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Design, Synthesis and Biological Evaluation of Pyridyltriazole Derivatives as Potent Antitubercular Agents

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In current investigation, a new series of pyridyltriazole derivatives were synthesized by conventional methods and characterized through spectroscopic techniques such as IR, NMR (^{1}H) and mass spectroscopy. Based on colour observation and percentage of inhibition, all 24 pyridyltriazole derivatives were subjected to *in vitro* anti-mycobacterial testing using MABA techniques against the *Mycobacterium tuberculosis* (H37Rv) strain. It was found that all of the synthesized compounds were efficient in suppressing the *M. tuberculosis* H37Rv strain at concentrations of 1, 5 and 10 µg/mL. Among all the synthesized compounds, only **2**, **3**, **6**, **7**, **8**, **11**, **14**, **15**, **18**, **20**, **21**, **22**, **23**, **24** has good anti-TB efficacy in contrast to the standard medication. According to the *in silico* ADME prediction, every synthesized molecule has drug-like qualities and is appropriate for oral bioavailability. Furthermore, research utilizing molecular docking have been conducted to get mechanistic understanding and molecular interactions in opposition to the mycobacterial InhA enzyme. Utilizing a molecular docking analysis, hits against specific molecular targets were found. This *in silico* study delved into the molecular interactions between potential compounds and the Mtbenoyl-reductaseInhA (PDB 5JFO).

Keywords: *In silico*, Molecular docking, Pyridyltriazole, *Mycobacterium tuberculosis*, Tuberculosis, Mtbenoyl-reductaseInhA.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is one of the most deadly and ancient infectious illnesses [1]. Tuberculosis, a persistent global human health problem, with considerable morbidity and mortality rates globally [2]. According to the World Health Organization's 2022 global tuberculosis report, 10.6 million new tuberculosis cases are predicted to result in about 1.3 million fatalities worldwide by 2022 [3]. The burden of tuberculosis in India remains a substantial public health problem, highlighting its global prevalence and the worrisome incidence of drug-resistant infections inside the country [4]. India is one of the top eight countries, accounting for more than two-thirds of all tuberculosis cases [5]. Furthermore, one in every four cases of multidrug-resistant tuberculosis (MDR-TB) in the world is reported in India. In 2022, India registered an expected 119,000 MDR-TB cases [6,7]. However, these figures may be significantly underestimated due to testing limitations and the fact that only 76% of newly diagnosed TB cases and 73% of patients who have previously received treatment have been tested for rifampicin resistance [6]. In India, the number of patients who received further therapy for MDR-TB and extensively drug-resistant (XDR)-TB was very low, at 4 per 100,000 and 1 per 100,000 in 2021, respectively [8-10]. Alarmingly, the total success rates of tuberculosis treatment in India were only 57% in 2019. These worrying numbers from India highlight the critical need for improved diagnostics, proper treatment and expanded measures to prevent drug-resistant tuberculosis [11].

Enoyl-reductase InhA is a crucial enzyme required for Mtb survival [12]. InhA regulates the formation of mycolic acid, which is an important component of the mycobacterial cell wall [13]. InhA catalyzes the final step of fatty acid elongation and eliminates double bonds in fatty acids to produce mycolic acid and maintain bacterial cell wall integrity [14]. Isoniazid, a first-line anti-TB medicine, inhibits its activity and prevents the generation of mycolic acid [15]. The emergence of drug-resistant strains has highlighted the need of

understanding the complicated processes controlling InhA and its role in drug resistance [16]. The current investigation used *in silico* docking to uncover potential hits against Mtbenoyl-reductase InhA [17]. A molecular docking study can be used to evaluate the drugs' binding orientation and affinity for their respective targets [18]. The selection of PDB 5JFO for molecular docking in the study of pyridyltriazole derivatives was a deliberate choice, shaped by several structural attributes and its relevance to the biology of *Mycobacterium tuberculosis* (Mtb) and therapeutic advancement.

The key interactions between pyridyltriazole derivatives and the InhA enzyme's active site can occur. Pyridyltriazole derivatives have the ability to bind to the active site of InhA and hinder its function by competing or mimicking substrates (or inhibitors like isoniazid). Targeting InhA with pyridyl-triazole derivatives might be a sensible strategy for tuberculosis medication development, considering its crucial role in Mtb's fatty acid production pathway. The development of pyridyl-triazole derivatives for tuberculosis treatment would rely on a rational drug design approach that addresses the specific challenges posed by *M. tuberculosis*, a causative agent of tuberculosis [19,20].

Triazoles are extremely stable heterocyclic compounds and due to their bioisosteric qualities, triazole rings have been utilized in antimicrobial activities [22-24]. In medicinal chemistry, pyridine and triazole rings are recognized scaffolds because of their capacity to interact molecularly with biological targets in certain ways. The pyridine ring is well-known for its basicity, capacity to form hydrogen bonds with biological substrates and nitrogen coordination. It can enhance a drug's pharmacokinetic qualities, such as its solubility and membrane permeability, which makes it appropriate for treating tuberculosis (TB).

By hybridizing these rings to form pyridyltriazole derivatives, substances that may bind to Mtb's molecular targets efficiently may be produced, which may disrupt important enzymes or pathways that are essential to the bacterium's survival and growth. Derivatives of pyridyltriazoles may inhibit these enzymes [21-27]. Derivatives of pyridyltriazole may be designed to specifically target enzymes or biochemical processes essential to the survival of Mtb's. Targeting important enzymes in the fatty acid production pathway, such as InhA, may help in preventing the development of this barrier of defense.

EXPERIMENTAL

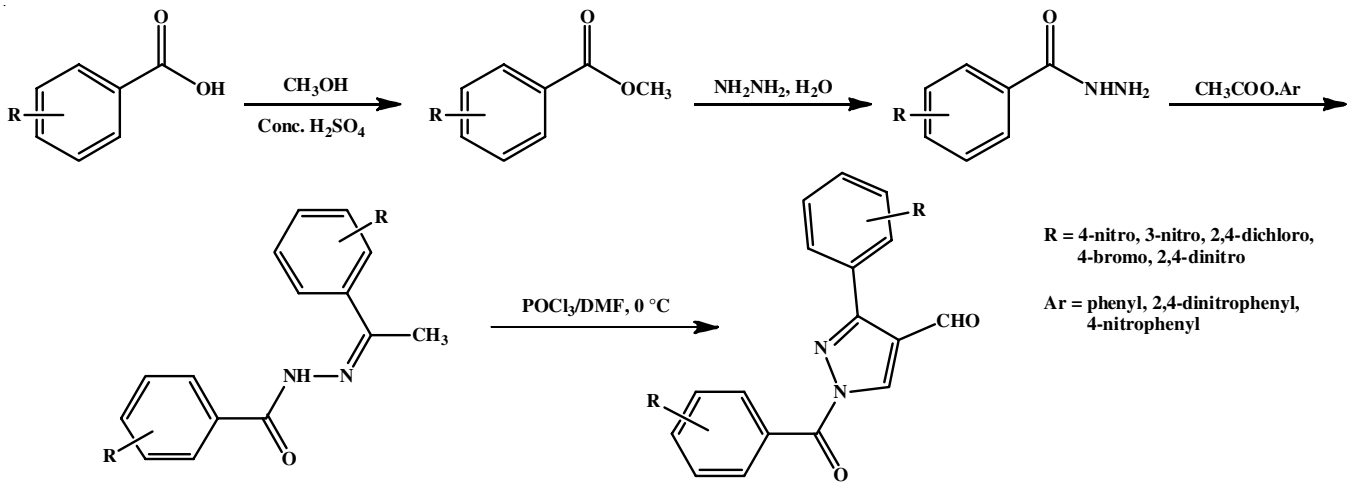
The synthetic grade chemicals and solvents *viz*. 4-methyl pyridine, hydrazide hydrate, aromatic aldehydes, potassium hydroxide, sodium hydroxide, ethanol, methanol, acetic acid, anhydrous ether, carbon disulfide, dimethyl sulfoxide, hydrogen chloride, chloroform, petroleum ether, silica gel-G were purchased from Lobachemie Pvt. Ltd. Using an open capillary technique, the melting points of the synthesized compounds were measured and are uncorrected. The ALPHA II FTIR spectrometer (Bruker) was used to record the IR spectra. Bruker FT-NMR spectrometer ^{1}H NMR spectra were acquired at 300 MHz using TMS as an internal standard. ESI employed Jeol SX-102 (FAB) mass spectrometer to record mass spectra.

Synthesis of substituted aromatic aldehydes (Scheme-I)

a) Synthesis of esters of substituted benzoic acid: Both esters were prepared using Fischer's esterification procedure, which entails refluxing substituted aromatic acids for 30 min with methanol while employing conc. H_2SO_4 as catalyst (colour: yellow, yield: 54%, m.p.: 130-131 ºC, R_f: 0.86).

b) Synthesis of substituted benzhydarzide and 4-bromobenzhydrazide from methyl benzoate and 4-bromomethyl benzoate: Both esters were treated to hydrazinolysis with hydrazine hydrate in alcohol. After refluxing the solution for 2 h, the solid that resulted was the same amount of hydrazide (colour: white, yield: 46%, m.p.: 129-130 ºC, R_f: 0.79).

c) Synthesis of substituted acetophenonephenyl-1-carbonyl hydrazone: For 0.5 h, a solution of 0.01 mol benzhydrazide and 0.01 mol substituted acetophenone was refluxed in 30 mL of methanol with a drop of glacial acetic acid as a catalyst. The solid that separated at the end of the reflux process was the equivalent hydrazone (colour: faint yellow, yield: 62%, m.p.: 128-129 ºC, R_f: 0.75).



