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First identification of xanthone sulfonamides as potent acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors

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ABSTRACT

Inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT) would be useful anti-atherogenic agents, since an absence of ACAT affects the absorption and transformation of cholesterol, indirectly resulting in the reduction of cholesteryl ester accumulation in blood vessels. This report discloses xanthone sulfonamides as novel class small molecule inhibitors of ACAT. A series of xanthone sulfonamides were synthesized and evaluated to result in the identification of several potent ACAT inhibitors, among which **2n** proved to be more potent than the positive control Sandoz58-35. Moreover, a molecular model for the binding between **2n** and the active site of ACAT-2 was provided based computational docking results.

Esterification of intracellular cholesterol has been proved to be catalyzed by acyl-CoA:cholesterol acyltransferase (ACAT; EC 2.3.1.26).[1] In hepatocytes and intestinal cells, cholesterol is esterified by ACAT and incorporated into apo B-containing lipoproteins, which are subsequently secreted into circulation. After lipolytic conversion, these lipoproteins are taken up by the cells of various organs, where their cholesteryl esters are hydrolyzed in lysosomes to liberate free cholesterol, which is re-esterified by ACAT again for storage in cytoplasmic lipid droplets.[2,3] Recent research shows that ACAT could mediate foam cell formation, an initial event of atherosclerosis.[4] Therefore, it is possible that inhibition of ACAT could result in a protective effect against atherosclerosis. In addition, ACAT inhibitors could be useful for the treatment of Alzheimer's disease, for they can decrease the generation of amyloid β peptide in animal models.[5]

A cell based high-throughput screening (HTS) assay has been successfully developed by Rudel's group to measure ACAT activity.[6] In recent years, our group has been focusing on synthesizing and evaluating the biological activities of novel xanthone compounds.[7,8] During the screening, compound **1** (Fig. 1), a xanthone sulfonamide, was identified to show ACAT inhibitory activity.

Compound **1**[9] showed a good inhibitory rate of 32.4% at 10 μg/ml concentration in the cell based assay, compared to 55.0% for the positive control (Sandoz58-35, an ACAT inhibitor, from Sigma). This finding prompted us to undertake further investigation. Herein, we describe our efforts to optimize the inhibitory effects of this compound by analyzing structure–activity relationships.

Compounds required for the establishment of structure–activity relationship were prepared as shown in Scheme 1 starting from 2,4,5-trimethoxybenzoic chloride (**3**). Friedel–Crafts acylation of the 1,3,5-trimethoxybenzene (**4**) afforded benzophenone (**5**) and the free OH group in **5** was formed by demethylation in extra amount of aluminium chloride.[10] Treatment of **5** with tetrabutylammonium hydroxide in pyridine and water under

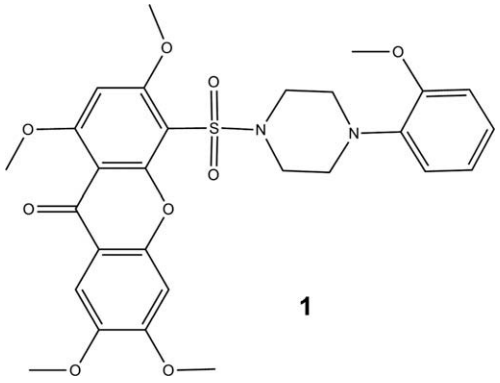

Figure 1. ACAT inhibitor identified through HTS.

