Research Inventy: International Journal of Engineering And Science
Vol.10, Issue 6 (June 2020), PP 09-14
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

Calculation analysis and biological evaluation of a new *Helicobacter pylori* urease inhibitor

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ABSTRACT: *Urease is found as a key target to Helicobacter pylori, which is the main pathogenic factor of various gastric diseases. The inhibiton activity and interaction mechanism of (2Z,3R,6S)-4-hydrazono- 3,6-dimethyl- 2-(3-methylbutylidene)octahydrobenzofuran-3-ol (compound **1**) as a new Helicobacter pylori urease inhibitor (IC_{50} = 1.56 μM) were studied by molecular docking, MM/GBSA binding free energy analysis and biological evaluation methods. The calculated ΔG_{bind} of compound **1** was -73.94 kcal/mol. By the decomposed energy comparisons of residues in binding sites, the hydrazine group of compound **1** would be the important group interacting with the key site Ni3001 and Ni3002 in urease. Compound **1** also has H^+,K^+-ATPase inhibition activity (IC_{50}=2.60 μM) in our previous studies. So these results could help for further rational design of the novel urease and H^+,K^+-ATPase dual inhibitor.*

KEYWORDS: *urease; Helicobacter pylori; Molecular docking; Binding free energy.*

Date of Submission: 18-06-2020 Date of Acceptance: 06-07-2020

I. INTRODUCTION

*Helicobacter pylor*i is the main pathogenic factor of various gastric diseases, including chronic gastritis, gastric lymphoma, peptic ulcers, and stomach cancer (Parsonnet et al., 1994), affecting more than half of the world's population (Conteduca et al., 2013). Now the first-line therapy for *Helicobacter pylori* infection has comprised a combination of a proton-pump (H^+,K^+-ATPase) inhibitor and two antibiotics, usually amoxicillin and clarithromycin. However, the eradication failure of *Helicobacter pylori* infection with this treatment regimens has been reported in many countries (Figura et al., 2016), because of the growing resistance of *Helicobacter pylori* to the antibiotics (Graham and Fischbach et al., 2010; Megraud et al., 2012).

Urease (urea amidohydrolase EC 3.5.1.5), a Ni-containing hyperactive metalloenzyme, is found as a key enzyme in *Helicobacter pylori*, which accelerating the hydrolysis of urea to ammonia and carbon dioxide (Krajewska et al., 2009). Then the protective ammonium cloud is released from urea, allowing *Helicobacter pylori* to survive in a hostile acidic environment (Maroney et al., 2014). So strategies based on urease inhibition are considered as a promising treatment for *Helicobacter pylori* infection (Azizian et al., 2012). Although hundreds of urease inhibitors have been determined, only acetohydroxamic acid (AHA) was approved by U.S. Food and Drug Administration in May, 1983 (Yu XD et al., 2015). However, its relatively moderate inhibitory activity requires rather large doses (about 1000 mg/day for adults) (Kosikowska et al., 2011).

Recently (2Z,3R,6S)-4-hydrazono-3,6-dimethyl-2-(3-methylbutylidene)octahydrobenzofuran-3-ol (compound **1** in Fig. 1) was synthesized and evaluated as a H^+,K^+-ATPase inhibitor (IC_{50}=2.60 μM) by our group (Jin et al., 2011; She et al., 2018), which was modified from bisabolangelone, a bioactive sesquiterpene in the roots of *Angelica polymorpha* (Chinese Tujia nationality medicine) (Wang et al., 2009 ; Luo et al., 2012). Does it have urease inhibition activity and become a novel dual inhibitor for *Helicobacter pylori* infection? Therefore, in this paper the interaction mechanism between compound **1** and urease was analyzed by molecular docking method, and *Helicobacter pylori* urease inhibition activity was then evaluated.

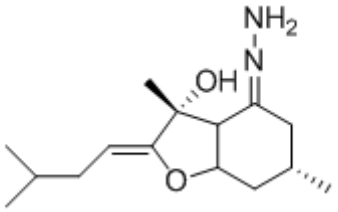


Fig1. Chemical structure of compound 1

II. MATERIALS AND METHODS

2.1 Molecular docking

The docking simulation was performed using induced-fit docking (IFD) method (Sherman et al., 2006; Luo et al., 2013) in the Schrödinger software suite (Schrödinger et al., 2010). The 3D structure of *Helicobacter pylori* urease with AHA (PDB code: 1E9Y, resolution: 3 Å) (Ha et al., 2001) was subject to the Protein Preparation Wizard module in Schrödinger using the OPLS-2005 force field (Jorgensen et al., 1996). The dimension for the cubic boundary box centered on the centroid of the ligand was set to 20 Å × 20 Å × 20 Å, and the docking mode was set in Glide XP (Friesner et al., 2006). Finally, An IFD score (IFD score = 1.0 Glide_Gscore + 0.05 Prime_Energy) was calculated and used to rank the docking poses.

