Synthesis of 1-Nonanoyl/octadecanoyl-4-substituted Thiosemicarbazides and substituted 1,2,4-Trizoles as biological active compounds

Katica Colanceska-Ragenovic[1], Vesna Dimova[1], Vlado Kakurinov[2], and Dora Molnar Gabor[3]

[1] Faculty of Technology and Metallurgy, The "Sv. Kiril & Metodij" University, R. Boskovic 16, 1000 Skopje, Macedonia
[2] Faculty of Agriculture, Department of microbiology, The "Sv. Kiril & Metodij" University, bul. Aleksandar Makedonski, bb, 1000 Skopje, Macedonia
[3] Institute of Chemistry, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 3, 21000 Novi Sad, Yugoslavia
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4-Alkyl/aryl-5-nonanoyl/octadecanoyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thiones were synthesized as potential antimicrobial agents. The course of synthesis included the reaction of nonanoyl/octadecanoyl hydrazines with selected alkyl/aryl isothiocyanates. The prepared thiosemicarbazides gave by cyclization the required 1,2,4-triazoles. A number of synthesized compounds were subjected to *in vitro* testing against two gram-positive, two gram-negative bacteria and two fungi.

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Various 1-acyl/aroyl-4-substituted thiosemicarbazides are known to exhibit significant tuberculostatic [1,2], antifungal [3,4], antiviral [5] and anticonvulsant [6] activities. Certain 1,2,4-triazole derivatives have also been reported to have the similar effects [7-11].

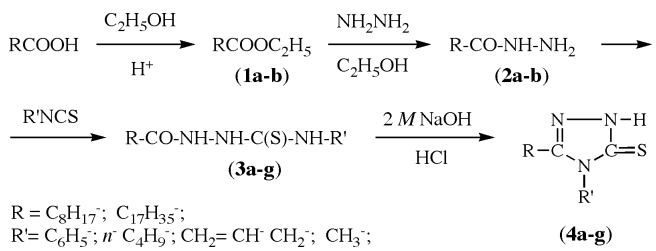
The literature reports that the antiviral [12] and the antibacterial [13-14] activities of thiourea derivatives were due to the –NH-C(S)-NH- function in the molecule and that the positive/negative changes in the biological activity depended on the nature of its substituents. It is also known that N-substituted fatty acid amides exhibit biological activity [15,16]. A large number of N, O and N, S-heterocyclic compounds prepared by condensation of fatty acids alkyl amides with carbonyl compounds and phosphorous penta sulfide, respectively, have shown excellent bacterostatic and fungistatic action [17].

Those observations prompted us to combine the fatty acid amides moiety with thiosemicarbazide structure, to synthesize substituted 1,2,4-triazoline-3-thiones with the same pharmacophoric group and investigate their antibacterial and antifungal activities.

Chemistry.

The synthetic route to thiosemicarbazides and triazole derivatives is presented in Scheme 1.

Scheme 1



R = $C_8H_{17}^-$; $C_{17}H_{35}^-$;
R'= $C_6H_5^-$; *n*- $C_4H_9^-$; CH_2= CH^- CH_2^-; CH_3^-;

Ethyl esters of nonanoic and octadecanoic acids (**1a-b**) were prepared following the standard method [18] by refluxing the acids and the alcohol in presence of sulfuric acid.

Further, the desired nonanoyl/octadecanoylhydrazines (**2a-b**) were obtained treating compounds (**1**) with hydrazine in ethanol for 12 hours [19,20]. This period of reflux leads to hydrazides with excellent purity and yield. The same hydrazides (**2a-b**) were converted into 1-nonanoyl/octadecanoyl-4-alkyl/aryl-thiosemicarbazides (**3a-b**) by refluxing with suitable alkyl/aryl isothiocyanates in ethanolic solution [21,22,23].

The reaction of cyclization [24,25] of the thiosemicarbazides with 2 *M* NaOH solution under reflux for about 4 hours produced 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazoline-3-thiones (**4a-g**) in a pure state and in good yield. The new compounds (**3a-g, 4a-g**) were characterized using ir and ^{1}H nmr spectroscopy together with elemental analysis.

Antibacterial Activites.

The filter paper disc method [26,27] was employed for the *in vitro* study of antibacterial and antifungal effects against *Escherichia coli*, *Bacillus subtilis*, *Salmonela enteritidis*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans* of the compounds (**3d-g, 4d-g**).

