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Synthesis, biological evaluation, and molecular docking studies of 2,5-substituted-1,4-benzoquinone as novel urease inhibitors

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ABSTRACT

A series of 2,5-substituted-1,4-benzoquinone (**1**–**6**) were prepared and structurally characterized by elemental analysis, IR spectra, ^{1}H and ^{13}C NMR spectra, and single crystal X-ray determination. The urease inhibitory activities of the compounds against *H. pylori* urease were studied. Among the compounds, 2,5-bis(2-morpholin-4-ylethylamino)-[1,4]benzoquinone (**2**) shows the most effective activity with IC_{50} value of 27.30 ± 2.17 μM. Docking simulation was performed to insert compound **2** into the crystal structure of *H. pylori* urease at the active site to determine the probable binding mode. As a result, compound **2** may be used as a potential urease inhibitor.

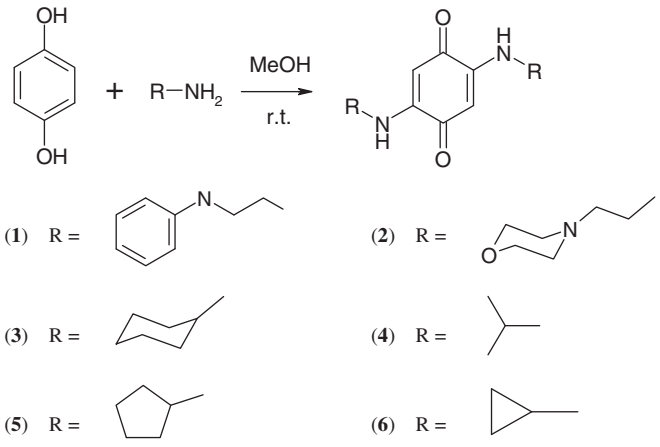
1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5) is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbamate.[1,2] The resulting carbamate spontaneously decomposes to yield ammonia and carbon dioxide. High concentration of ammonia arising from the reaction, as well as the accompanying pH elevation, has important negative effects in the fields of medicine and agriculture.[3–6] Control of the activity of urease through the use of inhibitors could counteract these negative effects. In recent years, a variety of urease inhibitors have been studied, including acetohydroxamic acid (AHA), humic acid, 1,4-benzoquinone, and inorganic metal salts, etc.[7–12] Among the inhibitors, 1,4-benzoquinone and its derivatives have been extensively studied for their inhibition on urease,[13,14] however, no rational structure–activity relationships have been achieved so far. As an extension of the work on the exploration of effective urease inhibitors related to quinone derivatives, in this paper, a series of 2,5-substituted-1,4-benzoquinones, 2,5-bis(2-phenylaminoethylamino)-[1,4]benzoquinone (**1**), 2,5-bis(2-morpholin-4-ylethylamino)-[1,4]benzoquinone (**2**), 2,5-biscyclohexylamino-[1,4]benzoquinone (**3**), 2,5-bisisopropylamino-[1,4]benzoquinone (**4**), 2,5-biscyclopentylamino-[1,4]benzoquinone (**5**), and 2,5-biscyclopropylamino-[1,4]benzoquinone (**6**), were synthesized and structurally characterized. The urease inhibitory activities of the compounds were investigated from both experimental and molecular docking study.

2. Results and discussion

2.1. Chemistry

The compounds **1**–**6** were readily synthesized by the reaction of 1:2 molar ratio of hydroquinone with *N*-phenylethylene-1,2-diamine, 2-morpholin-4-ylethylamine, cyclohexylamine, isopropylamine, cyclopentylamine, and cyclopropylamine, respectively, in



Scheme 1. Synthesis of the compounds.

