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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 1

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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**). We have successfully developed novel PPPA derivatives with a 7-*O*-substituted benzoyl substituent that significantly exhibit more potent ACAT2 inhibitory activity and higher ACAT2 isozyme selectivity than **1**.

Acyl-CoA:cholesterol acyltransferase (ACAT) is known to have an important role in cholesterol metabolism in mammals. Accordingly, numerous synthetic ACAT inhibitors such as ureas, imidazoles, and amides have been reported.[1,2] Unfortunately, these efforts have failed to lead to new types of cholesterol-lowering or anti-atherosclerotic agents.

Subsequently, since the 1990s, we have searched for ACAT inhibitors of natural origin to obtain ACAT inhibitors with novel chemical structures. Among the various natural ACAT inhibitors examined,[3] pyripyropenes of fungal origin exhibited the most potent ACAT inhibitory effects in enzyme assays using rat liver microsomes.[4–6] Accordingly, a primary structure–activity relationship (SAR) study was carried out by preparing approximately 200 semisynthetic derivatives of pyripyropene A (PPPA, **1**). Several derivatives exhibited higher in vitro potency than the parent compound **1**.[7–12]

Recent molecular biological studies revealed the presence of two ACAT isozymes with different functions in mammals, ACAT1 and ACAT2.[13–16] ACAT1 is ubiquitously expressed in tissues and cells such as sebaceous glands, steroidogenic tissues, and macrophages, while ACAT2 is predominantly expressed in the liver and intestine.[17]

To evaluate the isozyme-selectivity of ACAT inhibitors, cell-based assays using ACAT1- and ACAT2-expressing Chinese hamster ovary (CHO) cells have been developed.[18,19] Most known ACAT inhibitors of synthetic and natural origins inhibited both ACAT1 and ACAT2 or selectively inhibited ACAT1, but only pyripyropenes showed potent and selective inhibition toward ACAT2. Furthermore, among 200 semisynthetic PPPA derivatives, **1** was found to be the most ACAT2-selective inhibitor.[20,21]

Recently, synthetic avasimibe and pactimibe, which can inhibit both ACAT1 and ACAT2, failed to inhibit the progression of atherosclerosis in clinical studies.[22,23] One of the reasons may be that inhibition of ACAT1 in vascular cells, including macrophages, is cytotoxic because of the excessive accumulation of free cholesterol in these cells.[23] However, the in vivo efficacy of ACAT2 selective inhibitors had not been studied. Very recently, **1** was proven to be orally active in an in vivo atherogenic mouse model.[24] 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. Herein, we report the discovery and SAR studies of 7-*O*-substituted benzoyl PPPA derivatives, a new group of PPPA derivatives, with better potency and higher selectivity against ACAT2 than **1**.

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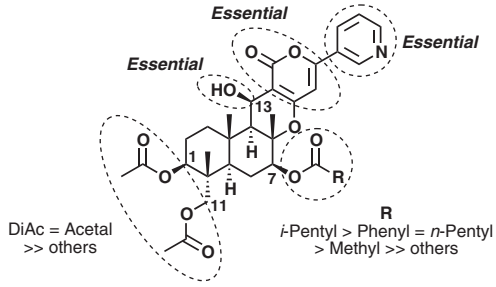
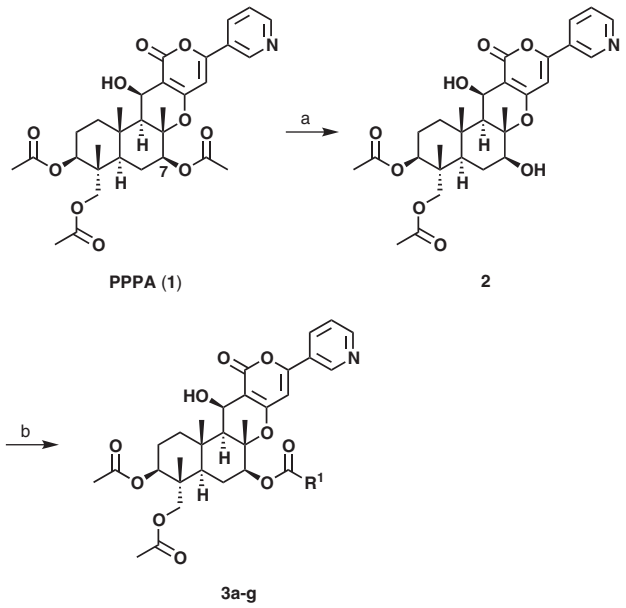


Figure 1. Summary of the primary SAR study of the synthetic PPPA derivatives.



