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Design and synthesis of simple, yet potent and selective non-ring-A pyripyropene A-based inhibitors of acyl-coenzyme A: cholesterol acyltransferase 2 (ACAT2)†

Yang Zhan,‡[a] Xiao-Wei Zhang,‡[b] Ying Xiong,[b] Bo-Liang Li*[b] and Fa-Jun Nan*[a]

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A series of pyripyropene A-based compounds were designed and synthesized by opening the upper section of the A-ring, which significantly simplifies the structure and synthesis from commercially available starting materials. Representative compound **(–)-3** exhibited potent activity against ACAT2 and greater selectivity for ACAT2 than for ACAT1.

Introduction

Acyl-coenzyme A: cholesterol acyltransferase (ACAT) is the exclusive intracellular enzyme that catalyzes the formation of cholesteryl esters from free cholesterol and long-chain fatty acyl-CoA. The ACAT family includes two members, ACAT1 and ACAT2. ACAT1 contributes significantly to macrophage-derived foam cell formation in atherosclerosis. ACAT1 is expressed ubiquitously in almost all human tissues, and the steryl esters catalyzed by ACAT1 incorporate into cellular lipid droplets exclusively. Whereas, ACAT2 is responsible for absorption of dietary cholesterol from the intestine and lipoprotein production by the liver, the steryl esters catalyzed by ACAT2 can incorporate into both intracellular lipid droplets and extracellular lipoproteins.[1–3] Therefore, ACAT is regarded as a potential therapeutic target for cardiovascular diseases.[4–6] However, synthetic ACAT inhibitors failed to show efficacy or cause severe side effects in clinical trials. In 2006, Fazio *et al.* observed[7] that inhibition of ACAT1 activity led to cytotoxicity toward macrophages and vascular cells, whereas ACAT2 inhibition decreased the synthesis of cholesteryl esters and provided therapeutic benefits. More recently, Lu *et al.* reported[8] that by investigating tissue samples from hepatocellular carcinoma (HCC) patients and HCC cell lines, a specific cholesterol metabolic pathway, involving induction of ACAT2 and esterification of excess oxysterols for secretion to avoid cytotoxicity, is established in a subset of HCCs for tumor growth. ACAT2 is also an attractive target for the treatment of HCCs, and selective ACAT2 inhibitors will be potential anti-HCC candidates with novel modes of action compared with current chemotherapeutics.

To date, natural product pyripyropene A (PPPA) and its analogues are the only chemical type ACAT2-specific inhibitors.[9] PPPAs were isolated from the fungal strain *A. fumigatus* FO-12897[10] and characterized by Omura *et al.* in detailed structure–activity relationship (SAR) studies.[11–16] PPPAs exhibited efficacy in an atherosclerosis animal model.[17] However, the acquisition of PPPA from either natural sources[10] or chemical synthesis[18] is difficult.

According to the total synthesis of PPPA reported[18,19] by Atsuki *et al.*, eleven steps are required to construct the A-ring, and these steps account for the majority of the synthesis procedure. Satoshi *et al.* reported structure–activity relationships (SARs) of PPPA modifications through simple replacement of small substituents.[11–16] Their results suggested that the pyridine pyrone moiety, free hydroxyl and three acetyl segments are essential for potent activity. However, A-ring, which is the most difficult portion of the molecule to synthesize, does not appear to be essential for maintaining ACAT2 activity. We analyzed these key pharmacophores in complicated natural products and analogues and rationally designed some simplified PPPA-based compounds by opening the upper portion of ring A (red part in Fig. 1) and maintaining the remaining acetyl esters. As shown in Fig. 1, to prove our initial concept, we selected a method to prepare a racemic mixture (**1**) as the first target molecule.

The synthesis of **1** is summarized in Scheme 1. The hydroxycarvone (±)-**6**,[20] which was prepared from **(*R*)-carvone**, was sulfonated with triflic anhydride to produce enol triflate (±)-**7**

[a]*State Key Laboratory of Drug Research, The National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China. E-mail: fjnan@simm.ac.cn*
[b]*State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Bio-logical Sciences, Chinese Academy of Sciences, Shanghai, 200031, China. E-mail: blli@sibs.ac.cn*
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‡These authors contributed equally to this work.

