*Send Orders for Reprints to reprints@benthamscience.net*

*Letters in Drug Design & Discovery,* **XXXX,** *XX,* **1-10**



**RESEARCH ARTICLE**

# Physicochemical, ADMET Properties, and Molecular Docking Studies of *N*-benzoyl-*N'*-naphtylthiourea Derivatives for Anti-Breast Cancer Activity

Dewi Sinta Megawati[1,2], Juni Ekowati[3] and Siswandono Siswodihardjo[3,*]

[1]*Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Jl Dr Ir H Soekarno Mulyorejo, Surabaya, 60115, East Java, Indonesia;* [2]*Department of Pharmacy, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia;* [3]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Jl Dr Ir H Soekarno Mulyorejo, Surabaya, 60115, East Java, Indonesia*

**Abstract:** ***Background*****:** *N*-benzoyl-*N'*-naphthylthiourea (BNTU) is a thiourea-derived compound that is predicted to have anti-breast cancer activity. However, their physicochemical properties, ADMET, and receptor-specific targets for their anti-breast cancer activity have not been reported.

***Objective*****:** This study aimed to predict the physicochemical properties, ADMET, and anti-breast cancer activity of BNTU and its derivatives by *in silico* approach.

***Methods*****:** The physicochemical and ADMET properties were predicted using the pkCSM online program and ProTox-II online tool. While the anti-breast cancer activity was predicted using the molecular docking method through the Molegro Virtual Docker (MVD) program on the HER-2 receptor. The parameter observed in the molecular docking method was the bond energy value or rerank score (RS). Compounds with small RS values were predicted to have a great activity.

***Results*****:** Most BNTU derivatives had lower RS values than BNTU, especially 4TBBNTU, and 4CFBNTU, although their RS values were still higher than lapatinib and TAK-285. As for the reference ligand hydroxyurea, the RS value of BNTU and its derivatives was much lower. The physicochemical and pharmacokinetic properties (ADMET) of lapatinib and TAK-285 were not better than that of BNTU and its derivatives.

***Conclusion*****:** Five compounds that deserve to be synthesized and tested for anti-breast cancer activity *in vitro* and *in vivo* are 4TBBNTU, 3CFBNTU, 4CFBNTU, 4OCBNTU, and the lead compound BNTU.

**ARTICLE HISTORY**

Received: November 14, 2022
Revised: June 21, 2023
Accepted: July 17, 2023

*DOI:*
*10.2174/1570180820666230817101819*

**Keywords:** Physicochemical properties, ADMET, docking, BNTU, anti-breast cancer, *in silico*.

## 1. INTRODUCTION

In the early stages of drug discovery, safety and toxicity issues are problems that must be addressed early [1]. ADMET (absorption, distribution, metabolism, excretion, and toxicity) is one of the benchmarks in assessing the effect or risk of a compound on the human body [2, 3]. The poor physicochemical and ADMET properties cause many drugs that fail in their development, so they are withdrawn from the market. Therefore, it needs to be considered early in the drug discovery process. The drug discovery process, which takes a very long time and is expensive, can be accelerated using the *in silico* method [4].

*In silico* is an approach to a natural condition or situation in a computer simulation using a specific program analogous to *in vitro* and *in vivo* drug discovery studies. Using computer simulations can reduce drug design and development costs by up to 50% [5].

Synthesis of compound derivatives with known activity and then testing the activity of these derivatives is one of the crucial strategies in developing new drugs. Thiourea and its derivatives have extensive applications in various fields, and have diverse activities, including as anticancer agents [6], due to their inhibition of receptor tyrosine kinases (RTKs), protein tyrosine kinases (PTKs), and NADH oxidase [7], acts as a topoisomerase inhibitor, somatostatin agonist, sirtuin, and carbonic anhydrase (CA) [8], and inhibits the expression of proteins that play an important role in cell proliferation [9]. The presence of anticancer activity of thiourea derivatives inspired medicinal chemists to design and synthesize new thiourea derivatives. Research on thiourea as an anti-breast cancer has been reported [9, 10].

The design of the new compound in this study was carried out by modifying the structure by including naphthyl

*Address correspondence to this author at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Jl Dr Ir H Soekarno Mulyorejo, Surabaya, 60115, East Java, Indonesia; Tel: +62 812-3206-328; E-mail: prof.sis@ff.unair.ac.id

and benzoyl groups in the thiourea pharmacophore, namely the compound *N*-benzoyl-*N'*-naphthylthiourea (BNTU) as the lead compound. The naphthyl and benzoyl groups are expected to increase the activity of these compounds as anti-breast cancer candidates by increasing their lipophilic and electronic properties. Substitution is carried out on the benzene ring (benzoyl group) of the side chain (R group). Substituents were selected based on the Topliss approach model into several derivative compounds.

This study aimed to determine the physicochemical properties, ADMET, and anti-breast cancer activity of BNTU compounds *in silico*. The physicochemical and ADMET properties were predicted using the pkCSM online program and ProTox-II online tool. While the anti-breast cancer activity was predicted using the molecular docking method through the Molegro Virtual Docker program on the HER-2 receptor with the PDB code 3RCD. The reference ligands used are hydroxyurea, lapatinib, and TAK-285 (3RCD ligand) (Fig. **1**). TAK-285 is the native ligand of the HER-2 receptor with the PDB code 3RCD [11].

Parameters observed in the molecular docking method were the root mean square deviation (RMSD) value for the validation of the docking method [6] and the bond energy value or rerank score (RS) for activity prediction [12]. The acceptable RMSD is 2 Å [13]. The re-docking results are more accurate because they approach the crystallographic ligands if the RMSD value gets smaller [14]. The RS is the basis for determining the best activity of the test ligand in the docking method. Prediction of anticancer activity is good if the RS value is lower, which means the ligand and receptor binding stability is higher [15]. Information related to physicochemical properties and ADMET will influence the decision to synthesize the BNTU test compound and its derivatives.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The hardware used was the HUAWEI-IM01SOUS with an AMD Ryzen 7 3700U processor with Radeon Vega Mobile Gfx based on Windows 10 Home Single Language operation with the following programs: ChemOffice 2020 (ChemDraw 20.0 and Chem3D 20.0) for molecular modeling, energy minimization and SMILES translator, pkCSM online server (http://biosig.unimelb.edu.au/pkcsm/prediction) for prediction of physicochemical and ADMET properties, ProTox-II online server (https://tox-new.charite.de/protox_II/) for toxicity prediction, Molegro Virtual Docker (MVD) 5.5 for validation and docking process.

