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MOLECULAR DOCKING OF 2-[(MORPHOLIN-4-YL) (PYRIDIN-3-YL) METHYL] HYDRAZINECARBOXAMIDE (MPH) AGAINST EXTENDED SPECTRUM OF BETA LACTAMASE ENZYME INHIBITION

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ABSTRACT: The aim of the present study is to synthesize and study the interaction of Morpholine derivatives against the receptor ESBL of *E.coli* by molecular modeling. Morpholine derivative was synthesized and characterized by H^1 and C^{13} NMR. *In Vitro* antibacterial activity of MPH along with Copper and Cobalt reveals that the MPH-Cu was found to be more potent than MPH-Co and at 25µg against ESBL producing *E.coli* and *Klebsiella* sp. Molecular docking studies revealed that MPH-Cu has strong binding affinity for these selected targets in comparison to a number of most commonly used antibiotics. The ability of MPH-Cu to bind the active sites on the proteins indicated that it is functionally similar to the commercially available drugs with strong broad spectrum antibacterial activity. The docking score of MPH-Cu reaches a value of -5.2 kcal/mol. The molecular docking results suggest a favorable hydrogen bond interaction between GLN and HIS indicates the higher affinity of ligand than standard antibiotic.

KEYWORDS: Pharmacology, Morpholine, β-lactamase, docking.

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1. INTRODUCTION

The number of newly approved drugs has been significantly decreasing hence the therapeutic dogma has prevailed to overcome target-specific 'drugs' against each and every disease. The pharmacology relies the fact on drug that can modulate its activity by interacting with multiple targets rather than just one [1]. The emergence of antibiotic-resistant strains of pathogenic bacteria is an increasing threat to global health that underscores an urgent need for an expanded antibacterial armamentarium. Resistance also evolves simultaneously and independently to these classes of antibiotics in most bacteria, so that most bacteria are multidrug-resistant [2]. Gram-negative bacteria, such as *Escherichia coli*, have become increasingly important clinical pathogens with limited treatment. The increased prevalence of pathogenic bacteria with resistance to clinically useful antibiotics is a growing threat to public health. Despite this urgent, the aim of docking is the search of the most suitable positions and orientations of the ligands in the Ligand Binding Centre (LBC) of the receptor or enzyme, as well as identification of factors that may lead to improvement of the ligand-receptor interaction. The result of the simulation is conformation of the ligand, which interacts with the protein binding site in the best way [3]. Molecular docking is most frequently used method in Structure-Based Drug Design because of its ability to predict, degree of accuracy and conformation of ligand within the target binding site [4]. Molecular docking is the key for drug modeling, which allows to provide agonism/antagonism to the biological target selected and the most favourable orientation for formation of a stable complex and the position between ligand and target. Active ligand molecules with known structural information against targets, can help to study the pharmacophore modeling and quantitative structure activity relationship (QSAR) methods [5]. Heterocyclic chemistry is a very important branch of organic chemistry accounting wide range of biological activity [6]. In addition, many pyridopyrimidine derivatives have a variety of effects of chemical and biological significance such as antimicrobial, analgesic, anti-allergic, antitumor, antihypertensive, antileishmanial**,** antifolate, anti inflammatory, antituberculostic**,** anticonvulsant, diuretic, potassium sparing, and anti-aggressive activities [7-9]. Docking allows reducing expenses and timing due to carrying out the procedure that is similar to high-performance biological screening [10]. Knowing the spatial structure of the target receptor or enzyme and the spatial structure of the ligand it is possible to explain the mechanism of interaction between them at the molecular level and calculate the strength of binding affinity between them [11]. The measure of the biological activity is such ligand concentration at which the cell response is equal to half the maximum. Therefore, ligands with the highest affinity provided will block or activate the molecular target in biological experiments best of all [12]. Affinity of the ligand in relation to the receptor is assessed both by geometric criteria of surface complementarity of the ligand in relation to the cavity of the receptor and by physico-chemical criteria.

