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STUDIES ON SYNTHESIS, ANTIMICROBIAL ACTIVITY AND IN-SILICO ANALYSIS OF SUBSTITUTED PYRIMIDINES

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

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

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

ABSTRACT

A series of substituted pyrimidines were synthesized from corresponding chalcones. The chalcones were prepared via Claisen Schimdt condensation between *p*-chloroacetophenone and substituted benzaldehyde. These chalcones were then cyclised with guanidine nitrate via Michael's addition to obtain substituted pyrimidines. Subsequently, the pyrimidines were acetylated to yield substituted pyrimidine derivatives. All pyrimidine derivatives were characterized by IR, NMR spectral studies. Their *in-vitro* antimicrobial activity was evaluated using the cup-plate method. In silico target studies were conducted using PyRx and other computational tools. The compound 3a,3b,3f,3g exhibited significant activity compared to the standard drug ciprofloxacin.

KEYWORDS: Chalcone, Pyrimidine, Antimicrobial activity, Molecular docking.

1. INTRODUCTIONS

Antimicrobial agents play a crucial role in controlling and preventing the spread of bacterial infections. In recent years, the increasing tolerance of microorganisms to antimicrobial agents has become a serious health concern, highlighting the need for safe, effective, and novel antimicrobial agents.[1] So, in this research work, we attempted to synthesize pyrimidine derivatives for better antimicrobial activities.

Chalcone have a many biological activities and is a well-known precursor for the synthesizing various heterocyclic compounds.[2] Cyclization of chalcones leads to

heterocyclic compounds containing nitrogen-bearing rings, like pyrimidine.[3] So, this was the rationale behind synthesizing chalcones. Chalcones can be synthesized by Claisen-Schmidt condensation between an aldehyde, ketone in the presence of base or acid followed by dehydration process to form α, β unsaturated carbonyl compounds.[4]

The Pyrimidine ring is an aromatic heterocyclic structure with nitrogen atoms at the 1st and 3rd positions, serving as a crucial core for various biologically active compounds.[5] Pyrimidine is fundamental structural component of DNA and RNA and which play crucial role in various processes of existence. Pyrimidines are one of the three isomeric diazines. Most abundant pyrimidines are uracil, cytosine, thymine which are building blocks of nucleic acids. These derivatives are also known as cyclic amine of 1-3 diazine or m-diazine.[6] Pyrimidine serves as a key precursor for the synthesis of wide variety of heterocyclic compounds and as a raw material for the synthesis of novel molecule.[7] Pyrimidine ring complexes with various heterocyclic moiety are essential components of natural products, agrochemicals and veterinary products.[8] Various analogs of pyrimidines are found to possess diverse Pharmacological activities such as antimicrobial[9], antimalarial[10], anti-inflammatory[11], anti-tumour[12], anti-convulsant[13], anti-cancer, anti-bacterial[14], analgesic[15], anti-oxidant[16], anti-tubercular[17], anti-pyretic[18], They also acts as calcium channel blockers.[19] The pyrimidines can be synthesized by condensing chalcones with guanidine nitrate in the presence of base via Michael's addition reaction[20] (**Fig no.1**).

