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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Discovery of new antitubercular agents by combining pyrazoline and benzoxazole pharmacophores: design, synthesis and insights into the binding interactions

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Abstract The present study is aimed at combining two well-known pharmacophores (pyrazoline and benzoxazole nucleus) to design and synthesize a series of new benzoxazole-based pyrazoline derivatives. In vitro antitubercular evaluation against *Mycobacterium tuberculosis* $H_{37}Rv$ and multidrug-resistant TB (MDR-TB) strains showed that most of the target compounds displayed potent activity (MIC ~0.625–25 μg/mL). Few compounds were found to be better than isoniazid against MDR-TB (MIC = 3.25 μg/mL). In order to gain insights into the plausible binding motifs, the target compounds were docked into enoyl-acyl carrier protein (ACP) reductase, a molecular target of isoniazid. Many compounds were successfully docked into the active site of enoyl-ACP reductase and all the docked compounds occupied the same hydrophobic binding pocket and interacted mostly by dispersion interactions with the neighboring residues Met103, Met155, Tyr158, Met199, Ile202, Ile215, and Leu218. Contribution of the three pharmacophoric fragments (pyrazoline, benzoxazole and aryl ring) towards protein–ligand binding was evaluated at semi-empirical quantum mechanics level. The interaction energies suggested that most of the binding was governed by the benzoxazole moiety followed by pyrazoline and aryl rings.

Keywords Antitubercular · Benzoxazole · Interaction energy · Molecular docking · Pharmacophore · Pyrazoline

Introduction

The designation of tuberculosis as a global public health crisis by the World Health Organization in the mid-1990s has underscored severe challenges facing the research community globally. It has been estimated by the World Health Organization (WHO) that almost one-third of the world's population, around two billion people, are infected with the disease (Dye *et al.*, 2009). Every year, more than eight million people develop an active form of the disease, which subsequently claims the lives of nearly two million. In 2002, the WHO estimated that if the worldwide spread of tuberculosis was left unchecked, then the disease would be responsible for approximately 36 million more deaths by the year 2020. Further, the emergence of multidrug-resistant TB (MDR-TB), a form of TB that does not respond to the first-line TB drugs and extensively drug-resistant TB (XDR-TB), an MDR-TB with resistance to fluoroquinolones and aminoglycosides, have become a serious threat to TB control and its treatment. On top of this, the recent cases of totally drug-resistant tuberculosis have raised alarming concerns on the existing drug regimen implying urgent need to discover newer antitubercular

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agents with newer molecular mechanisms (Udwadia *et al.*, 2012).

The benzoxazole scaffold has provided the basis for the design of biologically relevant molecules with broad therapeutic importance. Several benzoxazole derivatives have been reported to possess antitubercular activity (Fig. 1, right pane) (Vinsova *et al.*, 2006; Temiz-Arpaci *et al.*, 2008; Kočí *et al.*, 2002). Compounds with N–N bond are of primary importance due to the known fact that living organism finds it difficult to construct 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 (Ahsan *et al.*, 2012; Siddiqui *et al.*, 2011; Bandgar *et al.*, 2012; Abdel-Wahab *et al.*, 2012) including antimycobacterial activity (Fig. 1, left pane) (Manna and Agrawal, 2010; Shaharyar *et al.*, 2006; Sharma *et al.*, 2011). In light of these facts, we thought of incorporating the two scaffolds to come out with chemically new antimycobacterial agents (Fig. 1, compound **5a–5h** and **6a–6h**). The synthesized compounds were then subjected to antimycobacterial screening against *Mycobacterium tuberculosis* $H_{37}Rv$ and MDR-TB strains. We also evaluated compounds **4a–4h**, which are immediate precursors of compounds **5a–5h** and **6a–6h**.

To gain insights into the plausible binding interactions, we carried out docking of the designed compounds into the crystal structure of *M. tuberculosis* enoyl reductase (InhA) (PDB Code 2H7I) (He *et al.*, 2006). We also investigated contribution of the three fragments/pharmacophores of the target compounds (pyrazoline core, benzoxazole moiety, and aryl ring) towards protein binding.

Materials and methods

Chemistry

All chemicals and reagents used in the study were of analytical grade and were used directly. Melting points were determined in an open glass capillary and are uncorrected. Thin layer chromatography (TLC) was performed on Silica Gel G (Merck) and spots were visualized under UV radiation, exposure to iodine vapors and with various spray reagents. IR spectra were recorded on FT-IR Spectrometer Shimadzu 8201 PC instrument in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz BRUKER ULTRASHIELD using DMSO-d_6 as solvent and chemical shift values were expressed in δ ppm downfield from the internal standard tetramethylsilane.

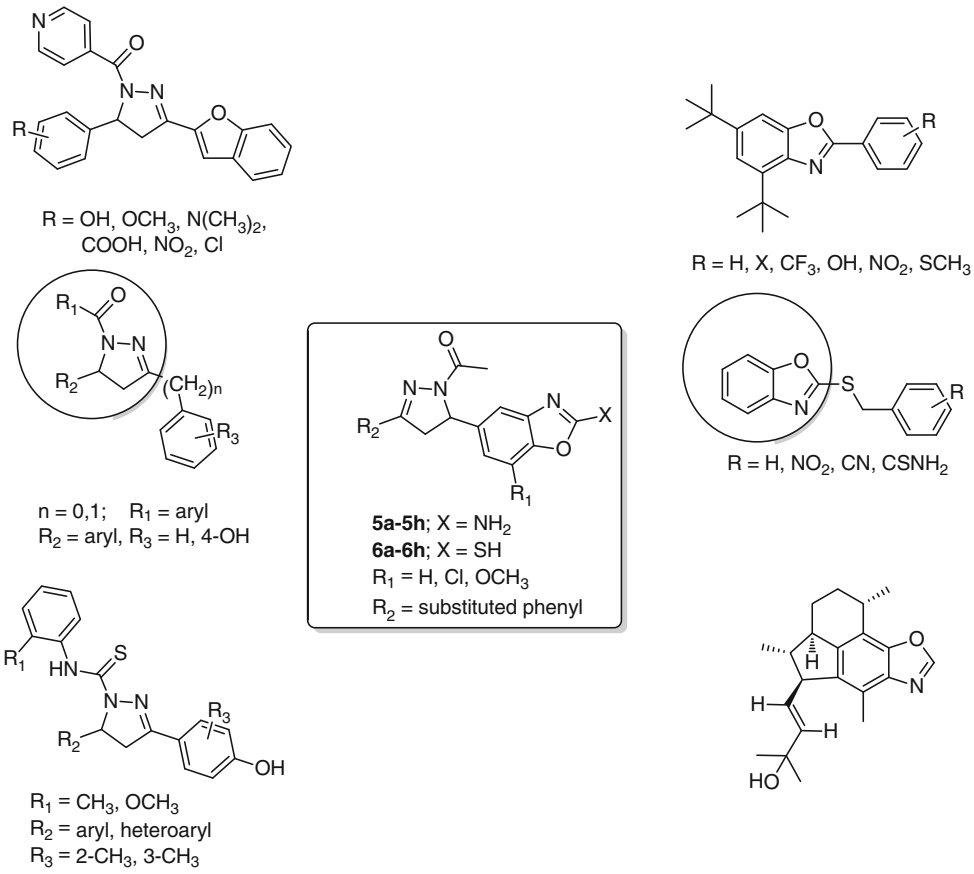


Fig. 1 Structures of some representative pyrazoline, benzoxazole derivatives, and designed compounds. Pyrazoline and benzoxazole moieties are *encircled*

*General procedure for synthesis of 1-aryl-3-(4-hydroxy-3-methoxy-5-nitrophenyl)-2-propenone (**2a–2h**)*

To a solution of 4-hydroxy-3-substituted-5-nitrobenzaldehyde (**1a**–**1c**, 50.8 mmol) and acetophenone (50.8 mmol) in methanol (75 mL) was added 25 % aqueous NaOH solution (75 mL) slowly while maintaining the temperature below 25 °C. The resulting reaction mixture was stirred for 20 h at room temperature. The reaction mixture was then acidified with 5 N HCl solution and the resulting orange-yellow precipitates were filtered off to afford 1-aryl-3-(4-hydroxy-3-substituted-5-nitrophenyl)-2-propenone (**2a**–**2h**) in good yield (75–90 %).

