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# Synthesis and Computational Study of Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) as Anticancer Candidate

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Abstract

Cancer is a disease characterized by cells forming abnormally so that a buildup can cause lumps. Drug compounds used for anticancer treatment by chemotherapy become a severe problem because they have dangerous side effects and can affect patient's quality of life. This study aims to discover new drug compounds with lowered toxicity effects. This was achieved by modifying their structures through synthesis, characterization, and estimating the interactions of the synthesized compounds with specific target receptors, utilizing a docking method. The result obtained was a synthesis yield of 36.2%. The characterization of complex compounds was characterized by the presence of a maximum wavelength of 273 nm and a molecular weight of 652 g/mmol, indicating the absorption of Co-O and Co-S at respective wavenumbers of 498 $cm^{-1}$ and 604 $cm^{-1}$. The docking results showed that the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) complex had the best activity on human estrogen receptor alpha (hER alpha) with a binding affinity value of - 9.40 kcal/mol and an inhibition constant of 0.129 M, which was lower than the comparison compound (cisplatin) and had a better pharmacokinetic profile than cisplatin. This study shows that the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) complex is predicted to have potential as an anticancer candidate.

## 1. Introduction

Cancer is one of the most common diseases causing death after cardiovascular which is defined as a non-communicable disease characterized by the formation of cells that abnormally develop so that there is a buildup and can cause lumps [1]. Based on data from the World Health Organization (WHO) in 2020, the number of causes of death from cancer accounted for almost 10 million people. Even 70 percent of deaths occur in developing countries, including Indonesia. Data from the Globocan from the World Health Organization (WHO) noted that the total number of cancer cases in Indonesia in 2020 reached 396,914 cases and total deaths of 234,511 cases.

Cancer has been managed through the most common treatment, namely chemotherapy, utilizing platinum-based drugs such as cisplatin. However, these drugs are limited due to their potential to cause severe and dangerous side effects, including neurotoxicity, ototoxicity, and nephrotoxicity, which can affect the patient's quality of life [2]. This has increased researchers' interest in finding new anticancer drugs with better activity and lower side effects in non-platinum organometallic complexes. One approach to achieve that involves the structural modification in synthesizing these anticancer drugs.

One type of metal with low toxicity and anticancer activity is cobalt (Co) [2]. Cobalt metal is an element needed in the human body, whose main function is a transport membrane in human blood cells and can prevent the growth of bacteria and fungi [3]. In addition, cobalt (II) is a metal cofactor of coenzyme B12 and vitamin B12 (cobalamin), which is essential for health, maintaining the metabolic system, and helping digest food [4]. This is evidenced by Zhang *et al.* [2], who conducted research involving synthesizing and

characterizing cobalt complexes from 5-chloro-8-hydroxyquinolin (HClQ). The results indicated that cobalt exhibits low toxicity properties and has the potential to serve as an anticancer agent in addition to its notable effectiveness. Moreover, cobalt offered the advantage of lower toxicity than platinum-based anticancer agents. In this current study, the compound complexed with cobalt metal was a thiourea derivative. Thiourea is one of the substances useful in drug research. The results of a previous study stated that thiourea derivatives have potent pharmacological activity as an anticancer [5]. Kesuma *et al.* [6] synthesized two novel compounds derived from N-benzoyl-N'phenylthiourea, specifically N-(3-chloro)benzoyl-N'-phenylthiourea and N-(3,4-dichloro) benzoyl-N'phenylthiourea. The research findings revealed that these compounds exhibit significantly greater anticancer activity against human breast cancer cells compared to the anticancer drug hydroxyurea.

To optimize the anticancer effect, the structure of phenylthiourea compounds is modified based on changes in the compound's lipophilic, steric, and electronic properties. Lipophilic and electronic modifications were carried out according to the Topliss model, which requires the addition of substituents to the benzene ring with the expectation of producing compounds that show greater activity than the initial compound [7]. Adding a chloride group that binds directly to the benzene nucleus and phenyl group improves the lipophilic and electronic properties of the compound 1-(3-chlorobenzoyl)-3-phenylthiourea. Therefore, it increases the ligand's binding affinity to the receptor and improves penetration into biological membranes, leading to increased anticancer activity [6].

Several pathways have been hypothesized for the possible anticancer effects of cobalt complexes, such as reactive oxygen species production, DNA interaction, anti-angiogenic qualities, protein interaction, and signal pathway modification. Cobalt complexes can bind to DNA, obstruct transcription and replication, and cleave DNA, leading to cell death. They have the ability to accelerate the formation of reactive oxygen species, which can lead to oxidative stress and may harm biological components. Certain cobalt complexes play a vital role in impeding angiogenesis, a mechanism essential for tumor growth and dissemination. They can also alter signal transduction pathways and inhibit important enzymes involved in DNA repair. Hypoxia-inducible factor (HIF), a transcription factor connected to tumor growth and metastasis, can also be stabilized by them. Moreover, they have the ability to cause immunogenic cell death, which may strengthen the body's defense against cancer. Research also exists in ligand design [2, 8].

This study aims to modify 1-(3-chlorobenzoyl)-3-phenylthiourea compounds with cobalt metal to increase the activities of these compounds. The resulting cobalt-thiourea complex is expected to establish a stable interaction with cancer receptors and achieve favorable pharmacokinetic properties and low toxicity levels, thereby rendering it a potential candidate for anticancer agents. The resulting complex compounds were characterized and computationally tested.

