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Novel 1,4-diarylpiperidine-4-methylureas as anti-hyperlipidemic agents: Dual effectors on acyl-CoA:cholesterol *O*-acyltransferase and low-density lipoprotein receptor expression

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ABSTRACT

A family of 1,4-diarylpiperidine-4-methylureas were designed and synthesized as novel dual effectors on ACAT and LDL receptor expression. We examined SAR of the synthesized compounds focusing on substitution at the three aromatic parts of the starting compound **1** and succeeded in identifying essential substituents for inhibition of ACAT and up-regulation of hepatic LDL receptor expression. Especially, we found that compound **12f**, which can easily be prepared, has biological properties comparable to those of SMP-797, a promising ACAT inhibitor. In addition, the in vitro effects of **12f** on lipid metabolism were substantially superior to those of a known ACAT inhibitor, Avasimibe.

Hyperlipidemia and related cardiovascular diseases, such as atherosclerosis, are risk factors for stroke and coronary heart diseases, which are among the leading causes of death in many industrialized countries. Acyl-coenzyme A:cholesterol acyltransferase (ACAT), which catalyzes intracellular cholesterol esterification,[1] plays important roles in several physiological processes, such as absorption of dietary and biliary cholesterol in the small intestine,[2] secretion of very low-density lipoprotein (VLDL) in the liver,[3] and accumulation of cholesteryl esters in macrophages in the arterial wall.[4] It is therefore believed that inhibition of ACAT may lower plasma cholesterol level and help prevent atherosclerosis.

It is known that expression of hepatic low-density lipoprotein (LDL) receptor is extremely important for lipid homeostasis. Statins, which are HMG-CoA reductase inhibitors that increase hepatic LDL receptor expression, are known to be effective in lowering total cholesterol and LDL cholesterol levels in hyperlipidemic patients.[5] From these findings, it is believed that dual effectors on ACAT and LDL receptor expression may be promising agents in the treatment of hyperlipidemia and related cardiovascular diseases.

We have previously reported a novel ACAT inhibitor, SMP-797 (Fig. 1), with potent cholesterol-lowering activity and direct regressive effect on atherosclerotic lesions.[6] We have also shown that SMP-797 up-regulate hepatic LDL receptor expression, an effect not reported with known ACAT inhibitors. Furthermore, SMP-797 at a concentration much higher than that needed for LDL receptor up-regulation, that is, 1 μM, had no effect on cholesterol synthesis in HepG2 cells. From these findings, we expect SMP-797 to be a next generation anti-hyperlipidemic agent.

Although SMP-797 is a promising compound, its preparation requires eight reaction steps.[7] Our aim is therefore to find back-up compounds with different mother templates and easy synthetic routes. Based on previous studies of SMP-797 (Fig. 1), the 2,6-diisopropylphenylurea part is essential for ACAT inhibitory activity. Therefore we examined replacement of the 1,8-naphthyridine moiety with other hydrophilic groups and succeeded in finding com-

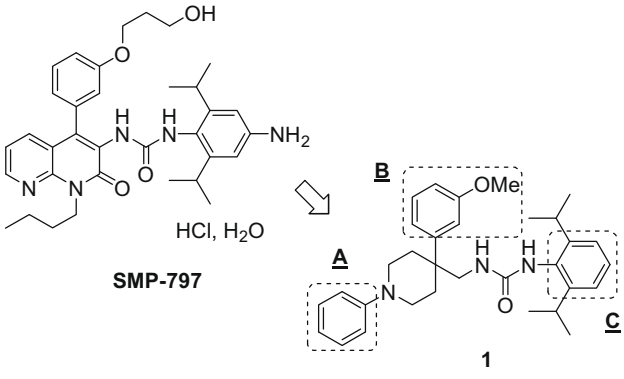

Figure 1. Structures of SMP-797 and compound **1**.

