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Synthesis, Characterization and Molecular Docking of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) Iron (III) Complex as Anticancer Candidate

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Abstract

The Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) complex has been synthesized from the reaction between 1-(2,4-dichlorobenzoyl)-3-methylthiourea and Fe (III) metal ion by reflux method with ethanol solvent at a temperature of 75°C for 7 hours. It was characterized by a hot stage microscope (HSM), UV-Vis, FT-IR, and mass spectroscopy. The % yield of the synthesis result was 97.58%. From the docking study on the ribonucleotide reductase enzyme, the binding affinity value was -7.76 kcal/mol, and the inhibition constant was 2.11 mM. The Bis-(1-(2,4-dichlorobenzoyl)-3 methylthiourea) iron (III) complex compounds can be synthesized and predicted as anticancer candidates.

1. Introduction

According to the World Health Organization (WHO), cancer is one of the leading causes of death worldwide. Global cancer statistics by world region for 2022, based on updated projections from the International Agency for Research on Cancer (IARC). In 2022, there were about 20 million new cancer diagnoses (including nonmelanoma skin cancers (NMSCs)), as well as 9.7 million cancer-related fatalities. According to estimates, one in every five men and women will develop cancer at some point in their lives. Furthermore, the mortality rate due to cancer is estimated to be one in every nine men and one in every twelve women [1].

Ruswanto [2] synthesized the composition of 1- benzoyl-3-methilthiourea and conducted an in-vitro study, demonstrating its anticancer activity against breast cancer. In 2017, docking was carried out between the complexity of its derivatives 1-(2,4-dichlorobenzoyl)-3-methylthiourea against the ribonucleotide reductase enzyme and showed enhanced activity in terms of absorption, distribution, and toxicity profiles [3].

Some deficiencies 1-(2,4-dichlorobenzoyl)-3-methylthiourea are permeability and lipophilicity, which are less supportive for interacting with biological membranes. Therefore, modification involving complexation with metals, such as iron (III), is necessary. Zheng *et al.* [4] have proven that complex compounds made with iron (III) metal have better antitumor activity against K562 leucocythemia cells and BEL7402 liver cancer cells than the free ligand, 2-acetylpyrazine N(4)–methylthiosemicarbazone, with IC_{50} values of 13.7 µM and 38.6 µM, respectively. Mardianingrum *et al.* [5] have also proven that Fe (III) complexes have better thermal compatibility than free ligands.

The selection of Fe(III) for synthesizing thiourea complexes in anticancer applications is based on several compelling reasons. Research has shown significant antitumor activity of Fe(III) complexes, as evidenced in studies that observed enhanced cytotoxic effects against leukemia cells and liver cancer cells compared to the free ligand. As an essential element in various biological processes, including DNA synthesis and oxygen transport, iron can directly affect cancer cell proliferation.

Moreover, Fe(III) can interact with critical enzymes in cancer processes, such as ribonucleotide reductase, which is essential for DNA synthesis, inhibiting it more effectively and preventing DNA replication in cancer cells. Pharmacologically, Fe(III) complexes often exhibit good

stability and solubility in biological solvents. Additionally, they tend to have lower toxicity compared to other anticancer-active heavy metals, such as platinum, making Fe(III) a safer choice that potentially reduces treatment-related side effects [6].

Based on this background, researchers conducted research in developing anticancer drugs by synthesizing complex compositions of compounds 1-(2,4-dichlorobenzoyl)-3-methylthiourea with iron (III) metal. Thus, the synthesis of thiourea-Fe (III) complexes as anticancer agents is grounded in their chemical capability to form stable, biologically active compounds that effectively target and inhibit essential processes in cancer cells. These complexes leverage the biological importance of iron and the functional versatility of thioureas to offer promising pathways in cancer treatment. The synthesized compounds were characterized and validated using Hot-Stage Microscopy (HSM), UV-Vis spectrophotometry, infrared spectrophotometry, and mass spectrometry. Comprehensive testing was also conducted, including docking studies and predictions of absorption, distribution, and toxicity properties.

