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Novel 3-Arylamino- and 3-Cycloalkylamino-5,6-diphenyl-pyridazines Active as ACAT Inhibitors

A new series of pyridazine derivatives, structurally related to the previously reported ACAT inhibitors 3-(cyclo)alkylamino-5,6-diphenyl-pyridazines, were synthesized and tested for their inhibitory properties. Substitution of the 3-alkylamino chain with a phenylamino group maintains activity. In contrast, the presence of either substituents on the phenylamino group or aliphatic rings having more or less than six carbon atoms lowers it.

Key Words: Hypercholesterolemia, Acyl-CoA:cholesterol acyltransferase (ACAT), ACAT inhibitors, 5,6-Diphenylpyridazine derivatives

Received: May 31, 2002 [FP704]

Full Paper

Introduction

Acetyl-CoA:cholesterol *O*-acyl transferase (EC 2.3.1.26; ACAT) is a microsomal enzyme that catalyzes the formation of long chain fatty acid cholesterol esters [1, 2]. Inhibition of ACAT reduces intracellular cholesterol esters that are substrates for steroidogenesis in adrenal cells [3]. Though the adrenal side effects of ACAT inhibitors remain a key point for their development as antiatherosclerotic agents, a number of them are currently in preclinical and clinical studies [4]. With the aim of finding compounds possibly devoid of such unwanted side effects, we have recently undertaken a study on a series of pyridazine derivatives (**I**, Chart 1), which combine two main characteristics of the reported ACAT inhibitors, namely a long alkylic side chain and an *ortho*-diphenyl system [5]. Further investigation on the model **I** demonstrated the ability of substituents on the 5- and 6-phenyl rings to modulate the inhibitory properties of this series [6, 7]. In addition, the substitution of the *n*-alkylamino group at C-3 with a cyclohexylamino group retained the activity [8]. We have now directed our studies to verify the importance of the *n*-alkyl- or cyclohexylamino side chain at position 3 of **I**. For this purpose, we synthesized a new series of derivatives (**1a**–**p**), where either a cycloalkyl moiety of size different from cyclohexyl or a (substituted)phenyl was present. This paper reports their synthesis and the results of the enzyme assay. In addition, attempts to correlate the activity of the compounds to their structural features are commented upon.

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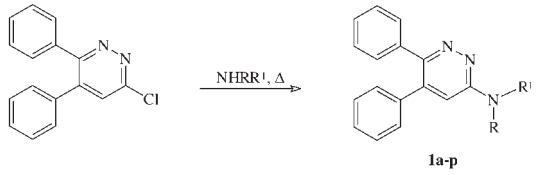
Chemistry

The compounds were prepared from 3-chloro-5,6-diphenylpyridazine [9] by heating at 140 °C in a sealed tube with the appropriate amine in a molar ratio of 1:2. Usual workup gave the desired compounds which were finally crystallized from an appropriate solvent (see Scheme 1).

 0365-6233/11/0563 $ 17.50+.50/0

Chart 1



Scheme 1

Enzyme assay

All the compounds obtained were tested for their potency to inhibit ACAT extracted from rat liver microsomes, according to a previously reported method [10]. **GERI-BP001 M** was used as reference. Inhibition values are listed in Tables 1 and 2.

Results and discussion

The substitution of the *n*-alkylamino with a cycloalkylamino chain, which was reported by us to retain the ACAT inhibition in the case of the cyclohexyl derivative **1 a** [8], was further evaluated in the series of compounds reported in Table 1. The compounds bearing a cyclopentyl (**1 b**) and a cycloheptyl (**1 c**) moiety show a

Table 1. Chemical and biological data of compounds **1 a**–**e**.

Compd.	R	R^1	Reaction time [h]	Yield [%]	Formula	Mp [°C]	IC_{50}[a] [μM]
1 a	H	cyclohexyl	5	42	$C_{22}H_{23}N_3$	182–184	3.6
1 b	H	cyclopentyl	4	95	$C_{21}H_{21}N_3$	180–182	46
1 c	H	cycloheptyl	4	60	$C_{23}H_{25}N_3$	198–199	25
1 d	CH_3	cyclohexyl	7	80	$C_{23}H_{25}N_3$	160–162	31
1 e	H	C_6H_5	3	95	$C_{22}H_{17}N_3$	232–234	4.5

[a] In vitro ACAT inhibition determined in rat liver microsomes. IC_{50} values are from three experiments which agreed within 10 %. In the same test **GERI-BP001 M** showed an IC_{50} value of 42 μM.

Table 2. Chemical and biological data of compounds **1 f**–**p**.

