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Synthesis and biological evaluation of isoflavone amide derivatives with antihyperlipidemic and preadipocyte antiproliferative activities

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ABSTRACT

A series of isoflavone amides were designed with isoflavone in place of the scaffold of 2-arylbenzoxazole as cholesterol ester transfer protein (CETP) inhibitors. Twelve new compounds were synthesized, and their inhibitory activities of CETP and preadipocyte proliferation were assayed. The hypolipidemic potency of the most effective compound **HY-2c** was further tested in vivo by hamster. The results indicate that **HY-2c** exhibited favorable antihyperlipidemic and preadipocyte antiproliferative activities.

1. Introduction

Cardiovascular diseases (CVD's) are among the major causes of morbidity and mortality globally today.[1] Dyslipidemia is considered a major risk factor in the progression of cardiovascular diseases. Epidemiological studies have established that both high low-density-lipoprotein cholesterol (LDL-C) level and low high-density-lipoprotein cholesterol (HDL-C) level are contributors to the progression of atherosclerosis and the underlying chronic disorder of CVD.[2,3]

Current clinical strategies of lipid regulation for the treatment of atherosclerosis have primarily focused on lowering circulating levels of LDL-C via the use of agents such as statins. However, CVD risk remains for some patients, and low HDL-C level could contribute to the residual risk.[4] HDL promotes reverse cholesterol transport (RCT), a process by which cholesterol is transferred from peripheral tissues and cells to the liver for excretion. Thus, elevating the concentration of HDL-C appears to be an attractive strategy of reducing atherosclerosis risk for patients with low HDL-C level. To date, two classes of HDL-C elevating drugs, fibrates and niacins, are currently used in clinical applications. However, they still go beyond the clinical requirement with the limited efficacy of fibrates and the gastric intolerance of niacins.[5] Therefore, studies on novel therapeutic agents aiming to effectively elevating HDL-C levels are needed.

CETP is a plasma protein that naturally transfers cholesterol from high-density lipoproteins to very low-density lipoproteins (VLDLs) or low-density lipoproteins (LDLs).[6] CETP has an additional role in the uptake of HDL-C by human adipocytes. Thus, inhibition of CETP may raise the concentration of HDL-C and lower plasma LDL-C levels thereby reduce the risk of CVD.[7]

Obesity can cause various health problems including diabetes, cardiovascular diseases and dyslipidemia. Increased production of CETP in obese status could directly result in reduced HDL-C levels. In obese patients, plasmatic CETP exhibits stronger bioactivity due to its higher concentration.[8] Adipose tissue which secretes CETP, may be an important source of plasmatic CETP in humans.[9] Preadipocytes are the initial formation of adipose depots and possess the ability to undergo complete differentiation into mature adipocytes. Thus, the inhibition of preadipocytes proliferation is an early target to reduce adipose tissue mass.[10] Therefore, inhibition of preadipocyte proliferation could reduce the activity of plasma CETP and treat dyslipidemia indirectly. The preadipocyte proliferation could be reduced by (1) antagonizing the actions of a gene whose activation is essential for cell growth; (2) limiting substrate availability for growth; (3) causing growth arrest; or (4) being cytotoxic.[11]

Soy isoflavones, such as genistein, daidzein and formononetin (Fig. 1) which are found in soybean and soybean-derived products, are a major source of phytoestrogens in human diet. They have a similar scaffold as estrogen, which enables them to bind estrogen

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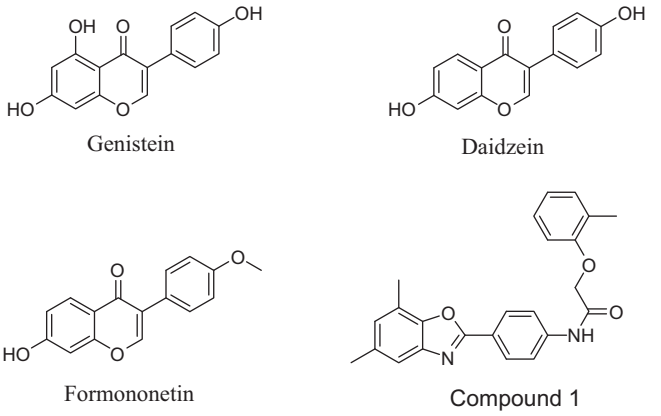


Figure 1. Structures of some isoflavone compounds and compound **1**.

receptor (ER), and act as estrogen agonists. It has been shown that genistein has anti-3T3-L1 preadipocyte proliferation activity.[12] Studies in humans and rodents support the hypothesis that soy isoflavones may be beneficial to the prevention of obesity and dyslipidemia.[13–15]

Compound **1** (Fig. 1) was identified as a CETP inhibitor after screening using the BODIPY-CE fluorescence assay by Bristol-Myers Squibb (BMS).[16] Its IC_{50} value of CETP inhibition in human whole plasma assay is 10 μM. Further efforts to optimize the structures of 2-arylbenzoxazole class of CETP inhibitors were made through the substitution of the benzoxazole moiety and modification of the α-alkoxyamide side chain by Merck.[17–21] The 2-arylbenzoxazole moiety of compound 1 shows a structural similarity to the molecular framework of cholesterol.[16] Isoflavone is similar to 17-β-estradiol in chemical structure and it is a common parent nucleus for scaffold hopping of steroids. Thus, isoflavone may mimic cholesterol better than 2-arylbenzoxazole moiety. In addition, isoflavones can also inhibit preadipocyte proliferation as mentioned above. In this paper, by replacing the 2-arylbenzoxazole with isoflavone scaffold 12 isoflavone amide derivatives were synthesized and they were anticipated to have CETP inhibition and preadipocyte antiproliferative activities.

