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FULL PAPER

# *In Silico* study of the effect of substituents on the structure of *N*-benzoyl-*N'*-naphthylthiourea as anti-breast cancer HER-2 positive candidates

**Dewi Sinta Megawati[a,b] | Juni Ekowati[c] | Siswandono Siswodihardjo[c,*] | A. Ghanaim Fasya[d]**

[a]*Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya-60115, (East Java) Indonesia*

[b]*Department of Pharmacy, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang-65144, (East Java) Indonesia*

[c]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya-60115, (East Java) Indonesia*

[d]*Department of Chemistry, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang-65144, (East Java) Indonesia*

***Corresponding Author:**
Siswandono Siswodihardjo
E-mail: prof.sis@ff.unair.ac.id
Tel.: +62 812-3206-328

One of the critical strategies in developing new drugs is to design drugs through structural modification. The structural modification leads to changes in the structure of a compound, thereby changing the compound's physicochemical properties, including lipophilic, electronic, and steric properties. These changes will cause changes in the biological activity of the compound. This study aimed to determine the effect of substituents on the benzoyl group of the compound *N*-benzoyl-*N'*-naphthylthiourea (BNTU) on the anti-breast cancer activity of HER-2 *in silico*. Molecular docking of BNTU lead compound and derivatives using Autodock tools software against HER-2 receptors (PDB ID: 3RCD). Compared to lipophilic and steric properties, electronic properties influence the anti-breast cancer activity of HER-2 on BNTU-derived compounds. The binding score (ΔG) of BNTU-derived compounds with strong electronic substituents was more negative than lipophilic and steric substituents. However, there is an anomaly in BNTU derivatives with fluoro (F) substituents because they have the lowest anticancer activity compared to the other BNTU derivatives. Anti-breast cancer activity of BNTU-derived compounds is influenced by variations in substituents, especially by the electronic properties (electron withdrawing groups) of these substituents. Compounds with Br substituents have a better affinity for HER-2 receptors than the lead compound BNTU and other BNTU derivatives.

**KEYWORDS**

Structural modification; BNTU; lipophilic properties; electronic properties; steric properties; *in silico*.

## Introduction

Cancer is the second leading cause of death in the world after cardiovascular disease and is a major health issue, both in developed and developing countries [1,2]. According to Globocan, in 2020, breast cancer was the leading cancer case in the last five years [3]. Approximately 20-35% of breast cancer is caused by over-expression of the human epidermal growth factor (HER-2) receptor. Positive HER-2 breast cancer has a tendency to grow faster than negative HER-2 breast cancer [4,5]. Overexpression of HER-2 is associated with increased aggression, relapse, poor prognosis, and decreased survival [4,6-7]. The main function of HER-2 is to suppress apoptosis to promote cellular survival, thus leading to proliferation and uncontrolled

tumour growth [5,8]. Therefore, inhibition of HER-2 activation at ATP binding sites is a target for developing molecular-targeted drug discovery in breast cancer cells [9]. Conventional cancer therapies such as chemotherapy and radiation therapy have serious side effects on normal cells. Selective chemopreventive agents are needed to reduce side effects and enhance the effectiveness of cancer therapy [10,11]. The goal of cancer therapy is to destroy cancer cells without harming normal cells [12]. Hence, there is a necessity to develop advanced anticancer agents that are more effective and exhibit greater selectivity [13].

Several studies have shown that thiourea derivatives have anticancer activity, including breast cancer [5,14-16]. Thiourea pharmacophores have specific binding sites, namely hydrogen binding area (NH), complement area (S), and additional binding area (1,3-substituent) [17, 18]. The derivative of thiourea that will be developed through this research is *N*-benzoyl-*N'*-naphtylthiourea (BNTU). BNTU is used as a lead compound in drug development through structural modification.

Structural modification is a method that can be used in drug design to obtain new drugs with better activity and lower toxicity [19]. The structure modification leads to some changes in the structure of a compound, thereby changing the compound's physicochemical properties, including lipophilic, electronic, and steric properties. Changes in these physicochemical properties will cause changes in the biological activity of the compound [20]. Lipophilic properties affect drug penetration through the cell membrane and increase the amount of drug bound to a receptor, which increases its activity. Whereas electronic properties play a role in drug solubility in drug distribution and drug-receptor interaction [21,22].

