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# Graphical abstract:

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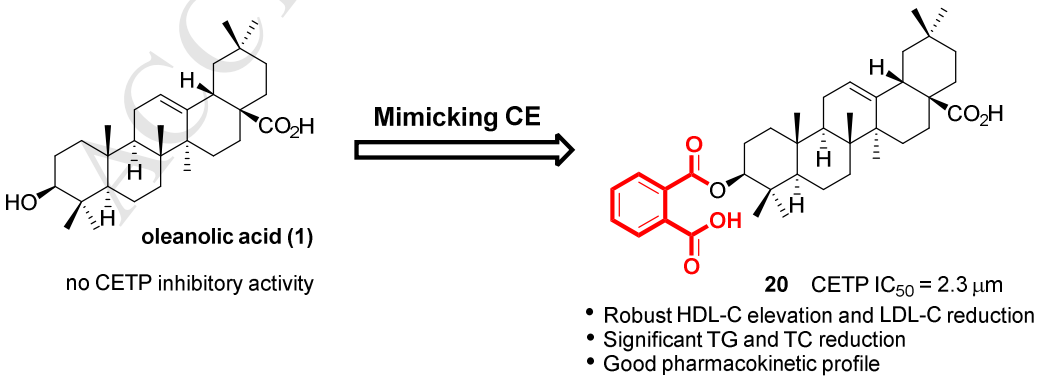

# Discovery of pentacyclic triterpene 3β-ester derivatives as a new class of cholesterol ester transfer protein inhibitors

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**Abstract**

A series of pentacyclic triterpene 3β-ester derivatives were designed, synthesized and evaluated as a new class of cholesteryl ester transfer protein (CETP) inhibitors for the treatment of dyslipidemia. In vitro screening assay showed that 5 out of 30 compounds displayed moderate inhibiting human CETP activity with $IC_{50}$s less than 10 μM. Among them, compound **20** ($IC_{50}$ = 2.3 μM) had the most potent biological activity, and effectively ameliorated plasma lipid levels of human adipose tissue specific CETP transgenic (ap2-CETPTg) mice and guinea pigs. Additional safety evaluation (no blood pressure elevation in guinea pigs) and pharmacokinetics studies indicated that the potential druggability for compound **20** which is a promising lead for development of a new class of CETP inhibitors for the treatment of dyslipidemia.

**Keywords**

CETP inhibitor, pentacyclic triterpenes, non-HDL-C, dyslipidemia

**Abbreviations used**

CVDs, cardiovascular diseases; CETP, cholesteryl ester transfer protein; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; VLDL, very-low-density lipoprotein; TC, total cholesterol; TG, triglyceride; CE, cholesteryl ester; ACAT, acyl-CoA: cholesterol acyl transferase; PPARs, peroxisome proliferator-activated receptors; PLs, pancreatic lipase; FXR, farnesoid X receptor; LXR, liver X receptor; PTs, pentacyclic triterpenes; SAR, structure activity relationship; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; H-mont, proton-exchanged montmorillonite; TLC, thin layer chromatography; HPLC, high performance liquid chromatography

## 1. Introduction

Cardiovascular diseases (CVDs) continue to be the leading cause of mortality worldwide over the past two decades [1, 2]. Atherosclerosis, a progressive disease characterized by the accumulation of lipids and the formation of atheroma plaque within the arteries, which is the primary cause of CVDs [3]. Numerous epidemiological evidences confirmed that lowering low-density lipoprotein cholesterol (LDL-C) levels as well as raising high density lipoprotein-cholesterol (HDL-C) levels in plasma appeared as a promising treatment strategy for cardiovascular pathologies [4]. Additional clinical studies indicated that non-HDL-C (total cholesterol minus HDL-C) and triglyceride (TG) could not be neglected for the prediction and treatment of CVDs, and even had many other compelling advantages over LDL-C and other traditional lipid parameters [5]. Currently, statin drugs are widely used in the prevention and treatment of CVDs by significantly lowering serum LDL-C levels; however, part of patients receiving statin therapy suffer from serious adverse events, such as muscle pain, myopathy and even fatal rhabdomyolysis [6]. It is undeniable that intensive-dose statin therapy is associated with an increased risk of new-onset diabetes [7]. Niacin, one of the oldest HDL-C-elevating drugs tested in clinical trials, has less benefit in patients at high risk of vascular events, and possesses adverse effects (cutaneous flushing and hyperglycemia) [8]. Therefore, the reasonable therapeutic strategy for patients with cardiovascular disease should effectively accommodate the balance between HDL-C and LDL-C, meanwhile positively ameliorate the other risk factors for atherosclerosis including total cholesterol (TC) and TG.

Cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein containing 476 amino acids, plays an unfavorable physiological action by transferring cholesteryl esters (CE) from HDL to very-low-density lipoprotein (VLDL) or LDL particles in exchange for TG, thereby

raising proatherogenic LDL-C and lowering atheroprotective HDL-C [9]. Accordingly, inhibition of CETP has been considered as a promising approach for the prevention and treatment of CVDs [10]. To date, a number of structurally distinct CETP inhibitors have been developed, and four of them entered phase III clinical trials [11]. (Fig. 1) Torcetrapib, as the first CETP inhibitor tested in a long-term outcomes clinical trial, discontinued in 2006 due to the elevation of blood pressure and aldosterone levels [12]. In 2015 Evacetrapib's phase III trials was terminated because of the lack of sufficiently clinical efficacy for patients with 'high-risk' coronary disease [13]. However, the Dal-GenE randomized trial is currently being conducted in patients with a recent acute coronary syndrome to evaluate the effects of Dalcetrapib on cardiovascular risk in a genetically defined population [14]. The large phase III clinical outcome trial of Anacetrapib is also ongoing to determine the safety and efficacy in patients with atherosclerotic cardiovascular diseases [14]. Recent research findings showed that Anacetrapib reduced progression of atherosclerosis by systematically reducing non-HDL-cholesterol, improved lesion stability and added to the beneficial effects of atorvastatin [15]. Nevertheless, almost all the reported CETP inhibitors exhibited highly lipophilic and poorly aqueous soluble characteristics [11], which might be an unfavorable factor for their discovery and clinical development [16]. Consequently, it is in urgent need to develop novel CETP inhibitors with different structural scaffolds to overcome the adverse effects and improve the efficacy.

<<**Fig.1** here>>

In order to find new structural CETP inhibitor candidates, a literature research was carried out to screen active components from natural Chinese herbal medicine. Pentacyclic triterpenes (PTs), a group of widespread natural products that synthesized by the cyclization of squalene [17], attracted our attention, that were observed to possess hypolipidemic activity more than thirty

years ago [18]. In China, the tablet of fine extract of hawthorn containing oleanolic acid (**1**) and ursolic acid (**3**) as the main triterpene ingredients has been marketed as an OTC drug for the treatment of hyperlipidemia. Many clinical studies had confirmed that there was a significant decrease in the serum TC, TG and LDL levels, and increase in HDL levels after treatment with the plant extracts containing various natural pentacyclic triterpene compounds [19]. However, the mechanism of hypolipidemic activity of PTs is still poorly understood. Given the similar structures of natural PTs with that of cholesterol and CE, it was proposed that PTs might exert hypolipidemic action through the regulation of cholesterol transport and metabolism [19].

Based on the above findings, we believed that PTs and their derivatives should be explored as novel CETP inhibitors for the treatment of dyslipidaemia. In fact, we have previously reported a new class of CETP inhibitors designed by linking the PTs scaffolds and the active fragment from Torcetrapib with the linker methylene chain [20]. In this manuscript, we first describe the design and synthesis of pentacyclic triterpene 3β-ester derivatives as novel CETP inhibitors, as well as structure-activity relationship (SAR) analysis, in vitro and in vivo biological testing, preliminary safety evaluation and pharmacokinetic studies.

## 2. Results and discussion

### *2.1. Design of pentacyclic triterpene 3β-ester derivatives as novel CETP inhibitors*

To design our compounds, oleanolic acid (**1**) was selected as a potential lead compound. As we have observed, **1** bears a hydroxyl group at C-3 position and a rigid pentacyclic skeleton with a large surface area of hydrophobic environment, which is quite similar with that of cholesterol. (Fig. 2) In addition, a carboxyl group at C-17 position of **1** might be a potential hydrogen bond site (HBS) that form interaction with CETP. In our previous studies, molecular docking of **1** into the active site of CETP confirmed that it almost occupied the binding position of the endogenous

ligand CE. Besides, hydrogen bond interactions between **1** and Ser191, Ser230 were observed [20]. (Fig. 2) Based on these findings, we hypothesized that PTs might be substrate analogue inhibitors of CETP, mimicking the protein-ligand interactions between the active site and CE scaffold. This could provide a reasonable explanation for the hypolipidemic effect of PTs.

<<**Fig.2** here>>

To better simulate the interactions between endogenous ligand CE and CETP, we proposed the strategy for molecular design of novel CETP inhibitors by linking the pentacyclic scaffold of **1** with some hydrophobic and flexible alkyl chains at C-3 position. Besides, some hydrophilic segments introduced into the another end of these alkyl chains could form crucial hydrogen bond interactions with several polar residues in the active site region of CETP, such as Gln-199, Ser-230, and His-232 [21]. (Fig. 3) In addition, the length of alkyl chains and different pentacyclic skeletons would also be investigated to point us toward the most promising candidates.

