NOVEL PYRIMIDINE DERIVATIVES AS POTENTIAL INHIBITOR OF *MYCOBACTERIUM TUBERCULOSIS*: SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING STUDIES

Usha Rani Peddaboina[1], Anitha Sadhula[1], Ravinder Nath Anisetti[2*]

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Abstract

In this study a novel series of nitrogen containing imidazole linked pyrimidine 4-aryl-6-(4-(2-aryl-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)pyrimidin-2(1*H*)-one **(9a-d)** have been synthesized by the condensation of chalcones **(7a-d)** with urea **(8).** The precursor was synthesized by the reaction of 1-(4-(2-substituted-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethenone **(5a-c)** with arylaldehydes. The structures of the newly synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass spectral studies. MABA (Microplate alamar Blue Assay) assay was employed for assessing the antitubercular activity against the *Mycobacterium tuberculosis H37Rv* strain. Among the synthesized compounds **9b**, **9c** and **9d** showed excellent anti-tubercular activity than the reference (MIC-3.125 μg/ml) isoniazid. The results indicated that most of the synthesised derivatives exhibited prominent inhibitory activity against tuberculosis. The molecular docking analysis of the compounds **(9a-d)** by using PyRx 0.8 software. The molecular docking studies were conducted against the target of InhA compared the same with standard drug isoniazid. Among all the compounds **9d** (-11.8 kcal/mol) highest and **9b**, **9c** (-11.5 kcal/mol) showed excellent binding affinity against InhA. Binding affinity higher than the standard drug isoniazid.

Keywords: pyrimidine, imidazole, InhA, antituberculosis activity,

[1]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad-500 007, India.
[2*]Central University of Kashmir, Jammu &Kashmir - 191201, India. E-mail: ravindernathaniseti@yahoo.com, Tel.: 9912058076

***Corresponding Author:** Ravinder Nath Anisetti
*Central University of Kashmir, Jammu &Kashmir - 191201, India. E-mail: ravindernathaniseti@yahoo.com, Tel.: 9912058076

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INTRODUCTION

The development of drug resistance and multidrug resistance has risen momentously. Over the past five decades, varying degrees of resistance to existing TB drugs have emerged. Therefore, efforts have been directed toward exploring new, potent anti-TB agents with low toxicity profiles when compared with currently used anti-TB drugs [1-4]. This highlights the need to develop novel InhA inhibitors [5]. Mycobacterial enoyl reductase is a well-known target of Isoniazid (INH), a front-line anti-TB drug necessary for mycolic acids synthesis [6, 7]. Inhibiting mycolic acid synthesis is the fundamental way to kill the infectious agent mycobacterium tuberculosis.[7]

Heterocyclic compounds are more abundant and useful in synthetic and semi-synthetic chemistry due to their derivatives possesses unique biological properties which enable them to be used as drugs [8]. Pyrimidine is one such six membered basic nitrogenous heterocyclic rings of pharmacological significance. The derivatives of pyrimidines possess several activities such as anti-bacterial [9], anti-fungal [10], anti-oxidant [11-13], anti-cancer [14], anti-diabetic [15], antitubercular [16,17], anti-HIV [18], antimalarial [19], anti-protozoal [20] and anti-inflammatory [21] activities.

Compounds with imidazole moiety have many pharmaceutical activities. Diverse biological activities such as potent antibacterial activity, anti-inflammatory, anti-tubercular and antiviral activities have been found to be associated with 5-imidazolone derivatives. Recently, 1,2,4-trisubstituted-5-imidazolones have been reported to possess Mono Amino Oxidase [MAO] inhibitory and anticonvulsant activities [22-28]. The combination of two or additional structural fragments prompted to extend the potency of activity enlarged pharmacokinetic and dynamic properties than precursor as well as completely different /or twin response and reduced unwanted side effects [29].

Based on the above facts, we here reported the synthesis and in-vitro antimycobacterial activity of novel imidazole linked pyrimidine derivatives including binding affinity through molecular docking was described.

