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Molecular Modeling Approach for the Discovery of *Escherichia coli* O157:H7 Interaction Inhibitors of Natural Product Compounds

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The aim of this study is to report the development of *Escherichia coli* O157:H7 template for structure-based drug design. This template was validated by redocking with crystal ligand I. The results showed a good matching of docked and the crystallographic binding orientations with root mean square deviation (RMSD) less than 2.0 Å. Moreover, the developed template was applied to predict binding mode of 19 known *E. coli* inhibitors and 40 natural products. The results showed that the binding energy of almost *E. coli* inhibitors was related to their biological activity. The use of developed *E. coli* O157:H7 template in automated docking could speed up the process of novel drug discovery by allowing designed inhibitors to be tested computationally before the compounds are synthesized.

Keywords: *Escherichia coli* O157:H7, Docking, Drug design, Antibacterial activity.

INTRODUCTION

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium. *E. coli* O157:H7 is an important food-borne bacterial pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome in humans and animals [1]. *E. coli* O157:H7 can survive passage through the extremely acidic gastric stomach (pH < 1.5) [2,3], enhance virulence expression at the gastrointestinal milieu *i.e.* induced expression of genes in the LEE pathogenicity island [4,5] and persist in various environmental sources such as soil [6], raw manure [7] and farm water [8]. Therefore, it is likely that coordinated regulation of a set of *E. coli* O157:H7 genes might be required for this environmental flexibility although the underlying mechanisms are not clearly understood [9].

Several molecular modeling studies of X-ray crystal complexes of *E. coli* O157:H7 have been reported [10-12]. However, there has been no investigation on the validation of *E. coli* O157:H7 template by docking with *E. coli* O157:H7 inhibitors. A template based on the protein target of *E. coli* O157:H7 inhibitors can be used for structure-based drug design or for virtual screening studies. The template in the present work was developed by using the visualization program AutoDock Tools (ADT) using automated docking program, AutoDock4.2. For validation, the developed template was applied to predict binding mode and binding affinity of nineteen known *E. coli* O157:H7 inhibitors. Therefore, the validated template was docked with selected natural products including alkaloids, flavonoids, phenolics and terpenes in order to search for bioactive principles.

EXPERIMENTAL

Template preparation and validation: The crystallographic structure of *E. coli* O157:H7 chosen as *E. coli* O157:H7 template was its complex with ligand I (phenol), *E. coli* O157:H7 inhibitor. This structure was obtained from the Protein Data Bank (PDB codes: 3KMH) [13]. It was solved by X-ray diffraction techniques with a resolution of 1.58 Å. Prior to docking, ligand and crystallographic water were removed. Hydrogens and Gasteiger charges were added by using AutoDockTools (ADT)

[14,15]. Atomic solvation parameters based on Stouten model and fragmental volumes were added in accordance with AutoDock force field [16]. Cubic affinity grid maps for each atom type in the ligand set (plus an electrostatics map) centered on the cavity with dimensions of 40 Å × 40 Å × 40 Å and 0.375 Å spacing between grid points were computed using AutoGrid 4.2 for each protein model [14]. Lennard-Jones parameters 12-10 and 12-6 were used for modeling H-bonds and van der Waals interactions, respectively. To validate the constructed *E. coli* O157:H7 template, native crystal structure of *E. coli* O157:H7 inhibitor obtained from the Protein Data Bank was used to dock with the template.

Ligand preparation: Structures of 19 known inhibitors (Table-2) and all studied compounds (Fig. 1) were generated using ChemOffice10.0 and energy was minimized by MM2. The deprotonated form at the acetate position was assigned. Atomic charge was added using Gasteiger-Marsili formalism.

Molecular docking: AutoDock4.2 was employed to perform the docking calculation. Each ligand was docked to the developed *E. coli* O157:H7 template by using a Lamarkian genetic algorithm search (LGA). Due to the stochastic nature of genetic search algorithms, each rigid ligand was docked in 150 trials. Docking trials for each ligand were initiated with a randomly generated population of 150 ligand orientations and the highest affinity configuration was selected after 2.5 million energy evaluations. Standard AutoDock parameters were used for the genetic algorithm: 2 % point mutation; 80 % cross over rate; 6 % local search rate [14]. The resulting ligand configurations from 150 trials within a 2.0 RMSD tolerance of each other were grouped together in clusters. The results of the docking experiments were evaluated by calculating the positional root mean square deviation (RMSD) of the corresponding atoms of each conformation. The final docked structure, RMSD from the bound crystal structure, docked energy and predicted free energy of binding were used for the analysis of its interaction with the active site.

