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# Anti-*Helicobacter pylori* Potential of Podophyllotoxin: In Silico Study

**Nakisa Zarrabi Ahrabi[1] (PhD), Yasin SarveAhrabi[1]*(PhD), Zahra Hashem Zadeh[1], Ayda Sharifiyan[1], Mohammad Reza Ebrahimi[1]**

*1. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran*

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ABSTRACT

*Helicobacter pylori* is probably the most common chronic bacterial infection in humans and has infected almost half of the world's population. Podophyllotoxin is a lignan compound of plant origin and has great medicinal importance due to its various biological properties. The aim of this study was to investigate the podophyllotoxin as *Urease*, *VacA* and *cagA* inhibitor of *Helicobacter pylori*. The crystallized structure of podophyllotoxin was received from the Zinc database and used as a ligand. The structure of the ligand was optimized by the $mm^2$ method with Chem3D v20.1.1.125 software. The ligand was evaluated as an inhibitor against the active site of the *urease enzyme*, *VacA*, and *cagA* by AutodackVina software. The output results were analyzed and evaluated by Discovery Studio v16.1.0 software. The best affinity was obtained against *VacA* = -8.1 kcal/mol. The highest diversity of links was also reported in *VacA*. Hydrogen bonds established with *VacA* against tyrosine=729 and threonine=672, indicating the effectiveness of podophyllotoxin against *VacA*. Podophyllotoxin against *cagA* and *Urease* also showed a variety of hydrogen bonds respectively with lysine, serine, and tyrosine - glutamine, arginine, and asparagine. These results demonstrate the excellent inhibition of podophyllotoxin against *Helicobacter pylori*. *Helicobacter pylori* plays a key role in the development of gastric cancer. The transplant conformations predicted in this study showed that podophyllotoxin has valuable inhibitory potential. Therefore, podophyllotoxin may be considered as an anti- *Helicobacter pylori* agent for further research into drug development.

## 1. Introduction

*Helicobacter pylori* (*H. pylori*) is the most common human pathogen (Li & Perez., 2018). The bacterium is specifically colonized in the epithelium below the gastric mucosa and is associated with a range of gastrointestinal diseases such as gastric and duodenal ulcers, gastritis, chronic atrophic gastritis, and finally gastric cancer (Piscione et al., 2021). *H. pylori* has been classified as a carcinogen and is a major known risk factor for gastric cancer (Su et al., 2019). According to GLOBOCAN 2018 statistics, *H. pylori* is associated with 90% of gastric cancer cases (Rawla & Barsouk., 2019). This bacterium is currently the most common cause of infections-related cancers; it is known to cause more than 5% of all cancers worldwide (Wang et al., 2019). Acute *H. pylori* infection usually occurs in childhood, and if left untreated, the infection can remain chronic for the rest of your life (Lucero et al., 2021). More than 50% of the world's population suffers from *H. pylori* infection, and the rate of infection is higher in developing countries than in developed countries (Smith et al., 2019). Direct person-to-person

*Corresponding author: Yasin SarveAhrabi
E-mail address: yas.sarveahrabi.sci@iauctb.ac.ir.

transmission is the main route of transmission of *H. pylori* infection and generally occurs through the oral-oral cycle (via saliva) and the fecal-oral cycle (Zamani et al., 2017). In the treatment of this important gastrointestinal pathogen, common antibiotics such as metronidazole, amoxicillin, clarithromycin, tetracycline, furazolidone, ciprofloxacin and rifamycin are used in combination with proton pump inhibitors and bismuth salts (Yousefi-Avarvand et al., 2018). However, in some cases, we encounter antibiotic resistance of this bacterium, so due to the high resistance of this bacterium to common antibiotics used for treatment, the need to replace new drugs is increasing day by day (Coelho et al., 2018). *Urease* is the most important protein component of *H. pylori* and is known as an important antigen for the diagnosis of this bacterium (Keikha et al., 2019). By hydrolyzing urea and producing ammonia as a neutralizer of stomach acid, this enzyme creates the conditions for bacterial survival at the acidic pH of the stomach (Ansari & Yamaoka., 2017). Also, ammonium hydroxide produced by this enzyme plays a major role in the degradation of host tissues (Graham & Miftahussurur., 2018). One of the most important pathogens in *H. pylori* is gene-related *cytotoxin A* (*VacA*) and *cytotoxin vacuolating* (*cagA*) (Vaira et al., 2001). The *cagA* gene encodes a 120-kDa protein with unknown function in 70-60% of *H. pylori* strains. In general, the data indicate that infection with strains containing *cagA* increases the risk of certain clinical symptoms, but the occurrence of these clinical symptoms is not predictable (Backert & Blaser., 2016). The *VacA* gene encodes another important pathogenic factor that causes a vacuole to form on the surface of eukaryotic cells under certain conditions *in vitro* (Lin et al., 2021). The compound podophyllotoxin (PTOX), known as podophyllux, is a non-alkaloid toxin in the lignan family, which is isolated from the podophyllum plant (*Podophyllum peltatum*) from the barberry family (Berberidaceae) and is used as one of the main treatments for viral agents (Sharma et al., 2018). PTOX and its derivatives are precursors to important anti-cancer compounds called Etoposide and Teniposide, which are used in the treatment of various cancers such as lung cancer, testicular cancer and lymphoma (Bashir et al., 2020). Research has also been done on the effect of PTOX and its derivatives on bacterial and fungal samples (Patwari et al., 2018). Despite numerous studies on the mechanism of action of podophyllotoxin against cancers and the proposed mechanisms, the exact mechanism of this drug is unknown (Singh et al., 2013). Studies have shown that the sugar derivative podophyllotoxin induces death Programmed cell (apoptosis) on ovarian carcinoma (Hela) and lung adenocarcinoma (A2) (Qi et al., 2005). Also, the study of apoptosis analysis using flow cytometry showed that podophyllotoxin induces apoptosis of human bladder cancer cell line 5637 (Sadeghi et al., 2015). The increasing need to produce pharmaceutical compounds and replace compounds with drugs with resistance has become a necessity, so the purpose of this study was to investigate the molecular docking studies of PTOX as potential *urease*, *VacA* and *cagA* inhibitors of *H. pylori*.

