Novel Acyl-CoA:Cholesterol Acyltransferase Inhibitors. Synthesis and Biological Activity of 3-Quinolylurea Derivatives

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A series of 3-quinolylurea derivatives (1) was synthesized and evaluated for acyl-CoA:cholesterol acyltransferase (ACAT) inhibitory activity. For *in vitro* studies, the most potent inhibitory activity was found in derivatives having substituents at the 6,7- or 6,8-positions and an ortho-substituted phenyl group at the 4-position of quinoline ring. The 2,4-difluorophenyl group appeared to be the optimum *N'*-substituent of the urea moiety. The IC_{50} values of compounds **52–54** and **59** were in the nanomolar order. Plasma cholesterol-lowering activity of compounds **50**, **52**, and **54** was observed at less than 1 mg/kg/day in cholesterol-fed rats. Compound **52** was also hypocholesterolemic in hamsters fed a diet without loading cholesterol.

Introduction

Elevated plasma cholesterol levels and the resultant intracellular accumulation of esterified cholesterol are widely accepted as major risk factors of atherosclerotic disease such as cerebrovascular and coronary heart diseases.[1] Therefore, there have been many efforts to find new drugs that lower the plasma cholesterol. As a part of our search for a hypocholesterolemic and antiatherosclerotic agent, we paid attention to acyl-CoA:cholesterol acyltransferase (ACAT) which plays a key role in the intracellular cholesterol esterification.[2] Cholesterol esterification is an essential step in cholesterol absorption in the intestine, cholesterol secretion from the liver, and cholesterol accumulation and the foam cell formation in the vascular wall.[2] Therefore, an ACAT inhibitor may exhibit both hypocholesterolemic and antiatherosclerotic effects by blocking cholesterol esterification.

A number of ACAT inhibitors have been reported previously[3] and are classified into two major chemical types, one being long-chain aliphatic carboxylic acid amide derivatives and the other being urea derivatives as exemplified in Figure 1. The amide derivatives seem to mimic structurally acyl-CoA, one of the substrates of ACAT. Synthesis of substrate analogs is a versatile way for finding an enzyme inhibitor. However, it was anticipated that limitation might exist in this case for further derivatization because a long-chain acyl moiety was thought to be essential.[4] On the other hand, the urea compounds looked attractive since they had little structural correlation with the substrates of this enzyme, *i.e.*, long-chain acyl-CoA and cholesterol. Various chemical modification would also be possible based on the urea structure to explore a novel class of ACAT inhibitors. On the basis of the SAR data reported by DeVries *et al.*,[3b] we conceived that the *N*-phenylurea portion might be a crucical structure for the enzyme inhibition and the lipophilic substituent(s) played an important role for the inhibitors to bind the enzyme probably through a hydrophobic interaction. Particular interests were directed toward derivatives with a heterocycle as an *N*-substituent

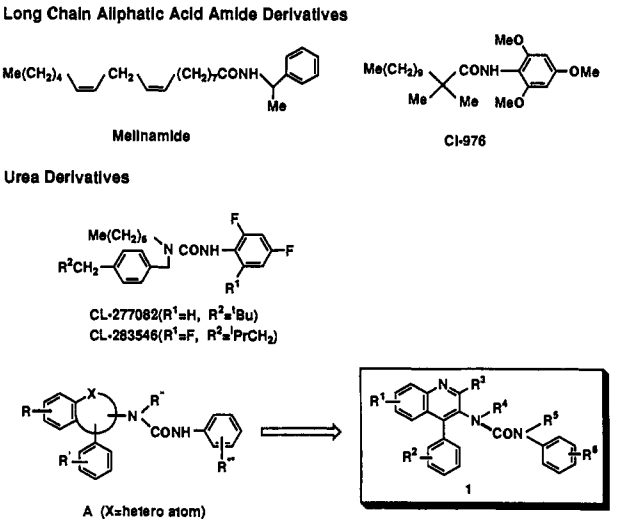

Figure 1. ACAT inhibitors.

since attempts along this concept had not been made previously as far as we were aware. Thus we designed phenylurea derivatives (A) as having as the *N*-substituent various benzoheterocycles with another phenyl group as a lipophilic substituent as shown in Figure 1. This paper describes the synthesis and ACAT inhibitory activity of novel quinolylurea derivatives[5] (1). Their cholesterol-lowering effects in animal models are also described.

Chemistry

3-Aminoquinoline derivatives[5,6] (7) and 3-carboxyquinoline derivatives[5,7] (16) shown in Schemes 1 and 2 were chosen as key intermediates to prepare the target 3-quinolylurea derivatives (1) by the procedure depicted in Scheme 3. Condensation of 2-aminobenzophenones[8] (2) with methazonic acid[6] (3) or 1-morpholino-2-nitroethene[9] (4) in the presence of aqueous HCl in acetone provided nitro enamine derivatives (5), which were cyclized by treatment with aqueous NaOH to afford 3-nitroquinoline derivatives (6). Reduction of 6 with $SnCl_2$–HCl gave the key intermediates 7 (method A, Scheme 1).

For the preparation of another key intermediate, 16, 2 was condensed with diethyl (ethoxymethylene)malonate