Compd.	R	R^1	Reaction time [h]	Yield [%]	Formula	Mp [°C]	% Inhibition[a]
1 f	H	3-OCH_3-C_6H_4	3	72	$C_{23}H_{19}N_3O$	200–202	83
1 g	H	4-OCH_3-C_6H_4	48	68	$C_{23}H_{19}N_3O$	227–229	79
1 h	H	3-CF_3-C_6H_4	2	14	$C_{23}H_{16}N_3F_3$	>300	69
1 i	H	4-CF_3-C_6H_4	3	92	$C_{23}H_{16}N_3F_3$	>300	92 (36)
1 j	H	3-Cl-C_6H_4	3	57	$C_{22}H_{16}N_3Cl$	233–235	76
1 k	H	4-Cl-C_6H_4	2.5	65	$C_{22}H_{16}N_3Cl$	276–279	77
1 l	H	4-Br-C_6H_4	6.5	33	$C_{22}H_{16}N_3Br$	278–280	79
1 m	H	4-*i*-C_3H_7-C_6H_4	3.5	36	$C_{25}H_{23}N_3$	153–155	80
1 n	H	4-*n*-C_7H_{15}-C_6H_4	3	80	$C_{29}H_{31}N_3$	154–157	72
1 o	CH_3	C_6H_5	10	95	$C_{23}H_{19}N_3$	139–141	94 (39)
1 p	H	$CH_2C_6H_5$	48	82	$C_{23}H_{19}N_3$	178–180	36

[a] In vitro percent inhibition of ACAT from rat liver microsomes at a final concentration of 200 μg/mL. In parenthesis IC_{50}/μM. Values are from three experiments, which agreed within 10 %. In the same test **GERI-BP001 M** showed an inhibition percentage of 88 and **1 e** showed an inhibition percentage of 85.

decrease in activity by about one degree of magnitude with respect to **1a**. A similar decrease also results in the case of the tertiary amino compound **1d**. Conversely, aromatization of the ring to **1e** maintains activity (IC_{50} = 4.5 μM as against 3.6 μM in the case of **1a**). Attempts to modulate the activity by introduction of substituents on the 3-phenylamino moiety were also undertaken. For this purpose, a series of substituted-phenyl derivatives were tested at a concentration of 200 μg/mL. The values of their inhibition percentages are reported in Table 2. At this concentration two compounds, **1i** and **1o**, presented good values of the inhibition percentages while the other compounds were poorer inhibitors than **1e**. However, a net decrease of activity was evident also for **1i** and **1o** when their IC_{50} values were determined. Thus, the overall data in Table 2 show that the electronic nature of the substituents on the phenyl ring of the side chain does not play an important positive role. This was also confirmed by the fact that theoretical studies on the compounds failed to establish any correlation of the molecular electrostatic potential on several points of their molecular surface (Table 3) with the inhibitory activity. It should be noted that the substitution of the phenyl ring by a benzyl group led to an almost inactive compound (**1p**), thus indicating a possible conjugation of the system. However, this would not explain why the phenyl- and the cyclohexyl derivatives have almost the same activity. On the contrary, the importance of the secondary amine seems to be clearly evidenced in both classes, as shown by the considerable loss of potency of the N-Me derivatives **1d** and **1o** compared to **1a** and **1e**, respectively.

Table 3. Molecular electrostatic potential on the molecular surface of compounds **1e**–**n**, **p**. Values of the electrostatic potential minima (*V*, kcal/mol) generated by the two nitrogen atoms of the pyridazine ring (N1 and N2) and of the maximum generated by the NH group.

Compd.	*V*(NH)	*V*(N1)	*V*(N2)
1e	38.7	–65.4	–65.6
1f	39.4	–63.5	–62.8
1g	38.3	–65.4	–65.3
1h	43.4	–61.2	–59.0
1i	45.4	–60.7	–58.6
1j	40.7	–63.5	–62.7
1k	41.5	–63.5	–62.6
1l	42.0	–63.2	–62.2
1m	38.2	–65.6	–66.2
1n	38.4	–65.5	–66.1
1p	32.9	–66.4	–64.3

In conclusion, the data of these new series of ACAT inhibitors seem to suggest that the long alkyl chain, which is supposed to mimic the natural substrate of ACAT [11], is not an essential requirement for this class of pyridazine inhibitors. In fact, they indicate a high tolerance of the enzyme both to phenyl and cyclohexyl moieties. However, the inhibitory activity is lowered by substituents on the phenylamino group and by cycloalkyl derivatives of ring size different from six. This seems to suggest that some limitations connected to steric hindrance are operative in the modulation of activity.

Table 4. ^{1}H-NMR data of compounds **1a**–**p** ($CDCl_3$; chemical shifts in ppm).

