Scientific paper

Synthesis, Crystal Structures and Urease Inhibition of *N'*-(2-Bromobenzylidene)-2-(4-nitrophenoxy) acetohydrazide and *N'*-(4-Nitrobenzylidene) -2-(4-nitrophenoxy)acetohydrazide

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Abstract

Two new hydrazone compounds, *N'*-(2-bromobenzylidene)-2-(4-nitrophenoxy)acetohydrazide (**1**) and *N'*-(4-nitrobenzylidene)-2-(4-nitrophenoxy)acetohydrazide (**2**), were prepared and characterized by elemental analysis, IR, UV-Vis and ^{1}H NMR spectroscopy, and single-crystal X-ray diffraction. Compound **1** crystallizes in the monoclinic space group $P2_1/n$ with unit cell dimensions of a = 5.3064(5) Å, b = 18.202(2) Å, c = 15.970(2) Å, β = 95.866(3)°, V = 1534.4(2) Å^3, Z = 4, R_1 = 0.0457, and wR_2 = 0.0975. Compound **2** crystallizes in the monoclinic space group $P2_1/c$ with unit cell dimensions of a = 4.6008(7) Å, b = 14.451(2) Å, c = 23.296(3) Å, β = 93.620(2)°, V = 1545.8(4) Å^3, Z = 4, R_1 = 0.0441, and wR_2 = 0.0985. Structures of the compounds are stabilized by hydrogen bonds and π···π interactions. The urease inhibitory activities of the compounds were studied. Both compounds show strong urease inhibitory activities, with IC_{50} values of 8.4 and 20.2 μM, respectively.

Keywords: Hydrazone; Crystal structure; Hydrogen bonds; X-ray diffraction; Urease inhibition

1. Introduction

Urease is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbamate.[1] The resulting carbamate spontaneously decomposes to yield a second molecule of ammonia and carbon dioxide. High concentration of ammonia arising from these reactions, as well as the accompanying pH elevation, have important negative implication in medicine and agriculture.[2] Control of the activity of urease through the use of inhibitors could counteract these negative effects. Aslam and co-workers reported that hydrazone compounds derived from thiosemicarbazide possess urease inhibitory activities.[3] Recently, our research group has reported some urease inhibitors with various types of organic compounds or metal complexes[4] and some metal complexes derived from hydrazone ligands.[5] 2-(4-Nitrophenoxy)acetohydrazide is a flexible compound, which can form hydrazones with aldehydes. In order to explore new urease inhibitors, in the present paper, a pair of structurally similar hydrazone compounds, *N'*-(2-bromobenzylidene)-2-(4-nitrophenoxy)acetohydrazide (**1**) and *N'*-(4-nitrobenzylidene)-2-(4-nitrophenoxy)acetohydrazide (**2**) (Scheme 1), is presented.

2. Experimental

2. 1. General

Starting materials, reagents and solvents with AR grade were purchased from commercial suppliers and used without further purification. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000–400 cm^{-1} region. UV-Vis spec-

tra were recorded on a Lambda 900 spectrometer. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument.

2. 1. 1. Synthesis of *N'*-(2-bromobenzylidene)-2-(4-nitrophenoxy)acetohydrazide, 1

2-Bromobenzaldehyde (1.0 mmol, 0.185 g) and 2-(4-nitrophenoxy)acetohydrazide (1.0 mmol, 0.211 g) were mixed in methanol and stirred at room temperature for 1 h. The methanol was evaporated to obtain colorless crystalline product, which was washed with methanol, and dried in air. Yield: 87%. Single crystals of the compound suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Anal. calcd. for $C_{15}H_{12}BrN_3O_4$: C, 47.6; H, 3.2; N, 11.1; Found: C, 47.8; H, 3.2; N, 11.0%. IR data (KBr, cm^{-1}): 1698 (s), 1596 (m), 1503 (s), 1417 (m), 1337 (s), 1263 (s), 1235 (m), 1177 (w), 1108 (w), 1062 (w), 1021 (w), 847 (w), 751 (w), 525 (w). UV-Vis (methanol) λ_{max} (logε) 258 (4.04); 308 (4.11) nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.39 (d, 2H), 8.18 (d, 2H), 8.04 (d, 1H), 7.73 (d, 1H), 7.50 (t, 1H), 7.41 (t, 1H), 4.87 (s, 2H).

