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In silico and *In vitro* Antitubercular Studies for Nitrogen Rich Hybrids of homopiperazine-pyrimidine-Pyrazole Adducts

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Novel homopiperazine-pyrimidine-pyrazole hybrids (**3a-j**) were synthesized using ethyl 2-cyanoacetate and 4,6-dichloropyrimidine as starting materials by a multi-step process to afford ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate in good yields using polar protic media. The intermediate **1**, in two steps, chloroamine condensation followed by acid amine coupling, furnished the title compounds ethyl 5-amino-1-(6-(4-substituted aryl-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**3a-j**). The synthesized compounds were docked in the crystal structure of *Mycobacterium tuberculosis* (PDB ID: 4TRO) to get insights into structural requirements for antitubercular activity. *In vitro* antitubercular activity against *M. tuberculosis* H37Rv strains showed that compounds **3a, 3d, 3e** and **3g** were found to be the most potent (Docking score: > -21; MIC = 1.6 µg/mL) 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.

Keywords: Pyrazole, Pyrimidine, Homopiperazine, Molecular docking, Antitubercular activity.

INTRODUCTION

From the discovery of *Mycobacterium tuberculosis*, causes for the TB by Robert Koch to till date [1], its infectious nature, complex immunological response, chronic progression and the need for long-term treatment, it has always been a major health burden. In more recent years, the appearance of multi-drug resistant forms and the current TB-HIV epidemic, associated with its severe social implications, treating and preventing TB have represented a permanent challenge throughout human history [2,3]. The discovery of new antitubercular agents with potential activity, less toxicity, broader spectrum and safer therapeutic proles, apart from the available first-line and second-line TB drugs, is an urgent need [4].

A very common fragment of several active pharmaceutical ingredients, nitrogen heterocycles or various positional combinations of nitrogen atoms, sulphur and oxygen in five or six and recently seven-membered rings can be found [5]. In this context, pyrazole and pyrimidine-based scaffolds attracted organic chemists very much due to their biological and chemotherapeutic importance [6]. Pyrazolopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. They are known to exhibit pharmacological activities such as CNS depressant, neuroleptic, the general class of adenosine receptors, tuberculostatic and many others [7-14]. The recent advances in the seven-membered ring system *i.e.* piperazine derivatives of ursolic acid analogues synthesized by Shao *et al.* [15] were found to be effective anticancer agents. In addition, piperazine moiety is included in many synthetic drugs such as antianginals, antidepressants, antihistamines, antiserotonergics and antipsychotics [16-18].

Keeping in mind the biomedical applications and to further assess the pharmacological prole of the nitrogen-bearing class of compounds, we envisioned our approach toward the synthesis of a novel series of ethyl 5-amino-1-(6-(4-substituted phenyl-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate derivatives by incorporating the three nitrogen-rich heterocycles *i.e.* homopiperazine, pyrazole and pyrimidine

in a single molecular framework with a potential spectrum of bio responses. The antitubercular activities of the newly synthesized compounds against *M. tuberculosis* H37Rv strain were studied using Alamar blue dye using microplate Alamar blue assay (MABA) method and the results were supported by *in silico* computational screening (PDB ID: 4TRO).

EXPERIMENTAL

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. TLC on silica gel plates (Merck, $60F_{254}$) was used for reaction monitoring. Elemental analysis (% C, H, N) was carried out on a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Bruker FT-IR alpha-t spectrophotometer in ATR. ^{1}H NMR (400 MHz) and ^{13}C NMR (101.1 MHz) spectra were recorded on Bruker AVANCE II spectrometer using tetramethyl silane (TMS) as the internal reference, with dimethyl sulfoxide (DMSO-d_6) as solvent. All chemical shifts were expressed in ppm. Mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer. Anhydrous reactions were carried out in oven-dried glassware in a nitrogen atmosphere.

Synthesis of 2-(ethoxymethyl)-3-methoxyacrylonitrile (C): In a round bottom flask (moisture-free), containing ethyl 2-cyanoacetate (**A**, 1 mmol) and triethoxymethane (**B**, 1 mmol) in acetic anhydride were refluxed at 140 ºC for 7 h. After completion of the reaction, acetic anhydride was distilled off and cooled to room temperature. The reaction mixture was poured into ice-water, filtered off and washed with ice-cold water. The product obtained was dried and recrystallized from alcohol. The obtained product was used directly for the next step. The formation of this intermediate **C** was identified based on slight orange colour solid at room temperature.

Synthesis of 4-chloro-6-hydrazinylpyrimidine (E): A solution of 4,6-dichloropyrimidine (**D**, 1 mmol) and hydrazine hydrate (0.95 mmol) in ethanol was stirred at room temperature for 2 h. After completion of the reaction, ethanol was distilled off and then cooled to room temperature. Isolated solid was filtered off and washed with ice-cold water. The product obtained was dried and recrystallized from alcohol.

Synthesis of ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (1): To a solution of 2-(ethoxymethyl)-3-methoxyacrylonitrile (**C**, 1 mmol) and 4-chloro-6-hydrazinylpyrimidine (**E**, 1 mmol) was charged and refluxed at 80 ºC for 8 h to generate a reactive intermediate **1**. After completion of the reaction, the reaction mass was poured into ice-cold water and stirred for 15 min at room temperature to isolate free product. The isolated product was dried for the next 12 h at room temperature.

