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Research Article

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Synthesis, Characterization and Anticancer Studies of Cu(II) Isoleucine Dithiocarbamate Complexes

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ABSTRACT

Complex metal compounds such as copper (Cu) have not been studied as alternative anticancer drugs from cisplatin. In addition, the use of ligands from complex compounds such as ditiokarbamat which has the potential as a metal poisoning drug. The combination of Cu essential metal with ditiokarbamat ligand was synthesized, namely Cu(II) Isoleucine Dithiocarbamate which was then characterized using Infra Red (IR) and Ultraviolet-Visible (UV-Vis) spectroscopy, melting point and conductivity. The anticancer activity of complex compounds was determined by in vitro using MCF-7 breast cancer cells. The test results showed that the complex (IC_{50}=98.17 µg/mL) had significant anticancer activity comparable to cisplatin (IC_{50}=50 µg/mL).

Keywords: Cisplatin; Complexes; Isoleucine; Dithiocarbamate; MCF-7.

INTRODUCTION

Since its discovery 5 decades ago, Cisplatin has shown its success in treating various types of cancer even used in the treatment of about 70% of all cancer patients [1]. Although cisplatin can be said to be the most successful anticancer drug in the world [2-5], cisplatin and the clinical value of platinum-based drugs have non-negligible side effects such as high toxicity to the human body, drug selectivity and intrinsic resistance [6-8].

One cancer cell such as MCF-7 (breast cancer) has so far been treated using cispaltin because the half-maximal inhibitory concentration (IC_{50}) is 13-36 µM [9]. This is very worrying because the highest cancer data in the world for women is breast cancer (38 per 100,000 women) [10]. Indonesia, including from 7 countries, namely Japan, Malaysia, the Philippines, Singapore, Sri Lanka and Taiwan that have breast cancer fall into the category of deadly cancer [11]. Therefore, the search for alternative anticancer drugs especially breast cancer without side effects is very necessary.

To overcome this shortcoming, many researchers use new metal compounds as potential anticancer agents [12]. *In vitro* and *in vivo* evaluations of some metal compounds have reached clinical trials but reducing side effects and targeting the benefits of drugs are still challenging [13]. There are several metal compounds that have benefits for the body and are needed by the body, namely essential metals. Many studies have also reported the use of essential metals such as anticancer, simple ferronicum salts or iron that have the potential to inhibit cancer cell growth [14]. One of the essential metals is copper (Cu) where the complexes of Co(II), Ni(II), Cu(II) and Zn(II) have potential as anticancer drugs [15]. Based on its oxidative activity and properties, Cu is an important element for human physiological functions so Cu may be promising as a metal-based anti-cancer compound [16,17].

Besides metals, using ligands also greatly affects the increase in activity. Potential anti-cancer agents can also be seen from ligands that are active in their biological processes [18]. Dithiocarbamate compounds have many uses depending on the chelating properties of ligands on metal ion types [19]. Potentials generated in the health sector can be used for metal poisoning drugs based on their ability as good chelating agents [20]. Can also be a radio chemotherapy target agent in tumors [21].

Ditiokarbamat compound has an S group so that it can donate electrons in monodentate or bidentate, so to make the metal complex can be used transition metals [22]. The diiocarbamate complex has been synthesized using main metal elements and transition metal elements as its central atom [23]. Ditiokarbamat ligands if used for additional donor groups, such as oxygen and nitrogen groups such as amines (Isoleucine), can increase the diversity of structures and affect the biological activity of complex compounds [24].

At present, no one has examined the diciocarbamate compound as a ligand of Cu(II) essential metal complex compounds which is expected to be one of the solutions for anticancer drugs. Thus to confirm our hypothesis about anticancer treatment especially MCF-7 cancer cells, a study using Cu(II) complex compounds with a combination of Isoleusinditiokarbamat ligands is expected to produce anti-cancer drugs that have high toxicity against cancer cells but suppress the side effects caused.