2. Experimental

2.1. Instruments and Materials

The tools used in this research included analytical scales, measuring cups, watch glasses, a reflux set, a magnetic stirrer, a hot plate, a 250 mL beaker, a steam cup, and a water bath. For characterization and analysis, a Hot-Stage Microscope, a UV-Vis Agilent Technologies Cary 60 UV-Vis Spectrophotometer (5 ppm in DMSO solution), and a Perkin Elmer Spectrum 100 FT-IR Spectrometer (with KBr pressing and scanned at 400-4000 cm^{-1}) were utilized. Computational and software tools included a Lenovo B490 laptop with an Intel® Core™ i3-2348M CPU @ 2.30GHz, 2.00 GB RAM, 32-bit operating system, along with ChemDraw Ultra 8.0, MarvinSketch 5.2, AutodockTools-1.5.6, Discovery Studio version 16.1, Molegro Molecular Viewer 2.5, and PreADMET for molecular modeling and analysis.

The chemicals used were 1-(2,4-dichlorobenzoyl)-3-methylthiourea, $FeCl_3.6H_2O$ (analytical grade), ethanol (analytical grade), HCl (analytical grade), distilled water, structure of 1-(2,4-dichlorobenzoyl)-3-methylthiourea, Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III), hydroxyurea, dimethyl sulfoxide (DMSO), and enzyme ribonucleotide reductase with code 2EUD.

2.2. Synthesis of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) Iron (III)

A solution of 1.333 mmol of 1-(2,4-dichlorobenzoyl)-3-methylthiourea in 30 mL of ethanol (solution A) was prepared. Separately, 0.564 mmol of $FeCl_3.6H_2O$ was dissolved in 10 mL of ethanol (solution B) and slowly added dropwise to solution A under consistent low-temperature conditions. The resulting mixture was refluxed for 7 hours at 75°C with agitation using a magnetic stirrer. Subsequently, the solvent was evaporated using a rotary evaporator, and the precipitate obtained was dried [5].

2.3. Characterization of Compounds

2.3.1. The Purity Test

The sample (±5 mg) was placed in the designated sample holder. Subsequently, the melting point was determined using hot-stage microscopy, which was continuously monitored by a connected computer from the beginning of the process until complete melting occurred.

2.3.2. UV-Visible Spectrophotometer

The 200 ppm sample was dissolved in DMSO and then analyzed using UV-Vis spectrophotometer at a wavelength range of 200-800 nm. The maximum wavelength was seen to have the maximum absorbance [7].

2.3.3. FTIR Spectroscopy

The KBr (15 mg) and 5-10% samples were ground in a mortar until a smooth and homogeneous mixture was achieved. This mixture was then loaded into a pelletizer that had been cleaned and dried with chloroform. The resulting pellet was expected to exhibit transparency upon completing the pelletization process. After opening the pelletizer, the sample pellet was analyzed within the wavenumber range of 4000-400 cm^{-1} [8].

2.3.4. Mass Spectrometer (MS)

A total of 10 mg of sample was inserted into the instrument and subsequently evaporated. Following this, an electron beam with an energy of 70 volts was directed onto the sample, and the resulting spectrum was analyzed [9].

Table 1. Melting point distance of the compounds

Compound	Melting point (°C)
$FeCl_3.6H_2O$	37
1-(2,4-dichlorobenzoyl)-3-methylthiourea	201-203
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	140-142

Table 2. The UV-Vis spectrum result

Compound	A	λ_{max} (nm)
$FeCl_3.6H_2O$	3.609	242
1-(2,4-dichlorobenzoyl)-3-methylthiourea	4.479	291.54
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	1.893	278.82

