

Synthesis and virtual screening of bis-(4-(tert-butyl)-N-(methylcarbamothioyl) benzamide)-Iron (III) complex as an anticancer candidate

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Submitted: 31-08-2020

Reviewed: 30-10-2020

Accepted: 12-03-2021

ABSTRACT

Thiourea derivatives were much used in drug discovery and drug-making, such as for an anticancer. The formation of drug complexes can increase lipophilicity through chelation formation, and the drug action is significantly upward due to the effective permeability to the center. In another study, the alteration of the compound becomes the complex with metal will grow in its activity so recently we have synthesized the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide)-Iron (III) complex. The synthesis of Fe (III) metal with the 4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide in ethanol by reflux at 75°C for 7 hours. Hot Stage Microscopy, UV-Visible Spectrophotometry Infrared Spectrophotometry, and Massa Spectrophotometry were used to characterize the complex. This study concerns representing, inferring, and predicting pharmacokinetics and toxicity and molecular docking complexes. The complex weight was 0.29469 g. Its purity has been tested using the melting point determination and has obtained its range was 113°-115°C. The Characteristics of Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide)-Iron(III) complex have a maximum wavelength of 260 nm and provide absorption of Fe-O vibrations at wavenumbers 478.2 cm⁻¹ and 588 cm⁻¹, and the m/z complex of spectrophotometry mass was 559.31. The molecular docking process was performed using AutodockTools-1.5.6 software. It showed that Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III) complex could interact with ribonucleotide reductase enzyme, and it has better interaction than the 4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide with the binding affinity energy (ΔG) of -8.52 kcal/mole and the constant inhibition (K_i) of 568.55 nM.

Keywords: *Complex, Docking, Fe (III), Synthesis, 4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide*

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INTRODUCTION

The World Health Organization (WHO) defines cancer as a large group of diseases that occur because cells grow beyond normal limits. Cancer can spread and infect other parts of the body. The disease has many anatomical and molecular subtypes, each of which requires special treatment. Based on global data in 2018, cancer is the second largest killer with an estimated 9.6 million deaths. (Organization, 2018; RI, 2019).

Thiourea is described as an organic compound containing carbon, nitrogen, hydrogen, and sulfur atoms. Thiourea has a similar structure to urea, but it has an S atom which replaces the O atom in the urea structure. In previous studies, drug discovery researches were frequently use urea as an anticancer. (Lan et al., 2018; Ren et al., 2014), anti-tuberculosis (Brown et al., 2011; North et al., 2013), antimalarial (Domínguez et al., 2005; Schwartz et al., 2015) and as an analgesic (Sartori et al., 1994). Thiourea is considerably used as a discovery of new drugs for anticancer and antitumor (Karakuş et al., 2009; Manjula et al., 2009; Pingaew et al., 2018; Yang et al., 2013; Yao et al., 2012), antibacterial, antimicrobial, and anti-tuberculosis (Bielenica et al., 2018; Binzet et al., 2018; Khan et al., 2008; Seth et al., 2004; Upadhayaya et al., 2009; Vega-Pérez et al., 2012) soluble epoxide hydrolase inhibitor and also as an antiviral (Burmistrov et al., 2018).

In the previous study, we have synthesized an in vitro test to cancer cells of the 4-(tert-butyl)-N-(methylcarbamothioyl) benzamide the IC₅₀ of 111 µg/mL. The 4-(tert-butyl)-N-(methylcarbamothioyl) benzamide is a compound derived from 1-benzoyl-3-methylthiourea (Ruswanto et al., 2018). One of the many advances to drug production is to convert compounds into metal complexes, making their absorption and distribution and their pharmacological activities better (Lin et al., 2008; Mardianingrum et al., 2019; Ruswanto et al., 2019).

The findings of anticancer drugs is done by synthesizing a complex between 4- (Tert-Butyl) -N-Methylcarbamothioyl) Benzamide with Fe (III) metal. It is a new compound in the thiourea derivative. Those were described and characterized using Hot Stage Microscopy (HSM), Infrared Spectrophotometry, UV-Vis Spectrophotometry, Mass Spectrophotometry, Molecular Docking, ADME Toxicity Prediction.

