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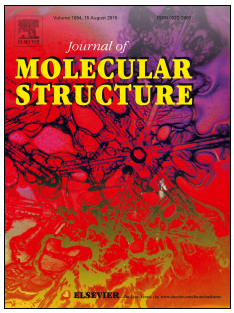

Novel *N*-thioamide analogues of pyrazolylpyrimidine based piperazine: Design, synthesis, characterization, *in-silico* molecular docking study and biological evaluation

Mayur K. Vekariya, Dhaval B. Patel, Pranav A. Pandya, Rajesh H. Vekariya, Prapti U. Shah, Dhanji P. Rajani, Nisha K. Shah

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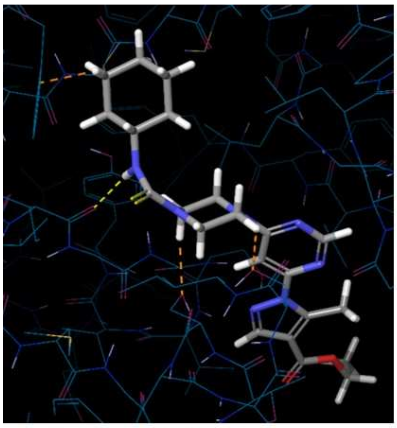

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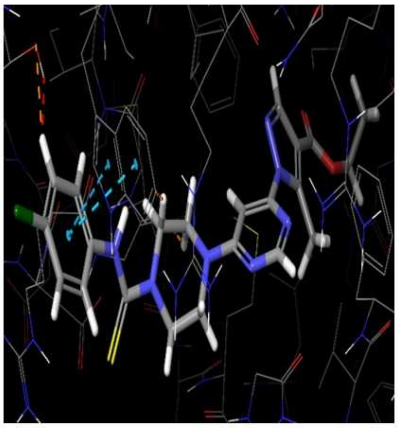

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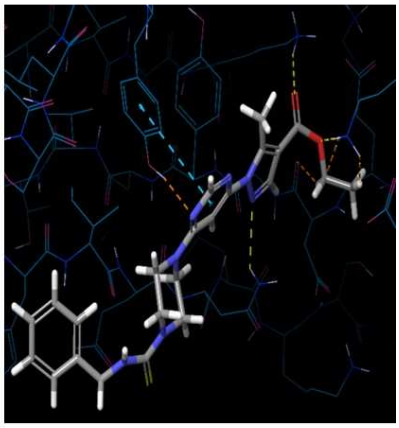

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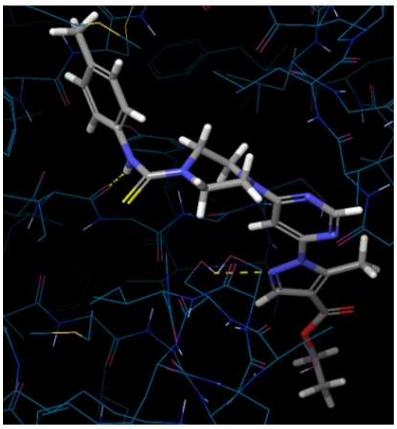

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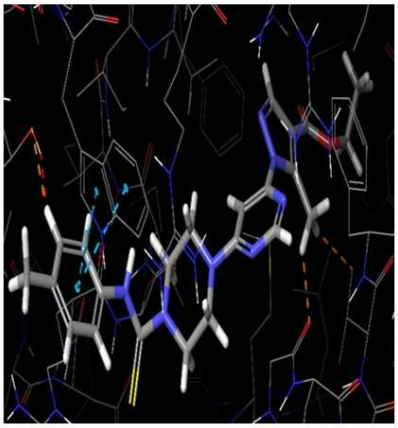

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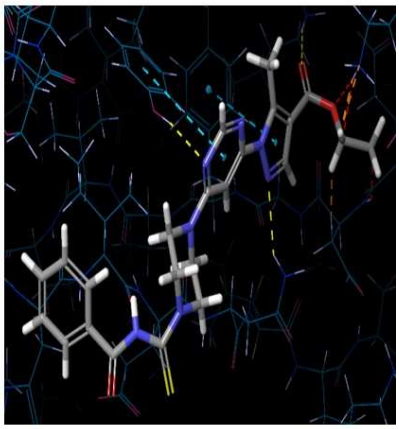

D20

PDB: 4CJN

Novel *N*-thioamide analogues of Pyrazolylpyrimidine based Piperazine: Design, Synthesis, Characterization, I*n-silico* molecular docking study and Biological evaluation

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Abstract:

Utilizing molecular hybridization approach, a progression of novel pyrazolylpyrimidine based *N*-thioamide derivatives of piperazine were identified in an effort to develop newer antibacterial and antitubercular agents against the cumulative bacterial resistance. Spectral analysis using Mass, ^{1}H-NMR and ^{13}C-NMR spectral techniques have been studied in order to affirm the structure of synthesized end molecules. Biological evaluation of all synthesized molecules were studied *in-vitro* for their antibacterial, antituberculosis and antimalarial efficacy against various bacterial and fungal strains, H37Rv and Plasmodium falciparum respectively. Molecular docking and ADME properties prediction study were also carried out for better insights of responsible proteins with the synthesised molecules. Interestingly, some of the pyrazolylpyrimidine based piperazine *N*-thioamide derivatives exhibited potential antibacterial, antifungal and antimalarial potency.

Keywords: Antimicrobial agents, docking, hybrids, piperazines, pyrazole, pyrimidine

Introduction

As indicated by WHO report 2016, more cases of tuberculosis occurs in the world than is beforehand expected[1]. As per this report sum of 10.4 million new cases of tuberculosis (TB) were stated in 2016. Thus, TB is on ninth rank in the list of death causing diseases worldwide. Recent approach to cure from tuberculosis involves DOTS (directly observed treatment, short-course) treatment. DOTS treatment consist of six month usage of four first-line TB-drugs i.e. Isoniazid, Rifampicin, Ethambutol and Pyrazinamide[2]. However, the growth of XDR and MDR TB forms turn out to be a dangerous issue leads to **an** increment of complications to cure from TB and therefore there is a need to find novel anti-TB pharmacophores for the development of harmless and fast acting antitubercular candidates.[3] Apart from TB, Malaria is also stated as most perilous life affecting disease as 21.60 Crores malaria cases were added in WHO report 2016[4]. Thus, both of TB and malaria remained as dangerous diseases till date and current research lead towards the identification of novel effective and safe antimalarial candidates to fight against the issue. Moreover, many classes of antibiotics were failing to show their efficacy due to the continuous increment of bacterial resistance occurred mainly because of random prescription of antibiotic drugs and its non-compliance treatment. To overcome from an existing situation obtained because of bacterial resistance, there is a need for the advancement of novel antibacterial and antifungal candidates.

Molecular hybridization is well known concept for the design and development of drug molecules which includes the combination of different pharmacophores of bioactive natural or synthetic substances into a single new hybrid compound with improved affinity and potency as compared to the parent drug molecules.[5] Additionally, this approach can result in compounds having different and/or dual modes of action, altered selectivity profile and decreased undesired side effects.[6] In the medicinal research field, major significance was

attained by Pyrazole derivatives among all heterocycles by presenting wide range of biological potency [7–10] like antitumor[9,11,12], antiviral[13,14], and anti-inflammatory agents[15]. Along with pyrazole, pyrimidine moiety came across in fame from various literature as it was stated as a core unit in thymine, cytosine and uracil i.e. building blocks of nucleic acid. Further, it's derivatives were stated as broad spectrum of biological active agents such as anti-HIV[16], antimicrobial[16], antidiabetic[17] and antiinflammatory[18]. Apart from these, several antiviral (idoxuridine and trifluridine), antibacterial (trimethoprim, sulphamethiazine and sulfadiazine), antimalarial (sulphadoxin), anti HIV (zidovudine, Retrovir and stavudine), antibiotic (Bacimethrin), antitubercular (Viomycin) and anticancer (5-fluorouracil) drugs comprises of pyrimidine motif in their structural formula. Furthermore, *N*-heteroaryl compounds have been the focus of interest as pharmaceutical agents and as agrochemicals in recent decades due to their ability to demonstrate a significant role in certain biological process. In particular, Pyrimidine moieties attached with nitrogen atom of pyrazole are the best example as *N*-Pyrazolyl pyrimidines have been reported as hA_{2A} receptor antagonists with excellent aqueous solubility[19], as antidiabetic agents (agonistic activity against human GPR142)[20], as anti-inflammatory agents[21], as kinase inhibitors[22,23], as antiulcer agents[24,25]. Thus, *N*-Pyrazolyl pyrimidine fragment was used for Molecular hybridization in the present research.

Furthermore, Piperazine is the most common heterocyclic secondary amine candidate as many piperazine comprised compounds have demonstrated extensive range of biological activities [26] including anticancer[27], antianginal[28,29], antidepressant[30–32], antihistamine[33], antipsychotic[34,35], analgesic and anti-inflammatory[36]. From literature survey, it was also observed that among piperazine containing compounds, *N*-thioamide derivatives of piperazine exhibited a wide range of biological activities as these derivatives were reported as potent DNA as gyrase inhibitors[37–39], as potent β-glucuronidase inhibitor[40], as potent arora kinase inhibitor[41], as potent antimicrobial agent[42], as potent phosphoglycerate dehydrogenase inhibitor[43], as potent FLT-3 inhibitor[44], as selective PDGFR inhibitor[45] and as Potent bacterial Sfp-PPTase inhibitor[46]. In all of these potent piperazine *N*-thiomide derivatives, one hydrophobic core unit attached with the other nitrogen of piperazine was found which may lead it towards more efficacy. So, piperazine *N*-thioamides were used as second fragment for Molecular hybridization. In addition, many of above bioactive piperazines, pyrimidine was found to be attached with piperazine. So in light of the aformentioned facts, pyrazolyl pyrimidine was selected as hydrophobic moiety along with *N*-thioamides of pipearzine to obtain new potent antimicrobial chemical entities through Molecular hybridization.

<<<<< Insert Figure 1>>>>>

Finally, *N*-pyrazolylpyrimidine based piperazine *N*-thioamide motif was selected in order to design novel bioactive piperazine derivatives. As a result of the aforementioned delineation **(Figure 1)**, *N*-pyrazolylpyrimidine based piperazine *N*- thioamide analogues were designed and synthesized to investigate of the potential ability of novel designed hybrid molecules.

Biological evaluation of all synthesized molecules were studied *in-vitro* for their antibacterial efficacy against two gram positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442), two gram negative bacterial strains (*Escherichia coli* MTCC 443,*Pseudomonas aeruginosa* MTCC 1688) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282), antituberculosis efficacy against H37Rv and antimalarial efficacy against Plasmodium falciparum respectively. On the basis of biological assay results, structure-activity relationship (SAR) and molecular interaction studies of *N*-pyrazolylpyrimidine based piperazine *N*- thioamide analogues were also described.

Experimental protocol:

Material and Methods

All the required reagents and crude materials were procured from known marketed sources and were utilized without any refinement. Reaction progress was checked on TLC of Merck (pre-covered silica gel 60F254 on aluminium sheets) and envisioned by UV light and iodine. Melting point determination using Optimelt MPA100, an automated apparatus and spectroscopic analysis i.e. Mass and NMR spectra were utilized to characterize all synthesized compounds. Mass spectra were analyzed by Water's SQD detector, Waters USA using 10mM ammonium acetate in water:Methanol(60:40) as mobile phase with electron spray ionization (ESI). ^{1}H-NMR spectra were recorded on Bruker AV 400MHz spectrometer (Bruker Avance III, Germany) using DMSO-d6 and TMS as a solvent and internal reference solvent respectively. Similarly ^{13}C-NMR spectra were recorded on a Bruker AV 100 MHz spectrometer DMSO-d6 and TMS as a solvent and internal reference solvent respectively. ADME prediction was done on QikProp and Molecular docking study was performed using Glide on Schrodinger Maestro 11.

Synthetic protocol for Targeted molecules

*Ethyl 2-((dimethylamino)methylene)-3-oxobutanoate (**Enaminone Intermediate**)*

Ethylacetoacetate (26 g, 200 mmol) and DMF-DMA (26.2 g, 220 mmol) were charged in 100 ml three-necked flask at room temperature and stirred for 5 h. The reaction mixture was then treated with *n*-pentane to evacuate unreacted material in n-pentane. Product formation was affirmed with boiling point determination and on TLC plate visualization in iodine. Obtained crude dark brown liquid was used further without any purification as *Enaminone Intermediate.* Yield 95%; b.p. 180°C.

