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MONO- AND DI-SUBSTITUTED 5,6-DIPHENYL-3-ALKYLAMINO-PYRIDAZINES ACTIVE AS ACAT INHIBITORS

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Abstract - A series of mono- or di-*para*-substituted 5,6-diphenyl-3-alkylaminopyridazines were synthesized and their inhibitory activity against acyl-CoA:cholesterol acyltransferase (ACAT) was tested on the enzyme prepared from rat liver microsomes. The compound which combines a chlorine atom on the 6-phenyl ring and a *n*-hexylamino chain showed a significant enhancement of activity with respect to the unsubstituted derivative. Attempts to correlate the activity of the compounds to their structural features, also through theoretical calculations, are reported.

INTRODUCTION

Hypercholesterolemia is recognized as an important risk factor for the development of coronary heart disease (CHD).[1] Since acyl-CoA:cholesterol acyltransferase (ACAT) is responsible for the production of cholesteryl esters, ACAT inhibitors have been identified as useful targets in the treatment of hypercholesterolemia.[2-7] As a continuation of previous studies[8] on 5,6-diphenyl derivatives active as ACAT inhibitors, we recently reported[9] the synthesis and the inhibiting properties of a series of 5,6-dinitrophenyl- and 5-aminophenyl-6-nitrophenyl-3-alkylaminopyridazines. Theoretical studies on these compounds suggested that an enhancement of their inhibition properties could be attained by introduction of substituents that make the pyridazine ring more electron-rich. To verify this hypothesis, we have now synthesized a new series of derivatives, having methoxy- or chloro-substituents in the *para* position of either one or both the phenyl rings. Moreover, the *n*-hexyl- and *n*-nonylamino groups were chosen as

substituents on the 3-position since the most interesting compounds in the unsubstituted series were found to have these side-chains.[8] We report here the synthesis of a number of derivatives that were tested for their inhibition of ACAT from rat liver microsomes. In addition, attempts to correlate their activity to their structural features through a theoretical investigation are discussed.

CHEMISTRY

The series of derivatives (**1a-f**) and (**2a-f**), *para*-substituted on one or both the phenyl rings with groups which are notoriously electron-releasing, namely the methoxy and chloro substituents, were synthesized according to the procedure reported in Scheme 1. The suitable phenylacetic acid was transformed into the corresponding acyl chloride and condensed with the appropriately substituted benzene in the presence of aluminum chloride at 0 °C. The so obtained keto derivative (**3**) easily led to the corresponding keto ester (**4**) by treatment with ethyl bromoacetate in dimethyl sulfoxide and in the presence of sodium hydride. Hydrolysis in basic medium to **5** and subsequent cyclization by hydrazine hydrate in refluxing ethanol led to the desired dihydropyridazinones (**6**), which were dehydrogenated to **7** by stirring at 100 °C in an alkaline medium with sodium *m*-nitrobenzenesulfonate. Finally, heating with $POCl_3$ at 60 °C gave the desired chloropyridazine (**8**), which was directly condensed with *n*-hexylamine to give **1** or with *n*-nonylamine to give **2**.