2. Results and discussion

2.1. Chemical synthesis

Synthesis of these isoflavone amide derivatives is outlined in Scheme 1. The isoflavone scaffold and the side chains were synthesized respectively.

Synthesis of the side chains was performed by a facile two-step procedure. The phenols (**2a**–**f**) reacted with ethyl chloroacetate (**3**) to give the corresponding ethers (**4a**–**f**). Then the acids (**5a**–**f**) were obtained through the hydrolysis of the ethers. The acyl chloride intermediates (**6a**–**f**) were synthesized by treating **5a**–**f** with thionyl chloride.

As a key intermediate, 7-hydroxy-4′-nitroisoflavone (**9**) was prepared with resorcinol (**8**) and 4-nitrobenzoic acid (**7**) as starting materials by Friedel–Crafts Acylation using boron trifluoride etherate as solvent and catalyst. The intermediate was then directly treated with Vilsmeier reagent to give the isoflavone scaffold. 7-Hydroxy-4′-aminoisoflavone (**10**) was prepared from the reduction of 7-hydroxy-4′-nitroisoflavone (**9**).

Compounds **HY-1a**–**HY-1f** were obtained by condensation of acyl chloride intermediates **(6a**–**f)** with 7-Hydroxy-4′-aminoisoflavone (**10**). Acetylation of **HY-1a**–**HY-1f** with acetyl chloride yielded **HY-2a**–**HY-2f**.

2.2. Biological evaluation

The commercially available CETP Inhibitor Drug Screening Kit (BioVision, USA) was used to assay CETP inhibitory efficacy of our compounds according to the manufacturer's protocol. The screening kit uses a donor molecule containing a fluorescent self-quenched neutral lipid (NBD-labeled Cholesterol in HDL) that is transferred to an acceptor molecule (LDL) in the presence of CETP (rabbit serum). CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence (ExEm = 465/535 nm). CETP Inhibitors will inhibit the lipid transfer and therefore decrease fluorescence intensity. Anacetrapib, which was developed by Merck and now in phase III clinical trial, was selected as positive control drug. The assay results are shown in Figure 2. The result showed that some compounds with an acetyl group at 7-OH of the isoflavone moiety, including **HY-2c**, **HY-2d** and **HY-2f**, had better activity than the corresponding unesterified one. While other acetylized compounds such as **HY-2a**, **HY-2b** and **HY-2e** had no activity. Methyl substitution at the 2, 3, or 4 positions of ring B would lead to reduction of activities. 2-OCH_3 substitution led to a loss of activity, while **HY-2c** with no substitution at ring B displayed the best activity. The inhibition rate of **HY-2c** and **HY-2f** was over 70% at the concentration of 100 μM. Then the IC_{50} value of the two compounds was obtained as 1.52 μM and 8.42 μM respectively by testing the activity of inhibition in seven concentration gradients (Fig. 5).

3T3-L1 cells were selected to assess the effects of the compounds in this study on preadipocyte proliferation using the standard MTT assay. Oleoyl Formononetin, previously reported by our lab,[22] was selected as the positive control. **HY-2c** and **HY-2f** exhibited satisfactory inhibition efficacy (Fig. 3). The mechanisms mediating such inhibition are not clear, but this effect is not due to a cytotoxic activity, since **HY-2c** was also tested on other cell types and it was found to have no significant effect on cell viability. Figure 4 showed that **HY-2c** had no proliferation inhibition effect on HepG2 cells.

We further investigated the hypolipidemic activities of **HY-2c** in vivo using male Syrian hamster dyslipidemia model induced by high lipid diet. The results were presented in Figure 6. After oral administration of **HY-2c** for 28 days, LDL-C was reduced by 44.6% and HDL-C was increased by 66%. This result suggested that **HY-2c** had a good lipid regulating potency.

