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New indole derivatives as ACAT inhibitors: synthesis and structure–activity relationships

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Summary — A series of ureas containing the indole group were synthesized and assessed for their ability to inhibit arterial and intestinal ACAT and to lower plasma total cholesterol in a cholesterol-fed rat model. The structural modulations carried out in this series led to compounds which proved to be very active in both the inhibition of aortic ACAT in vitro and the inhibition of rat cholesterol intestinal absorption in vivo. Several compounds from this series exhibit a remarkable hypocholesterolaemic effect with ED_{25} less than 0.1 mg/kg po.

indole derivative / aortic ACAT inhibition / intestinal ACAT inhibition / hypocholesterolaemic effect

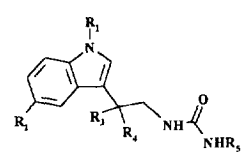
Introduction

Since Framingham's study [1], hypercholesterolaemia has been known as an independent risk factor in the development of atherosclerosis. Many clinical studies have demonstrated that lowering plasma cholesterol (total cholesterol and LDL cholesterol) reduces cardiovascular morbidity and mortality [2–4].

Acyl-CoA cholesterol O-acyl transferase (ACAT, EC 2.3.1.26) is the enzyme responsible for cholesterol intracellular esterification [5]. The essential function of this enzyme was shown in cholesterol intestinal absorption and thus in very low density lipoprotein (VLDL) packaging and lipid secretion in the lymphatic system [5–8]. ACAT is also present in the liver, where it is responsible for the formation of cholesterol esters prior to their inclusion in VLDLs, which themselves precede low density lipoproteins (LDL) [9]. ACAT is also involved in macrophage-induced cholesterol ester accumulation in the artery, and thus contributes to the development of atherosclerotic lesions [10]. Many studies have demonstrated that feeding animals with a cholesterol-enriched diet causes lesions in the arterial wall, especially in rabbits and monkeys [11–14]. These lesions are characterized by macrophage accumulation when ACAT activity is significantly enhanced.

Because of these three levels of action, ACAT inhibition has become a target in the development of new hypolipidaemic and anti-atherosclerotic agents. The various classes of currently known ACAT inhibitors have been summarized in two recent reviews [15, 16].

In the present article, we will describe the preparation, biological properties and structure–activity relationships of a new series of formula **1** ureas derived from indole. Some of the compounds prepared proved to be very active in the in vitro inhibition of ACAT and the in vivo inhibition of cholesterol intestinal absorption. They were characterized by a remarkable hypocholesterolaemic effect by oral route in the rat with extremely low effective doses 25 (ED_{25}), ie, sometimes less than 0.1 mg/kg.



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Chemistry

Compounds **1** (table I) were prepared by reaction of amines **2** with the suitable isocyanate or the corresponding trichloroacetanilide, or by treatment of the corresponding morpholinomethyl-1-substituted compounds **1** with gaseous hydrochloric acid in solution in ethanol (scheme 1). Amines **2** (table II) were pre-

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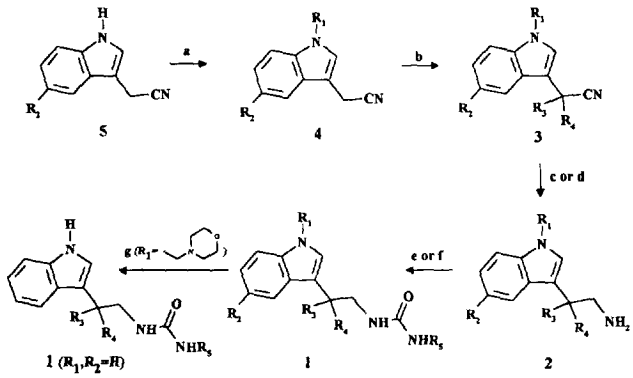
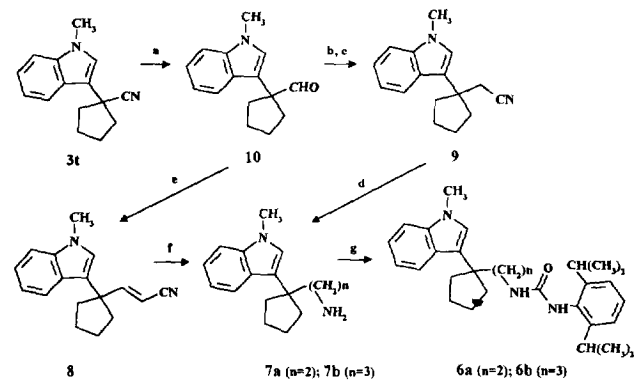
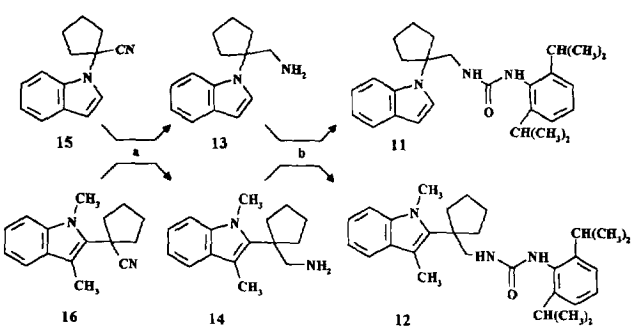


Scheme 1. (a) R_1X/base/phase-transfer reagent; (b) R_3X/NaH or R_3X/NaH then R_4X/NaH or $X\text{-}(CH_2)_n\text{-}X$/NaH; (c) $LiAlH_4$/ether/Δ; (d) H_2/Ni/NH_3; (e) R_5NCO; (f) $R_5NH\text{-}COCCl_3/K_2CO_3$/DMF/Δ; (g) HCl/$C_2H_5OH$/Δ.



Scheme 2. (a) Diisobutylaluminium hydride; (b) 2,4,6-triisopropylbenzenesulfonylhydrazide; (c) KCN/Δ; (d) $LiAlH_4$/Δ; (e) $(C_2H_5O)_2P(O)CH_2CN$/NaH; (f) H_2/Ni/NH_3; (g) 2,6-diisopropylphenylisocyanate.



