

Synthesis and structure–activity relationships of new ACAT inhibitors

JY Nioche, J Decerprit, D Festal*

Lipha Research & Development Centre, 115, avenue Lacassagne, 69003 Lyon, France

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Summary — A series of heterocyclic ureas were synthesized and their ability to inhibit arterial and intestinal ACAT was assessed in animals. The structural modifications carried out in this series led to *N*₂-(2,4-difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylurea **21**, which proved to be very active on both the inhibition of aortic ACAT and the inhibition of rat cholesterol intestinal absorption, thus exhibiting a strong hypocholesterolemic effect *po* in the rat (ED₂₅ = 0.2 mg/kg).

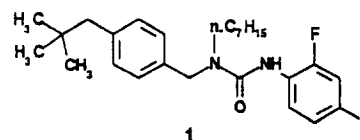
aortic ACAT / intestinal inhibition / benzoxepin / urea / cholesterol / hypocholesterolaemic activity

Introduction

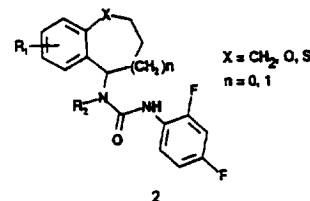
Acyl-CoA, cholesterol O-acyl transferase (ACAT, EC 2.3.1.26), is the enzyme naturally responsible for cholesterol intracellular esterification [1]. Many pharmacological studies demonstrated that feeding animals with a cholesterol-enriched diet caused atherosclerotic lesions in the arterial wall, especially in rabbits and monkeys [2–5]. These lesions are characterized by macrophage accumulations in which ACAT activity is enhanced, causing ester accumulation. In humans, large amounts of cholesteryl esters have been found in atherosclerotic plaques [6] with macrophagic tissues [7], thus reflecting the process observed in animals. As a consequence, the pharmacological inhibition of arterial ACAT is expected to stop or reduce the development of the disease.

Other works have demonstrated the essential function of ACAT in cholesterol intestinal absorption, mainly in the jejunum [8, 9] where the highest ACAT activity and most of the cholesterol absorption are observed. The inhibition of jejunal ACAT should help reduce this absorption.

A number of compounds described in the literature and especially a series of *N*-benzyl-*N*-alkylureas [10] were identified as ACAT inhibitors. A large number of animal and human studies [11] have been performed on compound **1**.



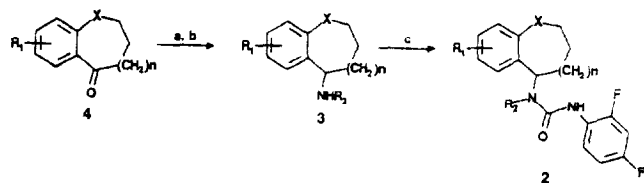
We decided to modify the benzyl part of compound **1** and study the effect of these modifications on the ACAT-inhibiting activity. We first tried to reduce the number of degrees of freedom of the benzyl group towards the urea function, which led us to prepare the bicyclic ureas **2**. Substitution variations in the aromatic part of these bicyclic compounds and in the adjacent nitrogen atom finally gave ACAT-inhibiting molecules with a level of activity significantly higher than that of compound **1**. Some of these compounds exhibited a strong hypocholesterolemic effect *in vivo*.



Chemistry

The analogs of compound **2** (table I) were prepared by reaction of 2,4-difluorophenylisocyanate with the corresponding amines **3** (table II), which were ob-

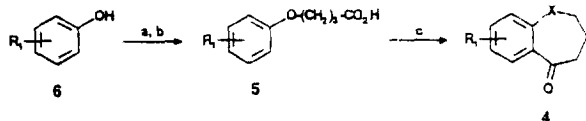
*Correspondence and reprints



Scheme 1. (a) R_2NH_2 /toluene; (b) $NaBH_4$ /THF; (c) 2,4-(F) $_2$ -C $_6$ H $_3$ NCO.

tained by amination reduction of the carbonyl derivatives **4** (scheme 1).

The compounds **4**, for which $X = O$ and $n = 0$ and $X = CH_2$ or S and $n = 1$, are commercially available or described in the literature. Benzoxepin-5-ones (table III) were prepared according to a process described by Fontaine [12] which consists of cyclizing the corresponding 4-phenoxybutyric acid **5** (table IV) with polyphosphoric acid (PPA). Compounds **5** were obtained by condensation of γ -butyrolactone on phenols **6** (scheme 2).



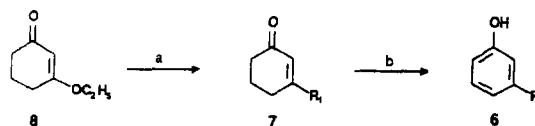
Scheme 2. (a) $NaOH$ / γ -butyrolactone; (b) HCl ; (c) PPA/toluene.

Most of the phenols **6** used are commercially available except *meta*-arylphenols (table V) which were prepared by aromatization [13] of 3-arylcyclohex-2-enones **7** (scheme 3, table VI). Compounds **7** were obtained by addition of a Grignard reagent to 3-ethoxycyclohex-2-enone [14].

