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Inhibitors of Acyl-CoA:Cholesterol Acyltransferase: Novel Trisubstituted Ureas as Hypocholesterolemic Agents[1]

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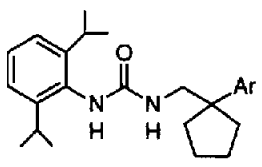
Abstract—Our continued interest in developing novel, potent acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors, and our discovery of several active series of disubstituted urea ACAT inhibitors, have led us to investigate a series of trisubstituted ureas that are structural hybrids of our disubstituted series and of a trisubstituted urea ACAT inhibitor series disclosed by scientists at Lederle. This investigation has led to the discovery of novel trisubstituted ureas, several of which inhibit ACAT in the nanomolar range and effectively lower total plasma cholesterol when administered as a diet admixture in an acute model of hypercholesterolemia in rats. One analogue (**35**) also lowered total cholesterol as efficaciously as CL 277,082 in our chronic hypercholesterolemic rat model. The most notable finding of this study is that the SAR of the trisubstituted ureas diverges from that seen in our previously disclosed disubstituted urea series. This series showed optimal activity with 2,4-difluoro and 2,4,6-trifluoro substitution on the urea *N*-phenyl, whereas the disubstituted series showed optimal activity with bulky 2,6-disubstitution on the phenyl ring. © 1997 Elsevier Science Ltd.

Introduction

Acyl–coenzyme A:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is an intracellular enzyme responsible for the esterification of cholesterol in all mammalian cells.[2] Substantial evidence implicates ACAT in the regulation of lipoprotein secretion in the liver.[3] It has been further shown that inhibition of arterial macrophage ACAT has a direct inhibitory effect on atherosclerotic lesion progression.[4] This evidence has sparked considerable interest in the design and development of novel ACAT inhibitors, both in our own laboratories[5–9] and in others.[10,11] The work presented here derives from our previously published disubstituted urea series,[6,8] of which some representative compounds are shown in Figure 1. Our intention was to determine whether the potent ACAT inhibition and hypocholesterolemic activity of these disubstituted ureas could be extrapolated to a series of trisubstituted ureas which incorporated features from our disubstituted series as well as from Lederle's ACAT inhibitor series, shown in Figure 2, such as di- and tri-fluorophenyl, *n*-heptyl, and benzyl substituents.

Chemistry

Most of the compounds were prepared from spiroalkylated primary amines. These amines were obtained by the method of Butler and Pollatz[12] from commercially available phenyl acetonitriles, as we have previously reported.[6,8] Other primary amines used are commercially available.



1 Ar = Ph
2 Ar = 4-NMe_2-Ph
3 Ar = 4-NMe_2-Ph·HCl (PD 132301-2)
4 Ar = 2-pyridyl

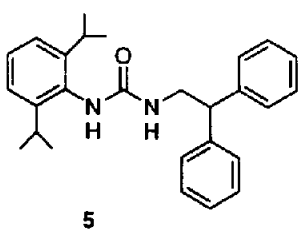


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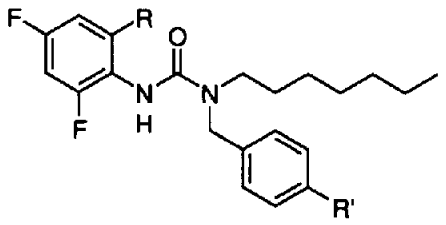
Figure 1.



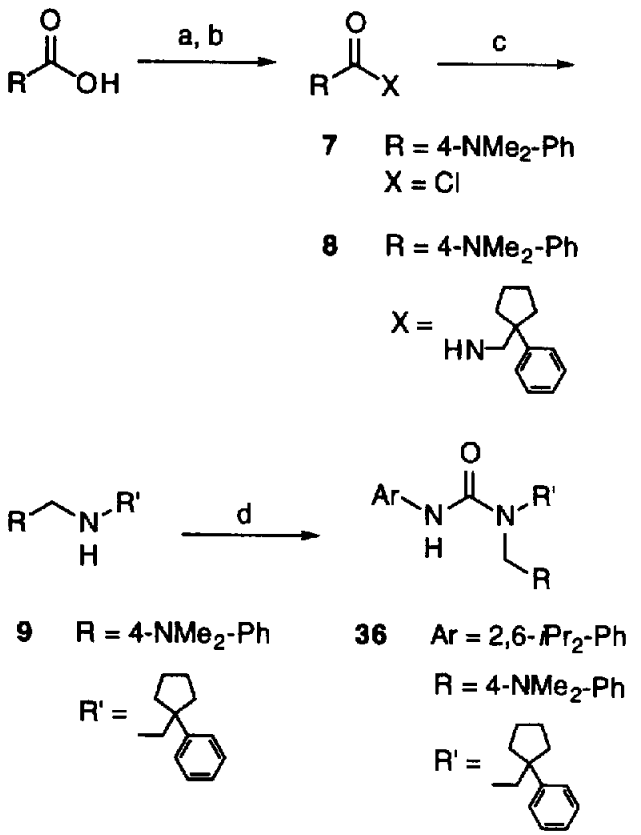
6 CL 277,082: R = H, R' = $CH_2C(CH_3)_3$
CL 283,546: R = F, R' = $(CH_2)_2CH(CH_3)_2$

Figure 2.

