RESEARCH ARTICLE

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A New Complex Design of Fe (II) Isoleucine Dithiocarbamate as a Novel Anticancer and Antivirus against SARSCOV-2 (COVID-19)

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Abstract

Background: This study was carried out to synthesize a new complex of Fe(II) with isoleucine dithiocarbamate ligand and to determine its potential as an anticancer and antiviral agent for SARSCOV-2. **Methods:** The synthesized complexes were then characterized by UV-vis and FT-IR spectroscopy and their melting points. The value of the conductivity of the complex compound is also determined. Anti-cancer activity was tested in vitro and molecular docking. Its potential as an antiviral against SARSCOV-2 was also carried out by molecular docking. Pharmacokinetics/ADMET properties were also carried out on the complex. **Result:** Spectral results showed the successful synthesis of Fe(II) isoleucine dithiocarbamate complex. The complex produced UV-vis spectra at 268 and 575 nm, and the IR data at 399–599 cm-1 showed the coordination between the Fe(II) atoms with sulphur, nitrogen and oxygen of the isoleucine dithiocarbamate ligand. Fe(II) isoleucine dithiocarbamate had a cytotoxicity effect on the MCF-7 cell line (IC_{50} =613 µg/mL). The complex significantly caused morphological changes in the breast cancer cell line, finally leading to cell apoptosis. **Conclusion:** Cytotoxic test of Fe(II) isoleucine dithiocarbamate showed moderate anticancer activity on MCF-7 cancer cells and showed antiviral activity against SARSCOV-2 by interfering with spike glycoprotein –ACE2 receptors, and inhibiting major proteases and 3Clpro.

Keywords: Complex- isoleucine- dithiocarbamate- MCF-7 cell lines- Fe (II)

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Introduction

Cancer is one of the leading causes of death worldwide (Momenimovahed, 2017). Approximately 7.5 million people who have died from cancer generally lived in poor and developing countries (WHO, 2020). The major causes of cancer are unhealthy living habits (e.g. smoking and wrong diet) and air pollution (WHO, 2020). Breast cancer is a neoplasm that is the main cause of death in women, especially those over the age of 40 years (Seidler and Huber, 2020). Various therapies, such as surgery, partial irradiation, endocrine therapy and modern medicine, have been applied to treat cancer (Schneeweiss, et al., 2020).

Metal-organic complex-based chemotherapeutic agents have attracted attention as alternatives to current treatments because of their enhanced therapeutic potential. The metal and the appropriate ligand are important in the formation of such complexes (Hwang and Jung, 2020). In cells, about 40% of the metal ions are assumed to be the active form of the antitumor agent (Kontoghiorghes and Kontoghiorghe, 2020). Metal ions help adjust reactivity, conformational changes, are catalytically active for many biochemical reactions (Zoroddu et al., 2019). Iron (Fe) is an essential metal for all living organisms. This metal has many roles in cellular and physiological metabolism due to the discovery of Fe with ligands, such as proteins or other biomolecules (Kontoghiorghes and Kontoghiorghe, 2020). The use of appropriate ligands can increase the biological activity of

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complex compounds (Ritacco et al., 2015). Ligands that are active in biological processes have attracted much attention to the design of potential antitumor agents (Kamaludin et al., 2013). The introduction of additional donor groups such as isoleucine dithiocarbamate into the ligand framework could facilitate increased bioavailability of potential metal complexes in chemotherapy (Radisavljevic et al., 2018). Isoleucine can serve as a structural model for the design of anticancer agents with better inhibition (Devi, 2021). Dithiocarbamate compounds are synthesized from the reaction of primary amines such as isoleucine, carbon disulfide (CS2) and alkyl halides under catalyzed conditions or sometimes using a base. Dithiocarbamate is important because of its wide application in medicinal chemistry (Shinde et al., 2020; Prihantono et al., 2021).

Dithiocarbamate is a metal chelating chemical that has a wide range of medical applications. It has also been used to treat bacterial and fungal infections, as well as AIDS and, more recently, cancer. Because current chemotherapeutic drugs are so toxic, their efficacy in eradicating tumors is restricted. As a result, many scientists have joined the search for new targeted medicines in the hopes of minimizing toxicity while increasing the therapeutic potential (Boschi and Martini, 2017; Boschi and Uccelli, 2019; Daniela, 2012; Malaguarnera et al., 2003).

Dithiocarbamate contains an R2CNS2- or RNHCS2- group, which acts as a counter ion. The dithiocarbamate group can be a bidentate ligand by the coordination of sulphur with metal ions (Kamoon et al., 2020). The newest dithiocarbamate derivative containing the chromene group presents good anti-bacterial activity. One of its derivatives can cause cell rupture in bacteria (Jiang et al., 2020). The chemo- protective and anti-tumor properties of gold metal ions and dithiocarbamate compounds make gold dithiocarbamate complexes a potential agent for cancer management (Adakah, 2020). The addition of complex compounds into the plasmid DNA accompanied by photo-irradiation caused the release of a single plasmid and rapid degradation of the DNA complex. Based on observations, complex compounds simultaneously act as switches and photoactive "scissors" against DNA (Saeed et al., 2020). The structure of the substrate is the main factor affecting the DNA binding. Modification of ligands and metals to synthesized complex compounds has exhibited varying DNA binding affinities (Xi et al., 2020). The Fe (II) complex shows electrostatic interactions when it binds to DNA. This interaction causes condensation on the plasmid such that the DNA structure appears like a clump. This phenomenon shows that iron can be applied in biology (Dinda et al., 2020; Irfandi et al., 2019). The dithiocarbamate complex has a cytotoxicity effect (IC_{50}=211.53 g/mL) against the MCF-7 breast cancer cell line and has a potential as an effective anti-cancer agent (Prihantono et al., 2020). The dithiocarbamate ligand acts as a selective inhibitor of mitochondrial respiration. Most of the dithiocarbamate complexes show specific toxicity to cancer cells without affecting normal epithelial cells (Mertens et al., 2020). The use of dithiocarbamate ligands with additional donor groups, such as oxygen and nitrogen groups (amines), can increase the structural diversity of dithiocarbamate complexes and change the nature of the complex compounds' biological activity (Ferreira et al., 2015).