*General procedure for synthesis of 1-(5-(4-hydroxy-3-substituted-5-nitrophenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**3a–3h**)*

To a solution of 1-aryl-3-(4-hydroxy-3-substituted-5-nitrophenyl)-2-propenone (**2a**–**2h**, 16.7 mmol) and glacial acetic acid (50 mL) was added hydrazine hydrate (80 % w/v) (33.4 mmol) solution drop wise. After completion of addition, the reaction mixture was refluxed for 3 h. The reaction mixture was then poured into ice-water. Resulting yellow residues were filtered off and dried to get the crude product which was further purified by recrystallization from ethanol to afford 1-(5-(4-hydroxy-3-substituted-5-nitrophenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**3a**–**3h**) in good yield (55–85 %).

*General procedure for synthesis of 1-(5-(3-amino-4-hydroxy-5-substituted-phenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**4a–4h**)*

To a solution of 1-(5-(4-hydroxy-3-substituted-5-nitrophenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**3a**–**3h**, 7.04 mmol) in methanol (25 mL) was added sodium dithionite (6.13 g, 35.2 mmol) portion wise at 55–60 °C to avoid vigorous frothing. The color of the reaction mixture slowly turned orange to colorless with the progress of the reaction. The colorless reaction mixture was further refluxed for additional 30 min to ensure the completion of reaction after which the reaction mixture was poured into ice-water. The resulting off-white residues were filtered off and dried to get the crude product which was further recrystallized from isopropyl alcohol to yield 1-(5-(3-amino-4-hydroxy-5-substituted-phenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**4a**–**4h**) as pure product.

*1-(5-(3-Amino-4-hydroxy-5-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone (**4a**)* Yield, 60.8 %; mp 137–139 °C; IR (KBr): 3500–3213 (O–H), 3360 (N–H), 3054 (Ar–H), 2924 (aliphatic C–H), 1684 (C=O), 1599 (C=N), 1514 (C=C), 1241 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.686–7.626 (m, 2H), 7.364–7.348 (m, 3H), 6.183 (d, 2H, $J = 2.12$ Hz), 5.357 (q, 1H, $J_{HH'} = 11.64$ Hz, $J_{HH''} = 4.16$ Hz), 3.741 (s, 3H), 3.694–3.620 (m, 2H), 2.315 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.65, 40.01, 42.19, 55.47, 59.50, 77.86, 78.18, 78.38, 78.51, 98.54, 104.60, 126.20, 128.32, 129.78, 131.10, 131.68, 133.12, 136.33, 147.27, 153.56, 167.46; MS (*m*/*z*); 326.2 (M+1).

*1-(5-(3-Amino-4-hydroxy-5-methoxyphenyl)-3-(2,4-dichlorophenyl)-4,5-dihydropyrazol-1-yl)ethanone (**4b**)* Yield, 64.6 %; mp 156–158 °C; IR (KBr): 3450–3210 (O–H), 3315 (N–H), 3037 (Ar–H), 2918 (aliphatic C–H), 1678 (C=O), 1593 (C=N), 1533 (C=C), 1138 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.711 (d, 1H, $J_{ab} = 8.52$ Hz), 7.461 (d, 1H, $J_{bc} = 1.9$ Hz), 7.348 (dd, 1H, $J_{ab} = 8.52$ Hz, $J_{bc} = 1.9$ Hz), 6.201 (d, 2H, $J = 10.56$ Hz), 5.341 (dd, 1H, $J_{HH'} = 11.64$ Hz, $J_{HH''} = 4.2$ Hz), 3.861 (q, 1H, $J_{HH'} = 12.16$ Hz, $J_{H'H''} = 18.44$ Hz), 3.738 (s, 3H), 3.200 (dd, 1H, $J_{HH''} = 4.28$ Hz, $J_{H'H''} = 18.24$ Hz), 2.275 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.68, 44.80, 55.45, 59.76, 98.12, 104.81, 127.17, 128.99, 130.10, 131.30, 131.66, 132.78, 132.83, 135.02, 136.54, 147.41, 151.62, 167.63; MS (*m*/*z*); 394.4 (M+1, 100 %), 396.2 (34 %), 398.2 *m*/*z* (7 %).

*1-(5-(3-Amino-4-hydroxy-5-methoxyphenyl)-3-(4-fluorophenyl)-4,5-dihydropyrazol-1-yl)ethanone (**4c**)* Yield, 47.8 %; mp 143–145 °C; IR (KBr): 3498–3228 (O–H), 3334 (N–H), 3042 (Ar–H), 2947 (aliphatic C–H), 1682 (C=O), 1602 (C=N), 1545 (C=C), 1227 (C–N); ^{1}H NMR (DMSO-d_6): 7.976 (broad s, 1H), 7.799–7.764 (sextet, 2H), 7.185 (t, 2H), 6.168 (s, 2H), 5.393 (dd, 1H, $J_{HH''} = 4.4$ Hz, $J_{HH'} = 11.7$ Hz), 4.583 (broad s, 1H), 3.780 (s, 3H), 3.765 (q, 1H, $J_{H'H''} = 17.84$ Hz, $J_{HH'} = 11.78$ Hz), 3.128 (dd, 1H, $J_{H'H''} = 17.8$ Hz, $J_{HH''} = 4.48$ Hz), 2.345 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.65, 42.29, 55.47, 59.61, 98.37, 104.58, 115.32, 115.53, 127.63, 128.38, 131.62, 133.08, 136.52, 147.33, 152.66, 161.90, 164.38, 167.33; MS (*m*/*z*); 344.2 (M+1).

*1-(5-(3-Amino-4-hydroxy-5-methoxyphenyl)-3-(2-bromophenyl)-4,5-dihydropyrazol-1-yl)ethanone (**4d**)* Yield, 43.5 %; mp 147–149 °C; IR (KBr): 3519–3218 (O–H), 3364 (N–H), 3071 (Ar–H), 2943 (aliphatic C–H), 1677 (C=O), 1599 (C=N), 1519 (C=C), 1123 (C–N); ^{1}H NMR (DMSO-d_6): δ 10.316 (s, 1H), 7.787 (q, 2H, $J = 4.32$ Hz, $J = 6.00$ Hz), 7.463 (t, 2H), 7.332 (d, 1H, $J = 1.96$ Hz), 7.100 (d, 1H, $J = 1.96$ Hz), 5.580 (dd, 1H, $J_{HH''} = 5.2$ Hz, $J_{HH'} = 12.4$ Hz), 4.083 (q, 1H, $J_{H'H''} = 18.39$ Hz, $J_{HH'} = 12.51$ Hz), 3.894 (s, 3H), 3.234 (dd, 1H, $J_{H'H''} = 18.47$ Hz, $J_{HH''} = 5.13$ Hz), 2.374 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.56, 56.28, 58.78, 59.66, 99.86, 104.38, 112.24, 114.32, 126.29, 128.34, 130.05, 130.60, 134.63, 144.88, 153.69,

160.31, 168.10; MS (*m/z*); 404.9 (M+1, 98 %), 407.1 (100 %).

