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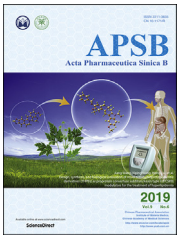

ORIGINAL ARTICLE

Design, synthesis, and biological evaluation of novel tetrahydroprotoberberine derivatives (THPBs) as proprotein convertase subtilisin/kexin type 9 (PCSK9) modulators for the treatment of hyperlipidemia

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KEY WORDS

PCSK9;
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Abstract Proprotein convertase subtilisin/kexin type 9 (PCSK9) modulators may attenuate PCSK9-induced low-density lipoprotein receptor (LDLR) degradation in lysosome and promote the clearance of circulating low-density lipoprotein cholesterol (LDL-C). A novel series of tetrahydroprotoberberine derivatives (THPBs) were designed, synthesized, and evaluated as PCSK9 modulators for the treatment of hyperlipidemia. Among them, eight compounds exhibited excellent activities in downregulating

Abbreviations: ADH, autosomal dominant hypercholesterolemia; AUC, area under the plasma concentration–time curve; BBR, berberine; CHD, coronary heart disease; CL, clearance; C_{max}, maximum concentration; CVDs, cardiovascular diseases; DiI-LDL, low-density lipoprotein, labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate; F, oral bioavailability; FDA, food and drug administration; hERG, human *ether-à-go-go* related gene; HFD, high-fat diet; LDL-C, low-density lipoprotein-cholesterol; LDLR, low-density lipoprotein receptor; mAbs, monoclonal antibodies; MRT, mean residence time; PCSK9, proprotein convertase subtilisin/kexin type 9; PK, pharmacokinetic; $POCl_3$, phosphoryl trichloride; $t_{1/2}$, half-life; TC, total cholesterol; THPBs, tetrahydroprotoberberine derivatives.

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cholesterol;
Lipid-lowering;
PCSK9 expression;
Low-density lipoprotein receptor;
Total cholesterol;
Hyperlipidemia hamster

hepatic PCSK9 expression better than berberine in HepG2 cells. In addition, five compounds **15**, **18**, **22**, (*R*)-**22**, and (*S*)-**22** showed better performance in the low-density lipoprotein, labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI-LDL) uptake assay, compared with berberine at the same concentration. Compound **22**, selected for *in vivo* evaluation, demonstrated significant reductions of total cholesterol (TC) and LDL-C in hyperlipidemic hamsters with a good pharmacokinetic profile. Further exploring of the lipid-lowering mechanism showed that compound **22** promoted hepatic LDLR expression in a dose-dependent manner in HepG2 cells. Additional results of human *ether-à-go-go* related gene (hERG) inhibition assay indicated the potential druggability for compound **22**, which is a promising lead compound for the development of PCSK9 modulator for the treatment of hyperlipidemia.

1. Introduction

Cardiovascular diseases (CVDs) are a major cause of death throughout the world[1]. Elevation of circulating low-density lipoprotein cholesterol (LDL-C) leads to an increased incidence of cardiovascular diseases[2]. Proprotein convertase subtilisin/kexin type 9 (PCSK9), which originally found in patients with autosomal dominant hypercholesterolemia (ADH), could adjust the circulating LDL-C levels *via* inducing low-density lipoprotein receptor (LDLR) endocytosis and lysosomal degradation in hepatic cell[3,4]. Loss-of-function mutations in PCSK9 in humans perform significantly lower LDL-C levels and CVDs morbidity[5], which indicates that PCSK9 may act as a therapeutic target for LDL-C-lowering.

Owing to the promise of its therapeutic potential, multiple PCSK9 monoclonal antibodies (mAbs) have been evaluated in clinical trials. Among them, two PCSK9 mAbs (alirocumab and evolocumab), have been approved by food and drug administration (FDA) for the treatment of coronary heart disease (CHD)[6,7]. In addition, a small interfering RNA ALN-PCSSC, which inhibited the synthesis of PCSK9 also demonstrated lower LDL-C levels in patients administered in phase 3 clinical trial[8]. However, both of these therapeutic drugs need to be administered by injection and costly, which may restrict their clinical promotion. Therefore, an orally available small molecule targets PCSK9 would be a highly desirable alternative therapeutic agent, based on its ease of administration and lower cost.

Small molecules target PCSK9 have been identified (Fig. 1). Compound **1** decreased PCSK9 protein expression in HepG2 cells with the IC_{50} value of 29.7 nmol/L[9]. Compound **2** (PF-06446846) developed by Pfizer reduced secreted PCSK9 level with the IC_{50} value of 0.5 μmol/L[10−13]. Natural products, such as moracin C (**3**), adenosine (**4**), and manglisin E (**5**), also exhibited potent down-regulation on PCSK9 mRNA expression in HepG2 cells, with IC_{50} values of 16.8, 18.46, and 3.15 μmol/L, respectively[14−16].

Berberine (BBR, **6**, Fig. 1), the main bioactive ingredient of *Coptis chinensis*, reduced serum total cholesterol (TC) by 29% and LDL-C by 25% in 32 hypercholesterolemic patients after 3 months of treatment[17]. It was also identified reducing PCSK9 protein expression in HepG2 cells[18], and decreasing PCSK9 mRNA level with the IC_{50} value of 8.04 μmol/L[15]. However, there are some factors that limit its wide prescription, such as its high oral dose of 1.0−1.5 g/day, poor bioavailability ($F<1\%$) and high hERG channel inhibitory activity (IC_{50} = 3.1 μmol/L)[17,19−24]. Therefore, with the rapid rise in the number of patients with hyperlipidemia worldwide, discovering novel therapeutics to treat hyperlipidemia with better efficacy and fewer side effects has been a research focus in academia and industry.

It is known that tetrahydroprotoberberine deviratives (THPBs), which are the primary components of natural isoquinoline alkaloids, also exhibit a wide range of bioactivities[25−27]. Our group is dedicated to performing chemical modification of THPBs and constructing a library of small molecules with different scaffolds[28−33]. Herein, we described a novel series of indole-containing THPB derivatives with anti-hyperlipidemia activity. We screened our in-house THPB library through PCSK9 expression in HepG2 cells. Fortunately, compound **7** featuring a novel scaffold, down-regulating PCSK9 expression assay at 5 μmol/L. In this study, based on the core structure of hit compound **7**, a series of novel indole-containing THPBs as PCSK9 modulators were designed and synthesized, as well as the detailed structure−activity relationship (SAR) analysis, *in vitro* and *in vivo* biological evaluation, and pharmacokinetic studies.

2. Results and discussion

2.1. Chemistry

In order to obtain novel compounds to reduce the PCSK9 expression in HepG2 cells, we screened our in-house THPB library, and compound **7** showed biological activity (PCSK9 protein level 0.84 @5 μmol/L). To improve the potency, a series of novel indole-containing THPBs were designed based on the hit compound **7** (Scheme 1). Firstly, the oxacyclopentene of ring A was opened, we introduced various alkoxy groups at the 2- and 3-position on the ring A and maintained the ring D, and novel compounds **8–13** were designed. Then, in order to reduce the potential hERG channel inhibition and improve the bioavailability, by introducing the methoxyl group at the 12-position of the ring D and investigating different substituents on the ring A, novel compounds **14–26** were designed. In addition, chiral compounds (*R*)- and (*S*)-**22** were also synthesized to explore the influence of the molecular configuration. Finally, introduction of the chlorine and bromine at the 13-position of the ring D, we obtained compounds **27** and **28**.

On the basis of the above design, indole-containing THPBs (**7–28**) were synthesized through the route shown in Scheme 1. The

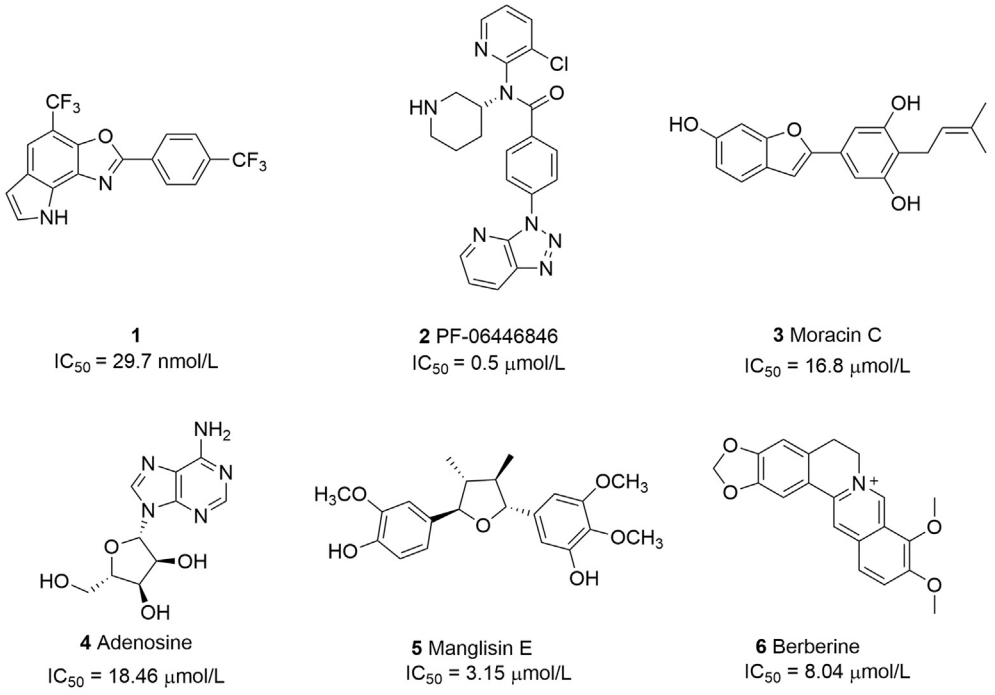


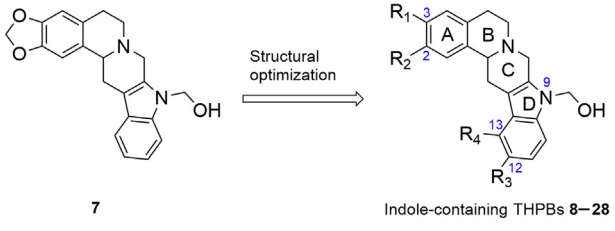
Figure 1 Reported PCSK9 modulators.

procedures of Henry reaction was used to obtain the corresponding nitrostyrene **30**. Reduction of the resulting nitrostyrenes with $LiAlH_4$ produced the phenylethanamine intermediate **31**. Then, phenylethanamine **31** and the substituted indole-3-acetic acid **32** were employed to generate amide **33**, which was further cyclized under the presence of phosphoryl trichloride ($POCl_3$) to give imine **34** in excellent yields, according to the procedures of the Bischler−Napieralski reaction. Reduction of the resulting imine **34** with sodium borohydride produced the key amine intermediate **35**. Cyclization of amine **35** *via* the Pictect−Spengler reaction with formaldehyde resulted in the target products **7–28** (Scheme 2).

Additionally, chiral compounds (*R*)- and (*S*)-**22** were prepared according to the procedure outlined in Scheme 3. Asymmetric hydrogenation of imine **35p**, catalyzed by a chiral Ru(II) complex (Noyori's catalyst)[34−36] produced chiral amines (*R*)-**35p**, and (*S*)-**35p**, followed by cyclization with formaldehyde to give (*R*)- and (*S*)-**22**.

