

Podophyllotoxin, Deoxypodophyllotoxin and Ursolic Acid as Potential Inhibitors of *tcpA*, *ompW*, and *ctxB* Genes in *Vibrio cholerae*: An *in-Silico* Study

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ARTICLE INFO

Original Article

Keywords: *Vibrio cholerae*, Ursolic Acid, Podophyllotoxin, *tcpA*, *ompW*

Received: 14 Aug. 2022

Received in revised form: 19 Jan. 2023

Accepted: 26 Dec. 2022

DOI: 10.52547/JoMMID.11.1.41

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ABSTRACT

Introduction: Cholera is a highly contagious disease that causes severe diarrhea and dehydration. This study investigated podophyllotoxin, deoxypodophyllotoxin, and ursolic acid as inhibitors of *tcpA*, *ompW*, and *ctxB* genes in *Vibrio cholerae*. **Methods:** We obtained the crystallized structure of podophyllotoxin, deoxypodophyllotoxin, and ursolic acid from the PubChem database for use as a ligand. The mm² method in Chem3D v20.1.1.125 was used to optimize the structure of the ligands. We used AutodockVina v.1.2.0 to evaluate the ligands as inhibitors against the active site of the *tcpA*, *ompW*, and *ctxB* proteins. The output results were analyzed and assessed by BIOVIA Discovery Studio 2016 V16.1.0 X64. **Results:** The reported affinities ranged from -6.8 and -8.7 kcal/mol. The highest diversity of links was found in *tcpA* and *ctxB*. Hydrogen bonds were established with Threonine (91, 111), Glycine (113, 114, 94), and Alanine (92) of *tcpA*, indicating the effectiveness of ligands against *tcpA*. The ligands podophyllotoxin, deoxypodophyllotoxin, and ursolic acid showed a variety of hydrogen bonds against *ompW* and *ctxB*, respectively, with Arginine, Isoleucine, Histidine, Glycine, and Glutamine. These results demonstrate the excellent inhibitory effects of the ligands against *Vibrio cholerae*. **Conclusion:** *Vibrio cholerae* plays a crucial role in causing pandemic cholera in humans. The predicted conformations of the ligands in this study showed that podophyllotoxin and deoxypodophyllotoxin have higher inhibitory potential than ursolic acid. Therefore, podophyllotoxin and deoxypodophyllotoxin can be potential agents for further research in developing Anti-*Vibrio cholerae* drugs.

INTRODUCTION

Cholera is a disease specific to humans and has caused seven outbreaks so far, resulting in thousands of deaths and significant social and economic damage [1]. *Vibrio cholerae*, the bacterium responsible for Cholera, infects 3 to 5 million people worldwide annually, leading to the death of 100,000 to 120,000 people. For many years, it has been recognized as a dangerous infectious disease [2]. Research indicated that during the last two centuries, this disease has been the leading cause of death and significant complications in many developing countries [3]. Even today, there continue to be reports of conflicts with this disease worldwide [4]. *V. cholerae* serogroup O₁ has two biotypes (classical and El Tor) and three serotypes (Ogawa, Inaba, and Hikojima). Classic cholera is caused by cholera and El Tor biotypes due to their toxin production, and they are part of serogroup O₁. However, some biotypes of serogroup O₁ do not produce toxins and do not cause disease [5]. The *Albenis* and *Proteus* biotypes are typically called non-agglutinating or non-

cholera strains, as they do not cause agglutination with specific antibodies [6]. The *V. cholerae* bacterium has three serotypes based on its antigenic structure: Inaba, Ogawa, and Hicojima [7]. The protein *tcpA* (coregulated toxin pilus) is a critical factor that plays a significant role in the binding and accumulation of *V. cholerae* in the intestine. The large subunit of bacterial pili, *tcpA*, is responsible for this function. As *tcpA* is located on the surface of bacteria, it is highly susceptible to the immune system's response. This protein weighs approximately 22 kDa [8].