MATERIALS AND METHOD

Materials and instruments

We used graduated cylinder, watch glass, analytical scales, Erlenmeyer Bulb, reflux sets, magnetic stirrers, thermometers, filter paper, hot plates, 50 mL and 100 mL beakers, crucible, 30 mL vials, Hot Stage Microscopy, UV-Vis Spectrophotometer, Genesys 10S, Perkin Elmer Spectrum 100 FT-IR Spectrometer, and Mass Spectrophotometry (MS). The laptop we used having hardware specifications with AMD Ryzen 3 2200U specifications with Radeon Vega Mobile Gfx (4 CPUs), 2.5GHz 4GB 64-bit RAM. And its software were ChemDraw Ultra 12.0, MarvinSketch 5.2, AutodockTools-1.5.6, Discovery Molecular Viewer 16.1, Molegro Molecular Viewer 2.5, and PreADMET.

The materials, we used the 4-(tert-butyl)-N-(methylcarbamothioyl)benzamide, ethanol p.a, FeCl₃.6H₂O p.a, Bis-(4-(tert-butyl)-N-(methylcarbamothioyl) benzamide)-Iron (III) complex, Dimethyl sulfoxide (DMSO), and 2EUD.pdb.

Synthesis of metal complex

The 4-(tert-butyl)-N-(methyl carbomethyl)benzamide as much as 0.992 moles liquefied in 30 mL ethanol (solution A) and 0.496 moles of metal FeCl₃.6H₂O in ethanol (solution B), then it (B) was dropped into solution A little by little. The drip process was under low heating and done gradually. For about 7 hours at 75°C we reflux it while stirring it using a magnetic stirrer. After that, we evaporate it slowly (Mardianingrum et al., 2019).

The purity test with hot stage microscopy

The Bis-(4-(tert-butyl)-N-(methylcarbamothioyl) benzamide)-Iron (III) complex on the slide was set on a hot stage. Then the temperature is gradually increased at a constant speed, and the melting process is observed with a polarization microscope (Šimek et al., 2014).

UV-Vis spectrophotometry

DMSO with a concentration of 100 ppm and 200 ppm, is used to dematerialize standards and samples. Then used the electronic spectrum from a UV-Vis spectrophotometer to measure it in the 200 nm - 800 nm area, and the absorbance was observed based on the maximum wavelength (Ruswanto et al., 2019).

Infrared spectrophotometry

The next process is to take 5 mg of KBr powder to be crushed on a mortal until homogeneous crushed. A cleaned pellet maker which has been dried with chloroform, ready to be used to make pellets transparently. The resulting pellets were then measured in the 4400-400 cm^{-1} wave area to be used as spectra (Ruswanto et al., 2018).

Mass spectrophotometer (MS)

MS settings were executed on capillary 3 kV, sampling cone 20, Extraxtion Cone 2, source temperature 120, Desolvation temperature 250, Flow Cone gas 50 L / hour, Flow Desolvation Gas 800 L / hour, and sampling flow 20 micro L / min. Figure 4 presents the mass spectrum. The molecular ion peak at m/z 559.31, equal to the complex molecular weight, which was detected in the mass spectrum of Bis- (4- (tert-butyl) -N- (methylcarbamothioyl) benzamide) -Iron (III) (Mardianingrum et al., 2019).

Molecular docking

AutodockTools-1.5.6 software was used to create ligands with receptors. Then using 2EUD to retrieve ribonucleotide reductase enzyme data from the PDB website <http://rcsb.org> (Xu et al., 2006). This was done to obtain the value of root-mean-square-deviation (RMSD), Gibbs free energy (ΔG)/ binding affinity for compounds/ ligands and complex compounds. After that, observing the interaction of receptors with ligands and receptors with complex compounds, and using Discovery Studio software version 16.1 to create its 2D visualization (Pal et al., 2019).

PREADMET and toxicity

The process was continued by utilizing PreADMET, a web-based application on <http://preadmet.bmdrc.org/>. After the structure of the compound was converted into molfile *.mol (molfile) format, PreADMET will calculate the predicted absorption for Caco2 cells, HIA (Human Intestinal Absorption), plasma protein binding (PPB) and their toxicity parameters through the Ames test automatically. (Rozano et al., 2017; Yamashita et al., 2000; Yee, 1997; Zhao et al., 2001).

