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Synthesis and pharmacological evaluation of clubbed isopropylthiazole derived triazolothiadiazoles, triazolothiadiazines and mannich bases as potential antimicrobial and antitubercular agents

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ABSTRACT

A series of novel clubbed Isopropylthiazole derivatives triazolothiadiazines **2a**–**g**, dihydro triazolothiadiazoles **3a**–**g**, thioxotriazoles **4a**–**d**, triazolothiadiazole **5**, arylideneamino triazolethiones **7a**–**h** and oxadiazolethiones **11a**–**b** were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental and mass spectral analysis. These compounds were evaluated for their preliminary in vitro antibacterial, antifungal and antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv strain by broth dilution assay method.

All the compounds exhibited moderate to significant antibacterial and antifungal activities. Results of the antitubercular screening against *M. tuberculosis* H_{37}Rv showed compounds **7c** and **7d** exhibited good antitubercular activity (MIC 4 and 8 μg/mL) respectively, when compared with first line drug such as isoniazid (0.25 μg/mL).

1. Introduction

Tuberculosis (TB) still remains a major public health threat and recent world health organization (WHO) reports show that TB is responsible for more than three million deaths annually worldwide [1]. The raise is attributed to increase in emergence of drug-resistant strains of *Mycobacterium tuberculosis*, multi drug-resistant (MDR) TB and extensively drug-resistant (XDR) TB. For this reason it is critical to discover new drugs acting with different mechanism from those drugs used in current therapy [2,3].

One of the important strategies for designing effective antitubercular agents is to develop inhibitors of Mycobacterial cell-wall biosynthesis. The cell-wall of Mycobacteria consists of a wide array of complex fatty acids, such as mycocerosic acid, mycolic acid, arabinogalactans and peptidoglycans [4,5]. Azole derivatives have demonstrated interesting antimycobacterial activity in addition to antimicrobial activity. It is established that these compounds reach target by transmembrane diffusion because of its lipophilic property and target the sterol demthylase, a mixed function oxidase involved in sterol synthesis in eukaryotic organism [6,7]. Isopropylthiazole moiety has already been identified for its antimicrobial activity and its coupling with other heterocyclic rings furnishes novel biologically active compounds [8].

Multi-component reactions (MCRs) constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, increased reaction rates to higher yields and reproducibility [22,9]. Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a secondary amine [10]. The aminoalkylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [11,12]. Considering above facts and in continuation of our research on thiazoles [13–17], it was contemplated to synthesize a series of clubbed isopropylthiazole derivatives triazolothiadiazoles, triazolothiadiazines and mannich bases, study their cytotoxicity, antimicrobial (bacterial and fungal) and antitubercular activity against H37Rv strain. The synthetic route and the sequence of reactions are depicted in Schemes 1 and 2.

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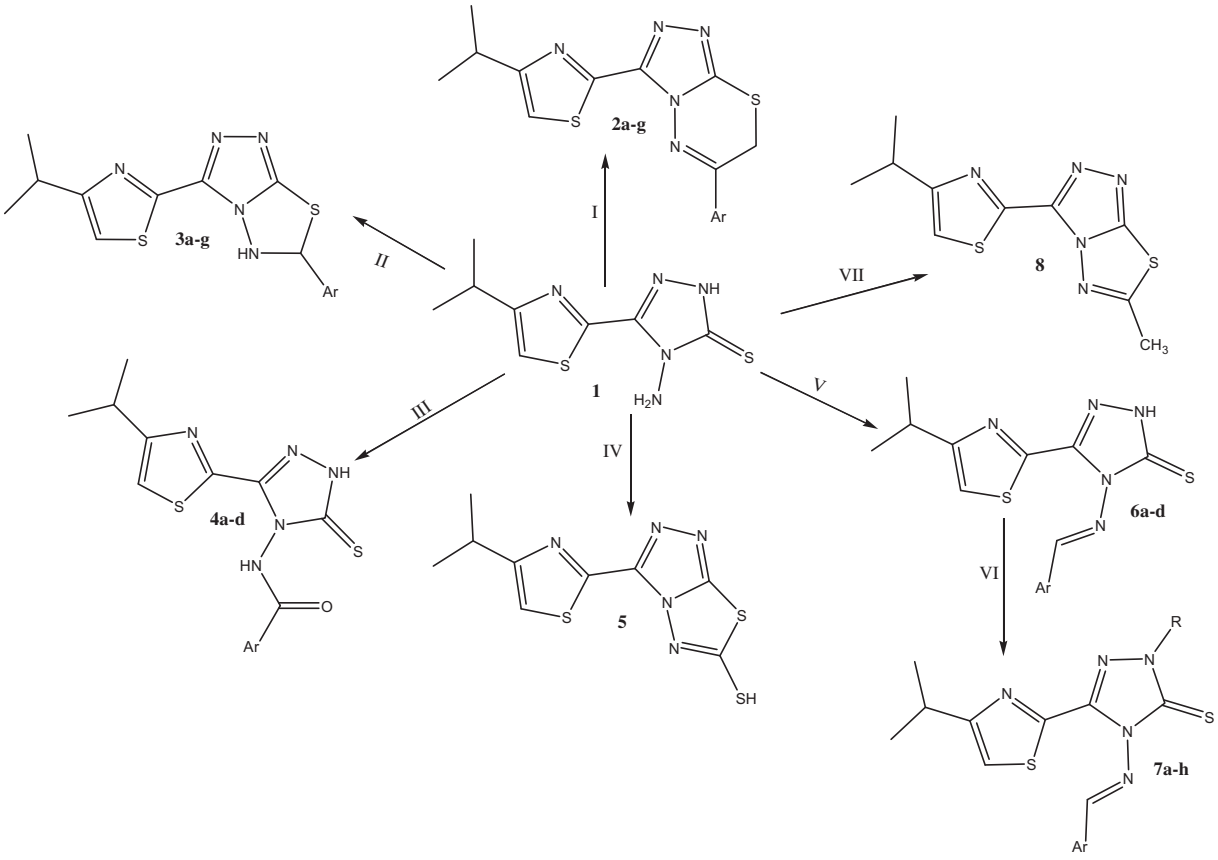
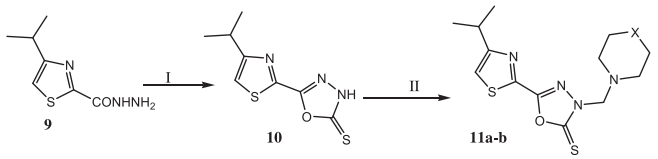


Scheme 1. Synthesis of 3-(4-isopropylthiazol-2-yl)-6-substituted triazolothiadiazines, triazolothiadiazoles and substituted benzylideneamino-triazole derivatives.

2. Chemistry

The reaction sequences employed for synthesis of target compounds are shown in Schemes 1 and 2, and their physical properties are depicted in Table 1. The key intermediates in the present study 4-amino-5-(4-isopropyl-1,3-thiazol-2-yl)-4*H*-1,2,4-triazole-3-thiol **1**, 4-arylideneamino-1,2,4-triazol-3(2H)-thione derivatives **6a**–**d**, 4-isopropylthiazole-2-carbohydrazide **9** and 5-(4-isopropylthiazol-2-yl)-1,3,4-oxadiazole-2(3H)-thione **10** were prepared as per the literature [18,19].

