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MOLECULAR MODELING AND DOCKING ANALYSIS OF PSEUDOMONAL BACTERIAL PROTEINS WITH EUGENOL AND ITS DERIVATIVES

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ABSTRACT:

Pseudomonas aeruginosa (PA) exhibits antibiotic resistance to several classes of antibiotics. This has limited the therapeutic options for its infection. Eugenol (4-allyl-2-methoxyphenol) is the main phytoconstituents found in cloves. It shows an excellent bactericidal activity. In the current study, an attempt was established to identify Eugenol and its ester derivatives as the potential inhibitors for the proteins, Penicillin Binding Protein 3 (PBP3), DNA gyrase and Topoisomerase IV from PA by docking analyses. The 3D structure of PBP3 was retrieved from Protein Data Bank (PDB). For DNA gyrase and Topoisomerase IV, the structures were determined using Homology Modeling method. The ligand structure of eugenol ester derivatives were simulated using ChemDraw Pro suite v3.5. Receptor-Ligand docking was carried out using Glide. Of the six compounds used for the study, 4- allyl-2-methoxyphenyl 2 pyridine carboxylate binds effectively when compared to the other derivatives with a docking energy score of -7.4, -5.2 and -3.8 Kcal/mol to the proteins, PBP3, DNA gyrase and Topoisomerase IV respectively. The comparative docking analysis of eugenol and its ester derivatives to the PA bacterial proteins shows that compound 4-alkyl-2-methoxyphenyl 2 pyridine carboxylate as the most promising agent against the target protein, PBP3 with minimum binding energy value. It could be investigated further using *in vitro* models for its anti-pseudomonal property and other pharmacological activities.

KEYWORDS: Eugenol, Penicillin Binding Protein 3, Type IIA Topoisomerases, Homology Modeling, Molecular Docking.

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1. INTRODUCTION

Pseudomonas aeruginosa (PA) is a motile, Gram-negative pathogenic bacterium. Due to its potential pathogenicity towards human beings, it differs from the other members of the same genus. It causes infections in immunocompromised individuals such as patients suffering from cancer, Cystic Fibrosis (CF), AIDS, Burn wounds (Lyczak*et al*., 2000, Ciofu, 2003). PA

has developed resistance to many classes of antibiotics. This has made it hard to tackle the infections caused by PA with existing therapeutic options. Thus improving the therapy for its infection has been designated a priority by the Antimicrobial Availability Task Force of the Infectious disease Society of America (Talbot*et al*., 2005).With the recent progresses in the technology in areas such as proteomics, genomics and microscopy, there is a more serious and rapid understanding of the PA pathogenicity. In the future there is a need for novel approaches to control infections caused by the PA (Veesenmeyer*et al*., 2009).Penicillin Binding Proteins (PBPs) are enzymes involved in the biosynthesis of peptidoglycan layer. Inhibiting the activity of PBP3 has shown to have impact on a large number of genes involved in antibiotics resistance and cell division. It also affects the genes that implicate the DNA repair and transcription of pyocin genes (Sauvage*et al*., 2008). PBP3 is a potential therapeutic target in PA infections. Another well studied target in the Gram-negative bacterial infections includes the Type IIA Topoisomerase – DNA gyrase and Topoisomerase IV. They are two highly homologous enzymes and play an important role in chromosome segregation, bacterial DNA replication and compaction. The role of DNA gyrase is to facilitate the unwinding of DNA at the replication forks. Topoisomerase IV functions in mediating the decatenation of interlocked daughter chromosomes. DNA gyrase and Topoisomerase IV has two A and two B subunits (A2B2) making them a heterotetramers. The A-subunits contains the active-site Tyrosine. It is involved in DNA breakage-reunion activity. The B-subunits catalyzes the hydrolysis of ATP (Sissi*et al*., 2010). Eugenol (4-allyl-2 methoxyphenol) is a naturally occurring phenol. It is extracted as essential oil from clove. It is known to exhibit neuroprotective properties. Studies show that eugenol is a known antioxidant and inhibits Monoamine Oxidase (MAO) (Jirovetz*et al*., 2006). In addition, eugenol is known to possess an excellent bactericidal activity against a broad range of Gram-negative bacteria such as *E.coli*, *S.typhii*, *S.aureus*, and *L.monocytogenes*. Eugenol acts by disrupting the cytoplasmic membrane of the Gram- negative bacteria (Gill *et al*., 2006). Eugenol is hydrophobic in nature. It enables eugenol to penetrate the lipopolysaccharide in the cell membrane of the Gram-negative bacteria. The process alters the cell structure and results in the leakage of the intracellular constituent thus killing the bacterial cell (Walsh *et al*., 2003). A molecular docking study was undertaken in order to evaluate eugenol and its ester derivatives (Sadhegian*et al*., 2008) as potential inhibitors of PA proteins. For the current study, three proteins, PBP3, DNA gyrase and Topoisomerase IV from PA were used as receptors. The three dimensional structural coordinates for DNA gyrase and Topoisomerase IV of PA is not