ENZYME ASSAY

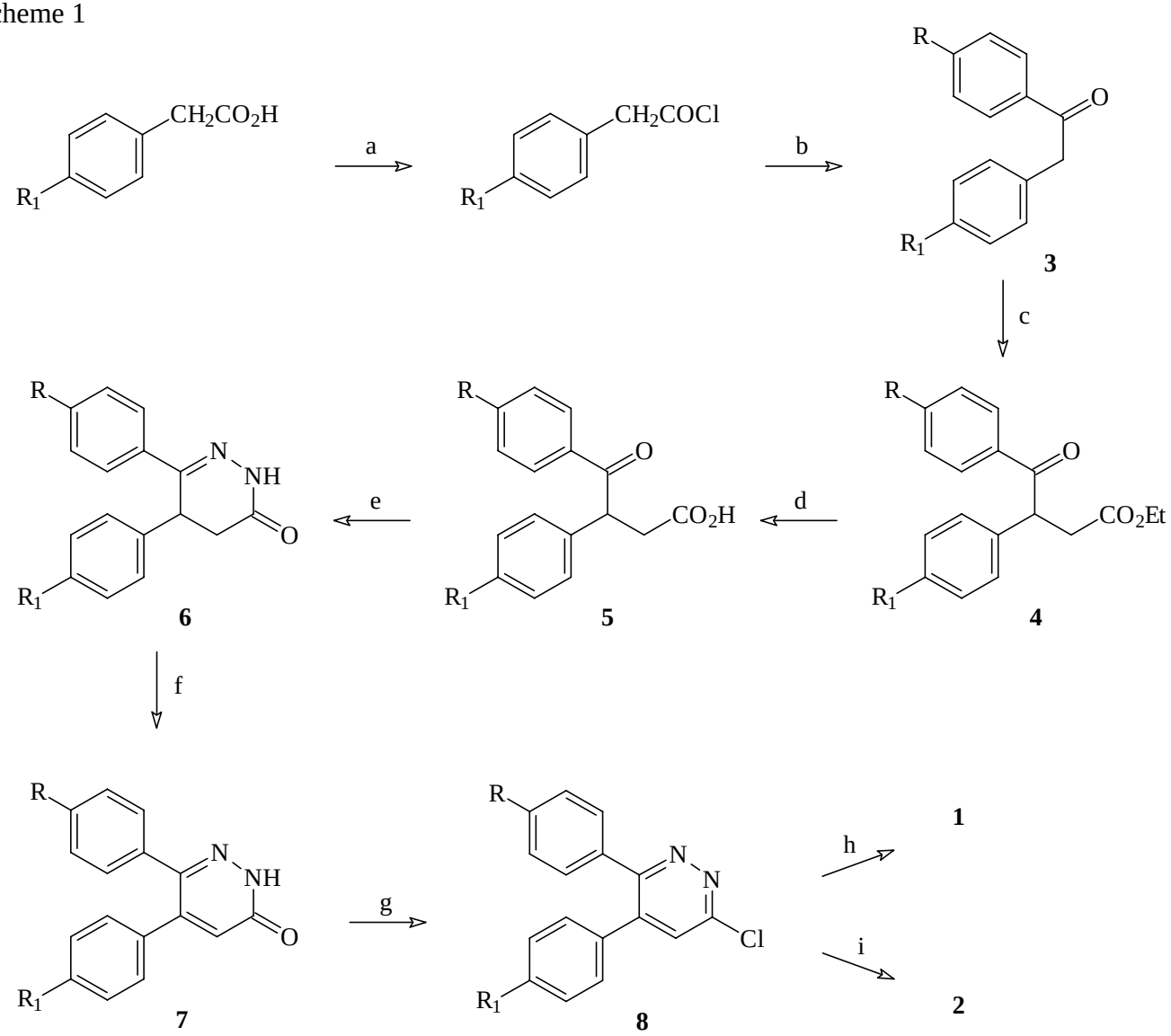
All the compounds obtained were tested for their potency to inhibit ACAT extracted from rat liver microsomes, according to a previously reported method.[10] The unsubstituted derivatives **(1g**) and (**2g**) were used as reference. IC_{50} values are listed in Table 1.

All the tested compounds were active at micromolar range. They all presented about the same activity ($IC_{50} \approx$ 10-30 μM), the only positive exceptions being in the *n*-hexyl series the compound chlorinated on the 6-phenyl group (**1e**) (IC_{50} = 1.0 μM) and in the *n*-nonyl series the doubly chlorinated compound (**2f**) (IC_{50} = 2.5 μM). A negative exception is represented by **2e** (IC_{50} = 80 μM) the compound corresponding to **1e** but with a longer *n*-alkyl chain.