2.2. *Structure−activity relationship of indole-containing THPBs*

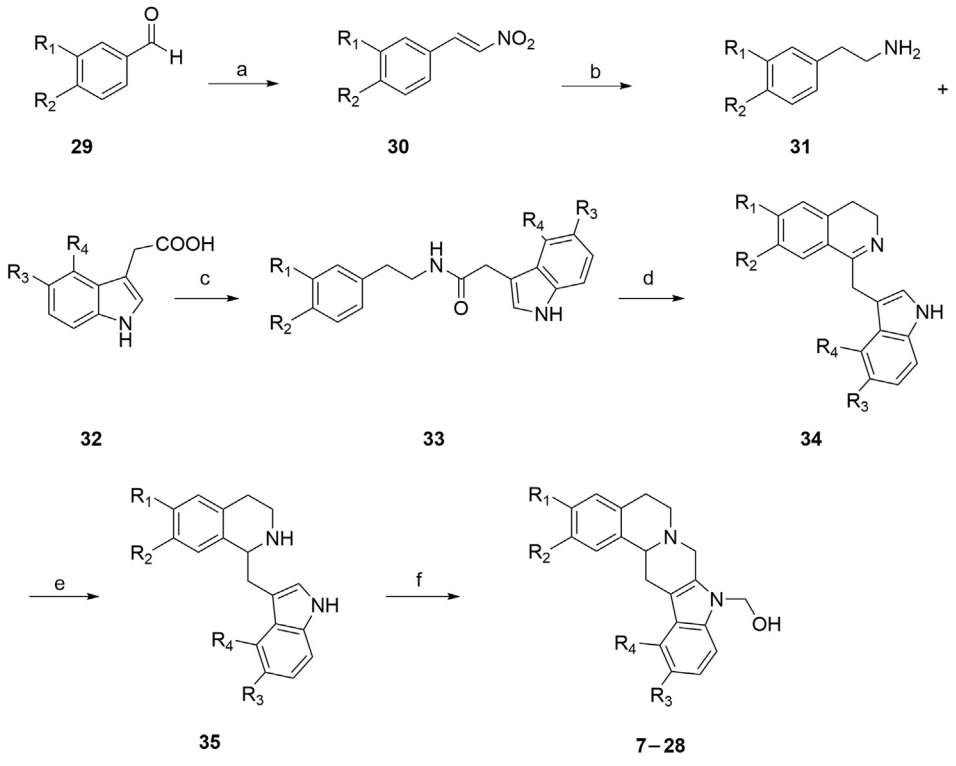
The human hepatoma cell line HepG2 cells were used to investigate the PCSK9 expression. All indole-containing THPBs were screened to examine their ability to down-regulate PCSK9 expression in HepG2 cells. Structures of the indole-containing THPBs (compounds **7–28**) and their regulatory activity on PCSK9 protein expression are shown in Table 1 (data of PCSK9 protein level is expressed as a mean of the fold of vehicle). Initial screening was carried out of each compound at a concentration of 5 μmol/L using Western blot assay (Supporting Information Fig. S1).



Scheme 1 Design of indole-containing THPBs.

The SAR study was first focused on the effect of the side chains at the 2- and 3-position of the aromatic ring A (Table 1). The oxacyclopentene was opened, and a methoxyl or substituted-benzyloxy group was attached at the 2- and 3-position of the compound **7**. The obtained compounds **8** and **13** showed higher activity on the down-regulation of PCSK9 expression than berberine and hit compound **7** (0.68 and 0.69 *vs.* 0.71 and 0.84, respectively). Compounds **9–12** bearing 2-F-OBn, 3-F-OBn, 4-F-OBn, or 3-Me-OBn at the 2-position, respectively, reduced their activities on the down-regulation of PCSK9 (1.33, 0.82, 0.81, and 0.93).

Next, on the indole ring D of compound **7**, the hydrogen atom at the 12-position was replaced with electron-donating methoxyl group. Compound **14** exhibited the similar biological activity with compound **7** (0.86 *vs.* 0.84). Then, retaining the methoxyl group at the 2-position, different substituted-benzyloxy group was introduced at the 3-position of the ring A. Compounds **15** and **18** displayed better biological activity than berberine on the down-regulation of PCSK9 (0.50 and 0.56 *vs.* 0.71). Replacement the substituted benzyloxy group to the trifluoroethoxyl group, compound **21** also exhibited good activity in down-regulating PCSK9 expression (0.58). Introduction of different substituted-benzyloxy group at the 2-position, compounds **22–26** were obtained. Compound **22** displayed significant down-regulation of PCSK9 expression (0.24), which is much better than berberine. Therefore, we also synthesized the chiral compounds (*R*)- and (*S*)-**22**, and further evaluated their biological activities on the down-regulation of PCSK9. The *R*-configured enantiomer (*R*)-**22** was more effective than the *S*-configured compound (*S*)-**22** (0.21 *vs.* 0.51).



35a: R_1,R_2 = -OCH_2O-; R_3 = R_4 = H
35b: R_1= OCH_3; R_2 = 2-F-OBn; R_3 = R_4 = H
35c: R_1= 2-F-OBn; R_2 = OCH_3; R_3 = R_4 = H
35d: R_1= 3-F-OBn; R_2 = OCH_3; R_3 = R_4 = H
35e: R_1= 4-F-OBn; R_2 = OCH_3; R_3 = R_4 = H
35f: R_1= 3-Me-OBn; R_2 = OCH_3; R_3 = R_4 = H
35g: R_1= 4-Me-OBn; R_2 = OCH_3; R_3 = R_4 = H
35h: R_1,R_2 = -OCH_2O-; R_3 = OCH_3; R_4 = H
35i: R_1= OCH_3; R_2 = OBn; R_3 = OCH_3; R_4 = H
35j: R_1= OCH_3; R_2 = 2-F-OBn; R_3 = OCH_3; R_4 = H
35k: R_1= OCH_3; R_2 = 3-F-OBn; R_3 = OCH_3; R_4 = H
35l: R_1= OCH_3; R_2 = 4-F-OBn; R_3 = OCH_3; R_4 = H
35m: R_1= OCH_3; R_2 = 3-OMe-OBn; R_3 = OCH_3; R_4 = H
35n: R_1= OCH_3; R_2 = 4-Me-OBn; R_3 = OCH_3; R_4 = H
35o: R_1 = OCH_3; R_2 = OCH_2CF_3; R_3 = OCH_3; R_4 = H
35p: R_1= OBn; R_2 = OCH_3; R_3 = OCH_3; R_4 = H
35q: R_1= 2-F-OBn; R_2 = OCH_3; R_3 = OCH_3; R_4 = H
35r: R_1= 3-F-OBn; R_2 = OCH_3; R_3 = OCH_3; R_4 = H
35s: R_1= 3-Me-OBn; R_2 = OCH_3; R_3 = OCH_3; R_4 = H
35t: R_1= 4-Me-OBn; R_2 = OCH_3; R_3 = OCH_3; R_4 = H
35u: R_1= OBn; R_2 = OCH_3; R_3 = H; R_4 = Cl
35v: R_1= OBn; R_2 = OCH_3; R_3 = H; R_4 = Br

Scheme 2 Synthesis of compounds **7–28**. Reagents and conditions: (a) CH_3NO_2, CH_3COONH_4, AcOH, 80 °C, 4 h; (b) $LiAlH_4$, THF, 0–65 °C, 4 h; (c) EDCI, Et_3N, CH_2Cl_2, rt, 8 h; (d) $POCl_3$, CH_3CN, reflux; (e) $NaBH_4$, methanol, rt, 8 h; (f) HCOOH, HCHO, 90 °C, 4 h.

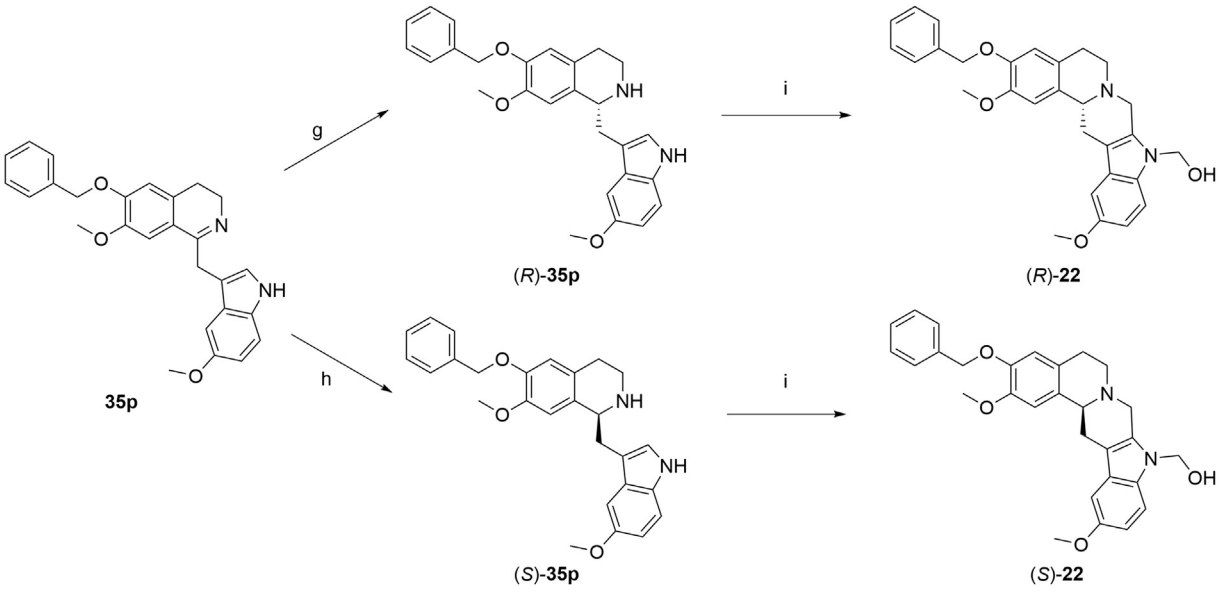
Finally, by replacing the hydrogen to the chlorine and bromine at the 13-position, compounds **27** and **28** reduced their activities on the down-regulation of PCSK9 (0.86 and 0.97). We concluded that the methoxyl group at the 12-position played a significant role in down-regulating PCSK9 expression.

2.3. *DiI-LDL uptake assays*

Whether down-regulation of PCSK9 expression could promote LDL-C clearance in hepatic cells was determined by low-density lipoprotein, labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI-LDL) uptake assay as previously described[20]. Eight compounds **8**, **15**, **18**, **21**, **22**, (*R*)-**22**, (*S*)-**22**, and **24** which exhibited better activity in decreasing hepatic cell PCSK9 expression than berberine, were co-incubated with HepG2 cells for the LDL-uptake assay. The results are summarized in Table 2. Firstly, berberine showed its LDL uptake rates with 1.38, compared to vehicle (DMSO). Subsequently, the above eight biological active compounds **8**, **15**, **18**, **21**, **22**, (*R*)-**22**, (*S*)-**22**, and **24** were tested, and among them, five compounds **15**, **18**, **22**, (*R*)-**22** and (*S*)-**22** displayed good LDL uptake rate (1.83, 1.78, 2.37, 2.19, and 1.83, respectively), which is much better than berberine (LDL uptake rate 1.38). The racemate **22** showed was slightly more potent than these two enantiomers (*R*)- and (*S*)-**22**, so the racemate **22** were used in the pharmacokinetic evaluation and *in vivo* biological assay. All selected compounds (<20 μmol/L) tested here showed no significant cytotoxicity in HepG2 cells (Supporting Information Table S1).

2.4. *Selectivity evaluation*

As a natural product, berberine and its derivatives exhibited various biological activities, including anti-depressant, anti-diabetes, and anti-inflammatory effects. Six compounds (**8**, **15**, **18**, **21**, **22**, and **24**) which exhibited better activity in decreasing hepatic cell PCSK9 expression than berberine, were selected to evaluate the biological selectivity, such as β_1 adrenergic receptor antagonist activity, dopamine D_1 and D_2 antagonist activity, and antagonistic activity on the serotonin 5-HT_{1B} (Table 3). Among them, compound **22** exhibited good selectivity against



Scheme 3 Synthesis of chiral compounds (*R*)- and (*S*)-**22**. Reagents and conditions: (g) (*S,S*)-Noyori's catalyst, HCOONa, $AgSbF_6$, $La(OTf)_3$, CTAB, H_2O, 40 °C, 12 h; (h) (*R,R*)-Noyori's catalyst, HCOONa, $AgSbF_6$, $La(OTf)_3$, CTAB, H_2O, 40 °C, 12 h; (i) HCOOH, 40% HCHO, CH_3CN, 80 °C, 4 h.