MATERIALS AND METHODS

Materials

Copper(II) sulfate, Ethanol (95%) methanol (95%), Acetone (95%), n-hexane (95%), Acetonitrile (95%), Carbon disulfide, Isoleucine, Cisplatin, Roswell Park Memorial Institute Medium, and DMSO.

Instruments

Magnetic stirer, Electrotermal 9100 melting point, conductometer, UV-Vis spectrometer, SHIMOZU Fourier Transform Infrared spectrometer and a set of cancer cell test kits (Biosafety Cabinet (BSC), Centrifuge), CO_2 Incubator, Microscope, and Multimode Reader), spray bottles and glass tools commonly used in laboratories.

Isoleucine Dithiocarbamate Ligan Synthesis

The synthesis of isoleucine dithiocarbamate ligand was carried out "*in situ*" by weighing 0.6559 gram (5 mmol) isoleucine which was then dissolved in 10 mL ethanol plus a CS_2 solution of 0.3 mL (5 mmol) slowly at a temperature 10°C.

Sintesis Cu(II) Dengan Ligan Isoleucine Dithiocarbamate

The synthesis of Cu(II) with isoleucine dithiocarbamate ligand was carried out by dissolving 0,7339 gram (3 mmol) $CuSO_4$ with 10 mL ethanol. The solution was added with an isoleucinedithiocarbamate ligand and stirred for 30 minutes. The resulting precipitate is filtered and dried with a desiccator. After drying, crystallization is carried out with the appropriate solvent. Crystals were then analyzed and characterized.

Complex Characterization

The electronic spectra were obtained using Jenwey UV-Vis spectrophotometer 200-1100 nm and infrared spectra performed using the Infra red SHIMADZU spectrophotometer, in the frequency range 4000-300 cm^{-1}. The melting point is measured by Electrothermal IA 9100, and conductivity is measured by a conductometer.

Cytotoxic Test on Breast Cancer Cells (MCF-7)

MCF-7 cell cultures were transferred into 96 well plates, and then incubated at 37°C and 5% CO_2 gas until the cell growth percentage reached 70%. The cells were then treated with dithiocarbamate complex and then incubated (for 24 hours at 37°C and 5% CO_2 gas). To facilitate the absorbance reading, presto blue reagents are added to the cell. Absorbance is measured using Multimode Reader.

RESULTS AND DISCUSSION

The synthesis of Cu(II) isoleucine dithiocarbamate complex was 57.28% with a melting point obtained 230°C-232°C and a conductivity value of 0,07 mS/cm.

UV-Vis Characterization

Characterization of electronic spectra results obtained using the Jenwey UV-Vis spectrophotometer 200-1100 nm can be seen in Table 1.

Table 1. UV-Vis data of Cu(II) isoleucine dithiocarbamate (IsoleuDtc=Isoleusindithiocarbamate)

Compund	λ maksimum (nm)	Electronic Transition
Cu(II)IsoleuDtc	268	$\pi \rightarrow \pi^*$
	353	$n \rightarrow \pi^*$

Characterization with UV-Vis in water solvents for Cu(II) isoleusinditiokarbamat complex compounds obtained by CS_2 group results from intraligan transitions $\pi \rightarrow \pi^*$ at 268 nm wavelength absorption which is shown in band I and in the absorption area of 250-300 nm group R against Nitrogen atoms undergo the effect of hyperconjugation [25,26]. The shift in band II shows the intraligan transition $n \rightarrow \pi^*$ for group N=C=S at a wavelength of 353 nm for complex compounds. The graph of UV-Vis results can be seen in Figure 1.