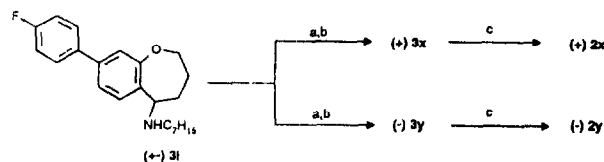
To study and compare the biological and pharmacological activity exhibited by the enantiomers of compounds **2**, two enantiomers **2x** and **2y** of the most active compound **21** (scheme 4) were prepared from the corresponding enantiomeric amines **3x** and **3y** obtained by resolution of the racemic compound **31** with (+) and (-)mandelic acids. The enantiomeric excess of compounds **3x** and **3y** was determined by HPLC assay of the diastereoisomer ureas prepared by condensation with *S*-(-)- α -methylbenzylisocyanate.

Biology

The arterial and intestinal ACAT inhibiting effects of compound **2** were assessed in three pharmacological tests. One was representative of the arterial ACAT



Scheme 3. (a) R_1MgX /ether; (b) $Pd/C/p$ -cymene.



Scheme 4. (a) (+)Mandelic acid for **3x** or (-)mandelic acid for **3y**; (b) $NaHCO_3$; (c) 2,4-(F) $_2$ -C $_6$ H $_3$ NCO.

activity, while the others represented the intestinal ACAT activity. To assess the arterial impact, the *in vivo* effect of the studied compounds was directly measured on a rabbit aorta microsomal preparation.

The intestinal impact was assessed by measuring the *in vivo* effect on cholesterol absorption in the rat according to two methods: 1) the absorption of 3H -labelled cholesterol in the normolipidemic animal (basic intestinal ACAT); and 2) hypercholesterolemic animals fed a cholesterol-enriched diet (induced intestinal ACAT).

Results and discussion

Table VII shows the results of the *in vitro* pharmacological activities of compounds **2** obtained for the rabbit microsomal ACAT and the *in vivo* absorption of 3H -labelled cholesterol in the normolipidemic rat. The results for compound **1** are given for comparison.

The preliminary modifications aiming at constraining the benzyl part of compound **1** led to benzopyran and benzothiapyran (**2a** and **2b**). These compounds both inhibit *in vitro* aortic ACAT and *in vivo* cholesterol intestinal absorption, but only compound **2b** shows a higher aortic ACAT inhibiting activity than compound **1**. Unfortunately, these compounds proved to have little or no activity on rat cholesterolemia (table VIII). Among the oxygen **2c**, sulphur **2d** and carbon **2e** homocycles, benzoxepin **2c** showed an effect equivalent to that of compound **1** on the absorption of 3H -labelled cholesterol (6.2 mg/kg) and proved to be significantly more active on the inhibition of aortic ACAT (0.75 μM). The fivefold increase of *in vitro* ACAT inhibition exhibited by **2c** in comparison with compound **1** led us to study the variations of substitu-

Table I. Physical data for compounds 2.

No	X	n	R ₁	R ₂	Yield (%)	MP (°C)	Cryst solvent ^a	Formula ^c
2a	O	0	H	n.C ₇ H ₁₅	11	oil	b	C ₂₃ H ₂₈ F ₂ N ₂ O ₂
2b	S	0	H	n.C ₇ H ₁₅	52	79-81	A	C ₂₃ H ₂₈ F ₂ N ₂ OS
2c	O	1	H	n.C ₇ H ₁₅	75	oil	b	C ₂₄ H ₃₀ F ₂ N ₂ O ₂
2d	S	1	H	n.C ₇ H ₁₅	84	67-70	b	C ₂₄ H ₃₀ F ₂ N ₂ OS
2e	CH ₂	1	H	n.C ₇ H ₁₅	24	oil	b	C ₂₅ H ₃₂ F ₂ N ₂ O ^d
2f	O	1	8-Br	n.C ₇ H ₁₅	95	77-79	B	C ₂₄ H ₂₉ BrF ₂ N ₂ O ₂
2g	O	1	8-F	n.C ₇ H ₁₅	81	oil	b	C ₂₄ H ₂₉ F ₃ N ₂ O ₂
2h	O	1	8-Cl	n.C ₇ H ₁₅	70	65-68	B	C ₂₄ H ₂₉ ClF ₂ N ₂ O ₂
2i	O	1	7-Cl	n.C ₇ H ₁₅	41	oil	b	C ₂₄ H ₂₉ ClF ₂ N ₂ O ₂
2j	O	1	9-Cl	n.C ₇ H ₁₅	49	93-96	C	C ₂₄ H ₂₉ ClF ₂ N ₂ O ₂
2k	O	1	8-C ₆ H ₅	n.C ₇ H ₁₅	56	89-91	A	C ₃₀ H ₃₄ F ₂ N ₂ O ₂
2l	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	81	91-93	D	C ₃₀ H ₃₃ F ₃ N ₂ O ₂
2m	O	1	8-(4-Cl-C ₆ H ₄)	n.C ₇ H ₁₅	56	85-87	A	C ₃₀ H ₃₃ ClF ₂ N ₂ O ₂
2n	O	1	8-(4-Me-C ₆ H ₄)	n.C ₇ H ₁₅	54	101-103	B	C ₃₁ H ₃₆ F ₂ N ₂ O ₂
2o	O	1	8-(4-MeO-C ₆ H ₄)	n.C ₇ H ₁₅	81	127-129	E	C ₃₁ H ₃₆ F ₂ N ₂ O ₃
2p	O	1	8-(3-F-C ₆ H ₄)	n.C ₇ H ₁₅	80	82-84	A	C ₃₀ H ₃₃ F ₃ N ₂ O ₂
2q	O	1	8-(3,4-F ₂ -C ₆ H ₃)	n.C ₇ H ₁₅	40	88-90	A	C ₃₀ H ₃₂ F ₄ N ₂ O ₂
2r	O	1	8-(4-F,3-Me-C ₆ H ₃)	n.C ₇ H ₁₅	25	97-99	A	C ₃₁ H ₃₅ F ₃ N ₂ O ₂
2s	O	1	8-(4-F-C ₆ H ₄)	n.C ₆ H ₁₃	57	87-89	A	C ₂₉ H ₃₁ F ₃ N ₂ O ₂
2t	O	1	8-(4-F-C ₆ H ₄)	n.C ₅ H ₁₁	59	135-137	F	C ₂₈ H ₂₉ F ₃ N ₂ O ₂
2u	O	1	8-(4-F-C ₆ H ₄)	n.C ₉ H ₁₉	50	oil	b	C ₃₂ H ₃₇ F ₃ N ₂ O ₂
2v	O	1	8-(4-F-C ₆ H ₄)	1-Me-C ₇ H ₁₄	30	108-110	D	C ₃₁ H ₃₅ F ₃ N ₂ O ₂
2w	O	1	8-(4-F-C ₆ H ₄)	PrO-(CH ₂) ₄	75	104-106	A	C ₃₀ H ₃₃ F ₃ N ₂ O ₃
2x	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	56	85-92	b	C ₃₀ H ₃₃ F ₃ N ₂ O ₂
2y	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	50	72-86	b	C ₃₀ H ₃₃ F ₃ N ₂ O ₂