Scheme 3. (a) H_2/Ni/NH_3; (b) 2,6-diisopropylphenylisocyanate/THF.

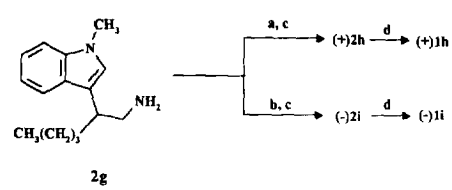
pared by reduction with lithium and aluminium hydride or by catalytic hydrogenation of nitriles **3** (table III), the latter being obtained by the mono- or dialkylation of nitriles **4** (table III). Nitriles **4** were prepared by *N*-alkylation of the corresponding indol-3-ylacetonitriles **5** with the suitable halide in the presence of a base and a phase-transfer reagent. Indol-3-ylacetonitrile was prepared classically from the gramine [17] and the 5-substituted indol-3-ylacetonitriles **5** ($R_2 \neq H$) were prepared by Fischer synthesis from the corresponding *para*-substituted phenylhydrazine chlorhydrate and from 4,4-diethoxybutyronitrile [18–20].

Compound **1t** ($R_1 = R_2 = H$, $R_3\text{-}R_4 = \text{-}(CH_2)_4\text{-}$, R_5 = 2,6-diisopropylphenyl) proved to be one of the most active compounds of the series. The homologues **6a** and **6b** (scheme 2, table I) were therefore prepared to determine the influence of the lateral chain lengthening on the level of activity of this compound.

Amine **7a**, which leads to compound **6a**, was prepared by reduction of nitrile **9** with lithium and aluminium hydride. The nitrile **9** was obtained by successive reaction of aldehyde **10** with 2,4,6-triisopropylbenzenesulfonylhydrazide and then potassium cyanide. Amine **7b**, which leads to compound **6b**, was prepared by catalytic hydrogenation of nitrile **8**. Compound **8** which was obtained by Wittig–Horner reaction of aldehyde **10**, which was prepared by reduction of nitrile **3t** with diisobutylaluminium hydride.

The influence exerted by the position of substitution in the lateral chain on the biological activity of compound **1t** was also assessed by preparing the analogous compounds **11** and **12**, which were substituted in the 1 and 2 positions of the indole respectively (table I). These two compounds were obtained (scheme 3) from the corresponding indol-1- [21] and indol-2-ylacetonitriles [22] via the amines **13** and **14** (table II) and nitriles **15** and **16** (table III).

The butyl compound **1g** ($R_1 = CH_3$, $R_2 = R_4 = H$, $R_3 = n\text{-}C_4H_9$, R_6 = 2,6-diisopropylphenyl) also proved to be very active, and so it was interesting to compare the activity of the corresponding enantiomers **1h** and **1i**. These were prepared from the enantiomeric amines **2h** and **2i** obtained by resolution of the racemic amine **2g** with the (+)- and (–)-mandelic acids (scheme 4).



Scheme 4. (a) (+)-Mandelic acid; (b) (–)-mandelic acid; (c) NaOH/H_2O/ether; (d) 2,6-diisopropylphenylisocyanate.

Table I. Physical data for ureas **1**, **6**, **11** and **12**.

