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Synthesis, Characterization, Docking Study and Biological Evaluation of Substituted Pyrimidines

Narendra B Gowda*, Nisarga Prakash M, Rachana M L

Department of Pharmaceutical Chemistry, Visveswarapura Institute of Pharmaceutical Sciences, Bengaluru, Karnataka, India 560070.

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Abstract

Objective: Synthesis and antimicrobial evaluation of a series of substituted pyrimidines.

Methods: A series of substituted pyrimidines (4a-g) were synthesized from the corresponding chalcones. The chalcones were prepared via Claisen Schimdt condensation between p-Chloroacetophenone and substituted benzaldehyde. These chalcones were then cyclized with thiourea in methanol medium via Michael's addition to obtain substituted pyrimidines. All pyrimidine derivatives were characterized by IR (Infrared radiation), 1 HNMR (Proton nuclear magnetic resonance) spectral studies. Their in-vitro antimicrobial activity was evaluated using the cup-plate method. Molecular docking studies were conducted using PyRx and other computational tools.

Results: Some of the compounds have exhibited promising antimicrobial activities.

Conclusion: According to the activity studies, it is observed that the synthesis and antimicrobial activity of substituted pyrimidines have been shown better activity. Furthermore, the findings from molecular docking offer guidance on how to further modify the lead compound to enhance its inhibitory effectiveness.

Keywords: Chalcone, 2-thioPyrimidine, Anti-microbial activity, Ciprofloxacin, Molecular docking.

***Corresponding Author**
Narendra B Gowda
Department of Pharmaceutical Chemistry,
Visveswarapura Institute of Pharmaceutical Sciences, Bengaluru, Karnataka, India 560070
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INTRODUCTION

Microbial resistance to various antimicrobial agents has become a serious challenge in the healthcaresector within the last few decades around the globe. According to the survey of World HealthOrganization, approximately 50 000 people across the world are dying every day as a consequenceof microbial infections [1].

Chalcones are a, b-unsaturated ketones, important precursors for synthetic work and major component of the natural products. Chalcones and their derivatives show significant biological activities which are very important and helpful in drug designing due to the presence of double bond in conjugation with carbonyl group. Chalcones can be synthesized conveniently by ClaisenSchmidt condensation and chalcone compounds are highly biologically active molecules [2]. A significant class of both natural and non-natural chemicals are heterocycles with a pyrimidine moiety due to their numerous biological and therapeutic uses. An integral part of nucleic acids like Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA), pyrimidine is vital for the storage and transfer of genetic information. Pyrimidine is found in three primary compounds: uracil (U), thymine (T), and cystosine (C). Whereas uracil is present in RNA, cytosine and thymine are found in DNA. Building blocks of DNA and RNA are created when these nucleotides combine with purine bases (adenine and guanine) [3].

One important class of pyrimidine is 2-thiopyrimidine (2-TP) and its derivatives, which are also well known as 2-mercaptopyrimidine compounds. In 2-TP ring sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base [4].

Molecular docking is the most common computational structure based drug design (SBDD) method and has been widely used ever since the early 1980s.The process of predicting the best position, orientation and conformation of a small molecule (drug candidate) when bound to a protein, provides the additional benefit of simplifying future lead optimization.

One of the most crucial tools in prevailing against bacterial strain-induced infections is antimicrobial drugs. The need for safe, effective, and innovative

antimicrobial agents has been brought to light by the growing resistance of bacteria to antimicrobial agents in recent years [5].Therefore, we tried to create pyrimidine derivatives in this study to improve their antibacterial properties.
The pyrimidines can be synthesized by condensing chalcones with thiourea in the presence of base via Michael's addition[6].**(Fig. 1)**.

