บทความวิจัย

การยับยั้งเชื้อแบคทีเรียของสารประกอบโคออร์ดิเนชันคอปเปอร์ (II) และนิกเกิล (II) ที่มีลิแกนด์พอลิเอซาวงใหญ่

The Antibacterial Activity of Copper(II) and Nickel(II) Coordinated Compounds Containing a Polyaza Macrocyclic Ligand

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บทคัดย่อ

สารประกอบ $[CuL](ClO_4)_2$ (1) และ $[NiL](ClO_4)_2$ (2) เตรียมได้จากการทำปฏิกิริยาของ 1,2-ไดอะมิโนโพรเพน แอลฟา-เมทิลเบนซิลลามีน และฟอร์มัลดีไฮด์ เมื่อ L คือ 6,13-ไดเมทิล-3,10-บิส(1-ฟีนิลเอทิล)-1,3,5,8,10,12-เฮกซะเอซาไซโคลเตตระเดคเคน ได้ยืนยันโครงสร้างสารประกอบโคออร์ดิเนชัน 1 และ 2 โดยเทคนิค คือ การวิเคราะห์ปริมาณธาตุ ลิคควิดโครมาโทกราฟี-แมสสเปกโทรเมตรี อินฟราเรด และยูวี-วิสิเบิล สเปกโทรสโกปี พบว่า สารประกอบ 1 และ 2 ประกอบด้วยไอออนหลัก ๆ คือ $[ML]^{2+}$ จำนวน 1 ไอออน และเปอร์คลอเรตจำนวน 2 ไอออน อะตอมไนโตรเจน 4 อะตอมของลิแกนด์พอลิเอซาวงใหญ่ได้สร้างพันธะโคออร์ดิเนตโคเวเลนต์กับโลหะ(II) ไอออนอะตอมกลางมีรูปร่างเป็นสี่เหลี่ยมแบนราบ สารประกอบโคออร์ดิเนชันทั้งสองมีประสิทธิภาพในการยับยั้งเชื้อแบคทีเรีย *Escherichia coli* และ *Staphylococcus aureus*

คำสำคัญ : สารประกอบโคออร์ดิเนชัน พอลีเอซา แอลฟา-เมทิลเบนซิลลามีน

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Abstract

$[CuL](ClO_4)_2$ (1) and $[NiL](ClO_4)_2$ (2) compounds containing a polyaza macrocyclic ligand have been prepared via the reaction of 1,2-diaminopropane, α-methylbenzylamine and formaldehyde in the presence of metal(II) ions, where L = 6,13-dimethyl-3,10-bis(1-phenylethyl)-1,3,5,8,10,12-hexaazacyclotetradecane. The compound 1 and 2 have been characterized by elemental analysis, LC-MS, IR and UV-Vis spectroscopy. The essential structure of two compounds consist of $[ML]^{2+}$ (M = Cu^{2+} and Ni^{2+}) cation and two perchlorate anions. Metal-ion center, which is Ni(II) and Cu(II), in each compound is coordinated by four secondary amine nitrogen donors of the macrocyclic ligand and has a square-planar geometry forming five- and six-membered rings. The investigation on the antibacterial activity of newly synthesized Ni(II) and Cu(II) compounds were found to be active against the tested antibacterial of *Escherichia coli* and *Staphylococcus aureus.*

Keywords : Coordination Compounds, Polyaza, α- Methylbenzylamine

Introduction

Coordination compounds containing polyaza macrocyclic ligand have attracted much attention in chemistry, material science and the chemical industry because of their applications for catalytic activity [1] antimicrobial activity [2] and magnetic properties [3] However, primary amine was side chain the most and it has been long chain of alkane group, such as methylamine [4] benzylamine [2] and 1-(3-aminopropyl)- imidazole [5].

Recently, it has been found the synthesized method of coordination compounds containing polyaza macrocyclic ligand simple route, convenient and high percent yield. There has been called "One pot template condensation reaction". Typical methods of condensation reaction consist of transition metals salt, formaldehyde and primary amine with reflux.