Compound	R_1	R_2	R_3	R_4	R_5	*Yield (%)*	*Mp (°C)*	*Crystallization solvent*[a]	*Formula*[b]
1a	H	H	H	H	$CH_3(CH_2)_2$	82	96–99	B	$C_{14}H_{19}N_3O$
1b	H	H	H	H	4-$MeOC_6H_4$	44	200–204	A	$C_{18}H_{19}N_3O_2$
1c	H	H	H	H	2,6-(*i*-Pr)$_2C_6H_3$	86	160–162	I	$C_{23}H_{29}N_3O$
1d	CH_3	H	CH_3	H	2,6-(*i*-Pr)$_2C_6H_3$	70	207–209	C	$C_{25}H_{33}N_3O$
1e	CH_3	H	C_2H_5	H	2,6-(*i*-Pr)$_2C_6H_3$	68	159–161	C	$C_{26}H_{35}N3O$
1f	CH_3	H	*n*-C_3H_7	H	2,6-(*i*-Pr)$_2C_6H_3$	78	133–135	C	$C_{27}H_{37}N_3O$
1g	CH_3	H	*n*-C_4H_9	H	2,6-(*i*-Pr)$_2C_6H_3$	35	143–145	C	$C_{28}H_{39}N_3O$
1h	CH_3	H	*n*-C_4H_9	H	2,6-(*i*-Pr)$_2C_6H_3$	88	138–142	D	$C_{28}H_{39}N_3O$
1i	CH_3	H	*n*-C_4H_9	H	2,6-(*i*-Pr)$_2C_6H_3$	87	138–142	D	$C_{28}H_{39}N_3O$
1j	H	H	*n*-C_4H_9	H	2,6-(*i*-Pr)$_2C_6H_3$	52	128–132	E	$C_{27}H_{37}N_3O$
1k	CH_3	H	*n*-C_7H_{15}	H	2,6-(*i*-Pr)$_2C_6H_3$	41	120–122	B	$C_{31}H_{45}N_3O$
1l	CH_3	H	*i*-Pr	H	2,6-(*i*-Pr)$_2C_6H_3$	57	156–157	C	$C_{27}H_{37}N_3O$
1m	CH_3	H	c-C_5H_9	H	2,6-(*i*-Pr)$_2C_6H_3$	79	169–171	C	$C_{29}H_{39}N_3O$
1n	CH_3	H	allyl	H	2,6-(*i*-Pr)$_2C_6H_3$	87	148–150	B	$C_{27}H_{35}N_3O$
1o	CH_3	H	$(CH_3)_2N(CH_2)_2$	H	2,6-(*i*-Pr)$_2C_6H_3$	78	172–173	C	$C_{28}H_{40}N_4O$
1p	CH_3	H	CH_3	CH_3	2,6-(*i*-Pr)$_2C_6H_3$	94	172–174	C	$C_{26}H_{35}N_3O$
1q	CH_3	H	C_2H_5	C_2H_5	2,6-(*i*-Pr)$_2C_6H_3$	58	183–184	C	$C_{28}H_{39}N_3O$
1r	CH_3	H	*n*-Bu	*n*-Bu	2,6-(*i*-Pr)$_2C_6H_3$	14	93–96	B	$C_{32}H_{47}N_3O$
1s	CH_3	H	Allyl	Allyl	2,6-(*i*-Pr)$_2C_6H_3$	50	121–123	G	$C_{30}H_{39}N_3O$
1t	CH_3	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	46	193–195	F	$C_{28}H_{37}N_3O$
1u	CH_3	H	-$(CH_2)_5$-		2,6-(*i*-Pr)$_2C_6H_3$	85	187–189	F	$C_{29}H_{39}N_3O$
1v	CH_3	H	-$(CH_2)_6$-		2,6-(*i*-Pr)$_2C_6H_3$	41	182–184	C	$C_{30}H_{41}N_3O$
1w	CH_3	H	-$(CH_2)_2O(CH_2)_2$-		2,6-(*i*-Pr)$_2C_6H_3$	53	197–201	C	$C_{28}H_{37}N_3O_2$
1x	CH_3	H	-$(CH_2)_4$-		C_6H_5	80	214–216	A	$C_{22}H_{25}N_3O$
1y	CH_3	H	-$(CH_2)_4$-		2,4-$F_2C_6H_3$	69	226–228	C	$C_{22}H_{23}F_2N_3O$
1z	CH_3	H	-$(CH_2)_4$-		2,6-(*i*-PrO)$_2C_6H_3$	64	145–147	C	$C_{28}H_{37}N_3O_3$
1aa	CH_3	H	-$(CH_2)_4$-		2,5-(t-Bu)$_2C_6H_3$	38	184–186	C	$C_{30}H_{41}N_3O$
1ab	CH_3	H	-$(CH_2)_4$-		2,4-(*i*-Pr)$_2C_6H_3$	63	205–208	A	$C_{28}H_{37}N_3O$
1ac	CH_3	H	-$(CH_2)_4$-		2-Pr-6-*i*-PrC_6H_3	54	180–183	A	$C_{28}H_{37}N_3O$
1ad	CH_3	H	-$(CH_2)_4$-		2,6-$Cl_2C_6H_3$	50	199–201	I	$C_{22}H_{23}Cl_2N_3O$
1ae	CH_3	H	-$(CH_2)_4$-		2,6-$(C_2H_5)_2C_6H_3$	83	171–173	C	$C_{26}H_{33}N_3O$
1af	CH_3	H	-$(CH_2)_4$-		2,4,6-$(MeO)_3C_6H_2$	79	176–178	C	$C_{25}H_{31}N_3O_4$
1ag	H	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	28	95–107	G	$C_{27}H_{35}N_3O$
1ah	C_2H_5	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	80	166–168	D	$C_{29}H_{39}N_3O$
1ai	*n*-C_4H_9	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	31	128–130	B	$C_{31}H_{43}N_3O$
1aj	*n*-C_7H_{15}	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	73	74–80	G	$C_{34}H_{49}N_3O$
1ak	*i*-Pr	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	82	168–170	F	$C_{30}H_{41}N_3O$
1al	allyl	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	21	131–133	J	$C_{30}H_{39}N_3O$
1am	$(CH_3)_2NCH_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	62	203–205[c]	A	$C_{30}H_{43}ClN_4O$[d]
1an	$(CH_3)_2N(CH_2)_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	84	228–230[c]	K	$C_{31}H_{45}ClN_4O$[d]
1ao	$(CH_3)_2N(CH_2)_3$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	44	122–130[c]	F	$C_{32}H_{47}ClN_4O$[d]
1ap	CH_3OCH_2	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	71	135–137	C	$C_{29}H_{39}N_3O_2$
1aq	$C_6H_5CH_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	39	83–86	A	$C_{34}H_{41}N_3O$
1ar	4F-$C_6H_4CH_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	52	68–74	A	$C_{34}H_{40}FN_3O$
1as	$C_6H_5CH_2$	H	-$(CH_2)_5$-		2,6-(*i*-Pr)$_2C_6H_3$	82	151–153	C	$C_{35}H_{43}N_3O$
1at	2-Morpholino$(CH_2)_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	83	241–243[c]	A	$C_{33}H_{47}ClN_4O_2$[d]
1au	2-Pyrrolidino$(CH_2)_2$	H	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	77	216–221[c]	K	$C_{33}H_{47}ClN_4O$[d]
1av	CH_3	CH_3	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	58	188–190	G	$C_{29}H_{39}N_3O$
1aw	CH_3	CH_3O	-$(CH_2)_4$-		2,6-(*i*-Pr))$_2C_6H_3$	57	157–160	C	$C_{29}H_{39}N_3O_2$
1ax	CH_3	Br	-$(CH_2)_4$-		2,6-(*i*-Pr)$_2C_6H_3$	66	186–191	C	$C_{28}H_{36}BrN_3O$
6a						25	239–241	F	$C_{29}H_{39}N_3O$
6b						75	194–196	A	$C_{30}H_{41}N_3O$
11						63	188–190	F	$C_{27}H_{35}N_3O$
12						45	206–208	G	$C_{29}H_{39}N_3O$

[a]A = ethanol; B = *i*-Pr_2O; C = $CH_3CO_2C_2H_5$; D = $CH_3CO_2C_2H_5$/*i*-Pr_2O; E = *i*-Pr_2O/hexane; F = CH_3COCH_3/*i*-Pr_2O; G = hexane; H = *i*-PrOH/*i*-Pr_2O; I = CH_3COCH_3; J = pentane; K = $CH_3CN/CH_3CO_2C_2H_5$; [b]C, H, N were analyzed for all the compounds as well as Cl, Br, F when present; the values obtained are at ± 0.4% of the theoretical values; [c]mp of the HCl salt; [d]analyzed as the

Table II. Physical data for amines **2**, **7**, **13** and **14**.

