Design, Molecular Docking, Synthesis and Biological Study of Pyrazole Containing Hydrazinyl Pyrimidine Derivatives with Potent Antitubercular Activity

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***Abstract*—A series of novel pyrazole-containing hydrazinyl pyrimidine derivatives were designed and subjected to molecular docking studies to investigate the binding interactions of the designed compounds with Protein (PDB ID: 5V3Y) involved in the survival of *Mycobacterium tuberculosis*. The Compounds 2a-2f shows favourable binding affinities and provided insight into the mechanisms of action. Those good binding energy compounds were synthesized from substituted acetophenone and substituted benzaldehyde to get the pyrazole-containing hydrazinyl pyrimidine derivatives and characterized by analytical IR, ^{1}HNMR and ESI-Mass spectrometry. Later, the synthesized compounds were evaluated for *in-vitro* anti-tubercular activity against *Mycobacterium tuberculosis* for MTCC 300 strains, where the Compound 2a, 2b and 2d revealed the good antitubercular activity when compared with Rifampicin as the standard drug, suggests that these pyrazole-pyrimidine derivatives hold potential as effective candidates for the development of new antitubercular agents.**

***Index Terms*—Pyrazole, Hydrazinyl Pyrimidine, Molecular docking, Antitubercular Activity.**

I. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major global health challenge claiming millions of lives each year [1]. Despite the availability of treatment, the emergence of multidrug-resistant and extensively drug-resistant strains has highlighted the need for novel and more effective antitubercular agents. In response to this urgent need, the design of new compounds targeting key biological pathways in *M. tuberculosis* has become a priority in drug discovery [2].

Pyrazole-containing compounds have garnered attention for their diverse pharmacological activities including antimicrobial and antitubercular effects. The incorporation of hydrazinyl pyrimidine moieties into such structures is particularly promising as pyrimidine derivatives are known for their role in inhibiting enzymes critical to the survival of mycobacteria. By combining these two bioactive scaffolds, we aim to develop novel molecules with enhanced antitubercular potency [3].

This study focuses on the synthesis of a series of pyrazole-containing hydrazinyl pyrimidine derivatives, followed by biological testing to evaluate their antitubercular activity against *M. tuberculosis* [4]. In addition, molecular docking studies were conducted to predict the binding affinity of these compounds to key target enzymes, providing valuable insight into their mechanism of action. The findings of this research could lead to the identification of promising drug candidates, contributing to the development of more effective treatments for tuberculosis, especially against resistant strains [6].

II. MATERIALS AND METHODOLOGY

A. Synthesis:

Step 1: Synthesis of N-acetyl pyrazole (1a, 1b, 1c, 1d, 1e, 1f): A solution of substituted acetophenones (0.01mol) in minimum quantity of ethanol (10 mL) was added to a benzaldehyde (0.01mol) in ethanol (10 mL) stir the mixture. To that add hydrazine hydrate (0.01M) with KOH solution. The reaction mixture was refluxed for 8hours, cool, diluted with water and acidified with conc. HCl. The product was filtered, dried and crystallized from ethanol.

