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Original article

The synthesis, structure and activity evaluation of pyrogallol and catechol derivatives as *Helicobacter pylori* urease inhibitors

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ABSTRACT

Some pyrogallol and catechol derivatives were synthesized, and their urease inhibitory activity was evaluated by using acetohydroxamic acid (AHA), a well known *Helicobacter pylori* urease inhibitor, as positive control. The assay results indicate that many compounds have showed potential inhibitory activity against *H. pylori* urease. 4-(4-Hydroxyphenethyl)phen-1,2-diol (**2a**) was found to be the most potent urease inhibitor with IC_{50}s of 1.5 ± 0.2 μM for extracted fraction and 4.2 ± 0.3 μM for intact cell, at least 10 times and 20 times lower than those of AHA (IC_{50} of 17.2 ± 0.9 μM, 100.6 ± 13 μM), respectively. This finding indicate that **2a** would be a potential urease inhibitor deserves further research. Molecular dockings of **2a** into *H. pylori* urease active site were performed for understanding the good activity observed.

1. Introduction

Urease is widely distributed in nature and is found in a variety of plants, algae, fungi and bacteria [1,2]. This enzyme is directly involved in the formation of infection of stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation [3,4].

Urease (urea amidohydrolase EC3.5.1.5) is a large heteropolymeric enzyme with the active site containing two nickel (II) atoms [5] and accelerates hydrolysis of urea by at least 10^{14} over the spontaneous reaction [6]. The amino acid sequences of the active site are principally conserved in all known ureases, and the catalytic mechanism of their action is believed to be the same. On the basis of several crystal structures of complexes of *Bacillus pasteurii* urease, Ciurli and his co-workers proposed the most reliable enzymatic reaction mechanism [7]. The active site of the native enzyme binds three water molecules and a hydroxide ion bridged between two nickel ions. In the course of enzymatic reaction, urea replaces these three water molecules and bridges the two metal ions [8]. The bidentated urea molecule is anchored tightly by a hydrogen-bonding network. This arrangement strongly activates the inert urea molecule, which is subsequently attacked by the Ni-bridging hydroxide ion, forming a tetrahedral transition state. As a result, ammonia is released from the active site followed by the negatively charged carbamate. The latter decomposes rapidly and spontaneously, yielding a second molecule of ammonia. These reactions in return cause significant increase of solution pH, which is known as a major cause of pathologies induced by *Helicobacter pylori* (*H. pylori*) and allows bacteria to survive at acidic pH of the stomach during colonization. The urease activity of *H. pylori* therefore plays an important role in the pathogenesis of gastric and peptic ulcer (including cancer) [6].

Several classes of compounds show significant inhibitory activity against urease with hydroxamic acids being the best recognized inhibitors [9,10] and with phosphoramidates being the most active [11,12]. Degradation of phosphoramidates at low pH [13] and teratogenicity of hydroxamic acids in rats [14] prevented them from using *in vivo*. Current efforts are therefore focused on seeking novel urease inhibitors with good bioavailability and low toxicity. Meanwhile, the studies on novel urease inhibitors are essential not only for the basic research on urease biochemistry but also for the possible development of a highly needed therapy for urease mediated bacterial infections.

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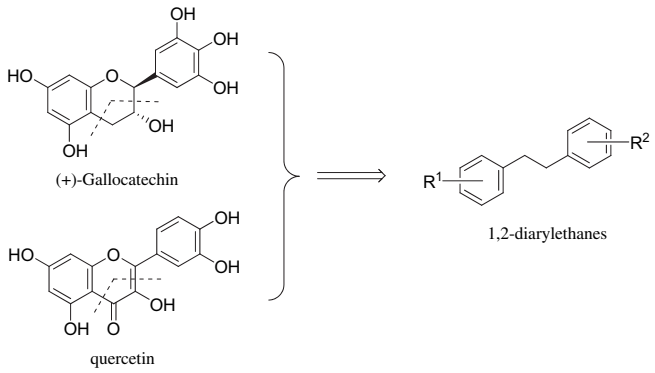


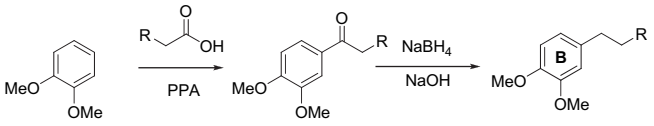
Scheme 1. The evolution process of the aimed urease inhibitors.

