Bis(2-(Acylamino)phenyl) Disulfides, 2-(Acylamino)benzenethiols, and *S*-(2-(Acylamino)phenyl) Alkanethioates as Novel Inhibitors of Cholesteryl Ester Transfer Protein

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A series of bis(2-(acylamino)phenyl) disulfides, 2-(acylamino)benzenethiols, *S*-(2-(acylamino)-phenyl) alkanethioates, and related compounds were synthesized, and their inhibitory effect on cholesteryl ester transfer protein activity in human plasma was evaluated. This study elucidated the structural requirements for inhibitory activity and determined that the optimum compound was *S*-(2-((1-(2-ethylbutyl)cyclohexane)carbonylamino)phenyl) 2-methylpropanethioate **(27)** (JTT-705). This compound achieved 50% inhibition of CETP activity in human plasma at a concentration of 9 μM and 95% inhibition of CETP activity in male Japanese white rabbits at an oral dose of 30 mg/kg. It increased the plasma HDL cholesterol level by 27% and 54%, respectively, when given at oral doses of 30 or 100 mg/kg once a day for 3 days to male Japanese white rabbits.

Introduction

Cholesteryl ester transfer protein (CETP) is a plasma protein that transfers neutral lipids among the lipoproteins.[1,2] Its most important action is the exchange of cholesteryl esters (CE) in high-density lipoprotein (HDL) for triglycerides in very low-density lipoprotein (VLDL).[1,2] The net effect of this process is to reduce the level of antiatherogenic HDL cholesterol while increasing proatherogenic VLDL and low-density lipoprotein (LDL).[1,2] Thus, CETP is a potentially atherogenic protein, and its atherogenicity has been supported by many studies.[3−6] On the other hand, a possible antiatherogenic role of CETP and the participation of CETP in reverse cholesterol transport (RCT) have also been suggested.[7−10] RCT is the movement of cholesterol from the peripheral cells through the plasma compartment (including HDL) to the liver for catabolism.[11] Thus, the role of CETP in the progression of atherosclerosis is still not defined. A powerful CETP inhibitor could be useful in studies to elucidate the role of CETP in atherosclerosis, so we have attempted to synthesize such an inhibitor. If the antiatherogenicity of CETP inhibition were confirmed, we should also be able to obtain a novel antiatherogenic drug from among the synthetic inhibitors of CETP.

Here we describe the structure−activity relationship (SAR) and optimization studies performed on a series of bis(2-(acylamino)phenyl) disulfides, 2-(acylamino)-benzenethiols, *S*-(2-(acylamino)phenyl) alkanethioates, and related compounds. These studies determined the structural requirements for CETP inhibition and eventually led to the optimum compound, *S*-(2-((1-(2-ethylbutyl)cyclohexane)carbonylamino)phenyl) 2-methylpropanethioate **(27)** (JTT-705), which achieved marked inhibition of CETP in human plasma and caused significant elevation of HDL cholesterol in male Japanese white rabbits (JW rabbits). The mechanism of action was considered to involve the formation of a disulfide bond between the thiol form of compound **27** and the cysteine residue at position 13 (Cys^{13}) of CETP.

Chemistry

The routes used for synthesis of the compounds assessed in this study are shown in Schemes 1−4. Scheme 1 shows the preparation of disulfides, thiols, thioesters, and a sulfide. Bis(2-(acylamino)phenyl) disulfides (**1**−**9** and the precursors of **17**−**24**) were synthesized by N-acylation of bis(2-aminophenyl) disulfide with the corresponding acyl chloride. Reduction of the disulfide bond with triphenylphosphine gave 2-acylaminobenzenethiols (**16**−**24**). The benzenethiols were coupled with the corresponding acyl chloride to give thioesters (**14** and **26**−**28**). The sulfide **25** was synthesized by methylation of 2-((1-(2-ethylbutyl)cyclohexane)carbonylamino)benzenethiol with iodomethane.

Bis(2-(*N*-methyl-*N*-pivaloylamino)phenyl) disulfide **(11)**, bis(3-(pivaloylamino)phenyl) disulfide **(12)**, or bis(4-(pivaloylamino)phenyl) disulfide **(13)** was also prepared by N-acylation of bis(2-(methylamino)phenyl) disulfide, bis(3-aminophenyl) disulfide, or bis(4-aminophenyl) disulfide, respectively, with pivaloyl chloride.

Commercially unavailable 1-alkylcyclohexanecarbonyl chlorides were synthesized by dianion coupling between cyclohexanecarboxylic acid and the corresponding alkyl bromide, followed by treatment with oxalyl chloride (Scheme 2).

The reversed amide **10** was prepared by coupling between 2,2′-dithiodibenzoic acid and *tert*-butylamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl; water-soluble carbodiimide hydrochloride) and 1-hydroxybenzotriazole (HOBt) (Scheme 3).

