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Synthesis, anti-tubercular evaluation and molecular docking studies of Nitrogen-rich piperazine-pyrimidine-pyrazole Hybrid Motifs

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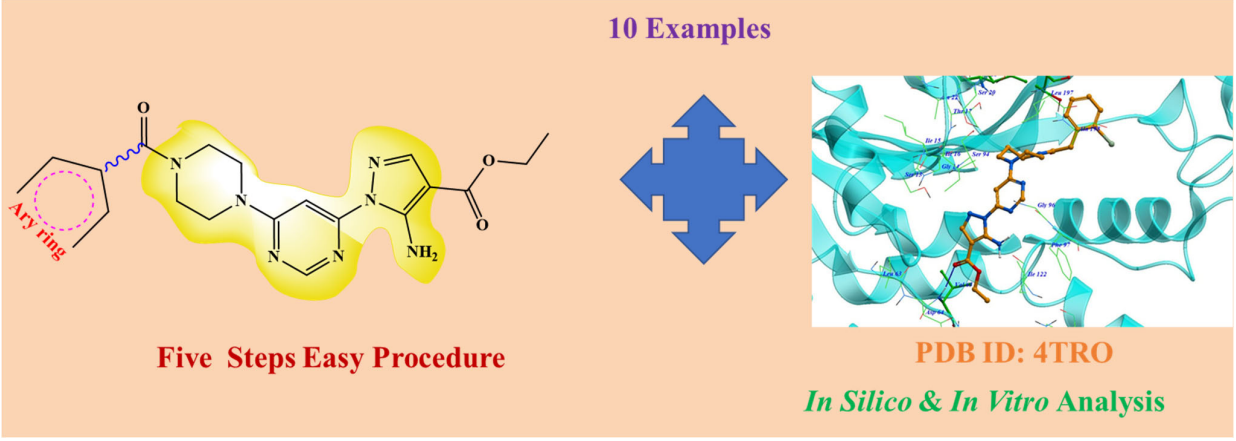
A B S T R A C T

A convenient and efficient synthesis of a series of ethyl-1-(6-(4-substitutedacetylatedpiperazin-1-yl)pyrimidin-4-yl)-5-amino-1*H*-pyrazole-4-carboxylate **(8a-8j)** has been developed by five steps which include activation of a methylene group, hydrazinolysis, cyclisation and chloro-amine coupling reactions. Moreover, our proposed mechanism was confirmed in this study demonstrating that ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate is the key intermediate to fulfill the desired outcomes. *In silico* and *in vitro* studies were carried out to identify the active agents among the developed adducts against mycobacterium tuberculosis (PDB ID:**4TRO**). Compound **8a** (Docking Score: **-26.81** and MIC: **1.6 ug/mL**) was found to be the most potent among the synthesized molecules. All the synthesized compounds showed acceptable drug-like properties which make them suitable for further lead modification using *in silico* design approaches.

Graphical Abstract



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1. Introduction

Tuberculosis (TB) is among the ten leading causes of death worldwide and the leading cause of a single infectious bacterium[1]. Though enormous success has been achieved in progressively reducing tuberculosis (TB) associated mortality in the past two decades, the magnitude of the problem remains significant. The spread of drug-resistant TB and the concomitant interaction with the human immunodeficiency virus (HIV) epidemic, increases the challenges associated with TB control and treatment[2]. New diagnostic tools, more effective drugs and adjunct therapies are urgently needed to improve the treatment outcome[3]. After looking at the WHO END TB strategy, it signifies the importance of developing new anti-tubercular agents to properly address the problem we are now facing[4]. One of the potential classes of compounds to become anti TB drugs is nitrogen-rich hybrids[5].

Heterocyclic core systems are fascinating scaffolds for the drug discovery and development process because of their potential to constitute various and essential bonds within the active site of their targets[6]. Among heteroatoms, nitrogen is of greater importance due to its high degree of electronegativity and ease of formation of polar bonds. Also, nitrogen atoms can play roles as both hydrogen bond acceptor and donor (in the form of NH) which makes this atom a key element in lead discovery[7].
Among various nitrogen-rich heterocycles, pyrazole, imidazole, 1,2,3- and 1,2,4-triazole, tetrazole, pyrimidines, piperazines, 1,3,5- and 1,2,4-triazine and their fused systems are considered as a fundamental structure of numerous synthetic drugs and pharmacologically active agents[8]. Of these, three heterocycles i.e. pyrimidines, piperazines and pyrazoles, are some of the most widely seen heterocycles in most of the marketed drug molecules. Piperazines have a long and distinguished history extending from the days of their discovery as important pharmacophores in chemotherapy. The medicinal value of piperazine derivatives is significant among various heterocycles, as they are found to possess various biological activities[9]. The pyrazole scaffold represents a common nucleus in many pharmaceutically active compounds and signifying a wide range of pharmacological activities; such as anti-inflammatory, antibacterial antifungal, hypoglycemic, anti-hyperlipidemic, cyclooxygenase-2 inhibitors, p38 MAP kinaseandCDK2/Cyclin A inhibitors, antiangiogenic. They also represent an elegant choice as a starting material for the synthesis of pharmaceutical compounds[10-19]. The pyrimidines represent one of the most active classes of compounds possessing a wide spectrum of biological activities like significant in vitro activity against unrelated DNA and RNA, viruses including polioherpes viruses, diuretic, antitumor, anti-HIV, and cardiovascular properties[20-26].