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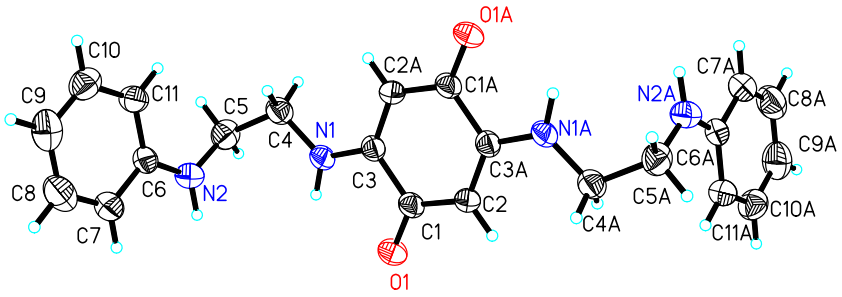

Figure 1. A perspective view of the molecular structure of **1** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

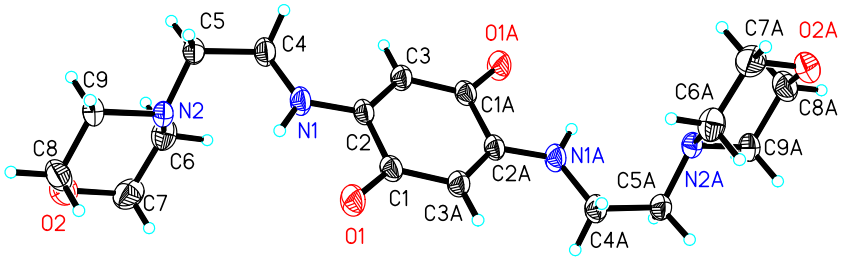

Figure 2. A perspective view of the molecular structure of **2** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

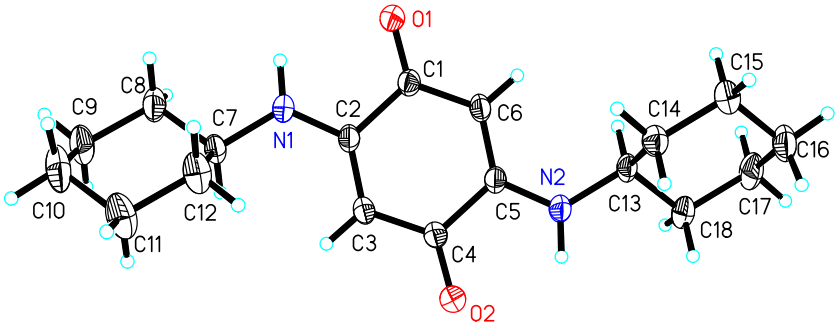

Figure 3. A perspective view of the molecular structure of **3** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

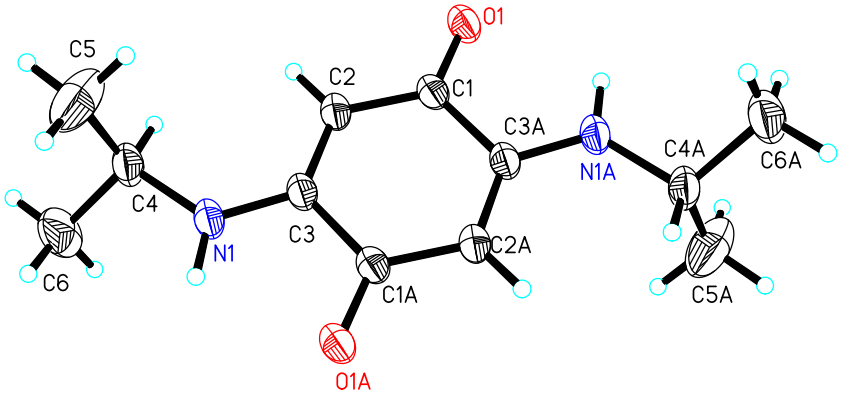

Figure 4. A perspective view of the molecular structure of **4** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

methanol, at room temperature (Scheme 1), with high yields (over 90%) and purity. The color of the reaction mixtures was turned from colorless to red. The compounds have been characterized by elemental analyses, IR spectra, ^{1}H and ^{13}C NMR spectra. Structures of the compounds **1**–**4** were further confirmed by single crystal X-ray crystallography (CCDC—840475 for **1**, 840476 for **2**, 840477 for **3**, and 840478 for **4**). We have tried to prepare the well-shaped single crystals of the compounds **5** and **6**, but only thin schistose-shaped products were formed, which were not suitable for single crystal X-ray diffraction.

