Vol. 12(27), pp. 421-426, 8 October, 2018
DOI: 10.5897/AJPP2018.4927
Article Number: 6D47B4B59059
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Synthesis, characterization, and anticancer activity against human breast cancer cell-line T47D studies of metal ion Cu(II) complex with 2,4,5-triphenylimidazole ligand

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Received 4 April, 2018; Accepted 8 October, 2018

The complex of metal ion Cu(II) with the ligand 2,4,5-trifenilimidazol has been successfully synthesized with mole ratio of metal and ligand 1:2 in N,N-dimethylformamide as a solvent. Complex synthesis results obtained green light crystalline solid. Complex absorbs UV-Vis light at 529 nm. Fourier transform infrared (FTIR) characterization results indicate occurrence of bonding of metals and ligand that is Cu-N in region 422.38 cm^{-1}. Results of elemental analyzer and Atomic Absorption Spectroscopy (AAS) analysis show the complex formed has the formula $[Cu(L)_2(H_2O)_2].Cl_2$. The molecular formula is also supported by the Thermal Gravimetric Analyzer data. Thermal Gravimetric Analyzer (TGA) analysis results showed that there was no water in the crystalline complex compounds. The cytotoxicity test complex compounds made by the method of 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromide (MTT) and the IC_{50} value of complex obtained 72.139 µg/ml.

Key words: Copper(II), 2,4,5-trifenilimidazol, complex compound, characterization, anticancer.

INTRODUCTION

Complex compounds continue to be developed into useful tools in the field of medicine. Some synthesized compounds exhibit good activity in the field of medicinal chemistry. Cisplatin has been proven as a very effective chemotherapy agent to treat various types of cancer (Reedijk and Lohman, 1985). However, these platinum-based complex compounds cause side effects at certain doses and provide drug resistance during the therapeutic process (Renny et al., 2013). This led to the development of the discovery of new non-platinum-based complex

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compounds, in the hope of improving pharmacological properties, reducing side-effects, and obtaining different drug-specific targets (Qiao et al., 2011). Some of the transition metals used in the synthesis of anticancer complex compounds include Co(II), Ni(II), Cu(II), Pd(II), Ru(II) and Pt(II) (Budzisz et al., 2009; Ali et al., 2013).

Copper(II) metal is an essential element and plays an important role in the biological system of the human body, as a constituent of redox and hemocyanin enzymes (Linder and Maryam, 1996). As previously reported, complexes synthesized from Cu(II) metal ions with 2-(4'-thiazolyl)benzimidazole and 2-(2-pyridyl)benzimidazole ligands have a cytotoxicity to liver cancer cells (hepatocellular carcinoma) (Devereux et al., 2007).

Ligands commonly used and continuously developed in studies of anticancer drug compounds have bound atoms of nitrogen and oxygen atoms, including derivatives of imidazole, benzamide, pyridine, and pyrazole (Goncalves et al., 2013; El Boraey, 2012; Tiwari et al., 2011; Budzisz et al., 2009). N-containing aromatic ligands such as pyridine, imidazole, and their derivatives (which are as electron donors similar to purine and pyrimidine bases) have been reported to possess *in vitro* anticancer properties such as Cisplatin (Deegan et al., 2006). The imidazole-based complex compounds showed anticancer activity against SK-MEL-31 skin cancer cells and tongue cancer cells CAL-27. The imidazole-based compounds also show cytotoxic effects on HepG2 liver cancer cells and A-498 bowel cancer, MCF-7 breast cancer, cervical cancer HeLa, and HL-60 blood cancers (Devereux et al., 2004; Bhat et al., 2011). Therefore, in this study, synthesized complex compounds of Cu (II) metal ion with 2,4,5-trifenylimidazole ligand and tested anticancer activity by MTT method *in vitro* assay against breast cancer cell T74D.

