Full Paper

Synthesis of New 3-Substituted-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2*H*-l,3,5-thiadiazine-2-thione Derivatives with Potential Antimicrobial Activity

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The purpose of this study is based upon design and synthesis of a new series of flexible molecules of 3,4,5,6-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (THTT) derivatives depending upon incorporation of 2-aminoethanol as a part of the polar moiety in this nucleus. Thirteen derivatives of 3-substituted-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione were synthesized by reaction of the appropriate alkyl, cycloalkyl, aralkyl amine, or glycine with carbon disulphide, formaldehyde, and 2-aminoethanol. The structures of the target compounds were elucidated using spectral methods as well as elemental analyses. A mass-spectrometry study was carried out on representatives of the synthesized derivatives. The title compounds were tested for their antibacterial activity *in vitro* against some *gram* positive and *gram* negative bacteria. The *in-vitro* antifungal activity was tested against dermatophytic, saprophytic, phytopathogenic, and antagonistic fungi. In most cases, the newly synthesized compounds **4–16** exhibited a considerable inhibitory effect on the growth of some of the tested organisms in comparison to that of ampicillin or muconazole as reference drugs. Moreover, the results indicated that the polar hydroxyethyl group at the N5- and the lipophilic one at the N3-positions are essential for the antimicrobial activity of the tested compounds.

Keywords: Antimicrobial / Synthesis / Thiadiazine-2-thione

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Introduction

Tetrahydro-2*H*-l,3,5-thiadiazine-2-thione (THTT) derivatives are known to display important biological activities. Numerous studies have been published on their antibacterial [1–10], antifungal [11–16], anthelmintic [17], antiprotozoal [18], and antituberculous [5] activity as prodrugs [19–21]. The antimicrobial activity of these compounds has been suggested to be based on isothiocyanates and dithiocarbamic acids, which are formed by hydrolysis of the tetrahydro-2*H*-l,3,5-thiadiazine-2-thione ring [15–21]. Some studies have pointed out the significance of the nature of the substituents at the 3- and 5-positions [3–5]. Lipophilic groups at both, the 3- and 5-positions, lead to compounds with high antimicrobial activities but also high toxicity. The toxicity of such compounds was greatly altered by using polar groups at the 5-position [13] with the optimum structure-activity illustrated in Fig. 1. The previous studies revealed that insertion of the carboxymethyl group at the 5-position would reduce both the lipophilicity and the antimicrobial activ-

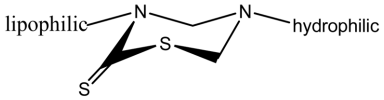

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Figure 1. Lead structure **I** of synthesized compounds.

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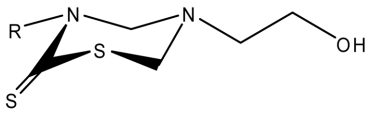
Abbreviations: Tetrahydro-2*H*-l,3,5-thiadiazine-2-thione (THTT)

Table 1. Physicochemical data of compounds **4–16**.