Polyphenols, such as flavones and isoflavones, constitute one of the most represented classes of compounds in higher plants including medicinal and edible plants. Extensive epidemiological studies and *in vitro* experiments with polyphenols have indicated their broad variety of biological activities, including anticancer [15], anti-inflammatory [16], antibacterial [17], cardioprotective [18], anti-osteoporotic [19] and enzyme-inhibitory [20] activities. Recently, some polyphenols from green tea ((+)-gallocatechin) [21] and several naturally occurring flavonoids (quercetin) [22] were shown to be *H. pylori* urease inhibitors in micromolar range. On the basis of these researches, we have reported some urease inhibitors derived from flavonoid by splitting C-ring (Scheme 1), in which 4-(4-hydroxyphenethyl)phen-1,2,3-triol (**1a**) showed the highest inhibitory activity against *H. pylori* urease [23]. In view of these positive results, we recently focused our efforts to develop urease inhibitors based on **1a**. In this paper, we present the synthesis of **1a** analogues and their evaluation on *H. pylori* urease inhibitory activity. Obtained results showed high activity of the synthesized structures, with IC_{50} of 1.5 ± 0.2 μM for the most active compound.

2. Results and discussion

2.1. Chemistry

Using 4-(4-hydroxyphenethyl)phen-1,2,3-triol (**1a**) as lead compound, twenty-nine compounds were designed and synthesized for *H. pylori* urease inhibitors by the routes outlined in Schemes 2 and 3. In our previous work [23], we found that the two ortho-hydroxyl groups were essential for urease inhibitory activity of a polyphenol. Consequently, the inhibitor design was based on catechol and pyrogallol derivatives, and the reforming focused on the B-ring. Structure-activity relationship (SAR) studies were also performed by replacement of B-ring with alkyl chains to determine if the subunits displayed urease inhibitory activity. The catechol and pyrogallol derivatives were all synthesized by the method described elsewhere [23] and compounds **3g** to **6g** were synthesized by the method reported by Percec et al. [24] with some



Scheme 3. Synthetic route of **3g**–**6g**.

modifications. All newly reported compounds were fully characterized by spectroscopic methods and elemental analysis together with a crystal structure (**1b**). Out of the compounds, fifteen (**1b**–**1h**, **1m**, **2b**–**2c**, **2e**–**2g** and **5g**–**6g**) were reported for the first time.

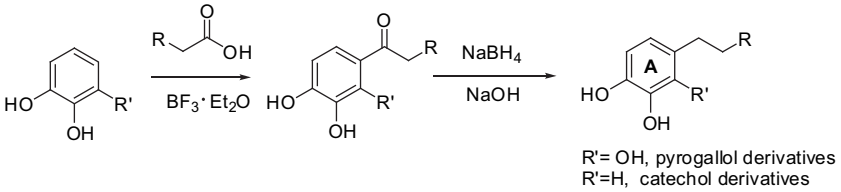
2.2. Description of the crystal structure and determination of central bond

Compound **1b** was determined by X-ray diffraction analysis. The crystal data are presented in Table 1, and Fig. 1 gave perspective views of compound **1b** with the atomic labeling system. The bond length of C7–C8 (1.416(6) Å) is significantly shorter than that in other reported 1,2-diphenylethane derivatives [25], and much longer than that in stilbenes [26]. However, four proton signals at δ_H 2.84 strongly indicated that C7–C8 is a single bond. In fact, the shortening is commonly observed for the central bond in 1,2-diphenylethane derivatives [27,28]. Harada and Ogawa attributed the shortening to an artifact caused by the torsional vibration of the C–Ph bonds in crystals [29]. Rings A and B are consequently linked by an $-CH_2-CH_2-$ group, which is consistent with the target structure described in Table 2. Based on this result, the structure of compounds **1c**–**1h** were distinctly determined to have $-CH_2-CH_2-$ linkage.

In the crystal structure of compound **1b**, C1–C6 (ring A) form a plane with the mean deviation of 0.0020 Å, and C9–C14 (ring B) form an other plane with the mean deviation of 0.0026 Å. The two planes make a dihedral angle of 55.8(1)°. The torsion angle of C4, C7, C8 and C9 is −178.5(4)°, which clearly indicated ring A and ring B are in the antiperiplanar conformation. There are two classical intramolecular hydrogen bonds occurring the three hydroxyl groups, and there is a non-classical intramolecular hydrogen bonds between C7 and O3 (Fig 1).

2.3. Urease inhibitory activity

The inhibitory potential of the synthesized compounds was assessed against urease from *H. pylori* with some also against in intact cell. Urease activity was evaluated using Berthelot color reaction procedure, as other methods of ammonia quantification based on pH changes (e.g., phenol red assay) lead to artificial results due to strong buffering properties of the studied inhibitors [30]. The results for all assayed compounds are given in Table 2. Acetohydroxamic acid (AHA) was used as the reference compound for the assay, and its value is also included in Table 2. Originally designed lead compound **1a** exhibited moderate activity with IC_{50} of 32 ± 7 μM. Further modification of this structure caused



Scheme 2. Synthetic route of catechol and pyrogallol derivatives.

Table 1
Crystal structure data for **1b**.