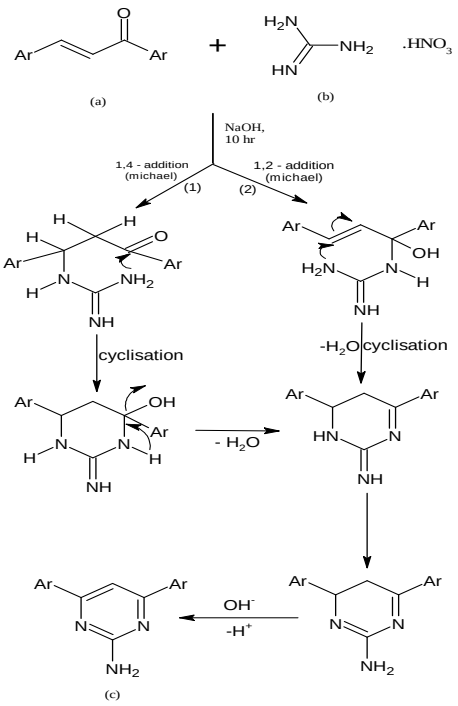


Figure No.1 - (a) Chalcone, (b) Guanidine nitrate, (c) Pyrimidine

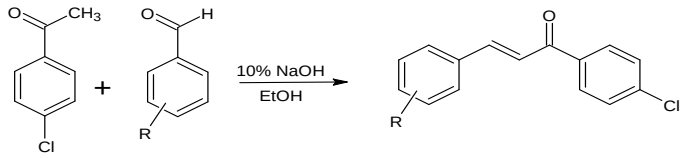
2. MATERIALS AND METHODS

2.1 Chemistry

Laboratory chemicals was provided by SPECTRUM, CENTRAL DRUG HOUSE (CDH), S.D. Fine Chem. Ltd. Melting points was evaluated by MEPA melting point apparatus by LABINDIA. The purity of a compound was checked by thin layer chromatography (TLC) by using silica gel G as a stationary phase 60 F_{254} pre-coated plates by Merck in solvent system petroleum ether: ethyl acetate (7:3). The spots was visualized by exposure to iodine Vapours or UV light. IR spectra was recorded by FT-IR model Bruker alpha 2 in the ranges of 400-4000 cm^{-1}. ^{1}H-NMR spectra was documented by Bruker 400MHz using $CDCl_3$ and chemical shifts (δ) are reported as parts per million downfield from internal reference Tetramethylsilane (TMS).

2.1.1. Synthesis of substituted Chalcones

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 (0.01 mol) 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.



R = 4-CH_3, 3-Cl, 4-$CH(CH_3)_2$, 3,4,5 $(OCH_3)_3$, 4-OCH_3, 4-NO_2, 3-NO_2

1-(4-chlorophenyl)-3 (4-methylphenyl) prop 2-en-1-one (1a)

MF: $C_{16}H_{13}OCl$; M W:256; Yield 90%; MP :122°C; Rf value: 0.8; FTIR cm^{-1} :1653 (C=O), 1586 (C=C), 807 (C-Cl).

3-(3-chlorophenyl)-1-(4-chlorophenyl) prop- 2-en-1-one(1b)

MF: $C_{15}H_{10}OCl_2$; M W: 277; Yield:88%; MP:102°C; Rf value:0.78; FTIR cm^{-1} : 1659 (C=O), 1603 (C=C), 787 (C-Cl).

1-(4- chlorophenyl)-3- [4- (propan-2- yl) phenyl] prop-2-en-1- one (1c):

MF: $C_{18}H_{16}OCl$; M W: 283; Yield:90%; MP:85°C; Rf value:0.68; FTIR cm^{-1} : 1660 (C=O), 1594 (C=C), 817 (C-Cl).

1-(4-chlorophenyl)-3- (3,4,5-trimethoxy phenyl) prop-2-en-1-one (1d)

MF: $C_{17}H_{15}O_3Cl$; M W: 332; Yield:89%; MP:130°C; Rf value:0.63; FTIR cm^{-1} : 1664 (C=O), 1587 (C=C), 781 (C-Cl), 1126 (C-O-C).

1-(4-chlorophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one (1e)

MF:$C_{16}H_{13}O_2Cl$; M W: 272; Yield:95%; MP:120°C; Rf value:0.65; FTIR cm^{-1} : 1654 (C=O), 1586 (C=C), 809 (C-Cl), 1087 (C-O-C).

1-(4-chlorophenyl)-3- (4-nitrophenyl) prop-2-en-1-one (1f)

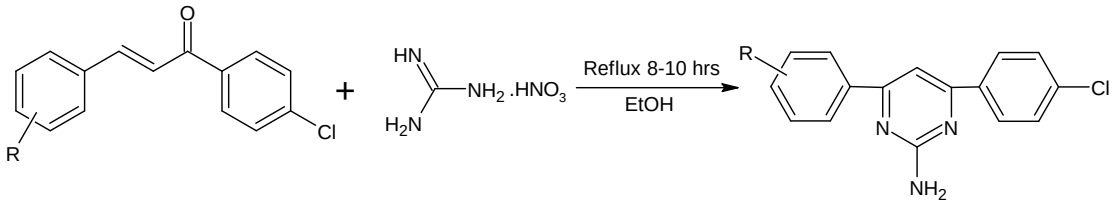
MF:$C_{15}H_{10}O_3NCl$; M W: 287; Yield:89%; MP:160°C; Rf value:0.71; FTIR cm^{-1} : 1659 (C=O), 1586 (C=C), 754 (C-Cl).

