Full Paper

3-Heptylamino-5-Phenylpyridazine Derivatives as Analogues of Acyl-CoA: Cholesterol Acyltransferase Inhibitors Containing the *N*-Heptyl-*N*′-Arylureidic Moiety

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A series of novel Acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors **8a – f** was synthesized; the substances were characterized by the presence of a 2,5-dimethylpyrazin-3-yl moiety at one end and a 3-heptylamino-5-phenylpyridazine system at the other one, linked through linear alkyl spacers of different length. The new derivatives were designed based on the hypothesis that the 3-amino-5-phenylpyridazine moiety could mimic the aryl substituted urea, which was present in a number of ACAT inhibitors previously described. The choice of the 2,5-dimethylpyrazin-3-yl substituent was supported by a preliminary investigation, which indicated that this moiety is the most powerful in conferring ACAT inhibitory properties to the new series. The pharmacological results proved the idea to be sound. Finally, compounds **9a – c**, lacking the phenylpyridazine moiety were prepared and tested to further strengthen our hypothesis.

Keywords: ACAT inhibitors / Hypercholesterolemia / Pyridazine derivatives / Ureido analogues

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Introduction

Acyl-CoA: cholesterol acyltransferase (E.C.2.3.1.26, ACAT) is an integral membrane protein, localized in the endoplasmic reticulum that catalyzes the intracellular esterification of cholesterol by using CoA-activated fatty acids [1]. ACAT-mediated esterification plays a role in intestinal cholesterol absorption, hepatic production of very low density lipoproteins (VLDL) and unregulated deposition of cholesteryl esters in atherosclerotic lesions [2]. Therefore, ACAT inhibition represents an attractive target for the treatment of hypercholesterolemia, atherosclerosis, coronary diseases [3, 4] and, possibly, also for Alzheimer's disease [5].

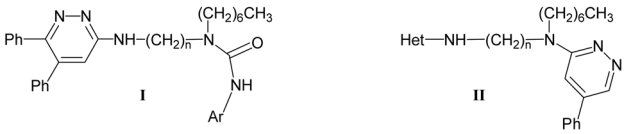

Figure 1. Structures of the ureido **I** and pyridazino **II** derivatives.

In previous papers [6 – 10], we reported our results on the design, the synthetic approach, the enzyme inhibition assay and the conformational study of novel ACAT inhibitors. In particular, a number of 5,6-diphenylpyridazines carrying an *n*-alkylamino substituent at position 3 were investigated. They resulted, in several cases, endowed with good inhibitory activity. Further studies showed that the alkyl chain of the substituent at position 3 could end with a group such as a substituted urea. This gave rise to compounds of the general formula **I,** which were still equipped with interesting inhibitory properties (Fig. 1).

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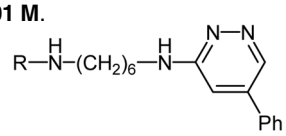
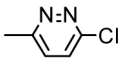
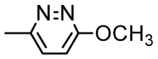
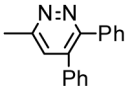
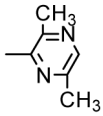
Table 1. Chemical data and ACAT inhibition of compounds **1–5** and **GERI-BP001 M**.



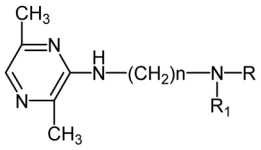
Compound	R	% Yield	Formula	% Inh.[a] (100 μg/mL)
1	H	55	$C_{16}H_{22}N_4$	72
2		16	$C_{20}H_{23}ClN_6$	83
3		3	$C_{21}H_{26}N_6O$	83
4		4	$C_{32}H_{32}N_6$	88
5		31	$C_{22}H_{28}N_6$	89
GERI-BP001 M				82

[a] Inhibition determined in rat liver microsomes. All values are means from three experiments, which differ by less than 10%.

Based on the hypothesis that the aryl substituted urea could be mimicked by a 3-amino-5-phenyl-pyridazine moiety in the binding ability and in the orientation of the groups, we planned the synthesis of a series of novel pyridazinyldiaminoalkyl derivatives of the general formula **II** (Fig. 1).