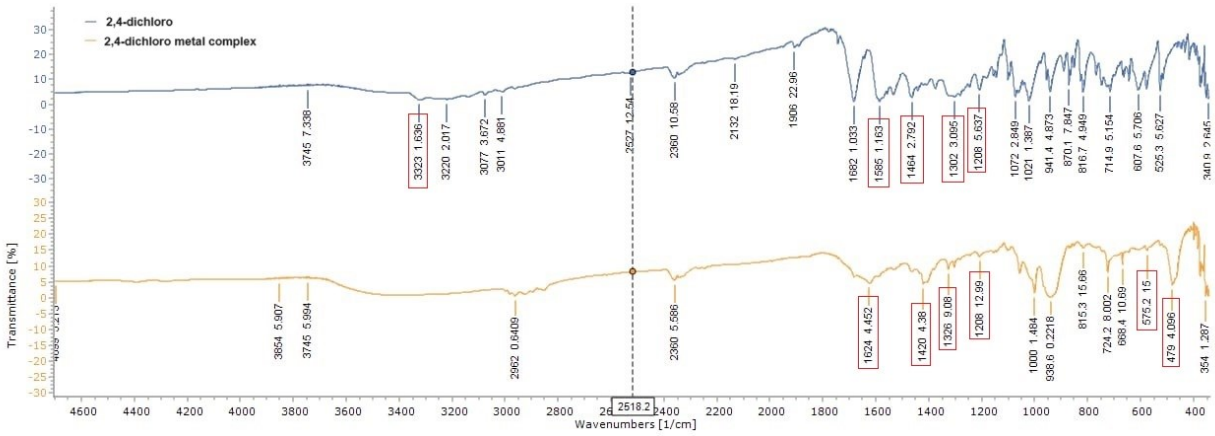

Figure 1. The FTIR spectra of complex compound (orange line) and 1-(2,4-dichlorobenzoyl)-3-methylthiourea (blue line)

2.4. Computational Study

2.4.1. Preparation of Protein Structure and Ligand

The ribonucleotide reductase crystal structure complexed with Gemcitabine (PDB entry 2EUD) [10] was utilized. The structures of the ligands were drawn using MarvinSketch software [11], where they were optimized in 3D format and adjusted for low energy state (protonation at pH 7). Subsequently, the resulting structures were saved in ".pdb" file format for use in molecular docking experiments [3].

2.4.2. Molecular Docking

The molecular docking was conducted using AutodockTools 1.5.6 software, employing a grid box centered at coordinates x: 24.165, y: 54.022, z: 15.925, with a spacing of 0.375 Å. Key parameters analyzed included root-mean-square deviation (RMSD), binding energy values (ΔG), and the formation of hydrogen bonds between the ligands and the receptor [11].

2.4.3. Predicting the Absorption, Distribution, and Toxicity Properties

According to Ruswanto *et al.* [3], the PreADMET program's website can be accessed at http://preadmet.bmdrc.org/. The structures of all compounds were converted into an SDF file format (*.sdf). The program automatically calculated predictive absorption in Caco-2 cells, human intestinal absorption (HIA), plasma protein binding, and toxicity predictions (Ames test).

3. Results and Discussion

3.1. Synthesis of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) Iron (III)

The complexation of 1-(2,4-dichlorobenzoyl)-3-methylthiourea with Fe (III) metal using the reflux method at 75°C for 7 hours with continuous stirring. The stirring process is crucial as it influences the kinetic energy of the molecules, facilitating particle collisions and thereby enhancing kinetic energy [5]. The complex compound yielded 97.85%. It is in powder form, yellow in color, emits a distinctive odor, is slightly soluble in water, and dissolves in ethanol.

3.2. The Purity Test Result

The data in Table 1 reveals that the iron (III) complex has a lower melting point distance than the ligand 1- (2,4-dichlorobenzoyl)-3-methylthiourea. Complex compounds often have lower boiling points than their original ligands due to weaker or more polar bonds within the complex, reduced intermolecular interactions, changes in polarity, and increased volatility. These factors reduce the packing force of molecules and facilitate evaporation [12].

3.3. Identification and Characterization of Metal Complex

The result of the UV-Vis spectrophotometer was the maximum wavelength value (λ_{max}), and the absorbance value (A) of a complex in DMSO solvent was shown in Table 2. The significant difference in λ_{max} values between $FeCl_3.6H_2O$, 1-(2,4-dichlorobenzoyl)-3-methylthiourea and Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) indicates a successful synthesis of the targeted complex compound. Besides that, conjugation involving the lone pair electrons of nitrogen was removed, facilitating charge transfer from the metal to the ligand [13]. The magnitude of the shift of λ_{max}, absorbance (A) and molar absorptivity (ε), and the cleavage energy (10 Dq) of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) complexes are shown in Table 3.

