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Synthesis and antimicrobial activities of some1,2,4-triazolo[3,4-b][1,3,4] thiadiazoles and 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines bearing bistrifluoromethylphenyl moiety

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ABSTRACT

*The reaction of 3,5-bistrifluoromethyl phenyl acid hydrazide with carbon disulfide and potassium hydroxide followed by treatment with hydrazine hydrate afforded 5-(3, 5-bis(trifluoromethyl) phenyl) -4-amino-4H-1,2,4-triazole-3-thiol. Condensation of (**6**) with various aromatic carboxylic acids or with phenacyl bromides afforded two series of fused heterocycles namely 6-(substituted aryl)-3-(3,5-bis(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadizoles (**7**) and 6-(substituted aryl)-3-(3,5-bis(trifluoromethyl)phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**8**), respectively. All the synthesized compounds were screened for their antibacterial and antifungal activities. Some of the compounds exhibited promising antimicrobial activities.*

Keywords: Bis-trifluoromethyl phenyl, Triazolothiadiazole, Triazolothiadiazine, Antibacterial, Antifungal activities.

INTRODUCTION

Synthesis of heterocyclic systems containing nitrogen atom has been attracting increasing interest over the past decade because of their utility in various pharmaceutical applications. 1,2,4-trizole derivatives are reported to possess a broad spectrum of biological activities such as antimicrobial [1-3], hypoglycaemic [4], analgesic [5], anti-inflammatory [6] and anticancer [7]. Among the fused heterocycles, the most common systems are triazoles combined to thiadiazoles or thiadiazines. Both the nuclei are biologically active with a broad spectrum of biological activities. The fusion of these nuclei results in the enhancement of biological activities of the resulting nucleus as compared to individual nuclei. Most of the 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole and 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities such as antibacterial and antifungal [8-10], antihelmintic [11], anti-inflammatory [11], analgesic [13], antitubercular [14], antiviral [15], hypoglycemic agents [4], anticancer [16], antitumer [17], antioxident [18] and diuretics [19]. Literature reveals that the halogen atoms at various positions in substituent aryl group enhance biological activity of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines [20]. Furthermore, the introduction of fluorine atom or CF_3 group into an organic molecule largely enhances the pharmacological properties as compared with the non-fluorinated analogues [21].

EXPERIMENTAL SECTION

Chemicals were procured from Aldrich Chemical Co. Reactions were monitored and purity of the products was checked by thin layer chromatography (TLC). TLC was performed on Merck 60F-254 silica gel plates with visualization by UV-light. Melting points were determined on a Buchi Melting Point B-545 apparatus. The IR spectra (in KBr pellets) were recorded on a Nicolet 6700 FT-IR spectrometry. ^{1}H NMR were recorded on Bruker Avance (400 MHz) spectrometer instruments in CDCl3 and DMSO-d6 solvents. Chemical shifts were recorded in

parts per million downfield from tetramethylsilane. Mass spectra were recorded on LC–MS QP trap spectrophotometer. Elemental analysis was performed on a Carlo Erba Perkin-Elmer model 240 analyzer. Analysis results were within 0.4% of the calculated value. Column chromatography was performed on silica gel (230–400 mesh) supplied by Acme Chemical Co. Ltd. The chemicals and solvents used were laboratory grade.

General procedure for the synthesis of 3,5-bis(trifluoromethyl)phenylcarbohydrazide (2)

To a stirred solution of 3,5-bis(trifluoromethyl)phenyl carboxylic acid (**1**) (2.58 g, 10 mmol) and diisopropyl ethyl amine (1.55 g, 12 mmol) in THF (20 mL) was added isobutyl chloroformate (1.5 g, 11 mmol) at 0-5˚C. The reaction mixture stirred for 2h at 0-5˚C. To this mixture hydrazine hydrate (98%) (0.1 g, 20 mmol) was added at 0-5˚C and further stirred for 4h. Progress of the reaction was monitored by TLC. After completion of the reaction, solvent was evaporated under vacuum and water (30 ml) was added to the residue. The solid separated out was filtered and washed with chilled water. The product was dried at 50-55˚C to obtain 3,5-bis(trifluoromethyl)phenyl carbohydrazide **(2)** Yield= 2.31g (85%).