Scheme-I: Reaction scheme of the substituted aromatic aldehydes

d) Synthesis of substituted acetophenonephenyl-1-carbonyl hydrazone from benzhydrazide: For 0.5 h, a solution containing 4-bromo benzhydrazide (0.01 mol) and 4-methoxy acetophenone (0.01 mol) was refluxed in 30 mL of methanol with a drop of glacial acetic acid acted as a catalyst. After the reflux process, the solid was filtered and washed thoroughly (colour: dark brown, yield: 53%, m.p.: 145-146 ºC, R_f: 0.89).

e) Synthesis of 1-(substituted phenyl-4-formyl pyrazole-1-carbonyl)substituted benzene: 4-Methoxy acetophenone phenyl-1-carbonyl hydrazine (3.048 g, 0.012 mol) was added in small aliquots to the Vilsmeier-Haack reagent prepared from 30 mL of DMF and 3.3 mL (0.036 mol) $POCl_3$ and the reaction mixture was refluxed over a boiling water bath for 10 h. The reaction mixture was then poured into ice cold water, the solid separated on neutralization with sodium acetate trihydrate, was filtered, washed with water and recrystallized with chloroform (colour: brown, yield: 46%, m.p.: 132-133 ºC, R_f: 0.84).

Synthesis of isonicotinic acid: In a double necked RBF containing 1 L of distilled water and 4-methylpyridine was heated on water bath for 3-4 h reflux at 70 ºC. Make 10 equal portions of $KMnO_4$. Added first five portions of $KMnO_4$ in RBF at 70 ºC followed by the addition of next five portions of $KMnO_4$ in RBF by raising the temperature at 85-90 ºC of water bath. Decolourization starts occurring then wash it with 20-25 mL water. After complete decolourization, increase the temperature of water bath to 95 ºC and then further hot reaction mixture filter out with suction pump and cake formation takes place. Then 200 mL hot water was poured in four portion on cake by filtering out without using suction pump combined the filtrate portion and evaporate it. Washed again with about 1500 mL water. Further adjust the pH at 3.6 by adding conc. HCl which next precipitate formation takes place and further recrystallized with hot water (colour: white, yield: 70%, m.p.: 309-310 ºC, R_f: 0.63).

Synthesis of isonicotinic acid hydrazide: After refluxing 2.4 g of isonicotinic acid and 1 g of hydrazide hydrate for 7-8 h in the presence of NaOH solution, the mixture was filtered, the filtrate was evaporated and the crude product of 6-amino-nicotinic acid hydrazide was obtained (colour: white, yield: 56%, m.p.: 171-172 ºC, R_f: 0.46).

Synthesis of dithiocarbazinate salt: Isonicotinic acid hydrazide (0.10 mol) was added gradually to an ethanolic KOH solution (0.15 mol). The reaction mixture was diluted with 50 mL of absolute ethanol and constantly swirled on a magnetic stirrer at room temperature for 16 h. After filteration, the precipitated potassium dithiocarbazinate salt was washed with anhydrous ether and dried (colour: reddish brown, yield: 62%, m.p.: 250-251 ºC, R_f: 0.70).

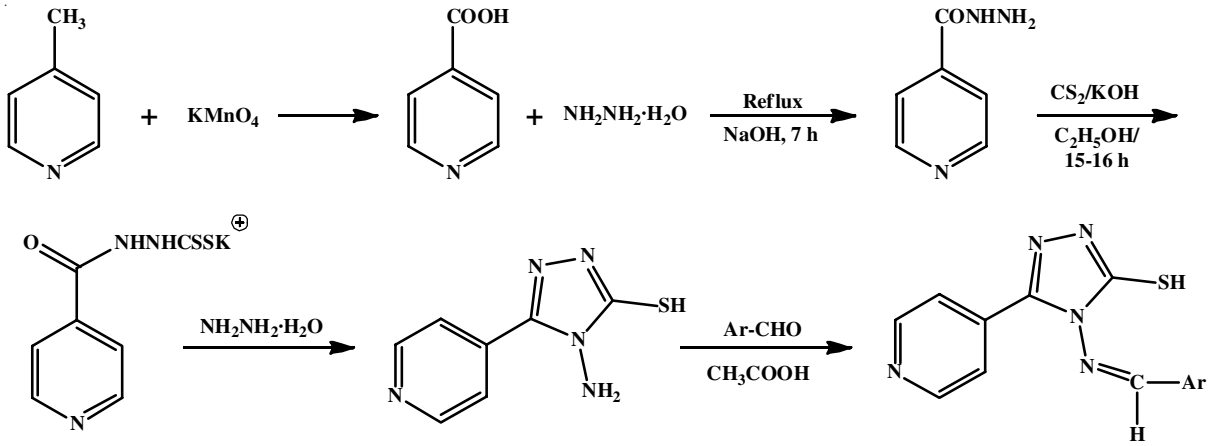
Synthesis of 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol: Hydrazine hydrate (10.3 g, 0.1 M), potassium dithiocarbazinate salt and 100 mL water were mixed in RBF. Reflux it for 8 h on water bath until H_2S gas evolved and the colour of reaction changed to deep green. Further it cooled down and acidified with HCl upto pH 1. The precipitate was filtered, recrystallized with ethanol and dried (colour: white, yield: 48%, m.p.: 268-269 ºC, R_f: 0.61).

Synthesis of pyridyltriazole derivatives (1-24): Pyridyltriazole (0.01 M) and different aldehydes (0.01 M) were mixed with ethanol (25 mL) and HCl (0.5 mL) in RBF and refluxed for 2 h. The solution was then evaporated and recrystallized from ethanol (**Scheme-II**).

(*E*)-4-((3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-methylene amino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole (1): Yield: 71.84%, m.p.: 231-233 ºC; m.f.: $C_{23}H_{17}N_8O_2S$; m.w.: 469.5; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): 3081.11 (-CH *str.*), 2582.81 (S-H *str.*), 606.25 (HC=N *str.*), 1525.07, 1317.31 (N-O), 1501.23 (C=C *str.*), 1221.48 (C-N *str.*), 1095.98 (N-N *str.*), 689.09 (C-S *str.*),

(*E*)-4-(4-Chlorobenzylideneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (2): Yield: 64.93%, m.p.: 205-207 ºC; m.f.: $C_{14}H_{10}ClN_5S$; m.w.: 315.78; TLC (R_f value): 0.67; IR (KBr, ν_{max}, cm^{-1}): 3163.14 (C-H *str.*), 2585.42 (S-H *str.*), 1588.49 (HC=N *str.*), 1501.23 (C=C *str.*), 1217.16 (C-N *str.*), 1093.73 (N-N *str.*), 698.71 (C-S *str.*), 671.98 (C-Cl *str.*); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.75 (s, 1H, N=CH), 6.91-8.06 (m, 8H, aromatic C-H),3.58 (s, 1H, SH), Mass (*m/z*): 317 (M+2), 315 (M^+) for $C_{14}H_{10}ClN_5S$.

(*E*)-4-((3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-methyleneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole (3): Yield: 71.84%, m.p.: 231-233 ºC; m.f.: $C_{23}H_{17}N_8O_2S$; m.w.: 469.5; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): 2999.81 (C-H



Scheme-II: Reaction scheme of pyridyltriazole derivatized compounds (**1-24**) [the details of Ar is given in Table-1]

str.), 2580.84 (S-H *str.*), 1663.53 (HC=N *str.*), 1572.17, 1315.31 (N-O), 1469.50 (C=C *str.*), 1255.54 (C-N *str.*), 936.70 (N-N *str.*), 664.18 (C-S *str.*).

((*E*)-4-(4-(Dimethylamino)benzylideneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole (4): Yield: 65.04%, m.p.: 212-213 ºC; m.f.: $C_{16}H_{16}N_6S$; m.w.: 324.4; TLC (R_f value): 0.65; IR (KBr, ν_{max}, cm^{-1}): 3233.34 (N-CH_3 *str.*), 3140.91 (C-H *str.*), 2815.39 (S-H *str.*), 1666.01 (HC=N *str.*), 1538.39 (C=C *str.*), 1317.81 (C-N *str.*), 1167.94 (N-N *str.*), 679.65 (C-S *str.*),

(*E*)-4-((3-(2,4-Dichlorophenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (5): Yield: 54.04%, m.p.: 170-171 ºC; m.f.: $C_{23}H_{15}Cl_2N_9O_4S$; m.w.: 584.39; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): 3156.97 (C-H *str.*), 2585.42 (S-H *str.*), 1606.19 (HC=N *str.*),1530.60 (C=C *str.*), N-O (1540.60, 1328.70), 1318.70 (C-N *str.*), 1174.66 (N-N *str.*), 723.74 (C-Cl *str.*), 689.71 (C-S *str.*),

(*E*)-4-(3,4-Dimethoxybenzylideneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (6): Yield: 67.25%, m.p.: 162-163 ºC; m.f.: $C_{16}H_{15}N_5O_2S$; m.w.: 341.39; TLC (R_f value): 0.65; IR (KBr, ν_{max}, cm^{-1}): 3157.16 (C-H *str.*), 2885.83 (O-CH_3 *str.*), 2587.31 (S-H *str.*), 1607.09 (HC=N *str.*), 1528.21 (C=C *str.*), 1319.24 (C-N *str.*), 1225.91 (C-O *str.*), 1089.43 (N-N *str.*), 687.39 (C-S *str.*); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.99 (s, 1H, N=CH), 7.07-8.28 (m, 7H, aromatic C-H), 4.32 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.25 (s, 1H, SH); Mass (*m/z*): 341 (M+) for $C_{16}H_{15}N_5O_2S$.

(E)-4-((3-Mercapto-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)phenol (7): Yield: 72.28%, m.p.: 186-187 ºC; m.f.: $C_{14}H_{11}N_5OS$; m.w.: 297.34; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3085.53), C=C stretching (1456.35), C-N stretching (1312.19), C-S stretch (675.53), S-H stretching (2582.43), HC=N stretching (1593.09), N-N stretching (1116.72), O-H stretching (3585.32), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.78 (s, 1H, N=CH), 6.76-7.90 (m, 8H, aromatic C-H), 5.21 (s, 1H, OH), 3.43 (s, 1H, SH); Mass (*m/z*): 297 (M^+) for $C_{14}H_{11}N_5OS$.