3. Conclusions

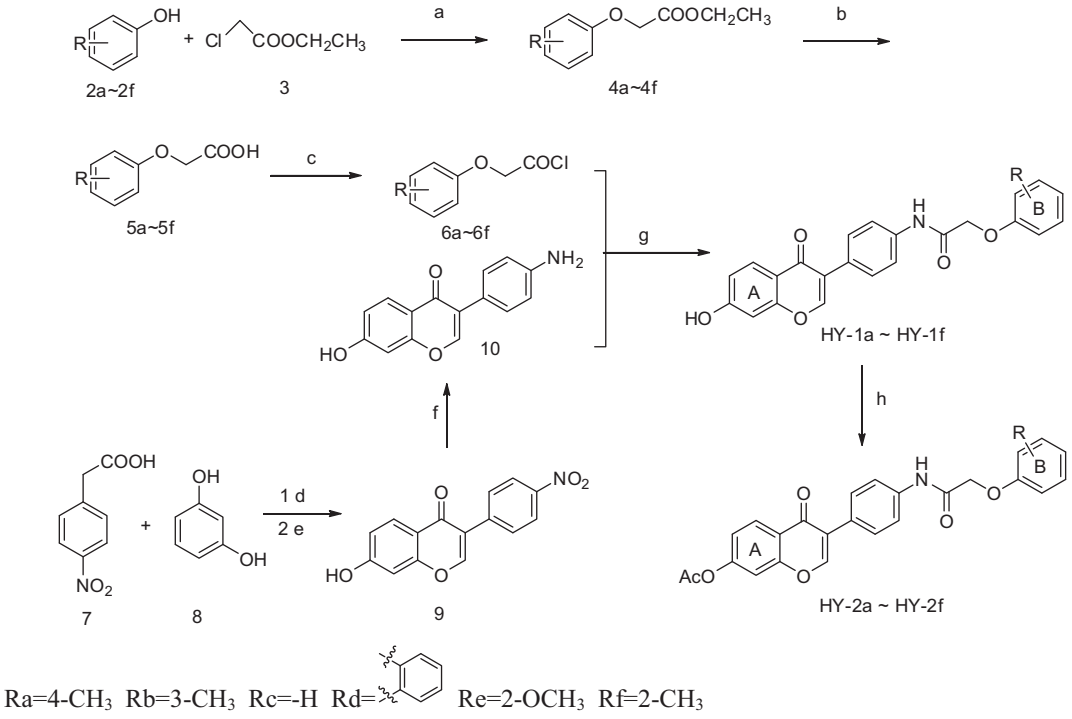
In order to maintain the hypolipidemic activities, we changed the 2-arylbenzoxazole scaffold into isoflavone and synthesized 12 isoflavone amide derivatives. Two of them, **HY-2c** and **HY-2f**, embodied remarkable CETP inhibition in vitro and preadipocyte antiproliferation activities. Furthermore, **HY-2c** exerted remarkable lipid regulating activity in vivo.

4. Experimental

4.1. Chemical synthesis

4.1.1. Materials

Most chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standard methods. Reactions were monitored by thin-layer chromatography (TLC) using precoated silica gel plates (silica gel GF/UV 254), and spots were visualized under UV light (254 nm). Melting points were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^1H NMR spectra were recorded with a Bruker



Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, 75 °C, 12 h; (b) 7.2% KOH, EtOH, rt, 1.5 h; (c) $SOCl_2$, THF, DMF, 50 °C, 5 h; (d) BF_3–Et_2O, 85 °C, 1.5 h; (e) PCl_5, DMF, 25 °C, 2 h; (f) Fe, H_2O, NH_4Cl, EtOH, reflux, 4 h; (g) THF, pyridine, 50 °C, 3 h; (h) CH_3COCl, THF, pyridine, reflux, 2 h.

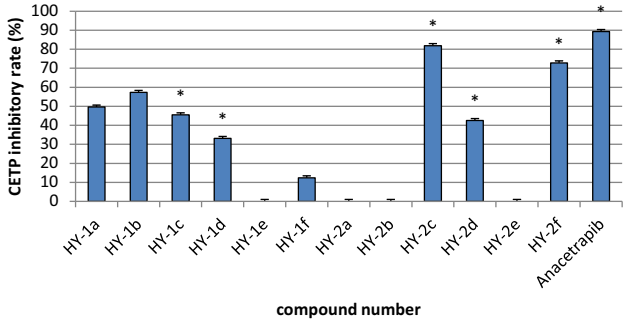


Figure 2. CETP inhibition activity of the synthetic compounds (100 μM) with Anacetrapib (100 μM) as positive control. $^*P \leqslant 0.05$ versus control.

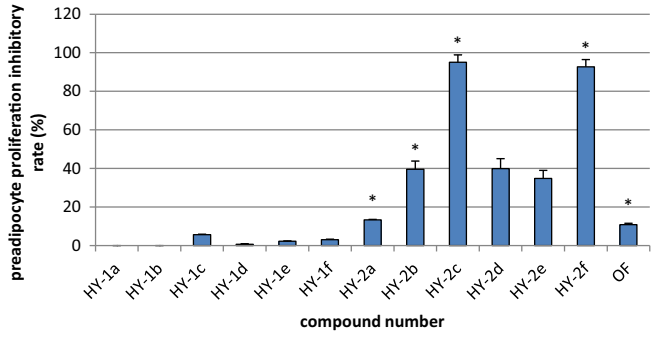


Figure 3. The antiproliferation effect of the synthetic compounds (100 μM) on preadipocytes by MTT assay. Cells were treated with the synthetic compounds for 48 h. Values represent % of the control (the control group, absence of synthetic compounds). $^*P \leqslant 0.05$ versus control group.