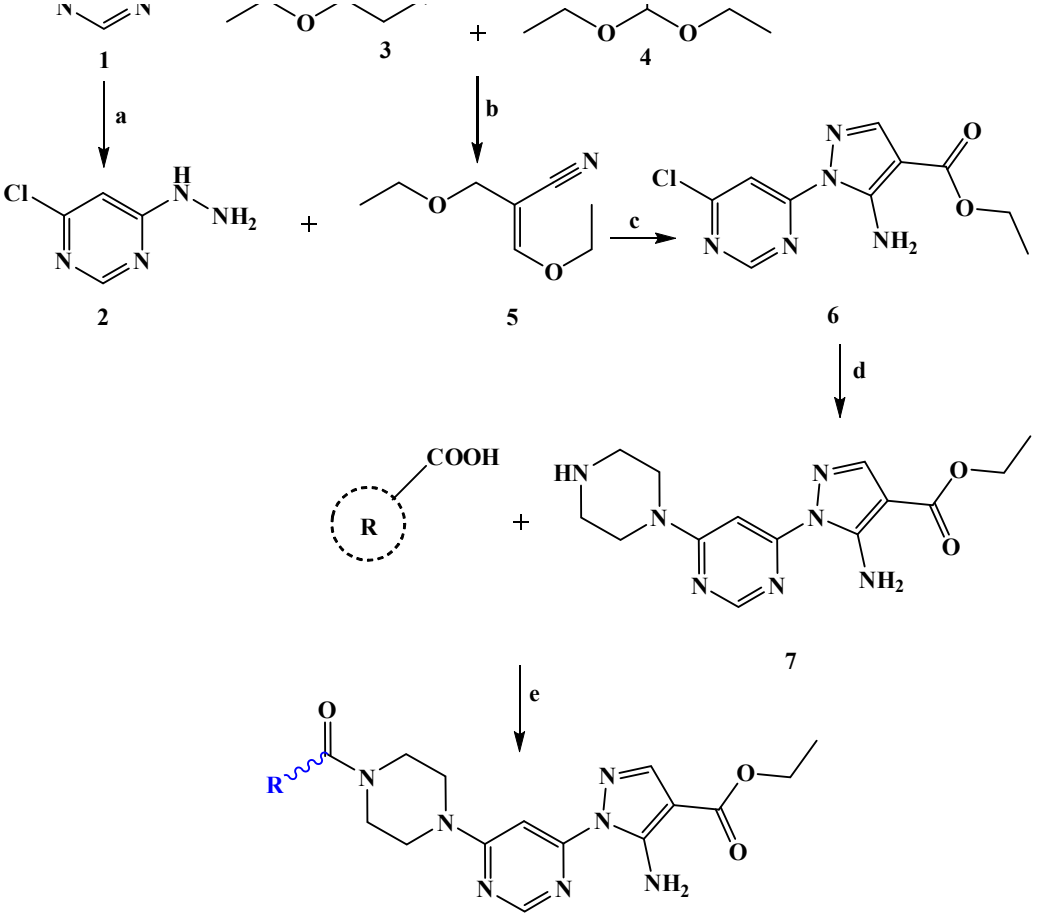
Nowadays, plenty of synthetic methods have been reported for the synthesis of various hybrids possessing active heterocycles in the core to enhance the biocompatibility of scaffolds[27]. Pyrazolo[3,4-d]pyrimidines are important bioactive heterocyclic molecules that have attracted attention as potential drugs or molecular tools. In particular, 6-aminopyrazolo[3,4-*d*]pyrimidine analogues exhibit pharmacological activities[28]. Piperazine and pyrimidine-based scaffolds were well defined for their numerous biological activities such as anti-cancer, anti-microbial and many more[29].

In this paper, we report a simple and efficient protocol for the synthesis of diverse molecules containing pyrimidine, pyrazole and piperazine scaffolds based on the molecularhybridizationconcept[30], which makes it possible to assemble biologically active scaffolds, i.e. piperazine-pyrimidine-pyrazole, into a single molecular framework. We also tested their biological importance using *in silico* and *in vitro* studies.

2. Results and Discussion

2.1 Chemistry

The modified synthetic methods for the formation of the pyrimidine-pyrazole-piperazine hybrids (**8a-8j**) synthesis are represented in **Scheme-1**. Two parallel reactions were undertaken simultaneously, of which compound **2** was formed by 4,6-dichloropyrimidine reacting with hydrazine hydrate at room temperature, for 1.5 h. On the other hand, 2-(ethoxymethyl)-3-methoxyacrylonitrile (**5**) was synthesized under anhydrous solvent acetic anhydride reflux, 140 ^{o}C for 8 h, each step the yield reached more than 70%. The targeted molecules ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**6**) were obtained by refluxing**2** with **5** at for 7 h in ethanol as a solvent. Compound **7**wassynthesized by the reaction of ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**6**) with piperazine and a catalytic amount of triethylamine using ethanol as solvent. Target compounds (**8a-8j**) were synthesized by reacting **7** with corresponding substituted acids using EDC-HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride): HOBT (hydroxy benzotriazole): DIPEA (N,N-diisopropylethylamine) [1:1:2 proportion] in the presence of DMF (*N*,*N*-Dimethylformamide) as a solvent.