Table 3. The electronic spectrum and 10 Dq value

Compound	Mr (g/mol)	λ_{max} (nm)	υ (cm^{-1})	A	ε ($L\ mol^{-1}\ cm^{-1}$)	10 Dq ($KJ\ mol^{-1}$)
$FeCl_3.6H_2O$	270.3	242	41322.34	3.609	4877.03	494.401
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	584.15	278.82	35865.43	1.893	5258.33	429.112

Table 4. The comparison of FTIR data of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) and 1- (2,4-dichlorobenzoyl)-3-methylthiourea compound

Functional group	υ (cm^{-1})	
	1-(2,4-dichlorobenzoyl)-3-methylthiourea	Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)
-NH	3323	-
C-O	1585	1624
C=C	1464	1420
C-N	1208	1208
$-CH_3$	1320	1327
Fe-O	-	479
Fe-N	-	575

The cleavage energy is a significant parameter in the study of transition metal complexes, particularly those of octahedral coordination geometry. The 10 Dq value is crucial for understanding various chemical and physical properties of metal complexes. Specifically, the difference in 10 Dq between the reactant and the product complex provides significant insights into how the electronic environment surrounding the metal ion has been modified through complexation. This alteration influences the stability, color, and reactivity of the complex. Such information is particularly valuable in the fields of materials science, catalysis, and coordination chemistry research.

The vibration of Fe-O at 479.41 cm^{-1} and Fe-N at 575.22 cm^{-1} are characteristic of complex compounds [14]. The C-O vibration shifts from 1683.6 cm^{-1} in the Fe complex to 1669.6 cm^{-1} in the free ligand [15]. The theoretical vibration range for N-H is typically 3500-3100 cm^{-1}, yet in the complex, no distinct N-H vibration is observed at 3323.16 cm^{-1}. This absence suggests that upon coordination with the central metal ion, the electronic environment around the nitrogen atoms from N-H groups is altered. Coordination likely causes a shift in electron density towards the metal, reducing the polarity of the N-H bonds and thereby hindering their typical vibration at N-H stretching frequencies in the IR spectrum. Additionally, the methyl vibration is observed at 1326.91 cm^{-1} in the complex compound, whereas in the free ligand, it occurs at 1302.31 cm^{-1}. Detailed infrared data are provided in Table 4, and the corresponding FTIR spectra can be viewed in Figure 1.

Table 5. The ΔG values of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III), 1-(2,4-dichlorobenzoyl)-3-methylthiourea, and hydroxyurea

Compound	Binding affinity (kcal/mol)	Inhibition constant (μM)
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	-7.74	2.11
1-(2,4-dichlorobenzoyl)-3-methylthiourea	-6.66	13.10
Hydroxyurea	-3.38	3340

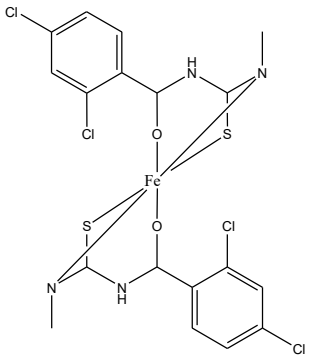

Figure 2. The structure of Bis-(1-(2,4-dichlorobenzoyl)-3 methylthiourea) iron (III)

The determination of molecule weight of complex compounds was based on the result of mass spectrometry [16]. The molecule weight of the complex compound is 585.98 g/mol. The structure of complex compound and mass spectrometry data can be seen in Figure 2 and Figure 3. Based on Figure 2, the methyl group located near the nitrogen atom can affect its electronegativity and electron density. Methyl groups are weak electron-withdrawing groups, and their presence can slightly increase the electron-donating character of nitrogen through inductive effects. This makes the nitrogen more effective in donating electrons to the Fe center, which is crucial for forming a stable coordination bond. The nearest methyl group can influence the polarization properties of nitrogen through hyperconjugation and inductive effects, making it more receptive to the metal center like Fe.

Based on Figure 3, the mass spectrum provides critical data that supports the assertion that the molecular weight of the complex compound is approximately 585.98 g/mol. In the spectrum, the most relevant peak appears at an m/z (mass-to-charge ratio) of 585.9772, which closely matches the theoretical mass of the Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) complex.

This peak is significant as it likely represents the $[M+H]^+$ ion of the complex, where the molecule has been ionized by the addition of a proton during the electrospray ionization process used in the mass spectrometry. The proximity of this observed m/z value to the calculated molecular weight of the complex validates our chemical synthesis and supports the integrity of the molecular structure postulated.

