Bull. Pharm. Sci., Assiut University, Vol. 28, Part 1, June 2005, pp. 9-15.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 3,5-DISUBSTITUTED-TETRAHYDRO-2H-1,3,5-THIADIAZINE-2-THIONE DERIVATIVES

Samia G. Abdel Moty

Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut - 71527, Egypt

أمكن في هذا البحث تخليق إثنى عشـــر مركب جديد من ٣ (ايزوبيوتيل) ٥ مستبدل تتراهيدرو ٢H ١،٣،٥ ثياديازين ٢ ثيون وذلك بعمل تفاعل بين ايزوبيوتيل أمين مع ثانى كبريتيد الكربون وهيدروكسيد البوتاسيوم. وتبع ذلك إضافة فورمالدهيد والأمين المناسب أو الحمض الأمينى أو حمض ايزونيكوتينك هيدرازيد وذلك إلى خليط التفاعل.

المركبات الناتجة تم التعرف عليها من خلال نتائج التحليل الطيفى وتحليل العناصر. المركبات تم اختبار فاعليتها ضد الميكروبات البكتيرية والفطرية. أعلى فاعلية تم الحصول عليها مع المركبات التى تحتوى على مجموعة قابلة للتأين في المكان N5 مثل مركب 4a , 4k وهذه الفاعلية تتأثر بحجم المجموعة الجانبية وعلى قابلية التأين .

*Twelve new 3-(isobutyl)-5-substituted-tetrahydro-2H-1,3,5-thiadiaz- ine-2-thiones were synthesized by the reaction of isobutylamine with carbon disulfide and potassium hydroxide, followed by formaldehyde and appropriate alkyl, cycloalkyl aralkyl amines, amino acid, and INH. Their structures have been elucidated by spectral data and elemental analysis. The title compounds were tested for antimicrobial activity in vitro against gram-positive bacteria (Staphylococcus aureus,and Micrococcus leuteus), gram-negative bacteria (Serratia marcescens and Escherichia coli) and some fungi (Candida albicans, Scopulariopsis brevicalus, Geotrichum candidum, Macrophomina phaseolina, Fusarium oxysporum and Trichoderma harzianum) using agar cup diffusion method. The antimicrobial activity was found to be greatly affected by the bulkiness of the side chain and the presence of polar carboxylic group. Highest activity was obtained with compounds **4a** and **4k** (R= CH_3, CH_2-COOH).*

INTRODUCTION

Tetrahydro-2H-1,3,5-thiadiazine-2-thione and related compounds displayed numerous pharmacological activities. Many reports were published on their antibacterial,[1-5] antifungal,[6-11] antiviral,[1,2] antihelmintic[3] and antituberculous activity[12]; as prodrugs.

The antimicrobial activity of these compounds was suggested to be attributed to the intact tetrahydro-1,3,5-thiadiazine-2-thione ring or to some biological modification of this ring. In addition to, isothiocyanates and/or dithiocarbamic acids, which were in vivo formed after hydrolysis of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring.[13-15]

Reiche *et al.*[1] pointed out the significance of the nature of the substituent at the 3rd position on antimicrobial activity, and at the 5th position on the toxicity of these compounds. Optimum activities were obtained for compounds bearing lipophilic groups at the 3rd and hydrophilic moieties at the 5th position.[16]

In the present work, isobutyl group (lipophilic) was incorporated into tetrahydrothiadiazine-2-thione (THTT) structure at N^3 and different polar and nonpolar groups at N^5 (**4a-l**) in order to study the effects on the microbiological activity.

Received in 5/5/2004 & Accepted in 30/1/2005

EXPERIMENTAL

Materials and equipments

Melting points were determined on an electrothermal melting point apparatus [Sturat Scientific, UK], and are all uncorrected. Precoated silica gel plates (Kiesel gel 0.25 mm, 60G F254, Merck) were used for thin layer chromatography. Developing solvent system of chloroform/methanol (9:1) was used and the spots were monitored by ultraviolet light.

IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan. ^{1}HNMR spectra were scanned on a Varian EM-360L NMR spectrometer (60 MHz) USA at Faculty Of Pharmacy Assiut University. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ as solvent. Elemental analyses were performed at the Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt.

The antimicrobial activity was performed at the Botany Department, Faculty of Science, Department of Plant Pathology, Faculty of Agriculture, and at the Department of Microbiology, Faculty of Medicine, Assiut University.

