2-Substitiuted Thio- and Amino-5,8-dimethoxy-1,4-naphthoquinones as a Novel Class of Acyl-CoA: Cholestrol Acyltransferase Inhibitors

Gui-Nan Shen, Jung Ho Choi,[†] Kondaji Gajulapati,[‡] Jee Hyun Lee, Young Kook Kim,[†] Mun Chal Rho,[†] Sang-Hun Jung, Kyeong Lee,[†] Sung Sik Han,[‡] Gyu-Yong Song,[*] and Yongseok Choi[‡,*]

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. [*]E-mail: gysong@cnu.ac.kr
[†]Korea Research Institute of Biosciences and Biotechnology, Daejeon305-733, Korea
[‡]School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea. [*]E-mail: ychoi@korea.ac.kr
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A series of 2-alkyl or 2-arylthio-5,8-dimethoxy-1,4-naphthoquinones (2-Thio-DMNQ, **5a-s**) and 2-alkylamino-5,8-dimethoxy-1,4-naphthoquinones (2-Amino-DMNQ, **6a-k**) was synthesized and evaluated for their ACAT inhibitory activities. Among them, the 2-dodecylthio-DMNQ **5l** and 2-isobutylamidoundodecylthio-DMNQ **5r** showed the most potent ACAT inhibitory activities with IC_{50} value of 22.8 and 24.4 μM, respectively. In a structure-activity relationship study, 2-thio-DMNQs with side chains of carbon number 11 ~ 15 exhibited significant ACAT inhibitory activities.

Key Words: Naphthoquinones, Mast cells, Acyl CoA: Cholestrol Acyl Transferase (ACAT), Structure-activity relationship

Introduction

Cholesterol is an important biological molecule as a precursor for the synthesis of the steroid hormones and bile acids. The cholesterol regulation, namely cholesterol homeostasis, is kept by preventing accumulation of cholesterol by transporting through the circulation in blood plasma and artery. The two third of cholesterol in blood plasma is stored as cholesteryl ester form. This cholesteryl ester is synthesized by activation of high density lipoprotein (HDL)-associated enzyme lecithin cholesterol acyltransferase (LCAT) or acyl-CoA:cholesterol acyltransferase (ACAT).[1,2] Increment of cholesteryl ester by high activation of ACAT enzyme brings about the formation of foam cell originated from macrophage. The massive accumulation of cholesterol, particularly cholesteryl ester in foam cells, causes hypercholesterinemia. Long term maintenance of this condition eventually leads to atherosclerosis.[3,4] Thus, the inhibitor of ACAT activity could be the competitive drug in the prevention and treatments of hypercholesterinemia and atherosclerosis by inhibiting the formation of cholesteryl ester within the cell.

So far, many ACAT inhibitors have been developed such as VULM 1457,[5] K-604,[6] CS-505,[7] and F12511,[8] *etc.* It was recently reported that shikonin derivatives with 5,8-dihydroxy-1,4-naphthoquinone, naphthazarin, (**1-3**, Figure 1), isolated from the roots of *Lithospermum erythrorhizon*, were tested for inhibitory effect against human ACAT-1 (hACAT-1) and human ACAT-2 (hACAT-2).[9] Among them, the shikonin derivative with isobutyl moiety (**2**) showed moderate inhibitory activities, with IC_{50} value of 57.7 μM against hACAT-2 and 32% inhibition at 120 μM against hACAT-1. Among the shikonin analogues, a derivative with 3-methylbutanoyl moiety **4**, which was semi-synthesized from isolated shikonin, showed the most potent ACAT inhibitory activity with IC_{50} value of 13.8 μM and 25.1 μM against hACAT-1 and hACAT-2, respectively.

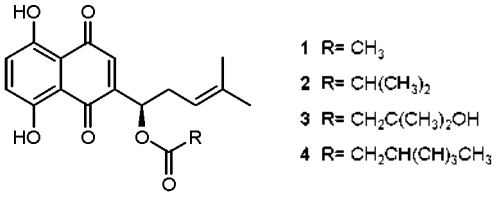


Figure 1. Structure of shikonin derivatives **1-4** known to ACAT inhibitor.

