

RESEARCH ARTICLE

Antioxidant Activities and Molecular Docking of *N*-benzoyl-*N*'-naphthylthiourea Derivatives

Dewi Sinta Megawati^{1,2}, Juni Ekowati^{3,4*}, Siswandono Siswodihardjo^{3,4,5}, A. Ghanaim Fasya⁶

¹Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy,
Universitas Airlangga, Surabaya-60115, (East Java) Indonesia.

²Department of Pharmacy, Faculty of Medicine and Health Sciences,
Maulana Malik Ibrahim State Islamic University, Malang-65144, (East Java) Indonesia.

³Department of Pharmaceutical Sciences, Faculty of Pharmacy,
Universitas Airlangga, Surabaya-60115, (East Java) Indonesia.

⁴Drug Development Research Group, Faculty of Pharmacy,
Universitas Airlangga, Surabaya-60115, (East Java) Indonesia.

⁵Faculty of Pharmacy, Bhakti Wiyata Institute of Health Sciences, Kediri-64114, (East Java) Indonesia.

⁶Department of Chemistry, Faculty of Science and Technology,
Maulana Malik Ibrahim State Islamic University, Malang-65144, (East Java) Indonesia.

*Corresponding Author E-mail: juni-e@ff.unair.ac.id

ABSTRACT:

Oxidative stress occurs when the body accumulates reactive oxygen species (ROS), which can be generated through enzymatic reactions or when oxygen interacts with cellular molecules. Antioxidants are frequently employed to impede, postpone, or minimize oxidative stress within the human body. These antioxidants supply electrons to free radicals, thereby counterbalancing their harmful effects. Compounds containing thiourea groups have attracted attention due to their diverse pharmaceutical properties, including their roles as antioxidants. This study aims to determine the antioxidant potential of the *N*-benzoyl-*N*'-naphthylthiourea (BNTU) and its four derivatives. This research evaluates the antioxidant activity of BNTU and its derivatives using the scavenging method of DPPH radicals. A docking study revealed potential interactions between binding models (human ROS1 kinase, PDB ID 3ZBF) and the antioxidant properties of the target compounds. Based on the results of the DPPH test, it was found that the antioxidant activity (IC₅₀) for 4CFBNTU was recorded at 189.6 ppm, whereas 3CFBNTU showed a measurement of 294.5 ppm. Conversely, BNTU, 4TBBNTU, and 4OCBNTU displayed IC₅₀ values surpassing 400 ppm. As for the Antioxidant Activity Index (AAI), The BNTU and its derivatives have AAI values < 0.56. The docking results obtained are consistent with the conclusions drawn from the antioxidant investigation. The four derivatives of BNTU have better antioxidant activity than the lead compound. Among them, 4CFBNTU exhibits the best antioxidant activity, although its antioxidant properties are relatively weak.

KEYWORDS: ROS, BNTU, Antioxidant, DPPH, Molecular docking.

Received on 26.02.2024 Revised on 11.05.2024
Accepted on 13.07.2024 Published on 24.12.2024
Available online from December 27, 2024
Research J. Pharmacy and Technology, 2024;17(12):6063-6069.
DOI: 10.52711/0974-360X.2024.00919
© RJPT All right reserved

This work is licensed under a Creative Commons
Attribution-NonCommercial-ShareAlike 4.0
International License. Creative Commons License.



INTRODUCTION:

Oxidative processes play crucial roles in the metabolism of all living organisms. Nevertheless, the generation of free radicals during chain reactions within the oxidation process is responsible for causing cellular damage.

When there is a discrepancy between the generation of reactive oxygen/nitrogen species (ROS/RNS) and the mitigating antioxidant actions of associated enzymes, oxidative stress arises¹⁻³. Oxidative stress is a primary

factor in cell damage through oxidation. It depends on both the rate at which oxidative damage occurs (input) and the rate at which it is effectively repaired and eliminated (output)⁴. ROS, comprising free radicals like superoxide anion radicals, hydroxyl radicals, singlet oxygen, and non-radical species such as hydrogen peroxide, represent diverse forms of activated oxygen. They are frequently produced as oxidation byproduct of biological reactions⁵.

