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Convenient Synthesis of Some Triazolothiadiazoles and Triazolothiadiazines Carrying 4-Methylthiobenzyl Moiety as Possible Antimicrobial Agents

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Abstract: 4-Amino-3-(4-methylthiobenzyl)-5-mercapto-1,2,4-triazole 5 reacts with various substituted acids (aryl/aryloxy) in presence of phosphorus oxychloride and with various substituted phenacyl bromides in presence of anhydrous sodium acetate to give two series of fused heterocycles namely, 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles 6 and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines 7, respectively. The structures of the newly synthesized compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral studies. All the newly synthesized compounds were tested for their antibacterial and antifungal activity against a variety of microorganisms.

Key words: Triazole, thiadiazole, thiadiazine, antibacterial, antifungal

INTRODUCTION

A large number of N-bridged heterocycles derived from 1,2,4-triazoles are important pharmacological agents and a significant amount of research has been directed towards this class of compounds. 1,3,4-Thiadiazole nucleus is associated with a wide variety of biological activities namely; antitumor, anticonvulsant, antibacterial, antifungal, antiinflammatory, antihypertensive, anesthetic, cardiotonic, antimycobacterial, antitrypanosomal and leishmanicidal (Hill, 1980; Vio *et al*., 1989; Nomoto *et al*., 1991; Mazzone *et al*., 1993; Rollas *et al*., 1996; Souy *et al*., 1999; Dogan *et al*., 2002; Samir *et al*., 2004; Alireza *et al*., 2005a, b). Similarly the 1,3,4-thiadiazine nucleus is known to be pharmacologically (Trepanier *et al*., 1967) important and possess antiplatelet and antithrombotic properties (Rehse *et al*., 1998). Various substituted 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles and 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines were synthesized and reported for their antimicrobial, antiviral, antiinflammatory, analgesic and anticancer activities (Prasad *et al*., 1989; Holla *et al*., 2001; Swamy *et al*., 2006; Holla *et al*., 2006). As a continuation of present study to explore potent biologically active molecules, we have synthesized some new 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines and screened them for their antimicrobial activities.

MATERIALS AND METHODS

The melting points were determined by an open capillary method and are uncorrected. IR spectra in KBr (ν_{max} in cm^{-1}) were recorded on a Shimadzu-FTIR Infrared spectrophotometer. ^{1}H NMR spectra were recorded in DMSO/$CDCl_3$ on a Bruker AMX-400 (400 MHZ) spectrometer using TMS as an internal standard. FABMS spectra were recorded on a JEOL SX 102/DA-6000 Mass spectrometer using argon/xenon (6 kv, 10 mA) as the FAB gas. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates.

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Procedure for the Synthesis of (4-Methylthiophenyl) Acetic Acid Hydrazide (3)

A mixture of (4-methylthiophenyl) acetic acid ester 2 (0.5 mol) and hydrazine hydrate (95%, 1 mol) in ethanol was heated to reflux on a water bath for 8 h. The excess of ethanol was distilled out and the residue obtained was poured into ice cold water. The solid separated was filtered, washed with water, dried and recrystallized from ethanol. Yield 85%, m.p. 136-38°C.

IR (KBr, ν in cm^{-1}): 3344, 3203 ($NHNH_2$), 3022, 2918 (Ar-H), 1622 (C = O), ^{1}H-NMR ($CDCl_3$) δ: 2.49 (s, 3H, SCH_3), 3.53 (s, 2H, CH_2), 3.82 (bs, 2H, NH_2), 6.67 (bs, 1H, NH), 7.18 (d, 2H, J = 8.36 Hz, 4-methylthiophenyl), 7.26 (d, 2H, J = 8.36 Hz, 4-methylthiophenyl), FABMS (m/z, %): 197 (M^++1, 28), 196 (M^+,15), 154 (21), 149 (8), 138 (20), 137 (100), 107 (14), 105 (10), 91 (11), 81 (14), 77 (15), 69 (21), 55 (20).