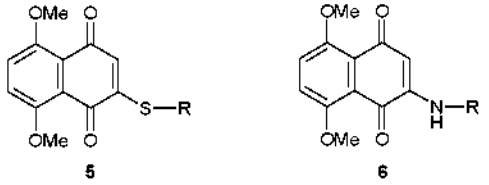
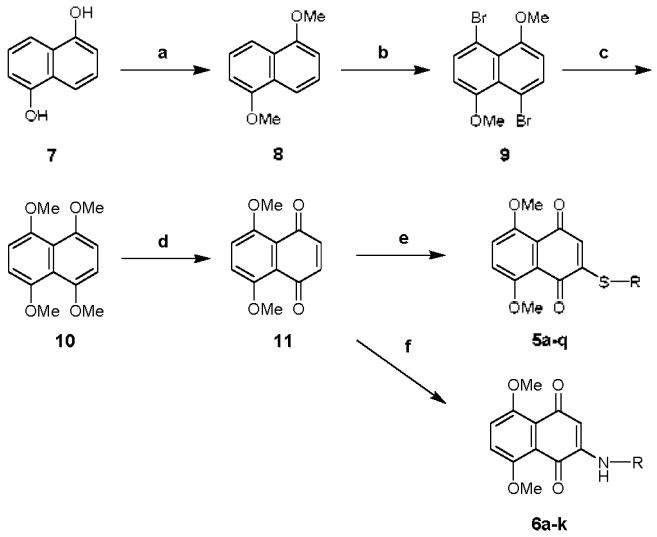


Figure 2. Structure of thio-DMNQ **5** and amino-DMNQ **6**.

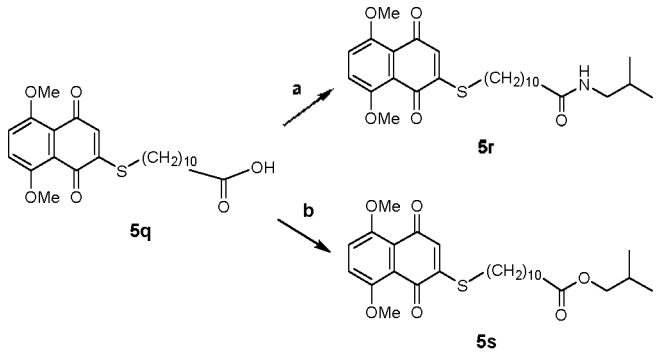
On the basis of ACAT inhibitory effect of the 1,4-naphthoquinone derivatives, it was of great interest to synthesize 1,4-naphthoquinone derivatives with different side chains to understand the structural requirement for ACAT inhibitory activity of naphthoquinone derivatives. Herein we describe the initial structure-activity relationships (SAR) in this series, particularly 2-alkyl and 2-arylthio-5,8-dimethoxy-1,4-naphtoquinones (**5**, thio-DMNQ) and 2-alkylamino-5,8-dimethoxy-1,4-naphthoquinones (**6**, amino-DMNQ) as novel ACAT inhibitors.

Results and Discussion

The synthetic routes for 2-substituted thio-DMNQ derivatives and 2-substituted amino-DMNQ derivatives were summarized in Scheme 1 and Scheme 2. 1,5-Dihydroxynaph-



Scheme 1. Reagents and conditions: (a) NaOH, $(CH_3)_2SO_4$, N_2, rt, 2 h; (b) NBS, rt, 3h, CH_3CN; (c) CH_3ONa, CuI, reflux, 30 h, DMF, MeOH; (d) CAN, rt, 1 h, CH_3CN, $CHCl_3$ (3:1); (e) HS-R or HS-$(CH_2)_{10}$-COOH, $Na_2Cr_2O_7$, H_2SO_4, rt, 4 h, MeOH; (f) NH_2-R, rt, 4 h, MeOH.



Scheme 2. Reagents and conditions: (a) DCC, DMAP, isobutyl amide, dry CH_2Cl_2, 0 °C, 4h; (b) DCC, DMAP, isobutyl alcohol, dry CH_2Cl_2, 0 °C, 4 h.

thalene, as a starting material, was reacted with sodium hydroxide and dimethyl sulfate under nitrogen followed by bromination with *N*-bromosuccinimide (NBS) in room temperature for 3 h to afford 1,5-dibromo-4,8-dimethoxynaphthalene (**9**). After methoxylation with sodium methoxide and copper (I) iodide in *N,N*-dimethyl formamide/methanol solution, oxidative demethylation of the 1,4,5,8-tetramethoxynaphthalene (**10**) with cerium (IV) ammonium nitrate (CAN) gave the key intermediate 5,8-dimethoxy-1,4-naphthoquine (DMNQ, **11**). Direct 1,4-type addition of various alkylthiols or arylthiols to the quinone moiety of DMNQ **11** yielded the appropriated 2-thio-DMNQs, **5a-q**, with yields varying from 10.6 to 77.9%. Treatment of compound **5q** with isobutylamine or isobutylalcohol in the presence of DCC and DMAP yielded the amide compound **5r** and acylated compound **5s**, respectively. Also, direct 1,4-type addition of various alkylamines to the quinone moiety of DMNQ **11** yielded the appropriated 2-amino-DMNQs, **6a-k**.

