STUDY IN SILICO OF THIOUREA-DERIVED COMPOUNDS AS TYROSINE KINASE RECEPTOR INHIBITORS

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Abstract

Cancer is a disease caused by protein mutations, which cause cells to proliferate uncontrollably. Inhibiting the action of protein kinases is one method of preventing the signal that initiates the process of uncontrolled cell proliferation. This research aimed to determine the affinity of thiourea-derived compound ligands with the protein tyrosine kinase enzyme (PDB ID: 5LMA). The binding energy between each ligand and the tyrosine kinase receptor ranged from -87,62 to -95,26 kcal/mol. The percentage of ligand interactions varies above 80%. On the active site of the amino acid residues Leu 456, Leu 495, Ala 496, Ala 497, Arg 498, and Val 500, the tyrosine kinase enzyme binds to the ligands of thiourea-derived compounds via hydrogen, pi alkyl, and alkyl bonds. Pharmacokinetic, toxicity, and Lipinski regulation of thiourea-derived compounds yielded significant results as anticancer drug candidates.

Keywords*: anticancer, inhibitor, tyrosine kinase, molecular docking, PLANTS*

Introduction

Cancer is a disease caused by the irregularity of hormone travel which results in the growth of flesh in body tissues. The development of cancer cells is highly dependent on angiogenesis, namely the formation of new blood vessels that supply oxygen and food needed for tumor cell proliferation and the development of metastases. Two important receptors on growth factors that influence angiogenesis are the receptor tyrosine kinase or tyrosine kinase receptor (TKR) and VEGFR-2 (Novi, 2021).

Tanjung,2019 conducted a study on the anticancer activity test of alkaloid compounds, and Fahmi 2016 designed candidate anticancer compounds using dimethoxy amino chalcone nanoencapsulation (Tanjung et al., 2019) (Fahmi et al., 2016).

Research conducted by Rajabto, 2021, on metastatic lung adenocarcinoma patients with favorable EGFR mutations, drug therapy targets the tyrosine kinase group shows an excellent response to treatment good with tolerable side effects (Rajabto & Angkasa, 2021). PDB 5 LMA is Human Spleen Tyrosine Kinase Domain In Complex With Azanaphthyridine Inhibitor.

Several computational studies have shown that thiourea-derived compounds can be used as anticancer agents. The thiourea derivatives under investigation were evaluated for their antineoplastic activity. They were shown to possess potential anticancer activity (Kirishnamaline et al., 2021). The results of research that Mardianingrum has carried out are that thiourea derivatives have been proven to inhibit the activity of Sirtuin-1 (SIRT-1) through computational studies (Mardianingrum & Yanuar, 2022).

The method of modeling molecules that is widely used at present is docking. One of the docking applications is PLANTS. PLANTS software has several

advantages; free software includes determining the critical area between the ligand and the receptor, being simple to use, and producing docking results with the same quality as Gold software and other paid docking software (Korb, 2016). An *in silico* approach related to the potential as an effective medical material is the structure of physicochemical properties with application analysis (Muchtaridi et al., 2018).

The development of modern medicine is generally carried out first with a computational approach (Purnomo, 2013). One of the software that supports the docking of protein ligands is PLANTS. PLANTS is an easy-to-use but quality software on par with other paid docking software. PLANTS software does not provide protein preparation functions, ligands, or visualization of receptor and ligand docking results, so additional applications are needed, namely YASARA (Purnomo, 2019). Various device software has been developed for 3D structure drawing, molecular docking, and result visualization. In line with docking molecular, general drug candidate evaluation is carried out through the analysis of similarity properties with drug (drug-likeness) and profile absorption, distribution, metabolism, excretion, and toxicity (ADMET). Evaluation of drug-like properties is generally carried out according to Lipinski's five rules. ADMET projections can give information regarding a drug's pharmacokinetic properties and pharmacodynamics, oral bioavailability, cell permease, metabolism, elimination, and toxicity (Kalita et al., 2019).

