Section A -Research paper

SYNTHESIS, SPECTRAL CHARACTERIZATION, *IN-VITRO* AND *IN-SILICO* ANTI-TUBERCULAR ACTIVITY SCREENING OF NOVEL SERIES OF IMIDAZO [2,1-b] [1,3,4]-THIADIAZOLE DERIVATIVES

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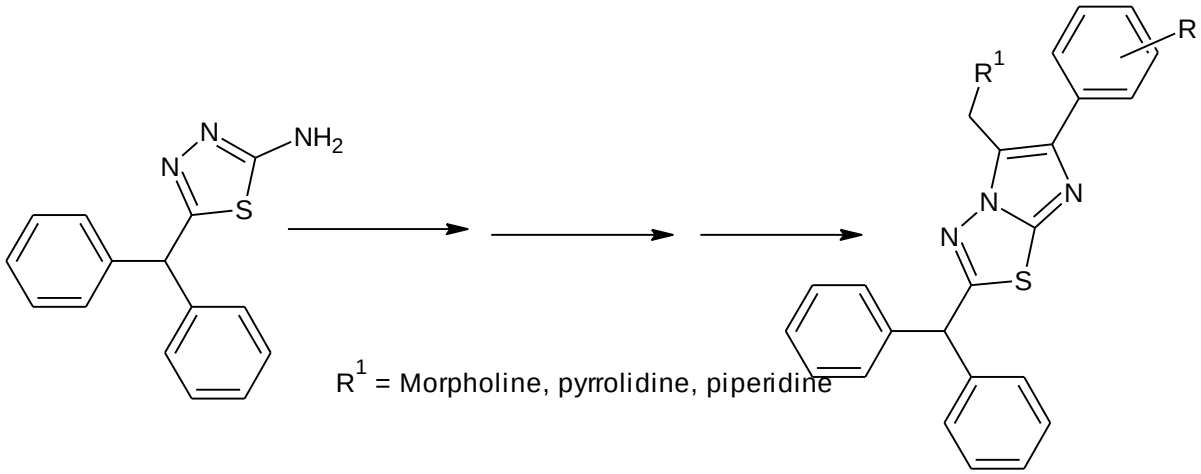
ABSTRACT

With the aim of synthesizing novel anti-tubercular agents, the present study is concentrated on the designing and synthesis of novel 2-benzhydryl-6-(4-substituted phenyl) imidazo[2,1-b] [1,3,4]thiadiazole derivatives by incorporating the two different substituted moieties together by reaction of 5-benzhydryl-1,3,4-thiadiazole-2-amine with appropriate α-haloaryl ketones. Formed products (2a-f) further subjected for reaction with different secondary amines such as morpholine, pyrrolidine and piperidine with formaldehyde, acetic acid in methanol, resulting in the formation of target products (**3a1-a6** and **3c1-c6**). Structure of synthesized compounds were confirmed by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectras). Further these compounds were subjected for anti-tubercular activity screening by MABA method using *Mycobacterium tuberculosis* H37Rv strain. Compounds **3a2, 3a6, 3b2, 3b4 and 3b5** were found to be more effective with MIC value of 12.5 µgm / mL. To enhance in vitro efficacy of these synthesizedcompounds, molecular docking experiments have been performed with pantothenate synthetase and cyclopropane mycolic acid synthase for Tuberculosis with docking scores-7.142 and-7.248 Kcal/mol.

GRAPHICAL ABSTRACT



KEYWORDS: Imidazo-thiadiazole, Pantothenate synthetase, anti-tubercular. MABA method.

INTRODUCTION

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB) and the leading cause of death from a single infectious disease agent in the world(World Health Organization: 2019). Nearly 2 million deaths have been estimated by the World Health Organization (WHO) in recent years as a global burden of TB. Besides, more than 1.7 billion people are known to be latently infected with about 10% of them redeveloping active TB in their lifetime. Poor choice of regimen, poor supply of drugs and poor adherence of patients to six-month treatment leading to the drug-resistant Mycobacterium tubercular strains development, including MDR (Multi-Drug Resistance) for various drugs (Nahid *et al*., 2019). Lessened duration of treatment showed increased dependence to treatment and reduced the growth of multi and extensive drug resistant TB. In 2018, the WHO reported 484,000 cases of MDR / rifampicin-resistant TB in 378,000 cases of MDR-TB with 214,000 deaths and an average of 6.2 % of MDR-TB and XDR-TB cases. 50 % world's burden is due to MDR-TB.This heavy burden of TB or MDR-TB in 24 countries is due to second-line drugs (Mustazzolu*et al*., 2018: Bernard *et al*., 2013).

Intensive investigations of thiadiazole and imidazole compounds have been conducted in recent years with versatile therapeutical properties such as anti-tubercular (Harun *et al.*, 2017: Manjoor *et al.*,2018: Manjoor *et al.*,2017: Rattan *et al.*, 1998: Gadad *et al.*, 2004: Ramprasad *et al.*, 2015), antibacterial(Behzad *et al*., 2015: Desai *et al*., 1992: Gawande *et al*., 1987) antifungal (Debarshi *et al*., 2018: Manjoor *et al*., 2013: Mamolo*et al*., 1996: Gadad *et al*., 2000: Alireza *et al*., 2003), anti-inflammatory (Murat *et al*., 2013: Song *et al*., 1999: Labanauskas*et al*., 2001),anticonvulsant (Chapleo*et al*., 1986: Chapleo*et al*., 1998),antihypertensive (Turner *et al*., 1988: Turner *et al*., 1988),anticancer (Chon *et al*., 2003: Tegginamath*et al*., 2013) and anticoagulants (Regis *et al*., 2005).

In the last two decades these compounds have attracted the interest of anti-tuberculosis research. The family of 5-benzhydryl-1,3,4-thiadiazole-2-amine shows good therapeutic efficacy against *M. tuberculosis* H37Rv strain. The objective of this research was to develop new molecules with an enhanced efficacy for tuberculosis treatment in less time.

In extension of our research on designing and developing new antitubercular agents(Pradeep Kumar*et al*., 2014: Pradeep Kumar *et al*., 2014: Pradeep Kumar *et al*., 2015: Rajesh *et al*., 2016: Shrinivas *et al*., 2016), in this paper, we have reported the synthesis and anti-TB screening of some novel 2-benzhydryl-6-(4-substituted phenyl) imidazo [2,1-b][1,3,4]thiadiazole derivatives.

