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ORIGINAL ARTICLE

Synthesis and *in vitro* study of some fused 1,2,4-triazole derivatives as antimycobacterial agents

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Abstract Because of the highly therapeutic nature of 1,2,4-triazoles, a new class of fused pyrazolo [3′,4′:4,5] thiazolo [3,2-b] [1,2,4]-triazole, isoxazolo [3′,4′:4,5] thiazolo [3,2-b] [1,2,4]-triazole moieties were prepared from the novel conventional methods via the reaction of 4-methyl benzoyl thiosemicarbazide with the appropriate chemical reagents. These compounds were screened for their antimicrobial activity against various bacterial and fungal strains. With the reference of antimicrobial activity data the synthesized compounds were further screened for their antimycobacterial activity against *Mycobacterium tuberculosis H37Rv* by the conventional methods. Among the synthesized compounds 4b, 4d, 4h, 5d and 5h have shown more activity compared to the standard drugs.

1. Introduction

The rapid development of mycobacterial resistance to conventional drugs is one of the major difficulties in the treatment of tuberculosis. The incidence of tuberculosis is increasing worldwide, partly due to poverty and inequity and partly to the HIV/AIDS pandemic, which greatly increase the risk of infection proceeding to overt disease. Moreover the development of drug resistant strains of mycobacterium species, has contributed to the inefficiency of the conventional antituberculosis therapy, thus it is still necessary to search for new antimicrobial agents. Since the discovery of hetero cyclic nucleus the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. The huge number of 1,2,4-triazoles containing systems exhibits anti-inflammatory activity (Mullican et al., 1993; Tozkoparan et al., 2007), antibacterial (Foroumadi et al., 2003), antimycobacterial (Kucukguzel et al., 2001), anticonvulsant (Kelley et al., 1995), antifungal (Heubach et al., 1979), antidepressant (Chiu and Huskey, 1998), and plant growth regulator anti coagulants (Eliott et al., 1987). On the other hand thiazoles are also a familiar class of heterocyclic compounds possessing a wide variety of biological activities and their utility in medicine is very much established (Andrew et al., 2008). Several physiological activities of various thiazole derivatives have proved the efficacy and efficiency in combating various diseases and noticed to have good therapeutic agents such as anti tubercular agents (El-Shaaer et al., 1998), antimicrobial agents (Gouda et al., 2010; Liaras et al., 2011), anti-Candida spp. agents (Chimenti et al., 2011).

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Furthermore, diverse chemotherapeutic activities have been ascribed to fused 1,2,4-triazolo thiazole moieties as antimicrobial agents (Guzeldemirci and Kucukbasmac, 2010). Literature survey revealed that some pyrazoles have been implemented as antiviral (Genin et al., 2000), antimicrobial (Foks et al., 2005; Prakash et al., 2008), anti-neoplastic (Farag et al., 2008), anti-tumour (Xia et al., 2008) and analgesic agents (Khode et al., 2009).

In view of these above findings it was contemplated to design and synthesize a new class of heterocyclic derivatives in which 1,2,4-triazole, pyrazolo-thiazole or isoxazolo-thiazole derivatives in a single molecular frame work act as antimicrobial and anti tuberculosis agents.

2. Experimental

All melting points were measured on the open capillary method. IR spectra were recorded for KBr disc on Schimadzu-8400 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker Avance II 400 spectrometer, operating at 400, 100.6 MHz respectively. Chemical shifts (δ) are reported in parts per million (ppm) and TMS as an internal standard. Molecular weights were determined with TOF MS ES Mass spectra. Reactions were monitored by thin layer chromatography (TLC) on silica gel, plates were visualized with ultraviolet light or iodine. Column chromatography was performed on silica gel 60(0.043–0.06 mm) Merck.

*2.1. 4-Methyl benzoyl thiosemicarbazide (**1**)*

A mixture of Ethyl-p-methyl-benzoate (0.01 mol) and thiosemicarbazide (0.01 mol) in methanol (25 ml) was refluxed for 10 h. The solvent was removed under reduced pressure and the viscous mass poured over ice water, filtered and recrystallized from methanol–water to afford compound **1**.

*2.2. 5-Mercapto-3-p-tolyl-s-triazole (**2**)*

P-methyl benzoyl thiosemicarbazide (2 g) in 8% NaOH Solution (30 ml) was heated under reflux temperature for 5 h. The reaction mixture was cooled to room temperature and acidified with dil. acetic acid. The product thus separated was filtered, washed with excess of water and recrystallized from ethanol to afford compound **2**.

*2.3. (Z)-5-(substituted-benzylidene)-2-(p-tolyl) thiazolo [3,2-b] [1,2,4] triazol-6(5H)-one (**3**)*

A mixture of compound **2** (0.01 mol), chloro acetic acid (0.01 mol), appropriate aromatic aldehyde (0.01 mol) fused sodium acetate (0.02 mol) in acetic anhydride (20 ml) and glacial acetic acid (30 ml) was refluxed on a heating mantle for 4 h, concentrated and cooled. The brown solid separated was filtered, washed well with water and recrystallized from glacial acetic acid as brown crystals. The compounds **3a**–**h** were prepared similarly by treating with corresponding aldehydes.

