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Computational Study of 1-(3-Nitrobenzoyloxymethyl)-5-Fluorouracil Derivatives as Colorectal Cancer Agents

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Abstract

Cancer is one of the chronic diseases with a reasonably high increase at this time. One type of cancer with the highest mortality rate is colorectal cancer. Colorectal cancer is cancer that occurs in the colon and rectum. Based on GLOBOCAN data (2018), cases of colorectal cancer in Indonesia reached 8.6% or 30,017 people and were the second most common cause of death in men and the third in women. The development of cancer drugs to obtain drugs with better activity, lower toxicity, and working more selectively through structural modifications is still being carried out until now. This study aims to determine the pharmacokinetic properties and stable interactions between the thymidylate synthase and one of the 78 derivatives of 1-(3-nitrobenzoiloximethyl)-5-fluorouracil (NB5FU) by in silico, namely molecular docking, and molecular dynamics simulations. The result shows that the NB5FU78 derivative compounds have better pharmacokinetic properties than NB5FU. Lipinski's rules of five criteria that fill the requirements have a smaller free bond energy value than NB5FU. Based on the results of molecular dynamics simulations carried out for 5 ns, the NB5FU78 derivative has a stable interaction with the thymidylate synthase (TS) receptor with total bond energy of -36.36 kcal/mol.

***Keywords**:* Colorectal cancer, 1-(3-nitrobenzoiloksimetil)-5-fluorouracil, molecular docking, molecular dynami

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1. INTRODUCTION

Cancer is a chronic disease with a high increase nowadays. According to GLOBOCAN (2019), cancer has become the world's second most prominent cause of death, with approximately 9.6 million deaths in 2018. In contrast, in Indonesia, the death due to cancer reached 103,000 deaths in men and 92,200 in women's deaths (WHO, 2014), with one of the most common types of cancer being colorectal cancer.

Colorectal cancer is a malignant cell that grows in the large intestine (colon) and rectum (Sander, 2012). Based on GLOBOCAN (2018) data, colorectal cancer cases in Indonesia reached 8.6% or 30,017 of the population, which occupies the fourth position and the second leading cause of death in men and third place in women. The increase in the number of people with cancer was caused by the lifestyle of Indonesians toward westerners (Westernization) (Kemenkes RI, 2018).

Due to the increase in cancer cases, drug development is needed to obtain drugs with better activity, lower toxicity, and work more selectively through structural modification (Siswandono, 2014). The most commonly used drug to treat colorectal cancer is 5-Fluorouracil (5-FU). Many studies synthesized 5-fluorouracyl derivatives and tested their activity as an anticancer (Bollag & Hartmann, 1980; Pat *et al.*, 2011; Tian *et al.*, 2007; Ozaki *et al.*, 1977; Sun *et al.*, 2013). However, Pan *et al.* (2011) research showed that 5-FU has low efficacy and relatively high toxicity, so it is necessary to develop drugs employing molecular modeling or in silico.

Based on Oktavianawati *et al.* (2014) researched the development of a 5-fluorouracil derivative drug through a benzoylation reaction with one of the substituted derivatives, 3-nitrobenzoyl, in silico. The result showed that 1-(3-nitrobenzoiloximethyl)-5-fluorouracil (NB5FU) had better bond affinity than 5-FU.

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Nevertheless, the value of the bond energy (ΔG) that can be assessed is still low for further analysis, which is -6.98 kcal/mol. Therefore, it is necessary to develop molecular modeling of NB5FU derivative compounds.

The macromolecule used in this analysis is thymidylate synthase (TS), which is the only enzyme responsible for the de novo biosynthesis of thymidylate (TMP) and is essential in regulating the balanced supply of 4 DNA precursors in normal DNA replication (Chen *et al.*, 2017). The obtained macromolecules were seen based on the similarity between the structure of the test ligand compound and the receptor.

This study aims to determine the pharmacokinetic properties and stable interactions between the thymidylate synthase and one of the 78 in-silico NB5FU derivatives, molecular docking, and molecular dynamics simulation.

