Eur J Med Chem (1995) 30, 39–46

Preliminary communication

Novel aryloxyalkylthioimidazoles as inhibitors of acyl-CoA: cholesterol-*O*-acyltransferase

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(Received 11 April 1994; accepted 6 September 1994)

Summary — A series of aryloxyalkylthioimidazoles have been synthesized and evaluated for their ability to interfere with the enzyme acyl-CoA (cholesterol-*O*-acyltransferase) (ACAT, EC 2.3.1.26). Most of the molecules possessed a good *in vitro* ACAT inhibitory activity with IC_{50} values ranging between 0.1 and 2.0 μM. Some of them, *eg*, 2-{5-[(4-isobutoxycarbonyl)phenoxy]-pentylthio}-4,5-diphenylimidazole **13**, 2-{3-[(4-isobutoxycarbonyl)phenoxy]-2-oximinopropylthio}-4,5-diphenylimidazole **21**, 2-{3-[(4-isobutoxycarbonyl)phenoxy]-2-hydrazonecarboxamidepropylthio}-4,5-diphenylimidazole **26**, 2-[5-(2-pyridoxy)-pentylthio]-4,5-diphenylimidazole **40** and 2-{5-[(3,5-diterbutyl-4-hydroxy)phenylthio]pentylthio}-4,5-diphenylimidazole **42**, were more potent (range of activity 10–90 nM). They were also more potent with respect to the reference CI-976. When administered orally in hyperlipemic rats, at 10 and 50 mg/kg doses, some representative compounds, like 2-{3-[(4-isobutoxycarbonyl)phenoxy]-2-hydroxypropylthio}-4,5-diphenylimidazole **1**, **13** and **26**, reduced VLDL/LDL-associated cholesterol levels by 30–50% and increased HDL cholesterol levels by 15–50%. In addition, liver accumulation of esterified cholesterol was counteracted (50–80% reduction) and liver ACAT *ex vivo* activity was decreased by 70–85%. Finally, the good efficacy displayed in an endogenous model of hypertriglyceridemia strongly supports the hypothesis of a good systemic availability, which constitutes one of the principal properties of a valuable ACAT inhibitor.

ACAT / cholesterol / hypolipemic activity / atherosclerosis

Introduction

Hyperlipemia and related atherosclerotic processes constitute a serious risk factor for ischemic heart disease [1], since a prominent feature of atherosclerosis is the accumulation of cholesterol and its esters in coronary arteries.

As a part of our search for antihyperlipemic agents endowed with a broad spectrum of activity for the treatment of various forms of dyslipoproteinemia, a series of purine derivatives has been reported to reduce serum triglycerides, to decrease very low density lipoprotein/low density lipoprotein (VLDL/LDL) cholesterol and to increase high density lipoprotein (HDL) cholesterol levels [2–4]. The mechanism of action of these purine compounds [4] appears to be due to interference with lipolysis processes, and is actively supported by the chemical features of the side chain, structurally related to aryloxycarboxylic esters.

Extending our research in this field, a series of molecules with the same side chain as the purine compounds, but characterized by different heteroaromatic nuclei, were tested [5]. Among them, some bicyclic thioethers possessed good hypolipemic activity, possibly acting on lipoprotein catabolism without a direct influence on lipolysis in the adipose tissue.

One possible antiatherosclerotic therapy, currently receiving much attention, is the treatment of hypercholesterolemia by inhibiting the ACAT enzyme. The enzyme acyl-CoA (cholesterol-*O*-acyltransferase) (ACAT, EC 2.3.1.26) is responsible for the intracellular esterification of cholesterol [6, 7] and plays a key role in the intestinal absorption of cholesterol [8] and in hepatic VLDL secretion [9]. Moreover, since the accumulation of cholesteryl esters is a distinctive phenomenon of atherosclerotic plaque formation, the regulation of ACAT might also play an important part in the progression of atheromata [9, 10]. Since ACAT inhibitors might have beneficial effects in preventing both dyslipidemic and atherosclerotic pathologies, we decided to approach this field using

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an aryloxyalkylthioethereal side chain linked to imidazole, a heteroaromatic nucleus previously used for the preparation of molecules that can interfere with the ACAT enzyme [11–17].

An SAR study for *in vitro* ACAT inhibitory activity of a series of aryloxyalkylthioimidazoles was undertaken. To this purpose, their structure was divided into 3 regions: (A) left-hand part; (B) bridging portion; and (C) right-hand part (for example, see chart 1). Moreover, the *in vivo* hypolipemic effects of some representative molecules were evaluated in comparison with a well-known ACAT inhibitor, CI-976.

The aim of the present study was to search for potent compounds in terms of both *in vitro* ACAT inhibitory activity and *in vivo* hypocholesterolemic effect. An additional goal was represented by the identification of a possible systemic effect. The preliminary results are presented here.

Chemistry

Table I lists all the molecules synthesized and their physical characteristics. Four synthetic methods were used to obtain the compounds (schemes 1–3).