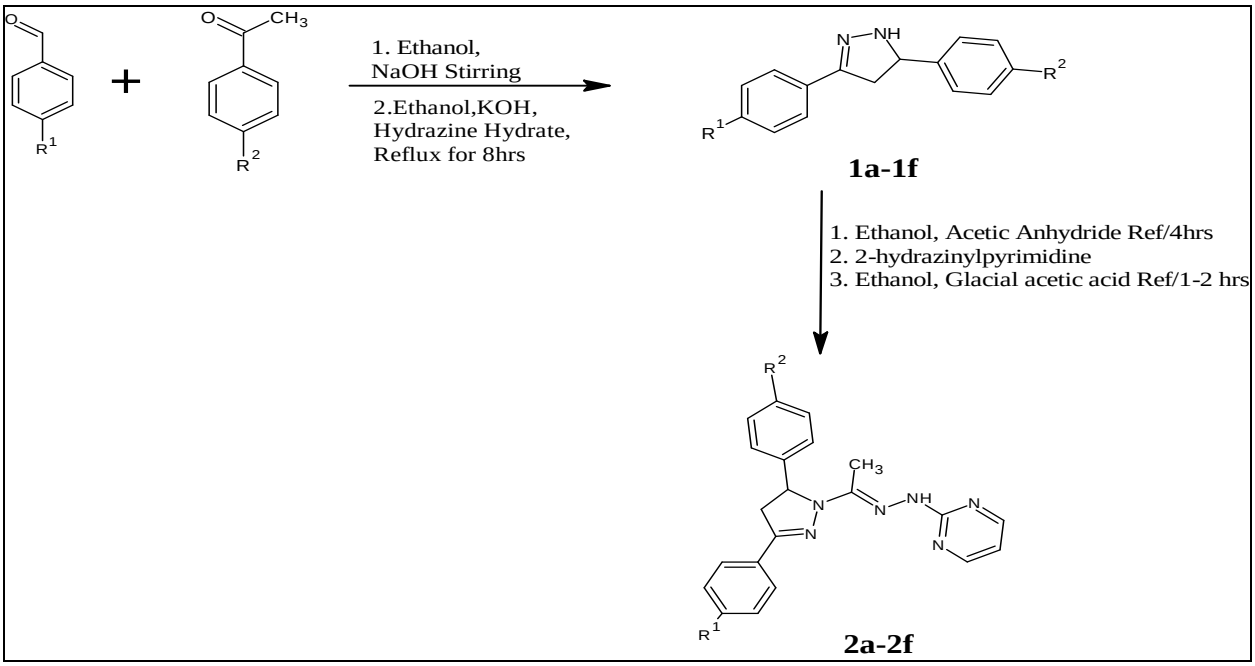


Fig no 1: Scheme for the synthesis of pyrazole contains hydrazinyl pyrimidine derivatives

Step-2: Synthesis of novel pyrazole contains hydrazinyl pyrimidine derivatives (2a, 2b, 2c, 2d, **2e** and 2f): To take 0.005mol of compound (Pyrazole derivatives), 5.1ml of acetic anhydride was added and warm hydrazine hydrate (0.01mol) solution, 2-amino pyrimidine (0.01mol) in alcohol (30ml) and NaOH (5ml, 0.01mol) solution was added and refluxed for 6h in the presence of glacial acetic acid. The progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and kept in refrigerator for overnight and extracted the product by using ethyl acetate. This was filtered off and recrystallized from ethanol to give crystalline solid.

III. MOLECULAR DOCKING

A. Dataset ligands and Ligand Optimization

The 2D structures of 6 compounds were generated from the ACD/Chemsketch Software. The generated ligands cleaned and performed 3D optimization then saved in the MDL Molfile format. The ligands were then converted to a PDBQT file format using the Open Babel chemistry toolbox [6].

B. Molecular Docking Studies

The three-dimensional structure of Pokeweed Antiviral Protein (PDB ID: 5V3Y) was downloaded from Brook heaven Protein Data Bank (https://www.rcsb.org) and saved as a Brookhaven protein data bank file and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using AUTODOCK suite of MGL Tools [7].

Auto dock Vina was used for molecular docking studies. A grid was generated around the co-crystallized ligand. The co-ordinates (x = 32.63, y =26.25, z = 16.59) were generated with the help of MGL Tools & Pharmit: interactive exploration of chemical space (http://pharmit.csb.pitt.edu/). Prepared pdbqt files for both target & ligands. Created in house batch file of ligands & amp; target and docking performed in the absence of water molecules for all 15 molecules. The molecules were analyzed after docking and visualized in the discovery studio for the interactions with the active site amino acids [8].

Binding interactions and efficiency of the binding were calculated in terms of dock Score, which is a combination of hydrophilic, hydrophobic, metal binding groups, Van der Waals energy, freezing rotatable bonds and polar interactions with receptor [9,10].

