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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and pharmacological evaluation of novel 4-isopropylthiazole-4-phenyl-1,2,4-triazole derivatives as potential antimicrobial and antitubercular agents

G. V. Suresh Kumar · Y. Rajendra Prasad · S. M. Chandrashekar

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Abstract A series of novel 4-isopropylthiazol-4-phenyl-1,2,4-triazol derivatives, *N*′-(substituted benzylidene)-2-(5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-acetohydrazides **4a–e**, 4-isopropylthiazol-2-yl-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio (substituted methyl benzylidene) acetohydrazides **5a–f**, 3-(4-isopropylthiazol-2-yl)-4-phenyl-5-(5-substituted-1,3,4-oxadiazol-2-ylthio)-4H-1,2,4-triazole derivatives **6a–f** and *N*-acetyl-5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1,2,4-triazol-3′-ylthio) acetohydrazide **7** were synthesized and characterized by spectroscopy, elemental, and mass spectral analysis. These compounds were evaluated for their preliminary in vitro antibacterial, antifungal, and antitubercular activity against *Mycobacterium tuberculosis* (*M. tuberculosis*) H37Rv strain by broth dilution assay method. All the compounds exhibited moderate to significant antibacterial and antifungal activities. Results of the antitubercular screening against *Mycobacterium tuberculosis* H37Rv showed that compounds **4c** and **6c** exhibited good antitubercular activity when compared with first line drug isoniazid.

Keywords 4-Isopropylthiazole · 1,2,4-Triazole · Cytotoxicity · Antimicrobial · Antitubercular activity

Introduction

Tuberculosis (TB), a contagious infection caused by *Mycobacterium tuberculosis* (MTB), still remains the leading cause of the worldwide death among the infectious disease (Espinal, 2003; Mitchison, 1993). The synergy between tuberculosis and the AIDS epidemic as well as the surge of multidrug-resistant isolates of MTB has reaffirmed tuberculosis as a primary public health threat (Young and Cole, 1993; Bloom and Murray, 1992).The current threat in TB treatment lies in the emergence of strains resistant to two of the best antitubercular drugs, isoniazid (INH) and rifampicin (RIF). The current TB treatment comprises of 3–4 drugs for a period of 6–9 months. Novel drugs are urgently required which can shorten this long-treatment period and target multidrug-resistant strains of TB.

A key target for antimycobacterial chemotherapy is cell wall biosynthesis. The cell envelope of MTB comprises four classes of polymer (peptidoglycan, arabinogalactan, mycolic acids, and lipoarabinomanan). This complex lipoglycan calyx on cell surface provides a significant physical barrier to intracellular-acting drugs (Kuo *et al.*, 2003). Azole derivatives have demonstrated interesting antitubercular activity in addition to its antimicrobial activity. It is established that these compounds reach target by transmembrane diffusion because of its lipophilic property and target the sterol demethylase, a mixed

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G. V. Suresh Kumar (✉)
Department of Medicinal Chemistry, St Johns Pharmacy College, 6, 2nd Stage Vijaynagar, R.P.C Layout, Bangalore 560 040, Karnataka, India
e-mail: gvsureshkumar@yahoo.com

Y. Rajendra Prasad
AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India
e-mail: dryrp_au@rediffmail.com

S. M. Chandrashekar
Poornaprajna Institute of Scientific Research (PPISR), Poornaprajnapura, Post: Bidalur, Ta: Devanahalli, Near Woodrich Resort, Bangalore 562 110, Karnataka, India
e-mail: Chandra_jan25@yahoo.co.in

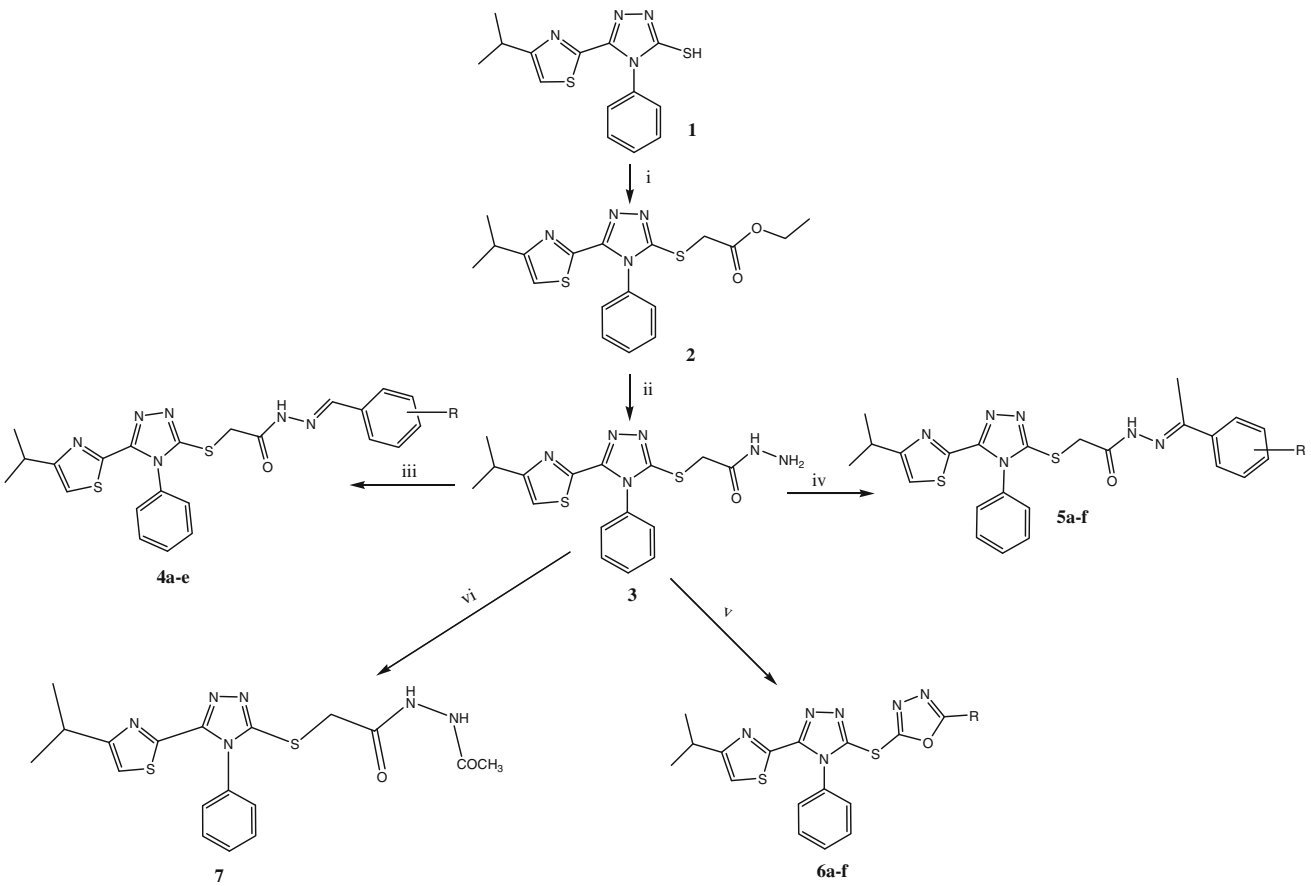
function oxidase involved in sterol synthesis in eukaryotic organism (Walczak *et al.*, 2004; Babaoglu *et al.*, 2003).