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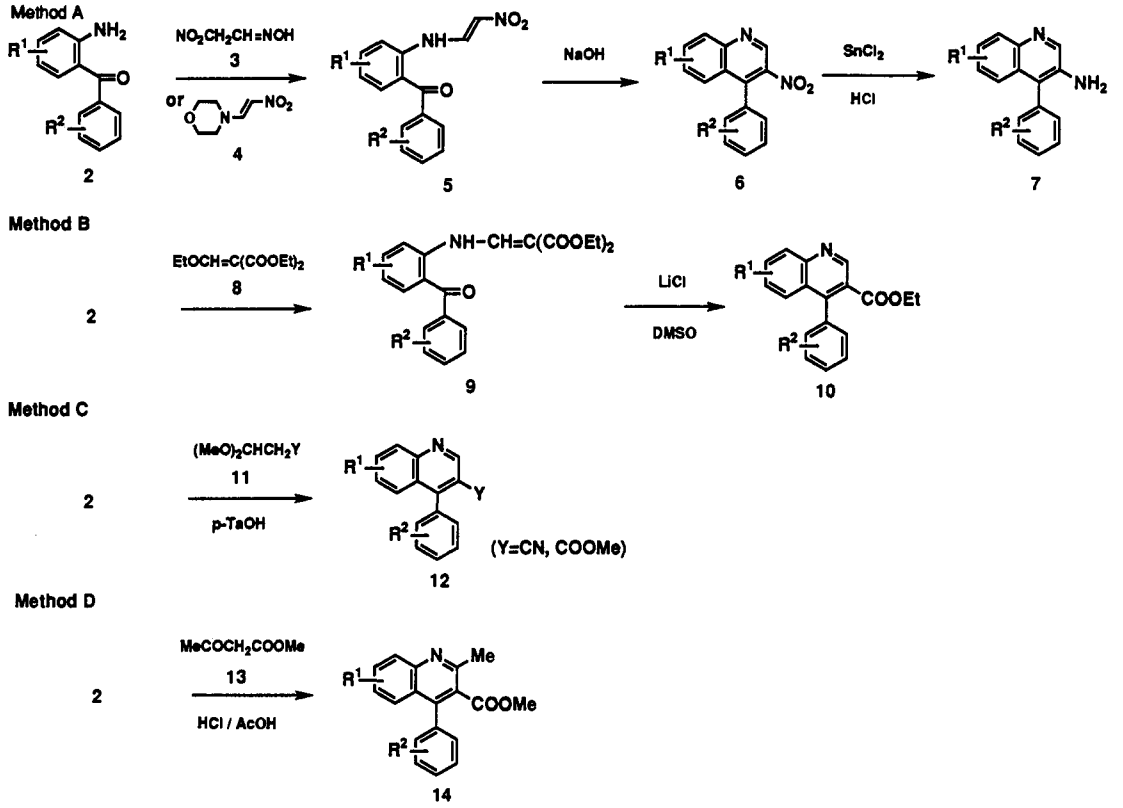
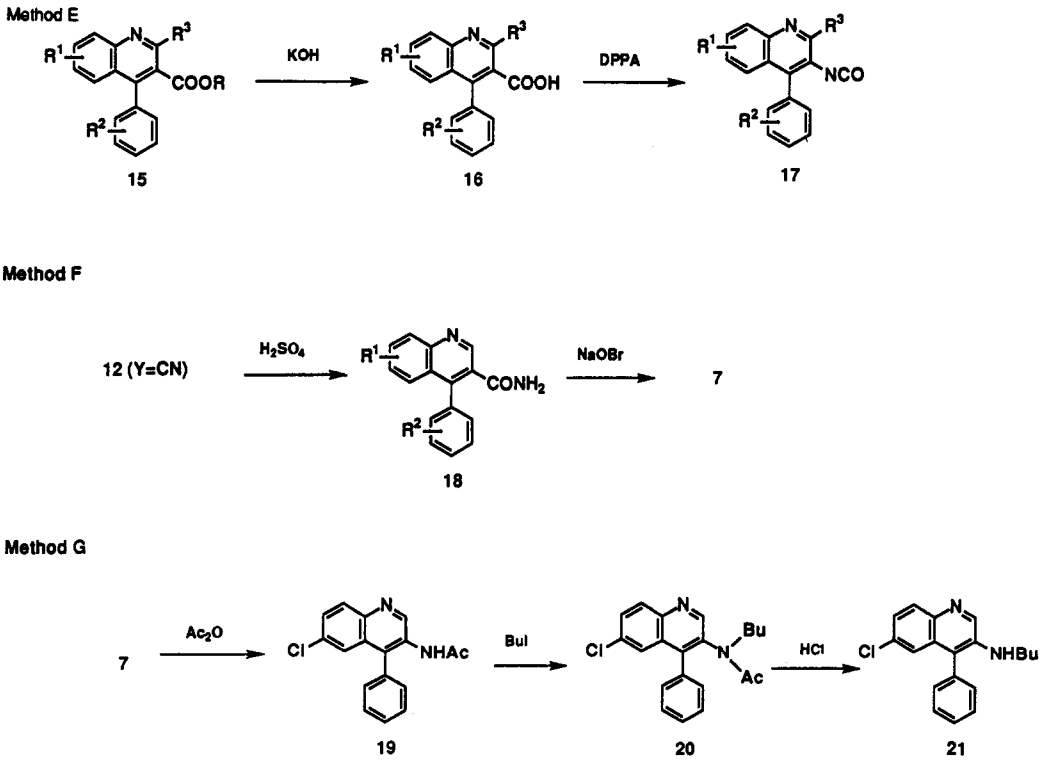
Scheme 1



Scheme 2



(8) to provide **9**. Decarbalkoxylation and cyclization of **9** with LiCl in DMSO at 180 °C gave the ester **10** (method B). In an alternative and more convenient approach, compound **2** was condensed with dimethoxyethane derivatives **11** in the presence of *p*-toluenesulfonic acid in toluene or with methyl acetoacetate in the presence of HCl in AcOH to give **12** (Y = CN, COOMe) or **14** (methods C and D). As shown in Scheme 2, alkaline saponification of **15** in refluxing aqueous EtOH provided the free carboxylic acid **16** in quantitative yield. Compound **16** was converted to 3-quinolylisocyanate (**17**) by reaction with diphenyl phosphorazidate (DPPA) in refluxing benzene (method E). Compound **17** was usually used in the next reaction without isolation (see the Experimental Section). Partial hydrolysis of **12** (Y = CN) in concentrated H_2SO_4 gave the 3-quinolinecarboxamides **18**, which were converted to the amines **7** by reaction with NaOBr in MeOH.