Condensation of triazole **1** with phenacyl bromides in presence of anhydrous sodium acetate and absolute ethanol produced a series of 3-(4-isopropylthiazol-2-yl)-6-substituted phenyl-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazines **2a**–**g**, condensation of compound **1** with appropriate substituted aldehydes in presence of *p*-toluenesulphonic acid produced a series of 5,6-dihydro fused triazolothiadiazoles **3a**–**g**. Triazole **1** when treated with substituted benzoyl chloride at 0 °C provided compounds **4a**–**d** in good yields. Chemical transformation of compound **1** to **5** was achieved by treating it with carbon disulfide and potassium hydroxide. Compounds **7a**–**h** was synthesized by reacting Schiff bases **6a**–**d** in one pot multi-component mannich reaction in presence of formaldehyde and morpholine or N-methylpiperazine in ethanol-dioxane medium.



Scheme 2. Synthesis of 5-(4-isopropylthiazol-2-yl)-3-substituted-1,3,4-oxadiazole-2 (3H)-thiones.

The synthesis of 3-(4-isopropylthiazol-2-yl)-6-methyl-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole **8** was achieved by reacting (4-amino-5-(4-isopropyl-1,3-thiazol-2-yl)-4*H*-1,2,4-triazole-3-thiol) triazole **1** with phosphorus oxychloride and acetic acid.

The Scheme 2 depicts synthesis of 5-(4-isopropylthiazol-2-yl)-3-(4-methylpiperazin-1-yl)-1,3,4-oxadiazole-2(3H)-thione **11a** and 5-(4-isopropylthiazol-2-yl)-3-(morpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione **11b** by reacting compound **10** with appropriate cyclic amines.

3. Biological activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of the synthesized compounds (**2a**–**g**, **3a**–**g**, **4a**–**d**, **5**, **7a**–**h**, **8** and **11a**–**b**) was performed by broth dilution method [20,21] against the following standard bacterial strains *Staphylococcus aureus* (ATCC 11632), *Streptococcus faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia* and *Pseudomonas aeruginosa* (ATCC 10145) and antifungal activity against Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT), mould: *Aspergillus niger* (ATCC 6275). MIC of compounds was determined against *M. tuberculosis* $H_{37}Rv$ strain by using broth dilution assay method. The cytotoxicity of the synthesized compounds were evaluated for their cytotoxic potential using A_{549} (lung adenocarcinoma) cell lines in the presence of fetal bovine serum.

Table 1
Analytical and physico-chemical data of synthesized compounds **2a**–**g**, **3a**–**g**, **4a**–**d**, **5**, **7a**–**h** and **11a**–**b**.

Compound	*X*	*R*	Molecular Formula	M.W.[a]	M.p. (°C)[b]/crystallization solvent	Yield (%)	% Analysis of C, H, N found (Calc.)[c]		
							C	H	N
2a	–	–	$C_{16}H_{15}N_5S_2$	341.45	224–227 Ethanol	81	56.28 (56.24)	4.43 (4.42)	20.51 (20.54)
2b	–	4-CH_3	$C_{17}H_{17}N_5S_2$	355.48	232–235 Ethanol	75	57.44 (57.43)	4.82(4.87)	19.70(19.71)
2c	–	4-OCH_3	$C_{17}H_{17}N_5OS_2$	371.48	216–219 Ethanol	72	54.96 (54.98)	4.61(4.65)	18.85 (18.82)
2d	–	4-OH	$C_{16}H_{15}N_5OS_2$	357.45	208–210 Ethanol	83	53.76 (53.78)	4.23(4.25)	19.59 (19.62)
2e	–	4-Cl	$C_{16}H_{14}ClN_5S_2$	375.9	202–205 Ethanol	80	51.12 (51.15)	3.75(3.77)	18.63 (18.65)
2f	–	4-NO_2	$C_{16}H_{14}N_6O_2S_2$	386.45	229–231 Ethanol	75	49.73 (49.76)	3.65(3.67)	21.75 (21.78)
2g	–	4-Br	$C_{16}H_{14}BrN_5S_2$	420.35	224–226 Ethanol	89	45.72 (45.70)	3.36(3.38)	16.66 (16.67)
3a	–	2-Cl	$C_{15}H_{14}ClN_5S_2$	363.04	238–240 Ethanol	74	49.51 (49.08)	3.88 (3.91)	19.25 (19.28)
3b	–	4-CH_3	$C_{16}H_{17}N_5OS_2$	359.09	222–225 Ethanol	83	53.46 (53.48)	4.77 (4.76)	19.48 (19.44)
3c	–	4-$N(CH_3)_2$	$C_{17}H_{20}N_6S_2$	372.12	230–234 Ethanol	71	54.81 (54.84)	5.41 (5.44)	22.56 (22.59)
3d	–	3,4,5-OCH_3	$C_{18}H_{21}N_5O_3S_2$	419.52	218–221 Ethanol	69	51.53 (51.55)	5.05 (5.08)	16.69 (16.67)
3e	–	2,4-F	$C_{15}H_{13}F_2N_5S_2$	365.06	254–257 Ethanol	74	49.30 (49.34)	3.59 (3.58)	10.40 (10.42)
3f	–	4-OH	$C_{15}H_{15}N_5OS_2$	345.44	221–225 Ethanol	70	52.15 (52.09)	4.38 (4.34)	20.27 (20.29)
3g	–	4-NO_2	$C_{15}H_{14}N_6O_2S_2$	374.44	234–236 Ethanol	87	48.11 (48.16)	3.77 (3.75)	22.44 (22.47)
4a	–	–	$C_{15}H_{15}N_5OS_2$	345.07	281–285 Ethanol	74	52.15 (52.16)	4.38 (4.36)	20.27 (20.31)
4b	–	4-CH_3	$C_{16}H_{17}N_5OS_2$	359.47	265–268 Ethanol	75	53.46(53.44)	4.77 (4.79)	19.48 (19.51)
4c	–	4-OH	$C_{15}H_{15}N_5O_2S_2$	361.44	254–256 Ethanol	79	49.84 (49.88)	4.18 (4.19)	19.38 (19.37)
4d	–	4-OCH_3	$C_{16}H_{17}N_5O_2S_2$	375.47	248–250 Ethanol	72	51.18 (51.14)	4.56 (4.19)	18.65 (18.66)
5	–	–	$C_9H_9N_5S_3$	283.4	212–214 Ethanol	82	38.14 (38.13)	3.20 (3.21)	24.71 (24.75)
7a	N–CH_3	2-Cl	$C_{21}H_{26}ClN_7S_2$	476.06	156–158 Ethanol: dioxane (2:1)	65	52.98 (52.94)	5.50 (5.53)	20.60 (20.66)
7b	N–CH_3	4-OCH_3	$C_{22}H_{29}N_7OS_2$	471.19	173–175 Ethanol: dioxane (2:1)	68	56.02 (56.08)	6.20 (6.24)	20.79 (20.81)
7c	N–CH_3	4-$N(CH_3)_2$	$C_{23}H_{32}N_8S_2$	484.68	150–152 Ethanol: dioxane (2:1)	65	57.00 (57.03)	6.65 (6.66)	23.12 (23.10)
7d	N–CH_3	3,4,5-OCH_3	$C_{24}H_{33}N_7O_3S_2$	531.69	161–163 Ethanol: dioxane (2:1)	67	54.21 (54.25)	6.26 (6.24)	18.44 (18.45)
7e	O	2-Cl	$C_{20}H_{23}ClN_6OS_2$	463.02	148–150 Ethanol: dioxane (2:1)	64	51.88 (51.89)	5.01 (5.09)	18.15 (18.10)
7f	O	4-OCH_3	$C_{21}H_{26}N_6O_2S_2$	458.6	154–157 Ethanol: dioxane (2:1)	68	55.00 (55.05)	5.71 (5.78)	18.33 (18.39)
7g	O	4-$N(CH_3)_2$	$C_{22}H_{29}N_7OS_2$	471.64	157–159 Ethanol: dioxane (2:1)	70	56.02 (56.09)	6.20 (6.23)	20.79 (20.80)
7h	O	3,4,5-OCH_3	$C_{23}H_{30}N_6O_4S_2$	518.65	159–161 Ethanol: dioxane (2:1)	67	53.26 (53.28)	5.83 (5.88)	16.20 (16.18)
8	–	–	$C_{10}H_{11}N_5S_2$	265.05	255–258 Dimethylsulfoxide/water (1:1)		45.26 (45.28)	4.18(4.19)	26.39(26.35)
11a	N–CH_3	–	$C_{13}H_{19}N_5OS_2$	325.1	218–220 Ethanol: dioxane (2:1)	81	47.98 (47.89)	5.88 (5.87)	21.52 (21.57)
11b	O	–	$C_{13}H_{18}N_4O_2S_2$	326.09	226–229 Ethanol: dioxane (2:1)	83	47.83 (47.86)	5.56 (5.59)	17.16 (17.19)