*2.3.1. 3-Phenyl-6-(p-tolyl)-3, 3a-dihydro-2H-pyrazolo [3′,4′:4,5] thiazolo [3,2-b] [1,2,4] triazole (**4a**)*

A mixture of compound **3** (0.005 mol), hydrazine hydrate (0.005 mol) and anhydrous CH_3COONa (0.01 mol) in glacial acetic acid was heated under reflux conditions for 5 h, cooled to room temperature and poured into ice cold water. The dark brown solid thus separated was filtered, washed with excess of water and recrystallized from acetic acid to obtain the desired compound. Yield 61.8%, m.p. 218–220 °C; IR (KBr) in cm^{-1}: 3325.39, 3078.49, 3064.99, 2951.23, 1558.54, 1236.41, 1030.02, 698.25; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.01–7.45 (m, 9H, Ar–H), 7.87 (s, 1H, N–H), 4.99 (d, 1H, N–CH), 4.47 (d, 1H, S–CH), 2.81 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.17, 41.3, 53.8, 121.8, 126.1, 128.5, 139.4, 147.2, 157.9; MS: m/z 334.38 [$M+1$].

The other compounds (**4b**–**h**) were also prepared similarly by treating with corresponding compounds **3b**–**h**. The physical and analytical data were given in Table 1.

*2.3.2. 3-(4-Chlorophenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4b**)*

Yield 64.7%, m.p. 254–257 °C; IR (KBr) cm^{-1}: 3296.18, 3078.49, 3064.99, 2953.11, 1600.97, 1558.54, 1236.41, 1107.18; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.91 (s, 1H, N–H), 7.09–7.41 (m, 6H, Ar–H), 7.01 (d, 2H, Ar–H near-Cl), 4.99 (d, 1H, N–CH), 4.32 (d, 1H, S–CH), 2.69 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 41.3, 53.6, 131.2, 118.3, 127.5, 130.1, 147.2, 156.5; MS: m/z 369.21 [$M+2$], 368.38 [$M+1$].

*2.3.3. 3-(3-Nitrophenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4c**)*

Yield-62.5%, m.p. 219–222 °C; IR (KBr) cm^{-1}: 3294.23, 3078.49, 3065.57, 2953.11, 1600.97, 1551.58, 1528.04, 1236.41; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.01 (s, 1H, N–H), 7.69 (d, 2H, Ar–H near NO_2), 7.03–7.41 (m, 6H, Ar–H), 5.09 (d, 1H, N–CH), 4.51 (d, 1H, N–CH), 2.78 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 41.3, 53.6, 119.6, 129.3, 133.7, 138.3, 147.2, 149.03, 156.9; MS: m/z 379.46 [$M+1$].

*2.3.4. 3-(4-Nitrophenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4d**)*

Yield-64.1%, m.p. 267–269 °C; IR (KBr) cm^{-1}: 3296.08, 3071.12, 3065.57, 2953.11, 1600.97, 1558.19, 1521.86; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.01 (s, 1H, N–H), 7.69 (d, 2H, Ar near NO_2), 7.12–7.53 (m, 6H, Ar–H), 5.06 (d, 1H, N–CH), 4.57 (d, 1H, S–CH), 2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.7, 41.3, 53.6, 119.6, 129.3, 134.2, 138.3, 146.9, 149.1, 157.41; MS: m/z 379.53 [$M+1$].

*2.3.5. 3-(2-Methoxyphenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4e**)*

Yield-59.7%, m.p. 237–239 °C; IR (KBr) cm^{-1}: 3287.11, 3071.12, 3065.68, 2959.45, 1600.97, 1558.16, 1249.98, 1236.41; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.91 (s, 1H, N–H), 7.1–7.46(m, 7H, Ar–H), 6.85 (d, 1H, Ar–H near OCH_3), 4.96 (d, 1H, N–CH), 4.67 (d, 1H, S–CH), 3.38 (s, 3H, OCH_3), 2.69 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6) δ ppm; 22.3, 41.3, 53.1, 55.8, 115.7, 127.4, 136.9, 147.2, 158.1, 159.6; MS: m/z 364.33 [$M+1$].

*2.3.6. 3-(4-Methoxyphenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4f**)*

Yield. 61.3%, m.p. 258–260 °C; IR (KBr) cm^{-1}: 3287.11, 3071.49, 3065.68, 2954.81, 1600.97, 1543.27, 1249.98,

Table 1 Physical and analytical data of synthesized compounds **4a–h** and **5a–h**.