(E)-4-(4-Nitrobenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (8): Yield: 61%, m.p.: 152-153 ºC; m.f.: $C_{14}H_{11}N_6O_2S$; m.w.: 327.34; TLC (R_f value): 0.67; IR (KBr, ν_{max}, cm^{-1}): N-O (1518.58, 1337.16), C-H stretching (3109.13), C=C stretching (1518.58), C-N stretching (1337.16), C-S stretch (666.15), S-H stretching (2560.31), HC=N stretching (1649.46), N-H stretching (3310.09), N-N stretching (1104.39).

(E)-4-(3-Chlorobenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (9): Yield: 315.78%, m.p.: 232-233 ºC; m.f.: $C_{14}H_{10}ClN_5S$; m.w.: 315.78; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3156.99), C=C stretching (1468.25), C-N stretching (1221.75), C-S stretch (681.73), S-H stretching (2556.23), HC=N stretching (1566.68), N-N stretching (1085.73), N=N stretching (1595.47), C-Cl stretching (732.53), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 9.01 (s, 1H, N=CH), 7.10-8.38 (m, 8H, aromatic C-H), 2.96 (s, 1H, SH), Mass (*m/z*): 317 (M^{+2}), 315 (M+) for $C_{14}H_{10}ClN_5S$.

(E)-3-((3-Mercapto-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)phenol (10): Yield: 63%, m.p.: 210-211 ºC; m.f.: $C_{14}H_{11}N_5OS$; m.w.: 297.34; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3033.43), C=C stretching (1561.44), C-N stretching (1226.50), C-S stretch (681.11), S-H stretching (2565.61), HC=N stretching (1615.86), N-N stretching (1011.91), N=N stretching (1675.84), O-H stretching (3613.26)

(E)-4-(3-Nitrobenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (11): Yield: 58%, m.p.: 190-191 ºC; m.f.: $C_{14}H_{11}N_6O_2S$; m.w.: 327.34; TLC (R_f value): 0.65; IR (KBr, ν_{max}, cm^{-1}): N=O stretching (1531.13), C-H stretching (3156.42), C=C stretching (1450.53), C-N stretching (1221.18), C-S stretch (688.37), S-H stretching (2570.63), HC=N stretching (1607.13), N-N stretching (1088.05).

(E)-4-(3,4,5-Trimethoxybenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (12): Yield: 66%, m.p.: 218-219 ºC; m.f.: $C_{17}H_{17}N_5O_3S$; m.w.: 371.41; TLC (R_f value): 0.64; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3151.15), C=C stretching (1528.14), C-N stretching (1228.80), C-S stretch (690.79), S-H stretching (2588.01), HC=N stretching (1606.01), N-N stretching (1003.95), C-O stretch(1119.94),O-CH_3 bending (2885.23).

((E)-N-(4-Bromophenyl)-N'-(3-mercapto-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-yl)acetamidine (13): Yield: 69%, m.p.: 226-227 ºC; m.f.: $C_{15}H_{13}BrN_6S$; m.w.: 389.27; TLC (R_f value): 0.0.63; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3000.15), C=C stretching (1570.50), C-N stretching (1219.96), C-S stretch (672.90), S-H stretching (2521.15), HC=N stretching (1607.50), N-N stretching (1083.94), C-Brstretching (672.90).

(E)-4-(2-Methoxybenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (14): Yield: 58%, m.p.: 206-207 ºC; m.f.: $C_{15}H_{13}N_5OS$; m.w.: 311.36; TLC (R_f value): 0.62; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3083.25), C=C stretching (1467.91), C-N stretching (1314.66), C-S stretch (690.97), S-H stretching (2491.13), HC=N stretching (1603.83), N-N stretching (1006.66), C-O stretch (1231.68), O-CH_3 bending (2841.33), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.62 (s, 1H, N=CH), 7.18-8.26 (m, 8H, aromatic C-H), 3.90 (s, 3H, OCH_3), 3.37 (s, 1H, SH), Mass (*m/z*): 311 (M^+) for $C_{15}H_{13}N_5OS$.

(E)-2-((3-Mercapto-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)phenol (15): Yield: 72%, m.p.: 210-211 ºC; m.f.: $C_{14}H_{11}N_5OS$; m.w.: 297.34; TLC (R_f value): 0.65; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3158.66), C=C stretching (1519.32), C-N stretching (1317.24), C-S stretch (680.70), S-H stretching (2613.61), HC=N stretching (1597.68), N-N stretching (1095.44), O-H stretching (3256.26).

(E)-4-(*p*-Methylbenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (16): Yield: 67%, m.p.: 236-237 ºC; m.f.: $C_{15}H_{13}N_5S$; m.w.: 295.36; TLC (R_f value): 0.61; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3080.60), C=C stretching (1557.08), C-N stretching (1312.78), C-S stretch (690.73), S-H stretching (2496.32), HC=N stretching (1603.20), N-N stretching (1082.48), C-H stretching (methyl group) (2856.41).

(E)-4-(2-Nitrobenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (17): Yield: 57%, m.p.: 212-213 ºC; m.f.: $C_{14}H_{11}N_6O_2S$; m.w.: 327.34; TLC (R_f value): 0.63; IR (KBr, ν_{max}, cm^{-1}): N=O stretching (1531.13), C-H stretching (3002.06), C=C stretching (1464.92), C-N stretching

(1313.90), C-S stretch (672.66), S-H stretching (2554.90), HC=N stretching (1584.73), N-N stretching (1155.01).

(E)-4-(4-Ethylbenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (18): Yield: 58%, m.p.: 180-181 ºC; m.f.: $C_{16}H_{15}N_5S$; m.w.: 309.39; TLC (R_f value): 0.58; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3156.89), C=C stretching (1446.51), C-N stretching (1315.70), C-S stretch (694.79), S-H stretching (2587.00), HC=N stretching (1604.46), N-N stretching (1095.14), C-H stretching (methyl group) (2878.61), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.68 (s, 1H, N=CH), 7.27-8.51 (m, 8H, aromatic C-H), 3.34 (s, 1H, SH), 2.72-2.99 (q, 2H, CH_2), 1.45-1.60 (t, 3H, CH_3), Mass (*m/z*): 309 (M+) for C16H15N5S.

(E)-5-((3-Mercapto-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)-2-methoxyphenol (19): Yield: 59%, m.p.: 220-221 ºC; m.f.: $C_{15}H_{13}N_5O_2S$; m.w.: 327.36; TLC (R_f value): 0.67; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3125.37), C=C stretching (1465.35), C-N stretching (1318.96), C-S stretch (673.74), S-H stretching (2502.21), HC=N stretching (1657.27), N-N stretching (1022.09), C-H stretching (methyl group) (2884.92) .

(E)-4-((3-(4-Bromophenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (20): Yield: 61%, m.p.: 214-215 ºC; m.f.: $C_{23}H_{16}BrN_9O_4S$; m.w.: 594.4; TLC (R_f value): 0.62; IR (KBr, ν_{max}, cm^{-1}): N=O stretching (1526.42, 1316.98), C-H stretching (3145.25), C=C stretching (1450.42), C-N stretching (1227.82), C-S stretch (691.32), S-H stretching (2579.13), HC=N stretching (1605.27), N-N stretching (1089.99), C-Br stretching (751.21).

(E)-4-((1-(2,4-Dinitrophenyl)-3-(4-nitrophenyl)-1*H*-pyrazol-4-yl)methyleneamino)-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (21): Yield: 67%, m.p.: 243-244 ºC; m.f.: $C_{23}H_{17}N_{10}O_6S$; m.w.: 561.51; TLC (R_f value): 0.66; IR (KBr, ν_{max}, cm^{-1}): N=O stretching (1590.42, 1295.37), C-H stretching (3053.62), C=C stretching (1466.32), C-N stretching (1312.23), C-S stretch (663.82), S-H stretching (2522.19), HC=N stretching (1647.11), N-N stretching (1158.29), C-Br stretching (751.21), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.77 (s, 1H, N=CH), 7.20-8.48 (m, 12H, aromatic C-H), 2.93 (s, 1H, SH), Mass (*m/z*): 558(M^+) for $C_{23}H_{14}N_{10}O_6S$.

(E)-7-Hydroxy-8-((3-mercapto-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)-4-methyl-2*H*-chromen-2-one (22): Yield: 62%, m.p.: 188-189 ºC; m.f.: $C_{18}H_{13}N_5O_3S$; m.w.: 379.39; TLC (R_f value): 0.60; IR (KBr, ν_{max}, cm^{-1}): C=O stretching (1771.27), C-H stretching (3152.97), C=C stretching (1452.33), C-N stretching (1317.99), C-S stretch (715.1), S-H stretching (2535.39), HC=N stretching (1555.47), N-N stretching (1018.65), O-H stretching (3418.08), C-O stretching (1771.27), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.59 (s, 1H, N=CH), 7.28-8.49 (m, 6H, aromatic C-H), 6.21 (s, 1H, C3-H of chromen), 5.17 (s, 1H, OH), 3.25 (s, 1H, SH), 2.12 (s, 3H, CH_3), Mass (*m/z*): 379 (M^+) for $C_{15}H_{13}N_5OS$.

(E)-4-(4-Methoxybenzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (23): Yield: 63%, m.p.: 258-259 ºC; m.f.: $C_{15}H_{13}N_5OS$; m.w.: 311.36; TLC (R_f value): 0.65; IR (KBr, ν_{max}, cm^{-1}): C-H stretching (3073.65), C=C stretching (1477.27), C-N stretching (1226.80), C-S stretch (697.34), S-H stretching (2635.73), HC=N stretching (1594.23), N-N stretching (1226.80), C-O stretch (methoxy) (1075.47), ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.71 (s, 1H, N=CH), 7.23-8.40 (m, 8H, aromatic C-H), 3.70 (s, 3H, OCH_3), 3.29 (s, 1H, SH), Mass (*m/z*): 311 (M^+) for $C_{15}H_{13}N_5OS$.

(*E*)-4-(Benzylideneamino)-5-(pyridin-3-yl)-4*H*-1,2,4-triazole-3-thiol (24): Yield: 59%, m.p.: 231-232 ºC; m.f.: $C_{14}H_{11}N_5S$; m.w.: 281.34; TLC (R_f value): 0.68; IR (KBr, ν_{max}, cm^{-1}): 3012.02 (C-H *str.*), 2635.82 (S-H *str.*), 1565.72 (HC=N *str.*), 1467.01 (C=C *str.*), 1275.62 (N-N *str.*), 1222.07 (C-N *str.*), 617.18 (C-S *str.*).