Compound	**1b**
Formula	$C_{14}H_{13}FO_3$
Mr	248.24
Crystal size/mm^3	0.30 × 0.20 × 0.20
Crystal system	Triclinic
Space group	*P*-1
a/Å	5.981(3)
b/Å	7.447(3)
c/Å	14.287(6)
α/°	86.810(10)
β/°	84.165(12)
γ/°	73.009(10)
V/Å^3	605.2(4)
Z	2
Dc/(g/cm^3)	1.362
μ/mm^{-1}	0.105
F(000)	260
Max. and min. trans.	0.9793 and 0.9691
θ range/°	1.43/25.49
Index range (*h*, *k*, *l*)	−7/6, −8/9, −15/17
Reflections collected/unique	3230/2219
Data/restraints/parameters	2219/0/160
R_{int}	0.0217
Goodness-of-fit on F^2	1.047
R_1, wR_2 [$I > 2\sigma(I)$]	0.0753/0.2110
R_1, wR_2	0.1313/0.2453
Extinction coefficient	–
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (e/Å^3)	0.335/−0.425

$R = \sum||F_o|-|F_c||/\sum|F_o|$.
$wR = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$.

considerable activity improvement for compounds **2a**, **2b** and **2i** toward chosen enzyme (with $IC_{50} = 1.5 \pm 0.2$, 6.2 ± 0.7 and 4.9 ± 0.4 μM, respectively). Five of the 25 catechol and pyrogallol derivatives, **2a**, **2b**, **2c**, **2i** and **2j**, significantly decreased the mean activity of *H. pylori* urease with IC_{50} below 10 μM. **4g**–**6g**, Without pyrogallol or catechol moiety, show very low inhibitory activity against *H. pylori* urease, which is consistent with our previous work [23].

In general, the compounds with 1,2,3-trihydroxy groups (**1a**–**1o**) in A-ring are much less active than those analogues with 1,2-dihydroxy groups against *H. pylori* urease. The dropped urease inhibitory activity of compound **1a** as compared to structurally related compounds **2a** may be due to the presence of a hydroxyl group at position 3 of A-ring which may provide some resistance to A-ring to bind at the active site. This was supported by the molecular docking study, which disclosed that the binding energy of **1a** (−9.89 kcal/mol) with *H. pylori* urease is higher than that of **2a** (−11.48 kcal/mol). Out of the compounds, **2a** was found to show the most potent activity, with IC_{50} values up to 10-fold lower than the reference compound, AHA. Substitution of hydroxyl group (**1a** and

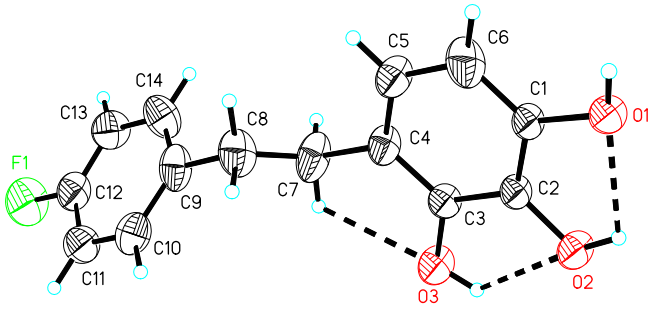
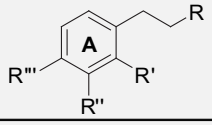


Fig. 1. Molecular structure of compound **1b**, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. Solid dashed lines indicate hydrogen bonds.

Table 2
Inhibitory activity (IC_{50}) of the synthetic compounds against *H. pylori* urease.



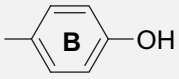
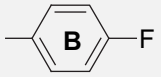
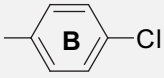
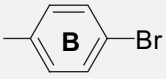
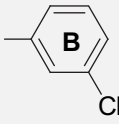
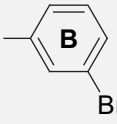
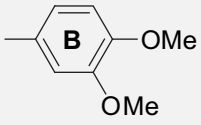
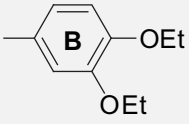
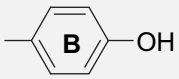
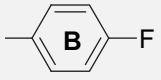
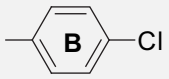
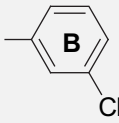
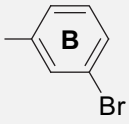
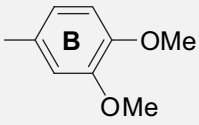
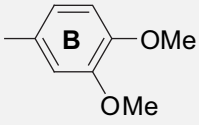
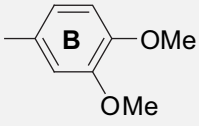
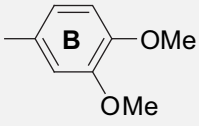
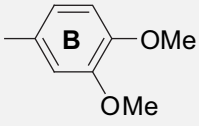
Entry	R	R′	R″	R‴	IC_{50}, μM: Cell-free urease	IC_{50}, μM: Urease in intact cell
1a		OH	OH	OH	32 ± 7	ND
1b		OH	OH	OH	43 ± 5	ND
1c		OH	OH	OH	47 ± 11	ND
1d		OH	OH	OH	48 ± 9	ND
1e		OH	OH	OH	50 ± 14	ND
1f		OH	OH	OH	52 ± 10	ND
1g		OH	OH	OH	71 ± 16	ND
1h		OH	OH	OH	69 ± 13	ND
1i	H	OH	OH	OH	34 ± 6	ND
1j	*n*-ethyl	OH	OH	OH	79 ± 11	ND
1k	*n*-butyl	OH	OH	OH	91 ± 15	ND
1l	*n*-hexyl	OH	OH	OH	103 ± 18	ND
1m	*n*-octyl	OH	OH	OH	124 ± 20	ND
1n	*n*-decyl	OH	OH	OH	168 ± 23	ND
1o	*n*-dodecyl	OH	OH	OH	250 ± 27	ND
2a		H	OH	OH	1.5 ± 0.2	4.2 ± 0.3
2b		H	OH	OH	6.2 ± 0.7	32.4 ± 2.5

