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Synthesis of novel 4,5-diphenylthiazole derivatives as potential acyl-CoA : cholesterol *O*-acyltransferase inhibitors[3]

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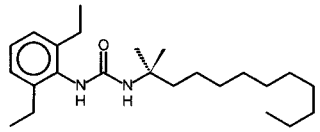
Several novel *N*-(4,5-diphenylthiazol-2-yl)-*N*′-aryl or alkyl (thio)ureas and *N*-(4,5-diphenylthiazol-2-yl)alkanamides were prepared as potential acyl-CoA : cholesterol *O*-acyltransferase (ACAT) inhibitors. Synthesis was accomplished by reaction of 2-amino-4,5-diphenylthiazole with the suitable isocyanate, isothiocyanate or acyl chloride. Some analogues without the 5-phenyl substituent or both the phenyl groups in 4 and 5 position of the thiazole ring were also prepared. Moreover, some bioisosters of the title compounds in which the thiazole ring was replaced by an imidazole were synthesized starting from the 2-amino-4,5-diphenyl-1*H*-imidazole. The ability of synthesized compounds to inhibit ACAT was evaluated *in vitro* by measuring the formation of cholesteryl[^{14}C]oleate from cholesterol and [1-^{14}C]oleoyl-CoA in rat liver microsomes. Among the tested compounds, only some thiazole ureas were able to inhibit ACAT in a reasonable degree. *N*-(4,5-diphenylthiazol-2-yl)-*N*′-[2,6-bis(2-methylethyl)phenyl] urea (**11**) and *N*-(4,5-diphenylthiazol-2-yl)-*N*′-n-butyl urea (**16**) were the most active compounds in the series showing IC_{50} values in the low micromolar range.

1. Introduction

Hypercholesterolemia has been identified as one of the major risk factors for coronary heart disease and many efforts have been directed towards the discovery of new and effective hypocholesterolemic drugs. The acyl-CoA : cholesterol *O*-acyltransferase (ACAT) is a microsomal enzyme that catalyzes the formation of long chain fatty acid cholesterol esters and plays a pivotal role in cholesterol metabolism. It represents an interesting biochemical target for novel hypolipidemic and antiatherosclerotic agents since its inhibition leads to a reduction of intestinal cholesterol absorption, to a decrease in liver secretion of very low density lipoprotein (VLDL) particles and to a reduced accumulation of cholesterol esters in the arterial wall cells, a key step in the atherosclerotic process [1, 2].

From a chemical point of view, known ACAT inhibitors represent a structurally heterogeneous class [2]; nevertheless an alkyl substituted urea (or amide) group seems to be a common structural feature in some of them such as in the potent inhibitors **1** [3] and **2** [4]. Moreover, some 2-alkylthio-4,5-diphenyl-1*H*-imidazoles **3** have been recently reported as potent inhibitors of this enzyme [5].

Structure-activity relationships studies in this series of compounds suggested that the presence of both the aryl groups as well as of the imidazole NH was essential for activity. With the aim to obtain new and potent ACAT inhibitors, we now report the synthesis and *in vitro* pharmacological evaluation of a new series of 2-substituted-4,5-diphenyl thiazoles **7**–**23**; the structure of these compounds contains an *ortho* diphenyl substituted heterocyclic nucleus coupled to an urea, thiourea, amide or urethane group in position 2. Although the bioisosteric substitution imidazole/thiazole leads to the loss of the endocyclic NH function, the presence of the urea or amide provides an exocyclic NH group that could mimic the imidazole NH and be important for activity. Moreover, we prepared some 2-substituted-4-phenyl thiazoles **24**–**26** and the *N*-thiazol-2-yl-*N*′-butyl urea (**27**) which does not bear any substituent in 4 and 5 positions of the thiazole ring, to test the importance of the two phenyl groups for activity. Finally, we also synthesized the bioisosteric analogues **35**–**39** of some active 4,5-diphenyl thiazole derivatives in which the thiazole was substituted with an imidazole ring.