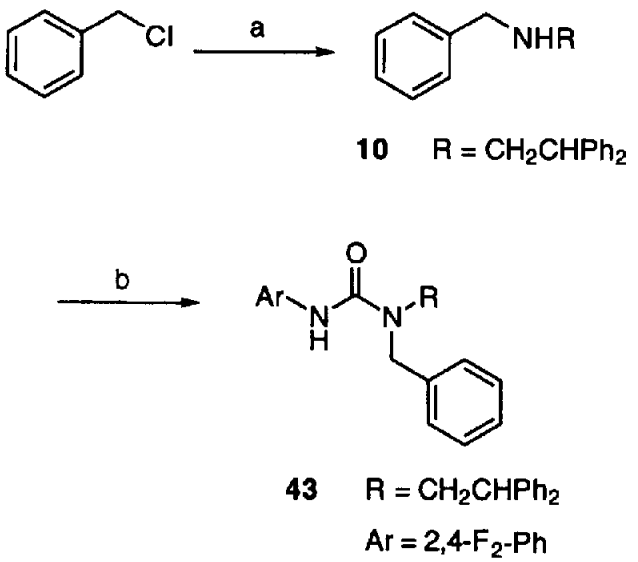
The compounds containing *N*-alkyl substituents were prepared as shown in Scheme 1 (Method A). Acid chlorides, obtained from acids by treatment with thionyl or oxalyl chloride, were allowed to react with the appropriate amines to afford the corresponding amides, which were then reduced using borane or lithium aluminum hydride. These secondary amines were then converted to the target compounds by treatment with various aryl isocyanates.

Most of the *N*-benzyl derivatives were prepared as illustrated in Scheme 2 (Method B) by the representative synthesis of **43**. In most cases, the primary amines were easily converted to the desired secondary amines by treatment with benzyl chloride in the presence of triethylamine. The benzyl amines were then allowed to react with various aryl isocyanates to afford the desired trisubstituted ureas. In a few cases, the primary amines were not nucleophilic enough to react with benzyl chloride. These analogues were prepared by Method A as shown in Scheme 1. The primary amines were treated with benzoyl chloride, reduced with borane or lithium aluminum hydride to give the desired secondary amines, and then allowed to react with aryl isocyanate. The preparation of **36** by this method (Scheme 1) is representative.

The 2,4,6-trifluorophenyl ureas could not be made by the conventional route, since 2,4,6-trifluorophenyl



Scheme 1. (a) Oxalyl chloride or thionyl chloride, dichloromethane, reflux; (b) $R'NH_2$, triethylamine, tetrahydrofuran, reflux; (c) borane or lithium aluminum hydride, tetrahydrofuran, reflux; (d) ArNCO, ethyl acetate, room temperature.

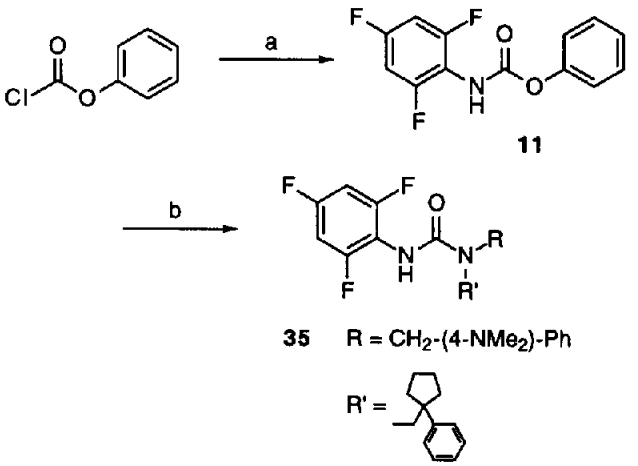


Scheme 2. (a) RNH_2, triethylamine, tetrahydrofuran, reflux; (b) ArNCO, ethyl acetate, room temperature.

isocyanate is neither commercially available nor accessible by the standard synthetic route utilizing phosgene. Instead, these analogues were synthesized via the trifluorophenyl carbamate,[13] as shown by the representative synthesis of **35** in Scheme 3 (Method C). First, trifluoroaniline was treated with phenyl chloroformate in the presence of *N,N*-dimethylaniline to give the corresponding phenyl carbamate. The carbamate was then heated with various secondary amines to afford the desired trisubstituted ureas.

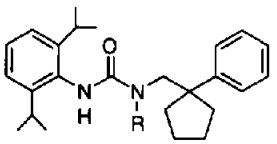
Results and Discussion

We initially synthesized a series of *N'*-phenyl-*N*-(1-phenyl cyclopentyl)-methyl ureas in which the *N*-hydrogen was replaced by an alkyl or arylalkyl moiety (Table 1). This study suggested that *n*-heptyl (**13**) and benzyl (**14**) substituents were both tolerated reasonably well with respect to ACAT inhibitory activity, causing



Scheme 3. (a) 2,4,6-Trifluoroaniline, *N,N*-dimethylaniline, toluene, room temperature; (b) NHRR', toluene, reflux.