The aim of this study was to synthesize and characterize a novel complex of Fe (II) with isoleucine dithiocarbamate ligand and to determine its potential as an anticancer and antiviral agent for SARSCOV-2.

Materials and Methods

CS2 (99.5%), cisplatin, Roswell Park Memorial Institute Medium, DMSO, $FeCl_2$, isoleucine, ethanol (95%), acetone (95%) and acetonitrile (95%) (Ajax Chemical Ltd).

Synthesis of Fe(II) complex with isoleusine ditiocarbamate ligand

$FeCl_2$ (3 mmol; 0.5964 g) was dissolved in 10 mL of ethanol. The resulting mixture was denoted as solution 1. Isoleucine (0.6559 g, 5 mmol) was dissolved in 10 mL of ethanol. Then, 0.302 mL of the CS2 solution (5 mmol) was slowly added at a cold temperature, and the mixture (solution 2) was stirred for 30 min. Solution 1 was added slowly to solution 2 whilst stirring for 30 min. The precipitate was filtered, dried in a desiccator and then purified by using a suitable solvent crystallisation method. The pure crystals were further characterized and analyzed. The synthesis of Fe(II) isoleucine dithiocarbamate is depicted in Figure 1.

Cytotoxic test against MCF-7 cell line

Cell cultures were prepared in 96-well plates and then incubated at 37 °C and 5% CO_2 gas, until the percentage of cell growth reached 70%. Cells were sampled and then incubated under the same conditions. Presto blue reagent was added into the wells. The absorbance was measured using a multimode reader.

In silico analysis

Canonical SMILES of Fe(II) isoleucine dithiocarbamate predicted the pharmacokinetic properties using Pkcsm online server (http://biosig.unimelb.edu.au/pkcsm/prediction) (Pires et al., 2015). The 3D structure of Fe (II) isoleucine dithiocarbamate was generated from MolView (https://molview.org/), then was docked by Molegro Virtual Docker 5 software to conduct anticancer and anti-viral activities (Annuar et al., 1874). Anticancer targeted proteins, caspase-8 and O6alkylguanine-DNA alkyltransferase, and antiviral proteins involved Spike glycoprotein of SARSCOV2 – ACE2 receptor, main protease, and 3CL- protease were retrieved from Protein Data Bank with ID 1qdu, 1qnt, 7dmu, 2gz9, and 2op9, respectively. Cisplatin (CID 5702198) and remdesivir (CID 121304016) were used as control for anticancer and antiviral. Both of their structure were obtained from PubChem database. The ligands – proteins interactions were analyzed and visualized by PyMol and Discovery Studio version 21.1.1. The binding energy of complex interactions was performed in kJ/mol.

Results

The synthesized Fe (II) isoleucine dithiocarbamate had a yield of 53.33% and melting point at 238–240°C, indicating that this Fe (II) complex had high purity. The conductivity of the Fe (II) complex was 0.19 mS/cm, which showed that the Fe (II) complex was a non-electrolyte compound.

UV-vis characterisation

The UV VIS spectrum showed the specific characteristics of Fe (II) Isoleucine dithiocarbamate

