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SUBSTITUTED N,N'-DIPHENYL UREAS AS POTENT INHIBITORS OF ACYL-CoA:CHOLESTEROL ACYLTRANSFERASE (ACAT)

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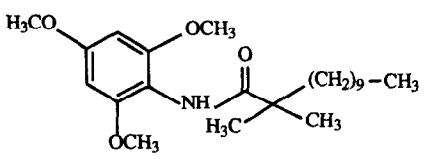
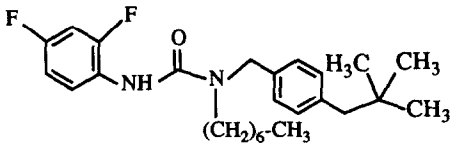
Abstract: A simple yet novel series of N,N'-diphenyl ureas has been synthesized and evaluated as ACAT inhibitors. These compounds not only inhibit ACAT *in vitro* but also effectively lower plasma cholesterol in an *in vivo* model of hypercholesterolemia.

Acyl-CoA: cholesterol acyltransferase (ACAT, EC 2.3.1.26) is the intracellular enzyme responsible for catalyzing the esterification of free cholesterol in various tissues including intestine and liver. It has been shown that potent inhibitors of ACAT reduce total plasma cholesterol concentrations in several cholesterol-fed animal models of hypercholesterolemia.[1] Furthermore, it has been reported that systemically available ACAT inhibitors will prevent the progression of atherosclerotic lesions in cholesterol-fed rabbits.[2] There are a significant number of lipophilic ACAT inhibitors reported in the literature which are being developed as clinical candidates for the treatment of hypercholesterolemia.[1] We have recently reported a fatty acid anilide derivative (CI-976)[3] which not only lowers plasma total cholesterol in cholesterol-fed rats but also induces the regression of arterial lesions in cholesterol-fed rabbits.[4] Due to this interesting biological activity, we have continued our efforts to identify novel ACAT inhibitors. This has led to the identification of a series of 2,6-alkylanilides[3] and phenyl ureas[5] of fatty acids as potent ACAT inhibitors. Based on these observations, and the activity displayed by the trisubstituted urea ACAT inhibitor (CL 277,082) developed by Lederle,[6] we chose to evaluate a series of simple disubstituted ureas.



CI-976

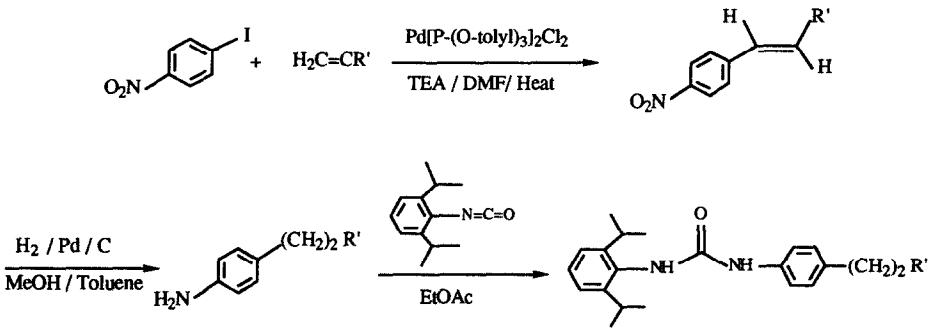


Lederle CL-277082

We synthesized a series of N,N'-diphenylurea derivatives with an optimal 2,6-diisopropyl phenyl moiety for ACAT inhibitory activity[5,7] and simple alkyl substituents on the N'-phenyl ring.[8] Most of these compounds were synthesized by the reaction of 2,6-diisopropyl phenylisocyanate with commercially available aniline derivatives in ethyl acetate at room temperature. The requisite amines

for compounds **14** and **16** were synthesized via Heck reaction (Scheme I). Reaction of p-nitro iodobenzene with the appropriate alkene in the presence of dichlorobis(tri-o-tolylphosphine)palladium(II) in DMF and triethylamine gave the corresponding styryl derivatives which were catalytically reduced using 20% Pd/C to give the required anilines. Reaction of these anilines with 2,6-diisopropyl phenyl isocyanate gave the corresponding ureas.

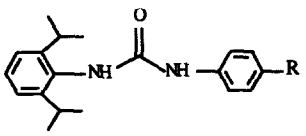
Scheme I: Synthesis of N,N'-diphenylurea



These compounds were first evaluated in an *in vitro* assay to measure ACAT inhibition (IAI).[3] The initial SAR suggested that the addition of an alkyl group, such as n-propyl, in the para position of the N'-phenyl moiety improved ACAT inhibitory activity 24 fold (**4** vs **1**). Incorporation of a branched alkyl group (**5,7**) onto the phenyl ring rendered these compounds less active. However, increasing the size of the chain length improved ACAT inhibitory activity. Analogs having side chains of greater than seven carbon atoms were the most potent ACAT inhibitors, with IC_{50} values of 9-15 nM. Surprisingly, the higher homolog **17** was significantly less active.