^aA = hexane; B = pentane; C = pentane/*i*Pr₂O; D = hexane/pentane; E = *i*Pr₂O; F = hexane/*i*Pr₂O. ^bPurified by chromatography on silica gel. ^cC, H, N were analyzed for all the compounds as well as Cl, Br, F and S when present; the values obtained are at $\pm 0.4\%$ of the theoretical values. ^dCalculated for C₂₅H₃₂F₂N₂O₂·1/2 H₂O: C = 70.89, H = 7.85, F = 8.97, N = 6.61; found C = 71.20, H = 7.88, F = 8.97, N = 6.58.

tion in the benzoxepin cycle to increase the inhibitory effect on ³H-cholesterol absorption. This is the reason why we chose it as a starting point to study the variations of substitution.

The substitution of position 8 in the benzoxepin cycle by a halogen atom led to compounds that are very active on both the inhibition of aortic ACAT and

the intestinal absorption of cholesterol (2f, 2g, 2h). A substituent effect is observed on *in vitro* and *in vivo* ACAT inhibition (F = Cl < Br) [15]. However, the reverse effect is obtained on intestinal absorption. The substitution of positions 7 or 9 led to compounds as active or less active than 2h on *in vitro* and *in vivo* ACAT inhibition, but with no antihypercholesterolo-

Table II. Physical data for compounds 3.

No	X	n	R ₁	R ₂	Yield (%)	M P (°C) ^a	Hydrochloride cryst. solvent ^c
3a	O	0	H	n.C ₇ H ₁₅	62	oil	-
3b	S	0	H	n.C ₇ H ₁₅	65	140-143	A
3c	O	1	H	n.C ₇ H ₁₅	53	157-159	A
3d	S	1	H	n.C ₇ H ₁₅	48	127-129	A
3e	CH ₂	1	H	n.C ₇ H ₁₅	92	145-147	A
3f	O	1	8-Br	n.C ₇ H ₁₅	63	138-140	A
3g	O	1	8-F	n.C ₇ H ₁₅	74	148-151	A
3h	O	1	8-Cl	n.C ₇ H ₁₅	72	142-144	A
3i	O	1	7-Cl	n.C ₇ H ₁₅	72	142-144	A
3j	O	1	9-Cl	n.C ₇ H ₁₅	61	172-174	A
3k	O	1	8-C ₆ H ₅	n.C ₇ H ₁₅	53	126-129	A
3l	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	70	oil	-
3m	O	1	8-(4-Cl-C ₆ H ₄)	n.C ₇ H ₁₅	83	oil	-
3n	O	1	8-(4-Me-C ₆ H ₄)	n.C ₇ H ₁₅	87	oil	-
3o	O	1	8-(4-MeO-C ₆ H ₄)	n.C ₇ H ₁₅	57	oil	-
3p	O	1	8-(3-F-C ₆ H ₄)	n.C ₇ H ₁₅	57	137-140	A
3q	O	1	8-(3,4-F ₂ -C ₆ H ₃)	n.C ₇ H ₁₅	80	oil	-
3r	O	1	8-(4-F,3-Me-C ₆ H ₃)	n.C ₇ H ₁₅	73	oil	-
3s	O	1	8-(4-F-C ₆ H ₄)	n.C ₆ H ₁₃	36	oil	-
3t	O	1	8-(4-F-C ₆ H ₄)	n.C ₅ H ₁₁	52	oil	-
3u	O	1	8-(4-F-C ₆ H ₄)	n.C ₉ H ₁₉	51	oil	-
3v	O	1	8-(4-F-C ₆ H ₄)	1-Me-C ₇ H ₁₄	59	132-135	B
3w	O	1	8-(4-F-C ₆ H ₄)	(CH ₂) ₄ -OPr	42	oil	-
3x	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	27	oil ^b	-
3y	O	1	8-(4-F-C ₆ H ₄)	n.C ₇ H ₁₅	22	oil ^b	-