In order to define which heterocycle should be introduced in this new series, we performed a preliminary investigation aimed at exploring the efficiency of various systems at N_1. Since the data we obtained indicated the 2,5-dimethylpyrazin-3-yl derivative **5** as the best compound both in terms of inhibitory activity and chemical yield (Table 1 and Scheme 1), we synthesized compounds **8a–f**, all bearing this group on the left side and the 3-heptylamino-5-phenylpyridazine system on the right side, the two moieties being linked through a linear alkyl spacer of different length. To better verify the real role of the phenylpyridazine ring, a few derivatives **9a–c** lacking this moiety were also prepared (Tables 2, 3 and Scheme 1). In addition, compounds **12a, b** having either a piperazinyl or a homopiperazinyl linker between the two heterocyclic moieties, instead of the linear alkyl chain, were synthesized to evaluate the role of the linker flexibility vs. rigidity (Table 4).

Table 2. Chemical data of compounds **6** and **7**.



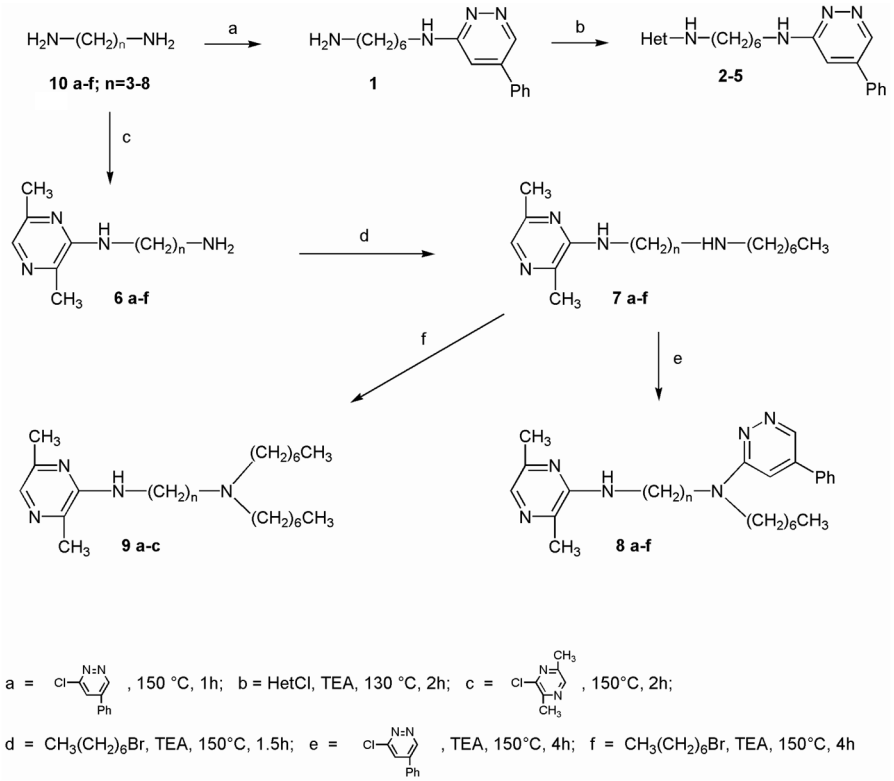
Compound	n	R	R_1	% Yield	Formula
6a	3	H	H	90	$C_9H_{16}N_4$
6b	4	H	H	88	$C_{10}H_{18}N_4$
6c	5	H	H	89	$C_{11}H_{20}N_4$
6d	6	H	H	87	$C_{12}H_{22}N_4$
6e	7	H	H	29	$C_{13}H_{24}N_4$
6f	8	H	H	23	$C_{14}H_{26}N_4$
7a	3	H	$(CH_2)_6CH_3$	73	$C_{16}H_{30}N_4$
7b	4	H	$(CH_2)_6CH_3$	71	$C_{17}H_{32}N_4$
7c	5	H	$(CH_2)_6CH_3$	70	$C_{18}H_{34}N_4$
7d	6	H	$(CH_2)_6CH_3$	69	$C_{19}H_{36}N_4$
7e	7	H	$(CH_2)_6CH_3$	28	$C_{20}H_{38}N_4$
7f	8	H	$(CH_2)_6CH_3$	34	$C_{21}H_{40}N_4$

Finally, to confirm the validity of our preliminary investigation on the efficiency of different heterocycles at N_1, we prepared and tested the analogue of the most potent compound of the series **8d**, still retaining the 5,6-diphenylpyridazine system (compound **15**), which characterized the ureido derivatives **I** [11] (Table 3 and Scheme 2).