The structural modification was carried out by including the naphthyl and benzoyl groups in the thiourea structure. It is expected that the naphthyl and benzoyl groups will help improve lipophilic and electronic properties. Previous studies have shown that adding a benzoyl group to thiourea can increase the activity of these compounds by increasing their lipophilic properties [23]. The lead compound of BNTU was modified by adding a substituent to the benzene ring of the benzoyl group. Ruswanto (2015) also reported that compounds without substituents on the benzene ring (1-benzoyl-3-methylthiourea) had lower anticancer activity in the Hela cell line than their derivatives [24]. The substituent is chosen at the -para position of the benzoyl group because it has a minimal steric effect. Various types of substituents with lipophilic, electronic, and steric properties in the benzoyl group are expected to increase its activity. Substituent changes in a drug will have a significant effect on these properties and biological activity. A study on structural modifications to investigate the impact of lipophilic and electronic properties has previously been conducted by Kesuma *et al.* (2010). In their research, they found a correlation between *in silico* and *in vitro* cytotoxic activity of *N*-(phenylcarbamothyoil) benzamide (PCTB) derivatives against the T47D cell line. The electronic effects were found to play a more significant role in cytotoxic activity compared to the lipophilic effects [22].

This research analyzes the effects of substituents (lipophilic, electronic, and steric properties) on BNTU compounds by *in silico* for breast cancer anti-activity through molecular docking. The goal is to predict the effects of these properties on anti-breast cancer activity, with the hope of assisting in obtaining thiourea derivative compounds, especially BNTU derivatives with the best potential as anti-breast cancer agents. There is integration between docking and *in vitro* studies to accelerate the discovery of cancer drugs with good consistency [25]. *In silico* approach is the initial step in designing more effective and profitable drugs, both in terms of

time and cost savings, before conducting *in vivo* and *in vitro* studies [26].

## Materials and methods

### *Materials*

The hardware used is 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 modelling, energy minimization and SMILES translator, AutoDockTools 1.5.6 software for validation and docking process, and Discovery Studio Visualizer 2020 for visualization and observation of docking results.

### *HER2 protein target download*

Docking studies were performed on the active site of the HER2 receptor (PDB code: 3RCD). The molecular structure of the receptor was obtained from the RSCB protein data bank (PDB) (https://www.rcsb.org/). HER2 Kinase domain complexed with TAK-285 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 (ligand code: 03P).

### *Macromolecule (receptor) preparation*

HER-2 receptor was prepared by separating the receptor from its native ligand using the AutoDockTools-1.5.6 program to obtain the receptor's protein structure without native ligands and the separate native ligand structure as shown in Figure 1 (visualization using the Discovery Studio Visualizer 2020 program). The HER-2 protein with the code 3RCD had four chains: A, B, C, and D. However, only chain A was used in the docking process. Furthermore, the water was removed, and the native ligand was separated, so that only the receptor protein with chain A remains. Polar hydrogen was added; Kollman charges were added and stored in pdbqt.

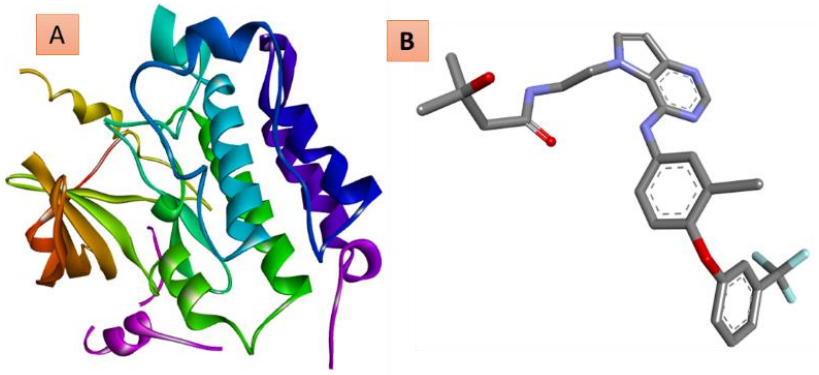



**FIGURE 1** (A) 3-Dimensional structure of the HER-2 receptor protein and (B) Native ligand

### *Ligands preparation*

The structure of BNTU and its derivatives (Table 1) was drawn using the ChemDraw 20.0 program, and copied to Chem3D 20.0, and energy minimization was performed using the MMFF94 method [21,27], and then stored in the mol2 [SYBYL2 (*.mol2)] and smi [SMILES (*.smi)] format The ligand in mol2 format was opened with the AutoDockTools-1.5.6 program, hydrogen atom were removed (edit hydrogen-merge nonpolar), Gasteiger charges were added, and then it was saved in the *.pdbqt format.

