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Antilipidemic agents, Part IV: Synthesis and antilipidemic testing of some heterocyclic derivatives of hexadecyl and cyclohexyl hemisuccinate esters

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Two main classes of novel esters containing a hexadecyl or cyclohexyl group and various heterocyclic rings, like quinazolines, triazoles, thiadiazoles and pyrazoles have been synthesized. The first class involves the synthesis of the hexadecyl ester derivatives; namely 3-substituted-2-(hexadecyloxycarbonylpropionylthio)-4(3*H*)-quinazolinones **4a–c**; 4-substituted-3-(4-pyridyl)-5-(hexadecyloxycarbonylpropionylthio)-4*H*-1,2,4-triazoles **6a–c**; 5-substituted-2-(hexadecyloxycarbonylpropionylamino)-1,3,4-thiadiazoles **8a–c**, 4-[4-(hexadecyloxycarbonyl)phenyl]azo-5-hydroxy-3-methyl-1-phenylpyrazole **16**; and 1-[4-(hexadecyloxycarbonyl) phenyl]-3-methyl-2-pyrazolin-5-one **19**. The second class comprises the synthesis of the cyclohexyl ester derivatives; namely 3-substituted-2-(cyclohexyloxycarbonylpropionylthio)-4(3*H*)-quinazolinones **11a**, **b**, 4-substituted-3-(4-pyridyl)-5-(cyclohexyloxycarbonylpropionylthio)-4*H*-1,2,4-triazoles **12a**, **b** and 5-isopropylthio-2-(cyclohexyloxycarbonylpropionylamino)-1,3,4-thiadiazole **13**. The antihypercholesterolemic as well as antihyperlipidemic activities of representative compounds have been studied. All the compounds tested resulted in a decrease in the lipid indices (cholesterol, LDL-cholesterol, HDL-cholesterol and serum triglycerides levels) studied in mice. Compounds **4b**, **11a** and **12a** showed the highest antihyperlipidemic activity; their activities were almost equal to that of β-sitosterol which was used as a standard.

1. Introduction

The major cause of death nowadays is vascular diseases, of which the most prevalent form is atherosclerotic heart disease. Although many causative factors of this disease are recognized, hyperlipidemia and elevated cholesterol levels are the most prevalent indicator for susceptibility to atherosclerotic heart disease. Atherosclerosis results in degenerative changes in the intima of medium and large arteries. These degenerative changes include the accumulation of lipids, cholesterol, cholesterol esters, complex carbohydrates, blood and blood products. These deposits or plaques, decrease the lumen of the artery, reduce its elasticity and may create foci for thrombi and subsequent occlusion of the blood vessel [1].

It has been proved that reduction of cholesterol levels with HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors, like statins [2, 3] lowers the risk of fatal or non fatal stroke [4].

Statins contain an ester moiety attached to various hydrophilic and hydrophobic groups for example pravastatin, lovastatin, mevastatin and simvastatin. Pravastatin is used as the active hydroxy acid, while the other three compounds are administered as lactone prodrugs [3].

Recently some heterocyclic derivatives have been reported to display potent cholesterol absorption inhibitory activity [5, 6].

β-Sitosterol has also received great attention as a hypocholesterolemic agent [7], as it lowers the levels of cholesterol in plasma [8] and tissues [9], and also decreases the cholesterol absorption from the gastrointestinal tract [10, 11].

In our previous work [12–14] we have shown the synthesis and antilipidemic effect of some β-sitosterol derivatives. The good results obtained by some of these derivatives [14] prompted us to persue our research in this field.

The present investigation deals with the synthesis of new leads in this field including heterocyclic thioesters, esters and amides containing hexadecyl and cyclohexyl moieties as hydrophobic parts, the heterocyclic moieties containing quinazolines, triazoles, thiadiazoles and pyrazoles. The hypocholesterolemic and hypolipidemic activity of some of the compounds prepared was studied.