Synthesis of ethyl 1-(6-(1,4-diazepan-1-yl)pyrimidin-4-yl)-5-amino-1*H*-pyrazole-4-carboxylate (2): A mixture of **1** (1 mmol) and homopiperazine (1.5 mmol) was made soluble in ethanol. The reaction mass was stirred well and added a catalytic amount of triethylamine after 30 min of stirring. The reaction mixture was stirred at 0-5 ºC. The mixture thus obtained was stirred for 4 h at room temperature and then the solution containing precipitates was poured on to crushed ice. The product was filtered and crystallized in methanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.89 (s, 1H, -NH, homopiperazine), 8.50 (s, 1H, imidazole-H),7.79 (s, 1H, pyrimidine-H), 7.73 (s, 2H, $-NH_2$), 6.93 (s, 1H, pyrimidine-H), 4.24-3.12 (m, 10H, homopiperazine-H), 2.07-1.91 (q, 2H, $-C\underline{H}_2-CH_3$), 1.29-1.23 (t, 3H, $-CH_2-C\underline{H}_3$);

General procedure for the synthesis of ethyl 5-amino-1-(6-(4-substitutedphenyl-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3a-j): To a solution of **2** (1 mmol) and substituted aromatic acid (1 mmol) in DMF was stirred well for 15 min at room temperature. To this reaction mixture, EDC·HCl:HoBt:DIPEA (in a ratio of 1:1:2) were added by maintaining the temperature below 0-5 ºC and vigorously stirred to get a clear solution. Reaction mass was then stirred for 3 h at room temperature. The separated solid was filtered, dried and recrystallized from ethyl acetate.

Ethyl 5-amino-1-(6-(4-(2-(4-methoxyphenyl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3a): Yield: 81%; m.p.: 192 ºC; IR (ATR, ν_{max} cm^{-1}): 3471 (N-H, 1º amine), 3269 (C-H, aromatic ring), 2929 (-C-H, methylene group), 1671 (>C=O ester), 1645 (>C=O amide), 1544 (>C=C<, aromatic ring), 1348 (C-N, carbon nitrogen linkage); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.50 (s, 1H, imidazole-H), 7.80-7.71 (d, 3H, Ar-H), 7.19 (s, 2H, $-NH_2$), 6.99-6.88 (d, 3H, Ar-H), 4.21 (s, 2H, $-CH_2$ near cyclic amide), 3.33-3.73 (m, 13H, $-OCH_3$, homopiperazine-H), 1.82 (q, 2H, $-C\underline{H}_2-CH_3$), 1.28 (t, 3H, $-CH_2-C\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 17.10, 32.15, 43.55, 48.26, 49.12, 51.12, 54.02, 55.65, 67.26, 85.56, 93.35, 115.11, 115.11, 122.95, 132.45, 132.45, 142.65, 157.95, 159.32, 161.95, 163.12, 164.78, 168.20, 173.06; MS: *m/z* 479 (M^+); Elemental analysis of $C_{24}H_{29}N_7O_4$ calcd. (found) %: C, 60.11 (60.15); H, 6.10 (6.07); N, 20.45 (20.39); O, 13.35 (13.37).

Ethyl 5-amino-1-(6-(4-(2-(2-bromophenyl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3b): Yield: 75%; m.p.: 178 ºC; IR (ATR, ν_{max} cm^{-1}): 3396 (N-H, 1º amine), 3271 (C-H, aromatic ring), 2956 (-C-H, methylene group), 1681 (>C=O ester), 1647 (>C=O amide), 1537 (>C=C<, aromatic ring), 1361 (C-N, carbon nitrogen linkage), 669 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.52 (s, 1H, imidazole-H), 7.91 (s, 1H, pyrimidine-H),7.51 (s, 2H, $-NH_2$), 7.18-7.49 (m, 4H, Ar-H), 7.10 (s, 1H, pyrimidine-H), 4.08-4.18 (m, 2H, homopiperazine-H), 3.74 (s, 2H, $-CH_2$ near cyclic amide), 3.35-3.41 (m, 6H, homopiperazine-H), 3.18-3.30 (m, 2H, homopiperazine-H), 1.72-1.81 (q, 2H, $-C\underline{H}_2-CH_3$), 1.22-1.29 (t, 3H, $-CH_2-C\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 18.45, 28.36, 42.62, 46.02, 47.95, 50.67, 56.02, 64.23, 84.65, 92.25, 122.65, 125.22, 127.03, 131.96, 135.95, 137.03, 143.69, 152.68, 159.62, 162.30, 163.55, 167.21, 172.47; MS: *m/z* 528 (M^+); Elemental analysis of $C_{23}H_{26}N_7O_3Br$ calcd. (found) %: C, 55.28 (55.33); H, 4.96 (4.99); N, 18.56 (18.51); O, 9.08 (9.03).