Scheme 3

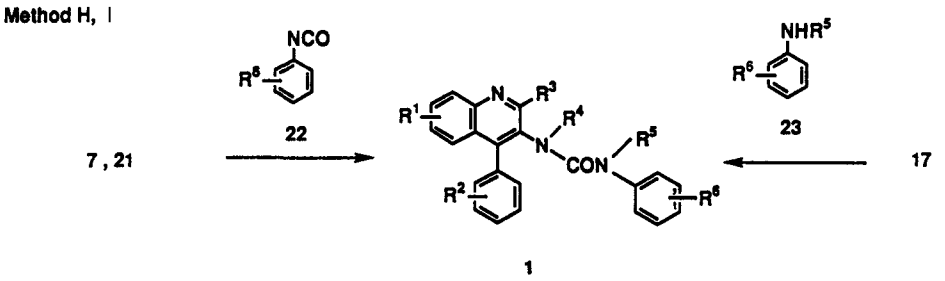
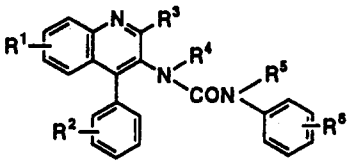


Table 1. Physical and Biological Properties of 24–59



compd no.	R^1	R^2	R^3	R^4	R^5	R^6	formula[a]	recrystn[b] solvent	mp (°C)	method[c]	yield (%)	ACAT inh (10^{-6} M)	ACAT inh IC_{50} (nM)
24	6-Cl	H	H	H	H	H	$C_{22}H_{16}ClN_3O$	EA	184–185	H	–[d]	5.0	>1000
25	6-Cl	H	H	H	H	4-Cl	$C_{22}H_{15}Cl_2N_3O$	E	224–225	H	–[d]	51.5[e]	1296
26	6-Cl	H	H	H	H	2-F	$C_{22}H_{15}ClFN_3O$	A–H	197–198	H	77.3	75.0	929
27	6-Cl	H	H	H	H	4-F	$C_{22}H_{15}ClFN_3O$	E	205–207	H	66.5	66.5	800
28	6-Cl	H	H	H	H	2,4-F_2	$C_{22}H_{14}ClF_2N_3O$	E	206–207	H	77.8	88.3	215
29	6-Cl	H	H	H	H	2,4-$(MeO)_2$	$C_{24}H_{20}ClN_3O_3$	A–E	210–211	H	41.5	47.6	864
30	6-Me	H	H	H	H	2,4-F_2	$C_{23}H_{17}F_2N_3O$	A–I	189–191	H	72.0	84.5	–
31	6-iPr	H	H	H	H	2,4-F_2	$C_{25}H_{21}F_2N_3O$	E	211–212	H	74.5	91.6	–
32	6-Br	H	H	H	H	2,4-F_2	$C_{22}H_{14}BrF_2N_3O$	A–I	195–197	H	82.1	85.5	–
33	6-CF_3	H	H	H	H	2,4-F_2	$C_{23}H_{14}F_5N_3O$	E	203–204	H	65.3	90.1	–
34	6-MeO	H	H	H	H	2,4-F_2	$C_{23}H_{17}F_2N_3O_2$	A–I	126–130	H	87.7	85.9	–
35	6-MeS	H	H	H	H	2,4-F_2	$C_{23}H_{17}F_2N_3OS$	M	117–120	H	89.4	87.8	–
36	6-Cl	2-Cl	H	H	H	2,4-F_2	$C_{22}H_{13}Cl_2F_2N_3O$	A-H_2O	210–212	H	86.7	98.0	21.2
37	6-Cl	3-Cl	H	H	H	2,4-F_2	$C_{22}H_{13}Cl_2F_2N_3O$	A–H_2O	207–208	H	68.1	88.5	90.6
38	6-Cl	4-Cl	H	H	H	2,4-F_2	$C_{22}H_{13}Cl_2F_2N_3O$	E	215–217	H	74.1	77.0	534
39	6-Cl	2-Me	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O$	A–H	219–220	H	72.6	97.2	28.7
40	6-Cl	4-Me	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O$	E	188–189	H	79.9	87.4	160
41	6-Cl	2-MeO	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O_2$	E	221–222	H	65.9	98.2	24.0
42	6-Cl	4-MeO	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O_2$	E	208–209	H	82.7	92.7	121
43	6-Cl	H	H	Bu	H	2,4-F_2	$C_{26}H_{22}ClF_2N_3O$	H	66–69	H	59.5	61.6	–
44	6-Cl	H	H	H	Me	2,4-F_2	$C_{23}H_{16}ClF_2N_3O$	A	181–182	I	62.6	9.4	>1000
45	6,8-Me_2	2-Cl	Me	H	H	2,4-F_2	$C_{25}H_{20}ClF_2N_3O$	A	234–235	I	86.9	85.4	–
46	H	2-Cl	H	H	H	2,4-F_2	$C_{22}H_{14}ClF_2N_3O$[f]	E	198–200	H	85.5	91.8	125
47	5-Me	2-Cl	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O$	E	218–220	H	64.3	87.3	149
48	6-Me	2-Cl	H	H	H	2,4-F_2	$C_{23}H_{16}ClF_2N_3O$	A	225–226	H	64.7	98.5	16.8
49	6-Me	2-Me	H	H	H	2,4-F_2	$C_{24}H_{19}F_2N_3O$	A–B	218–220	H	65.7	96.9	21.7
50	6-Et	2-Cl	H	H	H	2,4-F_2	$C_{24}H_{18}ClF_2N_3O$	A	228–229	H	75.4	99.2	14.0
51	6-iPr	2-Cl	H	H	H	2,4-F_2	$C_{25}H_{20}ClF_2N_3O$	A	232–233	H	79.3	99.3	14.0
52	6,7-Me_2	2-Cl	H	H	H	2,4-F_2	$C_{24}H_{18}ClF_2N_3O$	A	220–222	H	73.8	99.6	5.8[g]
53	6-Me-7-Cl	2-Me	H	H	H	2,4-F_2	$C_{24}H_{18}ClF_2N_3O$	A	221–223	H	69.8	98.6	9.3
54	6,8-Me_2	2-Cl	H	H	H	2,4-F_2	$C_{24}H_{18}ClF_2N_3O$	A–B	198–200	H, I	61.4[h]	98.9	9.2
55	6,8-Me_2	2-Me	H	H	H	2,4-F_2	$C_{25}H_{21}F_2N_3O$	E	200–201	H	66.0	98.5	12.0
56	6,8-Me_2	2-Cl	H	H	H	2,4,6-F_3	$C_{24}H_{17}ClF_3N_3O$	E	219–220	I	61.4	97.3	15.0
57	6,8-Me_2	2-Cl	H	H	H	2,6-iPr_2	$C_{30}H_{32}ClN_3O$	E	257–258	I	63.3	98.0	28.0
58	6-Cl-8-Me	2-Cl	H	H	H	2,4-F_2	$C_{23}H_{15}Cl_2F_2N_3O$	EE–B	199–201	H	67.8	97.6	12.0
59	6-Me-8-Cl	2-Cl	H	H	H	2,4-F_2	$C_{23}H_{15}Cl_2F_2N_3O$	A–B	220–222	H	63.0	97.6	8.9

