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ORIGINAL ARTICLE

Synthesis, characterization, molecular docking studies of Mn(II) Prolinedithiocarbamate and its potential as anticancer agents

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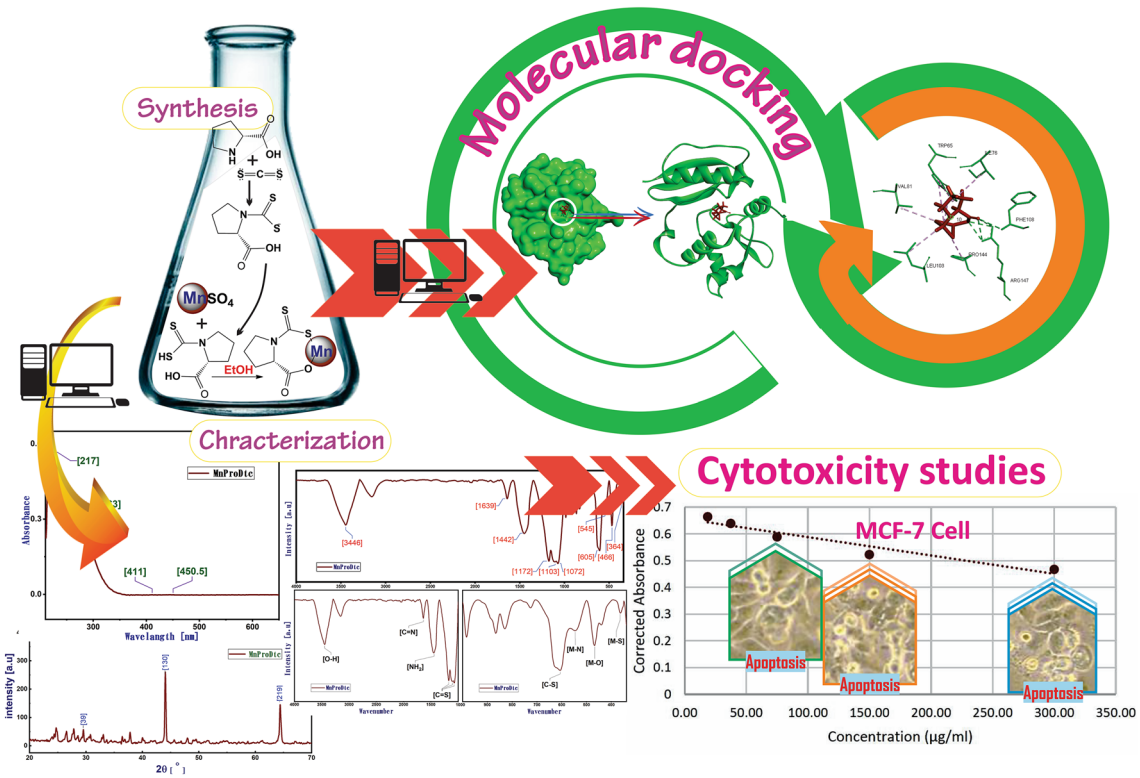
Abstract

Breast cancer is a non-communicable disease but dangerous for women, and research on anti-breast cancer drug compounds is being investigated. Mn(II)Prolinedithiocarbamate (MnProDtc) complex was synthesized and characterized in cytotoxicity and in silico assay by molecular docking. Dithiocarbamate ligand plays an important role as an anticancer agent. Melting point determination, conductivity, UV–Vis spectroscopy, FT-IR spectroscopy, XRD, and HOMO–LUMO have been studied. The binding of MnProDtc to cancer cells was examined by molecular docking, showing that the active sites of the MCF-7 strain, namely the protein O(6)-methylguanine-DNA methyltransferase (MGMT), caspase-8, and the estrogen receptor, bind to the complex. The results of the cytotoxic test of MCF-7 cancer cells undergoing apoptosis at a concentration of 37.50 µg/ml with an IC_{50} value of 453.96 µg/ml showed moderate anticancer activity in MCF-7 cancer cells.

Graphical abstract



Keywords Cytotoxicity · Breast cancer · Dithiocarbamate · Molecular docking

Extended author information available on the last page of the article

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Introduction

Breast cancer is a type of cancer that occurs in the breast glandular tissue and is the number two killer disease globally for women [1]. In 2020, there will be 2.3 million women diagnosed with breast cancer and 685,000 deaths globally [2]. Chemotherapy is one of the most widely used and highly effective cancer treatments [3]. Types of drugs often used in chemotherapy are platinum-derived compound, such as Cisplatin. The cisplatin complex is rectangular in shape, and has two labile chloride ligands and two inert ammonia ligands bonded to the central platinum(II) atom in a cis 5 configuration. [4] Cisplatin works as a cytostatic by inhibiting DNA synthesis, and subsequently, cancer cells undergo apoptosis [5]. However, cisplatin drugs still have some weaknesses such as lack of selectivity, unfavorable side effects, resistance, and toxicity in the body, kidney, hearing, and digestive disorders [6, 7]. The use of complex compound as non-platinum-based chemotherapeutic agents has potential as an anticancer and can reduce the side effects it causes.

Researchers have studied many complex compound as anticancer because they can easily predict the nature of their interactions with DNA [8]. The use of dithiocarbamate ligands with different donor groups such as oxygen and nitrogen in amino acids to synthesize complex compound can increase anticancer activity [9]. The choice of the amino acid proline is because it can selectively deliver cytotoxic units into aggressive cancer cells [10]. Natural and synthetic proline have been reported to exhibit high cytotoxicity against cancer cells and have been used as antineoplastic agents in chemotherapy. Drugs such as dolastatine 10, symplostatins 1 and 3, malevamide D and isodolastatin H, have an L-Proline moiety in their structure and have proven to be very effective against cancer proliferation [11].