The applications of coordination compounds containing polyaza macrocyclic ligand in the international journals showed that coordination compound can be used practically. Most of the tested microorganisms, such as bacteria are an inhibit *E. coli, P. aeruginosa, B. thuringiensis* and *S. typhimurium* etc., fungi including *P. chrysogenum, C. albicans and C. neoformans* [6]. The present is found that the many of antibiotic resistance, which are adjustment to the drug by various methods in order to eliminate or reduce the effectiveness of antibiotic resistance. The drug resistant may be naturally having microorganisms that may occur under the pressure of antibiotics [7].

In this work, $[CuL](ClO_4)_2$ (1) and $[NiL](ClO_4)_2$ (2) compounds containing polyaza macrocyclic ligand using $Cu(CH_3COO)_2•H_2O$, $Ni(CH_3COO)_2•4H_2O$, HCOH and $C_8H_{11}N$ were synthesized as side chains in which N-donor atoms coordinated to metal(II) center. In addition, these coordination compounds were studied for the antibacterial activity of *Escherchia coli* as gram negative and *Staphylococcus aureus* as gram positive by measuring of clear zone (millimeter) and comparing with tetracycline as standard drug.

Experimental

Materials and Method

$Cu(CH_3COO)_2•H_2O$, $Ni(CH_3COO)_2•4H_2O$, 1,2-diaminopropane, formaldehyde, α-methylbenzylamine and perchloric acid were all purchased from commercial source and used as received. *Caution!* Perchlorate salts of metal complexes are potentially explosive. Only a small amount material should be prepared, and it should be handled with care. The IR spectra (4000-400 cm^{-1}) were recorded with FT-IR spectrometer as a KBr disk and visible absorption spectra were recorded with a Shimadzu Lambda-1600 UV-Vis spectrophotometer in DMSO. The CHN-O elemental analysis was analyzed by dynamic flash combustion technique and Liquid Chromatograph-Mass Spectrometer (LC-MS) and it was recorded with Electron Spray Ionization Positive mode (ESI^+) in acetonitrile.

Synthesis of $[CuL](ClO_4)_2$ (1)

To a ethanolic solution of $Cu(CH_3COO)_2•H_2O$ (1.9970 g, 10 mmol) in 99% ethanol (10 mL) were added 98% 1,2-diaminopropane (1.3 mL, 20 mmol) in 99% absolute ethanol (10 mL). After the mixture was completely clear purple solution. Then 37% formaldehyde (2.2 mL, 40 mmol) was added with stirring until the mixture was completely dark blue solution and then α-methylbenzylamine (1.27 mL, 20 mmol) was added with continuous stirring. The mixture of reactants was refluxed for 48 hours and the purple - red solution was cooled to room temperature and with filtering to remove any insoluble solids. After the amount of concentrated perchloric acid was added dropwise to the mixture, it was filtered and stored at room temperature to form purple-red solids. Yield: ≈ 57% *Anal.* Calc. for $C_{26}H_{42}N_6Cl_2O_8Cu$: C, 43.46; H, 5.87; N, 12.01; O, 23.09. Found: C, 43.30; H, 5.83; N, 12.04; O, 23.05%.

Synthesis of $[NiL](ClO_4)_2$ (2)

The yellow solids of compound 2 were prepared by a similar procedure of complex 1 with an proximate yield of 27% but using $Ni(CH_3COO)_2•4H_2O$ instead of $Cu(CH_3COO)_2•H_2O$. *Anal.* Calc. for $C_{26}H_{42}N_6Cl_2O_8Ni$: C, 44.91; H, 6.08; N, 12.08; O, 20.09. Found: C, 44.89; H, 6.05; N, 12.06; O, 20.05%.