General procedure for synthesis of 3-isobutyl-5-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thiones (4a-l)

Carbon disulfide (60 mmol, 3.6 ml) was portionwise added to a well stirred mixture of isobutylamine (10 mmol, 0.99 ml) and potassium hydroxide (40%, 20 mmol, 1.4 ml), stirring was continued at ambient temperature for 3 h. Formaldehyde solution (35%, 22 mmol, 1.63 ml) was added to the mixture and the stirring was continued for further 3 h and the excess carbon disulfide was separated. The resulting clear solution was portionwise added, during 15 min, to a stirred solution of the appropriate alkylamine, cycloalkylamine, aralkylamine, glycine or isonicotinic acid hydrazide (10 mmol) in phosphate buffer (pH 7.8). After stirring for 6 hr at room temperature, the reaction mixture was acidified with dilute HCl (4N) to pH 4 and stirring was continued for further 30 min. The product was collected by filtration, washed with aqueous methanol, dried and crystallized from chloroform / methanol mixture. Yields, melting points, physical and spectral data are given in Tables I and II.

Antimicrobial activity (organisms and culture conditions)

The standard agar cup diffusion method[17] was used to evaluate the antimicrobial activity. Four bacterial species represent both Gram +ve and Gram -ve strains were used to test the antibacterial activities of the target compounds. *Micrococcus leuteus* and *Staphylococcus aureus* as representatives of Gram +ve strains where *Serratia marcesceus* and *Escherichia coli* as a representative of Gram -ve strains. Cultures were grown on nutrient agar.

Six fungal species were used in the present study: *Candida albicans, Scopulariopsis brevicauls, Geotrichum candidum, Macrophomina phaseolina* (Tassi), *Fusarium oxysporum* f.sp. lycopersici and *Trichoderma harzianum* (Rifai). Spore suspension in sterile distilled water was prepared from 3-5 days old culture of the test fungi growing on 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 1 ml of spore suspension. Plates were shaken gently to homogenize the inoculum. Sterile 6 mm filter paper disks (Whatman) were impregnated with solutions of the tested compounds (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 into the center of each plate. The seeded plates were incubated at 30±2° for 24 h for bacteria and for 7 days for fungi. The radius of the inhibition zones (in mm) was measured at successive intervals during the incubation period. Triplicate sets were applied for each treatment. Results are given in Table III.

Table I: Physicochemical data of 3-isobutyl-5-substituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives **4a-l**.

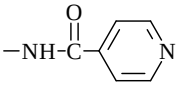
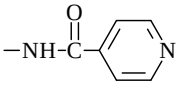
Compd. No.	R	Yield %	Formula M.wt.	M.p,°	Elemental analysis (Calc./found)			
					C	N	H	S
4a	$-CH_3$	78	$C_8H_{16}N_2S_2$ 204.08	73	47.02 46.43	13.71 13.70	7.89 7.79	- -
4b	$-C_2H_5$	45	$C_9H_{18}N_2S_2$ 218.09	46	49.50 48.81	12.83 12.87	8.31 8.62	29.37 29.80
4c	$-i-C_3H_7$	88	$C_{10}H_{20}N_2S_2$ 232.11	109	51.68 51.45	12.05 12.10	8.67 8.47	- -
4d	$-i-C_4H_9$	67	$C_{11}H_{22}N_2S_2$ 246.12	56	53.61 53.58	11.37 11.58	9.00 8.80	26.02 25.57
4e	$-t-C_4H_9$	55	$C_{11}H_{22}N_2S_2$ 246.12	82	53.61 53.33	11.37 11.40	9.00 9.03	- -
4f	$n-C_5H_{11}$	50	$C_{12}H_{24}N_2S_2$ 260.14	51	55.34 55.56	10.76 11.06	9.29 9.23	- -
4g	$c-C_6H_{11}$	46	$C_{13}H_{24}N_2S_2.3H_2O$ 320.12	69	- -	8.74 8.75	- -	19.97 19.86
4h	$CH2-C_6H_5$	80	$C_{14}H_{20}N_2S_2$ 280.11	88	59.96 59.63	9.99 9.68	7.19 7.12	- -
4i	$-(CH_2)_2-C_6H_5$	85	$C_{15}H_{22}N_2S_2$ 294.12	67	61.18 60.85	9.51 9.55	7.53 8.17	21.78 21.44
4j	$-CH(CH_3)-C_6H_5$	77	$C_{15}H_{22}N_2S_2$ 294.12	83	- -	9.51 9.50	- -	21.78 21.91
4k	$-CH_2-COOH$	88	$C_9H_{16}N_2O_2S_2$ 248.37	136	- -	11.28 11.13	- -	25.82 25.67
4l		50	$C_{13}H_{18}N_4OS_2.3H_2O$ 364.12	181	42.84 42.89	15.38 15.29	6.84 6.52	17.56 17.65

Table II: The ^{1}H-NMR chemical shifts of the synthesized compounds **4a-l** in $CDCl_3$.