## 2. Experimental

The research was conducted by synthesizing Bis-(1-(3-chlorobenzoyl)-3-phenylthiourea) Cobalt (III) complex compound and then carried out purity tests using Hot stage Microscopy and Thin Layer Chromatography (TLC). It was characterized and identified using three instruments: UV-Vis spectrophotometer, infrared spectrophotometer, and mass spectrophotometer and followed by computational studies of the synthesis of Bis-(1-(3-chlorobenzoyl)-3-phenylthiourea) Cobalt (III) complex.

### 2.1. Materials and Tools

The materials were 1-(3-chlorobenzoyl)-3-phenylthiourea) ($C_{14}H_{11}ClN_2S$) compound, Metal Cobalt (II) tetrafluoroborate hexahydrate ($Co(BF_4)_2.6H_2O$), silica gel plate $GF_{254}$. Ethanol, methanol, chloroform, ethyl acetate, and n-hexane were of analytical grade. Distilled water, DMSO (Dimethylsulfoxide), technical-grade ethanol, filter paper, breast cancer receptor (PDB: 3ERT), prostate cancer receptor (PDB: 1Z95), and cervical cancer receptor (PDB: 5UU1).

The tools and instruments were a set of reflux, magnetic stirrer (Thermo Scientific Cimarec), hot plate (Thermo Scientific Cimarec), beaker 100 mL (Pyrex), oven (Memmert), Hot-stage Microscopy, UV-Vis Spectrophotometer (Agilent Technologies Cary 60), FTIR Spectrophotometer, hardware and software such as Laptop Asus X441N Processor Intel(R) Celeron(R) CPU N3350 @ 1.10GHz RAM 4 GB, Protein Data Bank (PDB), MarvinSketch 22.19 for preparation the compounds, Autodock Tools 1.5.7. for molecular docking simulation, Biovia Discovery Studio 2021 (Discovery studio visualizer) for visualization 2D/3D of docking results, Molegro Molecular Viewer 2.5. for preparation of the compounds, Desmond Free Academic Version 2019-2 for molecular dynamic simulation, and pkCSM for ADMET prediction.

### 2.2. Synthesis of Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) Complex

A total of 149.8 mg (0.515 mmol) of the 1-(3-chlorobenzoyl)-3-phenylthiourea) compound was dissolved in 20 mL of analytical-grade ethanol in a flat bottom flask, then added drop by drop 102.2 mg of $Co(BF_4)_2.6H_2O$ (0.3 mmol) dissolved in 10 mM of analytical-grade ethanol. The mixture was refluxed for 7 hours at 75°C with a stirring speed of 300 rpm. Applying a temperature of 75°C with continuous stirring was intended to accelerate and optimize the reaction [9]. Subsequently, the refluxed solution was crystallized on a hot plate at 100°C until only a quarter of the solution remained. Afterward, it was left to cool at room temperature for 24 hours, leading to the formation of a precipitate. This precipitate was then filtered, washed with analytical-grade ethanol, and subsequently dried to yield crystalline solids.

### 2.3. Purity Test

Purity tests were conducted using two methods to determine the purity of the synthesized compound. One of these methods involved Hot Stage Microscopy, which evaluated the compound's purity based on its melting distance. A compound was considered pure if its melting distance was ≤ 2°C [10]. The second method was thin- layer chromatography, where purity was confirmed by the presence of a single stain formation [11].

### 2.4. Characterization and Identification of Samples

The results of the study were characterized using several instruments. These included a UV-Vis spectrophotometer to ascertain the maximum wavelength and electronic spectrum of complex compounds, an FTIR Spectrophotometer to identify the presence of typical functional groups in complex compounds, and mass analysis to determine the molecular mass of synthesized compounds.

### 2.5. Receptor Analysis

The receptor was downloaded from the Protein Data Bank (PDB) website at https://www.rcsb.org/structure/3ERT. Subsequently, an analysis of the protein profile was performed to assess the quality of the target protein, which is considered suitable for proceeding to the docking stage if it has a resolution of < 2 Å, a disallowed region of < 0.8%, and a most favored region of > 90% as stated on the Ramachandran plot website at https://www.ebi.ac.uk/pdbsum/ [12].

### 2.6. Ligand Preparation

Marvin sketch 5.2 was used to draw the 2D structure of the test ligand, then protonated to adjust the pH of human blood at pH 7.4 and conformed to determine the most stable structure using the MMFF94 method (Merck Molecular Force Field 94 or energy minimization-based on the force field method). This method can make ligands more stable, close to the initial state during molecular docking, and improve molecular docking results' performance [13].

### 2.7. Validation of Molecular Docking Method

Validation was done by re-docking the native ligand against the active receptor with a grid setting of 40 x 40 x 40 Å, spacing 0.375 Å, and grid center coordinates (x,y,z) for the receptor used. The parameter under examination was the RMSD (Root Mean Square Deviation) value, and it is considered valid if the value is < 2 Å [14].

### 2.8. Molecular Docking

Test ligands were docking against target receptors using a grid box with the same settings at the time of method validation, using an Autodock Tools program with the Lamarckian GA algorithm (GA run = 100). The Lamarckian Genetic Algorithm is frequently used in docking simulations due to its robustness in handling a wide range of datasets, empirical success in numerous investigations, and capacity to explore and optimize the ligand conformational space accurately and quickly [15, 16]. The parameters used were binding affinity and inhibition constant. It is considered good and stable if it has the lowest binding affinity value and inhibition constant [17]. The bond between ligands and amino acid residues in target receptors in 2D and 3D form can be known through visualization using Discovery Studio visualizer software. The results were interactions between amino acid residues, ligands, and hydrogen bonds.

