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Synthesis and structure–activity relationship of pyripyropene A derivatives as potent and selective acyl-CoA:cholesterol acyltransferase 2 (ACAT2) inhibitors: Part 3

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ABSTRACT

In an effort to develop potent and selective inhibitors toward ACAT2, structure–activity relationship studies were carried out using derivatives based on pyripyropene A (PPPA, **1**). In particular, we investigated the possibility of introducing appropriate 1,11-*O*-benzylidene and 7-*O*-substituted benzoyl moieties into PPPA (**1**). The new *o*-substituted benzylidene derivatives showed higher selectivity for ACAT2 than PPPA (**1**). Among them, 1,11-*O*-*o*-methylbenzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivative **7q** and 1,11-*O*-*o*,*o*-dimethylbenzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivative **7z** proved to be potent ACAT2 inhibitors with unprecedented high isozyme selectivity.

Acyl-CoA:cholesterol acyltransferase (ACAT) plays an important role in cholesterol metabolism in mammals. Recent molecular biological studies revealed the presence of two ACAT isozymes, ACAT1 and ACAT2, which have different functions in mammals.[1–4] ACAT1 is ubiquitously expressed in tissues and cells such as sebaceous glands, steroidogenic tissues, and macrophages, whereas ACAT2 is predominantly expressed in the liver and intestine.[5] Consequently, ACAT2-selective inhibitors could be employed as effective cholesterol-lowering or anti-atherosclerotic agents, with fewer side-effects than ACAT1-selective inhibitors. A newly developed cell-based assay using ACAT1- or ACAT2-expressing CHO cells[6,7] confirmed that pyripyropene A (PPPA, **1**) is a potent and selective inhibitor of ACAT2. Recent clinical studies showed that synthetic avasimibe and pactimibe, which can inhibit both ACAT1 and ACAT2, do not attenuate the progression of atherosclerosis.[8,9] This may be because the inhibition of ACAT1 in vascular cells, including macrophages, causes the excessive accumulation of free cholesterol in the cells and thus cytotoxicity.[9] Very recently, **1** was proven to be orally active in an in vivo atherogenic mouse model.[10,11] Therefore, our group re-investigated the synthesis of ACAT2-selective inhibitors based on PPPA derivatives for the development of cholesterol-lowering or anti-atherosclerotic agents.

We have previously described structure–activity relationship (SAR) studies of PPPA derivatives with a variety of substituted benzoyl groups at the 7-position[12] and 7-*O*-*p*-cyanobenzoyl PPPA derivatives with various acyl groups at the 1- and 11-positions.[13] As shown in Figure 1, 7-*O*-*p*-cyanobenzoyl PPPA derivative **2**, which exhibited higher ACAT2-innibitory activity (77 times) and isozyme selectivity (4.6 times) than PPPA (**1**), was developed based on results from our previous SAR studies. To the best of our knowledge, **2** is the most potent ACAT2-inhibitor known, with higher isozyme selectivity than **1**.

For the current SAR study, we focused on new 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives. The ACAT2 inhibitory activity of 1,11-*O*-benzylidene acetal derivatives **3** and **4** synthesized during earlier SAR studies[14,15] was comparable to **1**, whereas the PPPA derivatives showed lower isozyme selectivity than **1**. Herein, we report SAR studies of 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives, as well as the discovery of new PPPA derivatives with more potent ACAT2 inhibitory activity than **1** and unprecedented high isozyme selectivity.

The 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives **7** were prepared from pyripyropene tetraol **5** in two steps as shown in Scheme 1.[14]

