SYNTHESIS AND ANTIFUNGAL ACTIVITY OF SOME NEW 1,2,4-TRIAZOLO[3,4-b] [1,3,4]THIADIAZINES

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ABSTRACT

A new series of 5-aryl-3-(2-furyl)-(7H)-s-triazolo [3,4-b] [1,3,4] thiadiazines were prepared by the reaction of 4-amino-5-(2-furyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione with α-halocarbonyl compounds in refluxing ethanolic potassium hydroxide. The compounds were tested against a variery of fungal strains in comparison to clotrimazole. Some compounds exhibited moderate activity against *Candida albicans* and some of the fungi.

Keywords: 1,2,4- Triazolo[3,4-b][1,3,4] thiadiazine, Heterocycles, Fungicides.

INTRODUCTION

The triazole nucleus has a broad spectrum of antimicrobial activity (1). The (3,6)-disubstituted-(7H)-s-triazolo[3,4-b] [1,3,4] thiadiazine derivatives have been reported as antiparasite (2).

The antimicrobial activity of 1,2,4- triazolo[3,4-b] [1,3,4] thiadiazoles derivatives are also reported (3). Moreover, some (7H)-s-triazolo[3,4-b] [1,3,4] thiadiazines and s-trtiazolo[3,4-b] [1,3,4]thiadia-zoles have shown antibacterial and antifungal activity (4).

In view of the biological importance of these heterocycles a novel series of 5-aryl-3-(2-furyl)-(7H)-s-triazolo[3,4-b]-[1,3,4]-thia-diazine were synthesized and evaluated for in-vitro antifungal activity.

MATERIALS AND METHODS

Melting points were taken on a MEL-TEMP II apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker AC-80 or Varian 400 Unity plus spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The mass spectra were run on a Finigan TSQ- 70 spectrometer at 70 *eV*. MIC were determined by the agar dilution method on solid cultivation media (3).

5-(2-furyl)-1,3,4-oxadiazole-2-(3H)thione

To a mixture of furan-2-carboxylic acid hydrazide **1** (6.3 g, 0.05 mole) and KOH 85% (3.22g, 0.05 mole) in ethanol (10 ml), was added an excess of carbon disulfide (7.6g, 0.1 mole) and the mixture was refluxed for 7 hrs. The solvent was removed under reduced pressure and water was added to the residue and the mixture was filtered. The solution was poured into ice and dilute HCl and the product was collected by filtration, washed with water and crystallized from ethanol to give 6.3g (75%) of **3**. m.p. 173-174 °C (7).

4-Amino-5 -(2-furyl)-2,4-dihydro-1,2,4-triazole-3-thione (4)

To a mixture of 5-(2-furyl)-1,3,4-oxadiazole-2(3H)-thione **3** (22g, 0.13 mole) in ethanol (100 ml), 12 ml of 24% hydrazine hydrate was added dropwise and the mixture was refluxed for 8 hrs. After cooling water was added and the mixture was acidified by excess of 3N HCl, the separated solid was filtered off, washed with water and crystallized from ethanol to give 18.35g (77%) of **4**, m.p. 202-203 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 12.05 (s,1H, NH), 7.19-6.55 (m, 3H, furan), 5.62(broad s, 2H, NH_2).

5-Phenyl-3-(2-furyl)-(7H)-s-triazolo[3,4-b] [1,3,4]thiadiazine (5a)

A mixture of 4-amino-5-(2-furyl)-2,4-dihydro-1,2,4-triazole-3-thione **4** (2g, 11 mmoles) and phenacyl bromide (2.3g, 11.5 mmoles) in ethanol (50 ml) was refluxed for 4-5 hrs. The solvent was removed under reduced pressure and the residue was treated with HCl (2N, 100 ml). The precipitate was filtered and the filtrate was treated with NaOH (2N,100 ml). The product was filtered and crystallized from ethanol-water to

