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SYNTHESIS, ANTIMICROBIAL SCREENING AND MOLECULAR DOCKING STUDY OF SOME NEW PYRIMIDIN-2-AMINE DERIVATIVES AS POSSIBLE BIOACTIVE AGENTS

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ABSTRACT

A novel series of 4, 6-disubstituted pyrimidin-2-amine derivatives **(5a–f)** was synthesized in good yields via a cyclocondensation reaction of chalcone-based intermediates **(4a–f)** with guanidine hydrochloride using sodium methoxide as the base. The key intermediates, *N*'-(5-(3-(2,4-disubstituted phenyl)-3-oxoprop-1-enyl)-4-(4-bromophenyl)-6-(3,4-disubstituted phenyl) pyrimidin-2-yl)-*N*, *N*-dimethylformamidine derivatives **(4a–f),** were prepared from aryl and heteroaryl acetophenones **(3a–c)** and 4,6-diaryl-substituted pyrimidine-5-carboxaldehydes **(2a–b)** via Claisen–Schmidt condensation. The structures of the synthesized compounds were confirmed through spectroscopic techniques, including IR, ^{1}HNMR, ^{13}CNMR, mass spectrometry, and elemental analysis. The synthesized compounds were evaluated for their *in vitro* antibacterial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). Notably, compounds exhibited significant inhibitory activity against *E. coli*, with compound **5a** demonstrating superior performance, whereas moderate to poor activity was observed against *S. aureus*. Furthermore, molecular docking studies were conducted using the *E. coli* DNA gyrase B protein (PDB ID: 1KZN) to investigate the binding affinities of the ligands. Compounds **5a** and **5b** showed the highest docking scores and favourable binding interactions at the active site, correlating well with their biological activity. These results suggest that pyrimidin-2-amine derivatives, particularly **5a** and **5b**, represent promising candidates for the development of potent antibacterial agents targeting Gram-negative pathogens.

Keywords: Pyrimidin-2-amine, Guanidine Hydrochloride, Prop-2-en-1-one, Acetophenone, Pyrimidin-5-Carboxaldehyde.

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INTRODUCTION

Since the discovery of penicillin, antibiotics have revolutionized modern medicine, significantly reducing morbidity and mortality associated with bacterial infections.[1] However, the widespread and often indiscriminate use of these agents has accelerated the development of antibiotic-resistant bacterial strains, giving rise to multidrug-resistant infections. This growing resistance threatens to undermine decades of medical progress, as resistance now exists to nearly all known classes of antibiotics.[2] The diminishing time gap between antibiotic discovery and the emergence of resistance underscores the urgent need for novel antimicrobial agents. Without the development of new and effective antibiotics, the anticipated post-antibiotic era could render routine infections increasingly difficult to treat.[3-4] In this context, heterocyclic compounds, particularly Pyrimidines, have gained attention due to their structural versatility and broad biological relevance. Pyrimidine scaffolds are integral to many biologically active molecules, including pharmaceuticals, antibiotics, agrochemicals, and diagnostic agents, making them promising candidates in the search for next-generation antimicrobial therapeutics.[5-6] Pyrimidines represent a fundamental class of nitrogen-containing heterocycles ubiquitous in living organisms, due to their structural stability, synthetic accessibility, and the diverse pharmacological profiles of their derivatives; hence have garnered significant attention in medicinal chemistry due to their broad biological activities and versatile chemical properties. The pyrimidine ring is capable of forming efficient hydrogen bonds and serves as a bioisostere for phenyl and other aromatic π-systems,[7] enabling interaction with a wide array of biological targets, which often enhances both pharmacokinetic and pharmacodynamic properties of drug molecules.[8] Characterized by an

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electron-rich six-membered ring containing two nitrogen atoms, pyrimidine derivatives are integral components of nucleobases and are also found in various antibiotics (Fig.-1).