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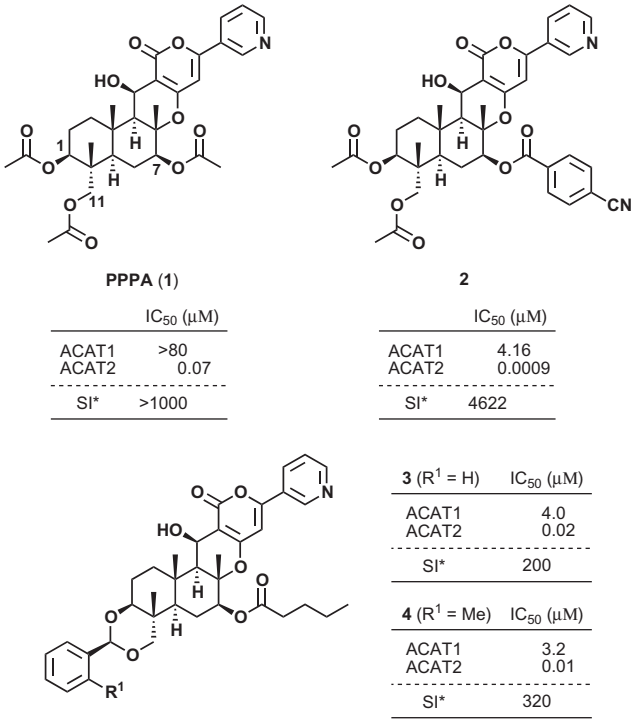
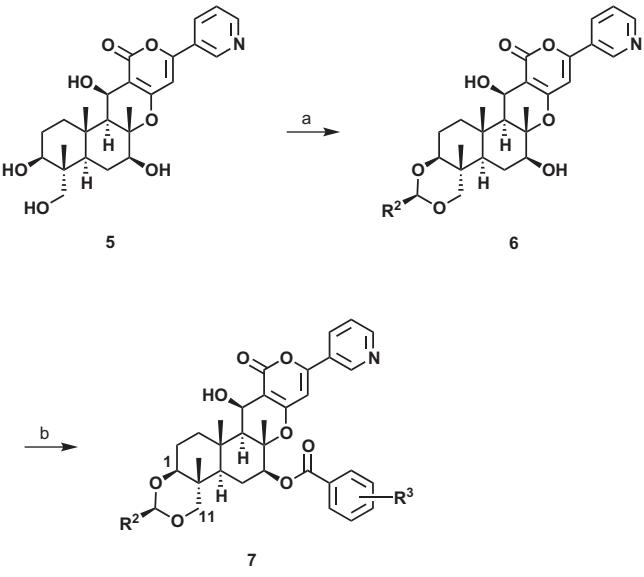


Figure 1. Structures of PPPA (**1**), 7-*O*-*p*-cyanobenzoyl PPPA derivative **2**, and 1,11-*O*-benzylidene-7-*O*-*p*-pentanoyl PPPA derivatives **3** and **4** (*Selectivity Index (SI): IC_{50} (ACAT1)/(IC_{50} (ACAT2)).

The ACAT2 inhibitory activity (IC_{50} values) and isozyme selectivity (SI values) of **1** and synthetic derivatives **7a**–**p** are listed in Table 1. Most 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl derivatives **7** showed higher ACAT2 inhibitory activity than **1**, except **7n**–**7p**, which have an *ortho*-substituent on the phenyl group in the benzoyl moiety. Among PPPA derivatives **7a**–**m**, 7-*O*-*p*-cyanobenzoyl derivative **7a** provided the best ACAT2 inhibitory activity (IC_{50} = 0.0060 μM). 7-*O*-*p*-Halobenzoyl derivatives **7b**–**d**, and especially 7-*O*-*p*-fluorobenzoyl derivatives **7b**, also showed high isozyme selectivity (SI = 885.7) comparable to that of **1**. The results indicate that 7-*O*-*p*-cyanobenzoyl and 7-*O*-*p*-halobenzoyl groups are requisite for high ACAT2 inhibitory activity and isozyme selectivity, and that the position of the substituent on the phenyl group in the benzoyl group is also critical: the ACAT2 inhibitory activity decreased in the order of *para*-, *meta*-, and *ortho*-substitution. These trends are consistent with those observed in our previous SAR study.[12]

The new 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl derivatives **7a**–**m** exhibited higher ACAT2 inhibitory activity than **1**, but their SI value was lower. Therefore, the phenyl group in the benzylidene acetal was chemically modified and additional SAR studies were conducted. These studies lead to the selection of the *p*-cyanobenzoyl and *p*-fluorobenzoyl groups as the 7-*O*-substituent.[12]

Table 2 shows the ACAT2 inhibitory activity and isozyme selectivity of new PPPA derivatives **7q**–**aa**, synthesized as described in Scheme 1. Derivatives **7q**–**v**, with a methyl group on the phenyl moiety in the benzylidene acetal, showed higher ACAT2 inhibitory activity than **1**, although only 1,11-*O*-*o*-methylbenzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivative **7q**[16] showed higher isozyme selectivity (SI >6161) than PPPA (**1**) and **2**. We assumed that the



Scheme 1. Reagents and conditions: (a) corresponding aldehydes or dimethylacetals, PPTS, DMF, rt; (b) corresponding aromatic carboxylic acids, EDCI, cat. DMAP, CH_2Cl_2, rt, 7**a**–**aa**: 20–67%.