Based on background, this study aims to identify the functional groups that play an active role in the interaction of ligands of thiourea-derived compounds, as well as the type of interaction that occurs between ligands and receptors, so that anticancer agent candidates developed in silico can be used as a reference for the clinical development of anticancer drugs (Mardianingrum & Yanuar, 2022). Modification of thiourea-derived compounds to be used as ligands as Figure 1.

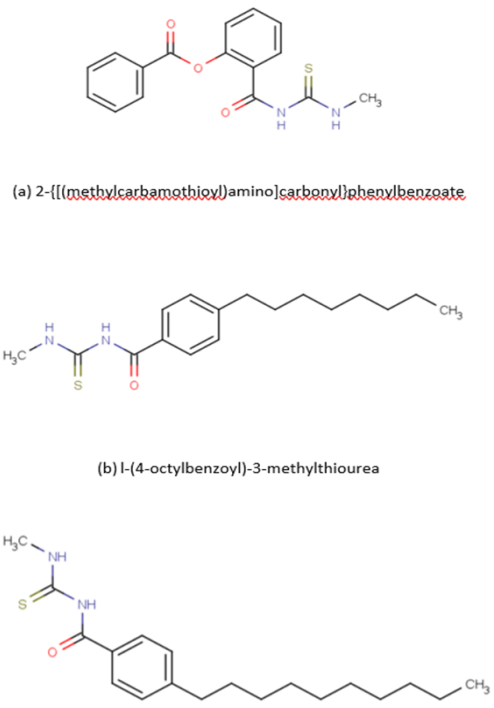

Figure 1. 2D Structure of Thiourea derivative compounds

Research Methods

Software Material: PLANTS, Marvinsketch, Marvinspace, Biovia Discovery Studios, YASARA. The enzyme structure retrieves data from the online program PDB (Protein Data Bank).

Hardware Material: A set of computers with processorCore™ i5-3230M2 Cores chip, 4 Threads@2.6GHz, 4.00 GB DDR3 1600 MHz random access memory, 2GB DDR3 Radeon HD 8670M video graphics array, supported by internet access to download protein data

Protein preparation

The research began by taking the structure of the protein Tyrosine Kinase complex in the format (.pdb) obtained from the Protein Data Bank (PDB) with the code 5LMA is then downloaded from the site http://www.rscb.Org/ prepared with the YASARA program. This preparation is intended to purify proteins from ligands or from other elements that are not subject to the process of bound proteins and ligands. The natural ligands (~{N}'-[7-(4-metil fenil) pirido[3,4-b]pirazin-5-il]butana-1,4-diamin), glycerol and dimethylsulfoxide that have been separated.

Method validation

Natural ligands (~{N}'-[7-(4-metil fenil)pirido[3,4-b]pirazin-5-il]butana-1,4-diamin) that have been separated from the protein are prepared using Marvin sketch. Following the pH of the human body, which is 7.4, ten conformations of ligands are created and stored in the form of ligand ligands as part of natural ligand production. Proteins separated from their natural ligands are stored as proteins.mol2 used YASARA software. Furthermore, natural ligands and proteins were tested for docking simulations. Grid box size values for tyrosine kinase receptors are X = 25.667, Y = - 42.3398, and Z = 37.0446, with a radius of 11.4269. The result of the molecular docking simulation is selected as the lowest energy than the calculated RMSD. If the RMSD result is less than 2 Å, the hardware used can be continued to use test ligands. The RMSD value becomes invalid if the compound is changed in the YASARA application (Purnomo, 2019).

Ligand preparation

The ligand to be tested is then prepared using a Marvin sketch. According to the body, such ligands are conditioned at a pH of 7.4. Furthermore, the ligands are composed of ten conformations determined by the ligands with the lowest energy. Likewise, ten conformations of such ligands are stored in the form of ligand.mol2. Ten conformations of such ligands tested their molecular docking simulations using PLANTS software. The results of the molecular docking of ligand molecules and proteins with the lowest energy are selected and then prepared using YASARA to be stored as a new PDB.

Simulation docking of receptor and ligand

The receptor and ten conformational ligands that have been prepared are simulated for docking at the binding site determined in the receptor validation test. The results of the docking simulation are in one results folder. The ligand conformation with the lowest energy is selected, and a new PDB is created with the receptor.