Table 1. Urea-*N* substitution

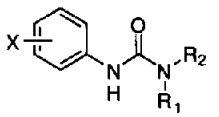


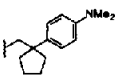
Compd	R	mp (°C)	Formula[a]	IAI[b] IC_{50} (μM)	APCC[c] (% Δ in TC)	CLOGP
1[d]	H			0.017	−47****	6.33
12	CH_3	159–161	$C_{26}H_{36}N_2O$	1.2	−5	6.13
13	*n*-heptyl	95–97	$C_{32}H_{48}N_2O$	0.11	−13	8.98
14	Bn	124–127	$C_{32}H_{40}N_2O$	0.17	NC	7.82
15	$(CH_2)_2Ph$	130–132	$C_{33}H_{42}N_2O$	1.7	−24***	7.92

[a]Analyses are within ±0.4%.
[b]Intestinal ACAT inhibition (see refs 5 and 11).
[c]Denotes percent change in total cholesterol relative to controls. All compounds dosed at 50 mg/kg by gavage. Statistical significance denoted as follows: **** = extremely significant ($p < 0.001$); *** = very significant ($p < 0.005$); ** = significant ($p < 0.05$); * = marginally significant ($p < 0.1$). All other values considered to be not significantly different from controls. NC = no change relative to controls.
[d]Melting point and analytical data previously published (see refs 6 and 8).

activity to drop by only 10-fold or less, while a 70- to 100-fold drop in activity was observed for the other analogues. The same trend had been observed earlier by scientists at Lederle.[10]

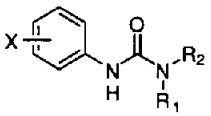
Table 2. *N*-Heptyl substitution

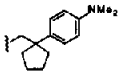


Compd	X	R^1	R^2	mp (°C)	Formula[a]	IAI[b] IC_{50}, μM	APCC[c] % Δ in TC	CLOGP
1[d]	2,6-(*i*Pr)$_2$		H			0.017	−47****	6.33
16	H	"	*n*-heptyl	87–89	$C_{26}H_{36}N_2O$	1.6	−34***	7.25
17	2,4-(F)$_2$	"	"	88–89	$C_{26}H_{34}F_2N_2O{\cdot}0.33H_2O$	0.31	−30***	7.48
18	2,4,6-(F)$_3$	"	"	100–102	$C_{26}H_{33}F_3N_2O$	0.052	−35****	7.20
19	2,6-(Me)$_2$	"	"	153–154	$C_{28}H_{40}N_2O$	1.6	−25**	7.13
20	2,4,6-(Me)$_3$	"	"	162–164	$C_{29}H_{42}N_2O$	0.057	−25**	7.63
21	2,4,6-(OMe)$_3$	"	"	84–86	$C_{29}H_{42}N_2O_4$	0.21	−35****	7.33
13	2,6-(*i*Pr)$_2$	"	"	95–97	$C_{32}H_{48}N_2O$	0.11	−13	8.98
2[d]	2,6-(*i*Pr)$_2$		H			0.055	65****	6.53
22	2,4,6-(F)$_3$	"	*n*-heptyl	99–101	$C_{28}H_{38}F_3N_3O$	0.29	−47****	7.39
23	2,6-(Me)$_2$	"	"	126–128	$C_{30}H_{45}N_3O$	0.16	−53****	7.32
24	2-Me-6-*i*Pr	"	"	146–147	$C_{32}H_{49}N_3O$	0.12	−13	8.25
25	2,6-(*i*Pr)$_2$	"	"	123–125	$C_{34}H_{53}N_3O$	0.25	−6	9.18
26	2,4,6-(F)$_3$	"	*n*-tetradecyl	60–62	$C_{35}H_{52}F_3N_3O$[e]	0.11	NC	11.10
5[d]	2,6-(*i*Pr)$_2$	CH_2CHPh_2	H			0.026	−51****	6.07
27	2,4-(F)$_2$	"	*n*-heptyl	76–78	$C_{38}H_{53}F_2N_3O{\cdot}0.5H_2O$	0.17	−31***	7.22
28	2,6-(Me)$_2$	"	"	171–173	$C_{30}H_{38}N_2O$	1.3	−28**	6.86

[a]Analyses are within ±0.4%, unless otherwise noted.
[b]Intestinal ACAT inhibition (see refs 5 and 11).
[c]Denotes percent change in total cholesterol relative to controls. All compounds dosed at 50 mg/kg by gavage. Statistical significance denoted as follows: **** = extremely significant ($p < 0.001$); *** = very significant ($p < 0.005$); ** = significant ($p < 0.05$); * = marginally significant ($p < 0.1$). All other values considered to be not significantly different from controls. NC = no change relative to controls.
[d]Melting point and analytical data previously published (see refs 6 and 8).
[e]C Anal calcd = 71.52; found = 71.04.

