5,6-Diphenylpyridazine Derivatives as Acyl-CoA:Cholesterol Acyltransferase Inhibitors

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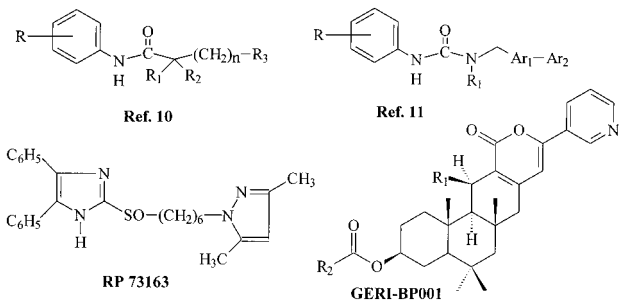
Alkyl-5,6-diphenylpyridazine derivatives combining several main features of ACAT inhibitors, such as a long alkyl side chain linked to a heterocycle and the *o*-diphenyl system, were synthesized and tested. Moreover, modeling studies on representative terms were performed. Some compounds displayed ACAT inhibition in the micromolar range, both on the enzyme isolated from rat liver microsomes and in cell-free homogenate of murine macrophages.

Introduction

Coronary heart disease is the major cause of death in western industrialized countries,[1] and hypercholesterolemia was found to be the leading risk factor for its development.[2] Acyl-CoA:cholesterol O-acyltransferase (ACAT) is an intracellular enzyme responsible for the catalysis of the esterification of free cholesterol in various tissues.[3] Moreover it plays an important role in the absorption[4] of cholesterol, in secretion of very low-density lipoproteins (VLDL),[5] and in the accumulation of cholesteryl ester in foam cells, which were recovered in atheromatous plaques.[6] Therefore the inhibition of ACAT enzyme could represent a good therapeutic approach.[7] Among different chemical classes of ACAT inhibitors,[8,9] interesting activity was shown by anilidic derivatives of fatty acids, in which the long alkylic chains mimic the natural substrate of ACAT, and by ureas derivatives.[10,11] Moreover, quite interesting results were obtained by Rhone-Poulenc Rorer with the synthesis of a class of 2-substituted-4,5-diphenyl-1*H*-imidazole in which a long side alkyl chain, which can be also embodied in a cycle, is linked to the heteroaromatic ring through a sulfur atom with different oxidation state.[12] Among these derivatives, compound RP73163 is a representative member.[13] In addition, several compounds with the same pharmacological activity such as GERI-BP001,[14] were identified from microbial origin (Chart 1). The pyridazine ring has often proved to be a suitable moiety for biologically active compounds,[15,16] but to our knowledge, it has never been exploited as a possible substrate for ACAT inhibitors. On these bases we report here the synthesis and biological properties of new compounds (**2**, **3**, **5**, **7**−**10**) which combine some of the characteristics of the reported ACAT inhibitors, namely a long alkylic side chain linked to a heterocyclic ring by a heteroatom of different nature and the *o*-diphenyl system.

Chart 1



Chemistry

2-Substituted-5,6-diphenylpyridazin-3(2*H*)-ones (**2**) were obtained from **1**[17] by alkylation with the appropriate bromo derivative in dimethylformamide and in the presence of anhydrous potassium carbonate. Alkaline hydrolysis of the ester **2k**−**m** and subsequent acidification led to the corresponding acids **3a**−**c**. The pyridazine derivatives **5a**−**j** were obtained by condensation of **4**[17] and the required alkylamines or sodium alcoholates. The amidic derivative **5e** was obtained from **4** by replacement of the chlorine in 3-position by an amino group using aqueous ammonia, followed by acylation with hexanoyl chloride. The regiochemistry of the last step was assigned on the basis of the ^{1}H NMR data of the compound, and eventually confirmed by reduction of **5e** to **5a** by $LiAlH_4$ in anhydrous diethyl ether. (See Scheme 1.)

The thioderivatives **7**−**10** were prepared from thione (**6**).[18] Alkylation with the appropriate bromo derivative gave compounds **7**. Treatment of **7a**−**d** by 30% H_2O_2 in CH_3COOH at room temperature afforded the sulfoxides **8**, which were further oxidized to **9** by *m*-chloroperbenzoic acid in dichloromethane at room temperature. Compounds **10** were easily obtained from **7e**−**h** by alkaline hydrolysis (Scheme 2).