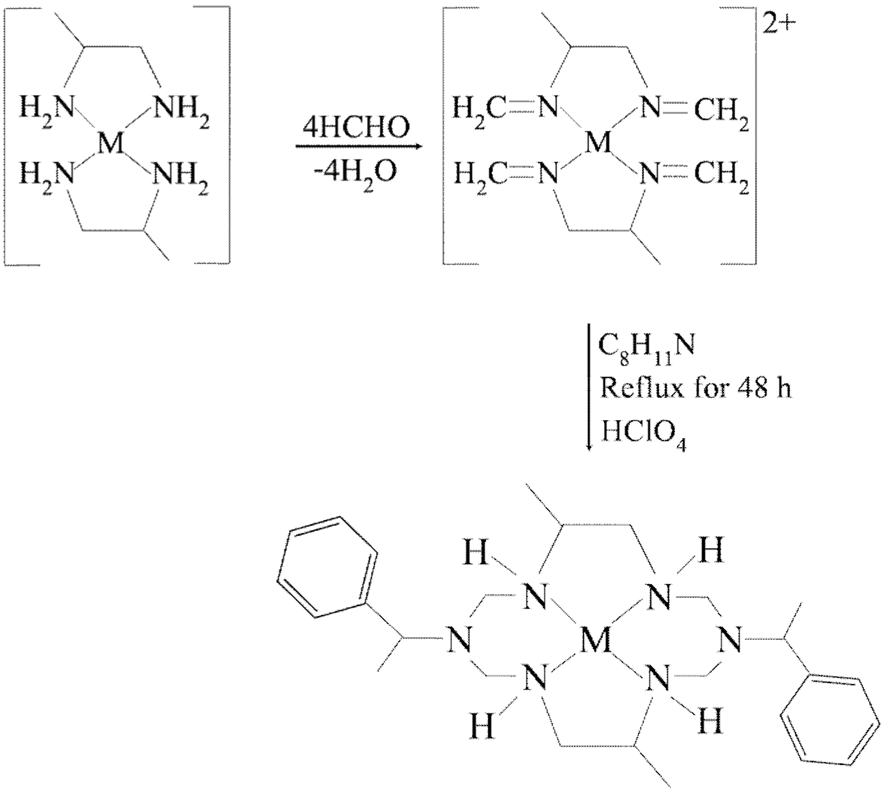
In *vitro* antibacterial assays

The agar diffusion method was followed for antibacterial test. Plates were prepared by pouring 20 mL of Nutrient Agar (NA), and allowed to solidify. These agar plates were inoculated with 0.1 mL of a McFaland standardized bacterial suspension (10^{-5}-10^{-7} cells/mL) and uniformly spread. A 6 mm paper disc was placed on agar plate in the center and the well was filled with a solution of the 1 and 2 compounds at three concentration (25, 50 and 100 mg/10 mL). The diameter of the clear zone observed around the well was measured for each bacterium. After 24 h of incubation at 37 $^{\circ}$C about 24 hours, a well was filled with DMSO solvent to serve as a control and compared with standard drug as tetracycline.

Results and Discussion

The synthesis route of coordination compounds with polyazamacrocyclic ligand

The coordination compounds with polyaza macrocyclic ligand were synthesized in a one – pot reaction involving both nickel acetate tetrahydrate and copper acetate monohydrate, 1,2-diaminopropane, formaldehyde and α-methylbenzylamine as shown in Scheme 1. In the begining, the metal ion (Cu^{2+}or Ni^{2+}) coordinated to 1,2-diaminopropane for the formation of complex then reacted with formaldehyde to form an imine. The imine formation was attacked by the α-methylbenzylamine of 1,2-diaminopropane to obtained a *gem-diamine*, then the condensation of other imines was formed to have the first six-membered ring. Consequently, the imine can be attacked by the α-methylbenzylamine of a free 1,2-diaminopropane in the solution to obtain a *gem-diamine*. Finally, the second six-membered ring was formed through imine condensation and the compound 1 and 2 are obtained and they are similar to previously reported [8].



Scheme 1. Reaction scheme for the formation of compound 1 and 2, where M = Cu (II) and Ni (II)

Compound 1 and 2 are stable in air and insoluble in water but they are soluble in some polar solvents, such as MeCN, DMF and DMSO. The analytical data for compound 1 and 2 with some physical properties are summarized in Table 1.

Table 1. Physical properties and analytical data for compound 1 and 2 containing polyaza macrocyclic ligand.