2. MATERIALS AND METHODS

Materials

The equipment used was in the form of hardware and software. The hardware used is a Personal Computer Index processor Intel® Celeron® CPU 10070@1.50GHz, 2.00 GB of 32-bit RAM. The free software used were MarvinSketch, Molegro Molecular Viewer, Discovery Studio Visualizer, Command Prompt, AutoDock Tools 1.5.6., and AMBER 16, available at http://www.ambermd.org/ (University of California, San Fransisco) University Padjadjaran license. The derivatives of 1-(3-nitrobenzoiloximethyl)-5-fluorouracil (**Figure 1**) and thymidylate synthase (TS) receptor with the PDB ID 5X67 were used as the materials. All of the compound structures can be seen in the Supplementary (**Table S1**).

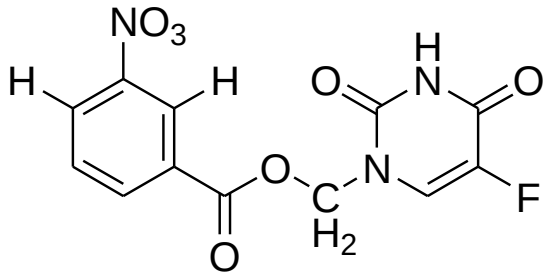

Figure 1. Chemical structure of 1-(3-nitrobenzoiloxymethyl)-5-fluorouracyl

Procedure

The scheme of research methods is presented in **Figure 2**.

Preparation of Receptor

The crystal structure of Thymidylate Synthase (TS) (PDB entry 5X67) (Xu *et al.*, 2006) recovered from the Brookhaven Protein Data Bank was used as a target for molecular docking using AutoDock Tools 1.5.6.

Preparation of Ligand

The structure of ligands was drawn using ChemDraw Ultra 8.0 software. The structure was cleaned in 3D format, and the energy was minimized using Marvin Sketch software and then saved in ".pdb" file formats for molecular docking studies.

Molecular Docking Validation Method

The molecular docking method is validated to prove and ensure that the method used meets the validity requirements and can minimize errors. The validation step is carried out by re-docking the ligand to the receptor that has been separated first. The docking method is said to be good if it has the resulting Root Mean Square Deviation (RMSD) value ≤ 2 (Puratchikody *et al.*, 2016).

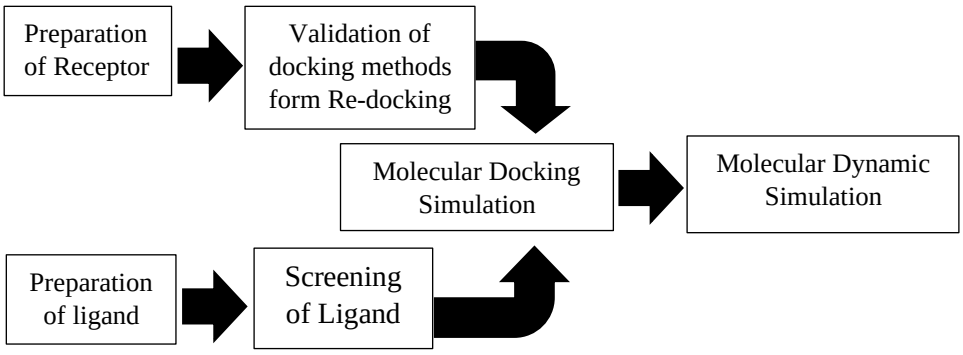

Figure 2. The scheme of methods research

Lipinski's Rule of Five

Drug observations were carried out on all ligands by observing good drug rules (Lipinski's Rule of Five), which included molecular weight <500 g/mol, LogP <5, hydrogen bond donor <5, hydrogen bond acceptor <10, molar refractory between 40–130. Lipinski's Rule of Five parameters can be determined using the MarvinSketch software (Tambunan *et al.*, 2012).

Pharmacokinetic and Toxicity Prediction

PreADMET, a web-based application available at http://preadmet.bmdrc.org/, was used to continue the procedure. PreADMET will automatically compute the expected absorption for CaCo-2 cells, HIA (Human Intestinal Absorption), plasma protein binding (PPB), and their toxicity characteristics through the Ames test after the structure of the chemical has been transformed into molfile *.mol (molfile) format (Rozano *et al.*, 2017; Yamashita *et al.*, 2000; Yee, 1997; Zhao *et al.*, 2001).

