Bull. Pharm. Sci., Assiut University, Vol. 28, Part 2, December 2005, pp. 255-260.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 3-(1-PHENYLETHYL)-5-SUBSTITUTED-2H-TETRAHYDRO-1,3,5-THIADIAZINE-2-THIONE DERIVATIVES

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من أجل البحث عن مركبات ذات فاعلية مضادة للميكروبات تم تخليق عدد ثلاثة عشر مركبا جديداً من مشتقات الـــ ٣ (١ فينيل إيثيل) ٥ مشتق ٢-يد رباعى هيدرو ١،٣،٥ ثياديازين ٢ ثيون. تم تخليق هذه المركبات من خلال تفاعل dl-α-1 فينيل إيثيل أمين مع الكربون ثنائي السلفيد والفورمالدهيد والألكيل، سيكلوالكيل، أرالكيل أمين وجليسين وإيثيل جليسين إستر. وقد تم التأكد من التراكيب البنائية للنواتج النهائية إعتمادا على نتائج التحاليل الطيفية المختلفة إلى جانب التحاليل الدقية لعناصرها المكونة.هذا وقد تم إختبار تأثير المشتقات المذكورة على تثبيط نمو بعض الكائنات البكتيرية والفطرية. وقد أشارت نتائج هذه الدراسة إلى أن فاعلية المركبات المختلفة كمثبطات للميكروبات تكون بدرجة كبيرة مع وجود مشتق صغير فى الحجم وغير متفرع أو مشتق مستقطب على ن٥.

In a search for potential antimicrobial compounds thirteen new 3-(1-phenylethyl)-5-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thiones were synthesized by the reaction of α-phenethylamine with carbon disulfide and potassium hydroxide, followed by formaldehyde and the appropriate alkyl, aralkylamines, glycine or ethyl glycinate (Scheme 1). The chemical structure of the synthesized compounds was elucidated by spectral data and elemental analysis. The title compounds were tested, in vitro, for antimicrobial activity against Gram-positive, Gram-negative bacteria, and some fungi, using agar disc method. The antimicrobial activity was found to be affected by the bulkiness of the side chain and presence of polar group at N^5 position. The highest activity was obtained with compounds ***4l*** *and* ***4m*** *(R= CH_2-COOH, CH_2-$COOC_2H_5$).*

INTRODUCTION

Systemic mycosis in patients suffering from debilitating diseases such as neopliasis and in persons on long term total parentral nutrition,[1] is becoming critical for the need of more and better antifungal agents. It is well established that tetrahydro-1,3,5-thiadiazine-2-thione (THTT) moiety possesses a significant antimicrobial activity which may take place by production of isothiocyanate and/or dithiocarbamic acids.[2-11] In our effort to shed light on the structural requirements for the antifungal and anti-bacterial activity of 1,3,5-thiadiazine-2-thione nucleus, we prepared several new derivatives of this ring 4a-m. The study was based on fixing lipophilic substituent at N^3 position and incorporation of a variety of substituents at N^5 position. The compounds were screened for their antifungal and antibacterial activities.

EXPERIMENTAL

Materials and equipment

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, UK], and are uncorrected. Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merk) were used for thin layer chromatography. Developing solvent system of chloroform/methanol (10:3) was used and the spots were detected by ultraviolet light. IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz) USA at Faculty of Pharmacy Assiut University. Chemical shifts

Received in 11/4/2005 & Accepted in 21/7/2005

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are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were performed at the Department of Chemistry, Faculty of Science. Antimicrobial activity was performed at the Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt.

General procedure for synthesis of 3-(1-phenylethyl)-5-substituted tetrahydro-2H-1,3,5-thiadiazine-2-thione; 4a-m

Carbon disulfide (60 mmol) was added portion-wise to a stirred mixture of ∝-phenethylamine **1** (10 mmol) and potassium hydroxide (40%, 20 mmol) in ethanol (10 ml) and stirring was continued for **3h**. at ambient temperature. To the reaction mixture, formaldehyde solution (35%, 22 mmol), was added and stirring was continued for further 1 h. The resulting clear solution was added portion-wise during 15 min to a stirred solution of the appropriate alkyl-, cycloalkyl,-aralkylamine, glycine or ethyl glycinate (10 mmol) in phosphate buffer (pH 7.8, 20 ml). After stirring for 6 h. at ambient temperature, the reaction mixture was acidified with dilute hydrochloric acid (5%, ~ 15-18 ml) to pH 2 and stirring was continued for further 30 min. The formed precipitate was collected by filtration, washed with 0.5% hydrochloric acid and dried. The crude solid was crystallized from ethanol-chloroform (1:1). Yields, physical and spectral data are given in Tables 1 and 2.