General procedure for the synthesis of 5-(3, 5-bis(trifluoromethyl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol (4).

3,5-bis(trifluoromethyl)phenyl carbohydrazide (**2**) (2.72 g, 10 mmol) was treated with a solution of potassium hydroxide (0.84g, 15mmol) in methanol (50 mL) at 0-5 ºC under stirring. Carbon disulfide (1.14 g, 15 mmol) was added slowly and the reaction mixture stirred for 12 hours at room temperature. The solid product of potassium dithiocarbazinate (**3**), was filtered, washed with chilled diethyl ether and dried. It was directly used for next step without further purification. To a stirred suspension of potassium dithiocarbazinate in water (20 mL) was added hydrazine hydrate (0.1g, 20 mmol) and refluxed 6h. The reaction mixture turned green with evolution of hydrogen sulphide and finally it became homogeneous. The reaction mixture was then poured into ice and neutralized with concentrated hydrochloric acid. The white precipitate was filtered, washed with cold water and recrystallized from ethanol to afford 5-(3, 5-bis(trifluoromethyl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol(**4**)**.** Yield =2.95g (80%) Mp=158-159˚C; MS (EI): m/z=329.2 (M+1) IR (KBr, cm^{-1}) 3307 (NH_2), 2645(S-H) , 2997 (Ar C-H), 1612 (C=N); ^{1}H NMR (400MHz, DMSO-*d6)*: δ 13.76 (s, 1H), 8.40(s,2H), 7.92 (s,1H). 5.27(s, 2H)

General procedure for the synthesis of 3-(3,5-bis(trifluoromethyl)phenyl)-6-aryl substituted-[1,2,4] triazolo[3,4b][1,3,4] thiadiazole (5a-f):

To a stirred solution of phenyl 5-(3, 5-bis(trifluoromethyl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol(**4**) (3.28 g, 10 mmol) in $POCl_3$ (10 mL), substituted aromatic/heterocyclic carboxylic acid (10 mmol) was added. The reaction mixture was heated to reflux for 6h. The reaction mixture was quenched in ice water and neutralized with sodium bicarbonate. The solid separated out was filtered and washed with cold water. The wet product was dried at 45-50 ˚C to afford corresponding 3-(3,5-bis(trifluoromethyl)phenyl)-6-aryl substituted-[1,2,4]triazolo[3,4b][1,3,4] thiadiazole **(5a-g)**. The crude product was purified by silica gel flash column chromatography using chloroform and ethanol as eluent.

3-(3,5-bis(trifluoromethyl)phenyl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5a):

White solid; mp: 160-162˚C; IR(KBr) ν/cm^{-1}: 3065.72, 1645, 1455, 1480, 1136; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.85 (s, 2H), 8.28(s, 1H), 7.35-7.59 (m, 5H); MS(EI): m/z= 415.3 (M+H); *Anal. Calcd* for $C_{17}H_8F_6N_4S$: C, 49.28; H, 1.95; N, 13.52; Found: C, 49.47; H, 2.02; N, 13.36.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(2-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5b):**

White solid. mp: 196-199˚C; IR(KBr) ν/cm^{-1}: 3089, 3001, 1621, 1458, 1478, 1138.^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.82 (s, 2H), 8.44 (d, 1H, J=7.8Hz), 8.38(s, 1H), 7.34(d, 1H, J=7.8 Hz), 7.97(t, 1H), 7.81(d, 1H, J=7.8 Hz); MS(EI): m/z=492.1(M-1), 494.1 (M+H); *Anal. Calcd* for $C_{17}H_7BrF_6N_4S$: C, 41.40; H, 1.43; N, 11.36; Found: C, 41.29; H, 1.51; N, 11.44.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5c):