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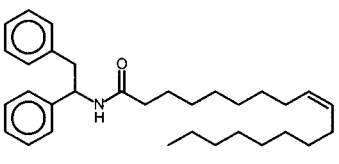

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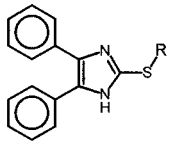

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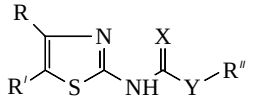
R = alkyl, cycloalkyl

2. Investigations, results and discussion

2.1. Chemistry

The synthesis of the thiazole derivatives **7**–**33** (Table 1) was accomplished as shown in Scheme 1. The starting materials were 2-amino-4,5-diphenylthiazole (**4**), 2-amino-4-phenylthiazole (**5**) and 2-aminothiazole (**6**). Compound **4** [6] was easily prepared, following a literature method, by heating at 120 °C deoxybenzoin with thiourea in presence of iodine. Compound **5** [7] was synthesized by the reaction of 2-bromoacetophenone with thiourea in DMF whereas compound **6** is commercially available. Reaction of 2-aminothiazoles **4**–**6** with the opportune aryl or alkyl iso(thio)cyanate in toluene at reflux gave the *N*-[(substituted)thiazol-2-yl]-*N*′-aryl(alkyl)ureas **7**–**20**, **24**–**27** and the corresponding thioureas **21**–**23** in good yields. Compound **4** was also reacted with alkanoic acid chlorides of different chain length to afford the alkanamides **28**–**31** in good yields. Some of them showed quite low melting points (**29** and **30** are waxes at room temperature) and, although the work-up of the reaction mixtures gave crude products containing only little amounts of side com-

Table 1: Physical properties of compounds 7–33



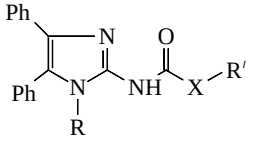
Compd.	R	R′	X	Y	R″	m.p. (°C)	Yield (%)	Recryst. solvent	IR (KBr, cm^{-1})	
									C=O	NH
7	Ph	Ph	O	NH	C_6H_5	120–121	79	EtOH/H_2O	1685	3540, 3270
8	Ph	Ph	O	NH	2-FC_6H_4	114–115	78	MeOH	1675	3390, 3190
9	Ph	Ph	O	NH	2-$(CH_3O)C_6H_4$	204–205	82	EtOH/H_2O	1675	3190
10	Ph	Ph	O	NH	2,4-$F_2C_6H_3$	193–194	81	MeOH	1670	3190
11	Ph	Ph	O	NH	2,6-(*iso*-$C_3H_7)_2C_6H_3$	178–179	85	Cyclohexane	1700	3390, 3240
12	Ph	Ph	O	NH	$CH_2C_6H_5$	164–165	59	Cyclohexane	1685	3380, 3160
13	Ph	Ph	O	NH	C_6H_{11}	214–215	54	EtOH	1695	3400, 3190
14	Ph	Ph	O	NH	*n*-C_3H_7	137–138	60	EtOH/H_2O	1680	3410, 3160
15	Ph	Ph	O	NH	*iso*-C_3H_7	192–193	45	EtOH/H_2O	1675	3490, 3160
16	Ph	Ph	O	NH	*n*-C_4H_9	149–150	87	Cyclohexane	1675	3390, 3150
17	Ph	Ph	O	NH	*n*-C_5H_{11}	138–139	80	Cyclohexane	1700	3410, 3180
18	Ph	Ph	O	NH	*n*-C_7H_{15}	118–119	58	Cyclohexane	1690	3410, 3160
19	Ph	Ph	O	NH	*n*-C_8H_{17}	131–132	62	Petroleum ether	1670	3380, 3160
20	Ph	Ph	O	NH	*n*-$C_{12}H_{25}$	69–70	60	Petroleum ether	1670	3370, 3280
21	Ph	Ph	S	NH	C_6H_5	188–190	72	EtOH/H_2O	–	3160
22	Ph	Ph	S	NH	$CH_2C_6H_5$	222–223	82	CH_3CN	–	3170
23	Ph	Ph	S	NH	*n*-C_4H_9	198–200	45	EtOH	–	3165
24	Ph	H	O	NH	C_6H_5	206–207	48	EtOH	1710	3390, 3200
25	Ph	H	O	NH	C_6H_{11}	139–140	89	Cyclohexane	1685	3410, 3170
26	Ph	H	O	NH	*n*-C_4H_9	135	78	Cyclohexane	1690	3370, 3200
27	H	H	O	NH	*n*-C_4H_9	108–109	91	Cyclohexane	1695	3400, 3200
28	Ph	Ph	O	CH_2	*n*-C_4H_9	125–126	40	Cyclohexane	1690	3150
29	Ph	Ph	O	CH_2	*n*-C_8H_{17}	wax	71	a	1680	3160
30	Ph	Ph	O	CH_2	*n*-$C_{10}H_{21}$	wax	72	a	1680	3175
31	Ph	Ph	O	CH_2	*n*-$C_{12}H_{25}$	78–79	40	a	1680	3160
32	Ph	Ph	O	O	*n*-C_4H_9	99–100	47	Cyclohexane	1720	3140
33[b]	–	–	–	–	–	193–194	43	CH_3CN	1680	3400, 3190

