



Anti-*Helicobacter pylori* Activity of New Derivatives of 1, 3, 4-Oxadiazole: In Silico Study

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Abstract

Background: The growing spread of drug resistance in *Helicobacter pylori* has caused concern. Urease is one of the most important enzymes associated with *H. pylori* activity. Oxadiazoles have a wide range of inhibitory activities. The aim of this study was to investigate new oxadiazole compounds as urease inhibitors of *H. pylori*.

Methods: The synthesized compounds were reused as ligands in the previous study, and the initial structure of the compounds was optimized by the Molecular Mechanics Models method. Then, the compounds were evaluated as inhibitors on the active site of the urease enzyme by AutoDock Vina software, and the output results were analyzed and evaluated using soft Discovery Studio software.

Results: All compounds, especially (4c) with flour groups, exhibited powerful inhibitory activity against the urease enzyme of *H. pylori*.

Conclusions: The present findings indicated the inhibitory potential of the novel synthetic 1, 3, 4-oxadiazole compounds.

Keywords: *Helicobacter pylori*, Urease, Oxadiazoles, Molecular docking simulation

Background

Helicobacter pylori is a gram-negative, spiral-shaped bacterium of the genus Epsilonproteobacteria (1). More than 50% of the world's population is infected with the bacterium, and the infection usually appears in the early years of life (2). The spiral shape allows this bacterium to enter between the mucous layers that cover the stomach. The bacterium breaks down urea molecules, a chemical that produces ammonia and carbon dioxide. Ammonia forms a sheath around the bacteria that protects it against stomach acid. Therefore, the production of large amounts of urease enzyme is essential for the survival and pathogenicity of *H. pylori* (3), which has been identified by the World Health Organization as a human carcinogen of group 1. This bacterium causes severe gastritis and peptic ulcers in all infected people (4). The prevalence of *H. pylori* varies based on race, social class, socioeconomic status, health, and age (5). Today, there are several diagnostic and therapeutic methods for *H. pylori*, there is also treatment resistance that calls for the introduction of new drug combinations (6). In a study conducted in Iran, resistance to metronidazole was very high (57.4%), which is almost consistent with the results of other Asian countries (46.6%); however, the average resistance in Iran is lower than that in African countries (97.55%). Furthermore, the average resistance to ciprofloxacin in Iran is 18.5% due to the small number of studies (7). The enzyme urease has a molecular weight of 540 kDa and consists of two large subunits: UreA with 26.50 kDa and UreB with 61 kDa with

a gene size of 1.70 kPa. Urease is a nickel metallic enzyme that hydrolyzes the conversion of urea to ammonium and carbon dioxide. *H. pylori* activated urease depends on the presence of UreA/B gene structures to form the 550 kDa holoenzyme while UreIE/F subgenes and UreB/I/G genes are required for high expression of urease activity and bacterial establishment in the stomach, respectively.

UreB is the most effective and common immunogen of all *H. pylori* strains that can elicit a protective immune response in the body against *H. pylori* (8). Oxadiazole derivatives have a wide range of biological activities including inhibitory properties (9). The increasing need to produce pharmaceutical compounds and replace compounds with resistant drugs has become a necessity; therefore, the purpose of this study was to investigate the molecular docking studies of oxadiazole compounds as potential urease inhibitors of *H. pylori*.

Methods

Ligand Preparation

Our previously synthesized compounds were reused (10). The initial structure of the compounds was plotted by ChemDraw Pro 12.0 software, then the crystallographic structure of the compounds was drawn using Chem3D 17.0 software, and finally optimized with the Molecular Mechanics Models in kcal.mol⁻¹ command (11). The total energy of each compound after optimization is specified and listed in Figure 1.

