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MOLECULAR DOCKING AND *IN-VITRO* ANTIMICROBIAL ACTIVTY OF SOME NOVEL AMINOPYRIMIDINE DERIVATIVES

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ABSTRACT

The emergence of drug-resistant microbes left us with a great need for new antimicrobial agents. Aminopyrimidines, with their wide range of biological activities, are good candidates in this respect. The derivatives of the title scaffold had proven to possess great potential, inhibiting and cidal activity of microbes even at concentrations below 1 μg ml^{-1}. The substitution pattern of these aminopyrimidines often includes hydroxy groups, halogens or other heteroaromic rings, In this article, we aim to report the molecular docking and *in-vitro* antimicrobial activity of our novel derivatives **AD** **(1-6)**. Among all six novel derivatives, two of them AD_3 & AD_4 were showing significant antimicrobial activity against both the gram positive and gram negative bacteria.

KEYWORDS: 2-Aminopyrimidine derivatives, Molecular docking, Antimicrobial activity.

1.INTRODUCTION

Heterocycles are ubiquitous to among pharmaceutical compounds [1]. Pyrimidine moiety is an important class of N-containing heterocycles widely used as key building blocks for pharmaceutical agents. It exhibits a wide spectrum of pharmacophore as it acts as bactericidal, fungicidal [2], analgesic [3], antihypertensive [4] and anti-tumor agents [5]. Among these; aminopyrimidine derivatives are similarly used as for anti-inflammatory and virucidal agents [6]. Also, preclinical data from literature survey indicates continuing research in polysubstituted pyrimidine as potential anti-tumor agents [7]. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

Heterocyclic compounds plays an important role in medicinal chemistry, serving as key templates central to the development of numerous important therapeutic agents [8]. Since the 2-aminopyrimidine ring (Fig.1) is a fragment of nucleotide bases in DNA and RNA, which are the most important components of living cells, the significance of these compounds in the nature can scarcely be over estimated. Since the various novel derivatives prepared based on 2-aminopyrimidine have a broad spectrum biological activities, the organic synthesis of these compounds has attracted attention for many decades, and the development of new methods for the synthesis of these compounds is of great interest [9]. Novel, inexpensive, and relatively

expeditious procedures are available to achieve the synthesis of different 2-aminopyrimidine and derivatives. Starting from readily available compounds such as guanidine hydrochloride, urea, 1,3-dialkylurea, or thiourea [10].

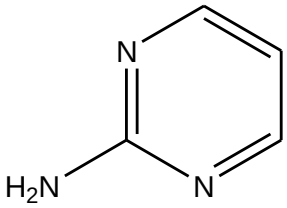


Fig.1: 2-aminopyrimidine ring

2-aminopyrimidine also called as pyrimidine-2-amine having molecular weight of 95.1 g/mol with molecular formula $C_4H_5N_3$. The chemical properties of 2-aminopyrimidine derivatives are determined by the presence of the amino group, the amidine moiety, and the aromatic character of the pyrimidine ring. Formally, pyrimidine is a cyclic amidine, which is responsible for the behavior of certain functional pyrimidine derivatives in chemical reactions. The carbon atoms in positions 2, 4, and 6 have similar reactivity, but their reactivity differs from that of the C atom in position 5 [11]. Some 2-aminopyrimidines were reported to be biologically active in low micromolar concentration to submicromolar concentration [12].

In the present scenario antibacterial agents are the greatest contribution of chemotherapy. They have great importance in the developing countries where infectious diseases predominate. 2-aminopyrimidines exhibit a wide spectrum of pharmacological activities like, antimicrobial, antitumor, cardiovascular, anti-inflammatory and antiviral. Antimicrobial drugs are the greatest contribution of 20th century to therapeutics. These are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic-resistant pathogens, fuelling an ever increasing need for new drugs. Reducing the inappropriate antibiotic use is thought to be the best way to control resistance. Although, awareness of the consequences of antibiotic misuse is increasing, overprescribing remains widespread, driven largely by patient demand.