[a] Purified by flash chromatography; [b] See structure in Scheme 1

pounds, a flash chromatography purification step had to be preferred over the less time-consuming recrystallization. Carbamic acid butyl ester derivative **32** was obtained by reaction of **4** with butylchloroformate in dry toluene. Starting from the bifunctional 1,6-diisocyanatohexane and **4**, the *N*,*N*″-1,6-hexanediylbis urea derivative **33** was also prepared.

The synthesis of compounds **35**–**39** (Table 2), which contain an imidazole ring instead of the thiazole, was accomplished, as shown in Scheme 2, starting from the 2-amino-4,5-diphenyl-1*H*-imidazole (**34**). Its synthesis was performed in two steps following the Nishimura-Kitajiama's method [8] in which the 2-amino-4,5-diphenyl-4-hydroxy-4*H*-imidazole is obtained by the reaction of benzil with guanidine. Subsequent catalytic hydrogenation of the 4-hydroxy derivative produces **34** in good yield. As already seen for the 2-aminothiazole derivatives **4**–**6**, compound **34** was then reacted, in the opportune experimental conditions, with the suitable isocyanate to give the ureas **35**–**37** or with the hexanoyl chloride to give the hexanamide derivative **39**. Methylation of the imidazole ring in **35** at the 1 position with methyl iodide in acetone afforded compound **38**. The presence of a singlet for the CH_3 group at δ 3.23 in the ^1H NMR spectrum of **38** and the lack of the singlet signal at δ 11.24 for the imidazole NH, as seen in the ^1H NMR spectrum of **35**, are in agreement with the methylation of the imidazole NH and not of the urea NH functions of **35**.

Table 2: Physical properties of compounds 35–39



Compd.	R	X	R′	m.p. (°C)	Yield (%)	Recryst. solvent	IR (KBr, cm^{-1})	
							C=O	NH
35	H	NH	*n*-C_4H_9	170–172	60	Ethyl acetate	1680	3320, 3220
36	H	NH	C_6H_{11}	182–184	50	Ethyl acetate	1680	3400, 3280
37	H	NH	C_6H_5	198–200	35	a	1690	3350, 3060
38	CH_3	NH	*n*-C_4H_9	190–192	60	Ethyl acetate	1680	3150
39	H	CH_2	*n*-C_4H_9	159	35	a	1680	3300

[a] Purified by flash chromatography

Table 3: Inhibitory activities on ACAT of compounds 7–33 and 35–39

Compd.	IC_{50} (μM)	Compd.	IC_{50} (μm)
7	80	**24**	60
8	>200	**25**	>200
9	>200	**26**	100
10	>200	**27**	>200
11	6	**28**	50
12	>200	**29**	>200
13	10	**30**	100
14	>200	**31**	>200
15	200	**32**	>200
16	5	**33**	30
17	>200	**35**	>200
18	200	**36**	>200
19	>200	**37**	100
20	>200	**38**	>200
21	>200	**39**	>200
22	>200	**40**	0.1
23	>200		

All the proposed structures were confirmed by elemental analyses, IR, ^{1}H NMR and, for some compounds, MS.

2.2. Pharmacological results and discussion

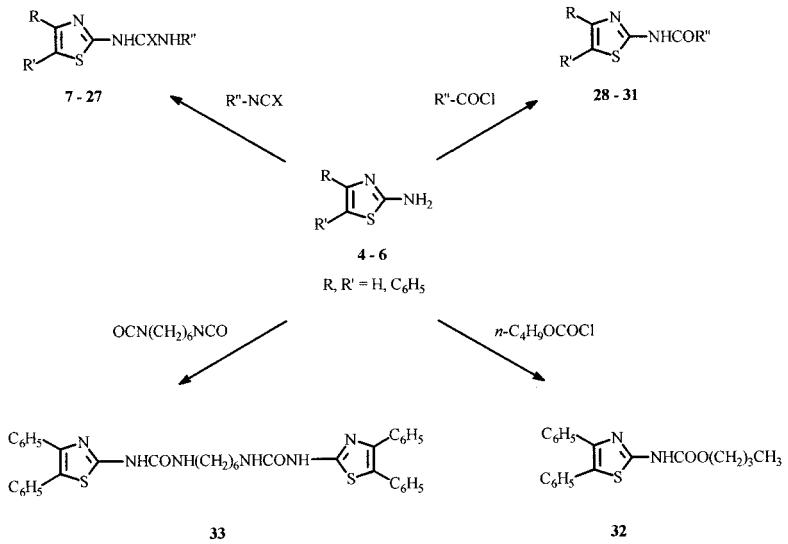
Synthesized compounds **7**–**33** and **35**–**39** were evaluated *in vitro* in the microsomal ACAT assay. Tests for ACAT activity were performed by measuring the formation of cholestery[^{14}C]oleate from cholesterol and [1-^{14}C]oleoyl-CoA in rat liver microsomes. ACAT inhibition activity of each compound was expressed as IC_{50}. 2-(Cyclohexylmethyl)thio-4,5-diphenyl-1*H*-imidazole **40** was used as reference compound. Its measured IC_{50} value (Table 3) was in accordance with the literature [5].

