



# **Synthesis, Characterization, Anti-Mycobacterial Evaluation and *In-Silico* Molecular Docking of Novel Isoxazole Clubbed Pyrimidine Derivatives**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Author RS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. This total research work was managed by author NP. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

In the present research, we tend to ready a series of novel pyrimidine-linked isoxazole derivatives (11-20). The molecular structure and the elemental composition of these compounds were confirmed by spectroscopic studies and elemental analysis. MABA (Microplate alamar Blue Assay) assay was employed for assessing the antitubercular activity against the Mycobacterium tuberculosis H37Rv strain. Among the ten synthesized compounds, 18 and 20 showed excellent anti-tubercular activity than the reference (MIC-3.125 µg/ml) at 0.78 µg/mL. The compounds were found to possess good binding affinity than a standard against thymidylate kinase enzyme (PDB-1MRS) as evidenced by the molecular docking studies. Additionally, the bioactivity was conducted by Mol-inspiration software tool and the drug-likeness property was evaluated on Lipinski's rule of five by SCFBio online software. The lead compounds identified through these studies could be useful for the furtherance of the drug discovery process in the area of antitubercular research.

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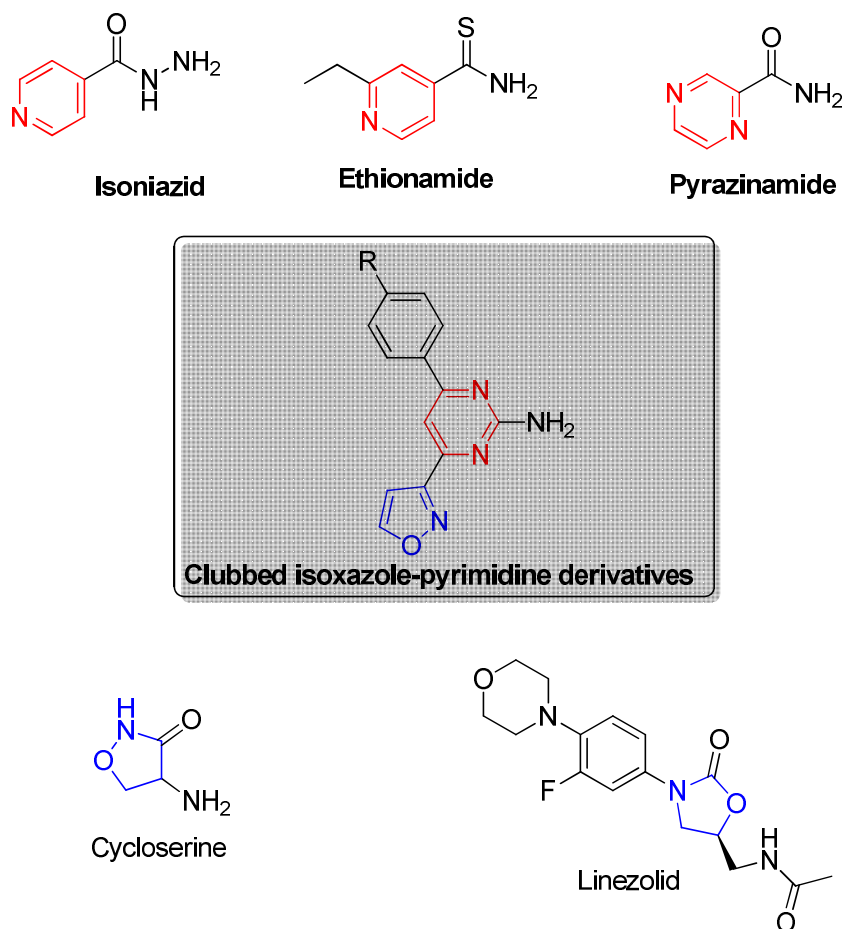
## 1. INTRODUCTION

Mycobacterium Tuberculosis (TB) is the second most noteworthy sickness that infected nearly 10 million folks and roughly 2 million folks of the planet population die due to tuberculosis. The expansion of recent cases recognitions to drug resistance of Mycobacterium TB and limitations with the marketed medication had directed analysis interest within the style and development of novel antitubercular agents [1].

In TB drug revelation network actually experiences a deficiency of approved restorative procedures involved novel organic targets and great little particles that regulate them [2]. Antitubercular drugs including isoniazid, pyrazinamide, and ethionamide contain six-

membered heterocyclic nitrogen rings making these types of scaffolds attractive in the synthesis of newer agents with improved anti-tubercular activities [3].