<<**Fig.3** here>>

*2.2. Chemistry*

Synthesis of the target compounds **7-11** is shown in Scheme 1. Esterification of the 3β-hydroxyl group of **1** with corresponding anhydrides in anhydrous pyridine gave the target products **7-9** in 52-90% yield. As described in our previous report [22], benzylation of **1** with benzyl bromide or 4-methoxy benzyl chloride in the presence of $K_2CO_3$ in DMF at room temperature, followed by condensation with *n*-nonylic acid or oleic acid in the present of EDCI and DMAP afforded the corresponding benzyl esters **33** and **34** in 81% and 68% yield over two steps, respectively. Debenzylation of **33** with hydrogen over palladium/carbon produced target compound **10**. According our previous reported method [23], compound **11** was obtained in 70%

yield by deprotection of the *p*-methoxybenzyl group of **34** with proton-exchanged montmorillonite (H-mont).

<<**Scheme 1** here>>

Actually, the new drug research and development of PTs for prevention and treatment of metabolic and vascular diseases has been an important part of our work, and a large amount of triterpenoid derivatives have been synthesized and formed a chemical compound library. Compounds **12-16** were prepared according to the procedures described in our previous studies [24]. Remarkably, heterocyclic, sulfonic and carboxyl group at the end of these compounds could form crucial hydrogen bond interactions with the active site region of CETP.

As shown in Scheme 2, esterification of the 3β-hydroxyl group of **1** separately with succinic anhydride, glutaric anhydride, and phthalic anhydride to furnish the corresponding target compounds **17**, **18** and **20** in 68-80% yield. Alternatively, benzylation of **1** with benzyl bromide, followed by condensation with adipic acid in presence of EDCI and DMAP, and then debenzylation with hydrogen over palladium/carbon in THF generated target product **19** in 65% yield over three steps. According to the above procedures, benzylation of **1** with benzyl bromide, followed by esterification separately with succinic anhydride and glutaric anhydride to give key intermediates **35** and **36**. Treatment of **35** and **36** with sulfinyl chloride in $CH_2Cl_2$, followed by amidation with separately ammonia, morpholidine, and 2-hydroxyethylamine, and further debenzylation over palladium/carbon gave the corresponding target products **21-26** in 32-94% yield over three steps.

<<**Scheme 2** here>>

Additionally, target compounds **27-32** were synthesized to investigate the roles of carboxyl group at C-17 position and different pentacyclic skeletons. As outlined in Scheme 3, compounds

**27** and **28** were synthesized by esterification of the 3β-hydroxyl group of β-amyrin (**5**) separately with succinic anhydride and glutaric anhydride. In similar fashions, target product **29** was synthesized from carboxylic acid **6**, whose preparation was described in our previous report [25]. As shown in Scheme 4, acylation of the 3β-hydroxyl groups of δ-oleanolic acid (**2**) [26], ursolic acid (**3**) and betulinic acid (**4**) using glutaric anhydride provided target compounds **30-32** containing three different pentacyclic skeletons. The details of the synthetic procedures and structural characterizations of target compounds are described in the Experimental Section.

<<**Scheme 3** here>>

<<**Scheme 4** here>>

*2.3. Screening assay of natural PTs and their 3β-ester derivatives as CETP inhibitors and SAR analysis.*

The inhibition of CETP mediated CE transfer for natural PTs **1-4** and all target compounds **7-32** were characterized in vitro using a fluorescence transfer assay with human recombinant CETP. The initial screening was carried out at a concentration of 10 μM for each compound, and compounds that displayed >30% inhibition at 10 μM were further evaluated for their $IC_{50}$s. The results are summarized in Table 1, and the details of the bioassay procedures are described in the Experimental Section. As shown in Table 1, thirteen compounds demonstrated moderate inhibitory activities, with >30% inhibition at a concentration of 10 μM for each compound. Three natural PTs such as oleanolic acid (**1**), δ-oleanolic acid (**2**) and betulinic acid (**4**) failed to show their inhibitory activity in vitro, but ursolic acid (**3**) displayed a moderate inhibitory potency against CETP with an $IC_{50}$ of 46.6 μM. It gives us great encouragement to explore a series of pentacyclic triterpene 3β-ester derivatives (see compounds **7-11**, as shown in Table 1 and Scheme 1), which better mimicking the structure of endogenous ligand CE. Unfortunately,

the introduction of these hydrophobic alkyl groups at C-3 position caused a loss in inhibitory activity, which suggested that these compounds with high lipid solubility might not be a potentially strong contender to CE in the active site region of CETP. However, the introduction of heterocyclic groups into the end of the alkyl chain of compound **8** afforded the new compounds **12-14**, which brought us some exciting surprises. Among them, compound **12** displayed a moderate inhibitory effect toward CETP with an $IC_{50}$ of 68.5 μM, but compounds **13** and **14** have hardly any inhibitory potency. Additionally, compound **15** with sulfonic group in the end exhibited $IC_{50}$ of 23.1 μM, while succinic acid ester **17** displayed an excellent inhibitory effect toward CETP ($IC_{50}$ = 4.3 μM), which was nearly 6-fold better than that of compound **15**. However, malic acid ester **16** showed no inhibitory activity against CETP, since the stable intramolecular hydrogen bond between the carboxyl group and hydroxyl group disturbed its binding to the active site region. This unexpected result indicates that the significant impact of hydrophilic segments in the end of these alkyl chains on the potency.

On the basis of above results, we held the carboxyl groups at opposite ends on the molecular scaffolds of compounds **18-20**, which all displayed an excellent CETP inhibitory activity. Among them, phthalic acid ester **20** exhibited the most potent CETP inhibitory activity; its $IC_{50}$ reached 2.3 μM. Remarkably, glutaric acid ester **18** exhibited $IC_{50}$ of 3.5 μM, while adipic acid ester **19** showed a faint loss in inhibitory potency against CETP, with an $IC_{50}$ of 7.0 μM. This indicates the significant impact of the carbon chain length on the potency. To further investigate the importance of carboxyl group, we attempted to introduce several amides to the end of the carbon chain of compounds **17** and **18**, which caused a complete loss in inhibitory activity (see compounds **21-26**, as shown in Table 1 and Scheme 2). Besides, we further substituted the carboxyl group from C-17 position of compounds **17** and **18** with methyl group to form

compounds **27** and **28**, which almost completely ablated their activities. However, compound **29** with a carboxylmethoxycarbonyl group at C-17 position had a moderate inhibitory potency, and its $IC_{50}$ value was 38.9 μM. The above results suggest that carboxyl groups at opposite ends on the molecular scaffold are both important to maintain the inhibitory potency of these compounds toward CETP.

Based on the above findings, we also synthesized compounds **30**-**32** with different pentacyclic skeletons, and further evaluated their biological activities toward CETP. As shown in Table 1, a decrease in CETP inhibitory activity was observed with the bioassay of olean-13(18)-ene-type derivative **30** and lupane-type derivative **32**, which exhibited $IC_{50}$ of 10.3 μM and 38.0 μM, respectively. However, ursane-type derivative **31** exhibited excellent CETP inhibitory activity, with an $IC_{50}$ of 3.4 μM, which was as well as that of the corresponding oleanane-type derivative **18**. These data show that the different pentacyclic scaffolds have an important influence on the CETP inhibitory activities of these compounds; oleanane-type and ursane-type scaffolds might be more suitable to fit the active pocket of CETP than other pentacyclic scaffolds. Consequently, according to the preliminarily SAR analysis, compound **20** was identified as a potent CETP inhibitor for an in-depth study in vivo.

<<**Table 1** here>>

*2.4. In vivo studies of compound **20**.*

Compound **20** was first tested in human adipose tissue specific CETP transgenic (ap2-CETPTg) mice pharmacodynamic model. Adipose tissue of ap2-CETPTg mice has a high level of CETP, which secrets into the blood circulation, reduces HDL-C, and increases non-HDL-C significantly; the lipid profile is very similar with lipid metabolism of type 2 diabetic [27]. Compound **20** was dosed in ap2-CETPTg mice at 30 mg/kg, once-a-day (q.d.); plasma samples

were obtained at 2 h after dosing on day 14, and then HDL-C, LDL-C, TG, and TC levels were measured. As shown in Fig. 4, compound **20** increased HDL-C level by 11.1% and decrease LDL-C level by 34.2% versus control, while an increase of 51.0% in HDL-C and a decrease of 40.4% in LDL-C were observed after dosing with Anacetrapib. Although compound **20** showed a slightly weaker activity in raising HDL-C and lowering LDL-C than Anacetrapib, it could effectively reduce TG and TC levels by 29.3% and 5.8%, respectively. Quite unexpectedly, 4.7% increase in TG level and 17.8% increase in TC level were observed after dosing of Anacetrapib for two weeks in ap2-CETPTg mice, which is worthy of further investigation.

<<**Fig.4** here>>

In view of reasonably regulation of lipid profile in CETP transgenic mice, we further evaluated compound **20** in a species that naturally expresses CETP. Since guinea pigs (unlike mice and rats) express CETP, and the lipid metabolism of which has some similarities to that of human, compound **20** was next tested in the normal fed and high-fat fed guinea pig models. As depicted in Fig. 5, compound **20** produced elevation in HDL-C of approximately 32.3% ($P < 0.05$, compared with control) and reduction in LDL-C of 19.8% in normal fed guinea pigs after dosing 30 mg/kg (q. d.) for two weeks. In our further studies, high-fat fed guinea pig models have a higher level of LDL-C, TG and TC, and a lower level of HDL-C than the control guinea pigs. (Fig. 6) An increase of 72.8% in HDL-C and a decrease of 40.8% in LDL-C versus model were observed after dosing with compound **20**, while Anacetrapib increased HDL-C level by 82.2% and decreased LDL-C level by 30.2%. (Fig. 6A and 6B) Much to our satisfaction, compound **20** could also reduce TG and TC levels by 27.3% and 20.3% compared with model, respectively. (Fig. 6C and 6D)

<<**Fig.5** here>>

<<**Fig.6** here>>

According the above results, compound **20** showed similar effects on modulating lipid profile as Anacetrapib in the normal fed and high-fat fed guinea pig models, in spite of its moderate inhibiting human CETP activity in vitro. However, one thing that needs to be addressed is that, natural PTs are often mild and sometimes even weak modulators for their targets, which can interact with multiple target proteins in lipid metabolism pathways [19]. For example, oleanolic acid (**1**) showed mild ACAT inhibitory activity ($IC_{50}$ = 77.9 μM) [28] and weak PL inhibitory activity ($IC_{50}$ = 83 μg/mL) [29]. Therefore, compound **20** with a natural product scaffold may be a multi-site modulator of lipid metabolism at a holistic level. To better explain its hypolipidemic effects, we would investigate the interaction of compound **20** with some other target proteins in the lipid metabolism pathways, such as ACAT, PPARs, PLs, FXR and LXR, etc. In our opinion, the mild interaction of compound **20** with these related target proteins may be the key to systematically modulate lipid metabolism.