Compound	R_1	R_2	R_3	R_4	*Yield*[a] *(%)*	*Mp (°C)*	*Crystallization solvent*
2d	CH_3	H	CH_3	H	63	Oil	–
2e	CH_3	H	C_2H_5	H	72	Oil	–
2f	CH_3	H	n-C_3H_7	H	77	Oil	–
2g	CH_3	H	n-C_4H_9	H	58	Oil	–
2h	CH_3	H	n-C_4H_9	H	29[b]	Oil	–
2i	CH_3	H	n-C_4H_9	H	15[b]	Oil	–
2j	H	H	n-C_4H_9	H	92	Oil	–
2k	CH_3	H	n-C_7H_{15}	H	64	Oil	–
2l	CH_3	H	i-Pr	H	74	Oil	–
2m	CH_3	H	c-C_5H_9	H	76	Oil	–
2n	CH_3	H	allyl	H	92	Oil	–
2o	CH_3	H	$(CH_3)_2N(CH_2)_2$	H	57	Oil	–
2p	CH_3	H	CH_3	CH_3	76	Oil	–
2q	CH_3	H	C_2H_5	C_2H_5	83	Oil	–
2r	CH_3	H	n-C_4H_9	n-C_4H_9	75	Oil	–
2s	CH_3	H	Allyl	Allyl	75	Oil	–
2t	CH_3	H	-$(CH_2)_4$-		88	Oil	–
2u	CH_3	H	-$(CH_2)_5$-		53	Oil	–
2v	CH_3	H	-$(CH_2)_6$-		60	87–90	Hexane
2w	CH_3	H	-$(CH_2)_2O(CH_2)_2$-	57	Oil	–	
2ah	C_2H_5	H	-$(CH_2)_4$-		82	Oil	–
2ai	n-C_4H_9	H	-$(CH_2)_4$-		86	Oil	–
2aj	n-C_7H_{15}	H	-$(CH_2)_4$-		85	Oil	–
2ak	i-Pr	H	-$(CH_2)_4$-		66	Oil	–
2al	allyl	H	-$(CH_2)_4$-		78	Oil	–
2am	$(CH_3)_2NCH_2$	H	-$(CH_2)_4$-		62	Oil	–
2an	$(CH_3)_2N(CH_2)_2$	H	-$(CH_2)_4$-		77	Oil	–
2ao	$(CH_3)_2N(CH_2)_3$	H	-$(CH_2)_4$-		96	Oil	–
2ap	CH_3OCH_2	H	-$(CH_2)_4$-		95	Oil	–
2aq	$C_6H_5CH_2$	H	-$(CH_2)_4$-		52	Oil	–
2ar	4F-$C_6H_4CH_2$	H	-$(CH_2)_4$-		97	Oil	–
2as	$C_6H_5CH_2$	H	-$(CH_2)_5$-		67	Oil	–
2at	2-morpholino$(CH_2)_2$	H	-$(CH_2)_4$-		76	Oil	–
2au	2-pyrrolidino$(CH_2)_2$	H	-$(CH_2)_4$-		83	Oil	–
2av	CH_3	CH_3	-$(CH_2)_4$-		63	Oil	–
2aw	CH_3	CH_3O	-$(CH_2)_4$-		70	Oil	–
2ax	CH_3	Br	-$(CH_2)_4$-		70	Oil	–
7a					74	Oil	–
7b					83	Oil	–
13					81	Oil	–
14					57	Oil	–

[a]Calculated after purification by dissolution in pentane followed by filtration then evaporation; [b]calculated from the corresponding racemic amine.

Biology

The arterial and intestinal ACAT inhibiting effect of compound **1** was assessed in three pharmacological tests. One was representative of the arterial ACAT activity, while the others represented the intestinal ACAT activity. To assess the arterial impact, the in vitro 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 was monitored in the normolipidaemic animal (basic intestinal ACAT); and 2) hypercholesterolemic animals were fed a cholesterol-enriched diet (induced intestinal ACAT).

Results and discussion

Table IV shows the pharmacological results obtained with compounds **1** in vitro on rabbit aortic ACAT

inhibition and in vivo (po) on rat ^{3}H-cholesterol absorption and on cholesterolaemia in diet-induced hyperlipidemic rats. The lack of activity observed with compounds **1a**, **1b** and **1x** confirmed the conclusions from previous work [23, 24], ie, the radical R of the -CONHR pharmacophore group must meet two conditions to exhibit a satisfactory in vitro effect on ACAT: it must be aromatic and substituted in at least one of the *o*,*o*' positions.

In the present case, the condition is not sufficient as shown by the negative results of compound **1c**. The comparison of the results obtained with compounds **1d** to **1k** demonstrated the importance of an alkyl substituent (R_3) in α position of the indole. The compounds α-ethyl **1e**, α-propyl **1f** and α-butyl **1g** are extremely potent on in vitro ACAT inhibition and in vivo cholesterol intestinal inhibition. This action was characterized by a strong hypocholesterolaemic effect

Table III. Physical data for nitriles **3**, **4**, **8**, **9**, **15** and **16**.