This method was performed using Sabouraud dextrose broth and Mueller Hinton broth. These agar media were inoculated with 0.5 ml of the 24 h liquid cultures containing 10^7 microorganisms/mL. Standard 5 mm diameter paper discs impregnated with solutions of each compound (concentrations: 1mg/mL; 5mg/mL and 10mg/mL of DMSO) were placed on the indicated agar mediums. The incubation time was 24 h at 37 °C for bacterial and 48 h at 30 °C for *Candida* species. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones. The screening results given in Table 3 indicate that

Table 1

Physical Data of Compounds (**3a-g**)

Comp. No.	R	R'	Mp [°C]	Yield [%]	Molecular formula Mol. wt.
3a	C_8H_{17} -	C_6H_5 -	124-6	72	$C_{16}H_{25}N_3OS$ (307.30)
3b	C_8H_{17} -	*n*-C_4H_9 -	131-2	77	$C_{14}H_{29}N_3OS$ (287.42)
3c	C_8H_{17} -	CH_2=CH-CH_2-	101-2	80	$C_{13}H_{25}N_3OS$ (271.43)
3d	$C_{17}H_{35}$ -	C_6H_5 -	116	60	$C_{25}H_{43}N_3OS$ (433.77)
3e	$C_{17}H_{35}$ -	*n*-C_4H_9 -	117-9	83	$C_{23}H_{47}N_3OS$ (413.71)
3f	$C_{17}H_{35}$ -	CH_2=CH-CH_2-	111	73	$C_{22}H_{43}N_3OS$ (397.74)
3g	$C_{17}H_{35}$ -	CH_3 -	119-21	78	$C_{20}H_{41}N_3OS$ (371.63)

3a	ir: 3269-3133 (NH); 1713 (C=O); 1195 (C=S) cm^{-1}. nmr: 0.87 (t, 3H, CH_3, J=6.7Hz); 1.24 (s, 10H, 5xCH_2); 1.66 (m, 2H, CH_2); 2.34 (t, 2H, CH_2C=O, J=7.6Hz); 7.23-7.48 (m, 5H, $H_{arom.}$); 8.86 (s, 1H, NH); 9.32 (s, 1H, NH); 9.96 (s, 1H, NH) ppm
3b	ir: 3298-3188 (NH); 1689 (C=O); 1196 (C=S) cm^{-1}. nmr: 0.88 (t, 3H, CH_3, J=6.9Hz); 0.94 (t, 3H, CH_3, J=7.3Hz); 1.27-1.68 (m, 16H, 8xCH_2); 2.30 (t, 2H, CH_2C=O, J=7.8Hz); 3.55 (m, 2H, CH_2-NH); 7.02 (s, 1H, NH); 8.80 (s, 1H, NH); 9.31 (s, 1H, NH) ppm
3c	ir: 3503-3173 (NH); 1695 (C=O); 1234 (C=S) cm^{-1}. nmr: 0.87 (t, 3H, CH_3, J=6.8Hz); 1.26 (s, 10H, 5xCH_2); 1.61 (m, 2H, CH_2); 2.28 (t, 2H, CH_2C=O, J=7.7Hz); 4.21 (t, 2H, CH_2, J=5.1Hz); 5.15 (dd, 1H, =CH_2, J=10.4Hz, J=1.2Hz); 5.21 (dd, 1H, =CH_2, J=19.2Hz, J=1.2Hz); 5.86 (m, 1H, CH); 7.15 (s, 1H, NH); 8.97 (s, 1H, NH); 9.39 (s, 1H, NH) ppm
3d	ir: 3350-3250 (NH); 1680 (C=O); 1285 (C=S) cm^{-1} nmr: 0.89 (t, 3H, CH_3); 1.26 (m, 28H, 14xCH_2); 1.68 (m, 2H, CH_2); 2.34 (t, 2H, CH_2C=O, J=7.6Hz); 7.25-7.43 (m, 5H, $H_{arom.}$); 8.83 (s, 1H, NH); 9.82 (bs, 2H, NHNH) ppm
3e	ir: 330-3200 (NH); 1650 (C=O); 1230 (C=S) cm^{-1} nmr: 0.89 (t, 3H, CH_3, J=7.1Hz); 0.95 (t, 3H, CH_3, J=7.0Hz); 1.26 (m, 30H, 15xCH_2); 1.60 (m, 4H, 2xCH_2); 2.31 (t, 2H, CH_2C=O, J=7.8Hz); 3.55 (m, 2H, NHCH_2); 8.3 (s, 1H, NH); 9.86 (bs, 2H, NHNH) ppm
3f	ir: 3280-3250 (NH); 1660 (C=O); 1270 (C=S) cm^{-1}. nmr: 0.89 (t, 3H, CH_3, J=7.0Hz); 1.26 (m, 28H, 14xCH_2); 1.64 (m, 2H, CH_2); 2.29 (m, 2H, CH_2C=O); 4.23 (m, 2H, NHCH_2); 5.20 (m, 2H, =CH_2); 5.87 (m, 1H, CH=); 7.16 (s, 1H, NH); 8.86 (s, 1H, NH); 9.50 (s, 1H, NH) ppm
3g	ir: 3310-3215 (NH); 1680 (C=O); 1228 (C=S) cm^{-1}. nmr: 0.89(t, 3H, CH_3, J=6.8Hz); 1.26 (m, 28H, 14xCH_2); 1.66 (m, 2H, CH_2); 2.30 (t, 2H, CH_2C=O, J=7.6Hz); 3.11 (d, 3H, NHCH_3,J=7.3Hz); 6.94 (s, 1H, NH); 8.48 (s, 1H, NH); 8.96 (s, 1H, NH) ppm

not all compounds exhibited antibacterial and antifungal activities. None of the compounds were active against the *Bacillus subtilis* and *Aspergillus niger*.

