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Authors: Dursun KISA, Yusuf CEYLAN, Merve YILDIRIM, Sümeyra DURGUN, Nesrin KORKMAZ

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Metal-containing Coordination Complexes (MCCs): Assessment of Biological Activity with *Helicobacter pylori* Caused Gastric Ulcer

Dursun KISA*[1], Yusuf CEYLAN[1], Merve YILDIRIM[1],
Sümeyra DURGUN[1], Nesrin KORKMAZ[2]

Abstract

The ulcer is a vital disease that unfavorably affects human health globally. *Helicobaster pylori*, which causes ulcers and some stomach disorders, survives by using the urease enzyme. Metal coordination complexes (MCCs) are used in numerous industrial areas and the health field. Cyanide bridged metal complexes are also applied in several applications. In the current study, antibacterial characteristic of (C1), $[Ni(bishydeten)_2Ag(CN)_2][Ag(CN)_2].H_2O$, and (C2), $([Ni(hydeten)_2Ag(CN)_2][Ag(CN)_2]$, MCCs were analyzed by disk diffusion, broth dilution and urease enzyme inhibition assays were performed. As a result of both antibacterial tests, C1 and C2 were observed to provide favorable effects. The synthesized compounds have effective inhibitory potential with IC_{50} values between 26.65±1.21 and 12.37±0.87 µM for urease.

Keywords: Metal coordination complexes, *Helicobacter pylori*, antibacterial, urease, enzyme inhibition

1. INTRODUCTION

H. pylori is a pathogenic Gram (-) bacterium that attacks the gastric epithelial surface and results in lifelong chronic gastritis unless treated with antimicrobial drugs. *H. pylori* is a microorganism that influences approximately 50% of the global population, and research on its eradication has been rising in previous years [1].

The bacterial urease enzyme is of particular impact, a potent virulence factor. The enzyme secreted by *H. pylori* in the mucus layer of the stomach converts urea into ammonia and carbamate [2]. In the presence of the urease, the hydrolysis of urea raises approximately tenfold. The bacterium thus preserves itself from the harmful impacts of stomach acid by establishing an alkaline habitat [3]. Urea is the main source of pathologies caused by *H. pylori* and therefore plays a fundamental role in the pathology of

* Corresponding author: dkisa@bartin.edu.tr
[1] Bartın University, Faculty of Science, Department of Molecular Biology and Genetics
E-mail: ysf.cyln@gmail.com, mervee.yildirim9@gmail.com, sdurgun141@gmail.com
ORCID: https://orcid.org/0000-0002-7681-2385, https://orcid.org/ 0000-0001-8186-7252, https://orcid.org/0000-0003-1427-9666, https://orcid.org/ 0000-0001-6437-5512
[2] Yozgat Bozok University, Hemp Research Institute
E-mail: nesrinokumus@gmail.com
ORCID: https://orcid.org/0000-0002-7896-1042

gastric and peptic ulcers. Excessive urease activity gives rise to cell death, kidney disorders, renal calculus formation, ulcer, hepatic, and urinary system diseases [4].

Enzymes, biological molecules responsible for the metabolic process, are synthesized by living cells and perform chemical reactions [5]. Enzymes reach optimum pH, temperature, substrate concentration, enzyme amount, activator, and inhibitory substances. Enzymes are used as biomarkers in hospital routine tests to diagnose various diseases [6].

Enzyme inhibition is a crucial step in pharmaceutical research since it has led to the detection of several curative drugs for many diseases [5]. Specific inhibitors interact with enzymes and block their activity. Urease (Ureamidohydrolase; EC 3.5.1.5), a crucial factor of the nitrogen cycle, is a nickel-containing metalloenzyme found in animals, plants, bacteria, and fungi [1,4].

Metal-containing coordination complexes (MCCs) have been used in several fields long ago, and one of the most critical areas of that is in the medical field. Papers have risen in this direction by increasing MCC availability in the industry. MCC is among the compounds researchers notice with this approach [7].

