

THE POTENCY OF 4-NITROBENZOYL-3-ALLYLTHIOUREA AS AN AGENT OF BREAST CANCER WITH EGFR/HER2: IN SILICO AND IN VITRO STUDY

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ABSTRACT

The objectives of this research were to predict the ADMET, docking, and investigate the cytotoxicity activity of 4-nitrobenzoyl-3-allylthiourea (BATU-11) on MCF7 and MCF7/HER2 cells. The docking was carried out through the interaction of ligands into the binding site of EGFR and HER2, to predict their affinity for this target with PDB codes: 1M17 and 3PP0. The docking simulation resulted in Rerank Score on EGFR and HER2 of -90.6421 kcal/mol and -91.0365 kcal/mol, respectively. The cytotoxicity activity of the BATU-11 on cell lines MCF7 and MCF7/HER2 was determined by MTT assay. The BATU-11 had a higher antiproliferative effect on MCF7/HER2 cells than on MCF7 cells, with IC₅₀ values of 85 μ M and 225 μ M, respectively. In the meanwhile, the CC₅₀ value is 1097 μ M. The result of the in silico study are in line with the in vitro results, as well as good ADMET prediction results from the BATU-11. Based on this study, 4-nitrobenzoyl-3-allylthiourea is potential as a breast cancer drug candidate with EGFR/HER2.

Keywords: Breast Cancer, Docking, Cytotoxicity, In Silico, EGFR/HER2, 4-nitrobenzoyl-3-allylthiourea.

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INTRODUCTION

Cancer is the second leading cause of death globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. Based on profile cancer data in Indonesia a total of 348,809 cases caused 207,210 deaths, where breast cancer ranked first in incidence and mortality.¹ In cases of breast cancer, 15-30% of the incidence is caused by overexpression of HER2 which is a member of the EGFR.^{2,3,4} The treatment that is currently widely used in the treatment of breast cancer cases with HER2 positive is Lapatinib as a chemotherapy agent, which has been reported to be resistant.^{5,6} The development of cancer drugs through inhibition of the kinase activity of EGFR/HER2 which plays a role in signal transduction pathways in cell cycle regulation is very interesting to be developed. One of them is the development of thiourea analog compounds that have a pharmacophore urea group and have anticancer activity.^{7,8} The thiourea derivative is one of the most promising groups. Studies on the development of thiourea derivatives as anticancer have been conducted by several researchers in the past year.⁹⁻¹² Research has also been conducted among others on derivatives of 3-benzoyl allylthiourea (BATU) showing high cytotoxic activity in breast cancer cells MCF7 and MCF7/HER2. Further research shows that BATU compounds can increase the expression of HER2 along with increased concentration.^{7,12} In line with the background, especially in an effort to find solutions to the problem of resistance to the use of Lapatinib, the BATU derivative, namely BATU-11, is very potential to be developed as a prospective breast cancer drug that has a synergistic effect with cancer drugs that work to suppress the expression of HER2. Therefore cytotoxic activity tests and selectivity tests in normal cells are very interesting to study. In this study, the prediction of pharmacokinetic properties of BATU-11 included absorption, distribution, metabolism, and excretion. The importance of ADME properties has been considered in the early stages of drug development. The docking approach can be done by using MVD (Molegro Virtual Docker) computer program. Prediction of anticancer activity indicated by the bond energy value of ligand-receptor interaction.¹³ This study selected the EGFR/HER2 receptor target with

codes 1M17.pdb and 3PP0.pdb, which contain standard ligand Erlotinib and SYR127063, respectively. This study also observed the cytotoxicity effect on human breast cancer cell lines (MCF7 and MCF7/HER2) and its selectivity in normal cells (Vero) using the MTT assay method.

EXPERIMENTAL

In-silico

The pharmacokinetic properties and toxicity of the compounds were predicted using the pkCSM program from Biosig Lab–University of Melbourne, Australia. Docking is done using molegro Virtual Docker program computer version 5.5. The test involved human epidermal growth factor receptor-2 (HER2), code PDB: 3PP0 and 1M17, which contains ligand standard SYR127063 and Erlotinib and taken from Protein Data Bank <https://www.rcsb.org/structure/3PP0> and <https://www.rcsb.org/structure/1M17>. The test was conducted using a set of laptops (hp®, intel core i7). Docking begins with creating 2D and 3D structures using the ChemBioOffice Ultra 16.0 program. Furthermore, energy minimization with MMFF94 is carried out. It is then stored in the *mol2/SYBYL2 extension. The next step is to use the MVD 5.5 program for the docking process.

Cell Culture

This study used MCF7, MCF7/HER2, and Vero cells obtained from the collection of the Faculty of Pharmacy, Airlangga University, and the Faculty of Medicine, Gadjah Mada University. Cultured cells use DMEM and M119 media containing 10% FBS.

