Molecular Docking Studies, Synthesis, Characterisation, and Evaluation of Azetidine-2-One Derivative

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ABSTRACT:
Azetidinone derivatives were reported to possess antibacterial, antifungal, antitumor, antitubercular activity, anti-HIV, analgesic, anti-inflammatory, and ulcerogenic activity. Molecular docking has become an important process in the course of drug discovery and docking aims to predict the binding mode and binding affinity of the protein-ligand complex. AutoDock is a popular non-commercial docking program that docks a ligand to its target protein and performs well (accurate and computationally fast). The Discovery Studio Visualizer is a free viewer that can be used to open data generated by other software in the Discovery Studio product line. A new series of Aztifdine-2-one derivative were synthesized by the reaction of Schiff base((Z)-N_(4-dimethylamino) benzylidene) pyrimidine-2-amine) with chloroacetyl chloride respectively. The chemical structures of the synthesized compounds were confirmed using IR, 1H-NMR. The synthesized compounds showed mild antibacterial activity against Staphylococcus aureus and Escherichia coli. The 5NI9 protein that was extracted from the protein database website all the compounds were drawn using the Chem draw Ultra 8.0 and Biovia draw 2018. It was saved in Mol format and then converted to PDB format using the Smiles Online Translator. All the files were logged in the Cygwin toolings. The conformation by ranking was calculated. This shows whether the compound has good binding energy with the ligand and hydrogen bonding and hydrophobic binding with the receptor. From the above analysis, it showed that compound 1 showed hydrogen bonding interactions when compared to that Standard compounds Rifampicin and isoniazid which had lesser hydrogen bonding interactions. The compound 2 did not show any hydrogen bonding. This shows that the Azetidine-2-ones shows antitubercular activity with the Enoyl-acyl carrier protein (enoyl-ACP) reductase enzyme.

KEYWORDS: Molecular docking, Azetidine-2-one derivatives, antitubercular activity, Enoyl-acyl carrier protein. Antibacterial, Chloroacetyl chloride, Schiff Base.

1. INTRODUCTION:

Azetidinone and Thiazolidinone derivatives were reported to possess antibacterial[1-2], antifungal[1-2]. Pyridine derivatives were reported to possess antimicrobial[3] activities. Therefore it was envisaged that compounds containing both the chemical moieties would result in compounds of interesting biological activities.

In this present study, The Schiff bases were subjected to addition reaction with chloroacetyl chloride in the presence of 1,4dioxane, triethylamine to produce azetidinone derivative respectively[6]. The chemical structures of the synthesized compounds were confirmed using IR, 1H-NMR. The synthesized compounds were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*.

Molecular docking has become an important process in the course of drug discovery and docking aims to predict the binding mode and binding affinity of the protein-ligand complex.

Insilico Docking studies means conducted or produced utilizing computer modeling or computer simulation. Based on the literature survey, one enzyme was selected for the insilico docking studies namely the Enoyl-acyl carrier protein (enoyl-ACP) reductase enzyme. The protein would be downloaded from the Protein database and the necessary studies would be done systematically and methodically.

Shrinivas D. Joshi [4] stated that a New series of quinoline derivatives were synthesized from 2-chloroquinoline-3-carbaldehydes. In the reaction sequence, substituted acetanilides were cyclized to give 2-chloroquinoline-3-carbaldehydes which were cyclized to give azetidinones. The key scaffolds viz., 2-methoxy derivatives obtained were converted to target Schiff bases and azetidinones with good yields. Structures of these compounds were established by FTIR, 1H NMR, 13C NMR, and mass spectrometry. The compounds were evaluated for in-vitro antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Vibrio cholera* and antitubercular activity against *Mycobacterium tuberculosis* H37Rv.. The Schiff bases and azetidinone derivatives exhibited good antibacterial and antitubercular activities. Bacterial enoyl ACP-reductase catalyzes the final step in each cycle of bacterial fatty acid biosynthesis and is an attractive target for the development of new antimicrobial agents. Molecular docking into the active site of enoyl ACP-reductase was performed on 2H7M.PDB and 4JX8.PDB files to understand ligand-protein interactions. The compounds obtained from the present research can be used as scaffolds in the fragment-based design of new potent drugs. Graphical Abstract Molecular modeling, synthesis, spectral, antimicrobial studies of quinolinyl Schiff bases and azetidinones using the crystal structure of E. coli and M. tuberculosis enoyl ACP-reductase (4JX8/2H7M PDB) and compared with invitro antimicrobial activity.