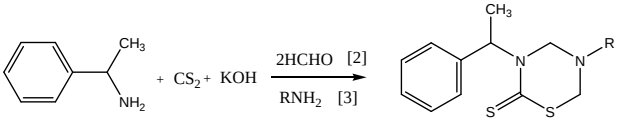
Organisms, culture conditions, and antimicrobial activity

Six pathogenic fungal species were used in the present study: Aspergillus fumigatus, Penicillium oxalicum, Trichophyton rubrum(Robin) Berkhout (a cause of candidiasis),[18] Microsporum canis, Chrysosporium tropicum and Candida albicans T. rubrum and C. albicans were isolated from clinical cases in the Assiut University hospitals.[19] Spore suspension in sterile distilled water was prepared from 3-5 days old culture of the test fungi growing on Sabouraud agar dextrose (SAD) medium. The final spore concentration was $5x10^4$ spores/ml. About 20 ml of growth medium was introduced on sterilized plates of 9 cm diameter and inoculated with 1ml of spore suspension. Plates were shaken gently to homogenize the inoculum. Antifungal activity of the tested compounds was performed by the standard agar disk diffusion method[17] as follow: Sterile 6 mm filter paper disks (Whatman) were impregnated with solutions of the tested compound (100 μM/ml in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as control. The impregnated disks were then dried for 1 hour and placed in the center of each plate. The seeded plates were incubated at 30 ± 2° for 7 days. The radii of inhibition zones (in mm) were measured at successive intervals during the incubation period. Triplicate set were applied for each treatment. Results are given in Table 3.

RESULTS AND DISCUSSION

Chemistry

Target compounds **4a-m** were synthesized adopting a previously reported[11-16] procedure. ∝-phenethylamine (**1**) was treated with carbon disulphide and potassium hydroxide, then formaldehyde was added, followed by the appropriate alkyl-, cycloalkyl-, aralkyl- amine, glycine, or ethyl glycinate in presence of phosphate buffer (pH = 7.8) to give (**4a-m**) (Scheme 1).[11-16] Structures of the synthesized compounds were verified by spectral and elemental analyses, Tables 1 and 2. IR spectra of compounds **4a-m** showed bands at 2840-2960 cm^{-1} (aliphatic C-H stretching); 3030-3060 cm^{-1} (aromatic C-H stretching) and at about 1420-1455 cm^{-1} (C=S stretching)



[1] [4a-m]

R= CH_3 (**a**), C_2H_5 (**b**), C_3H_7 (**c**), i-C_3H_7 (**d**), n-C_4H_9 (**e**), i-C_4H_9 (**f**), tert- C_4H_9 (**g**), cyclo-C_6H_{11} (**h**), C_6H_5-CH_2 (**i**), C_6H_5-$(CH_2)_2$ (**j**), C_6H_5-$CH(CH_3)$ (**k**), CH_2COOH (**l**), $CH_2COOC_2H_5$ (**m**).

Scheme 1

Moreover, compound **4l** showed the characteristic stretching absorption of the carboxylic group at the range 2500-3200 cm^{-1} (OH) and at 1705-1715 cm^{-1} (for the

carboxylic C=O). Compound **4m** showed the ester C=O stretching at 1745 cm^{-1} and C-O stretching at 1240 cm^{-1}.

^{1}H-NMR spectra revealed a common pattern for the N^{3}-1-phenylethyl [1.65 (d, 3H, $C_6H_5CH(C\underline{H}_3)$, 7.66 (5H, s, $C_6\underline{H}_5$), 7.80 (q, 1H, $C_6H_5C\underline{H}(CH_3)$].

Antimicrobial Activity

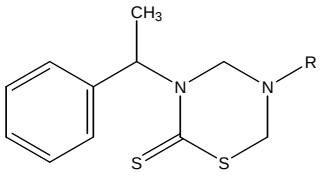
The synthesized compounds (**4a-m**) were tested for their antifungal activity *in vitro* against (*Aspergillus fumigatus, Penicillium oxalicum, Trichophyton rubrum, Microsporum canis, Chrysosporium tropicum and Candida albicans*) fungi using agar diffusion method[17] and mycostatin as standard.[20] The same compounds were tested, *in vitro*, for their antibacterial activity against *Micrococcus roseus, Micrococcus luteus, Escherichia coli* and Serratia rhodeni using chloramphenicol[10] as standard Table 3.