Compound	R_1	R_2	R_3	R_4	*Yield (%)*	*Mp (°C)*	*Crystallization solvent*[b]
3d	CH_3	H	CH_3	H	19	61–64	A
3e	CH_3	H	C_2H_5	H	40	Oil	–
3f	CH_3	H	n-C_3H_7	H	26	43–46	B
3g	CH_3	H	n-C_4H_9	H	41	63–66	B
3j	H	H	n-C_4H_9	H	47	Oil	–
3k	CH_3	H	n-C_7H_{15}	H	28	43–45	B
3l	CH_3	H	i-Pr	H	57	80–81	C
3m	CH_3	H	c-C_5H_9	H	37	76–78	C
3n	CH_3	H	Allyl	H	23	60–62	B
3o	CH_3	H	$(CH_3)_2N(CH_2)_2$	H	32	Oil	–
3p	CH_3	H	CH_3	CH_3	51	44–46	B
3q	CH_3	H	C_2H_5	C_2H_5	60	58–60	C
3r	CH_3	H	n-C_4H_9	n-C_4H_9	26	73–75	D
3s	CH_3	H	Allyl	Allyl	30	76–78	A
3t	CH_3	H	-$(CH_2)_4$-		54	118–120	C
3u	CH_3	H	-$(CH_2)_5$-		58	128–130	A
3v	CH_3	H	-$(CH_2)_6$-		10	99–101	C
3w	CH_3	H	-$(CH_2)_2O(CH_2)_2$-	62	178–180	A	
3ah	C_2H_5	H	-$(CH_2)_4$-		46	76–78	C
3ai	n-C_4H_9	H	-$(CH_2)_4$-		57	Oil	–
3aj	n-C_7H_{15}	H	-$(CH_2)_4$-		20	Oil	–
3ak	i-Pr	H	-$(CH_2)_4$-		50	69–71	C
3al	Allyl	H	-$(CH_2)_4$-		38	56–58	C
3am	$(CH_3)_2NCH_2$	H	-$(CH_2)_4$-		48	111–113	E
3an	$(CH_3)_2N(CH_2)_2$	H	-$(CH_2)_4$-		46	56–58	C
3ao	$(CH_3)_2N(CH_2)_3$	H	-$(CH_2)_4$-		45	Liquid[a]	–
3ap	CH_3OCH_2	H	-$(CH_2)_4$-		77	46–48	B
3aq	$C_6H_5CH_2$	H	-$(CH_2)_4$-		87	144–146	A
3ar	4F-$C_6H_4CH_2$	H	-$(CH_2)_4$-		56	80–82	A
3as	$C_6H_5CH_2$	H	-$(CH_2)_5$-		44	139–141	A
3at	2-Morpholino$(CH_2)_2$	H	-$(CH_2)_4$-		58	110–112	B
3au	2-Pyrrolidino$(CH_2)_2$	H	-$(CH_2)_4$-		46	73–75	B
3av	CH_3	CH_3	-$(CH_2)_4$-		94	Oil	–
3aw	CH_3	CH_3O	-$(CH_2)_4$-		53	100–101	F
3ax	CH_3	Br	-$(CH_2)_4$-		50	149–151	B
4a	CH_3	H	H	H	48	59–60	C
4b	CH_3	CH_3	H	H	90	Oil	–
4c	CH_3	CH_3O	H	H	51	104–106	F
4d	CH_3	Br	H	H	50	118–120	B
8					73	57-59	B
9					25	Oil	–
15					77	Oil	–
16					60	135–137	B

[a]$bp_{0.4}$ = 175–180°C. A = i-Pr_2O; B = hexane; C = pentane; D = heptane; E = ether; F = $CH_3CO_2C_2H_5$.

Table IV. In vitro/in vivo activities on ACAT and in vivo antihypercholesterolaemic effect of compounds **1**, **6**, **11** and **12**.

Compound	*Inhibition of rabbit aortic ACAT, IC_{50} (nM)*	*Inhibition of ^{3}H-cholesterol intestinal absorption ED_{50} (μg/kg) po*	*Antihypercholesterolaemic effect in rat, ED_{25} (mg/kg) po*
1a	Inactive at 1000	Inactive at 1000	Inactive at 3
1b	Inactive at 1000	Inactive at 1000	Inactive at 3
1c	Inactive at 1000	Inactive at 1000	Inactive at 5
1d	~ 750	Inactive at 1000	Inactive at 5
1e	68	94	0.58
1f	~ 50	167	0.51
1g	~ 380	83	0.63
1h	~ 450	90	1.94
1i	25.2	77	0.086
1j	~ 120	147	0.42
1k	~ 350	Inactive at 1000	Inactive at 3
1l	~ 110	198	1.41
1m	~ 330	290	Inactive at 3
1n	~ 400	380	0.142
1o	Inactive at 1000	Inactive at 1000	Inactive at 3
1p	~ 250	Inactive at 1000	~ 2.9
1q	~ 100	800	~ 2.8
1r	~ 750	Inactive at 1000	~ 1.8
1s	68	420	Inactive at 3
1t	46	176	0.098
1u	340	60	0.063
1v	~ 340	590	1.66
1w	~ 370	160	0.680
1x	Inactive at 1000	Inactive at 5000	Inactive at 5
1y	Inactive at 1000	Inactive at 5000	Inactive at 5
1z	~ 370	Inactive at 1000	Inactive at 3
1aa	Inactive at 1000	Inactive at 1000	Inactive at 3
1ab	Inactive at 1000	Inactive at 1000	Inactive at 3
1ac	99	~ 1100	Inactive at 3
1ad	Inactive at 1000	Inactive at 3000	Inactive at 3
1ae	~ 350	~ 150	1.6
1af	Inactive at 1000	Inactive at 1000	Inactive at 3
1ag	~ 170	~ 180	~ 1
1ah	99	101	0.53
1ai	13.5	208	1.43
1aj	~ 440	Inactive at 1000	~ 2.9
1ak	~ 710	490	1
1al	13	93	0.57
1am	71	280	0.6
1an	22	168	0.097
1ao	38	510	0.65
1ap	42	250	0.62
1aq	32	82	0.37
1ar	32.1	340	0.066
1as	~ 330	840	0.86
1at	~ 70	~ 800	~ 0.8
1au	Not determined	~ 800	Inactive at 3
1av	~ 110	145	1.5
1aw	~ 390	~ 700	Inactive at 3
1ax	~ 50	600	~ 1.1
6a	Inactive at 1000	Inactive at 3000	Inactive at 3
6b	Inactive at 1000	Inactive at 3000	Inactive at 3
11	~ 250	950	0.63
12	~ 370	Inactive at 3000	2.67

in rats per os with an ED_{25} value lower than 1 mg/kg for each of them. The enantiomers **1h** and **1i** of compound **1g** were both active in the three tests but the (–) isomer proved to be the most active with an ED_{25} value lower than 0.1 mg/kg.

The introduction of an unsaturation in **1n** markedly improved the hypocholesterolaemic effect with an ED_{25} = 0.14 versus 0.58 mg/kg for the corresponding saturated homologue α-propyl **1f**.

There is a limit to increasing size or lipophilicity since the lengthening to seven carbon atoms (**1k**) or the substitution by the cyclopentyl radical (**1m**) abolished the hypocholesterolaemic effect. Compound **1k** also proved to be inactive on ACAT in vivo. Unlike the observation made by Trivedi et al [25] in the phenyl series, adding a second substituent (**1p** to **1s**) was not favourable either and according to the comparison of compounds **1f** and **1l** the ramification was also characterized by a loss of activity in the three tests.