The thiosemicarbazides (**3d-f**) showed some more inhibition against *Escherichia coli*. Their cyclised product triazoles (**4d-g**), that one, with alkyl substituent in position four showed some more inhibition against *Candida albicans*, *Staphylococcus aureus* and *Salmonela enteritidis*.

The 4-allyl-5-heptadecyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione (**4f**) showed the highest inhibition zone diameter against *Escherichia coli* (8mm) compared with all test organisms and all tested compounds.

EXPERIMENTAL

General Procedure.

The melting points of synthesized compounds were determined on a Büchi melting point apparatus and are uncorrected. The ir spectra were recorded on the range of 4000-400 cm^{-1} using KBr pellets on a Perkin Elmer 297 spectrophotometer. The nmr spectra were measured in $CDCl_3$ using TMS as an internal reference on a Bruker AC 2500 nmr spectrometer at 60MHz.

Ethyl Nonanoate/octadecanoate (**1a-b**).

The esters were prepared following the standard procedure mentioned in the literature [18], (**1a**) yield 70%, bp 225 °C (lit. 227 °C, 216-19 °C) and (**1b**) yield 95%, mp 33-5°C (lit. 30.5-33°C).

Nonanoyl/octadecanoylhydrazine (**2a-b**).

These compounds were obtained treating the corresponding ethyl esters (**1**) with hydrazine in ethanol with modification of the literature procedures [19,20], (**2a**) yield 96%, mp 95 °C (lit. 94.5-5.5 °C) and (**2b**) yield 95%, mp 116-18 °C (lit. 115-116.2 °C). It is important to emphasize that excellent yield and purity are obtained with refluxing the reactants for 12 hours (ester:hydrazine in reaction 1:2 mole).

1-Nonanoyl/octadecanoyl-4-alkyl/aryl-thiosemicarbazides (**3a-b**).

A mixture of 0.01 mole of corresponding acyl hydrazines (**2a-b**) and 0.01 mole of alkyl/aryl isothiocyanate in 120 mL ethanol

was heated under reflux for 1.5-3 hours. The excess of ethanol was removed and the crystalline product that appeared on cooling was filtered and several times was washed with cold ethanol, dried and recrystallized from ethanol or acetone. The physical data of (**3a-g**) are given in Table 1.

4-Alkyl/aryl-5-octyl/heptadecyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thiones (**4a-g**).

A mixture of 0.005 mole of 1-nonanoyl/octadecanoyl-4-alkyl/aryl-thiosemicarbazides (**3a-b**) and 15 mL NaOH solution (2 *M*) was refluxed for 4-5 hours. The resulting viscous solution was poured into cool water and acidified with concentrated HCl (pH~ 4-5). The solid, which appeared, was collected by filtration, washed with water (neutral pH), dried and recrystallized from dilute ethanol (**4a-c**) and from ethanol (**4d-g**). The melting points, yield and elemental microanalysis are given in Table 2.

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Table 2

Physical Data of Compounds (**4a-g**)

Compound No.	R	R'	Mp [°C]	Yield [%]	Elemental analysis Calcd./Found C	H	N	Molecular formula Mol. wt.
4a	C_8H_{17} -	C_6H_5 -	124-6	72	66.44 66.22	7.96 7.71	14.53 14.84	$C_{16}H_{23}N_3S$ (289.45)
4b	C_8H_{17} -	*n*-C_4H_9 -	131-2	77	62.45 62.46	10.04 9.67	15.61 15.47	$C_{14}H_{27}N_3S$ (269.45)
4c	C_8H_{17} -	CH_2=CH-CH_2-	101-2	80	61.66 61.88	9.09 8.73	16.60 16.39	$C_{13}H_{23}N_3S$ (253.41)
4d	$C_{17}H_{35}$ -	C_6H_5 -	116	59	72.23 72.24	9.94 9.76	10.11 10.33	$C_{25}H_{41}N_3S$ (415.44)
4e	$C_{17}H_{35}$ -	*n*-C_4H_9 -	117-9	83	69.85 70.31	11.46 11.45	10.62 10.66	$C_{23}H_{45}N_3S$ (395.48)
4f	$C_{17}H_{35}$ -	CH_2=CH-CH_2-	111	73	69.61 69.07	10.88 10.54	11.07 11.28	$C_{22}H_{41}N_3S$ (379.44)
4g	$C_{17}H_{35}$ -	CH_3 -	119-21	78	/	/	/	$C_{20}H_{39}N_3S$ (353.47)