Table 3. *N*-Benzyl substitution



Compd	X	R_1	R_2	mp (°C)	Formula[a]	IAI[b] IC_{50}, μM	APCC[c] %Δ in TC	CLOGP
1[d]	2,6-(*i*Pr)$_2$		H			0.017	−47****	6.33
29	H	"	Bn	122–126	$C_{26}H_{28}N_2O$	2.3	−17	6.08
30	2,4-(F)$_2$	"	"	119–122	$C_{26}H_{26}F_2N_2O$	0.09	−29***	6.31
31	2,4,6-(F)$_3$	"	"	143 145	$C_{26}H_{25}F_3N_2O$	0.020	−41****	6.03
32	2,6-(Cl)$_2$	"	"	125–127	$C_{26}H_{26}Cl_2N_2O$	0.051	19	6.78
33	2,6-(Me)$_2$	"	"	200–202	$C_{28}H_{32}N_2O$	0.059	−19*	5.96
34	2,4,6-(Me)$_3$	"	"	212–214	$C_{29}H_{34}N_2O$	0.08	−23**	6.46
14	2,6-(*i*Pr)$_2$	"	"	124–127	$C_{32}H_{40}N_2O$	0.17	NC	7.82
35	2,4,6-(F)$_3$	"	CH_2-(4-NMe_2-Ph)	188–191	$C_{28}H_{30}F_3N_3O$	0.022	−29***	6.23
36	2,6-(*i*Pr)$_2$	"	"	122–127	$C_{34}H_{45}N_3O$	0.044	−27**	8.01
2[d]	2,6-(*i*Pr)$_2$		H			0.055	−65****	6.53
37	2,4-(F)$_2$	"	Bn	127–129	$C_{28}H_{31}F_2N_3O$	0.12	−19***	6.51
38	2,4,6-(F)$_3$	"	"	180–182	$C_{28}H_{30}F_3N_3O$	0.15	−38***	6.23
39	2,6-(Me)$_2$	"	"	174–175	$C_{30}H_{37}N_3O$	0.58	−16*	6.16
40	2,6-(*i*Pr)$_2$	"	"	145–146	$C_{34}H_{45}N_3O$	0.46	−24*	8.01
4[d]	2,6-(*i*Pr)$_2$		H			0.016	−45****	4.84
41	2,4,6-(F)$_3$	"	Bn	81–85	$C_{25}H_{24}F_3N_3O$	0.43	−40****	4.54
42	2,6-(*i*Pr)$_2$	"	"	127–130	$C_{31}H_{39}N_3O$	0.90	3	6.32
5[d]	2,6-(*i*Pr)$_2$	CH_2CHPh_2	H			0.026	−51****	6.07
43	2,4-(F)$_2$	"	Bn	120–123	$C_{28}H_{24}F_2N_2O$	0.29	−31***	5.89
44	2,4,6-(F)$_3$	"	"	137–140	$C_{28}H_{22}F_3N_2O$	0.24	−28**	5.61
45	2,6-(Me)$_2$	"	"	205–208	$C_{30}H_{30}N_2O$	1.4	NC	5.54
46	2,6-(*i*Pr)$_2$	"	"	153–155	$C_{34}H_{38}N_2O$	1.3	NC	7.39

[a]Analyses are within ±0.4%, unless otherwise noted.
[b]Intestinal ACAT inhibition (see refs 5 and 11).
[c]Denotes percent change in total cholesterol relative to controls. All compounds dosed at 50 mg/kg by gavage. Statistical significance denoted as follows: **** = extremely significant ($p < 0.001$); *** = very significant ($p < 0.005$); ** = significant ($p < 0.05$); * = marginally significant ($p < 0.1$). All other values considered to be not significantly different from controls. NC = no change relative to controls.
[d]Melting point and analytical data previously published (see refs 6 and 8).

This information was utilized in ensuing studies. We synthesized a series of trisubstituted ureas in which various substituents on the *N'*-phenyl moiety, chosen from among our own and Lederle's preferred substitution patterns, were investigated. The various arylalkyl sidechains were chosen from our active disubstituted urea series and the corresponding parent compound in each series was compared (Tables 2 and 3).

Heptyl substitution on the urea nitrogen is illustrated in Table 2. The *N*-heptyl derivatives were generally less potent than the parent, disubstituted compounds yet retained modest ACAT inhibitory activity. For this series there was also no clear trend with respect to optimal *N'*-phenyl substitution for in vitro potency, although the data suggests a preference for 2,4,6-trisubstitution (**18**, **20**).

Table 2 shows two compounds that interestingly displayed moderate to good in vivo activity despite having low in vitro potency (**16** and **19**). However, none of the compounds have in vivo activity surpassing that of the corresponding parent compound. Qualitative comparison of **13**, **24**, **25**, and **26** with the other analogues suggests that there may be an optimal lipophilicity required for in vivo activity that has been exceeded by these compounds. This assertion is supported by the CLOGP[14] data shown. All of these compounds have CLOGPs of greater than 8; the other compounds have CLOGPs ranging from approximately 6 to 8.

Table 3 illustrates the SAR of *N*-benzyl substituted ureas. In general we found that, analogous to the SAR found in the Lederle series, 2,4-difluoro (**30**, **37**, and **43**) and 2,4,6-trifluoro (**31**, **35**, **38**, **41**, and **44**) substituents

Table 4. Chronic hypercholesterolemic rat screen (CPCC)

Compd	IAI (IC_{50}, μM)	%Δ in TC[a]	%Δ in HDLC[b]	%Δ in non-HDLC[c]
3 (PD 132301-2)	0.052	−68***	160.3***	−76***
6 (CL 277,082)	0.20	−49**	5.2	−51
18	0.052	−23*	12.1	−24
21	0.21	−23*	1.7	−23
22	0.29	−45*	27.6	−48*
31	0.020	17	−27.6	−17
35	0.022	−54**	31.0	57**
38	0.15	−11	−13.8	−10
44	0.24	−12	17.2	−12

[a]Denotes percent change in total cholesterol relative to controls. All compounds dosed at 30 mg/kg by gavage. Statistical significance denoted as follows: *** = highly significant ($p < 0.001$); ** = significant ($p < 0.05$); * = marginally significant ($p < 0.1$). All other values considered to be not significantly different from controls.
[b]Denotes percent change in high density lipoprotein cholesterol relative to controls. Statistical significance denoted as previously described in footnote a.
[c]Denotes percent change in non-high density lipoprotein cholesterol relative to controls. Statistical significance denoted as previously described in footnote a.

were optimal for in vitro activity. These small, electron-withdrawing substituents were preferred over bulky, electron-donating 2,6-disubstitution, which in most cases was observed to be detrimental to activity (**14**, **39**, **40**, **42**, **45**, and **46**). This is markedly divergent from the SAR seen in our studies of disubstituted urea ACAT inhibitors.

