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-dihydrodibenz[*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 (+)-1 without racemization, respectively. The enantiomer (−)-1 showed potent ACAT inhibitory activity *in vitro* with an IC_{50} value of 8 nM 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

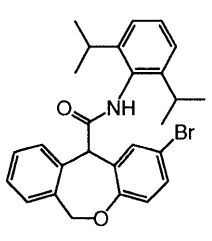
Acyl-CoA: cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a primary enzyme responsible for the intracellular esterification of cholesterol. This enzyme is thought to play an important role in the absorption of dietary cholesterol from the intestine, the metabolism of cholesterol in the liver and the accumulation of cholesteryl esters in arterial lesions.[1] 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.[4] 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. Melinamide was the first drug for which the optical isomers were individually examined for ACAT inhibitory and hypocholesterolemic effect.[5] In this case, the D-isomer was more effective than the L-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.[6,7]

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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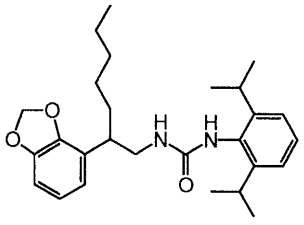

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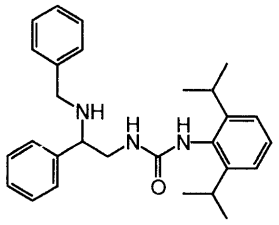

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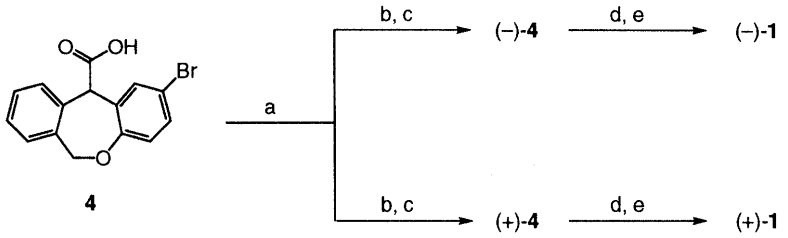

(a) cinchonidine; (b) recrystallization; (c) HCl; (d) $SOCl_2/CH_2Cl_2$; (e) 2,6-diisopropylaniline, pyridine/CH_2Cl_2.

Chart 1.

tively. 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 [^{14}C]oleoyl-CoA and liver microsomes from cholesterol-fed rabbits.[4] The hypocholesterolemic 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 hypocholesterolemic activity are shown in Table 1. The enantiomer (−)-**1** showed potent ACAT inhibitory activity *in vitro* with an IC_{50} value of 8 nM and was approximately 10-fold more active than (+)-**1**.

Furthermore, (−)-**1** showed strong hypocholesterolemic activity *in vivo*, whereas (+)-**1** was inactive at a dose of 10 mg/kg. Hypocholesterolemic activity of (−)-**1** *in vivo* was further evaluated in cholesterol-fed rats.[4] Both serum and hepatic total cholesterol levels were decreased significantly when (−)-**1** was administered orally at doses of more than 1 and 0.3 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 *N*-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-dihydrodibenz[*b*,*e*]oxepin-11-carboxamide **5**.[4] 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 1. ACAT Inhibitory Activity and Hypocholesterolemic Activity of **1**

Compd.	ACAT inhibitory activity[a] IC_{50} (nM)	Hypocholesterolemic activity[b] % reduction (10 mg/kg, *p.o.*)
(±)-**1**	23	101
(+)-**1**	77	5
(−)-**1**	8	116

a) Liver microsomes isolated from cholesterol-fed rabbits were used. IC_{50} values were determined by a single experiment. Each assay was performed in triplicate. *b*) Percent reduction of the increase in serum total cholesterol in golden hamsters fed with a diet containing 2% cholesterol ($n=5$): % reduction $=(B-A)/(B-C) \times 100$ (A, B, C represent serum cholesterol levels in drug-treated, control, and normal groups, respectively).[4]

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

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

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_5–C_6- 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

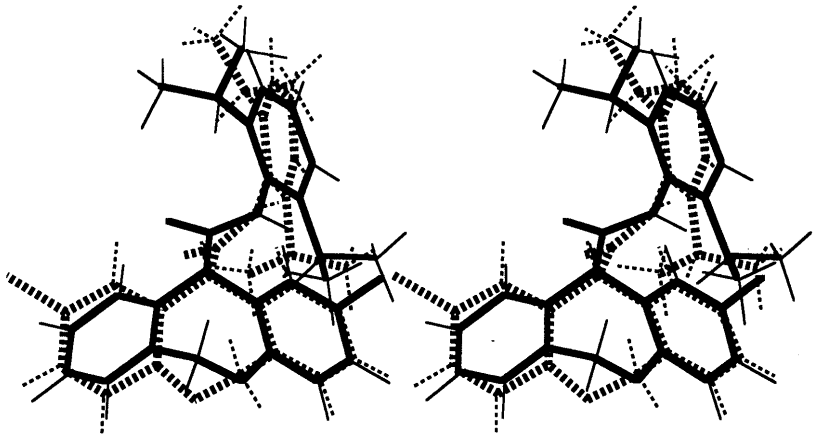

Fig. 1. Stereographic Drawing of the Superposition of (11*S*)-**1** (Conformation **1**A, Solid Line) and (11*R*)-**1** (Conformation **1**B, Dotted Line)