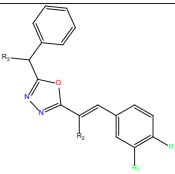
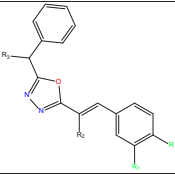
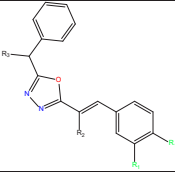
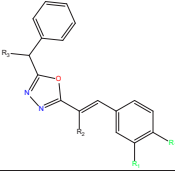
Compound	2D Structure	Total Energy (kcal/mol)
[4a] R ₁ - -Cl R ₂ - -COHNCH ₃ R ₃ - -OH		50.4298
[4b] R ₁ - -Bromide R ₂ - -COHNCH ₃ R ₃ - -OH		51.8095
[4c] R ₁ - -Flour R ₂ - -COHNCH ₃ R ₃ - -OH		47.9225
[4d] R ₁ - -Methoxy R ₂ - -COHNCH ₃ R ₃ - -OH		59.4552

Figure 1. 2D Structural and Total Energy of New Derivatives of 1, 3, 4-Oxadiazoles (10). Note. 2D: Two dimensional.

Protein Preparation

Crystallized structure of the urea enzyme of *H. pylori* was downloaded from the protein database with code (PDB ID: 1e9z) and the resolution of 3 angstroms (Å). To ensure the structure's health, its crystallography was observed by Visual Molecular Dynamics software. Further, the second structure of the protein was examined through Chimera software.

Molecular Docking

The docking process of compounds 4a-4d to the urease binding site was performed using AutoDock Vina software and Discovery Studio 4.5. Polar hydrogens were added to the compounds and proteins, then the partial charge of the compounds was added, and the partial charge of the protein was added by the Kollman method. Finally, 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 was 25 000 000, and an initial population of 150 was randomly assigned. In addition, the maximum number of generations, the mutation rate, and the crossover rate were 27 000, 0.02, and 0.8, respectively, along with 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 and the ligand as inflexible and flexible, respectively. Grid box with dimensions 120 × 120 × 120 which includes the whole protein, the distance of grade 1 Å, and other parameters (X-Center: 120.00, Y-Center: 100.00, Z-Center: 66.00) was considered, and the best conformity with the lowest amount of binding energy was selected as a result after docking (12).

Results

According to Table 1, all affinities of the compounds were calculated, and the best compounds with low ΔG (-ΔG) were selected to continue experiments and investigate chemical interactions. As demonstrated in Figure 2, the highest amount of hydrogen bonds was related to the 4c compound with the amino acid serine: 567, glutamic acid: 371, threonine: 374, and glutamine: 378, among which the compound bonds were the case. Regarding the 4d compound, the highest amount of carbon-hydrogen bonds was related to Lysine: 445 and Glutamine: 471.

Discussion

Compounds with 1,3,4-oxadiazole cores have occupied a specific place in medicinal and synthetic chemistry because of their extensive range of biological activities such as antibacterial, antifungal, anti-inflammatory, antiviral, anticancer, enzyme inhibitor, anticonvulsant, and anti-diabetic properties (13). These properties make it a desirable medicinal backbone that can be used to construct biologically-useful molecules. This study evaluated the *in silico* effects of 4 syntheses of 1, 3, 4-oxadiazole derivatives against the human pathogen *H. pylori*. Drug resistance of *H. pylori* has been increasing over the past few decades (14). According to a study by Moran-Gilad et al, *H. pylori* resistance rate was 54%, 31%, 10%, 4%, and 2% to clarithromycin, metronidazole, amoxicillin,

Table 1. AutoDock Vina Results of 1, 3, 4-Oxadiazole Compounds (4a-4d)

No.	Affinity (kcal/mol)	Carbon-Hydrogen Bond	Pi-Alkyl Bond	Pi-Anion Bond	Hydrogen Bond	Halogen Bond	Pi-Cation Bond
4a	-7.3	Asparagine: 111	Alanine: 94 Lysine: 92	Glutamic acid: 64	-	-	-
4b	-6.8	-	-	-	-	-	-
4c	-8.3	-	Alanine: 563 Valine: 560	-	Serine: 567 Glutamic acid: 371 Threonine: 374 Glutamine: 378	Valine: 444	Arginine: 375
4d	-7.0	Lysine: 445 Glutamine: 471	Alanine: 16 Valine: 33 Valine: 473	Isoleucine: 568	-	-	-