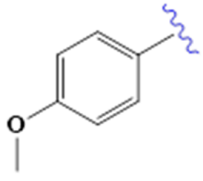
Reaction Conditions: (a) Ethanol, NH_2NH_2, RT, 1.5 h; (b) Acetic anhydride, Reflux, 140°C, 8h; (c) Ethanol, Reflux, 80°C, 7h; (d) Ethanol, TEA, Piperazine, RT, 3 h; (e) DMF, EDC-HCl, HOBT, DIPEA, RT, 2 h.

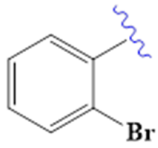
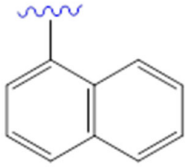
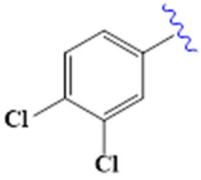
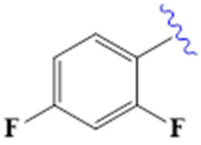
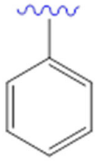
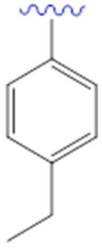
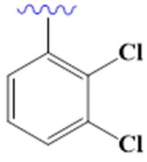
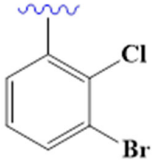
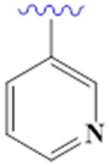
Scheme 1. Synthetic pathway of Pyrimidine-Pyrazole-Piperazine hybrids**(8a-8j)**

2.2 In Vitro Anti-tubercular Activity

All the synthesized compounds (**8a-8j**) were screened for their *in vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv strain. The minimum inhibitory concentration (MIC) values were determined by the serial dilution technique using Alamar Blue Dye using the microplate Alamar Blue assay (MABA) method. Results (**Table 1**) show that compounds displayed acceptable MIC values. It was encouraging to see that while some compounds (**8c**, **8d** and **8f**) showed MIC values equivalent or close to that of standard drugs. Compound **8a** stands out as the most potent compound with a MIC of 1.6 µg/mL.

Table 1. *In vitro* anti-tubercular activity (MIC) with Docking Score of Pyrimidine-Pyrazole-Piperazine adducts **(8a-8j)**

4	Compound ID	R	Docking Score	MIC (µg/mL)
	8a		-26.81	1.6

8b		-20.87	12.5
8c		-21.94	6.25
8d		-24.72	6.25
8e		-18.91	12.5
8f		-20.28	6.25
8g		-9.65	100
8h		-21.08	25
8i		-20.08	12.5
8j		-14.79	50
Pyrazinamide	-	-	3.125
Ciprofloxacin	-	-	3.125
Streptomycin	-	-	6.25

2.3 In silico drug design

Molecular docking of the synthesized compounds in the crystal structure of *Mycobacterium tuberculosis* (PDB ID: 4TRO) revealed interesting observations. Most of the compounds occupied a similar binding site as the native substrate of 4TRO. Compound **8a** showed the highest fit (Docking score: -26.81). This compound made very good interactions with the surrounding residues which included H-bonds with Ile122, Val65 and Leu197 (**Figure 1**). Also, the compound showed good Vander Walls hydrophobic interactions with residues like Ala198, Gly96, Phe97 and Ser94. It was encouraging to see that this docking protocol could distinguish between active and inactive molecules. Upon our analysis of **8g** (the lowest active compound with docking score of -9.65 and MIC value of 100); we could understand the reasons for its low activity. The compound's 4-ethyl phenyl ring occupied a completely different orientation to that of **8a**. Due to this, the compound lost H-bonding interactions with Ile122, Val65 and Leu197. This clearly shows that these H-bonds are essential for the binding with M. tuberculosis and in turn, to show the desired activity. Binding scores of all the compounds are shown in **Table 2** and the binding pose of two representative compounds (**8a** and **8g**) are shown in **Fig. 1**.

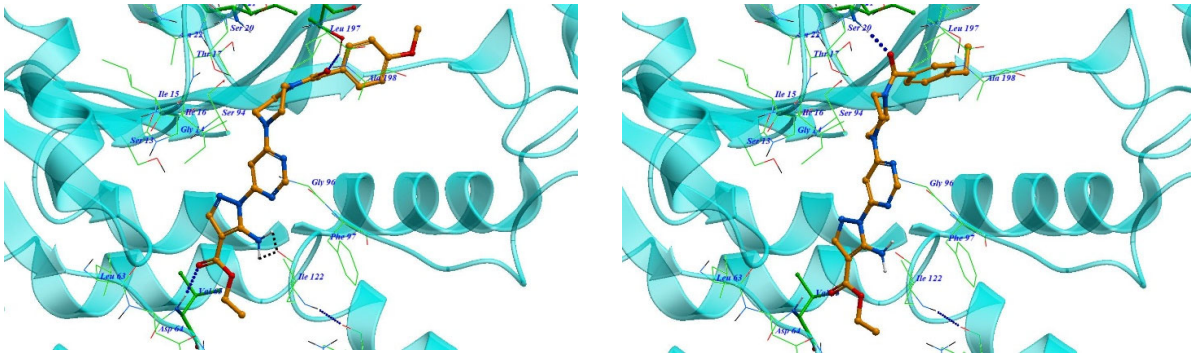


Fig. 1. Binding interactions of docked compounds (panel a: **8a**, panel b: **8g**). Ligand carbon in orange, protein (in cartoon) carbon in cyan, nitrogen in blue, oxygen in red. Hydrogen bonds are shown with dotted lines.

