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# Design, synthesis, and biological evaluation of novel tetrahydroprotoberberine derivatives to reduce SREBPs expression for the treatment of hyperlipidemia

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ABSTRACT

Statins play an important role in the treatment of hyperlipidemia, but drug resistance and adverse effects greatly limits their application. To discover new lipid-lowering drugs, three different series of tetrahydroprotoberberine derivatives (THPBs) were designed and synthesized. These compounds were first tested for their effects on viability of HepG2 cells and 21 compounds with the percent of cell viability over 90% were further screened to evaluate their ability to reduce total cholesterol (TC) and triglyceride (TG) levels. Among these derivatives, two compounds displayed significant down-regulation both intracellular of TC and TG content, especially compound **49** exhibited the greatest efficacy. Mechanistically, compound **49** promoted proteasomal degradation of SREBPs. Importantly, compound **49** displayed superior bioavailability (F = 65.1%) and obvious efficacy in the treatment of high fat diet induced obesity *in vivo*. Therefore, compound **49** is a promising candidate to develop new treatment of hyperlipidemia.


## 1. Introduction

With the development of the society and improvement of people's living standards, the diet structure has been remarkably changed, resulting in significantly increased prevalence of hyperlipidemia. Hyperlipidemia is one of the most important risk factors responsible for many cardiovascular and cerebrovascular diseases [1,2], such as atherosclerosis [3], hypertension, stroke, coronary heart disease and so on [4,5]. Hyperlipidemia is characterized by high levels of cholesterol and increased levels of serum triglyceride. Currently, drugs against hyperlipidemia mainly consist of fibrates that promote fatty acid oxidation by PPARα (Peroxisome proliferator-activated receptor α) [6], statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme inhibitors) [7], and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [8]. But even statins, the mainstay treatment for hyperlipidemia, still have many limitations including drug resistance and intolerance due to adverse effects. Therefore, it is an urgent need to develop new drugs with unique mechanism of action.

Sterol regulatory element-binding proteins (SREBPs), including SREBP-1a, SREBP-1c and SREBP-2, play important roles in the uptake and synthesis of cholesterol and fatty acids [9]. SREBP-1a and -1c are processed in Golgi to generate the mature protein, which mainly regulate the expression of genes required for fatty acid synthesis. SREBP-2 is responsible for the synthesis of cholesterol [10]. Accordingly, regulation of SREBPs is a potential strategy to develop novel lipid-lowing drugs [11].

Tetrahydroprotoberberine derivatives (THPBs) are the primary active components originally isolated from the Chinese herb *Corydalis yanhusuo* and various species of *Stephania* [12]. The previous study about THPBs mainly focused on central nervous system diseases [13], while lipid-lowering effects were rarely reported [14].

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0223-5234/

But within a high-throughput screening to discover novel lipid-lowering ligands [15], compound **GZ-1** (Fig. 1A) bearing a THPB scaffold was identified as a top hit, which could reduce total cholesterol (TC) and triglyceride (TG) levels in HepG2 cells (Fig. 1B and C). This result provided us a good lead compound to develop new lipid-lowering ligands. In this work, a series of THPBs with various substituents were synthesized and screened based on their ability to reduce TC and TG levels in HepG2 cells. Compound **49** was discovered as the top candidate which exhibited the most significant effects of reducing TC and TG levels in HepG2 cells. Further mechanistic investigation demonstrated that compound **49** reduced lipid contents through degrading SREBPs. Meanwhile, **49** displayed excellent pharmacokinetic profiles including superior bioavailability and high stability *in vivo*.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic routes for target compounds are illustrated in Scheme 1. The key intermediates **1** and **2** were synthesized through pyrolysis monodemethylation and reduction reactions of palmatine hydrochloride and berberine hydrochloride, respectively, and both has a rotation value of 0, as the previous report [16]. Compounds **3**–**28** and **29**–**45** were synthesized according to the general acetylation reaction procedure by intermediates **1** or **2** and selected acyl chlorides or sulfonyl chlorides. Compounds **46**–**50** were obtained by alkylation reaction of compound **1** and corresponding bromides.

### 2.2. Biological evaluation

#### 2.2.1. Evaluation of all compounds on cell viability

As obese patients tend to take medicine for a long time, the compound toxicity is critical. Firstly, all synthesized THPBs derivatives were screened at a concentration of 10 μM to test their cytotoxicity in HepG2 cells. The structures of THPB derivatives and their corresponding effects on cell viability are summarized in Tables 1–3. The study of structure–activity relationships (SARs) was first focused on the substituent groups of the aromatic ring D. Compounds **3**–**15** (Table 1) bearing different carboxyl substituent groups were designed and synthesized, which displayed little influence on the cell viability in the range of 80.9%–99.6%. Especially, compound **14** bearing a chloro-substituted *tert*-butyl group has almost no toxicity on HepG2 cells. Therefore, compounds **3**, **9**, **11**, and **14** with more 90% of cell viability were chosen for further evaluation on cellular lipid levels and expression of SREBPs.

Besides the modification of ring D, oxacyclopentene of ring A was also opened, and two methoxy groups were introduced to explore their structure–activity relationships. The replacement of oxacyclopentene with two methoxy groups provided compounds **16**–**23**, **26**, and **28** (Table 1), which displayed lower inhibition on cell viability than compounds **3**–**10**, **13**, and **15**, respectively. Especially, cell viability reminded 99.3% at a concentration of 10 μM of compound **26**, which indicated its extremely low toxicity.

After these carboxylate derivatives **3**–**28** were synthesized, a series of sulfonate substituent groups were introduced on the aromatic ring D. Overall, most of THPBs sulfonate derivatives (Table 2) showed stronger inhibition on cell viability when compared to the THPBs carboxylate derivatives, and only compounds **31**, **33**, **42**, **43**, and **45** displayed less than 10% inhibition on the cell viability, which were selected for the further studies. Besides the introduction of a carboxylic ester group and a sulfonate group on the aromatic ring D, a new series of ether derivatives **46**–**50** (Table 3) were designed and synthesized. Generally, these THPB ether derivatives showed little inhibition on cell viability and compounds **46**, **48**, and **49** retained cell viability more than 90%. Such as compound **46**, bearing a propyl ether, displayed almost no toxicity on HepG2 cells at a concentration of 10 μM.

#### 2.2.2. Cellular lipid levels and expression of SREBPs

According to the cell viability assay, 20 compounds with the percent of cell viability over 90% were further assessed for their regulatory efficacy on cellular TC and TG levels (Tables 1–3). HepG2 cells were first treated with these 20 compounds at the concentration of 10 μM, and their TC and TG content were subsequently measured and shown in Fig. 2A and B. Among these 20 compounds, there are 12 THPB carboxylate derivatives. Compared with the dioxymethylene substitution on the ring A, THPB carboxylate derivatives with two methoxy groups are more effective in reducing TC levels. But the overall effect of THPB carboxylate derivatives in reducing TC levels was minimal. Only compound **22** displayed similar effects in reducing TC levels with lead compound **GZ-1**.

For THPB sulfonate derivatives, most of them did not exhibit obvious activity on decreasing TC levels, but compound **43** with two methoxy groups on the ring A displayed potent activity and reduced the TC level by 15%. Compared with compound **33** bearing a dioxymethylene substitution on the ring A, compound **43** with two methoxy groups showed much better activity. This provides us a good strategy for further chemical modification.

Interestingly, THPB ether derivatives showed much better TC-lowering activity than THPB carboxylate and sulfonate derivatives. Especially, compounds **46** and **49** were more effective than lead compound **GZ-1**, which reduced the TC level by 18% and 17%, respectively.

Similar as the regulation of TC levels, THPB carboxylate derivatives with two methoxy groups on the ring A showed more

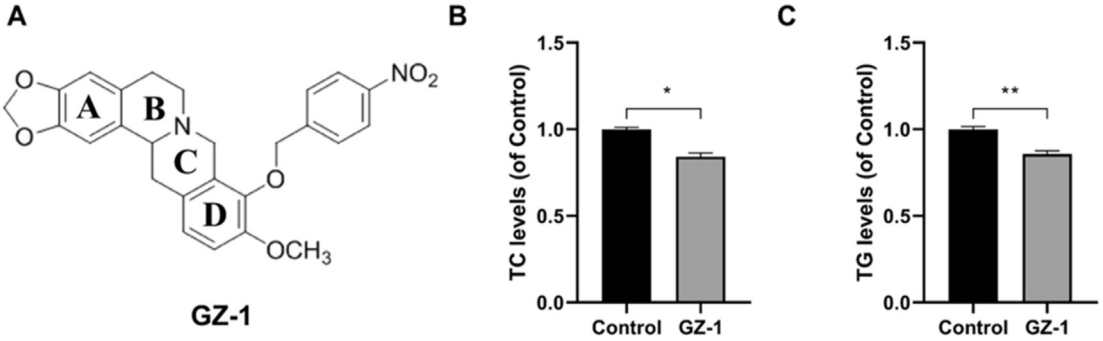
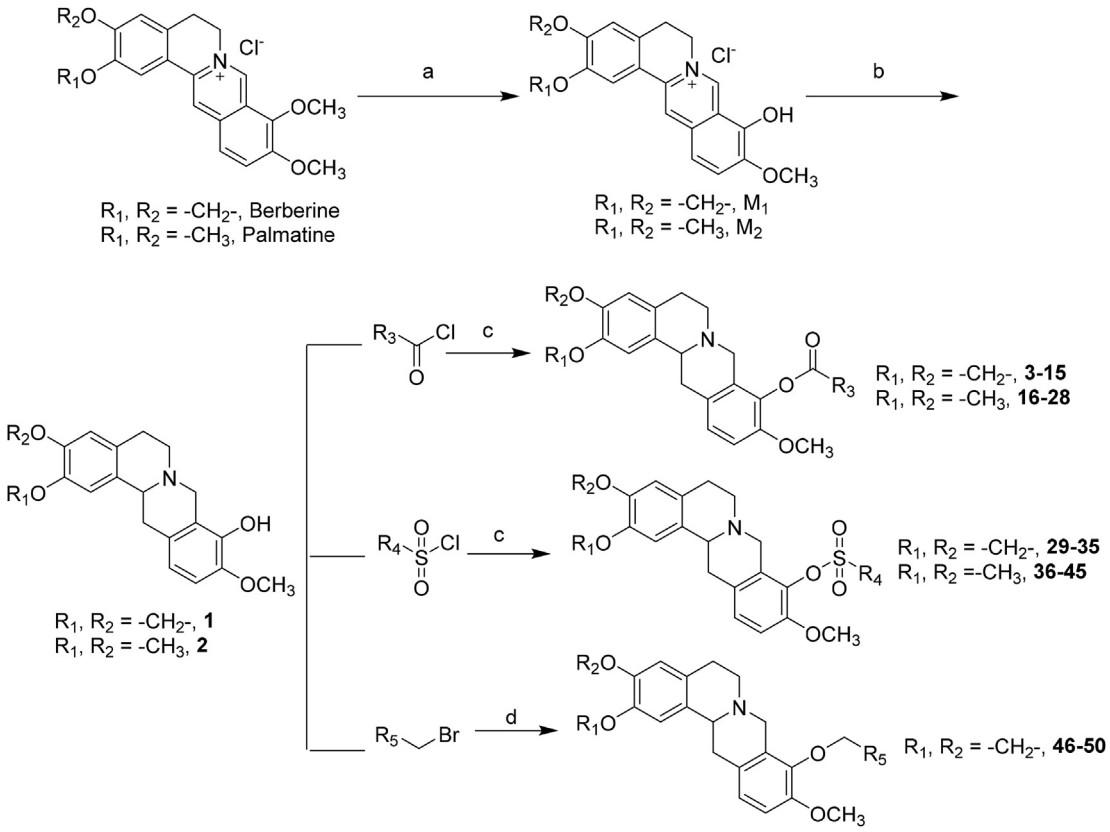



**Fig. 1.** (A) The structure of compound **GZ-1**. (B) Intracellular TC levels assayed in HepG2 cells administered with DMSO or compound **GZ-1**. (C) Intracellular TG levels assayed in HepG2 cells administered with DMSO or compound **GZ-1**.




**Scheme 1.** Synthesis of compounds **3**–**50**. Reagents and conditions: (a) (1) 195–205 °C, 30 min; (2) EtOH-HCl (95:5), rt, 1 h; (b) $NaBH_4$, MeOH, rt, 3 h; (c) $Et_3N$, $CH_2Cl_2$, rt, 4 h; (d) $K_2CO_3$, DMF, 80 °C, 4 h.

remarkable activity on the down-regulation of TG levels than dioxymethylene substitution on the ring A. Among these THPB carboxylate derivatives, only compound **20** displayed better effects in reducing TG levels compared with lead compound **GZ-1**. Compound **43**, which displayed potent activity in reducing TC levels, also demonstrated better TG-lowering effects than lead compound **GZ-1**. All THPB ether derivatives **46**, **48** and **49** had great effects in the down-regulation of TG levels and compound **49** showed the best effect.

