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N-(1-PHENYL-2-BENZIMIDAZOLYL)-*N'*-PHENYLUREA DERIVATIVES AS POTENT INHIBITORS OF ACYL-COA:CHOLESTEROL ACYLTRANSFERASE (ACAT).

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Abstract: A novel series of *N*-(1-phenyl-2-benzimidazolyl)-*N'*-phenylurea derivatives were prepared as ACAT inhibitors. These compounds showed potent ACAT inhibitory activity in vitro (liver microsomes from cholesterol-fed rabbits) and hypocholesterolemic activity in vivo (cholesterol-fed golden hamsters).

Acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a primary enzyme responsible for the intracellular esterification of free cholesterol. This enzyme is thought to play a key role in intestinal absorption of dietary cholesterol, hepatic production of lipoproteins and the accumulation of cholesteryl esters in the arterial lesions.[1] Inhibition of this enzyme would thus be expected to reduce plasma cholesterol concentration, to reduce the secretion of very low density lipoproteins (VLDL) into the plasma, and to prevent the formation of foam cells in the arterial walls. Therefore, ACAT inhibitors offer potential as hypocholesterolemic and antiatherosclerotic agents.[2]

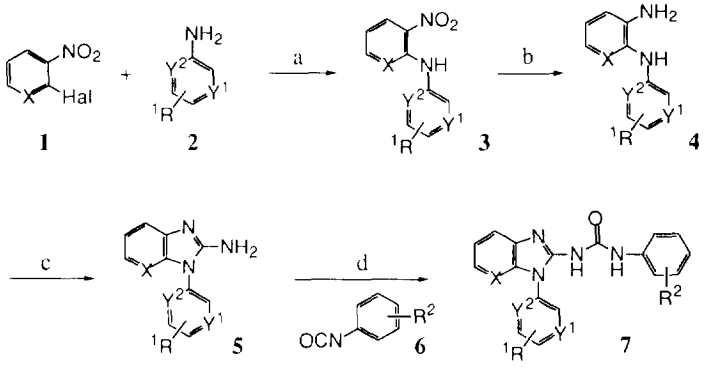
Recently, a number of potent ACAT inhibitors, of widely diverse structures, have been described.[3] Typically, they consist of two lipophilic moieties linked with an amide or a urea and most of them are highly lipophilic. In the course of our investigation to find novel ACAT inhibitors, we attempted to introduce heterocycles to improve in the lipophilicity and synthesized a series of *N*-(1-phenyl-2-benzimidazolyl)-*N'*-phenylurea derivatives.[4]

Compounds prepared were tested for their ability to inhibit ACAT in vitro and to decrease serum total cholesterol in vivo. In the present paper, we have described the synthesis, structure-activity relationships and biological evaluation of this novel class of ACAT inhibitors.

Chemistry

The general synthetic method of the compound **7** is shown in Scheme I. The key intermediate **5** were prepared according to the methods previously reported.[5,6] Thus, 2-bromonitrobenzene or 2-chloro-3-nitropyridine **1** was reacted with aniline **2** in the presence of K_2CO_3 and CuO to afford **3**. Reduction of **3** followed by the cyclization with BrCN gave 2-aminobenzimidazole **5**. Reaction of **5** with phenyl isocyanate **6** gave the corresponding urea **7**.

Scheme I.



a) CuO, K_2CO_3, 180°; b) $Na_2S_2O_4$, EtOH-H_2O, reflux or cat. $FeCl_3$, Fe powder, EtOH-H_2O, reflux; c) BrCN, 1,4-dioxane-H_2O, 0° - r.t.; d) CH_2Cl_2, 0° - r.t.

Results and Discussion

The compounds prepared were evaluated in the ability to inhibit ACAT in vitro by incubation with [^{14}C]oleoyl-CoA and liver microsomes from cholesterol-fed rabbits.[7] The results are shown in Table I.

2,6-Diisopropyl substitution on the benzene ring of aryl urea resulted in potent ACAT inhibitory activity. Although it has been reported that 2,4-difluorine and 2,4,6-trimethyl substitutions in aryl ureas provide a good profile of the inhibitory activity,[8,9] similar substitutions showed weak activity in this study (**7p**, **7q** and **7r**).