The in vivo activities of compounds found in Table 3 correlated favorably with the in vitro potencies in most cases. Compounds **30**, **31**, **35**, **38**, **41**, **43** and **44** all show cholesterol-lowering activity in the 30–40% range, and all are 2,4-difluoro or 2,4,6-trifluoro substituted. Exceptions are **32** and **33** that, despite good in vitro potency, fail to show significant in vivo potency; both compounds have bulky 2,6-disubstitution. As seen with the *N*-heptyl compounds, the *N*-benzyl trisubstituted ureas are not as active in vivo as the parent disubstituted ureas in this acute screen.

Comparison of *N*-heptyl versus *N*-benzyl substitution shows that the in vivo activities are approximately equivalent, the only exception being **23** vs **39**. Comparison of in vitro activities, however, shows that in general, *N*-benzyl substitution confers more potency than *N*-heptyl (**17** vs **30**, **18** vs **31**, **19** vs **33** and **22** vs **38**).

It is interesting to note that the incorporation of dimethylamino functionality onto the R^1 phenyl ring allowed retention or improvement of hypocholesterolemic activity despite conferring decreased in vitro potency (**18** vs **22**, **31** vs **38**). This supports our previous assertion (**1** vs **2**)[8] that such functionality improves the in vivo activity of our compounds, possibly as a result of improved pharmacokinetic properties.

Selected compounds were also tested in a chronic model of hypercholesterolemia in rats. This model differs from the acute paradigm in several respects. Firstly, hyperlipidemia is established prior to dosing in the chronic model. Thus lipid is already present in the GI tract, which may result in better absorption of lipophilic compounds in the chronic model. Secondly, the drug is given in multiple doses and is therefore more likely to accumulate in the intestine. Thirdly, it is important to note that in the acute model animals are dosed at 50 mg/kg, whereas in the chronic model they are dosed at 30 mg/kg. Thus, compounds that appear better in the acute screen may not necessarily be more efficacious here. The pre-establishment of hyperlipidemia in the chronic model represents a greater challenge to the cholesterol-lowering ability of the compound.

Results of the chronic screen are shown in Table 4, with in vitro results shown for comparison. Of these compounds, only **35** and CL 277,082 (**6**) produce a significant decrease in total cholesterol relative to controls ($p < 0.05$). Neither compound is as effective as our best disubstituted urea ACAT inhibitor, PD 132301-2 (**3**).

Here again, incorporation of a dimethylamino functionality is seen to improve efficacy in two parameters: percent change in total cholesterol and percent change in non-HDL cholesterol (**18** vs **22**, **31** vs **35**). In the case of **18** vs **22**, this improved efficacy is seen despite diminished in vitro potency.

None of the compounds caused a significant increase in HDL-cholesterol in the chronic screen, with the exception of PD 132301-2. This may be an indication that the trisubstituted ureas did not penetrate the liver in significant quantities.[15]

Conclusions

In this study, we have attempted to further delineate the SAR surrounding our potent series of urea ACAT inhibitors (Fig. 1) by adding a urea-*N* substituent. Our attempt to retain the potency and hypocholesterolemic activity previously seen in our disubstituted ureas in the trisubstituted analogues met with moderate success.

Several analogues inhibited ACAT in the range of 20–100 nM and lowered total plasma cholesterol in our acute model of hypercholesterolemia in rats. The best compound to emerge from the series, **35**, potently inhibited ACAT in vitro (IC_{50} of 22 nM) and efficaciously lowered cholesterol in a chronic animal model of hypercholesterolemia (−54% change in total cholesterol). This compound rivaled the efficacy of the Lederle compound, CL 277,082, but did not lower cholesterol as effectively as PD 132301-2, our best disubstituted urea ACAT inhibitor.

Of the urea-*N* substituents investigated, *N*-benzyl substituted compounds were generally more potent than *N*-heptyl compounds. Furthermore, this study suggests a divergent pharmacophore from that seen in our disubstituted series. Compounds having 2,4-difluorophenyl and 2,4,6-trifluorophenyl substitution on the urea-*N'* phenyl ring were generally more potent than were compounds containing bulky 2,6-disubstitution. This SAR is considerably different from our findings regarding disubstituted ureas, and supports our previous assertion[6] that the trisubstituted ureas interact with the enzyme in a different way than the disubstituted ureas.

Finally, incorporation of a dimethylamino functionality on a phenyl ring was observed to improve in vivo activity in several cases, possibly by improving the physicochemical properties of our compounds.