ACAT inhibition activities of compounds **7**–**33** and **35**–**39** are summarized in Table 3. Among the 4,5-diphenylthiazole derivatives, ureas **11**, **13** and **16**, bearing a 2,6-bis(2-methylethyl)phenyl, a cyclohexyl or a *n*-butyl group as substituent at the urea nitrogen distal from the thiazole ring, displayed IC_{50} values in the low micromolar range and were the most active derivatives in the series. However they are less active than 2-cyclohexylmethyl-4,5-diphenyl-1*H*-imidazole (**40**), a potent ACAT inhibitor used as reference compound in the enzymatic assay. The nature of the substituent at the urea nitrogen seems critical for activity. Among the *N*′-phenyl substituted ureas, the 2,6-bis(2-methylethyl)phenyl derivate **11** showed a good activity with a IC_{50} value of 6 μM, whereas the phenyl analog **7** was a poor inhibitor ($IC_{50} = 80$ μm) and the 2-fluorophenyl, the 2-methoxyphenyl and the 2,4-difluorophenyl derivatives (**8**, **9** and **10**, respectively) were completely inactive ($IC_{50} > 200$ μM). The insertion of a methylene group between the urea nitrogen and the phenyl group in **7** was detrimental for activity since the *N*′-benzyl derivative **12** was also inactive. With reference to the *N*′-alkyl ureas, the *n*-butyl derivative **16** showed the best activity ($IC_{50} = 5$ μM), followed by the *N*′-cyclohexyl derivative **13** ($IC_{50} = 10$ μM). Quite surprisingly, all the other *N*′-alkyl derivatives were completely inactive. In fact, either the lower homologues **14** and **15** of the *n*-butyl derivative **16**, either its higher homologues **17** displayed IC_{50} values >200 μM. Moreover, compounds **18**, **19**, and **20**, bearing longer alkyl chains at the urea nitrogen, were also inactive; this result was unexpected since a long alkyl chain is a structural feature of some potent ACAT inhibitors such as **1** and **2**.

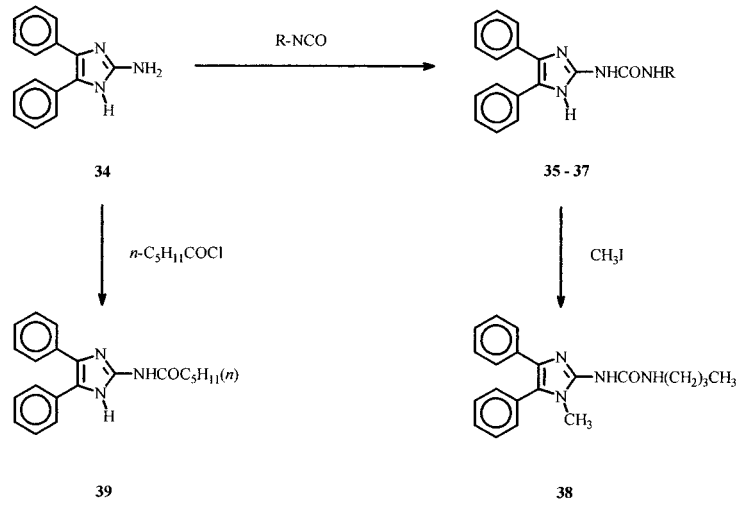
A drastic decrement in activity was observed when the urea oxygen was substituted by a sulfur atom, being thioureas **21**–**23** inactive. This is evident in the comparison between compound **16**, the most active in the series, and its thiourea analog **23**, which showed a $IC_{50} > 200$ μM. These data suggest that the urea oxygen is one of the structural features important for activity in this series of molecules. Some other bioisosteric variation of the urea group did not give better results. The urethane derivative **32**, in which an oxygen atom substitutes the urea nitrogen distal to the thiazole ring in **16**, showed no activity on ACAT. With reference to the amide derivatives, only the pentanamide **28** showed a moderate activity whereas homologues **29**–**31** with longer alkyl chains displayed higher IC_{50} values. Compound **28** can be considered as analogue of **16** and **32** in which a CH_2 group has substituted the distal urea nitrogen or the urethane ester oxygen, respectively. Comparison of the IC_{50} values for these three compounds shows how, in that position, the NH group of **16** ($IC_{50} = 5$ μM) is preferred either to the CH_2 of **28** ($IC_{50} = 50$ μM), either and even more to the oxygen of **32** ($IC_{50} > 200$ μM).