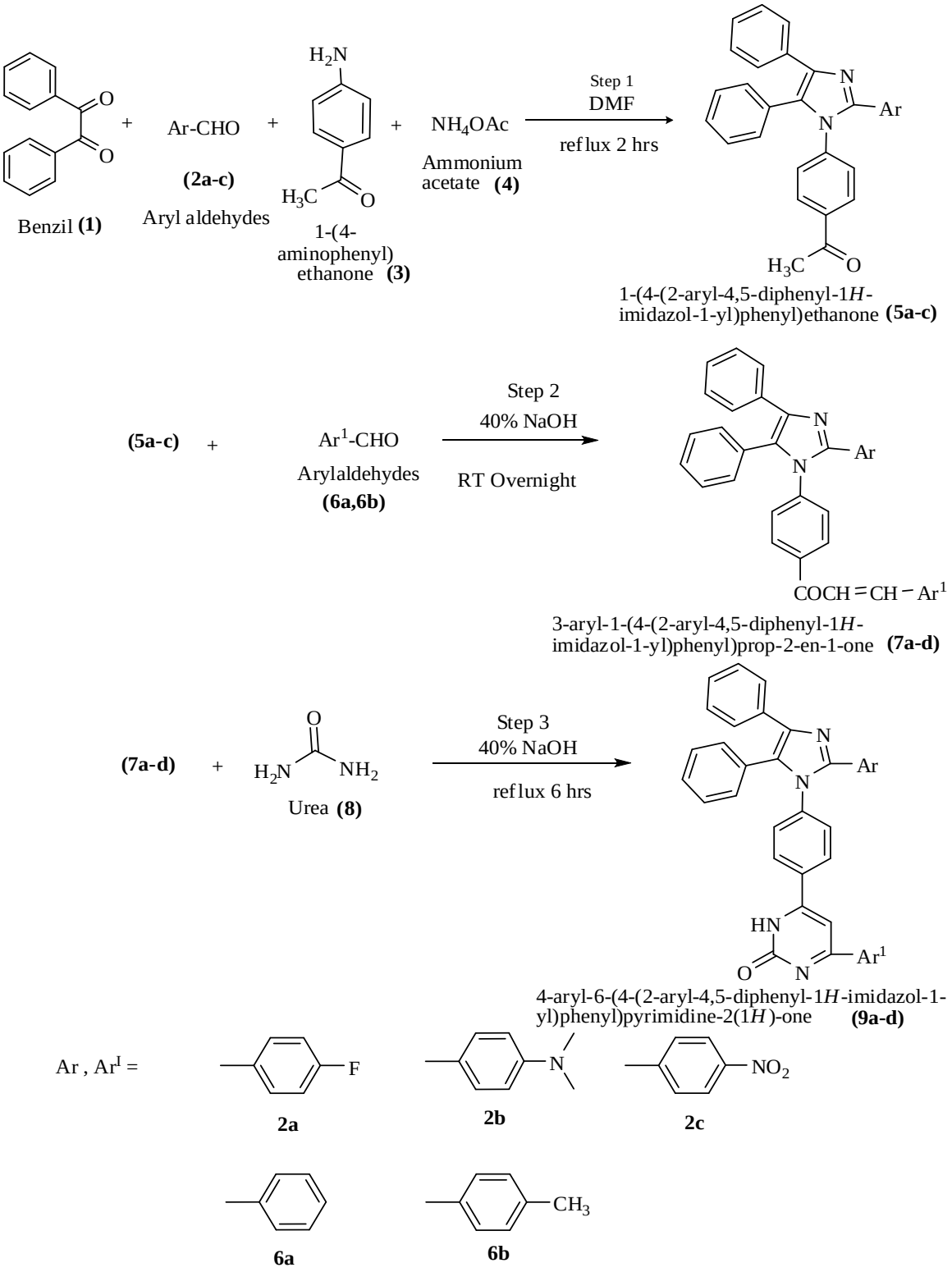
MATERIALS AND METHODS

All the chemicals were of AR grade and were obtained from Sigma–Aldrich and Merck. Melting points (m. p) were determined in open capillaries on Opti Melt automated melting point system and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) with silica gel F_{254} (Merck) with visualization by UV-light. The compounds are purified by using column chromatography on silica gel (60-120 mesh). The instruments used for obtaining the spectroscopic data were: OPTIZEN3220 UV-Visible spectrophotometer, FT-IR spectrophotometer SHIMADZU-435, ^{1}H NMR ($CDCl_3$, Avance300 MHz), ^{13}C NMR (CDCl3, Inova 75 MHz). Mass spectral analysis using electrospray ionization (ESI).

EXPERIMENTAL/ METHODOLOGY

The key intermediate 3-aryl-1-(4-(2-aryl-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)prop-2-en-1-one **(7a-d)** obtained by the reaction of 1-(4-(2-substituted-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethanone **(5a-c)** with aryl aldehydes **(6a,6b)** in presence of ethanolic sodium hydroxide solution. Reaction of **(7a-d)** with **(8)** in presence of ethanolic NaOH resulted in the formation of titled compounds i. e., 4-aryl-6-(4-(2-aryl-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)pyrimidin-2(1*H*)-one **(9a-d).** The synthetic approach is outlined in

Scheme-1.