Molecular Docking Simulation

AutoDock Tools 1.5.6 was used to prepare for docking simulation between NB5FU derivative compounds, and TS receptors downloaded on Protein Data Bank with PDB ID 5X67 with the grid box point x=37.733; y=10.76; and z=12.01 (Chen *et al.*, 2017; Jarmula, 2010; Glovannetti *et al.*, 2007; Gmeiner, 2005; Taricani *et al.*, 2010). The molecular docking parameter file is according to the Lamarckian Genetic Algorithm (LGA) to get the best conformation between ligands and receptors. The ligands with the lowest free energy (ΔG) value and inhibition constant (Ki) will be selected for the next step, namely molecular dynamics simulations by AMBER (Ruswanto *et al.*, 2021; Mardianingrum *et al.*, 2021; Ruswanto, 2015; Ruswanto *et al.*, 2018).

Molecular Dynamics Simulation

The AMBER ff14SB force field for protein was used in the MD simulations. The ligands were given the general AMBER force field (GAFF), and TIP3P water was placed in the box with a minimum distance of 10 between the protein outer section and the box edge. The next step is determining the system's initial coordinate point and minimization in three-step with the addition of ions and solvation. Then, the system equilibration is carried out through a gradual heating of 0º to 310K, accompanied by a decrease in resistance and a constant change of resistance for 5 ns (Dermawan *et al.*, 2019; Case *et al.*, 2014; Mardianingrum *et al.*, 2021).

3. RESULT AND DISCUSSION

Lipinski's Rule of Five, Pharmacokinetic, and Toxicity Prediction

The 1-(3-nitrobenzoyloxymeryl)-5-fluorouracyl and its derivatives have been prepared and screened by parameterizing the characteristics of Lipinski's Rules of Five, pharmacokinetic and toxicity predictions. The result is presented in **Table 1** and **Table 2**. 25 NB5FU derivative compounds were selected to do molecular docking simulation, and its result is presented in **Table 3**.

The log P value corresponds to hydrophobicity which is the ability of a chemical compound to dissolve in oil, fat, and non-polar solvents. The drug should be hydrophobic enough to penetrate the lipid bilayer, but it should not be too hydrophobic; causing the drug not to penetrate the membrane that will cause the drug to be toxic because it lasts longer in the body. The value of donor and acceptor hydrogen bonding is related to the biological activity of a drug molecule which can affect the chemical-physical properties of compounds such as melting point, boiling point, water solubility, ability to form chelating, and acidity. Molar Refractory is related to the total polarizability value of drug molecules that are heavily dependent on temperature, refractive index, and pressure. This polarizability is associated with the molecular form and the relative molecular mass, which usually increases the number of electrons, the more easily polarized.

Based on **Table 1**, it is known that NB5FU78 derivative compounds have been qualified according to Lipinski's rules. It can be predicted that the compounds have good permeability, quickly absorbed, resulting in more compounds interacting with more receptors and more significant activity.

Based on **Table 2**, The ADME can be explained that all compounds with CaCo-2 cell parameters are spanned 4 to 70 nm/sec (medium permeability category), HIA% values are stretched 20-100% (medium and good category), so it can be predicted that all compounds will be absorbed well, and %PBB with grades less than 90% means weakly bound which means these compounds will distribute

well in the body. Meanwhile, the toxicity test through PreADMET with the Amest test can be concluded that all of the compounds are predicted to be mutagenic.

Table 1. The Characteristic of 1-(3-nitrobenzoiloxymethyl)-5-fluorouracyl and its derivatives Lipinski;s rules of five

No	Compounds	Lipinski's Rules of Five				
		LogP	MW	Acceptor H	Donor H	Ref. Molar
		< 5	< 500 g/mol	< 10	< 5	40 – 130
	NB5FU	1.42	309.03	11	1	69.32
1	NB5FU7	2.05	355.02	11	1	82.08
2	NB5FU37	2.38	337.07	11	1	78.96
3	NB5FU41	2.83	351.08	11	1	83.56
4	NB5FU43	3.27	365.10	11	1	88.16
5	NB5FU44	4.60	407.14	11	1	101.96
6	NB5FU45	4.16	393.13	11	1	97.36
7	NB5FU46	5.05	421.16	11	1	106.57
8	NB5FU47	5.94	449.19	11	1	115.77
9	NB5FU49	2.45	337.07	11	1	79.40
10	NB5FU52	2.96	351.08	11	1	84.44
11	NB5FU78	2.85	391.02	10	1	91.34

Table 2. The prediction of 1-(3-nitrobenzoiloxymethyl)-5-fluorouracyl and its derivatives pharmacokinetic properties and toxicity.