[a] Molecular weight of the compound.
[b] Melting point of the compound at their decomposition.
[c] Elelmental analysis of C, H, and N were with in ±0.4% of theoretical value.

Table 2
Antibacterial and antifungal activity of compounds **2a**–**g**, **3a**–**g**, **4a**–**d**, **5**, **7a**–**h** and **11a**–**b** expressed as MIC (μg/mL).

Compounds	Gram-positive organisms[a]			Gram-negative organisms[b]			Fungi[c]		
	Sa	Sf	Bs	Kp	Ec	Pa	Sc	Ct	An
2a	125	125	62.5	125	125	62.5	62.5	125	62.5
2b	16	31.25	31.25	8	8	8	62.5	31.25	31.25
2c	8	4	8	16	16	8	16	31.25	16
2d	62.5	31.25	31.25	16	31.25	62.5	16	31.25	31.25
2e	125	8	8	16	125	16	16	62.5	31.25
2f	125	125	16	31.25	16	125	16	31.25	62.5
2g	125	31.25	31.25	16	31.25	62.5	62.5	31.25	125
3a	31.25	31.25	125	31.25	62.5	62.5	125	16	31.25
3b	31.25	16	62.5	250	125	62.5	62.5	125	125
3c	16	31.25	62.5	125	62.5	62.5	31.25	31.25	31.25
3d	31.25	31.25	31.25	62.5	125	31.25	16	16	31.25
3e	8	4	4	31.25	16	16	62.5	125	31.25
3f	62.5	62.5	31.25	62.5	62.5	62.5	62.5	125	125
3g	125	125	62.5	31.25	62.5	125	16	8	8
4a	16	31.25	31.25	125	31.25	16	62.5	31.25	16
4b	62.5	125	62.5	62.5	62.5	125	62.5	125	62.5
4c	125	62.5	31.25	31.25	31.25	31.25	31.25	125	125
4d	62.5	125	62.5	16	8	8	4	8	16
5	125	31.25	16	31.25	62.5	31.25	16	8	16
7a	16	31.25	125	16	125	31.25	125	125	62.5
7b	31.25	16	8	31.25	31.25	16	16	31.25	31.25
7c	31.25	4	8	4	16	31.25	16	31.25	8
7d	16	8	4	16	8	31.25	125	62.5	16
7e	31.25	62.5	125	16	62.5	62.5	125	125	31.25
7f	125	62.5	125	31.25	125	16	62.5	31.25	16
7g	16	31.25	16	31.25	31.25	31.25	62.5	31.25	16
7h	125	62.5	62.5	16	125	62.5	125	62.5	62.5
8	62.5	31.25	31.25	16	125	62.5	62.5	31.25	31.25
11a	62.5	31.25	31.25	16	31.25	16	62.5	31.25	16
11b	31.25	16	16	31.25	31.25	16	31.25	31.25	62.5
Ciprofloxacin	≤5	≤5	≤1	≤1	≤1	≤5	–	–	–
Norfloxacin	≤5	≤5	≤1	≤1	≤1	≤5	–	–	–
Flucanozole	–	–	–	–	–	–	≤1	≤1	≤1

[a] The screening organisms. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632, Sa), *Streptococus faecalis* (ATCC 14506, Sf), and *Bacillus subtilis* (ATCC 60511, Bs).
[b] The screening organisms. Gram-negative bacteria: *Klebsiella penumoniae* (ATCC 10031, Kp), *Escherichia coli* (ATCC 10536, Ec), and *Pseudomonas aeruginosa* (ATCC 10145, Pa).
[c] The screening organisms. Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, Ct), mould: *Aspergillus niger* (ATCC 6275, An).

4. Results and discussion

The results of antimicrobial testing of synthesized compounds against selected gram +ve, gram −ve bacteria, yeasts, moulds and *M. tuberculosis* H_{37}Rv are illustrated in Tables 2 and 3 respectively.

The prepared compounds **2a**–**g**, **3a**–**g**, **4a**–**d**, **5**, **8** and **11a**–**b** exhibited interesting trends in Structure Activity Relationship (SAR) studies. The linkage between NH_2 and SH groups depicted moderate antimicrobial activity (3**a**–**g**), whereas the aforementioned moieties linked through methylene spacer (**2a**–**g**) exhibited significant activity against both Gram-positive and gram-negative strains. Interestingly, compound **2c** comprising *p-methoxy* substitution demonstrated improved antimicrobial activity against tested bacterial and fungal species and excellent activity against *M. tuberculosis* H37Rv at MIC 4 μg/mL.

The SAR studies and antimicrobial activity of thiadiazole series **3a**–**g** illustrate compound **3e** comprising *di fluoro* substitution exhibited excellent inhibition against *M. tuberculosis* H37Rv compared to its antifungal inhibition, this increased activity is attributed to presence of fluorine atoms (highly electro negative) in the molecule which increases the lipophilicity and affects the partitioning of a molecule into membranes and facilitates hydrophobic interactions of the molecule with specific binding sites on either receptor or enzymes [22–24]. Compound **3g** comprising electron withdrawing nitro group showed increased antifungal inhibition, but loss of activity against tested bacterial species. Although, compounds **3a** and **3f** which are having inductively electron withdrawing but mesomerically electron donating substituent's on phenyl group were found to be less active compounds against the tested microorganisms.

Comparing the antimicrobial activity of the synthesized 3-(4-isopropylthiazol-2-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl substituted benzamide derivatives **4a**–**d**, it was ascertained that the compounds were more effective against tested gram -ve bacteria and antifungal species compared to gram +ve bacteria. Particularly, compound **4d** comprising para-*methoxy* (electron withdrawing) substitution exhibited excellent inhibition at MIC 4–16 μg/mL against tested gram −ve bacteria. This excellent inhibition of compound **4d** is attributed to participation of the free electron pairs on the oxygen by resonance and increased electron density in the aromatic system. In contrarily compound **4b** containing electron donating para-*methyl* substitution exhibited loss of activity.

The Mannich bases **7a**–**h** exhibited varying degrees of inhibition against the tested microorganisms. The presence of N-methyl-piperazine moiety was found instrumental in contributing to the net biological activity of system and to increase antimicrobial activity of parent compound. Compounds **7c** and **7d** showed comparatively good activity against all tested microbial strains and excellent inhibition towards *M. tuberculosis* H_{37}Rv at MIC 4 and 8 μg/mL respectively. Compounds **7b** and **7g** possessing para-*methoxy* and para-*di methyl amino* substitution exhibited moderate activity at MIC 8–31.25 μg/mL. Compounds having oxadiazole rings **11a** and **11b** exhibited good to moderate antimicrobial activity at 16–62.5 μg/mL.