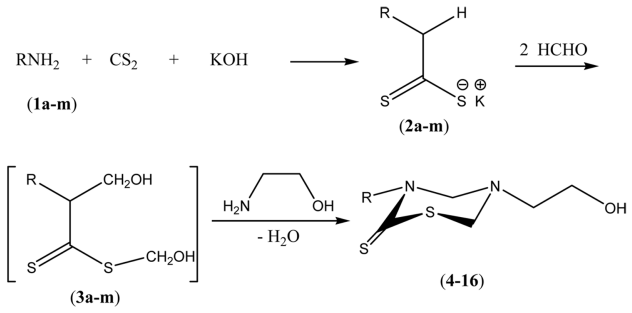


Compound	R	Molecular formula (MW)	Yield[a] (%)	Mp[b] (°C)	R_f[c]	Clog P
4	CH_3	$C_6H_{12}N_2OS_2$ (192.30)	78	liquid	0.31	0.448
5	C_2H_5	$C_7H_{14}N_2OS_2$ (192.30)	75	liquid	0.32	1.027
6	*n*-C_3H_7	$C_8H_{16}N_2OS_2$ (192.30)	90	118–119	0.35	1.556
7	*i*-C_3H_7	$C_8H_{16}N_2OS_2$ (192.30)	90	121–122	0.41	1.330
8	*n*-C_4H_9	$C_9H_{18}N_2OS_2$ (192.30)	83	93	0.51	2.085
9	*i*-C_4H_9	$C_9H_{18}N_2OS_2$ (192.30)	80	85–86	0.52	1.865
10	*n*-C_5H_{11}	$C_{10}H_{20}N_2OS_2$ (192.30)	70	110–111	0.58	2.614
11	*n*-C_6H_{13}	$C_{10}H_{22}N_2OS_2$ (192.30)	83	127–128	0.56	3.143
12	*c*-C_6H_{13}	$C_{11}H_{20}N_2OS_2$ (192.30)	80	144–145	0.52	2.439
13	$C_6H_5CH_2$	$C_{12}H_{16}N_2OS_2$ (192.30)	92	125–126	0.49	2.138
14	2-$C_6H_5C_2H_4$	$C_{13}H_{18}N_2OS_2$ (192.30)	85	98–99	0.45	2.445
15	*dl*-1-$C_6H_5C_2H_4$	$C_{13}H_{18}N_2OS_2$ (192.30)	80	145–146	0.43	2.447
16	$HOOCCH_2$	$C_7H_{12}N_2O_3S_2$ (192.30)	65	178–179	0.27	−1.027

[a] The developing solvent system used is $CHCl_3$: CH_3OH (5 : 3).
[b] The boiling points of compounds **4** and **5** are 215°C/10 mm Hg and 218°C/10 mm Hg, respectively.
[c] The crystallization solvent for the solid compounds is ethanol.

ity [19–21]. Accordingly, further tuning of the polarity of the substituent group at the 5-position of the THTT moiety seemed to be of interest. This work is concerned with the incorporation of the hydroxyethyl group in the 5-position of the flexible THTT nucleus. This will afford analogues with modified lipophilic properties and the testing of their antimicrobial activities, if present.

It is also of interest to investigate the mass-spectral behavior of the representatives of the synthesized derivatives for further exploration of the reported compounds [22, 23].



R: **4** = CH_3, **5** = C_2H_5, **6** = C_3H_7, **7** = *i*-C_3H_7, **8** = *n*-C_4H_9, **9** = *i*-C_4H_9, **10** = *n*-C_5H_{11}, **11** = *n*-C_6H_{13}, **12** = *cyclo*-C_6H_{13}, **13** = $C_6H_5CH_2$, **14** = 2- $C_6H_5C_2H_4$, **15** = 1-$C_6H_5C_2H_4$, **16** = CH_2COOH.

Scheme 1. Synthesis route of compounds **4–16**.