Ethyl 5-amino-1-(6-(4-(2-(naphthalen-1-yl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3c): Yield: 69%; m.p.: 180 ºC; IR (ATR, ν_{max} cm^{-1}): 3400 (N-H, 1º amine), 3273 (C-H, aromatic ring), 2924 (-C-H, methylene group), 1683 (>C=O ester), 1643 (>C=O amide), 1510 (>C=C<, aromatic ring), 1348 (C-N, carbon nitrogen linkage); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.48 (s, 1H, imidazole-H), 7.98-

7.89 (m, 2H, Ar-H), 7.87-7.80 (m, 2H, pyrimidine-H), 7.73 (s, 2H, -NH_2), 7.55-7.51 (d, 2H, Ar-H), 7.50-7.45 (m, 1H, Ar-H), 7.40-7.35 (m, 1H, Ar-H), 6.99 (s, 1H, Ar-H), 4.24-4.16 (m, 4H, homopiperazine-H), 3.75-3.61 (m, 6H, homopiperazine-H), 3.34 (s, 2H, -CH_2 near cyclic amide), 1.83 (q, 2H, -C$\underline{H}_2$-CH_3), 1.20 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 17.45, 29.71, 44.45, 45.96, 47.02, 48.69, 50.12, 62.54, 83.06, 92.78, 121.65, 124.02, 126.01, 127.95, 130.45, 131.02, 132.68, 134.44, 136.39, 138.36, 138.74, 151.98, 158.32, 160.25, 161.58, 167.03, 171.65; MS: *m/z* 499 (M^+); Elemental analysis of $C_{27}H_{29}N_7O_3$ calcd. (found) %: C, 64.91 (64.94); H, 5.85 (5.89); N, 19.63 (19.58); O, 9.61 (9.64).

Ethyl 5-amino-1-(6-(4-(2-(3-fluorophenyl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3d): Yield: 70%; m.p.: 168 °C; IR (ATR, ν_{max} cm^{-1}): 3408 (N-H, 1° amine), 3282 (C-H, aromatic ring), 2945 (-C-H, methylene group), 1683 (>C=O ester), 1639 (>C=O amide), 1541 (>C=C<, aromatic ring), 1417 (C-N, carbon nitrogen linkage), 1014 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.43 (s, 1H, imidazole-H), 7.79 (s, 2H, -NH_2), 6.77-7.25 (m, 6H, Ar-H), 4.19-4.25 (m, 2H, homopiperazine-H), 3.82 (s, 2H, -CH_2 near cyclic amide), 3.39-3.66 (m, 6H, homopiperazine-H), 3.16-3.29 (m, 2H, homopiperazine-H), 1.67-1.75 (q, 2H, -C$\underline{H}_2$-CH_3), 1.23-1.30 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 19.54, 30.25, 39.65, 42.95, 47.69, 51.98, 52.03, 57.51, 85.69, 92.96, 110.36, 119.20, 125.08, 127.65, 138.35, 141.65, 153.39, 160.22, 160.56, 162.28, 167.01, 169.32, 172.35; MS: *m/z* 467 (M^+); Elemental analysis of $C_{23}H_{26}N_7O_3F$ calcd. (found) %: C, 59.09 (59.12); H, 5.61 (5.65); N, 20.97 (20.93); O, 10.97 (10.25).

Ethyl 5-amino-1-(6-(4-(2-(3,4-dichlorophenyl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3e): Yield: 85%; m.p.: 202 °C; IR (ATR, ν_{max} cm^{-1}): 3397 (N-H, 1° amine), 3302 (C-H, aromatic ring), 2931 (-C-H, methylene group), 1676 (>C=O ester), 1635 (>C=O amide), 1539 (>C=C<, aromatic ring), 1348 (C-N, carbon nitrogen linkage), 823 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.49 (s, 1H, imidazole-H), 7.80 (s, 1H, pyrimidine-H), 7.72 (s, 2H, -NH_2), 7.55-7.57 (d, 1H, Ar-H), 7.22-7.24 (m, 1H, Ar-H), 7.51 (s, 1H, pyrimidine-H), 7.21 (s, 1H, Ar-H), 4.19-4.24 (m, 2H, homopiperazine-H), 3.81 (s, 2H, -CH_2 near cyclic amide), 3.74-3.75 (m, 2H, homopiperazine-H), 3.63-3.67 (m, 4H, homopiperazine-H), 3.60-3.61 (m, 2H, homopiperazine-H), 1.82-1.89 (q, 2H, -C$\underline{H}_2$-CH_3), 1.26-1.29 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 18.45, 29.25, 40.85, 43.96, 48.92, 50.33, 51.65, 57.40, 82.62, 87.78, 121.68, 132.08, 133.61, 135.00, 136.48, 138.62, 140.92, 151.62, 159.30, 160.62, 163.09, 167.25, 173.34; MS: *m/z* 518 (M^+); Elemental analysis of $C_{23}H_{25}Cl_2N_7O_3$ calcd. (found) %: C, 53.29 (53.35); H, 4.86 (4.91); N, 18.91 (18.94); O, 9.26 (9.21).

