Docking Studies of Benzimidazole Derivatives on Peptide Deformylase and Heptosyltransferase Waac As Antibacterial Agent

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ABSTRACT

A series of benzimidazole containing isoxazoline-5-one and pyrazoline-5-one compounds were computationally designed and optimized with the Auto Dock 4.0.1 to investigate the interactions between the target compounds and the amino acid residues of the *Escherichia coli* PDF enzyme and *Escherichia coli* heptosyltransferase WaaC. In this study, the docking studies were done using auto dock between computationally designed benzimidazole derivatives and peptide deformylase (PDF) and also with heptosyltransferase WaaC. The free energies of binding (Δ G) and inhibition constants (Ki) of the docked ligands were calculated by the Lamarckian Genetic Algorithm (LGA). These values suggested that the designed benzimidazole derivatives are excellent inhibitor of both *Escherichia coli* PDF enzyme and heptosyltransferase WaaC.

Key words: Benzimidazole, isoxazoline-5-one, peptide deformylase and heptosyltransferase WaaC.

INTRODUCTION

The emergence of resistance in the commonly occurring Gram-positive and Gram-negative bacterial pathogens to antimicrobial agents has become a significant medical crisis[1]. The limited number of antimicrobial classes and the common occurrence of resistance within and between classes reinforce the urgent need to identify new potent compounds with novel mechanisms of action. Therefore, there is an urgent need for antibiotics with novel mechanisms of action. Bacterial peptide deformylase (PDF) belongs to a subfamily of metalloproteases catalysing the removal of the N-terminal formyl group from newly synthesized proteins. PDF removes the formyl group from the first methionine of the nascent polypeptide through Fe^{2+}-mediated catalysis. PDF is essential in prokaryotes and appears to be conserved throughout the eubacteria. This enzyme is absent in mammalian cells and provides a unique target for antimicrobial chemotherapy [2-5]. Thus, it may be suitable as a target for new chemotherapeutic agents.

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Lipopolysaccharides constitute the outer leaflet of the outer membrane of Gram-negative bacteria and are therefore essential for cell growth and viability. The heptosyltransferase WaaC is a glycosyltransferase (GT) involved in the synthesis of the inner core region of Lipopolysaccharides. It catalyzes the addition of the first l-glycero-d-manno-heptose molecule to one 3-deoxy-d-manno-oct-2-ulosonic acid (Kdo) residue of the Kdo2-lipid A molecule. These heptose is an essential component of the Lipopolysaccharides core domain; its absence results in a truncated lipopolysaccharide associated with the deep-rough phenotype causing a greater susceptibility to antibiotic. Thus, WaaC represents a promising target in antibacterial drug design[6].

In order to investigate the interactions between our designed compounds and the amino acid residues of the *Escherichia coli* PDF enzyme and heptosyltransferase WaaC, a molecular docking study was performed. From this optimization of the designed compounds, in future we planned to

synthesize these designed compounds and test their antibiotic activities in vitro against various gram positive and negative bacteria.

TOOLS AND MATERIALS AND METHODS

Auto Dock

Auto Dock is an automated docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. Auto Dock actually consists of two main programs: one performs the docking of the ligand to a set of grids describing the target protein; and the other Auto Grid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualized. A graphical user interface called Auto Dock Tools or ADT was utilized to generate grids, calculate dock score and evaluate the conformers.

Molecular docking study

In order to gain more insight on the binding mode of the compounds with peptide deformylase (PDF) and heptosyltransferase WaaC docking studies using Auto Dock 4.0.1 were carried out [7]. Top scoring molecules from the largest cluster were considered for interaction studies. The crystallographic structure of *Escherichia coli* peptide deformylase (PDF) and heptosyltransferase WaaC, which are retrieved from the RCSB Protein Data Bank (PDB code 1G2A, 2GT1 respectively) serves as docking receptor,[8] and all the designed compounds are selected as ligand molecules. Before docking the screened ligands in to the protein active site, the protein was prepared by deleting the substrate cofactor as well as the crystallographically observed water molecules and then protein was defined for generating the grid. All molecules were drawn using ChemDraw Ultra 8.0 tool and energy minimized using Chem 3D Ultra 8.0 software. Automated docking was used to locate the appropriate binding orientations and conformations of various inhibitors into the 1G2A binding pocket. To perform the task, the powerful genetic algorithm method implemented in the program Auto Dock 4.0.1 was employed. Grid maps were generated by AutoGrid program. Each grid was centered at the crystal structure of the corresponding 1G2A and 2GT1 separately. Lamarckian Genetic Algorithm was employed as the docking algorithm. The grid dimensions were 60 Å X 60 Å X 60 Å with points separated by 0.375 Å. For all ligands, random starting positions, random orientations and torsions were used. During docking, grid parameters were specified for x, y and z axes as 38.808, 30.946 and 42.249 respectively. The Docking parameters Number of Genetic Algorithm (GA) runs: 10, Population size: 150, Maximum number of evaluation: 2,500,000, Maximum number of generation: 27,000 were used for this study. The structure with the lowest binding free energy and the most cluster members was chosen for the optimum docking conformation.