2. Investigation, results and discussion

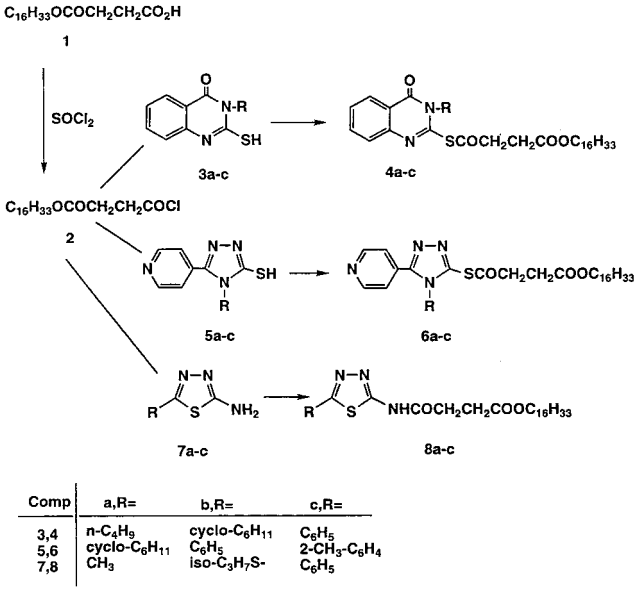
2.1. Synthesis and characterization

To prepare the target compounds the steps outlined in Schemes 1, 2 and 3 were followed. Thus hexadecylhemisuccinate **1** was treated with $SOCl_2$ to give the acid chloride **2** which was reacted with 3-substituted-4(3*H*)-quinazolinone-2-thiols **3a–c** [15], 4-substituted-3-(4-pyridyl)-5-mercapto-4*H*-1,2,4-triazoles **5a–c** [16], and 2-amino-5-substituted-1,3,4-thiadiazoles **7a–c** [17] to give the required new products 3-substituted-2-(hexadecyloxycarbonylpropionylthio)-4(3*H*)-quinazolinones **4a–c**; 4-substituted-3-(4-pyridyl)-5-(hexadecyloxycarbonylpropionylthio)-4*H*-1,2,4-triazoles **6a–c**; and 5-substituted-2-(hexadecyloxycarbonylpropionylamino)-1,3,4-thiadiazoles **8a–c**, respectively (Scheme 1). On the other hand, 3-substituted-2-(cyclohexyloxycarbonylpropionylthio)-4(3*H*)-quinazolinones **11a**, **b**; 4-substituted-3-(4-pyridyl)-5-(cyclohexyloxycarbonylpropionylthio)-4*H*-1,2,4-triazoles **12a**, **b**; and 5-isopropylthio-2-(cyclohexyloxycarbonylpropionylamino)-1,3,4-thiadiazole **13** were prepared by the reaction of the acid chloride **10** (which was obtained from cyclohexylhemisuccinate **9** and $SOCl_2$) with the corresponding quinazolinones **3**; triazoles **5** and thiadiazole **7**, respectively (Scheme 2).

The pyrazole derivatives were obtained according to scheme 3. Thus, the reaction of 4-(4-carboxyphenyl)azo-5-hydroxy-3-methyl-1-phenylpyrazole **14** [18] with $SOCl_2$ gave the corresponding acid chloride **15** which was esterified with hexadecyl alcohol to produce the required 4-[4-(hexadecyloxycarbonyl)phenyl]azo-5-hydroxy-3-methyl-1-phenylpyrazole **16**. Reaction of 1-(4-carboxyphenyl)-3-methyl-2-pyrazolin-5-one **17** with $SOCl_2$ gave the corresponding acid chloride **18** which was esterified with hexadecyl alcohol to produce the target 1-[4-(hexadecyloxycarbonylphenyl]-3-methyl-2-pyrazolin-5-one **19**.

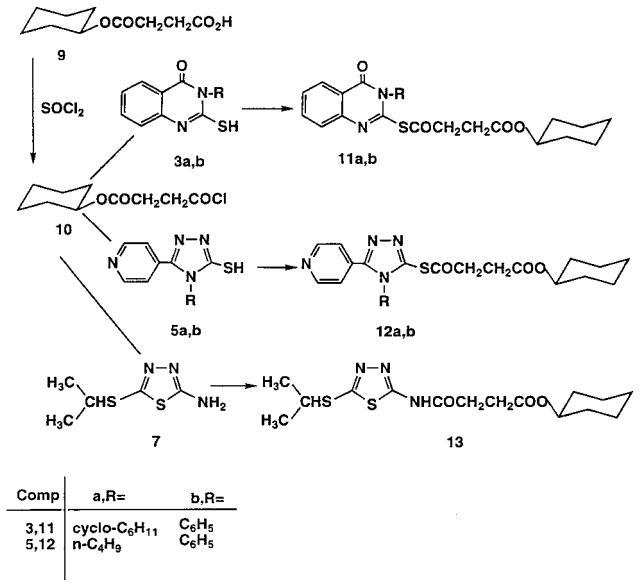
The new compounds were characterized by microanalyses, IR, ^{1}H NMR spectra and MS. The IR of the quinazolinone derivatives **4a–c** and **11a**, **b** showed three absorption bands due to the three C=O groups at 1735–1730 (C=O ester); 1731–1720 (C=O thioester) and 1693–1665 cm^{-1} (C=O quinazolinone). The ^{1}H NMR of **4c** showed the

