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SYNTHESIS, EVALUATION AND BIOLOGICAL SCREENING OF CYCLOHEXANONE DERIVATIVES BEARING AZETIDINE SCAFFOLD AS AN ANTIMICROBIAL AGENT

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ABSTRACT

Synthesized compounds were screened for antimicrobial activity by poar plate method. The potency of this present work deals with synthesis, characterization and biological activity of some new Azetidinones derivatives derived from cyclohexanones. Different Schiff bases derivatives were obtained from different substituted or unsubstituted anilines upon treatment acetamide with toluene. A Schiff base on reaction with chloroacetyl chloride gives the different azetidinones. The final derivatives of Azetidinones were obtained by reaction with chloro acetyl chloride which gives different types of Azetidinones derivatives. The structure of the newly synthesized compounds has been established on the basis of their spectral data and elemental analysis. The docking study has done of all compounds by using 3U2D *S. aureus* GyrB ATPase domain in complex with the small molecule inhibitor. The synthesized compounds were determined against standard drug Amoxicillin by measuring the zone of inhibition and calculating the MIC.

Keyword: Azetidinones, Schiff Base, Antimicrobial activity

1. INTRODUCTION

One of the milestones in medicinal chemistry is the discovery of simple four membered β-lactam ring which a lactam with a heteroatomic ring structure, consisting of three carbon atom and one nitrogen atom **[1-5]**. A lactam is a cyclic amide which is a part of the structure of several antibiotic families, principally the penicillins, cephalosporins, carbapenems and monobactams, which are therefore also called β- lactam antibiotics **[5-10]**. A large number of 3-chloro monocyclic β-lactams possess powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant, antitubercular activity. They also function as enzyme inhibitors. The activities of a large number of 2-azitididones containing β-lactam moieties are greatly influenced by different substituents **[11-15]**. The 2-azetidinone derivatives had reported to possess various types of biological activities i.e. antimicrobial, anticancer, antitubercular, anti-inflammatory, anticonvulsant, anti-diabetic, antiviral, cholesterol absorption inhibitor, tryptase and chymase inhibitor, vasopressin via antagonists and fatty acid amide hydrolase and a large number of 3-chloro monocyclic beta lactum possess powerful antibacterial, anticonvulsant, antitubercular, and analgesic Properties **[16, 17]**. Schiff bases are the most widely used organic compounds having a wide variety of applications in many fields, e.g. biological, inorganic and analytical chemistry. Schiff bases, derived mostly from variety of heterocyclic rings, have been reported to possess a broad spectrum of pharmacological activities. The development of new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemist because they are known to exhibit a variety of potent activities **[18-25]**.

2. MATERIALS AND METHODS

2.1 Materials:

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avra labs. The progress of reactions was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F254 aluminum sheets and visualized by UV light. The melting points were determined using melting point apparatus VEEGO MODEL VMP-D and are uncorrected. IR spectra were recorded on FT-IR8400S, Fourier Transform (Prestige) Infrared spectrophotometer (Schimadzu, Kyoto, Japan). The 1H-NMR spectra of synthesized compounds were recorded on Bruker Advance II 400 NMR

Spectrometer (Billerica, MA, USA) at 400 MHz frequency in deuterated DMSO with tetramethylsilane as an internal standard **[26-32]**.

2.2 Methods:

2.2.1 Preparation of Parent Compounds:

Charge 30 gm 4-aminocyclohexanone (1.0 Mol) in dry 150 ml DCM in RBF and cool to 0-5°C added 29.5 gm triethylamine (1.1 Mol) under stirring, then added 22.36 gm acetyl chloride in 90 ml DCM in 30 min, stirred for 3 hr. after completion of reaction by TLC (Ethyl acetate: Hexane :2:8) 240 ml added water, collect organic layer and again wash with 150 ml aq. 5% sodium bicarbonate and 150 ml water, after distillation of DCM get off white color solid compound **[33]**.

2.2.2 Preparation of Imine Compounds:

Charge 2 gm N-(4-oxocyclohexyl) acetamide (1.0 Mol) and aniline derivative (1.0 Mol) in 10 ml toluene, reflux for 12 hr. after completion of reaction by TLC (Ethyl acetate: Hexane :2:8) 240 ml added water, collect organic layer and distilled out toluene to get compound **[34]**.

2.2.3 Preparation of Azetidinone Compounds:

Charge 1 gm Schiff base derivative in 5 ml DCM and added Triethyl amine (2.2 Mol) in RBF then added chloro acetyl chloride (1.1 Mol) in 3 ml DCM in 15 min, stir for 3 hrs. After completion of reaction by TLC (Ethyl acetate: Hexane: 2:8) 240 ml added water, collect organic layer and distilled out DCM to get compound **[35]**.

2.2.4 Antimicrobial susceptibility testing:

Synthesized azetidinone derivative were subjected to antimicrobial susceptibility testing by well diffusion method against Gram positive *Staphyllococcus aureus,* and Gram negative *Escherichia coli* and *Pseudomonas aeruginosa. By* well diffusion method and minimal inhibitory concentration was determined **[36-38]**.

