Inhibitors of Acyl-CoA: Cholesterol Acyltransferase. II.
Preparation and Hypocholesterolemic Activity of Optically Active
Dibenz[b,e]oxepin-11-carboxanilides

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In a previous paper, we reported a novel inhibitor of acyl-CoA: cholesterol acyltransferase (ACAT), 2-bromo-N-(2,6-diisopropylphenyl)-6,11-dihydrodibenzen[b,e]oxepin-11-carboxamide (1). In this work, we prepared both enantiomers and tested them for ability to inhibit ACAT (liver microsomes from cholesterol-fed rabbits) in vitro and to decrease serum total cholesterol in cholesterol-fed golden hamsters in vivo. The precursor carboxylic acid 4 was optically resolved with cinchonidine. The obtained (−)- and (+)-4 were converted to (−)- and (+)-I without racemization, respectively. The enantiomer (−)-1 showed potent ACAT inhibitory activity in vitro with an IC_{50} value of 8 μM and was approximately 10-fold more active than (+)-1. Furthermore, (−)-1 showed strong hypocholesterolemic activity in vivo, whereas (+)-1 was inactive.

A molecular modeling study showed that the difference of ACAT inhibitory activity between the enantiomers was derived from the spatial alignment of the bromine. Compound (−)-1 was selected for further evaluation as KW-3033.

Key words acyl-CoA: cholesterol acyltransferase; hypocholesterolemic amide; antiatherosclerotic amide; optical resolution; molecular modeling study; dibenz[b,e]oxepin-11-carboxanilide

Acyl-CoA: cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a primary enzyme responsible for the intracellular esterification of cholesterol. This enzyme is thought to play an important role in the absorption of dietary cholesterol from the intestine, the metabolism of cholesterol in the liver and the accumulation of cholesteryl esters in arterial lesions.11 Inhibition of this enzyme would 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 have potential as hypocholesterolemic and antiatherosclerotic agents.2,3

In a previous paper, we reported a novel series of ACAT inhibitory dibenz[b,e]oxepin-11-carboxanilides.49 Among the compounds tested, compound 1 showed the most promising activities both in vitro and in vivo. Since compound 1 possesses an asymmetric carbon at position 11 of the dibenz[b,e]oxepin ring system, it is of interest to test the enantiomers separately. Melamin was the first drug for which the optical isomers were individually examined for ACAT inhibitory and hypocholesterolemic effect.5 In this case, the β-isomer was more effective than the α-isomer. Recently, two groups have independently reported that N-2,6-diisopropylphenylurea ACAT inhibitors (e.g. compounds 2 and 3) show small optical isomeric differences in activity.5,7

We therefore prepared both enantiomers of 1 and tested them for ability to inhibit ACAT in vitro and to decrease serum total cholesterol in vivo.

Chemistry

Since compound 1 does not have a convenient functional group for optical resolution, the precursor carboxylic acid 4 was chosen as a target. Screening of optically active amines showed that cinchonidine has a good ability to resolve 4. Recrystallization of the salt of 4 with cinchonidine from isopropanol was repeated four times to give the optically pure salt with >99.6% ee.8 After treatment of the salt with 0.5 N HCl, the optically pure carboxylic acid (−)-4 was obtained (>99.6% ee). Repeated recrystallization from isopropanol of the (−)-4 rich salt that was obtained by concentration of the combined fractions of mother liquor gave the salt of the enantiomer with >99.6% ee. This salt also gave the optically pure carboxylic acid (−)-4 (>99.6% ee). The obtained optically pure carboxylic acids (−)-4 and (+)-4 were each treated with thionyl chloride to give carboxylic chlorides, which were reacted with 2,6-diisopropylaniline in the presence of pyridine to give the anilides (−)-1 and (+)-1, respec-

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Racemization did not occur and the optically pure anilides (−)-1 and (+)-1 were obtained (>99.6% ee). 8

**Results and Discussion**

The compounds prepared were evaluated in *in vitro* and *in vivo* assays. The ability to inhibit ACAT *in vitro* was determined by incubation with [14C]oleoyl-CoA and liver microsomes from cholesterol-fed rabbits. 4 The hypcholesterolemic effect 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. After 3 d of feeding, serum total cholesterol was measured and the percent change vs. the control was determined. 4 The results of ACAT inhibitory activity and hypcholesterolemic activity are shown in Table 1. The enantiomer (−)-1 showed potent ACAT inhibitory activity *in vitro* with an IC50 value of 8 nm and was approximately 10-fold more active than (+)-1.