Table 3
Comparison of in vitro antimycobacterial activity of compounds **2a**–**g**, **3a**–**g**, **4a**–**d**, **5**, **7a**–**h** and **11a**–**b** against drug-sensitive Mycobacterium tuberculosis H37Rv strain.

Compound	MIC values (μg/mL) of *M. tuberculosis* H_{37}Rv
2a	125
2b	16
2c	4
2d	125
2e	16
2f	31.25
2g	62.5
3a	62.5
3b	31.25
3c	4
3d	62.5
3e	4
3f	125
3g	62.5
4a	16
4b	31.25
4c	62.5
4d	16
5	31.25
7a	31.25
7b	16
7c	4
7d	8
7e	31.25
7f	31,25
7g	16
7h	31.25
8	31.25
11a	16
11b	31.25
Isoniazid	0.25

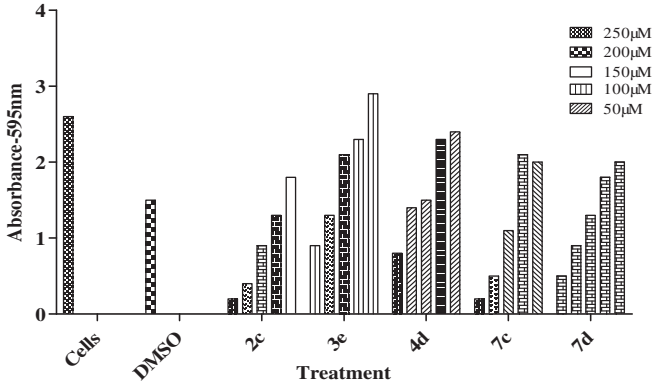


Fig. 1. Cytotoxic activity of compounds **2c**, **3e**, **4d**, **7c** and **7d** tested in A_{549} cells by MTT assay. The bars reflect the viable cells in each treatment. Cells represents, cells alone without any treatment, DMSO denote the vehicle control. The experiment was done in duplicate with triplicate readings of each experiment.

The most active antitubercular compounds **2c**, **3e**, **4d**, **7c** and **7d** were tested for their cytotoxic potential using A_{549} (lung adenocarcinoma) cell lines in the presence of fetal bovine serum. As shown in Fig. 1 compound **3e** showed maximum cytotoxicity at the concentration of 250 μM. The other compounds **2c**, **4d**, **7c** and **7d** showed appreciable cytotoxicity of about 50% of the vehicle control at a concentration of 250 μM.

5. Conclusion

In conclusion, this work demonstrates the synthesis of series of novel clubbed Isopropylthiazole derivatives triazolothiadiazines, dihydro triazolothiadiazoles, thioxotriazoles, triazolothiadiazoles, arylideneamino triazolethiones, oxadiazolethiones and in vitro evaluation of antimicrobial (bacterial and fungal) and antitubercular activity against H37Rv strain. Antimicrobial study revealed that compounds **2c**, **4d** and **7b** demonstrated significant activity against tested gram +ve and gram −ve bacteria and fungal species. The in vitro antituberculosis screening of these series showed that all compounds were active, in particular compounds **7c** and **7d** exhibited excellent antitubercular activity at MIC 4 and 8 μg/mL respectively, when compared with first line drug such as Isoniazid. The promising in vitro antimicrobial activity and low-toxicity

profile of clubbed Isopropylthiazole class of compounds make them certain promising molecules for further lead optimization in the development of novel antimycobacterial agents.

6. Experimental

6.1. Chemical protocols

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 157, ^{1}H NMR and ^{13}C NMR spectra were recorded (in $CDCl_3$/DMSO-d_6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. Mass spectra (EI) on (AMD-604) mass spectrometer operating at 70 eV. Elemental analysis was performed on Thermo Finnigan Flash (EA 1112 CHNS Analyzer).

Thin layer chromatography (TLC) was performed throughout the reaction on Merck silica gel GF_{254} aluminium sheets using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent.

6.1.1. General procedure for the synthesis of 3-(4-isopropylthiazol-2-yl)-6-substituted-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**2a**–**g**)

A mixture of triazole **1** (1.0 mmol) and substituted phenacyl bromide (1.2 mmol) in 15 ml of absolute ethanol and sodium acetate was refluxed for 6–7 h. The reaction mixture was cooled and slowly quenched onto crushed ice with stirring and neutralized with solid sodium bicarbonate. The solid which separated after standing overnight was filtered, washed with cold water, dried and recrystallized.

*6.1.1.1. 3-(4-Isopropylthiazol-2-yl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**2a**).* IR (KBr) ν max, cm^{-1}: 3087 (aromatic C–H), 1619 (C=N), 1237 (N–N=C), 684 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (5H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 3.06 (s, 2H, C_6 thiadiazine), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.43 (C_5-thiadiazine), 162.48 (thiazole-C_5), 145.24 (triazole-C_5), 135.76 (C_6-thiadiazine), 133.65 (C_4-phenyl), 131.76 (C_1-phenyl), 129.11 (phenyl C_3 and C_5), 127.87 (phenyl C_2 and C_6), 112.20 (thiazole-C_2), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 341.45 (100.0%), 342.08 (19.1%), 343.07 (9.1%), 342.07 (1.8%), 344.08 (1.6%).

*6.1.1.2. 3-(4-Isopropylthiazol-2-yl)-6-p-tolyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**2b**).* IR (KBr) ν max, cm^{-1}: 3075 (aromatic C–H), 1617 (C=N), 1266 (N–N=C), 689 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (4H, Ar-H), 7.23 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 3.11 (s, 2H, C_6 thiadiazine), 2.56 (s, 3H, CH_3 at C_4-phenyl), 1.24 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.43 (C_5-thiadiazine), 163.43 (thiazole-C_5), 144.43 (triazole-C_5), 135.76 (C_1-phenyl), 133.63 (C_4-phenyl), 128.43 (phenyl C_3 and C_5), 126.11 (phenyl C_2 and C_6), 111.48 (thiazole-C_2), 35.43 (C_6-thiadiazine), 32.43 (tertiary-1C-isopropyl), 24.16 (CH_3 at C_4-phenyl), 21.43 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 355.48 (100.0%), 356.10 (18.6%), 357.09 (9.4%), 356.09 (3.4%).

*6.1.1.3. 3-(4-Isopropylthiazol-2-yl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine (**2c**).* IR (KBr) ν max, cm^{-1}: 3086 (aromatic C–H), 1615 (C=N), 1268 (N–N=C), 683 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (4H, Ar-H), 7.31 (s, 1H, thiazole-C_5), 3.74 (s, 3H, OCH_3 at C_4-phenyl), 3.25 (m, 1H, isopropyl), 3.11(s, 2H, C_6 thiadiazine), 1.12 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.58 (C_5-thiadiazine), 163.54 (thiazole-C_5), 142.44 (triazole-C_5), 133.81 (C_4-phenyl), 133.25 (C_1-phenyl), 128.25 (phenyl C_3 and C_5), 126.87 (phenyl C_2 and C_6), 111.34 (thiazole-C_2), 55.94 (OCH_3 at C_4-phenyl), 35.25 (C_6-thiadiazine), 32.11 (tertiary-1C-isopropyl), 21.55 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 371.48 (100.0%), 372.09 (20.2%), 373.08 (9.1%), 374.09 (1.8%).

