Research article

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Future Medicinal Chemistry

Synthesis, characterization and biological screening of pyrazole-conjugated benzothiazole analogs

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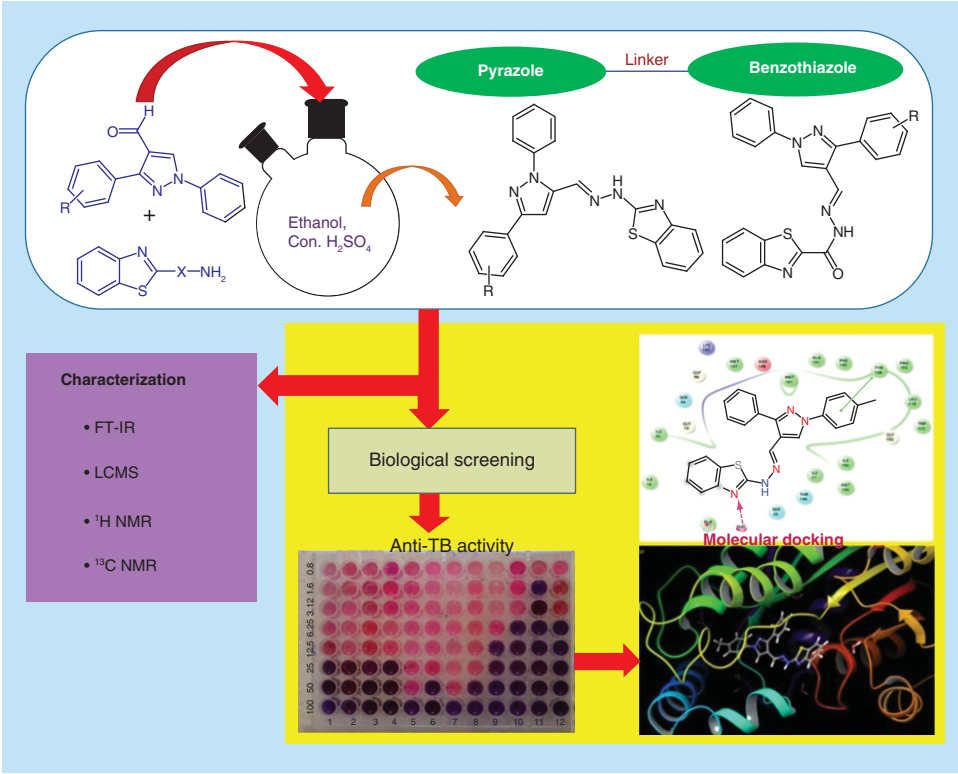
Aim: Benzothiazole and pyrazoles are two important pharmacophores, the activity can be enhanced by conjugating them. Here, two novel series of the pyrazole-conjugated benzothiazole derivatives were synthesized. **Results:** Synthesized compounds were characterized by Fourier-transform infrared, LC-MS, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. Synthesized compounds exhibited moderate antimicrobial, antioxidant and excellent anti-TB activities. In *in vitro* anti-TB activity, **4d** and **4e** exhibited 1.6 μg/ml minimum inhibitory concentration value. In order to rationalize the anti-TB activity, molecular docking studies were carried out and they were correlated with the *in vitro* results. **Conclusion:** Compounds containing electron donating groups show the promising antimicrobial and antioxidant activities, compounds with CH_3 and Cl substitution show excellent anti-TB activity. Synthesized molecules may become potential candidates for the clinical trials.



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Nitrogen containing heterocyclics are abundant in natural products and also in synthetic drug molecules, because of the variety of applications and superior pharmacological-profile actions. Pyrazole is the 5-membered simple aromatic–heterocyclic moiety, characterized by the two adjacent nitrogen atoms in the ring and they belong to the alkaloids groups. Pyrazoles are the integral architects of many of the heterocyclic compounds with superior biological activity. Pyrazofurin is the well-known antibiotic and it contains the pyrazole moiety in their structure. Pyrazole linked with the other heterocyclic analogs create endeavor in higher pharmaceutical activity with lower toxic effects [1,2]. Benzothiazole is one of the prestigious scaffolds and plays a major role in designing the new pharmacological action of drug molecules because of the diversified activity [3]. In addition to this, pyrazole derivatives exhibit a variety of biological activities like anti-inflammatory [4], antitumor, antihypotensive [5], etc. The synthesis of pyrazole moiety linked with the benzothiazole scaffold may help to increase the biological activities. Some of the pyrazole-based drugs are shown in Figure 1.

The hybrid molecular designing is an effective method in modern medicinal chemistry to get highly active molecules. This hybridization may result in the complementary pharmacophoric functions or emerge as new mechanism of action. In continuation to this ideology, we designed pyrazole-conjugated benzothiazole moieties [6]. In this study, conjugation was achieved by imine (azomethine) linkage. These azo-methine linkages are widespread in natural, natural derived or synthesized compounds and are marked for the various biological activities [7]. Based on the rich biological reports on pyrazole derivatives and continuation of our benzothiazole drug development work [8,9], we planned for the synthesis of molecules with pyrazole and benzothiazole through the imine linkage and screened for the antimicrobial, antitubercular, antioxidant activities.

Methods

All the substituted acetophenones, 2-aminothiophenol, diethyl oxalate and $POCl_3$ were purchased from the SD Fine Chem Ltd (India) and solvents were procured from the Rankem Pvt. Ltd (India). Phenyl hydrazine was obtained from the Loba Chemie (India) and all the chemicals were used without further purification. LC-MS, NMR and FT-IR spectrometric techniques were adopted for the confirmation of the structures of products. Different acetophenones were condensed with phenyl hydrazine followed by the Vilsmeier–Haack cyclization reaction. The obtained intermediates were condensed with the 1,3-benzothiazole-2-carbohydrazide and 2-hydrazinyl-1,3-benzothiazole through azomethine linkage as shown in Figure 2. The monitoring of the reaction was carried out by thin layer chromatography (TLC), using an aluminum plate, coated with silica, which was obtained from Merck (Mumbai, India), using appropriate solvent mixtures. The melting points (m.p.'s) were determined by the open capillary method and were uncorrected. The products were confirmed by FT-IR, LC-MS and ^{1}H NMR spectral studies. FT-IR spectrum was recorded on Perkin Elmer spectrophotometer version 10.03.09 instrument over the range of 600 to 4000 cm^{-1}, ^{1}H NMR spectrum was recorded with 400 MHz and ^{13}C NMR with 100 MHz from Agilent 400 RDD2 spectrometer and LC-MS was recorded on mass spectrometer (Waters, USA) by positive mode, using solvents 0.1% formic acid in acetonitrile.

Synthesis of the pyrazole aldehyde via Vilsmeier–Haack cyclization reaction

General procedure for the synthesis of 1-phenyl-2-(1-phenylethylidene) hydrazine (2a–i)

Phenyl hydrazine (0.014 mol) and different acetophenones (**1a–i**, 0.014 mol) were dissolved in ethanol (20 ml) taken in a 50 ml round bottomed flask and 2–3 drops of glacial acetic acid was added at 0°C. The resultant reaction mixture was heated to 70°C for 2 h. After completion of the reaction (confirmed by TLC), the reaction mixture was poured into ice cold water and filtered the solid product, dried to get the intermediate (85–90% yield) [10].

Synthesis of pyrazole derivatives (3a-i)

Dimethylformamide (DMF; 3 ml) was taken in a 100 ml RB flask and cooled to 0°C, to that 1 ml of $POCl_3$ was added at the same temperature, stirred for 15 min to get the Vilsmeier–Haack reagent. To the reaction mixture, cold solution of 1-phenyl-2-(1-phenyl ethylidene) hydrazine derivatives in DMF (5 ml) was added drop wise and heated the reaction mixture for 4 h. After completion of the reaction (confirmed by TLC), the reaction mixture was quenched with the aqueous solution of sodium bicarbonate (up to pH = 7) and product was precipitated out. The precipitated solid was filtered, dried in a hot air oven and purified by recrystallization from ethanol (yield 82–88%) [11].

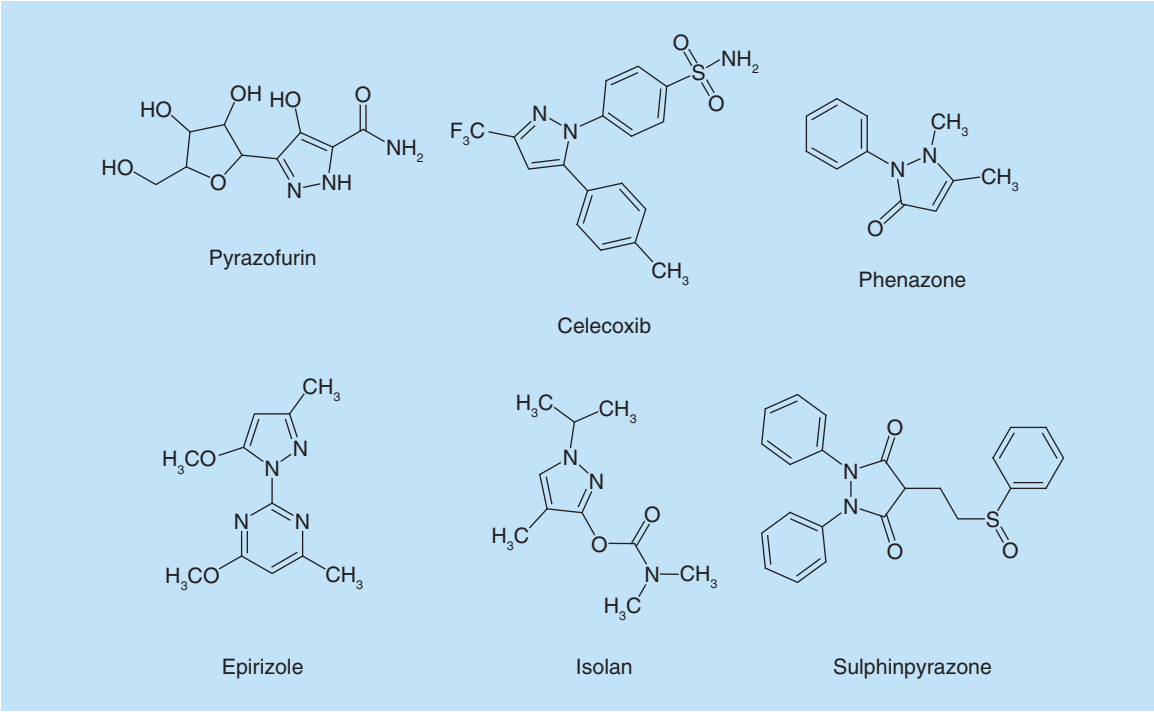

Figure 1. Pyrazole-based drug molecules in medicine.
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General procedure for the synthesis of pyrazole-conjugated benzothiazole derivatives (4a–i)

An equimolar mixture of pyrazole aldehyde derivatives and 1,3-benzothiazole-2-carbohydrazide were dissolved in ethanol and 3–4 drops of the Con. H_2SO_4 added. The reaction mixture was heated to 70°C and stirred for 3 h. To the reaction mixture, crushed ice was added after completion of the reaction and precipitated solid was filtered, dried and the crude material was purified by recrystallization.