β_1 adrenergic receptor, dopamine D_1 and D_2 receptor ($IC_{50} \geq 100$ μmol/L). Compound **22** showed moderate antagonistic activity on the serotonin 5-HT_{1B} (IC_{50} = 13.43 μmol/L). The selectivity of compound **22** is better than berberine[21].

2.5. *Preliminary pharmacokinetic (PK) evaluation of compound **22***

To explore the further druggability of the new identified indole-containing THPB derivatives, compound **22** was further evaluated for its pharmacokinetic properties in hamsters after oral administration and intravenous, respectively. As shown in Table 4, compound **22** given orally at 20 mg/kg displayed a half-life ($t_{1/2}$) of 6.35 h, the maximum plasma concentration (C_{max}) of 169 ng/mL, and an $AUC_{0-\infty}$ value of 1005 ng/mL·h. Besides, compound **22** showed an oral bioavailability of 21.9%. Taken together, compared to the poor PK of the natural product berberine with low oral bioavailability (~0%)[22,23], compound **22** exhibited significant improvements on overall PK properties.

2.6. *Oral efficacy evaluation of compound **22** in hyperlipidemic hamsters in vivo*

In order to verify the hypolipidemic effect of compound **22** *in vivo*, high-fat diet (HFD)-induced hyperlipidemic hamster model was employed. Fenofibrate was used as the positive control during the *in vivo* studies[37]. After 21 days treatment, compared to the HFD control group, HFD hamsters given compound **22** 30 mg/kg by oral gavage showed 36.7% reduction in serum TC (Fig. 2A) and 41.4% reduction in serum LDL-C, respectively (Fig. 2B), which confirmed the hypolipidemic effect of compound **22** *in vivo*. The efficacy data showed that fenofibrate (*p.o.*, 100 mg/kg) decreased TC and LDL-C in 71.3% and 79.4%, respectively, compared to hamsters in the HFD control group. Also, serum TC and LDL-C in the HFD control group increased 376% and 751%, respectively, as compared to the normal control group, which indicated that the high-fat-diet-induced hyperlipidemic hamster model was successful.

2.7. *Effect of compound **22** on LDLR expression in hepatic cells*

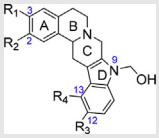
Mechanisms' studies demonstrated that PCSK9 could bind to the LDL receptor and target it for lysosomal degradation in hepatic cells[3]. Lower PCSK9 may promote LDLR expression and increase LDL-C clearance. Since compound **22** decreases PCSK9 protein expression in HepG2 cells with an IC_{50} value of 1.34 μmol/L and PCSK9 mRNA expression with an IC_{50} value of 1.10 μmol/L (Supporting Information Figs. S2 and S3), for the purpose of exploring the lipid-lowering mechanism of compound **22**, we tested the effect of compound **22** on LDLR expression in HepG2 cells.

HepG2 cells were treated with increasing concentrations of compound **22** (0.1, 0.5, 1, 2.5, and 5 μmol/L) for 24 h. Results demonstrated that compound **22** promotes hepatic cell LDLR expression in a dose-dependent manner with optimal 2.06-fold at 5 μmol/L compared to DMSO (Fig. 3), consistent with 2.37-fold LDL-uptake rate in DiI-LDL uptake assay (Table 2), which suggested that the increase of LDL-uptake in HepG2 cells mainly depended on the promotion of LDLR expression by compound **22**.

2.8. *hERG testing of compound **22***

Blockade of the human hERG channel was a significant hurdle encountered in drug discovery. Because compound **22** was efficacious *in vivo*, it was subjected to hERG testing using a patch-clamp experiment. The result showed that the IC_{50} value of compound **22** on hERG inhibition was 7.99 μmol/L. Compared to berberine (IC_{50} = 3.1 μmol/L)[24], compound **22** exhibited lower inhibitory activity on the hERG channel *in vitro*.

Table 1 Effects of test compounds on PCSK9 expression in HepG2 cells[a].



Compd. (5 μmol/L)	R_1	R_2	R_3	R_4	PCSK9 protein level (compared to vehicle, mean ± SEM)
7	$-OCH_2O-$		H	H	0.84±0.02*
8	OCH_3	2-F-OBn	H	H	0.68±0.06*
9	2-F-OBn	OCH_3	H	H	1.33±0.06*
10	3-F-OBn	OCH_3	H	H	0.82±0.09
11	4-F-OBn	OCH_3	H	H	0.81±0.07
12	3-Me-OBn	OCH_3	H	H	0.93±0.03
13	4-Me-OBn	OCH_3	H	H	0.69±0.17
14	$-OCH_2O-$		OCH_3	H	0.86±0.06
15	OCH_3	OBn	OCH_3	H	0.50±0.04**
16	OCH_3	2-F-OBn	OCH_3	H	1.02±0.15
17	OCH_3	3-F-OBn	OCH_3	H	0.84±0.08
18	OCH_3	4-F-OBn	OCH_3	H	0.56±0.09*
19	OCH_3	3-OMe-OBn	OCH_3	H	1.04±0.16
20	OCH_3	4-Me-OBn	OCH_3	H	0.92±0.09
21	OCH_3	OCH_2CF_3	OCH_3	H	0.58±0.07*
22	OBn	OCH_3	OCH_3	H	0.24±0.02***
(*R*)-**22**	OBn	OCH_3	OCH_3	H	0.21±0.01***
(*S*)-**22**	OBn	OCH_3	OCH_3	H	0.51±0.03**
23	2-F-OBn	OCH_3	OCH_3	H	0.87±0.06
24	3-F-OBn	OCH_3	OCH_3	H	0.48±0.02**
25	3-Me-OBn	OCH_3	OCH_3	H	0.94±0.03
26	4-Me-OBn	OCH_3	OCH_3	H	0.95±0.08
27	OBn	OCH_3	H	Cl	0.86±0.09
28	OBn	OCH_3	H	Br	0.97±0.11
Berberine	–				0.71±0.05*
Vehicle	–				1.00±0.03

–Not applicable.

[a]Effects of test compounds on PCSK9 expression in HepG2 cells. Test compounds (5 μmol/L) were co-incubated with HepG2 cells for 24 h. Then the cells were collected and lysed in RIPA buffer containing protease inhibitor cocktail. Western blot was utilized to measure PCSK9 expression in HepG2 cells. The abundance of PCSK9 was quantified using imageJ software with normalization by signals of β-actin. Data are presented as mean ± SEM of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; as compared to the vehicle group.

3. Conclusions

In this study, a novel series of indole-containing THPBs were designed, synthesized, and evaluated as PCSK9 modulators for the treatment of hyperlipidemia. SAR exploration led to the identification of a highly potent PCSK9 modulator **22** (IC_{50} = 1.34 μmol/L), which promoted 2.37-fold (5 μmol/L) LDL uptake in HepG2 cells. Notably, as a lead compound, compound **22** showed excellent *in vivo* hypolipidemic potency and a good pharmacokinetic profile, which is much better than berberine. To conclude, results we have earned suggest that compound **22** worths further investigation as an anti-hyperlipidemic agent.

4. Experimental

4.1. General metods of chemistry

Chemicals and solvents were purchased from commercial sources (Alfa, Acros, Sigma−Aldrich, and Shanghai Chemical Reagent Company) and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15−0.2 mm thickness, YantaiHuiyou Company, China). Column chromatography was performed with CombiFlash Companion system (Teledyne Isco, Inc. Lincoln, NE, USA). Nuclear magnetic resonance spectra was recorded on a 400 MHz instrument (TMS as IS). Chemical shifts were reported in parts per million (ppm). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (MS and HR-MS) were given with electrospray ionization (ESI) produced by Q-TOF mass spectrometer. All target compounds were confirmed with over 95% purity (Supporting Information Table S2), which were determined by Agilent-1100 HPLC with binary pump, photodiode array detector (DAD), using Agilent Extend-C18 column (15 cm × 0.46 cm, 5 μm), CH_3OH/H_2O [0.1% triethylamine was added in CH_3OH = 70/30 (*v*/*v*)] or CH_3OH/H_2O [0.1% triethylamine was added in CH_3OH = 60/40 (*v*/*v*)] at 1.0 mL/min, and calculating the peak areas at 280 nm. Berberine was purchased from meilun biotechnology (Dalian, China).

Table 2 Effects of test compounds on the regulation of LDL uptake by HepG2 cells[a].

Compd. (5 μmol/L)	DiI-LDL uptake rate (compared to vehicle, mean±SEM)
8	1.04±0.04
15	1.83±0.04**
18	1.78±0.06**
21	1.45±0.04**
22	2.37±0.07***
(*R*)-**22**	2.19±0.07***
(*S*)-**22**	1.83±0.03**
24	1.62±0.05**
Berberine	1.38±0.03*
Vehicle	1.00±0.02

[a]Effects of test compounds on the regulation of LDL uptake by HepG2 cells. HepG2 cells were treated with test compounds (5 μmol/L) for 24 h. Then the culture medium was changed to DMEM supplemented with 2% LPDS and 20 μg/mL DiI-LDL for another 4 h incubation. DiI-LDL uptaken by HepG2 cells was extracted by isopropanol and measured by microplate reader. Results are shown as mean ± SEM of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; as compared to the vehicle group.

4.2. *General synthetic procedures for the target compounds **7–28** (compound **7** as example)*

4.2.1. *(E)-5-(2-Nitrovinyl)benzo[d][1,3]dioxole (**30a**)*

To a solution of benzo[*d*][1,3]dioxole-5-carbaldehyde (**29a**, 20 mmol) and ammonium acetate (3.08 g, 40 mmol) in nitromethane (20 mL) was added acetic acid (10 mL), and the resulting mixture was heated to 80 °C for 4 h. Then the mixture was concentrated, and the concentrate was added saturated $NaHCO_3$ to obtained yellow solid, which was washed with isopropanol to get the intermediate (*E*)-5-(2-nitrovinyl)benzo[*d*][1,3]dioxole (**30a**) (3.67 g, 95%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 13.5 Hz, 1H), 7.51 (d, J = 13.5 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 7.04 (s, 1H), 6.91 (d, J = 7.9 Hz, 1H), 6.10 (s, 2H). ESI-MS *m/z*: 194 $[M+H]^+$.

4.2.2. *2-(Benzo[d][1,3]dioxol-5-yl)ethanamine (**31a**)*

Lithium aluminium hydride (2.16 g, 57 mmol) was slowly added into dry tetrahydrofuran (50 mL) to form suspensions. A solution of **30a** (3.67 g, 19 mmol) in tetrahydrofuran (50 mL) was added dropwise to suspensions at 0 °C, and the mixture was stirred for 4 h at 65 °C. The reaction mixture was cooled to room temperature (RT) and diluted with CH_2Cl_2 (150 mL), and then the reaction mixture was quenched with appropriate ice–water and filtered. The filtrate was evaporated under reduced pressure to get the product (**31a**) (2.19 g, 71%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76–6.71 (m, 1H), 6.69 (d, J = 4.5 Hz, 1H), 6.63 (dd, J = 7.9, 1.5 Hz, 1H), 5.91 (s, 2H), 2.90 (t, J = 6.8 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H), 1.40–1.13 (m, 2H). ESI-MS *m/z*: 166 $[M+H]^+$.