2. 1. 2. Synthesis of *N'*-(4-nitrobenzylidene)-2-(4-nitrophenoxy)acetohydrazide, 2

4-Nitrobenzaldehyde (1.0 mmol, 0.151 g) and 2-(4-nitrophenoxy)acetohydrazide (1.0 mmol, 0.211 g) were mixed in methanol, and stirred at room temperature for 1 h. The methanol was evaporated to obtain yellow crystalline product, which was washed with methanol, and dried in air. Yield: 93%. Single crystals of the compound suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Anal. calcd. for $C_{15}H_{12}N_4O_6$: C, 52.3; H, 3.5; N, 16.3; Found: C, 52.4; H, 3.6; N, 16.1%. IR data (KBr, cm^{-1}): 1680 (s), 1596 (m), 1518 (s), 1406 (w), 1343 (s), 1263 (s), 1230 (m), 1177 (w), 1108 (m), 1075 (w), 935 (w), 852 (w), 751 (w), 690 (w), 623 (w), 508 (w), 440 (w). UV-Vis (methanol) λ_{max} (logε) 258 (4.28); 325 (4.05); 400 (3.60) nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.99 (s, 1H), 8.41 (s, 1H), 8.27 (d, 2H), 8.17 (d, 2H), 8.00 (d, 2H), 7.20 (d, 2H), 4.91 (s, 2H).

2. 2. Data Collection, Structural Determination and Refinement

Diffraction intensities for the compounds were collected at 298(2) K using a Bruker D8 VENTURE PHOTON diffractometer with Mo Kα radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program,[6] and multi-scan absorption corrections were performed using the SADABS program.[7] The structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL.[8] All of the non-hydrogen atoms were refined anisotropically. The amino H atoms were located in difference Fourier maps and refined isotropically, with N–H distances restrained to 0.90(1) Å. All other H atoms were placed in idealized positions and constrained to ride on their parent atoms. Crystallographic data for the compounds are summarized in Table 1. Hydrogen bonding information is given in Table 2.

Table 1. Crystallographic and experimental data for the compounds

Compound	1	2
Formula	$C_{15}H_{12}BrN_3O_4$	$C_{15}H_{12}N_4O_6$
M_r	378.2	344.3
T (K)	298(2)	298(2)
Crystal shape/color	block/colorless	block/yellow
Crystal size (mm^3)	0.23 × 0.20 × 0.20	0.17 × 0.13 × 0.10
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$
a (Å)	5.3064(5)	4.6008(7)
b (Å)	18.202(2)	14.451(2)
c (Å)	15.970(2)	23.296(3)
β (°)	95.866(3)	93.620(2)
V (Å^3)	1534.4(2)	1545.8(4)
Z	4	4
D_c (g cm^{-3})	1.637	1.479
μ (Mo-Kα) (mm^{-1})	2.703	0.117
F(000)	760	712
Reflections collected	14602	12373
Unique reflections	2930	3343
Observed reflections ($I \geq 2\sigma(I)$)	1904	2193
Parameters	212	229
Goodness-of-fit on F^2	1.066	1.020
R_1, wR_2 [$I \geq 2\sigma(I)$]^a	0.0457, 0.0975	0.0441, 0.0985
R_1, wR_2 (all data)^a	0.0877, 0.1162	0.0762, 0.1138

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

Table 2. Hydrogen bond distances (Å) and bond angles (°) for the compounds

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	Angle (*D*–H···*A*)
1				
N2–H2···O1^{i}	0.90(1)	1.96(1)	2.851(3)	176(3)
2				
N3–H3A···O3^{ii}	0.90(1)	1.98(1)	2.881(2)	176(2)

Symmetry codes: i) 1 – x, 1 – y, 1 – z; ii) – x, 1 – y, 1 – z.