Diacylation of 2-aminophenol with 1-methylcyclohexanecarbonyl chloride and subsequent hydrolysis gave 1-((1-methylcyclohexane)carbonylamino)phenol. Cou-

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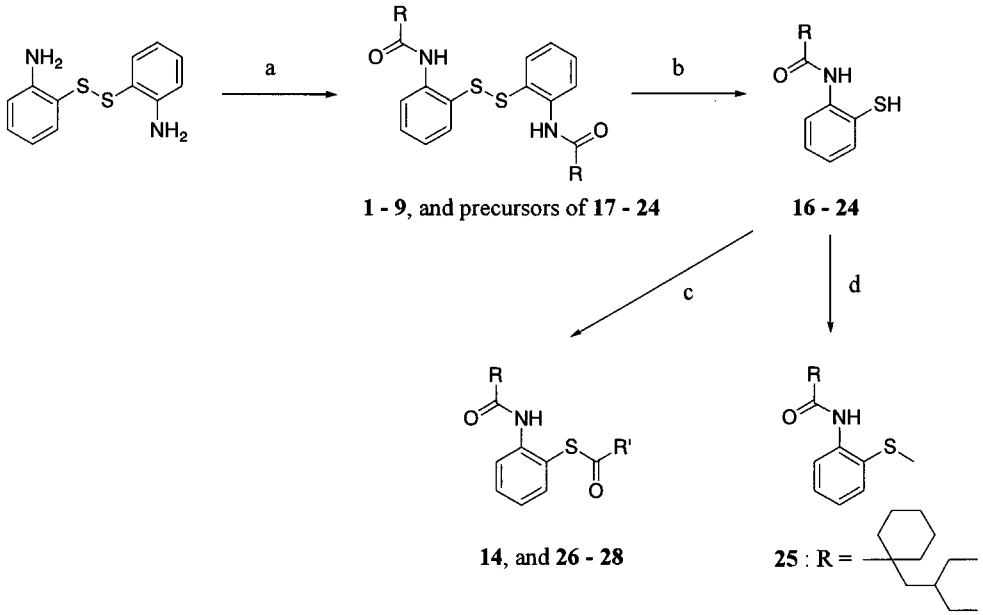
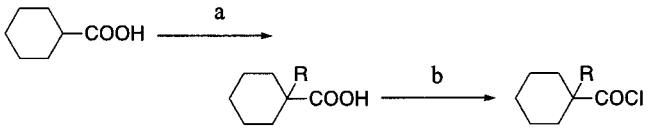
Scheme 1[a]



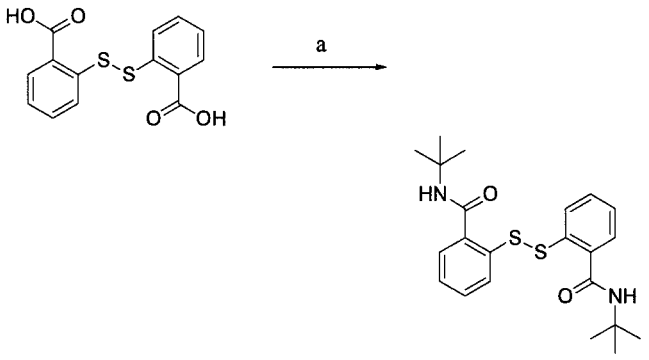
[a] Reagents: (a) R-COCl, pyridine, 100 °C; (b) triphenylphosphine; (c) R′-COCl, pyridine; (d) iodomethane, K_2CO_3.

Scheme 2[a]



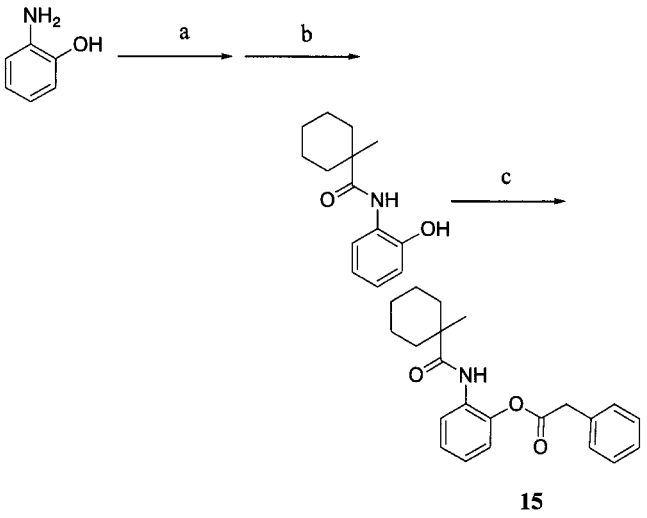
[a] Reagents: (a) 2 equiv of lithium diisopropylamide, R-Br; (b) oxalyl chloride.

Scheme 3[a]



[a] Reagents: (a) *tert*-butylamine, WSCD·HCl, HOBt.

Scheme 4[a]



[a] Reagents: (a) R-COCl, Et_3N; (b) KOH; (c) phenylacetyl chloride, pyridine.

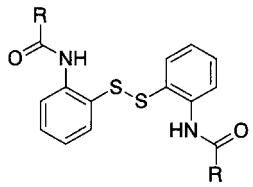
pling between the phenol and phenylacetyl chloride gave the ester **15** (Scheme 4).

Results and Discussion

The goal of this study was to synthesize a specific CETP inhibitor that showed activity when administered orally. We selected bis(2-(acetylamino)phenyl) disulfide (**1**) as the initial lead compound in our chemical library, because it showed 50% inhibition of CETP activity in human plasma at a concentration of 500 μM. The structure of this lead compound was systematically varied in order to elucidate the structural requirements for inhibition of CETP and to synthesize the optimum compound. The various compounds synthesized were primarily evaluated for their ability to inhibit CETP-mediated CE transfer in human plasma. Selected compounds were further evaluated for their ability to inhibit CETP and to increase HDL cholesterol in JW rabbits.