Procedure for the Synthesis of Potassium Dithiocarbazinate (4)

To a continuously stirred mixture of potassium hydroxide (0.3 mol) and 3 (0.3 mol) in ethanol, carbon disulphide (0.3 mol) was added drop wise. The resulting mixture was agitated for 12-16 h. It was then diluted with dry ether and the precipitated solid was collected by filtration, washed with ether and dried. The potassium salt was obtained in quantitative yield and used for further reaction without purification.

Procedure for the Synthesis of 4-Amino-3-(4-methylthiobenzyl)-5-mercapto-1,2,4-triazole (5)
Method A

To a suspension of 4 (0.1 mol) in water was added hydrazine hydrate (0.1 mol). The reaction mixture was refluxed for 1 h. It was then diluted with cold water and acidified with concentrated hydrochloric acid. The solid thus obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield 90%, m.p.198-200°C.

Method B

An equimolar mixture of (4-methylthiophenyl) acetic acid 1 and thiocarbohydrazide were heated in an oil bath till the contents melted. The mixture was maintained at this temperature for 10-15 min. The product obtained on cooling was treated with dilute sodium bicarbonate solution in order to remove any unreacted acid left. It was then washed with water, filtered, dried and recrystallized from ethanol.

IR (KBr, ν in cm^{-1}): 3273, 3165 (NH_2), 3033, 2933 (Ar-H), 1625 (C = N), ^{1}H-NMR (DMSO-d_6) δ: 2.44 (s, 3H, SCH_3), 4.02 (s, 2H, CH_2), 5.58 (s, 2H, NH_2, exchangeable with D_2O), 7.21 (s, 4H, 4-methylthiophenyl), 13.51 (s, 1H, SH, exchangeable with D_2O), ^{13}C-NMR (DMSO-d_6) δ: 166, 151, 136, 132, 129, 126, 30, 15, FABMS (m/z, %): 253(M^++1, 10), 210 (8), 176 (14), 165 (12), 154 (100), 137 (73), 136 (95), 120 (18), 107 (34), 105 (21), 91 (34), 88 (51), 77 (44).

General Procedure for the Synthesis of 6-(Substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (6a-h)

A mixture of 5 (0.01 mol), substituted benzoic acids/aryloxy acetic acids (0.01 mol) and phosphorus oxychloride (10 mL) were heated on a water bath for 8 h. The resulting reaction mass was poured into crushed ice with stirring. The solid obtained was filtered, washed with dilute sodium bicarbonate solution, dried and recrystallized from ethanol. The characterization data of these compounds are given in Table 1.

6b. 6-(4-Methoxyphenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole

IR (KBr, cm^{-1}): 2920, 2831 (Aliph.CH), 1605 (C = N), 1115 (C-O), ^{1}H-NMR ($CDCl_3$) δ: 2.47 (s, 3H, SCH_3), 3.91 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2), 7.02 (d, 2H, J = 8.56 Hz, 4-methoxyphenyl),

Table 1: Characterization data of triazolothiadiazoles (6a-h)

Compound No.	R/R'	Molecular formula	MP (°C)	Yield (%)	Elemental analysis found (Calc.) C	H	N
6a	H	$C_{17}H_{14}N_4S_2$	166-68	76	60.37 (60.33)	4.14 (4.17)	16.58 (16.55)
6b	4-OCH_3	$C_{18}H_{16}N_4OS_2$	186-88	73	58.61 (58.67)	4.39 (4.38)	15.28 (15.20)
6c	4-Cl	$C_{17}H_{13}ClN_4S_2$	190-92	80	54.72 (54.76)	3.47 (3.51)	15.06 (15.02)
6d	2,4-Dichloro	$C_{17}H_{12}Cl_2N_4S_2$	150-52	82	50.12 (50.13)	2.98 (2.97)	13.78 (13.75)
6e	H	$C_{18}H_{16}N_4OS_2$	134-36	78	58.61 (58.67)	4.42 (4.38)	15.28 (15.20)
6f	4-CH_3	$C_{19}H_{18}N_4OS_2$	138-40	76	59.67 (59.66)	4.72 (4.74)	14.69 (14.65)
6g	4-Cl	$C_{18}H_{15}ClN_4OS_2$	154-56	79	53.68 (53.66)	3.71 (3.75)	13.96 (13.90)
6h	2,4-Dichloro	$C_{18}H_{14}Cl_2N_4OS_2$	168-70	80	49.48 (49.43)	3.22 (3.23)	12.87 (12.81)

