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Synthesis and structure–activity relationship studies on a novel series of naphthylidinoylureas as inhibitors of acyl-CoA:cholesterol *O*-acyltransferase (ACAT)

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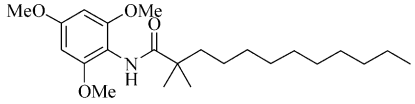
Abstract—The synthesis and structure–activity relationships of *N*-phenyl-*N*′-[3-(4-phenylnaphthylidinoyl)]urea derivatives **3** as a novel structural class of potent ACAT inhibitors is described. A 3-methoxy group substituted on the naphthylidinone 4-phenyl ring, together with a 1-*N*-^{n}butyl substitution, SM-32504 (**3m**), gave a potent ACAT inhibitor, in vitro, respectively. The most potent compound, SM-32504 (**3m**), decreased the serum cholesterol level significantly in a high fat and high cholesterol-fed mouse model.

Hypercholoesterolemia and cardiovascular diseases (atherosclerosis, etc.) are major health problems in many industrialized countries. Acyl-CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is the primary enzyme responsible for the intracellular esterification of free cholesterol with fatty acyl-CoA to produce cholesterol esters.[1] In the small intestine, ACAT facilitates the absorption of exogenous cholesterol, which is incorporated into chylomicrons.[2,3] In the liver, ACAT plays an important role in the assembly of very low density lipoprotein (VLDL), which is secreted into the blood.[4–6] Thus, ACAT inhibitors are being investigated as potent therapeutic agents for the treatment of hypocholesterolemia and atherosclerosis.[7]

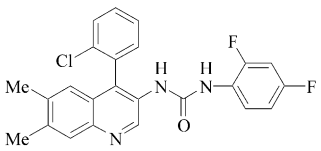
In recent years, a number of ACAT inhibitors have been reported and their pharmacological activities have been evaluated in animals and humans.[7–10] Most are structurally classified into two major groups, one being fatty acid anilide derivatives designed to mimic acyl-CoA represented by **1** (CI-976),[9] and the other being phenyl urea derivatives.[10] In the latter group, bulky lipophilic substituents on the phenyl ring such as 2,6-diisopropyl are known to have beneficial effect on the activity.[10e–g] TMP-153 (**2**) and the corresponding 2-quinolone analogues have also been reported as potent ACAT inhibitors.[10a,b]

Based on the phenyl urea derivatives,[10a,b] we designed *N*-phenyl-*N*′-[3-(4-phenylnaphthylidinoyl)]urea derivatives **3** as a novel structural class of ACAT inhibitor. We describe herein their synthesis and structure–activity relationships (Fig. 1).

The general procedure for the synthesis of naphthylidinoylureas is shown in Scheme 1. Treatment of the dianion of 2-(pivaloylamino)pyridine with substituted benzaldehydes followed by oxidation with manganese(IV)oxide afforded the aroylpyridines **4**.[11] Removed of the pivaloyl group on **4** followed by cyclization with diethylmalonate, in the presence of pyridine, gave the 2-naphthylidinones **5**.[12] The crucial step to synthesize the target compounds **3** from **5** or **6** was a Curtius rearran-



1:CI-976



2:TMP-153

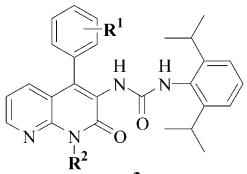


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Figure 1.