*6.1.1.4. 4-(3-(4-Isopropylthiazol-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)phenol (**2d**).* IR (KBr) ν max, cm^{-1}: 3078 (aromatic C–H), 1616 (C=N), 1274 (N–N=C), 677 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (4H, Ar-H), 7.25 (s, 1H, thiazole-C_5), 5.02 (s, OH at C_4-phenyl) 3.91 (s, 2H, C_6 thiadiazine), 3.40 (m, 1H, isopropyl), 1.39 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.21 (C_5-thiadiazine), 163.54 (thiazole-C_5), 143.02 (triazole-C_5), 133.58 (C_4-phenyl), 133.51 (C_1-phenyl), 128.58 (phenyl C_3 and C_5), 126.11 (phenyl C_2 and C_6), 111.02 (thiazole-C_2), 35.64 (C_6-thiadiazine), 32.45 (tertiary-1C-isopropyl), 21.79 (terminal $2CH_3$-isopropyl)ppm.

MS (%) 357.45 (100.0%), 238.02 (17.5%), 152.05 (1.7%).

*6.1.1.5. 6-(4-chlorophenyl)-3-(4-isopropylthiazol-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**2e**).* IR (KBr) ν max, cm^{-1}: 3085 (aromatic C–H), 1613 (C=N), 1232 (N–N=C), 674 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.4–7.9 (4H, Ar-H), 7.02 (s, 1H, thiazole-C_5), 3.97 (s, 2H, C_6 thiadiazine), 3.25 (m, 1H, isopropyl), 1.25 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.74 (C_5-thiadiazine), 163.24 (thiazole-C_5), 143.09 (triazole-C_5), 133.24 (C_4-phenyl), 131.51 (C_1-phenyl), 128.26 (phenyl C_3 and C_5), 126.58 (phenyl C_2 and C_6), 111.02 (thiazole-C_2), 32.56 (tertiary-1C-isopropyl), 35.21 (C_6-thiadiazine), 21.11 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 375.9 (100.0%), 377.03 (41.0%), 376.03 (3.2%), 376.03 (1.8%), 378.03 (1.3%).

*6.1.1.6. 3-(4-Isopropylthiazol-2-yl)-6-(4-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**2f**).* IR (KBr) ν max, cm^{-1}: 3097 (aromatic C–H), 1614 (C=N), 1236 (N–N=C), 674 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (4H, Ar-H), 7.58 (s, 1H, thiazole-C_5), 3.43 (m, 1H, isopropyl), 3.31 (s, 2H, C_6 thiadiazine), 1.25 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 164.35 (C_5-thiadiazine), 163.11 (thiazole-C_5), 143.12 (triazole-C_5), 133.58 (C_1-phenyl), 133.26 (C_4-phenyl), 128.14 (phenyl C_3 and C_5), 126.00 (phenyl C_2 and C_6), 111.18 (thiazole-C_2), 38.61 (C_6-thiadiazine), 32.55 (tertiary-1C-isopropyl), 21.21 (terminal $2CH_3$-isopropyl), ppm.

MS (%) 386.4 (100.0%), 377.03 (41.0%), 378.04 (7.2%), 379.03 (3.2%).

*6.1.1.7. 6-(4-bromophenyl)-3-(4-isopropylthiazol-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**2g**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C–H), 1613 (C=N), 1233 (N-N=C), 679 (C–S–C).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.4–7.9 (4H, Ar-H), 7.65 (s, 1H, thiazole-C_5), 3.43 (s, 2H, C_6 thiadiazine), 3.22 (m, 1H, isopropyl), 1.22 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.74 (C_5-thiadiazine), 163.11 (thiazole-C_5), 143.05 (triazole-C_5), 111.17 (thiazole-C_2), 128.24 (phenyl C_3 and C_5), 126.54 (phenyl C_2 and C_6), 133.24 (C_4-phenyl), 131.51 (C_1-phenyl), 32.95 (tertiary-1C-isopropyl), 35.21 (C_6-thiadiazine), 21.11 (terminal $2CH_3$-isopropyl), ppm.

MS (%) 418.99 (100.0%), 420.99 (99.3%), 422.98 (9.1%), 423.98 (1.8%).

*6.1.2. General procedure for the synthesis of 3-(4-isopropylthiazol-2-yl)-6-substituted 5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles (**3a**–**g**)*

An equimolecular mixture of triazole (0.02 mol) **1** and appropriate aldehyde (0.02 M), dry DMF (30 ml) and a catalytic amount of p-toluenesulphonic acid (10 mg) was taken in a round bottom flask. The mixture was refluxed for about 10–12 h, concentrated to half of its volume and cooled to room temperature. The cooled mixture was poured gradually onto crushed ice cubes with stirring. The mixture was allowed to stand and solid was separated. It was filtered, washed thoroughly with cold water, dried and recrystallized.

*6.1.2.1. 3-(4-Isopropylthiazol-2-yl)-6-(2-chlorophenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**3a**).* IR (KBr) ν max, cm^{-1}: 3370 (NH stretching), 3056 (aromatic CH stretching), 1618 (C=N stretching), 1580, 1535 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71–7.78 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.91(s, 1H, NH–**CH**–S), 3.26 (m, 1H, isopropyl), 2.11(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl)ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 141–105 (phenyl C_1 to C_6), 148.2 (C_3 of triazole), 145.24 (triazole-C_5), 115.48 (thiazole-C_5), 53.4(NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 363.04 (100.0%), 365.03 (41.0%), 364.04 (18.0%), 366.04 (6.8%), 367.03 (3.2%).

*6.1.2.2. 3-(4-Isopropylthiazol-2-yl)-6-(4-methoxyphenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**3b**).* IR (KBr) ν max, cm^{-1}: 3374 (NH stretching), 3052 (aromatic CH stretching), 1612 (C=N stretching), 1578, 1531 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71–7.78 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.91(s, 1H, NH–**CH**–S), 3.73(s, 3H, OCH_3), 3.26 (m, 1H, isopropyl), 2.15(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.2(C_3 of triazole), 145.44 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 55.82(OCH_3), 53.9(NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl), ppm.

MS (%) 359.09 (100.0%), 360.09 (19.1%), 361.08 (9.1%), 362.09 (1.6%).

*6.1.2.3. 4-(3-(4-Isopropylthiazol-2-yl)-5,6-dihydro-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)-N,N-dimethylbenzenamine (**3c**).* IR (KBr) ν max, cm^{-1}: 3377 (NH stretching), 3058 (aromatic CH stretching), 1615 (C=N stretching), 1582, 1537 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 6.3–6.8 (4H, Ar-H), 4.91(s, 1H, NH–**CH**–S), 3.26 (m, 1H, isopropyl), 2.81(s, 6H, N–$(CH_3)_2$), 2.13(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.24 (C_3 of triazole), 145.24 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 53.42(NH–C–S), 40.34 (N-$(CH_3)_2$), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 372.12 (100.0%), 373.12 (22.2%), 374.11 (9.1%), 375.12 (1.7%).

*6.1.2.4. 3-(4-Isopropylthiazol-2-yl)-6-(3,4,5-trimethoxyphenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**3d**).* IR (KBr) ν max, cm^{-1}: 3370 (NH stretching), 3052 (aromatic CH stretching), 1612 (C=N stretching), 1589, 1536 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 6.1 (2H, Ar-H), 4.91(s, 1H, NH–**CH**–S), 3.81(s, 9H, $3OCH_3$), 3.26 (m, 1H, isopropyl), 2.11(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.27(C_3 of triazole), 145.24 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 56.83($3OCH_3$), 53.4 (NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 419.51 (100.0%), 420.11 (23.3%), 421.10 (9.1%), 422.11 (1.9%).