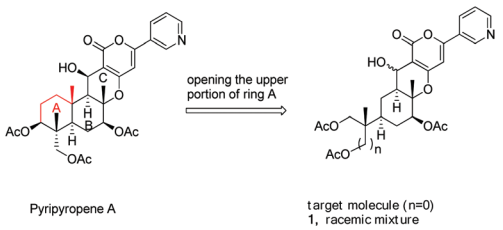
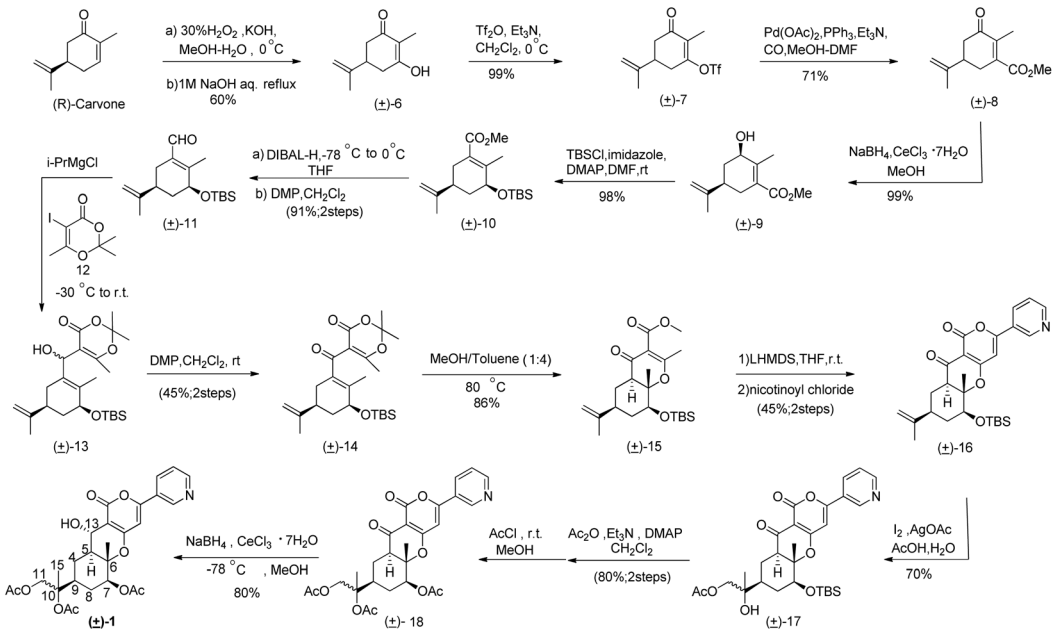


Fig. 1 Rational design of simplified pyripyropene A-based compounds *via* opening the upper portion of the A-ring.

followed by carbonylation using a procedure described by Stille[21] to produce the methyl ester **(±)-8**. Stereoselective Luche reduction[22] provided **(±)-9** quantitatively, which was protected as a TBS ether to form product **(±)-10**. Coupling reaction of the 5-iodo-1,3-dioxin-4-one derivative **12** with **(±)-11**, which was generated from reduction with DIBALH of the ester **(±)-10** followed by oxidation with DMP, afforded intermediate **(±)-13** under conditions reported by Knochel *et al.*[23] Subsequent Dess–Martin oxidation formed **(±)-14** in 45% yield over two steps. Deprotection of **(±)-14** followed by cyclization was realized under methanolysis conditions, producing **(±)-15**. Enolization of **(±)-15** by LHMDS, γ-H acylation with nicotinoyl chloride, and treatment with an additional portion of LHMDS by intramolecular cyclization formed **(±)-16** from **(±)-15** in one pot. Prevost dihydroxylation of **(±)-16** furnished **(±)-17** as a 1 : 1 mixture of diastereomers at the newly formed stereocenter C_{10}. Protecting group manipulations of **(±)-17** by two steps resulted in **(±)-18**, which was subjected to Luche reduction to give **1** as the final product. Synthetic racemic mixture **1** was obtained as a mixture of four isomers. ACAT2 inhibition in the NBD22-steryl ester fluorescence assay indicated that compound **1** exhibited activity comparable to PPPA, with an IC_{50} value of 0.155 μM, whereas the IC_{50} of PPPA was 0.185 μM under the same assay conditions. We also test the selectivity index (SI) by using the cholesterol oxidase assay with an Amplex red cholesterol assay kit, compound **1** exhibited high selectivity to ACAT2 isoform, with a selectivity index (SI) of 733 (ACAT1-IC_{50}: 107.7 μM; ACAT2-IC_{50}: 0.147 μM), which was also comparable to that of PPPA (Table 1).