First, the effect of the acyl groups on the inhibition of CETP was examined (Table 1). The propionyl compound **2** was about 2.8-fold more potent than the acetyl compound **1**, while the isobutyryl compound **3** was about 12-fold more potent and the valeryl compound **4** was 20-fold more potent. These results indicated that the improvement of potency was attributable to an increase in the lipophilicity of the acyl moiety. However, the potency of the cyclohexanecarbonyl compound **6** and the octanoyl compound **7** was almost equivalent to that of the valeryl compound **4**. This showed that a further increase of lipophilicity in the acyl moiety did not lead to a further improvement in potency. Thus, the effect of acyl moiety lipophilicity on potency showed a plateau. On the other hand, the pivaloyl compound **5** and the 1-methylcyclohexanecarbonyl compound **8** with a ter-

Table 1. Bis(2-(Acylamino)phenyl) Disulfides



compd	R	CETP inhibition in human plasma IC_{50} (μM)[a]
1	CH_3	500
2	C_2H_5	180
3	$(CH_3)_2CH$	41
4	n-C_4H_9	25
5	$(CH_3)_3C$	13
6		22
7	n-C_7H_{15}	21
8		8
9		101

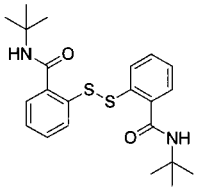
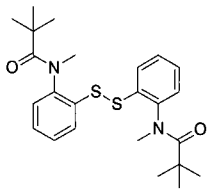
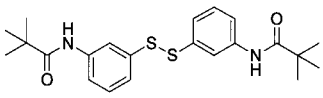
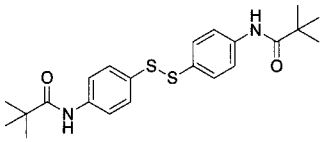
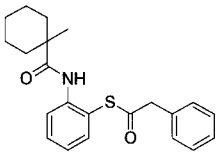
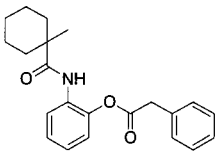
[a] Concentration achieving 50% inhibition of CETP-mediated CE transfer from HDL to VLDL and LDL.

tiary alkyl group next to the carbonyl group displayed higher potency than the other alkyl compounds shown in Table 1. Replacement of the cyclohexane ring of compound **6** with a phenyl ring (compound **9**) caused a decrease in potency. Accordingly, not only lipophilicity but also the shape of the acyl moiety seemed to influence the potency, and tertiary alkyl carbonyl groups were found to be specific for high potency.

Next, the amide moiety was examined (Table 2). The reversed amide **10** showed no activity, and introduction of a methyl group into the amide nitrogen (**11**) caused loss of activity. These results indicated that the benzamide structure was important for activity and that the amide proton may be essential.

Subsequently, the relative position between the disulfide moiety and the amide group on the benzene ring of compound **5** was changed (Table 2). Regioisomers **12** and **13**, in which the disulfide group was moved from the ortho-position of the pivaloylaminobenzene to the meta- or para-position, showed loss of activity. Thus, both the disulfide group and the amide group substituted at the next position were essential for activity. On the other hand, the thioester **14** also showed activity, indicating that the thioester group could play the same role as the disulfide group and that the dimeric structure was not essential. Since the ester **15** did not show activity, the sulfur atom was essential. Because the thioester was readily hydrolyzed to a thiol, we suspected

Table 2. Analogues of Compounds **5** and **8**

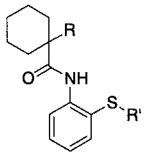
compd	structure	CETP inhibition in human plasma IC_{50} (μM)[a]
10		>500
11		>300
12		>500
13		>500
14		23
15		>300

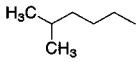
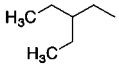
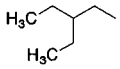
[a] Concentration achieving 50% inhibition of CETP-mediated CE transfer from HDL to VLDL and LDL.

that it was the precursor of an active thiol. In fact, thiol **16** showed activity, as anticipated (Table 3). The disulfide would also be active because it was not observed to undergo transformation to the thiol in vitro. Therefore, the disulfide group and the thiol group were functionally equivalent. Because both disulfides and thiols have the ability to form a disulfide bond with another thiol group, we considered that formation of a disulfide bond between the test compounds and a cysteine residue of CETP may have been related to the mechanism of inhibition. Therefore, we performed a study using point mutations of recombinant human CETP and showed that Cys^{13} of CETP was targeted when the compounds synthesized in this study exhibited an inhibitory effect.[14] We also confirmed that the inhibitory activity was maintained after washout of the test compounds.

Since we found that the 1-methylcyclohexanecarbonyl structure of the acyl moiety was specific for high potency in the series of disulfide compounds (Table 1), synthesis of various 1-alkylcyclohexanecarbonyl compounds was carried out for further optimization using a series of thiol compounds (Table 3). Introduction of alkyl groups

Table 3. 2-Acylaminobenzenethiols



compd	R	R'	CETP inhibition in human plasma IC_{50} (μM)[a]
16	CH_3	H	17
17	C_2H_5	H	12
18	n-C_3H_7	H	8
19	n-C_4H_9	H	9
20	n-C_5H_{11}	H	7
21	n-C_6H_{13}	H	6
22		H	6
23		H	3
24	n-C_7H_{15}	H	7
25		CH_3	>300

[a] Concentration achieving 50% inhibition of CETP-mediated CE transfer from HDL to VLDL and LDL.

with 2−7 carbon atoms at the 1-position of the cyclohexane ring was performed. Introducing an alkyl group with 6 carbon atoms was found to be the most effective modification, and the 2-ethylbutyl compound **23** showed the highest potency. This compound achieved 50% inhibition of CETP activity in human plasma at a concentration of 5 μM and was about 100-fold more potent than lead compound **1**. The *S*-methyl compound **25**, which was unable to form disulfide bonds with Cys of CETP, showed loss of activity as expected. Several studies have already demonstrated that CETP is inhibited by certain cysteine-modifying reagents under serum-free conditions,[12,13] but such compounds have not been confirmed to be effective in plasma or in vivo. Nonspecific reactions between these compounds and endogenous cysteine-containing compounds, such as glutathione, could decrease their inhibitory activity under in vivo conditions.