## 2. Materials and Methods

### *2.1. Ligand preparation*

The Crystallized structure of PTOX as a ligand was downloaded from the ZINC database (The ZINC database is a curated collection of commercially available chemical compounds prepared especially for virtual screening) with code Zinc1532024, Chemical Formula: $C_{22}H_{22}O_8$, Molecular Weight: 414.4kD and PubChem ID: 11384429 (Fig 1). Then optimized ligand with the $MM^2$ Job command by Chem3D v20.1.1.125 software. The total energy, Stretch, bend and Torsion of ligand after optimization, respectively, 36.5151 kcal/mol, 3.4127, 23.0434 and -16.6882 (Outeiral et al., 2021).

### *2.2. Protein preparation*

Crystallized structure of *urease* (*PDB ID: 1E9Z-chain B*), *VacA* (*PDB ID: 2QV3-chain A*) and *cagA* (*PDB ID: 4DVY-chain P*) of *H. pylori* were downloaded from Protein Data Bank (The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids) with resolution of 3°A angstrom. Gastiger charge and polar hydrogens added with AutoDockTools-1.5.6 software. All water molecules and ligands were deleted from main chains of each structures. Grid box for *1W9Z* = 90×90×90, *2QV3*= 86×86×86 and *4DVY*=

114×114×114 with spacing 1°A (Ghameshlouei et al., 2021).

*2.3. Molecular docking*

The docking process of PTOX to the *urease*, *VacA* and *cagA* binding sites were performed using AutoDockVina software and Discovery Studio 4.5. Ligand and junction interactions were investigated and analyzed by Discovery Studio 4.5 Client software. All docking calculations were performed using the genetic optimization algorithm and Lamarck traits, which are configured as follows: The maximum number of energy assessments is 25,000,000, an initial population of 150 is randomly assigned, the maximum number of generations is 27,000, the mutation rate is 0.02, and the crossover rate is 0.8 and an elitism value. For local search, the so-called Solis algorithm was used with a maximum of 1000 repetitions per search. This process was performed by considering the protein as inflexible and the ligand as flexible (Morris & Lim-Wilby., 2008).

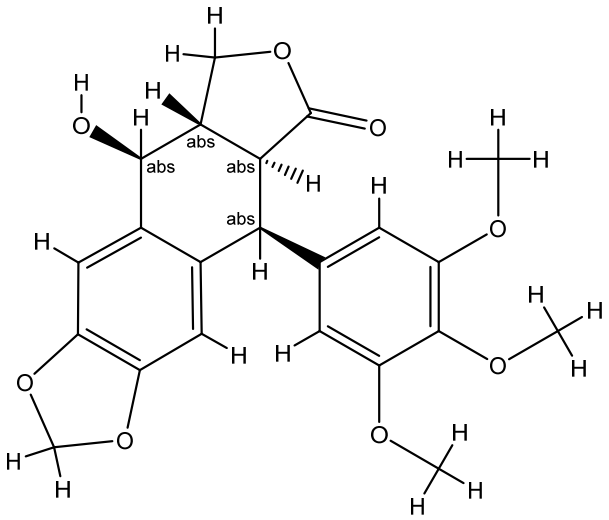



**Figure 1.** Chemical structure of PTOX

**3. Results**

According to Table 1, All affinities were calculated, the best affinity for each receptor with low ΔG ($-\Delta G_{bind}$) were selected to continue for Auto dock interactions. PTOX interactions within the active site *1E9Z-chain B*, *2QV3-chain A*, and *4DVY-chain P* are shown in Table 1 and Figure 2. PTOX various links in order with two strong hydrogen bonds with the amino acids Tyrosine: 729 and Threonine: 672, two stronger carbon-hydrogen bonds with Asparagine: 732 and Tyrosine: 675, two van der Waals bonds balanced with Proline: 677 and Phenylalanine: 674. And two Alkyl bonds with Valine: 739 and Arginine: 734, it forms the *2QV3* receptor.

