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Original Article

Synthesis, Characterization, Antitubercular Activity and Docking Studies of 2-(benzo [d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(substituted aryl)-4,5-dihydro-1H-pyrazol-1-yl) ethanone

Muralidharan Venugopal*, Raja Sundararajan, Asha Deepti Choppala
Department of Pharmaceutical Chemistry, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh-500035, INDIA.

ABSTRACT

A new series of 2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(substituted aryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**6a-6j**) were designed and synthesized from the intermediate chalcones (**1a-1j**). The synthesized compounds were characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and bases of elemental analysis. The agar dilution method *(In vitro M. tuberculosis* method) was employed for anti-tubercular screening. From the study, it was revealed that compounds **6b and 6h** showed increased potency. In phenyl ring attached to 4,5-dihydropyrazole ring presence of electron donating substituent like dimethylamino, hydroxy and methoxy moiety mightbe responsible for the powerful anti-mycobacterial activity displayed by derivatives **6b and 6h**. Further docking studies were performed to predict the interactions of the target compounds **6a-6j** within the *Mycobacterium tuberculosis* enoyl reductase enzyme by their scores and mode of binding to amino acids. In addition, drug-likeness score and molecular properties responsible for a good pharmacokinetic profile were calculated by Osiris property explorer and Molinspiration online toolkit, respectively. From the results, it was revealed that the synthesized compounds with electron releasing groups showed the most potent activity compared to that of standard drug.

Key words: Anti-tubercular activity, Benzo[d]oxazole-2-thiol, Chalcones, Docking studies, Pyrazolines.

Correspondence

Muralidharan V,

Department of Pharmaceutical Chemistry, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh-500035, INDIA.

Phone: +91 9866807070

Email: vmd1213@gmail.com

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INTRODUCTION

Tuberculosis (TB) is the most powerful communicable bacterial diseases caused by *Mycobacterium tuberculosis*.[1,2] It has a good impact on the history of infection on humankind and was called "Captain Among These Men of Death" in 18th and 19th century. It was declared as "Global Public Health Emergency" by WHO.[3,4] Highest total number of TB cases worldwide in 2010 was reported in India, in part due to poor disease management within the public and private health care sector. Programs such as the Revised National Tuberculosis Control Program are working to reduce TB levels among people receiving public health care.[5] The increase in the numbers of death cases were based upon the decrease in the efficacy of first four line drugs and increase in resistance to at least isoniazid and rifampicin which was called Multidrug Resistance tuberculosis (MDR-TB).

As a consequence of multiple mutations in specific resistant-associated genes of *Mycobacterium tuberculosis* (*inhA,katG, rpoB, gyrA, rrs, tlyA* and *eis*), extensively drug-resistant (XDR) TB has arisen.[6] It was shown that treatment of XDR-TB by using isoniazid and rifampicin plus a fluoroquinolone derivative and amikacin, kanamycin or capreomycin is ineffectual. In view of the frequency and emergence of MDR and XDR tuberculosis and consequences of acquired resistance to clinically employed drugs, researchers have persisted in performing synthesis and anti-tuberculosis evaluation of novel compounds bearing various chemical entities.

Pyrazoline (Dihydropyrazole) is a heterocyclic scaffold of interest to a synthetic chemist, as it has a broad spectrum of pharmacological activity and its ease of synthesis. Pyrazolines possess a sundry of activities including antimicrobial,[7] anti-diabetic,[8] antitumor,[9] antiarrythmic,[10] anti-inflammatory,[11] analgesic,[12] anti-malaria,[13] anti-oxidant,[14] CNS activity,[15] carbonic anhydrase inhibition,[16] as glycogen synthase kinase (GSK) inhibitors,[17] cyclin dependent kinase (CDK) inhibitors,[18] epidermal growth factor receptor (EGFR) inhibitors,[19] dual src/Ab1 kinase inhibitors.[20] Considering the findings above and in continuation of our efforts for the development of anti-tubercular agents, we undertook the design and synthesis of some novel prototypes which possess advantage of the two pharmacophores of pyrazolines and benzo*[d]*oxazole in single molecular backbone

MATERIALS AND METHODS

The organic solvents such as methanol, ethanol, acetone, chloroform, n-hexane and ethyl acetate were of spectral grade and used as such without further purification. Some of the solvents were purchased from the local distributors of S.D. Fine Chem. Ltd., Mumbai, India.

All the chemicals used in the synthesis were obtained from standard commercial sources. 4-fluoro-3-methyl-acetophenone was purchased from Avra chemicals. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade)

has been used for column chromatography. Each fraction of 100 ml was collected. The separations of the compounds were checked on TLC under UV lamp and also by spraying the plates with 10 % sulphuric acid.

All the melting points were determined in open capillaries, using Boitus digital melting point apparatus, expressed in °C and are uncorrected. The ^{1}H NMR spectra of the compounds were recorded on Bruker Ultra Shield (400 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [$(CH_3)_4Si$] as the internal standard. Chemical shift (δ) are expressed in ppm.

The Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 240c analyzer and were within ±0.4% of the theoretical values.

Experimental

Preparation of (E)-3-(substituted phenyl)-1-(4-fluoro-3-methylphenyl)prop-2-en-1-one (3a-3j)

The key intermediates (E)-3-(substituted phenyl)-1-(4-fluoro-3-methylphenyl)prop-2-en-1-one (3a-3j) were prepared according to the reported literature.[21] The starting material 4-fluoro-3-methyl acetophenone (2.5 mmol) was treated with aromatic aldehydes (2.5 mmol) in presence of catalytic amount of lithium hydroxide. Ethanol (20ml) was used as a solvent. The reaction mixture was kept for constant stirring using a multistage magnetic stirrer at room temperature until the solution turns turbid. The reaction was monitored by TLC (n-hexane: acetone - 7:3). Then the reaction mixture was poured into crushed ice and neutralized with the help of dil. HCl. The precipitate was filtered under vacuum, washed with cold ethanol and distilled water. The obtained chalcones were purified by recrystalization and column chromatography.

(E)-1-(4-fluoro-3-methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3a)

Greenish yellow crystals (EtOH), Yield = 81%; mp 101-103°C. FT-IR (KBr) cm^{-1}: 1657 (C=O, Chalcone), 1585 (C=C), 1149 (C-F), 2949 (C-CH_3), ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.358(s, 3H, CH_3), 7.430 (d, 1H, α-H), 7.921 (d, 1H, β-H), 7.42 (s, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.848 (d, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.433 (d, 1H, Ar-H), 7.972 (s, 1H, Ar-H). MS (EI) m/z: 247 (M^+). Anal. Calcd for $C_{14}H_{11}FOS$: C, 68.27; H, 4.50; F, 7.71; O, 6.50; S, 13.02.

(E)-1-(4-fluoro-3-methylphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3b)

Lemon yellow crystals (EtOH), Yield = 86%; mp 95-97°C. FT-IR (KBr) cm^{-1}: 1651 (C=O, Chalcone), 1585(C=C), 1244 (C-O-CH_3), 2924 (C-CH_3), 1122 (C-F). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.38(s, 3H, -CH_3), 3.952 (d, 9H, -OCH_3), 7.758 (d, 1H, α-H), 7.926 (d, 1H, β-H), 7.719 (s, 1H, Ar-H), 7.14(d, 1H, Ar-H), 6.889 (d, 1H, Ar-H), 7.12(s, 1H, Ar-H), 7.28(s, 1H, Ar-H). MS (EI) m/z: 331 (M^+). Anal. Calcd for $C_{19}H_{19}FO_4$: C, 69.08; H, 80; F, 5.75; O, 19.37.