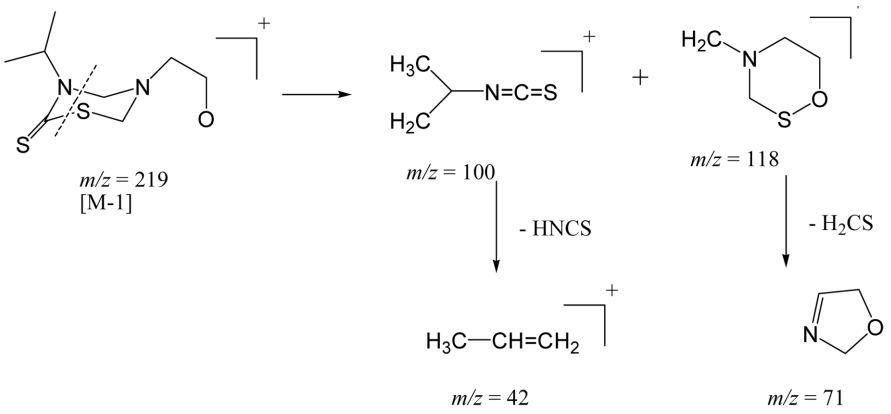
Results and discussion

Chemistry

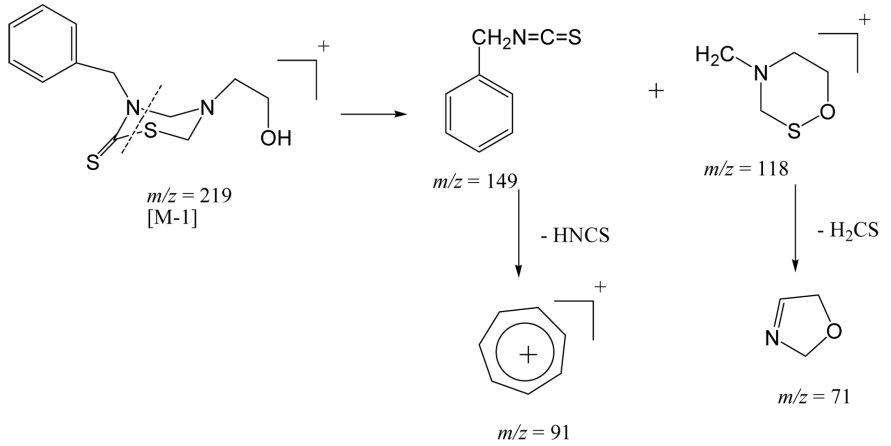
The target compounds were synthesized by reaction of the appropriate alkyl, cyc1oalkyl, aralkyl amine, or glycine **la–m**, with carbon disulphide and potassium hydroxide. The resulting alkyl dithiocarbamate salts **2** were subjected to cyc1o-condensation reaction with formaldehyde and 2-aminoethanol to provide the target derivatives **4–16**, Scheme 1. It was suggested that the reaction proceeded through the formation of the intermediate **3** *in situ* [l0–18]. The structures of the synthesized compounds were verified on the basis of spectral and elemental analyses. Table 1 shows the physicochemical data of compounds **4–16**.

All the spectral data are in accordance with the proposed structures. IR spectra of compounds **4–16** show prominent strong absorption bands around 3500–3450 cm^{-1} (OH), in addition, an absorption band at about 1450 cm^{-1} was attributed to a C=S bond. ^{1}H-NMR spectra revealed a common pattern of the THTT backbone and the N5-substituent (CH_2CH_2OH). The differences in sets and patterns were only attributed to the N3 alkyls, cycloalkyl, or aralkyls, where they showed pattern in accordance with the expected structures of the target compounds (see Experimental).

On the other hand, trials for the preparation of some derivatives with the hydroxyethyl moiety in position 3 were unsuccessful. The difficulty of preparation of such compounds might be attributed to the salt formation of 2-aminoethanol in strong alkaline medium [3].



Scheme 2. Fragmentation patterns of compounds **7**.



Scheme 3. Fragmentation patterns of compounds **13**.

Moreover, the structures of the target compounds were further confirmed on the basis of the MS (El) data of two representative compounds as given in the fragmentation patterns of compounds **7** and **13** of Schemes 2 and 3, respectively. The mass spectrum of **7** showed a weak M^+-1 peak at *m/z* 219 (11.8%) as the deprotonation of the substituent attached at N5 proceeded very easily, while the mass spectrum showed the prominent peaks at *m/z* 118 (12.4%), 100 (39.5%), and 42 (100%) (base peak corresponding to the fragment C_3H_6). On the other hand, the mass spectrum of compound **13** showed a weak molecular ion peak at *m/z* 268 (4.6%) (Scheme 3).

Lipophilicity

The lipophilicity of the target compounds **4–16** is expressed in the term of Clog P values (Table 1). The values were computed with a routine method called calculated log P (Clog P) contained in a PC-software package as mentioned in Experimental [24]. This work is devoted to study the effect of the lipophilicity on the antimicrobial activity.

Antimicrobial activity

The synthesized compounds **4–16** were evaluated *in vitro* for their antibacterial and antifungal activities using the standard agar diffusion method [25] according to the protocol mentioned below. Table 2, showed the antimicrobial activity of the tested compounds expressed as the inhibition zone in mm.

The antibacterial activities of the target compounds were tested against *Bacillus cereus* and *Micrococcus roseus* as *gram*-positive and *Serratia rodenii* and *Escherichia coli* as *gram*-negative bacterial species. The antifungal activity of the tested compounds was investigated *in vitro* against dermatophytes (*Candida albicans, Microsporum canis, Microsporum gypseum,* and *Trichophyton rubrum*), Saprophytes (*Aspergillus terreus* and *Penicillim chrysogenum*), phytopathogens (*Drechslera spicifera* and *Fusarium oxyspo-*

Table 2. Antimicrobial activity of the tested compounds **4 – 16** and the reference drugs. (All values are the average of three observations.)