The specialty of dithiocarbamate has a sulfur group (S-) which can bind metal ions strongly and selectively to form organometallic complexes. Dithiocarbamate can form monodentate or bidentate complexes with metals by coordination bonds, depending on the nature of the amine. Dithiocarbamate ligands can bind metals with various oxidation numbers firmly and stably [12]. Radio-chemotherapeutic treatments may also target tumors with these chemicals. In addition, dithiocarbamate has low toxicity in the body [13]. As an enzyme cofactor, the metal Mn(II) is a vital ingredient for intracellular action. The role of Mn(II) metal in development, digestion, reproduction, antioxidant, energy generation, immunological response, and brain function is critical [14]. This research is the first to synthesize and characterize the essential metal complex Mn(II) reacted with prolinedithiocarbamate. The anticancer activity of the MnProDtc complex against MCF-7 cancer cell lines was investigated in vitro and in silico. In silico molecular docking analysis was used to understand the affinity of complex compound with target biomolecules such as DNA and cell proteins and showed that MnProDtc complex compound could inhibit the growth of MCF-7 through Protein O(6)-methylguanine-DNA methyltransferase (MGMT), Caspase 8, and Estrogen receptors.

Experimental

Materials and general techniques

Manganese(II)sulfate ($MnSO_4$), Carbon disulfide (99.5%) (Ajax Chem. Ltd), Roswell Park MIM, Proline, Cisplatin, Acetone (95%), DMSO, Ethanol (95%), and Acetonitrile (95%) (chemistry laboratory, Hasanuddin University, Indonesia).

The characterization of complex MnProDtc using Electrothermal IA 9100 to measure the melting point and a conductometer to measure the conductivity. Analysis of the FT-IR spectrum of complex used SHIMADZU FT-IR Spectrophotometer in the wavelength range of 300–4000 cm^{-1}. Shimadzu XRD 7000 was used for XRD analysis, and Jenwey's UV–Vis spectrophotometer was used to examine UV–Vis Spectrum from 200 to 700 nm. The bandgap energy is also determined by the energy difference ΔE (E_{LUMO}-E_{HOMO}).

Synthesis of Mn(II) with proline dithiocarbamate ligand

$MnSO_4$, as much as 0.7380 g (3 mmol), was put in 100 ml Erlenmeyer, dissolved with 10 ml ethanol (solution 1). Then 0.580 g (5 mmol) of Proline was added in a 100 ml Erlenmeyer, then dissolved with 10 ml of ethanol. Next, 0.302 ml (5 mmol) CS_2 solution was added slowly at −4 °C then stirred for 30 min (solution 2). KOH with a concentration of 0.06 M was added to the preparation of proline dithiocarbamate ligand (solution 2) as a catalyst. In solution (2), solution (1) adds dropwise while stirring with a magnetic stirrer for 30 min. After that, the precipitate obtained was filtered and dried in a desiccator, crystallized using ethanol solvent to make pure crystals, then examined and described; Fig. 1 shows the schematic of the synthesis reaction of MnProDtc**.**

Breast cancer cell cytotoxicity test (MCF-7)

Media preparation, positive control, and sample

PrestoBlue™ Cell Viability Reagent was utilized in research. It contains 10% Fetal Bovine Serum (FBS) and

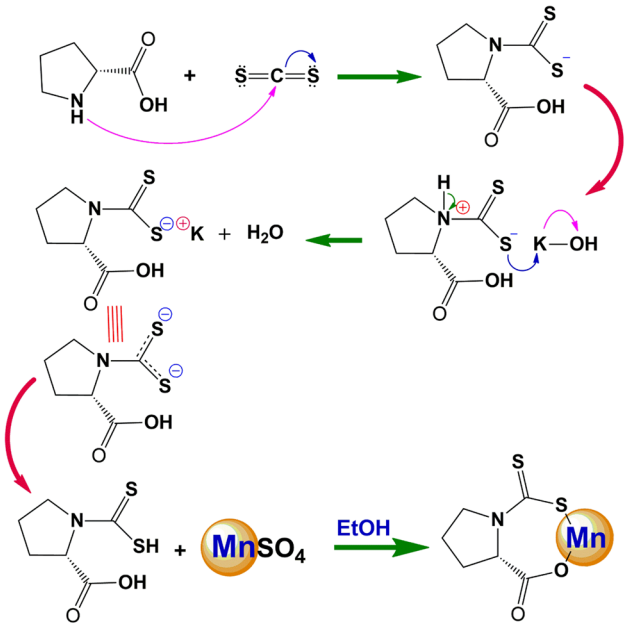

Fig. 1 Schematic reaction for the synthesis of MnProDtc (−4 °C, 30 min and yield 23.7%)

50 µl/50 ml antibiotics from the Roswell Park Memorial Institute (RPMI), a liquid culture medium for all types of bacteria. Only 53 µM of positive control (Cisplatin) was utilized in this study, although the sample had between 2.34 and 300 µg/ml Cells are not harmed by the solvent employed in this procedure at all.

Preparation of cells

Cells must be at least 70% confluent, the medium on the plate must be removed, and the cells should be washed twice with 1 ml PBS. It was incubated for 5 min to distribute the layers of cells supplemented with 1 ml of Trypsin–EDTA solution. The cells were placed into a medium tube, centrifuged at 3000 g for 5 min, and dissolved the pellet into a tube containing media.

Preparation of 96-well plates for cell culture

Except for trypan blue, the viability of the cells was assessed, and cell suspensions were returned with a final cell density of 170.000 cells/ml in medium (17.000 cells/well). Using sterile microtubes, 10 µl of Trypan blue dye was made. We added 10 µl of the cell suspension to the trypan blue solution and then homogenized the mixture. The hemacytometer was cleaned using 70% ethanol, and the slip closed before being dried. Place 10 µl of trypan blue cell solution on one side of the chamber. The number of healthy cells and cells per ml were counted. Incubation at 37 °C and 5% CO_2 gas for 24 h (or until the cells are 70% confluent) followed by seeding/cell culture on 96 well plates.

Treatment of cells with a sample, positive control, and negative control cells

The stock sample was diluted to eight concentrations ranging from 2.34 to 300 µg/ml) on eight 1.5 ml microtiter plates, each labeled with appropriate concentrations. They removed cells from the incubator, labeled them, and removed the medium from each well. A micropipette was used to transfer 100 µl of each sample and cisplatin positive control from a microtube into each of the matching wells on a well plate 96 containing cells and incubated again for 48 h.