In conclusion, compounds **43** and **49** reduced both intracellular TC and TG content, especially compound **49** exhibited the greatest effects. Therefore, compound **49** was chosen for further *in vitro* and *in vivo* biological evaluation and pharmacokinetic studies.

After confirming the favorable effects on cell viability and lipid regulation of compound **49**, its underlying mechanism of down-regulation in intracellular cholesterol and triglyceride levels were next investigated. The Western blot analysis manifested that compound **49** downregulated the precursor and mature forms of SREBP-1 and SREBP-2, in a concentration dependent manner at protein levels in HepG2 cells (Fig. 2C). In addition, compound **49** showed no effects on the expression of PCSK9 (Fig. 2D). As compound **49** reduced the SREBPs in the nucleus, the expression of their target genes, 1-aminocyclopropane-1-carboxylic acid (*ACC*), fatty acid synthase (*FASN*), low-density lipoprotein receptor (*LDLR*) and stearoyl-CoA desaturase (*SCD*) were also significantly reduced (Fig. 2E), which means compound **49** can decrease lipid synthesis.

To further elucidate the mechanism of compound **49** in reducing SREBPs expression, HepG2 cells were treated with cycloheximide, an inhibitor of synthesis on both DNA and protein, then SREBPs amount was measured in different time point to calculate SREBPs degradation rate. Compound **49** displayed notable reduction of SREBPs compared to cells treated with cycloheximide and vehicle, which meant compound **49** increased SREBPs degradation (Fig. 2F). MG-132, a proteasome inhibitor, reversed the reduction of SREBPs in HepG2 cells treated with compound **49** (Fig. 2G). These results indicated that compound **49** promoted SREBPs proteasomal degradation. Moreover, the immunoprecipitation results demonstrated that compound **49** increased the ubiquitination of SREBPs, leading to accelerated degradation of SREBPs though the ubiquitin proteasome system (Fig. 2H).

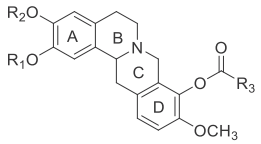
### 2.3. Pharmacokinetic properties of Compound *49*

The pharmacokinetic evaluation of compound **49** was conducted to further examine its drugability. SD rats were orally administrated (10 mg/kg) or intravenous injected (1 mg/kg) **49**, and the pharmacokinetic results are shown in Table 4. When compound **49** was administered by intravenous injection, its maximum plasma concentration ($C_{max}$) was 900.7 ng/mL and **$AUC_{0-\infty}$** value was 658.9 h*ng/mL. The oral administration of compound **49** demonstrated $C_{max}$ was 428.3 ng/mL and $AUC_{0-\infty}$ value was 4290 h*ng/mL. Meanwhile, compound **49** displayed great half-life ($t_{1/2}$) both through intravenous and oral administration, with the values of 4.95 and 5.36 h, respectively. Moreover, compound **49** exhibited a good oral bioavailability of 65.1%. All these pharmacokinetic parameters indicated the good drugability of compound **49**, which deserved further research.

### 2.4. Effect of compound *49* on high fat diet induced obesity

Due to the good *in vitro* activity and favorable drugability, we further investigated the lipid-lowing effects of compound **49**

**Table 1**
Effects of THPB carboxylate derivatives on HepG2 cell viability.



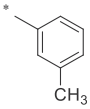
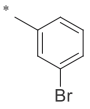
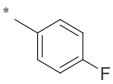
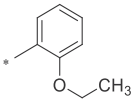
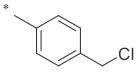
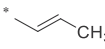
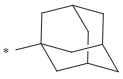
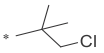
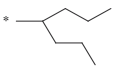
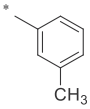
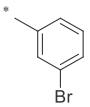
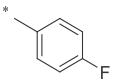
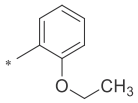
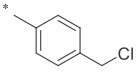
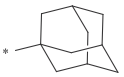
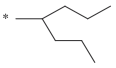

| ID | $R_1$ | $R_2$ | $R_3$ | Cell Viability |
|---|---|---|---|---|
| **3** | **-$CH_2$-** | |  | 90.6% |
| **4** | **-$CH_2$-** | |  | 88.2% |
| **5** | **-$CH_2$-** | |  | 85.5% |
| **6** | **-$CH_2$-** | |  | 80.9% |
| **7** | **-$CH_2$-** | |  | 87.7% |
| **8** | **-$CH_2$-** | |  | 85.0% |
| **9** | **-$CH_2$-** | |  | 90.7% |
| **10** | **-$CH_2$-** | |  | 83.9% |
| **11** | **-$CH_2$-** | |  | 90.1% |
| **12** | **-$CH_2$-** | |  | 85.0% |
| **13** | **-$CH_2$-** | |  | 85.6% |
| **14** | **-$CH_2$-** | |  | 99.6% |
| **15** | **-$CH_2$-** | |  | 86.3% |
| **16** | **-$CH_3$** | **-$CH_3$** |  | 96.0% |
| **17** | **-$CH_3$** | **-$CH_3$** |  | 97.8% |
| **18** | **-$CH_3$** | **-$CH_3$** |  | 84.3% |
| **19** | **-$CH_3$** | **-$CH_3$** |  | 80.2% |

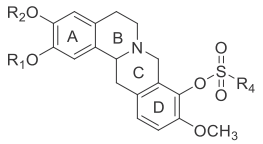
**Table 1** (*continued*)

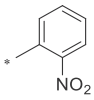
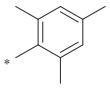
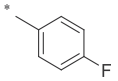
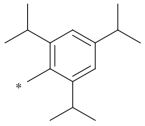
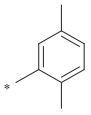
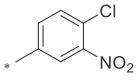
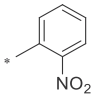
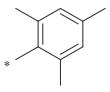
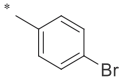
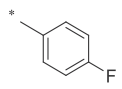
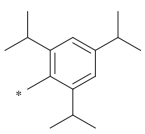
| ID | $R_1$ | $R_2$ | $R_3$ | Cell Viability |
|---|---|---|---|---|
| **20** | **-$CH_3$** | **-$CH_3$** |  | 95.1% |
| **21** | **-$CH_3$** | **-$CH_3$** |  | 95.4% |
| **22** | **-$CH_3$** | **-$CH_3$** |  | 92.1% |
| **23** | **-$CH_3$** | **-$CH_3$** |  | 84.6% |
| **24** | **-$CH_3$** | **-$CH_3$** |  | 88.3% |
| **25** | **-$CH_3$** | **-$CH_3$** |  | 81.7% |
| **26** | **-$CH_3$** | **-$CH_3$** |  | 99.3% |
| **27** | **-$CH_3$** | **-$CH_3$** |  | 91.2% |
| **28** | **-$CH_3$** | **-$CH_3$** |  | 97.9% |

*in vivo*. C57BL/6J mice were fed with chow or high fat diet (HFD) for 15 weeks. After that, HFD-fed mice were treated with vehicle (1% CMC-Na solution) or compound **49** (low-dose 170 mg/kg/day and high-dose 340 mg/kg/day) for additional 6 weeks (Fig. 3A). During the treatment, no obvious toxicity was observed and mice fed with HFD had similar food intake (Fig. 3B and D). Compound **49** dramatically reduced bodyweight of HFD-fed mice, and high dosage of compound **49** treated mice were even lighter than the chow diet-fed mice (Fig. 3B and C). Notably, liver weight in compound **49** treated mice decreased (Fig. 2B and E). Consistently, epididymal fat/body weight ratio also dropped in compound **49** treated mice, while brown fat/body weight ratio slightly raised only in high dosage of compound **49** treated mice (Fig. 3B, F and 3G). H&E and Oil red O section staining revealed that the livers of compound **49** treated mice contained lower lipid accumulation compared with the livers of vehicle treated mice (Fig. 3H). Histological analysis showed that compound **49** reduced the cell size of both white adipocyte tissue (WAT) and brown adipocyte tissue (BAT) (Fig. 3H). The H&E staining of heart, spleen, lung, and kidney showed none obvious side effects on compound **49** treated mice (Fig. 3I).

Glucose tolerance and insulin resistance were markedly ameliorated in compound **49** treated HFD-fed mice (Fig. 4A and B). The serum TC and TG levels in compound **49** treated mice were significantly lower than those of vehicle treated mice (Fig. 4C and D). Consistent with the results in HepG2 cells above, SREBPs were significantly decreased in livers of compound **49** treated mice, compared to vehicle treated mice (Fig. 4E). The expression of their target genes, *Acc*, *Fasn*, *Ldlr* and *Scd* were also reduced (Fig. 4F). Collectively, these data demonstrated that compound **49** improves high fat diet induced hyperlipidemia in C57BL/6J mice through reducing SREBPs.

**Table 2**
Effects of THPB sulfonate derivatives on HepG2 cell viability.



| ID | $R_1$ | $R_2$ | $R_4$ | Cell Viability |
|---|---|---|---|---|
| **29** | **-$CH_2$-** | |  | 88.8% |
| **30** | **-$CH_2$-** | |  | 80.7% |
| **31** | **-$CH_2$-** | |  | 93.4% |
| **32** | **-$CH_2$-** | |  | 86.8% |
| **33** | **-$CH_2$-** | |  | 92.8% |
| **34** | **-$CH_2$-** | |  | 89.5% |
| **35** | **-$CH_2$-** | |  | 84.5% |
| **36** | **-$CH_3$** | **-$CH_3$** |  | 84.8% |
| **37** | **-$CH_3$** | **-$CH_3$** |  | 73.3% |
| **38** | **-$CH_3$** | **-$CH_3$** |  | 86.5% |
| **39** | **-$CH_3$** | **-$CH_3$** |  | 80.2% |
| **40** | **-$CH_3$** | **-$CH_3$** |  | 87.7% |
| **41** | **-$CH_3$** | **-$CH_3$** |  | 89.2% |
| **42** | **-$CH_3$** | **-$CH_3$** |  | 93.4% |
| **43** | **-$CH_3$** | **-$CH_3$** |  | 91.1% |

(*continued on next page*)

**Table 2** (*continued*)

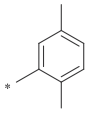
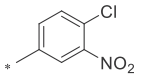
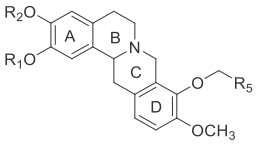
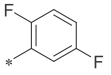
| ID | $R_1$ | $R_2$ | $R_4$ | Cell Viability |
|---|---|---|---|---|
| **44** | **-$CH_3$** | **-$CH_3$** |  | 81.4% |
| **45** | **-$CH_3$** | **-$CH_3$** |  | 94.7% |

**Table 3**
Effects of THPB ether derivatives on HepG2 cell viability.



| ID | $R_1$ | $R_2$ | $R_5$ | Cell Viability |
|---|---|---|---|---|
| 46 | -$CH_2$- | |  | 101.5% |
| **47** | **-$CH_2$-** | |  | 87.7% |
| **48** | **-$CH_2$-** | |  | 98.1% |
| **49** | **-$CH_2$-** | |  | 93.0% |
| **50** | **-$CH_2$-** | |  | 87.7% |

## 3. Conclusions

In this study, three different series of THPBs were designed, synthesized and preliminary screened based on effects on viability of HepG2 cells. The further screen by examining ability of decreasing TC and TG in HepG2 cells led to the identification of compound **49**, which exhibited greatest downregulating effects on TC and TG and reduced SREBPs expression through promoting proteasomal degradation of SREBPs. In particularly, compound **49** displayed an excellent pharmacokinetic profile and great effects in improving high fat diet induced obesity *in vivo*. In conclusion, compound **49** deserves further research in battle with hyperlipidemia.

## 4. Experimental section

### *4.1. 4.1 Chemistry*

Thin-layer chromatography (TLC) was performed on silica gel GF254 plates of 0.5 mm of thickness for analysis. Layers were air dried and activated at 110 °C for 0.5 h before use. NMR spectra ($^1$H NMR, $^{13}$C NMR) were recorded on a Bruker Avance 500 or 400 or 300 MHz spectrometer with tetramethylsilane as the internal standard, and chemical shifts were expressed in $\delta$ (parts per million). HR-ESI-MS experiments were performed on Waters Xevo G2-XS QTOF mass spectrometer.