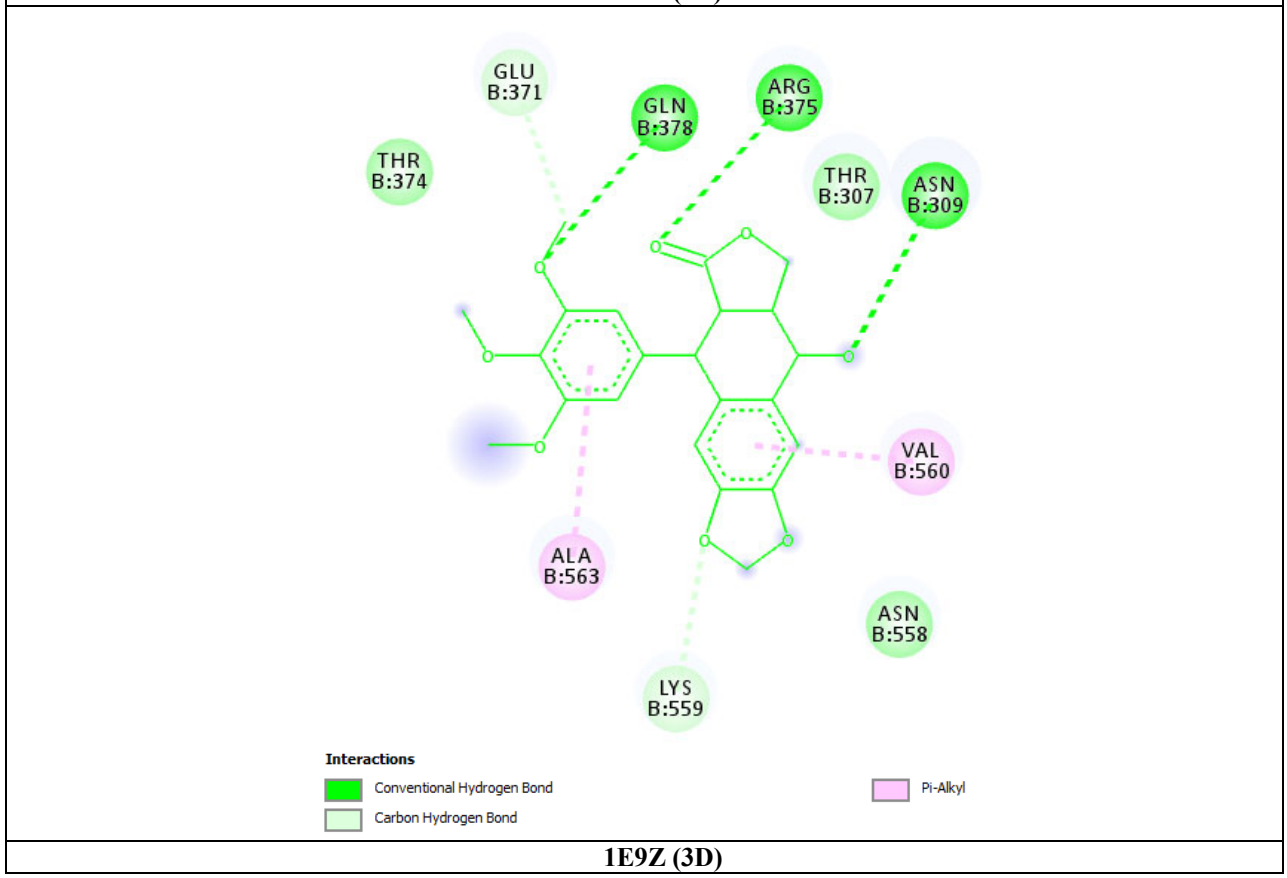
Docking's results showed that PTOX in a favorable conformation and by creating stronger bonds causes better and more inhibition of *2QV3* receptor than other receptors. In addition, PTOX forms three strong hydrogen bonds with the amino acids Lysine: 499, Serine: 497 and Tyrosine: 473 of the *4DVY* receptor. Also, PTOX forms three strong hydrogen bonds with the amino acids Glutamine: 378, Arginine: 375 and Asparagine: 309 of the *1E9Z* receptor. According to the results of the predicted conformational conformations, it can be claimed that the combination PTOX has valuable inhibitory potential and can be used as an agent to develop alternative drug structures in the treatment of *H. pylori* infections in future research.

**Table 1.** AutoDockVina results of PTOX as an inhibitor of *H.pylori*

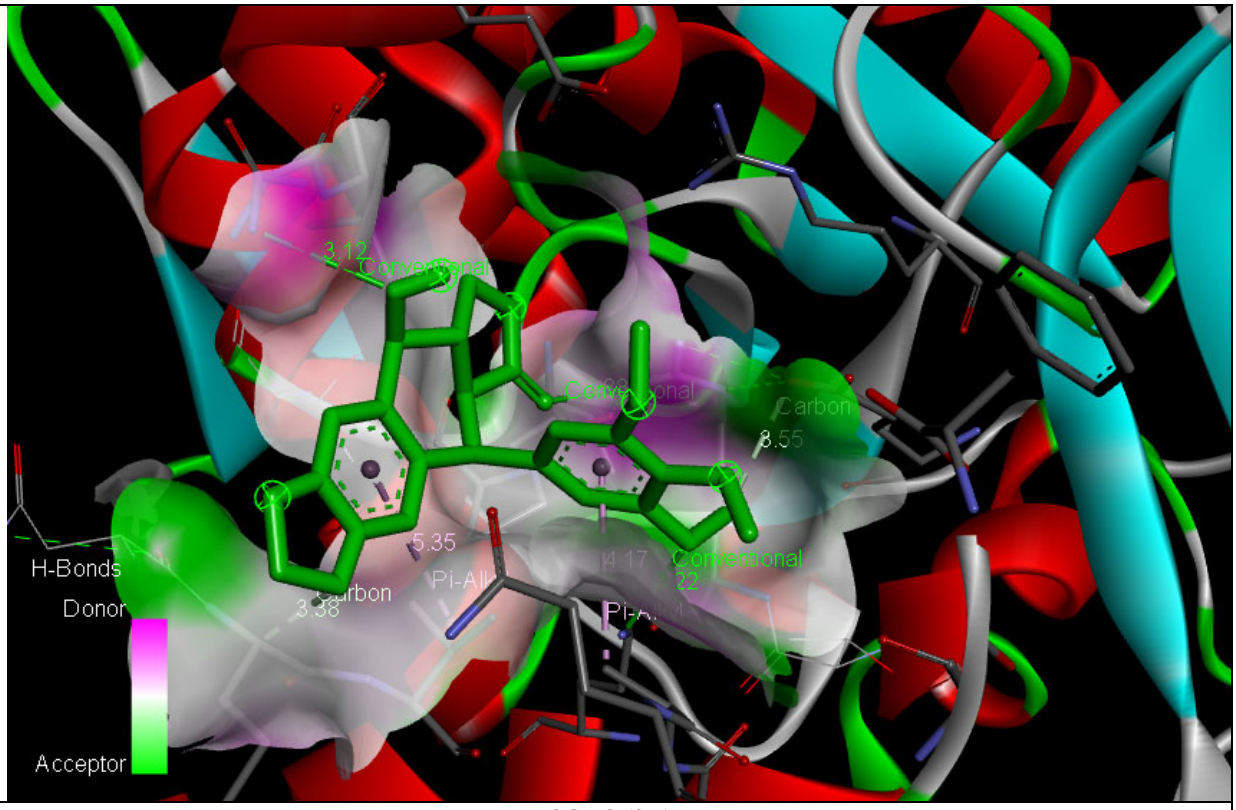
| Ligand | Receptor | Affinity (kCal/mol) | Hydrogen Bond | Carbon-Hydrogen Bond | Pi-Alkyl Bond | Van Der Waals |
|---|---|---|---|---|---|---|
| PTOX | ***1E9Z-chain B*** | -7.5 | Glutamine: 378<br>Arginine: 375<br>Asparagine: 309 | Glutamic acid: 371<br>Lysine: 559 | Valine: 560<br>Alanine: 563 | - |
| | ***2QV3-chain A*** | -8.1 | Tyrosine: 729<br>Threonine: 672 | Asparagine:732<br>Tyrosine: 675 | Valine: 739<br>Arginine: 734 | Proline: 677<br>Phenylalanine:674 |
| | ***4DVY-chain P*** | -7.9 | Lysine: 499<br>Serine: 497<br>Tyrosine: 473 | Glutamic acid: 422<br>Asparagine:597<br>Glycine:496 | - | - |