2.2. Structure description of the compounds 1–4

Single crystal structural X-ray crystallography reveals that the compounds **1**–**4** are 2,5-substituted-1,4-benzoquinones. The substitute groups are *N*-phenylethyl for **1** (Fig. 1), 2-morpholin-4-ylethyl

Table 1
Hydrogen bond distances (Å) and bond angles (°) for the compounds **1**–**4**

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	Angle (*D*–H···*A*)
1				
N2–H2A···O1^{i}	0.90(1)	2.22(3)	2.963(5)	140(3)
N1–H1···O1^{i}	0.90(1)	2.31(2)	3.156(4)	158(3)
2				
N1–H1···O1	0.90(1)	2.18(2)	2.628(2)	110(2)
3				
N2–H2···O1^{ii}	0.90(1)	2.15(2)	2.946(3)	146(3)
N1–H1···O2^{iii}	0.90(1)	2.36(2)	3.203(3)	157(3)
4				
N1–H1···O1^{iv}	0.90(1)	2.16(2)	3.018(2)	158(2)

Symmetry codes: (i) 1−*x*, 1−*y*, −*z*; (ii) *x*, 1/2−*y*, 1/2 + *z*; (iii) *x*, 1/2−*y*, −1/2 + *z*; (iv) 1/2 + *x*, 1/2−*y*, 1/2 + *z*.

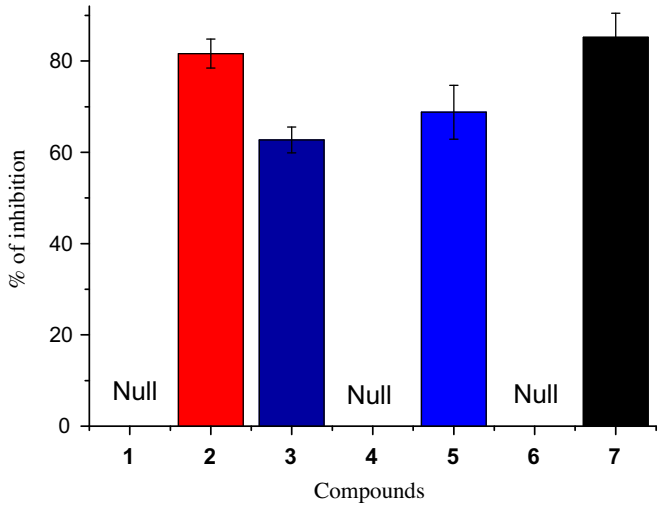

Figure 5. Inhibition rates of the tested materials to the urease. Compound **7** represents AHA.

for **2** (Fig. 2), cyclohexyl for **3** (Fig. 3), and isopropyl for **4** (Fig. 4). There are crystallographic inversion centers in the compounds **1**, **2**, and **4**, which are located at the midpoints of the quinone groups. The dihedral angle between the quinone ring and the benzene ring in **1** is 77.8(3)°. The morpholine rings in **2** and the cyclohexyl rings in **3** are in chair conformation. The distances between the C and O atoms of the quinone groups in the compounds are range from 1.23 to 1.24 Å, confirming them as typical double bonds. Close examination of the structures of the compounds reveals that the bond lengths are comparable to each other, and also comparable to those observed in the substituted quinone derivatives.[15–17] The crystal structures of the compounds are stabilized by hydrogen bonds (Table 1).

2.3. Pharmacology

The measurement of *Helicobacter pylori* urease inhibitory activity was carried out for three parallel times. The percents of inhibition at the concentration of 100 μM for the compounds against urease are 81.63 ± 3.18 (**2**), 62.71 ± 2.82 (**3**), and 68.79 ± 5.91 (**5**). There are no or very weak inhibition observed for compounds **1**, **4**, and **6** at the same condition. The AHA was used as a reference[7] with the percent of inhibition of 85.21 ± 5.27 at the concentration of 100 μM. The results are depicted in Figure 5. Compound **2** shows the most effective activity with the IC_{50} value of 27.30 ± 2.17 μM. Compounds **3** and **5** also show effective activity with the IC_{50} values of 41.50 ± 3.22 and 35.36 ± 4.03 μM, respectively. It should be notable that the IC_{50} values of **2** and **5** are even lower than the AHA (46.27 ± 0.73 μM).