2. CHEMISTRY:

The melting points were taken in an open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on Win-Bommen B-104 IR Spectrophotometer with KBr pellets. 1H-NMR spectra were recorded on Bruker A VIII 500 MHz NMR Facility using DMSO-d6 as a solvent. The chemical shifts are reported as parts per million downfield from tetramethylsilane (Me4Si). The purity of the compounds was checked by TLC on precoated aluminum sheets(Silica gel 60 F254) using benzene and alcohol as mobile phase and visualized by iodine vapors.

3. General Methods of Synthesis of Azetidine-2-one derivative (A1)[5-7]

Chloroacetyl chloride (0.01mol) was added dropwise to a mixture of Schiff base (0.01mol) and triethylamine (0.02mol) in dioxane (25ml) at room temperature. The mixture was stirred for 8 hours and allowed to stand at room temperature for 3 days. The contents were poured on crushed ice and the precipitate obtained was filtered, washed with 10% w/v sodium bicarbonate solution, vacuum dried and recrystallized using absolute ethanol.

Synthetic Scheme

IR (KBr) cm^{-1}:3423(NH),1638(C=O amide),1400 (CH bending), 1119(CH-N)

603(w), 748(w),1 119(s), 1400(s), 1638(s), 2089(m) 2264(w), 2502(w), 2688(w), 2796(w), 3423 (w)

1H-NMR
(DMSO-d6) : 7.2-7.6(m; CH Pyrimidine), 6.7-6.9(m, CH benzene),4.2 (s, 1H; CH), 8.5(s; NH amide),3.5 (s.CH methyl), 4.2 (s,CH methyl)

4. ANTIMICROBIAL ACTIVITY:

The antibacterial activity of the synthesized compounds was tested against gram(+) bacteria (Staphylococcus aureus and gram(-) bacteria (Escherichia coli) using Nutrient agar medium.

4.1 Cup Plate method[9]:

Inoculate a previously liquefied medium appropriate to the assay with the requisite quantity of a suspension of the microorganism, add the suspension to the medium at a temperature between 40 to 50^0C and immediately pour the inoculated medium into Petri dishes to give a depth of 3-4mm. Ensure that the layers of a medium are uniform in thickness by placing the dishes or plates on a level surface. The prepared dishes must be stored in a manner to ensure that no significant growth or death of the test organism occurs before the dishes are used and that the surface

of the agar layer is dry at the time of use. The cavities in the agar plates are prepared by using a metal borer. The cavities formed must be uniform throughout the dish. Apply the solutions to the surface of the solid medium in sterile cavities prepared in the agar medium. The volume of solution added to each cavity must be uniform and sufficient almost to fill those holes when these are used. Leave the dishes standing for 1-4 hrs at room temperature or at 4^0C as appropriate as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. Then the plates are incubated at 37 ± 1^0C for 24 hrs and observed for antibacterial activity. The diameter of the zone of inhibition was measured for the plates in which the zone of inhibition was observed. The average area of inhibition in millimeter (mm)was calculated and compared with that of the standards as shown in the Table 1 below.

Table 1: Zone of Inhibition in mm Antibacterial activity

		Antibacterial activity	
S. No	Compound	*Staphylococcus aureus* (Gram+ve)	*Escherichia coli* (Gram -ve)
		Zone of Inhibition in mm	Zone of Inhibition in mm
1	Standard 50 µg/ml	40mm	36mm
2	Sample A1 100 µg/ml	20mm	25mm
2	Sample A1 200 µg/ml	22mm	27mm

The antibacterial activity of the Synthesised compound A1 was performed using ciprofloxacin as standard. The concentration of ciprofloxacin used was 50 µg/ml and the concentration of synthesized A1 used was 100 µg/ml and 200 µg/ml respectively. The antibacterial activity was done against *Staphylococcus aureus*(Gram +ve) and *Escherichia coli* (Gram –ve). The compound A1 showed mild activity against both the organisms.