2. MATERIALS AND METHODS

Chemistry of synthesis [13]

10 m moles (1.70 g) of Morpholine were dissolved in a 15 ml of Ethanol in a 100 ml 2-neck round bottom flask equipped with a reflux condenser protected by a calcium chloride drying tube and a quick fit thermometer. Then 10 m mole of semicarbazide and 10 m mole (1.36g) of Pyridine-3-carboxaldehyde were dissolved with constant stirring. To this reaction mixture, 0.2 to 0.4 ml HCl were added with cooling on an ice bath. Yellow colour solid separates just after the addition of HCl. The mixture was stirred for 1-2 hours at room temperature. The reaction mixture was refluxed on a water bath at 90-95˚C for 3-4 hours. The reaction was examined by TLC with time to time till completion. The excess of solvent was removed under reduced pressure. The product was washed with water and recrystallized with 95% Ethanol respectively. The recrystallized product was dried over vacuum.

In vitro antibacterial activity [14]

Different concentration (25, 50, 75 and 100 µg/ disc) of 1:1 ratio of MPH and metal complex were loaded on sterile disc and dried. Test pathogens *E.coli, S. aureus, Bacillus* sp. and *Klebsiella* sp. were swabbed over Mueller Hinton agar plates. Sample loaded disc, a positive control ciprofloxacin disc, and negative control DMSO was placed on the agar surface and kept incubation for 24 h/overnight at incubator. Zone of inhibition was taken after 24 h.

Target frequency prediction

A target protein for synthesized compound was evaluated with Swissdock software against Homosepiens protein data base.

Molecular docking

Protein preparation [15]

AutoDock is a suite of automated docking tool. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. The protein retrieved from PDB database. The protein structure of Extended-spectrum β-lactamase (PDB ID: 4LEN). All water molecules removed from all protein structure and added with Kollmann charges was assigned. The energy minimized protein was then saved in PDB format. Using MGLTools-1.4.6 nonpolar hydrogens were merged, AutoDock atom type AD4 and Gasteiger charges were assigned and finally saved in protein.pdbqt format.

Ligand preparation

Structure of ligands were drawn using Chem Sketch, optimized with 3D-geometry and the two-dimensional structures of synthetic were converted into 3-D structure using the open Babel format molecule converter and saved in PDB format for Auto Dock compatibility. MGLTools-1.4.6. The Sripps Research Institute was used to convert ligand.pdb files to ligand.pdbqt files.

Docking protocol-MGL tools [16]

Grid parameter files (protein.gpf) and docking parameter files (ligand.dpf) have written using MGLTools-1.4.6. Receptor grids were generated using 80x80x60 grid points in xyz with grid spacing of 0.375 Å. Grid box was centred co crystallized ligand map types were generated using autogrid4. Docking of macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm, with an initial population of 250 randomly placed individuals, a maximum number of 106 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. One hundred independent docking runs were performed for each ligand. Results differing by 2.0 Å in positional root-mean square deviation (RMSD) were clustered together and represented by the result with the most favourable free energy of binding**.**

Docking protocol-Hex tools [17]

The molecular docking between the target receptor and ligand was performed by Hex tool. This tool is for interactive docking and can able to run in any operating system. There are advanced versions in this tool and it provides the energy values for all the models. The model can visualize in any forms with docked parameters. The structure for the ligand MPH can be visualized in the phymol viewer and docked with the structure of Ebola virus and the target receptor for cancer. The ligand structure was drawn by Chemsketch and subjected to docking. Both the structures were docked and showed energy values such as E-max, E-min, E-shape, and E-total. These values were calculated. The Net charges with number of orientations were also calculated. Finally, the results were compared based on the docking parameters.