Method 1 (scheme 1) involved the reaction of 2-mercapto-4,5-diphenylimidazole with epoxides in refluxing ethanol, under 2,6-lutidine catalysis, to give compounds **1**, **8** and **9**. The epoxides were obtained by reacting epichlorohydrin, in the presence of K_2CO_3 in refluxing methylethylketone, or commercially available *S*(+) or *R*(−) glycidyltosylates in the presence of NaH/DMF with isobutyl 4-hydroxybenzoate. The substituted phenols were either commercially available or synthesized by esterifying the corresponding hydroxy benzoic acids by standard methods.

Method 2 (scheme 2) is the reaction of 2-mercapto-4,5-diphenylimidazole with the appropriate halides, performed with sodium isobutoxide in isobutanol or potassium *t*-butoxide in DMF or 2,6-lutidine in ethanol and was exploited for the preparation of compounds **10–19** and **27–42**. The halides were obtained by reacting appropriately substituted phenols (see above) with commercially available α,ω-dihalogenoalkanes in DMF/K_2CO_3.

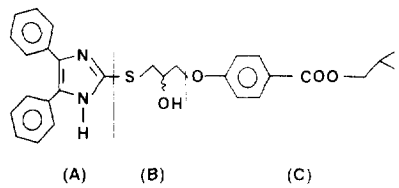


Chart 1. Structural regions examined in SAR studies (example of compound **1**).

Method 3 (scheme 3) was used to synthesize compound **20** by reaction of 2-mercapto-4,5-diphenylimidazole with 3-(4-isobutoxycarbonyl)phenoxy-1-bromo-acetone. The intermediate 3-(4-isobutoxycarbonyl)phenoxy-1-bromoacetone was prepared from 4-isobutoxycarbonylphenoxy-2,3-epoxypropane (see *Method 1*) by ring opening with hydrobromic acid in acetic acid/water, followed by oxidation with chromic anhydride in acetone.

Method 4 (scheme 3) was employed to transform the same available starting material, ketone **20**, into the series of imino derivatives **21–26**, by means of the appropriate hydroxylamines or semicarbazide in isobutanol at 100°C. Medium- and long-chain *O*-alkyl hydroxylamines were prepared from *N*-hydroxyphthalimide and the appropriate halides [18], followed by hydrazinolysis in refluxing ethanol.

Finally, starting from thiourea and benzoin, 2-mercapto-4,5-diphenylimidazole was readily prepared in 72% yield by fusion at about 180°C [19].

Results and discussion

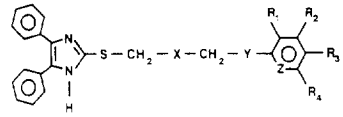
The efficacy of the compounds to interfere with ACAT was determined using liver microsomes prepared from normolipemic rats as the enzyme source. The potency was expressed as the micromolar concentration of a compound required to inhibit the enzyme activity by 50%. These resulting IC_{50} values were used for the SAR studies.

To do this, modifications at the bridging portion (B) and the right-hand part (C) (chart 1) were introduced: in fact, region A was constantly kept as 4,5-diphenylimidazole since this nucleus, unlike the other tested imidazoles *N*-methylimidazole **2** and benzimidazole **3**, showed good *in vitro* ACAT inhibitory activity (table II). Similar heteroaromatic structures, like benzthiazole **4**, 5-chlorobenzthiazole **5**, benzoxazole **6** or close analogs like 4,5-diphenyl oxazole **7** [20], were endowed with hypolipemic efficacy, but were inactive in the ACAT inhibition test.

In the region of the bridging portion (B), the optimal chain length and the desirable functional groups were examined (table I). The test used did not discriminate between the 2 enantiomeric alcohols **8** and **9**, apparently negating any importance of this chiral center on the inhibitory activity. Furthermore, the hydroxyl itself seems to be irrelevant to *in vitro* activity (compare **1** with **11**). Moreover, when the hydroxyl was oxidized, the resulting ketone **20** was almost as active as alcohol **1**.

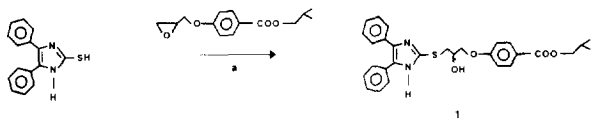
More interesting were some of the derivatives of ketone **20**: oxime **21** was 50-fold more active (IC_{50} = 20 nM), while short- and medium-length chain alkylated oximes **22** and **23** retained good, even if lower, activity. The presence of polarity (a hydroxyl in **25**)

Table I. Physical data and biological activity of derivatives **1** and **8–42**.