Characterization of the synthesized compounds

Synthesis of *(E)*-2-{2-[(1,3-diphenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d] thiazole (**4a**): white solid., m.p. 156–158°C, FT-IR: γ (cm^{-1}): 3205 (NH), 3068 (Ar C–H), 2780 (=C–H), 1619(Ar-C=C), 1599 (C=N), 1360 (Ar C–N), 959 (pyrazole N–N), 1058 (benzothiazole), 694 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.23 (s, 1H, NH), 8.89 (s, 1H, pyrazole), 8.23 (s, 1H), 7.98–7.96 (d, J = 6.0 Hz, 2H), 7.79–7.77 (d, J = 5.6 Hz, 2H), 7.74–7.73 (d, J = 7.2 Hz, 1H), 7.53–7.46 (m, 5H), 7.39–7.36 (d, J = 14.8 Hz, 2H), 7.26 (s, 1H), 7.08 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 166.9, 151.5, 139.5, 138.2, 132.6, 130.1, 129.1, 128.9, 128.3, 127.4, 126.4, 122.0, 119.2, 117.3. LC-MS: (ESI, *m/z*) found 396.10 for $(M + 1)^+$ peak at R_t 3.82 and calculated for $C_{23}H_{17}N_5S$ is 395.12.

Synthesis of *(E)* 2-{2-[(3-(4-methoxy phenyl)-1-phenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d] thiazole (**4b**): brown solid, m.p. 216–218°C, FT-IR: γ (cm^{-1}): 3195 (NH), 3054 (Ar C–H), 2815 (=C–H), 1599 (C=N), 1553 (Ar–C=C), 1458 (Ar C–N), 960 (pyrazole N–N), 1044 (benzothiazole), 685 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.27 (s, 1H, NH), 8.84 (s, 1H, pyrazole), 8.19 (s, 1H), 7.97–7.95 (d, J = 8.0 Hz, 2H), 7.89–7.87 (d, J = 8.4 Hz, 1H), 7.74–7.72 (d, J = 8.4 Hz, 3H), 7.53–7.49 (t, J = 7.6, 16 Hz, 2H), 7.39–7.38 (d, J = 3.2 Hz, 1H), 7.36–7.32 (t, J = 7.2, 14.8 Hz, 1H), 7.28–7.24 (t, J = 7.2, 14.8 Hz, 1H), 7.08–7.06 (d, J = 8.8 Hz, 2H), 7.05–7.02 (d, J = 8.8 Hz, 1H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 165.2, 152.7, 141.4, 139.8, 134.7, 133.2, 131.6, 129.9, 129.3, 128.4, 127.6, 123.6, 120.0, 119.4, 56.8. LC-MS: (ESI, *m/z*) found 426.05 for $(M + 1)^+$ peak at R_t 3.75 and calculated for $C_{24}H_{19}N_5OS$ is 425.13.

Synthesis of *(E)*-4-{5-[(2-(benzo[d] thiazol-2-yl) hydrazono] methyl)-1-phenyl-1H-pyrazol-3-yl} phenol (**4c**): brown solid, m.p. 240–242°C, FT-IR: γ (cm^{-1}): 3215 (OH), 3151 (NH), 3072 (Ar C–H), 2958 (=C–H), 1573 (Ar–C=C), 1456 (Ar C=N), 1324 (C–N), 961 (pyrazole N–N), 1057 (benzothiazole), 684 (C–S). ^{1}H NMR

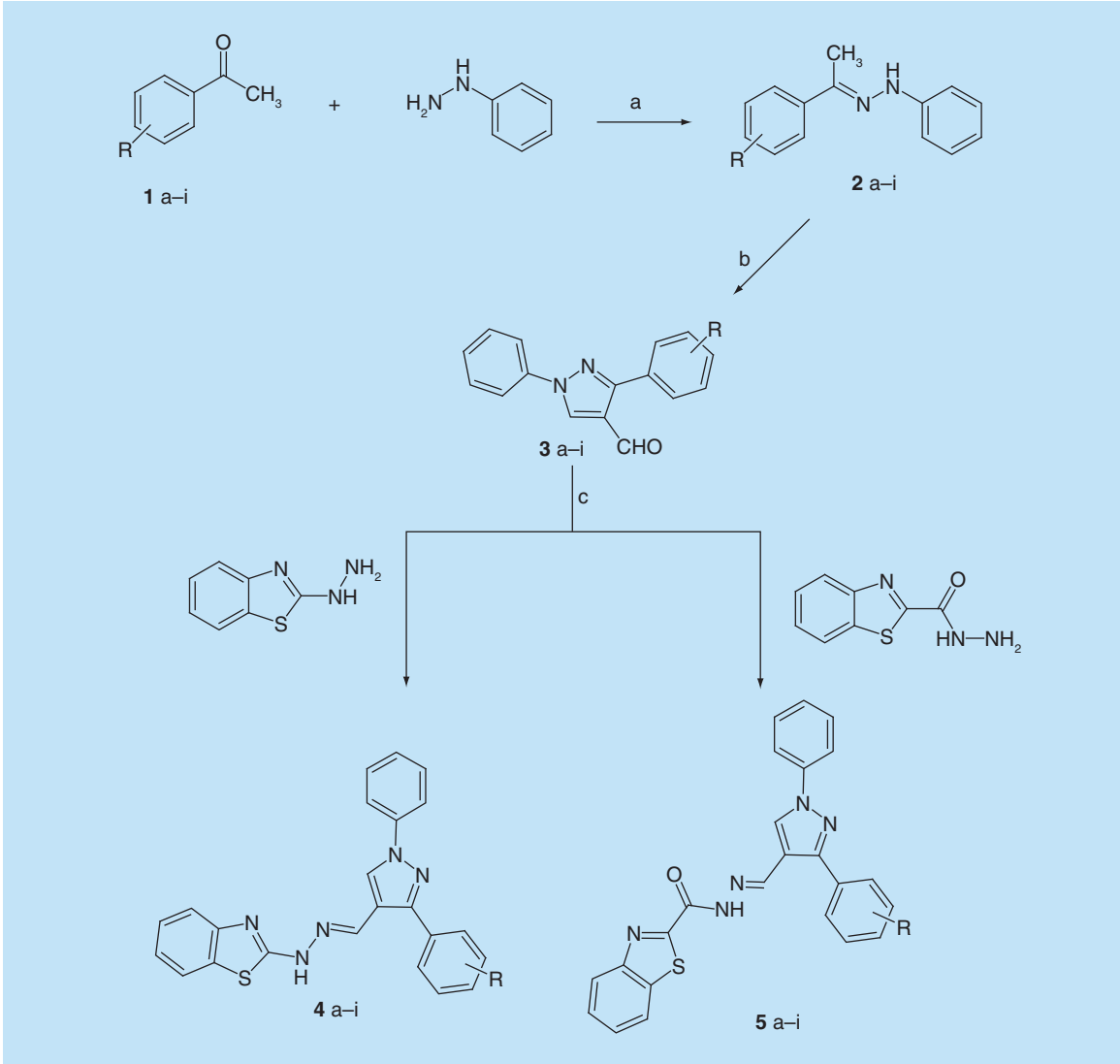

Figure 2. Synthesis of pyrazole-conjugated benzothiazole derivatives.
Reagents: **(A)** Ethanol, glacial acetic acid, heat, **(B)** $POCl_3$, Reflux, **(C)** Ethanol, Con. H_2SO_4. Where R = H, *p*-OCH_3, *p* -OH, *p* -CH_3, *p* -Cl, *p* -Br, *p* -NO_2, *m*-NO_2, *p* -$N(CH_3)_2$

(400 MHz, DMSO-d_6), δ 11.8 (s, 1H, OH), 9.70 (s, 1H, pyrazole), 7.60–7.58 (d, J = 6.8 Hz, 2H), 7.53–7.49 (t, J = 8.0, 15.6 Hz, 3H), 7.38 (s, 1H), 7.35–7.32 (t, J = 7.6, 14.8 Hz, 1H), 7.27–7.24 (t, J = 7.2, 14.8 Hz, 1H), 7.08–7.04 (t, J = 6.8, 15.2 Hz, 1H), 6.90–6.88 (d, J = 8.8, Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 163.4, 151.2, 1425, 140.3, 135.6, 134.5, 133.8, 130.5, 130.1, 129.5, 128.5, 124.4, 121.3, 118.3. LCMS: (ESI, *m/z*) found 412.5 for $(M + 1)^+$ peak at R_t 3.38 and calculated for $C_{23}H_{17}N_5OS$ is 411.12.