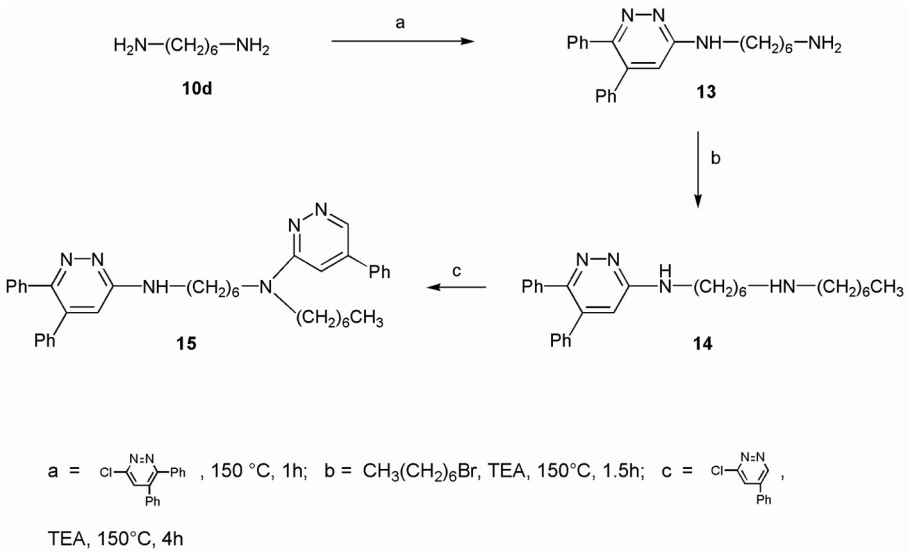
Results and discussion

All compounds were tested for their inhibitory properties towards ACAT extracted from rat liver microsomes. Their activity, expressed as inhibition percentage at 100 μg/mL, is reported in Tables 1, 3, and 4. GERI-BP001 M was used as reference compound [12].

As shown in Table 3, the length of the chain proved to have some influence on the ACAT inhibitory activity, though it could not easily be rationalized through an expression of activity as a function of chain length. In particular, the medium-length tertiary amine **8d** was the most active (95% inhibition) when compared to its higher and lower homologues (**8a–c, 8e–f**) which showed percentage inhibition in the range 58–87%. By contrast, the presence of a ring (**12a, b**) instead of the flexible spacer dramatically reduced the activity. In fact, inhibition values of 57 and 62% were found for **12a** and **12b**, respectively (Table 4). However, this could also depend on the distance between the two nitrogen atoms, even shorter than in **8a**, the compound with the shortest alkyl linker,



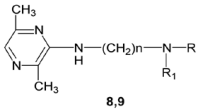
Scheme 1. Synthesis of N_1-substituted-N_6-(5-phenylpyridazin-3-yl)-1,6-diaminohexane derivatives **1 – 5** and N_1-(2,5-dimethylpyrazin-3-yl)-diaminoalkyl-N_ω-substituted derivatives **6 – 9**.



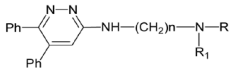
Scheme 2. Synthesis of N_1-(5,6-diphenylpyridazin-3-yl)-N_6-(5-phenylpyridazin-3-yl)-N_6-hepthyl-1,6-diaminohexane **15**.

which was in turn one of the weakest representatives of the series. It should be noted that, when the pyridazine moiety was replaced by a second *n*-heptyl residue (**9** series), the activity always dropped significantly and inhibition percentages lower than 60% were found, thus confirming the effective role of the heterocycle as a potential substitute for the ureido group in this series of ACAT inhibitors. Finally, the 5,6-diphenylpyridazinyl deriv-

Table 3. Chemical data and ACAT inhibition of compounds **8, 9, 15,** and **GERI-BP001 M**.