Table 2 (*continued*)

Entry	R	R′	R″	R‴	IC_{50}, μM: Cell-free urease	IC_{50}, μM: Urease in intact cell
2c		H	OH	OH	8.3 ± 0.5	50.1 ± 4.9
2e		H	OH	OH	15.7 ± 1.0	94.7 ± 14
2f		H	OH	OH	16.9 ± 1.2	88.9 ± 11
2g		H	OH	OH	22.7 ± 2.6	ND
2i	H	H	OH	OH	4.9 ± 0.4	17.3 ± 1.2
2j	*n*-ethyl	H	OH	OH	7.9 ± 0.6	38.1 ± 3.4
2k	*n*-butyl	H	OH	OH	12.3 ± 0.9	77.8 ± 6.7
2l	*n*-hexyl	H	OH	OH	58 ± 9	ND
3g		OH	H	H	227 ± 19	ND
4g		H	H	OH	204 ± 28	ND
5g		H	Cl	H	235 ± 25	ND
6g		H	H	Cl	254 ± 32	ND
AHA	–	–	–	–	17.2 ± 0.9	100.6 ± 13

Note: ND = no determination.

2a) with fluorine (**1b** and **2b**) at 4-position in B-ring resulted in a slight decrease in inhibitory activity, while the activity was maintained when the fluorine is replaced by bromine and chlorine, respectively. Shifting the halogen atom from the *para*- to *meta*-position in B-ring led to some drop in *H. pylori* urease inhibitory potency. Compounds **1g**, **1h** and **2g**, bearing two methoxy groups (or ethoxy groups) at positions 3 and 4 in B-ring, show the lowest activity in their own analogues.

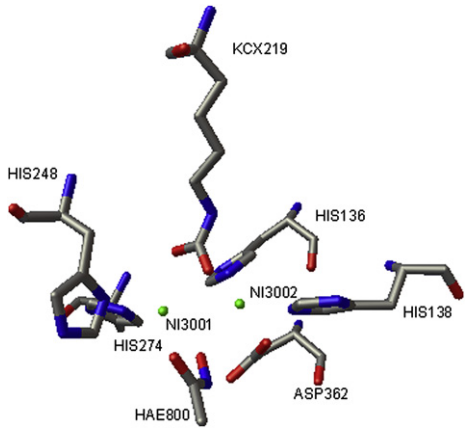


Fig. 2. The active site residues of the AHA-inhibited *H. pylori* urease, and the molecule of AHA is labeled with HAE800.

Kubo and his co-workers reported anacardic acids, salicylic acid analogues with a long alkyl chain, as Jack bean urease inhibitors [31]. Based on this discovery, we substituted a series of alkyl chains for B-ring to probe the available space around the catechol or pyrogallol moiety. The results clearly showed that changing the length of the alkyl chain could significantly affect the activity of the molecule against *H. pylori* urease. The longer the side chain, the lower the activity. It is noted that compounds having 1–3 methylene units such as **1i** and **1j** show inhibitory activity compared to 1,2-diarylethane derivatives (**1a** to **1h**) as seen by same range of IC_{50} values. In comparison with **1k** to **1o**, the enhanced potency of **1a** to **1h** may be due to a decrease in conformational flexibility, suggesting that the space around the catechol or pyrogallol is limited.

