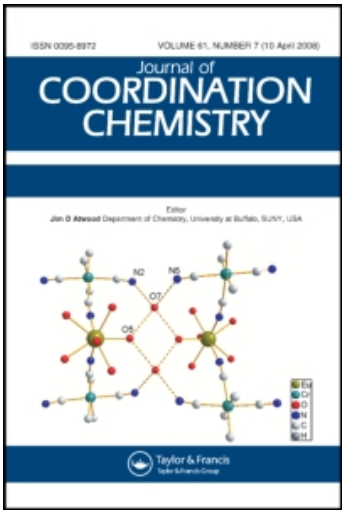

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Synthesis, molecular docking, and inhibitory activity of a Ni Schiff-base complex against urease

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A Ni(II) Schiff-base complex, $Ni(C_{14}H_{10}NOBr_2)_2$, was synthesized and structurally characterized by single-crystal X-ray analysis. Its inhibitory activity against *Helicobacter pylori* urease was evaluated *in vitro* and showed strong inhibitory activity against *H. pylori* urease compared with acetohydroxamic acid ($IC_{50} = 42.12\,\mu mol\,L^{-1}$), which is a positive reference. A docking analysis using the autodock 4.0 program could explain the inhibitory activity of the complex against urease.

Keywords: Schiff base; Molecular docking; Urease; Inhibitor

1. Introduction

Schiff-base transition metal complexes have been extensively investigated for many years due to their novel structures and potential applications in many fields [1]. Many Schiff-base transition metal complexes possess anticancer and antimicrobial activities [2–4]; some drugs have greater activity when administered as metal complexes than that as free organic compounds [5]. Therefore, Schiff-base transition metal complexes may be an untapped reservoir for drugs.

Urease, the first enzyme crystallized to be shown to possess nickel ions, is an important enzyme in both agriculture and medicine, rapidly catalyzing the hydrolysis of urea to form ammonia and carbamic acid [6, 7]. The end-product of such decomposition also results in a pH increase, responsible for the negative effects of urease activity in human health, such as causing peptic ulcers, stomach cancer, etc. [8], and in agriculture, for example, the efficiency of soil nitrogen fertilization with urea decreases due to ammonia volatilization and root damage caused by an increase in soil pH [9].

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It is of interest to control the activity of urease through the use of inhibitors in order to counteract these negative effects. Some materials possessing inhibitory activity against urease have been reported, such as fluoride [10], α-hydroxy ketones [11], and boric acid [12]. Recently, our group reported that some salen-type Schiff-base complexes possess good inhibitory activities against xanthine oxidase and excellent antibacterial activities [13].

In order to have insight into the potential application of complexes of Schiff bases as an enzyme inhibitor against urease, we designed and synthesized a new Ni complex containing the Schiff-base ligand derived from the condensation of 3,5-dibromosalicylaldehyde with benzylamine (HL, $C_{14}H_{11}NOBr_2$), and investigated its inhibitory activity against *Helicobacter pylori* urease. Docking simulation was performed to position the complex into the *H. pylori* urease active site to determine the probable binding conformation using autodock 4.0 [14].

2. Experimental

2.1. *Materials and methods*

3,5-Dibromosalicylaldehyde and benzylamine were purchased from Aldrich and used without purification. Elemental analyses (C, H, and N) were carried out on a Perkin-Elmer 2400 analyzer. Infrared (IR) spectra were recorded using KBr pellets (4000–400 cm^{-1}) on a Nexus 870 FT-IR spectrophotometer. Electronic spectra from 200 to 800 nm were measured using DMSO-H_2O (1 : 1 v/v) solution on a Shimadzu UV-160 spectrophotometer. All chemicals and reagents used in the current study were of analytical grade. Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannhein, Germany) and brucella broth was from [Becton–Dickinson] (Cockeysville, MD). Horse serum was from Hyclone (Utah, America).