Oxidative stress manifests when there is an excess of ROS in the body, which can arise from enzymatic processes, oxygen interaction with cellular molecules, or external sources like airborne pollutants, ozone, and industrial chemicals. ROS disturbs normal cell function by harming vital biomolecules such as proteins, lipids, and DNA^{2,5}. This damage can progressively lead to various conditions, including cancer, inflammation, neurodegenerative diseases, cardiovascular disorders, and diabetes mellitus. The advancement of these ailments can be alleviated through antioxidant intervention^{2,3,6}.

Antioxidants contribute to various essential biological functions, including immune response, safeguarding tissues from damage, reproductive processes, and growth or development. They help maintain cellular functionality amidst disruptions to homeostasis, such as those induced by septic shock, aging, and oxidative stress-related mechanisms^{7,8}. Antioxidants are compounds that assist in neutralizing free radicals and prevent their generation^{9,10}. Antioxidants are used extensively in inhibiting, delaying, or reducing oxidative stress within the human body¹¹. They are categorized based on their origin as either endogenous agents (produced by the body itself) or exogenous agents (originating from outside the body). Antioxidants work by scavenging free radicals, inhibiting pro-oxidant enzymes, and chelating metals. Antioxidants donate electrons to free radicals, consequently neutralizing their harmful effects^{1,12}.

Polyphenols are indeed recognized as a prominent group of antioxidant compounds. Nevertheless, various other compounds can function as antioxidants. These encompass compounds containing aromatic rings adorned with additional electron-donating substituents aside from hydroxy and dimethylamino groups⁶. Recently, compounds containing thiourea groups have gained attention due to their diverse pharmaceutical properties, including their roles as anticancer¹³⁻¹⁷ and antioxidant agents^{6,18}. Thiourea (TU) is an organic compound with the $SC(NH_2)_2$ formula. It contains two nitrogen atoms, which can have either identical or different substituents, and acts as an active source of nitrogen atoms¹⁹. Thiourea pharmacophores possess

specific binding regions, consisting of a hydrogen-binding area (NH), a complementary region (S), and an extra binding area (1,3-substituent)^{20,21}.

Many researchers have extensively explored studies on thiourea derivative compounds as antioxidants. Huong *et al.* analyzed the antioxidant capabilities of DPTU (1,3-diphenyl-2-thiourea) and BPTU (1-benzyl-3-phenyl-2-thiourea) compounds using color change measurements of 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS^{•+}). The results indicated that DPTU exhibited better free radical scavenging ability than BPTU²². Oleiwi *et al.* synthesized ten new thiourea derivative compounds based on 4-methoxybenzoyl chloride as antioxidants using the DPPH method, with IC₅₀ values ranging from 5.8 to 126.9 µg/mL. Compound 8 showed the best antioxidant activity²³. Maryoosh and Al-Jeilawi also synthesized eight new derivatives containing the thiourea moiety, and overall, all synthesized compounds demonstrated good antioxidant activity. Among compounds (II), (VIII), (III), and (V), each exhibited the highest antioxidant activity with IC₅₀ values of 34.75, 36.89, 37.55, and 45.5 ppm, respectively, while the remaining compounds showed moderate activity¹⁹. Sudzhaev *et al.* synthesized various thiourea derivatives, including 1-(2-aminoethyl) thiourea, *N,N'*-(iminodiethane-2,1-diyl)bis(thiourea), and 1-[1-methyl-2-(phenylamino)ethyl] thiourea. These compounds were then assessed for their antioxidant properties using cumene oxidation reactions. The derivatives may decrease cumene autoxidation by scavenging cumylperoxy radicals and influencing the catalytic decomposition of cumyl hydroperoxide²⁴. Thiourea and its derivatives have also been proven effective as scavengers of O₂^{•-} and OH⁻ radicals^{22,23}.