*2.5. Safety evaluation of compound **20**.*

In view of excellent biological activities of compound **20**, we further evaluated its effects on the systolic blood pressure in normal fed and high-fat fed guinea pig models. As shown in Fig. 7A and 7B, the systolic blood pressure of normal fed and high-fat fed guinea pigs had hardly any change versus control and model after dosing with 30 mg/kg (q. d.) compound **20** for two weeks. In addition, a comparison of physiochemical property profiles of compound **20** and four typical CETP inhibitors are shown in Table 2. To our satisfaction, compound **20** with two carboxyl groups exhibited better aqueous solubility, with a partition coefficient of 0.49, which might be an advantage for drug absorption and drug safety over the existing CETP inhibitors.

<<**Fig.7** here>>

<<**Table 2** here>>

*2.6. Pharmacokinetic evaluation of compound **20**.*

Compound **20** was further evaluated for its preliminary pharmacokinetic profile in normal fed guinea pig models, compared with **1** at the same oral dose, and the relevant pharmacokinetic parameters are listed in Table 3. The results showed that compound **20** had a good pharmacokinetic profile, with an $AUC_{0-48}$ (area under the plasma concentration-time curve from 0 to 48 hours) of 9337.83 ± 2152.23 ng/mL*h and a good half-life ($t_{1/2}$ = 18.75 ± 5.15 h). After oral administration of compound **20** at 30 mg/kg dose, plasma concentrations reached its peak ($C_{max}$ = 1266.46 ± 406.61 ng/mL) at 1.46 ± 0.40 h. It is noteworthy that only a very small amount of compound **20** metabolized into **1** in normal fed guinea pigs. (Fig. 8) On the basis of its favorable in vitro and in vivo properties, compound **20** was selected as a candidate for further development.

<<**Table 3** here>>

<<**Fig.8** here>>

## 3. Conclusions

In the present study, a series of pentacyclic triterpene 3β-ester derivatives were designed and synthesized, and their inhibitory activities in vitro were evaluated using a fluorescence transfer assay with human recombinant CETP. Among them, five compounds displayed moderate inhibitory activities against CETP, with $IC_{50}$s < 10 μM. Compound **20** showed the best biological activity, with an $IC_{50}$ of 2.3 μM. The preliminary SAR analysis indicated that hydrophilic segments at opposite ends on the molecular scaffold are indispensable to maintain the CETP inhibitory potency of these compounds, and different pentacyclic skeletons play a key role for the inhibitory activities. Further studies in vivo showed that compound **20** provided an 11.1%

increase in HDL-C levels and a 34.2% decrease in LDL-C levels versus control in ap2-CETPTg mice. Remarkably, compound **20** could effectively reduce TG and TC levels by 29.3% and 5.8%, which was superior to the positive control drug Anacetrapib. Furthermore, compound **20** also showed robust potential to regulate the lipid profile both in the normal fed and high-fat fed guinea pig models, which is very comparable to Anacetrapib. Additionally, no significant changes on the systolic blood pressure in normal fed and high-fat fed guinea pigs when compound **20** was administered orally for two weeks. Preliminary pharmacokinetics studies in guinea pigs indicated that compound **20** has a good pharmacokinetic profile. In summary, the studies described herein demonstrate that these pentacyclic triterpene 3β-ester derivatives with hydrophilic segments at opposite ends on the molecular scaffold, especially compound **20**, have promising potential as novel generation of CETP inhibitors for the treatment of dyslipidemia.

## 4. Experiments

### *4.1. Chemistry*

All commercially available solvents and reagents were used without further purification. Reactions were monitored by TLC on Silica Gel 60 F254 plates (Qingdao Ocean Chemical Company, China). Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Ocean Chemical Company, China). $^{1}H$ and $^{13}C$ NMR spectra were recorded on an ACF* 300 Q Bruker or ACF* 500 Q Bruker spectrometer in $CDCl_3$, or in DMSO-$d_6$ with $Me_4Si$ as the internal reference. Low- and high- resolution mass spectra (LRMS and HRMS) were recorded in electron impact mode. Chemical shifts were reported in parts per million (ppm). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br). In addition, compound **20** was confirmed with over 98% purity, which was determined by Agilent 1260 with binary pump, photodiode array detector, using Agilent Extend-

C18 column (0.46 cm × 15 cm, 5 μm), $CH_3OH/H_2O$ = 80/20 (v/v) at 1.0 mL/min, and calculated the peak areas at 254 nM.

*4.1.1. General procedure for the synthesis of compounds **7**-**9**, **17**, **18** and **20**.*

To a solution of **1** (100 mg, 0.219 mmol) dissolved in dry pyridine (3 mL) was added anhydrides (0.876 mmol) and DMAP (27 mg, 0.219 mmol). The reaction mixture was stirred at room temperature (or heated to reflux) until the TLC indicated the consumption of starting material, and then concentrated in vacuo to dryness. Then water (10 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography ($SiO_2$, petroleum ether/ethyl acetate 30 : 1 to 5 : 1) to give the desirable product.

*4.1.1.1. 3β-Acetyloxy-olean-12-en-28-oic acid (**7**)* [30]. Compound **7** (98 mg, 90%) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.28 (s, 1H), 4.56-4.42 (m, 1H), 2.82 (dd, $J$ = 13.3, 2.9 Hz, 1H), 2.04 (s, 3H), 1.99-0.65 (m, 22H), 1.13 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H), 0.85(s, 3H), 0.75 (s, 3H); ESI-MS *m*/*z*: 521.3 $[M + Na]^+$.

*4.1.1.2. 3β-Propionyloxy-olean-12-en-28-oic acid (**8**)* [30]. Compound **8** (92 mg, 79%) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.27 (s, 1H), 4.58-4.40 (m, 1H), 2.81 (d, $J$ = 13.5 Hz, 1H), 2.32 (q, $J$ = 7.5 Hz, 2H), 2.06-0.70 (m, 25H), 1.13 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.85 (s, 6H), 0.75 (s, 3H); ESI-MS *m*/*z*: 511.4 $[M - H]^-$.

*4.1.1.3. 3β-Butyryloxy-olean-12-en-28-oic acid (**9**)* [30]. Compound **9** (60 mg, 52%) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.28 (s, 1H), 4.57-4.43 (m, 1H), 2.81 (d, $J$ = 12.9 Hz, 1H), 2.28 (t, $J$ = 7.3 Hz, 2H), 2.06-0.65 (m, 27H), 1.13 (s, 3H), 0.93 (s, 6H), 0.90 (s, 3H), 0.85 (s, 6H), 0.75 (s, 3H); ESI-MS *m*/*z*: 525.4 $[M - H]^-$.

*4.1.1.4. 3β-[3-(Carboxyl)propionyloxy]-olean-12-en-28-oic acid (**17**)* [30]. Compound **17** (98 mg, 80%) was obtained as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 5.29 (s, 1H), 4.56 (t, $J$ = 7.8 Hz, 1H), 2.90-2.79 (m, 1H), 2.78-2.53 (m, 4H), 2.00-0.70 (m, 22H), 1.13 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H); ESI-MS *m/z*: 579.3 $[M + Na]^+$.

*4.1.1.5. 3β-[(4-Carboxyl)butyryloxy]-olean-12-en-28-oic acid (**18**)* [30]. Compound **18** (85 mg, 68%) was obtained as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.09 (brs, 1H), 5.13 (s, 1H), 4.39-4.36 (m, 1H), 2.73-2.69 (m, 1H), 2.29-2.27 (m, 2H), 2.21 (t, $J$ = 6.93, 2H), 2.00-0.70 (m, 24H), 1.08 (s, 3H), 0.84 (s, 9H), 0.78 (s, 6H), 0.69 (s, 3H); ESI-MS *m/z*: 569.4 $[M - H]^-$.

*4.1.1.6. 3β-[(2-Carboxyphenyl)carbonyloxy]-olean-12-en-28-oic acid (**20**)* [30]. Compound **20** (99 mg, 75%) was obtained as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.70-7.67 (m, 1H), 7.60-7.57 (m, 3H), 5.15 (s, 1H), 4.63-4.57 (m, 1H), 2.74-2.71 (m, 1H), 2.00-0.70 (m, 22H), 1.10 (s, 3H), 0.88 (s, 6H), 0.85 (s, 6H), 0.80 (s, 3H), 0.71 (s, 3H); ESI-MS *m/z*: 627.3 $[M + Na]^+$.

*4.1.2. 3-[(2-Carboxyethyl)carbonyloxy]-β-amyrin (**27**)* [31].