As shown in Table 1 and 2, the synthesized 2-thio-DMNQ

Table 1. ACAT inhibitory activities of 2-Thio-DMNQ derivatives (**5a-s**) *in vitro* assay system

Compound	R	IC_{50} (μM)
5a	$-CH_3$	>110
5b	$-CH_2CH_3$	>110
5c	$-(CH_2)_2CH_3$	>110
5d	$-(CH_2)_3CH_3$	>110
5e	$-(CH_2)_4CH_3$	>110
5f	$-(CH_2)_5CH_3$	>110
5g	$-(CH_2)_6CH_3$	106.7
5h	$-(CH_2)_7CH_3$	107.3
5i	$-(CH_2)_8CH_3$	88.65
5j	$-(CH_2)_9CH_3$	76.50
5k	$-(CH_2)_{10}CH_3$	39.77
5l	$-(CH_2)_{11}CH_3$	22.78
5m	$-(CH_2)_{14}CH_3$	39.66
5n	$-(CH_2)_{17}CH_3$	>110
5o	-Ph	>110
5p	$-(CH_2)_{10}CH_2OH$	>110
5q	$-(CH_2)_{10}CH_2COOH$	>110
5r	$-(CH_2)_{10}CH_2CONHCH_2CH(CH_3)_2$	24.40
5s	$-(CH_2)_{10}CH_2COOCH_2CH(CH_3)_2$	>110

Table 2. ACAT inhibitory activities of 2-Amino-DMNQ derivatives (**6a-k**) *in vitro* assay system

Compound	R	IC_{50} (μM)[a]
6a	$-CH_3$	>110
6b	$-CH_2CH_3$	>110
6c	$-(CH_2)_2CH_3$	>110
6d	$-(CH_2)_3CH_3$	>110
6e	$-(CH_2)_4CH_3$	>110
6f	$-(CH_2)_5CH_3$	>110
6g	$-(CH_2)_6CH_3$	>110
6h	$-(CH_2)_7CH_3$	>110
6i	$-(CH_2)_8CH_3$	>110
6j	$-(CH_2)_9CH_3$	>110
6k	$-(CH_2)_{10}CH_3$	>110

[a]IC_{50} indicates concentrations for 50% inhibition of ACAT activitity.

analogues (**5a-s**) and 2-amino-DMNQ analogues (**6a-k**) were evaluated for their inhibition of ACAT in human hepatoma HepG2 cells. It was notable that the inhibitory effect of the 2-thio-DMNQs against ACAT was dependent on the carbon length of alkyl groups. Compounds with alkyl chains of intermediate length (C_7-C_{15}) possessed significant ACAT inhibitory activity with IC_{50} values of 22.78 to 107.3 μM. However, the elongation of the alkyl moiety beyond C_{18} or shortening below C_6 decreased the potency. Thus, there seems to be a relationship between inhibitory ACAT activity and the carbon length of the substituents. 1,4-Naphthoquinone derivative **5l** with dodecyl alkyl chain exhibited the most potency with IC_{50} value of 22.78 μM.

Next, we synthesized and assayed the 2-phenylthio-DMNQ (**5o**) with aromatic ring as substituent instead of aliphatic linear chain. However, compound **5o** did not exhibit inhibition of ACAT activity. In addition, in order to improve solubility of

DMNQ derivatives in water, we introduced hydroxyl (**5p**) or carboxylic group (**5q**) instead of methyl group in the terminal of 2-dodecylthio-DMNQ (**5l**) with the most potency against ACAT. However, introduction of hydroxyl and carboxylic group of **5l** completely lost inhibitory effect on ACAT activity. These results indicate that the liphophic groups are more suitable than the hydrophilic groups in the terminal of DMNQ analogues.