**Figure 2.** 2D and 3D results of AutoDockVina

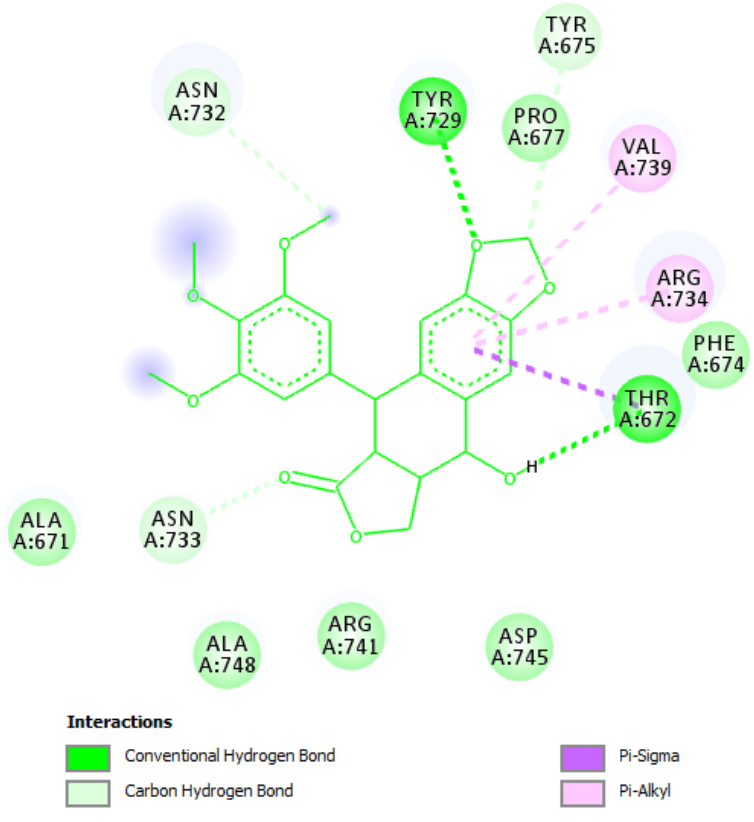
**1E9Z (2D)**



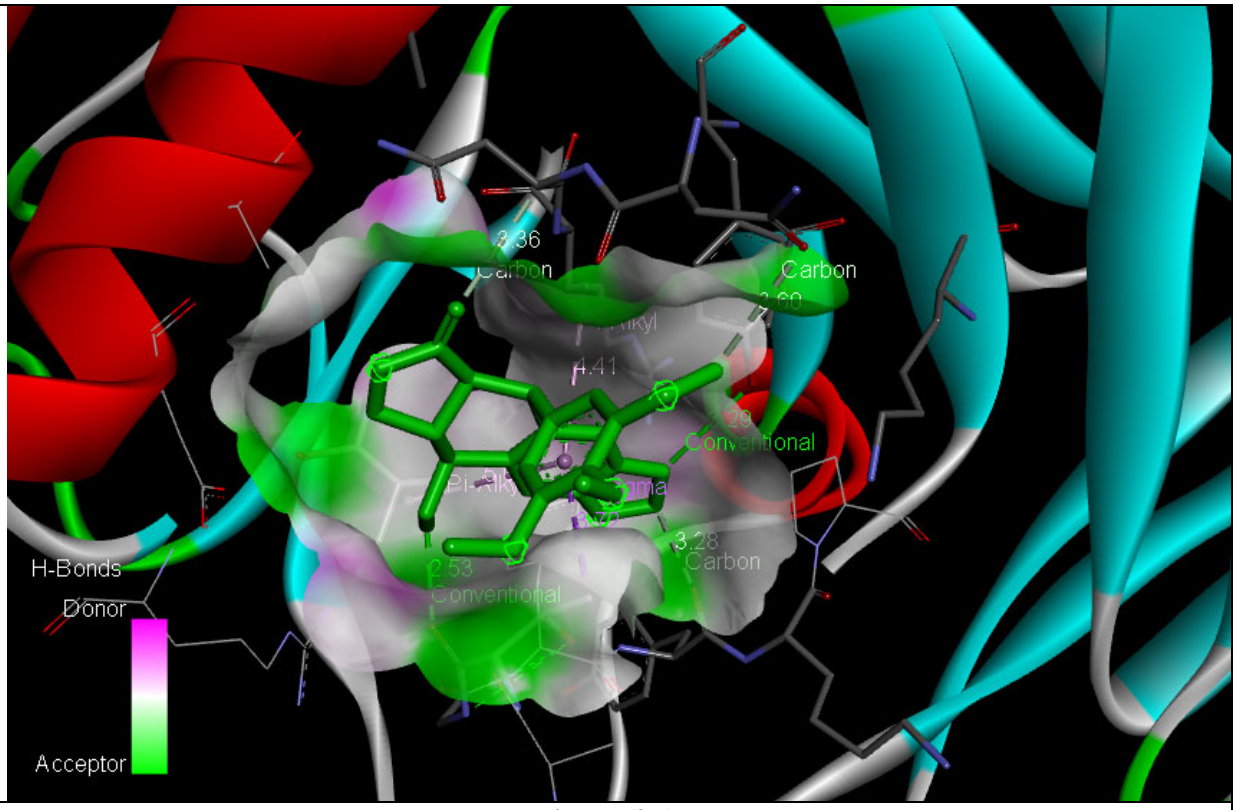

**1E9Z (3D)**



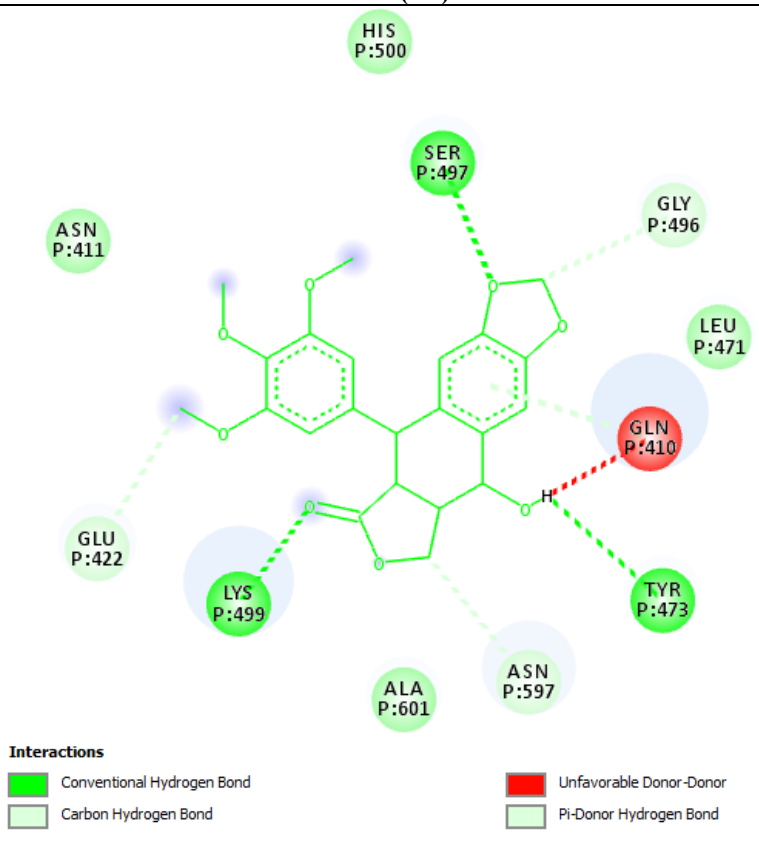

**2QV3 (2D)**




**2QV3 (3D)**




**4DVY (2D)**




**4DVY (3D)**

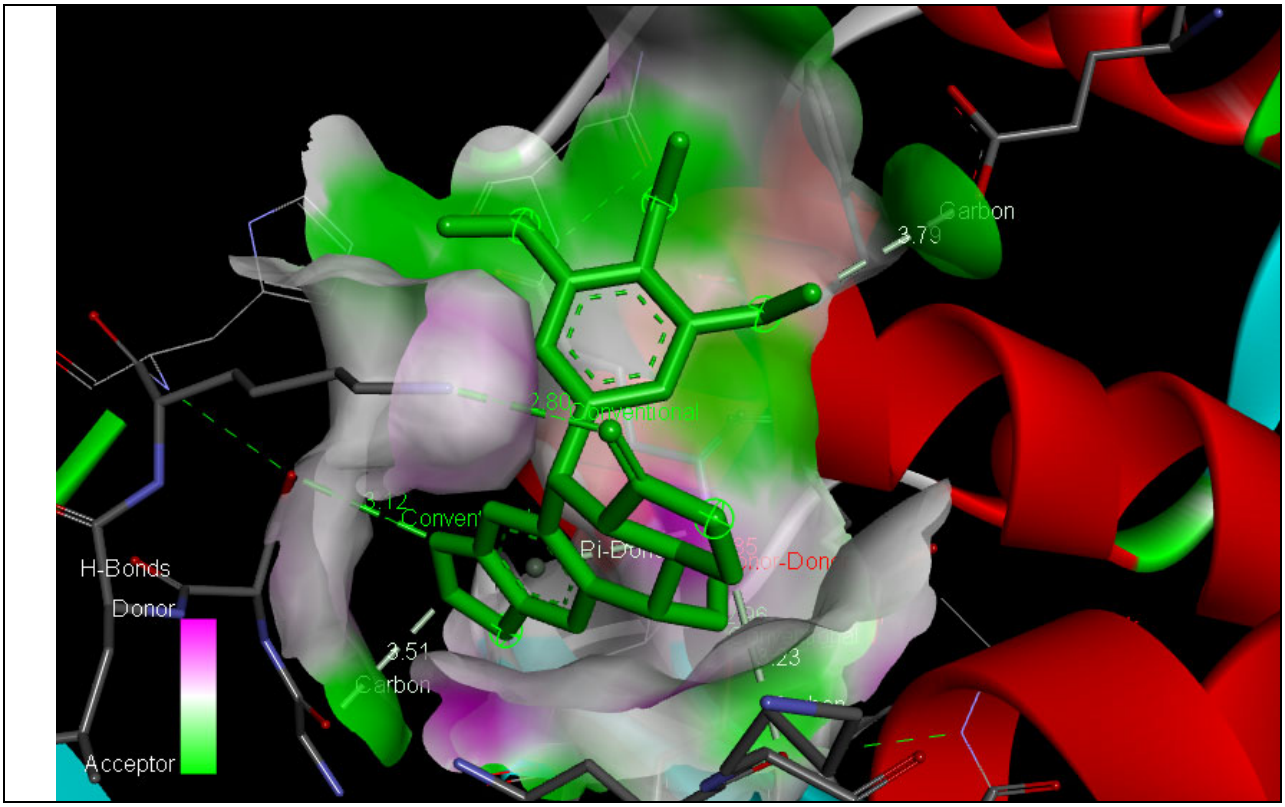



**Figure 4**. M-PCR test results for samples 1 to ۳۰, from left to right: Ladder 100bp - positive control - negative control of mep1 (1650 bp) and mep2 (1400 bp) genes. (Types 1, 3, 4, 5, 6, 9, 10, , 11,13 , 14 ,15, 16, 17, 19, 20, 21, 22, 23,27 and 28 had the Mep2 gene, and strains 7 had the Mep1 gene.)

## 4. Discussion

Cancer and its incidence is one of the most important current problems for human society (Smith et al., 2017). Unfortunately, in recent years, there is a growing trend in the world and in Iran. Finding therapeutic effects of natural compounds that can be used as drugs to kill cancer cells is one of the key priorities. Natural compounds have many advantages over chemical compounds such as fewer side effects (Demain & Vaishnav., 2011). Lignans are a group of phenylpropanoid compounds that are formed by the separation of two units of phenylpropane (Guo et al., 2019). Some studies has been shown the ecological and physiological function of lignans. However, that these compounds participate in the plant defense apparatus against plant and human pathogens (Sharma et al., 2020). Lignans are also part of phytoestrogen compounds and act like human estrogens. These compounds are structurally similar to estrogenic hormones and prevent the development of cancer when ingested by intestinal bacteria (Tanwar et al., 2021). Lignans are also important in the treatment of diseases