From the results, it can be seen that the flexible substituted groups with suitable sizes such as 2-morpholin-4-ylethyl, cyclohexyl, and cyclopentyl are preferred units for the urease inhibition. However, the substituted groups with rigid or very small sizes in the compounds can severely decrease the urease inhibitory activities.

2.4. Molecular docking study

The molecular docking study was performed to investigate the binding effects between the compounds and the active sites of the *H. pylori* urease. In the X-ray structure available for the native *H. pylori* urease, two nickel atoms were coordinated by His136, His138, Kcx219, His248, His274, Asp362 and water molecules, while in the AHA-inhibited urease, the water molecules were replaced by AHA.[11] Figures 6–8 are the binding models for the compounds **2**, **3**, and **5**, respectively, in the enzyme active site of the urease. The docking scores are −4.75 for **1**, −5.34 for **2**, −6.17 for **3**, −3.96 for **4**, −5.88 for **5**, and −4.16 for **6**. As a comparison, the docking score for the AHA is −5.01. The values of the docking scores are roughly agreed with the inhibitory activities observed from the experiment, indicating the results are believable.

From the docking results, it can be seen that compound **1** is located far away from the active site of the urease, as a result of the large bulk of the compound. The molecule of **1** is bind with the urease through two N–H···O hydrogen bonds. For **2**, the molecule is located at the active site of the urease. There form two hydrogen bonds among the amino NH groups and the quinone O atoms of the compound with the Asp223 and His221 residues of the urease (Fig. 6). For **3** and **5**, the binding models are similar to each other. They are well filled in the active pocket of the urease. For **3**, there form three hydrogen bonds among the amino NH groups and the quinone O atoms of the compound with the Arg338, Met317 and His322 residues of the urease (Fig. 7). For **5**, there form two hydrogen bonds among the amino NH groups and the quinone O atoms of the compound with the Met317 and His322 residues of the urease (Fig. 8). For the molecules of **4** and **6**, even though their sizes are much smaller than those of the other four compounds, they are located far away from the active site of the urease. The molecule of **4** is bind with the urease through two N–H···O hydrogen bonds. The molecule of **6** is bind with the urease with no obvious hydrogen bonds. The results of the molecular docking study could explain the activities of the compounds against *H. pylori* urease.

3. Conclusion

The present study reports the synthesis, structures and urease inhibitory activities of a series of 2,5-substituted-1,4-benzoquinones. Three of the compounds have effective urease inhibition. Among the compounds, **2** may be used as a potential urease inhibitor, which deserves further study. The urease inhibitory activities and the molecular docking studies of the compounds against *H. pylori* urease indicate that suitable size and flexibility of the substituted groups of the benzoquinone rings are important factors for the exploration of effective urease inhibitors.

4. Experimental protocols

4.1. General

Starting materials, reagents and solvents with AR grade were purchased from commercial suppliers and used without further