Synthesis of *(E)*-2-{2-[(1-phenyl-3-*p*-tolyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d] thiazole (**4d**), white solid, m.p. 265–267°C, FT-IR: γ (cm^{-1}): 3224 (NH), 3112 (Ar C–H), 2982 (=C–H), 1599 (C=N), 1572 (Ar–C=C), 1373 (Ar C–N), 956 (pyrazole N–N), 1055 (benzothiazole), 693 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.28 (s, 1H, NH), 8.86 (s, 1H, pyrazole), 8.42 (s, 1H), 8.25–8.23 (d, J = 8.0 Hz, 1H), 8.11–8.09 (d, J = 7.6 Hz, 1H), 7.94–7.92 (d, J = 8.4 Hz, 2H), 7.75–7.73 (d, J = 8.0 Hz, 2H), 7.61–7.56 (m, 4H), 7.42–7.40 (d, J = 5.6 Hz), 7.35–7.31 (t, J = 7.2, 14.8 Hz, 1H), 2.31(s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 168.2, 152.5, 150.1, 139.7, 139.2, 131.7, 130.8, 130.0, 1295, 127.9, 126.5, 126.2, 125.7, 125.3, 124.5, 121.8, 119.2, 114.3, 21.3. LCMS: (ESI, *m/z*) found 409.98 for $(M + 1)^+$ peak at R_t 3.89 and calculated for $C_{24}H_{19}N_5S$ is 409.14.

Synthesis of *(E)*-2-{2-[(3-(4-chloro phenyl)-1-phenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d] thiazole (**4e**). Off-white solid, m.p. 258–260°C, FT-IR: γ (cm^{-1}): 3192 (NH), 3108 (Ar C–H), 2995 (=C–H), 1598 (C=N), 1572 (Ar–C=C), 1429 (Ar C–N), 1057 (benzothiazole), 961 (pyrazole N–N), 749 (C–Cl), 684 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.53 (s, 1H, NH), 8.86 (s, 1H, pyrazole), 8.45 (s, 1H), 8.79–8.77 (d, J = 7.6 Hz, 1H), 8.52–8.50 (d, J = 8.0 Hz, 1H), 8.16–8.14 (d, J = 8.4 Hz, 2H), 7.87–7.85 (d, J = 9.2 Hz, 2H), 7.58–7.56 (d, J = 7.2 Hz, 2H), 7.59–7.46 (m, 4H), 7.29–7.25 (t, J = 5.6, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 169.1, 153.1, 150.1, 139.7, 139.2, 143.3, 131.1, 130.8, 129.5, 126.4, 126.2, 125.3, 124.5, 121.8, 118.3, 114.2. LCMS: (ESI, m/z) found 430.01 for $(M + 1)^+$ peak at R_t 4.13 and calculated for $C_{23}H_{16}ClN_5S$ is 429.08.

Synthesis of *(E)*-2-{2-[(3-(4-bromo phenyl)-1-phenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d]thiazole (**4f**), pale yellow solid, m.p. 262–264°C, FT-IR: γ (cm^{-1}): 3210 (NH), 3118 (Ar C–H), 2990 (=C–H), 1597 (C=N), 1573 (Ar–C=C), 1429 (Ar–C–N), 963 (pyrazole N–N), 1056 (benzothiazole), 683 (C–S), 632 (C–Br). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.81 (s, 1H, NH), 8.73 (s, 1H, pyrazole), 8.51 (s, 1H), 8.78–8.76 (d, J = 8.0 Hz, 1H), 8.61–8.59 (d, J = 7.6 Hz, 1H), 8.15–8.13 (d, J = 8.4 Hz, 2H), 8.05–8.01 (t, J = 6.4, 16.4 Hz, 2H), 7.88–7.86 (d, J = 8.0 Hz, 2H), 7.49–7.38 (m, 2H), 7.28–7.24 (t, J = 5.6, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 168.8, 153.2, 150.5, 139.7, 139.2, 132.3, 132.1, 130.8, 1293, 128.3, 127.9, 126.4, 126, 125.3, 124.5, 123.1, 121.8, 118.3, 114.7. LCMS: (ESI, m/z) found 473.93 for $(M + 1)^+$ peak at R_t 3.68 and calculated for $C_{23}H_{16}BrN_5S$ is 473.03.

Synthesis of *(E)*-2-{2-[(3-(3-nitro phenyl)-1-phenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d]thiazole (**4g**), off-white solid, m.p. 233–235°C, FT-IR: γ (cm^{-1}): 3208 (NH), 3117 (Ar C–H), 3008 (=C–H), 1600 (C=N), 1579 (Ar–C=C), 1533 & 1303(NO_2), 1350 (Ar C–N), 971 (pyrazole N–N), 1018 (benzothiazole), 688 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.88 (s, 1H, NH), 8.78 (s, 1H, pyrazole), 8.45 (s, 1H), 8.17–8.15 (d, J = 8.0 Hz, 1H), 8.06–78.04 (d, J = 7.6 Hz, 1H), 7.93–7.91 (d, J = 8.8 Hz, 2H), 7.75–7.73 (d, J = 7.2 Hz, 2H), 7.61–7.56 (m, 4H), 7.53–7.51 (t, J = 6.8, 13.6 Hz, 1H), 7.43(s, 1H), 7.38–7.34 (t, J = 7.6, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.0, 153.5, 151.3, 149.4, 140.7, 140.2, 134.9, 134.6, 131.8, 131.6, 130.3, 128.9, 127.4, 127.2, 126.3, 125.5, 124.9, 123.7, 122.8, 119.3, 112.7 LCMS: (ESI, m/z) found 441.02 for $(M + 1)^+$ peak at R_t 3.80 and calculated for $C_{23}H_{16}N_6O_2S$ is 440.11.

Synthesis of *(E)*-2-{2-[(3-(4-nitro phenyl)-1-phenyl-1H-pyrazol-5-yl) methylene] hydrazinyl} benzo[d]thiazole (**4h**), pale yellow solid, m.p. 188–190°C, FT-IR: γ (cm^{-1}): 3198 (NH), 3115 (Ar C–H), 2998 (=C–H), 1575 (C=N), 1530 (Ar–C=C), 1445 & 1320 (NO_2), 1345 (Ar C–N), 860 (pyrazole N–N), 1112 (benzothiazole), 690 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.25 (s, 1H, NH), 8.63 (s, 1H, pyrazole), 8.29 (s, 1H), 8.15–8.13 (d, J = 7.6 Hz, 2H), 8.07–8.05 (d, J = 8.8 Hz, 1H), 7.87–7.85 (d, J = 8.0 Hz, 2H), 7.82-7.78 (t, J = 7.2, 14.6 Hz, 1H), 7.64-7.62 (d, J = 7.6 Hz, 2H), 7.59-7.46 (m, 4H), 7.36-7.32 (t, J = 7.2, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.9, 152.5150.1, 139.7, 130.8, 129.3, 128.2, 126.7, 126.4, 126.2, 125.6, 124.4, 121.9, 118.3, 114.3. LCMS: (ESI, m/z) found 441.03 for $(M + 1)^+$ peak at R_t 4.063 and calculated for $C_{23}H_{16}N_6O_2S$ is 440.11.

Synthesis of *(E)*-4-{5-[(2-(benzo[d] thiazol-2-yl) hydrazono) methyl]-1-phenyl-1H-pyrazol-3-yl}-*N,N*-dimethyl aniline (**4i**), off-white solid, m.p. 244–246°C, FT-IR: γ (cm^{-1}): 3208 (NH), 3108 (Ar C–H), 2990 (=C–H), 2793 (CH3), 1599 (C=N), 1566 (Ar–C=C), 1350 (Ar C–N), 1053 (benzothiazole), 956 (pyrazole N–N), 682 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.42 (s, 1H, NH), 8.89 (s, 1H, pyrazole), 8.45 (s, 1H), 8.16–8.12 (t, J = 5.6, 12.8 Hz, 2H), 8.09–8.07 (d, J = 8.8 Hz, 1H), 7.98-7.96 (d, J = 8.0 Hz, 1H), 7.83–7.81 (d, J = 8.4 Hz, 2H), 7.69–7.67 (d, J = 7.2 Hz, 2H), 7.54–7.48 (m, 2H), 7.06–7.03 (d, J = 9.2 Hz, 2H), 6.85–6.83 (t, J = 7.2, 14.8 Hz, 1H), 2.88 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 168.2, 155.3, 152.5, 139.7, 139.2, 130.8, 129.3, 128.4, 127.9, 126.4, 126.2, 125.3, 124.5, 122.5, 121.8, 120.4, 118.5, 115.6. LCMS: (ESI, m/z) found is 439.21 for $(M + 1)^+$ peak at R_t 3.72 and calculated for $C_{25}H_{22}N_6S$ is 438.16.

Synthesis of *(E) N'*-[(1,3-diphenyl-1H-pyrazol-4-yl) methylene) benzo[d] thiazole-2-carbohydrazide (**5a**), white solid, m.p. 188–190°C, γ(cm^{-1}): 3215 (>CONH), 3018 (Ar C–H), 2992 (=C–H), 1655 (>CONH), 1598 (C=N), 1551 (Ar–C=C), 1338 (Ar C–N), 959 (pyrazole N–N), 1057 (benzothiazole), 693 (C–S). ^{1}H NMR (400 MHz, $CDCl_3$), δ 10.39 (s, 1H, NH), 8.72 (s, 1H, pyrazole), 8.37 (s, 1H), 8.08–8.06 (d, J = 8.0 Hz, 1H), 8.01–7.99 (d, J = 8.0 Hz, 1H), 7.82–7.80 (d, J = 8.0 Hz, 2H), 7.69–7.67 (d, J = 7.2 Hz, 2H), 7.59–7.46 (m, 8H), 7.36–7.32 (t, J = 7.2, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 163.8, 157.2, 154.3, 152.2, 145.4, 139.8, 137.1, 133.4, 131.8, 131.2, 129.1, 128.3, 128.0, 125.6, 124.4, 122.8, 119.7, 118.1. LCMS: (ESI, m/z) found 423.98 for $(M + 1)^+$ peak at R_t 3.68 and calculated for $C_{24}H_{17}N_5OS$ is 423.49.