Compound	Bacteria							Fungi					
	Gram positive		*Gram* negative		Dermatophytes			Saprophytes		Phytopathogens		Antagonist	
	B. cereus	*M. roseus*	*S. rodenii*	*E. coli*	*C. albicans*	*T. rubrum*	*M. gypseum*	*M. canis*	*A. terreus*	*P. chrysogenum*	*F. oxysporum*	*D. spicifera*	*T. harzianum*
4	8[a]	0[b]	0	10	6	10	7	10	8	15	7	12	0
5	11	13	12	8	10	15	10	15	8	13	10	13	7
6	0	15	10	7	10	10	10	7	10	12	10	12	7
7	0	10	15	10	12	17	7	10	12	15	11	15	8
8	0	10	12	10	20	20	20	22	12	18	13	18	8
9	8	16	11	12	0	7	10	15	0	12	0	12	0
10	0	12	7	9	0	7	15	13	0	9	0	9	8
11	11	12	10	15	15	15	15	16	9	15	10	15	13
12	15	15	9	16	20	20	20	18	15	18	15	18	18
13	0	0	7	15	20	22	23	21	13	20	10	20	22
14	11	10	13	12	13	18	20	20	14	15	12	15	12
15	12	0	15	10	8	7	15	16	0	9	0	9	0
16	8	7	15	8	0	0	0	0	0	0	0	0	0
Ampicillin	14	12	18	10	–[c]	–	–	–	–	–	–	–	–
Muconazole	–	–	–		7	25	26	27	25	25	25	25	15

[a] Inhibition zone in mm; disc diameter is 6 mm.
[b] 0 = no inhibition.
[c] – = not tested.

rum), and an antagonist (*Trichoderma harzianum*). The results of the antimicrobial activity of the tested compounds are given in Table 2, in comparison with the reference drugs.

The antibacterial and antifungal activities vary with the variation of the N3 substituents of the THTT moiety against the tested microbial strains. Most of the compounds gave antibacterial activity comparable to the reference drug ampicillin, further, some showed potency against *Micrococcus roseus* and *Escherichia coli*.

Again, highly antifungal activities were obtained for the tested compounds on comparison to the reference drug muconazole with no constant pattern. The study revealed an interesting finding that compound **16** has no inhibitory effect on the utilized fungal species, the structure of this compound includes two polar groups at N3 (CH_2COOH) and N5 (CH_2CH_2OH). This finding indicates that one of the two substituents at N3 or N5 must be nonpolar for activity, a result which is in accordance with the lipophilicity concept. Generally, compounds such as **7**, **8**, **13**, and **14** of high Clog P values showed the best antimicrobial activities, because of the ease of penetration into the lipophilic cell wall, whereas compound **16** with the lowest Clog P value had nearly of the lowest antimicrobial activity among the tested compounds. Interestingly, the antifungal activities of the tested compounds are more pronounced than their antibacterial activity.

However, most of the tested compounds revealed no or weak inhibitory activity against *Trichoderma harzianum*, though they have a reasonable antifungal activity against most of the studied fungi. Accordingly, the tested compounds can be considered as promising candidates for antifungal agents or for the use in plant-diseases integrated control programs.

Conclusion

We conclude that a new series of 3-substituted-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2*H*-l,3,5-thiadiazine-2-thione derivatives was synthesized and tested for their antimicrobial activity. The chemical structures of the target compounds were elucidated utilizing the spectral as well as the elemental methods of analysis. Moreover, MS assured the expected structures. The antimicrobial activity varied with the N-3 substituent groups on THTT, and the microorganisms used in comparison with the reference drugs. Most of the tested compounds exhibited pronounced antimicrobial activities with no or weak inhibitory activity against *Trichoderma harzianum*. Accordingly, they can be considered as promising candidates to be used either as antimicrobial agents and/or in plant-diseases integrated control programs. Moreover, the results of study revealed the essentiality of the lipophilic group at N3 and a polar substituent at N5 positions for good antimicrobial activity.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined on an electrothermal melting point apparatus (Sturat Scientific, UK) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60G F254; Merck, Germany) were used for thin layer chromatography. As developing solvent system chloroform/methanol (5 : 3) was used and the spots were detected by ultraviolet light and/or iodine. IR spectra (KBr disc) were recorded on an IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H-NMR spectra were measured on a Varian EM-360 L NMR spectrometer (60 MHz), (Varian, USA). Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ as a solvent. Mass spectra were made on a JEOL JMS600 (El) mass spectrometer (JEOL, Japan) at Assiut University Central Laboratory, Assiut, Egypt. Elemental analyses were performed at the Department of Chemistry, Faculty of Science, Assiut University, and the Micro Analytical Center, Faculty of Science, Cairo University, Egypt. The antimicrobial activity was performed at the Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt.