The extramembrane protein *OmpW*, weighing 22 kilodaltons, makes *V. cholerae* resistant to environmental shocks, including pH changes, osmotic shock, and detergents. Additionally, this protein enables the bacteria to colonize the intestine and cause disease. The binding subunit of chlorotoxin contains five parts consisting of 103 identical amino acids each, forming a ring-like arrangement that binds to the ganglioside GM1 receptor

of intestinal epithelial cells [9]. On the other hand, the *Cholera* toxin B subunit (*ctxB*) is responsible for binding the toxin to the cytoplasmic membrane receptors of the host cell and is non-toxic [10]. Lignans are a diverse group of plant secondary metabolites found in various plant species and show different biological activities [11]. Among these lignan compounds, podophyllotoxin (PPT) and its derivatives, such as deoxypodophyllotoxin (DPT), possess antibacterial [12], antifungal [13], antiviral [14], and anticancer [15] properties. Ursolic acid (UA) is a naturally occurring chemical compound in herbs such as rosemary and apples. This substance has antibacterial [16], antifungal [17], antioxidant [18], and anticancer [19] effects. Belonging to the triterpene class of compounds naturally produced in plants and animals, UA can have various effects on the body. One of its fundamental mechanisms is the ability to block cellular signaling pathways [20]. As the demand for novel pharmaceutical compounds continues to rise, this study aimed to

investigate the molecular docking studies of PPT, DPT, and UA as inhibitors of *tcpA*, *ompW*, and *ctxB* in *V. cholerae*.

MATERIAL AND METHODS

Ligand preparation. The PPT, DPT, and UA crystallized structures were downloaded as ligands from the PubChem database. PPT identified by the code CID10607 has a chemical formula of $C_{22}H_{22}O_8$, and a molecular weight of 414.4kD, as shown in Fig 1 (a). Fig 1 (b) shows DPT, identified by the code CID345501, with a chemical formula of $C_{22}H_{22}O_7$ and a molecular weight of 398.4kD. Fig 1(c) shows UA, identified by the code CID64945, with a chemical formula of $C_{30}H_{48}O_3$ and a molecular weight of 456.7kD. The ligands were optimized using Chem3D v20.1.1.125 software for optimizing the ligands with the MM² method [21].

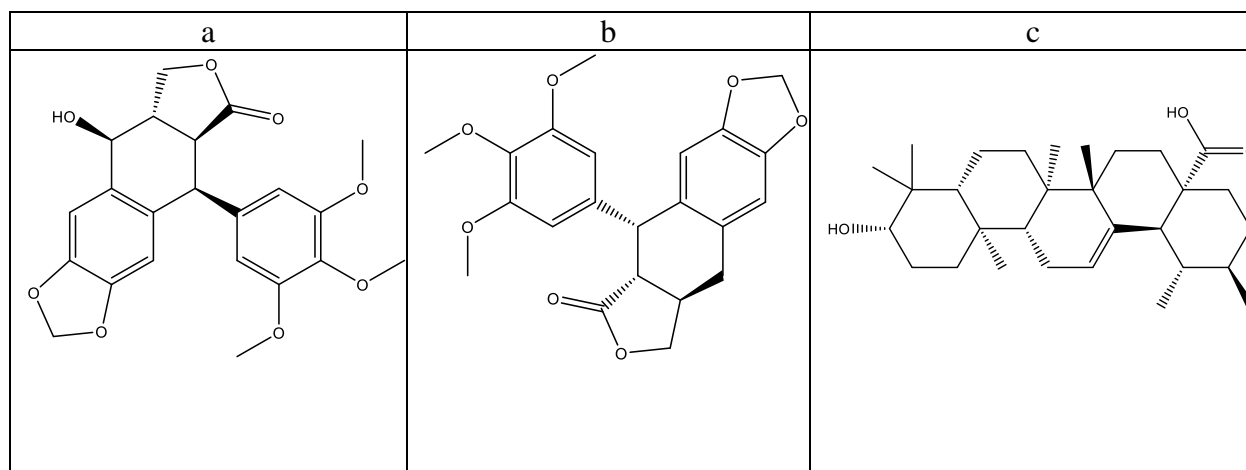


Fig. 1. Chemical structure of PPT (a), DPT (b), and UA (c).