Presto Blue reagent addition and absorbance measurement

Each well's media was removed using "PrestoBlue™ Cell Viability Reagent" in a ratio of 10 µl per 90 µl of media, the solution was diluted to 100 µl per well in the microplate and incubated for 1–2 h until a color change was observed. (PrestoBlue reagent is reduced from a blue compound resazurin without an intrinsic fluorescent value to a red compound resorufin that is highly fluorescent). The number of metabolically active cells is directly related to the conversion value; hence it can be objectively quantified. Resazurin and resorufin absorbance spectra are used to measure absorbance. A multimode reader was used to measure the absorbance at 570 nm (reference: 600 nm).

Molecular docking of Mn(II)Prolinedithiocarbamate

MnProDtc complex was predicted canonical SMILE by application with (http://www.cheminfo.org/flavor/malaria/Utilities/SMILES_generator_checker/index.html), and a three-dimensional Structure was created using online Corina modeling https://www.mn-am.com/online_demos/corina_demo [15]. The complication compound's Structure interacted with protein O(6)-methylguanine-DNA methyltransferase (MGMT), caspase-8, and estrogen receptor alpha to assess its potential anticancer action [16–18]. 3D Structure of the protein was downloaded from the Protein Data Bank (PDB) database with the respective IDs, respectively 1QNT, 1QDU, and 6d0F. The Structure of the complex compound MnProDtc obtained canonical smile code, namely O = C2O[Mn]1SC(S1)N3CCCC23.

Preparation of the Molegro virtual docker five software included eliminating undesired ligands, proteins, or proteins from the complex molecules. Furthermore, the target protein was predicted to be a cavity (active site of the protein) with a maximum expand van der Waals parameter of 10. The compound were docked in the protein cavity respectively

O(6)-methylguanine-DNA methyltransferase (MGMT) X 1.29 A; Y 46.8 A; Z 55.6 A; Volume 35.84 A3; and Surface 131.84 A2, caspase-8 X-2.4 A; Y 29.5 A; Z 77.4 A; Volume 459,776 A3 and surface 1177,6 A2 and Estrogen receptor alpha X -17.2 A; Y 6.5 A; Z 49.3 A; Volume 196.6 A3; surface 451.84 A2. Each radius used is 15. A MolDock Score Grid 0.30A, a total MolDock Score, and a Rerank Score for docking. The energy of the bond in kJ/mol, as measured by the docking result [19]. PyMol software is used to superimpose the docking results with the prepared protein.

Results and discussion

MnProDtc physical and chemical characteristics

The synthesis of MnProDtc was successfully carried out, and the yield obtained was 23.70% with a melting point of 229–230 which indicates the complex has a high purity level. The conductivity value of the Mn(II) complex is 0.02 mS/cm, which shows the MnProDtc complex is not an electrolyte. The schematic of the reaction for the synthesis of MnProDtc can be seen in Fig. 1.

The reaction steps starting from CS2 are reacted with the amino acid proline. The resulting reaction forms the unstable iminium ion, KOH base is added to stabilize the iminium ion formed. The H^+ ion coupled to the iminium ion will attract the OH^- ion from the KOH base to produce an H_2O molecule, while the K^+ from the KOH base will interact with the sulfide. The sulfide group experiences electron resonance during the contact, enabling the K^+ and sulfide interactions to be separated. $MnSO_4$ was added to ethanol under identical circumstances to generate the MnProDtc complex, the final product of the synthesis. The bond between the metal and the ligands of the complex compound formed can be predicted by looking at the Molecular Structure of the angle and length of the bond. Bond length and angle can be seen in Fig. 2 and Table 1. The bond between Mn-S has a bond length of 2.304 Å which shows the properties of a covalent bond; Mn-C2 bond length is 1.865 Å with a C-Mn-S bond angle of 110.6°. This bond angel is compatible with S-Cd-S from ligand dithiocarbamate [20].

Spectroscopy UV–Vis characterization

Spectroscopy UV–Vis analysis of the MnProDtc complex in the band I revealed an absorption band at 217–263 nm, corresponding to the CS2 group $\pi \rightarrow \pi^*$ Intra ligand transition. Precisely in the 250–300 nm absorbance range, this is owing to the nitrogen atom's hyperconjugation activity by the R group [21]. In the MnProDtc complex, band II shifts to 411 nm, an intra-ligand transition $n \rightarrow \pi^*$ of the

Table 1 MnProDtc structural characteristics (Å, °) are of particular interest

Bond lengths		Bond angles	
S1-C5	1.442	S1-C5-S2	115.4
S2-C5	1.816	S2-Mn-C2	110.6
S2-Mn	2.304	C2-C3-C1	106.0
Mn-C2	1.865	C3-N-C5	126.3
C2-C3(inter ligand)	1.351		
C3-N	1.370		
N-C2	1.445		
N-C5	1.461		

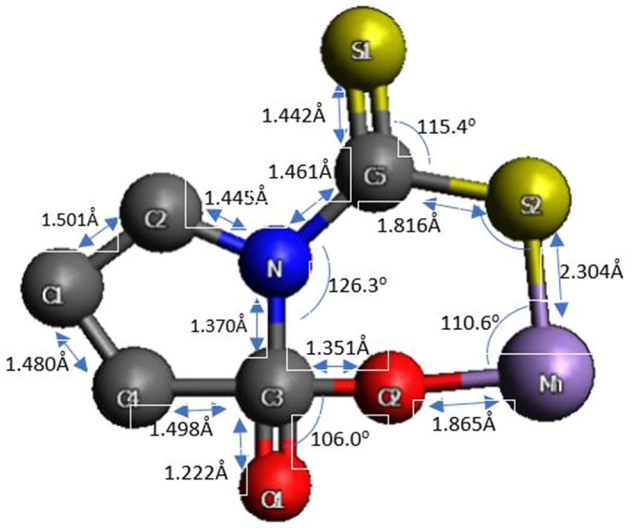

Fig. 2 Complex MnProDtc bond length and angle schematic (Avogadro's application)

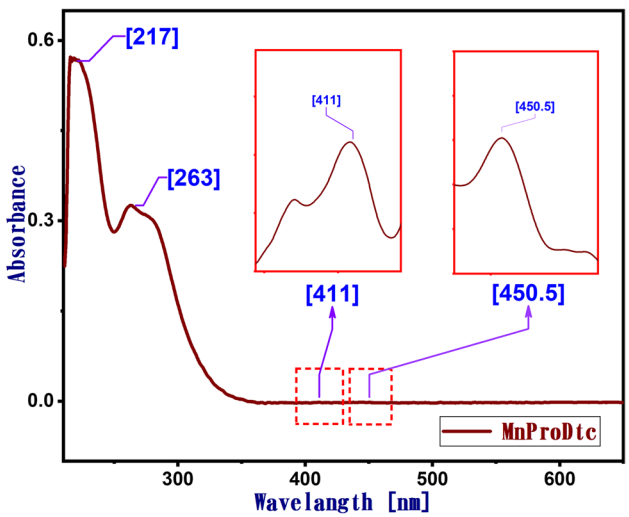

Fig. 3 UV–Vis spectrum of MnProDtc

N=C=S group. In the absorption spectrum of the UV–Vis, the complex's more extensive conjugation system than the ligand causes band III to emerge at a wavelength of 667 nm as well (Fig. 3).