Previously, it was reported that piperchabamide D, an alkamide compound isolated from *Piper nigrum*, strongly inhibited ACAT activity.[10] This compound has a benzodioxol ring as a basic skeleton with the alkenyl side chain including isobutyl amide moiety in the terminal of alkene moiety. On the basis of this observation, isobutyl amide was introduced to the terminal carboxylic acid moiety of the 2-thio-DMNQ analogue **5q** to afford compound **5r** which turned out to show potent ACAT inhibitory effect (IC_{50}, 24.4 μM) as potent as 2-dodecylthio-DMNQ (**5l**), the most potent compound (IC_{50}, 22.78 μM) in this series of compounds. This result suggests that isobutyl amide moiety at terminal alkyl chain appears to play an important role for ACAT inhibitory activity.

On the other hand, replacement of sulfur atom of 2-thio-DMNQ analogues with nitrogen atom, 2-amino-DMNQ analogues (**6a-k**), resulted in complete loss of the inhibitory activity regardless of carbon length at side chain. This result may suggest that hydrophobic groups such as sulfur atom are more suitable for the inhibition of ACAT activity than hydrophilic group like -NH (**6a-k**), -OH (**5p**) or -COOH (**5q**) group.

In conclusion, we synthesized the 2-substituted thio/amino-DMNQ analogues and evaluated their ACAT inhibitory activities. Preliminary structure-activity relatioships identified novel ACAT inhibitors represented by analogue **5l**. It is noticeable that the ACAT enzyme activity of DMNQ analogues is dependent on the carbon length and lipophilicity of DMNQ's substituents. Taken together, 2-thio-DMNQ analogues may prove useful for the design of new potent ACAT inhibitors.

Experimental Section

Chemical reagents were obtained from Aldrich Chemical Company. Solvents were of reagent grade and used without further purification. Melting points were determined on an Electrothermal capillary melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a JNM-AL 400 (400 MHz). Chemical shifts (δ) are given in ppm downfield from tetramethylsilan as the internal standard. MS spectra were collected with a PE SCIX API 2000 MS/MS.

1,5-Dimethoxynaphthalene (8): Under ice cooling, dimethyl sufate (156 g, 1.24 mol) was dropwise to a solution of 1,4-dihydroxynaphthalene **7** (100 g, 0.62 mol) in 10% aqueous NaOH (500 mL), and stirred for 2 h. The precipitate was collected by filtration, washed with 5% aqueous KOH (200 mL × 2) and water (200 mL × 3), and then dried in oven. The crude product was recrystallized from benzene to give compound **8** (73.0 g, 63%) as a white solid: m.p. 181-182 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, J = 8.8 Hz, 2H), 7.38 (t, J = 8.0 Hz, 2H), 6.98 (d, J = 8.0 Hz, 2H), 3.94 (s, 6H).

4,8-Dibromo-1,5-dimethoxynaphthalene (9): To a solution of **8** (10.0 g, 0.053 mol) in acetonitrile (160 mL) was dropwise solution of *N*-bromosuccinimide (21.0 g, 0.118 mol) in acetonitrile (180 mL). The resulting mixture was stirred at room temperature under nitrogen for 2.5 h. The solid was collected filtration, washed with acetonitrile (50 mL × 2) and then with 20 mL of hexane to give compound **9** (12.7 g, 69%) as a white solid: m.p. 187-188 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, J = 8.4 Hz, 2H), 6.72 (d, J = 8.4 Hz, 2H), 3.91 (s, 6H).

1,4,5,8-Tetramethoxynaphthalene (10): To a solution of **9** (14.5 g, 0.04 mol) in *N*,*N*-dimethylformamide (300 mL) and MeOH (300 mL) was added copper iodide (26.6 g, 0.14 mol) and sodium methoxide (7.47 g, 0.14 mol). The resulting mixture was reflux for 30 h. 500 mL of ice water was added to the solution. The mixture was filtered, washed with water (100 mL), and dried in oven, separated with chloroform. The crude product was recrystallized from benzene to give compound **10** (6.50 g, 62.5%) as a white solid: m.p. 168-169 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.85 (s, 4H), 3.90 (s, 12H).

5,8-Dimethoxynaphthalene-1,4-dione (11): To a solution of **10** (10 g, 40.3 mmol) in acetonitrile (450 mL) and chloroform (150 mL) was added dropwise a solution of cerium ammonium nitrate (54 g, 98.5 mmol) in water (300 mL). The resulting mixture was stirred at room temperature for 1 h, after solution was added water (600 mL) and $CHCl_3$ (600 mL). The organic layer was separated, dried over sodium sulfate, and concentrated in vacuo. The residue was recrystallized from MeOH to give compound **11** (4.80 g, 54.6%) as a red solid: m.p. 122-123 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (s, 2H), 6.79 (s, 2H), 3.97 (s, 6H).