Experimental

Materials used were obtained from commercial suppliers, unless otherwise noted, and were used without purification. Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. High-field nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 spectrometer. All chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Combustion analyses were determined on a Perkin–Elmer Model 240C elemental analyzer.

Method A

The following synthesis of **36** represents a general scheme for the synthesis of related analogues as shown in Scheme 1.

4-(Dimethylamino)benzoyl chloride (7). 4-Dimethylaminobenzoic acid (20.0 g, 0.121 mol) was suspended in 500 mL dichloromethane and heated to reflux, at which time the heat source was removed. Oxalyl chloride (30.7 g, 0.242 mol) was added dropwise to the reaction mixture, cautiously at first so as to avert violent reflux. After addition was complete, the reaction mixture was heated at reflux for 2 h, then stirred at room temperature for 20 h. The clear solution was concentrated in vacuo. The solid residue was suspended in ether and collected, then oven-dried overnight to afford 20.5 g (92%) of **7**, a shiny white solid, mp 144–150 °C, which was carried on without further purification. ^{1}H NMR ($CDCl_3$) δ 3.11 (s, 6H), 6.64–6.70 (m, 2H), 7.94–8.00 (m, 2H). Anal. ($C_9H_{10}ClNO$) C (calcd, 58.87; found, 58.42), H, Cl, N.

4-Dimethylamino-*N*-(1-phenylcyclopentylmethyl)benzamide (8). 1-Phenyl-cyclopentane methanamine (19.7 g, 0.10 mol) and triethylamine (30.4 g, 0.30 mol) were dissolved together in 100 mL tetrahydrofuran. Compound **7** (20.2 g, 0.11 mol) was suspended in 200 mL tetrahydrofuran and added slowly to the stirring solution, which became warm during addition. The reaction mixture was heated at reflux for 2 h, cooled to room temperature, and then concentrated under vacuum; the residue was taken up in 300 mL chloroform and washed with 3 × 100 mL water followed by 100 mL brine. The organic solution was dried over Na_2SO_4, filtered, and concentrated. The resulting waxy solid was oven-dried to give 33.5 g (104%) of **8**, a pale tan crystalline solid, mp 99–106 °C, which was carried on directly without purification. ^{1}H NMR ($CDCl_3$) δ 1.73–1.78 (m, 2H), 1.85–1.94 (m, 4H), 2.02–2.06 (m, 2H), 2.99 (s, 6H), 3.56 (d, 2H, J = 6.0 Hz), 5.62 (bt, 1H), 6.63 (d, 2H, J = 8.7 Hz), 7.24–7.28 (m, 1H), 7.33–7.40 (m, 4H), 7.48–7.51 (m, 2H). Anal. ($C_{21}H_{26}N_2O$) C, H, N.

Dimethyl-[4-[[(1-phenylcyclopentylmethyl)amino]methyl]phenyl]amine (9). Compound **8** (11.3 g, 0.035 mol) was dissolved in 150 mL tetrahydrofuran and heated to reflux under nitrogen atmosphere. The heat source was removed, and 105 mL of a 1.0 M solution of BH_3 in THF was added slowly. The reaction mixture was heated at reflux for 3 h, then stirred at room temperature for 20 h. The reaction was then cooled to 10 °C and quenched by adding 65 mL 1 N HCl. This mixture was filtered and concentrated under vacuum to afford crude material as a yellow oil, which was purified by flash-column chromatography in ethyl acetate/hexane to give 8.6 g (80%) of **9**, a clear yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (bs, 1H), 1.66–1.71 (m, 4H), 1.88–2.06 (m, 4H), 2.66 (s, 2H), 2.92 (s, 6H), 3.57 (s, 2H), 6.67 (d, 2H, J = 9.0 Hz), 7.03 (d, 2H, J = 8.6 Hz), 7.12–7.33 (m, 5H). Anal. ($C_{21}H_{28}N_2$) C (calcd, 81.77; found, 80.95), H, N (calcd, 9.08; found, 8.63).

***N'*-[2,6-Bis(1-methylethyl)phenyl]-*N*-[[4-(dimethylamino)phenyl]methyl]-*N*-[(1-phenylcyclopentyl)methyl]urea (36).** 2,6-Diisopropylphenyl isocyanate (1.6 g, 8.0 mmol) was added to a solution of **9** (2.5 g, 8.0 mmol) in 100 mL ethyl acetate in a single portion. The reaction mixture was stirred at room temperature for 20 h, then concentrated in vacuo. The residue was dissolved in hexane and left to stand at room temperature until a solid precipitate had formed. The precipitate was collected, washed with hexane, and oven-dried to give 2.9 g (71%) of **36**, a yellow solid, mp 122–127 °C. ^{1}H NMR ($CDCl_3$) δ 0.95 (m, 4H), 1.16 (m, 4H), 1.59–1.66 (m, 2H), 1.91 (m, 4H), 2.14–2.19 (m, 2H), 2.74 (septet, 2H, J = 6.9 Hz), 2.90 (s, 2H), 3.64 (s, 2H), 3.75 (s, 2H), 5.46 (s, 1H), 6.63 (d, 2H, J = 8.7 Hz), 6.95 (d, 2H, J = 8.6 Hz), 7.04–7.07 (m, 2H), 7.13–7.41 (m, 6H). Anal. ($C_{34}H_{45}N_3O$) C, H, N.

Method B

The following synthesis of **43** represents a general scheme for the synthesis of related analogues as shown in Scheme 2.