Scheme-1.

Synthesis of 1-(4-(2-substituted-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethanone (5a-c)

Multi component reaction of Benzil **(1)** (0.01 mol), Aromatic aldehyde **(2a-c)** (0.01 mol), *p*- Amino acetophenone **(3)** (0.05 mol), Ammonium acetate **(4)** (0.05 mol) in presence of DMF (5 ml) was refluxed for 2hr. The completion of the reaction was monitored for every 1hr by TLC. Upon completion the reaction mixture was poured into crushed ice and stirred continuously to obtain a solid precipitate. The resulting crystalline precipitate of imidazole was filtered and washed with cold water and dried. The dried product was

recrystallized with ethanol. Purification of the compounds was done by column chromatography.

Synthesis of 3-aryl-1-(4-(2-aryl-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)prop-2-en-1-one (7a-d)

The obtained compound of 1-(4-(2-(substituted)-4,5-diphenyl-*1H*-imidazol-1-yl)phenyl)ethanone **(5a-c)** (0.01 mol) and arylaldehydes **(6a,6b)** (0.01 mol) was then stirred in Ethanol (20 ml) and then aqueous solution of 40% sodium hydroxide (10 ml) was added to it. The mixture was stirred for 6-8hr and kept overnight. After completion of reaction contents of the beaker were poured in ice-cold water and kept aside for few minutes. The separated solid was filtered, washed with cold water. Purification of the compounds was done by column chromatography.

Synthesis of 4-aryl-6-(4-(2-aryl-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)pyrimidin-2(1*H*)-one (9a-d)

condensation of chalcones **(7a-d)** with urea **(8)** in dilute ethanolic NaOH solution. Mixture was refluxed for 6hr. Completion of the reaction was monitored by TLC. The separated solid was filtered, washed with cold water followed by methanol. Purification of the compounds was done by column chromatography.

1-(4-(2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethanone (5a)

Red colour solid; Yield: 78%; m. p: 164-166 °C; IR (KBr, cm^{-1}): 3040 (Ar-H str), 2962 (aliphatic-CH str), 1720(C=O), 1620 (C=C str), 1484 (C=N str), 1046(C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm) : 7.81 (m, 6H, Ar-H), 7.50 (m, 6H, Ar-H), 7.23 (m, 6H, Ar-H), 6.98 (d, 1H, Ar-H), 2.50 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 198.54, 169.88, 166.49, 153.83, 144.46, 135.88, 131.82, 130.22, 129.40, 128.35, 128.29, 127.53, 125.82, 120.73, 116.92, 109.00, 26.44; ESI-MS (m/z): 432 $(M)^+$;

1-(4-(2-(4-(dimethylamino)phenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethanone (5b)

Red colour solid; Yield: 83%; m. p: 180-182 °C; IR (KBr, cm-1): 3091 (Ar-H str), 2928 (aliphatic-CH str), 1711(C=O), 1611 (C=C str), 1488 (C=N str), 1083 (C-N str); 1H NMR (300 MHz, CDCl3, δ/ppm) : 8.16 (d, 2H, Ar-H, J = 7.75 Hz), 7.70 (m, 2H, Ar-H), 7.45 (m, 7H, Ar-H), 7.05 (m, 3H, Ar-H), 6.80 (m, 5H, Ar-H), 3.03 (s, 3H, N-(CH3)2), 2.50 (s, 3H, -CH3); 13C NMR (75 MHz, CDCl3, δ/ppm): 198.51, 169.67, 166.08, 153.93, 142.00, 132.21, 128.58, 127.38, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 41.36, 27.51 ; ESI-MS (m/z): 457 (M)+;

1-(4-(2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethanone (5c)

Brown colour solid; Yield: 80%; m. p: 252-254 °C; IR (KBr, cm^{-1}): 3045 (Ar-H str), 2929 (aliphatic-CH str), 1717(C=O), 1599 (C=C str), 1464 (C=N str), 1052 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm) : 8.00 (m, 5H, Ar-H), 7.70 (m, 4H, Ar-H), 7.30 (m, 6H, Ar-H), 2.42 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 198.51, 167.19, 166.08, 142.00, 136.83, 131.76, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 27.51; ESI-MS (m/z): 461 $(M+2)^+$;

1-(4-(2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-3-phenylprop-2-en-1-one (7a)