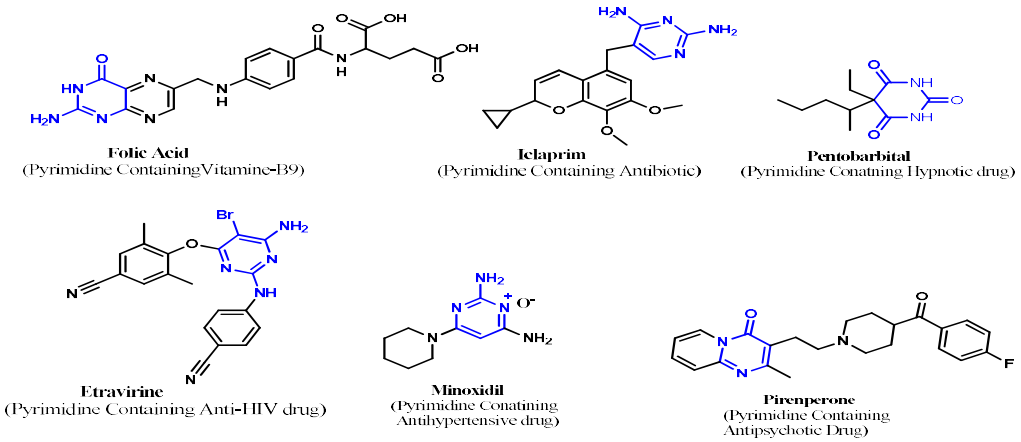


Fig.-1: Clinically Used Drugs with Pyrimidine as an Integral Component

Substituted pyrimidines also constitute an important category of *N*-heterocycles widely explored across therapeutic domains such as antimicrobial[9-10], anticancer[11-14], anti-HIV[15-16], and antiviral[17-18] treatments. In addition, their roles in the development of antitumor[19-20], anti-tubercular[21], anti-inflammatory[22-23], and antioxidant agents [24] further emphasize their prominence in modern drug discovery efforts. The adaptability of the pyrimidine scaffold allows for the incorporation of multiple pharmacophores, making it a valuable core structure in the design of novel therapeutic agents. Among the various pyrimidine derivatives, pyrimidin-2-amines are of considerable interest and are frequently synthesized via cyclization reactions involving chalcones and guanidine salts, such as guanidine hydrochloride or guanidine nitrate, under acidic or basic conditions.[25] Several synthetic approaches have been reported, including conventional reflux methods, microwave irradiation[26], ultrasonication[27], and phase-transfer catalysis.[28] These reactions have been carried out in diverse media such as NaOH[29], piperidine, sodium hydride[30], ethanol, methanol, 1,4-dioxane, DMF, and PEG-400.[31] Additionally, the cycloaddition of chalcones with amidines has proven to be a viable and efficient route for the synthesis of pyrimidines.[32-33] In light of the pharmacological importance of pyrimidine-based compounds, the present study focuses on the synthesis, molecular docking, and *in vitro* biological evaluation of a series of novel 4, 6-disubstituted pyrimidin-2-amines. Initially, 4, 6-diaryl-substituted pyrimidin-5-carboxaldehydes **(2a–b)** were synthesized via Vilsmeier–Haack formylation, followed by cyclocondensation with chalcone derivatives **(4a–f)** using guanidine hydrochloride and sodium methoxide as a base. This approach yielded six new compounds **(5a–f),** which are currently being evaluated for their potential therapeutic activities. The findings from this investigation may pave the way for the development of novel drug candidates targeting various disease conditions.

EXPERIMENTAL

Materials and Methods

All the chemicals used were from Himedia, Aldrich, Merck, and Loba. TLC aluminum sheet silica gel 60F254 was used to monitor the reactions, and a UV chamber was used to observe them. Purity of the product was also checked by using the thin-layer chromatographic technique. The open capillary technique was used to obtain the melting points, which are uncorrected. Using KBr discs, IR spectra were captured using a Shimadzu FT-IR spectrophotometer. PMR and 13C NMR spectra were recorded at 500 MHz using $CDCl_3$ solvent. Chemical shift values were mentioned in ppm, and tetramethylsilane (TMS) was used as an internal standard.

General Procedure for Synthesis of [4-(3,4-disubstituted phenyl)-2-formamidinyl-6-(4-substituted phenyl] pyrimidine-5-carboxaldehydes (2a-b)

Vilsmeier-Haack formylation: $POCl_3$ (1.1 mole eq.) was added dropwise in a precooled anhydrous DMF (1.1 mole eq.) at -5°C. In that viscous reaction mass, a solution of 4,6-diaryl-substituted pyrimidin-2-amine **(1)** in DMF was added slowly with stirring. The reaction mixture was kept stirring for 1 hour at room temperature and then heated at 80°C for 2 hours. Reaction completion was checked on TLC. Following

completion, the reaction mixture was cooled on ice, the resultant solid was subjected to filtration and subsequently vacuum-dried **(2a-b)**[33], and pure aldehyde was acquired.

Spectral Data

***N′*-(4-(4-bromophenyl)-6-(3,4-dimethoxyphenyl)-5-formylpyrimidin-2-yl)-*N, N*-dimethylformamidine (2a)**

M. P.: 195°C, Yield: 69%, FT-IR (KBr cm^{-1}) 1680 (aldehydic C=O stret.), 2834 (aldehydic C-H stret.), 1583 (C=N stret.), 1140 (C-O-C stret.), 1071 (sym O-CH_3 stret.) and 1259 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 3.1 (s, 6H, -CH_3), 8.48 (s, 1H, imine), 3.04 (s, 6H, -O$\underline{C}H_3$), 10.4(s, 1H, aldehyde -H), 6.94-7.87 (m, 7H, aromatic), LCM(*m/z)*: 470 $[M+2]^+$, 491 $[M+Na]^+$, 492 $[M+Na+H]^+$, Elemental Analytical calculations for $C_{22}H_{21}BrN_4O_3$; calculated: C, 56.30; H, 4.51; N, 11.94; Found: C, 56.24; H, 4.48; N, 11.91.

***N′*-(4-(4-bromophenyl)-5-formyl-6-(4-methoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (2b)**

M. P.: 210°C, Yield: 74%, FT-IR (KBr cm^{-1}) 1701 (aldehydic C=O stret.), 2845 (aldehydic C-H stret.), 1594 (C=N stret.), 1143 (C-O-C stret.), 1080 (sym O-CH_3 stret.) and 1256 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 3.1 (s, 6H, -CH_3), 8.48 (s, 1H, imine), 3.01 (s, 3H, -O$\underline{C}H_3$), 10.35 (s, 1H, aldehyde -H), 6.92-7.87 (m, 8H, aromatic), LCM(*m/z)*: 440 $[M+2]^+$, 461 $[M+Na]^+$, 462 $[M+Na+H]^+$, Elemental calculations for $C_{21}H_{19}BrN_4O_2$: calculated: C, 57.41; H, 4.36; N, 12.75; Found: C, 57.34; H, 4.31; N, 12.62

General Procedure for Synthesis of 1-aryl-3- {[6-(4-substituted phenyl)4-(3,4-disubstituted phenyl)2-formamidinyl] pyrimidinyl} prop-2-en-1-ones (4a-f)

