



Synthesis of novel 2-(4-clorophenyl)-4,5-diphenyl-1*H*-imidazole and its cobalt(II), nickel(II), copper(II) and Cu(II) complexes

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Abstract The discovery of *cisplatin*s new metallodrugs shows higher efficiencies and lower side effects. In this work, 2-(4-clorophenyl)-4,5-diphenyl-1*H*-imidazole schiff base ligand and complexes of 2-(4-clorophenyl)-4,5-diphenyl-1*H*-imidazole ligand with Co(II), Ni(II) and Cu(II) ions were synthesized. 2-Dimensional TLC results one node that reveal the ligand is pure. The melting point of the ligand is 197-199°C. Identification the result of 2-(4-clorophenyl)-4,5-diphenyl-1*H*-imidazole is showed by ¹H-NMR spectrometer. All complexes were characterized by analytical techniques and their molecular formulae established. The result showed that Co(II) complex and Ni(II) complex has octahedral geometry and Cu(II) compound has tetrahedral geometry. Their cytotoxic activity against cancer celllines Human Colorectal (HCT 116) was tested. The complexes provide good activity (IC₅₀=17.9 μM for Co(II) complex, 29.6 μM for Ni(II) complex and 16.5 μM for Cu(II) complex.

Keywords Cobalt(II) complex, nickel(II) complex, copper(II) complex, schiff base ligand and 2-(4-clorophenyl)-4,5-diphenyl-1*H*-imidazole, cytotoxic activity.

1. Introduction

Imidazole is an aromatic heterocyclic with “1,3-diazole” and is classified as an alkaloid. The imidazole-ring can be found in the biological blocks such as histidine, histamine. Imidazole and its derivatives are widely used as intermediates in syntheses of organic target compounds including pharmaceuticals like anticancer, anti-aging agents, anticoagulants, anti-inflammatory, antibacterial, antifungal, antiviral, anti-tubercular, anti-diabetic and anti-malaria [1]. This makes imidazole very beneficial for developing new drugs and potential medicinal treatment in next era.

The increasing concern about antibiotic resistance caused by the clinical use of antibiotics or the occurrence and fate of antibiotic residues in the environment has motivated researchers to discover more effective and lower side effect drugs with the potential to extend over the anticancer spectrum and to overcome drug resistance. One promising class of compounds to achieve this goal is metallodrug compounds. The coordination chemistry of metallodrug compounds is attracting considerable interest from chemists and pharmacists, owing to their wide application in many fields, particularly in designing more biologically active drugs. Several metallodrugs have been proven to possess interesting pharmaceutical properties such as antimicrobial, antiviral, and anticancer activity, and have been extensively used as antimicrobial chemotherapeutics. Transition metals with different oxidation states that can bind with some binding states in the organism cells have led to the development of metal-based drugs with a unique therapeutic opportunities. The action of metal complexes on living cells is different from usual organic drugs. This has, for instance, led to clinical applications as chemotherapeutic agents for cancer treatment, such as cisplatin [2].

Complex compounds with different metal ions have different potential as anti-microbial agents and showed selective and specific activities as anti-cancer agents. Cytotoxic assay of complex with ligand S-2-methyl-benzyl-β-N-(di-2-



pyridyl)methylenedithiocarbamat (NNS') [Cu(NNS'), [Zn(NNS')] and [Ni(NNS')] against cancer cell lines MCF-7 is 12, 15 and 5 $\mu\text{g/mL}$ [3].

Therefore, this work aims to synthesize complexes Co(II), Ni(II) and Cu(II) with 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole ligand. The complex has been characterized by several tools of analyses such as elemental analysis, thermal analysis, Atomic Absorption Spectroscopy and FTIR. The complexes were screened for anticancer activity against Human Colorectal (HCT 116) cell lines.

2. Experiments

Chemicals

All chemicals used in this research were analytical grade and were used as purchased from chemical store. 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole chemically 1,2-diphenyletana-1,2-dion ($\text{C}_{14}\text{H}_{10}\text{O}_2$) (benzil) and *p*-chlorobenzaldehyde was obtained from Sigma-Aldrich, and it was used as such without further purification. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, methanol, ammonium acetate, ethyl acetate, glacial acetic acid and NaOH was purchased from Merck.

