RESEARCH PAPER

Novel Pyrimidine-5-Carbonitrile: Synthesis, Docking, in-Silico Prediction, and Evaluation for Antibacterial Activity

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1. INTRODUCTION :

In chemotherapeutic treatment, antimicrobial resistance is a threat globally, so the discovery of a novel antibacterial agents is a global priority[1]. Pyrimidine, a two-nitrogen heterocycle, is a widely used key building block for the development of many pharmaceutical agents with diverse pharmacophoric modifications. Recently, many researchers focused on the development of Pyrimidine Carbonitrile with various biological potentials, such as Antibacterial[2-5], Anticancer[6-10], Anti-inflammatory[11-12], Anticonvulsant[13], Antiplasmodial[14], Antinociceptive[15], etc. Many methods have been published for the synthesis of pyrimidine-5-carbonitrile, which have been intensively studied in recent decades[16-18]. Hence, building on these findings, we synthesized a pyrimidine-5-carbonitrile **(3)** by reacting acrylate **(1)** with benzimidine hydrochloride **(2)** in the presence of a readily available catalyst, anhydrous K_2CO_3. The resulting pyrimidine-5-carbonitrile was then subjected to derivatization with different heterocyclic amines to give the final derivatives, **4a-h.** This research aims to design and synthesize a series of pyrimidine-5-carbonitriles derivatives as antibacterial agents. Some already reported pyrimidine-5-carbonitrile derivatives with potential antibacterial activity[19-23], showing their zone of inhibition against the bacterial strain *E. coli,* along with the newly discovered ones, are shown in **Figure 1**.

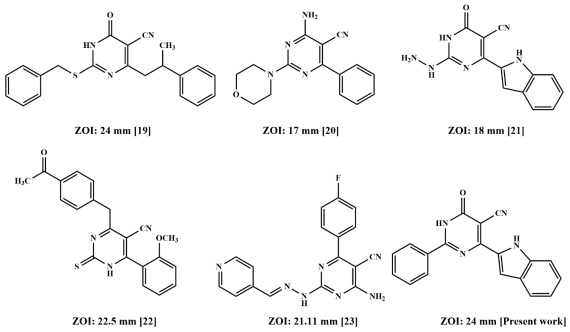


Figure 1. Reported pyrimidine-5-carbonitrile compounds showing promising antibacterial activity against *E. coli.*

In this study, we designed and synthesized novel target compound; Pyrimidine-5-carbonitrile derivatives **(4a-h)**, demonstrating their significant antibacterial potential. The compounds **(4a-h)** were screened for their in vitro antibacterial efficacy against the Gram+ve and Gram-ve bacteria. Additionally, docking & *in silico* ADMET prediction were also evaluated for all the synthesized compounds **(4a-h)**.

2. MATERIAL AND METHODS

2.1 General

Chemicals were obtained from BLD Chemicals Ltd, Sigma-Aldrich and Merck. Synthesized compounds were characterized and confirmed by spectral analysis like FTIR, NMR (^{1}H and ^{13}C), and Mass spectroscopy.

2.2 Instrumentation

The purity of all synthesized derivatives was verified by using thin-layer chromatography on a silica gel-percolated aluminium plate (Silica Gel 60 $F_{254,}$ Merck), and UV light was used for visualization of this spots.

OptiMelt automated melting point system were used for the checking of melting points (Stanford Research System) and was uncorrected. Solvents were evaporated using a rotary evaporator (Heidolph). A Shimadzu IR-435 spectrophotometer (KBr, cm^{-1}) were used to record an FTIR spectra. A LC-MS Q- ToF Micro Water spectrophotometer was used to record exact mass of the synthesized compounds. FT NMR Spectrophotometer Cryo-magnet 500 MHz (Bruker) used to record the ^{1}H and ^{13}C NMR spectra, using tetramethylsilane as standard.

2.2.1 Synthesis

2.2.1.1 Synthesis of 4-(methylthio)-6-oxo-2-phenyl-1, 6-dihydropyrimidine-5-carbonitrile (3): An equimolar mixture of ethyl-2-cyno-3, 3-bis (methylthio) acrylate **1** (10 mmol) and benzimidine hydrochloride **2** (10 mmol) in a DMF as solvent (10 ml) in the presence of a catalytic amount of anhydrous K_2CO_3 was heated to reflux for 4 h. After completion of the reaction (TLC) the mixture was cooled to room temperature and then poured into the ice cold water, solid obtained was collected by filtration, purified by recrystallization from ethanol to yield the titled compound **3**. The compound **3** found to be turmeric yellow colored; yield: 92%; m.p.: 146 -147 °C; **FTIR** (KBr, cm^{-1}): 2935.66 (Aromatic CH), 2220.07 (Nitrile C=N), 1658.78 (Ketone C=O), 1533.41 (C=N); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 3.48 (s, 3H, SCH_3), 7.5 (m, 5H, Ar-H), 8.22 (S, 1H, NH of pyrimidine); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 08.34, 38.91, 39.42, 45.30, 113.36, 123.16, 128.43, 129.61, 130.49, 131.14, 132.02, 166.90, 181.23; **ESI-MS:** m/z calculated for $C_{16}H_{16}N_4O$ **(M+H)**: 243.14; found: 243.99 (M+1).

