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 dynamics

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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 (Siswando, 2014). The most commonly used drug to treat colorectal cancer is 5-Fluorouracil (5-FU). Many studies synthesized 5-fluorouracil 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.
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 Francsico) University Padjadjaran license. The derivatives of 1-(3-nitrobenzoiloxymethyl)-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-fluorouracil](image)

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](image)
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 refractivity 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; Giovannetti 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 0° 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-nitrobenzoyloxymethyl)-5-fluorouracil and its derivatives have been prepared and screened by parameterizing the characteristics of Lipinski's Rule 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 %PPB 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-nitrobenzoioxymethyl)-5-fluorouracyl and its derivatives Lipinski's rules of five

| No | Compounds | LogP | MW | Acceptor H | Donor H | Ref. Molar |
|----|-----------|------|----|------------|---------|------------|
|    |           |      |    | < 5        | < 10    | < 5        | 40 – 130   |
| 1  | NB5FU     | 1.42 | 309.03 | 11 | 1 | 69.32 |
| 2  | NB5FU7    | 2.05 | 355.02 | 11 | 1 | 82.08 |
| 3  | NB5FU37   | 2.38 | 337.07 | 11 | 1 | 78.96 |
| 4  | NB5FU41   | 2.83 | 351.08 | 11 | 1 | 83.56 |
| 5  | NB5FU43   | 3.27 | 365.10 | 11 | 1 | 88.16 |
| 6  | NB5FU44   | 4.60 | 407.14 | 11 | 1 | 101.96 |
| 7  | NB5FU45   | 4.16 | 393.13 | 11 | 1 | 97.36 |
| 8  | NB5FU46   | 5.05 | 421.16 | 11 | 1 | 106.57 |
| 9  | NB5FU47   | 5.94 | 449.19 | 11 | 1 | 115.77 |
| 10 | NB5FU49   | 2.45 | 337.07 | 11 | 1 | 79.40 |
| 11 | NB5FU52   | 2.96 | 351.08 | 11 | 1 | 84.44 |
| 12 | NB5FU58   | 17.85 | 98.84 | 82.92 | + | + |
| 13 | NB5FU71   | 3.09 | 61.18 | 82.96 | + | + |
| 14 | NB5FU72   | 5.28 | 70.13 | 81.24 | + | + |
| 15 | NB5FU74   | 17.84 | 89.51 | 88.86 | + | + |
| 16 | NB5FU77   | 0.41 | 51.58 | 84.51 | + | + |
| 17 | NB5FU80   | 0.65 | 80.66 | 100 | + | - |

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

| No | Compounds | Pharmacokinetic | Toxicity |
|----|-----------|-----------------|----------|
|    |           | CaCo-2          | HIA (%)  | PPB (%) | Amest_test | Carcino mouse | Carcino rat |
| 1  | NB5FU     | 14.95           | 60.37    | 86.08   | +          | +            | +          |
| 2  | NB5FU6    | 6.404           | 48.20    | 69.28   | +          | +            | +          |
| 3  | NB5FU38   | 17.98           | 98.84    | 82.92   | +          | +            | +          |
| 4  | NB5FU42   | 17.98           | 98.84    | 82.92   | +          | +            | +          |
| 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-nitrobenzoyloxymethyl)-5-fluorouracil and its derivatives on Thymidylate Synthase receptor obtained that one of 1-(3-nitrobenzoyloximethyl)-5-fluorouracil derivatives have a free binding affinity (∆G) which is lower than 1-(3-nitrobenzoyloximethyl)-5-fluorouracil. Free binding affinity (∆G) is strength of binding affinity parameter from the compound to the receptor. The lower the ∆G, the more potent, and the higher the binding affinity ligand-receptor is more stable. ∆G values of the docking results can be shown in Table 3.

Table 3 shows that NB5FU78 has a lower bond-free energy value (∆G) 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 (∆G) measures the drug's ability to bind to the receptor. A small value of ∆G indicates a higher affinity between the receptor and the ligand. In contrast, a significant value of ∆G means that the affinity between the receptor and the ligand is lower. The more negative the value of ∆G 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 ∆G, 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.

| No | Compounds | ∆G (kcal/mol) | Ki (µM) | Residues Interaction |
|----|-----------|---------------|--------|----------------------|
|    | NB5FU     | -6.98         | 7.59   | Lys50, Ile81          |
| 1  | NB5FU4    | -7.50         | 3.17   | Lys77, Gln214, Cys195, Asn226 |
| 2  | NB5FU6    | -7.12         | 8.80   | Trp109, Lys77, Phe80  |
| 3  | NB5FU7    | -7.38         | 6.08   | Arg78, Gly220, Phe91, Gly195 |
| 4  | NB5FU13   | -6.73         | 11.66  | Trp109, Lys77, Phe80  |
| 5  | NB5FU21   | -6.79         | 10.61  | Lys77, Asp218, Gly220, Phe91, Asn226 |