### 2.9. Molecular Dynamic Analysis

Molecular dynamics were performed on one of the best receptors docked with cobalt-thiourea complex compounds using the Desmond program to test the stability of ligand-receptor complex bonds with TIP3P and NaCl 0.15 M model water. "TIP3P" means "Transferable Intermolecular Potential with 3 Points". TIP3P is one of the most popular models for simulating water in molecular dynamics investigations. It is a three-point model because it depicts water as a stiff molecule with three interaction sites, which stand in for the oxygen and two hydrogen atoms. "Transferable" means using it in different situations and substances. TIP3P offers a molecular simulation solution that balances computational efficiency and physical correctness in water modeling, making it appropriate for various applications [18, 19].

The minimization stage was conducted for 100 ps, at 300K temperature and standard pressure (1.01325 bar) for 100 ns with an orthorhombic box, 10x10x10 dimension buffer, and NTP ensemble. The choice of simulation box geometry in molecular dynamics (MD) simulations can significantly impact computational efficiency and simulation accuracy. Orthorhombic boxes offer advantages such as simplicity, computational efficiency, minimal image distance, grid calculations, and ease in handling planar systems. They are straightforward to define and visualize, making them easier to set up and analyze.

However, orthorhombic boxes may be less volume-efficient than other box types, causing shape-related artifacts in small solutes. Non-orthorhombic boxes, like truncated octahedrons, may provide more isotropic pressure coupling for NPT (constant Number of particles, Pressure, and Temperature) simulations. The box type choice depends on the simulation specifics and computational strategy [20]. The energy was recorded at intervals of 1.2 ps. The protein-ligand complex compound was neutralized by adding Na and Cl to maintain the temperature during the MD process. Used dynamic algorithms Noose Hoover and Martyna-Tobias-Klein [21].

### 2.10. Pharmacokinetic and Toxicity Predictions

The structure of the test compound was converted in SMILES format and then analyzed using pKCSM online tools through the https://biosig.lab.uq.edu.au/pkcsm/ website to predict the pharmacokinetic profile of the test compound, including absorption, distribution, metabolism, excretion, and toxicity.

## 3. Results and Discussion

### 3.1. Synthesis of Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) Complex

Cobalt metal is a transition metal with empty orbitals and can accept electron pairs when bonding to ligands to form complex compounds [22]. The complex compound resulting from the reaction between thiourea compounds and cobalt metal is a blue crystalline solid with a yield of 36.2%, shown in Figure 1.

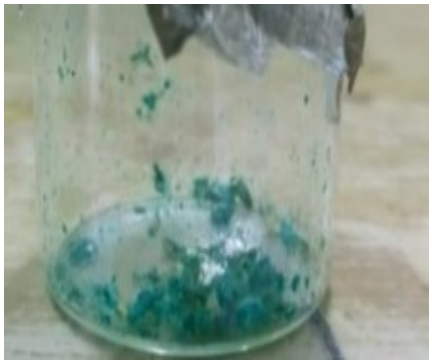

**Figure 1**. The physical form of complex compounds

At the stage of synthesis of complex compounds, there is a coordination covalent bond between 1-(3-Chlorobenzoyl)-3-phenylthiourea and cobalt metal where the 1-(3-Chlorobenzoyl)-3-phenylthiourea act as Lewis bases (electron pair donors) because they have free electron pairs, while cobalt metal acts as Lewis acids (free electron pair recipients). In the resulting complex, the $Co^{2+}$ ion can readily oxidize to the $Co^{3+}$ ion. This is because the crystal field stabilization energy of the $Co^{3+}$ ion, which has a $d^6$ electron configuration, is higher than that of the $Co^{2+}$ ion with a $d^7$ configuration. Therefore, $Co^{3+}$ is more stable in its complex form than $Co^{2+}$ [23].

### 3.2. Purity Test

#### 3.2.1. Hot stage Microscopy

A compound is considered pure when a melting distance of ≤ 2°C is calculated when the compound partially melts to melt completely. The melting distance result data are shown in Table 1.

Table 1 shows that the two compounds have different melting distances, where the melting distance of complex compounds is lower than the initial compound. This shows that intermolecular bonds in complex compounds are weaker than in early compounds, giving elements a lower melting point. In addition, both compounds are said to be pure because they have a melting distance of ≤ 2°C [5].

**Table 1**. Melting range

| Compound | Melting distance |
|---|---|
| 1-(3-Chlorobenzoyl)-3-phenylthiourea | 158-160°C |
| Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | 135-137°C |

#### 3.2.2. Thin-layer Chromatography

Silica gel $GF_{254}$ was employed as the stationary phase, and the mobile phase consisted of a combination of three eluent mixtures with varying polarities. The reason for using mixed eluents as the mobile phase is that it allows for convenient adjustment of the elution strength by blending two solvents. This adjustment enables the optimization of the separation process [24].

**Table 2**. Thin-layer chromatography data

| Compound | Eluent | $R_f$ | Number of Stain |
|---|---|---|---|
| 1-(3-Chlorobenzoyl)-3-phenylthiourea | Chloroform: n-hexane (7:3) | 0.35 | 1 |
| | Chloroform: ethyl acetate (9:2) | 0.525 | 1 |
| | Methanol: ethyl acetate (3:1) | 0.9 | 1 |
| Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | Chloroform: n-hexane (7:3) | 0.475 | 1 |
| | Chloroform: ethyl acetate (9:2) | 0.65 | 1 |
| | Methanol: ethyl acetate (3:1) | 0.875 | 1 |

Table 2 states a difference in $R_f$ values between the initial and complex compounds. This shows that the synthesized new compound has been formed and can be said to be pure because there is a single stain on the TLC plate.