The compounds were also evaluated for their physicochemical properties to see their drug-likeness. It was encouraging to see that all the compounds passed the basic filter (Lipinski rule of 5) and showed an acceptable permeability profile (CACO2 and PAMPA) with fewer hERG concerns. The results are summarized in **Table 2**.

Table 2. Physicochemical properties of designed compounds (**8a-8j**)

Name	MW	Log P	Log S	PSA	Drug Likeness	HBA	HBD	CACO-2 cells	HERG	PAMPA	PGP	PGP inhibitor	PGP substrate
8a	465.21	3.16	-3.22	99.97	0.93	9.00	2.00	-5.07	0.09	-5.12	1.00	0.10	1.00
8b	513.11	3.91	-3.91	92.43	0.53	8.00	2.00	-5.08	0.04	-5.21	0.98	0.03	0.98
8c	485.22	4.32	-4.33	92.15	0.87	8.00	2.00	-5.20	0.05	-5.20	0.98	0.09	0.98
8d	503.12	4.54	-4.58	92.43	0.98	8.00	2.00	-5.06	0.17	-5.31	0.98	0.04	0.98
8e	471.18	3.45	-3.54	92.43	0.92	8.00	2.00	-5.03	0.07	-5.27	0.99	0.10	0.99
8f	421.19	2.79	-3.24	92.95	0.75	8.00	2.00	-4.54	0.14	-5.20	0.97	0.23	0.97
8g	449.22	3.82	-3.88	92.95	0.98	8.00	2.00	-4.52	0.15	-5.19	0.97	0.08	0.97
8h	489.11	4.23	-4.51	92.95	0.95	8.00	2.00	-4.78	0.23	-5.28	0.98	0.03	0.98
8i	457.17	3.35	-3.44	92.95	0.69	8.00	2.00	-4.76	0.17	-5.32	0.97	0.10	0.97
8j	422.18	1.60	-1.73	102.47	1.09	9.00	2.00	-4.64	0.08	-5.18	0.90	0.65	0.90

Where, MW= Molecular Weight; PSA= Polar Surface Area; HBA= Hydrogen-Bond Acceptor; CACO-2 cells= human colon adenocarcinoma cells; HBD= Hydrogen-Bond Donor; HERG= Human Ether-a-go-go-Related Gene; PAMPA= Parallel Artificial Membrane Permeation Assay; PGP= P-glycoprotein.

In the above stated study based on *in silico* and *in vitro*, it had directed us to identify the structure–activity relationship (SAR) analysis and was revealed that the presence of an anisole (**8a**), a simple phenyl ring (**8f**) or a naphthalene (**8c**) as acid substituents at the 4th position of piperazine ring core was favourable to attain good anti-tubercular activity. Surprisingly, the presence of the multi/single halogens at the benzoyl ring were found to be least potent in the series but it should be notified that the multi chloro group at the 3rd and 4th position shown comparable potency with **8a**, **8c** and **8f** and well to maintain the activity against the H37Rv MTB strain, whilst the substitution with ethyl and 3-pyrydyl group in compounds **8g** and **8j**, respectively, completely abolished the activity against both the MTB strains.

The above findings and observations supported the view that further molecular modelling study was carried out to elucidate the potential mode of action of the tested compounds as well as to rationalize their SAR which represents one of the potential MTB therapeutic targets.

3. Conclusions

A convenient protocol was designed for the synthesis of new pyrimidine as core having amino-pyrazole and halogen-containing piperazine scaffolds using five steps procedure under conventional reaction setup. *In vitro,* an anti-tubercular assay showed that compound **8a** proved to be the most potent (MIC = 1.6 µg/mL). Compounds **8c**, **8d** and **8f** also showed decent activity. Although, they were found to be less active than the reference drugs; it was encouraging to see that compound **8a** was found to be better than the reference drugs. The functionalization of the piperazine moiety at the N-4 position by methoxyphenyl, phenyl groups and a naphthyl substituent, as well as the presence of dichloro group in the meta, para position were essential for displaying significant first-line anti-TB agents (rifampicin and isoniazid). Also, it was encouraging to see that we could get a good correlation between predicting activity (docking score) and actual MIC values, which further suggests that *in silico* design can be used effectively before synthesizing further analogues.

Acknowledgements

Authors acknowledge the support from Shishir Rohit, Kashiv Biosciences Pvt. Ltd., Ahmedabad for the *in silico* design work.