Elimination of the phenyl substituent in 5-position of the

Scheme 1



Scheme 2



thiazole ring (compounds **24**–**26**) resulted in a decrease of inhibitory activity. The 4-phenyl thiazole derivative **26**, bearing a *n*-butyl chain at the distal urea nitrogen, was 20-fold less active than its analogue **16**. Interestingly, among the 4-phenylthiazole derivatives, the *N′*-phenyl derivative **24** was the only one showing activity, even if moderate. In the 4,5-diphenylthiazole series, on the contrary, the *N′*-butyl and the *N′*-cyclohexyl derivatives **16** and **13** have shown higher ACAT inhibitory activity than the *N′*-phenyl analogue **7**. Finally, when the two phenyls of the thiazole ring were replaced by hydrogens (compound **27**) no ACAT inhibition was observed. These data suggest that the contemporary presence of both phenyl groups on the heterocyclic ring is important for activity, as already seen in a series of 2-alkylthio-4,5-diphenylimidazoles [5]. If only one is present in 5-position, the structure-activity relationships for these derivative do not overlap those for the 4,5-diphenylthiazole series.

In order to evaluate the importance of the nature of the heterocyclic ring for activity, in compounds **35**–**37** thiazole was substituted by an imidazole. Imidazole is present in the structure of known ACAT inhibitors [5] and its endocyclic NH could act as a hydrogen bond donor in addition to the ureic or amidic ones in the inhibitor-enzyme complex. Unfortunately, either the urea derivatives **35** and **36**, or the amide **39** were inactive with $IC_{50} > 200\,\mu M$. The only compound showing a certain degree of ACAT inhibitory activity was the *N′*-phenyl urea derivative **37**, which however, with a $IC_{50} = 100\,\mu M$ is a very poor inhibitor. Inactivity was also shown by compound **38** which represents the methylated analogue of **35** at the imidazole nitrogen.

Finally, the "dimeric" compound **33**, which presents two *N*-(4,5-diphenylthiazol-2-yl)urea moieties connected by an alkyl chain of six methylene units, showed a moderate activity with $IC_{50} = 30\,\mu M$. It is interesting to note that other bisureas with a connecting alkyl chain, such as BAYo2752 [13] had shown ACAT inhibitory activity *in vitro* and hypocholesterolemic activity *in vivo*.

In conclusion, a series of novel *N*-(4,5-diphenylthiazol-2-yl)-*N′*-aryl or alkyl ureas were synthesized and some of them were active in the ACAT inhibition assay in the low micromolar range. Moreover, several analogues which present some structural variations of the group in 2-position of the thiazole ring (thiourea, amide, urethane), of the substituents in 4- and 5-position (phenyl or hydrogen) and in the nature of the heterocyclic ring (thiazole or imidazole) were also prepared and tested. However, most of these structural modifications were detrimental for activity and only some *N*-(4,5-diphenylthiazol-2-yl)urea derivatives showed good ACAT inhibitory activity. *N*-(4,5-diphenylthiazol-2-yl)-*N′*-[2,6-bis(2-methylethyl)phenyl] urea (**11**) and *N*-(4,5-diphenylthiazol-2-yl)-*N′*-n-butyl urea (**16**) were the most active compounds in the series showing IC_{50} values in the low micromolar range. These compounds, however, are less potent than other known ACAT inhibitors such as DUP128 [2] which shows IC_{50} values in the nanomolar range.