2. 3. Urease Inhibitory Activity Assay

Helicobacter 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 condition (5% O_2, 10% CO_2, and 85% N_2). The preparation method of *Helicobacter pylori* urease by Mao was followed. Briefly, broth cultures (50 mL, 2.0 × 10^8 CFU mL^{-1}) were centri-

fuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *Helicobacter pylori* precipitate was stored at –80 °C. While the *Helicobacter pylori* was returned to room temperature, and 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 stored at 4 °C until used in the experiment. The mixture, containing 25 μL (4U) of *Helicobacter 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 for three parallel times by measuring ammonia production using the indophenol method as described by Weatherburn.[9]

2. 4. Molecular Docking Study

Molecular docking of the compounds into 3D X-ray structures of *Helicobacter pylori* urease structure (entry 1E9Y in the Protein Data Bank) was carried out by using AutoDock 4.2 software as implemented through the graphical user interface AutoDockTools (ADT 1.5.4). The graphical user interface AutoDockTools was employed to setup the enzymes: all hydrogens were added, Gasteiger charges were calculated and non-polar hydrogens were merged to carbon atoms. The Ni initial parameters are set as r = 1.170 Å, q = +2.0, and van der Waals well depth of 0.100 kcal/mol.[10] The molecule of the complex was transferred to a pdb file with ChemBio3D. The pdb file was further transferred to pdbqt files with AutoDockTools. AutoDockTools was used to generate the docking input files. In the docking a grid box size of 60 × 60 × 80 points in x, y, and z directions was built, the map was centered on the original ligand molecule in the catalytic site of the protein. A grid spacing of 0.375 Å and a distances-dependent function of the dielectric constant were used for the calculation of the energetic map. 100 runs were generated by using Lamarckian genetic algorithm searches. 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. The results of the most favorable free energy of binding were selected as the resultant complex structure.

3. Results and Discussion

3. 1. Synthesis and Characterization

Both compounds were readily synthesized by reaction of 1:1 molar ratio of 2-(4-nitrophenoxy)acetohydrazide with 2-bromobenzaldehyde and 4-nitrobenzaldehyde, respectively, in methanol at room temperature, with high yields and purity. Single crystals suitable for X-ray diffraction were obtained by slow evaporation of the methanol solutions containing the compounds in air. The compounds have been characterized by elemental analyses, IR, UV-Vis and ^{1}H NMR spectroscopy. Structures of the compounds were further confirmed by single-crystal X-ray diffraction. The C, H, N analyses are in accordance with the chemical formulae proposed by the single-crystal X-ray analysis.

The characteristic intense bands located at 1698 cm^{-1} for **1** and 1680 cm^{-1} for **2** are generated by the ν(C=O) vibration, whereas the bands located at 1596 cm^{-1} for both **1** and **2** are assigned to the ν(C=N) vibration.[11] The bands indicative of the $\nu_{as}(NO_2)$ and $\nu_s(NO_2)$ vibrations are observed at 1503 and 1337 cm^{-1} for **1**, and at 1518 and 1343 cm^{-1} for **2**, respectively.

The electronic spectra of the compounds are quite similar. The strong bands centered at 308 nm for **1** and 325 nm for **2**, as well as those centered at 258 nm for both compounds are attributed to the $\pi \rightarrow \pi^*$ absorptions. The weak absorption centered at 400 nm in **2** can be assigned to the $n \rightarrow \pi^*$ absorptions.

The ^{1}H NMR spectra of compounds **1** and **2** were recorded in dimethyl sulfoxide. The typical signals of the CH=N protons are observed at 8.85 ppm for **1** and 8.41 ppm for **2**.

3. 2. Crystal Structure Description

Figures 1 and 2 give perspective views of compounds **1** and **2**, respectively, with atomic labeling systems. X-ray

Table 3. Parameters among planes for the compounds

Cg	Distance between ring centroids (Å)	Dihedral angle (°)	of *Cg*(I) on *Cg*(J) (Å) Perpendicular distance	of *Cg*(J) on *Cg*(I) (Å) Perpendicular distance	Beta angle (°)	Gamma angle (°)
1						
Cg(1)-*Cg*(2)^{iii}	4.145	9	3.588	3.204	39.4	30.1
2						
Cg(3)-*Cg*(3)^{iv}	4.601	0	3.460	–3.460	41.2	41.2
Cg(4)-*Cg*(4)^{iv}	4.601	0	3.201	–3.201	45.9	45.9

Symmetry codes: iii) ½ + x, ½ – y, ½ + z; iv) –1 + x, y, z. *Cg*(1) and *Cg*(2) are the centroids of C1–C6 and C10–C15 of **1**; *Cg*(3) and *Cg*(4) are the centroids of C1–C6 and C10–C15 of **2**, respectively.

crystallography reveals that the molecules of the compounds adopt *E* configuration with respect to the methylidene units. The distances of the C7–N1 bond in **1** and C7–N2 bond in **2**, ranging from 1.26 to 1.28 Å, confirm them as typical double bonds. The bond lengths and angles in the compounds are comparable to each other, and are within normal ranges.[12] The dihedral angles between the two aromatic rings are 75.9(4)° for **1** and 70.3(3)° for **2**. Crystal structures of the compounds are stabilized by hydrogen bonds and π···π interactions (Table 3; Figures 3 and 4).

