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SYNTHESIS OF NOVEL SUBSTITUTED PYRAZOLYL-2-TOLUIDINOMETHANETHIONE AND PYRAZOLYL-2-METHOXYANILINOMETHANETHIONE AS POTENTIAL ANTITUBERCULAR AGENTS

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Abstract: In the present investigation, a series of 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione and 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione were synthesized and were tested for their antimycobacterial activity *in vitro* against isoniazid (INH) resistant *Mycobacterium tuberculosis* (INHR-MTB). Compound **6i** was found to be the most potent derivative of the series with an MIC value of 0.90 mg/mL. When compared to INH, compound **6i** was ca. 2-fold more active against INH resistant *Mycobacterium tuberculosis* (INHR-MTB).

Keywords: pyrazolines, antimycobacterial

Tuberculosis (TB), the world's leading killer among infectious diseases, has not faced an effective new class of drugs for more than 30 years. It is estimated that worldwide 8.2 million new TB cases occurred in the year 2000, and more than 95% of those were in developing countries (1). The global resurgence of tuberculosis and development of drug resistant populations have rekindled the need for and interest in the development of new antitubercular drugs. However, no new antituberculosis agents have been developed since the introduction of rifampin into clinical use. There is still a need for new therapeutic agents with high efficacy in antituberculosis activity.

Mycobacterium tuberculosis is the primary cause of mortality due to the infectious disease in the world. It is ubiquitous organism that is becoming increasingly important intracellular pathogen that establishes an infection in oxygen-rich macrophage of the lung (2). A resistance of *Mycobacterium tuberculosis* strains to antimycobacterial agents is an increasing problem worldwide (3-5). However, powerful new anti-TB drugs with new mechanisms of action have not been developed in the last forty 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). The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The synthesis of novel pyrazoline derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades for biological, medicinal and agricultural reasons. Living organisms find difficulty in construction of N-N bonds which limits the natural abundance of compounds having such bonds. Pyrazoline and their derivatives, a class of compounds containing the N-N bond exhibit a wide range of biological activities. A literature survey has revealed that pyrazoline derivatives are active against many Mycobacterias (7-13). The current work describes the synthesis of novel pyrazoline moiety with encouraging antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$.

EXPERIMENTAL

The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography

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(TLC) on silica gel G in the solvent system toluene-ethyl formate-formic acid (5:4:1, v/v/v) and benzene-methanol (8:2, v/v), the spots were located under iodine vapors or UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr discs). ^{1}H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as an internal standard in DMSOd_6. Mass spectra were recorded on a Bruker Esquire LCMS apparatus using ESI. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

General procedure for 1-(4-hydroxy-3-methylphenyl)-3-(substituted) phenyl-2-propen-1-ones

A mixture of 4-hydroxy-3-methyl acetophenone (1.5017 g, 0.01 mmol), appropriate aldehyde (0.01 mmol) in ethanol and sodium hydroxide (30%, 5 mL) in presence of 10 mL of petroleum ether was stirred under room temperature for 4 h. The resulting solution was allowed to stand overnight and poured into ice-cold water, then it was neutralized with hydrochloric acid. The solid so obtained was filtered, dried and crystallized from ethanol.

1-(4'-Hydroxy-3'-methylphenyl)-3-[(substituted) phenyl-2-propen-1-one (**3a-k**)

These derivatives were synthesized by condensing 4-hydroxy-3-methylacetophenone with appropriate aromatic aldehydes according to Claisen-Schmidt condensation.

1-(4-Hydroxy-3-methylphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**3a**)

IR (KBr, cm^{-1}): 3210 (OH), 3030 (CH), 1682 (C=O); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (3H,s, CH_3), 3.9 (3H,s, OCH_3), 6.8 -7.5(1H × 2, dd *J* = 7.5 Hz, 8.5 Hz CH=CH), 7.7-8.2 (7H, s, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl)-3-(4-cholorophenyl)-2-propen-1-one (**3b**)

IR (KBr, cm^{-1}): 3210 (OH), 3042 (CH), 1680 (C=O), 782 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (3H, s, CH_3), 6.7-7.2 (1H × 2, dd *J* = 8.34 Hz, 6.79 Hz, CH=CH), 7.7-8.0 (7H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl)-3-(4-dimethylaminophenyl)-2-propen-1-one (**3c**)

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1684 (C=O); ^{1}H-NMR (DMSO-d6), δ (ppm): 2.2 (3H, s, CH_3), 3.9 (6H, s, N(CH_3 × 2)), 6.8-7.5 (1H × 2, dd *J* = 7.61 Hz, 7.63 Hz, CH=CH), 7.6-8.1 (7H, m, aromatic), 9.2 (1H, s, OH).