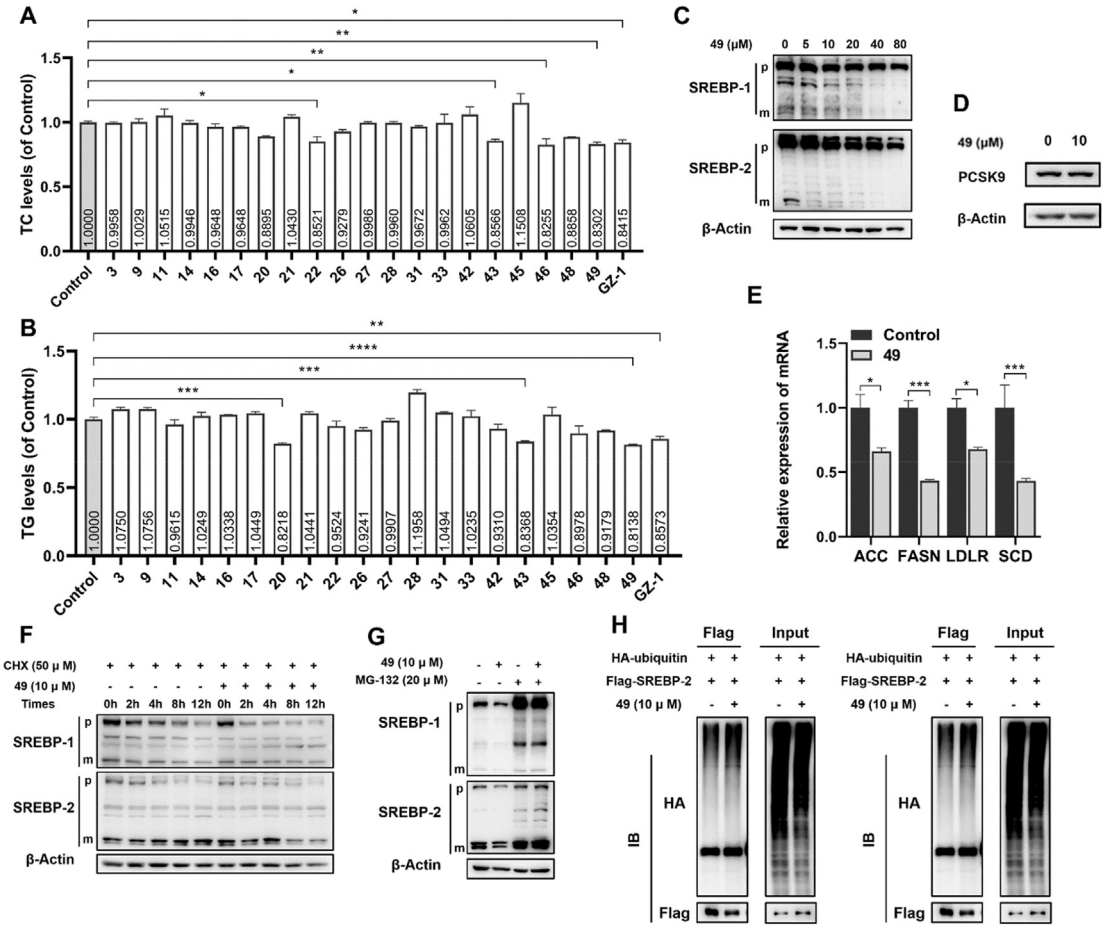



**Fig. 2.** (A) Intracellular TC levels assayed in HepG2 cells. (B) Intracellular TG levels assayed in HepG2 cells. (C) The expression of SREBP-1 and SREBP-2 in HepG2 cells with compound **49** at the indicated doses for 24 h was detected by Western blot. β-actin was used as an endogenous reference. (D) The expression of PCSK9 in HepG2 cells with compound **49** at the indicated doses for 24 h was detected by Western blot. β-actin was used as an endogenous reference. (E) The expression of *ACC*, *FASN*, *LDLR* and *SCD* was assessed by qRT-PCR in HepG2 cells. Expression was normalized to *GAPDH*. Error bars are represented as mean ± SEM. Statistical analyses were done with one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001 vs control. (F) After incubation with 50 μM cycloheximide for 1 h, cells were switched to medium supplemented with 50 μM cycloheximide plus vehicle, or 10 μM compound **49** for indicated period of time. Cells were lysed and SREBPs degrading rate were measured by Western blot. (G) After HepG2 cells were treated with 10 μM MG-132 for 1 h, cells were switched to medium supplemented with 10 μM MG-132 plus vehicle, or 10 μM compound **49** for 4 h. Cells were lysed and SREBPs were detected by Western blot. (H) 293T cells were transfected with flag-SREBP-1 or flag-SREBP-2 and HA-ubiquitin for 24 h, after the treatment, the cells were incubated with medium containing compound **49** for another 24 h. Cells were lysed and pulled down by flag antibody. Ubiquitylated SREBPs were detected by Western blot.

**Table 4**
Pharmacokinetic parameters of compound **49** in SD rats.

| Compd. | Admin. | $C_{max}$ (ng/mL) | $AUC_{0-\infty}$ (h*ng/mL) | $MRT_{0-\infty}$ (h) | $T_{max}$ (h) | $t_{1/2}$ (h) | CL (mL/h/kg) | F (%) |
|---|---|---|---|---|---|---|---|---|
| 49 | IV | 900.7 | 658.9 | 3.18 | 0.033 | 4.95 | 1545 | – |
| | PO | 428.3 | 4290 | 8.03 | 6.67 | 5.36 | 511 | 65.1 |

[a]Values are the average of three runs. $C_{max}$, maximum concentration; AUC, area under the plasma concentrationtime curve; MRT, mean residence time; $t_{1/2}$, half-life; CL, clearance; F, oral bioavailability. Dose: p.o. at 10 mg/kg. Dose: i.v. at 1 mg/kg.

### *4.1.1. General synthetic procedures for the target compounds **3**–**28** (compound **3** as the example)*

***4.1.1.1.*** *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl cyclohexanecarboxylate (**3**).* The intermediate 1 (0.5 g, 1.5 mmol) was added to dichloromethane (DCM, 30 mL) and stirred for dissolving at room temperature, Cyclohexanecarboxylic acid chloride (0.2 mL, 1.5 mmol), 2 mL triethylamine and slight 4-dimethylamiopryidine were added to the solution. The mixture was stirred for 4 h at room temperature, then washed three times by water, saturated $NaHCO_3$ solution and saturated NaCl solution, respectively. The organic layer was dried over sodium sulfate, filtered, the solvent was evaporated in vacuum. The residues were purified via silica gel chromatography ($CH_2Cl_2$/MeOH) to give the product **3** (0.46 g, 71%).

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.41–1.25 (m, 3H), 1.63–1.49 (m, 3H), 1.76–1.72 (m, 2H), 2.00–1.97 (m, 2H), 2.49–2.43 (m, 1H), 2.66–2.53 (m, 3H), 2.92–2.86 (m, 1H), 3.06–3.03 (m, 1H), 3.25–3.28 (m, 1H), 3.37–3.33 (m, 1H), 3.42–3.40 (m, 1H), 3.72 (s, 3H), 3.82 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H),6.92 (s, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 172.66, 148.60, 145.67, 145.41, 135.48, 130.64, 127.88, 127.43, 127.39, 126.02, 110.71, 107.99, 105.65, 100.47, 58.82, 55.86, 52.76, 50.47, 41.83, 40.33, 35.42, 28.90, 28.56, 28.56, 25.22, 24.59, 24.59. HR-ESI-MS: ($m/z$) 436.2138 $[M+H]^+$, (Calcd:

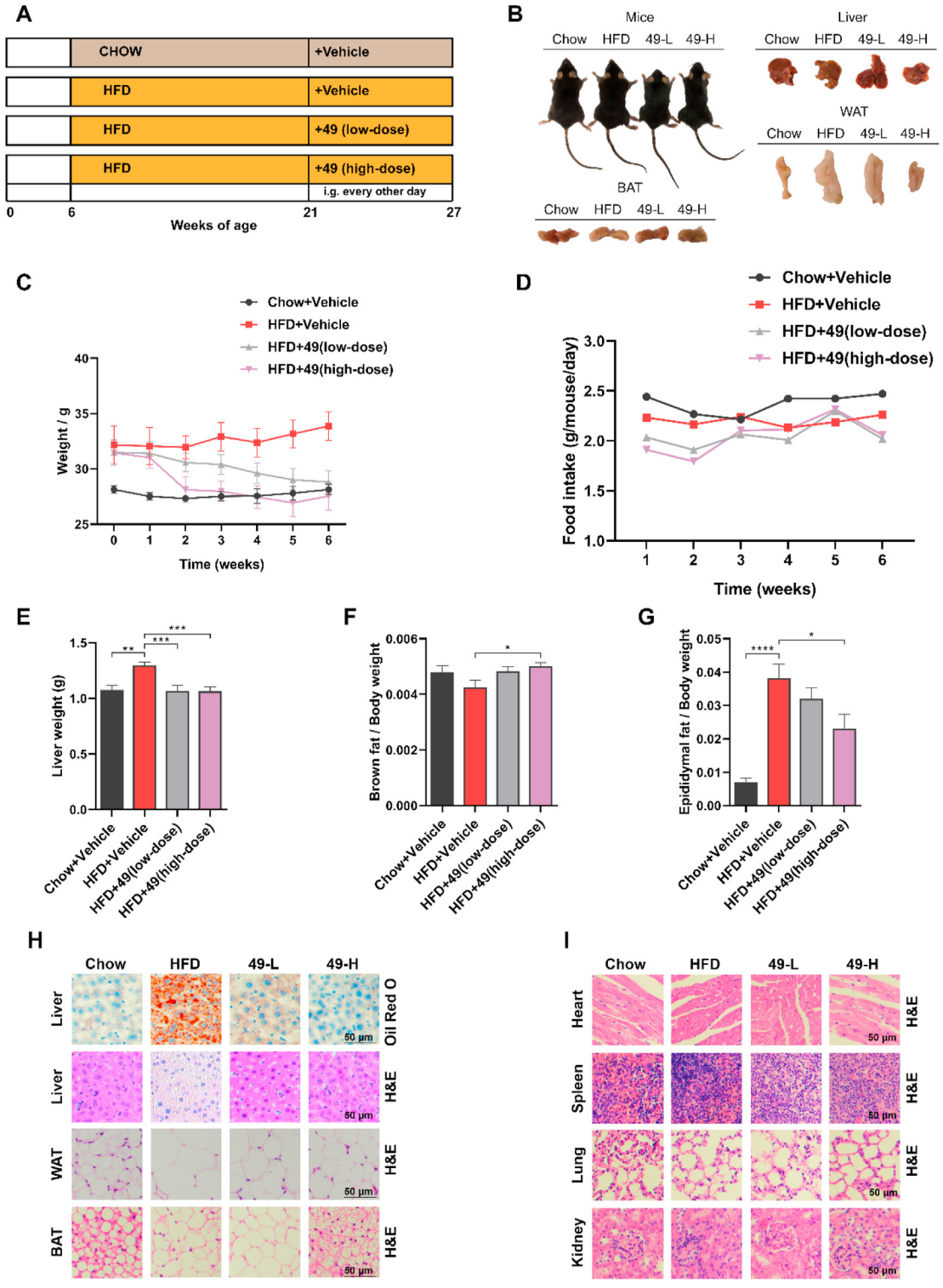



**Fig. 3.** (A) Male C57BL/6J mice at 6 weeks of age were fed with chow or HFD for 15 weeks. Then vehicle, 49(low-dose) and **49**(high-doge) were administrated to mice by oral gavage for another 6 weeks. (B) Macroscopic images of mice, their respective livers, WAT and BAT. (C) Body weight per mouse, (D) Food intake per mouse, (E) Liver weight, (F) epididymal fat/body weight ratio and (G) brown fat/body weight ratio during the experiments. (H) Histologic analysis of liver, white adipose tissue (WAT), and white adipose tissue (BAT). Liver tissues sections were stained with oil red O or H&E to visualize lipid contents. WAT and BAT were stained with H&E. Bars = 50 μm. (I) Histologic analysis of heart, spleen, lung, and kidney. The tissues sections were stained with H&E. Bars = 50 μm.