*4-chloro-6-hydrazinylpyrimidine (**A**)*

A solution of 4,6-Dichloropyrimidine (15 g, 100 mmol) in ethanol was cooled to 0-5°C in 250 ml three-necked flask. To the above flask Hydrazine hydrate (4.7 ml, 120 mmol) was added dropwise under cooling atmosphere. After that, the reaction mixture was stirred for 90 minutes at room temperature. Reaction progress was checked on TLC and poured into

water to get crude product. Pure 4-chloro-6-hydrazinylpyrimidine (**A**) *as* Pale yellow solids[47] obtained by recrystallization in ethanol. Yield 95%; Yellowish white solid; Rf = 0.5 (hexane:ethyl acetate, 4:1); m.p. 164 °C, ^{1}H NMR (d_6-DMSO, 400 MHz) δ = 8.83 (s, 1H), 8.17 (s, 1H),6.76 (s, 1H), 4.50 (s, 2H); EI-MS,(m/z): 145.02 (M+1).

*Ethyl 1-(6-chloropyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate (**B**)*

Intermediate-**A** (13.0 mL, 69.2 mmol) and 1N HCl (10mL) were added dropwise in the solution of **C** (10.0 g, 69.2 mmol) in ethanol (100 mL) at 0-5 ^{0}C and was stirred for 1h. Completion of reaction was confirmed on TLC after 1h. Then reaction mixture was poured on ice water to afford crude product. Filtration and recrystallization from ethanol of crude product yielded ethyl 1-(6-chloropyrimidin-4-yl)-5-methyl-1*H*-pyrazole-4-carboxylate (**B**) as white crystals.[47] Yield 90%; Rf = 0.8 (hexane:ethyl acetate, 4:1); m.p. 180°C; ^{1}H NMR 8.94 (s, 1H), 8.42 (s, 1H), 7.238 (s, 1H), 4.216 (q, *J*=7.2 Hz, 2H), 2.782 (s, 3H), 1.312 (t, *J*=7.2 Hz, 3H). EI-MS (m/z): 268.07 (M+2).

*Ethyl 5-methyl-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**C**)*

To a solution of **B** (20.0g, 74.9 mmol) in DMF (50 mL) was added piperazine (7.6 mL, 75 mmol) dropwise at 0-5^{0}C and the mixture was stirred at 80 ^{0}C for 1 h. After complete consumption of the reactant **B** observed on TLC, reaction mixture was poured on ice-cold water. Resultant white precipitates were filtered off, dried and recrystallized with ethanol followed by tituration with hexane to afford Ethyl 5-methyl-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**C**) as white solid. Yield 80%; Rf = 0.35 (hexane:ethyl acetate, 1:1); m.p. 188-192°C; ^{1}H NMR 8.52 (s, 1H, pyridine CH), 8.07 (s, pyrazol, 1H CH), 7.10 (s, pyrimidine, 1H CH), 4.81 (s, 1H, piperazine NH), 4.25 (q, *J* = 6.8 Hz, 2H, ethoxy CH_2), 3.82-3.43 (m, 8H, piperazine CH_2), 2.88 (s, 3H, CH_3-Pyrazole), 1.29 (t, *J* = 7.2 Hz, 3H, ethoxy CH_3); EI-MS (m/z): 338.47 (M+1).

Novel *N*-thiomide analogues of *Ethyl 5-methyl-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate* **(D1-D24)**

To the solution of **C** (1.0 mmol) in 5 mL DMF and corresponding isothiocyanates (1.0 mmol) were added dropwise in 25-mL RBF in cooling atmosphere. After addition reaction mixture was stirred at room temperature for 2-3 h. Reaction progress was monitored by TLC plate by using hexane: ethylacetate solvent system as mobile phase. After completion of reaction mixture was poured into cold water (30 mL) and the resulting solid was filtered off, washed with cold water. Crude products were recrystallized from ethanol (95%) to afford pure compounds. The product formation were further confirmed by spectral data (^{1}H–NMR, ^{13}C–NMR and ESI-MS).

Ethyl 5-methyl-1-(6-(4-(phenylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**D1**)

Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 231-233 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.391 (s, -NHCS, 1H), 8.561 (s, pyrimidine, 1H), 8.084 (s, pyrazol, 1H), 7.342-7.287 (m, 4H), 7.138-7.108 (m, 2H), 4.265 (q, J= 7.2 Hz, 2H), 4.072 (broad s, 4H), 3.852 (broad s, 4H), 2.892 (s, 3H), 1.304 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.04(CS), 162.51, 158.46, 157.27, 145.38, 142.35, 133.31, 132.69, 129.88, 124.51, 113.91, 93.29, 59.84(CH_2 ester), 47.01(piperazine), 42.77(piperazine), 14.21(Me-pyrazole), 13.08(Me-ester); EI-MS (m/z): 359.5 (M+1).

Ethyl 5-methyl-1-(6-(4-(methylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**D2**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 215-217 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.541 (s, -NHCS, 1H), 8.078 (s, pyrimidine, 1H), 7.780 (s, pyrazol, 1H), 7.077 (s, pyrimidine, 1H), 4.261 (q, J= 7.6 Hz, 2H), 3.928 (broad s, 4H), 3.773 (broad s, 4H), 2.940 (s, 3H), 2.880 (s, 3H), 1.304 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.91, 162.51, 158.43, 157.24, 145.35, 142.21, 113.87, 93.29, 59.82, 45.94, 44.49, 42.77, 32.51, 14.19, 13.05; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(ethylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate (**D3**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 182-184 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.544 (s, -NHCS, 1H), 8.080 (s, pyrimidine, 1H), 7.730 (s, pyrazol, 1H), 7.077 (s, pyrimidine, 1H), 4.262 (q, J= 6.8 Hz, 2H), 3.930 (broad s, 4H), 3.772 (broad s, 4H), 3.540 (s, 2H), 2.880 (s, 3H), 1.302 (t, J = 7.6 Hz, 3H), 1.117 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 180.99, 162.51, 158.43, 157.25, 145.35, 142.22, 113.88, 93.35, 59.83, 45.94, 42.78, 14.34, 13.03; EI-MS (m/z): 359.5 (M+1).

Ethyl 5-methyl-1-(6-(4-(propylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (**D4**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 166-168 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.547 (s, -NHCS, 1H), 8.084 (s, pyrimidine, 1H), 7.740 (s, pyrazol, 1H), 7.080 (s, pyrimidine, 1H), 4.262 (q, J= 7.2 Hz, 2H), 3.933 (broad s, 4H), 3.772 (broad s, 4H), 3.458 (q, J= 6.4 Hz, 2H), 2.880 (s, 3H), 1.560 (q, J= 7.2 Hz, 2H), 1.301 (t, J = 7.2 Hz, 3H), 0.856 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.21, 162.55, 158.43, 157.27, 145.35, 142.33, 113.88, 93.40, 59.83, 47.07, 46.00, 42.77, 21.88, 14.20, 13.02, 11.32; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(butylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate (**D5**)
Yield; 85%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 160-162 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.542 (s, -NHCS, 1H), 8.079 (s, pyrimidine, 1H), 7.713 (s, pyrazol, 1H), 7.074 (s, pyrimidine, 1H), 4.262 (q, J= 6.8 Hz, 2H), 3.933 (broad s, 4H), 3.768 (broad s, 4H), 3.505 (q, J= 6.4 Hz, 2H), 2.880 (s, 3H), 1.543 (q, J= 7.2 Hz, 2H), 1.320 – 1.248 (m, 5H), 0.894 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.19, 162.58, 158.43, 157.24, 145.34, 142.21,

113.88, 93.33, 59.82, 46.00, 45.04, 42.77, 30.81, 19.57, 14.19, 13.78, 13.03; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(isopropylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate (**D6**)
Yield; 85%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 182-184 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.548 (s, -NHCS, 1H), 8.085 (s, pyrimidine, 1H), 7.370 (s, pyrazol, 1H), 7.078(s, pyrimidine, 1H), 4.571-4.522 (m, 1H),4.263 (q, *J*=7.2 Hz, 2H), 3.927 (broad s, 4H), 3.767 (broad s, 3H), 2.880 (s, 3H), 1.301 (t, *J*=7.2 Hz, 3H), 1.160 (d, *J*=6.4 Hz, 6H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.21, 162.55, 158.43, 157.27, 145.35, 142.33, 113.88, 93.40, 59.83, 47.07, 46.00, 43.87, 21.88, 14.20, 13.02; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(tert-butylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D7**)
Yield; 85%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 160-162 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.548 (s, -NHCS, 1H), 8.088 (s, pyrimidine, 1H), 7.059 (s, pyrazol, 1H), 6.073(s, pyrimidine, 1H), 4.264 (q, *J*=7.6 Hz, 2H), 3.894 (broad s, 4H), 3.765 (broad s, 3H), 2.882 (s, 3H), 1.465 (s, 9H), 1.300 (t, *J*=6.8 Hz, 3H), 1.160 (d, *J*=6.4 Hz, 6H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.19, 162.58, 158.43, 157.24, 145.34, 142.21, 113.88, 93.33, 59.82, 46.00, 45.04, 42.77, 30.81, 13.78, 13.03; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(allylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D8**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 191-193 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.543 (s, -NHCS, 1H), 8.075 (s, pyrimidine, 1H), 7.910 (s, pyrazol, 1H), 7.079 (s, pyrimidine, 1H), 5.900 (dt, J= 16.8, 5.6 Hz, 1H), 5. 146 (d, *J*= 17.2 Hz, 1H), 5.072 (d, *J*= 10.0 Hz, 1H), 4.288-4.200 (m, 4H), 3.961 (broad s, 4H), 3.781 (broad s, 4H), 2.880 (s, 3H), 1.303 (t, *J*= 6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.37, 162.58, 162.48, 158.44, 157.25, 145.33, 142.22, 135.40, 115.38, 113.88, 93.35, 59.83, 47.67, 46.17, 42.77, 14.20, 13.03; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(cyclohexylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D9**)
Yield; 92%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 196-199 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.539 (s, -NHCS, 1H), 8.075 (s, pyrimidine, 1H), 7.337 (s, pyrazol, 1H), 7.069 (s, pyrimidine, 1H), 4.270-4.236 (m, 3H), 3.929 (broad s, 4H), 3.763 (broad s, 4H), 2.878 (s, 3H), 1.877-1.589 (m, 5H), 1.318-1.079 (m, 8H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 180.24, 162.58, 162.47, 158.42, 157.24, 145.34, 142.21, 113.88, 93.32, 59.82, 54.51, 42.77, 40.12, 32.05, 25.19, 14.19, 13.03; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(benzylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate (**D10**)
Yield; 93%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 182-184 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.544 (s, -NHCS, 1H), 8.332 (s, pyrimidine, 1H), 8.079 (s, pyrazol, 1H),