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

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

1-(4-Hydroxy-3-methylphenyl)-3-(3,4-dimethoxyphenyl)-2-propen-1-one (**3e**)

IR (KBr, cm^{-1}): 3210 (OH), 3030 (CH), 1686 (C=O); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (3H, s, CH_3), 3.9 (6H, s, OCH_3 × 2), 6.9 -7.3 (1H × 2, dd *J* = 7.45 Hz, 7.29 Hz, CH=CH), 7.6-8.2 (6H, m, aromatic), 9.2 (1H, s, OH).

1-(4-Hydroxy-3-methylphenyl)-3-(3,4,5trimethoxyphenyl)-2-propen-1-one (**3f**)

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1680 (C=O); ^{1}H-NMR (DMSO-d_6), δ (ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7-8.2 (5H, m, aromatic), 3.9 (9H, s, OCH_3 × 3), 6.9-7.5 (1H × 2, dd *J* = 7.55 Hz, 7.27 Hz, CH=CH).

1-(4-Hydroxy-3-methylphenyl)-3-(4-fluorophenyl) -2-propen-1-one (**3g**).

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1680 (C=O), 670 (C-F); ^{1}H-NMR (DMSO-d_6), δ (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, dd *J*= 7.24 Hz, 7.29 Hz, CH=CH).

1-(4-Hydroxy-3-methylphenyl)-3-(2-chlorophenyl)-2-propen-1-one (**3h**)

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

1-(4-Hydroxy-3-methylphenyl)-3-(2,6-dichlorophenyl)-2-propen-1-one (**3i**)

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1680 (C=O), 770 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7-8.2 (6H, m, aromatic), 6.9-7.5 (1H × 2, dd *J* = 5.41 Hz, 15.68 Hz, CH=CH).

1-(4-Hydroxy-3-methylphenyl)-3-(3-nitrophenyl)-2-propen-1-one (**3j**)

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1680 (C=O); ^{1}H-NMR (DMSO-d_6), δ (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, dd *J* = 5.46 Hz, 16.3 Hz, CH=CH).

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

IR (KBr, cm^{-1}): 3200 (OH), 3040 (CH), 1680 (C=O); ^{1}H-NMR (DMSO-d_6), δ (ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH_3), 7.7-8.2 (6H, m, aromatic), 7.43-7.48 (3H, m, furan), 6.9-7.5 (1H × 2, dd *J*= 3.0 Hz, 8.36 Hz, CH=CH).

General procedure for 4-[5-(substituted) phenyl-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenols (**4a-k**)

To a solution of chalcone (**3a-k**) in ethanol, hydrazine hydrate (99%) was added dropwise. 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.

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); ^{1}H–NMR (DMSO-d_6) δ (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-[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); ^{1}H-NMR (DMSO-d_6), δ (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-[5-(4'- Dimethylaminophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4c**)

IR (KBr, cm^{-1}): 3307 (OH), 1580 (C=N), 1324 (C-N); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 2.9 (6H, 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).

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); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 4.24 (1H, s, CH), 5.54 (1H, s, NH), 7.3-7.6 (8H, m, aromatic), 9.5 (1H, s, OH).

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); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 3.7 (6H, s, $OCH_3 \times 2$), 4.24 (1H, s, CH), 5.50 (1H, s, NH), 7.2-7.8 (6H, m, aromatic), 9.2 (1H, s, OH).

4-[5-(3',4',5'-Trimethoxyphenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4f**)

IR (KBr, cm^{-1}): 3307 (OH), 1596 (C=N), 1320 (C-N); ^{1}H-NMR (DMSO-d_6), δ (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-[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); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2). 3.4 (3H, s, CH_3), 4.24 (1H, s, CH), 5.42 (1H, s, NH), 7.3-7.8 (7H, m, aromatic), 9.4 (1H, s, OH).

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

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

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); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 3.4 (3H, s, CH_3), 4.24 (1H, s, CH), 5.54 (1H, s, NH), 7.3-7.8 (6H, m, aromatic), 9.5 (1H, s, OH).

4-[5-(3'-Nitrophenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methylphenol (**4j**)

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

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); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 3.42 (3H, s, CH_3), 4.20 (1H, s, CH), 5.52 (1H, s, NH), 7.3-7.8 (3H, m, aromatic), 7.8-8.2 (3H, m, furan), 9.2 (1H, s, OH).