Thiazole and its derivatives are reported to be physiologically and pharmacologically active heterocycle and utilized in treatment of several diseases like epilepsy, diabetes, antifertility, and sedative-hypnotic or anesthetic action. Isopropyl thiazole is an important pharmacophore and privileged structure in medicinal chemistry encompassing a diverse range of biological activities including antibacterial and antitubercular activity (Khalaf *et al.*, 2004).

1,2,4-Triazoles represent an overwhelming and rapid developing field in modern heterocyclic chemistry. For instance, some biheterocyclic compounds, consisting of clubbed 1,2,4-triazole and thiazoles or 1,3,4 oxadiazole rings are reported as novel azole class of antimycobacterials, which are proved to be highly active both in vitro and in vivo experiments (Demirbas *et al.*, 2004; Bayrak *et al.*, 2009; Turan-Zitouni *et al.*, 2005; Rida *et al.*, 1986). So, after extensive literature search and in continuation of our research on thiazoles, (Shiradkar *et al.*, 2007; Mallikarjun *et al.*, 2007; Mallikarjuna *et al.*, 2009; Suresh Kumar *et al.*, 2010a) it was contemplated to synthesize a series of novel 4-isopropylthiazol-4-phenyl-1,2,4-triazol derivatives to study their cytotoxicity, antimicrobial (bacterial and fungal), and antitubercular activity against H37Rv strain.

Chemistry

The general synthetic strategy employed to obtain the title compounds with excellent yields is depicted in Scheme 1 and their physical properties are depicted in Table 1. The key intermediate in the present study 5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol **1** was prepared as per the literature (Suresh Kumar *et al.*, 2010a). Acetylation of compound **1** by refluxing with ethyl bromoacetate in the presence of sodium hydroxide and absolute ethanol yielded compound **2**. The important intermediate 5′-(4-isopropylthiazol-2-yl)-4-phenyl-4′H-1,2,4-triazol-3-ylthio) acetohydrazide **3** was



i. ethyl bromoacetate, NaOH, ethanol, reflux 4h **ii.** hydrazine hydrate, ethanol, reflux 4h **iii.** aryl aldehyde, ethanol, glacial acetic acid, reflux 2h

iv. acetophenone, ethanol,glacial acetic acid reflux 2h **v.** aromatic acid, POCl3, reflux 6h

Scheme 1 Synthesis of compounds **2**, **3**, **4a**–**4e**, **5a**–**5f**, **6a**–**6f** and **7**

Table 1 Analytical and physico-chemical data of synthesized compounds CHCl3:CH3OH (9:1)

Compound	*R*	Molecular formula	M.W	Solvent system	M.p (°C)[a]/ crystallization solvent	Yield (%)	% Analysis of C, H ,N found (Calc.)[b]		
							C	H	N
2	–	$C_{18}H_{20}N_4O_2S_2$	388	–	98–100 (Ethanol)	85	55.65 (55.62)	5.19 (5.16)	14.42 (14.43)
3	–	$C_{16}H_{18}N_6OS_2$	374	$CHCl_3$:CH_3OH (9:1)	218–220 (Ethanol)	89	51.32 (51.38)	4.84 (4.85)	22.44 (22.47)
4a	3,4-OCH_3	$C_{22}H_{26}N_6O_3S_2$	522	$CHCl_3$:CH_3OH (9:1)	193–195 (Ethanol)	82	59.86 (59.88)	5.22 (5.23)	13.43 (13.46)
4b	4-Cl	$C_{23}H_{21}ClN_6OS_2$	496	$CHCl_3$:CH_3OH (9:2)	148–150 (Ethanol)	92	58.11 (58.16)	4.47 (4.49)	14.12 (14.13)
4c	4-OH	$C_{23}H_{22}N_6O_2S_2$	478	$CHCl_3$:CH_3OH (9:1)	203–205 (Ethanol)	86	60.36 (60.35)	4.85 (4.82)	14.66 (14.61)
4d	3-OCH_3, 4-Cl	$C_{24}H_{24}N_6O_3S_2$	507	$CHCl_3$:CH_3OH (9:2)	160–162 (Ethanol)	93	59.15 (55.16)	4.96 (4.97)	13.80 (13.85)
4e	3-NO_2	$C_{23}H_{21}N_7O_3S_2$	506	$CHCl_3$:CH_3OH (9:1)	223–225 (Ethanol)	84	56.90 (55.92)	4.38 (4.37)	16.59 (16.54)
5a	4-NO_2	$C_{24}H_{23}N_7O_3S_2$	521	$CHCl_3$:C_2H_5OH (9:3)	187–189 (Ethanol)	84	57.67 (57.65)	4.65 (4.67)	16.14 (16.11)
5b	4-OCH_3	$C_{25}H_{26}N_6O_2S_2$	506	$CHCl_3$:C_2H_5OH (9:2)	175–178 (Ethanol)	79	61.76 (61.75)	5.38 (5.39)	13.85 (13.86)
5c	4-OH	$C_{24}H_{24}N_6O_2S_2$	492	$CHCl_3$:C_2H_5OH (9:3)	171–173 (Ethanol)	81	61.08 (61.09)	5.13 (5.11)	14.25 (14.21)
5d	4-CH_3	$C_{25}H_{26}N_6OS_2$	490.16	$CHCl_3$:C_2H_5OH (9:2)	162–165 (Ethanol)	81	61.20 (61.23)	5.34 (5.39)	17.13 (16.11)
5e	4-Br	$C_{24}H_{23}BrN_6OS_2$	554.06	$CHCl_3$:C_2H_5OH (9:3)	154–157 (Ethanol)	74	51.89 (51.84)	4.17 (4.15)	15.13 (15.17)
5f	4-Cl	$C_{24}H_{23}ClN_6OS_2$	510.11	$CHCl_3$:C_2H_5OH (9:1)	163–165 (Ethanol)	68	56.40 (56.44)	4.54 (4.51)	16.44 (16.43)
6a	C_6H_5	$C_{23}H_{20}N_6OS_2$	460	$CHCl_3$:CH_3OH (9:1)	117–120 (Ethanol)	69	59.98 (59.90)	4.38 (4.37)	18.25 (18.22)
6b	CH_2Cl	$C_{18}H_{17}ClN_6OS_2$	432	$CHCl_3$:CH_3OH (9:2)	190–193 (Ethanol)	74	49.93 (49.97)	3.96 (3.97)	19.41 (19.46)
6c	C_6H_4Cl	$C_{23}H_{19}ClN_6OS_2$	475	$CHCl_3$:CH_3OH (9:1)	183–185 (Ethanol)	72	55.81 (55.79)	3.87 (3.81)	16.98 (16.95)
6d	$C_6H_4NO_2$	$C_{23}H_{19}N7O_3S_2$	505.1	$CHCl_3$:CH_3OH (9:2)	192–194 (Ethanol)	68	54.64 (55.66)	3.79 (3.83)	19.39 (19.33)
6e	$C_6H_4CH_3$	$C_{24}H_{22}N_6OS_2$	474.13	$CHCl_3$:CH_3OH (9:1)	182–185 (Ethanol)	73	60.74 (60.72)	4.67 (4.66)	17.71 (17.65)
6f	C_6H_4OH	$C_{23}H_{20}N_6O_2S_2$	476.11	$CHCl_3$:CH_3OH (9:2)	171–174 (Ethanol)	69	57.97 (57.89)	4.23 (4.22)	17.63 (17.64)
7	–	$C_{18}H_{20}N_6O_2S_2$	416	$CHCl_3$:CH_3OH (9:1)	198–200 (Ethanol)	67	51.90 (51.89)	4.84 (4.82)	20.18 (20.15)