In silico docking study

Ligand preparation: Using ACD/Chemdraw software, the chemical structures and SMILES of the designed compounds were created [19]. In order to rectify the tautomeric and ionization states, produced structures were protonated using BIOVIA Discovery Studio [20]. The Avogadro program was utilized to minimize energy in the created chemical structures [21]. The force field MMFF94 with the steepest descent algorithm was applied to the developed compounds in order to minimize their energy [22].

Protein preparation: The RCSB Protein Data Bank [23] provided the previously published crystal form structure of Mtbenoyl-reductaseInhA (PDB 5JFO), which has a resolution of 2.91 Å [26]. All of the hetero atoms and water molecules were eliminated from the downloaded protein crystal structure in order to improve it for docking study [27]. To protonate the residues of amino acids in a pristine protein crystal structure, polar hydrogen atoms were added [26]. The protein structure enhancement protocol was performed using BIOVIA Discovery Studio.

Molecular docking: Virtually designed compounds were subjected to docking study against (PDB 5JFO). Docking protocol was executed using the PyRx 0.8 program [26]. The AutoDockVina wizard unit of PyRx 0.8 was used to import and choose prepared protein and ligand structures [27]. The blind docking protocol was used to explore the binding ability of docked compounds on entire protein surface [28,29]. Grid box was focused at center coordinates as X: -38.776, Y: -29.025, Z: 25.0202 and the dimension of grid was selected as X: 93.7654, Y: 92.0372, Z: 73.3332 coordinates. By default, the exhaustiveness was set to 8 [30,31]. Each compound's docked pose with the highest negative binding affinity was stored in pdb format and BIOVIA Discovery Studio was used to examine other binding interactions.

In vitro anti TB activity

Preparation of inoculum for test organism: The H37Rv strain of Mycobacterium TB (purchased from Pune, India) was chosen and cultured for 18 days at 37 ºC on Lowenstein Jensen medium (LJ). The bacterial solution was then homogenized and the turbidity was adjusted to match the McFarland no. 1 scales (3.2×10^6 cfu/mL) in the tube using a vortex shakeup. A 1:20 dilution of the bacterial solution was used to create the inoculum and from that 100 µL suspension was inoculate in each microwell of the plate.

Anti-mycobacterial activity: The anti-mycobacterial activity using resazurin as an indicator, a susceptibility test were conducted in 96 microplates for all chemicals (**1-24**) in the activity. The growth inhibition was recorded. Isoniazid was used as standard drug, dissolved in DMSO. Middle Brook 7H9 broth and the test inoculum were added to 100 µL of each testing well as well as the drug-free control wells. Following that, 100 mL of the synthesized compounds were added to the first well of each row and the micro plate column was used to create a two-fold dilution series from those compounds. Each micro dish was parafilm-coated and then incubated at 37 ºC for 7 days. After the incubation period, 25 µL of resazurin 0.02% w/v was added to each well and the mixture was then incubated again for 24 h at 37 ºC to develop colour. The minimal drug concentration that prevented the resazurin reagent from becoming pink instead of blue was known as the visual minimum inhibitory concentration. The minimum inhibitory concentration (MIC) is defined as the concentration at which the resazurin reagent did not become pink instead of blue [32].

RESULTS AND DISCUSSION

All the synthesized pyridyltriazole derivatives (**1-24**) were verified using spectroscopic (IR, ^{1}H NMR and mass spectrometry) and chromatographic techniques. To obtain the substituted aromatic aldehydes, the synthesis approach is depicted in **Scheme-I**. **Scheme-II** illustrates the synthesis approach used to produce the desired pyridyltriazole derivatives. IR spectra supporting the structures assigned to the aromatic substituted pyrazole aldehydes **1**, **2**, **3**, **4** and **5** showed absorption bands between 1600-1400 cm^{-1} (C-C arom.), 3180-2960 cm^{-1} (C-H arom.), 2990-2850 cm^{-1} (C-H *str.* arom.), 1740-1720 cm^{-1} (C=O *str.*), 3500-3300 cm^{-1} (N-H *str.*) and 1600-1200 cm^{-1} (C-N *str.*). The structures attributed to pyridyltriazole derivatives **1-24** were corroborated by infrared spectra that revealed absorption bands between 1360 and 1290 cm^{-1} (asymmetrical N-O *str.*), 1300 and 1360 cm^{-1} (symmetrical N-O *str.*) and 2550 and 2600 cm^{-1} (S-H *str.*). aromatic C=C *str.* band between 1450 and 1600 cm^{-1}, aromatic C-N *str.* band between 1200 and 1360 cm^{-1} (arom. C-N *str.*, N-N *str.*), aromatic C=C *str.* band between 1591 and 1669 cm^{-1} and 600 to 700 cm^{-1} (C-S *str.*). The ^{1}H NMR reveals a singlet at δ 8.75 (s, 1H, N=CH) ppm because of the hydrogen atom (H) that is directly bound to the nitrogen in the imine; the presence of 3H; a multiplet between 7.91 and 8.06 because of the triazole and aromatic hydrogen; and a singlet at δ 3.0-3.5 ppm because of the SH group. The remaining aromatic and aliphatic protons were all found to be within the anticipated ranges. The synthesized chemicals' structures were also validated by mass spectra.

Molecular docking studies: The molecular docking of the designed molecules *i.e.* pyridyltriazole derivatives were docked with the 5JFO protein of InHA enzyme (2-*trans*-enoyl-acyl carrier protein reductase). The binding affinity of the compounds is observed between the ranges of -10.2 to -7.1. Amongst all 24 molecules **1**, **3**, **5**, **11**, **16**, **21** & **22** was observed to be the most potent molecule with the docking score of -9.7, -9.4, -10.1, -8.0, -8.2, -10.2 and -9.3, respectively (Table-1).

The negative binding affinities reflected the thermodynamic favourability of binding interactions indicating a potentially stable complex formation between compound **21** and the targeted enzyme. Compound **21** demonstrated the highest negative

TABLE-1
BINDING AFFINITY OF PYRIDYLTRIAZOLE DERIVATIVE STRUCTURE AGAINST PDB 5JFO ALONG WITH INTERACTING RESIDUE AND TYPE OF INTERACTION

Comp. Code	Ar	Binding affinity	Interacting residues	Type of interaction	Distance
1	3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazole	-9.7	THR 196	Conventional hydrogen bond	3.03
			SER 94		3.16
			ALA 22		3.29
			ILE 21		3.01, 3.36
			SER 20	Carbon hydrogen bond	3.59
			ILE 95	pi-sigma	3.90
			PHE 41	pi-pi stacked	3.86
			ALA 198	pi-alkyl	4.05
			LEU 197		5.02
			Val 65		5.18
			ILE 122		4.82
2	Chlorobenzene	-7.6	PHE 97	pi-pi stacked	4.33
			GLY 14	pi-sigma	3.37
			PHE 41	pi-alkyl	5.29
			ILE122		5.24
			ILE 16		4.91
			ILE 95		4.96
3	3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazole	-9.4	SER 94	Conventional hydrogen bond	3.02
			THR 196		3.18
			ILE 85	pi-sigma	3.81
			PHE 41	pi-pi stacked	3.97
			ILE 122	pi-alkyl	4.69
			VAL 65		5.28
			ALA 198		4.47
			ILE 16		5.18