436.2118);

***4.1.1.2.*** *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl furan-2-carboxylate (**4**).* Light yellow solid, Yield: 24%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.45–2.40 (m, 1H), 2.62–2.56 (m, 2H), 2.91–2.85 (m, 1H), 3.04–3.02 (m, 1H), 3.35–3.32 (m, 1H), 3.36–3.40 (m, 1H), 3.43–3.45 (m, 1H), 3.74 (s, 3H), 3.90 (d, $J$ = 16.0 Hz, 1H), 5.95 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H),

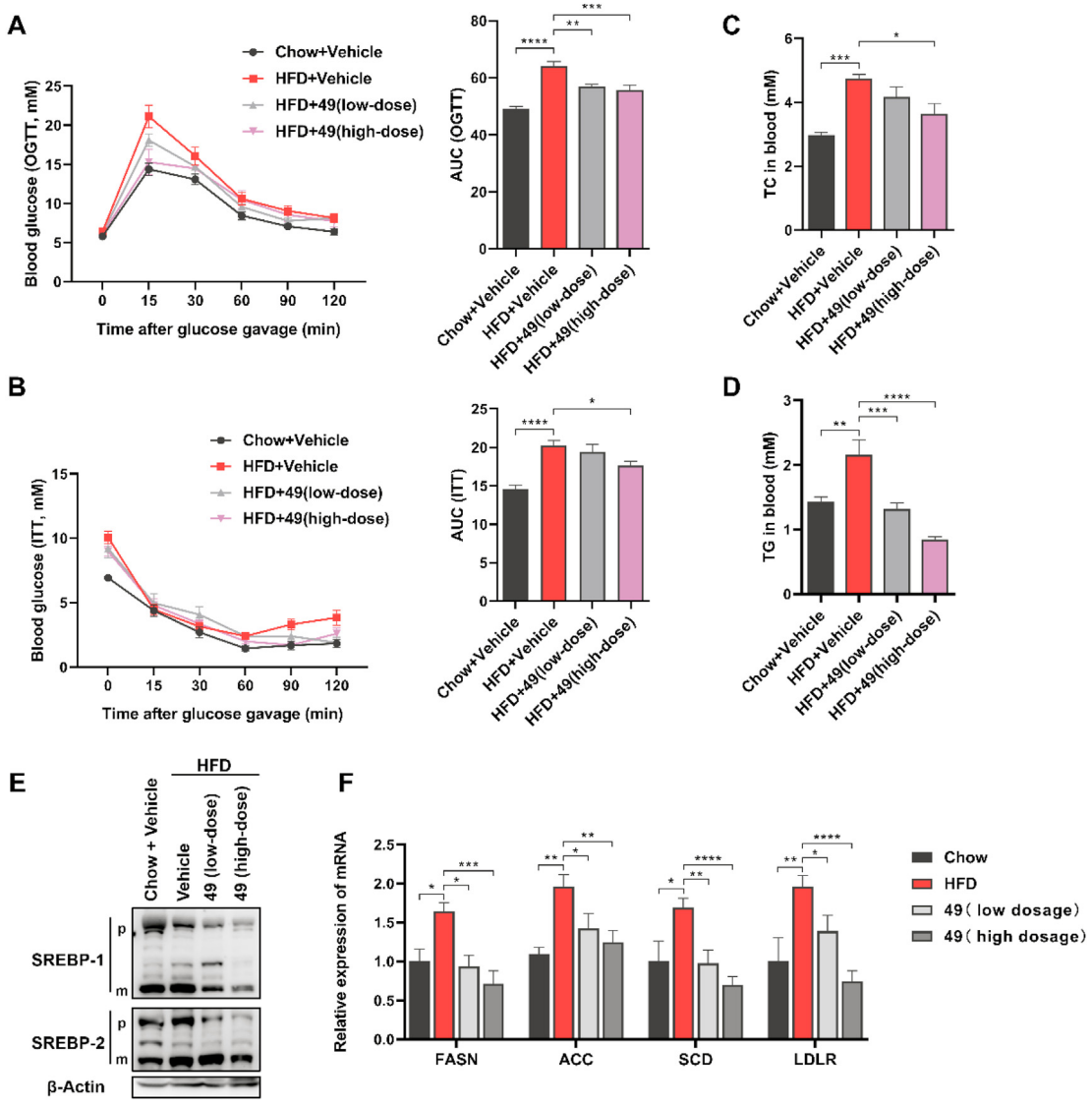



**Fig. 4.** (A) Effect of compound **49** on oral glucose tolerance in HFD-fed mice as determined by oral glucose tolerance test (OGTT). Quantification of the area under the curve (AUC) from the OGTT. (B) Effect of compound **49** on insulin resistance as determined by insulin tolerance test (ITT). Quantification of the AUC of the ITT. (C) TC levels in serum. (E) TG levels in serum. (G) The Western blot analysis of SREBPs in mouse liver tissues. (H) The relative levels of SREBPs target genes in mouse liver tissues. Error bars are represented as mean ± SEM. Statistical analyses were done with one-way ANOVA (Dunnett's post test). *p < 0.05, **p < 0.01, ***p < 0.001 vs HFD.

6.81–6.80 (m, 1H), 6.93 (s, 1H), 7.02 (d, $J$ = 8.0 Hz, 1H), 7.09 (d, $J$ = 8.0 Hz, 1H), 7.58 (d, $J$ = 3.0 Hz, 1H), 8.10 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 155.29, 148.68, 145.69, 145.41, 142.56, 134.61, 130.58, 128.89, 128.15, 127.39, 127.62, 107.98, 126.60, 120.21, 112.67, 110.80, 105.65, 100.47, 58.83, 55.85, 52.69, 50.35, 48.59, 35.42. HR-ESI-MS: ($m/z$) 420.1437 [M+H] $^+$, (Calcd: 420.1442).

***4.1.1.3***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 3-methylbenzoate (**5**).* White solid, Yield: 56%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.43–2.39 (m, 1H), 2.63–2.55 (m, 2H), 2.91–2.85 (m, 1H), 3.04–3.02 (m, 1H), 3.46–3.34 (m, 3H), 3.72 (s, 3H), 3.92 (br, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H), 6.93 (s, 1H), 7.02 (d, $J$ = 8.5 Hz, 1H), 7.09 (d, $J$ = 8.5 Hz, 1H), 7.50 (t, $J$ = 8.0 Hz, 1H), 7.57 (t, $J$ = 8.0 Hz, 1H), 7.94 (d, $J$ = 8.0 Hz, 1H), 7.96 (s, 1H). $^{13}$CNMR (75 MHz, DMSO) δ 163.63, 148.69, 145.70, 145.43, 138.52, 135.45, 134.62, 130.62, 130.12, 128.88, 128.41, 128.09, 127.57, 127.42, 126.98, 126.41, 110.76, 108.01, 105.68, 100.50, 58.88, 55.84, 52.76, 50.41 35.49, 28.91, 20.72. HR-ESI-MS: ($m/z$)444.1808 [M+H] $^+$, (Calcd: 444.1805).

***4.1.1.4***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 3-bromobenzoate (**6**).* White solid, Yield: 68%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.42–2.39 (m, 1H), 2.63–2.55 (m, 2H), 2.91–2.85 (m, 1H), 3.04–3.02 (m, 1H), 3.46–3.37 (m, 3H), 3.73 (s, 3H), 3.96 (br, 1H), 5.95 (d, $J$ = 1.0 Hz, 2H), 6.65 (s, 1H), 6.93 (s, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.10 (d, $J$ = 8.5 Hz, 1H), 7.6 (t, $J$ = 8.0 Hz, 1H), 7.97 (d, $J$ = 8.0 Hz, 1H), 8.15 (d, $J$ = 8.0 Hz, 1H), 8.25 (s, 1H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 162.27, 148.52, 145.69, 145.42, 136.79, 135.22, 132.08, 131.28, 130.61, 130.60, 128.83, 128.06, 127.63, 127.41, 126.61, 122.05, 110.78, 107.99, 105.66, 100.48, 58.84, 55.89, 52.59, 50.33, 35.44, 28.89. HR-ESI-MS: ($m/z$) 508.0746 [M+H] $^+$, (Calcd: 508.0754).

***4.1.1.5***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 4-fluorobenzoate (**7**).* White solid, Yield: 88%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.43–2.39 (m, 1H),

2.63–2.55 (m, 2H), 2.91–2.84 (m, 1H), 3.02–3.00 (m, 1H), 3.46–3.37 (m, 3H), 3.73 (s, 3H), 3.92 (br, 1H), 5.95 (s, 2H), 6.65 (s, 1H), 6.93 (s, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.09 (d, $J$ = 8.5 Hz, 1H), 7.45 (d, $J$ = 8.5 Hz, 2H), 8.23 (d, $J$ = 8.0 Hz, 2H). $^{13}$CNMR (125 MHz, DMSO-$d_6$) $\delta$: 166.5, 162.6, 148.6, 145.7, 145.4, 135.3, 132.8, 132.7, 130.6, 128.1, 127.6, 127.4, 126.5, 125.0, 116.2, 116.1, 110.8, 107.9, 105.6, 100.5, 58.8, 55.9, 52.7, 50.4, 35.4, 28.9. HR- ESI-MS: ($m/z$) 448.1567 [M+H] $^+$, (Calcd: 448.1555);

***4.1.1.6***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl methacrylate (**8**).* Light yellow solid, Yield: 56%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.01 (s, 3H), 2.44–2.41 (m, 1H), 2.60–2.55 (m, 2H), 2.92–2.86 (m, 1H), 3.06–3.03 (m, 1H), 3.43–3.31 (m, 3H), 3.73 (s, 3H), 3.84 (br, 1H), 5.89 (s, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.30 (s, 1H), 6.66 (s, 1H), 6.92 (s, 1H), 6.98 (d, $J$ = 8.5 Hz, 1H), 7.05 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 164.21, 148.65, 145.69, 145.42, 135.41, 134.76, 130.62, 127.96, 127.72, 127.50, 127.39, 126.24, 110.71, 107.99, 105.65, 100.49, 58.85, 55.83, 52.70, 50.43, 35.45, 28.92, 18.03. HR-ESI-MS: ($m/z$) 394.1631 [M+H] $^+$, (Calcd: 394.1649).

***4.1.1.7***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl cyclopropanecarboxylate (**9**).* White solid, Yield: 46%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.07–1.01 (m, 4H), 1.88–1.93 (m, 1H), 2.47–2.43 (m, 1H), 2.61–2.53 (m, 2H), 2.93–2.87 (m, 1H), 3.07–3.05 (m, 1H), 3.28–3.30 (m, 1H), 3.36–3.33 (m, 1H), 3.42–3.40 (m, 1H), 3.73 (s, 3H), 3.82 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.91 (s, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 171.73, 148.73, 145.69, 145.42, 135.37, 130.62, 127.99, 127.43, 127.39, 126.12, 110.67, 108.00, 105.65, 100.49, 58.81, 55.81, 52.75, 50.45, 35.44, 28.92, 12.25, 8.71, 8.71. HR-ESI-MS: ($m/z$) 394.1631 [M+H] $^+$, (Calcd: 394.1649).

***4.1.1.8***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2-ethoxybenzoate (**10**).* Light yellow solid, Yield: 72%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.37 (t, $J$ = 7 Hz, 3H), 2.48–2.43 (m, 1H), 2.63–2.57 (m, 2H), 2.94–2.87 (m, 1H), 3.04–3.02 (m, 1H), 3.47–3.37 (m, 3H), 3.75 (s, 3H), 3.96 (br, 1H), 4.18 (q, 2H, $J$ = 7.0 Hz), 5.95 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.93 (s, 1H), 7.0 (d, $J$ = 8.5 Hz, 1H), 7.08 (m, 2H), 7.21 (d, $J$ = 8.5 Hz, 1H), 7.61 (m, 1H), 7.85 (d, $J$ = 8.0 Hz, 1H).$^{13}$C NMR (75 MHz, DMSO) δ 163.28, 157.92, 148.74, 145.67, 145.39, 135.56, 134.10, 131.11, 130.60, 128.05, 127.50, 127.34, 126.18, 120.12, 119.12, 113.66, 110.74, 107.96, 105.64, 100.46, 64.08, 58.86, 55.84, 53.06, 50.50, 35.47, 28.89, 14.59. HR-ESI-MS: ($m/z$) 474.1912 [M+H] $^+$, (Calcd: 474.1911).

***4.1.1.9***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 4-(chloromethyl)benzoate (**11**).* White solid, Yield: 26%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.44–2.39 (m, 1H), 2.63–2.51 (m, 2H), 2.92–2.85 (m, 1H), 3.04–3.01 (m, 1H), 3.46–3.40 (m, 3H), 3.73 (s, 3H), 3.95 (br, 1H), 4.91 (s, 2H), 5.96 (d, $J$ = 2.0 Hz, 2H), 6.67 (s, 1H), 6.96 (s, 1H), 7.04 (d, $J$ = 8.5 Hz, 1H), 7.11 (d, $J$ = 8.5 Hz, 1H), 7.68 (d, $J$ = 8.2 Hz, 2H), 8.17 (d, $J$ = 8.2 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO) δ 163.68, 149.19, 146.26, 145.99, 144.22, 135.89, 131.15, 130.76, 130.76, 130.76, 129.85, 129.85, 129.85, 128.72, 127.95, 127.04, 111.32, 108.55, 106.23, 101.06, 59.43, 56.41, 53.30, 50.93, 45.65, 36.01, 29.44. HR-ESI-MS: ($m/z$) 478.1423 [M+H] $^+$, (Calcd: 478.1416).

***4.1.1.10***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl (E)-but-2-enoate(**12**).* White solid, Yield: 59%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.96–1.95 (m, 3H), 2.45–2.40 (m, 1H), 2.59–2.54 (m, 2H), 2.88–2.86 (m, 1H), 3.04–3.02 (m, 1H), 3.27–3.25 (m, 1H), 3.37–3.34 (m, 1H), 3.42–3.40 (m, 1H), 3.72 (s, 3H), 3.82 (m, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.15 (dd, $J$ = 1.2 Hz, 12.5 Hz, 1H), 6.65 (s, 1H), 6.92 (s, 1H), 6.96 (d, $J$ = 8.5 Hz, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.12 (m, 1H). $^{13}$C NMR (126 MHz, DMSO) δ 163.66, 149.25, 148.39, 146.24, 145.97, 135.83, 131.17, 128.56, 127.99, 127.95, 126.70, 121.55, 111.18, 108.54, 106.21, 101.05, 59.42, 56.30, 53.39, 51.01, 36.02, 29.47, 18.43. HR-ESI-MS: ($m/z$) 394.1631 [M+H] $^+$, (Calcd: 394.1649).