These results encouraged us to perform detailed SAR studies for these simplified PPPA-based ACAT2 inhibitors. Because racemic **1** was obtained as a mixture of four isomers, acquisition of the single enantiomer was different from the procedure for the racemic mixture (**1**). We changed several steps to obtain chiral aldehyde **11** from natural **(*R*)-carvone** or **(*S*)-carvone**, followed by a similar procedure to afford the single isomers.

The synthesis of **(+)-2** and **(+)-3** is outlined in Scheme 2. The known allylic alcohol **(+)-19**,[24] which was prepared from commercially available **(*R*)-carvone**, was protected as a TBS ether to form product **(+)-20**. The nitrile **(+)-20** was reduced with DIBAL-H to give aldehyde **(+)-11** in 84% yield.[25] The subsequent five steps were the same as those used in the synthetic method shown in Scheme 1. Prevost dihydroxylation of **(+)-16** resulted in **(+)-17A** and **(+)-17B** as a 1 : 1 mixture of diastereomers at the newly formed stereocenter C_{10}, and these diastereomers were isolated and identified by 2d-NMR (Fig. 2). As shown in Fig. 2, on the basis of the evidence of the methyl NOESY cross-correlation with both methylene (H4 and H8), and the configural-superiority effect, we obtained two stable Newman forms. The NOE between methylene (H11) and methylene (H4 or H8) protons was consistent with **(*S*)-(+)-17A**

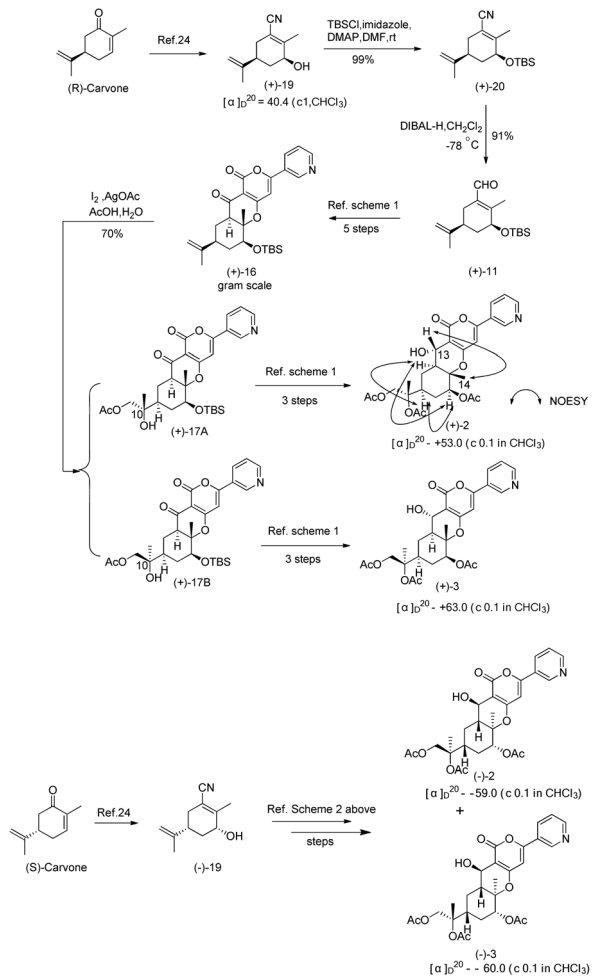


Scheme 1 Preparation of simplified pyripyropene A-like racemic mixture 1.