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 [4]. Pyrimidine is one such six-membered basic nitrogenous heterocyclic rings of pharmacological significance. The derivatives of pyrimidines possess several activities such as anti-bacterial [5], anti-fungal [6], anti-oxidant [7-9], anti-cancer [10], anti-diabetic [11], anti-tubercular [12,13], anti HIV [14], antimalarial [15], anti-protozoal [16], anti-inflammatory [17] and anti-fungal [18] activities.



**Fig. 1. Structures of marketed antitubercular drugs with heterocyclic ring**

According to previously reported that several authors prepared different pyrimidine derivatives i.e. tri substituted pyrimidines [19], pyrrole [3, 4-d] pyrimidines [20] and 2, 4-diamino-5-[2-methoxy-5-( $\omega$ -carboxyalkyloxy) benzyl] pyrimidines [21] were shown considerable anti-mycobacterial activity. Amino group present in 2<sup>nd</sup> position of pyrimidine ring reacts with different aldehydes produced imine (-C=N-) was shown potent activity, alongside 4<sup>th</sup> and 6<sup>th</sup> substitution plays major role in anti-TB activity [22,23]. Fortunately, varieties of latest potential antitubercular candidate's medication with heterocyclic rings, that are presumably to be effective against resistant strains, have entered clinical trials in current years [24].

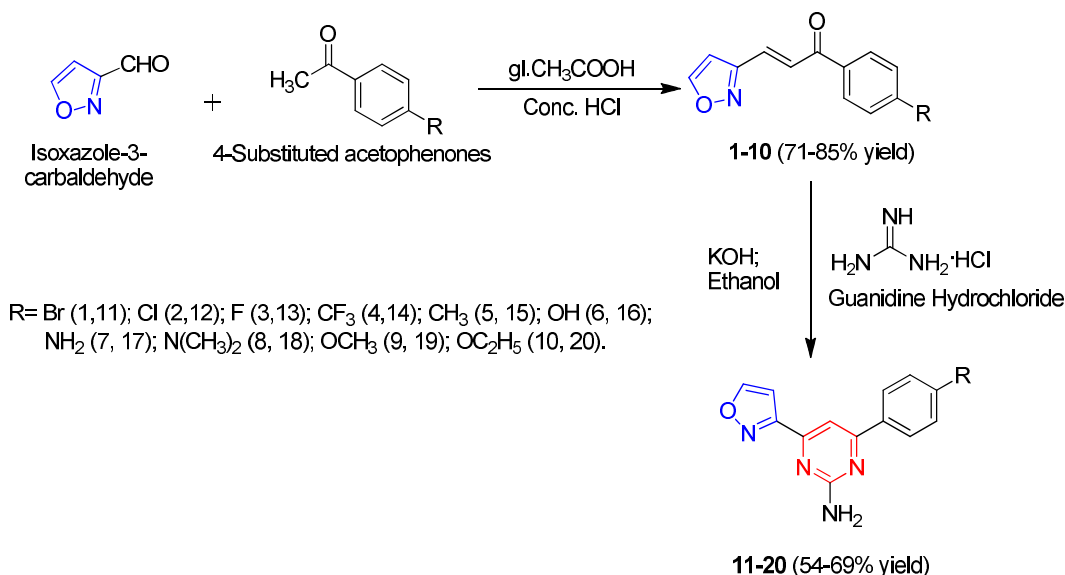
The reduced isoxazole ring i.e. isoxazolidine and its isomer, oxazolidine rings are seen in drugs like cycloserine and linezolid (Fig. 1) [25]. The linezolid is structurally clubbed oxazolidine (5-membered heterocyclic) derivative approved in the treatment of multidrug resistance TB by the FDA approved in year of 2016 [26]. 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 [27].

Because of the above facts, we here reported the synthesis and antitubercular evaluation of

pyrimidine clubbed isoxazole derivatives to develop better agents against tuberculosis. Herein the synthesis and in-vitro anti-mycobacterial activity of novel clubbed isoxazole-pyrimidine derivatives (Fig. 1) including drug-likeness, bioactivity score studied, and binding affinity through molecular docking was described.