1.1 DISC DIFFUSION METHOD

Disc diffusion methods, have great popularity in busy clinical microbiology laboratories because of their relative simplicity and ability to easily test multiple antimicrobial agents on each bacterial isolate. The method involves the placing of antimicrobial impregnated paper discs onto the surface of agar which has previously been seeded with the bacteria to be tested. The antimicrobial agent subsequently diffuses into the agar where it may inhibit bacterial growth in a zone surrounding the disk.

Principle: The extent of diffusion of the antimicrobial agent into the agar is influenced by many physiochemical properties of both the agent and the agar including its solubility, the pH, temperature and the agar concentration and depth within the plate. This diffusibility differs greatly between individual antimicrobial agents and is unique to each agent. The antimicrobial agent concentrations in the agar are the result of dynamic events with multiple variables and, hence, bear no numerical relationship to the amount of agent impregnated into the disc. The established diffusion gradient is fairly steep for up to six hours, after which progression toward equilibrium results in gradual falling off of the concentration near the disc edge. A zone of inhibition of bacterial growth results when an antimicrobial concentration in the agar equal to or greater than the minimal inhibitory concentration acts upon a critical population of bacterial organisms which have been inoculated on the agar surface. Following inoculation of the organisms on the agar surface there is a variable lag time of two to four hours before active bacterial proliferation begins. It is during this period that the zone diameter is formed and its size determined by the effective concentration of an antimicrobial agent at some specific distance from the disc edge. Beyond this specific distance, the lower antimicrobial

concentration allows the bacteria to proliferate. Subsequent incubation produces minimal change in the zone radius until the gradient drops to the point at which decreasing inhibition, in the absence of bactericidal effects, allows multiplication of the population The following conditions must be met for the screening of antimicrobial activity:

- There should be intimate contact between the test organisms and substance to be evaluated.
- Required conditions should be provided for the growth of microorganisms.
- Conditions should be same through the study.
- Aseptic / sterile environment should be maintained.
- Various methods have been used from time to time by several workers to evaluate
- the antimicrobial activity.

The evaluation can be done by the following methods:

- Turbidometric method.
- Agar streak dilution method.
- Serial dilution method.
- Agar diffusion method.

Following techniques are used as agar diffusion method:

- Agar Cup method.
- Agar Ditch method.
- Paper Disc method.

We have used the Agar cup Method to evaluate the antibacterial activity. It is one of the non automated *i-vitro* bacterial susceptibility tests. This classic method yields a zone of inhibition in mm result for the number of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in Petri plates [13]