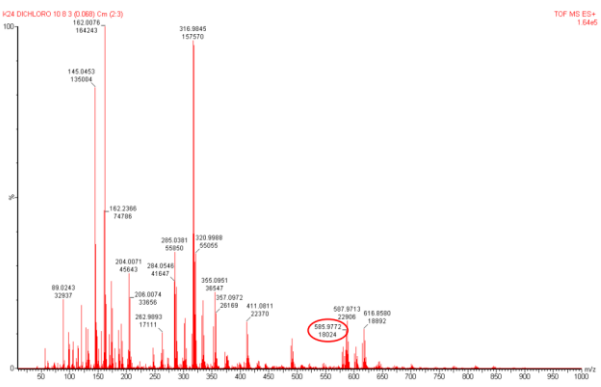

Figure 3. The mass spectrum of complex compound

Table 6. Interaction between ligand and amino acid residues in the receptor

Compound	Hydrophobic bond	Hydrogen bond
1-(2,4-dichlorobenzoyl)-3-methylthiourea	Arg A:293, Ser A:610, Tyr A:155, Met A:606, Ser A:217, Ala A:201, His A:200, Leu A:445, Ala A:446, Thr A:608	Thr A:611, Ser A:202
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	Ala A:245, Gly A:246, Cys A:247, Gly A:247, Leu A:445, Met A:606, Ser A:610, Thr A:611, Ser A:202, Ala A:201, Pro A:203, Asn A:291	Arg A:293, Ser A:217, Thr A:608, Cys A:428
Hydroxyurea	Ser A:217, Pro A:203, Ala A:201, Met A:606, Leu A:445, Thr A:608, Ala A:609, Ser A:612, Tyr A:155	Ser A:202, Thr A:611, Ser A:610

Additionally, the presence of this peak as one of the more intense signals in the spectrum indicates the complex's high purity and stability under the conditions used in the mass spectrometric analysis. This observation confirms the molecular weight of the complex and implies that the complex maintains its structure without significant fragmentation in the mass spectrometer, which is critical for accurate mass determination and further studies on the biological activity of the compound.

In conclusion, the mass spectrum distinctly supports the identification of the complex compound with a molecular weight of 585.98 g/mol, confirming the successful synthesis and characterization of this potential anticancer agent.

3.4. Computational Study

The ligand underwent protonation at a pH of 7.4, corresponding to the physiological blood pH in the body. This protonation process was essential to achieve conformational alignment with the receptor, ensuring an optimal structure where the potential energy is minimized. The water molecules in the structure of receptor RNR were eliminated to expedite the docking process, as the presence of water could extend the duration of the interaction. Additionally, hydrogen atoms were added to the receptor to depict potential interactions with the ligand [17].

The docking method was validated using AutodockTools 1.5.6 to ensure its reliability for future docking research. The method is considered valid if the RMSD value is ≤ 2. A lower RMSD value indicates that the predicted ligand pose closely resembles that of the native ligand [18, 19]. In this validation, an RMSD value of 1.19 Å was obtained, indicating a high similarity between the docked ligand and the native ligand pose [20].

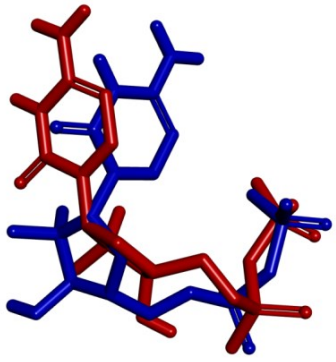

Figure 4. Native ligands (blue), docking ligands (red)

The docking result of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) on ribonucleotide reductase compared with ligand 1-(2,4-dichlorobenzoyl)-3-methylthiourea and hydroxyurea which is a class of anticancer drugs. Based on the docking results, Bis-(1-(2,4-dichlorobenzoyl)-3 methylthiourea) iron (III) exhibits a ΔG value that is lower than that of 1-(2,4-dichlorobenzoyl)-3 methylthiourea and hydroxyurea. The ΔG value indicates the binding affinity of the ligand to the receptor, with lower values indicating higher binding affinity and greater stability of the ligand-receptor complex [21]. Table 5 presents the ΔG values obtained from the docking studies.