EXPERIMENTAL

Chemicals used for present research were procured from Sigma Aldrich Pvt Ltd, Spectrochem Pvt Ltd, and S.D. Fine Chem. Pvt Ltd. Melting points have been determined by digital melting point apparatus and are uncorrected. The IR spectra were recorded on the Nicolet Impact 410 FT-IR spectrophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR were recorded on the Bruker 300-MHz FT NMR spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as an internal standard. Mass spectra were recorded by using electron impact technique on the Quattro II Micromass Walter instrument [UK Ltd.].

General procedure for preparation of 2-benzhydryl-6-(4-substitutedphenyl) imidazo[2,1-b][1,3,4] thiadiazoles[2a-f]

The equimolar quantities of 5-benzhydryl-1,3,4-thiadiazole-2-amine [0.01 mol] and bromoacetyl compound [0.01 mol] was refluxed in dry ethanol at 95-100^{o}C for 8 hours. The excess of solvent was distilled off and the solid hydrobromide, isolated by filtration, suspended in water and eventually neutralized using aqueous sodium carbonate solution. The crude solid, isolated by filtration, was washed with water, dried and recrystallized from a suitable solvent.[12]

General procedure for preparation of 2-benzhydryl-5- (morpholin-1-ylmethyl or pyrrolidin-1-ylmethyl or piperidin-1-ylmethyl)-6-arylimdazo (2,1-b)-1,3,4-thiadiazoles. (3a1-a6 to 3c1-c6)

The equimolar quantities of a mixture of secondary amines (0.006 mol) (morpholine or pyrrolidine or piperidine), formalin (1 mL), acetic acid (1 mL) and 2-alkyl / aryl-6-arylimidazo[2,1-b][1,3,4]thiadiazole (0.005 mmol) in 20 mL of ethanol was refluxed for 8 h (monitored by TLC). The mixture was diluted with water (10 mL) and extracted with chloroform (5mL each for three times). The combined extract was washed with water, and dried over anhydrous sodium sulfate. Using a vacuum pump the solvent was evaporated and the resulting solid product was recrystallized using appropriate solvent. [29,30]

2-benzhydryl-5-(morpholinomethyl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole [3a1]

Brownish yellow crystals (65%), M.P. 140-142 °C, IR (KBr) ν cm-1 (Ar-CH) 3020, (C=N) 1600, (C-N) 1110, (C-H) 2801;^{1}H NMR (400MHz, DMSO-d_6). δ 8.20 (d, 2H, Ar-H, C_2, C_6), δ 7.84 (d, 2H, Ar-H, C_3,C_5) δ 7.14 (m, 2H, C_2, C_6), δ 7.19 (m, Ar-H, C_3, C_5),δ 7.16 (m, 2H, Ar-H, C_4), δ 4.22 (s, CH, methine) δ 3.98 (S, 2H, CH_2), δ 3.90 (t, 4H, C_3, C_5-H, morpholine), δ 2.40 (t, 4H, C_2, C_6-H, morpholine), δ 4.30 (s, CH, methine); Mass, m/z (%), 511.59 (M+1, 100).Anal. Calculated for $C_{28}H_{25}N5O_3S$: C (65.74%), H(4.93%), N(13.69%), O(9.38%), S(6.27%). Found: C(64.62%), H(3.99%), N(14.93%), O(8.99%), S(7.57%).

2-benzhydryl-5-(morpholinomethyl)-6-p-tolylimidazo[2,1-b] [1,3,4]thiadiazole [3a2]

Brownish yellow solid crystals (72%), M.P. 120-125 °C, IR (KBr) ν cm-1 (Ar-CH) 3015, (C=N) 1588, (C-N) 1099, (C-H) 2880; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.35 (d, 2H, Ar-H, C_2, C_6), δ 7.10 (d, 2H, Ar-H, C_3,C_5) δ 7.17 (m, 2H, C_2, C_6), δ 7.23 (m, Ar-H, C_3, C_5),δ 7.11 (m, 2H, Ar-H, C_4), δ 2.46 (s, CH_3), δ 4.25 (s, CH, methine) δ 3.89 (S, 2H, CH_2) δ 3.90 (t, 4H, C_3, C5-H, morpholine), δ 2.40 (t, 4H, C_2, C_6-H, morpholine);Mass, m/z (%), 480.62 (M+1, 100). Anal. Calcd for $C_{29}H_{28}N_4OS$:

C(72.47%), H(5.87%), N(11.66%), O(3.33%), S(6.67%). Found: C(69.97%), H(7.56%), N(12.51%), O(2.98%), S(6.98%).

2-benzhydryl-6-(4-methoxyphenyl)-5-(morpholinomethyl)imidazo[2,1-b][1,3,4] thiadiazole [3a3]

Dark Brown solid crystals (70%), M.P. 130-135 °C, IR (KBr) ν cm-1 (Ar-CH) 3027, (C=N) 1577, (C-N) 1078, (C-H) 2809; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.38 (d, 2H, Ar-H, C_2, C_6), δ 7.00 (d, 2H, Ar-H, C_3,C_5) δ 7.16 (m, 2H, C_2, C_6), δ 7.12 (m, Ar-H, C_3, C_5),δ 7.04 (m, 2H, Ar-H, C_4), δ 3.86 (s, OCH_3), δ 4.22 (s, CH, methine) δ 3.85 (S, 2H, CH_2) δ 3.77 (t, 4H, C_3, C_5-H, morpholine), δ 2.35 (t, 4H, C_2, C_6-H, morpholine), δ 4.20 (s, CH, methine);Mass, m/z (%), 496.19 (M+1, 100). Anal. Calcd for $C_{29}H_{28}N_4O_2S$: C(70.14%), H(5.68%), N(11.28%), O(6.44%), S(6.46%). Found: C(71.74%), H(6.01%), N(10.26%), O(5.98%), S(6.01%).

2-benzhydryl-6-(4-chlorophenyl)-5-(morpholinomethyl)imidazo[2,1-b] [1,3,4]thiadiazole [3a4]

Brownish yellow solid crystals (62%), M.P. 135-139 °C, IR (KBr) ν cm^{-1} (Ar-CH)3042, (C=N) 1577, (C-N) 1076, (C-H) 2870;^{1}H NMR (400MHz, DMSO-d_6). δ 7.31 (d, 2H, Ar-H, C_2, C_6), δ 7.40 (d, 2H, Ar-H, C_3,C_5) δ 7.12 (m, 2H, C_2, C_6), δ 7.16 (m, Ar-H, C_3, C_5),δ 7.14 (m, 2H, Ar-H, C_4), δ 4.01 (s, CH, methine) δ 3.67 (S, 2H, CH_2), δ 3.69 (t, 4H, C_3, C_5-H), δ2.39 (t, 4H, C_2, C_6-H); Mass, m/z (%), 500.14 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}ClN_4OS$: C(67.12%), H(5.03%), Cl(7.08%), N(11.18%), O(3.19%), S(6.40%).