due to their antifungal, antimicrobial and antiviral properties. One of the most important lignans is PTOX from the aryltetraline group, whose semi-synthetic compounds are used in the treatment of cancer (Barbary et al., 2010). Few studies have been reported on the antibacterial activity of PTOX (Rocha et al., 2018). However, Nanjundaswamy et al. (2007) synthesized precursors of PTOX and examined their antibacterial activity. Their results showed that ethyl-2-(3′-methyl-4′-methoxybenzoyl) -3- (4-methoxyphenol) -cyclopropane-1-carboxylic acid and Ethyl-2-(3′-methyl-4′-methoxybenzyol-3-1- dimethoxyphenyl)-cyclopropane-carboxylic acid has significant antibacterial activity against *Citrobacter* sp., *Escherchia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and *Streptococcus faecalis*. They also pointed out that the PTOX activities less than ciprofloxacin and equal to gentamicin and more than penicillin and streptomycin (Nanjundaswamy et al., 2007). In another study, Umesha et al. (2015) evaluated the antibacterial properties of synthesized

pyrazoles containing PTOX analogues, which showed that PTOX derived compounds had favorable antibacterial properties against *E. faecalis*, *E. aerugenes*, *K. pneumonia* and *P. aeruginosa* (Umesha et al., 2015). In another study, Umesha and Basavaraju (2014) synthesized new pyrazole analogues of PTOX. Among their synthesized compounds, compound 7-(methylthio)-5-(4-(methylthio) phenyl)-4,5-dihydro-2 H-benzo-indazole is more active than of all derivatives, Also compound 7-(methylthio)-5-(4-(methylthio)phenyl)-4,5-dihydro-2H-benzo-indazole had favorable antibacterial and antifungal activity (Umesha & Basavaraju., 2014). In this study, PTOX was evaluated