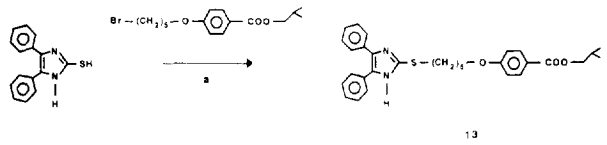


Compound	X	Y	Z	R_1	R_2	R_3	R_4	Mp (°C)	Yield (%)	Molecular formula	Anal[a]	Synthetic method	IC_{50}[b] (μM)
1	(R,S)CHOH	O	CH	H	H	COO*i*-Bu	H	135–138	86	$C_{29}H_{30}N_2O_4S$	C, H, N	1	0.26
8	(R)CHOH	"	"	"	"	"	"	100–102	75	$C_{29}H_{30}N_2O_4S$	C, H, N	1	0.28
9	(S)CHOH	"	"	"	"	"	"	68–69	68	$C_{29}H_{30}N_2O_4S$	C, H, N	1	0.12
10	–	"	"	"	"	"	"	163–164	19	$C_{28}H_{28}N_2O_3S$	C, H, N	2	5.50
11	CH_2	"	"	"	"	"	"	154–155	23	$C_{29}H_{30}N_2O_3S$	C, H, N	2	0.40
12	$(CH_2)_2$	"	"	"	"	"	"	152–155	37	$C_{30}H_{32}N_2O_3S$	C, H, N	2	0.09
13	$(CH_2)_3$	"	"	"	"	"	"	122–124	56	$C_{31}H_{34}N_2O_3S$	C, H, N	2	0.06
14	$(CH_2)_4$	"	"	"	"	"	"	135–136	61	$C_{32}H_{36}N_2O_3S$	C, H, N	2	0.42
15	$(CH_2)_6$	"	"	"	"	"	"	113–115	18	$C_{34}H_{40}N_2O_3S$	C, H, N	2	1.35
16	$(CH_2)_8$	"	"	"	"	"	"	108–109	17	$C_{36}H_{44}N_2O_3S$	C, H, N	2	19.20
17	$(CH_2)_{10}$	"	"	"	"	"	"	88–90	23	$C_{38}H_{48}N_2O_3S$	C, H, N	2	15.00
18	CH=CH	"	"	"	"	"	"	126–128	53	$C_{30}H_{30}N_2O_3S$	C, H, N	2	8.20
19	C≡C	"	"	"	"	"	"	100–101	51	$C_{30}H_{28}N_2O_3S$	C, H, N	2	11.00
20	C=O	"	"	"	"	"	"	149–150	89	$C_{29}H_{28}N_2O_4S$	C, H, N	3	0.83
21	C(NOH)	"	"	"	"	"	"	156–158	85	$C_{29}H_{29}N_3O_4S$	C, H, N	4	0.02
22	C(NOMe)	"	"	"	"	"	"	50–52	73	$C_{30}H_{31}N_3O_4S$	C, H, N	4	0.52
23	$C(NOC_5H_{11})$	"	"	"	"	"	"	88–90	89	$C_{34}H_{39}N_3O_4S$	C, H, N	4	0.39
24	$C(NOC_{10}H_{21})$	"	"	"	"	"	"	oil	54	$C_{39}H_{49}N_3O_4S$	C, H, N	4	≫10
25	$C(NOCH_2CH_2CH_2OH)$	"	"	"	"	"	"	67–68	53	$C_{32}H_{35}N_3O_5S$	C, H, N	4	0.88
26	$C(NNHCONH_2)$	"	"	"	"	"	"	172–174	55	$C_{30}H_{31}N_5O_4S$	C, H, N	4	0.09
27	$(CH_2)_3$	"	"	"	"	COOEt	"	129–130	36	$C_{29}H_{30}N_2O_5S$	C, H, N	2	0.14
28	"	"	"	"	"	COOMe	"	85–86	36	$C_{28}H_{28}N_2O_5S$	C, H, N	2	0.26
29	"	"	"	"	"	COOH	"	187–190	74	$C_{27}H_{26}N_2O_5S$	C, H, N	2	1.70
30	"	"	"	OMe	"	COOMe	"	103–106	54	$C_{29}H_{30}N_2O_4S$	C, H, N	2	0.23
31	"	"	"	H	"	H	"	112–113	21	$C_{26}H_{26}N_2OS$	C, H, N	2	5.10
32	"	"	"	"	"	OMe	"	118–120	28	$C_{27}H_{28}N_2O_2S$	C, H, N	2	0.39
33	"	"	"	"	OMe	H	"	103–105	22	$C_{27}H_{28}N_2O_2S$	C, H, N	2	1.15
34	"	"	"	OMe	H	"	"	108–109	64	$C_{27}H_{28}N_2O_2S$	C, H, N	2	0.70
35	"	"	"	H	"	*tert*-Bu	"	132–133	39	$C_{30}H_{34}N_2OS$	C, H, N	2	0.09
36	"	"	"	"	"	Cl	"	151–152	36	$C_{26}H_{25}ClN_2OS$	C, H, N	2	0.79
37	"	"	"	"	"	F	"	93–94	68	$C_{26}H_{25}FN_2OS$	C, H, N	2	1.30
38	"	"	"	"	"	NO_2	"	130–132	62	$C_{26}H_{25}N_3O_3S$	C, H, N	2	0.65
39	"	"	"	F	"	F	"	96–97	57	$C_{26}H_{24}F_2N_2OS$	C, H, N	2	1.90
40	"	"	N	H	"	H	"	120–124	76	$C_{25}H_{25}N_3OS$	C, H, N	2	0.01
41	"	S	CH	"	"	"	"	105–108	20	$C_{26}H_{26}N_2S_2$	C, H, N	2	1.90
42	"	"	"	"	*tert*-Bu	OH	*tert*-Bu	45–50	30	$C_{34}H_{42}N_2OS_2$	C, H, N	2	0.01
CI-976													0.14