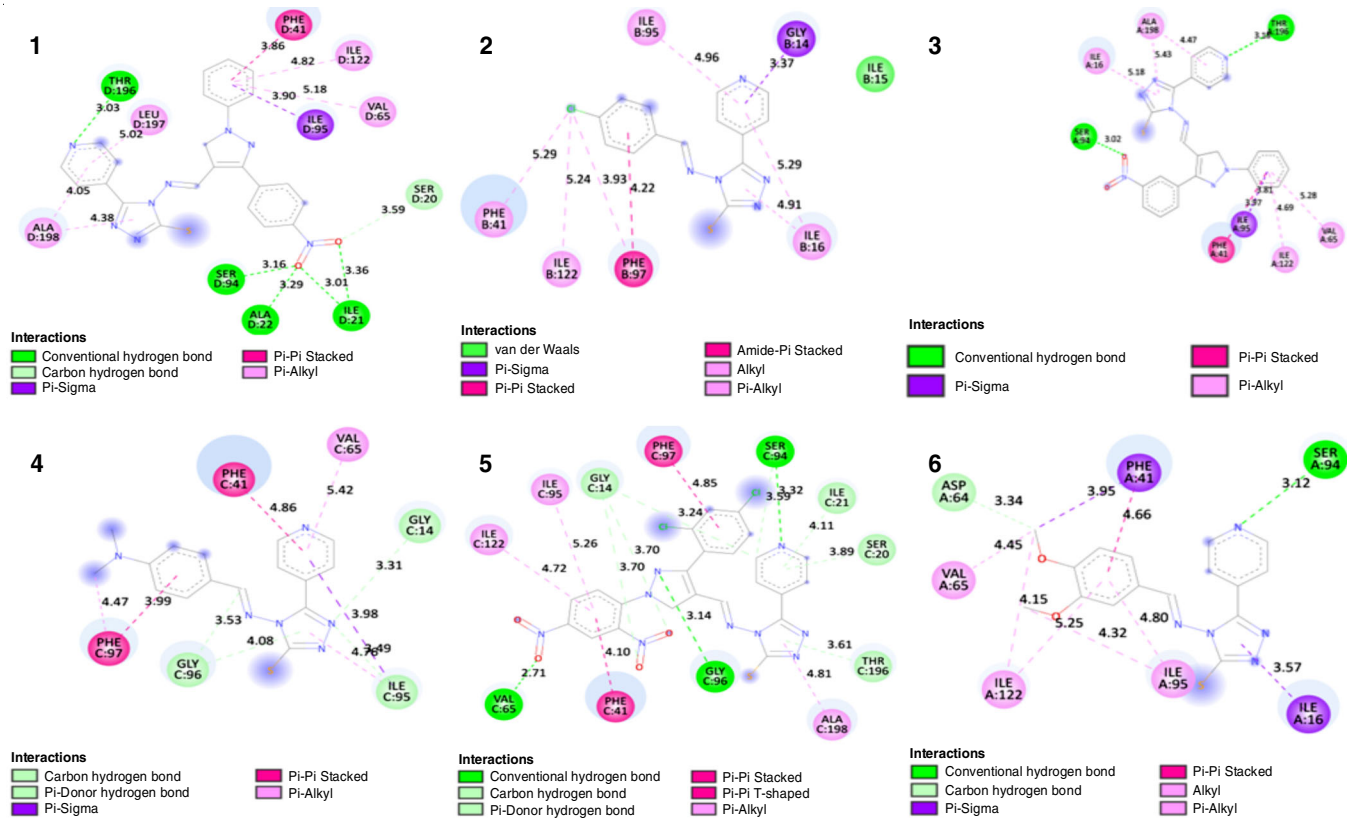
4	N,N,2-Trimethylbenzenamine	7.5	GLY 96	Carbon hydrogen bond	3.53
			GLY 14		3.31
			ILE 95	pi-sigma	3.98
			PHE 41	pi-pi stacked	4.86
			PHE 97		3.99
			VAL 65	pi-alkyl	5.42
5	3-(2,4-Dichlorophenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazole	-10.1	SER 94	Conventional hydrogen bond	3.32
			GLY 96		3.14
			VAL 65		2.71
			ILE 21	Carbon hydrogen bond	3.89
			SER 20		4. 11
			THR 196	pi donor hydrogen bond	3.61
			PHE 41	pi-pi stacked	4.10
			PHE 97		4.85
			ILE 95	pi-alkyl	5.26
			ILE 122		4.72
			ALA 198		4.81
6	3,4-Dimethoxybenzene	-7.1	SER 94	Conventional hydrogen bond	3.12
			ASP 64	Carbon hydrogen bond	3.34
			ILE 16	pi-sigma	3.57
			PHE 41	pi-pi stacked	4.66
			ILE 122	pi-alky	4.15, 5.25
			ILE 95		4.32, 4.80
			VAL 65		4.45
7	*p*-Hydroxybenzene	-7.9	LYS 118	Conventional hydrogen bond	3.07
			GLY 14	Carbon hydrogen bond	3.69
			ILE 95	pi-sigma	3.88
			PHE 97	pi-pi stacked	3.87
			PHE 41		4.38
			VAL 65	pi-alkyl	5.11
8	*p*-Nitrobenzene	-7.9	SER 94	Conventional hydrogen bond	3.18
			VAL 65		3.10, 3.31
			GLY 96	Carbon hydrogen bond	3.41
			ILE 95	pi-sigma	3.82
			PHE 41	pi-pi stacked	4.52
			ILE 122	pi-alkyl	5.35
9	*m*-Chlorobenzene	-7.7	GLY 14	Carbon hydrogen bond	3.37
			SER 94		3.38
			ILE 16	pi-sigma	3.91
			ILE 95		3.84
			PHE 41	pi sulfur	5.19
			ILE 16	pi-alkyl	3.91
			VAL95		3.84
10	*m*-Hydroxybenzene	-7.7	ASP 64	Conventional hydrogen bond	2.20
			ILE 95	pi-sigma	3.70
			PHE 41	pi-pi stacked	4.08
			ILE 122	pi-alkyl	4.89
			VAL 65		5.15
11	*m*-Nitrobenzene	-8.2	SER 94	Conventional hydrogen bond	3.32
			THR 39		3.03
			GLY 14		3.08
			SER 13	Carbon hydrogen bond	3.51
			ILE 95	pi-sigma	3.90
			ILE 16		3.55
			PHE 41	pi-pi stacked	4.73
12	3,4,5-Trimethoxybenzene	-7.1	LYS 118	Conventional hydrogen bond	3.89
			ILE 95	pi-sigma	3.68
			PHE 41	pi-pi stacked	5.35
			PHE 97		3.86
			VAL 65	pi-alkyl	5.09

13	4-Bromo-*N*-ethylbenzenamine	-7.2	SER 20	Conventional hydrogen bond	2.91
			THR 196		3.66
			ILE 16	pi-sigma	3.58
			PHE 97	pi-pi stacked	4.21
			GLY 14		4.33
			ILE 95	pi-alkyl	5.12
			ILE 122		5.08
			PHE 41		4.68
14	2-Methoxybenzene	-7.8	GLY 14	Carbon hydrogen bond	3.57
			ILE 95	pi-sigma	3.91
			PHE 41	pi-pi stacked	4.46
			PHE 97		3.86
			VAL 65	pi-alkyl	5.12
15	2-Hydroxybenzene	-7.5	GLY 96	Conventional hydrogen bond	2.29
			ILE 95	pi-sigma	3.99
			PHE 41	pi-pi stacked	3.79
			PHE 41	pi-pi t shaped	5.31
			ILE 16	pi-alkyl	4. 61, 4.61
			ILE 122		4.75
			VAL 65		5.13
16	Toluene	-8.0	ILE 95	Carbon hydrogen bond	4.74
			GLY 14		3.20
			GLY 96	pi- donor hydrogen bond	4.06
			PHE 97	pi-sigma	3.99
			PHE 41	pi-pi stacked	4.87
			VAL 65	pi-alkyl	5.48
17	2-Nitrobenzene	-8.0	GLY 14	Carbon hydrogen bond	3.69
			ILE 95	pi-sigma	3.74
			PHE 97	pi-pi stacked	3.78
			PHE 41		5.29
			ILE 16	pi-alkyl	5.19
			VAL 65		5.33
18	4-Ethylbenzene	-7.9	SER 94	Conventional hydrogen bond	3.18
			GLY 14	Carbon hydrogen bond	3.45
			ILE 16	pi-sigma	3.96
			ILE 95		3.95
			PHE 41	pi-pi stacked	4.60
			VAL 65	pi-alkyl	4.50
			ILE 122		3.87
19	2-Methoxyphenol	-7.6 -10	SER 94	Conventional hydrogen bond	2.96, 2.57
			GLY 96	Carbon hydrogen bond	3.69
			GLY 14	pi-sigma	3.83
			PHE 41	pi-pi stacked	5.56
			ILE 16	pi-alkyl	5.27
			ILE 21		4.10
			ILE 95		5.14
			MET 147		4.17
			SER 94	Conventional hydrogen bond	3.34
20	3-(4-Bromophenyl)-1-(2,4-dinitrophenyl)-1*H*-pyrazole		GLY 96		3.14
			VAL 65		2.73
			ILE 21	Carbon hydrogen bond	4.09
			SER 20		3.87
			THR 196	pi-donor hydrogen bond	3.59
			GLY 14		3.72
			PHE 97	pi-pi stacked	4.87
			PHE 41	pi-pi t shaped	4.12
			ILE 95	pi-alkyl	5.27
			ILE 122		4.71
			ALA 198		4.78
21	1-(2,4-Dinitrophenyl)-3-(4-nitrophenyl)-1*H*-pyrazole	-10.2	ILE 21	Conventional hydrogen bond	3.33
			ILE 20		2.91
			SER 94		3.24
			VAL 65		3.30
			ILE 95	pi-sigma	3.46
			PHE 97	pi-pi stacked	3.84
			PHE 41	pi-pi t shaped	4.95

22	7-Hydroxy-4-methyl-2*H*-chromen-2-one	-9.3	SER 94	Conventional hydrogen bond	3.05
			ILE 95	pi- sigma	3.71
			ILE 122		3.82
			PHE 41	pi-pi stacked	5.44, 4.21, 3.67
			ILE 16	pi-alkyl	4.58
			VAL 65		5.37
23	4-Methoxy-benzene	-7.9	LYS 118	Conventional hydrogen bond	3.06
			GLY 14	Carbon hydrogen bond	3.47
			ILE 95	pi- sigma	4.48
			PHE 41	pi-pi stacked	4.67
			PHE 97		3.84
			ILE 16	pi-alkyl	4.90
			VAL 65		5.43
24	Benzene	-7.9	ILE 95	pi-sigma	3.91
			PHE 41	pi-pi stacked	5.17
			PHE 97		3.87
			ILE 16	pi-alkyl	4.81

binding affinity with value of -10.2 kcal/mol. Compound **21** formed a conventional hydrogen bond with ILE21, SER 20, SER 94AND VAL 65 at a bond distance of 3.33, 2.91, 3.24, 3.30, 3.46 Å, respectively. Compound **21** established a π-σ bond with ILE 95 while this residue was also involved in forming diverse interactions including π-π stacked, π-donor hydrogen bond with compound **21**. Amino acid residues such as PHE 41 and PHE 97 participated in the formation of π-π stacked and π-donor hydrogen bond interactions with compound **21**. The 3D binding orientation of compound **21** with Mtbenoyl-reductaseInhA (PDB 5JFO) is depicted in Figs. 1 and 2. The diverse interactions described between compound **21** and the amino acid residues of InhA highlighted its possible potential as a strong inhibitor.