Bandgap energy calculated from the difference between HOMO and LUMO is shown in Fig. 4 with a value of 1.447 eV. Where this value includes a semiconductor that has similarities with NPS ZnO synthesized by Abdolmohammadi et al. as a crucial feature that influences its toxicological reaction at the cellular level due to its semiconductor bandgap energy and the ability to deliver more radicals to diffuse into cancer cell membranes [22]

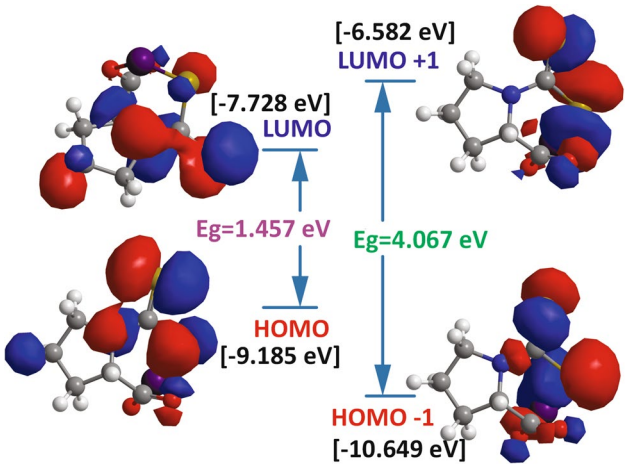

Fig. 4 The HOMO, LUMO energy gap of Complex MnProDtc

FT-IR characterization

FT-IR analysis aims to identify the functional groups in the synthesized complex compound and identify the bonds between metals and ligands [23]. FT-IR analysis was carried out at a wavenumber of 300–4000 cm^{-1} (Fig. 5). The ligand's tion group (C=S) interacts with Mn metal, as shown by an infrared absorption peak of 364 cm^{-1}. Oxygen ligands interact with Mn metal, as demonstrated by an absorption peak with a wavenumber of 466 cm^{-1}. Absorption at