Compounds	Reaction	Melting point (^{o}C)	Color
$[CuL](ClO_4)_2$ **(1)**	$Cu(CH_3COO)_2$•$H_2O + 2C_3H_{10}N_2 + 4CH_2O + 2C_8H_{11}N$	~ 259	Purple - red
$[NiL](ClO_4)_2$ **(2)**	$Ni(CH_3COO)_2$•$4H_2O + 2C_3H_{10}N_2 + 4CH_2O + 2C_8H_{11}N$	~ 277	Yellow

Infrared spectra

The preliminary identification of compound 1 and 2 has been characterized by IR spectroscopy. The main infrared spectra of both compounds are presented in Table 2. The IR data reveal the spectra of compound 1 and 2 are shown the presence of sharp band in the range 3206-3239 cm^{-1} which are assigned to the coordination of secondary amine stretching mode. The absence of a band in the regions 1720–1740 cm^{-1}, which are characteristic ν(C=O) carbonyl of aldehyde moiety, further confirms occuring condensation. The ν(C-N) and ν(C–H) modes are found in the range 1283-1452 and 2975-2976 cm^{-1}, respectively. In addition, it is apparent that there is considerable broadening and splitting in the region centered around 1100 cm^{-1} with two peaks at 1062 and 1111 cm^{-1} in which these regions are assigned to perchlorate ions. An important feature is the appearance of a new band of peak intensity in the range 467-511 cm^{-1} which is attributable to ν(M-N) is that assigned to aza-nitrogen as coordinate with metal ions. In polyaza macrocyclic ligand, similar spectra of IR were also observed and it was previously reported [9].

Table 2. IR spectra data (cm^{-1}) for the compound 1 and 2.

Compounds	ν(N-H)	ν(C-N)	ν(C–H)	ν(Cl–O)	δ(Cl-O)	δ(aromatic ring)	ν(M-N)
$[CuL](ClO_4)_2$ **(1)**	3209(s)	1283(w)	2975(m)	1111(s)	624(vs)	750(m)	467(w)
$[NiL](ClO_4)_2$ **(2)**	3206(s)	1289(w)	2976(m)	1091(s)	624(vs)	752(m)	511(w)

vs very strong; s strong; m medium; w weak

ESI mass spectra

The electron spray ionization mass spectra of compound 1 and 2 were studied in positive mode and the *m/z* values are shown in Table 3. The most abundant ion observed in the compound 1 and 2 shows an intense signal corresponding to $[\text{M-anion}]^+$at *m/z* 595.3 and *m/z* 600.3, respectively. The proposed formula of compound 1 and 2 were confirmed by ESI mass spectrum. The different fragments of compound 1 and 2 give peaks with various intensities at different *m/z* values. The mass spectrum data of compound 1 and 2 are given in Table 3.

Table 3. Mass spectrum data for compound 1 and 2.

Compounds	Precursor ion	Mass (*m/z*)		Fragment ions (*m/z*)
		Theoretical	Observed	
$[\text{CuL}](\text{ClO}_4)_2$ **(1)**	$[\text{M-ClO}_4]^+$	597.3	595.3	495.3, 362.2, 229.1, 185.1
$[\text{NiL}](\text{ClO}_4)_2$ **(2)**	$[\text{M-ClO}_4]^+$	602.3	600.3	500.3, 367.2, 190.1

Visible absorption data

The electronic absorption data (Figure 1.) of compound 1 (violet-red) and 2 (yellow) measured in dimethyl sulfoxide shows one *d-d* transition band with absorption maxima (λ_{max}) at 516 and 450 nm, respectively and are comparable with those reported for square-planar Cu(II) and Ni(II) complexes [10].