Benzyl-(2,2-diphenylethyl)amine (10). 2,2-Diphenylethylamine (10.0 g, 0.051 mol) and triethylamine (28.2 mL, 0.203 mol) were dissolved together in 200 mL tetrahydrofuran. Benzyl chloride (7.1 g, 0.056 mol) dissolved in 100 mL tetrahydrofuran was added to the amine solution with stirring. The reaction mixture was heated at reflux 48 h, cooled to room temperature, and concentrated under vacuum. The residue was taken up in 100 mL chloroform and stirred with 100 mL 2 N NaOH for 0.5 h. The layers were separated and the aqueous portion was extracted with 2 × 100 mL chloroform. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash-column chromatography in ethyl acetate/hexane to give 5.8 g (40%) of **10**, a clear-gold oil, which was characterized as its HCl salt, mp 250–253 °C. ^{1}H NMR ($CDCl_3$) δ 3.37 (bd, 2H), 3.75 (bt, 2H), 4.71 (t, 1H, J = 7.8 Hz), 7.14–7.42 (m, 15H), 9.73 (bs, 2H). Anal. ($C_{21}H_{21}N$.HCl) C, H, Cl, N.

***N'*-(2,4-Difluorophenyl)-*N*-(2,2-diphenylethyl)-*N*-(phenylmethyl)-urea (43).** 2,4-Difluorophenyl isocyanate (0.81 g, 5.2 mmol) and **10** (1.5 g, 5.2 mmol) were dissolved together in 100 mL ethyl acetate. The reaction mixture was stirred 20 h at room temperature. The mixture was concentrated under vacuum, and the solid residue was suspended in hexane and collected. The white solid was oven-dried overnight to give 2.2 g (96%) of a fine white powder (**43**), mp 120–123 °C. ^{1}H NMR ($CDCl_3$) δ 4.07 (d, 2H, J = 7.6 Hz), 4.24 (s, 2H), 4.48 (t, 1H, J = 7.7 Hz), 6.14 (m, 1H), 6.69–6.83 (m, 2H), 7.14–7.37 (m, 15H), 7.84–7.94 (m, 1H). Anal. ($C_{28}H_{24}F_2N_2O$) C, H, F, N.

Analytical data

Compound	Formula	Microanalysis C Calcd	C Found	H Calcd	H Found	N Calcd	N Found
12	$C_{26}H_{36}N_2O$	79.55	79.52	9.24	9.29	7.14	6.91
13	$C_{32}H_{48}N_2O$	80.62	80.29	10.15	10.15	5.88	5.63
14	$C_{32}H_{40}N_2O$	82.01	82.02	8.60	8.64	5.98	5.80
15	$C_{32}H_{48}N_2O$	82.11	82.25	8.77	9.16	5.80	5.58
16	$C_{26}H_{36}N_2O$	79.55	79.78	9.24	9.40	7.13	7.15
17	$C_{26}H_{34}F_2N_2O{\cdot}0.33H_2O$[a]	71.86	71.91	8.04	7.92	6.44	6.12
18	$C_{26}H_{33}F_3N_2O$[b]	69.93	69.59	7.45	7.40	6.27	6.10
19	$C_{28}H_{40}N_2O$	79.95	79.83	9.59	9.74	6.66	6.66
20	$C_{29}H_{42}N_2O$	80.13	79.81	9.74	9.62	6.44	6.37
21	$C_{29}H_{42}N_2O_4$	72.17	71.93	8.77	9.03	5.80	5.66
22	$C_{28}H_{38}F_3N_3O$[c]	68.68	68.45	7.82	7.85	8.58	8.47
23	$C_{30}H_{45}N_3O$	77.71	77.16	9.78	9.86	9.06	9.01
24	$C_{32}H_{49}N_3O$	78.16	77.89	10.04	10.07	8.54	8.29
25	$C_{34}H_{53}N_3O$	78.56	78.42	10.28	10.20	8.08	8.03
26	$C_{35}H_{52}F_3N_3O$[d]	71.52	71.04	8.92	8.77	7.15	6.86
27	$C_{28}H_{32}F_2N_2O{\cdot}0.5H_2O$[e]	73.17	73.10	7.23	7.10	6.09	6.05
28	$C_{30}H_{38}N_2O$	81.40	81.12	8.65	8.70	6.33	6.20
29	$C_{26}H_{28}N_2O$	81.21	81.16	7.34	7.27	7.29	7.12
30	$C_{26}H_{26}F_2N_2O$[f]	74.27	74.02	6.23	6.13	6.66	6.56
31	$C_{26}H_{25}F_3N_2O$[g]	71.22	71.10	5.75	5.70	6.39	6.27
32	$C_{26}H_{26}Cl_2N_2O$[h]	68.88	68.84	5.78	5.65	6.18	6.01
33	$C_{28}H_{32}N_2O$	81.51	81.69	7.82	7.95	6.79	6.87
34	$C_{30}H_{34}N_2O$	81.65	81.72	8.03	8.09	6.57	6.60
35	$C_{28}H_{30}F_3N_3O$[i]	69.84	69.79	6.28	6.26	8.73	8.81
36	$C_{34}H_{45}N_3O$	79.80	79.59	8.86	9.04	8.21	8.13
37	$C_{28}H_{31}F_3N_3O$[j]	72.55	72.45	6.74	7.03	9.06	9.01
38	$C_{28}H_{30}F_3N_3O$[k]	69.83	70.03	6.28	6.35	8.72	8.56
39	$C_{30}H_{37}N_3O$	79.08	78.42	8.18	8.03	9.22	8.87
40	$C_{34}H_{45}N_3O$	79.80	79.61	8.86	9.06	8.21	8.06
41	$C_{25}H_{24}F_3N_3O$[l]	68.33	68.34	5.50	5.46	9.56	9.41
42	$C_{31}H_{39}N_3O$	79.28	79.51	8.37	8.45	8.95	8.91
43	$C_{28}H_{24}F_2N_2O$[m]	76.00	76.08	5.47	5.53	6.33	6.27
44	$C_{28}H_{23}F_3N_2O$[n]	73.03	72.95	5.03	5.16	6.08	5.90
45	$C_{30}H_{30}N_2O$	82.91	82.79	6.96	7.00	6.45	6.19
46	$C_{34}H_{38}N_2O$	83.22	83.32	7.81	7.78	5.71	5.56

