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Short communication

Synthesis, structural activity relationship and anti-tubercular activity of novel pyrazoline derivatives

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Abstract

In the present investigation, a series of 5-(-4-(substituted) phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidino methane thione and 5-(substituted) phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxyanilino methane thione were synthesized by the reaction between hydrazine hydrate and chalcones (**3a–k**) followed by condensation with appropriate aryl isothiocyanate which yielded *N*-substituted pyrazoline derivatives. Newly synthesized compounds were tested for their in vitro anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system. Among the synthesized compounds, compound anilino-3-(4-hydroxy-3-methylphenyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-1-pyrazolylmethanethione (**6i**) was found to be more active agent against *M. tuberculosis* H37Rv with minimum inhibitory concentration of 0.0034 μM.

Keywords: Pyrazoline; Isothiocyanate; Anti-tubercular; *Mycobacterium tuberculosis*

1. Introduction

Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis*, is the primary cause of mortality in the world. Mycobacteria are ubiquitous organisms that are becoming increasingly important intracellular pathogens that establish an infection in oxygen-rich macrophage of the lung [1]. The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries [2]. Resistance of *M. tuberculosis* strains to anti-mycobacterial agents is an increasing problem worldwide [3–5]. However, powerful new anti-TB drugs with new mechanism of action have not been developed in the last 40 years. In spite of severe toxicity on repeated dosing of isoniazid (INH), it is still considered to be a first line drug for chemotherapy of tuberculosis [6]. Literature survey reveals that pyrazoline derivatives are active against many mycobacteria [7–10]. The current work describes the synthesis of novel pyrazoline moiety with encouraging anti-mycobacterial activity against *M. tuberculosis* H37Rv.

2. Results and discussion

2.1. Chemistry

5-(-4-(Substituted) phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidino methane thione and 5-(substituted) phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxyanilino methane thione (**5a–k**) and (**6a–k**) described in this study are shown in Tables 1, 2 and 3, and a reaction sequence for the preparation is outlined in Scheme 1. The chalcones were prepared by reacting 3-methyl-4-hydroxy acetophenone with appropriate aldehyde in the presence of a base by conventional Claisen–Schmidt condensation. Reaction between newly synthesized chalcones and hydrazine hydrate in ethanol led to synthesis of novel pyrazolines (**4a–k**), which on treatment with various

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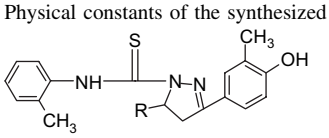
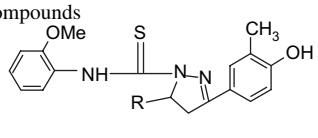
Table 1
Physical constants of the synthesized compounds



5a-k



6a-k

Compound	R	Yield (%)	Melting point (°C)	Molecular formula	Molecular weight
5a	4-Methoxy phenyl-	74	144	$C_{25}H_{25}N_3O_2S$	431.55
5b	4-Chloro phenyl-	70	131	$C_{24}H_{22}N_3OSCl$	435.97
5c	4-Dimethylamino phenyl-	72	104	$C_{26}H_{28}N_4OS$	444.59
5d	Phenyl-	80	121	$C_{24}H_{23}N_3OS$	401.52
5e	3,4-Dimethoxy phenyl-	82	102	$C_{26}H_{27}N_3O_3S$	461.57
5f	3,4,5-Trimethoxy phenyl-	85	103	$C_{27}H_{29}N_3O_4S$	491.60
5g	4-Fluoro phenyl-	92	196	$C_{24}H_{23}N_3OSF$	420.52
5h	2-Chloro phenyl-	85	115	$C_{24}H_{22}N_3OSCl$	435.97
5i	2,6-Dichloro phenyl-	77	164	$C_{24}H_{21}N_3OSCl_2$	470.41
5j	3-Nitro phenyl-	82	104	$C_{24}H_{22}N_4O_3S$	446.52
5k	Furfuryl-	90	205	$C_{22}H_{21}N_3O_2S$	392.48
6a	4-Methoxy phenyl-	82	124	$C_{25}H_{25}N_3O_3S$	447.55
6b	4-Chloro phenyl-	80	153	$C_{24}H_{22}N_3O_2SCl$	451.96
6c	4-Dimethylamino phenyl-	75	115	$C_{26}H_{28}N_4O_2S$	460.59
6d	Phenyl-	80	234	$C_{24}H_{23}N_3O_2S$	417.52
6e	3,4-Dimethoxy phenyl-	77	197	$C_{26}H_{27}N_3O_4S$	477.57
6f	3,4,5-Trimethoxy phenyl-	72	106	$C_{27}H_{29}N_3O_5S$	507.60
6g	4-Fluoro phenyl-	82	142	$C_{23}H_{20}N_3O_2S$	436.52
6h	2-Chloro phenyl-	72	156	$C_{24}H_{22}N_3O_2SCl$	451.96
6i	2,6-Dichloro phenyl-	70	172	$C_{24}H_{21}N_3O_2SCl_2$	486.41
6j	3-Nitro phenyl-	44	194	$C_{24}H_{22}N_4O_4S$	462.52
6k	Furfuryl-	80	169	$C_{22}H_{21}N_3O_3S$	407.48