Scheme 1



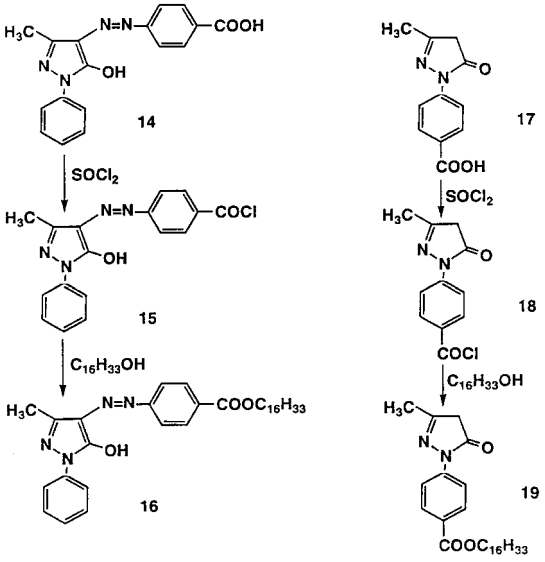
Comp	a,R=	b,R=	c,R=
3,4	n-C_4H_9	cyclo-C_6H_{11}	C_6H_5
5,6	cyclo-C_6H_{11}	C_6H_5	2-CH_3-C_6H_4
7,8	CH_3	iso-C_3H_7S-	C_6H_5

Scheme 2



Comp	a,R=	b,R=
3,11	cyclo-C_6H_{11}	C_6H_5
5,12	n-C_4H_9	C_6H_5

Scheme 3



hexadecyl moiety as a multiplet at 1–2 ppm due to $-(CH_2)_{14}$-CH_3 and a dist. triplet at 4.6 ppm due to $-OCH_2$. It also showed two dist. triplets at 2.4 and 2.5 ppm due to $-CH_2CH_2-$ of the hemisuccinate moiety; the two doublets due to quinazolinone-$C_{8,5}$-H appeared at 8.3 and 8.6 ppm. The ^{1}H NMR of **11b** showed a multiplet at 1.1–1.7 due to the cyclohexyl moiety; the cyclohexyl-C_1-H was under DMSO, in addition to the other protons at their expected regions.

The MS of **4a** showed the absence of the molecular ion peak at m/z 558, however it showed $M^{+\cdot}-CO$ at m/z 530. Also **11a** did not show a molecular ion peak at m/z 442 but it showed $M^{+\cdot}-C_2H_2$ at m/z 416.

The triazole derivatives **6a–c** and **12a**, **b** were characterized by their IR spectra which showed two C=O stretching bands 1729 (C=O ester) and 1716–1714 (C=O thioester); they also showed C=N at 1663–1645 cm^{-1}. The ^{1}H NMR of **6c** showed the hexadecyl moiety as a dist. triplet at 0.9 ppm due to CH_3; a multiplet at 1.3–1.5 ppm due to $-(CH_2)_{14}-$ and a triplet at 4 ppm due to OCH_2. It was also characterized by a doublet at 8.6 ppm due to pyridine $-C_{2,6}-$H, this beside the other protons at their expected regions.

The ^{1}H NMR of **12b** showed three multiplets at 1–1.5, 1.6–1.8 and 4.6–4.7 ppm due to the cyclohexyl protons; it also showed a doublet at 8.4 ppm due to pyridine $C_{2,6}-$H. The MS of **6b** showed the absence of molecular ion peak, but it showed $M^{+\cdot}-C_5H_4N$ at m/z 500.

The thiadiazoles derivatives **8a–c** and **13** were characterized by their IR spectra which showed two C=O stretching vibration bands 1742–1732 (C=O ester) and 1700–1699 (C=O amide); in addition to the bands characteristic for NH, C=N and C=C. The ^{1}H NMR of **8b** showed the signals due to the hexadecyl and isopropyl moieties at their expected regions. It showed a D_2O exchangeable signal at 7.3 ppm due to NH. Compound **13** showed in its ^{1}H NMR the cyclohexyl moiety as three multiplets at 1.3–1.45, 1.55–1,8 and 4.4–4.5 ppm and the isopropyl moiety as two doublets at 1.1, 1.2 ppm due to $2CH_3$ magnetically non equivalent and a septet at 3.6 ppm due to CH; in addition to other protons at their expected regions. The MS of **8b** showed the molecular ion peak at m/z 499 and $M^{+\cdot}$ + 2 at m/z 501; the base peak was at m/z 91.1 corresponding to $CHNS_2$. The MS of **13** did not show $M^{+\cdot}$ at m/z 357, but it showed $M^{+\cdot}-CH_3$ at m/z 342 and the base peak at m/z 91.1 due to $CHNS_2$.

The pyrazolones **16** and **19** showed in their ^{1}H NMR the hexadecyl moiety and a singlet at 2.2–2.5 ppm due to pyrazolone-C_3-CH_3; compound **19** showed also a signal at 3.3 ppm due to pyrazolone-C_4-H_2. The MS of **16** showed the molecular ion peak at m/z 546.

2.2 Antihyperlipidemic activity

The antihypercholesterolemic and antihyperlipidemic effect of representative compounds **4b**, **6b**, **8a**, **8c**, **11a**, **12a** and **16** were studied on serum total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol) and triglycerides levels (mg/dl) in mice (mean ± standard error from 10 mice) (Table 1).