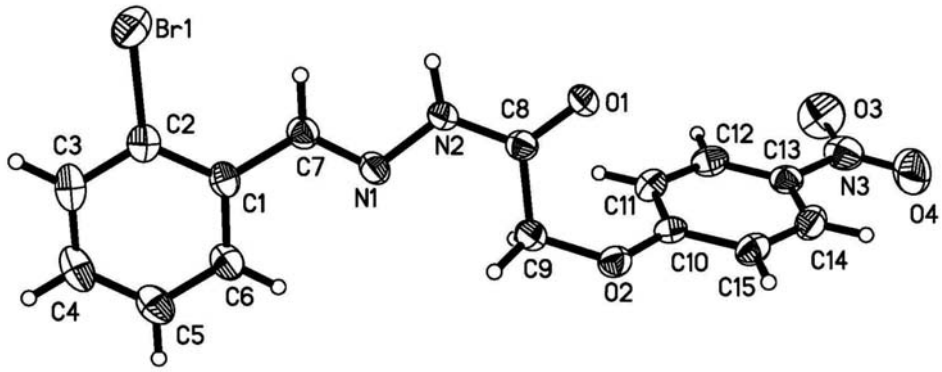

Figure 1. A perspective view of the molecular structure of **1** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

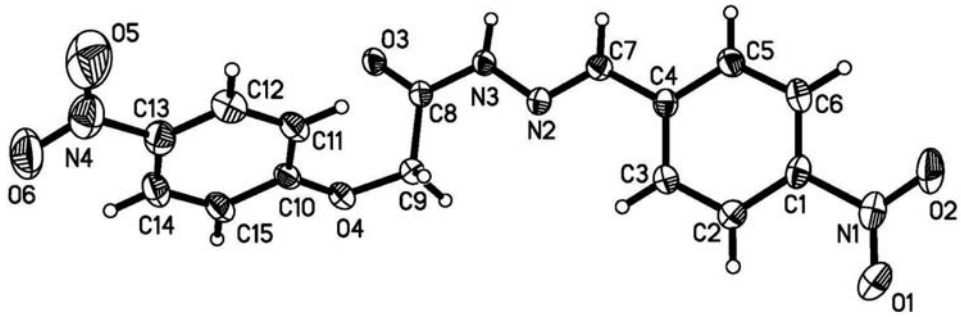

Figure 2. A perspective view of the molecular structure of **2** with the atom labeling scheme. Thermal ellipsoids are drawn at the 30% probability level.