4.2.3. *N-(2-(Benzo[d][1,3]dioxol-5-yl)ethyl)-2-(1H-indol-3-yl) acetamide (**33a**)*

2-(1*H*-Indol-3-yl)acetic acid (**32a**, 3.5 g, 19.95 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.8 g, 19.95 mmol), 1-hydroxybenzotriazole (3.23 g, 23.94 mmol), and triethylamine (2.01 g, 19.95 mmol) were dissolved in 50 mL of dichloromethane, and the mixture was stirred for 1 h at room temperature. Then a solution of 2-(benzo[*d*][1,3]dioxol-5-yl) ethanamine (**31a**) (13.3 mmol) in dichloromethane (20 mL) was added into the mixture and stirred for 8 h, water was added, the mixture was extracted with ethyl acetate, and the organic extracts were washed with brine, dried over Na_2SO_4, and concentrated.

Table 3 Selectivity of six compounds on the typical biological targets of berberine[a].

Compd.	IC_{50} (μmol/L)			
	β_1	D_1	D_2	5-HT_{1B}
8	>100	5.56±0.08	17.87±0.86	47.16±1.22
15	>100	1.61±0.13	>100	40.83±0.80
18	~100	1.71±0.10	>100	>100
21	~100	2.35±0.07	~50	>100
22	>100	~100	>100	13.43±0.51
24	>100	>100	>100	15.70±0.59
Berberine	–	15.5[b]	17.1[b]	–

–Not applicable.

[a]Values are the average of 3 independent experiments. β_1, beta-1 adrenergic receptor antagonist activity; D_1, dopamine D_1 antagonist activity; D_2, dopamine D_2 antagonist activity; 5-HT_{1B}, serotonin 5-HT_{1B} receptor antagonist activity.

[b]Ref. 21.

Table 4 Pharmacokinetic parameters of compound **22** in hamsters[a].

Compd.	Admin.	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng/mL·h)	MRT (h)	$t_{1/2}$ (h)	CL (mL/min/kg)	F (%)
22	*p.o.*	169	1005	10.3	6.35	–	21.9
	i.v.	–	2088	4.13	3.61	84.1	

–Not determined.

[a]Values are the average of three runs. C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve; MRT, mean residence time; $t_{1/2}$, half-life; CL, clearance; F, oral bioavailability. Dose: *p.o.* at 20 mg/kg. Dose: i.v. at 10 mg/kg.

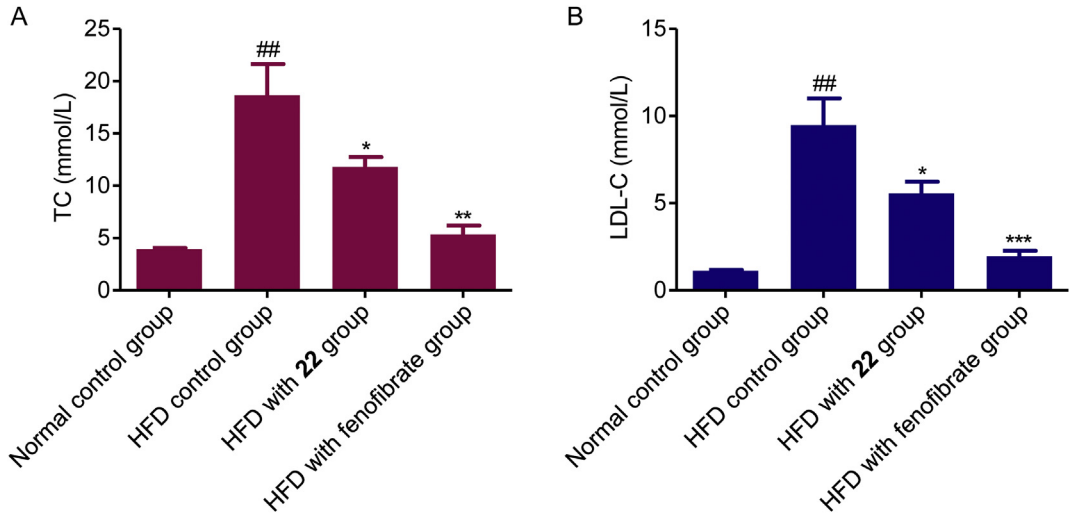


Figure 2 Hypolipidemic effect of compound **22** and fenofibrate in the hyperlipidemic hamsters. Hamsters involved in the experiment were divided into normal control group ($n = 7$) and HFD group ($n = 21$), and then the HFD group was switched into a high-fat diet (0.5% cholesterol) to induce hyperlipidemia. One week later, hamsters in the HFD group were divided into HFD control group and test groups ($n = 7$ per group). Test groups were given compound **22** at a daily dose of 30 mg/kg or fenofibrate at a daily dose of 100 mg/kg by oral gavage for 21 days. Blood samples of hamsters were collected at the end of experiment course. Serum TC levels (A) and LDL-C levels (B) of hamsters were measured by commercially available kits. Data are presented as mean ± SEM ($^{\#\#}P < 0.01$ as compared to the normal control group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; as compared to the HFD control group).

The residue was purified by flash chromatography to give **33a** (3.39 g, 79%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.49 (d, $J = 7.9$ Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 1H), 7.28−7.19 (m, 1H), 7.17−7.10 (m, 1H), 7.06 (d, $J = 2.1$ Hz, 1H), 6.51 (d, $J = 7.9$ Hz, 1H), 6.42 (d, $J = 1.6$ Hz, 1H), 6.26 (dd, $J = 7.9$, 1.7 Hz, 1H), 5.88 (s, 2H), 5.76 (s, 1H), 3.71 (s, 2H), 3.36 (q, $J = 6.7$ Hz, 2H), 2.56 (t, $J = 6.8$ Hz, 2H). ESI-MS *m/z*: 323 $[M+H]^+$.

*4.2.4. 3-((7,8-Dihydronaphtho[2,3-d][1,3]dioxol-5-yl)methyl)-1H-indole (**35a**)*

N-(2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl)-2-(1*H*-indol-3-yl)acetamide (**33a**) (3.39 g, 10.5 mmol) was dissolved in 20 mL of acetonitrile, and $POCl_3$ (5.9 mL, 63.3 mmol) was added. The solution was heated to reflux under argon for 1 h. The solvents were evaporated under reduced pressure. The pH of the mixture was adjusted to alkalinity with the addition of saturated $NaHCO_3$. The mixture was extracted with dichloromethane. The combined organic phase was evaporated under reduced pressure to get the crude product (**34a**) which was used in the next step without further purification. ESI-MS *m/z*: 305 $[M+H]^+$. The intermediate **34a** was dissolved in 50 mL of methanol, and $NaBH_4$ (3.97 g, 105 mmol) was added in batches at 0 °C. The mixture was stirred for 2 h at room temperature. The reaction mixture was quenched with ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, and the combined organic phase was evaporated under reduced pressure to get the crude product, which was purified by flash chromatography on silica gel to get intermediate **35a** (1.19 g, 3.89 mmol, 37% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.59 (d, $J = 7.8$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.18 (dd, $J = 11.1$, 4.0 Hz, 1H), 7.15−7.08 (m, 2H), 6.76 (s, 1H), 6.55 (s, 1H), 5.92 (d, $J = 0.9$ Hz, 2H), 4.32 (dd, $J = 9.5$, 3.8 Hz, 1H), 3.38 (dd, $J = 14.8$, 3.8 Hz, 2H), 3.27 (s, 1H), 3.13 (dd, $J = 14.8$, 9.6 Hz, 2H), 2.87−2.75 (m, 2H), 2.74−2.63 (m, 1H). ESI-MS *m/z*: 307 $[M+H]^+$.

*4.2.5. (5,6,14,14a-Tetrahydro-[1,3]dioxolo[4,5-g]indolo[3′,2′:4,5]-pyrido[2,1-a]isoquino-lin-9(8H)-yl)methanol (**7**)*

The intermediate **35a** (0.5 g, 1.63 mmol), 0.5 mL of formaldehyde, and 0.1 mL of formic acid were dissolved in 30 mL of acetonitrile.

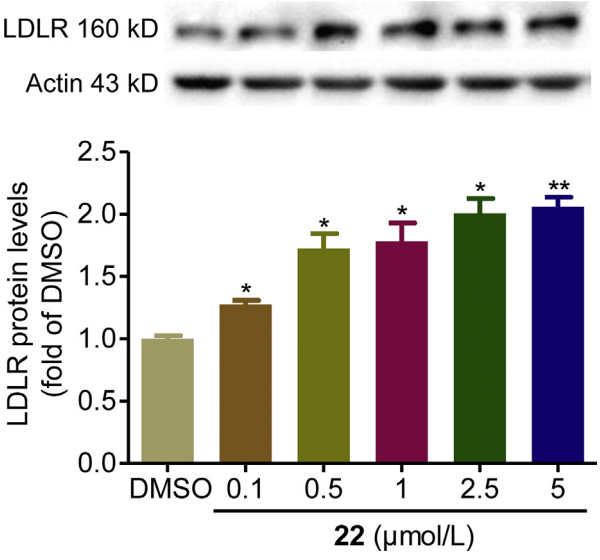


Figure 3 Compound **22** promotes HepG2 cell LDLR expression in a dose-dependent manner. Total protein of HepG2 cells treated with increasing concentrations of compound **22** (0.1, 0.5, 1, 2.5, and 5 μmol/L) for 24 h was isolated by RIPA buffer. Western blot was utilized to measure LDLR expression in HepG2 cells. The protein abundance of LDLR was quantified using imageJ software with normalization by signals of β-actin. Results are shown as mean ± SEM of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; as compared to the DMSO group.

The mixture was stirred for 4 h at 90 °C. Then the mixture was evaporated under reduced pressure to get the residue. With water added, the mixture was extracted with ethyl acetate, and the organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. Then chromatographed on silica gel to give the target compound **7** (0.2 g). Yellow solid, m.p. = 258–260 °C; Yield: 35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (dd, J = 14.2, 7.8 Hz, 2H), 7.15−7.06 (m, 1H), 7.05−6.99 (m, 2H), 6.69 (s, 1H), 6.25 (t, J = 7.1 Hz, 1H), 5.96 (dd, J = 5.3, 0.9 Hz, 2H), 5.48−5.36 (m, 2H), 4.19 (d, J = 15.1 Hz, 1H), 3.69 (d, J = 15.1 Hz, 1H), 3.58 (dd, J = 10.4, 3.3 Hz, 1H), 3.37 (dd, J = 15.2, 2.7 Hz, 1H), 3.15−3.06 (m, 1H), 2.93 (m, 1H), 2.74−2.56 (m, 2H), 2.49−2.39 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.74, 145.45, 136.01, 133.11, 131.42, 127.49, 126.85, 120.63, 119.02, 117.61, 109.73, 108.05, 107.67, 106.03, 100.56, 65.29, 59.52, 51.13, 50.87, 29.41, 29.20; ESI-MS *m/z*: 349 $[M+H]^+$. ESI-HR-MS Calcd. for $C_{21}H_{21}N_2O_3^+$ $[M+H]^+$ 349.1547, Found 349.1553. HPLC analysis: MeOH/H_2O = 70:30 (*v/v*), 7.12 min, 98.44% purity.