Ethyl 5-amino-1-(6-(4-(2-(2,4-difluorophenyl)acetyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3f): Yield: 67%; m.p.: 182 °C; IR (ATR, ν_{max} cm^{-1}): 3454 (N-H, 1° amine), 3066 (C-H, aromatic ring), 2929 (-C-H, methylene group), 1670 (>C=O ester), 1620 (>C=O amide), 1508 (>C=C<, aromatic ring), 1363 (C-N, carbon nitrogen linkage), 1018 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.52 (s, 1H, imidazole-H), 7.78 (s, 1H, pyrimidine-H), 7.74 (s, 2H, -NH_2), 7.57-7.69 (d, 1H, Ar-H), 7.45 (s, 1H, pyrimidine-H), 6.78-6.92 (d, 1H, Ar-H), 6.12 (s, 1H, Ar-H), 4.08-4.18 (m, 6H, homopiperazine-H), 3.51-3.64 (m, 4H, homopiperazine-H), 3.32 (s, 2H, -CH_2 near cyclic amide), 1.80-1.83 (q, 2H, -C$\underline{H}_2$-CH_3), 1.21-1.30 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 16.45, 26.95, 41.02, 45.66, 47.38, 50.23, 56.95, 60.82, 84.12, 92.65, 101.65, 116.73, 121.09, 127.53, 140.18, 152.62, 158.06, 159.02, 159.81, 161.65, 162.68, 168.36, 171.32; MS: *m/z* 485 (M^+); Elemental analysis of $C_{23}H_{25}N_7O_3F_2$ calcd. (found) %: C, 56.90 (56.87); H, 5.19 (5.15); N, 20.20 (20.15); O, 9.89 (9.93).

Ethyl 5-amino-1-(6-(4-(furan-2-carbonyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3g): Yield: 83%; m.p.: 214 °C; IR (ATR, ν_{max} cm^{-1}): 3423 (N-H, 1° amine), 3307 (C-H, aromatic ring), 2924 (-C-H, methylene group), 1685 (>C=O ester), 1647 (>C=O amide), 1534 (>C=C<, aromatic ring), 1365 (C-N, carbon nitrogen linkage), 669 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.46 (s, 1H, imidazole-H), 7.82-7.79 (d, 2H, Furan-H), 7.71 (s, 2H, -NH_2), 6.99-6.91 (d, 2H, pyrimidine-H), 6.60 (s, 1H, Furan-H), 4.24-3.58 (m, 10H, homopiperazine-H), 1.99-1.88 (q, 2H, -C$\underline{H}_2$-CH_3), 1.31-1.23 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 18.21, 31.69, 42.65, 45.25, 50.69, 51.63, 58.12, 78.69, 88.58, 109.23, 115.95, 132.24, 142.98, 144.36, 151.59, 160.02, 161.36, 162.69, 167.24, 167.56; MS: *m/z* 425 (M^+); Elemental analysis of $C_{20}H_{23}N_7O_4$ calcd. (found) %: C, 56.46 (56.53); H, 5.45 (5.40); N, 23.05 (23.09); O, 15.04 (15.01).

Ethyl 5-amino-1-(6-(4-(2,3-dichlorobenzoyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3h): Yield: 66%; m.p.: 168 °C; IR (ATR, ν_{max} cm^{-1}): 3442 (N-H, 1° amine), 3311 (C-H, aromatic ring), 2924 (-C-H, methylene group), 1683 (>C=O ester), 1637 (>C=O amide), 1508 (>C=C<, aromatic ring), 1367 (C-N, carbon nitrogen linkage), 713 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.54 (s, 1H, imidazole-H), 7.81 (s, 2H, -NH_2), 7.61 (s, 1H, 1H, pyrimidine-H), 7.21-7.35 (d, 2H, Ar-H), 7.10 (s, 1H, pyrimidine-H), 7.45-6.56 (d, 1H, Ar-H), 4.02-4.14 (m, 2H, homopiperazine-H), 3.67-3.74 (m, 2H, homopiperazine-H), 3.31-3.35 (m, 4H, homopiperazine-H), 3.21-3.28 (m, 2H, homopiperazine-H), 1.70-1.79 (q, 2H, -C$\underline{H}_2$-CH_3), 1.25-1.28 (t, 3H, -CH_2-C$\underline{H}_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 17.45, 26.35, 39.11, 40.78, 45.69, 47.42, 57.69, 78.02, 89.69, 120.72, 122.63, 126.95, 130.62, 132.62, 134.30, 147.86, 151.23, 157.32, 158.62, 160.44, 167.21, 171.51; MS: *m/z* 518 (M^+); Elemental analysis of $C_{22}H_{23}N_7O_3Cl_2$ calcd. (found) %: C, 53.39 (53.43); H, 4.60 (4.63); N, 19.44 (19.39); O, 9.52 (9.48).