ADMET Prediction

The determined physicochemical properties include absorption parameters such as intestinal absorption and skin permeability; distribution parameters such as volume of distribution at steady state (VDss), fraction unbound, BBB permeability; metabolic parameters as cytochrome P450 inhibitor with CYP2D6 substrate and inhibitor; and excretion parameters including total clearance and renal OCT2 substrate. Meanwhile, the toxicity parameters observed included hepatotoxic.^{14,15}

Docking

The docking test begins with ligand preparation by creating 2-D and 3-D structures using the ChemBioOffice Ultra 16.0 program and minimizing energy with MMFF94.^{16,17} Then downloaded 3PP0 and 1M17 receptors containing the standard ligands SYR127063 and Erlotinib, respectively. After that, the method validation process was carried out by determining the RMSD value $< 2\text{\AA}$, and docking was carried out for the test compound to obtain a rerank score and amino acid interactions.¹⁸

Cell Culture

Cells were cultured in a tissue culture dish (TCD) and incubated in a 5% CO₂ incubator, at 37°C until they were confluent. After the cells were confluent, the cells were added with trypsin-EDTA 0.25% to release the cells and incubated for 3 minutes in a CO₂ incubator. Cells were counted with a hemocytometer. The cell suspension was added with a certain amount of medium to obtain a cell concentration of 5×10^3 cells/ml.¹⁹

MTT Assay

Cell lines were distributed into 96 well plates, then incubated in a 5% CO₂ incubator for 24 hours. Next, the series concentration of the test solution, positive control, and negative control was added and incubated again for 24 hours. Next, 100 μL of MTT with a concentration of 0.5 mg/mL was added. Incubation was continued for 3 hours then the MTT reaction was stopped by adding 100 μL of 10% SDS in 0.01 N HCl. The 96 well plates were wrapped in aluminum foil and incubated overnight. The absorption was read with an ELISA reader at a wavelength of 570 nm.¹²

RESULTS AND DISCUSSION

Prediction of ADMET

Predicted results of ADMET (absorption, distribution, metabolism, excretion, and toxicity) of BATU-11 and lapatinib (LP) for comparison are shown in Table-1.

Table-1: ADMET Predictions

Pharmacokinetic Properties	Parameter	BATU-11	LP
Absorption	Permeability of skin ($\log K_p$, cm/h)	-2.800	-2.735
	Absorption of Intestinal (%)	92.198	95.160
Distribution	Permeability of BBB ($\log BB$)	-0.418	-0.737
	Volume Dss ($\log L/kg$)	-0.104	-0.293
Metabolism	Substrate of CYP2D6	No	No
	Inhibitor of CYP2D6	No	No
Excretion	Total Clearance ($\log ml/min/kg$)	-0.046	0.557
	Substrate of Renal OCT2	No	No
Toxicity	Hepatotoxic	No	No

The importance of ADME properties has been considered in the early stages of drug development. Most drugs evaluated in clinical trials cannot be marketed due to lack of effectiveness or unacceptable side effects. This has led to the need for new approaches to understand, explore, and predict ADME properties to improve compound quality and efficacy.^{14,20} The pharmacokinetic predictions of the BATU-11 compound can be seen in Table-1. The BATU-11 compounds have good intestinal absorption, so they can be used for oral use. Regarding toxicity, the BATU-11 compound is not hepatotoxic. This is in line with the results of cytotoxicity in Vero cells, which showed that the BATU-11 compound was non-toxic against normal cells. However, it is toxic to breast cancer cells.

Docking

The result of obtaining docking value (Rerank Score) from BATU-11 compound in 1M17 and 3PP0 and the interaction between ligands-amino acids can be seen in Table-2 and Table-3.

Table-2: Docking Results on 1M17 and 3PP0 Receptors

Compounds	Rerank Score (kcal/mol)	
	EGFR (1M17)	HER2 (3PP0)
BATU-11	-90.6421	-91.0365
Native Ligand (SYR127063)	-	-143.2220
Native Ligand (Erlotinib)	-115.425	-

Table-3: Interaction between Ligand-Amino acids

Compounds	3PP0		1M17	
	Hydrogen	Steric	Hydrogen	Steric
BATU-11	Thr862, Met801	Leu796, Val797, Ala751, Gln799, Thr798, Met801, Gly804	Gly705	Val718, Lys704, Leu768, Ala719, Met769, Gln767, Asp831
SYR127063	Thr862, Met801, Asp863	Asp863, Ala751, Asn850	-	-
Erlotinib	-	-	Met769	Pro770, Gly772, Met769, Gln767, Asp831