Aromatic substituted pyrimidin-5-carboxaldehyde (1 mole eq.) **(2a-b)** and aromatic acetophenone (1 mole eq.) **(3a-c)** were taken in a round-bottom flask. 40% aq. NaOH was added dropwise to the pre-cooled mixture of aldehyde and acetophenone by keeping the temperature between 10-15°C. The reaction mixture was kept stirring and monitored by TLC for its completion. Following reaction completion after 14 hours, the reaction mixture was poured over crushed ice, and the solid was separated, filtered, cleaned with water, dried, and recrystallized using ethanol to produce **(4a-f).**

General Procedure for Synthesis of 1-aryl-3- {[6-(4-substituted phenyl-4-(3,4-disubstituted phenyl)-2-formamidinyl] pyrimidinyl} pyrimidin-2-amines (5a-f)

Guanidine hydrochloride (3 mole eq.) and sodium methoxide (1 mole eq.) were taken in 5ml methanol in RBF and kept stirring for 10 min at room temperature. After this, **4a** (1 mole eq.) was added to this reaction mixture, and it was refluxed for 14 hours. The progress of the reaction was monitored on TLC. To get pure pyrimidin-2-amines **5a,** the reaction mixture was cooled to room temperature and then poured over crushed ice. The solid was subsequently filtered, dried, and recrystallized using ethanol. Similarly, other derivatives were synthesized using the same process to obtain **(5b-f).**

Spectral Data

***N*'-(5-(3-(benzofuran-2-yl)-3-oxoprop-1-enyl)-4-(4-bromophenyl)-6-(3,4-dimethoxyphenyl)pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4a)**

M. P. 166°C, Yield: 60%, FT-IR (KBr cm^{-1}) 1651 (conjug C=O stret.), 1563 (conjug C=C stret.), 1614 (aromatic C=C stret.), 1678 (C=N stret.), 1135 (aromatic C-O-C stret.), 3484 (alkene C-H stret.), 1022 (sym O-CH_3 stret.), 1216 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.87 (d, 1H, -CH), 6.83 (d, 1H, -CH ene), 8.47 (s, 1H, imine), 2.59 (s, 6H, -CH_3), 7.85 (s, 1H, benzofuran), 7.01-8.02 (m, 11H), 3.84 (s, 3H), 3.47 (s, 3H). LCM(*m/z),*688 $[M+2]^+$, 709 $[M+Na]^+$, 710 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{32}H_{27}BrN_4O_4$; calculated: C, 62.85; H, 4.45; N, 9.16; Found: C, 62.80; H, 4.44; N, 9.12.

***N′*-(4-(4-bromophenyl)-5-(3-(4-bromophenyl)-3-oxoprop-1-enyl)-6-(3,4-dimethoxyphenyl)pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4b)**

M. P. 172°C, Yield: 75%, FT-IR (KBr cm^{-1}) 1651 (conjug C=O stret.), 1563 (conjug C=C stret/), 1614 (aromatic C=C stret.), 1678 (C=N stret.), 3484 (alkene C-H stret.), 1022 (sym O-CH_3 stret.), 1216 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.91 (d, 1H, -CH), 6.72 (d, 1H, -CH ene), 8.47 (s, 1H, imine), 2.57 (s, 6H, -CH_3), 3.27 (s, 3H), 3.49 (s, 3H), 6.99-8.01 (m, 11H). LCM(*m/z),*602 $[M+2]^+$, 623 $[M+Na]^+$, 624 $[M+Na+H]^+$ Elemental Anal. Calcd. $C_{30}H_{26}Br_2N_4O_3$; calculated: C, 55.40; H, 4.03; N, 8.61; Found C, 55.35; H, 4.02; N, 8.58.

***N'*-(4-(4-bromophenyl)-6-(3,4-dimethoxyphenyl)-5-(3-(2-hydroxy-4-methylphenyl)-3-oxoprop-1-enyl)pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4c)**

M. P. 181°C, Yield: 59%, FT-IR (KBr cm^{-1}) 1651 (conjug C=O stret.), 1563 (conjug C=C stret.), 1614 (aromatic C=C stret.), 1681 (C=N stret.), 1009 (phenolic C-O stret), 3364 (O-H stret.), 3487 (alkene C-H stret.), 1023 (sym O-CH_3 stret.), 1217 (asym O-CH_3 stret.), 2933 (methyl C-H stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.91 (d, 1H, -CH), 7.08 (d, 1H, -CH ene), 8.47 (s, 1H, imine), 2.57 (s, 6H, -CH_3), 11.32 (s, 1H, -OH), 2.44 (s, 3H), 3.49 (s, 3H), 3.27 (s, 3H), 6.99-7.97 (m, 11H). LCM(*m/z)*601$[M+1]^+$, 623 $[M+Na]^+$, 624 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{31}H_{29}BrN_4O_4$; calculated: C, 61.90; H, 4.86; N, 9.31; Found: C, 61.92; H, 4.84; N, 9.28.

***N'*-(5-(-3-(benzofuran-2-yl)-3-oxoprop-1-enyl)-4-(4-bromo phenyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4d)**

M. P. 194°C, Yield: 67%, FT-IR (KBr cm^{-1}) 1649 (conjug C=O stret.), 1576 (conjug C=C stret.), 1609 (aromatic C=C stret.), 1671 (C=N stret.), 1105 (aromatic C-O-C stret.), 3472 (alkene C-H stret.), 1023 (sym O-CH_3 stret.), 1217 (asym O-CH_3 stret.) H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.87 (d, 1H, -CH), 6.81 (d, 1H, -CH enol), 8.47 (s, 1H, imine), 2.58 (s, 6H, -CH_3), 7.85 (s, 1H, benzofuran), 7.01-8.48 (m, 12H), 3.88 (s, 3H). ^{13}C NMR ($CDCl_3$ 100MHz) δ ppm: 171.9, 129.7, 144.6, 134.8, 157.8, 165.9, 164.7, 163.5, 161.8, 160.7, 138.4, 137.1, 136.8, 135.5, 129.9, 128.6, 124.9, 123.9, 114.1, 103.2, 55.4, 43.7. LCM(*m/z):* 582 $[M+2]^+$, 603 $[M+Na]^+$, 604 $[M+Na+H]^+$, Elemental Anal. Calcd.$C_{31}H_{25}BrN_4O_3$; calculated: C, 64.03; H, 4.33; N, 9.64; Found: C, 64.01; H, 4.31; N, 9.60.