THEORETICAL CALCULATIONS

Since previous studies suggested[9] a correlation between the activity of this class of compounds and the electrostatic properties near the nitrogen atoms of the pyridazine ring, we similarly decided to determine the same properties in the series of compounds (**1a-f)** and (**2a-f**). Thus, the conformational space of all the compounds was explored at the semiempirical AM1 level[11] allowing to determine their energy minima. Their geometrical features appeared quite similar as the presence of one or two substituents on the *para*

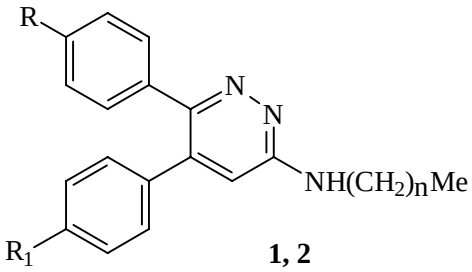
Scheme 1



a) $SOCl_2$, CH_2Cl_2, reflux; b) R-C_6H_5, $AlCl_3$, CH_2Cl_2, 0 °C, then rt; c) NaH, $BrCH_2CO_2Et$, DMSO, toluene; d) 25% NaOH in EtOH/H_2O 1:1; e) $NH_2NH_2 \cdot H_2O$, EtOH, reflux; f) sodium *m*-nitrobenzensulfonate, NaOH, reflux; g) $POCl_3$, 60 °C; h) *n*-hexylamine; i) *n*-nonylamine.

positions of the phenyl rings does not influence the conformational behaviour of the molecules. For the global minimum of each compound the molecular electrostatic potential (MEP) was calculated on the molecular surface of the optimized conformations at the AM1 level. The values of the electrostatic potential minima generated by the two nitrogen atoms of the pyridazine ring and the maximum generated by the NH group, taken as significant points of the core region of the molecules potentially involved in favorable interactions at the active site of the enzyme, resulted in all the cases quite similar (Table 1). These results confirmed our initial hypothesis that a more electron-rich pyridazine ring is necessary to confer ACAT inhibitory properties to this class. In fact, the average values of the electrostatic potentials of this new series are quite close to those of the most potent derivatives in the previously reported amino/

Table 1. Biological data and molecular electrostatic potential data of compounds (**1a-g**) and (**2a-g**).



1, 2

compd	n	R	R_1	IC_{50} [a]	V(NH) [b]	V(N1) [b]	V(N2) [b]
1a	5	H	OMe	33	35.2	-65.9	-65.2
2a	8	H	OMe	22	34.9	-65.9	-65.6
1b	5	OMe	H	11	35.4	-65.3	-64.3
2b	8	OMe	H	8.9	35.0	-65.4	-64.2
1c	5	OMe	OMe	25	34.4	-65.1	-65.5
2c	8	OMe	OMe	11	34.4	-64.8	-64.9
1d	5	H	Cl	19	37.1	-64.3	-63.0
2d	8	H	Cl	18	37.1	-64.2	-62.9
1e	5	Cl	H	1.0	36.9	-62.8	-62.7
2e	8	Cl	H	80	36.2	-62.7	-62.6
1f	5	Cl	Cl	19	38.6	-61.2	-61.2
2f	8	Cl	Cl	2.5	38.6	-61.2	-61.2
1g	5	H	H	24	35.8	-66.4	-74.8
2g	8	H	H	18	35.7	-65.9	-65.0

a) *In vitro* ACAT inhibition determined in rat liver microsomes. IC_{50} values are from three experiments, which agreed within 10%.

b) MEP values (V, kcal/mol) on the molecular surface, calculated for the AM1 optimized conformations.

nitro-substituted analogs.[9] In addition, the data of activity seem to suggest a very important role of the chlorine atom in the interaction with the active site of the enzyme. This interaction might be influenced by the length of the *n*-alkyl chain, which could control the way of presentation of the molecules in the active site. In fact, though compounds (**1e**) and (**2e**) differ only in the length of the alkyl chain and hence have practically identical electrostatic properties, they are, respectively, the most and the least active compounds. The combination present in **1e**, *i. e.* a chlorine atom on the *para*-position of the 6-phenyl ring together with the shorter (n=5) alkyl chain, seems to be very favorable, since it produces a significant enhancement in activity with respect to the reference compounds (**1g**) and (**2g**). On the contrary, the longer homolog (**2e**) (n=8) is less active with respect to both **1e** and the reference compounds, though by different degrees.