Found: C(66.32%), H(5.06%), Cl(6.87%), N(10.91%), O(4.01%), S(6.83%).

2-benzhydryl-6-(4-bromophenyl)-5-(morpholinomethyl)imidazo[2,1-b][1,3,4]thiadiazole [3a5]

Brownish yellowsolid crystals (60%), M.P. 137-141 °C, IR (KBr) ν cm^{-1} (Ar-CH) 3029, (C=N) 1577, (C-N) 1077, (C-H) 2805;^{1}H NMR (400MHz, DMSO-d_6). δ 7.50 (d, 2H, Ar-H, C_2, C_6), δ 7.42 (d, 2H, Ar-H, C_3,C_5) δ 7.14 (m, 2H, C_2, C_6), δ 7.18 (m, Ar-H, C_3, C_5),δ 7.16 (m, 2H, Ar-H, C_4), δ 4.03 (s, CH, methine) δ 3.69 (S, 2H, CH_2), δ 3.70 (t, 4H, C_3, C_5-H), δ 2.41 (t, 4H, C_2, C_6-H); Mass, m/z (%), 545.49 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}BrN_4OS$: C(61.65%), H(4.62%), Br(14.65%), N(10.27%), O(2.93%), S(5.88%). Found: C(60.78%), H(5.01%), Br(15.13%), N(11.03%), O(3.94%), S(4.11%).

2-benzhydryl-5-(morpholinomethyl)-6-(3-nitrophenyl) imidazo[2,1-b][1,3,4]thiadiazole [3a6]

Brown yellow solid crystals (69%), M.P. 140-145 °C, IR (KBr) ν cm-1 (Ar-CH) 3013, (C=N) 1588, (C-N) 1083, (C-H) 2809;^{1}H NMR (400MHz, DMSO-d_6). δ 8.31 (d, 2H, Ar-H, C_2, C_4), δ 7.84 (d, 2H, Ar-H, C_5,C_6) δ 7.17 (m, 2H, C_2, C_6), δ 7.21 (m, Ar-H, C_3, C_5),δ 7.15 (m, 2H, Ar-H, C_4), δ 4.31 (s, CH, methine) δ 3.88 (S, 2H, CH_2), δ 3.87 (t, 4H, C_3, C_5-H, morpholine), δ 2.37 (t, 4H, C_2, C_6-H, morpholine); Mass, m/z (%), 511.59 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}N_5O_3S$: C(65.74%), H(4.93%), N(13.69%), O(9.38%), S(6.27%). Found: C(66.97%), H(5.98%), N(11.71%), O(8.98%), S(6.36%).

2-benzhydryl-6-(4-nitrophenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazole [3b1]

Yellowish brown solid crystals (67%), M.P. 125-129 °C, IR (KBr) ν cm-1 (Ar-CH) 3041, (C=N) 1620, (C-N) 1100, (C-H) 2808;^{1}H NMR (400MHz, DMSO-d_6). δ 8.20 (d, 2H, Ar-H, C_2, C_6), δ 7.84 (d, 2H, Ar-H, C_3,C_5) δ 7.14 (m, 2H, C_2, C_6), δ 7.19 (m, Ar-H, C_3, C_5),δ 7.16 (m, 2H, Ar-H, C_4), δ 4.22 (s, CH, methine) δ 3.98 (S, 2H, CH_2), δ 2.60 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.50 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 495.59 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}N_5O_2S$: C(67.86%), H(5.08%), N(14.13%), O(6.46%), S(6.47%). Found: C(68.78%), H(6.11%), N(12.23%), O(5.99%), S(6.89%).

2-benzhydryl-5-(pyrrolidin-1-ylmethyl)-6-p-tolylimidazo[2,1-b] [1,3,4] thiadiazole [3b2]

Brown solid crystals (62%), M.P. 110 - 115 °C, IR (KBr) ν cm-1 (Ar-CH) 3022, (C=N) 1566, (C-N) 1088, (C-H) 2807; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.35 (d, 2H, Ar-H, C_2, C_6), δ 7.10 (d, 2H, Ar-H, C_3,C_5) δ 7.17 (m, 2H, C_2, C_6), δ 7.23 (m, Ar-H, C_3, C_5),δ 7.11 (m, 2H, Ar-H, C_4), δ 2.39 (s, CH_3), δ 4.25 (s, CH, methine) δ 3.76 (S, 2H, CH_2), δ 2.40 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.60 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 464.62 (M+1, 100). Anal. Calcd for $C_{29}H_{28}N_4S$: C(74.97%), H(6.07%), N(12.06%), S(6.90%).

Found: C(73.89%), H(7.19%), N(11.99%), S(6.93%).

2-benzhydryl-6-(4-methoxyphenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole [3b3]

Dark brownish yellow solid crystals (60%), M.P. 116-121 °C, IR (KBr) ν cm-1 (Ar-CH) 3023, (C=N) 1567, (C-N) 1055, (C-H) 2817; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.38 (d, 2H, Ar-H, C_2, C_6), δ 7.00 (d, 2H, Ar-H, C_3,C_5) δ 7.16 (m, 2H, C_2, C_6), δ 7.12 (m, Ar-H, C_3, C_5),δ 7.04 (m, 2H, Ar-H, C_4), δ 3.86 (s, OCH_3), δ 4.22 (s, CH, methine) δ 3.85 (S, 2H, CH_2) δ 2.39 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.59 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 480.62 (M+1, 100).Anal. Calcd for $C_{29}H_{28}N_4OS$: C(72.47%), H(5.87%), N(11.66%), O(3.33%), S(6.67%). Found: C(73.17%), H(4.73%), N(11.55%), O(4.21%), S(6.34%).

2-benzhydryl-6-(4-chlorophenyl)-5-(pyrrolidin-1-ylmethyl) imidazo[2,1-b] [1,3,4] thiadiazole [3b4]

Brownish yellow solid crystals (66%), M.P. 123-127 °C, IR (KBr) ν cm^{-1} (Ar-CH) 3031, (C=N) 1555, (C-N) 1066, (C-H) 2846;^{1}H NMR (400MHz, DMSO-d_6). δ 7.31 (d, 2H, Ar-H, C_2, C_6), δ 7.40 (d, 2H, Ar-H, C_3,C_5) δ 7.12 (m, 2H, C_2, C_6), δ 7.16 (m, Ar-H, C_3, C_5),δ 7.14 (m, 2H, Ar-H, C_4), δ 4.65 (s, CH, methine) δ 3.67 (S, 2H, CH_2), δ 2.42 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.67 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 485.04 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}ClN_4S$: C(69.33%), H(5.20%), Cl(7.31%), N(11.55%), S(6.61%). Found: C(71.01%), H(4.40%), Cl(6.91%), N(10.67%), S(7.01%).