8,9



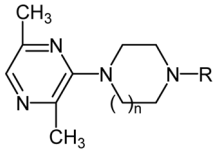
15

Compound	n	R	R_1	% Yield	Formula	% Inh.[a] (100 μg/mL)
8a	3		$(CH_2)_6CH_3$	17	$C_{26}H_{36}N_6$	70
8b	4		$(CH_2)_6CH_3$	15	$C_{27}H_{38}N_6$	87
8c	5		$(CH_2)_6CH_3$	17	$C_{28}H_{40}N_6$	79
8d	6		$(CH_2)_6CH_3$	17	$C_{29}H_{42}N_6$	95
8e	**7**		$(CH_2)_6CH_3$	21	$C_{30}H_{44}N_6$	58
8f	8		$(CH_2)_6CH_3$	31	$C_{31}H_{46}N_6$	76
9a	3	$(CH_2)_6CH_3$	$(CH_2)_6CH_3$	40	$C_{23}H_{44}N_4$	52
9b	4	$(CH_2)_6CH_3$	$(CH_2)_6CH_3$	43	$C_{24}H_{46}N_4$	55
9c	5	$(CH_2)_6CH_3$	$(CH_2)_6CH_3$	39	$C_{25}H_{48}N_4$	58
15	6		$(CH_2)_6CH_3$	18[b]	$C_{39}H_{46}N_6$	79
GERI-BP001 M						82

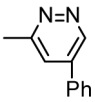
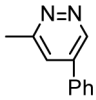
[a] *In vitro*% ACAT inhibition determined in rat liver microsomes. All values are means from three experiments, which differ by less than 10%.

[b] From **14**.

Table 4. Chemical data and ACAT inhibition of compounds **12** and **GERI-BP001 M.**



11,12

Compound	n	R	% Yield	Formula	% Inh.[a] (100 μg/mL)
11a	1	H	91	$C_{10}H_{16}N_4$	
11b	2	H	88	$C_{11}H_{18}N_4$	
12a	1		46	$C_{20}H_{22}N_6$	57
12b	2		46	$C_{21}H_{24}N_6$	62
GERI-BP001 M					82

[a] *In vitro*% ACAT inhibition determined in rat liver microsomes. All values are means from three experiments, which differ by less than 10%.

ative (**15**, 79% inhibition) was much weaker than its 2,5-dimethylpyrazinyl analogue (**8d**, 95% inhibition).

In conclusion, our initial hypothesis that the pyridazino moiety could possibly replace the ureido moiety present in a series of ACAT inhibitors we previously described [11], was supported fairly well by the data shown in Table 3. The new 2,5-dimethylpyrazin-3-yl derivatives **8** have ACAT inhibitory activity fully comparable to that previously reported for ureido counterparts. In addition, the choice based on a preliminary screening of the 2,5-dimethylpyrazinyl moiety instead of the 5,6-diphenylpyridazinyl ring, which characterized the ureido derivatives, proved to be successful, as clearly evidenced by the comparison between **15** and **8d**.

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Experimental

Chemistry

^{1}H-NMR spectra were recorded on a Bruker AC200 spectrometer (Bruker); chemical shifts are reported as δ (ppm), using the solvent as internal standard. Merck F-254 commercial plates (Merck, Germany) 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. The structures of all compounds were consistent with their analytical and spectroscopic data.

*General synthesis of **N_1**-substituted-**N_6**-(5-phenylpyridazin-3-yl)-1,6-diaminohexane derivatives **1 – 5***

3-Chloro-5-phenylpyridazine (6.8 mmol, 1.30 g) was added, under stirring to 1,6-diaminohexane **10d** (68 mmol, 7.90 g) and the mixture was heated for 1 h at 150°C. After cooling, the residue was purified by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 9 : 1) to give **1** (1.00 g, 55%). The appropriate heterocyclic chloride (2.1 mmol) and TEA (2.1 mmol) were added to **1** (2.1 mmol, 0.57 g) and the mixture stirred at 130°C for 2 h. Compounds **2 – 5** were purified by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 98 : 2) (for details see Tables 1, 5 and Scheme 1).

*General synthesis of **N_1**-(2,5-dimethylpyrazin-3-yl)-diaminoalkyl-**N_ω**-substituted derivatives **6 – 9***

3-Chloro-2,5-dimethylpyrazine (5.0 mmol, 0.71 g) was added under stirring to the required amine (**10a – f**, 0.05 mol) and the mixture was heated at 150°C for 2 h. After cooling, the residue was purified by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 9 : 1) to give the mono substituted amines **6a – f** which were heated at 150°C for 1.5 h, under stirring, with equimolar 1-bromoheptane and TEA. After purification by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 98 : 2), the intermediates **7a – f** were treated with equimolar amounts of either 3-chloro-5-phenylpyridazine or 1-bromoheptane in the presence of excess TEA at 150°C for 4 h. Compounds **8a – f** and **9a – c** were finally purified by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 98 : 2) (for details see Tables 2, 3, 5 and Scheme 1).