***N'*-(4-(4-bromophenyl)-5-(-3-(4-bromo phenyl)-3-oxoprop-1-enyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4e)**

M. P. 162°C, Yield: 72%, FT-IR (KBr cm^{-1}) 1651 (conjug C=O stret.), 1562 (conjug C=C stret.), 1609 (aromatic C=C stret.), 1680 (C=N stret.), 3289 (alkene C-H stret.), 1032 (sym O-CH_3 stret.), 1300 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.71 (d, 1H, -CH), 6.46 (d, 1H, -CH ene), 8.47 (s, 1H, imine), 2.59 (s, 6H), 3.24 (s, 3H), 7.02-8.01 (m, 12H). LCM(*m/z):* 620 $[M+2]^+$, 641 $[M+Na]^+$, 642 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{29}H_{24}Br_2N_4O_2$; calculated: C, 56.15; H, 3.90; N, 9.03; Found: C, 56.12; H, 3.88; N, 9.01.

***N'*-(4-(4-bromo phenyl)-5-(-3-(2-hydroxy-4-methyl phenyl)-3-oxoprop-1-enyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (4f)**

M. P. 188°C, Yield: 70%, FT-IR (KBr cm^{-1}) 1649 (conjug C=O stret.), 1576 (conjug C=C stret.), 1609 (aromatic C=C stret.), 1682 (C=N stret.), 1008 (phenolic C-O stret.), 3289 (O-H stret.), 3282 (alkene C-H stret.), 1032 (sym O-CH_3 stret.), 1359 (asym O-CH_3 stret.), 2833 (methyl C-H stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 7.71 (d, 1H, -CH), 6.50 (d, 1H, -CH ene), 8.47 (s, 1H, imine), 2.59 (s, 6H, -CH_3), 3.88 (s, 3H, -CH_3), 11.34 (s, 1H, -OH), 2.34 (s, 3H, -CH_3), 3.24 (s, 3H, -CH_3), 6.99-8.01 (m, 11H). LCM(*m/z):* 572 $[M+2]^+$, 593 $[M+Na]^+$, 594 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{30}H_{27}BrN_4O_3$; calculated: C, 63.05; H, 4.76; N, 9.80; Found: C, 63.01; H, 4.71; N, 9.78.

***N'*-(5-(2-amino-6-(benzofuran-2-yl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(3,4-dimethoxyphenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5a)**

M. P. 137°C, Yield: 54%, FT-IR (KBr cm^{-1}) 1671 (C=N stret.), 1614 (aromatic C=C stret.), 1137 (aromatic C-O-C stret.), 2836 (N-H stret.), 1262 (aromatic primary C-N stret.), 1023 (sym O-CH_3 stret.), 1217 (asym O-CH_3 stret). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.71 (s, 2H, -NH_2), 7.42 (s, 1H, pyrimidine ring), 8.48 (s, 1H, imine), 2.66(s, 6H, -CH_3), 7.34 (s, 1H, benzofuran), 7.01-8.02 (m, 11H), 3.89 (s, 3H, -CH_3), 3.42 (s, 3H, -CH_3). LCM(*m/z):* 651 $[M+2]^+$, 672$[M+Na]^+$, 673 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{33}H_{28}BrN_7O_3$ calculated: C, 60.93; H, 4.34; N, 15.07; Found: C, 60.90; H, 4.31; N, 15.02.

***N'*-(5-(2-amino-6-(4-bromophenyl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(3,4-dimethoxyphenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5b)**

M. P. 159°C, Yield: 58%, FT-IR (KBr cm^{-1}) 1680 (C=N stret.), 1640 (aromatic C=C stret.), 2837 (N-H stret.), 1365 (aromatic primary C-N stret.), 1023 (sym O-CH_3 stret.), 1217 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.62 (s, 2H, -NH_2), 7.42 (s, 1H, pyrimidine ring), 8.48 (s, 1H, imine), 2.64 (s, 6H,

-CH_3), 7.02-7.94 (m, 11H), 3.64 (s, 3H, -CH_3), 3.35 (s, 3H, -CH_3). LCM(*m/z):* 689 $[M+2]^+$, 691 $[M+2]^+$, 710 $[M+Na]^+$, 711 $[M+Na+H]^+$ Elemental Anal. Calcd. $C_{31}H_{27}Br_2N_7O_2$; calculated: C, 54.01; H, 3.95; N, 14.22; Found: C, 54.03; H, 3.92; N, 14.18.

***N′*-(5-(2-amino-6-(2-hydroxy-4-methylphenyl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(3,4-dimethoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5c)**

M. P. 144°C, Yield: 62%, FT-IR (KBr cm^{-1}) 1678 (C=N stret.), 1615 (aromatic C=C stret.), 2837 (N-H stret.), 1365 (aromatic primary C-N stretching), 1009 (phenolic C-O stretching), 3364 (O-H stretching), 1023 (sym O-CH_3 stret.), 1217 (asym O-CH_3 stret.), 2933 (methyl C-H stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.78 (s, 2H, -NH_2), 7.42 (s, 1H pyrimidine ring), 8.48 (s, 1H, imine), 2.64 (s, 6H, -CH_3), 7.92-7.01 (m, 10H), 2.34 (s, 3H, -CH_3), 3.64 (s, 3H, -CH_3), 3.35 (s, 3H, -CH_3), 11.33 (s, 1H, -OH). LCM(*m/z):* 641 $[M+2]^+$, 662 $[M+Na]^+$, 711 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{32}H_{30}BrN_7O_3$; calculated C, 60.00; H, 4.72; N, 15.31; Found: C, 60.03; H, 4.70; N, 15.28.