Since the disulfides in Tables 1 and 2 were not orally bioavailable and the thiols in Table 3 were unstable, orally bioavailable thioesters were used for the in vivo assays (Table 4). In vitro, the acetyl compound **26** was more potent than the isobutyryl compound **27**, and the pivaloyl compound **28** was much weaker than **26** or **27**. The differences in the IC_{50} values of these thioesters were probably due to differences in the rate of hydrolysis. In contrast, the isobutyryl compound **27** showed 95% inhibition of CETP activity at an oral dose of 30 mg/kg in JW rabbits and was the most potent of these compounds invivo (**26**−**28**). Thus, compound **27** had a higher absorption than compound **26**. After the in vivo inhibitory effect of compound **27** on CETP was confirmed, its effect on the plasma HDL cholesterol level was examined in JW rabbits. As a result, a 27% or 54% increase of plasma HDL cholesterol was respectively observed when 30 or 100 mg/kg of compound **27** was administered orally once a day for 3 days.

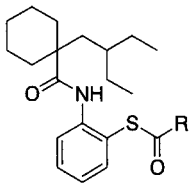
Conclusions

SAR studies on disulfide, thiol, and thioester compounds elucidated the structural requirements for inhibition of CETP and led to the optimum compound **27**. This compound showed marked inhibition of CETP in human plasma and in JW rabbits and caused a significant increase of the plasma HDL cholesterol level in JW rabbits. The mechanism of action was considered to be formation of a disulfide bond between the inhibitor and Cys[13] of CETP. Since 6 months of treatment with compound **27** (JTT-705) increased the HDL cholesterol level, decreased the LDL level, and prevented the progression of atherosclerosis in rabbits,[14] development of this compound as an antiatherosclerosis drug is currently underway in clinical trials.

Experimental Section

Chemistry. Melting points were obtained with a Yanagimoto micro melting point apparatus or a Mettler-Toledo FP62 melting point instrument and are uncorrected. Elemental

Table 4. *S*-(2-(Acylamino)phenyl) Alkanethioates



compd	R	CETP inhibition in human plasma IC_{50} (μM)[a]	CETP inhibition in JW rabbits (%)[b] 10 mg/kg	CETP inhibition in JW rabbits (%)[b] 30 mg/kg	HDL elevation in JW rabbits (%)[c] 30 mg/kg × 3	HDL elevation in JW rabbits (%)[c] 100 mg/kg × 3
26	CH_3	4		40		
27	$(CH_3)_2CH$	6	35	95	27	54
28	$(CH_3)_3C$	41		32		

[a] Concentration achieving 50% inhibition of CETP-mediated CE transfer from HDL to VLDL and LDL. [b] Percent inhibition of CETP was measured 3 h after administration of the test compound and was calculated from CETP-mediated CE transfer in the plasma of JW rabbits administered the test compounds at oral doses of 10 and 30 mg/kg relative to the transfer in plasma from rabbits given the vehicle. Inhibition was shown to persist for 9 h. [c] Percent elevation was calculated from the plasma HDL levels in JW rabbits administered the test compounds at oral doses of 30 and 100 mg/kg once a day for 3 days relative to the levels in rabbits given the vehicle.

analysis was performed with a Perkin-Elmer 2400 series II CHNS/O analyzer, and all values were within ±0.4% of the calculated values. FAB mass spectra were recorded on a Finnigan TSQ 700 spectrometer. High-resolution mass spectra were obtained with a JEOL SX 102A spectrometer. ^{1}H NMR spectra were recorded on a JEOL JNM-A300W or Bruker AMX 300 spectrometer in a solution of either $CDCl_3$ or DMSO-d_6 using tetramethylsilane as the internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. All compounds gave spectra consistent with their assigned structures.

Bis(2-acylaminophenyl) Disulfides 1−9. A typical run (**8** in Table 1) was as follows. A mixture of 1-methylcyclohexanecarboxylic acid (680 mg, 4.78 mmol) and oxalyl chloride (0.5 mL, 5.73 mmol) in methylene chloride (5 mL) was stirred at room temperature for 1 h and concentrated to a crude acid chloride. This crude 1-methylcyclohexanecarbonyl chloride in methylene chloride (5 mL) was added dropwise to a solution of bis(2-aminophenyl) disulfide (500 mg, 2.01 mmol) and pyridine (0.4 mL, 4.95 mmol) in methylene chloride (5 mL) at room temperature for 1 h. The mixture was diluted with chloroform (50 mL), washed with water (30 mL) and with brine (30 mL), dried over sodium sulfate, and concentrated. The crude product was subjected to silica gel chromatography and eluted with ethyl acetate/*n*-hexane (1:5) to give bis(2-((1-methylcyclohexane)carbonylamino)phenyl) disulfide **(8)** (980 mg, 98%) as an amorphous powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, 6H), 1.25−1.70 (m, 16H), 1.80−2.00 (m, 4H), 6.95 (dt, J = 1.5 and 7.8 Hz, 2H), 7.22 (dd, J = 1.5 and 7.8 Hz, 2H), 7.40 (ddd, J = 1.5, 7.8, and 8.4 Hz, 2H), 8.48 (dd, J = 1.5 and 8.4 Hz, 2H), 8.51 (br s, 2H); MS (FAB) m/z 497 $(M + H)^+$; HRMS calcd for $C_{28}H_{37}N_2O_2S_2$, 497.2296; found, 497.2288.