available in PDB. Their structures were not experimentally determined using X-ray crystallography or NMR methods. Hence their structures were predicted using Homology modeling methods and were used in the molecular docking studies.

2. MATERIALS AND METHODS

2.1. Homology Modeling

The amino acid sequences of DNA gyrase and Topoisomerase IV for PAwere retrieved from UniProtKB in FASTA format. The template proteins for carrying out the homology modeling of DNA gyrase and Topoisomerase IV were selected based on the BLAST result for each sequence. The 3D structure of template proteins for DNA gyrase and Topoisomerase IV were retrieved from PDB with the PDB ID: 1ZVU and 2Y3P respectively. The sequence alignment for DNA gyrase and Topoisomerase IV along with their respective template protein sequences were carried out using ClustalW. Their homology models were predicted using MODELLER v9.12. The stereochemical quality of the predicted models was validated using PROCHECK.

2.2. Molecular Docking

The structure of eugenol was retrieved from PubChem. For the eugenol ester derivatives, the structures were drawn with the reference from the literature using ChemDraw Pro v13.0. The generated structures were then saved to a file in .mol format for using them in docking later. The 3D structure of the eugenol and its ester derivatives used as ligands are shown in Fig.1.

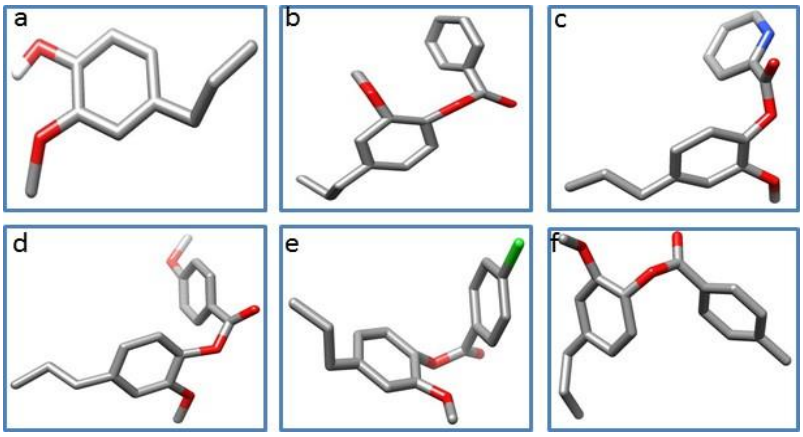

Fig. 1 - Ligands used in the docking calculations. a - 4-allyl-2-methoxyphenol (Eugenol), b - 4-allyl-2-methoxyphenyl benzoate, c - 4-allyl-2-methoxyphenyl 2 pyridine carboxylate, d - 4-allyl-2-methoxyphenyl 4 methoxybenzoate, e - 4-allyl-2-methoxyphenyl 4 chlorobenzoate, f - 4-allyl-2-methoxyphenyl 4 methylbenzoate.