as a ligand to inhibit the *urease*, *VacA*, and *cagA* receptors. The results showed that the ability of PTOX to inhibit *VacA* was higher than others due to its high and strong hydrogen bonds. In this regard, Jouimyi et al. (2020) evaluated the molecular binding of flavonoid compounds with *cagA* and *VacA* of *H. pylori* virulence factors. For the *cagA* protein, the licochalcone-A molecule showed the highest binding affinity (-8 kcal/mol). For the *VacA* protein, the galangin, luteolin, and apigenin molecules showed the highest binding affinity (-8.9, -8.5, and -8.2 kcal/mol, respectively) (Jouimyi et al., 2020). The PTOX combination used in our study showed a higher affinity from the study of Jouimyi et al. *H. pylori* uses a needle-shaped organ to inject *CagA* and *VacA* at the junction of two cells lining the stomach. *CagA* is a toxin that produces the cytotoxin associated with the *VacA* gene. Not all strains of *H. pylori* carry the *CagA* and *VacA* genes, but only those classified as *CagA* and *VacA* positive. These toxins alter the structure of stomach cells, making it easier for bacteria to attach to them (Miernyk et al., 2011). In this study, computer data showed that the combination of PTOX with high levels of *VacA* inhibition could prevent them from being injected into stomach wall cells and even high adhesion. This possibility is confirmed when *In Vitro* and *In vivo* tests are also confirmed. In general, the present theoretical data suggest that PTOX can inhibit *H. pylori* pathogens. Molecular docking studies have provided good information on the binding tendency of PTOX to receptors and helped to understand the interactions that require further biochemical testing.