## 2. MATERIALS AND METHODS

Laboratory and analytical grade synthetic chemical reagents and solvents were purchased from National Scientific in Guntur, Andhra Pradesh, India. Detection of compounds by using Thin Layer Chromatography (TLC) which are pre-coated commercial silica gel aluminium plates (Merck-F524). Melting points of synthesized compounds are detected by using the melting point apparatus (Bio-Technics, Mumbai, India) by the open capillary method. Further elemental and spectral analysis carried by the elemental analyzer and then Infrared spectra were recorded by the KBR pellet method by using FTIR as expressed in cm<sup>-1</sup> which is manufactured by Bruker. Advanced NMR (1H and 13C) used to evaluate the nature of hydrogens and carbons (hydrocarbons) with suitable solvent i.e., chloroform and TMS as an internal standard and expressed in parts per million ( $\delta$ ppm). The molecular mass of synthetic structures was derived on (Carlo Erba-1108) micro instrument by mass spectra analyzer and elemental analyzer used to quantify C, H, N, O and extra elements.



**Scheme 1. Synthesis of clubbed isoxazole-pyrimidine derivatives**

## 2.1 Chemistry

*General procedure for the synthesis of target compounds (11-20):* Acid catalyzed condensation of 4-substituted acetophenones (1 mmol) with Isoxazole-3-carbaldehyde afforded chalcones [28] (1-10). To an equimolar solution of previously reported [29] (*E*)-1-(4-substitutedphenyl)-3-(isoxazol-3-yl)prop-2-en-1-one derivatives (1 mmol) (1-10) and guanidine hydrochloride (1 mmol) in absolute ethanol (20 mL), alcoholic potassium hydroxide (0.3 mL) was added drop wise at room temperature. The reaction mixture was refluxed for 8-10h and the solvent was evaporated completely. Then, the reaction mixture was poured into ice-cold water and the solid that separated out was filtered, dried and purified by column chromatography with ethyl acetate/hexane and crystallized from chloroform to give compounds [30] **11-20** (Scheme 1).

**4-(4-bromophenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (11):** Yield 64%, m.p.: 210-212°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3346 ( $\text{NH}_2$ ), 1628 ( $\text{C}=\text{N}$ ), 1585 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.31 (1H, s, C-5-H), 5.31 (2H, s, C-2-NH<sub>2</sub>), 7.19-7.95 (6H, Ar-H). Elemental analysis for  $\text{C}_{13}\text{H}_9\text{BrN}_4\text{O}$ : Cal: C, 49.28%; H, 2.91%; Br, 25.28%; N, 17.71%; O, 5.09%; found: C, 49.23%; H, 2.86%; Br, 25.20%; N, 17.67%; O, 5.05%.

**4-(4-chlorophenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (12):** Yield 65%, m.p.: 222-224°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3349 ( $\text{NH}_2$ ), 1629 ( $\text{C}=\text{N}$ ), 1582 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.34 (1H, s, C-5-H), 5.33 (2H, s, C-2-NH<sub>2</sub>), 7.21-7.98 (6H, Ar-H). Elemental analysis for  $\text{C}_{13}\text{H}_9\text{ClN}_4\text{O}$ : Cal: C, 57.29%; H, 3.37%; Cl, 13.14%; N, 20.58%; O, 5.85%; found: C, 57.26%; H, 3.32%; Cl, 13.0%; N, 20.55%; O, 5.87%.

**4-(4-fluorophenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (13):** Yield 67%, m.p.: 229-231°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3350 ( $\text{NH}_2$ ), 1632 ( $\text{C}=\text{N}$ ), 1589 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.36 (1H, s, C-5-H), 5.35 (2H, s, C-2-NH<sub>2</sub>), 7.19-7.99 (6H, Ar-H). Elemental analysis for  $\text{C}_{13}\text{H}_9\text{FN}_4\text{O}$ : Cal: C, 60.78%; H, 3.89%; F, 7.43%; N, 21.82%; O, 6.29%; found: C, 60.70%; H, 3.92%; F, 7.39%; N, 21.78%; O, 6.22%.

**4-(4-trifluoromethylphenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (14):** Yield 69%, m.p.: 202-204°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3352 ( $\text{NH}_2$ ), 1632 ( $\text{C}=\text{N}$ ), 1591 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.37 (1H, s, C-5-H), 5.38 (2H, s, C-2-

NH<sub>2</sub>), 7.22-8.02 (6H, Ar-H). Elemental analysis for  $\text{C}_{14}\text{H}_9\text{F}_3\text{N}_4\text{O}$ : Cal: C, 54.97%; H, 2.99%; F, 18.67%; N, 18.38%; O, 5.24%; found: C, 54.91%; H, 2.96%; F, 18.61%; N, 18.30%; O, 5.22%.