*1-(5-(3-Amino-4-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone (**4e**)* Yield, 58.9 %; mp 86–88 °C; IR (KBr): 3522–3186 (O–H), 3311 (N–H), 3062 (Ar–H), 2912 (aliphatic C–H), 1676 (C=O), 1598 (C=N), 1510 (C=C), 1180 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.681–7.623 (m, 2H), 7.380–7.350 (m, 3H), 6.676 (d, 1H, $J_{ab} = 7.92$ Hz), 6.591 (dd, 1H, dd, 1H, $J_{ab} = 7.91$ Hz, $J_{bc} = 2.01$ Hz), 6.487 (d, 1H, d, 1H, $J_{bc} = 1.99$ Hz), 5.388 (q, 1H, $J_{HH''} = 3.92$ Hz, $J_{HH'} = 11.35$ Hz), 3.878–3.633 (m, 2H), 2.327 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 24.62, 43.11, 59.63, 72.28, 107.94, 113.76, 123.47, 126.07, 129.25, 132.81, 134.04, 136.37, 138.14, 146.16, 154.12, 168.10; MS (*m/z*); 296.3 (M+1).

*1-(5-(3-Amino-4-hydroxyphenyl)-3-(2-bromophenyl)-4,5-dihydropyrazol-1-yl)ethanone (**4f**)* Yield, 55.6 %; mp 95–97 °C; IR (KBr): 3512–3225 (O–H), 3305 (N–H), 3044 (Ar–H), 2932 (aliphatic C–H), 1658 (C=O), 1610 (C=N), 1542 (C=C), 1215 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.775–7.751 (m, 2H), 7.454–7.432 (m, 2H), 6.721 (d, 1H, $J_{ab} = 8.13$ Hz), 6.661 (dd, 1H, dd, 1H, $J_{ab} = 8.19$ Hz, $J_{bc} = 1.93$ Hz), 6.538 (d, 1H, d, 1H, $J_{bc} = 2.04$ Hz), 5.589 (dd, 1H, $J_{HH''} = 5.7$ Hz, $J_{HH'} = 12.66$ Hz), 4.185 (q, 1H, $J_{H'H''} = 18.78$ Hz, $J_{HH'} = 12.66$ Hz), 3.486 (dd, 1H, $J_{H'H''} = 18.71$ Hz, $J_{HH''} = 5.72$ Hz), 2.372 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.73, 59.24, 59.94, 99.62, 105.84, 113.30, 115.71, 126.35, 128.34, 130.65, 132.54, 135.67, 144.93, 155.42, 160.38, 168.23; MS (*m/z*); 374.1 (M+1, 100 %), 376.1 (95 %).

*1-(5-(3-Amino-4-hydroxy-5-chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone (**4g**)* Yield, 72.7 %; mp 103–105 °C; IR (KBr): 3478–3247 (O–H), 3289 (N–H), 3080 (Ar–H), 2910 (aliphatic C–H), 1611 (C=O), 1591 (C=N), 1499 (C=C), 1182 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.692–7.635 (m, 2H), 7.396–7.315 (m, 3H), 6.719 (s, 1H), 6.541 (s, 1H), 5.595 (dd, 1H, $J_{HH''} = 5.2$ Hz, $J_{HH'} = 12.4$ Hz), 3.905 (q, 1H, $J_{H'H''} = 18.39$ Hz, $J_{HH'} = 12.51$ Hz), 3.201 (dd, 1H, $J_{H'H''} = 18.47$ Hz, $J_{HH''} = 5.13$ Hz), 2.388 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.59, 40.17, 42.16, 60.41, 80.14, 78.62, 104.94, 126.81, 129.55, 130.47, 131.73, 132.26, 133.12, 137.09, 147.34, 154.16, 167.83; MS (*m/z*); 330.2 (M+1).

*1-(5-(3-Amino-4-hydroxy-5-chlorophenyl)-3-(2-bromophenyl)-4,5-dihydropyrazol-1-yl)ethanone (**4h**)* Yield, 57.9 %; mp 112–114 °C; IR (KBr): 3556–3311 (O–H), 3317 (N–H), 3077 (Ar–H), 2931 (aliphatic C–H), 1653 (C=O), 1596 (C=N), 1507 (C=C), 1201 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.831–7.795 (m, 2H), 7.414–7.398 (m, 2H), 6.732 (s, 1H), 6.663 (s, 1H), 5.559 (dd, 1H, $J_{HH''} = 4.82$ Hz, $J_{HH'} = 11.62$ Hz), 3.843 (q, 1H, $J_{H'H''} = 17.93$ Hz, $J_{HH'} = 11.69$ Hz), 3.463 (dd, 1H, $J_{H'H''} = 17.91$ Hz, $J_{HH''} = 4.79$ Hz), 2.391 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.86, 58.72, 60.13, 105.23, 113.15, 115.12, 126.97, 128.69, 129.22, 130.41, 131.81, 132.40, 133.09, 134.63, 144.54, 154.47, 168.18; MS (*m/z*); 408.2 (M+1, 100 %), 410.0 (99 %).

*General procedure for synthesis of 1-(5-(2-amino-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**5a**–**5h**)*

To a suspension of 1-(5-(3-amino-4-hydroxy-5-substituted-phenyl)-3-aryl-4,5-dihydropyrazol-1-yl)ethanone (**4a**–**4h**, 1.54 mmol) in 1:1 mixture of methanol and water (20 mL) was added a solution of CNBr (0.18 g, 1.54 mmol) in methanol (5 mL) drop wise. The resulting solution was then stirred for the next 30 min. The resulting reaction mixture was then neutralized to pH ~8 using sodium bicarbonate and the reaction mixture was stirred for 30 min. The reaction mixture was then poured into ice-water and the resulting off-white residues were filtered off, dried, and recrystallized from ethanol to yield 1-(5-(2-amino-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**5a**–**5h**).

*1-(5-(2-Amino-7-methoxybenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**5a**)* Yield, 65.0 %; mp 115–117 °C; IR (KBr): 3474 (N–H), 3079 (Ar–H), 2935 (aliphatic C–H), 1712 (C–O), 1632 (C=O), 1615 (C=N), 1468 (C=C), 1203 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.477–7.460 (m, 2H), 7.152–7.136 (m, 3H), 6.785 (s, 2H), 6.353 (s, 1H), 6.203 (s, 1H), 5.283 (dd, 1H, $J_{HH''} = 11.74$ Hz, $J_{HH'} = 4.4$ Hz), 3.623 (s, 3H), 3.571 (q, 1H, $J_{H'H''} = 17.8$ Hz, $J_{HH'} = 11.6$ Hz), 2.893 (dd, 1H, $J_{H'H''} = 17.78$ Hz, $J_{HH''} = 4.52$ Hz), 2.090 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.62, 42.41, 55.79, 59.72, 102.07, 104.77, 126.27, 128.36, 129.88, 130.99, 135.15, 138.37, 142.66, 145.41, 153.63, 162.90, 167.55; MS (*m/z*): 351.2 (M+1).