2.2. *Synthesis of ([Ni(L)$_2$])*

3,5-Dibromosalicylaldehyde (56 mg, 0.2 mmol) and benzylamine (21 mg, 0.2 mmol) were dissolved in an aqueous acetonitrile solution (5 mL). The mixture was stirred for 5 min to give an orange solution, which was added to a methanol solution (1 mL) of $Ni(NO_3)_2 \cdot 6H_2O$ (29 mg, 0.1 mmol). The mixture was stirred for another 5 min at room temperature to give a kelly-green solution and then filtered. The filtrate was kept in air for 3d, forming brown flake crystals. The crystals were isolated, washed three times with distilled water, and dried in a vacuum desiccator containing anhydrous calcium chloride. Yield: 86%. Anal. Calcd for $C_{28}H_{20}Br_4N_2NiO_2$ (%): C, 42.31; H, 2.54; N, 3.52. Found (%): C, 42.20; H, 2.99; N, 3.41. IR (KBr, cm^{-1}): 457, 555, 607, 1168, 1444, 1496, 1517, 1618, 2868, 2926, 3028. UV-Vis (DMSO-H_2O, k nm^{-1}): 243, 267, 376.

2.3. *Crystal structure determinations*

Diffraction intensities for the complex were collected at 291(2) K using a Bruker SMART CCD area detector with Mo-Kα radiation ($\lambda = 0.71073$ Å). The collected data

were reduced using the SAINT program [15], and empirical absorption corrections were performed using SADABS [16]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares using SHELXTL version 5.1 [17]. All non-hydrogen atoms were refined anisotropically. All hydrogens were placed in geometrically ideal positions and constrained to ride on their parent atoms. The crystallographic data for the complex are summarized in table 1. Selected bond lengths and angles are given in table 2.

2.4. *Measurement of inhibitory activity against H. pylori urease*

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 conditions (5% O_2, 10% CO_2, and 85% N_2), as previously described [18].

The method of preparation of *H. pylori* urease by Mao [19] was followed. Briefly, 50 mL broth cultures (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. *Helicobacter 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 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 use in the experiment. The mixture, containing 25 μL (4U) of *H. pylori* urease and 25 μL of the test compound, was pre-incubated for 3 h at room temperature in a ninety-six-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [20].

Table 1. Crystal data for the complex.

Empirical formula	$C_{28}H_{20}Br_4NiN_2O_2$
Molecular weight	794.81
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions (Å, °)	
a	10.6062(6)
b	6.1056(4)
c	20.7125(15)
α	90
β	102.431(3)
γ	90
Temperature (K)	291(2)
Volume (Å^3), Z	1309.84(15), 2
Calculated density (g cm^{-3})	2.015
F(000)	772
Absorption coefficient (Mo-Kα) (mm^{-1})	6.872
Data/restraint/parameters	2712/0/169
Goodness-of-fit on F^2	1.049
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0432$, $wR_2 = 0.1081$

Table 2. Selected bond lengths (Å) and angles (°) in the complex.

Ni(1)–O(1)	1.847(3)	Ni(1)–O(1a)	1.847(3)
Ni(1)–N(1)	1.944(4)	Ni(1)–N(1a)	1.944(4)
O(1)–Ni(1)–O(1a)	180.00(18)	O(1)–Ni(1)–N(1a)	88.03(15)
O(1)–Ni(1)–N(1)	91.97(15)	O(1a)–Ni(1)–N(1a)	91.97(15)
O(1a)–Ni(1)–N(1)	88.03(15)	N(1)–Ni(1)–N(1a)	180.0(2)

Complex (a: $1-x$, $1-y$, $-z$).

2.5. *Docking simulations*

Molecular docking of the complex into the 3-D X-ray structure of *H. pylori* urease (entry 1E9Z in the Protein Data Bank) was carried out using the AUTODOCK software package (version 4.0) as implemented through the graphical user interface AutoDockTools (ADT 1.4.6) [21].

