Organic &
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Chemistry

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Volume 1 | Number 1 | Jan 2013 | Pages 1–100

ROYAL SOCIETY
OF CHEMISTRY

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ARTICLE

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]
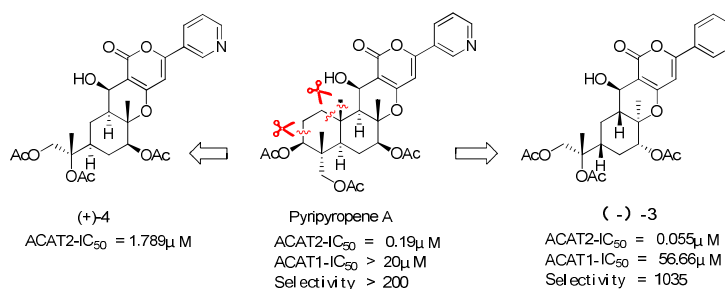
(+)-4
ACAT2-IC$_{50}$ = 1.789 μM

Pyripyropene A
ACAT2-IC$_{50}$ = 0.19 μM
ACAT1-IC$_{50}$ > 20 μM
Selectivity > 200

(-)-3
ACAT2-IC$_{50}$ = 0.055 μM
ACAT1-IC$_{50}$ = 56.66 μM
Selectivity = 1035

A series of pyripyropene A-based compounds were designed and synthesized by opening the upper section of 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-HCCs 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 Satoshi 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,

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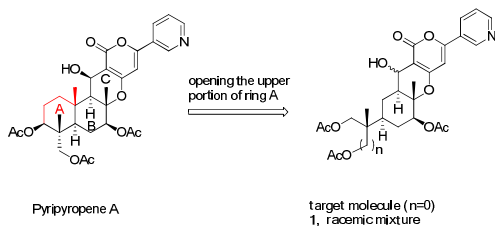

Figure 1. Rational design of simplified pyripyropene A-based compounds via opening the upper portion of the A-ring.

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 Figure 1) and maintaining the remaining acetyl esters. As shown in Figure 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 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 in same assay condition. We also test the selectivity index (SI) by determining cholesterol oxidase assay with 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
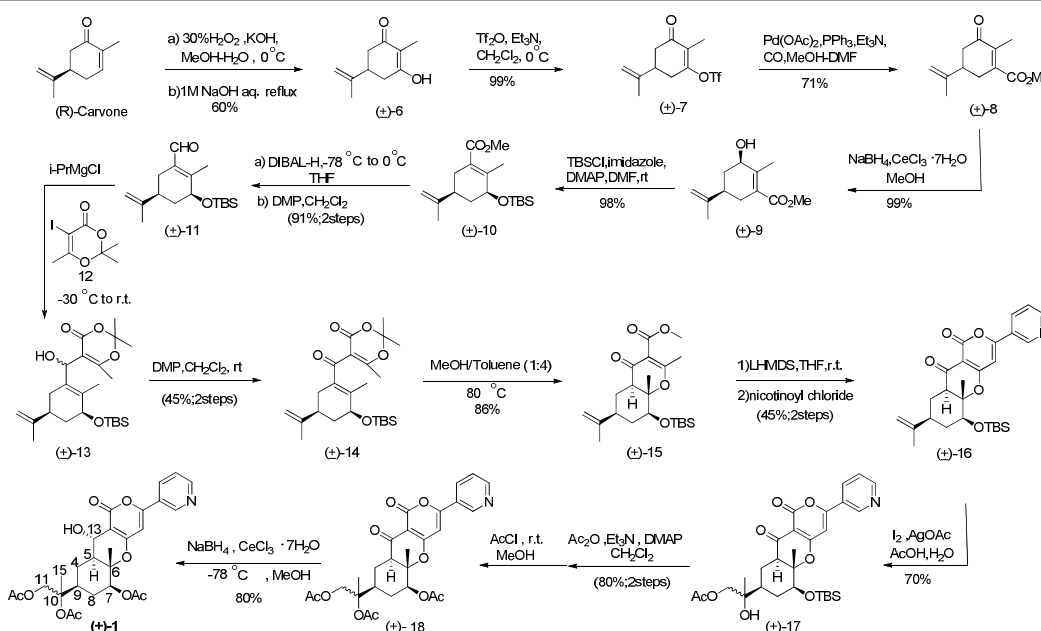
Scheme 1. Preparation of simplified Pyripyropene A -like racemic mixture 1.