4. Experimental

4.1 Materials

The required chemicals and solvents for the synthesis were purchased from Merck Ltd., SD fine chemicals, and Sigma-Aldrich Chemical. Most of the reactions were carried out by standard techniques for the exclusion of moisture. The open-end capillary method was used to determine the synthesized derivatives' melting points, and the results were reported and uncorrected. Thin Layer Chromatographywas accomplished on 0.2 mm Precoated plates of Silica gel G60 F_{254} (Merck)and visualized in UV light (254 and 365 nm). IR spectra of all compounds were recorded on a "Shimadzu, Japan IR-435 Spectrophotometer" using the ATR technique. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on the "Bruker AVANCE II Spectrometer" using DMSO-d_6 as solvent and TMS as the internal reference. Mass spectra were recorded on a Jeol-JMSD 300 mass spectrometer at 70ev.

4.2 Synthetic methodology

*4.2.1 4-Chloro-6-hydrazinylpyrimidine (**2**)*

In a solution of 4,6-dichloropyrimidine (**1**,1 mmol) in ethanol (60 ml), hydrazine hydrate (0.95 mmol) was added dropwise at 0˚C. The reaction mixture was allowed to stir at room temperature for 1.5 h. After completion of the reaction, it was poured into crushed ice and stirred well for 15 min. The solid separated was filtered and washed with cold water. The product obtained was dried and recrystallized from ethanol.

*4.2.2 2-(Ethoxymethyl)-3-methoxyacrylonitrile (**5**)*

Ethyl 2-cynoacetate (**3,** 1 mmol) and triethoxymethane (**4,** 1 mmol) was added to acetic anhydride and refluxed at 140˚C for 8 h. After completion of the reaction, the remaining acetic anhydride was distilled off and the reaction mass was cooled to room temperature. The reaction mixture was poured into ice-crushed water and filtered off. The separated solid was washed with cold water and dried to get **5** as light orange colour solid.

*4.2.3 Ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**6**)*

4-Chloro-6-hydrazinylpyrimidine (**2,** 1 mmol) and 2-(ethoxymethyl)-3-methoxyacrylonitrile (**5,** 1 mmol) in ethanol (20 ml) was refluxed at 80˚C for 7 h. The reaction mixture was cooled to room temperature and poured into water. The solid obtained was filtered, dried and recrystallized using ethanol.

4.2.4 Ethyl 5-amino-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (7)

To a solution of **6** (1 mmol) and piperazine (1.5 mmol) in ethanol (30 ml) was added triethylamine (0.01 mmol) and the reaction mixture was stirred at 5˚C for 0.5 h. It was further stirred at room temperature for 3 h followed by quenching with ice water to afford precipitates. The solid separated was well stirred overnight and filtered. The product was crystallized from methanol.

*4.2.5General procedure for the synthesis of ethyl 5-amino-1-(6-(4-substitutedphenylpiperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate **(8a-8j)***

Substituted acid (1 mmol) and **7** (1 mmol) was dissolved in DMF (20 ml) and the reaction mixture was cooled at 5°C. To this reaction mixture, EDC-HCl (1.5 mmol), HOBT (1.5 mmol) and DIPEA (3 mmol) were added by maintaining the temperature below 5°C and the reaction mixture was stirred well to get a clear solution which was further stirred for 2 h at room temperature. After completion of the reaction, the reaction mixture was poured into ice-cold water, filtered, and dried. The crude product was further purified by column chromatography using silica gel (60-120 mesh) in ethyl acetate: n-hexane **(7:3)** as a mobile phase.