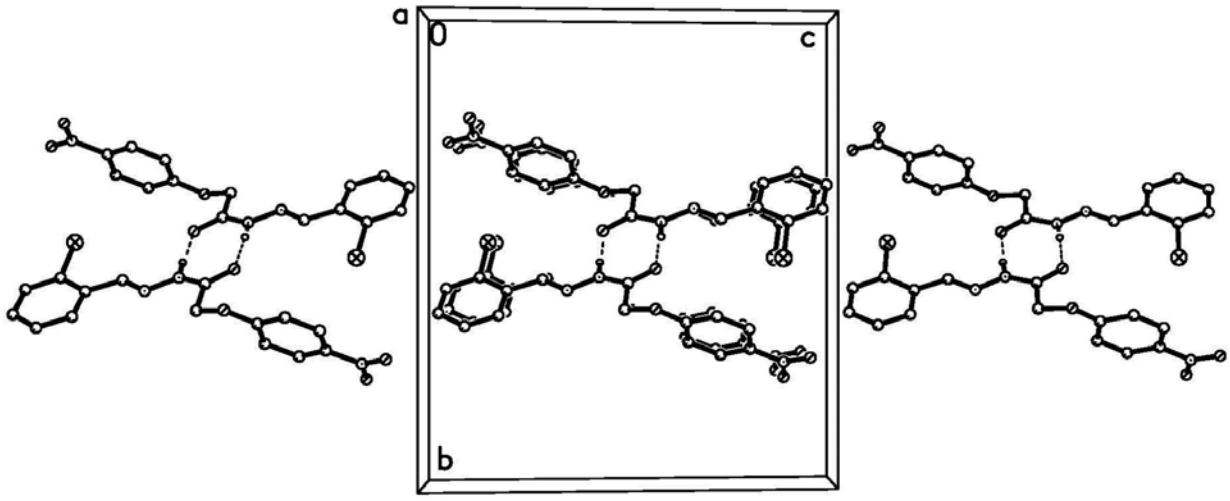

Figure 3. Molecular packing diagram of **1**. Hydrogen bonds are shown as dashed lines.

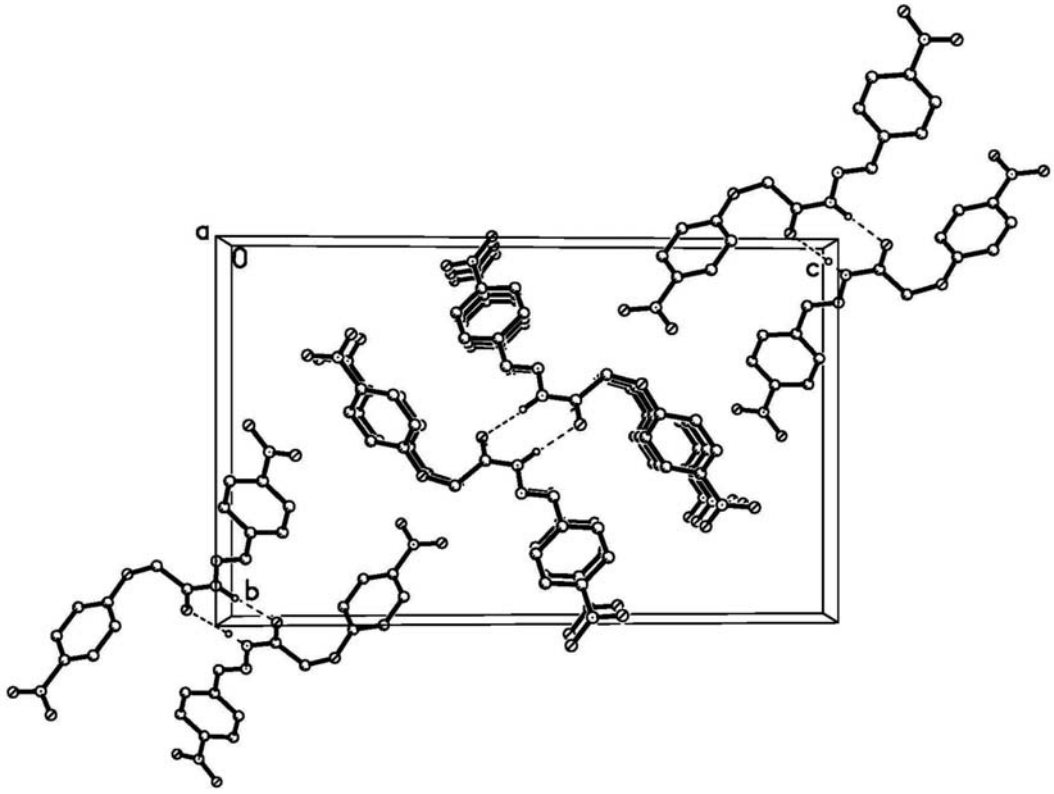

Figure 4. Molecular packing diagram of **2**. Hydrogen bonds are shown as dashed lines.