Recrystallization: Ethanol, acetic acid.

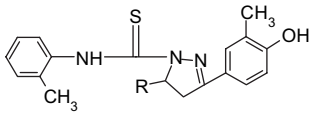
aryl isothiocyanates afforded respective 3,5-substituted pyrazolines (**5a**–**k**) and (**6a**–**k**) in 65–92% yield after recrystallization with glacial acetic acid and ethanol. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H NMR, IR) of all the synthesized compounds were in full agreement with the proposed structures. Final compounds in general, in the infrared spectra (IR), revealed OH, NH, C=O, C=N, C–N, and C=S peaks at 3307, 3220, 1640, 1590, 1320 and 1130 cm^{-1}, respectively. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed singlet at δ 2.2 ppm corresponding to C4-methylene group; singlet at δ 2.8 ppm corresponding to methyl group; singlet at δ 3.9 ppm for methoxy proton; singlet at δ 5.78 ppm corresponding to C5 proton; multiplet at δ range 7.2–8.4 ppm for aromatic proton; singlet at δ range 8.7–9.5 ppm for OH and singlet at δ range 10.0–10.5 ppm for NH proton. The elemental analysis results were within ±0.4% of the theoretical values.

2.2. Anti-mycobacterial activity

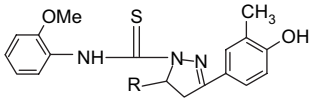
Among the ring substituted pyrazoline derivatives (**5a**–**k**) and (**6a**–**k**) were tested for their anti-mycobacterial activity in vitro against *M. tuberculosis* H37Rv using the BACTEC 460 radiometric system. The results are summarized in Tables 1 and 2 with INH, a standard used for comparison. Among the 22 newly synthesized compounds, compound anilino-3-(4-hydroxy-3-methylphenyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-1*H*-1-pyrazolylmethanethione (**6i**) produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0034 μM followed by (**6f**), (**6a**) and (**6e**) which showed moderate inhibitory activity with 0.0084 μM, 0.0092 μM and 0.0118 μM, respectively. The 2,6-dichloro group substitution (**6a**–**k**) derivatives (**6i**) displayed relatively higher inhibitory activity in general. However, the electron rich groups such as, 4-chloro, 2-chloro, 2,6-dichloro and 3-nitro substituted analogue compounds—**5b**, **5h**, **5i**, **5j**, **6b**, **6h**, **6j**—produced significant decrease in inhibitory activity against *M. tuberculosis* H37Rv. On the other hand pyrazoline analogues with 4′-methoxy phenyl substitution (**6a**), 3′,4′-dimethoxy phenyl substitution (**6e**) and 3′,4′,5′-trimethoxy phenyl substitution (**6f**) showed relatively moderate anti-tubercular activity. But among (**5a**–**k**) derivatives, compounds with 3′,4′-dimethoxy phenyl substitution (**5e**) and 3′,4′,5′-trimethoxy phenyl substitution (**5f**) exhibited relatively low inhibitory activity against *M. tuberculosis* H37Rv. Instead of (CH_3) group, (OCH_3) group substitution at N^1 phenyl ring in pyrazoline analogue worsens the anti-mycobacterial activity. These reports clearly showed that the increases in the presence of dichloro substitution at 2,6 position pyrazoline (**6a**–**k**) derivatives (**6i**) causes remarkable improvement in anti-mycobacterial activity.

