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Original article

Synthesis of novel β-propanamides to inhibit cholesteryl ester transfer protein (CETP)

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ABSTRACT

A novel series of β-propanamide derivatives as inhibitors of cholesteryl ester transfer protein (CETP) were synthesized. Previously, **H3** (IC_{50} 2 μmol/L) was observed to inhibit CETP moderately (Xie et al., 2016). Structural modifications based on **H3** led to discovery of the successful CETP inhibitor, known as 1-methyl-4-arylpyrazole. Using a similar approach, compound **Q08** was identified as a highly potent CETP inhibitor with an IC_{50} of 490 nmol/L *in vitro*.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in industrialized and developing countries [1,2]. Epidemiological studies have identified two key risk factors that increased the likelihood of CVD events: decreased levels of high-density lipoprotein-cholesterol (HDL-C) and increased levels of low-density lipoprotein-cholesterol (LDL-C) [3–5]. Several clinical studies have established the inverse relationship between cardiovascular events and HDL-C serum levels [6–8]. Niacin elevates HDL-C levels in clinical treatments but is not used often due to considerable side effects [9,10]. Thus, there is a high demand for a specific HDL-C raising therapies with better efficacy and safety.

Plasma cholesteryl ester transfer protein (CETP) regulates the inverse transport of cholesterol esters and also facilitates the transfer of cholesteryl esters from HDL-C to both LDL-C and very low density lipoprotein-cholesterol (VLDL-C) [11,12]. CETP reduces atheroprotective HDL-C levels. Conversely, CETP inhibitors help prevent retrograde cholesterol transport and therefore increase HDL-C levels. Four small molecule inhibitors have entered phase III clinical trials: torcetrapib, anacetrapib, evacetrapib and dalcetrapib (Scheme 1). Torcetrapib is the first inhibitor of phase III clinical trials with a potent CETP IC_{50} of 50 nmol/L [13,14]. However, torcetrapib was prematurely terminated due to a higher mortality rate in the torcetrapib/atorvastatin group than the atorvastatin group. Dalcetrapib also showed modest potency in phase III clinical trials and was terminated because it failed to exhibit a clinically relevant reduction in cardiovascular events [15]. Recently, potent CETP inhibitors anacetrapib and evacetrapib entered phase III and overcame the issues with torcetrapib and dalcetrapib [16,17]. Clinical trial data for both anacetrapib and evacetrapib showed elevated HDL-C and lowered LDL-C without side effects, such as torcetrapib.

We previously reported a series of *N*,*N*-3-phenyl-3-benzylaminopropionanilide derivatives as CETP inhibitors. Structure–activity optimization from screening **L10** identified compound **H16** (IC_{50} 0.15 μmol/L) as a potent CETP inhibitor and **H3** (IC_{50} 2 μmol/L) as a modest CETP inhibitor [18]. The structure of **H16** was notably flexible and might be the basis for its poor bioavailability. To identify novel CETP inhibitors, we explored β-propanamide derivatives with low flexibility and reduced synthetic complexity. To begin our optimization, we methodically replaced β-propanamide moieties of **H3** with a focus on amide terminus substructure. Simultaneously, we focused on benzyl moiety modifications with 3-1,1,2,2-tetrafluoroethoxyl and 4-F. After further optimization of **H3**, we discovered compound **Q08** (IC_{50} 490 nmol/L) to inhibit CETP *in vitro*, as measured by BODIPY-CE fluorescence assay.

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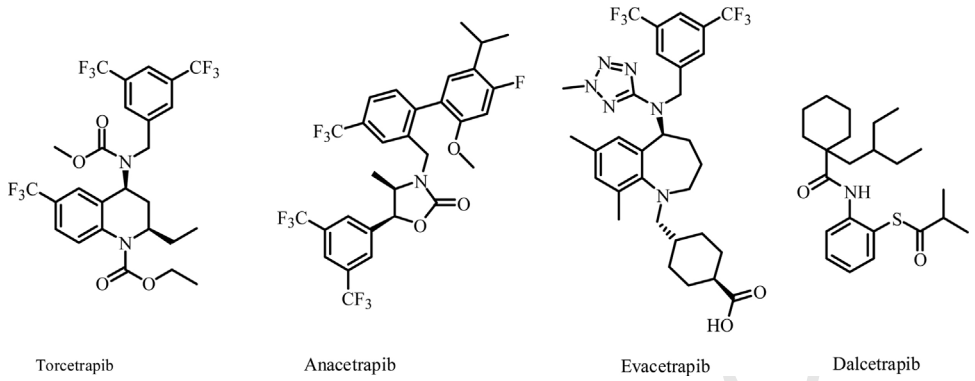


Scheme 1. The four CETP inhibitors that have advanced to phase III clinical trials.