MATERIALS AND METHODS

The materials used in this study were copper(II)chloride dihydrate ($CuCl_2.2H_2O$) (Merck 99.0%), N,N-dimethylformamide (DMF) (Merck 99.8%), dimethyl sulfoxide (DMSO) (Merck 99.8%), 2,4,5-triphenylimidazole (Sigma-Aldrich 90%), methanol (Sigma-Aldrich 98%), breast cancer cell T74D (CVCL_0553), RPMI 1640 Medium (Gibco), Phospate-buffered saline 1X (PBS 1X) (Gibco), and Thiazoyl blue tetrazolium bromide (MTT) (Bio Basic).

Determination of maximum wavelength of $[Cu(L)_2(H_2O)_2].Cl_2$

The wavelength of the Cu(II) complex compound with 2,4,5-trifenyl imidazole was determined by continuous variation method. Continuous variation begins with a 1 mole molecular synthesis of $CuCl_2.2H_2O$ and 2,4,5-triphenylimidazole, 1 mole with a volume ratio of 0:10, 1:9, 3:7, 5:5, 7:3, 9:1, and 10:1. Each of these solutions is in DMF and heated 3 h at 120°C. The formed solid is decanted and dried. The solid was dissolved in DMSO and measured the maximum wavelength (λ_{max}) with a UV-Vis spectrophotometer, then graphed between absorbance as ordinate and mole fraction of metal as abscess.

Synthesis and characterization of $[Cu(L)_2(H_2O)_2].Cl_2$

The synthesis of these complex compounds was performed by the mole of metal and ligand 1:2. Watched the copper(II)chloride dihydrate and 2,4,5-triphenylimidazole in DMF.[15] (Han et al., 2012). The complex solution was put into a vial, distilled for 30 min and heated at 120°C for 3 h. The mixture was then cooled to room temperature in vial covered with aluminium foil which has been given several small holes and left for 7 days to form solids and every day was washed with methanol to remove impurities contained in the mixture. The formed solid is decanted and dried. Subsequently, was characterized by UV-Vis Spectrophotometer, FTIR Spectroscopy, Atomic Absorption Spectroscopy (AAS), Thermal Gravimetric Analyzer (TGA), and CHN analyzer.

Anticancer activity

The breast cancer cell T74D with a density of 5×10^3 cells/well was distributed into 96 wells plate, incubated for 24 h at a 37°C CO_2 to attach. The medium was then replaced with fresh complete medium containing DMSO 0.1% (control), compound complex at concentration of 50, 25, 12.5, 6.25, 3.13, and 1.56 µg/ml, and incubated for 20 h (37°C/CO_2). Then into each well was added 100 µl RPMI containing MTT reagent and the plates incubated for an additional 4 h. Living cells react with MTT to form formazan crystals (Mosmann, 1983). After 4 h, the medium containing MTT was discarded and then added 50 µl DMSO solutions to dissolve the formazan crystals, homogenized on top of shaker for 10 min, then read with Microplate reader at wavelength 595 nm.

RESULTS AND DISCUSSION

Maximum wavelength of $[Cu(L)_2(H_2O)_2].Cl_2$

The solids of $CuCl_2.2H_2O$ and 2,4,5-triphenylimidazole with a 1:2 mole ratio reacted with DMF solution and heated for 3 h at 120°C. The complexes obtained are light green solids (72.127% of yield) and maximum wavelength complex with UV-VIS Spectrophotometer at 529 nm as shown in Figure 1. The result of 10x magnification photograph shows that the obtained solid is in the form of a needle as shown in Figure 2.

Analysis of functional groups with FTIR Spectroscopy

Characterization using FTIR spectroscopy was used to determine the presence of functional groups in complex and new bonds formed between metal and ligand. Comparison of FTIR spectrum of ligand and complex is as shown in Figure 3.