Synthesis of *(E) N'*-[3-(4-methoxy phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene) benzo[d]thiazole-2-carbohydrazide (**5b**), reddish solid, m.p. 192–194°C, γ(cm^{-1}): 3295 (>CONH), 3025 (Ar C–H), 2996 (=C–H), 1667 (>CONH), 1595 (C=N), 1556 (Ar–C=C), 1351 (Ar C–N), 964 (pyrazole N–N), 1150 (C–O), 1055 (benzothiazole), 698 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 12.59 (s, 1H, NH), 8.97 (s, 1H, pyrazole), 8.83 (s, 1H), 8.25–8.23 (t, *J* = 7.6, 8.4 Hz, 1H), 8.17–8.15 (d, *J* = 8.0 Hz, 1H), 8.01–7.99 (d, *J* = 7.6 Hz, 2H), 7.71–7.68 (t, *J* = 2.4, 9.2 Hz, 2H), 7.64–7.56 (m, 2H), 7.53–7.49 (t, *J* = 7.6, 15.6 Hz, 2H), 7.36–7.34 (d, *J* = 7.6 Hz, 1H), 7.09–7.07 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ 164.4, 160.1, 157.5, 153.2, 152.4, 144.7, 139.5, 136.5, 130.2, 127.7, 127.5, 127.4, 124.7, 124.5, 123.5, 119.3, 116.8, 114.6, 55.73. LCMS: (ESI, *m/z*) found 454.09 for $(M + 1)^+$ peak at R_t 3.688 and calculated for $C_{25}H_{19}N_5O_2S$ is 453.13.

Synthesis of *(E)-N'*-{[3-(4-hydroxy phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5c**) off-white solid, m.p. 218–219°C, γ(cm^{-1}): 3215 (>CONH), 3018 (Ar C–H), 2992 (=C–H), 1655 (>CONH), 1598 (C=N), 1551 (Ar–C=C), 1338 (Ar C–N), 959 (pyrazole N–N), 1057 (benzothiazole), 693 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.31 (s, 1H, NH), 9.62 (s, 1H, OH), 9.06 (s, 1H, pyrazole), 8.63 (s, 1H), 7.99–7.97 (d, *J* = 6.4 Hz, 2 H), 7.75–7.73 (d, *J* = 7.6 Hz, 1H), 7.65–7.63 (d, *J* = 8.4 Hz, 2H), 7.38 (s, 1H), 7.42–7.39 (t, *J* = 7.6, 14.8 Hz, 1H), 7.37–7.34 (t, *J* = 7.2, 14.8 Hz, 1H), 7.28–7.24 (d, *J* = 8.0 Hz, 1H), 7.14–7.12 (*J* = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 163.2, 159.1, 155.2, 151.3, 150.4, 143.6, 138.5, 165.4, 129.3, 128.8, 126.7, 126.4, 126.2, 123.6, 123.3, 123.1, 122.5, 118.3, 117.9, 115.6. LCMS: (ESI, *m/z*) found 440.06 for $(M + 1)^+$ peak at R_t 3.17 and calculated for $C_{24}H_{17}N_5O_2S$ is 439.49.

Synthesis of *(E)-N'*-{[1-phenyl-3-(*p*-tolyl)-1H-pyrazol-4-yl] methylene} benzo [d]thiazole-2-carbohydrazide (**5d**), off-white solid, m.p. 260–262°C, γ(cm^{-1}): 3408 (>CONH), 3108 (Ar C–H), 2952 (=C–H), 1682 (>CONH), 1598 (C=N), 1556 (Ar–C=C), 1372 (Ar C–N), 1050 (benzothiazole), 956 (pyrazole N–N), 689 (C–S), ^{1}H NMR (400 MHz, DMSO-d_6), δ 12.12 (s, 1H, NH), 8.91 (s, 1H, pyrazole), 8.42 (s, 1H), 8.15–8.13 (d, *J* = 8.0 Hz, 1H), 8.11–8.09 (d, *J* = 7.6 Hz, 1H), 7.92–7.88 (t, *J* = 5.6, 11.6 Hz, 2H), 7.88–7.86 (d, *J* = 8.0 Hz, 2H), 7.75–7.53 (d, *J* = 7.2 Hz, 2H), 7.59–7.46 (m, 3H), 7.41–7.39 (d, *J* = 8.8 Hz, 1H), 7.36–7.32 (t, *J* = 7.2, 14.8 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 168.3, 160.5, 153.4, 150.4, 142.3, 140.2, 137.3, 132.5, 131.3, 131.0, 129.7, 129.5, 129.2. 127.3, 126.4, 125.6, 125.3, 124.5, 122.4, 119.8, 116.5, 21.3. LCMS: (ESI, *m/z*) found 437.13 for $(M + 1)^+$ peak at R_t: 3.28 and calculated for $C_{25}H_{19}N_5OS$ is 438.12.

Synthesis of *(E)-N'*-{[3-(4-chloro phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5e**), white solid, m.p. 232–234°C, γ(cm^{-1}): 3321 (>CONH), 3095 (Ar C-H), 2958 (=C-H), 1665 (>CONH), 1600 (C=N), 1572 (Ar-C=C), 1404 (Ar C-N), 991 (pyrazole N-N), 1052 (benzothiazole), 685 (C-S), 605 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.45 (s, 1H, NH), 8.89 (s, 1H, pyrazole), 8.42 (s, 1H), 8.25–8.23 (d, *J* = 7.6 Hz, 1H), 8.18–8.16 (d, *J* = 8.0 Hz, 1H), 8.11–8.09 (d, *J* = 9.2, Hz, 2H), 7.84–7.82 (d, *J* = 7.6 Hz, 2H), 7.76–7.74 (d, *J* = 8.8 Hz, 2H), 7.69–7.67 (d, *J* = 7.2 Hz, 2H), 7.52–7.46 (m, 3H), 7.38–7.34 (t, *J* = 7.2, 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.3, 161.5, 154.6, 151.7, 144.3, 139.4, 137.2, 135.3, 132.1, 131.3, 129.6, 129.3, 128.9, 127.2, 126.3, 124.5, 122.6, 120.9, 115.6. LCMS: (ESI, *m/z*) found 458.02 for $(M + 1)^+$ peak at R_t 4.08 and calculated for $C_{24}H_{16}ClN_5OS$ is 457.02

Synthesis of *(E)-N'*-{[3-(4-bromo phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5f**), pale yellow solid, m.p. 186–188°C, γ(cm^{-1}): 3262 (>CONH), 3032 (Ar C–H), 2991 (=C–H), 1655 (>CONH), 1599 (C=N), 1550 (Ar–C=C), 1373 (Ar C–N), 1072 (pyrazole N–N), 1007 (benzothiazole), 693 (C–S), 682 (C–Br), ^{1}H NMR (400 MHz, $CDCl_3$), δ 12.6 (s, 1H, NH), 9.02 (s, 1H, pyrazole), 8.82 (s, 1H), 8.24–8.23 (d, *J* = 7.6 Hz, 1H), 8.16–8.14 (d, *J* = 8.0 Hz, 1H), 8.02–7.99 (d, *J* = 7.6 Hz, 2H), 7.75–7.69 (q, *J* = 8.8 Hz, 4H), 7.63–7.58 (m, 8H), 7.53–7.51 (t, *J* = 7.6, 15.6 Hz, 2H), 7.35(s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 164.3, 156.2, 153.2, 151.2, 144.1, 139.4, 136.5, 132.1, 130.9, 130.0, 128.3, 127.7, 127.6, 127.5, 124.5, 123.5, 122.5, 119.4, 117.2. LCMS: (ESI, *m/z*) found 503.97, 504.97 for $(M + 1)^+$, $(M + 2)^+$ peaks at R_t 4.02 and calculated for $C_{24}H_{16}BrN_5OS$ is 502.39.

Synthesis of *(E)-N'*-{[3-(3-nitro phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5g**), pale yellow solid, m.p. 177–179°C, γ(cm^{-1}): 3279 (>CONH), 3065 (Ar C–H), 2971 (=C–H), 1689 (>CONH), 1599 (C=N), 1544 (Ar–C=C), 1544 & 1319 (NO_2), 1350 (Ar C–N), 961 (pyrazole N–N), 1061 (benzothiazole), 689 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.28 (s, 1H, NH), 8.93 (s, 1H, pyrazole), 8.45 (s, 1H), 8.25–8.23 (d, *J* = 7.6 Hz, 1H), 8.15–8.13 (d, *J* = 8.8 Hz, 2H), 7.95–7.91 (t, *J* = 5.2, 10.8 Hz, 2H), 7.72–7.70 (d, *J* = 8.0 Hz, 2H), 7.59–7.46 (m, 4H), 7.43–7.7.41 (t, *J* = 7.2, 14.8 Hz, 2H), 7.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.5, 162.3, 154.5, 151.4, 149.4, 144.3, 140.7, 1372, 134.6, 130.6,

130.3, 129.5, 129.3, 127.2, 126.3, 125.5, 124.9, 123.7, 122.6, 120.9, 115.9. LCMS: (ESI, *m/z*) found 469.06 $(M + 1)^+$ peak at R_t 4.30 and calculated for $C_{24}H_{16}N_6O_3S$ is 468.10.