*4.2.6. (2-((2-Fluorobenzyl)oxy)-3-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**8**)*

Compound **8** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 180–183 °C; Yield: 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (dd, J = 8.3, 6.8 Hz, 1H), 7.52−7.37 (m, 3H), 7.33−7.20 (m, 2H), 7.08 (dt, J = 29.0, 7.3 Hz, 3H), 6.74 (s, 1H), 6.27 (s, 1H), 5.43 (d, J = 7.2 Hz, 2H), 5.14 (q, J = 11.7 Hz, 2H), 4.20 (d, J = 12.1 Hz, 1H), 3.75 (s, 3H), 3.73−3.65 (m, 1H), 3.59 (m, 1H), 3.42 (d, J = 13.9 Hz, 1H), 3.15 (d, J = 7.8 Hz, 1H), 3.03−2.86 (m, 1H), 2.69 (d, J = 15.0 Hz, 2H), 2.45−2.26 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.45 (d, $J_{C\text{-}F}$ = 246.1 Hz), 147.64, 145.89, 136.01, 131.05 (d, $J_{C\text{-}F}$ = 4.0 Hz), 130.34 (d, $J_{C\text{-}F}$ = 8.2 Hz), 126.75, 124.48 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.16, 124.01, 120.67, 119.06, 117.57, 115.32 (d, $J_{C\text{-}F}$ = 21.1 Hz), 111.90, 111.75, 109.76, 107.64, 65.27, 64.44 (d, $J_{C\text{-}F}$ = 3.3 Hz), 59.23, 55.46, 51.08, 51.04, 28.90, 28.86; ESI-MS *m/z*: 459 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}FN_2O_3^+$ $[M+H]^+$ 459.2078, Found 459.2090. MeOH/H_2O = 70:30 (*v/v*), 15.04 min, >99% purity.

*4.2.7. (3-((2-Fluorobenzyl)oxy)-2-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**9**)*

Compound **9** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 167–169 °C; Yield: 38%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (td, J = 7.5, 1.4 Hz, 1H), 7.52−7.40 (m, 3H), 7.32−7.21 (m, 2H), 7.13−7.07 (m, 1H), 7.03 (dd, J = 10.4, 3.4 Hz, 2H), 6.86 (s, 1H), 6.26 (t, J = 7.1 Hz, 1H), 5.52−5.35 (m, 2H), 5.12−5.06 (m, 2H), 4.20 (d, J = 15.2 Hz, 1H), 3.79 (s, 3H), 3.70 (d, J = 15.1 Hz, 1H), 3.61 (dd, J = 10.3, 3.2 Hz, 1H), 3.46 (dd, J = 15.0, 2.7 Hz, 1H), 3.20−3.05 (m, 1H), 2.95 (m, 1H), 2.73−2.59 (m, 2H), 2.44 (d, J = 13.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.40 (d, J = 246.0 Hz), 147.57, 146.06, 136.02, 133.19, 131.02, 130.82 (d, $J_{C\text{-}F}$ = 4.1 Hz), 130.36 (d, $J_{C\text{-}F}$ = 8.1 Hz), 126.87, 126.43, 124.55 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.10 (d, $J_{C\text{-}F}$ = 14.6 Hz), 120.62, 119.02, 117.68, 115.37 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.43, 110.03, 109.76, 107.77, 65.30, 64.15 (d, $J_{C\text{-}F}$ = 3.4 Hz), 59.33, 55.87, 51.24, 51.08, 29.09, 28.97; ESI-MS *m/z*: 459 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}FN_2O_3$ $[M+H]^+$ 459.2078, Found 459.2090. MeOH/H_2O = 70:30 (*v/v*), 12.75 min, 98.83% purity.

*4.2.8. (3-((3-Fluorobenzyl)oxy)-2-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**10**)*

Compound **10** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 149–151 °C; Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52−7.42 (m, 3H), 7.35−7.23 (m, 2H), 7.17 (td, J = 8.6, 2.2 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.02 (dd, J = 8.8, 6.2 Hz, 2H), 6.81 (s, 1H), 6.26 (t, J = 7.0 Hz, 1H), 5.50−5.37 (m, 2H), 5.14−5.03 (m, 2H), 4.20 (d, J = 15.1 Hz, 1H), 3.81 (s, 3H), 3.69 (d, J = 15.1 Hz, 1H), 3.61 (dd, J = 10.3, 3.0 Hz, 1H), 3.46 (dd, J = 15.0, 2.3 Hz, 1H), 3.19−3.07 (m, 1H), 3.00−2.90 (m, 1H), 2.64 (dd, J = 11.0, 7.7 Hz, 2H), 2.44 (d, J = 13.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.20 (d, $J_{C\text{-}F}$ = 243.4 Hz), 147.61, 145.99, 140.35 (d, $J_{C\text{-}F}$ = 7.5 Hz), 136.02, 133.17, 131.00, 130.45 (d, $J_{C\text{-}F}$ = 8.4 Hz), 126.86, 126.38, 123.54, 120.62, 119.01, 117.66, 114.52 (d, $J_{C\text{-}F}$ = 20.9 Hz), 114.18 (d, $J_{C\text{-}F}$ = 21.8 Hz), 113.57, 110.06, 109.75, 107.76, 69.13, 65.30, 59.32, 55.94, 51.23, 51.06, 29.08, 28.97; ESI-MS *m/z*: 459 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}FN_2O_3^+$ $[M+H]^+$ 459.2078, Found 459.2081. MeOH/H_2O = 70:30 (*v/v*), 13.63 min, 96.21% purity.

*4.2.9. (3-((4-Fluorobenzyl)oxy)-2-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**11**)*

Compound **11** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 149–151 °C; Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58–7.41 (m, 4H), 7.23 (t, J = 8.9 Hz, 2H), 7.10 (t, J = 7.4 Hz, 1H), 7.06–6.96 (m, 2H), 6.81 (s, 1H), 6.26 (t, J = 7.0 Hz, 1H), 5.43 (d, J = 6.7 Hz, 2H), 5.04 (s, 2H), 4.20 (d, J = 15.1 Hz, 1H), 3.80 (s, 3H), 3.69 (d, J = 15.1 Hz, 1H), 3.64–3.57 (m, 1H), 3.52–3.43 (m, 1H), 3.19–3.08 (m, 1H), 2.94 (m, 1H), 2.68–2.60 (m, 2H), 2.44 (d, J = 12.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.77 (d, $J_{C\text{-}F}$ = 243.5 Hz), 147.59, 146.12, 136.02, 133.58, 133.55, 133.18, 130.83, 130.03, 129.94, 126.87, 126.35, 119.01, 117.67, 115.33, 115.12, 113.49, 109.98, 109.76, 107.77, 69.22, 65.30, 59.33, 55.89, 51.24, 51.08, 29.09, 28.98; ESI-MS *m/z*: 459 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}FN_2O_3^+$ $[M+H]^+$ 459.2078, Found 459.2090. MeOH/H_2O = 70:30 (*v/v*), 12.73 min, 95.53% purity.

*4.2.10. (2-Methoxy-3-((3-methylbenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**12**)*

Compound **12** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 152–154 °C; Yield: 41%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (dd, J = 7.8, 2.8 Hz, 2H), 7.27 (p, J = 7.5 Hz, 3H), 7.15 (d, J = 7.2 Hz, 1H), 7.11 (t, J = 7.3 Hz, 1H), 7.07−6.99 (m, 2H), 6.26 (t, J = 7.0 Hz, 1H), 5.43 (d, J = 7.7 Hz, 2H), 5.01 (s, 2H), 4.20 (d, J = 15.1 Hz, 1H), 3.80 (s, 3H), 3.70 (d, J = 15.5 Hz, 1H), 3.61 (d, J = 7.0 Hz, 1H), 3.46 (d, J = 14.5 Hz, 1H), 3.14 (d, J = 6.5 Hz, 1H), 3.02−2.87 (m, 1H), 2.65 (d, J = 13.2 Hz, 2H), 2.45 (d, J = 12.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.59, 146.32, 137.52, 137.21, 136.03, 133.17, 130.67, 128.45, 128.40, 128.32, 126.85, 126.32, 124.94, 120.64, 119.02, 117.68, 113.37, 109.97, 109.76, 107.76, 70.01, 65.30, 59.34, 55.89, 51.23, 51.08, 29.05, 28.98, 21.04; ESI-MS *m/z*: 455 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{31}N_2O_3^+$ $[M+H]^+$ 455.2329, Found 455.2330. MeOH/H_2O = 70:30 (*v/v*), 20.71 min, 98.07% purity.

*4.2.11. (2-Methoxy-3-((4-methylbenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**13**)*

Compound **13** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 158–160 °C; Yield: 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (dd, J = 7.8, 3.3 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 7.10 (t, J = 7.1 Hz, 1H), 7.02 (dd, J = 13.6, 5.8 Hz, 2H), 6.80 (s, 1H), 6.26 (t, J = 7.1 Hz, 1H), 5.43 (d, J = 6.6 Hz, 2H), 5.09−4.96 (m, 2H), 4.20 (d, J = 15.2 Hz, 1H), 3.79 (s, 3H), 3.69 (d, J = 15.0 Hz, 1H), 3.65−3.57 (m, 1H), 3.46 (dd, J = 15.0, 2.6 Hz, 1H), 3.18−3.08 (m, 1H), 3.00−2.85 (m, 1H), 2.70−2.59 (m, 2H), 2.44 (d, J = 12.9 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.58, 146.29, 137.02, 136.02, 134.28, 133.18, 130.62, 128.95, 127.90, 126.87, 126.34, 120.62, 119.01, 117.67, 113.37, 109.99, 109.76, 107.78, 69.80, 65.30, 59.34, 55.90, 51.24, 51.11, 29.09, 28.99, 20.82; ESI-MS *m/z*: 455 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{31}N_2O_3^+$ $[M+H]^+$ 455.2329, Found 455.2342. MeOH/H_2O = 70:30 (*v/v*), 19.28 min, >99% purity.

*4.2.12. (12-Methoxy-5,6,14,14a-tetrahydro-[1,3]dioxolo[4,5-g]indolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**14**)*

Compound **14** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 169–171 °C; Yield: 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (d, J = 8.8 Hz, 1H), 7.01 (s, 1H), 6.97 (d, J = 2.4 Hz, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.69 (s, 1H), 6.19 (t, J = 7.0 Hz, 1H), 5.96 (d, J = 1.6 Hz, 2H), 5.40−5.33 (m, 2H), 4.16 (d, J = 15.2 Hz, 1H), 3.76 (s, 3H), 3.66 (d, J = 14.1 Hz, 1H), 3.57 (d, J = 6.5 Hz, 1H), 3.36 (d, J = 15.6 Hz, 1H), 3.12 (d, J = 6.0 Hz, 1H), 3.02−2.86 (m, 1H), 2.66 (d, J = 15.8 Hz, 2H), 2.47−2.35 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.52, 145.73, 145.44, 133.59, 131.38, 131.09, 127.41, 127.21, 110.33, 110.01, 108.01, 107.43, 105.97, 100.54, 100.08, 65.37, 59.54, 55.31, 51.16, 50.86, 29.31, 29.23; ESI-MS *m/z*: 379 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{22}H_{23}N_2O_4^+$ $[M+H]^+$ 379.1652, Found 379.1661. MeOH/H_2O = 70:30 (*v/v*), 5.63 min, 96.36% purity.

*4.2.13. (2-(Benzyloxy)-3,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1 a]isoquinolin-9(8H)-yl)methanol (**15**)*

Compound **15** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 182–183 °C; Yield: 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52−7.47 (m, 2H), 7.45−7.40 (m, 2H), 7.38−7.32 (m, 2H), 7.05 (s, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.74 (dd, J = 8.7, 2.4 Hz, 2H), 6.21 (t, J = 7.1 Hz, 1H), 5.42−5.32 (m, 2H), 5.16−5.03 (q, J = 11.8 Hz, 2H), 4.16 (d, J = 15.1 Hz, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 3.64 (d, J = 14.9 Hz, 1H), 3.55 (dd, J = 12.8, 5.0 Hz, 1H), 3.32 (m, 1H), 3.18−3.05 (m, 1H), 2.95 (m, 1H), 2.71−2.57 (m, 2H), 2.40−2.25 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.57, 147.62, 146.07, 137.47, 133.77, 131.10, 130.17, 128.38, 127.95, 127.77, 127.23, 126.92, 111.89, 110.44, 110.10, 107.54, 99.87, 70.37, 65.39, 59.33, 55.52, 55.34, 54.94, 51.31, 51.20, 29.18, 29.00; ESI-MS *m/z*: 471 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{31}N_2O_4^+$ $[M+H]^+$ 471.2278, Found 471.2286. MeOH/H_2O = 70:30 (*v/v*), 10.10 min, 97.37% purity.