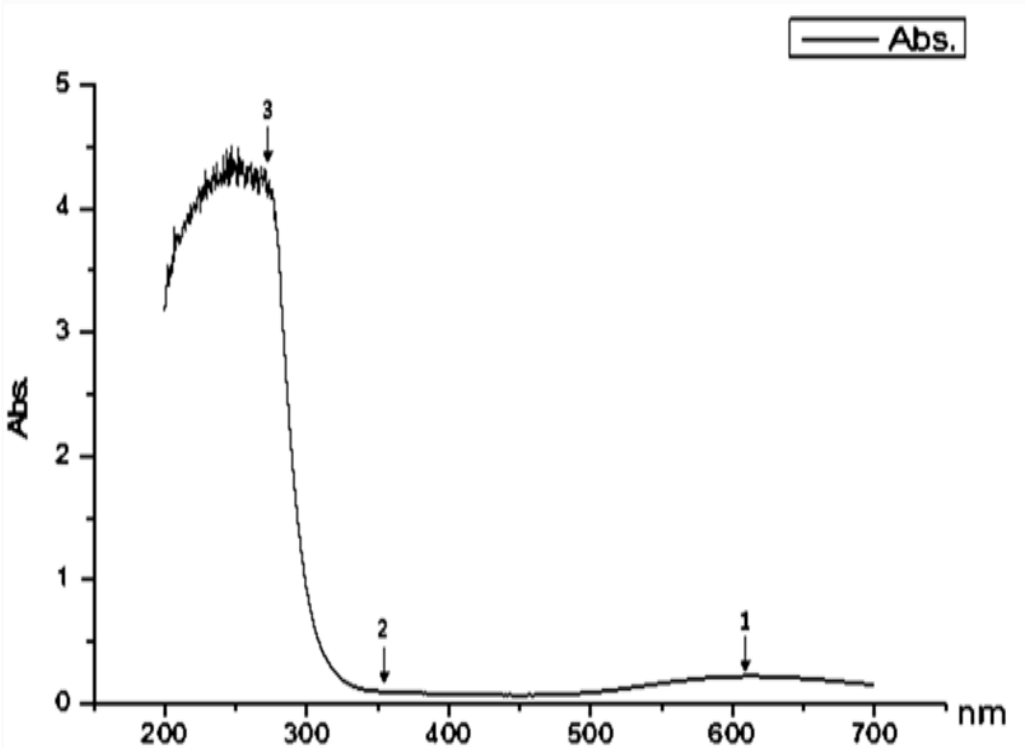

Figure 1. UV-Vis spectrum of Cu(II)isoleucine dithiocarbamate

Infrared characterization of spectra using Infrared SHIMADZU spectrophotometer, in the frequency of 4000-300 cm^{-1} can be seen in Table 2.

Table 2. Data on IR absorption complex compounds with isoleucine dithiocarbamate ligands (s=strong; m=medium; w=weak)

Compound	ν(C=N)	ν(C=S)	ν(M-S)	ν(M-O)	ν(M-N)
Cu(II)IsoleuDtc	1662 s	1105 m	393 w	474 w	592 m

Identification of dithiocarbamate compounds can be seen from infrared peak absorption, namely the existence of two main types of bonds C=N and C=S [27]. There are two types of coordination that have absorption peaks ν (C-S), namely monodentate and bidentate. The type of bidentate coordination is seen at single absorption peaks ν (C-S) while monodentat coordination is seen at double absorption peaks [28].

The dithiocarbamate complex compound has a ν (C=N) bond obtained from ν uptake (C-N) which lies in the wave number between single bonds (1350-1250) cm^{-1} and double bonds (1690-1640) cm^{-1}. Whereas for C-S uptake lies in the wavelength number between C-S (550-800) cm^{-1} single bonds and double bonds C=S (1050-1200) cm^{-1} so that the bonds are written as ν (C=S) [26,29]. Strain of sulfur metal bonds from ditiocarbamate ligands and metal bonds with nitrogen from bipyridyl or phenantroline ligands shows the bond between metals and the ligand observed from far infrared absorption (400-100) cm^{-1} [30,31].

Based on Table 2. Infrared absorption peaks at wave number 393 cm^{-1} indicate the interaction of S atoms with Cu metal ions. The absorption peak at wave number 474 cm^{-1} shows the interaction of O atoms of complex compounds with Cu metal ions. The absorption peak at wave number 592 cm^{-1} shows the interaction of N atoms of complex compounds with each Cu metal ion. The appearance of absorption at wave number 1105 cm^{-1} shows a single

absorption peak that shows bidentate coordination between groups (C=S) with Cu metal ions. Then there is a strong absorption at the wave number 1662 cm^{-1} which indicates that it is derived from the amine group (C=N). The results of the spectrum of complex compounds have been synthesized, Figure 2.