*6.1.2.5. 6-(2,4-difluorophenyl)-3-(4-isopropylthiazol-2-yl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**3e**).* IR (KBr) ν max, cm^{-1}: 3370 (NH stretching), 3055 (aromatic CH stretching), 1619 (C=N stretching), 1589, 1539 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 6.6–6.9 (3H, Ar-H), 4.91(s, 1H, NH–**CH**–S), 3.26 (m, 1H, isopropyl), 2.11(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.25(C_3 of triazole), 145.24 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 53.42(NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 365.06 (100.0%), 366.06 (19.8%), 367.05 (9.1%), 368.06 (1.5%).

*6.1.2.6. 3-(3-(4-Isopropylthiazol-2-yl)-5,6-dihydro-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)phenol (**3f**).* IR (KBr) ν max, cm^{-1}: 3377 (NH stretching), 3053 (aromatic CH stretching), 1612 (C=N stretching), 1586, 1532 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 6.6–7.9 (4H, Ar-H), 5.11(s, 1H, OH), 4.91(s, 1H, NH–**CH**–S), 3.26 (m, 1H, isopropyl), 2.11(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.2 (C_3 of triazole), 145.24 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 53.43(NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl), ppm.

MS (%) 345.44 (100.0%), 347.07 (9.6%), 348.07 (1.6%).

*6.1.2.7. 3-(4-Isopropylthiazol-2-yl)-6-(4-nitrophenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**3g**).* IR (KBr) ν max, cm^{-1}: 3375 (NH stretching), 3057 (aromatic CH stretching), 1613 (C=N stretching), 1586, 1530 (C=C ring stretch).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.3–8.4 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.91(s, 1H, NH–**CH**–S), 3.26 (m, 1H, isopropyl), 2.11(s, 1H, N–NH), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl), ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.2(C_3 of triazole), 145.24 (triazole-C_5), 115–135 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 53.4(NH–C–S), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 374.44 (100.0%), 375.07 (16.5%), 376.06 (9.7%), 377.06 (1.6%).

*6.1.3. General procedure for the synthesis of 3-(4-isopropylthiazol-2-yl)-5-thioxo-1H-(1,2,4-triazol-4(5H)-yl) substituted benzamides (**4a**–**d**)*

The triazole **1** (0.01 mol) in 20 mL of 10% NaOH was treated dropwise with an equimolar amount of the substituted benzoyl chloride at 0 °C, which was stirred for 30–45 min. At the end of stirring, obtained precipitate was filtered, washed thoroughly with water and recrystallized.

*6.1.3.1. 3-(4-Isopropylthiazol-2-yl)-5-thioxo-1H-(1,2,4-triazol-4 (5H)-yl)benzamide (**4a**).* IR (KBr) ν max, cm^{-1}: 3049 (aromatic CH stretching), 3257 (NH), 1694 (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C−SH), 8.23 (s, 1H, N−NH), 7.7−7.8 (5H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 164.12 (C=O), 120−140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 345.07 (100.0%), 346.08 (15.1%), 348.07 (1.6%), 347.08 (1.5%).

*6.1.3.2. 3-(4-Isopropylthiazol-2-yl)-5-thioxo-1H-(1,2,4-triazol-4 (5H)-yl)-4-methylbenzamide (**4b**).* IR (KBr) ν max, cm^{-1}: 3040 (aromatic CH stretching), 3248 (NH), 1691 (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C−SH), 8.23 (s, 1H, N−NH), 7.7−7.8 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.32 (m, 3H, $-CH_3$), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 164.12 (C=O), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120−140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 32.49 (tertiary-1C-isopropyl), 24.15 (CH3), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 359.09 (100.0%), 360.09 (16.1%), 361.08 (7.1%), 362.09 (1.4%).

*6.1.3.3. 4-Hydroxy-N-(3-(4-isopropylthiazol-2-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzamide (**4c**).* IR (KBr) ν max, cm^{-1}: 3043 (aromatic CH stretching), 3251 (NH), 1696 (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C−SH), 7.62 (s, 1H, thiazole-C_5), 8.23 (s, 1H, N−NH), 7.7−7.8 (4H, Ar-H), 5.21 (s, 1H, −OH), 3.26 (m, 1H, isopropyl), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 164.12 (C=O), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120−140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 361.44 (100.0%), 362.07 (18.1%), 363.07 (2.2%), 364.07 (1.6%).

*6.1.3.4. 3-(4-Isopropylthiazol-2-yl)-5-thioxo-1H-(1,2,4-triazol-4 (5H)-yl)-4-methoxybenzamide (**4d**).* IR (KBr) ν max, cm^{-1}: 3045 (aromatic CH stretching), 3255 (NH), 1692 (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.59 (s, 1H, N=C−SH), 8.23 (s, 1H, N−NH), 7.7−7.8 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 3.71 (s, 1H, $-OCH_3$), 13.26 (m, 1H, isopropyl), 21.7 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 164.12 (C=O), 115.48 (thiazole-C_5), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120−140 (phenyl C_1 to C_6), 55.15 ($-OCH_3$), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 375.08 (100.0%), 376.09 (14.6%), 377.08 (7.4%), 377.09 (1.1%).

*6.1.4. General preparation of 3-(4-isopropylthiazol-2-yl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione (**5**)*

Carbon disulfide (0.015 mol) was added dropwise with constant stirring to the solution of **1** (0.01 mol) in methanolic potassium hydroxide. Refluxed for 6-7h, mixture was cooled and then product was extracted with dry methanol, which was then poured onto ice and acidified with dil. hydrochloric acid and recrystalized.

IR (KBr) ν max, cm^{-1}: 3375 (NH stretching), 2598 (−SH), 1608, 1587, 1543 and 1432 (C=N stretching).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 13.50 (s, −SH), 7.71 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 223.4(C−SH), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.2(C_3 of triazole), 145.24 (triazole-C_5), 115.48 (thiazole-C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 283.00 (100.0%), 285.00 (14.0%), 284.01 (9.8%).

*6.1.5. General procedure for the synthesis of 4-(substituted benzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-substituted-2H-1,2,4-triazole-3(4H)-thione (**7a**–**h**)*

The appropriate compounds **6a**–**d** (10 mmol) was dissolved in a mixture of ethanol and dioxane (2:1). Then, formaldehyde (40%, 1.5 ml) and suitable cyclic amine (10 mmol) in ethanol was added to the solution. The mixture was stirred for 2−3 h and kept overnight at room temperature. The resulting solid that separated was collected by filtration, washed with ethanol and recrystalised.

*6.1.5.1. 4-(2-chlorobenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-((4-methylpiperazin-1-yl)methyl)-2H-1,2,4-triazole-3(4H)-thione (**7a**).* IR (KBr) ν max, cm^{-1}: 3087 (aromatic C−H), 2949 (CH_2), 1618 (CH=N), 1243 (C=S), 1179 (N−CH_2−N), 1065 (CH_2−O−CH_2), 1042 (N−N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.12 (s, 1H, N=CH), 7.4−7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.52 (s, 2H, N−CH_2−N), 3.26 (m, 1H, isopropyl), 3.00 (t, 4H, CH_2−N−CH_2), 2.68 (t, 4H, CH_2−N−CH_2), 2.46 (s, 3H, N−CH_3 of piperazine), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl), ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 134.12 (s, 1H, N=CH), 120−140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 61.1 (N−CH_2−N), 54.9 (piperazine C_3 and C_5), 50.4 (piperazine C_2 and C_6), 44.9 (piperazine N−CH_3), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 475.14 (100.0%), 477.14 (35.4%), 113.04 (9.6%), 479.13 (3.2%), 478.13 (1.6%).

