6.98 (d, J = 7.1 Hz, 1H), 6.74 (d, J = 8.4 Hz, 1H), 6.63 (d, J = 7.4 Hz, 1H), 5.37 (s, 2H), 3.97–3.93 (m, 2H), 2.57–2.48 (m, 2H), 2.24 (d, J = 13.3 Hz, 9H), 2.09 (s, 3H), 1.80-1.70 (m, 4H); ESI–MS *m/z*: 383 $[M - H]^-$.

2-(3,5-Dibromo-4-hydroxyphenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy)pentanoate **(B-6)** Yield 54.7%; mp: 8 white solid, 88.9–90.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.16 (s, 2H), 6.97 (d, J = 7.4 Hz, 1H), 6.72 (s, 1H), 6.62

(d, J = 7.4 Hz, 1H), 4.90 (s, 2H), 3.93 (t, J = 6.0 Hz, 2H), 2.30 (t, J = 7.1 Hz, 2H), 2.25 (s, 3H), 2.09 (s, 3H), 1.76–1.66 (m, 4H); ESI–MS *m/z*: 513 $[M - H]^-$.

***2-(4-Hydroxy-3-nitrophenyl)-2-oxoethyl-5-(2,5-dimethylphenoxy) pentanoate* (B-7)** Yield 63.2%; white solid, mp: 59.8–61.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.57–8.39 (m, 1H), 8.23–8.03 (m, 1H), 7.28–7.21 (m, 1H), 6.96 (d, J = 7.4 Hz, 1H), 6.71 (s, 1H), 6.61 (d, J = 7.4 Hz, 1H), 4.87 (s, 2H), 3.92 (t, J = 5.9 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 2.24 (s, 3H), 2.08 (s, 3H), 1.77–1.63 (m, 4H); ESI–MS *m/z*: 400 $[M - H]^-$.

General procedure for the synthesis of compound 10

A solution of ethyl isobutyrate (**8**, 1.13 mol) and 1,3-dimethylpropyleneurea (5 mL) in THF (160 mL) was added a solution of lithium diisopropylamide in THF (790 mL, 2 mol/L) at -50 to -78 °C. The mixture was stirred for 0.5 h at -78 °C, and 1-bromo-3-chloropropane **9** (1.58 mol) was added, stirred overnight at room temperature. After the reaction was completed, aqueous HCl (6 N, 250 mL), water (500 mL), ice (500 g) and saturated NH_4Cl solution (400 mL) were added into the reaction mixture. The mixture was extracted with methyl tert-butyl ether (250 mL × 2), washed with saturated NaCl solution (200 mL), dried with $MgSO_4$, filtered and concentrated, distillation under vacuum to get compound **10**, yield 52.7%, waxy oil, ESI–MS *m/z*: 192$[M - H]^-$.

General procedure for the synthesis of compounds 13a−13q

A solution of appropriate phenol derivative **11a** (10 mmol) and K_2CO_3 (12 mmol) in DMF (25 mL) was stirred at room temperature for 0.5 h, and then compound **10** (12 mmol) and TBAB (0.1 mmol) were added into the above solution. The reaction mixture was stirred overnight at 90 °C; after the reaction was completed, water was added and extracted with DCM (40 mL × 3). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to get **12a**. Then, compound **12a** was dissolved in EtOH (10 mL), the reaction was stirred at room temperature and added a solution of NaOH (20 mmol) in EtOH. The reaction was allowed to 80 °C and stirred for 2 h. After the reaction was completed, it was added water (150 mL), extracted with DCM (40 mL × 3), concentrated at reduced pressure to afford **13a**, which was used in the next step without further purification. Compounds **13b**–**13q** were prepared in a similar manner to the synthesis of compound **13a**.

General procedure for the synthesis of C1–C24

Compounds **C1**–**C24** were prepared in a similar manner to that described in the synthesis of compound **A-1**.