^aCharacterized as the hydrochloride salt; the melting point indicated is that of the hydrochloride salt. ^bMelting point of the mandelate salt = 149–151°C (*i*Pr₂O/AcOEt); ^cA = *i*Pr₂O/*i*PrOH; B = Et₂O.

lemic effect (2i, 2j). These results show the importance of the substitution of benzoxepin in the *para* position of the urea function by a large halogen (Br).

These results led us to increase the steric bulk of position 8 in the benzoxepin cycle and increase lipophilicity with the introduction of a phenyl group. The replacement of the chlorine atom 2h with a phenyl group 2k causes an eightfold increase in the inhibiting

effect of cholesterol absorption. Substitution of position 4 on the phenyl ring by a fluorine atom yielded the most active compound of the series 21 in all three tests. Several analogs of 21, produced by replacement of the fluorine atom with a chlorine, methyl or methoxy group (2m, 2n and 2o respectively), a fluorine atom in the 3 position (2p) or difluoro substitution (2q, 2r), significantly reduce

Table III. Physical data for compounds 4.

<i>No</i>	<i>R</i> ₁	<i>Yield</i> (%)	<i>MP or</i> <i>BP</i> _{mm} (°C)	<i>Cryst</i> <i>solvent</i> ^b
4f	8-Br	74	B P _{0.4} =109-113	-
4j	9-Cl	36	B P _{0.2} =112-114	-
4k	8-C ₆ H ₅	66	81-83	A
4l	8-(4-F-C ₆ H ₄)	78	107-110	B
4m	8-(4-Cl-C ₆ H ₄)	60	141-143	C
4n	8-(4-Me-C ₆ H ₄)	64	127-129	D
4o	8-(4-MeO-C ₆ H ₄)	56	88-92	A
4p	8-(3-F-C ₆ H ₄)	90	oil ^a	-
4q	8-(3,4-F ₂ -C ₆ H ₃)	49	79-81	C
4r	8-(4-F,3-Me-C ₆ H ₃)	55	oil ^a	-

^aPurified by chromatography on silica gel; ^bA = hexane; B = EtOH; C = *i*Pr₂O.

Table IV. Physical data for compounds 5.

<i>No</i>	<i>R</i> ₁	<i>Yield</i> (%)	<i>MP</i> (°C)	<i>Cryst</i> <i>solvent</i> ^b
5f	3-Br	66	43-47	A
5j	2-Cl	25	85-88	A
5k	3-C ₆ H ₅	55	94-98	C
5l	3-(4-F-C ₆ H ₄)	79	77-79	B
5m	3-(4-Cl-C ₆ H ₄)	36	oil ^a	-
5n	3-(4-Me-C ₆ H ₄)	82	97-100	C
5o	3-(4-MeO-C ₆ H ₄)	39	99-101	D
5p	8-(3-F-C ₆ H ₄)	66	57-62	A
5q	8-(3,4-F ₂ -C ₆ H ₃)	96	oil ^a	-
5r	8-(4-F,3-Me-C ₆ H ₃)	24	oil ^a	-

^aPurified by chromatography on silica gel; ^bA = hexane/*i*Pr₂O; B = *i*Pr₂O; C = hexane; D = pentane/*i*Pr₂O.

anti-ACAT activity and therefore the hypocholesterolemic activity.

These results show both the importance of the substitution of a small halogen (F) at position 4 on the

phenyl ring and the limited amount substitution in the other positions (perhaps because of steric effects).

Compounds **2f**, **2i** and **2m** are effective on ³H-cholesterol absorption but not on diet-induced hyper-

Table V. Physical data for compounds 6.

No	R ₁	Yield (%)	M P (°C)	Cryst solvent
6n	3-(4-Me-C ₆ H ₄)	47	76-78	hexane
6q	3-(3,4-F ₂ -C ₆ H ₃)	52	67-69	hexane/iPr ₂ O
6r	3-(4-F,3-Me-C ₆ H ₃)	85	oil ^a	-

^aPurified by chromatography on silica gel.

Table VI. Physical data for compounds 7.

No	R ₁	Yield (%)	M P or B P _{mm} (°C)	Cryst solvent
7n	3-(4-Me-C ₆ H ₄)	75	50-55	pentane
7q	3-(3,4-F ₂ -C ₆ H ₃)	64	34-39	hexane/iPr ₂ O
7r	3-(4-F,3-Me-C ₆ H ₃)	72	B P _{0.6} =122-125	-

cholesterolemia. This can be explained by the fact that intestinal ACAT has been largely induced in the latter (in contrast to the former) and the inhibitory effect of these compounds is then probably overcome.