C. Characterisation:

Melting point of the synthesized compounds was determined by an open-end capillary tube method using electrically heated melting point apparatus. The

respective values were expressed in oC and were uncorrected. Reaction progress and compounds purity was ascertained by thin layer chromatography (TLC). The structures of the synthesized compounds were elucidated by Fourier Transform IR spectrometer (Thermo Nicolet Nexus 670) in the range of 400-4000 cm^{-1} using KBr pellets and values are reported in cm^{-1} and the spectra were interpreted. ^{1}H-NMR spectra were recorded on Bruker-Topspin NMR spectrometer using DMSO-d6 and chemical shift (δ) are reported in parts per million down field from internal reference Tetramethylsilane (TMS). Mass spectra were recorded on Shimadzu by LC-MS-8030 mass spectrometer and the spectra were interpreted.

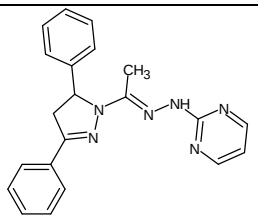
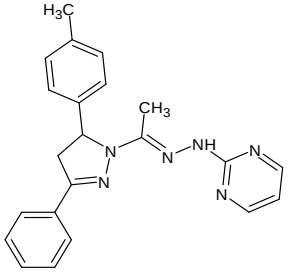
D. Antitubercular Activity:

The *M. tuberculosis* (MTCC 300) strain was used in the present study for the assessment of antimycobacterial activity. The strain used for the study was procured from Microbial Type Culture and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), India. The mentioned strain was sub-cultured and persevered as per the earlier depicted method on the Lowenstein Jensen medium. The Agar diffusion method was used to assess the sensitivity of *M. tuberculosis* strain against the synthesized compounds. Different stock solutions such as 0.1, 0.5, and 1.0 mg/mL of all compounds were prepared in dimethyl sulfoxide (DMSO). A sterile corn borer of 9-mm diameter was further employed to prepare holes into the Middlebrook 7H9 agar, already inoculum seeded and solidified. Firstly, the wells were labelled appropriately according to the compounds, and afterward a volume of 40 µL of each compound was added by using a sterile pipette. The test was executed in triplicates. To achieve the sample pre-diffusion, the plates were stored in the refrigerator and further incubated at room temperature for 48 h. After the incubation, the growth of the mentioned strain was detected and the diameter of the inhibition zone was measured. Rifampicin was used as a positive control for the mentioned experiment [11].

IV. RESULT AND DISCUSSION

A. Molecular Docking:

Molecular docking studies were performed in order to find the possible protein ligand interactions of the dataset ligands. The potential active site amino acids of 5V3Y complex were predicted using CASTp. The target protein and inhibitors were geometrically optimized. All the 6 compounds were docked against active site of target protein using AUTODOCK VINA. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About 100 different protein-ligand complex conformations for each docked complex were generated through AUTODOCK suite of MGL Tools, the confirmation with lowest binding energy was displayed as the best binding energy. Binding energy of the dataset ligands were shown in Table 1 along with the interaction amino acids and number of amino acids.

Compound name	Structure	Binding energy	No of Hydrogen bonding	Hydrogen Bonding Amino acids residues	Van der Waal's Interaction
2a		-10.2	1	TYR;1582	VAL;1611,ALA;1586, TRP;1579,ILE;1594, ALA;1583,ILE;1597, ARG;1581,MET;1669
2b		-9.8	1	TYR;1582	PHE;1590,ALA;1586 ,TRP;1579,ILE;1594, ALA;1583,ILE;1597, MET;1669, ARG;1581