Compounds **12a** and **12b**, having a cyclic spacer instead of the linear alkyl chain, were prepared in a similar manner starting from piperazine or homopiperazine, respectively (for details see Tables 4, 5).

*Synthesis of **N_1**-(5,6-diphenylpyridazin-3-yl)-**N_6**-(5-phenylpyridazin-3-yl)-**N_6**-hepthyl-1,6-diaminohexane **15***

a) To 1,6-diaminohexane **10d** (18.7 mmol, 2.18 g), 3-chloro-5,6-diphenylpyridazine [11] (1.87 mmol, 0.50 g) was added under stirring and the mixture was heated for 1 h at 150°C. After cooling, the residue was purified by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 9 : 1) to give **13** (78%). $C_{22}H_{26}N_4$.

b) Compound **13** (1.46 mmol, 0.5 g) was heated at 150°C for 1.5 h, under stirring, with 1-bromoheptane (1.46 mmol, 0.26 g) and TEA (1.46 mmol, 0.15 g). After purification by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 98 : 2), **14** was obtained (16%). $C_{29}H_{40}N_4$.

c) Compound **14** (0.23 mmol, 0.10 g) was treated with 3-chloro-5-phenylpyridazine (0.23 mmol, 0.044 g) in the presence of excess TEA (0.46 mmol, 0.047 g) at 150°C for 4 h. After purification by flash chromatography (eluent: CH_2Cl_2/CH_3OH-NH_3, ratio 98 : 2) **15** was obtained (18%). $C_{39}H_{46}N_6$ (see Table 5 and Scheme 2 for details).

Table 5. ^{1}H-NMR data of compounds **1 – 15** ($CDCl_3$; chemical shifts in ppm).