We have also studied the effect of the length or branching of the *n*-heptyl chain (substituent R₂) on ACAT inhibition. Reducing or lengthening the alkyl chains (**2s**, **2t** and **2u**), branching the chain **2v** and insertion of an oxygen atom **2w** all reduced ACAT inhibition. The two enantiomers (**2x** and **2y**) of compound **2l** were equally active *in vitro*, but the (–) isomer **2y** appeared to be slightly more potent than **2x** in both tests *in vivo*.

Conclusion

This study led us to synthesize a series of heterocyclic ureas which inhibited aortic and intestinal ACAT. *N*₂-(2,4-Difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptyl urea **2l** proved to be particularly active both *in vitro* (rabbit aortic ACAT) and *in vivo* (intestinal absorption of ³H-labelled cholesterol). These activities were characterized by a strong *in vivo* hypocholesterolemic effect with a *po* effective dose 25 of 0.18 mg/kg. This compound was chosen to be developed as a potential hypocholesterolemic and antiatherosclerotic drug.

Experimental protocols

Chemistry

The melting points were determined in a capillary tube with a Gallenkamp apparatus and are not corrected. The IR spectra

were recorded with a Perkin-Elmer spectrophotometer type 881 and are expressed in wavenumber (cm⁻¹). The ¹H NMR spectra were recorded in a Brücker spectrometer WP60 CW at 60 MHz using tetramethylsilane as an internal standard. The chemical shifts are expressed in ppm. The abbreviations s, d, t, q and m are used for singlet, doublet, triplet, quartet and multiplet respectively. Optical rotations were determined with a Gyromat apparatus. The elementary analyses were carried out by the Service Central d'Analyses du CNRS (Vernaison, France). The thin-layer chromatography (TLC) was performed on silica-gel sheets 60F254 Merck and column chromatography over silica gel 60 (Merck, 230–400 mesh). The HPLC analyses were conducted on a Shimadzu LC-9A instrument using a Spherisorb column 5 μm of 25 cm (mobile phase: ethyl acetate/hexane: 1:3).

The operating conditions described in the following examples can be applied to the various compounds shown in tables I–VI.

The following phenols **6** are described in the literature: 1,1'-biphenyl-3'-fluoro-3-ol [16], 1,1'-biphenyl-4'-chloro-3-ol [17], 1,1'-biphenyl-4'-methoxy-3-ol [18], 1,1'-biphenyl-4'-fluoro-3-ol [13], 1,1'-biphenyl-3-ol [19]. The 3,4-dihydro-2*H*-1-benzoxepin-5-ones **3** are also described in the literature. Their substituent R₁ is as follow: 8-Cl [20]; H [21]; 8-F [22]; 7-Cl [23]; 8-CH₃ [24]; and 3,4-dihydro-2*H*-1-benzothiapin-5-one [25].

3-(4-Methylphenyl)cyclohex-2-enone **7n**

Under a nitrogen atmosphere, 4-bromotoluene (45.5 g, 0.266 mol) was added over a 45 min period to a stirred suspension of 6.4 g (0.266 atom-gram) of magnesium turnings in 130 ml dry diethylether. Once the reaction had started, the addition of 4-bromotoluene was adjusted to obtain a slight reflux of the reaction medium. After the addition, the mixture was further stirred for 1 h at room temperature, and then 25 g (0.178 mol) of 3-ethoxycyclohex-2-enone **8** was added within 15 min. The reaction medium was stirred for 2 h at room temperature, and then acidified by addition at 15°C of 1 N hydrochloric acid. The mixture was extracted with diethylether, and then the extract was washed with water, dried over Na₂SO₄ and the solvent evaporated. A yellow solid (25.9 g, 78%) was thus isolated

mp = 50–55°C (pentane). IR (KBr): 1650 cm⁻¹. ¹H NMR (CDCl₃): 2.35 (s, 3H), 1.70–3.05 (m, 6H), 6.35 (s, 1H), 7.1 (d, *J* = 7.5 Hz, 2H), 7.4 (d, *J* = 7.5 Hz, 2H).

1,1'-Biphenyl-4'-methyl-3-ol 6n

A suspension of 24 g (0.129 mol) of 3-(4-methylphenyl)cyclohex-2-enone 7n and 8.7 g of 5% Pd/C in 77 ml of *p*-cymene was heated at reflux under nitrogen atmosphere for 2 h. The organic phase was then filtered, the solvent evaporated, and the residue dissolved in an 1 N ethanol potassium hydroxide solution. This solution was evaporated to dryness. The raw potassium phenate thus obtained was dissolved in water. The aqueous phase was then washed with diethylether, acidified with 1 N hydrochloric acid and then extracted with diethylether. This extract was dried over Na₂SO₄ and the solvent evaporated. Phenol 6n was thus isolated as an oil which crystallized at room temperature (11.6 g, 47%), mp = 76–78°C (hexane). IR (KBr): 3280 cm⁻¹. ¹H NMR (CDCl₃): 2.42 (s, 3H), 4.75 (s, 1H), 6.5–7.75 (m, 8H).