### *Validation of protocol docking*

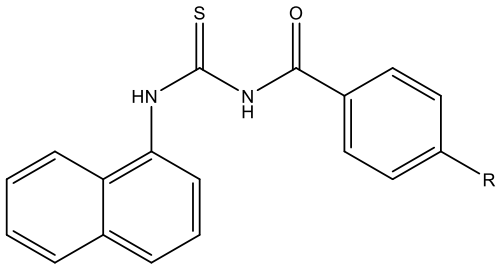
The validation of the docking protocol was carried out before the docking process for the test ligands. The redocking process was

carried out using co-crystal ligands from the HER2 receptor. The co-crystal ligands were extracted, a polar hydrogen group was added, given the charge, torque, and rotational bonds were adjusted, then stored in the *.pdbqt. Co-crystal ligand re-docking was carried out at the position and size of grid box that had been determined from the orientation results [28]. Based on the orientation results, the grid box size is 30 x 34 x 24 with grid box position x: 12.480, y: 2, .964, z: 28.015. The number of genetic algorithm (GA) runs used in the docking process is 60 [29]. The root-mean-square deviation (RMSD) value of less than 2 Å is a parameter observed in the docking protocol validation process [28-30].

*Molecular docking*

Molecular docking of the test compound or ligand was carried out using the same method as the one for validation of the docking protocol. The grid box size and grid box position correspond to the co-crystal ligands. However, the parameters used in the docking process are free energy of binding (ΔG) and amino acid residues [31].

**TABLE 1** The chemical structure of BNTU and its derivatives




*N*-Benzoyl-*N*'-Naphtylthiourea

| No. | Compounds name | Code | Functional groups (R) | Properties of substituent |
|---|---|---|---|---|
| 1 | *N*-benzoyl-*N'*-naphtylthiourea | BNTU | H | Es(+) |
| 2 | *N*-(4-fluorobenzoyl)-*N'*-naphtylthiourea | 4FBNTU | 4-F | π(+)σ(+)Es(++++) |
| 3 | *N*-(4-chlorobenzoyl)-*N'*-naphtylthiourea | 4ClBNTU | 4-Cl | π(+++)σ(+)Es(+) |
| 4 | *N*-(4-bromobenzoyl)- *N'*-naphtylthiourea | 4BrBNTU | 4-Br | π(++++)σ(+)Es(+) |
| 5 | *N*-(4-trifluoromethylbenzoyl)-*N'*-naphtylthiourea | 4CFBNTU | 4-$CF_3$ | π(+++)σ(++)Es(-) |
| 6 | *N*-(4-tribromomethylbenzoyl)-*N'*-naphtylthiourea | 4CBrBNTU | 4-$CBr_3$ | π(+++)σ(++)Es(-) |
| 7 | *N*-(4-trichloromethylbenzoyl)-*N'*-naphtylthiourea | 4CClBNTU | 4-$CCl_3$ | π(+++)σ(++)Es(-) |
| 8 | *N*-(4-nitrobenzoyl)-*N'*-naphtylthiourea | 4NBNTU | 4-$NO_2$ | π(+)σ(++++)Es(-) |
| 9 | *N*-(4-metoxybenzoyl)-*N'*-naphtylthiourea | 4OCBNTU | 4-$OCH_3$ | π(-)σ(-)Es(+++) |
| 10 | *N*-(4-ethoxybenzoyl)-*N'*-naphtylthiourea | 4OC2BNTU | 4-$OCH_2CH_3$ | π(-)σ(-)Es(+++) |
| 11 | *N*-(4-propoxybenzoyl)-*N'*-naphtylthiourea | 4OC3BNTU | 4-$OCH_2CH_2CH_3$ | π(-)σ(-)Es(+++) |
| 12 | *N*-(4-isopropoxybenzoyl)-*N'*-naphtylthiourea | 4IOC3BNTU | 4-$OCH(CH_3)_2$ | π(-)σ(-)Es(+++) |
| 13 | *N*-(4-methylbenzoyl)-*N'*-naphtylthiourea | 4CBNTU | 4-$CH_3$ | π(++)σ(-) |
| 14 | *N*-(4-ethoxybenzoyl)-*N'*-naphtylthiourea | 4C2BNTU | 4-$CH_2CH_3$ | π(++)σ(-) |
| 15 | *N*-(4-propoxybenzoyl)-*N'*-naphtylthiourea | 4C3BNTU | 4-$CH_2CH_2CH_3$ | π(++)σ(-) |
| 16 | *N*-(4-*tert*-butylbenzoyl)-*N*'-naphtylthiourea | 4TBBNTU | 4-$C(CH_3)_3$ | π(+++++)σ(-)Es(-) |