Visualization of molecular docking

The new PDB that has been obtained then visualizes the interaction between the ligand and receptor to determine the active side of the protein bound to the ligand and the type of bond used in *the Biovia Discovery Studies* software.

Pharmacokinetic prediction analysis used ADMET

The ligands used were screened to determine the process of adsorption, distribution, metabolism, excretion, and toxicity using ADMET and Lipinski's

Rules of Five. A good ADMET profile determines successful drug development. Due to a related failure, pharmacokinetics is standard in clinical trials (Moroy et al., 2012).

Results and Discussion

Protein preparation

Natural ligands that have been separated from the receptors conditioned at pH seven as in the body and created ten conformations to find the position with the lowest energy in the Marvin sketch. Energy minimization can simplify and stabilize the bond arrangement during molecular tethering, the addition of hydrogen, and the conversion of two-dimensional structures into three-dimensional (Ganesan et al., 2020). When a compound performs optimization through properly and precisely performed energy minimization can improve the accuracy and performance of molecular tethering results (Hanif et al., 2020). The conformation with the lowest energy of 54,57 kcal/mol is shown in Figure 2.

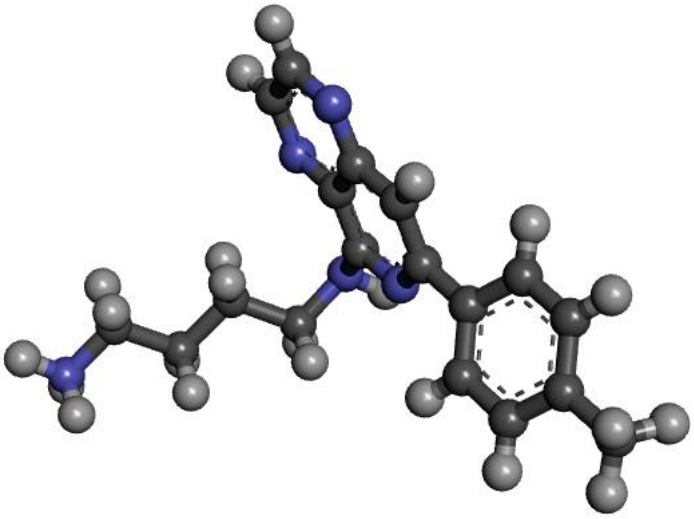

Figure 2. Conformation of a Natural Ligand with the best energy

RMSD calculation

This study began with the validation of receptors. Process validation is a tool parameter that will be used worthy of research. This validation process started with retrieving receptors through the PDB Bank web with the code 5LMA. Furthermore, by using YASARA software, natural ligands are separated first. Natural ligands are then protonated at pH 7.4 according to the pH of the human body used Marvin sketch software and made ten conformations to find the most stable position.

Furthermore, the ligands that have been protonated are docked with receptors used PLANTS. The lowest energy from this molecular docking simulation result is calculated as RMSD. RMSD is the distance of shift from the position of the natural ligand with the protonated ligand; if RMSD < 2Å, it can be concluded that the calculation of the tool used is more accurate (Purnomo, 2011). The result of the docking study of the 5LMA receptor was 0.3940 Å (Figure 3). These RMSD results are eligible for further research used.

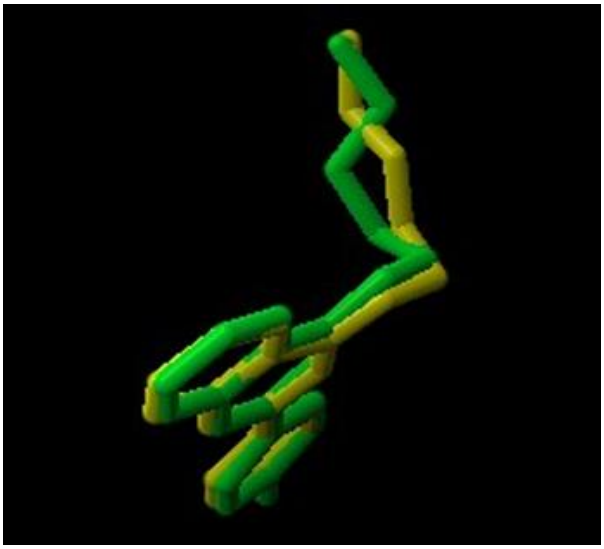

Figure 3. RMSD result 0.3940 Å. The natural ligand is yellow, and the protonated ligand is green.