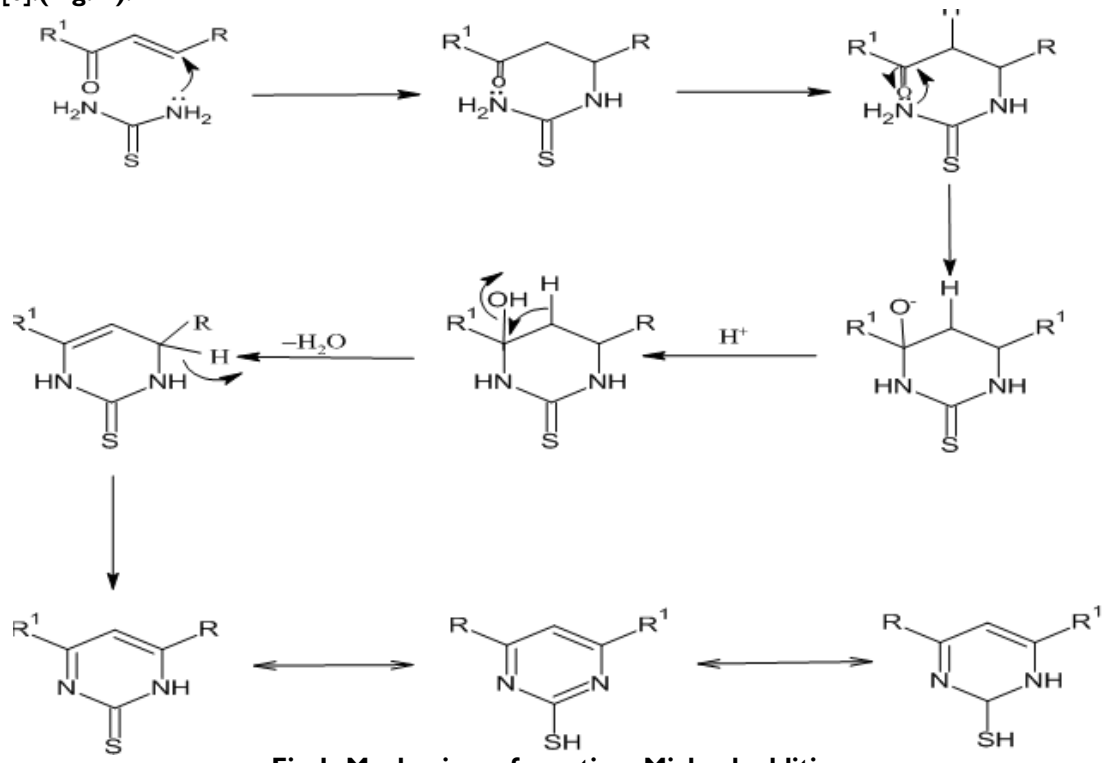


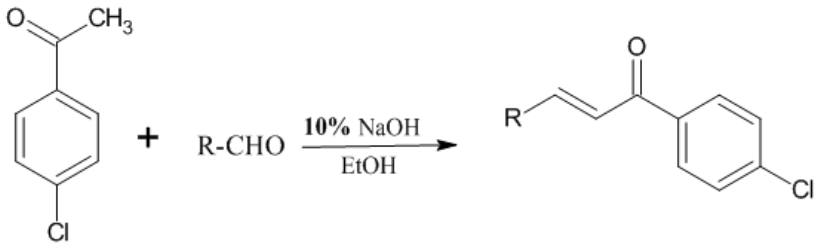
Fig.1: Mechanism of reaction: Michael addition

MATERIALS AND METHODS

Laboratory chemicals were provided by SPECTRUM, CENTRAL DRUG HOUSE (CDH), S.D. Fine Chem. Ltd. Melting points was determined by MEPA melting point apparatus by LABINDIA. The purity of the compound was checked by thin layer chromatography (TLC) using silica gel Gasstationary phase 60 F254 pre-coated plates by Merck in the solvent system petroleum ether: ethyl acetate (7:3). The spots were observed by exposure to iodine Vapours or by UV light. The IR spectra were recorded on FTIR-8400S modelShimadzu in the ranges of 400-4000 cm^{-1}. ^{1}H-NMR spectra were recorded in Bruker 400MHz using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS).

Synthesis of Substituted Chalcones(3a-g)

p-Chloroacetophenone (0.01 mol) was dissolved in 15 ml of ethanol in a round bottomed flask, stirred on magnetic stirrer. 4 ml of 10% NaOH was added slowly. Immediately, the reaction mixture turned golden yellow colour. Then, aromatic aldehyde (0.01mol) was added through dropping funnel. The stirring was continued at room temperature for about 3-4 hours. The precipitate obtained was filtered, washed with water and recrystallized from ethanol [7].