3. Experimental

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrometer (Perkin Elmer, Überlingen, Germany) with KBr disks. Elemental analyses for C, H, N and S were performed on a Carlo Erba EA 1108 elemental analyzer (Carlo Erba, Milano, Italy) and were within ±0.4% of the theoretical values.

All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel 60 F_{254}, E. Merck, Darmstadt, Germany) and visualized by UV ($\lambda = 254$ and 366 nm). Column flash chromatography was performed on silicagel 60 (0.040–0.063 mm). The ^{1}H NMR spectra were recorded on a 250 MHz Bruker instrument or a 200 MHz Varian instrument in DMSO-d_6 or in $CDCl_3$. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and signals were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signals). MS (m/z) were recorded on a Kratos MS-50 instrument fitted with a standard EI source (ionization energy = 70 eV).

2-(Cyclohexylmethyl)thio-4,5-diphenyl-1 *H*-imidazole (**40**) was prepared following a literature method [5]. Compounds **21** [9], **24** [10] and **26** [10] were already described in literature.

3.1. Synthesis of the compounds

3.1.1. N-(4,5-Diphenylthiazol-2-yl)-N′-heptyl urea (**18**)

Preparation of this compound is presented as an example of the general synthesis of compounds **7**–**20**.

Heptyl isocyanate (0.615 g, 4.36 mmol) was added to a hot solution of **4** (1.0 g, 3.96 mmol) in toluene (15 ml) and the mixture was refluxed for 1 h. Then the solvent was removed in vacuo and the oily residue was suspended in petroleum ether (40–60 °C) (20 ml). After stirring for 15 min, the solid material was filtered off, washed with petroleum ether and dried. Recrystallization from cyclohexane gave **18** as a white powder (0.9 g, 58%): m.p. 118–119 °C. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 6.2 Hz, 3 H, CH_3), δ 1.26 (m, 8 H, $(CH_2)_4$), δ 1.35–1.50 (m, 2 H, $NHCH_2CH_2$), δ 3.0–3.2 (m, 2 H, $NHCH_2$), δ 6.53 (br t, 1 H, $NHCH_2$), δ 7,23–7.41 (m, 10 H, aromatic), δ 10.55 (br s, 1 H, NH); MS *m/z* 393 (M^+); IR (cm^{-1}, selected lines): 3410, 3160, 2920, 1690, 1565, 1530, 1295, 1230, 750, 685. $C_{23}H_{27}N_3OS$

Thiourea derivatives **21**–**23** were prepared following the same procedure using the appropriate isothiocyanate. Compounds **24**–**26** and **27** were synthesized following the same procedure starting from the suitable 2-aminothiazole derivatives **5** and **6**, respectively.

3.1.2. N-(4,5-Diphenylthiazol-2-yl)tetradecanamide (**31**)

Preparation of this compound is presented as an example of the general synthesis of compounds **28**–**31**.
Myristoyl chloride (1.07 g, 4.36 mmol) was added to a solution of **4** (1.0 g, 3.96 mmol) and TEA (0.441 g, 4.36 mmol) in tetrahydrofuran (20 ml) with an immediate formation of a white precipitate (TEA · HCl). The reaction mixture was refluxed for 6 h and, after cooling, the solid material was filtered off, washed with cold tetrahydrofuran and discharged. The combined filtrates were evaporated in vacuo and the red oily residue was then purified by flash chromatography on silica gel (eluent: cyclohexane/ethylacetate 9.25 : 0.75, v/v). Homogeneous fractions were combinerd and the volatiles evaporated in vacuo to give **31** as crystals (0.719 g, 40%): m.p. 78–79 °C. ^{1}H NMR (DMSO-d_6) δ 0.82 (t, J = 6.8 Hz, 3 H, CH_3), δ 1.21 (m, 20 H, $(CH_2)_{10}$), δ 1.50–1.67 (m, 2 H, $COCH_2CH_2$), δ 2.43 (t, J = 7.24 Hz, 2 H, $COCH_2$), δ 7.27–7.43 (m, 10 H, aromatic), δ 12.25 (br s, 1 H, NH which exchanges with D_2O); IR (cm^{-1}, selected lines): 3160, 3020, 2910, 2845, 1680, 1530, 1440, 1270, 740, 680. $C_{29}H_{38}N_2OS$

3.1.3. (4,5-Diphenylthiazol-2-yl)carbamic acid butyl ester (**32**)