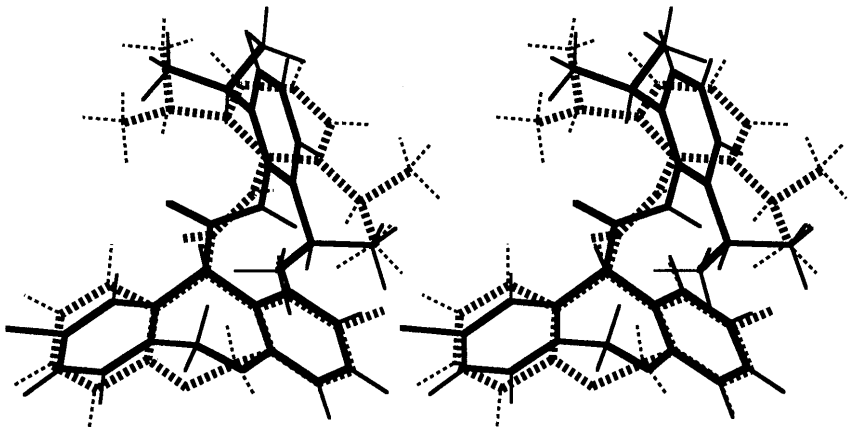

Fig. 2. Stereographic Drawing of the Superposition of (11*S*)-**5** (Conformation **5**A, Solid Line) and (11*R*)-**5** (Conformation **5**B, Dotted Line)

enough to explain the observed 10-fold activity difference between (−)-**1** and (+)-**1**. Thus, the difference of activity may be derived mainly from steric and electrostatic effects.

The lowest energy conformer (comformer **5**A, heat of formation = $-5.140\,\text{kcal mol}^{-1}$) and second-lowest energy conformer (conformer **5**B, heat of formation = $-4.510\,\text{kcal mol}^{-1}$) of **5** are pseudo-enantiomers, as in the case of compound **1** (Fig. 2). The conformations of **5**A and **5**B are similar to those of **1**A and **1**B, respectively, except for the bromine orientation. According to above hypotheses, the activity of (11*R*)-**5** might be stronger than that of (11*S*)-**5**, since the absolute configuration of (−)-**1** was determined as (11*S*)[10] and the alignment of bromine in (11*R*)-**5** and (11*S*)-**5** corresponds to that in (11*S*)-**1** and (11*R*)-**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-diisopropylphenyl)-6,11-dihydrodibenz-[*b*,*e*]oxepin-11-carboxamide (**1**). The enantiomer (−)-**1** showed potent ACAT inhibitory activity *in vitro* and strong hypocholesterolemic activity *in vivo*, and was selected for further evaluation as KW-3033.

Experimental

Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter.

Optical Resolution of 2-Bromo-6,11-dihydrodibenz[*b*,*e*]oxepin-11-carboxylic Acid (4). (−)-2-Bromo-6,11-dihydrodibenz[*b*,*e*]oxepin-11-carboxylic Acid [(−)-4] A solution of 5.24 g (16.4 mmol) of **4** and 4.83 g (16.4 mmol) of cinchonidine in 250 ml of MeOH was evaporated and the residue was recrystallized from isopropanol four times to give 2.92 g of the cinchonidine salt of (−)-**4** (>99.6% ee) (29%). This salt was suspended in 50 ml of ethyl acetate and 50 ml of water. After addition of 50 ml of 0.5 N HCl, the mixture was stirred at room temperature for 30 min. The organic layer was washed with saturated brine, dried, and evaporated *in vacuo* to give 1.52 g of (−)-**4** (>99.6% ee) (29%) as colorless crystals: mp 166.5—167 °C; $[\alpha]_D^{20}$ −139.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{15}H_{11}BrO_3$: C, 56.45; H, 3.47. Found: C, 56.59; H, 3.43.

(+)-2-Bromo-6,11-dihydrodibenz[*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 (+)-rich **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 °C; $[\alpha]_D^{20}$ +143.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{15}H_{11}BrO_3$: C, 56.45; H, 3.47. Found: C, 56.47; H, 3.34.

(−)-2-Bromo-*N*-(2,6-diisopropylphenyl)-6,11-dihydrodibenz[*b*,*e*]oxepin-11-carboxamide [(−)-1] A solution of 1.47 g of (−)-**4** (4.5 mmol) in 30 ml of CH_2Cl_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 °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_2Cl_2 and a solution of 0.81 g of 2,6-diisopropylaniline (4.5 mmol) and 0.37 ml of pyridine (4.5 mmol) was added dropwise at −5 °C. The mixture was

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; $[\alpha]_D^{20}$ −94.2° (c=0.8, MeOH). *Anal.* Calcd for $C_{27}H_{28}BrNO_2$: C, 67.78; H, 5.90; N, 2.93. Found: C, 67.81; H, 5.95; N, 2.88.

(+)-2-Bromo-*N*-(2,6-diisopropylphenyl)-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; $[\alpha]_D^{20}$ +92.4° (c=0.8, MeOH). *Anal.* Calcd for $C_{27}H_{28}BrNO_2$: C, 67.78; H, 5.90; N, 2.93. Found: C, 67.91; H, 5.85; N, 2.88.

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