Hypolipidemic effect of SIPI-7623, a derivative of an extract from oriental wormwood, through farnesoid X receptor antagonism

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[ABSTRACT] Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. As a metabolic regulator, FXR plays key roles in bile acid and cholesterol metabolism and lipid and glucose homeostasis. Therefore, FXR is a potential drug target for several metabolic syndromes, especially those related to lipidemia disorders. In the present study, we identified small molecule SIPI-7623, a derivative of an extract from Oriental wormwood (Artemisia capillaris), and found that it specifically upregulated the expression of cholesterol-7-alpha-hydroxylase (CYP7A1), downregulated the expression of sterol-regulatory element-binding protein 1c (SREBP-1c) in the liver, and inhibited the expression of ileal bile acid binding-protein (IBABP) in the ileum of rats. We found that inhibition of FXR by SIPI-7623 decreased the level of cholesterol and triglyceride. SIPI-7623 reduced the levels of cholesterol and triglyceride in vitro HepG2 cell models, ameliorated diet-induced atherosclerosis, and decreased the serum lipid content on rats and rabbits model of atherosclerosis in vivo. Furthermore, SIPI-7623 decreased the extent of atherosclerotic lesions. Our results demonstrated that antagonism of the FXR pathway can be employed as a therapeutic strategy to treat metabolic diseases such as hyperlipidemia and atherosclerosis. In conclusion, SIPI-7623 could be a promising lead compound for development of drugs to treat hyperlipidemia and atherosclerosis.

[KEY WORDS] Farnesoid X receptor antagonist; Hypolipidemic; Bile acid enterohepatic circulation; Cholesterol-7-alpha-hydroxylase; Sterol-regulatory element-binding protein 1c


Introduction

Farnesoid X receptor (FXR) is a multifunctional nuclear receptor that is expressed mainly in the liver, intestine, kidney, and adrenal gland [1]. It plays an important role in maintaining bile acid [2], in cholesterol metabolism [3], and in lipid [4] and glucose homeostasis [5]. FXR is the receptor mostly dedicated to signaling by bile acids [6]. In the liver, FXR activation reduces conversion of cholesterol to bile acids by downregulating the expression of enzymes involved in bile acid synthesis, such as cholesterol-7-alpha-hydroxylase (CYP7A1). Within the intestine, FXR activation involves bile acid absorption via promoting bile acid movement across the enterocytes via ileal bile acid binding-protein (IBABP). Furthermore, FXR activation decreases lipogenesis via inhibition of sterol-regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS). In recent years, the FXR agonist obeticholic acid has entered into phase III clinical trials for treatment of fatty liver, and the U.S. Food and Drug Administration (FDA) has approved it as a drug for treatment of primary biliary cirrhosis. However, clinical trials have shown that obeticholic acid increases blood lipid levels [7].

Development of the FXR antagonist has long been neglected, but progress has been made in recent years. Z-Gugulsterone, as the first discovered natural steroidal FXR antagonist, has been shown to reduce total cholesterol (TC) and triglycerides (TGs) [8], another FXR antagonist, 16-dehy-
dro-pregnenolone, has been shown to have the ability of reducing TC in a high-fat rat model.\(^{[10]}\)

Oriental wormwood (Artemisia capillaris Thunb./Yin Chen Hao) is a traditional Chinese medicine that can suppress “moist heat” and eliminate jaundice. In a previous study, we found that an extract of Oriental wormwood exerted a cholagogue effect. Its constituent, \(p\)-hydroxyacetophenone (PHA), can effectively promote bile secretion\(^{[10]}\), decrease cholesterol concentration and total amount, and increase bile acid salt concentration and the total amount of salts in bile, suggesting that the compound can convert cholesterol to bile salts. We speculated that PHA might be involved in \textit{de novo} bile acid synthesis, promoting the conversion of cholesterol to bile salts and inhibiting bile acids from returning to the liver, which finally leads to lower plasma cholesterol levels. However, because PHA has a small molecular weight and can easily cross the blood-brain barrier, it is thought to be not appropriate for clinical use as a cholesterol-lowering agent. Therefore, we decided to study derivatives of PHA as drug candidates for hyperlipidemia.