(E)-3-(2-bromophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (3c)

Yellow crystals (EtOH), Yield = 76%; mp 100-102°C. FT-IR (KBr) cm^{-1}: 1658 (C=O, Chalcone), 1588(C=C), 2924 (C-CH_3), 1153(C-F), 752,9 (C-Br). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.384 (s, 3H, -CH_3), 7.374 (d, 1H, α-H), 8.155 (d, 1H, β-H), 7.119-7.93 (d, 7H, Ar-H). MS (EI) m/z: 319 (M^+). Anal. Calcd for $C_{16}H_{12}BrFO$: C, 60.21; H, 3.79; F, 5.95; O, 5.01; Br, 25.04.

(E)-1-(4-fluoro-3-methylphenyl)-3-(2-nitrophenyl)prop-2-en-1-on (3d)

Orange crystals (EtOH), Yield = 77%; mp 94-96°C. FT-IR (KBr) cm^{-1}: 1674 (C=O, Chalcone), 1513(C=C), 2878 (C-CH_3), 1292 (C-F), 1341 (C-NO_2). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.39 (s, 3H, CH_3), 7.86 (d, 1H, α-H), 8.09 (d, 1H, β-H), 7.585 (s, 1H, Ar-H), 7.173 (d, 1H, Ar-H), 7.71 (d, 1H, Ar-H) 8.15 (s, 1H, Ar-H), 8.112 (d, 1H, Ar-H), 7.582 (d, 1H, Ar-H), 7.928 (d, Ar-H). MS (EI) m/z: 284 (M^+). Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; F, 6.66; N, 4.91;O,16.83.

(E)-3-(4-bromophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (3e)

Yellow crystals (EtOH), Yield = 76%; mp 100-102°C. FT-IR (KBr) cm^{-1}: 1645 (C=O, Chalcone), 1593(C=C), 2941 (C-CH_3), 1103(C-F), 759 (C-Br). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.371 (s, 3H, -CH_3), 7.342 (d, 1H, α-H), 8.015 (d, 1H, β-H), 7.109-7.92 (d, 7H, Ar-H). MS (EI) m/z: 319 (M^+). Anal. Calcd for $C_{16}H_{12}BrFO$: C, 60.21; H, 3.79; F, 5.95; O, 5.01; Br, 25.04.

(E)-3-(3,4-dimethoxyphenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (3f)

Lemon yellow crystals (EtOH), Yield = 81%; mp 85-87°C. FT-IR (KBr) cm^{-1}: 1656 (C=O, Chalcone), 1583(C=C), 2930 (C-CH_3), 1254(C-F), 1142 (C-OCH_3). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 3.936 (d, 6H, -OCH_3), 7.739 (d, 1H, α-H), 7.905 (d, 1H, β-H), 7.38 (s, 1H, Ar-H), 7.25 (d, 1H, Ar-H), 7.864 (d, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.080 (d, 1H, Ar-H), 6.913 (d, 1H, Ar-H). MS (EI) m/z: 301 (M^+). Anal. Calcd for $C_{18}H_{17}FO_3$: C, 71.99; H, 5.71; F, 6.33; O, 15.98.

(E)-3-(4-chlorophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (3g)

Lemon yellow crystals (EtOH), Yield = 77%; mp 101-103°C. FT-IR (KBr) cm^{-1}: 1663 (C=O, Chalcone), 1591(C=C), 2960 (C-CH_3), 1243(C-F), 819 (C-Cl). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2,38 (s, 3H, CH_3), 7,591 (d, 1H, α-H), 7,93 (d, 1H, β-H), 7,58 (s, 1H, Ar-H), 7,433 (d, 1H, Ar-H), 7,61 (d, 1H, Ar-H), 7,60 (s, 1H, Ar-H), 7,75 (d, 1H, Ar-H), 7,47 (d, 1H, Ar-H). MS (EI) m/z: 275 (M^+). Anal. Calcd for $C_{16}H_{12}ClFO$: C, 69.95; H, 4.40; Cl, 12.91; F, 6.92; O, 5.82.

(E)-3-(4-(dimethylamino)phenyl)-1-(4-fluoro-3-methylphenyl)prop-2-en-1-one (3h)

Bright red crystals (EtOH), Yield = 83%; mp 93-95°C. FT-IR (KBr) cm^{-1}: 1651 (C=O, Chalcone), 1593(C=C), 2923 (C-CH_3), 1243(C-F). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 2.37 (s, 3H, CH_3), 3.06 (s, 6H, N(CH_3), 7.913 (d, 1H, α-H), 7.577 (d, 1H, β-H), 7.34 (s, 1H, Ar-H), 7.03 (d, 1H, Ar-H), 7.849 (d, 1H, Ar-H), 7.78 (s, 2H, Ar-H), 6.734 (d, 2H, Ar-H). MS (EI) m/z: 284 (M^+). Anal. Calcd for $C_{18}H_{18}FNO$: C, 76.30; H, 6.40; F, 6.71; O, 5.65; N, 4.94.

(E)-1-(4-fluoro-3-methylphenyl)-3-(4-hydroxy-3-methoxy-5-nitrophenyl) prop-2-en-1-one. (3i)

Pale yellow crystals (EtOH), Yield = 78%; mp 91-93°C. FT-IR (KBr) cm^{-1}: 1684 (C=O, Chalcone), 1546(C=C), 2944 (C-CH_3), 1103(C-F), 1230 (C-OCH_3), 1366 (NO_2), 3200 (OH). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) CH_3: 2.186 (s, 3H, CH_3), 1.727 (s, 1H, OH) 4.043 (s, 3H, -OCH_3), 7.664 (d, 1H, α-H), 8.245 (d, 1H, β-H), 7.285-8.245(m, 5H, Ar-H). MS (EI) m/z: 331,09 (M^+). Anal. Calcd for $C_{17}H_{14}NO_5$: C, 61.63; H, 4.26; F, 5.73; N, 4.23; O, 24.15.

(E)-3-(5-bromo-2-hydroxy-3-methoxyphenyl)-1-(4-fluoro-3-methylphenyl)prop-2-en-1-one (3j)

Orange crystals (EtOH), Yield = 69%; mp 93-95°C, FT-IR (KBr) cm^{-1}: 1656 (C=O, Chalcone), 1581(C=C), 2923 (C-CH_3), 1195 (C-F), C-OH (3243), C-OCH_3 (1255), C-Br (705): ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm): 1,617 (s, 3H, CH_3), 7,34 (d, 1H, α-H), 9,879 (s, 1H, β-H), 7,203 (d, 5H, Ar-H), 7,285(s, 1H, OH), 3,943(s, 3H, C-OCH_3): MS (EI) m/z: 365 (M^+): Anal. Calcd for $C_{17}H_{14}FBrO_3$: C, 55.91; H, 3.83; Br, 21.88; F, 5.20; O, 13.14.

