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Ureidopyridazine Derivatives as Acyl-CoA:cholesterol acyltransferase Inhibitors

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Abstract

A series of *N*-(2,4-difluorophenyl)-*N'*-heptyl-*N'*-{4-[(substituted)-pyridazin-3-yl)thio]pentyl}urea derivatives having a phenyl ring at positions 5 and/or at position 6 of the heterocycle, as well as the corresponding sulfones, were synthesized. Their inhibitory activity against acyl-CoA:cholesterol acyltransferase (ACAT) was tested on the enzyme prepared from rat liver microsomes. Theoretical studies were performed to correlate their activity to their structural features.

Keywords

Hypercholesterolemia, ACAT inhibitors, pyridazine derivatives, ureido derivatives

Introduction

Hypercholesterolemia has been identified as one of the major risk factors for coronary heart diseases. Many efforts have been directed towards the discovery of new and effective hypocholesterolemic drugs [1, 2]. Acyl-CoA: cholesterol

acyltransferase (ACAT) is a microsomial enzyme that catalyzes the formation of long chain fatty acid cholesterol esters [3, 4, 5]. It represents an attractive target to design novel hypolipidemic and anti-atherosclerotic drugs. Its inhibition produces a reduction in intestinal absorption of cholesterol, liver secretion of very low-density lipoprotein (VLDL) particles, and reduced accumulation of cholesteryl esters in the arterial wall cells, the latter being a key step in the atherosclerotic process [6].

In previous papers [7-11] we reported our results on the design, the synthetic approach, the enzyme inhibition assay, and the conformational study of several mono- and diphenylpyridazine derivatives as novel ACAT inhibitors, including a series of compounds [11], structurally related to the potent ureido derivative DuP 128 [12], in which a linear pentamethylene chain links the pyridazine moiety to a substituted ureido group.

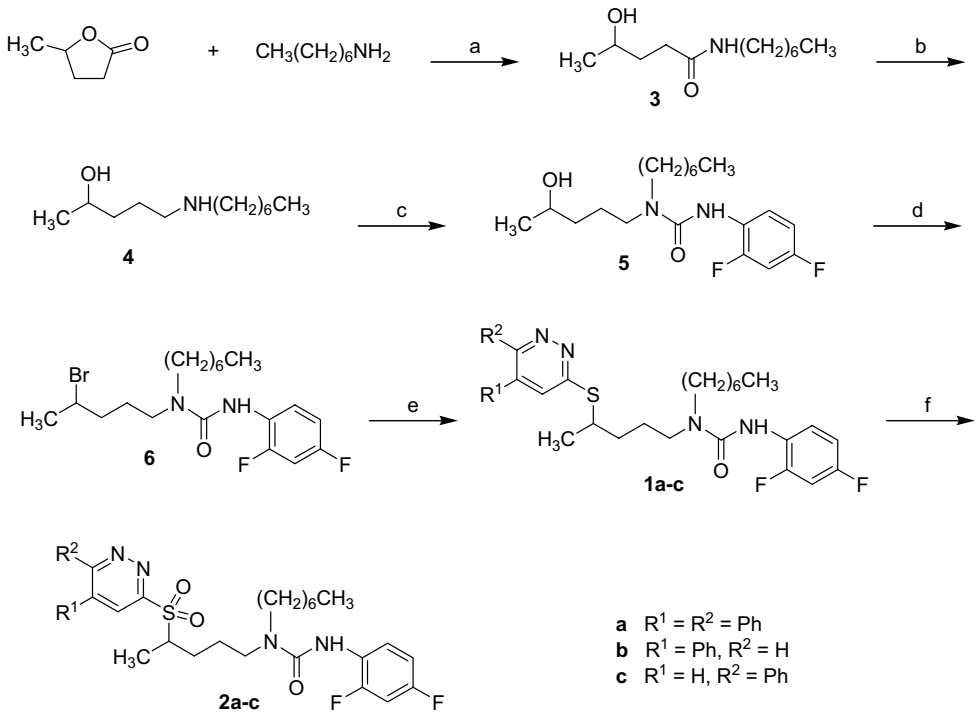
We have now undertaken a SAR study on the linker, in order to get information on the essential requirements of this moiety for activity. As a first approach, branching of the linker was considered. This paper reports on the synthesis and ACAT inhibition activity of compounds **1a-c** and **2a-c**. In addition, attempts to correlate their activity to their structural features through theoretical calculations are presented.