*Ethyl 5-amino-1-(6-(4-(2-(4-methoxyphenyl)acetyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8a**)*
Off white solid. Yield: 79%. mp (^{0}C): 177. IR (cm^{-1}): 3415 (N-H Primary amine stretching), 3302 (C-H Aromatic stretching), 2914 (C-H Alkane stretching), 1685 (>C=O Ester stretching), 1637 (>C=O Amide stretching), 1419 (C=C Aromatic stretching), 1284 (C-N Linkage stretching).^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.48 (s, 1H, Ar-H Pyrazole), 7.79 (s, 2H, Ar-H Pyrimidine), 7.71 (s, 1H, Ar-H), 7.17 (s, 2H, -NH_2), 6.87-6.98 (d, 3H, Ar-H), 3.33-3.73 (m, 13H, -OCH_3, Ar-H Piperazine, -CH_2 near Piperazine ring), 4.21-4.22 (q, 2H, -C<u>H$_2$</u>-CH_3), 1.28 (t, 3H, -CH_2-C<u>H$_3$</u>). ^{13}C NMR: (DMSO-d_6, 101.1 MHz) δppm: 173.12, 168.16, 165.52, 158.17, 156.52, 154.02, 151.78, 141.80, 134.55, 134.55, 129.69, 117.25, 117.25, 86.30, 80.25, 64.58, 53.09, 45.16, 45.16, 43.78, 43.78, 38.41, 18.26. Mass (m/z): 465.52 (M^+). Anal. $C_{23}H_{27}N_7O_4$ requires: C, 59.34%; H, 05.85%; N, 21.06%; O, 13.75%; Found: C, 59.32%; H, 05.87%; N, 21.11%; O, 13.71%.
*Ethyl 5-amino-1-(6-(4-(2-(2-bromophenyl)acetyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8b**)*
Off white solid. Yield: 67%. mp (^{0}C): 132. IR (cm^{-1}): 3444 (N-H Primary amine stretching), 3321 (C-H Aromatic stretching), 3013 (C-H Alkane stretching), 1668 (>C=O Ester stretching), 1633 (>C=O Amide stretching), 1423 (C=C Aromatic stretching), 1242 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.50 (s, 1H, Ar-H Pyrazole), 7.72 (s, 1H, Ar-H Pyrimidine), 7.66-7.59 (d, 2H, Ar-H), 7.31-7.34 (d, 1H, Ar-H), 7.31 (s, 2H, -NH_2), 7.20-7.22 (d, 1H, Ar-H), 7.02-7.19 (d, 1H, Ar-H), 4.19-4.25 (q, 2H, -C<u>H$_2$</u>-CH_3), 3.62-3.84 (s, 2H, -CH_2 near Piperazine ring), 3.34 (m, 8H, Ar-H Piperazine), 1.23-1.30 (t, 3H, -CH_2-C<u>H$_3$</u>).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 172.45, 166.45, 164.75, 161.58, 157.96, 153.32, 140.69, 138.75, 135.48, 132.52, 128.27, 123.69, 122.59, 100.69, 87.58, 72.69, 46.14, 46.14, 42.95, 42.95, 39.26, 20.15. Mass (m/z): 514.41 (M^+). Anal. $C_{22}H_{24}BrN_7O_3$ requires: C, 51.37%; H, 04.70%; N, 19.06%; O, 09.33%; Found: C, 51.42%; H, 04.67%; N, 19.02%; O, 09.37%.
*Ethyl 5-amino-1-(6-(4-(2-(naphthalen-1-yl)acetyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8c**)*
Off white solid Yield: 82%. mp (^{0}C): 186. IR (cm^{-1}): 3441 (N-H Primary amine stretching), 3226 (C-H Aromatic stretching), 2948 (C-H Alkane stretching), 1681 (>C=O Ester stretching), 1593 (>C=O Amide stretching), 1419 (C=C Aromatic stretching), 1282 (C-N Linkage stretching).^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.49 (s, 1H, Ar-H Pyrazole), 7.92-7.98 (d, 2H, Ar-H), 7.80-7.87 (d, 2H, Ar-H), 7.48-7.53 (d, 2H, Ar-H), 7.73 (s, 2H, -NH_2), 7.47 (s, 1H, Ar-H Pyrimidine), 7.40 (d, 1H, Ar-H), 7.01 (s, 1H, Ar-H Pyrimidine), 4.19-4.25 (m, 4H, -C<u>H$_2$</u>-CH_3, -CH_2Near Piperazine ring), 3.61-3.75 (m, 8H, Ar-H Piperazine), 1.20-1.29 (t, 3H, -CH_2-C<u>H$_3$</u>). ^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 172.25, 168.69, 164.02, 161.69, 159.68, 157.85, 142.89, 138.36, 135.48, 134.02, 133.86, 132.60, 128.16, 124.92, 123.21, 121.61, 118.92, 87.32, 82.62, 67.62, 49.52, 49.52, 41.71, 41.71, 38.45, 18.35. Mass (m/z): 485.50 (M^+). Anal. $C_{26}H_{27}N_7O_3$ requires: C, 64.32%; H, 05.60%; N, 20.19%; O, 09.89%; Found: C, 64.36%; H, 05.65%; N, 20.15%; O, 09.84%.
*Ethyl 5-amino-1-(6-(4-(2-(3,4-dichlorophenyl)acetyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8d**)*
Off white solid. Yield: 74%. mp (^{0}C): 164. IR (cm^{-1}): 3448 (N-H Primary amine stretching), 3319 (C-H Aromatic stretching), 2978 (C-H Alkane stretching), 1737 (>C=O Ester stretching), 1676 (>C=O Amide stretching), 1384 (C=C Aromatic stretching), 1286 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.49 (s, 1H, Ar-H Pyrazole), 7.80 (s, 1H, Ar-H), 7.72 (s, 2H, -NH_2), 7.57 (s, 1H, Ar-H Pyrimidine), 7.51-7.55 (d, 1H, Ar-H), 7.22-7.29 (d, 1H, Ar-H Pyrimidine), 7.00 (s, 1H, Ar-H), 4.19-4.24 (q, 2H, -C<u>H$_2$</u>-CH_3), 3.81 (s, 2H, -CH_2 near Piperazine ring), 3.60-3.75 (m, 8H, Ar-H Piperazine), 1.26-1.29 (t, 3H, -CH_2-C<u>H$_3$</u>). ^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 172.52, 164.25, 163.82, 159.64, 157.02, 156.75, 137.25, 135.10, 134.82, 132.36, 130.15, 128.92, 124.26, 97.30, 82.58, 63.65, 49.12, 49.12, 41.74, 41.74,37.25, 21.58. Mass (m/z): 504.41 (M^+). Anal. $C_{22}H_{23}Cl_2N_7O_3$ requires: C, 52.39%; H, 04.60%; N, 19.44%; O, 09.52%; Found: C, 52.43%; H, 04.56%; N, 19.47%; O, 09.56%.
*Ethyl 5-amino-1-(6-(4-(2-(2,4-difluorophenyl)acetyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8e**)*
Off white solid. Yield: 65%. mp (^{0}C): 204. IR (cm^{-1}): 3450 (N-H Primary amine stretching), 3321 (C-H Aromatic stretching), 2991 (C-H Alkane stretching), 1685 (>C=O Ester stretching), 1653 (>C=O Amide stretching), 1348 (C=C Aromatic stretching), 1282 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.50 (s, 1H, Ar-H Pyrazole), 7.80 (s, 1H, Ar-H), 7.73 (s, 2H, -NH_2), 7.31-7.33 (d, 1H, Ar-H), 7.17-7.21 (d, 1H, Ar-H), 7.01 (s, 2H, Ar-H Pyrimidine), 4.21-4.22 (q, 2H, -C<u>H$_2$</u>-CH_3), 3.60-3.79 (m, 10H, Ar-H Piperazine, -CH_2 near Piperazine ring), 1.26-1.29 (t, 3H, -CH_2-C<u>H$_3$</u>).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 173.15, 169.95, 167.25, 160.69, 158.02, 157.96, 155.32, 154.92, 142.02, 135.58, 120.08, 115.30, 100.69, 92.58, 80.40, 58.19, 48.54, 49.54, 43.15, 43.15, 37.11, 19.30. Mass (m/z): 471.50 (M^+). Anal. $C_{22}H_{23}F_2N_7O_3$ requires: C, 56.05%; H, 04.92%; N, 20.80%; O, 10.18%; Found: C, 56.10%; H, 04.86%; N, 20.77%; O, 10.14%.
*Ethyl 5-amino-1-(6-(4-benzoylpiperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8f**)*
Off white solid. Yield: 84%. mp (^{0}C): 214. IR (cm^{-1}): 3458 (N-H Primary amine stretching), 3298 (C-H Aromatic stretching), 2976 (C-H Alkane stretching), 1693 (>C=O Ester stretching), 1633 (>C=O Amide stretching), 1382 (C=C Aromatic stretching), 1276 (C-N Linkage stretching).^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.50 (s, 1H, Ar-H Pyrazole), 7.80 (s, 1H, Ar-H Pyrimidine), 7.50 (s, 2H, -NH_2), 7.45-7.48 (m, 5H, Ar-H), 7.03 (s, 1H, Ar-H Pyrimidine), 4.21-4.25 (q, 2H, -C<u>H$_2$</u>-CH_3), 3.74-4.19 (m, 8H, Ar-H Piperazine), 1.26-1.30 (t, 3H, -CH_2-C<u>H$_3$</u>).^{13}C NMR (DMSO-d_6, 101.1 MHz)