As can be seen from Table 1 cholesterol administration resulted in significant increases in serum total cholesterol level as compared to the normal control group (73.22 ± 5.21 mg/dl vs. 41.63 ± 4.28 mg/dl, $P < 0.001$). β-Sitosterol administration resulted in significant decreases in serum total cholesterol level as compared to the cholesterol

Table 1: Effect of some of the synthesized compounds on serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels in mice

Group	Comp.	Serum cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Serum triglycerides (mg/dl)
I	Cholesterol	73.22 ± 5.21	40.28 ± 2.23	35.55 ± 1.96	61.93 ± 4.62
II	Olive oil	41.63 ± 4.28	12.70 ± 1.11	25.82 ± 2.21	28.64 ± 3.16
III	β-Sitosterol	42.98 ± 3.92	13.54 ± 1.56	26.74 ± 2.87	29.53 ± 2.88
IV	**4b**	43.62 ± 3.19	14.01 ± 1.42	27.52 ± 2.91	30.56 ± 2.91
V	**6b**	70.18 ± 4.76	39.96 ± 1.55	33.68 ± 1.53	59.20 ± 3.86
VI	**8a**	69.57 ± 5.23	39.16 ± 1.98	33.13 ± 1.57	58.18 ± 3.97
VII	**8c**	51.68 ± 2.84	22.61 ± 2.32	32.21 ± 1.82	47.23 ± 3.15
VIII	**11a**	46.21 ± 2.58	15.22 ± 1.87	27.83 ± 1.92	34.23 ± 3.05
IX	**12a**	44.35 ± 3.06	14.12 ± 1.66	27.66 ± 1.88	32.96 ± 2.86
X	**16**	58.27 ± 2.95	28.73 ± 2.30	31.66 ± 1.93	50.18 ± 2.73
F test		10.3520	43.3884	2.6443	16.0400
LSD		11.0925	5.4423	5.9662	9.5335
P		<0.001	<0.001	<0.05	<0.001

mean ± standard error from 10 mice

group (42.98 ± 3.92 mg/dl vs. 73.22 ± 5.21 mg/dl, $P < 0.001$). The synthesized compounds **4b**, **8c**, **11a**, **12a** and **16** significantly decreased serum total cholesterol levels as compared the to cholesterol group 43.62 ± 3.19, 51.68 ± 2.84, 46.21 ± 2.58, 44.35 ± 3.06, 58.27 ± 2.95 vs. 73.22 ± 5.21, $P < 0.001$). Compounds **4b**, **11a** and **12a** showed hypocholesterolemic activity almost equal to that of β-sitosterol.

Cholesterol administration also resulted in significant increases in serum LDL-cholesterol level as compared to the normal control group (40.28 ± 2.23 mg/dl vs. 12.70 ± 1.11 mg/dl, $P < 0.001$). β-sitosterol administration resulted in significant decreases in serum LDL-cholesterol level as compared to the cholesterol group (13.54 ± 1.56 mg/dl vs. 40.28 ± 2.23 mg/dl, $P < 0.001$). The synthesized compounds **4b**, **8c**, **11a**, **12a** and **16** resulted in significant decreases in serum LDL-cholesterol level as compared to the cholesterol group (14.01 ± 1.42, 22.61 ± 2.32, 15.22 ± 1.87, 14.12 ± 1.66 and 28.73 ± 2.9 mg/dl vs. 40.28 ± 2.23 mg/dl, $P < 0.001$). Compounds **4b**, **11a** and **12a** showed antilipidemic activity almost equal to that of β-sitosterol.

Furthermore, cholesterol administration resulted in significant increases in serum HDL-cholesterol level as compared to normal control (35.55 ± 1.96 mg/dl vs. 25.82 ± 2.21 mg/dl, $P < 0.05$). β-Sitosterol administration resulted in significant decreases in serum HDL-cholesterol level as compared to the cholesterol group (26.74 ± 2.87 mg/dl vs. 35.55 ± 1.96 mg/dl, $P < 0.05$). The synthesized compounds **4b**, **11a** and **12a** resulted in significant decreases in serum HDL-cholesterol level as compared to the cholesterol group (27.52 ± 2.91, 27.83 ± 1.92 and 27.66 ± 1.88 mg/dl vs. 35.55 ± 1.96 mg/dl, $P < 0.05$). In addition, cholesterol administration resulted in significant increases in serum triglycerides as compared to the normal control group (61.93 ± 4.62) mg/dl, vs. 28.64 ± 3.16 mg/dl, $P < 0.001$). β-Sitosterol administration resulted in significant decreases in serum triglycerides level as compared to the cholesterol group (29.53 ± 2.88 mg/dl vs. 61.93 ± 4.62 mg/dl, $P < 0.001$). The synthesized compounds **4b**, **8c**, **11a**, **12a** and **16** resulted in significant decreases in serum triglycerides level as compared to the cholesterol group (30.56 ± 2.91, 47.23 ± 3.15, 34.23 ± 3.05, 32.96 ± 2.86 and 50.18 ± 2.73 mg/dl vs. 61.93 ± 4.62 mg/dl, $P < 0.001$). Compounds **4b**, **11a** and **12a** showed antilipidemic activity almost equal to that of β-sitosterol.