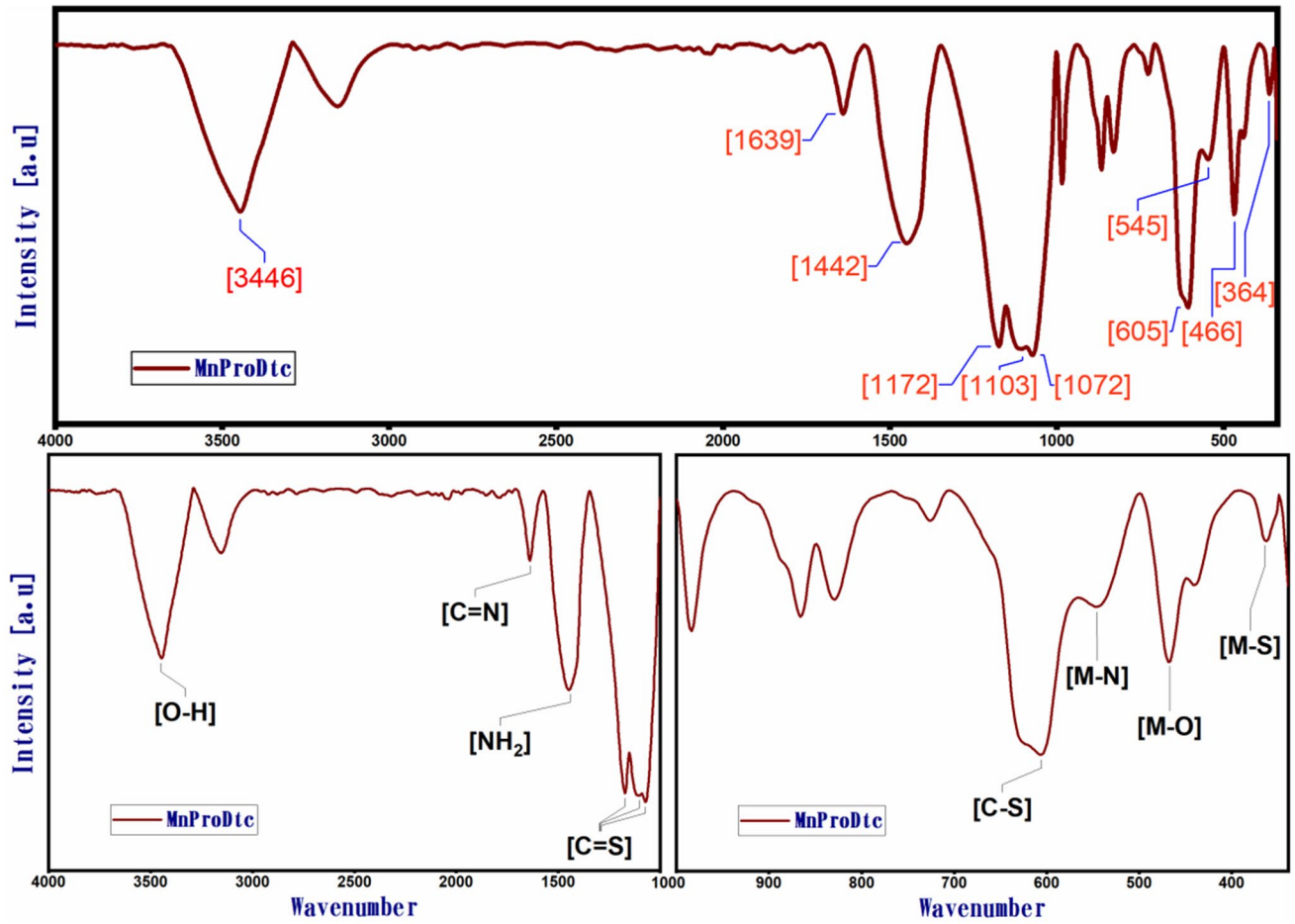

Fig. 5 FT-IR Spectrum of MnProDtc

545 cm^{-1} reflects the binding of Mn metal ions to the N ligand atom [23]. The absorption bands at wavenumbers 1072, 1103, and 1172 cm^{-1} indicate the presence of a functional group C=S in the dithiocarbamate ligand, which suggests bidentate properties of the dithiocarbamate moiety [24, 25]. There is a C=N group present, as seen by the absorption peak at 1639 cm^{-1} [26, 27]. The FT-IR results of the MnProDtc complex generally showed the characteristics of the synthesized complex and proved the success of complex synthesis.

Analysis of XRD data

MnProDtc complex compound were analyzed by X-ray diffraction (XRD), and the results were analyzed using the Match software (Fig. 6). There are two polycrystalline phases, namely MnO (manganese(II) oxide) and MnS (manganese(II) sulfide). X-ray diffraction peaks at 2θ values, 29.70°, 30.96°, 48.18°, 51.74°, and 61.42° corresponded with hkl value 111, 200, 202, 311, and 222 identified of MnS based on X-ray diffraction patterns from standards to powders (PDF file 96-900-5940) and has similarities to those published by Li et al. [28]. The diffraction peaks at 2θ values, 37.98, 44.22, and 64.58 with hkl values respectively 111, 202, and 202 identified MnO by the X-ray diffraction powder pattern standards (PDF files 96-400-1900) and comparable findings by Touqeer et al. [29].

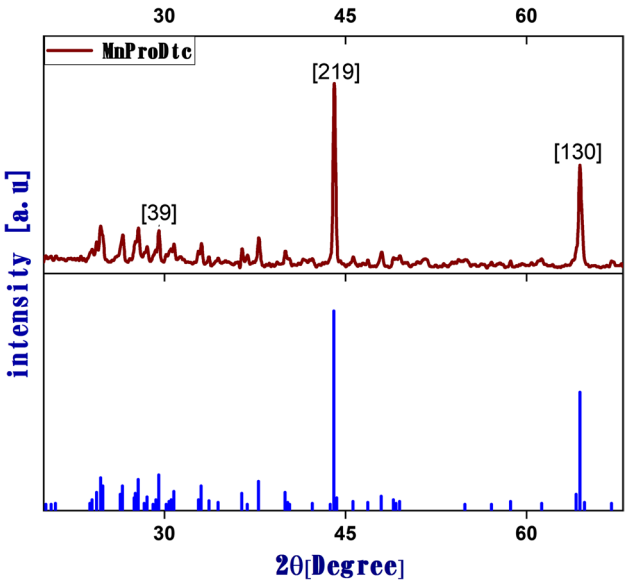

Fig. 6 XRD Spectrum of MnProDtc

MCF-7 cell cytotoxicity test results

We performed an in vitro cytotoxicity assay using MnProDtc complex against MCF-7 breast cancer cells to see how it compares to Cisplatin, The most commonly used drug against breast cancer cells. Test for complex compound were carried out within 48 h with various concentrations ranging from 2.3 to 300 µg/ml (Table 2). MnProDtc obtained from the regression equation $Y = -0.0007\ X + 0.6563$ (Fig. 7) was 453.96 µg/ml, and the regression equation of cisplatin $Y = -0.0061\ X + 0.6383$ (Fig. 8). The IC_{50} value of the complex MnProDtc obtained was 453.96 µg/ml while the IC_{50} of Cisplatin was 53.48 µg/ml.

MCF-7 cells underwent apoptosis with the addition of MnProDtc, which is illustrated in Fig. 9. The figure shows that MCF-7 cells began to undergo apoptosis at a concentration of 37.50 µg/ml), indicating the MnProDtc complex has activity against cancer cells. The IC_{50} value obtained

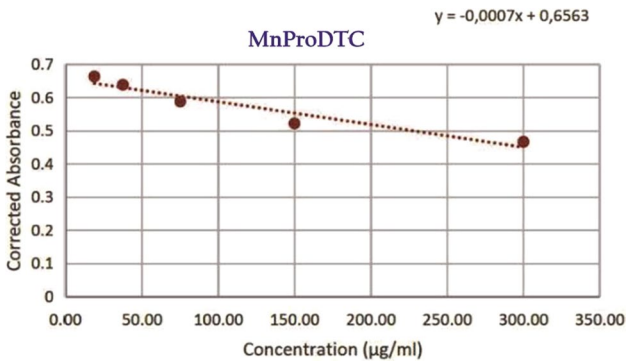

Fig. 7 Cytotoxicity Curve of MnProDTC

Table 2 Absorbance Results of MnProDTC against MCF7 cells

Wavelength/nm	Media	Media + Cell	Cisplatin	DMSO 2%	Sample Concentration (µg/ml)							
					300.00	150.00	75.00	37.50	18.75	9.38	4.69	2.34
570	0.4811	0.8023	0.6349	0.8284	0.7117	0.7336	0.7486	0.7570	0.7783	0.8035	0.7947	0.7998
	0.4859	0.7878	0.6149	0.7871	0.7210	0.7290	0.7618	0.7866	0.7784	0.8103	0.7988	0.7955
600	0.6179	0.2915	0.4804	0.2820	0.3814	0.3381	0.3097	0.2756	0.2638	0.2536	0.2388	0.2466
	0.6227	0.2667	0.4911	0.2531	0.3898	0.3521	0.2969	0.2633	0.2374	0.2450	0.2370	0.2427
Corrected absorbance	−0.1368	0.6477	0.2913	0.6833	0.4672	0.5324	0.5759	0.6183	0.6514	0.6867	0.6927	0.6902
		0.6579	0.2607	0.6708	0.4681	0.5137	0.6017	0.6601	0.6779	0.7021	0.6987	0.6896

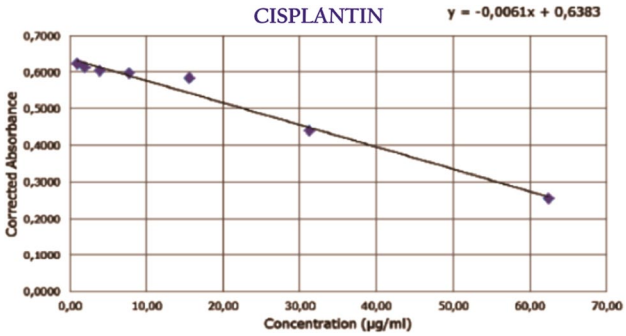

Fig. 8 Cytotoxicity curve of cisplatin

belongs to moderate cytotoxicity, which is 100–1000 µg/ml [30], so MnProDtc has moderate cytotoxicity to MCF-7 cancer cells. Figure 10 shows the results of the MnProDtc well plate test against MCF-7.