General procedure for 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5a-k**)

2-Methylaryl isothiocyanate (0.01 mol) was added to a solution of pyrazoline (**4a-k**) (0.01 mol) in ethanol (20 mL). The reaction mixture was refluxed for 4 h and after cooling it was poured onto crushed ice. Then, the separated solid mass was filtered, washed with water and crystallized from ethanol.

5-(4-Methoxy phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5a**)

IR (KBr, cm^{-1}): 3307 (OH), 3224 (NH), 1596 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3 \times 2$), 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^+); Analysis: calc. 69.58 %C, 5.84 %H, 9.74 %N; found: 69.52 %C, 5.84 %H, 9.73 %N.

5-(4 Chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5b**)

IR (KBr, cm^{-1}): 3317 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 770 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.6 (6H, s, $CH_3 \times 2$), 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 $(M+1)^+$; Analysis: calc.: 66.12 %C, 5.09 %H, 9.64 %N; found: 66.22 %C, 5.04 %H, 9.60 %N.

5-(4-Dimethylaminophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5c**)

IR: (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 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^+); Analysis: calc.: 70.24 %C, 6.35 %H, 12.60 %N; found: 70.22 %C, 6.32 %H, 12.64 %N.

5-(Phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5d**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 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 $(M+1)^+$; Analysis: calc: 71.79 %C, 5.77 %H, 10.46 %N; found: 71.70 %C, 5.77 %H, 10.45 %N.

5-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2- toluidinomethanethione (**5e**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 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 $(M+1)^+$; Analysis: calc. 67.66 %C, 5.90 %H, 9.10 %N; found: 67.66 %C, 5.90 %H, 9.12 %N.

5-(3,4,5-Trimethoxyphenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5f**)

IR (KBr, cm^{-1}): 3317 (OH), 3220 (NH),1596 (C=N), 1320 (C-N), 1132 (C=S); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.0 (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^+); Analysis: calc.: 65.97 %, 5.95 %H, 8.55 %N; found: 65.94 %C, 5.94 %H, 8.53 %N.

5-(4-Fluorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5g**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 724 (C-F); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 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^+); Analysis: calc.: 68.71 %C, 5.29 %H, 10.02 %N; found: 68.70, 5.29 %H, 10.06 %N.

5-(2-Chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5h**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH) 1590 (C=N), 1320 (C-N), 1130 (C=S), 770 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 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^+); Analysis: calc.: 66.12 %C, 5.09 %H, 9.64 %N; found: 66.10 %C, 5.05 %H, 9.64 %N.

5-(-2,6-Dichlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5i**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 770 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.7 (6H, s, $CH_3\times 2$), 5.3 (1H, s, CH), 7.2-7.4 (10 H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH); EISMS: m/z: 471 $(M+1)^+$; Analysis: calc.: 61.28 %C, 4.50 %H, 8.93 %N; found: 61.26 %C, 4, 4.52 %H, 8.93 %N.

5-(3-Nitrophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5j**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.1(2H, s, CH_2), 2.4 (6H, s, $CH_3\times 2$), 5.3 (1H, s, CH), 7.2-7.4 (11 H, m, aromatic), 8.6 (1H, s, OH), 13.5 (1H, s, NH): EISMS: m/z: 446 (M^+); Analysis: calc.: 64.56 %C, 4.07 %H, 12.55 %N; found: 64.54 %C, 4.59 % N, 12.53 %H.

5-(Furyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5k**)

IR (KBr, cm^{-1}): 3317 (OH), 3220 (NH), 1580 (C=N), 1310 (C-N), 1136 (C=S); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.2 (2H, s, CH_2), 2.8 (6H, s, $CH_3\times 2$), 5.3 (1H, s, CH), 7.2-7.4 (7H, m, aromatic), 7.8-8.0 (furan), 9.2 (1H, s, OH), 10.0 (1H, s, NH); EISMS: m/z: 392 $(M+1)^+$; Analysis: calc.: 67.50 %C, 5. 41 %H, 10.73 %N, found: 67.51 %C, 5.41 %H, 10.72 %N.

General procedure for 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethiones (**6a-k**)

To a solution of pyrazoline **(4a-k)** in ethanol (20 mL) 2-methoxyaryl isothiocyanate (0.01 mol) was added and the reaction mixture was refluxed for 4 h. Then, after cooling, the reaction mixture was

poured onto crushed ice and the separated solid mass was filtered, washed with water and crystallized from ethanol.