***4.1.1.11***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl (3r,5r,7r)-adamantane-1-carboxylate (**13**).* White solid, Yield: 66%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$1.74 (s, 6H), 2.01 (s, 6H), 2.04 (s, 3H), 2.46–2.42 (m, 1H), 2.60–2.55 (m, 2H), 2.92–2.87 (m, 1H), 3.05–3.03 (m, 1H), 3.27–3.26 (m, 1H), 3.36–3.33 (m, 1H), 3.42–3.40 (m, 1H), 3.71 (s, 3H), 3.78 (br, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.92 (s, 1H), 6.94 (d, $J$ = 8.5 Hz, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO) δ 174.65, 149.16, 146.24, 145.97, 136.08, 131.21, 128.41, 127.99, 127.96, 126.52, 111.29, 108.55, 106.21, 101.04, 59.38, 56.51, 53.25, 51.09, 49.08, 41.02, 38.85, 38.85, 38.85, 36.38, 36.38, 35.97, 29.46, 27.81, 27.81, 27.81. HR-ESI-MS: ($m/z$) 488.2433 [M+H] $^+$, (Calcd: 488.2431).

***4.1.1.12***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 3-chloro-2,2-dimethylpropanoate(**14**).* White solid, Yield: 62%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.39 (s, 6H), 2.47–2.42 (m, 1H), 2.61–2.54 (m, 2H), 2.89–2.86 (m, 1H), 3.01–2.98 (m, 1H), 3.42–3.32 (m, 3H), 3.72 (s, 3H), 3.93–3.89 (m, 3H), 5.94 (s, 2H), 6.66 (s, 1H), 6.92 (s, 1H), 6.96 (d, $J$ = 8.5 Hz, 1H), 7.04 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO) δ 172.68, 149.05, 146.24, 145.98, 135.88, 131.17, 128.50, 128.01, 127.95, 126.86, 111.31, 108.56, 106.21, 101.05, 59.38, 56.49, 53.48, 52.23, 51.14, 45.16, 35.94, 29.44, 23.37, 23.37. HR-ESI-MS: ($m/z$) 444.1592 [M+H] $^+$, (Calcd: 444.1572).

***4.1.1.13***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2-propylpentanoate (**15**).* Light yellow solid, Yield: 51%. $^1$H NMR (300 MHz, DMSO) δ 0.97–0.92 (m, 6H), 1.56–1.38 (m, 6H), 1.70–1.64 (m, 2H), 2.49–2.40 (m, 1H), 2.65–2.55 (m, 3H), 2.99–2.92 (m, 2H), 3.41–3.24 (m, 3H), 3.70 (s, 3H), 3.84–3.79 (m, 1H), 5.94 (s, 2H), 6.65 (s, 1H), 6.91 (s, 1H), 6.94 (d, $J$ = 8.5 Hz, 1H), 7.02 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 172.92, 148.55, 145.68, 145.42, 135.43, 130.55, 127.73, 127.41, 127.32, 126.12, 110.68, 107.97, 105.62, 100.48, 58.76, 55.67, 53.06, 50.56, 44.43, 35.41, 34.15, 34.15, 28.86, 19.90, 19.84, 13.76, 13.76. HR-ESI-MS: ($m/z$) 452.2417 [M+H]$^+$, (Calcd: 452.2431).

***4.1.1.14***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino[3,2-a]isoquinolin-9-ylcycloh exanecarboxylate (**16**).* White solid, Yield: 42%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.41–1.23 (m, 3H), 1.64–1.51 (m, 3H), 1.76–1.73 (m, 2H), 2.00–1.98 (m, 2H), 2.48–2.43 (m, 1H), 2.66–2.58 (m, 3H), 2.95–2.89 (m, 1H), 3.06–3.04 (m, 1H), 3.28–3.26 (m, 1H), 3.43–3.40 (m, 2H), 3.72 (s, 6H), 3.75 (s, 3H), 3.83 (d, $J$ = 16.0 Hz, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 7.04 (d, $J$ = 8.5 Hz, 1H). $^{13}$CNMR (125 MHz, DMSO-$d_6$) $\delta$: 172.57, 148.57, 147.18, 135.48, 129.46, 127.94, 127.54, 126.27, 125.95, 111.72, 110.70, 109.45, 58.54, 55.84, 55.69, 55.36, 52.83, 50.64, 41.80, 35.30, 28.53, 28.49, 28.43, 25.18, 24.55, 24.54. HR-ESI-MS: ($m/z$) 452.2417 [M+H] $^+$,(Calcd: 452.2431).

***4.1.1.15***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino[3,2-a]isoquinolin-9-yl furan-2-carboxylate(**17**).* White solid, Yield: 22%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.45–2.41 (m, 1H), 2.63–2.57 (m, 2H), 2.93–2.88 (m, 1H), 3.05–3.03 (m, 1H), 3.36–3.33 (m, 1H), 3.46–3.44 (m, 2H), 3.72 (s, 3H), 3.74 (s, 3H), 3.76 (s, 3H), 3.91 (d,

$J$ = 15.0 Hz, 1H), 6.67 (s, 1H), 6.81–6.80 (m, 1H), 6.89 (s, 1H), 7.02 (d, $J$ = 8.5 Hz, 1H), 7.11 (d, $J$ = 8.5 Hz, 1H), 7.58 (d, $J$ = 3.5 Hz, 1H), 8.10 (s, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 155.87, 149.19, 149.16, 147.73, 147.73, 143.10, 135.15, 129.95, 128.78, 128.30, 127.12, 126.83, 120.80, 113.24, 112.23, 111.31, 109.97, 59.14, 56.37, 56.25, 55.92, 53.36, 51.11, 35.91, 29.01. HR-ESI-MS: ($m/z$) 436.1753 [M+H] $^+$, (Calcd: 436.1755).

***4.1.1.16***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 3-methylbenzoate (**18**).* White solid, Yield: 78%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.42–2.40 (m, 1H), 2.43 (s, 1H), 2.64–2.56 (m, 2H), 2.92–2.88 (m, 1H), 3.03–3.01 (m, 1H), 3.47–3.35 (m, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.76 (s, 3H), 3.91 (br, 1H), 6.67 (s, 1H), 6.90 (s, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H),7.50 (t, $J$ = 7.5 Hz, 1H), 7.57 (d, $J$ = 7.5 Hz, 1H), 7.95 (d, $J$ = 7.5 Hz, 1H), 7.97 (s, 1H) .$^{13}$C NMR (101 MHz, DMSO) δ 164.16, 149.20, 147.74, 147.74, 139.06, 136.00, 135.17, 130.66, 129.99, 129.42, 128.95, 128.71, 128.24, 127.52, 126.90, 126.83, 112.23, 111.27, 109.97, 59.17, 56.36, 56.25, 55.92, 53.40, 51.14, 35.95, 29.02, 21.25. HR-ESI-MS: ($m/z$) 460.2126 [M+H] $^+$, (Calcd: 460.2118).

***4.1.1.17***. *2,3,10-trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 3-bromo benzoate (**19**).* White solid, Yield: 63%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.48–2.44 (m, 1H), 2.67–2.59 (m, 2H), 2.93–2.91 (m, 1H), 3.08–3.07 (m, 1H), 3.51–3.48 (m, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.99 (br, 1H), 6.70 (s, 1H), 6.93 (s, 1H), 7.07 (d, $J$ = 8.5 Hz, 1H), 7.16 (d, $J$ = 8.5 Hz, 1H), 7.63 (t, $J$ = 8.0 Hz, 1H), 8.01 (d, $J$ = 8.0 Hz, 1H), 8.18 (d, $J$ = 8.0 Hz, 1H), 8.28 (s, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 162.86, 148.89, 147.68, 137.28, 135.80, 133.70, 132.54, 131.86, 131.10, 129.92, 129.39, 128.64, 128.30, 127.13, 126.83, 122.64, 112.25, 111.21, 109.98, 66.65, 59.16, 56.42, 56.26, 55.90, 53.31, 36.00, 29.01. HR-ESI-MS: ($m/z$) 524.1049 [M+H] $^+$, (Calcd: 524.1067).

***4.1.1.18***. *2,3,10-trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 4-fluorobenzoate (**20**).* White solid, Yield: 83%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.44–2.40 (m, 1H), 2.64–2.56 (m, 2H), 2.92–2.88 (m, 1H), 3.04–3.01 (m, 1H), 3.47–3.34 (m, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.76 (s, 3H), 3.93 (br, 1H), 6.67 (s, 1H), 6.90 (s, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H), 7.45 (t, $J$ = 8.5 Hz, 2H), 8.24–8.21 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 167.34, 164.83, 163.14, 149.16, 147.74, 135.88, 133.38, 133.28, 129.97, 128.70, 128.27, 127.00, 126.83, 125.57, 116.83, 116.61, 112.23, 111.28, 109.96, 59.15, 56.37, 56.24, 55.91, 53.35, 51.12, 35.94, 29.01. HR-ESI-MS: ($m/z$) 464.1889 [M+H] $^+$, (Calcd: 464.1868).

***4.1.1.19***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-ylmethacry late (**21**).* Light yellow solid, Yield: 21%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.01 (s, 3H), 2.45–2.42 (m, 1H), 2.61–2.58 (m, 2H), 2.93–2.88 (m, 1H), 3.07–3.05 (m, 1H), 3.45–3.42 (m, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 3.86 (br, 1H), 5.90 (s, 1H), 6.31 (s, 1H), 6.68 (s, 1H), 6.89 (s, 1H), 6.98 (d, $J$ = 8.0 Hz, 1H), 7.07 (d, $J$ = 8.0 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 164.76, 149.17, 147.74, 135.96, 135.30, 129.98, 128.57, 128.31, 128.17, 126.82, 126.76, 112.24, 111.26, 109.97, 59.15, 56.37, 56.25, 55.93, 53.34, 51.17, 35.90, 29.02, 18.58. HR-ESI-MS: ($m/z$) 410.1971 [M+H] $^+$, (Calcd: 410.1962).

***4.1.1.20***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl cyclopro panecarboxylate (**22**).* Light yellow solid, Yield: 46%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.10–1.05 (m, 4H), 1.96–1.89 (m, 1H), 2.52–2.49 (m, 1H), 2.60–2.53 (m, 2H), 3.00–2.91 (m, 1H), 3.10–3.09 (m, 1H), 3.34–3.29 (m, 1H), 3.48–3.44 (m, 2H), 3.75 (s, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 3.85 (d, $J$ = 16.0 Hz, 1H), 6.71 (s, 1H), 6.91 (s, 1H), 6.98 (d, $J$ = 8.5 Hz, 1H), 7.08 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 172.30, 149.25, 147.73, 135.91, 129.99, 128.61, 128.10, 126.82, 126.63, 112.24, 111.22, 109.97, 59.12, 56.35, 56.25, 55.92, 53.39, 51.20, 35.91, 29.03, 12.79, 9.29, 9.24. HR-ESI-MS: ($m/z$) 410.1971 [M+H] $^+$, (Calcd: 410.1962).

***4.1.1.21***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2-ethoxy benzoate (**23**).* White solid, Yield: 81%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.38 (t, $J$ = 7 Hz, 3H), 2.48–2.45 (m, 1H), 2.64–2.59 (m, 2H), 2.96–2.91 (m, 1H), 3.06–3.04 (m, 1H), 3.40–3.37 (m, 1H), 3.48–3.444 (m, 2H), 3.73 (s, 3H), 3.75 (s, 3H), 3.76 (s, 3H), 3.97 (br, 1H), 4.20–4.16 (m, 2H), 6.68 (s, 1H), 6.90 (s, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H), 7.11–7.07 (m, 2H), 7.22 (d, $J$ = 8.5 Hz, 1H), 7.62–7.59 (m, 1H), 7.84 (d, $J$ = 8.0 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 163.88, 158.50, 149.28, 147.71, 147.71, 136.12, 134.72, 131.72, 129.98, 128.70, 128.19, 126.78, 126.73, 120.70, 119.63, 114.22, 112.19, 111.28, 109.94, 64.63, 59.20, 56.40, 56.24, 55.90, 53.72, 51.28, 35.97, 29.03, 15.19. HR-ESI-MS: ($m/z$) 490.2209 [M+H] $^+$, (Calcd: 490.2224).

***4.1.1.22***. *2,3,10-trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 4-(chloro methyl)benzoate (**24**).* White solid, Yield: 74%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.44–2.41 (m, 1H), 2.64–2.59 (m, 2H), 2.90–2.88 (m, 1H), 3.03–3.02 (m, 1H), 3.44–3.32 (m, 1H), 3.48–3.45 (m, 2H), 3.72 (s, 3H), 3.73 (s, 3H), 3.76 (s, 3H), 4.02–3.83 (m, 1H), 4.89 (s, 2H), 6.67 (s, 1H), 6.90 (s, 1H), 7.03 (d, $J$ = 8.0 Hz, 1H), 7.12 (d, $J$ = 8.0 Hz, 1H), 7.68 (d, $J$ = 8.0 Hz, 2H), 8.16 (d, $J$ = 8.0 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO) δ 163.69, 149.18, 147.75, 144.24, 135.92, 130.77, 130.77, 130.77, 129.86, 129.86, 129.86, 129.86,128.73, 128.27, 127.01, 126.88, 112.23, 111.32, 109.95, 59.20, 56.41, 56.26, 55.94, 53.37, 51.12, 45.65, 35.95, 29.00. HR-ESI-MS: ($m/z$) 494.1744 [M+H] $^+$, (Calcd: 494.1729).