Compound **27** (98 mg, 79%) was prepared from **5** as a white solid following the similar procedure carried out for compound **17**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.17 (s, 1H), 4.53-4.51 (m, 1H), 2.68-2.62 (m, 4H), 1.97-0.70 (m, 23H), 1.12 (s, 3H), 0.95 (s, 6H), 0.86 (s, 9H), 0.82 (s, 6H); ESI-MS *m/z*: 549.4 $[M + Na]^+$.

*4.1.3. 3-[(3-Carboxypropyl)carbonyloxy]-β-amyrin (**28**)* [31].

Compound **28** (102 mg, 81%) was prepared from **5** as a white solid following the similar procedure carried out for compound **18**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.20 (s, 1H), 4.66-4.45 (m, 1H), 2.48-2.38 (m, 4H), 2.09-0.80 (m, 25H), 1.15 (s, 3H), 0.99 (s, 6H), 0.89 (s, 12H), 0.85 (s, 3H); ESI-MS *m/z*: 539.4 $[M - H]^-$.

*4.1.4. Carboxymethyl 3β-[(4-carboxy)butyryloxy]-olean-12-en-28-oate (**29**).*

Compound **29** (14 mg, 11%) was prepared from **6** as a white solid following the similar procedure carried out for compound **18**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.26 (s, 1H), 4.62-4.47 (m, 2H), 4.47-4.45 (m, 1H), 2.91-2.87 (m, 1H), 2.41-2.32 (m, 4H), 2.15-0.70 (m, 24H), 1.18 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H); ESI-MS *m/z*: 674.4 $[M + 2Na]^{2+}$.

*4.1.5. 3β-[(4-Carboxyl)butyryloxy]-olean-13(18)-en-28-oic acid (**30**).*

Compound **30** (107 mg, 86%) was prepared from **2** as a white solid following the similar procedure carried out for compound **18**. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.08 (brs, 1H), 4.43 (dd, *J* = 11.8, 5.1 Hz, 1H), 2.70 (d, *J* = 15.2 Hz, 1H), 2.37-2.29 (m, 2H), 2.30-2.17 (m, 4H), 2.08-2.03 (m, 1H), 1.84-0.7 (m, 22H), 1.14 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H), 0.71 (s, 3H); ESI-MS *m/z*: 569.3 $[M - H]^-$.

*4.1.6. 3β-[(4-Carboxyl)butyryloxy]-urs-12-en-28-oic acid (**31**)* [32].

Compound **31** (106 mg, 85%) was prepared from **3** as a white solid following the similar procedure carried out for compound **18**. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.02 (brs, 1H), 5.13 (s, 1H), 4.45-4.39 (m, 1H), 2.35-2.30 (m, 1H), 2.26-2.21 (m, 4H), 2.11 (d, *J* = 11.1 Hz, 1H), 1.98-0.68 (m, 23H), 1.06 (s, 3H), 0.91 (s, 6H), 0.82 (s, 9H), 0.76 (s, 3H); ESI-MS *m/z*: 569.4 $[M - H]^-$.

*4.1.7. 3β-[(4-Carboxyl)butyryloxy]-lup-20(29)-en-28-oic acid (**32**)* [33].

Compound **32** (101 mg, 82%) was prepared from **4** as a white solid following the similar procedure carried out for compound **18**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.73 (s, 1H), 4.61 (s, 1H), 4.51-4.46 (m, 1H), 3.03-2.96 (m, 1H), 2.46-2.39 (m, 4H), 2.29-0.78 (m, 26H), 1.69 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.85 (s, 6H), 0.82 (s, 3H); ESI-MS *m/z*: 569.3 $[M - H]^-$.

*4.1.8. 3β-Nonanoyloxy-olean-12-en-28-oic acid (**10**).*

As described in our previous report [22], benzylation of **1** with benzyl bromide afforded the key intermediate benzyl ester derivative. To a solution of this benzyl ester (200 mg, 0.366 mmol) dissolved in $CH_2Cl_2$ (10 mL), was added *n*-nonylic acid (70 mg, 0.439 mmol), EDCI (140 mg, 0.732 mmol) and DMAP (45 mg, 0.366 mmol). The reaction mixture was stirred at 40 $^oC$ until the TLC indicated the consumption of starting material. Then water (20 mL) was added to the reaction mixture and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography ($SiO_2$, petroleum ether/ethyl acetate 100 : 1) to give compound **33** (203 mg, 81% for two steps) as a colorless oil.

To a solution of **33** (200 mg, 0.291 mmol) was dissolved in THF (5 mL) and treated with 10% Pd/C (20 mg). The mixture was stirred at room temperature under $H_2$ atmospheric pressure until the TLC indicated the consumption of starting material. The reaction mixture was filtered through celite and the insoluble substance was washed with THF. The filtrate was concentrated in vacuo to give **10** (121 mg, 70%) as a white solid. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 5.27 (s, 1H), 4.59-4.41 (m, 1H), 2.82 (d, $J$ = 10.9 Hz, 1H), 2.29 (t, $J$ = 7.2 Hz, 2H), 2.01-0.65 (m, 37H), 1.13 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.85 (s, 6H), 0.74 (s, 3H); ESI-MS *m*/*z*: 595.5 $[M - H]^-$.

*4.1.9. 3β-Oleoyloxy-olean-12-en-28-oic acid (**11**).*

Following the similar procedure carried out for compound **33**, benzylation of **1** with 4-methoxybenzylchloride, and then condensation with oleic acid in the presence of EDCI and DMAP, gave key intermediate **34** (208 mg, 68% for two steps) as a colorless oil. To a solution of **34** (90 mg, 0.107 mmol) dissolved in $CH_2Cl_2$ (3 mL) was added H-mont (40 mg) and anisol (1

mL). The mixture was stirred at room temperature until the TLC indicated the consumption of starting material. The reaction mixture was filtered to remove H-mont. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography ($SiO_2$, petroleum ether/ethyl acetate 100 : 1) to give **11** (50 mg, 70%) as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.37 (s, 2H), 5.31 (s, 1H), 4.58-4.44 (m, 1H), 2.85 (d, $J$ = 13.4 Hz, 1H), 2.32 (t, $J$ = 7.3 Hz, 2H), 2.12-0.70 (m, 51H), 1.16 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.91 (s. 3H), 0.89 (s, 6H), 0.79 (s, 3H); ESI-MS *m*/*z*: 719.6 $[M - H]^-$.

*4.1.10. 3β-[(5-Carboxy)valeryloxy]-olean-12-en-28-oic acid (**19**)* [34].

Following the similar procedure carried out for compound **33**, benzylation of **1** with benzyl bromide, and then condensation with adipic acid in the presence of EDCI and DMAP, gave key intermediate **37** (247 mg, 72% for two steps) as a colorless oil. Following the procedure described for preparation of **10**, compound **19** (156 mg, 90%) was prepared from **37** as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 12.01 (brs, 1H), 5.16 (s, 1H), 4.51-4.33 (m, 1H), 2.77-2.72 (m, 1H), 2.31-2.27 (m, 2H), 2.23-2.19 (m, 2H), 1.99-0.70 (m, 26H), 1.11 (s, 3H), 0.89 (s, 3H), 0.87 (s, 6H), 0.81 (s, 6H), 0.72 (s, 3H); ESI-MS *m*/*z*: 583.4 $[M - H]^-$.

*4.1.11. General Procedure for the Synthesis of compounds **21-26**.*

As described in our previous report [24], benzylation of **1** with benzyl bromide, followed by acylation with succinic anhydride and glutaric anhydride respectively, gave key intermediate **35** and **36** in a good yield. To a solution of **35** (100 mg, 0.155 mmol) dissolved in $CH_2Cl_2$ (5 mL) was added thionyl chloride (1 mL). The reaction mixture was refluxed for 4 h, and then concentrated in vacuo to dryness. The residue was dissolved in $CH_2Cl_2$ (3 mL). Amine (0.155 mmol) and $Et_3N$ (0.186 mmol) were added into the reaction mixture, and then stirred at room temperature until the TLC indicated the consumption of starting material. Water (20 mL) was

added to the reaction mixture and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was dissolved in THF (5 mL) and treated with 10% Pd/C (20 mg). The mixture was stirred at room temperature under $H_2$ atmospheric pressure until the TLC indicated the consumption of the starting material. The reaction mixture was filtered through celite and the insoluble substance was washed with THF. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography ($SiO_2$, petroleum ether/ethyl acetate 3 : 1) to give desirable products **21-23**. Following the similar procedure carried out for **21-23**, Compounds **24-26** were prepared from key intermediate **36**.

*4.1.11.1. 3β-(4-Amino-4-oxobutyryloxy)-olean-12-en-28-oic acid (**21**).* Compound **21** (93 mg, 94% for two steps) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.25 (s, 1H), 5.73 (s, 1H), 5.26 (s, 1H), 4.55-4.49 (m, 1H), 2.84-2.78 (m, 1H), 2.68-2.64 (m, 2H), 2.54-2.49 (m, 2H), 2.06-0.70 (m, 22H), 1.12 (s, 3H), 0.92 (s, 6H), 0.90 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.75 (s, 3H); ESI-MS *m*/*z*: 668.5 $[M + Na]^+$.

*4.1.11.2. 3β-(4-Morpholino-4-oxobutyryloxy)-olean-12-en-28-oic acid (**22**).* Compound **22** (57 mg, 59% for two steps) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.27 (s, 1H), 4.53-4.48 (m, 1H), 3.70-3.65 (s, 4H), 3.63-3.60 (s, 2H), 3.50-3.48 (s, 2H), 2.85-2.79 (m, 1H), 2.68-2.61 (m, 4H), 2.04-0.70 (m, 22H), 1.13 (s, 3H), 0.93 (s, 6H), 0.90 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.75 (s, 3H); ESI-MS *m*/*z*: 624.5 $[M - H]^-$.