Further work appears necessary to better define the required structural features of this class of ACAT inhibitors, but compound (**1e**) could represent a good model for the search of more active compounds.

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. Silica gel 60 (Merck; 230-400 mesh) was used for flash chromatography. The structures of all compounds were consistent with their analytical and spectroscopic data. Compound (**3c**) (desoxyanisoin) was purchased from Aldrich Co.

General method for the synthesis of compounds (3)

A solution of the required phenylacetic acid (0.012 mol) and $SOCl_2$ (2.1 g, 0.029 mol) in CH_2Cl_2 (5 mL) was refluxed overnight. After evaporating the excess thionyl chloride, the obtained acyl chloride was dissolved in CH_2Cl_2 (20 mL) together with the appropriately substituted benzene (0.040 mol), added dropwise to an ice-cooled suspension of $AlCl_3$ (4.9 g, 0.037 mol) in CH_2Cl_2 (10 mL), and stirred overnight at rt. The reaction mixture was poured onto a mixture of ice (27 g) and HCl (11 mL). The organic phase was separated, dried over sodium sulfate, the solvent evaporated, and the crude product purified by flash chromatography by eluting with cyclohexane/ethyl acetate 9:1 (see Table 2 for data). For **3a**, ^{1}H-NMR ($CDCl_3$) δ: 3.8 (s, 3H), 4.2 (s, 2H), 6.8 (d, 2H, J= 8 Hz), 7.2 (d, 2H, J=8 Hz), 7.4-7.6 (m, 3H), 8.0 (d, 2H, J=7.5 Hz).

General method for the synthesis of compounds (4)

To a suspension of dry NaH (95%; 0.16 g, 0.0064 mol) in anhydrous DMSO (3.3 mL) under nitrogen atmosphere, a solution of the appropriate ketone (**3**) (0.0064 mol) in anhydrous DMSO (8.2 mL) was added dropwise. After the reaction subsides, ethyl bromoacetate (0.7 mL, 0.0064 mol) in toluene (5 mL) was added dropwise and the mixture stirred overnight. The mixture was then acidified by 2N HCl, diluted with water (30 mL) and exctracted by dichloromethane (3 × 20 mL). After drying over sodium sulfate, the solvent was evaporated and the residue purified by flash chromatography, eluting with cyclohexane/ethyl acetate 9:1, to give as the first run the unreacted **3**, followed by the desired **4** (see Table 2 for data). For **4a**, ^{1}H-NMR ($CDCl_3$) δ: 1.2 (t, 3H, J=7 Hz), 2.7 (dd, 1H, J=17.5, 5 Hz), 3.3 (dd, 1H, J=17.5, 9.5 Hz), 3.7 (s, 3H), 4.2 (q, 2H, J=7 Hz), 5.1 (dd, 1H, J=9.5, 5 Hz), 6.8 (d, 2H, J=8 Hz), 7.2 (d, 2H, J=8 Hz), 7.3-7.6 (m, 3H), 8.0 (d, 2H, J=7.5 Hz).

General method for the synthesis of compounds (6)

a) The appropriate ester (**4**) (0.0023 mol) was hydrolized by 25% NaOH in ethanol/water 1:1 (15 mL, 0.094 mol), by stirring at rt for 2 h. After evaporation of ethanol, the residue was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The aqueous layer was then acidified by 6N HCl and extracted with CH_2Cl_2 (3 × 20 mL). After drying over sodium sulfate, evaporation of the solvent gave the acid (**5**) which was used as such for the next step.