The studies mentioned above indicate the significant potential of thiourea derivatives in capturing free radicals and displaying antioxidant properties. However, the antioxidant potential of thiourea derivatives such as *N*-benzoyl-*N'*-naphthylthiourea (BNTU) and its derivatives have yet to be extensively studied. In this study, the antioxidant ability of BNTU and its four derivatives was evaluated for their antioxidant activity using experimental methods (DPPH[•] radical scavenging assay) and predicted for their antioxidant activity *in silico* using molecular docking methods. A common approach to assess antioxidant activity involves measuring the scavenging activity of DPPH radicals, owing to its high sensitivity, cost-effectiveness, and quick results¹⁹. Computational molecular docking has become an effective strategy for forecasting molecular targets and supporting experimental results²⁵.

MATERIALS AND METHODS:

Materials:

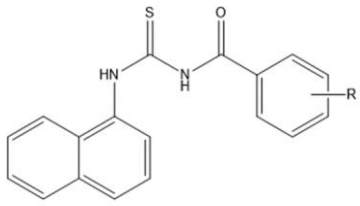
Chemicals:

All chemicals utilized in the study, including DPPH (1,1-diphenyl-1-picrylhydrazyl), BHT (butylated hydroxytoluene), vitamin C (ascorbate), and absolute ethanol for analysis, were procured from Sigma Chemical Company (St. Louis, MO, USA). These chemicals were of analytical grade and sourced from Sigma, Merck, and Aldrich. The test compounds used were synthesized products, namely *N*-benzoyl-*N'*-naphthylthiourea, *N*-(4-trifluoromethylbenzoyl)-*N'*-naphthylthiourea, *N*-(3-trifluoromethylbenzoyl)-*N'*-naphthylthiourea, *N*-(4-methoxybenzoyl)-*N'*-naphthylthiourea, *N*-(4-tert-butylbenzoyl)-*N'*-naphthylthiourea (Table 1).

Instrumentations:

Antioxidant activity was analyzed using a microplate reader spectrophotometer (Spektrostar Nano). IC₅₀ values were determined using GraphPad Prism 8 software. The hardware utilized in the study comprised the HUAWEI-IM01S0US equipped with an AMD Ryzen 7 3700U processor featuring Radeon Vega Mobile Gfx. The operating system employed was Windows 10 Home Single Language. The following software programs were utilized: ChemOffice 2020 (comprising ChemDraw 20.0 and Chem3D 20.0) for molecular modeling and energy minimization, PyRx-virtual screening tool software for the docking process, and Discovery Studio Visualizer 2020 for the visualization and examination of docking results.

Table 1. The structure of BNTU and derivatives

 N-benzoyl-<i>N'</i>-naphthylthiourea			
NO	Compound code	R	Compound name
1	BNTU	H	<i>N</i> -benzoyl- <i>N'</i> -naphthylthiourea
2	4CFBNTU	4-CF ₃	<i>N</i> -(4-trifluoromethylbenzoyl)- <i>N'</i> -naphthylthiourea
3	3CFBNTU	3-CF ₃	<i>N</i> -(3-trifluoromethylbenzoyl)- <i>N'</i> -naphthylthiourea
4	4OCBNTU	4-OCH ₃	<i>N</i> -(4-methoxybenzoyl)- <i>N'</i> -naphthylthiourea
5	4TBBNTU	4-C(CH ₃) ₃	<i>N</i> -(4-tert-butylbenzoyl)- <i>N'</i> -naphthylthiourea

Investigating antioxidant activity:

DPPH assay:

The test sample (BNTU and four derivatives) was dissolved in a 98% ethanol solvent to create various concentrations of the sample solution (400, 200, 100, 50, and 25 ppm). The standard (positive control) sample (BHT and ascorbic acid) was dissolved in a 98% ethanol solvent to create various concentrations of the sample solution (50; 25; 12.5; 6.25; and 3.125 ppm for BHT); (6.25; 3.13; 1.56; 0.78; and 0.39 ppm for Ascorbic acid). In a 96-well microplate, various dilutions of the test and standard samples (100 µL) were prepared, and then 100 µL DPPH (0.2 mM) was added to each well. After incubating for 30 minutes in the dark at room temperature, the absorbance was determined at a wavelength of 517 nm using a microplate reader. For control purposes, the absorbance of the DPPH radicals without antioxidants was determined using 98% ethanol as the blank reference. Following this, The absorbance measurements were compared to the blank controls. The Radical Scavenging Antioxidant (RSA) percentage was determined using the following formula: ^{1,26,27}

$$\% \text{ RSA} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100\% \quad (1)$$