R=C_6H_5, 3-Cl-C_6H_5, 4-Cl-C_6H_5, 4-OCH_3-C_6H_5, 2,4-OCH_3-C_6H_5, 3-NO_2-C_6H_5, C_4H_3O

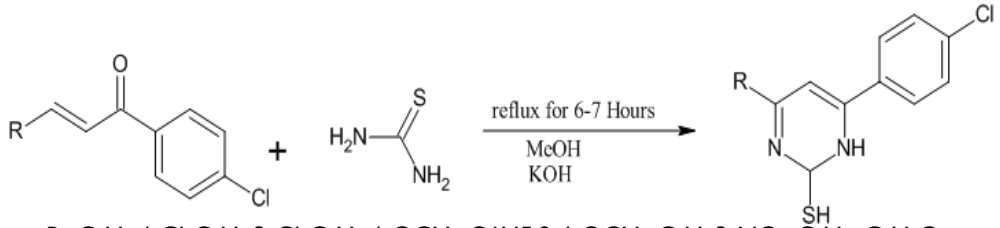
ANALYTICAL DATA

3a:1-(4-chlorophenyl)-3-phenylprop-2-en-1-one; M.F: $C_{15}H_{11}OCl$;M.W: 242; mp :100°C; Yield: 79%; Rf value: 0.5; **FTIR (cm^{-1}):**1658(C=O),1598(C=C), 761 (C-Cl). **3b**: 1-(4-chlorophenyl)-3-(3-nitrophenyl) prop-2-en-1-one; M.F: $C_{15}H_{10}O_3NCl$; M.W:287; mp:145°C;Yield: 71%; Rf value :0.47; **FTIR (cm^{-1}):**1608(C=O),1667(C=C),742(C-Cl).

3c:1-(4-chlorophenyl)-3-(furan-3-yl)prop-2-en-1-one; M.F:$C_{13}H_9O_2Cl$; M.W:232;mp:103° C; Yield: 78%; Rf value: 0.42; **FTIR (cm^{-1}):**1545(C=O),1654(C=C),817(C-Cl).
3d: 1-(4-chlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one; M.F: $C_{17}H_{15}O_3Cl$; M.W: 302; mp:126 ° C; Yield: 69%; Rf value: 0.7; **FTIR (cm^{-1})**:1557(C=O),1650(C=C),815(C-Cl), 1207 (C-O-C) asymmetric, 1025 (C-O-C) symmetric.
3e:1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one; M.F: $C_{16}H_{13}O_2Cl$; M.W: 272; mp:115 °C; Yield: 75%; Rf value:0.6; **FTIR (cm^{-1})**:1654(C=O),1585(C=C),809(C-Cl), 1168 (C-O-C) asymmetric, 1087 (C-O-C) symmetric.
3f:1,3 bis(4-chlorophenyl)prop-2-en-1-one,M.F: $C_{15}H_{10}OCl_2$; M.W: 277; mp :158°C; Yield:70%; Rf value: 0.7**;FTIR (cm^{-1})**:1651(C=O),1583(C=C),742(C-Cl).
3g:3-(3-chlorophenyl)-1-(4-chlorophenyl)prop-2-en-1-one, M.F: $C_{15}H_{10}OCl_2$; M.W: 277; mp: 104 ° C; Yield: 73%; Rf value: 0.6; **FTIR (cm^{-1})**: 1659(C=O),1584(C=C),787(C-Cl).

Synthesis of Substituted pyrimidines (4a-g)

Chalcone (0.001 mol) was dissolved in 4ml methanol in a round bottom flask and then added potassium hydroxide(0.001mol) and thiourea (0.0012mol) then refluxed for 6-7 hours,thencooled the mixture in room temperature. Then transfer the reaction mixture into beaker and dilute the few drops of concentrated hydrochloric acid with 5 ml of water and then add dilute HCl into the beaker containing reaction mixture. The obtained product was filtrated and washed with water and the product was dried and recrystallized by ethanol[8].