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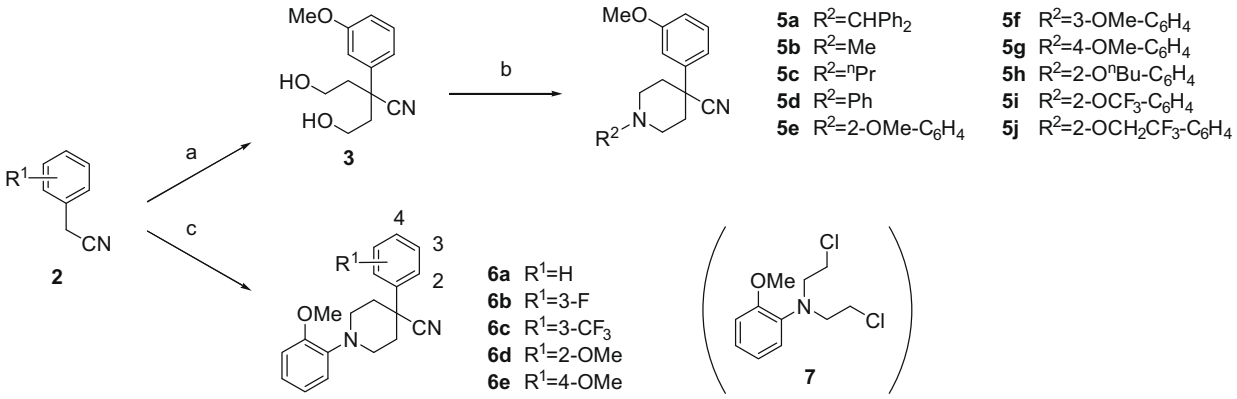
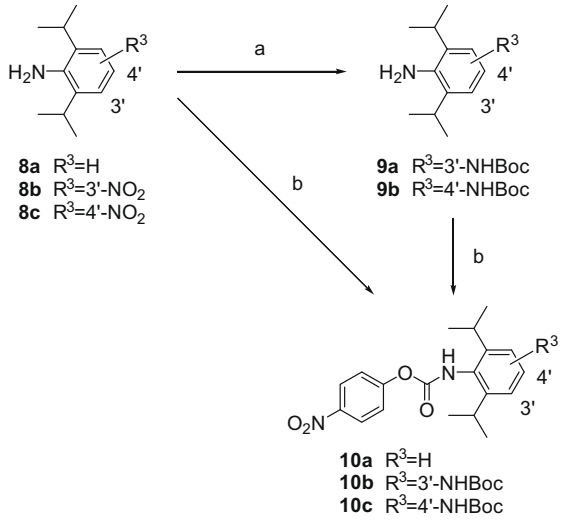


Scheme 1. Synthesis of Nitriles **5a–j** and **6a–e**. Reagents and conditions: (a) 1—NaH, $BrCH_2CH_2OTHP$, DMF, 0 °C → rt; 2—*p*-TsOH·H_2O, MeOH, rt, 74%; (b) 1—triflic anhydride, DIPEA, EtOAc, −30 °C; 2—R^2NH_2 **4**, DIPEA, −30 °C → rt, 26–89%; (c) **7**, $CH_3(CH_2)_{15}P^+Bu_3{\cdot}Br^-$, 50% NaOHaq, 100 °C, 52–68%.

pound **1** as a potent ACAT inhibitor. The IC_{50} of **1** toward ACAT (IC_{50} = 35 nM) was comparable to that of SMP-797 (IC_{50} = 31 nM). Herein, we reported the design, synthesis, and biological activity of a novel series of 1,4-diarylpiperidine-4-methylureas as anti-hyperlipidemic agents.

We initially synthesized the precursor nitriles **5** and **6** to afford the ureas **11** and **12**. These nitriles were prepared by two synthetic methods using different functional groups (Scheme 1). The first synthetic method was conversion of the *N*-substituted piperidines in part A, Figure 1. The diol **3** was prepared by common dialkylation of the 3-methoxyphenylacetonitrile with the commercially available 2-(2-bromoethoxyl)tetrahydro-2*H*-pyran followed by methanolysis. The diol **3** thus obtained was converted to the nitriles **5a–j** by one-pot preparation via bis-triflate with the appropriate amines **4** (R^2NH_2).[8] The second synthetic method was conversion of the substituent on the phenyl ring in part B, Figure 1. The nitriles 6a–**e** were prepared in moderate yields by dialkylation of the commercially available phenylaceto-nitriles **2** with *N*,*N*-bis(2-chloroethyl)anisidine[9] **7** in the presence of a catalytic amount of phosphonium bromide.[10]