5. MOLECULAR DOCKING STUDIES:

The lists of Azetidine structures and the standard compounds were shown in Table No 1

5.1 Methodology Performed For The Insilico Activity Of The Azetidine -2-One Derivatives

Table No 2-The Azetidinone structures and the standard compounds that are to used for the study are as follows:

Azetidine-2-one	Compound	PDB structure
	1	
	2	
STANDARD ISONIAZID		
STANDARD RIFAMPICIN		

Materials and methods:

Windows XP or Windows and were required[18-19]

The MGL tools , Cygwin, Autodock 4.2 Discovery Visualizer were also required

5.2 DISCOVERY STUDIO:

Table No 3 : shows the Molecular Docking Study of compound ligands with the protein 5NI9 using AutoDock 4.2 Software

S. No	Compound	Conformation	binding energy	Ligand Efficiency	Inhibition Constant/ Units
1.	Compound 1	1st	-7.51	-0.36	3.12
2	Compound 2	3	-7.08	-0.39	6.42
3	Standard Isoniazid	2	-4.78	-0.48	313.68
4	Standard Rifampicin	2	-0.69	-0.1	311.65

Discovery Studio Pictures:

Compound 1 binding with receptor
Fig 1-Protein Ligand Interaction With The Receptor

Compound 1 binding without the receptor
Fig 2-Protein Ligand Interaction Without The Receptor

Standard Isoniazid
Fig 3-Protein Ligand Interaction Without The Receptor

Fig 4- Protein Ligand Interaction With The Receptor

Discovery Studio

Table 4: Molecular Docking Study of compound ligands with the protein 5NI9 using

S. No	Compound	Conformation	No of Hydrogen Bonds	Hydrogen Contacts
1.	Compound 1	1st	1	139A PRO
2	Compound 2	3	0	NIL
3	Standard Isoniazid	2	1	147B LEU
4	Standard Rifampicin	2	1	3A GLU

6. SUMMARY AND CONCLUSION:

The present work describes the synthesis of azetidinone derivatives along with their antibacterial activities The azetidinone derivatives were prepared by the method of S.Ramachandran et al. The reaction completion was confirmed by TLC and the synthesized compounds were purified by recrystallization. The structures of the synthesized compounds were assigned based on the spectral data. The infrared, nuclear magnetic resonance spectra of these azetidinone compounds showed the expected frequencies and signals. The antibacterial activity of the Synthesised compound A1 was performed using ciprofloxacin as standard. The concentration of ciprofloxacin used was 50µg/ml and the concentration of synthesized A1 used was 100µg/ml and 200µg/ml respectively. The antibacterial activity was done against Staphylococcus aureus(Gram +ve) and Escherichia coli (Gram –ve). The compound A1 showed mild activity against both the organisms.

Auto Dock is a popular non-commercial docking program that docks a ligand to its target protein and performs well (accurate and computationally fast). In this study, we propose an easier user-friendly docking protocol for docking ligands with target protein that utilizes AutoDock and Cygwin for docking operations. Thus we can claim that a researcher with no previous background in bioinformatics research would be able to perform molecular docking using Auto- Dock 4.2 program by following stepwise guidelines.

The Discovery Studio Visualizer is a free viewer that can be used to open data generated by other software in the Discovery Studio product line.

Complete broad-spectrum literature was done using various search engines such as Google general, Google scholar, and SciFinder databases.

The literature survey was done according to the following means- namely Azetidine-2-one derivatives synthesis and its antimicrobial activity, Usage of Auto dock vina 4.2 and Cygwin tools for the docking study, Usage of Discovery studio for the visualization of the receptor-ligand interactions.

5NI9 was the protein that was extracted from the Protein database website.

The 2 compounds were drawn using the Chem draw Ultra 8.0 and Biovia draw 2018. It was saved in Mol format and then converted to PDB format using the Smiles Online Translator.

All the files were logged in the Cygwin tooling and the various attributes were performed and then docked file was saved dlg files. These dlg files were seen in the visual mode in the Discovery studio website and the conformation by ranking was calculated and the results were tabulated. Generally, 10 conformations by ranking were taken into consideration. This shows whether the compound has good binding energy with the ligand and hydrogen bonding and hydrophobic binding with the receptor.

From the above analysis, the following results were found out
Compound 1 showed hydrogen bonding and compound 2 showed no hydrogen bonding. The binding energy of the compound 1,2 was more or else similar binding energy of the standard drug Isoniazid.

Standard drug Isoniazid showed good hydrogen bonding and binding energy with the receptor.

Standard drug Rifampicin showed very less hydrogen bonding and lower binding energy with the receptor compared with the compounds. This shows that the Azetidine-2-ones shows antitubercular activity with the Enoyl-acyl carrier protein (enoyl-ACP) reductase enzyme.

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