Table 2. Chemical properties of compounds (**1-8**).

compound	R	R_1	Yield %	mp/°C
1a	H	OMe	77.7	102-104
2a	H	OMe	71.4	105-106
3a	H	OMe	68.0	92-93
4a	H	OMe	43.7	oil
5a	H	OMe	88.7	115-116
6a	H	OMe	46.0	202-203
7a	H	OMe	94.2	210-213
8a	H	OMe	87.8	95-96
1b	OMe	H	82.0	99-100
2b	OMe	H	84.0	94-96
3b	OMe	H	30.0	74-75
4b	OMe	H	50.6	oil
5b	OMe	H	96.7	118-120
6b	OMe	H	36.4	175-176
7b	OMe	H	90.0	182-185
8b	OMe	H	92.2	85-86
1c	OMe	OMe	56.7	112-114
2c	OMe	OMe	85.7	110-112
4c	OMe	OMe	43.4	oil
5c	OMe	OMe	65.5	120-122
6c	OMe	OMe	41.0	155-156
7c	OMe	OMe	99.0	235-237
8c	OMe	OMe	81.6	145-148
1d	H	Cl	95.1	97-99
2d	H	Cl	94.0	94-96
3d	H	Cl	55.7	130-131
4d	H	Cl	33.9	oil
5d	H	Cl	81.0	145-146
6d	H	Cl	63.6	197-198
7d	H	Cl	85.7	205-206
8d	H	Cl	83.0	120-122
1e	Cl	H	72.9	135-136
2e	Cl	H	65.4	133-134
3e	Cl	H	12.0	135-136
4e	Cl	H	37.9	oil
5e	Cl	H	78.4	132-133
6e	Cl	H	56.5	208-209
7e	Cl	H	89.7	221-222
8e	Cl	H	85.4	114-115
1f	Cl	Cl	83.2	138-140
2f	Cl	Cl	75.3	132-134
3f	Cl	Cl	21.0	97-98
4f	Cl	Cl	46.3	oil
5f	Cl	Cl	73.0	118-119
6f	Cl	Cl	48.5	172-173
7f	Cl	Cl	56.7	236-238
8f	Cl	Cl	82.0	94-95

b) A mixture of the required acid (**5**) (0.0017 mol) and hydrazine monohydrate (0.17 mL, 0.0035 mol) in ethanol (13 mL) was refluxed overnight. After cooling, the precipitated dihydropyridazinone (**6**) was filtered under suction and thoroughly washed with ethanol (see Table 2 for data). For **6a**, ^{1}H-NMR ($CDCl_3$) δ: 2.8 (br d, 1H, J=17 Hz), 3.0 (dd, 1H, J=17, 7.5 Hz), 3.7 (s, 3H), 4.4 (br d, 1H, J=7.5 Hz), 6.8 (d, 2H, J=8.5 Hz), 7.1 (d, 2H, J=8.5 Hz), 7.4 (m, 3H), 7.7 (m, 2H), 8.6 (s, 1H, exch. with D_2O).

General method for the synthesis of compounds (7)

A mixture of the required dihydropyridazinone (**6**) (0.0007 mol), sodium *m*-nitrobenzensulfonate (0.156 g, 0.00069 mol), and 0.5N NaOH (8 mL, 0.0040 mol) was refluxed for 4 h. After cooling, the solution was acidified to pH 1 by 6N HCl. The so formed precipitate was collected by suction and thoroughly washed with water (see Table 2 for data). For **7a**, ^{1}H-NMR ($CDCl_3$) δ: 3.8 (s, 3H), 6.8 (d, 2H, J=8 Hz), 6.9 (s, 1H), 7.1 (d, 2H, J=8 Hz), 7.2-7.4 (m, 5H), 11.0 (s, 1H, exch. with D_2O).