Compd.	^{1}H-NMR
1a	1.20–2.20 (m, 10 H); 3.70–3.80 (m, 1 H); 5.60 (br s, 1 H, exch with D_2O); 6.70 (s, 1 H); 7.10–7.40 (m, 10 H)
1b	1.40–2.20 (m, 8 H); 4.00–4.20 (m, 1 H); 4.90 (br s, 1 H, exch with D_2O); 7.0 (s, 1 H); 7.20–7.80 (m, 10 H)
1c	1.40–2.20 (m, 12 H); 3.90–4.00 (m, 1 H); 6.0 (br s, 1 H, exch with D_2O); 6.75 (s, 1 H); 7.10–7.50 (m, 10 H)
1d	1.00–2.00 (m, 10 H); 3.10 (s, 3 H); 4.50–4.75 (m, 1 H); 6.75 (s, 1 H); 7.10–7.40 (m, 10 H)
1e	7.05 (s, 1 H); 7.20–7.60 (m, 15 H); 11.90 (br s, 1 H, exch with D_2O)
1f	3.80 (s, 3 H); 6.85–7.15 (m, 5 H); 7.20–7.50 (m, 10 H); 11.70 (br s, 1 H, exch with D_2O)
1g	3.90 (s, 3 H); 6.95–7.20 (m, 5 H); 7.25–7.50 (m, 10 H); 11.70 (br s, 1 H, exch with D_2O)
1h[a]	7.20–7.50 (m, 15 H); 11.50 (br s, 1 H, exch with D_2O)
1i[a]	6.65 (d, 2 H); 7.20–8.00 (m, 13 H); 11.80 (br s, 1 H, exch with D_2O)
1j	7.00 (s, 1 H); 7.10–7.55 (m, 14 H); 12.00 (br s, 1 H, exch with D_2O)
1k	6.95 (s, 1 H); 7.00–7.25 (m, 14 H); 11.40 (br s, 1 H, exch with D_2O)
1l	7.00 (s, 1 H); 7.15 (d, 2 H); 7.20–7.50 (m, 10 H); 7.60 (d, 2 H); 11.90 (br s, 1 H, exch with D_2O)
1m	1.30 (d, 6 H); 2.90 (m, 1 H); 7.00–7.50 (m, 15 H); 11.55 (br s, 1 H, exch with D_2O)
1n	0.95 (m, 3 H); 1.20–1.40 (m, 8 H); 1.50–1.70 (m, 2 H); 2.60 (t, 2 H); 7.10–7.45 (m, 15 H); 8.65 (br s, 1 H, exch with D_2O)
1o	3.80 (s, 3 H); 6.85 (s, 1 H); 7.00–7.55 (m, 15 H)
1p	4.70 (d, 2 H); 6.90 (s, 1 H); 7.00–7.50 (m, 15 H); 7.80 (br s, 1 H, exch with D_2O)

[a] DMSO-d_6.

Experimental

Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane. TLC on silica gel plates was used to check product purity. The structures of all compounds were consistent with their analytical and spectroscopic data.

General method for the synthesis of compounds **1 a**–**p**

A mixture of 3-chloro-5,6-diphenylpyridazine [9] (1 mmol) and the appropriate amine (2 mmol) was heated at 140 °C in a sealed tube for a time ranging from 2.5 to 48 hours, depending on the nature of the amine. After cooling, the mixture was poured onto water and extracted with CH_2Cl_2 (3 × 20 mL). The solvent was dried over Na_2SO_4, filtered, and evaporated under vacuum. The resulting crude **1 a**–**p** were crystallized from ethanol. See Tables 1 and 2 for details and Table 4 for NMR data.

Enzyme assay

Microsomes prepared from rat liver were used as a source of the enzyme. The activity of the ACAT inhibitors was measured according to a previously described method [10]. Accordingly, the reaction mixture containing 4 µL of microsomes (10 mg/mL protein), 20 µL of 0.5 M potassium-phosphate buffer, 15 µL of bovine serum albumin, 2 µL of cholesterol in acetone, 41 µL of water, and 10 µL of the test sample was preincubated for 30 minutes at 37 °C. The reaction was initiated by the addition of 8 µL of [1-^{14}C]oleoyl-CoA solution and stopped after 15 minutes by the addition of 1.0 mL of isopropanol-heptane (4:1; *v/v*) solution. After work-up, the radioactivity was measured using a liquid scintillation counter (Packard Delta-2000). Background values were obtained by preparing heat-inactivated microsomes.

Theoretical calculations

Theoretical calculations were performed with the SPARTAN package [12] using the AM1 [13] semiempirical method and were carried out at the RHF level. The geometry of all the compounds investigated was fully optimized and energy minimized. Several optimizations from different starting geometries were performed to take into account the possible conformers of each compound. The molecular electrostatic potential (MEP) was calculated on the molecular surface of the global minimum of each compound at the AM1 level.

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