All the newly synthesized compounds (**5a**–**k**) and (**6a**–**k**) were tested for cytotoxicity (IC_{50}) in VERO cells at

Table 2
Primary anti-mycobacterial activity of the synthesized compounds against *M. tuberculosis* H37Rv



5a-k

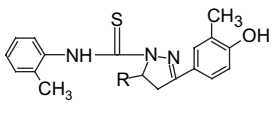


6a-k

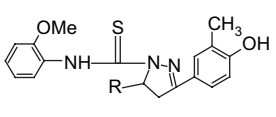
Compound	R	Primary screen (6.25 μg/ml)	% Inhibition	Concentration (μM)
5a	4-Methoxy phenyl-	>6.25	06	0.0403
5b	4-Chloro phenyl-	>6.25	44	0.0143
5c	4-Dimethylamino phenyl-	>6.25	12	0.0140
5d	Phenyl-	>6.25	29	0.0354
5e	3,4-Dimethoxy phenyl-	>6.25	72	0.0977
5f	3,4,5-Trimethoxy phenyl-	>6.25	44	0.1467
5g	4-Fluoro phenyl-	6.25	32	0.0148
5h	2-Chloro phenyl-	>6.25	33	0.0150
5i	2,6-Dichloro phenyl-	>6.25	21	0.0143
5j	3-Nitro phenyl-	>6.25	14	0.0132
5k	Furfuryl-	>6.25	26	0.1706
6a	4-Methoxy phenyl-	<6.25	94	0.0092
6b	4-Chloro phenyl-	>6.25	04	0.0138
6c	4-Dimethylamino phenyl-	6.25	28	0.0135
6d	Phenyl-	>6.25	06	0.0149
6e	3,4-Dimethoxy phenyl-	<6.25	88	0.0118
6f	3,4,5-Trimethoxy phenyl-	<6.25	92	0.0084
6g	4-Fluoro phenyl-	>6.25	16	0.0164
6h	2-Chloro phenyl-	>6.25	44	0.0202
6i	2,6-Dichloro phenyl-	**6.25**	**98**	**0.0034**
6j	3-Nitro phenyl-	>6.25	08	0.2080
6k	Furfuryl-	>6.25	18	0.0201

Isoniazid (0.025–0.05 μg/ml).

Table 3
Anti-mycobacterial activity of the synthesized compounds against *M. tuberculosis* H37Rv



5a-k



6a-k

Compound	R	Actual MIC (μg/ml)
5a	4-Methoxy phenyl-	–
5b	4-Chloro phenyl-	6.25
5c	4-Dimethylamino phenyl-	6.25
5d	Phenyl-	–
5e	3,4-Dimethoxy phenyl-	–
5f	3,4,5-Trimethoxy phenyl-	–
5g	4-Fluoro phenyl-	6.25
5h	2-Chloro phenyl-	6.25
5i	2,6-Dichloro phenyl-	6.25
5j	3-Nitro phenyl-	6.25
5k	Furfuryl-	–
6a	4-Methoxy phenyl-	4.12
6b	4-Chloro phenyl-	6.25
6c	4-Dimethylamino phenyl-	6.25
6d	Phenyl-	6.25
6e	3,4-Dimethoxy phenyl-	5.67
6f	3,4,5-Trimethoxy phenyl-	–
6g	4-Fluoro phenyl-	–
6h	2-Chloro phenyl-	–
6i	2,6-Dichloro phenyl-	**1.66**
6j	3-Nitro phenyl-	–
6k	Furfuryl-	–

Isoniazid (0.025–0.05 μg/ml).

concentrations of 62.5 μg/ml or 10 times. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method. Most of the active compounds were found to be non-toxic till 62.5 μg/ml.

Among the newer derivatives, it is conceivable that derivatives showing anti-mycobacterial activity can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about quantitative structure–activity relationships (QSAR) are in progress in our laboratory. The pyrazoline derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of anti-tubercular diseases.