Based on the obtained data, the appearance of a new peak at a wavelength 422.38 cm^{-1} can be seen. The peak is observed as a vibration of metals and ligands. This is in line with previous research showing that new peaks of

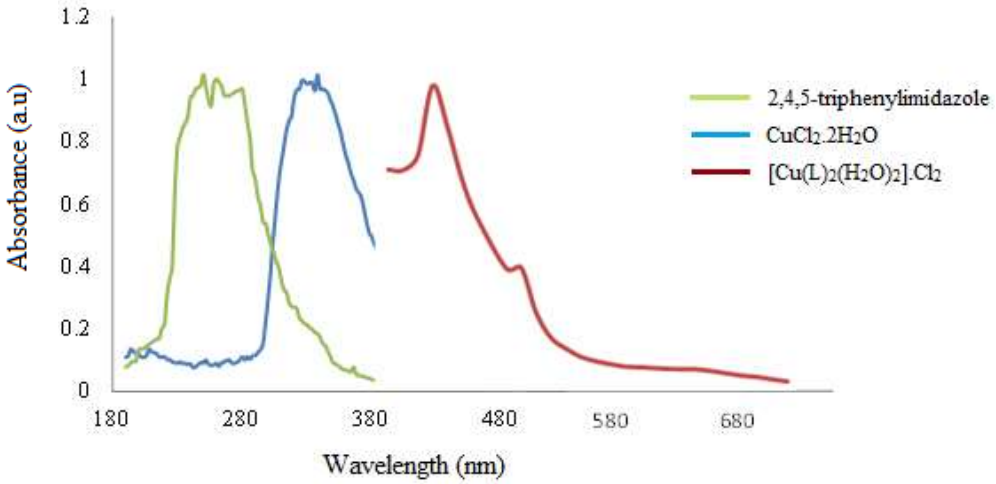

Figure 1. Maximum wavelength spectrum of $CuCl_2.2H_2O$ (blue light), $[Cu(L)_2(H_2O)_2].Cl_2$ (red), and 2,4,5-triphenylimidazole ligand (green light). There was a maximum wavelength shift from the 2,4,5-triphenylimidazole ligand to the $[Cu(L)_2(H_2O)_2].Cl_2$ complex of 243 nm to 529 nm, and the maximum wavelength of $CuCl_2.2H_2O$ at 338 nm. This shows that the complex $[Cu(L)_2(H_2O)_2].Cl_2$ has been formed.

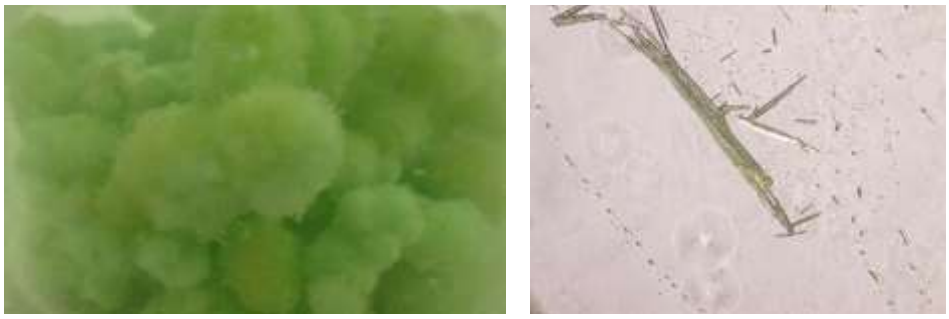

Figure 2. The compounds of $[Cu(L)_2(H_2O)_2].Cl_2$ (right); 10x magnification (left).

complexes (metal bonds and ligands (Cu-N)) appear on a wavelength of 453 cm^{-1}, whilst Cu metal bonds with H_2O ligands appear at the of 534.25 cm^{-1} wavelength indicating the presence of Cu-O (Gomathi and Murugan, 2014).

This indicates that the N-H bond detected is a tertiary N-H. While the complexes appear to peak with weak intensity in the area of 3400.27 cm^{-1}; this peak is a characteristic of secondary amines located in the 2,4,5-trifenylimidazole ligand (Marzouk et al., 2013). The formation of secondary amine peaks is due to the synthesized complex structure [Cu(II)-2,4,5-triphenylimidazole] possessing two moles of ligand, so that the intensity of the secondary NH bond is stronger and therefore it can be detected even when the intensity is weak. In the spectra of complex, located in an area above 3000 cm^{-1}, it is slightly larger than the ligand because of the presence of a coordinated O-H bond in the water as a ligand (Ullah et al., 2016). The uptake that appears at 1600 to 1700 cm^{-1} is a C=N vibration in a 2,4,5-triphenylimidazole ligand coordinate with Cu metal ions.