7.322-7.078 (m, 5H), 7.078 (s, pyrimidine, 1H), 4.833 (s, 2H), 4.254 (broad s, 2H), 4.008 (broad s, 4H), 3.801 (broad s, 4H), 2.884 (s, 3H), 1.302 (broad s, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.69, 162.58, 162.46, 158.43, 157.22, 145.37, 142.22, 139.59, 128.04, 127.21, 126.54, 113.88, 93.24, 59.82, 48.34, 46.28, 42.79, 14.19, 13.07; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((2-methoxyphenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D11**)
Yield; 96%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 191-193 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 8.863 (s, -NHCS, 1H), 8.553 (s, pyrimidine, 1H), 8.075 (s, pyrazole, 1H), 7.279-6.918 (m,5H), 4.261 (broad s, 2H), 4.074 (broad s, 4H), 3.841-3.774 (m, 7H), 2.891 (s, 3H), 1.305 (broad s, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 182.02, 162.59, 162.48, 158.46, 157.25, 154.11, 145.37, 142.22, 129.32, 129.25, 126.94, 119.79, 113.90, 111.67, 93.31, 59.83, 55.47, 46.74, 42.81, 14.20, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((3-methoxyphenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D12**)
Yield; 93%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 175-177 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.381 (s, -NHCS, 1H), 8.562 (s, pyrimidine, 1H), 8.089 (s, pyrazol, 1H), 7.208 (t, J=8.0 Hz, 1H), 7.108 (s, 1H), 6.964 (s,1H, pyrazole), 6.927 (d, J=8.0Hz, 1H), 6.699 (d, J=7.2 Hz, 1H), 4.264 (q, J=7.2 Hz, 2H), 4.056 (broad s, 4H), 3.847 (broad s, 4H), 3.734 (s, 3H), 2.891 (s, 3H), 1.303 (q, J=7.2 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.82, 162.65, 162.58, 158.86, 157.45, 154.31, 145.35, 142.42, 129.29, 129.05, 126.83, 119.69, 113.50, 111.46, 93.21, 59.53, 55.87, 46.84, 42.40, 14.30, 13.08; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((4-methoxyphenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D13**)
Yield; 91%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 221-223 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.275 (s, -NHCS, 1H), 8.562 (s, pyrimidine, 1H), 8.090 (s, pyrazol, 1H), 7.192 (d, J=8.4 Hz, 2H), 7.107 (s, 1H), 8.879 (d, J=8.4 Hz, 2H), 4.264 (q, J=6.8 Hz, 2H), 4.061 (broad s, 4H), 3.842 (broad s, 4H), 3.748 (s, 3H), 2.891 (s, 3H), 1.305 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 182.02, 162.59, 162.48, 158.46, 157.25, 154.11, 145.37, 142.22, 129.32, 129.25, 126.94, 119.79, 113.90, 111.67, 93.31, 59.83, 55.47, 46.74, 42.81, 14.20, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((3-chlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D14**)
Yield; 90%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 206-208 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.497 (s, -NHCS, 1H), 8.566 (s, pyrimidine, 1H), 8.092 (s, pyrazol, 1H), 7.473 (s, 1H), 7.322-7.115 (m, 4H), 4.266 (q, J=6.8 Hz, 2H), 4.072 (broad s, 4H), 3.859 (broad s, 4H), 2.893 (s, 3H), 1.304 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.95, 162.18, 162.66, 158.37, 157.17, 145.28, 142.14, 139.17, 128.22, 127.64, 126.70, 113.61, 93.21, 59.73, 48.46, 46.88, 42.72, 14.19, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((4-chlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D15**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 214-216 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.461 (s, -NHCS, 1H), 8.559 (s, pyrimidine, 1H), 8.084 (s, pyrazol, 1H), 7.365 (m, 4H), 7.105 (s, pyrimidine, 1H), 4.266 (q, J=6.8 Hz, 2H), 4.077 (broad s, 4H), 3.855 (broad s, 4H), 2.893 (s, 3H), 1.306 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.25, 162.58, 162.46, 158.47, 157.27, 145.38, 142.24, 139.87, 128.32, 127.84, 126.90, 113.91, 93.31, 59.83, 48.56, 46.96, 42.80, 14.19, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((4-fluorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D16**)
Yield; 90%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 218-220 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.388 (s, -NHCS, 1H), 8.560 (s, pyrimidine, 1H), 8.084 (s, pyrazol, 1H), 7.329-7.107 (m, 5H), 4.266 (q, J=6.8 Hz, 2H), 4.078 (broad s, 4H), 3.855 (broad s, 4H), 2.892 (s, 3H), 1.305 (t, J=6.4 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.50, 162.58, 160.41, 158.46, 157.27, 145.38, 142.24, 137.15, 127.72, 114.48, 93.31, 59.83, 46.83, 42.77, 14.19, 13.07; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((2,4-dichlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D17**)
Yield; 94%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 215-217 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.362 (s, -NHCS, 1H), 8.566 (s, pyrimidine, 1H), 8.088 (s, pyrazol, 1H), 7.656 (s, 1H), 7.420 (d, J= 7.6 Hz, 1H), 7.345 (d, J=8.4 Hz,1H), 7.121 (s, pyrimidine, 1H), 4.267 (q, J=6.8 Hz, 2H), 4.089 (broad s, 4H), 3.863 (broad s, 4H), 2.893 (s, 3H), 1.305 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.64, 162.55, 158.48, 157.28, 145.38, 142.25, 137.49, 133.02, 132.27, 128.79, 127.36, 113.91, 93.43, 59.84, 46.96, 42.82, 14.21, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((3-iodophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D18**)
Yield; 92%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 228-230 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.454 (s, -NHCS, 1H), 8.560 (s, pyrimidine, 1H), 8.086 (s, pyrazol, 1H), 7.747 (s, 1H), 7.468 (d, J= 6.8 Hz, 1H), 7.398 (d, J= 7.2 Hz, 1H), 7.109 (s, 2H), 4.264 (q, J=6.4 Hz, 2H), 4.067 (broad s, 4H), 3.853 (broad s, 4H), 2.892 (s, 3H), 1.305 (t, J=6.4 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.04, 162.52, 158.46, 157.27, 145.38, 142.30, 133.31, 132.69, 129.88, 124.51, 113.91, 93.29, 59.84, 47.01, 42.77, 14.21, 13.08; EI-MS (m/z): 359.5 (M+1).

Ethyl 5-methyl-1-(6-(4-(p-tolylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate **(D19**)
Yield; 93%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 233-235 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.303 (s, -NHCS, 1H), 8.555 (s, pyrimidine, 1H), 8.079 (s, pyrazol, 1H), 7.203-7.101 (m, 5H), 4.273 (q, J=6.4 Hz, 2H), 4.059 (broad s, 4H), 3.841 (broad s, 4H), 2.890 (s, 3H), 2.281(s, 3H), 1.304 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.48, 162.54,

158.47, 157.28, 145.37, 142.24, 138.25, 133.61, 128.46, 125.56, 113.90, 93.36, 59.83, 46.85, 42.82, 40.12, 20.51, 14.20, 13.05; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-(benzoylcarbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D20**)
Yield; 95%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 180-182 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 10.954 (s, -NHCS, 1H), 8.564 (s, pyrimidine, 1H), 8.085 (s, pyrazol, 1H), 7.970 (t, J=7.2 Hz, 2H), 7.623 (d, J=7.6 Hz, 1H), 7.526 (t, J= 7.2 Hz, 2H), 7.092 (s, 1H), 4.260 (q, J=7.2 Hz, 2H), 3.943 (broad s, 4H), 3.815 (broad s, 4H), 2.891 (s, 3H), 1.299 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 180.12, 164.00, 162.58, 162.41, 158.49, 157.49, 157.29, 145.41, 142.28, 132.52, 128.43, 127.03, 113.93, 93.34, 59.83, 49.44, 48.78, 42.88, 14.20, 13.08; EI-MS (m/z): 359.5 (M+1).

Ethyl 5-methyl-1-(6-(4-((4-nitrophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-carboxylate **(D21**)
Yield; 94%; Yellow solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 265-267 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.298 (s, -NHCS, 1H), 8.562 (s, pyrimidine, 1H), 8.175 (d, J=8.0 Hz, 2H), 8.081 (s, pyrazol, 1H), 7.658 (d, J=8.0 Hz, 2H), 7.114 (s, pyrimidine, 1H), 4.266 (q, J=6.4 Hz, 2H), 4.099 (broad s, 4H), 3.877 (broad s, 4H), 2.895 (s, 3H), 1.308 (t, J=6.4 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 180.74, 162.49, 158.48, 157.26, 147.81, 145.40, 142.25, 123.89, 122.69, 113.85, 93.27, 59.83, 47.51, 44.65, 42.78, 14.18, 13.08; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((2,3-dichlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D22**)
Yield; 90%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 203-205 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.488 (s, -NHCS, 1H), 8.571 (s, pyrimidine, 1H), 8.091 (s, pyrazol, 1H), 7.545 (d, J=7.6 Hz, 1H), 7.382-7.304 (m, 3H), 7.126 (s, pyrimidine, 1H), 4.267 (q, J=6.8 Hz, 2H), 4.094 (broad s, 4H), 3.871 (broad s, 4H), 2.895 (s, 3H), 1.305 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 163.61, 161.23, 157.79, 143.90, 140.60, 139.24, 120.14, 117.54, 95.30, 65.5, 56.4, 43.8, 40.1, 13.16; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((2,6-dichlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D23**)
Yield; 90%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 253-255 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.366 (s, -NHCS, 1H), 8.564 (s, pyrimidine, 1H), 8.087 (s, pyrazol, 1H), 7.548 (d, J=8.0 Hz, 2H), 7.382-7.304 (m, 2H), 7.112 (s, pyrimidine, 1H), 4.267 (q, J=6.8 Hz, 2H), 4.089 (broad s, 4H), 3.863 (broad s, 4H), 2.893 (s, 3H), 1.305 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.64, 162.55, 158.48, 157.28, 145.38, 142.25, 137.49, 133.02, 132.27, 128.79, 127.36, 113.91, 93.43, 59.84, 46.96, 42.82, 14.21, 13.06; EI-MS (m/z): 359.5 (M+1).

Ethyl 1-(6-(4-((2-chlorophenyl)carbamothioyl)piperazin-1-yl)pyrimidin-4-yl)-5-methyl-1H-pyrazole-4-carboxylate **(D24**)

Yield; 92%; white solid; Rf = 0.4 (hexane:ethyl acetate, 1:1); m.p.: 228-230 ^{0}C; ^{1}H NMR (400 Hz, DMSO-d6) δ: 9.454 (s, -NHCS, 1H), 8.560 (s, pyrimidine, 1H), 8.086 (s, pyrazole, 1H), 7.279-6.918 (m,5H), 4.273 (q, J=6.4 Hz, 2H), 4.059 (broad s, 4H), 3.841 (broad s, 4H), 2.890 (s, 3H), 1.304 (t, J=6.8 Hz, 3H); ^{13}C NMR (100 Hz, DMSO-d6) δ: 181.64, 162.55, 158.48, 157.28, 145.38, 142.25, 137.49, 133.02, 132.27, 128.79, 127.36, 113.91, 93.43, 59.84, 47.01, 42.77, 14.21, 13.08; EI-MS (m/z): 359.5 (M+1).

In-vitro Biological screening

Biological screening has been performed at Microcare Laboratory and TRC, Surat, India as per earlier reported methods.[47–50] All synthesized compounds were evaluated *in-vitro* for their antimicrobial efficacy against two gram positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442), two gram negative bacterial strains (*Escherichia coli* MTCC 443,*Pseudomonas aeruginosa* MTCC 1688) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282), antituberculosis efficacy against H37Rv and antimalarial efficacy against Plasmodium falciparum respectively. In antibacterial evaluation, ampicillin, ciprofloxacin and chloramphenicol were used whereas in antifungal evaluation, nystatin and greseofulvin were used as standard control drugs. Similarly, isoniazide for antituberculosis and Chloroquine as well as quinine for antimalarial evaluation were used as standard control drugs.

Docking protocol

From the RCSB Protein Data Bank, protein structure coordinates (PDB Code 4DPD[51], 4CJN[52] & 1HNJ[53]) were procured. Docking study was performed as per previously reported route.[47]

Results and Discussion

Synthesis and structural characterization

In present work, novel distinct *N*-thioamide derivatives of pyrazolylpyrimidine based piperazine are prepared through a plausible conversion way. The synthesis route for the *N*-thioamides of pyrazolylpyrimidine based piperazine is delineated below as **Scheme 1**.

As depicted in **Scheme 1**, 4,6-dichloropyrimidine selected as a primary reactant which when reacted with hydrazine hydrate yielded Compound **A**. Enaminone intermediate were obtained by the reaction of *N*,*N*-Dimethylformamide dimethyl acetal (DMF-DMA) [54] with Ethylacetoacetate which was key step to formulate Compound **B** i.e. ethyl 1-(6-chloropyrimidin-4-yl)-5-methyl-1*H*-pyrazole-4-carboxylate. Compound **B** was formed by the reaction of Enaminone intermediate with Compound **A** catalysed by dilute HCl.[55] Nucleophilic substitution of Compound **B** by piperazine resulted in formation of monosubstitued *Ethyl 5-methyl-1-(6-(piperazin-1-yl)pyrimidin-4-yl)-1H-pyrazole-4-*

carboxylate (**C**) without using any base as catalyst. Pyrazole ring formation from enaminone intermediate using dilute HCl and monosubstitution of piperazine without using any base are the best protocol developed here to get products in high yield without further refinement as previously reported methods associated with the formation of low yielded product. Different isothiocyanates were used in final step to afford to the final targeted compounds **(D1-D24)** with good yield and easy workup processes.

<<<<< Insert Scheme 1>>>>>

Structures of all synthesized compounds (**D1-D24**) were confirmed by their ^{1}H-NMR, ^{13}C-NMR and Mass spectra analysis, and the obtained spectra for each compound are found as per the proposed molecular structures. In mass spectra, molecular ion peak i.e. M+1 or M+2 peak confirms the formation of product as it was found appropriate to the molecular weight of the compound. In **Figure 2,** δ values found from ^{1}H-NMR and ^{13}C-NMR spectra which were denoted are in good accordance with those of specific protons and specific carbons of common scaffold found in all compounds. In case of aromatic thioamide derivatives, all protons were detected more deshielded as compared to aliphatic thioamides. In ^{13}C-NMR, carbon of thioamide group and carbonyl carbon of ester group (except compound **D20**) were observed more deshielded than the other carbons present in respective compound at around 181ppm and 162ppm respectively. Overview of NMR spectral date are represented below as **Figure 2** and spectral data i.e. ^{1}H-NMR, ^{13}C-NMR and Mass spectra of all compounds were provided in supporting data.

