Biphenyl versus Phenylpyridazine Derivatives: The Role of the Heterocycle in a Series of Acyl-CoA:Cholesterol Acyl Transferase Inhibitors

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A series of alkylamido- (**1**) and alkylaminobiphenyl (**2**) derivatives were synthesized as possible bioisosters of the reported ACAT inhibitors phenylpyridazine analogues (**I**). Both **1** and **2** were tested on the human ACAT-1 and ACAT-2 isoforms. The amino derivatives **2** were found to be inactive, contrary to the related pyridazine derivatives. By contrast, the ortho-substituted amides **1a** and **1d** showed an interesting activity. These results support the essential role of the pyridazine nucleus. Modeling studies were also performed.

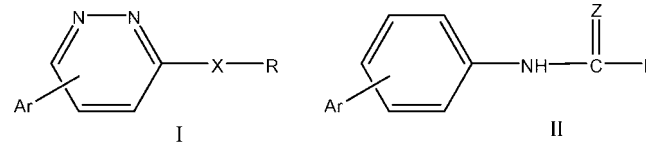
Introduction

Acyl-CoA:cholesterol acyl transferase (E.C.2.3.1.26, ACAT) is an intracellular enzyme that catalyzes the esterification of cholesterol with long chain fatty acylCoA.[1] In mammalian species, two isoforms have been identified: ACAT-1 is predominant in human liver, macrophages, and adrenal gland, while ACAT-2 is present exclusively in hepatocytes and intestinal cells.[2,3] Though there has been intensive research on ACAT inhibitors as potential agents for the treatment of hypercholesterolemia, atherosclerosis, and coronary diseases, recent negative findings in clinical trials seem to lower the chance for this class of compounds to offer cardiovascular protection.[3]

However, there is a strong need for selective ACAT1/ACAT2 inhibitors in order to fully elucidate the roles of the two isozymes in humans and to answer a very important question on whether specific inhibitors could treat or prevent atheroschlerosis. In addition, ACAT inhibitors could find application in Alzheimer's disease, since they have been shown to decrease the generation of amyloid β peptide in mice.[3,4]

Recently, we synthesized a series of pyridazine derivatives (**I**),[5] structurally related to a class of imidazolyl compounds which were reported as potent ACAT inhibitors.[6] Our aim was to verify the effects of the substitution of the aryl moiety on the activity of this class. Although the pyridazine derivatives were less potent than the imidazolyl model, they retained interesting properties. To verify the importance of different parameters, subsequent SAR studies were carried out in our laboratories both on the alkyl side chain and on the two phenyl groups in positions meta and para with respect to it.[7–12] Beside giving information on several structural requirements for the optimum activity of these compounds, evidence was provided that in our series the two adjacent phenyl groups were not essential and only one of them was required on the pyridazine ring. In addition, in all of the compounds we prepared, the best results were obtained when the linker between the aryl moiety and the side chain was a secondary amino group (**I**, X = NH. See Chart 1). Substitution of this group with the corresponding amide always brought about a complete loss of activity.[5]

Chart 1



Ar = 5-Ph, 6-Ph, 5,6-Ph_2 Ar = *o*.Ph, *m*.Ph, *p*.Ph

X = NH, O, S, SO, SO_2 Z = H_2, O

As a part of our ongoing studies on this class, we have now addressed our interest on the role of the pyridazine ring by designing a new series where the heterocyclic moiety of **I** was replaced by a benzene ring (**II**) (Chart 1). Since it is well-known from the literature[13] that fatty acid anilide derivatives possess significant ACAT inhibition, we planned to investigate, besides the influence of the position of the phenyl substituent, the role of the amino vs the amido function in **I** and **II**, respectively. On these bases, we synthesized a series of alkylamido (**1**) and alkylamino (**2**) biphenyl derivatives and determined their potential as ACAT inhibitors.

Chemistry

Compounds were prepared by condensing *o*-, *m*-, or *p*-phenylaniline with octanoyl or nonanoyl chloride at room temperature in dichloromethane for 20 h. Reduction of the so obtained amides **1a–f** by lithium aluminum hydride in anhydrous THF gave the corresponding amines **2a–f** (Scheme 1).

Results and Discussion

The amines **2** as well as the amides **1** were tested on the human ACAT-1 and ACAT-2 isoforms, according to a previously reported assay.[14] Their activity is expressed as percentage inhibition at 50 μM. For the most interesting terms, the IC_{50} (μM) values are also reported. The 2,6-diisopropyl derivative of oleic acid anilide (0.5 μM) was used as positive control.