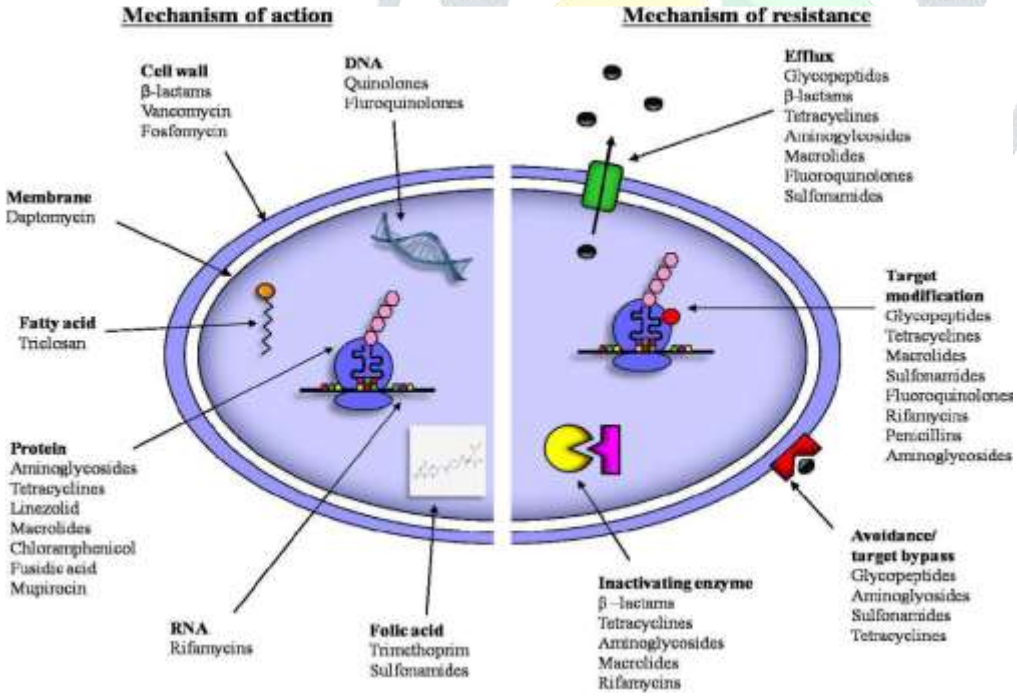


FIG 2 :Mechanism of action of antimicrobial agents

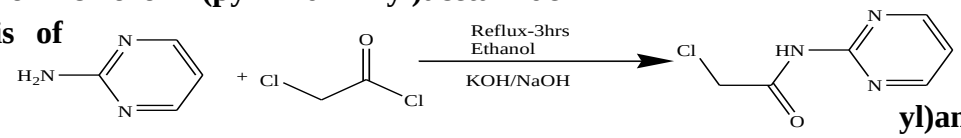
2.MATERIALS AND METHODS

2.1 CHEMICAL SYNTHESIS

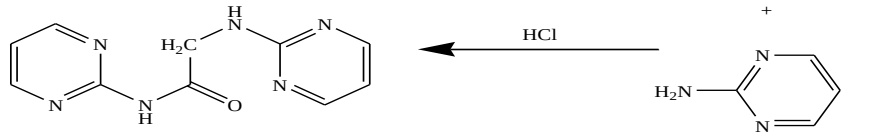
SCHEME 1

Step 1:synthesis of 2-chloro-N-(pyrimidin-2-yl)acetamide

Step 2:synthesis of N-(pyrimidine-2-yl)-2-(pyrimidine-2-yl)amino)acetamide



pyrimidin-2-amine chloroacetyl chloride 2-chloro-*N*-(pyrimidin-2-yl)acetamide

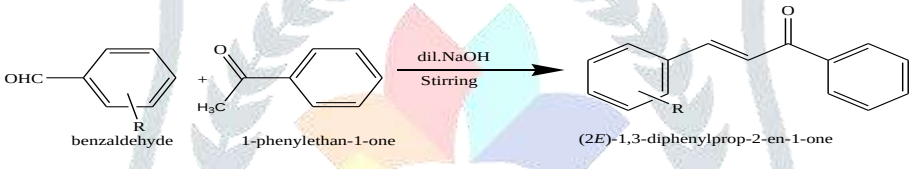


N-(pyrimidin-2-yl)-2-[(pyrimidin-2-yl)amino]acetamide pyrimidin-2-amine

SCHEME 2

step 1:Chalcone preparation

Step 2:synthesis of 2-aminopyrimidine derivtaives



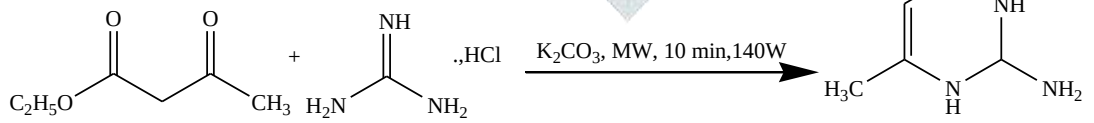
benzaldehyde 1-phenylethan-1-one (2*E*)-1,3-diphenylprop-2-en-1-one

R=H,No$_2$,OH,Cl

2-Aminopyrimidine derivatives guanidine hydrochloride

SCHEME 3

Synthesis of 2-amino-6-methyl-2,3-dihydropyrimidine-4(1H)-one



ethyl 3-oxobutanoate guanidine hydrochloride 2-amino-6-methyl-2,3-dihydropyrimidin-4(1*H*)-one