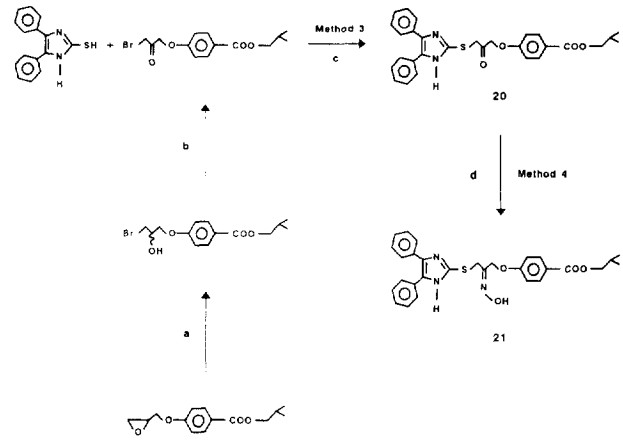
[a]Analytical results are within ± 0.4% of theoretical values unless otherwise noted; [b]ACAT inhibition *in vitro* in liver microsomes isolated from normolipemic rats. Each determination was performed in triplicate; control values 18.4 ± 0.8 nmol/h/mg protein.



Scheme 1. *Method 1.* Reagents: a) 2,6-lutidine, EtOH, reflux.



Scheme 2. *Method 2.* Reagents: a) *i*-BuOH, *i*-BuONa.



Scheme 3 *Methods 3* and *4*. Reagents: a) HBr/AcOH; b) CrO_3/H_2SO_4; c) 2,6-lutidine, EtOH; d) NH_2OH·HCl, *i*-BuOH.

on the terminus of the alkyl chain was apparently uninfluential. The longer alkylic chain of oxime **24** made it completely inactive. Semicarbazone **26** was also quite active, with an IC_{50} (90 nM) comparable to that of oxime **21**.

To check the importance of the chain length many analogs from C_2 (**10**) to C_{12} (**17**) were prepared and tested. A bell-shaped curve, which is often found for different classes of enzyme inhibitors, was obtained. The lowest activities were found for the C_2 and C_{10} compounds, the highest for C_4 (IC_{50} = 90 nM) and C_5 (IC_{50} = 60 nM) analogs. When unsaturations were introduced, *eg* the *trans*-double bond of **18** and the triple bond of **19**, a significant drop in activity was observed, showing that a flexibility of the chain is required.

Since the C_5 chain length turned out to be ideal and oxime seemed to be the most effective functional group, it is presumable that a molecule characterized by a C_5 chain, bringing the oxime to the middle carbon atom, could be very promising. On the other

Table II. Hypolipemic activity on cholesterol-fed rats[a] and *in vitro* ACAT inhibition of *Het-S-CH_2-CHOH-CH_2-O-∅-(p-COOi-Bu)* in the 5-d test, 100 mg/kg•d dose, 10 ml/kg 0.5% CMC *po*.

Compound	*Het*	*% change (vs controls[b])*			*IC_{50} (μM)[c]*
		Triglyceride	*VLDL/LDL cholesterol*	*HDL cholesterol*	
1	4,5-Diphenylimidazol-2-yl	−25 ± 4*	−65 ± 3*	+32 ± 1*	0.26
2	1-Methylimidazol-2-yl	−6 ± 2	+9 ± 2	−3 ± 3	> 1000
3	Benzimidazol-2-yl	−19 ± 1*	0 ± 1	+3 ± 2	> 1000
4	Benzthiazol-2-yl	−16 ± 2*	−19 ± 2*	+8 ± 2	> 1000
5	5-Chlorobenzthiazol-2-yl	−9 ± 1	−20 ± 1*	+5 ± 1	> 1000
6	Benzoxazol-2-yl	−23 ± 1*	−26 ± 2*	+18 ± 1*	> 1000
7	4,5-Diphenyloxazol-2-yl	−35 ± 2*	−18 ± 2*	+9 ± 2	460

[a]Nath's diet: 0.5% cholesterol + 0.5% cholic acid; [b]controls given orally 10 ml/kg of 0.5% w/v carboxymethylcellulose (CMC), serum parameters evaluated 24 h after last administration; [c]ACAT inhibition *in vitro* in liver chromosomes isolated from normolipemic rats. Each determination performed in triplicate. Control values 18.4 ± 0.8 nmol/h/mg protein; *$p < 0.05$ Dunnett *t* test, after ANOVA determination; control value levels (± SE, 3 experiments): triglycerides 402 ± 13 mg/dl; VLDL/LDL cholesterol 271 ± 11 mg/dl; HDL cholesterol 22.6 ± 1 mg/dl.

hand, for modifications in region C of the molecule, analogs characterized by a plain C_5 polymethylenic bridging portion were utilized, on the basis of the ease of synthesis.