<<<<< Insert Figure 2>>>>>

***In-silico* ADMET prediction and Drug likeness study**

For drug discovery in earlier clinical step, Lipinski rule of five becomes the most influential concept to check the Drug likeness properties of novel molecules.[56] Drug likeness properties of synthesized compounds (**D1-D24**) were calculated by free Molinspiration-molecular property calculation services online. Marvinsketch tool was used to obtain molar refractivity of all novel designed target molecules using 3d structure followed by energy minimization. Obtained results were depicted in supporting data as **Table A**. All compounds displayed good drug likeness parameters without Lipinski's rule of five violation and thereby shown decent bioavailability. Alternative to this rule certain other pharmacokinetic parameters or molecular descriptors like polar surface area were also becomes the powerful concept for drug discovery in preclinical stage.

Thus, ADME properties were also predicted by Jorgensen's Method[57] using Qikprop tool (Shrodinger) for novel molecules and the obtained values are provided in supporting data as **Table B**. All molecules found to have acceptable pharmacokinetic properties (ADME). All compound have depicted decent human oral absorption percentage with in permissible range (75.57‑100%) and also good blood‑brain barrier permeability (QP log BB) values within acceptable range of -3 to 1.2. Similarly all compounds exhibited great intestinal

Absorption Prediction as predicted value of Caco-2 cell permeability (QPPCaco) for the all compounds were found greater than 500nm/sec. All compound shown admirable range IC_{50} value for HERG K+ channel blockage (QP logHERG) below -5. PSA values for designed molecules were also obtained in the range of 82.367-140.333 Å which guarantees good influence on bioavailability of molecules.[58] Morever, the predicted results were found to be differ in case of aqua solubility parameter (QPlogS) from the acceptable range i.e. -6.5 to 0.5. Number of methods available like Particle size reduction, pH adjustment, Hydrotrophy, Solid dispersion etc to improve the aqua solubility of drug content.[59] The essential pharmacokinetic parameters are delineated along with their permissible ranges in Table B (Supporting Data). ADME prediction may found to facilitate assessment of the eligible molecules.

***In-vitro* Biological evaluation:**

All compounds were evaluated *in-vitro* for their biological potency against various microorganisms and the resulted MIC or IC_{50} values were presented in **Table 1** and **Table 2**. As from the bioassay results, Compound **D19** and **D9** demonstrated good efficacy against malarial organism with IC_{50} values of 0.07 and 0.09 µg/mL respectively as compare to standard drugs i.e quinine and chloroquine. As compared to Quinine many of synthesized thioamide derivatives exhibited good potency with IC_{50} values ranges from 0.15 - 0.26 µg/mL against *P.falciparum.*

<<<<< Insert Table 1>>>>>

Amongst all compounds, two Compounds i.e. **D15** and **D19** against *E.Coli*, one compound i.e. **D20** against *P. aeruginosa* and *S. pyogenes,* two Compounds i.e. **D20** and **D10** against *S. aureus* emerged out from *in-vitro* antimicrobial assay as more potent antibacterial agents as compare to all three standard drugs i.e. Ampicillin, Ciprofloxacin and Chloramphenicol (**Table 2**). Compound **D20** exhibited excellent antibacterial activity against all bacterial strain except *E.Coli* compared to standard drugs i.e. Ampicillin, Chloramphenicol and Ciprofloxacin. Compound **D15** demonstrated excellent activity with MIC value of 25 µg/mL equipotent to standard drug ciprofloxacin. Among the three fungal strains, *C. Albicans* was only found to be sensitive towards the targeted compounds (**Table 2**). Four of synthesized compounds i.e. **D5**, **D6**, **D19** and **D20** found to be more potent antifungal agent against the *C. Albicans* organisms as compare to Greseofulvin. **D9** and **D19** were emerged out as potent antimalarial agents with IC_{50} value of 0.09 and 0.07 µg/mL respectively. Thus, these compounds found to be more potent than standard antimalarial drug Quinine. In case of anti-tubercular assay, all thioamides were found to have moderate MIC values ranging from 25 to 1000 µg/mL. Only compound **D6** exhibited good antitubercular activity (**Table 1**). From all biological assay, the results revealed that synthesized *N*-thioamides of pyrazolylpyrimidine based piperazine showed significant antibacterial and antimalarial activity.

<<<<< Insert Table 2>>>>>

***In-silico* Molecular docking studies**

To recognise the probable interaction of the bioactive molecules as ligands with the parent protein i.e. enzyme, Molecular docking of the synthesized molecules were studied against responsible enzymes i.e. *P. falciparum* dihydrofolate reductase (Pf-DHFR), β-Ketoacyl-acyl carrier protein (ACP) synthase i.e. *E.coli* FabH and *S.Aureus* hydrolase using PDB ID 4DPD, 1HNJ & 4CJN respectively as respective inhibitors of such enzymes are found responsible for the potency. Further, favourable interaction of many bioactive molecules against these enzymes were found in literature[60–62] which encouraged us for the selection of the aforementioned enzymes in present research. Enzyme i.e. protein 3D structures were taken from protein data bank and Glide tool in Maestro 11 (Schrodinger, LLC, New York, NY, 2015) was used to calculate docking scores of all synthesized molecules. Docking scores for all synthesized compounds obtained were shown in supplementary data. Docking poses in 2D and 3D view of most potent molecules in the active site of key enzymes are depicted in **Figures 3-5**. 3D docking poses were generated only with those amino acid residue which are in 4 Å range from ligand with the use of custom preset option. All potent ligands exhibited strong interaction with respective enzymes with good docking scores. Common π-π interaction with amino acid residue TRP 32 was observed by potent **D15** and **D19** in case of FabH. Likewise **D10** and **D20** also interacted with amino acid residue TYR 105 via common π-π interaction. Ligand protein interaction for some potent compounds with the respective key enzyme were represented in **Table 3** along with the docking score. Overall, the *in-vitro* results found in accordance with the molecular docking results and the docked ligands shown good docking score by showing good interactions with amino acid residues of the corresponding enzymes. Hence, the potential behavior of these bioactive molecules (**D9**, **D19**, **D10**, **D15** and **D20**) may expected due to their inhibitory efficacy against responsible enzymes. Various ligand protein interactions obtained were depicted along with docking score and bond length in **Table 3**.

<<<<< Insert Figure 3>>>>>

<<<<< Insert Figure 4>>>>>

<<<<< Insert Figure 5>>>>>

<<<<< Insert Table 3>>>>>

Structure-activity relationship (SAR)

Substituted Arylamine at distinct position found to affect biological potency. The electronic configuration of the various functional group led to promising effect on bioactivity. Compounds comprising -OCH_3 like electron-releasing groups on aromatic ring led to better antitubercular activity. Additionally, ortho substitution of chloro group to phenyl ring was led to be more biopotency. Moreover, compounds possessing -NO_2 like electron withdrawing groups attached on benzene ring resulted in decreasing antitubercular efficacy. Compounds possessing electron-withdrawing and halogenated groups excluding iodo group on aromatic ring led to increasing the antimalarial activity. It was also observed that ortho

and para substitution of the methyl and methoxy groups on phenyl ring led to increasing bioactivity. Meta substitution of iodo group phenyl ring exhibited excellent anti-malarial activity.

Among aliphatic amine substitution, ethylamine and methylamine substitution led to decrease in antitubercular and antimalarial efficacy. While compounds containing isopropyl amine and butylamine substituents led to excellent antitubercular activity but less antimalarial activity. Compounds possessing cyclohexyl amine and propyl amine substituents led to excellent antimalarial activities. Furthermore, less antitubercular potency was detected by isobutyl amine and propylamine substituted compounds.

Most compounds found active against *S. aureus* bacterial strain. Compounds comprising of electron-releasing groups (like halogen) on aromatic ring found to be potent against Gram positive bacterial strains. Another side, an electron withdrawing group like nitro on aromatic ring directed increasing Gram positive bacterial inhibition. Para subtitution of the fluoro group on aromatic ring with the lowest lipophilicity (Lowest Log P) and benzoyl substituent led to an increase inhibition against both Gram negative as well as Gram positive bacterial strains. In addition, compound possessing $–CH_3$ and $–OCH_3$ groups on phenyl ring decreased bacterial inhibition.

Compounds possessing halogenated groups like -Cl and -F on aromatic ring contained excellent inhibition against *E. coli* i.e. Gram negative bacterial strain. Compounds comprising substituents like isobutyl amine, prop-2-en-1-amine and cyclohexyl amine led to more potent Gram positive antibacterial activity while compounds comprising butyl amine, isopropyl amine and propyl amine led to decrease inhibition against Gram positive bacterial strain. Furthermore, compounds with methyl amine substitution expressed excellent Gram negative *E. coli* bacterial inhibition activity. **Figure 6** demonstrates the outcome of SAR study in graphical form.

<<<<< Insert Figure 6>>>>>

Conclusion:

The present study demonstrated synthesis, spectroscopic evaluation, molecular docking studies and *in-vitro* biological evaluation i.e. antimicrobial, antimalarial and anti-tubercular assay of N-thioamide derivatives of pyrazolylpyrimidine based piperazine. A convenient and most efficient synthetic pathway had been reported to synthesize novel hybrid molecules of pyrazole, pyrimidine and piperazine. All compounds were obtained in good yield and high purity. The formation of all compounds were unambiguously seen from the spectral data. Notably, antibacterial studies demonstrate that four compounds **D10**, **D15**, **D19** and **D20** exhibit superior antibacterial activity against distinct bacterial strain. Compound **D20** i.e. benzoyl thioamide derivative was found most potent against all bacterial strains *except E. Coli.* Furthermore, Compound **D9** and **D19** exhibit superior antimalarial activity among all

compounds. Molecular docking studies results show that all these potent compounds were also found to have favourable interaction with the responsible enzymes that means compounds with good docking scores have shown good potency. Thus, the *in-silico* studies and the biological assay can be specifically related with each other and optimization of potent molecules identified in the present study can be conveyed out for further advancement.

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Conflicts of interest

The authors declare that they have no competing interests.

References

[1] WHO, WHO | Global tuberculosis report 2016. http://www.who.int/tb/publications/global_report/en/ (accessed July 13, 2017).

[2] D.A. Nugiel, A. Vidwans, A.-M. Etzkorn, K.A. Rossi, P.A. Benfield, C.R. Burton, S. Cox, D. Doleniak, S.P. Seitz, Synthesis and Evaluation of Indenopyrazoles as Cyclin-Dependent Kinase Inhibitors. 2. Probing the Indeno Ring Substituent Pattern, J. Med. Chem. 45 (2002) 5224–5232. doi:10.1021/jm020171+.

[3] R.C. Goldman, K.V. Plumley, B.E. Laughon, The evolution of extensively drug resistant tuberculosis (XDR-TB): history, status and issues for global control, Infect. Disord. Drug Targets. 7 (2007) 73–91.

[4] WHO, WHO | Fact Sheet: World Malaria Report 2016, WHO. (n.d.). http://www.who.int/malaria/media/world-malaria-report-2016/en/ (accessed July 13, 2017).

[5] C. Lazar, A. Kluczyk, T. Kiyota, Y. Konishi, Drug Evolution Concept in Drug Design: 1. Hybridization Method, J. Med. Chem. 47 (2004) 6973–6982. doi:10.1021/jm049637+.

[6] Claudio Viegas-Junior, Eliezer J. Barreiro, Carlos Alberto Manssour Fraga, Molecular Hybridization: A Useful Tool in the Design of New Drug Prototypes, Curr. Med. Chem. 14 (2007) 1829–1852. doi:10.2174/092986707781058805.

[7] U. Madsen, F.A. Sløk, T.B. Stensbøl, H. Bräuner-Osborne, H.-C.H. Lützhøft, M.V. Poulsen, L. Eriksen, P. Krogsgaard-Larsen, Ionotropic excitatory amino acid receptor ligands. Synthesis and pharmacology of a new amino acid AMPA antagonist, Eur. J. Med. Chem. 35 (2000) 69–76. doi:10.1016/S0223-5234(00)00104-5.

[8] D.L. Selwood, D.G. Brummell, R.C. Glen, M.C. Goggin, K. Reynolds, M.A. Tatlock, G. Wishart, Solution-Phase parallel synthesis of 5-carboxamido 1-benzyl-3-(3-dimethylaminopropyloxy)-1H-pyrazoles as activators of soluble guanylate cyclase with improved oral bioavailability, Bioorg. Med. Chem. Lett. 11 (2001) 1089–1092.doi:10.1016/S0960-894X(01)00141-X.