π: Lipophilic parameter, σ : electronic parameter, and Es : steric parameter.

## Results and discussion

*Validation of protocol docking*

The docking method's accuracy was evaluated by docking the co-crystal ligand of the HER-2 receptor (PDB ID 3RCD). The RMSD value is an observed parameter to assess whether the docking method can be used to dock the test compound. RMSD is the deviation distance from the binding position of the native ligand to the protein after docking to the actual binding position of the native ligand [32]. RMSD values less than 2.0 Å generally indicate that the docking protocol can accurately predict the binding orientation of co-crystal ligands [33]. The RMSD value obtained in this study's validation of the docking protocol was 1.25 Å. This value is less than 2 Å, so the docking method can be used for docking BNTU and their derivatives. The 3RCD ligand overlay before and after docking can be seen in Figure 2.

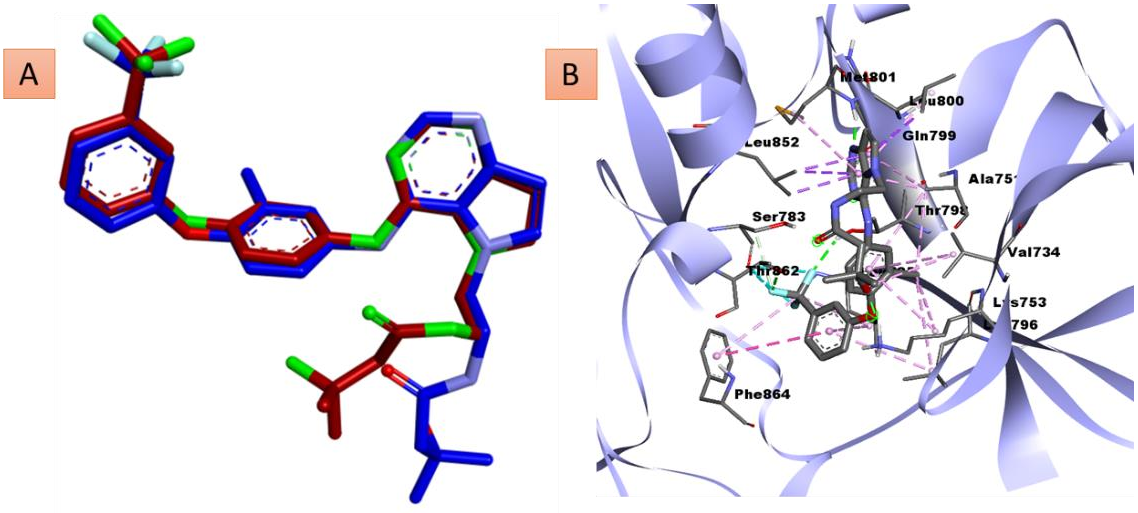



**FIGURE 2** (A) Co-crystal ligand overlay before re-docking (blue) and after re-docking (red) and (B) Co-crystal Ligand Interaction HER2 (TAK-285) in Amino Acid Residues from The 3RCD Receptor

*Molecular docking of BNTU and its derivatives*

The docking results were evaluated based on the parameter of free bond energy (ΔG). The results of docking between BNTU compounds and their derivatives against the HER-2 receptor can be seen in Figure 3.