*4.1.11.3. 3β-{4-[(2-Hydroxyethyl)amino]-4-oxobutyryloxy}-olean-12-en-28-oic acid (**23**).* Compound **23** (40 mg, 43% for two steps) was obtained as a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.18 (s, 1H), 5.27 (s, 1H), 4.54-4.48 (m, 1H), 3.72-3.68 (m, 2H), 3.43-3.39 (m, 2H),

2.85-2.78 (m, 1H), 2.69 (t, *J* = 6.2 Hz, 2H), 2.49 (t, *J* = 6.6 Hz, 2H), 2.04-0.70 (m, 22H), 1.12 (s, 3H), 0.93 (s, 6H), 0.90 (s, 3H), 0.84 (s, 6H), 0.74 (s, 3H); ESI-MS *m*/*z*: 622.4 $[M + Na]^+$.

*4.1.11.4. 3β-(5-Amino-5-oxovaleryloxy)-olean-12-en-28-oic acid (**24**).* Compound **24** (33 mg, 38% for two steps) was obtained as a light yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.18 (brs, 1H), 5.53 (brs, 1H), 5.28 (s, 1H), 4.54-4.49 (m, 1H), 2.86-2.80 (m, 1H), 2.43-2.38 (m, 2H), 2.32-2.27 (m, 2H), 2.31-0.70 (m, 24H), 1.14 (s, 3H), 0.94 (s, 6H), 0.91 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.76 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 183.1, 175.7, 172.8, 143.8, 122.3, 81.0, 55.2, 47.5, 46.5, 45.9, 41.6, 41.0, 39.3, 38.0, 37.7, 37.0, 34.8, 33.8, 33.7, 33.1, 32.5, 32.4, 30.7, 29.7, 28.1, 27.7, 25.9, 23.6, 23.4, 22.9, 20.8, 18.2, 17.0, 16.8, 15.4; ESI-MS *m*/*z*: 568.4 $[M - H]^-$; HRMS calcd for $C_{35}H_{54}NO_5$ $[M - H]^-$: 568.4002, found: 568.4015.

*4.1.11.5. 3β-(5-Morpholino-5-oxovaleryloxy)-olean-12-en-28-oic acid (**25**).* Compound **25** (35 mg, 32% for two steps) was obtained as a light yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.28 (s, 1H), 4.57-4.40 (m, 1H), 3.72-3.64 (m, 4H), 3.63-3.56 (m, 2H), 3.52-3.42 (m, 2H), 2.92-2.77 (m, 1H), 2.43-2.36 (m, 4H), 2.20-0.70 (m, 24H), 1.14 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.76 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 183.1, 173.1, 171.0, 143.6, 122.5, 81.0, 66.9, 66.7, 55.3, 47.5, 46.5, 46.0, 45.8, 41.9, 41.6, 41.0, 39.3, 38.0, 37.7, 37.0, 33.8, 33.0, 32.5, 32.4, 32.1, 30.6, 29.7, 28.1, 27.7, 25.9, 23.6, 23.4, 22.9, 20.6, 18.2, 17.1, 16.7, 15.4; ESI-MS *m*/*z*: 638.4 $[M - H]^-$; HRMS calcd for $C_{39}H_{60}NO_6$ $[M - H]^-$: 638.4421, found: 638.4438.

*4.1.11.6. 3β-{5-[(2-Hydroxyethyl)amino]-5-oxovaleryloxy}-olean-12-en-28-oic acid (**26**).* Compound **26** (46 mg, 43% for two steps) was obtained as a light yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.08 (brs, 1H), 5.29 (s, 1H), 4.55-4.50 (m, 1H), 3.75-3.72 (m, 2H), 3.46-3.39 (m, 2H), 2.89-2.79 (m, 1H), 2.40 (t, *J* = 6.9 Hz, 2H), 2.32-2.23 (m, 2H), 2.12-0.70 (m, 24H), 1.15 (s,

3H), 0.95 (s, 6H), 0.92 (s, 3H), 0.87 (s, 6H), 0.77 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 183.5, 173.5, 173.2, 143.7, 122.5, 81.1, 62.2, 55.3, 47.5, 46.5, 45.8, 42.4, 41.5, 40.9, 39.3, 38.0, 37.7, 37.0, 35.5, 33.8, 33.0, 32.5, 30.6, 29.7, 28.1, 27.7, 25.9, 23.6, 23.4, 22.8, 21.1, 18.2, 17.2, 16.7, 15.4; ESI-MS *m/z*: 612.4 $[M - H]^-$; HRMS calcd for $C_{37}H_{58}NO_6$ $[M - H]^-$: 612.4264, found: 612.4278.

*4.2. CETP inhibitory assay in vitro.*

The CETP inhibitory bioactivities of natural PTs and their 3β-ester derivatives were determined by using a standard fluorescent-CE transfer assay with CETP activity assay kit (Roar Biomedical Inc.) and human recombinant CETP (Roar Biomedical Inc.). Assay system was prepared according the instruction for the CETP activity assay kit and human recombinant CETP. The assay uses synthetic HDL donor particles that contain a fluorescent CE with an excitation maximum at 465 nm and emission maximum at 535 nm. As the fluorescent CE is transferred from a donor molecule to an acceptor molecule by human recombinant CETP, fluorescence is observed and quantified. Inhibition of CETP mediated CE transfer is characterized by a decrease in levels of fluorescence observed relative to control. Anacetrapib was used as the positive control drug.

The assay procedure can be described briefly as follows. All testing compounds were totally dissolved in 100% DMSO and stored at nitrogen cabinet. Reconstitute the 80 μg/mL human recombinant CETP in total protein with assay buffer and dilute the stocking compounds with DMSO. A solution that contains no human recombinant CETP was as background and that contains human recombinant CETP but no testing compounds as the positive control. Donor molecule (4 mL), acceptor molecule (4 mL) and testing compound (1 mL) with human recombinant CETP (30 ng) were mixed in assay buffer (200 mL). After incubation at 37 $^{o}$C for 3

h, fluorescence intensity was read in a fluorimeter (Flex Station III) and the inhibition ratio was also calculated. The initial screening was carried out at a concentration of 10 μM for each testing compound. Compounds that displayed >30% inhibition at 10 μM were diluted with DMSO for 8 points titration (1 : 5 serial dilutions) in 96-well dilution plate, and the inhibition ratios were also calculated according to the above methods. $IC_{50}$ values of these compounds were determined from a curve fit of the data with each concentration tested. The curve was fitted using "Sigmoidal dose-response (variable slope)" in GraphPad Prism software.

*4.3. In vivo sudies of compound **20** in animal models.*

For the transgenic mice pharmacodynamic assay, ap2-CETPTg mice expressing human CETP were used. Blood samples were collected by retro-orbital bleed. Compound **20** was formulated in DMSO/cremophor/saline at a 3 : 4 : 93 ratio and oral dosing at 30 mg/kg, once-a-day, for two weeks. Blood was drawn 2 h postdose on day 14. HDL-C, LDL-C, TG and TC levels in ap2-CETPTg mice were measured using an automated lipid analyzer (COBAS), which were reported as the mean ± SD ($n \geq 6$), and compared to the values measured in control group. Anacetrapib was used as the positive control drug.

For the normal fed guinea pig study, the male guinea pigs (12 weeks of age) were used. Blood samples were collected by retro-orbital bleed. The male guinea pigs were dosed with compound **20** by gavage, 30 mg/kg, once-a-day, for two weeks, with normal diet simultaneously. At 2 h after dosing on day 14, plasma samples were collected. HDL-C and LDL-C levels in normal fed guinea pigs were measured, which were reported as the mean ± SD ($n \geq 6$) and compared to the values measured in control group. Anacetrapib was used as the positive control drug.

For the high-fat fed guinea pig study, the male guinea pigs (12 weeks of age) were used and initially fed a high fat diet (2.5% coconut oil and 0.25% cholesterol) for two weeks. Blood

samples were collected by retro-orbital bleed. Then, the high-fat fed guinea pigs were dosed with compound **20** by gavage, 30 mg/kg, once-a-day, for two weeks, with high-fat diet simultaneously. At 2 h after dosing on day 14, plasma samples were obtained. HDL-C, LDL-C, TG and TC levels in high-fat fed guinea pigs were measured, which were reported as the mean ± SD ($n \geq 6$), and compared to the values measured in model group. Anacetrapib was used as the positive control drug.

For the pharmacokinetic evaluation of compound **20**, the male guinea pigs (12 weeks of age) were used. Compound **20** dissolved in DMSO/cremophor/saline at a 3 : 4 : 93 ratio was administered to normal fed guinea pigs at dose of 30 mg/kg (n = 6) by oral gavages. Blood samples (300 μL) were collected before and after administration by retro-orbital bleed at 0.25 (at the end of oral gavage), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 36 and 48 h after drug administration. After centrifugation of blood samples, 100 μL aliquots of plasma samples were collected. After adding 10 μL internal standard (I.S.) glycyrrhetic acid (100 ng/mL), the samples were extracted with 1 mL of ethyl acetate and centrifuged at 4000 r/min for 10 minutes. The organic layer (900 μL) was transferred and evaporated to the dryness by SPD 2010 vacuum concentrator (Thermo Electron Corporation, MA, USA). The residues were dissolved by methanol and then injected into the LC-MS/MS system. The LC-MS/MS was performed by triple quadrupole system TSQ Quantum Ultra (Thermo Scientific, MA, USA). Analyses were carried out using electrospray ionization (ESI) in negative-ion mode using selective reactions monitoring (SRM): *m/z* 603.3→453.2 for compound **20**, *m/z* 455.4→407.3 for **1** and *m/z* 469.3→425.3 for I.S.. Separation of the test compounds was achieved using a 150 mm × 2.1 mm i.d., 3.5 μm, Agilent ZORBAX Elipse Plus C8 column. The mobile phase composition was (A) water with 1 mM ammonium acetate and (B) methanol. The column temperature was maintained at 35 °C. Mobile

phase B maintained to 15% for 1 minute and then increased to 95% in half a minute. Over next 6.5 minutes, mobile phase B went to 100% smoothly. At 8.01 minute, mobile phase B was decreased to 15% rapidly and final equilibration held for 2 minutes.