[9] D. Raffa, G. Daidone, B. Maggio, S. Cascioferro, F. Plescia, D. Schillaci, Synthesis and antileukemic activity of new 3-(1-phenyl-3-methylpyrazol-5-yl)-2-styrylquinazolin-4(3H)-ones, Il Farm. 59 (2004) 215–221. doi:10.1016/j.farmac.2003.10.004.

[10] L. Liu, J. Yang, Z. Zhao, P. Shi, X. Liu, Solvent-free synthesis of indole-based thiosemicarbazones under microwave irradiation, J. Chem. Res. 34 (2010) 57–60. doi:10.3184/030823410X12628833270368.

[11] A.M. Farag, A.S. Mayhoub, S.E. Barakat, A.H. Bayomi, Regioselective synthesis and antitumor screening of some novel N-phenylpyrazole derivatives, Bioorg. Med. Chem. 16 (2008) 881–889. doi:10.1016/j.bmc.2007.10.015.

[12] S.A.F. Rostom, Polysubstituted pyrazoles, part 6. Synthesis of some 1-(4-chlorophenyl)-4-hydroxy-1H-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents, Bioorg. Med. Chem. 18 (2010) 2767–2776. doi:10.1016/j.bmc.2010.02.006.

[13] M.J. Genin, C. Biles, B.J. Keiser, S.M. Poppe, S.M. Swaney, W.G. Tarpley, Y. Yagi, D.L. Romero, Novel 1,5-Diphenylpyrazole Nonnucleoside HIV-1 Reverse Transcriptase Inhibitors with Enhanced Activity versus the Delavirdine-Resistant P236L Mutant: Lead Identification and SAR of 3- and 4-Substituted Derivatives, J. Med. Chem. 43 (2000) 1034–1040. doi:10.1021/jm990383f.

[14] A.E. Rashad, M.I. Hegab, R.E. Abdel-Megeid, J.A. Micky, F.M.E. Abdel-Megeid, Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives, Bioorg. Med. Chem. 16 (2008) 7102–7106. doi:10.1016/j.bmc.2008.06.054.

[15] R.V. Ragavan, V. Vijayakumar, N.S. Kumari, Synthesis and antimicrobial activities of novel 1,5-diaryl pyrazoles, Eur. J. Med. Chem. 45 (2010) 1173–1180. doi:10.1016/j.ejmech.2009.12.042.

[16] N.C. Desai, G.M. Kotadiya, A.R. Trivedi, Studies on molecular properties prediction, antitubercular and antimicrobial activities of novel quinoline based pyrimidine motifs, Bioorg. Med. Chem. Lett. 24 (2014) 3126–3130. doi:10.1016/j.bmcl.2014.05.002.

[17] A. Barakat, S.M. Soliman, A.M. Al-Majid, G. Lotfy, H.A. Ghabbour, H.-K. Fun, S. Yousuf, M.I. Choudhary, A. Wadood, Synthesis and structure investigation of novel pyrimidine-2,4,6-trione derivatives of highly potential biological activity as anti-diabetic agent, J. Mol. Struct. 1098 (2015) 365–376. doi:10.1016/j.molstruc.2015.06.037.

[18] H. Kaur, M. Machado, C. de Kock, P. Smith, K. Chibale, M. Prudêncio, K. Singh, Primaquine–pyrimidine hybrids: Synthesis and dual-stage antiplasmodial activity, Eur. J. Med. Chem. 101 (2015) 266–273. doi:10.1016/j.ejmech.2015.06.045.

[19] D.H. Slee, X. Zhang, M. Moorjani, E. Lin, M.C. Lanier, Y. Chen, J.K. Rueter, S.M. Lechner, S. Markison, S. Malany, T. Joswig, M. Santos, R.S. Gross, J.P. Williams, J.C. Castro-Palomino, M.I. Crespo, M. Prat, S. Gual, J.-L. Díaz, J. Wen, Z. O'Brien, J. Saunders, Identification of Novel, Water-Soluble, 2-Amino-N-pyrimidin-4-yl Acetamides as A2A Receptor Antagonists with In Vivo Efficacy, J. Med. Chem. 51 (2008) 400–406. doi:10.1021/jm070623o.

[20] N. Toda, X. Hao, Y. Ogawa, K. Oda, M. Yu, Z. Fu, Y. Chen, Y. Kim, M. Lizarzaburu, S. Lively, S. Lawlis, M. Murakoshi, F. Nara, N. Watanabe, J.D. Reagan, H. Tian, A. Fu, A. Motani, Q. Liu, Y.-J. Lin, R. Zhuang, Y. Xiong, P. Fan, J. Medina, L. Li, M. Izumi, R. Okuyama, S. Shibuya, Potent and Orally Bioavailable GPR142 Agonists as Novel Insulin Secretagogues for the Treatment of Type 2 Diabetes, ACS Med. Chem. Lett. 4 (2013) 790–794. doi:10.1021/ml400186z.

[21] K. Kobayashi, S. Chono, H. Yamada, Mepirizole, a non-steroidal antiinflammatory compound, its ulcerogenicity and inhibitory action on lesions induced by acidic antiinflammatory agents in the rat stomach, Gastroenterol. Jpn. 15 (1980) 427–432. doi:10.1007/BF02773903.

[22] J.-S. Choi, H. Hwang, S.-W. Kim, B.I. Lee, J. Lee, H.-J. Song, J.S. Koh, J.-H. Kim, P.H. Lee, Highly potent and selective pyrazolylpyrimidines as Syk kinase inhibitors, Bioorg. Med. Chem. Lett. 25 (2015) 4441–4446. doi:10.1016/j.bmcl.2015.09.011.

[23] L.N. Casillas, S.J. Chakravorty, P. Eidam, P.A. Haile, T.V. Hughes, A.L. Shah, L.K. Leister, N.A. Miller, A. Rahman, C.A. Sehon, G.Z. Wang, D. Zhang, Pyrazolyl-pyrimidines as kinase inhibitors, US20130023534A1, 2013. https://patents.google.com/patent/US20130023534A1/en.

[24] M. Ikeda, K. Maruyama, Y. Nobuhara, T. Yamada, S. Okabe, Cytoprotective Effects of 4, 6-Bis(1H-pyrazol-1-yl)pyrimidine and Related Compounds on HCl·Ethanol-Induced Gastric Lesions in Rats, Chem. Pharm. Bull. (Tokyo). 45 (1997) 549–551. doi:10.1248/cpb.45.549.

[25] M. Ikeda, K. Maruyama, Y. Nobuhara, T. Yamada, S. Okabe, Synthesis and Cytoprotective Antiulcer Activity of 2- or 4-(1H-Pyrazol-1-yl)pyrimidine Derivatives Related to Mepirizole and Dulcerozine, Chem. Pharm. Bull. (Tokyo). 44 (1996) 1700–1706. doi:10.1248/cpb.44.1700.

[26] A.K. Rathi, R. Syed, H.-S. Shin, R.V. Patel, Piperazine derivatives for therapeutic use: a patent review (2010-present), Expert Opin. Ther. Pat. 26 (2016) 777–797. doi:10.1080/13543776.2016.1189902.

[27] R. Capdeville, E. Buchdunger, J. Zimmermann, A. Matter, Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug, Nat. Rev. Drug Discov. 1 (2002) 493–502. doi:10.1038/nrd839.

[28] S.L. Hale, R.A. Kloner, Ranolazine, an inhibitor of the late sodium channel current, reduces postischemic myocardial dysfunction in the rabbit, J. Cardiovasc. Pharmacol. Ther. 11 (2006) 249–255. doi:10.1177/1074248406294607.

[29] G. Fragasso, A. Palloshi, P. Puccetti, C. Silipigni, A. Rossodivita, M. Pala, G. Calori, O. Alfieri, A. Margonato, A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure, J. Am. Coll. Cardiol. 48 (2006) 992–998. doi:10.1016/j.jacc.2006.03.060.

[30] M.H. Trivedi, M. Fava, S.R. Wisniewski, M.E. Thase, F. Quitkin, D. Warden, L. Ritz, A.A. Nierenberg, B.D. Lebowitz, M.M. Biggs, J.F. Luther, K. Shores-Wilson, A.J. Rush, STAR*D Study Team, Medication augmentation after the failure of SSRIs for depression, N. Engl. J. Med. 354 (2006) 1243–1252. doi:10.1056/NEJMoa052964.

[31] R.J. Fanelli, T. Schuurman, T. Glaser, J. Traber, Ipsapirone: a novel anxiolytic and selective 5-HT1A receptor ligand, Prog. Clin. Biol. Res. 361 (1990) 461–467.

[32] B. Cusack, A. Nelson, E. Richelson, Binding of antidepressants to human brain receptors: focus on newer generation compounds, Psychopharmacology (Berl.). 114 (1994) 559–565. doi: 10.1007/BF02244985.

[33] J.A. Grant, J.-M. Riethuisen, B. Moulaert, C. DeVos, A double-blind, randomized, single-dose, crossover comparison of levocetirizine with ebastine, fexofenadine, loratadine, mizolastine, and placebo: suppression of histamine-induced wheal-and-flare response during 24 hours in healthy male subjects, Ann. Allergy. Asthma. Immunol. 88 (2002) 190–197. doi:10.1016/S1081-1206(10)61995-3.

[34] L. Tang, P.K. Shukla, Z.J. Wang, Trifluoperazine, an orally available clinically used drug, disrupts opioid antinociceptive tolerance, Neurosci. Lett. 397 (2006) 1–4. doi:10.1016/j.neulet.2005.11.050.

[35] L. Rees, Chlorpromazine and Allied Phenothiazine Derivatives, Br. Med. J. 2 (1960) 522–525. doi: 10.1136/bmj.2.5197.522.

[36] P.M. Manoury, A.P. Dumas, H. Najer, D. Branceni, M. Prouteau, F.M. Lefevre-Borg, Synthesis and analgesic activities of some (4-substituted phenyl-1-piperazinyl)alkyl 2-aminobenzoates and 2-aminonicotinates, J. Med. Chem. 22 (1979) 554–559. doi: 10.1021/jm00191a017.

[37] V.U. Jeankumar, R.S. Reshma, R. Vats, R. Janupally, S. Saxena, P. Yogeeswari, D. Sriram, Engineering another class of anti-tubercular lead: Hit to lead optimization of an intriguing class of gyrase ATPase inhibitors, Eur. J. Med. Chem. 122 (2016) 216–231. doi:10.1016/j.ejmech.2016.06.042.

[38] M. Chandran, J. Renuka, J.P. Sridevi, G.S. Pedgaonkar, V. Asmitha, P. Yogeeswari, D. Sriram, Benzothiazinone-piperazine derivatives as efficient Mycobacterium tuberculosis DNA gyrase inhibitors, Int. J. Mycobacteriology. 4 (2015) 104–115. doi:10.1016/j.ijmyco.2015.02.002.

[39] K.I. Reddy, K. Srihari, J. Renuka, K.S. Sree, A. Chuppala, V.U. Jeankumar, J.P. Sridevi, K.S. Babu, P. Yogeeswari, D. Sriram, An efficient synthesis and biological screening of benzofuran and benzo[d]isothiazole derivatives for Mycobacterium tuberculosis DNA GyrB inhibition, Bioorg. Med. Chem. 22 (2014) 6552–6563. doi:10.1016/j.bmc.2014.10.016.

[40] M. Taha, N.H. Ismail, W. Jamil, K.M. Khan, U. Salar, S.M. Kashif, F. Rahim, Y. Latif, Synthesis and evaluation of unsymmetrical heterocyclic thioureas as potent β-glucuronidase inhibitors, Med. Chem. Res. 24 (2015) 3166–3173. doi:10.1007/s00044-015-1369-x.

[41] S.L. Warner, S. Bashyam, H. Vankayalapati, D.J. Bearss, H. Han, D. Mahadevan, D.D. Von Hoff, L.H. Hurley, Identification of a lead small-molecule inhibitor of the Aurora kinases using a structure-assisted, fragment-based approach, Mol. Cancer Ther. 5 (2006) 1764–1773. doi:10.1158/1535-7163.MCT-05-0524.

[42] K. Reddy, R. Chenna, S. Rasheed, D. Subba Rao, S. Adam, Y. Venkata Rami Reddy, C.N. Raju, New Urea and Thiourea Derivatives of Piperazine Doped with Febuxostat: Synthesis and Evaluation of Anti-TMV and Antimicrobial Activities, Sci. World J. (2013). doi:10.1155/2013/682603.