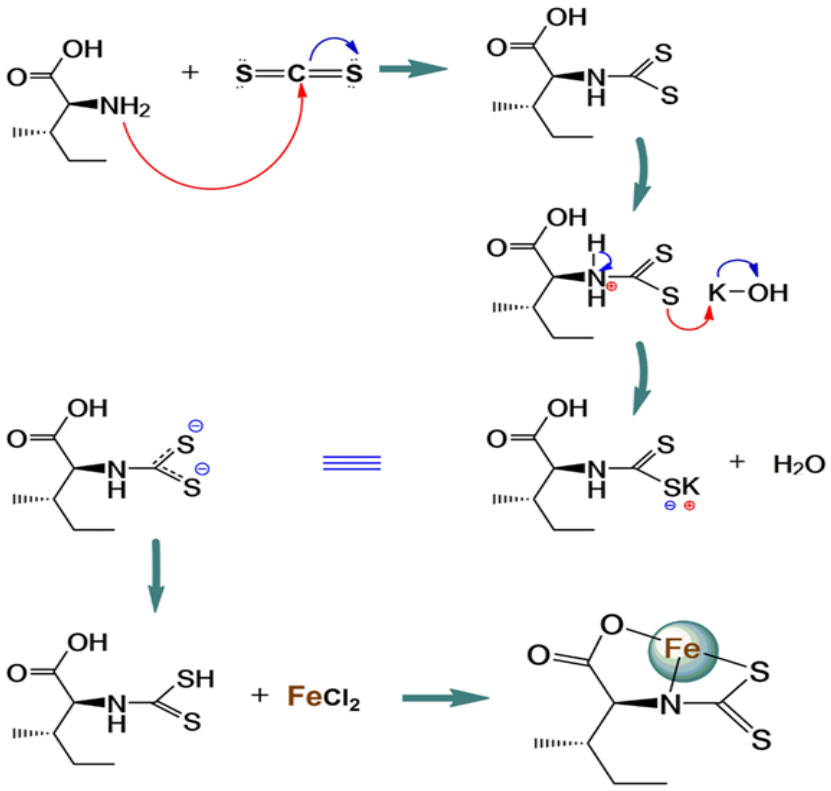

Figure 1. Synthesis route of Zn (II) Prolinedithiocarbamate

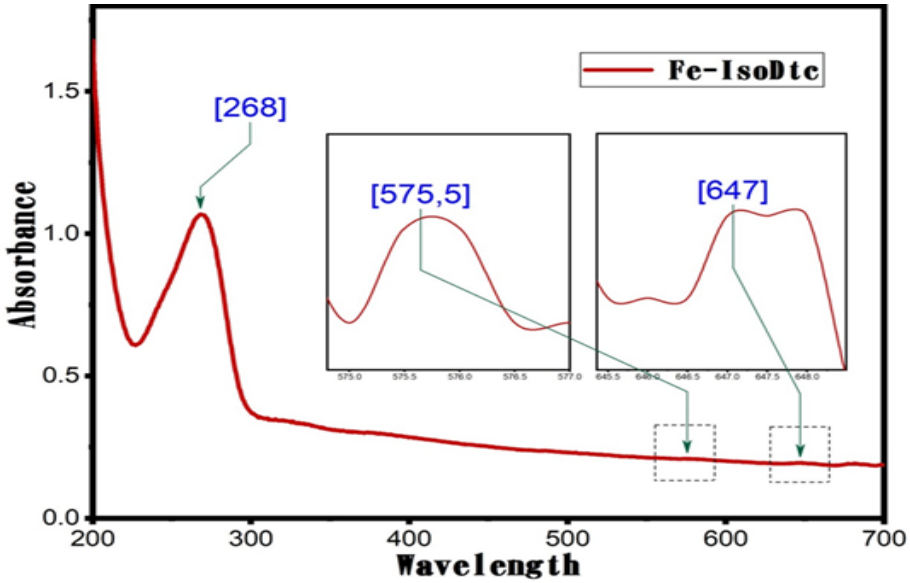

Figure 2. UV-Vis Spectrum of Fe (II) Isoleucine Dithiocarbamate

Table 1. IC_{50} values of the Fe (II) Isoleucine dithiocarbamate Complexes

Compounds	t (h)	IC_{50} (µg/mL)
Fe (II) Isoleucine Dithiocarbamate	48	613,00
FeCl2	48	9302,50
Cisplatin	48	53,48

Table 2. Pharmacokinetic Characteristic of Fe (II) Isoleucine Dithiocarbamate

Parameter		Fe (II) Isoleucine dithiocarbamate	Parameter		Fe (II) Isoleucine dithiocarbamate
Absorption	Water Solubility (log mol/L)	-2.39	Metabolism	CYP3A4 substrate	No
	Caco2 permeability (log Papp in 10-6 cm/s)	1.63		CYP1A2 inhibitor	No
	Intestinal absorption (% absorbed)	95.66		CYP2C19 inhibitor	No
	Skin permeability (log Kp)	-2.8	Toxicity	AMES Toxicity	Yes
	P-glycoprotein substrate	No		Max tolerated dose (log mg/kg/day)	0.39
	P-glycoprotein I inhibitor	No		hERG I inhibitor	No
	P-glycoprotein II inhibitor	No		hERG II inhibitor	No
Distribution	VDss (log L/kg)	-0.04		Oral Rat Acut Toxicity (LD50) (mol/kg)	2.84
	Fraction unbound (Fu)	0.61		Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	0.5
	BBB Permeability (log BB)	-0.01		Hepatotoxicity	Yes
	CNS permeability (log PS)	-2.87		Skin Sensitisation	Yes
Excretion	Total clearance (log ml/min/kg)	0.02		T. pyriformis toxicity (log ug/L)	0.93
	Renal OCT2 substrate	No		Minnow toxicity (log mM)	1.63

(Figure 2). The UV-vis spectrum of the synthesized in band I showed an absorption band at 268 nm. This band was an intra-ligand transition π→π* of the CS2 group, which was influenced by the hyperconjugation effect of the R group on the nitrogen (N) atom in the region (Prihantono et al., 2020). The shift in band II, which was an intra-ligand transition n → π* of the N=C=S group at 575 nm, was also observed in the Fe(II) isoleucine dithiocarbamate complex. The UV-vis spectrum of the complex also showed band III at 647 nm, and this band was due to the fact that the complex had a larger conjugation system than the ligand.

IR characterization

The IR absorption peak at 399 cm^{-1} indicated an interaction between the CS2 group and Fe metal ions (Mertens et al., 2020). The absorption peak at 462 cm^{-1} could be attributed to the interaction of O atoms of the complex with Fe, and that at 599 cm^{-1} was the interaction of the N atom of the complex compound with the Fe metal

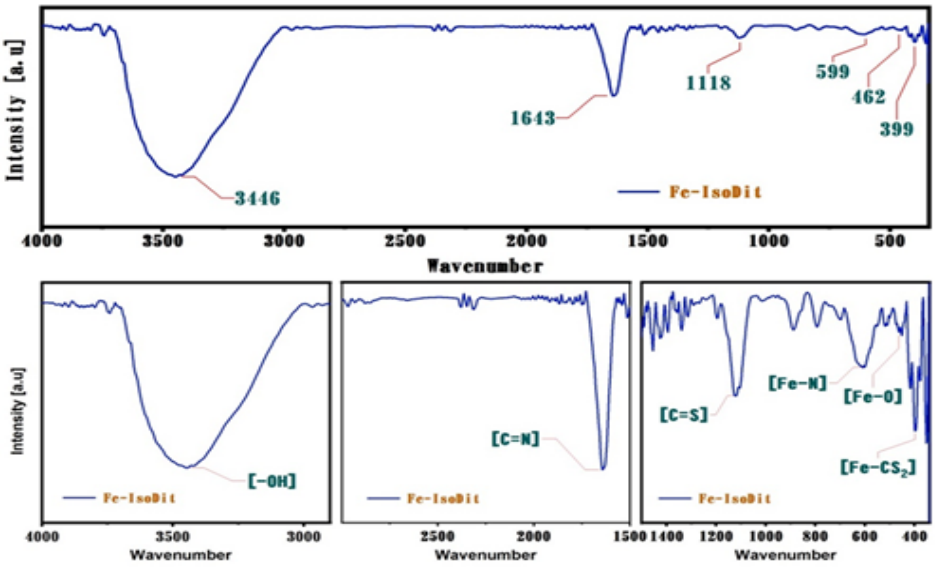

Figure 3. IR Spectrum of Fe (II) Isoleucine Ditiocarbamate

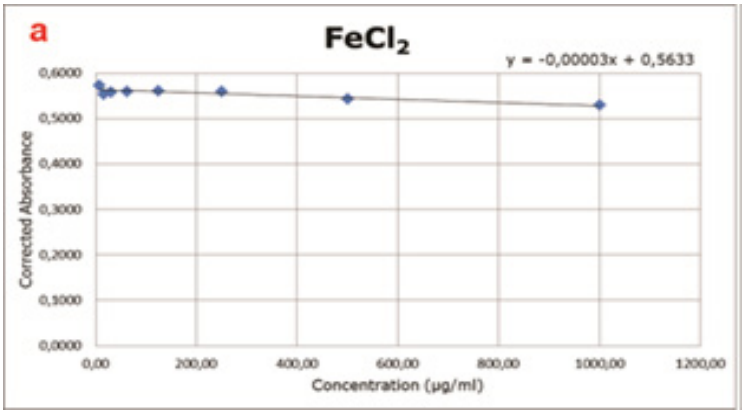

Figure 4. Cytotoxicity Curve of Fe (II) Isoleucine Dithiocarbamate.