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give 1.34g (43%) **5a**, m.p. 142-143 °C; MS(m/z %): 283 (M^+,100), 172(6), 121(4), 117(14), 103 (22), 77(28), 58(22). ^{1}H-NMR($CDCl_3$) δ (400 MHz) ppm: 7.97-7.55 (m, 6H, phenyl and H_5-furyl), 7.23-7.20 (m, 1H, H_3-furyl), 6.58-6.56 (m, 1H, H_4-furyl) and 4.02 (s, 2H, CH_2). ^{13}C-NMR ($CDCl_3$) δ ppm: 22.3(C_6-thiadiazine), 116(C_4-furyl), 113.1 (C_3-furyl), 127 (C_3 and C_5-phenyl), 129.2(C_2 and C_6-phenyl), 132.1 (C_4-phenyl), 133.5 (C_1-phenyl), 140.8 (C_5-thiadiazine), 141.0 (C_{7a}-thiadiazine), 144.5 (C_5-furyl), 145.9 (C_3-triazole), 153.9 (C_2-furyl).

*5-(4-Flourophenyl)-3- (2-furyl)-(7H)-s- triazolo [3,4-b] [1,3,4] thiadiazine (**5b**)*

This compound was prepared according to the method described for **5a** in 49% yield, m.p. 164-165°C. MS(m/z %): 300(M^+,100),179(8),135(36), 121(64), 95(85), 58(70).

^{1}H-NMR ($CDCl_3$) δ (400 MHz) ppm: 8.07-8.03 (m, 2H, phenyl), 7.69-7.65 (m, 1H, H_5-furyl), 7.29-7.25 (m, 2H, phenyl), 7.21-7.19 (m, 1H, H_3-furyl), 6.65-6.63 (m, 1H, H_4-furyl) and 3.98 (s, 2H, CH_2). ^{13}C-NMR ($CDCl_3$) δ ppm: 22.4 (C_6-thiadizine), 110.8 (C_4-furyl), 112.1 (C_3-furyl), 115.4 (d, C_3 and C_5- phenyl, J_{CF}: 22Hz), 128.9 (d, C_1-phenyl, J_{CF}= 4Hz), 129.0 (d, C_2 and C_6-phenyl J_{CF}= 10.7 Hz), 139.8 (C_5-thiadiazine), 140.5 (C_{7a}-thiadiazine), 143.48 (C_5-furyl), 144.6 (C_3-triazole), 152.8 (C_2-furyl), 163.88 (d, C_4-phenyl, J_{CF}= 251 Hz).

*5-(2,4-Diflourophenyl)-3-(2-furyl)-(7H)-s-triazolo[3,4-b] [1,3,4]thiadiazine (**5c**)*

This compound was prepared according to the method described for **5a** in 66% yield; m.p. 205-207 °C. ^{1}H-NMR ($CDCl_3$) δ (400 MHz) ppm:7.91-7.82 (m, 1H, phenyl), 7.65-7.63 (m, 1H, H_5-furyl), 7.13 (m, 1H, H_3-furyl), 7.11-6.95 (m, 2H, phenyl), 6.58-6.55 (m, 1H, H_4-furyl) and 3.99 (s, 2H, CH_2).^{13}C-NMR ($CDCl_3$) δ ppm: 22.5 (C_6-thiadiazine), 105.0 (dd, C_3-phenyl, J_{CF}= 25.4 Hz), 114 (C_4-furyl), 112.7 (dd, J_{CF}= 22 Hz and J_{CF}= 3.5 Hz), 112.9 (C_3-furyl), 118.9 (dd, C_1-phenyl, J_{CF}= 22 Hz and J_{CF}= 4 Hz), 131.6 (dd, C_6-phenyl, J_{CF}= 12 Hz and J_{CF}= 10 Hz), 140.6 (C_5-thiadiazine), 141.2 (C_{7a}-thiadiazine), 144.7 (C_5-furyl), 145.8 (C_3-triazole), 151.3 (C_2-furyl), 161.6 (dd, C_2-phenyl, J_{CF}=253 Hz and J_{CF}=12.5 Hz), 165.2 (dd, C_4-phenyl, J_{CF}= 255 Hz and J_{CF}= 13 Hz).