5-(4- Methoxyphenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6a**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1592 (C=N), 1320(C-N), 1132 (C=S); ^{1}H-NMR (DMSO-d_6), δ (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^+); Analysis: calc.: 67.09 %C, 5.63 %H, 9.39 %N, found: 67.05 %C, 5.63 %H, 9.36 %N.

5-(4-Chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6b**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1598 (C=N), 770 (C-Cl), 1310 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (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^+); Analysis: calc.: 63.78 %C, 4.91 %H, 9.30 %N, found: 63.78 %C, 4.90 %H, 9.32 %N.

5-(4-Dimethylaminophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6c**)

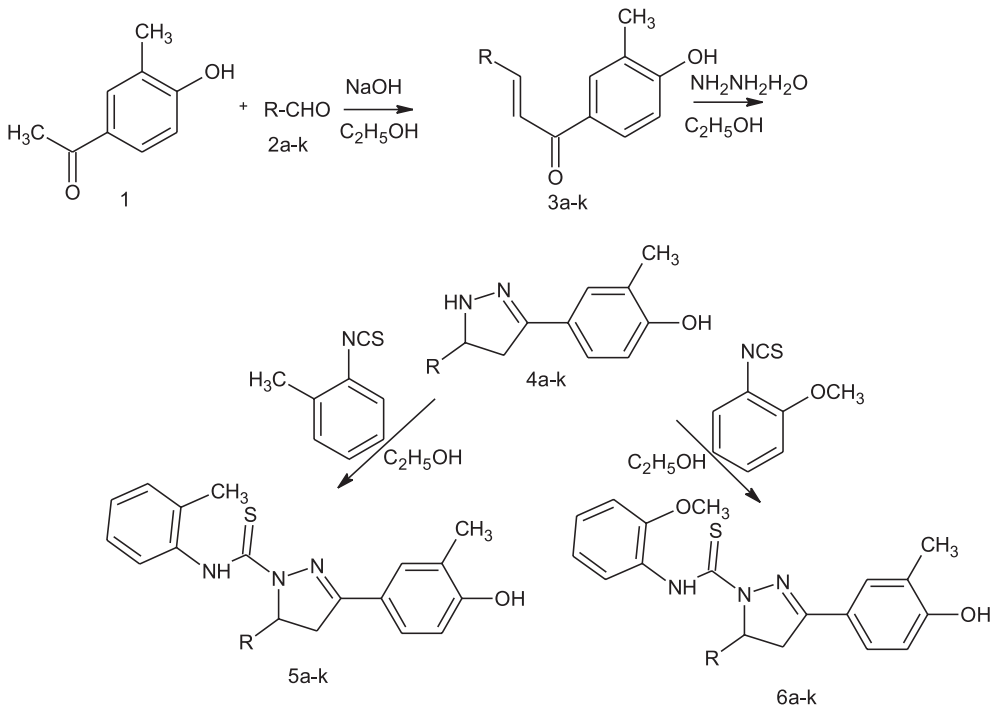
IR (KBr, cm^{-1}): 3307 (OH), 1596 (C=N), 1320 (C-N), 1140 (C=S), 3222 (NH); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (3H, s, CH_3), 2.9 (6H, s, $N(CH_3)_2$), 3.9 (3H, s, OCH_3), 4.4 (1H, s, CH) 7.2-7.8 (11H, m, aromatic), 9.7 (1H, s, OH), 10.0 (1H, s, NH); EISMS: m/z: 461 $(M+1)^+$; Analysis: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

5-(Phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6d**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1324 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (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^+); Analysis: calc.: 69.04 %C, 5.55 %H, 10.06 %N, found: 69.00 %C, 5.54 %H, 10.06 %N.

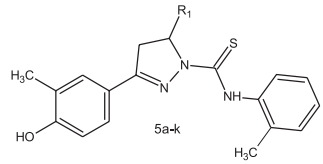
5-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6e**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1310 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (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 (11 H, m, aromatic), 8.7 (1H, s, OH), 10.10 (1H, s, NH); EISMS: m/z: 478 $(M+1)^+$; Analysis: calc.: 65.39



Scheme 1. Synthesis of 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione (**5a-k**) and 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione and (**6a-k**)

Table 1. Physical constants and antimycobacterial activity of the newly synthesized 5-[(substituted) phenyl]-3-(4-hydroxy-3-methyl-phenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione[a] **(5a-k).**