***N′*-(5-(2-amino-6-(benzofuran-2-yl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(4-methoxyphenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5d)**

M. P. 172°C, Yield: 58%, FT-IR (KBr cm^{-1}) 1678 (C=N stret.), 1614 (aromatic C=C stret.), 1068 (aromatic C-O-C stret.), 2836 (N-H stret.), 1364 (aromatic primary C-N stret.), 1023 (sym O-CH_3 stret.), 1216 (asym O-CH_3 stret.) H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.76 (s, 2H, -NH_2), 7.38 (s, 1H, pyrimidine ring), 3.22 (s, 6H, -CH_3), 7.34 (s, 1H, benzofuran), 8.48 (s, 1H, imine), 7.01- 8.04 (m, 12H). ^{13}C NMR ($CDCl_3$ 500MHz) δ ppm: 167.11, 161.79, 164.71, 124.90, 133.54, 161.79, 165.92, 163.53, 47.55, 131.93, 136.77, 128,65, 141.70, 114.16, 165.38, 55.44, 164.04, 103.17, 131.93, 126.89, 129.91, 110.18, 164.09. LCM(*m/z):* 621 $[M+2]^+$, 642 $[M+Na]^+$, 643 $[M+Na+H]^+$, Elemental Anal. Calcd. $C_{32}H_{26}BrN_7O_2$; calculated C, 61.94; H, 4.22; N, 15.80. Found: C, 61.90; H, 4.20; N, 15.75.

***N′*-(5-(2-amino-6-(4-bromophenyl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(4-methoxyphenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5e)**

M. P. 148°C, Yield: 61%, FT-IR (KBr cm^{-1}) 1678 (C=N stret.), 1614 (aromatic C=C stret.), 2836 (N-H stret.), 1262 (aromatic primary C-N stret.), 1068 (sym O-CH_3 stret.), 1216 (asym O-CH_3 stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.64 (s, 2H, NH_2), 7.41 (s, 1H, pyrimidine ring), 8.48 (s, 1H, imine), 2.71 (s, 6H, -CH_3), 7.92-7.10 (m, 12H), 3.31 (s, 3H, -CH_3). LCM(*m/z):* 659 $[M+2]^+$, 661 $[M+4]^+$ 680 $[M+Na]^+$, 681 $[M+Na+H]^+$ Elemental Anal. Calcd. $C_{30}H_{25}Br_2N_7O$; calculated C, 54.65; H, 3.82; N,14.87; Found: C, 54.62; H, 3.80; N,14.85

***N′*-(5-(2-amino-6-(2-hydroxy-4-methylphenyl)pyrimidin-4-yl)-4-(4-bromophenyl)-6-(4-methoxy phenyl) pyrimidin-2-yl)-*N, N*-dimethyl formamidine (5f)**

M. P. 153°C, Yield: 51%, FT-IR (KBr cm^{-1}) 1680 (C=N stret.), 1614 (aromatic C=C stret.), 2836 (N-H stret.), 1262 (aromatic primary C-N stret.), 1008 (phenolic C-O stret.), 3364 (O-H stret.), 1022 (sym O-CH_3 stret.), 1216 (asym O-CH_3 stret.), 2933 (methyl C-H stret.). H^1 NMR ($CDCl_3$ 500MHz) δ ppm: 5.72 (s, 2H, -NH_2), 7.34(s, 1H, pyrimidine ring), 8.48 (s, 1H, imine), 2.71 (s, 3H, -CH_3), 3.31 (s, 3H, -CH_3), 2.37 (s, 3H, -CH_3), 11.37 (s, 1H, -OH). LCM (*m/z):* 611 $[M+2]^+$, 632 $[M+Na]^+$, 633 $[M+Na+H]^+$ Elemental Anal. Calcd. $C_{31}H_{28}BrN_7O_2$ calculated C, 60.99; H, 4.62; N, 16.06; Found: C, 60.95; H, 4.58; N, 16.10.

Antibacterial Screening

The antibacterial activity of the newly synthesized pyrimidin-2-amine derivatives **(5a–f)** was assessed using the cup plate diffusion method. The evaluation was conducted against two bacterial strains: *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Fresh bacterial cultures were prepared by incubating each strain for 18 hours at 37°C in the nutrient broth. Following incubation, 10 µL of the bacterial suspension was added to 10 mL of sterile nutrient agar maintained at 40°C. The mixture was then poured into sterile Petri dishes and allowed to solidify. Sterile paper discs were placed onto the surface of the solidified agar plates. Stock solutions of the synthesized compounds were prepared by dissolving 1 mg of each compound in ethanol. From these, working solutions were prepared at concentrations of 25, 50, and 100 µg/µL. Each concentration was carefully pipetted onto the discs (in triplicate) and the plates were incubated at 36°C for 18 hours. After incubation, the antibacterial activity was evaluated by measuring the zone of inhibition (in

millimeters) surrounding each disc. The diameter of the inhibition zone indicated the efficacy of the compounds against the tested bacteria. Results for each compound at varying concentrations are presented in Table-1.