Ethyl 5-amino-1-(6-(4-(2,4-difluorobenzoyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (3i): Yield: 78%; m.p.: 174 °C; IR (ATR, ν_{max} cm^{-1}): 3392 (N-H, 1° amine), 3280 (C-H, aromatic ring), 2924 (-C-H, methylene group), 1689 (>C=O ester), 1627 (>C=O amide), 1516 (>C=C<, aromatic ring), 1348 (C-N, carbon nitrogen linkage), 1064 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.47 (s, 1H, imidazole-H), 7.83 (s, 1H, pyrimidine-H), 7.76 (s, 2H, -NH_2), 7.58 (s, 1H, Ar-H), 7.43 (s, 1H, pyrimidine-H), 7.40 (s, 1H, Ar-H), 7.34-7.38 (d, 1H, Ar-H), 4.15-4.25(m, 8H, homo-

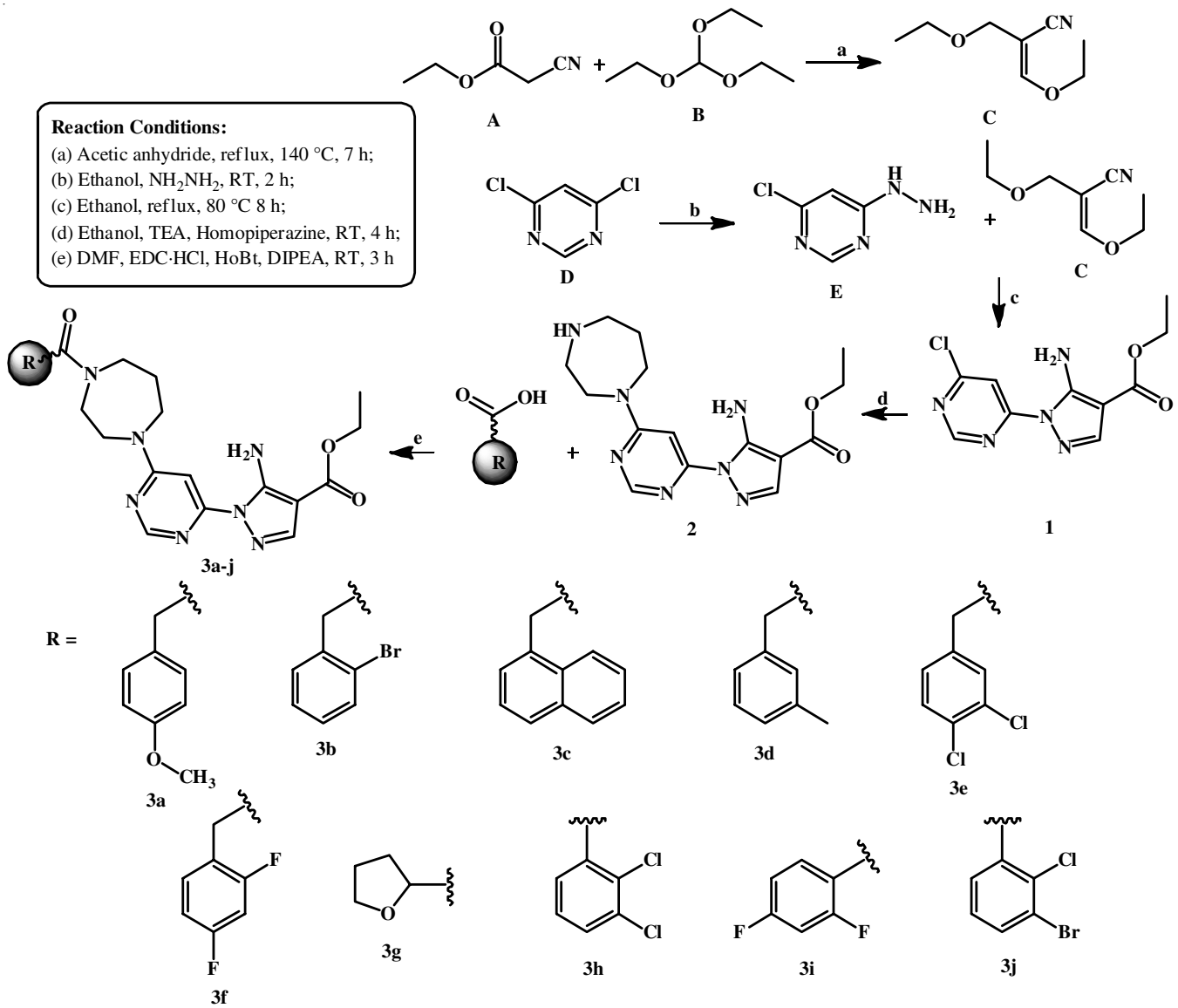
piperazine-H), 3.58-3.73 (m, 2H, homopiperazine-H), 1.80-1.86 (q, 2H, -C<u>H</u>$_2$-CH$_3$), 1.18-1.24 (t, 3H, -CH$_2$-C<u>H</u>$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 19.40, 29.58, 45.69, 47.65, 50.89, 48.69, 60.52, 83.62, 88.62, 100.21, 108.36, 120.25, 130.52, 135.69, 154.15, 158.95, 160.69, 161.36, 162.55, 165.49, 167.28, 172.91; MS: *m/z* 471 (M$^+$); Elemental analysis of $C_{22}H_{23}N_7O_3F_2$ calcd. (found) %: C, 56.05 (56.08); H, 4.92 (4.87); N, 20.80 (20.83); O, 10.18 (10.21).