Molecular Docking of MnProDtc

DNA docking simulation In-silico molecular docking was used to determine the most likely DNA binding site for metal complex interaction and to determine their binding affinity. Molecular Docking is an initial tool to examine the anticancer properties of metal complexes theoretically. Molecular docking analysis in silico has been extensively used to understand the molecular affinity of small anticancer agents with targeted biomolecules like DNA and cell proteins [31]. In this study, in vitro cytotoxicity of the MnProDtc complex was compared with the results of the in silico test through molecular docking studies. The in vitro and the in silico tests showed that the MnProDtc complex compound could inhibit the growth of MCF-7. Target ligand and protein binding sites were determined by examining the in silico data using Discovery Studio ver 21.1.1.

As a result of averaging the total of grid scores, moldock scores, and rerank scores, the bond energies were reported as the mean standard deviation. The in silico test showed that

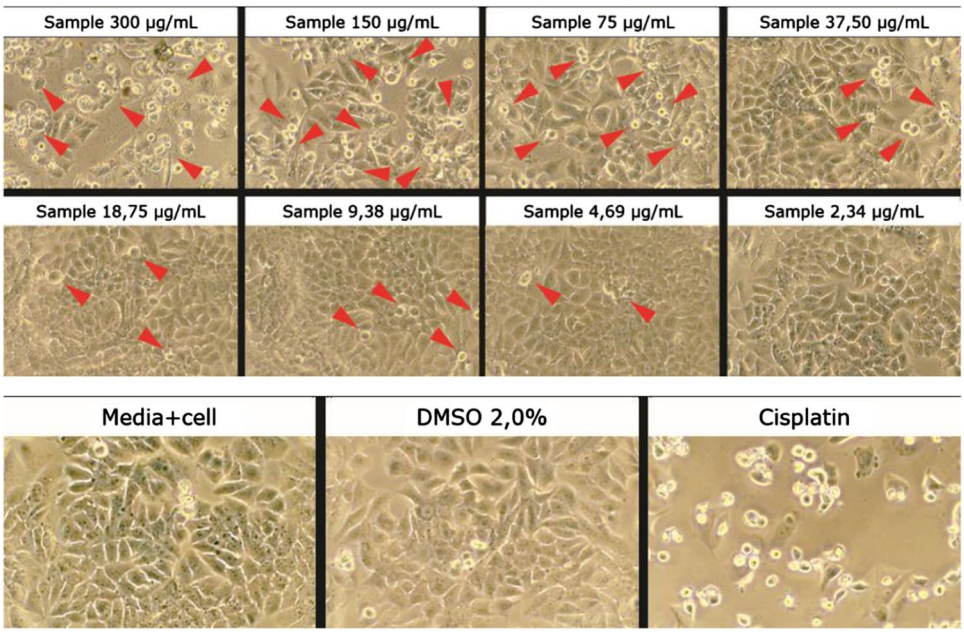

Fig. 9 Apoptosis of MCF-7 cells induced by MnProDtc

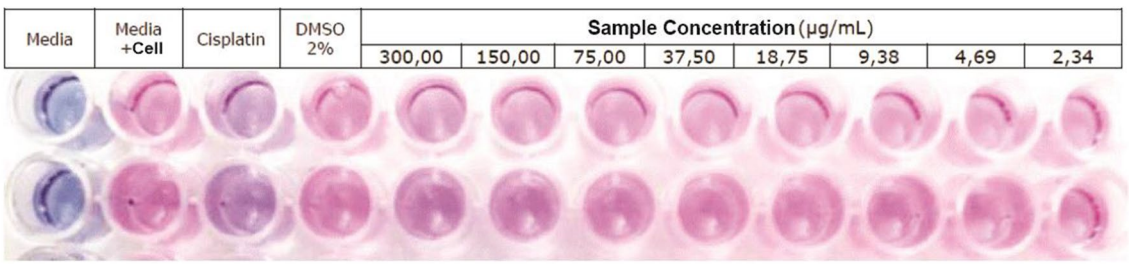

Fig. 10 Well plate documentation of MnProDTC test results against MCF-7

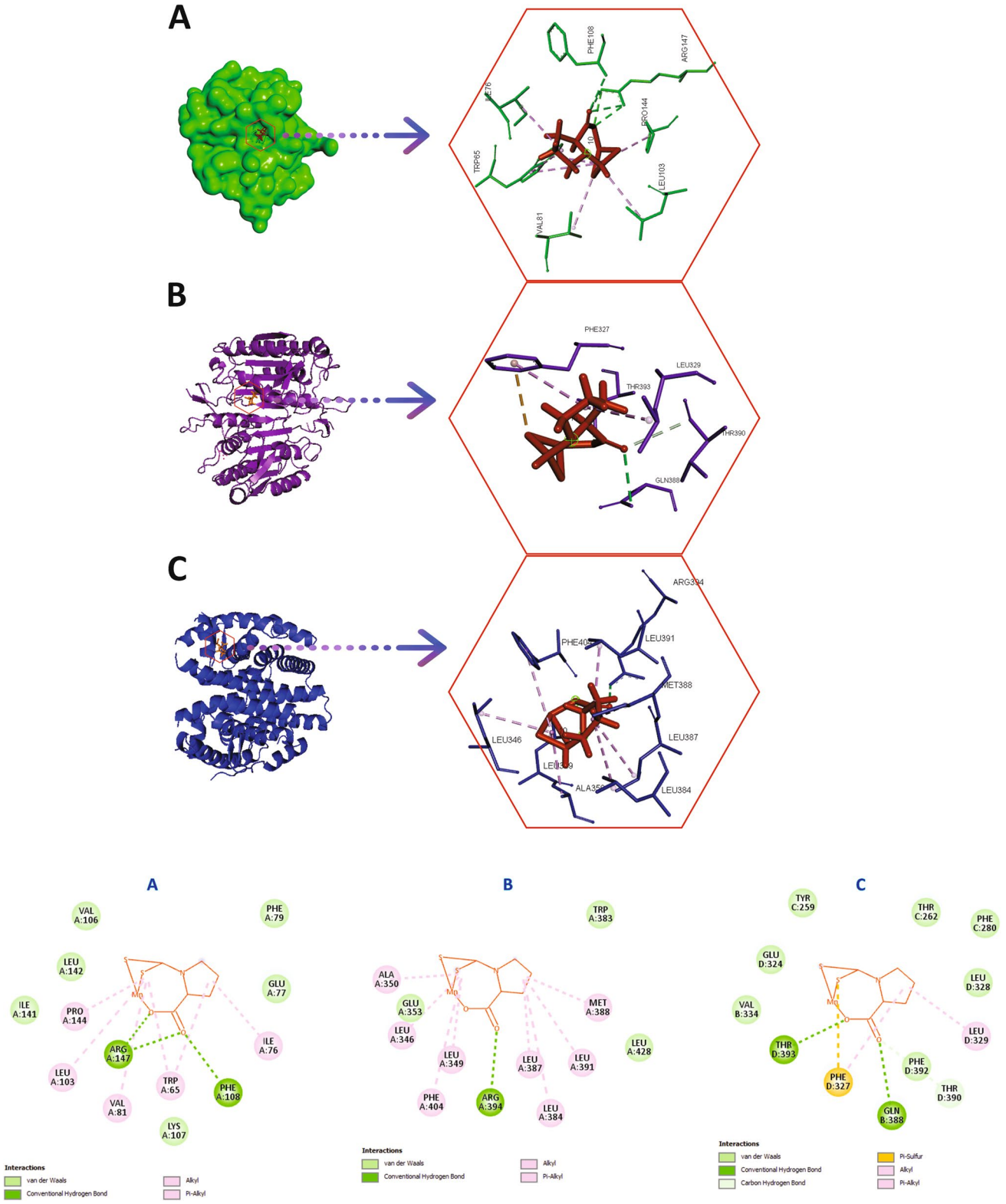

Fig. 11 Interaction between MnProDtc (**A**). O(6)-methylguanine-DNA methyltransferase (MGMT) protein, green indicates protein, red indicates compound-complex (**B**). Caspase-8 protein, purple denotes the caspase-8 protein, whereas red denotes a compound complex (**C**). Estrogen receptor protein, the color blue denotes an estrogen receptor protein, whereas the color red denotes a complex