3. Experimental

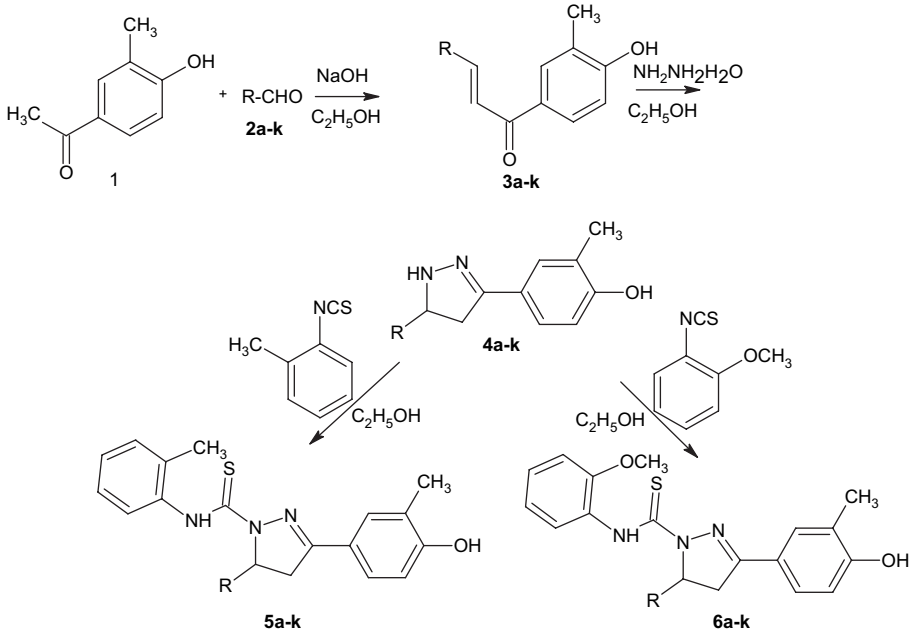
The entire chemicals were supplied by E. Merck (Germany) and SD Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene–ethyl formate–formic acid (5:4:1) and benzene–methanol (8:2), the spots were located under iodine vapors or UV light. IR spectra were obtained on a Perkin–Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/$CDCl_3$, mass spectra were recorded on a Bruker Esquire LC–MS using ESI and elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

3.1. General procedure

4-Hydroxy-3-methyl acetophenone (0.01 mol) was dissolved in ethanol. Then, a solution of potassium hydroxide (30%, 5 ml) and suitable substituted aldehydes (0.01 mol) in 10 ml of petroleum ether was added to the resulting solution with continuous stirring. The resulting solution was allowed to stand overnight. After 4 h of stirring, it was poured into ice-cold water, then neutralized with hydrochloric acid. The solid separate was filtered off, dried and purified from ethanol (**3a–k**).

3.1.1. 1-(4′-Hydroxy-3′-methylphenyl)-3-[(substituted) phenyl]-2-propen-1-one

1-(4′-Hydroxy-3′-methylphenyl)-3-[(substituted) phenyl]-2-propen-1-one derivatives were synthesized by condensing 4-hydroxy-3-methyl acetophenone with appropriate aromatic aldehydes according to Claisen–Schmidt condensation.



Scheme 1. Protocol for the synthesis.

*3.1.2. 1-(4-Hydroxy-3-methylphenyl-3-(4-methoxy phenyl)-2-propen-1-one (**3a**)*

IR: (KBr, cm^{-1}) 3210 (OH), 1682 (C=O), 3030 (CH). ^1H NMR (DMSO-d_6, ppm): 2.2 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 6.8–6.9 (1H × 2, d, $J = 7.5$, 8.5 Hz, CH=CH), 7.2–7.9 (7H, s, aromatic), 9.2 (1H, s, OH).

*3.1.3. 1-(4-Hydroxy-3-methylphenyl-3-(4-chloro phenyl)-2-propen-1-one (**3b**)*

IR: (KBr, cm^{-1}) 3210 (OH), 782 (C–Cl), 1680 (C=O), 3042 (CH). ^1H NMR (DMSO-d_6, ppm): 2.2 (3H, s, CH_3), 6.7–6.8 (1H × 2, d, $J = 8.34$, 6.79 Hz, CH=CH), 7.7–8.0 (7H, m, aromatic), 9.2 (1H, s, OH).

*3.1.4. 1-(4-Hydroxy-3-methylphenyl-3-(4-dimethyl amino phenyl)-2-propen-1-one (**3c**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1684 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6, ppm): 2.2 (3H, s, CH_3), 2.83 (6H, s, N ($CH_3 \times 2$)), 6.8–6.9 (1H × 2, d, $J = 7.61$, 7.63 Hz, CH=CH), 7.6–8.1 (7H, m, aromatic), 9.2 (1H, s, OH).

*3.1.5. 1-(4-Hydroxy-3-methylphenyl-3-phenyl-2-propen-1-one (**3d**)*

IR: (KBr, cm^{-1}) 3210 (OH), 1670 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6, ppm): 2.2 (3H, s, CH_3), 6.8–7.4 (1H × 2, d, $J = 8.28$, 6.70 Hz, CH=CH), 7.7–8.2 (8H, m, aromatic), 9.2 (1H, s, OH).