*5-(4-Bromophenyl)-3-(2-furyl)-(7H)-s-triazolo[3,4-b] [1,3,4]thiadiazine (**5d**)*

It was prepared according to the method described for **5a** in 55% yield; m.p. 218-220 °C, MS (m/z %): 362(M^+,21), 197(12), 157(12), 109(72), 93(100), 58(41). ^{1}H-NMR ($CDCl_3$) δ (400 MHz) ppm: 7.81 (d, 2H, H_2 and H_6-phenyl, J= 8 Hz), 7.69 (d, 2H, H_3 and H_5-phenyl J=8 Hz), 7.65-7.63 (m, 1H, H_5-furyl), 7.17-7.15 (m, 1H, H_3-furyl), 6.61-6.58 (m, 1H, H_4-furyl) and 4.01 (s, 2H, CH_2). ^{13}C-NMR ($CDCl_3$) δ ppm: 22.3 (C_6-thiadiazine), 111.6 (C_4-furyl), 113.1 (C_3-furyl), 127.2 (C_4-phenyl), 128.6 (C3 and C_5-phenyl), 132.3 (C_1-phenyl), 132.5 (C_2 and C_6-phenyl), 140.8 (C_{7a} and C_5-thiadiazine), 144.6 (C_5-furyl), 145.8 (C_3-triazole), 152.9 (C_2-furyl).

*5-(2,4-Dichlorophenyl)-3-(2-furyl)-(7H)-s-triazolo[3,4-b] [1,3,4]thiadiazine (**5e**)*

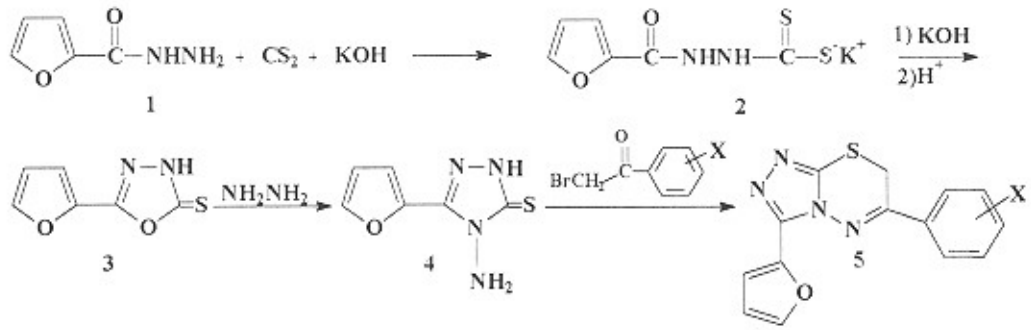
This compound was prepared according to the method described for **5a** in 46% yield, m.p. 242-244°C.MS (m/z%):351(M^+,17), 269(100), 223(21), 195(72), 182(14), 171(7), 109(70) 94(50), 58(21). ^{1}H-NMR ($CDCl_3$) δ (400 MHz) ppm:7.68-7.60 (m, 1H, H_5-furyl), 7.59 (d, H_6-phenyl, J=8 Hz), 7.55 (d, H_3-phenyl, J= 4 Hz), 7.43 (m, H_5-phenyl), 7.11-7.05 (m, 1H, H_3-furyl), 6.54-6.52 (m, 1H, H_4-furyl) and 4.00 (s, 2H, CH_2). ^{13}C-NMR ($CDCl_3$) δ ppm: 26.2 (C_6-thiadiazine), 111.6 (C_4-furyl), 113.3 (C_3-furyl), 128 (C_5-phenyl), 136.4 (C_3-phenyl), 131.9 (C_6-phenyl), 132.8 (C_2-phenyl), 133.3 (C_4-phenyl), 138.6 (C_2-phenyl), 140.5 (C5-thiadiazine), 141.4 (C_{7a}-thiadiazine), 144.7 (C_5-furyl), 145.8 (C_3-triazole), 154.6 (C_2-furyl).

*5-(4-Methoxyphenyl)-3-(2-furyl)-(7H)-s-triazolo[3,4-b][1,3,4]thiadiazine (**5f**)*

This compound was prepared according to the method described for **5a** in 64% yield, m.p. 161-163°C. ^{1}H-NMR ($CDCl_3$) δ (80 MHz) ppm: 7.91(d, 2H, phenyl), 7.73 (d, 2H, phenyl),7.26-6.57(m, 3H, furan), 3.98(s, 3H, OCH_3) and 3.90(s, 2H, CH_2).