2.2.1.2 General Procedure for the synthesis mixture **of 6-oxo-2-phenyl-4-(substituted heteroaryl)-1, 6-dihydropyrimidine-5-carbonitrile derivatives (4a-h):** An equimolar mixture of compound **3** (10 mmol) and substituted heterocyclic amines (10 mmol) in a solution of sodium ethoxide and reflux for 5-24 h. Completion of the reaction was monitored by TLC. After the completion of the reaction, the mixture was cooled to room temperature, and the solid obtained was collected by filtration and purified by recrystallization from ethanol to get the titled compounds **(4a-h)** in good yield.

6-oxo-2-phenyl-4-(piperidine-1-yl)-1, 6-dihydropyrimidine-5-carbonitrile (4a): The compound 4a found to be white solid, Yield: 85%; m.p.: 280–283 °C; **FTIR** (KBr, cm^{-1}): 3398.57 (NH stretching), 2918.30 (CH stretching of aromatic), 2196.92 (CN stretching), 1635.64 (C=O stretching), 1495.99 (CH bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 2.4 (t, 6H, -CH_2), 4.41 (t, 4H, -CH_2), 7.40 (m, 5H, CH=CH), 8.30 (s, 1H,>NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 12.02, 18.64, 39.51, 61.27, 89.83, 97.78, 115.86, 128.17, 130.95, 133.60, 136.31, 161.35, 165.68, 167.80, 171.67, 181.37; **ESI-MS:** m/z calculated for $C_{16}H_{16}N_4O$ (M+H): 281.14; found: 282.01 (M+2); Elemental analysis calculated for $C_{16}H_{16}N_4O$ C, 68.55; H,5.75; 19.99; O, 5.71; Observed C, 68.51; H, 5.70; N, 19.93; O, 5.68

4-morpholino-6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carbonitrile (4b): The compound 4b found to be white solid, Yield: 89%; m.p.: 290–294 °C; **FTIR** (KBr, cm^{-1}): 3398.53 (NH stretching), 2933.67 (CH stretching of aromatic), 2187.80 (CN stretching), 1632.35 (C=O stretching), 1481.01 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 2.5 (t, 2H, -CH_2), 4.01 (t, 2H, -CH_2), 7.10 (m, 5H, CH=CH), 8.36 (s,1H,>NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 13.93, 18.64, 39.51, 61.93, 89.83, 97.78, 115.88, 117.26, 127.98, 128.19, 130.95, 133.65, 161.37, 165.62, 167.88, 181.83; **ESI-MS:** m/z calculated for $C_{15}H_{14}N_4O_2$ (M+H+): 283.12; found: 283.01 (M+1); Elemental analysis calculated for $C_{15}H_{14}N_4O_2$ C, 63.82; H, 5.00: N, 19.85; O, 11.34: Observed C, 63.78; H, 5.01; N, 19.81; O, 11.30.

6-oxo-2-phenyl-4-(piperazin-1-yl)-1,6-dihydropyrimidine-5-carbonitrile (4c): The compound 4c found to be white solid, Yield: 82%; m.p.: 253–255 °C; **FTIR** (KBr, cm^{-1}): 3332.45 (NH stretching), 2955.67 (CH stretching of aromatic), 2166.76 (CN stretching), 1657.78 (C=O stretching), 1488.94 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 1.62 (s, 1H, NH), 2.35 (t, 4H, -CH2), 4.42 (t, 4H, CH_2, 7.41 (m, 5H, CH=CH), 8.33 (s,1H, >NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 18.45, 39.51, 62.42, 89.43, 116.77, 128.19, 130.49, 132.21, 165.73, 167.88, 182.87; **ESI-MS:** m/z calculated for $C_{15}H_{15}N_5O$ (M+H+): 282.13; found: 282.17 (M+1); Elemental analysis calculated for $C_{15}H_{15}N_5O$ C, 64.04; H, 5.37; N, 24.90; O, 5.69; Observed C, 64.01; H, 5.34; N, 24.84; O, 5.65.

4-(4-(2-hydroxyethyl) piperazine-1yl)-6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carbonitrile (4d): The compound 4d found to be white solid, Yield: 94%; m.p.: 250-252 °C; **FTIR** (KBr, cm^{-1}): 3663.64 (OH stretching), 3312 (NH stretching), 2916.09 (CH stretching of aromatic), 2225.21 (CN stretching), 1660.76 (C=O stretching), 1443.31 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 2.42 (t, 4H, CH_2), 3.32 (t, 2H, CH_2), 3.62 (t, 4H, CH_2), 4.32 (t, 2H,

CH_2), 7.65 (m, 5H, CH=CH), 8.40 (s, 1H, >NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 19.56, 39.51, 40.89, 60.34, 89.32, 102.34, 115.76, 127.15, 131.43, 133.92, 134.54, 165.45, 167.76, 183.54; **ESI-MS:** m/z calculated for $C_{17}H_{19}N_5O_2$ (M+H+): 326.16; found: 326.17 (M+1); Elemental analysis calculated for $C_{17}H_{19}N_5O_2$ C, 62.75: H, 5.19: N, 21.52: O, 9.83; Observed C, 62.71; H, 5.82; N, 21.45; O, 9.03