Ethyl 5-amino-1-(6-(4-(3-bromo-2-chlorobenzoyl)-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (5j): Yield: 68%; m.p.: 198 ºC; IR (ATR, ν_{max} cm^{-1}): 3446 (N-H, 1º amine), 3311 (C-H, aromatic ring), 2924 (-C-H, methylene group), 1683 (>C=O ester), 1635 (>C=O amide), 1539 (>C=C<, aromatic ring), 1348 (C-N, carbon nitrogen linkage), 829 (C-Cl), 669 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.49 (s, 1H, imidazole-H), 7.81 (s, 2H, -NH$_2$), 7.45 (s, 2H, pyrimidine-H), 6.92-7.19 (m, 1H, Ar-H), 6.78-7.81 (m, 2H, Ar-H),3.97-4.12 (m, 2H, homopiperazine-H), 3.81-3.89 (m, 4H, homopiperazine-H), 3.71-3.74 (m, 2H, homopiperazine-H), 3.57-3.68 (m, 2H, homopiperazine-H), 1.80-1.84 (q, 2H, -C<u>H</u>$_2$-CH$_3$), 1.25-1.32 (t, 3H, -CH$_2$-C<u>H</u>$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 21.87, 29.69, 39.26, 45.62, 50.68, 51.41, 60.65, 85.85, 93.21, 120.77, 124.59, 132.67, 137.03, 139.54, 141.66, 142.69, 156.58, 160.95, 161.58, 162.35, 168.12, 172.65; MS: *m/z* 548 (M$^+$); Elemental analysis of $C_{22}H_{23}N_7O_3BrCl$ calcd. (found) %: C, 48.15 (48.15); H, 4.22 (4.19); N, 17.87 (17.83); O, 08.75 (8.70).

***In vitro* antitubercular activity:** Synthesized compounds (**3a-j**) were evaluated for their whole-cell anti-TB activity against H37Rv strains o *M. tuberculosis*. The minimum inhibitory concentration (MIC) values were determined by the serial dilution technique using Alamar blue dye using the micro plate Alamar blue assay (MABA) method.

RESULTS AND DISCUSSION

The synthesis of a novel hybrid of homopiperazine-pyrazole derivatives described in this study is outlined in **Scheme-I**. Title compounds (**3a-j**) were synthesized in five steps. Compound **C** was synthesized by a mixture of ethyl 2-cyanoacetate



Scheme-I: Synthetic route for hybrid homopiperazine-pyrimidine-pyrazole adducts (**3a-j**)

(**A**) and 2-(diethoxymethoxy)ethan-1-ylium (**B**) which was refluxed at 80 ºC using acetic anhydride as a solvent to give 3-ethoxy-2-(ethoxymethyl)acrylonitrile (**C**). 4,6-Dichloropyrimidine (**D**) was treated with hydrazine hydrate at room temperature to give intermediate 4-chloro-6-hydrazinylpyrimidine (**E**). In the third step, intermediate **C** and intermediate **E** was refluxed in the presence of ethanol as a solvent to give ethyl 5-amino-1-(6-chloropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**1**). In the fourth step, compound **1** was treated with homopiperazine and a catalytic amount of triethylamine in ethanol to give ethyl 1-(6-(1,4-diazepan-1-yl)pyrimidin-4-yl)-5-amino-1*H*-pyrazole-4-carboxylate (**2**). In the final step, compounds **3a-j** were generated by intermediate **2** with EDC-HCl: HOBT: DIPEA (1:1:2 proportion) in the presence of DMF as a solvent. ^{1}H NMR, IR, mass of synthesized compounds were recorded and found in full agreement with the proposed structures. The elemental analysis results were within ± 0.5% of the theoretical values.

The structural assignment for the synthesized compounds **3a-j** was confirmed based on IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The IR spectrum of synthesized compounds **3a-j** gave stretching vibration at ~3454 cm^{-1}, indicating the primary amine attached in the final motif. The Ar-H presence in the structure was seen by a stretching frequency obtained at ~3311 cm^{-1} in the molecules. A sharp absorption band at ~2931 cm^{-1} helped to conclude the targeted compounds' >CH_2 functional group. The intense absorption peaks at ~1676 cm^{-1} and ~1639 cm^{-1} were obtained due to the presence of >C=O functionality of ester and cyclic amide respectively. Moreover, the absorption band appeared in compounds **3a-j** at ~1541 and ~1348 cm^{-1} indicated C=C, C-N linkage present in the compound. In the ^{1}H NMR spectrum for the compounds, **3a-j** were confirmed by the singlet signal's appearance at δ = ~8.50 ppm due to the presence of a pyrazole ring proton. The definite peak for the pyrimidine proton shows at δ ~7.80 ppm. The protons present on the aromatic nucleus showed the absorption details between δ ~6.60 to ~7.98 ppm. Compounds **3a-j** was also conforming broad singlet peak showed at δ ~7.19 ppm due to the presence of primary amine group of the pyrazole ring system. Moreover, compounds **3a-j** homopiperazine ring proton shows a characteristic value between at δ ~3.58 to ~4.24 ppm. The remaining substituents' protons were in fair agreement with theoretical values. ^{13}C NMR spectra helped us to identify the formation of the final adducts. ^{13}C NMR Spectra has also confirmed the presence of two peaks at δ ~60 δ ppm and ~39 ppm demonstrating the methylene carbon terminal part of the compound and the one adjacent to phenyl ring respectively. The homopiperazine ring and pyrimidine carbons show the peak at δ ~52 and ~156 ppm, respectively confirms the cyclization as it is in the range of aromatic value. Moreover, the characteristic value δ ~172 ppm showed the presence of the carbonyl group present in compounds **3a-j**. The aromatic ring carbon and heterocyclic ring carbons were in decent agreement with the theoretical values. The mass spectrum revealed a molecular ion peak in compounds **3a-j** shown between *m/z* =~548 to ~425 in mass spectra, molecular ion peak was in agreement with proposed molecular weight and elemental analysis.