It is known that MCCs demonstrate various biological activities such as antitumor, antimicrobial, antifungal, analgesic, anti-inflammatory, anti-HIV, carbonic anhydrase inhibition, and local anesthetic [8]. Enzymes are responsible for almost all biochemical reactions to maintain homeostasis. Metal complexes carrying –OH groups on their active surfaces have more enzyme inhibition effects. The effects of MCC on the inhibition of enzymes have been initiated to be examined in recent years [9]. Cyano bridged metal complexes among the complex coordination compounds are one-, two- or three-dimensional macromolecules arranged in the form of metal-metal or metal-ligand-metal. Cyano complexes containing transition metals take part in material chemistry due to their various bonding, structural properties, and broad usage areas [10].

Cyano-bridged homo- and heteronuclear compounds are among the molecules that attract attention due to their different properties and application areas. Various coordination compounds are still used as anticancer drugs [11-16]. In conclusion, coordination compounds are promising complexes in anticancer and antimicrobial therapies.

H. pylori is sensitive to many antibiotics *in vitro*, although it is resistant to *in vivo* conditions. Currently, antibiotics such as lansoprazole, amoxicillin, metronidazole, and clarithromycin and inhibitory agents such as urea C-13 are applied for the eradication of *H. pylori* and the inhibition of the urease enzyme. It has been reported that the urease secreted by *H. pylori* is found in the cytoplasm in freshly prepared cultures and the outer membrane in old cultures [17-18].

Urease inhibitors are one of the targets for new antiulcer drugs. Studying the synthesis of new urease inhibitors that can suppress the urease enzyme and new coordination complexes with antimicrobial effects against *H. pylori* to treat such diseases is a promising approach to disease treatment. This study aimed to minimize the formation of ammonia, which has a vital role in the *H. pylori* life cycle, by inhibiting the urease function of the newly synthesized MCC and analyzing its usage as an antibacterial agent. Our research group will examine the effect of MCCs, which were previously shown to have inhibitory effects on AChe, BChE, and carbonic anhydrase on urease, for the first time with this study [19].

2. EXPERIMENTAL

2.1. Disc Diffusion Method

Previously synthesized MCCs ($[Ni(hydeten)_2Ag(CN)_2][Ag(CN)_2]$ and $[Ni(bishydeten)_2Ag(CN)_2][Ag(CN)_2].H_2O$) were dissolved in sterile distilled water and five different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL and 50 µg/mL) were prepared under sterile conditions. Different concentrations of MCCs were absorbed into sterile discs. *Helicabter pyolori* (ATCC 700824)

used in the current study was obtained from the Microbiology Reference Laboratories and Biological Products Department of the Ministry of Health of the Republic of Turkey. Modification of Kirby and Bauer's disk diffusion assay was practiced because of the use of blood agar instead of Mueller Hinton agar [20]. Bacterial growth was calibrated 0.5 McFarland turbidity ($1.5x10^{8}$ cell/mL). Bacteria were seeded into blood agar (LabM), and discs were placed on the petri dishes and incubated overnight at 37°C. After incubation, the inhibition zones around the discs were measured, and the study was performed with three replicates.

2.2. Broth Microdilution Assay

The modified Minimal Inhibition Concentration (MIC) test was applied for antibacterial activity of MCCs against *H. pylori* that were cultured in Tryptic Soy Broth (LabM). The concentration of bacterial suspension was adjusted to 0.5 McFarland in %9 sterile NaCl. Chemicals were dispersed in 10% DMSO and diluted to 500 µg/mL concentration, and serial two-fold dilution was performed among 15.625 µg/mL. MIC and Minimal Bactericidal Concentration (MBC) values of MCCs were identified against *H. pylori* [21].