Based on the docking data in Figure 3, it is known that the lead compound BNTU with a benzoyl group without substituent has the highest ΔG value after 4OC3BNTU. This indicates that the anticancer activity of BNTU is lower than its derivatives, except for 4OC3BNTU. This means that the presence of a substituent bound to the benzoyl group in BNTU is predicted to cause an increase in anticancer activity. Meanwhile, 4OC3BNTU has the largest ΔG value, meaning its activity is the lowest. This possibility is caused by the presence of steric factors so that the compound cannot occupy the active site/binding pocket of the HER-2 receptor. This is in accordance with the research conducted by Bai *et al.* (2020) that there is an effect of the substituent bound to the thiourea moiety on antiproliferative activity against cell lines, where the phenyl group which has no substituent and is attached to the thiourea moiety has no antiproliferative activity. This indicates the importance of the substituents on the aromatic ring [16]. Ruswanto (2015) also reported that compounds without substituents on the benzene ring (1-benzoyl-

3-methylthiourea) showed lower cytotoxic activity than their derivatives [23].

Substituents with electron-withdrawing groups, namely 4BrBNTU and 4CBrBNTU, had the greatest activity, followed by 4CClBNTU compounds. Electronegative substituents will change the electronic properties of the compound. In 4BrBNTU and 4CBrBNTU compounds, the ΔG value was the highest compared to other BNTU derivatives. Based on Tables 2 and 3, and Figure 4, it is known that there are 734-Val amino acids with alkyl/pi-alkyl interactions that are not owned by the lead compound (BNTU), so it is predicted that 734-Val amino acids are the key amino acids that have the greatest influence on the affinity of BNTU derivative compounds at the HER-2 receptor. Although the compounds 4BrBNTU and 4CBrBNTU have the same ΔG, the interactions of the amino acid residues are slightly different. There are amino acid residues 751-Ala with alkyl/pi-alkyl interaction and amino acid 863-Asp with hydrogen bond interactions in 4BrBNTU compounds, which 4CBrBNTU does not have. This means amino acids 751-Ala and 863-Asp do not affect the compound's affinity.