2-benzhydryl-6-(4-bromophenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazole [3b5]

Brownish yellow solid crystals (61%), M.P. 118-123 °C, IR (KBr) ν cm^{-1} (Ar-CH) 3038, (C=N) 1561, (C-N) 1048, (C-H) 2890;^{1}H NMR (400MHz, DMSO-d_6). δ 7.59 (d, 2H, Ar-H, C_2, C_6), δ 7.48 (d, 2H, Ar-H, C_3,C_5) δ 7.18 (m, 2H, C_2, C_6), δ 7.29 (m, Ar-H, C_3, C_5),δ 7.17 (m, 2H, Ar-H, C_4), δ 4.54 (s, CH, methine) δ 3.59 (S, 2H, CH_2), δ 2.56 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.66 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 529.49 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}BrN_4S$: C(63.51%), H(4.76%), Br(15.09%), N(10.58%), S(6.06%). Found: C(64.63%), H(5.16%), Br(14.01%), N(11.61%), S(5.976%).

2-benzhydryl-6-(3-nitrophenyl)-5-(pyrrolidin-1-ylmethyl)imidazo[2,1b][1,3,4] thiadiazole[3b6]

Yellowish brown solid crystals (60%), M.P. 125-130 °C, IR (KBr) ν cm-1 (Ar-CH) 3040, (C=N) 1600, (C-N) 1101, (C-H) 2837;^{1}H NMR (400MHz, DMSO-d_6). δ 8.31 (d, 2H, Ar-H, C_2, C_4), δ 7.84 (d, 2H, Ar-H, C_5,C_6) δ 7.17 (m, 2H, C_2, C_6), δ 7.21 (m, Ar-H, C_3, C_5),δ 7.15 (m, 2H, Ar-H, C_4), δ 4.31 (s, CH, methine) δ 3.88 (S, 2H, CH_2), δ 2.56 (t, 4H, C_2, C_5-H, pyrrolidine), δ 1.69 (t, 4H, C_3, C_4-H, pyrrolidine); Mass, m/z (%), 511.59 (M+1, 100).

Anal. Calcd for $C_{28}H_{25}N5O_3S$: C(65.74%), H(4.93%), N(13.69%), O(9.38%), S(6.27%). Found: C(66.01%), H(4.96%), N(12.01%), O(11.08%), S(5.94%).

2-benzhydryl-6-(4-nitrophenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazole[3c1]

Yellowish brown solid crystals (68%), M.P. 150-154 °C, IR (KBr) ν cm-1 (Ar-CH) 3035, (C=N) 1581, (C-N) 1099, (C-H) 2809;^{1}H NMR (400MHz, DMSO-d_6). δ 8.44 (d, 2H, Ar-H, C_2, C_6), δ 7.92 (d, 2H, Ar-H, C_3,C_5) δ 7.19 (m, 2H, C_2, C_6), δ 7.18 (m, Ar-H, C_3, C_5),δ 7.19 (m, 2H, Ar-H, C_4), δ 4.33 (s, CH, methine) δ 3.69 (S, 2H, CH_2), δ 2.51 (t, 4H, C_2, C_6-H, piperidine), δ 1.59 (t, 4H, C_3, C_5, C_4-H, piperidine); Mass, m/z (%), 509.62 (M+1, 100).Anal. Calcd for $C_{29}H_{27}N_5O_2S$: C(68.35%), H(5.34%), N(13.74%), O(6.28%), S(6.29%). Found: C(66.34%), H(6.00%), N(14.04%), O(6.89%), S(7.03%).

2-benzhydryl-5-(piperidin-1-ylmethyl)-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazole[3c2]

Brownish yellow solid crystals (59%), M.P. 130-135 °C, IR (KBr) ν cm-1 (Ar-CH) 3024, (C=N) 1573, (C-N) 1051, (C-H) 2807; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.29 (d, 2H, Ar-H, C_2, C_6), δ 7.16 (d, 2H, Ar-H, C_3,C_5) δ 7.07 (m, 2H, C_2, C_6), δ 7.10 (m, Ar-H, C_3, C_5),δ 7.11 (m, 2H, Ar-H, C_4), δ 2.24 (s, CH_3), δ 4.16 (s, CH, methine) δ 3.78 (S, 2H, CH_2), δ 2.66 (t, 4H, C_2, C_6-H, piperidine), δ 1.66 (t, 4H, C_3, C_5, C_4-H, piperidine); Mass, m/z (%), 478.65 (M+1, 100).Anal. Calcd for $C_{30}H_{30}N_4S$: C(75.28%), H(6.32%), N(11.71%), S(6.70%). Found: C(74.51%), H(7.41%), N(12.33%), S(5.90%).

2-benzhydryl-6-(4-methoxyphenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole [3c3]

Dark brownish solid crystals (60%), M.P. 136-141 °C, IR (KBr) ν cm-1 (Ar-CH)3022, (C=N) 1604, (C-N) 1062, (C-H) 2862; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.22 (d, 2H, Ar-H, C_2, C_6), δ 7.33 (d, 2H, Ar-H, C_3,C_5) δ 7.16 (m, 2H, C_2, C_6), δ 7.05 (m, Ar-H, C_3, C_5),δ 7.04 (m, 2H, Ar-H, C_4), δ 3.90 (s, OCH_3), δ 4.44 (s, CH, methine) δ 3.84 (S, 2H, CH_2) δ 2.55 (t, 4H, C_2, C_6-H, piperidine), δ 1.59 (t, 4H, C_3, C_5, C-H, piperidine); Mass, m/z (%), 494.65 (M+1, 100).Anal. Calcd for $C_{30}H_{30}N_4OS$: C(72.84%), H(6.11%), N(11.33%), O(3.23%), S(6.48%). Found: C(69.94%), H(7.14%), N(12.03%), O(4.01%), S(6.88%).

2-benzhydryl-6-(4-chlorophenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazole[3c4]

Brown solid crystals (66%), M.P. 139-145 °C, IR (KBr) ν cm^{-1} (Ar-CH) 3024, (C=N) 1599, (C-N) 1103, (C-H) 2820; ^{1}H NMR (400MHz, DMSO-d_6). δ 7.41 (d, 2H, Ar-H, C_2, C_6), δ 7.31 (d, 2H, Ar-H, C_3,C_5) δ 7.13 (m, 2H, C_2, C_6), δ 7.15 (m, Ar-H, C_3, C_5),δ 7.14 (m, 2H, Ar-H, C_4), δ 4.65 (s, CH, methine) δ 3.67 (S, 2H, CH_2), δ 2.57 (t, 4H, C_2, C_6-H, piperidine), δ 1.61 (t, 4H, C_3, C_5, C_4-H, piperidine); Mass, m/z (%), 499.06 (M+1, 100).