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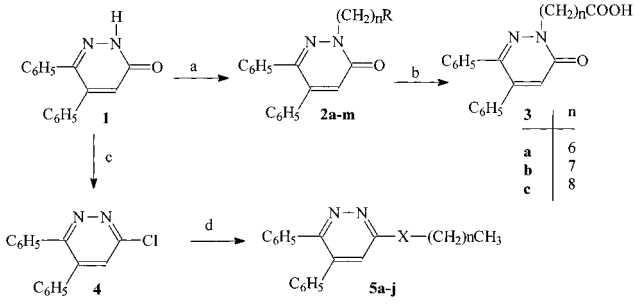
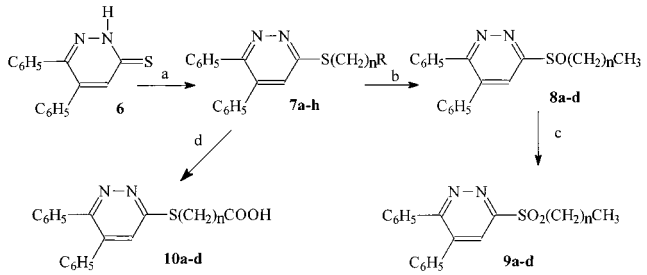
Scheme 1[a]



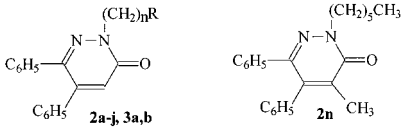
(a) R(CH2)nBr, DMF, K2CO3, 20-50°C; (b) for **2k-m** (n=6-8, R=COOEt): 6 N NaOH, EtOH, rt; (c) POCl3, Δ; (d) for X=NH, NH2(CH2)nCH3, 160°C; for X=NHCO, 30% aq NH3, 150°C and successively CH3(CH2)4COCl, rt; for X=O, CH3(CH2)nONa, toluene.

Scheme 2[a]



(a) R(CH2)nBr, DMF, K2CO3, rt; (b) for **7a-d** (n=5-8, R=CH3) CH3COOH, H2O2 30%, rt; (c) MCPBA, CH2Cl2, rt; (d) for **7e-h** (n=5-8, R=COOEt) 6 N NaOH, EtOH, rt.

Table 1. Chemical Data and ACAT Inhibition of Pyridazinone Derivatives



compd	R	*n*	% yield[a]	mp (°C)	formula[b]	IC_{50} (μM)[c]
2a	CH_3	2	55	oil	$C_{19}H_{18}N_2O$	230 ± 15
2b	$CH(CH_3)_2$	0	56	138−140	$C_{19}H_{18}N_2O$	152 ± 8.5
2c	CH_3	3	58	oil	$C_{20}H_{20}N_2O$	176 ± 11
2d	CH_3	4	59	oil	$C_{21}H_{22}N_2O$	66 ± 4.6
2e	CH_3	5	86	oil	$C_{22}H_{24}N_2O$	57 ± 2.2
2f	CH_3	6	72	oil	$C_{23}H_{26}N_2O$	86 ± 5.1
2g	CH_3	7	87	54−56	$C_{24}H_{28}N_2O$	80 ± 5.2
2h	CH_3	8	77	oil	$C_{25}H_{30}N_2O$	85 ± 3.7
2i	cyclopentyl	0	55	155−157	$C_{21}H_{20}N_2O$	119 ± 9.6
2j	cyclohexyl	0	35	161−163	$C_{22}H_{22}N_2O$	>300
2n			58	oil	$C_{23}H_{26}N_2O$	56 ± 2.5
3a	COOH	6	70	135−137	$C_{23}H_{24}N_2O_3$	>300
3b	COOH	7	75	112−115	$C_{24}H_{26}N_2O_3$	>300
RP73163						0.1
GERI-BP001M						42 ± 1.7

[a] From **1**. [b] C, H, N analysis within ±0.4%. [c] In vitro ACAT inhibition determined in rat liver microsomes. IC_{50} values are mean from three experiments.