Table 2: Characterization data of triazolothiadiazines (7a-h)

Compound No.	R	Molecular formula	MP (°C)	Yield (%)	Elemental analysis found (Calc.) C	H	N
7a	H	$C_{18}H_{16}N_4S_2$	138-40	76	61.37 (61.34)	4.54 (4.58)	15.88 (15.89)
7b	4-CH_3	$C_{19}H_{18}N_4S_2$	148-50	73	62.21 (62.27)	4.96 (4.95)	15.28 (15.29)
7c	4-OCH_3	$C_{19}H_{18}N_4OS_2$	122-24	80	59.72 (59.66)	4.72 (4.74)	14.66 (14.65)
7d	4-F	$C_{18}H_{15}FN_4S_2$	178-80	82	58.32 (58.36)	4.09 (4.08)	15.18 (15.12)
7e	4-Cl	$C_{18}H_{15}ClN_4S_2$	152-54	78	55.81 (55.88)	3.92 (3.91)	14.48 (14.48)
7f	4-Br	$C_{18}H_{15}BrN_4S_2$	160-62	76	50.17 (50.12)	3.52 (3.50)	12.96 (12.99)
7g	4-NO_2	$C_{18}H_{15}N_5O_2S_2$	156-58	79	54.38 (54.39)	3.71 (3.80)	17.66 (17.62)
7h	4-OH-3-$CONH_2$	$C_{19}H_{17}N_5O_3S_2$	222-24	80	55.48 (55.46)	4.22 (4.16)	17.07 (17.02)

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7.22 (d, 2H, J = 8.36 Hz, 4-methylthiophenyl), 7.38 (d, 2H, J = 8.36 Hz, 4-methylthiophenyl), 7.80 (d, 2H, J = 8.56 Hz, 4-methoxyphenyl), FABMS (m/z, %): 369(M^++1, 100), 368(M^+, 80), 355 (10), 307 (10), 289 (5), 154 (40), 136 (30), 107 (10).

6c. 6-(4-Chlorophenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole

^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 4.43 (s, 2H, CH_2), 7.21 (d, 2H, J = 8.32 Hz, 4-methylthiophenyl), 7.35 (d, 2H, J = 8.24 Hz, 4-methylthiophenyl), 7.51 (d, 2H, J = 8.60 Hz, 4-chlorophenyl), 7.78 (d, 2H, J = 8.60 Hz, 4-chlorophenyl), FABMS (m/z, %): 373 (M^+, 70), 371 (10), 308 (10), 307 (55), 289 (30), 165 (10), 154 (100), 136 (60), 120 (20), 107 (20), 88 (10).

6f. 6-(4-Methylphenoxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole

IR (KBr, cm^{-1}): 2919, 2860 (Aliph.CH), 1585 (C=N), 1104 (C-O), ^{1}H-NMR ($CDCl_3$) δ: 2.30 (s, 3H, CH_3), 2.45 (s, 3H, SCH_3), 4.38 (s, 2H, CH_2), 5.25 (s, 2H, OCH_2), 6.86 (d, 2H, J = 8.52 Hz, 4-methylphenoxy), 7.12 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.20 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.30 (d, 2H, J = 8.24 Hz, 4-methylphenoxy), FABMS (m/z, %): 383 (M^++1, 100), 382 (M^+, 50), 307 (5), 289 (5), 276 (20), 154 (50), 136 (40), 107 (10), 90 (10).