Preparation of 3-(3-chloro-4-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole (4a-4j)

The previously synthesized (E)-3-(substituted phenyl)-1-(4-fluoro-3-methyl phenyl) prop-2-en-1-one (2.5mmol) was refluxed with hydrazine hydride (5mmol) upto 6 to 8h in presence of 20ml of glacial acetic acid.[22] Further, the reaction was monitored by TLC and the solvent was evaporated in vacuum. The crushed ice was added to the residue while mixing thoroughly after completion of the reaction. The precipitate formed was filtered under vacuum. The crude products were recrystallized from a suitable solvent to yield the analytically pure product.

3-(4-fluoro-3-methylphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole. (4a)

Dark greenish crystals (MeOH), Yield = 70%; mp 94 – 96°C. FT-IR (KBr) cm^{-1}: 1008 (C-F), 2941 (C-CH_3 Str), 1590 (C=N), 1463 (CH_2 bend), 3657 (Ar-CH). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.332 (s, 3H, CH_3), 1.213-1.304 (d, 2H, C_4-H Pyrazoline), 3.491 (t, 1H, C_5-H pyrazoline), 4.928 (d, 1H, NH), 6.87 – 7.38 (6H, Ar-H). MS (EI) m/z: 260 (M^+): Anal. Calcd for $C_{14}H_{13}FN_2S$: C, 71.40; H, 5.09; N, 8.33. Found: C, 71.25; H, 4.97; N, 8.24.

3-(4-fluoro-3-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole (4b)

Reddish crystals (MeOH), Yield = 62%; mp 102 – 104°C. FT-IR (KBr) cm^{-1}: 992 (C-F), 2924 (C-CH_3 Str), 1561 (C=N), 1468 (CH_2 bend), 3481 (Ar-CH), 1132 (O-CH_3). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.219 (s, 3H, CH_3), 1.246-1.251 (d, 2H, C_4-H Pyrazoline), 4.081 (t, 1H, C_5-H Pyrazoline), 3.813, 3.663, 3.522 (s, 9H, O-CH_3), 5.126 (d, 1H, NH), 6.75-7.72 (5H, Ar-H). MS (EI) m/z: 344 (M^+). Anal. Calcd for $C_{19}H_{21}FN_2O_3$: C, 66.27; H, 6.15; N, 8.13. Found: C, 65.86; H, 6.07; N, 8.04.

5-(2-bromophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazole (4c)

Reddish brown colour crystals (MeOH), Yield = 64%; mp 98 - 100°C. FT-IR (KBr) cm^{-1}: 1013 (C-F), 2944 (C-CH_3 Str), 1564 (C=N), 1355 (CH_2 bend), 3425 (Ar-CH), 771 (C-Br). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.311 (s, 3H, CH_3), 1.610-1.241 (d, 2H, C_4-H Pyrazoline), 3.911-3.938 (t, 1H, C_5-H Pyrazoline), 4.843 (d, 1H, NH), 7.08-7.68 (7H, Ar-H). MS (EI) m/z: 332 (M^+). Anal. Calcd for $C_{16}H_{14}BrFN_2$: C, 57.68; H, 4.24; N, 8.41. Found: C, 57.62; H, 4.01; N, 8.31

3-(4-fluoro-3-methylphenyl)-5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazole (4d)

Red crystals (MeOH), Yield = 70%; mp 89 - 91°C. FT-IR (KBr) cm^{-1}: 1081 (C-F), 2926 (C-CH_3 Str), 1574 (C=N), 1352 (CH_2 bend), 3309 (Ar-CH), 1527 and 1495 (C-NO_2, Str). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.335 (s, 3H, CH_3), 1.625 (d, 2H, C_4-H Pyrazoline), 4.059 (t, 1H, C_5-H Pyrazoline), 5.213 (d, 1H, NH), 6.92-7.72 (7H, Ar-H). MS (EI) m/z: 299 (M^+). Anal. Calcd for $C_{16}H_{14}FN_3O_2$: C, 64.21; H, 4.71; N, 14.04. Found: C, 64.18; H, 4.64; N, 14.01

5-(4-bromophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazole (4e)

Maroon colour crystals (MeOH), Yield = 68%; mp 104 - 106°C. FT-IR (KBr) cm^{-1}: 1171 (C-F), 2941 (C-CH_3 Str), 1583 (C=N), 1391 (CH_2 bend), 3321 (Ar-CH), 776 (C-Br). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.333 (s, 3H, CH_3), 1.610-1.243 (d, 2H, C_4-H Pyrazoline), 3.981-3.938 (t, 1H, C_5-H Pyrazoline), 5.016 (d, 1H, NH), 6.921-7.867 (7H, Ar-H). MS (EI) m/z: 333 (M^+). Anal. Calcd for $C_{16}H_{14}BrFN_2$: C, 57.68; H, 4.24; N, 8.41. Found C, 57.57; H, 4.16; N, 8.35.

5-(3,4-dimethoxyphenyl)-3-(4-fluoro-3-methylphenyl)-l-4,5-dihydro-1H-pyrazole (4f)

Yellowish crystals (MeOH), Yield = 70%; mp 84 - 86°C. FT-IR (KBr) cm^{-1}:1057 (C-F), 2981 (C-CH_3 Str), 1531 (C=N), 1383 (CH_2 bend), 3434 (Ar-CH), 1030 (O-CH_3). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.244 (s, 3H, CH_3), 3.944 (d, 6H, OCH_3), 1.551 (d, 2H, C_4-H Pyrazoline), 2.396 (t, 1H, C_5-H Pyrazoline), 4.964 (d, 1H, NH), 6.87-7.76 (6H, Ar-H). MS (EI) m/z: 314 (M^+). Anal. Calcd for $C_{18}H_{19}FN_2O_2$: C, 68.77; H, 6.09; N, 8.91. Found: C, 68.72; H, 5.99; N, 8.84

5-(4-chlorophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazole (4g)

Orange crystals (MeOH), Yield = 68%; mp 100 - 102°C. FT-IR (KBr) cm^{-1}: 1070 (C-F), 2924 (C-CH_3 Str), 1542 (C=N), 1436 (CH_2 bend), 3361 (Ar-CH). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.905 (s, 3H, CH_3), 2.44-2.680 (d, 2H, C_4-H Pyrazoline), 4.41-4.52 (t, 1H, C_5-H Pyrazoline), 5.121 (d, 1H, NH), 7.31-8.01 (7H, Ar-H). MS (EI) m/z: 288 (M^+), 290 (M^{+2}): Anal. Calcd for $C_{16}H_{14}ClFN_2$: C, 66.55; H, 4.89; N, 9.70. Found: C, 66.52; H, 4.81; N, 9.61

4-(3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylaniline (4h)

Orange red crystals (MeOH), Yield = 72%; mp 89 - 91°C, FT-IR (KBr) cm^{-1}:1024 (C-F), 2966 and 1357 (C-CH_3 Str), 1684 (C=N), 1609 and 1472 (C=C), 1392 (CH_2 bend), 3060 (Ar-CH). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.38 (s, 3H, CH_3), 3.15 (s, 6H, N-CH_3), 1.70-1.85 (d, 2H, C_4-H Pyrazoline), 3.61-3.84 (t, 1H, C_5-H Pyrazoline), 4.861 (d, 1H, NH), 6.71-7.72 (m, 7H, Ar-H). MS (EI) m/z: 297 (M^+). Anal. Calcd for $C_{18}H_{20}FN_3$: C, 72.70; H, 6.78; F, 6.39; N, 14.13. Found C, 72.64; H, 6.28; N, 14.06.