### 3.3. Characterization and Identification

The purpose of UV-Vis analysis in this study is to determine the maximum wavelength and electronic spectrum of the synthesized compound.

Table 3 shows that new complex compounds are successfully synthesized, characterized by a shift in the maximum wavelength to a shorter region (Hypochromic). Hypochromic shifting occurs due to the removal of conjugation, namely the conjugation of the lone pair of electrons, the reduction of the auxochrome group in the compound, and the transfer of charge from the metal to the ligand [25]. In addition, in the complex compounds formed, the cobalt ion binds to the 1-(3-Chlorobenzoyl-3-Phenylthiourea ligand, which is a polydentate ligand and is chelate. The coordination of metal ions with strong ligands will cause the division of the d orbital to increase, thus requiring greater transition energy, resulting in absorption peaks appearing in smaller regions [26].

**Table 3**. Maximum wavelength data of test compounds

| Compound | $\lambda_{max}$ | A | ε ($L.mol^{-1} cm^{-1}$) | 10 Dq (kJ $mol^{-1}$) |
|---|---|---|---|---|
| 1-(3-Chlorobenzoyl)-3-phenylthiourea | 276 | 0.4853 | $28.197 \times 10^3$ | 433.496 |
| Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | 273 | 0.3324 | $42.724 \times 10^3$ | 438.260 |

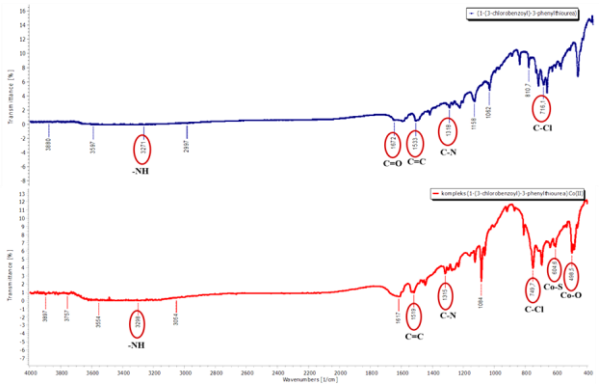


**Figure 2.** Infrared spectrum of the 1-(3-Chlorobenzoyl)-3-Phenylthiourea) and the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt(III) complex

Furthermore, the determination of the electronic spectrum aimed at determining the electronic transition that occurs from the synthesized compound, the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) has a molar absorptivity price of 42.724 x $10^3$ L $mol^{-1}$ $cm^{-1}$. The relatively large molar absorptivity (ε) price indicates that this compound has a strong fluorescence intensity; the greater the molar absorptivity value of the compound, the greater its ability to absorb light [27].

In comparison, the value of 10 Dq is the energy needed to separate d orbitals or excited electrons to a higher energy level when given light energy. The greater the number of ligands attached to the central atom, the stronger the crystal field and the greater the price of 10 Dq. If the value of 10 Dq increases, the maximum absorption will have a shorter wavelength [28].

FTIR analysis aims to predict specific types of typical functional groups of bonds contained in complex compounds. The spectrum FTIR can be seen in Figure 2.

Table 4 shows that the formation of Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) is characterized by vibrations between cobalt metal and ligands in the fingerprint region, Co-S bonds are found in the 604 $cm^{-1}$ wavenumber region and for Co-O bonds are found in the 498 $cm^{-1}$ wavenumber region. This is in accordance with the literature, which states that vibrations of metallic bonds with S groups of ligands typically occur in the region of 650-500 $cm^{-1}$, while vibrations of metals with O groups of ligands tend to appear in wavenumbers between 600-400 $cm^{-1}$ [29].

**Table 4.** FTIR analysis data

| Functional group | υ ($cm^{-1}$) | |
|---|---|---|
| | 1-(3-Chlorobenzoyl)-3-Phenylthiourea | Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) |
| -NH | 3271 | 3298 |
| C=O | 1672 | - |
| C-Cl | 716.1 | 749.7 |
| C-N | 1318 | 1315 |
| C=C | 1533 | 1519 |
| Co-S | - | 604.6 |
| Co-O | - | 498.5 |

### 3.4. Characterization of test compounds by mass spectrophotometry

Mass analysis aims to determine the molecular mass of synthesized compounds, which are then compared with molecular mass predictions using the Marvin sketch application.

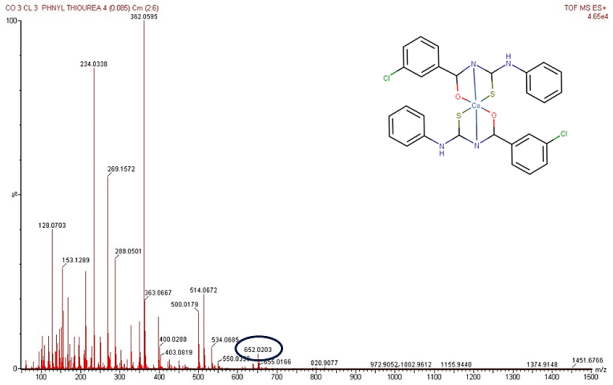


**Figure 3**. Analysis results of mass spectrophotometer

Based on Figure 3, it can be seen that the results of characterization by mass spectrophotometry, the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) obtained a molecular weight of 652.0203 g/mol [M+10H]. This shows that there are identified fragments in the form of element H, contributing to an increase in their molecular mass. The analysis results are similar to the predicted molecular mass of Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) in Marvin sketch, which was 642.48 g/mol.