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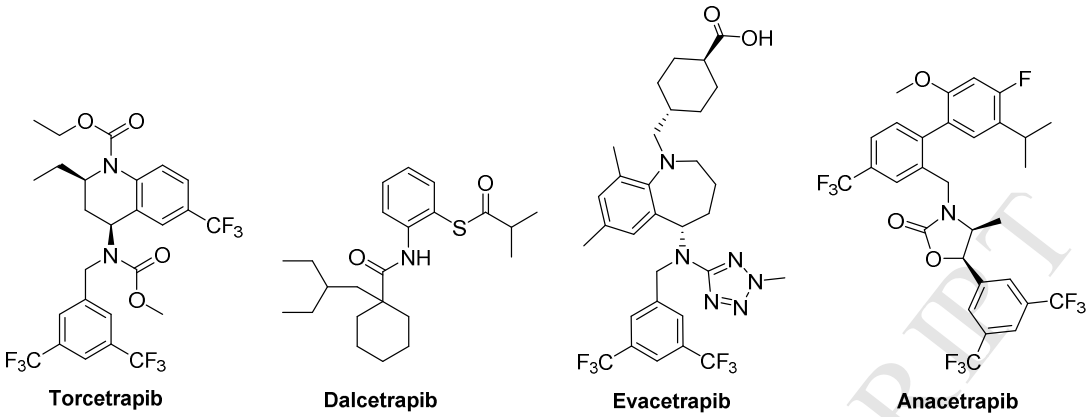



**Fig.1.** Four typical CETP inhibitors having reached phase III clinical trials.

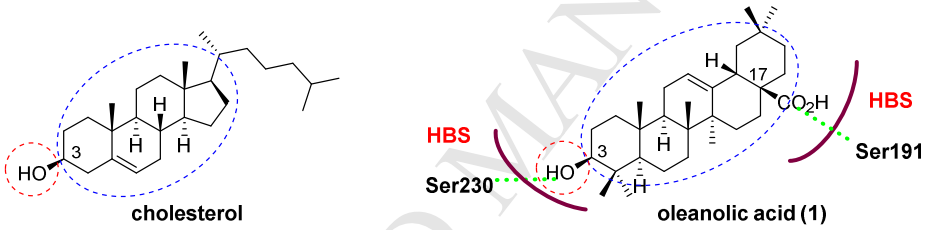



**Fig. 2.** Similar structural characteristics of cholesterol and oleanolic acid (**1**). The possible hydrogen bond interactions between **1** and the active site of CETP.

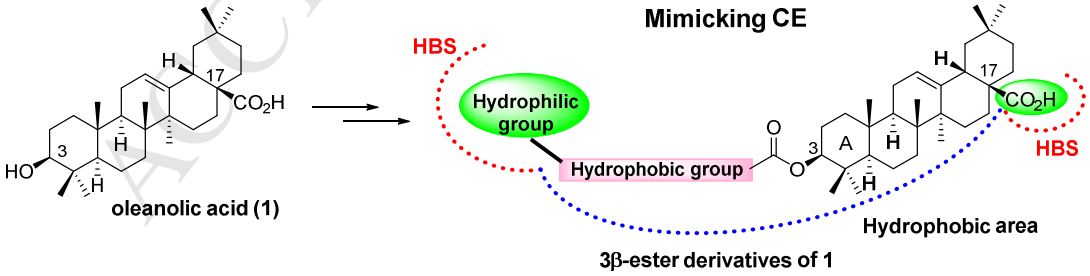
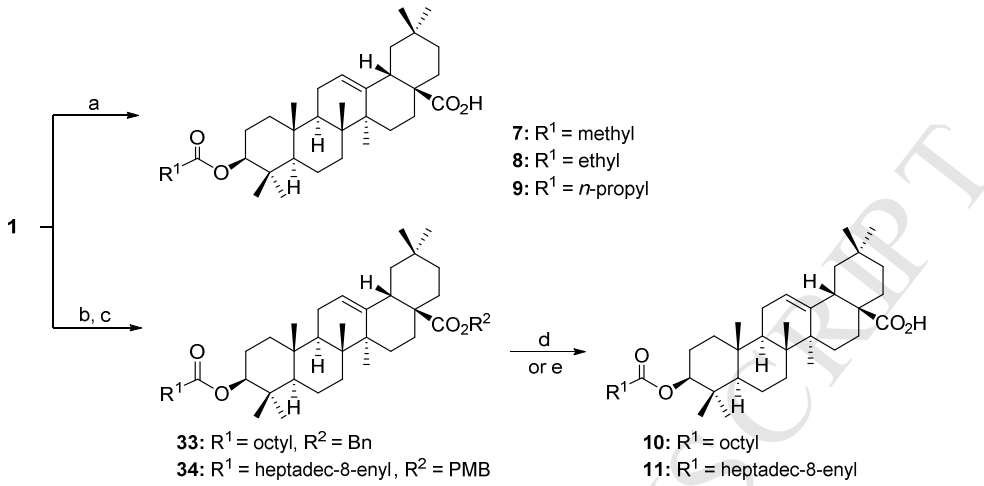


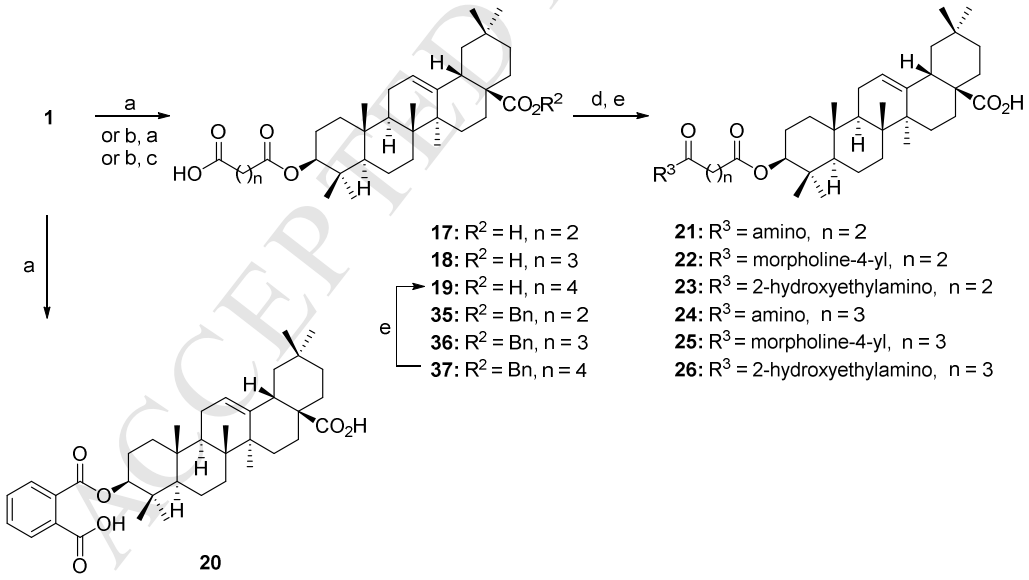

**Fig. 3.** Design of 3β-ester derivatives of **1** as a new class of CETP inhibitors.

**Scheme 1.** Synthesis of compounds **7-11**. [a]



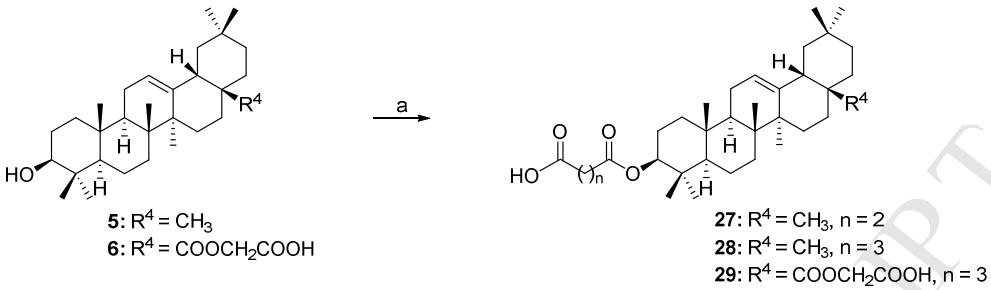

[a] Reagents and conditions: (a) anhydride, pyridine, DMAP, r.t, 52-90%; (b) BnBr or PMBCl, $K_2CO_3$, DMF, r.t; (c) *n*-nonylic acid or oleic acid, EDCI, DMAP, $CH_2Cl_2$, reflux, 68-81% for two steps; (d) $H_2$, Pd/C, THF, r.t, 70% for **10**; (e) H-mont, $CH_2Cl_2$, r.t, 70% for **11**.