[a] All compounds were analyzed for C, H, and N; analytical results obtained for these elements were within ±0.4% of calculated value. [b] A = acetone, B = benzene, E = ethyl alcohol, H = hexane, I = isopropyl ether, M = methyl alcohol, EA = ethyl acetate, EE = ethyl ether. [c] See the Experimental Section. [d] See ref 10. [e] Mean ± SD of three independent experiments: 51.5 ± 4.2. [f] 1/2 ethanol solvated. [g] Mean ± SD of three independent experiments: 5.8 ± 2.4. [h] Yield of method H.

The *N*-alkylated quinolylurea derivatives 1 (R^4 = butyl) were prepared by the route shown in method G. Acetylation of 7 with acetic anhydride gave 19. Alkylation of 19 in the presence of NaH in DMF followed by hydrolysis with aqueous HCl provided 3-(butylamino)quinoline derivative 21.

The desired 3-quinolylurea derivatives 1 were prepared using methods H and I shown in Scheme 3. Reaction of 3-aminoquinoline derivatives 7 and 21 with substituted phenyl isocyanates 22 (method H) or alternatively reaction of 3-quinolylisocyanates (17) with aniline derivatives 23 (method I) gave 1 in satisfactory yields.

Results and Discussion

The structure, physical constants, and biological data of the quinolylurea derivatives are shown in Table 1. The ACAT inhibitory effect is presented in terms of percent inhibition at the concentration of 10^{-6} M or IC_{50} value. For convenience in discussing structure–activity relationships of the urea moiety of 1, the nitrogen atom that binds

at the 3-position of quinoline ring and the one on the phenyl ring are designated as N and N′, respectively.

The substituent effects in the N′-phenyl ring of N-(6-chloro-4-phenylquinolyl) derivatives were examined with compounds **24–29**. It is noteworthy that compounds **25–29** possessing substituents (R^6) such as halogen and alkoxy in the N′-phenyl ring showed considerable activity despite the lack of activity with nonsubstituted N′-phenyl derivative **24**. In particular, 2,4-difluorophenyl derivative **28** had the most potent inhibitory activity in this series.

Effects of substituents (R^1) at the 6-position of quinoline ring in N′-(2,4-difluorophenyl) series were evaluated with compounds **30–35**. These compounds showed more than 84% inhibition at the concentration of 10^{-6} M irrespective of the electronic property of the substituent, *i.e.*, an electron-withdrawing group such as halogen (**32**) and trifluoromethyl (**33**), and an electron-donating group such as alkyl (**30**, **31**), methoxy (**34**), and methylthio (**35**). Substitution at the 6-position seems to be better than unsubstitution (**36**, **48**, **50**, **51** vs **46**) or substitution at the 5-position (**48** vs **47**), though more data should be accumulated for further discussion.

Compounds **36–42** represent variation of the position and the nature of substituents (R^2) on the phenyl group at the 4-position of the quinoline ring possessing the 6-chloro atom and the N′-(2,4-difluorophenyl) group. Among the derivatives having a chloro atom at the ortho, meta, or para position in the 4-phenyl ring (**36**, **37**, **38**), ortho derivative **36** showed the most favorable activity. A similar effect was also observed for the methyl analogue (**39** vs **40**) and the methoxy analogue (**41** vs **42**). This effect was also independent of the electronic nature of the substituent as in the case of the 6-position of the quinoline ring. Furthermore, it should be noted that the introduction of these ortho substituents gave rise to marked improvement of the inhibitory potency (**36**, **39**, **41** vs **28**). This result was probably caused by restricted rotation of the phenyl group due to the steric bulk of the ortho substituent, the plane of the benzene ring being oriented to a proper angle for a hydrophobic interaction with the enzyme.

Alkyl substituents on N and N′ of the urea moiety tended to decrease ACAT inhibitory activity as observed in compounds **43** and **44**. In particular, N′-methyl compound **44** (R^5 = Me) lost ACAT inhibitory property, suggesting that the hydrogen on N′ is important for interaction of these inhibitors with the enzyme probably through a hydrogen bond.

Biologically more potent compounds were obtained when 6,7- or 6,8-disubstituted derivatives were synthesized. Compounds **52–54** and **59** had IC_{50} values of 10^{-9} M level as shown in Table 1.

Among these compounds, **50**, **52**, and **54** were subjected to *in vivo* evaluation. Compound **50** (0.8 mg/kg/d) and **54** (0.83 mg/kg/d) significantly decreased plasma cholesterol level in cholesterol-fed rats by 45.4% and 39.2%, respectively (Figure 2). Compound **52** reduced plasma cholesterol level dose-dependently in cholesterol-fed rats (Figure 2), and the effective dose to reduce plasma cholesterol level by 50% (ED_{50}) was calculated to be 0.25 mg/kg/d. Thus hypocholesterolemic effect of compound **52** was further examined in Golden hamsters. The ED_{50} value of **52** (Table 2) were 0.81 mg/kg/day in hamsters fed a stock diet and 8.0 mg/kg/day in hamsters fed a cholesterol-diet, respectively. It is noteworthy that this compound is hypocholesterolemic in hamsters fed the stock diet without loading cholesterol. This fact suggests that mechanisms other than inhibition of intestinal cholesterol absorption underlie its cholesterol-lowering effect. However, this series of compounds did not inhibit activities of other cholesterol-related enzymes, such as HMG-CoA reductase, cholesterol 7α-hydroxylase, and lecitin:cholesterol acyltransferase (data not shown). IC_{50} value of **52** for hamster liver ACAT was 6.4 nM and more than 242 nM of the compound was found in the hamster liver when 10 mg/kg of **52** was orally given to hamsters (unpublished data). Therefore, one possible explanation is that inhibition of hepatic ACAT by **52** largely contributes to the hypocholesterolemic effect in hamsters. Discussion on this topic will be reported in detail elsewhere.