### 3.5. Computational Studies of Complex Compounds

#### 3.5.1. Receptor Analysis

Receptor analysis aims to determine the quality of the protein amino acids used and whether they are qualified and feasible to continue in the docking process. Receptors were analyzed using Ramachandran plots with the PROCHECK program. PROCHECK analyses the entire geometric model with residues based on residual geometry and gives the stereochemical quality of the predicted model. The parameter viewed is resolution ≤ 2 Å, Disallowed regions < 0.8%, and Most Favoured regions > 90% [6].

Table 5 shows that all three target receptors have a resolution of < 2Å and have residues in the Most Favoured regions of more than 90%. The number of amino acid residues in the prohibited disallowed regions [X, X] is less than 0.8%. From these data, it can be said that the structure of all three receptors is valid and has good quality.

**Table 5.** Receptor analysis

| Plot Ramachandran | Receptor | | |
|---|---|---|---|
| | 3ERT | 1Z95 | 5UU1 |
| Resolution | 1.9 Å | 1.8 Å | 2.0 Å |
| Most favored regions | 91.2% | 94.4% | 90.3% |
| Disallowed regions | 0.0 | 0.0 | 0.0 |

### 3.5.2. Preparation of Receptors and Ligands

Generally, the structure of macromolecules downloaded from the protein data bank site is not pure because it still contains water molecules and other residues. Therefore, it is essential to perform preparation procedures to make these macromolecules used in the docking process run optimally. Receptor preparation separates native ligands from target receptors because native ligands bind to the active side of the receptor to prevent other ligands from binding. In addition, the presence of water molecules can influence binding results, as water may interact with the compounds meant to bind to receptors, necessitating their removal [30]. At this stage, the ligand in protonation adjusts the pH in human blood to about 7.4. Then, it is confirmed by choosing the lowest and most stable binding energy to interact with the active side of the receptor.

### 3.5.3. Docking Method Validation

Docking method validation aims to ensure that the chosen method meets the validity requirements, can be used for testing other compounds, and can minimize errors by looking at the produced RMSD value. RMSD (Root Mean Square Deviation) is the distance deviation from the binding position of native ligands with proteins after docking against the actual position of native ligand bonds [31]. The overlay of crystallographic and redocking results can be seen in Figure 4. The docking method is considered good if it has a value of (RMSD) < 2 Å. If the RMSD value is low, the docking result is more like the crystallography [12].

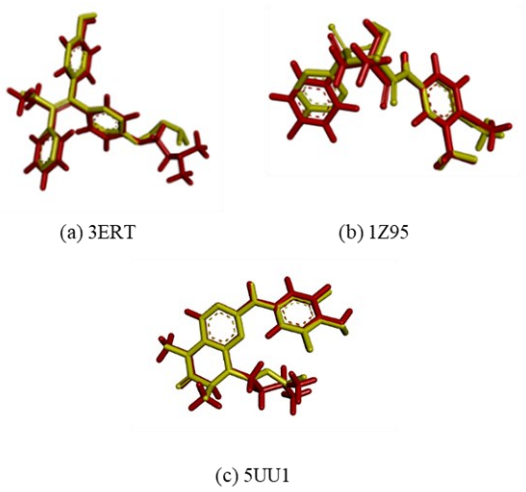


**Figure 4**. The overlay of crystallographic (red) and redocking results (yellow)

**Table 6**. Docking method validation

| Receptor | RMSD (Å) | Binding affinity (kcal/mol) |
|---|---|---|
| Human estrogen receptor alpha (3ERT) | 0.74 | -11.19 |
| Topoisomerase VI (1Z59) | 0.59 | -8.63 |
| Vaccinia-related protein kinases (5UU1) | 0.46 | -8.96 |

Table 6 shows that the validation results of the docking method on the three target receptors have RMSD values of < 2 Å, which states that the three receptors are valid and have met the validity criteria of the docking method so that the receptors can be used further for docking test compounds.

### 3.5.4. Molecular Docking Results

The docking process of the test ligand against the target receptor is conducted using an identical grid box configuration as was established during the method validation phase. The Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) was compared with 1-(3-Chlorobenzoyl)-3-Phenylthiourea and cisplatin. The parameters considered when calculating molecular docking results include binding affinity, which indicates stability and how strong the bond is between proteins and ligands. Good affinity is associated with the lowest ΔG value, indicating that the compound requires less energy for binding. Consequently, this implies a higher potential for the compound to interact effectively and establish strong bonds with the target protein. The inhibition constant is responsible for indicating the concentration required for the ligand to inhibit the activity of the target receptor; a good inhibition constant is in the form of a smaller KI value [13].

The data presented in Table 7 reveals that the ΔG and KI values for Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) and 1-(3-Chlorobenzoyl)-3-Phenylthiourea) against the 3ERT, 1Z95, and 5UU1 receptors are lower when compared to ΔG values among these receptors with cisplatin. This suggests that the complex compounds and compounds initially predicted to have more favorable ΔG and KI values than cisplatin. Among the three receptors used, the 3ERT receptor yielded lower ΔG and KI values than the other receptors. Therefore, the docking of test ligands to these receptors proceeded to the molecular dynamics stage to obtain data in the form of RMSD and RMSF, allowing us to assess the stability of the test ligands.