Synthesis of 3-substituted-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives ***4–16***

Carbon disulfide (4.8 mL, 80 mmol) was added portionwise to a well-stirred mixture of alkyl, cycloalkyl, aralkyl amine, or glycine **la–m**, (20 mmol) and potassium hydroxide (5.5 mL, 40% aqueous solution, 40 mmol); stirring was continued at 0°C for 2 h. To the above mixture, formaldehyde solution (3.8 mL, 35%, 44 mmol) was added and the stirring was continued for further 3 h. The resulting solution was added dropwise to a solution of 2-aminoethanol (1.2 mL, 20 mmol) in phosphate buffer (pH 7.8, 5 mL) at 0°C. After stirring for 5 h at ambient temperature, the medium was rendered nearly neutral and the formed precipitates were collected by filtration, washed with aqueous methanol, dried, and crystallized from ethanol. On the other hand, liquid products were obtained by extraction of the reaction mixture with chloroform. The combined organic extract was washed with brine and dried (Na_2SO_4). Concentration and column chromatography ($CHCl_3$/MeOH, 10 : 2) afforded the target compounds. Yields, melting points, and physical data are given in Table 1.

5-(2-Hydroxyethyl)-3-methyl-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***4***

^{1}H-NMR: 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.65 (3H, s, CH_3), 3.85–4.25 (3H, m, CH$_2$C*H*$_2$O*H*), 4.75 (4H, s, 2CH_2). Anal. calcd. for $C_6H_{12}N_2OS_2$: C, 37.47; H, 6.29; N, 14.57; Found: C, 37.40; H, 6.04; N, 14.55.

3-Ethyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***5***

^{1}H-NMR: 1.25 (3H, t, CH_3), 2.85 (1H, br s, OH), 3.05 (2H, t, C*H*$_2$CH$_2$OH), 3.80–4.15 (4H, m, CH$_2$C*H*$_2$OH and C*H*$_2$CH$_3$), (4H, s, 2 × CH_2). Anal. calcd. for $C_7H_{14}N_2OS_2$: C, 40.75; H, 6.84; N, 13.58. Found: C, 41.05; H, 6.73; N, 13.55.

5-(2-Hydroxyethyl)-3-n-propyl-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***6***

^{1}H-NMR: 1.00 (3H, t, CH_3), 1.50–2.10 (2H, m, CH$_2$C*H*$_2$CH$_3$), 2.85 (1H, br s, OH), 3.10 (2H, t, C*H*$_2$CH$_2$OH), 3.75–4.30 (4H, m, C*H*$_2$CH$_2$CH$_3$ and CH$_2$C*H*$_2$OH), 4.65 (4H, s, 2CH_2). Anal. calcd. for $C_8H_{16}N_2OS_2$: C, 43.60; H, 7.32; N, 12.71; Found: C, 43.64; H, 7.63; N, 12.60.

5-(2-Hydroxyethyl)-3-i-propyl-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***7***

^{1}H-NMR: 1.35 (6H, d, 2CH_3), 2.40 (1H, br s, OH), 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.95 (2H, t CH$_2$C*H*$_2$OH), 4.65 (4H, s, 2CH_2), 6.20-6.80 (1H, m, C*H*Me$_2$). Anal. calcd. for $C_8H_{16}N_2OS_2$: C, 43.60; H, 7.32; N, 12.71; Found: C, 43.67; H, 7.83; N, 12.64.