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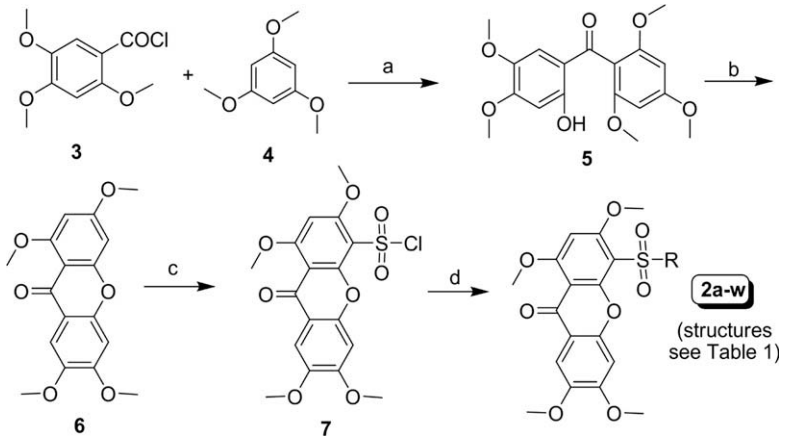


Scheme 1. Synthesis of compounds **2a**–**w**. Reagents and conditions: (a) $AlCl_3$, Et_2O, rt, 48 h, 72%; (b), TBAOH (25% in H_2O), Py-H_2O (1:1), reflux, 4 h, 95%; (c) HSO_3Cl, 0 °C, 30 min, 80%; (d) amine, 1,4-dioxane, rt, 1 h, 90–98%.

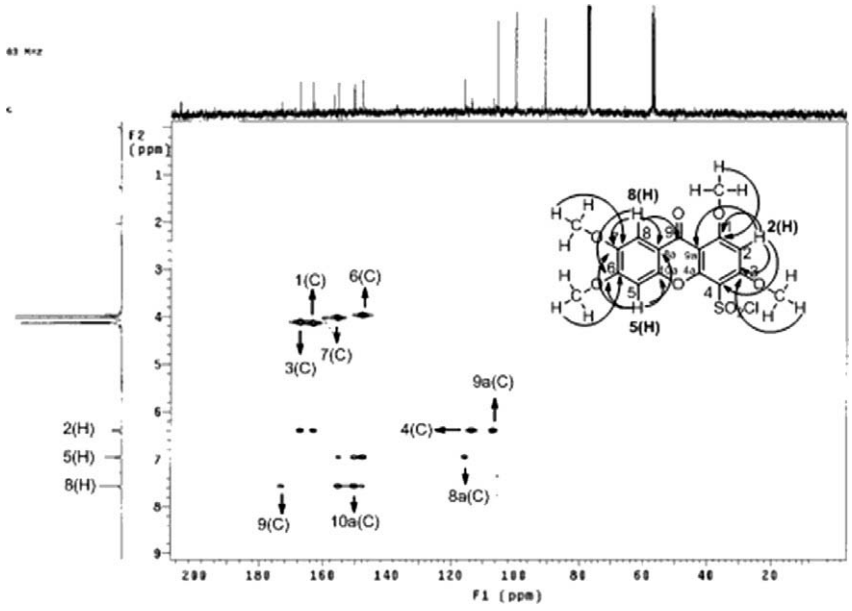


Figure 2. The main HMBC correlations of compound **7**.

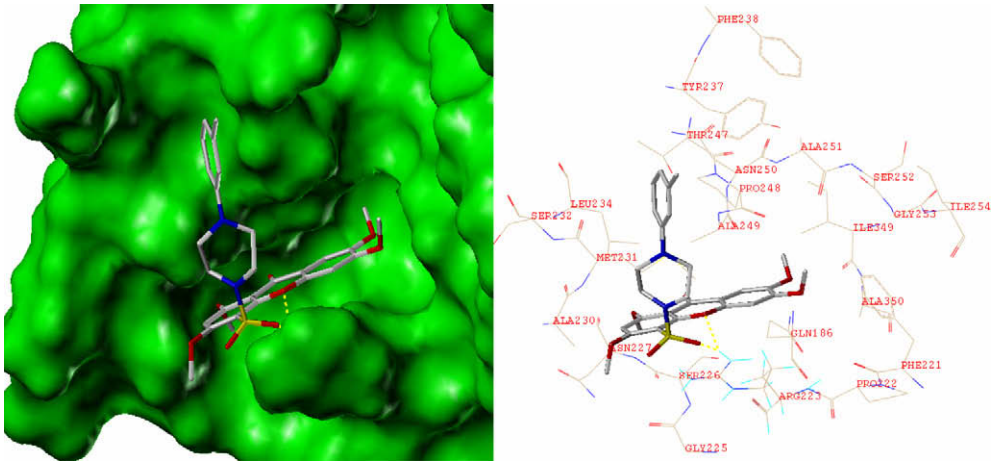
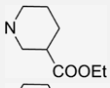
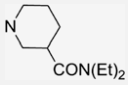
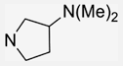
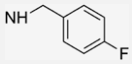
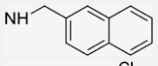
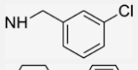
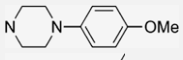
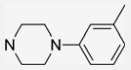
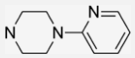
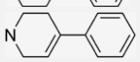
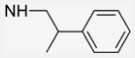
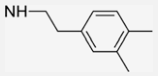
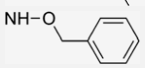
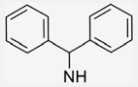
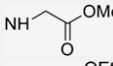
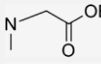
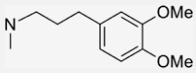