3. RESULTS AND DISCUSSION

Synthesis, Characterization and antibacterial

As a result of docking, a number of values of Consensus scoring functions has been obtained. These values assess the quality and energy of binding of the structures studied 2-[(morpholin-4-yl) (pyridin-3-yl) methyl] hydrazinecarboxamide (MPH) with the molecules of Homo sapiens bio targets. The results of target sit prediction shows that the synthesized compound was found to target 40% receptor and 33% enzymes (Fig 1). The 3D structure of tested ligand reveals more than 75 structurally similar compounds were found to be affinity towards Mu-type opioid receptor, Delta-type opioid receptor, Kappa-type opioid receptor, three compound similarly targeting Histamine H3 receptor and 19 similar compound were found to be enzyme as target against Carbonic anhydrases. The opioid system controls pain, reward and addictive behaviors. By acting at opioid receptors, the ligand shows extremely potent pain-killers, but may also highly addictive drugs. Antibacterial activity of MPH-Copper and MPH-Cobalt reveals that the both the tested compound are active against *E.coli, S. aureus, Bacillus* sp. and *Klebsiella* sp. Of these two tested compound MPH-Co complex was moderately active with the maximum of 14 mm zone of inhibition against *E.coli* at 75µg. Ligand MPH-Cu complex exhibited potent antibacterial activity against

all tested pathogens with maximum of 18 mm zone of inhibition against *E.coli, S. aureus* and *Klebsiella* sp. were greater than ligand alone. The relative inhibitory zone RIZD was found to be 82%. Metal ion complex alter the permeability of the cell membrane and reduce the potential [18-19] resulting death of cells. The cobalt, copper and nickel metal complexes showed increased activity against the test bacteria, compared with the ligand with inhibitory zone [20].

Table 1. The molecular docking metal ligand with Extended-spectrum β-lactamase

S. No.	Compound name	Docking score	Inhibitory Concentration	H-Bond Interaction	Distance
1	**Compound Co**	-5.11	4.2	SER237 N-H…O	2.125
2	**Compound Cu**	-5.2	2.3	GLN192 N-H…O HIS197 N-H…O	2.111 2.146
3	**Ciprofloxacin**	-4	389	SER235 N-H…O SER130 N-H…O	1.793 1.958