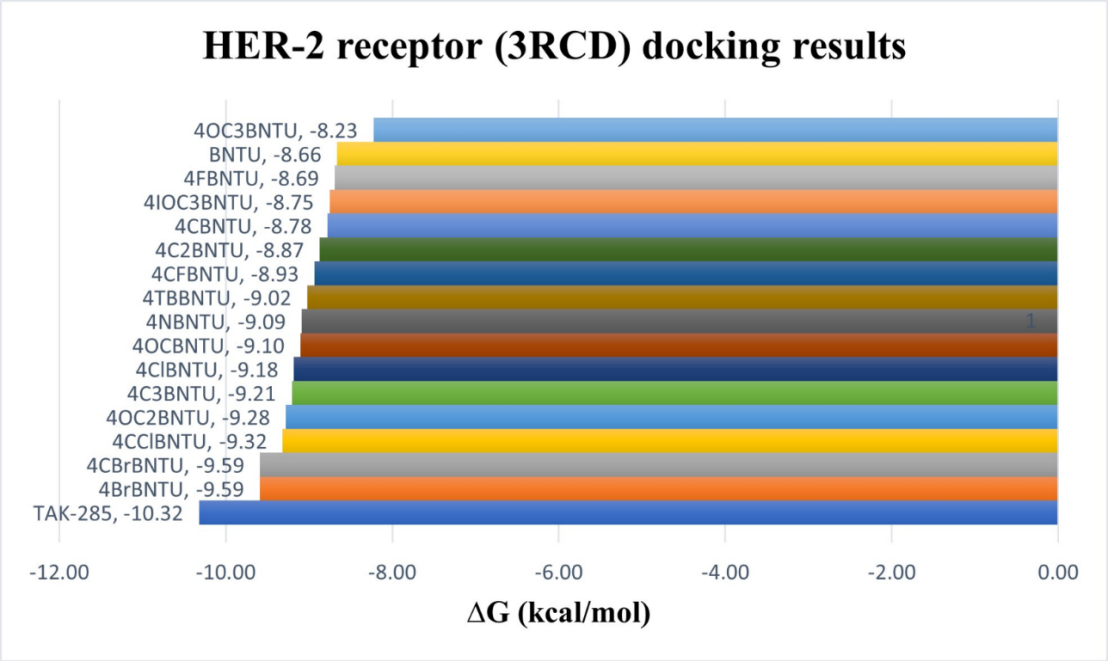



**FIGURE 3** The results of the docking (binding energy) between the BNTU and its derivatives against the HER-2 receptor

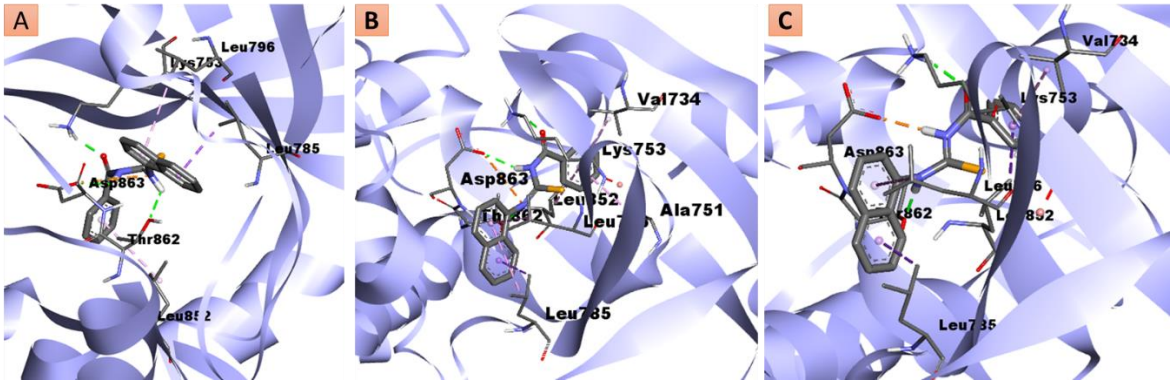



**FIGURE 4** Interaction of BNTU (A), 4BrBNTU (B), and 4CBrBNTU (C) in amino acid residues from the 3RCD receptor

In compounds with Cl substituent, the ΔG value of 4CClBNTU is lower than 4ClBNTU, meaning that the activity of 4CClBNTU is better than 4ClBNTU. This is because the Cl atom has a positive value of (lipophilic parameter) and (electronic parameter), so the increase in activity with the addition of Cl substituent on the lead compound of BNTU is caused by an increase in the lipophilic and electronic properties of the compound. This is in accordance with the research results of Ruswanto *et al*. (2015) that the presence of an electron-withdrawing group Cl on the aromatic ring of the compound structure increases the compound's lipophilicity and is responsible for the increase in cytotoxicity in the MTT model [23,24]. The increase in the lipophilic nature of the compound causes the drug absorption through the biological membrane to increase. In the context of pharmacokinetics, for a drug to be absorbed orally, normally, it must pass through a biological membrane (lipid bilayer) in the intestinal epithelium, so the drug should be sufficiently lipophilic to penetrate the lipid bilayer. However, the drug should not be too lipophilic because it will be difficult to pass through the biological membrane. The BNTU derivative compounds with the most potent activity are 4BrBNTU and 4CBrBNTU compounds because their ΔG values are the most negative. In the compound 4CClBNTU, the ΔG value was lower, with a difference of 0.27 compared to 4BrBNTU and 4CBrBNTU. Table 2 presents 4CClBNTU has 726-Leu amino acid with alkyl/pi-alkyl interaction. The amino acid may be the cause of the decreased affinity of the compound, meaning that the amino acid with its interaction is not expected. The 4ClBNTU compound has a lower affinity than the 4CClBNTU compound, which is predicted because the 4ClBNTU compound does not have 805-Cys amino acid with alkyl/pi-alkyl interaction.

In the compound 4TBBNTU, 850-Asn amino acids with hydrogen bond interactions are not owned by the lead compound BNTU. It is predicted that the amino acid with the hydrogen bond interaction will cause its affinity to be better than the lead compound. The nonpolar tertiary butyl substituent will increase the lipophilic properties of the compound, while the electronegative substituent (trifluoromethyl) will change the compound's electronic properties. There is an anomaly in compound 4FBNTU, where the activity is the lowest after compound 4OC3BNTU. Based on the substituent constants used in the Topliss approach for aromatic substitution, it is known that the 4F derivative has low values for lipophilic parameter (π) and steric parameter (Es), which are 0.15 and 0.06 respectively, while its steric parameter (Es) has a high value of 0.78 [34]. This steric parameter value is the highest compared to other substituents. Therefore, the decrease in anticancer activity is likely due to the presence of a significant steric factor.