### 2.2. Ligands Preparation

The chemical structure of BNTU and its derivatives (Table **1**), as well as the reference ligands (Fig. **1**), was drawn in 2D and 3D using the ChemOffice 2020 program (ChemDraw 20.0 and Chem3D 20.0). The minimum energy (Etot) was measured using the MMFF94 (Merck molecular force field 94) method, then stored in mol2 form [SYBYL2 ( *.mol2)] and smi [SMILES (*.smi)].

## 3. EXPERIMENTAL

### 3.1. Prediction of the Physicochemical and ADMET Properties

Prediction of the physicochemical and ADMET properties BNTU and its derivatives in SMILES format predicted physicochemical properties such as molecular weight (MW), the logarithm of octanol/water partition coefficient (log P), rotatable bond (ROT), hydrogen bond acceptors (HBA), and

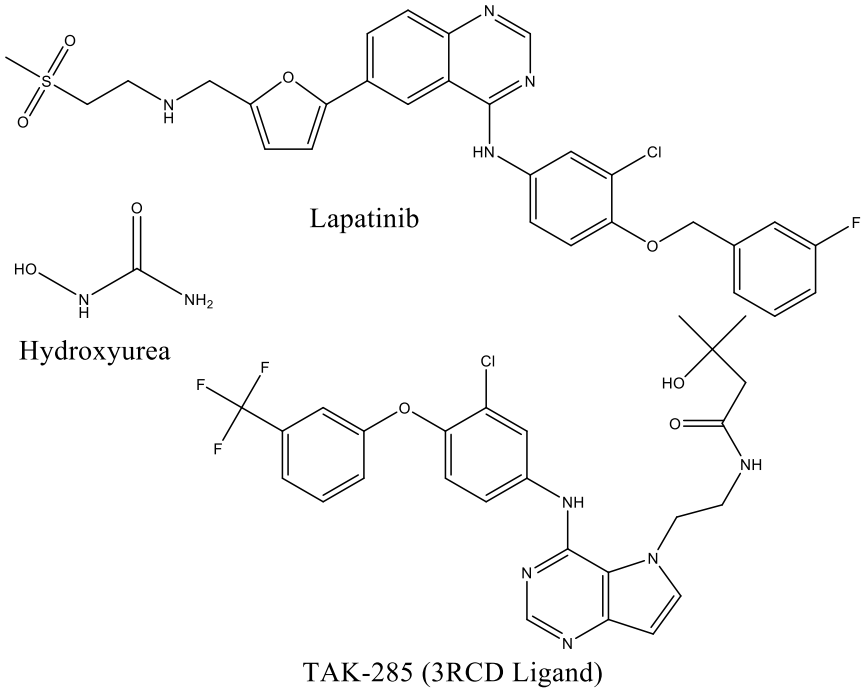
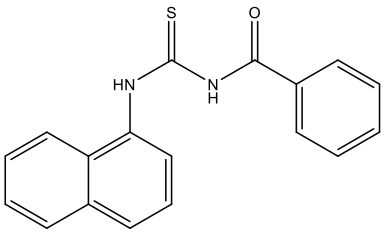



**Fig. (1).** Two-dimensional structure of the reference ligands.

**Table 1. The chemical structure of *N*-benzoyl-*N'*-naphtylthiourea derivatives and references.**




| No | Ligand Code | R | Ligand Name |
|---|---|---|---|
| 1 | BNTU | H | *N*-benzoyl-*N'*-naphtylthiourea |
| 2 | 2ClBNTU | 2-Cl | *N*-(2-chlorobenzoyl)-*N'*-naphtylthiourea |
| 3 | 3ClBNTU | 3-Cl | *N*-(3-chlorobenzoyl)- *N'*-naphtylthiourea |
| 4 | 4ClBNTU | 4-Cl | *N*-(4-chlorobenzoyl)-*N'*-naphtylthiourea |
| 5 | 34ClBNTU | 3,4-Cl | *N*-(3,4-dichlorobenzoyl)-*N'*-naphtylthiourea |
| 6 | 24ClBNTU | 2,4-Cl | *N*-(2,4-dichlorobenzoyl)-*N'*-naphtylthiourea |
| 7 | 4BrBNTU | 4-Br | *N*-(4-bromobenzoyl)-*N'*-naphtylthiourea |
| 8 | 4FBNTU | 4-F | *N*-(4-fluorobenzoyl)-*N'*-naphtylthiourea |
| 9 | 3CFBNTU | 3-$CF_3$ | *N*-(3-trifluoromethylbenzoyl)-*N'*-naphtylthiourea |
| 10 | 4CFBNTU | 4-$CF_3$ | *N*-(4-trifluoromethylbenzoyl)-*N'*-naphtylthiourea |
| 11 | 3NBNTU | 3-$NO_2$ | *N*-(3-nitrobenzoyl)-*N'*-naphtylthiourea |
| 12 | 4NBNTU | 4-$NO_2$ | *N*-(4-nitrobenzoyl)-*N'*-naphtylthiourea |
| 13 | 4CBNTU | 4-$CH_3$ | *N*-(4-methylbenzoyl)-*N'*-naphtylthiourea |
| 14 | 4OCBNTU | 4-$OCH_3$ | *N*-(4-metoxybenzoyl)-*N*'-naphtylthiourea |
| 15 | 4TBBNTU | 4-$C(CH_3)_3$ | *N*-(4-*tert*-butylbenzoyl)-*N*'-naphtylthiourea |
| 16 | HU | - | Hydroxyurea |
| 17 | LT | - | Lapatinib |
| 18 | TAK-285 | - | Native ligand of HER-2 receptor (3RCD) |

hydrogen bond donors (HBD) using the pkCSM online tool. Prediction of ADMET properties using the pkCSM online program.

### 3.2. Molecular Docking Study

Docking studies were performed on the active site of the HER-2 receptor (PDB code: 3RCD). The molecular structure of the receptor was obtained from the RSCB protein data bank (PDB) (https://www.rcsb.org/).

The original ligand from the HER2 receptor was first validated by the docking method by measuring the RMSD value of the native ligand. The structure of BNTU and its derivatives, as well as reference ligands that have been stored in the form of mol2 {SYBYL2(*.mol2)} in the Chem 3D 20.0 program carried out the docking process against the HER-2 receptor target using the Molegro computer program Virtual Docker version 5.5. The results obtained were in the form of RS. The test results were repeated three times.