Scheme 1. Reagents and conditions: (a) DBU, aq MeOH, 0 °C, 52%; (b) corresponding aromatic carboxylic acids, EDCI, cat. DMAP, CH_2Cl_2, rt, **3a–g**: 92–96% (Table 1), **3h–ai**: 65–98% (Table 2), **3aj–aq**: 73–79% (Table 3).

Figure 1 summarizes the ACAT2 inhibition results from a SAR study of previously synthesized PPPA derivatives. In brief, (1) the 3-pyridinyl, α-pyrone, and 13-hydroxy groups are necessary; (2) the diacetyl and acetal groups at the 1,11-dihydroxy position are suitable for modification; and (3) the acyl group on the 7-*O*-hydroxyl position is necessary—the effect of the R group follows *i*-pentyl > phenyl = *n*-pentyl > methyl ≫ others.

The present SAR study focused on PPPA derivatives with various aromatic substituents on the 7-*O*-acyl group that have not yet been sufficiently synthesized.[25] The 7-*O*-aromatic acyl PPPA derivatives **3a–g** were prepared from **1** in 2 steps as shown in Scheme 1.[9]

The ACAT2 inhibitory activity (IC_{50} values) and isozyme selectivity (SI values) for **1** and synthetic derivatives **3a–g** are listed in Table 1. Surprisingly, all of the new derivatives except **3f** exhibited higher ACAT2 inhibitory activity than **1** (IC_{50}, 0.07 μM). Among them, the 7-*O*-*p*-methylbenzoyl derivative **3c** had the most potent ACAT2 inhibitory activity, with a 77-fold higher potency than **1** (IC_{50}, 0.0009 μM). Although the SI values of **3a–g** were lower than that of **1**, these results encouraged further investigations by preparing various 7-*O*-monosubstituted benzoyl derivatives.

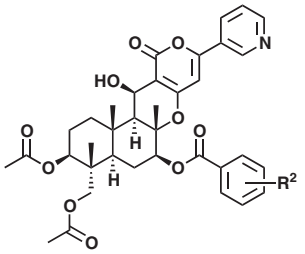
7-*O*-Monosubstituted benzoyl derivatives **3h–ai** were synthesized in 65–98% yield according to the procedures described in Scheme 1.[9] Remarkably, as shown in Table 2, the monosubstituted benzoyl groups contributed to significantly higher ACAT2 inhibitory activities. Results indicated that the position of the substituent on the phenyl group was critical: the ACAT2 inhibitory activity decreased in order of *para*-, *meta*-, and *ortho*-substitution. In contrast, differences between the electron-withdrawing and -donating substituents on the phenyl group did not affect the ACAT2 inhibitory

Table 1
ACAT1 and 2 inhibitory activities and isozyme selectivity for 7-*O*-aromatic acyl PPPA derivatives **3a–g**

Compound		IC_{50} (μM)		
No.	R^1	ACAT1	ACAT2	SI[a]
3a	Phenyl	0.68	0.0100	68.0
3b	4-Biphenyl	0.70	0.0095	73.7
3c	*p*-Methylphenyl	0.27	0.0009	300.0
3d	2-Naphthyl	0.71	0.0190	37.4
3e	2,2-Difluoro-1,3-benzodioxole-5-yl	0.46	0.0200	23.0
3f	1-Naphthyl	3.20	0.2700	11.9
3g	2-Benzo[*b*]thio-phenyl	3.80	0.0400	95.0
1	Methyl	>80	0.0700	>1000.0

[a] Selectivity index (SI): IC_{50} (ACAT1)/IC_{50} (ACAT2).

Table 2
ACAT1 and 2 inhibitory activities and isozyme selectivity for 7-*O*-monosubstituted benzoyl PPPA derivatives **3h–ai**



Compound		IC_{50} (μM)		
No.	R^2	ACAT1	ACAT2	SI[a]
3h	*p*-OMe	0.51	0.0008	637.0
3i	*p*-CN	4.16	0.0009	4622.0
3c	*p*-Me	0.27	0.0009	300.0
3j	*m*-F	0.23	0.0009	256.0
3k	*p*-Cl	0.69	0.0010	690.0
3l	*p*-Et	0.19	0.0010	190.0
3m	*m*-Br	0.12	0.0012	100.0
3n	*p*-F	0.79	0.0017	465.0
3o	*p*-Br	0.83	0.0020	415.0
3p	*p*-SMe	0.54	0.0027	200.0
3q	*p*-I	1.17	0.0040	292.5
3r	*p*-NO_2	7.41	0.0050	1482.5
3s	*p*-Vinyl	0.71	0.0050	142.0
3t	*m*-Me	0.65	0.0050	130.0
3u	*m*-I	0.25	0.0050	50.0
3v	*o*-I	0.19	0.0050	38.0
3w	*p*-N_3	0.66	0.0050	132.0
3x	*m*-Cl	0.21	0.0070	30.0
3y	*m*-SMe	0.11	0.0090	12.2
3z	*m*-CN	2.23	0.0100	223.0
3a	H	0.68	0.0100	68.0
3aa	*o*-Me	0.42	0.0100	42.0
3ab	*m*-Vinyl	0.15	0.0110	13.6
3ac	*m*-OMe	0.15	0.0150	10.0
3ad	*o*-F	1.00	0.0300	33.3
3ae	*o*-Cl	1.00	0.0600	16.7
1		>80	0.0700	>1000.0
3af[b]	*p*-NH_2	5.10	0.2000	25.5
3ag	*o*-OMe	2.69	0.2300	11.7
3ah	*o*-CN	1.62	0.2900	5.6
3ai[b]	*p*-OH	2.80	0.2700	10.4