Figure 3. Computed binding geometry of **2n** in the active site of ACAT-2.

reflux gave **6** in high yield (95%).[11] Chlorosulfonic acid was added dropwise into the flask containing **6** at 0 °C to give key intermediate **7** as a brown powder, which was characterized by 1D and 2D NMR. The main HMBC correlations of **7** are shown in Figure 2. Reaction of **6** with different amines formed the final xanthone sulfonamides **2a–w** in quantitative yield.[12,13] The reactions were carried out in parallel. All new compounds were characterized by NMR and MS.

Table 1
Structure and ACAT inhibition rate of **2a–w** at 10 μg/mL concentration

Sample	R	%Inh.
Sandoz58-35[a]		55.00
2a		–[b]
2b		–
2c		–
2d		10.53
2e		7.99
2f		12.59
2g		14.50
2h		20.96
2i		8.84
2j		10.24
2k		25.00
2l		15.76
2m		36.92
2n		64.80
2o		43.56
2p		51.34
2q		10.78
2r		11.86
2s		16.76
2t		–
2u		5.13
2v		16.79
2w		30.06

[a] Sandoz58-35, an inhibitor towards ACAT, as a positive control, at 10 μg/mL.
[b] No inhibition activity observed at 10 μg/mL.

The effect of the synthesized compounds on ACAT was first studied at a concentration of 10 μg/mL. As shown in Table 1, except compounds **2a–c** and **2t**, all tested compounds showed certain ACAT inhibitory activity (5.13–64.8%). Compounds **2d–h** which had five- or six-member rings possessed moderate inhibitory activities. Compounds containing 1-(substituted-aryl)-piperazines (**2m–o**) showed strong inhibition to ACAT. It should be noted that their aryl groups at one position of piperazine led to a significant enhancement of potency. With the similar replacement groups of **2m–o**, compound **2p** also had a good potency. Among all these compounds, compound **2n** showed higher inhibitory activity than the positive control.

To explain the results, we proposed a likely binding mode for **2n** to the active site of ACAT-2 based on computational docking results (Fig. 3).[14] The designed compound could fit into the hydrophobic pocket formed by Gln186, Arg223, Ser226, Asn227, Ala230, Met231, Leu234, Thr247, Ala249, Ile349 and Ala350 (PDB structure 1WL4). Moreover, the sulfonamide group and xanthone ring would generate a hydrogen bond interaction with the Arg223. These results may provide some guidance for the development of novel ACAT inhibitory lead structures.

In summary, a series of 1,3,6,7-tetramethoxyxanthone-4-sulfonamide derivatives **2a–w** as potential ACAT inhibitors were synthesized in high yields. The biological screening of these compounds resulted in the identification of several potent ACAT inhibitors, particularly compound **2n** which is more potent than the established ACAT inhibitor Sandoz58-35. This observation was explainable by a molecular model resulting from the computational docking simulation, which showed that **2n** could fit into the hydrophobic pocket of ACAT-2. Effort aimed at further optimization, as well as in-depth biological investigations, of the identified lead compounds is continuing in our laboratories, and results will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.101.