## Conclusion

The results showed that the potential anti-H. Pylori effect of PTOX against the factors involved in the pathogenicity of this bacterium could be due to inhibition of various receptors, including VacA. The results of this research can play a key role in the development of therapeutic drugs for this bacterium.

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**Ethics approval:** This research has not been performed on human samples or laboratory animals.

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## Refereces

Ansari, S., Yamaoka, Y., 2017. Survival of Helicobacter pylori in gastric acidic territory. Helicobacter. 22, 12386. https://doi.org/10.1111/hel.12386.

Backert, S., Blaser, MJ., 2016. The role of CagA in the gastric biology of Helicobacter pylori. Cancer. Res. 76, 4028-4031. https://doi.org/10.1158/0008-5472.CAN-16-1680.

Bashir, MK., Mustafa, YF., Oglah, MK., 2020. Antitumor, antioxidant, and antibacterial activities of glycosylconjugated compounds: A review. Syst. Rev. Pharm. 11, 175-185. https://doi.org/: 10.31838/srp.2020.4.26.

Barbary, O., El-Sohaimy, S., El-Saadani, M., Zeitoun, A., 2010. Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. Am. J. Agric. Biol. Sci. 6, 247-256.

Coelho, LGV., Marinho, JR., Genta, R., Ribeiro, LT., Passos, MD., Zaterka, S., Assumpção, PP., Barbosa, AJ., Barbuti, R., Braga, LL., Breyer, H., 2018. IVth Brazilian consensus conference on Helicobacter pylori infection. Arq.

Gastroenterol. 55, 97-121. https://doi.org/10.1590/S0004-2803.201800000-20.

Demain, A., & Vaishnav, P., 2011. Natural products for cancer chemotherapy. Microb. Biotechnol. 4, 687-699. https://doi.org/10.1111/j.1751-7915.2010.00221.x.

Graham, DY., Miftahussurur, M., 2018. Helicobacter pylori urease for diagnosis of Helicobacter pylori infection: A mini review. J. Adv. Res. 13, 51-57. https://doi.org/10.1016/j.jare.2018.01.006.

Ghameshlouei, S., Ahrabi, NZ., Souldozi, A., SarveAhrabi, Y., 2021. Evaluation of the Antibacterial and Investigation of the Molecular Docking of New Derivatives of 1, 3, 4-Oxadiazole as Inhibitors of Quorum Sensing System in the Human Pathogen Pseudomonas Aeruginosa. Avicenna. J. Clin. Microbiol. Infect. 8, 27-33. https://doi.org /10.34172/ajcmi.2021.06.

Guo, K., Tong, C., Fu, Q., Xu, J., Shi, S., Xiao, Y., 2019. Identification of minor lignans, alkaloids, and phenylpropanoid glycosides in Magnolia officinalis by HPLC□DAD□QTOF-MS/MS. J. Pharm. Biomed. 170, 153-160. https://doi.org/10.1016/j.jpba.2019.03.044.

Jouimyi, MR., Bounder, G., Essaidi, I., Boura, H., Zerouali, K., Lebrazi, H., Kettani, A., Maachi, F., 2020. Molecular docking of a set of flavonoid compounds with Helicobacter pylori virulence factors CagA and VacA. J. Herbmed. Pharmacol. 9, 412-419. https://doi.org/10.34172/jhp.2020.52.

Keikha, M., Eslami, M., Yousefi, B., Ghasemian, A., Karbalaei, M., 2019. Potential antigen candidates for subunit vaccine development against Helicobacter pylori infection. J. Cell. Physiol. 234, 21460-21470. https://doi.org/10.1002/jcp.28870.

Li, J., Perez-Perez, GI., 2018. Helicobacter pylori the latent human pathogen or an ancestral commensal organism. Front. Microbiol. 9, 609-609. https://doi.org/10.3389/fmicb.2018.00609.

Lucero, Y., Lagomarcino, AJ., Torres, JP., Roessler, P., Mamani, N., George, S., Huerta, N., Gonzalez, M., O'Ryan, M., 2021. Helicobacter pylori, clinical, laboratory, and noninvasive biomarkers suggestive of gastric damage in healthy school-aged children: A case-control study. Int. J. Infect. Dis. 103, 423-430. https://doi.org/10.1016/j.ijid.2020.11.202.

Lin, TY., Lan, WH., Chiu, YF., Feng, CL., Chiu, CH., Kuo, CJ., Lai, CH., 2021. Statins' Regulation of the Virulence Factors of Helicobacter pylori and the Production of ROS May Inhibit the Development of Gastric Cancer. Antioxidants. 10, 1293. https://doi.org/10.3390/antiox10081293.

Morris, GM., Lim-Wilby, M., 2008. Molecular docking. J. Mol. Model. 443, 365-382. https://doi.org/10.1007/978-1-59745-177-2_19.

Miernyk, K., Morris, J., Bruden, D., McMahon, B., Hurlburt, D., Sacco, F., Parkinson, A., Hennessy, T., Bruce, M., 2011. Characterization of Helicobacter pylori cagA and vacA genotypes among Alaskans and their correlation with clinical disease. J. Clin. Microbiol. 49, 3114-3121. https://doi.org/10.1128/JCM.00469-11.

Nanjundaswamy, N., Satishi, S., Rai, KL., Shashikanth, S., Raveesha, KA., 2007. Antibacterial activity of synthetic precursors of podophyllotoxin. Int. J. Biomed. Sci. 3, 112-115. PMID: 23675032; PMCID: PMC3614634.

Outeiral, C., Strahm, M., Shi, J., Morris, GM., Benjamin, SC., Deane, CM., 2021. The prospects of quantum computing in computational molecular biology. Wiley. Interdiscip. Rev. Comput. Mol. Sci. 11, 1481. https://doi.org/10.1002/wcms.1481.