2.2 MOLECULAR DOCKING

Software used

In the present investigation, molecular docking methodology was implemented by AutoDock tools 1.4.6 and MGL tools 1.5.4 packages (The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037). Construction and energy minimization of ligands were done with Chem. Draw Ultra 8.0 and Chem3D ultra 8.0 (Cambridge Soft.Com, 100 Cambridge park drive, Cambridge, MA 02140, USA) respectively. Missing residues of the enzyme were corrected by PDB2PQR online software. In this study, AUTODOCK4.2 software was used to establish a ligand-based computer-modeling algorithm for

the prediction of binding energy and calculation of inhibition constants of the designed derivatives were done.The Pyrazinamide and 5-flurouracil were kept as the standards. The docking results provided the binding affinities and corresponding predicted inhibition constants (Ki) of the designed 2-Aminopyrimidine analogues could be compared with standard gentamycin.

Preparation of Enzyme Structure [15]

Preparation of Ligands [16]

Docking methodology [17]

AutoDock Vina, a new program for molecular docking and virtual screening is presented. Autodock vina is done by two methods

a) Autodock vina using PyRx virtual screening tools

b) Autodock vina using mgltools

AUTODOCK VINA USING PyRx virtual screening tools

PyRx is open source software to perform virtual screening, it's a combination of several softwares such as Autodock Vina, Autodock 4.2, Mayavi, Openbabele.t.c. PyRx uses Vina and Autodock 4.2 as docking softwares.

Step 1: Loading molecules into PyRx workspace

Step 2: Converting .pdb files to .pdbqt files (vina input file format)

Step 3: Selecting Vina search space

Step 4: Exporting vina results

AUTODOCK VINA BY USING vina.exe

Softwares used in this method are vina.exe, is docking software execute docking and give binding energy of ligand with protein. And also uses pymol for chemical visualization and preparation of protein pdb files for docking.

Step 1: Pymol Protein Preparation

Step 2: Preparation of .pdbqt files from pdb files using ADT

Step 3: Preparation of input file for docking (vina run) using a word file: conf.txt

Step 4: Vina Run Procedure

For both Autodock vina and Autodock vina with PyRx, it is visualized by Biovia Discovery Studio client 2020.

2.2.ANTIMICROBIAL ACTIVITY

IN-VITRO BIOLOGICAL SCREENING

The entire synthesized substituted pyrimidine derivatives (**AD_{1-6}**) were subjected to *in-vitro* biological screening to study their effects on the microbial flora. The activities were studied under this category as follows.

ANTIBACTERIAL ACTIVITY

Materials and methods

All the synthesized test compounds were tested for their antimicrobial activity against various bacterial strains like gram positive bacteria's; *Staphylococcus aureus*, gram negative bacteria's; *Escherichia coli* (NCIM 2809). All the strains used for bacteria were collected from the Department of Biotechnology, Grace college of pharmacy, Palakkad, 678004. All the strains used were pure cultures purified and preserved by the method of Raistrick and Hetherington as stab slant cultures at a temperature of 4ºC. The test compounds were tested for their zone of inhibition against gram positive bacteria; *Staphylococcus aureus*, gram negative bacteria; *Escherichia coli*. The present investigations were undertaken to test whether there is any antimicrobial activity. Finally, the antimicrobial potency of these compounds was assured by standard method (agar

diffusion or disc diffusion technique) against the same gram positive and gram-negative strains and the results so obtained with the standard antibiotic Gentamycin.

Nutrient media get prepared.All the appropriately weighed quantities of ingredients were dissolved in the specified quantity of distilled water. The pH was adjusted to 7.2–7.4 and was sterilized in an autoclave at 15 psi pressure and 121 ºC for 20 minutes.

Nutrient agar media were prepared.All the appropriately weighed quantities of ingredients were dissolved in the specified quantity of distilled water. The pH was adjusted to 7.2-7.4 and was sterilized in an autoclave at 15 psi pressure and 121 °C for 20 minutes. Then slant was prepared by keeping the hot sterilized test tubes containing Nutrient Agar at 10-15 °C.