***In vitro* antitubercular activity:** The growth inhibition of the tested compounds is given in Table-1. It was encouraging to observe that several tested compounds displayed attractive antitubercular activity. Compounds **3a**, **3d**, **3e** and **3g** were found to be the most potent compounds amongst the series. They were found to be even more active than the standard drugs used *i.e.* pyrazinamide, ciprofloxacin and streptomycin.

TABLE-1
In vitro ANTITUBERCULAR ACTIVITY (MIC) WITH *in silico* STUDY (DOCKING SCORE) OF PYRIMIDINE-PYRAZOLE-PIPERAZINE ADDUCTS (**3a-j**)

Compound	Docking score	MIC (µg/mL)
3a	-28.50	1.6
3b	-9.83	50
3c	-16.55	12.5
3d	-21.99	1.6
3e	-27.17	1.6
3f	-14.60	50
3g	-22.98	1.6
3h	-15.39	6.25
3i	-22.10	25
3j	-20.69	3.12
Pyrazinamide	–	3.125
Ciprofloxacin	–	3.125
Streptomycin	–	6.25

***In silico* drug design:** Molecular docking of the compounds into crystal structure of *M. tuberculosis* (PDB ID 4TRO) revealed the interesting observations. Firstly, it was encouraging to see that most of the compounds occupied nearly same binding site as the native substrate of 4TRO. Compound **3a** showed highest fit (Docking score: -28.50). This compound made very good interactions with the surrounding residues which included H-bonds with Ile122, Val65 and Leu197 (Fig. 1). The other reason of good binding and in turn, good antitubercular activity was found to be van der Waals hydrophobic interactions with residues like Ala198, Gly96, Phe97 and Ser94. The active compounds **3e** also displayed similar interactions and as a result, was predicted as highly potent *in silico* (Docking Score: -27.17). Additionally, it was encouraging to see that our docking protocol could distinguish between active and inactive compounds. Upon our analysis of compounds **3b** and **3f** (two lowest active compounds with docking score of -9.83 and -14.60 and MIC value of 50); the reasons of its low activity could be understood. These two compounds' phenethyl ring occupied completely different orientations to that of compounds **3a** and **3e**. Due to this, compound lost H-bonding interactions with Ile122 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. Docking scores of all the compounds are shown in Table-1.

In order to check the compounds' drug-like properties, they were also evaluated for their physico-chemical properties. It was encouraging to see that all the compounds showed acceptable profile; which includes passing the basic filter (Lipinski rule of 5) and decent permeability profile (CACO2 and PAMPA) with less of hERG concerns. The results are summarized in Table-2.