The results were expressed as IC₅₀ (the concentration ppm of BNTU and its derivatives that scavenge 50 % of DPPH· radical)²⁸. IC₅₀ values were calculated using GraphPad Prism 8.0. The antioxidant activity was quantified using the antioxidant activity index (AAI), calculated as follows as: ^{29,30}

$$\text{AAI} = \frac{\text{Final concentration of DPPH } (\mu\text{g.mL}^{-1})}{\text{IC}_{50} (\mu\text{g.mL}^{-1})} \quad (2)$$

Molecular docking studies:

The chemical structures of BNTU and its derivatives (as presented in Table 1) were depicted in 2D and 3D formats utilizing the ChemOffice 2020 program (ChemDraw 20.0 and Chem3D 20.0). Subsequently, the minimum energy (E_{tot}) was determined using the MMF9 method, and the data were saved in mol2 format [SYBYL2 (*.mol2)]. Molecular docking was performed on the BNTU and its derivatives against the human ROS1 kinase receptor. The human ROS1 kinase receptor with PDB ID: 3ZBF from the Protein Data Bank was visualized by BIOVIA Discovery Studio Visualizer. Molecular docking was performed using AutoDock Vina within the PyRx Virtual Screening Tool software. The validation of the docking protocol was carried out by redocking the native ligand from the 3ZBF receptor³¹. Molecular docking was validated using the Pyrx-vina tool, which involves setting up a site for

ligands to bind to the receptor. This can be achieved by positioning the grid box either on the active site of the receptor or by encompassing the entire molecule. The grid box was adjusted according to the specified coordinates (center x, y, z)³². The RMSD values were obtained during the validation process of the docking method, with a value less than 2 Å signifying a successful docking result³³. The interactions between the receptor molecules, BNTU, and derivatives were examined using BIOVIA Discovery Studio Visualizer.

RESULT:

Antioxidant activity using the DPPH method:

The antioxidant activity of BNTU and its derivatives is represented as IC₅₀. The reported IC₅₀ was calculated from a plot between sample concentration and % RSA. The percentage of antioxidant activity increases with the rising concentration of the sample because at higher concentrations (Figure 1), more antioxidant compounds donate hydrogen atoms to the DPPH radical, forming stable DPP-H compounds. Therefore, there is a decrease in color intensity from purple to yellow³⁴. The DPPH radical scavenging activity of the test sample was measured and compared with that of BHT and ascorbic acid (Figure 2) to evaluate the *in vitro* antioxidant activity of BNTU and its derivatives.

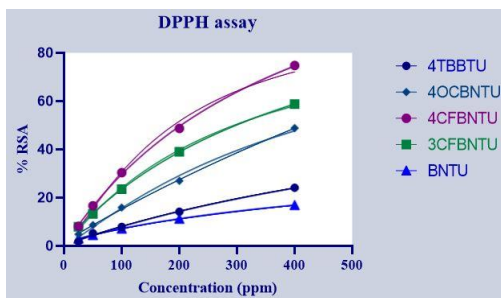


Figure 1. The radical scavenging antioxidant of BNTU and derivatives

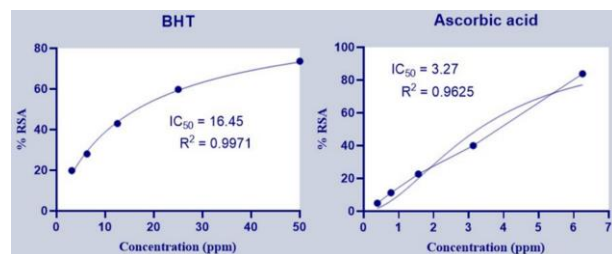


Figure 2. Radical scavenging antioxidant and IC₅₀ of BHT and ascorbic acid

The absorbance of the test sample-DPPH mixture was measured at 517 nm in darkness to avoid light-induced duplication of the DPPH free radicals, which could impact the accuracy of the reading²³. According to the DPPH test results, BHT and ascorbic acid, used as standard compounds (positive controls), have