*1-(5-(2-Amino-7-methoxybenzoxazol-5-yl)-3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**5b**)* Yield, 75.3 %; mp 139–141 °C; IR (KBr): 3459 (N–H), 3062 (Ar–H), 2947 (aliphatic C–H), 1696 (C–O), 1627 (C=O), 1603 (C=N), 1484 (C=C), 1145 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.897 (broad s, 2H), 7.717 (d, 1H, $J = 8.44$ Hz), 7.459 (s, 1H), 7.345 (d, 1H, $J = 8.48$ Hz), 6.603 (s, 1H), 6.435 (s, 1H), 5.506 (dd, 1H, $J_{HH''} = 4.04$ Hz, $J_{HH'} = 11.88$ Hz), 3.939 (q, 1H, $J_{H'H''} = 18.83$ Hz, $J_{HH'} = 11.88$ Hz), 3.230 (dd, 1H, $J_{H'H''} = 18.24$ Hz, $J_{HH''} = 4.32$ Hz), 2.289 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.62, 44.98, 55.75, 59.98, 101.93, 104.89, 127.13, 128.84, 130.09,

131.20, 132.89, 135.17, 135.24, 137.95, 142.71, 145.38, 151.66, 162.91, 167.85; MS (*m/z*): 419.3 (M+1, 100 %), 421.1 (30 %), 423.0 (12 %).

1-(5-(2-Amino-7-methoxybenzoxazol-5-yl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5c)*** Yield, 65.3 %; mp 132–134 °C; IR (KBr): 3512 (N–H), 3103 (Ar–H), 2955 (aliphatic C–H), 1687 (C–O), 1662 (C=O), 1547 (C=N), 1461 (C=C), 1213 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.841 (sextet, 2H), 7.328 (s, 2H), 7.276 (t, 2H), 6.556 (d, 1H, $J = 0.92$ Hz), 6.509 (d, 1H, $J = 1.08$ Hz), 5.547 (dd, 1H, $J_{HH'} = 11.72$ Hz, $J_{HH''} = 4.52$ Hz), 3.868 (s, 3H), 3.850 (q, 1H, $J_{H'H''} = 17.88$ Hz, $J_{HH'} = 11.72$ Hz), 3.175 (dd, 1H, $J_{H'H''} = 18.02$ Hz, $J_{HH''} = 4.64$ Hz), 2.316 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.56, 42.43, 55.76, 59.82, 87.04, 99.48, 102.06, 104.83, 115.26, 115.48, 128.31, 130.04, 131.08, 138.17, 152.74, 164.27, 168.16; MS (*m/z*): 369.4 (M+1).

1-(5-(2-Amino-7-methoxybenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5d)*** Yield, 75.6 %; mp 141–143 °C; IR (KBr): 3571 (N–H), 3068 (Ar–H), 2956 (aliphatic C–H), 1707 (C–O), 1671 (C=O), 1626 (C=N), 1495 (C=C), 1178 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.904-7.819 (m, 2H), 7.731–7.622 (m, 2H), 7.473 (s, 2H), 7.331 (d, 2H, $J = 1.11$ Hz), 7.173 (d, 2H, $J = 1.09$ Hz), 5.848 (dd, 1H, $J_{HH''} = 5.2$ Hz, $J_{HH'} = 12.4$ Hz), 4.208 (q, 1H, $J_{H'H''} = 18.39$ Hz, $J_{HH'} = 12.51$ Hz), 3.931 (s, 3H), 3.339 (dd, 1H, $J_{H'H''} = 18.47$ Hz, $J_{HH''} = 5.13$ Hz), 2.418 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.54, 58.12, 70.23, 98.31, 102.20, 105.63, 118.07, 121.26, 126.71, 128.92, 131.35, 131.94, 140.15, 153.22, 164.31, 168.42; MS (*m/z*); 429.1 (M+1, 100 %), 431.1 (95 %).

1-(5-(2-Aminobenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5e)*** Yield, 66.4 %; mp 93–95 °C; IR (KBr): 3532 (N–H), 3097 (Ar–H), 2953 (aliphatic C–H), 1721 (C–O), 1626 (C=O), 1550 (C=N), 1534 (C=C), 1088 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.709–7.686 (m, 2H), 7.594–7.488 (m, 2H), 7.527 (d, 1H, $J_{bc} = 2.17$ Hz), 7.424 (s, 2H), 7.315–7.227 (m, 3H), 7.279 (dd, 1H, $J_{ab} = 8.13$ Hz, $J_{bc} = 2.21$ Hz), 5.365 (dd, 1H, $J_{HH''} = 4.12$ Hz, $J_{HH'} = 11.27$ Hz), 3.715–3.675 (m, 2H), 2.372 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.45, 69.32, 100.92, 102.64, 104.59, 126.30, 129.13, 131.81, 133.87, 144.39, 153.71, 165.02, 168.64; MS (*m/z*); 321.1 (M+1).

1-(5-(2-Aminobenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5f)*** Yield, 58.3 %; mp 106–108 °C; IR (KBr): 3496 (N–H), 3087 (Ar–H), 2941 (aliphatic C–H), 1763 (C–O), 1625 (C=O), 1549 (C=N), 1515 (C=C), 1191 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.844–7.790 (m, 3H), 7.753–7.531 (m, 4H), 7.475 (d, 1H, $J = 1.64$ Hz), 7.351 (d, 1H, $J = 1.73$ Hz), 5.559 (dd, 1H, $J_{HH''} = 3.70$ Hz, $J_{HH'} = 11.45$ Hz), 3.709–3.547 (m, 2H), 2.361 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.41, 69.37, 98.14, 103.32, 105.42, 109.51, 117.86, 120.45, 126.35, 129.03, 131.12, 132.09, 140.33, 154.03, 164.24, 167.91; MS (*m/z*); 399.2 (M+1), 401.1 (90 %).

1-(5-(2-Amino-7-chlorobenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5g)*** Yield, 55.0 %; mp 97–99 °C; IR (KBr): 3528 (N–H), 3067 (Ar–H), 2976 (aliphatic C–H), 1720 (C–O), 1631 (C=O), 1563 (C=N), 1498 (C=C), 1239 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.667–7.632 (m, 2H), 7.587 (s, 2H), 7.296–7.252 (m, 3H), 6.569 (s, 1H), 6.429 (s, 1H), 5.372 (dd, 1H, dd, 1H, $J_{HH''} = 4.36$ Hz, $J_{HH'} = 11.40$ Hz), 3.962 (q, 1H, $J_{H'H''} = 17.48$ Hz, $J_{HH'} = 11.37$ Hz), 3.140 (dd, 1H, $J_{H'H''} = 17.52$ Hz, $J_{HH''} = 4.29$ Hz), 2.428 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.66, 70.35, 102.28, 102.97, 105.16, 129.42, 132.70, 137.63, 143.69, 153.88, 165.54, 168.31; MS (*m/z*); 354.9 (M+1, 100 %), 356.8 (30 %).