R=C_6H_5,4-Cl-C_6H_5,3-Cl-C_6H_5,4-OCH_3-C6H5,2,4-OCH_3-C_6H_5,3-NO_2-C_6H_5, C_4H_3O

ANALYTICAL DATA

4a:6-(4-chlorophenyl)-4-phenyl-1,2-dihydropyrimidine-2-thiol; M.F:$C_{16}H_{13}N_2SCl$; M.W:300; mp:218°C; Yield:77%; Rf value:0.19; **FTIR(cm^{-1})**: 3250(NH), 2855(SH), 1572(C=N);^{1}**HNMR, $CDCl_3$, 400MHz**: δ 7.81(s,1H,Ar-H),7.46-7.30(m,8H,Ar-H),7.03(s,1H,Ar-H),5.31-5.22(d-1H,CH),1.72(s,1H,NH),1.29(s,1H,SH).
4b:6-(4-Chlorophenyl)-4-(3-nitrophenyl)-1,2-dihydropyrimidine-2-thiol;M.F:$C_{16}H_{12}N_3O_2SCl$; M.W:345;mp:225°C; Yield:65%; Rf value: 0.21; **FTIR(cm^{-1})**: 3250(NH),2925(SH),1529(C=N);^{1}**HNMR: $CDCl_3$,400MHz**: δ7.49-7.29(m,9H,ArH),4.35-4.25(d,1H,CH),1.7 (s,1H,NH),1.3 (s,1H,SH).
4c: 6-(4-Chlorophenyl)-4-(furan-2-yl)-1,2-dihydropyrimidine-2thiol; M.F:$C_{14}H_{11}N_2OSCl$; M.W: 290;mp :212°C; Yield: 73%;Rf value: 0.19;**FTIR(cm^{-1})**: 3211(NH),2364(SH), 1477(C=N)^1**HNMR, $CDCl_3$,400MHz**:δ7.9-7.29(m,8H,ArH), 5.33-5.23 (d,1H,CH),1.28(s,1H,SH),1.68 (s,1H,NH)
4d:6-(4-Chlorophenyl)-4-(2,4-dimethoxyphenyl)-1,2-dihydropyrimidine-2-thiol;M.F:$C_{18}H_{17}N_2O_2SCl$; M.W: 360;mp : 277° C; Yield: 74%; Rf value: 0.34;**FTIR(cm^{-1})**: 3230(NH),2316(SH),1548(C=N); ^{1}**HNMR, $CDCl_3$, 400MHz**: δ 7.4-7.0(m,8H,ArH),5.5-5.2(d,1H,CH)3.9- 3.7 [m,6H,[$(OCH_3)_2$],1.8(m,1H,NH), 1.3(s,1H,SH).
4e:6-(4-Chlorophenyl)-4-(4-methoxyphenyl)1,2-dihydropyrimidine-2-thiol;M.F: $C_{17}H_{15}N_2OSCl$; M.W: 330;mp: 243° C; Yield: 69% ; Rf value: 0.16;**FTIR (cm^{-1})**:3186(NH),2833(SH),1569(C=N); ^{1}**HNMR, $CDCl_3$, 400MHz**: δ 7.81(s,1H,Ar-H), 7.22-7.11(m,7H,Ar-H), 6.78-6.76(d,1H,Ar-H), 5.07-5.01(d,1H,CH), 3.66(s,3H,OCH_3),1.72(s,1H,NH), 1.29(s,1H,SH).
4f:4,6-bis(4-Chlorophenyl)-1,2-dihydropyrimidine-2-thiol;M.F: $C_{16}H_{12}N_2SCl_2$; M.W: 335;mp : 221°C; Yield: 71%; Rf value: 0.36;**FTIR (cm^{-1}):**3180(NH),2316(SH),1548(C=N)^1**HNMR, $CDCl_3$, 400MHz**: δ7.4-7.1(m,9H,ArH),5.2-5.1 (d,1H,CH),1.65(s,1H,NH), 1.23(s,1H,SH).
4g:4-(3-chlorophenyl)-6-(4-chlorophenyl)-1,2-dihydropyrimidine-2-thiol; M.F: $C_{16}H_{12}N_2SCl$; M.W: 335;mp: 238° C; Yield: 73%; Rf value: 0.24;**FTIR (cm^{-1}):**3189(NH),2984(SH),1578(C=N);^{1}**HNMR, $CDCl_3$, 400MHz**: δ 7.2-7.6(m,9H,ArH),5.2-5.1(d,1H,CH),1.58(s,1H,NH),1.23(s,1H,SH).

Antimicrobial activity

The *In-vitro* Anti-microbialactivityscreening of the newly synthesized 2- thio Pyrimidines (4a-g) was carried out against Gram-positive organisms *(Enterococcus faecalis and Staphylococcus aureus),*Gramnegative organisms (*Klebsiella pneumoniae and Escherichia Coli*) by cup plate method and compared with that of the standard drug Ciprofloxacin. A stock solution of drug with concentration 200 and 400 µL concentrationwas prepared in Dimethylformamide (DMF). The tubes were incubated for 24hrs and observed for zone of inhibition. The antimicrobial activity of the compounds (4a-l) is given in table-1.