Docking of Thiourea derivative receptors and ligands

The three ligands of the thiourea-derived compounds were reacted with the receptors using the PLANTS software after the ligands and receptors were prepared. The PLANTS software has the advantage of estimating the grid box or binding area between the ligand and the receptor, and the radius, which is the active side area of the receptor area. The grid box X = 25.667, Y = -42.3398, and this study determined Z = 37.0446 with a radius of 11.4269. The lower the docking score, the easier the receptor and ligand will bind (Menendez, 2015). Ten conformations were made for each ligand to keep the docking score low. The best energy is selected, then the new PDB using YASARA software. Factors that affect the binding value of the ligand and the receptor are the type of bond between the ligand and the amino acid residue, which is the receptor's active site, and the bond distance.

As shown in Table 1, in natural ligands, a salt bridge on the Asp 554 residue causes an increase in protein stability. The hydrogen bonds in the salt bridge are usually more potent than the hydrogen bonds in the uncharged groups. The lone pair of electrons from the nitrogen on the ligand binds to the Phe 513 residue, causing the protein to become more stable. The lone pair on the nitrogen is delocalized to the carbonyl, forming a partial double bond between the N and the carbonyl carbon. Other bonds that support the binding of receptors and ligands are alkyl bonds, pi alkyl bonds, hydrogen bonds, and unfavorable bump bonds. In pi-alkyl interactions, there is a pi-electron cloud interaction between the aromatic group and the electron groups of an alkyl group (Figure 4). The number of bonds on the receptor and the ligand indicate the stability of the docking of the ligand, resulting in a low energy of -104.766 kcal/mol. In compound (a), there is an attractive charge bond on the positively charged protonated amine from the ligand compound with the negatively charged deprotonated oxygen from residue Asp 494. This bond is a non-covalent interaction between groups that carry opposite charges. Other bonds that are formed are carbon-hydrogen bonds at residue Leu 495 and alkyl groups from residues Ile 432, Leu 485, and Ile 510, which are electron donor groups to pi bonds in ligands (a) so that a small dipole moment can be formed (Figure 5).

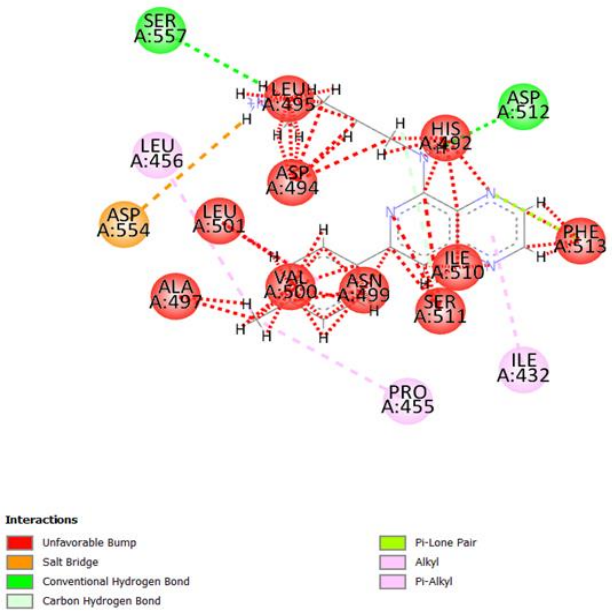

Figure 4. Visualization of the interaction between Natural Ligand and Tyrosine Kinase