Bis(2-(acetylamino)phenyl) disulfide (1): mp 162−163 °C; NMR (300 MHz, $CDCl_3$) δ 1.98 (s, 6H), 7.03 (dt, J = 1.5 and 7.8 Hz, 2H), 7.37−7.45 (m, 4H), 7.90 (br s, 2H), 8.35 (d, J = 8.1 Hz, 2H). Anal. ($C_{16}H_{16}N_2O_2S_2 \cdot 0.5H_2O$) C, H, N.

Bis(2-(propionylamino)phenyl) disulfide (2): mp 139−140 °C; NMR (300 MHz, $CDCl_3$) δ 1.15 (t, J = 7.5 Hz, 6H), 2.19 (q, J = 7.5 Hz, 4H), 7.00 (dt, J = 1.5 and 7.8 Hz, 2H), 7.37−7.42 (m, 4H), 7.96 (br s, 2H), 8.38 (dd, J = 1.5 and 8.4 Hz, 2H). Anal. ($C_{18}H_{20}N_2O_2S_2$) C, H, N.

Bis(2-(isobutyrylamino)phenyl) disulfide (3): mp 147−148 °C; NMR (300 MHz, $CDCl_3$) δ 1.18 (d, J = 6.9 Hz, 12H), 2.32 (sep, J = 6.9 Hz, 2H), 6.96 (dt, J = 1.5 and 7.8 Hz, 2H), 7.31 (dd, J = 1.5 and 7.8 Hz, 2H), 7.40 (ddd, J = 1.5, 7.8, and 8.4 Hz, 2H), 8.07 (br s, 2H), 8.40 (dd, J = 1.5 and 8.4 Hz, 2H). Anal. ($C_{20}H_{24}N_2O_2S_2$) C, H, N.

Bis(2-(valerylamino)phenyl) disulfide (4): mp 68−70 °C; NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.2 Hz, 6H), 1.30−1.75 (m, 8H), 2.17 (t, J = 7.2 Hz, 4H), 7.00 (dt, J = 1.5 and 7.8 Hz, 2H), 7.36−7.48 (m, 4H), 7.94 (br s, 2H), 8.40 (dd, J = 1.5 and 8.4 Hz, 2H). Anal. ($C_{22}H_{28}N_2O_2S_2 \cdot 0.15C_6H_{14}$) C, H, N.

Bis(2-(pivaloylamino)phenyl) disulfide (5): mp 86−87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 18H), 6.94 (dt, J = 1.5 and 7.8 Hz, 2H), 7.21 (dd, J = 1.5 and 7.8 Hz, 2H), 7.40 (ddd, J = 1.5, 7.8, and 8.4 Hz, 2H), 8.46 (dd, J = 1.5 and 8.4 Hz, 2H), 8.52 (br s, 2H). Anal. ($C_{22}H_{28}N_2O_2S_2$) C, H, N.

Bis(2-(cyclohexanecarbonylamino)phenyl) disulfide (6): mp 167−170 °C; NMR (300 MHz, $CDCl_3$) δ 1.10−2.10 (m, 22H), 6.99 (dt, J = 1.5 and 7.8 Hz, 2H), 7.36−7.45 (m, 4H), 8.09 (br s, 2H), 8.44 (dd, J = 1.5 and 8.4 Hz, 2H). Anal. ($C_{26}H_{32}N_2O_2S_2$) C, H, N.

Bis(2-(octanoylamino)phenyl) disulfide (7): mp 79−81 °C; NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 6H), 1.20−1.40 (m, 16H), 1.50−1.70 (m, 4H), 2.16 (t, J = 7.2 Hz, 4H), 7.00 (dt, J = 1.5 and 7.8 Hz, 2H), 7.35−7.43 (m, 4H), 7.97 (br s, 2H), 8.40 (dd, J = 1.5 and 8.4 Hz, 2H). Anal. ($C_{28}H_{40}N_2O_2S_2$) C, H, N.

Bis(2-(benzoylamino)phenyl) disulfide (9): mp 144−145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (dt, J = 1.5 and 7.8 Hz, 2H), 7.31 (dt, J = 1.5 and 8.4 Hz, 2H), 7.40−7.60 (m, 8H), 7.69 (dd, J = 1.5 and 8.4 Hz, 4H), 8.50 (dd, J = 1.5 and 8.4 Hz, 2H), 8.93 (br s, 2H). Anal. ($C_{26}H_{20}N_2O_2S_2$) C, H, N.

Bis(2-(*N*-methyl-*N*-pivaloylamino)phenyl) disulfide (11): mp 192−193 °C; NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 18H), 3.42 (s, 6H), 7.14−7.33 (m, 6H), 7.44−7.58 (m, 2H). Anal. ($C_{24}H_{32}N_2O_2S_2$) C, H, N.

Bis(3-(pivaloylamino)phenyl) disulfide (12): mp 155−157 °C; NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 18H), 7.16−7.27 (m, 4H), 7.35−7.48 (m, 4H), 7.75 (s, 2H). Anal. ($C_{22}H_{28}N_2O_2S_2$) C, H, N.

Bis(4-(pivaloylamino)phenyl) disulfide (13): mp 200−201 °C; NMR (300 MHz, DMSO -d_6) δ 1.19 (s, 18H), 7.41 (d, J = 8.7 Hz, 4H), 7.60 (d, J = 8.7 Hz, 4H), 9.30 (br s, 2H). Anal. ($C_{22}H_{28}N_2O_2S_2 \cdot 0.3C_6H_{14}$) C, H, N.