The *p*-nitrophenylcarbamates **10a–c** were obtained as illustrated in Scheme 2. The carbamates were synthesized in a few reaction steps from the 2,6-diisopropylanilines **8a**, **9a**,[11] and **9b**,[7b]



Scheme 2. Synthesis of phenylcarbamates **10a–c**. Reagents and conditions: (a) 1—H_2, 10% Pd/C, MeOH, rt; 2—Boc_2O, 2 N NaOHaq, THF, rt, 75–87%; (b) *p*-$NO_2C_6H_4OCOCl$, THF, rt, 78–95%.

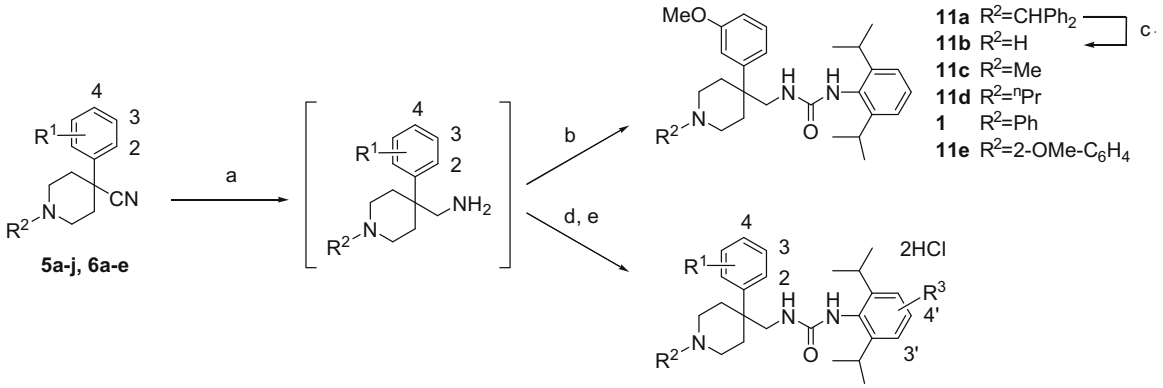
which were either commercially available or synthesized by known literature procedures. The stable key intermediates **9a** and **9b** were prepared by catalytic hydrogenation of the corresponding **8b** and **8c** followed by selective protection of the less hindered amino group by *tert*-butylcarbonyl group (Boc). Thus, the desired *p*-nitrophenylcarbamates **10a–c** could be obtained in excellent yields.

The ureas **1**, **11**, and **12** were achieved as illustrated in Scheme 3. These ureas were prepared in excellent yields by coupling reaction of the primary amines with the corresponding phenylcarbamates **10a–c**. The primary amines provided by reduction of the nitrile moiety were used following the coupling reaction without purification because of their polarity. In the case of compound **11b**, the benzohydryl group of compound **11a** was removed by palladium-catalyzed hydrogen transfer reaction with ammonium formate. In the case of urea **12**, Boc group was removed by 10% methanolic HCl in the final step to give the desired compounds as HCl salts.

Compounds **1**, **11b–e**, and **12a–e** inhibitory activity for ACAT and up-regulation of LDL receptor expression are shown in Table 1. As compared to **1** or **11e**, the inhibitory activity of **11b**, **11c**, or **11d** for ACAT decreased more than 10-fold. These findings suggest that the aryl substituent on the nitrogen of the piperidine (A-part) is important for ACAT inhibition. Compounds **1**, **11e**, and **12a–e** inhibited ACAT with similar IC_{50} values, however, only **12c** up-regulated LDL receptor expression. From these results it is assumed that the 2-methoxyphenyl group in A-part (Fig. 1) and the 4-amino-2,6-diisopropylphenyl group in C-part are essential for the dual inhibition of ACAT and up-regulation of LDL receptor expression.