Instruments

TLC analyses were carried out on silica gel F254 chromaplates with the developing solvent systems. Checking the spots were detected by exposure to UV-lamp at 254 nm or 366 nm. Some instruments needed to identify and characterize included spectrophotometer FTIR 8400S (SHIMADZU). The ^1H NMR Spectra were recorded with a JNM-ECA (500 MHz, DMSO). Microanalyses (C, H and N) were performed using a Perkin Elmer 2400 series CHN elemental analyzer and the metals were performed using Atomic Absorption Spectroscopy. Thermogravimetric analysis (TGA) were conducted using a STAR SW 10.00 thermal analyzer with standard platinum TG pans.

Syntheses of 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole

A mixture of benzyl (10 mmol) and ammonium acetate (0,1 mol) (immediately fused) in glacial acetic acid (25 mL) was stirred at 80-100 $^\circ\text{C}$ for 1 h under nitrogen atmosphere (To prevent incorporation of any atmospheric impurities and moisture). Substituted aldehydes (10 mmol) in glacial acetic acid (5 mL) was added drop-wise over a period of 15-20 min at the same temperature and stirred for another 4 h, the progress of the reaction was monitored by TLC test using ethyl acetate as eluent. After completion of reaction, mixture was allowed to stand at room temperature. The resulting homogenous solution was poured over crushed ice (200 g). The yellow precipitate was collected by filtration and washed with cold water and dried under vacuum. Product was recrystallized from ethyl acetate thrice to afford the pure compound.

Syntheses of Metal Complexes

Metal complexes of Co(II), Ni(II) and Cu(II) ions with 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole were prepared by refluxing the 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole ligand (2 mmol) and NaOH (1 mmol) with each metal chloride salt (1 mmol) in methanol on a hotplate for 24 h at ~ 70 $^\circ\text{C}$. The mixtures were left undisturbed overnight until the solid products completely precipitated. The colored solid precipitates were isolated. The products were then collected and dried in vacuum.

3. Result and Discussion

FTIR of 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole

2-(4-chlorophenyl)-4,5-diphenyl-1*H*-Imidazole Ligand have special characteristics as compared with the initial precursor is benzyl. Imidazole structure with three aromatic rings, which is reinforced by the presence of aromatic C=C bonds phenyl at wave number 1485.24 cm^{-1} [1], the C=N bond of the imidazole in the area around 1600.97 cm^{-1} [3]. Synthesis of 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-Imidazole ligand have C-Cl functional group with identical absorption peak appears on the uptake 1128.39 and 1091.75 cm^{-1} . The structure of the ligand 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-Imidazole at wave number 3028.34 cm^{-1} are CH sp^2 which explains their aromatic substituent derived from phenyl. Wave number 1327.07 cm^{-1} identifies the group C-N [4]. Wave number 1437.02 cm^{-1} as



bending double NH stretching vibration of the C=C and C=N [5]. N-H group (secondary amines) which neighbors aromatic often do not appear in the IR spectra, because secondary amines with aromatic bending experience thus appear very small absorption intensity and the energy produced below 2000 cm^{-1} . Cl substituted benzene the finger print area lies in wave numbers from 769.62 to 835.21 cm^{-1} . Spectrum ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole is shown in **Figure 1**.

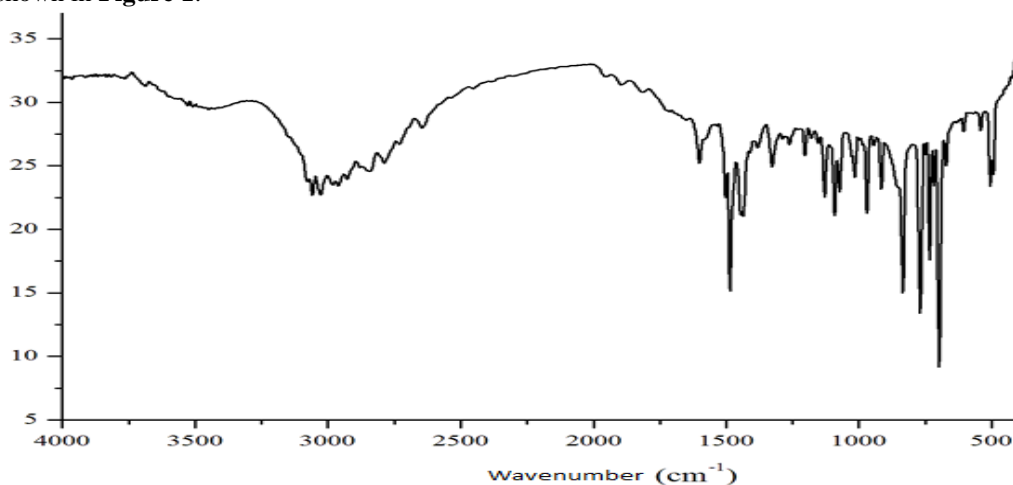


Figure 1: FTIR Spectrum of Ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole

^1H NMR of 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole

^1H NMR analysis supports the FTIR spectra in reading the structure of the ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole. ^1H NMR spectrum ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole is shown in **Figure 2**. ^1H NMR analysis carried out by counting the number of protons in the spectrum matched to the number of protons in the structure of the ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole. In the analysis showed the presence of three signals that indicate the presence of three types of protons. The number of protons were identified from the total signal integration is as much as 15 protons. Proton on a chemical shift of 7.28 to 7.55 ppm and multiplicity multiplet showed proton of the phenyl group binding two imidazole. Proton area shifts from 8.09 to 8.10 ppm with integrity of 2H aromatic protons that are an environment chloro substituent on the phenyl group, so that it is located more downfield. Singlet signal chemical-shift in 12.78 ppm a proton belonging N-H group.

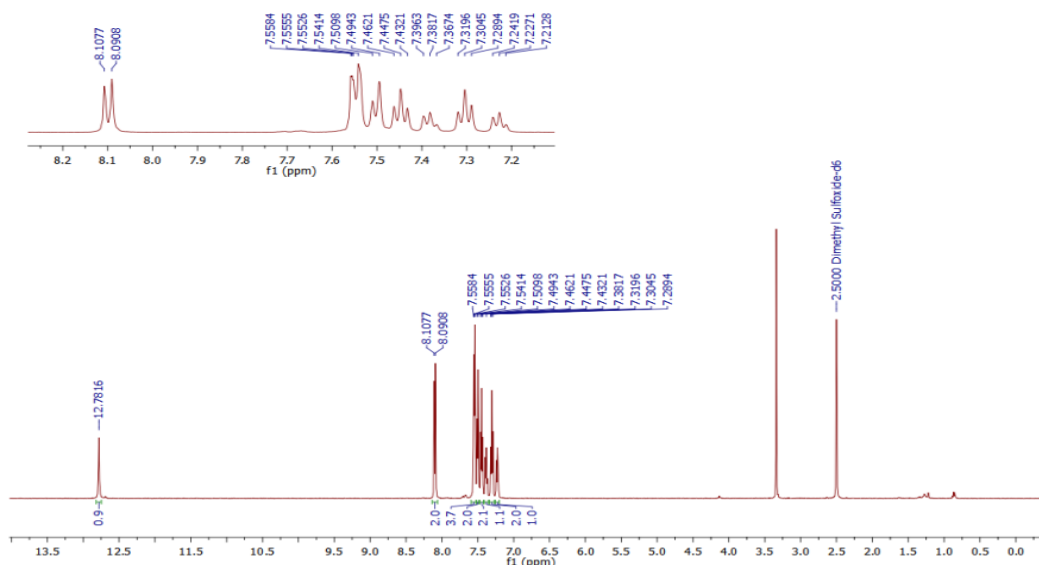


Figure 2: Spectrum ^1H NMR of Ligand 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole

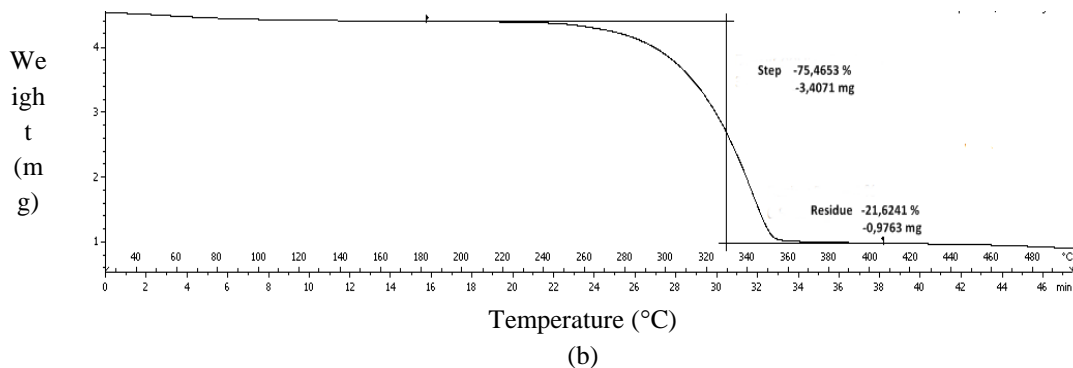
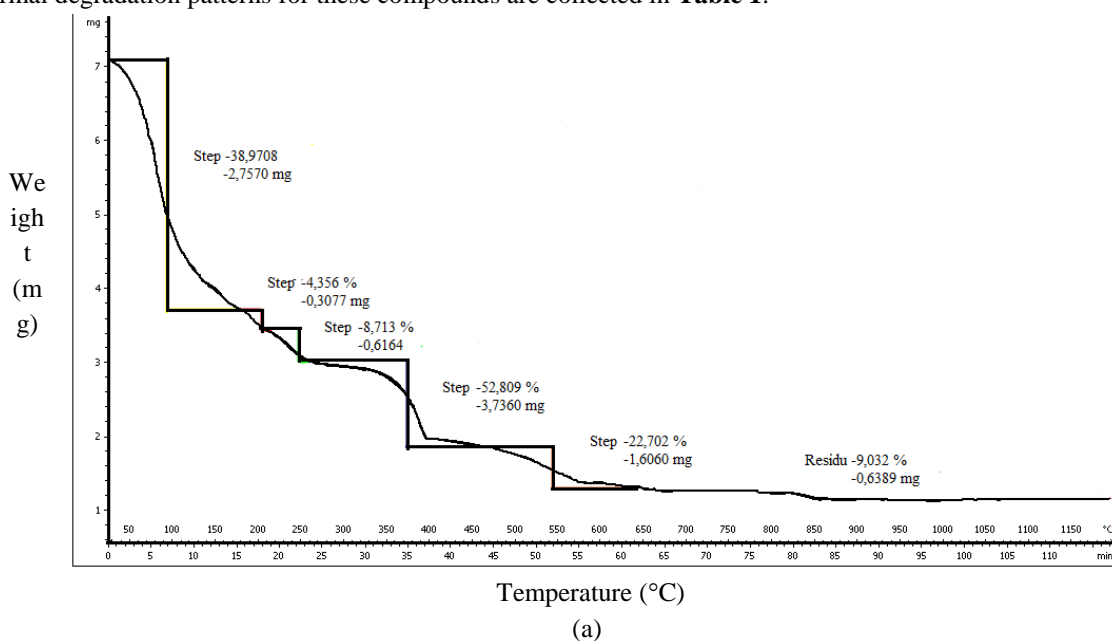


FTIR of Co(II), Ni(II) and Cu(II) Complexes

The reaction of ligand with the metal ions were conducted in basic media solution (NaOH in MeOH), so the ligand molecule was deprotonated at the N-H position. The bands observed between approximately 3390 and 3360 cm^{-1} are assigned to the $\nu(\text{O-H})$ stretching vibrations of the coordinated or uncoordinated water molecules. The weak to very weak bands observe in the range 586-499 cm^{-1} could be assigned to the stretching vibration of the $\nu(\text{M-N})$ band. The bands observed at approximately 450 cm^{-1} of the complexes could be assigned to the $\nu(\text{M-O})$ vibration. The IR spectrum observed at 362 and 310 cm^{-1} in the spectrum of the complexes are assigned to the $\nu(\text{M-Cl})$ vibration.

Thermal characteristics

Complexes provided several observations. The Co(II), Ni(II) and Cu(II) complexes were stable up to 20 until 600 $^{\circ}\text{C}$, respectively. The observed weight losses in each decomposition step were in agreement with the calculated weight losses. The complexes underwent several decomposition steps resulting in metal oxides (Co-O, Ni-O and Cu-O) as the final decomposition products. Their representative thermograms are illustrated in **Figure 3**. The possible thermal degradation patterns for these compounds are collected in **Table 1**.