1-(4-chlorophenyl)-3- (3-nitrophenyl) prop-2- en-1-one (1g)

MF:$C_{15}H_{10}O_3NCl$; M W: 287; Yield:86%; MP:140°C; Rf value:0.70; FTIR cm^{-1} : 1667 (C=O), 1608 (C=C), 742 (C-Cl).

2.1.2. Synthesis of Substituted Pyrimidine derivatives

Chalcone (0.01 mol) and guanidine nitrate (0.01 mol) was taken in a three necked flask, 30 ml of ethanol was added. The above mixture was refluxed on a magnetic stirrer. After the contents were dissolved in the alcohol, an aqueous solution of NaOH (40%, 5ml) was added fraction wise during a period of 3 hours. Reflux was continued for 7 hours. The solvent was reduced to half of its volume and on cooling the solid product was separated out. Later it was filtered, washed with cold aqueous ethanol followed by water and recrystallized from ethanol.



R = 4-CH_3, 3-Cl, 4-$CH(CH_3)_2$, 3,4,5 $(OCH_3)_3$, 4-OCH_3, 4-NO_2, 3-NO_2

4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-amine (2a)

MF: $C_{17}H_{14}ClN_3$; M W: 295.77;Yield:80%; MP: 174°C; Rf value: 0.7; FTIR cm^{-1}: 3301,3170 (NH_2), 1634(C=N),800 (C-Cl); ^{1}H -NMR, $CDCl_3$, 400 MHz: δ 8.0-7.9 (m,4H, Ar-H), 7.2-7.5 (m, 5H, Ar-H, 5.2 (s, 2H, NH_2), 2.45 (s, 3H, CH_3).

4-(3-chlorophenyl)-6-(4-chlorophenyl) pyrimidin-2-amine (2b)

MF: $C_{16}H_{11}Cl_2N_3$; M W: 316.185;Yield:77% MP: 178°C; Rf value: 0.62; FTIR cm^{-1} : 3305, 3177 (NH_2), 1638(C=N),803 (C-Cl); ^{1}H-NMR, $CDCl_3$, 400 MHz: δ 8.0-7.9 (m,4H, Ar-H), 7.5-7.2 (m, 5H, Ar-H), 5.2 (s, 2H, NH_2).

4-(4-chlorophenyl)-6-[4-(propan-2-yl) phenyl] pyrimidin-2-amine (2c)

MF: $C_{19}H_{18}ClN_3$; M W: 323.824;Yield:75% MP: 139°C; Rf value: 0.52; FTIR cm^{-1} : 3302, 3198 (NH_2), 1607 (C=N), 811 (C-Cl); ^{1}H-NMR, $CDCl_3$, 400 MHz: δ 8.0-7.9(m, 4H, Ar-H), 7.5-7.2 (m,5H, Ar-H), 5.2 (s, 2H, NH_2),3.0-2.9 (m, 1H CH), 1.32-1.31[(d,6H, $CH_3)_2$].

4-(4-chlorophenyl)-6-(3,4,5-trimethoxyphenyl) pyrimidin-2-amine (2d)

MF: $C_{19}H_{18}ClN_3O_3$; M W: 371.321;Yield:78% MP: 167°C; Rf value: 0.6; FTIR cm^{-1} : 3376 (NH_2),1615 (C=N), 800 (C-Cl),1123(C-O-C); ^{1}H -NMR, $CDCl_3$, 400 MHz: δ 8.04-8.02 (d,2H, Ar-H), 7.5-7.4 (d, 2H, Ar-H), 7.3-7.2 (m, 3H, Ar-H), 5.2(s, 2H, NH_2),4.0 [(s, 6H, $0CH_3)_2$], 3.9 (s, 3H, OCH_3).