General procedure for the synthesis of compounds (5a-5q). To a solution of **11** (1.38 mmol) in MeOH (30 mL) were added corresponding alkyl thiols (1.65 mmol), respectively. The mixture was stirred at room temperature for 4 h and to the solution was added dropwise a solution of sodium dichromate (0.23 mmol) and sulfuric acid (0.76 mmol) in water. The resulting mixture was stirred for a few minute and the acidic solution was then extracted with dichloromethane (50 mL × 3). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was chromatography (hexane:EtOAc = 2:1) to give the title compounds **5a-5q**.

2-Methylthio-5,8-dimethoxy-1,4-naphthoquinone (5a): Obtained as a red solid (64 mg, 28.2%): m.p. 167-169 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.2 Hz, 1H), 7.27 (d, J = 9.2 Hz, 1H), 6.41 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 2.31 (s, 3H); m/z 286.9 $(M+Na)^+$.

2-Ethylthio-5,8-dimethoxy-1,4-naphthoquinone (5b): Obtained as a red solid (235 mg, 61.2%): m.p. 130-131 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 6.44 (s, 1H), 3.94 (s, 6H), 2.78 (q, J = 7.2 Hz, 2H), 1.38 (t, J = 7.2 Hz, 3H); m/z 301.0 $(M+Na)^+$.

2-Propylthio-5,8-dimethoxy-1,4-naphthoquinone (5c): Obtained as a red solid (242 mg, 60%): m.p. 80-81 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.2 Hz, 1H), 7.27 (d, J = 9.2

Hz, 1H), 6.45 (s, 1H), 3.96 (s, 6H), 2.74 (t, J = 7.2 Hz, 2H), 1.81-1.72 (m, 2H), 1.08 (t, J = 7.2 Hz, 3H); m/z 315.0 $(M+Na)^+$.

2-Butylthio-5,8-dimethoxy-1,4-naphthoquinone (5d): Obtained as a red solid (226 mg, 53.5%): m.p. 104-105 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.6 Hz, 1H), 6.44 (s, 1HHH), 3.95 (s, 6H), 2.76 (t, J = 7.2 Hz, 2H), 1.76-1.68 (m, 2H), 1.54-1.45 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); m/z 329.0 $(M+Na)^-$.

2-Pentylthio-5,8-dimethoxy-1,4-naphthoquinone (5e): Obtained as a red solid (186 mg, 42.1%): m.p. 101-102 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.6 Hz, 2H), 1.77-1.33 (m, 6H), 0.92 (t, J = 7.2 Hz, 3H); m/z 343.0 $(M+Na)^-$.

2-Hexylthio-5,8-dimethoxy-1,4-naphthoquinone (5f): Obtained as a red solid (194 mg, 42.1%): m.p. 139-140 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.2 Hz, 1H), 7.27 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.6 Hz, 2H), 1.73-1.48 (m, 2H), 1.47-1.30 (m, 6H), 0.90 (t, J = 7.2 Hz, 3H); m/z 356.9 $(M+Na)^+$.

2-Heptylthio-5,8-dimethoxy-1,4-naphthoquinone (5g): Obtained as a red solid (51 mg, 10.6%): m.p. 125-126 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 6.45 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.75-1.26 (m, 10H), 0.89 (t, J = 6.8 Hz, 3H); m/z 371.0 $(M+Na)^-$.

2-Octylthio-5,8-dimethoxy-1,4-naphthoquinone (5h): Obtained as a red solid (221 mg, 44.2%): m.p. 109-110 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33(d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.77-1.27 (m, 12H), 0.88 (t, J = 6.8 Hz, 3H); m/z 384.8 $(M+Na)^-$.

2-Nonylthio-5,8-dimethoxy-1,4-naphthoquinone (5i): Obtained as a red solid (400 mg, 77%): m.p. 75-76 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.77-1.27 (m, 14H), 0.89 (t, J = 7.2 Hz, 3H); m/z 398.8 $(M+Na)^-$.

2-Decylthio-5,8-dimethoxy-1,4-naphthoquinone (5j): Obtained as a red solid (348 mg, 64.5%): m.p. 97-98 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.2 Hz, 1H), 6.45 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.76-1.26 (m, 16H), 0.88 (t, J = 7.2 Hz, 3H); m/z 412.9 $(M+Na)^-$.