the MnProDtc complex compound could inhibit the growth of MCF-7 through Protein O(6)-methylguanine-DNA methyltransferase (MGMT), Caspase 8, and Estrogen receptors. MGMT with MnProDtc complex showed an active site against Phe108, Arg147, Val81, Leu103, Pro144, Ile76, and Trp65 which could affect the DNA methylation process so that it could inhibit cancer cells (Fig. 11).

The bond energy of the MnProDtc complex showed low energy of -199 ± 7.07 kJ/mol. Negative ΔG° value indicates that the protein-complex formation is spontaneous [32]. In summary, it can be proposed that the MnProDtc complex with MGMT can eventually stop the growth of cancer cells. MGMT consists of an N-terminal at residues 4–85 and a C-terminal at residues 92–176. The N terminal consists of

Table 3 Interaction between MnProDtc on O(6)-methylguanine-DNA methyltransferase (MGMT) protein

Ligand–Protein Complex/Binding energy (kJ/mol)	Interaction	Distance (A)	Category	Types	Donor	From chemistry	Acceptor	To chemistry
MnProDtc–O(6)-methylguanine-DNA methyltransferase (MGMT)–199 ± 7.07	A:PHE108:N–:10:O1	3.14316	Hydrogen Bond	Conventional Hydrogen Bond	A: PHE108:N	H-Donor	:10:O1	H-Acceptor
	A:ARG147:NH1–:10:O1	2.88846	Hydrogen Bond	Conventional Hydrogen Bond	A: ARG147:NH1	H-Donor	:10:O1	H-Acceptor
	A:ARG147:NH1–:10:O2	2.80596	Hydrogen Bond	Conventional Hydrogen Bond	A: ARG147:NH1	H-Donor	:10:O2	H-Acceptor
	A:ARG147:NH2-:10:O1	3.08587	Hydrogen Bond	Conventional Hydrogen Bond	A: ARG147:NH2	H-Donor	:10:O1	H-Acceptor
	A:ARG147:NH2-:10:O2	2.89764	Hydrogen Bond	Conventional Hydrogen Bond	A: ARG147:NH2	H-Donor	:10:O2	H-Acceptor
	:10–A:VAL81	5.01146	Hydrophobic	Alkyl	:10	Alkyl	A:VAL81	Alkyl
	:10–A:LEU103	5.25018	Hydrophobic	Alkyl	:10	Alkyl	A:LEU103	Alkyl
	:10–A:PRO144	4.90183	Hydrophobic	Alkyl	:10	Alkyl	A:PRO144	Alkyl
	:10–A:ILE76	4.75311	Hydrophobic	Alkyl	:10	Alkyl	A: ILE76	Alkyl
	A:TRP65–:10	5.33411	Hydrophobic	Pi-Alkyl	A: TRP65	Pi-Orbitals	:10	Alkyl
	A:TRP65–:10	5.10318	Hydrophobic	Pi-Alkyl	A: TRP65	Pi-Orbitals	:10	Alkyl
	A:TRP65–:10	4.92483	Hydrophobic	Pi-Alkyl	A: TRP65	Pi-Orbitals	:10	Alkyl