Results and Discussion

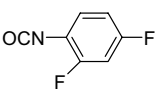
All compounds were tested for their inhibitory properties towards ACAT extracted from rat liver microsomes. Their activity, expressed as inhibition percentage at 50 μg/ml, is shown in Table 1. Amongst the thioderivatives, the 5-phenyl derivative (**1b**) showed the best activity with an inhibition percentage of 78. Both the 5,6-diphenyl (**1a**) and the 6-phenyl derivative (**1c**) were weaker inhibitors (67% and 62%, respectively). Oxidation of **1b** to its corresponding sulfone (**2b**) led to a significant loss of potency. In the case of **1c**, the same transformation did not bring about any significant change (**2c** inhibition value was 64%). By contrast, oxidation

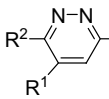
of **1a** to the corresponding sulfone **2a**, led to a compound provided with much higher inhibitory activity (78%).

Scheme 1. Synthetic pathway to compounds **1a-c, 2a-c**.

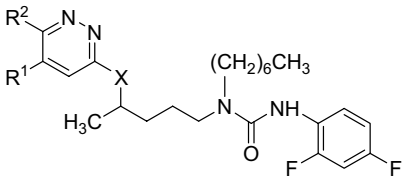


a) $PhCH_3$, N_2, 18h, reflux; b) $LiAlH_4$, THF, N_2, 18h, reflux;

c)  , CH_2Cl_2, N_2, 1h, 0 °C; d) CBr_4, $P(Ph)_3$, CH_2Cl_2, 3h, rt;

e)  (**7a-c**), K_2CO_3, DMF, 6h, 80°C; f) Oxone, CH_3OH, 4h, rt.

Tab. 1. Chemical data and rat ACAT inhibition of compounds **1a-c** and **2a-c** and GERI-BP001M.



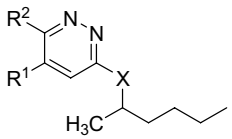
Compd	R^1	R^2	X	% Yield	Formula[a]	% Inhibition at 50 μg/mL
1a	Ph	Ph	S	52[b]	$C_{35}H_{40}F_2N_4OS$	67
1b	Ph	H	S	80[b]	$C_{29}H_{36}F_2N_4OS$	78
1c	H	Ph	S	40[b]	$C_{29}H_{36}F_2N_4OS$	62
2a	Ph	Ph	SO_2	42[c]	$C_{35}H_{40}F_2N_4O_3S$	78
2b	Ph	H	SO_2	22[c]	$C_{29}H_{36}F_2N_4O_3S$	66
2c	H	Ph	SO_2	23[c]	$C_{29}H_{36}F_2N_4O_3S$	64
GERI-BP001M						83

a) In vitro % ACAT inhibition determined in rat liver microsomes. All values are means from three experiments, which differ by less than 10%. *b)* From **7a**-**c**. *c)* From **1a**-**c**.

A modeling study of compounds **1a-c** and **2a-c** was performed in order to attempt a rationalization of the inhibitory activity of the two series of compounds on geometrical grounds. As the substituted ureido group was invariant in all the cases, this group was omitted in the theoretical calculations and the simplified structures **8a-c** and **9a-c** were modeled. The energy profiles for rotation around the C3-X (X=S, SO_2) bond were initially determined on models unsubstituted at the 5 and 6 positions of the heterocyclic ring. Then, after addition of the 5- and/or the 6-phenyl groups to the minima located in the profiles, the structures were optimized allowing

to determine the minimum energy conformations reported in Table 2. The data show that the presence of the phenyl groups at C5 and C6 does not influence the behavior of the substituent at C3 (compare, for example, the $\tau 1$ and $\tau 2$ values of **8aA** with those of **8bA** and **8cA**); the only geometrical effect of having one phenyl group instead of two is a smaller deviation from planarity of the phenyl with respect to the pyridazine ring. The phenyl groups can assume two orientations characterized by opposite values of $\tau 5$ and $\tau 6$ yielding, however, almost isoenergetic conformations (compare, for example, **8aA** with **8aB** or **8aC** with **8aD**). Two possible orientations of the C3 substituent were found in the case of **8** as well as of **9**. For the sulfides **8** the differences in energy between the two orientations (see, for example, **8aA** vs. **8aC**) is large enough (about 3.8 kcal/mol) to ensure that only one orientation gives conformers significantly populated, those characterized by $\tau 1 \approx 3°$; in the case of the sulfones **9**, instead, the second orientation (see, for example, **9aC**), characterized by $\tau 1 \approx -50°$ gives a not negligible contribution to the overall population being only about 1.2 kcal/mol less stable than the preferred one characterized by $\tau 1 \approx 45°$ (see, for example, **9aA**).