**Table 7**. Docking test ligands against receptors

| PDB | Compound | ΔG (kcal/mol) | KI (μM) |
|---|---|---|---|
| 3ERT | 1-(3-Chlorobenzoyl)-3-Phenylthiourea | -7.09 | 6.39 |
| | Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | -9.40 | 0.129 |
| | Cisplatin | -5.94 | 44.39 |
| 1Z95 | 1-(3-Chlorobenzoyl)-3-Phenylthiourea | -8.68 | 471.27 |
| | Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | -8.79 | 360.40 |
| | Cisplatin | -3.02 | 6120 |
| 5UU1 | 1-(3-Chlorobenzoyl)-3-Phenylthiourea | -7.23 | 5.04 |
| | Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | -7.58 | 2.80 |
| | Cisplatin | -4.52 | 488.55 |

### 3.5.5. Visualization of Docking Results

The visualization of docking results aims to observe the bond between ligands and amino acid residues from breast cancer receptors PDB 3ERT. In addition to the binding affinity value and the value of the inhibition constant, the parameters that must be considered are the interaction between amino acid residues and ligands and the presence of hydrogen bonds. Hydrogen bonds play an essential role in protein structure because they affect the stability of protein structure. More hydrogen bonds in the ligand-receptor interaction result in greater stability [32].

Besides that, there are also hydrophobic bonds, defined as bonds that avoid the liquid environment and tend to cluster within the globular structure of the protein, where the formation of hydrophobic bonds can minimize the interaction of nonpolar residues with water [33]. In ligand-receptor interactions, hydrophobic interactions are crucial, especially in maintaining the stability of the ligand within the receptor's binding pocket. Although they are not bonds, these interactions help determine the specificity and affinity of molecular binding interactions. The hydrophobic effect, van der Waals forces, molecular form and fit, and hydrophobic pockets are important features of hydrophobic interactions. They majorly affect the receptors' conformational alterations, binding affinity, and selectivity. Hydrophobic interactions can improve a candidate molecule's potency and specificity in drug creation. Hydrophobic interactions are essential in proteins to interact with hydrophobic residues within the binding pocket and stabilize the three-dimensional folded structures. Among the hydrophobic interactions are ligand-binding domains and leucine zipper motifs [34].

The interaction between the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt(III) complex compound and amino acid residues resulted in 15 bonds, while the interaction of the initial compound with amino acid residues revealed as many as 17 bonds, with two hydrogen bonds forming ( MET A: 343, LEU A: 346) (Figure 5). The presence of the same residual bond between native ligands, comparators, complex compounds, and starting compounds shows that the active side of the binding site to the 3ERT receptor is the same. Consequently, this consistency leads to a similar affinity for inhibiting the 3ERT receptor as the native ligand [35].