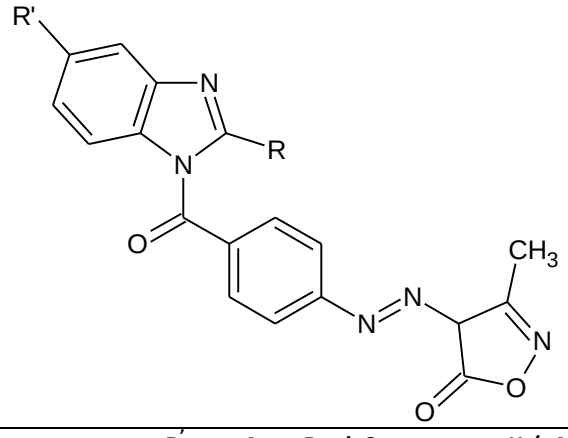
RESULTS AND DISCUSSION

The newly designed molecules are energy minimized and the resulting molecules are considered for docking analysis using Auto Dock 4.0.1. At the end of each run, docked orientations are saved and the resultant molecules are checked for geometry and number of hydrogen bonds. Auto Dock is employed to study the docking molecules within active site region of 1G2A, 2GT1 and the H-bond interaction. Docked scores of newly designed molecules along with inhibition constant (Ki) and hydrogen bonds are represented in Table-1.

A docking energy of -8.80 kcal/mol with two hydrogen bonds and -9.87 kcal/mol with two hydrogen bonds and inhibition constants (Ki) of 0.354 and 0.057 were showed by compound 6 with peptide deformylase and heptosyltransferase WaaC receptor sites respectively. Figure 1a, 1b, 1c and 2a, 2b, 2c shows the interaction mode of compound 6 with peptide deformylase and heptosyltransferase WaaC receptor sites respectively. All the analogs share a common binding mode and occupy similar position in the active site.

Docking results show that all the designed molecules have similar orientations in the binding pocket of PDF enzyme, except that the terminal substituents on the benzimidazole ring have relatively large conformational differences because of their diversity on atomic composition and chemical property. The binding modes of the compound 6 bound to active site of (PDF) enzyme and heptosyltransferase WaaC are shown in Figure 1a, 1b, 1c and Figure 2a, 2b, 2c respectively. From the binding model, we can see that compound 6 is bound in to peptide deformylase receptor and heptosyltransferase WaaC receptor site via hydrophilic binding by hydrogen bond between O of Hydroxyl…N-H of Arg 66 (1.804 Å), O of

Table 1 Docked scores of newly designed compounds



Comp.	R	R'	Auto Dock Score (Kcal/mol)		K_i (µM)		No of H-bonds	
			1G2A	2GT1	1G2A	2GT1	1G2A	2GT1
1	H	H	-7.04	-7.41	6.93	3.67	2	2
2	CH_3	H	-7.60	-6.98	2.69	7.68	1	1
3	$-CH_2C_6H_5$	H	-7.49	-8.76	3.24	0.382	1	1
4	-CH(OH)CH(OH)-COOH	H	-7.56	-8.08	2.86	1.19	0	4
5	$-CH=CH-C_6H_5$	H	-7.48	-8.45	3.28	0.635	2	1
6	**$-C_6H_4$(o-OH)**	**H**	**-8.80**	**-9.87**	**0.354**	**0.057**	**2**	**2**
7	$-C_6H_4$(o-COOH)	H	-6.33	-7.58	22.91	2.80	2	1
8	$-CH_2CH_2$-COOH	H	-5.93	-7.63	44.96	2.57	0	3
9	H	$-NO_2$	-6.28	-8.32	25.1	0.945	1	2
10	CH_3	$-NO_2$	-6.37	-8.95	17.22	0.274	2	3
11	$-CH_2C_6H_5$	$-NO_2$	-7.15	-8.81	10.34	0.355	1	2
12	-CH(OH)CH(OH)-COOH	$-NO_2$	-5.25	-6.25	91.25	31.26	0	1
13	$-CH=CH-C_6H_5$	$-NO_2$	-5.66	-6.77	60.76	8.06	1	2
14	$-C_6H_4$(o-OH)	$-NO_2$	-6.96	-7.05	20.12	6.12	1	1
15	$-C_6H_4$(o-COOH)	$-NO_2$	-6.32	-5.61	24.87	76.94	2	2
16	$-CH_2CH_2$-COOH	$-NO_2$	-4.22	-8.65	831.9	0.458	2	2

K_i = inhibition constant

isoxazole moiety ----N-H of Lys 18 (1.942 Å) and O of Hydroxyl…N-H of Arg 298 (1.934 Å), O of isoxazole moiety ----N-H of Lys 32 (1.944 Å) respectively. These strong hydrogen-bonding interactions are concomitant with the introduction of the 2-(o-hydroxy phenyl) moiety at benzimidazole ring, which means that this portion can increase the binding affinity between the target molecule and the *Escherichia coli* PDF and heptosyltransferase WaaC.