Based on previous experimental results\(^{[10]}\) and published studies\(^{[11]}\), the PHA fragment was identified as the pharmacophore of the FXR antagonist. Both FXR and PPAR\(\alpha\) are nuclear receptors, and their crystal structures are similar. Therefore, using fribrates as molecular scaffolds and the natural product PHA as a pharmacophore will help identify targeted compounds that can enter the active pocket of FXR. On this basis, we designed and synthesized a batch of structurally related compounds and screened out SIPI-7623 (Fig. 1), which is a new compound with an issued patent (ZL201110174070.5). In the present study, SIPI-7623 was proven to regulate lipid metabolism as an FXR antagonist \textit{in vitro} and \textit{in vivo}.

Fig. 1 Structure of SIPI-7623

**Materials and Methods**

**Animals**

Male Sprague-Dawley (SD) rats, weighing 180–200 g, were provided by the Shanghai SIPPR/BK Experimental Animal Co., Ltd., Shanghai, China. Male rabbits, weighing 1.5–1.8 kg, were purchased from Jiagan Biotechnology Co., Ltd., Shanghai, China. The animals were housed in a temperature- and humidity-controlled, specific-pathogen-free environment with a 12 h/12 h light/dark cycle (lights on at 7:00 am) and acclimatized for 3–7 d before the experiment. The animals were given food and filtered water \textit{ad libitum}. All the animal studies were approved by the Shanghai Institute of Pharmaceutical Industry Animal Care and Use Committee [Pharmaceutical Research Division (2017) No.9.2017-9-21]. The laboratory animal facility was accredited by Shanghai Committee of Science and Technology [Approval ID: SKXY (Shanghai): 2014-0018].

**Drugs and reagents**

SIPI-7623 was synthesized by the Shanghai Institute of Pharmaceutical Industry (99% purity), Shanghai, China. The synthetic pathway of SIPI-7623 was described by the patent (ZL201110174070.5). HepG2 cells (ATCC: HB-8065) were purchased from the Chinese Academy of Sciences cell bank, Shanghai, China; the FXR protein was obtained from CloudClone Crop (purity > 98%, catalog number: RPC042-Hu01), Houston, TX, USA; the filipin staining kit and Nile red staining kit were obtained from GenMed Sciences Inc. Arlington, VA, USA; and Gemfibrozil was obtained from Zhejiang Excel Pharma Co., Ltd., China (purity of 99%), Hangzhou, Zhejiang, China. Simvastatin was obtained from Shanghai Xinyi Pharmaceutical Co., Ltd., Shanghai, China. Obeticholic acid (FXR agonist with anticholeretic activity, CAS# 459789-99-2) was provided by the Shanghai Institute of Pharmaceutical Industry, Shanghai, China.

**SIPI-7623 target confirmation**

The effect of SIPI-7623 on FXR and PPAR\(\alpha\) was monitored by the time-resolved fluorescence resonance energy transfer TR-FRET assay technique using the double luciferase reporter gene method. The concentrations of compound for the FXR antagonist were 50 \(\mu\)mol\(\cdot\)L\(^{-1}\) as the initial concentration and 100 \(\mu\)mol\(\cdot\)L\(^{-1}\) for the PPAR\(\alpha\) agonist, with 10 concentrations prepared using a 3-fold serial dilution.