2-Undecanthio-5,8-dimethoxy-1,4-naphthoquinone (5k): Obtained as a red solid (280 mg, 50.1%): m.p. 83-84 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 6.44 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.74-1.69 (m, 2H), 1.47-1.42 (m, 2H), 1.35-1.25 (m, 14H), 0.87 (t, J = 6.8 Hz, 3H); m/z 405.1 $(M+H)^+$.

2-Dodecanthio-5,8-dimethoxy-1,4-naphthoquinone (5l): Obtained as a red solid (234 mg, 40.5%): m.p. 74-75 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.6 Hz, 1H), 7.26 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.74-1.70 (m, 2H), 1.47-1.42 (m, 2H), 1.35-1.25 (m, 16H), 0.87 (t, J = 6.8 Hz, 3H); m/z 441.1 $(M+Na)^-$.

2-Pentadecanthio-5,8-dimethoxy-1,4-naphthoquinone (5m): Obtained as a red solid (325 mg, 51.1%): m.p. 78-80 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.2 Hz, 1H), 7.26 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.75 (t, J = 7.2 Hz, 2H), 1.74-1.69 (m, 2H), 1.47-1.42 (m, 2H), 1.35-1.25 (m, 22H), 0.87 (t, J = 7.2 Hz, 3H); m/z 483.1 $(M+Na)^-$.

2-Octadecanthio-5,8-dimethoxy-1,4-naphthoquinone (5n): Obtained as a red solid (264 mg, 38.1%): m.p. 97-98 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.6 Hz, 1H), 7.26 (d, J = 9.6 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 2.75 (t, J = 7.6 Hz, 2H), 1.76-1.69 (m, 2H), 1.47-1.42 (m, 2H), 1.35-1.25 (m, 28H), 0.88 (t, J = 7.2 Hz, 3H); m/z 525.1 $(M+Na)^-$.

2-Phenylthio-5,8-dimethoxy-1,4-naphthoquinone (5o): Obtained as a red solid (261 mg, 58%): m.p. 105-106 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.52-7.47 (m, 5H), 7.31 (s, 2H), 5.97 (s, 1H), 3.98 (s, 3H), 3.92 (s, 3H); m/z 348.7 $(M+Na)^-$.

2-(11-Hydroxyundecanthio)-5,8-dimethoxy-1,4-naphthoquinone (5p): Obtained as a red solid (390 mg, 67.2%): m.p. 67-68 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.6Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.64 (t, J = 6.8 Hz, 2H), 2.75 (t, J = 7.2 Hz, 2H), 1.75-1.69 (m, 2H), 1.47-1.42 (m, 2H), 1.35-1.25 (m, 14H); m/z 442.9 $(M+Na)^+$.

11-(5,8-Dimethoxy-1,4-dioxo-naphthalen-2-ylthio)undecanoic acid (5q): Obtained as a red solid (467 mg, 77.9%): m.p. 146-147 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.6 Hz, 1H), 6.46 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.34 (t, J = 7.2 Hz, 2H), 2.75 (t, J = 2.7 Hz, 2H), 2.41-2.32 (m, 5H), 2.06-2.00 (m, 2H), 1.76-1.56 (m, 5H), 1.48-1.39 (m, 2H), 0.97-0.88 (m, 2H); m/z 435 $(M+H)^+$.

11-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)isobutyl amide (5r): To a solution of **5q** (250 mg, 0.575 mmol) in dry dichloromethane (30 mL) was added DCC (142 mg, 0.69 mmol), DMAP (28.1 mg, 0.23 mmol), and isobutyl amide (0.069 mL, 0.69 mmol). The mixture was stirred at 0 °C for 4 h and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (hexanes:EtOAc = 3:1) to give compound **5r** (219 mg, 77.9%) as a red solid: m.p. 74-75 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 9.2 Hz, 1H), 6.44 (s, 1H), 5.55 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.10 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 7.6 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.78-1.7 (m, 2H), 1.63 (m, 2H), 1.45 (m, 1H), 0.92 (s, 3H), 0.90 (s, 3H); m/z 490.0 $(M+H)^+$.