RESULT AND DISCUSSION

Synthesis of the Bis-(4-(tert-butyl)-N-(methylcarbamothioyl) benzamide)-Iron (III) Complex

Ligands are electron pair donors. Each atom has a lone pair that coordinates covalently with the iron. The iron that was reacted to become a ligand will become complex, with the ratio of mole metal:ligand is 1:2. One of them must be significantly larger so that the equilibrium of the reaction changes to the right because $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ can react absolutely with 4-(tert-butyl)-N-(methylcarbamothioyl)benzamide. The result of the reaction scheme is in Figure 1.

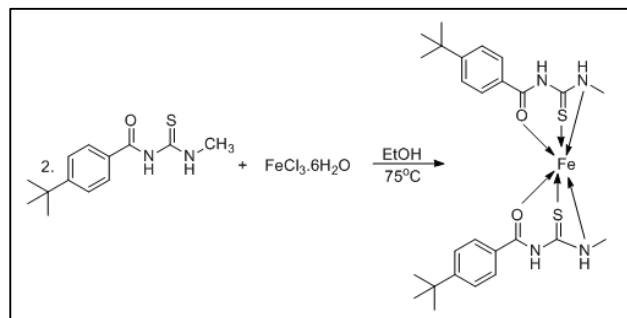


Figure 1. The reaction proposed of ligand and iron (III)

The reflux process needed 7 hours at 75°C. For better results, the process must be longer. The stirring process used a magnetic stirrer. The process will cause the particles to collide with each other, thereby affecting the increase in the kinetic energy of the molecules.

To obtain crystals complex, the reflux result was then slowly evaporated. It was reached 85.85% in powder and brownish-yellow.

The purity test results

The melting point distance check was used to make sure that the compound produced was a new and not a starting material. The compound was assumed to be pure if the melting point difference reach about 2°C. Check on [Table 1](#) for the result.

Table 1. Melting point of the compounds

Compounds	Melting point (°C)
<i>4-(tert-butyl)-N-(methylcarbamothioyl)benzamide</i>	109-111
<i>Bis-(4-(tert-butyl)-N-(methylcarbamothioyl)benzamide)-Iron(III)</i>	113-115

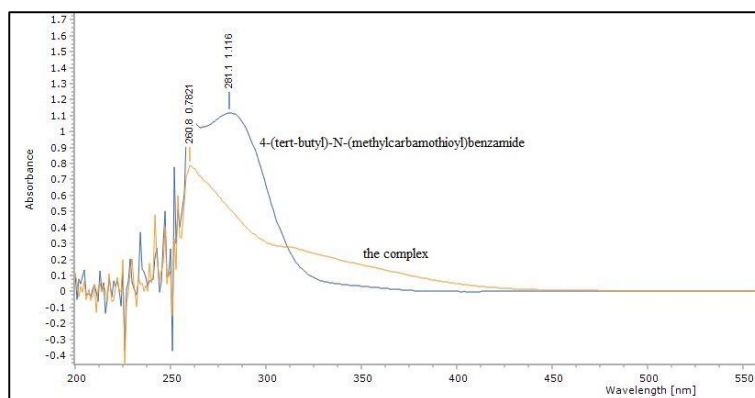
The melting point diversion between the synthesis product and a starting material can illustrate that the new compound has been synthesized powerfully, and it was predicted as the complex.

Characterization and Identification of Complex

As the first structure, the synthesized compounds (MS) were then characterized and identified structurally using IR spectrophotometry, UV-Vis spectrophotometry, and mass spectrophotometry. That was done to set the maximum shift in wavelength to release the spectral profile of it, which it was then compared to the spectral profile of compound 4-(tert-butyl)-N-(methylcarbamothioyl)benzamide and Bis-(4-(tert-butyl)-N-(methylcarbamothioyl) benzamide)-Iron-benzamide (III). Then the results were obtained as in [Table 2](#) which shows the value of the maximum wavelength (λ max) absorbance (A) of the compounds in the DMSO solvent and [Figure 2](#), which is an overlay of the UV-Vis spectra of the starting material and the complex.