Comp′d	R	Yield (%)	m.p. (°C)	Mol. Formula/(M.wt)	C, H, N analysis (calcd/found)		
					C	H	N
4a	H	61.8	218–220	$C_{18}H_{15}N_5S$/333.41	64.84 (64.87)	4.53 (4.49)	21.01 (21.05)
4b	4-Cl	64.7	254–257	$C_{18}H_{14}ClN_5S$/367.86	58.77 (58.74)	3.84 (3.87)	19.04 (19.00)
4c	3-NO_2	62.5	219–222	$C_{18}H_{14}N_6O_2S$/378.41	57.13 (57.09)	3.73 (3.69)	22.21 (22.25)
4d	4-NO_2	64.1	267–269	$C_{18}H_{14}N_6O_2S$/378.41	57.13 (57.10)	3.73 (3.77)	22.21 (22.24)
4e	2-OCH_3	59.7	237–239	$C_{19}H_{17}N_5OS$/363.44	62.79 (62.83)	4.71 (4.75)	19.27 (19.31)
4f	4-OCH_3	61.3	258–260	$C_{19}H_{17}N_5OS$/363.44	62.79 (62.75)	4.71 (4.74)	19.27 (19.30)
4g	4-$N(CH_3)_2$	63.5	241–243	$C_{20}H_{20}N_6S$/376.48	63.81 (63.84)	5.35 (5.39)	22.32 (22.36)
4h	4-Br	58.6	226–228	$C_{18}H_{14}BrN_5S$/412.31	52.43 (52.48)	3.42 (3.46)	16.99 (16.95)
5a	H	68	249–251	$C_{18}H_{14}N_{4z}OS$/334.39	64.65 (64.69)	4.22 (4.18)	16.75 (16.79)
5b	4-Cl	67.1	265–267	$C_{18}H_{13}ClN_4OS$/368.84	58.61 (58.66)	3.55 (3.59)	15.19 (15.23)
5c	3-NO_2	69.5	225–227	$C_{18}H_{13}N_5O_3S$/379.39	56.98 (57.02)	3.45 (3.49)	18.46 (18.50)
5d	4-NO_2	70.1	261–263	$C_{18}H_{13}N_5O_3S$/379.39	56.98 (56.95)	3.45 (3.49)	18.46 (18.51)
5e	2-OCH_3	65.6	241–243	$C_{19}H_{16}N_4O_2S$/364.42	62.62 (62.66)	4.43 (4.47)	15.37 (15.33)
5f	4-OCH_3	68	273–275	$C_{19}H_{16}N_4O_2S$/364.42	62.62 (62.67)	4.43 (4.47)	15.37 (15.41)
5g	4-$N(CH_3)_2$	71	256–258	$C_{20}H_{19}N_5OS$/377.46	63.64 (63.68)	5.07 (5.03)	18.55 (18.59)
5h	4-Br	60.8	218–220	$C_{18}H_{13}BrN_4OS$/413.29	52.31 (52.27)	3.17 (3.22)	13.56 (13.60)

1236.41; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.03 (s, 1H, N–H), 7.08–7.48 (m, 6H, Ar–H), 6.93 (d, 2H, Ar–H near OCH_3), 4.99 (d, 1H, N–CH), 4.67 (d, 1H, S–CH), 3.38 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm; 22.5, 40.91, 53.6, 55.8, 115.7, 127.3, 136.1, 147.3, 158.1, 159.6; MS: m/z 364.28 [$M+1$].

*2.3.7. N-N-dimethyl-4-(6-(p-tolyl)-3, 3a-dihydro-2H-pyrazolo[3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazol-3-yl)aniline (**4g**)*

Yield 63.5%, m.p. 241–243 °C, IR (KBr) cm^{-1}: 3291.68, 3078.48, 3064.99, 2951.23, 2936.18, 1600.97, 1558.08, 1236.73; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.98 (s, 1H, N–H), 7.1–7.53 (m, 6H, Ar–H), 7.68 (d, 2H, Ar–H near NMe_2), 4.96 (d, 1H, N–CH), 4.51 (d, 1H, N–CH), 2.86 (s, 6H, CH_3), 2.69 (s, 3H, CH_3).^{13}C NMR (DMSO-d_6) δ ppm; 23.8, 40.91, 53.6, 128.6, 135.2, 139.7, 147.2, 150.8, 158.3; MS: m/z 377.32 [$M+1$].

*2.3.8. 3-(4-Bromophenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**4h**)*

Yield: 58.6%; m.p. 226–228 °C; IR (KBr) cm^{-1}: 3285.27, 3073.51, 3064.99, 2951.23, 1600.97, 1558.08, 1236.73; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm: 7.98 (s, 1H, N–H), 7.05–7.61(m, 6H, Ar–H), 6.91 (d, 2H, Ar–H), 4.81 (d, 1H, N–CH), 4.55 (d, 1H, S-CH), 2.79 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.9, 41.5, 52.9, 121.8, 124.5, 131.09, 133.6, 142.3, 147.2, 158.7; MS: m/z 413.92 [$M+2$], 412.87 [$M+1$].