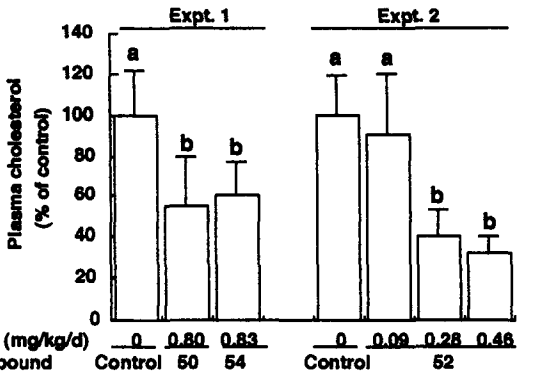


Figure 2. Hypocholesterolemic activities of 3-quinolylurea derivatives. Seven-week-old, male Sprague–Dawley rats were given compounds as a dietary admixture of a cholesterol diet for 7 days, and plasma cholesterol was measured [mean ± SD (n = 6)]. Different scripts in the same experiment indicate a significant difference ($p < 0.05$) between the respective data.

Table 2. Hypocholesterolemic Activity of **52** (TMP-153) in Hamsters[a]

cholesterol load	plasma cholesterol (mg/dL)				ED_{50} (mg/kg/d)
		compound **52**			
	control	0.0001%	0.0003%	0.001%	
−	181 ± 4[a]	169 ± 4[b]	−	95 ± 6[c]	0.81
+	288 ± 23[a]	279 ± 23[a]	245 ± 19[b]	213 ± 25[c]	8.0

[a] Compound **52** was given to 10-week-old, male Golden hamster fed a stock diet or a cholesterol diet as a dietary admixture (0.0001%, 0.0003%, or 0.001%) for 14 days. Data represent mean ± SD of five animals. Different superscripts in the same row indicate a significant difference ($p < 0.05$) between the respective data.

On the basis of the pharmacological and toxicological evaluation, N-[6,7-dimethyl-4-(2-chlorophenyl)-3-quinolyl]-N′-(2,4-difluorophenyl)urea (**52**, TMP-153) was selected as a candidate for further development.

In summary, we have described on quinolylurea derivatives as potent ACAT inhibitors. These are the first urea-type ACAT inhibitors having a nitrogen heterocycle as the N-substituent.[4] On the basis of the results presented in this work, our efforts at optimizing ACAT inhibitory activity have been further expanded to various heterocycles, which will be reported in the forthcoming papers.

Experimental Section

Pharmacological Method: (1) ACAT Inhibitory Effect (Table 1). The intestines were removed from 6-week-old, male Sprague–Dawley rats and placed on ice. The first 15 cm of the intestine from the stomach was discarded. The next 30 cm was washed with ice-cold saline solution, and the wall was scraped with a microscope slide. The mucosa was placed in 10 mL of 0.25 M sucrose solution (pH 6.2) and homogenized with a motor-driven Teflon pestle at 0–4 °C. The mucosal homogenate was

centrifuged at 12000g for 15 min, and then the supernatant was centrifuged at 100800g for 30 min to isolate the microsomes.

The ACAT activity in the microsomes was determined by the formation of labeled cholesteryl ester from [1-^{14}C]oleoyl-CoA and endogenous cholesterol according to the slightly modified method of Helgerud *et al.*[10] The microsomes with 0.2 mg of protein were preincubated in a vial containing 0.46 mL of 0.154 M phosphated buffer with 42 nmol of bovine serum albumin (fatty acid free) at 37 °C for 15 min. Thereafter, the reaction was initiated by addition of 10 nmol of oleoyl-CoA containing 3.7 kbq of [1-^{14}C]oleoyl-CoA (20 μL) and 2 min later was stopped by adding 6 mL of chloroform–methanol (2:1). Lipids were extracted by the method of Folch *et al.*[11] The lipid extract was taken quantitatively for separation by thin-layer chromatography on silica gel 60–F_{254} (Merck). The area cotaining cholesteryl ester was scraped, and the radioactivity was counted by liquid scintillation counter. The test compound dissolved with dimethyl sulfoxide was added into the vial before the preincubation at 1 μM of the final concentration. The inhibitory action of the test compound on the ACAT activity was explained as inhibition rate (%) at 10^{-6} M. To show the variability of data, data were represented as mean ± SD of three independent experiments on a typical compound (compound **25**).

IC_{50} values, which are the concentration to reduce the enzyme activity by 50%, were calculated from their inhibition curves. To indicate the variability of the values, data were represented as mean ± SD of three independent experiments on a typical compound (compound **52**).

(2) Hypocholesterolemic Effect. Animals and Diet. Animals were individually housed in metal cages in a room with controlled temperature (23 ± 1 °C), humidity (55 ± 5%), and light (7 a.m.–7 p.m.). They were weaned at 4 weeks of age, maintained freely on water and a stock diet, CE-2 (Clea Japan Inc., Tokyo), and used at 7–10 weeks of age without fasting.

Hypocholesterolemic Activity. Seven-week-old, male Sprague–Dawley rats were given compounds as a dietary admixture of a cholesterol diet containing 1% cholesterol, 0.5% cholic acid, and 5% olive oil in CE-2 for 7 days. Compound **52** was also given to 10-week-old, male Golden Syrian hamsters fed CE-2 or the cholesterol diet as a dietary admixture for 14 days. Blood was taken from the tail vein (for rats) or from the orbital sinus (for hamsters), and plasma cholesterol was mesured enzymatically using commercially available assey kits (Iatron Laboratories Inc., Tokyo). Drug intake was calculated from the diet intake. Effective doses to reduce plasma cholesterol by 50% (ED_{50} values) were calculated from the regression lines of dose–response curves.