*3.1.6. 1-(4-Hydroxy-3-methylphenyl-3-(3,4-dimethoxy phenyl)-2-propen-1-one (**3e**)*

IR: (KBr, cm^{-1}) 3210 (OH), 1686 (C=O), 3030 (CH). ^1H NMR (DMSO-d_6, ppm): 2.2 (3H, s, CH_3), 3.9 (6H, s, $OCH_3 \times 2$), 6.9–7.3 (1H × 2, d, $J = 7.45$, 7.29 Hz, CH=CH), 7.6–8.1 (6H, m, aromatic), 9.2 (1H, s, OH).

*3.1.7. 1-(4-Hydroxy-3-methylphenyl-3-(3,4,5-tri-methoxy phenyl)-2-propen-1-one (**3f**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.1 (5H, m, aromatic), 3.9 (9H, s, $OCH_3 \times 3$), 6.9–7.5 (1H × 2, d, $J = 7.55$, 7.27 Hz, CH=CH).

*3.1.8. 1-(4-Hydroxy-3-methylphenyl-3-(4-fluoro phenyl)-2-propen-1-one (**3g**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH), 670 (C–F). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 (1H × 2, d, $J = 7.24$, 7.29 Hz, –CH=CH).

*3.1.9. 1-(4-Hydroxy-3-methylphenyl-3-(2-chloro phenyl)-2-propen-1-one (**3h**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH), 770 (C–Cl). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.6–8.0 (7H, m, aromatic), 6.9–7.5 (1Hx × 2, d, $J = 8.35$, 3.63 Hz, –CH=CH).

*3.1.10. 1-(4-Hydroxy-3-methylphenyl-3-(2,6-dichloro phenyl)-2-propen-1-one (**3i**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH), 770 (C–Cl). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.0 (6H, m, aromatic), 6.9–7.5 (1H × 2, d, $J = 5.41$, 15.68 Hz, CH=CH).

*3.1.11. 1-(4-Hydroxy-3-methylphenyl-3-(3-nitro phenyl)-2-propen-1-one (**3j**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 (1H × 2, d, $J = 5.46$, 16.3 Hz, CH=CH).

*3.1.12. 1-(4-Hydroxy-3-methylphenyl-3-furfuryl-2-propen-1-one (**3k**)*

IR: (KBr, cm^{-1}) 3200 (OH), 1680 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6, ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7–8.2 (6H, m, aromatic), 6.4–7.4 (3H, m, furan), 6.8–6.9 (1H × 2, d $J = 3.0$, 8.36 Hz, CH=CH).

4. General procedure

*4.1. 4-[5-(Substituted) phenyl-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4a–k**)*

To a solution of chalcone (**3a–k**) in ethanol, hydrazine hydrate (99%) was added drop wise. The reaction mixture was heated under reflux for 7 h and then cooled and poured onto crushed ice. The solid pyrazoline product was filtered and recrystallized from ethanol (**4a–k**).

*4.1.1. 4-[5-(4′-Methoxyphenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4a**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 4.24 (1H, s, CH), 5.52 (1H, s, NH), 7.3–7.8 (7H, m, aromatic), 9.5 (1H, s, OH).

*4.1.2. 4-[5-(4′-Chlorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4b**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 770 (C–Cl). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 4.24 (1H, s, CH), 5.50 (1H, s, NH), 7.2–7.6 (7H, m, aromatic), 9.5 (1H, s, OH).

*4.1.3. 4-[5-(4′-Dimethylaminophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4c**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1580 (C=N), 1324 (C–N). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 2.9 (3H × 2, s, $N(CH_3)_2$), 3.4 (3H, s, CH_3), 4.24 (1H, s, CH), 5.52 (1H, s, NH), 7.4–8.0 (7H, m, aromatic), 9.5 (1H, s, OH).

*4.1.4. 2-Methyl-4-(5′-phenyl-4,5-dihydro-1H-3-pyrazolyl) phenol (**4d**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 5.54 (1H, s, NH), 4.24 (1H, s, CH), 7.3–7.6 (8H, m, aromatic), 9.5 (1H, s, OH).