4-(1H-imidazol-1-yl)-6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carbonitrile (4e): The compound 4e found to be white solid, Yield: 88%; m.p.: 278–280 °C; **FTIR** (KBr, cm^{-1}): 3332.45 (NH stretching), 2955.57 (CH stretching of aromatic), 2193.76 (CN stretching), 1655.53 (C=O stretching), 1455.64 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 7.65 (m, 2H, Ar-CH), 7.74 (m, 5H, CH=CH), 7.80 (m, 1H, Ar-CH), 8.38 (s, 1H,>NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 20.13, 39.53, 61.42, 90.87, 98.25, 116.56, 128.11, 130.47, 133.34, 133.32, 160.69, 167.23, 183.57; **ESI-MS:** m/z calculated for $C_{14}H_9N_5O$ (M+H+): 264.08; found: 264.07 (M=1); Elemental analysis calculated for $C_{14}H_9N_5O$ C, 63.87; H, 3.45; N,26.60; O,6.08; Observed C, 63.80; H, 3.41; N, 26.55; O, 6.01

4-(1H-benzo[d]imidazol-1-yl)-6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carbonitrile (4f): The compound 4f found to be white solid, Yield: 87%; m.p.: 274–278 °C; **FTIR** (KBr, cm^{-1}): 3371.35 (NH stretching), 2917.60 (CH stretching of aromatic), 2175.34 (CN stretching), 1642.34 (C=O stretching), 1489.54 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 7.60 (m, 4H, CH=CH), 7.75 (m, 5H, CH=CH),7.95 (m, 1H, Ar-CH), 8.53 (s, 1H, >NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 21.36, 40.52, 60.56, 89.21, 98.29, 114.45, 129.18, 130.95, 132.12, 136.45, 165.65, 167.82, 182.54; **ESI-MS:** m/z calculated for $C_{18}H_{11}N_5O$ (M+H+): 314.10; found: 314.14 (M+1); Elemental analysis calculated for $C_{18}H_{11}N_5O$ C, 69.00; H, 3.54; N, 22.35; O, 5.11; Observed C, 69.01; H, 3.51; N, 22.32; O, 5.10

6-oxo-2-phenyl-4-(p-tolylamino)-1,6-dihydropyrimidine-5-carbonitrile (4g): The compound 4g found to be white solid, Yield: 91%; m.p.: 295–298 °C; **FTIR** (KBr, cm^{-1}): 3357.23 (NH stretching), 2915.67 (CH stretching of aromatic), 2195.13 (CN stretching), 1650.56 (C=O stretching), 1485.34 (CH Bending of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 1.89 (s, 3H, -CH3), 4.43 (s, 1H, -NH), 7.39 (m, 4H, CH=CH), 7.87 (m, 5H, CH=CH), 8.34 (s, 1H, >NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 20.96, 41.55, 60.23, 90.09, 101.26, 117.78, 128.14, 130.47, 132.90, 136.38, 161.60, 165.63, 180.64; **ESI-MS:** m/z calculated for $C_{18}H_{14}N_4O$ (M+H+): 303.33; found: 303.12 (M+1); Elemental analysis calculated for $C_{18}H_{14}N_4O$ C, 71.51; H, 4.67; N, 18.53; O, 5.29; Observed C, 71.48; H, 4.671; N, 18.55; O; 5.24

4-(benzylamino)-6-oxo-2-phenyl-1-6-dihydropyrimidine-5-carbonitrile (4h): The compound 4h found to be white solid, Yield: 95%; m.p.: 272–275 °C; **FTIR** (KBr, cm^{-1}): 3357.23 (NH stretching), 2915.67 (CH stretching of aromatic), 2195.13 (CN stretching), 1650.56 (C=O stretching of CH_2); **^{1}H NMR** (DMSO d6, 500 MHz) δ: 2.15 (s, 1H, -NH), 2.41 (t, 2H, -CH_2), 7.44 (m, 5H, CH=CH),7.95 (m,5H, CH=CH), 1485.34 (CH Bending CH), 8.43 (s,1H,>NH); **^{13}C NMR** (DMSO d6, 125 MHz) δ: 19.65, 40.84, 60.76, 89.09, 101.26, 116.87, 128.13, 131.43, 132.68, 133.33, 164.62, 165.25, 166.84, 182.65; **ESI-MS:** m/z calculated for $C_{18}H_{14}N_4O$ (M+H+): 303.33; found: 303.01 (M+1); Elemental analysis calculated for $C_{18}H_{14}N_4O$ C, 71.51; H,4.67: N, 18.53; O, 5.29; Observed C, 71.50; H, 4.65;N, 18.51; O, 5.25

2.3 In Vitro Antibacterial Activity

All the synthesised compounds were screened for antibacterial activity against four bacterial strains: *Staphylococcus aureus* (NCTC 10788), *Bacillus subtilis* (NCTC 10400), *E. Coli* (ATCC 8739), and *Pseudomonas aeruginosa* (NCIM 2200), using the agar well-diffusion method[24]. The experiment began with the preparation of sterile media, which were poured into the plates to a uniform depth and allowed to solidify. Each plate was incubated with a bacterial suspension at 10^6 colony-forming units per ml, spread uniformly across the agar surface area using a sterile cotton swab. Well, 8 mm in diameter were made in the agar using a sterile borer. Then, 50 µL of each sample, along with the standard (Positive control) and blank (Negative control), were added to the respective wells. The plates were then incubated at 37 °C ±2 °C for 24 h. Activity was assessed by measuring the zone of inhibition (ZOI) on agar in mm. All test strains were received from the microbiology laboratory, Dr. Rafiq Zakaria campus, Chh. Sambhajinagar, India.