***2-(4-Hydroxyphenyl)-2-oxoethyl-5-(4-(4-chlorobenzoyl)phenoxy)-2,2-dimethylpentanoate* (C-1)** Yield 70.4%; white solid, mp: 114.1–116.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.9 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 5.37 (s, 2H), 4.09 (t, J = 5.8 Hz, 2H), 1.82–1.65 (m, 4H), 1.23 (s, 6H); ESI–MS *m/z*: 493 $[M - H]^-$, 517 $[M + Na]^+$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(4-(4-chlorobenzoyl)phenoxy)-2,2-dimethylpentanoate* (C-2)** Yield 64.7%; white solid, mp: 130.3–131.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 6.77 (d, J = 2.1 Hz, 2H), 6.52 (t, J = 2.1 Hz, 1H), 5.34 (s, 2H), 4.09 (t, J = 6.0 Hz, 2H), 1.85–1.76 (m, 2H), 1.74–1.68 (m, 2H), 1.23 (s, 6H); ESI–MS *m/z*: 511 $[M + H]^+$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(4-acetylphenoxy)-2,2-dimethylpentanoate* (C-3)** Yield 61.5%; white solid, mp: 121.5–124.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.77 (s, 2H), 6.51 (s, 1H), 5.33 (s, 2H), 4.06 (t, J = 5.7 Hz, 2H), 2.51 (s, 3H), 1.83–1.66 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 415 $[M + H]^+$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2,5-difluorophenoxy)-2,2-dimethylpentanoate* (C-4)** Yield 58.6%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (s, 2H), 7.24 (dd, J = 9.6, 4.6 Hz, 1H), 7.10 (dd, J = 9.6, 3.7 Hz, 1H), 6.78 (d, J = 2.1 Hz, 2H), 6.72 (dd, J = 8.7, 3.4 Hz, 1H), 6.52 (t, J = 2.1 Hz, 1H), 5.34 (s, 2H), 4.05–4.01 (m, 2H), 1.81–1.71 (m, 2H), 1.71–1.67 (m, 2H), 1.22 (s, 6H); ESI–MS *m/z*: 407 $[M - H]^-$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2,5-dichlorophenoxy)-2,2-dimethylpentanoate* (C-5)** Yield 68.7%; white solid, mp: 151.3–153.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 7.44 (d, J = 8.5 Hz, 1H), 7.23 (d, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.5, 2.3 Hz, 1H), 6.78 (d, J = 2.2 Hz, 2H), 6.52 (t, J = 2.1 Hz, 1H), 5.34 (s, 2H), 4.09 (t, J = 5.9 Hz, 2H), 1.83–1.68 (m, 4H), 1.22 (s, 6H); ESI–MS *m/z*: 439 $[M - H]^-$.

***2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(4-ethylphenoxy)-2,2-dimethylpentanoate* (C-6)** Yield 67.1%; white solid, mp: 82.5–84.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 7.10 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H),

6.78 (d, J = 2.1 Hz, 2H), 6.52 (t, J = 2.1 Hz, 1H), 5.33 (s, 2H), 3.92 (t, J = 5.7 Hz, 2H), 2.52 (t, J = 5.9 Hz, 2H), 1.79–1.63 (m, 4H), 1.22 (s, 6H), 1.14 (t, J = 7.6 Hz, 3H); ESI–MS m/z: 399 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-2,2-dimethyl-5-((5,6,7,8-tetrahydronaphthalene-2-yl)oxy)pentanoate **(C-7)** Yield 61.2%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 6.92 (d, J = 8.3 Hz, 1H), 6.77 (d, J = 2.1 Hz, 2H), 6.64 (dd, J = 8.2, 2.7 Hz, 1H), 6.60–6.58 (m, 1H), 6.52 (t, J = 2.1 Hz, 1H), 6.40 (d, J = 2.7 Hz, 1H), 5.32 (s, 2H), 3.89 (t, J = 5.7 Hz, 2H), 2.64 (d, 4H), 1.69 (d, 8H), 1.22 (s, 6H); ESI–MS m/z: 425 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-2,2-dimethyl-5-(naphthalen-2-yloxy)pentanoate **(C-8)** Yield 58.8%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.74 (s, 2H), 7.81 (t, J = 7.6 Hz, 3H), 7.45 (t, J = 7.6 Hz, 1H), 7.36–7.30 (m, 2H), 7.17 (dd, J = 8.9, 2.5 Hz, 1H), 6.79 (d, J = 2.1 Hz, 2H), 6.53 (t, J = 2.1 Hz, 1H), 5.35 (s, 2H), 4.08 (t, J = 6.1 Hz, 2H), 1.86–1.72 (m, 4H), 1.24 (s, 6H); ESI–MS m/z: 421 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-2,2-dimethyl-5-((5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl) oxy)pentanoate **(C-9)** Yield 67.5%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 7.81 (d, J = 8.2 Hz, 1H), 6.90 (s, 1H), 6.87 (s, 1H), 6.76 (d, J = 2.1 Hz, 2H), 6.50 (s, 1H), 5.33 (s, 2H), 4.05 (t, J = 6.2 Hz, 2H), 2.89 (t, J = 5.7 Hz, 2H), 2.51 (d, J = 1.9 Hz, 2H), 1.99 (q, 2H), 1.79–1.68 (m, 4H), 1.22 (s, 6H); ESI–MS m/z: 439 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2-acetyl-3-hydroxyphenoxy)-2,2-dimethylpentanoate **(C-10)** Yield 54.9%; mp: 92.3–94.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 9.73 (s, 2H), 7.34 (s, 1H), 6.78 (d, J = 2.0 Hz, 2H), 6.56–6.47 (m, 3H), 5.34 (s, 2H), 4.03 (t, J = 5.3 Hz, 2H), 2.58 (s, 3H), 1.86–1.69 (m, 4H), 1.22 (s, 6H); ESI–MS m/z: 429 $[M - H]^-$, 431 $[M + H]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(3-acetyl-5-hydroxyphenoxy)-2,2-dimethylpentanoate **(C-11)** Yield 67.4%; white solid, mp: 162.6–162.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.80 (s, 1H), 9.71 (s, 2H), 6.93 (s, 2H), 6.77 (d, J = 1.8 Hz, 2H), 6.58 (s, 1H), 6.51 (s, 1H), 5.34 (s, 2H), 3.97 (t, J = 5.7 Hz, 2H), 2.51 (s, 3H), 1.78–1.64 (m, 4H), 1.23 (s, 6H); ESI–MS m/z: 429 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-([1,1′-biphenyl]-2-yloxy)-2,2-dimethylpentanoate **(C-12)** Yield 55.7%; white solid, mp: 112.9–116.1 °C; ^{1}H NMR (400 MHz, DMSO) δ: 9.74 (s, 2H), 7.53 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 (d, J = 7.3 Hz, 3H), 7.09 (d, J = 8.1 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 6.79 (s, 2H), 6.54 (s, 1H), 5.31 (s, 2H), 3.98 (t, J = 4.8 Hz, 2H), 1.74–1.60 (m, 4H), 1.16 (s, 6H); ESI–MS m/z: 447 $[M - H]^-$.