This research requires experimental studies such as *in vitro* testing to prove that the predicted breast cancer anticancer activity of BNTU compounds and their derivatives *in silico* correlates with *in vitro* anticancer activity. The results of this research are only preliminary predictions to assist in selecting compounds that are a priority for further synthesis.

**TABLE 2** The docking results of test ligands at the binding site of 3RCD receptor

| Ligand | TAK-285 | BNTU | 4Br | 4CBr | 4CCl | 4OC2 | 4C3 | 4Cl | 4OC | 4N |
|---|---|---|---|---|---|---|---|---|---|---|
| **ΔG (kcal/mol)** | -10.32 | -8.66 | -9.59 | -9.59 | -9.32 | -9.28 | -9.21 | -9.18 | -9.10 | -9.09 |
| **SD** | 0.13 | 0.04 | 0.06 | 0.26 | 0.27 | 0.12 | 0.15 | 0.13 | 0.03 | 0.09 |
| **Amino acid residues** | - | - | - | - | 726-Leu[a] | - | 726-Leu[a] | 726-Leu[a] | - | - |
| | 734-Val[a] | - | 734-Val[a] | 734-Val[a] | 734-Val[a] | 734-Val[a] | - | 734-Val[e] | - | - |
| | 751-Ala[a] | - | 751-Ala[a] | - | 751-Ala[a] | - | 751-Ala[a] | 751-Ala[a] | 751-Ala[b] (3.60 Å) | 751-Ala[a] |
| | 753-Lys[b] (1.92 Å) | 753-Lys[b] (1.76 Å) | 753-Lys[b] (1.99 Å) | 753-Lys[b] (2.15Å) | 753-Lys[b] (1.67Å) | 753-Lys[b] (1.74 Å) | 753-Lys[g] | 753-Lys[b] (1.75 Å) | - | 753-Lys[b] (2.76 Å) |
| | - | - | - | - | - | - | 774-Met[a] | - | - | - |
| | 783-Ser[c] | - | - | - | - | - | 783-Ser[b] (2.07 Å) | - | - | - |
| | 785-Leu[a] | 785-Leu[e] | 785-Leu[a,e] | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] | - | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] |
| | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | - | - |
| | 798-Thr[b] (2.18 Å) | - | - | - | - | - | - | - | - | - |
| | 799-Gln[c] | - | - | - | - | - | - | - | - | 799-Gln[h] |
| | 800-Leu[e] | - | - | - | - | 800-Leu[a] | - | - | 800-Leu[a] | - |
| | 801-Met[b] (1,77 Å) | - | - | - | - | - | - | - | 801-Met[b] (1.94 Å) | 801-Met[b] (1.80 Å) |
| | - | - | - | - | 805-Cys[a] | - | - | - | - | - |
| | 852-Leu[e] | 852-Leu[a] | 852-Leu[a] | 852-Leu[e] | 852-Leu[a] | 852-Leu[a] | 852-Leu[d] | 852-Leu[a] | 852-Leu[e] | 852-Leu[e] |
| | 862-Thr[b] (2.14 Å) | 862-Thr[b] (2.14 Å) | 862-Thr[b] (1.97 Å) | 862-Thr[b] (1.70Å) | 862-Thr[b] (2.00 Å) | 862-Thr[b] (2.01 Å) | 862-Thr[b] (2.12 Å) | 862-Thr[b] (1.89 Å) | 862-Thr[b] (2.12 Å) | 862-Thr[b] (2.95 Å) |
| | - | 863-Asp[f] | 863-Asp[f] | 863-Asp[f] | 863-Asp[f] | 863-Asp[f] | - | 863-Asp[f] | 863-Asp[f] | - |
| | 864-Phe[d] | 863-Asp[b] (2.04 Å) | 863-Asp[b] (2.16 Å) | - | - | - | 864-Phe[d] | - | 864-Phe[d] | 864-Phe[d] |
| | | | | | | | 1004-Phe[a] | | | |

[a] Alkyl/pi-alkyl interaction; [b] Conventional Hydrogen bond; [c] Carbon hydrogen bond; [d] Pi-Pi T-shaped; [e] Pi-sigma; and [f] Attractive charge interaction; Salt bridge.