4-(3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxy-6-nitrophenol (4i)

Orange crystals (MeOH), Yield = 70%; mp 93-95°C. FT-IR (KBr) cm^{-1}: 1108 (C-F), 2921 and 1299 (C-CH_3 Str), 1651 (C=N), 1374 (CH_2 bend), 1017 (OCH_3), 3116 (Ar-CH), 3317(OH), 1554 and 1374 (C-NO_2, Str). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.365 (s, 3H, CH_3), 1.684-1.80 (d, 2H, C_4-H Pyrazoline), 4.42-4.58 (t, 1H, C_5-H Pyrazoline), 3.39(s, 3H, OCH_3), 5.27 (s, 1H, NH), 7.19-7.91 (5H, Ar-H). MS (EI) m/z: 346 (M^+). Anal. Calcd for $C_{17}H_{16}FN_3O_4$: C, 59.13; H, 4.67; N, 12.17. Found: C, 59.03; H, 4.56; N, 12.09

3-bromo-2-(3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-6-methoxyphenol (4j)

Greenish crystals (MeOH), Yield = 70%; mp 102 - 104°C. FT-IR (KBr) cm^{-1}:991 (C-F), 2882 (C-CH_3 Str), 1512 (C=N), 1464 (CH_2 bend), 3212 (Ar-CH). ^{1}HNMR(400 MHz, $CDCl_3$, δ ppm) 2.09 (s, 3H, CH_3), 1.41-1.90 (d, 2H, C_4-H Pyrazoline), 4.55-4.71 (t, 1H, C_5-H Pyrazoline), 3.412 (s, 3H, O-CH_3), 5.102 (d, 1H, NH), 5.87 (s, 1H, OH), 6.84-7.94 (5H, Ar-H). MS (EI) m/z: 378 (M^+). Anal. Calcd for $C_{17}H_{16}BrFN_2O_2$: C, 53.84; H, 4.25; N, 7.39; Found: C, 53.76; H, 4.18; N, 7.31

Preparation of 2-(benzo [d] oxazol-2-ylthio) - 1 - (3-(4-fluoro-3-methyl phenyl) -5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6a-6j)

3,5-Diaryl-2-pyrazoline (4a-4j) (2mmol) and triethylamine (2mmol) were added to acetone (30 mL) and dissolved with constant stirring. Later, the mixture was cooled in an ice bath and chloroacetylchloride (0.02 mol) was added drop wise with constant stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature. Benzo[d]oxazole-2-thiol (0.01 mol) and K_2CO_3 (0.01 mol) was added to the reaction mixture and refluxed for 8 h.[23] The reaction was monitored using TLC. After completion of the reaction, the reaction mixture was cooled and the solution was evaporated until dryness. The residue was washed with water and recrystallized from ethanol.

2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6a)

Yield = 76 %, m.p. 261-263°C. IR (KBr) cm^{-1}: 3029 (Ar-CH), 2970 (CH_3-CH), 1732 (C=O), 1639 (C=N), 1608 (C=C), 1254 (C-F) and 1026 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.06-8.12 (m, 7H, Ar-CH), 6.38-6.60 (m, 3H, CH of thiazole), 5.10-5.19 (t, 1H, C_5-H of pyrazole), 4.85 (s, 2H, SCH_2CO), 2.93 (s, 3H, CH_3), 1.98-2.07 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 451 (M^+). *Anal*. Calcd for $C_{23}H_{18}FN_3O_2S_2$: C, 61.18; H, 4.02; N, 9.31. Found: C, 61.07; H, 3.95; N, 9.12.

2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6b)

Yield = 74 %, m.p. 244-246°C. IR (KBr) cm^{-1}: 3058 (Ar-CH), 2963 (CH_3-CH), 1710 (C=O), 1648 (C=N), 1612 (C=C), 1223 (C-F) and 1032 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.09-8.04 (m, 9H, Ar-CH), 4.98-5.11 (t, 1H, C_5-H of pyrazole), 4.36 (s, 2H, SCH_2CO), 3.91 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 2.12 (s, 3H, CH_3), 1.58-1.80 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 535 (M^+). *Anal*. Calcd for $C_{28}H_{26}FN_3O_5S$: C, 62.79; H, 4.89; N, 7.85 Found: C, 62.68; H, 4.75; N, 7.75.

2-(benzo[d]oxazol-2-ylthio)-1-(5-(2-bromophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6c)

Yield = 72 %, m.p. 234-236°C. IR (KBr) cm^{-1}: 3095 (Ar-CH), 2961 (CH_3-CH), 1721 (C=O), 1649 (C=N), 1623 (C=C), 1229 (C-F), 1013 (C-O-C) and 623 (C-Br). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.09-8.05 (m, 11H, Ar-CH), 4.68-4.94 (t, 1H, C_5-H of pyrazole), 4.73 (s, 2H, SCH_2CO), 2.52 (s, 3H, CH_3), 1.69-1.94 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 525 (M^{+2}), 523 (M^+). *Anal*. Calcd for $C_{25}H_{19}BrFN_3O_2S$: C, 57.26; H, 3.65; N, 8.01. Found: C, 57.14; H, 3.60; N, 7.93.

2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6d)

Yield = 78 %, m.p. 256-258°C. IR (KBr) cm^{-1}: 3039 (Ar-CH), 2937(CH_3-CH), 1757 (C=O), 1642 (C=N), 1619 (C=C), 1536 and 1340 (NO_2), 1225 (C-F) and 1022 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 6.91-7.99 (m, 11H, Ar-CH), 4.67-4.90 (t, 1H, C_5-H of pyrazole), 3.71 (s, 2H, SCH_2CO), 2.36 (s, 3H, CH_3), 1.68-1.92 (d, 2H, C_4-H of pyrazole). EI-MS m/z: 490 (M^+). *Anal*. Calcd for $C_{25}H_{19}FN_4O_4S$: C, 61.22; H, 3.90; N, 11.42. Found: C, 61.15; H, 3.81; N, 11.36.

2-(benzo[d]oxazol-2-ylthio)-1-(5-(4-bromophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6e)

Yield = 76 %, m.p. 230-232°C. IR (KBr) cm^{-1}: 3045 (Ar-CH), 2926 (CH_3-CH), 1738 (C=O), 1645 (C=N), 1628 (C=C), 1154 (C-F), 1021 (C-O-C) and 684 (C-Br). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.04-8.12 (m, 11H, Ar-CH), 4.68-5.06 (t, 1H, C_5-H of pyrazole), 4.30 (s, 2H, SCH_2CO), 2.12 (s, 3H, CH_3), 1.57-1.92 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 525 (M^{+2}), 523 (M^+). *Anal*. Calcd for $C_{25}H_{19}BrFN_3O_2S$: C, 57.26; H, 3.65; N, 8.01. Found: C, 57.16; H, 3.61; N, 7.92.