The binding constants of the compound and the target protein were obtained using the Octet system developed by ForteBio Corporation (Pall ForteBio LLC, California, USA) with Bio-Layer Interferometry (BLI). PBST (phosphate buffered saline with 0.2% Tween 20) was prepared as an appropriate assay buffer or dilution. For the column of sensors to be used, 200 \(\mu\)L of assay buffer was added per well in a column of the 96-well plate, with concentrations of SIPI-7623 at 6.25, 12.5, and 25 \(\mu\)mol\(\cdot\)L\(^{-1}\) and FXR protein at 20 \(\mu\)g\(\cdot\)mL\(^{-1}\). The BIGS sensor chips were prewashed with 30 min in 200 \(\mu\)L of assay buffer and then rinsed with 200 \(\mu\)L of assay buffer containing 0.2% Tween 20. For each measurement, the BIGS sensors were washed with 200 \(\mu\)L of assay buffer three times, after the signal had returned to baseline, 200 \(\mu\)L of SIPI-7623 or FXR protein was added into the column, and the sensors were washed with 100 \(\mu\)L of PBS at 25 \(\mu\)mol\(\cdot\)L\(^{-1}\) and FXR protein at 20 \(\mu\)g\(\cdot\)mL\(^{-1}\). The binding was visualized using a horseradish peroxidase-conjugated secondary antibody (Abcam, Cambridge, UK). For Western blotting, antibody binding was visualized using a horseradish peroxidase-conjugated secondary antibody (CST, Massachusetts, USA) and a 3, 3’-diaminobenzidine (DAB, Sigma, USA) detection system.

Analysis of effect of SIPI-7623 on the FXR downstream target in the liver and ileum

To establish a rat model of hyperlipidemia (the control rats consumed normal rat chow while the model rats were supplemented with intralipid), obeticholic acid (OCA, 10 mg\(\cdot\)kg\(^{-1}\)) was chosen as a positive drug, and after administration of SIPI-7623 at 80 mg\(\cdot\)kg\(^{-1}\) for 21 days, liver and ileal tissues were extracted for Western blotting analyses. Tissues (100 mg) was immediately used for total protein extraction using Trizol (Invitrogen, USA) by Bead Ruptor 12 Homogenizer (Omni, NW Kennesaw, USA). Tissues were lysed for 30 min, and spun for 10 min at 8 000 \(g\) at 4 °C.

The primary antibodies were purchased from Abcam (Abcam, Cambridge, UK). For Western blotting, antibody binding was visualized using a horseradish peroxidase-conjugated secondary antibody (CST, Massachusetts, USA) and a 3, 3’-diaminobenzidine (DAB, Sigma, USA) detection system.

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The bands were scanned using an image scanning densitometer (Tanon Science & Technology Co., Shanghai, China).

**Analysis of effect of SIPI-7623 on high cholesterol and high triglyceride models in vitro**

HepG2 cells grown to 90% confluence were adjusted to the appropriate cell density. The cells were inoculated into 6-well culture plates, and the high cholesterol model of HepG2 cells was simulated with 10 μg·mL−1 of cholesterol and 1 μg·mL−1 of 25-hydroxycholesterol to observe the cholesterol-reducing effect of SIPI-7623 in 24 h. The intracellular cholesterol distribution was observed by Filipin staining. The high triglyceride model of HepG2 cells was simulated with 150 μmol·L−1 of oleic acid to study the triglyceride-reducing effect of SIPI-7623 in 24 h. The intracellular distribution of esters was observed by Nile red staining.

Filipin Staining and Nile-Red Staining: After fixation with 4% paraformaldehyde (PFA) in PBS, cells were washed twice with PBS and stained with 50 mg·mL−1 filipin or 0.5 mg mL−1 Nile red in the dark for 10 min at room temperature. Fluorescence signals of stained cells were analyzed with a microscope (Nikon, Japan) using the excitatory wavelength of 340 nm and 488 nm, respectively [12].