As shown in Table 1, all of the amino biphenyl derivatives **2** were found to be inactive toward both ACAT isoforms,

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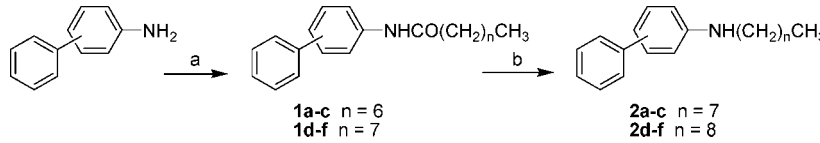
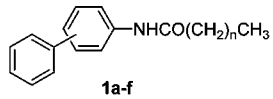
Scheme 1[a]

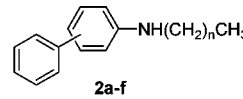


[a] Key: (a) $ClCO(CH_2)_nCH_3$, NEt_3, CH_2Cl_2, rt, 20 h; (b) $LiAlH_4$, anhyd THF, N_2, Δ, 20 h.

Table 1. Chemical Data and Human ACAT Inhibition of Biphenyl Derivatives **1a–f–2a–f**



1a-f



2a-f

Compound	Isomer	n	Yield (%)	Formula[a]	% inh.[b]/ IC_{50}(μM) *h*ACAT-1	% inh.[b]/ IC_{50}(μM) *h*ACAT-2
1a	*o*-	6	70	$C_{20}H_{25}NO$	92/60.9	39/230
1b	*m*-	6	75	$C_{20}H_{25}NO$	13	-4
1c	*p*-	6	60	$C_{20}H_{25}NO$	0	11
1d	*o*-	7	71	$C_{21}H_{27}NO$	84/64.6	27/414
1e	*m*-	7	75	$C_{21}H_{27}NO$	18	-6
1f	*p*-	7	57	$C_{21}H_{27}NO$	21	20
2a	*o*-	7	29	$C_{20}H_{27}N$	36	-4
2b	*m*-	7	27	$C_{20}H_{27}N$	11	-20
2c	*p*-	7	26	$C_{20}H_{27}N$	0	6
2d	*o*-	8	16	$C_{21}H_{29}N$	31	-1
2e	*m*-	8	31	$C_{21}H_{29}N$	14	-8
2f	*p*-	8	17	$C_{21}H_{29}N$	29	18
Positive control[c]					53	47

[a] C,H,N analysis within ± 0.4%. [b] At a concentration of 50 μM. All values are means from three experiments, which differ by less than 10%. [c] At a concentration of 0.50 μM.

contrary to the related pyridazine derivatives which were provided with significant inhibitory properties. In contrast, as expected from literature data, the ortho-substituted amides **1a** and **1d** showed a noticeable activity, whereas their meta and para analogues were completely inactive. The chain length did not influence the activity in any case. All of these results strongly evidence that the pyridazine nucleus of **I** plays an essential role for the inhibitory properties of this series and that this role cannot be played by the central benzene ring of **II**.

It should be noted that both **1a** and **1d** were more potent toward hACAT-1 over hACAT-2. Presently, it is still controversial whether pharmacological inhibition of ACAT-1, which plays a critical role in foam cell formation in macrophages, would cause reduction or exacerbation of atheroschelorosis.[2,3] However, a recent paper[15] reported on a selective ACAT-1 inhibitor able to suppress atherosclerosis in fat-fed hamsters, without affecting plasma cholesterol levels. On these bases, ACAT-1 could represent an efficient target for ACAT inhibitors.

In order to give theoretical support to the above results, modeling studies were performed. A conformational study of the three couples **1d/2d**, **1e/2e**, **and 1f/2f**, as representative terms of all the synthesized compounds, was carried out. In order to obtain all of the possible conformations, rotation around the single bonds close to the amide or the amine groups was evaluated, and all of the geometries were optimized at the B3LYP/6-31G(d) level.[16] The energy of the optimized conformations was recalculated in water using a continuous solvent model (C-PCM);[17] however, solvation did not significantly affect the relative stability of the various conformations. In Figure 1 are reported the preferred conformations of all the compounds in a superimpositions of each amido derivative with the corresponding amino derivative obtained through rms fit of the atoms of the central benzene ring. The superimposition of the active amide **1d** with the corresponding inactive amine **2d** does not show any significant difference in the overall shape of these compounds. This suggests that the carbonyl group present in **1a** and **1d** should have, in the interaction at the active site of the enzyme, a specific role that is not allowed to the corresponding amines. Moreover, the absence of inhibition properties in the meta and para compounds shows that the alkyl chain and the phenyl group are able to confer activity only when these substituents are ortho oriented on the central ring.