In the present study, cholesterol administration for 6 days significantly increased all the measured lipid indices in mice. This is in agreement with previous reports indicating that feeding animals with high cholesterol diets significantly increases the serum levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides [14, 19]. The rise in serum cholesterol observed here was particularly evident in the LDL fraction, probably as a result of suppression of LDL-receptor activity under the effect of high cholesterol concentrations. Studies in tissue culture demonstrated that increasing the cholesterol content of

Table 2: Physicochemical data of compounds 4, 6, 8

Comp. No.	R	Yield %	M.p. °C	Mol. Formula (Mol. wt.)
4a	n-C_4H_9	75	159–160	$C_{32}H_{50}N_2O_4S$ (558.8)
4b	C_6H_{11} (cyclo)	71	117–120	$C_{34}H_{52}N_2O_4S$ (584.9)
4c	C_6H_5	77	137–139	$C_{34}H_{46}N_2O_4S$ (578.8)
6a	C_6H_{11} (cyclo)	85	195–198	$C_{33}H_{52}N_4O_3S$ (584.9)
6b	C_6H_5	91	177–179	$C_{33}H_{46}N_4O_3S$ (578.8)
6c	2-CH_3–C_6H_4	90	257–259	$C_{34}H_{48}N_4O_3S$ (592.9)
8a	CH_3	88	230–232	$C_{23}H_{41}N_3O_3S$ (439.7)
8b	iso-C_3H_7S	87	214–216	$C_{25}H_{45}N_3O_3S_2$ (499.8)
8c	C_6H_5	92	215–220	$C_{28}H_{43}N_3O_3S$ (501.7)

Table 3: Physicochemical data of compounds 11, 12, 13

Comp. No.	R	Yield %	M.p. °C	Mol. Formula (Mol. wt.)
11a	C_6H_{11} (cyclo)	65	183–185	$C_{24}H_{30}N_2O_4S$ (442.6)
11b	C_6H_5	60	113–115	$C_{24}H_{24}N_2O_4S$ (436.5)
12a	n-C_4H_9	35	173–175	$C_{21}H_{28}N_4O_3S$ (416.6)
12b	C_6H_5	60	180–181	$C_{23}H_{24}N_4O_3S$ (436.5)
13	–	55	158–160	$C_{15}H_{23}N_3O_3S_2$ (357.5)

cells will down regulate synthesis of LDL receptors [20, 21].

It can be concluded that the synthesized compounds **4b**, **8c**, **11a**, **12a** and **16** resulted in significant decreases in all the measured lipid indices in mice. Compounds **4b**, **11a** and **12a** showed the highest antilipidemic activities. Their activities were almost equal to that of β-sitosterol.

3. Experimental

M.P's were uncorrected and determined in open glass capillaries. IR spectra were measured as KBr discs on a Perkin Elmer 1430 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM-390, 90 MHz spectrometer, in DMSO-d_6 or $CDCl_3$ using TMS as internal standard. The chemical shifts are given in δ ppm values and the exchangeable protons were confirmed by D_2O. The MS were run on a Finnigan mat spectro-meter model SSQ/7000 (70 ev). The microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University, Egypt and the values were within ±0.4% of the theoretical data.

3.1. Synthesis of the compounds

3.1.1. 3-Hexadecyloxycarbonylpropionyl chloride (**2**)*, 3-Cyclohexyloxycarbonylpropionyl chloride* (**10**)

To a solution of hexadecylhemisuccinate **1** or cyclohexylhemisuccinate **9** (0.01 mole) in dry benzene (20 ml) $SOCl_2$ (3.5 ml, 0.03 mole) was added. The reaction mixture was heated under reflux for 2 h, and excess $SOCl_2$ and benzene were evaporated in vaccum. The acid chloride produced was sufficiently pure and was used as such for the next step. IR (nujol, cm^{-1}): 1780–1770 (C=O, acid chloride), 1730–1728 (C=O ester).

3.1.2. 4-(4-Chloroformylphenyl)azo-5-hydroxy-3-methyl-1-phenylpyrazole (**15**) *and 1-(4-chloroformylphenyl)-3-methyl-2-pyrazolin-5-one* (**18**)

To a solution of the acid **14** or **17** (0.01 mol) in dry $CHCl_3$ (20 ml), $SOCl_2$ (3.5 ml, 0.03 mole) was added. The reaction mixture was heated under reflux for 2 h. Excess $SOCl_2$ and $CHCl_3$ were evaporated in vacuum. Compound **18** was used as such for the next step. Compound **15** was crystallized from dry benzene, yield 2.13 g, 98%, m.p. 117–118ºC. IR (nujol, cm^{-1}): 1780 (C=O) acid chloride), 1710 (C=O pyrazolone), 1640 (C=N), 1600, 1500 (C=C).