Tab. 2. Relative energy and selected geometrical data of the preferred conformations of compounds **8a-c** and **9a-c**.



8a-c, **9a-c**

Conformation	E_{rel} (kcal/mol)	R^1	R^2	X	$\tau 1^a$ (°)	$\tau 2^b$ (°)	$\tau 5^c$ (°)	$\tau 6^d$ (°)
8aA	0.00	Ph	Ph	S	3	-158	-50	-41
8aB	0.06				4	-158	50	42
8aC	3.76				-169	-167	-50	-41

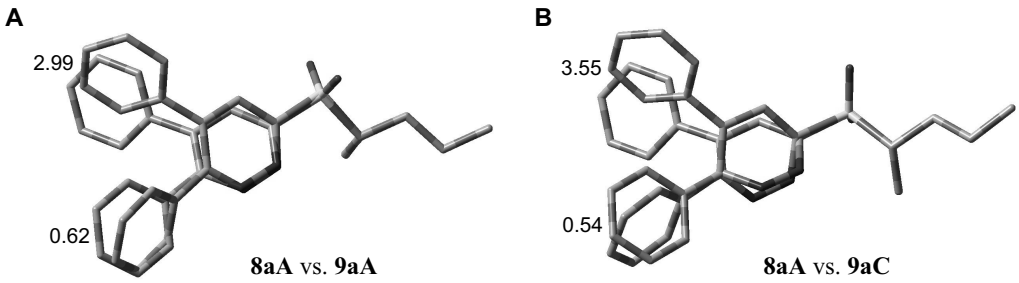
8aD	3.90				-168	-165	50	42
8bA	0.00	Ph	H	S	5	-158	-36	-
8bB	0.01				3	-157	37	-
8bC	3.90				-172	-164	-37	-
8bD	3.99				-168	-166	37	-
8cA	0.00	H	Ph	S	1	-157	-	-22
8cB	0.01				3	-158	-	20
8cC	3.80				-167	-167	-	-20
8cD	3.86				-168	-166	-	20
9aA	0.00	Ph	Ph	SO_2	44	-168	-48	-42
9aB	0.01				46	-167	49	42
9aC	1.15				-47	-156	49	42
9aD	1.19				-53	-154	-48	-41
9bA	0.00	Ph	H	SO_2	44	-168	-34	-
9bB	0.08				43	-168	33	-
9bC	1.22				-51	-155	34	-
9bD	1.24				-48	-154	-34	-
9cA	0.00	H	Ph	SO_2	46	-168	-	21
9cB	0.04				44	-167	-	-21
9cC	1.15				-52	-155	-	-20
9cD	1.19				-50	-155	-	21

a) Torsional angle defined by N2-C3-X-CH. *b)* Torsional angle defined by C3-X-CH-CH_2. *c)* Torsional angle defined by C4-C5-C1'-C2'. *d)* Torsional angle defined by C5-C6-C1''-C2''.

In the hypothesis that the ureido function of all the compounds interacts in the same way at the binding site inducing a similar orientation of the alkyl chain, we performed a comparison of the geometry of the conformation **8aA** of **8a** with the two conformations **9aA** and **9aC** of **9a** by superimposing the alkyl chain and

evaluating the differences in the orientation of the diphenylheterocyclic moiety (Figure 1). The distances between the centroids of the phenyl groups of the molecules in comparison were measured and are reported in Figure 1. The comparison of **8aA** with **9aA** (Figure 1A) as well as with **9aC** (Figure 1B) shows a correspondence of the 6-phenyl group of the two molecules. On the contrary, the 5-phenyl group is oriented in different ways.

Fig. 1. Superimposition of conformation **8aA** with conformations **9aA** and **9aC** (the numbers near the phenyl groups represent the distances in Å between their centroids).