Preparation of standard and test stock solution:

Test compounds and standard drug gentamycin were dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 2000µg/mL.

Determination of Zones of Inhibition by disc Diffusion Technique

In this method, pure Gentamycin was taken as the standard antibiotic for the comparison of the results. Stock solutions (each of 1 mg/mL concentration) of both compounds and that of the standard antibiotic were prepared. From these stock solutions, two dilutions (100µg/mL) of compounds in DMSO and Gentamycin (100µg/mL in sterile distilled water) were prepared in sterile McCartney bottles. Antibacterial activity was determined by disc diffusion method employing 24 h cultures of test organisms. Sterile nutrient agar plates were prepared and incubated 35-37ºC for 24 hours to check. *Staphylococcus aureus* (ATCC-6633), gram negative bacteria's; *Escherichia coli* (NCIM 2118), for contamination if any. Each sterile nutrient agar plate was then flooded with the corresponding peptone culture of the test organism, dried for 30 dminutes and after drying off the flooded plate, wells were made using a cork borer (size-3) on the solidified medium. Prepared wells were filled with respective dilutions (100 µg/mL) of the test and standard compounds and marked as quadrants at the back of the plates. The same technique was repeated in the case of the remaining test organisms for both the compounds and the standard antibiotic. All the flooded plates were incubated at 35-37ºC for 24 hours. The diameters of the zones of inhibition were measured in mm and their means were compared accordingly

3. RESULTS AND DISCUSSION

3.1. Docking studies for Anmicrobial activity

Docking of 1AB4 protein with 6 compounds were done by AUTODOCK VINA software and dock scores of these molecules were represented in (Table 5.1), with their binding affinity and types of bonds with which different amino acids bonded to the ligand's different functional groups. Binding affinity of the protein-ligand interactions are important to describe how fit the drug binds to the target macromolecules.

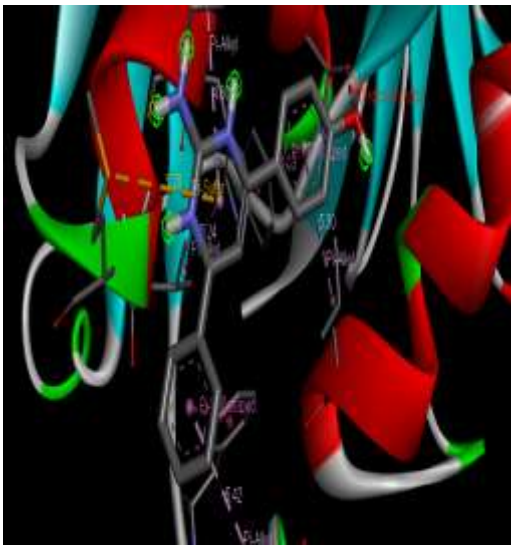
In Auto dock study of 6 compounds were screened against specific target protein 1AB4 was carried out. The compounds were docked at the active site of the protein and by analysing it we can calculate the compounds that have maximum binding energy compare with standard (Gentamycin).

Table.3.1.1:. The interaction energies (kcal mol-1) of 1AB4 and ligands obtained from the molecular docking with AUTODOCK