Computationally designed ligands along with the compounds were pre-filtered for their drug like properties by lipinski's rule [9].

CONCLUSION

The predicted binding free energy that includes the intermolecular energy and torsional free energy was used as the criterion for ranking. Furthermore, the intermolecular hydrogen bonds, whose effect has already been counted in the binding energy, were also investigated in order to find useful information for drug design. Among all designed compounds, compound 6 exhibited the lowest free energy (ΔG) on both peptide deformylase and heptosyltransferase WaaC receptors (-8.80 kcal/mol and -9.87 kcal/mol respectively) than the remaining analogues. In other words, they possess the highest potential binding affinity into the binding site of the 3D

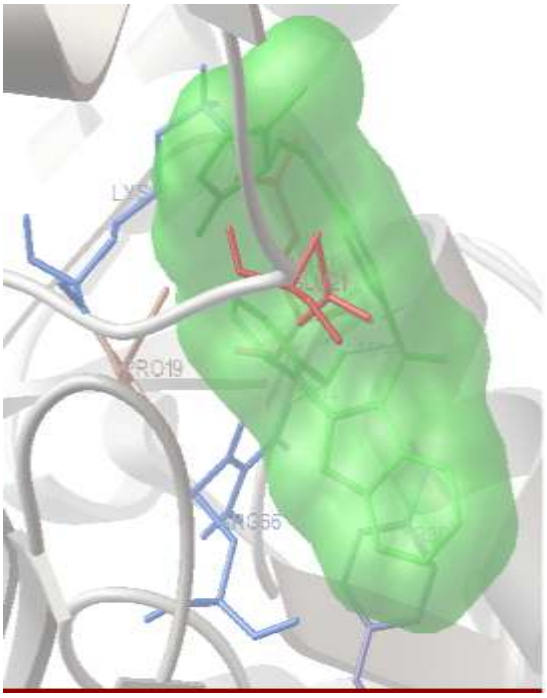


Figure 1a. Binding mode of compound 6 (colored in green) with peptide deformylase (PDF) viewed through Auto Dock 4.0.1 software

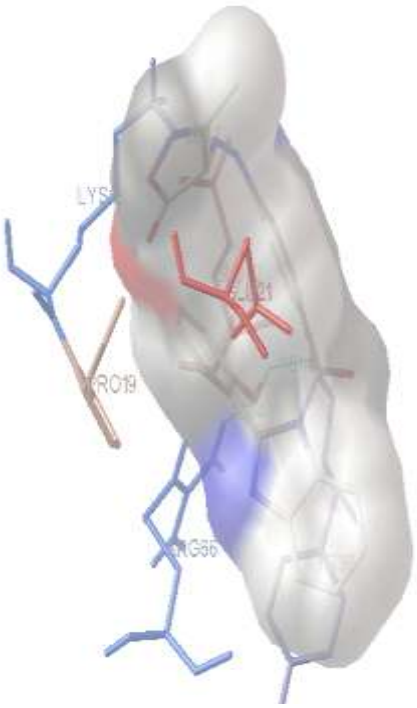


Figure 1b. Binding mode of compound 6 in the active site of peptide deformylase (PDF) along with interacting amino acids viewed through Auto Dock 4.0.1 software

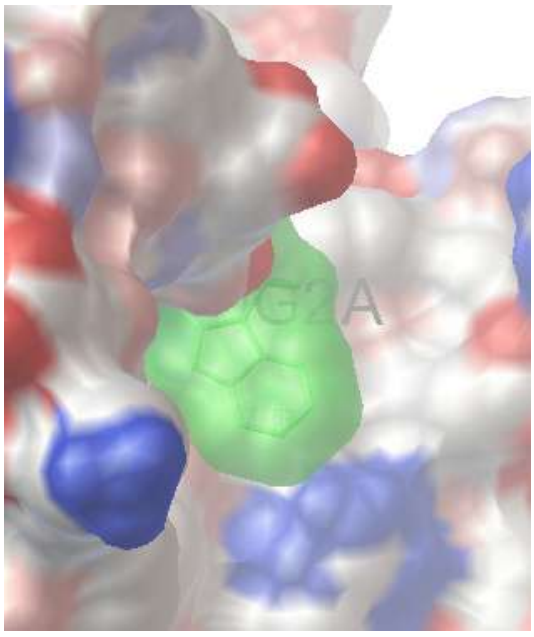


Figure 1c. Compound 6 (colored in green) is extending in to the mouth of the substrate tunnel of peptide deformylase (PDF) viewed through Auto Dock 4.0.1 software