In the same way as in phenyl series [25, 26], the introduction of a geminal ring in C4 and C5 conferred a remarkably potent hypocholesterolaemic activity to the corresponding compounds **1t** and **1u** as shown by the ED_{25} values of 0.098 and 0.063 mg/kg respectively. On the other hand, adding another link (**1v**) or introducing an oxygen atom (**1w**) markedly reduced this activity and the ACAT-inhibiting effect in vitro and in vivo.

Compound **1t**, which was the most active of the series, was chosen as starting point in the study of structure–activity relationships according to the nature of the substituent R_1, R_2 and R_5. The replacement of the methyl group in position 1 by a hydrogen atom (**1ag**) markedly reduced the ACAT-inhibiting effect and hypocholesterolaemic activity of compound **1t**. The activity in the overall tests was also decreased when the carbon chain was lengthened (**1ah–1aj**) except for the in vitro ACAT-inhibiting effect of the butyl compound **1ai**. In an unexplained manner, the latter was one of the most potent in vitro ACAT inhibitors of the series (IC_{50} = 13.5 nM). This was also the case for the substituted allyl compound **1al** (IC_{50} = 13 nM).

The replacement of the methyl group in position 1 by an *N,N*-dimethylaminoethyl radical (**1an**) generally maintained the activity of the compound **1t** in the tests. On the other hand, the lower and upper homologues **1am** and **1ao** were significantly less active in vivo. The inclusion of the nitrogen atom into a pyrrolidine or morpholine ring (**1at** and **1au**) reduced the hypocholesterolaemic activity and the in vivo ACAT-inhibiting effect of the parent compound **1an**. The replacement of the methyl group in position 1 by a methoxymethyl (**1ap**) or benzyl (**1aq**) radical reduced the hypocholesterolaemic activity of compound **1t**. However, these two compounds still had a good ACAT-inhibiting effect in vitro and in vivo. The introduction of a fluorine atom in position 4 of the benzyl radical gave compound **1ar** an excellent aortic ACAT-inhibiting activity (IC_{50} = 32 nM) with a strong hypocholesterolaemic activity (ED_{25} = 0.066 mg/kg).

The few substitutions attempted in position 5 of the indole core (**1av**, **1aw** and **1ax**) were also characterized by a marked reduction of the activity. The corresponding methylated and brominated compounds were even inactive at 3 mg/kg po on rat cholesterolemia.

The R_5 variations (compounds **1y–1af**) were characterized by a marked reduction or loss of activity in the overall tests. This was especially the case of the 2,4-difluorophenyl and 2,4,6-trimethoxyphenyl substituents, although these substituents are known to confer a good level of activity in other series of ACAT inhibitors [27].

We have also studied how lengthening the supporting chain of the pharmacophore group and its substitution site could influence the ACAT-inhibiting and hypocholesterolaemic properties of compound **1t**. Lengthening the lateral chain by 1 or 2 links (**6a** and **6b**) led to compounds inactive in the three tests and changing it to position 1 or 2 was not favourable either.

Conclusion

This study investigated new indoles derivatives as ACAT inhibitors. The structural modulations carried out in this series gave compounds which proved to be very active on both the inhibition of aortic ACAT in vitro and the inhibition of cholesterol intestinal absorption in vivo with IC_{50} < 100 nM and ED_{25} < 0.1 μg/kg po. These compounds exhibited a very potent hypocholesterolaemic effect in the rat with ED_{25} < 0.1 mg/kg po. Compound **1t** and the corresponding *N,N*-dimethylaminoethyl-1-substituted derivative **1an** was chosen to be developed as a potential hypocholesterolaemic 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 wavenumbers (cm^{-1}). The ^1H NMR spectra were recorded on 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). 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 μ 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–III.

1-Methylindole-3-acetonitrile ***4a***
To a stirred mixture of 492 g (3.15 mol) of indol-3-acetonitrile, 894 g (6.3 mol) of methyl iodide and 55.7 g of a 35% methanolic solution of triton B, was added dropwise 1260 mL of a 50% aqueous solution of sodium hydroxide. The mixture was further stirred for 3 h at room temperature then extracted with diethylether. The extract was washed to neutrality, dried over Na_2SO_4, filtered and concentrated to 1500 mL. The precipitate of nitrile **4a** formed upon cooling was filtered and dried (324 g, 60%), mp = 59–60 °C. IR (KBr): 2240 cm^{-1}, ^1H NMR ($CDCl_3$): 3.65 (s, 3H), 3.70 (s, 2H), 6.9–7.6 (m, 5H).

1-(1-Methylindol-3-yl)cyclopentanecarbonitrile ***3t***
A mixture of 164 g (0.96 mol) of nitrile **4a**, 228 g (0.96 mol + 10%) of 1,4-dibromobutane, 150 mL of DMSO and 2400 mL of diethylether was added dropwise to a stirred suspension of 84.5 g (1.92 mol + 10%) of sodium hydride (60% in oil) in 1200 mL of DMSO under a dry nitrogen atmosphere. The addition was adjusted to obtain a slight reflux of the reaction medium. After the addition, the mixture was further stirred and heated for 4 h; cold water (2000 mL) was then added and the mixture was extracted with diethylether. The extract was washed with water to neutrality, dried over Na_2SO_4 and the solvent evaporated. The residue thus obtained was washed with diisopropyloxide to give nitrile **3t** as a white solid (133.2 g, 62%), mp = 118–120 °C. IR (KBr): 2223 cm^{-1}; ^1H NMR ($CDCl_3$): 1.8–2.2 (m, 4H), 2.2–2.7 (m, 4H), 3.7 (s, 3H), 6.9–7.8 (m, 5H).

1-(1-Methylindol-3-yl)cyclopentylmethylamine ***2t***
Under a nitrogen atmosphere, a solution of 101 g (0.45 mol) of nitrile **3t** in 760 mL of tetrahydrofuran was added dropwise to a suspension of 25.6 g (0.45 mol) of $LiAlH_4$ in 680 mL of diethylether so as to maintain a slight reflux of the reaction medium. The mixture was heated at reflux for 4 h, and a 20% aqueous solution of sodium hydroxide was then added. The organic phase was decanted, dried over Na_2SO_4 and the solvent was evaporated. The amine **2t** was thus isolated as an oil (70.5 g, 69%). IR (film): 3370 and 3380 cm^{-1}.