Table 3. The result of molecular docking simulation
| No. | Sequence | Start | End | Mean |
|-----|----------|-------|-----|------|
| 6   | NB5FU31  | -8.16 | 1.05| Ser216, Gly217, Val223, Leu192, Pro194, Trp109, Phe91, Ile108, Phe225 |
| 7   | NB5FU37  | -7.77 | 2.01| Asn226, Pro224, Val79, Arg78, Thr306, Ile307, Phe80, Gly222, Met311 |
| 8   | NB5FU38  | -6.47 | 18.10| Asn112, Leu192, Phe225, Gly222, Gly217, Ser216, Asn226 |
| 9   | NB5FU41  | -7.51 | 3.11| Ser216, His196, Phe91, Trp109, Pro194, Leu192, Ile108, Asn226, Phe225, Gly222, Leu221 |
| 10  | NB5FU42  | -7.75 | 2.09| Met311, Pro224, Val79, Gly222, Lys77, Phe80, Asn226, Gly222, Gln214, Asp218 |
| 11  | NB5FU43  | -7.32 | 4.32| Lys77, Phe80, Ile108, Met311, Lys77, Pro224, Val79, Gly222, His196, Gly217, Ser216, Val225, Arg78, Phe80, Asn226, Gly222, Asp218 |
| 12  | NB5FU44  | -6.67 | 12.96| His256, Asp218, Cys195, Tyr135, Asn226, Gln214 |
| 13  | NB5FU45  | -7.01 | 7.31| Ser216, Gly222, Asn226, Pro194, Tyr135, Phe91, Phe225, Ile108, Leu221, Met311, Asn112, Tyr258, Ala312, Gly217, Gln214, Asn12, Leu192, Leu221, Ala312, Arg50, Ser216, Arg215, Tyr258, Gln214, Gly217, His196, Phe225 |
| 14  | NB5FU46  | -6.62 | 14.04| Tyr258, Asn112, Ser218, Tyr258, His196, Gly217, Ser216, Val225, His258, Leu192, Asn112, Met311, Phe225 |
| 15  | NB5FU47  | -6.37 | 21.32| Ser18, Arg78, Phe80, Asn226, Gly222, Leu221, Val313, Leu192, Ala312, Trp109, Pro194, Leu192, Ser216, Gly217, Asn112, Ala312, Tyr258, Leu221, Phe225, Ile108, Phe91, Pro224, Val79, Thr306, Arg78, Met311, Trp109, His196, Tyr231, Ala285, Ser189, Gly195, Phe64 |
| 16  | NB5FU48  | -6.86 | 9.38| Gln214, Asp218, Cys195, Tyr135, Gln214, Asn226, Cys195, Tyr135, Pro194, Tyr135, Gln214, Asn226, Cys195, Tyr135 |
| 17  | NB5FU49  | -6.80 | 10.31| Lys77, Phe80, Asp218, Gln214, Asp218, Tyr135, Cys195, Asp218, Gln214, Asp218 |
| 18  | NB5FU52  | -6.42 | 19.73| His256, Asp218, Gln214, Asp218, Tyr135, Cys195, Asp218, Gln214, Asp218 |
| 19  | NB5FU58  | -6.62 | 14.09| Tyr135, Gln214, Tyr135, Pro194, Tyr135, Phe91, Trp109, Pro194, Leu192, Ile108, Phe225, Gly222, Asn226, Gly217, Ser216, Tyr258, Met311, Ser216, Met311, Tyr258, Met311, Gln214, Tyr135, Pro194, Leu192, Ser216, Phe80, Thr306, Val79, Pro224, Gly220, Gly222, Asn226 |
| 20  | NB5FU59  | -6.98 | 7.70| Ile108, Lys77, Ile108, Lys77, Met311, Phe80, Thr306, Val79, Pro224, Gly220, Gly222, Asn226 |
| 21  | NB5FU61  | -6.27 | 25.29| Gly217, Ser216, Tyr258, Met311, Phe91, Trp109, Pro194, Leu192, Ile108, Phe225, Gly222, Gln214, Asn122, Pro224, Val79, Gly220, Thr306, Asn226, Met311, Gly217, Cys195, Gln214, Val79, Ile108, Met311, Tyr258 |
| 22  | NB5FU71  | -6.94 | 8.24| Phe80, Lys77, Phe225, Phe80, Lys77, Phe225, Phe80, Lys77, Phe225, Phe80, Lys77, Phe225, Phe80, Lys77, Phe225 |
| 23  | NB5FU72  | -6.93 | 8.32| Asn226, Phe80, Lys77, Asn226, Phe80, Lys77, Asn226, Phe80, Lys77, Asn226, Phe80, Lys77, Asn226, Phe80, Lys77, Asn226 |
| 24  | NB5FU77  | -7.41 | 3.71| Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191 |
| 25  | NB5FU78  | -8.01 | 1.35| Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191, Tyr108, Cys68, Asp191 |
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, lle81, Asn199, Phe198, Ser189, Gln187, His229 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.
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_{EEL} (VdW + EEL)        | -77.21 | -84.05  | -37.90  |
| ΔG_{GB} (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    | 1.21–1.4| 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 (∆G_{TOTAL}) that is close to the ∆G_{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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