3.1.3. 3-Substituted-2-(hexadecyloxycarbonylpropionylthio)-4(3 H)-quinazolinones **4a–c***; 3-Substituted-2-(cyclohexyloxycarbonylpropionylthio)-4(3 H)-quinazolinones* **11a**, **b**

To a solution of the appropriate **3a–c** (0.001 mole) in dry dioxane (20 ml), a small piece of sodium metal (0.001 mole) was added and the solution was stirred until the sodium dissolved. Then a solution of the acid chloride **2** or **10** (0.001 mole) in dry benzene (10 ml) was added. The reaction mixture was stirred for 12 h, concentrated in vaccum and left at RT overnight, whereupon a crystalline product was obtained. It was filtered, washed with H_2O, dried and crystallized from dry dioxane (Tables 2, 3).

IR (KBr, cm^{-1}): 1735–1730 (C=O ester); 1731–1720 (C=O thioester); 1693–1665 (C=O quinazolinone); 1648–1630 (C = N); 1600–1588, 1519–1500 (C=C); 1270–1237, 1180–1169, 1080–1070 (C–O–C); 1220–1204, 1034–1020 (C–S–C). ^{1}H NMR (**4c**)(DMSO-d_6, δ ppm): 1–2 (m, 31 H, $-(CH_2)_{14}-CH_3$); 2.4, 2.5 (two dist. t, each 2 H, CH_2-CH_2); 4.6 (t, 2 H, J = 7 Hz, OCH_2); 6.9–8.2 (m, 7 H, Ar–H); 8.3, 8.6 (two d, each 1 H, J = 8.2 Hz, quinazolinone $-C_{8,5}-$H). ^{1}H NMR (**11b**)(DMSO-d_6, δ ppm): 1.1–1.7 (m, 10 H, cyclohexyl); 2.4, 2.5 (two dist. t, each 2H, CH_2-CH_2, one of them under DMSO); 3.4–3.5 (m, 1 H, cyclohexyl-C_1-H); 7–7.5 (m, 8H, Ar–H); 8.1 (d, 1 H, quinazolinone-c_5-H).

MS (**4a**) m/z (%): 558 (absent ($M^{+\cdot}$); 530 (1) $M^{+\cdot}-CO$; 468 (2); 409 (2); 391 (2); 325 (3); 297 (1); 296 (1); 279 (3); 268 (2); 252 (2); 235 (7); 234 (24); 225 (2); 218 (37); 217 (11); 202 (8); 201 (61); 189 (6); 179 (23); 178 (9); 177 (4); 176 (29); 163 (52); 162 (100); 147 (11); 146 (82); 134 (8); 120 (24); 119 (75); 92 (21); 91 (7); 90 (23); 76 (3); 64 (8); 63 (7).

MS (**11a**) m/z (%): 442 (absent) $M^{+\cdot}$; 416 (1) $M^{+\cdot}-C_2H_2$; 405 (10); 372 (1); 363 (10); 352 (15); 343 (20); 337 (10); 323 (10); 315 (2); 305 (5); 282 (5); 279 (10); 272 (2); 264 (20); 256 (20); 245 (20); 236 (15); 220 (25); 218 (100); 183 (2); 163 (40); 146 (60); 134 (40); 120 (58); 119 (44); 101 (25); 99 (40); 98 (40); 91 (24); 85 (5); 83 (15); 82 (18); 81 (18); 70 (6); 65 (10); 57 (7); 56 (30).

3.1.4. 4-Substituted-3-(4-pyridyl)-5-(hexadecyloxycarbonylpropionylthio)-4 H-1,2,4-triazoles **6a–c***; and 4-substituted-3-(4-pyridyl)-5-(cyclohexyloxycarbonylpropionylthio)-4 H-1,2,4-triazoles* **12a**, **b**

To a solution of the appropriate mercaptotriazole **5a–c** (0.001 mole) in dry dioxane (20 ml), small pieces of sodium (0.001 mole) was added and the reaction mixture was stirred until the sodium dissolved. Then a solution of the acid chloride **2** or **10** (0.001 mol) in dry benzene (10 ml) was added. The reaction was then treated as above; the products were crystallized from acetone (Table 2, 3).

IR (KBr, cm^{-1}: 1729 (C=O ester); 1716-1714 (C=O thioester); 1663–1645 (C=N); 1613–1605, 1587–1567, 1519–1510 (C=C); 1291–1285, 1198–1190, 1085–1072 (C–O–C); 1264–1262, 1005–1001 (C–S–C).