1-(5-(2-Amino-7-chlorobenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(5h)*** Yield, 69.0 %; mp 121–123 °C; IR (KBr): 3501 (N–H), 3106 (Ar–H), 2934 (aliphatic C–H), 1736 (C–O), 1627 (C=O), 1572 (C=N), 1453 (C=C), 1193 (C–N); ^{1}H NMR (DMSO-d_6): δ 7.861–7.835 (m, 2H), 7.562 (s, 2H), 7.426–7.386 (m, 2H), 6.761 (s, 1H), 6.594 (s, 1H), 5.396 (dd, 1H, dd, 1H, $J_{HH''} = 4.92$ Hz, $J_{HH'} = 11.86$ Hz), 3.868 (q, 1H, $J_{H'H''} = 17.97$ Hz, $J_{HH'} = 11.86$ Hz), 3.833 (dd, 1H, $J_{H'H''} = 17.96$ Hz, $J_{HH''} = 4.90$ Hz), 2.335 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.73, 60.11, 61.47, 106.11, 113.75, 116.01, 127.30, 128.71, 129.54, 131.18, 132.24, 133.49, 134.10, 136.15, 145.42, 154.49, 165.27, 168.63; MS (*m/z*); 432.3(M+1, 100 %), 434.1 (98 %).

*General procedure for synthesis of 1-(5-(2-mercapto-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**6a–6h**)*

To a solution of potassium hydroxide (1.54 mmol) in methanol (15 mL) was added carbon disulfide (0.1 mL, 1.54 mmol) drop wise. The reaction mixture turned to light yellow upon addition of carbon disulphide. To the resulting solution, 1-(5-(3-amino-4-hydroxy-5-substituted-phenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)ethanone (**4a**–**4h**, 1.54 mmol) was added and the reaction mixture was refluxed for the 3 h. The reaction mixture was acidified with 2 N HCl solution. The resulting off-white residues were filtered off, dried, and recrystallized from ethanol to afford 1-(5-(2-mercapto-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**6a**–**6h**).

1-(5-(2-Mercapto-7-methoxybenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6a)*** Yield, 73.0 %; mp 207–209 °C; IR (KBr): 3366 (S–H), 3010 (Ar–H), 2928 (aliphatic C–H), 1719 (C–O), 1659 (C=O), 1577 (C=N), 1445 (C=C), 1199 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.554 (s, 1H), 7.730–7.706 (m, 2H), 7.414–7.390 (m, 3H), 6.684 (d, 1H, $J = 1.28$ Hz), 6.546 (d, 1H, $J = 1.24$ Hz), 5.561 (dd, 1H, $J_{HH''} = 4.92$ Hz, $J_{HH'} = 11.86$ Hz), 3.857 (q, 1H, $J_{HH'} = 17.96$ Hz, $J_{H''H'} = 11.92$ Hz), 3.148 (dd, 1H, $J_{HH''} = 5.04$ Hz, $J_{H''H'} = 17.98$ Hz), 2.325 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.60, 42.18, 56.23, 59.45, 99.38, 105.09, 126.34, 128.41, 130.05, 130.75, 132.88, 135.92, 140.54, 142.82, 153.70, 167.74, 180.16; MS (*m*/*z*): 368.4 (M+1).

1-(5-(2-Mercapto-7-methoxybenzoxazol-5-yl)-3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6b)*** Yield, 75.9 %; mp 212–214 °C; IR (KBr): 3347 (S–H), 3076 (Ar–H), 2939 (aliphatic C–H), 1739 (C–O), 1651 (C=O), 1542 (C=N), 1435 (C=C), 1205 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.615 (s, 1H), 7.769 (d, 1H, $J_{ab} = 8.48$ Hz), 7.537 (d, 1H, $J_{bc} = 2.08$), 7.419 (dd, 1H, $J_{ab} = 8.46$ Hz, $J_{bc} = 2.12$ Hz), 6.643 (d, 1H, $J = 1.23$ Hz), 6.708 (d, 1H, $J = 1.21$ Hz), 5.600 (dd, 1H, $J_{HH''} = 4.84$ Hz, $J_{HH'} = 11.92$ Hz), 4.01–3.916 (m, 4H), 3.309 (dd, 1H, $J_{H'H''} = 18.24$ Hz, $J_{HH''} = 4.96$ Hz), 2.352 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.60, 44.74, 56.19, 59.67, 99.61, 104.86, 127.19, 128.63, 130.12, 131.27, 132.87, 132.91, 135.31, 136.01, 140.11, 142.89, 151.72, 168.01, 180.19; MS (*m*/*z*): 436.7 (M+1, 100 %), 438.0 (37 %), 440.1 (10 %).

1-(5-(2-Mercapto-7-methoxybenzoxazol-5-yl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6c)*** Yield, 80.2 %; mp 200–202 °C; IR (KBr): 3359 (S–H), 3025 (Ar–H), 2948 (aliphatic C–H), 1748 (C–O), 1672 (C=O), 1580 (C=N), 1448 (C=C), 1254 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.841 (s, 1H), 7.638 (dd, 1H, $J = 8.48$ Hz, $J = 1.48$ Hz), 7.572 (s, 1H), 7.448–7.418 (m, 2H), 7.274-7.251 (m, 2H), 7.076–7.053 (m, 1H), 5.848 (dd, 1H, $J_{HH''} = 4.96$ Hz, $J_{HH'} = 11.92$ Hz), 3.991 (q, 1H, $J_{H'H''} = 17.88$ Hz, $J_{HH'} = 11.96$ Hz), 3.109 (dd, 1H, $J_{H'H''} = 17.88$ Hz, $J_{HH''} = 4.96$ Hz), 2.406 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 21.62, 42.26, 56.25, 59.55, 99.45, 105.09, 115.43, 115.65, 127.30, 128.60, 132.84, 135.87, 140.58, 142.83, 152.85, 167.62, 180.14; MS (*m*/*z*): 385.9 (M+1).

1-(5-(2-Mercapto-7-methoxybenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6d)*** Yield, 76.5 %; mp 218–220 °C; IR (KBr): 3334 (S–H), 3028 (Ar–H), 2932 (aliphatic C–H), 1755 (C–O), 1684 (C=O), 1581 (C=N), 1442 (C=C), 1247 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.795 (s, 1H), 7.447–7.405 (m, 1H), 7.274–7.224 (m, 3H), 7.117 (d, 1H, $J = 0.92$ Hz), 7.071–7.048 (m, 1H), 5.863 (dd, 1H, $J_{HH''} = 4.96$ Hz, $J_{HH'} = 11.96$ Hz), 4.019 (s, 3H), 3.964 (q, 1H, $J_{H'H''} = 17.88$ Hz, $J_{HH'} = 12.0$ Hz), 3.120 (dd, 1H, $J_{H'H''} = 17.88$ Hz, $J_{HH''} = 5.0$ Hz), 2.423 (s, 3H); ^{13}C NMR (DMSO-d$_6$): δ 21.73, 58.43, 71.20, 98.65, 102.56, 107.05, 118.58, 121.32, 129.25, 131.32, 133.74, 140.17, 141.79, 153.49, 165.56, 180.42; MS (*m*/*z*); 446.0 (M+1, 100 %), 448.2 (95 %).

1-(5-(2-Mercaptobenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6e)*** Yield, 73.6 %; mp 123–125 °C; IR (KBr): 3392 (S–H), 3022 (Ar–H), 2923 (aliphatic C–H), 1732 (C–O), 1677 (C=O), 1582 (C=N), 1440 (C=C), 1276 (C–N); ^{1}H NMR (DMSO-d$_6$): δ 13.807 (s, 1H), 7.748–7.532 (m, 4H), 7.446 (d, 1H, $J_{bc} = 2.17$ Hz), 7.364–7.251 (m, 3H), 5.606 (dd, 1H, $J_{HH''} = 5.04$ Hz, $J_{HH'} = 12.49$ Hz), 3.824 (q, 2H, $J_{H'H''} = 18.21$ Hz, $J_{HH'} = 12.42$ Hz), 3.190 (dd, 1H, $J_{H'H''} = 18.25$ Hz, $J_{HH''} = 5.06$ Hz), 2.354 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.31, 69.76, 100.45, 105.71, 108.28, 126.10, 126.13, 129.44, 132.07, 134.21, 149.16, 153.82, 166.72, 180.51; MS (*m*/*z*); 338.1 (M+1).