2.2 MM/GBSA calculation

Using molecular mechanics-generalized Born surface area (MM/GBSA) method in Prime program (Jacobson et al., 2004; Kollman et al., 2011), the binding free energy (ΔG_{bind}) calculations were performed for the best docking pose complex according to the following equations (Massova et al., 2000):

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv}$$

ΔE_{MM} - the difference of the gas phase MM energy between the complex and the sum of the energies of the protein and inhibitor; ΔG_{solv} - the change of the solvation free energy upon binding. To analyze the key residues related to the detailed interaction mechanism, the binding free energy between ligand and urease was decomposed into the contribution of each residue through Prime program (Jacobson et al., 2004; Kollman et al., 2011).

2.3 *Helicobacter pylori* urease inhibition activity measurement

Helicobacter pylori (ATCC 43504; American Type Culture Collection) was grown on Columbia agar supplemented with bovine serum albumin (BSA) for 72 h at 37℃ under a microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). *Helicobacter pylori* urease was then prepared (Matsubara et al., 2003). 50 mL broth cultures (2.0 × 108 CFU/mL) were centrifuged (5000 g, 4℃) to collect the bacteria. After washing twice with phosphate-buffered saline (pH 7.4), the *Helicobacter pylori* precipitation was prepared and then added of 3 mL distilled water and protease inhibitors under sonication for 1 min. The supernatant was desalted through Sephadex G-25 column. Subsequently, centrifugation (12,000g, 4℃) was performed. Finally, the resultant urease solution was added to an equal volume of glycerol and stored at 4℃ for the experiment.

The assay mixtures comprising 25 μL (10 U) of *Helicobacter pylori* urease and 25 μL of the test compound, was pre-incubated for 1 h at 37℃ in a 96-well assay plate. Urease activity was determined by measuring the absorbance of ammonia production and using the indophenol method described by Weatherburn (Weatherburn et al., 1967). The inhibitory rate (%) was determined by the following equation: % inhibition = [(activity without inhibitor - activity with inhibitor) / activity without inhibitor] × 100% , and the 50% inhibitory concentration (IC_{50}) of the urease activity was determined (AHA as positive drug). The experiments were triply performed.

III. RESUITS AND DISCUSSION

The IC_{50} value of compound **1** to urease was measured as 1.56 μM (Fig. 2), while IC_{50} of AHA was 20.10 μM. Molecular docking between urease and compounds was simulated by IFD method, and Glide Gscores, IFD scores and ΔG_{bind} were listed in Table 1. From the calculation results, the order of favorable binding interaction with urease is compound **1** > AHA. It is in agreement with the experiment data of inhibition activity.

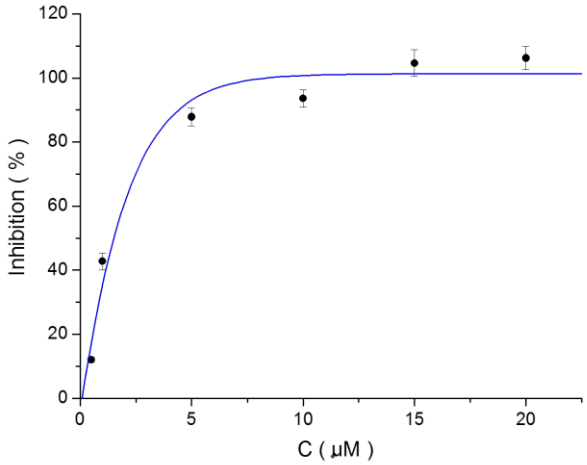
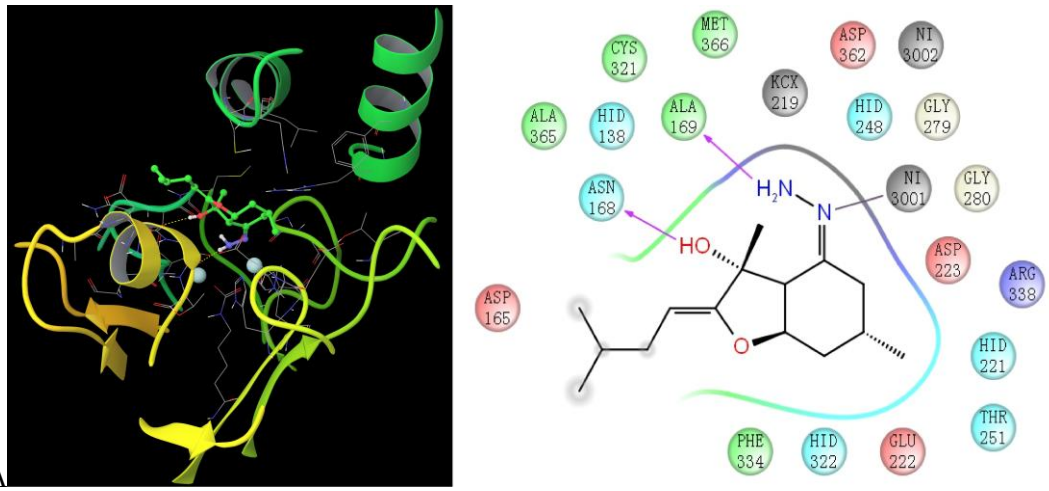