A series of substitutions were also introduced in the right-hand part (C) mostly at the *para*-position of the phenoxy ring. Both electron-donating (**32–35**) and electron-withdrawing substituents (**13**, **27–29**, **36–39**) are important for activity (compare with **31**), but no significant difference between the 2 groups was observed. The addition of a second substituent was uninfluential (see **30**, **39**); *para*, *meta* and *ortho* isomers showed comparable activity (see **32–34**).

Considerable lipophilicity is apparently necessary for optimal inhibition of ACAT *in vitro*. Compounds **35** and **42**, which are more lipophilic molecules, possessed a high activity (IC_{50} = 90 and 10 nM, respectively). Esters (**13**, **27** and **28**) were better than the corresponding acid (**29**) and, in the ester series, activity seems correlated to lipophilicity, since it increased in the order methyl < ethyl < *i*-butyl. The significant influence of a heteroatom on the aromatic ring is shown by comparing the phenoxy (**31**) with the pyridoxy derivative (**40**); the latter is about 300-fold more active than **31**. Phenoxy was a feature present in the large majority of the compounds, even if it has been verified that oxygen can be profitably replaced with sulfur (**31** *vs* **41**).

The *in vivo* activity of some of the more potent and representative compounds was evaluated on hyperlipemic animals in comparison with CI-976 [21–23], a well-studied ACAT inhibitor (tables III and IV).

Feeding a high fat/cholesterol diet to rats resulted in an enhancement of VLDL/LDL-associated cholesterol and triglyceride levels (up to 400%), with an additional strong decrease in HDL-associated cholesterol levels. Circulating non-esterified fatty acids (NEFA) levels were also high. Moreover, it was observed here and elsewhere [24] that cholesterol-fed rats showed a net increase in liver cholesterol concentration. Such an accumulation was nearly linear for esterified cholesterol over the 15-d time course of the experiment. Consequently, it was possible to use this parameter as a predictive index of the cholesterol absorption and accumulation.

All the tested compounds, when coadministered with a modified Nath's diet [25], could interfere dose-dependently with the changes in plasma lipoprotein

Table III. Hypolipemic activity on cholesterol-fed rats[a] and *ex vivo* liver ACAT activity of aryloxyalkylthioimidazoles in 15-d test, 2 doses, 10 ml/kg 0.5% CMC *po*.

Compound	*Dose (mg/kg d)*	*% change (vs controls[b])*							
		Serum				*Liver*			*Liver ACAT ex vivo activity*
		Triglyceride	*VLDL/LDL cholesterol*	*HDL cholesterol*	*NEFA[c]*	*Free cholesterol*	*Esterified cholesterol*	*Triglyceride*	
1	10	−17 ± 3	−29 ± 2*	+16 ± 1*	+7 ± 1	−18 ± 4	−59 ± 4*	−3 ± 1	nd
	50	+20 ± 1*	−38 ± 3*	+33 ± 2*	−15 ± 2	−22 ± 2*	−64 ± 2*	−13 ± 3	−73%
13	10	+ 3 ± 1	−48 ± 4*	+43 ± 5	−4 ± 2	−41 ± 6*	−77 ± 7*	+7 ± 2	nd
	50	−11 ± 2	−52 ± 2*	+49 ± 1*	−31 ± 3*	−30 ± 2*	−79 ± 4*	−8 ± 5	−85%
21	10	−15 ± 3	−6 ± 2	+6 ± 1	−29 ± 5*	−21 ± 4*	−35 ± 2*	+17 ± 3	nd
	50	+2 ± 4	−22 ± 1*	+7 ± 2	−27 ± 2*	−21 ± 3*	−44 ± 4*	+2 ± 4	−54%
26	10	−18 ± 2*	−30 ± 4*	+21 ± 2*	−31 ± 4*	−26 ± 3*	−49 ± 3*	+12 ± 4	nd
	50	−20 ± 2*	−43 ± 3*	+25 ± 2*	−35 ± 4*	−27 ± 3*	−70 ± 6*	−9 ± 3	−76%
CI-976	10	−7 ± 3	+1 ± 2	+2 ± 1	+10 ± 2	−14 ± 2	−19 ± 5	−8 ± 2	nd
	50	−20 ± 2*	−38 ± 3*	+15 ± 1*	+2 ± 3	+ 3 ± 5	−74 ± 4*	−26 ± 2*	−79%

[a]Nath's diet: 0.5% cholesterol + 0.5% cholic acid; [b]controls given orally 10 ml/kg of 0.5% w/v CMC, serum parameters evaluated 24 h after last administration; [c]non-esterified fatty acids; *$p < 0.05$ Dunnett *t* test, after ANOVA determination; the typical mean control values (± SE, 3 experiments) were as follows: serum triglycerides 679 ± 34 mg/dl, serum VLDL/LDL cholesterol 296 ± 20 mg/dl, serum HDL cholesterol 24.6 ± 1.2 mg/dl, NEFA 4.677 ± 0.145 m eq/l, liver free cholesterol 5.7 ± 1.0 mg/g, esterified cholesterol 5.2 ± 0.6 mg/g, triglycerides 26.3 ± 2.2 mg/g tissue and liver ACAT *ex vivo* activity 8.3 ± 0.4 nmol/h/mg protein. nd = not determined.