## 4. RESULTS

### 4.1. Prediction of the Physicochemical and ADMET Properties

Prediction of physicochemical properties of BNTU and its derivatives, as well as reference ligands, were carried out using Lipinski's "rules of Fives" and can be seen in Table **2**. Meanwhile, the ADMET prediction results are presented in Table **3**.

### 4.2. Molecular Docking Study

Molecular docking studies of BNTU and its derivatives against the HER-2 receptor (PDB ID 3RCD) were carried out to predict the anti-breast cancer activity of these compounds. The parameters observed in the validation process are listed in Table **4**. The docking results and amino acid interactions of the HER2 receptor that interact with BNTU and its derivatives can be seen in Tables **5** and **6**.

**Table 2. Physicochemical properties prediction of BNTU derivatives and reference ligands.**

| No | Ligands | MW (g/mol) | Log P | ROT | HBA | HBD |
|---|---|---|---|---|---|---|
| 1 | BNTU | 306.39 | 3.9666 | 2 | 2 | 3 |
| 2 | 2ClBNTU | 340.835 | 4.62 | 2 | 2 | 2 |
| 3 | 3ClBNTU | 340.835 | 4.62 | 2 | 2 | 2 |
| 4 | 4ClBNTU | 340.835 | 4.62 | 2 | 2 | 2 |
| 5 | 34ClBNTU | 375.28 | 5.2734 | 2 | 2 | 2 |
| 6 | 24ClBNTU | 375.28 | 5.2734 | 2 | 2 | 2 |
| 7 | 4BrBNTU | 385.286 | 4.7291 | 2 | 2 | 2 |
| 8 | 4FBNTU | 324.38 | 4.1057 | 2 | 2 | 2 |
| 9 | 3CFBNTU | 374.387 | 4.9854 | 2 | 2 | 2 |
| 10 | 4CFBNTU | 374.387 | 4.9854 | 2 | 2 | 2 |
| 11 | 3NBNTU | 351.387 | 3.8748 | 3 | 4 | 2 |
| 12 | 4NBNTU | 351.387 | 3.8748 | 3 | 4 | 2 |
| 13 | 4CBNTU | 320.417 | 4.27502 | 2 | 2 | 2 |
| 14 | 4OCBNTU | 336.416 | 3.9752 | 3 | 3 | 2 |
| 15 | 4TBBNTU | 362.498 | 5.2641 | 2 | 2 | 2 |
| 16 | HU | 76.055 | -0.9561 | 0 | 2 | 3 |
| 17 | LT | 581.069 | 6.1391 | 11 | 8 | 2 |
| 18 | TAK-285 | 547.965 | 5.9166 | 9 | 7 | 3 |

**Table 3. *In silico* prediction ofADMET of BNTU derivatives and reference ligands.**

| No | Ligands | Absorption | | Distribution | | Metabolism | | Excretion | | Toxicity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Caco2 Permeability (log Papp; $10^{-6}$ cm/s) | Intestinal Absorption (Human; %) | VDss (Human; Log L/kg) | BBB Permeability (Log BB) | CYP2D6 Substrate | CYP3A4 Substrate | Total Clearance (Log mL/min/kg) | Renal OCT2 Substrate | Hepatotoxicity | $LD_{50}$ (mg/kg) | Class |
| 1 | BNTU | 1.674 | 90.953 | -0.137 | 0.427 | Yes | Yes | -0.226 | No | No | 2850 | V |
| 2 | 2ClBNTU | 1.683 | 89.292 | -0.119 | 0.426 | Yes | Yes | -0.091 | No | No | 2885 | V |
| 3 | 3ClBNTU | 1.522 | 90.163 | -0.049 | 0.364 | Yes | Yes | -0.297 | No | No | 2885 | V |
| 4 | 4ClBNTU | 1.522 | 89.571 | -0.099 | 0.384 | Yes | Yes | -0.359 | No | No | 2885 | V |
| 5 | 34ClBNTU | 0.977 | 89.24 | 0.023 | 0.334 | Yes | Yes | -0.174 | No | No | 2885 | V |
| 6 | 24ClBNTU | 0.955 | 88.997 | -0.031 | 0.322 | Yes | Yes | -0.168 | No | No | 2885 | V |
| 7 | 4BrBNTU | 0.983 | 89.504 | -0.083 | 0.383 | Yes | Yes | -0.38 | No | No | 2850 | V |
| 8 | 4FBNTU | 1.469 | 90.425 | -0.163 | 0.536 | Yes | Yes | -0.371 | No | No | 2885 | V |
| 9 | 3CFBNTU | 0.975 | 89.737 | -0.015 | 0.279 | Yes | Yes | -0.244 | No | Yes | 2850 | V |
| 10 | 4CFBNTU | 0.997 | 88.697 | -0.084 | 0.402 | Yes | Yes | -0.309 | No | Yes | 2850 | V |
| 11 | 3NBNTU | 0.892 | 89.918 | -0.2 | 0.007 | No | Yes | -0.146 | No | No | 2850 | V |

**(Table 3) Contd….**

| No | Ligands | Absorption | | Distribution | | Metabolism | | Excretion | | Toxicity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Caco2 Permeability (log Papp; $10^{-6}$ cm/s) | Intestinal Absorption (Human; %) | VDss (Human; Log L/kg) | BBB Permeability (Log BB) | CYP2D6 Substrate | CYP3A4 Substrate | Total Clearance (Log mL/min/kg) | Renal OCT2 Substrate | Hepatotoxicity | $LD_{50}$ (mg/kg) | Class |
| 12 | 4NBNTU | 0.878 | 89.224 | -0.252 | -0.063 | No | Yes | -0.21 | No | No | 2850 | V |
| 13 | 4CBNTU | 1.515 | 91.03 | -0.073 | 0.397 | Yes | Yes | -0.285 | No | Yes | 2850 | V |
| 14 | 4OCBNTU | 1.339 | 91.918 | -0.053 | 0.421 | Yes | Yes | -0.191 | No | No | 2885 | V |
| 15 | 4TBBNTU | 0.974 | 89.455 | -0.02 | 0.345 | Yes | Yes | -0.344 | No | Yes | 2885 | V |
| 16 | HU | 0.487 | 73.704 | -0.888 | -0.955 | No | No | 0.655 | No | No | 5760 | VI |
| 17 | Lapatinib | 0.235 | 100 | 0.276 | -1.196 | No | Yes | 0.569 | No | Yes | 1500 | IV |
| 18 | TAK-285 | 0.819 | 88.927 | 0.429 | -1.845 | No | Yes | 0.156 | No | Yes | 1368 | IV |