*2.3.9. 3-Phenyl-6-(p-tolyl)-3, 3a-dihydro-isoxazolo [3′,4′: 4,5] thiazolo [3,2-b] [1,2,4] triazole (**5a**)*

A mixture of compound 3a (0.005 mol), hydroxylamine hydrochloride (0.005 mol) and anhydrous CH_3COONa (0.01 mol) in glacial acetic acid was heated under reflux conditions for 6 h. Concentrated and cooled, the brown colour solid separated was filtered, washed well with water and recrystallized from glacial acetic acid as brown crystals. Yield ≅ 68%, m.p. 249–51 °C; IR (KBr) cm^{-1}: 3038.63, 3057.16, 2929.95, 1537.08, 1238.68, 920.42, 677.21; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.09–7.48 (m, 9H, Ar–H), 5.37 (d, 1H, CH–O), 4.71 (d, 1H, S–CH), 2.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 71.7, 124.3, 126.8, 130.4, 134.2, 138.9, 142.3, 151.9, 165.8; MS: m/z 335.26 [$M+1$].

The other compounds (**5b–h**) were also prepared similarly by treating with corresponding compounds **3b–h**. The physical and analytical data were given in Table 1.

*2.3.10. 3-(4-Chlorophenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5b**)*

Yield: 67.1%, m.p. 265–267 °C; IR (KBr) cm^{-1}: 3057.16, 3038.63, 2931.18, 1537.08, 1239.01, 1086.8, 920.42; ^{1}H NMR

(400 MHz, DMSO-d_6) δ ppm: 7.08 (d, 2H, Ar–H near Cl), 7.12–7.41 (m, 6H, Ar–H), 5.37 (d, 1H, O–CH), 4.71 (d, 1H, S–CH), 2.41 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 71.7, 126.8, 129.9, 131.9, 134.2, 138.9, 139.7, 151.9, 165.8; MS: m/z 370.16 [M + 2], 369.08 [M + 1].

*2.3.11. 3-(3-Nitrophenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5c**)*

Yield: 69.5%, m.p. 225–227 °C; IR (KBr) cm^{-1}: 3059.11, 3037.01, 2931.18, 1537.08, 1515.63, 1239.01, 918.97; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.09–7.43 (m, 6H, Ar–H), 7.59 (d, 2H, Ar–H near NO_2), 5.39 (d, 1H, O–CH), 4.68 (d, 1H, S–CH), 2.45 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 71.7, 121.4, 126.8, 129.9, 130.4, 134.2, 138.9, 141.6, 148.7, 149.1, 151.9, 165.8; MS: m/z 380.19 [M + 1].

*2.3.12. 3-(4-Nitrophenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5d**)*

Yield: 70.1%; m.p. 261–263 °C; IR (KBr) cm^{-1}: 3059.11, 3037.01, 2933.27, 1537.08, 1515.63, 1239.01, 1041.85, 920.03; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.09–7.45 (m, 6H, Ar–H), 7.61 (d, 2H, Ar–H near NO_2), 5.41 (d, 1H, O–CH), 4.87 (d, 1H, S–CH), 2.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 71.7, 121.4, 126.8, 129.9, 130.4, 134.2, 138.9, 148.7, 149.1, 151.9, 165.8; MS: m/z 380.07 [M + 1].

*2.3.13. 3-(2-Methoxyphenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3',4':4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5e**)*

Yield: 65.6%, m.p. 241–243 °C; IR (KBr) cm^{-1}: 3064.52, 3037.01, 2938.16, 1537.08, 1239.01, 1228.78, 1041.85, 918.64; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.05–7.47 (m, 7H, Ar–H), 6.87 (d, 1H, Ar–H near OCH_3), 5.41 (d, 1H, O–CH), 4.87 (d, 1H, S–CH), 3.29 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.1, 35.1, 54.8, 71.8, 117.9, 121.4, 126.2, 129.9, 134.2, 138.9, 151.9, 159.3, 165.8; MS: m/z 365.29 [M + 1].

*2.3.14. 3-(4-Methoxyphenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5f**)*

Yield: 68%, m.p. 273–275 °C; IR (KBr) cm^{-1}: 3064.52, 3037.01, 2938.16, 1537.08, 1239.01, 1228.78, 1038.12, 918.64; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.05–7.47 (m, 7H, Ar–H), 6.93 (d, 1H, Ar–H near OCH_3), 5.41 (d, 1H, O–CH), 4.69 (d, 1H, S–CH), 3.36 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.1, 35.1, 54.8, 71.8, 117.9, 121.4, 126.2, 129.9, 133.2, 138.9, 151.9, 159.3, 163.2; MS: m/z 365.4 [M + 1].

*2.3.15. N-N-dimethyl-4-(6-(p-tolyl)-3, 3a-dihydro isoxazolo[3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazol-3-yl)aniline(**5g**)*

Yield: 71%, m.p. 256–258 °C; IR (KBr) cm^{-1}; 3057.12, 3039.81, 2938.16, 2951.08, 1537.08, 1239.33, 1038.12, 919.27; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.71 (d, 1H, Ar–H near NMe_2), 7.11–7.38 (m, 6H, Ar–H), 5.41 (d, 1H, O–CH), 4.68 (d, 1H, S–CH), 2.78 (s, 6H, CH_3), 2.37 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 43.8, 71.2, 111.7, 126.2, 129.9, 131.8, 134.2, 138.9, 145.1, 151.9, 165.1; MS: m/z 378. 27 [M + 1].