RESULTS AND DISCUSSION

Furan 2-carboxylic acid hydrazide **1** was condensed with carbon disulfide, in ethanolic potassium hydroxide to yield the potassium 3-(2-furyl)-dithiocarbazate **2** which was cyclized to give 5-(2-furyl)-1,3,4-oxadiazole-2(3H)-thione **3** (5).



5a: X=H 5d: X=4-Br
5b: X=4-F 5e: X=2.4-Cl_2
5c: X=2.4F_2 5f: X=4-OCH_3

Scheme I

Table 1. Characterization and antifungal activity of compounds **5a-f,** expressed as the minimum inhibitory concentration (MIC)

Compound	Antifungal Activity (MIC, mg/mL^{-1})			
	Candida albicans **PTCC*: 5027**	Aspergillus fumigatus **PTCC: 5008**	Aspergillus niger **PTCC: 5011**	Microsporum gypseum **PTCC: 5070**
5a	6.25	12.5	12.5	12.5
5b	12.5	12.5	6.25	50
5c	>100	100	50	50
5d	12.5	25	6.25	100
5e	>100	>100	100	25
5f	6.25	12.5	6.25	6.25
Clotrimazole	1.56	6.25	3.12	0.78

*Persian Type Culture Collection

Treatment of the latter with hydrazine hydrate in refluxing ethanol gave 4-amino-5-(2-furyl)-2,3-dihydro -1,2,4- triazine-3- thione **4** (5).

Cyclocondensation of **4** with α-halocarbonyl compounds in refluxing ethanol (6) gave the corresponding fused systems (7H)-s-triazolo [3,4-b] [1,3,4] thiadiazine **5a-f** (Scheme I) in moderate yields (Table 1). The in-vitro anti-fungal activity of the synthesized compounds against some fungi and candida albicans are reported in Table 1. Clotrimazole was used as reference drug. The results of antifungal activity test revealed that most of the prepared compounds exhibit moderate activity. The most active compound, expressed as minimum inhibitory concentration (MIC), was 6-(4-methoxyphenyl)-3- (2-furyl)-(7H)-s-triazolo[3,4-b][1,3,4]thiadiazine **5f**.

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REFERENCES

1. Eweiss, N.F., Bahajaj, A.A., Elsherbini, E.A. (1986) Synthesis of heterocycles (part VI [1]): Synthesis and Antimicrobial Activity of 4-Amino-5-aryl-1,2,4-trizole-3-thiones and their Derivatives. J. Heterocyclic. Chem. 23:1451-1458.

2. EL-Dawy, M.A., Omar, A.M.M.E., Ismail, A.M., Hazzaa, A.A.B. (1983) Potential broad spectrum anthelmintics IV: design, synthesis and antiparasitic screening of certain 3,6-Disubstituted-(7H)-s-trizolo-[3,4-b][1,3,4]thiadiazine derivatives. J. Pharm. Sci. 43(72):45-50.
3. Dwivedi, V., Agarwal, P.K. (1991) Antimicrobial activity of some new 1,2,4-triazolo [3,4-b] [1,3,4]-thiadiazoles. Indian. J. Pharm. Sci. 53(3): 82-84.
4. Eweiss, N.F., Bahajaj, A.A. (1987) Synthesis of heterocycles (part VII[1]); synthesis and antimicrobial activity of some 7H-s-triazolo[3,4-b] [1,3,4] thiadiazine and s-triazolo [3,4-b] [1,3,4] thiadiazole derivatives. J. Heterocyclic. Chem . 24:1173-1182.
5. Reid, J.R., Heindel, N.D. (1976) Improved syntheses of 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazoles. J. Heterocyclic. Chem. 13: 925-926.
6. Golgolab, H., Lalezari, I., Hosseini-Gohari, L. (1972) 1,3,4-thiadiazolo[2,3-c]-as-triazines and s-triazolo[3,4-b]-1,3,4-thiadiazoles (1). J. Heterocyclic. Chem. 10(3):387-390.
7. Mndzhoyan, A.L., Afrikyan, V.G. (1957) Benzylfuran and some of its derivatives. Doklady. Akad. Nauk. Armyan. S. S. R. 25(4):201-205 (Chem .Abstr. 52: 8116b).