The 3D structure of PBP3 was retrieved from PDB with the PDBID:3OC2. The active sites for PBP3 and the modelled structures of DNA gyrase and Topoisomerase IV were calculated using CASTp, an online server for active site prediction. The molecular docking was carried out using Grid-based ligand docking with energetics (Glide). Glide is a docking program that comes as a part of Schrödinger suite of programs. The calculations were carried out on a system with a configuration of Intel (R) Xenon (R) Quadcore CPU E3-1225 V2 @3.20 GHz with Linux Operating system. All the steps involved in ligand preparation, protein preparation and Glide docking was carried out using the Maestro v9.0 Graphical User Interface (GUI) workspace. The ligands were prepared using the default setting of LigPrep module in Schrödinger. This generated various tautomers of the ligand molecules. The water molecules and the bound heteroatom structures were removed from the protein structure prior to protein preparation step in Schrödinger. Proteins were prepared using the default settings of the PrepWizard in Schödinger. During this step, the polar hydrogen atoms were added to the protein structure and then it was energy minimized and optimized. The PrepWizard uses the OPLS-AA force field for energy minimization. The structures were energy minimized until it reached an RMSD value less than 1Å. During the optimization of the structures, the bond angles, bond orders and the topology were assigned. After protein preparation, a grid was generated using 25Å box with the center of the grid defined by the CASTp prediction of active site of the protein. The default parameter in Glide Standard Precision (SP) docking was changed to eXtra Precision (XP) docking mode for the docking calculation.

2.3 Visualization and Analysis

UCSF Chimera is a free molecule visualizer used for visualizing the homology modeling results from MODELLER. The docking results were visualized and analysed using Biovia Discovery Studio 4.1 Visualizer. For analyzing the interaction in a protein-ligand complex, a surface is created close to the ligand in DS visualizer. These receptor surfaces provide a unique insight into the inner working of a receptor. The surface is colored by a number of receptor atom properties that includes the hydrophobicity density map derived based on the Kyte-Doolittle scale where the hydrophobic region is shown in brown and the hydrophilic region in blue.

3. RESULTS AND DISCUSSION

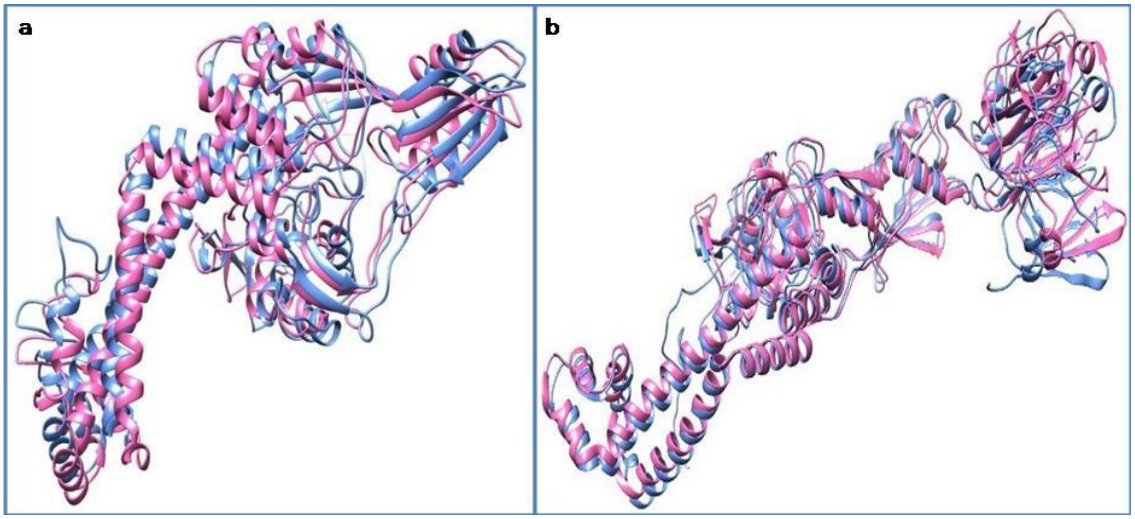

Fig. 2 - Superimposed image of the predicted homology models for DNA gyrase (a) and Topoisomerase IV (b) shown in Blue along with their templates shown in Pink.