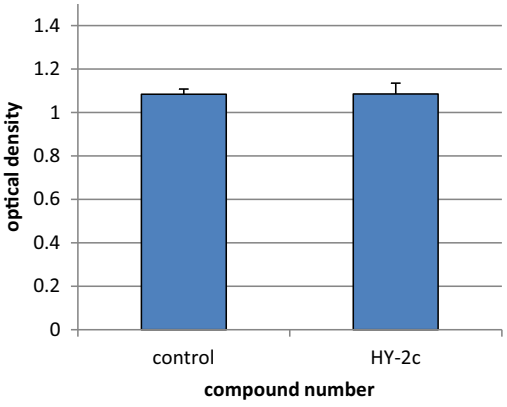


Figure 4. The MTT assay result of **HY-2c** (100 μM) on HepG2 cells. Cells were treated with **HY-2c** for 48 h. The control group, absence of **HY-2c**. The **HY-2c** group was not significantly different from control (p>0.05).

Avance 500 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spectrum (ESI), or a LC/MSD TOF HR-MS Spectrum. Chemical shifts (d) were expressed in parts per million relative to tetramethylsilane, which was used as an internal standard, coupling constants (*J*) were in hertz (Hz), and the signals were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet.

4.1.2. 7-Hydroxy-3-(4-nitrophenyl)-4*H*-benzopyran-4-one (9)

A mixture of 4-nitrophenylacetic acid (**7**; 1.0 g, 6.0 mmol), resorcinol (**8**; 0.72 g, 6.5 mmol) and BF_3/Et_2O (10 ml) was heated at 85 °C for 1.5 h with stirring, then cooled down to 10 °C and 10 ml DMF was added slowly. In another flask, 10 ml DMF was added and cooled to 10 °C, PCl_5 (2.0 g, 9 mmol) was added in batches, afterwards the mixture was heated to 55 °C and stirred for 0.5 h, after that cooled to 10 °C. Then the mixture was added by droplet into the first flask and stirred at 25 °C for 2 h. Then

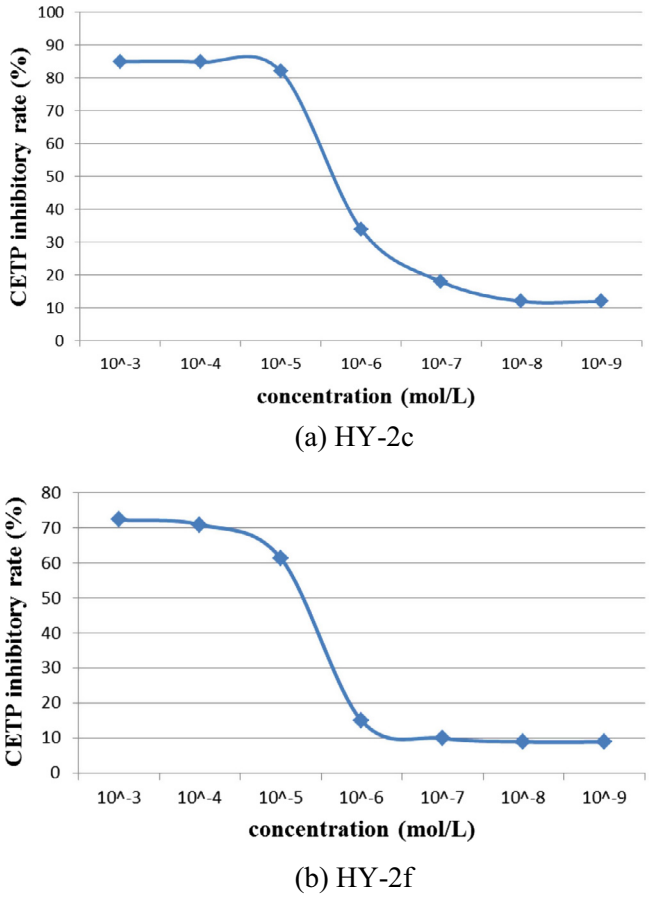


Figure 5. CETP inhibition activity of **HY-2c** and **HY-2f** in seven concentration gradients.