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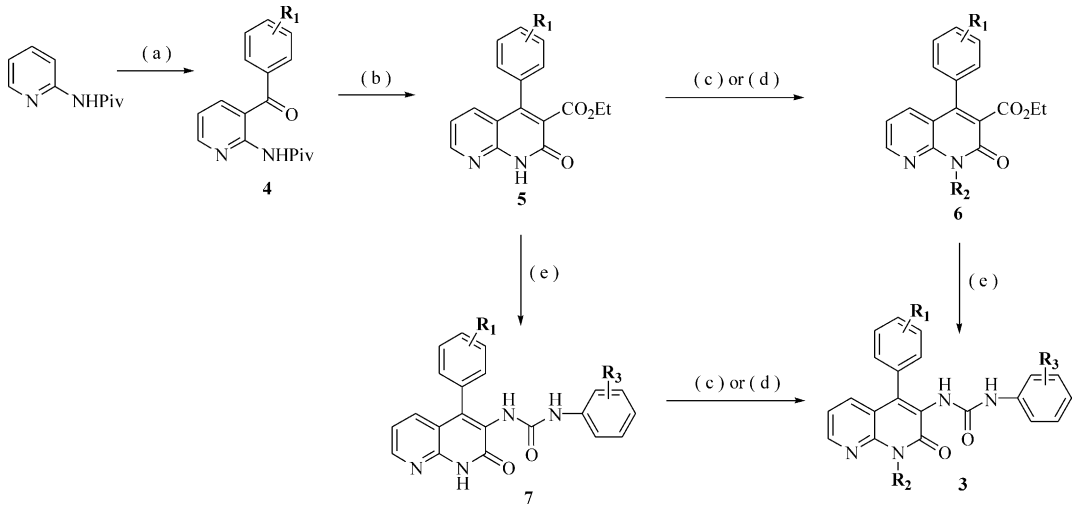


Scheme 1. (a) (1) *n*-BuLi, TMEDA/THF then $\mathbf{R_1}C_6H_4CHO$, (2) MnO_2/CH_2Cl_2; (b) (1) 6N HCl, (2) $CH_2(CO_2Et)_2$/pyridine; (c) NaH, $\mathbf{R_2}$-I/DMF; (d) $\mathbf{R_2}$-I, K_2CO_3/DMF; (e) (1) NaOH/EtOH, (2) DPPA, Et_3N/DMF then $\mathbf{R_3}C_6H_4NH_2$ or $\mathbf{R_3}C_6H_3NH_2$.

gement of the acylazides synthesized from the carboxylic acids derived from esters **5** or **6** using diphenylphosphorylazide (DPPA) and triethylamine[13] followed by reaction of the intermediate isocyanates (not isolated) the treatment with substituted anilines, in DMF. The reaction proceeded in high yield when either $\mathbf{R^3}$ was alkyl (**5**→**6**→**3**) or hydrogen (**5**→**7**→**3**).

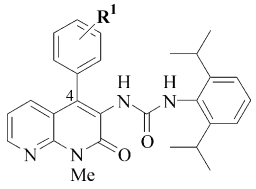
The compounds prepared were evaluated in both an enzyme assay and a cell-based assay. In the enzyme assay, ACAT activity was determined by measuring the production of cholesteryl ^{14}C-oleate while incubating with ^{14}C-oleoyl-CoA and rabbit liver microsomes.[14] In the cell-based assay, whole cell ACAT activity in rat macrophages was determined by the incorporation of an extracellular ^{3}H-oleic acid-BSA complex into the intracellular cholesteryl ester.[15]

The ACAT inhibitory activity of each compound in these assays was expressed by an IC_{50} value. Desirable compounds were required to show potent inhibitory activities in both assay systems.

First, we examined the effect of a substituent on the phenyl ring at the 4-position of the 2-naphthylidinone. The result are shown in Table 1. Compounds **3a**–**e** possessed strong inhibitory activities in the enzyme assay and moderate inhibitory activities in the cell-based assay. We had expected that **3a**, having the same substituent as **2** would have given the highest activity, however, it was slightly inferior to **3c** and **3d**. Regarding the nature of the substituent at the 2-position of the phenyl ring, the electron-donating methoxy group was effective in the enzyme assay and the presence of a substituent itself was important in the cell-based assay (see **3a**, **3b** and **3c**). Regarding the position of the methoxy group on the phenyl ring, the 3-position was effective in the enzyme assay and the 2-position was effective in the cell-based assay (see **3c**, **3d** and **3e**).

Next, we examined the effect of substituent(s) ($\mathbf{R^3}$) on the phenyl ring of the phenylureido moiety. The results are shown in Table 2. Although it was reported that the 2,4-difluorophenyl moiety brought about potent inhibition in **2** and in some other ACAT inhibitors,[10a–d] **3f** showed only weak inhibitory activity. Steric hinderance of the substituents at the *ortho*-positions of the phenyl

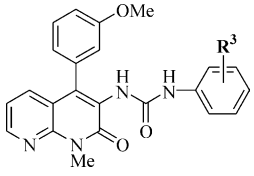
Table 1. Effect of 4-phenyl ring substitution on the biological activities



Compd	R_1	Enzyme assay IC_{50}[a] (nM)	Cell-based assay IC_{50}[b] (nM)
3a	2-Cl	56	140
3b	H	40	562
3c	2-OMe	21	100
3d	3-OMe	8	158
3e	4-OMe	63	447

[a] In vitro ACAT inhibition determined in rabbit liver microsomes.
[b] In vitro ACAT inhibition determined in rat macrophages.