**4-(4-methylphenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (15):** Yield 54%, m.p.: 198-200°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3341 ( $\text{NH}_2$ ), 1622 ( $\text{C}=\text{N}$ ), 1581 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.25 (1H, s, C-5-H), 5.29 (2H, s, C-2-NH<sub>2</sub>), 7.15-7.85 (6H, Ar-H). Elemental analysis for  $\text{C}_{14}\text{H}_{13}\text{N}_4\text{O}$ : Cal: C, 66.35%; H, 5.21%; N, 22.18%; O, 6.35%; found: C, 66.39%; H, 5.17%; N, 22.12%; O, 6.32%.

**4-(4-hydroxyphenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (16):** Yield 59%, m.p.: 235-237°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3346 ( $\text{NH}_2$ ), 1628 ( $\text{C}=\text{N}$ ), 1585 ( $\text{C}=\text{C}$ ), 3322 (Ar-OH);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.31 (1H, s, C-5-H), 5.31 (2H, s, C-2-NH<sub>2</sub>), 7.19-7.95 (6H, Ar-H), 5.12 (1H, s, Ar-OH). Elemental analysis for  $\text{C}_{13}\text{H}_{11}\text{N}_4\text{O}_2$ : Cal: C, 61.2%; H, 4.37%; N, 22.01%; O, 12.58%; found: C, 61.17%; H, 4.34%; N, 21.95%; O, 12.54%.

**4-(4-aminophenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (17):** Yield 61%, m.p.: 229-231°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3356 ( $\text{NH}_2$ ), 1632 ( $\text{C}=\text{N}$ ), 1584 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  7.24 (1H, s, C-5-H), 5.36 (2H, s, C-2-NH<sub>2</sub>), 7.19-7.95 (6H, Ar-H), 5.21 (2H, s, Ar-NH<sub>2</sub>). Elemental analysis for  $\text{C}_{13}\text{H}_{12}\text{N}_5\text{O}$ : Cal: C, 61.44%; H, 4.79%; N, 27.57%; O, 6.31%; found: C, 61.41%; H, 4.76%; N, 27.54%; O, 6.29%.

**4-(4-dimethylaminophenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (18):** Yield 62%, m.p.: 185-187°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3352 ( $\text{NH}_2$ ), 1633 ( $\text{C}=\text{N}$ ), 1591 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  3.12 (6H, s, -N(CH<sub>3</sub>)<sub>2</sub>), 7.35 (1H, s, C-5-H), 5.33 (2H, s, C-2-NH<sub>2</sub>), 7.29-8.05 (6H, Ar-H). Elemental analysis for  $\text{C}_{15}\text{H}_{15}\text{N}_5\text{O}$ : Cal: C, 64.10%; H, 5.38%; N, 24.93%; O, 5.67%; found: C, 64.04%; H, 5.37%; N, 24.90%; O, 5.69%.

**4-(4-methoxyphenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (19):** Yield 69%, m.p.: 165-167°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3341 ( $\text{NH}_2$ ), 1622 ( $\text{C}=\text{N}$ ), 1579 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm): $\delta$  3.92 (3H, s, Ar-OCH<sub>3</sub>), 7.28 (1H, s, C-5-H), 5.36 (2H, s, C-2-NH<sub>2</sub>), 7.17-7.86 (6H, Ar-H). Elemental analysis for  $\text{C}_{14}\text{H}_{13}\text{N}_4\text{O}_2$ : Cal: C, 62.47%; H, 4.91%; N, 20.88%; O, 11.85%; found: C, 62.44%; H, 4.87%; N, 20.81%; O, 11.88%.

**4-(4-ethoxyphenyl)-6-(isoxazol-3-yl)pyrimidin-2-amine (20):** Yield 58%, m.p.: 175-177°C; FT-IR (KBr,  $\text{cm}^{-1}$ ): 3343 ( $\text{NH}_2$ ), 1626 ( $\text{C}=\text{N}$ ), 1582 ( $\text{C}=\text{C}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  4.26 (2H, q, Ar-OCH<sub>2</sub>CH<sub>3</sub>), 2.25 (3H, t, Ar-OCH<sub>2</sub>CH<sub>3</sub>), 7.24 (1H, s, C-5-H), 5.36 (2H, s, C-2-NH<sub>2</sub>), 7.15-7.81 (6H, Ar-H). Elemental analysis for  $\text{C}_{15}\text{H}_{15}\text{N}_4\text{O}_2$ : Cal: C, 63.56%; H, 5.39%; N, 19.82%; O, 11.34%; found: C, 63.59%; H, 5.34%; N, 19.78%; O, 11.30%.