***4.1.1.23***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl (E)-but-2-enoate (**25**).* Light yellow solid, Yield: 66%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.99 (d, $J$ = 6.9 Hz, 3H), 2.52–2.47 (m, 1H), 2.65–2.60 (m, 2H), 2.98–2.89 (m, 1H), 3.09–3.06 (m, 1H), 3.34–3.28 (m, 1H), 3.54–3.45 (m, 2H), 3.75 (s, 6H), 3.78 (s, 3H), 3.75 (s, 3H), 3.85 (d, $J$ = 15.0 Hz, 1H), 6.19 (dd, $J$ = 16.0 Hz, 1.6 Hz, 1H), 6.71 (s, 1H), 6.92 (s, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H), 7.22–7.09 (m, 2H). $^{13}$C NMR (126 MHz, DMSO) δ 163.68, 149.24, 148.41, 147.74, 147.74, 135.84, 129.97, 128.63, 128.10, 126.81, 126.67, 121.55, 112.22, 111.20, 109.95, 59.18, 56.29, 56.25, 55.93, 53.48, 51.20, 35.93, 29.02, 18.44. HR-ESI-MS: ($m/z$) 410.1971 [M+H] $^+$, (Calcd: 410.1962).

***4.1.1.24***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl (3r,5r,7r)-adamantane-1-carboxylate (**26**).* White solid, Yield: 61%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.74 (s, 6H), 1.99 (s, 6H), 2.05 (s, 3H), 2.48–2.43 (m, 1H), 2.62–2.56 (m, 2H), 2.91–2.88 (m, 1H), 3.06–3.04 (m, 1H), 3.27–3.26 (m, 1H), 3.43–3.3.40 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.75 (s, 3H), 3.78 (br, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 7.04 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO) δ 174.65, 149.14, 147.72, 147.72, 136.07, 130.02, 128.49, 128.12, 126.83, 126.48, 112.24, 111.30, 109.96, 59.14, 56.52, 56.24, 55.93, 53.38, 51.29, 41.03, 38.85, 38.85, 38.85, 36.38, 36.38, 35.89, 29.03, 27.80, 27.80, 27.80. HR-ESI-MS: ($m/z$) 504.2724 [M+H] $^+$, (Calcd: 504.2744).

***4.1.1.25***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 3-chloro-2,2-dimethylpropanoate (**27**).* White solid, Yield: 69%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.42 (s, 6H), 2.2.53–2.45 (m, 1H), 2.66–2.61 (m, 2H), 3.07–2.92 (m, 2H), 3.34–3.26 (m, 1H), 3.51–3.46 (m, 2H), 3.75 (s, 6H), 3.79 (s, 3H), 4.02–3.91 (m, 3H), 6.71 (s, 1H), 6.93 (s, 1H), 7.01 (d, $J$ = 8.0 Hz, 1H), 7.11 (d, $J$ = 8.0 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO) δ 172.67, 149.03,

147.72, 147.72, 135.88, 129.98, 128.57, 128.13, 126.82, 126.82, 112.23, 111.31, 109.94, 59.14, 56.49, 56.24, 55.92, 53.58, 52.24, 51.34, 45.17, 35.86, 29.00, 23.39. HR-ESI-MS: (*m/z*) 460.1907 [M+H] $^{+}$, (Calcd: 460.1885).

***4.1.1.26***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2-propyl pentanoate (**28**).* Light yellow solid, Yield: 46%. $^{1}$H NMR (300 MHz, DMSO) $\delta$ 0.97–0.92 (m, 6H), 1.59–1.36 (m, 6H), 1.73–1.65 (m, 2H), 2.49–2.43 (m, 1H), 2.66–2.58 (m, 3H), 3.02–2.88 (m, 2H), 3.31–3.26 (m, 1H), 3.45–3.41 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.75 (s, 3H), 3.85–3.79 (m, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 6.96 (d, $J$ = 8.5 Hz, 1H),7.05 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 172.97, 148.55, 147.21,147.21, 135.45, 129.40, 127.83, 127.56, 126.24, 126.11, 111.71, 110.72, 109.43, 58.54, 55.70, 55.7, 55.37, 53.18, 50.79, 44.44, 35.34, 34.17, 34.17, 28.44, 19.92, 19.85, 13.79, 13.79. HR-ESI-MS: (*m/z*) 468.2753 [M+H] $^{+}$, (Calcd: 468.2744).

***4.1.2***. *General synthetic procedures for the target compounds **29**–**45** (compound **29** as example)*

***4.1.2.1***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2-nitrobenzenesulfonate (**29**).* The starting material 1 (0.4501 g, 1.4 mmol) was added to DCM (20 mL) and stirred for dissolving at room temperature, 2-nitrobenzenesulfonyl chloride (0.3270 g, 1.6 mmol), 2 mL triethylamine and slight 4-dimethylamiopryidine were added to the solution. The mixture was stirred for 4 h at room temperature, then washed three times by water, saturated $NaHCO_3$ solution and saturated NaCl solution, respectively. The organic layer was dried over sodium sulfate, filtered, the solvent was evaporated in vacuum. The residue was purified via silica gel chromatography ($CH_2Cl_2$/MeOH) to give the product **29** (0.4003 g, 56%).

White solid. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.45–2.41 (m, 1H), 2.62–2.54 (m, 2H), 2.92–2.87 (m, 1H), 2.99–2.97 (m, 1H), 3.38 (s, 3H), 3.45–3.38 (m, 3H), 3.99 (d, $J$ = 16.0 Hz, 1H), 5.95 (d, $J$ = 3.0 Hz, 2H), 6.67 (s, 1H), 6.91 (s, 1H), 6.96 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H), 7.96 (t, $J$ = 8.0 Hz, 1H), 8.07 (t, $J$ = 8.0 Hz, 1H), 8.11 (d, $J$ = 8.0 Hz, 1H), 8.19 (d, $J$ = 8.0 Hz, 1H). $^{13}$CNMR (125 MHz, DMSO-$d_6$) $\delta$: 148.90, 147.44, 145.69, 145.47, 136.03, 134.78, 132.81, 130.73, 130.40, 129.36, 129.23, 128.20, 128.08, 127.30, 124.95, 111.24, 100.49, 108.00, 105.60, 58.68, 55.41, 53.19, 50.35, 35.15, 28.80. HR-ESI-MS: (*m/z*) 511.1182 [M+H] $^{+}$, (Calcd: 511.1170).

***4.1.2.2***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2,4,6-trimethylbenzenesulfonate (**30**).* White solid, Yield: 39%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.32 (s, 3H), 2.42–2.38 (m, 1H), 2.53 (s, 6H), 2.61–2.58 (m, 2H), 2.92–2.86 (m, 1H), 2.97–2.94 (m, 1H), 3.35–3.33 (m, 4H), 3.41–3.37 (m, 2H), 3.98 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 3.0 Hz, 2H), 6.66 (s, 1H), 6.89 (d, $J$ = 8.5 Hz, 1H), 6.90 (s, 1H), 7.05 (d, $J$ = 8.5 Hz, 1H), 7.15 (s, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 149.45, 145.68, 145.44, 143.16, 138.82, 134.75, 132.99, 131.25, 130.53, 129.81, 127.82, 127.55, 127.30, 110.94, 107.99, 105.62, 100.48, 58.82, 55.30, 53.4, 50.42, 35.22, 28.85, 22.08, 20.44. HR-ESI-MS: (*m/z*) 508.1806 [M+H] $^{+}$, (Calcd: 508.1788).

***4.1.2.3***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl thiophene-2-sulfonate (**31**).* White solid, Yield: 57%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.44–2.39 (m, 1H), 2.55–2.53 (m, 1H), 2.62–2.56 (m, 1H), 2.92–2.85 (m, 1H), 3.00–2.97 (m, 1H), 3.36–3.33 (m, 2H), 3.42–3.90 (m, 1H), 3.55 (s, 3H), 3.96 (d, $J$ = 16.0 Hz, 1H), 5.95–5.94 (m, 2H), 6.66 (s, 1H), 6.91 (s, 1H), 6.97 (d, $J$ = 8.0 Hz, 1H), 7.10 (d, $J$ = 8.0 Hz, 1H), 7.32–7.30 (m, 1H), 7.86 (dd, $J$ = 15.0 Hz, 4 Hz, 1H), 8.21 (d, $J$ = 5.0 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 149.47, 145.69, 145.45, 136.34, 135.67, 135.48, 134.49, 130.49, 129.66, 127.91, 127.91, 127.27, 111.16, 111.16, 108.00, 105.60, 100.49, 58.76, 55.57, 53.29, 50.39, 35.21, 28.83. HR-ESI-MS: (*m/z*) 472.0862 [M+H] $^{+}$, (Calcd: 472.0883).

***4.1.2.4***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 4-fluorobenzenesulfonate (**32**).* White solid, Yield: 67%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.46–2.41 (m, 1H), 2.63–2.53 (m, 2H), 2.90–2.86 (m, 1H), 3.01–2.99 (m, 1H), 3.42–3.35 (m, 3H), 3.44 (s, 3H), 4.01 (d, $J$ = 16.0 Hz, 1H), 5.95 (d, $J$ = 2.5 Hz, 2H), 6.67 (s, 1H), 6.91 (s, 1H), 6.93 (d, $J$ = 8.5 Hz, 1H), 7.08 (d, $J$ = 8.5 Hz, 1H), 7.56–7.53 (m, 2H), 8.03–8.01 (m, 2H). $^{13}$C NMR (75 MHz, DMSO) δ 167.00, 163.64, 149.17, 145.70, 145.46, 134.51, 132.84, 131.26, 131.13, 130.49, 129.73, 127.95, 127.87, 127.29, 116.68, 116.38, 111.07, 108.01, 105.59, 100.51, 58.76, 55.30, 53.46, 50.42, 35.23, 28.86. HR-ESI-MS: (*m/z*) 484.1245 [M+H] $^{+}$, (Calcd: 484.1225).

***4.1.2.5***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2,4,6-triisopropylbenzenesulfonate (**33**).* White solid, Yield: 27%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.20 (d, $J$ = 6.5 Hz, 6H), 1.25 (d, $J$ = 6.5 Hz, 6H), 1.90 (d, $J$ = 6.5 Hz, 6H), 2.37–2.32 (m, 1H), 2.59–2.53 (m, 2H), 2.86–2.82 (m, 2H), 3.01–2.98 (m, 1H), 3.27–3.26 (m, 1H), 3.33 (s, 3H), 3.38–3.35 (m, 2H), 3.85 (d, $J$ = 16.0 Hz, 1H), 4.03–3.97 (m, 2H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H), 6.90 (s, 1H), 6.98 (d, $J$ = 8.0 Hz, 1H), 7.05 (d, $J$ = 8.5 Hz, 1H), 7.35 (s, 2H). $^{13}$C NMR (75 MHz, DMSO) δ 153.54, 149.54, 149.16, 149.16, 145.68, 145.42, 134.96, 132.36, 130.45, 129.62, 127.72, 127.50, 127.23, 123.50, 123.50, 110.83, 107.98, 105.61, 100.47, 58.80, 55.20, 53.52, 50.48, 35.23, 33.46, 29.36, 29.36, 28.79, 24.14, 24.14, 24.14, 24.14, 23.33, 23.33. HR-ESI-MS: (*m/z*) 592.2731 [M+H] $^{+}$, (Calcd: 592.2727).

***4.1.2.6***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 2,5-dimethylbenzenesulfonate (**34**).* White solid, Yield: 54%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.34 (s, 3H), 2.40–2.37 (m, 1H), 2.61–2.54 (m, 2H), 2.65 (s, 3H), 2.91–2.85 (m, 1H), 2.94–2.93 (m, 1H), 3.31 (m, 1H), 3.33 (s, 3H), 3.40–3.37 (m, 2H), 3.91 (d, $J$ = 16.0 Hz, 1H), 5.95–5.94 (m, 2H), 6.66 (s, 1H), 6.89 (s, 1H), 6.9 (d, $J$ = 8.5 Hz, 1H), 7.05 (d, $J$ = 8.5 Hz, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 7.49 (d, $J$ = 8.0 Hz, 1H), 7.61 (s, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 149.40, 145.67, 145.43, 135.85, 135.83, 134.79, 134.79, 134.51, 132.32, 130.48, 129.68, 128.78, 127.85, 127.65, 127.26, 111.10, 107.98, 105.59, 100.48, 58.76, 55.27, 53.33, 50.39, 35.19, 28.82, 20.09, 19.54. HR-ESI-MS: (*m/z*) 494.1653 [M+H] $^{+}$, (Calcd: 494.1632).