Molecular docking study

PyRx virtual screening software 0.8 was used for molecular docking in order to assess the interaction between two molecules and determine the ideal ligand orientation that creates a stable complex with the least amount of energy. Using DNA gyrase and beta lactamase as target proteins, all of the produced compounds were docked. The Protein data bank (PDB)(www.rcsb.org/pdb) provides the protein structure file (PDB ID: 6BSQ and 5FAT) in three-

dimensional (3D) format for download. Biovia Discovery Studio Visualizer 16.1.0 was used to process the downloaded protein structures, eliminating any existing heterocyclic ligands, adding polar hydrogens, and saving them in PDB format. After being sketched in ChemSketch and saved as.mol files, the compound structures were translated to pdbqt format in PyRx by selecting the Open Babel option. Around the target protein, a grid was created. After the ligands and protein docked, Biovia Discovery Studio was used to visualize the outcomes. An Excel sheet including the binding energies and the protein-ligand interactions was saved in two-dimensional format[9].

RESULTS AND DISCUSSION

A series of substituted Pyrimidine **(4a-g)** derivatives have been synthesized and screened for their *In-vitro* antibacterial activity.Compounds **4a** and **4g** exhibit strong antibacterial activity against gram-positive bacteria, while compounds **4b** and **4f** exhibit good antimicrobial activity against gram-negative bacteria at a concentration of 200µg/ml, according to antimicrobialstudies.

Specifically, compounds **4c, 4d, and 4e** demonstrated moderate to significant antimicrobial activity against every organism used at a 200 µg/ml concentration, which is equivalent to that of the standard drug ciprofloxacin.The antimicrobial activity of the compounds **(4a-g)** is given in table-1.

Table 1: Antimicrobial activity of substituted pyrimidine derivatives (4a-g) by cup plate method

Compound code	Zone of inhibition (mm)							
	Escherichia Coli		*Staphylococcus aureus*		*Klebsiella pneumoniae*		*Enterococcus faecalis*	
	200 µg/ml	400 µg/ml	200 µg/ml	400 µg/ml	200 µg/ml	400 µg/ml	200 µg/ml	400 µg/ml
4a	20	18	21	20	23	25	30	27
4b	28	22	22	25	-	20	24	22
4c	21	20	15	25	-	20	23	24
4d	20	11	21	22	-	22	22	23
4e	20	23	25	20	25	18	21	25
4f	25	20	20	20	-	12	25	21
4g	23	20	21	20	27	-	27	22
Ciprofloxacin[a] 100µg/ml 100 µg/ml	27		30		29		30	
Control	-		-		-		-	

[a]Standard drug- Ciprofloxacin

In vitro studies of Antimicrobial activity of substituted pyrimidine derivatives (4a-g) by cup plate method

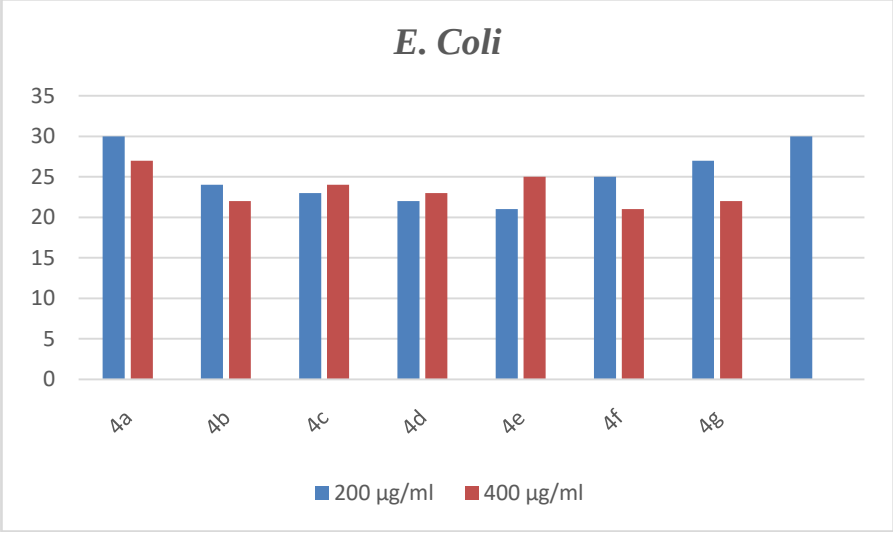


Fig 01:

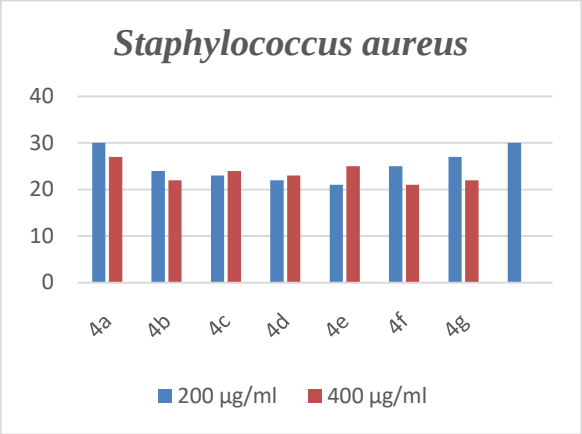


Fig 02:

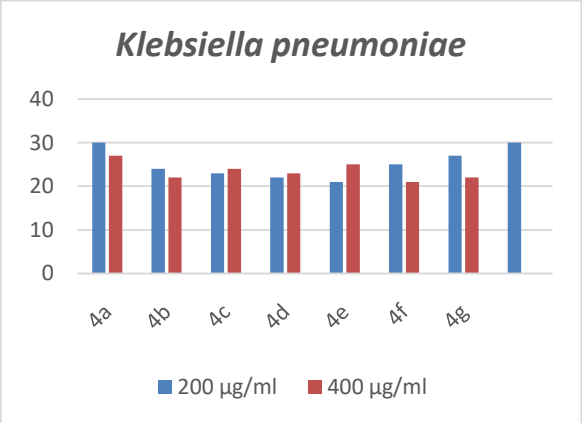


Fig 03:

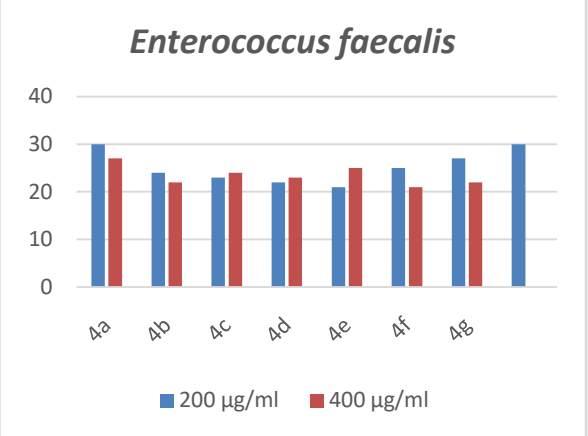


Fig 04:

All of the synthesized compounds have good docking scores, and all of them exhibit good interactions and binding affinities in docking studies. Molecular docking studies of the synthesized compound were conducted in order to rationalize the obtained biological results. Molecular docking studies also aid in the detailed understanding of the various interactions between ligand and enzyme active sight.

According to the results of the molecular docking investigation, **4a** and **4g**, two of the synthesized compounds, had a substantial binding affinity with binding energy values ranging from -9.1 to -10.1 kcal/mol, in comparison to the reference standard drug ciprofloxacin (-7.0 kcal/mol) with PDB ID: 5FAT. On the other hand, molecules like **4b** and **4f** demonstrated strong binding affinity with binding energy values ranging from -8.2 to -8.5 kcal/mol, in contrast to the ciprofloxacin (-7.0 kcal/mol) with PDB ID: 6BSQ. The molecular docking of the compounds **(4a-g)** is given in table-2.

Table 2: Docking score of the synthesized compounds by using PyRx Software

Compound code	Anti-bacterial studies	
	PDB ID-5FAT Binding affinity (Kcal/mol)	PDB ID-6BSQ Binding affinity (Kcal/mol)
4a	**-9.1**	- 8.1
4b	-8.1	**-8.2**
4c	-7.7	-7.4
4d	-7.2	-8.2
4e	-7.4	-8.1
4f	-7.8	**-8.5**
4g	**-10.1**	-8.1
Standard (ciprofloxacin)	-7.0	-7.0

CONCLUSION

Thus, the derivatives of substituted pyrimidines have significant antibacterial activity, and additional study on lead optimization is necessary to improve the antimicrobial characteristic. For a future perspective, additional synthesis of a few more derivatives, characterisation, and in-vivo experiments must be conducted.

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