^{1}H NMR (**6c**) DMSO-d_6, δ ppm): 0.9 (dist. t, 3H CH_3); 1.3–1.5 (m, 28 H, $-(CH_2)_{14}-$); 2 (s, 3 H, $C_6H_4-CH_3$), 2.2,2.4 (two dist. t, each 2H, CH_2-CH_2); 4 (t, 2 H, J = 7 Hz, OCH_2); 7–7.6 (m, 6 H, Ar–H); 8.6 (d, 2 H, J = 5.5 Hz, pyridine-$C_{2,6}$-H). ^{1}H NMR (**12b**)(DMSO-d_6, δ ppm): 1–1.5 (m, 6 H, cyclohexyl-$C_{3,4,5}-$H); 1.6–1.8 (m, 4 H, cyclohexyl-$C_{2,6}-$H); 2.3,2.4 (two t, each 2H, J = 7Hz, CH_2CH_2); 4.6–4.7 (m, 1 H, cyclohexyl-C_1-H); 7–7.6 (m, 7 H, Ar–H); 8.4 (d, 2 H, J = 5.5 Hz, pyridine-$C_{2,6}-$H).

MS (**6b**) m/z (%): 578 (absent) $M^{+\cdot}$; 500 (2) $M^{+\cdot}-C_5H_4N$; 424 (1); 383(1); 371 (3); 353 (1); 345 (2); 344 (20); 343 (85); 325 (2); 279 (2); 269 (10); 256 (10); 255 (25); 254 (99); 252 (80); 241 (3); 224 (30); 196 (30); 168 (20); 149 (30); 140 (30); 139 (33); 125 (10); 119 (100); 111 (10); 101 (90); 97 (16); 83 (20); 70 (90); 69 (20); 57 (20); 55 (26).

3.1.5. 5-Substituted-2-(hexadecyloxycarbonylpropionylamino-1,3,4-thiadiazoles **8 a–c***; and 5-isopropylthio-2-(cyclohexyloxycarbonylpropionylamino)-1,3,4-thiadiazole* **13**

To a suspension of the appropriate **7a–c** (0.001 mole) in benzene (20 ml), TEA (0.5 ml) was added, followed by dropwise addition of a solution of the corresponding acid chloride **2** or **10** in benzene (10 ml). The reaction mixture was stirred at RT for 48 h, concentrated, cooled and the crystalline precipitate obtained was filtered, washed with H_2O, dried and crystallized from acetone/pet. ether 40–60 ºC (Tables 2, 3).

IR (KBr, cm^{-1}): 3167–3157 (NH); 1742–1732 (C=O ester); 1700–1699 (C=O amide); 1663, 1645 (C=N); 1570 (δ-NH); 1628–1624, 1504–1501 (C=C); 1260–1249, 1181–1169, 1053–1043 (C–O–C); 1224–1213, 1036–1022 (C–S–C). ^{1}H NMR (**8b**)(DMSO-d_6, δ ppm): 1 (dist. t, 3 H, CH_3); 1.1–1.3 (m, 34 H, $-(CH_2)_{14}$ and $CH(CH_3)_2$); 2.7–2.8 (two t, each 2 H, CH_2CH_2); 4 (septet $CH(CH_3)_2$); 4.2 (dist. t, 2 H, OCH_2); 7.3 (s, 1 H, NH, D_2O exchangeable). ^{1}H NMR (**13**)(DMSO-d_6, δ ppm): 1.1, 1.2 (two d, each 3 H, J = 6.5 Hz, $CH(CH_3)_2$; 1.3–1.45 (m, 6 H, cyclohexyl-$C_{3,4,5}-$H); 1.55–1.8 (m, 4 H, cyclohexyl-$C_{2,6}-$H); 2.65, 2.7 (two t, each 2H, J = 7Hz, CH_2-CH_2); 3.6 (septet, 1H, CH $(CH_3)_2$); 4.4–4.5 (m,1H, cyclohexyl-C_1-H); 7.3–7.5 (m, 1 H, NH, D_2O exchangeable).

MS (**8b**) m/z (%): 501 (0.01) $M^{+\cdot}$ + 2, 499 (0.2) $M^{+\cdot}$; 484 (0.2); 470 (0.2); 467 (1); 456 (2); 429 (5); 424 (20); 406 (5); 396 (2); 383 (1); 369 (1); 306 (15); 305 (57); 279 (2); 272 (11); 250 (2); 224 (7); 223 (13); 196 (5); 190 (5); 177 (1); 149 (7); 148 (63); 125 (9); 119 (2); 111 (18); 101 (9); 97 (26); 91 (100); 86 (5); 85 (11); 84 (1); 71 (17); 70 (15); 69 (23); 65 (12); 57 (26); 55 (32).