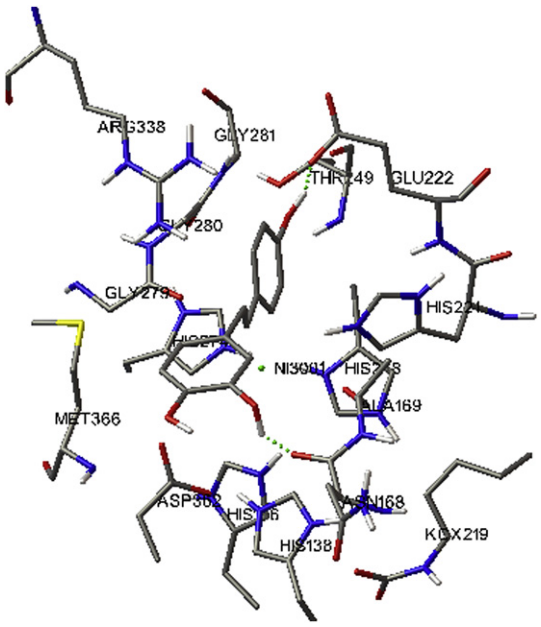


Fig. 3. Binding mode of compound **2a** with *H. pylori* urease. For clarity, only interacting residues are displayed. The hydrogen bond is displayed as green dashed line.

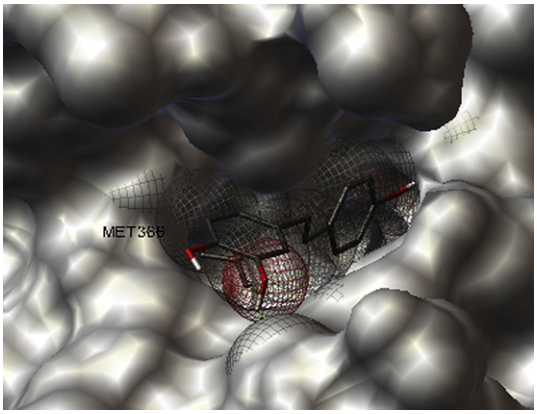


Fig. 4. Binding mode of compound **2a** with *H. pylori* urease. The enzyme is shown as surface; while **2a** docked structures are shown as sticks and close contacts are shown as wireframe sphere.

Compounds **2a**–**c**, **2e**,**f**, **2i**–**k** and AHA, with relatively low IC_{50} values against cell-free urease, were selected for inhibiting urease activity in intact *H. pylori*. As shown in Table 2, the IC_{50} values of the test compounds against urease in intact cell were 3- to 6-fold higher than those for extracted urease. The higher IC_{50}s may reflect the permeability barrier of the cell surface to the compounds by *H. pylori*. Another interesting finding of this assay is that **2a** also significantly inhibited the urease activity in intact cell. This indicates that **2a** would be a potential urease inhibitor deserves further research.

2.4. Molecular docking study

In the X-ray structure available for the native *H. pylori urease* (entry 1E9Z in the Protein Data Bank), the two nickels were coordinated by His136, His138, Kcx219, His248, His274, Asp362 and water molecules, while in the AHA-inhibited *H. pylori urease* (entry 1E9Y in the Protein Data Bank), these water molecules were replaced by AHA (Fig. 2, labeled with HAE800). In order to give an explanation and understanding of good activity observed, molecular docking of the most potent inhibitor **2a** into AHA binding site of *H. pylori urease* was performed on the binding model based on the *H. pylori urease* complex structure (1E9Y.pdb). The binding model of compound **2a** and *H. pylori* urease was depicted in Fig. 3 and the enzyme surface model was showed in Fig. 4, which revealed that the molecular is well filled in the active pocket. There are two novel interactions, in comparison to AHA-urease complex, anchored the ligand to this enzyme, namely two hydrogen bonds formed by 2-hydroxyl group in A-ring and 4-hydroxyl group in B-ring of **2a** with Asn168 and Glu222, respectively. The docking calculations revealed that compound **2a** has lower free energy of binding (−11.48 kcal/mol) than that of AHA (−10.01 kcal/mol), which may explain the excellent inhibitory activity of **2a** against *H. pylori* urease.

3. Conclusions

Some catechol and pyrogallol derivatives were prepared and tested for their inhibitory activity against *H. pylori* urease. Compound **2a**, 4-(4-hydroxyphenethyl)phen-1,2-diol, showed the most potent inhibitory activity with IC_{50} of 1.5 ± 0.2 μM against this enzyme. Substitution of hydroxyl group with halogen at 4-position in B-ring resulted in a drop in inhibitory activity. On the other hand, replacement B-ring with a long alkyl chain significantly weakened the activity of the molecule against *H. pylori* urease. Molecular dockings of the most potent compound **2a** into *H. pylori* urease active site were performed. The binding pattern of **2a** may explain its inhibitory activity against *H. pylori* urease.

4. Experimental section

4.1. Biological activity

4.1.1. Materials

Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannheim, Germany) and Brucella broth was from Becton–Dickinson (Cockeysville, MD). Horse serum was from Hyclone (Utah, American).