11-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)isobutyl ester (5s): To a solution of **5q** (250 mg, 0.575 mmol) in dry dichloromethane (30 mL) was added DCC (142 mg, 0.69 mmol), DMAP (28 mg, 0.23 mmol), and isobutyl alcohol (63.8 mL, 0.69 mmol). The mixture was stirred at 0 °C for 4 h and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (hexanes: EtOAc = 3:1) to give compound **5s** (138 mg, 48.8%) as a red solid: m.p. 58-59 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.32 (d, J = 9.6 Hz, 1H), 7.26 (d, J = 9.6Hz, 1H), 6.44 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.85 (d, J = 6.8 Hz, 2H), 2.75 (t, J = 7.6 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 1.96-1.89 (m, 1H), 1.72-1.68 (m, 4H), 1.29-1.25 (m, 12H), 0.94 (s, 3H), 0.92 (s, 3H); m/z 491 $(M+H)^+$.

General procedure for the synthesis of compounds (6a-6k). To a solution of **11** (301 mg, 1.38 mmol) in methanol (30 mL) was added the corresponding alkyl amine (2.07 mmol). The mixture was stirred at room temperature for 4 h and evaporated under reduced pressure. The crude product was purified by column chromatography (hexanes:EtOAc = 2:1) to give the titled compounds **6a-6k**.

2-Methylamino-5,8-dimethoxy-1,4-naphthoquinone (6a): Obtained as a red solid (194 mg, 56.7%): m.p. 203-204 °C; ^{1}H-NMR ($CDCl_3$, 400MHz) δ 7.34 (d, J = 9.6 Hz), 7.19 (d, J = 9.2 Hz, 1H), 5.75 (br s, 1H), 5.60 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 2.87 (d, J = 5.2 Hz, 3H); m/z 248 $(M+H)^+$.

2-Ethylamino-5,8-dimethoxy-1,4-naphthoquinone (6b): Obtained as a red solid (85 mg, 23.6%): m.p. 172-173 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.2 Hz, 1H), 7.19 (d, J = 9.6 Hz, 1H), 5.63 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.18-3.127 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H); m/z 262.1 $(M+H)^+$.

2-Propylamino-5,8-dimethoxy-1,4-naphthoquinone (6c): Obtained as a red solid (177 mg, 46.5%): m.p. 175-176 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.2 Hz, 1H), 7.19 (d, J = 9.2 Hz, 1H), 5.72 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.09 (q, 2H), 1.68 (m, J = 6.8 Hz, 2H), 0.99 (t, J = 7.6 Hz, 3H); m/z 276 $(M+H)^+$.

2-Butylamino-5,8-dimethoxy-1,4-naphthoquinone (6d): Obtained as a white solid (185 mg, 46.2%): m.p. 104-105 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.6 Hz, 1H), 7.19 (d, J = 9.6 Hz, 1H), 5.70 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.64 (m, 2H), 1.46-1.38 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); m/z 290 $(M+H)^+$.

2-Pentylamino-5,8-dimethoxy-1,4-naphthoquinone (6e): Obtained as a red solid (234 mg, 55.9%): m.p. 106-107 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.2 Hz, 1H), 7.19 (d, J = 9.2 Hz, 1H), 5.70 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H); m/z 303.6 $(M+H)^+$.

2-Hexylamino-5,8-dimethoxy-1,4-naphthoquinoe (6f): Obtained as a red solid (207 mg, 47.3%): m.p. 83-84 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.6 Hz, 1H), 7.18 (d, J = 9.6 Hz, 1H), 5.69 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.66-1.58 (m, 4H), 1.32-1.30 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H); m/z 318 $(M+H)^+$.

2-Heptylamino-5,8-dimethoxy-1,4-napthoquinone (6g): Obtained as a red solid (191 mg, 41.8%): m.p. 74-75 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.6 Hz, 1H), 7.33 (d, J = 9.6 Hz, 1H), 7.18 (d, J = 9.6 Hz, 1H), 5.69 (br s, 1H), 5.60 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.66-1.61 (m, 2H), 1.35-1.29 (m, 8H), 0.89 (t, J = 6.4 Hz, 3H); m/z 332 $(M+H)^+$.

2-Octylamino-5,8-dimethoxy-1,4-naphthoquinone (6h): Obtained as a red solid (214 mg, 45.1%): m.p. 81-82 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.6 Hz, 1H), 7.18 (d, J = 9.6 Hz, 1H), 5.69 (br, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.68-1.61 (m, 2H), 1.41-1.20 (m, 10H), 0.88 (t, J = 6.4 Hz, 3H); m/z 346 $(M+H)^+$.