2-(benzo[d]oxazol-2-ylthio)-1-(5-(3,4-dimethoxyphenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro -1H-pyrazol-1-yl)ethanone (6f)

Yield = 70 %, m.p. 239-241°C. IR (KBr) cm^{-1}: 3066 (Ar-CH), 2915 (CH_3-CH), 1752 (C=O), 1679 (C=N), 1619 (C=C), 1204 (C-F) and 1010 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.14-8.09 (m, 9H, Ar-CH), 4.86-5.06 (t, 1H, C_5-H of pyrazole), 4.58 (s, 2H, SCH_2CO), 3.61 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 2.04 (s, 3H, CH_3), 1.51-1.86 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 505 (M^+). *Anal*. Calcd for $C_{27}H_{24}FN_3O_4S$: C, 64.14; H, 4.78; N, 8.31. Found: C, 64.06; H, 4.70; N, 8.21.

2-(benzo[d]oxazol-2-ylthio)-1-(5-(4-chlorophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6g)

Yield = 78 %, m.p. 233-235°C. IR (KBr) cm^{-1}: 3091 (Ar-CH), 2946 (CH_3-CH), 1781 (C=O), 1621 (C=N), 1608 (C=C), 1161 (C-F), 1026 (C-O-C) and 684 (C-Cl). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.04-8.12 (m, 11H, Ar-CH), 4.68-5.06 (t, 1H, C_5-H of pyrazole), 4.30 (s, 2H, SCH_2CO), 2.12 (s, 3H, CH_3), 1.57-1.92 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 525 (M^{+2}), 523 (M^+). *Anal*. Calcd for $C_{25}H_{19}ClFN_3O_2S$: C, 62.56; H, 3.99; N, 8.76. Found: C, 62.45; H, 3.87; N, 8.67

1-(5-(4-dimethylaminophenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydropyrazol-1-yl)-2-(benzo[d]oxazol-2-ylthio)ethanone (6h)

Yield = 71 %, m.p. 226-228°C. IR (KBr) cm^{-1}: 3351 and 3220 (NH_2), 3044 (Ar-CH), 2934 (CH_3-CH), 1731 (C=O), 1664 (C=N), 1625 (C=C), 1228 (C-F) and 1045 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 6.80-7.51 (m, 11H, Ar-CH), 4.99-5.20 (t, 1H, C_5-H of pyrazole), 4.17 (s, 2H, NH_2), 3.82 (s, 2H, SCH_2CO), 3.11 (s, 6H, N-CH_3), 2.17 (s, 3H, CH_3), 1.68-1.85 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 460 (M^+). *Anal*. Calcd for $C_{25}H_{21}FN_4O_2S$: C, 65.20; H, 4.60; N, 12.17. Found: C, 65.12; H, 4.55; N, 12.10

2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(4-hydroxy-3-methoxy-5-nitro phenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethanone (6i)

Yield = 73 %, m.p. 233-235°C. IR (KBr) cm^{-1}: 3467 (OH), 3032 (Ar-CH), 2952 (CH_3-CH), 1739 (C=O), 1636 (C=N), 1627 (C=C), 1545 and 1352 (NO_2), 1234 (C-F) and 1031 (C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.23-8.19 (m, 9H, Ar-CH), 5.46 (s, 1H, OH), 4.82-5.11 (t, 1H, C_5-H of pyrazole), 4.09 (s, 2H, SCH_2CO), 3.50 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3), 1.89-2.04 (d, 2H, C_4-H of pyrazole). EI-MS m/z: 536 (M^+). *Anal*. Calcd for $C_{26}H_{21}FN_4O_6S$: C, 58.20; H, 3.95; N, 10.44. Found: C, 58.11; H, 3.85; N, 10.36.

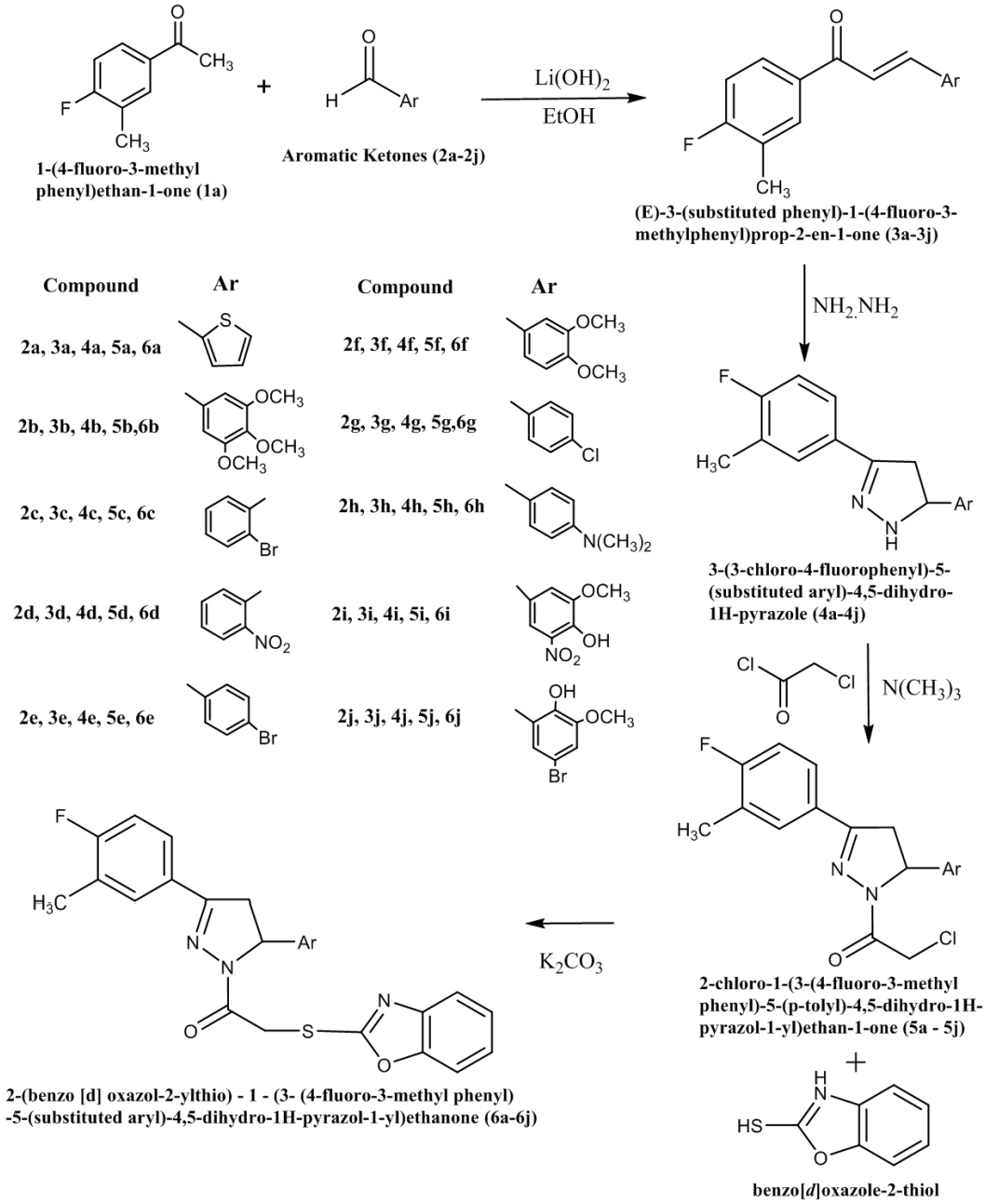
2-(benzo[d]oxazol-2-ylthio)-1-(5-(6-bromo-2-hydroxy-3-methoxyphenyl)-3-(4-fluoro-3-methyl phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6j)