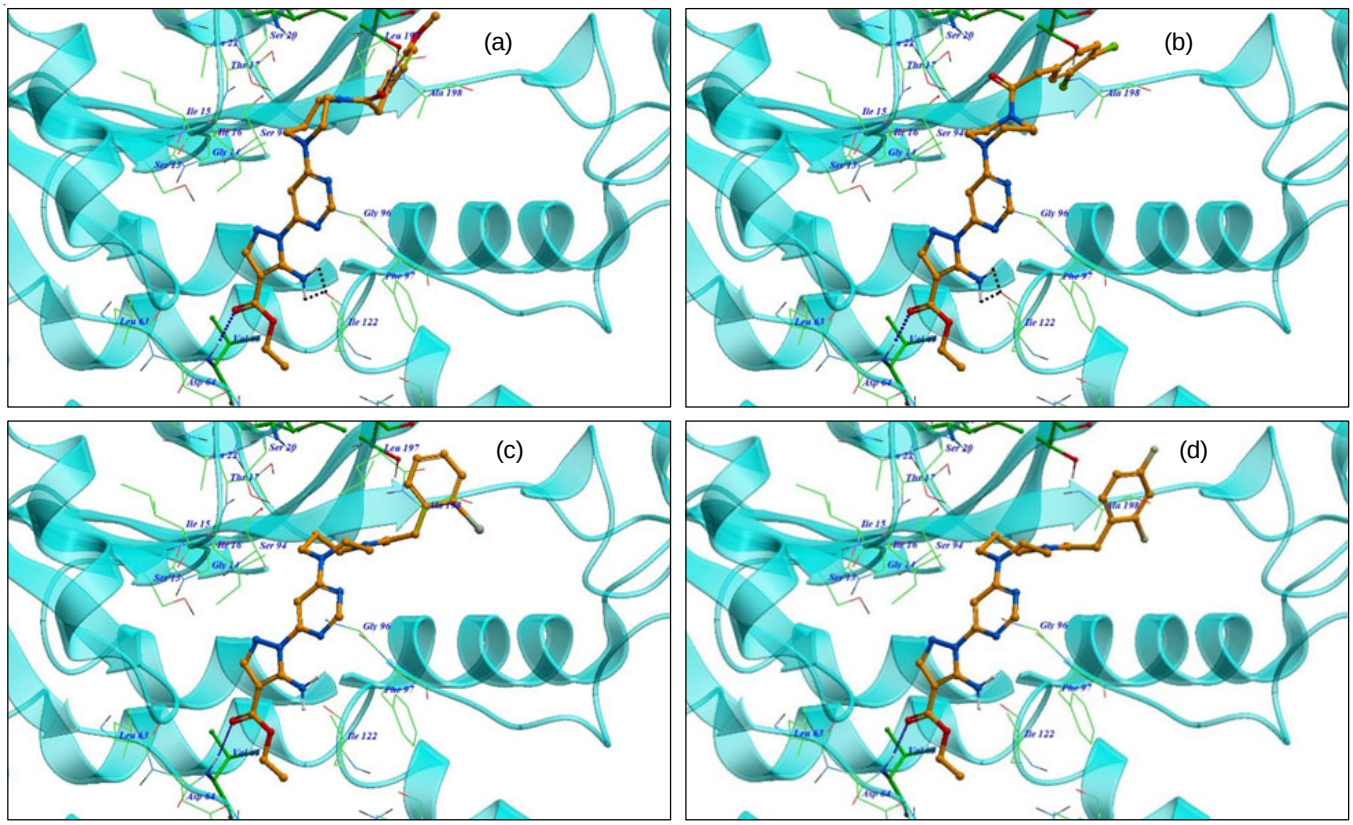


Fig. 1. Binding interactions of docked compounds (panel a: **3a**, panel b: **3e**, panel c: **3b**, panel d: **3f**). Ligand carbon in orange, protein carbon in cyan (cartoon) or green (stick), nitrogen in blue, oxygen in red. Hydrogen bonds is shown with dotted lines

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF DESIGNED COMPOUNDS (**3a-j**)

Compd. No.	MW	log P	log S	PSA	Drug likeness	HBA	HBD	CACO2	HERG	PAMPA	PGP	PGP inhibitor	PGP substrate
3a	479.23	3.42	-3.59	100.30	0.87	9.00	2.00	-5.07	0.11	-5.08	1.00	0.13	1.00
3b	527.13	4.18	-4.23	92.76	0.46	8.00	2.00	-5.10	0.07	-5.18	0.97	0.16	0.97
3c	499.23	4.59	-4.69	92.49	0.79	8.00	2.00	-5.21	0.08	-5.17	0.98	0.16	0.98
3d	467.21	3.61	-3.71	92.76	0.89	8.00	2.00	-5.10	0.17	-5.17	0.98	0.10	0.98
3e	517.14	4.80	-4.86	92.76	0.91	8.00	2.00	-5.08	0.24	-5.25	0.99	0.02	0.99
3f	485.20	3.71	-3.89	92.76	0.85	8.00	2.00	-5.03	0.12	-5.23	0.99	0.11	0.99
3g	425.18	2.58	-2.81	101.86	0.56	9.00	2.00	-4.79	0.21	-5.21	0.91	0.07	0.91
3h	503.12	4.50	-4.60	93.28	0.89	8.00	2.00	-4.76	0.30	-5.21	0.97	0.02	0.97
3i	471.18	3.62	-3.70	93.28	0.65	8.00	2.00	-4.74	0.25	-5.27	0.98	0.12	0.98
3j	547.07	4.54	-4.73	93.28	0.65	8.00	2.00	-4.73	0.19	-5.20	0.98	0.22	0.98

Conclusion

A series of new ethyl 5-amino-1-(6-(4-substituted aryl-1,4-diazepan-1-yl)pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**3a-j**) was synthesized and their antitubercular activity have been evaluated. Four compounds, **3a**, **3d**, **3e** and **3g** were found to be the most potent (MIC = 1.6 µg/mL). Molecular docking studies revealed that the major driving force for binding to the crystal structure of *M. tuberculosis* and in turn, to show *in vitro* antitubercular activity is H-bonding and some van der Waals interactions. Not only the actives, but inactive compounds **3b** and **3f** were also successfully identified by the docking porotocols as true negatives. The most potent compounds were found to be even better than the reference drugs (streptomycin, pyrazinamide and ciprofloxacin). Indeed, this further opens new avenues for lead modifications to prepare a series of such hybrid molecules which could have increased potency and more drug like properties so as to be taken further in the drug discovery pipeline. Also, it was encouraging to observe a good correlation between *in silico* prediction (docking score) and actual MIC values, which further suggests that *in silico* design can be used well before attempting synthesis of similar molecules to rationalize the drug discovery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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