4.1.1.5 3-n-Butyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***8***

^{1}H-NMR: 1.05 (3H, t, CH_3), 1.30-2.15 (4H, m, CH$_2$(C*H*$_2$)$_2$CH$_3$), 2.90 (1H, br s, OH), 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.65-4.40 (4H, m, C*H*$_2$(CH$_2$)$_2$CH$_3$ and CH$_2$C*H*$_2$OH), 4.75 (4H, s, 2 × CH_2). Anal. calcd. for $C_9H_{18}N_2OS_2$: C, 46.12; H, 7.74 ; N, 11.95; Found: C, 46.01; H, 8.38; N, 12.19.

3-i-Butyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***9***

^{1}H-NMR: 1.15 (6H, d, CH_3), 2.20–2.80 (2H, m, CH and OH), 3.25 (2H, t, C*H*$_2$CH$_2$OH), 3.80-4.35 (4H, m, C*H*$_2$CH and CH$_2$C*H*$_2$OH), and 4.75 (4H, s, 2CH_2). Anal. calcd. for $C_9H_{18}N_2OS_2$: C, 46.12; H, 7.74; N, 11.95; Found: C, 46.55; H, 7.83; N, 11.56.

5-(2-Hydroxyethyl)-3-n-pentyl-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***10***

^{1}H-NMR: 1.00 (3H, t, CH_3), 1.25–2.05 (6H, m, CH$_2$(C*H*$_2$)$_3$CH$_3$), 2.85 (1H, br s, OH), 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.65–4.40 (4H, m, C*H*$_2$(CH$_2$)$_3$CH$_3$ and CH$_2$C*H*$_2$OH), 4.75 (4H, s, 2 × CH_2). Anal. calcd. for $C_{10}H_{20}N_2OS_2$: C, 48.35; H, 8.12; N, 11.28; Found: C, 48.50; H, 8.00; N, 11.50.

3-n-Hexyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***11***

^{1}H-NMR: 0.95 (3H, t, CH_3), 1.20–2.00 (8H, m, CH$_2$(C*H*$_2$)$_4$CH$_3$), 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.54 (1H, br s, OH), 3.70–4.35 (4H, m, C*H*$_2$(CH$_2$)$_4$CH$_3$ and CH$_2$C*H*$_2$OH), 4.65 (4H, s, 2 × CH_2). Anal. calcd. for $C_{11}H_{22}N_2OS_2$: C, 50.34; H, 8.45; N, 10.67; Found: C, 50.44; H, 8.34; N, 10.78.

3-Cyclohexyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***12***

^{1}H-NMR: 1.00–2.25 (10H, m, *c*-hexyl), 2.45 (1H, br s, OH), 3.15 (2H, t, C*H*$_2$CH$_2$OH), 3.85 (2H, t, CH$_2$C*H*$_2$OH), 4.55 (4H, s, 2CH_2), 5.75–6.30 (1H, m, C*H*N of *c*-hexyl). Anal. calcd. for $C_{11}H_{20}N_2OS_2$: C, 50.73; H, 7.74; N, 10.76; Found: C, 50.51; H, 8.41; N, 10.55.

3-Benzyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***13***

^{1}H-NMR: 1.95 (1H, br s, OH), 2.90(2H, t, C*H*$_2$CH$_2$OH), 3.70 (2H, t, CH$_2$C*H*$_2$OH), 4.50 (2H, s, CH_2 ring), 4.55 (2H, s, CH_2 ring), 5.55 (2H, s, CH_2Ph), 7.60 (5H, s, C_6H_5). Anal. calcd. for $C_{12}H_{16}N_2OS_2$: C, 53.70; H, 6.01; N; 10.44; Found: C, 53.83; H, 6.11; N, 10.23.

5-(2-Hydroxyethyl)-3-phenacyl-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***14***

^{1}H-NMR: 2.35 (1H, br s, OH), 2.80 – 3.45 (4H, m, C*H*$_2$CH$_2$OH and CH$_2$C*H*$_2$Ph), 3.80 (2H, t, CH$_2$C*H*$_2$OH), 4.20 – 4.65 (6H, m, 2CH$_2$ ring and C*H*$_2$CH$_2$Ph), 7.50 (5H, s, C$_6$H$_5$). Anal. calcd. for $C_{13}H_{18}N_2OS_2$: C, 55.28; H, 6.42; N, 9.92; Found: C, 54.95; H, 6.50; N, 9.71.