4-(4-chlorophenyl)-6-(4-methoxyphenyl) pyrimidin-2-amine (2e)

MF: $C_{17}H_{14}ClN_3O$; M W: 311.769;Yield:84% MP: 161°C; Rf value: 0.67; FTIR cm^{-1} : 3324, 3202 (NH_2), 1641 (C=N), 810 (C-Cl), 1171(C-O-C); ^{1}H - NMR, $CDCl_3$, 400 MHz: δ 8.06-8.01 (m,4H, Ar-H), 7.49-7.47 (m,3H, Ar-H), 7.3-7.0 (m, 2H, Ar-H), 5.2 (s, 2H, NH_2), 3.9 (s, 3H, OCH_3).

4-(4-chlorophenyl)-6-(4-nitrophenyl) pyrimidin-2-amine (2f)

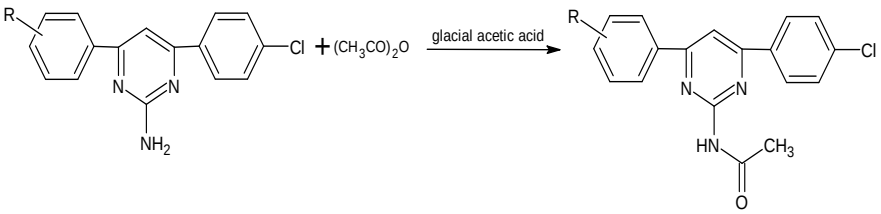
MF: $C_{16}H_{11}ClN_4O_2$; M W: 326.74; Yield:69% MP: 200°C; Rf value: 0.6; FTIR cm^{-1} : 3300,3201 (NH_2),1577 (C=N), 822 (C-Cl).

4-(4-chlorophenyl)-6-(3-nitrophenyl) pyrimidin-2-amine (2g)

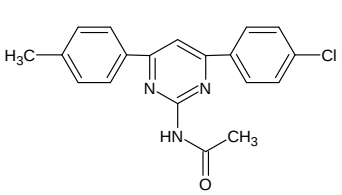
MF: $C_{16}H_{11}ClN_4O_2$; M W: 326.74; Yield:67% MP: 186°C; Rf value: 0.55; FTIR cm^{-1}: 3298,3189(NH_2), 1580(C=N),830(C-Cl).

2.1.3. Synthesis of Acetylated Pyrimidine derivatives

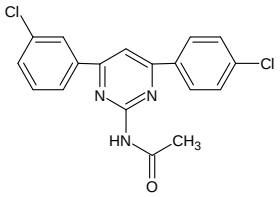
Substituted pyrimidine (0.01 mol), glacial acetic acid (5ml) and acetic anhydride (0.02 mol) were taken in a round bottomed flask. The above mixture was refluxed on a heating mantle for 2 hours. After the reaction was completed, the reaction mixture was cooled and poured into a beaker containing cold water. Solid product obtained was filtered, washed with water and recrystallized from ethanol.



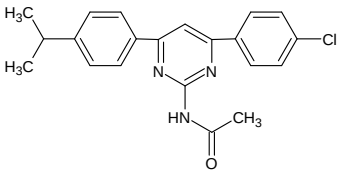
R = 4-CH_3, 3-Cl, 4-$CH(CH_3)_2$, 3,4,5 $(OCH_3)_3$, 4-OCH_3, 4-NO_2, 3-NO_2



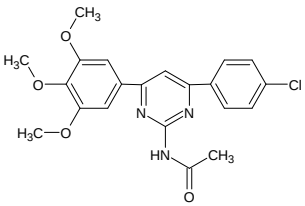
3a



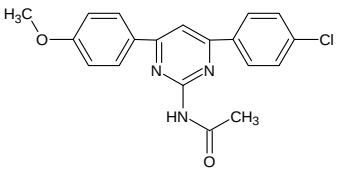
3b



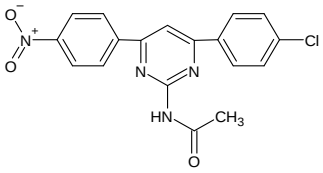
3c



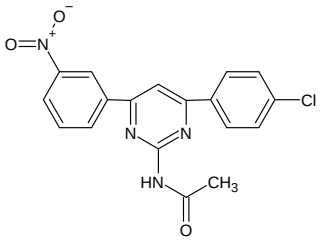
3d



3e



3f



3g

N-[4-(4-chlorophenyl)-6-(4-methylphenyl) pyrimidin-2-yl] acetamide (3a)