4.1.2. Bacteria

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in Brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as previously described [32].

4.1.3. Preparation of H. pylori urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80 °C. *H. pylori* was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000 g, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.1.4. Measurement of urease activity

The assay mixture, containing 25 μL (10U) of *H. pylori* urease which was replaced by 25 μL of cell suspension (4.0×10^7 CFU/mL) for the urease assay of intact cells and 25 μL of the test compound, was pre-incubated for 1.5 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [30].

4.2. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 60 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the

most favorable free energy of binding were selected as the resultant complex structures.

4.3. Crystallographic studies

X-ray single-crystal diffraction data for compound **1b** was collected on a Bruker SMART APEX CCD diffractometer at 296(2) K using MoKα radiation ($\lambda = 0.71073$ Å) by the ω scan mode. The program SAINT was used for integration of the diffraction profiles. All the structures were solved by direct methods using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares methods with SHELXL [33]. All non-hydrogen atoms of compound **1b** were refined with anisotropic thermal parameters. All hydrogen atoms were generated theoretically onto the parent atoms and refined isotropically with fixed thermal factors.

4.4. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A.) and Sinopharm Chemical Reagent Co., Ltd (China). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on a Waters GCT mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker AV-300 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

4.4.1. General procedure for the Preparation of catechol and pyrogallol derivatives

5 mmol of pyrogallol (catechol) and 5 mmol of appropriately substituted phenylacetic acid (aliphatic acid) were dissolved into 10 mL of fresh distilled $BF_3 \cdot Et_2O$. The mixture was stirred and heated on the oil-bath at 80–85 °C for about 3 h. After cooling, the contents were poured into 150 mL of ice-cold aqueous sodium acetate (w% = 10%) with stirring. Then, the precipitate was filtered and washed thrice with water. Crystallization from methanol-water gave the products, ketones. For some cases, the products were necessary to be purified over a silica gel column.

The resulting ketone (3 mmol) was dissolved in 0.33 M NaOH (21 mL), and 24 mmol of $NaBH_4$ was subsequently added. The mixture was heated under reflux for 3 h. After cooling, 5 M hydrochloric acid was added to decompose the excess $NaBH_4$ on an ice-water bath. The mixture was extracted with EtOAc. After removal of the solvent, the resulting residue was purified over a silica gel column eluting with EtOAc–petroleum ether.

*4.4.1.1. 4-(4-Fluorophenethyl)phen-1,2,3-triol (**1b**).* White powder, yield 62%, mp 132–133 °C, ^{1}H NMR ($CDCl_3$): 2.84 (s, 4H); 4.91 (s, 1H); 4.99 (s, 1H); 5.20 (s, 1H); 6.37 (d, J = 8.2 Hz, 1H); 7.48 (d, J = 8.2 Hz, 1H); 6.94 (t, J = 8.6 Hz, 2H); 7.11 (t, J = 8.4 Hz, 2H); EIMS m/z 248 (M^+). Anal. Calcd for $C_{14}H_{13}FO_3$: C, 67.73; H, 5.28; Found: C, 67.79; H, 5.26.

*4.4.1.2. 4-(4-Chlorophenethyl)phen-1,2,3-triol (**1c**).* White powder, yield 80%, mp 148–149 °C, ^{1}H NMR ($CDCl_3$): 2.84 (s, 4H); 4.86 (s, 1H); 5.03 (s, 1H); 5.18 (s, 1H); 6.36 (d, J = 8.4 Hz, 1H); 7.46 (d, J = 8.4 Hz, 1H); 7.08 (d, J = 8.2 Hz, 2H); 7.22 (d, J = 8.4 Hz, 2H); EIMS m/z 264 (M^+). Anal. Calcd for $C_{14}H_{13}ClO_3$: C, 63.52; H, 4.95; Found: C, 63.53; H, 4.97.

*4.4.1.3. 4-(4-Bromophenethyl)phen-1,2,3-triol (**1d**).* White powder, yield 60%, mp 147–149 °C, ^{1}H NMR ($CDCl_3$): 2.83 (s, 4H); 4.87 (s, 1H); 5.01 (s, 1H); 5.19 (s, 1H); 6.36 (d, J = 8.2 Hz, 1H); 7.46 (d, J = 8.4 Hz, 1H); 7.04 (d, J = 8.4 Hz, 2H); 7.37 (d, J = 8.2 Hz, 2H); EIMS m/z 308 (M^+). Anal. Calcd for $C_{14}H_{13}BrO_3$: C, 54.39; H, 4.24; Found: C, 54.31; H, 4.27.