Compound no.	R_1	Yield (%)	M.p (^oC)	Mol. formula	Mol. weight	MIC[b] (µg/mL)
5a	4-Methoxyphenyl	74	144	$C_{25}H_{25}N_3O_2S$	431.55	6.25
5b	4-Chlorophenyl	70	131	$C_{24}H_{22}N_3OSCl$	435.97	1.98
5c	4-Dimethylaminophenyl	72	104	$C_{26}H_{28}N_4OS$	444.59	5.25
5d	Phenyl	80	121	$C_{24}H_{23}N_3OS$	401.52	
5e	3,4-Dimethoxyphenyl	82	102	$C_{26}H_{27}N_3O_3S$	461.57	6.25
5f	2,3,4-Trimethoxyphenyl	85	103	$C_{27}H_{29}N_3O_4S$	491.60	6.12
5g	4-Fluorophenyl	92	196	$C_{24}H_{22}N_3OSF$	419.51	3.12
5h	2-Chlorophenyl	85	115	$C_{24}H_{22}N_3OSCl$	435.97	2.76
5i	2,6-Dichlorophenyl	77	164	$C_{24}H_{21}N_3OSCl_2$	470.41	**1.80**
5j	3-Nitrophenyl	82	104	$C_{24}H_{22}N_4O_3S$	446.52	4.12
5k	Furyl	90	205	$C_{22}H_{21}N_3O_2S$	391.48	6.25
INH	-	-	-	-	-	**1.86**

[a]Recrystallization: ethanol, acetic acid; [b] INH resistant *Mycobacterium tuberculosis*

%C, 5.70 %H, 8.80 %N, found: 65.29 %C, 5.72 %H, 8.84 %N.

5-(3,4,5-Trimethoxyphenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6f**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6), δ (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 $(M+1)^+$; Analysis: calc.: 63.89%C, 5.76 %H, 8.28 %N, found: 63.86 %C, 5.75 %H, 8.29 %N.

5-(4-Fluorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6g**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 820 (C-F); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.3 (2H, s, CH_2), 2.7 (3H, 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^+); Analysis: calc.: 66.19 %C, 5.09 %H, 9.65 %N, found: 66.17 %C, 5.07 %H, 9.63 %N.

5-(2-Chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6h**)

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

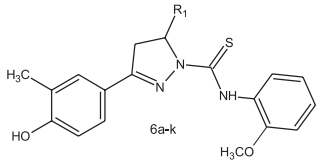
5-(2,6-Dichlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6i**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 770 (C-Cl); ^{1}H-NMR (DMSO-d_6), δ (ppm): 2.2 (2H, s, CH_2), 2.8 (3H, 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^+); Analysis: calc.: 59.26 %C, 4.35 %H, 8.64 %N, found: 59.25 %C, 4.35 %H, 8.63 %N.

5-(3-Nitrophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6j**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S), ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.2 (2H, s, CH_2), 2.8 (3H, 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 $(M+1)^+$; Analysis: calc.: 62.32 %C, 4.79 %H, 12.11 %N, found: 62.32 %C, 4.79 %H, 12.10 %N.

Table 2. Physical constants and antimycobacterial activity the newly synthesized 5-[(substituted) phenyl]-3- (4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione[a] **(6a-k).**



Compound no.	R_1	Yield (%)	M.p[a]. (^oC)	Mol. formula	Mol. weight	MIC[b] (µg/mL)
6a	4-Methoxyphenyl	82	124	$C_{25}H_{25}N_3O_3S$	447.55	6.10
6b	4-Chlorophenyl	80	153	$C_{24}H_{22}N_3O_2SCl$	451.96	**1.20**
6c	4-Dimethylaminophenyl	75	115	$C_{24}H_{23}N_3O_2S$	460.59	4.89
6d	Phenyl	80	234	$C_{26}H_{27}N_3O_4S$	417.52	6.25
6e	3,4-Dimethoxyphenyl	77	197	$C_{27}H_{29}N_3O_5S$	477.57	6.12
6f	2,3,4-Trimethoxyphenyl	72	106	$C_{24}H_{22}N_3O_2SF$	507.60	5.78
6g	4-Fluorophenyl	82	142	$C_{24}H_{22}N_3O_2SCl$	435.51	**0.98**
6h	2-Chlorophenyl	72	156	$C_{24}H_{21}N_3O_2SCl_2$	451.96	2.20
6i	2,6-Dichlorophenyl	70	172	$C_{24}H_{22}N_4O_4S$	486.41	**0.90**
6j	3-Nitrophenyl	44	194	$C_{22}H_{21}N_3O_3S$	462.52	3.76
6k	Furyl	80	169	-	407.48	6.10
INH	-	-	-		-	**1.86**