Table 1
ACAT1 and 2 inhibitory activity and isozyme selectivity of 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives **7a**–**p** (R^2 = Ph)

Compound		IC_{50} (μM)		
No.	R^3	ACAT1	ACAT2	SI*
7a	*p*-CN	2.80	0.0060	466.7
7b	*p*-F	6.20	0.0070	885.7
7c	*p*-Cl	5.80	0.0090	644.4
7d	*p*-Br	6.90	0.0100	690.0
7e	*m*-F	3.00	0.0130	230.8
7f	*p*-OMe	6.90	0.0140	492.9
7g	*m*-Cl	2.70	0.0190	142.1
7h	*m*-CN	2.20	0.0300	73.3
7i	*m*-Br	4.30	0.0300	143.3
7j	*p*-CHO	5.50	0.0300	183.3
7k	*o*-F	2.70	0.0500	54.0
7l	*p*-NO_2	13.90	0.0600	231.7
7m	*m*-OMe	4.30	0.0700	61.4
PPPA (**1**)	—	>80	0.0700	>1000.0
7n	*o*-Cl	1.78	0.1100	16.2
7o	*o*-CN	0.79	0.3200	2.5
7p	*o*-OMe	3.40	0.3200	10.5

The PPPA derivatives are sorted in descending order based on ACAT2 inhibitory activity.
* Selectivity Index (SI): IC_{50} (ACAT1)/IC_{50} (ACAT2).

Table 2
ACAT1 and 2 inhibitory activity and isozyme selectivity of 1,11-*O*-*o*-substituted benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives **7q**–**aa**

Compound			IC_{50} (μM)		
No.	R^2	R^3	ACAT1	ACAT2	SI*
7q	*o*-MePh	*p*-CN	>72.8	0.0118	>6161
7r	*o*-MePh	*p*-F	13.9	0.0230	604.3
7s	*m*-MePh	*p*-CN	3.73	0.0078	478.2
7t	*m*-MePh	*p*-F	9.51	0.0476	199.8
7u	*p*-MePh	*p*-CN	8.11	0.0097	836.1
7v	*p*-MePh	*p*-F	5.54	0.0151	366.9
7w	*o*-MeOPh	*p*-CN	>71.0	0.0368	>1929
7x	*o*-FPh	*p*-CN	0.33	0.0068	48.5
7y	*o*,*p*-diMePh	*p*-CN	10.0	0.0066	1515
7z	*o*,*o*-diMePh	*p*-CN	>71.2	0.0072	>9916
7aa	α-Naphthyl	*p*-CN	>69.1	0.0232	>2978
PPPA (**1**)	—	—	>80	0.0700	>1000.0

* Selectivity Index (SI): IC_{50} (ACAT1)/IC_{50} (ACAT2).

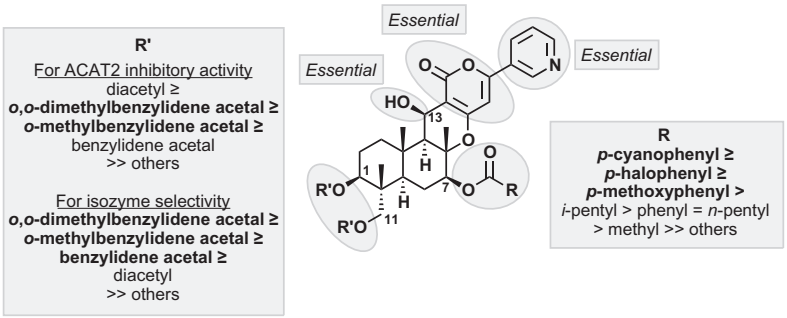


Figure 2. Summary of our earlier and our latest SAR studies on synthetic PPPA derivatives.

presence of the *o*-substituent on the phenyl group in the benzylidene acetal plays an important role in high isozyme selectivity and thus synthesized other 1,11-*O*-*o*-substituted benzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivatives. Unfortunately, all attempts to synthesize derivatives with substituents other than methyl, methoxy or fluoro groups failed due to steric hindrance at the *ortho* position. Although derivative **7x**, with an *o*-fluoro group, showed low isozyme selectivity, derivative **7w**, with an *o*-methoxy group, exhibited higher isozyme selectivity than **1**, as expected. In addition, we designed and synthesized new derivatives **7y** (with *o*,*p*-dimethyl groups) and **7z** (with *o*,*o*-dimethyl groups). Both derivatives exhibited better ACAT2 inhibitory activity and isozyme selectivity than **1**. In particular, *o*,*o*-dimethylbenzylidene derivative **7z** proved to be a potent ACAT2 inhibitor with the highest isozyme selectivity (SI >9916) observed to date. The α-naphthyl derivative **7aa** also showed higher isozyme selectivity than **1**. These results show that the *o*-substituent on the phenyl group in the benzylidene acetal, and especially a methyl group, contributes significantly to high isozyme selectivity.