N-(2,6-Diisopropylphenyl)-N'-[1-(1-methylindol-3-yl)]cyclopentylmethylurea ***1t***
A mixture of 4.8 g (0.02 mol + 5%) of the amine **2t**, 6.45 g (0.02 mol) of 2,2,2-trichloro-*N*-(2,6-diisopropylphenyl)acetamide and 8.3 g (0.06 mol) of K_2CO_3 in 30 mL of DMF was heated at 110 °C for 30 min. Water (200 mL) was then added and the precipitate formed was filtered, washed with water and dried (6.8 g, 79%), mp = 198–199 °C ($CH_3CO_2C_2H_5$). IR (Kbr): 1638 cm^{-1}; ^1H NMR ($CDCl_3$): 0.9 (d, 12H), 1.5–2 (m, 8H), 3 (hept, 2H), 3.4 (qs, 2H), 3.4 (s, 3H), 3.8 (s, 1H), 5.8 (qs, 1H), 6.2 (s, 1H), 6.8–7.6 (m, 7H). Analysis: $C_{28}H_{37}N_3O$ (C, H, N).

(±)-α-Butyl-1-methylindole-3-acetonitrile ***3g***
To a suspension of 12 g (0.3 mol) of sodium hydride (60% in oil) in 200 mL of DMF was added a solution of 51.1 g (0.3 mol) of 1-methylindol-3-acetonitrile **4a** in 200 mL of DMF. During the addition, the temperature of the medium was kept at 25 °C by ice cooling. The mixture was further stirred for 3 h, a solution of 41.1 g (0.3 mol) of 1-bromobutane in 400 mL of DMF was then added dropwise while ice cooling. The addition was adjusted to maintain the temperature of the medium between 25 and 35 °C. The mixture was further stirred for 4 h then cooled to 10 °C. Water was added until the precipitation was complete; the precipitate was filtered, washed with water and dried. The nitrile **3g** was isolated as a white solid by chromatography on silica gel (hexane/CH_2Cl_2: 5:1; 44.3 g, 65%), mp = 63–66 °C (hexane). IR (KBr): 2235 cm^{-1}; ^1H NMR ($CDCl_3$): 0.7–2.3 (m, 9H), 3.7 (s, 3H), 4 (t, 1H), 7–7.8 (m, 5H).

(±)-2-(1-Methylindol-3-yl)hexylamine ***2g***
A solution of 44.2 g (0.195 mol) of nitrile **3g** in 300 mL of diethylether was added dropwise to a suspension of 11.4 g (0.3 mol) of $LiAlH_4$ in 1000 mL of diethylether under dry nitrogen atmosphere. The addition was adjusted to obtain a slight reflux of the reaction medium. The mixture was then heated at reflux for 2 h and treated with a 20% aqueous solution of sodium hydroxide. The organic phase was then decanted, dried over sodium sulfate and distillated. Amine **2g** was thus obtained as a beige oil (37.9 g, 84%), $bp_{0.5}$ = 138–140 °C. IR (film): 3372 and 3297 cm^{-1}; ^1H NMR ($CDCl_3$): 0.5–2 (m, 9H), 1 (s, 2H), 2.5–3.2 (m, 3H), 3.65 (s, 3H), 6.8 (s, 1H), 6.8–7.7 (m, 4H).

(±)-N-(2,6-Diisopropylphenyl)-N'-[2-(1-methylindol-3-yl)-hexyl]urea ***1g***
A mixture of 3.05 g (0.015 mol) of 2,6-diisopropylphenylisocyanate and 3.6 g (0.0156 mol) of amine **2g** in 30 mL of diisopropylether was stirred at room temperature for 16 h. The precipitate obtained was filtered then washed with diisopropylether. The urea **1g** was thus isolated as a white solid (2.5 g, 38.5%), mp = 143–145 °C (ethylacetate). IR (KBr): 1648 cm^{-1}; ^1H NMR ($CDCl_3$): 0.7–2 (m, 21H), 2.8–3.6 (m, 5H), 3.6 (s, 3H), 4 (s, 1H), 5.6 (s, 1H), 6.4 (s, 1H), 6.8–7.6 (m, 7H). Analysis: $C_{28}H_{39}N_3O$ (C, H, N).

(–)-N-(2,6-Diisopropylphenyl)-N'-[2-(1-methylindol-3-yl)-hexyl]urea ***1i***
A 24 g (0.104 mol) portion of racemic amine **2g** and 15.8 g (0.104 mol) of *R*-(–)-mandelic acid were dissolved into 600 mL of diethylether. The solution obtained was stirred at 20 °C for 3 h; the precipitate formed was filtered off and then crystallized twice from ethyl acetate while the filtrate was kept in order to prepare the corresponding enantiomeric amine **2h**. The (–)-2-(1-methylindol-3-yl)hexylamine mandelate salt was thus isolated as a white solid (6.8 g, 15%, mp = 115–116 °C). Neutralization of the mandelate salt gave the levogyre enantiomer **2i** of **2g**. (83.3%), mp = 88–90 °C, $[\alpha]_D^{20}$ = –22.9° (C = 3, $CHCl_3$).

The enantiomeric purity of **2i** was measured from the HPLC chromatogram of the urea formed by condensation with *S*-(–)-α-methylbenzylisocyanate (83.3%), mp = 88–90 °C, RT = 14.79 min, ee = 98%.

(–)-*N*-(2,6-Diisopropylphenyl)-*N'*-[2-(1-methylindol-3-yl)hexyl]urea **1i** was prepared by reaction with 2,6-diisopropylphenylisocyanate according to the method described for compound **1g** (86.7%), mp = 138–142 °C (diisopropyloxide/$CH_3CO_2C_2H_5$ 3:1), $[\alpha]_D^{20}$ = –23° (C = 3, $CHCl_3$). Analysis: $C_{28}H_{39}N_3O$ (C, H, N).