Compound	^{1}H-NMR
1	1.30 – 1.55 (m, 6H + 2H, ex), 1.65 – 1.75 (m, 2H), 2.65 – 2.75 (t, 2H), 3.40 – 3.50 (m, 2H), 4.80 – 4.90 (brs, 1H, ex), 6.75 (s, 1H), 7.40 – 7.55 (m, 3H), 7.55 – 7.65 (d, 2H), 8.80 (s, 1H)
2	1.30 – 1.55 (m, 4H), 1.60 – 1.80 (m, 4H), 3.30 – 3.50 (m, 4H), 4.85 (brs, 2H, ex), 6.60 (d, 1H), 6.70 (s, 1H), 7.05 (d, 1H), 7.25 – 7.55 (m, 3H), 7.60 – 7.70 (m, 2H), 8.80 (s, 1H)
3	1.45 – 1.55 (m, 2H), 1.55 – 1.75 (m, 6H), 3.35 – 3.50 (m, 4H), 3.95 – 4.00 (s, 3H), 4.30 (brs, 1H, ex), 4.80 (brs, 1H, ex), 6.65 (d, 1H), 6.70 (s, 1H), 7.05 (d, 1H), 7.45 – 7.55 (m, 3H), 7.60 – 7.70 (m, 2H), 8.80 (s, 1H)
4	1.50 – 1.60 (m, 4H), 1.65 – 1.80 (m, 4H), 3.40 – 3.50 (m, 4H), 5.00 (brs, 2H, ex), 6.60 (s, 1H), 6.75 (s, 1H), 7.10 – 7.35 (m, 11H), 7.40 – 7.50 (m,2H), 7.50 – 7.60 (m,2H), 8.75 (s,1H)
5	1.45 – 1.55 (m, 4H), 1.60 – 1.80 (m, 4H), 3.30 (s, 3H), 3.35 (s, 3H), 3.40 – 3.50 (m, 4H), 4.20 (brs, 1H, ex), 4.85 (brs, 1H, ex), 6.75 (s, 1H), 7.45 – 7.50 (m, 3H), 7.55 (s, 1H), 7.60 – 7.65 (m, 2H), 8.80 (s, 1H)
6a	1.75 – 1.85 (m, 2H + 2H, ex), 2.30 (s, 3H), 2.35 (s, 3H), 2.60 (t, 2H), 3.40 (t, 2H), 5.95 (brs, 1H, ex), 7.50 (s, 1H)
6b	1.50 – 1.70 (m, 4H + 2H, ex), 2.30 (s, 3H), 2.35 (s, 3H), 2.55 (t, 2H), 3.40 (t, 2H), 4.50 (brs, 1H, ex), 7.50 (s, 1H)
6c	1.35 – 1.40 (m, 2H + 2H, ex), 1.50 – 1.55 (m, 4H), 2.30 (s, 3H), 2,35 (s, 3H), 2.60 (t, 2H), 3.40 (t, 2H), 4.20 (brs, 1H, ex), 7.55 (s, 1H)
6d	1.30 – 1.45 (m, 6H + 2H, ex), 1.50 – 1.65 (m, 2H), 2.20 (s, 3H), 2.25 (s, 3H), 2.50 – 2.70 (m, 2H), 3.30 – 3.45 (m, 2H), 4.25 (brs, 1H, ex), 7.45 (s, 1H)
6e	1.30 – 1.50 (m, 6H), 1.50 – 1.70 (m, 4H + 2H, ex), 2.30 (s, 3H), 2.35 (s, 3H), 2.60 – 2.70 (t, 2H), 3.35 – 3.50 (m, 2H), 4.20 (brs, 1H, ex), 7.50 (s, 1H)
6f	1.20 – 1.50 (m, 10H + 2H, ex), 1.55 – 1.70 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.60 – 2.70 (t, 2H), 3.35 – 3.45 (m, 2H), 4.20 (brs, 1H, ex), 7.50 (s, 1H)
7a	0.85 (t, 3H), 1.20 – 1.40 (m, 8H), 1.50 – 1.60 (m, 2H + 1H, ex), 1.75 – 1.90 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.60 (t, 2H), 2.80 (t, 2H), 3.50 – 3.60 (m,2H), 6.05 (brs, 1H,ex), 7.75 (s, 1H)
7b	0.85 (t, 3H), 1.20 – 1.35 (m, 8H), 1.40 – 1.50 (m, 2H), 1.50 – 1.75 (m, 4H + 1H, ex), 2.30 (s, 3H), 2.35 (s, 3H), 2.60 (t, 2H), 2.65 – 2.75 (t, 2H), 3.40 – 3.50 (m, 2H), 4.55 (brs, 1H, ex), 7.55 (s, 1H)
7c	0.85 (t, 3H), 1.20 – 1.40 (m, 8H), 1.40 – 1.60 (m, 6H + 1H, ex), 1.60 – 1.70 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.55 – 2.65 (m, 4H), 3.40 – 3.50 (m, 2H), 4.20 (br s, 1H, ex), 7.55 (s, 1H)
7d	0.85 (t, 3H), 1.20 – 1.30 (m, 8H), 1.35 – 1.40 (m, 4H), 1.50 – 1.60 (m, 4H), 1.60 – 1.70 (m, 2H + 1H, ex), 2.25 (s, 3H), 2.30 (s, 3H), 2.50 – 2.70 (m,4H), 3.35 – 3.50 (m, 2H), 4.20 (brs, 1H, ex), 7.55 (s, 1H)
7e	0.80 – 0.90 (t, 3H), 1.20 – 1.40 (m, 12H + 1H, ex), 1.40 – 1.50 (m, 4H), 1.55 – 1.70 (m, 4H), 2.30 (s, 3H), 2.35 (s, 3H), 2.55 – 2.69 (m, 4H), 3.40 – 3.50 (m, 2H), 4.20 (brs, 1H, ex), 7.55 (s, 1H)
7f	0.85 (t, 3H), 1.20 – 1.40 (m, 16H), 1.40 – 1.55 (m, 4H), 1.60 – 1.65 (m, 2H), 1.90 (brs, 1H, ex), 2.30 (s, 3H), 2.35 (s, 3H), 2.55 – 2.65 (m, 4H), 3.40 – 3.50 (m, 2H), 4.20 (brs, 1H, ex), 7.45 (s, 1H)