Fig 2. The urease inhibition curve of compound 1

Table1 Docking scores, binding free energies (kcal/mol) and IC_{50} values of compounds with urease

Compounds	Gscore	IFD score	ΔGbind	IC_{50} /μM
1	-5.09	-1508.29	-73.94	1.56
AHA	-3.37	-1024.30	-56.21	20.10

A



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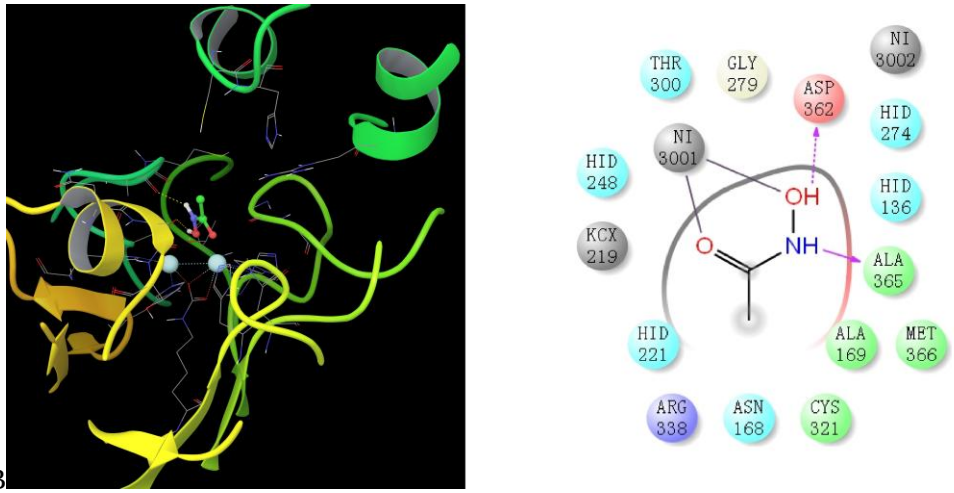

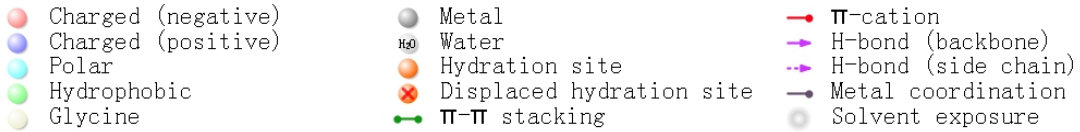

Fig3. Interaction modes of ligands with urease, (A): compound 1; (B): AHA

The interaction modes of compound **1** and AHA (the best pose) were compared in Fig. 3 using Ligand Interactions module embedded in Maestro 9.3 (Maestro et al., 2012). Compound **1** was docked in the binding sites of urease near Ni atoms, specially the nitrogen atom of hydrazine group interacting with Ni3001 by metal coordination. Hydroxyl group and hydrazine group of compund **1** have hydrogen bond interactions with Asn168 (the distance: 2.007 Å) and Ala169 (the distance: 2.401 Å), respectively. The residues (Hid138, Asn168, Hid221, Hid248, Thr251 and Hid322) in the binding pocket mostly have polar interactions with the ligand. Asp165, Glu222, Asp223 and Asp362 contact with the ligand by negative charged interaction (Fig. 3). AHA has metal coordination interaction with Ni3001 through carbonyl and hydroxyl groups, and has two H-bonds with Asp362 (the distance: 1.643 Å) and Ala365 (the distance: 2.243 Å).