*4.1.5. 4-[5-(3′,4′-Dimethoxyphenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4e**)*

IR: (KBr, cm^{-1}) 3310 (OH), 1590 (C=N), 1320 (C–N)). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 3.7 (6H, s, 2 × OCH_3), 7.2–7.8 (6H, m, aromatic), 5.50 (1H, s, NH), 4.24 (1H, s, CH), 9.2 (1H, s, OH).

*4.1.6. 4-[5-(3′,4′,5′-Trimethoxy phenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4f**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1596 (C=N), 1320 (C–N). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 3.6 (9H, s, OCH_3), 4.24 (1H, s, CH), 5.48 (1H, s, NH), 7.3–7.8 (5H, m, aromatic), 9.5 (1H, s, OH).

*4.1.7. 4-[5-(4′-Fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4g**)*

IR: (KBr, cm^{-1}) 3312 (OH), 1590 (C=N), 1320 (C–N), 700 (C–F). ^1H NMR (DMSO-d_6, ppm): 9.4 (1H, s, OH), 7.3–7.8 (7H, m, aromatic), 5.42 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH_3), 2.3 (2H, s, CH_2).

*4.1.8. 4-[5-(2′-Chlorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4h**)*

(KBr, cm^{-1}) 3306 (OH), 1586 (C=N), 1320 (C–N), 774 (C–Cl). ^1H NMR (DMSO-d_6, ppm): 9.5 (1H, s, OH), 7.6–8.2 (7H, m, aromatic), 5.50 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH_3), 2.3 (2H, s, CH_2).

*4.1.9. 4-[5-(2′,6′-Dichlorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4i**)*

IR: (KBr, cm^{-1}) 3317 (OH), 1594 (C=N), 1320 (C–N), 770 (C–Cl). ^1H NMR (DMSO-d_6, ppm): 9.5 (1H, s, OH), 7.3–7.8 (6H, m, aromatic), 5.54 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH_3), 2.3 (2H, s, CH_2).

*4.1.10. 4-[5-(3′-Nitrophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylpheno (**4j**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N). ^1H NMR (DMSO-d_6, ppm): 9.4 (1H, s, OH), 7.8–8.4 (7H, m, aromatic), 5.56 (1H, s, NH), 4.20 (1H, s, CH), 3.2 (3H, s, CH_3), 2.7 (2H, s, CH_2).

*4.1.11. 4-[5-(2′-Furyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4k**)*

IR: (KBr, cm^{-1}) 3317 (OH), 1590 (C=N), 1320 (C–N). ^1H NMR (DMSO-d_6, ppm): 7.3–7.8 (3H, m, aromatic), 6.8–7.9 (3H, m, furan), 5.52 (1H, s, NH), 4.20 (1H, s, CH), 3.42 (3H, s, CH_3), 2.3 (2H, s, CH_2), 9.2 (1H, s, OH).

5. General procedure

*5.1. 3-(4-Hydroxy-3-methylphenyl)-5-(substituted) phenyl-4,5-dihydro-1H-1-pyrazolyl-2-toluidino methane thione (**5a–k**)*

To a solution of pyrazoline (**4a–k**) (0.01 mol) in ethanol (20 ml) was added 2-methyl aryl isothiocyanate (1.501 ml, 0.01 mol) and the reaction mixture was refluxed for 4 h. The reaction mixture was cooled and then poured onto crushed ice. The obtained solid was filtered, washed with water and purified from ethanol to give compounds (**5a–k**).

*5.1.1. Compound (**5a**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1596 (C=N), 1320 (C–N), 1130 (C=S), 3224 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 3.9 (3H, s, OCH_3), 5.3 (1H, s, CH), 7.2–7.4 (11H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH), EISMS: *m/z*: 431 (M^+); Calcd/Anal. [C 69.58/69.52, H 5.84/5.84, N 9.74/9.73].

*5.1.2. Compound (**5b**)*

IR: (KBr, cm^{-1}) 3317 (OH), 1590 (C=N), 770 (C–Cl), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.6 (6H, s, $2 \times CH_3$), 5.2 (1H, s, CH), 7.2–7.4 (11H, m, aromatic), 9.5 (1H, s, OH), 10.0 (1H, s, NH), EISMS: *m/z*: 436 (M^{+1}); Calcd/Anal. [C 66.12/66.22, H 5.09/5.04, N 9.64/9.60].

*5.1.3. Compound (**5c**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 3.9 (6H, s, $-N(CH_3)_2$), 4.9 (1H, s, CH), 7.2–7.8 (11H, m, aromatic), 8.7 (1H, s, OH), 10.2 (1H, s, NH), EISMS: *m/z*: 444 (M^+); Calcd/Anal. [C 70.24/70.22, H 6.35/6.32, N 12.60/12.64].