Statistical analysis: The data were expressed as mean ± SD and statistically analysed by Student's *t* test or Duncan's multiple range test.

Chemistry. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi IR-215 spectrometer in Nujol. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-390 or Varian Gemini-200 spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are given in ppm with tetramethylsilane as the internal standard and coupling constants (*J*) are given in hertz. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublets of doublet, b = broad, bs = broad singlet, and bt = broad triplet.

Method A: General Procedure for the Preparation of 3-Aminoquinolines (7). (1) 2-[(2-Nitroethenyl)amino]benzophenones (5).[5,6] A mixture of **2** (0.01 mol), methazonic acid (**3**, 0.015–0.02 mol), or 1-morpholino-2-nitroethene (**4**, 0.015–0.02 mol), 6 N HCl (10 mL), and acetone (30 mL) was stirred at ambient temperature for 1–15 h. The mixture was diluted with H_2O to give **5** as a yellow solid which was collected by filtration and washed with H_2O. The crude crystals were used in the next reaction without further purification.

(2) 3-Nitroquinolines (6).[5,6] To a stirred suspension of **5** (0.01 mol) in MeOH (30 mL) was added dropwise 2 N NaOH (6.0 mL). The mixture was stirred at ambient temperature for 3–7 h and diluted with H_2O. The resulting crystals were collected, washed with H_2O, and recrystallized.

(3) 3-Aminoquinolines (7).[5,6] To a stirred mixture of **6** (0.01 mol), concentrated HCl (10 mL), and dioxane (30 mL) was added portionwise $SnCl_2{\cdot}2H_2O$ (0.03–0.04 mol), during which period an exothermic reaction occurred. The mixture was stirred at ambient temperature for 1–5 h, made alkaline with 6 N NaOH with ice-cooling, diluted with H_2O, and extracted with $CHCl_3$. The usual workup of the extract gave **7** as crystals.

The following typical examples are given to illustrate the general procedure for methods B–G.[5]

Method B: Ethyl 6-Chloro-4-phenyl-3-quinolinecarboxylate. (1) A mixture of 2-amino-5-chlorobenzophenone (4.62 g) and diethyl (ethoxymethylene)malonate (**8**, 5.18 g) was heated at 120–130 °C for 2 h with stirring. After cooling, the mixture was crystallized by addition of isopropyl ether to give diethyl [[(2-benzoyl-4-chlorophenyl)amino]methylene]malonate (7.33 g, 91.4%). Recrystallization from EtOH gave pale yellow plates: mp 119–120 °C; ^{1}H NMR 1.31 (3H, t, J = 7.5), 1.35 (3H, t, J = 7.5), 4.25 (2H, q, J = 7.5), 4.38 (2H, q, J = 7.5), 7.25–7.81 (8H, m), 8.48 (1H, d, J = 13.5). Anal. ($C_{21}H_{20}ClNO_5$): C, H, N. (2) A mixture of diethyl [[(2-benzoyl-4-chlorophenyl)amino]methylene]malonate (7.0 g), LiCl (3.7 g), and DMSO (70 mL) was heated at 180 °C for 1.5 h. The mixture was diluted with H_2O to give ethyl 6-chloro-4-phenyl-3-quinolinecarboxylate as crystals (3.8 g, 70.0%). Recrystallization from EtOH gave pale brown needles: mp 123–124 °C; IR 1720; ^{1}H NMR 0.98 (3H, t, J = 7.5), 4.10 (2H, q, J = 7.5), 7.23–7.77 (7H, m), 8.13 (1H, d, J = 9), 9.30 (1H, s). Anal. ($C_{18}H_{14}ClNO_2$) C, H, N.

Method C-1: 4-(2-Chlorophenyl)-6,8-dimethyl-3-quinolinecarbonitrile. A mixture of 2-amino-2′-chloro-3,5-dimethylbenzophenone (20.0 g), 3,3-dimethoxypropionitrile (11.5 g), *p*-TsOH·H_2O (1.46 g), and toluene (200 mL) was refluxed for 3 h azeotropic removal of water using a Dean–Stark apparatus. The mixture was washed with an aqueous $NaHCO_3$ solution and H_2O, dried ($MgSO_4$) and concentrated to give 4-(2-chlorophenyl)-6,8-dimethyl-3-quinolinecarbonitrile as crystals which were collected and washed with hexane (20.7 g, 92.0%). Recrystallization from EtOH gave colorless prisms: mp 153–154 °C; IR 2200; ^{1}H NMR 2.38 (3H, s), 2.80 (3H, s), 7.03–7.70 (6H, m), 9.03 (1H, s). Anal. ($C_{18}H_{13}ClN_2$) C, H, N.

Method C-2: Methyl 4-(2-Chlorophenyl)-6,8-dimethyl-3-quinolinecarboxylate. A mixture of 2-amino-2′-chloro-3,5-dimethylbenzophenone (2.59 g), methyl 3,3-dimethoxypropionate (3.7 g), *p*-TsOH·H_2O (0.19 g), and toluene (30 mL) was refluxed for 16 h with azeotropic removal of water using a Dean–Stark apparatus. The mixture was washed with an aqueous $NaHCO_3$ solution and H_2O, dried ($MgSO_4$), and concentrated to give an oil which was chromatographed on silica gel using hexane–AcOEt (9:1) as an eluent. The eluate was concentrated to give methyl 4-(2-chlorophenyl)-6,8-dimethyl-3- quinolinecarboxylate as an oil which was crystallized from hexane (1.90 g, 58.5%). Recrystallization from isopropyl ether gave colorless prisms: mp 117–118 °C; IR 1720; ^{1}H NMR 2.37 (3H, s), 2.82 (3H, s), 3.72 (3H, s), 6.97–7.60 (6H, m), 9.40 (1H, s). Anal. ($C_{19}H_{16}ClNO_2$) C, H, N.