Elementals analysis

Determination of the molecular formula of the complex can be obtained through the theoretical calculation approach of the composition of the formation of the complex. In this case, a complex formed of one metal and two ligands with the formula molecule $[(H_2O)_xCu-(L)_2(Cl)_y].Cl_y$ can be seen. The addition of Cl outside the complex serves to neutralize the complex charge, so the amount of Cl will adjust to its charge. The theoretical

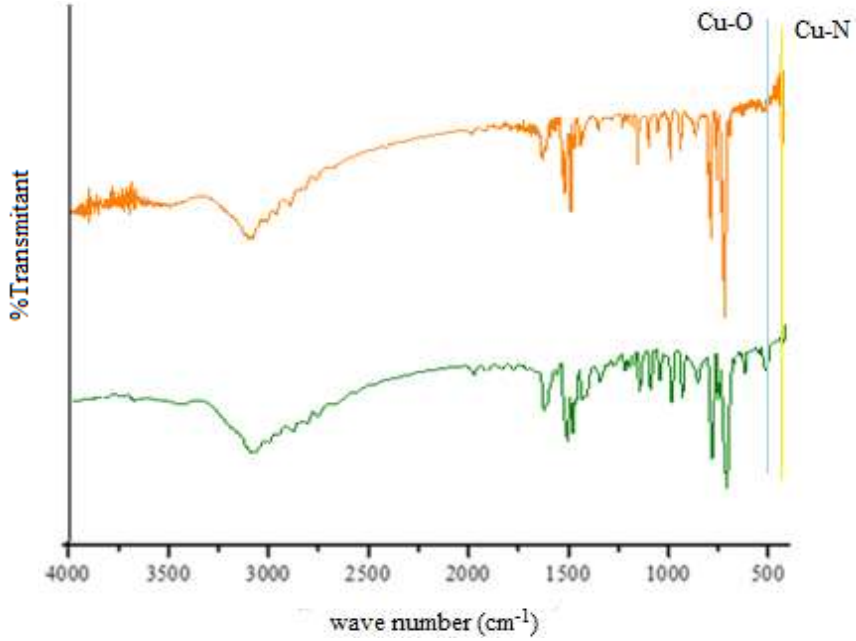

Figure 3. Comparison of FTIR spectrum of 2,4,5-triphenylimidazole ligand (orange) and complex compound $[Cu(L)_2(H_2O)_2].Cl_2$ (green).

approach is done by calculating the percentages of Cu, C, H, and N on the complex. Theoretically, the complex formed has the formula $[Cu(L)_2(H_2O)_2].Cl_2$ where two ligands bind one Cu metal according to continuous variation.

The result of the analysis shows that the synthesized complex contains Cu elements of 8.334%, C element of 66.2751%, H element 4.7284% and N element equal to 7.3861%. Based on the data, the percentage level of each element in theory from some complex structures that may be formed was calculated. The result of the calculation of elemental content theoretically approaching experimental measurement result is the prediction result of molecule formula of complex compound. Matches show results appropriate for the complex formula $[Cu(L)_2(H_2O)_2].Cl_2$. These results indicate that the 2,4,5-triphenylimidazole ligand only binds one Cu. The presence of a steric hindrance causes the 2,4,5-triphenyilimidazole ligand to be very difficult to bind metals and can only bind one metal. This shows that H bound to N is not all replaced by Cu.