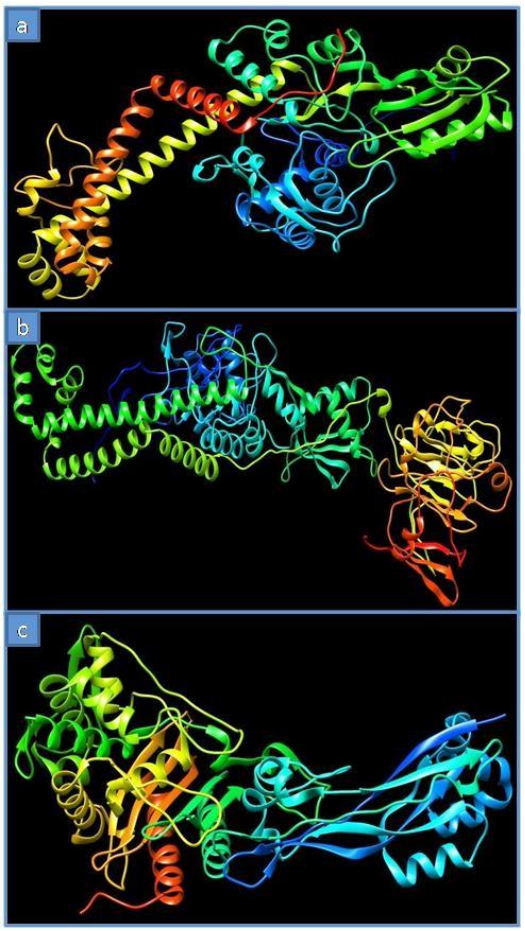

Fig. 3 - The three dimensional structure of the receptor proteins taken for the study. A - DNA gyrase, B - Topoisomerase IV & C – Penicillin Binding Protein 3.