4a ir: 3095 (NH); 1572 (C=N); 1335 (C=S) cm^{-1}
nmr: 0.85 (t, 3H, CH_3, J=7.0Hz); 1.19 (s, 10H, 5xCH_2); 1.55 (m, 2H, CH_2); 2.47 (t, 2H, CH_2C=N, J=7.8Hz); 7.31-7.35 (m, 2H, $H_{arom.}$); 7.54-7.61 (m, 3H, $H_{arom.}$); 12.41 (s, 1H, NH) ppm

4b ir: 3105 (NH); 1568 (C=N); 1358 (C=S) cm^{-1}
nmr: 0.88 (t, 3H, CH_3); 0.98 (t, 3H, CH_3, J=7.0Hz); 1.28-1.42 (m, 12H, 6xCH_2); 1.73 (bs, 4H, 2xCH_2); 2.62 (t, 2H, CH_2C=N, J=7.3Hz); 3.95 (t, 2H, CH_2-N, J=7.6Hz); 11.86 (s, 1H, NH) ppm

4c ir: 3110 (NH);1570 (C=N); 1353 (C=S) cm^{-1}
nmr: 0.85 (t, 3H, CH_3, J=6.8Hz); 1.24 (s, 10H, 5xCH_2); 1.71 (m, 2H, CH_2); 2.59 (t, 2H, CH_2C=N, J=8.0Hz); 4.64 (m, 2H, CH_2); 5.09 (d, 1H, =CH_2, J=17.1Hz); 5.25 (d, 1H, =CH_2, J=10.4Hz); 5.87 (m, 1H, CH); 12.29 (s, 1H, NH) ppm

4d ir: 3000-2800 (NH) cm^{-1};
nmr: 0.89 (t, 3H, CH_3, J=7.1Hz); 1.26 (m, 28H, CH_2x14); 1.57 (m, 2H, CH_2); 2.51 (t, 2H, CH_2C=N, J=7.7Hz); 7.35-7.60 (m, 5H, arom.) ppm;

4e ir: 3120-3030 (NH) cm^{-1}
nmr: 0.89 (t, 3H, CH_3, J=7.2Hz); 0.99 (t, 3H, CH_3, J=7.1Hz); 1.26 (m, 2H, CH_2x14); 1.40 (m, 4H, CH_2x2); 1.72 (m, 4H, CH_2x2); 2.67 (t, 2H, CH_2C=N, J=7.2Hz); 4.00 (t, 2H, NCH_2, J=7.7Hz) ppm;

4f ir: 3350-3030 (NH) cm^{-1}
nmr: 0.88 (t, 3H, CH_3,J=7.0Hz); 1.26 (m, 28H, CH_2x14); 1.75 (m, 2H, CH_2); 2.62 (t, 2H, CH_2C=N, J=7.3Hz); 4.68 (d, 2H, NCH_2, J=7.9Hz); 5.13 (d, 1H, Htrans-vinyl, J=17.2Hz); 5.29 (d, 1H, Hcis-vinyl, J=10.3); 5.90 (m. 1H, CH=) ppm

4g ir: 3300-3250 (NH) cm^{-1}
nmr: 0.88 (t, 3H, CH_3, J=7.0Hz); 1.26 (m, 28H, CH_2x14); 1.73 (m, 2H, CH_2); 2.64 (t, 2H, CH_2C=N, J=7.3Hz); 3.54 (s, 3H, NCH_3) ppm;

Table 3

Inhibition Zones (mm)

Compound	Concentration [mg/mL]	*Escherichia coli*	*Bacillus subtilis*	*Salmonella enteritidis*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
3d	5	6.5	-	-	-	-	-
	10	6.5	-	-	-	-	-
	1	5.5	-	-	-	-	-
3e	5	5.5	-	6.5	-	-	-
	10	5.5	-	7	-	-	-
	1	-	-	-	-	-	-
3f	5	5.5	-	-	-	-	-
	10	5.5	-	-	-	-	-
	1	-	-	-	-	-	-
3g	5	-	-	-	-	-	-
	10	-	-	-	-	-	5.5
	1	-	-	-	-	-	-
4d	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	1	-	-	7.5	-	-	6.5
4e	5	-	-	-	7	-	6.5
	10	-	-	-	7	-	6.5
	1	-	-	-	7	-	-
4f	5	8	-	5.5	7	-	5.5
	10	8	-	5.5	-	-	7
	1	-	-	-	-	-	-
4g	5	-	-	-	-	-	-
	10	-	-	-	-	--	5.5

- No inhibition zone

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