2.4 Determination of MIC:

The most active compounds 4e and 4f were tested to determine their minimum inhibitory concentrations (MICs) by agar well diffusion method against the two bacterial strains, *Bacillus subtilis* and *E. Coli* Compounds **4e** and **4f** were dissolved separately in DMSO and water according to their solubility, to give a

concentration of 800 µg/ml. Two-fold serial dilutions were prepared (800, 400, 200, 100, and 50 µg/ml). The bacterial suspension at 10^6 colony-forming units/ml was incubated. Using the same agar well-diffusion method procedure described above, MIC determinations were carried out. The MIC was recorded as the lowest concentration that produced a zone of inhibition.

2.5 Docking studies

The target protein the *E. Coli* DNA Gyrase B **(PDB ID: 4WUB)** and the *B. Subtilis* RNASE P Protein **(PDB ID: 1A6F),** are imported independently from the protein data bank. The protein preparation started by removal of water molecules and the bound ligand from the receptor protein. The Compute gasteiger and Kollman charges and polar hydrogen charges were added. Then the protein get converted to PDBQT format in AutoDock vina software. Then ligand preparation, defining grid box, ligand-receptor interaction were carried out, and the molecular docking score were recorded and 2D and 3D interactions were visualised in Discovery Studio Visualizer.

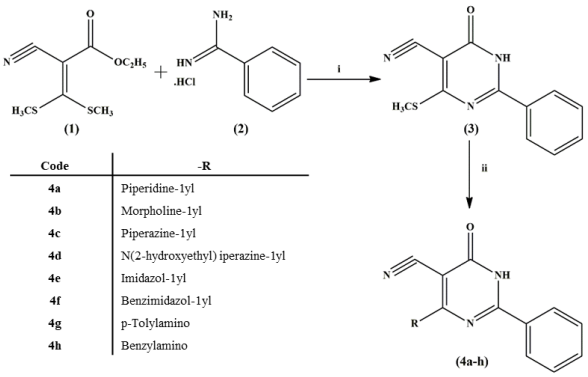
2.6 In-Silico ADMET studies

The oral bioavailability of all the synthesized derivatives **(4a-h)** was predicted by using online software ADMET lab 2.0. This predicted bioavailability of the synthesized compounds was shown to be the acceptable % ABS, ranging from **75.79 % to 83.89 %,** as shown in **Table 3**. The % ABS was calculated by using the formula: % ABS = 109 - (0.345 × TPSA). Here, we predicted all the parameters by using ADMET Lab 2.0.

3. RESULT AND DISCUSSION

3.1 Chemistry

The synthesis pathway for preparing the target compounds, 6-oxo-2-phenyl-4-(substituted heteroaryl)-1,6-dihydropyrimidine-5-carbonitrile **(4a-h)** is depicted in **Scheme 1.** The compound **3** is the precursor for the newly synthesized compounds **(4a-h)**; it was synthesized by reacting ethyl-2-cyno-3, 3-bis (methylthio) acrylate **(1)** with benzimidine hydrochloride **(2)** in the presence of anhydrous K_2CO_3 in dimethyl formamide (DMF) as solvent. Then target derivatives **(4a-h)** were obtained by reacting compound **3** with appropriate aromatic or heteroaryl amines in the presence of sodium ethoxide, in good yield.



Code	-R
4a	Piperidine-1yl
4b	Morpholine-1yl
4c	Piperazine-1yl
4d	N(2-hydroxyethyl) iperazine-1yl
4e	Imidazol-1yl
4f	Benzimidazol-1yl
4g	p-Tolylamino
4h	Benzylamino

Scheme 1: Synthetic pathway of compound 4a-h. **Reagent and conditions:** i) Anhydrous K_2CO_3, DMF, Reflux, ii) Sodium ethoxide, Reflux.

The purity of all the synthesized compounds was checked by thin-layer chromatography (TLC). Melting points were determined using the OptiMelt automated melting system and were uncorrected. All the synthesized derivatives were characterized and confirmed by spectral analysis, viz., FTIR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy, and Elemental analysis. The physical constant data for compounds **3** and **(4a-h)** are shown in **Table 1.**

Table 1. Physical constant data of novel synthesized compounds **3** and **(4a-h).**

Code	-R	Molecular formula	Molecular weight	[a]Yield (%)	[b]m.p. (°C)
3	Thiomethyl	$C_{12}H_9N_3SO$	246.13	93	140 - 142
4a	Piperidine-1yl	$C_{16}H_{16}N_4O$	280.13	85	280 - 283
4b	Morpholine-1yl	$C_{15}H_{14}N_4O_2$	282.11	89	290 - 294
4c	Piperazine-1yl	$C_{15}H_{15}N_5O$	281.13	82	253 - 255
4d	N(2-hydroxyethyl) Piperazine-1yl	$C_{17}H_{19}N_5O_2$	325.15	94	250 - 252
4e	Imidazol-1yl	$C_{14}H_9N_5O$	263.08	88	278 - 280

4f	Benzimidazol-1yl	$C_{18}H_{11}N_5O$	313.10	87	274 - 278
4g	p-Tolylamino	$C_{18}H_{14}N_4O$	302.33	91	295 - 298
4h	Benzylamino	$C_{18}H_{14}N_4O$	302.33	95	272 - 275

[a]Isolated Yield; [b]Observed melting point.