Synthesis of *(E)-N'*-{[3-(4-nitro phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5h**), off-white solid, m.p. 216–217°C, γ(cm^{-1}): 3256 (>CONH), 3072 (Ar C–H), 2965 (=C–H), 1739 (>CONH), 1592 (C=N), 1562 (Ar–C=C), 1371 (Ar C–N), 1532 & 1327 (NO_2), 995 (pyrazole N–N), 1062 (benzothiazole), 690 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6), δ 12.12 (s, 1H, NH), 9.01 (s, 1H, pyrazole), 8.79 (s, 1H), 8.36–8.34 (d, J = 8.0 Hz, 1H), 8.25–8.23 (d, J = 7.6 Hz, 1H), 8.14–8.12 (d, J = 8.8 Hz, 2H), 7.76–7.70 (q, J = 8.8 Hz, 2H), 7.65–7.60 (m, 2H), 7.60–7.58 (d, J = 8.0 Hz, 2H), 7.53–7.51 (t, J = 7.6, 15.6 Hz, 2H), 7.35(s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.9, 162.5, 154.5, 151.4, 148.9, 144.3, 140.7, 140.1, 137.2, 131.3, 130.3, 126.4, 126.2, 125.5, 124.5, 121.6, 120.7, 116.7. LCMS: (ESI, *m/z*) found 469.06 for $(M + 1)^+$ peak at R_t 3.92 and calculated for $C_{24}H_{16}N_6O_3S$ is 468.10

Synthesis of *(E)-N'*-{[3-(4-(dimethyl amino) phenyl)-1-phenyl-1H-pyrazol-4-yl] methylene} benzo[d] thiazole-2-carbohydrazide (**5i**), white solid, m.p. 188–190°C, γ(cm^{-1}): 3245 (>CONH), 3081 (Ar C–H), 2975 (=C–H), 1685 (>CONH), 1585 (C=N), 1568 (Ar–C=C), 1385 (Ar C–N), 992 (pyrazole N–N), 1054 (benzothiazole), 685 (C–S), ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.60 (s, 1H, NH), 8.72 (s, 1H, pyrazole), 8.20 (s, 1H), 8.08–8.06 (d, J = 8.0 Hz, 1H), 7.98–7.96 (d, J = 8.0 Hz, 2H), 7.68–7.66 (d, J = 8.0 Hz, 2H), 7.63–7.61 (d, J = 7.2 Hz, 2H), 7.59–7.48 (m, 3H), 7.41–7.39 (d, J = 9.2 Hz, 2H), 7.34–7.30 (t, J = 7.2, 14.8 Hz, 1H), 2.82 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6), δ 169.1, 160.5, 155.3, 153.5, 150.4, 143.3, 139.7, 136.2, 130.3, 129.3, 128.4, 126.2, 125.3, 124.5, 122.5, 121.6, 120.3, 118.1, 41.3. LCMS: (ESI, *m/z*) found 467.23 for $(M + 1)^+$ peak at R_t: 3.02 and calculated for $C_{26}H_{22}N_6OS$ is 466.16.

Anti-TB activity using Alamar Blue dye

The antimycobacterial tubercular activity of compounds was assessed against *M. tuberculosis* using microplate Alamar Blue assay [12]. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. To the 96-well plate, 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were added. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After incubation, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 were added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color scored as growth. The minimum inhibitory concentration (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink.

Molecular docking

Molecular docking was performed with the aid of Maestro 9.3.5 version of the Schrodinger software suite, 2011. The 3D crystallographic structure of proteins (PDB ID: 1ZID) was retrieved from Protein Data Bank [20]. The protein structures were preprocessed and refined by Protein Preparation Wizard. Further, it was minimized by Optimized Potential for Liquid Simulations (OPLS)-2005 force field until the root mean square deviation reached 0.3 Å. The ligands were optimized by LigPrep program using the OPLS-2005 force field to generate lowest energy state of ligands. The molecular docking studies of the ligand and protein were carried out by GLIDE. The activity was ranked on the basis of docking score, those which were based on how the ligands fit with the target protein [13].

Antimicrobial assay

Microbial cultures used in the study were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The cultures used were *Escherichia coli* (MTCC-40), *Staphylococcus aureus* (MTCC 7443), *Candida albicans* (MTCC-183) and *Aspergillus niger* (MTCC-1344). Stock cultures of bacteria were maintained on nutrient agar (Himedia, Mumbai) slants at 4°C with periodical sub-culturing.

The antimicrobial activities of compounds were screened by disc diffusion method (Makut *et al.*, 2008) [14]. Briefly, the 500 µg solutions of the synthesized compounds were placed on the preseeded disc on agar plates. 100 µl of 24 h cultures of test microorganisms in broth was used for the seeding. Chloramphenicol (500 µg solution) and Bavistin (500 µg/ml solution) were used as positive controls for bacteria and fungi respectively, while the discs with only the solvent (200 µl/disc) was used as negative control. The plates were preincubated for 1 h at 4°C.

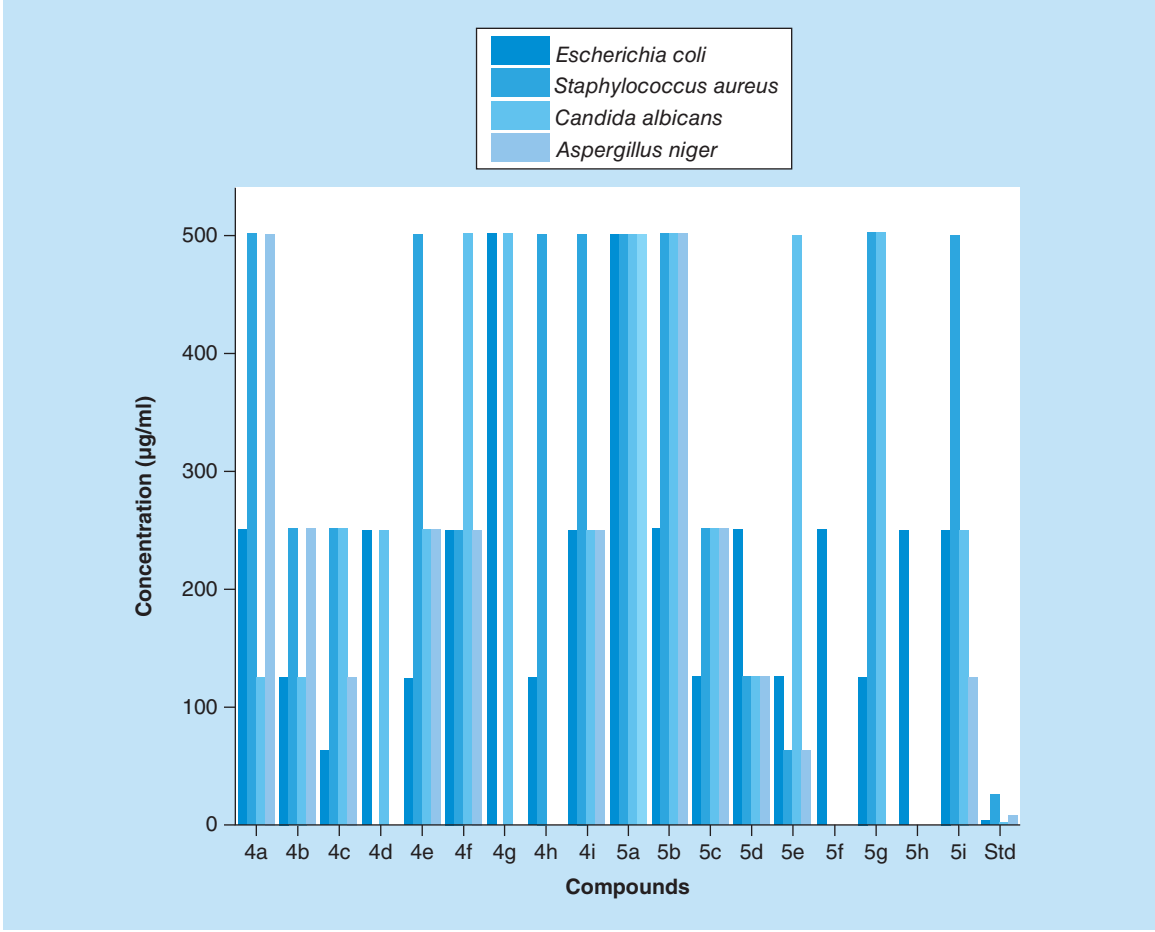

Figure 3. Minimum inhibition concentrations of the antimicrobial activity.

The bacterial plates were incubated at 37°C for 24 h and fungal plates for 5 days at 30°C. The diameters of the inhibition zones (in mm) were measured after the incubation and values are shown in Figure 3.

Determination of minimum inhibitory concentration

A broth micro-dilution method was used to determine the MIC of the isolated compounds (Raj *et al.*) [15]. A serial twofold dilution of the compounds was prepared over the range 500 to 0.08 µg/ml. Each of the discs was inoculated with synthesized compounds. The bacterial plates were incubated at 37°C for 24 h and those of fungi at 28°C for 72 h. The diameters of the inhibition zones (in mm) were measured and the inhibition zone appears for the minimum concentration was taken as MIC of the compound.

Antioxidant activity

Free radical scavenging ability by DPPH radical assay (1,1-diphenyl-2-picryl hydrazyl)

The DPPH radical scavenging activity of purified compounds was determined by Raj *et al.* [15]. Various concentrations of test samples in aliquots of 100 µl were mixed with 100 µl of 40 µM methanolic solutions of DPPH (Himedia, Mumbai, India) in a 96-well micro titer plates. The decrease in absorbance at 517 nm was recorded after the incubation of 15 min at room temperature. The absorbance of the DPPH solution with only methanol and without sample was used as the control. The ascorbic acid (AA, Himedia, Mumbai) was used as a standard to compare the activity. Appropriate blank readings at 517 nm were recorded for each tested dilutions. The assay was carried out in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according

to the formula.

$$\text{Percentage of inhibition} = [\frac{Ac - As}{Ac}] \times 100 \quad (1)$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample/standard after 15 min. The IC_{50} value was calculated graphically based on the capacity of compound concentration to scavenge 50% of free radicals.