(+)-N-(2,6-Diisopropylphenyl)-N'-[2-(1-methylindol-3-yl)-hexyl]urea ***1h***
The dextrogyre amine **2h** was prepared with a 99.6% enantiomeric excess in the same way as for **2i** by neutralization of the

mother liquors obtained from the preparation of the (–)-2-(1-methylindol-3-yl)hexylamine followed by salification with (+)-mandelic acid and then neutralization. The urea **1h** was obtained in a 87.7% yield according to the operating sequence used to prepare the urea **1i**, mp = 138–142 °C (diisopropyloxide/$CH_3CO_2C_2H_5$ 3:1), $[\alpha]_D^{20}$ = +23.6° (C = 3, $CHCl_3$). Analysis: $C_{28}H_{39}N_3O$ (C, H, N).

1-(1-Methylindol-3-yl)cyclopentanecarboxaldehyde ***10***
A 94.4 mL (0.094 mol) portion of a 1 N toluenic solution of diisobutylaluminium hydride was added at –60 °C under dry nitrogen atmosphere to a stirred solution of 13.3 g (0.059 mol) of nitrile **3t** in 230 mL of toluene. The mixture was further stirred until its temperature reached room temperature then 50 mL of methanol and 230 mL of 3 N hydrochloric acid were added. The solution was extracted with dichloromethane, the extract was then washed with water, dried over sodium sulfate and the solvent evaporated. A white solid was thus isolated (7.1 g, 53%), mp = 69–70 °C. IR (KBr): 1714 cm^{-1}; ^1H NMR ($CDCl_3$): 1.4–3 (m, 8H), 3.7 (s, 3H), 6.9 (s, 1H), 6.9–7.6 (m, 4H), 9.4 (s, 1H).

1-(1-Methylindol-3-yl)cyclopentaneacetonitrile ***9***
A solution of 2.9 g (0.0134 mol) of the aldehyde **10** and 5 g of 2,4,6-triisopropylbenzenesulfonylhydrazide in 30 mL of tetrahydrofuran was stirred at room temperature for 3 h. The tetrahydrofuran was then evaporated and replaced by 30 mL of methanol and 2.6 g (0.0402 mol) of KCN was added. The mixture was heated at reflux for 4.5 h and then extracted with dichloromethane/water. The extract was washed with aqueous $NaHCO_3$, dried over Na_2SO_4 and the solvent evaporated. The residue was chromatographed on silica-gel (dichloromethane). Compound **9** was thus isolated as an oil (0.8 g, 25%). IR (film): 2250 cm^{-1}; ^1H NMR ($CDCl_3$): 1.5–2.7 (m, 8H), 2.8 (s, 2H), 3.7 (s, 3H), 6.8–8 (m, 5H).

E,Z-3-[1-(1-Methylindol-3-yl)cyclopentyl]acrylonitrile ***8***
Under a dry nitrogen atmosphere, diethylcyanomethylphosphonate (5.3 mL, 0.033 mol) was added between 20 and 25 °C to a stirred suspension of 1.4 g (0.033 mol + 5%) of sodium hydride (60% in oil) in 40 mL of tetrahydrofuran. A solution of 7.5 g (0.033 mol) of aldehyde **10** in 40 mL of tetrahydrofuran was then added and the mixture further stirred at room temperature for 1 h and then heated at reflux for 2 h 30 min. Cold water was added, the mixture was then extracted with diethylether, the extract washed with water, dried over sodium sulfate and the solvent evaporated. The residue was crystallized by dispersion into hexane (6 g, 73%), mp = 57–59 °C. ^1H NMR ($CDCl_3$): 1.5–2.5 (m, 8H), 3.7 (s, 3H), 4.95 and 5.1 (dd, J_1 = 16.5 Hz, J_2 = 12 Hz, 1H), 6.6–7.7 (m, 6H).

3-[1-Methylindol-3-yl)cyclopentyl]propylamine ***7b***
A solution of 1.92 g (0.0077 mol) of nitrile **8** in 50 mL of ethanol saturated with NH_3 was hydrogenated (150 bars) in the presence of Raney nickel (1 g) at 50 °C for 5 h. The amine **7b** was isolated as an oil (1.65 g, 83.6%). IR (film): 2360 cm^{-1}; ^1H NMR ($CDCl_3$): 0.8–3 (m, 16H), 3.6 (s, 3H), 6.7 (s, 1H), 6.8–7.6 (m, 4H).

Biology

Measurement of rabbit aortic microsomal ACAT
Male New-Zealand rabbits weighing 2–2.5 kg (Elevage Scientifique des Dombes, Châtillon-sur-Chalaronne) were fed for 2 weeks a 1.25% cholesterol-enriched diet to activate ACAT in vivo. After sacrifice, 20 mL of 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 HCl 1 N (*Buffer A*).

The homogenate was first centrifuged for 10 min at 18 000 *g* and at +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 [28].

ACAT was measured according to Gillies and coworkers [29]. 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 of 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 [30]: the organic phase containing ^{14}C-labelled lipids was collected. ^{14}C-Oleyl 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 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 normolipidaemic rat
Male Wistar rats weighing 200–220 g were randomized into groups of six (one cage per group). Following an overnight fasting, each animal was treated orally with a bolus of ^3H-labelled cholesterol (1α, 2α-^3H-cholesterol, 750 kBq/kg) dissolved in a 10% aqueous solution of bovine bile (Sigma B3883). Three hours later, 1 mL of blood was withdrawn on heparine from each animal, through the retroorbitary 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 measurement of the basic intestinal ACAT activity (normolipidaemic animals).

Effect on the hypercholesterolaemic rat
Male Wistar rats weighing 160–180 g (IFFA Credo, Les Oncins, Saint-Germain-sur-l'Arbresle) were randomized into groups of six (one cage per group). They were fed a 2.5% cholesterol-enriched diet for 8 days without restriction in order to increase their blood cholesterol level and saturate their intestinal ACAT activity.

The last 2 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 coagulation, the blood was centrifuged for 30 min at 2000 *g* and the supernatant was withdrawn to assay the plasmatic cholesterol level by cholesterol oxidase. Hypercholesterolaemia was used as measurement of the 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 [31].

The active compounds 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 [31].

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