**Analysis of effect of SIPI-7623 on a rat model of atherosclerosis**

The rats were randomly divided into eight groups (n = 10/group). The rats in the model group, except normal group, were treated with vitamin D3 at a dose of 700 000 IU·kg−1 by intra-peritoneal injection. The rat atherosclerosis model was established by daily gastric perfusion of 1 mL/100 g of intralipid for 21 days [13] and administration of different dosages of SIPI-7623 (20, 40, 80, and 160 mg·kg−1), simvastatin (10 mg·kg−1), or gemfibrozil (60 mg·kg−1) from the 22nd day to the 8-week feeding. The rabbits of the atherosclerosis model were injected into the ear vein of rabbits at the first week of an 8-week feeding. The parent structure of SIPI-7623 was determined to be p-hydroxycetophenone, and the effect of PHA on bile changes has also been demonstrated in previous experiments [10]. Therefore, it was speculated that the bile acid receptor was an action target of SIPI-7623, and verification was performed in the present study.

**SIPI-7623 as an FXR inhibitor**

The double luciferase reporter assay showed that SIPI-7623 was an FXR antagonist with an IC50 of 16.41 μmol·L−1, while the IC50 of the positive control Z-guggulsterone was 26.79 μmol·L−1. No agonist activity with PPARα was observed for SIPI-7623.

**The binding constant of SIPI-7623 and FXR protein**

SIPI-7623 was combined with FXR protein at different concentrations according to the Octet system, showing a binding constant Kd of 8.51 × 10−7 mol·L−1 (Fig. 2).

**Effects of SIPI-7623 on FXR and downstream targets in the liver and ileum**

The liver and ileum in hyperlipidemia SD rats were subjected to the action of SIPI-7623 at 80 mg·kg−1 for 3 weeks, which was compared with the model group. The changes in the FXR-related target protein in rat liver and ileum under SIPI-7623 are shown in Fig. 3.

Western blot analysis of the downstream target genes associated with FXR was performed with liver tissues. The results showed that the expression of CYP7A1 was increased when FXR was inhibited, and the expression of SREBP-1c decreased (Fig. 3A).

Additionally, FXR in the intestinal tract was inhibited by SIPI-7623, and the expression of IBABP was decreased in the ileum (Fig. 3B).

**Results**

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**Hypolipidemic effect of SIPI-7623 in vitro**

SIPI-7623 was observed to have certain TC- and TG-reducing effects on high cholesterol and high triglyceride models of HepG2 cells. In the high cholesterol model, comparison among the SIPI-7623 (60 ng·mL⁻¹) group, the simvastatin group, and the model group showed a significant difference in the amount of TC and TG in the cells (P < 0.05), while P < 0.01 (ANOVA) for the SIPI-7623 120 ng·mL⁻¹ group. In the high triglyceride model, a comparison between the SIPI-7623 60 ng·mL⁻¹ dose group and model group showed significant difference (P < 0.05), while P < 0.01 (ANOVA) for the SIPI-7623 120 ng·mL⁻¹ group (Table 1).

**Table 1 Effects of SIPI-7623 on TC and TG in HepG2 cells (means ± SD, n = 6)**

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol·L⁻¹)</th>
<th>TG (mmol·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15 ± 0.01*</td>
<td>0.04 ± 0.01**</td>
</tr>
<tr>
<td>Model</td>
<td>0.19 ± 0.02</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>SIPI-7623 30 ng·mL⁻¹</td>
<td>0.19 ± 0.04</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>SIPI-7623 60 ng·mL⁻¹</td>
<td>0.15 ± 0.03*</td>
<td>0.11 ± 0.02**</td>
</tr>
<tr>
<td>SIPI-7623 120 ng·mL⁻¹</td>
<td>0.12 ± 0.01**</td>
<td>0.08 ± 0.02**</td>
</tr>
<tr>
<td>Simvastatin 200 ng·mL⁻¹</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gemfibrozil 40 ng·mL⁻¹</td>
<td>—</td>
<td>0.11 ± 0.04</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs model group