*4.2.14. (2-((2-Fluorobenzyl)oxy)-3,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**16**)*

Compound **16** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 182–183 °C; Yield: 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (t, J = 7.2 Hz, 1H), 7.44 (dd, J = 13.3, 6.2 Hz, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.27 (dd, J = 12.7, 6.0 Hz, 2H), 7.09 (s, 1H), 6.96 (d, J = 1.9 Hz, 1H), 6.86−6.66 (m, 2H), 6.22 (t, J = 7.0 Hz, 1H), 5.36 (t, J = 9.6 Hz, 2H), 5.14 (q, J = 11.6 Hz, 2H), 4.16 (d, J = 15.1 Hz, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.65 (d, J = 15.0 Hz, 1H), 3.57 (d, J = 7.8 Hz, 1H), 3.40 (s, 1H), 3.20−3.07 (m, 1H), 2.97 (dd, J = 18.8, 7.2 Hz, 1H), 2.65 (dd, J = 21.8, 13.3 Hz, 2H), 2.43−2.31 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.48 (d, $J_{C\text{-}F}$ = 246.1 Hz), 153.56, 147.63, 145.92, 133.76, 131.11, 131.10, 131.07, 130.24, 127.29, 127.23, 124.50 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.16 (d, $J_{C\text{-}F}$ = 14.6 Hz), 115.44, 115.23, 111.94, 110.42, 110.09, 107.54, 99.88, 65.39, 64.60 (d, $J_{C\text{-}F}$ = 3.3 Hz), 59.30, 55.48, 55.34, 51.31, 51.15, 29.16, 29.03. ESI-MS *m/z*: 489 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{30}FN_2O_4^+$ $[M+H]^+$ 489.2194, Found 489.2193. MeOH/H_2O = 70:30 (*v/v*), 11.45 min, 95.77% purity.

*4.2.15. (2-((3-Fluorobenzyl)oxy)-3,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**17**)*

Compound **17** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 176–178 °C; Yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (m, 1H), 7.39−7.27 (m, 3H), 7.18 (td, J = 8.4, 2.3 Hz, 1H), 7.05 (s, 1H), 6.93 (d, J = 2.4 Hz, 1H), 6.80−6.64 (m, 2H), 6.21 (t, J = 7.1 Hz, 1H), 5.44−5.31 (m, 2H), 5.20−5.05 (m, 2H), 4.16 (d, J = 15.1 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.64 (d, J = 15.0 Hz, 1H), 3.54 (dd, J = 10.1, 3.0 Hz, 1H), 3.31 (d, J = 2.6 Hz, 1H), 3.21–3.07 (m, 1H), 3.00−2.89 (m, 1H), 2.81−2.57 (m, 2H), 2.36−2.28 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.21 (d, $J_{C\text{-}F}$ = 243.4 Hz), 153.56, 147.63, 145.72, 140.48 (d, $J_{C\text{-}F}$ = 7.4 Hz), 133.76, 131.08, 130.42 (d, $J_{C\text{-}F}$ = 8.2 Hz), 130.15, 128.17 (d, $J_{C\text{-}F}$ = 42.0 Hz), 127.19, 127.16, 123.72 (d, $J_{C\text{-}F}$ = 2.7 Hz), 114.60, 114.38, 112.00 (d, $J_{C\text{-}F}$ = 16.3 Hz), 110.45, 110.13, 107.47, 99.74, 69.44, 65.38, 59.31, 55.54, 55.31, 51.30, 51.20, 29.16, 28.99; ESI-MS *m/z*: 489 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{30}FN_2O_4^+$ $[M+H]^+$ 489.2184, Found 489.2181. MeOH/H_2O = 70:30 (*v/v*), 10.63 min, >99% purity.

*4.2.16. (2-((4-Fluorobenzyl)oxy)-3,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**18**)*

Compound **18** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 151–153 °C; Yield: 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (dd, J = 8.5, 5.7 Hz, 2H), 7.37 (d, J = 8.8 Hz, 1H), 7.24 (t, J = 8.9 Hz, 2H), 7.04 (s, 1H), 6.94 (d, J = 2.2 Hz, 1H), 6.74 (dd, J = 7.8, 3.1 Hz, 2H), 6.23 (t, J = 6.7 Hz, 1H), 5.37 (d, J = 7.5 Hz, 2H), 5.09 (q, J = 11.7 Hz, 2H), 4.17 (d, J = 14.8 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.61 (dd, J = 26.4, 16.6 Hz, 1H), 3.13 (d, J = 8.0 Hz, 1H), 2.99−2.93 (m, 1H), 2.69−2.63 (m, 2H), 2.35 (t, J = 11.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.78 (d, $J_{C\text{-}F}$ = 243.4 Hz), 153.57, 147.67, 145.92, 133.70, 133.67, 131.09, 130.19, 130.10, 127.17, 127.06, 115.28, 115.07, 112.07, 111.88, 110.47, 110.14, 107.48, 99.80, 69.70, 65.39, 59.33, 55.51, 55.33, 51.28, 51.16, 29.16, 29.00; ESI-MS *m/z*: 489 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{30}FN_2O_4^+$ $[M+H]^+$ 489.2184, Found 489.2185. MeOH/H_2O = 70:30 (*v/v*), 10.30 min, >99% purity.

*4.2.17. (3,12-Dimethoxy-2-((3-methoxybenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**19**)*

Compound **19** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 85–86 °C; Yield: 43%. ^{1}H

NMR (400 MHz, DMSO-d_6) δ 7.51−7.20 (m, 2H), 7.21−7.00 (m, 3H), 7.01−6.85 (m, 2H), 6.83−6.66 (m, 2H), 6.20 (t, J = 6.9 Hz, 1H), 5.37 (d, J = 7.6 Hz, 2H), 5.09 (q, J = 12.1 Hz, 2H), 4.16 (d, J = 14.9 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.77 (s, 3H), 3.64 (d, J = 14.8 Hz, 1H), 3.54 (d, J = 8.2 Hz, 1H), 3.35 (d, J = 2.9 Hz, 1H), 3.13 (d, J = 9.5 Hz, 1H), 3.03–2.84 (m, 1H), 2.64 (m, 2H), 2.33 (t, J = 12.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.32, 153.56, 147.62, 145.99, 139.07, 133.77, 131.10, 130.15, 129.48, 127.22, 126.94, 120.01, 113.36, 113.23, 111.94, 111.90, 110.43, 110.07, 107.50, 99.90, 70.19, 65.38, 59.33, 55.54, 55.37, 55.08, 51.31, 51.20, 29.16, 28.99; ESI-MS *m/z*: 501 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{30}H_{33}N_2O_5^+$ $[M+H]^+$ 501.2384, Found 501.2395. MeOH/H_2O = 70:30 (*v/v*), 9.60 min, 95.32% purity.

*4.2.18. (3,12-Dimethoxy-2-((4-methylbenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**20**)*

Compound **20** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 205–207 °C; Yield: 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (dd, J = 8.4, 3.0 Hz, 3H), 7.22 (d, J = 7.8 Hz, 2H), 7.06 (s, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.73 (dd, J = 9.2, 2.8 Hz, 2H), 6.21 (t, J = 7.1 Hz, 1H), 5.37 (d, J = 6.6 Hz, 2H), 5.05 (q, J = 11.6 Hz, 2H), 4.16 (d, J = 15.1 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.64 (d, J = 15.1 Hz, 1H), 3.55 (dd, J = 10.5, 2.9 Hz, 1H), 3.38 (d, J = 2.9 Hz, 1H), 3.17−3.06 (m, 1H), 3.02−2.86 (m, 1H), 2.73−2.56 (m, 2H), 2.36 (d, J = 12.8 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.55, 147.58, 146.13, 137.00, 134.37, 133.77, 131.10, 130.15, 128.90, 128.12, 127.23, 126.81, 111.86, 111.77, 110.43, 110.08, 107.54, 99.91, 70.22, 65.38, 59.35, 55.50, 55.37, 51.32, 51.20, 29.16, 29.00, 20.84. ESI-MS *m/z*: 501 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{30}H_{33}N_2O_4^+$ $[M+H]^+$ 485.2435, Found 485.2432. MeOH/H_2O = 70:30 (*v/v*), 16.71 min, >99% purity.

*4.2.19. (3,12-Dimethoxy-2-(2,2,2-trifluoroethoxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl) methanol (**21**)*

Compound **21** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 180–182 °C; Yield: 41%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (d, J = 8.8 Hz, 1H), 7.13 (s, 1H), 6.96 (d, J = 2.4 Hz, 1H), 6.80 (s, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.21 (t, J = 7.0 Hz, 1H), 5.37 (d, J = 6.4 Hz, 2H), 4.93–4.59 (m, 2H), 4.17 (d, J = 15.1 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.66 (d, J = 15.0 Hz, 1H), 3.56 (dd, J = 10.2, 2.8 Hz, 1H), 3.44 (dd, J = 15.0, 2.8 Hz, 1H), 3.19–3.10 (m, 1H), 3.05–2.90 (m, 1H), 2.72–2.60 (m, 2H), 2.47–2.35 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.56, 147.55, 144.68, 133.70, 131.09, 130.39, 128.95, 127.19, 124.14 (d, $J_{C\text{-}F}$ = 278.1 Hz), 113.02, 112.31, 110.42, 110.12, 107.48, 99.86, 66.10 (d, $J_{C\text{-}F}$ = 33.8 Hz), 65.38, 59.25, 55.62, 55.35, 51.28, 51.06, 29.10, 29.06; ESI-MS *m/z*: 463 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{24}H_{26}F_3N_2O_4^+$ $[M+H]^+$ 463.1839, Found 463.1842. MeOH/H_2O = 70:30 (*v/v*), 5.61 min, 95.21% purity.

*4.2.20. (3-(Benzyloxy)-2,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**22**)*

Compound **22** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 145–146 °C; Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49−7.44 (m, 2H), 7.41 (m, 2H), 7.38−7.32 (m, 1H), 7.06−6.95 (m, 2H), 6.82 (s, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.20 (t, J = 7.1 Hz, 1H), 5.43−5.36 (m, 2H), 5.06 (s, 2H), 4.17 (d, J = 15.2 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.66 (d, J = 15.1 Hz, 1H), 3.59 (dd, J = 10.5, 3.0 Hz, 1H), 3.43 (dd, J = 15.0, 2.7 Hz, 1H), 3.19–3.04 (m, 1H), 2.94 (m, 1H), 2.70–2.58 (m, 2H), 2.47–2.37 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.54, 147.57, 146.26, 137.32, 133.74, 131.08, 130.74, 128.40, 127.80, 127.78, 127.24, 126.38, 113.40, 110.38, 110.10, 110.05, 107.56, 100.01, 69.92, 65.38, 59.39, 55.95, 55.37, 51.33, 51.13, 29.16, 28.99; ESI-MS *m/z*: 471 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{31}N_2O_4$ $[M+H]^+$ 471.2278, Found 471.2288. MeOH/H_2O = 70:30 (*v/v*), 9.86 min, 96.68% purity.