Red colour solid; Yield: 69%; m. p (°C): 236-237; IR (KBr, cm^{-1}): 3045 (Ar-H str), 2950 (aliphatic-CH str), 1717 (C=O), 1643 (CH=CH str), 1094 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 8.00 (d, 1H, Ar-H,), 7.57 (m, 6H, Ar-H), 7.30 (m, 10H, Ar-H), 7.00 (m, 5H, Ar-H), 6.70 (d, 3H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 189.00, 163.24, 143.67, 141.36, 135.88, 134.91, 127.91, 127.82, 125.88, 120.95, 116.77, 107.57; ESI-MS (m/z): 521$(M+1)^+$

1-(4-(2-(4-(dimethylamino)phenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-3-p-tolylprop-2-en-1-one (7b)

Brick red colour solid; Yield: 80%; m. p (°C): 273-275; IR (KBr, cm^{-1}): 3054 (Ar-H str), 2924 (aliphatic-CH str), 1719 (C=O), 1625 (CH=CH str), 1064 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 8.19 (d, 1H, Ar-H,), 7.80 (m, 8H, Ar-H), 7.58 (m, 6H, Ar-H), 7.23 (m, 4H, Ar-H), 7.00 (m, 5H, Ar-H), 3.11 (s, 6H, -N(CH_3)$_2$), 2.30 (s, 3H, -CH_3),; ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 181.53, 164.46, 142.00, 136.83, 129.34, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 40.08, 21.79; ESI-MS (m/z): 560$(M+1)^+$

1-(4-(2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-3-p-tolylprop-2-en-1-one (7c)

Yellow Red colour solid; Yield: 74%; m. p (°C): 270-272; IR (KBr, cm^{-1}): 3023 (Ar-H str), 2935 (aliphatic-CH str), 1725 (C=O), 1637 (CH=CH str), 1091 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 7.90 (m, 5H, Ar-H), 7.60 (m, 8H, Ar-H), 7.40 (m, 6H, Ar-H), 7.20 (m, 6H, Ar-H), 2.40 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 188.32, 166.65, 131.18, 128.32, 126.37, 123.25,

121.68, 118.97, 111.35, 110.96, 21.68; ESI-MS (m/z): 561(M+)$^+$

1-(4-(2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-3-p-tolylprop-2-en-1-one (7d)

Yellow Red colour solid; Yield: 74%; m. p (°C): 270-272; IR (KBr, cm^{-1}): 3060 (Ar-H str), 2945 (aliphatic-CH str), 1735 (C=O), 1659 (CH=CH str), 1092 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 7.90 (m, 9H, Ar-H), 7.61 (m, 4H, Ar-H), 7.40 (m, 5H, Ar-H), 7.25 (m, 6H, Ar-H), 2.33 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 188.09, 163.12, 144.71, 138.73, 137.25, 130.05, 129.26, 129.20, 128.09, 127.82, 127.22, 121.46, 112.42, 112.00, 21.63; ESI-MS (m/z): 534(M+1)$^+$

6-(4-(2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-4-phenylpyrimidin-2(1*H*)-one (9a)

Dark red colour powder; Yield: 67%; m. p (°C): 220-222; IR (KBr, cm^{-1}): 3387 (N-H str), 3072 (Ar-H str), 2956 (aliphatic-CH str), 1672 (C=O str), 1593(CH=CH str), 1092 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 7.80 (m, 5H, Ar-H), 7.55 (m, 3H, Ar-H), 7.25 (m, 10H, Ar-H), 6.98 (m, 6H, Ar-H), 4.85 (s, 1H, -CH of Pyrimidine ring); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 164.54, 153.83, 144.46, 139.05, 131.23, 129.40, 128.35, 128.29, 127.53, 125.82, 120.73, 120.64, 116.92, 109.17, 109.00; ESI-MS (m/z): 560 (M)$^+$.

6-(4-(2-(4-(dimethylamino)phenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-4-p-tolylpyrimidin-2(1*H*)-one (9b)

Red colour powder; Yield: 72%; m. p (°C): 285-287; IR (KBr, cm^{-1}): 3330 (N-H str), 3050 (Ar-H str), 2941 (aliphatic-CH str), 1674(C=O str), 1591(CH=CH str), 1120 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 8.13 (s,1H,-NH), 7.82 (m, 6H, Ar-H), 7.56 (m, 8H, Ar-H), 7.30 (m, 4H, Ar-H), 7.06 (m, 5H, Ar-H), 4.91 (s, 1H, -CH of Pyrimidine ring), 3.01(s, 6H, -N(CH_3)$_2$), 2.40 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 164.01, 163.20, 156.17, 153.00, 150.94, 142.36, 135.01, 133.20, 132.85, 129.20, 128.17, 127.51, 125.60, 123.02, 119.75, 105.73, 40.34, 21.94; ESI-MS (m/z): 600 (M+1)$^+$.