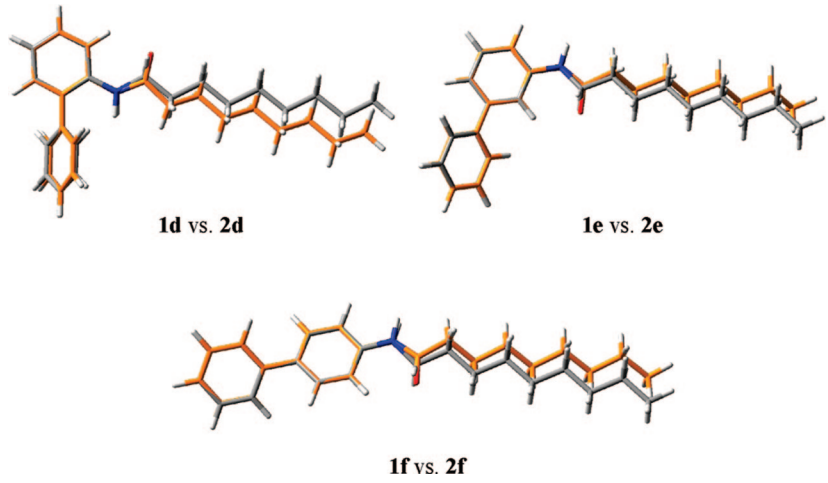


Figure 1. Overlay of amides **1d–f** (orange shading) with the corresponding amines **2d–f** obtained through rms fit of the central benzene ring.

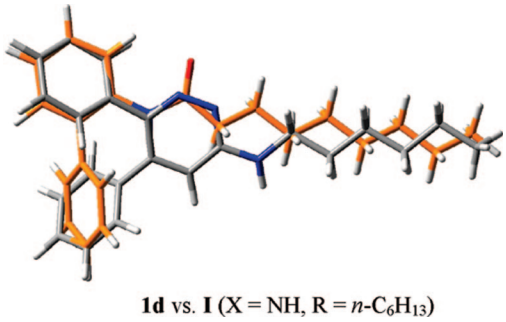


Figure 2. Overlay of amide **1d** (orange shading) with 3-hexylamino-5,6-diphenylpyridazine (**I**, X = NH, R = *n*-C_6H_{13}).

Finally, in order to find possible analogies between the active compounds **1a/1d** and the pyridazine derivatives **I**, we tried to superimpose the preferred conformation of **1d** with that of 3-hexylamino-5,6-diphenylpyridazine, which was shown to be the most active among compounds of general formula **I**.[5] When we allowed the best fit of the biphenyl system of **1d** with the two phenyl groups of **I**, the two structures matched very well (Figure 2). This indicates that the pyridazine ring of **I** does not mimic the central benzene ring of **1d**. By contrast, the carbonyl group of **1d** is oriented in about the same direction of the nitrogen atom(s) of the heterocycle in compounds **I**. This suggests that these moieties could play the same role in the interaction with the enzyme.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a 300 MHz Oxford-Varian spectrometer; chemical shifts are reported as δ (ppm), using the solvent as internal standard. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction and to check product purity. Silica gel 60 (Merck, 230–400 mesh) was used for flash chromatography. Elemental analyses of all new compounds were within ±0.4 of the theoretical values. The structures of all compounds were consistent with their analytical and spectroscopic data.

General Procedure for the Synthesis of Biphenyl Amides (1a–f). To a solution of the appropriate amine (3×10^{-3} mol) in CH_2Cl_2 (75 mL) was added NEt_3 (0.84 mL), and then a solution of the required acyl chloride (3×10^{-3} mol) in CH_2Cl_2 (12 mL) was dropped. The solution was stirred at rt for 20 h and washed twice with water (2 × 30 mL) and the organic phase dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (eluent: cycloexane/dichloromethane 7/3) to give the required amide (see Scheme 1 and Tables 1 and 2 for data).