MF: $C_{19}H_{16}ClN_3O$; M W: 337.80; Yield:77% MP: 212°C; Rf value: 0.58; FTIR cm^{-1} : 3280 (NH), 1655 (C=O), 821 (C-Cl); ^1H -NMR, $CDCl_3$, 400 MHz : δ 8.1-8.0 (m, 5H, Ar-H), 7.7 (s, 1H, NH), 7.5 (d,2H,Ar-H), 7.5-7.2 (d, 2H, Ar-H), 2.7(s, 3H, CH_3) 2.4 (s,3H, CH_3).

N-[4-(3-chlorophenyl)-6-(4-chlorophenyl) pyrimidin-2-yl] acetamide (3b)

MF: $C_{18}H_{13}Cl_2N_3O$; M W: 358.22; Yield:70% MP: 189°C; Rf value: 0.57; FTIR cm^{-1} : 3354 (NH), 1669(C=O), 776(C-Cl); ^1H -NMR, $CDCl_3$, 400 MHz : δ 8.1-8.0(m, 4H,Ar-H), 7.7 (s, 1H,NH), 7.5-7.2 (m, 5H,Ar-H), 2.7 (s,3H, CH_3).

N-{4-(4-chlorophenyl)-6-[4-(propan-2-yl) phenyl] pyrimidin-2-yl} acetamide (3c)

MF: $C_{21}H_{20}ClN_3O$; M W: 365.86; Yield:69% MP: 178°C; Rf value: 0.55; FTIR cm^{-1} : 3250 (NH), 1663(C=O), 825(C-Cl); ^{1}H-NMR, $CDCl_3$, 400 MHz : δ 8.1-7.9 (m, 4H, Ar-H), 7.7(s, 1H, NH), 7.5-7.1 (m,5H, Ar-H), 3.0-2.9 (s, 1H, CH) 2.7 (s, 3H, CH_3), 1.3-1.2 [(d, 6H, $CH_3)_2$].

N-[4-(4-chlorophenyl)-6-(3,4,5-trimethoxyphenyl) pyrimidin-2-yl] acetamide (3d)

MF: $C_{21}H_{20}ClN_3O_4$; M W: 413.85; Yield:70% MP: 234°C; Rf value: 0.37; FTIR cm^{-1} : 3200 (NH), 1671(C=O), 824(C-Cl); ^{1}H -NMR, $CDCl_3$, 400 MHz : δ 8.1-8.0 (m, 3H, Ar-H), 7.7(s, 1H,NH), 7.5-7.2(m, 4H, Ar-H),4.0-3.9 [(d, 9H, $OCH_3)_2$], 2.7(s, 3H,CH_3).

N-[4-(4-chlorophenyl)-6-(4-methoxyphenyl) pyrimidin-2-yl]acetamide (3e)

MF: $C_{19}H_{16}ClN_3O_2$; M W: 353.80; Yield:75% MP: 205°C; Rf value: 0.5; FTIR cm^{-1} : 3107 (NH), 1661 (C=O), 827 (C-Cl); ^{1}H- NMR, $CDCl_3$, 400 MHz : δ 8.11-8.03 (m, 5H, Ar-H), 7.70 (s, 1H, NH), 7.51-7.02(m, 4H, Ar-H), 3.89 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3).

N-[4-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]acetamide (3f)

MF: $C_{18}H_{13}ClN_4O_3$; M W: 368.77; Yield:65% MP: 256°C; Rf value: 0.4; FTIR cm^{-1}: 3280(NH), 1676 (C=O), 828 (C-Cl).

N-[4-(4-chlorophenyl)-6-(3-nitrophenyl)pyrimidin-2-yl]acetamide (3g)

MF: $C_{18}H_{13}ClN_4O_3$; M W: 368.77; Yield:62% MP: 220°C; Rf value: 0.35; FTIRcm^{-1}: 3297(NH), 1666 (C=O), 820 (C-Cl).