**Table 4. Method validation result of HER-2 receptor.**

| Parameters | Results |
|---|---|
| PDB ID | 3RCD |
| Ligan code | 03P_9001[A] |
| Native ligand | *N*-{2-[4-({3-chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl]ethyl}-3-hydroxy-3-methylbutanamide |
| Cavity | 1 |
| Center | X: 13.44<br>Y: 2.65<br>Z: 27.45 |
| Radius | 10 |
| Number of runs | 10 |
| RMSD | 1.26 |
| Amino acid residues (hydrogen bonds) | Met 801 |
| Amino acid residues (steric interaction) | Asp 863, Leu 726, Ala 751, Ser 783, Phe 864 |

## 5. DISCUSSION

The "Rule of Five" predicts the suitability of drug candidates but was developed primarily using orally administered drugs. The "Rule of Five" states that poor absorption or permeation is expected when (MW>500, the number of HBD>5, the number of HBA>10, or log P>5 [16, 17]. Table **2** shows that the BNTU and its derivatives complied with Lipinski's rules, except for 34ClBNTU, 24ClBNTU, and 4TBBNTU, because the log P value was greater than 5. However, according to Chander *et al*. (2017), 95% of clinically approved drugs have the following range of physicochemical properties: MW (130–725), HBD (0–6), HBA (2–20), log P (-2–6.5), and ROT (0–15)(18), so 34ClBNTU, 24ClBNTU, and 4TBBNTU fall into that category. In contrast, the reference ligands lapatinib and TAK-285 did not comply with Lipinski's rule because their MW was greater than 500, and their log P value was higher than 5. However, it is still a clinically approved drug, according to Chander (2017). It can be concluded that the BNTU and some of its derivatives have better absorption and permeability than lapatinib and TAK-285, so they are potential drug candidates, as predicted.

**Table 5. Docking results of BNTU and its derivatives with HER-2 receptors.**

| No | Ligands | Average RS(kcal/mol) |
|---|---|---|
| 1 | BNTU | -94.9564 ± 0.033 |
| 2 | 2ClBNTU | -91.2791 ± 0.543 |
| 3 | 3ClBNTU | -95.2371 ± 0.200 |
| 4 | 4ClBNTU | -92.8024 ± 0.317 |
| 5 | 34ClBNTU | -95.2439 ± 0.203 |
| 6 | 24ClBNTU | -89.5517 ± 0.021 |
| 7 | 4BrBNTU | -94.1807 ± 1.812 |
| 8 | 4FBNTU | -91.7899 ± 0.999 |
| 9 | 3CFBNTU | -99.1661 ± 0.659 |
| 10 | 4CFBNTU | -104.090 ± 0.363 |
| 11 | 3NBNTU | -96.5193 ± 0.718 |
| 12 | 4NBNTU | -96.1716 ± 0.205 |
| 13 | 4CBNTU | -90.9751 ± 0.648 |
| 14 | 4OCBNTU | -98.8942 ± 1.653 |
| 15 | 4TBBNTU | -106.137 ± 0.894 |
| 16 | HU | -34.0187 ± 0.007 |
| 17 | LT | -125.98 ± 0.452 |
| 18 | TAK-285 | -119.514 ± 0.749 |

**Table 6. HER2 receptor amino acids (PDB ID 3RCD) involved in interaction with BNTUderivatives.**

| No | Ligands | Hydrogen Bond | Steric Interaction |
|---|---|---|---|
| 1 | BNTU | Ala 751 | Asp 863, Ala 751, Leu 796, Leu 785, Val 734, Ile 752, Lys 753 |
| 2 | 4TBBNTU | Lys 753 | Thr 862(2), Ala 751, Lys 753, Phe 864, Thr 798, Leu 796 |
| 3 | 4CFBNTU | Ala 751 | Asp 863, Ala 751, Thr 862, Val 734, Ile 752, Lys 753 |
| 4 | 3CFBNTU | Thr 862 | Asp 863, Ala 751, Thr 862 |
| 5 | 4OCBNTU | Thr 862, Asp 863 | Asp 863, Thr 862, Lys 753, Thr 798 |
| 6 | HU | Thr 862(2), Asp 863, Ser 783, Thr 798 | Thr 862, Asp 863, Thr 798, Ser 783, Leu 785 |
| 7 | LT | Met 801 | Phe 864, Ala 751(2), Lys 753, Asp 863, Met 801(2) |
| 8 | TAK-285 | Gly 865 | Leu 852, Met 801(2), Phe 1004, Phe 731, Gly 865, Thr 862(2), Asp 863, Lys 753, Ala 751, Ile 752 |

Drug candidates are triaged early during drug development based on computer modeling, high-throughput screening, and cell-based assays that predict pharmacologic activity. It is, however, much more difficult to predict drug ADME, which typically requires evaluation *in vivo*. *In vivo* studies are slow and expensive, so it is desirable to have simple methods to predict the ADME properties of drug candidates [17, 18]. Predicting pharmacokinetic profiles of BNTU and its derivatives, reference ligands hydroxyurea, lapatinib, and TAK-285 can be seen in Table **3**.

The absorption parameters used were Caco2 permeability and human intestinal absorption. Caco-2 cells are one of the *in vitro* models derived from human colorectal carcinoma. They are most widely used to understand intestinal absorption and decipher transport mechanisms for orally administered drugs [2, 19]. Calculation *via* log Papp (the apparent permeability coefficient) measurements. Compounds with Papp > $8x10^{-6}$ cm/s are considered to have high Caco2 permeability. High Caco-2 permeability would translate into predicted log Papp values > 0.90 cm/s for the pkCSM predictive model [20]. BNTU and its derivatives have log Papp values > 0.9 cm/s, except for 3NBNTU and 4NBNTU. However, both compounds have log Papp values close to 0.9 cm/s. Reference ligands hydroxyurea, lapatinib, and TAK-285 have log Papp values < 0.9 cm/s. Therefore, it can be concluded that BNTU and its derivatives are predicted to have high and better permeability than the reference ligands, except 3NBNTU dan 4NBNTU.