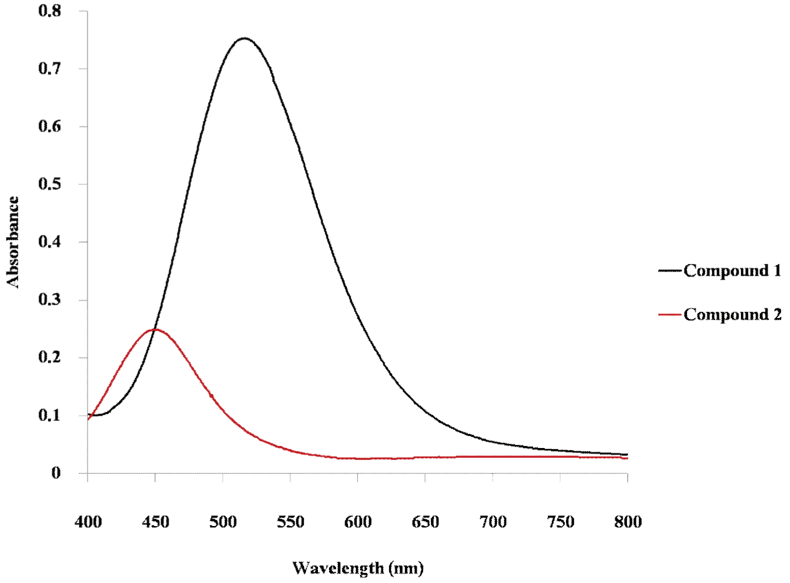


Figure 1. The visible spectra for compound 1 and 2.

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In *vitro* Antibacterial activity

Antibacterial activities of the synthesized compound 1 and 2 were tested by the agar diffusion method using nutrient agar. The compounds were tested against the bacterial, *viz*., gram positive *Staphylococcus aureus* and gram negative *Escherichia coli.* The in *vitro* antibacterial activities are expressed as diameter of clear zones and compared to the tetracycline standard drug. The growth of the colony was recorded on completion of the incubation, and the average diameter for each compound at a concentration of 25, 50 and 100 mg/10 mL was recorded. DMSO and standard tetracycline were used as a control. The average of clear zone values, which was the investigated compounds, is summarized in Table 4 and 5.

Table 4. Antibacterial screening data for compound 1 and 2 as tested against the *Escherichia coli.*

Compounds	Zone of inhibition (mm) ± *SD*.			
	25 mg/10mL	50 mg/10mL	100 mg/10mL	Tetracycline
$[CuL](ClO_4)_2$ **(1)**	19.50 ± 0.58	24.07 ± 0.58	27.00 ± 0.00	30.00 ± 0.00
$[NiL](ClO_4)_2$ **(2)**	18.70 ± 0.58	23.67 ± 0.58	25.00 ± 0.00	30.00 ± 0.00

Table 5. Antibacterial screening data for compound 1 and 2 as tested against the *Staphylococcus aureus.*

Compounds	Zone of inhibition (mm) ± *SD*.			
	25 mg/10mL	50 mg/10mL	100 mg/10mL	Tetracycline
$[CuL](ClO_4)_2$ **(1)**	16.00 ± 0.21	22.12 ± 0.15	25.00 ± 0.40	30.00 ± 0.00
$[NiL](ClO_4)_2$ **(2)**	14.70 ± 0.08	20.67 ± 0.50	22.00 ± 0.00	30.00 ± 0.00

Antibacterial activity of the compound 1 and 2 were against selected bacteria both *Escherichia coli* and *Staphylococcus aureus.* It was found that compound 1 showed better antibacterial against compound 2. They however showed less antibacterial activity than standard drug, tetracycline. According to Tweedy's chelation theory [11] the lipid memberane, which surrounds the cell, favors the passage of only the lipid-soluble materials owing to liposolubility is an important factor that controls the antibacterial activity. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system of a polyaza macrocyclic ligand formed in the period of coordination. This system increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the microorganism thus destroying them more affressively.

Conclusion

In this work, a newly synthesized of coordination compounds containing a polyaza macrocyclic ligand has been synthesized in a one – pot reaction by reflux method. The characterizations of the proposed structures have been studies by spectroscopic technique containing elemental analysis, IR, LC-MS and Visible spectroscopy. The antibacterial activity revealed that the coordination compounds exhibited an inhibitory effect against *Escherichia coli,* gram negative bacteria tested and *Staphylococcus aureus* as gram positive bacteria tested. Compound 1 and 2 showed less antibacterial activity than standard drug.

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