Protein preparation. The crystal structures of *tcpA* (PDB ID: 3HRV-chain A), *ompW* (PDB ID: 2F1T-chain A), and *ctxB* (PDB ID: 1EEI) proteins from *V. cholerae* were downloaded from the Protein Data Bank. The Protein Data Bank (PDB) database contains three-dimensional structural data of large biological molecules, such as proteins and nucleic acids, with a resolution of 2°A angstrom. AutoDockTools-1.5.6 software was used to incorporate Gastiger charges and polar hydrogens. The main chains of each structure were stripped of all water molecules and ligands. Grid box for 3HRV = 48×48×48 (size_x = 48, size_y = 48, size_z = 48), 2F1T= 72×72×72 (size_x = 72, size_y = 72, size_z = 72) and 1EEI= 72×72×72 (size_x = 72, size_y = 72, size_z = 72) with spacing 1°A [21].

Molecular docking. AutoDock Vina software and Discovery Studio 4.5 were used to conduct the PPT, DPT, and UA docking procedure to the binding sites of *tcpA*, *ompW*, and *ctxB*. Discovery Studio 4.5 Client software was utilized to investigate and analyze the ligands and junction interactions. The docking calculations were

conducted by our previous research [22]. It is important to note that this research did not involve human samples or laboratory animals.

RESULTS

The affinity values for all receptors were calculated, and the best affinity with low ΔG ($-\Delta G$ bind) was chosen for further AutoDock interactions, as indicated in Table 1. The PPT, DPT, and UA interactions within the active site of 3HRV-chain A, 2F1T-chain A, and 1EEI are presented in Table 1 and Figure 2. The PPT molecule forms three strong hydrogen bonds with the 3HRV-chain A receptor, specifically with amino acids Threonine at position 91, Glycine at position 113, and Glycine at position 114, as well as one Alkyl bond with Alanine at position 63. Additionally, the PPT molecule forms one strong hydrogen bond with Arginine at position 146, one strong carbon-hydrogen bond with Lysine at position 95, and one van der Waals bond with Asparagine at position 145 in the 2F1T-chain A receptor. In addition, the PPT molecule

creates two strong hydrogen bonds with the 1EEI receptor, specifically with the amino acids Arginine at position 73. The molecule also forms one strong carbon-

hydrogen bond with Aspartic acid at position 70 and one Alkyl bond with Isoleucine at position 74.

Table 1. AutoDock Vina results of PPT, DPT, and UA as an inhibitor of *V. cholerae*.