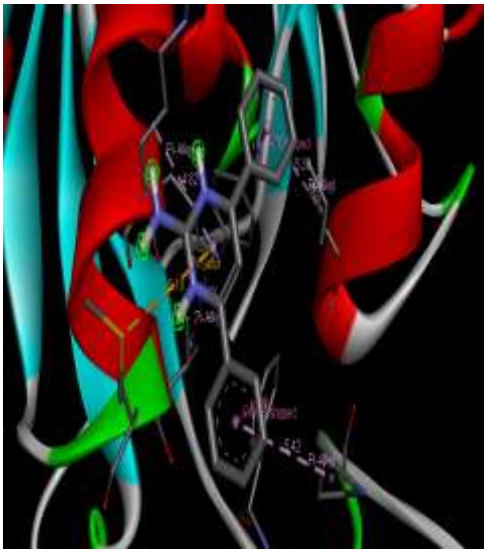
Compound code	Autodock vina(Pyrex)	H-bonds	Vander waal forces	Unfavourable donor-donor	Pi-Pi T shaped	Pi-alkyl	Pi-sulphur	RMSD
AD1	-6.0	GLUA:296,SERA:2,GLNA:3, ARGA:293.	ARGA:289,PROA:4,SERA:5, ALAA:7,GLYA:299,LEUA:295 , LEUA:295					0.00
AD2	-4.8	ARGA:293,GLUA:296,GLNA:3,PROA:4,SERA:5.	LEUA:297,SERA:2,ALAA:7					0.00
AD3	-7.0				PHEA:11,TRPA:182.	VALA:186,ARGA:183,LEUA:297,PROA:4	META:187	0.00
AD4	-7.7		GLYA:269,ARGA:293,ILEA:270.		PHEA:11,TRPA:182	PROA:4, VALA:186,LEUA:297,ARGA:183	META:187	0.00
AD5	-6.8	SERA:2,LEUA:300.		ASNA:8,GLNA:3.		ALAA:7 ,PROA:302,LEUA:295.		0.00
AD6	-6.9	ARGA:183	ILEA:270,GLYA:269,ALAA:268.		PHEA:11,TRPA:182	PROA:4, VALA:186,LEUA:297,ARGA:183	META:187	0.00
STANDARD	-6.8	GLUA:296,ARGA:293,GLNA:3.	ARGA:289,PROA:4,ASNA:8, SERA:2,SERA:5					0.00

Fig 3.1. Docking Poses of AUTODOCK VINA (Docking pose between lead compounds& standard and 1AB4)