2-((1-(2-Ethylbutyl)cyclohexane)carbonylamino)benzenethiol (23). Lithium diisopropylamide (2.0 M) in tetrahydrofuran (100 mL, 200 mmol) was added dropwise to a solution of cyclohexanecarboxylic acid (10.3 g, 80 mmol) in tetrahydrofuran (100 mL) at 0 °C. After stirring for 1.5 h at room temperature, a solution of 1-bromo-2-ethylbutane (21.1 g, 128 mmol) in tetrahydrofuran (20 mL) was added dropwise at 0 °C. Then the mixture was stirred overnight at room temperature. The solution was poured into water (50 mL), acidified with 2 N HCl (150 mL), and extracted with ethyl acetate (200 mL). The combined extracts were washed with water (100 mL), dried over sodium sulfate, and concentrated to a crude 1-(2-ethylbutyl)cyclohexanecarboxylic acid. A mixture of this crude acid and oxalyl chloride (8.0 mL, 91.7 mmol) in methylene chloride (100 mL) was stirred for 1 h at room temperature. After the solvent was evaporated under a vacuum, the residue was distilled to give the acid chloride (6.97 g, 38%) as a colorless liquid (bp 135 °C, 10 mmHg). The acid chloride (2.72 g, 11.8 mmol) was added dropwise to a solution of bis(2-aminophenyl) disulfide (1.46 g, 5.88 mmol) in pyridine (15 mL). Then the mixture was heated overnight at 100 °C. After evaporation of the solvent, water (50 mL) was added to the residue. The aqueous solution was extracted with ethyl acetate (2 × 100 mL), after which the extract was washed with brine (50 mL), dried over sodium sulfate, and concentrated. A mixture of this disulfide, triphenylphosphine (3.10 g, 11.8 mmol) in dioxane (25 mL) and water (25 mL) was stirred for 1 h at 50 °C. After cooling to room temperature, 1 N aqueous sodium hydroxide (50 mL) was added to the mixture. This aqueous solution was washed with *n*-henxane (30 mL), acidified to pH 3−4 with 10% hydrochloric acid, and extracted with ethyl acetate (2 × 100 mL). The organic layer was washed with brine (50 mL), dried over sodium sulfate, and concentrated. Chromatography of the residue on a silica gel column eluted with ethyl acetate/*n*-hexane (1:10) gave **23** as a colorless solid (2.66 g, 71%): mp 68.5−74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (t, J = 6.9 Hz, 6H), 1.20−1.80 (m, 15H), 2.05−2.25 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.51 (dd, J = 1.5 and 7.8 Hz, 1H), 8.33 (dd, J = 1.5 and 8.4 Hz, 1H), 8.45 (br s, 1H). Anal. ($C_{19}H_{29}NOS$) C, H, N.

2-((1-Methylcyclohexane)carbonylamino)benzenethiol (16): mp 82−83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 3H), 1.25−1.75 (m, 8H), 2.04−2.20 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.50 (dd, J = 1.5 and 7.8 Hz, 1H), 8.31 (dd, J = 1.5 and 8.4 Hz, 1H), 8.42 (br s, 1H). Anal. ($C_{14}H_{19}NOS$) C, H, N.

2-((1-Ethylcyclohexane)carbonylamino)benzenethiol (17): mp 84−85 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 7.2 Hz, 3H), 1.20−1.70 (m, 10H), 2.05−2.19 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.50 (dd, J = 1.5 and 7.8 Hz, 1H), 8.32 (dd, J = 1.5 and 8.4 Hz, 1H), 8.38 (br s, 1H). Anal. ($C_{15}H_{21}NOS$) C, H, N.

2-((1-Propylcyclohexane)carbonylamino)benzenethiol (18): mp 93−94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.2 Hz, 3H), 1.20−1.70 (m, 12H), 2.05−2.20 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.30 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.50 (dd, J = 1.5 and 7.8 Hz, 1H), 8.32 (dd, J = 1.5 and 8.4 Hz, 1H), 8.38 (br s, 1H). Anal. ($C_{16}H_{23}NOS$) C, H, N.

2-((1-Butylcyclohexane)carbonylamino)benzenethiol (19): mp 97−98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.2 Hz, 3H), 1.20−1.70 (m, 14H), 2.05−2.20 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.30 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.50 (dd, J = 1.5 and 7.8 Hz, 1H), 8.31 (dd, J = 1.5 and 8.4 Hz, 1H), 8.37 (br s, 1H). Anal. ($C_{17}H_{25}NOS$) C, H, N.

2-((1-Pentylcyclohexane)carbonylamino)benzenethiol (20): NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.7 Hz, 3H), 1.20−1.70 (m, 16H), 2.05−2.20 (m, 2H), 3.07 (s, 1H), 7.00 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.51 (dd, J = 1.5 and 7.8 Hz, 1H), 8.31 (dd, J = 1.5 and 8.4 Hz, 1H), 8.36 (br s, 1H). Anal. ($C_{18}H_{27}NOS$) C, H, N.

2-((1-Hexylcyclohexane)carbonylamino)benzenethiol (21): NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3H), 1.17−1.70 (m, 18H), 2.05−2.18 (m, 2H), 3.07 (s, 1H), 7.01 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.51 (dd, J = 1.5 and 7.8 Hz, 1H), 8.31 (dd, J = 1.5 and 8.4 Hz, 1H), 8.37 (br s, 1H). Anal. ($C_{19}H_{29}NOS$) C, H, N.