Figure 2 summarizes all our ACAT2 inhibitory activity and isozyme selectivity SAR results on synthetic PPPA derivatives. Our initial SAR study demonstrated that the 3-pyridinyl, α-pyrone, and 13-hydroxy groups are essential. The study also showed that some acyl groups at the 7-hydroxy position, and diacetyl groups and benzylidene acetal at the 1,11-dihydroxy position, augment ACAT2 inhibitory activity, but decrease isozyme selectivity. These groups are shown in plain text encircled by pale color (Fig. 2). In contrast, our latest SAR study revealed that the *p*-cyanobenzoyl group at the 7-hydroxy position and *o*-substituted benzylidene acetal at the 1,11-dihydroxy position are superior to other substituents, and are shown in bold text encircled by pale color (Fig. 2).

In conclusion, novel 1,11-*O*-benzylidene-7-*O*-monosubstituted benzoyl PPPA derivatives were prepared and evaluated in cell-based assays to measure ACAT1 and ACAT2 inhibition.[17] Most of the synthetic PPPA derivatives showed more potent ACAT2 inhibitory activity than natural PPPA (**1**). Among them, four derivatives (**7q**, **7w**, **7z**, and **7aa**) with an *o*-substituent on the phenyl group in the benzylidene acetal exhibited higher isozyme selectivity than **1**. In particular, 1,11-*O*-*o*-methylbenzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivative **7q** and 1,11-*O*-*o*,*o*-dimethylbenzylidene-7-*O*-*p*-cyanobenzoyl PPPA derivative **7z** proved to be potent ACAT2 inhibitors with unprecedented high isozyme selectivity (SI: >6161 and >9916, respectively). The in vivo antiatherosclerotic activity of these derivatives will be reported elsewhere. Further SAR studies of the PPPA analogues are currently underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.075.