[a] F: calcd 8.73; found 8.73. [b] F: calcd 12.76; found 12.68. [c] F: calcd 11.64; found 11.40. [d] F: calcd 9.70; found 9.98. [e] F: calcd 8.43; found 8.79. [f] F: calcd 9.04; found 8.74. [g] F: calcd 13.00; found 13.38. [h] Cl: calcd 15.64; found 15.79. [i] F: calcd 11.84; found 11.86. [j] F: calcd 8.19; found 8.50. [k] F: calcd 11.83; found 11.45. [l] F: calcd 12.97; found 13.31. [m] F: calcd 8.59; found 8.55. [n] F: calcd 12.38; found 12.20.

Method C

The following synthesis of **35** represents a general scheme for the synthesis of related analogues as shown in Scheme 3.

Phenyl (2,4,6-trifluorophenyl)-carbamate (11). 2,4,6-Trifluoroaniline (15.0 g, 0.102 mol) and *N,N*-dimethylaniline (14.8 g, 0.122 mol) were dissolved together in 300 mL toluene. Phenyl chloroformate (16.0 g, 0.102 mol) was dissolved in 100 mL toluene and added in a steady stream to the stirring amine solution. The reaction was stirred at room temperature for 20 h. Reaction was worked up by adding 100 mL ethyl acetate and 200 mL water. The layers were separated, and the aqueous portion was extracted with 100 mL ethyl acetate. The combined organics were washed with 2×150 mL 1 N HCl followed by 2×150 mL brine, then dried ($MgSO_4$), filtered, and concentrated. The residue was suspended in hexane, collected, and oven-dried to give 24.9 g (91%) of **11**, a white crystalline solid, mp 126–129 °C. ^{1}H NMR ($CDCl_3$) δ 6.30 (bs, 1H), 6.71–6.82 (m, 2H), 7.18–7.26 (m, 3H), 7.34–7.41 (m, 2H). Anal. ($C_{13}H_8F_3NO_2$) C, H, F, N.

***N*-[[4-(Dimethylamino)phenyl]methyl]-*N*-[(1-phenylcyclopentyl)methyl]-*N'*-(2,4,6-trifluorophenyl)-urea (35).** Compounds **9** (2.2 g, 7.0 mmol) and **11** (1.9 g, 7.0 mmol) were dissolved together in 100 mL toluene and heated at reflux for 2 h, then stirred at room temperature for 20 h. The reaction mixture was concentrated under vacuum; the residue was taken up in 100 mL chloroform and washed with 2×100 mL 1 N NaOH followed by 100 mL brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 2.6 g (77%) of **35**, a white, granular solid, mp 188–191 °C. ^{1}H NMR ($CDCl_3$) δ 1.61–1.78 (m, 2H), 1.80–2.01 (m, 4H), 2.03–2.19 (m, 2H), 2.91 (s, 6H), 3.56 (s, 2H), 3.76 (bs, 2H), 5.45 (bs, 1H), 6.60–6.67 (m, 4H), 6.92 (d, 2H, J = 8.7 Hz), 7.24–7.39 (m, 5H). Anal. ($C_{28}H_{30}F_3N_3O$) C, H, F, N.

Biological methods

In vitro ACAT assay. ACAT inhibitory activity was determined in vitro by the method previously described using intestinal microsomes from cholesterol-fed rabbits and [1-^{14}C] oleyl-CoA.[5,7]

Acute cholesterol-fed rat model

Cholesterol-lowering activity was assessed in an acute in vivo screen, designated acute peanut oil/cholesterol/cholic acid (APCC), as has been previously described,[8] with n = 5–7 rats/group. Animals were dosed at 50 mg/kg, and the data are expressed as percentage decrease in total cholesterol relative to controls. Statistically significant changes were determined using unpaired, two-tailed Student's t-tests.

Chronic cholesterol-fed rat model

Selected compounds were evaluated for cholesterol-lowering activity in a chronic in vivo screen, designated chronic peanut oil/cholesterol/cholic acid (CPCC), as has been previously described,[8] with n = 6 rats/group. Animals were dosed at 30 mg/kg, and the data are expressed as percentage change relative to vehicle controls. Determinations of statistical significance relative to controls were made using unpaired, two-tailed t-tests.

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