M.W Molecular weight of the compounds

[a] Melting point range of the compounds

[b] Elemental analysis of C, H, and N were with in ±0.4 % of theoretical value

obtained on treating compound **2** with hydrazine hydrate in the presence of absolute ethanol.

The treatment of acetohydrazide derivative **3** with substituted aromatic aldehydes affords schiff bases **4a–e**. The compounds **4a–e** having arylidene hydrazide structure may exist as E/Z geometrical isomers about –C=N double bond and as cis/trans amide conformers. According to the literature (Galic *et al.*, 2001), the compounds containing imine bond are present in higher percentage in dimethyl-d6 sulfoxide solution in the form of geometrical E isomer about –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In the present study, the spectral data were obtained in dimethyl-d6 sulfoxide solution, hence no signal belonging to Z isomer for compounds **4a–e** was observed.

The treatment of compound **3** with substituted acetophenones in the presence of catalytic amount of glacial acetic acid affords acetohydrazides **5a–f**. Compound **3** on reflux with appropriate aromatic acids yielded a series of 3-(4-isopropylthiazol-2-yl)-4-phenyl-5-((5-substituted-1,3,4-oxadiazol-2-yl)methylthio)-4H-1,2,4-triazoles **6a–f**. Compound **7**, *N*-acetyl-5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio) acetohydrazide was synthesized on treating compound **3** with acetic anhydride in presence of ethanol.

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 (**3**, **4a–e**, **5a–f**, **6a–f**, and **7**) was performed by broth dilution method (Eweiss *et al.*, 1986) against the following standard bacterial strains: *Staphylococcus aureus* (ATCC 11632), *S. faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC 10031), and *Escherichia* and *Pseudomonas aeruginosa* (ATCC 10145); and antifungal activity against yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT); and mold: *Aspergillus niger* (ATCC 6275). MIC of compounds was determined against MTB H37Rv strain by broth dilution assay method. The cytotoxicity of the synthesized compounds was evaluated for their cytotoxic potential using A549 (lung adenocarcinoma) cell lines in the presence of fetal bovine serum.

Results and discussion

Chemistry

The –SH proton of compound **1** is acidic enough and substitution reaction could be achieved on this group in the presence of a base (Klimesova *et al.*, 2004; Pomarnacka and Kornicka 2001). In the present study, compound **2**, an acetic acid ester derivative of compound **1,** was obtained in good yield. ^{1}H NMR spectrum of compound **2** depicts additional signals derived from ester group at 1.33 (–$OCH_2\underline{CH_3}$), 3.91 (–SCH_2), and 4.12 (–$O\underline{CH_2}CH_3$) ppm integrating for three protons, two protons, and two protons, respectively. The signals of ^{13}C NMR spectrum of the compound **2** belonging to the same groups were recorded at 13.24, 32.5, and 63.24 ppm, respectively.

The ^{1}H NMR spectrum of compound **3** displayed signals derived from hydrazide structure appeared at 4.52 and 9.35 (–$NHNH_2$) ppm integrating for two protons and one proton, respectively (controlled by changing D_2O). The ^{1}H and ^{13}C NMR spectra of compounds **4a–e** displayed additional signals due to the aromatic ring derived from aldehyde moiety at aromatic region, while the signal belonging to –NH_2 group of hydrazide structure was absent. The signals belonging to –SCH_2, –N=CH, and –NH groups were observed between 3.32 and 3.72, 8.23 and 8.71, and 11.05 and 11.71 ppm, respectively.

^{1}H NMR of compound **5a** showed a signal at δ 2.32 due to methyl group and peaks between δ 7.2 and δ 7.5 accountable for aromatic benzyl group. Further, mass spectrum of compound **5a** showed a molecular ion peak m/z 520.09 which confirmed its molecular weight. The structure of compound **6a** was confirmed by lack of resonances corresponding to NH and NH_2 in the ^{1}H NMR spectrum and appearance of peaks around 144.11 and 164.32 due to C_2 and C_5 of oxadiazole in ^{13}C NMR spectra. Further, molecular ion base peak at m/z 460.11 confirmed structure of compound **6a**.

Biological activity

The results of antimicrobial testing of synthesized compounds (**3**, **4a–e**, **5a–f**, **6a–f**, and **7**) against selected Gram-positive, Gram negative bacteria, yeasts, molds, and MTB H37Rv are illustrated in Tables 2 and 3, respectively.

Several recent experiments indicate that the incorporation of hydrophobic moieties into the framework of acetohydrazide enhance penetration of the drug into the tissues of the mammalian host and into the waxy cell wall of the bacterium. This strategy of drug design has been proposed as a vehicle for controlled study of the growth cycle of the pathogen as well as a means of augmenting fundamental drug activity.