*2.3.16. 3-(4-Bromophenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole (**5h**)*

Yield: 60.8%, m.p. 218–220 °C; IR (KBr) cm^{-1}; 3057.12, 3039.81, 2936.33, 1537.08, 1241.03, 1043.78, 917.26; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.01–7.41 (m, 8H, Ar–H), 5.39 (d, 1H, O–CH), 4.73 (d, 1H, S–CH), 2.39 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ ppm: 22.3, 35.1, 71.2, 123.6, 126.2, 129.9, 132.6, 134.2, 138.8, 140.3, 150.1, 164.9; MS: m/z 415.16 [M + 2], 414.01 [M + 1].

3. Results and discussion

3.1. Chemistry

The synthesis of 3-(substituted-phenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole **(4a–h)** and 3-(substituted-phenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole **(5a–h)** compounds was prepared by the use of key intermediate **2**, condensation of Ethyl-p-methyl benzoate with thiosemicarbazide at reflux temperature gave **1**. The synthesis of 5-mercapto-3-p-tolyl-S-triazole **2** was carried out by the cyclization reaction of compound **1** with 8% NaOH at reflux temperature (Scheme 1). Compound **2** was then reacted with chloroaceticacid, appropriate aldehydes and acetic anhydride in the presence of anhydrous sodium acetate in glacial AcOH at reflux temperature to give chalcone derivatives of 2-(p-tolyl) thiazolo [3,2-b] [1,2,4] triazol-6(5H)-one **(3a–h)**. Thus resulted compounds **3a–h** were then converted into title compounds **4a–h** and **5a–h** by condensation with hydrazine hydrate and hydroxylamine hydrochloride respectively followed by the literature method[25]. The structures of newly synthesized compounds were confirmed by their IR, ^{1}H NMR, ^{13}C NMR and MS analysis.

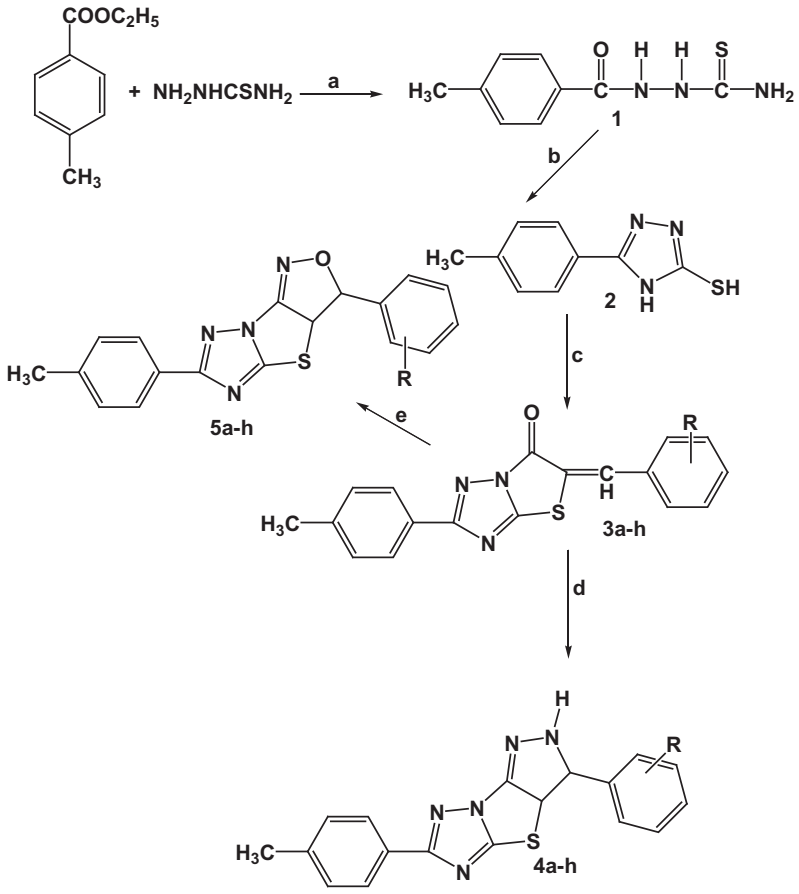
In the IR Spectra of compounds **4a–h** and **5a–h** disappearance of the carbonyl group absorption band at ~1700 cm^{-1}, which was present in compound **3** confirmed the cyclization (or) involvement of α, β-unsaturated carbonyl system. The N–H band of the pyrazole moiety was observed at about 3290 cm^{-1}, and N–O band of isoxazole moiety was observed at about ~920 cm^{-1}.

In the ^{1}H NMR spectra of compounds **4a–h** and **5a–h** recorded in DMSO-d_6, the signals due to CH–N, CH–O protons appeared at about δ 5 ppm and 5.4 ppm as doublets. On the other hand the signal due to N–H protons of pyrazole moiety appeared at about 8 ppm as a singlet. These signals demonstrate that the cyclization step had occurred as pyrazoles and isoxazoles. All the other aromatic protons of **4a–h** and **5a–h** were observed at the expected regions.