AD_3

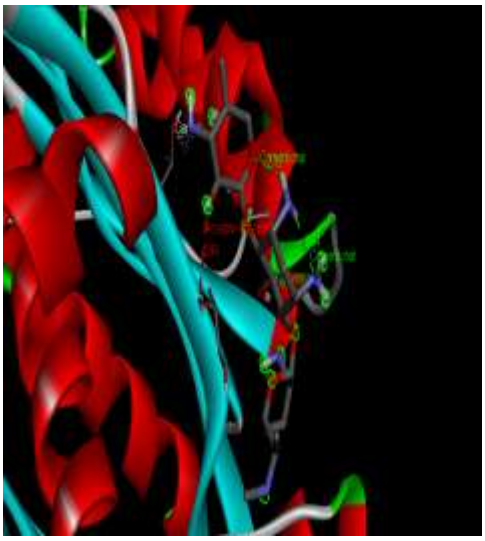


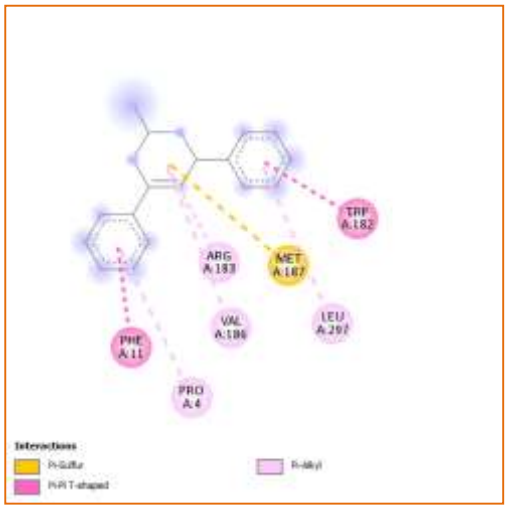
AD_4



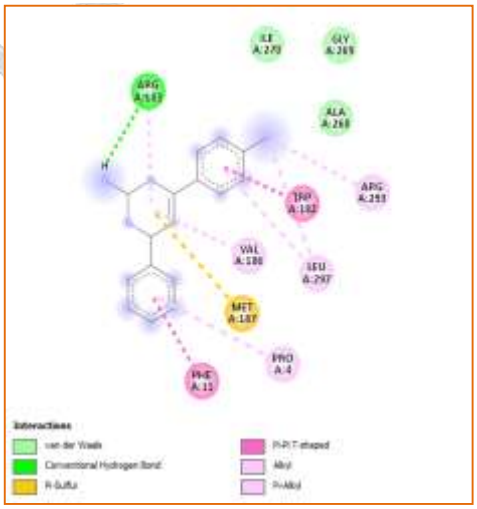
AD_6

STANDARD

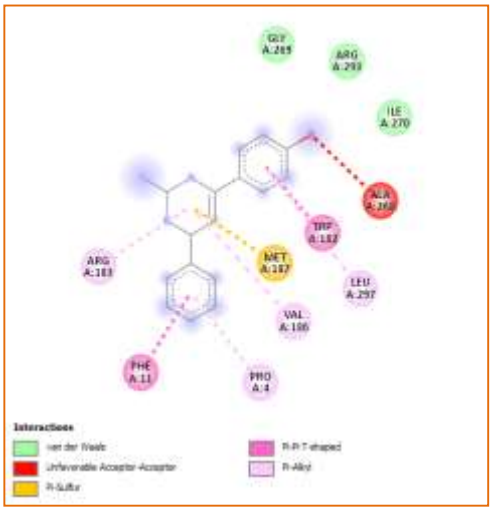




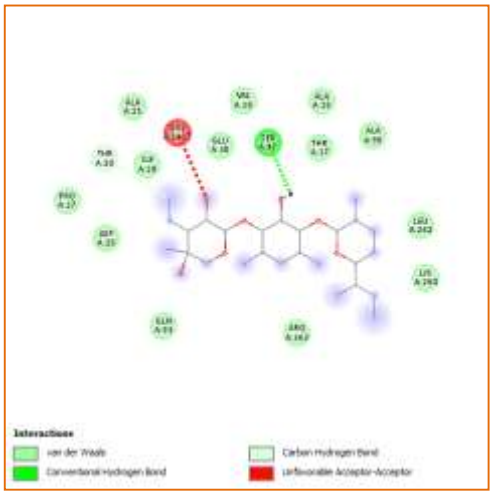
AD_3



AD_4



AD6



STANDARD:

3.2.*In-vitro* antimicrobial activity

Table 3.2.1: Antibacterial activity of test compounds (AD3,AD4,&AD6)against *Escheria.coli*

Sl no	Compound code	Concentration (µg/mL)	Zone of inhibition (mm)	
1	AD3	100	17	15
		200	15	13
		300	13	10
		400	10	8
2	AD4	100	8	8
		200	8	8
		300	8	8
		400	8	8
3	AD6	100	11	11
		200	9	10
		300	7	11
		400	7	11
4	STANDARD (Gentamycin)	100	18	-
5	DMSO/CONTROL	2000	8	-
6	BUFFER		6	-

Table 3.2.2: Antibacterial activity of test compounds (AD3,AD4 &AD6)against *Styphylococcus aureus*

Sl no	Compound code	Concentration (µg/mL)	Zone of inhibition (mm)	
1	AD3	100	7	7
		200	6	6
		300	6	7
		400	7	5
2	AD4	100	6	6
		200	6	6
		300	6	6
		400	6	6
3	AD6	100	6	7
		200	6	6
		300	7	6
		400	7	7
4	STANDARD (Gentamycin)	100	12	-
5	DMSO/CONTROL	2000	6	-
6	BUFFER		8	-

CONCLUSION

In the present attempt, all the newly synthesized 2-aminopyrimidine derivatives were having antibacterial activity against both the strains of gram positive and gram negative bacteria including *Etyphylococcus aureus* and *Escheria coli*.In our 6 derivatives , AD_3, AD_4 ,AD_6 were having significant antibacterial activity.AD_3 and AD_6 were having high activity at 100 µg/mL against both gram positive and gram negative bacteria *styphylococcus aureus* and *escheria coli* .