The parameter of intestinal absorption can predict the percent of absorption in the human intestine. The compound has good absorption if the absorption value is > 80%, and the absorption is bad if < 30%. Table **3** shows that the BNTU and its derivatives have absorption values > 80%, so it is predicted to have good absorption, as well as lapatinib and TAK-285. In comparison, hydroxyurea has an absorption value of < 80%, so it is predicted that its absorption will not be better than that of BNTU and its derivatives.

The distribution of BNTU and its derivatives was predicted based on VDss and BBB permeability parameters. The (VDss) is the theoretical volume in which the total dose of a drug must be distributed evenly to give the same concentration as in blood plasma. Drugs will be distributed more in the tissues than plasma if the VDss value is higher. Compounds are said to have a low volume of distribution if the VDss value is < -0.15 L/kg and high if it is> 0.45 L/kg. The ability of a drug to cross the blood-brain barrier is an important parameter that needs to be considered to help reduce side effects and toxicity or to increase the efficacy of drugs whose pharmacological activity is present in the brain. A compound is said to be able to penetrate the blood-brain barrier well if it has a BB value > 0.3 and cannot be properly distributed if the BB log < -1 [20]. Based on Table **3**, it can be seen that the VDss value of BNTU and its derivatives is greater than -0.15, except for 4FBNTU, 3NBNTU, 4NBNTU, which means that the distribution volume is good. The reference ligands are smaller than -0.15, which means that the volume of distribution is low. While the log BB value ranges from -0.063 to 0.427, it can be said that all BNTU and its derivatives can penetrate the blood-brain barrier well. Not so with hydroxyurea, lapatinib, and TAK-285 as reference ligands.

Metabolism is concerned with drug biotransformation (the process by which drugs are chemically converted in the body into metabolites) involving enzymes [2]. Cytochrome P450 is a detoxification enzyme that works through the oxidation of foreign organic compounds, including drugs and helps excrete these compounds CYP2D6 and CYP3A4 are the major isoforms of cytochromes involved in drug biotransformation in humans. All BNTU and its derivatives (except 3NBNTU and 4NBNTU) were predicted to inhibit CYP2D6 and CYP3A4, while the reference ligands did not, which means that all ligands that affect or inhibit CYP2D6 and CYP3A4.

Parameters to measure the compound excretion process are the total clearance value and renal OCT2 substrate value. The BNTU and all its derivatives had a lower total clearance value than the reference ligands, meaning the reference ligands were excreted more quickly than the test ligands. The total clearance value is important to determine the dose level in achieving steady-state concentrations [15]. OCT2 is a transporter protein that plays a role in renal absorption, disposition, and clearance of drug compounds. Helpful information regarding not only its clearance but also its potential

contraindications [21]. All test and reference ligands are predicted not to be OCT2 substrates because they do not affect OCT2 substrates.

The toxicity parameters calculated were hepatotoxicity predicted using the pkCSM online tool program, $LD_{50}$, and toxicity class using the ProTox-II online tool. The $LD_{50}$ values of BNTU and its derivatives are 2850 and 2885 mg/kg. However, four BNTU derivatives are hepatotoxic: 3CFBNTU, 4CFBBNTU, 4OCBNTU, 4TBBNTU, but belongs to class V GHS (Globally Harmonized System), toxicity class V GHS which means the compound has a low acute toxicity effect, so it is predicted to have safer toxicity than lapatinib and TAK-285, which are included in class IV according to the GHS [22].

Method validation and re-docking were carried out to ensure that the method to be used was appropriate and aimed at finding pocket cavity receptors where the drug/test ligand would be attached [23]. The results of re-docking were the default results from native ligands (03P_9001[A]) with receptors. The validation parameter is the RMSD value less than 2 Å [24]. The RMSD value from the validation method is 1.26 Å, which means the method used is acceptable because the value is less than 2 Å (Table **4** and (Fig. **2**).

The results of the *in silico* test using the docking method on the BNTU and its derivatives against the HER-2 receptor can be seen in Tables **5** and **6**. Based on Table **5**, it is known that lapatinib has a lower RS value than the native ligand. This shows that the affinity of lapatinib is higher than the native ligand of the HER-2 receptor (PDB ID 3RCD) itself, which means that lapatinib has better anti-breast cancer activity than its native ligand. The lower the RS value indicates that the bond between the ligand-receptor is more stable, so the interaction is stronger. Therefore it can be predicted that biological activity will also increase [25]. All BNTU and its derivatives had greater RS values than lapatinib and TAK-285, but the physicochemical and ADMET properties of lapatinib and TAK-285 were not better than BNTU and its derivatives. As for the reference ligand hydroxyurea, the value of RS BNTU and its derivatives is much lower, so the test ligand's activity is predicted to be better than hydroxyurea. 3-D descriptions of amino acid interactions of BNTU and its derivatives can be seen in Figs. (**3**-**6**) and Table **6**. The four ligands with the best anti-breast cancer activity based on the RS value are 4TBBNTU, 3CFBNTU, 4CFBNTU, and 4OCBNTU. Although 4TBBNTU, 3CFBNTU, and 4CFBNTU are predicted to be hepatotoxic, they have a low acute toxicity effect according to GHS, so they must be proven *in vitro* and *in vivo*. Therefore, these four ligands, along with the lead compound BNTU were feasible to be synthesized and continued for *in vitro* and *in vivo* tests.