No.	Compounds	Pharmacokinetic			Toxicity		
		CaCo-2	HIA (%)	PPB (%)	Amest_test	Carcino mouse	Carsino rat
	NB5FU	14.95	60.37	86.08	+	+	+
1	NB5FU6	6.404	48.20	69.28	+	+	+
2	NB5FU38	14.98	46.52	86.14	+	+	+
3	NB5FU42	17.85	98.84	82.92	+	+	+
4	NB5FU49	16.23	73.23	78.63	+	-	+
5	NB5FU58	7.41	49.75	66.48	+	-	+
6	NB5FU59	6.80	55.34	69.92	+	-	+
7	NB5FU61	12.73	55.34	79.36	+	+	+
8	NB5FU71	3.09	61.18	82.96	+	+	+
9	NB5FU72	5.28	70.13	81.24	+	+	+
10	NB5FU74	17.84	89.51	88.86	+	+	+
11	NB5FU77	0.41	51.58	84.51	+	+	-
12	NB5FU4	20.45	83.57	94.45	+	-	-
13	NB5FU13	4.13	72.47	96.89	+	-	-
14	NB5FU21	4.07	70.28	92.19	+	-	-
15	NB5FU31	19.95	51.24	91.72	+	-	-
16	NB5FU52	19.43	72.16	76.35	+	-	-
17	NB5FU78	0.65	80.66	100	+	-	-

Note: Caco-2 = < 4 Bad, 4 – 70 Medium, 70 Good; HIA (%) = 0 – 20 Bad, 20 – 70 Medium, 70 – 100 Good; PPB (%) = > 90 Strong bond, < 90% Weak bond; (+): toxic; (-): non-toxic

Validation Method

The receptor has been done Re-docking of 7Z9 (native ligand) with an RMSD value is 0.52Å. It has been valid and capable of performing the docking calculation due to filling the validity criteria of the RMSD value ≤2Å. The visualization results of Re-docking are presented in **Figure 3**.

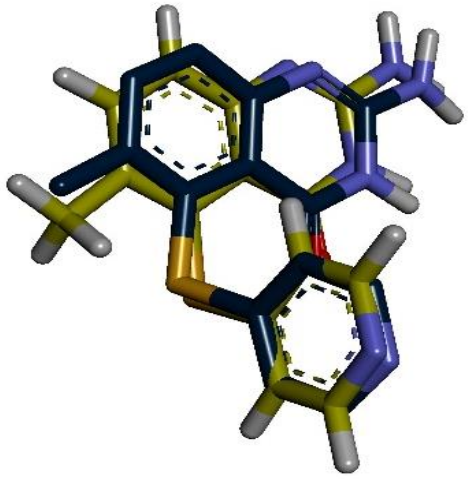

Figure 3. The visualization result of validation method

Molecular Docking Results

The docking results of 1-(3-nitrobenzoiloximethyl)-5-fluorouracyl and its derivatives on Thymidylate Synthase receptor obtained that one of 1-(3-nitrobenzoiloximethyl)-5-fluorouracil derivatives have a free binding affinity (ΔG) which is lower than 1-(3-nitrobenzoiloximethyl)-5-fluorouracil. Free binding affinity (ΔG) is strength of binding affinity parameter from the compound to the receptor. The lower the ΔG, the more potent, and the higher the binding affinity ligand-receptor is more stable. ΔG values of the docking results can be shown in **Table 3**.

Table 3 shows that NB5FU78 has a lower bond-free energy value (ΔG) than the NB5FU compound, which is -8.01 kcal/mol with an inhibition constant (Ki) of 1.35 nM. Therefore, it will be carried out in the molecular dynamic simulation. Free energy (ΔG) measures the drug's ability to bind to the receptor. A small value of ΔG indicates a higher affinity between the receptor and the ligand. In contrast, a significant value of ΔG means that the affinity between the receptor and the ligand is lower. The more negative the value of ΔG produced, the better the affinity between the ligand and the receptor.