antioxidant capacity (IC₅₀) values of 16.45 ppm and 3.27 ppm, respectively. Meanwhile, the test compounds 4CFBNTU and 3CFBNTU have IC₅₀ values of 189.6 ppm and 294.5 ppm, respectively. Conversely, BNTU, 4TBBNTU, and 4OCBNTU show IC₅₀ values exceeding 400 ppm or fall outside the concentration range of the prepared sample solutions. A smaller IC₅₀ value indicates stronger antioxidant potency and vice versa. 4CFBNTU has the highest IC₅₀ value compared to the lead compound and other BNTU derivatives. However, its value is not higher than that of the standard compounds BHT and ascorbic acid (Table 2).

The Antioxidant Activity Index (AAI) values are used to classify antioxidant properties. If AAI < 0.56, it indicates weak antioxidant activity; when AAI ranges from 0.5 to 1, it suggests moderate antioxidant activity; AAI between 1 and 2 indicates strong antioxidant activity, and AAI > 2 signifies very strong antioxidant activity³⁰. The AAI values for the test compounds can be seen in Table 2. The BNTU and its derivatives have AAI values < 0.56, categorizing them as having weak antioxidant properties.

Table 2. Antioxidant (DPPH scavenging) activity of BNTU and its derivatives, and standard substance presented as IC₅₀ values (ppm) and appropriate AAI values IC₅₀

Compounds	IC ₅₀ (ppm)	AAI
BNTU	> 400	< 0.56
4TBBNTU	> 400	< 0.56
4OCBNTU	> 400	< 0.56
4CFBNTU	189.6	0.42
3CFBNTU	294.5	0.27
BHT	16.45	4.79
Ascorbic acid	3.27	24.09

Molecular docking:

Docking validation was performed using the re-docking technique. Following the docking process, the ligand was preserved and compared against the crystallographic ligand³⁵. The visualization overlay of ligand results from redocking with the native ligand from crystallography is presented in Figure 3. The ligand redocked exhibits a comparable orientation to the crystallographic ligand³⁶. The validation of molecular docking involves the utilization of grid box configurations. The grid box parameters were set to X = 35.6845; Y = 11.4127; Z = 1.5107, with dimensions (Å) at X = 49.7954, Y = 62.9755, and Z = 57.9121. The measurement provided Root Mean Square Deviation (RMSD) values for the ligands, with RMSD < 2 Å, indicating their eligibility for the subsequent process^{35,37}.

Docking simulations were conducted using the PyRx-Virtual Screening Tool software to analyze the interaction between Human ROS1 Kinase Receptor and BNTU, and its derivatives are shown in Table 3.

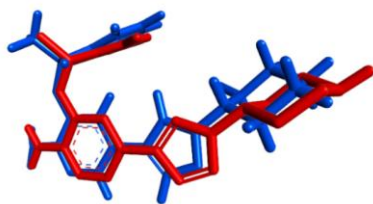


Figure 3. Overlay of redocking ligands (red) with native ligand from crystallography (blue) at 3ZBF receptor

Based on Table 3, it is known that the BNTU derivatives have lower binding affinity than BNTU as a lead compound. Crizotinib, as the native ligand, has a binding affinity of -8.23 kcal/mol, which is higher compared to 4OCBNTU (-7.73 kcal/mol) and 4TBBNTU (-7.60 kcal/mol). However, its affinity is the same as 3CFBNTU (-8.23 kcal/mol) and higher than BNTU (-7.40 kcal/mol). The *in silico* prediction suggests that 4CFBNTU is predicted to have the highest antioxidant activity. Therefore, it can be concluded that 4CFBNTU has a better binding affinity for the Human ROS1 kinase.