Off White solid, mp: 258-259˚C; IR(KBr) ν/cm^{-1}: 3091, 1589, 1437, 1473, 1132; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.86 (s, 2H), 8.17 (s, 1H), 7.96 (d, 2H, J=8.64 Hz), 7.85 (d, 2H, J=8.64 Hz); MS(EI): m/z= 460.2 (M+H); *Anal. Calcd* for $C_{17}H_7F_6N_5O_2S$: C, 44.45; H, 1.54; N, 15.25;Found: C, 44.61; H, 1.70; N, 15.32.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(2-methyl-3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5d):

Off white solid; mp: 219-222˚C; IR(KBr) ν/cm^{-1}: 3078, 1608, 1477, 1456, 1531, 1136;

^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 8.87 (s, 2H), 8.04 (d, 1H, J=7.8 Hz), 8.02 (s, 1H), 7.87 (d, 1H, J=7.2 Hz), 7.62 (t, 1H), 2.73 (s, 3H); MS (m/z): 474.2(M+H), 496.1 (M+Na); *Anal. Calcd* for $C_{18}H_9F_6N_5O_2S$: C, 45.67; H, 1.92; N, 14.80; Found: C, 45.39; H, 1.88; N, 14.66.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(2-methyl-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5e):
Off white solid; mp: 189-191˚C; IR(KBr) ν/cm^{-1}: 3093, 1605, 1455, 1472, 1137; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.85 (s, 2H), 8.26 (s, 1H), 7.81(d, 1H, J=7.6 Hz), 7.57 (d, 1H, J=7.6 Hz) 7.46-7.53 (m, 2H); MS(EI): m/z= 429.2 (M+H); *Anal. Calcd* for $C_{18}H_{10}F_6N_4S$: C, 50.47; H, 2.35; N, 13.08; Found: C, 50.71; H, 2.46; N, 13.28.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(4-methyl-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5f):
white solid**;** mp: 217-219˚C; IR(KBr) ν/cm^{-1}:3082, 3042, 2878, 1625, 1456, 1475, 1139; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.86 (s, 2H), 8.30 (s, 1H), 7.84(d, 2H, J=7.2 Hz), 7.27 (d, 2H, J=7.2 Hz), 2.38 (s, 3H); MS(EI): m/z= 429.3 (M+H); *Anal. Calcd* for $C_{18}H_{10}F_6N_4S$: C, 50.47; H, 2.35; N, 13.08; Found: C, 50.68; H, 2.41; N, 13.14.

3,6-bis(3,5-bis(trifluoromethyl)phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5g):
Gray solid; mp: 238-239˚C; IR(KBr) ν/cm^{-1}: 3091, 3023, 1622, 1440, 1479, 1134; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.80 (s, 2H), 8.64 (s, 2H), 8.43(s, 1H), 8.29 (s, 1H); MS(EI): m/z= 429.3 (M+H); *Anal. Calcd* for $C_{19}H_6F_{12}N_4S$: C, 41.47; H, 1.10; N, 10.18; Found: C, 41.09; H, 1.02; N, 9.98.

General procedure for the synthesis of 3-(3,5-bis(trifluoromethyl)phenyl)-6-arylsubstituted-7H-[1,2,4] triazolo[3,4b][1,3,4] thiadiazine (6a-f):
To a stirred solution of 5-(3, 5-bis(trifluoromethyl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol(**4**) (3.28 g, 10 mmol) in absolute ethanol (20 mL), substituted α-halo aromatic/heterocyclic ketone (12 mmol) was added. The reaction mixture was refluxed for 6h. The reaction was monitored by TLC. After completion of reaction, reaction mixture cooled to 25˚C and neutralized with sodium bicarbonate. The solid was filtered, washed with water to afford 3-(3,5-bis(trifluoromethyl)phenyl)-6-arylsubstituted-7H-[1,2,4]triazolo[3,4b][1,3,4] thiadiazine (**6a-f**). The crude product was purified by silica gel column chromatography using chloroform and ethanol.