Yield = 77 %, m.p. 263-265°C. IR (KBr) cm^{-1}: 3429 (OH), 3027 (Ar-CH), 2949 (CH_3-CH), 1715 (C=O), 1640 (C=N), 1601 (C=C), 1242 (C-F) 1027 (C-O-C) and 629 (C-Br). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ ppm: 7.18-8.35 (m, 9H, Ar-CH), 5.41 (s, 1H, OH), 4.83-5.06 (t, 1H, C_5-H of pyrazole), 4.06 (s, 2H, SCH_2CO), 3.49 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3), 1.73-1.98 (d, 2H, C_4-H of pyrazole). EI-MS *m/z*: 571 (M^{+2}), 569 (M^+). *Anal*. Calcd for $C_{26}H_{21}BrFN_3O_4S$: C, 54.74; H, 3.71; N, 7.37. Found: C, 54.57; H, 3.61; N, 7.25

Docking studies

To know the plausible mode of interactions of the target compounds 6a-6j within the *Mycobacterium tuberculosis* enoyl reductase enzyme, docking studies[24] were performed using the X-ray crystal structure of *Mycobacterium tuberculosis* enoyl reductase (INHA) complexed with reference inhibitor 1-cyclohexyl-n-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide having resolution 1.62 Å obtained from Protein Data Bank (PDB ID: 4TZK). Docking studies were performed using Autodoc 4.2 software. Preparation of enzymes (Addition of polar hydrogens, addition of AD4 type atoms, removal of water molecules and heteroatoms) were done with Autodoc 4.2 software as per default settings. Binding site was determined using the previous knowledge of the original ligand's interaction site. For the stimulation runs, each ligand was kept flexible, but the aminoacid residues of active site were kept rigid and default parameters values were taken.

The docking results reveals the energy associated with intermolecular interactions (affinity in kcal/mol) obtained upon computational docking



Scheme 1: Synthesis of Pyrazoline derivatives (6a-6j).

for all compounds (6a-6j) within INHA active site and hydrogen bonding interactions between the amino acid residues and functional groups of compounds are summarized in Table 2.

Calculation of pharmacokinetic parameters

Molinspiration online property calculation toolkit and Osiris property explorer were used to check the pharmaceutical fidelity of the drug candidates. Molecular descriptors, such as number of hydrogen bond donors, the number of hydrogen bond acceptors, the molecular mass of the compounds, topological polar surface area (TPSA), number of rotatable bonds, were calculated using Molinspiration online property calcula-tion toolkit. Percentage of absorption (%ABS) was calculated by: %ABS =109-[0.345× TPSA]

Calculation of toxicity potential

Osiris Property Explorer was used to analyze various attributes of the drugs, such as toxicity, drug-likeness, and drug score.

Biological Activity

Anti-tubercular activity

Anti-tubercular potency of title compounds were estimated by agar dilution method *(In vitro M. tuberculosis* method).[25,26] Using OADC growth supplement in Middle brook 7H11 agar slants each test analogs were incorporated in 10 fold serial dilutions. *M. tuberculosis* $H_{37}RV$ inoculums were prepared using OADC growth supplement in fresh Middle brook 7H11 agar slants adjusted to 1 mg/ml in 0.05 % tween 80 saline diluted to 10^{-2} (10^{7} cfu/ml concentration approximately). In 7H11 agar tubes per ml 10 fold serial dilutions of test analogs 5 µl

bacterial suspension was added. At 37°C incubated the tubes and after 28 days final readings were measured. Results obtained on test tubes (Test analog, medium and $H_{37}RV$) were compared with control tubes (Medium and $H_{37}RV$). The concentration at which complete inhibition of *M. tuberculosis* growth occurs is known as MIC (minimum inhibitory concentration). INH (Isoniazid), Rifampicin and Ethambutol was used as standard drug for comparing MIC of the title analogs and the obtained results are depicted in Table 1.

RESULTS AND DISCUSSION

Chemistry

The protocol for the synthesis of target compounds 6a-6j was shown in Scheme 1. In this study, a series of novel pyrazoline derivatives 6a–6j were synthesized by substituting various aromatic rings at 5-position and 2-benzo[d]oxazol-2-ylthio ethanone moiety at 1-position. By a multistep synthesis, a sequence of new 2-(benzo[d]oxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone 6a-6j were synthesized from aromatic aldehydes 2a-2j and 4-fluoro-3-methyl acetophenone 1a. Initially 4-fluoro-3-methyl acetophenone 1a was treated with various aromatic aldehydes 2a-2j to obtain chalcones 3a-3j by Clasein-Schmidt condensation reaction. Latter, obtained chalcones 3a-3j undergone reaction with hydrazine hydrate in Presence of glacial acetic acid and produced 3-(3-chloro-4-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole (4a-4j).

Table 1: MIC (Minimum inhibitory concentration in µg/ml) of synthesized compounds.

Compounds	*M. tuberculosis*
1	> 125
2	1.95
3	62.5
4	125
5	62.5
6	3.9
7	31.25
8	1.95
9	7.81
10	3.9
Isoniazid	0.12
Rifampicin	0.12
Ethambutol	1.95

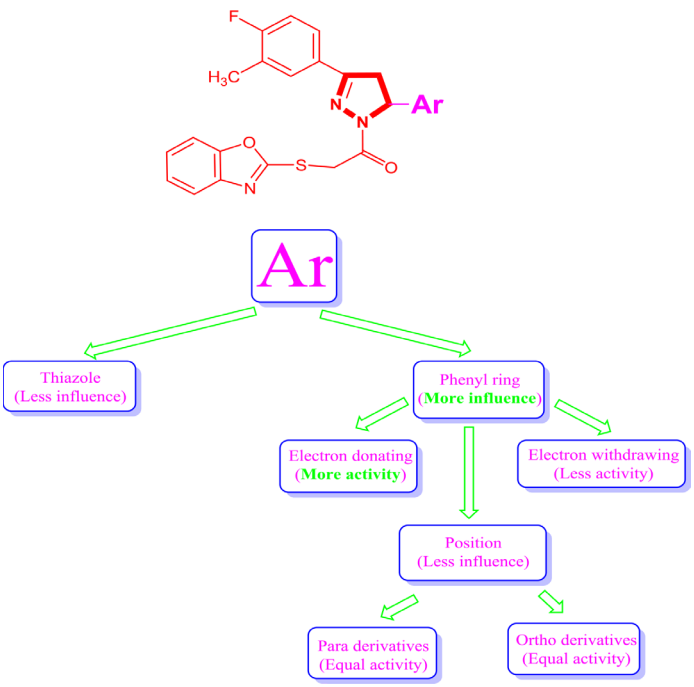


Figure 1: SAR of novel benzoxazole substituted 4,5-dihydropyrazoles (1-10).