2-((1-Isohexylcyclohexane)carbonylamino)benzenethiol (22): mp 76−79 °C; NMR (300 MHz, $CDCl_3$) δ 0.83 (d, J = 6.6 Hz, 6H), 1.07−1.73 (m, 15H), 2.05−2.20 (m, 2H), 3.07 (s, 1H), 7.01 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.51 (dd, J = 1.5 and 7.8 Hz, 1H), 8.29 (dd, J = 1.5 and 8.4 Hz, 1H), 8.35 (br s, 1H). Anal. ($C_{19}H_{29}NOS$) C, H, N.

2-((1-Heptylcyclohexane)carbonylamino)benzenethiol (24): NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3H), 1.10−1.70 (m, 20H), 2.05−2.18 (m, 2H), 3.07 (s, 1H), 7.01 (dt, J = 1.5 and 7.8 Hz, 1H), 7.31 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.51 (dd, J = 1.5 and 7.8 Hz, 1H), 8.31 (dd, J = 1.5 and 8.4 Hz, 1H), 8.37 (br s, 1H); MS (FAB) m/z 334 $(M + H)^+$; HRMS calcd for $C_{20}H_{32}NOS$, 334.2204; found, 334.2195.

1-Methylthio-2-((1-(2-ethylbutyl)cyclohexane)carbonylamino)benzene (25). Iodomethane (0.12 mL, 1.88 mmol) was added to a mixture of **23** (100 mg, 1.88 mmol) and potassium carbonate (260 mg, 1.88 mmol) in dimethylformamide (10 mL). The mixture was stirred at room temperature for 15 min, after which the solution was poured into water (20 mL) and extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with water (20 mL), dried over sodium sulfate, and concentrated to give **25** as a colorless oil (585 mg, 93%): NMR (300 MHz, $CDCl_3$) δ 0.78 (t, J = 7.2 Hz, 6H), 1.15−1.70 (m, 15H), 2.05−2.25 (m, 2H), 2.38 (s, 3H), 7.04 (dt, J = 1.5 and 7.8 Hz, 1H), 7.30 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 7.49 (dd, J = 1.5 and 7.8 Hz, 1H), 8.42 (dd, J = 1.5 and 8.4 Hz, 1H), 8.78 (br s, 1H); MS (FAB) m/z 334 $(M + H)^+$; HRMS calcd for $C_{20}H_{32}NOS$, 334.2204; found, 334.2206.

***S*-(2-((1-(2-Ethylbutyl)cyclohexane)carbonylamino)-phenyl) 2-Methylpropanethioate (27).** Isobutyryl chloride (904 mg, 8.48 mmol) was added dropwise to a solution of **23** (2.66 g, 8.33 mmol) and pyridine (1.7 mL, 21 mmol) in chloroform (30 mL). The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was chromatographed on silica gel eluted with ethyl acetate/*n*-hexane (1:15) to give **27** as a colorless solid (3.15 g, 97%): mp 63−63.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78 (t, J = 6.9 Hz, 6H), 1.15−1.75 (m, 15H), 1.30 (d, J = 6.9 Hz, 6H), 1.95−2.20 (m, 2H), 2.94 (sep, J = 6.9 Hz, 1H), 7.11 (dt, J = 1.5 and 7.8 Hz, 1H), 7.38 (dd, J = 1.5 and 7.8 Hz, 1H), 7.45 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 8.12 (br s, 1H), 8.40 (dd, J = 1.5 and 8.4 Hz, 1H). Anal. ($C_{23}H_{35}NO_2S$) C, H, N.

***S*-(2-((1-Methylcyclohexane)carbonylamino)phenyl) 2-phenylethanethioate (14):** NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 3H), 1.21−1.64 (m, 8H), 1.73−1.88 (m, 2H), 3.94 (s, 2H), 7.10 (dt, J = 1.2 and 7.8 Hz, 1H), 7.30−7.50 (m, 7H), 7.89 (br s, 1H), 8.35 (dd, J = 1.2 and 8.4 Hz, 1H); MS (FAB) m/z 368 $(M + H)^+$; HRMS calcd for $C_{22}H_{26}NO_2S$, 368.1684; found, 368.1683. Anal. C, H, N.

***S*-(2-((1-(2-Ethylbutyl)cyclohexane)carbonylamino)-phenyl) ethanethioate (26):** mp 76.5−79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (t, J = 6.9 Hz, 6H), 1.15−1.70 (m, 15H), 2.00−2.15 (m, 2H), 2.46 (s, 3H), 7.12 (dt, J = 1.5 and 7.8 Hz, 1H), 7.40 (dd, J = 1.5 and 7.8 Hz, 1H), 7.47 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 8.13 (br s, 1H), 8.38 (dd, J = 1.5 and 8.4 Hz, 1H). Anal. ($C_{21}H_{31}NO_2S$) C, H, N.

***S*-(2-((1-(2-Ethylbutyl)cyclohexane)carbonylamino)-phenyl) 2,2-dimethylpropanethioate (28):** mp 64.5−66.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (t, J = 6.9 Hz, 6H), 1.10−1.75 (m, 15H), 1.36 (s, 9H), 1.95−2.15 (m, 2H), 7.10 (dt, J = 1.5 and 7.8 Hz, 1H), 7.36 (dd, J = 1.5 and 7.8 Hz, 1H), 7.45 (ddd, J = 1.5, 7.8, and 8.4 Hz, 1H), 8.11 (br s, 1H), 8.42 (dd, J = 1.5 and 8.4 Hz, 1H). Anal. ($C_{24}H_{37}NO_2S$) C, H, N.