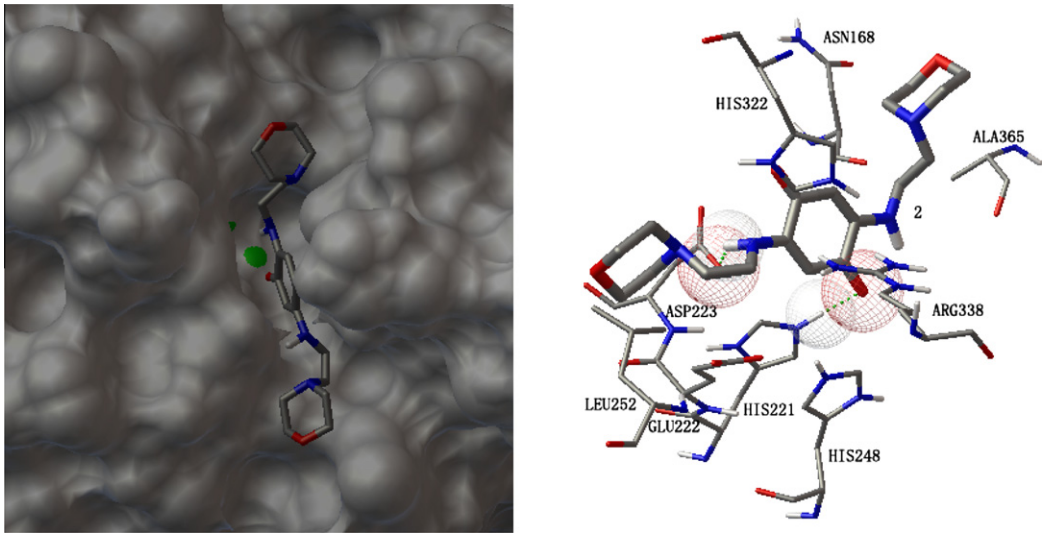

Figure 6. 3D (left) and 2D (right) binding mode of **2** with *H. pylori* urease. Hydrogen bonds are shown as dashed lines.

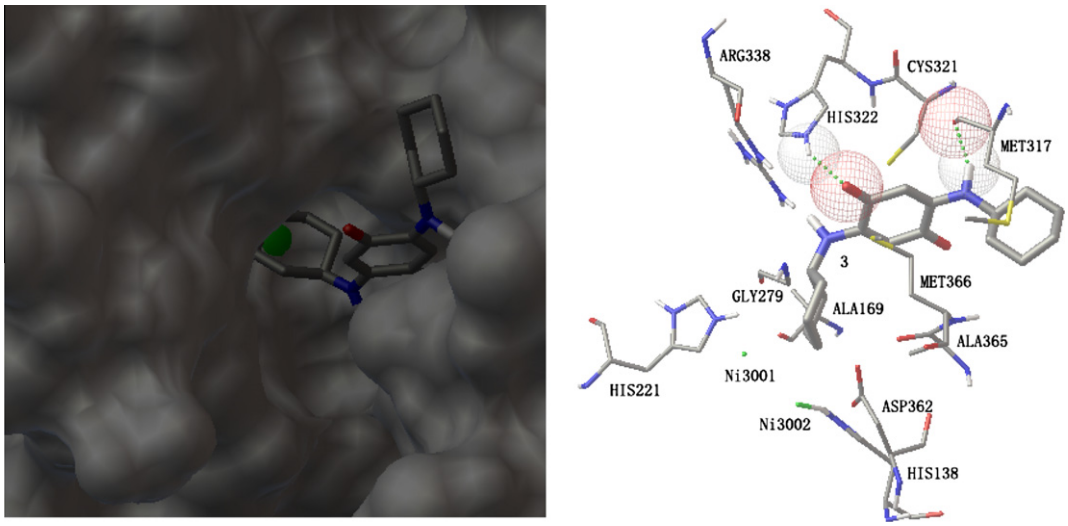

Figure 7. 3D (left) and 2D (right) binding mode of **3** with *H. pylori* urease. Hydrogen bonds are shown as dashed lines.

purification. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. The IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000–400 cm^{-1} region. Single crystal structural X-ray diffraction was carried out at a Bruker SMART 1000 CCD area diffractometer equipped with MoKα radiation, and the structures were solved by direct method using SHELXTL package.[18] The crystallographic data for the compounds are summarized in Table 2.

4.2. Synthesis

4.2.1. Synthesis of 2,5-bis(2-phenylaminoethylamino)-[1,4]benzoquinone (1)

Hydroquinone (1.0 mmol, 0.11 g) and *N*-phenylethylene-1,2-diamine (1.0 mmol, 0.14 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product of **1**, which was washed with methanol, and dried in air. Yield: 93%. Anal. Calcd for $C_{22}H_{24}N_4O_2$: C, 70.2; H, 6.4; N, 14.9. Found: C, 70.0; H, 6.4; N, 15.0%. ^{1}H NMR (DMSO-d_6): 3.22 (t, 4H), 3.30 (t, 4H), 3.33 (s, 2H), 5.31 (s, 2H), 6.56 (m, 6H), 7.08 (t, 4H). ^{13}C NMR (DMSO-d_6): 40.8, 92.1, 111.9, 115.9, 128.9, 148.2, 151.1, 177.3. IR data (KBr, cm^{-1}): 3397 (w), 3276 (m, sh), 1639 (vw), 1602 (m), 1550 (s), 1497 (s), 1443 (s), 1369 (w), 1301 (m), 1257 (w), 1179 (w), 1130 (w), 1096 (w), 875 (w), 816 (w), 753 (m), 698 (w), 670 (w), 515 (vw), 463 (vw). Single crystals of **1** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol.