[43] J.M. Rohde, K.R. Brimacombe, L. Liu, M.E. Pacold, A. Yasgar, D.M. Cheff, T.D. Lee, G. Rai, B. Baljinnyam, Z. Li, A. Simeonov, M.D. Hall, M. Shen, D.M. Sabatini, M.B. Boxer, Discovery and optimization of piperazine-1-thiourea-based human phosphoglycerate dehydrogenase inhibitors, Bioorg. Med. Chem. (2018). doi:10.1016/j.bmc.2018.02.016.

[44] M.D. Gaul, G. Xu, J. Kirkpatrick, H. Ott, C.A. Baumann, 4-Amino-6-piperazin-1-yl-pyrimidine-5-carbaldehyde oximes as potent FLT-3 inhibitors, Bioorg. Med. Chem. Lett. 17 (2007) 4861–4865. doi:10.1016/j.bmcl.2007.06.046.

[45] J.A. Heath, M.M. Mehrotra, S. Chi, J.-C. Yu, A. Hutchaleelaha, S.J. Hollenbach, N.A. Giese, R.M. Scarborough, A. Pandey, Identification of 4-piperazin-1-yl-quinazoline template based aryl and benzyl thioureas as potent, selective, and orally bioavailable inhibitors of platelet-derived growth factor (PDGF) receptor, Bioorg. Med. Chem. Lett. 14 (2004) 4867–4872. doi:10.1016/j.bmcl.2004.07.041.

[46] T.L. Foley, G. Rai, A. Yasgar, T. Daniel, H.L. Baker, M. Attene-Ramos, N.M. Kosa, W. Leister, M.D. Burkart, A. Jadhav, A. Simeonov, D.J. Maloney, 4-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)-N-(4-methoxypyridin-2-yl)piperazine-1-carbothioamide (ML267), a potent inhibitor of bacterial phosphopantetheinyl transferase that attenuates secondary metabolism and thwarts bacterial growth, J. Med. Chem. 57 (2014) 1063–1078. doi:10.1021/jm401752p.

[47] M.K. Vekariya, R.H. Vekariya, K.D. Patel, N.P. Raval, P.U. Shah, D.P. Rajani, N.K. Shah, Pyrimidine-Pyrazole Hybrids as Morpholinopyrimidine-Based Pyrazole Carboxamides: Synthesis, Characterisation, Docking, ADMET Study and Biological Evaluation, ChemistrySelect. 3 (24) 6998–7008. doi:10.1002/slct.201801011.

[48] R.H. Vekariya, K.D. Patel, M.K. Vekariya, N.P. Prajapati, D.P. Rajani, S.D. Rajani, H.D. Patel, Microwave-assisted green synthesis of new imidazo[2,1-b]thiazole derivatives and their antimicrobial, antimalarial, and antitubercular activities, Res. Chem. Intermed. (2017) 1–25. doi:10.1007/s11164-017-2985-5.

[49] P.E. Palmer, H.J. Wolfe, C.-I. Kostas, MULTISYSTEM FIBROSIS IN ALPHA-1-ANTITRYPSIN DEFICIENCY, The Lancet. 311 (1978) 221–222. doi:10.1016/S0140-6736(78)90669-4.

[50] C.K. Stover, P. Warrener, D.R. VanDevanter, D.R. Sherman, T.M. Arain, M.H. Langhorne, S.W. Anderson, J.A. Towell, Y. Yuan, D.N. McMurray, B.N. Kreiswirth, C.E. Barry, W.R. Baker, A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis, Nature. 405 (2000) 962–966. doi:10.1038/35016103.

[51] Y. Yuthavong, B. Tarnchompoo, T. Vilaivan, P. Chitnumsub, S. Kamchonwongpaisan, S.A. Charman, D.N. McLennan, K.L. White, L. Vivas, E. Bongard, C. Thongphanchang, S. Taweechai, J. Vanichtanankul, R. Rattanajak, U. Arwon, P. Fantauzzi, J. Yuvaniyama, W.N. Charman, D. Matthews, Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target, Proc.Natl.Acad.Sci.USA. 109 (2012) 16823–16828. doi:10.2210/pdb4dpd/pdb.

[52] R. Bouley, M. Kumarasiri, Z. Peng, L.H. Otero, W. Song, M.A. Suckow, V.A. Schroeder, W.R. Wolter, E. Lastochkin, N.T. Antunes, H. Pi, S. Vakulenko, J.A. Hermoso, M. Chang, S. Mobashery,

Discovery of Antibiotic (E)-3-(3-Carboxyphenyl)-2-(4-cyanostyryl)quinazolin-4(3H)-one, J. Am. Chem. Soc. 137 (2015) 1738–1741. doi:10.1021/jacs.5b00056.
[53] X. Qiu, C.A. Janson, W.W. Smith, M. Head, J. Lonsdale, A.K. Konstantinidis, Refined structures of β-ketoacyl-acyl carrier protein synthase III11Edited by I. A. Wilson, J. Mol. Biol. 307 (2001) 341–356. doi:10.1006/jmbi.2000.4457.
[54] S. Al-Mousawi, E. John, M.M. Abdelkhalik, M.H. Elnagdi, Enaminones as building blocks in heterocyclic syntheses: A new approach to polyfunctionally substituted cyclohexenoazines, J. Heterocycl. Chem. 40 (2003) 689–695. doi:10.1002/jhet.5570400421.
[55] M.K. Vekariya, R.H. Vekariya, P.S. Brahmkshatriya, N.K. Shah, Pyrimidine-based pyrazoles as cyclin-dependent kinase 2 inhibitors: Design, synthesis, and biological evaluation, Chem. Biol. Drug Des. (2018). doi:10.1111/cbdd.13334.
[56] C. Abad-Zapatero, Chapter 5 - Analysis of the Content of SAR Databases, in: Ligand Effic. Indices Drug Discov., Academic Press, San Diego, 2013: pp. 67–79. doi:10.1016/B978-0-12-404635-1.00005-0.
[57] E.M. Duffy, W.L. Jorgensen, Prediction of Properties from Simulations: Free Energies of Solvation in Hexadecane, Octanol, and Water, J. Am. Chem. Soc. 122 (2000) 2878–2888. doi:10.1021/ja993663t.
[58] A. Lu, H. Luo, M. Shi, G. Wu, Y. Yuan, J. Liu, F. Tang, Design, synthesis and docking studies on benzamide derivatives as histone deacetylase inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 4924–4927. doi:10.1016/j.bmcl.2011.06.001.
[59] S. Sareen, G. Mathew, L. Joseph, Improvement in solubility of poor water-soluble drugs by solid dispersion, Int. J. Pharm. Investig. 2 (2012) 12–17. doi:10.4103/2230-973X.96921.
[60] P.-C. Lv, J. Sun, Y. Luo, Y. Yang, H.-L. Zhu, Design, synthesis, and structure–activity relationships of pyrazole derivatives as potential FabH inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 4657–4660. doi:10.1016/j.bmcl.2010.05.105.
[61] S.S. Thakkar, P. Thakor, H. Doshi, A. Ray, 1,2,4-Triazole and 1,3,4-oxadiazole analogues: Synthesis, MO studies, in silico molecular docking studies, antimalarial as DHFR inhibitor and antimicrobial activities, Bioorg. Med. Chem. 25 (2017) 4064–4075. doi:10.1016/j.bmc.2017.05.054.
[62] K.D. Patel, R.H. Vekariya, N.P. Prajapati, D.B. Patel, H.D. Patel, T. Shaikh, D.P. Rajani, S. Rajani, N.S. Shah, D. Jhala, Synthesis of N'-(Quinazolin-4-yl)isonicotinohydrazides and their biological screening, docking and ADME studies, Arab. J. Chem. (2018). doi:10.1016/j.arabjc.2018.02.017.

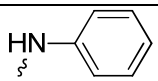
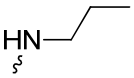
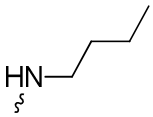
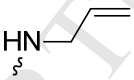
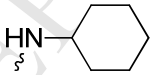
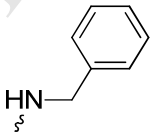
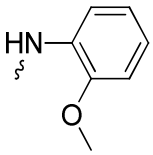
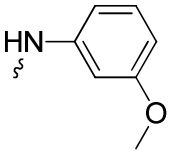
Figures and Scheme captions

Scheme 1: Reagents and conditions: **(i)** $NH_2NH_2.H_2O$, EtOH, rt;
(ii) Enaminone intermediate, 1N HCl, EtOH, rt;
(iii) Piperazine, DMF, 80^0 C;
(iv) different isothiocynates, DMF;

Figure 1: hybridization approach to design targeted molecules
Figure 2: Overview of NMR spectral data for all compounds
Figure 3: Docking pose of **D9** and **D19** with pf-DHFR (4DPD)
Figure 4: Docking pose of **D15** and **D19** with *E.Coli* FabH (1HNJ)
Figure 5: Docking pose of **D10** and **D20** with S. Aureus Hydrolase (4CJN)

Figure 6: Graphical presentation of Structure-activity relationship study

Table 1: Antitubercular and Antimalarial activity of D1-D24

Comp.	R Group	Antitubercular activity $H_{37}Rv$ MTCC200 MICs,(µg/mL)	Antimalarial activity P.falciparum (IC_{50}, µg/mL)
D1		100	0.26
D2		125	1.57
D3		100	1.12
D4		250	0.16
D5		50	1.12
D6		25	0.97
D7		100	1.62
D8		500	0.84
D9		125	0.09
D10		100	0.53
D11		250	0.15
D12		62.5	0.38

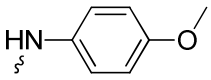
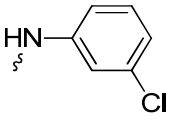
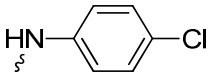
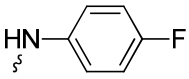
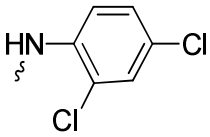
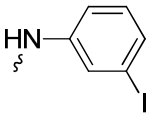
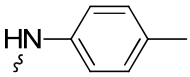
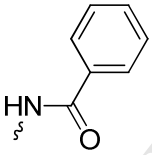
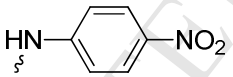
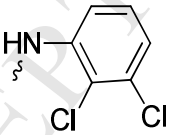
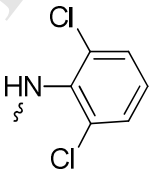
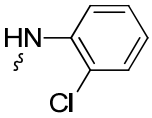
D13		50	0.43
D14		250	1.08
D15		100	0.26
D16		250	0.68
D17		1000	0.23
D18		500	0.20
D19		125	0.07
D20		250	0.94
D21		100	1.47
D22		250	0.30
D23		125	0.32
D24		62.5	0.47
Drug	**Isoniazide**	0.20	--
Drug	**Chloroquin**	--	0.02
Drug	**Quinine**	--	0.26

Table 2 : Antibacterial and antifungal activity (MICs, µg/ml)

Compounds	Antibacterial activity				Antifungal activity		
	Gram negative bacteria		Gram positive bacteria				
	E.C. MTCC 443	P.A. MTCC 1688	S.A. MTCC 96	S.P. MTCC 442	C.A. MTCC 227	A.N. MTCC 282	A.C. MTCC 1323
D1	62.5	500	250	500	500	1000	1000
D2	62.5	250	250	500	500	1000	1000
D3	250	500	500	250	500	1000	1000
D4	100	500	500	500	250	1000	1000
D5	100	250	500	500	250	1000	1000
D6	125	100	500	500	500	1000	1000
D7	125	100	100	250	1000	500	500
D8	100	250	62.5	100	1000	1000	1000
D9	250	500	100	125	500	1000	1000
D10	100	500	50	500	1000	1000	1000
D11	100	500	250	500	500	1000	1000
D12	125	250	100	500	500	1000	1000
D13	100	250	500	500	500	1000	1000
D14	100	500	500	250	500	1000	1000
D15	25	500	250	250	500	1000	1000
D16	62.5	250	250	125	500	1000	1000
D17	500	500	100	200	500	500	500
D18	250	500	200	200	500	500	500
D19	50	200	250	250	200	1000	500
D20	100	12.5	50	25	250	100	100
D21	100	100	200	100	500	1000	1000
D22	62.5	100	100	500	1000	1000	1000
D23	62.5	500	125	500	1000	1000	1000
D24	200	250	250	250	500	500	500
Ampicillin	100	100	250	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