## 2.2 Biological Activity

The antitubercular activity was done against *Mycobacterium tuberculosis H37Rv* strain. Pyrazinamide (PYZ) was used as standard drug employing the procedure prescribed elsewhere [31,32,33]. A frozen culture in Middlebrook 7H9 broth with the addition of 0.2% glycerol and 10% albumin-dextrose-catalase was melted and diluted in broth to  $10^5$  CFU  $\text{mL}^{-1}$  (colony forming unit/mL) dilutions. Individual test compound was dissolved separately in DMSO followed by dilution with broth to attain a concentration which was two times the required concentration. The final concentration of DMSO of the experimental medium was 1.3% during the assay. Later, each U-tube was inoculated with 0.05 ml of standardized culture and then incubated at 37°C for 21 days. The growth of the organism in the U-tubes was compared with pyrazinamide (positive control) and without drug and inoculum (negative control). Minimum inhibitory concentration (MIC) of each compound was determined by means of broth dilution assay. MIC is defined as the lowest concentration of drug or a compound that inhibits  $\leq 99\%$  of the bacteria present at the start of the assay (Table 1).

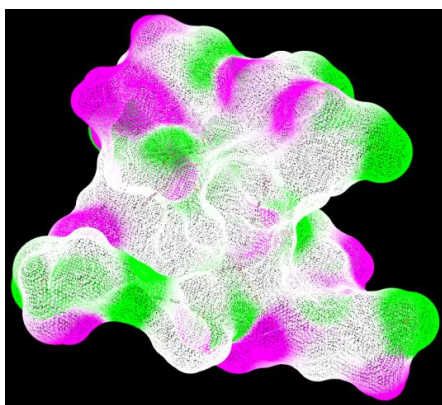


Fig. 2(a). Hydrogen acceptor-donor surface

## 2.3 Molecular Docking

Molecular docking was done to establish a possible mode of action for the prepared isoxazole clubbed pyrimidine derivatives (11-20) in anti-mycobacterial potential. It is used to predict ligand conformation which was produced best pose towards to favorable region in the active binding site of the receptor. Schrodinger Maestro software [34] is in the identification of the best binding pose which expressed as a negative numerical value and explained confirmation of protein-ligand interactions in angstroms ( $\text{A}^\circ$ ).

### 2.3.1 Ligands and protein preparation

Thymidylate kinase is an essential key enzyme in DNA synthesis and cellular growth via phosphoryl donor and can be download from protein data bank ([www.rcsb.org](http://www.rcsb.org)) in RCSB website (PDB ID-1MRS) with good resolution as .pdb format and adjust by delete exit ligand, water molecules, adjust zero-order bonds to metal ions and added polar hydrogens using prime by applying OPLS3 in the Maestro Protein Wizard. Grid parameters of protein active site of receptor adjusted by pointing any atom of co-crystal protein and generated grid X, Y and Z coordinates of the protein of 1MRS.

### 2.3.2 Docking procedure

The dynamic ligand is docked with Skim first in the Instigated Fit convention. The strategy uses diminished van der Waals radii to produce a wide variety of ligand depictions, and it can also remove highly adaptable side chains during the

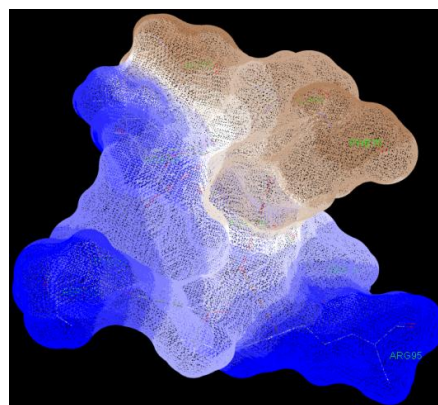


Fig. 2(b). Hydrophobic surface

docking step. An Excellent design expectation is then used for each represent to oblige the ligand by reorienting close-by-side chains. These deposits, as well as the ligand, are then constrained. Finally, each ligand is re docked into its corresponding low energy protein structures, and the subsequent structures are positioned by a scoring capacity that combines Glide score and energies.

Molecular descriptors are fundamental parameters that explained structural characteristics expressed as numerical values in the design of drugs. ADME and bioactivity studies of ligands were conducted by free online software respectively. Additionally, a drug-likeness property that is very helpful in drug discovery was explained by Lipinski's rule of five with help of an online tool (<http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>). Bioactivity of compounds checked by calculating activity scores of GPCP ligand, ion channel modulator, nuclear receptor ligand, a kinase inhibitor, a protease inhibitor, and enzyme inhibitor using Mol-inspiration online web software [35].