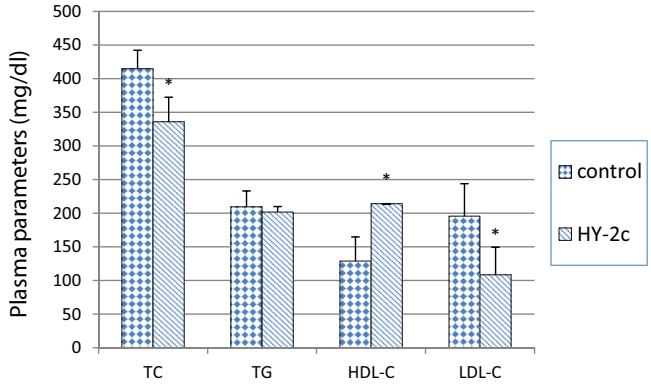


Figure 6. The biochemistry analysis of hamster treated with **HY-2c** for 4 weeks. (ig). Control: high-fat diet-fed group untreated with **HY-2c**; **HY-2c**: high-fat diet-fed hamster treated with **HY-2c** (50 mg/kg/d). $^{*}P \leqslant 0.05$, **HY-2c** group versus control.

the reaction mixture was poured into heated dilute hydrochloric acid (0.5 mol/L). The product was filtered, and purified by recrystallization from methanol to yield **9** (0.36 g, 1.26 mmol, 21%) as a white solid. ^{1}H NMR (DMSO, 300 MHz): δ 8.48 (s, 1H, H-2), 8.28 (2H, J = 8.8 Hz, H-3′, H-5′), 8.07 (d, 1H, J = 8.8 Hz, H-5), 7.96 (d, 2H, J = 8. 8 Hz, H-2′, H-6′), 7.05 (dd, 1H, J = 8.8, 2.4 Hz, H-6), 6.97 (d, 1H, J = 2.4 Hz, H-8); MS (ESI): m/z = 282 $[M-H]^{-}$.

4.1.3. 7-Hydroxy-3-(4-aminophenyl)-4*H*-benzopyran-4-one (10)

To a solution of **9** (3 g, 10 mmol) in 30 ml ethanol, iron powder (6 g, 100 mmol) and NH_4Cl (1 g in 3 ml water) solution were added. Then the mixture was refluxed for 4 h. The hot solution was filtered, and the filtrate was concentrated under reduced pressure to give **10** (2 g, 0.75 mmol, 75%) as a white solid. ^{1}H NMR (DMSO, 300 MHz): δ 8.14 (s, 1H, H-2), 8.05 (d, 1H, J = 8.8 Hz, H-5), 7.46 (d, 2H, J = 8. 8 Hz, H-2′, H-6′), 6.99 (dd, 1H, J = 8.8, 2.4 Hz, H-6), 6.90 (d, 1H, J = 2.4 Hz, H-8), 6.88 (2H, J = 8.8 Hz, H-3′, H-5′); MS (ESI): m/z = 254 $[M+H]^{+}$.

4.1.4. General procedure for the synthesis of 5a–f

A mixture of phenols (0.3 mmol), acetone (10 ml), dimethylformamide (10 ml), ethyl chloroacetate (2.5 ml, 0.26 mmol), potassium carbonate (2 g) and potassium iodide (0.15 g) was heated at 75 °C for 12 h. Afterwards, the solid was filtered and the solvent of the filtrate was removed in vacuo. The colorless liquid (**4a**–**f**) obtained was dissolved in ethanol (20 ml). A solution of potassium hydroxide (7.2%, 5 ml) was added and stirred at room temperature for 1.5 h. The mixture was poured into hydrochloric acid (1 mol/L) and a white precipitate was formed. Purification by recrystallization from methanol afforded the corresponding aryloxyl acid (**5a**–**f**).

4.1.4.1. 4-Methyl phenyloxyl acid (5a). Compound (**5a**) was obtained as a white solid. Yield: 80.7%. ^{1}H NMR (DMSO, 300 MHz): δ 7.12 (m, 2H, H-3, H-5), 6.90 (m, 2H, H-2, H-6), 4.62 (s, 2H, $-OCH_2CO-$), 2.22 (s, 3H, Ar-CH_3); MS (ESI): m/z = 165 $[M-H]^{-}$.

4.1.4.2. 3-Methyl phenyloxyl acid (5b). Compound (**5b**) was obtained as a white solid. Yield: 83%. δ 7.17 (m, 1H, H-5), 6.60-6.84 (m, 3H, H-2, H-4, H-6), 4.66 (s, 2H, $-OCH_2CO-$), 2.28 (s, 3H, Ar-CH_3); MS (ESI): m/z = 165 $[M-H]^{-}$.

4.1.4.3. Phenyloxyl acid (5c). Compound (**5c**) was obtained as a white solid. Yield: 84.6%. δ 7.35 (m, 2H, H-3, H-5), 6.84-7.05 (m, 3H, H-2, H-4, H-6), 4.69 (s, 2H, $-OCH_2CO-$); MS (ESI): m/z = 151 $[M-H]^{-}$.

4.1.4.4. 1-Naphthyloxyl phenyloxyl acid (5d). Compound (**5d**) was obtained as a white solid. Yield: 76.2%. δ 7.80–7.91 (m, 3H, H-4, H-5, H-8), 7.49 (m, 1H, H-7), 7.39 (m, 1H, H-6), 7.33 (m, 1H, H-1), 7.28 (m, 1H, H-3), 4.88 (s, 2H, $-OCH_2CO-$); MS (ESI): m/z = 201 $[M-H]^{+}$.