ABTS [2,2'-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid)] radical scavenging activity

ABTS assay was performed with a modification of the Samaga *et al.* method [16]. The ABTS stock solution was prepared by mixing equal volumes of 7.4 mM ABTS solution and 2.6 mM potassium per sulfate solution followed by incubation for 12 h at room temperature in the dark. The reaction mixture consisted of 1 ml of the purified compound at different concentrations (0.5–500 µg/ml in respective solvents) and 3 ml of standardized ABTS solution. The decrease in absorbance was measured at 734 nm after 15 min of incubation. Data for each assay were recorded in triplicate. Ascorbic acid (NICE Chemicals Pvt. Ltd, Cochin, India) was used as positive control. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula.

$$\text{Percentage of scavenging} = [\frac{Ac - As}{Ac}] \times 100 \quad (2)$$

Where A_C is absorbance of control, A_S is absorbance of tested sample/standard solution after 15 min. The IC_{50} value was calculated graphically based on the capacity of compound concentration to scavenge 50% of free radicals.

In vitro cytotoxicity assay

Toxicity of the most active anti-TB compounds (**4b**, **4d** and **4e**) was determined on the basis of measurement of *in vitro* growth of the normal cell line (HEK-293) using MTT assay. It is a human embryonic cell and selected for toxicity evaluation of the synthesized compounds [17]. The IC_{50} values (50% inhibitory concentration) were calculated.

Results & discussion

Syntheses of 4-fromyl pyrazole derivatives were achieved by the Vilsmeier–Haack reaction and the generated pyrazole formyl derivatives were conjugated with the 2-amino benzothiazole-hydrazine and benzothiazole-hydrazides to get the benzothiazole derivatives of the pyrazole. The formation of the product was primarily identified by the TLC and recorded the melting points. The synthesized compounds were characterized by FT-IR, LC-MS, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. The peak at 1598 cm^{-1} (>C=N-) indicates the formation of the azo methane linkage in the synthesized compounds. In ^{1}H NMR spectroscopy, singlet at 8.89 p.p.m. indicates the pyrazole proton, which confirms the presence of 5-membered pyrazole ring and a singlet at 7.1 p.p.m. indicates the protons of azomethine linkage (-imine formation), the singlet at 12.5 p.p.m. confirms the presence of NH from benzothiazole.

Biological activity studies

Antimicrobial study

The synthesized compounds were tested for *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* to know the efficiency of the compounds to act as antimicrobial agents by disc diffusion method at 500 µg/ml. The activity was measured in diameters of the zone of inhibition and values are shown in Table 1.

Synthesized compounds showed the promising activity for the *E.coli* and moderate activity to the others tested microorganisms. Compounds **4b**, **4c**, **4f**, **5c** and **5d** had remarkable activity in the series. Compounds with -OH, -OMe, -Me and -Br groups showed the superior activity. MIC of the synthesized compounds was determined by serial twofold dilution method. The majority of the compounds showed the activity in higher concentrations. In the series **4c** had 62.5 µg/ml as MIC value, followed by **4b**, **4e**, **5c** which had the 125 µg/ml MIC concentrations for the *E.coli*. For the *Staphylococcus aureus* **5e** had 62.5 µg/ml MIC value and **5d** had 125 µg/ml, which were

Table 1. Antimicrobial activity of the synthesized compounds at 500 μg/ml.

Entry	Zone of inhibition (mm)			
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
4a	18	12	19	12
4b	20	14	22	14
4c	17	17	23	17
4d	11***	–	15***	–
4e	16*	15*	09*	15*
4f	16	16	16	16
4g	17***	–	09***	–
4h	15***	09***	–	–
4i	16	10	12	13
5a	16*	10*	10*	10*
5b	19	11	09	11
5c	17	16	16	16
5d	14	15	11	15
5e	19	19	10	19
5f	15***	–	–	–
5g	17**	09**	09**	–
5h	17***	–	–	–
5i	14	12	13	14
Std	21	30	20	30

ANOVA analysis followed by Tukey's test, significance levels.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with the respective standard.

comparable with standard Chloramphenicol (25 μg/ml). For the *Candida albicans* **4a**, **4b**, **5c** exhibit the 125 μg/ml MIC values. **5c** was the most potent anti-*A. niger* compound, which had 62.5 μg/ml as MIC value.

Structure–activity relationship reveals that, compound with hydroxyl, methoxy group showed the superior activity, in other words, compounds containing electron-donating groups on fourth position of the *N*-substituted phenyl ring show the superior activity.

Antioxidant activity

The reactive oxygen species is generated from metabolism or interactions of the external sources on the biological systems. The uncontrolled formation of reactive oxygen species directly related to many diseases at the molecular level. So, effective scavenging of these reactive species may be helpful to defeat many diseases. The substance scavenges the reactive oxygen species, anions, radicals, peroxides, etc. which are called antioxidants. The ability of the synthesized benzothiazole derivatives to act as antioxidants is estimated by DPPH radical scavenging assay and ABTS assay.

Both the DPPH and ABTS *in vitro* antioxidant assay of pyrazole-conjugated benzothiazole derivatives exhibited almost similar activities. In the present study, benzothiazole hydrazide compounds show better activities compared with the benzothiazole carbohydrazide derivatives. The presence of the hydrazine group (-NH-N-) showed the antioxidant activities. The hydrazine compound has the ability to give the proton by resonance, so, they show the notable activities as shown in Figure 4. Compounds **4c** and **5c** have -OH group on *N*-phenyl group conjugated with the pyrazole and marked for the superior activity in the series, **4c** has 168 and 180 μg/ml concentration in ABTS and DPPH assay. Compound **5c** has IC_{50} values at 210 and 205 μg/ml concentrations in DPPH and ABTS assay, respectively. In **4i** and **5i** have *p-N,N* dimethyl amino group which shows the ABTS assay IC_{50} values 210 and 242.9 μg/ml, respectively. The remaining compounds showed the moderate to lower activity and have IC_{50} values at higher concentrations.

Anti-TB activity

Synthesized compounds were screened for the *in vitro* antimycobacterium tubercular activity against *Mycobacterium tuberculosis* by Alamar Blue assay. All the synthesized compounds were active against *M. tuberculosis* at 100 μg/ml

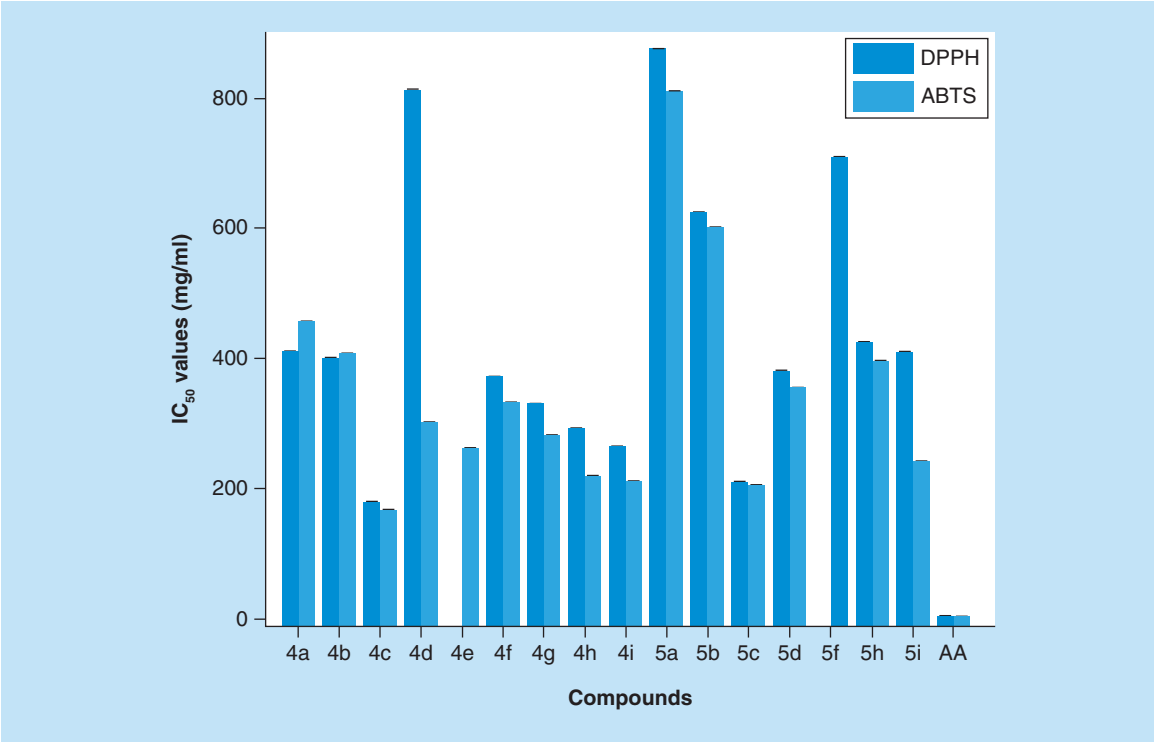

Figure 4. Antioxidant activity of the synthesized compounds.

Table 2. Antitubercular activity of the pyrazole-conjugated benzothiazole derivatives.

Compound	MIC (in μg/ml)	Compound	MIC (in μg/ml)
4a	12.5	**5a**	25
4b	6.25	**5b**	25
4c	6.25	**5c**	25
4d	1.6	**5d**	25
4e	1.6	**5e**	100
4f	6.25	**5f**	50
4g	6.25	**5g**	100
4h	50	**5h**	50
4i	25	**5i**	50
Pyrazinamide	3.125	Ciprofloxacin	3.125

MIC: Minimum inhibitory concentration.

and their values are shown in Table 2. So, they are screened for lower concentrations to know their minimum inhibition concentration in the range of 100 to 0.8 μg/ml. In the series, benzothiazole hydrazine compounds showed the superior activity (MIC values 25 to 1.6 μg/ml) compared with the benzothiazole carbohydrazide derivatives (MIC values 100 to 25 μg/ml), which was also shown in the antimicrobial study. In the synthesized compounds *p*-CH_3 and *p*-Cl (in hydrazine derivative) showed excellent activity. In the present work, Pyrazinamide and Cifrofloxacin were employed as standard drugs for Anti-TB activity, both of them showed 3.25 μg/ml as MIC value.