No.	R	4 & 6CH_2	$(CH_3)_2CH$-CH_2-N^3	-N^5-R
4a	-CH_3	4.57, s, 4H	1.03 (d, 6H, 2CH_3) 2.00-2.63 (m, 1H, CH) 4.02 (d, 2H, CH_2)	2.80 (s, 3H, CH_3)
4b	-C_2H_5	4.53, s, 4H	1.02 (d, 6H, 2CH_3) 1.94-2.60 (m, 1H, CH) 4.00 (d, 2H, CH_2)	1.24 (t, 3H, CH_3) 3.02 (q, 2H, CH_2)
4c	-i-C_3H_7	4.45, s, 4H	0.96 (d, 6H, 2CH_3) 2.00-2.64 (m, 1H, CH) 3.90 (d, 2H, CH_2)	1.20 (d, 6H, 2CH_3) 2.94-3.60 (m, 1H, CH)
4d	-i-C_4H_9	4.56, s, 4H	1.00 (d, 6H, 2CH_3) 1.53-2.53 (m, 2H, CH at N^3 + CH at N^5) 4.03 (d, 2H, CH_2)	1.98 (d, 6H, 2CH_3) 2.73 (d, 2H, CH_2)
4e	-t-C_4H_9	4.73, s, 4H	1.00 (d, 6H, 2CH_3) 1.93-2.82 (m, 1H, CH) 4.07 (d, 2H, CH_2)	1.36 (s, 9H, 3CH_3)
4f	n-C_5H_{11}	4.54, s, 4H	0.97 (br.d, 9H, 2CH_3 at N^3 + CH_3 at N^5) 2.00-2.63 (m, 1H, CH) 4.03 (d, 2H, CH_2)	1.20-1.95 (br.m, 6H, 3CH_2) 2.95 (t, 2H, CH_2-N^5)
4g	c-C_6H_{11}	4.64, s, 4H	1.03 (d, 6H, 2CH_3) 1.23-2.63 (br.m, 11H, CH at N^3 + 5CH_2 of cyclohexyl) 4.05 (d, 2H, CH_2)	2.73-3.28 (m, 1H, CH of cyclohexyl)
4h	CH2-C_6H_5	4.48, s, 4H	1.03 (d, 6H, 2CH_3) 2.00-2.54 (m, 1H, CH) 3.91 (d, 2H, CH_2)	4.08 (s, 2H, CH_2-ph) 7.51 (s, 5H, C_6H_5)
4i	-$(CH_2)_2$-C_6H_5	4.47, s, 4H	1.05 (d, 6H, 2CH_3) 1.90-2.56 (m, 1H, CH) 3.94 (d, 2H, CH_2)	3.03 (t, 2H, CH_2-ph) 3.13 (t, 2H, CH_2-N^5) 7.51 (s, 5H, C_6H_5)
4j	-$CH(CH_3)$-C_6H_5	4.60, s, 4H	0.96 (d, 6H, 2CH_3) 1.85-2.48 (m, 1H, CH) 3.50-4.45 (m, 3H, 2H of CH_2)	1.56 (d, 3H, CH_3) 7.61 (s, 5H, C6H_5)
4k	-CH_2-COOH	4.48, s, 4H	1.00 (d, 6H, 2CH_3) 1.96-2.62 (m, 1H, CH) 3.95 (d, 2H, CH_2)	3.84 (s, 2H, CH_2) 9.45 (br.s, 1H, COOH)*
4l**		4.80, s, 4H	0.90 (d, 6H, 2CH_3) 1.93-2.50 (m, 1H, CH) 3.93 (d, 2H, CH_2)	7.90 (d, 2H, H at 3', C5' of pyridyl ring) 8.98 (d, 2H, H at C2', C6' of pyridyl ring) 10.96 (s, 1H, NH)*

* Exchangeable proton by D_2O.

** Using DMSO-d_6.

Table III: Antimicrobial activity[(a)].