Table 3. Docking result of test compounds with Human ROS1 kinase receptor (3ZBF) active site

No.	Compounds	Binding affinity (kcal/mol)	Amino acid residues
1	4CFBNTU	-8.33 ± 0.06	Leu1951, Val1959, Leu2086, Ala1978, Leu2026, Gly2032, Met2029
2	3CFBNTU	-8.23 ± 0.21	Leu1951, Val1959, Leu2086, Ala1978, Gly2101, Asp2102, Leu2026, Lys1980
3	4OCBNTU	-7.73 ± 0.21	Leu1951, Val1959, Leu2086, Ala1978, Gly2101, Asp2102, Leu2026, Lys1980
4	4TBBNTU	-7.60 ± 0.10	Val1959, Gly1952, Asp2033, Lys1980
5	BNTU	-7.40 ± 0.17	Val1959, Leu2086, Ala1978, Gly2101, Lys1980
6	Crizotinib (native ligand)	-8.23 ± 0.06	Leu1951, Val1959, Leu2086, Ala1978, Glu1961

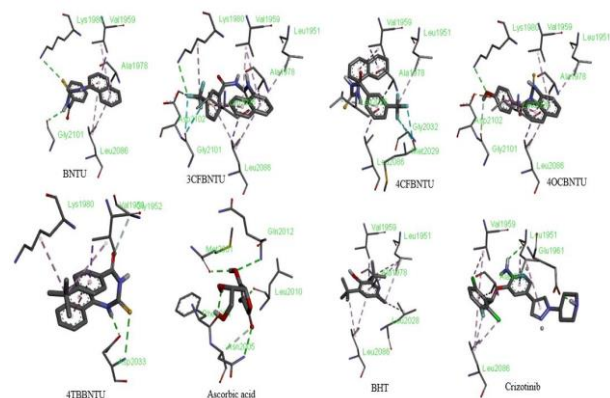


Figure 4. Three-dimensional comparison of docking results from BNTU and derivatives at Human ROS1 Kinase Receptor binding site

DISCUSSION:

The antioxidant activity of BNTU and its derivatives is assessed using an ethanol solution of DPPH reagent. The DPPH method is employed to investigate the antioxidant effects of test compounds and their capability to diminish free radical activity. The activity is quantified by observing the reduction in absorbance of the sample compared to the standard DPPH solution³⁰. DPPH represents a stable free radical capable of receiving an electron or hydrogen radical, leading to its transformation into a diamagnetic molecule³⁸. The DPPH assay assesses the capacity of antioxidants to counteract the stable radical DPPH by reacting with suitable reducing agents. This pairs electrons, causing the solution to undergo a color change proportionate to the number of electrons absorbed^{39,40}. Vitamin C was chosen as a standard because of its widely recognized antioxidant properties, attributed to its hydroxyl groups that stabilize free radicals, thereby enhancing its own considerable inhibitory capacity²³. Ascorbic acid demonstrates a robust ability to eliminate DPPH (3.27 ppm). The outcome of the DPPH assay is expressed as the quantity of antioxidants required to decrease the initial concentration of DPPH radicals by 50% (IC₅₀) within 30 minutes. Consequently, lower IC₅₀ values indicate higher antioxidant activity⁴¹.

BNTU derivatives differ in the substituents attached to the benzoyl group side chain. Olewi *et al.* (2023) found that the compound with the third highest antioxidant activity among the thiourea derivatives based on 4-methoxybenzoyl chloride is the one with a naphthyl group attached to the thiourea group²³. BNTU and its derivatives also contain a naphthyl group attached to the thiourea group. Research on antioxidant activity by Sudhamani *et al.* (2019) on urea and thiourea derivatives of 4-hydroxytryptophan indicates that compounds 3a, 3g, 3h, and 3l demonstrate significantly higher antioxidant activity compared to other synthesized compounds. The presence of electron-withdrawing groups such as chloro and fluoro groups on the phenyl ring of urea and thiourea derivatives may be the reason for their elevated activity⁴². 4CFBNTU has an electron-withdrawing group in the benzoyl group in the thiourea moiety (Fluor group), so the electrons are attracted toward the phenyl ring. As a result, the proton in the thiourea group is easily donated.