In conclusion, the presence and the orientation of the 5-phenyl group emerge as the main factors which modulate activity. Additional influence on the activity might derive from the different tendency of the phenyl groups to deviate from planarity with respect to the pyridazine ring.

Experimental

^{1}H-NMR spectra were recorded on a Bruker AC200 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 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 the thioderivatives 1a-c and 2a-c.

A solution of γ-valerolactone (4.8 mL, 50 mmol) and *n*-heptylamine (9 mL, 60 mmol) in toluene (10 mL) was refluxed for 18 h in a nitrogen atmosphere. After cooling, ethyl acetate (60 mL) was added to the mixture, which was washed in sequence with 1 N HCl (3 × 10 mL) and brine (3 × 10 mL). After drying over sodium sulfate and evaporation of the solvent, *N*-heptyl-4-hydroxypentanamide (**3**) was obtained (8.9 g, 82%). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20 (d, 3H); 1.25-1.35 (m, 9H); 1.40-1.55 (m, 2H); 1.60-1.85 (m, 2H); 2.35 (t, 2H); 3.20 (q, 2H); 3.78-3.85 (m, 1H); 6.06 (m, 1H).

To a mixture of $LiAlH_4$ (0.26 g, 6.9 mmol) in anhydrous THF (12 mL) under nitrogen a solution of **3** (0.75 g, 3.5 mmol) in anhydrous THF (4 mL) was added dropwise. The mixture was then refluxed for 18 h. After cooling to 0 °C, a 10% aqueous solution of Na_2SO_4 (3 mL) was added, the solid filtered off and the mixture extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with aqueous NaCl (3 × 5 mL), dried over sodium sulfate and evaporated under vacuum to give *N*-heptyl-4-hydroxypentanamine (**4**) (0.56 g, 80%). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.18 (d, 3H); 1.20-1.35 (m, 8H); 1.40-1.55 (m, 4H); 1.70-1.80 (m, 2H); 2.48-2.65 (m, 3H); 2.75-2.85 (m, 1H); 3.68-3.78 (m, 1H).

To a solution of **4** (0.35 g, 1.74 mmol) in CH_2Cl_2 (2.25 mL) cooled to 0 °C, 2,4-difluorophenyl isocyanate (0.21 mL, 1.74 mmol) was slowly added under nitrogen. The mixture was stirred for 1 h and then poured into 1 N HCl (5.5 mL) and extracted with ethyl acetate (3 × 5 mL). The organic layer was washed in sequence with water and brine (3 × 5 mL). After drying over sodium sulfate and evaporation of

the solvent, the oily yellow residue was purified by flash chromatography (eluent CH_2Cl_2/CH_3OH 98/2) to give *N*-(2,4-difluorophenyl)-*N'*-heptyl-*N'*-(4-hydroxypentyl)urea (**5**) (0.34 g, 55%). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.23 (d, 3H); 1.25-1.85 (m, 15H); 3.25-3.45 (m, 4H); 3.82-3.95 (m, 1H); 6.65-6.70 (br s, 1H); 6.80-6.87 (m, 2H); 7.98-8.08 (m, 1H).

To a solution of **5** (0.310 g, 0.87 mmol) and CBr_4 (0.348 g, 1.05 mmol) in CH_2Cl_2 (7.2 mL) under nitrogen, a solution of PPh_3 (0.275 g, 1.05 mmol) in CH_2Cl_2 (2.0 mL) was added slowly and the mixture stirred for 3 h at room temperature. The so obtained oily mixture was purified by flash chromatography (eluent petroleum ether/EtOAc 9/1) to give *N*-(2,4-difluorophenyl)-*N'*-heptyl-*N'*-(4-bromopentyl)urea (**6**) (0.170 g, 47%). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-1.90 (m, 17H); 3.25-3.40 (m, 4H); 4.10-4.25 (m, 1H); 6.40.6.45 (br s, 1H); 6.80-6.85 (m, 2H); 8.00-8.08 (m, 1H).