*4.4.1.4. 4-(3-Chlorophenethyl)phen-1,2,3-triol (**1e**).* White powder, 58%, mp 108–110 °C, ^{1}H NMR ($CDCl_3$): 2.85 (s, 4H); 4.92–5.50 (bs, 3H); 6.37 (d, J = 8.2 Hz, 1H); 7.48 (d, J = 8.2 Hz, 1H); 7.05 (dd, J = 8.3 Hz, J = 1.9 Hz, 1H); 7.12–7.23 (m, 3H); EIMS m/z 264 (M^+). Anal. Calcd for $C_{14}H_{13}ClO_3$: C, 63.52; H, 4.95; Found: C, 63.57; H, 4.94.

*4.4.1.5. 4-(3-Bromophenethyl)phen-1,2,3-triol (**1f**).* White powder, 69%, mp 112–114 °C, ^{1}H NMR ($CDCl_3$): 2.84 (s, 4H); 4.90–5.51 (bs, 3H); 6.36 (d, J = 8.4 Hz, 1H); 7.48 (d, J = 8.2 Hz, 1H); 7.03 (dd, J = 8.2 Hz, J = 2.0 Hz, 1H); 7.15–7.27 (m, 3H); EIMS m/z 308 (M^+). Anal. Calcd for $C_{14}H_{13}BrO_3$: C, 54.39; H, 4.24; Found: C, 54.30; H, 4.28.

*4.4.1.6. 4-(3,4-Dimethoxyphenethyl)phen-1,2,3-triol (**1g**).* White powder, 61%, mp 144–145 °C, ^{1}H NMR ($CDCl_3$): 2.83 (s, 4H); 3.80 (s, 3H); 3.85 (s, 3H); 4.83 (s, 1H); 5.07 (s, 1H); 5.27 (s, 1H); 6.40 (d, J = 8.2 Hz, 1H); 6.52 (d, J = 8.4 Hz, 1H); 6.63 (d, J = 1.8 Hz, 1H); 6.72 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H); 6.82 (d, J = 7.9 Hz, 1H); EIMS m/z 290 (M^+). Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25; Found: C, 66.25; H, 6.22.

*4.4.1.7. 4-(3,4-Diethoxyphenethyl)phen-1,2,3-triol (**1h**).* White powder, 77%, mp 122–123 °C, ^{1}H NMR ($CDCl_3$): 1.39 (t, J = 7.0 Hz, 3H); 1.42 (t, J = 7.0 Hz, 3H); 2.81 (s, 4H); 3.98–4.09 (m, 4H); 4.40–5.61 (bs, 3H); 6.38 (d, J = 8.4 Hz, 1H); 6.50 (d, J = 8.2 Hz, 1H); 6.64 (d, J = 2.0 Hz, 1H); 6.69 (dd, J = 8.2 Hz, J = 2.0 Hz, 1H); 6.80 (d, J = 8.0 Hz, 1H); EIMS m/z 318 (M^+). Anal. Calcd for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97; Found: C, 67.85; H, 6.97.

*4.4.1.8. 4-Decylphen-1,2,3-triol (**1m**).* White powder, 86%, mp 110–112 °C, ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 7.0 Hz, 3H); 1.18–1.38 (m, 16H); 2.53 (t, J = 7.5 Hz, 2H); 4.88 (s, 1H); 5.08 (s, 1H); 5.20 (s, 1H); 6.39 (d, J = 8.2 Hz, 1H); 6.54 (d, J = 8.2 Hz, 1H); EIMS m/z 266 (M^+). Anal. Calcd for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84; Found: C, 72.06; H, 9.87.

*4.4.1.9. 4-(4-Fluorophenethyl)phen-1,2-diol (**2b**).* White powder, 65%, mp 83–85 °C, ^{1}H NMR ($CDCl_3$): 2.74–2.87 (m, 4H); 5.09 (bs, 2H); 6.57 (dd, J = 8.0 Hz, J = 1.8 Hz, 1H); 6.66 (d, J = 1.8 Hz, 1H); 6.76 (d, J = 8.0 Hz, 1H); 6.94 (t, J = 8.7 Hz, 2H); 7.08 (dd, J = 8.4 Hz, J = 5.5 Hz, 2H); EIMS m/z 232 (M^+). Anal. Calcd for $C_{14}H_{13}FO_2$: C, 72.40; H, 5.64; Found: C, 72.46; H, 5.62.

*4.4.1.10. 4-(4-Chlorophenethyl)phen-1,2-diol (**2c**).* White powder, 71%, mp 134–136 °C, ^{1}H NMR ($CDCl_3$): 2.74–2.87 (m, 4H); 4.51–5.92 (bs, 2H); 6.56 (dd, J = 8.0 Hz, J = 1.8 Hz, 1H); 6.66 (d, J = 1.8 Hz, 1H); 6.75 (d, J = 8.0 Hz, 1H); 7.05 (d, J = 8.2 Hz, 2H); 7.22 (d, J = 8.0 Hz, 2H); EIMS m/z 248 (M^+). Anal. Calcd for $C_{14}H_{13}ClO_2$: C, 67.61; H, 5.27; Found: C, 67.54; H, 5.28.