Table 2. UV-Visible spectrum results

Compounds	Absorbance	λ_{\max} (nm)
FeCl ₃ .6H ₂ O	3.602	243.00
	3.626	243.00
	3.602	240.00
4-(tert-butyl)-N-(methylcarbamothioyl)benzamide	1.117	281.00
	1.116	281.00
	1.117	281.00
Bis-(4-(tert-butyl)-N-(methylcarbamothioyl)benzamide)-Iron(III)	0.788	260.00
	0.802	260.00
	0.825	260.00

**Figure 2. The overlay of the UV-Vis spectra of the starting material and the complex**

The hypochromic shift occurs due to a more polar solvent that is moving $n \rightarrow \pi^*$ where the organic molecule has an unsaturated functional group so that the double bonds in the group provide the required π orbitals. The ground state in some of the transitioning particles becomes more polar than the excited state. (Santos et al., 2015). There is a shift in the maximum wavelength towards the smaller or hypochromic (blue shift) spectrum of Fe³⁺ (260.00 nm) from the FeCl₃.6H₂O solution to the Fe³⁺ spectrum from Bis-(4-(tert-butyl)-N-(methylcarbamothioyl)benzamide) Iron (III) solution. This indicates a complex formation.

Table 3 shows the size of λ_{\max} shift, absorbance (A) and molar absorption (ϵ), and cleavage energy (10 Dq) of the Bis-(4-(tert-butyl)-N-(methylcarbamothioyl)benzamide) Iron (III) complex.

Table 3. Electronic spectrum and value 10 Dq [13]

Compound	Mr (g/mol)	λ_{\max} (nm)	ν (cm ⁻¹)	A	ϵ (L.mol ⁻¹ . cm ⁻¹)
FeCl ₃ .6H ₂ O	270	242.00	41322.314	3.602	4878.378
4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide	250.36	281.00	35587.188	1.117	1329.365
Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III)	556.56	260.00	38461.538	0.805	2150.309

The next test is infrared spectrophotometry which was carried out to obtain functional group information on the synthetic compounds produced. This complex showed that the C=O vibration absorption is located in 1627 cm⁻¹, which the vibration frequency decreased due to the mesomeric effect. Based on the theory, the C=C vibration absorption is in 1680-1600 cm⁻¹, while the C-N bond strain absorption is at 1326 cm⁻¹. The absorption of metal vibrations with the O group of the ligand will also appear at the wave number 600-400 cm⁻¹. Based on the theory, the metal vibration

absorption with an O group of the ligands will appear at a wavenumber of 600-400 cm^{-1} . The Fe-O vibration absorption analysis result of the complex compound Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron (III) appeared in the 478.2 cm^{-1} and 588 cm^{-1} , and The Fe-N vibration absorption analysis result of the complex compound Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron (III) appeared in the 356.5 cm^{-1} (Rao and Venkataraghavan, 1962; Santos et al., 2015; Saratovskikh et al., 2013; Wang and Andrews, 2006). Table 4 shows the infrared data, and Figure 3 shows the infrared spectrum data.

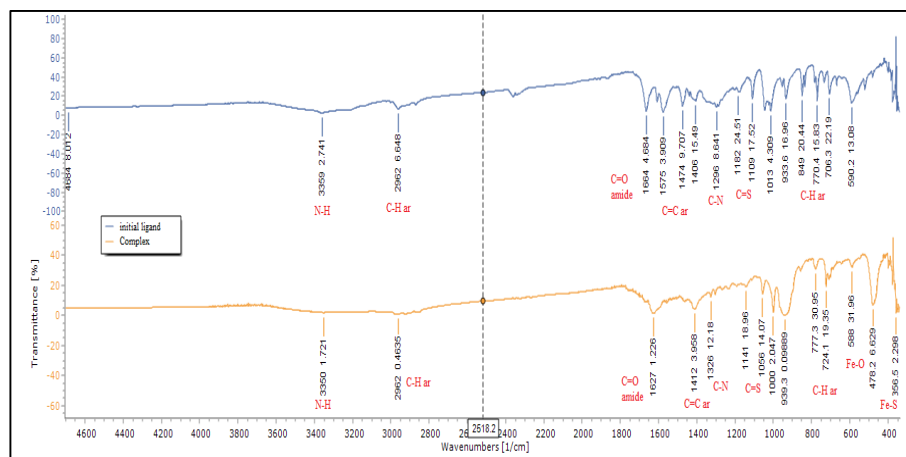


Figure 3. Infrared Spectrum for the ligand 4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide (blue); the Infrared Spectrum for the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III) complex (Orange)

Table 4. The comparison of infrared data for Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III) and 4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide complex

Functional Groups	ν (cm^{-1})	
	4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide	Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III)
NH stretch	3359	3350
C=O amide	1664	1627
C=C aromatic	1474	1412
C-N amine	1296	1326
Fe-O	-	588; 478
Fe-S	-	356

The next characterization was Mass Spectrophotometry (MS). It proved that the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III) complex was obtained in a pure condition. In Figure 3, we can see that the result of molecular ion peak has an m/z value of 559.31. Theoretically, the calculation of the complex molecular weight is 556.56. It indicates that the complex compounds from the one-step acylation synthesis are by the approximate structure in Figure 3.