8a	0.85 (t, 3H), 1.20 – 1.40 (m, 8H), 1.60 – 1.70 (m, 2H), 1.90 – 2.00 (m, 2H), 2.30 (s, 3H), 2.50 (s, 3H), 3.40 – 3.50 (m, 2H), 3.50 – 3.55 (m, 2H), 3.85 (t. 2H), 5.90 (brs, 1H, ex), 6.80 (s,1H), 7.45 – 7.55 (m, 4H), 7.55 – 7.60 (m,2H), 8.85 (s, 1H)
8b	0.85 (t, 3H), 1.20 – 1.40 (m, 8H), 1.60 – 1.70 (m, 4H) 1.70 – 1.80 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 3.45 – 3.60 (m, 4H), 3.80 (t, 2H), 4.50 (brs, 1H, ex), 6.80 (s, 1H), 7.45 – 7.60 (m, 6H), 8.75 (s, 1H)
8c	0.85 (t, 3H), 1.20 – 1.40 (m, 8H),1.40 – 1.50 (m, 6 H), 1.60 – 1.80 (m, 2 H), 2.30 (s, 3H), 2.35 (s, 3H), 3.40 – 3.55 (m, 4H), 3.65 (t, 2H), 4.20 (brs, 1H, ex), 6.80 (s, 1H), 7.45 – 7.55 (m, 4H), 7.55 – 7.60 (m, 2H), 8.75 (s, 1H)
8d	0.85 (t, 3H), 1.20 – 1.40 (m, 8H), 1.40 – 1.50 (m, 4H), 1.60 – 1.80 (m, 6H), 2.25 (s, 3H), 2.30 (s, 3H), 3.40 – 3.50 (m, 2H), 3.50 – 3.60 (m, 2H), 3.60 – 3.70 (m, 2H), 4.20 (brs, 1H, ex), 6.75 (s, 1H), 7.45 – 7.50 (m, 3H), 7.55 (s, 1H), 7.60 – 7.65 (m, 2H), 8.70 (s, 1H)
8e	0.85 (t, 3H), 1.20 – 1.50 (m, 14H) 1.50 – 1.70 (m, 6H), 2.30 (s, 3H), 2.35 (s, 3H), 3.40 – 3.50 (m, 2H), 3.50 – 3.70 (m, 4H), 4.20 (brs, 1H, ex), 6.80 (s, 1H), 7.45 – 7.50 (m, 3H), 7.55 (s, 1H), 6.60 – 7.65 (m, 2H), 8.75 (s, 1H)
8f	0.85 (t, 3H), 1.20 – 1.40 (m, 16H), 1.40 – 1.55 (m, 4H), 1.55 – 1.65 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 3.40 – 3.45 (m, 2H), 3.50 – 3.60 (m, 4H), 4.20 (brs, 1H, ex), 6.75 (s, 1H), 7.40 – 7.50 (m, 3H), 7.55 (s, 1H), 7.60 – 7.65 (m, 2H), 8.70 (s, 1H)
9a	0.85 (t, 6H), 1.15 – 1.35 (m, 16H), 1.40 – 1.50 (m, 4H), 1.70 – 1.85 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.40 – 2.50 (m, 4H), 2.55 – 2.65 (m, 2H), 3.45 – 3.55(m, 2H), 6.35 (brs, 1H, ex), 7.30 (s,1H)
9b	0.85 (t, 6H), 1.20 – 1.35 (m,16H), 1.35 – 1.55 (m, 4H), 1.55 – 1.70 (m, 4H), 2.30 (s, 3H), 2.35 (s.3H), 2.35 – 2.50 (m, 6H), 3.40 – 3.50 (m, 2H), 4.35 (brs, 1H, ex), 7.55 (s, 1H)
9c	0.85 (t, 6H), 1.20 – 1.35 (m, 16H), 1.35 – 1.55 (m, 8H), 1.60 – 1.70 (m, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.35 – .2.45 (m, 6H), 3.40 – 3.50 (m, 2H), 4.20 (brs, 1H, ex), 7.55 (s, 1H)
11a	1.85 (s, 1H, ex), 2.35 (s, 3H), 2.45 (s, 3H), 2.95 – 3.00 (m, 4H), 3.10 – 3.15 (m, 4H), 7.85 (s, 1H)
11b	1.80 – 1.90 (m, 2H), 2.20 (brs, 1H, ex), 2.30 (s, 3H), 2.45 (s, 3H), 2.80 – 2.85 (m, 2H), 3.00 – 3.10 (m, 2H), 3.50 – 3.65 (m, 4H), 7.75 (s, 1H)
12a	2.40 (s, 3H), 2.55 (s, 3H), 3.30 – 3.40 (m, 4H), 3.85 – 3.90 (m, 4H), 7.10 (s, 1H), 7.45 – 7.55 (m, 3H), 7.60 – 7.70 (m, 2H), 7.95 (s, 1H), 8.85 (s, 1H)
12b	2.05 – 2.15 (m, 2H), 2.35 (s, 3H), 2.45 (s, 3H), 3.45 – 3.50 (m, 2H), 3.70 – 3.80 (m, 2H), 3.80 – 3.90 (m, 2H), 4.10 – 4.15 (m, 2H), 6.90 (s, 1H), 7.45 – 7.55 (m, 3H), 7.60 – 7.65 (m, 2H), 7.80 (s, 1H), 8.80 (s, 1H)
13	1.30 – 1.50 (m, 6H), 1.60 – 1.70 (m, 2H), 1.75 – 1.90 (brs, 3H, ex), 2.65 (t, 2H), 3.35 – 3.45 (m, 2H), 6.60 (s, 1H), 7.10 – 7.35 (m, 10H)
14	0.85 (t, 3H), 1.20 – 1.35 (m, 10H), 1.40 – 1.55 (m, 4H + 1H, ex), 1.60 – 1.75 (m, 4H), 2.50 – 2.65 (m, 4H), 3.40 – 3.50 (m, 2H), 4.85 (brs, 1H, ex), 6.60 (s, 1H), 7.10 – 7.35 (m, 10H)
15	0.85 (t, 3H), 1.20 – 1.40 (m, 10H), 1.40 – 1.60 (m, 4H), 1.60 – 1.80 (m, 4H + 1H, ex), 3.40 – 3.55 (m, 4H), 3.60 – 3.70 (m, 2H), 6.70 (s, 1H), 6.80 (s, 1H), 7.10 – 7.35 (m, 11H), 7.45 – 7.55 (m, 2H), 7.55 – 7.60 (m, 2H), 8.70 (s, 1H)