The approach of the test in silico is based on the influence of electronic and steric properties on the bonding power of drug receptors. In silico activity test is conducted to predict BATU derivative compounds have cytotoxic activity. Receptor 1M17 is a model of EGFR and 3PP0 is a model of human epidermal growth factor receptor (HER2) which is one of the families of EGFR. The standard ligand of 3PP0 is SYR127063, a compound that works to inhibit tyrosine kinase that plays a role in the HER2 pathway.^{21,22} It is also known that thiourea derivatives act as EGFR inhibitors by inhibiting tyrosine kinase receptors (RTKs) in the intracellular region.²³ This in silico study is intended to reduce the trial and error factor in designing a new drug development.²⁴ From the results of the docking study, it can be seen which amino acids are involved in the interaction between the ligand and the receptor. It can also be obtained the value of the energy bond between the compound 4-nitrobenzoyl-3-allylthiourea and the HER2 receptor.

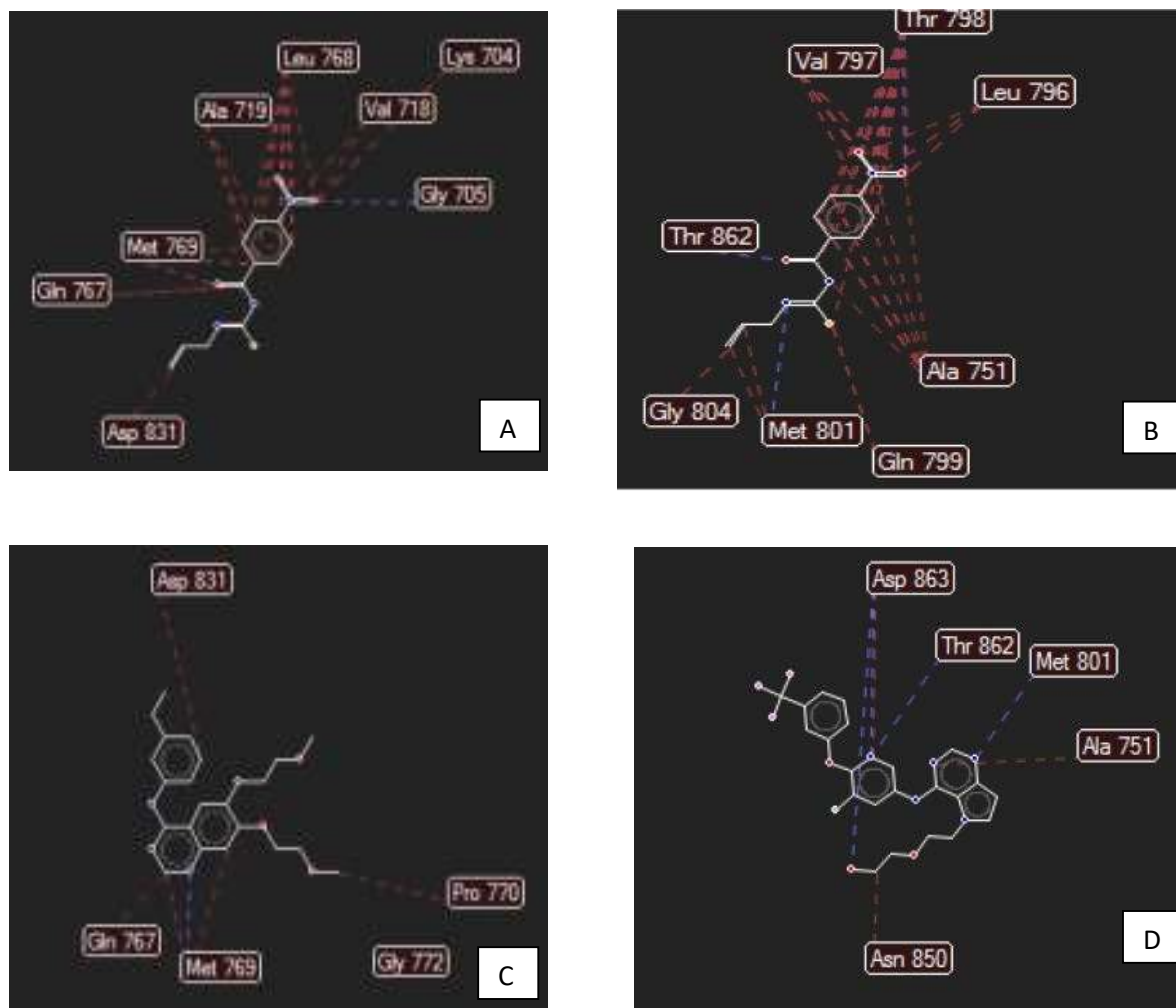


Fig.-1: Interaction between Compound 4-nitrobenzoyl-3-allylthiourea and Ligand Standard with Amino Acids in EGFR (A) and HER2 (C) receptors. Native ligand C) Erlotinib and D) SYR127063. The Blue Line Indicates The Hydrogen Bond And The Red Line of Van Der Walls.