Table 3 : Various Ligand protein Interactions

Protein (PDB)	Comp Code	Dock Score	Atom[a]	H-bond Aminoacid[b]	B.L. (Å)[c]	π – π interaction
Pf-DHFR (4DPD)	**D9**	-7.460	H atom (piperazine)	O(OH) of SER 11	2.37	--
			H atom (Cyclohexyl)	H(Ph) of PHE 58	2.40	--
			H atom (piperazine)	O(OH) of SER 108	2.31	--
			H atom (NHCS)	O(CO) of ILE 164	2.12	--
	D19	-7.320	H atom (NHCS)	H(OH) of ILE 164	1.98	--
			N atom (pyrazole)	H(OH) of SER 108	2.56	--
***E.coli* FabH (1HNJ)**	**D15**	-7.175	H atom (Phenyl)	O(OH) of THR 28	2.36	--
			H atom (Phenyl)	H(OH) of THR 28	1.48	--
			H atom (piperazine)	O(CO) of GLY152	2.30	--
			Phenyl ring	--	--	TRP32
	D19	-6.551	H atom (tolyl)	O(OH) of THR 28	2.34	--
			H atom (tolyl)	H(OH) of THR 28	1.45	--
			H atom (piperazine)	O(CO) of GLY152	2.40	--
			Phenyl ring	--	--	TRP32
***S.Aureus* hydrolase (4CJN)**	**D10**	-3.726	N atom (pyrimidine)	H(OH) of TYR 105	2.15	--
			H atom (ethyl)	O(CO) of ASP 295	2.40	
			N atom (pyrazole)	$H(NH_2)$ of LEU 147	2.64	--
			H atom (ethyl)	$H(NH_3)$ of LYS 273	1.97	--
			pyrimidine ring	--		TYR105
	D20	-3.384	N atom (pyrimidine)	H(OH) of TYR 105	2.15	--
			O atom (CO)	$H(NH_3)$ of LYS 316	2.01	--
			pyrimidine ring	--		TYR105
			Pyrazole ring	--		TYR295

[a]Atom of ligand participate in interaction with residue
[b]Interactive amino acid residue
[c]Bond distance

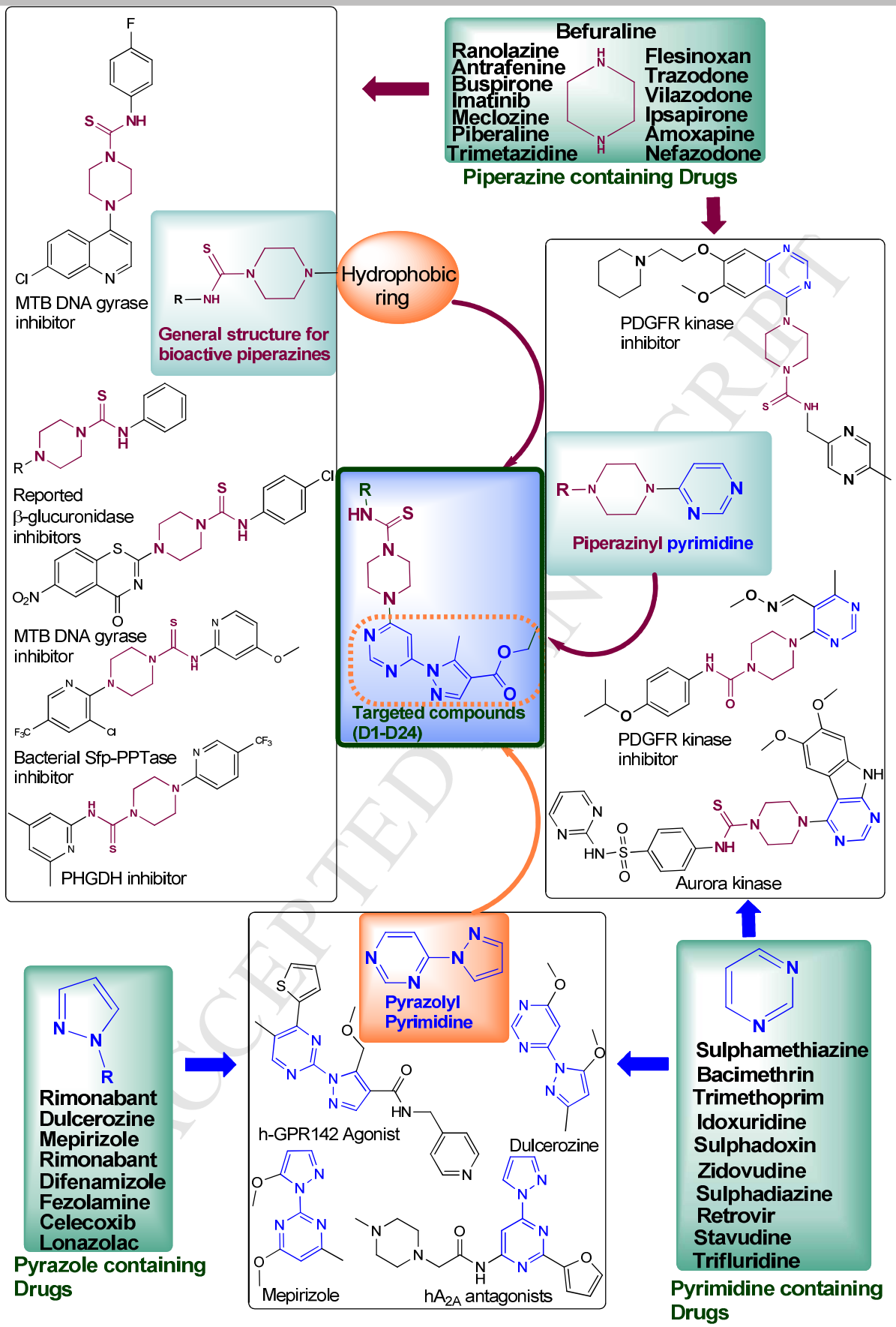
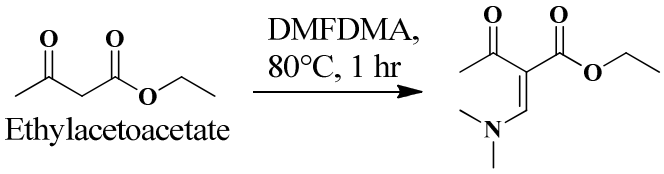
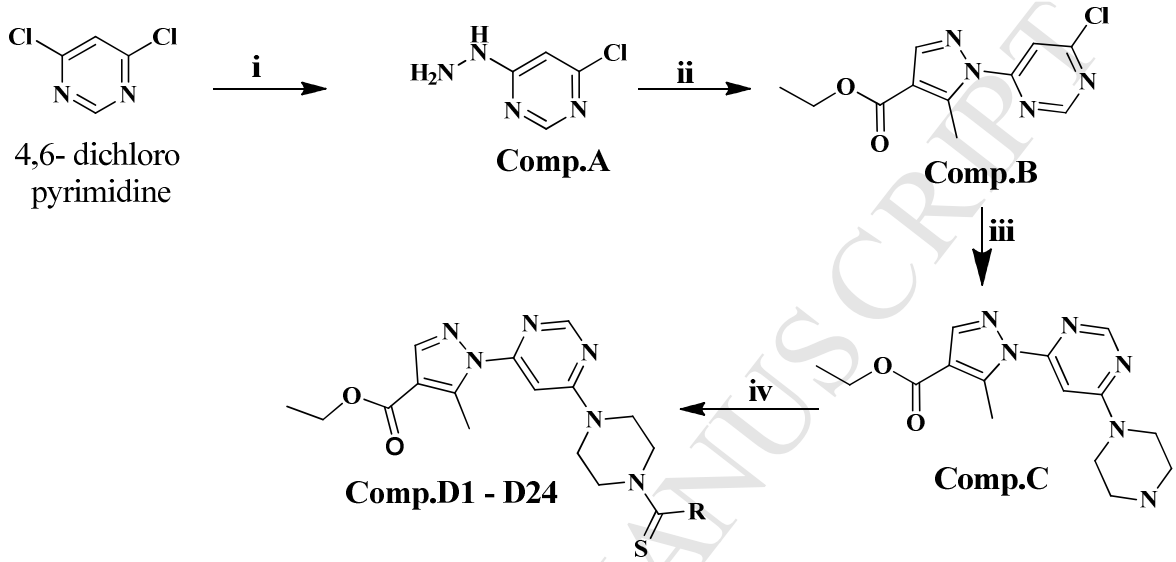

Befuraline
Ranolazine
Antrafenine
Buspirone
Imatinib
Meclozine
Piberaline
Trimetazidine
Flesinoxan
Trazodone
Vilazodone
Ipsapirone
Amoxapine
Nefazodone
Piperazine containing Drugs
MTB DNA gyrase inhibitor
General structure for bioactive piperazines
Hydrophobic ring
PDGFR kinase inhibitor
Piperazinyl pyrimidine
Reported β-glucuronidase inhibitors
MTB DNA gyrase inhibitor
Bacterial Sfp-PPTase inhibitor
PHGDH inhibitor
Targeted compounds (D1-D24)
PDGFR kinase inhibitor
Aurora kinase
Pyrazolyl Pyrimidine
h-GPR142 Agonist
Dulcerozine
Mepirizole
hA2A antagonists
Rimonabant
Dulcerozine
Mepirizole
Rimonabant
Difenamizole
Fezolamine
Celecoxib
Lonazolac
Pyrazole containing Drugs
Sulphamethiazine
Bacimethrin
Trimethoprim
Idoxuridine
Sulphadoxin
Zidovudine
Sulphadiazine
Retrovir
Stavudine
Trifluridine
Pyrimidine containing Drugs