Butylchloroformate (0.811 g, 5.94 mmol) was added to a warm solution of **4** (1.0 g, 3.96 mmol) in dry toluene (40 ml) and the reaction mixture was refluxed for 4 h. After cooling, the solvent was removed in vacuo and the oily residue was suspended in petroleum ether (40–60 °C) (20 ml). After stirring for 1 h, the solid material was filtered off, washed with petroleum ether and dried. Recrystallization from cyclohexane gave **32** as a white powder (0.66 g, 47%): m.p. 99–100 °C. ^{1}H NMR (DMSO-d_6) δ 0.90 (t, J = 7.3 Hz, 3 H, CH_3), δ 1.30–1.41 (m, 2 H, CH_3CH_2), δ 1.55–1.67 (m, 2 H, OCH_2CH_2), δ 4.17 (t, J = 6.6 Hz, 2 H, OCH_2), δ 7.26–7.42 (m, 10 H, aromatic), δ 11.92 (br s, 1 H, NH which exchanges with D_2O); IR (cm^{-1}, selected lines): 3140, 3040, 2960, 2920, 1720, 1550, 1300, 1240, 1065, 760, 695. $C_{20}H_{20}N_2O_2S$

3.1.4. N,N″-1,6-Hexanediylbis[N′-(4,5-diphenylthiazol-2-yl)urea] (**33**)

1,6-Diisocyanatohexane (0.333 g, 1.98 mmol) was added to a hot solution of **4** (1.0 g, 3.96 mmol) in toluene (15 ml) and the reaction mixture was refluxed for 1 h. After cooling, the volatiles were evaporated in vacuo and the residue was suspended in ethyl acetate (10 ml). After stirring for 1 h, the solid material was filtered off, washed with a little amount of cold ethyl acetate and dried. Recrystallization from CH_3CN afforded **33** as a white powder (0.57 g, 43%): m.p. 193–194 °C. ^{1}H NMR (DMSO-d_6) δ 1.34–1.52 (m, 8 H, $(CH_2)_4$), δ 3.13–3.20 (m, 4 H, $NHCH_2$), δ 6.52 (t, J = 5.5 Hz, 2 H, $NHCH_2$), δ 7.24–7.43 (m, 20 H, aromatic), δ 10.36 (br s, 2 H, NHCO); IR (cm^{-1}, selected lines): 3400, 3260, 3180, 3060, 2930, 1680, 1560, 1440, 1300, 1240, 745, 680. $C_{38}H_{36}N_6O_2S_2$

3.1.5. N-(4,5-Diphenyl-1 H-imidazol-2-yl)-N′-butyl urea (**35**)

Preparation of this compound is presented as an example of the general synthesis of compounds **35**–**37**.
Butylisocyanate (0.23 g, 2.3 mmol) was added to a solution of 2-amino-4,5-diphenylimidazole **34** (0.5 g, 2.12 mmol) and the reaction mixture was heated under reflux for 4 h. After cooling, the solvent was removed in vacuo and the crude residue collected. Recrystallization from ethyl acetate afforded **35** as a white powder (0.43 g, 60%): m.p. 170–172 °C. ^{1}H NMR (DMSO-d_6) δ 0.90 (t, J = 7.1 Hz, 3 H, CH_3), δ 1.28–1.49 (m, 4 H, $CH_3CH_2CH_2$), δ 3.12–3.21 (m, 2 H, $NHCH_2$), δ 7.27–7.55 (m, 10 H, aromatic), δ 9.19 (s, 2 H, NHCONH), δ 11.24 (s, 1 H, imidazole NH); IR (cm^{-1}, selected lines): 3320, 3220, 3080, 2950, 2920, 1680, 1600 1585 1560, 1350, 755, 700. $C_{20}H_{22}N_4O$

3.1.6. N-(4,5-Diphenyl-1-methyl-1 H-imidazol-2-yl)-N′-butyl urea (**38**)

A mixture of **35** (0.28 g, 0.8 mmol), K_2CO_3 (0.22 g, 1.6 mmol) and CH_3I (0.68 g, 4.8 mmol) in acetone (20 ml) was stirred at room temperature for 48 h. Then, the solvent was removed in vacuo and the residue was suspended in H_2O. The solid material was filtered off and dried. Recrystallization from ethyl acetate gave **38** as a white powder (0.175 g, 60%): m.p. 190–192 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (t, J = 7.0 Hz, 3 H, CH_2CH_3), δ 1.36–1.53 (m, 4 H, $CH_3CH_2CH_2$), δ 3.17–3.26 (m, 2 H, $NHCH_2$), δ 3.23 (s, 3 H, NCH_3), δ 7.09–7.53 (m, 10 H, aromatic), δ 8.58 (br s, 1 H, $NHCH_2$), δ 9.1 h (s, 1 H, $NHCONHCH_2$); IR (cm^{-1}, selected lines): 3150, 3030, 2955, 2860, 1680. 1580, 1545. $C_{21}H_{14}N_4O$