Anal. Calcd for $C_{29}H_{27}ClN_4S$: C(69.79%), H(5.45%), Cl(7.10%), N(11.23%), S(6.42%). Found: C(68.87%), H(5.09%), Cl(8.21%), N(12.02%), S(5.81%).

2-benzhydryl-6-(4-bromophenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole[3c5]

Brown solid crystals (61%), M.P. 141-146 °C, IR (KBr) ν cm^{-1} (Ar-CH) 3020, (C=N) 1560, (C-N) 1086, (C-H) 2850;^{1}H NMR (400MHz, DMSO-d_6). δ 7.59 (d, 2H, Ar-H, C_2, C_6), δ 7.48 (d, 2H, Ar-H, C_3,C_5) δ 7.17 (m, 2H, C_2, C_6), δ 7.30 (m, Ar-H, C_3, C_5),δ 7.18 (m, 2H, Ar-H, C_4), δ 4.54 (s, CH, methine) δ 3.59 (S, 2H, CH_2), δ 2.60 (t, 4H, C_2, C_6-H, piperidine), δ 1.58 (t, 4H, C_3, C_5, C_4-H, piperidine); Mass, m/z (%), 543.52 (M+1, 100).

Anal. Calcd for $C_{29}H_{27}BrN_4S$: C(64.08%), H(5.01%), Br(14.70%), N(10.31%), S(5.90%). Found: C(66.13%), H(4.05%), Br(12.65%), N(11.06%), S(6.11%).

2-benzhydryl-6-(3-nitrophenyl)-5-(piperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazole [3c6]

Yellowish brown solid crystals (63%), M.P. 149-150 °C, IR (KBr) ν cm-1 (Ar-CH)3012, (C=N) 1581, (C-N) 1104, (C-H) 2819;^{1}H NMR (400MHz, DMSO-d_6). δ 8.29 (d, 2H, Ar-H, C_2, C_4), δ 7.88 (d, 2H, Ar-H, C_5,C_6) δ 7.22 (m, 2H, C_2, C_6), δ 7.24 (m, Ar-H, C_3, C_5),δ 7.23 (m, 2H, Ar-H, C_4), δ 4.71 (s, CH, methine) δ 3.79 (S, 2H, CH_2), 2.73 (t, 4H, C_2, C_6-H, piperidine), δ 1.61 (t, 4H, C_3, C_5, C_4-H, piperidine); Mass, m/z (%), 509.62 (M+1, 100).Anal. Calcd for $C_{29}H_{27}N_5O_2S$: C (68.35%), H(5.34%), N(13.74%), O(6.28%), S(6.29%).

Found: C (67.31%), H(6.43%), N(14.04%), O(5.12%), S(7.01%).

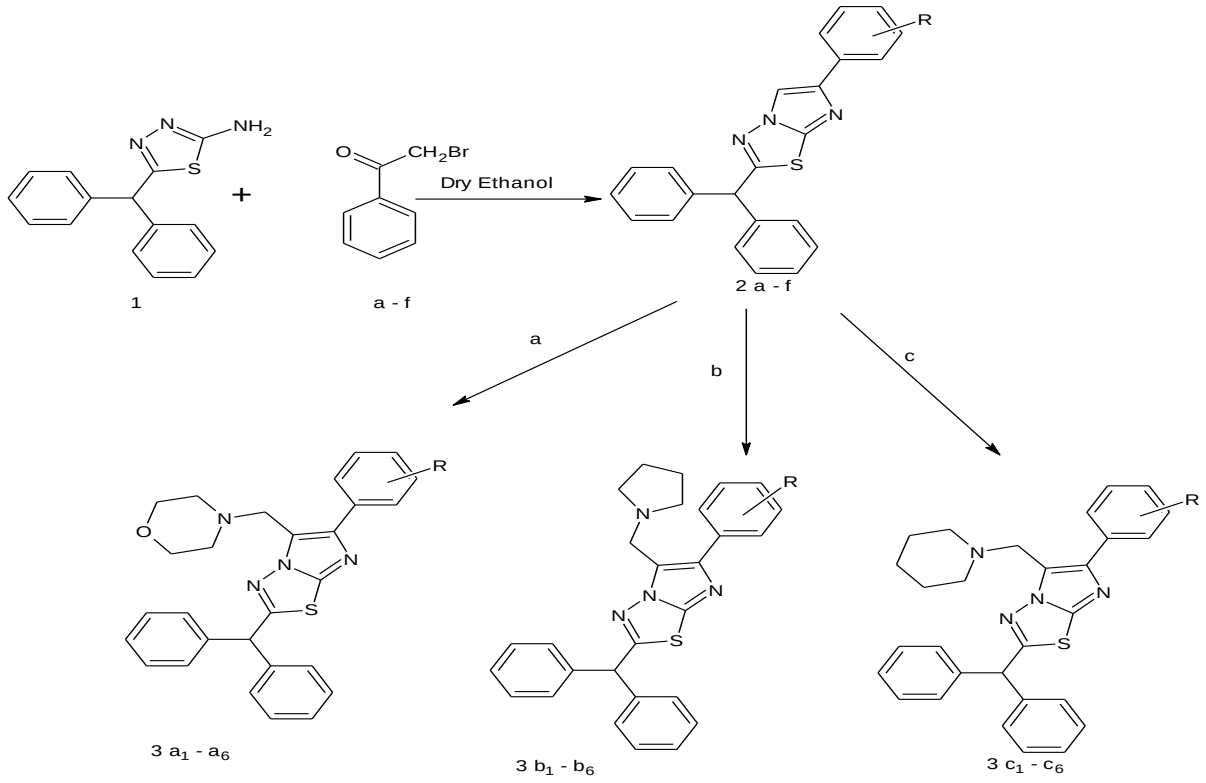
Antitubercular Activity

MIC values were determined for all the synthesized compounds against *M. tuberculosis* strain H_{37}Rv using the Microplate Alamar Blue assay (MABA) using isoniazid as the standard drug (Franzblau *et al.*,1998). The result of antitubercular activity shown in Table 2.