Table 1 Effect of inhibitors on cholesteryl ester (CE) synthesis and selectivity of inhibitors toward ACAT isoforms

Compound	IC_{50} ± SD (μM)			Selectivity index (IC_{50} for ACAT1[b]/IC_{50} for ACAT2[b])
	ACAT2[a]	ACAT2[b]	ACAT1[b]	
Pyripyropene A	0.185 ± 0.007	0.198 ± 0.016	>20[c]	>200[c]
1	0.155 ± 0.010	0.147 ± 0.018	107.7	733
(+)-2	0.743 ± 0.098	—[d]	—	—
(+)-3	1.272 ± 0.149	—	—	—
(+)-4	1.789 ± 0.084	—	—	—
(+)-5	3.059 ± 0.222	—	—	—
(−)-2	1.700 ± 0.062	—	—	—
(−)-3	0.077 ± 0.006	0.055 ± 0.007	56.66	1035
(−)-4	2.456 ± 0.161	—	—	—
(−)-5	0.416 ± 0.052	—	—	—

[a] By using the NBD22-steryl ester fluorescence assay. [b] By using the cholesterol oxidase assay. IC_{50} values were means ± SD of three replications. [c] From Ref. 29. [d] Not test.



Scheme 2 Preparation of one single enantiomer **(+)-2**, **(+)-3** and **(−)-2**, **(−)-3**.

and **(*R*)-(+)-17B** at stereocenter C_{10}. The following three steps imitated the route of the racemic mixture to access **(+)-2** and **(+)-3** as the final product. The relative configuration of the stereocenters was determined by irradiation of the methyl-H_{14} and H_{13} (Scheme 2: NOESY). The synthetic method of **(−)-2** and **(−)-3** is similar to the method shown in Scheme 2. The known allylic alcohol **(−)-19**[24] was prepared from commercially available **(S)-carvone**.

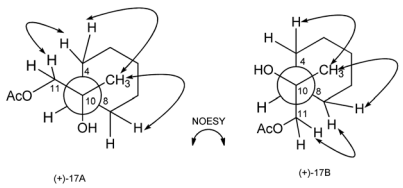
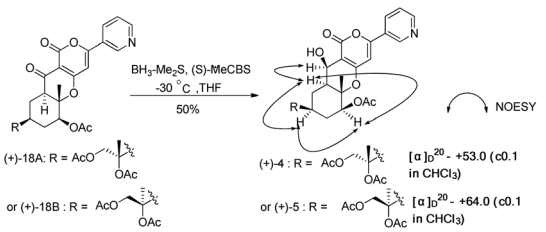


Fig. 2 Identification of the configuration at stereocenter C_{10}.

Four single isomers, **(+)-2**, **(+)-3**, **(−)-2** and **(−)-3**, were tested for ACAT2 inhibition. Surprisingly, compounds **(+)-2** and **(+)-3**, which have the same core-structure stereochemistry as the natural product PPPA, showed weak inhibitory activity of ACAT2, with IC_{50} values of 0.743 and 1.272 μM, respectively. However, **(−)-3** was the most potent ACAT2 inhibitor among all of the investigated isomers, with an IC_{50} value of 0.077 μM in NBD22-steryl ester fluorescence assay and a much higher SI value of 1035 (ACAT1-IC_{50}: 56.66 μM; ACAT2-IC_{50}: 0.055 μM, by assaying steryl esters) (Table 1). Isomer **(−)-2** also exhibited weak inhibitory activity of ACAT2, with an IC_{50} value of 1.700 μM. The latter two isomers actually exhibit opposite stereochemistry in the B, C ring compared with the natural product PPPA.



Scheme 3 Preparation of **(+)-4** and **(+)-5**.