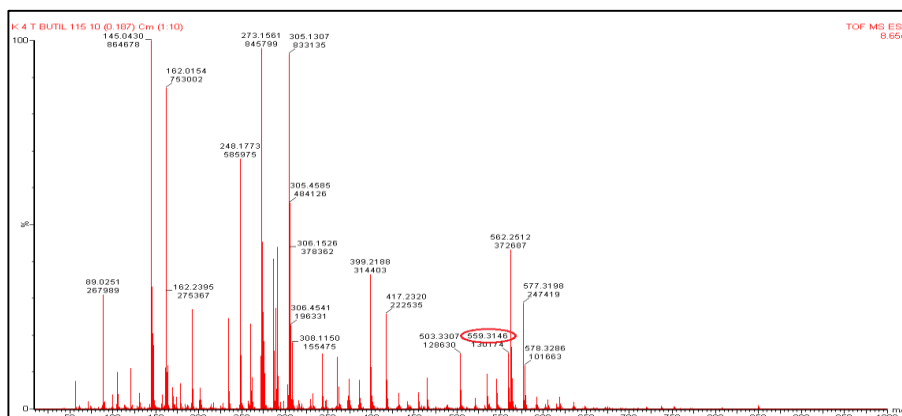


Figure 3. Molecular mass spectrum analysis of Bis- (4- (Tert-Butyl) -N-(Methylcarbamothioyl) Benzamide) -Iron (III) Complex Compounds

Based on the characterization in IR and MS spectrophotometry, the approximate structure of the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide) -Iron (III) complex is shown in Figure 4. In this study, the complex's crystal structure has been described and it will not be discussed further here. However, the metal ion is coordinated to N, O and S-chelating anionic ligands for this complex and exhibits a distorted square planar geometry of coordination. (Do Couto Almeida et al., 2016).

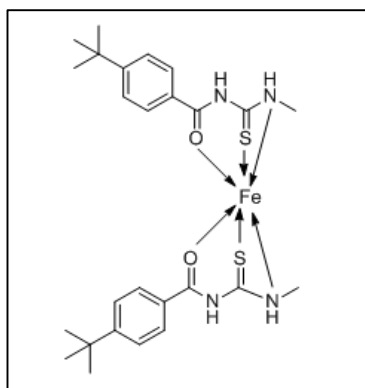


Figure 4. The proposed of the Bis-(4-(Tert-Butyl)-N (Methylcarbamothioyl) Benzamide)-Iron (III) structure complex

From Figure 4 we will find the formation between 4-(Tert-Butyl) -N- (Methylcarbamothioyl) Benzamide with Fe (III) metal which occurs in O, S, and one N groups of NH₂, from the lone electron pair derived from of these atoms, and used to bond with the metal Fe (III) to form the Bis-(4-(Tert-Butyl)-N (Methylcarbamothioyl) Benzamide)-Iron (III).

Complex molecular docking

In the molecular docking process, the first step we must prepare the 4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide ligand and the receptors (2EUD.pdb) (Xu et al., 2006). Molecular docking aims to predict the conformation of bond and binding affinity. The Gibbs free energy (ΔG) value/ binding affinity will be pull out from the docking process. This process was carried out using AutodockTools-1.5.6 software between Bis- (4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide) -Iron (III) ligands with anticancer receptors. This process has several

stages, including ligand preparation, receptor preparation, validation of the docking method, docking of test ligands against the target receptor, analysis of docking results, PreADMET test. The recapitulation of docking results can be check in [Table 5](#).

Table 5. Recapitulation of docking result

Compounds	Binding affinity (kcal/mol)	Inhibition constant
Natural ligands 2EUD	-8.23	32.21 μ M
4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide	-6.47	18.03 μ M
Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III)	-8.52	568.55 nM

The complex compound Bis- (4- (Tert-Butyl) -N- (Methylcarbamothioyl) Benzamide) -Iron (III) has a binding affinity of -8.52 kcal.mol and an inhibition constant of -8.52 kcal.mol 568.55 nM. This is lower than the native ligand or 4- (Tert-Butyl) -N- (Methylcarbamothioyl) Benzamide. The differences were in the value of the binding affinity and the inhibition constant. Therefore, [Table 5](#) concludes that the produced complex compounds are predicted to have the most stable interactions.