3.2 In vitro Antibacterial Activity

The newly synthesized compounds **3** and **4a-h** were assessed for in vitro antibacterial activity against four bacterial strains: two gram-positive, namely *Staphylococcus aureus* and *Bacillus subtilis,* and two gram-negative, namely *E. Coli* and *Pseudomonas aeruginosa,* using the agar well diffusion method[24]. The well-known gentamycin (Aargenta®) was used as a standard, and DMSO as a blank. The evaluation was carried out by measuring the zone of inhibition (ZOI) on agar media, in mm, as shown in **Table 2.**

Among the series, two compounds, **4e** and **4f,** displayed excellent antibacterial activity with the ZOI, 25 mm and 35 mm, against *Bacillus subtilis* and 20 mm and 24 mm against *E. coli,* respectively, while compounds 3, 4a, 4c, and 4e show some antibacterial activity against the same bacterial strains, as shown in **Figure 2** and **Table 2.** However, the series generally exhibited little activity against the other two bacterial strains, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The data shown in **Table 2** reveals that, the compound with imidazole substitusion, **4e** and benzimidazole substitution**, 4f** shows the remarkable antibacterial activity with ZOI, 25 and 35 mm respectively against *bacillus subtilis* and 20 mm and 24 mm against *E-coli,* while the compound with piperidine substitusion, **4a**, piperazine substitusion, **4c** and N (2-hydroxyethyl) Piperazine substitusion, **4d** exhibits activity with ZOI 25 mm, 18 mm and 20 mm respectively against *Bacillus subtilis* and the compound with methylthio substitusion, **3** and N (2-hydroxyethyl) Piperazine substitusion, **4d** showed antibacterial activity against *E. Coli* with the ZOI, 18 mm and 15 mm as compare with the standard gentamycin.

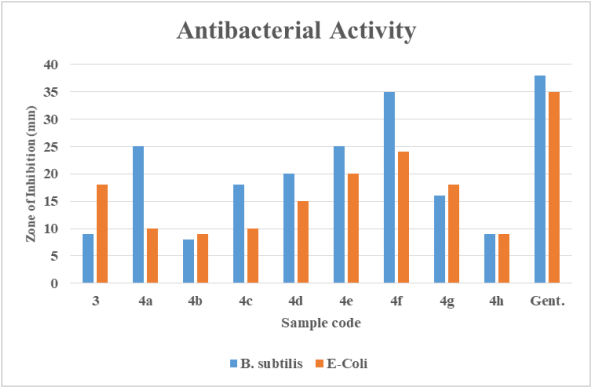


Figure 2: Bar graph representing the zone of inhibition (in mm) of the synthesized compounds 3 and (4a-h), against *B. Subtilis* and *E. Coli* as compared with standard Gentamycin.

To assess bacterial susceptibility, the Minimum Inhibitory Concentration (MIC) of the two most active compounds **4e** and **4f**, was determined against *Bacillus subtilis* and *E. Coli*; MIC for both compounds was found to be < 50 µg/ml, as shown in **Table 2.**

Table 2. In-vitro antibacterial evaluation data of novel synthesized compounds **3** and **(4a-h).**

Code	-R	Zone of Inhibition in mm/ (MIC in µg/ml)			
		[a]S. aureus	***[a]B. subtilis***	***[b]E-Coli***	***[b]P. aeruginosa***
3	Thiomethyl	<10	<10	**18**	<10
4a	Piperidine	<10	**25**	<10	<10
4b	Morpholine	<10	<10	<10	<10
4c	Piperazine	<10	**18**	<10	<10
4d	N(2-hydroxyethyl) Piperazine	<10	**20**	15	<10
[c]4e	Imidazole	<10	**25/ (<50)**	**20/ (<50)**	<10
[c]4f	Benzimidazole	<10	**35/ (<50)**	**24/ (<50)**	<10
4g	p-Tolylamino	<10	16	18	<10
4h	Benzylamino	<10	<10	<10	<10

[cd]Gentamycin	-	35	38 (<50)	35 (<50)	30

[a]Gram-positive bacteria; [b]Gram-negative bacteria; [c]Samples undergo MIC determination; [d]Used as standard.

3.3 In silico ADMET prediction

The acceptable ADMET profile play an important role in the success of a drug along with good efficacy. The QSAR method and related to the data modeling methods were used to investigate the molecular structure features that influence the parameters like Absorption, Distribution, Metabolism, Excretion, and Toxicity of the drug candidates[25]. We have calculated the various parameters using ADMET lab 2.0 to predict the ADMET profile of all the derivatives, as shown in **Table 3**. All the design target compound **(4a-h)** prediction data are within the range of acceptable limits, and they exhibited a good drug like property based upon Lipinski's rule of five[26]. None of the newly synthesized compounds violated the Lipinski's rule of five. The data about the polar surface area (PSA), Log P, and H/C ratio of the synthesized compounds showed good oral bioavailability. By using molinspiration predicted that a good % of absorption (% ABS) ranging from **75.79 % to 83.89 %**, as shown in **Table 3.**

Table 3. Pharmacokinetic parameters of novel synthesized compounds **(4a-h)** for a good oral bioavailability study.