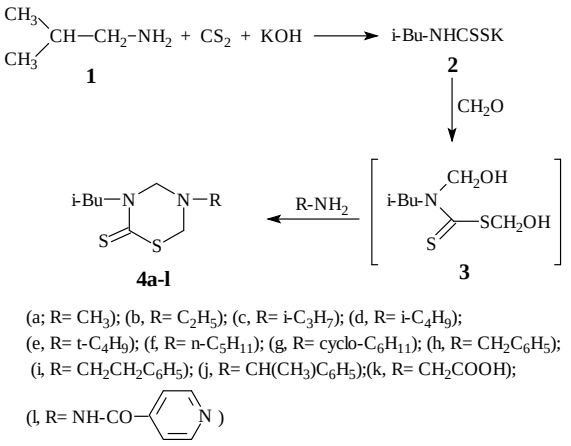
Organism / Compd. No.	Bacteria				Fungi					
	Micrococus luteus	*Staph. aureus*	*Serratia marcescens*	*E. coli*	*Candida albicans*	*Scopulariopsis brevicauls*	*Geotrichum candidum*	*Macrophomina phaseolina*	*Fusarium oxysporum*	*Trichoderma harzianum*
4a	0	0	7 mm	10 mm	0	0	0	13 mm	12 mm	9.5 mm
4b	0	0	8 mm	13 mm	6 mm	0	0	-	-	-
4c	0	0	8 mm	16 mm	0	0	7 mm	8.5 mm	8 mm	10.13 mm
4d	0	7 mm	9 mm	5 mm	0	0	6 mm	5.9 mm	6.3 mm	10.1 mm
4e	7 mm	0	7 mm	15 mm	0	7 mm	6 mm	0	0	0
4f	8 mm	13 mm	8 mm	3 mm	0	0	0	0	1.1 mm	10.5 mm
4g	8 mm	0	7 mm	17 mm	0	0	6 mm	6.3 mm	6.6 mm	10.8 mm
4h	0	0	0	3 mm	0	0	0	0	0	3.0 mm
4i	6 mm	0	7 mm	0	0	8 mm	0	0	0	9.3 mm
4j	0	7 mm	8 mm	3 mm	0	0	0	0	0	7.8 mm
4k	8 mm	0	13 mm	13 mm	0	0	0	2.5 mm	3.0 mm	10.6 mm
4l	8 mm	0	9 mm	3 mm	0	0	0	0	0	6.5 mm
Fluconazole	-	-	-	-	-	-	-	7 mm	0	0
Cansten 1%	-	-	-	-	19 mm	20 mm	18 mm	-	-	-
Streptomycin	60 mm	50 mm	45 mm	-	-	-	-	-	-	-

a) Average of three observations. Inhibition zone in mm. Disc diameter is 6 mm.
"0" no inhibition zone "-" not tested

RESULTS AND DISCUSSION

Chemistry

Isobutylamine was allowed to react with carbon disulfide in the presence of KOH to yield the corresponding potassium dithiocarbamate salt **2**. Addition of formaldehyde to the dithiocarbamate afforded compound **3** (in situ), which was allowed to react with the appropriate alkylamine, cycloalkylamine, aralkyl amine, glycine or isoniazid (INH) in the presence of phosphate buffer (pH= 7.8) to provide the desired derivatives (**4a-l**) (Scheme 1). Structure of these compounds was verified on the bases of spectral and elemental methods of analyses. Table I shows the physicochemical constants of the newly synthesized derivatives **4a-l**. All spectral data are in accordance with the expected structures. The IR spectra of compounds **4a-l** showed bands around 2855-2950 cm^{-1} (C-H stretching aliphatic); 3030-3050 cm^{-1} (C-H stretching aromatic) and at about 1400-1450 cm^{-1} (C=S stretching).



Scheme 1

In addition, compound **4k** showed the characteristic stretching absorption of the carboxylic acid at the range 3500-3200 cm^{-1} (OH) and at 1715 cm^{-1} (for the carboxylic C=O). Also, compound **4l** showed the amidic NH at 3340 cm^{-1}, the amidic C=O stretching at 1660, pyridine C=N stretching at 1485-1496 cm^{-1} and amide band at around 1550-1520 cm^{-1}.

In the ^{1}H-NMR spectra, a common pattern for the methylenes at C4 and C6 and for the isobutyl group of all derivatives was found. The differences in sets and patterns were only attributed to the N5 substituent where they give patterns in accordance with the structure of the concerned compounds (Table II).