2.3. Urease Inhibition Assay

The urease inhibition study of MCCs was carried out using the indophenols method to quantify ammonia [22]. In brief, the reaction mixtures comprise 50 µL of phosphate buffer (100 mM, pH 8.2), which contains 100 mM urea µL, 25 µL of jack bean urease solution, and 20 µL of MCCs were incubated in a 96 well microplate at 37 °C for 15 min. Then, the ammonia liberated was allowed to complex with 45 µL of a phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside - $Na_2[Fe(CN)_5NO]\cdot 2H_2O$), and 70 µL of an alkali reagent (0.5% w/v NaOH and 0.1% NaOCl). After the mixture was incubated for 20 min, the absorbance of the color change was spectrophotometrically measured at 630 nm using the microplate reader. (MultiskanGO Microplate Reader, Thermo Scientific).

3. RESULTS AND DISCUSSION

3.1. Disc Diffusion Method

H. pylori is one pathogenic bacteria that significantly influence human health globally. Since *H. pylori* are sensitive to antibiotics *in vivo* conditions, research on inhibiting or eradicating the bacteria continues. In current paper, different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL and 50 µg/mL) of **C1** (bishydeten) and **C2** (hydeten) compounds were analyzed and tetracycline antibiotic was used as positive control (Table 1). The largest zone was measured at the concentration of 1000 µg/mL concentration, the highest dose for both compounds (Figure 1). The highest dose for compound **C1** and **C2** was measured at 1000 µg/mL concentrations at 10±0,24 mm and 14±1,00 mm, respectively. On the other hand, tetracycline formed a zone diameter of 27 mm. When previous studies were viewed, it was observed that chemical compounds with similar structures had a lower zone diameter than the antibiotic used as a positive control [10, 23-25]. As the concentrations of **C1** and **C2** compounds of *H. pylori* reduced, it was revealed that the diameter of the effect zone narrowed. Both chemical compounds were assessed effectively against bacteria.

Table 1 Inhibitory zone diameter (mm) for different concentration of C1 and C2 against *H. pylori* by disc diffusion method

Conc. (µg/mL)	C1	C2
1000	10±0.24	14±1.00
500	8±0.47	10±0.31
250	8±0.28	8±0.20
100	7±0.16	NE

NE: No effect

Figure 1 Photograph showing antibacterial C1 and C2 screening on blood agar

3.2. Broth Microdilution Assay

C1 and **C2** were subjected to MIC and MBC analysis. The results are served in Figures 2 and 3. As seen in Figure, both chemicals demonstrated MBC and MIC characteristics at 500 µg/mL and 250 µg/mL concentrations, respectively. Like the disc diffusion method, it was observed that the bacteriostatic and bacterial effects increased with increasing chemical concentration. According to MIC results of EUCAST Clinical Breakpoint, Fluoroquinolones and tetracyclines shows MIC breakpoints at 1 mg/mL concentration [26]. Antibacterial properties of the MCCs possibly be clarified by the many essential factors like hydrophilicity, hydrophobicity, metal ion, metal ion coordination site can possess an appreciable effect on the antibacterial effect. The activities of MCCs can be ascribed to their susceptibility to experience different ligand substitute reactions with the biological ligands such as DNA and protein [27-29].

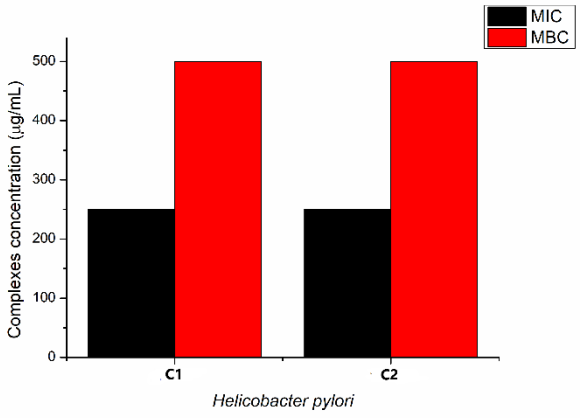

Figure 2 Minimum inhibition and minimum bacteriostatic/bactericidal concentration of C1 and C2 against *H. pylori*