6-(4-(2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-4-p-tolylpyrimidin-2(1*H*)-one (9c)

Red colour powder; Yield: 70%; m. p (°C): 264-266; IR (KBr, cm^{-1}): 3345 (N-H str), 3053 (Ar-H str), 2921 (aliphatic-CH str), 1656(C=O str), 1597(CH=CH str), 1107 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 8.20 (m,6H, Ar-H), 7.80 (m, 6H, Ar-H), 7.50 (m, 8H, Ar-H), 7.20 (m, 6H, Ar-H), 4.98 (s, 1H, -CH of Pyrimidine ring), 3.91(s, 6H, -N(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 164.71, 143.45, 138.97, 132.31, 131.84, 129.69, 128.32, 127.59, 126.90, 126.51, 125.15, 123.36, 120.97, 116.64, 114.78, 110.19, 97.24, 21.84 ; ESI-MS (m/z): 603 (M+2)$^+$.

6-(4-(2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)-4-p-tolylpyrimidin-2(1*H*)-one (9d)

Red colour powder; Yield: 65%; m. p (°C): 218-220; IR (KBr, cm^{-1}): 3375 (N-H str), 3072 (Ar-H str), 2965 (aliphatic-CH str), 1653(C=O str), 1609(CH=CH str), 1084 (C-N str); ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 8.20 (m, 4H, Ar-H), 7.96 (m, 4H, Ar-H), 7.56 (m, 8H, Ar-H), 7.11 (m, 7H, Ar-H), 4.99 (s, 1H, -CH of Pyrimidine ring), 2.50(s, 6H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm): 164.40, 158.58, 156.65, 142.00, 135.93, 131.76, 129.34, 128.54, 127.51, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 18.58; ESI-MS (m/z): 578 (M+2)$^+$.

ANTITUBERCULAR ACTIVITY

The antitubercular activity of all the test compounds was tested for against the *Mycobacterium tuberculosis H37Rv* strain, employing isoniazid as the reference standard according to the standard procedure described in the literature [30-33]. A frozen culture in Middlebrook 7H9 broth with the addition of 0.2% glycerol and 10% albumin-dextrose-catalase was thawed and diluted in broth to 10^5 CFU mL^{-1} (colony forming unit/mL) dilutions. Each test compound was dissolved separately in DMSO followed by dilution with broth to attain a concentration which was two times the required concentration. During this experiment, the final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and later incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility in opposition to a positive control (with Isoniazid) and negative control (without drug and inoculum). A broth dilution assay was utilized to determine the minimum inhibitory concentration (MIC) of each compound. MIC is defined as the lowest concentration of drug or a compound that inhibits ≤ 99% of the bacteria present at the start of the assay

MOLECULAR DOCKING

Molecular docking was done to establish a possible mode of action for the prepared imidazole linked pyrimidine derivatives **(9a-d)** in anti-

mycobacterial potential. It is used to predict ligand conformation which was produced best pose towards to favourable region in the active binding site of the receptor. Molecular docking studies were performed using PyRx 0.8 software

Setup of the system

Proteins InhA (PDB ID: 5G0W) from mycobacterium tuberculosis was retrieved from the RCB PDB bank. Proteins were prepared using protein wizard in AutoDock 4.2 (MGLtools 1.5.6) by deleting water molecules and ligands and adding polar hydrogen atoms and charges. The compounds chemical structures were drawn using the Chemoffice tool (Chemdraw 16.0) proper 2D orientation is assigned, energy minimization was done using ChemBio3D. drugs Isoniazid and tamoxifen were downloaded in .SDF format from Pubchem database. They were converted to PDB format by open babel.

Docking

The ligands with protein docking were performed using Pyrx software. The target is loaded into the software and set as macromolecules. Then all the ligands were loaded and docked with macromolecule in the PDBQT format. Grid box was generated for InhA and 7JXQ with proper dimensions. The position of grid box was set to InhA (X= -5.782, Y= 12.318, Z= 28.620). A maximum of 9 conformers were generated during the docking process.