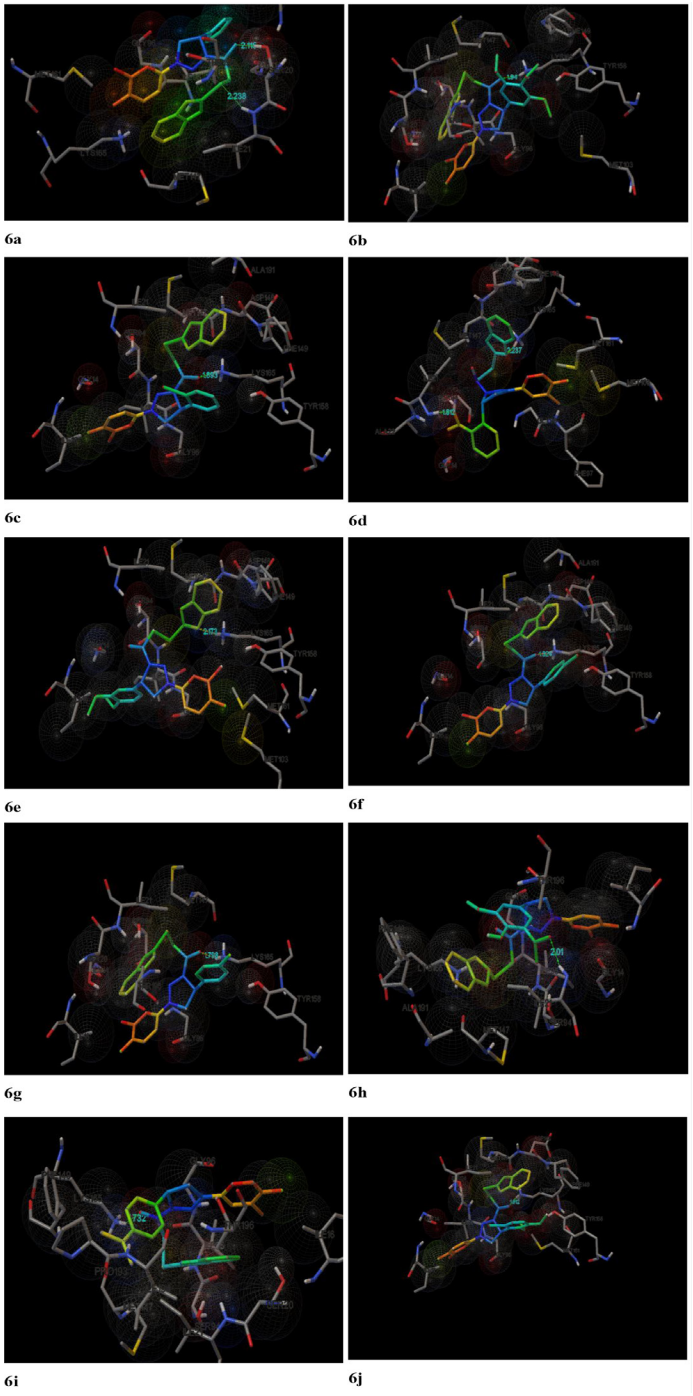


Figure 2: A view of docked poses between the synthesized compounds and the enzyme.

Table 2: Docking scores of the synthesized compounds.

S.No.	Compound	Docked energy (kcal/mol)	No.of hydrogen bonds	Distance (Å) from main residue	Aminoacid interactions
	6a	-8.02	2	2.115	Ser20
				2.238	Ile21
	6b	-9.12	2	2.032	Lys165
				1.94	Ser94
	6c	-8.6	1	1.893	Lys165
	6d	-9.92	2	1.812	ILE21
				2.237	Lys165
	6e	-8.56	1	1.824	Ser20
	6f	-8.99	1	2.173	Lys165
	6g	-8.92	1	1.798	Ser20
	6h	-10.48	2	2.01	Ser94
				2.238	Ser20
	6i	-7.96	1	2.01	Ile21
	6j	-8.25	1	1.92	Lys165

Table 3: Drug-likeness/scores and toxicity calculations of ginger compounds based on Osiris property explorer

Compound	TPSA	% ABS	HBD	HBA	n-ROTB	Drug-likeness	Drug Score	Mutagenic	Tumerigenic	Irritant	Reproductive effect
6a	112	70.36	0	5	5	1.39	0.51	green	green	green	green
6b	111.69	70.46	0	8	8	3.07	0.48	green	green	green	green
6c	84	80.02	0	5	5	-3.57	0.22	green	green	green	green
6d	129.82	64.21	0	8	6	-7.08	0.30	green	green	green	green
6e	84	80.02	0	5	5	-0.52	0.30	green	green	green	green
6f	102.46	96.8	0	7	7	1.83	0.48	green	green	green	green
6g	84	80	0	5	5	2.43	0.46	green	green	green	green
6h	87.24	78.9	0	6	6	-0.21	0.23	green	red	green	green
6i	159.28	54.14	1	10	7	-4.40	0.30	green	green	green	green
6j	113.46	69.85	1	7	6	-1.35	0.25	green	green	green	green

Note: topological polar surface area (TPSA); percentage of absorption (%ABS); number of hydrogen bond donors (HBD); number of hydrogen bond acceptors (HBA); number of rotatable bonds (n-ROTB).

In the succeeding step, compounds (4a-4j) were treated with triethyl amine, chloroacetylchloride and benzo[d]oxazole-2-thiol to yield the title compound (6a-6j). Thin-layer chromatography (TLC) was performed throughout the reactions to optimize the reactions for purity and completion.

Infrared (IR), nuclear magnetic resonance (NMR), mass spectra and elemental analyses of the synthesized compounds are in accordance with the assigned structures. The IR spectra of all synthesized compounds showed some characteristic peaks indicating the presence of particular groups. The formations of intermediate chalcones (2a-2j) were confirmed by the presence of IR peak in the region of 1700 to 1600 cm^{-1} range (CH=CH-C=O). The presence of fluoro group in the chalcones was characterized by the appearance of a strong band in its IR spectrum at 1149 cm^{-1}. The presence of methyl group was confirmed by ^{1}H NMR peak at δ 2.3 ppm and IR peak in the region of 2924 cm^{-1}. The IR peak in the region of 1530 cm^{-1} to1597 cm^{-1} range corresponds to C=N stretching vibrations with medium intensity indicates the formation of pyrazolines (4a-4j). Its ^{1}H NMR spectrum showed a singlet peak at a range of δ 1.226 – 2.70 ppm due to the proton at the fourth position in the pyrazoline ring confirms its formation. The appearance of IR peak at 1022 cm^{-1} reveals the attachment of 2-benzo[d]oxazol-2-ylthio ethanone to pyrazoline ring in the final product. The ^{1}H NMR spectrum showed a singlet peak at δ 4.09 ppm also supports thio ethanone linkage between pyrazoline and 2-benzo[d]oxazol. The disappearance of ^{1}H NMR peak at 4.96 proved the formation of the title compound (6a-6j). Further mass spectrum confirmed their purity and molecular weight.