Structurally, the modifications engendering these desirable drug properties (enhanced penetration and decreased resistance) are optimally made at N2 of the hydrazide framework (Fig. 1). Such modifications block the resulting molecule against the action of *N*-arylaminoacetyl transferases (NATs). These enzymes are found in both mycobacteria and their mammalian hosts, and they deactivate hydrazides by means of reaction at N2. NATs have been implicated in the development of resistance, particularly among those patients known as the ''fast acetylators,'' for whom there is an inherent problem of chronic underdosing of INH (isonicotonic hydrazide) under genetic control. Improving serum drug concentrations should narrow this range (Nuermberger and Grosset, 2004; Kinzig-Schippers *et al.*, 2005; Balcells *et al.*, 2006), and structurally blocking hydrazide toward the actions of NATs at N2 may thus combat the rise of resistance. In line with the above discussion, our rationale has been to prepare various Schiff bases (**4a–e**, **5a–f**) with enhanced lipophilicity and to examine their efficacy against Gram-positive, Gram-negative bacteria, yeasts, molds, and MTB H37Rv by inducting halogens, which are known for inductive, conjugative, steric and/or electronic effects that can be directly involved in the biological activity (i.e., antimicrobial).

The antimicrobial activity of acetohydrazide derivatives **4a–e**, **5a–f** divulged that compound **4a** comprising 3,4-di methoxy and **5b** comprising 4-methoxy substitution (electron withdrawing) showed improved antimicrobial activity against tested bacterial and fungal species. This excellent inhibition of compound **4a** is attributed to participation of the free electron pairs on the oxygen by resonance and increased electron density in the aromatic system. Compounds **4c** possessing 4-OH on phenyl ring exhibited moderate antimicrobial activity and excellent antitubercular activity against MTB H37Rv at MIC 8 mg/mL.

In drug design, halogen atoms are used to improve penetration through lipid membranes and tissues. They may also present a significant reactivity depending on the structure of the molecule. In this work, the introduction of a para-halogen substituent in the *N*-phenyl ring of 4b (4-Cl), 5f (4-Cl), or 5e (4-Br) presented a negative effect as they reduced the antitubercular activity (Table 2). Probably, the halogen reactivity at *R* position compromised the original interactions of the non-substituted compounds with the bacterial target. The SAR analysis also pointed the volume of the halogen as a feasible restrictive factor since bromine, the largest halogen, is more deleterious to the antitubercular activity than chlorine. Based on these data, we may infer that substituent (*R*) is a steric and/or restricted position that should be carefully considered in the future design of antitubercular targets.

Evaluating the antimicrobial activity of the synthesized 2-(4-isopropylthiazol-2-yl)-5-substituted-1,3,4-oxadiazole

Table 2 Antimicrobial activity Table 2 Antimicrobial activity of synthesized compounds **2**, **3**, **4a–e**, **5a–f**, **6a–f** and **7** expressed as MIC (μg/mL) 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
2	125	125	62.5	125	125	62.5	62.5	125	62.5
3	16	31.25	31.25	8	8	8	62.5	31.25	31.25
4a	8	12	8	16	16	31.25	16	31.25	16
4b	62.5	31.25	31.25	62.5	31.25	62.5	16	31.25	31.25
4c	125	16	125	16	125	62.5	162.5	62.5	31.25
4d	125	125	16	8	8	16	16	62.5	31.25
4e	125	31.25	31.25	16	31.25	62.5	62.5	31.25	125
5a	31.25	31.25	125	31.25	62.5	62.5	125	31.25	125
5b	31.25	31.25	62.5	250	125	62.5	62.5	125	125
5c	62.5	31.25	62.5	31.25	16	16	31.25	8	8
5d	125	62.5	31.25	16	125	62.5	62.5	125	31.25
5e	16	125	62.5	31.25	62.5	125	31.25	125	31.25
5f	31.25	31.25	125	62.5	16	31.25	31.25	31.25	125
6a	31.25	31.25	31.25	62.5	125	31.25	16	16	31.25
6b	8	16	31.25	31.25	16	16	62.5	125	31.25
6c	8	8	4	62.5	62.5	62.5	62.5	125	125
6d	31.25	31.25	62.5	31.25	62.5	31.25	62.5	31.25	62.5
6e	62.5	31.25	125	62.5	31.25	62.5	125	31.25	125
6f	31.25	62.5	31.25	125	62.5	31.25	31.25	31.25	31.25
7	125	125	62.5	31.25	62.5	125	16	8	8
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), *Streptococcus 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), mold: *Aspergillus niger* (ATCC 6275, An)

derivatives **6a–f** revealed that compounds were more effective against Gram-positive bacteria at MIC 4–31.25 mg/mL. Compounds **3**, **4c**, **5c**, and **6c** were tested for their cytotoxic potential using A549 (lung adenocarcinoma) cell lines in the presence of fetal bovine serum. As shown in Fig. 2, compound **4c** showed maximum cytotoxicity at a concentration of 250 μM. The other compounds **3, 5c**, and **6c** showed appreciable cytotoxicity of about 50 % of the vehicle control at a concentration of 250 μM.

Conclusion

In conclusion, this work demonstrates the synthesis of a series of novel 4-isopropylthiazol-4-phenyl-1,2,4-triazol derivatives (**3**, **4a–e**, **5a–f**, **6a–f**, and **7)** and their in vitro evaluation of antimicrobial (bacterial and fungal) and antitubercular activity against H37Rv strain. Antimicrobial study revealed that compounds **4a**, **4c**, **5c**, and **6c** demonstrated significant activity against tested Gram-positive and Gram-negative bacteria and fungal species. The in vitro antituberculosis screening of these series showed that all compounds were active, in particular compounds **4c** and **6c** exhibited excellent antitubercular activity at MIC 8 and 4 μg/mL, respectively, when compared with first line drug 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.

Table 3 Primary antitubercular activity of synthesized compounds **2**, **3**, **4a–e**, **5a–f**, **6a–f**, and **7**

Compound	MIC values (μg/mL) of MTB $H_{37}Rv$
2	125
3	16
4a	8
4b	125
4c	8
4d	31.25
4e	62.5
5a	62.5
5b	8
5c	16
5d	62.5
5e	31.25
5f	31.25
6a	62.5
6b	16
6c	4
6d	125
6e	62.5
6f	62.5
7	62.5
Isoniazid	0.25

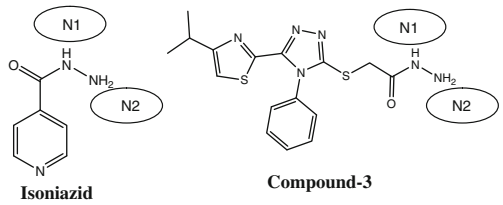


Fig. 1 Structures of isoniazid and synthesized compound **3** showing the position N2

Experimental

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-d6) 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 GF254 aluminum sheets using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent.