*6.1.5.2. 4-(4-methoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-((4-methylpiperazin-1-yl)methyl)-2H-1,2,4-triazole-3(4H)-thione (**7b**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C−H), 2954 (CH_2), 1608 (CH=N), 1227 (C=S), 1179 (N−CH_2−N), 1072 (CH_2−O−CH_2), 1022 (N−N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.12 (s, 1H, N=CH), 7.4−7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.56 (s, 1H, N−CH_2−N), 3.74 (s, 3H, OCH_3 at C_4-phenyl), 3.26 (m, 1H, isopropyl), 3.12 (t, 4H, CH_2−N−CH_2), 2.74 (t, 4H, CH_2−N−CH_2), 2.46 (s, 3H, N−CH_3 of piperazine), 1.27 (d, J = 8.5 Hz, 6H, terminal $2CH_3$ of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120−140 (phenyl C_1 to C_6), 134.12 (s, 1H, N=CH), 115.48 (thiazole-C_5), 63.1 (N−CH_2−N), 55.9 (OCH_3 at C_4-phenyl), 50.4 (piperazine C_2 and C_6), 44.9 (piperazine N−CH_3), 44.1 (piperazine C_3 and C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 471.19 (100.0%), 472.19 (25.8%), 113.0 (9.6%), 473.19 (3.9%), 472.18 (2.6%), 474.19 (2.3%).

*6.1.5.3. 4-(4-(dimethylamino)benzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-((4-methylpiperazin-1-yl)methyl)-2H-1,2,4-triazole-3(4H)-thione (**7c**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C−H), 2954 (CH_2), 1608 (CH=N), 1227 (C=S), 1179 (N−CH_2−N), 1072 (CH_2−O−CH_2), 1022 (N−N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.12 (s, 1H, N=CH), 7.4–7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.55 (s, 2H, N–CH_2–N), 3.26 (m, 1H, isopropyl), 3.15 (t, 4H, CH_2–N–CH_2), 2.84(s, 6H, N(CH_3)$_2$ at C_4-phenyl), 2.65 (t, 4H, CH_2–N–CH_2), 2.46 (s, 3H, N–CH_3 of piperazine), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 134.12 (s, 1H, N=CH), 120–140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 62.8 (N–CH_2–N), 50.4 (piperazine C_2 and C_6), 44.9 (piperazine N–CH_3), 43.9 (piperazine C_3 and C_5), 40.5 (N(CH_3)$_2$ at C_4-phenyl), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 484.22 (100.0%), 485.22 (29.4%), 113.02 (9.1%), 486.21 (9.1%).

*6.1.5.4. 4-(3,4,5-trimethoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-((4-methylpiperazin-1-yl)methyl)-2H-1,2,4-triazole-3(4H)-thione (**7d**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C–H), 2954 (CH_2), 1608 (CH=N), 1227 (C=S), 1179 (N–CH_2–N), 1072 (CH_2–O–CH_2), 1022 (N–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl), 3.26 (m, 1H, isopropyl), 8.12 (s, 1H, N=CH), 7.71 (s, 1H, thiazole-C_5), 7.4–7.9 (2H, Ar-H), 4.58 (s, 2H, N–CH_2–N), 3.84 (s, 3H, 3OCH_3 at $C_{3,4,5}$-phenyl), 3.62 (t, 4H, CH_2–N–CH_2), 3.03 (t, 4H, CH_2–N–CH_2), 2.46 (s, 3H, N–CH_3 of piperazine) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120–140 (phenyl C_1 to C_6), 115.48 (thiazole-C_5), 134.12 (s, 1H, N=CH), 62.3 (N–CH_2–N), 56.5 (3OCH_3 at $C_{3,4,5}$-phenyl), 50.4 (piperazine C_2 and C_6), 47.2 (piperazine C_3 and C_5), 44.9 (piperazine N–CH_3), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 531.21 (100.0%), 532.21 (30.6%), 113.03 (9.3%), 533.22 (3.4%), 534.21 (2.6%), 533.21 (1.8%).

*6.1.5.5. 4-(2-chlorobenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7e**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C–H), 2954 (CH_2), 1608 (CH=N), 1227 (C=S), 1179 (N–CH_2–N), 1072 (CH_2–O–CH_2), 1022 (N–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.12 (s, 1H, N=CH), 7.4–7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.12 (s, 2H, N–CH_2–N), 3.65 (t, J = 4.5 Hz, 4H, morpholine residue), 3.26 (m, 1H, isopropyl), 2.46 (t, J = 4.5 Hz, 4H, morpholine residue), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 174.12 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 120–140 (phenyl C_1 to C_6), 145.24 (triazole-C_5), 144.12 (N=CH), 115.48 (thiazole-C_5), 65.4 (N–CH_2–N), 50.4, 66.3 (morpholine residue), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl)ppm.

MS (%) 462.11 (100.0%), 464.10 (41.1%), 113.1 (9.3%), 463.10 (2.2%).

*6.1.5.6. 4-(4-methoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7f**).* IR (KBr) ν max, cm^{-1}: 3090 (aromatic C–H), 2954 (CH_2), 1610 (CH=N), 1224 (C=S), 1173 (N–CH_2–N), 1076 (CH_2–O–CH_2), 1027 (N–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.12 (s, 1H, N=CH), 7.4–7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.17 (s, 2H, N–CH_2–N), 3.67 (t, J = 4.5 Hz, 4H, morpholine residue), 3.74(s, 3H, OCH_3 at C_4-phenyl), 3.26 (m, 1H, isopropyl), 2.42 (t, J = 4.5 Hz, 4H, morpholine residue), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 171.12 (C=S of triazole), 164.48 (thiazole-C_4), 152.20 (thiazole-C_2), 139.42 (s, 1H, N=CH), 120–140 (phenyl C_1 to C_6), 145.24 (triazole-C_5), 62.8 (N–CH_2–N), 113.48 (thiazole-C_5), 55.9 (OCH_3 at C_4-phenyl), 50.2, 66.5 (morpholine residue), 32.49 (tertiary-1C-isopropyl), 21.14 (terminal 2CH_3-isopropyl) ppm.

MS (%) 458.16 (100.0%), 459.16 (24.7%), 460.15 (9.1%), 459.15 (2.2%).

*6.1.5.7. 4-(4-(dimethylamino)benzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-morpholinomethyl-2H-1,2,4-triazole-3(4H)-thione (**7g**).* IR (KBr) ν max, cm^{-1}: 3082 (aromatic C–H), 2963 (CH_2), 1610 (CH=N), 1224 (C=S), 1173 (N–CH_2–N), 1076 (CH_2–O–CH_2), 1027 (N–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.12 (s, 1H, N=CH), 7.71 (s, 1H, thiazole-C_5), 7.4–7.9 (4H, Ar-H), 4.25 (s, 2H, N–CH_2–N), 3.67 (t, J = 4.5 Hz, 4H, morpholine residue), 3.26 (m, 1H, isopropyl), 2.84 (s, 6H, N(CH_3)$_2$ at C_4-phenyl), 2.42 (t, J = 4.5 Hz, 4H, morpholine residue), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 173.72 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120–140 (phenyl C_1 to C_6), 134.12 (s, 1H, N=CH), 115.48 (thiazole-C_5), 66.8 (N–CH_2–N), 50.1, 66.9 (morpholine residue), 40.5 (N(CH_3)$_2$ at C_4-phenyl), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 471.19 (100.0%), 472.19 (25.8%), 473.18 (9.1%), 474.19 (2.3%).