Additionally, compound **5** exhibited the second-highest negative binding affinity of -10.1 kcal/mol against Mtbenoyl-reductase InhA (PDB 5JFO). Binding profile compound **5** showed formation of a carbon-hydrogen interaction with ILE 21, SER 20, along with a π-stacked bond with the PHE 41, PHE 97 residue. Furthermore, a π-donor hydrogen bond binding interaction was observed between compound **5** and THR 197. Compound **5** engaged in π-alkyl interactions with multiple amino acid residues, namely ILE 95, ILE 122 and ALA 198. Formation of diverse range of interactions demonstrated by compound **5** with specific amino acid residues within the active site of InhA underscores its possibility of potential as a potent inhibitor. The formation of specific interactions like conventional hydrogen bond, π-σ, π-π stacked and π-donor hydrogen



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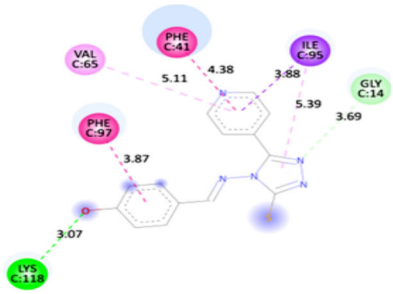
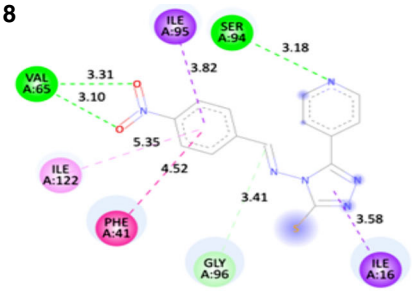


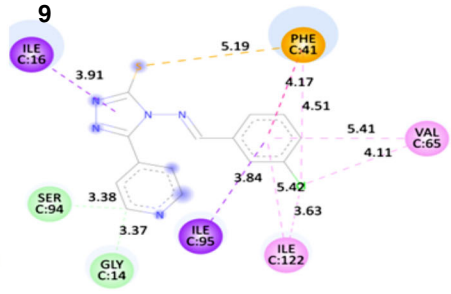
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

8



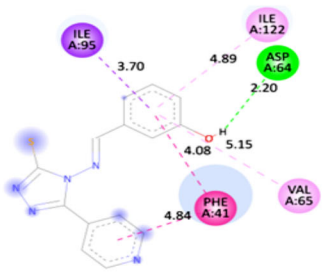
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

9



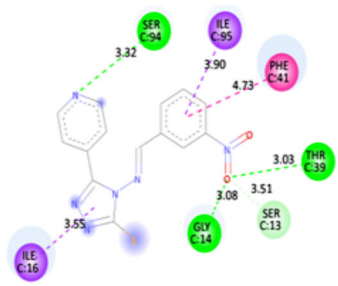
Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Sulfur
Pi-Pi Stacked
Alkyl
Pi-Alkyl

10



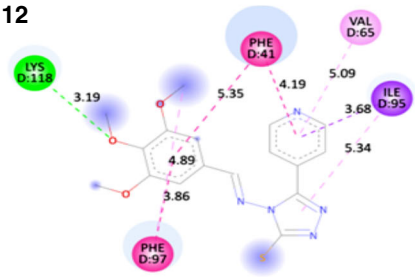
Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

11



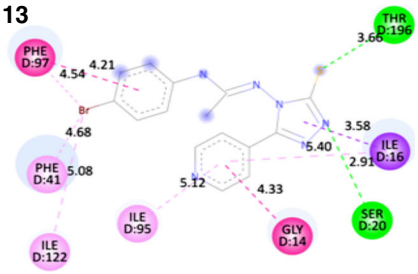
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Sigma
Pi-Pi Stacked

12



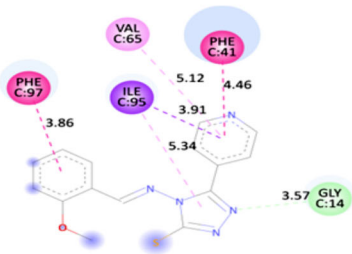
Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

13



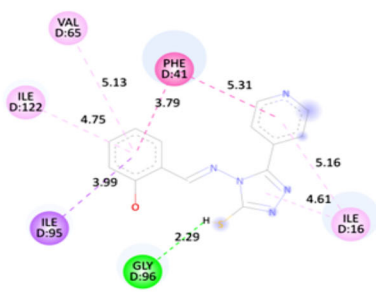
Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Amide-Pi Stacked
Alkyl
Pi-Alkyl

14



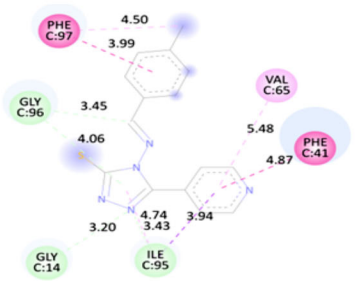
Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

15



Interactions
Conventional hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl

16



Interactions
Carbon hydrogen bond
Pi-Donor hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

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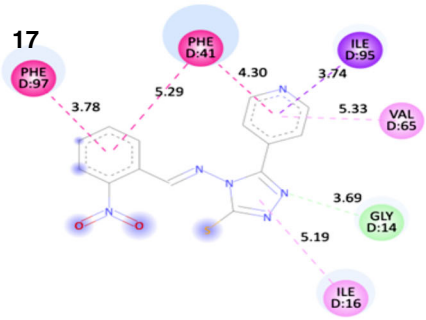

Interactions
Carbon hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

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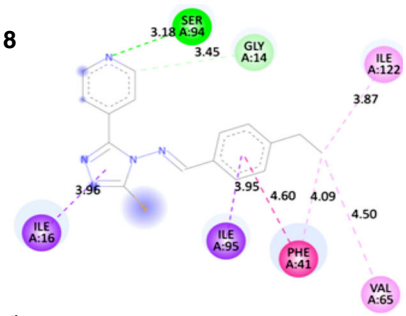


Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Sigma
Pi-Pi Stacked
Alkyl
Pi-Alkyl