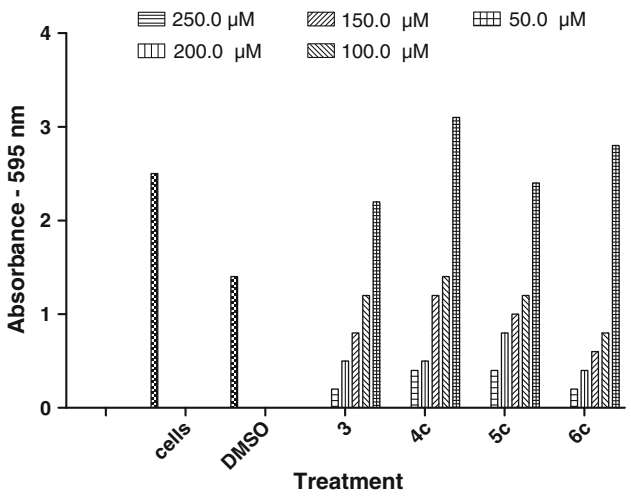


Fig. 2 Cytoxic activity of compounds **3**, **4c**, **5c**, and **6c** tested in A549 cells by MTT assay. The *bars* reflect the viable cells in each treatment. Cells represents, cells alone without any treatment, DMSO denote the vehicle control. The experiment was done in duplicate with triplicate readings of each experiment

Synthesis of 5′-(4-isopropylthiazol-2-yl)-ethyl-2-(4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio)acetate **2**

Compound **2** was synthesized by refluxing an equimolar mixture of compound **1** (0.01 mol), NaOH, and ethyl bromoacetate for 4 h. The reaction mixture was cooled and crystalline mass obtained was recrystallised.

IR (KBr) *v* max, cm^{-1}: 3,068 (Ar C–H str), 1,730 (C=O), 1,189 (O–C_2H_5), 765 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) *δ*: 7.2–7.5 (*m*, 5H, Ar–H), 7.32 (*s*, 1H, thiazole-C_5), 4.12 (*m*, 2H, O-CH_2), 3.91 (*s*, 2H, S-CH_2), 3.26 (*m*, 1H, isopropyl), 1.33 (*s*, 3H, terminal CH_3), 1.23 (*d*, 6H, terminal CH_3), 0.97 (*d*, 6H, terminal $2CH_3$) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) *δ*: 166.11 (acetyl C=O), 163.98 (thiazole C_4), 153.29 (thiazole-C_2), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 123.09 (thiazole-C_5), 113.43–138.08 (Ar–C), 63.24 (CH_2), 33.12 (tertiary-1C-isopropyl), 32.54 (S–CH_2), 21.24 (terminal $2CH_3$-isopropyl), 13.24 (acetyl CH_3) ppm.

MS (%) 388.10 (M + 100.0 %), 389.11 (M + 1, 19.8 %), 390.10 (9.4 %), 390.11 (2.6 %).

Synthesis of 5′-(4-isopropyl thiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio) aceto hydrazide **3**

The mixture of Compound **2** (0.1 mol) an ester and 2.5 equivalents of hydrazine hydrate in 30 mL ethanol was

refluxed for 4 h. Cooled to room temperature, the precipitate obtained was filtered and purified by recrystallization.

IR (KBr) *v* max, cm^{-1}: 3,423 (NH str of amide), 3,328 (NH_2 str), 3,080 (Ar H str), 2,921 (Ali C–H str), 1,450 Ar (C=C), 1,419 (C=C), 760 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 9.35 (*s*, 1H, NH of hydrazine hydrate), 7.40 (*s*, 1H, thiazole-C_5), 7.3–7.6 (*m*, 5H, Ar–H), 4.52 (*d*, 2H, NH_2 of hydrazine hydrate), 3.93 (*s*, 2H, S–CH_2), 3.61 (*m*, 1H, isopropyl), 0.95 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 165.9 (C=O), 161.98 (thiazole C_4), 160.29 (thiazole-C_2), 114.09 (thiazole-C_5), 149.21 (C_5 of triazole), 145.40 (C_3 of triazole), 113.21–138.08 (Ar–C), 43.41 (S–CH_2), 33.21 (tertiary-1C-isopropyl), 21.28 (terminal 2CH_3-isopropyl) ppm.

MS (%) 375.20 (M + 1 100.0 %), 348.30, 271.3.

General method for the synthesis of 5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio) (substituted benzylidene)acetohydrazide ***4a–e***

Aceto hydrazide **3** (0.05 mol) and the substituted aryl aldehyde (0.05 mol) in 20 ml of ethanol along with catalytic amount of glacial acetic acid (1 mL) was refluxed for 2 h in water bath at 90 °C; the mixture was cooled to room temperature, the separated solid was filtered, washed with alcohol dried, and recrystallized.

5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4H-1′,2′,4′-triazol-3′-yl thio-(3″,4″-dimethoxy benzylidene) acetohydrazide ***4a*** IR (KBr) *v* max, cm^{-1}: 3,200 (NH str), 3,104 (Ar C–H str), 1,671 (C=N str), 1,655 (C=N str) imines, 1,601 (C=C), 1,265 (Ar–OCH_3), 763 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 11.05 (*s*, 1H, NH), 8.23 (*s*, 1H, N=CH), 7.32 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 8H, Ar–H), 3.81 (*m*, 6H, OCH_3), 3.72 (*s*, 2H, S–CH_2), 3.62 (*m*, 1H, isopropyl), 0.95 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 166.3 (C=O), 163.98 (thiazole C_4), 161.29 (thiazole-C_2), 149.11 (C_5 of triazole), 143.92 (N=CH), 114.11 (thiazole-C_5), 111.23–138.08 (Ar–C), 145.32 (C_3 of triazole), 56.23 (2 OCH_3), 43.21 (S–CH_2), 33.16 (tertiary-1C-isopropyl), 21.21 (terminal 2CH_3-isopropyl) ppm.

MS (%) 523.3 (M + 2), 329.2, 255.2.

5′-(4-isopropylthiazol-2-yl)-4-phenyl-4′H-1′,2′,4′-triazol-3′-yl thio-(4″-chloro benzylidene) acetohydrazide ***4b*** IR (KBr) *v* max, cm^{-1}: 3,413 (NH str), 3,084 (Ar CH str), 2888 (Ali C–H str), 1,673 (C=N str) imines, 763 (C–S), 698 (C–Cl).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 11.71 (*s*, 1H, NH), 8.26 (*s*, 1H, N=CH), 7.31 (*s*, 1H, thiazole-C_5), 7.3–7.7 (*m*, 9H, Ar–H), 3.62 (*s*, 2H, S–CH_2), 3.43 (*m*, 1H, isopropyl), 0.98 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 165.91 (C=O), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 114.19 (thiazole-C_5), 149.13 (C_5 of triazole), 145.34 (C_3 of triazole), 143.41 (N=CH), 111.54–138.28 (Ar–C), 43.28 (S–CH_2), 33.31 (tertiary-1C-isopropyl), 21.30 (terminal 2CH_3-isopropyl) ppm.