4.2.2. Synthesis of 2,5-bis(2-morpholin-4-ylethylamino)-[1,4]benzoquinone (2)

Hydroquinone (1.0 mmol, 0.11 g) and 2-morpholin-4-ylethylamine (1.0 mmol, 0.13 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product of **2**, which was washed with methanol, and dried in air. Yield: 95%. Anal. Calcd for $C_{18}H_{28}N_4O_4$: C, 59.3; H, 7.7; N, 15.4. Found: C, 59.2; H, 7.8; N, 15.2%. ^{1}H NMR (DMSO-d_6): 2.40 (t, 8H), 2.51 (s, 2H), 3.24 (t, 4H), 3.36 (t, 4H), 3.56 (t, 8H), 5.27 (s, 2H). ^{13}C NMR (DMSO-d_6): 38.8, 52.9, 55.3, 66.1, 92.1, 150.9, 177.2. IR data (KBr, cm^{-1}): 3355 (m, sh), 1646 (s), 1613 (s), 1495 (m), 1454 (m), 1354 (m), 1334 (m), 1293 (m), 1143 (m), 1115 (s),

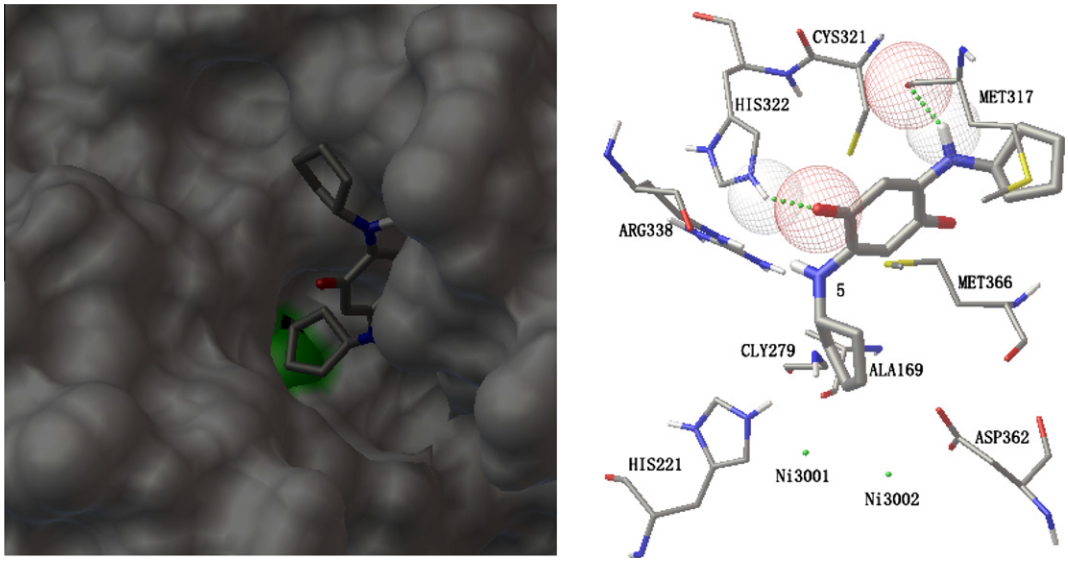

Figure 8. 3D (left) and 2D (right) binding mode of **5** with *H. pylori* urease. Hydrogen bonds are shown as dashed lines.