**Effects of SIPI-7623 on the cholesterol and lipid levels as measured by Filipin and Nile staining**

SIPI-7623 decreased the steady-state levels of cellular cholesterol and neutral lipids (mainly triglyceride). Filipin staining showed that SIPI-7623 reduced the cholesterol level in the high TC model of HepG2. Cholesterol aggregates were visible around the cell nucleus, showing uniform green fluorescence in the model group, while SIPI-7623 120 ng·mL⁻¹ intracellular cholesterol aggregates were significantly decreased, with weakened green fluorescence (Fig. 4). Nile red staining showed that SIPI-7623 reduced the triglyceride level in the high TG model of HepG2. The cytoplasm was filled with a large number of red fluorescent lipid droplets, while the cytoplasm was filled with a small amount of orange-red fluorescent lipid droplets in the SIPI-7623 120 ng·mL⁻¹ group (Fig. 5).

**Effects of SIPI-7623 on atherosclerosis in rats**

After the animals were treated with SIPI-7623 at 20, 40, 80, and 160 mg·kg⁻¹, their TC levels were reduced by 21.1%, 30.6%, 32.5%, and 43.1% in a dose-dependent manner. Reduction under a dosage of 160 mg·kg⁻¹ was superior to that of simvastatin; under an SIPI-7623 dosage of 20, 40, 80, and 160 mg·kg⁻¹, TG was reduced by 42.0%, 44.8%, 54.1%, and 59.1% in a dose-dependent manner, while gemfibrozil produced a reduction rate of 55.9%. Under the dosage of 160 mg·kg⁻¹, SIPI-7623 reduced LDL-C by 44.9%, which was superior to 38.3% by simvastatin.

The biochemical results of serum TC, TG, HDL-C, and LDL-C in the groups are shown in Table 2. According to the score criteria for the Roberts & Thompson method (Fig. 6), as well as histological analysis, the endangium of the aortic arch was smooth and intact in the normal rats. The tissue was in...

Fig. 4 Filipin Staining (200 ×). A: blank group; B: model group; C: SIPI-7623 (120 ng·mL−1); D: SIPI-7623 (60 ng·mL−1); E: SIPI-7623 (30 ng·mL−1); F: simvastatin (200 ng·mL−1) group; G: SIPI-7623 decreases the cellular cholesterol level. The results are shown as means ± SD (n = 6 fields/group). *P < 0.05 vs model group.

Fig. 5 Nile Staining (200 ×). A: Blank group; B: model group; C: SIPI-7623 (120 ng·mL−1) group; D: SIPI-7623 (60 ng·mL−1) group; E: SIPI-7623 (30 ng·mL−1) group; F: gemfibrozil (40 μg·mL−1) group; G: SIPI-7623 decreases the cellular neutral lipid level. The results are shown as means ± SD (n = 6 fields/group). *P < 0.05, **P < 0.01 vs model group.

Fig. 6 Histology of aortic arch in atherosclerosis rats treated with SIPI-7623. A: Control; B: Model; C: SIPI-7623 (160 mg·kg−1); D: SIPI-7623 (80 mg·kg−1); E: SIPI-7623 (40 mg·kg−1); F: gemfibrozil (60 mg·kg−1); G: simvastatin (10 mg·kg−1); H: the scores of aortic arch. The results are shown as means ± SD (n = 10). *P < 0.05, **P < 0.01 vs model group.

Regular and neat arrangement, and pathological changes were not observed. Atherosclerotic plaques were found in the endangium of the aortic arch in model group, with plaque upheaval and necrosis, lipid deposition, calcium salt deposition, and fibrous tissue hyperplasia. The SIPI-7623 80 mg·kg−1 group exhibited the mildest lesions; the SIPI-7623 160 mg·kg−1 group was second to the 80 mg·kg−1 group but superior to the simvastatin and gemfibrozil groups (Fig. 6).