Code	[a]%ABS	TPSA	[b]nROTB	[c]MV	[d]MW	[e]LogP	[f]nON	[g]nOHN	nviolations	Toxicity
Rules	-	-	-	-	<500	<5	<10	<5	<1	-
4a	83.89	72.78	2	291.30	280.13	3.257	5	1	0	Non toxic
4b	80.70	82.01	2	288.80	282.11	1.836	6	1	0	Non toxic
4c	79.74	84.81	2	285.00	281.13	1.589	6	2	0	Non toxic
4d	75.79	96.25	4	328.39	325.15	1.551	7	2	0	Non toxic
4e	78.86	87.36	2	262.44	263.08	1.338	6	1	0	Non toxic
4f	78.86	87.36	2	317.79	313.10	2.226	6	1	0	Non toxic
4g	80.88	82.38	2	328.79	302.33	2.07	7	3	0	Non toxic
4h	82.77	76.02	2	302.30	302.33	1.93	6	1	0	Non toxic

[a]Percentage of absorption; [b]Topological polar surface area, [c]Numbers of rotatable bonds; [d]Molecular volume; [e]Molecular weight; [f]Logarithm of partision coefficient; [g]Number of hydrogen bond acceptors; [h]Number of hydrogen bond donors.

3.4 Molecular Docking

Molecular docking of the newly synthesized compounds was performed to predict the ligand interaction with the target receptor at the molecular level. The synthesized compounds having the pyrimidine scaffold in their structures as popular and potent antibacterial drugs like Trimethoprim, Sulfadiazine, and Sulfamethazine offer a broad spectrum of activity[27]. The Trimethoprim is an antibacterial drug that works by inhibiting the bacterial dihydrofolate reductase (DHFR), Sulfadiazine and Sulfamethazine inhibit the bacterial dihydropteroate synthatase (DHPS). Thymidylate synthatase (TH) disrupts the production of deoxythymidine monophosphate (dTMP).

The docking of synthesized compounds was performed with the *E. Coli* DNA Gyrase B (PDB ID: **4WUB**) and *B. Subtilis* RNASE P Protein (**PDB ID: 1A6F**) to determine molecular interaction and specificity of the synthesized compounds; the results are shown in **Table 4.** Docking score against targets *E. Coli* DNA Gyrase B and *B. Subtilis* RNASE P Protein were assessed by using AutoDock Vina[28]. **Figure 3** and **Figure 4** show the 2D and 3D docking poses of the most potent compounds **4e** and **4f** with the above two targets, along with the standard ciprofloxacin.

Table 4. Molecular docking of the synthesized compounds **(4a-h)** with DNA Gyrase B (PDB ID: **4WUB**) and RNASE P Protein (**PDB ID: 1A6F**).

Code	Docking Score (kcal/mol)	
	PDB ID: 4WUB	PDB ID: 1A6F
4a	-8.1	-7.1
4b	-7.8	-7.1
4c	-7.8	-7.2
4d	-8.0	-6.8
4e	-7.4	-6.1
4f	-8.9	-7.8
4g	-9.1	-6.8
4h	-8.8	-7.0
[a]Ciprofloxacin	7.8	-5.7

[a]Use as standard.

The docking study of the target DNA Gyrase B (PDB ID: **4WUB**) and RNASE P Protein (**PDB ID: 1A6F**) demonstrated that the synthesized compounds, 4a-h, have a superior binding affinity for these target proteins, with key hydrophilic and hydrophobic interactions being essential for effective inhibition of DNA Gyrase B and RNASE P Protein. The chemical interactions between the most active compounds, 4e and 4f, with their target protein are considered to be a crucial factor in their molecular activity. These chemical interactions include the hydrogen bond, potential hydrophobic interactions, pi-pi and T-shaped interactions, as shown in **Figure 3** and **Figure 4**.

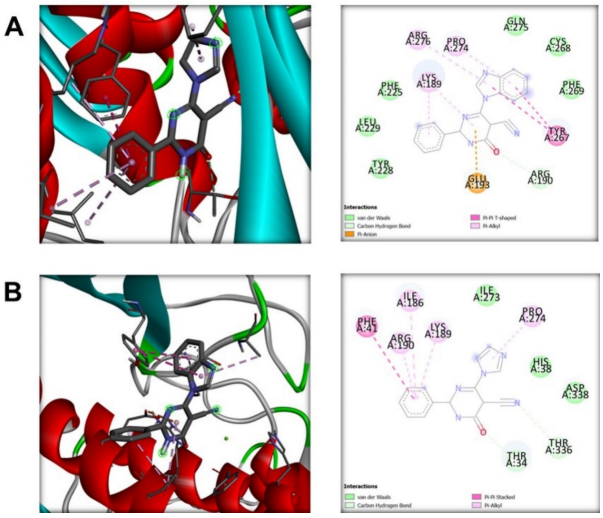


Figure 3. Docking poses of compounds on the target receptors *E. Coli* DNA Gyrase B (**PDB ID Code: 4WUD**): 2D and 3D interaction representation of co-crystalline ligand 4**e (A)** and **4f (B).**

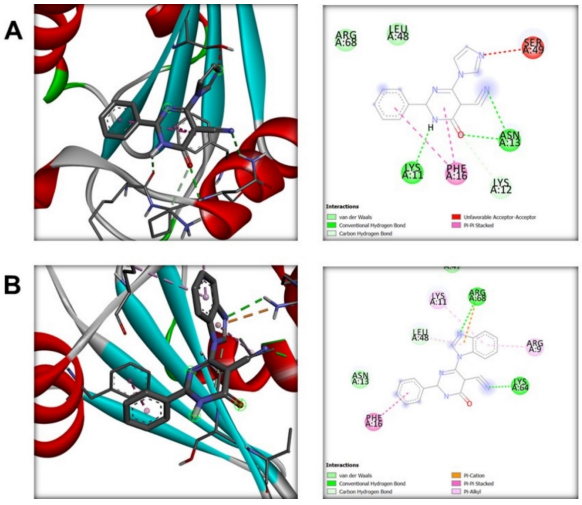


Figure 4. Docking poses of compounds on the target receptors *B. Subtilis* RNASE P Protein (**PDB ID Code: 1A6F**): 2D and 3D interaction representation of co-crystalline ligand **4e (A)** and **4f (B).**