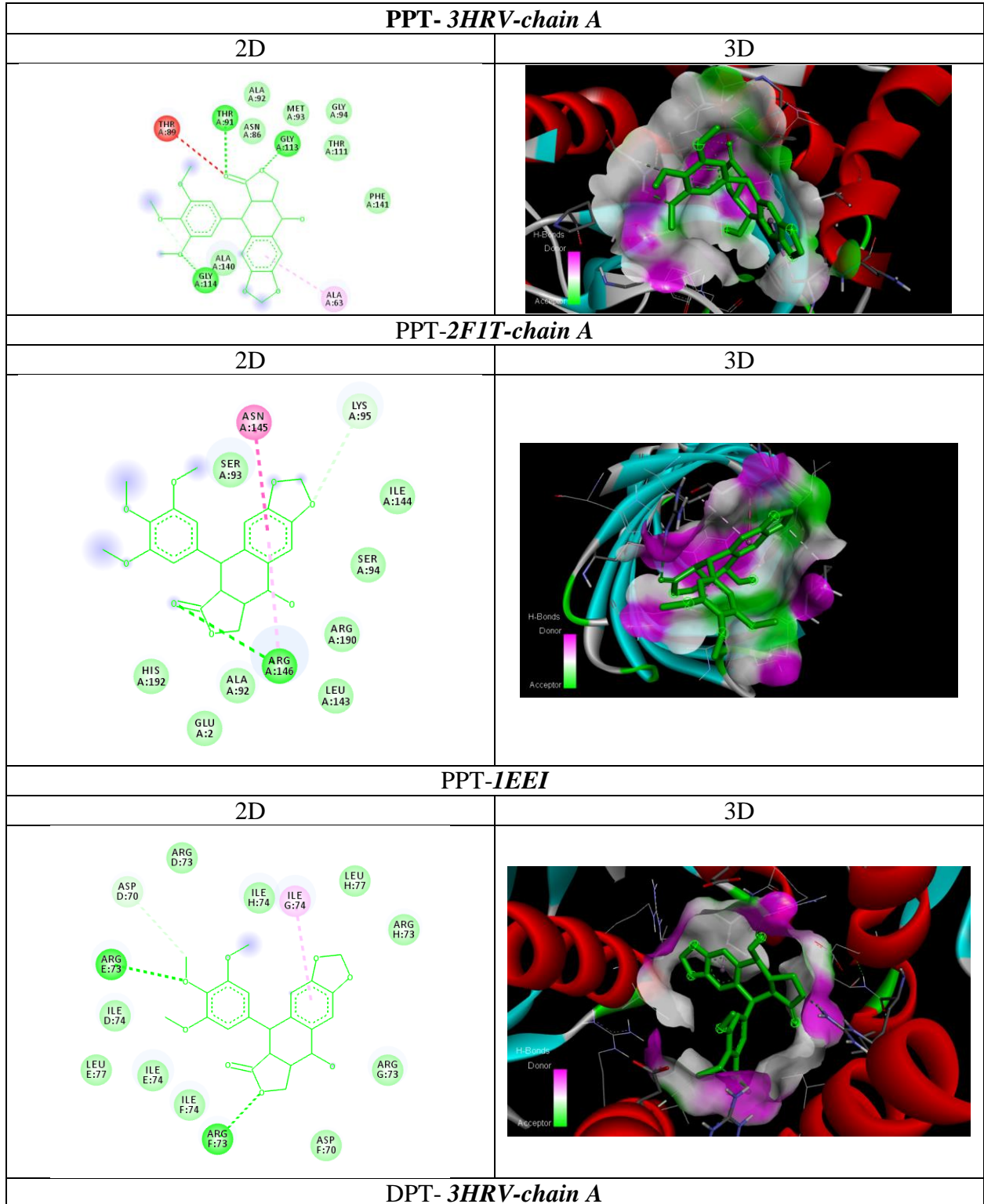
Ligand	Receptor	Affinity (kCal/mol)	Hydrogen Bond	Carbon-Hydrogen Bond	Pi-Alkyl Bond	Van Der Waals
PPT	3HRV-chain A	-7.2	Threonine: 91 Glycine: 113 Glycine: 114	-	Alanine: 63	-
	2F1T-chain A	-6.0	Arginine: 146 Arginine: 73	Lysine: 95	-	Asparagine: 145
	1EEI	-7.8	Arginine: 73 Glycine: 94	Aspartic acid: 70	Isoleucine: 74	-
DPT	3HRV-chain A	-7.0	Threonine: 111 Isoleucine: 104	Alanine: 140 Proline: 81	Alanine: 140	Threonine: 89 Threonine: 67 Tyrosine: 105
	2F1T-chain A	-6.3	Histidine: 13 Glycine: 33 Glutamine: 67	-	Isoleucine: 58 Tyrosine: 12	Glutamic acid: 51
	1EEI	-7.1	Alanine: 92	-	Alanine: 140 Phenylalanine: 31	-
UA	2F1T-chain A	-7.5	-	-	Leucine: 123 Isoleucine: 73 Alanine: 121	-
	1EEI	-8.7	Arginine: 67 Glutamine: 66	Lysine: 63	Lysine: 63 Arginine: 67	-