6h. 6-(2,4-Dichlorophenoxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole

^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 4.43 (s, 2H, CH_2), 5.33 (s, 2H, OCH_2), 6.92 (d, 1 H, J_o = 8.72 Hz, 2,4-dichlorophenoxy), 7.19 (d, 2 H, J = 8.20 Hz, 4-methylthiophenyl), 7.23 (dd, 1H, $J_{o/m}$ = 8.76 Hz, 2.44 Hz, 2,4-dichlorophenoxy), 7.30 (d, 2 H, J = 8.04 Hz, 4-methylthiophenyl), 7.43 (d, 1 H, J_m = 2.38 Hz, 2,4-dichlorophenoxy), FABMS (m/z, %): 439 (M^++2, 70), 437 (M^+, 100), 436 (30), 307 (10), 289 (10), 276 (30), 154 (30), 137 (20), 136 (20), 107 (10), 88 (5).

General Procedure for the Synthesis of 7*H*-6-(Substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (7a-h)

A mixture of 5 (0.01 mol), substituted phenacyl bromides (0.01 mol) and anhydrous sodium acetate (0.01 mol) in ethanol was heated to reflux for 4 h. The resulting reaction mass was cooled and the precipitated solid was filtered, dried and recrystallized from ethanol. The characterization data of these compounds are given in Table 2.

7b. 7*H*-6-(4-Methylphenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine

IR (KBr, cm^{-1}): 3020, 2988 (Ar-H), 2918 (Aliph.CH), 1595 (C = N), ^{1}H-NMR ($CDCl_3$) δ: 2.43 (s, 3H, CH_3), 2.46 (s, 3H, SCH_3), 3.90 (s, 2H, CH_2), 4.29 (s, 2H, SCH_2), 7.18 (d, 2H, J = 8.32 Hz, 4-methylthiophenyl), 7.28 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.33 (d, 2H, J = 8.56 Hz, 4-methylphenyl), 7.74 (d, 2H, J = 8.56 Hz, 4-methylphenyl), FABMS (m/z, %): 367 (M^++1, 100), 366 (M^+, 50), 307 (20), 289 (10), 250 (10), 220 (3), 205 (2), 167 (5), 154 (80), 149 (35), 136 (55), 120 (5), 107 (10), 105 (5).

7c. 7*H*-6-(4-Methoxyphenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine

IR (KBr, cm^{-1}): 3025, 2981 (Ar-H), 2917 (Aliph.CH), 1585 (C = N), 1112 (C-O), ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2), 4.28 (s, 2H, SCH_2), 7.01 (d, 2H, J = 8.56 Hz, 4-methoxyphenyl), 7.18 (d, 2H, J = 8.04 Hz, 4-methylthiophenyl), 7.27 (d, 2H, J = 8.40 Hz, 4-methylthiophenyl), 7.80 (d, 2H, J = 8.60 Hz, 4-methoxyphenyl).

7d. 7*H*-6-(4-fluoro phenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine

IR (KBr, cm^{-1}): 3035, 2989 (Ar-H), 2919 (Aliph.CH), 1592 (C = N), 1105 (C-F), ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 4.01 (s, 2H, CH_2), 4.34 (s, 2H, SCH_2), 7.18 (d, 2H, J = 8.20 Hz, 4-methylthiophenyl), 7.23 (d, 2H, J = 8.36 Hz, 4-methylthiophenyl), 7.30 (d, 2H, J = 7.96 Hz, 4-fluorophenyl), 7.85-7.88 (m, 2H, J = 8.28 Hz, 5.08 Hz, 3.20 Hz, 4-fluorophenyl).