Based on Table 5, it is evident that the complex compound Bis- (1-(2,4-dichlorobenzoyl) -3-methyl thiourea) iron (III) exhibits the highest binding affinity compared to the other compounds. Furthermore, the docking results were further analyzed using Discovery Studio Visualization to examine interactions such as hydrogen bonds and hydrophobic interactions between the compound and the receptor [19]. The hydrogen and hydrophobic bonds are shown in Table 6. Additionally, a two-dimensional representation of these interactions for the complex can be visualized in Figure 5.

Table 6 can be explained that Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) compound-complex interaction with four hydrogen bonding with Arg 293 Ser 217, Thr 608, Cys 428 and have interaction with twelve hydrophobic bonding. Hydrogen and hydrophobic bonds influence the physicochemical of drugs and conformation stability between ligands and receptors [11].

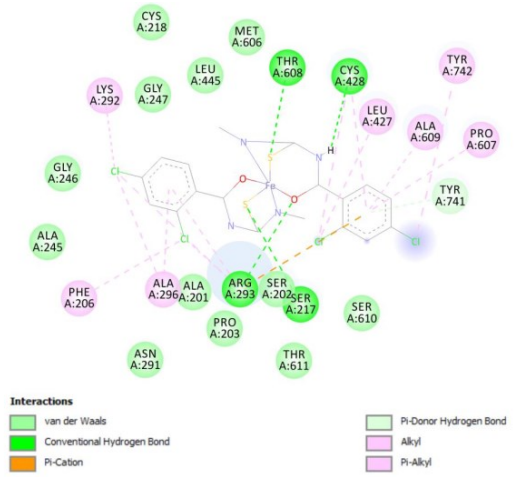

Figure 5. The 2D visualization of the Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)

Table 7. The absorption, distribution and toxicity prediction of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)

Compound	Absorption		Distribution	Toxicity
	HIA (%)	Caco2 (%)	PPB (%)	Ames test
Hydroxyurea	61.07	2.36	4.84	Mutagenic
1-(2,4-dichlorobenzoyl)-3-methylthiourea	94.80	39.18	85.38	Mutagenic
Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III)	97.80	53.64	100	Mutagenic

The absorption and distribution parameters (%Caco2, %PPB, %HIA) were assessed using http://preadmet.bmdrc.kr/. Additionally, the toxicity prediction parameter (Amest test) indicates the mutagenic properties of the compound. The predicted value of the absorption, distribution, and toxicity of Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) can be shown in Table 7.

Table 7 indicates that the %HIA value of the complex compound is 97.80%, categorizing it as well-absorbed, which is superior to the 70% threshold for well-absorbed compounds. The %Caco2 value is 53.64%, indicating moderate permeability, which is higher compared to the other compounds [22]. However, the %PPB value is 100%, indicating strong binding to plasma proteins and suggesting potential challenges in distribution. Regarding toxicity prediction, all compounds are classified as mutagenic, indicating their potential to increase mutation genes and potentially contribute to cancer development [23]. Despite this mutagenic classification, these compounds can still be utilized as anticancer agents with careful dose management [24].

4. Conclusion

The synthesis of the Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) complex was successfully achieved by reacting 1-(2,4-dichlorobenzoyl)-3-methylthiourea with $FeCl_3.6H_2O$, yielding a recovery rate of 97.58%. This complex exhibited significant interaction with the 2EUD receptor in molecular docking studies, demonstrating superior binding affinity (-7.64 kcal/mol) and a lower inhibition constant (2.11 µM) than its precursors. Notably, the complex formed four crucial hydrogen bonds with amino acid residues Arg 293, Ser 217, Thr 608, and Cys 428, and established twelve hydrophobic interactions, highlighting its potential stability and effectiveness as an anticancer agent. The complex exhibits a high HIA rate of 97.80% and moderate permeability, as indicated by a Caco2 value of 53.64%, supporting its potential as a highly bioavailable oral anticancer treatment. Despite showing mutagenic properties in the Ames test, a detailed analysis suggests that the therapeutic benefits of the compound could significantly outweigh its risks with appropriate dosing strategies. These findings provide a promising outlook for the Bis-(1-(2,4-dichlorobenzoyl)-3-methylthiourea) iron (III) complex as an anticancer candidate, warranting further investigation and development to explore its full clinical potential.

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