Anti-tubercular Activity

In vitro anti-tubercular activity of all title analogs were screened against *M. tuberculosis* (H_{37}Rv strain) and MIC of entire tested analogs were determined and presented in Table 1. Simultaneously MIC of Isoniazid, Rifampicin and Ethambutol was also measured in order to control the sensitivity of the test organisms. From the results it was found that in varying degree synthesized compounds inhibited the growth of *M. tuberculosis*. Among various tested compounds, analogs such as 6b and 6h inhibited the growth of *M. tuberculosis* at a low concentration (MIC: 1.95 µg/ml) which is equal to standard drug Ethambutol. Compound 6b possess three methoxy groups and compound 6h possess strong

electron donating dimethylamino moiety at phenyl ring attached to 4,5-dihydropyrazole ring. MIC of test compounds 6f and 6j were found to be 3.9 µg/ml may be due to presence of hydroxy and methoxy substituent in phenyl ring. Even though, derivative 6i containing methoxy and hydroxyl moiety completely inhibited the growth of *M. tuberculosis* only at 7.81 µg/ml concentration because it also contains strong electron withdrawing nitro group. The MIC of analogs 6g was found to be 31.25 µg/ml and test compounds 6c and 6e were found to be 62.5 µg/ml. Only at higher concentration compounds 6a and 6d displayed activity (MIC: ≥125 µg/ml). The MIC of standard drugs isoniazid, Rifamipicin and Ethambutol were found to be 0.12 µg/ml, 0.12 µg/ml and 1.95 µg/ml, respectively.

Structural Activity Relationship

SAR of synthesized compounds is depicted in Figure 1. In this study overall it was found that title analogs 6b and 6h exhibited good anti-tubercular activity; title analogs 6f and 6j showed moderate anti-tubercular activity; whereas all other title analogs (6a, 6c, 6d, 6e, 6g and 6i) produced only less anti-tubercular activity. In phenyl ring attached to 4,5-dihydropyrazole ring presence of electron donating substituent like dimethylamino, hydroxy and methoxy moiety might be responsible for the powerful anti-bacterial activity displayed by derivatives 6b and 6h. Generally from the SAR study it was found that 4,5-dihydropyrazole derivatives possessing electron releasing group 6b, 6f and 6h, 6j exhibited superior anti-tubercular potency than corresponding 4,5-dihydropyrazole derivatives possessing electron withdrawing moieties 6c, 6d, 6e and 6g. while, thiazole analog 6a displayed least activity. In addition it was also found that position of the substituent doesn't play any important role in anti-microbial activity because ortho substituted analogs 6c showed equal activity to corresponding para substituted analogs 6e. Out of ten title compounds, the potent compounds was found to be 2-(benzoxazol-2-ylthio)-1-(3-(4-fluoro-3-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone 6b and 1-(5-(4-dimethylamino phenyl)-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-(benzoxazol-2-ylthio) ethanone 6h.

Docking Studies

The docking studies, of the title compounds were carried out with *Mycobacterium tuberculosis* enoyl reductase (INHA) (4TZK.pdb) by using Autodock 4.2 software. The interaction of molecule with *Mycobacterium tuberculosis* enoyl reductase (INHA) enzyme is shown in Figure 2. The docking results of the target compounds revealed that all the compounds were energetically favorable in terms of Autodock score (Table 2). The Autodock score of the most potent compounds, 6b and 6h were −9.12 and −10.48 respectively. The dock score of the less active compounds was found more when compared to the active compounds. These results indicate that the more potent compounds require less energy for good binding interaction with the receptor enzyme, whereas the less active compounds require more energy than the high active molecules. The binding interaction of the most active compound, 6b showed that the ketone linkage at N1 of pyrazoline binds with the amino acid residues LYS165 through hydrogen bonding. These binding interactions reveal the importance of the N1 ketone linkage for favorable binding interaction, so that better *Mycobacterium tuberculosis* enoyl reductase inhibitory activity is expected.

Toxicity risks and drug score assessment

Nowadays, it is much more convenient to predict the toxicity risks of compounds through reliable bioinformatics tools. In the present study, toxicity risks parameters such as mutagenicity, tumorigenicity, irritation, and reproductive or developmental toxicity of all the synthesized compounds (6a-6j) was calculated by using Osiris property explorer (Table 3). The predictions are based on the functional group similarity for the query derivatives with the *in vitro* and *in vivo* validated compounds present in the database of this online program. The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. The results can be visualized using color codes; green color shows low tendency of toxicity, yellow shows the mediocre, and red color shows potent tendency of toxicity. However, compound 6h indicated high risk of tumerogenicity. In addition, the bioavailability of synthesized compounds was judged through TPSA analysis.

This descriptor has been reported to correlate with passive molecular transport through membranes and therefore, allows prediction of transport properties of drugs and has been linked to drug bioavail¬ability. Veber's rule states that for good oral bioavailability of a drug, the number of rotatable bond must be ≤10, and TPSA values ≤ 140 Å2.27 The number of rotatable bonds has been shown to be a very good descriptor of oral bioavailability of the drugs. Rotatable bond is defined as any single non-ring bond, bounded to non-terminal heavy (ie, non-hydrogen) atom. Amide C–N bonds are not considered because of their high rotational energy barrier. Number of rotatable bonds was found to be appropriate in all the compounds. Percentage of absorption was estimated using the equation: %ABS =109-0.345× TPSA, according to Zhao *et al*.[28] TPSA was also calculated using Molinspiration online property calculation toolkit according to the fragment-based method of Ertl *et al*.[29]

CONCLUSION

In conclusion, we synthesized new 2-pyrazoline derivatives bearing 2-benzo[d]oxazol ring and aromatic substitutions by the reaction of chalcones with hydrazine hydride and 2-benzo[d]oxazol thiol. The structures of obtained compounds were confirmed by spectroscopic methods. All newly obtained pyrazoline derivatives were tested *in vitro* against *Mycobacterium* strain: $H_{37}Rv$. The most active compopunds against *Mycobacterium tuberculosis* are compounds 6b and 6h with trimethoxy phenyl group and 4-dimethylamino phenyl group. The molecular docking studies investigating pyrazoline derivatives using the enzyme *Mycobacterium tuberculosis* enoyl reductase (INHA) as their potential biological target indicated that the substituted group on pyrazoline spacer play an important role in interactions with the active site of INHA, LYS165, PHE149, ASP148, MET147, TYR158, ILE21 and SER20 as the most active amino acid residues. In the *in silico* studies, these test compounds showed minimum binding energy with *Mycobacterium tuberculosis* enoyl reductase (INHA) enzyme. So the present study provides us insight for the further development of better antitubercular agents as *Mycobacterium tuberculosis* enoyl reductase (INHA) inhibitors

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

FT:IR: Fourier Transmission Infra Red; **^{1}H:NMR:** Proton Nuclear Magnetic resonance; **TB:** Tuberculosis; **XDR:** extensively drug:resistant; **MDR:** Multiple drug:resistant; **CNS:** Central Nervous System; **TLC:** Thin Layer chromatography; **TPSA:** Topical Polar Surface Area; **INHA:** *Mycobacterium tuberculosis* enoyl reductase.

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