2-((1-Methylcyclohexane)carbonylamino)phenyl Phenylacetate (15). 1-Methylcyclohexanecarbonyl chloride (6.48 g, 40.3 mmol) was added to a solution of 2-aminophenol (2.00 g, 18.3 mmol) and triethylamine (8 mL, 57.4 mmol) in chloroform (30 mL) at room temperature. Then the mixture was stirred at room temperature for 2 h and washed with water (50 mL), after which the solvent was evaporated under a vacuum. The residue was added to a solution of potassium hydroxide (3.0 g, 45.5 mmol) in tetrahydrofuran (10 mL), methanol (10 mL), and water (2 mL). This mixture was stirred at room temperature for 1 h, diluted with water (50 mL), acidified with 10% hydrochloric acid, and extracted with ethyl acetate (2 × 100 mL). The combined extracts were washed with saturated aqueous sodium bicarbonate (30 mL), water (30 mL), and brine (30 mL). After evaporation of the solvent, the residue was crystallized from ether/*n*-hexane to give 2-((1-methylcyclohexane)carbonylamino)phenol as an off-white solid (3.98 g, 93%). Phenylacetyl chloride (0.48 mL, 3.62 mmol) was added to a solution of the phenol (0.8 g, 3.43 mmol) and pyridine (0.85 mL, 10.5 mmol) in chloroform (10 mL). Then the mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was subjected to silica gel chromatography and eluted with ethyl acetate/*n*-henxane (1:10), after which it was recrystallized from ether/*n*-henxane to give **15** as a colorless solid (576 mg, 48%): mp 104−106 °C; NMR (300 MHz, $CDCl_3$) δ 1.03 (s, 3H), 1.15−1.60 (m, 8H), 1.66−1.78 (m, 2H), 3.90 (s, 2H), 7.02−7.43 (m, 9H), 8.24 (dd, J = 1.2 and 7.8 Hz, 1H). Anal. ($C_{22}H_{25}NO_3 \cdot 0.75H_2O$) C, H, N.

2,2′-Dithiobis(*N*-*tert*-butylbenzamide) (10). A mixture of 2,2′-dithiodibenzoic acid (1 g, 3.26 mmol), WSCD·HCl (1.5 g, 7.82 mmol), HOBt (1.06 g, 6.92 mmol), and *tert*-butylamine (1.05 mL, 9.99 mmol) in dimethylformamide (15 mL) was stirred overnight at room temperature. Then the mixture was diluted with ethyl acetate (100 mL) and washed with 5% hydrochloric acid (20 mL), 1 N aqueous sodium hydroxide (20 mL), water (20 mL), and brine (20 mL). The resulting organic solution was dried over sodium sulfate and concentrated. Chromatography of the residue on a silica gel column eluted with ethyl acetate/*n*-hexane (1:1) gave **10** as a slightly yellow solid (193 mg, 14%): mp 203−204 °C; NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 18H), 5.87 (br s, 2H), 7.21 (dt, J = 1.5 and 7.8 Hz, 2H), 7.33 (ddd, J = 1.5, 7.8, and 8.1 Hz, 2H), 7.43 (dd, J = 1.5 and 7.8 Hz, 2H), 7.73 (dd, J = 1.5 and 8.1 Hz, 2H). Anal. ($C_{22}H_{28}N_2O_2S_2 \cdot 0.15C_6H_{14}$) C, H, N.

Bioassays. 1. In Vitro CETP Activity. In vitro CETP activity was determined form the rate of [^{3}H]cholesteryl ester transfer from HDL to apoprotein B-containing lipoproteins.[13,14] Blood was collected from normolipidemic volunteers into tubes containing heparin (VT-100H, Terumo Inc., Japan). Plasma was isolated by centrifugation at 1500*g* for 15−30 min at 4 °C. An appropriate amount of each test compound, dissolved in a 1:1 mixture of *N*-methyl-2-pyrrolidinone and poly(ethylene glycol) (with an average molecular weight of 400), was added to the plasma. The final concentration of the organic solvent was 2%. After the reaction mixture with HDL containing [^{3}H]-CE was incubated at 37 °C for 4 h, apoprotein B-containing lipoproteins were precipitated using dextran sulfate and magnesium chloride (at final concentrations of 0.075% and 37.5 mM, respectively), and 100 μL of the supernatant containing HDL was used for counting the radioactivity. CETP-mediated transfer was determined by the decrease in radioactivity of the supernatant, and the concentration achieving 50% inhibition of CETP activity was estimated from the dose−response curves obtained with five doses.

2. Ex Vivo CETP Activity. Ex vivo CETP activity was measured in whole plasma obtained from male JW rabbits (11−15 weeks old, Kitayama Labes Co., Ltd., Japan), which were orally administered the test compounds (10 or 30 mg/kg).[15] Plasma was isolated by centrifugation at 11000*g* for 5 min at 4 °C. Whole plasma (1.8 μL) from control or treated rabbits was diluted to 600 μL with Tris-buffered saline (pH 7.4) containing 0.1 mg/mL of bovine serum albumin and was incubated at 37 °C for 15 h in the presence of HDL containing [^{3}H]CE and VLDL/LDL. After precipitation of VLDL/LDL using dextran sulfate and magnesium chloride, the radioactivity of the supernatant containing HDL was counted. CETP activity was determined by the decrease in radioactivity versus that of the blank sample without plasma.

3. In Vivo Plasma HDL Cholesterol Level. HDL cholesterol levels were measured in the plasma after precipitation of apoprotein B-containing lipoproteins with 13% poly(ethylene glycol) (at an average molecular weight of 6000) by an enzyme assay (Liquitec-TC, Boehringer Mannheim, Germany). Plasma was obtained from male JW rabbits (11−15 weeks old, N = 5), which were orally administered compound **27** (30 or 100 mg/kg) once a day for 3 days.

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