2-(3,5-dihydroxyphenyl)-2-oxoethyl-5-([1,1′-biphenyl]-4-yloxy)-2,2-dimethylpentanoate **(C-13)** Yield 64.4%; white solid, mp: 127.4–128.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (s, 2H), 7.60 (t, J = 7.8 Hz, 4H), 7.43 (t, J = 7.8 Hz, 2H), 7.30 (t, J = 7.4 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 6.79 (d, J = 2.1 Hz, 2H), 6.52 (t, J = 2.1 Hz, 1H), 5.35 (s, 2H), 4.01 (t, J = 5.9 Hz, 2H), 1.82–1.65 (m, 4H), 1.24 (s, 6H); ESI–MS m/z: 447 $[M - H]^-$, 471 $[M + Na]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-([1,1′-biphenyl]-3-yloxy)-2,2-dimethylpentanoate **(C-14)** Yield 59.2%; white solid, mp: 84.6–87.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, J = 7.2 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.40–7.30 (m, 2H), 7.21–7.11 (m, 2H), 6.88 (dd, J = 8.1, 2.1 Hz, 1H), 6.82 (d, J = 2.0 Hz, 2H), 6.51 (t, J = 2.0 Hz, 1H), 5.18 (s, 2H), 4.03 (t, J = 5.9 Hz, 2H), 1.86 (dd, J = 14.2, 6.3 Hz, 4H), 1.33 (s, 6H); ESI–MS m/z: 447 $[M - H]^-$, 471 $[M + Na]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(3-(benzyloxy)phenoxy)-2,2-dimethylpentanoate **(C-15)** Yield 53.8%; white solid, mp: 84.3–86.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.74 (s, 2H), 7.44 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.1 Hz, 1H), 7.17 (t, J = 8.5 Hz, 1H), 6.79 (d, J = 2.1 Hz, 2H), 6.58 (d, J = 6.6 Hz, 2H), 6.53 (d, J = 7.6 Hz, 2H), 5.34 (s, 2H), 5.08 (s, 2H), 3.94 (t, J = 5.6 Hz, 2H), 1.79–1.66 (m, 4H), 1.22 (s, 6H); ESI–MS m/z: 477 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(4-(benzyloxyphenoxy)-2,2-dimethylpentanoate **(C-16)** Yield 53.5%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (s, 2H), 7.36 (m, 6H), 6.84 (d, J = 2.0 Hz, 2H), 6.81 (s, 1H), 6.68 (d, J = 8.9 Hz, 2H), 6.52 (d, J = 2.1 Hz, 1H), 4.98 (s, 2H), 4.81 (s, 2H), 3.89 (t, J = 5.9 Hz, 2H), 1.76–1.63 (m, 4H), 1.21 (s, 6H); ESI–MS m/z: 477 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2-(benzyloxy)phenoxy)-2,2-dimethylpentanoate **(C-17)** Yield 57.4%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.74 (s, 2H), 7.47 (d, J = 7.6 Hz, 2H), 7.37 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.2 Hz, 1H), 7.01 (dd, J = 17.8, 7.6 Hz, 2H), 6.90 (dd, J = 15.0, 7.3 Hz, 2H), 6.79 (d, J = 2.0 Hz, 2H), 6.54 (s, 1H), 5.32 (s, 2H), 5.09 (s, 2H), 3.98 (s, 2H), 1.75 (s, 4H), 1.21 (s, 6H); ESI–MS m/z: 477 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-(2-methoxy-5-methylphenoxy)-2,2-dimethylpentanoate **(C-18)** Yield 55.1%; waxy oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 6.82 (d, J = 8.1 Hz, 1H), 6.77 (d, J = 2.2 Hz, 3H), 6.67 (d, J = 8.2 Hz, 1H), 6.51 (t, J = 2.1 Hz, 1H), 5.33 (s, 2H), 3.92 (t, J = 6.0 Hz, 2H), 3.71 (s, 3H), 2.22 (s, 3H), 1.78–1.65 (m, 4H), 1.22 (s, 6H); ESI–MS m/z: 415 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-2,2-dimethyl-5-((5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)oxy)pentanoate **(C-19)** Yield 56.3%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, J = 8.7 Hz, 1H), 7.72 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 6.75 (dd, J = 8.7, 2.0 Hz, 1H), 6.64 (s, 1H), 5.19 (s, 2H), 3.97 (t, J = 5.8 Hz, 2H), 2.84 (t, J = 5.8 Hz, 2H), 2.55 (t, J = 6.4 Hz, 2H), 2.04–1.98 (m, 2H), 1.90–1.68 (m, 4H), 1.25 (s, 6H); ESI–MS *m/z*: 423 $[M - H]^-$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-2,2-dimethyl-5-((5,6,7,8-tetrahydronaphthalen-2-yl)oxy)pentanoate **(C-20)** Yield 64.2%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.3 Hz, 1H), 6.84 (dd, J = 9.2, 2.3 Hz, 2H), 6.68–6.59 (m, 2H), 5.23 (s, 2H), 3.94 (t, J = 5.6 Hz, 2H), 2.77–2.63 (m, 4H), 1.85–1.74 (m, 8H), 1.32 (s, 6H); ESI–MS *m/z*: 433 $[M + Na]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-([1,1′-biphenyl]-4-yloxy)-2,2-dimethylpentanoate **(C-21)** Yield 54.1%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, J = 8.8 Hz, 2H), 7.57–7.49 (m, 5H), 7.40 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 5.23 (s, 2H), 4.03 (t, J = 5.8 Hz, 2H), 1.84 (dd, J = 12.0, 7.2 Hz, 4H), 1.33 (s, 6H); ESI–MS *m/z*: 433 $[M + H]^+$.