3. 3. Urease Inhibition

The urease inhibition assay was carried out. Compounds **1** and **2** have IC_{50} (half maximal inhibitory concentration) values of 8.4 and 20.2 μM, respectively. As a comparison, the reference inhibitor acetohydroxamic acid, a commercial urease inhibitor, has the IC_{50} value of 37.0 μM under the same experimental condition. Recently, we have reported an acetylhydroxamate-coordinated oxovanadium(V) complex derived from *N'*-(5-bromo-2-hydroxybenzylidene)-3-nitrobenzohydrazide. The complex shows strong urease inhibitory activity, with IC_{50} value of 8.3 ± 1.6 μM, however, the hydrazone itself has no activity on urease.[13] The substituent groups Br and NO_2 in the previously reported hydrazone compound are similar as in compound **1**. The difference of the urease inhibitory activity may come from the flexibility of the molecules. Thus, the present compounds are effective urease inhibitors, which deserve further study.

3. 4. Molecular Docking Study

Molecular docking study was performed to investigate the binding effects between the compounds and the active sites of the *H. pylori* urease. Figures 5 and 6 are the binding models for compounds **1** and **2**, respectively, in the enzyme active site of the urease. The docking scores are –12.61 for **1** and –9.55 for **2**. As a comparison, the docking score for acetohydroxamic acid is –5.01. The values of the docking scores agree well with the inhibitory activities observed from the experiment. From the docking results, it can be seen that the molecules of the compounds resides well in the cavity of the active center of the urease due to their flexibility. Even though the two hydrazone molecules are similar, only with Br and NO_2 in the benzene rings different from each other, they adopt different configuration in the active center of the urease. The molecule of **1** binds with the urease through a N–H···O hydrogen bond with *His*221. The molecule of **2** binds with the urease through N–H···O hydrogen bonds with *His*221, *Ala*365 and *Arg*168. In addition, there are a lot of interactions including van der Waals forces, hydrophobic interactions, *etc.* among the substrates and the enzyme. The results of the molecular docking study could explain the activities of the compounds against *H. pylori* urease.

4. Supplementary Material

CCDC–1012079 for **1**, and 1012080 for **2** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at

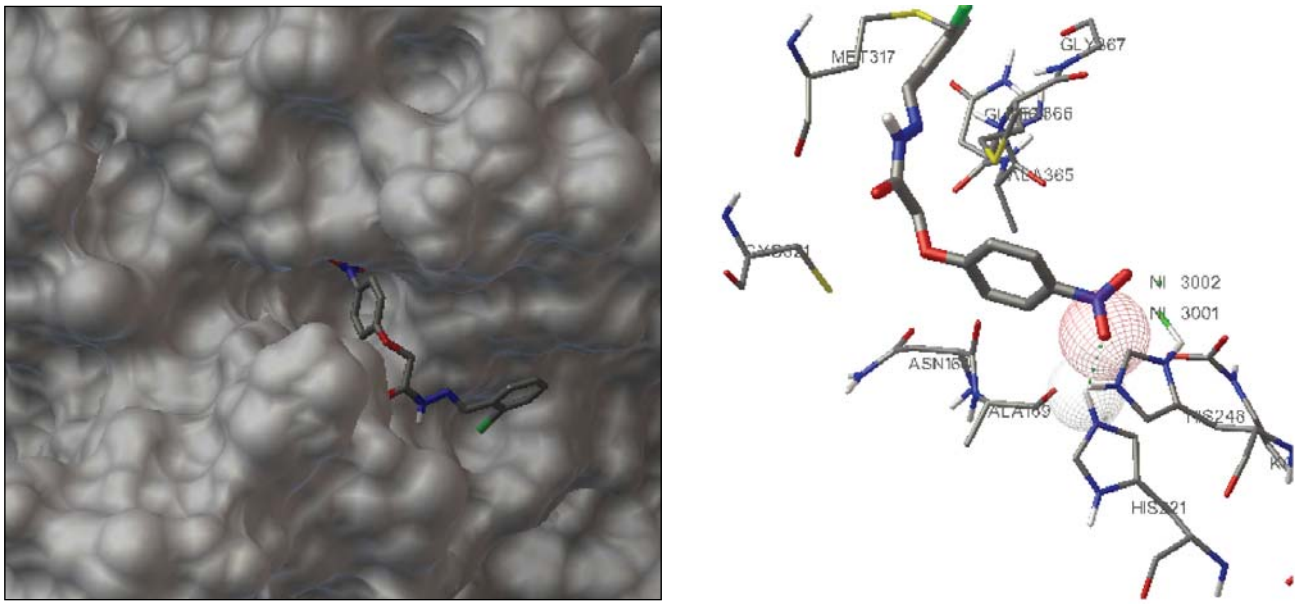

Figure 5. Binding mode of compound **1** with *H. pylori* urease. The enzyme is shown as surface, and molecule of **1** is shown as sticks (left). The hydrogen bond is displayed as a dashed line (right).