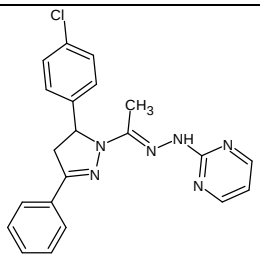
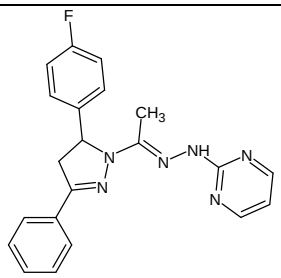
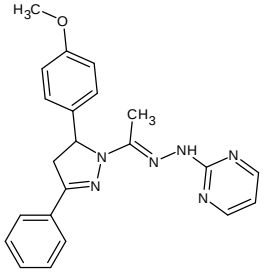
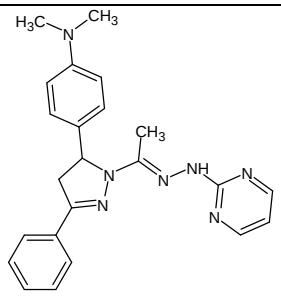
2c		-9.6	1	TYR;1582	TYR;1637,PHE;1590,ALA;1586,TRP;1579,ILE;1594,ALA;1583, ILE;1597,ARG;1581, MET;1669
2d		-10.1	1	TYR;1582	VAL;1611,PHE;1590, TRP;1579,ALA;1586,ILE;1594,ALA;1583, ILE;1597,ARG;1581
2e		-9.6	1	TYR;1582	ARG;1634,GLN;1633,ALA;1586,TRP;1579,ILE;1594,ALA;1583, ILE;1597,ARG;1581, MET;1669
2f		-9.7	1	TYR;1582	ARG;1634,LEU;1615,ALA;1586,ILE;1594,ALA;1583,ILE;1597, TRP;1579, ARG;1581, MET;1669

Table no 1: Molecular Docking results with β-Tubulin.