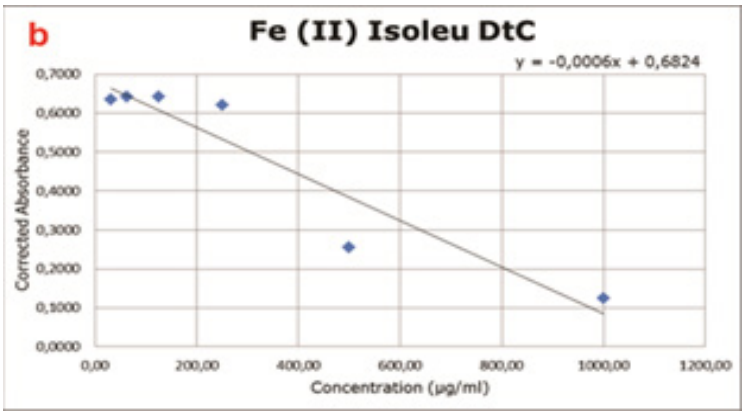

Figure 5. Cytotoxicity Curve of $FeCl_2$

ions (Ferreira et al., 2015). The appearance of absorption at 1,118 cm^{-1} indicated the presence of a C=S functional group from the dithiocarbamate ligand. Then, a strong absorption 200 300 400 500 600 700 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 Abs. Uv-Vis spectrum of Fe (II) IsoleuDTC Abs. nm was observed at 1643 cm^{-1}, which could be attributed to the C=N group (Pires et al., 2015). IR absorption showed the characteristics of Fe (II) isoleucine

Table 3. The Detailed Interactions of Fe (II) Isoleucine Dithiocarbamate and Cisplatin with O6alkylguanine-DNA alkyltransferase and Caspase-8 Proteins

Complex	Binding Energy (kJ/mol)	Interaction
Cisplatin - O6alkylguanine-DNA alkyltransferase	-171.6	Halogen;Other (Metal-Acceptor;Halogen (Cl, Br, I)): (:LIG3:PT - :LIG3:CL)
		Hydrogen Bond: (:LIG2:N - A:HIS146)
Fe (II) Isoleucine dithiocarbamate - O6alkylguanine-DNA alkyltransferase	-194.3	Hydrogen Bond: (A:TRP65:HE1 - :LIG1:O), (:LIG1:S - A:LEU142:O), (:LIG1:O - A:ILE76:O), (:LIG1:H - A:GLU77:OE2)
		Metal-Acceptor: (:LIG1:FE - :LIG1:O)
Cisplatin - Caspase-8	-175.8	Hydrogen Bond;Electrostatic;Halogen (Salt Bridge;Attractive Charge;Halogen (Cl, Br, I)): (H:LYS397:HZ3 - :LIG3:CL)
		Hydrogen Bond: (E:GLN283:HE22 - :LIG1:N), (F:TYR349:HN - :LIG1:N), (F:GLN385:HE22 - :LIG1:N), (:LIG1:N - F:TYR340:O), (:LIG2:N - F:LEU370:O), (:LIG2:N - F:ASN374:OD1), (F:SER339:CB - :LIG1:N), (F:THR347:CB - :LIG1:N)
		Conventional Hydrogen Bond;Halogen (Cl, Br, I): (F:THR371:HG1 - :LIG3:CL), (H:LEU398:HN - :LIG3:CL)
		Metal-Acceptor; Halogen (Cl, Br, I): (:LIG3:PT - :LIG3:CL)
Fe (II) Isoleucine dithiocarbamate - Caspase-8	-204.1	Metal-Acceptor: (:LIG1:FE - :LIG1:O)
		Hydrophobic: (:LIG1:C - F:VAL334)

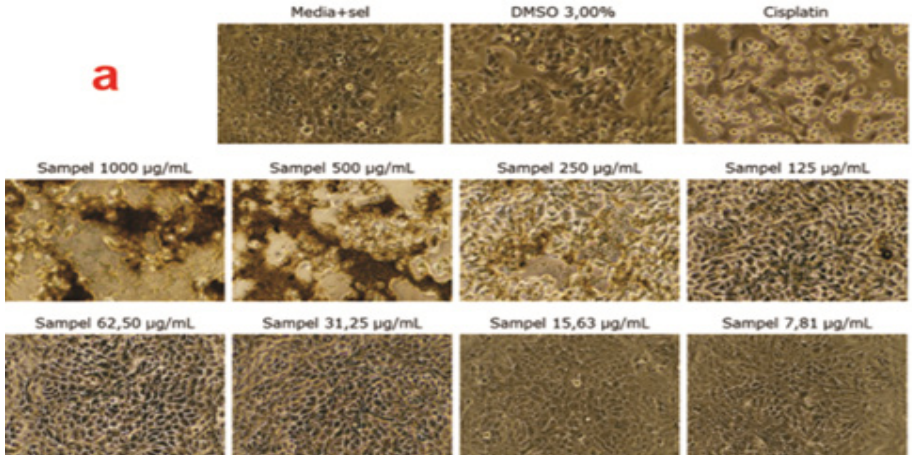

Figure 6. Apoptosis of MCF-7 Cells Induced by Fe (II)Isoleucine Dithiocarbamate

dithiocarbamate. The results showed the successful synthesis of the desired complex. The spectral results of the synthesized complex is shown in Figure 3.