The PPPA derivatives were sorted in descending order of ACAT2 inhibitory activity.
[a] Selectivity index (SI): IC_{50} (ACAT1)/IC_{50} (ACAT2).
[b] See Ref. 26.

Table 3
ACAT1 and 2 inhibitory activities and isozyme selectivity for 7-*O*-di- and trisubstituted benzoyl PPPA derivatives **3aj**–**aq**

Compound		IC_{50} (μM)		
No.	R^2	ACAT1	ACAT2	SI[a]
3aj	*m*-F, *p*-Br	0.49	0.0050	98.0
3ak	*m*-F, *p*-NO_2	4.14	0.0060	690.0
3al	*m*-F, *p*-OMe	0.96	0.0061	157.4
3am	*m*-F, *p*-Me	0.43	0.0063	68.3
3an	*o*-F, *p*-Br	1.15	0.0070	164.3
3ao	*m*-F, *p*-CN	0.65	0.0078	83.3
3ap	*o*, *m*, *p*-F	1.43	0.0081	176.5
3aq	*o*-I, *p*-Cl	0.89	0.0390	22.8
1		>80	0.0700	>1000.0

The PPPA derivatives were sorted in descending order of ACAT2 inhibitory activity.
[a] Selectivity index (SI): IC_{50} (ACAT1)/IC_{50} (ACAT2).

activity. The *p*-cyanobenzoyl (**3i**) and *p*-nitrobenzoyl (**3r**) derivatives showed not only more potent ACAT2 inhibitory activity but also higher isozyme selectivity than **1**. To the best of our knowledge, **3i**[27] is the most potent ACAT2 inhibitor with the highest isozyme selectivity.

Our investigations also included 7-*O*-di- and tri-substituted benzoyl derivatives (Table 3, **3aj**–**aq**). Although such di- or trisubstituted derivatives, which were prepared following Scheme 1, possess higher ACAT2 inhibitory activity than **1**, their isozyme selectivities fell short of our expectations.

In conclusion, novel PPPA derivatives with a 7-*O*-substituted benzoyl substituent were prepared and evaluated in cell-based assays for measuring ACAT1 and ACAT2 inhibition. Among them, 21 derivatives showed ACAT2 inhibitory activity with selectivity indexes (SI) of >100, and 4 derivatives strongly inhibited ACAT2 with pico molar IC_{50} values. It is important to note that 7-*O*-*p*-cyanobenzoyl (**3i**) and 7-*O*-*p*-nitrobenzoyl (**3r**) derivatives exhibited higher isozyme selectivity than **1**.[28] 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.