2.2. *In-vitro* Antimicrobial activity

Antibacterial activity of the synthesized compounds was evaluated by cup-plate method[21] against Gram-positive bacteria (Staphylococcus aureus MTCC 3160, Enterococcus faecalis MTCC 9845) and Gram-negative bacteria (Escherichia coli MTCC 1687, Klebsiella pneumoniae MTCC 3040). Preparation of nutrient broth, subculture, inoculation, incubation done as per standard procedure. All synthesized compounds (1mg) was dissolved in 1 ml of dimethyl formamide (1000 µg/ml). Volumes of 50 µg/ml and 100 µg/ml of each compound was used for testing. Cups, each 9 mm in diameter, was made in the medium using a sterilized borer in a petri dish streaked with the organisms. Solutions of each test compound (50 µL and 100 µL) was added separately into the cups, and the petri dishes were then incubated at 37°C for 24h. Ciprofloxacin were used as standard drug and dimethyl

formamide used as a control which did not show any inhibition. Zone of inhibition was determined.

2.3. In-Silico studies

Molecular docking was performed using PyRx virtual screening software 0.8 to evaluate the interaction between two molecules and identify the optimal ligand orientation that forms a stable complex with minimum energy.[22] All the synthesized compounds was docked using DNA gyrase and Beta lactamase as target proteins. The protein structure file (PDB ID: 3G7E and 5FAT) are downloaded in 3D format from PDB (www.rcsb.org/pdb). The downloaded protein structures were processed in Biovia Discovery Studio Visualizer 16.1.0 by removing existing heterocyclic ligands, adding polar hydrogens, and saving them in PDB format. The compound structures were drawn in ChemSketch and saved as .mol files, then converted to. pdbqt format in PyRx using the Open Babel option. The grid was drawn around the target protein. The ligands were then docked with the protein, and the results were visualized in Biovia Discovery Studio. The protein-ligand interactions were saved in 2D format, and the binding energies were saved into an in an Excel sheet.[23]

3. RESULT AND DISCSSION

The synthesis of substituted pyrimidine derivatives carried out in three steps, in the first step chalcones were synthesized by Claisen-Schimdt condensation between *p*-chloroacetophenone and substituted aldehydes. In second step, the chalcones were then cyclised with guanidine nitrate to form corresponding pyrimidines via Michael addition reaction, in order to reduce excess ethanol usage during filtration, a little more quantity of ethanol was used in reaction mixture so that the crystals formed were almost free from the red coloured impurity. In third step, the free $–NH_2$ group of substituted pyrimidine were acetylated with acetic anhydride to get substituted pyrimidine derivatives (3a, 3b,3c, 3d, 3e,3f,3g). All final compounds were identified by analytical and spectral techniques such as thin layer chromatography, melting point, Fourier transform infrared (FTIR) and ^{1}H-NMR spectroscopy.

The IR band of 3400-3500cm^{-1} for primary amine is disappeared and conversion of secondary amine is obtained at 3350-3200cm^{-1} is an indication for formation of acetylated pyrimidine derivatives also there is a presence of C=O group confirms the sharp peak at 1660cm^{-1}. The ^{1}H NMR spectroscopy all the aromatic protons were observed between 8.1 to 7.0 ppm and NH was found merged with aromatic protons, because NH is bonded to carbonyl group so, it causes deshielding effect.

Table No 1: Antibacterial evaluation of synthesized pyrimidine derivatives.

Compound code	Zone of inhibition (mm)							
	Staphylococcus aureus		Enterococcus faecalis		Escherichia Coli		Klebsiella pneumoniae	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
3a	6	13	8	14	9	17	9	14
3b	8	15	10	17	8	12	5	11
3c	7	10	5	10	7	10	6	12
3d	-	-	7	12	6	13	6	11
3e	7	11	6	11	7	11	7	13
3f	9	14	6	13	8	14	9	16
3g	11	16	9	15	7	13	7	14
Standard 50µg/ml	22		24		20		20	
Control	-		-		-		-	

The compounds was screened for their antimicrobial activity, by cup-plate method given in table No 1. All the synthesized compounds show significant amount of activity in 100 µg/ml concentrations. The compounds 3b, 3g demonstrated significant activity against gram-positive and 3a, 3f showed significant activity against gram-negative bacteria. Compounds 3c, 3d, and 3e have a moderate activity against both gram-positive and gram-negative bacteria. The standard ciprofloxacin showed better activity than the synthesized compounds at a 50µg/ml concentration. Hence, the synthesized compounds are not as effective as the standard reference drug.