1-(5-(2-Mercaptobenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6f)*** Yield, 59.1 %; mp 149–151 °C; IR (KBr): 3370 (S–H), 3015 (Ar–H), 2931 (aliphatic C–H), 1723 (C–O), 1662 (C=O), 1502 (C=N), 1446 (C=C), 1262 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.812 (s, 1H), 7.596 (dd, 1H, $J = 1.87$ Hz, $J = 8.40$ Hz), 7.574–7.571 (d, 1H, $J = 8.43$ Hz), 7.190–7.064 (m, 5H), 5.816 (dd, 1H, $J_{HH''} = 4.92$ Hz, $J_{HH'} = 12.16$ Hz), 3.997 (q, 2H, $J_{H'H''} = 18.24$ Hz, $J_{HH'} = 12.21$ Hz), 3.050 (dd, 1H, $J_{H'H''} = 18.27$ Hz, $J_{HH''} = 5.02$ Hz), 2.373 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.47, 69.58, 104.29, 109.63, 117.83, 121.31, 126.50, 129.26, 130.44, 132.12, 134.14, 148.76, 155.19, 165.23, 180.86; MS (*m*/*z*); 415.4 (M+1, 100 %), 417.4 (90 %).

1-(5-(2-Mercapto-7-chlorobenzoxazol-5-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(6g)*** Yield, 74.8 %; mp 122–124 °C; IR (KBr): 3345 (S–H), 3060 (Ar–H), 2930 (aliphatic C–H), 1731 (C–O), 1701 (C=O), 1533 (C=N), 1442 (C=C), 1230 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.798 (s, 1H), 7.570–7.568 (d, 1H), 7.512–7.445 (m, 2H), 7.314–7.312 (d, 1H), 7.292–6.195 (m, 3H), 5.873–5.831 (dd, 1H, dd, 1H, $J_{HH''} = 4.86$ Hz, $J_{HH'} = 12.55$ Hz), 3.974–3.892 (q, 1H, $J_{H'H''} = 18.10$ Hz, $J_{HH'} = 12.53$ Hz), 3.216–3.159 (dd, 1H, $J_{H'H''} = 18.13$ Hz, $J_{HH''} = 4.91$ Hz), 2.426 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.54, 69.75, 118.04, 121.67, 123.05, 129.35, 130.44, 132.79, 134.53, 137.85, 143.71, 149.21, 153.79, 165.68, 168.46; MS (*m*/*z*); 371.8 (M+1).

*1-(5-(2-Mercapto-7-chlorobenzoxazol-5-yl)-3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**6h**)* Yield, 77.0 %; mp 136–138 °C; IR (KBr): 3382 (S–H), 3018 (Ar–H), 2934 (aliphatic C–H), 1751 (C–O), 1658 (C=O), 1576 (C=N), 1439 (C=C), 1235 (C–N); ^{1}H NMR (DMSO-d_6): δ 13.774 (s, 1H), 7.398 (d, 1H, $J = 8.84$ Hz), 7.353 (s, 1H), 7.263 (t, 2H), 7.183 (d, 1H, $J = 7.8$ Hz), 7.115 (d, 1H, $J = 1.24$ Hz), 5.579 (dd, 1H, $J_{HH''} = 4.8$ Hz, $J_{HH'} = 11.92$ Hz), 4.033 (s, 3H), 3.888 (q, 1H, $J_{H'H''} = 17.92$ Hz, $J_{HH'} = 12.0$ Hz), 3.218 (dd, 1H, $J_{H'H''} = 17.92$ Hz, $J_{HH''} = 4.88$ Hz), 2.394 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 23.73, 67.47, 110.11, 119.89, 121.23, 125.00, 127.37, 129.73, 131.05, 132.92, 135.56, 140.18, 144.82, 155.71, 165.32, 180.68; MS (*m*/*z*); 450.2 (M+1, 100 %), 452.1 (99 %).

Antitubercular screening

The in vitro antitubercular activity was carried out by measuring the growth of *M. tuberculosis* ($H_{37}R_V$) using Lowensteine–Jensen medium (L. J. medium) (Stover *et al.*, 2000). Briefly, eggs were broken aseptically to obtain 200 mL of egg solution. The solution was filtered through a sterile muslin cloth into a sterile conical flask containing glass beads. Sterilized mineral salt solution (120 mL) (consisting of 4.0 g potassium phosphate, 0.4 g of magnesium sulfate, 1.6 g magnesium citrate, 6.0 g of asparagine, 20 mL of glycerol, distilled water makeup up to 1,000 mL) and 4 mL of sterilized malachite green solution (2.0 %) were added to the 200 mL of egg solution. The contents were mixed well to form a uniform medium. Compounds (10 mg) were dissolved in 10.0 mL of dimethyl sulfoxide (DMSO) and were diluted with DMSO to make 250 and 10 mg/mL stock solutions. An aliquot (0.8 mL) of each concentration was transferred into different McCartney bottles. To this, 7.2 mL of L. J. medium was added and mixed well. Isoniazid was considered as a reference standard for the comparison of antitubercular activity. The drug was dissolved in DMSO and diluted as described above. The bottles were incubated at 75–80 °C for 3 days for solidification and sterilization.

Procedure for inoculation

A sweep from multidrug-resistant H_{37}Rv strain of *M. tuberculosis* culture was transferred with the help of 22 S.W. nichrome wire loop of 3-mm external diameter into a sterile bijou bottle containing six 3-mm glass beads and 4 mL of sterile distilled water. Each loop of culture delivered approximately 4 mg of bacilli cells. The bottle was shaken with the help of vertex mixture for 2 min. The suspension was inoculated on the surface of each L. J. medium containing test compounds using 27 S.W.G nichrome wire loop of 3 mm external diameter and L. J. medium containing isoniazid. The medium containing DMSO (control) was inoculated with the test organism for positive and negative controls. Medium without any test compound/DMSO was also inoculated with the test organism to check whether the media supports the growth of the tubercle bacilli or not. The inoculated bottles were incubated at 37 °C for 6 weeks, at the end of which readings were taken. Bacterial counts were measured and compared with the standard drugs and controls (vehicle-treated).

Docking studies

The complex was subjected to preparation steps using the Protein Preparation Wizard in Maestro using the default settings. First, the waters beyond 5 Å from the ligand were removed, bond orders assigned and hydrogens added. Next, the orientation of amide (Asn and Gln), hydroxyl (Ser, Thr, and Tyr), and thiol groups (Cys), and the protonation and tautomeric state of the His residues were optimized using the exhaustive sampling option. A grid box of 20 × 20 × 20 Å^3 for the receptor was generated with a default inner box of 10 × 10 × 10 Å^3, which was centered on the corresponding ligand. The default parameters were used, and no constraints were included. Docking calculations were performed using the Glide Extra Precision (XP) algorithm (Friesner *et al.*, 2006). In the protocol, Glide was set to determine the five best poses per ligand.