Piscione, M., Mazzone, M., Marcantonio, MC., Muraro, R., Mincione, G., 2021. Eradication of Helicobacter pylori and gastric cancer: a controversial relationship. Front. Microbiol. 12, 630852. https://doi.org/10.3389/fmicb.2021.630852.

Patwari, SB., Murali, MS., Jadhav, VB., 2018. Microwave□Assisted Synthesis, Antimicrobial, and Cytotoxicity Activity of N‐tert‐butyl‐3‐{[2‐(arylamino) pyrimidin‐4‐yl] amino} Benzenesulfonamides. J. Heterocycl. Chem. 55, 2911-2918. https://doi.org/10.1002/jhet.3364.

Qi, YL., Liao, F., Zhao, CQ., Lin, YD., Zuo, MX., 2005. Cytotoxicity, apoptosis induction, and mitotic arrest by a novel podophyllotoxin glucoside, 4DPG, in tumor cells. Acta Pharmacol. Sin. 26, 1000-1008. https://doi.org/10.1111/j.1745-7254.2005.00148.x.

Rawla, P., Barsouk, A., 2019. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz. Gastroenterol. 14, 26-38. https://doi.org/10.5114/pg.2018.80001.

Rocha, MP., Campana, PR., Scoaris, DD., Almeida, VL., Lopes, JC., Shaw, JM., Silva, CG., 2018. Combined in Vitro Studies and in Silico Target Fishing for the Evaluation of the Biological Activities of Diphylleia cymosa and Podophyllum hexandrum. Molecules. 23, 3303. https://doi.org/10.3390/molecules23123303.

Su, T., Li, F., Guan, J., Liu, L., Huang, P., Wang, Y., Qi, X., Liu, Z., Lu, L., Wang, D., 2019. Artemisinin and its derivatives prevent Helicobacter pylori-induced gastric carcinogenesis via inhibition of NF-κB signaling. Phytomedicine. 63, 152968. https://doi.org/10.1016/j.phymed.2019.152968.

Smith, S., Fowora, M., Pellicano, R., 2019. Infections with Helicobacter pylori and challenges encountered in Africa. World. J. Gastroenterol. 25, 3183-3195. https://doi.org/10.3748/wjg.v25.i25.3183.

Sharma, S., Sharma, YP., Bhardwaj, C., 2018. Development and validation of HPLC□UV method for quantification of podophyllotoxin in Podophyllum hexandrum Royle. J. Pharmacogn. Phytochem. 7, 3748-3752.

Singh, M., Chaudhry, P., Fabi, F., Asselin, E., 2013. Cisplatin-induced caspase activation mediates PTEN cleavage in ovarian cancer cells: a potential mechanism of chemoresistance. BMC cancer. 13, 1-9. https://doi.org/10.1186/1471-2407-13-233.

Sadeghi, I., Behmanesh, M., Chashmi, NA., Sharifi, M., Soltani, BM., 2015. 6-Methoxy podophyllotoxin induces apoptosis via inhibition of TUBB3 and TOPIIA gene expressions in 5637 and K562 cancer cell lines. Cell Journal (Yakhteh). 17, 502-509. https://doi.org/10.22074/cellj.2015.10.

Smith, R., Andrews, K., Brooks, D., Fedewa, SA., Manassaram□Baptiste, D., Saslow, D., Brawley, OW., Wender, RC., 2017. Cancer screening in the United States, 2017: a review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J. Clin. 67, 100-121. https://doi.org/10.3322/caac.21392.

Sharma, A., Sidhu, GP., Araniti, F., Bali, AS., Shahzad, B., Tripathi, DK., Brestic, M., Skalicky, M., Landi, M., 2020. The role of salicylic acid in plants exposed to heavy metals. Molecules. 25, 540. https://doi.org/10.3390/molecules25030540.

Tanwar, AK., Dhiman, N., Kumar, A., Jaitak, V., 2021. Engagement of phytoestrogens in breast cancer suppression: Structural classification and mechanistic approach. Eur. J. Med. Chem. 213, 113037. https://doi.org/10.1016/j.ejmech.2020.113037.

Umesha, B., Basavaraju, YB., Mahendra, C., 2015. Synthesis and biological screening of pyrazole moiety containing analogs of podophyllotoxin. Med. Chem. Res. 24, 142-151. https://doi.org/10.1007/s00044-014-1100-3.

Umesha, B., Basavaraju, Y., 2014. Synthesis and pharmacological studies of new pyrazole analogues of podophyllotoxin. Russ. J. Bioorganic. Chem. 40, 467-476. https://doi.org/10.1134/S106816201404013X.

Vaira, D., Gatta, L., Ricci, C., D'Anna, L., Miglioli, M., 2001. Helicobacter pylori: diseases, tests and treatment. Dig. Liver. Dis. 33, 788-794. https://doi.org/10.1016/S1590-8658(01)80697-6.

Wang, D., Guo, Q., Yuan, Y., Gong, Y., 2019. The antibiotic resistance of Helicobacter pylori to five antibiotics and influencing factors in an area of China with a high risk of gastric cancer. BMC. Microbiol. 19, 1-10. https://doi.org/10.1186/s12866-019-1517-4.

Yousefi-Avarvand, A., Vaez, H., Tafaghodi, M., Sahebkar, AH., Arzanlou, M., Khademi, F., 2018. Antibiotic resistance of Helicobacter pylori in Iranian children: a systematic review and meta-analysis. Microb. Drug. Resist. 24, 980-986. https://doi.org/10.1089/mdr.2017.0292.

Zamani, M., Vahedi, A., Maghdouri, Z., Shokri-Shirvani, J., 2017. Role of food in environmental transmission of Helicobacter pylori. Caspian. J. Intern. Med. 8, 146-152. https://doi.org/10.22088/cjim.8.3.146