*5.1.4. Compound (**5d**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 5.1 (1H, s, CH), 7.2–7.7 (12H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH), EISMS: *m/z*: 402 (M^{+1}); Calcd/Anal. [C 71.79/71.70, H 5.77/5.77, N 10.46/10.45].

*5.1.5. Compound (**5e**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 3.9 (6H, s, $OCH_3 \times 2$), 5.0 (1H, s, CH), 7.2–7.4 (10H, m, aromatic), 8.7 (1H, s, OH), 10.2 (1H, s, NH): EISMS: *m/z*: 462 (M^{+1}); Calcd/Anal. [C 67.66/67.66, H 5.90/5.90, N 9.10/9.12].

*5.1.6. Compound (**5f**)*

IR: (KBr, cm^{-1}) 3317 (OH), 1596 (C=N), 1320 (C–N), 1132 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2 (2H, s, CH_2), 2.8 (6H, s, CH_3), 3.9 (9H, s, $OCH_3 \times 3$), 5.3 (1H, s, CH), 7.2–7.4 (9H, m, aromatic), 8.7 (1H, s, OH), 10.4 (1H, s, NH): EISMS: *m/z*: 491 (M^+); Calcd/Anal. [C 65.97/65.94, H 5.95/5.94, N 8.55/8.53].

*5.1.7. Compound (**5g**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 724 (C–F), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 5.4 (1H, s, CH), 7.2–7.9 (11H, m, aromatic), 8.7 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 420 (M^+); Calcd/Anal. [C 68.71/68.70, H 5.29/5.29, N 10.02/10.06].

*5.1.8. Compound (**5h**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 770 (C–Cl), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 5.2 (1H, s, CH), 7.2–7.4 (11H, m, aromatic), 9.4 (1H, s, OH), 10.0 (1H, s, NH), EISMS: *m/z*: 435 (M^+); Calcd/Anal. [C 66.12/66.10, H 5.09/5.05, N 9.64/9.64].

*5.1.9. Compound (**5i**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 770 (C–Cl), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.7 (6H, s, $2 \times CH_3$), 5.3 (1H, s, CH), 7.2–7.4 (10H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 471 (M^{+1}); Calcd/Anal. [C 61.28/61.26, H 4.50/4.52, N 8.93/8.93].

*5.1.10. Compound (**5j**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.1 (2H, s, CH_2), 2.4 (6H, s, $2 \times CH_3$), 5.3 (1H, s, CH), 7.2–8.2 (11H, m, aromatic), 8.6 (1H, s, OH), 10.5 (1H, s, NH): EISMS: *m/z*: 446 (M^+); Calcd/Anal. [C 64.56/64.54, H 4.97/4.95, N 12.55/12.53].

*5.1.11. Compound (**5k**)*

IR: (KBr, cm^{-1}) 3317 (OH), 1580 (C=N), 1310 (C–N), 1136 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $2 \times CH_3$), 5.3 (1H, s, CH), 7.2–7.4 (7H, m, aromatic), 6.8–7.9 (Furan), 9.2 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 392 (M^{+1}); Calcd/Anal. [C 67.50/67.51, H 5.41/5.41, N 10.73/10.72].

6. General procedure

*6.1. 3-(4-Hydroxy-3-methylphenyl)-5-(substituted) phenyl-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilino methane thione (**6a–k**)*

To a solution of pyrazolines (0.01 mol) (**4a–k**) in ethanol (20 ml) was added 2-methoxy aryl isothiocyanate (1.661 ml, 0.01 mol) and the reaction mixture was refluxed for 4 h. The reaction mixture was cooled and then poured onto crushed ice. The obtained solid was filtered, washed with water and purified from ethanol to give compounds (**6a–k**).

*6.1.1. Compound (**6a**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1592 (C=N), 1320 (C–N), 1132 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 1.6 (2H, s, CH_2), 2.5 (3H, s, CH_3), 3.3 (6H, s, $OCH_3 \times 2$), 5.2 (1H, s, CH), 6.5–8.4 (11H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH), EISMS: *m/z*: 447 (M^+); Calcd/Anal. [C 67.09/67.05, H 5.63/5.63, N 9.39/9.36].