Cytotoxicity test on MCF-7 cell line

The cytotoxicity of Fe (II) isoleucine dithiocarbamate against MCF-7 was determined in vitro. The cells were treated with different concentrations of the synthesized complex (7.81–1000 µg/mL) for 48 h. The IC_{50} value of the synthesized complex was determined from the regression equation y = −0.0006x + 0.6824 (Figure 5), and the IC_{50} value of $FeCl_2$ was determined from the

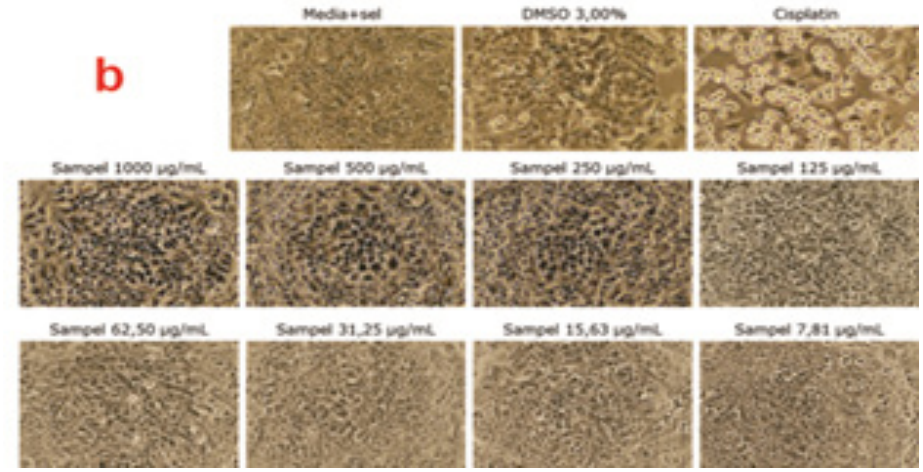

Figure 7. Apoptosis of MCF-7 Cells induced by $FeCl_2$.

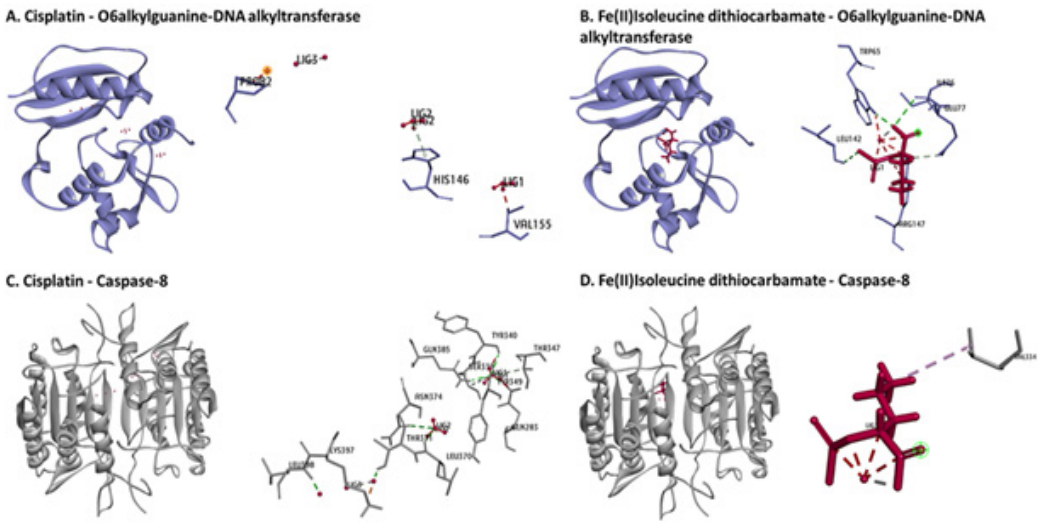

Figure 8. The Complex Structure of Fe (II) Isoleucine Dithiocarbamate and Cisplatin to O6alkylguanine-DNA alkyltransferase and Caspase-8 Proteins

Table 4. The Interaction of Fe (II) Isoleucine Dithiocarbamate and Remdesivir Complexed with SARSCOV-2 Viral Proteins

Complex	Binding Energy (kJ/mol)	Interactions
Remdesivir - Spike Glycoprotein SARSCOV2-ACE2	-397	Hydrogen Bond : (B:ARG403:NH1 - :10:O6), (B:TYR505:OH - :10:O4), (:10:H6 - B:ASP405:OD2), (:10:H8 - A:ALA386:O), (:10:H10 - A:ASN33:O), (:10:H10 - A:GLU37:OE2), (B:ARG408:CD - :10:N5), (:10:H2 - B:TYR505:OH)
		Electrostatic (Pi-Cation) : B:ARG403:NH2 - :10
		Electrostatic (Pi-Anion): (B:ASP405:OD2 - :10), B:ASP405:OD2 - :10
		Hydrophobic: (:10:C17 - A:PRO389), (A:PHE390 - :10:C19)
Fe (II) Isoleucine dithiocarbamate - Spike Glycoprotein SARSCOV2-ACE2	-229	Hydrogen Bond: (A:LYS353:NZ - :10:O2), (B:GLY496:N - :10:O2)
		Pi-Sulfur: :10:S1 - A:HIS34
		Hydrophobic: (:10:C1 - B:ARG403), (B:TYR495 - :10:C1), (B:PHE497 - :10:C1), (B:TYR505 - :10:C1)
Remdesivir - 3CL Pro	-412,2	Hydrogen Bond: (B:LYS5:N - :10:N4), (B:LYS5:NZ - :10:O5), (:10:H20 - B:VAL125:O), (:10:H21 - B:VAL125:O), (B:ARG4:CD - :10:N4), (:10:H13 - A:GLN127:O), (:10:H14 - A:PHE3:O)
		Electrostatic: B:GLU290:OE2 - :10
		Hydrophobic: (:10:C16 - B:LYS137), (:10 - A:LYS5), (:10 - A:LYS5), (:10 - B:LYS5)
Fe (II) Isoleucine dithiocarbamate - 3CL Pro	-240,4	Hydrogen Bond: (B:ARG4:NH1 - :10:S2), (B:ALA7:N - :10:O2), (B:GLN127:N - :10:O2), (:10:H8 - B:LYS5:O)
		Hydrophobic: (A:ALA7 - :10:C1), (B:ALA7 - :10:C1), (:10:C1 - A:VAL125), (:10:C1 - B:VAL125)
Remdesivir - MPro	-384,2	Hydrogen Bond: (A:ASN142:ND2 - :10:O1), (A:ASN142:ND2 - :10:N2), (A:SER144:OG - :10:O3), (A:CYS145:N - :10:O3), (:10:H6 - A:HIS163:NE2), (A:HIS41:CE1 - :10:O6), (:10:H3 - A:LEU141:O), (A:ASN142:ND2 - :10)
		Hydrophobic: (A:ASN142:CB - :10), (:10:C16 - A:MET49), (:10:C16 - A:MET165)
Fe (II) Isoleucine dithiocarbamate - MPro	-218,75	Hydrogen Bond: (A:ASN142:ND2 - :10:N1), (A:GLY143:N - :10:O2), (A:SER144:N - :10:O2), (A:CYS145:N - :10:O1), (A:CYS145:N - :10:O2)
		Pi-Sulfur: (:10:S1 - A:HIS163), (:10:S1 - A:HIS172)
		Hydrophobic: (:10:C1 - A:CYS145), (A:HIS41 - :10:C1)