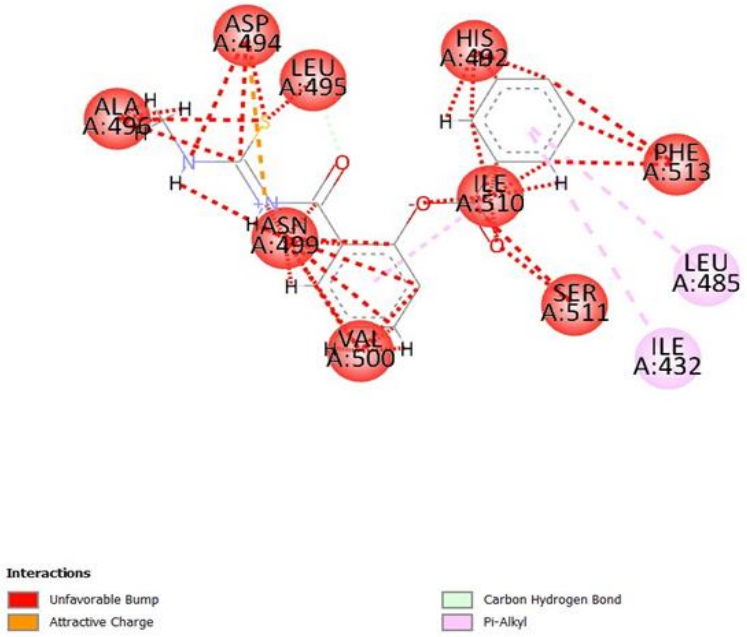

Figure 5. Visualization of the Interaction of Thiourea-derived compounds (a) with Tyrosine Kinase

Table 1. Data on the results of docking receptors and ligands of Thiourea derivative compounds

Ligand	Energy Calculation Results	Interaction Percentage	Bond Type	Bond Distance	Amino Acid Residues
Natural Ligands	-104,77	100%	Conventional Hydrogen Bond	2,06 Å	Asp 512
			Salt Bridge	2,92 Å	Ser 557
			Pi Lone Pairs	3,23 Å	Asp 554
				2,79 Å	Phe 513
				5,33 Å	Pro 455
			Alkyl	3,18 Å	Ala 497
				4,39 Å	Leu 456
			Pi-Alkil	5,00 Å	Ile 432
				5,15 Å	Leu 501
			Unfavorable Bump		Leu 495, His 492, Asp 494, Leu 501. Ala 497, Val 500, Asn 499, Ile 510
(a)	-87, 62	83,63%	Attractive Charge	5,29 Å	Asp 494
			Pi Alkil	5,45 Å	Ile 432
				5,26 Å	Leu 4,85
				5,08 Å	Ile 5,10
			Unfavorable Bump		Leu 495, Ala 496, Asn 499, Val 500, His 492, Ile 510, Ser 511, Phe 513
			Hydrogen Bonding	2,27 Å	Leu456
				3,05 Å	Ala 497
				2,69 Å	Glu 564
(b)	-92,01%	87,82%	Alkyl	5,46Å	Leu 485
			Pi Alkil	3,89 Å	Phe 513
				4,15 Å	Val 500
			Unfavorable Bump		Ile 510, Ser 511, Ala 496, Arg 498, His 492
			Hydrogen Bonding	2,27	Leu 456
				3,31	Ala 497
			Unfavorable Donor-donor	2,34	Asn 457
(c)	-95,26%	90,92%	Pi Alkyl	3,85	His 4,92
				5,10	Phe 513
				5,25	Leu 495
			Alkyl	4,27	Leu 485
				4,38	Ile 510
			Conventional Hydrogen Bond	2,27	Leu 456
				2,31	Asn 499
			Unfavorable Bump		Ala 496, Ala 497, Arg 498, Asn 499,