4.1.4.5. 2-Methoxy phenyloxyl acid (5e). Compound (**5e**) was obtained as a white solid. Yield: 82.4%. δ 6.84–7.02 (m, 4H, Ar-5), 4.70 (s, 2H, $-OCH_2CO-$), 3.85 (s, 3H, $-OCH_3$); MS (ESI): m/z = 181 $[M-H]^{-}$.

4.1.4.6. 2-Methyl phenyloxyl acid (5f). Compound (**5f**) was obtained as a white solid. Yield: 81.3%. δ 7.14 (m, 1H, H-3), 6.81–6.89 (m, 3H, H-4, H-5, H-6), 4.73 (s, 2H, $-OCH_2CO-$), 2.26 (s, 3H, Ar-CH_3); MS (ESI): m/z = 165 $[M-H]^{-}$.

4.1.5. General procedure for the synthesis of HY-1a–HY-1f

A mixture of aryloxyl acid (**5a**–**f**) (10 mmol), thionyl chloride (15 mmol), tetrahydrofuran (50 ml) and dimethylformamide (1 ml) was stirred at 50 °C for 5 h. Then tetrahydrofuran and thionyl chloride were removed in vacuo. Yellow liquid (**6a**–**f**) obtained and pyridine (2.5 ml) were then added to a solution of **10** (10 mmol in 25 ml tetrahydrofuran). The solution was stirred at 50 °C for 3 h. When the reaction was completed, the solution was poured into hydrochloric acid (1 mol/L) and extracted with CH_2Cl_2 (20 ml × 3). The combined organic phase was washed with brine (20 ml × 3), dried over Na_2SO_4, concentrated to afford yellow solid. Purification by silica gel column chromatography (PE:EA = 15:1) yielded the isoflavone amide derivatives **HY-1a**–**HY-1f**.

4.1.5.1. 7-Hydroxy-3-[4-(4-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-1a). Compound **HY-1a** was obtained as a white solid. Yield: 56.8%. Mp 170–172 °C; IR (cm^{-1}): 3382, 3127, 2365, 2324, 1655, 1588, 1509, 1400, 1260, 1236; ^1H NMR (DMSO, 300 MHz): δ 10.80 (s, 1H, NH), 10.11 (s, 1H, OH), 8.37 (s, 1H, H-2), 7.96 (d, 1H, J = 8.91 Hz, H-5), 7.56 (m, 4H, Ar-H), 7.12 (d, 2H, J = 8.58 Hz, H-2′, H-6′), 6.90 (m, 4H, Ar-H), 4.62 (s, 2H, –OCH_2CO–), 2.22 (s, 3H, Ar-CH_3); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 402.1336, found 402.1330.

4.1.5.2. 7-Hydroxy-3-[4-(3-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-1b). Compound **HY-1b** was obtained as a white solid. Yield: 37.5%. Mp 168–170 °C; IR (cm^{-1}): 3394, 3100, 2363, 2326, 1686, 1587, 1522, 1265, 1171; ^1H NMR (DMSO, 300 MHz): δ 10.79 (s, 1H, NH), 10.12 (s, 1H, OH), 8.38 (s, 1H, H-2),7.97 (d, 1H, J = 8.91 Hz, H-5), 7.57 (m, 4H, Ar-H), 6.96 (t, 2H, J = 8.48 Hz, H-2′, H-6′), 6.85 (m, 4H, Ar-H), 4.68 (s, 2H, –OCH_2CO–), 2.28 (s, 3H, Ar-CH_3); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 402.1336, found 402.1336.

4.1.5.3. 7-Hydroxy-3-(4-phenyloxyacethyphenyl)-4*H*-benzopyran-4-one (HY-1c). Compound **HY-1c** was obtained as a white solid. Yield: 46.8%. Mp 166–168 °C; IR (cm^{-1}): 3125, 2578, 2365, 2348, 1734, 1705, 1670, 1585, 1497, 1437, 836, 756, 685; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.85 (s, 1H, NH), 10.22 (s, 1H, OH), 8.43 (s, 1H, H-2),8.34 (d, 1H, J = 8.81 Hz, H-5), 7.57 (m, 4H, Ar-H), 6.96 (t, 2H, J = 8.48 Hz, H-2′, H-6′), 6.85 (m, 4H, Ar-H), 4.68 (s, 2H, –OCH_2CO–); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 388.1179, found 388.1176.

4.1.5.4. 7-Hydroxy-3-(4-naphthyloxyacethyphenyl)-4*H*-benzopyran-4-one (HY-1d). Compound **HY-1d** was obtained as a white solid. Yield: 50.8%. Mp 166–168 °C;IR (cm^{-1}): 3403, 3133, 2353, 2342, 1682, 1622, 1577, 1533, 1508, 1398, 1266, 1240, 1108; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.80 (s, 1H, NH), 10.30 (s, 1H, OH), 8.34 (m, 2H, H-2 Ar-H),7.88 (m, 2H, H-5 Ar-H), 7.64 (m, 8H, Ar-H), 6.95 (d, 2H, J = 8.28 Hz, H-2′, H-6′), 6.88 (s, 1H, Ar-H), 4.90 (s, 2H, –OCH_2CO–); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 438.1336, found 438.1332.