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 (Figure 2). As shown in Figure 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 were consistent with (S)-(+)-17A and (R)-(+)-17B at stereocenter C_{10}. The following three steps imitated the route of 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.
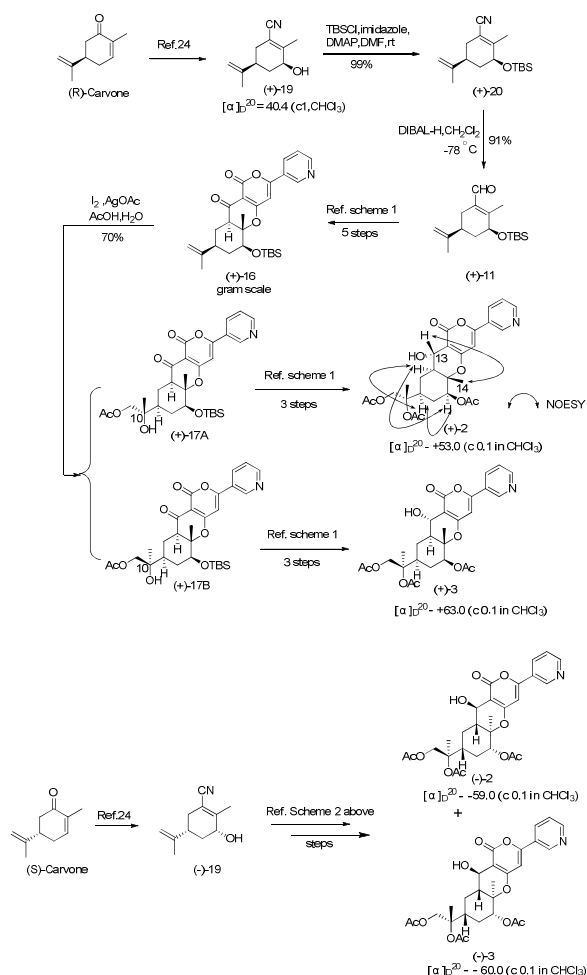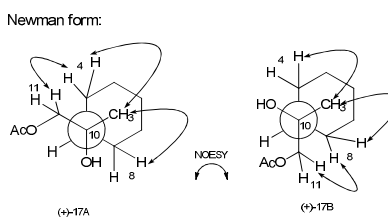

Figure 2. Identification of the configuration at stereocenter C_{10}.

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.

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.

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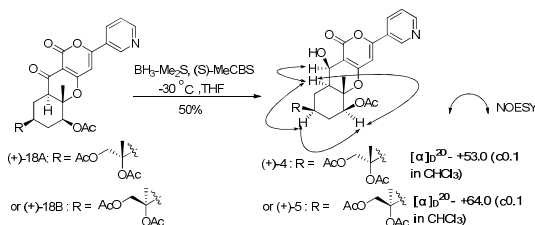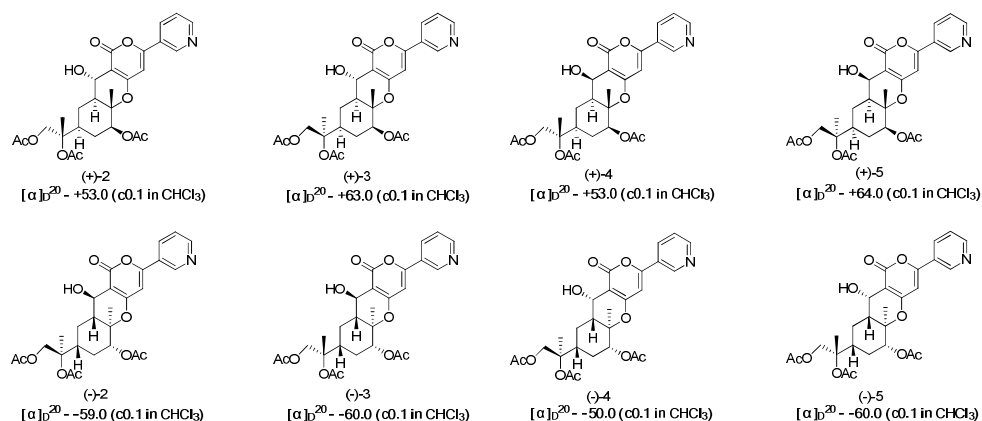

Scheme 3. Preparation of (+)-4 and (+)-5.

Figure 3. The structure of eight isomers: (+)/(-)-2-5.

Recently, Nagamitsu *et al.* reported[27,28] several non-ring-A PPPA-based analogues, which shared same core-structure of 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 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 druglike and selective ACAT2 inhibitors with potential therapeutic use in atherosclerosis and HCC. Further studies assessing the SARs of these compounds are in progress.

Acknowledgements

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Table1. 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 determining the NBD22-Steryl ester fluorescence. b: By determining the cholesterol oxidase assay. IC$_{50}$ values were means ± SD of three replications. c : From Ref.29.   d: Not test.

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