Pyridazine derivatives as novel acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors

Arianna Gelain

Istituto di Chimica Farmaceutica e Tossicologica, Viale Abruzzi 42, 20131 Milano (Italy)

Acyl-CoA:cholesterol acyltransferase (E.C.2.3.1.26, ACAT) is a microsomial enzyme that catalyses the formation of cholesteryl esters by acylation of cholesterol with long chain fatty acylCoA [1].

ACAT plays important roles in cellular homeostasis and in the early stages of atherosclerosis.

Therefore, ACAT inhibitors have been identified as useful agents in the treatment of hypercholesterolemia, atherosclerosis and coronary diseases [2]. In addition, recently their application has been proposed for Alzheimer's disease [3].

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In mammalian species two isoforms are present: though their role is not completely clarified, their distribution seems to be peculiar: in particular, ACAT1 is predominant in human liver, macrophage and adrenal gland while ACAT2 is mainly present in the intestine [4].

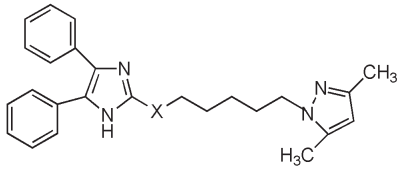
The first studies on ACAT inhibitors were performed in the early 70s. Since that time a large number of ACAT inhibitors have been discovered and it is possible to classify these compounds in two major groups, on the basis of their chemical structure:

- Fatty acid anilide derivatives
- Urea derivatives

Several representative examples are:

- among fatty acids anilide derivatives: **CI-976** [5], **F12511** [6], **TEI 6522** [7].
- among urea derivatives: **PD132301-2** [8], **FR190809** [9], **YM 17E** [10].

In addition, several interesting compounds not belonging to the previous classes have been considered. Among others **CI-1011**(Avasimibe) [11] and a series of 4,5-diphenylimidazole derivatives, e.g. **RP70676** [12], **RP73163** [13]. In particular, the latter attracted our attention (Fig.1).



X=S RP 70676
[Rhone-Poulenc Rorer, IC50 = 0.040 μM]

X=SO RP 73163
[Rhone-Poulenc Rorer, IC50 = 0.086 μM]

Figure 1

In fact, given the interest of our group for the pyridazine moiety, whose biological properties are well known, we thought it challenging to study a class of structurally related compounds, in which the imidazole ring was substituted by a pyridazine ring (Fig.2).

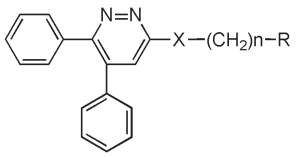
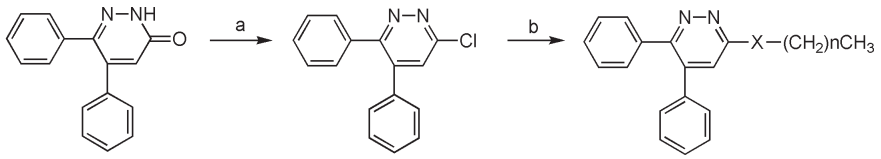


Figure 2

Such compounds would retain several main characteristics of ACAT inhibitors, namely a long alkyl chain linked through an heteroatom to an heterocycle, in turn substituted by an *ortho*-biphenyl system.

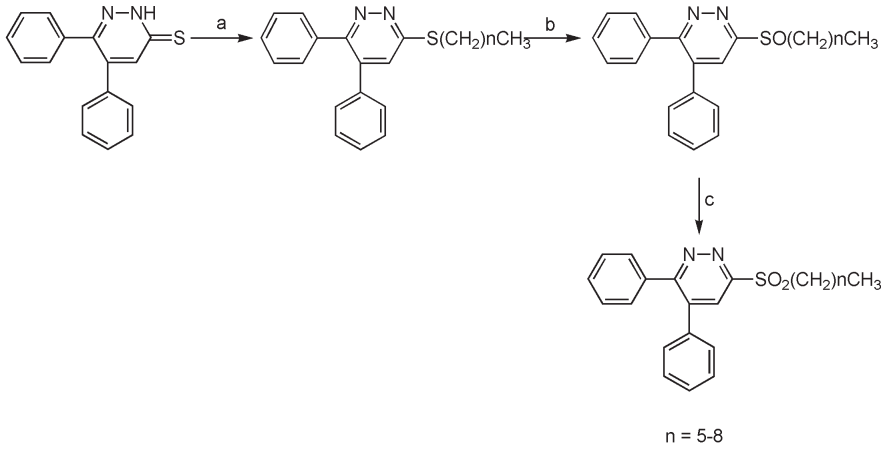
We considered nitrogen, oxygen and sulphur in different oxidation states as linkers between the heterocycle and alkyl chain. Compounds where X = NH, NHCO and O were synthesized following Scheme 1. The known piridazin-3-one in turn obtained starting from desoxybenzoin [14], was converted into the corresponding chloropyridazine that under appropriate conditions gave the desired products [15].