2. Experimental

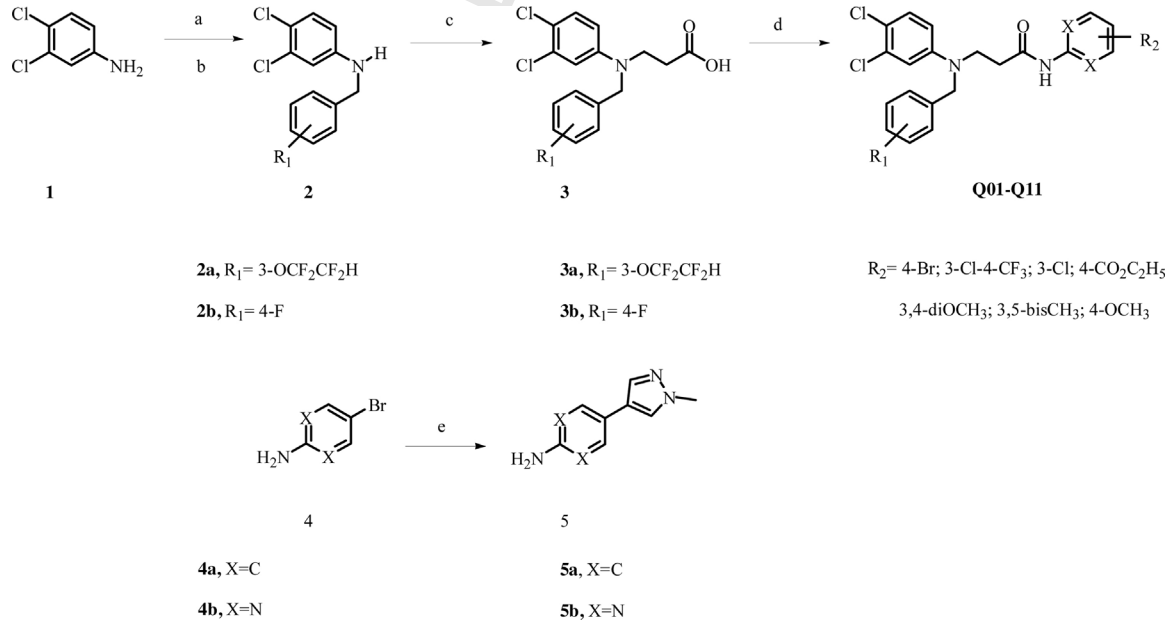
2.1. Chemistry

Compounds **Q01**–**Q11** were prepared according to Scheme 2. Overall, the compounds were prepared in three steps. The key intermediate **2** was prepared by standard reductive amination using commercially available benzaldehydes. The secondary amine 2 was treated with acrylic acid through a Michael reaction at 50 °C for 6 h to generate the key linker **3**. Next, compound **3** was treated with HOBT, EDCI and DIEA in DMF at 25 °C for 2 h, followed by substitution of various aliphatic amines or by condensation reactions to obtain the desired target **5** compounds. Compound **5** was prepared by aromatic amines with 1-methyl-4-(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole through a Suzuki reaction.

All solvents were purchased from Aladdin (Shanghai, China) and were not purified further, and all of the chemicals were purchased from commercial sources with purity >98%. Melting points (mp) were determined in open capillaries on a Buchi 353 melting point apparatus (Buchi Labortechnik, Flawil, Switzerland) and were uncorrected. Solvents used for moisture sensitive reactions were distilled and used in an argon atmosphere. Purity and homogeneity of the compounds were measured by chromatography on a glass column using silica gel (100–200 mesh) assessed by TLC and HPLC chromatography. Mass spectra were taken in ESI mode on an Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). NMR spectra were recorded at 400 MHz for ^{1}H and 151 MHz or 101 MHz for ^{13}C on a Bruker spectrometer with TMS as an internal standard, $CDCl_3$ or DMSO-d_6 as solvent, and 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. These data can be found in the Supporting information.

2.2. In vitro *CETP inhibitory assay*

The CETP RP Activity Assay Kit (Catalogue # RB-RPAK; Roar) uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor by CETP (Catalogue # R8899; Roar). CETP-mediated transfer of the fluorescence neutral lipid to the acceptor molecule results in an increase in fluorescence (ExEm = 465/535 nm). Inhibition of CETP prevents lipid transfer and therefore decreases the fluorescence intensity. The testing compounds were entirely dissolved using 100% DMSO. Vibrating hard on oscillator for more than 30 s helped significantly before storing in a nitrogen cabinet. Stocking compounds (10 mmol/L)



Scheme 2. Synthetic routes. Reagents and conditions: (a) AcOH, substituted benzald ehydes; (b) $NaBH_4$, r.t.; (c) 10% HCl, acrylic acid, 50 °C; (d) substituted amines, HOBT, EDCI, DIEA, r.t.