4.1.5.5. 7-Hydroxy-3-[4-(2-methyloxyphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-1e). Compound **HY-1e** was obtained as a white solid. Yield: 58.3%. Mp 168–170 °C; IR (cm^{-1}): 3385, 3133, 2355, 2334, 1697, 1532, 1504, 1400, 1263, 1219, 1126; ^1H NMR (DMSO, 300 MHz): δ 9.09 (s, 1H, OH), 8.19 (d, 1H, J = 8.91 Hz, H-5), 7.95 (s, 1H, H-2), 7.63 (m, 4H, Ar-H), 7.02 (m, 5H, J = 8.48 Hz, H-2′, H-6′), 6.86 (s, 1H, Ar-H), 4.70 (s, 2H, –OCH_2CO–), 3.97 (s, 3H, Ar-OCH_3); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 418.1285, found 418.1298.

4.1.5.6. 7-Hydroxy-3-[4-(2-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-1f). Compound **HY-1f** was obtained as a white solid. Yield: 40%. Mp 168–170 °C; IR (cm^{-1}): 3397, 3126, 2355, 2334, 1623, 1590, 1529, 1493, 1455, 1400, 1243, 1183; ^1H NMR (DMSO, 300 MHz): δ 10.80 (s, 1H, NH), 10.13 (s, 1H, OH), 8.38 (s, 1H, H-2), 7.96 (d, 1H, J = 8.91 Hz, H-5), 7.60 (m, 4H, Ar-H), 6.16 (t, 2H, J = 8.48 Hz, H-2′, H-6′), 6.89 (m, 4H, Ar-H), 4.73 (s, 2H, –OCH_2CO–), 2.26 (s, 3H, Ar-CH_3); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 402.1336, found 402.1342.

4.1.6. General procedure for the synthesis of HY-2a–HY-2f

To a solution of **HY-1a**–**HY-1f** (1 mmol in 10 ml tetrahydrofuran), pyridine (0.3 ml) and acetyl chloride (0.3 ml) were added and stirred at reflux for 2 h. Then the reaction mixture was poured into aqueous HCl (1 mmol/L) to give the white solid. The mixture was filtered, dried and the product was obtained.

4.1.6.1. 7-Acetoxy-3-[4-(4-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-2a). Compound **HY-2a** was obtained as a white solid. Yield: 85%. Mp 165–167 °C; IR (cm^{-1}): 3119, 2909, 2585, 1738, 1608, 1593, 1510, 1430, 1400, 1379, 1339, 1302, 1254; ^1H NMR (DMSO, 300 MHz): δ 8.37 (s, 1H, H-2), 7.96 (d, 1H, J = 8.91 Hz, H-5), 7.56 (m, 4H, Ar-H), 7.12 (d, 2H, J = 8.58 Hz, H-2′, H-6′), 6.90 (m, 4H, Ar-H), 4.62 (s, 2H, –OCH_2CO–), 3.43 (s, 3H, –$COCH_3$), 2.22 (s, 3H, Ar-CH_3–); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 444.1442, found 444.1441.

4.1.6.2. 7-Acetoxy-3-[4-(3-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-2b). Compound **HY-2b** was obtained as a white solid. Yield: 87.2%. Mp 160–163 °C; IR (cm^{-1}): 3391, 3126, 1767, 1682, 1635, 1618, 1533, 1441, 1221, 1178; ^1H NMR (DMSO, 300 MHz): δ 10.14 (s, 1H, NH) 8.54 (s, 1H, H-2), 8.18 (d, 1H, J = 8.91 Hz, H-5), 7.59 (m, 5H, Ar-H), 7.56 (m, 2H, H-2′, H-6′), 6.90 (m, 4H, Ar-H), 4.69 (s, 2H, –OCH_2CO–), 2.29 (s, 3H, –$COCH_3$), 2.26 (s, 3H, Ar-CH_3-); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 444.1442, found 444.1450.

4.1.6.3. 7-Acetoxy-3-(4-phenyloxyacethyphenyl)-4*H*-benzopyran-4-one (HY-2c). Compound **HY-2c** was obtained as a white solid. Yield: 86.3%. Mp 159–160 °C; IR (cm^{-1}): 3131, 2359, 2330, 1783, 1639, 1618, 1513, 1440, 1399, 1272, 1248, 1234, 1151; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.43 (s, 1H, H-2), 8.35 (m, 1H, J = 8.91 Hz, H-5), 7.71 (m, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.23 (d, 3H, J = 8.58 Hz, H-2′, H-6′,Ar-H), 6.88 (m, 3H, Ar-H), 4.65 (s, 2H, –OCH_2CO–), 2.38 (s, 3H, –$COCH_3$); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 430.1285, found 430.1286.