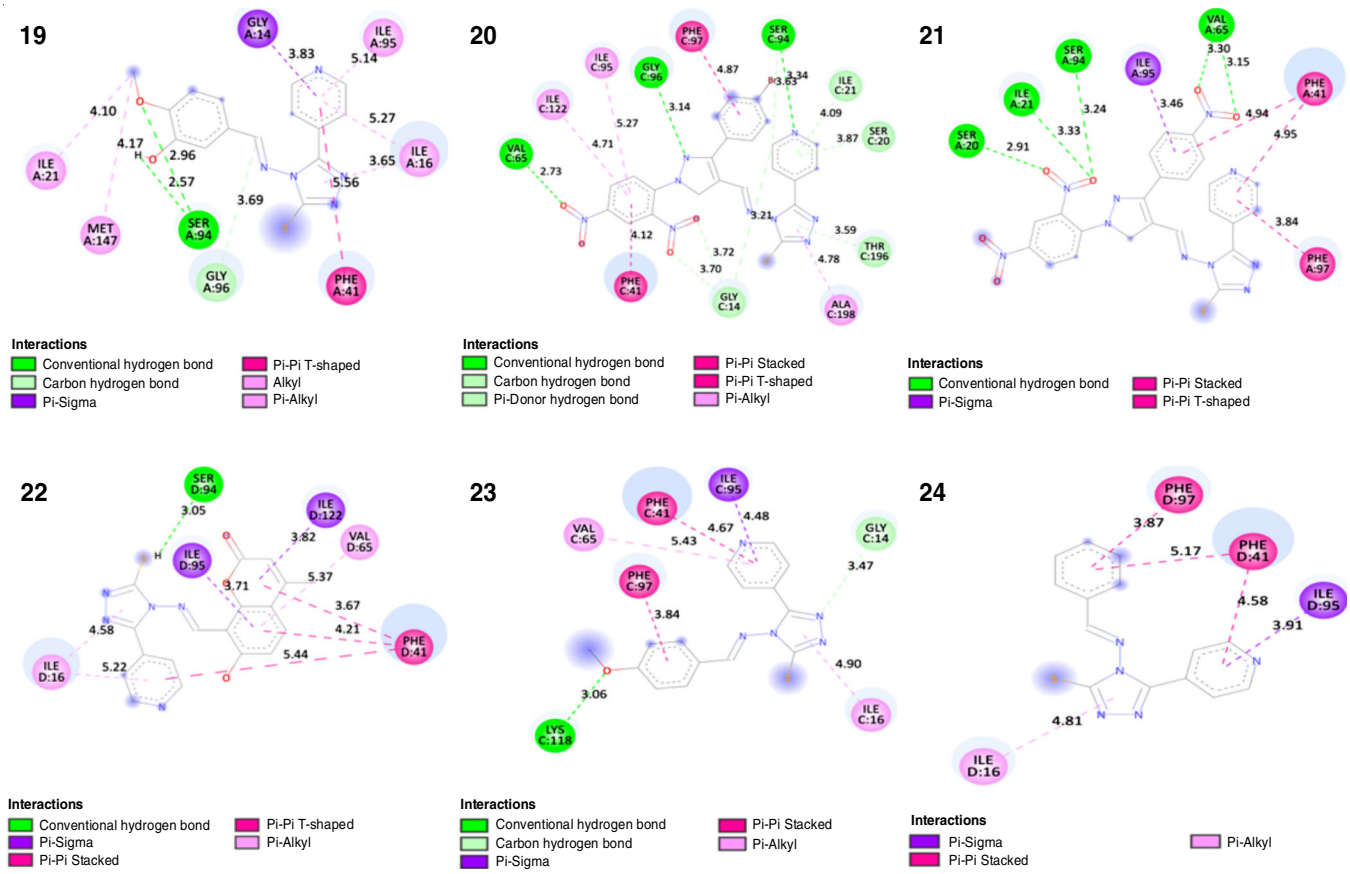
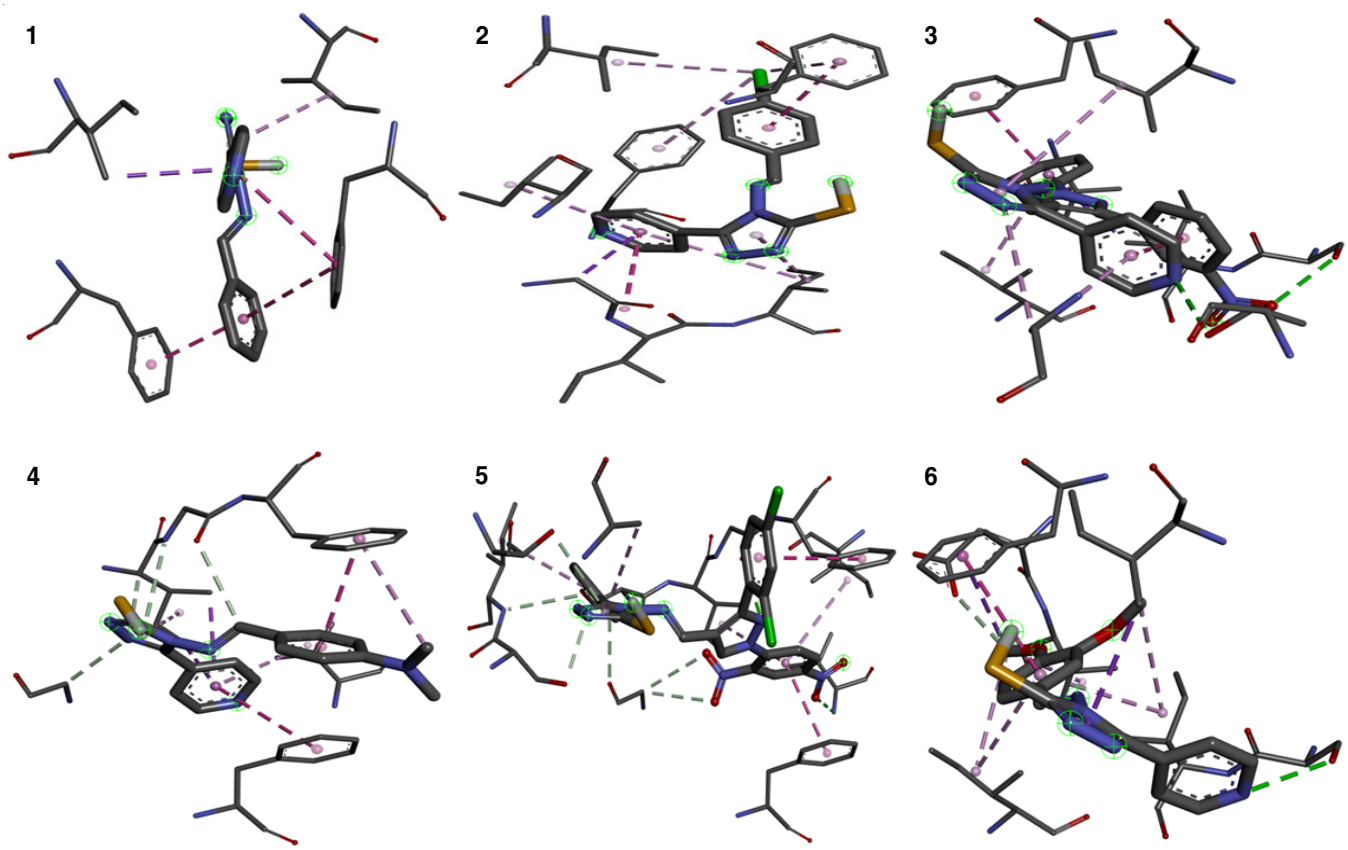
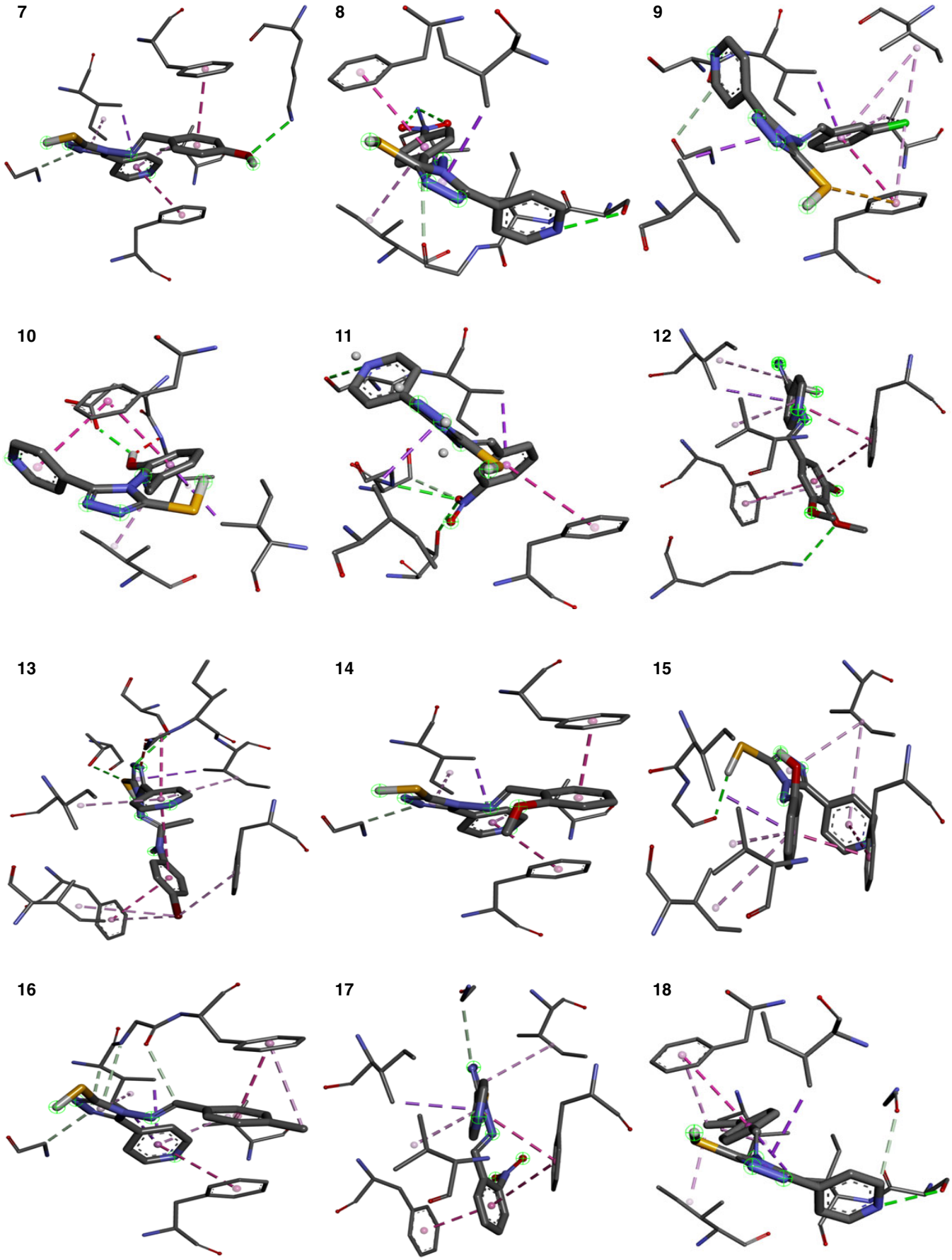


Fig. 1. 2D Interactions diagram of pyridyltriazole derivative structure against the 5JFO PDB

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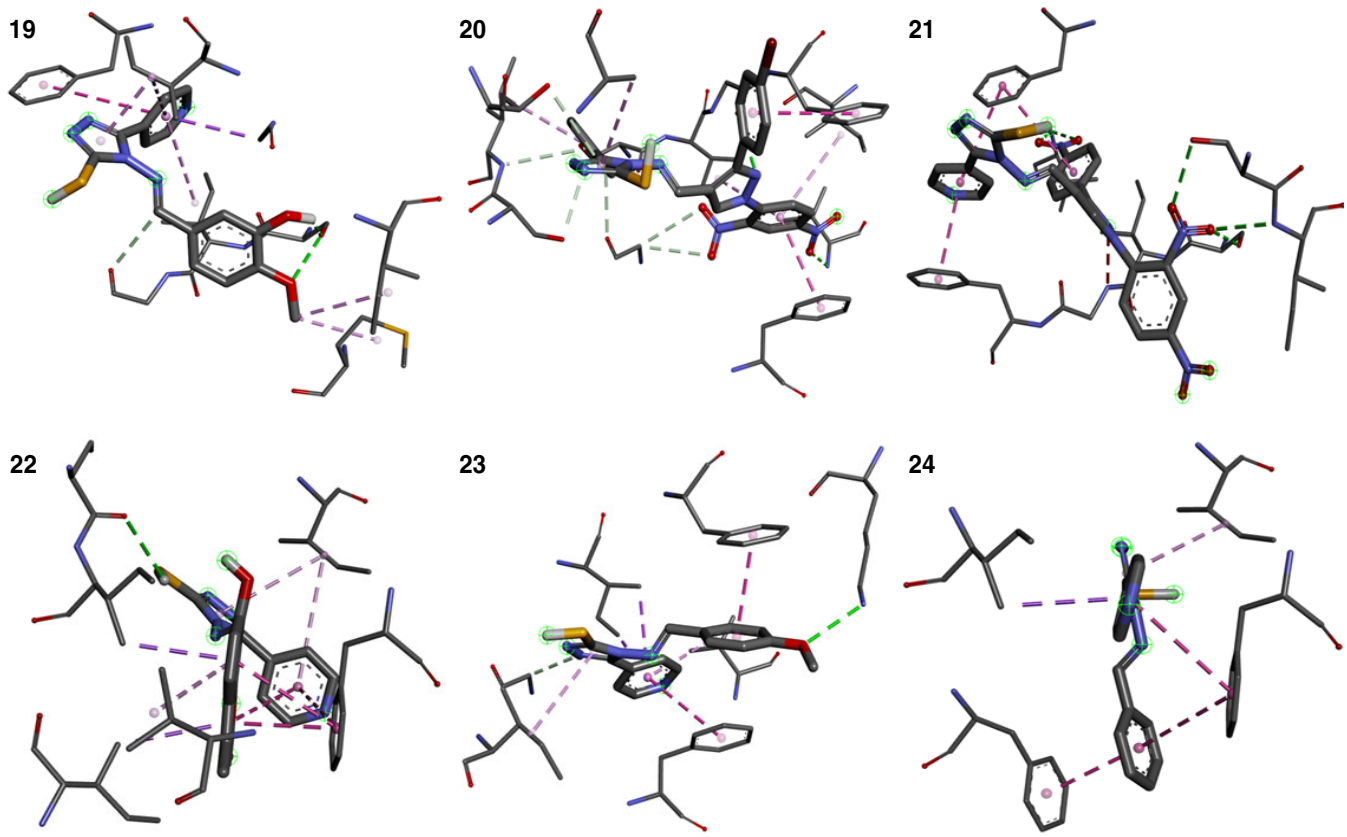

Fig. 2. 3D Interactions diagram of pyridyltriazole derivative structure against the 5JFO PDB

bond interactions may play a significant role in the molecular recognition and binding of small molecules with their respective TB targets.