3-(3-(3,5-bis(trifluoromethyl)phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)thiophene-2-sulfonamide (6a):
Off White solid; mp:207-209˚C; IR(KBr) ν/cm^{-1}: 3417.9, 3300.3, 3091, 3023, 1627.9, 1446.0, 1516, 1350.22,1134; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.80 (s, 2H), 8.64 (s, 2H), 8.43(s, 1H), 8.29 (s, 1H), 4.35 (s, 2H); MS(EI): m/z= 514.3 (M+H); *Anal. Calcd* for $C_{18}H_{10}F_6N_4S$: C, 37.43; H, 1.77; F, 22.20; N, 13.64; Found: C, 37.05; H, 1.63; N, 13.57.

3-(3,5-bis(trifluoromethyl)phenyl)-6-(thiophene-3-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadizine (6b):
White powder; mp: 218-220˚C; IR(KBr) ν/cm^{-1}: 3076, 1618, 1453, 1475, 1128; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.69 (s, 2H), 8.49 (dd, 1H, J=1.24, 2.8 Hz), 8.24(s, 1H), 7.76 (dd, 1H, J=2.8, 5.12Hz), 7.59 (dd, 1H, J=1.24, 5.12 Hz), 4.43 (s, 2H); MS(EI): m/z= 434 (M+H); *Anal. Calcd* for $C_{16}H_8F_6N_4S_2$: C, 44.24; H, 1.86; N, 12.90; Found: C, 44.42; H, 1.84; N, 12.95.

3-(3,5-bis(trifluoromethyl)phenyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (6c):
White powder; mp: 250-251˚C; IR(KBr) ν/cm^{-1}: 3107, 3020, 1618, 1176, 1455, 1478. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.66 (s, 2H), 8.30 (s, 1H), 7.05-7.25 (m, 5H), 4.51 (s, 2H); MS(EI): 429.3(M+1); *Anal. Calcd* for $C_{18}H_{10}F_6N_4S$: C, 50.47; H, 2.35; N, 13.08; Found: C, 50.51; H, 2.41; N, 13.20.

3,6-bis(3,5-bis(trifluoromethyl)phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (6d):
Gray solid; mp: 252-253˚C; IR(KBr) ν/cm^{-1}: 3099, 3022, 1642, 1417, 1469, 1132; ^{1}H NMR(400 MHz, DMSO-d_6, ppm): δ 8.68 (s, 2H), 8.65 (s, 2H), 8.28 (s, 1H), 8.23 (s, 1H), δ4.58(s,2H); MS(EI): 565(M+1); *Anal. Calcd* for $C_{20}H_8F_{12}N_4S$: C, 42.56; H, 1.43; N, 9.93; Found: C, 42.71; H, 1.53; N, 10.05.