**Scheme 2.** Synthesis of compounds **17-26**. [a]



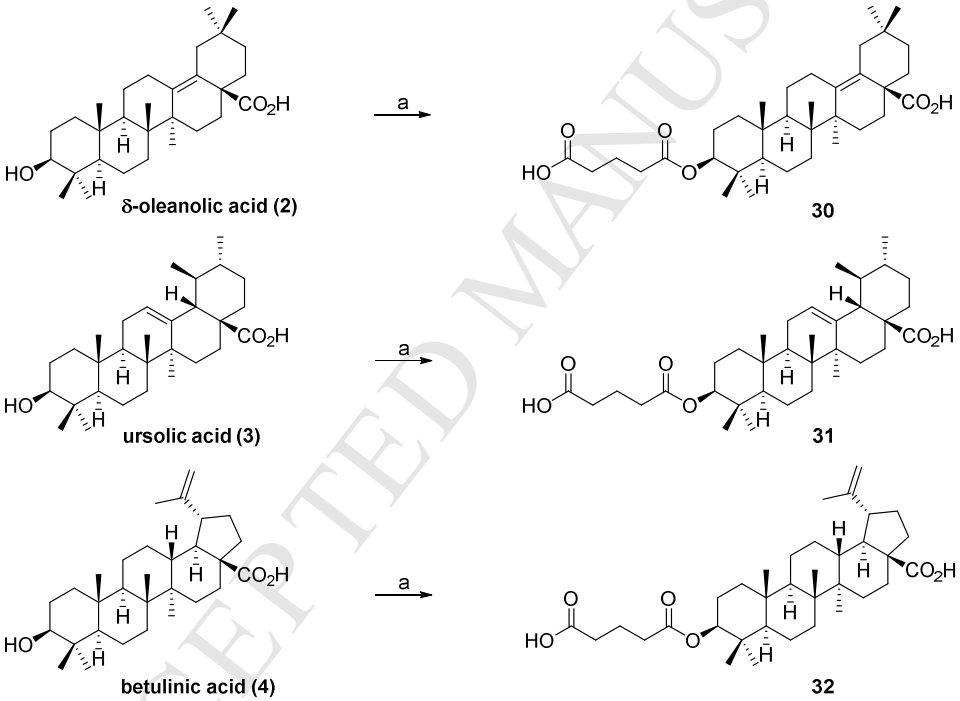

[a] Reagents and conditions: (a) anhydride, pyridine, DMAP, r.t, 68-80%; (b) BnBr, $K_2CO_3$, DMF, r.t, 90%; (c) EDCI, DMAP, $CH_2Cl_2$, reflux, 80%; (d) $SOCl_2$, $CH_2Cl_2$, 40 $^oC$; then $Et_3N$, amines, $CH_2Cl_2$, r.t; (e) $H_2$, Pd/C, THF, r.t, 32-94% for two steps.

**Scheme 3.** Synthesis of compounds **27-29**. [a]



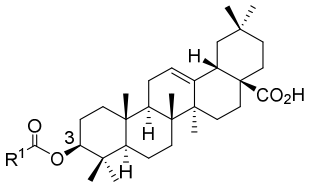
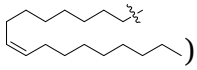
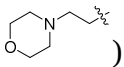
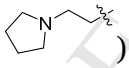
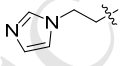
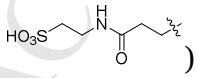
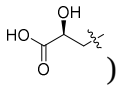
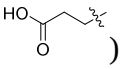

[a] Reagents and conditions: (a) anhydride, pyridine, DMAP, r.t, 11-81%.

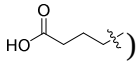
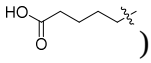
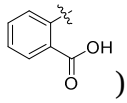
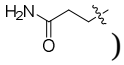
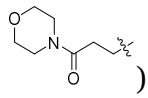
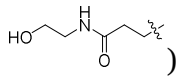
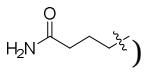
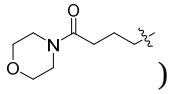
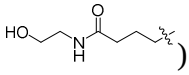
**Scheme 4.** Synthesis of compounds **30-32**. [a]




[a] Reagents and conditions: (a) anhydride, pyridine, DMAP, r.t, 82-86%.

**Table 1.** Screening assay of natural PTs and their derivatives as novel CETP inhibitors in vitro. [a]

| Compd. | In vitro CETP inhibitory activity | |
|---|---|---|
| | % Inhibition at 10 μM | $IC_{50}$ (μM) |
| **1** | 9.7 | / [b] |
| **2** | 0 | / |
| **3** | 31.9 | 46.6 |
| **4** | 31.4 | n.e. [c] |
|  | | |
| **7** ($R^1$ = Me) | 38.3 | n.e. |
| **8** ($R^1$ = Et) | 8.5 | / |
| **9** ($R^1$ = *n*-Pr) | 13.6 | / |
| **10** ($R^1$ =  ) | 12.6 | / |
| **11** ($R^1$ =  ) | 0 | / |
| **12** ($R^1$ =  ) | 30.9 | 68.5 |
| **13** ($R^1$ =  ) | 28.9 | / |
| **14** ($R^1$ =  ) | 16.5 | / |
| **15** ($R^1$ =  ) | 55.2 | 23.1 |
| **16** ($R^1$ =  ) | 4.7 | / |
| **17** ($R^1$ =  ) | 54.0 | 4.3 |

| | | |
|---|---|---|
| **18** ($R^1$ =  ) | 55.2 | 3.5 |
| **19** ($R^1$ =  ) | 53.7 | 7.0 |
| **20** ($R^1$ =  ) | 66.8 | 2.3 |
| **21** ($R^1$ =  ) | 0 | / |
| **22** ($R^1$ =  ) | 0 | / |
| **23** ($R^1$ =  ) | 0 | / |
| **24** ($R^1$ =  ) | 0 | / |
| **25** ($R^1$ =  ) | 0 | / |
| **26** ($R^1$ =  ) | 0 | / |
| **27** | 0 | / |
| **28** | 14.7 | / |
| **29** | 49.0 | 38.9 |
| **30** | 59.1 | 10.3 |
| **31** | 62.0 | 3.4 |
| **32** | 30.0 | 38.0 |
| **Anacetrapib** [d] | 84.1 | 0.0338 |

[a] The initial screening was carried out at a concentrations of 10 μM for each compound and $IC_{50}$s were measured for compounds that displayed >30% inhibition of CETP (average for two times at different time points). [b] "/" means that no experiment was conducted. [c] n.e. means that no effect. [d] The reference drug.

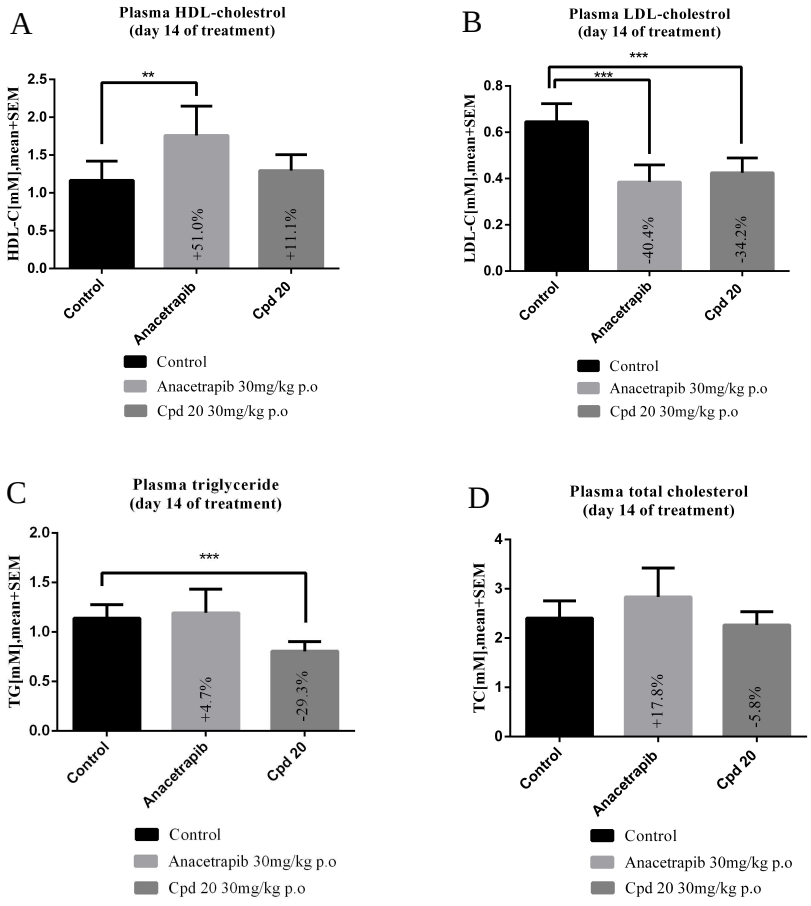



**Fig. 4.** In vivo efficacy data for compound **20** in ap2-CETPTg mice. (A) HDL-C, (B) LDL-C, (C) TG and (D) TC were measured. ** $p < 0.01$, *** $p < 0.001$ versus control.

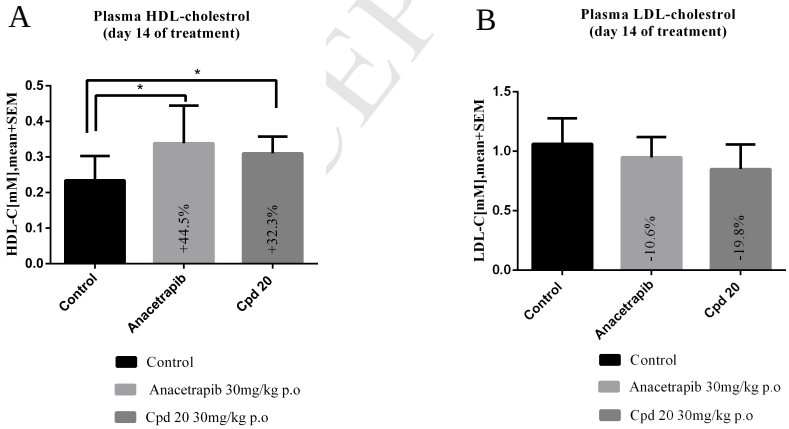



**Fig. 5.** In vivo efficacy data for compound **20** in normal fed guinea pigs. (A) HDL-C and (B) LDL-C were measured. * $p < 0.05$ versus control.