Table IV. Effect of aryloxyalkylthioimidazoles on serum lipid parameters in fructose-fed rats[a] in 3-d test, 50 mg/kg·d dose, 10 ml/kg 0.5% CMC *po*.

Compound	*Triglyceride (mg/dl)*	*VLDL/LDL cholesterol (mg/dl)*	*HDL cholesterol (mg/dl)*
Normolipemic controls[b]	122 ± 11	37 ± 4	48 ± 5
Fructose-fed controls	283 ± 29	32 ± 5	41 ± 2
1	174 ± 19*	22 ± 2*	52 ± 2*
13	172 ± 13*	20 ± 2*	55 ± 2*
21	218 ± 24	26 ± 3	52 ± 3*
26	153 ± 22*	20 ± 1*	50 ± 1*
CI-976	188 ± 15*	24 ± 2*	54 ± 4*

[a]15% fructose in drinking water, serum parameters evaluated 2 h after last administration; [b]controls given orally 10 ml/kg of 0.5% w/v CMC; *$p < 0.05$ Dunnett t test *vs* fructose-fed animals, after ANOVA determination.

concentrations induced by the diet. In particular, compounds **1**, **13** and **26** are potent hypocholesterolemic agents even at 10 mg/kg dose, lowering VLDL/LDL cholesterol levels by 30–50% and raising HDL cholesterol levels by about the same amount. Compound **21** shows a good *in vitro* activity (IC_{50} = 20 nM), but was less potent *in vivo*, possibly because of the different degree of lipophilicity (log P = 7.49, compared to 5.39 of **1** and 6.61 of **26** (Lundstedt and Thelin, unpublished results). Such a feature might account for a decreased absorption into mucosal intestinal cells or liver uptake.

As expected, liver esterified cholesterol was greatly reduced by aryloxyalkylthioimidazoles, with an almost complete prevention of accumulation. In fact, the 70% reduction of hepatic cholesterol shown by compounds **13** and **26** represents the normalization of this parameter. On the other hand, no significant effect on liver triglycerides was observed.

All the tested analogs showed lower efficacy in decreasing serum triglycerides and NEFA, with the exception of **26**, which significantly reduced these parameters even at 10 mg/kg dose. Since such activity is normally due to an interference with lipoprotein assembly and catabolism, this effect can be symptomatic of a good bioavailability.

The potent and well-characterized ACAT inhibitor CI-976 [23], used as reference standard, displayed a good hypolipemic efficacy at only a 50 mg/kg dose in our model.

Finally, to test the possible systemic efficacy of aryloxyalkylthioimidazoles, the *ex vivo* ACAT activity in hyperlipemic animals was evaluated. A 15-d treatment with a 50 mg/kg dose of compounds **1**, **13** and **26** caused 73–85% decrease of the hepatic ACAT activity (for comparison CI-976 reduced 79%). The analog **21** showed only a 48% reduction in activity, another possible clue to lower systemic availability.

None of the compounds caused a variation of normal body weight gain.

Although the hyperlipemic model is very useful for comparing the hypolipemic efficacy of new compounds, it may present some disadvantages in predicting if the target site of action is reached. In cholesterol-fed animals it is virtually impossible to draw a clear-cut distinction between a systemically acting ACAT inhibitor and a compound that inhibits the ACAT enzyme at the intestinal level only, interfering with dietary cholesterol. In fact, the reduction of ACAT enzyme activity might be secondary to the inhibition of cholesterol absorption. To partially solve this problem fructose-fed rats were used, which is a well-characterized model of endogenous hypertriglyceridemia due to the direct stimulation of liver VLDL synthesis [26, 27]. Since it has been demonstrated that VLDL synthesis and secretion are controlled by a cholesterol esterification/deesterification cycle [28], it is possible to evaluate the systemic interference on hepatic ACAT by monitoring the efficacy of the compounds in reducing serum triglycerides levels.

In fructose-fed rats, compounds **1**, **13**, **26** and CI-976 could decrease the almost doubled triglyceride levels to near normality. In addition, they significantly reduced VLDL/LDL cholesterol levels, a parameter not modified by fructose administration, while elevating HDL-associated cholesterol levels.

The combined data support the hypothesis that aryloxyalkylthioimidazoles interfere with both intestinal and liver ACAT. At the origin of this remarkable

activity, there could be a good systemic bioavailability, which, although it must be supported by direct pharmacokinetic data, represents, to our knowledge, a feature present in few ACAT inhibitors.

Conclusions

Preliminary SAR studies indicated that an effective moiety for region A was 4,5-diphenyl-1*H*-imidazole and evidenced the importance of region B, the modification of which resulted in significant changes in biological activity. The optimum chain length was five atoms and oxime was the best functional group. In addition high values of lipophilicity at region C seemed to be important for efficacy.

On the basis of the *in vitro* ACAT inhibitory activity and the antihyperlipemic profile of some selected representatives in different testing models, aryloxyalkylthioimidazoles seem to belong to a very interesting class of systemically available acyl-CoA inhibitors.