Scheme 1



a) $POCl_3$, Δ
a) for X=NH: RNH_2, Δ
for X=NHCO: 1) 30% aq. NH_3
2) RCOCl, r.t.
for X=O : RoNa, toluene, Δ

Scheme 2

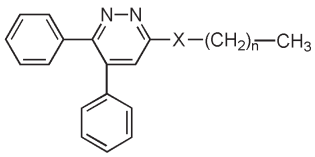


a) $R(CH_2)_nBr$, K_2CO_3, r.t.
b) CH_3COOH, 30% H_2O_2, r.t.
c) *m*.CPBA, r.t.

When X = S, the pyridazin-3-one was first converted into the corresponding thioderivative by treatment with Lawesson's reagent, then reacted with the suitable alkyl bromide. Oxidation of the sulfides gave the corresponding sulfoxides and solfones [15] (Scheme 2).

All the compounds were tested on ACAT extracted from rat liver microsomes. The natural inhibitor GERI-BP001 M, which in this assay shows IC_{50} = 42 μM, was used as reference compound (Table1). The best activity was obtained when X = NH and n = 8 as in derivative **6**. Shortening the side chain to n = 5 (compound **5**) still retained good activity. On the contrary, the insertion of an amide instead of the amino group in 3, brought about a complete loss of activity. The presence of any other heteroatom than nitrogen always led to less potent compounds.

Table 1



Compound	X	**n**	**IC_{50}** (μM) [a]
1	O	5	72
2	S	5	62
3	SO	5	75
4	SO_2	5	60
5	NH	5	24
6	NH	8	18
7	NHCO	4	>300

[a] IC_{50} evaluated toward ACAT extracted from rat liver microsomes (reference compound GERI-BP00 M IC_{50}= 42 μM).

On these bases, the subsequent SAR studies were limited to the following substrate (Fig. 3). In particular, we considered several parameters:

- length and nature of the side chain, e.g. linear, branched, cyclic
- introduction of an additional heterocycle in the side chain
- presence of substituents on the *ortho*-biphenyl system
- importance of the *ortho*-biphenyl system.

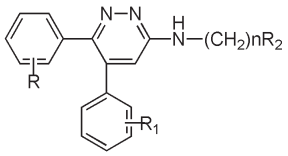
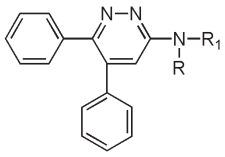


Figure 3

Table 2



Compound	R	R_1	IC_{50} (μM) [a]
8	H	cyclopentyl	46
9	H	cyclohexyl	3.6
10	H	cycloeptyl	25
11	H	phenyl	4.5
12	H	4-CF_3-phenyl	36
13	CH_3	cyclohexyl	31
Ref.	H	n-nonyl	18

[a] IC_{50} evaluated toward ACAT extracted from rat liver microsomes

Initially we investigated the substitution of the linear alkyl side chain with a cyclic mojety [16]. As shown (Table 2) compounds **9** and **11**, which present an alkyl and an aromatic six membered ring, respectively, showed the best activity. However it should be noted that both contracting (compound **8**) and enlarging (compound **10**) the ring size as well as introducing a substituent on the phenyl ring (**12**) caused a dramatic loss of activity. The same negative result was obtained by transforming the secondary amine of **9** into a tertiary amine as in **13**.

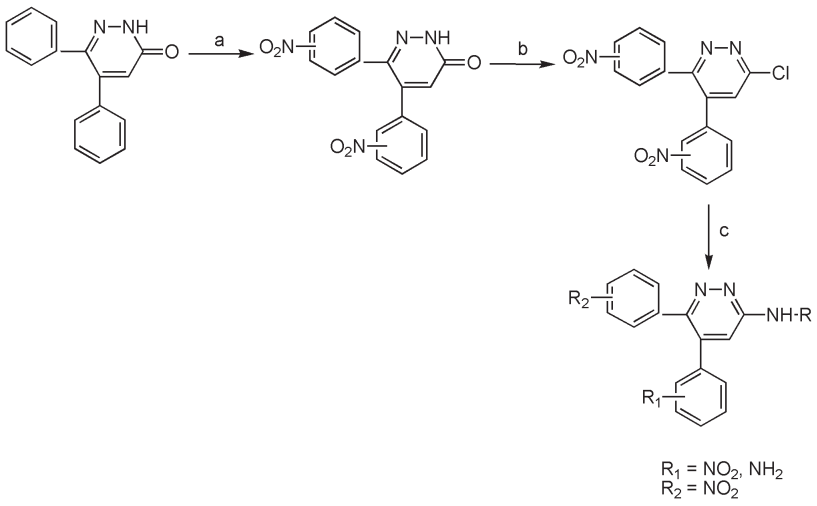
These data seem to suggest that the enzyme has good tolerance for electronic variations, but more stringent steric constrains.