Substituents at position 2 or 3 of the benzene ring attached to position 1 of the benzimidazole ring were crucial for potent inhibitory activity (**7b** and **7c**), while unsubstituted and 4-chloro derivatives showed weak activity (**7a** and **7d**). As for the nature of the substituent at position 2, an electronic effect did not seem to influence activity (**7b, 7e-7j**). Substitution with 2-hydroxy group, however, decreased the activity (**7k**). Incorporation of a carboxyl group at the 5 position of 2-chloro derivative **7b** resulted

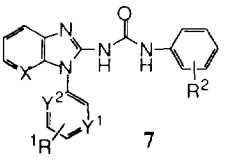


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Table I.

compd	X	Y^1	Y^2	R^1	R^2	*in vitro* ACAT IC_{50}(nM)[a]	*in vivo* reduction % 10 mg/kg, po[b]
7a	CH	CH	CH	H	2,6-iPr_2	66	N.T.[c]
7b	CH	CH	CH	2-Cl	2,6-iPr_2	18	80
7c	CH	CH	CH	3-Cl	2,6-iPr_2	20	N.T.
7d	CH	CH	CH	4-Cl	2,6-iPr_2	280	N.T.
7e	CH	CH	CH	2-Br	2,6-iPr_2	22	37
7f	CH	CH	CH	2-Me	2,6-iPr_2	19	N.T.
7g	CH	CH	CH	2-CF_3	2,6-iPr_2	72	N.T.
7h	CH	CH	CH	2,6-Cl_2	2,6-iPr_2	11	74
7i[e]	CH	CH	CH	2-NMe_2	2,6-iPr_2	41	102
7j	CH	CH	CH	2-OMe	2,6-iPr_2	20	N.T.
7k[e]	CH	CH	CH	2-OH	2,6-iPr_2	200	N.T.
7l[e]	CH	CH	CH	2-Cl, 5-COOH	2,6-iPr_2	(2%)[d]	N.T.
7m[e]	N	CH	CH	2-Cl	2,6-iPr_2	33	76
7n[e]	CH	CH	N	2-Cl	2,6-iPr_2	100	N.T.
7o[e]	CH	N	CH	2-Me	2,6-iPr_2	100	N.T.
7p	CH	CH	CH	2-Cl	2,4-F_2	(27%)[d]	N.T.
7q[e]	CH	CH	CH	2-Cl	2,6-Me_2	420	N.T.
7r	CH	CH	CH	2,6-Cl_2	2,4,6-Me_3	(24%)[d]	N.T.

[a] Liver microsomes isolated from cholesterol-fed rabbits. IC_{50} values were determined by a single experiment. Each assay was performed in triplicate. [b] Percent reduction in increased serum total cholesterol in golden hamsters fed with a diet containing 2% cholesterol (n=5): % reduction = (B - A)/(B - C) x 100 (A, B, and C represent serum cholesterol levels in drug-treated, control, and normal groups, respectively).[7] [c] Not tested. [d] Percent inhibition at 1 μM. [e] $MeSO_3H$ salt.

in a complete loss of inhibitory activity (**7l**). These suggest that a lipophilicity plays an important role in determining ACAT inhibitory activity. Replacement of the benzene ring by pyridine reduced the activity (**7n** and **7o**). On the other hand, conversion of benzimidazole to imidazo[4,5-*b*]pyridine almost retained the potency (**7m**).

Certain compounds that showed potent inhibitory activity in vitro were further evaluated in vivo to measure the hypocholesterolemic effect, which was assessed in golden hamsters fed with a diet containing 2 % cholesterol. Each test compound suspended in olive oil was administered orally once a day for 3 days. After 3 days of feeding, serum total cholesterol was measured and the percent change vs control was determined.[7] The results of hypocholesterolemic activity are shown in Table I. Compounds tested, except **7e**, showed potent hypocholesterolemic effect at a dose of 10 mg/kg (**7b**, **7h**, **7i** and **7m**).

In conclusion, we found a novel series of ACAT inhibitors with *N*-(1-phenyl-2-benzimidazolyl)-*N'*-phenylureas. In this series, 2,6-diisopropyl group on benzene ring and a substituent at position 2 or 3 of the benzene ring attached to position 1 of the benzimidazole ring were essential for potent ACAT inhibition in vitro.

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