References and notes

1. Brown, M. S.; Dana, S. E.; Goldstein, J. L. *J. Biol.Chem.* **1975**, *250*, 4025.
2. Lee, O.; Chang, C. Y.; Lee, W.; Chang, T. Y. *J. Lipid Res.* **1998**, *39*, 1722.
3. Meuwese, M. C.; Franssen, R.; Stroes, E. S. G.; Kastelein, J. J. P. *Curr. Opin. Lipidol.* **2006**, *17*, 426.
4. Miyazaki, A.; Kanome, T.; Watanabe, T. *Curr. Drug Targets Cardiovasc. Haematol. Disord.* **2005**, *5*, 463.
5. Puglielli, L.; Konopka, G.; pack-Chung, E.; Ingano, L. A. M.; Berezovska, O.; Hyman, B. T.; Chang, T. Y.; Tanzi, R. E.; Kovacs, D. M. *Nat. Cell Biol.* **2001**, *3*, 905.
6. Lada, A. T.; Davis, M.; Kent, C.; Chapman, J.; Tomoda, H.; Omura, S.; Rudel, L. L. *J. Lipid. Res.* **2004**, *45*, 378.
7. Hu, H. G.; Wang, M. J.; Zhao, Q. J.; Liao, H. L.; Cai, L. Z.; Song, Y.; Zhang, J.; Yu, S. C.; Chen, W. S.; Liu, C. M.; Wu, Q. Y. *Chem. Nat. Compd.* **2007**, *43*, 663.
8. Hu, H. G.; Wang, M. J.; Zhao, Q. J.; Yu, S. C.; Liu, C. M.; Wu, Q. Y. *Chin. Chem. Lett.* **2007**, *18*, 1323.
9. Compound **1**, ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.60 (s, 1H), 7.04 (m, 1H), 6.95 (s, 1H), 6.91 (m, 3H), 6.39 (s, 1H), 4.07 (s, 3H), 4.05 (s, 3H), 4.02 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.53 (m, 4H), 3.14 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.03, 165.17, 163.20, 157.59, 155.14, 152.30, 150.57, 147.34, 145.09, 123.84, 121.17, 118.84, 115.63, 111.48, 107.58, 107.06, 105.47, 99.57, 90.95, 56.83, 56.65, 56.59, 56.32, 50.82, 46.11. ESI, calcd for $C_{28}H_{31}N_2O_9S^+$, M+H$^+$, 571.2, found: 571.1.
10. Quillinan, A. J.; Scheinmann, F. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1329.

11. Lin, C. N.; Liou, S. S.; Ko, F. N.; Teng, C. M. *J. Pharm. Sci.* **1992**, *81*, 1109.
12. Elgamal, M. H. A.; Shalaby, M. M. M.; Duddeck, H.; Rosenbaum, D. *J. Heterocycl. Chem.* **1987**, *24*, 721.
13. Representative analytical data for compound **2n**, yield 98%, ^{1}HNMR (300 MHz, DMSO-d_6, TMS): δ 7.39 (s, 1H), 7.06 (t, 1H, J = 7.8 Hz), 6.97 (s, 1H), 6.73 (m, 3H), 6.59 (d, 1H, J = 6.9 Hz), 4.04 (s, 1H), 4.02 (s, 1H), 3.94 (s, 1H), 3.85 (s, 1H), 3.32 (m, 4H), 3.18 (m, 4H), 2.20 (s, 3H). ESI, calcd for $C_{28}H_{30}N_2O_8S^+$, M+H$^+$, 555.2, found: 555.1.
14. Kursula, P.; Sikkila, H.; Fukao, T.; Kondo, N.; Wierenga, R. K. *J. Mol. Biol.* **2005**, *347*, 189.