4-[3-(4-Fluorophenyl)phenoxy]butanoic acid 5l

A solution of 100 g (0.531 mol) of 1,1'-biphenyl-4'-fluoro-3-ol [13] and 21.2 g (0.531 mol) of solid sodium hydroxide in 40 ml

Table VII. *In vitro* and *in vivo* activities of compounds 2 on ACAT.

Compound	Inhibition of rabbit aortic ACAT, IC ₅₀ (μM)	Inhibition of intestinal absorption of ³ H-cholesterol, ED ₅₀ (mg/kg) po
2a	> 10	13.7
2b	0.51	22
2c	0.75	6.2
2d	1.1	~10
2e	~7	~20
2f	0.83	2.83
2g	1.13	5.7
2h	1.14	5.8
2i	5.6	4.32
2j	1.0	~10
2k	8.2	0.7
2l	0.79	0.97
2m	~7	5.5
2n	5.7	~10
2o	6	≥ 10
2p	7.8	1.81
2q	2.9	1.38
2r	> 5	> 5
2s	1.2	1.76
2t	2.9	2.65
2u	6.4	> 5
2v	~5	~5
2w	1.62	~5
2x	0.96	Inactive at 3
2y	1.28	< 3
1	3.54	4

Table VIII. Antihypercholesterolemic activity of compounds 2.

Compound	Rat, ED ₂₅ (mg/kg)
2a	< 10
2b	> 10
2c	> 10
2d	~10
2e	> 10
2f	> 10
2g	10.4
2h	8.3
2i	> 10
2j	> 10
2k	2.66
2l	0.18
2m	> 10
2n	> 10
2o	9.7
2p	1.91
2q	< 10
2r	< 5
2s	0.68
2t	< 3
2u	~5
2v	< 5
2w	< 5
2x	~3
2y	< 3
1	4.03

of ethanol was heated at reflux for 1 h. After evaporation of the solvent, 59.4 g (0.690 mol) γ-butyrolactone was rapidly added under a nitrogen atmosphere at 150°C. The solution obtained was heated for 4 h at 190°C, and then cooled, taken up with water, and acidified with concentrated hydrochloric acid. The solution was extracted with diethylether, dried (Na₂SO₄) and evaporated. Compound 5l was thus isolated in the form of a beige solid (35 g, 65%), mp = 77–79°C (hexane). IR (KBr): 1695 cm⁻¹.

8-(4-Fluorophenyl)-3,4-dihydro-2H-1-benzoxepin-5-one 4l

A solution of 95 g (0.346 mol) of 4-[3-(4-fluorophenyl)phenoxy]butanoic acid 5l was added over 45 min to a hot suspension of 570 g polyphosphoric acid in 1250 ml xylene and heated to reflux. It was then further heated at reflux for 4 h. The organic phase was then decanted and the xylene was evaporated under reduced pressure. A solid (69.2 g, 78%) is thus isolated, mp = 111–113°C (ethanol). IR (KBr): 1685 cm⁻¹. ¹H NMR (CDCl₃): 2.20 (q, *J* = 6.7 Hz, 2H), 2.92 (t, *J* = 6.7 Hz, 2H), 4.25 (t, *J* = 6.7 Hz, 2H), 6.75–8 (m, 7H). Analysis: C₁₆H₁₃FO₂ (C, H, F).

(±)-N-8-(4-Fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-N₁-n-heptylamine 3l

In a reactor equipped with a Dean–Stark apparatus, a mixture of 32.5 g (0.127 mol) 8-(4-fluorophenyl)-3,4-dihydro-2H-1-benzoxepin-5-one 4l in 31 ml of *n*-heptylamine, 490 ml xylene and

a trace of *para*-toluenesulfonic acid was heated at reflux for 14 h. The solvent was then evaporated and 560 ml ethanol and 13 ml water were added; 2.2 g of sodium borohydride were then added. This solution was stirred for 12 h at room temperature and then the solvent was evaporated and the residue was taken up with water, extracted with ether, dried (Na_2SO_4) and evaporated. The oil **3l** dissolved in ethanol was added to a solution of ethanol saturated with HCl. After evaporation of the solvent, the residue was dispersed into ethyl and then filtered (69.2 mg, 78%), mp = 142–144°C (diisopropylether). $^1\text{H-NMR}$ (CDCl_3): 0.8–1.5 (m, 13H), 1.7–2.2 (m, 4H), 2.3–2.6 (m, 3H), 3.5–4.5 (m, 3H), 6.8–7.7 (m, 7H).

The **3l** basis was obtained by neutralization of the hydrochloride salt formed above in an aqueous solution of sodium bicarbonate. After extraction of the aqueous phase with ethyl ether, then drying and evaporation of the organic phase, the amine **3l** was obtained as an oil with a quantitative yield.