Table 2
Crystallographic and experimental data for the compounds **1**–**4**

Compound	**1**	**2**	**3**	**4**
Formula	$C_{22}H_{24}N_4O_2$	$C_{18}H_{28}N_4O_4$	$C_{18}H_{26}N_2O_2$	$C_{12}H_{18}N_2O_2$
Mr	376.4	364.4	302.4	222.3
T (K)	298(2)	298(2)	298(2)	298(2)
Crystal shape/color	block/red	Block/red	Block/red	Block/red
Crystal size (mm^3)	0.18 × 0.17 × 0.15	0.20 × 0.20 × 0.18	0.23 × 0.20 × 0.20	0.23 × 0.20 × 0.20
Crystal system	Orthorhombic	Monoclinic	Monoclinic	Monoclinic
Space group	Pbca	$P2_1/c$	$P2_1/c$	$C2/c$
a (Å)	7.729(6)	17.118(1)	14.225(2)	10.783(2)
b (Å)	7.928(6)	5.131(1)	8.970(2)	9.109(2)
c (Å)	31.60(2)	11.124(1)	13.369(2)	12.867(3)
β (°)		107.987(3)	99.348(9)	106.472(15)
V ($Å^3$)	1936(2)	929.3(2)	1683.3(5)	1212.0(4)
Z	4	2	4	4
D_c (g cm^{-3})	1.291	1.302	1.193	1.218
μ (Mo-Kα) (mm^{-1})	0.085	0.093	0.078	0.084
F(000)	800	392	656	480
Reflections collected	12,573	5413	9218	3436
Unique reflections	1770	2033	3449	1297
Observed reflections ($I \geqslant 2\sigma(I)$)	741	1372	2163	805
Parameters	133	122	205	78
Restraints	2	1	2	1
Min. and max. transmission	0.985, 0.987	0.982, 0.983	0.982, 0.985	0.981, 0.984
Goodness-of-fit on F^2	1.023	1.025	1.009	1.059
R_1, wR_2 [$I \geqslant 2\ \sigma(I)$][a]	0.0725, 0.1210	0.0430, 0.1012	0.0847, 0.2165	0.0481, 0.1217
R_1, wR_2 (all data)[a]	0.1913, 0.1643	0.0689, 0.1181	0.1192, 0.2484	0.0850, 0.1413
Large diff. peak and hole (e$Å^{-3}$)	0.142, −0.116	0.133, −0.149	0.419, −0.261	0.135, −0.150

[a] $R_1 = F_o - F_c/F_o$, $wR_2 = [\ w(F_o^2 - Fc^2)/\ w(F_o^2)^2]^{1/2}$.

1071 (w), 1026 (w), 945 (w), 911 (w), 859 (w), 809 (m), 770 (w), 593 (m), 469 (vw). Single crystals of **2** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol.

4.2.3. Synthesis of 2,5-biscyclohexylamino-[1,4]benzoquinone (3)

Hydroquinone (1.0 mmol, 0.11 g) and cyclohexylamine (1.0 mmol, 0.10 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product of **3**, which was washed with methanol, and dried in air. Yield: 93%. Anal. Calcd for $C_{18}H_{26}N_2O_2$: C, 71.5; H, 8.7; N, 9.3. Found: C, 71.3; H, 8.7; N, 9.2%. ^{1}H NMR (DMSO-d_6): 1.15 (m, 2H), 1.37 (m, 8H), 1.59 (d, 2H), 1.67 (d, 4H), 1.77 (d, 4H), 2.51 (m, 2H), 5.32 (s, 2H), 7.25 (d, 2H). ^{13}C NMR (DMSO-d_6): 24.2, 24.9, 30.9, 50.7, 92.1, 149.8, 177.3. IR data (KBr, cm^{-1}): 3272 (m, sh), 1638 (m), 1590 (s), 1563 (s), 1493 (s), 1449 (m), 1343 (m), 1297 (s), 1250 (m), 1208 (m), 1143 (w), 1091 (w), 976 (w), 889 (w), 869 (w), 815 (m), 743 (w), 669 (m), 575 (w), 504 (w), 425 (w). Single crystals of **3** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol.