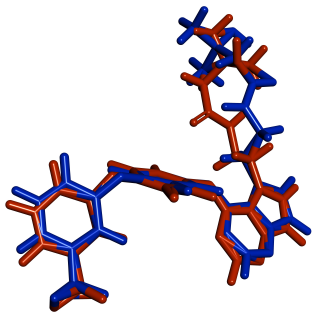


**Fig. (2).** Results of docking 3RCD receptors with native ligand: overlay between re-docking ligands (blue) and native ligands (red). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

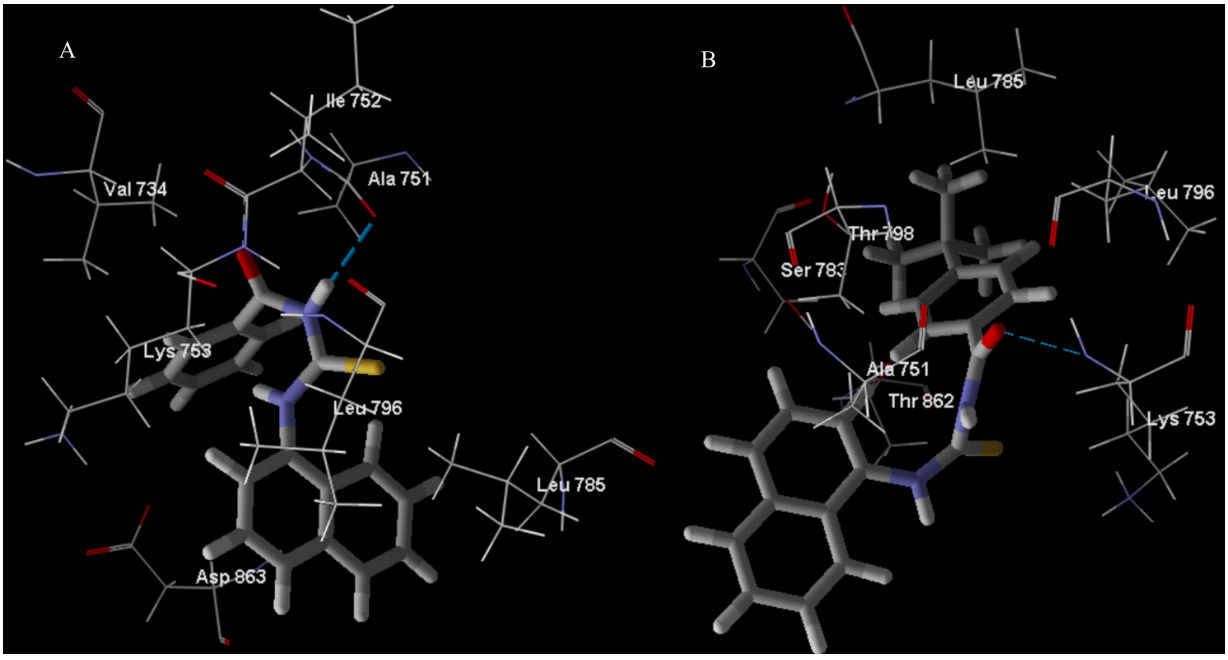



**Fig. (3).** 3-D description of amino acid interactions of HER-2 receptors with BNTU (**A**) and 4TBBNTU (**B**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

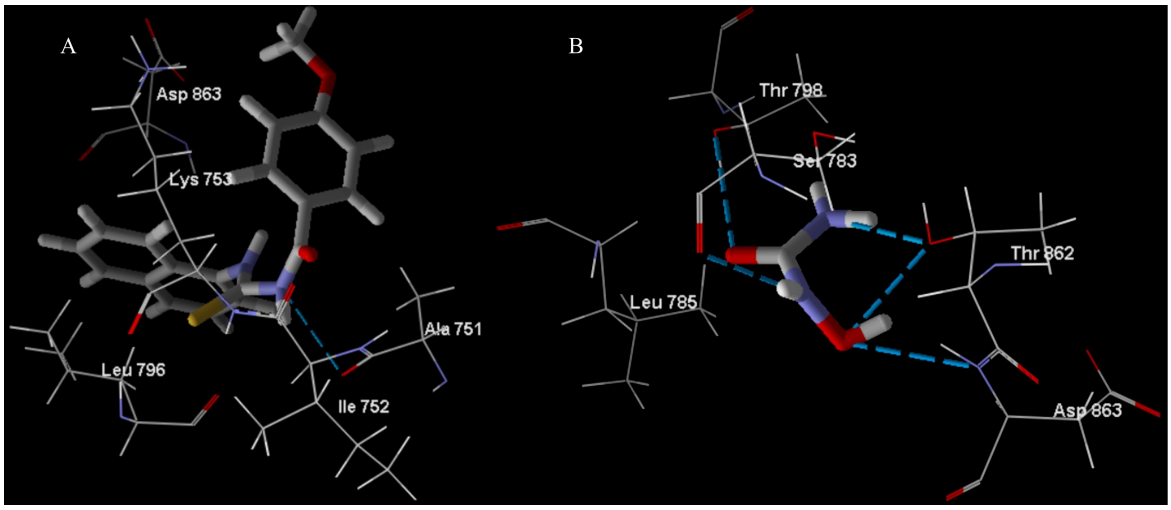



**Fig. (4).** 3-D description of amino acid interactions of HER-2 receptors with 4CFBNTU (**A**) and 3CFBNTU (**B**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

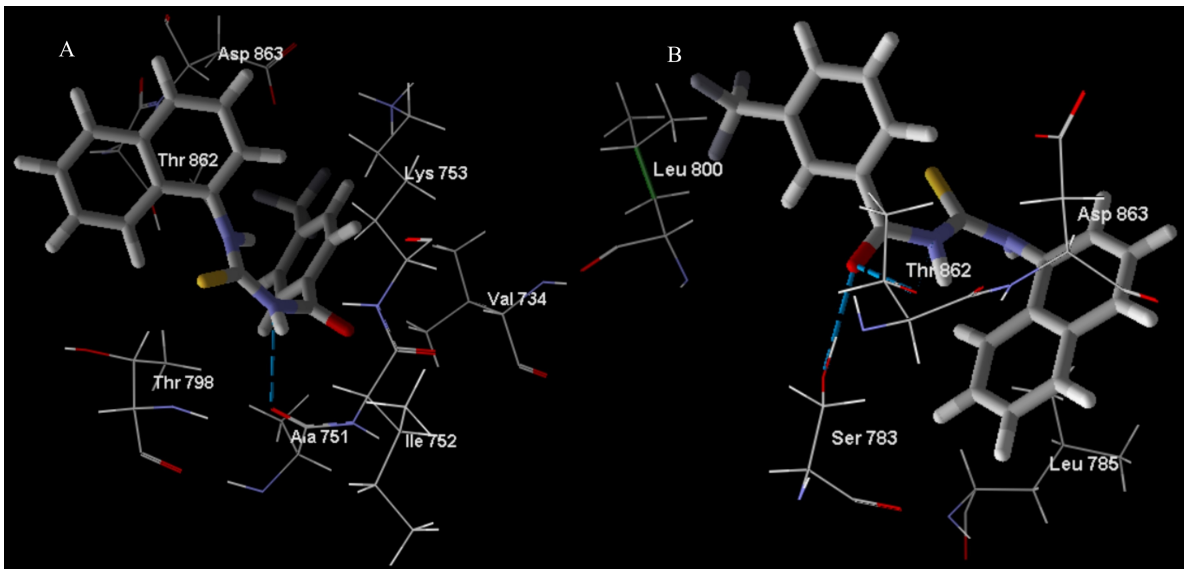



**Fig. (5).** 3-D description of amino acid interactions of HER-2 receptors with 4OCBNTU (**A**) and Hydroxyurea (**B**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