The antimicrobial study explored variable activities for variation at N^{5} position of 1,3,5-thiadiazine-2-thione nucleus. Results clearly indicate that introduction of a polar group (acetic acid or its ethyl ester **4l** and **4m** respectively) gave good to moderate antimicrobial activities. Compound **4l** is the most active against the sporulation of most of the tested species. Meanwhile, introduction of alkyl group showed a decrease in activity from moderate with methyl group **4a** to weak ethyl group **4b** to non-active propyl **4c** and butyl **4e** groups. However, branching of the alkyl group showed moderate **4d** to weak **4g**. Bulky hydrophobic group showed very weak activity like benzyl **4i** or showed no activity at all such as cyclohexyl, 2-phenylethyl or 1-phenylethyl **4h, j,k**.

Table 1: Physicochemical data of the newly synthesized derivatives, **4a-m**.

Compd. No.	R	Yield %	Molecular Formula[a]	M.P°	Elemental analyses (Calc/found)	
					N	S
4a	CH_3	45	$C_{12}H_{16}N_2S_2$	130-1	11.10 11.16	25.41 25.14
4b	C_2H_5	60	$C_{13}H_{18}N_2S_2$	153-5	10.51 10.54	24.07 24.33
4c	C_3H_7	57	$C_{14}H_{20}N_2S_2$	163-4	9.99 9.90	22.87 22.23
4d	*i*-C_3H_7	65	$C_{14}H_{20}N_2S_2$	171-2	9.99 9.92	22.87 22.23
4e	C_4H_9	62	$C_{15}H_{22}N_2S_2$	165-7	9.51 9.61	21.78 21.09
4f	i-C_4H_9	50	$C_{15}H_{22}N_2S_2$	196	9.51 9.55	21.78 21.88
4g	t- C_4H_9	55	$C_{15}H_{22}N_2S_2$	193-6	9.51 9.49	21.78 21.68
4h	cyclo-C_6H_{11}	50	$C_{17}H_{24}N_2S_2$. H_2O	215-7	8.27 8.54	18.94 18.91
4i	C_6H_5-CH_2	55	$C_{18}H_{20}N_2S_2$	128-131	8.53 8.50	19.52 19.30
4j	C_6H_5-$(CH_2)_2$	86	$C_{19}H_{22}N_2S_2$	122-3	8.18 8.19	18.72 19.12
4k	C_6H_5-$CH_2(CH_3)$	87	$C_{19}H_{22}N_2S_2$	106-8	8.18 8.15	18.72 18.80
4l	CH_2COOH	58	$C_{13}H_{16}N_2O_2S_2$	198-201	10.80 9.53	21.64 22.02
4m	$CH_2COOC_2H_5$	70	$C_{15}H_{20}N_2O_2S_2$. H_2O	128-30	8.18 8.59	18.73 18.60

Table 2: ^{1}H-NMR of **4a-m** (N^5-R) in DMSO-d_6.