MS (**13**) m/z (%) : 357 (absent) $M^{+\cdot}$; 342 (1) $M^{+\cdot}-CH_3$; 340 (1); 323 (20); 306 (10); 305 (8); 272 (5); 223 (40); 198 (2); 190 (10); 183 (10); 171 (33); 149 (35); 138 (9) 131 (3); 106 (5); 101 (85); 91 (100); 86 (99); 83 (23); 82 (40); 65 (11); 55 (32).

3.1.6. 4-[4-(Hexadecyloxycarbonyl)phenyl]azo-5-hydroxy-3-methyl-1-phenylpyrazole **16** *and 1-[4-(hexadecyloxycarbonyl)phenyl]-3-methyl-2-pyrazolin-5-one* **19**

To a solution of the appropriate acid chloride **15** or **18** (0.001 mole) in benzene (20 ml), a solution of hexadecyl alcohol (0.25 g, 0.001 mole) in benzene (10 ml) was added. The reaction mixture was refluxed for 2 h, cooled and pyridine (0.5 ml) was added. It was then stirred at RT for 12 h. H_2O was added to remove the pyridinium hydrochloride formed. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with H_2O (10 mol), dried over anh. Na_2SO_4, evaporated in vaccum and the product obtained was crystallized from $CHCl_3$/pet. ether 40–60 ºC.

Compound **16**: yield 90%, crystallized from $CHCl_3$, m.p. 63–65 ºC.

IR (KBr, cm^{-1}) (**16**): 3585 (OH enolic); 3444 (NH); 1726 (C=O ester); 1717 (C=O pyrazolone); 1663, 1647, 1629 (C=N); 1609, 1590, 1502 (C=C); 1568 (N=N); 1554 (δ NH); 1256, 1155, 1104, 1047 (C–O–C). ^{1}H NMR (**16**)(DMSO-d_6, δ ppm): 1.1–1.3 (m, 31 H, $-(CH_2)_{14}-CH_3$); 2.2 (s, 3 H, CH_3); 4.3 (dist. t, 2 H, OCH_2); 7–8 (m, 9 H, Ar–H).

MS (**16**) m/z (%): 546 (57) $M^{+\cdot}$; 518 (5); 504 (5); 426 (5); 375 (5); 361 (20); 322 (22); 305 (10); 304 (20); 277 (10); 224 (10); 201 (29); 187 (32); 173 (20); 161 (20); 149 (10); 137 (70); 121 (30); 111 (58); 105 (18); 97 (90); 83 (98); 71 (72); 57 (100).

$C_{33}H_{46}N_4O_3$ (546.8).

Compound **19**, yield 80%, crystallized from $CHCl_3$/pet. ether 40–60 ºC, m.p. 63–65 ºC.

IR (KBr, cm^{-1}) (**19**): 1731 (C=O ester and pyrazolone); 1647 (C=N); 1608, 1556, 1523 (C=C); 1273, 1175, 1102, 1052, 1016 (C–O–C). ^{1}H NMR (**19**) ($CDCl_3$, δ ppm): 6.7 (dist. t, 3H, CH_3); 1–1.5 (m, 28 H$-(CH_2)_{14}-$); 2.5 (s, 3 H, CH_3); 3.3 (br. s, 2 H, pyrazolone-C_4-H_2); 4.3 (dist. t, 2 H, OCH_2); 7.7–8.3 (m, 4 H, Ar–H).

$C_{27}H_{42}N_2O_3$ (442.65).

3.2. Antihyperlipidemic testing

3.2.1. Material and methods

One hundred male mice weighing 20–25 g were used throughout this work. They were allowed to adapt to the experimental animal facility for seven days before the experiment. The mice were housed under the same environmental conditions, fed normal laboratory diet and they had free access to tap water. The mice received a solution of the test compounds in olive oil at a dose of 150 m/kg body weight orally (gavage) for a period of six successive days. Cholesterol (150 mg/kg) [14] was added to all of the compounds tested. β-Sitosterol was used as a reference standard at the same dose level [14].

The mice were randomly divided into ten groups, each of ten mice as follows:

Group I received cholesterol (150 mg/kg) orally for 6 days.

Group II received olive oil orally for 6 days and served as a control.

Group III received β-sitosterol (150 mg/kg) together cholesterol (150 mg/kg) orally for 6 days.

Groups IV-X received the synthesized compounds **4b**, **6b**, **8a**, **8c**, **11a**, **12a** and **16** respectively (150 mg/kg) together with cholesterol (150 mg/kg) orally for 6 days.

3.2.2. Sample collection

At the end of the experiment, the mice were fasted for 18–20 h with free access to water. The mice were sacrified and the serum was separated and used for the estimation of the following parameters according to reported methods: total serum cholesterol [22], serum LDL cholesterol [23], serum HDL cholesterol [24], serum triglycerides [25].

3.2.3. Statistical methods

Data are expressed as means with their corresponding standard errors. Data were evaluated by the one way analysis of variance. The data were then subjected to the least significant difference (LSD) test. [26]. The results are given in Table 1.

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