Table 4 Interaction between MnProDtc on Caspase-8 protein

Ligand–Protein Complex Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types	Donor	From chemistry	Acceptor	To chemistry
Mn(II) Prolinedithiocarbamate–Caspase-8 –215.8 ± 4,08	B:GLN388:NE2–:10:O1	3.13906	Hydrogen Bond	Conventional Hydrogen Bond	B:GLN388:NE2	H-Donor	:10:O1	H-Acceptor
	D:THR393:OG1–:10:O2	2.65448	Hydrogen Bond	Conventional Hydrogen Bond	D:THR393:OG1	H-Donor	:10:O2	H-Acceptor
	D:THR390:C–:10:O1	3.74812	Hydrogen Bond	Carbon Hydrogen Bond	D:THR390:C	H-Donor	:10:O1	H-Acceptor
	:10:S1–D:PHE327	3.7089	Other	Pi-Sulfur	:10:S1	Sulfur	D:PHE327	Pi-Orbitals
	:10–D:LEU329	4.30057	Hydrophobic	Alkyl	:10	Alkyl	D:LEU329	Alkyl
	D:PHE327–:10	4.54314	Hydrophobic	Pi-Alkyl	D:PHE327	Pi-Orbitals	:10	Alkyl

Table 5 Interaction between MnProDtc on Estrogen Receptor protein

Ligand–Protein Complex Binding energy (kJ/mol)	Interaction	Distance (A)	Category	Types	Donor	From chemistry	Acceptor	To chemistry
Mn(II) Prolinedithiocarbamate–Estrogen Receptor α –190.25 ± 8.5	A:ARG394:NH2–:10:O1	2.82891	Hydrogen Bond	Conventional Hydrogen Bond	A:ARG394:NH2	H-Donor	:10:O1	H-Acceptor
	A:ALA350–:10	4.12256	Hydrophobic	Alkyl	A:ALA350	Alkyl	:10	Alkyl
	:10–A:LEU346	4.44881	Hydrophobic	Alkyl	:10	Alkyl	A:LEU346	Alkyl
	:10–A:LEU349	5.17795	Hydrophobic	Alkyl	:10	Alkyl	A:LEU349	Alkyl
	:10–A:LEU384	5.46437	Hydrophobic	Alkyl	:10	Alkyl	A:LEU384	Alkyl
	:10–A:LEU387	4.69449	Hydrophobic	Alkyl	:10	Alkyl	A:LEU387	Alkyl
	:10–A:MET388	4.2232	Hydrophobic	Alkyl	:10	Alkyl	A:MET388	Alkyl
	:10–A:LEU391	4.88713	Hydrophobic	Alkyl	:10	Alkyl	A:LEU391	Alkyl
	A:PHE404–:10	5.32427	Hydrophobic	Pi-Alkyl	A:PHE404	Pi-Orbitals	:10	Alkyl

a -sheet structure of three -strands, B1–B3. It is made up of just the H1 helix. Loops between Leu33 B3 and E57 H1 link the sheet and the helix. Residues E45–G55 are hydrophobic pockets with polar residues and have good electron density for sheet-like stabilization and the hydrophobic side chain of MGMT. The residues G37–A41 have no electron density, while L34–K36 and E42–V44 have weak electron density. C terminal consists of 4-helix (H2-H5), H3 and H4 are binding sites with DNA, helix turn helix elements. Between H4 and H5, there is an active site with a -C145HR- a motif which is a turn residue. The active site of the MGMT protein compound demonstrates binding to C and N termini of the DNA methylation process, which are affected by compound-complex (Table 3).

The complex compound MnProDtc with Caspase-8 showed active sites including Gln388, Thr393, Thr390, Phe327, Leu329, and Phe327. All those indicate that the complex binds to Caspase-8 protein in the same area. The types of bonds shown in complex compounds are hydrogen and hydrophobic bonds, and the interaction of MnProDtc with protein Caspase-8 indicates that apoptosis occurs in cancer cells (Fig. 10, Table 4).

The complex MnProDtc—Estrogen Receptor showed the active site are Arg394, Ala350, Leu346, Leu349, Leu384, Leu387, Met388, Leu391, and Phe404. At the same time, types of complex compound binding with Estrogen receptor alpha protein are hydrogen bonds and hydrophobic interactions. These two types of interactions have strong bonds with low bond energies (Fig. 10, Table 5). Complex binding with the Estrogen receptor can inhibit the Estrogen receptor, which works as a high-affinity cell surface receptor in cancer cells that can bind polypeptide growth factors, cytokines, and hormones [33] so that it has potential as an anti-breast cancer agent.

Molecular docking validated the Mn(II) complex's robust binding to DNA residues. According to the HSAB hypothesis, the Nitrogen atom in guanine, a component of DNA, is essential. At the same time, Mn(II) is acidic, implying a solid link between the Mn(II) and guanine complexes. This is compatible with the attachment of lanthanide metals (Tb and Eu) to the Nitrogen atom in DNA nucleotides [34, 35], and It is consistent with Hud and Feigon's findings that Mn(II) is covalently linked to DNA [36].

Generally, DNA base-pairs give adequate room for planar aromatic intercalators to enter. This mechanism stretches the DNA double helix structure, altering the electron density in the phosphate framework and modifying the conformation of the DNA sugars. Complexes with more alkyl and aryl groups bonded to the central metal give a better biological effect than complexes with fewer numbers. Therefore, the dithiocarbamate ligand significantly contributes to the cytotoxicity of the Mn metal complex against the cancer cell lines. The ligand functions as a carrier and contributes to the complex's lipophilicity, which facilitates the metal's movement to the site where its cytotoxicity properties are exerted [37–39].

Conclusions

The synthesis and characterization of Mn(II) complex with prolinedithiocarbamate ligand have been studied and showed the complex was successfully synthesized with a yield of 23.70%. The complex bandgap energy of the semiconductor category can provide more radicals to diffuse into the cancer cell membrane. in vitro cytotoxicity study of the complex

with MCF-7 breast cancer cells is in the moderate category. The in silico test showed that the MnProDtc complex compound could inhibit the growth of MCF-7 through Protein O(6)-methylguanine-DNA methyltransferase (MGMT), Caspase 8, and Estrogen receptors. MGMT with MnProDtc complex showed an active site against Phe108, Arg147, Val81, Leu103, Pro144, Ile76, and Trp65 which could affect the DNA methylation process so that it could inhibit cancer cells. The reported compound can be considered potential anticancer breast cancer candidate in the future.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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