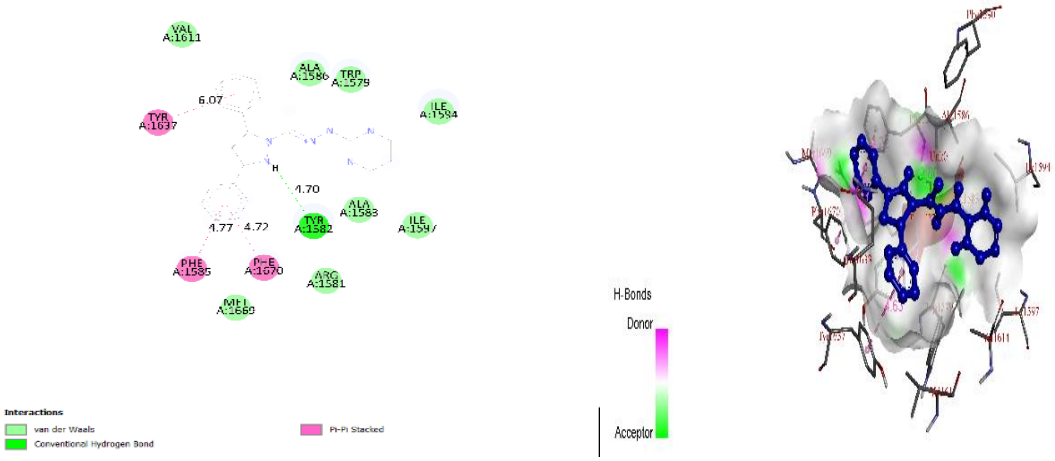


Fig no 2 :2D and 3D Structures of Compound 2a with PDB id: 5v3y

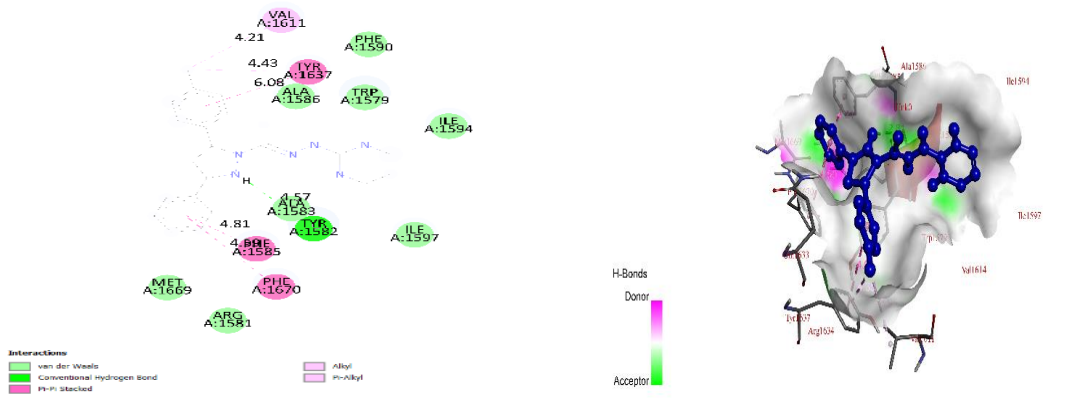


Fig no 3 :2D and 3D Structures of Compound 2b with PDB id: 5v3y

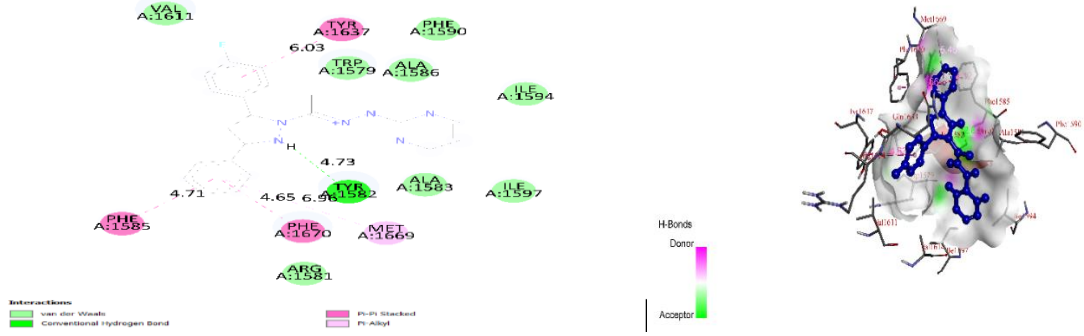


Fig no 4 :2D and 3D Structures of Compound 2d with PDB id: 5v3y

B. Characterization

Compound 2a: IR(vcm-1): 3325 (N-H *Str* in amine group), 3022 (C-H *Str,* aromatic), 2973, 2897 (C-H *Str* in aliphatic), 1646 (-C=N, *Str in* immine group), 1613 (-C=N, *Str* in rings), 1405 (CH=CH *Str* in aromatic ring), 1309 (C=C, *Str* in aromatic ring).

^{1}H-NMR(DMSO) δ ppm: 9.42992 (s, 1H, amine), 8.8090-8.5691 (d, 2H, aromatic H), 8.2210-8.2062 (d, 2H, aromatic -H), 8.1492-8.1292 (d, 2H,aromatic -H), 8.0568-8.0384 (t, 3H, aromatic -H), 7.8501-7.7337 (t, 3H, aromatic -H), 7.7285-7.5435 (t, 1H, aromatic - H), 4.5827-4.5802 (dd, 2H in pyrazole ring protons), 3.1528 (t, 1H in pyrazole ring protons), 1.8338 (s, 3H, -CNCH3).

Mass (ESI-MS): m/z 356.17 (M), 357.21 (M + 1, 100%).

Compound 2b:

IR(vcm-1): 3227 (N-H *Str* in amine group), 3017 (C-H *Str,* aromatic), 2915, 2873, 2741 (C-H *Str* in aliphatic), 1617 (-C=N, *Str in* immine group), 1513 (-C=N, *Str* in rings), 1430 (CH=CH *Str* in aromatic ring), 1322 (C=C, *Str* in aromatic ring).