δppm:171.11, 168.35, 164.18, 161.32, 156.20, 155.68, 147.02, 140.62, 132.05, 131.78, 131.78, 125.02, 125.02, 102.32, 92.15, 78.95, 49.15, 49.15, 38.58, 38.58, 22.62. Mass (m/z): 421.38 (M^+). Anal. $C_{21}H_{23}N_7O_3$ requires: C, 59.85%; H, 05.50%; N, 23.26%; O, 11.39%; Found: C, 59.81%; H, 05.53%; N, 23.21%; O, 11.42%.

*Ethyl 5-amino-1-(6-(4-(4-ethylbenzoyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8g**)*

Off white solid. Yield: 66%. mp (^{0}C): 176. IR (cm^{-1}): 3631 (N-H Primary amine stretching), 3388 (C-H Aromatic stretching), 2962 (C-H Alkane stretching), 1683 (>C=O Ester stretching), 1604 (>C=O Amide stretching), 1460 (C=C Aromatic stretching), 1271 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.49 (s, 1H, Ar-H Pyrazole), 7.80 (s, 1H, Ar-H Pyrimidine), 7.73 (s, 2H, -NH_2), 7.37-7.39 (d, 2H, Ar-H), 7.30-7.37 (d, 2H, Ar-H), 7.01 (s, 1H, Ar-H Pyrimidine)4.19-4.24 (q, 2H, -C<u>H</u>$_2$-CH_3), 3.29-3.74 (m, 8H, Ar-H Piperazine), 2.63-2.68 (q, 2H, -CH_2 near Piperazine ring),1.19-1.34 (m, 6H, -CH_2-C<u>H</u>$_3$).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 172.78, 168.02, 165.62, 163.19, 158.30, 154.52, 145.61, 140.26, 138.47, 132.12, 132.12, 129.65, 129.65, 88.32, 81.37, 74.30, 48.65, 48.65, 42.61, 42.61, 35.68, 28.11, 18.68. Mass (m/z): 449.62 (M^+). Anal. $C_{23}H_{27}N_7O_3$ requires: C, 61.46%; H, 06.05%; N, 21.81%; O, 10.68%; Found: C, 61.48%; H, 06.09%; N, 21.83%; O, 10.65%.