Acknowledgments

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References and notes

1. Roth, B. D. *Drug Discovery Today* **1998**, *3*, 19.
2. Kathawala, F. G.; Heider, J. C. In *Antilipidemic Drugs*; Witiak, D. T., Newman, H. A. I., Feller, D. R., Eds.; Elsevier: New York, 1992; p 159.
3. Tomoda, H.; Ōmura, S. *Pharmacol. Ther.* **2007**, *115*, 375.
4. Ōmura, S.; Tomoda, H.; Kim, Y. K.; Nishida, H. *J. Antibiot.* **1993**, *46*, 1168.
5. Tomoda, H.; Kim, Y. K.; Nishida, H.; Masuma, R.; Ōmura, S. *J. Antibiot.* **1994**, *47*, 148.
6. Kim, Y. K.; Tomoda, H.; Nishida, H.; Sunazuka, T.; Obata, R.; Ōmura, S. *J. Antibiot.* **1994**, *47*, 154.
7. Obata, R.; Sunazuka, T.; Li, Z.; Tomoda, H.; Ōmura, S. *J. Antibiot.* **1995**, *48*, 749.
8. Obata, R.; Sunazuka, T.; Tomoda, H.; Harigaya, Y.; Ōmura, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2683.
9. Obata, R.; Sunazuka, T.; Li, Z.; Tian, Z.; Harigaya, Y.; Tabata, N.; Tomoda, H.; Ōmura, S. *J. Antibiot.* **1996**, *49*, 1133.
10. Obata, R.; Sunazuka, T.; Kato, Y.; Tomoda, H.; Harigaya, Y.; Ōmura, S. *J. Antibiot.* **1996**, *49*, 1149.
11. Obata, R.; Sunazuka, T.; Tian, Z.; Tomoda, H.; Harigaya, Y.; Ōmura, S. *J. Antibiot.* **1997**, *50*, 229.
12. Obata, R.; Sunazuka, T.; Tian, Z.; Tomoda, H.; Harigaya, Y.; Ōmura, S.; Smith, A. B., III *Chem. Lett.* **1997**, *26*, 935.
13. Chang, C. C.; Huh, H. Y.; Cadigan, K. M.; Chang, T. Y. *J. Biol. Chem.* **1993**, *268*, 20747.
14. Anderson, R. A.; Joyce, C.; Davis, M.; Reagan, J. W.; Clark, M.; Shelness, G. S.; Rudel, L. L. *J. Biol. Chem.* **1998**, *273*, 26747.
15. 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.
16. Oelkers, P.; Behari, A.; Cromley, D.; Billheimer, J. T.; Sturley, S. L. *J. Biol. Chem.* **1998**, *273*, 26765.
17. 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**, *110*, 2017.
18. Lada, A. T.; Davis, M.; Kent, C.; Chapman, J.; Tomoda, H.; Ōmura, S.; Rudel, L. L. *J. Lipid Res.* **2004**, *45*, 378.
19. Ohshiro, T.; Rudel, L. L.; Ōmura, S.; Tomoda, H. *J. Antibiot.* **2007**, *60*, 43.
20. 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.
21. Ohshiro, T.; Tomoda, H. *Future Med. Chem.* **2011**, *3*, 2039.
22. 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.
23. 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. Eng. J. Med.* **2006**, *354*, 1253.
24. 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.
25. Only the derivatives with phenyl, 3-pyridinyl, and *p*-azidophenyl as aromatic substituents in 7-*O*-acyl group had been synthesized in the primary SAR study.
26. (a) *p*-Aminobenzoyl PPPA **3af** was synthesized from **3w** through the Staudinger reaction with Me_3P (89% yield). (b) *p*-Hydroxybenzoyl PPPA derivative **3ai** was synthesized via esterification between **2** and the corresponding *p*-triisopropylsilyloxybenzoic acid (65% yield), which was derived from *p*-hydroxybenzaldehyde by protection of the hydroxyl group as a TIPS ether and oxidation of the aldehyde with $NaClO_2$ (92% yield in 2 steps), followed by deprotection of the TIPS group (72% yield).
27. Selected spectra data for **3i**: $[\alpha]_D^{24}$ +72 (*c* 1.0, $CHCl_3$); IR (KBr) 3490, 2950, 2230, 1730, 1640, 1580, 1270, 1250, 1110, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.96 (dd, 1H, H-2″, *J* = 2.4, 0.6 Hz), 8.67 (dd, 1H, H-6″, *J* = 4.8, 1.5 Hz), 8.21 (d, 2H, H-Ar, *J* = 7.8 Hz), 8.09–8.05 (m, 1H, H-4″), 7.80 (d, 2H, H-Ar, *J* = 7.8 Hz), 7.41–7.36 (m, 1H, H-5″), 6.40 (s, 1H, H-5′), 5.29 (dd, 1H, H-7, *J* = 11.1, 6.0 Hz), 5.04 (d, 1H, H-13, *J* = 4.2 Hz), 4.82 (dd, 1H, H-1, *J* = 11.0, 6.0 Hz), 3.82 (d, 1H, H-11a, *J* = 12.0 Hz), 3.73 (d, 1H, H-11b, *J* = 12.0 Hz), 3.00 (br s, 1H, OH-13), 2.18–1.50 (m, 8H, H-2, 3, 5, 8, 9), 2.13 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.85 (s, 3H, Me), 1.50 (s, 3H, Me), 0.91 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.90, 170.50, 163.87, 162.00, 159.68, 157.47, 153.70, 151.60, 146.76, 133.90, 132.95, 132.33, 132.33, 130.23, 130.23, 127.04, 123.62, 117.84, 116.71, 102.99, 99.20, 83.15, 79.29, 73.48, 64.84, 60.16, 54.75, 45.43, 40.38, 37.91, 36.14, 25.24, 22.68, 21.11, 20.82, 17.49, 16.55; HRMS (FAB, *m*-NBA) $[M+H]^+$ Calcd for $C_{37}H_{39}N_2O_{10}$ 671.2605. Found 671.2600.
28. Tomoda, H.; Nagamitsu, T.; Ōmura, S.; Rudel, L. L. PCT Int. Appl. WO 2009081957, 2009.