Thermal gravimetric analysis (TGA)

In the determination of complex molecular formulas, thermo gravimeters can provide specific information of a complex that decompose when it is heated. The complex thermo gravimetric analysis of Cu(II)-2,4,5-triphenylimidazole was carried out at 25 to 600°C with a complex sample weight of 6.3670 mg. Based on the complex TGA curve of Figure 4, it can be seen that there is one stage of decomposition in the complex. Weight loss of 86.8791% occurring at 255.33 to 355.83°C indicates a complex decomposition consisting of 2 ligand molecules 2,4,5-triphenylimidazole, 2 molecules H_2O and 1 molecule Cl_2. This result corresponds to the theoretical weight that in weight reduction of 86.8791% is decomposition $((C_{21}H_{16}N_2)_2(H_2O)_2Cl_2)$. The residue of 13.1209% (theoretically 8.333%) can be predicted as Cu, as in the previous study that CuO is the final residue of the complex [Cu(6-hydroxypicolinate)$_2$(3-picolinate)$_2$] (Kukovec et al., 2012). Weight loss does not occur at temperatures of 75 to 147°C. This means the complex does not contain crystalline water (Tamaekong et al., 2014).

Anticancer activity

In this research, cytotoxicity test was done by MTT method using T74D breast cancer cell. This test is used to determine the cytotoxic effect of tested compound. The classification of toxicity level of the extract based on IC_{50}, which is very high category (highly toxic) if it can kill 50% of cells at concentrations of 1 to 10 µg/ml, medium category (medium toxic) at concentrations of 10 to 100 µg/ml, and low toxic concentration of 100 to 1000 µg/ml

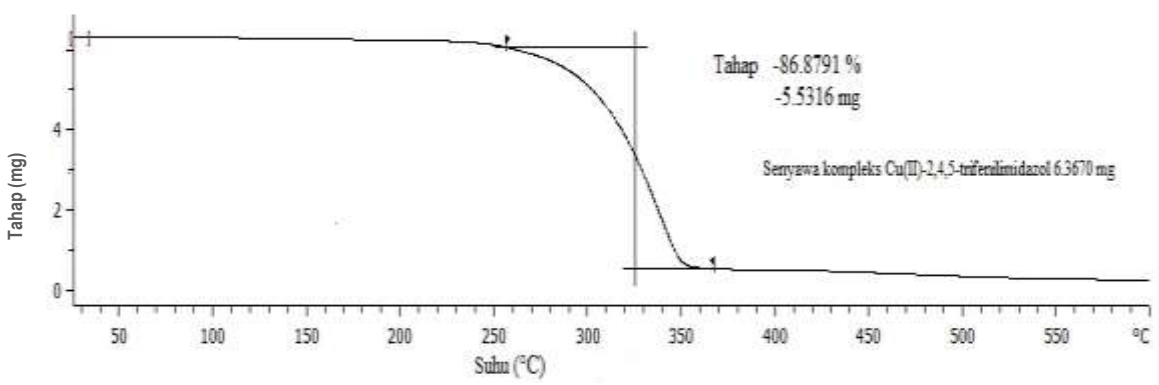

Figure 4. Thermal gravimetric analysis curve of $[Cu(L)_2(H_2O)_2].Cl_2$.

(Meyer et al., 1982). Based on the calculation, IC_{50} value for complex $[Cu(L)_2(H_2O)_2].Cl_2$ was obtained at 72.139 µg/ml. Based on the IC_{50}, the $[Cu(L)_2(H_2O)_2].Cl_2$ complex belongs to the category of medium toxicity compound (medium toxic). This is in consistent with the previous research in which the CuL complex (L = 3-(1,3-dioxoisoindolin-2-yl)-2,6-dioxopiperidine-1-carbodithioate) kills more cancer cells than its free ligands (Ali et al., 2013). The result of IC_{50} of $[Cu(L)_2(H_2O)_2].Cl_2$ complex in this study was greater than in previous study, where the MTT assay was performed on the complex $[Cu(TBZH)_2(BZA)]$-(BZA).0.5TBZH.H_2O wherein the TBZH ligand was 2-(4'-thiazole)benzimidazole and the BZA ligand was benzoic acid, IC_{50} of 32 µM (Devereux et al., 2007).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by the Institute of Tropical Disease (ITD), the Center of Excellence (COE) program by the Ministry of Research and Technology (RISTEK) Indonesia, Stem Cell Research and Development Center Universitas Airlangga, and Environment Agency of East Java Province.

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