**TABLE 3** The docking results of test ligands at the binding site of 3RCD receptor (*Continued*)

| Ligand | TAK-285 | BNTU | 4TB | 4CF | 4C2 | 4C | 4IOC3 | 4F | 4OC3 |
|---|---|---|---|---|---|---|---|---|---|
| **ΔG (kcal/mol)** | -10.32 | -8.66 | -9.02 | -8.93 | -8.87 | -8.78 | -8.75 | -8.69 | -8.23 |
| **SD** | 0.13 | 0.04 | 0.11 | 0.08 | 0.35 | 0.21 | 0.24 | 0.04 | 0.3 |
| **Amino acid residues** | - | - | - | - | 726-Leu[a] | - | 726-Leu[a] | - | - |
| | 734-Val[a] | - | - | 734-Val[a] | 734-Val[e] | - | 734-Val[a] | 734-Val[a] | 734-Val[a] |
| | 751-Ala[a] | - | 751-Ala[a] | 751-Ala[a] | 751-Ala[a] | 751-Ala[a] | - | 751-Ala[a] | 751-Ala[a] |
| | 753-Lys[b] (1.92Å) | 753-Lys[b] (1.76Å) | - | 753-Lys[b] (1.93 Å) | 753-Lys[b] (2.05 Å) | - | 753-Lys[b] (1.84 Å) | 753-Lys[b] (1.96 Å) | 753-Lys[b] (1.94 Å) |
| | - | - | - | - | - | - | - | - | - |
| | 783-Ser[c] | - | - | - | - | 783-Ser[b] (2.17 Å) | - | - | - |
| | 785-Leu[a] | 785-Leu[e] | 785-Leu[a] | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] | 785-Leu[e] |
| | 796-Leu[a] | 796-Leu[a] | - | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] | 796-Leu[a] |
| | 798-Thr[b] (2.18Å) | - | - | - | - | - | - | - | - |
| | 799-Gln[c] | - | - | - | - | - | - | - | - |
| | 800-Leu[e] | - | - | - | - | 800-Leu[a] | - | - | 800-Leu[a] |
| | 801-Met[b] (1,77Å) | - | - | - | - | - | - | - | 801-Met[a] |
| | - | - | 850-Asn[b] (3.78Å ) | - | - | - | - | - | - |
| | 852-Leu[e] | 852-Leu[a] | 852-Leu[e] | 852-Leu[a] | 852-Leu[a] | 852-Leu[e] | 852-Leu[e] | 852-Leu[a] | 852-Leu[a] |
| | 862-Thr[b] (2.14Å) | 862-Thr[b] (2.14Å) | 862-Thr[i] | 862-Thr[b] (2.04 Å) | 862-Thr[b] (1.98 Å) | 862-Thr[b] (2.15 Å) | 862-Thr[b] (1.87 Å) | - | 862-Thr[b] (2.17 Å) |
| | - | 863-Asp[f] | 863-Asp[f] | 863-Asp[f] | 863-Asp[b] (2.14 Å)[,f] | - | 863-Asp[f] | 863-Asp[f] | 863-Asp[f] |
| | 864-Phe[d] | 863-Asp[b] (2.04Å) | 864-Phe[a] | 864-Phe[d] | - | 864-Phe[d] | - | - | - |
| | | | | | | 1004 -Phe[a] | | | |

[a] Alkyl/pi-alkyl interaction; [b] Conventional Hydrogen bond; [c] Carbon hydrogen bond; [d] Pi-Pi T-shaped; [e] Pi-sigma; and [f] Attractive charge interaction; Salt bridge.

## Conclusion

Anti-breast cancer activity of BNTU-derived compounds is influenced by variations in substituents, especially by the electronic properties (electron withdrawing groups) of these substituents. Compounds with Br substituents have a better affinity for HER-2 receptors than the lead compound BNTU and other BNTU derivatives. We recommend for future research to consider comparing the results of this study using different molecular docking software or other methods such as molecular dynamics and QSAR, and validate the findings through *in vitro* testing.

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## Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all aspects of this work.

## Conflict of Interest

The author declared that they have no conflict of interest.

## *Orcid:*

*Dewi Sinta Megawati:*
https://orcid.org/0000-0003-2993-8230
*Juni Ekowati:*
https://orcid.org/0000-0002-4402-2039
*Siswandono Siswodihardjo:*
https://orcid.org/0000-0002-9579-8929
*A. Ghanaim Fasya:*
https://orcid.org/0000-0002-2273-4035

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