Table No 2: Drug likeness and non-drug likeness of investigational ligands.

Sl. No	Ligand	MW	HBD	HBA	LOG P	tPSA	MR	BBB	GIA
1	3a	337.80	1	3	4.66	54.18	97.19	Yes	High
2	3b	358.22	1	3	4.87	54.88	97.24	Yes	High
3	3c	365.86	1	3	3.62	54.88	106.81	Yes	High
4	3d	413.86	1	6	3.85	82.57	111.70	No	High
5	3e	353.81	1	4	4.27	64.11	98.72	Yes	High
6	3f	368.78	1	5	4.17	100.70	101.05	No	High
7	3g	368.78	1	5	4.15	100.70	101.05	No	High

MW: Molecular weight; HBD: Hydrogen bond donor; HBA: Hydrogen bond acceptor; LOG P: Lipophilicity; MR: Molar refractivity; tPSA: Tropical polar surface area; BBB: Blood brain barrier permeation; GIA: Gastrointestinal absorption.

Table No. 3: Binding energies of investigational ligands with appropriate proteins.

Sl. no	Ligand	3G7E		5FAT	
		Binding Affinity (Kcal/mol)	H-bond	Binding Affinity (Kcal/mol)	H- bond
1	3a	-8.9	-	-8.5	TRP 95
2	3b	-9.1	-	-8.4	TRP 95
3	3c	-8.8	VAL 43	-8.0	TRP 95
4	3d	-8.4	HIS116, GLY 101	-8.3	TRP 222, THR 197, MET 115
5	3e	-8.7	-	-8.1	TRP 95, VAL 85
6	3f	-9.0	THR 165, ASP 74, GLY 75, GLN 72	-8.6	LYS 192, ARG 189, ASN 179, ALA 207
7	3g	-9.8	THR 165, ASP 74, GLY 75	-8.7	PHE 126, TRP 95
8	Ciprofloxacin	-7.9	-	-6.4	TRP 222, THR 71, GLN 251

A simple method to evaluate the drug-like properties is to verify the compliance with Lipinski's rule (rule of 5), which specifies the active oral drug should have (i) not more than five hydrogen bond donors (OH and NH groups); (ii) not more than five hydrogen bond acceptors (notably N and O); (iii) molecular weight less than 500 Daltons; (iv) octanol–water partition coefficient (log P) less than 5. The tPSA values of all the compounds are within the limits indicating the cell permeability and all the synthesized compounds obey the Lipinski rule of five shown in Table No 2.

Table No 3. displays the in-silico studies were performed the synthesized compounds. The target proteins used for the study of interaction are DNA gyrase and Beta lactamase, because they are critical bacterial enzyme, beta-lactamase confers resistance by degrading antibiotics, while DNA gyrase is essential for DNA replication. Targeting them helps in designing effective inhibitors to combat bacterial infections and resistance. The two proteins (3G7E and 5FAT) were selected from literature survey and the ligand structure were drawn and made to interact with each other. The binding site of various pyrimidine derivatives with the protein is comparable to that of the standard marketed drug (ciprofloxacin), as the binding pockets of both are found at a similar site in the protein. Among the synthesized compounds 3b,3g have a better binding affinity (-9.1 and -9.8 kcal/mol), with the protein 3G7E. Compounds 3a, 3f, 3g showed good binding affinities (-8.5, -8.6 and -8.7 kcal/mol), with the protein 5FAT.