Interaction energies of the ligand fragments with enoyl-ACP reductase

The inhibitor structures were fragmented into three segments: pyrazoline ring, benzoxazole ring, and aryl ring. The separated side chains and the main chains were capped by hydrogens. PM6-D3H4X method developed in our laboratory (Brahmkshatriya *et al.*, 2013) in implicit solvent model was used to calculate interaction energies.

Results and discussion

Chemistry

The title compounds (**4a**–**4h**, **5a**–**5h**, and **6a**–**6h**) were synthesized in good yields by multistep chemical synthesis as outlined in the Fig. 2.

Aldol condensation of 4-hydroxy-3-nitro-5-substituted-benzaldehyde (**1a**–**1c**) with substituted acetophenones in presence of sodium hydroxide afforded corresponding chalcone derivatives (**2a**–**2h**). Cyclization of these chalcone derivatives (**2a**–**2h**) was carried out by reaction with hydrazine hydrate in glacial acetic acid to yield corresponding

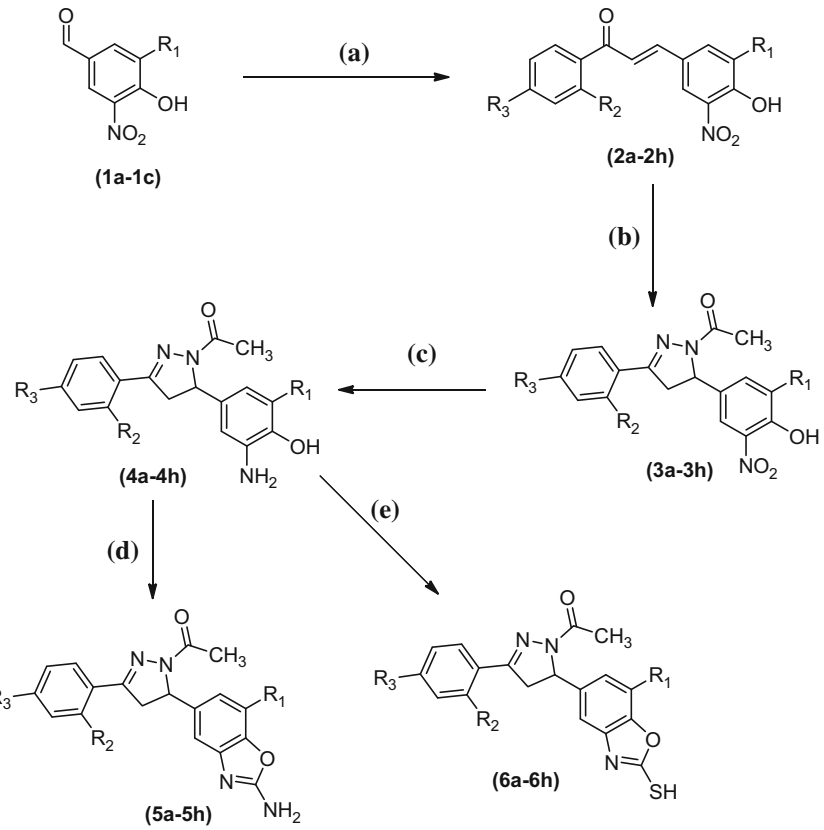


Fig. 2 Pathway for the synthesis of the title compounds. *a* Substituted acetophenones, 25 % aqueous NaOH solution, methanol, 24 h, rt; *b* hydrazine hydrate, glacial acetic acid, 3 h, reflux; *c* sodium dithionite, methanol, 30 min, reflux; *d* cyanogen bromide, sodium bicarbonate, methanol–water, 1 h, rt; *e* carbon disulphide, methanol, 3 h, reflux

pyrazoline derivatives (**3a**–**3h**) in good yields. Reduction of nitro group by sodium dithionite afforded respective *o*-aminophenol derivatives (**4a**–**4h**). Cyclization of these o-aminophenol derivatives was brought by two different ways: (a) treatment with cyanogen bromide in methanol–water mixture at room temperature to yield corresponding 2-aminobenzoxazole derivatives (**5a**–**5h**); and (b) treatment with carbon disulphide and potassium hydroxide in ethanol at reflux temperature to afford 2-mercaptobenzoxazole derivatives (**6a**–**6h**).

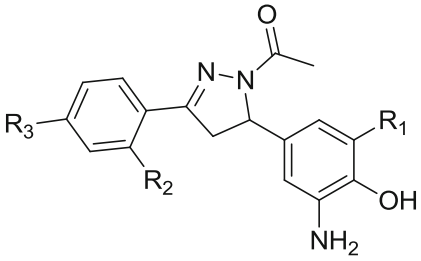
Antitubercular screening

Synthesized compounds (**4a**–**4h**, **5a**–**5h**, and **6a**–**6h**) were screened for their antitubercular activity against *M. tuberculosis* $H_{37}Rv$ and MDR-TB strains. Table 1 shows the results of the biological screening. Isoniazid was also screened as a standard drug for their antitubercular activity against both $H_{37}Rv$ and MDR-TB strains under similar experimental conditions. It was encouraging to see that majority of the compounds displayed satisfactory MIC values against *M. tuberculosis* $H_{37}Rv$ and MDR-TB strains. Compounds **4f** and **5a** were found to be potent as good as isoniazid against *M. tuberculosis* $H_{37}Rv$, while **4d** was found to be potent against MDR-TB stain. Although none of the compounds was found to be potent than isoniazid against *M. tuberculosis* $H_{37}Rv$, compound **4d** was found to be more potent than isoniazid against MDR-TB strain.

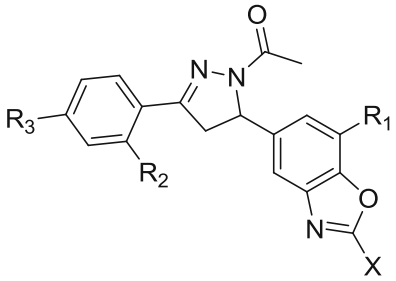
Docking study

In order to gain better insights into the plausible binding motifs of the target compounds, we carried out molecular docking. For this, we chose the crystal structure of *M. tuberculosis* enoyl reductase (InhA) (PDB Code 2H7I) (He *et al.*, 2006). We have shown earlier that Glide (Friesner *et al.*, 2006) performs better over other programs for the present target (Mohan *et al.*, 2012). We showed in that paper that Glide performed better in finding the docked geometry closer to experimental geometry (root mean square deviation = 0.47 Å for the native ligand of 2H7I). The docking studies revealed that most of the target compounds occupied the hydrophobic cavity of enoyl-ACP reductase. A visual analysis of the crystal structure of enoyl-ACP reductase (PDB Code 2H7I) suggests that chief interactions are of dispersion type. The ligand is less exposed to solvent and buried deep inside a hydrophobic pocket made up of Tyr158, Ile215, Met103, and Met199 residues. Most of the target compounds showed consistent orientations of the pyrazoline, aryl, and benzoxazole moieties within the active site (Fig. S1, Supplementary Material). Figure S1a shows detailed interactions of the most

Table 1 Structures of the synthesized target compounds and their in vitro antitubercular activity



(4a-4h)



(5a-5h, 6a-6h)