[Table 6](#) explains the interaction between the ligand 4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide with amino acid residues at the receptor and the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide) -Iron (III) complex with amino acid residues.

Table 6. The interaction between ligands with amino acid residues

Compounds	Hydrophobic Bond	Hydrogen Bond
Native ligand	ALA 245, GLY 247, LEU 427, MET 606, PRO 607, LYS 292, PHE 206, PRO 203, TYR 155, ALA 201	GLY 246, SER 217, CYS 218, THR 608, ALA 609, SER 610, THR 611, SER 202, ASN 291
4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide	ALA 296, GLY 247, PRO 203, SER 217, ALA 201, ALA 245, LYS 292, PHE 206, GLU 430	ARG 293, GLY 246, SER 202
Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III)	ASN 291, SER 610, PRO 203, ALA 201, THR 611, ALA 296, TYR 742, PHE 403, THR 608, LEU 445, SER 217, ALA 609, PRO 607, MET 606, GLY 290, LYS 292, SER 154, TYR 741	SER 202

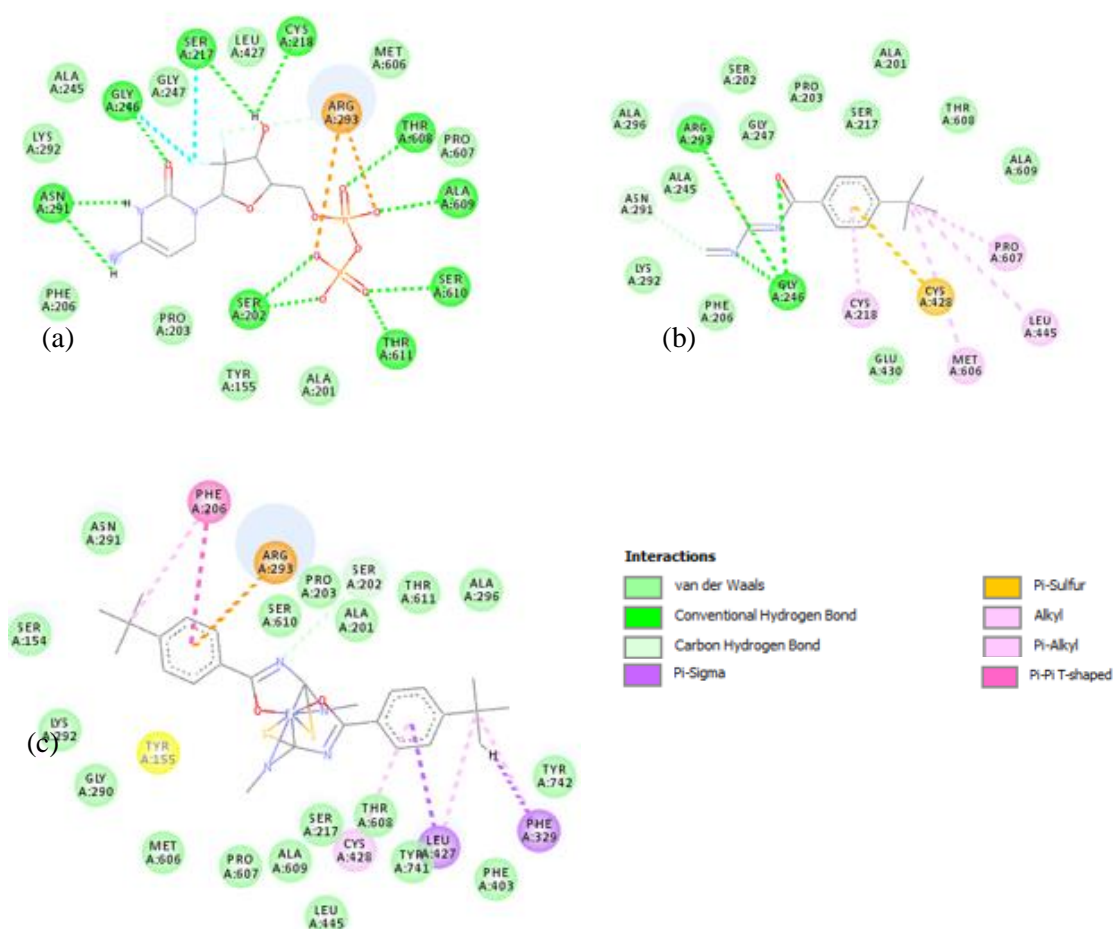


Figure 5. The 2D visualization of native ligand (a), the 2D visualization of 4-(Tert-Butyl) - (Methylcarbamothioyl) Benzamide (b), the 2D visualization of Bis-(4-(Tert-Butyl)-(Methylcarbamothioyl) Benzamide)-Iron (III) complex (c)