### 3. RESULTS AND DISCUSSION

To synthesized compounds (11-20) by reaction in between already we reported isoxazole chalcones (1-10) and guanidine hydrochloride in presence of basic sodium hydroxide solution for conventional method and recrystallized to get final compounds with the percentage of yield between 54-69%. Structures of purified compounds were untangled by IR and <sup>1</sup>H NMR spectra and determined the predicting structures by specific peaks. IR spectra showed different characteristic bands at different wavenumber regions 1579-1591 cm<sup>-1</sup> (C=C), 1622-1633 cm<sup>-1</sup> (C=N) and 3341-3356 cm<sup>-1</sup> (-NH<sub>2</sub>) respectively. Three different characteristic peaks range between  $\delta$ 7.25- 7.37 (-C-5-H),  $\delta$ 5.29-5.38 (-C-2-NH<sub>2</sub>), and  $\delta$ 7.15- 8.05 (Ar-H-6H) represented that structure of the 4-(4-substituted phenyl)-6-(1, 2-oxazol-3-yl) pyrimidin-2-amine. The chemical shift of the remaining peaks is explained by protons in the aromatic region. The molecular ion peak in the positive ion mass spectrum further depicts the formation of isoxazole clubbed pyrimidine compounds.

Based on the results, we evaluated that all synthesized compounds shown good to excellent (100  $\mu$ g/ml, 50  $\mu$ g/ml, 25  $\mu$ g/ml, 12.5  $\mu$ g/ml, 6.25  $\mu$ g/ml, 3.125  $\mu$ g/ml, 1.562  $\mu$ g/ml and 0.78  $\mu$ g/ml) anti-mycobacterial activity (H37RV strain) by

dilution technique against standard. Isoxazole clubbed pyrimidine compounds were shown potent activity than their precursor i.e. isoxazole chalcone derivative. The compound 18 & 20 was shown more potent activity with MIC (0.78  $\mu$ g/ml), some (Compounds 8, 10, 16, 17 & 19) with MIC (1.56  $\mu$ g/ml) were better than standard (PYZ) MIC (3.125  $\mu$ g/ml) and presented in Table 1.

Thymidylate kinase is a critical protein for pyrimidine blend that catalyzes the phosphorylation of thymidylate 5-monophosphate within the sight of ATP and magnesium ion particles, fundamental capacity in cell replication, enzymatic energy, and related constructions have been resolved in different organisms [36]. Previously, some authors reported docking studies on pyrazole-thiocyanatoethanone derivatives [37], phenyl and indole derivatives [38], propolis constituents [39] act as kinase inhibitors against Mycobacterium tuberculosis. Owono Owono LC et al. reported that to predict thymidine analogs (TMA) based on complexation methodology and validated by a Pharmacophore (PH<sub>4</sub>) analysis provided structural information helpful in the design of new analogs, in that one of the most potent new analog TMA12 is HB bonded to the selective Tyr39 making the best of TMAs a promising set for synthesis and evaluation [40]. Herein active binding site, favorable amino acids (Tyr 39, Arg95, Ash163, Arg160, Asn100) were present in the hydrophilic region for the stability of conformation and the active binding site can be optimized with grid parameters (X=25.12 A<sup>o</sup>, Y= 11.16A<sup>o</sup>, Z= 8.97A<sup>o</sup>) and RMSD value 0.578 A<sup>o</sup> stated that six bioactive compounds were estimated that showing good to excellent binding affinity, among that compounds 18 and 20 promising highest binding affinities (-5.69 Kcal/mol and -6.30 Kcal/mol) than that standard (PYZ) and its binding affinity is -4.45 Kcal/mol and represented in Table 2 & Fig. 1.

Lipinski rule of five applied to potent synthesized compounds and all are shown as drug candidates within their limits Table 3. In this analysis we have usually reported isoxazole chalcones (1-10) about their property in connection with oral bio-availability linked with drug-likeness. All synthesized compounds did not surpass Lipinski's rule of five and all are in the interim (LogP is not greater than 5, Molecular weight is not more than 500 Daltons, Number of hydrogen donors are not less than 5, Number of hydrogen donors are not less than 10 and molar refractivity is in between 40-130).