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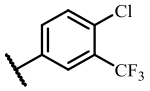
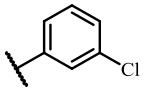
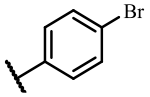
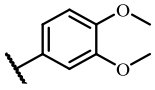
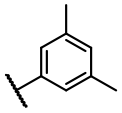
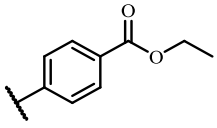
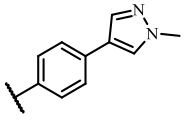
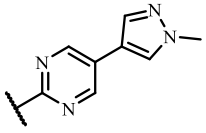
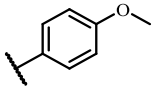
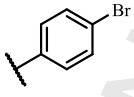
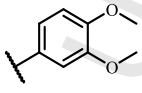
Table 1
Structure–activity relationship (SAR) of β-propanamides derivatives **Q01**–**Q11**.

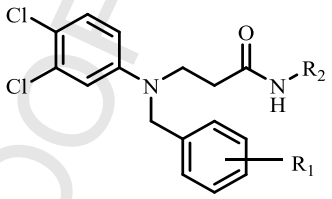
Compound	R_1	R_2	IC_{50} (μmol/L)
Q01	3-OCF_2CF_2H		12.8
Q02	3-OCF_2CF_2H		20.9
Q03	3-OCF_2CF_2H		21.3
Q04	3-OCF_2CF_2H		9.4
Q05	3-OCF_2CF_2H		21.8
Q06	3-OCF_2CF_2H		2.8
Q07	4-F		0.72
Q08	4-F		0.49
Q09	4-F		7.2
Q10	4-F		18.5
Q11	4-F		5.3
Anacetrapib[a]			0.06

[a] The positive control.

were diluted with DMSO for eight titration points (1:5 serial dilutions) in 96-well dilution plates. The assay was performed according to the instruction for the CETP inhibitor screening kit and recombinant CETP.

3. Results and discussion

3.1. In vitro *activity and structure–activity relationships*



To evaluate the ability of various β-propanamides derivatives to inhibit CETP, all of these new synthetic compounds were assayed using anacetrapib as a reference compound for their inhibitory effects against CETP by BODIPY-CE fluorescence assay with CETP RP Activity Assay Kit (Catalogue # RB-RPAK; Roar) (Table 1). Most of the target compounds have a mild effect on CETP inhibition activity, while several key structural changes indicated the general SAR of this series. First, the effect of the substituted aniline terminus on the inhibition of CETP was examined. Compound aniline termini substituted by 3-CF_3-4-Cl, 4-Br, 3-Cl, 3,5-bisCH_3 and 3,4-diOCH_3 exhibited modest CETP inhibition, while the one substituted by 4-$COOC_2H_5$ compound **Q06** exhibited an IC_{50} of 2.8 μmol/L. These results indicated that increased lipophilicity of the aniline moiety did not lead to increased potency. Compounds with 1-methyl-4-arylpyrazole **Q07** exhibited an IC_{50} of 0.72 μmol/L. When pyrimidines replaced benzene rings, we identified **Q08**, which exhibits IC_{50} 0.49 μmol/L and lower than **Q07**. These suggested that increased hydrophilicity was important to improve inhibition activity. The calculated LogP of **Q07** and **Q08** was 4.7 and 4.8, respectively. These results indicated that **Q07** and **Q08** physical properties fall within drug-like property space. However, the activity of compound **Q11** was higher than **Q04**, indicating that compounds with 4-F benzyls exhibited higher potency than compounds with 3-1,1,2,2-tetrafluoroethoxylbenzyl. These results indicated that 4-F benzyl is significant to inhibit CETP.

4. Conclusion

We report a novel CETP inhibitor based on the structure of our previously developed compound **H3**. Most of the newly synthetic compounds exhibited moderate inhibition CETP activity. Compounds **Q07** (IC_{50} 0.72 μmol/L) and **Q08** (IC_{50} 0.49 μmol/L) were identified to exhibit potent CETP inhibition. Compound **Q08** titration is flexible and has a remarkable CLogP 4.8; therefore, **Q08** was selected for further development. It is highly expected that this novel scaffold will produce promising CETP inhibition agents after further modifications.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2016.10.016.

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