MS (%) 496.80 (M + 1), 343.1, 315.0.

5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio-(4″-hydroxybenzylidene) acetohydrazide ***4c*** IR (KBr) *v* max, cm^{-1}: 3,438 (OH str), 3,204 (NH str), 3,074 (Ar C–H str), 2,863 (Ali C–H str), 1,667 (C=N str) imines, 1,601 (C=C), 1,186 (C–O), 768 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 11.68 (*s*, 1H, NH), 8.29 (*s*, 1H, N=CH), 7.32 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 5.02 (*s*, 1H, OH), 3.65 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S-CH_2), 1.02 (*d*, 6H, terminal 2CH_3)ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 165.34 (C=O), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 114.12 (thiazole-C_5), 149.31 (C_5 of triazole), 145.40 (C_3 of triazole), 143.32 (N=CH), 112.11–137.02 (Ar–C), 43.03 (S–CH_2), 33.23 (tertiary-1C-isopropyl), 21.16 (terminal 2CH_3-isopropyl) ppm.

MS (%) 477.13 (M + 100.0 %), 478.13 (M + 1, 29.5 %), 479.13 (10.4 %), 479.14 (3.3 %).

5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio-(3″-methoxy-4″-hydroxy benzylidene) acetohydrazide ***4d*** IR (KBr) *v* max, cm^{-1}: 3,431 (OH str), 3,294 (NH str), 3,089 (Ar C–H str), 1,676 (C=N str) imines, 1,241 (Ar OCH_3), 739 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.23 (*s*, 1H, N=CH), 11.71 (*s*, 1H, NH), 7.42 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 8H, Ar–H), 5.02 (*s*, 1H, S-OH), 4.46 (*s*, 1H, OH), 3.83 (*s*, 3H, OCH_3), 3.68 (*m*, 1H, isopropyl), 3.36 (*s*, 2H, S–CH_2), 0.94 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 165.90 (C=O), 163.98 (thiazole C_4), 163.41 (thiazole-C_2), 149.22 (C_5 of triazole), 145.26 (C_3 of triazole), 143.44 (N=CH), 114.09 (thiazole-C_5), 113.15–136.11 (Ar–C), 56.04 (OCH_3), 43.65 (S–CH_2), 33.15 (tertiary-1C-isopropyl), 21.28 (terminal 2CH_3-isopropyl) ppm.

MS (%) 507.14 (M + 100.0 %), 508.14 (M + 1, 30.6 %), 509.14 (10.6 %), 509.15 (3.6 %).

5′-(4′-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio-(3″-nitrobenzylidene) acetohydrazide ***4e*** IR (KBr) *v* max, cm^{-1}: 3,328 (NH str), 3,182 (Ar C–H str), 2,850 (Ali C–H str), 1,566 (C–NO_2), 1,186 (C–O), 740 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.36 (*s*, 1H, N=CH), 11.70 (*s*, 1H, NH), 7.34 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 3.63 (*m*, 1H, isopropyl), 3.34 (*s*, 2H, S–CH_2), 0.94 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 165.97 (C=O), 163.96 (thiazole C_4), 163.33 (thiazole-C_2), 149.25 (C_5 of triazole), 145.32 (C_3 of triazole), 143.66 (N=CH), 114.21 (thiazole-C_5), 114.11–132.38 (Ar–C), 43.17 (S–CH_2), 33.22 (tertiary-1C-isopropyl), 21.34 (terminal 2CH_3-isopropyl) ppm.

MS (%) 506.12 (M + 100.0 %), 507.12 (M + 1, 29.9 %), 508.12 (10.7 %), 508.13 (3.3 %).

General method for the synthesis of 5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′, 2′,4′-triazol-3′-ylthio) (substituted methyl benzylidene)acetohydrazide derivatives ***5a–f***

A mixture of compound **3** aceto hydrazide (0.01 mol) and appropriate acetophenone (0.01 mol) in ethanol (30 mL) and few drops of glacial acetic acid was refluxed for 2 h. The solid that separated out on cooling was filtered and recrystallised.

5′-(4-Isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio-(4″-nitromethylbenzylidene) acetohydrazide ***5a*** IR (KBr) ν max, cm^{-1}: 3,453 (NH str), 3,088 (Ar C–H str), 2,964 (C–H str of CH_3), 1,687 (C=N str) imines, 1,560 (C–NO_2).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.23 (*s*, 1H, NH), 7.35 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 4.27 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S–CH_2), 2.32 (*m*, 3H, CH_3), 0.97 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 168.93 (C=O), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.30 (C_5 of triazole), 145.43 (C_3 of triazole), 143.22 (N=C), 114.12 (thiazole-C_5), 111.54–138.03 (Ar–C), 43.91 (S–CH_2), 33.12 (tertiary-1C-isopropyl), 22.21 (CH_3), 21.24 (terminal 2CH_3-isopropyl) ppm.

MS (%) 522.13 (M + 2), 377.09, 343.1,

5′-(4-Isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-3′-yl thio-(4″-methoxy methylbenzylidene) acetohydrazide ***5b*** IR (KBr) ν max, cm^{-1}: 3,433 (NH str), 3,080 (Ar C–H str), 2,959 (C–H str of CH_3), 2,828 (CH_3–O), 1,670 (C=N str) imines.

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.01 (*s*, 1H, NH), 7.2–7.5 (*m*, 9H, Ar–H), 7.37 (*s*, 1H, thiazole-C_5), 3.74 (*s*, 3H, OCH_3), 3.63 (*m*, 1H, isopropyl), 3.34 (*s*, 2H, S–CH_2), 2.12 (*s*, 3H, CH_3), 0.92 (*d*, 6H, terminal CH_3)ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 168.9 (C=O), 163.96 (thiazole C_4), 163.21 (thiazole-C_2), 114.09 (thiazole-C_5), 149.33 (C_5 of triazole), 145.32 (C_3 of triazole), 143.23 (N=CH), 112.11–138.33 (Ar–C), 55.73 (OCH_3), 43.92 (S–CH_2), 33.16 (tertiary-1C-isopropyl), 22.24 (CH_3), 21.24 (terminal 2CH_3 -isopropyl) ppm.

MS (%) 505.16 (M + 100.0 %), 506.16 (M + 1, 31.6 %), 507.16 (10.5 %).