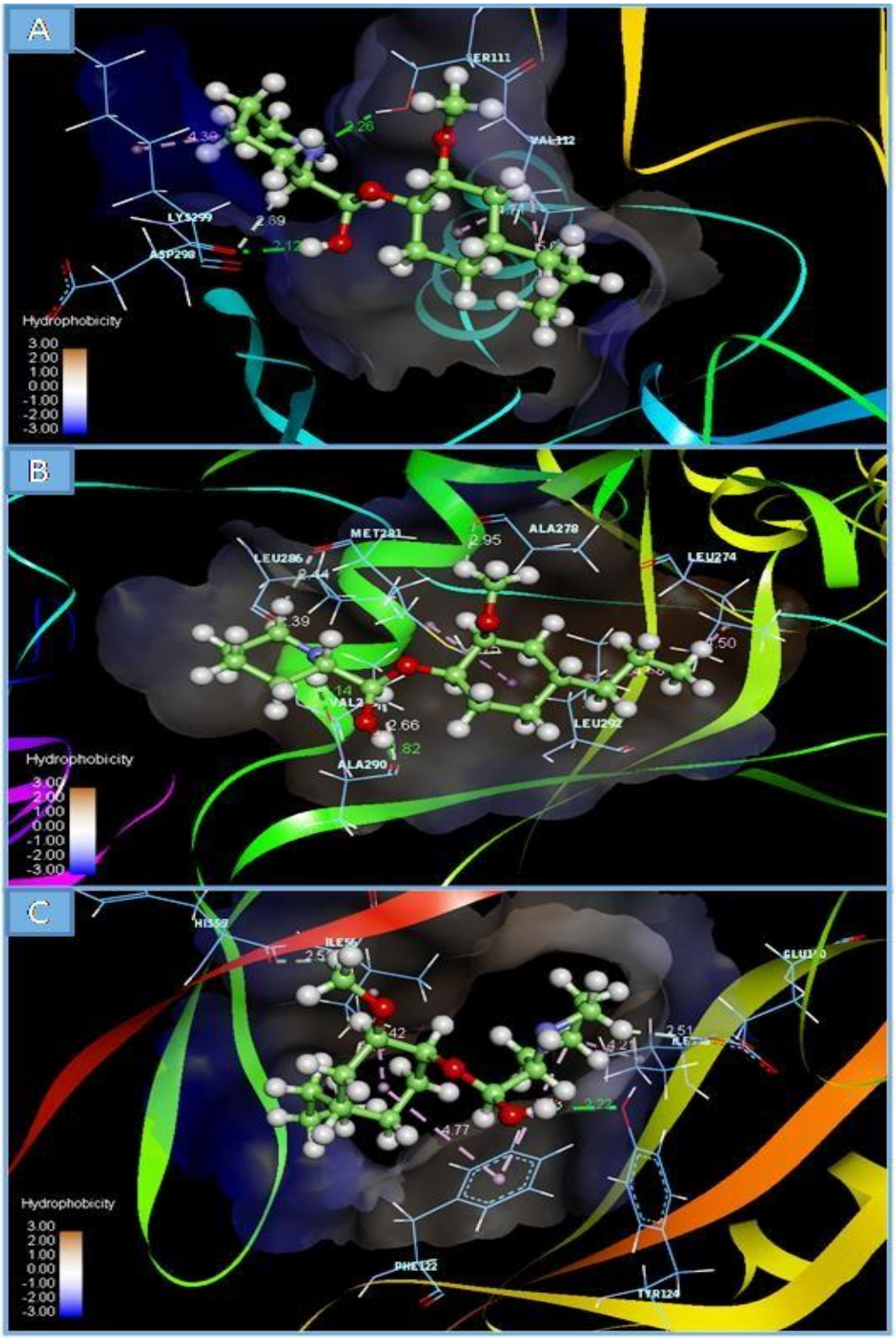

Fig. 4: Image shows the docked results of Eugenol ester derivative 4-allyl-2-methoxyphenyl 2 pyridine carboxylate to the receptors DNA gyrase (A), Topoisomerase IV (B) and Penicillin Binding Protein 3 (C). The ligand is shown as ball and stick model with carbon atom shown in olive green. The interacting receptors are shown as line with carbon atom in blue. A hydrophobic surface based on Kyte-Doolittle scale of hydrophobicity was created with hydrophobic region in brown and hydrophilic region in blue.

Table 1- Docking results for Eugenol derivatives against *Pseudomonas aeruginosa* proteins

Ligand name	Penicillin Binding Protein 3			DNA gyrase			Topoisomerase IV		
	H-Bond distance (Å)	Amino acid	Docking score (Kcal/mol)	H-Bond distance (Å)	Amino acid	Docking score (Kcal/mol)	H-Bond distance (Å)	Amino acid	Docking score (Kcal/mol)
4-allyl-2-methoxyphenol	2.89	ALA60	-5.428	1.83	THR219	-4.649	1.73	LEU496	-1.878
4-allyl-2-methoxyphenyl benzoate	2.10	GLU150	-6.205	2.43 3.09	ASP298 SER111	-4.407	2.28 2.08	ALA283 MET281	-2.198
4-allyl-2-methoxyphenyl 2-pyridine carboxylate	**2.51, 2.51 2.22**	**HIS55 TYR124**	**-7.399**	**2.12 2.26**	**ASP298 SER111**	**-5.212**	**1.82 2.14**	**ALA290 VAL289**	**-3.797**
4-allyl-2methoxyphenyl 4-methoxybenzoate	2.61, 2.32 2.42	GLU121 ALA57	-5.772	1.94	LYS299	-5.151	2.16	ARG309	-3.365
4-allyl-2-methoxyphenyl 4-chlorobenzoate	3.06, 2.59 2.41	ALA57 GLU150	-6.400	1.94 2.31	GLY110 PHE109	-5.048	2.05	VAL289	-2.858
4-allyl-2-methoxyphenyl 4-methylbenzoate	2.26	ALA57	-6.337	1.97	GLY110	-4.812	1.91	ALA290	-2.703