3. Results and discussion

3.1. *IR, UV analysis*

The complex and the Schiff-base HL have been characterized from elemental analytical and spectral data. The strong absorption at 1629 cm^{-1} for HL is assigned to ν(C=N) [22], which is shifted to lower wavenumber in the complex (1618 cm^{-1}), due to the coordination of the Schiff-base ligand. This conclusion is further supported by the presence of weak ν(Ni–N) at 555 cm^{-1} for the complex. A weak band indicative of Ni–O is observed at 457 cm^{-1}. For the complex, a broad, strong absorption at 1444, 1496, and 1517 cm^{-1} can be reasonably attributed to the presence of benzene ring C=C backbone stretching vibration. The complex exhibits the other absorptions: ν(C–Br) at 607, ν(Ph–O) at 1168, and ν(C–H) at 2868, 2926, and 3028 cm^{-1}. The IR spectra agree with single-crystal X-ray analysis.

UV-Vis spectra for the complex were obtained in assay condition (DMSO : H_2O, 1 : 1 v/v). Absorptions of amine are displaced due to the coordination of nitrogen to nickel. The complex shows a weak d–d band in the 500 nm region. The complex exhibits weak bands at 370–390 nm, attributed to a charge transfer (CT) transition [23, 24].

3.2. *Crystal structure description*

The coordination environment of Ni(II) and the crystal structure of the complex are shown in figure 1. X-ray single-crystal diffraction reveals that the complex crystallizes in monoclinic space group $P2_1/c$. The Ni(II) lies on an inversion center [symmetry codes: $1-x$, $1-y$, $-z$] and is four-coordinate by two nitrogens and two oxygens from two Schiff bases in a square planar *trans*-[NiN_2O_2]. The bond distances of Ni1–O1 and Ni1–N1 are 1.847(3) and 1.944(4) Å, respectively, comparable with corresponding values reported for analogous square planar Ni(II) species [25, 26].

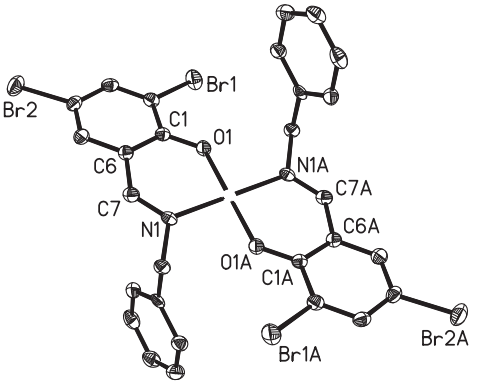

Figure 1. An ORTEP diagram showing the structure of the complex with selected atom labeling. The thermal ellipsoids are plotted at 30% probability and hydrogens omitted for clarity. Symmetry codes A: $1-x$, $1-y$, $-z$.

Table 3. Inhibition of *H. pylori* urease by the complex, Schiff-base ligand and Ni(II) ion.

Tested materials	IC_{50} ($\mu mol\,L^{-1}$)
HL	>100
Ni^{2+}	4.6 ± 0.3
Complex ($Ni(C_{14}H_{10}NOBr_2)_2$)	3.9 ± 0.2
Acetohydroxamic acid	42.3 ± 0.4

3.3. *Inhibitory bioactivity against urease*

The ability of the Schiff-base HL, Ni^{2+}, and the complex in inhibiting urease has been studied by IC_{50} values of the material (25 μL, 100 μg) tested against *H. pylori* urease (25 μL, 10 kU L^{-1}) using urea (500 mmol L^{-1}) in HEPES buffer (0.2 mL, 100 mmol L^{-1}; pH = 6.8). On reaction with *H. pylori* urease in the presence of phenol red, HL has no ability to inhibit urease ($IC_{50} > 100\,\mu mol\,L^{-1}$), indicating that the Schiff base has little influence on the activity of *H. pylori* urease. Under the same conditions, both Ni(II) and its Schiff-base complex showed potent urease inhibitory activities, compared with that of the standard inhibitor acetohydroxamic acid which had IC_{50} of 42.12 μmol L^{-1}, as shown in table 3. The results agree with those reported previously [25], but are much stronger than the IC_{50} value (13.0–41.1 mmol L^{-1}) of copper(II) complexes [27, 28].