Table-1: Antibacterial Screening of Pyrimidin-2-Amine Derivative (5a-f)

Compd. Code	Zone of inhibition in (mm)					
	E. coli (Gram-ve)			*S. aureus* (Gram + ve)		
	MIC Conc. (µg/mL)			MIC Conc. (µg/mL)		
	25µl	50 µl	100 µl	25µl	50 µl	100 µl
5a	15	17	15	09	10	14
5b	13	18	19	08	05	13
5c	17	16	17	10	09	12
5d	19	18	18	09	03	13
5e	16	17	18	07	06	14
5f	20	19	19	06	07	10
DMSO	-	-	-	-	-	-
Std. Drug Chloramphenicol	20	23	25	12	15	20

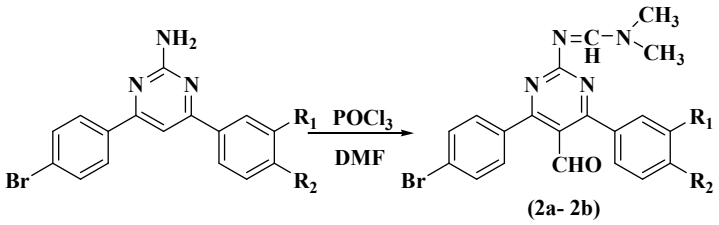
Molecular Docking Study

To gain insight into the possible molecular basis of the observed antibacterial activity, molecular docking studies were carried out for the synthesized pyrimidin-2-amine derivatives (**5a–f)** using AutoDock Tools 1.5.7. The docking protocol was designed to investigate the binding affinity of the ligands toward the *E. coli* DNA gyrase subunit B enzyme, a validated antibacterial target. The crystal structure of the target protein (*E. coli* DNA gyrase subunit B; PDB ID: 1KZN) was retrieved from the Protein Data Bank. The protein was prepared for docking by removing all water molecules and heteroatoms. Polar and aromatic hydrogen atoms were added, and Gasteiger partial charges were assigned using AutoDock Tools 1.5.7 following standard protocols. [35–37] The prepared structure was saved in PDBQT format for docking. The 2D structures of the synthesized compounds were initially sketched and then converted into energy-minimized 3D structures in PDB format using Open Babel GUI. The ligand structures were also prepared in PDBQT format by assigning torsion angles to remain fully flexible during the docking process. A grid box was generated around the active site of the protein to encompass the key amino acid residues. Grid coordinates along the X, Y, and Z axes were defined such that the entire active site pocket was covered. Docking simulations were performed using AutoDock 4.2, employing the Lamarckian Genetic Algorithm with default parameters. For each compound, ten docked conformations were generated, and the binding energy (kcal/mol) of the most favourable pose was recorded. The docking results were visualized and analyzed using Discovery Studio Visualizer 4.5, focusing on key interactions such as hydrogen bonding, π–π stacking, and hydrophobic interactions between the ligand and amino acid residues in the active site.

RESULTS AND DISCUSSION

A series of novel 4, 6-diaryl-substituted pyrimidin-2-amines **(5a–f)** were synthesized through a conventional multistep synthetic approach, as outlined in **Schemes-1** and **2**. The synthetic route commenced with the Vilsmeier–Haack formylation of appropriately substituted acetophenones, leading to the formation of pyrimidine-5-carboxaldehydes **(2a–b).** These intermediates were then subjected to Claisen–Schmidt condensation with aryl and heteroaryl ketones **(3a–c)** to afford α, β-unsaturated carbonyl derivatives **(4a–f).** Subsequent cyclocondensation with guanidine hydrochloride in the presence of sodium methoxide yielded the target pyrimidin-2-amine derivatives **(5a–f).** The structures of the synthesized compounds were confirmed through IR, ^{1}HNMR, ^{13}CNMR, and mass spectrometric analysis. The IR spectra of the final compounds **(5a–f)** exhibited a characteristic absorption band in the range of 1365–1262 cm^{-1}**,** corresponding to the C–N stretching vibration of an aromatic primary amine. The absence of a C=O stretching band typically found in α, β-unsaturated carbonyl compounds (1651–1649 cm^{1}) indicated successful cyclization to the pyrimidine core. The N–H stretching vibration appeared in the range of 2837–2836 cm^{1}, confirming the presence of the –NH_2 functionality on the pyrimidine ring. The ^{1}HNMR spectrum of compound **5d** is representative of the series. A singlet at δ 5.76 ppm confirmed the presence of two

protons of the –NH_2 group attached to the C_2 position of the pyrimidine ring. A distinct singlet at δ 7.38 ppm was assigned to the C_5 proton of the pyrimidine core. Aromatic protons appeared as multiplets in the range of δ 7.01–8.04 ppm, consistent with the substituted aryl rings. A singlet at δ 7.34 ppm confirmed the presence of a proton at the third position of the benzofuran ring. A downfield singlet at δ 8.48 ppm was attributed to the methine proton flanked by two nitrogen atoms within the pyrimidine moiety. Additionally, a singlet at δ 3.88 ppm corresponded to three protons of the methoxy substituent, while a singlet at δ 3.22 ppm accounted for six protons of the dimethylformamidine moiety.