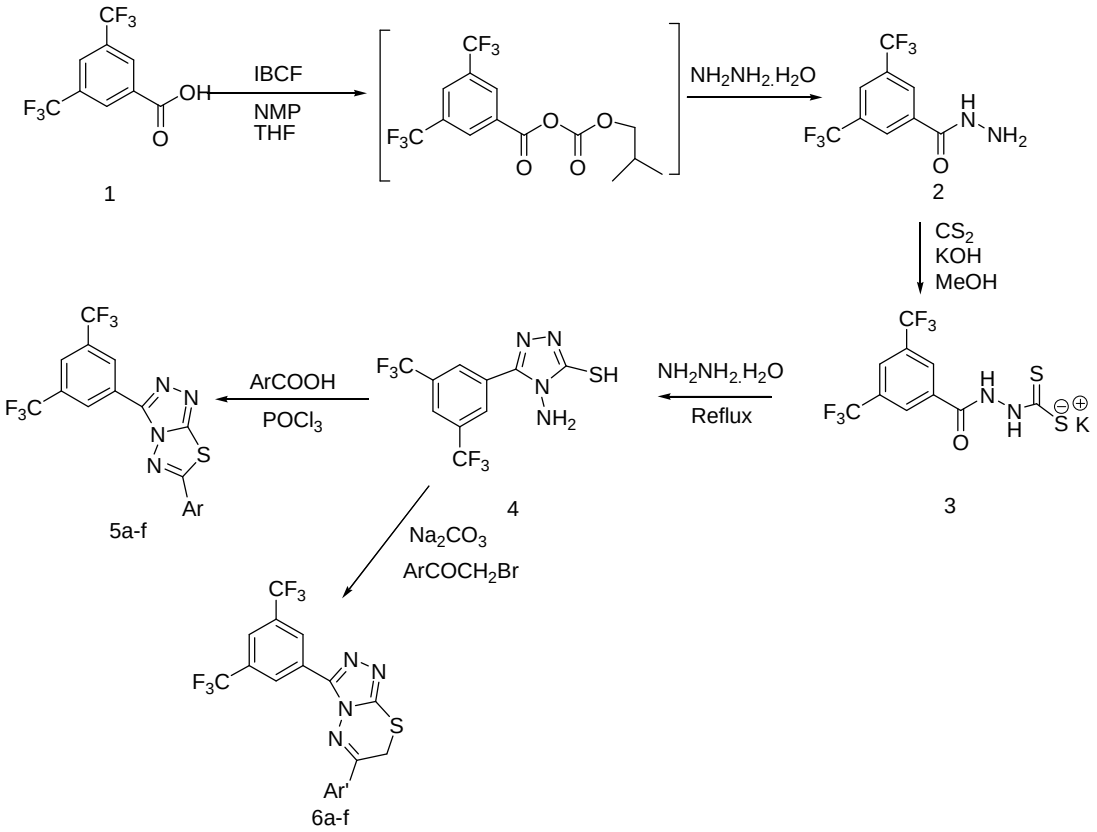
3-(3,5-bis(trifluoromethyl)phenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (6e):
White solid; mp: 235-236˚C; IR(KBr) ν/cm^{-1}: 3066, 2845, 1257, 1643; ^{1}H NMR(400 MHz, DMSO-d_6, ppm): δ 8.63 (s, 2H), 8.27 (s, 1H), 7.73 (d, 2H, J=8.8Hz) 7.00(d, 2H, J=8.8Hz), 4.47 (s, 2H) 3.83(s, 3H); MS(EI):m/z= 459.2(M+H); *Anal. Calcd* for $C_{19}H_{12}F_6N_4OS$: C, 49.78; H, 2.64; F, 24.87; N, 12.22; Found: C, 49.92; H, 2.71; N, 12.10.

3-(3-(3,5-bis(trifluoromethyl)phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)phenol(6f).
White solid; mp: 235-236˚C; IR(KBr) ν/cm^{-1}: 3078, 1606, 1433, 1474, 1236, 1132; ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.68 (s, 2H), 8.32 (s, 1H), 7.56 (d, 2H, J=10Hz), 6.90 (d, 2H, J=10Hz), 10.64(s,1H); MS(EI):m/z= 445.1(M+H); *Anal. Calcd* for $C_{18}H_{10}F_6N_4OS$: C, 48.65; H, 2.27; N, 12.61; Found: C, 48.51; H, 2.20; N, 12.69.