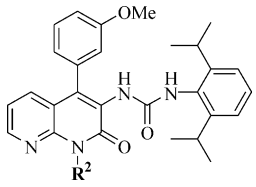
Table 2. Effect of varying phenyl substituents on the biological activities



Compd	R^3	Enzyme assay IC_{50}[a] (nM)
3f	2,4-F_2	>1000
3g	2-Et	>1000
3h	2-^{i}Pr	>1000
3i	2-^{i}Pr-6-Me	76
3d	2,6-^{i}Pr$_2$	8

[a] In vitro ACAT inhibition determined in rabbit liver microsomes.

Table 3. Effect of 1-*N*-substituent on the biological activities



Compd	R^2	Enzyme assay IC_{50}[a] (nM)	Cell based assay IC_{50}[b] (nM)
3j	H	100	>1000
3d	CH_3	8	158
3k	CH_2CH_3	9	170
3l	$(CH_2)_2CH_3$	13	50
3m	$(CH_2)_3CH_3$	25	11
3n	$CH(CH_3)_2$	68	230
3o	$(CH_2)_4CH_3$	13	35

[a] In vitro ACAT inhibition determined in rabbit liver microsomes.
[b] In vitro ACAT inhibition determined in rat macrophages.

ring was strongly effective in enhancing the activity and the best result was obtained with **3d**, as we expected.

Finally, the effect of the 1-*N*-alkylsubstituents (**R^2**) on the 2-naphthylidione ring was investigated. The results are shown in Table 3. Alkyl substitution was highly effective in enhancing the inhibitory activities in both assay systems. The effect of the length of the straight-chain alkyl substituents was not clear in the enzyme assay, though the effect was clearer in the cell-based assay. The best result was obtained with *n*-butyl substitution, **3m**. In contrast, the branched-chain alkyl compound **3n** showed diminished activity.

We also investigated the effect of the *N*-alkyl substituent in the 2-methoxy analogues of **3c**, however, no noteworthy result was obtained. Furthermore, 2-methoxy isomer **3p** (R^1 = 2-OMe, $R^2 = {}^n$Bu, R^3 = 2,6-$^i Pr_2$) showed only about 50% of the permeability in a Caco-2 membrane[16] than the corresponding 3-methoxy isomer **3m**.

Thus, compound **3m** (SM-32504) was identified as a potent ACAT inhibitor.[17] In vivo evaluation of **3m** showed that it decreased the serum cholesterol level by 38% (at a dosage of 10 mg/kg/d for 4 d) and 40% (at a dosage of 30 mg/kg/d for 4 d) in high fat and high cholesterol-fed mice, respectively.[18]

In conclusion, we found that naphthylidinoylureas **3** were a novel series of ACAT inhibitor. In paticular, compound **3m** showed potent inhibitory activity in vitro and excellent efficacy in vivo. Further investigation on these inhibitors is currently in progress and the results will be reported in the near future.