***4.1.2.7***. *10-Methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-9-yl 4-chloro-3-nitrobenzenesulfonate (**35**).* White solid, Yield: 61%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.50–2.48 (m, 1H), 2.64–2.55 (m, 2H), 2.91–2.88 (m, 1H), 3.06–3.04 (m, 1H), 3.41–3.37 (m, 1H), 3.45 (s, 3H), 3.48–3.46 (m, 2H), 4.05 (d, $J$ = 16.0 Hz, 1H), 5.95 (d, $J$ = 3.0 Hz, 2H), 6.98 (s, 1H), 6.91 (s, 1H), 6.97 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H), 8.12 (d, $J$ = 8.5 Hz, 1H), 8.23 (dd, $J$ = 2.0 Hz, 8.5 Hz, 1H), 8.66 (d, $J$ = 2.0 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO) δ 148.76, 147.65, 145.69, 145.47, 136.36, 134.33, 132.94, 132.37, 131.18, 130.35, 129.57, 128.23, 128.13, 127.25, 125.04, 111.16, 108.00, 105.55, 100.50, 58.69, 55.31, 53.32, 50.36, 35.15, 28.80. HR-ESI-MS: (*m/z*) 545.0790 [M+H]$^{+}$, (Calcd: 545.0780).

***4.1.2.8***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2-nitrobenzenesulfonate (**36**).* White solid, Yield: 62%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.46–2.42 (m, 1H), 2.63–2.58 (m, 2H), 2.95–2.89 (m, 1H), 3.00–2.98 (m, 1H), 3.38 (s, 3H), 3.45–3.40 (m, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 3.98 (d, $J$ = 16 Hz, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 6.96 (d, $J$ = 8.5 Hz, 1H), 7.15 (d,

$J$ = 8.5 Hz, 1H), 7.97 (t, $J$ = 8.0 Hz, 1H), 8.07 (t, $J$ = 8.0 Hz, 1H), 8.11 (d, $J$ = 8.0 Hz, 1H), 8.19 (d, $J$ = 8.0 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 149.43, 147.99, 147.78, 147.74, 136.60, 135.35, 133.38, 131.28, 129.98, 129.80, 129.75, 128.76, 128.73, 126.74, 125.51, 112.23, 111.77, 109.93, 59.01, 56.25, 55.95, 55.92, 53.86, 51.13, 35.65, 28.94. HR-ESI-MS: ($m/z$) 527.1491 [M+H]$^+$, (Calcd: 527.1483).

***4.1.2.9***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2,4,6-trimethylbenzenesulfonate (**37**).* Light yellow solid, Yield: 67%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.33 (s, 3H), 2.43−2.39 (m, 1H), 2.53 (s, 6H), 2.63−2.56 (m, 2H), 2.98−2.91 (m, 2H), 3.34 (s, 3H), 3.44−3.38 (m, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 3.98 (d, $J$ = 16.0 Hz, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 6.90 (d, $J$ = 8.0 Hz, 1H), 7.09 (d, $J$ = 8.0 Hz, 1H), 7.51 (s, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 149.98, 147.74, 143.73, 139.38, 139.38, 135.29, 133.52, 131.82, 131.82, 131.82, 130.44, 129.88, 128.49, 128.08, 126.73, 112.22, 111.46, 109.94, 59.16, 56.23, 55.92, 55.83, 54.10, 51.19, 35.74, 28.98, 22.65, 22.65, 21.00. HR-ESI-MS: ($m/z$) 524.2126 [M+H] $^+$, (Calcd: 524.2101).

***4.1.2.10***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 4-bromo benzenesulfonate (**38**).* White solid, Yield: 66%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.46−2.43 (m, 1H), 2.64−2.54 (m, 2H), 2.95−2.90 (m, 1H), 3.01−2.99 (m, 1H), 3.42−3.37 (m, 3H), 3.44 (s, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 3.99 (d, $J$ = 16.0 Hz, 1H), 6.69 (s, 1H), 6.87 (s, 1H), 6.94 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H), 7.87 (d, $J$ = 8.5 Hz, 2H), 7.93 (d, $J$ = 8.5 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 149.65, 147.77, 147.74, 136.25, 135.02, 132.97, 132.97, 130.37, 130.37, 130.28, 129.84, 129.14, 128.66, 128.47, 126.74, 112.23, 111.68, 109.93, 59.07, 56.25, 55.92, 55.85, 54.05, 51.17, 35.69, 28.98. HR-ESI-MS: ($m/z$) 560.0745 [M+H] $^+$, (Calcd: 560.0737).

***4.1.2.11***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl propane-1-sulfonate (**39**).* Light yellow solid, Yield: 45%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.08 (t, $J$ = 7.5 Hz, 3H), 1.95−1.90 (m, 2H), 2.49−2.47 (m, 1H), 2.64−2.51 (m, 2H), 2.93−2.90 (m, 1H), 3.08−3.05 (m, 1H), 3.45−3.41 (m, 2H), 3.50 (d, $J$ = 16.0 Hz, 1H), 3.58 (t, $J$ = 7.5 Hz, 2H), 3.73 (s, 3H), 3.75 (s, 3H), 3.83 (s, 3H), 4.08 (d, $J$ = 16.0 Hz, 1H), 6.68 (s, 1H), 6.88 (s, 1H), 7.03 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 149.72, 147.76, 147.74, 135.29, 130.58, 129.90, 128.67, 127.97, 126.77, 112.24, 111.75, 109.95, 59.06, 56.48, 56.24, 55.92, 54.16, 51.22, 35.76, 29.00, 17.67, 13.03. HR-ESI-MS: ($m/z$) 448.1783 [M+H] $^+$, (Calcd: 448.1788).

***4.1.2.12***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl ethane sulfonate (**40**).* White solid, Yield: 47%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.45 (t, $J$ = 7.5 Hz, 3H), 2.50−2.48 (m, 1H), 2.65−2.54 (m, 2H), 2.97−2.91 (m, 1H), 3.10−3.06 (m, 1H), 3.53−3.44 (m, 3H), 3.62 (q, $J$ = 7.5 Hz, 2H), 3.73 (s, 3H), 3.76 (s, 3H), 3.83 (s, 3H), 4.10 (d, $J$ = 16.0 Hz, 1H), 6.70 (s, 1H), 6.95 (s, 1H), 7.06 (d, $J$ = 8.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 149.72, 147.76, 147.74, 135.27, 130.58, 129.91, 128.66, 127.98, 126.78, 112.24, 111.74, 109.96, 59.06, 56.45, 56.25, 55.92, 54.17, 51.22, 47.44, 35.77, 29.01, 8.70. HR-ESI-MS: ($m/z$) 434.1651 [M+H] $^+$, (Calcd: 434.1632).

***4.1.2.13***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl thiophene -2-sulfonate (**41**).* Light yellow solid, Yield: 58%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.47−2.44 (m, 1H), 2.66−2.54 (m, 2H), 2.98−2.91 (m, 1H), 3.37 (d, $J$ = 16.0 Hz, 1H), 3.49−3.45 (m, 3H), 3.59 (s, 3H), 3.76 (s, 3H), 3.78 (s, 3H), 4.01 (d, $J$ = 16.0 Hz, 1H), 6.72 (s, 1H), 6.91 (s, 1H), 7.01 (d, $J$ = 8.5 Hz, 1H), 7.16 (d, $J$ = 8.5 Hz, 1H), 7.35−7.33 (m, 1H), 7.90−7.89 (m, 1H), 8.26−8.24 (m, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 150.01, 147.76, 147.74, 136.93, 136.24, 136.04, 135.06, 130.29, 129.87, 128.59, 128.49, 126.73, 112.23, 111.73, 109.93, 59.08, 56.25, 56.12, 55.92, 53.94, 51.16, 35.70, 28.96. HR-ESI-MS: ($m/z$) 488.1212 [M+H] $^+$, (Calcd: 488.1196).

***4.1.2.14***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 4-fluoro benzenesulfonate (**42**).* Light yellow solid, Yield: 38%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.45−2.43 (m, 1H), 2.64−2.55 (m, 2H), 2.95−2.90 (m, 1H), 3.02−2.99 (m, 1H), 3.45−3.39 (m, 3H), 3.45 (s, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 4.01 (d, $J$ = 16.0 Hz, 1H), 6.68 (s, 1H), 6.87 (s, 1H), 6.94 (d, $J$ = 8.5 Hz, 1H), 7.11 (d, $J$ = 8.5 Hz, 1H), 7.55 (t, $J$ = 9.0 Hz, 1H), 8.04−8.01 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 167.13, 164.60, 149.70, 147.77, 147.74, 135.07, 133.38, 131.81, 131.71, 130.34, 129.86, 128.63, 128.41, 126.75, 117.24, 117.01, 112.24, 111.66, 109.94, 59.08, 56.25, 55.92, 55.88, 54.10, 51.18, 35.71, 28.97. HR-ESI-MS: ($m/z$) 500.1551 [M+H] $^+$, (Calcd: 500.1538).

***4.1.2.15***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2,4,6-triisopropylbenzenesulfonate (**43**).* White solid, Yield: 37%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.20 (d, $J$ = 6.5 Hz, 12H), 1.25 (d, $J$ = 6.5 Hz, 6H), 2.37−2.32 (m, 1H), 2.59−2.52 (m, 2H), 2.89−2.83 (m, 2H), 3.01−2.98 (m, 1H), 3.27−3.24 (m, 1H), 3.34 (s, 3H), 3.44−3.41 (m, 2H), 3.72 (s, 3H), 3.74 (s, 3H), 3.85 (d, $J$ = 16.0 Hz, 1H), 4.03−3.98 (m, 2H), 6.67 (s, 1H), 6.87 (s, 1H), 6.91 (d, $J$ = 8.0 Hz, 1H), 7.08 (d, $J$ = 8.0 Hz, 1H), 7.35 (s, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 154.14, 150.12, 149.74, 149.74, 147.75, 147.75, 135.53, 132.92, 130.24, 129.82, 128.42, 128.03, 126.67, 124.09, 124.09, 112.22, 111.41, 109.92, 59.14, 56.23, 55.92, 55.77, 54.18, 51.27, 35.74, 34.03, 29.94, 28.92, 24.75, 24.75, 24.68,24.68, 23.90, 23.86. HR-ESI-MS: ($m/z$) 608.3036 [M+H] $^+$, (Calcd: 608.3040).

***4.1.2.16***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 2,5-dimethylbenzenesulfonate (**44**).* Light yellow solid, Yield: 46%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 2.37 (s, 3H), 2.45−2.39 (m, 1H), 2.59−2.54 (m, 2H), 2.60 (s, 3H), 2.97−2.89 (m, 2H), 3.34−3.30 (m, 1H), 3.36 (s, 3H), 3.48−3.36 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.95 (d, $J$ = 16.0 Hz, 1H), 6.71 (s, 1H), 6.90 (s, 1H), 6.94 (d, $J$ = 8.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 1H), 7.48 (d, $J$ = 8.0 Hz, 1H), 7.54 (d, $J$ = 8.0 Hz, 1H), 7.64 (s, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 149.94, 147.71, 136.42, 136.42, 135.34, 135.34, 135.11, 132.91, 130.30, 129.84, 129.33, 128.54, 128.20, 126.70, 112.18, 111.67, 109.89, 59.10, 56.23, 55.91, 55.83, 53.98, 51.17, 35.68, 28.95, 20.65, 20.11. HR-ESI-MS: ($m/z$) 510.1935 [M+H]$^+$, (Calcd: 510.1945).

***4.1.2.17***. *2,3,10-Trimethoxy-5,8,13,13a-tetrahydro-6H-isoquinolino [3,2-a]isoquinolin-9-yl 4-chloro-3-nitrobenzenesulfonate (**45**).* White solid, Yield: 23%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.50−2.46 (m, 1H), 2.65−2.58 (m, 2H), 2.93−2.90 (m, 1H), 3.06−3.04 (m, 1H), 3.48−3.45 (m, 6H) 3.73 (s, 3H), 3.75 (s, 3H), 4.04 (d, $J$ = 16.0 Hz, 1H), 6.69 (s, 1H), 6.88 (s, 1H), 6.97 (d, $J$ = 8.5 Hz, 1H), 7.15 (d, $J$ = 8.5 Hz, 1H), 8.12 (d, $J$ = 8.5 Hz, 1H), 8.25 (dd, $J$ = 2.0 Hz, 8.5 Hz, 1H), 8.67 (d, $J$ = 2.0 Hz, 1H). HR-MS: ($m/z$) 561.1111 [M+H] $^+$, (Calcd: 561.1093).

***4.1.3***. *General synthetic procedures for the target compounds **46**−**50** and GZ-1 (compound **46** as the example)*

***4.1.3.1***. *10-Methoxy-9-propoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a] isoquinoline (**46**).* The starting material 1 (0.5004 g, 1.5 mmol) was added to dimethylformamide (DMF, 20 mL) and stirred for dissolving at room temperature, 1-bromopropane (0.14 mL, 1.5 mmol) was added slowly, followed by anhydrous $K_2CO_3$ (0.4228 g, 3 mmol). The mixture was heated at 80 °C for 4 h, then cooled. The mixture was partitioned between water and DCM. The organic layers were washed three times by water and saturated NaCl solution, respectively. The extracts were dried over sodium sulfate, filtered, the solvent was evaporated in

vacuum. The residue was purified via silica gel chromatography ($CH_2Cl_2$/MeOH) to give the product **46** (0.1984 g, 36%).