4a-m

No.	R	^{1}H-NMR-chemical shifts (ppm)	
		CH_2 at C4 CH_2 at C6	N^5-R
4a	CH_3	4.0 (m, non equivalent 2H) 4.3 (m, non equivalent 2H)	2.4 (s, 3H, N-C<u>H</u>$_3$)
4b	C_2H_5	3.9-4.3 (m, non equivalent 2H) 4.3-4.75 (m, non equivalent 2H)	0.80 (t, 3H, CH_2C<u>H</u>$_3$), 2.55 (q, 2H, C<u>H</u>$_2CH_3$)
4c	C_3H_7	3.9-4.3 (m, non equivalent 2H) 4.3-4.75 (m, non equivalent 2H)	0.80 (t, 3H, CH_2CH_2-C<u>H</u>$_3$), 1.05 (m, 2H, CH_2C<u>H</u>$_2CH_3$), 2.5 (t, 2H, C<u>H</u>$_2CH_2CH_3$)
4d	*i*-C_3H_7	4.0-4.3 (m, non equivalent 2H) 4.3-4.80 (m, non equivalent 2H)	0.5 (d, 3H, (C<u>H</u>$_3$)CH(CH_3), 1.15 (d, 3H, (CH_3)CH(C<u>H</u>$_3$), 3.00 (m, 1H, C<u>H</u>(CH_3)$_2$)
4e	C_4H_9	3.9-4.3 (m, non equivalent 2H) 4.3-4.75 (m, non equivalent 2H)	0.75 (3H, t, butyl C<u>H</u>$_3$), 0.9-1.3 (4H, br.m, CH_2C<u>H</u>$_2$C<u>H</u>$_2CH_3$), 2.4 (2H, br. t, C<u>H</u>$_2CH_2CH_2CH_3$)
4f	i- C_4H_9	3.9-4.3 (m, non equivalent 2H) 4.3-4.75 (m, non equivalent 2H)	0.70 (t, 6H, i-butyl 2 C<u>H</u>$_3$), 1.2 (br.m,1H, CH methine of i-butyl), 2.3 (two dd, non equivalent 2H, C<u>H</u>$_2$ methylene of i-butyl)
4g	t- C_4H_9	3.3-4.5 (m, non equivalent 2H) 4.5-4.8 (m, non equivalent 2H)	1.00 (s, 9H, t-butyl 3 CH_3),
4h	cyclo-C_6H_{11}	4.0 -4.7 (m, 4H, two non equivalent 2H)	0.5-2.5 (14H, br.m, the cyclohexyl protons and C_6<u>H</u>$_5$CH(C<u>H</u>$_3$))
4i	C_6H_5-CH_2	3.7-4.6 (m, 4H, two non equivalent 2H)	3.3 and 3.7 (two s, non equivalent 2H, C_6H_5-C<u>H</u>$_2$), 7.5 (s, 5H, C_6<u>H</u>$_5$-CH_2)
4j	C_6H_5-$(CH_2)_2$	3.9-4.3 (m, non equivalent 2H) 4.3-5.8 (m, non equivalent 2H)	2.30-3.0 (br. m, 4H, C_6H_5-C<u>H</u>$_2$ C<u>H</u>$_2$).7.0-7.4 (br. m, 5H, C_6<u>H</u>$_5$-CH_2)
4k	C_6H_5-CH(CH_3)	3.5-4.3 (m, non equivalent 2H) 4.3-4.6 (m, non equivalent 2H)	1.50 (d, 3H, C_6H_5CH(C<u>H</u>$_3$), 7.0-7.4 (m, 6H, C_6<u>H</u>$_5$ and C_6H_5C<u>H</u>(CH_3)
4l	CH_2COOH	3.3-4.5 (m, non equivalent 2H) 4.5-4.8 (m, non equivalent 2H)	3.25 and 3.4 (2H, two s, C<u>H</u>$_2$ COOH), 12 (broad hump, 1H,COO<u>H</u>)
4m	$CH_2COOC_2H_5$	4.0-4.9 (m, 6H, two non equivalent 2H at C4 and C6 and N^5-CH_2COOC<u>H</u>$_2CH_3$)	1.2 (t, 3H, CH_2COOC<u>H</u>$_2$C<u>H</u>$_3$), 3.3 and 3.4 (2H, two s, C<u>H</u>$_2$COOC_2H_5).

Table 3: Antimicrobial activity of the tested compounds (expressed as the diameter of the inhibition zone[a] in mm).

Co No.	*Aspergillus fumigatus*	*Penicillium oxalicum*	*Trichophyton rubrum*	*Microsporum canis*	*Chrysosporium tropicum*	*Candida albicans*	*Micrococcus roseus*	*Micrococcus luteus*	*Escherichia coli*	Serratia *rhodeni*
4a	8	-	-	-	-	13	-	11	8	-
4b	-	-	-	-	-	9	14	-	-	-
4c	-	-	-	-	-	-	-	-	-	-
4d	9	-	-	-	-	12	22	13	-	-
4e	-	-	-	-	-	-	-	-	-	-
4f	-	-	-	-	-	-	-	-	-	-
4g	-	-	-	-	-	-	-	12	-	-
4h	-	-	-	-	-	-	-	-	-	-
4i	-	-	-	-	-	-	15	-	-	-
4j	-	-	-	-	-	-	-	-	-	-
4k	-	-	-	-	-	-	-	-	-	-
4l	12	9	-	8	12	27	22	23	11	14
4m	8	7	-	-	10	17	11	16	-	7
M	14	11	7	10	12	10	-	-	-	7
Chlo	-	-	-	-	-	-	11	32	-	50

[a)]Average of three determinations.

Conclusion

In this work a series of 3-(1-phenylethyl)-5-substituted-2H-tetrahydro-1,3,5-thiadiazine-2-thione derivatives was synthesized and tested for antimicrobial activity. The study showed that polar group at N^5 position is most favored for activity. For N^{5-}aliphatic groups, the smaller alkyl chain, the better activity. Alkyl group of length lager than two carbons showed no activity. Also aralkyl group showed no acitivity. Preparation of other derivatives with various types of side chain and testing of their antimicrobial activity is currently being carried out in our laboratory.

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