Effects of SIPI-7623 on a rabbit model of atherosclerosis

Under an SIPI-7623 dosage of 20, 40, and 80 mg·kg−1, TC was reduced by 27.6%, 25.3%, and 50.2% in a dose-dependent manner. The reduction rate at a dosage of 80 mg·kg−1 was superior to 30.2% of simvastatin. Under an SIPI-7623 dosage of 20, 40, and 80 mg·kg−1, TG was reduced by 72.8%, 64.9% and 71.2%, which was superior to 63.8% by gemfibrozil; under a dosage of 80 mg·kg−1, SIPI-7623 decreased LDL-C by more than 43.9%, which was superior to 25.4% by simvastatin.
The changes in the biochemical measurements of serum TC, TG, HDL-C, and LDL-C in the different groups are shown in Table 3. According to the score criteria of the Roberts & Thompson method (Fig.7), as well as histological analysis, the arterial surface of the normal group was smooth with a dense structure. There were plaque-like lesions in the model group that were protuberant from the blood vessel wall with a dense structure. There were plaque-like lesions in the arterial wall, with varying degrees of damage in the plaque lesions, with varying degrees of damage in the vascular wall membrane (Fig. 7).

### Table 2  Level of blood lipid with SIPI-7623 administered for 3 weeks (means ± SD, n = 10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>IR (%)</th>
<th>TC (mmol·L⁻¹)</th>
<th>TG (mmol·L⁻¹)</th>
<th>HDL-C (mmol·L⁻¹)</th>
<th>LDL-C (mmol·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>1.1 ± 0.3 *</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1 **</td>
<td>63.8 ± 8.2</td>
<td>0.7 ± 0.1 **</td>
<td>0.5 ± 0.2 **</td>
</tr>
<tr>
<td>Model</td>
<td>12.3 ± 6.4</td>
<td>0.6 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>11.4 ± 6.2</td>
<td>42.4 ± 14.9 *</td>
<td>27.6 ± 10.7 *</td>
</tr>
<tr>
<td>Sim</td>
<td>10</td>
<td>7.6 ± 1.7 *</td>
<td>43.1 ± 0.7</td>
<td>38.1 ± 0.1 **</td>
<td>71.2 ± 2.6</td>
<td>55.9 ± 4.3</td>
</tr>
<tr>
<td>Gem</td>
<td>160</td>
<td>7.0 ± 1.3 *</td>
<td>43.1 ± 0.7</td>
<td>44.8 ± 0.2</td>
<td>6.3 ± 1.4 **</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>SIPI-7623</td>
<td>80</td>
<td>8.3 ± 2.5</td>
<td>32.5 ± 0.7</td>
<td>1.4 ± 0.2</td>
<td>7.6 ± 2.4</td>
<td>33.3 ± 3.3</td>
</tr>
<tr>
<td>SIPI-7623</td>
<td>40</td>
<td>8.5 ± 2.4</td>
<td>30.6 ± 0.7</td>
<td>1.4 ± 0.2</td>
<td>7.6 ± 2.6</td>
<td>33.3 ± 3.3</td>
</tr>
<tr>
<td>SIPI-7623</td>
<td>20</td>
<td>9.7 ± 4.6</td>
<td>21.1 ± 0.7</td>
<td>1.4 ± 0.4</td>
<td>9.0 ± 5.0</td>
<td>21.6 ± 2.4</td>
</tr>
</tbody>
</table>