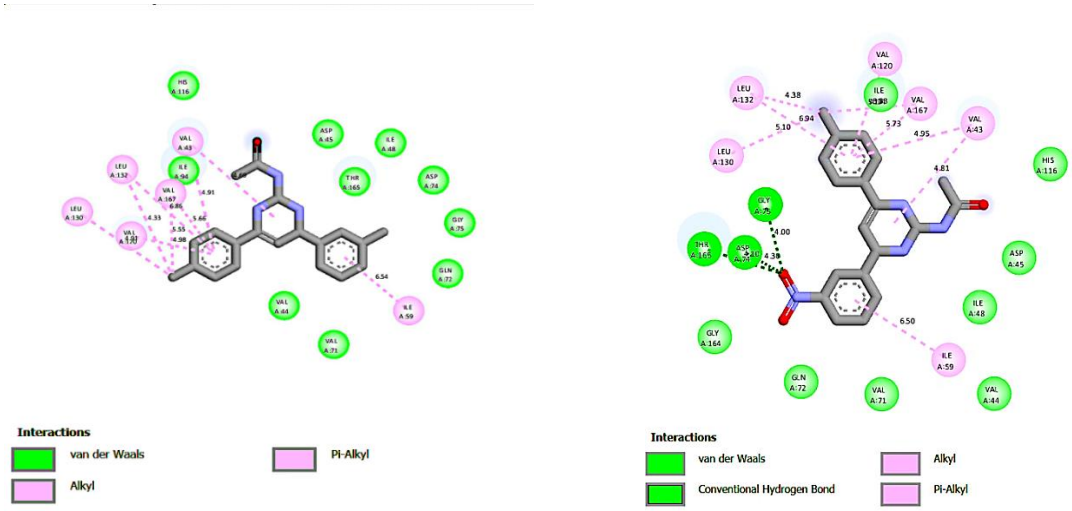


Fig 3: 2D interaction diagrams of 3b and 3g with protein (PDB ID: 3G7E).

The hydrogen bond primarily forms with the carbonyl (C=O) group of the pyrimidine nucleus, but if in case the molecule contains NO_2 the hydrogen bond prioritizes more there. Even the compounds which don't have the hydrogen bonding interaction also have a good binding affinity, this is mainly due to the van der Waals forces acting upon. The compounds containing nitro group has shown the best consistent binding affinity towards the protein. The compounds that have better binding affinity and good docking score with their target proteins. The *in-vitro* antimicrobial values are correlated with binding energy values.

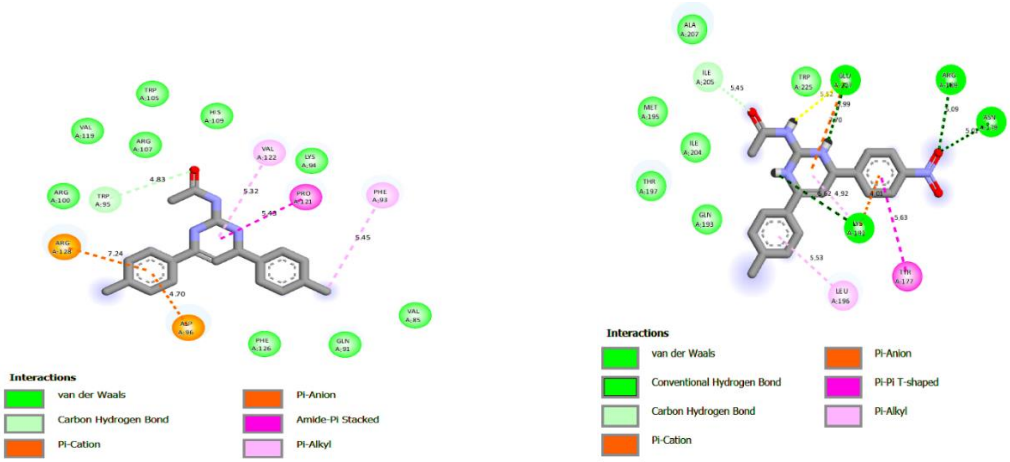


Fig 4: 2D interaction diagrams of 3a and 3f with protein (PDB ID: 5FAT).

4. CONCLUSION

Hence, the substituted pyrimidine derivatives have notable antimicrobial activity and further research should be conducted on lead optimization to enhance the antimicrobial property.

Further synthesis of few more derivatives, characterization and in-vivo activities to be carried out for the future perspective.

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