Meanwhile, the inhibitory constant (Ki), called the dissociation constant, can give an idea of the affinity between the compound and the decomposition. From the equilibrium reaction between the compound and the receptor, the smaller the Ki value means the reaction equilibrium tends toward the formation of the compound of the receptors. The value of Ki is directly proportional to the ΔG, so the lower the inhibitory constant (Ki), the more effective the inhibition shown by the ligand on the target protein activity. From the value Ki value, it can be seen that the Ki of compound NB5FU7 is lower than NB5FU, which can be predicted that the compound has a better inhibition than NB5FU.

Table 3. The result of molecular docking simulation

No	Compounds	ΔG (kcal/mol)	Ki (μM)	Residues Interaction	
				Hydrogen Bond	Hydrophobic Bond
	NB5FU	-6.98	7.59	Lys50, Ile81	Asn199, Gly193, Val52, Phe53, Gly195
1	NB5FU4	-7.50	3.17	Gln214, Cys195, Tyr135, Asn226	Leu221, Ser216, Asp218, Gly217, Val223, Leu192, Pro194, Trp109, Phe91, Phe225
2	NB5FU6	-7.12	8.80	Lys77	Arg78, Phe80, Val79, Pro224, Gly220, Met311, Gly222, Asn226
3	NB5FU7	-7.38	6.08	Trp109, Lys77, Phe80	Phe91, His196, Pro224, Val79
4	NB5FU13	-6.73	11.66	Asp218, Asn226, Phe80	Lys107, Tyr258, Ser216, Gly217, Cys195, Gln214, His195, Phe225, Val70, Lys77, Arg78
5	NB5FU21	-6.79	10.61	Lys77	Gly281, Pro224, Asn228, His106, Met331, Phe80, Thr106, Ile350

6	NB5FU31	-8.16	1.05	His256, Asp218, Cys195, Tyr135, Asn226, Gln214	Ser216, Gly217, Val223, Leu192, Pro194, Trp109, Phe91, Ile108, Phe225
7	NB5FU37	-7.77	2.01	Lys77, Ile108	Asn226, Pro224, Val79, Arg78, Thr306, Ile307, Phe80, Gly222, Met311
8	NB5FU38	-6.47	18.10	Gln214, Asp218, Cys195, Ile108	Asn112, Leu192, Phe225, Gly222, Gly217, Ser216, Asn226
9	NB5FU41	-7.51	3.11	Gln214, Asp218, Cys195, Tyr135	Ser216, His196, Phe91, Trp109, Pro194, Leu192, Ile108, Asn226, Phe225, Gly222, Leu221
10	NB5FU42	-7.75	2.09	Lys77	Met311, Pro224, Val79, Gly220, Arg78, Phe80, Asn226, Gly222, Glu87
11	NB5FU43	-7.32	4.32	Lys77, Phe80	Pro224, Val79, Glu87, Asn112
12	NB5FU44	-6.67	12.96	Gln214, An226, Asp218	Lys77, Phe80, Leu221, Gly222, His196, Gly217, Ser216, Val225, His258, Leu192, Asn112, Met311, Phe225
13	NB5FU45	-7.01	7.31	Cys195, Asp218	Ser216, Leu192, Gly222,Asn226, Pro194, Tyr135, Phe91, Phe225, Ile108, Leu221, Met311, Asn112, Tyr258, Ala312, Gly217, Gln214
14	NB5FU46	-6.62	14.04	Asn226, Ile108, Cys195, Asp218	Asn112, Leu192, Leu221, Ala312, Arg50, Ser216, Arg215,Tyr258, Gln214, Gly217, His196, Phe225
15	NB5FU47	-6.37	21.32	His256, Asp218	Tyr258, Asn112, Ser218, Trp109, His196, Tyr135, Gln214, Phe91, Glu87, Asn226, Phe225, Leu221, Val313, Leu192, Ala312
16	NB5FU48	-6.86	9.38	Cys195, Asp218, Gln214, Asn226, Tyr135	Trp109, Pro194, Leu192, Ser216, Gly217, Asn112, Ala312, Tyr258, Leu221, Phe225, Ile108, Phe91
17	NB5FU49	-6.80	10.31	Lys77, Phe80, Asn26	Pro224, Val79, Thr306, Arg78, Met311, Trp109, His196
18	NB5FU52	-6.42	19.73	Asp194, Gln187, Cys168, Glu60, Asn199	Tyr231, Ala285, Ser189, Gly195, Phe64
19	NB5FU58	-6.62	14.09	Glu87, Lys77, Asn226	Gly220, Pro224, Met311, Val79, Phe80, Thr306
20	NB5FU59	-6.98	7.70	Ile108, Lys77	Met311, Phe80, Thr306, Val79, Pro224, Gly220, Gly222, Asn226
21	NB5FU61	-6.27	25.29	Tyr135, Cys195, Asp218, Asn226	Gly217, Ser216, Tyr258, Met311, Phe91, Trp109, Pro194, Leu192, Ile108, Phe225, Gly222, Gln214
22	NB5FU71	-6.94	8.24	Phe80, Lys77, Phe225	Asn122, Pro224, Val79, Gly220, Thr306, Asn226, Met311
23	NB5FU72	-6.93	8.32	Asn226, Phe80, Lys77, Asp218	Gly217, Cys195, Gln214, Val79, Ile108, Met311, Tyr258
24	NB5FU77	-7.41	3.71	Tyr135, Cys195, Asp218, His256, Gln214, Asn226	Pro194, Pro193, Leu192, Ser216, Gly217, Arg50, Ala312, Met311, Leu221, Phe225, Ile108, Phe91
25	NB5FU78	-8.01	1.35	Tyr108, Cys68, Asp191	Leu194, Met284, Phe64, Trp82, Pro167, Leu165, Ile81, Asn199, Phe198, Ser189, Gln187, Hie229, Tyr231