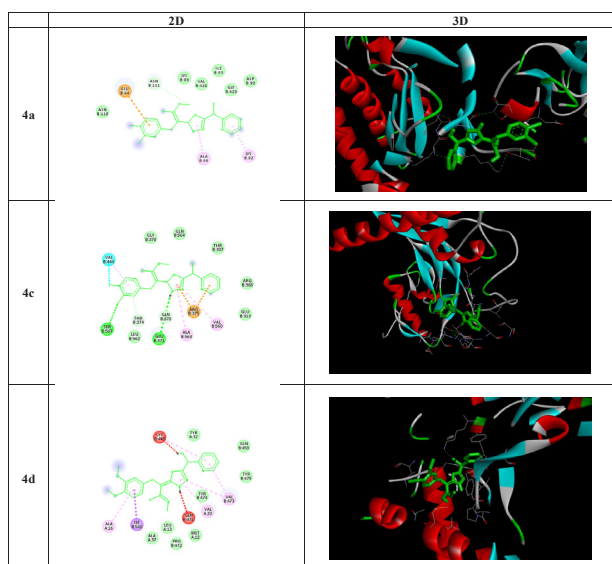


Figure 2. 2D and 3D Results of AutoDock Vina. Note. 2D: Two dimensional; 3D: Three dimensional.

rifampicin, and levofloxacin, respectively (15). Resistance was found to be an alarm to increase drug resistance to treatment. Therefore, compounds with central nuclei of 1, 3, 4-oxadiazole can be a good alternative to existing drugs. In the present study, compound 4c with the flour group appeared with a promising inhibitory activity that can be used as an inhibitor of this compound in the synthesis of anti-*H. pylori* drugs. In a study conducted by Khan et al, oxadiazole derivatives were evaluated as urease inhibitors, and the results showed that compound 6a had the lowest K_i among the series, while compounds 6d, 6e, 6g, and 6i were subsequently found to have significant K_i values after 6a. Molecular docking has supported their inhibition types and structure activity-relationship (16). In another study, Mao et al synthesized 14 metronidazole derivatives (compounds 3a–f and 4b–h) by coupling the metronidazole and salicylic acid derivatives. The docking results revealed that the AutoDock 4.0 program could explain the inhibitory activities of compound 4g (bind Cl group in structure) against *H. pylori* urease (17). The results also indicated that group Cl could inhibit the *H. pylori* urease enzyme, but results of the present study indicated that the flour group can inhibit the urease enzyme. Moreover, in their study, Kataria and Khatkar synthesized morin analogous with oxadiazole group as *H. pylori* urease inhibitors. Among their synthesized compounds, N-(2-chlorophenyl)-N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene) thiourea (M2b) and N-(4-bromophenyl)-N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene) thiourea (M2i) were found to be most potent urease inhibitors in silico. The results of this study confirmed the effects of chlorine-containing compounds on the inhibition of urease activity of *H. pylori* (18). The results of the above-mentioned studies are consistent with the results of the present study so that in many studies the main role of the 1, 3, 4-oxadiazole ring has been determined. Further,

the combination of 4a with chloride group and 4d with methoxy group showed inhibitory activity so that they had fewer bonding connections than the 4C combination. Moreover, the simple workup, tall abdicates, and brief response times make the strategy a valuable expansion for planning advanced pharmaceutical synthetics.

Conclusions

Helicobacter pylori urease enzyme is one of the most important enzymes associated with bacterial activity, so the inhibition of this enzyme will kill bacteria. Alternative drug structures are a basic and important need in the treatment of pathogenic strains of bacteria. Hence, the need to make and synthesize alternative drugs has increased gradually. Newly synthesized structures containing the central nucleus of 1, 3, 4-oxadiazole can be used for a variety of biological activities. It appears that oxadiazole subordinates will be a supportive structure for conceivable improvement of unused drugs, but this result must be confirmed by other broad clinical trials that will be a portion of our future plans.

Acknowledgments

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Conflict of Interests

None.

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