Scheme-1

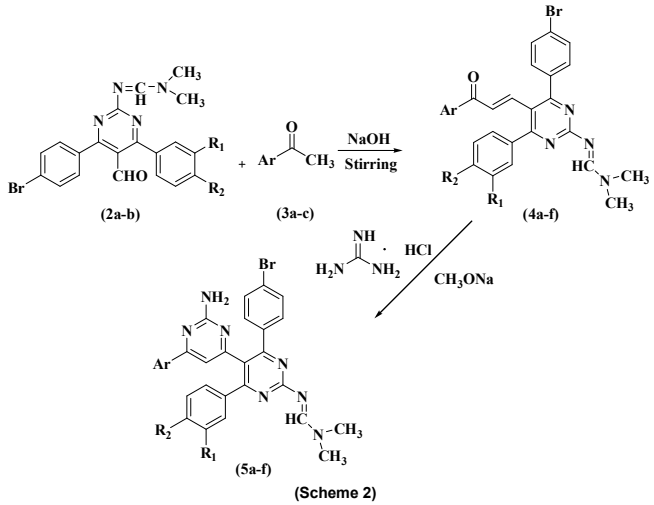


Fig-2: General Reaction Scheme: - Synthesis of Compounds Pyrimidin-2-Amines Derivatives (5a-f)
Ar = benzofuranyl (4a, 5a, 4d, 5d), 4-Br-C_6H_5 (4b, 5b, 4e, 5e) and 2-OH-4-CH_3-C_6H_5 (4c, 5c, 4f, 5f)

Compound Code	R_1	R_2
2a, 4a, 4b, 4c, 5a, 5b, 5c	-OCH_3	-OCH_3
2b, 4d, 4e, 4f, 5d, 5e, 5f	-H	-OCH_3

The ^{13}CNMR spectrum of compound **5d** further supported the structural assignments. The most deshielded signal at δ 167.11 ppm was attributed to the C_2 carbon of the pyrimidine ring. Other notable chemical shifts included δ 164.71 ppm and δ 161.79 ppm for the C_4 and C_6 carbons, respectively. The signal for C_5 appeared at δ 124.90 ppm**,** falling within the expected range for aromatic carbons adjacent to nitrogen atoms. Mass spectral analysis also corroborated the molecular structures of the synthesized compounds. For compound **5d**, a molecular ion peak was observed at m/z 621 [M+2]**,** consistent with the calculated molecular formula $C_{32}H_{26}N_7BrO_2$**.** This was in agreement with the structural transformation from precursor **4d**, which exhibited a molecular ion peak at m/z 582 [M+2]**,** corresponding to the formula $C_{31}H_{25}N_4BrO_3$. The increase in molecular weight further validated the successful cyclization and introduction of the guanidine moiety. Collectively, the spectroscopic data provided strong evidence for the successful synthesis and structural elucidation of the target pyrimidin-2-amine derivatives **(5a–f)**, thereby supporting their potential biological evaluation and docking studies.

Antibacterial Screening

The antibacterial efficacy of the synthesized pyrimidin-2-amine derivatives **(5a–f)** was evaluated *in vitro* using the cup-plate method against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacterial strains. The compounds were tested at concentrations of 25, 50, and 100 μg/μL, and the zone of inhibition (in mm)

was recorded after incubation at 37 °C for 18 hours. The antibacterial results are summarized in **Table-1**. The results indicated that the synthesized compounds exhibited moderate to excellent inhibitory activity against *E. coli*. Among them, compounds **5a** and **5b** demonstrated the highest antibacterial activity, producing the largest zones of inhibition at all tested concentrations. This enhanced activity may be attributed to the presence of electron-donating or electron-withdrawing substituents on the aromatic rings, which can influence the interaction with bacterial targets. In contrast, all compounds displayed weak to negligible activity against *S. aureus*. The reduced efficacy against this Gram-positive strain could be due to differences in the bacterial cell wall structure, permeability, or efflux mechanisms that limit the penetration or retention of the compounds. These findings suggest that the synthesized pyrimidin-2-amines possess selective antibacterial potential, particularly against Gram-negative strains, supporting their further exploration as potential antimicrobial agents.

Interaction of Compounds Against X-ray Crystal Structure of DNA Gyrase Enzyme PDB (1KZN)
Amino acid residues of the active pocket were VAL43, ASN46, ASP73ILE59, ASP73, ALA47, VAL71ILE90, MET91, GLN72, ILE78, MET166, VAL120VAL167, THR165, ASP49, GLU50, PRO79, HIS95, VAL43, ILE90, ALA96, VAL118, GLY119, MET166, ASP49, VAL67, VAL71, ILE78, MET95, ALA47, VAL67, ARG136. The dock calculations and the hydrogen bond interactions are shown in Table-2.

Table-2: Docking Studies of Pyrimidin-2-Amine Derivative (5a-f) against *E. coli* PDF Enzyme

Comp. Code	Binding energy (kcal/mol)	Ligand Efficiency	H-bonds Residue-Amino Acid (H-A Distance A^0)	Hydrophobic interaction Residue-Amino Acid (Distance A^0)
5a	-9.06	-0.24	ASN 46 (3.38)	ASN46(3.31), ASP49(3.99)
5b	-8.52	-0.22	GL42(3.02)	VAL118(3.47), VAL118(3.55)
5c	-13	-0.29	ARG136(1.98), (ARG136(3.14)	VAL43(3.74), ASN46(3.71), ALA47(3.55), GLU50(3.25), VAL71(3.71), ILE78(3.14), VAL 167(3.51), ILE78(3.61)
5d	-9.09	-0.25	ASN46(3.33), ASP 49(2.20)	
5e	-8.36	-0.23	ASN46(3.11), ASN46(2.57), GLY117(1.85)	ASP45(3.34), ILE90(3.96), HIS95(3.96)
5f	-9.17	-0.24	VAL118(3.27)	HIS38(3.93), VAL118(3.04)