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 following a previously described method [12]. **GERI-BP001 M** was used as reference compound. Accordingly, the reaction mixture, containing 4 μL of microsomes (10 mg/mL protein), 20 μL of 0.5 M potassium-phosphate buffer (pH 7.4, 10 mM dithiothreitol), 15 μL of bovine serum albumin (fatty acid free, 40 mg/mL), 2 μL of cholesterol in acetone (20 mg/mL, added last), 41 μL of water, and 10 mL of test sample in a total volume of 92 μL, was preincubated for 30 min at 37°C. The reaction was initiated by the addition of 8 μL of [1-^{14}C]oleoyl-CoA solution (0.05 μCi, final conc. 10 μM). After 15 min of incubation at 37°C, the reaction was stopped by the addition of 1.0 mL of isopropanol-heptane (4 : 1; v/v) solution. A mixture of 0.6 mL of heptane and 0.4 mL of 0.1 M potassium-phosphate buffer (pH 7.4, 2 mM dithiothreitol) was then added to the terminated reaction mixture. The above solution was mixed and allowed to phase separation under gravity for 2 min. Cholesterol oleate was recovered in the upper heptane phase (total volume 0.9 – 1.0 mL). The radioactivity in 100 mL of the upper phase was measured in a 7 mL liquid scintillation vial with 4 mL of scintillation cocktail (Lipoluma, Lumac Co., Basel, Switzerland) using a liquid scintillation counter (Packard Delta-2000; Hewlett Packard). Background values were obtained by preparing heat inactivated microsomes. Since some of the radioactivity recovered in the upper phase could be due to enzymatic incorporation of radioactive fatty acid into other products than cholesteryl oleate, the partitioned heptane phases were purified by preparative TLC (eluent *n*-hexane/diethyl ether/acetic acid; 90 : 10 : 1) and their radioactivity measured as described above. Percent inhibition of ACAT activity was calculated by subtracting the background values from both control and test sample values according to the following equation:

$$\% \text{ Inhibition} = 100 \times \left[1 - \frac{\text{Sample (cpm)} - \text{Background (cpm)}}{\text{Control (cpm)} - \text{Background (cpm]}}\right]$$

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