2-Nonylamino-5,8-dimethoxy-1,4-naphthoquinone (6i): Obtained as a red solid (218 mg, 44.0%): m.p. 85-86 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 9.6 Hz, 1H), 7.18 (d, J = 9.6 Hz, 1H), 5.69 (br s, 1H), 5.61 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 7.2 Hz, 2H), 1.66-1.61 (m, 2H), 1.41-1.20 (m, 12H), 0.88 (t, J = 6.4 Hz, 3H); m/z 360 $(M+H)^+$.

2-Decylamino-5,8-dimethoxy-1,4-naphthoquinone (6j): Obtained as a red solid (91 mg, 17.6%): m.p. 86-87 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.2 Hz, 1H), 7.18 (d, J = 9.2 Hz, 1H), 5.69 (br s, 1H), 5.60 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.66-1.61 (m, 2H), 1.40-1.20 (m, 14H), 0.88 (t, J = 6.4 Hz, 3H); m/z 374.0 $(M+H)^+$.

2-Dodecylamino-5,8-dimethoxy-1,4-naphthoquinone (6k): Obtained as a red solid (308 mg, 55.5%): m.p. 74-75 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 9.6 Hz, 1H), 7.18 (d, J = 9.2 Hz, 1H), 5.69 (br s, 1H), 5.60 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.11 (q, J = 6.8 Hz, 2H), 1.66-1.61 (m, 2H), 1.40-1.20 (m, 18H), 0.88 (t, J = 6.4 Hz, 3H); m/z 402.0 $(M+H)^+$.

ACAT inhibition assay. *In vitro* study for cellular cholesteryl ester formation: Target cells were seeded in a 6 well plate at the density of 1×10^6 cells/mL/well and cultured in the medium containing 10% FBS for 2 days and then cultured overnight in the medium containing 1% BSA. The medium was replaced and the cells were incubated with 2.5 μL of sample or 0.1% DMSO 0.1% as their vehicle and [1-^{14}C] oleic acid (0.5 μCi) for 6 h in 6 well plate. Then, the medium was removed and the cells were washed three times with PBS. The intracellular lipids of cells were extracted by hexanes/isopropanol (3/2) and the organic phase was evaporated under nitrogen. The total lipid was separated by silica gel TLC plate in petroleum ether/diethyl ether/acetic acid (90/10/1) and the amount of radioactivity was analyzed with a bioimaging analyzer (BAS-1500, FUJIFILM). Each experiment was performed at least in triplicate. Results are expressed or plotted as the mean value.

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References

1. Meiner, V. L.; Cases, S.; Myers, H. M. *et al. Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14041.
2. Sucking, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647.
3. Adameova, A.; Kuzelova, M.; Faberova, V.; Holub, P.; Svec, P. *Triglycerides and Cholesterol Research*; Welson, L. T., Ed.; Nova Science Publishers, Inc.: 2006; Chapter VII.
4. Ikenoya, M.; Yoshinaka, Y.; Kobayashi, H.; Kawamine, K.; Shibuya, K.; Sato, F.; Sawanobori, K.; Watanabe, T.; Miyazaki, A. *Atherosclerosis* **2007**, *191*, 290.
5. Terasaka, N.; Miyazaki, A.; Kasanuki, N.; Ito, K.; Ubukata, N.; Koieyama, T.; Kitayama, K.; Tanimoto, T.; Maeda, N.; Inaba, T. *Atherosclerosis* **2007**, *190*, 239.
6. Zamorano-Leon, J. J.; Fernandez-Sanchez, R.; Lopez Farre, A. J.; Lapuente-Tiana, L.; Alonso-Orgaz, S.; Sacristan, D.; Junquera, D.; Delhon, A.; Conesa, A.; Mateos-Caceres, P. J.; Macaya, C. *J. Cardiovas. Pharm.* **2006**, *48*, 128.
7. Ross, R. *New Engl. J. Med.* **1999**, *340*, 115.
8. Stein, O.; Stein, Y. *Atherosclerosis* **2005**, *178*, 217.
9. An, S.; Park, Y. D.; Paik, Y. K.; Jeong, T. S.; Lee, W. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1112.
10. Rho, M. C.; Lee, S. W.; Park, H. R.; Choi, J. H.; Kang, J. Y.; Kim, K.; Lee, H. S.; Kim, Y. K. *Phytochem.* **2007**, *68*, 899.