Furthermore, (−)-1 showed strong hypcholesterolemic activity *in vivo*, whereas (+)-1 was inactive at a dose of 10 mg/kg. Hypcholesterolemic activity of (−)-1 *in vivo* was further evaluated in cholesterol-fed rats. 8 Both serum and hepatic total cholesterol levels were decreased significantly when (−)-1 was administered orally at doses of more than 1 mg/kg/d, respectively, for 7 d (Table 2).

As stated in the introduction, a small difference (ca. 2-3 fold) in potency *in vitro* between the enantiomers of 2,6-diisopropylphenylurea ACAT inhibitors has been reported. 6, 7 To explain this difference a preliminary molecular modeling study of compound 2 has been conducted. 6 It was revealed that the enantiomers of 2 can be well superposed in a certain conformation, when the 2,6-diisopropylphenyl groups are overlapped. In our case, there was an about 10-fold difference in potency *in vitro* between the enantiomers of 1. We therefore performed a molecular modeling study of compound 1 and 9-bromo-N-(2,6-diisopropylphenyl)-6,11-dihydrodibenzo[b,e]pirepin-11-carboxamide 5, 4, 5 Compound 5 showed weaker activity than compound 1.

The conformational search of 1 and 5 was conducted using the "generate conformational model" utility of CATALYST Ver. 2.1 (Molecular Simulations Inc.) with the "best quality" option. From the inspection of the energy distribution of 40 conformers with the lowest conformational energy, the lowest energy and second-lowest energy conformers were selected. These four conformers were optimized by the AM1 method of MOPAC Ver. 6. 9

<table>
<thead>
<tr>
<th>Compd.</th>
<th>ACAT inhibitory activity (%)</th>
<th>Hypcholesterolemic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-1</td>
<td>23</td>
<td>101</td>
</tr>
<tr>
<td>(+)-1</td>
<td>77</td>
<td>5</td>
</tr>
<tr>
<td>(−)-1</td>
<td>8</td>
<td>116</td>
</tr>
</tbody>
</table>

(a) Liver microsomes isolated from cholesterol-fed rabbits were used. IC50 values were determined by single experiment. Each assay was performed in triplicate.

**Table 2. Effect of (−)-1 on Rat Cholesterol Levels**

<table>
<thead>
<tr>
<th>Dose of (−)-1 mg/kg per day, p.o.</th>
<th>Serum (mg/dl)</th>
<th>Liver (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>104.0 ± 5.0</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>229.3 ± 31.0</td>
<td>22.2 ± 0.9</td>
</tr>
<tr>
<td>0.3</td>
<td>199.8 ± 30.4</td>
<td>17.0 ± 0.8**</td>
</tr>
<tr>
<td>1</td>
<td>165.2 ± 17.4**</td>
<td>13.6 ± 0.8**</td>
</tr>
<tr>
<td>3</td>
<td>135.1 ± 8.3**</td>
<td>12.5 ± 1.1**</td>
</tr>
<tr>
<td>10</td>
<td>97.2 ± 4.6**</td>
<td>8.1 ± 1.0**</td>
</tr>
</tbody>
</table>

(a) Male Sprague-Dawley rats were fed a semisynthetic diet containing 1% cholesterol, 1% cholic acid, 50% sucrose, 12% coconut oil, and 20% casein for 7 d. Compound (−)-1 was orally administered once a day for 7 d. All values are mean ± S.E.M. All groups contained 5 animals each. b) Determined at day 8 of the experiment.  * p<0.05.  ** p<0.01.

The lowest energy conformer (conformer 1A, heat of formation = −5.457 kcal mol⁻¹) and the second-lowest energy conformer (conformer 1B, heat of formation = −4.946 kcal mol⁻¹) of 1 seem like pseudo-enantiomers. The atom-by-atom fitting of 1A and the mirror image of 1B confirms this finding (Fig. 1, all non-hydrogen atoms excluding bromine, −O−C− bridge and isopropyl groups are used). In this superposition, the main difference is the position of the bromine.

From this conformational study and the fact that both (+)-1 and (−)-1 show ACAT inhibitory activity *in vitro*, the following hypotheses are suggested:

a) The active conformation of (−)-1 can be closely reproduced by (+)-1 (e.g., conformers 1A and 1B).

b) Bromine substitution on position 2 enhances the activity through steric and electrostatic effects.