To evaluate the influence of the presence of substituents on the *ortho*-biphenyl system, a series of nitro/aminophenyl derivatives was initially considered. Their synthesis is reported in Scheme 3.

After nitration, the pyridazin-3-ones were converted into their corresponding chloropyridazines that were reacted with the required amine. The complex mixture obtained was purified by chromatography and the attribution of the structures was done mainly on ^{1}H-NMR bases [17].

The results of the biological tests (Table 3), reported only for two representative products, clearly showed that the presence of electronwithdrawing groups (**14**) caused a significant loss of activity, while the presence of an NH_2 group as in **15**, restored it. The values of the molecular electrostatic potentials, which were calculated both for the substituted and unsubstituted terms, suggested that the introduction of substituents that made the pyridazine ring

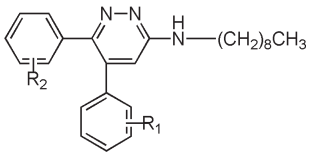
Scheme 3



a) HNO_3/H_2SO_4, r.t.
b) $POCl_3$, Δ
c) RNH_2, Δ

more electron-rich would lead to an enhancement of the activity.

Table 3

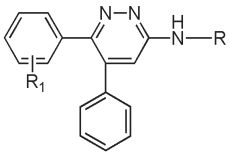


Compound	R_1	R_2	% inh. [a] (at 200 mg/ml)	V (NH)	V (N1)	V (N2)
14	*p*.NO_2	*m*.NO_2	53.5	46.6	-51.4	-52.6
15	*p*.NH_2	*m*.NO_2	77.6	77.6	-60.1	-62.6
Ref.	H	H	74.4	74.4	-65.9	-64.7

[a] % inh. evaluated toward ACAT extracted from rat liver microsomes

On these bases, we synthesized and tested a series of chloro and methoxy analogues [18] (Table 4). The significant activity showed by **16** with respect to its unsubstituted analogue seems to confirm our hypothesis, which is further validated by the values of the MEP of this new series. In addition, it should be noted that the chlorine atom of **16** is probably important in the interaction with the active site of the enzyme and that the length of the side chain might modulate this interaction, as is evident if we compare **16** and its longest analogue **17**. On the contrary, the length of the chain does not affect the activity of the methoxy substituted compounds.

Table 4

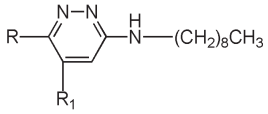


Compound	R	R_1	IC_{50} (μM) [a]	V(NH)	V(N1)	V(N2)
16	$(CH_2)_5CH_3$	*p*.Cl	1.0	36.9	-62.8	-62.7
17	$(CH_2)_8CH_3$	*p*.Cl	80	36.2	-62.7	-62.6
18	$(CH_2)_5CH_3$	*p*.OCH_3	11	35.4	-65.3	-64.3
19	$(CH_2)_8CH_3$	*p*.OCH_3	8.9	35.0	-65.4	-64.2
Ref.	$(CH_2)_5CH_3$	H	24	35.8	-66.4	-64.8

[a] IC_{50} evaluated toward ACAT extracted from rat liver microsomes.

Finally, the role of the *ortho*-biphenylic system on this series was evaluated, by eliminating alternatively the 5-and 6-phenyl. As can be seen in Table 5, while the 5-monosubstituted phenyl (**20**) retains some activity with respect to the biphenyl (**6**), the 6-phenyl derivative (**21**) is inactive.

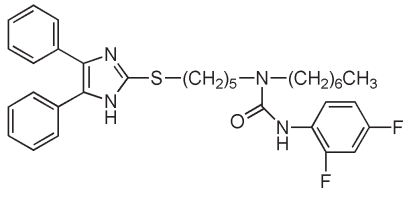
Table 5



Compound	R	R_1	IC_{50} (μM) [a]
20	H	Ph	105
21	Ph	H	N.A.
6	Ph	Ph	18

[a] IC_{50} evaluated toward ACAT extracted from rat liver microsomes.

More recently, we have extended our investigations to a new series of pyridazine derivatives, which present an ureido mojety in their structure. The rational is based on the observation that, as already seen, many very potent ACAT inhibitors, e.g. **DuP 128** [19] present this functionality (Figure 4).