*4.2.21. (R)-(3-(Benzyloxy)-2,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol ((R)-**22**)*

Compound **35p** was prepared in a similar manner as described for compound **7**. 6-(Benzyloxy)-7-methoxy-1-((5-methoxy-1*H*-indol-3-yl)methyl)-3,4-dihydroisoquinoline (**35p**, 2 mmol) dissolved in 10 mL of water, and (*S,S*)-Noyori's catalyst (0.04 mmol), $AgSbF_6$ (0.06 mmol), $La(OTf)_3$ (0.6 mmol), CTAB (2 mmol) and HCOONa (3 mmol) were added. The solution was heated to 40 °C under for 12 h. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine, and the combined organic phase was evaporated under reduced pressure to get the crude product, which was purified by flash chromatography on silica gel to get (*R*)-**35p** (441 g, 1.03 mmol, 51%). (*R*)-**35p** (428.5 mg, 1 mmol) was dissolved in 20 mL of acetonitrile, then excess of formaldehyde and 0.5 mL of formic acid were added into solution. The mixture was stirred for 2 h at 80 °C. The pH of the mixture was adjusted to alkalinity with the addition of saturated $NaHCO_3$. The organic layer was separated and washed with water. The combined organic phase was evaporated under reduced pressure and then chromatographed on silica gel to give the target product (*R*)-**22** (197 mg, 41%). Yellow solid, m.p. = 144–145 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 7.7 Hz, 2H), 7.40 (dd, J = 14.8, 7.1 Hz, 2H), 7.34 (dd, J = 8.4, 4.8 Hz, 1H), 7.02−6.96 (m, 2H), 6.82 (s, 1H), 6.73 (dd, J = 8.8, 1.6 Hz, 1H), 6.22 (t, J = 6.9 Hz, 1H), 5.37 (d, J = 6.5 Hz, 2H), 5.06 (s, 2H), 4.17 (d, J = 15.1 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.62 (dd, J = 29.8, 12.5 Hz, 2H), 3.44 (d, J = 14.9 Hz, 1H), 3.16−3.08 (m, 1H), 2.94 (t, J = 12.0 Hz, 1H), 2.61 (dd, J = 24.1, 15.4 Hz, 2H), 2.47−2.38 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.55, 147.57, 146.26, 137.32, 133.74, 131.08, 130.73, 128.41, 127.80, 127.24, 126.38, 113.37, 110.39, 110.11, 110.04, 107.57, 100.00, 69.91, 65.38, 59.41, 55.95, 55.37, 51.33, 51.14, 29.18, 29.00; ESI-MS *m/z*: 471 $[M+H]^+$. MeOH/H_2O = 70:30 (*v/v*), 10.03 min, 95.06% purity.

*4.2.22. (S)-(3-(Benzyloxy)-2,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]Pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol ((S)-**22**)*

This compound was prepared by replacement of (*S,S*)-Noyori's catalyst with (*R,R*)-Noyori's catalyst using a similar synthetic procedure of (*R*)-**22**. Yellow solid, m.p. = 146–147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 7.0 Hz, 2H), 7.41 (dd, J = 10.0, 4.7 Hz, 2H), 7.35 (dd, J = 8.0, 5.1 Hz, 1H), 6.99 (dd, J = 7.1, 3.2 Hz, 2H), 6.81 (s, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.21 (t, J = 7.0 Hz, 1H), 5.37 (d, J = 6.6 Hz, 2H), 5.06 (s, 2H), 4.17 (d, J = 15.1 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.70−3.55 (m, 2H), 3.49−3.39 (m, 1H), 3.18−3.10 (m,

1H), 3.02−2.87 (m, 1H), 2.72−2.55 (m, 2H), 2.47−2.38 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.55, 147.57, 146.26, 137.32, 133.74, 131.08, 130.72, 128.41, 127.80, 127.24, 126.38, 113.37, 110.39, 110.11, 110.03, 107.57, 100.00, 69.91, 65.38, 59.40, 55.94, 55.37, 51.33, 51.14, 29.17, 28.99; ESI-MS *m/z*: 471 $[M+H]^+$. MeOH/H_2O = 70:30 (*v/v*), 9.87 min, 95.07% purity.

*4.2.23. (3-((2-Fluorobenzyl)oxy)-2,12-dimethoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl) methanol (**23**)*

Compound **23** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 105–106 °C; Yield: 38%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (td, J = 7.5, 1.4 Hz, 1H), 7.49−7.40 (m, 1H), 7.36 (d, J = 8.8 Hz, 1H), 7.30−7.23 (m, 2H), 6.99 (dd, J = 9.3, 3.1 Hz, 2H), 6.86 (s, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.21 (t, J = 7.1 Hz, 1H), 5.44−5.32 (m, 2H), 5.20−5.01 (m, 2H), 4.17 (d, J = 15.1 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.66 (d, J = 15.0 Hz, 1H), 3.59 (dd, J = 10.3, 3.0 Hz, 1H), 3.43 (dd, J = 14.9, 2.8 Hz, 1H), 3.20−3.09 (m, 1H), 3.02−2.89 (m, 1H), 2.73−2.57 (m, 2H), 2.47−2.35 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.41 (d, J = 245.9 Hz), 153.56, 147.56, 146.07, 133.75, 131.09, 131.04, 130.83 (d, $J_{C\text{-}F}$ = 4.1 Hz), 130.36 (d, $J_{C\text{-}F}$ = 8.2 Hz), 127.25, 126.46, 124.55 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.10 (d, $J_{C\text{-}F}$ = 14.7 Hz), 115.37 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.42, 110.40, 110.12, 110.06, 107.57, 100.00, 65.39, 64.14 (d, $J_{C\text{-}F}$ = 3.6 Hz), 59.41, 55.91, 55.37, 51.34, 51.13, 29.18, 28.97; ESI-MS *m/z*: 489 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{30}FN_2O_4^+$ $[M+H]^+$ 489.2184, Found 489.2170. MeOH/H_2O = 70:30 (*v/v*), 10.88 min, 95.00% purity.

*4.2.24. (3-((3-Fluorobenzyl)oxy)-2,12-dimethoxy-5,6,14,14a tetrahydroindolo[3′,2′:4,5] pyrido[2,1-a]isoquinolin-9(8H)-yl) methanol (**24**)*

Compound **24** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 98–100 °C; Yield: 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50−7.39 (m, 1H), 7.38−7.25 (m, 3H), 7.17 (td, J = 8.6, 1.9 Hz, 1H), 7.00 (dd, J = 12.8, 3.5 Hz, 2H), 6.81 (s, 1H), 6.73 (dd, J = 8.8, 2.5 Hz, 1H), 6.21 (t, J = 7.1 Hz, 1H), 5.43−5.29 (m, 2H), 5.09 (s, 2H), 4.16 (d, J = 15.1 Hz, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.66 (d, J = 15.0 Hz, 1H), 3.59 (dd, J = 10.5, 3.3 Hz, 1H), 3.43 (dd, J = 15.0, 2.6 Hz, 1H), 3.21−3.05 (m, 1H), 3.18−3.07 (m, 1H), 3.00−2.87 (m, 1H), 2.71−2.57 (m, 2H), 2.48−2.36 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.20 (d, $J_{C\text{-}F}$ = 243.4 Hz), 153.55, 147.60, 146.00, 140.35 (d, $J_{C\text{-}F}$ = 7.4 Hz), 133.74, 131.05 (d, $J_{C\text{-}F}$ = 7.9 Hz), 130.46 (d, $J_{C\text{-}F}$ = 8.2 Hz), 128.11 (d, $J_{C\text{-}F}$ = 61.6 Hz), 127.25, 126.41, 123.54 (d, $J_{C\text{-}F}$ = 2.7 Hz), 114.53 (d, $J_{C\text{-}F}$ = 20.9 Hz), 114.20 (d, $J_{C\text{-}F=}$21.8 Hz), 113.54, 110.40, 110.12, 110.09, 107.56, 100.00, 69.11, 65.39, 59.40, 55.98, 55.37, 51.33, 51.11, 29.16, 28.98; ESI-MS *m/z*: 489 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{29}H_{30}FN_2O_4^+$ $[M+H]^+$ 489.2184, Found 489.2174. MeOH/H_2O = 70:30 (*v/v*), 10.72 min, 96.62% purity.

*4.2.25. (2,12-Dimethoxy-3-((3-methylbenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl) methanol (**25**)*

Compound **25** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 96–98 °C; Yield: 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37 (d, J = 8.8 Hz, 1H), 7.31−7.21 (m, 3H), 7.15 (d, J = 7.3 Hz, 1H), 7.01−6.97 (m, 2H), 6.81 (s, 1H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H), 6.26 (t, J = 6.9 Hz, 1H), 5.46−5.31 (m, 2H), 5.12−4.92 (m, 2H), 4.18 (d, J = 15.2 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.70–3.55 (m, 2H), 3.44 (d, J = 14.2 Hz, 1H), 3.13 (d, J = 7.5 Hz, 1H), 3.02–2.85 (m, 1H), 2.65 (d, J = 14.2 Hz, 2H), 2.47−2.39 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.55, 147.57, 146.33, 137.53, 137.21, 133.74, 131.10, 130.63, 128.46, 128.42, 128.33, 127.22, 126.32, 124.96, 113.34, 110.42, 110.13, 109.99, 107.53, 100.00, 69.99, 65.37, 59.41, 55.93, 55.38, 51.32, 51.13, 29.14, 28.99, 21.05; ESI-MS *m/z*: 485 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{30}H_{33}N_2O_4^+$ $[M+H]^+$ 485.2435, Found 485.2424. MeOH/H_2O = 70:30 (*v/v*), 15.58 min, 95.08% purity.

*4.2.26. (2,12-Dimethoxy-3-((4-methylbenzyl)oxy)-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**26**)*

Compound **26** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 103–105 °C; Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (dd, J = 17.5, 8.5 Hz, 3H), 7.26−7.13 (m, 2H), 7.07−6.95 (m, 2H), 6.80 (s, 1H), 6.73 (dd, J = 8.8, 2.5 Hz, 1H), 6.21 (t, J = 7.0 Hz, 1H), 5.36 (dd, J = 12.3, 10.8 Hz, 2H), 5.00 (s, 2H), 4.17 (d, J = 15.1 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.67 (d, J = 14.5 Hz, 1H), 3.59 (d, J = 9.8 Hz, 1H), 3.47−3.39 (m, 1H), 3.13 (d, J = 6.5 Hz, 1H), 2.94 (t, J = 11.5 Hz, 1H), 2.64 (d, J = 13.1 Hz, 2H), 2.47−2.37 (m, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.5, 147.57, 146.31, 137.02, 134.26, 131.09, 128.94, 127.90, 127.22, 126.32, 113.36, 110.39, 110.13, 110.03, 107.54, 100.01, 69.79, 65.38, 59.41, 55.94, 55.37, 51.31, 51.13, 29.13, 28.94. ESI-MS *m/z*: 485 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{30}H_{33}N_2O_4^+$ $[M+H]^+$ 485.2435, Found 485.2431. MeOH/H_2O = 70:30 (*v/v*), 14.63 min, 97.92% purity.

*4.2.27. (3-(Benzyloxy)-13-chloro-2-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**27**)*

Compound **27** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 150–152 °C; Yield: 42%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49−7.44 (m, 3H), 7.40 (t, J = 7.3 Hz, 2H), 7.35 (d, J = 7.1 Hz, 1H), 7.10−7.00 (m, 2H), 6.95 (s, 1H), 6.82 (s, 1H), 6.39 (t, J = 7.1 Hz, 1H), 5.44 (d, J = 7.0 Hz, 2H), 5.06 (s, 2H), 4.20 (d, J = 15.4 Hz, 1H), 3.78 (s, 3H), 3.75–3.68 (m, 2H), 3.63 (d, J = 10.0 Hz, 1H), 3.13 (dd, J = 6.6, 3.7 Hz, 1H), 2.98−2.86 (m, 1H), 2.76−2.58 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.55, 146.35, 137.31, 137.21, 134.68, 130.58, 128.40, 127.80, 127.76, 126.42, 124.13, 123.94, 121.39, 119.46, 113.54, 110.06, 109.04, 107.27, 69.94, 65.55, 59.09, 55.99, 51.01, 50.61, 30.87, 28.91; ESI-MS *m/z*:475 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}ClN_2O_3^+$ $[M+H]^+$ 475.1783, Found 475.1794. MeOH/H_2O = 60:40 (*v/v*), 6.31 min, 96.58% purity.