RESULTS AND DISCUSSION

2-Benzhydryl-6-(4-substituted phenyl) imidazo[2,1-b][1,3,4] thiadiazoles **[2a-f]** were prepared by the treatment of 5-benzhydryl-1,3,4-thiadiazole-2-amine with appropriate α-haloaryl ketone **[a-f]**. Mannich reaction of **2a-f** with different cyclic amines and formaldehyde in methanol with a catalytic amount of acetic acid produced target compounds **[3a1-a6 to 3c1-c6] (Scheme 1)**.

Synthetic scheme for target compounds



a) Morpholine, HCHO, CH3COOH, Methanol b) Pyrrolidine, HCHO, CH_3COOH, Methanol

c) Piperidine, HCHO, CH_3COOH, Methanol

R = 4 - NO_2 (a1,b1,c1), 4 - CH_3 (a2,b2,c2), 4-OCH_3 (a3, b3,c3), 4 - Cl (a4,b4,c4), 4 - Br (a5,b5,c5 3- NO_2 (a6, b6,c6)

Antitubercular Activity Results

Synthesized compounds **(2a-f and 3a1-a6 to 3c1-c6)** were tested for antitubercular activity against *Mycobacterium tuberculosis* H_{37} Rv strain. Compounds **3a2, 3a6, 3b2, 3b4,** and **3b5** exhibited potent antitubercular activity compared to standard drugs Isoniazid. The results are summarized in**Table 1.**

Table 1: Anti-TB screening of synthesized compounds by MABA method.

Compound code	MIC µgm/mL
3a1	25
3a2	12.5
3a3	25
3a4	25
3a5	25
3a6	12.5
3b1	25
3b2	12.5
3b3	25
3b4	12.5
3b5	12.5
3b6	25
3c1	25
3c2	25
3c3	25
3c4	25
3c5	25
3c6	25
Isoniazid	0.25

MOLECULAR DOCKING

In docking studies, the selection of various targets depending on its mechanism of action was made since the structural drug design depends on the function of the receptor. The drugs that are developed demonstrate the impact in the majority of cases against targets such as Pantothenate

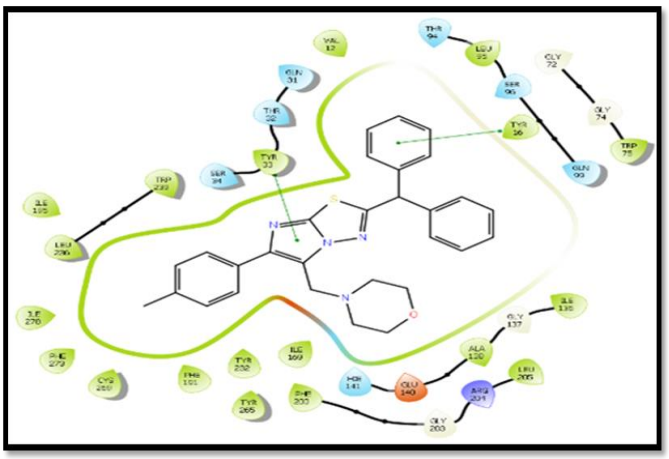
synthetase (1n2 g), Cyclopropane mycolic acid synthase (1-1kpg). PS (Pantothenate synthetase) catalyzes the ATP-dependent condensation of pantoate and β-alanine to produce pantothenate, which belongs to the Vitamin B complex group (Vitamin B5) and is a key precursor to the synthesis of coenzyme A and acyl carrier protein (Abiko *et al*., 1967)in Mycobacterium tuberculosis, Pantothenate binds to D-pantoate and ATP through uncompetitive inhibition and exhibits non-competitive inhibition towards β – alanine (Chopra *et al*., 2002).The main lipid component of mycobacterial cell walls are the mycolic acids responsible for the protection of tuberculosis bacilli, the mycolic acids are synthesized in the cytoplasm and transported to the outer membrane of the bacteria glycolipid (Jeffery *et al*., 2014). α-Alkylated β-hydroxylated fatty acids, i.e., mycolic acid, are the primary constituents of the mycobacterial cell wall responsible for the permeability, integrity, and virulence of the outer membrane (Marrakchi *et al*., 2008: Glickman *et al*., 2000: Dubnan*et al*., 2000).

Imidazole thiadiazoles are best known for their high potency against Mtb, which, in their molecular docking, revealed targets such as Pantothenate synthetase (1n2 g), Cyclopropane mycolic acid synthase (1-1kpg) for Mtb, due to the assessment of their represent static for compounds **3a2, 3a6, 3b1, 3b2, 3b4** and**3b5**. We were inspired by experimental data from MIC as compared to molecular docking values for various targets Pantothenate synthetase (1n2 g), Cyclopropane mycolic acid synthase (1-1kpg) for Mtb for selected Imidazo thiadiazole derivatives. To further rationalize the drug design for the different biological target for our compounds and to conduct molecular docking for all compounds, but we presented those compounds (**3a2, 3a6, 3b1, 3b2, 3b4**, and **3b5**) which showed better activity based on experimental data as experimental data consistent with data from molecular docking studies. In the present molecular docking analysis, our novel synthesized molecules were more potent against Mtb targets, with compounds (**3b1** and **3b4**) having very good activity against Cyclopropane mycolic acid synthase CmaA1 complexed with SAH (1-1kpg) but less active against pantothenate synthetase *M. Tuberculosis* in the AMPCPP (1N2G) cluster.The two compounds were docked at the binding site of a 1-1kpg protein and had strong docking scores of-7.142 and-7.248.Molecular docking result of synthesized compounds against Cyclopropane mycolic acid synthase and Pantothenate synthetase is shown in Table.2

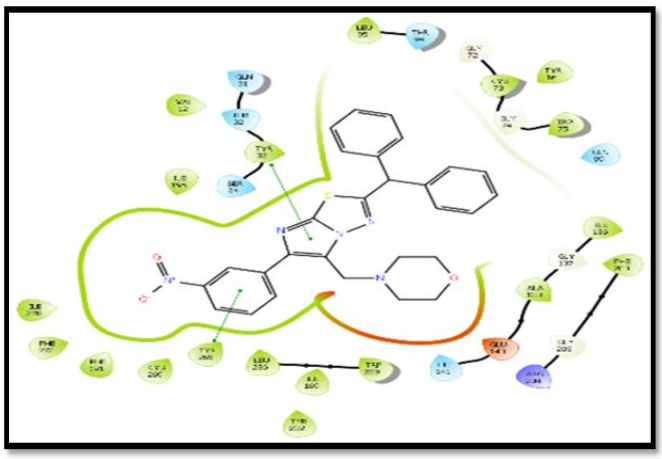
The active compounds **3a2, 3a6, 3b1, 3b2,3b4** and **3b5** binding analysis reveals that the nitro (at 3rd position), methyl, chloro, and bromo groups showed strong MIC in the para-position of the phenyl ring substituted for imidazothiadiazole at 6th position.