In ligand (b), there is an alkyl bond on the residue Leu 485 with a distance of 5.46 Å and two pi-alkyl bonds with a distance of 3.89 Å with residues Phe 513 and Val 500 with a bond distance of 4.15 Å to stabilize the receptor. Three carbon-hydrogen bonds in the residue Leu 4.56, Ala 497, and Glu 564, as well as several unfavorable bump bonds on residues Ile510, Ser 511, Ala 496, Arg 498, and His 492, strengthen the stability of the receptor so that the energy is lower than the bonds made by the ligand (a) (Figure 6). In compound (c), there is one unfavorable donor-donor bond with a distance of 2.34 Å with residue Asn 457, three alkyl bonds with residues Leu 485, Leu 495, and Ile 510 with bond lengths of 4.27 Å, 5.25 respectively Å and 4.38 Å. Two Pi-alkyl bonds with residues His 492 and Phe 513 with a bond distance of 3.85 Å and 5.10 Å. Two Hydrogen bonds with Ala 497 and Leu 456 with a bond distance of 3.31 Å and 2.27 Å. Five amino acid residues bond in an unfavorable bump: Val 500, Ala 497, Asn 499, Ala 496, and Arg 498 (Figure 7). Judging from the structure of the three compounds, the c ligand has the longest chain; there are ten long CH_2 chains, causing more effective binding to the receptor. The bond distance formed by the receptor and the ligand is, on average, below 5 Å, so the energy is lower, and the position of the ligand is more stable.

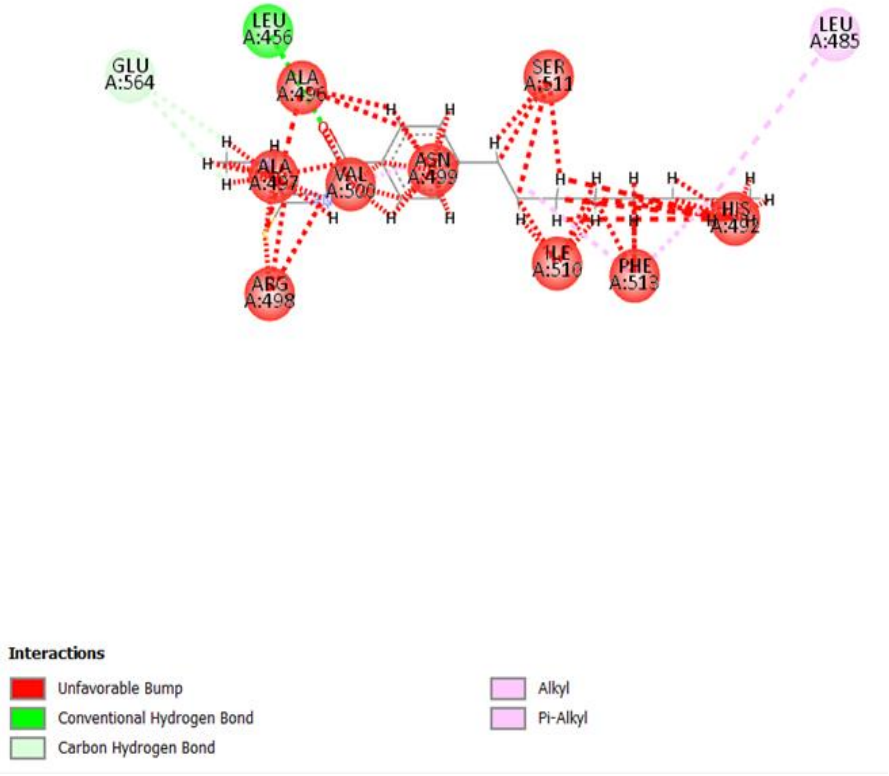

Figure 6. Visualization of the Interaction of Thiourea-derived Compounds (b) with Tyrosine Kinase