7e. 7*H*-6-(4-Chlorophenyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine

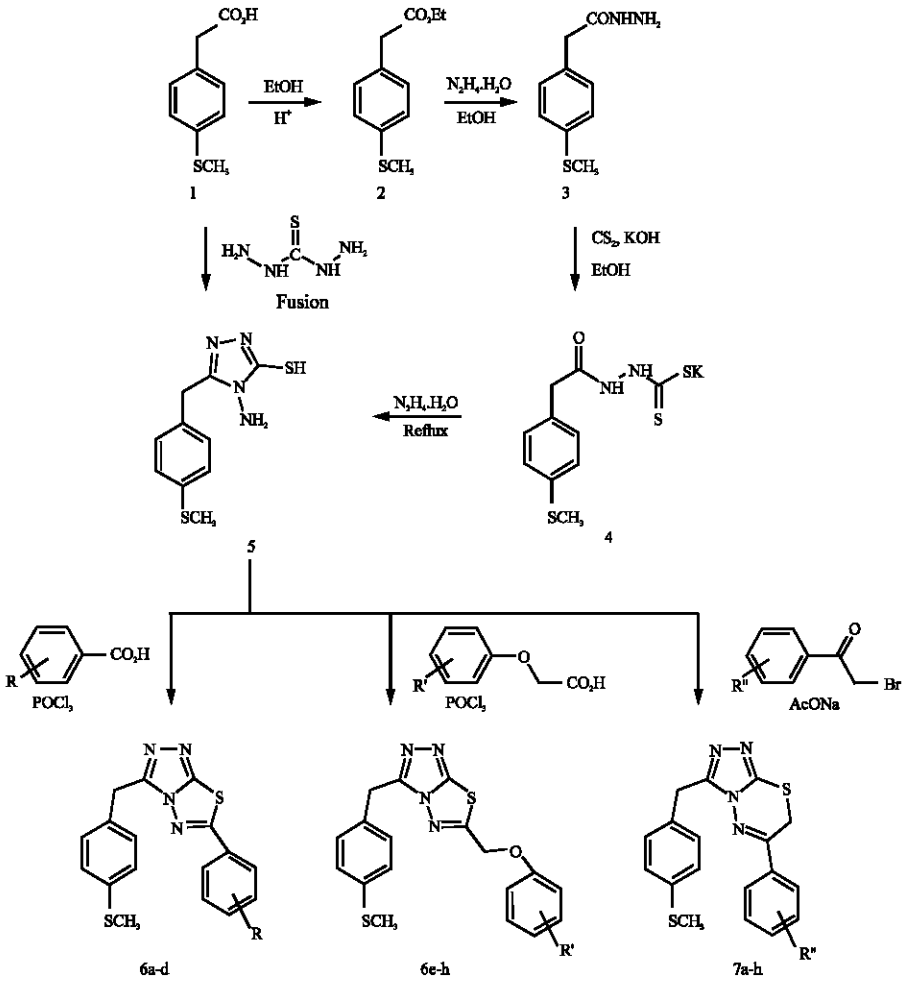
^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 3.89 (s, 2H, CH_2), 4.27 (s, 2H, SCH_2), 7.17 (d, 2H, J = 8.28 Hz, 4-methylthiophenyl), 7.27 (d, 2 H, J = 8.00 Hz, 4-methylthiophenyl), 7.49 (d, 2 H, J = 8.64 Hz, 4-chlorophenyl), 7.77 (d, 2H, J = 8.64 Hz, 4-chlorophenyl), FABMS (m/z, %): 389 (M^++2, 60), 387 (M^+, 100), 367 (2), 307 (15), 289 (10), 279 (2), 250 (10), 220 (5), 205 (2), 167 (3), 154 (55), 149 (20), 136 (35), 120 (5), 107 (10), 105 (5).