(\pm)-*N*₂-(2,4-Difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylurea **2l**
2,4-Difluorophenylisocyanate (15.2 g, 0.098 mol) was added dropwise to a solution of 36.2 g (0.102 mol) of (\pm)-*N*-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylamine **3l** in 730 ml hexane under a dry nitrogen atmosphere. The mixture was then further stirred for 1 h and the precipitate was filtered and dried (40 g, 79%), mp = 91–93°C (hexane). IR (KBr): 3460, 1670 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3): 0.7–1.7 (m, 13H), 1.7–2.5 (m, 4H), 3–3.6 (m, 2H), 3.8–4.7 (m, 3H), 5–5.2 (m, 1H), 6.2–7.7 (m, 9H), 7.7–8.6 (m, 1H). Analysis: $\text{C}_{30}\text{H}_{33}\text{F}_3\text{N}_2\text{O}_2$ (C, H, F, N).

(+)-*N*₂-(2,4-Difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylurea **2x**
To a solution of 18.8 g (0.051 mol) of (\pm)-*N*-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylamine **3l** in 150 ml ethanol was added a solution of 7.76 g (0.051 mol) of (+) mandelic acid in 50 ml ethanol. After 1 h stirring at room temperature, the precipitate (6.7 g, 26.7%) was filtered, mp = 149–151°C, $[\alpha]_{\text{D}}^{20} = +39^\circ$ (C = 2.5- CHCl_3).

Neutralization of the mandelate salt gave the dextrogyre enantiomer **3x** of **3l**. The enantiomeric purity of **3x** was measured from the HPLC chromatogram of the urea formed by condensation with *S*(-)- α -methylbenzylisocyanate (RT = 15.2 min; ee = 95.4%).

(+)-*N*₂-(2,4-Difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylurea **2x** was prepared by reaction with 2,4-difluorophenylisocyanate according to the method described for compound **2l** (56%), mp = 85–92°C, $[\alpha]_{\text{D}}^{20} = +39^\circ$ (C = 2.5- CHCl_3). Analysis: $\text{C}_{30}\text{H}_{33}\text{F}_3\text{N}_2\text{O}_2$ (C, H, F, N).

(-)-*N*₂-(2,4-Difluorophenyl)-*N*₁-8-(4-fluorophenyl)-2,3,4,5-tetrahydro-1-benzoxepin-5-yl-*N*₁-*n*-heptylurea **2y**

The levogyre amine **3y** was obtained in the same way as **3x** by replacing (+)mandelic acid by (-)mandelic acid with an enantiomeric excess of more than 96%. The urea **2y** was obtained with a yield of 50% according to the operating sequence used to prepare the urea **2x**, mp = 72–86°C, $[\alpha]_{\text{D}}^{20} = -37.4^\circ$ (C = 2.5- CHCl_3). Analysis: $\text{C}_{30}\text{H}_{33}\text{F}_3\text{N}_2\text{O}_2$ (C, H, F, N).

Biology

Measurement of rabbit aortic microsomal ACAT

Male New-Zealand rabbits weighing from 2 to 2.5 kg (Elevage Scientifique des Dombes, Châtillon-sur-Chalaronne) were fed for 2 weeks on a 1.25% cholesterol-enriched diet to activate

ACAT *in vivo*. After sacrifice, 20 ml blood was withdrawn from the ear vein and the thoracic aorta was removed. After coagulation, the plasma was isolated by centrifugation (30 min at 2000 g). The plasma was incubated for 30 min at 60°C to suppress the LCAT activity. After cooling, the plasma cholesterol level was measured enzymatically using cholesterol oxidase.

The aortic segment was opened longitudinally, rinsed with iced physiological salt solution (NaCl 154 mM +4°C), and then dissected to eliminate the adventice. The remaining tissue was homogenized in 5 ml of the following ice-cold buffer: Tris HCl 100 mM, saccharose 0.25%, KCl 150 mM, EDTA 2 mM, dithiothreitol 2 mM, adjusted to pH = 7.4 with 1 N HCl (buffer A). The homogenate was centrifuged for 10 min at 18 000 g and +4°C. The supernatant was centrifuged for 2 h at 150 000 g and +4°C. The microsome pellet was then taken up with 200 μl buffer A and kept in liquid nitrogen until use. An aliquot was used to determine the protein level by the Lowry method [26].

ACAT was measured according to Gillies and coworkers [27]. The microsomes were first activated by a 1 h incubation at 37°C in the presence of deactivated plasma (15–20 μg of microsomal proteins for 20 μg plasma cholesterol). The compound to be tested was then added (variable concentrations, adapted solvent and corresponding controls). Two minutes later, the enzymatic reaction was initiated by addition of 30 μM of ^{14}C -oleyl-CoA (1.96 GBq/mmol) and incubated for 90 min at 37°C.

The reaction was stopped by addition of Folch solvent [28]. The organic phase containing ^{14}C -labelled lipids was collected. ^{14}C -Oleoyl cholesterol was separated by TLC (silica gel G25-Merck) using diethylether/petroleum ether/acetic acid (10:90:1 v/v).

The radioactivity of the samples was measured by liquid scintillation (Dynagel 10 ml, on Packard counter 1900 CA). Each measurement was carried out four times at each concentration.

The final enzymatic activity was expressed in picomoles of ^{14}C -oleyl-cholesterol formed per minute and per milligram of microsomal proteins (pmol/min/mg).