Experimental protocols

Chemistry

Melting points were determined in a Büchi 510 apparatus and are uncorrected. IR spectra were obtained with a Biorad FTS7 spectrophotometer. NMR spectra were recorded on Bruker AC 200 spectrometer using tetramethylsilane as an internal standard. All the new compounds were analysed for C, H, N and the values found were within ± 0.4% of the theoretical values.

Reference standard CI-976 (2,2-dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide) was prepared as described in literature (mp 58–59°C, lit 59–60°C) [21].

Method 1. 2-{3-[4-Isobutoxycarbonyl)phenoxy]-2-hydroxypropylthio}-4,5-diphenylimidazole ***1***

A mixture of 1-phenoxy-2,3-epoxypropane (9.9 g, 39.6 mmol), 4,5-diphenyl-2-imidazolethiol (10 g, 39.6 mmol), 2,6-lutidine (2 ml) and absolute ethanol (150 ml) was refluxed for 2 h, concentrated and the residue, dissolved in ethylacetate, was washed with $NaHCO_3$ (sat aq sol), water, dried (Na_2SO_4) and concentrated. The residue was triturated with petroleum ether, filtered and crystallized from ethanol/*n*-heptane, to give **1** (17.2 g, 34.3 mmol, 86% yield), mp 135–138°C.

^{1}H-NMR ($CDCl_3$) δ: 0.97–1.00 (d, J = 6.7 Hz, 6H, $(CH_3)_2$), 2.01–2.08 (m, 1H, $CH(CH_3)_2$), 3.23–3.47 (m, 2H, SCH_2), 4.03–4.06 (d, J = 6.6 Hz, 2H, $COOCH_2$), 4.1–4.2 (m, 2H, OCH_2), 4.43 (m, 1H, $CHOH$), 6.87–6.92 (d, J = 9 Hz, 2H, arom *meta* to COO), 7.1–7.5 (m, 10H, $(C_6H_5)_2$), 7.94–7.99 (d, J = 9 Hz, 2H, arom *ortho* to COO), 9.9 (broad s, 1H, NH).

Method 2. 2-{5-[(4-Isobutoxycarbonyl)phenoxy]pentylthio}-4,5-diphenylimidazole ***13***

A mixture of 4-(5-bromopentoxy)isobutylbenzoate (5.4 g, 15.8 mmol), 4,5-diphenyl-2-imidazolethiol (4 g, 15.8 mmol), 2,6-lutidine (2.2 ml, 19 mmol) and absolute ethanol (50 ml) was refluxed for 5 h, concentrated and the residue, dissolved in ethyl acetate (50 ml), was washed with $NaHCO_3$ (sat aq sol), water, dried (Na_2SO_4) and concentrated. The residue was triturated with ether, filtered and crystallized from ethanol to give **13** (4.6 g, 8.9 mmol, 56% yield), mp 122–124°C.

^{1}H-NMR ($CDCl_3$) δ: 0.98–1.01 (d, J = 6.7 Hz, 6H, $(CH_3)_2$), 1.56–1.98 (m, 6H, $(CH_2)_3$), 2.01–2.08 (m, 1H, $CH(CH_3)_2$), 3.11–3.18 (t, J = 7 Hz, 2H, SCH_2), 3.96–4.02 (t, J = 6.2 Hz, 2H, OCH_2), 4.03–4.06 (d, J = 6.6 Hz, 2H, $COOCH_2$), 6.83–6.88 (d, J = 8.9 Hz, 2H, arom *meta* to COO), 7.23–7.57 (m, 10H, $(C_6H_5)_2$), 7.92–7.97 (d, J = 8.9 Hz, 2H, arom *ortho* to COO), 9.0 (broad s, 1H, NH).

Method 3. 2-{3-[(4-Isobutoxycarbonyl)phenoxy]-2-oxopropylthio}-4,5-diphenylimidazole ***20***

A solution of 1-bromo-3-[(4-isobutoxycarbonyl)phenoxy]-propan-2-one (6.5 g, 19.82 mmol) in absolute ethanol (110 ml) was added dropwise to a stirred suspension of 4,5-diphenyl-2-imidazolethiol (5 g, 19.82 mmol) and 2,6-lutidine (2.77 ml, 23.78 mmol) in absolute ethanol (50 ml). The reaction mixture was stirred at 60°C for 1 h and concentrated. The residue was dissolved in ethylacetate (250 ml) and was washed with $NaHCO_3$ (sat aq sol, 200 ml), water (2 x 200 ml), dried (Na_2SO_4) and concentrated. The resulting solid was washed with petroleum ether to yield the title compound (8.82 g, 17.6 mmol, 89% yield), mp 149–150°C.

^{1}H-NMR ($CDCl_3$) δ: 0.97–1.00 (d, J = 6.7 Hz, 6H, $(CH_3)_2$), 2.00–2.07 (m, 1H, $CH(CH_3)_2$), 4.02–4.05 (d, J = 6.6 Hz, 4H, $COOCH_2$ + SCH_2), 4.94 (s, 2H, OCH_2), 6.87–6.91 (d, J = 8.8 Hz, 2H, arom *meta* to COO), 7.0–7.5 (m, 10H, $(C_6H_5)_2$), 7.87–7.91 (d, J = 8.8 Hz, 2H, arom *ortho* to COO), 9.8 (broad s, 1H, NH).