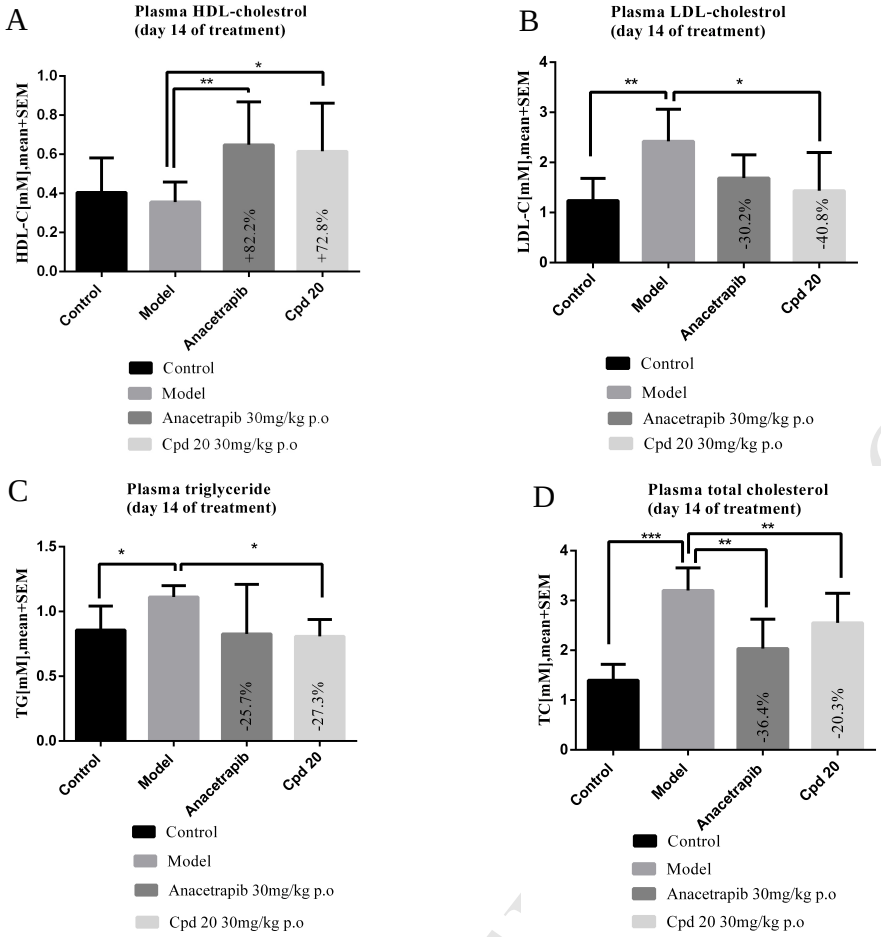



**Fig. 6.** In vivo efficacy data for compound **20** in high-fat fed guinea pigs. (A) HDL-C, (B) LDL-C, (C) TG and (D) TC were measured. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus model.

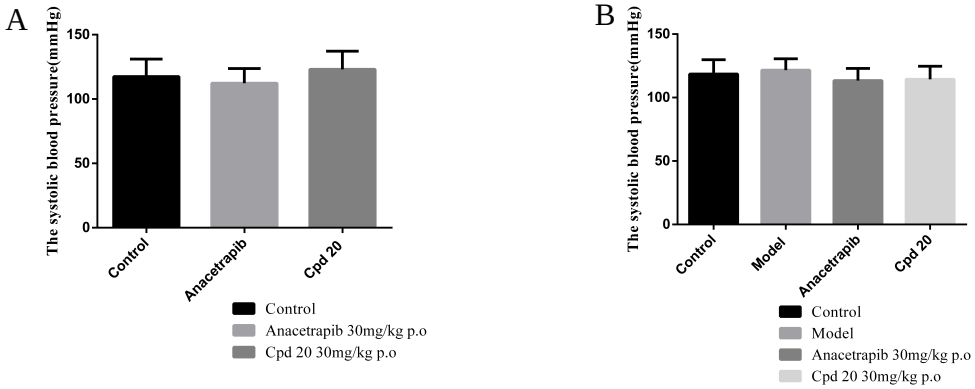



**Fig. 7.** Effects on the systolic blood pressure of compound **20** (30 mg/kg, once-a-day, for two weeks) in normal fed guinea pigs (A) and high-fat fed guinea pigs (B).

**Table 2.** Physiochemical properties of compound **20** and four typical CETP inhibitors.

| Compd. | Molecular formula/weight | Character | Log P (pH 7.4) [a] |
|---|---|---|---|
| **20** | $C_{38}H_{52}O_6$/604.83 | white powder | 0.49 |
| Torcetrapib | $C_{26}H_{25}F_9N_2O_4$/600.48 | colorless solid | 1.05 |
| Dalcetrapib | $C_{23}H_{35}NO_2S$/389.60 | white powder | 0.92 |
| Anacetrapib | $C_{30}H_{25}F_{10}NO_3$/637.52 | colorless solid | 0.73 |
| Evacetrapib | $C_{31}H_{36}F_6N_6O_2$/638.66 | white powder | 0.51 |

[a] Shake-flask method and HPLC were used to determine the partition coefficients of test compounds in octanol-water system at 25 $^oC$.

**Table 3.** Pharmacokinetic parameters of compounds **20** and **1** in normal fed guinea pigs. [a]

| Parameters [b] | Compd. **20** | Compd. **1** |
|---|---|---|
| $AUC_{0-48}$ (h*ng/mL) | 9337.83 ± 2152.23 | 291.47 ± 80.89 |
| $AUC_{0-\infty}$ (h*ng/mL) | 10851.60 ± 2578.21 | 312.41 ± 87.56 |
| $MRT_{0-48}$ (h) | 14.95 ± 1.86 | 11.72 ± 1.87 |
| $MRT_{0-\infty}$ (h) | 23.39 ± 2.74 | 14.97 ± 2.72 |
| $C_{max}$ (ng/mL) | 1266.46 ± 406.61 | 52.19 ± 27.89 |
| $T_{max}$ (h) | 1.46 ± 0.40 | 0.75 ± 0.32 |
| $t_{1/2}$ (h) | 18.75 ± 5.15 | 11.74 ± 3.51 |

[a] The oral doses were formulated in DMSO/cremophor/saline at a 3 : 4 : 93 ratio and given at 30 mg/kg to normal fed guinea pigs (n = 6). [b] AUC, area under the plasma concentration-time curve; MRT, mean residence time; $C_{max}$, observed maximal plasma concentration following oral dosing; $T_{max}$, time to reach the $C_{max}$; $t_{1/2}$, terminal half-life. Values expressed as mean ± SD.

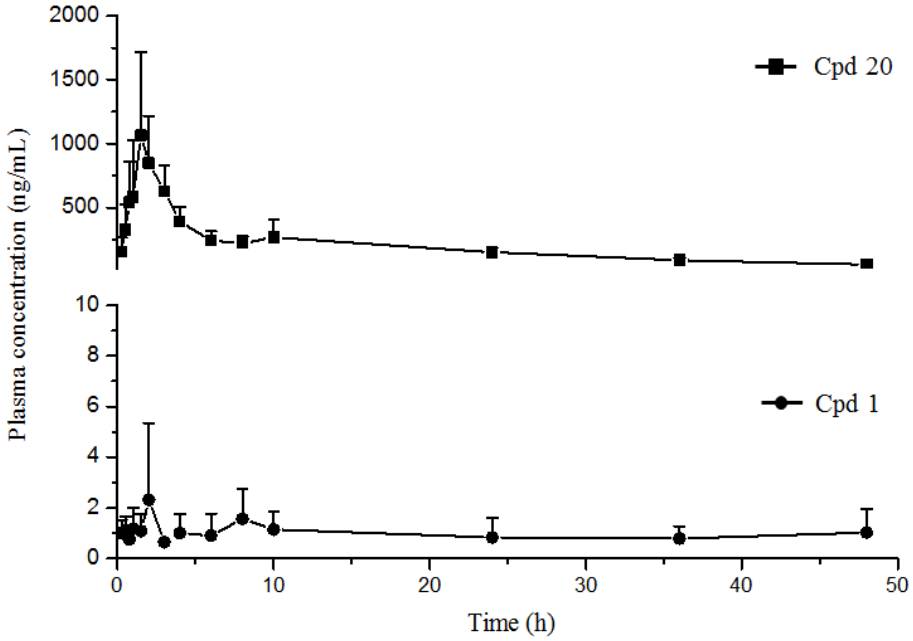



**Fig. 8.** Mean plasma concentration-time profiles of compound **20** and its metabolite **1** in normal fed guinea pigs after oral administration at 30 mg/kg dose of compound **20** (n = 6). Vertical bars represent standard deviation.

- A series of pentacyclic triterpene 3β-ester derivatives were designed, synthesized and evaluated as a new class of cholesteryl ester transfer protein (CETP) inhibitors.
- The most potent compound **20** displayed an $IC_{50}$ value of 2.3 μM against CETP in *vitro*.
- Compound **20** effectively ameliorated plasma lipid levels of human adipose tissue specific CETP transgenic mice and guinea pigs, and had no significant changes on the systolic blood pressure in guinea pigs.
- Compound **20** has a good pharmacokinetic profile.