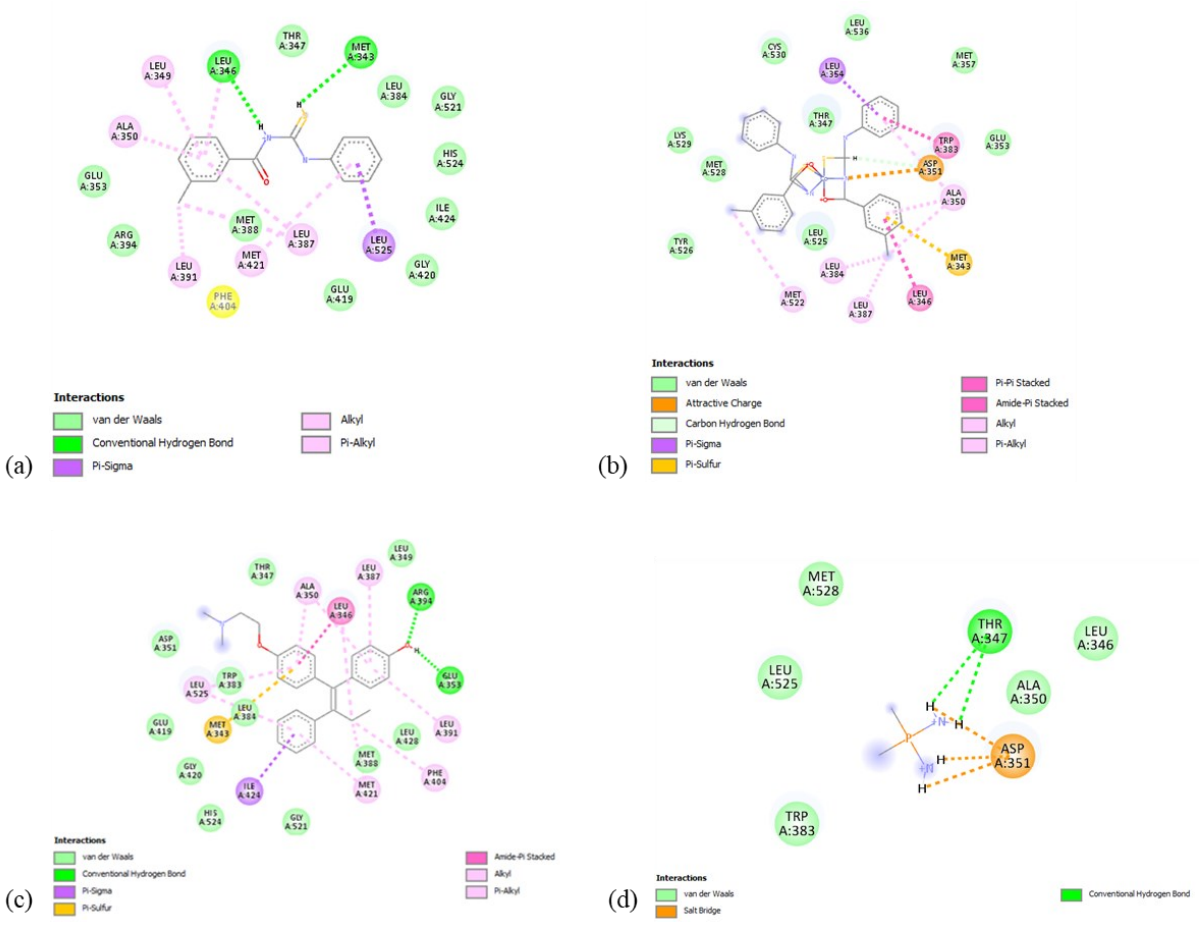


**Figure 5**. Visualization of docking results: a) 1-(3-Chlorobenzoyl)-3-Phenylthiourea, b) Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III), c) Native ligand, and d) Cisplatin

### 3.5.6. Molecular Dynamics

A molecular dynamics simulation was performed to understand better the interactions between ligands and proteins in a flexible state. This is essential because, during molecular docking, the protein is typically in a static conformation, which limits its ability to adapt its shape to accommodate ligands. Besides that, molecular dynamics are also used to evaluate changes in protein conformation, protein structure, and tethered ligands, which can analyze the stability and mechanism of complex-ligand interactions through RMSD at each simulation step based on a predetermined time [36]. The analysis of the results from molecular dynamics simulations focused on two key aspects: Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF).

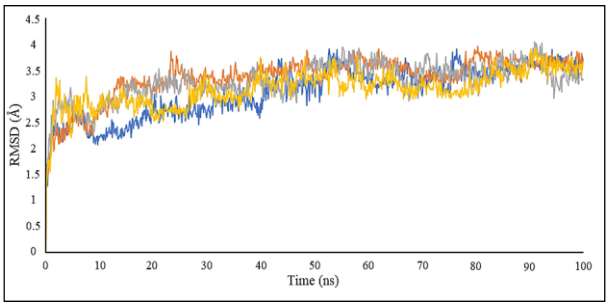


**Figure 6**. RMSD graph of the metal complex (blue), ligand (orange), native ligand (grey), and cisplatin (yellow)

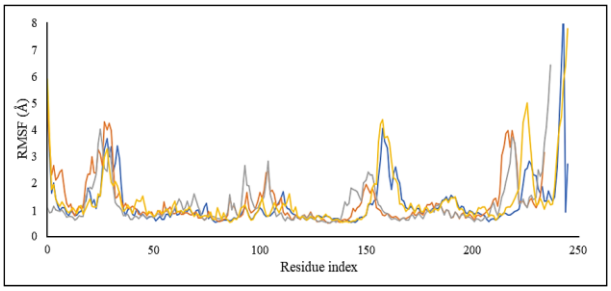


**Figure 7**. RMSF graph of the metal complex (blue), ligand (orange), native ligand (grey), and cisplatin (yellow)

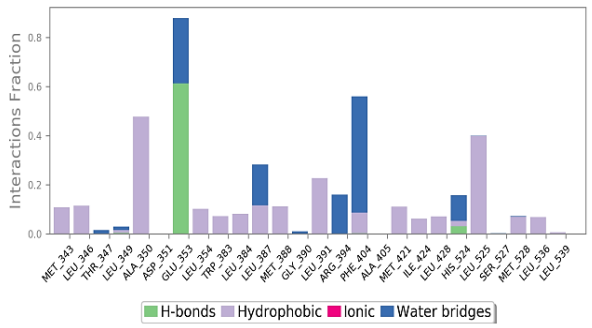


**Figure 8.** Histogram bar graph showing Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) – 3ERT in contact residue

RMSD value aims to show that the protein is stable and undenatured. An RMSD value is considered favorable when it demonstrates minimal fluctuation and typically remains below 0.1 nm. Any sharp fluctuations observed in the RMSD graph suggest the occurrence of protein bonds being stretched to a certain distance, potentially leading to protein denaturation [37]. The ligands selected for molecular dynamics simulation are native, comparison compounds (cisplatin), starting compounds, and the best complex compounds with the lowest breast cancer receptor binding affinity (PDB: 3ERT).

Figure 6 shows that the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) marked with orange lines have stable interactions with average, minimum, and maximum distances of 3.115 Å, 1.279 Å, and 3.941 Å, respectively. An increase in RMSD values signifies the initiation of protein structure unfolding, suggesting that ligands are actively searching for suitable binding sites or coordinates on the protein. In addition, the interaction between residues makes the protein tend to maintain its structure. Conversely, a stable RMSD value indicates that the protein has attained its maximum conformation when bound to the ligand, thereby maintaining its position [36]. Aside from the RMSD value, the representation of interaction stability can also be evaluated using data from the RMSF graph shown in Figure 7.

Root Mean Square Fluctuation (RMSF) shows fluctuations in receptor constituent amino acid residues during the simulation process that can represent residual flexibility. Unlike RMSD, RMSF is calculated against each protein constituent residue by looking at the extent of fluctuations in the movement of each residue during the simulation. RMSF also serves to evaluate fluctuations in sequence numbers of amino acid residues comprising proteins during simulations [38]. The RMSF chart for native ligands, initial ligands, metal complex, and cisplatin reveals that these compounds exhibit residual fluctuations in the same area.