^{1}H-NMR(DMSO) δ ppm: 9.4031 (s, 1H, -NH proton), 8.4787-8.3533 (d, 2H, aromatic H), 8.0122-8.0012 (d, 2H, aromatic -H), 7.9492-7.9267 (d, 2H, aromatic -H), 7.8287-7.7574 (d, 2H, aromatic -H), 7.1705-7.1463 (t, 3H, aromatic -H), 6.8819-6.8350 (t, 1H, aromatic -H), 4.3817- 4.3051 (dd, 2H in pyrazole ring protons), 3.0174 (t, 1H in pyrazole), 2.0714 (s, 3H, -CN-CH3), 1.8670 (s, 3H, Ar-CH3).

Mass (ESI-MS): m/z 370.19 (M), 371.3 (M + 1, 100%).

Compound 2c:

IR(vcm-1): 3205 (N-H *Str* in amine group), 3021 (C-H *Str,* aromatic), 2931, 2893 (C-H *Str* in aliphatic), 1616 (-C=N, *Str in* immine group), 1602 (-C=N, *Str* in rings), 1381 (CH=CH *Str* in aromatic ring), 1345 (C=C, *Str* in aromatic ring), 787 (C-Cl, *Str* in Ar-Cl ring).

^{1}H-NMR(DMSO) δ ppm: 9.1591 (s, 1H, -NH proton), 8.5001-8.3190 (d, 2H, aromatic H), 8.1546-8.1139 (d, 2H, aromatic -H), 8.0910-8.0142 (d, 2H, aromatic -H), 7.9400-7.9068 (d, 2H, aromatic -H), 7.8989-7.8040 (t, 3H, aromatic -H), 7.7940-7.6778 (t, 1H, aromatic -H), 4.5330-4.5078 (dd, 2H in pyrazole ring protons), 3.1726 (t, 1H in pyrazole), 2.0480 (s, 3H, Ar-CH3).

Mass (ESI-MS): m/z 390.14 (M), 391.03 (M + 1, 100%), 392.54 (M + 2, 30%)

Compound 2d:

IR(νcm-1): 3384 (N-H *Str* in amine group), 3087 (C-H *Str,* aromatic), 2993, 2821 (C-H *Str* in aliphatic), 1632 (-C=N, *Str in* immine group), 1598 (-C=N, *Str* in rings), 1365 (CH=CH *Str* in aromatic ring), 1301 (C=C, *Str* in aromatic ring), 821 (C-CF, *Str* in Ar-F ring).

^{1}H-NMR(DMSO) δ ppm: 9.5321 (s, 1H, -NH proton), 8.3452-8.2901 (d, 2H, aromatic H), 8.1032-8.1092 (d, 2H, aromatic -H), 7.8943-7.6753 (d, 2H, aromatic -H), 7.4132-7.3512 (d, 2H, aromatic -H), 7.2094- 7.1293 (t, 3H, aromatic -H), 6.9396-6.7832 (t, 1H, aromatic - H), 4.3523-4.2031 (dd, 2H in pyrazole ring protons), 3.0432 (t, 1H in pyrazole), 2.0934 (s, 3H, Ar-CH3).

Mass (ESI-MS): m/z 374.17 (M), 375.18 (M + 1, 100%), 376.03 (M + 2, 30%).

Compound 2e:

IR(νcm-1): 3239 (N-H *Str* in amine group), 3078 (C-H *Str,* aromatic), 2967, 2898, 2756 (C-H *Str* in aliphatic), 1631 (-C=N, *Str in* immine group), 1595 (-C=N, *Str* in rings), 1402 (CH=CH *Str* in aromatic ring), 1094 (C-O *Str* in Ar–OCH3), 1313 (C=C, *Str* in aromatic ring).

^{1}H-NMR(DMSO) δ ppm: 9.2093 (s, 1H, -NH proton), 8.2094-8.1732 (d, 2H, aromatic H), 8.1293-8.0021 (d, 2H, aromatic -H), 7.9845-7.8732 (d, 2H, aromatic -H), 7.5093-7.4321 (d, 2H, aromatic -H), 7.19943-7.1032 (t, 3H, aromatic -H), 6.9892-6.8773 (t, 1H, aromatic - H), 4.5643-4.4895 (dd, 2H in pyrazole ring protons), 3.6732-3.6003 (s, 3H, Ar-OCH3), 3.2187 (t, 1H in pyrazole), 1.9973 (s, 3H, Ar-CH3).