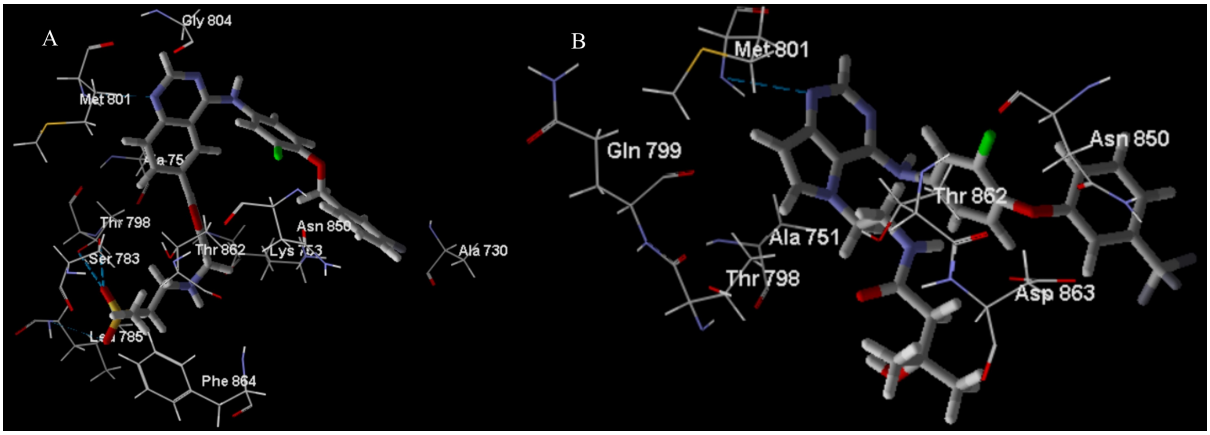



**Fig. (6).** 3-D description of amino acid interactions of HER-2 receptors with Lapatinib (**A**) and TAK-285 (**B**). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

## CONCLUSION

Based on the results of the *in silico* test, it is known that the ligands 4TBBNTU, 3CFBNTU, 4CFBNTU, and 4OCBNTU are the four best anti-breast cancer candidates that deserve to be synthesized and tested for their activity *in vitro* and *in vivo* because they have the lowest molecular docking RS value and has good physicochemical and ADMET properties.

## LIST OF ABBREVIATIONS

BNTU = *N*-benzoyl-*N′*-naphthylthiourea

CA = Carbonic Anhydrase

MVD = Molegro Virtual Docker

MVD = Molegro Virtual Docker

PTKs = Protein Tyrosine Kinases

RMSD = Root Mean Square Deviation

RS = Rerank Score

RTKs = Receptor Tyrosine Kinases

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

## FUNDING

This work is financially supported by the Project Management Unit (PMU) of Maulana Malik Ibrahim State Islamic University through the program "Masters (S2) and Doctoral (S3) Advanced Study Scholarships The Development of UIN Maulana Malik Ibrahim Malang Phase II, East Java Project".

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Conceptualization, D.S.M.; methodology, D.S.M; software, D.S.M., S.S; validation, J.E., S.S.; formal analysis, D.S.M., J.E., and S.S.; investigation, D.S.M., J.E., and S.S.; data curation, D.S.M.; writing - original draft preparation, D.S.M.; writing - review and editing, J.E. and S.S.; supervision, J.E., S.S.; project administration, D.S.M. All authors have read and agreed to the published version of the manuscript. Special thanks to Prof. Dr. apt. Siswandono, MS., who allowed the use of the license from Molegro Virtual Docking (MVD).

## REFERENCES

[1] Van de Waterbeemd, H. Improving compound quality through *In vitro* and *In Silico* physicochemical profiling. *Chem. Biodivers.,* **2009**, *6*(11), 1760-1766. http://dx.doi.org/10.1002/cbdv.200900056 PMID: 19937820

[2] Lin, J.; Sahakian, D.; de Morais, S.; Xu, J.; Polzer, R.; Winter, S. The role of absorption, distribution, metabolism, excretion and toxicity in drug discovery. *Curr. Top. Med. Chem.,* **2003**, *3*(10), 1125-1154. http://dx.doi.org/10.2174/1568026033452096 PMID: 12769713

[3] Cheng, F.; Li, W.; Liu, G.; Tang, Y. *In Silico* ADMET prediction: Recent advances, current challenges and future trends. *Curr. Top. Med. Chem.,* **2013**, *13*(11), 1273-1289. http://dx.doi.org/10.2174/15680266113139990033 PMID: 23675935

[4] van de Waterbeemd, H.; Gifford, E. ADMET *In Silico* modelling: Towards prediction paradise? *Nat. Rev. Drug Discov.,* **2003**, *2*(3), 192-204. http://dx.doi.org/10.1038/nrd1032 PMID: 12612645

[5] Geldenhuys, W.J.; Gaasch, K.E.; Watson, M.; Allen, D.D.; Van der Schyf, C.J. Optimizing the use of open-source software applications in drug discovery. *Drug Discov. Today,* **2006**, *11*(3-4), 127-132. http://dx.doi.org/10.1016/S1359-6446(05)03692-5 PMID: 16533710

[6] Pingaew, R.; Prachayasittikul, V.; Anuwongcharoen, N.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V. Synthesis and molecular docking of N,N′-disubstituted thiourea derivatives as novel aromatase inhibitors. *Bioorg. Chem.,* **2018**, *79*, 171-178. http://dx.doi.org/10.1016/j.bioorg.2018.05.002 PMID: 29758407

[7] Yang, W.; Hu, Y.; Yang, Y.S.; Zhang, F.; Zhang, Y.B.; Wang, X.L.; Tang, J.F.; Zhong, W.Q.; Zhu, H.L. Design, modification and 3D QSAR studies of novel naphthalin-containing pyrazoline derivatives with/without thiourea skeleton as anticancer agents. *Bioorg. Med. Chem.,* **2013**, *21*(5), 1050-1063. http://dx.doi.org/10.1016/j.bmc.2013.01.013 PMID: 23391364