Compound no.	R_1	R_2	R_3	X	MIC (μg/mL)	
					$H_{37}Rv$	MDR-TB
4a	$-OCH_3$	H	H	–	25	25
4b	$-OCH_3$	Cl	Cl	–	12.5	12.5
4c	$-OCH_3$	H	F	–	12.5	25
4d	$-OCH_3$	Br	H	–	12.5	3.25
4e	–H	–H	–H	–	1.25	25
4f	–H	–Br	–H	–	0.625	12.5
4g	–Cl	–H	–H	–	3.25	25
4h	–Cl	–Br	–H	–	12.5	25
5a	$-OCH_3$	H	H	$-NH_2$	0.625	6.25
5b	$-OCH_3$	Cl	Cl	$-NH_2$	12.5	12.5
5c	$-OCH_3$	H	F	$-NH_2$	6.25	25
5d	$-OCH_3$	Br	H	$-NH_2$	12.5	25
5e	–H	–H	–H	$-NH_2$	25	25
5f	–H	–Br	–H	$-NH_2$	25	12.5
5g	–Cl	–H	–H	$-NH_2$	25	25
5h	–Cl	–Br	–H	$-NH_2$	3.25	25
6a	$-OCH_3$	H	H	–SH	6.25	12.5
6b	$-OCH_3$	Cl	Cl	–SH	6.25	12.5
6c	$-OCH_3$	H	F	–SH	12.5	6.25
6d	$-OCH_3$	Br	H	–SH	12.5	25
6e	–H	–H	–H	–SH	25	25
6f	–H	–Br	–H	–SH	12.5	6.25
6g	–Cl	–H	–H	–SH	1.25	25
6h	–Cl	–Br	–H	–SH	6.25	12.5
Isoniazid	–	–	–	–	0.625	6.25

potent compound **5a** with the neighboring residues. It was encouraging to see that most of the ligands retained all the dispersion interactions found in the crystal structure (PDB Code 2H7I) with some additional interactions with residues Ile202, Met155, and Leu218. However, it should also be noted that for some compounds, we could not obtain satisfactory docking poses. Also, it was noteworthy that Glide_Score did not correlate with the biological activity ($r^2 = 0.04$) which is understandable because of the simplicity of the molecular mechanics-based scoring function.

Interaction energies of the ligand fragments with enoyl-ACP reductase

In order to investigate the pharmacophoric contribution of the target compounds, we investigated contribution of the

Table 2 Glide_Score of the target compounds and their interaction energies using PM6-D3H4X method (in kcal/mol)

Comp. no.	pMIC	Glide score	Total interaction energy	Substituted phenyl	Acetyl pyrazoline	(Mercapto/amino) benzoxazole
4a	7.11	−7.22	−0.20	8.53	1.54	−10.27
4b	7.50	−4.64	−1.67	6.44	−1.54	−6.57
4c	7.44	−6.23	−1.25	4.19	−3.49	−1.95
4e	8.37	−7.1	−3.84	6.51	−3.26	−7.09
4g	8.01	−7.13	−3.90	7.96	−1.25	−10.61
4h	7.51	−5.66	−0.41	6.94	3.56	−10.90
5a	8.75	−5.78	−7.87	3.30	−4.60	−14.67
5e	7.11	−6.87	−3.27	5.22	−0.25	−8.23
5f	7.20	−3.2	−1.22	5.29	3.25	−9.76
Isoniazid	8.44	−3.59	–	–	–	–
1-Cyclohexyl-5-oxo-*N*-phenypyrrolidine-3-carboxamide (2H7I-ligand)	–	−8.01	–	–	–	–

three fragments of the target compounds (pyrazoline core, benzoxazole moiety, and aryl ring) towards protein binding. We have shown earlier that the semi-empirical quantum mechanical scoring method with corrections for hydrogen bonding, dispersion and halogen bond, called PM6-D3H4X, developed in our laboratory, accurately describes all protein–ligand noncovalent interactions (Brahmkshatriya *et al.*, 2013). Thus, in consistent with our earlier study (Brahmkshatriya *et al.*, 2013), we carried out fragmentation of the ligands and measured the interaction of energy of these ligand subsystems with the neighboring residues using PM6-D3H4X method. Results clearly showed that benzoxazole moiety is found to be a critical pharmacophore as it interacted favorably with Met103, Phe149, Ala157, Tyr158, Ile202, and Ile215 (blue region, Fig. S1b). Pyrazoline ring interacted with Ala198 and Met199 by dispersion interactions (yellow region, Fig. S1b). However, the interaction energies were much lower than those for benzoxazole moiety. It was surprising to find that the aryl ring faces towards solvent and does not find any interacting partner (Fig. S1b) and hence, the interaction energies were found to be against the binding. This indicates that the fifth position on the pyrazoline ring need not be substituted with aryl ring and can be replaced with smaller substituents such as alkyl. Glide score values of the ligands and the interaction energies of the ligand fragments are summarized in Table 2 and Fig. S2, Supplementary Material. It was very encouraging to see that the total interaction energy (sum of all contributing interaction energies of the fragments) well correlated with the pMIC values of the compounds ($r^2 = 0.70$, Fig. 3). A very good predictive index (PI) (Pearlman and Charifson, 2001) of 0.83 indicates good rank ordering of the compounds based on the predicted interaction energy. Comparison of

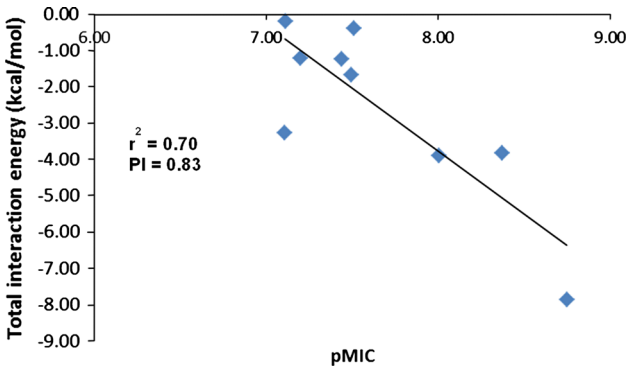


Fig. 3 The correlation of total interaction energy (in kcal/mol) with pMIC

the docking pose of isoniazid was quite surprising. The designed compounds interacted favorably with the active site residues than isoniazid which is reflected by their better score values. We think that this might be due to better fit of the target compounds in the active site cavity as compared to isoniazid (Fig. S1c, d). However, it should also be noted that the poor score of isoniazid might be due to two well-known issues with the docking: *sampling* and *force field-based scoring function*. We understand that the Glide_Score is a crude estimate of the binding interactions as it depends on the underlying force field (OPLS in Glide) and better chemical description (e.g. quantum mechanics-based scoring function) is necessary.

Conclusion

In the present study, a series of 1-(5-(2-amino-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1*H*-pyrazol-

1-yl)ethanones and 1-(5-(2-mercapto-7-substituted-benzoxazol-5-yl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanones were designed by combining the two pharmacophoric motifs (pyrazoline and benzoxazole) for antitubercular activity. Synthesized target compounds and their intermediate aminophenol derivatives were evaluated for their ability to inhibit *M. tuberculosis* H37Rv and MDR-TB strains in vitro. The biological screening provided interesting results where some of the compounds displayed significant antitubercular activity with a few analogs showing better activity than isoniazid against MDR-TB strains. In order to have insights into the nature of binding of the target compounds with the molecular target of tuberculosis (enoyl-ACP reductase), molecular docking was carried out. Docking results suggest that the compounds chiefly interact with the protein by dispersion interactions. The interaction energies of the ligand fragments (the three key cores) provided useful information about the key pharmacophores suggesting importance of benzoxazole core over the other two ring nucleuses. The present study provides a useful protocol to combine two pharmacophoric motifs in one compound to design potent antitubercular agents.

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