*4.4.1.11. 4-(3-Chlorophenethyl)phen-1,2-diol (**2e**).* White powder, 63%, mp 59–61 °C, ^{1}H NMR ($CDCl_3$): 2.74–2.87 (m, 4H); 4.53–5.47 (bs, 2H); 6.56 (dd, J = 8.0 Hz, J = 1.8 Hz, 1H); 6.66 (d, J = 1.8 Hz, 1H); 6.75 (d, J = 8.0 Hz, 1H); 7.04 (dd, J = 8.2 Hz, J = 1.9 Hz, 1H); 7.12–7.23 (m, 3H); EIMS m/z 248 (M^+). Anal. Calcd for $C_{14}H_{13}ClO_2$: C, 67.61; H, 5.27; Found: C, 67.57; H, 5.26.

*4.4.1.12. 4-(3-Bromophenethyl)phen-1,2-diol (**2f**).* White powder, 59%, mp 62–64 °C, ^{1}H NMR ($CDCl_3$): 2.76–2.86 (m, 4H); 4.65–5.38 (bs, 2H); 6.58 (dd, J = 8.0 Hz, J = 1.9 Hz, 1H); 6.67 (d,

$J = 1.9$ Hz, 1H); 6.76 (d, $J = 8.2$ Hz, 1H); 7.05 (d, $J = 8.0$ Hz, 1H); 7.13 (t, $J = 8.0$ Hz, 1H); 7.29–7.36 (m, 2H); EIMS m/z 292 (M^+). Anal. Calcd for $C_{14}H_{13}BrO_2$: C, 57.36; H, 4.47; Found: C, 57.29; H, 4.48.

*4.4.1.13. 4-(3,4-Dimethoxyphenethyl)phen-1,2-diol (**2g**).* White powder, 67%, mp 73–74 °C, ^{1}H NMR ($CDCl_3$): 2.75–2.85 (m, 4H); 3.83 (s, 3H); 3.85 (s, 3H); 4.64–5.38 (bs, 2H); 6.59 (dd, $J = 8.2$ Hz, $J = 1.8$ Hz, 1H); 6.64 (d, $J = 1.9$ Hz, 1H); 6.65–6.80 (m, 4H); EIMS m/z 274 (M^+). Anal. Calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61; Found: C, 70.12; H, 6.60.

*4.4.2. General procedure for the preparation of compounds **3g**–**6g***

8 mmol of veratrole and 5 mmol of appropriate substituted phenylacetic acid were dissolved into 20 g of polyphosphoric acid (PPA) under stirring. The mixture was heated on an oil-bath at 70–80 °C for about 3–5 h. After cooling, ice was added to the stirring. The solution was then extracted with EtOAc and washed with H_2O, saturated $NaHCO_3$ and saturated saline. The organic layer was dried with $MgSO_4$, filtered, and concentrated. Crystallization from EtOAc–petroleum ether gave the products, ketones. The resulting ketones were subsequently reduced under the above mentioned conditions to give **3g**–**6g** in good yields.

*4.4.2.1. 4-(3-Chlorophenethyl)-1,2-dimethoxybenzene (**5g**).* White powder, 79%, mp 60–62 °C, ^{1}H NMR ($CDCl_3$): 2.74–2.85 (m, 4H); 3.83 (s, 3H); 3.85 (s, 3H); 6.54 (dd, $J = 8.8$ Hz, $J = 1.5$ Hz, 1H); 6.60 (d, $J = 1.7$ Hz, 1H); 6.73 (d, $J = 8.6$ Hz, 1H); 7.06 (dd, $J = 8.6$ Hz, $J = 1.7$ Hz, 1H); 7.13–7.26 (m, 3H); EIMS m/z 276 (M^+). Anal. Calcd for $C_{16}H_{17}ClO_2$: C, 69.44; H, 6.19; Cl, 12.81; Found: C, 69.47; H, 6.17; Cl, 12.80.

*4.4.2.2. 4-(4-Chlorophenethyl)-1,2-dimethoxybenzene (**6g**).* White powder, 75%, mp 63–64 °C, ^{1}H NMR ($CDCl_3$): 2.73–2.87 (m, 4H); 3.82 (s, 3H); 3.84 (s, 3H); 6.55 (dd, $J = 8.6$ Hz, $J = 1.8$ Hz, 1H); 6.63 (d, $J = 1.9$ Hz, 1H); 6.71 (d, $J = 8.8$ Hz, 1H); 7.06 (d, $J = 8.8$ Hz, 2H); 7.38 (d, $J = 8.7$ Hz, 2H); EIMS m/z 276 (M^+). Anal. Calcd for $C_{16}H_{17}ClO_2$: C, 69.44; H, 6.19; Cl, 12.81; Found: C, 69.41; H, 6.20; Cl, 12.81.

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