Method D: Methyl 4-(2-Chlorophenyl)-2,6,8-trimethylquinoline-3-carboxylate. A mixture of 2-amino-2′-chloro-3,5-dimethylbenzophenone (2.6 g), methyl acetoacetate (2.32 g), concentrated HCl (0.1 mL), and AcOH (30 mL) was heated at 120 °C for 3 h. After removal of the solvent, the residue was neutralized with an aqueous $NaHCO_3$ solution and extracted with AcOEt. The usual workup of the extract gave methyl 4-(2-chlorophenyl)-2,6,8-trimethyl-3-quinolinecarboxylate as crystals (2.53 g, 74.4%). Recrystallization from EtOH gave colorless needles: mp 119–120 °C; IR 1730; ^{1}H NMR 2.33 (3H, s), 2.77 (6H, s), 3.53 (3H, s), 6.87 (1H, s), 7.17–7.57 (5H, m). Anal. ($C_{20}H_{18}ClNO_2$) C, H, N.

Method E: 4-(2-Chlorophenyl)-6,8-dimethyl-3-quinolinecarboxylic Acid. A mixture of methyl 4-(2-chlorophenyl)-6,8-dimethyl-3-quinolinecarboxylate (0.98 g), KOH (0.5 g), and 80% EtOH (10 mL) was refluxed for 15 min. The mixture was diluted with H_2O and adjusted to pH 2 with 2 N HCl to give the title compound as crystals (0.9 g, 96.8%). Recrystallization from EtOH gave colorless prisms: mp 234–235 °C; IR 1700; ^{1}H NMR (DMSO-d_6) 2.32 (3H, s), 2.75 (3H, s), 6.85 (1H, s), 7.20–7.63 (5H, m), 9.27 (1H, s). Anal. ($C_{18}H_{14}ClNO_2$) C, H, N.

Method F: 3-Amino-4-(2-Chlorophenyl)-6,8-dimethylquinoline. (1) A mixture of 4-(2-chlorophenyl)-6,8-dimethyl-3-quinolinecarbonitrile (17.6 g) and 97% H_2SO_4 (120 mL) was stirred for 2 h and then allowed to stand overnight at room

temperature. The mixture was poured into ice–water and extracted with AcOEt. The usual workup of the extract gave 4-(2-chlorophenyl)-6,8-dimethyl-3-quinolinecarboxamide as an oil which was crystallized by addition of MeOH (18.5 g, 90.2%). Recrystallization from MeOH gave the methanol solvate as crystals which showed double melting points at 99–100 °C and 163–164 °C: IR 3300, 3250, 1660, 1610; ^{1}H NMR 1.17 (1H, bt, J = 5), 2.37 (3H, s), 2.83 (3H, s), 3.46 (2H, d, J = 5), 5.67 (2H, bs), 6.93 (1H, s), 7.28 (5H, m), 9.20 (1H, s). Anal. ($C_{18}H_{15}ClN_2O\cdot CH_4O$) C, H, N.

(2) Br_2 (3.2 mL) was added dropwise to a stirred and ice-cooled solution of NaOH (10.4 g) in H_2O (100 mL). A solution of 4-(2-chlorophenyl)-6,8-dimethyl-3-quinolinecarboxamide methanolate (18.5 g) obtained in step 1 in dioxane (100 mL) was added dropwise thereto. The mixture was stirred at room temperature for 30 min and then at 90 °C for 40 min and adjusted to pH 1 with 6 N HCl. After gas evolution had ceased, the mixture was stirred for an additional 30 min, and the precipitate was filtered off. The filtrate was made alkaline with 6 N NaOH, diluted with H_2O, and extracted with AcOEt. The usual workup of the extract gave 3-amino-4-(2-chlorophenyl)-6,8-dimethylquinoline as an oil which was crystallized by addition of hexane (14.0 g, 91.7%). Recrystallization from EtOH gave colorless prisms: mp 155–156 °C; IR 3375, 3350, 3300, 1720, 1695, 1605; ^{1}H NMR 2.33 (3H, s), 2.77 (3H, s), 3.65 (2H, bs), 6.73 (1H, s), 7.17 (1H, s), 7.21–7.73 (4H, m), 8.58 (1H, s). Anal. ($C_{17}H_{15}ClN_2$) C, H, N.

Method G: 6-Chloro-3-(butylamino)-4-phenylquinoline (21). To a solution of 3-acetamido-6-chloro-4-phenylquinoline (19)[12] (1.0 g) in DMF (10 mL) was added portionwise NaH (0.15 g, 60% in oil) with ice-cooling. After the mixture was stirred for 30 min with ice-cooling, butyl iodide (0.58 mL) was added dropwise. The whole was stirred at room temperature for 1 h and poured into ice–water (50 mL) to give 3-(*N*-butylacetamido)-6-chloro-4-phenylquinoline (20) as crystals which were collected by filtration and washed with H_2O, ice-cooled EtOH, and finally hexane. Recrystallization from ether–hexane gave colorless crystals (0.96 g, 81.0%): mp 108–110 °C; ^{1}H NMR 0.78 (3H, t, J = 6), 0.93–1.67 (4H, m), 1.95 (3H, s), 2.32–2.63 (1H, m), 3.73–4.07 (1H, m), 7.13–7.75 (7H, m), 8.13 (1H, d, J = 6), 8.73 (1H, s). Anal. ($C_{21}H_{21}ClN_2O$) C, H, N. A mixture of the crystals (0.9 g) obtained above, concentrated HCl (5 mL), and MeOH (5 mL) was refluxed for 5 h. The mixture was diluted with H_2O, made alkaline with solid NaOH, and extracted with AcOEt. The usual workup of the extract gave 21 as crystals which were recrystallized from MeOH to afford light yellow crystals (0.58 g, 73.0%): mp 71–73 °C; IR 3350, 1606; ^{1}H NMR 0.90 (3H, t, J = 6), 1.12–1.70 (4H, m), 3.28 (2H, q, J = 6), 3.68 (1H, b), 7.18–7.72 (7H, m), 7.93 (1H, d, J = 9), 8.68 (1H, s). Anal. ($C_{19}H_{19}ClN_2$) C, H, N.

General Procedure for the Preparation of 3-Quinolylurea Derivatives (1, Table I).[5] Method H. To a stirred solution of 7 or 21 (0.01 mol) in THF (30 mL) was added dropwise 22 (0.012–0.02 mol). The mixture was stirred at room temperature for 3–20 h and concentrated. The residue was recrystallized from the solvent given in Table 1 to afford 1.