Mass (ESI-MS): m/z 386.19 (M), 387.02 (M + 1, 100%).

Compound 2f:

IR(νcm-1): 3342 (N-H *Str* in amine group), 3035 (C-H *Str,* aromatic), 2956, 2845, 2787 (C-H *Str* in aliphatic), 1643 (-C=N, *Str in* immine group), 1556 (-C=N, *Str* in rings), 1409 (CH=CH *Str* in aromatic ring), 1321 (C=C, *Str* in aromatic ring).

^{1}H-NMR(DMSO) δ ppm: 9.4320 (s, 1H, -NH proton), 8.4093-8.3823 (d, 2H, aromatic H), 8.1342-8.0932 (d, 2H, aromatic -H), 7.9874-7.7322 (d, 2H, aromatic -H), 7.8765-7.6433 (d, 2H, aromatic -H), 7.3231- 7.2093 (t, 3H, aromatic -H), 7.2013-7.0322 (t, 1H, aromatic - H), 4.3945-4.3803 (dd, 2H in pyrazole ring protons), 3.3092 (t, 1H in pyrazole), 2.8791-2.9932 (s, 6H, Ar-N(CH3)2), 2.0321 (s,3H, Ar-CH3).

Mass (ESI-MS): m/z 399.22 (M), 400.32 (M + 1, 100%).

Compound	R_1	R_2	Molecular Formula	Molecular Weight Gm/mol	Melting Point (°C)	% Yield	Rf Value
2a	H	H	$C_{21}H_{30}N_6$	356.42	177-179	76	0.81
2b	H	CH_3	$C_{22}H_{22}N_6$	370.04	243-245	80	0.67
2c	H	Cl	$C_{21}H_{19}N_6Cl$	390.14	213-215	74	0.81
2d	H	F	$C_{21}H_{19}N_6F$	374.17	209-211	74	0.73
2e	H	OCH_3	$C_{22}H_{22}N_6O$	386.19	201-203	69	0.76
2f	H	$N(CH3)_2$	$C_{23}H_{25}N$	399.13	219-221	78	0.72

Table No 2: Physical Characterisation data of the Synthesized Compounds (2a-2f)

C. Antitubercular Activity:

The results of the antimycobacterial activity of the synthesized compounds are summarized in Table 2, which clearly shows the differential sensitivity of Mycobacterial strain MTCC 300 toward the test compounds. The compounds **2b**(MIC-12.5±0.61µg/ml), **2b**(MIC-9.0±0.56µg/ml) and 2**d**(MIC-11.8±0.65µg/ml) were found to be most effective growth inhibitors of this strain when Compared with the Standard drug Rifampicin.

Sl No	Compound name	MIC(µg/ml)
1	2a	12.5 ± 0.61
2	2b	9.0 ± 0.56
3	2c	7.6 ± 0.83
4	2d	11.8 ± 0.65
5	2e	7.6 ± 0.72
6	2f	8.7 ± 0.48
7	Rifampicin	0.8±0.70

Table no 3: Antitubercular screening of compounds. The results are expressed as the mean values from three independent experiments ± standard deviation

V. CONCLUSION

In conclusion, molecular docking studies, synthesis and biological evaluation of pyrazole-containing hydrazinyl pyrimidine derivatives have demonstrated their promising antitubercular activity against

Mycobacterium tuberculosis. The molecular docking studies provided valuable insights into the compounds' binding interactions with PDB ID: 5V3Y crucial to the survival of *M. tuberculosis*, supporting the observed Antitubercular activity. The *in-vitro* antitubercular activity revealed that Compound 2a, 2d and 2b of the synthesized compounds exhibit potent inhibitory effects, comparable to or exceeding the activity of standard antitubercular drug Rifampicin. These findings suggest that pyrazole-pyrimidine derivatives are promising candidates for further development as novel antitubercular agents, particularly for combating drug-resistant strains.

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