Next, we turned our attention to the effects of a substitution on the phenyl ring of B-part (Fig. 1). The inhibitory activity for ACAT of compounds **12c** and **12i–m** and their up-regulation of LDL receptor expression are summarized in Table 2. Substitution on the phenyl ring of B-part was well tolerated for ACAT inhibitory activity. However, for up-regulation of LDL receptor expression, electron donating groups, such as a methoxy group, were preferred to electron-withdrawing groups.

Finally, in order to improve both the inhibitory activity for ACAT and the up-regulatory effect on LDL receptor expression, we carried out SAR studies on the 2-alkoxy groups of A-part. As shown in Table 3, 2-alkoxyl substituents on the phenyl ring were well tolerated with IC_{50} values for inhibition of ACAT ranging from 32 to 68 nM. Interestingly, the up-regulatory effect on LDL receptor expression was improved in the case of *n*-butoxy (**12f**)[12] and fluoroalkoxy (**12g** and **12h**) as compared to that of compound **12c**. These findings indicate that an increase in compounds hydrophobicity is effective in improving both the inhibitory activity for ACAT



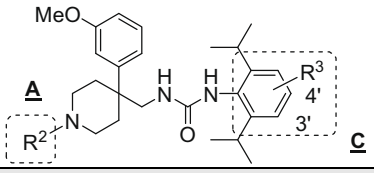
12a	R^1=3-OMe	R^2=Ph	R^3=4'-NH_2
12b	R^1=3-OMe	R^2=2-OMe-C_6H_4	R^3=3'-NH_2
12c	R^1=3-OMe	R^2=2-OMe-C_6H_4	R^3=4'-NH_2
12d	R^1=3-OMe	R^2=3-OMe-C_6H_4	R^3=4'-NH_2
12e	R^1=3-OMe	R^2=4-OMe-C_6H_4	R^3=4'-NH_2
12f	R^1=3-OMe	R^2=2-O^{n}Bu-C_6H_4	R^3=4'-NH_2
12g	R^1=3-OMe	R^2=2-OCF_3-C_6H_4	R^3=4'-NH_2
12h	R^1=3-OMe	R^2=2-OCH_2CF_3-C_6H_4	R^3=4'-NH_2
12i	R^1=H	R^2=2-OMe-C_6H_4	R^3=4'-NH_2
12j	R^1=3-F	R^2=2-OMe-C_6H_4	R^3=4'-NH_2
12k	R^1=3-CF_3	R^2=2-OMe-C_6H_4	R^3=4'-NH_2
12l	R^1=2-OMe	R^2=2-OMe-C_6H_4	R^3=4'-NH_2
12m	R^1=4-OMe	R^2=2-OMe-C_6H_4	R^3=4'-NH_2

Scheme 3. Synthesis of ureas **1**, **11a–e** and **12a–m**. Reagents and conditions: (a) $LiAlH_4$, THF, reflux; (b) **10a**, THF, rt, 85–92%; (c) 10% Pd/C, HCO_2NH_4, EtOH, reflux, 36%; (d) **10b** or **10c**, THF, rt; (e) 10% HCl/MeOH, rt, 70–91%.

and the up-regulatory effect on LDL receptor expression. Particularly, compound **12f** demonstrated biological properties comparable to those of SMP-797.

In summary, we disclose here a new class of piperidine-based ACAT inhibitors. These compounds, which like SMP-797 show potent ACAT inhibitory activity and remarkable up-regulation of hepatic LDL receptor expression, can easily be synthesized in three to five reaction steps. Furthermore, these compounds in vitro activity on lipid metabolism was substantially superior to that of a known ACAT inhibitor, Avasimibe (IC_{50} = 479 nM, in house data).[6,13] However, the solubility of these compounds, including the selected **12f** was not high enough (e.g., **12f**; 0.0003 mg/mL, at pH 7.4). In order to improve the solubility further optimization studies are necessary. In addition, studies to unveil the mechanism by which the synthesized compounds up-regulate hepatic LDL receptor expression are underway.