If we assume that the active conformations of (−)-1 and (+)-1 are 1A and 1B respectively, the existence ratio of the conformers (1A:1B = 70:30 at 300 K) is not
enough to explain the observed 10-fold activity difference between (-)-I and (+)-I. Thus, the difference of activity may be derived mainly from steric and electrostatic effects.

The lowest energy conformer (conformer 5A, heat of formation = -5.140 kcal mol\(^{-1}\)) and second-lowest energy conformer (conformer 5B, heat of formation = -4.510 kcal mol\(^{-1}\)) of 5 are pseudo-enantiomers, as in the case of compound 1 (Fig. 2). The conformations of 5A and 5B are similar to those of 1A and 1B, respectively, except for the bromine orientation. According to above hypotheses, the activity of (11R)-5 might be stronger than that of (11S)-5, since the absolute configuration of (-)-I was determined as (11S)\(^1\)) and the alignment of bromine in (11R)-5 and (11S)-5 corresponds to that in (11S)-1 and (11R)-1, respectively. When we establish which enantiomer of 5 has higher activity, we may be able to estimate the effects of the molecular conformation and substituents on the activity of ACAT inhibitors more precisely.

In conclusion, we prepared both enantiomers of 2-bromo-N-(2,6-dimethylphenyl)-6,11-dihydrodibenzo-[b,e]oxepin-11-carboxylic acid [(+)-4]. The combined fractions of the mother liquor of recrystallization of the cinchonidine salt were evaporated in vacuo to give 5.77 g of the cinchonidine salt of (+)-4 (67.4% ee). Recrystallization from isopropanol was repeated six times to give 1.44 g of the pure cinchonidine salt of (+)-4 (>99.6% ee). This salt was converted to 0.75 g of (+)-4 (>99.6% ee) in the same manner as described for (-)-4: mp 167.5-168.5\(^\circ\)C; [\(\alpha\)]\(D\)\(_{10}^\theta\) = +139.0\(^\circ\) (c = 1.0, MeOH).

Optical Resolution of 2-Bromo-6,11-dihydrodibenzo-[b,e]oxepin-11-carboxylic Acid [(+)-4]. A solution of 1.47 g of (+)-4 (4.5 mmol) in 30 ml of CH\(_2\)Cl\(_2\) was treated dropwise with 0.66 ml of SOCl\(_2\) (9.1 mmol) in the presence of a catalytic amount of pyridine at -10\(^\circ\)C and the reaction mixture was stirred at the same temperature for 1 h. The mixture was stirred at room temperature for 3 h, and evaporated in vacuo. The residue was dissolved in 10 ml of toluene, and further evaporated in vacuo. The residue was dissolved in 40 ml of CH\(_2\)Cl\(_2\) and a solution of 0.81 g of 2,6-dimethylpyridine (4.5 mmol) and 0.37 ml of pyridine (4.5 mmol) was added dropwise at -5\(^\circ\)C.
stirred at −5 °C for 1 h, then 50 ml of 1 N HCl was added. The separated organic layer was washed with 1 N HCl and saturated brine, dried, and evaporated in vacuo. The residue was chromatographed on silica gel with toluene-AcOEt (9:1) to give crystals, which were washed with isopropyl ether to give 1.96 g of (−)-1 (90%) (>99.6% ee; mp 158−159 °C; [α]D19 9.8
−94.2° (c = 0.8, MeOH). Anal. Calcd for C12H16BrNO2: C, 67.78; H, 5.90; N, 2.93. Found: C, 67.81; H, 5.95; N, 2.88.

(+)-2-Bromo-N-(2,6-dimethylphenyl)-6,11-dihydrodibenz[b,e]oxepin-11-carboxamide [(+)-1] Compound (+)-4 was converted to (+)-1 in the same manner as described for (+)-4 (78%); mp 158−159 °C; [α]D19 99.8 +92.4° (c = 0.8, MeOH). Anal. Calcd for C12H16BrNO2: C, 67.78; H, 5.90; N, 2.93. Found: C, 67.91; H, 5.85; N, 2.88.

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References and Notes
8) The enantiomeric excess was determined by HPLC analysis (column, Chiralcel OD®; solvent, hexane:2-propanol = 90:10). Compound 4 was esterified with Me2SiCHN2 for HPLC analysis.