5′-(4-Isopropylthiazol-2-yl)-4-phenyl-4′H-1′,2′,4′-triazol-3′-ylthio-(4″-hydroxy methylbenzylidene) acetohydrazide ***5c*** IR (KBr) ν max, cm^{-1}: 3,417 (OH str), 3,075 (Ar C–H str), 2,926 (C–H str of CH_3), 1,668 (C=N str) imines, 690 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.09 (*s*, 1H, NH), 7.37 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 5.07 (*s*, 1H, OH), 3.64 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S-CH_2), 2.13 (*s*, 3H, CH_3), 0.93 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 168.49 (C=O), 164.08 (thiazole C_4), 163.39 (thiazole-C_2), 149.25 (C_5 of triazole), 145.32 (C_3 of triazole), 143.43 (N=CH), 114.31 (thiazole-C_5), 115.31–135.08 (Ar–C), 43.19 (S-CH_2), 33.36 (tertiary-1C-isopropyl), 22.11 (CH_3), 21.26 (terminal 2CH_3 -isopropyl) ppm.

MS (%) 491.14 (M + 100.0 %), 492.15 (M + 1, 27.4 %), 493.14 (9.1 %).

5-(4-Isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-N′-(1-p-tolylethylidene)acetohydrazide ***5d*** IR (KBr) ν max, cm^{-1}: 3,125.38 (Ar C–H str), 2,933.3 (C–H str of CH_3), 2,841.18 (Ali C–H str), 1,662.72 (C=N str) imines, 694.55 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.09 (*s*, 1H, NH), 7.37 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 3.64 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S–CH_2), 2.13 (*s*, 3H, CH_3), 2.53 (*s*, 3H, CH_3), 0.93 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 168.49 (C=O), 164.08 (thiazole C_4), 163.39 (thiazole-C_2), 149.25 (C_5 of triazole), 145.32 (C_3 of triazole), 143.43 (N=CH), 114.31 (thiazole-C_5), 115.31–135.08 (Ar–C), 43.19 (S–CH_2), 33.36 (tertiary-1C-isopropyl), 22.11 (CH_3), 24.56 (CH_3), 21.26 (terminal 2CH_3 -isopropyl) ppm.

MS (%) 490.16 (M + 100.0 %), 491.16 (M + 1, 30.9 %), 493.16 (2.6 %).

1-(4-Bromophenyl)ethylidene)-2-(5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)acetohydrazide ***5e*** IR (KBr) ν max, cm^{-1}: 3,134 (Ar C–H str), 2,922 (C–H str of CH_3), 2,888 (Ali C–H str), 1,654 (C=N str) imines, 674 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 8.09 (*s*, 1H, NH), 7.37 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 3.64 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S–CH_2), 2.13 (*s*, 3H, CH_3), 0.93 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 168.49 (C=O), 164.08 (thiazole C_4), 163.39 (thiazole-C_2), 149.25 (C_5 of triazole), 145.32 (C_3 of triazole), 143.43 (N=CH), 114.31 (thiazole-C_5), 115.31–135.08 (Ar–C), 43.19 (S–CH_2),

33.36 (tertiary-1C-isopropyl), 24.56 (CH_3), 21.26 (terminal $2CH_3$ -isopropyl) ppm.

MS (%) 556.05 (M + 100.0 %), 554.06 (M + 1, 94.0 %), 555.06 (26.2 %), 557.06 (24.2 %), 558.05 (9.2 %).

1-(4-Chlorophenyl)ethylidene)-2-(5-(4-isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)acetohydrazide ***5f*** IR (KBr) *v* max, cm^{-1}: 3,133 (Ar C–H str), 2,955 (C–H str of CH_3), 1,649 (C=N str) imines, 645 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.85 (*s*, 1H, NH), 7.66 (*s*, 1H, thiazole-C_5), 7.2–7.8 (*m*, 9H, Ar–H), 3.55 (*m*, 1H, isopropyl), 3.32 (*s*, 2H, S-CH_2), 2.44 (*s*, 3H, CH_3), 0.97 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 162.49 (C=O), 163.55 (thiazole C_4), 162.11 (thiazole-C_2), 145.35 (C_5 of triazole), 142.32 (C_3 of triazole), 141.41 (N=CH), 113.66 (thiazole-C_5), 115.31–135.08 (Ar–C), 42.19 (S–CH_2), 32.11 (tertiary-1C-isopropyl), 22.33 (CH_3), 21.23 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 510.11 (M + 100.0 %), 512.10 (M + 1, 41.1 %), 511.11 (27.9 %), 513.11 (11.0 %), 512.11 (4.5 %).

General method for the synthesis of 3-(4-isopropylthiazol-2-yl)-4′-phenyl-5′-((5″-substituted-1″,3″,4″-oxadiazol-2″-yl)-methylthio)-4′H-1′,2′,4′-triazol ***6a–f***

A mixture of compound **3** aceto hydrazide (0.001 mol) and appropriate aromatic acid (0.001 mol) in phosphorus oxychloride was refluxed for 6 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, treated with dilute sodium bi carbonate, washed with water, and recrystallized.

3-(4-Isopropyl thiazol-2-yl)-4-phenyl-5-((5″-phenyl-1,3,4-oxadiazol-2-yl) methylthio)-4H-1,2,4-triazole ***6a*** IR (KBr) *v* max, cm^{-1}: 3,053 (Ar C–H str), 2,980 (Ar C–H str), 1,688 (C=N of oxadiazole), 1,078 (C–O of oxadiazole), 745 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.35 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 10H, Ar–H), 3.65 (*m*, 1H, isopropyl), 0.96 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 164.32 (C_5 of oxadiazole), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 144.11 (C_2 of oxadiazole), 114.09 (thiazole-C_5), 111.11–138.08 (Ar–C), 33.12 (tertiary-1C-isopropyl), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 460.11 (M + 100.0 %), 461.12 (M + 1, 25.1 %), 462.12 (3.6 %), 463.11 (2.5 %).

3-((5″-(Chloromethyl)-1″,3″,4″-oxadiazol-2″-yl)methylthio)-5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazole ***6b*** IR (KBr) *v* max, cm^{-1}: 2,963 (Ar C–H str), 1,711 (C=N of oxadiazole), 1,626 (C=C), 690 (C–Cl).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.35 (s, 1H, thiazole-C_5), 7.2-7.7 (m, 9H, Ar–H), 4.12 (s, 2H, S-CH_2), 3.67 (m, 1H, isopropyl), 0.98 (d, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 114.09 (thiazole-C_5), 164.32 (C_5 of oxadiazole), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 144.11 (C_2 of oxadiazole), 113.1–139.02 (Ar–C), 33.12 (tertiary-1C-isopropyl), 45.11 (CH_2), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 432.06 (M + 100.0 %), 434.06 (M + 1, 42.0 %), 433.06 (23.3 %), 435.06 (8.6 %).