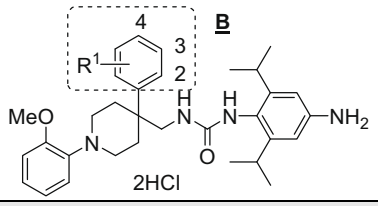
Table 1
Effects of a substitution on the nitrogen of piperidine (A-part) and 2,6-diisopropylphenyl (C-part) on biological activity



Compound	R^2	R^3	ACAT inhibition IC_{50}[a] (nM)	LDL receptor expression[b] (100 nM)
11b	H	H	>1000	nt[c]
11c	Me	H	664	nt[c]
11d	^{n}Pr	H	547	nt[c]
1	Ph	H	35	–[d]
11e	2-OMe-C_6H_4	H	47	–
12a[e]	Ph	4′-NH_2	40	–[d]
12b[e]	2-OMe-C_6H_4	3′-NH_2	81	–
12c[e]	2-OMe-C_6H_4	4′-NH_2	37	+
12d[e]	3-OMe-C_6H_4	4′-NH_2	48	–
12e[e]	4-OMe-C_6H_4	4′-NH_2	142	–
SMP-797			31	++

[a] Compounds inhibitory activity for ACAT in $HepG_2$.
[b] Effect of compounds on expression of LDL receptor in $HepG_2$.
[c] Not tested.
[d] 10 μM.
[e] 2HCl salts.

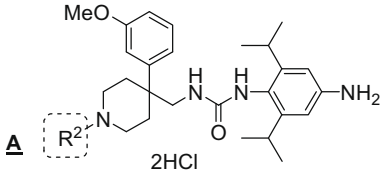
Table 2
Effects of a substitution on the phenyl ring (B-part) on biological activity



Compound	R^1	ACAT inhibition IC_{50}[a] (nM)	LDL receptor expression[b] (100 nM)
12i	H	82	–
12j	3-F	77	–
12k	3-CF_3	32	–
12l	2-OMe	43	+
12c	3-OMe	37	+
12m	4-OMe	49	+
SMP-797		31	++

[a] Compounds inhibitory activity for ACAT in $HepG_2$.
[b] Effect of compounds on expression of LDL receptor in $HepG_2$.

Table 3
Effects of the 2-alkoxy substituent on the phenyl ring (A-part) on biological activity



Compound	R^2	ACAT inhibition IC_{50}[a] (nM)	LDL receptor expression[b] (100 nM)
12c	2-OMe-C_6H_4	37	+
12f	2-O^{n}Bu-C_6H_4	32	++
12g	2-OCF_3-C_6H_4	68	++
12h	2-OCH_2CF_3-C_6H_4	48	++
SMP-797		31	++
Avasimibe		479[c]	–

[a] Compounds inhibitory activity for ACAT in $HepG_2$.
[b] Effect of compounds on expression of LDL receptor in $HepG_2$.
[c] In-house data.

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12. Compound **12f**; mp 168–170 °C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.88 (3H, t, J = 7.3 Hz), 1.08 (12H, d, J = 6.8 Hz), 1.30 (2H, m), 1.51 (2H, m), 2.39 (2H, m), 2.51 (2H, m), 3.08 (2H, qq, J = 6.8, 6.8 Hz), 3.45 (2H, m), 3.56 (2H, m), 3.60 (2H, m), 3.81 (3H, s), 3.98 (2H, m), 6.07 (1H, s), 6.89 (1H, m), 7.01 (3H, m), 7.10 (2H, s), 7.21 (1H, d, J = 8.1 Hz), 7.36 (2H, m), 7.67 (1H, s), 8.07 (1H, m); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 13.2, 18.4, 23.0, 27.7, 29.8, 29.9, 33.8, 49.0, 54.8, 68.4, 111.6, 112.6, 114.4, 117.3, 118.4, 120.7, 123.1, 129.3, 130.0, 130.3, 132.7, 148.2, 151.2, 156.6, 159.4; IR (ATR) ν 2960, 2568, 1653, 1606, 1558 cm^{-1}; HRMS (ESI) m/z calcd for $C_{36}H_{51}O_3N_4$ 587.3956; found 587.3936 (Δ = −3.39 ppm); Anal. Calcd for $C_{36}H_{50}N_4O_3$?2HCl?2.25H_2O: C, 61.75; H, 8.13; N, 8.00. Found: C, 61.62; H, 8.01; N, 7.99.
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