Acknowledgements

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References and notes

1. Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647.
2. Field, F. J.; Salome, R. G. *Biochim. Biophys. Acta* **1982**, *712*, 557.
3. Heider, J. G.; Pickens, C. E.; Kelly, L. A. *J. Lipid Res.* **1983**, *24*, 1127.
4. Drevon, C. A.; Engelhorn, S. C.; Steinberg, D. *J. Lipid Res.* **1980**, *21*, 1065.
5. Khan, B.; Wilcox, H. G.; Heimberg, M. *Biochem. J.* **1989**, *258*, 807.
6. Cianflone, K. M.; Yasruel, Z.; Rodriguez, M. A.; Sniderman, A. D. *J. Lipid Res.* **1990**, *31*, 2045.
7. (a) Sliskovic, D. R.; White, A. D. *Trends Pharmacol. Sci.* **1991**, *12*, 194. (b) Rodriguez, A.; User, D. C. *Atherosclerosis* **2002**, *161*, 45. (c) Hirakawa, Y.; Shimokawa, H. *Folia Pharamacaol. Jpn* **2001**, *118*, 389.
8. (a) Matsuda, K. *Med. Res. Rev.* **1994**, *14*, 271. (b) McCarthy, P. A. *Med. Res. Rev.* **1993**, *13*, 139.
9. (a) For examples of fatty acid anilides, see: Roth, B. D.; Stanfield, R. L. *J. Med. Chem.* **1992**, *35*, 1609. (b) McCathy, P. A. *J. Med. Chem.* **1994**, *37*, 1252. (c) Kataoka, K.; Yamaguchi, H. *J. Med. Chem.* **1995**, *38*, 3174. (d) Matsuyama, N. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2039. (e) Krause, B. R. *J. Lipid Res.* **1993**, *34*, 279. (f) Murakami, S. *Cell. Mol. Biol.* **1996**, *42*, 865. (g) Kataoka, K.; Yamaguchi, H. *J. Med. Chem.* **1996**, *39*, 1262.
10. (a) For examples of phenylureas, see: Meguro, K.; Tawada, H. *J. Med. Chem.* **1994**, *37*, 2079. (b) Tawada, H.; Meguro, K. *Chem. Pharm. Bull.* **1995**, *43*, 616. (c) Higley, C. A.; Wexler, R. R. *J. Med. Chem.* **1994**, *37*, 3511. (d) *J. Lipid Res.* **1989**, *30*, 681. (e) Sekiya, T.; Umezu, K. *Chem. Pharm. Bull.* **1994**, *42*, 586. (f) O'Brien, P. M.; Stanfield, R. L. *J. Med. Chem.* **1994**, *37*, 1810. (g) Trivedi, B. K.; Krause, B. R. *J. Med. Chem.* **1994**, *37*, 1652. (h) Kimura, T.; Yamatsu, I. *J. Med. Chem.* **1993**, *36*, 1641. (i) Tanaka, A.; Tanaka, H. *Bioorg. Med. Chem.* **1998**, *6*, 15.
11. Estel, L.; Linard, F.; Marsais, F.; Godard, A.; Queguiner, G. *J. Heterocyclic Chem.* **1989**, *26*, 105.
12. Bell, S. C.; Sulkowsky, T. S.; Gochman, C.; Childness, S. J. *J. Org. Chem.* **1962**, *27*, 562.
13. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.
14. Heider, J. G.; Pickens, C. E.; Kelly, L. A. *J. Lipid Res.* **1983**, *22*, 271.
15. Miyazaki, A.; Horiuchi, S. *Biochim. Biophys. Acta* **1992**, *1126*, 73.
16. Yamashita, S. *Eur. J. Pharm. Sci.* **2000**, *10*, 195 The apparent permeability coefficient, Papp value for 2-methoxy isomer **3p** was 15(nm/s) compared to 29(nm/s) for **3m**.
17. Selected spectroscopic data for **3m** (SM-32504): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (t, 3H, J = 7.34 Hz), 1.01 (br, 12H), 1.35–1.47 (m, 2H), 1.64–1.75 (m, 2H), 2.86–2.95 (m, 2H), 3.76 (s, 3H), 4.51 (t, 2H, J = 7.70 Hz), 6.91 (br, 2H), 7.02–7.04 (m, 2H), 7.12–7.17 (m, 1H), 7.24 (dd, 1H, J = 4.59, 7.89 Hz), 7.39 (dd, 1H, J = 7.89, 8.25 Hz), 7.61 (d, 1H, J = 6.60 Hz), 7.72 (d, 1H, J = 3.49 Hz), 8.60 (d, 1H, J = 4.59 Hz); IR (neat) 2960, 1678, 1646, 1600, 1585, 1562, 1456, 1249 cm^{-1}; APCI-MS m/z 527 (MH^+).
18. Ioriya, K.; Noguchi, T.; Muraoka, M.; Fujita, K.; Shimizu, H.; Ohashi, N. *Pharmacology* **2002**, *65*, 18.