The overall evaluation of the work indicates that the compound bearing electron-withdrawing groups like nitro, methoxy are very much active against *Mycobacterium tuberculosis.*

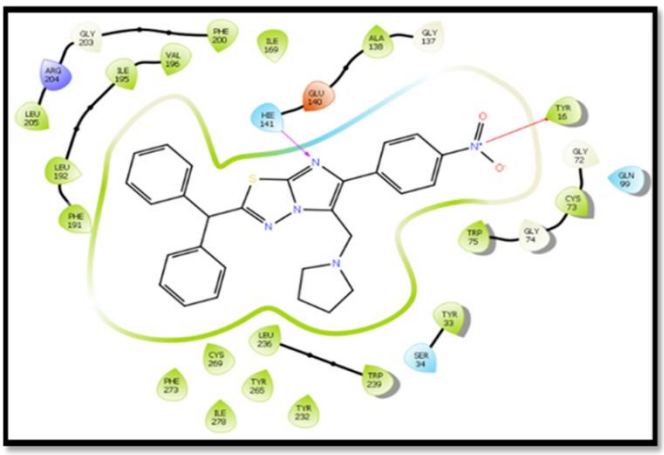
Cyclopropane mycolic acid synthase (1-1kpg)



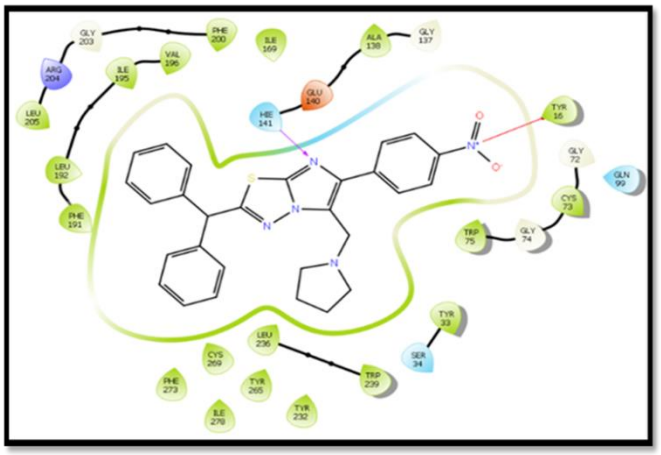
3a2



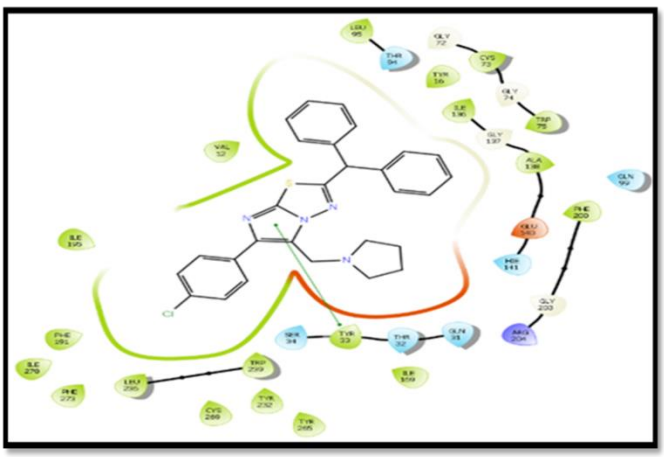
3a6



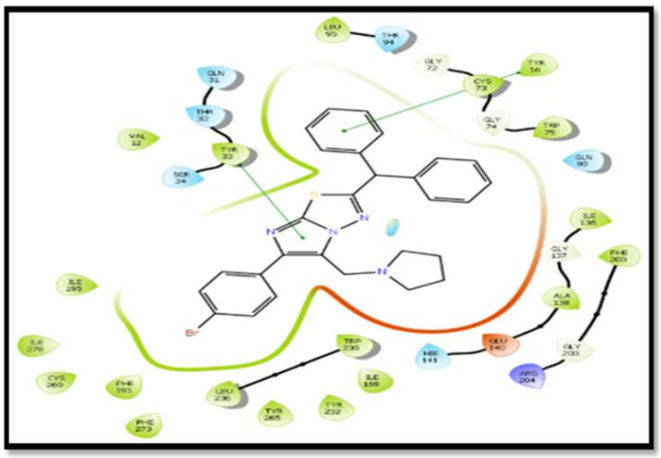
3b1



3b2

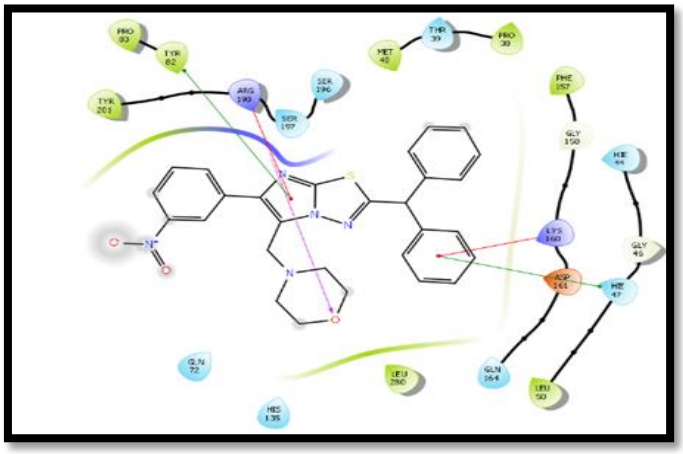


3b4

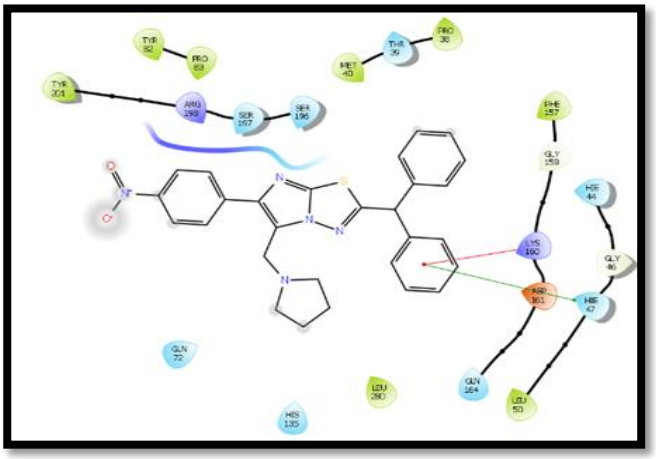


3b5

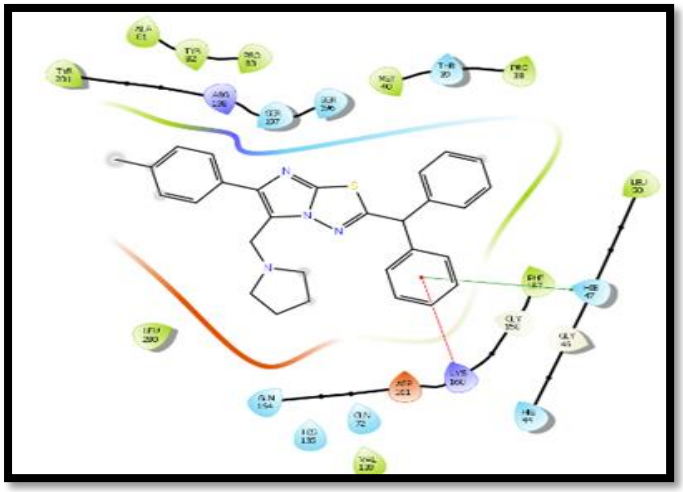
Pantothenatesynthetase (1n2 g)



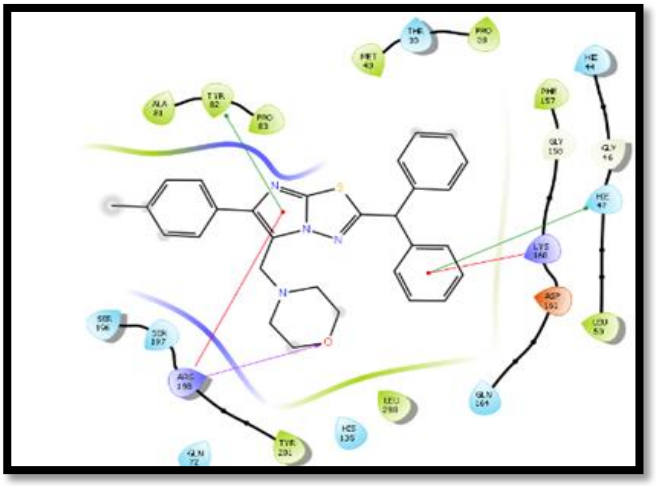
3a2



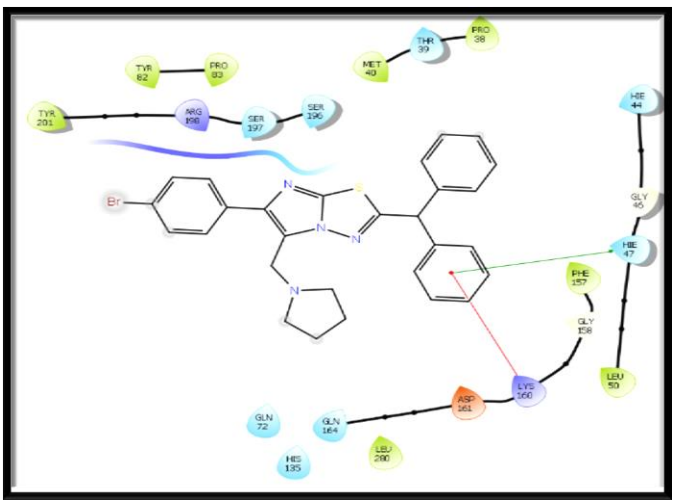
3b2

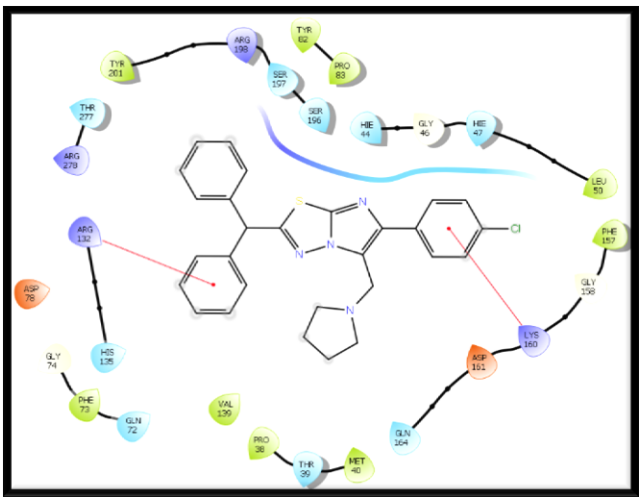


3a6



3b1





3b5 **3b4**

Table 2: Molecular docking result of synthesized compounds against Cyclopropane mycolic acid synthase and Pantothenate synthetase

	Cyclopropane mycolic acid synthase (1-1kpg)		Pantothenate synthetase (1n2 g)	
Compound	**Glide XP gscore**	**Glide Energy**	**Glide XP gscore**	**Glide Energy**
3a1	-6.04	-22.657	-5.453	-56.764
3a2	-6.95	-25.654	-5.538	-59.759
3a3	-6.87	-22.935	-5.112	-60.335
3a4	-6.85	-23.321	-5.324	-57.117
3a5	-6.95	-23.035	-5.436	-58.886