RESULTS AND DISCUSSION

E. coli O157:H7 template was developed by using AutoDock Tool suite software and the developed template was then validated by redocking with native ligand I. The results indicated a good agreement between crystallographic and docking results (Table-1). The redocked result was close to the crystallographic configuration with an RMSD of 1.94 Å. The binding modes of ligand I are given in Fig. 2.

Nineteen known *E. coli* O157:H7 inhibitors [17] were docked to the developed *E. coli* O157:H7 template in order to predict binding mode and binding affinity. Table-2 summarizes the results of docking study presented as binding energies compared with their EC_{50} values. The lowest energy docked conformation

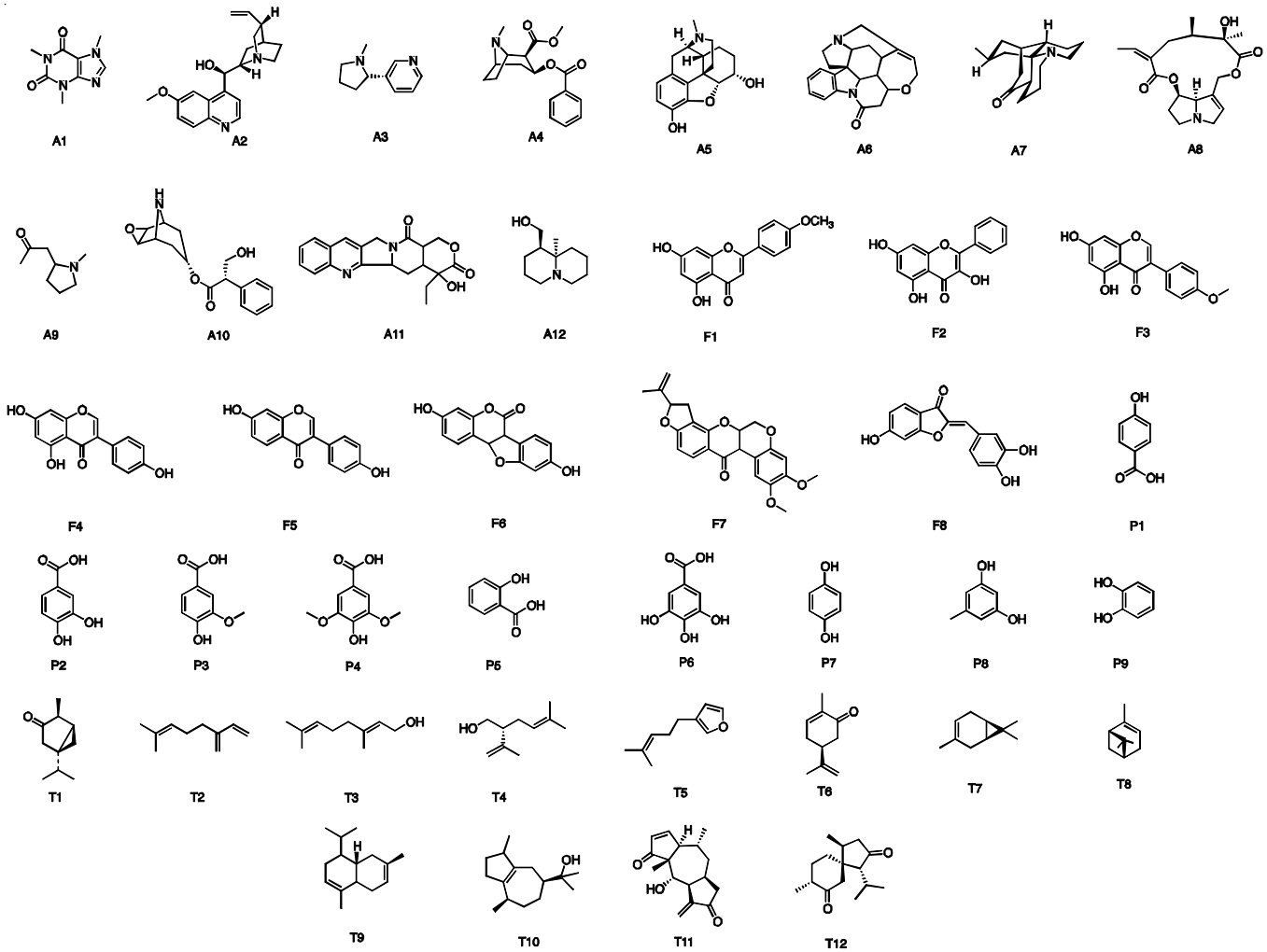


Fig. 1. Structures of studied alkaloids, flavonoids, phenolics and terpenes

TABLE-1
DOCKING ENERGIES, MEMBER IN THE HIGHEST CLUSTER AND RMS (Å) OF LIGAND **I** WITH *E. coli* O157:H7 TEMPLATE

PDB	Member in the highest cluster	$E_{binding}$ (kcal/mol)	RMS (Å)
3 kmh	149	-4.19	1.94