The structure–activity relationship study reveals that, the compounds with electron donating groups exhibit the superior activity in both the series, compound with hydrazine linked shows the superior activity than the carbohydrazide linked benzothiazole with pyrazole derivatives. Compounds with electron donating groups like (OH, OMe) show the MIC values 6.25 μg/ml. Compound **4e** has *p*-Cl substitution (electron withdrawing group)

Table 3. The *in vitro* cytotoxicity activity on normal cell line (HEK-293) by MTT assay at 48 h of exposure.

Compound	IC_{50}† in μg/ml
4b	>500
4d	>500
4e	96.39 ± 0.42
Cisplatin‡	>500

†Data represented in mean ± SD of three experiments.
‡Positive control.

exhibited MIC at 1.6 μg/ml, compared to the standard drug, **4e** has activity at lower concentration, hence it is a superior compound compared to the standard drug.

The present results can correlate with the earlier investigations and our results were comparable with the findings of the others. Telvekar *et al.* synthesized the 2-[2-(4-aryloxy benzylidene) hydrazinyl] benzothiazole derivatives and one of the compounds showed the MIC value up to 1.5 μg/ml [18]. Huang *et al.* synthesized the benzothiazole isoxazole derivatives and screened for tuberculosis and compounds showed potential activity with MIC value 1.9 μM [19].

Structure–activity relation correlates the biological activity and chemical structure of the molecule. In general, the activity of the compound is influenced by the electronic structure, size, shape, molecular arrangements, and electron donating/withdrawing groups, etc. Compounds with hydroxy, methoxy groups showed superior antimicrobial and antioxidant activities. In general, compounds containing electron donating groups at *p*-position showed superior antimicrobial activity. The synthesized hybrid molecule exhibited the excellent activity, in which benzothiazole and pyrazole linked by hydrazine (NH–N=CH) imine are superior to the carbohydrazide (CO–NH–N=CH) imine conjugation. Here, compounds with electron donating groups are marked for superior compound. In halogen substituted compounds, *p*-chloro derivatives are most potent.

Effects of the compounds on the viability of normal cell lines

In the synthesized series of the compound **4b**, **4d** and **4e** were most anti-TB active compounds, so toxicity toward the normal cell line (HEK-293) was evaluated by MTT assay. From the data as reported in Table 3, it is reveal that, compounds **4b** and **4d** were nontoxic to the normal cells, whereas the **4e** showed the toxicity at higher concentration with IC_{50} value 96.39 ± 0.42 μg/ml. These compounds show the anti-TB activity at lower concentration (**4b** has 6.25 μg/ml, **4d** and **4e** have 1.6 μg/ml MIC), so synthesized compound can act as anti-TB agent without toxic effect. In the tested compound it was observed that biological response is higher in halogenated compound (**4e**). Compound with electron donating groups like -OH (**4d**) and -OMe (**4b**) show the toxicity IC_{50} value at higher concentration (>500 μg/ml). Less toxic effect is major advantage of the synthesized pyrazole-conjugated benzothiazole derivatives.

Molecular docking studies

To know the molecular interactions of the benzothiazole compounds with the protein, we had carried out the molecular docking studies with enoyl acyl carrier reductase (Inha, PDB ID-1ZID) of *M. tuberculosis*. InhA is a key enzyme, which involves type II fatty acid biosynthesis pathway of *M. tuberculosis*. Here, compounds showed the remarkable docking score -5.83 to -9.6, which can be compared with the standard drug Isoniazid with -6.61 as docking score as shown in Table 4.

The docking studies of the benzothiazole hydrazine pyrozole conjugated derivatives showed the docking score over the range of -8.12 to -5.83, which can be comparable to the standard drug isoniazid and ciprofloxacin with docking score -6.61 and -5.3, respectively. The compound which has larger negative values is marked for the efficient binding for the ligand. In the series compound **4e** (Cl) showed the maximum docking score -8.125 followed by the **4f** has -7.7, **4d** has -7.68 and **4a** has -7.6 with Br, Me and H substitutions on *N*-phenyl ring of the pyrazole moiety. Compound **4g** has *p*-NO_2 group, which showed -5.9 least docking score.

The *in silico* results can be co-related with the *in vitro* anti-TB activity. Compound **4b**, **4c** and **4g** has average docking score (-5.8 to -5.9), they have moderate *in vitro* MIC value 6.25 μg/ml, compound **4d** and **4e** showed the MIC value at 1.6 μg/ml, they also showed superior docking score -7.68 and -8.12, respectively. Compound **4h** has

Table 4. Docking score of pyrazole-conjugated benzothiazole.

Compound	Docking score	Compound	Docking score
4a	-7.614	**5a**	-8.41
4b	-5.83	**5b**	-7.662
4c	-5.968	**5c**	-9.631
4d	-7.685	**5d**	-7.705
4e	-8.125	**5e**	-7.985
4f	-7.703	**5f**	-8.064
4g	-5.963	**5g**	-7.896
4h	-6.616	**5h**	-7.961
4i	-6.921	**5i**	-6.354
Isoniazid	-6.615	Ciprofloxacin	-5.345

average docking score (-6.6) and also average MIC value (50 μg/ml) in *in vitro* studies. The molecular docking 2D and 3D representations of the compound **4b** and **4f** are shown in Figure 5.

Benzothiazole carbohydrazide pyrazole-conjugated derivatives showed the docking score in the range of -9.9 to -7.66, which is superior compared with the tested standard drugs. Compound **5c** (OH) has highest docking score of -9.63 and compound **5b** (methoxy) has lowest docking score -6.3. The above results can be correlated to the *in vitro* studies. The compound **5c** has the OH group, which has the highest docking score and *in vitro* anti-TB activity shows the 25 μg/ml as MIC value. Compound **5d** (CH_3 substitution) and **5a** has superior docking score -7.7 and -8.41 and also MIC value at 25 μg/ml. The above results support the *in silico* molecular docking studies for the determination of biological activities of the compounds.

The molecular docking studies of the synthesized compounds with InhA of *M. tuberculosis*, the 2D and 3D molecular representation, show the molecular interactions with different amino acids. *N*-substituted phenyl interacts with the tyrosine (158) and aromatic ring of benzothiazole interact with phenylalanine (41). The -NO_2 group present in the **4g** and **4i** interacts with the lysine (165) and tyrosine (158), respectively, oxygen of the -NO_2 acts as the hydrophilic center, so they interact with water molecule through the hydrogen bonding.

The molecular orbitals of the of the benzothiazole carbohydrazide pyrazole-conjugated derivatives interact with many amino acids, as shown in the docking experiments when carried out for InhA proteins. The *N*-phenyl group of pyrazole moiety interacts with the phenylalanine (41), -NH of the amide and >C=O of amide interacts with glycine (14) and serine (20), respectively. In some of the compounds (**5e**, **5f** and **5h**) the nitrogen of the thiazole unit interacts with the Threonin (196) unit and amide oxygen and oxygen of the -NO_2 group act as hydrophobic center, which interact with water molecule through the hydrogen bonding. Molecular interactions of the compound **5a** and **5f** are shown in Figure 6 and Figure 7. The targeted compound was also carried out docking studies with DprE1 protein, which is the main precursor for the arabinogalactan and lipoarabinomannan synthesis in the mycobacterium cell wall. None of the synthesized compounds docked with the DprE1 protein. Hence, the synthesized compound was involved in the inhibition of mycolic acid pathway.

Conclusion

In the present study, two series of the pyrazole-conjugated benzothiazole derivatives were synthesized by the Vilsmeier–Haack reaction, followed by the Schiff's base formation. The newly synthesized compounds were screened for the antibacterial, antioxidant and anti-TB activities. They showed moderate antibacterial and antioxidant activities. Compounds containing -OH, -CH_3 and -Cl groups exhibited the superior antibacterial activity in the series. Majority of the compounds exhibit excellent *in vitro* anti-TB activity, showed the MIC values up to 1.6 μg/ml and they are appeared nontoxic to the normal cell line in that concentration. The synthesized compounds with *p*-CH_3 and *p*-Cl showed superior activity. The molecular docking studies were carried out to know the molecular interactions and they showed the superior docking score compared with the standard.

Statistical analysis

The values of antioxidant activity are expressed as mean ± standard deviation. The inhibition zone was measured from the antimicrobial activity of compound and analyzed using one way analysis of variance (ANOVA) followed by Tukey's test at $p < 0.05$. The software Origin Pro 9.0 was employed for the statistical analysis.