3a6	-6.148	-35.25	-5.249	-59.412
3b1	-7.142	-5.675	-5.151	-58.112
3b2	-6.95	-23.035	-5.331	-57.972
3b3	-6.86	-23.112	-5.254	-59.002
3b4	-7.248	-29.881	-4.462	-52.09
3b5	-6.762	-26.821	-5.141	-58.172
3b6	-6.82	-23.123	-5.101	-58.213
3c1	-6.44	-22.034	-5.151	-58.112
3c2	-6.75	-24.611	-5.235	-57.270
3c3	-6.77	-22.656	-4.254	-54.132
3c4	-6.76	-23.223	-4.061	-51.123
3c5	-6.83	-23.115	-5.261	-56.974
3c6	-6.144	-35.134	-5.224	-56.089

CONCLUSION

In the present study, a new series of imidazo[2,1-b][1,3,4]thiadiazole derivatives were designed, synthesized and screened for their *in-vitro* antitubercular activity. This study specifies that some of the compounds have shown effective antitubercular property. Molecular docking result of synthesized compounds against Cyclopropane mycolic acid synthase and Pantothenate synthetase also further confirms the potent efficacy of these compounds as anti-tubercular activity. Thus, with a minor modification of the fundamental structure, an improved antitubercular function can be accomplished. These conclusions will have a larger influence on young researchers for further study in the search for potent antimycobacterial scaffolds.

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