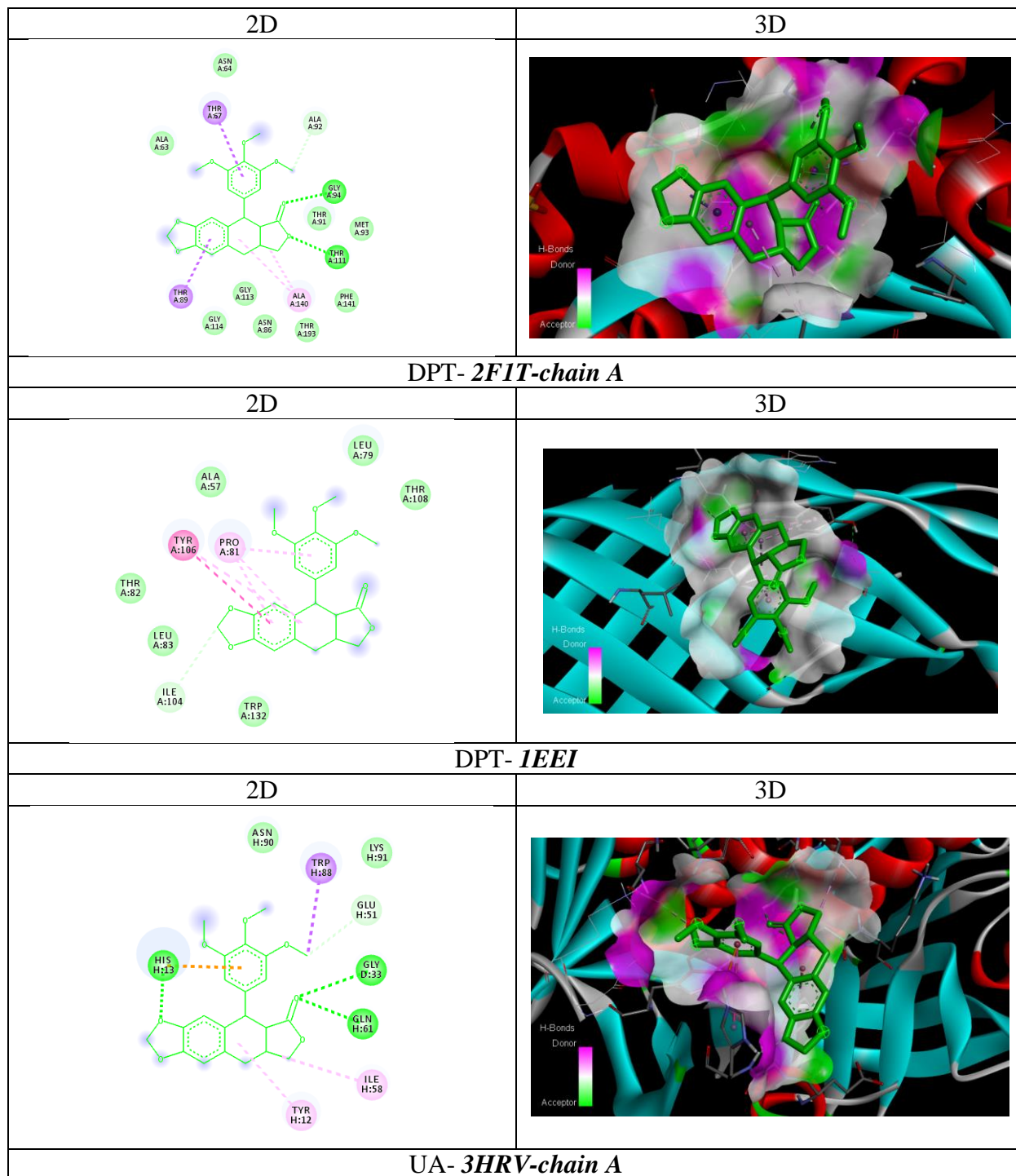
The docking results showed that PPT had a favorable conformation, and by creating stronger bonds, it achieved better inhibition of the 3HRV-chain A receptor compared to other receptors. DPT forms various bonds with the 3HRV-chain A receptor, including two strong hydrogen bonds with the amino acids Glycine at position 94 and Threonine at position 111, one carbon-hydrogen solid bond with the amino acid Lysine at position 95, and one Alkyl bond with amino acid Alanine at position 140. Additionally, it forms two van der Waals bonds with amino acids Threonine at position 89 and Threonine at position 67. Furthermore, DPT forms various bonds with the 2F1T-chain A receptor, including one strong hydrogen bond with the amino acid Isoleucine at position 104, one carbon-hydrogen solid bond with the amino acid Proline at position 81, and one van der Waals bond with amino acid Tyrosine at position 105. In addition, DPT makes various bonds with the 1EEI receptor, including three strong hydrogen bonds with the amino acids Histidine at position 13, Glycine at position 33, and Glutamine at position 67, two Alkyl bonds with amino acids Isoleucine at position 58 and Tyrosine at position 12, and one van der Waals bond with amino acid Glutamic acid at position 51. The docking results showed that DPT had a favorable conformation, and by creating stronger bonds, it achieved better inhibition of the 1EEI receptor than other receptors. UA inhibits the 3HRV-chain A receptor via one strong hydrogen bond with the amino acid Alanine at position 92 and one Alkyl bond with the amino acid Alanine at position 140. Furthermore, UA inhibits the 2F1T-chain A receptor via four strong Alkyl bonds with the amino acids Phenylalanine at position 31, Leucine at position 123, Isoleucine at position 73, and Alanine at position 121. In addition, UA inhibits the 1EEI receptor via various bonds, including two strong hydrogen bonds with the amino