Table 6 and Figure 5 explained that the amino acid residue that interacts the hydrogen bond with all compounds is SER 202, while the amino acids that have the most hydrophobic interactions are with complex compounds (about 18 amino acid residues: ASN 291, SER 610, PRO 203, ALA 201, THR 611, ALA 296, TYR 742, PHE 403, THR 608, LEU 445, SER 217, ALA 609, PRO 607, MET 606, GLY 290, LYS 292, SER 154, TYR 741). Meanwhile, 3 amino acid residues interact hydrophobically with all compounds, namely PRO 203, ALA 20 and LYS 292.

The Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide)-Iron (III) complex was tested for ADME (Absorption and Distribution) and toxicity using PreADMET server, which can be read at <https://preadmet.bmdrc.kr/>. The purpose of the ADME test is to shape the pharmacokinetic profile of the complex.

Table 7. The prediction of ADME and toxicity results

Compounds	Absorption		Distribution PPB (%)	Toxicity <i>Ames Test</i>
	HIA (%)	caco-2 (nm/sec)		
<i>4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide</i>	93.865	25.6117	92.643	Mutagen
<i>Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl)Benzamide)-Iron(III)</i>	97.83.87	24.128	100	Mutagen

Classification: HIA (%) 0-20 = not well absorbed. 20-70 = moderately absorbed. 70-100 = well absorbed; Caco-2 (nm/sec) <4 = low permeability. 4-70 = moderate permeability. > 70 = high permeability; PPB (%) > 90 = strongly bound. <90 = weakly bound.

Plasma protein binding (PPB) value is more than 90%. It caused the Bis- (4- (Tert-Butyl) -N- (Methylcarbamothioyl) benzamide) -Iron (III) complex and its comparison compounds have strong bonds with plasma proteins. It can be said to be lacking in distribution. In HIA, it is in the well-absorbed category, and based on Caco2 it has moderate permeability. All of these are presented in [Table 7](#).

Permeability coefficient as permeability to Caco2 single-layer cell culture, not only related to hydrogen bonding capacity and ionic charge, but also lipophilicity. Permeability is a function of various Physico-chemical parameters, namely permeability = f (lipophilicity, molecular size, hydrogen-bonding capacity, and charge). Descriptors have a positive correlation with% HIA. Those are the number of rotational bonds (Nrot), number of hydrogen bond donor groups (Hdon), number of hydrogen bond acceptor groups (Hacc), logarithm of octanol-water partition coefficient (Log P) and logarithm of solubility coefficient (Log S). Plasma protein binding (PPB) is a fraction of the available drug in free form for distribution to various tissues. Human plasma contains 70% protein, with albumin (HAS, human serum albumin), α -1-glycoprotein acid (AGP, alpha-acyd glycoprotein), and lipoproteins as the main components. It is easier to bind with acidic or neutral drugs, whereas AGP and lipoproteins with essential medications.

The toxicity parameter can be find from the mutagenic properties of the tested compound. Mutagens are compounds that can increase the rate of changes or mutations in genes so that they can trigger cancer development ([Nursamsiar et al., 2016](#)). The results of toxicity prediction using the PreADMET program showed that the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide) -Iron (III) complex and its comparators had mutagenic properties. Although these compounds are mutagenic, they can still be used as drug candidates at the right dosages ([Yamashita et al., 2000](#); [Yee, 1997](#); [Zhao et al., 2001](#)).

CONCLUSION

We concluded that the Bis-(4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide) -Iron (III) complex could be synthesized from the reaction between the 4-(Tert-Butyl)-N-(Methylcarbamothioyl) Benzamide with $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and the complex yield was obtained by 85.85%.

We analyzed the molecular docking and concluded that the complex's interaction was better than the initial ligand, so the complex could be used as an anticancer candidate.

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