3-(4-Isopropyl thiazol-2-yl)-4′-phenyl-5′-((5″-chloro phenyl-1″,3″,4″-oxadiazol-2″-yl) methylthio) -4′H-1′,2′,4′-triazole ***6c*** IR (KBr) *v* max, cm^{-1}: 3,162 (Ar C–H str), 1,653 (C=N of oxadiazole), 1,258 (Ar–C–O), 1,079 (C–O of oxadiazole), 760 (C–S), 674.02 (C–Cl).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.41 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 9H, Ar–H), 3.63 (*m*, 1H, isopropyl), 0.97 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 164.32 (C_5 of oxadiazole), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 144.11 (C_2 of oxadiazole), 116.11–132.08 (Ar–C), 114.09 (thiazole-C_5), 33.12 (tertiary-1C-isopropyl), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 494.08 (M + 100.0 %), 496.07 (M + 1, 41.1 %), 495.08 (25.1 %), 497.08 (8.2 %).

3-(4-Isopropylthiazol-2-yl)-5-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-4-phenyl-4H-1,2,4-triazole ***6d*** IR (KBr) *v* max, cm^{-1}: 3,162 (Ar C–H str), 1,653 (C=N of oxadiazole), 1,263 (Ar–C–O), 1,055 (C–O of oxadiazole), 760 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.33 (*s*, 1H, thiazole-C_5), 7.3–7.6 (*m*, 9H, Ar–H), 3.67 (*m*, 1H, isopropyl), 0.93 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 164.32 (C_5 of oxadiazole), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.21 (C_5 of triazole), 145.34 (C_3 of triazole), 144.21 (C_2 of oxadiazole), 116.11–132.08 (Ar–C), 114.89 (thiazole-C_5), 33.52 (tertiary-1C-isopropyl), 21.24 (terminal $2CH_3$-isopropyl) ppm.

MS (%) 505.10 (M + 100.0 %), 506.10 (M + 1, 29.2 %), 508.10 (2.4 %), 507.10 (1.7 %).

3-(4-Isopropylthiazol-2-yl)-4-phenyl-5-((5-p-tolyl-1,3,4-oxadiazol-2-yl)methylthio)-4H-1,2,4-triazole ***6e*** IR (KBr) *v* max, cm^{-1}: 3,162 (Ar C–H str), 1,673 (C=N of oxadiazole), 1,057 (C–O of oxadiazole), 767 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.34 (*s*, 1H, thiazole-C_5), 7.3–7.6 (*m*, 9H, Ar–H), 3.67 (*m*, 1H, isopropyl), 2.35 (*s*, 3H, CH_3), 0.93 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 164.32 (C_5 of oxadiazole), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.55 (C_5 of triazole), 145.33 (C_3 of triazole), 144.61 (C_2 of oxadiazole), 116.11–132.38 (Ar–C), 114.39 (thiazole-C_5), 33.82 (tertiary-1C-isopropyl), 24.44 (CH_3), 21.24 (terminal 2CH_3-isopropyl) ppm.

MS (%) 474.13 (M + 100.0 %), 475.13 (M + 1, 29.8 %), 476.13 (10.3 %), 476.14 (3.3 %).

4-(5-((5-(4-Isopropylthiazol-2-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)methyl)-1,3,4-oxadiazol-2-yl)phenol ***6f*** IR (KBr) *v* max, cm^{-1}: 3,162 (Ar C–H str), 1,644 (C=N of oxadiazole), 1,233 (Ar–C–O), 1,055 (C–O of oxadiazole), 760.58 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 7.34 (*s*, 1H, thiazole-C_5), 7.3–7.6 (*m*, 9H, Ar–H), 3.67 (*m*, 1H, isopropyl), 5.23 (*s*, 1H, OH), 0.95 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 164.32 (C_5 of oxadiazole), 163.98 (thiazole C_4), 163.29 (thiazole-C_2), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 144.11 (C_2 of oxadiazole), 116.11–132.08 (Ar–C), 114.59 (thiazole-C_5), 33.62 (tertiary-1C-isopropyl), 21.24 (terminal 2CH_3-isopropyl) ppm.

MS (%) 476.11 (M + 100.0 %), 477.11 (M + 1, 28.8 %), 478.10 (9.1 %), 478.12 (3.0 %).

Synthesis of N-acetyl-5′-(4-isopropylthiazol-2-yl)-4′-phenyl-4′H-1′,2′,4′-triazol-(3′-ylthio) acetohydrazide ***7***

Compound **3** aceto hydrazide (0.003 mol) was warmed with acetic anhydride for 2 h and then the mixture was allowed to attain room temperature. The deposited solid was filtered, washed, and recrystallized from ethanol.

IR (KBr) *v* max, cm^{-1}: 3,265 (NH str), 1,815 (C–O), 1,685 (C=O), 770 (C–S).

^{1}H NMR (DMSO-d6, 300 MHz) δ: 10.21 (*s*, 1H, NH of hydrazine hydrate), 8.24 (1H, NH_2 of hydrazine hydrate), 7.35 (*s*, 1H, thiazole-C_5), 7.2–7.5 (*m*, 5H, Ar–H), 3.82 (*s*, 2H, S–CH_2), 3.63 (*m*, 1H, isopropyl), 2.12 (*s*, 3H, CH_3), 0.95 (*d*, 6H, terminal CH_3) ppm.

^{13}C NMR (DMSO-d6, 300 MHz) δ: 171.54 (C=O), 169.44 (CH_3), 164.32 (C=O), 163.98 (thiazole C_4), 163.29 (C_2 of thiazole-), 149.21 (C_5 of triazole), 145.32 (C_3 of triazole), 114.09 (C_5 of thiazole), 114.1–136.2 (Ar–C), 40.91 (S–CH_2), 33.12 (tertiary-1C-isopropyl), 21.24 (terminal 2CH_3-isopropyl) ppm.

MS (%) 416.11 (M + 100.0 %), 417.11 (M + 1, 23.4 %), 418.12 (1.8 %).

Biological protocol

Antimicrobial activity

The antimicrobial susceptibility testing was performed in vitro by broth microdilution method (Hassan *et al.*, 1983; Khalil *et al.*, 1993). 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 bacteria (*K. penumoniae, E. coli. P. aeruginosa*). The antifungal activity was assayed against yeasts (*C. tropicalis, S. cerevisiae*) and molds (*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 105 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 °C for 24 h. 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.

Anti tubercular activity

The preliminary antitubercular screening for test compounds were obtained for MTB H37Rv, the MIC of each drug was determined by broth dilution assay (Goto *et al.*, 1981) 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 105 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.

MTT assay for cell viability

Toxicity of compounds **3, 4c, 5c**, and **6c** in A549 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 (Mosmann, 1983). The compounds were dissolved in DMSO at 10 mM concentration and stored at −20 °C. The dilutions were made in culture medium before treatment.

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