acids Arginine at position 67 and Glutamine at position 66, one Carbon-hydrogen bond with the amino acid Lysine at position 63, and two Alkyl bonds with the amino acids Lysine at position 63 and Arginine at position 67. The docking results showed that UA had a favorable conformation, and by creating stronger bonds, it achieved better inhibition of the 1EEI receptor than other receptors. According to this part of the results of the predicted conformational conformations, it can be concluded that the combination of PPT, DPT, and UA has valuable inhibitory potential and can be used as an agent to develop alternative drug structures for the treatment of *V. cholerae* pathogenicity in future research.

DISCUSSION

According to global statistics, diarrhea is the second leading cause of death among children worldwide, causing 1,600 deaths every day or more than 580,000 annually [23]. Pathogenic bacteria in rivers, including *V. cholerae*, are common causes of diarrhea. According to reports from various countries, there has been a surge in the resistance of *V. cholerae* to commonly used antibiotics. Several documented cases of *V. cholera* O1 exhibit resistance to nalidixic acid, cotrimoxazole, and furazolidone [24]. *tcpA* protein, which serves as the large subunit of pili and is located on the surface of bacteria, is a tempting target for developing anti-colonization drugs [9]. On the other hand, the *ctxB* protein has a homopentameric and non-toxic structure and is located on the large chromosome of *V. cholera* bacteria. Apart from its role in generating antibodies and protecting against cholera toxin, the *ctxB* protein also acts as a vital biological adjuvant to initiate immune responses, particularly mucosal immunity [25].