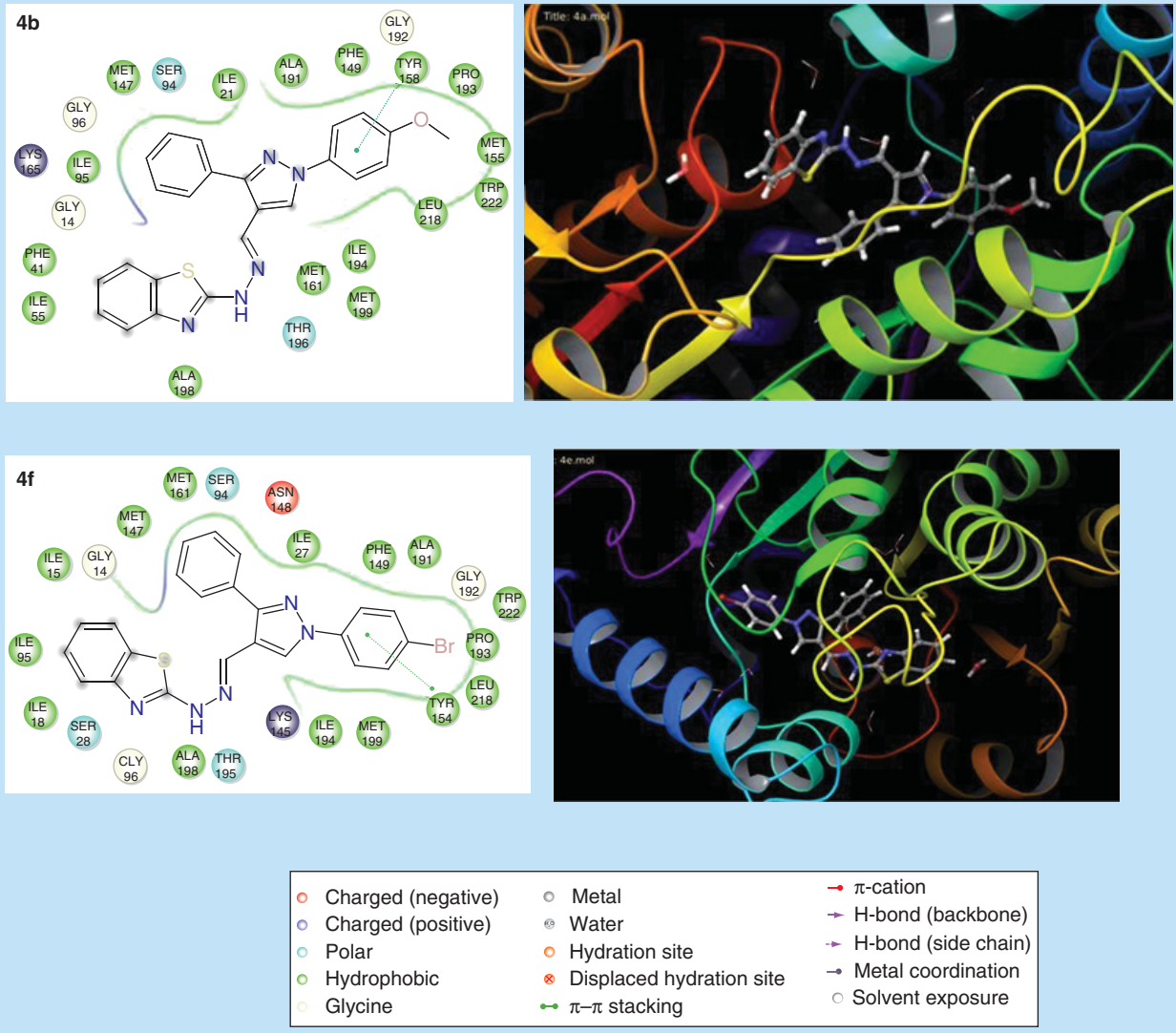

Figure 5. 2D and 3D representations of 4b and 4f molecular docking studies with InhA proteins.

Future perspective

Tuberculosis is a deadly disease that mainly affects lungs and causes respiratory-related problems. According to the WHO statistical data, a third of the world's population is infected by tuberculosis disease. Here, an attempt has been made to synthesize novel pyrazole-conjugated benzothiazole derivatives as anti-TB agent. The synthesized hybrid molecule (**4d** & **4e**) showed the MIC at 1.6 μg/ml. So, synthesized molecule may become the potential candidate for the clinical trials in the future.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

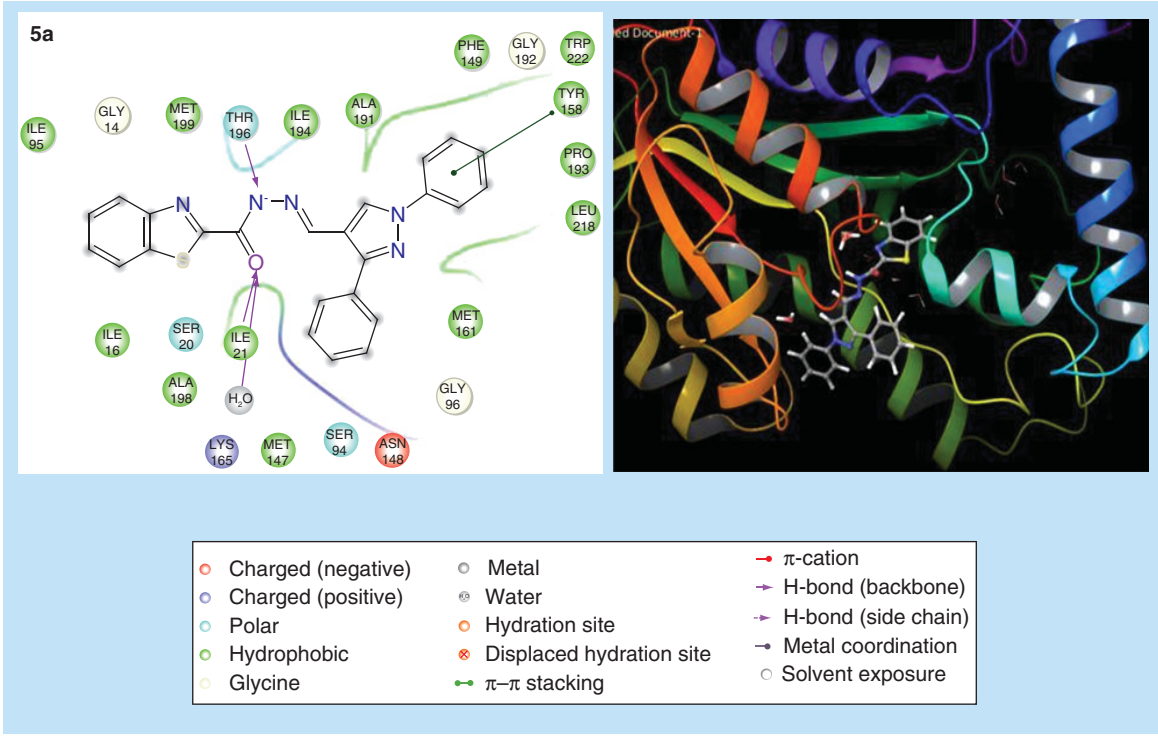

Figure 6. 2D and 3D representations of 5a in molecular docking studies with InhA proteins.

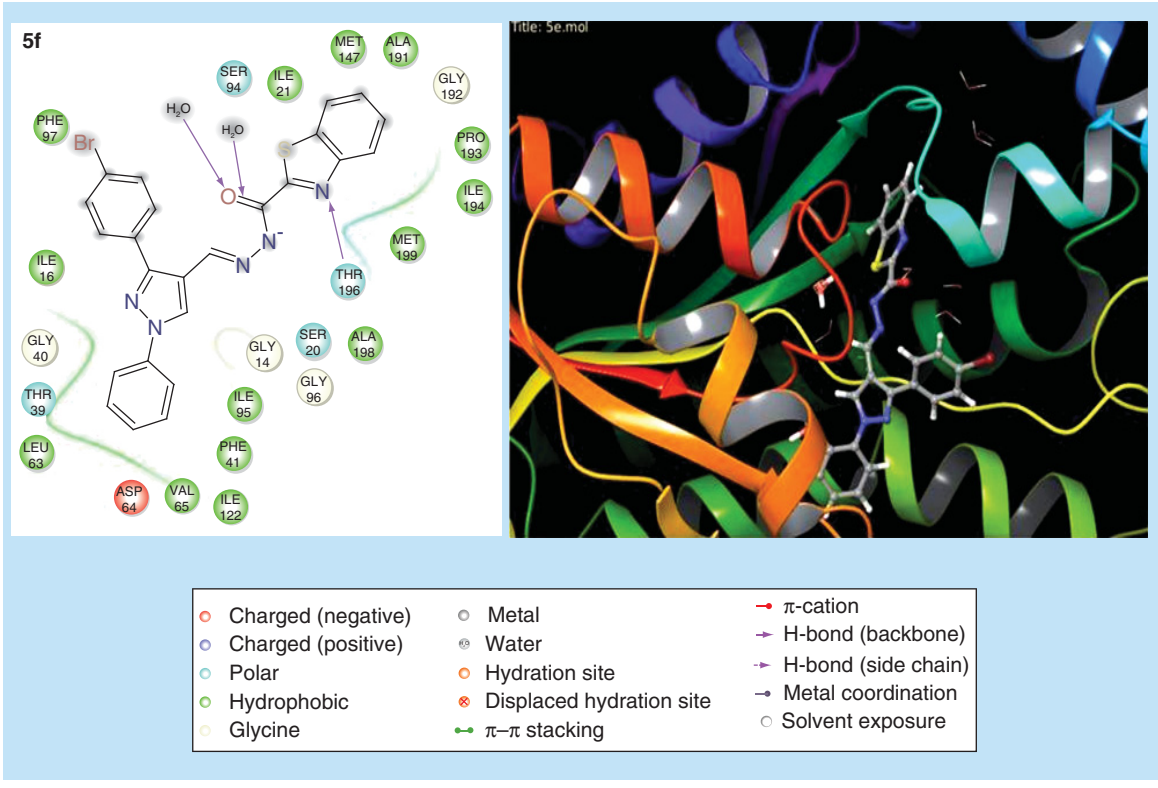

Figure 7. 2D and 3D representations of 5f in molecular docking studies with Inha proteins.

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Summary points

- Two novel series of the pyrazole-conjugated benzothiazole derivatives were synthesized by the Vilsmeier–Haack reaction, followed by the imine formation.
- Synthesized compounds were characterized by the FT-IR, LCMS, NMR, NOSEY spectral techniques.
- The newly synthesized compounds were screened for the antibacterial, antioxidant and antituberculosis (anti-TB) activities.
- Majority of the compounds exhibited excellent *in vitro* anti-TB activity and showed the minimum inhibitory concentration values up to 1.6 μg/ml.
- Level of toxicity of most active compounds was determined to HEK-293.
- The *in silico* molecular docking studies were carried out and co-related with the observed anti-TB activity.
- Some candidates would represent dual anti-TB and antimicrobial activity.

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