DISCUSSION

The 3D structure of DNA gyrase and Topoisomerase IV from PA were not reported in PDB. Their structures were predicted using homology modeling methods with MODELLER. The predicted models for DNA gyrase and Topoisomerase IV are shown along with their templates as superimposed images in Fig. 2 (a & b). Their 3D models were validated using PROCHECK program of Structural Analysis and Verification Server (SAVES). The stereochemical quality of the models was assessed with the Ramachandran plot derived from the PROCHECK analysis (Laskowski*et al*., 1993, Morris *et al*., 1992). For the predicted model of DNA gyrase residues in most favoured region are about 80.7% whereas for Topoisomerase IV it is around 91.7%. After validation using PROCHECK, the predicted models of DNA gyrase (Fig. 3A) and Topoisomerase IV (Fig. 3B) along with PBP3 (Fig. 3C) were used as receptors. The docking calculations were carried out separately for each protein with the same set of ligands using Glide (Friesner*et al*., 2006). The docking results were visualized and analyzed using Biovia Discovery Studio v4.1 visualizer. It enables to view the non-covalent interactions such as hydrogen bond (Classical and Non-Classical) and Hydrophobic contacts (pi, Alkyl) formed between the compound and the protein. This helps in understanding the binding modes, the affinities and the orientation of the docked compound at the active site of the receptor molecule. The scoring function is the process of evaluating a particular pose in the docking. It is done by counting the number of favourable intermolecular interaction such as hydrophobic contacts and number of H-bonds formed between the receptor and the ligand. If there is a hydrophobic-hydrophobic contact between the ligand and the receptor, the interaction would be favourable (Oda*et al*., 2006, Jain 2006). Table 1 shows the docking score obtained for each calculation along with the H-bond interactions. The results show that the compound 4-allyl-2-methoxyphenyl-2-pyridine carboxylate has a greater binding affinity to PBP3, DNA gyrase and Topoisomerase IV with docking energy values of -7.39, -5.212 and -3.797 Kcal/mol respectively. The compound formed two H- bonds with a distance radius of 2.12Å and 2.69Å to the residue ASP298 and one H-bond with a distance radius of 2.26Å to SER111 in the active site of DNA gyrase. The compound also made three hydrophobic contacts of type Alkyl with the residues VAL112 and LYS299 in DNA gyrase (Fig. 4A). For Topoisomerase IV (Fig. 4B), though the energy score is -3.797Kcal/mol, it formed five H-bond with a distance radius of 1.82Å, 2.66Å to ALA290, 2.14Å to VAL289, 2.39Å to LEU286 and 2.95Å to ALA278 in the active site. It made three alkyl hydrophobic contacts with the residues MET281, LEU292 and

LEU274. In PBP3 (Fig. 4C), the compound formed three H-bond with a distance 2.22Å, 2.51Å and 2.51Å to the residues TYR124, GLU150 and HIS55 respectively. Two types of hydrophobic contacts were made in the active site of PBP3. It formed three alkyl hydrophobic contacts with the residues ILE148 and ILE56 and two Pi-Alkyl contacts with the residue PHE122. Of all the compounds used as ligands, eugenol exhibited relatively weak binding affinity to Topoisomerase IV with a docking score of -1.9Kcal/mol and formed an H-bond with a distance 1.73Å to the residue LEU496. Based on the results obtained as gscore, it is evident that the compound 4-allyl-2-methoxyphenyl-2-pyridine carboxylate can be a promising one compared to the other studied ligands. The current study was undertaken in order to study the inhibitory property of eugenol and its esters derivatives on a structural basis to PAbacterial proteins. The result shows that the eugenol ester derivative 4-allyl-2-methoxyphenyl 2 pyridine carboxylate binds effectively to the receptor proteins PBP3, DNA gyrase and Topoisomerase IV when compared to the other derivatives from eugenol with a docking energy score of -7.4, -5.2 and -3.8 Kcal/mol respectively. We suggest that 4-allyl-2-methoxyphenyl-2-pyridine carboxylate could be investigated further using *in vitro* models for its anti-pseudomonal property and other pharmacological properties.

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