2-(3,5-Dihydroxyphenyl)-2-oxoethyl-5-([1,1′-biphenyl]-2-yloxy)-2,2-dimethylpentanoate **(C-22)** Yield 55.8%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, J = 8.8 Hz, 2H), 7.59–7.52 (m, 3H), 7.43–7.30 (m, 5H), 6.99 (dd, J = 12.5, 7.9 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 5.19 (s, 2H), 3.98 (d, J = 4.5 Hz, 2H), 1.72 (d, J = 9.9 Hz, 4H), 1.25 (s, 6H); ESI–MS *m/z*: 455 $[M + Na]^+$.

2-(3,5-dihydroxyphenyl)-2-oxoethyl-5-(2,5-dichlorophenoxy)-2,2-dimethylpentanoate **(C-23)** Yield 57.6%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, J = 8.8 Hz, 2H), 6.91–6.79 (m, 5H), 5.27 (s, 2H), 4.01 (t, J = 6.0 Hz, 2H), 1.95–1.78 (m, 4H), 1.33 (s, 6H); ESI–MS *m/z*: 425 $[M + H]^+$.

2-(3,5-dihydroxyphenyl)-2-oxoethyl-5-(4-acetylphenoxy)-2,2-dimethylpentanoate **(C-24)** Yield 60.2%; waxy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96–7.89 (m, 2H), 7.77 (d, J = 8.8 Hz, 2H), 6.90 (dd, J = 10.3, 8.9 Hz, 4H), 5.25 (s, 2H), 4.04 (t, J = 6.0 Hz, 2H), 2.56 (s, 3H), 1.94–1.76 (m, 4H), 1.32 (s, 6H); ESI–MS *m/z*: 399 $[M + H]^+$.

The characterization data for the synthesized compounds are deposited in Supporting information.

FXR TR-FRET assay [19, 20]

A 20-μL mixture of 3 nM GST-hFXR, 50 ng Eu-anti GST antibody, 500 nM Biotin-SRC1 and 50 ng streptavidin-APC in assay buffer was added into a 384-well black plate that contained 200 nL of 5 mM testing chemical and 5 nL of inducer, 520 μM GW4064. The final concentration of GW4064 was 130 nM in all wells except for DMSO negative control wells. The final chemical concentration was 50 μM, and the final DMSO concentration was 1% in all wells. The plates were then spun down after a brief shake and incubated for 3 h at room temperature. The TR-FRET signal was then collected for each well with an Envison plate reader using an excitation wavelength of 340 nm and emission wavelengths of 665 and 615 nm. The 665/615 nm ratio from each well was calculated and employed for % inhibition calculation. The % inhibition for each well was calculated using the following equation:

$$\%\text{Inhibition} = \frac{(130\ \text{nM GW4064}_{665\text{nm}/615\text{nm}} - \text{Chemical}_{665\text{nm}/615\text{nm}})}{(130\ \text{nM GW4064}_{665\text{nm}/615\text{nm}} - \text{DMSO}_{665\text{nm}/615\text{nm}})} \times 100\%$$

In the dose response hFXR TR-FRET assay, the general protocol used for the single point testing was followed with minor modification. Briefly, 1-to-3 titrated testing chemical from 20 mM to 60 μM with 100% DMSO and 5 nL of inducer, 520 μM GW4064 were transferred into a 384-well black plate, and then 20-μL mixture of 3 nM GST-hFXR, 50 ng Eu-antiGST antibody, 500 nM Biotin-SRC1 and 50 ng streptavidin-APC in assay buffer was added. The final concentration of GW4064 was 130 nM in all wells except for the DMSO negative control wells. The final DMSO concentration was 1% in all wells. The plates were then spun down after a brief shake and incubated for 3 h at room temperature. The TR-FRET signal was then collected for each well using an Envison plate reader using an excitation wavelength of 340 nm and emission wavelengths of 665 nm and 615 nm. The 665/615 nm ratio from each well was calculated and employed for % inhibition calculation. The % inhibition for each well was also calculated using the above-mentioned equation. The IC_{50} value and curve fitting analyses were calculated with Graphpad Prism 5.

Docking study

Receptor preparation

The co-crystallized structure of human FXR with GW4064 analog (PDB code: 3FXV, resolution: 2.26 Å) was selected for docking study. The structures were prepared by using the "Protein Preparation Wizard" module in Maestro [21]. The protein was prepared by adding missing hydrogen atoms, assigning ligand bond orders, determining ligand protonation state, removing waters, optimizing hydrogen bond network and performing a restrained minimization, etc. Docking grid files were then generated using the

"Receptor Grid Generation" module at their default settings.

Ligand preparation

Compounds were prepared with LigPrep to generate 3D structures including all possible stereoisomers and tautomers. Epik was chosen to generate all possible protonation states of the compounds at a pH from 5.0 to 9.0. OPLS_2005 was adopted as force field. The remaining parameters were used in their default values. The conformation of each structure was sampled using macromodel with "conformational CSearch" protocol. The top 10 representative conformations were obtained after a clustering analysis by RMSD.

Precise docking by Glide

The Glide software can perform high-accuracy docking. During grid generation, six hydrogen bond constraints were set up including His451 (HBA), Tyr365 (HBD), Tyr373 (HBD), Ser336 (HBA), Arg335 (HBD) and His298 (HBA). At least one hydrogen bond must match was set up for docking. All the docking calculations were performed by the standard precision mode. The predicted binding modes were chosen by combining with the docking score and the protein ligand interaction pattern.

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