*6.1.5.8. 4-(3,4,5-trimethoxybenzylideneamino)-5-(4-isopropylthiazol-2-yl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (**7h**).* IR (KBr) ν max, cm^{-1}: 3082 (aromatic C–H), 2963 (CH_2), 1610 (CH=N), 1224 (C=S), 1173 (N–CH_2–N), 1076 (CH_2–O–CH_2), 1027 (N–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.12 (s, 1H, N=CH), 7.4–7.9 (4H, Ar-H), 7.71 (s, 1H, thiazole-C_5), 4.52 (s, 2H, N–CH_2–N), 3.84(s, 9H, 3OCH_3 at $C_{3,4,5}$-phenyl), 3.67 (t, J = 4.5 Hz, 4H, morpholine residue), 3.26 (m, 1H, isopropyl), 2.42 (t, J = 4.5 Hz, 4H, morpholine residue), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 184.12 (C=S of triazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 145.24 (triazole-C_5), 120–140 (phenyl C_1 to C_6), 134.12 (s, 1H, N=CH), 115.48 (thiazole-C_5), 59.8 (N–CH_2–N), 56.5 ($3OCH_3$ at $C_{3,4,5}$-phenyl), 50.2, 66.3 (morpholine residue), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 518.18 (100.0%), 519.18 (27.0%), 520.17 (9.1%), 520.18 (4.8%), 521.18 (2.7%), 519.17 (2.2%).

*6.1.6. General method for the synthesis of 3-(4-isopropylthiazol-2-yl)-6-methyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **(8)***

To a mixture of compound **1** (0.01 mol) and acetic acid (0.01 mol), phosphorus oxychloride (10 mL) was added and the reaction contents were refluxed for 2 h on a water bath. After removing the excess of phosphorus oxychloride under reduced pressure, ice water was added to residue with vigorous stirring. The precipitate was filtered off and washed with 20% sodium bicarbonate solution and water and recrystalized.

IR (KBr) ν max, cm^{-1}: 1603, 1595, 1557 and 1446 (C=N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 3.26 (m, 1H, isopropyl), 2.62 (s, –CH_3), 1.27 (d, J = 8.5 Hz, 6H, terminal 2CH_3 of isopropyl), ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 148.2(C_3 of triazole), 145.24 (triazole-C_5), 115.48 (thiazole-C_5), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl), 13.55 (CH_3) ppm.

MS (%) 265.05 (100.0%), 266.05 (10.9%), 267.04 (9.1%).

*6.1.7. General procedure for the synthesis of 5-(4-isopropylthiazol-2-yl)-3-(4-substituted)-1,3,4-oxadiazole-2(3H)-thione **(11a–b)***

The compound **10** (10 mmol) was dissolved in a mixture of ethanol and dioxane (2:1). Then, formaldehyde (40%, 1.5 ml) and suitable secondary amine (10 mmol) in ethanol was added to the

solution. The mixture was stirred for 2–3 h and kept overnight at room temperature and resulting solid is washed with ethanol and recrystalized.

*6.1.7.1. 5-(4-Isopropylthiazol-2-yl)-3-(4-methylpiperazin-1-yl)-1,3,4-oxadiazole-2(3H)-thione (**11a**).* IR (KBr) ν max, cm^{-1}: 3092 (aromatic C–H), 2954 (CH_2), 1227 (C=S), 1179 (N–CH_2–N).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 4.52 (s, 2H, N–CH2–N), 3.26 (m, 1H, isopropyl), 2.46 (s, 8H, of piperazine), 2.26 (s, 3H, N–CH_3 of piperazine), 1.27 (d, $J = 8.5$ Hz, 6H, terminal 2CH_3 of isopropyl), ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 174.12 (C=S of oxadiazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 115.48 (thiazole-C_5), 72.8 (N–CH_2–N), 54.9 (piperazineC_3 and C_5), 50.4 (piperazineC_2 and C_6), 44.9 (piperazine N–CH_3), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 325.10 (100.0%), 326.11 (14.3%), 113.1 (9.3%), 327.11 (1.4%).

*6.1.7.2. 5-(4-Isopropylthiazol-2-yl)-3-(morpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione (**11b**).* IR (KBr) ν max, cm^{-1}: 2961 (CH_2), 1222 (C=S), 1171 (N–CH_2–N), 1072 (CH_2–O–CH_2).

^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.71 (s, 1H, thiazole-C_5), 3.72 (s, 2H, N–CH_2–N), 3.26 (m, 1H, isopropyl), 2.46 (s, 4H of morpholine), 3.31 (s, 4H of morpholine), 1.27 (d, $J = 8.5$ Hz, 6H, terminal 2CH_3 of isopropyl) ppm.

^{13}C NMR (DMSO-d_6, 300 MHz) δ: 174.12 (C=S of oxadiazole), 165.48 (thiazole-C_4), 152.20 (thiazole-C_2), 115.48 (thiazole-C_5), 66.1 (N–CH_2–N), 66.4 (morpholine C_2 and C_6), 50.9 (morpholine C_3 and C_5), 44.9 (piperazine N–CH_3), 32.49 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl)ppm.

MS (%) 326.09 (100.0%), 327.09 (15.9%), 328.08 (9.1%).

6.2. Biological protocol

6.2.1. Antimicrobial activity

The antimicrobial susceptibility testing was performed in vitro by broth microdilution method [25–27]. The MIC determination of the synthesized compounds was carried out in side-by-side comparison with ciprofloxacin and norfloxacin against Gram-positive bacteria (*S. aureus*, *S. faecalis*, *B. subtilis*) and Gram-negative (*Klebsiella penumoniae*, *Escherichia coli*. *P. aeruginosa*). The antifungal activity was assayed against yeasts (*C. tropicalis*, *S. cerevisiae*) and moulds (*A. niger*). The minimal inhibitory concentrations (MIC, μg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Test compounds (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL) then diluted in culture medium (Mueller-hinton broth for bacteria and Sabouraud liquid medium for fungi), further progressive dilutions to obtain final concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 μg/mL. DMSO never exceeded 1% v/v. The tubes were inoculated with 10^5 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 °C for 24h. The growth control consisting of media (positive control) and media with DMSO (negative control) at the same dilutions as used in the experiments were employed to compare with Ciprofloxacin and Norfloxacin (antibacterial control standards) and Flucanozole (antifungal control standard).

6.2.2. Antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for *M. tuberculosis* $H_{37}Rv$, the MIC of each drug was determined by broth dilution assay [28,29] and is defined as the lowest concentration of drug, which inhibits ≤99% of bacterial population present at the beginning of the assay. A frozen culture in Middlebrook 7H9 broth supplemented with 10% albumin–dextrose–catalase and 0.2% glycerol was thawed and diluted in broth to 10^5 cfu mL^{-1} (colony forming unit/mL) dilutions. Each test compound was dissolved in DMSO and then diluted in broth twice at the desired concentration. The final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard isoniazid.

6.2.3. MTT assay for cell viability

The toxicity of most active antitubercular compounds **2c**, **3e**, **4d**, **7c** and **7d** in A_{549} cell lines in the presence of 10% and 0.2% FBS, respectively, was determined using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide reduction assay [30,31]. The compounds were dissolved in DMSO at 10 mM concentration and stored at −20°. The dilutions were made in culture medium before treatment.

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