regression equation y = −0.00003x + 0.5633 (Figure 4). The IC_{50} value was obtained by replacing the y value with half the control value (DMSO). The IC_{50} values of Fe (II) isoleucine dithiocarbamate, FeCl2 and cisplatin complexes are shown in Table 1.

The apoptosis of the MCF-7 cells as a result of treatment with Fe (II) isoleucine dithiocarbamate ligand, Fe (II) metal solution without ligand and cisplatin is illustrated in Figures 6 and 7. Treatment with Fe (II) isoleucine dithiocarbamate from 7.81–62.5 µg/mL resulted in no visible cell death. Apoptosis started when the concentration of the synthesized complex was 250 µg/mL. By contrast, treatment with cisplatin showed a cytotoxic effect at 53.48 µg/mL. The Fe (II) metal solution without ligands showed a non-toxic effect on the MCF-7 cells.

Pharmacokinetic characteristic, anticancer and antiviral activities of Fe (II) Isoleucine dithiocarbamate

Pharmacokinetic properties of Fe (II) Isoleucine dithiocarbamate described at Table 2. Fe (II) Isoleucine dithiocarbamate has high absorption in intestine with 95.6% absorption and high $CaCO_2$ permeability. In the P-glycoprotein, Fe (II) Isoleucine dithiocarbamate inactive as substrate and inhibitor of P-glycoprotein. The steady state volume of Fe (II) Isoleucine dithiocarbamate distribution was low distribution with value less than -0.15. Fe (II) Isoleucine dithiocarbamate is also considered poorly absorbed to the blood-brain barrier and unable to penetrate the central nervous system (CNS). Total clearance of Fe (II) Isoleucine dithiocarbamate described as excreted synthetic complex by liver and kidney, which was low dose with the value 0.02 log

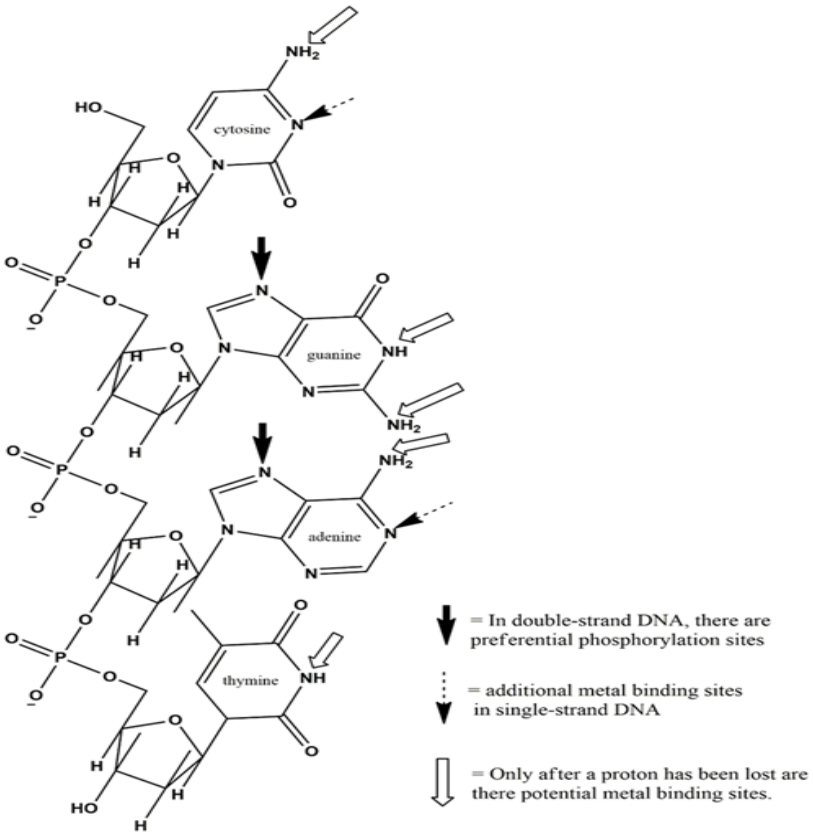

Figure 9. Prediction of the binding of Fe (II) Isoleucine Dithiocarbamate Complex to Guanine (nitrogen base that composes DNA).

ml/min/kg. The metabolism parameter showed that Fe (II) Isoleucine dithiocarbamate did not contraindicate with cytochrome P450. However, according to the AMES test, Fe (II) Isoleucine dithiocarbamate predicted causing mutagenicity. The maximum tolerated dose of Fe (II) Isoleucine dithiocarbamate and the oral LD50 was considered low.