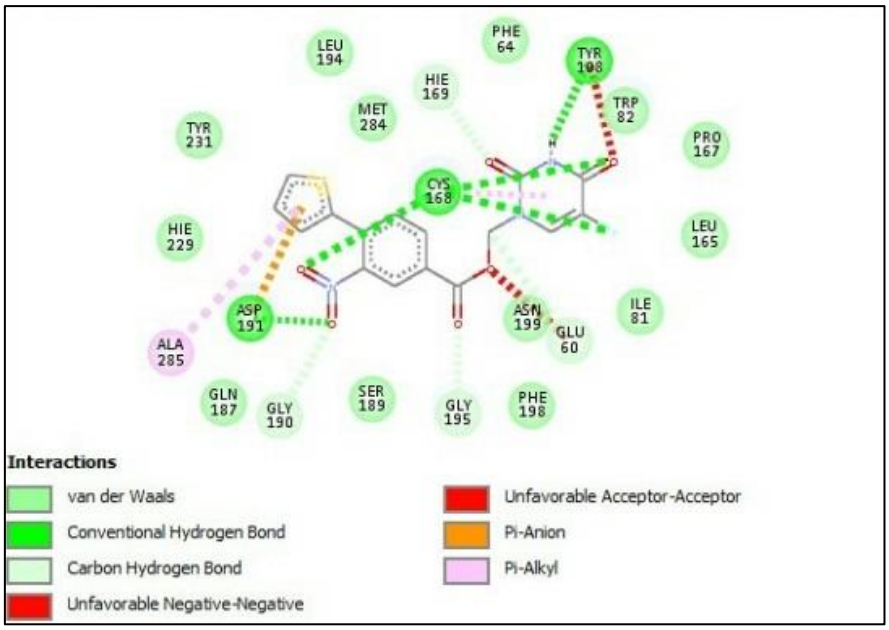

Figure 4. Interaction between NB5FU78 and TS receptor of docking result

In addition, the docking results can be analyzed by interactions between compounds to receptors employing visualization using the Molegro molecular viewer program. By visualization can be observed amino acid residues contact and the hydrogen bonds formed between the compounds to the receptor. The more hydrogen bond interactions between compounds and amino acid residues, it is predicted that the interactions will be more stable and better. NB5FU78 forms by three hydrogen bonds with Tyr108, Cys68, Asp191 and thirteen hydrophobic bonds with Leu194, met284, Phe64, Trp82, Pro167, Leu165, Ile81, Asn199, Phe198, Ser189, Gln187, Hie229 and Tyr231. The interaction between NB5FU78 and TS receptor is presented in **Figure 4**.