\( ^{*} P < 0.05, ^{**} P < 0.01 \) vs model group

### Table 3  Blood lipid levels of rabbits after SIPI-7623 (means ± SD, n = 8, 7, 8, 8, 8 and 7, respectively)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>IR (%)</th>
<th>TC (mmol·L⁻¹)</th>
<th>TG (mmol·L⁻¹)</th>
<th>HDL-C (mmol·L⁻¹)</th>
<th>LDL-C (mmol·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>1.7 ± 0.7 **</td>
<td>0.9 ± 0.5 **</td>
<td>0.8 ± 0.4 **</td>
<td>56.8 ± 8.2</td>
<td>3.1 ± 0.3</td>
<td>44.1 ± 8.5</td>
</tr>
<tr>
<td>Model</td>
<td>39.6 ± 12.2 **</td>
<td>30.2</td>
<td>2.6 ± 1.5</td>
<td>36.9</td>
<td>2.9 ± 0.5</td>
<td>32.9 ± 9.3 **</td>
</tr>
<tr>
<td>Gem</td>
<td>46.1 ± 11.5</td>
<td>18.8</td>
<td>1.5 ± 0.8 **</td>
<td>63.8</td>
<td>3.3 ± 0.4</td>
<td>38.6 ± 9.9</td>
</tr>
<tr>
<td>SIPI-7623</td>
<td>80</td>
<td>28.3 ± 12.8 **</td>
<td>50.2</td>
<td>1.2 ± 0.4 **</td>
<td>71.2</td>
<td>2.6 ± 0.7</td>
</tr>
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<td>40</td>
<td>42.4 ± 14.9 *</td>
<td>25.3</td>
<td>1.4 ± 0.8 **</td>
<td>64.9</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>SIPI-7623</td>
<td>20</td>
<td>41.1 ± 10.7 *</td>
<td>27.6</td>
<td>1.1 ± 0.8</td>
<td>72.8</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

\( ^{*} P < 0.05, ^{**} P < 0.01 \) vs model group

Unlike guggulsterone, SIPI-7623 is a non-steroidal lipid-lowering compound, with the structure of fibrate. RT-FRET and BLI results proved that it was a FXR antagonist without PPARs agonist activity.

Western blotting results showed that SIPI-7623 could inhibit the expression of FXR, increase the expression of CYP7A1, and promote the synthesis of bile acids in the liver. As a result, the level of cholesterol as the bile acid synthesis raw material would be reduced. After administration of SIPI-7623, the expression of IBABP in the ileum decreased, thereby reducing the reabsorption of bile acids and increasing the excretion of bile acids. In the ileum, FXR activation can inhibit the expression of IBABP [22]. Studies have found that there was a negative feedback regulation of IBABP by bile acids in humans and mice [23,24]. Unlike the cholesterol-lowering mechanism of statins, this cholesterol-lowering effect of SIPI-7623 was achieved by regulating the metabolic balance between bile acid and cholesterol [25].

FXR activation inhibits the production of the synthesis of triglycerides by inhibiting the transcription of SREBP-1c [26]. It has been reported that FXR activation inhibits the expression of CYP7A1 and regulates the expression of SREBP-1c [27] by affecting small heterodimer partner (SHP) in the liver. SIPI-7623 reduced the expression of triglyceride in the pharmacodynamics study in vivo, and results of the Western blotting analyses showed that SREBP-1c protein decreased under the action of SIPI-7623. Studies have shown that FXR inhibitors can have a synergistic effect with mouse intestinal
SIPI-7623 reduced TC and TG in vitro and in vivo, and the decrease in the high-dose group was significantly greater than that of the positive control drug simvastatin and gemfibrozil. With the current hypolipidemic drugs that are clinically used, there is no drug that can reduce TC and TG at the same time. In atherosclerotic rats and rabbits, SIPI-7623 reduced both TC and TG levels in serum, which was a feature of SIPI-7623 as a new lipid-lowering drug. Moreover, SIPI-7623 decreased the extent of atherosclerotic plaques as evidenced by the pathological changes, which indicated that it would be highly effective in clinical application as a hypo-

lipidemic drug. Currently, clinical studies of SIPI-7623 have been submitted for the approval of the China Food and Drug Administration (CFDA), and hopefully it will enter phase I clinical trials in the near future.

In conclusion, SIPI-7623, which was a derivative of an extract of Oriental wormwood, eliminated jaundice and promoted the secretion of bile. Our data indicated that SIPI-7623 was an FXR antagonist, and can reduce cholesterol by regulating the synthesis of bile acid through CYP7A1. There will be a great potential for this compound to be further developed as non-bile acid salt derivatives. These results from the present study also suggested that FXR antagonism could be a novel strategy for new drug discovery for the therapy of hyperlipidemia.

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