4.2.4. Synthesis of 2,5-bisisopropylamino-[1,4]benzoquinone (4)

Hydroquinone (1.0 mmol, 0.11 g) and isopropylamine (1.0 mmol, 0.06 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product

of **4**, which was washed with methanol, and dried in air. Yield: 87%. Anal. Calcd for $C_{12}H_{18}N_2O_2$: C, 64.8; H, 8.2; N, 12.6. Found: C, 65.1; H, 8.1; N, 12.7%. ^{1}H NMR (DMSO-d_6): 1.16 (d, 12H), 3.63 (m, 2H), 5.27 (s, 2H), 7.29 (d, 2H). ^{13}C NMR (DMSO-d_6): 21.1, 43.6, 92.2, 149.9, 177.4. IR data (KBr, cm^{-1}): 3210 (m, sh), 1637 (m), 1592 (s), 1559 (s), 1470 (s), 1446 (m), 1351 (m), 1303 (s), 1223 (m), 1207 (m), 1164 (w), 1139 (w), 967 (m), 872 (w), 841 (w), 765 (w), 732 (w), 516 (w), 415 (w). Single crystals of **4** suitable for X-ray diffraction were obtained by recrystallization of the product in methanol.

4.2.5. Synthesis of 2,5-biscyclopentylamino-[1,4]benzoquinone (5)

Hydroquinone (1.0 mmol, 0.11 g) and cyclopentylamine (1.0 mmol, 0.08 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product of **5**, which was washed with methanol, and dried in air. Yield: 92%. Anal. Calcd for $C_{16}H_{22}N_2O_2$: C, 70.0; H, 8.1; N, 10.2. Found: C, 70.2; H, 8.0; N, 10.4%. ^{1}H NMR (DMSO-d_6): 1.53–1.69 (m, 12H), 1.90 (m, 4H), 3.75 (m, 2H), 5.27 (s, 2H), 7.30 (d, 2H). ^{13}C NMR (DMSO-d_6): 23.7, 31.6, 53.3, 92.8, 150.5, 177.3. IR data (KBr, cm^{-1}): 3261 (m, sh), 1637 (m), 1592 (s), 1567 (s), 1491 (s), 1449 (m), 1344 (m), 1296 (s), 1245 (m), 1207 (m), 1136 (w), 1087 (w), 971 (w), 873 (w), 854 (w), 821 (m), 745 (w), 657 (m), 573 (w), 511 (w), 443 (w).

4.2.6. Synthesis of 2,5-biscyclopropylamino-[1,4]benzoquinone (6)

Hydroquinone (1.0 mmol, 0.11 g) and cyclopropylamine (1.0 mmol, 0.06 g) were mixed in methanol, and stirred at room temperature for 1 h. The solution was changed from colorless to red. The methanol was evaporated to obtain red crystalline product of **6**, which was washed with methanol, and dried in air. Yield: 87%. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.5; N, 12.8. Found: C, 65.9; H, 6.5; N, 13.0 %. ^{1}H NMR (DMSO-d_6): 0.63 (m, 4H), 0.75 (m, 4H), 2.45 (m, 2H), 5.46 (s, 2H), 7.72 (d, 2H). ^{13}C NMR (DMSO-d_6): 6.2, 24.2, 94.0, 152.3, 177.9. IR data (KBr, cm^{-1}): 3215 (m, sh), 1636 (m), 1592 (s), 1556 (s), 1472 (s), 1445 (m), 1351 (m), 1301 (s), 1222 (m), 1207 (m), 1163 (w), 1139 (w), 972 (m), 867 (w), 845 (w), 751 (w), 737 (w), 532 (w), 426 (w).

4.3. Measurement of the urease inhibitory activity

H. Pylori was grown in brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic condition (5% O_2, 10% CO_2, and 85% N_2). The method of the preparation of the *H. pylori* urease by Mao[19] was followed. Briefly, broth cultures (50 mL, 2.0×10^8 CFU mL^{-1}) 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. While the *H. pylori* was returned to room temperature, and was mixed with 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 SephadexG-25 column (PD-10 columns, Amersham–Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and was stored at 4 °C until use in the experiment. The mixture, containing 25 μL of the *H. pylori* urease and 25 μL of the test compound, was pre-incubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as that described by Weatherburn.[20]

4.4. Docking simulations

Molecular docking study of the compounds into the 3D X-ray structure of the *H. pylori* urease (entry 1E9Y in the Protein Data Bank) was carried out by using the AutoDock version 4.2. First, AutoGrid component of the program precalculates a 3D grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of $100 \times 100 \times 60$ Å^3 points in *x*, *y*, and *z* direction 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 biding 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 100 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. Give overall consideration of the most favorable free energy of biding and the majority cluster, the results were selected as the most probable complex structures.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.002.

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