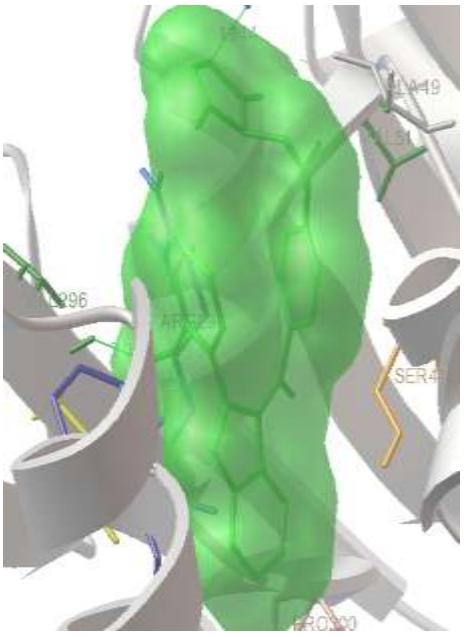


Figure 2a. Binding mode of Compound 6 with heptosyltransferase WaaC viewed through Auto Dock 4.0.1 software

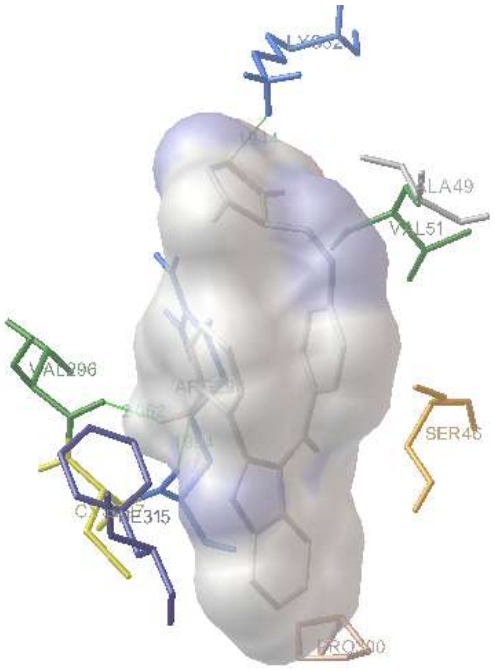


Figure 2b. Binding mode of compound 6 in the active site of heptosyltransferase WaaC along with interacting amino acids viewed through Auto Dock 4.0.1 software

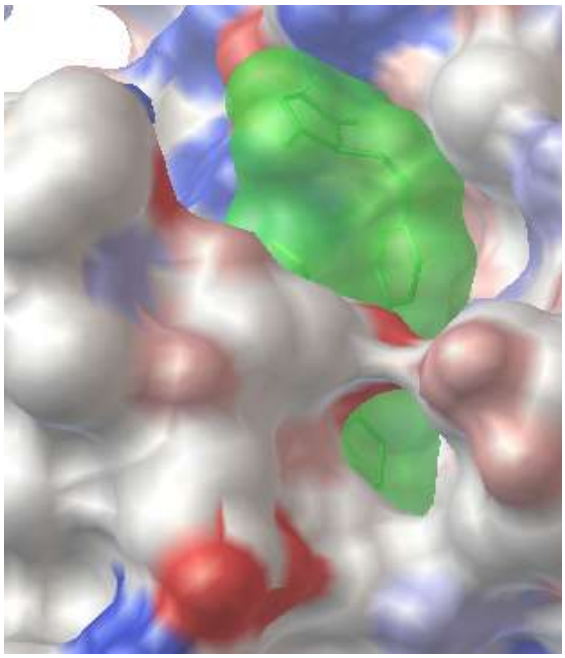


Figure 2c. Compound 6 (colored in green) is extending in to the mouth of the substrate tunnel of heptosyltransferase WaaC viewed through Auto Dock 4.0.1 software

macromolecule (peptide deformylase, 1G2A andheptosyltransferase WaaC, 2GT1). Applying Lipinski's rule of five to these benzimidazole derivatives to evaluate drug-likeness, there was no violation of the rule determining drugs pharmacological activity in the body[9]. Thus this study will be useful for the design of novel peptide deformylase and heptosyltransferase WaaC enzymes inhibitors based on docking method.

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