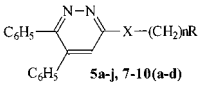
Results and Discussion

All compounds were tested for their inhibitory properties toward ACAT extracted from rat liver microsomes.[14] In the 2-substituted pyridazinone series (Table 1), activity was displayed by compounds bearing a long linear alkyl chain, the optimum being found for $n = 5$ (compound **2e**; $IC_{50} = 57$ μM). Introduction of a methyl group at position 4 of this compound (compound **2n**) left the activity completely unchanged, suggesting that a small lipophilic group can easily be accommodated. Longer chains up to 9 carbon atoms as well as shortening by one carbon atom were well tolerated (compounds **2f**−**h**, **2d**). On the contrary, the presence of shorter alkyl chains as well as cyclic residues and terminal carboxylic acid groups always led to very weak or completely inactive compounds. As far as the pyridazine series is concerned (see Table 2), compounds bearing an oxygen or a sulfur atom at position 3, as well as its oxidized derivative SO, showed a quite similar trend. In fact, the best activity was displayed when $n = 5$, 6 while it tended to decrease for longer alkyl substituents. Also in this case, the presence of a terminal carboxy group (compounds **10a**−**d**) led to inactive compounds. Further oxidation to SO_2 derivatives gave better inhibitors (**9b**, **9c**; $IC_{50} = 49$ and 36 μM, respectively). However, the most potent compounds were found in the amino derivative series, and the two most active (**5a**, **5d**; $IC_{50} = 24$ and 18 μM) have the shortest ($n = 5$) and the longest ($n = 8$) chains, respectively. Substitution of the amino by an amido group (compound **5e**) resulted in an inactive derivative.

To better define the pharmacological properties of this series, three significant compounds (**5c**,**d**; **5h**), were further evaluated on macrophages.[19] Compound 58-035[9] was used as a control.

The three compounds inhibit cholesterol esterification in macrophages enriched with cholesterol, an in vitro model of foam cells,[20] without affecting cholesterol efflux and cell viability[21] (Table 3). These results, together

Table 2. Chemical Data and ACAT Inhibition of Pyridazine Derivatives



compd	X	*n*	R	% yield[a]	mp (°C)	formula[b]	IC_{50} (μM)[c]
5a	NH	5	CH_3	64	120−122[d]	$C_{22}H_{25}N_3$	24
5b	NH	6	CH_3	68	104−107[g]	$C_{23}H_{27}N_3$	55
5c	NH	7	CH_3	50	113−115[d]	$C_{24}H_{29}N_3$	40
5d	NH	8	CH_3	61	111−113[d]	$C_{25}H_{31}N_3$	18
5e	NHCO	4	CH_3	81	217−219[h]	$C_{22}H_{23}N_3O$	>300
5f	O	4	CH_3	95	oil	$C_{21}H_{22}N_2O$	73
5g	O	5	CH_3	84	oil	$C_{22}H_{24}N_2O$	72
5h	O	6	CH_3	70	oil	$C_{23}H_{26}N_2O$	58
5i	O	7	CH_3	70	oil	$C_{24}H_{28}N_2O$	104
5j	O	8	CH_3	62	oil	$C_{25}H_{30}N_2O$	180
7a	S	5	CH_3	79	66−67[d]	$C_{22}H_{24}N_2S$	62
7b	S	6	CH_3	91	oil	$C_{23}H_{26}N_2S$	76
7c	S	7	CH_3	77	oil	$C_{24}H_{28}N_2S$	130
7d	S	8	CH_3	74	oil	$C_{25}H_{30}N_2S$	116
8a	SO	5	CH_3	77[e]	95−97[d]	$C_{22}H_{24}N_2OS$	75
8b	SO	6	CH_3	81[e]	oil	$C_{23}H_{26}N_2OS$	77
8c	SO	7	CH_3	79[e]	oil	$C_{24}H_{28}N_2OS$	92
8d	SO	8	CH_3	75[c]	oil	$C_{25}H_{30}N_2OS$	87
9a	SO_2	5	CH_3	91[f]	oil	$C_{22}H_{24}N_2O_2S$	60
9b	SO_2	6	CH_3	88[f]	oil	$C_{23}H_{26}N_2O_2S$	49
9c	SO_2	7	R=CH_3	80[f]	oil	$C_{24}H_{28}N_2O_2S$	36
9d	SO_2	8	CH_3	70[f]	oil	$C_{25}H_{30}N_2O_2S$	76
10a	S	5	COOH	62	120−122[d]	$C_{22}H_{22}N_2O_2S$	>300
10b	S	6	COOH	58	126−128[d]	$C_{23}H_{24}N_2O_2S$	>300
10c	S	7	COOH	48	97−100[g]	$C_{24}H_{26}N_2O_2S$	>300
10d	S	8	COOH	57	oil	$C_{25}H_{28}N_2O_2S$	>300
RP73163							0.1
GERI-BP001M							42

[a] From **5**. [b] C, H, N analysis within ±0.4%. [c] In vitro ACAT inhibition determined in rat liver microsomes. IC_{50} values are means from three experiments. [d] Ethanol. [e] From **6**. [f] From **7**. [g] Ethanol/water 1:1. [h] Cyclohexane.