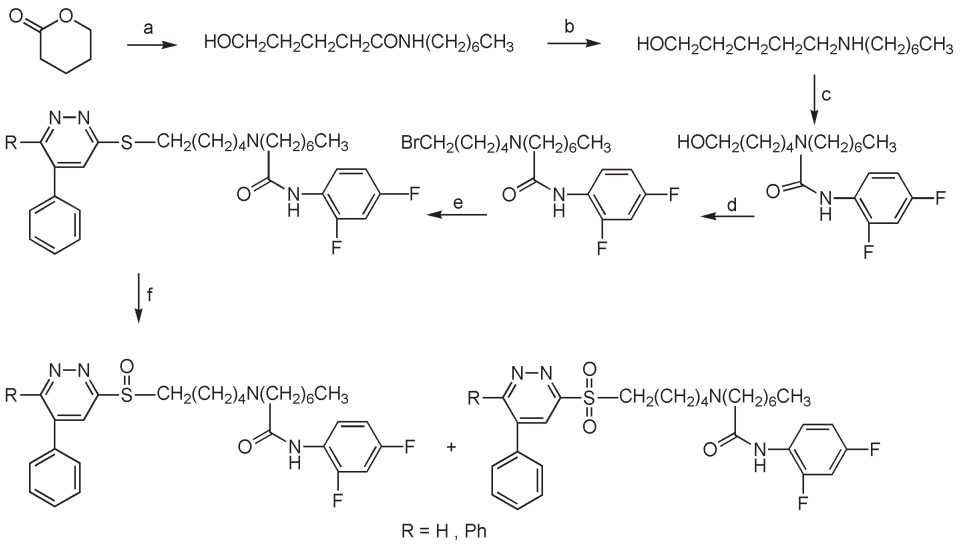
DuP 128

[DuPont Merck Lab., IC50 = 10 nM]

Figure 4

In analogy with the DuP model, we initially considered compounds where X = S, SO, SO_2; however, on the bases of our previous results, we also considered compounds where X = NH. The synthesis [20] of the first series was accomplished according to Scheme 4.

Scheme 4



a) $NH_2(CH_2)_6CH_3$, toluene, Δ
b) $LiAlH_4$, THF
c) 2,4-difluoro-phenylisocyanate, 0°C K_2C_3, Δ
d) CBr_4, PBr_3, r.t.
e) 5-phenyl or 5,6biphenyl-*2H*-pyridazin-3-thione, K_2CO_3, Δ
f) Oxone, r.t.

The thioderivatives were synthesized starting from δ-valerolacton, that was converted into its hydroxyamide and reduced to the corresponding amine. The latter reacted with 2,5-diF-phenylisocianate to give the hydroxy-ureido derivative and after substitution with PBr_3, the intermediate was reacted with the suitable pyridazin-3-one. The sulfides obtained, by oxidation gave the corresponding sulfoxides and sulfones . The compounds were purified by chromatography.

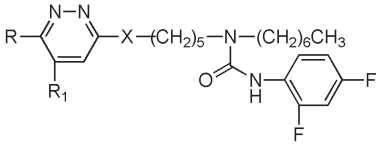
The results of the biological assays (Table 6) showed that both the mono- and the biphenyl sulfides (**22** and **25**) were slightly more active in comparison with their corresponding sulfoxides and sulfones. It should be noted that, as seen in the previous series, also in this case the 6-phenyl does not seem to be important for activity.

However, once more the best results were obtained with the substitution of S with N. In particular the 5,6-biphenyl derivative **30** and the 6-phenyl **29** were the most active, while the 5-phenyl was almost devoid of activity.

In conclusion, our investigations studied the effects of the substitution of the imidazole ring by a pyridazine ring in a series of potent ACAT inhibitors. Our hope was to find better features for these compounds, which are quite often accompanied by severe side effects.

In particular, we evaluated modifications on the heteroatom, the side chain and the *o*-biphenyl system and we evidenced several characteristics, that could represent an interesting enhancement of knowledge on the essential requirements for good inhibitors.

Table 6



Compound	X	R	R_1	% inh. [a] (at 50 μg/ml)
22	S	H	Ph	75
23	SO	H	Ph	62
24	SO_2	H	Ph	67
25	S	Ph	Ph	75
26	SO	Ph	Ph	65
27	SO_2	Ph	Ph	70
28	NH	H	Ph	46
29	NH	Ph	H	89
30	NH	Ph	Ph	87

[a] % inh. evaluated toward ACAT extracted from rat liver microsomes (reference compound GERI-BP001 M % inh. =83)

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