Method I. To a stirred mixture of 16 (0.01 mol), diphenyl phosphorazidate (DPPA, 0.012 mol), and benzene (40 mL) was added dropwise Et_3N (0.01 mol). The mixture was stirred at room temperature for 15–30 min and refluxed for 20–60 min to give 17. Then 23 (0.012–0.02 mol) was added, and the whole mixture was refluxed for 30 min to 7 h, washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was recrystallized from the solvent given in Table 1 to afford 1.

References

(1) The Lipid Research Clinics Program; The Lipid Research Clinics Colonary Primary Prevention Trial Results. *J. Am. Med. Assoc.* **1984**, *251*, 351–374.

(2) Suckling, K. E.; Stange, E. F. Role of acyl-CoA cholesterol acyltransferase in cellular cholesterol metabolism. *J. Lipid Res.* **1985**, *26*, 647–671.

(3) (a) DeVries, V. G.; Schaffer, S. A.; Largis, E. E.; Dutia, M. D.; Wang, C. H.; Bloom, J. D.; Katocs, A. S., Jr. Potential Antiatherosclerotic Agent. 5. An Acyl-CoA: Cholesterol O-Acyltransferase Inhibitor with Hypocholesterolemic Activity. *J. Med. Chem.* **1986**, *29*, 1131–1133. (b) DeVries, V. G.; Bloom, J. D.; Dutia, M. D.; Katocs, A. S., Jr.; Largis, E. E. Potential Antiatherosclerotic Agents. 6. Hypocholesterolemic Trisubstituted Urea Analogue. *J. Med. Chem.* **1989**, *32*, 2318–2325. (c) Gammill, R. B.; Bell, F. P.; Bisaha, S. N.; Wilson, G. J. Antiatherosclerotic Agents. A Structurally Novel Bivalent Inhibitor of AcylCoA: Cholesterol O-Acyltransferase with Systemic Activity. *J. Med. Chem.* **1990**, *33*, 2685–2687. (d) Drago, R. S.; Andrew, D. W. Therapentic potential of ACAT inhibitors as lipid lowering and antiatherosclerotic agents. *Trends Phamacol. Sci.* **1991**, 194–199. (e) Woolf, T. F.; Bjorge, S. M.; Black, A. E.; Halmes, A.; Chang, T. Metabolism of the Acyl CoA: Cholesterol Acyltransferase Inhibitor 2,2-Dimethyl-N-(2,4,6-trimethoxyphenyl)dodecanamide in Rat and Monkey. *Drug Metab. Dispos.* **1991**, *19*, 696–702. (f) Harris, N. V.; Smith, C.; Ashton, M. J.; Bridge, A. W.; Bush, R. C.; Coffee, E. C. J.; Dron, D. I.; Harper, M. F.; Lythgoe, D. J.; Newton, C. G.; Riddell, D. Acyl-CoA: Cholesterol O-Acyltransferase (ACAT) Inhibitors. 1. 2-(Alkylthio)-4,5-diphenyl-1H-imidazoles as Potent Inhibitors of ACAT. *J. Med. Chem.* **1992**, *35*, 4384–4392. (g) Trivedi, B. K.; Holmes, A.; Stoeber, T. L.; Blankley, C. J.; Roark, W. H.; Picard, J. A.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. Inhibitors of Acyl-CoA: Cholesterol Acyltransferase. 4. A Novel Series of Urea ACAT Inhibitors as Potential Hypocholesterolemic Agents. *J. Med. Chem.* **1993**, *36*, 3300–3307.

(4) Recently heterocyclic carboxanilide derivatives have been reported: (a) Kumazawa, T.; Yanase, M.; Harakawa, H.; Shirakura, S.; Ohishi, E.; Yamada, K. ACAT inhibitors: Synthesis and Hypocholesterolemic Activity of Dibenz[b,e]oxepin-11-carboxanilide. 206th ACS Natinal Meeting (1993), Abstracts of papers, Division of Medicinal Chemistry, 43. (b) Chucholowski, A. W.; Bolton, G. L.; Stanfield, R. L.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R. Tetrahydroisoquinoline Anilides, Inhibitors of Acyl-CoA: cholesterol Acyl Transferase (ACAT). 206th ACS Natinal Meeting (1993), Abstracts of Papers, Division of Medicinal Chemistry, 45.

(5) Meguro, K.; Ikeda, H. Quinoline derivatives, their production and use. Eur. Pat. 354994, 1990.

(6) Schafield, K.; Theobald, R. S. Reaction of Methazonic Acid. Part I. The Preparation of 3-Nitrolepidines and 3-Nitroquinolines. *J. Chem. Soc.* **1950**, *52*, 395–402.

(7) Toi, Y.; Isagawa, K.; Fushizaki, Y. o-Aminobenzophenone Derivatives II. Syntheses 2,3,4,6-tetrasubstituted and 2,4,6-trisubstituted quinoline derivatives. *Jpn. J. Chem.* **1969**, *90*, 81–85.

(8) Sternbach, L. H.; Fryer, R. I.; Metlesics, W.; Sach, G.; Stempel, A. Quinazolines and 1,4-Benzodiazepines. V. o-Aminobenzophenones. *J. Org. Chem.* **1962**, *27*, 3781–3788.

(9) Faulques, M.; Rene, L.; Royer, R. A One-Pot Synthsis of Nitroenamines. *Synthesis* **1982**, 260–261.

(10) Helgerud, P.; Saaren, K.; Norum, K. R. Acyl-CoA Cholesterol Acyltransferase in Human Intestine: Its Activity and Some Properties of the Enzyme reaction. *J. Lipid Res.* **1981**, *22*, 271–277.

(11) Folch, J.; Less, M.; Stanley, G. H. S. A Simple Methods for the Isolation and Purification of Total Lipid from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.

(12) Kuwada, Y.; Meguro, K.; Miyano, H. 3-Carbamoylamino-4-phenylquinoline Compounds. U.S. Pat. 3862152, 1975.