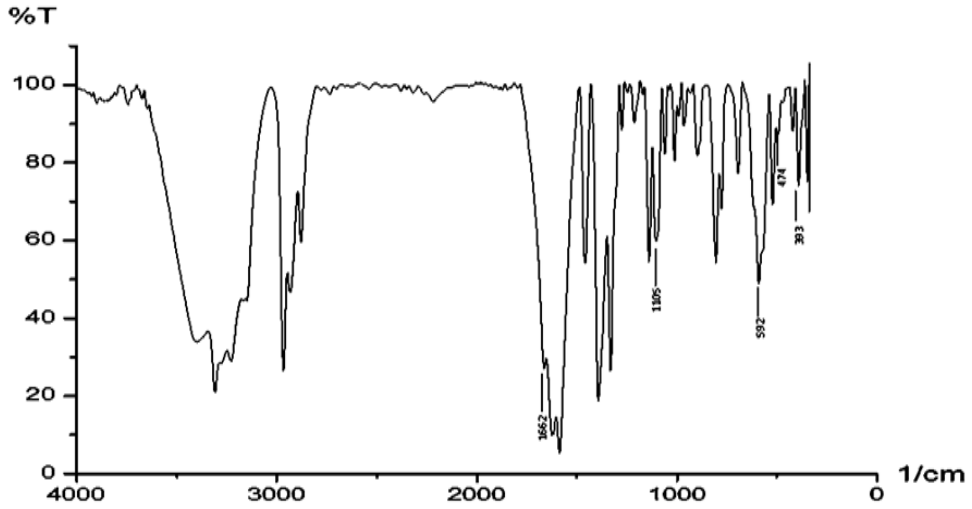

Figure 2. IR spectrum Of Cu(II) isoleucine dithiocarbamate

Cytotoxic Test on MCF-7 Cancer Cells

The results of the cytotoxicity test on MCF-7 cells for complex Cu(II) isoleucine dithiocarbamate compounds obtained IC_{50}=98.17 µg/mL while cisplatin (IC_{50}=50 µg/mL) was used as a comparison. This result can be seen that the IC_{50} value of complex Cu is very close to the ability of cisplatin, so that it can be used as a reference for anticancer drugs with its advantages having few side effects. The ability to kill cancer cells can be seen in Figure 3 which gradually changes morphology with increasing complex Cu concentration.

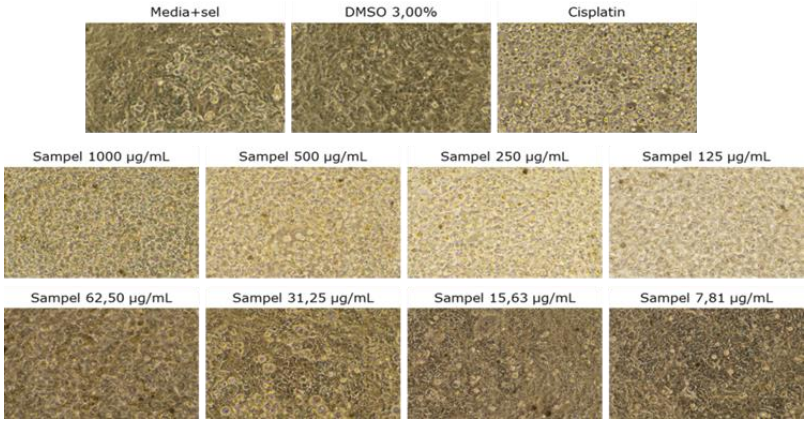

Figure 3. Morphological changes induced by Cu(II) isoleusindithiocarbamate in MCF-7 cells

CONCLUSION

Characterization of the complex using UV-Vis and IR showed that the dithiocarbamat Cu(II) isoleucine complex compound was successfully synthesized. The IC_{50} value=98,17 µg/mL for the Cu complex approached cisplatin ability, which showed that the Cu(II) complex also had inhibitory activity against cancer cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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