In the ^{13}C NMR spectrum of compounds **4a–h** and **5a–h** recovered in DMSO-d_6 the signals corresponding to the carbons of fused ring compounds observed nearly at about δ 22, 41, 53, 58, 121, 126, 128, 139, 141, 147, 158, 165 ppm are further evidence of their structures. Mass spectra of all of synthesized compounds showed $M^+/M^+ + 1$ peaks in agreement with their molecular formula.

3.2. Antimicrobial activity

The newly prepared synthesized compounds **4a–h** and **5a–h** were screened for their anti bacterial activity against *Bacillus subtilis, Bacillus thuringiensis, Escherichia coli*, *Pseudomonas*



Scheme 1 Synthesis of compounds **4a**–**h** and **5a**–**h**. Reaction conditions: (a) methanol, 10h; (b) 8% NaOH, 5h; (c) chloro acetic acid, fused CH3COONa, acetic anhydride, glac. AcOH, 4h; (d) hydrazine hydrate, anhyd., CH3COONa, glac. AcOH, 5h and (e) hydroxyl amine hydrochloride, anhyd., CH3COONa, glac. AcOH, 6h.

Table 2 Antibacterial activity data of synthesized compounds **4a**–**h** and **5a**–**h**.

S.l. No.	Minimal Inhibitory Concentration (MIC μg/mL)			
	B.subtilis[a]	*B.thuringiensis* [a]	*E.coli* [a]	*P. aeruginosa* [a]
4a	12.5	12.5	12.5	25
4b	3.125	3.125	3.125	3.125
4c	3.125	3.125	6.25	6.25
4d	3.125	3.125	3.125	3.125
4e	12.5	12.5	25	25
4f	12.5	25	25	25
4g	6.25	6.25	12.5	6.25
4h	3.125	3.125	6.25	6.25
5a	25	25	50	50
5b	3.125	6.25	6.25	6.25
5c	3.125	3.125	6.25	12.5
5d	3.125	3.125	3.125	6.25
5e	25	12.5	25	50
5f	25	25	25	25
5g	6.25	6.25	6.25	6.25
5h	3.125	3.125	6.25	6.25
Streptomycin	3.125	6.25	6.25	6.25
Chloramphencol	6.25	6.25	6.25	6.25

[a] *B. subtilis* (MTCC No.: 1133), *B. thuringiensis* (MTCC No.: 4714), *E. coli* (MTCC No.: 443), and *P.aeruginosa* (MTCC No.: 2297).

aeruginosa Candida albicans, Aspergillus fumigatus, Bortaris fabae and Fusarium Oxysporam strains. This activity was determined by the agar diffusion method and the compounds were dissolved in DMSO at concentration $lmgm^{-1}$. The activity was compared with streptomycin and chloramphenicol standard drugs.

The results depicted in Table 2 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of tested Gram-positive and Gram negative bacterial strains. As usual tested compounds revealed better activity against Gram-positive bacteria than Gram-negative bacteria. The antibacterial screened data showed moderate activity of test compounds. Among the screened 4b, 4c, 4d, 4h, 5b, 5c, 5d, 5g and 5h electron withdrawing groups (–Cl, NO2, Br) have shown high activity against all the bacterial strains employed. In this view 4b and 4d were 50% more potent to streptomycin and chloramphenicol against all the strains employed (MIC 3.125 μg/ml) except *B. subtilis* organism. In the same way 4c, 4h and 5h were more potent to streptomycin and chloramphenicol in inhibiting the growth of *B. thuringiensis* (MIC 3.125 μg/ml), while its activity was equipotent to streptomycin and chloramphenicol against *E. coli* and *P. aeruginosa* (MIC 6.25 μg/ml) but these compounds were equipotent to streptomycin and more potent to chloramphenicol against *B. subtilis* (MIC 3.125 μg/ml). On the other hand 5b was equipotent to streptomycin against all the strains employed (MIC 3.125 μg/ml, 6.25 μg/ml). Also 5d was more potent to streptomycin and chloramphenicol against *B. thuringiensis* and *E. coli* (MIC 3.125 μg/ml), while its activity was equipotent to Streptomycin against *B. subtilis* and *P. aeruginosa* (MIC 3.125 μg/ml and 6.25 μg/ml). In the same way 5c was more potent to Streptomycin and chloramphenicol against *B. thuringiensis* (MIC, 3.125 μg/ml) and 50% less potent than streptomycin and chloramphenicol against *P. aeruginosa* (MIC, 12.5 μg/ml), while its activity was equipotent to streptomycin against *B. subtilis* and *E. coli* (MIC 3.125 μg/ml and 6.25 μg/ml). In addition 5b, 5c and 5d were more potent to chloramphenicol against *B. subtilis* (MIC 3.125 μg/ml). The remaining compounds have also shown moderate to good antibacterial activity.

All the synthesized compounds of series **4a**–**h** and **5a**–**h** were evaluated for their antifungal activity against *Candida albicans, Aspergillus fumigatus, Bortaris fabae* and *fabae and Fusarium oxysporam* fungal strains. The activity was compared with standard drug Treflucan.