Table 2. ^{1}H NMR Data of Compounds **1a–f–2a–f** ($CDCl_3$; Chemical Shifts in ppm)

compd	^{1}H NMR (δ)
1a	0.80 (t, 3H), 1.10–1.40 (m, 8H), 1.50–1.60 (m, 2H), 2.20 (t, 2H), 7.05–7.20 (m, 2H), 7.30–7.60 (m, 7H), 8.30 (br s., 1H, exch. with D_2O)
1b	0.90 (t, 3H), 1.20–1.45 (m, 8H), 1.60–1.70 (m, 2H), 2.40 (t, 2H), 7.30–7.50 (m, 7H), 7.55–7.60 (m, 2H), 7.80 (br s., 1H, exch. with D_2O)
1c	0.90 (t, 3H), 1.20–1.45 (m, 8H), 1.60–1.70 (m, 2H), 2.40 (t, 2H), 7.20 –7.60 (m, 9H+1H exch. with D_2O)
1d	0.80 (t, 3H) 1.20–1.40 (m, 10H), 1.50–1.60 (m, 2H), 2.20 (t, 2H), 7.10–7.30 (m, 2H), 7.35–7.55 (m, 7H), 8.30 (br s., 1H, exch. with D_2O)
1e	0.90 (t, 3H), 1.20–1.40 (m, 10H), 1.60–1.70 (m, 2H), 2.40 (t, 2H), 7.30–7.50 (m, 7H), 7.55–7.60 (m, 2H), 7.80 (br s., 1H, exch. with D_2O)
1f	0.90 (t, 3H), 1.20–1.40 (m, 10H), 1.60–1.70 (m, 2H), 2.40 (t, 2H), 7.20–7.65 (m, 9H+1H exch. with D_2O)
2a	0.80 (t, 3H), 1.10–1.40 (m, 10H), 1.45–1.50 (m, 2H), 3.10 (t, 2H), 4.80 (br s., 1H, exch. with D_2O), 6.75–6.80 (m, 2H), 7.10 7.25 (m, 2H), 7.30–7.50 (m, 5H)
2b	0.90 (t, 3H), 1.20–1.50 (m, 10H), 1.60–1.70 (m, 2H), 3.10 (t, 2H), 4.60 (br s., 1H, exch. with D_2O), 6.60 (dd, 1H), 6.80 (s, 1H), 6.90 (d, 1H), 7.20–7.45 (m, 4H), 7.60 (d, 2H)
2c	0.90 (t, 3H), 1.20–1.55 (m, 10H), 1.60–1.70 (m, 2H), 3.10 (t, 2H), 4.60 (br s., 1H, exch. with D_2O), 6.70 (d, 2H), 7.20–7.45 (m, 5H), 7.55 (d, 2H)
2d	0.90 (t, 3H), 1.10–1.40 (m, 12H), 1.45–1.50 (m, 2H), 3.10 (t, 2H), 4.80 (br s., 1H, exch. with D_2O), 6.70–6.80 (m, 2H), 7.10 7.25 (m, 2H), 7.40–7.50 (m, 5H)
2e	0.80 (t, 3H), 1.20–1.50 (m, 12H), 1.55–1.60 (m, 2H), 3.10 (t, 2H), 4.70 (br s., 1H, exch. with D_2O), 6.60 (dd, 1H), 6.80 (s, 1H), 6.90 (d, 1H), 7.10–7.45 (m, 4H), 7.60 (d, 2H)
2f	0.90 (t, 3H), 1.20–1.50 (m, 12H), 1.55–1.60 (m, 2H), 3.10 (t, 2H), 4.80 (br s., 1H, exch. with D_2O), 6.75 (d, 2H), 7.20–7.45 (m, 5H), 7.60 (d, 2H)

General Procedure for the Synthesis of Biphenyl Amines (2a–f). To a suspension of $LiAlH_4$ (0.147 g; 3.88×10^{-3} mol) in anhydrous THF (15 mL), under nitrogen, was added dropwise a solution of the above-described amide (4.90×10^{-4} mol) in anhydrous THF (9 mL). The reaction mixture was refluxed for 20 h and then cooled to 0 °C, and a cooled solution of Na_2SO_4 was added. The suspension was filtered, under vacuum, on Celite. The filtrate was extracted with ethyl acetate (3 × 30 mL), washed with brine (30 mL), and dried over Na_2SO_4. The organic phase was concentrated under vacuum and the residue purified by flash chromatography (eluent: cycloexane/dichloromethane 7/3) to give the amines **2** (see Scheme 1 and Tables 1 and 2 for data).

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Supporting Information Available: Elemental analyses of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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