However, complex ligands display lower fluctuations when compared to initial ligands, with an average RMSF value of 1.250 Å for complex ligands, 1.267 Å for initial ligands, and 1.352 Å for cisplatin. The data proved that simulations between complex compounds with 3ERT proteins have better flexibility and interaction than between initial ligands and cisplatin with 3ERT proteins. Several interactions observed during molecular dynamics simulations encompass hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges, as depicted in Figure 8.

### 3.6. Pharmacokinetic and Toxicity Predictions

The pharmacokinetic analysis involves the assessment of several parameters obtained from Human Colon Adenocarcinoma (CaCo2) cells, Human Intestinal Absorption (HIA), VDss value (log L/kg), Blood-Brain Barrier (BBB) permeability (log BB), OCT2 substrate potential, Ames toxicity, hepatotoxicity, and $LD_{50}$ values.

CaCo2 cells are used as parameters to predict the absorption permeability of compounds in the intestine. A compound is considered to have high permeability if its permeability coefficient is > 0.90 and low permeability if the permeability coefficient is < 0.9 [38]. Table 8 indicates that complex and initial compounds are predicted to have a high permeability value of > 0.90. In comparison, cisplatin exhibits a low permeability value.

Human intestinal absorption (HIA) is a parameter used to predict the percentage of compounds the human intestine can absorb. A compound with an absorption rate of less than 30% is regarded as having poor absorption. Conversely, an absorption value > 80% indicates good absorption [39]. Table 8 states that the starting compound, complex compound, and cisplatin have good absorption values.

The volume distribution is the theoretical volume by which the total dose of a drug is evenly distributed to obtain a concentration equal to the blood plasma. The higher the volume of distribution, the more drugs are distributed in the tissue than in plasma. The compound is said to have a high VDss value when the log VD value > 0.45 and a low when the log VD value < −0.15 [40]. Table 8 shows that the volume of distribution for the Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) has a high value so that it can be predicted that complex compounds can be distributed evenly to give the same concentration as in blood plasma. In contrast, the initial compound and cisplatin have low VD values.

The ability of a drug to penetrate the blood-brain barrier is an important parameter that is considered to minimize adverse effects, toxicity, and enhance the efficacy of pharmaceutically active substances within the brain. If a compound has a logBB > 0.3, it is considered proficient at traversing the blood-brain barrier to the brain well, whereas a logBB value < -1 indicates inadequate distribution [34]. Table 8 states that initial compounds and complex compounds can be predicted to penetrate the blood-brain barrier well, while cisplatin is unable to penetrate the blood-brain well because it has a logBB < -1.

**Table 8**. Pharmacokinetic analysis result

| Parameters | Compound | | |
|---|---|---|---|
| | 1-(3-Chlorobenzoyl)-3-Phenylthiourea | Bis-(1-(3-Chlorobenzoyl)-3-Phenylthiourea) Cobalt (III) | Cisplatin |
| CaCo2 | 1.778 | 2.155 | 0.566 |
| HIA | 89.33 | 90.659 | 86.776 |
| VDss | -0.064 | 0.593 | -0.279 |
| BBB | 0.391 | 0.325 | -0.285 |
| OCT2 | No | No | No |
| Ames Toxicity | No | No | Yes |
| Hepatotoxicity | No | No | No |
| $LD_{50}$ | 2.172 | 2.532 | 3.565 |

Organic cation transporter 2 (OCT2) is a renal uptake transporter that plays an important role in renal transport, drug clearance, and endogenous compounds in the kidneys. OCT2 substrates can also cause unwanted interactions when administered together with OCT2 inhibitors. Evaluating potential candidates for transportation by OCT2 provides useful information regarding clearance and potential contraindications. The prediction results stated that the ligand, metal complex, and cisplatin are not renal OCT2 substrates [41].

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The Ames test is a method often used to predict the mutagenic potential of compounds characterized by positive tests that can act as carcinogens [35]. From the prediction results in Table 8, initial and complex compounds are predicted to have no mutagenic potential and do not act as carcinogens. Cisplatin has mutagenic potential and acts as a carcinogen.

The hepatotoxicity test aims to predict toxic compounds in the liver. Table 8 shows that the ligand, metal complex, and cisplatin are predicted to be nontoxic to the liver. $LD_{50}$ (Lethal Dose 50) is a parameter used as an acute toxicity standard to assess the relative toxicity of compounds. The value $LD_{50}$ indicates the dose of the compound that caused the death of 50% of the test animal group [35].

## 4. Conclusion

From the research that has been done, it can be concluded that the Bis 1-(3-Chlorobenzoyl-3-Phenylthiourea) Cobalt (III) was successfully synthesized from the reaction between compound 1-(3-Chlorobenzoyl-3-Phenylthiourea) with cobalt(II) metal with a yield of 36.2%, which is characterized by a shift towards shorter wavelengths (hypochromic) and a new absorption in the wavenumber regions of 498 and 604 $cm^{-1}$, which shows Co-O and Co-S bonds. Based on the results of molecular docking, Bis 1-(3-Chlorobenzoyl-3-Phenylthiourea) Cobalt(III) complex exhibits a stable interaction and demonstrates the most promising activity when interacting with the breast cancer receptor PDB 3ERT, with a binding affinity of -9.40 kcal/mol and inhibition constant of 0.129 µM lower than initial ligand and cisplatin. Moreover, it has a better pharmacokinetic profile prediction than cisplatin. This suggests that the synthesized compound has potential as an anticancer candidate.

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