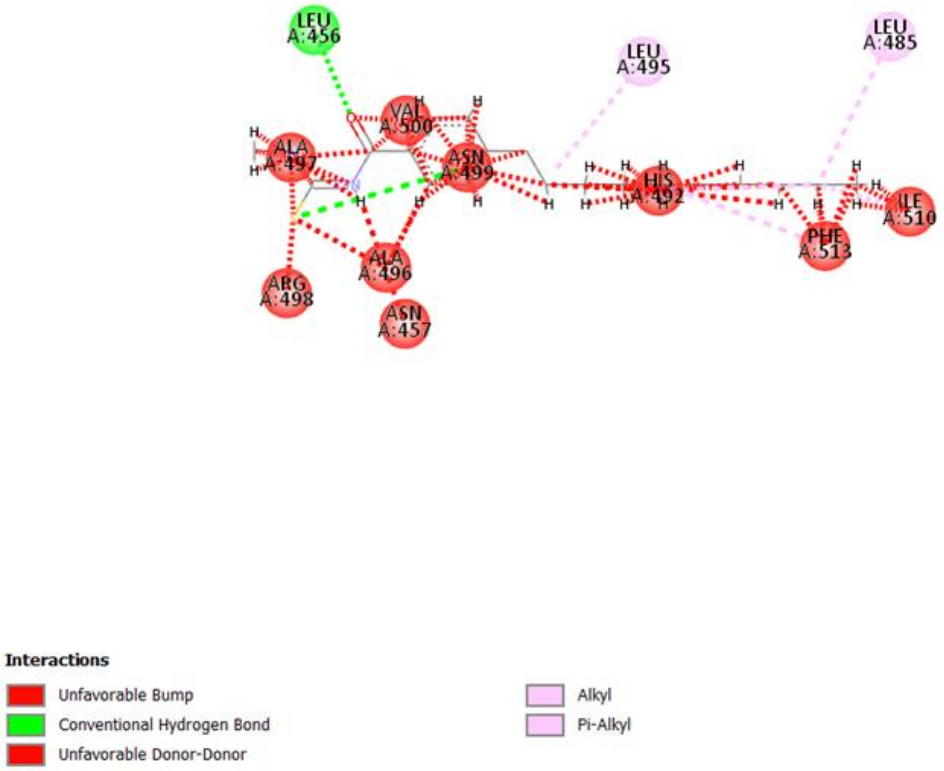

Figure 7. Visualization of the Interaction of Thiourea-derived Compounds (c) with Protein Tyrosine Kinase

Physicochemical screening of test compounds

As shown in Table 2, pharmacokinetic predictions were made using the PreADMET program with the parameters CaCo-2, human intestinal absorption (HIA), and plasma protein binding (PPB). CaCo-2 cells have a role as the transport pathway of the drug through the intestinal epithelium, which is a derivative of the colon of human adenocarcinoma. There are three classifications of CaCo-2 values: low permeable values of 0–4, medium permeable 4–70, and high absorbent values of 70 and above. A compound can have good biological activity if it can pass through appropriate cell membranes and reach the target tissue (Hamzah, Nursalam, et al., 2015). Table 2 shows that natural and test ligands have values between 4–70, meaning that thiourea derivative compounds used as ligands can penetrate medium cell membranes. HIA (Human Intestinal Absorption) is an approximate human intestinal absorption data that is the sum of bioavailability and absorption assessed from the ratio of excretion or cumulative excretion in urine, bile, and feces. This HIA value has been measured at pH 7.4 and adjusted to the condition of the human body. A value of 0–20 % is a compound absorbed slightly in the body, a value of 20%–70% is a medium-absorbed compound, while 70%–100% indicates that the compound is well absorbed in the body. According to the study, the body absorbs this thiourea derivative molecule quite efficiently, with a value between 94.72 %–95%.

ADMET can predict the percent of drugs bound in plasma proteins, such as human in vitro data, called plasma protein binding (PPB). A prescription is effective if the compound bonds penetrate the cell membrane well. According to Nursamsiar, 2016 there are two types of drugs in the blood: drugs in a solid bound form that have a value greater than 90 and are not firmly bound when the plasma value of protein binding is less than 90. In

the results of this study, a natural ligan with a PPB < 90 means that the compound is a ligand with a small bond, so the drug is more efficient in penetrating cell membranes. The results of the test ligand study (c) have a PPB value of < 90%, while compounds (b) and (c) have a PPB of > 90%, which means that the drug bound to plasma proteins will be inactive. Over-the-counter or unbound medications can produce biological activity by acting on target receptors. Ligand (c) has the highest plasma protein binding.

The Ames test is a simple method of testing the mutagenicity of a compound, suggested by Dr. Ames. It uses different Salmonella typhimurium strains because histidine production is affected by gene changes, and these strains need histidine to grow. Toxicology predictions assess test substances' toxicity levels and naturally occurring ligands. The Ames test, carcino mouse, and carcino rat were the three criteria used in the toxicity test. Utilizing such compounds as possible anticancer therapy possibilities is safe in large doses because all ligands have a mutagenic effect.