*4.2.28. (3-(Benzyloxy)-13-bromo-2-methoxy-5,6,14,14a-tetrahydroindolo[3′,2′:4,5]pyrido[2,1-a]isoquinolin-9(8H)-yl)methanol (**28**)*

Compound **28** was prepared in a similar manner as described for compound **7**. Yellow solid, m.p. = 163–165 °C; Yield: 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (d, J = 8.1 Hz, 1H), 7.49−7.38 (m, 4H), 7.38−7.30 (m, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.00 (t, J = 7.9 Hz, 1H), 6.94 (s, 1H), 6.83 (s, 1H), 6.40 (t, J = 6.9 Hz, 1H), 5.43 (d, J = 6.5 Hz, 2H), 5.06 (s, 2H), 4.19 (d, J = 15.3 Hz, 1H), 3.82 (s, 1H), 3.78 (s, 3H), 3.70 (d, J = 15.3 Hz, 1H), 3.61 (dd, J = 10.3, 2.7 Hz, 1H), 3.13 (dd, J = 6.4, 3.5 Hz,

1H), 3.00–2.85 (m, 1H), 2.78–2.57 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.53, 146.35 137.31, 137.08, 134.89, 130.62, 128.42, 127.81, 127.77, 126.42, 125.41, 122.75, 121.77, 113.53, 112.40, 110.01, 109.51, 107.89, 69.94, 65.50, 59.00, 55.95, 51.03, 50.59, 31.06, 28.90; ESI-MS *m/z*: 519 $[M+H]^+$; ESI-HR-MS Calcd. for $C_{28}H_{28}BrN_2O_3^+$ $[M+H]^+$ 519.1278, Found 519.1281. MeOH/H_2O = 60:40 (*v/v*), 6.26 min, 96.18% purity.

4.3. Pharmacology

4.3.1. Cell culture

The human hepatoma cell line HepG2 (ATCC® HB-8065™, Rockville, USA) cells were maintained in Dulbecco's modified Eagle's medium (DMEM, HyClone, cat. SH30243.01, Logan, USA) supplemented with 10% (*v/v*) fetal bovine serum (FBS, Gibco, cat. 10099141, Carlsbad, USA) at 37 °C in a 5% CO_2 incubator. For the analysis of PCSK9 and LDLR expression or DiI-LDL uptake assay, the medium was replaced with DMEM supplemented with 2% (*v/v*) lipoprotein-deficient serum (LPDS) after cells were grown to confluence.

4.3.2. Western blot analysis of PCSK9 and LDLR in HepG2 cells

HepG2 cells plated in 12-well plate were treated with various test compounds or berberine (Meilun Biotechnology, cat. MB6000, Dalian, China) in the presence of 2% LPDS for 24 h. Then the cells were lysed in RIPA buffer (Beyotime Biotechnology, cat. P0013B, Shanghai, China) containing protease inhibitor cocktail (Merck Millipore, cat. 539134, Darmstadt, Germany). Total protein in cell lysates equivalent to 15 μg was separated by gel electrophoresis using 8% SDS-PAGE and then transferred to a PVDF membrane for protein blotting. Non-specific binding sites were blocked in 5% Blotting Grade Blocker Non-Fat Dry Milk (Bio-Rad, cat. 170-6404, Hercules, USA) for 2 h. The membranes were incubated with antibodies specific for PCSK9 (Abcam, cat. ab181142, Cambridge, UK; 1:2000), LDLR (Proteintech, cat. 10785-1-AP, Wuhan, China; 1:2500) and β-actin (CST, cat. #3700, Danvers, MA, USA; 1:1000), respectively, at 4 °C overnight. After incubation with the primary antibodies, the membranes were washed in TBST (Tris-buffered saline, 0.1% Tween 20) 3 times and incubated with the secondary antibodies at room temperature for another 2 h. All bands were visualized with an ECL kit (Bio-Rad, cat. 170-5061, Hercules, USA) and chemiluminescence was detected on a ChemiDoc XRS (Bio-Rad). ImageJ (NIH, 1.50i, Rockville, MD, USA) was used for the quantification of band intensities.

4.3.3. DiI-LDL uptake assay

HepG2 cells plated in 24-well plate were used to implement DiI-LDL uptake assay[20]. In general, after incubation with test compounds or berberine in the presence of 2% LPDS for 24 h, cell culture medium was changed to DMEM supplemented with 2% LPDS and 20 μg/mL DiI-LDL. Following another 4 h co-incubation with DiI-LDL, HepG2 cells were washed with PBS containing 0.4% albumin 2 times, and then PBS (phosphate buffered saline) 3 times. For the fluorescence quantification, 400 μL of isopropanol was added into each well of the 24-well plate, and the plate was gently shaken on a shaker for 20 min at room temperature. Then 200 μL of isopropanol in each well was used for the analysis with a SpectraMax M5e Microplate Reader (Molecular Devices, San Jose, USA; excitation wavelength of 520 nm, emission wavelength of 570 nm). The remaining cells were lysed in NaOH (0.5 mol/L) for protein determination. DiI-LDL was prepared as previously described[20].

4.3.4. Calcium mobilization assay

HEK293 cells stably expressing β_1-AR/Gα_{16}, D_1/Gα_{16} or D_2/Gα_{16} were seeded onto 96-well plates and incubated for 24 h. Cells were loaded with 2 μmol/L Fluo-4 AM in Hanks balanced salt solution (HBSS, containing KCl 5.4 mmol/L, Na_2HPO_4 0.3 mmol/L, KH_2PO_4 0.4 mmol/L, $NaHCO_3$ 4.2 mmol/L, $CaCl_2$ 1.3 mmol/L, $MgCl_2$ 0.5 mmol/L, Mg_2SO_4 0.6 mmol/L, NaCl 137 mmol/L, BSA 5 g/L, glucose 5.6 mmol/L, sulfinpyrazone 250 μmol/L, pH 7.4) at 37 °C for 45 min. The excess dye was removed and 50 μL HBSS containing test compounds were added. After incubation at room temperature for 10 min, 25 μL HBSS containing the respective agonists were dispensed into the well using a FlexStation III microplate reader (Molecular Devices, San Jose, CA, USA) and intracellular calcium change was recorded with an excitation wavelength of 485 nm and emission wavelength of 525 nm. The half maximal inhibitory concentrations (IC_{50}) of compounds were determined with GraphPad Prism software by constructing their dose−response curves.

4.3.5. Whole-cell binding assay

CHO−K1 cells stably expressing 5-HT_{1B} were seeded at a density of 3×10^4 cells/well into 96-well culture plates and incubated overnight at 37 °C in 5% CO_2, and radioligand binding assay was carried out after seeding for 24 h. For homogeneous binding, the cells were incubated in binding buffer with constant concentration of ^{3}H-GR125743 (1 nmol/L) or ^{3}H-Mesulergine (1 nmol/L) and different concentrations of unlabeled compounds (1.28 nmol/L−100 μmol/L) at 4 °C overnight. Cells were washed three times with ice-cold PBS and lysed by 50 μL lysis buffer (PBS supplemented with 20 mmol/L Tris−HCl, 1% Triton X-100, pH 7.4). The plates were subsequently counted for radioactivity (counts per minute, CPM) in a scintillation counter (MicroBeta^2 Plate Counter, PerkinElmer, Waltham, MA, USA) using a scintillation cocktail (PerkinElmer, cat. 1200-439, Waltham, MA, USA).

4.3.6. Pharmacokinetic profiles in male hamsters

Compound **22** was subjected to PK studies on male hamsters (n = 3, each group). Hamsters in oral administration group were given 20 mg/kg test compound (0.5% carboxymethylcellulose sodium) by oral gavage. Hamsters in intravenous administration group were given 10 mg/kg test compound (5% DMSO, 10% cremophor EL in 85% saline) by intravenous administration. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after oral or intravenous administration. Serum samples were isolated by centrifuging for 10 min at 4000×g. The test compound concentrations in serum were measured by LC/MS/MS.

4.3.7. In vivo cholesterol-lowering assay

Male Syrian hamsters with body weights of 100−120 g were purchased from Beijing Vital River Laboratory Animal Technology and housed under controlled temperature (22±2 °C) and lighting (12 h light/dark cycle). All hamsters had free access to food and water. After 7 days of acclimatization, hamsters were divided into normal control group (n = 7) and HFD group (n = 21). Then the HFD group was switched into a high-fat diet (0.5% cholesterol; Research Diets, Inc. New Brunswick, NJ, USA)

to induce hyperlipidemia. One week later, hamsters of the HFD group were divided into HFD control group and test groups randomly ($n = 7$ per group). Test groups were given a daily dose test compound **22** 30 mg/kg or fenofibrate 100 mg/kg by oral gavage for 21 days. The control groups were given a vehicle (0.5% carboxymethylcellulose sodium).

At the end of the animal experiment, blood samples were collected from all the hamsters under anesthesia after 15 h of fasting (5 p.m.−8 a.m.). Serum was isolated by centrifuging for 10 min at 4000×*g*, and TC and LDL-C levels were measured using commercially available kits purchased from Maccura Biotechnology (Guangzhou, China).

The study was conducted in accordance with guidelines and ethics of Institutional Animal Care and Use Committee (IACUC), Shanghai Institute of Materia Medica, Chinese Academy of Sciences and the Ethics Committee of Shanghai Xuhui Central Hospital (China).

4.3.8. hERG testing using FluxOR™ thallium assay

Step 1: Growing cells. CHO-hERG-ZG cells are grown in 75 cm^2 flask with complete medium with 100 μg/mL G418 and 100 μg/mL hygromycin B until 80%−90% confluency. Wash cells with PBS once. Incubate cells with 1 mL 0.25% trypsin until all cells are rounded and can be easily dislodged from the surface. Add 10 mL complete medium to stop trypsin activity. Disassociate cells by thoroughly and repetitively pipetting. Transfer them to 50 mL Falcon tube and spin down at 1000 rpm (Labofuge 400 Centrifuge, Thermo Fisher, Germany) for 5 min. Aspirate medium and resuspend cells using a small volume of complete medium, like 0.5 mL. Count cell density.

Step 2: Cell seeding. CHO-hERG-ZG cells are plated into 96-well plates and after plating, tap plates on sides to separate cells and let plates sit in the dark at RT for 30 min before incubation at 37 °C for 16–18 h. Cells will reach 80% confluence. After overnight incubation, the media of cells are changed in loading buffer (old media is tapped out) and incubated in the dark at RT for 90 min. Remove the loading buffer and replace with assay buffer. Compounds were added to the cell plate. The cell plate is incubated with compound **22** for 20 min in the dark at RT. Load the cell plates on Functional Drug Screening System (FDSS). Fluorescent signals will be recorded every 2 s till 10 s. At 10 s, stimulus buffer will be added to cells. Then fluorescent signals will be recorded every second till 180 s on FDSS.

4.3.9. Real-time PCR

HepG2 cells were treated with the test compound in the presence of 2% LPDS for 24 h. The total RNA was isolated by Trizol (Invitrogen, cat. 10296010, Leawood, KA, USA) and reverse-transcribed to cDNA by reverse transcriptase (Promega, cat. M1705, Madison, USA). The expression of PCSK9 mRNA was assessed by real-time PCR using specific primers and SYBR® Green Supermix (Bio-Rad, cat. 1725124, Hercules, CA, USA). Primer sequences of PCSK9 gene are 5′-CCAAGCCTCTTCTTACTTCACC-3′ and 5′-GCATCGTTCTGCCATCACT-3′.

4.3.10. Statistical analysis

Data were analyzed with GraphPad Prism software. Results are presented as mean ± SEM. Nonlinear regression analysis was used to generate the dose−response curve and calculate a concentration of 50% inhibition (IC_{50}) value. Differences between treatment groups were assessed by Student's *t*-test or one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

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Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.06.006.

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