The results depicted in Table 3 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of tested fungal strains. The screened 4a, 4e, 4f, 5a, 5e and 5f compounds have shown a highly antifungal activity against all strains employed. In this view compound 4a was equipotent to Treflucan against *C. albicans* (MIC, 3.125 μg/ml), while its activity was 50% lower than that of Treflucan against all the strains employed (MIC, 6.25 μg/ml). On the other hand compound 4f, 5a and 5f were equipotent to Treflucan against all the strains employed (MIC, 3.125 μg/ml). Also compound 5e was 50% lower than Treflucan against *F. oxysporam* (MIC, 6.25 μg/ml), while its activity was equipotent to Treflucan against *C. albicans, A. fumigatus* and *B. fabae* (MIC, 3.125 μg/ml). The remaining compounds have also shown moderate to good antifungal activity against all the strains employed.

3.3. Antitubercular studies

All the synthesized compounds of series **4a**–**h** and **5a**–**h** were evaluated for their anti tubercular activity. Drug susceptibility and determination of MIC of the test compounds against *Mycobacterium tuberculosis H37Rv* were performed by the agar micro dilution method, where two fold dilutions of each test compound were added into 7H10 agars supplemented with OADC and organism. A culture of used microorganism *M. tuberculosis H37Rv* growing on the L–J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentrations of compound keeping the volume constant, that is, 0.1 ml medium was allowed to cool keeping the tubes in the slanting position. These tubes were then incubated at

Table 3 Antifungal activity data of synthesized compounds **4a**–**h** and **5a**–**h**.

Compound	Minimal Inhibitory Concentration (MIC μg/mL)			
	C. albicans[a]	*A. fumigatus*[a]	*B. fabae*[a]	*F. oxysporam*[a]
4a	3.125	6.25	6.25	6.25
4b	12.5	12.5	50	25
4c	6.25	12.5	12.5	12.5
4d	25	12.5	25	25
4e	3.125	6.25	3.125	3.125
4f	3.125	3.125	3.125	3.125
4g	12.5	25	25	12.5
4h	12.5	12.5	12.5	12.5
5a	3.125	3.125	3.125	3.125
5b	12.5	12.5	12.5	25
5c	6.25	6.25	12.5	12.5
5d	12.5	25	50	50
5e	3.125	3.125	3.125	6.25
5f	3.125	3.125	3.125	3.125
5g	6.25	12.5	12.5	12.5
5h	6.25	6.25	12.5	12.5
Treflucan	3.125	3.125	3.125	3.125

[a] *C. albicans* (MTCC No.: 183), *A. Fumigatus* (MTCC No.: 2550), *B. fabae* (ATCC No.: 14862) and *F. oxysporam* (MTCC No.: 7392).

Table 4 Antitubercular activity data of synthesized compounds **4a–h** and **5a–h**.

Compound	MIC (μg/ml)	Compound	MIC (μg/ml)
4a	> 12.5	**5a**	50
4b	3.125	**5b**	6.25
4c	6. 25	**5c**	> 6.25
4d	3.125	**5d**	3.125
4e	25	**5e**	> 50
4f	> 25	**5f**	> 50
4g	> 6.25	**5g**	> 12.5
4h	> 3.125	**5h**	> 3.125
Streptomycin	3.125	Streptomycin	3.125

37 °C for 24 h followed by streaking of *M. tuberculosis H37Rv* (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were controlled with control tubes where the medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of the test compound. Streptomycin was used as standard drug. The MIC levels of some active compounds (**4a–h** and **5a–h**) against these organisms were given in Table 4.

The results depicted in Table 4 revealed that the compounds, displayed variable inhibitory effects on the growth of the tested *M. tuberculosis H37Rv* strains.

The antitubercular screened data showed moderate activity of test compounds. Among the screened 4b, 4d, 4h, 5d and 5h electron withdrawing groups (–Cl, NO_2, Br) have shown high activity against *M. tuberculosis H37Rv*.

4. Conclusion

In this communication our aim has been verified by the synthesis of 3-(substituted-phenyl)-6-(p-tolyl)-3, 3a-dihydo-2H-pyrazolo [3′,4′:4,5] thiazolo[3,2-b] 1,2,4]-triazole **4a–h** and 3-(substituted-phenyl)-6-(p-tolyl)-3, 3a-dihydo isoxazolo [3′,4′:4,5] thiazolo[3,2-b] [1,2,4]-triazole 5a-h derivatives in which 1,2,4-triazole and thiazole moieties incorporated with pyrazole or isoxazole moieties in a single molecular framework. Obtained results revealed that most of the tested compounds showed moderate antimicrobial activity comparable with the standard drugs. On the other hand the titled compounds showed promising activity as anti-tuberculosis agents. After all the above findings it can be concluded that these molecules become lead molecules for further synthetic and biological evaluation.