Visualization using Discovery Studio Visualizer

Ligands and target receptor interactions were analyzed by selecting the conformations with least free binding energy. 2D and 3D interactions were analysed by Discovery Studio visualizer. H-bonds and the interacting residues are represented in ball and stick model, the ligands are represented in different colours **(Fig. 1 to 2)**. Molecular docking scores against compounds under study. Binding score, residual interactions, van der Waal interactions, and hydrogen bonds, of all the 4 compounds along with standard compounds against InhA (PDB ID: 5G0W) was tabulated in the **(Table 2).**

RESULTS AND DISCUSSIONS

The key precursors 1-(4-(2-substituted-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)ethenone**(5a-c)** required for the synthesis of target compounds was obtained by the reaction of benzil**(1)**, Aromatic aldehyde**(2a-c)**, *p*- Amino acetophenone**(3)** and Ammonium acetate **(4)**. IR spectrum of compounds showed absorption bands at 3091-3040 cm^{-1} due to aromatic C-H stretching, at 2962-2928 cm^{-1} due to aliphatic C-H, 1720-1711 cm^{-1} due to C=O group, 1620-1599 cm^{-1} due to C=C group and 1495-1484 cm^{-1} due to C=N stretching. ^{1}H NMR spectra of compounds showed singlet at δ 2.50-2.42 due to -COC<u>H</u>$_3$ protons, peaks at δ 8.16-6.80 integrated for aromatic protons and also some compounds **5b** showed singlet at δ 3.03 due to –N(C<u>H</u>$_3$)$_2$. In the ^{13}C NMR spectra of the compounds **(5a-c)**, the chemical shift values of carbon atoms appeared at δ 27.51-26.44 (aliphatic, -<u>C</u>H$_3$), δ 169.88-167.19 (cyclic <u>C</u>=O), δ 198.54-198.51 (aliphatic-<u>C</u>=O). In compound **5b** at δ 41.36 due to –N(<u>C</u>H$_3$)$_2$. The mass spectra of **(5a-c)** are in good agreement with the proposed structures. The key intermediates of 3-(4-(substituted)-1-(4-(2-(substituted)-4,5-diphenyl-*1H*-imidazol-1-yl)phenyl)prop-2-en-1-one **(7a-d)** were obtained by the reaction of 1-(4-(2-(substituted)-4,5-diphenyl-*1H*-imidazol-1-yl)phenyl)ethanone **(5a-c)** (0.01 mol) and Aromatic aldehyde **(6a,6b)** in presence of Ethanolic NaOH. IR spectra of compounds **(7a-d)** showed absorption bands in the region of 3060-3023 cm^{-1} due to aromatic C-H stretching, 2950-2924 cm^{-1} due to aliphatic C-H stretching, 1735-1719 cm^{-1} due to –C=O functional group. 1659-1625 cm^{-1} due to –CH=CH stretching and 1094-1064 cm^{-1} due to –C=N stretching. ^{1}H NMR spectra of compounds (**7a-d)** For all the compounds multiplet was observed between δ 8.19-6.70 due to aromatic protons (Ar-<u>H</u>), for compound **7b, 7c, 7d** singlet between δ 2.30, δ 2.40 and δ 2.33 respectively was observed due to Ar-C<u>H</u>$_3$ protons and for compound **7b** showed singlet at δ 3.11due to -N(C<u>H</u>$_3$)$_2$. In the ^{13}C NMR spectra of for all the compounds **(7a-d)** the chemical shift values of carbon atoms appeared at δ 166.65-163.12 due to cyclic amide-<u>C</u>ONH and δ 189.09–181.53 due to aliphatic –<u>C</u>=O functionality. Compounds **7b, 7c, 7d** showed chemical shift values of carbon appeared at δ 21.68, 21.79, δ 21.63 respectively due to –<u>C</u>H$_3$ and for compound **7b** at δ 40. 08 due to –N(<u>C</u>H$_3$)$_2$ clearly confirms the final products formation **(7a-d)**, moreover the mass spectrum of **(7a-d)**, agrees with the molecular weight of the proposed structure.