*6.1.2. Compound (**6b**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1598 (C=N), 770 (C–Cl), 1310 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.5 (3H, s, CH_3), 2.7 (2H, s, CH_2), 3.8 (3H, s,

OCH_3), 5.0 (1H, s, CH), 6.5–8.4 (11H, m, aromatic), 9.9 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 451 (M^+); Calcd/Anal. [C 63.78/63.75, H 4.91/4.90, N 9.30/9.32].

*6.1.3. Compound (**6c**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1596 (C=N), 1320 (C–N), 1140 (C=S), 3222 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 2.9 (6H, s, $-N(CH_3)_2$), 3.9 (3H, s, OCH_3), 4.4 (1H, s, CH), 7.2–7.8 (11H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 461 (M^{+1}); Calcd/Anal. [C 63.40/63.42, H 5.73/5.76, N 11.37/11.35].

*6.1.4. Compound (**6d**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1324 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 3.9 (3H, s, OCH_3), 4.24 (1H, s, CH), 7.2–7.4 (12H, m, aromatic), 9.7 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 417 (M^+); Calcd/Anal. [C 69.04/69.00, H 5.55/5.54, N 10.06/10.06].

*6.1.5. Compound (**6e**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1310 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 3.9 (9H, s, $OCH_3 \times 3$), 4.24 (1H, s, CH), 7.2–7.4 (11H, m, aromatic), 8.7 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 478 (M^{+1}); Calcd/Anal. [C 65.39/65.29, H 5.70/5.72, N 8.80/8.84].

*6.1.6. Compound (**6f**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 3.9 (12H, s, $OCH_3 \times 4$), 4.4 (1H, s, CH), 7.2–7.4 (11H, m, aromatic), 9.7 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 508 (M^{+1}); Calcd/Anal. [C 63.89/63.86, H 5.76/5.75, N 8.28/8.29].

*6.1.7. Compound (**6g**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 820 (C–F), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.3 (2H, s, CH_2), 2.7 (6H, s, CH_3), 3.9 (3H, s, OCH_3), 7.2–7.4 (11H, m, aromatic), 9.4 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 436 (M^+); Calcd/Anal. [C 66.19/66.17, H 5.09/5.07, N 9.65/9.63].

*6.1.8. Compound (**6h**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 770 (C–Cl), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 3.8 (9H, s, OCH_3), 7.2–7.5 (11H, m, aromatic), 12.0 (1H, s, OH), 12.10 (1H, s, NH): EISMS: *m/z*: 452 (M^{+1}); Calcd/Anal. [C 63.78/63.76, H 4.91/4.90, N 9.30/9.31].

*6.1.9. Compound (**6i**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 770 (C–Cl), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, CH_3), 3.9 (3H, s, OCH_3), 7.2–7.6 (10H, m, aromatic), 9.7 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 486 (M^+); Calcd/Anal. [C 59.26/59.25, H 4.35/4.35, N 8.64/8.63].

*6.1.10. Compound (**6j**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, CH_3), 3.9 (3H, s, OCH_3), 7.2–8.4 (11H, m, aromatic), 9.7 (1H, s, OH), 10.10 (1H, s, NH): EISMS: *m/z*: 463 (M^{+1}); Calcd/Anal. [C 62.32/62.32, H 4.79/4.79, N 12.11/12.10].

*6.1.11. Compound (**6k**)*

IR: (KBr, cm^{-1}) 3307 (OH), 1590 (C=N), 1320 (C–N), 1130 (C=S), 3220 (NH). ^1H NMR (DMSO-d_6, ppm): 2.2 (2H, s, CH_2), 2.5 (6H, s, CH_3), 3.8 (3H, s, OCH_3), 7.0–7.4 (7H, m, aromatic), 6.8–7.9 (Furan), 9.2 (1H, s, OH), 10.0 (1H, s, NH): EISMS: *m/z*: 408 (M^{+1}); Calcd/Anal. [C 64.85/64.84, H 5.19/5.18, N 10.31/10.30].

7. Biology

The primary screening was conducted at concentration of 6.25 μg/ml (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis* H37Rv (ATCC27294) in BACTEC 12B medium using the BACTEC 460 radiometric system [11,12]. Compounds demonstrating at least 90% inhibition in the primary screen were re-examined at lower concentration (MIC) in broth micro-dilution assay with Almar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC_{50}) in VERO at concentration equal to and greater than the MIC for *M. tuberculosis* H37Rv after 72 h of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation assay.

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