RESULTS AND DISCUSSION

In continuation to our studies related to the development of new bioactive heterocyclic compounds, we herein report an easy access to two series of fused heterocycles namely; triazolothiadiazoles and triazolothiadiazines in good yields. We report in this study the synthesis of a new triazole namely, 4-amino-3-(4-methylthiobenzyl)-5-mercapto-1,2,4-triazole (5) employing two different routes. The hydrazide route which involves four steps was found to be a better choice in comparison to the one stepped direct fusion route owing to its excellent yield. The triazole (5) undergoes cyclocondensation with various substituted benzoic acids/aryloxy acetic acids in presence of phosphorus oxychloride and with various substituted phenacyl bromides in presence of anhydrous sodium acetate to afford two series of fused heterocyclic systems namely; 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (6a-h) and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (7a-h) respectively (Scheme 1). All the compounds were isolated in good yields after recrystallization from ethanol. Few selected compounds were characterized by IR, ^{1}H NMR and mass spectral analysis.

Antibacterial Activity

We investigated the newly synthesized 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (6a-h) and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (7a-h) for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Psuedomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method



Scheme 1

(Cruickshank *et al*., 1975; Arthington-Skaggs *et al*., 2000). Ciprofloxacin was used as a standard drug. Solvent and growth controls were kept and the zone of inhibition in mm was noted. The results of such studies are given in Table 3. It can be seen that most of the compounds are moderately active against all the bacterial strains. The compounds 6c, 6d, 6g, 6h, 7e and 7f have exhibited maximum activity against the tested strains.

Antifungal Studies

Newly synthesized compounds 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (6a-h) and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (7a-h) were screened for their antifungal activity against *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigatus* (NCIM No.902), *Candida albicans* (NCIM No.3100) and *Penicillium marneffei* (recultured) in DMSO by serial plate dilution method (Cruickshank *et al*., 1975; Arthington-Skaggs *et al*., 2000). Antifungal activity was determined by measuring the diameter of the inhibition zone. The results of such studies are given in Table 4. Activity

Table 3: Antibacterial activity of compounds (6a-h) and (7a-h) at the conc. 10 μg mL^{-1} (Disc diffusion method)

Compound No.	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
6a	10	10	12	10
6b	10	12	12	10
6c	18	22	18	18
6d	18	23	18	18
6e	12	15	10	12
6f	12	15	10	11
6g	18	23	19	17
6h	18	24	19	18
7a	10	10	10	10
7b	10	10	12	10
7c	10	10	12	12
7d	15	20	16	16
7e	18	23	18	18
7f	18	22	19	18
7g	10	12	10	10
7h	10	10	10	10
Ciprofloxacin	19	25	20	18

Table 4: Antifungal activity of compounds (6a-h) and (7a-h) at conc. 10-100 μg mL^{-1} (Serial plate dilution method)

Compound No.	*A. fumigatus*	*A. flavus*	*C. albicans*	*P.marneffei*
6a	10	10	12	10
6b	10	10	10	10
6c	18	18	18	19
6d	20	18	19	18
6e	12	10	10	12
6f	12	10	10	10
6g	20	17	20	20
6h	20	18	20	18
7a	10	10	10	10
7b	10	10	10	12
7c	10	12	12	12
7d	12	12	15	12
7e	18	17	18	20
7f	18	17	18	18
7g	12	10	10	10
7h	10	10	10	10
Ciclopiroxolamine	22	18	20	20

of each compound was compared with Ciclopiroxolamine as standard drug. The study reveals that most of the compounds are moderately active against all the fungal strains. Once again the compounds 6c, 6d, 6g, 6h, 7e and 7f showed the highest inhibition.

CONCLUSIONS

Novel 6-(substituted aryl/aryloxymethyl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles and 7*H*-6-(substituted aryl)-3-(4-methylthiobenzyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines were prepared and screened for their antibacterial and antifungal activities. The compounds 6c, 6d, 6g, 6h, 7e and 7f exhibited maximum antibacterial as well as antifungal activity and hence can be recommended for further studies. Also the presence of one or more halogen atom in the nucleus has considerably increased the biological activity of the molecules.

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