General method for the synthesis of compounds (1a-f) and (2a-f)

a) A mixture of the appropriate pyridazinone (**7**) (0.00065 mol) and $POCl_3$ (1.5 mL, 0.016 mol) was stirred at 60 °C for 3 h. After cooling, the mixture was poured onto ice/water (10 mL), its pH was brought to 6 by 5N NaOH, and then it was extracted with CH_2Cl_2 (3 × 15 mL). After drying over sodium sulfate, evaporation of the solvent gave **8**, which was used as such for the next step (see Table 2 for data).

b) The required chloropyridazine (**8**) (0.1 mmol) was heated overnight at 160 °C with *n*-hexyl- or *n*-nonylamine (0.2 mmol). The crude product was purified by flash chromatography, eluting with cyclohexane/ethyl acetate 6:4 (see Table 1 for data). For **1a**, ^{1}H-NMR ($CDCl_3$) δ: 0.9 (t, 3H, J=6.5 Hz), 1.2-1.5 (m, 6H), 1.6-1.7 (m, 2H), 3.4 (q, 2H, J=6.5 Hz), 3.8 (s, 3H), 5.0 (t, 1H, J=5.5 Hz, exch. with D_2O), 6.6 (s, 1H), 6.8 (d, 2H, J=8.5 Hz), 7.1 (d, 2H, J=8.5 Hz), 7.3-7.4 (m, 5H).

1a: Anal. Calcd for $C_{23}H_{27}N_3O$: C, 76.42; H, 7.53; N, 11.62. Found: C, 76.73; H, 7.33; N, 11.69.

2a: Anal. Calcd for $C_{26}H_{33}N_3O$: C, 77.38; H, 8.24; N, 10.41. Found: C, 77.25; H, 8.09; N, 10.23.

1b: Anal. Calcd for $C_{23}H_{27}N_3O$: C, 76.42; H, 7.53; N, 11.62. Found: C, 76.38; H, 7.46; N, 11.54.

2b: Anal. Calcd for $C_{26}H_{33}N_3O$: C, 77.38; H, 8.24; N, 10.41. Found: C, 77.55; H, 8.40; N, 10.11.

1c: Anal. Calcd for $C_{24}H_{29}N_3O_2$: C, 73.63; H, 7.47; N, 10.73. Found: C, 73.50; H, 7.30; N, 10.67.

2c: Anal. Calcd for $C_{27}H_{35}N_3O_2$: C, 74.79; H, 8.14; N, 9.69. Found: C, 74.48; H, 8.21; N, 9.83.

1d: Anal. Calcd for $C_{22}H_{24}N_3Cl$: C, 72.22; H, 6.61; N, 11.48. Found: C, 72.01; H, 6.56; N, 11.60.

2d: Anal. Calcd for $C_{25}H_{30}N_3Cl$: C, 73.60; H, 7.41; N, 10.30. Found: C, 73.64; H, 7.22; N, 10.42.

1e: Anal. Calcd for $C_{22}H_{24}N_3Cl$: C, 72.22; H, 6.61; N, 11.48. Found: C, 72.15; H, 6.75; N, 11.55.

2e: Anal. Calcd for $C_{25}H_{30}N_3Cl$: C, 73.60; H, 7.41; N, 10.30. Found: C, 73.40; H, 7.35; N, 10.15.

1f: Anal. Calcd for $C_{22}H_{23}N_3\ Cl_2$: C, 66.00; H, 5.79; N, 10.50. Found: C, 66.22; H, 5.71; N, 10.68.

2f: Anal. Calcd for $C_{25}H_{29}N_3\ Cl_2$: C, 67.87; H, 6.61; N, 9.50. Found: C, 68.02; H, 6.53; N, 9.58.

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]

Theoretical calculations

Theoretical calculations were performed with the SPARTAN package[12] using the AM1 semiempirical method[11] 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. However, the *n*-hexyl and *n*-nonyl chains were always taken in the straight conformation.

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