Effect on the absorption of ^3H -labelled cholesterol in the normolipidemic rat

Male Wistar rats weighing from 200 to 220 g were randomized into groups of six (1 cage per group). Following an overnight fasting, each animal was treated orally with a bolus of ^3H -labelled cholesterol (1 α , 2 α - ^3H -cholesterol, 750 kBq/kg) dissolved in a 10% aqueous solution of bovine bile (Sigma B3883). Three hours later, 1 ml blood was withdrawn on heparine from each animal, through the retroorbital sinus and under ether anaesthesia. Plasma was then isolated by centrifugation (30 min at 2000 g). The plasmatic ^3H -radioactivity was measured on a 100 μl sample by liquid scintillation (Dynagel 10 ml, counter Packard 1900 CA). This value (dpm/ml) was used as a measure of the basic intestinal ACAT activity (normolipidemic animals).

Effect on the hypercholesterolemic rat

Male Wistar rats weighing from 160 to 180 g (IFFA Credo, Les Oncins, Saint-Germain-sur-l'Arbresle) were randomized into groups of six (1 cage per group). They were fed a 2.5% cholesterol-enriched diet for 8 d without restriction in order to increase their blood cholesterol level and saturate their intestinal ACAT activity. On the last two days of this diet period, each animal was orally treated with the compound to be tested, 24 and 4 h before the sacrifice by total exsanguination (abdominal aorta puncture under ether anaesthesia). After coagu-

lation, the blood was centrifuged for 30 min at 2000 g and the supernatant was withdrawn to assay the plasmatic cholesterol level by cholesterol-oxidase. Hypercholesterolemia was used as a measure of diet-induced intestinal ACAT activity.

Statistics

Our biological data were considered *a priori* as random variables. The comparison between groups was performed using Student-Fischer's *t*-tests and Mann and Whitney's *U*-test [29]. The active compound were taken up with several doses (or concentrations). An effective dose (or concentration) was calculated with 95% confidence limits and variance analysis to validate the regression models [29].

References

- 1 Suckling KE, Stange EF (1985) *J Lipid Res* 26, 647–671
- 2 Hashimoto S, Dayton S (1977) *Atherosclerosis* 28, 447–452
- 3 Bell F, Schaub RG (1986) *Arteriosclerosis* 6, 42–49
- 4 Del Boccio G, Lapenna D, Porreca E, Pennelli A, Savini F (1990) *Atherosclerosis* 81, 127–135
- 5 Ross R (1986) *New Eng J Med* 314, 488–500
- 6 Mc Grath LT, Elliott RJ (1990) *Anal Biochem* 187, 273–276
- 7 Roessner A, Herrera A, Höning HJ, Vollmere, Zwadlo G (1987) *Virchow's Archiv A* 412, 169–174
- 8 Heider JG, Pickens CE, Kelly LA (1983) *J Lipid Res* 24, 1127–1134
- 9 Helgerud P, Soarem K, Morum KR (1981) *J Lipid Res* 22, 271–277
- 10 De Vries VG, Bloom DJ, Dutia MD, Katocs AS, Largis EE (1989) *J Med Chem* 32, 2318–2325
- 11 De Vries VG, Schaffer SA, Largis EE *et al* (1986) *J Med Chem* 29, 1133–1134
- 12 Fontaine G, Maitte P (1964) *C R Acad Sci* 4583–4585
- 13 Horning EC, Horning MG (1947) *J Am Chem Soc* 69, 1359–1361
- 14 Dewar MJS, Marchand AP (1966) *J Am Chem Soc* 88, 3318–3327
- 15 Kumazawa T, Yanase M, Harakawa H *et al* (1994) *J Med Chem* 37, 804–810
- 16 Kelm J, Strauss K (1981) *Spectrochim Acta Part A* 689–692
- 17 Porowska N, Polaczko W (1970) *Rocz Chem* 44, 375–382
- 18 Kobayashi S, Ishibashi S, Tsuru S *et al* (1990) *Jpn Kokai Tokyo Koho* 02229131; *Chem Abstr* 114, 121751
- 19 Ames GR, Davey W (1957) *J Chem Soc* 3480–3487
- 20 Protiva M, Seidlova V, Svatek E, Hradil F (1972) *Collection of Czechoslovak Chemical Communications* 37, 868–886
- 21 Powell SG, Anderson L (1931) *J Am Chem Soc* 811
- 22 Freedman J, Steward KT (1989) *J Heterocyclic Chem* 26, 1547–1554
- 23 Thuillier G, Laforest J, Bessin P (1975) *Ger Offen* 2436075; *Chem Abstr* 83, 9834
- 24 Badilescu I (1975) *Rev Roum Chim* 20, 761–774
- 25 Cagniant P, Deluzarche A (1946) *C R Acad Sci* 223, 677–679
- 26 Lowry OH, Randall RJ, Rosenbrough NG, Farral L (1951) *J Biol Chem* 193, 265–275
- 27 Gillies PJ, Rathbert KA, Perri MA, Robinson CS (1986) *Exp Mol Pathol* 44, 329–339
- 28 Folch J, Lees M, Sloane-Stanley (1957) *J Biol Chem* 226, 497–509
- 29 Delaunois AL (1973) *In: Biostatistics in Pharmacology* Pergamon Press, UK