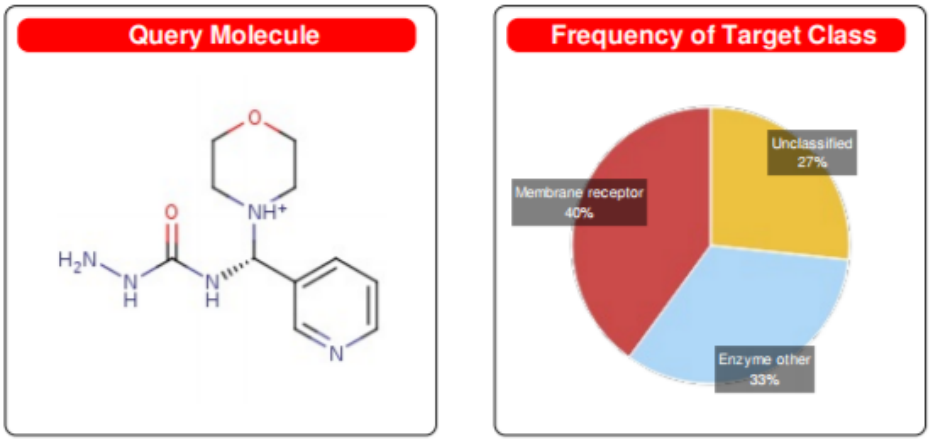

Fig. 1. Target site prediction against Homo sapiens

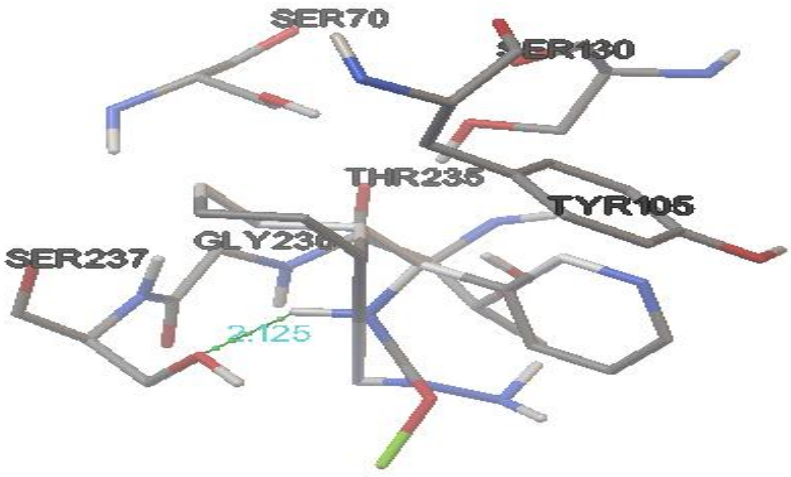

Fig. 2. ESBL with compound MPH-Co (II)

Docking study of Co^{II} complex of MPH with ESBL

Fig. 2 reveals that the amino acid residues SER237 was involved in interactions with compound Co (II) the active site of ESBL. The amino acid residues SER237 was involved in interactions with compound Co (II) the active site of ESBL. The length of hydrogen bond formed 2.125Å. The IC50 values of this compound have 4.2 µm and low docking score (-5.11). Docking score of the complexed inhibitor was found to be -5.11, with RMSD of 0.486. The molecular components include not only genes and gene products, but also other chemical substances in living cells [21].

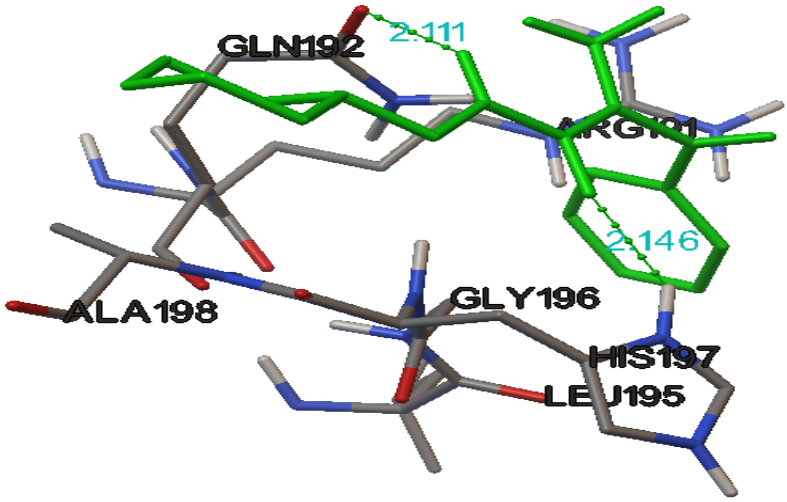

Fig. 3. ESBL with compound MPH-Cu (II)

Docking study of Cu^{II} complex of MPH with ESBL

Fig. 3. reveals that the amino acid residues GLN192 and HIS192 were involved in interactions with compound Cu (II) the active site of ESBL. The amino acid residues GLN192 and HIS192 were involved in interactions with compound Cu (II) the active site of ESBL. From the table it was noted that the length of hydrogen bond formed 2.111Å and 2.146Å. The IC_{50} values of this compound have 2.3(µm) and low docking score (-5.2). The interaction of tested metal ions with organic ligands shows better antibacterial activity compared to free ligand a choice for new drugs with Beta Lactam producing pathogenic bacteria. Studies show that metal complexes with copper ions penetrate more easily through the bacterial cell wall, due to denature protein with sulphydrile group [22] and destroying the bacterial cell wall.

4. CONCLUSION

Binding energies of the ESBL protein-ligand (drug) interactions are important to describe how fit the drug binds to the target macromolecule. We have attempted to locate ESBL inhibitors by performing molecular docking and molecular dynamics studies on ESBL with Morpholine derivative. Our docking simulation resulted in a very close target protein structure, which supports our findings. The compounds Co (II) and compound Cu (II) were highly interacting with ESBL. It is important to that more experimental studies are needed to find out the relationship

between MIC and interaction energy.

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CONFLICT OF INTEREST

The authors don't have any conflict of interest.

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