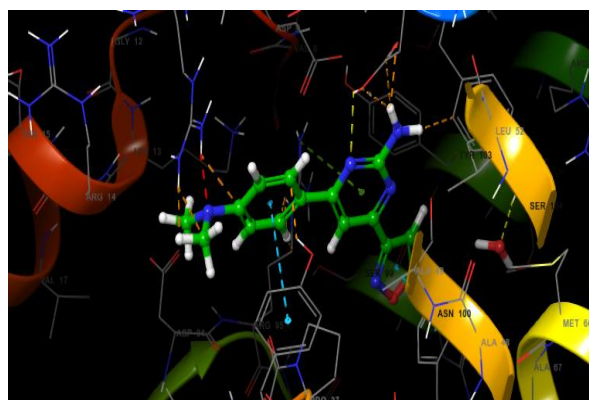


**Table 1. Antitubercular results of isoxazole clubbed pyrimidine derivatives (1-20)**

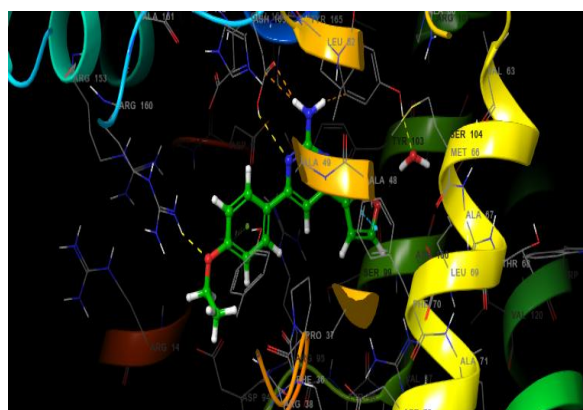
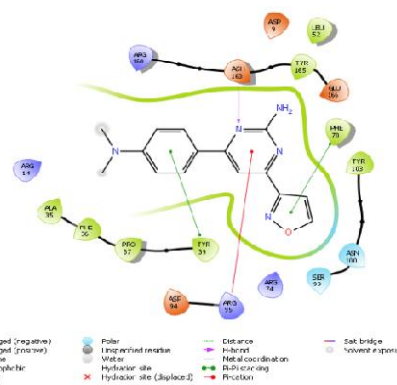
#	R	MIC values (µg/mL) of <i>M. tuberculosis</i> H37Rv	#	MIC values (µg/mL) of <i>M. tuberculosis</i> H37Rv
1	Bromo	25	11	12.5
2	Chloro	50	12	25
3	Fluoro	50	13	50
4	Trifluoromethyl	100	14	50
5	Methyl	6.25	15	3.125
6	Hydroxy	3.125	16	1.562
7	Amino	3.125	17	1.562
8	Dimethyl amino	1.562	18	0.78
9	Methoxy	3.125	19	1.562
10	Ethoxy	1.562	20	0.78
	Pyrazinamide	3.125		

**Table 2. Molecular docking scores and ligand interaction values of synthesized potent compounds**

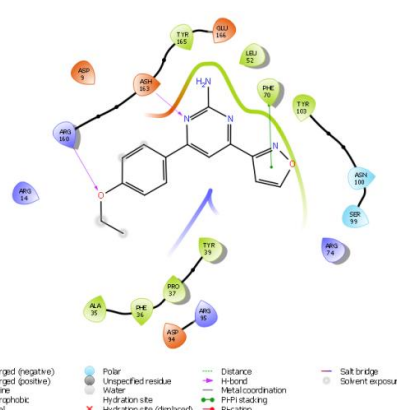
Compound code	Glide docking score (Kcal/mol)	Glide energy (Kcal/mol)	No. of rotational bonds	Interactions (A <sup>0</sup> )
				Hydrophilic
18	-5.69	-45.01	3	Tyr 39, Arg 95, Ash 163
20	-6.30	-30.94	4	Arg 160, Ash 163
PYZ	-4.45	-11.89	1	Asn 100