CONCLUSION:

A novel series of pyrimidine-5-carbonitriles was designed, synthesized, and evaluated for their in vitro antibacterial activity. The compounds **4e** and **4f** exhibit excellent antibacterial activity with the ZOI, 25 mm and 35 mm against *Bacillus subtilis* and 20 mm and 24 mm against *E. coli,* respectively, and MIC <50 µg/ml for both compounds. The molecular docking study shows target specificity with potential residual interactions. Finally, the ADME study showed that the synthesized compounds exhibit good drug-like properties and serve as a lead compound for further optimization studies.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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REFERENCES:

[1] Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet 2022; 399 (10325): 629–55. doi: 10.1016/S0140-6736(21) 02724-0.

[2] Aremu OS, Gopaul K, Kadam P, Singh M, Mocktar C, Singh P, and Koorbanally NA. Synthesis, characterization, anticancer and antibacterial activity of some novel pyrano [2,3-d] pyrimidinone carbonitrile derivatives. Anticancer Agents in Medicinal Chemistry. 2017; 17 (5): 719-725. https://doi.org/10.2174/1871520616666160813213245

[3] Tryambake PT. Synthesis, characterization and antimicrobial screening of 2-(2-arylidenehydrazinyl)-4-(3-chlorophenyl)-6-hydroxypyrimidine-5-carbonitrile. Rasayan Journal of Chemistry. 2018; 11(1): 69-73. doi: 10.7324/RJC.2018.1111615

[4] Khedkar SA, Patil JS, Sabale PM. Design and Synthesis, In Silico Analysis of Condensed Pyrimidine derivatives as Potent Antimicrobial Agents. Research Journal of Pharmacy and Technology. 2022; 15(6):2422-2426. doi: 10.52711/0974-360X.2022.0040

[5] Ahmed K, Choudhary MI, and Saleem RSZ. Heterocyclic pyrimidine derivatives as promising antibacterial agents. European Journal of Medicinal Chemistry. 2023; 259: 115701. doi: 10.1016/j.ejmech.2023.115701.

[6] Al-Mahmoudy A, Hassan A, Ibrahim T, Youssif B G M, Taher E, Tantawy M, Abdel-Aal E, and Osman N. Novel benzyloxyphenyl pyrimidine-5-carbonitrile derivatives as potential apoptotic antiproliferative agents. Anti-cancer agents in medicinal chemistry. 2022; *22*(5): 978–990. doi: 10.2174/1871520621666210612043812

[7] El-Dydamony NM, Abdelnaby RM, Abdelhady R, Ali O, Fahmy MI, R Fakhr Eldeen R, and Helwa AA. Pyrimidine-5-carbonitrile based potential anticancer agents as apoptosis inducers through PI3K/AKT axis inhibition in leukaemia K562. Journal of Enzyme Inhibition and Medicinal Chemistry. 2022; 37(1): 895–911. doi: 10.1080/14756366.2022.2051022

[8] Nasser AA, Eissa IH, Oun MR, El-Zahabi MA, Taghour MS, Belal A, Saleh AM, Mehany ABM, Luesch H, Mostafa AE, Afifi WM, Rocca JR, and Mahdy HA. Discovery of new pyrimidine-5-carbonitrile derivatives as anticancer agents targeting $EGFR^{WT}$ and $EGFR^{T790M}$. Organic and Biomolecular Chemistry. 2020; 18(38): 7608–7634. doi: 10.1039/d0ob01557a

[9] Reda N, Elshewy A, El-Askary HI, Mohamed KO, and Helwa AA. Design, synthesis, and biological evaluation of new pyrimidine-5-carbonitrile derivatives as novel anti-cancer, dual $EGFR^{WT}$/COX-2 inhibitors with docking studies. RSC advances. 2023; 13(46): 32296–32320. doi: 10.1039/d3ra06088h

[10] Saleh AM, Mahdy HA, El-Zahabi MA, Mehany ABM, Khalifa MM, and Eissa IH. Design, synthesis, in silico studies, and biological evaluation of novel pyrimidine-5-carbonitrile derivatives as potential anti-proliferative agents, VEGFR-2 inhibitors and apoptotic inducers. RSC advances. 2023; 13(32): 22122–22147. doi: 10.1039/d3ra04182d

[11] Alfayomy AM, Abdel-Aziz SA, Marzouk AA, Shaykoon MSA, Narumi A, Konno H, Abou-Seri SM, and Ragab FAF. Design and synthesis of pyrimidine-5-carbonitrile hybrids as COX-2 inhibitors: Anti-inflammatory activity, ulcerogenic liability, histopathological and docking studies. Bioorganic Chemistry. 2021; 108: 104555. doi: 10.1016/j.bioorg.2020.104555