In vitro and in silico data performed potential anticancer activity of Fe (II) Isoleucine dithiocarbamate. Fe (II) Isoleucine dithiocarbamate bound to 06-alkylguanine-DNA alkyltransferase (MGMT) and caspase-8 in some active site residues (Figure 8, Table 3). Fe (II) Isoleucine dithiocarbamate interacted with O6alkylguanine-DNA alkyltransferase in different residues and generated lower binding energy than cisplatin as control. Fe (II) Isoleucine dithiocarbamate and cisplatin interacted with targeted

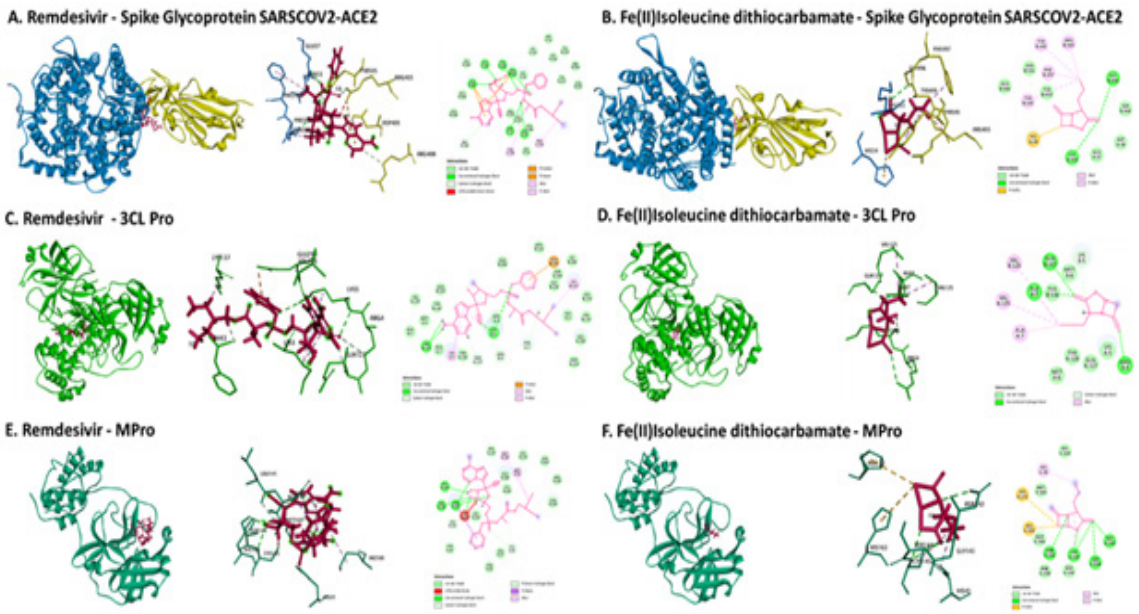

Figure 10. The 3D and 2D Representative of Fe (II) Isoleucine Dithiocarbamate and Remdesivir Complexed with SARSCOV-2 Viral Proteins

anticancer protein by metal acceptor and hydrogen bond. In comparison, Fe (II) Isoleucine dithiocarbamate showed interaction with a residue of caspase-8, Val334, by hydrophobic interaction. Cisplatin interacted with caspase-8 in several amino acid residues, performing tighter interaction than Fe (II) Isoleucine dithiocarbamate. However, the binding energy of cisplatin was higher than Fe (II) Isoleucine dithiocarbamate.

Molecular docking against SARSCOV-2 targetted proteins was performed in this study to evaluate antiviral activity. Fe (II) Isoleucine dithiocarbamate and remdesivir established several binding sites of SARSCOV-2 and angiotensin converting enzyme receptor (ACE-2) (Figure 10, Table 4). Fe (II) Isoleucine dithiocarbamate interfered spike glycoprotein and ACE2 interaction by binding at LYS353, GLY496, HIS34, ARG403, TYR495, PHE497, and TYR505. Interestingly, specific interaction, Pi-Sulfur, was conducted in HIS34 residue. Similar with Fe (II) Isoleucine dithiocarbamate, remdesivir interfered spike glycoprotein of SARSCOV-2 and ACE2 receptor by hydrogen bond and hydrophobic interactions. Similar position of protein binding was identified on Fe (II) Isoleucine dithiocarbamate and remdesivir, involved ARG403 and TYR505.

Fe (II) Isoleucine dithiocarbamate had 4 hydrogen bonds and 2 hydrophobic interactions with active residues of 3C-like protease. Moreover, Fe (II) Isoleucine dithiocarbamate also performed two catalytic residues of main protease, CYS145 and HIS41 by hydrophobic interaction. CYS145 was identified at Fe (II) Isoleucine dithiocarbamate – MPro with a hydrogen bond. Interestingly, Fe (II) Isoleucine dithiocarbamate disrupted protease activity by binding 3C-like protease and main protease of SARSCOV-2 in the same position with remdesivir. Those residues involved LYS5, VAL125, ARG4, and GLN127 of 3C-like protease. Similar binding sites of Fe(II)Isoleucine dithiocarbamate – MPro also identified on remdesivir – MPro, including ASN142, SER144, CYS145, HIS163, and HIS41. Similar binding sites of protease indicated that Fe(II)Isoleucine dithiocarbamate might be potentially substituted remdesivir as viral drug.

Discussion

The Fe (II) isoleucine dithiocarbamate complex was synthesized and tested in vitro against MCF-7 cancer cells. The cytotoxicity of Fe (II) complex is better than that of Dioscorea esculenta L. as reported by Haryoto (Annuar et al., 1874). According to the classification of Prayong (Baharum et al., 2014) the IC_{50} standard for cytotoxic samples, Fe (II) isoleucine dithiocarbamate could be classified under the moderate cytotoxic category, because its IC_{50} values were in the range of 100–1000 g/mL. The cytotoxic ability between the complex compounds and MCF-7 cancer cells can be considered according to the bioactivity of the metal itself in the body and the structural properties of a complex. In addition, if viewed from the nature of HSAB, Fe (II) can be included in the borderline acid category, resulting in a strong coordination bond between the Fe metal and the nitrogenous base (borderline) of guanine, which is the basic framework for DNA formation (Figure 9). In addition, Fe is an essential metal needed for all living organisms and found in many types of proteins. This metal plays a role in cellular and physiological metabolic pathways due to the formation of iron bonds with ligands, such as proteins or other biomolecules, compared to cisplatin, which has harmful side effects in the body. The high IC_{50} value of the synthesized complex was influenced by the isoleucine dithiocarbamate ligand as indicated by the comparative results of the IC_{50} values of the synthesized Fe (II) isoleucine dithiocarbamate and Fe metal without ligands. Therefore, metal complexes tended to coordinate not only covalently but also non-covalently.