4.1.6.4. 7-Acetoxy-3-(4-naphthyloxyacethyphenyl)-4*H*-benzopyran-4-one (HY-2d). Compound **HY-2d** was obtained as a white solid. Yield: 88.7%. Mp 149–151 °C; IR (cm^{-1}): 3125, 2571, 1728, 1601, 1399, 1266, 1224, 1155, 1100, 817, 521; ^1H NMR (DMSO, 300 MHz): δ 10.34 (s, 1H, NH), 8.54 (s, 1H, H-2), 8.17 (d, 1H, J = 8.34 Hz, H-5), 7.77 (d, 2H, Ar-H), 7.53 (m, 9H, H-2′, H-6′, Ar-H), 6.95 (m, 1H, Ar-H), 4.62 (s, 2H, –OCH_2CO–), 2.34 (s, 3H, –$COCH_3$); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 480.1436, found 480.1440.

4.1.6.5. 7-Acetoxy-3-[4-(2-methyloxyphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-2e). Compound **HY-2e** was obtained as a white solid. Yield: 84.4%. Mp 164–167 °C; IR (cm^{-1}): 3132, 1628, 1601, 1548, 1506, 1262; ^1H NMR (DMSO, 300 MHz): δ 10.20 (s, 1H, NH), 8.36 (s, 1H, H-2), 7.97 (d, 1H, J = 8.81 Hz, H-5), 7.60 (m, 4H, Ar-H), 6.95 (m, 6H, Ar-H, H-2′, H-6′), 4.70 (s, 2H, –OCH_2CO–), 3.81 (s, 3H, -OCH_3), 2.22 (s, 3H, $COCH_3$); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 460.1391, found 460.1397.

4.1.6.6. 7-Acetoxy-3-[4-(2-methylphenyloxyacethy)phenyl]-4*H*-benzopyran-4-one (HY-2f). Compound **HY-2f** was obtained as a white solid. Yield: 85.8%. Mp 169–160 °C; IR (cm^{-1}): 3126, 2579, 2481, 1739, 1704, 1637, 1602, 1497, 1458, 1423, 1268, 758; ^1H NMR (DMSO, 300 MHz): δ 10.16 (s, 1H, NH), 8.55 (s, 1H, H-2), 8.18 (d, 1H, J = 8.73 Hz, H-5), 7.59 (m, 5H, Ar-H, H-2′), 7.12 (d, 1H, J = 8.58 Hz, H-6′), 6.95 (m, 4H, Ar-H), 4.74 (s, 2H, –OCH_2CO–), 2.34 (s, 3H, –$COCH_3$), 2.26 (s, 3H, Ar-CH_3); HRMS (EI) calcd for $C_{24}H_{19}NO_5$ 444.1442, found 444.1439.

4.2. 3T3-L1 cell and HepG2 cells proliferation assay by MTT

3T3-L1 preadipocytes and HepG2 cells (5000 cells/well) were seeded in 96-well plates. After two days' conventional culture,

the cells were incubated with Dulbecco's Modified Eagle's medium (DMEM, Gibco) containing the selected compounds (100 μmol/L) for 48 h. The culture solution containing 0.1% (V/V) ethanol (96%) was given to the control group. Twenty microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was placed in each well for 4 h at 37 °C. Consecutively, 100 μL of dimethyl sulfoxide (DMSO) was added to extract the MTT formazan, and followed by agitation on a plate shaker for 15 min. The optical density (OD) was then measured on the multi-well enzyme-linked immunosorbent assay automatic spectrometer reader at 540 nm. The inhibition ratio was calculated through formula 1:

$$\text{Inhibition ratio} = (1 - \text{OD}_{\text{experimental group}}/\text{OD}_{\text{blank group}}) \times 100\% \quad (1)$$

4.3. CETP inhibitory assay

3 μl of Rabbit Serum was added to the testing sample solution (100 μM in 160 μl dH_2O). A solution that contains no rabbit serum was as background and that contains rabbit serum but no testing compounds as the positive control. Donor molecule (10 μl), acceptor molecule (10 μl) and CETP assay buffer (20 μl) were mixed and added to the testing sample solution. The mixture was then incubated at 37 °C for 1 h. Afterwards the fluorescence intensity of the blank, test samples and the positive control was measured using a fluorometer (ExEm = 465/535 nm). The inhibition ratio was also calculated through formula 1.

4.4. In vivo study in hamsters

Male Syrian golden hamsters (4 weeks old; Vital River Laboratory Animal Technology Co. Ltd, Beijing, China) were allocated to control and treated groups ($n = 5$). Hamsters were placed on a high-fat diet for a month, blood samples were drawn from orbit. After the establishment of the hyperlipidemia model, compound **HY-2c** was suspended in olive oil and administered at 50 mg/kg/day for 28 days to the treated group, and vehicle to the control group. The general condition was observed daily and body weight was measured. Again blood samples were drawn from orbit and the serum lipid levels were measured.

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Supplementary data

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