5-(2-Hydroxyethyl)-3-(1-dl-phenacyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***15***

^{1}H-NMR: 1.55 (3H, d, CH$_3$), 1.95 (1H, br s, OH), 2.35 – 2.95 (2H, t, C*H*$_2$CH$_2$OH), 3.35 (2H, t, CH$_2$C*H*$_2$OH), 4.25 – 4.65 (5H, m, 2CH$_2$ and CHPh), 7.50 (5H, s, C$_6$H$_5$). Anal. calcd. for $C_{13}H_{18}N_2OS_2$: C, 55.28; H, 6.42; N, 9.92; Found: C, 55.65; H, 6.50; N, 10.11.

3-Carboxymethyl-5-(2-hydroxyethyl)-3,4,5,6-tetrahydro-2H-1,3,5-thiadiazine-2-thione ***16***

^{1}H-NMR: 3.05 (2H, t, C*H*$_2$CH$_2$OH), 3.75 (2H, t, CH$_2$C*H*$_2$OH), 4.55-4.80 (6H, m, 2CH$_2$ and C*H*$_2$COOH), 5.45 (2H, br s, COOH and OH). Anal. calcd. for $C_7H_{12}N_2O_3S_2$: C, 35.58; H, 5.12; N, 11.85; Found: C, 35.50; H, 5.13; N, 12.05.

Calculation of the log P values

The log P values of the target compounds **4 – 16**, were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (McLogP 2.0, BioByte Corp., Claremont, CA, USA). A representation of the molecular structure where hydrogens are omitted or 'suppressed' (SMILES notation) is entered into the program, which computes the log P based on the fragment method developed by Leo (1993) [24]. The results are given in Table 1.

Organisms, culture conditions, and antimicrobial activity

To test the antibacterial activities of the target compounds: *B. cereus* and *M. roseus* were used as representatives of *gram*-positive species and S. *rodenii* and *E. coli* were used as representatives of *gram*-negative species.

Nine fungal species including dermatophytes, saprophytes, phytopathoges, and antagonists were used in the present study. These fungi are namely *T. rubrum, M. Canis,* and *M. gypseum* (dermatophytes causing tinea corporis, tinea pedis, tinea manuum, and onychomycosis); C. *albicans* (a cause of candidiasis [26, 27]); *P. chrysogenum* and *A. terreus* (food and foodstuff contaminants); *F. oxysporum, D. spicifera* (the common root pathogens in Egyptian soils), and *T. harzianum* (an antagonist of many plant pathogens [27]).

C. *albicans, M canis, M. Gypseum,* and *T. rubrum* were isolated from clinical cases in the Assiut University hospitals [28 – 30]. The other fungal species *(Aspergillus terreus, Drechslera spicifera, Fusarium oxysporum, Penicillum chrysogenum,* and *Trichoderma harzianum)* were isolated from diseased and healthy roots of some crop plants as well as from soil samples [31, 32]. These species were maintained on sabouraud dextrose agar (SDA) or potato dextrose agar (PDA) media in the collection herbarium of the Botany Department.

Spore suspension in case of fungi or cell suspension in case of bacteria in sterile distilled water were prepared from 3 – 5 days old culture of the test organisms growing on nutrient agar (in case of bacteria) and sabouraud dextrose agar (SDA) or potato dextrose agar (PDA) media (in case of fungi). The final spore concentration was approximately 10^5 spores/mL. About 15 mL of growth medium was introduced into sterilized plates of 9 cm diameter and inoculated with 1 mL of spore or cell suspension. Plates were shaken gently to homogenize the medium.

The antimicrobial activity of the tested compounds was performed by the standard agar disc diffusion method [25] as follows: Sterile 6 mm filter paper discs (Whatman) were impregnated with solutions of the tested compound as well as the reference drugs (10 μM/mL in DMSO). In addition, other discs were impregnated with the solvent (DMSO) and served as control. The impregnated discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28 ± 2°C for 7 days in case of fungi and 2 days at 37 ±2°C in case of bacteria. The radii of the inhibition zones (in mm) were measured at successive intervals during the incubation period. Triplicate sets were applied for each treatment and the results are given in Table 2.

References

[1] M. Lipsitch, *Trends Microbiol* **2001**, *9*, 438 – 444.

[2] D. W. Eugene in *Burger's Medicinal Chemistry,* 4th Ed., John Wiley and Sons, New York, **1979**, p. 531.