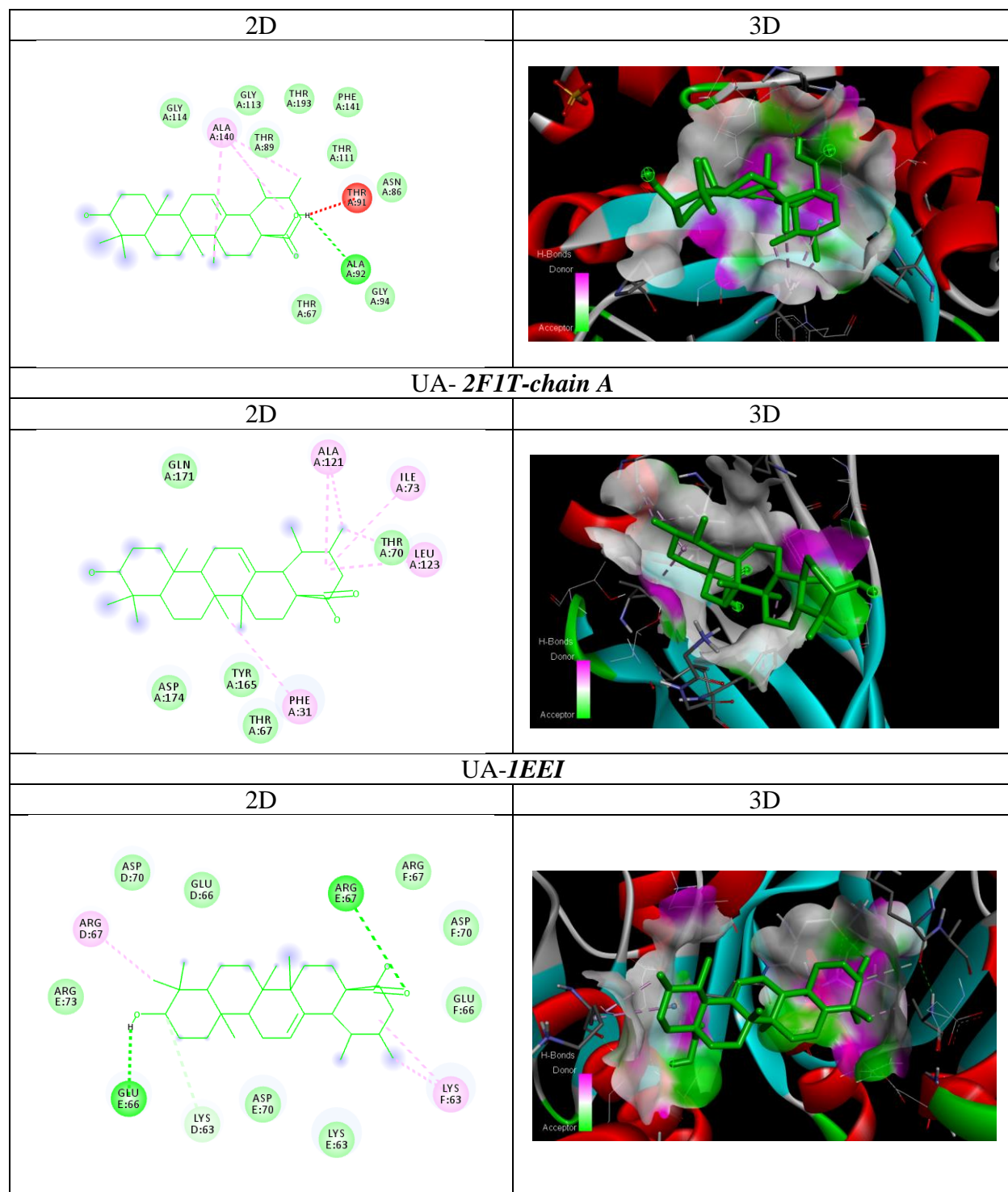


Fig 2. 2D and 3D results of AutoDock Vina. The output of AutoDock Vina shows the binding site residues of proteins with the ligands. The residues in the binding site are highlighted in green, and the ligands are represented in a green stick format.