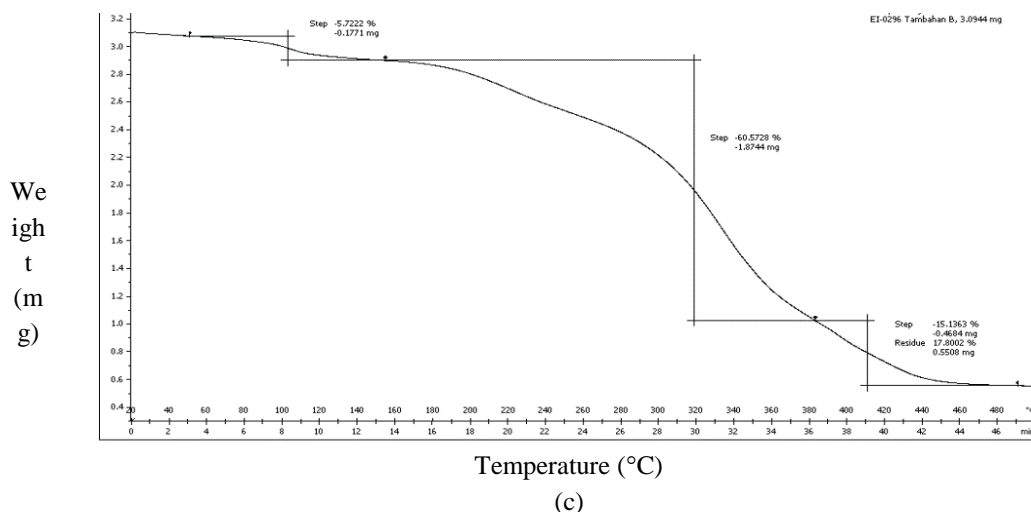


Figure 3: TGA thermogram (a) Complex Co(II) (b) Complex Ni(II) (c) Complex Cu(II)

Table 1: Thermal decomposition data for the complexes Co(II), Ni(II) and Cu(II)

Compound	TG range (°C)	TG % mass loss	Lost species
Complex Co(II)	50-120	38,97	Water
	160,75-180,71	4,356	Crystal water (2H ₂ O)
	216,15-263,18	6,778	Ligand water (4H ₂ O)
	356,36-401,13	52,809	4,5-Diphenyl-1 <i>H</i> -Imidazole
	448,16-578,87	22,702	2-(4-Clorophenyl)
	>600	9,032	Co-O
Complex Ni(II)	270-380	75,46	Water ligand (4H ₂ O) and 2-(4-Clorophenyl)-4,5-Diphenyl-1 <i>H</i> -Imidazole
	>400	21,26	Ni-O
Complex Cu(II)	20-120	5,722	Water
	120-380	60,573	Water crystal (2H ₂ O), water Ligand (2H ₂ O) and 2-(4-Clorophenyl)
	380-480	15,136	4,5-Diphenyl-1 <i>H</i> -Imidazole
	>400	17,805	Cu-O

C,H,N Elemental Analyzer and Atomic Absorption Spectroscopy

Elemental analysis data of the complexes and supported by Atomic Absorption Spectroscopy considered in this study is summarized in **Table 2**. As a result of the good agreement between experimental and calculated data can be hypothesized.



Table 2: Result of elemental analysis complexes

Complex	% C		% H		% N		% Metal	
	Found	Calculated	Found	Calculated	Found	Calculated	Found	Calculated
Co(II)	61.04	61.03	5.17	5.12	6.78	6.78	7.15	7.13
Ni(II)	63.83	63.62	4.75	4.79	7.09	7.06	7.46	7.41
Cu(II)	27.61	28.97	4.25	4.36	3.20	3.21	7.52	7.50

L = 2-(4-Chlorophenyl)-4,5-Diphenyl-1*H*-Imidazole

Cytotoxicity Studies

Anticancer activity of selected compound was assessed against HCT 116 colorectal carcinoma using a standard 48 hours MTT cytotoxicity assay. The results are summarized in **Table 3**.

Table 3: Cytotoxicity activity of complexes Co(II), Ni(II) and Cu(II) against HCT 116

Compounds	IC ₅₀ HCT 116 (μM)
Complex Co(II)	17.9 ± 0.9
Complex Ni(II)	29.6 ± 1,3
Complex Cu(II)	16.5 ± 0.7

Co(II) complex displayed apparently higher citotoxicity than Ni(II) complex. However, Cu(II) complex more cytotoxic than Co(II) HCT 116 cell.

4. Conclusion

The aims of this work was to understand the coordination behavior and biological properties of the metal ions Co(II), Ni(II) and Cu(II). The metal complexes were prepared and isolated and their structure were established based on a range of physicochemical methods, such the electronic and vibrational spectroscopies, as well as elemental and thermal analyses. The anticancer activity of the complexes was assessed against good activity of HCT 116 cell.

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