Bonds to guanine showed greater kinetic energy (Sun et al., 2016). This tendency resulted from the stronger basicity of nitrogen. The DNA structure was significantly distorted, resulting in a decrease in melting temperature, shortening, detachment and denaturation. The cell morphology of the MCF-7 line showed that the Fe (II)-DNA complex reaction disrupted the cell cycle. With cases of inadequate repair, the cell eventually underwent failed mitosis, leading to cell death via apoptotic mechanisms.

In silico approach revealed that Fe (II) Isoleucine dithiocarbamate interacted with anticancer and antiviral targeted protein therapy. Fe (II) Isoleucine dithiocarbamate bound to several active sites of O6alkylguanine-DNA alkyltransferase that not identified on cisplatin. A recent report revealed that Zn (II) prolinedithiocarbamate altered the conformational structure of MGMT by binding ARG147, LEU102, VAL106, ILE76, and GLU77 (Irfandi et al., 2022). Antitumor agent, combi-nitrosourea prodrug 3-(3- (2-amino-9H-purin-6-yl) oxy) methyl)benzyl)-1-(2-chloroethyl)-1-nitrosourea, reported inhibit the O6alkylguanine-DNA alkyltransferase at Try114, Cys145, Val148, and Ser159 (Sun et al., 2016). O6alkylguanine-DNA alkyltransferase or MGMT repaired the DNA by alkylation O6 of guanine to Cys145 of protein. MGMT was higher expression in tumour cells than normal cells. Inhibiting the DNA repair activity by MGMT was an alternative therapy for cancer (Chikan et al., 2015; Lin et al., 2017). Furthermore, in the current study, Fe (II) Isoleucine dithiocarbamate activated caspase-8 by binding in several binding site P12 of caspase-8. Activated caspase-8 promoted apoptosis cells mechanism and inhibited tumor cell proliferation. Terpenoid compounds, Albiziasaponin C, caratuberoside A, and canophyllal, reported exhibited caspase-3 and caspase-8 activation and induced apoptosis cells (Desai and Joshi, 2019). Some compounds from Clinacanthun nuthans also induced apoptosis by interacting with caspase-3 and caspase-8 (Zafirah et al., 2020). Conversly, some anthocyanins inhibited caspase-3 and might be promoted cell proliferation (Sari et al., 2020).

Our findings have been shown that Fe (II) Isoleucine dithiocarbamate was potentially as anticancer and antivirus against SARSCOV-2 protein. Fe (II) Isoleucine dithiocarbamate clearly disrupted the interaction between spike glycoprotein of SARSCOV-2 and ACE2 receptor by binding the amino acid residues both of spike glycoprotein and ACE2 receptor proteins. Fe (II)

Isoleucine dithiocarbamate also bound to main protease of SARSCOV-2 in catalytic site His41 and Cys145. Main protease (MPro) encoded polyprotein that played a role in replication and transcription viral genome of SARSCOV-2 (Shyr et al., 2020; Song et al., 2021). Main protease consisted of conserved domain that distributed in four subsite S1', S1, S2, and S4, the MPro catalytic site of SARSCOV-2 was HIS41 and Cys145. S1 subsite of MPro SARSCOV-2 composed Phe140, Leu141, Asn142, Gly143, Glu166, His163, and His172. His41, Met49, Tyr54, His164, Met165, and Asp187 were considered as S2 subsite of MPro, and S4 subsite consisted of Leu167, Phe185, Gln189, and Gln192 (Song et al., 2021; Cui et al., 2020). Recent study reviewed several peptide, proteins, compounds and drugs showed positive results for COVID-19 therapy. Convalescent plasma, monoclonal antibodies spike binding peptide, and small molecules blocked viral entry. Chlorpomazine and chloroquine inhibited endocytosis, niclosamide and chloroquine increased pH of viral endosome, and arbidol inhibited relasing viral genome (Muhammed, 2020; Su et al., 2021; Riva et al., 2020; Mei and Tan, 2021). Viral replication inhibitors involved lopinavir, remdesivir, favipiravir, and emetine inhibited catalization process of viral protease, such as Plpro, 3CLPro, and RdRp (Shyr et al., 2020; Cui et al., 2020; Su et al., 2021; Riva et al., 2020). Viral entry targeted proteins, viral genome replications protein, and protease were an effective therapy and management for COVID-19 (Shyr et al., 2020; Song et al., 2021; Muhammed, 2020; Mei and Tan, 2021; Fig et al., 2020). Fe(II)Isoleucine dithiocarbamate prevented SARSCOV-2 viral entry and inhibited viral replication.

In conclusion, Fe (II) isoleucine dithiocarbamate was synthesized in situ by reacting primary amine (isoleucine) with CS2 in ethanol and Fe metal as solvent. The results from the cytotoxicity test against MCF-7 cancer cells showed that the complex had IC_{50} value of 613.00 µg/mL. This value indicates that the synthesized complex has moderate cytotoxicity for the treatment of breast cancer. Fe (II) isoleucine dithiocarbamate presented antiviral activity against SARSCOV-2 by interrupted spike glycoprotein –ACE2 receptor, and inhibited main protease and 3Clpro. The challenge faced in this research is the difficulty of finding a single solvent in the recrystallization stage of the Fe (II) complex, while the solution is to use multiple solvents, namely acetonitrile and ethanol.

Author Contribution Statement

Rizal Irfandi: Conceptualization, Methodology, Investigation, Writing–original draft. Riswandi: Writing – review & editing, Validation. Indah Raya: Conceptualization, Methodology, Supervision. Ahyar Ahmad: Writing – review & editing, Validation. Ahmad Fudholi: Writing– review & editing. Sulistiani Jarre Dewi: Writing – review & editing, Validation. Ratih Tirto Sari: Formal analysis, Software. Santi Santi: Visualization, Data curation. Ronald Ivan Wijaya: Writing – review & editing, Validation. Prihantono: Writing – review & editing, Validation

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Ethical Declaration

This research does not involve humans or animals as research subjects.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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