[12] Abdel-Aziz SA, Taher ES, Lan P, Asaad GF, Gomaa HAM, El-Koussi NA, and Youssif BGM. Design, synthesis, and biological evaluation of new pyrimidine-5-carbonitrile derivatives bearing 1,3-thiazole moiety as novel anti-inflammatory EGFR inhibitors with cardiac safety profile. Bioorganic Chemistry. 2021; 111: 104890. doi: 10.1016/j.bioorg.2021.104890

[13] Shaquiquzzaman M, Khan SA, Amir M, and Alam MM. Synthesis and anticonvulsant activity of some 2-(2-{1-[substituted phenyl] ethylidene} hydrazinyl)-4-(4-methoxy-phenyl)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile. Journal of Enzyme Inhibition and Medicinal Chemistry. 2012; *27*(6): 825–831. doi: 10.3109/14756366.2011.618129

[14] Kaur H, Balzarini J, de Kock C, Smith P J, Chibale K, and Singh K. Synthesis, antiplasmodial

activity and mechanistic studies of pyrimidine-5-carbonitrile and quinoline hybrids. European Journal of Medicinal Chemistry. 2015; 101: 52–62. doi: 10.1016/j.ejmech.2015.06.024

[15] dos Anjos JV, Srivastava RM, Costa-Silva JH, Scotti L, Scotti MT, Wanderley AG, Leite ES, de Melo SJ, and Mendonça Junior FJ. Comparative computational studies of 3,4-dihydro-2,6-diaryl-4-oxo-pyrimidine-5-carbonitrile derivatives as potential antinociceptive agents. Molecules (Basel, Switzerland). 2012; 17(1): 809–819. doi: 10.3390/molecules17010809

[16] Aher JS, Kardel AV, Gaware MR, Lokhande DD, and Bhagare Am. One pot synthesis of pyrimidine-5-carbonitrile and pyrimidine-5-carboxamide using ammonium chloride under solvent free condition, Journal of Chemical Sciences. 2019; 131: 54. doi: 10.1007/s12039-019-1633-6

[17] Siahpour Z, and Hajjami M. Highly efficient synthesis of pyrimidine-5-carbonitrile derivatives over a robust biowaste bone char-bronsted solid acid catalyst. Scientific Reports. 2024; 14: 30411. doi: 10.1038/s41598-024-82040-3

[18] Val C, Crespo A, Yaziji A, Coelho A, Azuaje J, El Maatougui A, Carbajales C, and Sotelo E. Three-component assembly of structurally diverse 2-aminopyrimidine-5-carbonitriles, *ACS Combinatorial Science.* 2013; *15* (7): 370-378. doi: 10.1021/co4000503

[19] Al-Deeb, Omar A, Al-Turkistani, Abdulghafoor A, Al-Abdullah, Ebtehal S, El-Brollosy, Nasser R, Habib, Elsayed E, and El-Emam Ali A. Pyrimidine-5-carbonitriles – Part III: Synthesis and antimicrobial activity of novel 6-(2-substituted propyl)-2,4-disubstituted pyrimidine-5-carbonitriles. Heterocyclic Communications. 2013; 19(6): 411-419. doi: 10.1515/hc-2013-0139

[20] Keshk RM, Salama ZA, and Elsaedany SK. Synthesis, antimicrobial, anti-inflammatory, antioxidant and cytotoxicity of new pyrimidine and pyrimidopyrimidine derivatives. Scientific Reports. 2025; 15: 9328 https://doi.org/10.1038/s41598-025-92066-w

[21] Mohamed MS, Awad SM, and Ahmed NM. Synthesis and antimicrobial activities of new indolyl-pyrimidine derivatives, Journal Applied Pharmaceutical Science. 2011; 01 (05): 76-80.

[22] Fathalla OA, Zeid IF, Haiba ME, Soliman AM, Abd-Elmoez SI, and El-Serwy WS. Synthesis, antibacterial and anticancer evaluation of some pyrimidine derivatives. World Journal of Chemistry. 2009; 4 (2): 127-132.

[23] Aburzeza MM, Awheda IM, Kollab WA, Abdulnnour AO. 4-Amino pyrimidine derivatives: Design, synthesis, antiviral, and antibacterial investigation. Journal of Neonatal surgery. 2025; 14 (26): 521-527.

[24] Mounyr B, Moulay S, Saad K I. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6: 71–79. doi: 10.1016/j.jpha.2015.11.005

[25] Fu L, Shi S, Yi J , Wang N, He Y, Wu Z, Peng J, Deng Y, Wang W, Wu C, Lyu A, Zeng X, Zhao W, Hou T, Cao D. ADMETlab 3.0: An updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality, and decision support. *Nucleic Acids Research.* 2024; 52(W1): W422–W431. doi: 10.1093/nar/gkae236

[26] Lipinski CA. Lead- and drug-like compounds: The Rule-of-five revolution. Drug Discovery Today: Technologies. 2004; 1 (4): **337–**341. doi: 10.1016/J.ddtec.2004.11.007

[27] Selvam TP, James CR, Dniandev PV, Valzita SK. A mini review of pyrimidine and fused pyrimidine marketed drugs. Research in Pharmacy. 2012; 2(4): 22. https://updatepublishing.com/journal/index.php/rip/article/view/271

[28] Trott O, Olson AJ. Autodock Vina: Improving the speed and accuracy of docking with new scoring function. Efficient optimization and multithreading. Journal of Computational Chemistry. 2010; 31: 455-461. doi: 10.1002/Jcc.21334.