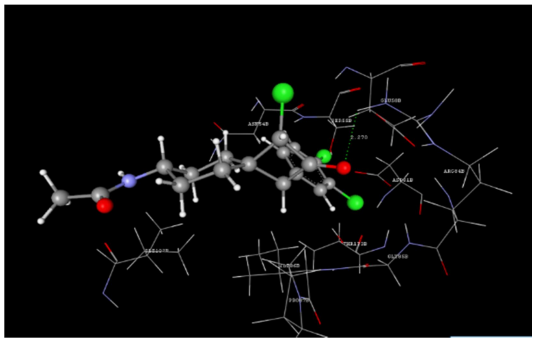
Well diffusion method: Well diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from well, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Well diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the well **[39]**.

2.2.5 Determination of minimum inhibitory concentration: Another approach to antimicrobial susceptibility testing is to determine minimum inhibitory concentration (MIC) using tube micro dilution procedure. Concentration of an antimicrobial agent that is effective in preventing the growth of the microorganism and gives an indication of the dosage of the

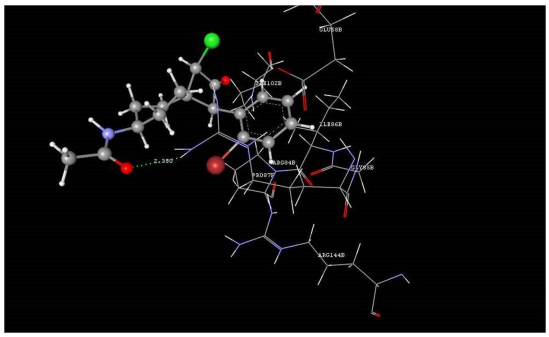
antimicrobial agent that should be effective in controlling the infection in the patient **[40-45]**.

3. RESULTS AND DISCUSSION

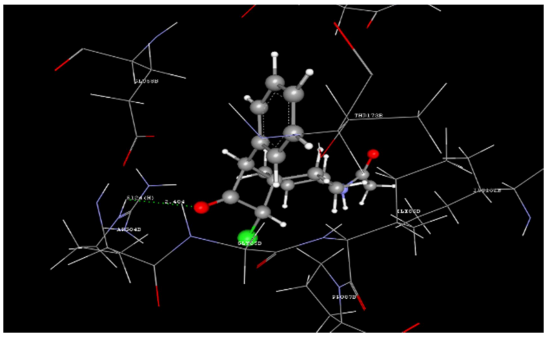
1. Docking study:



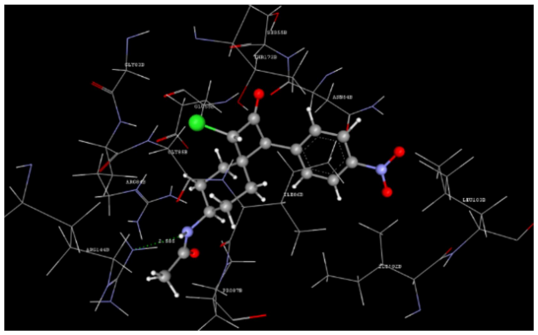
Compound A



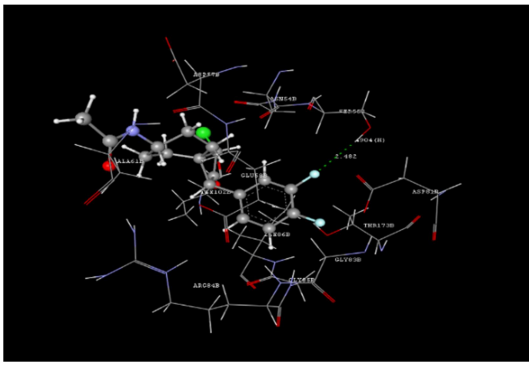
Compound B



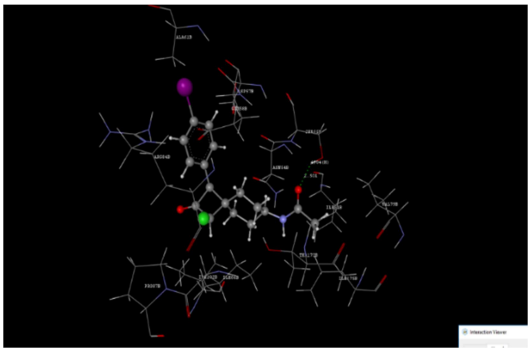
Compound C



Compound D



Compound E



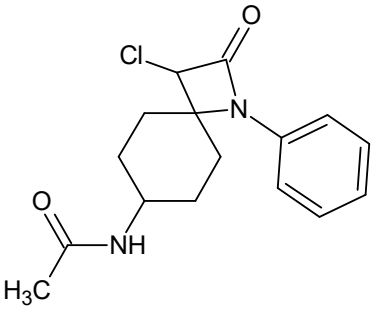
Compound F

Figure 1: Docking studies graphs

Table 1: Docking Protocol: Grip Based Docking

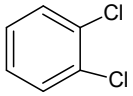
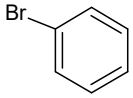
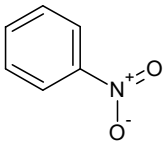
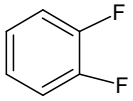
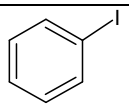
Molecule CODE	Interactions			Binding Energy kcal/mol
	Hydrogen Bond	Hydrophobic Interaction	Van der waal interaction	
A1	ARG84	ILE102 PRO87 ILE86	ASN54 SER55 GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 THR173	-57.96
B2	ARG84	PRO87	GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 ARG144	10
C3	ARG84	ILE102 PRO87 ILE86	ASN54 SER55 GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 THR173	-54.60
D4	ARG84	ASN54 SER55 GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 THR173	ASN54 SER55 GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 THR173	-67.42
E5	SER55	ASN54 SER55 ASP57 GLU58 ALA61 ASP81 ARG84 GLY85 ILE86	ASN54 SER55 GLU58 ASP81 ARG84 GLY85 ILE86 PRO87 ILE102 THR173	-55.43
F6	SER55	ASN54 SER55 ASP57 GLU58 GLY85 ILE86	ASN54 SER55 GLU58 GLY85 ILE86 PRO87 ILE102 THR173	-58.61

2. Characteristic data of Azetidinone :

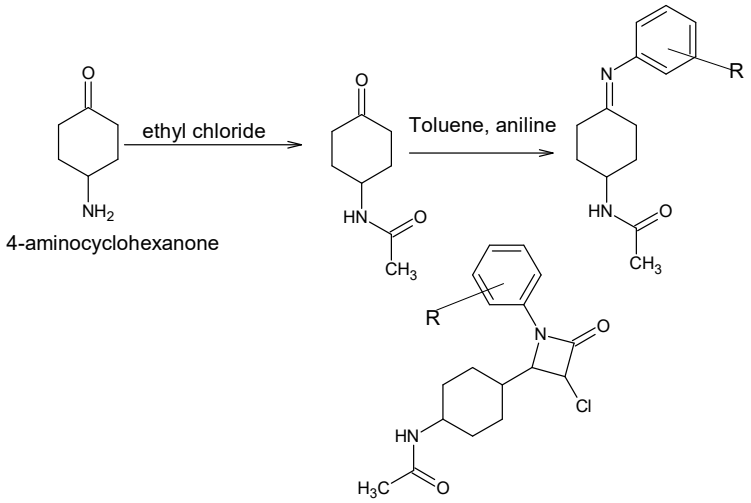


N-(3-chloro-2-oxo-1-phenyl-1-azaspiro[3.5]non-7-yl)acetamide

Table 2: Details of substituents [46-57]

S. No.	Compound code	R1	Molecular formula	Molecular weight	Melting Point
1	A		$C_{16}H_{17}Cl_3N_2O_2$	375.677	123^0C
2	B		$C_{16}H_{18}BrClN_2O_2$	309.201	80^0C
3	C		$C_{16}H_{19}ClN_2O_2$	306.78	162^0C
4	D		$C_{16}H_{18}ClN_3O_4$	351.78	135^0C
5	E		$C_{16}H_{17}ClF_2N_2O_2$	342.76	128^0C
6	F		$C_{16}H_{18}ClIN_2O_2$	432.68	140^0C

Synthesis Scheme:



4-aminocyclohexanone

N-[4-(3-chloro-4-oxo-1-phenylazetidin-2-yl)cyclohexyl]acetamide

DISCUSSION

The synthesized azetidinone derivatives shown good anti-bacterial activity. Here when the two moieties are fused and screened for anti-bacterial studies they showed a broad spectrum of antibacterial activity. They showed good activity against Gram (+ve) and Gram (-ve) bacteria. The azetidinone molecules are responsible for antibacterial activity, but it was also interesting to note that azetidinone moiety when fused with the other moieties showed broad spectrum antibacterial activity. The above results establish the fact that azetidinone moiety can be a rich source for various pharmacological activities. Therefore in search of new generation of antibiotics it bearing both Schiff bases and azetidine scaffold has the possibility to increase the potency of the moieties.

4. CONCLUSION

To design novel azetidine scaffolds, docking studies were carried out on the series of already synthesized azetidine derivatives. As protein-ligand interaction plays a significant role in structure-based drug design, we thought it worthwhile to dock the designed azetidine scaffolds against, a gyrase enzyme. Thus, in this study, molecular docking was applied to explore the binding and to correlate its docking score with the activity. The results of our present study can be useful

for the design and development of novel compounds having better inhibitory activity against several types of bacterial infection that involve overexpression of gyrase enzyme. This compound may serve as promising lead or candidate to design novel antibacterial agents. Thus, the designed compounds were subjected to synthesis compound its spectral characterization was done. All azetidine scaffolds derivatives were synthesized by various aromatic aniline in presence of chloroacetyl chloride, glacial acetic acid, triethylamine and 1, 4-dioxane.

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