Reaction of 3-(4-(substituted)-1-(4-(2-(substituted)-4,5-diphenyl-*1H*-imidazol-1-yl)phenyl)prop-2-en-1-one **(7a-d)** with urea **(8)** in the presence of ethanolic NaOH resulted in the formation of titled compounds i.e., 4- substituted 6-(4-(2-argio-4,5-diphenyl-1*H*-imidazol-1-yl)phenyl)pyrimidin-2(1*H*)-one **(9a-d).** IR spectra of compounds **(9a-d)** showed absorption bonds in the region of 3384-3330cm^{-1} due to N-H stretching, 3072-3050 cm^{-1} due to aromatic –CH- stretching. 2965-2921 cm^{-1}

due to aliphatic C-H stretching 1674-1653 cm^{-1} due to C=O functionality, 1609-1591 cm^{-1} due to C=C stretch and 1120-1084 cm^{-1} due to C-N stretching, indicating the evidence for the formation of the final compounds **(9a-d)**. ^{1}H NMR spectra for all the compounds showed singlet at δ 8.13- 8.00 due to Pyrimidine–NH, and δ 8.20-6.98 due to aromatic protons, and all compounds showed singlet in the region δ 4.99- 4.85 due to –CH of Pyrimidine. ^{1}H NMR spectra of compounds **9b, 9c, 9d** showed singlets at δ 2.40, δ 2.42 and δ 2.5 respectively was observed due to Ar-CH_3 protons and for compounds **9b** showed singlet t δ 3.01respectively due to -$N(CH_3)_2$. In ^{13}C NMR spectra of all the derivatives **(9a-d)** observed at δ 165.01-164.40 due to cyclic imine –C=N, δ163.20-158.58 due to cyclic –C-N, δ156.65-152.54 due to cyclic amide –CONH, functionality clearly confirms the final products formation **(9a-d)**. Compounds **9b, 9c, 9d** showed singlets at δ 21.94, δ 21.84 and δ 18.58 respectively was observed due to Ar-CH_3 protons and for compounds **9b** showed singlets at δ 40.34 due to -$N(CH_3)_2$. More over the mass spectrum of **(9a-d)** agrees with the molecular weights of the proposed structures.

Based on the results in (**Table 1)** that all synthesized compounds were shown excellent to good (100 µg /ml, 50 µg /ml, 25 µg /ml, 12.5 µg /ml, 6.25 µg /ml, 3.125 µg /ml, 1.562 µg /ml and 0.78 µg /ml) anti mycobacterial activity (H37RV strain) by dilution technique against standard drug Isoniazid. Imidazole linked pyrimidine compounds were shown potent activity. The compounds **9b, 9c & 9d** were shown more potent activity with MIC (0.78 µg/ml). Four folds more active than standard drug compound **9a** shown good activity with MIC (3.125 µg/ml) was equal to standard (Isoniazid) MIC (3.125 µg/ml).

Table 1: Antitubercular results of imidazole linked pyrimidine derivatives

Compound	Antitubercular activity (MIC in µg/mL) Of M. tuberculosis
9a	3.125
9b	0.78
9c	0.78
9d	0.78
Isoniazid	3.125

The PyRx 0.8 software was applied to study the interaction of compounds with the active site of InhA (PDB ID: 5G0W) against pyrimidine derivatives. The molecular docking analysis of the compounds under study was performed to know the binding pattern of compounds with InhA and compared with the standard drug isoniazid. **9d** being the highest binding affinity with the minimum binding energies (-11.8 kcal/mol), compounds **9b** and **9c** were shown good binding affinity (-11.5 kcal/mol) and compound **9a** shown (-11 kcal/mol). Compounds **9a, 9b, 9c** and **9d** were shown highest binding affinities than that standard drug isoniazid and its binding affinity is -5.7 Kcal/mol. Binding score, residual interactions, van der Waal interactions, and hydrogen bonds of all the 4 compounds along with standard compounds were depicted in the **table 2**. **Fig. 1** shown the Binding interactions of compound **9d** with InhA (5G0W). **Fig. 2** shown the Binding interactions of Isoniazid with InhA (5G0W)

Table 2: Molecular docking studies of InhA (PDB ID: 5G0W) against pyrimidine derivatives

Molecule	Binding score	Hydrogen bonds	Hydrophobic interactions	Vanderwaal interactions
9a	-11	Ala-198, Gln-100	Phe-41,Gly-14,Ile-95,Ile-16,Leu-197,Ile-122,Phe-97,Thr-196*,Gln-100,Ala-198	Met-199,Met-98,Met-103,Pro-99,Gly-96,Ile-202,Gly-40
9b	-11.5	Thr-196, Gly-14	Leu-197, Ala-198, Ile-16,Val-65,Ile-122,Phe-41,Ile-95,Met-199	Gln-66,Asp-64,Thr-17,Tyr-158,Pro-193,Ile-194,Lys-165,Ile-21,Ser-20,Gly-96,Met-147,Ser-94,Ile-15,Ile-47,Gly-40,Asp-94