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References

Andrew, S., Susan, M., Michael, F., Mathew, W., Penny, L., Paul, L., Anne, G., Chris, B., John, M., Donald, T., Dennis, S., Tesfaye, B., 2008. Synthesis and biological activity of anticoccidial agents: 5,6-Diarylimidazo[2,1-*b*][1,3]thiazoles. Bioorg. Med. Chem. Lett. 18, 5263–5267.

Chimenti, F., Bizzarri, B., Bolasco, A., Secci, D., Chimenti, P., Granese, A., Carradori, S., D'Ascenzio, M., Lilli, D., Rivanera, D., 2011. Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles as anti-Candida spp.. Agents Eur. J. Med. Chem. 46, 378–382.

Chiu, S.-H.L., Huskey, S.-E.W., 1998. Species differences in *N*-glucuronidation. Drug Metab. Dispos. 26, 838–847.

E1-Shaaer, H.M., Foltfnovfi, P., Lacova, M., Chovancova, J., Stankovicova, H., 1998. Synthesis, antimicrobial activity and bleaching effect of some reaction products of 4-oxo-4H-benzopyran-3-carboxaldehydes with amino benzothiazoles and hydrazides. I1 Farmaco 53, 224–232.

Eliott, R., Sunley, R.L., Griffin, D.A., 1987. Preparation of plant growth regulant triazoles and imidazoles, UK Pat Appl GB 1986, 2175, 301. Chem. Abstr. 107, 134310n.

Farag, A.M., Mayhoub, A.S., Barakat, S.E., Bayomi, A.H., 2008. Regioselective synthesis and antitumor screening of some novel *N*-phenyl pyrazole derivatives. Bioorg. Med. Chem. 16, 881–889.

Foks, H., Pancechowska-ksepko, D., Kedzia, A., Zwolska, Z., Janowiec, M., Augustynowicz-Kopec, E., 2005. Synthesis and antibacterial activity of 1H-pyrazolo[3,4-b]pyrazine and -pyridine derivatives. IL Farmaco 60, 513–517.

Foroumadi, A., Mansouri, S., Kiani, Z., Rahamani, A., 2003. Synthesis and in vitro antibacterial evaluation of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones. Eur. J. Med. Chem. 38, 851–854.

Genin, M.J., Biles, C., Keiser, B.J., Poppe, S.M., Swaney, S.M., Tarpley, W.G., Yagi, Y., Romero, D.L., 2000. Novel 1,5-diphenylpyrazole non nucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3- and 4-substituted derivatives. J. Med. Chem. 43, 1034–1040.

Gouda, M.A., Berghot, M.A., Abd El-Ghani, G.E., Khalil, A.M., 2010. Synthesis and antimicrobial activities of some new thiazole and pyrazole derivatives based on 4,5,6,7-tetrahydrobenzothiophene moiety. Eur. J. Med. Chem. 45, 1338–1345.

Guzeldemirci, N.U., Kucukbasmac, O., 2010. Synthesis and anti microbial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2, 1-b] thiazole moiety. Eur. J. Med. Chem. 45, 63–68.

Heubach, G., Sachse, B., Buerstell, H., 1979. 1,2,4-Triazole derivatives. Ger. Offen. 2, 826, 760; Chem. Abstr. 1975, 92, 181200h.

Kelley, J.L., Koble, C.S., Davis, R.G., Mc Lean, E.W., Soroko, F.E., Cooper, B.R., 1995. 1-(Fluorobenzyl)-4-amino-1H-1,2,3-triazolo[4,5-c]pyridines: synthesis and Anticonvulsant activity. J. Med. Chem. 38, 4131–4134.

Khode, S., Maddi, V., Aragade, P., Palka, M., Ronad, P.K., Mamledesai, S., Thippeswamy, A.H.M., Satyanarayana, D., 2009. Synthesis and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-Coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents. Eur. J. Med. Chem. 44, 1682–1688.

Kucukguzel, I., Kucukguzel, S.G., Rollas, S., Kiraz, M., 2001. Some 3-thioxo/alkylthio-1,2,4-triazoles with a substituted thiourea moiety as possible antimycobacterials. Bioorg. Med. Chem. Lett. 11, 1703–1707.

Liaras, K., Geronikaki, A., Glamočlija, J., Irić, A.C., Sokovic, M., 2011. Thiazole-based chalcones as potent antimicrobial agents. Synthesis and biological evaluation. Bioorg. Med. Chem. 19, 3135–3140.

Mullican, M.D., Wilson, M.W., Conner, D.T., Kostlan, C.R., Schrier, D.J., Dyer, R.D., 1993. Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles as orally active nonulcerogenic anti-inflammatory agents. J. Med. Chem. 36, 1090–1099.

Prakash, O., Kumar, R., Parkash, V., 2008. Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones. Eur. J. Med. Chem. 43, 435–440.

Tozkoparan, B., Kupeli, E., Yesilada, E., Ertan, M., 2007. Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity. Bioorg. Med. Chem. 15, 1808–1814.

Xia, Y., Fan, C.D., Zhao, J., Shin, D.S., Miao, J.Y., 2008. Synthesis and structure–activity relationships of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells. Eur. J. Med. Chem. 43, 2347–2353.