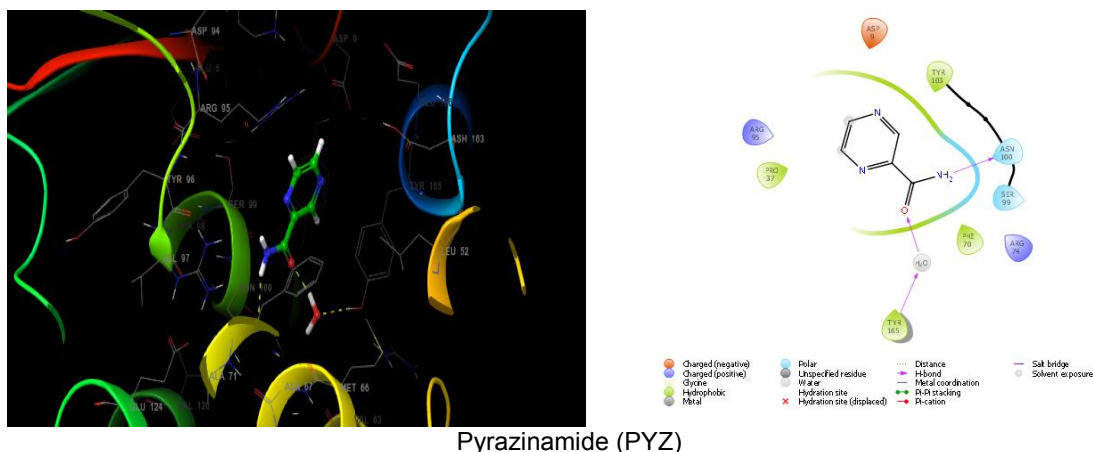


Compound 18



Compound 20





Pyrazinamide (PYZ)

**Fig. 3. Demonstrated that 2D and 3D molecular interaction of compounds 18 & 20 as well as standard (PYZ) against thymidylate kinase (PDB-1MRS) enzyme of H37Rv**

**Table 3. Drug-likeness values of synthesised compounds (11-20)**

Code	Log p	M.W	H.D	H.A	Refractive index
11	2.57	316	2	5	73.09
12	1.69	272.5	2	5	67.47
13	1.94	256	2	5	65.35
14	2.82	306	2	5	70.39
15	2.12	252	2	5	70.13
16	1.52	254	3	6	67.06
17	1.39	253	4	6	69.81
18	1.88	281	2	6	79.72
19	1.82	268	2	6	71.95
20	2.21	282	2	6	76.56

**Table 4. Bio-activity data of synthesised compounds (1-20)**

S. no.	GPCR receptor	Ion channel receptor	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
1	-0.42	-0.33	-0.80	-0.95	-0.78	-0.19
2	-0.27	-0.22	-0.76	-0.81	-0.66	-0.13
3	-0.26	-0.23	-0.68	-0.74	-0.64	-0.10
4	0.01	-0.04	-0.36	-0.28	-0.30	0.02
5	-0.34	-0.33	-0.79	-0.82	-0.69	-0.17
6	-0.20	-0.15	-0.66	-0.56	-0.58	0.00
7	-0.21	-0.14	-0.57	-0.88	-0.48	0.05
8	-0.13	-0.21	-0.48	-0.55	-0.46	-0.04
9	-0.26	-0.30	-0.68	-0.68	-0.59	-0.11
10	-0.24	-0.29	-0.66	-0.55	-0.53	-0.13
11	-0.07	0.09	0.29	-0.23	-0.44	0.07
12	0.06	0.18	0.33	-0.11	-0.34	0.12
13	0.07	0.17	0.39	-0.05	-0.32	0.14
14	0.22	0.25	0.46	0.21	-0.09	0.16
15	0.01	0.09	0.29	-0.12	-0.36	0.09
16	0.12	0.24	0.41	0.10	-0.27	0.23
17	0.08	0.23	0.42	-0.11	-0.23	0.22
18	0.13	0.13	0.43	0.02	-0.21	0.14
19	0.04	0.07	0.33	-0.05	-0.30	0.11
20	0.03	0.05	0.28	0.02	-0.27	0.06
PYZ	-1.97	-1.45	-1.71	-2.87	-1.84	-1.43



A computation of GPCR, protease inhibitor, enzyme inhibitor, kinase inhibitor, and ion channel modulator were used to measure bioactivity in twenty compounds. A compound possesses extensive activity when having a bioactivity score greater than 0.00, showed moderate activity when having a bioactivity score in between -0.5 to 0.00, produced a minor amount of activity, or presumed to be inactive 17 when having a bioactivity score lesser than -0.5. The present study revealed that compounds were moderate to considerable activity than standard compounds. Especially isoxazole clubbed pyrimidines (11-20) screened more considerably than isoxazole chalcones (1-10) and compounds 11 to 20 showed especially more potent bioactivity score i.e., greater than 0.25 on kinase inhibitor than others and represented in Table 4.

#### 4. CONCLUSION

In the present work, we prepared isoxazole clubbed 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 preceding chalcones and standard drug pyrazinamide. Molecular docking studies revealed that compounds 18 and 20 had more potential to interact with selected enzyme than standard which are expressed on docking score. They were possessed as drug-likeness through Lipinski's rule. Thus, based on the study, we state that compounds 18 and 20 should be promising compounds and can be later developed as potential drugs for the treatment of *Mycobacterium tuberculosis* infections.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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