RESULTS AND DISCUSSION

3,5-bis(trifluoromethyl)phenyl carboxylic acid (**1)** was converted to mixed anhydride by reacting with isobutyl chloroformate using NMP in THF at 0-5˚C. Mixed anhydride in-situ was reacted with hydrazine hydrate at 0-5˚C to obtain 3,5-bis(trifluoromethyl)phenyl carboxylic acid hydrazide (**2**) as white solid. 3,5-bis(trifluoromethyl)phenyl carboxylic acid hydrazide **(2)** was treated with carbon disulfide in a methanolic potassium hydroxide solution at 0-5 °C to obtain potassium dithiocarbazinate (**3**) in good yield and was directly used for the next step without purification. 5-(3,5-bis(trifluoromethyl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol (**4)** was synthesized by refluxing potassium dithiocarbazinate (**3**) with hydrazine hydrate in water for 6 h.

3-(3,5-bis(trifluoromethyl)phenyl)-6-aryl substituted-[1,2,4]triazolo[3,4b][1,3,4] thiadiazole (**5a-g**) were synthesized by refluxing 5-(3,5-bis(trifluoromethyl) phenyl)-4-amino-4H-1,2,4-triazole-3-thiol (**4**) and aromatic carboxylic acids in phosphorus oxychloride. 3-(3,5-bis(trifluoromethyl)phenyl)-6-arylsubstituted-7H-[1,2,4]triazolo[3,4b][1,3,4] thiadiazine (**6a-f**) were synthesized by refluxing 5-(3,5-bis(trifluoromethyl) phenyl)-4-amino-4H-1,2,4-triazole-3-thiol (**4**) and substituted phenacyl bromides in absolute ethanol for 6h.



Scheme 1: Synthesis of substituted triazolothiadiazoles and Triazolothadiazines

Biological evaluation: Antimicrobial Activity

Agar Diffusion Bioassay: The screening of antibiotics from synthetic or natural compounds, the agar diffusion bioassay *in vitro* is commonly used. Here a particular compound gets diffused in agar and inhibits the growth of selected test organisms, which creates the zone of inhibition. The bioassay was carried out in Nunc Bioassay plate of size 230 X 230 X 18 mm. All the screened compounds were dissolved in DMSO and tested at 100 μg/mL and 200 μg/mL concentration. In each well 50 μL sample was loaded. The zone of inhibition is expressed as diameter of zone in millimeter (mm). The compounds were evaluated for antimicrobial activity against panel of gram positive bacteria *Staphylococcus aureus* (*S. aures)* and *Bacillus subtilis* (*B. subtilis*), gram negative bacteria *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae*) and pathogenic fungus *Fusarium solani* (*F. solani), Aspergillus niger* (*A. niger) , Candida albicans(C. albicans*).

The antibiotic Ciprofloxacin and Ketoconazole were used as reference for antibacterial and antifungal substances, respectively for comparison. Dimethyl sulphoxide (1%) was used as a control. The antibacterial activities of synthesized compounds **(5a-g)** and **(6a-f)** are presented in **Table 1** while the results of anti-fungal activity in **Table 2**.

Table 1: Antibacterial activities of compounds (5a-g) and (6a-f)

Entry	Conc. (µg/mL)	*S. Aureus*	*B. Subtils*	*E. coli*	*K. pneumoniae*
5a	100	14	16	11	14
	200	16	18	13	15
5b	100	25	26	24	21
	200	28	29	27	26
5c	100	20	17	19	16
	200	22	18	22	18
5d	100	12	14	15	11
	200	14	15	17	12
5e	100	10	11	11	9
	200	11	11	11	10
5f	100	8	7	6	7
	200	10	11	10	10
5g	100	38	37	36	37
	200	43	41	40	39
6a	100	34	37	38	36
	200	37	39	39	38
6b	100	26	29	31	28
	200	29	31	34	31
6c	100	7	8	7	6
	200	9	9	9	8
6d	100	38	38	37	36
	200	41	42	43	39
6e	100	10	8	6	11
	200	13	10	9	13
6f	100	24	23	22	26
	200	28	29	27	29
Ciprofloxacine	100	34	36	40	37
	200	38	42	45	42

Table 2: Antifungal activities of compounds (5a-g) and (6a-f)