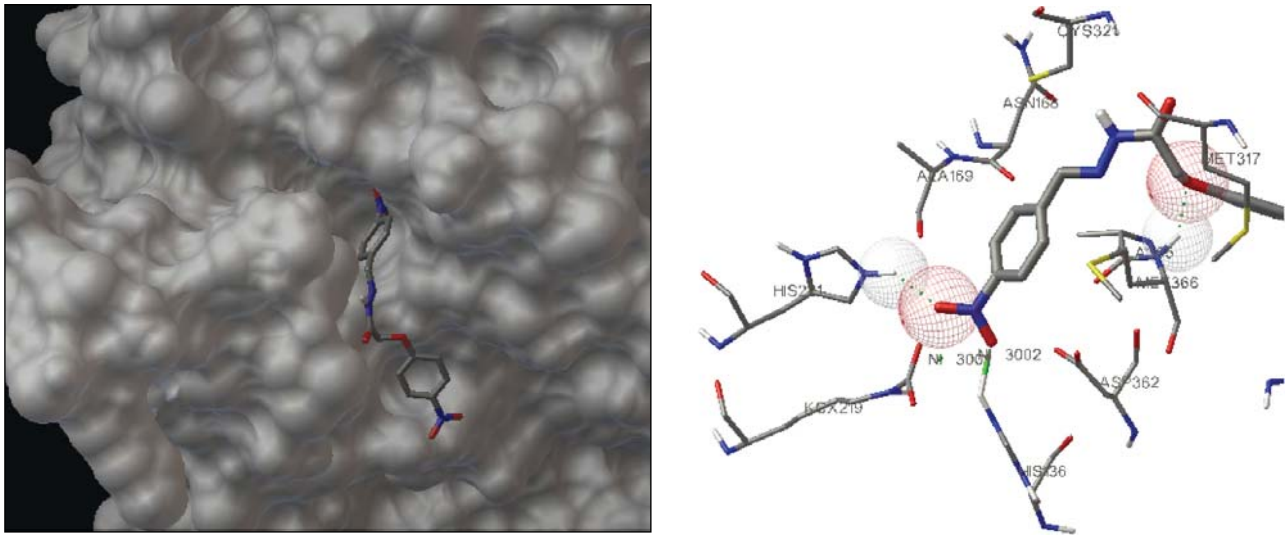

Figure 6. Binding mode of compound **2** with *H. pylori* urease. The enzyme is shown as surface, and molecule of **2** is shown as sticks (left). The hydrogen bonds are displayed as dashed lines (right).

http://www.ccdc.cam.ac.uk/const/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

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Povzetek

Sintetizirana sta dva nova hidrazona *N'*-(2-bromobenziliden)-2-(4-nitrofenoksi)acetohidrazid (**1**) in *N'*-(4-nitrobenziliden)-2-(4-nitrofenoksi)acetohidrazid (**2**), ki sta okarakterizirana z elementno analizo, IR, UV-Vis in ^{1}H NMR spektroskopijo in rentgensko monokristalno difrakcijo. Spojina **1** kristalizira v monoklinski prostorski skupini $P2_1/n$ z dimenzijami osnovne celice a = 5,3064(5) Å, b = 18,202(2) Å, c = 15,970(2) Å, β = 95,866(3)°, V = 1534,4(2) Å^3, Z = 4, R_1 = 0,0457 in wR_2 = 0,0975. Spojina **2** kristalizira v monoklinski prostorski skupini $P2_1/c$ z dimenzijami osnovne celice a = 4,6008(7) Å, b = 14,451(2) Å, c = 23,296(3) Å, β = 93,620(2)°, V = 1545,8(4) Å^3, Z = 4, R_1 = 0,0441 in wR_2 = 0,0985. Strukture spojin so stabilizirane z vodikovimi vezmi in π···π interakcijami. Določena je inhibitorna aktivnost na ureazi. Obe spojini izkazujeta močno inhibitorno aktivnost ureaze z IC_{50} vrednostjo 8,4 in 20,2 μM.