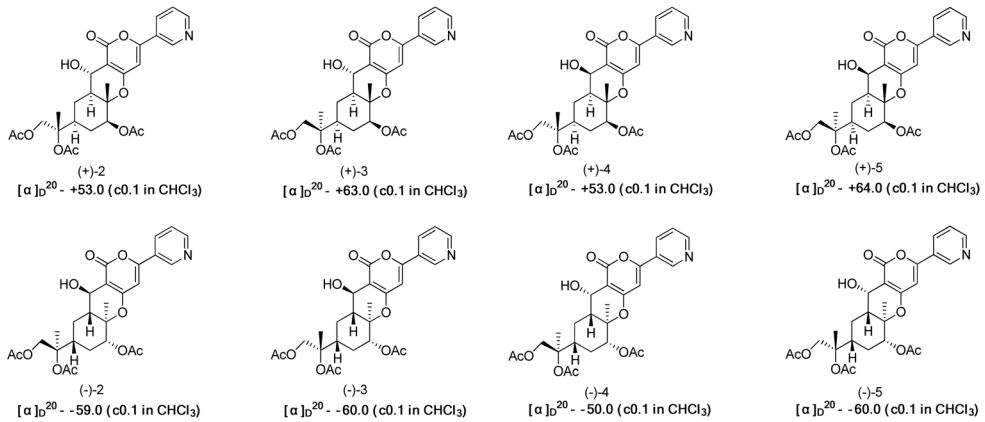


Fig. 3 The structure of eight isomers: **(+)/(−)-2–5**.

By comparing the stereochemistry of four single isomers with PPPA, we observed that the relative stereochemistry at the C_{13} position of the most potent compound, **(−)-3**, also differed from that at the same position of the natural product. These results suggested that, in non-A-ring analogs, an exact structure–activity relationship should be further assigned. Therefore, we synthesized **(+)/(−)-4** and **(+)/(−)-5** as four corresponding C_{13} *epi*-isomers of **(+)/(−)-2** and **(+)/(−)-3**, respectively.

The synthesis of **(+)-4** and **(+)-5** is outlined in Scheme 3. Starting from the intermediate **(+)-18A** or **(+)-18B**, respectively, the final asymmetric hydroxyl was introduced by reduction of the enone using (*S*)-Me-CBS-oxazaborolidine,[26] which provided allylic alcohol **(+)-4** or **(+)-5** in 50% yield. The corresponding enantiomers **(−)-4** and **(−)-5** were obtained using a similar procedure starting from **(−)-18A** and **(−)-18B**, respectively.

We next determined the ACAT2 inhibition of four isomers **(+)/(−)-4–5** using NBD22-steryl ester fluorescence assay. IC_{50} results showed that they all have weaker ACAT2 inhibitory activity compared with PPPA and **(−)-3**. Surprisingly, compound **(−)-5**, which is **C_{13}-*epi* (−)-3**, showed 5 fold decreased activity, indicating that non-ring-A compounds exhibit different SARs toward ACAT2 (Fig. 3).

Recently, Nagamitsu *et al.* reported[27,28] several non-ring-A PPPA-based analogues, which shared the same core-structure of the B, C ring of the PPPA, showed weak activity of ACAT2 inhibition and selectivity. Our results will possibly explain why their non-ring-A compounds are weak or inactive, because all of the compounds they reported have the same stereochemistry in the B, C ring with the natural product PPPA.

Conclusions

In conclusion, eight new simplified non-ring-A PPPA-based analogues were designed and synthesized in this article. Among these analogues, compound **(−)-3** exhibited more potent ACAT2 inhibition and higher selectivity toward ACAT2 compared with the natural product PPPA. Compound **(−)-3** also exhibited opposite stereochemistry in the B, C ring compared with PPPA, which suggests that the SAR profiles of these simplified analogues differ from the profile of the natural product. These non-ring-A analogues are novel compounds that can be easily assembled from naturally abundant carvone in several steps and in high yield. More detailed SARs are required to generate more drug-like and selective ACAT2 inhibitors with potential therapeutic use in atherosclerosis and HCC. Further studies assessing the SARs of these compounds are in progress.

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