A mixture of the appropriate pyridazinethiol [11] (**7a-c**, 0.12 mmol), in turn obtained from the corresponding pyridazinones [8, 13, 14], anhydrous K_2CO_3 (0.065 mg, 0.47 mmol), and **6** (0.10 g, 0.24 mmol) in anhydrous DMF (0.5 mL) was stirred at 80 °C for 6 h. To the cooled solution, water was added (2 mL) and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). After drying, the solvent was evaporated under vacuum to give the final compounds (**1a-c**), which were purified by flash chromatography (eluent cyclohexane/EtOAc 75/25).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(5,6-diphenylpyridazin-3-yl)thio]pentyl}urea (**1a**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-1.40 (m, 9H); 1.50-1.75 (m, 6H); 1.85-1.95 (m, 2H); 3.25-3.45 (m, 4H); 4.35-4.45 (m, 1H); 6.40-6.45 (br s, 1H); 6.75-6.85 (m, 2H); 7.15-7.20 (m, 2H); 7.25-7.40 (m, 9H); 7.98-8.08 (m, 1H).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(5-phenylpyridazin-3-yl)thio]pentyl}urea (**1b**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-1.90 (m, 17H); 3.25-3.40 (m, 4H); 4.35-4.40 (m, 1H); 6.40-6.45 (br s, 1H); 6.75-6.85 (m, 2H); 7.40-7.65 (m, 6H); 7.98-8.8.08 (m, 1H); 9.12 (s, 1H).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(6-phenylpyridazin-3-yl)thio]pentyl}urea (**1c**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-1.90 (m, 17H); 3.25-3.40 (m, 4H); 4.35-4.40 (m, 1H); 6.40-6.45 (br s, 1H); 6.75-6.85 (m, 2H); 7.35 (d, 1H); 7.45-7.55 (m, 3H); 7.62 (d, 1H); 7.95-8.05 (m, 3H).

To a solution of the appropriate **1a-c** (0.083 mmol) in methanol (2.5 mL) oxone (0.102 g, 0.17 mmol) was added portionwise and the mixture stirred for 4 h. The residue was treated with methanol (10 mL) and the insoluble filtered off. After evaporation of the solvent, **2** was purified by flash chromatography (eluent cyclohexane/EtOAc 60/40) (see Table 1).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(5,6-diphenylpyridazin-3-yl)sulfonyl]pentyl}urea (**2a**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-2.20 (m, 17H); 3.20-3.40 (m, 4H); 4.00-4.05 (m, 1H); 6.40-6.45 (br s, 1H); 6.75-6.85 (m, 2H); 7.10-7.50 (m, 10H); 7.95-8.05 (m, 1H); 8.18 (s, 1H).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(5-phenylpyridazin-3-yl)sulfonyl]pentyl}urea (**2b**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-2.10 (m, 17H); 3.20-3.40 (m, 4H); 3.95-4.05 (m, 1H); 6.40-6.45 (m, 1H); 6.75-6.85 (m, 2H); 7.55-7.60 (m, 3H); 7.65-7.70 (m, 2H), 7.95-8.05 (m, 1H); 8.35 (s, 1H); 9.60 (s, 1H).

N-(2,4-Difluorophenyl)-*N'*-heptyl-*N'*-{4-[(6-phenylpyridazin-3-yl)sulfonyl]pentyl}urea (**2c**), ^{1}H-NMR ($CDCl_3$) δ: 0.85 (t, 3H); 1.20-2.10 (m, 17H); 3.20-3.40 (m, 4H); 3.90-

4.00 (m, 1H); 6.40 (br s, 1H); 6.75-6.85 (m, 2H); 7.55-7.60 (m, 3H); 7.90-8.02 (m, 1H), 8.05-8.25 (m, 4H).

Enzyme assays

In vitro assay against rat ACAT. Microsomes prepared from rat liver were used as a source of the enzyme. The activity of the ACAT inhibitors against rat ACAT was measured according to a previously described method [15]. GERI-BP001M was used as reference compound.

Computational methods

All calculations were carried out using the Gaussian 03 [16] program package. The conformational space of compounds **8a-c**, **9a-c** was explored through optimizations at the B3LYP level with the 6-31G* basis set. The energy profiles for rotation around the C3-X bond were initially determined on simplified molecules unsubstituted at the 5 and 6 positions of the heterocyclic ring. Then, after addition of the 5- and/or 6-phenyl groups to the minima located in the profiles, the structures were fully optimized allowing to determine the minimum energy conformations of compounds **8-9**.

Acknowledgements

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