Figure 1

Preparation of Enaminone intermediate



Preparation of targeted compounds



Scheme 1

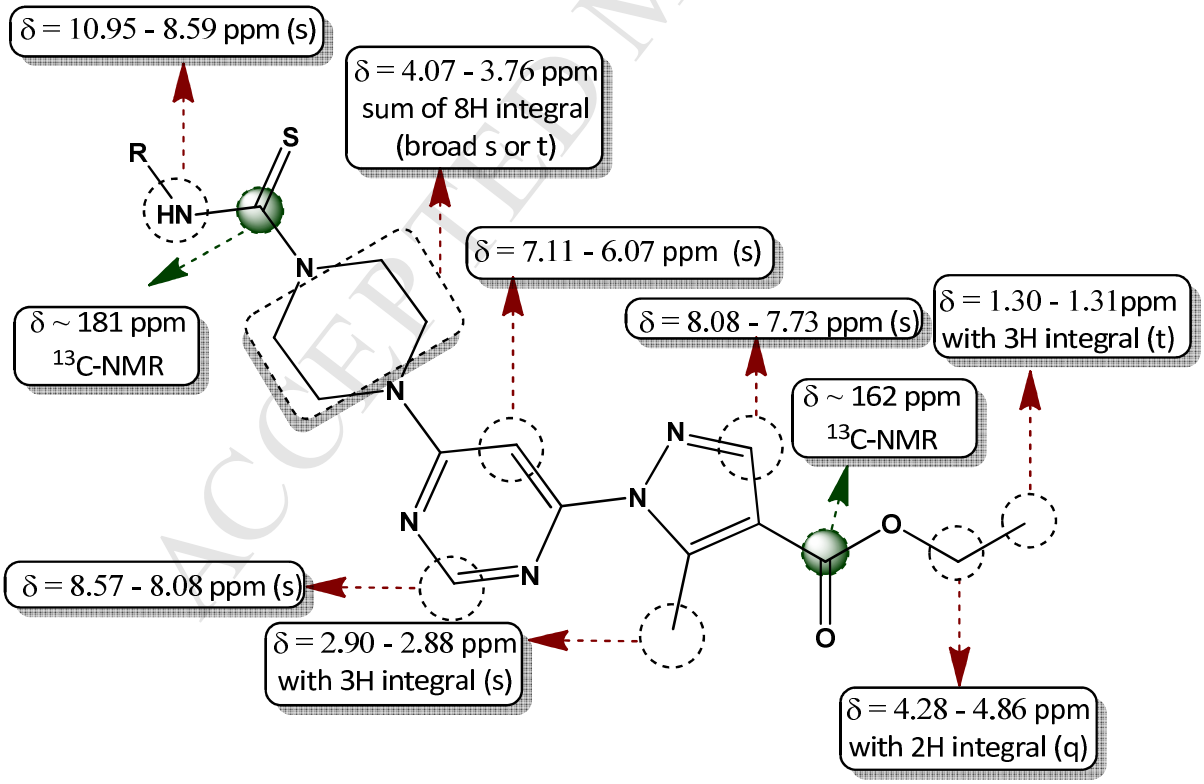


Figure 2

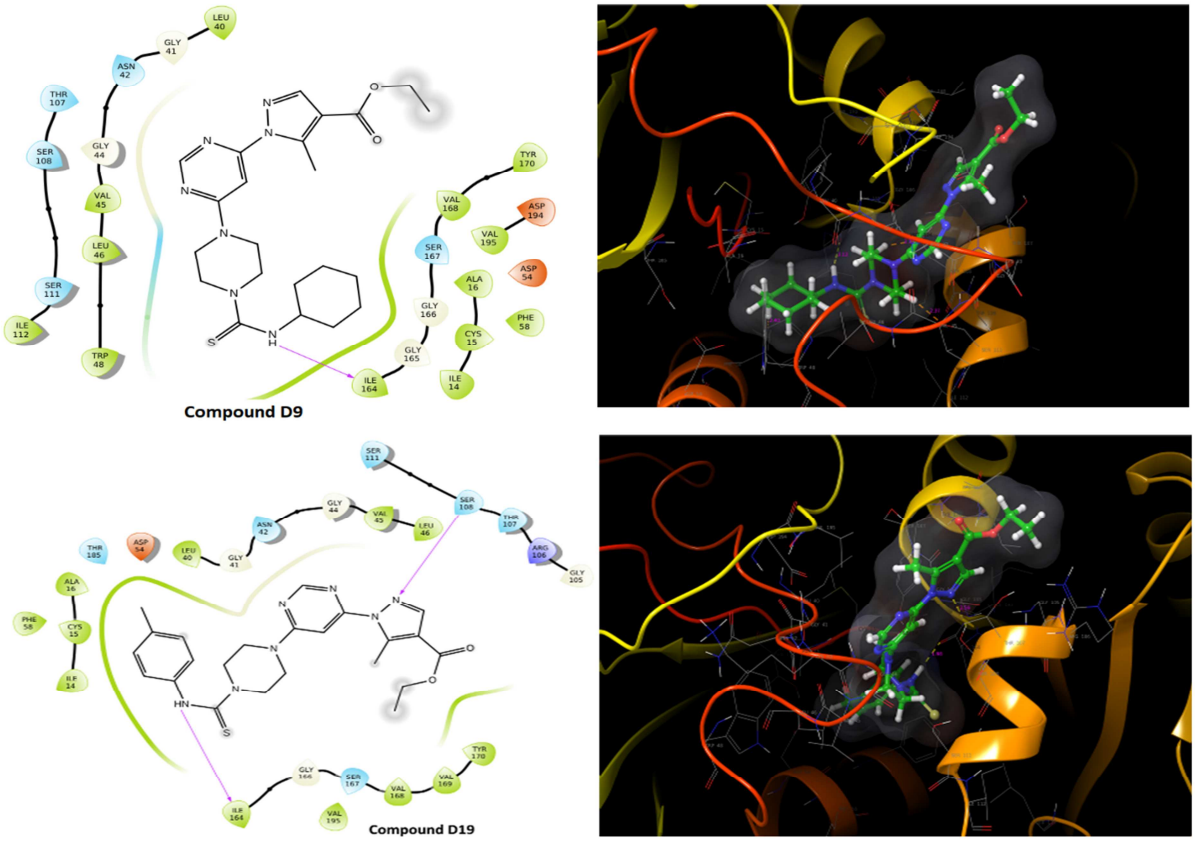


Figure 3

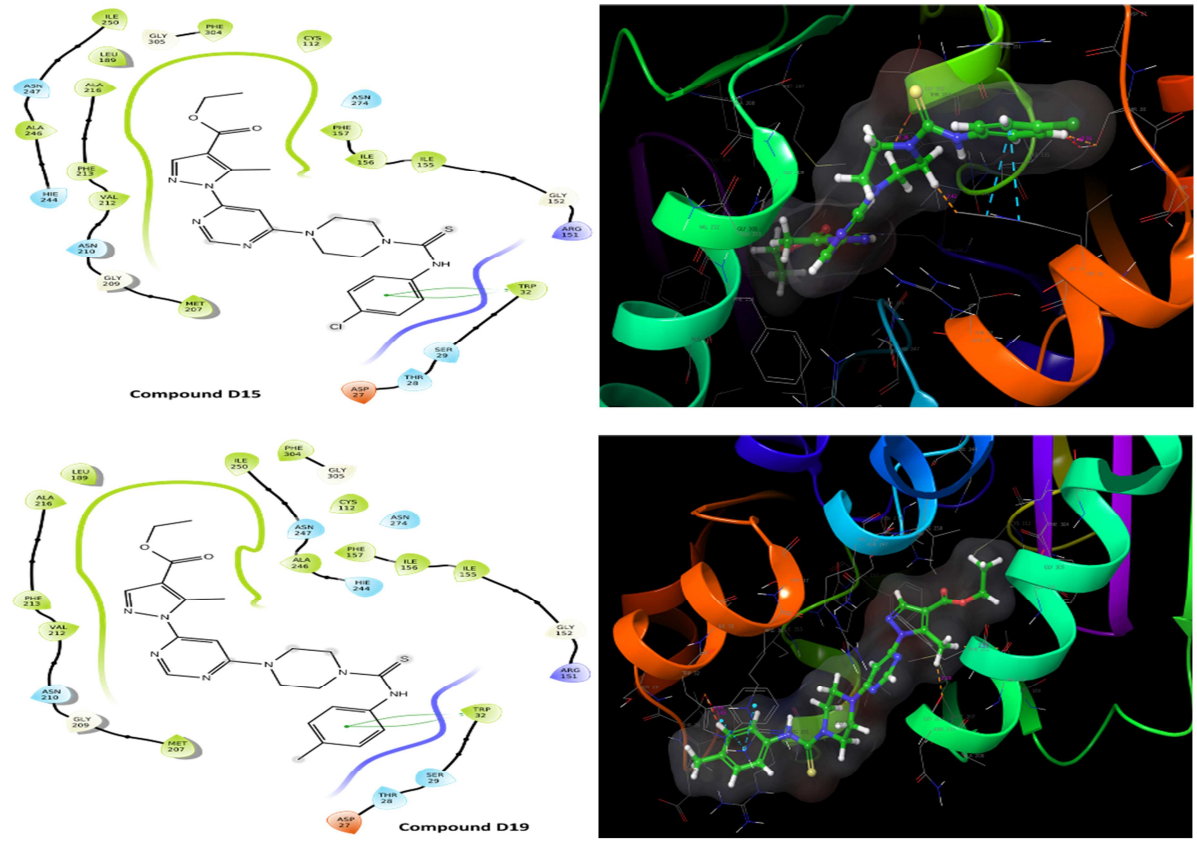


Figure 4

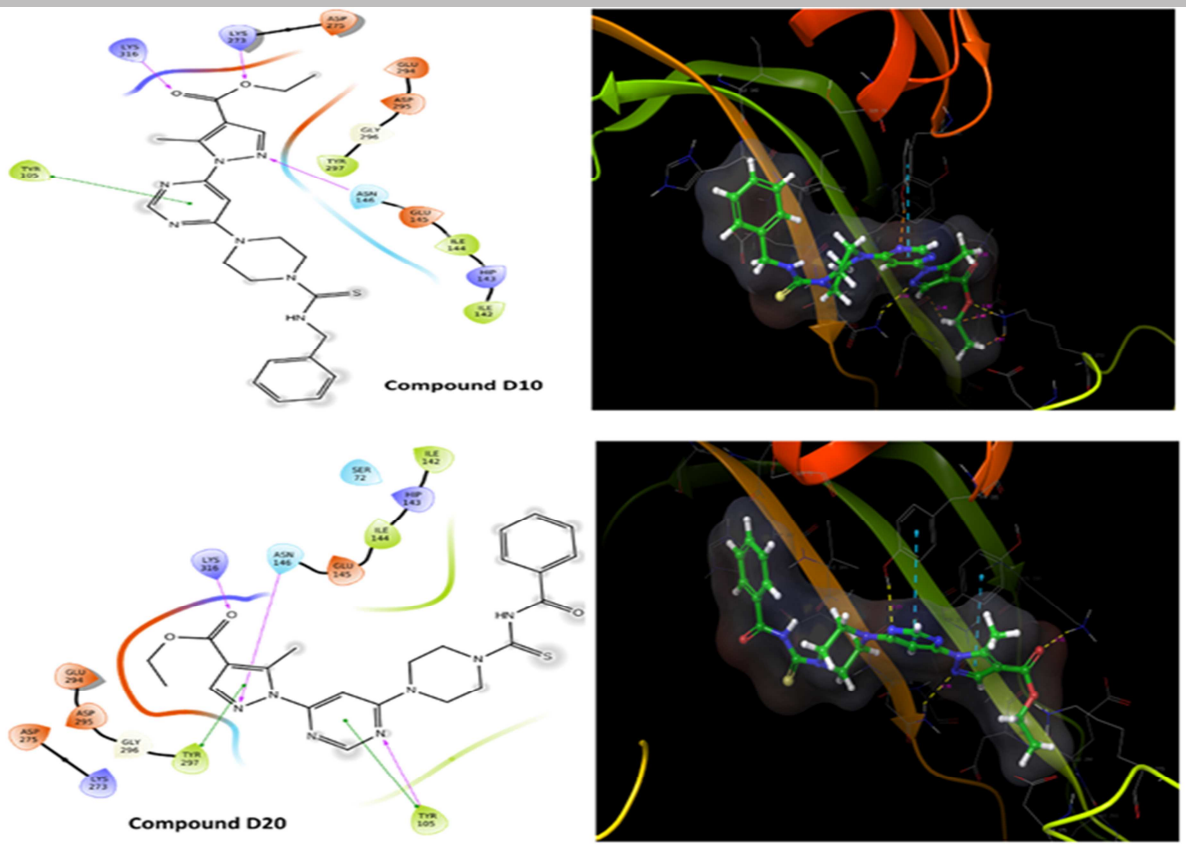


Figure 5

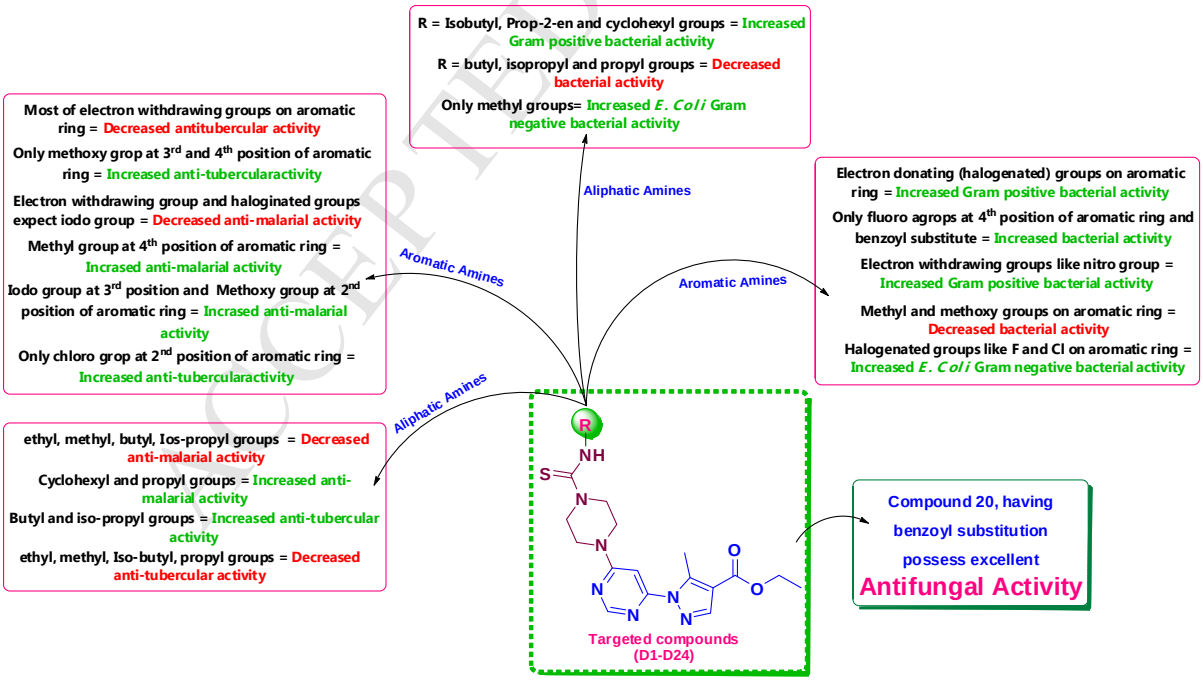


Figure 6

Highlights

- Synthesis and Characterization of novel *N*-thioamides of Pyrazolylpyrimidine based Piperazine.
- Molecular docking study of potent compounds against responsible target proteins using Glide tool in SCHRODINGER.
- Antimicrobial evaluation for antibacterial, antifungal, antimalarial and antitubercular efficacy.