[8] Kumar, V.; Chimni, S. Recent developments on thiourea based anticancer chemotherapeutics. *Anticancer. Agents Med. Chem.,* **2015**, *15*(2), 163-175. http://dx.doi.org/10.2174/1871520614666140407123526 PMID: 24712324

[9] Widiandani, T.; Siswandono, ; Meiyanto, E. Anticancer evaluation of *N*-benzoyl-3-allylthiourea as potential antibreast cancer agent through enhances HER-2 expression. *J. Adv. Pharm. Technol. Res.,* **2020**, *11*(4), 163-168. http://dx.doi.org/10.4103/japtr.JAPTR_77_20 PMID: 33425698

[10] Viswas, R.S.; Pundir, S.; Lee, H. Design and synthesis of 4-piperazinyl quinoline derived urea/thioureas for anti-breast cancer activity by a hybrid pharmacophore approach. *J. Enzyme Inhib. Med. Chem.,* **2019**, *34*(1), 620-630. http://dx.doi.org/10.1080/14756366.2019.1571055 PMID: 30727782

[11] Ishikawa, T.; Seto, M.; Banno, H.; Kawakita, Y.; Oorui, M.; Taniguchi, T.; Ohta, Y.; Tamura, T.; Nakayama, A.; Miki, H.; Kamiguchi, H.; Tanaka, T.; Habuka, N.; Sogabe, S.; Yano, J.; Aertgeerts, K.; Kamiyama, K. Design and synthesis of novel human epidermal growth factor receptor 2 (HER2)/epidermal growth factor receptor (EGFR) dual inhibitors bearing a pyrrolo[3,2-d]pyrimidine scaffold. *J. Med. Chem.,* **2011**, *54*(23), 8030-8050. http://dx.doi.org/10.1021/jm2008634 PMID: 22003817

[12] Nasyanka, A.L.; Siswodihardjo, S.; Hardjono, S. Docking, synthesis, and cytotoxic activity of N-4-methoxybenzoyl-N'-(4-fluorophenyl)thiourea on HeLa cell line. *Thaiphesatchasan,* **2017**, *41*(3), 99-102.

[13] Macari, G.; Toti, D.; Del Moro, C.; Polticelli, F. Fragment-based ligand-protein contact statistics: Application to docking simulations. *Int. J. Mol. Sci.,* **2019**, *20*(10), 2499. http://dx.doi.org/10.3390/ijms20102499 PMID: 31117183

[14] Pratama, M.R.F.; Poerwono, H.; Siswodihardjo, S. Design and molecular docking of novel 5-O-Benzoylpinostrobin derivatives as anti-breast cancer. *Thaiphesatchasan,* **2019**, *43*(4), 201-212.

[15] Hardjono, S.; Widiandani, T.; Purwanto, B.T.; Nasyanka, A.L. Molecular docking of N-benzoyl-N'-(4-fluorophenyl) thiourea derivatives as anticancer drug candidate and their ADMET prediction. *Res. J. Pharma. Technol.,* **2019**, *12*(5), 2160-2166. http://dx.doi.org/10.5958/0974-360X.2019.00359.7

[16] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.,* **2012**, *64*, 4-17. http://dx.doi.org/10.1016/j.addr.2012.09.019 PMID: 11259830

[17] Choy, Y.B.; Prausnitz, M.R. The rule of five for non-oral routes of drug delivery: Ophthalmic, inhalation and transdermal. *Pharm. Res.,* **2011**, *28*(5), 943-948. http://dx.doi.org/10.1007/s11095-010-0292-6 PMID: 20967491

[18] Chander, S.; Tang, C.R.; Al-Maqtari, H.M.; Jamalis, J.; Penta, A.; Hadda, T.B.; Sirat, H.M.; Zheng, Y.T.; Sankaranarayanan, M. Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro- 2H -1,5-benzodiazepin-2-one derivatives. *Bioorg. Chem.,* **2017**, *72*, 74-79. http://dx.doi.org/10.1016/j.bioorg.2017.03.013 PMID: 28371664

[19] Hardjono, S.; Siswandono, S.; Andayani, R. Evaluation of N-benzoylthiourea derivatives as popssible analgesic agents by predicting their hysicochemical and pharmacokinetic properties, toxicity, and analgesic activity. *Indones. J. Biotechnol.,* **2018**, *22*(2), 76-85. http://dx.doi.org/10.22146/ijbiotech.27171

[20] Pires, D.E.V.; Blundell, T.L.; Ascher, D.B. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J. Med. Chem.,* **2015**, *58*(9), 4066-4072. http://dx.doi.org/10.1021/acs.jmedchem.5b00104 PMID: 25860834

[21] Pratama, M.R.F.; Poerwono, H.; Siswodiharjo, S. ADMET properties of novel 5- *O* -benzoylpinostrobin derivatives. *J. Basic Clin. Physiol. Pharmacol.,* **2019**, *30*(6), 20190251. http://dx.doi.org/10.1515/jbcpp-2019-0251 PMID: 31851612

[22] United Nations. Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Available from: https://unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf(Accessed October 23, 2022)

[23] Harahap, Y.D.; Elya, B.; Bahtiar, A. Study of molecular docking of vitexin in binahong (Anredera cordifolia(Ten.) Steenis) leaves extract on Glibenclamide-CYP3A4 interaction. *Pharmacogn. J.,* **2019**, *11*(6), 1471-1476.

[24] Siswodihardjo, S.; Pratama, M.R.F.; Praditapuspa, E.N.; Kesuma, D.; Poerwono, H.; Widiandani, T. *Boesenbergia pandurata* as an anti-breast cancer agent: Molecular docking and ADMET study. *Lett. Drug Des. Discov.,* **2022**, *19*(7), 606-626. http://dx.doi.org/10.2174/1570180819666211220111245

[25] Widiandani, T.; Purwanto, B. The potency of 4-nitrobenzoyl-3-allylthiourea as an agent of breast cancer with EGFR/HER2: *In Silico* and *in vitro* study. *Rasayan J. Chem.,* **2022**, *15*(3), 2083-2088. http://dx.doi.org/10.31788/RJC.2022.1536976