[3] A. Reiche, G. Hilgetag, A. Martini, R. Philippson, *Arch. Pharm.* **1961,** *294*, **201 – 209.**

[4] T. Zsolanai, *Arzneim – Forsch./Drug Res.* **1968**, *18*, 1319 – 1324.

[5] T. Zsolanai, *Arzneim – Forsch./Drug Res.* **1961**, *21*, 121 – 127.

[6] M. Ertan, A. B. Tayhan, N. Yulug, *Acta Pharm. (Weinheim)* **1961**, *323*, 605 – 609.

[7] M. Ertan, S. Sarac, N. Yulug, *Arzneim – Forsch.* **1990**, *40,* 790 – 795.

[8] J. Bernat, P. Kristian, J. Imrich, I. Chomca, *Synth. Commun*. **1996**, *26*, 4343 – 4350.

[9] S. Sarac, M. Ertan, A. Balken, N. Yulug, *Arch. Pharm. (Weinheim)* **1991,** *324*, **449 – 453.**

[10] A. EI – Shorbagi, *Arch. Pharm. Pharm. Med Chem.* **2000**, *333*, 281 – 286.

[11] J. Goksor, *Acta Chem. Scand*. **1964**, *18*, 1341 – 1352.

[12] M. Ertan, H. G. Ayyildiz, N. Yulug, *Arzneim-Forsch.* **1991**, *41*, 1182 – 1185.

[13] M. Ertan, R. Bilgin, E. Palaska, N. Yulug, *Arzneim – Forsch.* **1992**, *42*, 160 – 163.

[14] T. Aboul – Fadl, M. A. Hussein, A. EI-Shrobagi, K. Abdel-Rauuf, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 438 – 442.

[15] A. H. Mostafa, A. EI – Shrobagi, K. Abdel-Rauuf, *Arch. Pharm. Med. Chem.* **2001**, *334,* 305 – 308.

[16] T. Aboul – Fadl, M. Osman, *Sci. Pharm.* **2002**, *70*, 359 – 378.

[17] M. Schorr, W. Durrcheimer, P. Klatt, G. Lommler, *et al, Arzneim – Forsch./Drug Res.* **1969**, *19*, 1807 – 1819.

[18] O. Carmen, P. Eduard, P. Roland, S. Margarita, *et al*., *Arzneim-Forsch.* **1999**, *49*, 764 – 769.

[19] A. EI-Shorbagi, *Eur. J. Med. Chem.* **1994**, *29*, **11 – 15.**

[20] T. Aboul-Fadl, A. EI – Shrobagi, *Eur. J. Med Chem.* **1999**, *31*, 165 – 169.

[21] T. Aboul-Fadl, K. Hassanin, *Pharmazie* **1999**, *54*, 244 – 247.

[22] I. Eser, C. Gultaze, E. Nedime, *Farmaco* **1999**, *50*, 787 – 790.

[23] M. A. Roberto, M. Nazario, S. Carlos, S. Margarita, *et al., Rapid Commun. Mass Spectrom.* **2001**, *15*, 758 – 762.

[24] A. J. Leo, *Chem. Rev*. **2001**, *93*, **1281 – 1306.**

[25] H. William in *Microbiological assay: An introduction to quantitative principles and evaluation*, Academic Press, New York, **1977**.

[26] S. A. Out, S. M. Sherif, *Folia Microbiol.* **1993**, *38*, 181 – 187.

[27] A. A. Moubasher, *Soil fungi in Qatar and other Arab countries,* Scientific and Applied Research Center, University of Qatar, Qatar, **1993.**

[28] T. P. West, *F. Microbios* **1993**, *56*, 226 – 227.

[29] K. J. Kwon-Chung, J. E. Bennett, *Medical Mycology Lea and Febiger,* Malvern, Pennsylvania, USA, **1992**, p. 866.

[30] A. A. Moubasher, M. B. Mazen, A. M. Moharram, A. EI-Shanawany, Proc. 10th *Ann. Sci. Coni* April **1992**.

[31] M. B. Mazen, A. Y. Abdel-Malek, A. D. Allam, M. Hashem, *Bul. Fac. Sci. Asiut Univ.* **1997**, *25 (I – D)*, 67 – 84.

[32] M. Hashem, *Czech Mycol.* **2002**, *56*, 223 – 238.