While the parameters of carcino mouse and carcino rat are used to see whether the ligands used can provide a carcinogenic effect against mice and mice, the results found that all test ligands did not have a carcinogenic impact on rats or mice. However, test ligands based on predictions can have a carcinogenic effect on mice. So natural and test ligands as anticancer candidates must have certain restrictions in their use.

Table 2. Prediction of pharmacokinetic properties and their toxicity

Compound	ADME Predictions			Toxicity Prediction		
	CaCo-2	HIA	PPB	Ames Test	Carcino Mouse	Carcino Rat
Natural Ligands	30,35	94,83	62,97	mutagen	positive	negative
(a)	22,16	94,93	87,54	mutagen	negative	negative
(b)	43,06	94,72	100,00	mutagen	negative	negative
(c)	45,32	94,89	100,00	mutagen	negative	negative

The requirement for a compound that has potential as a potential drug is that the molecular weight must be less than 500 Dalton. If the molecular weight is more than 500 Daltons, it will lead to a failure of the diffusion of molecules in penetrating the cell membrane. Another requirement is that a compound has five hydrogen bond donors and not more than ten hydrogen bond acceptors. This is because the smaller the hydrogen bond, the less energy is required for absorption (Sim et al., 2018). Log P value or partition coefficient to see the nature of lipophilicity or hydrophobicity is the ability of a compound to dissolve in water or fat. A high Log P value indicates that the molecule has very hydrophobic properties. The higher the hydrophobic value means that a compound, the higher the toxicity level of the compound. The test ligand has higher hydrophobic properties because its log P value is more than 5. A hydrophobic compound will be retained longer in the lipid bilayer. In addition, the compound will spread more widely in the body so that the selectivity of the bond to the target receptor is reduced (Lipinski, C.A., Lombardo F., Dominy B.W., 2001).

The molecular weight parameters of a compound can affect the distribution process of a drug by looking at its ability to penetrate biological membranes through the process of passive diffusion. The larger the molecular size of a compound, the more difficult it will be to penetrate the biological membrane. All

three and natural ligands have molecular weights that meet the requirements. If the compound has a small molecular size, the compound will more easily penetrate the biological membrane. All compounds that are small enough to dissolve in water can pass through the membrane channels. Most of the membranes (small intestinal epithelial cellular membranes and others) are small, namely 4–7 Å and can only be passed by compounds with small molecular weights, namely less than 150 for round compounds, or less than 400 if the compounds consist of long chains (Syukri, 2002).

The biological activity of the drug molecule is closely correlated with the rules of the hydrogen bond donor parameters of 5 and the acceptor of hydrogen bonds of 10. The physicochemical characteristics of a molecule can change if hydrogen bonding is present. Many hydrogen bond donors attach to the solvent in a chemical and produce hydrogen bonds. Hydrogen acceptors can also impact a compound's permeability because they interact well with solvents that have hydrogen bonds. Consequently, substances that have the propensity to interact favorably with polar solvents may reduce their capacity to permeate the lipid membrane of the bilayer.

The refractory molar value of all ligands meets the requirements, which is in the range of 40–130. This value measures the total polarizability value of a drug molecule that depends on the refractive index, temperature, and pressure.

Table 3. Data of *Lipinski's Rule of Five*

Compound	Molecular Weight (<500 g/mol)	Hydrogen Donor (<5)	Hydrogen Acceptor (<10)	Log P (<5)	Refractory Molar (40–130)
Natural ligands	293,37	4	2	2,65	89,20
a	314,36	2	5	2,14	86,97
b	306,5	2	1	5,22	93,51
c	334,52	2	1	6,11	102,72

Conclusions

Based on the research results, the three thiourea derivative compounds have good potential as receptor tyrosine kinase activity inhibitors, but compound (c) is the best because it has the lowest energy. The most important active sites are Leu 456, Leu 495, Ala 496, Ala 497, Arg 498, and Val 500.

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