These analogs were then evaluated in both an acute (APCC) and a chronic (CPCC) model of hypercholesterolemia for their ability to lower total plasma cholesterol *in vivo* (Table I). Most of the shorter chain analogs (**1-7**) showed only modest cholesterol-lowering effect when evaluated at 30 mg/kg orally in the APCC screen. The remaining analogs lowered total cholesterol by 50-77% in this screen. However, when these analogs were evaluated in a chronic CPCC screen, most had only a modest cholesterol-lowering effect. Compound **16** was most effective, lowering total cholesterol by as much as 70% when dosed at 30 mg/kg. Thus, for *in vivo* efficacy, a chain length of C-13 is optimal, whereas compounds with shorter chain length possess somewhat less activity in the *in vivo* screen. The lack of activity in the CPCC screen compared to the APCC screen for certain compounds suggests that reversal or correction of a pre-established hypercholesterolemia is more difficult than prevention of an acute hypercholesterolemic state. Whether this phenomenon is related to drug absorption and/or site of action (e.g. intestine vs liver) remains to be determined. It

Table I. *in vitro* and *in vivo* Activity of N,N'-Diphenylureas



Compound	R	IAI[a] IC_{50} (uM)	APCC (% Δ TC)[b] 30 mg/kg	CPCC (% Δ TC)[c] 30 mg/kg
1	H	0.91	-11	N.T.
2	CH_3	0.25	-32*	N.T.
3	Et	0.10	-7	N.T.
4	n-Pr	0.038	-29*	N.T.
5	i-Pr	0.066	-30*	N.T.
6	t-Bu	0.168	-35*	-10
7	n-Bu	0.029	-45*	-12
8	$(CH_2)_4CH_3$	0.032	-59*	N.T.
9	$(CH_2)_5CH_3$	0.026	-58*	-18
10	$(CH_2)_6CH_3$	0.019	-50*	-21
11	$(CH_2)_7CH_3$	0.011	-55*	-29
12	$(CH_2)_8CH_3$	0.009	-72*	-8
13	$(CH_2)_9CH_3$	0.013	-58*	-25
14	$(CH_2)_{10}CH_3$	0.009	-61*	-29
15	$(CH_2)_{11}CH_3$	0.015	-59*	-47*
16	$(CH_2)_{12}CH_3$	0.011	-77*	-70*
17	$(CH_2)_{13}CH_3$	0.50	-67*	-25
18	CL 277,082	0.12	-60*	-54*

[a] Intestinal ACAT Inhibition. The incorporation of ^{14}C-oleoyl-CoA into cholesteryl oleate was determined in the presence of intestinal microsomes from cholesterol-fed rabbits. In this *in vitro* screen compounds are added in dimethyl sulfoxide vehicle at four different concentrations in triplicate to calculate IC_{50} values.[3] [b] Acute Peanut Oil/ Cholesterol/ Cholic Acid Screen. Male, Sprague-Dawley rats (200-225 g, n=7/group) are given a single dose of compound in an aqueous suspension (1.5% carboxymethylcellulose, CMC/0.2 % Tween 20) vehicle at 3-4 pm and allowed to consume overnight normal rat chow supplemented with peanut oil (5.5%), cholesterol (1.5%) and cholic acid (0.5%). The following morning (8 am) blood samples are taken for determination of plasma cholesterol levels. [c] Chronic Peanut Oil/ Cholesterol/ Cholic Acid Screen. Rats as above are fed the PCC diet for two weeks and dosed with compounds by daily morning gavage during the second week using the CMC/Tween vehicle. Blood for plasma cholesterol determination is taken 24 hours after the last dose from nonfasted animals. *Statistically significant at $p < 0.05$. TC= Total Cholesterol. NT= Not Tested.

is noteworthy that the *in vitro* potencies and *in vivo* efficacies of certain analogs (**15, 16**) are greater than those observed for the Lederle ACAT inhibitor.

In conclusion, we have synthesized a series of N,N'-diphenyl ureas which are ACAT inhibitors *in vitro* . In this series, a para alkyl substituent of greater than n-pentyl is essential for potent ACAT inhibition *in vitro*. However, a certain lipophilicity is also required for optimal cholesterol lowering *in vivo*. Compound **16** represents a simple urea derivative with an excellent profile for development as a hypocholesterolemic agent.

Acknowledgement:

We would like to thank Ms. Maureen Anderson and Ms. Laura Minton for the evaluation of a few analogs in the *in vitro* IAI screen.

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8. During the course of this investigation, a similar disclosure was made by the scientists from Mitsubishi Kasei Corporation in the patent literature. EP 405233-A.