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CONFLICTS OF INTEREST: The authors declared that they have no conflicts of interest.

REFERENCE

1. Krakow G, Vennesland B. The Equilibrium Constant of the Dihydroorotic Dehydrogenase Reaction. J. Biol. Chem: 236, 142-144, 1961.
2. Brown D J, Katrizsky, A R Rees, C W Boulton, A J Mckilop A. Comprehensive Heterocyclic chemistry.1984;3: 57-155.
3. Kenner G W, Todd A, Elderfield, R C John Wiley. Heterocyclic Compounds. 1957:234.
4. McCall JM, Kloosterman D, Kamdar BV. Cyanoimine chem: New routes to pyrimidinones and (carbonylamino) iminopropamides. J org Chem. 1979, 44: 1562- 1563.
5. Vinayak ND, Wakefieldm BJ. Aminopyrimidine. J Chem Res. (synopsis) 1983: 200.
6. Butter M. Large scale synthesis of 4-ethylpyrimidine. J. Het Chem 1992;29 1369-1370 **7.** Butter M. Large scale synthesis of 4-ethylpyrimidine. J. Het Chem 1992; 29
7. Butter M. Large scale synthesis of 4-ethylpyrimidine. J. Het Chem 1992; 29 1369-1370.
8. V.M. Barot and S.D. Desai. Synthesis of 2-aminopyrimidine derivatives as
9. antimicrobial agents. International Journal of Chemical Science: 2013, 11(2), 865-872.

10. Williams M, Strategies for Drug Discovery, NIDA Research Monograph, 1993; 132: 122. -143. **10.** Soloman AK. Molecular modelling and drug design.2nd edition (Chennai), MB publisher, 2008
11. Alvarez, M.A., Zarelli, V.E.P., Pappano, N.B. and Debapttista, N.B. (2004) Bacteriostatic action of synthetic polyhydroxylated chalcones against Escherichia coli. Biocell 28, 31–34.
12. Ayman, M., El-Messery, S.M., Habib, E.E., Al-Rashood, S.T., Almehizia, A.A., Alkahtani, H.M. and Hassan, G.S. (2019) Targeting microbial resistance: synthesis, antibacterial evaluation, DNA binding and modeling study of new chalcone-based dithiocarbamate derivatives. Bioorg Chem 85, 282–292.
13. Avila, H.P., Smania, E.F.A., Monache, F.D. and Smania, A. Jr (2008) Structure–activity relationship of antibacterial chalcones. Bioorg Med Chem 16, 9790–9794.
14. Safaei-Ghomi J, Ghasemzadeh MA. AN EFFICIENT ROUTE TO THE SYNTHESIS OF PYRIMIDINE-2-ONES UNDER ULTRASOUND IRRADIATION. Digest Journal of Nanomaterials & Biostructures (DJNB). 2010;5(2).
15. Koroleva EV, Gusak KN, Ignatovich ZV. Synthesis and applications of 2-aminopyrimidine derivatives as key intermediates in chemical synthesis of biomolecules. Russian Chemical Reviews. 2010;79(8):655.
16. Bayramoğlu D, Kurtay G, Güllü M. Ultrasound-assisted rapid synthesis of 2-aminopyrimidine and barbituric acid derivatives. Synthetic Communications. 2020;50(5):649-58.
17. Hoffelner M, Hassan U, Seebacher W, Dolensky J, Hochegger P, Kaiser M, Maeser P, Saf R, Weis R. New 2-aminopyrimidine derivatives and their antitrypanosomal and antiplasmodial activities. Monatshefte für Chemie-Chemical Monthly. 2020;151(9):1375-85.
18. Singh N, Pandey SK, Anand N, Dwivedi R, Singh S, Sinha SK, Chaturvedi V, Jaiswal N, Srivastava AK, Shah P, Siddiqui MI. Synthesis, molecular modeling and bio-evaluation of cycloalkyl fused 2-aminopyrimidines as antitubercular and antidiabetic agents. Bioorganic & medicinal chemistry letters. 2011;21(15):4404-8.