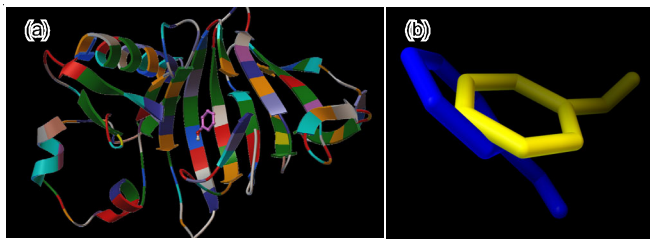
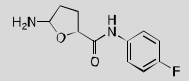
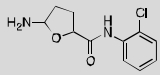
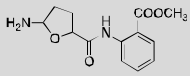
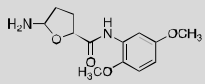
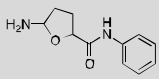
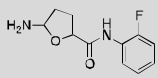
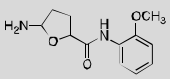
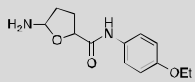
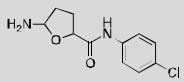
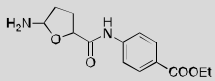
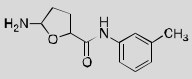
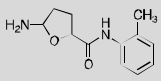
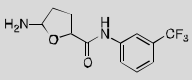
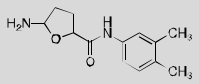
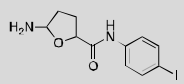
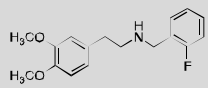
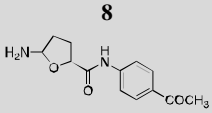
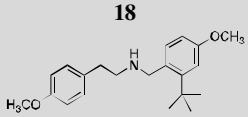
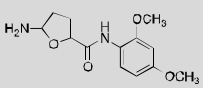


Fig. 2. (a) The active site structure and interaction between ligand **I** and *E. coli* O157:H7 template. (b) The superimposition of the X-ray (blue) and docked conformation of ligand **I** (yellow)

of the most populated cluster was selected and taken into account. The results showed that the compounds possessing potent *E. coli* O157:H7 inhibitory action with EC_{50} at micromolar concentrations had binding energies lower than ligand I (-4.19 kcal/mol) except compounds **12** and **15**. While compounds having less potency with EC_{50} higher than 12.5 µM had binding energies higher than ligand I. Only 2 out of 19 compounds showed false positive results. AutoDock, like other docking software, may have difficulty correctly ranking the binding free energy of all inhibitors unless the compounds are very similar structurally.

The *E. coli* O157:H7 template developed in this study was applied to dock with selected known natural products including alkaloids, flavonoids, phenolics and terpenes [18]. The docking results showed that alkaloids **A9** and **A12** possessed low binding energies -4.90 and -6.07 kcal/mol, respectively. Alkaloid **A9** bound *E. coli* O157:H7 template through one hydrogen bond at Glu186 (Fig. 3a). Alkaloid **A12** formed a hydrogen bond at Glu110 amino acid residue of *E. coli* O157:H7 template (Fig. 3b). The good docking results of phenolics **P5** and **P8** showed low binding energies -5.15 and -5.27 kcal/mol, respectively. The binding orientation of compound **P5** with *E. coli* O157:H7 template was through four hydrogen bonds at

TABLE-2
DOCKING ENERGIES OF 19 KNOWN *E. coli* INHIBITORS

Compound	EC_{50} (µM)	$E_{binding}$ (kcal/mol)	Compound	EC_{50} (µM)	$E_{binding}$ (kcal/mol)
 **1**	0.8	-5.42	 **11**	3.5	-8.21
 **2**	0.8	-5.28	 **12**	3.5	-2.99
 **3**	1.2	-5.40	 **13**	4.0	-5.31
 **4**	1.2	-5.20	 **14**	4.0	-5.16
 **5**	1.4	-5.72	 **15**	4.0	-4.11
 **6**	2.5	-5.14	 **16**	5.5	-5.21
 **7**	2.5	-4.69	 **17**	6.2	-5.35
 **8**	2.5	-5.67	 **18**	<12.5	4.09
 **9**	2.5	-5.49	 **19**	<12.5	17.66
 **10**	2.5	-4.87	–	–	–