Light yellow solid. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 0.99 (t, $J$ = 7.5 Hz, 3H), 1.71–1.65 (m, 2H), 2.46–2.44 (m, 1H), 2.61–2.54 (m, 2H), 2.94–2.88 (m, 1H), 3.08–3.05 (m, 1H), 3.31–3.29 (m, 1H), 3.39–3.36 (m, 2H), 3.76 (s, 3H), 3.90–3.82 (m, 2H), 4.06 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.82 (d, $J$ = 8.5 Hz, 1H), 6.86 (d, $J$ = 8.5 Hz, 1H), 6.89 (s, 1H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 150.34, 146.20, 145.91, 144.12, 131.39, 128.82, 128.09, 127.97, 123.93, 111.72, 108.53, 106.19, 101.01, 73.95, 59.56, 56.25, 54.07, 51.27, 36.26, 29.52, 23.70, 10.97. HR-ESI-MS: ($m/z$) 368.1868 [M+H]$^+$, (Calcd: 368.1856).

***4.1.3.2***. *9-Ethoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo [4,5-g]isoquino lino[3,2-a]isoquinoline (**47**).* Light yellow solid, Yield: 35%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.27 (t, $J$ = 7.0 Hz, 3H), 2.46–2.44 (m, 1H), 2.61–2.54 (m, 2H), 2.93–2.87 (m, 1H), 3.10–3.07 (m, 1H), 3.31–3.29 (m, 1H), 3.38–3.35 (m, 2H), 3.76 (s, 3H), 4.00–3.94 (m, 2H), 4.06 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.83 (d, $J$ = 8.5 Hz, 1H), 6.87 (d, $J$ = 8.5 Hz, 1H), 6.90 (s, 1H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 150.36, 146.20, 145.91, 143.94, 131.40, 128.94, 128.06, 127.97, 123.97, 111.64, 108.53, 106.19, 101.01, 67.95, 59.58, 56.22, 54.09, 51.23, 36.25, 29.52, 16.20. HR-ESI-MS: ($m/z$) 354.1701 [M+H]+, (Calcd: 354.1700).

***4.1.3.3***. *9-Butoxy-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo [4,5-g]isoquinolino[3,2-a]isoquinoline (**48**).* Light yellow solid, Yield: 32%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 0.94 (t, $J$ = 7.0 Hz, 3H), 1.49–1.41 (m, 2H), 1.68–1.63 (m, 2H), 2.47–2.43 (m, 1H), 2.61–2.53 (m, 2H), 2.93–2.87 (m, 1H), 3.08–3.05 (m, 1H), 3.27–3.26 (m, 1H), 3.38–3.35 (m, 2H), 3.75 (s, 3H), 3.95–3.86 (m, 2H), 4.06 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H), 6.82 (d, $J$ = 8.5 Hz, 1H), 6.86 (d, $J$ = 8.5 Hz, 1H), 6.89 (s, 1H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 150.36, 146.25, 145.91, 144.13, 131.41, 128.84, 128.10, 127.98, 123.96, 111.74, 108.54, 106.20, 101.02, 71.99, 59.57, 56.26, 54.05, 51.27, 36.25, 32.48, 29.51, 19.21, 14.24. HR-ESI-MS: ($m/z$) 382.1994 [M+H]$^+$, (Calcd: 382.2013).

***4.1.3.4***. *9-(Cyclohexylmethoxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinoline (**49**).* White solid, Yield: 45%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.10–1.03 (m, 2H), 1.31–1.17 (m, 3H), 1.73–1.64 (m, 4H), 1.84–1.82 (m, 2H), 2.46–2.44 (m, 1H), 2.62–2.53 (m, 2H), 2.93–2.88 (m, 1H), 3.06–3.04 (m, 1H), 3.30–3.29 (m, 1H), 3.39–3.36 (m, 2H), 3.71–3.67 (m, 2H), 3.75 (s, 3H), 4.05 (d, $J$ = 16.0 Hz, 1H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.66 (s, 1H), 6.82 (d, $J$ = 8.5 Hz, 1H), 6.86 (d, $J$ = 8.5 Hz, 1H), 6.89 (s, 1H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 150.38, 146.21, 145.92, 144.24, 131.41, 128.78, 128.13, 127.97, 123.96, 111.82, 108.54, 106.20, 101.02, 77.61, 59.52, 56.31, 54.04, 51.30, 38.71, 36.24, 29.83, 29.83, 29.51, 26.61, 25.83, 25.83. HR-ESI-MS: ($m/z$) 422.2306 [M+H]$^+$, (Calcd: 422.2326).

***4.1.3.5***. *9-((2,5-Difluorobenzyl)oxy)-10-methoxy-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoqu inolino[3,2-a]isoquinoline (**50**).* White solid, Yield: 42%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.43–2.38 (m, 1H), 2.60–2.54 (m, 2H), 2.91–2.85 (m, 1H), 3.02–2.99 (m, 1H), 3.29–3.26 (m, 1H), 3.35–3.30 (m, 2H), 3.80 (s, 3H), 4.02 (d, $J$ = 16.0 Hz, 1H), 5.02 (q, 2H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H),6.93–6.87 (m, 3H), 7.32–7.23 (m, 2H), 7.39–7.37 (m, 1H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 159.47, 157.80, 157.56, 155.87, 150.28, 146.21, 145.93, 143.21, 131.33, 128.99, 128.11, 127.93, 127.27, 127.21, 127.13, 127.07, 124.66, 117.47, 117.43, 117.35, 117.27, 117.23, 117.16, 117.06, 116.99, 116.87, 116.80, 111.66, 108.53, 106.20, 101.02, 67.08, 59.46, 56.29, 53.82, 51.13, 36.14, 29.46. HR-ESI-MS: ($m/z$) 452.1677 [M+H] $^+$, (Calcd: 452.1668).

***4.1.3.6***. *10-Methoxy-9-((4-nitrobenzyl)oxy)-5,8,13,13a-tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquino lino[3,2-a]isoquinoline (GZ-1).* Light yellow solid, Yield: 38%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.44–2.41 (m, 1H), 2.60–2.52 (m, 2H), 2.91–2.86 (m, 1H), 3.05–3.03 (m, 1H), 3.39–3.3.33 (m, 3H), 3.80 (s, 3H), 4.08 (d, $J$ = 16.0 Hz, 1H), 5.11 (s 2H), 5.94 (d, $J$ = 2.0 Hz, 2H), 6.65 (s, 1H), 6.95–6.90 (m, 3H), 7.75 (d, $J$ = 8.5 Hz, 2H), 8.26 (d, $J$ = 8.5 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO) $\delta$ 150.25, 147.48, 146.23, 145.94, 143.43, 131.33, 128.94, 128.94, 128.94, 128.18, 127.97, 124.69, 123.96, 123.96, 123.96, 111.67, 108.55, 106.21, 101.03, 72.65, 59.46, 56.31, 53.89, 51.14, 36.18, 29.47. HR-ESI-MS: ($m/z$) 461.1700 [M+H] $^+$, (Calcd: 461.1707).

### 4.2. Biological assays

#### 4.2.1. Cell culture

The cell line HepG2 was maintained in eagle's medium (DMEM, Gibco, UK), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/mL streptomycin and cultured in a 37 °C incubator with 5% $CO_2$ in the air.

#### 4.2.2. Animals and treatment

Animal experiments were conducted in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University, and the protocols were approved by the Animal Ethics Committee of this institution. Six-week-old male C57BL/6J mice were fed with high fat diet (HFD, 45% calories from fat, Medicience, China) for 15 weeks to induce obesity. The obese animals were then randomly separated into obesity model, low-dose **49** treatment (170 mg/kg, dissolved in 0.1% (w/v) carboxymethyl cellulose-Na (CMC-Na); purity > 98%, HPLC) and high-dose **49** treatment (340 mg/kg) group. The mice in both normal control and obesity model groups were given the same volume of 0.1% (w/v) CMC-Na. Mice were administered by gavage at the same time in each day for 6 weeks. During the gavage period, mice were continually given free access to water and normal diet (13.5% of energy from fat, normal diet, Qinglong Mountain, China) for normal control mice or high fat diet for the mice in model and drug treated groups. Body weight and food intake were recorded every week.

#### 4.2.3. Cell viability assay

HepG2 cells were plated in 96-well plates at the density of $2 \times 10^4$ cells/well (n = 6) containing 100 μL of DMEM +10% FBS treated with 10 μM compounds and cultured for 24 h, then incubated with CCK8 solution (10 μL/well) for 2 h. The absorbance was detected with a microplate reader at a test wavelength of 450 nm.

#### 4.2.4. TC and TG measurement

For the experimental high-throughput screening of intracellular TC and TG measurement, 480 in-house compounds were screened with PerkinElmer explorer G3 workstation iX20 incl. combined with full-automatic hander Flex 750 and JANUS G3 automatic liquid processing platform. The HepG2 cells were cultured in 96-well plates for 24 h, and then compounds at the concentration of 10 μM were incubated with cells for another 24 h. Before measurement, cell culture medium was removed and washed by PBS. Cells were lysed for 1 h with 2% Triton X-100 solution. The TC or TG measurement solution (Jiancheng, China) was added in cell lysis, cultured in 37 °C for 10 min, and measured at 510 nm wavelength.

For TC and TG measurement of newly synthesized compounds, the cells were cultured in six-well plates and collected in 200 μL PBS. After ultrasonic disruption of cells (energy 30%, on 5 s, off 10s, total 60s), the concentrations of TC or TG were measured using the TC or TG determination kit according to the manufacturer's instructions, respectively (SKHB, China). And the protein

quantification was operated after the manufacturer's instructions of BCA kit (Beyotime, China). TC and TG levels in blood were measured according to the manufacturer's instructions (SKHB, China).

*4.2.5. Quantitative PCR*

Total RNA was extracted from cells and tissue samples using TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). Equal amounts of RNA samples were used for cDNA synthesis with a kit (HiScript Q RT SuperMix for qPCR, Vazyme, China). Quantitative PCR analysis was carried out using qRT-PCR (Roche, Basel, Switzerland) with SYBR-green (AceQ qPCR SYBR Green Master Mix, Vazyme, China).

*4.2.6. Western blot analysis*

Protein from cells or mice liver tissues was extracted with RIPA lysis buffer (65 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) together with protease inhibitor cocktail tablets (#04693132001, Roche) and quantified with a BCA Protein Assay Kit (Beyotime, China). The protein samples were separated using 10% SDS-PAGE gels and then transferred to nitrocellulose filter (NC) membrane. After blocking with 5% skim milk, the membranes were incubated with the indicated primary antibodies overnight at 4 °C and then with secondary antibodies for 1 h at room temperature. The protein expression signals were detected on an Imaging System (Tanon 5200, China).

*4.2.7. Immunoprecipitation*

293T cells were seeded in three 100-mm dishes ($3 \times 10^6$ cells/dish) on day 0. The cells were transfected with indicated plasmids (flag-SREBP1, flag-SREBP2 and HA-ubiquitin) on day 1. DMSO or **49** was mixed with fresh medium to replace former culture medium on day 2. The cells were harvested on day 3 in PBS and lysed by IP lysis buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 5 mM EDTA, 1% NP-40, 10% glycerol, 50 mM NaF, 1 mM Na3VO4 and protease inhibitor PMSF). The cell lysate was centrifuged for 10 min at 12,000 rpm at 4 °C. About 10% of the supernatant was used for Western blot as inputs, while the rest of homogenates were incubated with indicated antibodies (flag and IgG) overnight at 4 °C. Protein A/G plus agarose beads (Santa Cruz) were added at 4 °C for another 2 h. The beads were washed with cold PBS for five times, followed by western blotting analysis.

*4.2.8. Oral glucose tolerance and insulin tolerance tests*

Oral glucose tolerance and insulin tolerance tests were performed on mice fasted overnight, and insulin tolerance tests were performed on mice fasted for 4 h. Mice were injected with either 2 g/kg glucose by i.g. or 0.75 U/kg insulin (Sigma) by i.p.. Glucose levels were measured from tail blood before and 15, 30, 60, or 120 min after the injection. Insulin tolerance and oral glucose tolerance tests were performed 1 and 2 weeks before their sacrifice.

*4.2.9. Histopathologic analysis*

WAT, BAT, heart, liver, spleen, lung and kidney were fixed in 4% paraformaldehyde at 4 °C overnight and embedded in paraffin wax. Paraffin sections were mounted on glass slides for H&E staining. Cryosections of livers were stained by oil red O and counterstained with hematoxylin to visualize the lipid droplets.

*4.2.10. Pharmacokinetic profiles in SD rats*

The PK study of compound **49** was conducted on SD rats. Six SD rats were divided into two groups, intravenous and oral administration group. SD rats were administrated 1 and 10 mg/kg of compound **49** in intravenous and oral administration groups, respectively. Blood samples of intravenous administration groups were collected at 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 12 h after intravenous administration. Blood samples of oral administration groups were collected at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h after oral administration. The concentrations of compound **49** in serum were measured by LC/MS/MS [17–19].

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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