Molecular Dynamics Simulation

RMSD analysis in **Figure 5** showed that NB5FU78 is the most stable movement. The stability of these fluctuations is due to the interaction of residues in the enzyme. Therefore the protein tends to maintain its structure. Ligand and protein complexes will attain maximum or stable conformation after binding with proteins that maintain their position. The low fluctuation of residues shown in **Figure 6**, namely Leu205 and Leu206, are the stable residues due to not taking many position changes during the simulation.

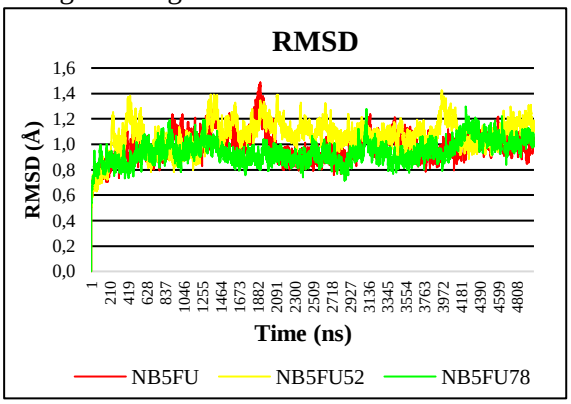

Figure 5. RMSD of complex

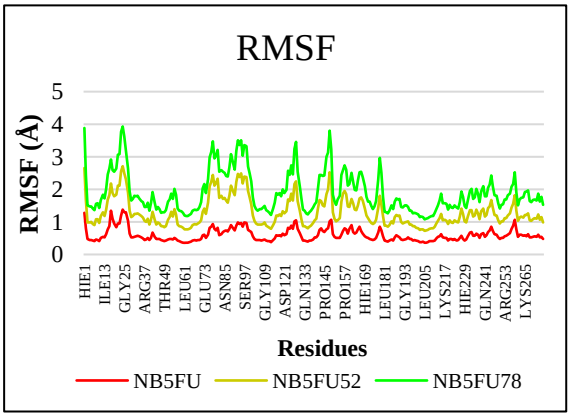

Figure 6. RMSF of residues

Table 4. Calculation results of bond energy method of molecular mechanics-generalized born surface area (MM-GBSA)

Energy Component (kcal/mol)	System		
	NB5FU	**NB5FU52**	**NB5FU78**
Van der Waals Interaction (VdW)	-41.89	-44.20	-44.35
Electrostatic Energy (EEL)	-35.32	-39.85	6.45
Electrostatic Contribution to Solvation Free Energy (E_{GB})	42.58	52.88	6.71
Non-Polar Contribtio to Solvation Free Energy (E_{SURF})	-4.68	-84.05	-5.17
ΔG_{gas} (VdW + EEL)	-77.21	-84.05	-37.90
ΔG_{solv} (E_{GB} + E_{SURF})	37.89	47.20	1.54
ΔG_{TOTAL} (VdW + EEL + E_{GB} + E_{SURF})	-39.31	-36.85	-36.36
RMSD (Å)	1.2	1.2–1.4	1.2

The results of MMGBSA bond energy calculations are presented in Table 4. It shows that the TS–NB5FU78 complex and the TS–NB5FU52 complex has a total bond-free energy value (ΔGTOTAL) that is close to the ΔG_{TOTAL} NB5FU value, which the Van der Waals interaction (VdW) contributes more than electrostatic energy (EEL). It shows that the VdW is the energy component with the most significant influence on the system. The enormous VdW interaction contribution to the system indicates that the amino acid residues that make up the side active of thymidylate synthase protein are dominated by hydrophobic residues. Therefore, it can be predicted that the TS–NB5FU78 complex and the TS–NB5FU52 complex has good stability.

4. CONCLUSIONS

The NB5FU78 derivative compounds have better pharmacokinetic properties than NB5FU. Lipinski's rules of five criteria fill the requirements and have a smaller free bond energy value than NB5FU. Based on the results of molecular dynamics simulations carried out for 5 ns, the NB5FU78 derivative has a stable interaction with the thymidylate synthase (TS) receptor with a total bond energy value of -36.36 kcal/mol.

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