***In vitro* anti TB activity:** The biological activity of each synthesized compounds against *M. tuberculosis* (Mtb) H37RV strain was assessed. Utilizing the microplate Almar blue assay, the anti-TB profile of compounds **1-24** were examined.It was found that most of the compounds demonstrated effective antimycobacterial activity, with mean minimum inhibitory concentrations (MIC) for compounds **2**, **3**, **6**, **7**, **8**, **11**, **14**, **15**, **18**, **20**, **21**, **22**, **23**, **24** being 2.6, 2.8, 3.1, 4.3, 2.6, 3.2, 3.58, 2.87, 3.1, 4.2, 3.4, 2.6, 3.6 and 3.2 µg/mL, respectively (Table-2). The activity was almost identical to that of an isoniazid as shown in Fig. 3.

TABLE-2
ANTITUBERCULAR ACTIVITY OF SYNTHESIZED COMPOUNDS (**1-24**)

Sample code	Concentration (µg/mL)	Absorbance at 600 nm			Mean	Growth inhibition (%)	IC_{50} (µg/mL)	MIC (µg/mL)
		Test 1	Test 2	Test 3				
Standard (isoniazid)	1	1.13	1.12	1.12	1.12	24.32	7.12	<1
	5	0.82	0.82	0.82	0.82	44.59		
	10	0.40	0.41	0.40	0.40	72.97		
1	1	1.51	1.52	1.54	1.52	NE	NE	5.3
	5	1.30	1.31	1.32	1.31	11.48		
	10	1.21	1.19	1.23	1.21	18.24		
2	1	1.07	1.05	1.09	1.07	27.70	NE	2.6
	5	0.91	0.92	0.93	0.92	37.83		
	10	0.83	0.81	0.85	0.83	43.91		
3	1	1.24	1.22	1.25	1.23	17.89	NE	2.8
	5	1.01	1.02	1.04	1.02	31.08		
	10	0.89	0.87	0.90	0.88	40.54		
4	1	1.93	1.91	1.94	1.92	NE	NE	NE
	5	1.57	1.55	1.58	1.56	NE		
	10	1.51	1.49	1.53	1.51	NE		
5	1	1.17	1.18	1.15	1.16	21.62	NE	NE
	5	1.12	1.10	1.14	1.12	24.32		
	10	0.99	0.97	0.99	0.98	33.78		

	1	1.10	1.12	1.10	1.10	25.67		
6	5	0.83	0.81	0.85	0.83	43.91	NE	3.1
	10	0.71	0.69	0.72	0.70	52.70		
	1	1.21	1.22	1.23	1.22	17.56		
7	5	1.09	1.07	1.08	1.08	27.02	NE	4.3
	10	0.78	0.80	0.81	0.79	47.62		
	1	1.30	1.31	1.32	1.31	11.48		
8	5	1.09	1.07	1.09	1.08	27.02	NE	2.6
	10	0.81	0.82	0.84	0.82	44.59		
	1	1.27	1.28	1.29	1.28	13.51		
9	5	1.18	1.20	1.19	1.19	19.59	NE	4.26
	10	0.93	0.91	0.94	0.92	37.83		
	1	1.59	1.60	1.57	1.58	–		
10	5	1.48	1.46	1.49	1.47	–	NE	7.9
	10	1.30	1.31	1.33	1.31	11.48		
	1	1.29	1.27	1.30	1.28	13.51		
11	5	1.02	1.04	1.02	1.02	31.08	8.26	3.2
	10	0.61	0.63	0.60	0.61	58.78		
	1	1.53	1.55	1.51	1.53	–		
12	5	1.47	1.45	1.48	1.46	–	NE	8.26
	10	1.04	1.06	1.02	1.04	29.72		
	1	1.17	1.18	1.19	1.18	20.27		
13	5	1.03	1.05	1.01	1.03	30.40	NE	2.65
	10	0.94	0.92	0.95	0.93	37.16		
	1	1.12	1.14	1.10	1.12	24.32		
14	5	0.77	0.78	0.79	0.78	47.29	9.33	3.58
	10	0.73	0.71	0.75	0.73	50.67		
	1	1.10	1.09	1.12	1.10	25.67		
15	5	0.92	0.93	0.91	0.92	37.83	NE	2.87
	10	0.75	0.73	0.77	0.75	49.32		
	1	1.93	1.91	1.94	1.92	NE		
16	5	1.71	1.69	1.73	1.71	NE	NE	9.2
	10	1.21	1.22	1.23	1.22	17.56		
	1	1.57	1.59	1.55	1.57	NE		
17	5	0.92	0.90	0.93	0.91	38.51	NE	4.20
	10	0.89	0.88	0.90	0.89	39.86		
	1	1.09	1.07	1.10	1.08	27.02		
18	5	0.97	0.99	0.95	0.97	34.45	NE	3.1
	10	0.78	0.76	0.79	0.76	48.64		
	1	1.53	1.54	1.51	1.52	NE		
19	5	1.47	1.48	1.45	1.46	NE	NE	8.2
	10	1.08	1.07	1.09	1.08	27.02		
	1	1.48	1.46	1.49	1.47	NE		
20	5	1.07	1.05	1.09	1.07	27.70	NE	4.2
	10	0.72	0.70	0.73	0.71	52.02		
	1	1.18	1.16	1.20	1.18	20.27		
21	5	1.09	1.07	1.11	1.09	27.35	9.33	3.4
	10	0.73	0.71	0.75	0.73	50.67		
	1	1.28	1.26	1.29	1.27	14.18		
22	5	1.09	1.06	1.11	1.08	27.02	NE	2.6
	10	0.86	0.88	0.84	0.86	41.89		
	1	1.373	1.371	1.375	1.372	14.41		
23	5	0.982	0.983	0.984	0.983	38.67	9.25	3.6
	10	0.756	0.754	0.758	0.756	52.83		
	1	1.357	1.358	1.359	1.358	15.28		
24	5	1.105	1.103	1.107	1.105	31.06	NE	3.2
	10	0.983	0.981	0.985	0.983	38.60		

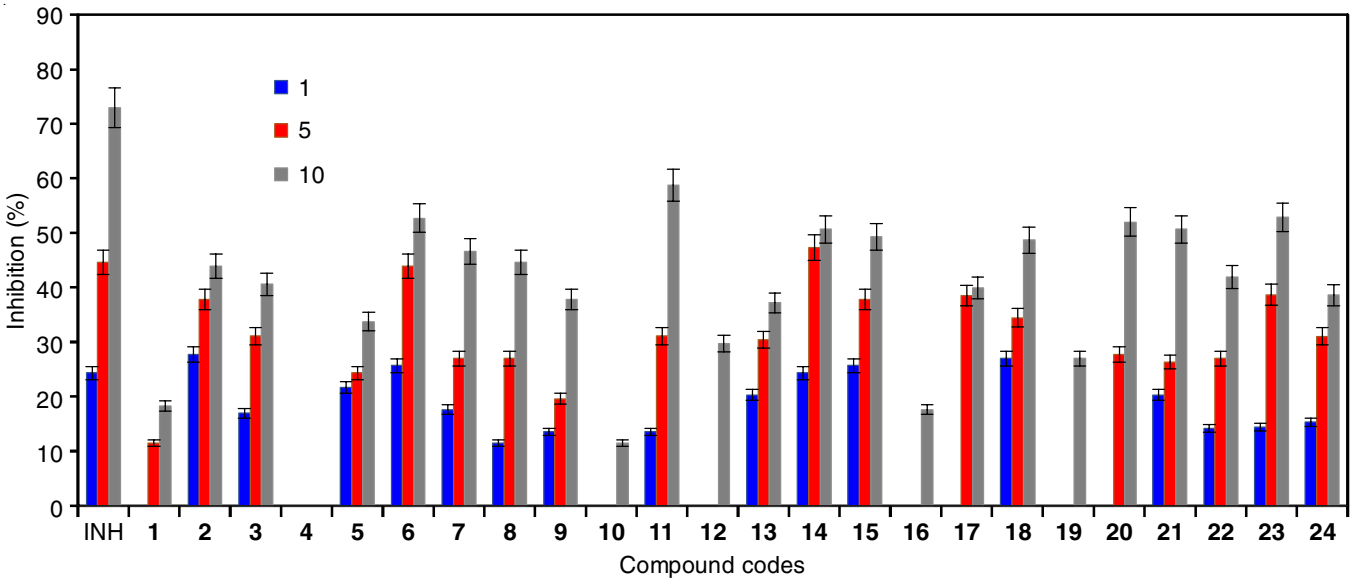

Fig. 3. The percentage of compounds **1-24** growth inhibition against the *M. tuberculosis* strain H37Rv

Conclusion

The title compounds were synthesized by condensing various aromatic aldehydes with 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol. IR, NMR and MS techniques were used to elucidate the newly synthesized 1,2,4-triazole derivatives. The synthesized compounds were all evaluated for their anti-tubercular properties. With mean MIC values of 2.6, 2.8, 3.1, 4.3, 2.6, 3.2, 3.58, 2.87, 3.1, 4.2, 3.4, 2.6, 3.6 and 3.2 µg/mL in comparison to the standard drug, compounds **2**, **3**, **6**, **7**, **8**, **11**, **14**, **15**, **18**, **20**, **21**, **22**, **23** and **24** exhibit good anti-TB activity. The *in silico* study delved into the molecular interactions between potential compounds and mtbenoyl-reductase InhA (PDB 5JFO), which is a crucial target in TB therapy. Compound **21** emerged with the highest negative binding affinity (-10.2 kcal/mol) further followed closely by compound **5** (-10.1 kcal/mol) and found exhibiting promising interactions within the active site of enoyl-reductase InhA. The elucidation of these compound-target interactions *via* docking studies contributes significantly to the rational drug design. It will offer insights crucial for refining hit compounds and ultimately fostering the creation of more effective treatments against TB.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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