Entry	Conc. (µg/mL)	*F. Solani*	*A. niger*	*C. albicans*
5a	100	22	26	25
	200	24	29	28
5b	100	24	20	22
	200	26	23	24
5c	100	28	23	26
	200	31	26	29
5d	100	17	17	16
	200	19	22	21
5e	100	16	16	17
	200	20	18	21
5f	100	15	16	14
	200	18	19	18
5g	100	39	37	34
	200	44	41	39
6a	100	22	24	25
	200	27	29	28
6b	100	32	30	39
	200	35	34	42
6c	100	20	19	16
	200	23	21	19
6d	100	32	30	39
	200	35	34	42
6e	100	28	25	20
	200	31	27	23
6f	100	22	20	19
	200	25	24	22
Ketoconazole	100	38	38	38
	200	42	42	42

In vitro antibacterial and antifungal activity assay (**Table 1** and **2**) indicated that all the synthesized compounds **(5a-g)** and **(6a-f)** shows moderate to excellent activity against all the tested bacterial strains and fungal pathogens determined at concentrations (100 and 200 µg/mL).

The investigation of antibacterial screening data of triazolothiadiazole **(5a-g)** revealed that compounds (**5g**) showed excellent activity against both gram positive bacteria as well as gram negative bacteria. The excellent activity is

attributed to the presence of pharmacologically active 3,5-bis(trifluoromethyl)phenyl moiety attached at 6 position of the triazolothidiazole ring. The compounds (**5b**) and (**5c**) showed moderate activity while other compounds (**5a**), (**5d**), (**5e**) and (**5f**) showed sharp decrease in antibacterial activity. These results indicate that presence of methyl moiety attached to any position of phenyl ring at 6 position of triazolothiadiazole substantially reduce the antibacterial activity. Triazolothiadiazole compound having two 3,5-bis(trifluoromethyl)phenyl at 3rd and 6th position showed antibacterial activity comparable to standard ciprofloxacin.

Antibacterial screening data of triazolothiadiazine **(6a-f)** showed that some of the compounds showed moderate to excellent activity against all bacterial strains. Compounds (**6a**) and (**6d**) showed excellent activity while compounds (**6b**) and (**6f**) showed moderate activity. The presence of thiophene-2-sulphonamide and 3,5-bis(trifluoromethyl)phenyl moiety at 6th position of triazolothiadizine enhance the antibacterial activity while 4-methoxy phenyl and phenyl moiety substantially decrease the activity.

Antifungal activity of triazolethiadiazole compounds (**5a-g**) and triazolothadiazine (**6a-f**) revealed that all the tested compounds showed excellent to moderate antifungal activity against all tested pathogens. Among the triazolothiadiazole compounds, (**5g**) showed the comparable antifungal activity with standard drug ketoconazole The structure of compound (**5g**) contains 3,5-bis(trifluoromethyl)phenyl moiety at 6th position of triazolothiadizole which is responsible for good antifungal activity. Among all the tested triazolothiadiazines, compounds (**6a**) showed comparable antifungal activity with the standard drug ketoconazole.

CONCLUSION

In conclusion, several substituted 3-(3,5-bis(trifluoromethyl)phenyl-[1,2,4]triazolo[3,4b][1,3,4] thiadiazole and 3-(3,5-bis(trifluoromethyl)phenyl)-7H-[1,2,4]triazolo[3,4b][1,3,4] thiadiazine were synthesized. The pharmacological study was undertaken to evaluate the effect of substituent on the antibacterial and antifungal activities. All the synthesized compounds showed good to excellent antibacterial and antifungal activities. The synthesized compounds exhibit better antifungal activity than antibacterial activity. Also Antimicrobial activity data showed that the substitutent group at 6th position of triazolothiadizole as well as triazolothiadizine has substantial effect on their activities. It is also noteworthy to mention that 3, 5-bis (trifluoromethyl)phenyl is pharmacologically active moiety which enhance the antimicrobial activities of the compounds.

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