Furthermore, the involvement of the *ompW* factor has been reported in the development of cholera. Drug resistance and its incidence represent some of society's most pressing issues. Unfortunately, in recent years, drug resistance has become increasingly prevalent worldwide [26]. Therefore, finding therapeutic effects of natural compounds capable of killing bacterial cells has become

a top priority. Natural compounds have many advantages over chemical compounds, such as fewer side effects [27]. Lignans, for instance, comprise a group of phenylpropanoid compounds created by separating two units of phenylpropane [28]. Several studies have demonstrated the ecological and physiological function of lignans as they play an essential role in the plant defense

apparatus against plant and human pathogens [29]. In addition, lignans are crucial in treating diseases because of their antimicrobial, anticancer, and antiviral properties. Two essential lignans, PPT and DPT from the aryltetraline group, have semi-synthetic compounds used in treating illnesses. Additionally, UA, a 5-ring triterpenoid that is lipophilic and has an isomer called oleanolic acid (OA) (-36 hydroxyl-olea-12-en-28-oic acid), shares many similar biological effects. However, there have been limited studies reporting the anti-*V. cholerae* activity of PPT, DPT, and UA, Ragunathan *et al.* (2018) selected 20 phytochemical compounds to screen potential inhibitors against *V. cholerae*. This approach was taken to avoid complications that arise from synthesizing small molecules. According to their findings, quercetin was identified as the most potential and stable inhibitor of *MurB* [30]. The results of the present study were consistent with those of their research. PPT, DPT, and UA were evaluated as ligands to inhibit the *tcpA*, *ompW*, and *ctxB* receptors. The findings revealed that PPT had a higher ability to inhibit 3HRV-chain A than the others due to its strong hydrogen bonds. To investigate the anti-*Vibrio cholerae* properties of medicinal plants, Perveen and Chaudhary (2015) conducted a study. The findings revealed that two compounds, Luteolin from Tulsi and Catechin from Green Tea, demonstrated good binding energy and Inhibitory against *ToxT* [31]. Based on their research, Sharavanan *et al.* (2019) found erythro-2-(4-allyl-2,6-dimethoxy phenoxy)-1-(4-hydroxy-3-methoxyphenyl) propane-1-ol and Leucasperone B could effectively serve as antimicrobial compounds to treat bacterial infections [32].

On the other hand, the results showed that DPT and UA had a higher ability to inhibit 1EEI due to their firm and high hydrogen bonds. These findings will be confirmed during the *in vitro* and *in vivo* tests. Based on current theoretical data, PPT, DPT, and UA show promise as inhibitors of *V. cholera* pathogens. Molecular docking studies have provided insightful data regarding the binding tendencies of PPT, DPT, and UA to receptors, and understanding these interactions will require further biochemical testing. The results showed that the potential anti-*V. cholerae* effects of PPT, DPT, and UA against factors involved in the pathogenicity of this bacterium could be due to the inhibition of various receptors, including *tcpA* and *ctxB*. The findings of this research can be instrumental in developing therapeutic drugs for this bacterium.

ACKNOWLEDGMENT

The authors would like to express their gratitude to all the professors who participated in this investigation voluntarily. It is worth mentioning that this article is an independent study; hence, it was not supported financially by any organization.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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Cite this article:

SarveAhrabi Y, Rostamiyan O, Nejati khoei S. Podophyllotoxin, Deoxypodophyllotoxin and Ursolic Acid as Potential Inhibitors of *tcpA*, *ompW*, and *ctxB* Genes in *Vibrio cholerae*: An *in-Silico* Study. *J Med Microbiol Infect Dis*, 2023; 11 (1): 41-48. DOI: 10.52547/JoMMID.11.1.41