Method 4. 2-{3-[(4-Isobutoxycarbonyl)phenoxy]-2-oximinopropylthio}-4,5-diphenylimidazole ***21***

A mixture of **20** (15 g, 30 mmol) and hydroxylamine•HCl (10.4 g, 150 mmol) in absolute ethanol (150 ml) was stirred for 15 min, concentrated and the residue, dissolved in ethyl acetate, was washed with $NaHCO_3$ (sat aq sol), water, and then dried (Na_2SO_4) and concentrated. Crystallization from ether gave 13.1 g (25 mmol, 85% yield) of **21**, as mixture of *syn*- and *anti*-oximes, mp 155–159°C.

^{1}H-NMR ($CDCl_3$) δ: 0.97–1.00 (d, J = 6.7 Hz, 6H, $(CH_3)_2$), 1.9–2.1 (m, 1H, $CH(CH_3)_2$), 3.85 (s, SCH_2, minor isomer), 3.99–4.02 (d, J = 5.7 Hz, 2H, $COOCH_2$), 4.06 (s, SCH_2, major isomer), 4.77 (s, OCH_2, major isomer), 5.07 (s, OCH_2, minor isomer), 6.87–6.91 (d, J = 8.8 Hz, 2H, arom *meta* to COO), 7.1–7.6 (m, 10H, $(C_6H_5)_2$), 7.90–7.94 (d, J = 8.8 Hz, 2H, arom *ortho* to COO), 10.1 (broad s, 1H, NH).

Biological methods

In vitro studies

The ACAT inhibitory activity was evaluated in microsomes from normolipemic rat liver. The assays were carried out according to the method described by Lichtenstein and Brecher [29] with some minor modifications: 150 μg microsomal proteins and 0.1 μCi [^{14}C]oleoyl-CoA, in a total volume of 0.2 ml, was used. The conversion of oleate into labelled cholesteryl esters was evaluated by scintillation counting after separation of the reaction products by high performance thin-layer chromatography. Results are given as the concentration (μM) which inhibits 50% of the activity (IC_{50}), derived by linear regression analysis of the data.

In vivo studies: 5-d test

Some of the substances were tested in male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 120–180 g. The

animals, 8 per group, maintained at 22 ± 1°C and 65 ± 10% relative humidity, were fed *ad libitum* with a modified Nath's diet [25] supplemented with 1% cholesterol (w/w), 1% cholic acid (w/w) (Dottori Piccioni, Gessate, Italy) for 5 d, concomitantly with test compound administration. Test compounds were suspended in 0.5% w/v carboxymethylcellulose (CMC) and administered daily by gavage between 9–11 am. On the fifth day, 2 h after the last treatment, the rats were exsanguinated by decapitation and blood samples collected.

The following analyses were performed on serum obtained by low speed centrifugation: 1) triglyceride and total cholesterol levels were determined by an enzymatic method (Poli kit); 2) HDL cholesterol was determined by an enzymatic method after removal of VLDL/LDL by precipitation (phosphotungstate method); and 3) VLDL/LDL cholesterol was evaluated as difference between total and HDL-associated cholesterol.

Fifteen-day test

Selected compounds were then evaluated for a longer period in male Sprague–Dawley rats, 8 per group, fed a Nath's diet supplemented with 0.5% cholesterol (w/w), 0.5% cholic acid (w/w) (Dottori Piccioni, Gessate, Italy) for 15 d, concomitantly with test compound administration. Test compounds were suspended in 0.5% w/v carboxymethylcellulose (CMC) and administered daily by gavage between 9 and 11 am. On the sixteenth day, 24 h after the last treatment, the rats were exsanguinated by decapitation and blood samples collected.

The same analyses as in the 5-d test, plus evaluation of non-esterified fatty acids (NEFA-Quick, Boehringer), were performed on the serum obtained by low-speed centrifugation. Body weight gain during the treatment period was also recorded.

Hepatic cholesterol and triglycerides were assayed by saponification of the liver [30], extraction of the lipid content into organic solvent (methanol/water 2:1), and colorimetric analysis. ACAT *ex vivo* activity was evaluated in crude homogenates rather than in microsomes as described in literature [31]. Free cholesterol was tested by an enzymatic kit (Boehringer). Esterified cholesterol was determined as the difference between total and free cholesterol.

Fructose-fed rats (3-d test)

Male rats, 10 per group, which were normally fed a commercial rat chow, were given a 15% fructose solution as drinking water for 3 consecutive days. Test compounds were suspended in 0.5% w/v carboxymethylcellulose (CMC) and concomitantly administered daily by gavage. On the third day, 2 h after the last treatment, the rats were exsanguinated and blood samples collected.

Acknowledgments

The authors are indebted to A Depaoli, N Colombo and staff for spectral determinations; G Fioriello and G Fachin for synthetic preparations; and L Palazzolo, C Ciceri and O Veneroni for pharmacological determinations.

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