To provide the quantitative interaction information of the key residues, the binding free energies between urease and ligands were decomposed into the contribution of each residue. From the energy comparison of residues in binding sites (Fig. 4 and Table 2), it can be observed that there is the distinct difference in interacting with Ni3001 between compound **1** and AHA. The hydrazine group of compound **1** interacts strongly with the key site Ni3001 by metal coordination interaction (-105.33 kcal/mol), while the interaction energy between Ni3001 and AHA is only -52.77 kcal/mol. For binding with another Ni atom, the ΔG_{bind} of compound **1** with Ni3002 (-10.46 kcal/mol) is also far higher than that of AHA (-2.08 kcal/mol) (Fig. 3, Table 2). In our previous studies (She et al., 2018), the hydrazine group of compound **1** has strong H-bond interaction with H^+,K^+-ATPase, too. So the hydrazine group could be an important group of the urease and H^+,K^+-ATPase dual inhibitor. Due to the H-bond interactions, there are high binding free energies of AHA with Asp362 (-21.69 kcal/mol) and Ala365 (-11.58 kcal/mol), while compound **1** with Asn168 (-8.35 kcal/mol) and Ala169 (-9.88 kcal/mol). In addition, AHA has strong interaction with Kcx219 (-24.75 kcal/mol). The ΔG_{bind} of compound **1** with Arg338 (14.39 kcal/mol), Hid248 (4.08 kcal/mol) and Hid274 (2.06 kcal/mol) are positive and unfavorable. Compound **1** has the potential for further modification.

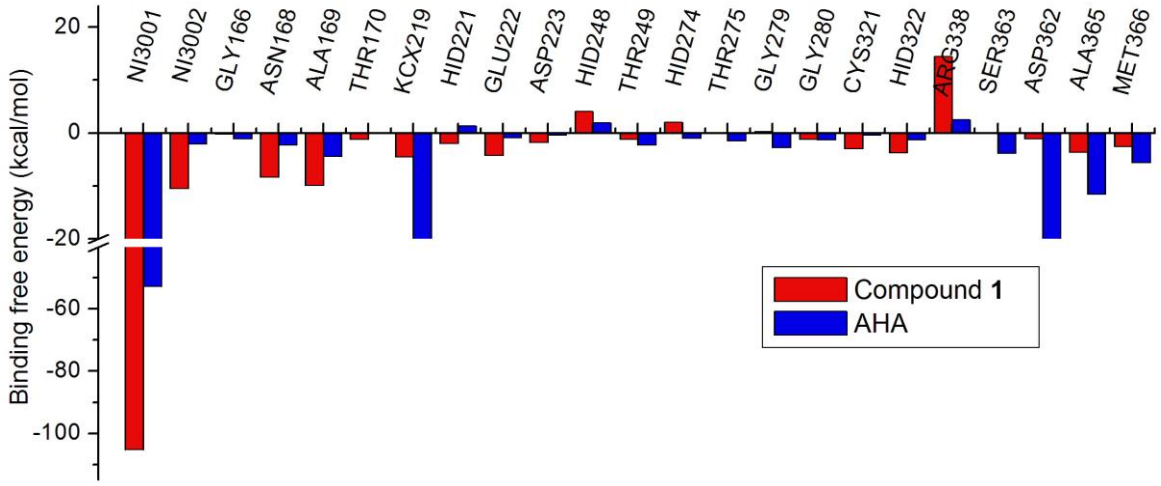

Fig4. The energy comparisons of residues in binding sites of compound 1 and AHA

Table2 The binding energies (kcal/mol) of residues in binding sites of urease

Residue	Compound 1	AHA
NI3001	-105.33	-52.77
NI3002	-10.46	-2.08
GLY166	-0.22	-1.07
ASN168	-8.35	-2.31
ALA169	-9.88	-4.38
THR170	-1.17	-0.05
KCX219	-4.54	-24.75
HID221	-1.96	1.36
GLU222	-4.25	-0.95
ASP223	-1.8	-0.4
HID248	4.08	1.93
THR249	-1.16	-2.31

HID274	2.06	-1.02
THR275	-0.16	-1.47
GLY279	0.28	-2.79
GLY280	-1.24	-1.27
CYS321	-2.98	-0.43
HID322	-3.73	-1.36
ARG338	14.39	2.47
SER363	-0.02	-3.89
ASP362	-1.11	-21.69
ALA365	-3.64	-11.58
MET366	-2.56	-5.61

IV. CONCLNSIONS

Compound **1** has the high inhibition activity of *Helicobacter pylori* urease (IC_{50} = 1.56 μM) by molecular docking calculations and biological evaluation. Using the binding free energy decomposition, we insight into the interaction mechanism of compound **1** with urease, and would conclude that the hydrazine group is the important group interacting with the key site Ni3001 and Ni3002 in urease. As a lead compound, compound **1** could be further modified to design the novel urease and H^+,K^+-ATPase dual inhibitor.

Conflict of interest

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China (No. 21776162).

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