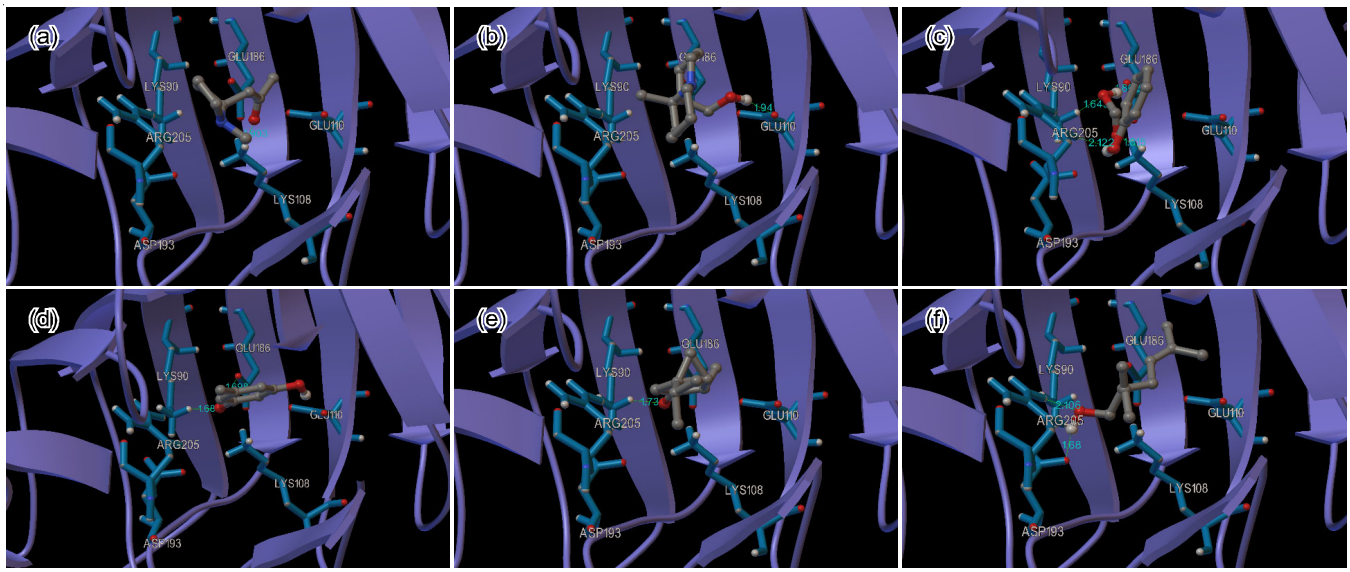


Fig. 3. Binding orientation of ligands with *E. coli* O157:H7 template (a) The active site structure of compound **A9** with enzyme. (b) Active site structure of compound **A12** with enzyme. (c) Active site structure of compound **P5** with enzyme. (d) Active site structure of compound **P8** with enzyme. (e) Active site structure of compound **T1** with enzyme. (f) Active site structure of compound **T4** with enzyme

Lys90, Lys108, Glu186 and Arg205 (Fig. 3c). While compound **P8** formed two hydrogen bonds at Lys90 and Lys108 amino acid residue of *E. coli* O157:H7 template (Fig. 3d). The docking results of terpenes **T1** and **T4** showed low binding energies -5.75 and -5.62 kcal/mol, respectively. Compound **T1** formed a hydrogen bond at Arg205 amino acid residue of *E. coli* O157:H7 template (Fig. 3e). Compound **T4** formed two hydrogen bonds at Asp193 and Arg205 amino acid residue of *E. coli* O157:H7 template (Fig. 3f). The results showed that *E. coli* O157:H7 template developed by AutoDock Tool suite software can be used to predict ligand binding orientations and binding affinities.

Conclusion

The development of *E. coli* O157:H7 template in this report was not only validated by redocking with crystallographic ligands but also by docking with 19 *E. coli* O157:H7 inhibitors. The results showed a compatibility between the calculation of binding energies and the values of biological activity. Moreover, this developed template also docked with selected known natural products such as alkaloids, flavonoids, phenolics and terpenes. All the results indicated that developed *E. coli* O157:H7 template is a good model for prediction of ligand binding orientations and binding affinities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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