[a] Recrystalization: ethanol, acetic acid; [b] INH resistant *Mycobacterium tuberculosis*

5-(Furyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6k**)

IR (KBr, cm^{-1}): 3307 (OH), 3220 (NH), 1590 (C=N), 1320 (C-N), 1130 (C=S); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.2 (2H, s, CH_2), 2.5 (3H, s, CH_3), 3.8 (3H, s, OCH_3), 7.0-7.4 (7H, m, aromatic), 7.8-8.2 (furan), 9.2 (1H, s, OH), 13.0 (1H, s, NH); EISMS: m/z: 408 $(M+1)^+$; Analysis: calc.: 64.85 %C, 5.19 %H, 10.31 %N, found: 64.84 %C, 5.18 %H, 10.30 %N.

RESULTS AND DISCUSSION

Chemistry

5-[(Substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidinomethanethione and 5-[(substituted) phenyl]-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione, (**5a-k**) and (**6a-k**), respectively, described in this study are shown in Tables 1 and 2, and the reaction sequence for the preparation is outlined in Scheme 1. The chalcones were prepared by reaction of 3-methyl-4-hydroxyacetophenone with appropriate aldehyde in a presence of base by conventional Claisen-Schmidt condensation. The reaction between newly synthesized chalcones with hydrazine hydrate in ethanol led to synthesis of novel pyrazolines (**4a-k**), which on treatment with various aryl isothiocyanates afforded 3,5-disubstituted pyrazolines (**5a-k**) and (**6a-k**), respectively, with 65-92% yield. The purity of the compounds was checked by TLC and elemental analyses. Spectral data (IR, MS and ^{1}H-NMR) fully confirmed the structures.

Antimycobacterial activity

Among the ring substituted pyrazoline derivatives (**5a-k**) and (**6a-k**) were tested for their antimycobacterial activity *in-vitro* against INH resistant *Mycobacterium tuberculosis* (INHR-MTB), using the BACTEC 460-radiometric system. The results are summarized in Tables 1 and 2 with INH, a standard used for comparison. Among the twenty-two newly synthesized compounds, the compound 5-(2,6-dichlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxyanilinomethanethione (**6i**) produced the highest efficacy and exhibited > 90% inhibition at MIC 0.90 µg/mL, followed by (**6g**) and (**6b**), which showed moderate inhibitory activity with MIC 0.98 µg/mL and 1.20 µg/mL, respectively. The 2,6-dichloro group substitution in (**6a-k**) derivatives, i.e. in (**6i**) displayed, in general, relatively higher inhibitory activity. However, an

electron rich group substituted analogues, such as 4-chlorophenyl, 2-chlorophenyl, 2,6-dichlorophenyl and 3-nitrophenyl, (**5b**), (**5h**), (**5i**), (**5j**), (**6h**) and (**6j**), showed moderate inhibitory activity against (INHR-MTB). On the other hand, the analogues substituted with 4'-methoxyphenyl (**6a**), 3',4'-dimethoxyphenyl (**6e**) and 3',4',5'-trimethoxyphenyl (**6f**) showed significant decrease in inhibitory activity. Among **5a-k** derivatives, compounds substituted with 4'-methoxyphenyl (**5a**), 3',4'-dimethoxyphenyl (**5e**) and 3',4',5'-trimethoxyphenyl (**5f**) exhibited relatively low inhibitory activity against (INHR-MTB). The substitution of OCH_3 group instead of CH_3 group at the phenyl ring in pyrazoline analogue improves the antimycobacterial activity. These reports clearly showed that the presence of dichloro substitution at pyrazoline C-5 phenyl ring among **6a-k** derivatives like in (**6i)** causes remarkable improvement in antimycobacterial activity and compounds **6g**, **6b** and **5i** exhibited moderate to good inhibitory activity against INH resistant *Mycobacterium tuberculosis* (INHR-MTB).

All the compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations 62.5 μg/mL i.e. 10 times higher that 6.25 mg/mL. After 72 h 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 (14). Most of the active compounds were found to be non-toxic below 62.5 μg/mL.

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

Biology

The primary screening was conducted at a concentration of 6.25 μg/mL (or molar equivalent of highest molecular weight compound in a series of congeners) against INH resistant *Mycobacterium tuberculosis* (INHR-MTB) in BACTEC 12B medium using the BACTEC 460 radiometric system (15, 16).

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