*Ethyl 5-amino-1-(6-(4-(2,3-dichlorobenzoyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8h**)*

Off white solid. Yield: 70%. mp (^{0}C): 212. IR (cm^{-1}): 3466 (N-H Primary amine stretching), 3342 (C-H Aromatic stretching), 2908 (C-H Alkane stretching), 1685 (>C=O Ester stretching), 1645 (>C=O Amide stretching), 1442 (C=C Aromatic stretching), 1284 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.49 (s, 1H, Ar-H Pyrazole), 7.95 (s, 1H, Ar-H Pyrimidine), 7.86 (s, 2H, -NH_2), 7.72-7.80 (d, 1H, Ar-H), 7.42-7.58 (m, 2H, Ar-H), 7.01 (s, 1H, Ar-H Pyrimidine) 4.18-4.24 (q, 2H, -C<u>H</u>$_2$-CH_3), 3.27-3.81 (m, 8H, Ar-H Piperazine), 1.20-1.29 (t, 3H, -CH_2-C<u>H</u>$_3$).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 171.32, 166.58, 160.30, 159.68, 157.15, 153.69, 143.95, 137.62, 135.92, 131.03, 130.20, 125.68, 122.39, 103.45, 84.09, 70.61, 44.03, 44.03, 39.15, 39.15, 23.58. Mass (m/z): 489.24 (M^+). Anal. $C_{21}H_{21}Cl_2N_7O_3$ requires: C, 51.44%; H, 04.32%; N, 20.00%; O, 09.79%; Found: C, 51.38%; H, 04.35%; N, 20.07%; O, 09.73.

*Ethyl 5-amino-1-(6-(4-(3-bromo-2-chlorobenzoyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8i**)*

Light brown solid. Yield: 87%. mp (^{0}C): 184. IR (cm^{-1}): 3448 (N-H Primary amine stretching), 3327 (C-H Aromatic stretching), 2922 (C-H Alkane stretching), 1685 (>C=O Ester stretching), 1633 (>C=O Amide stretching), 1444 (C=C Aromatic stretching), 1305 (C-N Linkage stretching).^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.53 (s, 1H, Ar-H Pyrazole), 7.97 (s, 1H, Ar-H Pyrimidine), 7.68 (s, 2H, -NH_2), 7.58-7.62 (d, 1H, Ar-H), 7.34-7.46 (t, 1H, Ar-H), 7.21-7.28 (d, 1H, Ar-H), 7.04 (s, 1H, Ar-H Pyrimidine) 4.18-4.31 (q, 2H, -C<u>H</u>$_2$-CH_3), 3.71-4.18 (m, 8H, Ar-H Piperazine), 1.21-1.30 (t, 3H, -CH_2-C<u>H</u>$_3$).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 173.78, 168.26, 164.02, 160.37, 157.64, 156.25, 143.68, 139.69, 136.58, 132.03, 128.68, 124.92, 120.07, 104.34, 92.38, 69.46, 46.52, 46.52, 41.06, 41.06, 20.68. Mass (m/z): 534.79 (M^+). Anal. $C_{21}H_{21}BrClN_7O_3$ requires: C, 47.16%; H, 03.96%; N, 18.33%; O, 08.98%; Found: C, 47.19%; H, 03.89%; N, 18.36%; O, 08.93%.

*Ethyl 5-amino-1-(6-(4-nicotinoylpiperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**8j**)*

Off white solid. Yield: 75%. mp (^{0}C): 220. IR (cm^{-1}): 3437 (N-H Primary amine stretching), 3315 (C-H Aromatic stretching), 2980 (C-H Alkane stretching), 1678 (>C=O Ester stretching), 1614 (>C=O Amide stretching), 1492 (C=C Aromatic stretching), 1280 (C-N Linkage stretching). ^{1}H NMR (DMSO-d_6, 400 MHz) δppm: 8.68 (s, 1H, Ar-H Pyrazole), 8.50 (s, 1H, Ar-H), 7.88-7.91 (t, 2H, Ar-H), 7.80 (s, 1H, Ar-H Pyrimidine), 7.73 (s, 2H, -NH_2), 7.50-7.53 (d, 1H, Ar-H), 7.02 (s, 1H, Ar-H Pyrimidine) 4.19-4.24 (q, 2H, -C<u>H</u>$_2$-CH_3), 2.93-3.82 (m, 8H, Ar-H Piperazine), 1.20-1.29 (t, 3H, -CH_2-C<u>H</u>$_3$).^{13}C NMR (DMSO-d_6, 101.1 MHz) δppm: 172.25, 167.68, 165.02, 161.03, 159.96, 154.34, 148.25, 143.48, 141.68, 137.36, 132.06, 122.68, 105.57, 89.29, 67.21, 46.58, 46.58, 41.32, 41.32, 23.65. Mass (m/z): 422.39 (M^+). Anal. $C_{20}H_{22}N_8O_3$ requires: C, 56.86%; H, 05.25%; N, 26.53%; O, 11.36%; Found: C, 56.81%; H, 05.29%; N, 26.48%; O, 11.39%.

4.3 Molecular docking

ICM version 3.9-2b of Molsoft was used to predict the binding mode and affinity of ligands against the protein. The designed 2D structures were directly incorporated into ICM Molsoft and were converted in 3D, followed by minimization to remove all strain from the molecular structure and ensure a well-defined conformer. High-resolution crystal structure of enoyl-ACP reductase of Mycobacterium tuberculosis (PDB ID:4TRO) was downloaded through the protein Data Bank PDB/RCSB and imported into ICM Molsoft and was converted into ICM object which includes-deleting the water molecules, optimizing the hydrogen and other amino acids such as Histidine, protein and glycine, cysteine etc., missing side chains were also treated before the receptor was used for the docking process. The receptor grid was generated around the bound ligand (Isonicotinic-Acetyl-Nicotinamide-Adenine Dinucleotide). Two poses were generated for each ligand. In all cases, the program's default parameters were used. The binding poses were analyzed using ICM-molsoft.

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