3.4. *Molecular docking study*

Molecular docking of *H. pylori* urease and the complex were performed on the binding model based on the *H. pylori* urease structure (entry 1E9Z in the Protein Data Bank). A superposition of the structures of uninhibited and inhibited *H. pylori* urease reveals a flap motion of the segment composed of residues α313–α346 forming a helix-turn-helix motif, which opens the active site when the inhibitor is bound and closes it when not bound [29]. *Helicobacter pylori* urease's active site contains two nickel(II) ions (NI3001 and NI3002) coordinated by His136, His138, Kcx219, His248, His274, Asp362, and water [30–32].

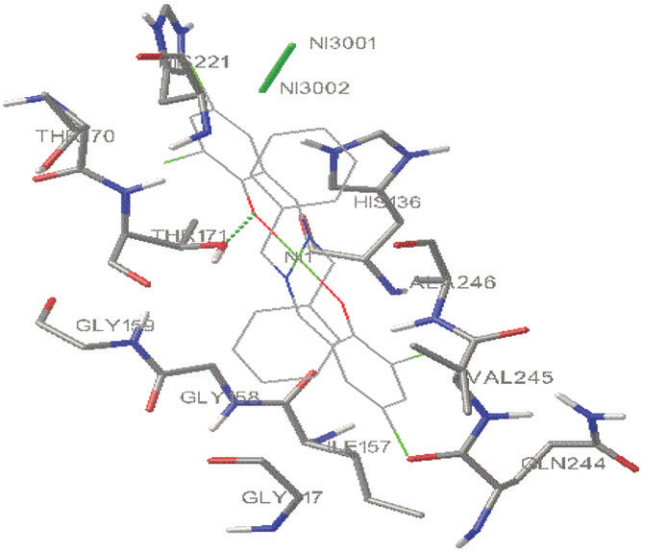

Figure 2. ($Ni(C_{14}H_{10}NOBr_2)_2$) bound into *H. pylori* urease (entry 1E9Z in the Protein Data Bank). The dotted line shows the hydrogen bond.

The binding mode of phenylphosphorodiamidate, a known efficient urease inhibitor, in the enzyme active site was modeled [33]. In the binding model, the oxygen of phenylphosphorodiamidate formed a hydrogen bond with the amino hydrogen of His221 (length of the hydrogen bond: $His221N–H\cdots O_{phenylphosphorodiamidate} = 2.167$ Å; angle of the hydrogen bond: $His221N–H\cdots O_{phenylphosphorodiamidate} = 171.6^{\circ}$). The benzene ring of phenylphosphorodiamidate probably forms hydrophobic interactions with Ala168, Ala169, and Ala365.

The binding model of the complex and *H. pylori* urease is depicted in figure 2. All the amino acid residues which had interactions with *H. pylori* urease are shown. In comparison to phenylphosphorodiamidate–urease complex and acetohydroxamic acid–urease complex, in the model, hydroxy of THR171 forms a hydrogen bond with the oxygen of the complex (length of the hydrogen bond: $THR171O–H\cdots O_{complex} = 2.495$ Å; angle of the hydrogen bond: $THR171O–H\cdots O_{complex} = 66.7^{\circ}$). Moreover, the complex may form hydrophobic interactions with Ile157, Val245, and Ala246 of *H. pylori* urease. The urease-inhibitory property possessed by the complex may be attributed to the above hydrogen bond and hydrophobic interactions formed with *H. pylori* urease. The docking calculations reveal that the complex has lower free energy of binding (-11.72 kcal mol^{-1}) than that of AHA (-10.07 kcal mol^{-1}), which may also explain the excellent inhibitory activity of the complex against *H. pylori* urease.

4. Conclusion

The present report describes the syntheses, X-ray crystal structure, and inhibitory enzyme activity of a Schiff-base nickel complex. The complex exhibits ability

to inhibit urease, although its Schiff-base ligand HL has no ability to inhibit urease ($IC_{50} > 100\,\mu mol\,L^{-1}$). Docking simulation was performed to position the complex into the *H. pylori* urease active site to determine the probable binding conformation, and the result indicated that the complex was a potent inhibitor of *H. pylori* urease. Detailed investigations are continuing to study the mechanisms of the inhibitory activity reported here.

Supplementary material

Crystallographic data in CIF format for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre No. 754514 for the complex ($[Ni(C_{14}H_{10}NOBr_2)_2]$). Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336 033; Email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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