9c	-11.5	Thr-196, Gly-14	Leu-197, Ala-198, Met-199,Val-65,Ile-122,Phe-41,Ile-95,Ile-16	Gln-66,Asp-64,Thr-17,Ile-194,Tyr-158,Ile-21,Lys-165,Ser-20,Gly-96,Ile-15,Ile-47,Gly-40
9d	-11.8	Thr-196, Gly-14	Leu-197,Ala-198,Met-199,Ile-16,Ile-95,Phe-41,Val-65,Ile-122	Gln-66,Asp-64,Gly-40,Ile-15,Ile-47,Gly-96,Ser-20,Ser-94,Lys-165,Ile-21,Ile-194,Pro-193,Tyr-158,Met-147
Isoniazid	-5.7	Thr-39,Leu-63	Ile-95,Ile-122,Phe-41,Val-65,	Ser-13,Asp-64,Gly-40,Gly-14

Fig. 1: Binding interactions of pyrimidine derivative **9d** with InhA (5G0W)

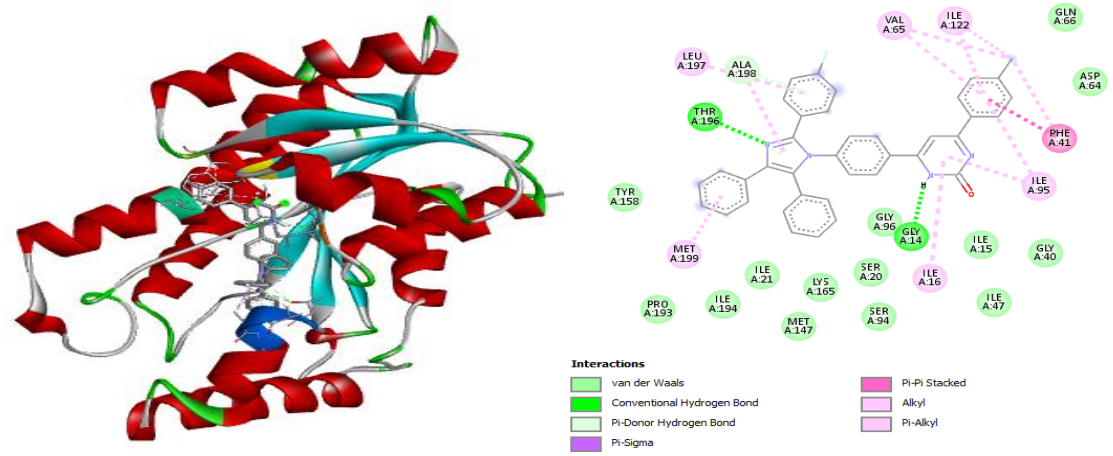
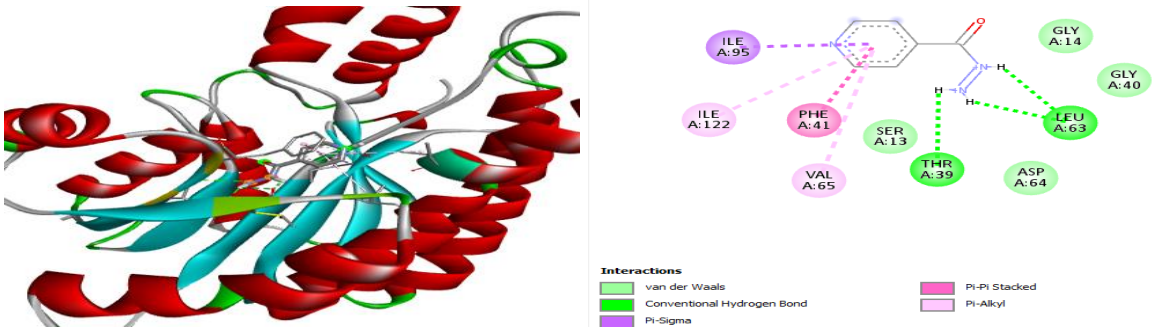


Fig. 2: Binding interactions of Isoniazid with InhA (5G0W)



CONCLUSIONS

In the present work, we prepared imidazole linked pyrimidine derivatives using the conventional method and then performed their antitubercular against *Mycobacterium tuberculosis* H37Rv strain. The in-vitro activity results were promising and they showed good to excellent activity than the standard drug isoniazid. Molecular docking studies revealed that compounds **9d, 9b** and **9c** had more potential to interact with selected InhA than standard which are expressed on docking score. We state that compounds **9d, 9b** and **9c** should be promising compounds and can be later developed as potential drugs for the treatment of *Mycobacterium tuberculosis* infections.

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