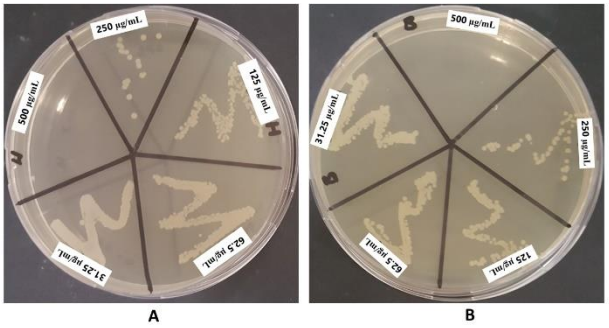

Figure 3 Results of minimum bactericidal concentration against *H. pylori*

3.3. Urease Inhibition Ability of MCCs

In this study, the inhibition effects of MCCs on urease enzyme associated with *H. pylori* were evaluated, and their IC_{50} values are shown in Figure 4 and Table 2 below. The synthesized compound C1 has effective inhibitory potential with an IC_{50} value at 12.37±0.87 µM. The urease inhibitory potential of the compound C2 was 12.37±0.87 µM for IC_{50} value. The compounds inhibited urease activity in micromolar concentration. A previous study on inhibition of urease with metal compounds of schiff base ligands demonstrated that transition metals had exhibited different abilities, and their IC_{50} value changed between 0.37-100 µM for urease inhibition [30]. The inhibitory activity of complexes containing metals such as Cu, Ni, Zn, and Co against urease activity declared that their synthesized complexes have IC_{50} values between 2.14± 0.12 – 100 µM for urease [31]. Another study expressed that some metal complexes show medium urease inhibitory ability, with IC_{50} values of 35.7±3.1, and 41.5±2.7 µmol·L^{-1}, respectively [32]. The obtained compounds should be assessed as urease inhibitors according to previous studies. The inhibitory potential of molecules against enzymes has been associated with the structure of compounds, allowing them to interact between enzymes and substances [33].

Table 2 Inhibition values of dicyanidosilver complexes on urease

Compounds	IC_{50} (µM)	r^2
$[Ni(hydeten)_2Ag(CN)_2][Ag(CN)_2]$ (C2)	26.65±1.21	0.897
$[Ni(bishydeten)_2Ag(CN)_2][Ag(CN)_2].H_2O$ (C1)	12.37±0.87	0.904

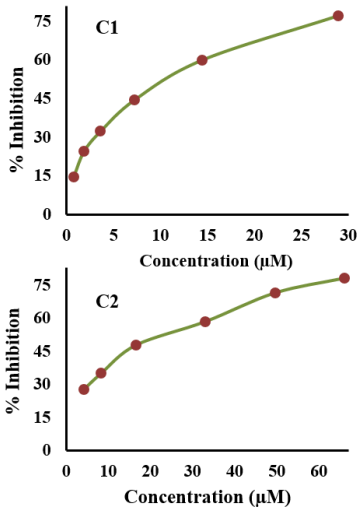

Figure 4 Inhibition ability of MCCs on the studied urease enzyme

4. CONCLUSION

Health issues caused by *H. pylori* are diseases that adversely affect many world populations. It is concluded that MCCs have antibacterial effects against *H. pylori* and anti-urease activity. The eradication of *H. pylori* by enzyme inhibition has prompted researchers to attempt new solutions to inhibit urease activity. MCCs are applied in several fields and the health sector, such as antifungal, antibacterial, anticancer, and antitumor. The current paper analyzed the antibacterial effect of C1 and C2 compounds against *H. pylori* by disc diffusion and broth dilution methods.

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The Declaration of Conflict of Interest/ Common Interest

The authors have declared no conflict of interest or common interest.

Authors' Contribution

The authors contributed equally to the study.

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The author declares that this document does not require approval from the ethics committee or any special permission.

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The authors of the paper declare that they comply with the scientific, ethical, and quotation rules of SAUJS in all the article processes and that they do not falsify the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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