Table 3. Other Biological Properties of Compounds **5c**,**d** and **5h**

compd	cholesterol efflux [^{3}H]-cholesterol in the medium (dpm × 10^3/mg protein) (μM)	% inhibition of cell viability murine macrophages (μM)	macrophage ACAT inhibitory activity[a] (IC_{50}; μM)
control	12.9 ± 0.1		
58-035[b]	12.8 ± 1.4 (2.15)		0.6 ± 0.01
5c	12.9 ± 1.6 (5)	11.5 ± 3.5 (1)	3.2 ± 0.32
	11.5 ± 0.8 (25)	9.9 ± 1.6 (5)	
		3.7 ± 1.7 (10)	
		4.8 ± 3.7 (25)	
5d	14.1 ± 1.8 (5)	5.9 ± 1.9 (1)	11.0 ± 0.34
	13.8 ± 4.8 (25)	2.7 ± 2.7 (5)	
		<1 (10)	
		<1 (25)	
5h	12.6 ± 1.3 (5)	<1 (1)	5.1 ± 1.16
	11.8 ± 0.6 (25)	<1 (5)	
		<1 (10)	
		<1 (25)	

[a] IC_{50} values are means from three experiments. [b] See ref 9 for its structure.

with the observed ability of the tested compounds to reduce cholesterol esterification when added to cell-free homogenates, suggest that they influence cholesterol metabolism by specifically inhibiting ACAT activity. The differences in the compounds' activity on ACAT assay in macrophages and in rat liver microsomes, could be interpreted on the basis of the different properties of ACAT isoenzymes.[22,23] Moreover, it was reported that disruption of the gene for ACAT (*Acact*) in mice resulted in a marked reduction in cholesteryl ester levels in the adrenal glands and peritoneal macrophages; in contrast, the livers of these mice still contained significant amount of cholesteryl esters and exhibited no reduction in cholesterol esterification activity and in sterol absorption in the intestine.[24]

We performed modeling studies on several representative terms. The effect of the chain length has been discussed above and appears quite intriguing; in fact, while most of the results suggest a linear C_5 or C_6 alkyl chain as the optimal length, in some cases this is not true. An explanation of this behavior could reside in the nature of the X atom (or group) that could exert its effects by forcing the alkyl chain to assume different orientations or influencing the movements of the same chain with respect to the heterocyclic ring. Thus, a different chain length could be necessary in each series to accommodate it into the same binding site. The modeling study of compounds **2e**/**2n**/**5a**/**5g**/**7a**/**8a**/**9a**/ was performed at the semiempirical level with the AM1 program;[25] the conformational space of each compound was fully explored, and the conformers of minimum energy were located. The 5- and 6-phenyl groups can rotate, but they present similar energy minima and similar barriers to rotation in all the cases; on the contrary, when we determined the energy profiles for rotation around the single bond N2−C1′ (for **2e** and **2n**) or C3−X (for the other compounds), we found a large heterogeneity in the energy profiles. The energy barriers to rotation ranged from about 3 kcal/mol in **5a** to about 6 kcal/mol in **7a**. It is not easy to correlate these profiles with activity; however, the most active compound (**5a**) is the one that presents the better easiness of rotation. A different effect of the atom X on activity could derive from its influence on the electrostatic properties of the heterocyclic moiety. For the global minima of all the modeled compounds, the molecular electrostatic potential was calculated on the molecular surface and the value of the electrostatic potential minima generated by the heteroatoms of the heterocyclic moiety was determined. Once again, no safe correlations appear possible, as can be seen, for example, in Table 4, where the value of the potential in proximity of the N1 nitrogen atom of pyridazine or pyridazinone is reported. However, **5a** was the one that presents the more negative electrostatic potential minimum near the N1 nitrogen atom of pyridazine.

Table 4. Molecular Electrostatic Potential Values (kcal/mol) on the Molecular Surface, Calculated for the AM1 Optimized Conformations

	2e	**2n**	**5a**	**5g**	**7a**	**8a**	**9a**
V(N1)	−50.1	−51.2	−66.7	−64.0	−62.5	−59.3	−54.7

In conclusion, though not very potent, the compounds presented show appreciable preference toward ACAT in macrophages than in rat liver. Since accumulation of cholesteryl esters in macrophages results in foam cell formation and in a final atherosclerotic lesion, this class could represent a novel model to be investigated. Finally, our results seem to suggest that an easier rotation of the alkyl chain and a more negative electrostatic potential near the pyridazine ring are elements that favor a good activity.

Experimental Section

Chemistry. All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4, and the solvents were removed under reduced pressure. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography.