3.1.7. N-(4,5-Diphenyl-1 H-imidazol-2-yl)hexanamide (**39**)

Hexanoyl chloride (0.114 g, 0.85 mmol) was added dropwise to a stirred mixture of **34** (0.20 g, 0.85 mmol) and TEA (0.10 g, 0.9 mmol) in dry tetrahydrofuran (20 ml). The reaction mixture was stirred overnight, then filtered and the filtrate evaporated at reduced pressure. The obtained residue was then purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate 7 : 3, v/v). Homogeneous fractions were combined and the volatiles removed in vacuo to give **39** (0.10 g, 35%). An analytical sample was recrystallized from EtOH: m.p. 159 °C. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, J = 7.0 Hz, 3 H, CH_3), δ 1.28–1.32 (m, 4 H, $CH_3CH_2CH_2$), δ 1.55–1.67 (m, 2 H, $COCH_2CH_2$), δ 2.34 (t, J = 7.2 Hz, 3 H, $COCH_2$), δ 7.13–7.46 (m, 10 H, aromatic), δ 11.13 (s, 1 H, NHCO), δ 11.60 (s, 1 H, imidazole NH); IR (cm^{-1}, selected lines): 3300, 3060, 2950, 2920, 1680, 1600, 765. $C_{21}H_{23}N_3O$

3.2. Pharmacology

3.2.1. Preparation of rat liver microsomes

Male Wistar rats, weighing 250 to 300 g, were killed by decapitation. Livers were perfused in situ with Na/K phosphate buffer (100 mM, pH 7.4) containing 1 mM EDTA and 1 mM dithiothreitol (Buffer I) at 4 °C. All subsequent steps were performed at 4 °C. The tissue was minced and homogenized in a Potter – Elvehjem Teflon – glass apparatus. Homogenate was firstly centrifuged at 10.000 g for 30 min and then the surnatant was centrifuged at 100.00 g for 60 min. The microsomial fraction was resuspended in Buffer I and then isolated by centrifugation at 100.00 g for 30 min. Microsomes were resuspended in Buffer I at a concentration of about 10 mg of microsomal protein/ml (determined following the method of Lowry modified by Peterson [11]). Aliquots were stored frozen at −80 °C for up to 8 weeks.

3.2.2. Acyl-CoA : cholesterol acyltransferase inhibition assay

Tests for ACAT activity were performed by measuring the formation of cholesteryl[^{14}C]oleate from cholesterol and [1-^{14}C]oleoyl-CoA (New England Nuclear Corp., Boston, MA) in rat liver microsomes following the method of Lichtenstein and Brecher [12] with some modifications. All experiments were routinely performed in duplicate.
Briefly, the incubation mixture contained Tween® 80 (0.3 mg/ml), bovine serum albumine (4 mg/ml; it enhances enzymatic activity [12]), rat hepatic microsomal proteins (0.55 mg/ml) and the test compound (0.5 μM, 2 μM and 30 μM) in Buffer I (total final volume 200 μl). Test compounds were dissolved in DMSO and the amount of DMSO in each test tube was 5% of the final volume. After preincubation at 37 °C for 15 min, 30 μM [1-^{14}C]oleoyl-CoA (0.05 μCi, spec. act. 7.6 μCi/μmol) were added in 20 μl and the incubation was continued at 37 °C for 20 min. Enzymatic reaction was stopped by the addition of CH_3OH (1.0 ml) and the reaction mixture was then extracted with petroleum ether (3 × 1 ml). Cholesteryl oleate (100 μg) (Sigma Chemical Co. St. Louis, MO) was added as internal standard to the combined petroleum layers, then the extract, evaporated under a stream of N_2 and redissolved in 20 μl of $CHCl_3$, was applied to glass-backed precoated Silica Gel G plates and developed in petroleum ether/ethyl ether/acetic acid 90 : 10 : 1, v/v/v. Conversion of radiolabeled oleoyl-CoA to cholesteryl[^{14}C]oleate was determined directly by scanning plates in a System 200 Imaging Scanner (Packard) equipped with a MPM computer. The percent inhibition at each drug concentration was the average of two experiments. Approximate IC_{50} values for ACAT inhibitory activity were then derived by linear regression analysis of the data.

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