Antimicrobial activity

The synthesized compounds **4a-l** were evaluated in vitro for their antibacterial and antifungal activities using the standard agar cup diffusion method[17] as mentioned in the experimental part. The study was carried out at concentration (100 µM /ml in DMSO) for each compound. Table III reveals the antimicrobial activity of the tested compounds expressed as the inhibition zone in mm.

As for the antibacterial screening, the synthesized compounds were tested against gram-positive (*Staphylococcus aureus* and *Micrococcus leuteus*) and gram-negative bacteria (*Serratia marcescens and Escherichia coli*). Streptomycin was used as reference drug. Results are given in Table III.

The antifungal activity was investigated against *Candida albicans, Scopulariopsis brevicauls, Geotrichum candidum, Macrophomina phaseolina, Fusarium oxysporum* and *Trichoderma harzianum*. Fluconazole and Cansten were used as a reference antifungal drugs. Results are shown in Table III.

Antimicrobial study revealed that, variable activities were obtained for the tested compounds according to variation at the N^5-substituent of the THTT moiety and the tested bacteria or fungi. Thus, introduction of groups (such as CH_2-COOH; CH_3; i-pr, i-Bu or c-hexyl) at N^5 of the THTT system afforded good to moderately active compounds for both antibacterial and antifungal potencies. Meanwhile, increasing the length or the bulkiness of the alkyl side chain (N^5-R) resulted in variable effects depending upon the tested bacteria and/or fungus. Aralkyl derivatives were found to be weakly active for most organisms.

Conclusion

In this work a series of 5-substituted-3-isobutyl-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives were synthesized and tested for antimicrobial activity. In spite of, the variations of the antimicrobial activity with the

different N-[5] substituent groups, some of the compounds, that exhibited a moderate antimicrobial activities, can be considered for further studies. Preparation of other derivatives with various types of side chain and testing of their antimicrobial activity is currently being carried out in our laboratory.

Acknowledgement

The author is indebted to Prof. Dr. M.Hassan at the department of Plant Pathology, Faculty of Agriculture, Assiut University for the facility provided to the antifungal screening of the prepared compounds.

REFERENCES

1- A. Reiche, G. Hilgetag, A. Martini, O. Nejedly and J. Schegel, Arch. Pharm., 293, 957 (1960).
2- A. Reiche, G. Hilgetag, A. Martini and R. Philippson, Arch. Pharm., 294, 201-209 (1961).
3- M. Schorr, W. Durckheimer, P. Klatt, G. Lammler, G. Nesemann and E. Schrinner, Arzneim-Forsch./ Drug Res., 19, 1807-1819 (1969).
4- T. Zsolnai, Arzneim-Forsch./ Drug Res., 21, 121-127 (1971).
5- M. Ertan, A.B. Tayhan and N. Yulug, Arch. Pharm. (Weinheim), 323, 605-609 (1990).
6- M. Ertan, S. Sarac and N. Yulug, Arzneim-Forsch./ Drug Res., 40, 790-795 (1990).
7- S. Sarac, M. Ertan, A. Balkan and N. Yulug, Arch. Pharm., (Weinheim), 324, 449-453 (1991).
8- M. Ertan, H.G. Ayyildiz and N. Yulug, Arzneim-Forsch./ Drug Res., 41, 1182-1185 (1991).
9- M. Ertan, A.A. Bilgin, E. Palaska and N. Yulug, Arzneim-Forsch./ Drug Res., 42, 160-163 (1992).
10- E. Ilhan, G. Capan, N. Ergene, M. Uzun, M. Kiraz and D. Kaya, Farmaco, 50, 787-790 (1995).
11- A. El-Shorbagi, Arch. Pharm., 333, 281-286 (2000).
12- T. Aboul-Fadl and K. Hassanin, Pharmazie, 54, 244-247 (1991).
13- C. Ochoa, E. Perez, R. Perez, M. Suarez, E. Ocho, H. Rodriguez, A.G. Barrio, S. Muelas, J.J. Nogal and R.A. Martinez, Arzneim-Forsch./ Drug Res., 49, 764-769 (1999).
14- J. Goksoyr, Acta Chem. Scand., 18, 1341 (1964).
15- W. Schade and A. Rieche, Arch. Pharm. (Weinheim), 299, 589 (1966).
16- W. Weuffen, D. Martin and W. Schade, Pharmazie, 18, 420 (1963).
17- S.T. Pai and M.W. Platt, "Letters in Applied Microbiology", 1995, 20, 14-18.