General Procedure for Compounds 2a−m, 7a−h, 3a−c, and 10a−d. A mixture of 5,6-diphenylpyridazin-3(2*H*)-one (**1**)[17] or its thioanalogue (**6**)[18] (1.6 mmol), anhydrous K_2CO_3 (1.8 g, 13 mmol), and the appropriate (cyclo)alkyl bromide (15 mmol) or alkyl bromoester in anhydrous DMF (6 mL) was heated at 40−80 °C under stirring for 2−48 h. After dilution with cold water (150 mL), the suspension was extracted with CH_2Cl_2 (3 × 50 mL). Removal of the solvent afforded a crude oil which crystallized by treatment with EtOH for compounds **2b**, **2i**, and **7a**; all other compounds were purified by column chromatography. Eluents: cyclohexane/ethyl acetate 2:1. Esters **2k−m** and **7e−h** were recovered as crude products and hydrolyzed in an alkaline medium to give compounds **3a−c** and **10a−d**.

2-Hexyl-4-methyl-5,6-diphenylpyridazin-3(2*H*)-one 2n. The 5,6-diphenyl-4,5-dihydropyridazin-3(2*H*)-one[17] (0.4 g, 1.6 mmol) was heated with 40% aqueous CH_2O in 5% ethanolic KOH (20 mL) at 100 °C for 30 min. After cooling, acidification with 6 N HCl afforded the crude 5,6-diphenyl-4-methylpyridazin-3(2*H*)-one,[26] which was recovered by suction and reacted with *n*-hexylbromide as above-reported for **2a−j** to give **2n**, finally purified by column chromatography (cyclohexane/ethyl acetate 2:1).

General Procedure for Amines 5a−d. 3-Chloropyridazine (**4**; 1.5 mmol) and the appropriate alkylamine (150 mmol) were heated at 160 °C in a sealed tube for 3 h. After dilution with water (10 mL), the mixture was extracted with CH_2Cl_2 (3 × 20 mL) and concentrated in vacuo. The oil residue

crystallized by treatment with ice−ethanol (**5a**,**b**) or ethanol/water 1:1 (**5c**,**d**).

***N*-(5,6-Diphenylpyridazin-3-yl)hexanoic Amide 5e.** 3-Chloropyridazine (**4**; 0.1 mmol) and NH_3 30% (6 mL) were heated in a reactor at 150 °C for 3 h. The 3-amino derivative was recovered by suction from the still hot solution and washed with a small amount of water. The dried compound was stirred at room temperature with hexanoyl chloride (10 mmol) for 22 h. The mixture was cautiously diluted with water and extracted with CH_2Cl_2 (3 × 30 mL). After evaporation of the solvent under vacuum, the oil residue crystallized by treatment with ethanol.

General Procedure for Ethers 5f−j. Sodium (0.2 mmol) was dissolved in the appropriate alcohol (0.5−0.8 mL) at room temperature. 3-Chloropyridazine (**4**, 0.1 mmol) was added under stirring for 1 h maintaining the temperature at 120−140 °C. After dilution with water, the mixture was extracted with CH_2Cl_2 (3 × 20 mL). Distillation under vacuum (10^{-2} mmHg at 140 °C) afforded compounds **5f−j**, which were purified by column chromatography. Eluents: cyclohexane/ethyl acetate 3:1.

General Procedure for Sulfoxides 8a−d. To a suspension of **7a−d** (0.5 mmol) in 50% (w/v) AcOH (10 mL) was added 35% (w/v) H_2O_2 (0.5 mL). The mixture was stirred for 11−30 h at room temperature. After standard workup of the reaction mixture, compounds **8a−d** were recovered and purified by column chromatography. Eluents: cyclohexane/ethyl acetate 1:2.

General Procedure for Sulfones 9a−d. A solution of **8a−d** (0.5 mmol) and MCPBA (1.5 mmol) in CH_2Cl_2 (6 mL) was stirred at room temperature for 1−3 h. The solution was extracted with 5% (w/v) $NaHCO_3$ (2 × 15 mL), and the organic layer was washed with water (15 mL) and dried. Removal of the solvent afforded the crude compounds **9a−d**, which were purified by column chromatography. Eluents: cyclohexane/ethyl acetate 3:1.

Biological Assays. Compounds were tested according to previously reported procedures: ACAT assay,[14,19] cholesterol esterification in cell culture,[19] cellular cholesterol efflux,[19] and evaluation of cell viability.[21]

Supporting Information Available: Additional spectral data for synthesized compounds and biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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