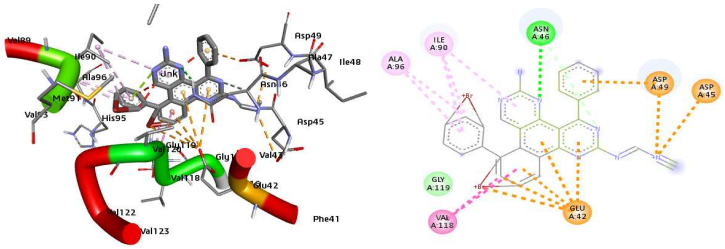


Fig.-3: Protein-Ligand Interaction 3D and 2D Structure of 5a

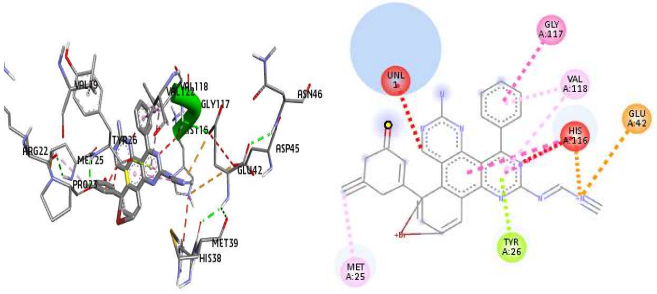


Fig.-4: Protein-Ligand Interaction 3D and 2D Structure of 5b

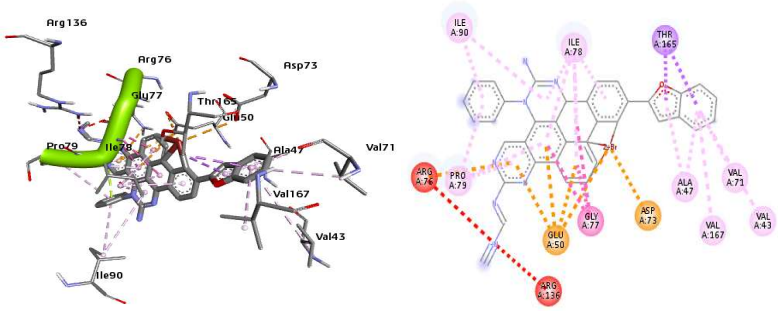


Fig.-5: Protein-ligand Interaction 3D and 2D Structure of 5c

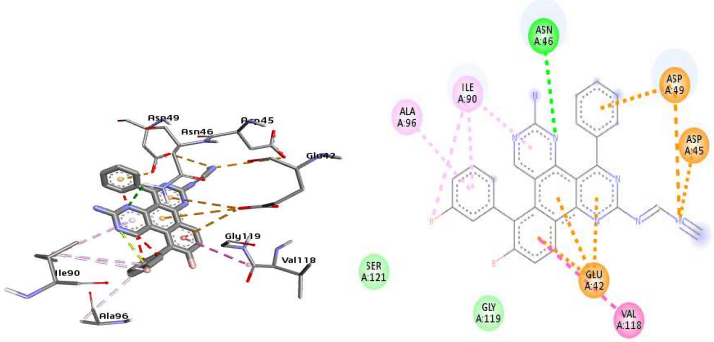


Fig.-6: Protein-Ligand Interaction 3D and 2D structure of 5d

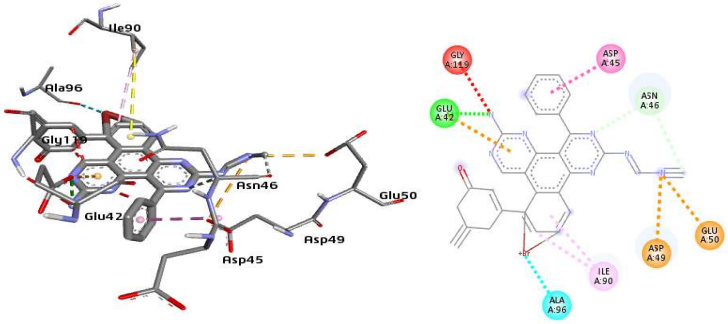


Fig.-7: Protein-Ligand Interaction 3D and 2D Structure of 5e

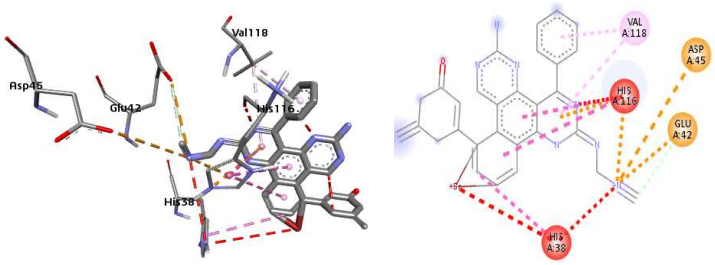


Fig.-8: Protein-Ligand Interaction 3D and 2D Structure of 5f

CONCLUSION

In conclusion, a novel series of 4,6-diaryl-substituted pyrimidin-2-amines **(5a–f)** was successfully synthesized through a multistep synthetic route involving Vilsmeier–Haack formylation, Claisen–Schmidt condensation, and cyclocondensation with guanidine hydrochloride. The structures of the synthesized compounds were confirmed through comprehensive spectroscopic techniques, including IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The *in vitro* antibacterial screening revealed that several derivatives, particularly **5a** and **5b**, exhibited moderate to significant inhibitory activity against *E. coli*, while limited activity was observed against *S. aureus*. These results indicate a degree of selectivity towards Gram-negative bacterial strains. Molecular docking studies further supported the biological findings, suggesting

that the synthesized compounds exert their antibacterial effect through strong binding interactions with the active site of the *E. coli* DNA gyrase enzyme (PDB ID: 1KZN). The observed binding affinities and docking scores provide mechanistic insights into the antimicrobial potential of these compounds. Overall, the study highlights the potential of pyrimidin-2-amine derivatives as promising candidates for the development of new antibacterial agents, particularly against Gram-negative pathogens.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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