Docking was carried out between ligands with receptors 1M17 and 3PP0. The energy strength that occurs in the interaction of the ligand and receptor is described by RS (Rerank Score) which is a measure of the binding energy of the ligand-receptor. The lower the RS value, the more stable the bond between the ligand-receptor so the stronger the interaction, therefore it can be predicted that the biological activity will also increase. In this study, the parameters measured in the docking process were the energy values involved in the drug-receptor interaction process, in the form of RS. To measure the bond strength of drug-receptor parameters that are commonly used is the RS value.¹⁶ The lower the RS value indicates the more stable the bond. The stability of the bonds indicates the strength of the interaction between drug-receptor bonds. Based on the graph of the in-silico test results, the results of the compound 4-nitrobenzoyl-3-allylthiourea at the HER2 receptor are -91.0365 kcal/mol. The RS value is still slightly lower than the RS value at the EGFR receptor.

Cytotoxicity Effect

Cytotoxicity activity test of MCF7 and MCF7/HER2 cells was conducted by incubating 5×10^3 cells in 96 well plates for 24 hours, then given sample treatment, and incubated again for 24 hours according to the procedure.

The cytotoxicity effect of the compound on MCF7 and MCF7/HER2 cell lines was observed by MTT assay. Based on the concentration vs. cell viability curve model, showed that there was a decrease in the viability

of MCF7 and MCF7/HER2 breast cancer cells with an increase in the concentration of the BATU-11 compound. From the calculation results obtained BATU-11 exhibited the antiproliferative effect on MCF7/HER2 cells with IC_{50} values of 85 μ M higher than MCF7 cells with IC_{50} values of 225 μ M. Meanwhile, the CC_{50} value is 1097 μ M. Thus, BATU-11 acts more selectively against breast cancer by overexpression of HER2 and has been shown to be not-toxic to normal cells.

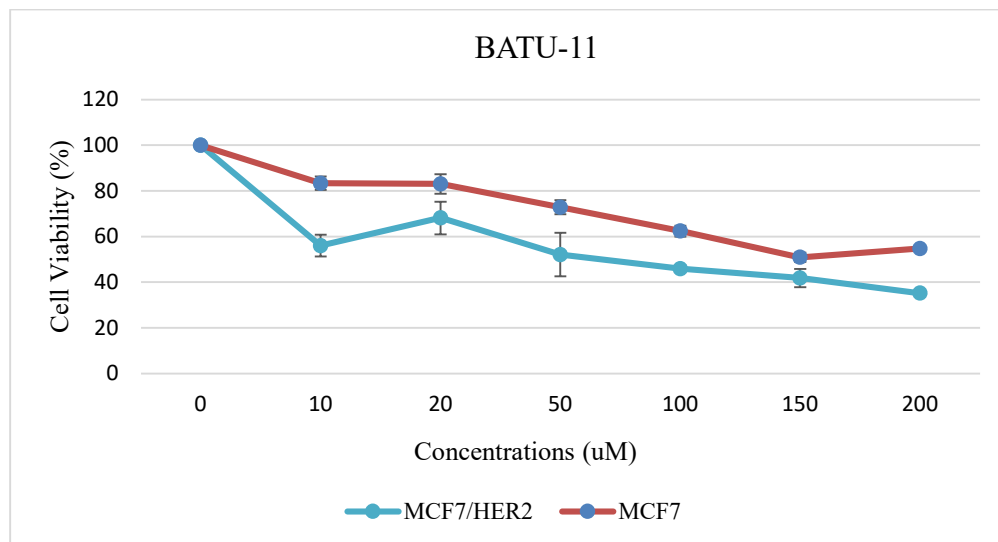


Fig.-2: The Curve of Cell Viability vs Concentration of BATU-11 in MCF7 and MCF7/HER-7

Table-3: IC_{50} and CC_{50} Value of BATU-11 on Cell Lines

Compounds	MCF7 (μ M)	MCF7/HER2 (μ M)	Vero (μ M)
4-nitrobenzoyl-3-allylthiourea	225 $\pm$ 1.64	85 $\pm$ 2.91	1097 $\pm$ 3.48
Lapatinib	160 $\pm$ 3.07	81 $\pm$ 2.49	-

CONCLUSION

Based on the study, it is known that the 4-nitrobenzoyl-3-allylthiourea compound has the potential to be further developed as an anticancer candidate for breast cancer with HER2 overexpression.

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