References and notes

1. Chang, C. C.; Huh, H. Y.; Cadigan, K. M.; Chang, T. Y. *J. Biol. Chem.* **1993**, *268*, 20747.
2. Anderson, R. A.; Joyce, C.; Davis, M.; Reagan, J. W.; Clark, M.; Shelness, G. S.; Rudel, L. L. *J. Biol. Chem.* **1998**, *273*, 26747.
3. Cases, S.; Novak, S.; Zheng, Y. W.; Myers, H. M.; Lear, S. R.; Sande, E.; Welch, C. B.; Lusis, A. J.; Spencer, T. A.; Krause, B. R.; Erickson, S. K.; Farese, R. V., Jr. *J. Biol. Chem.* **1998**, *273*, 26755.
4. Oelkers, P.; Behari, A.; Cromley, D.; Billheimer, J. T.; Sturley, S. L. *J. Biol. Chem.* **1998**, *273*, 26765.
5. Parini, P.; Davis, M.; Lada, A. T.; Erickson, S. K.; Wright, T. L.; Gustafsson, U.; Sahlin, S.; Einarsson, C.; Eriksson, M.; Angelin, B.; Tomoda, H.; Ōmura, S.; Willingham, M. C.; Rudel, L. L. *Circulation* **2004**, *2017*, 110.
6. Lada, A. T.; Davis, M.; Kent, C.; Chapman, J.; Tomoda, H.; Ōmura, S.; Rudel, L. L. *J. Lipid Res.* **2004**, *45*, 378.
7. Ohshiro, T.; Rudel, L. L.; Ōmura, S.; Tomoda, H. *J. Antibiot.* **2007**, *60*, 43.
8. Tardif, J. C.; Gregoire, J.; L'Allier, P. L.; Anderson, T. J.; Bertrand, O.; Reeves, F.; Title, L. M.; Alfonso, F.; Schampaert, E.; Hassan, A.; McLain, R.; Pressler, M. L.; Ibrahim, R.; Lesperance, J.; Blue, J.; Heinonen, T.; Rodes-Cabau, J. *Circulation* **2004**, *110*, 3372.
9. Nissen, S. E.; Tuzcu, E. M.; Brewer, H. B.; Sipahi, I.; Nicholls, S. J.; Ganz, P.; Schoenhagen, P.; Waters, D. D.; Pepine, C. J.; Crowe, T. D.; Davidson, M. H.; Deanfield, J. E.; Winsniewski, L. M.; Hanyok, J. J.; Kassalow, L. M. N. *Engl. J. Med.* **2006**, *354*, 1253.
10. Ohshiro, T.; Matsuda, D.; Sakai, K.; Degirolamo, C.; Yagyu, H.; Rudel, L. L.; Ōmura, S.; Ishibashi, S.; Tomoda, H. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1108.
11. Ohshiro, T.; Tomoda, H. *Future Med. Chem.* **2011**, *3*, 2039.
12. Ohtawa, M.; Yamazaki, H.; Ohte, S.; Matsuda, D.; Ohshiro, T.; Rudel, L. L.; Ōmura, S.; Tomoda, H.; Nagamitsu, T. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1285.
13. Ohtawa, M.; Yamazaki, H.; Matsuda, D.; Ohshiro, T.; Rudel, L. L.; Ōmura, S.; Tomoda, H.; Nagamitsu, T. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2659.
14. Obata, R.; Sunazuka, T.; Kato, Y.; Tomoda, H.; Harigaya, Y.; Ōmura, S. *J. Antibiot.* **1996**, *49*, 1149.
15. Ohshiro, T.; Ohte, S.; Matsuda, D.; Ohtawa, M.; Nagamitsu, T.; Sunazuka, T.; Harigaya, Y.; Rudel, L. L.; Ōmura, S.; Tomoda, H. *J. Antibiot.* **2008**, *61*, 503.
16. *Selected spectra data for* **7q**: $[\alpha]_D^{24}$ + 208 (*c* 1.0, $CHCl_3$); IR (KBr) 3434, 2947, 2863, 2233, 1708, 1643, 1579, 1414, 1273, 1219, 933, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.97 (dd, 1H, H-2″, *J* = 0.8, 3.2 Hz), 8.67 (dd, 1H, H-6″, *J* = 2.0, 6.4 Hz), 8.22 (d, 2H, H–Ar, *J* = 11.2 Hz), 8.09–8.05 (m, 1H, H-4″), 7.81 (d, 2H, H–Ar, *J* = 11.2 Hz), 7.65–7.62 (m, 1H, H–Ar), 7.40–7.36 (m, 1H, H-5″), 7.24–7.23 (m, 2H, H–Ar), 7.22–7.13 (m, 1H, H–Ar), 6.40 (s, 1H, H-5′), 5.70 (s, 1H, C*H*Ar), 5.30 (dd, 1H, H-7, *J* = 8.0, 12.8 Hz), 5.05 (dd, 1H, H-13, *J* = 3.2, 4.8 Hz), 3.90 (d, 1H, H-11a, *J* = 13.6 Hz), 3.58–3.52 (m, 2H, H-11b, *J* = 13.6 Hz), 3.00 (br d, 1H, OH-13, *J* = 2.8 Hz), 2.40 (s, 3H, Ar*Me*), 2.29 (d, 1H, H-3a, *J* = 17.2 Hz), 2.05–1.18

(m, 7H, H-2, 3b, 5, 8, 9), 1.86 (s, 3H, Me), 1.53 (s, 3H, Me), 1.26 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.06, 163.86, 162.00, 157.45, 151.62, 146.78, 135.97, 135.53, 133.85, 132.93, 132.39, 130.42, 130.26, 128.86, 127.03, 126.01, 125.95, 123.63, 117.85, 116.77, 103.03, 100.76, 99.24, 85.81, 83.34, 79.42, 78.29, 60.39, 60.01, 54.67, 48.83, 38.38, 37.07, 36.50, 25.04, 23.14, 21.06, 18.77, 18.21, 16.72, 13.39.
ESI-LRMS m/z 689 (MH^+); ESI-HRMS (TFA-Na) calcd for $C_{41}H_{41}N_2O_8$ 689.2863 (MH^+), found 689.2885 (MH^+).

17. Tomoda, H.; Nagamitsu, T.; Matsuda, D.; Ohshiro, T.; Ohtawa, M. Ōmura, S. PCT Int. Appl. WO 2011122468, 2011.