The BNTU and its four derivatives were docked into the active site of Human ROS1 Kinase (PDB id: 3ZBF). This was carried out to examine how these compounds interact with the receptor (Human ROS1 Kinase) and estimate their inhibitory activity as well as the free binding energy ΔG^0 , measured in kcal/mol. This energy encompasses hydrogen bonding, phi-sigma interaction, phi-alkyl interaction, Van der Waals forces, and other

types of interactions. The predicted interactions between Human ROS1 kinase protein residues and BNTU and its derivatives can be observed in Figure 4. The substituents attached to the benzoyl group of BNTU significantly influence its binding affinity with the active site of the Human ROS1 Kinase receptor. 4CFBNTU, 3CFBNTU, and 4OCBNTU exhibit interactions similar to crizotinib compared to 4TBBNTU and BNTU. However, 4CFBNTU has the highest docking score among them. This correlates with the results of antioxidant activity testing using the DPPH method. It is likely also due to the presence of the $-CF_3$ group, which is a powerful electron-withdrawing group. Low binding affinity suggests stability and alignment in the binding process with Human ROS1 Kinase Receptor compared to other compounds⁴³. In summary, 4CFBNTU shows the most promising inhibitory activity (theoretically) at the active site of Human ROS1 kinase. Once its inhibitory effects are confirmed through in vitro experiments, 4CFBNTU could be further developed as a Human ROS1 kinase inhibitor.

CONCLUSION:

The four derivatives of BNTU exhibit better antioxidant activity than the lead compound. Among them, 4CFBNTU exhibits the best antioxidant activity, although its antioxidant properties are relatively weak. The docking results obtained are consistent with the conclusions drawn from the antioxidant investigation.

CONFLICT OF INTEREST:

The authors have disclosed that there are no conflicts of interest related to this investigation.

ACKNOWLEDGMENTS:

The authors express their gratitude to the Project Management Unit (PMU) for the scholarship provided: "Magister (S2) and Doctor (S3) Program Development of UIN Maulana Malik Ibrahim Malang Phase II, East Java Project".

REFERENCES:

- Al-Salahi R, Taie HAA, Bakheit AH, Marzouk M, Almehizia AA, Herqash R et al. Antioxidant activities and molecular docking of 2-thioxobenzo[g]quinazoline derivatives. *Pharmacol Reports*. 2019; 71: 695–700. doi: 10.1016/j.pharep.2019.04.003
- Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur J Med Chem*. 2021; 209: 112891. doi: 10.1016/j.ejmech.2020.112891
- Tan BL, Norhaizan ME, Liew WPP, Rahman HS. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Front Pharmacol*. 2018; 9: 1162. doi: 10.3389/fphar.2018.01162
- Saha D, Tamrakar A. Xenobiotics, Oxidative Stress, Free Radicals Vs. Antioxidants: Dance Of Death to Heaven's Life. *Asian J Res Pharm Sci*. 2011; 1: 36–38.
- Muthukumaran P, Salami S, Umamaheshwari R. In vitro antioxidant activity of *Premna serratifolia* Linn. *Asian J Res Pharm Sci*. 2013; 3: 15–18.
- Bollinger A, Brandt ON, Stettler LD, Ream A, Kopysciansky VT, Nelson CA et al. Sulfide-linked 3,4,5-trimethoxyphenylthiosemicarbazide/triazole hybrids: Synthesis, antioxidant, antiglycation, DNA cleavage and DNA molecular docking studies. *Results Chem*. 2023; 5: 0–6. doi:10.1016/j.rechem.2023.100806
- Prakash CR, Saravanan G, Raja S, Kumar PD, Selvam TP. Synthesis and evaluation of antioxidant activities of some novel isatin derivatives and analogs. *Asian J Res Pharm Sci*. 2011; 1: 140–143.
- Patel RN, Patel UY, Sen DJ. Polyphenol antioxidants of green tea as free radical scavengers in green heart nanotechnology. *Res J Sci Technol*. 2010; 2: 89–94.
- Aitha S, Thumma V, Matta R, Ambala S, Jyothi K, Manda S et al. Antioxidant activity of novel 4H- chromene tethered 1,2,3-Triazole Analogues: Synthesis and molecular docking studies. *Results Chem*. 2023; 5: 1–8. doi: 10.1016/j.rechem.2023.100987
- Malathi R, John SA, Cholarajan A. Antioxidant activity of extract from the leaves of *Tylophora asthmatica*. *Asian J Res Pharm Sci*. 2012; 2: 80–82.
- Ali KWR, Mayur C, Mansi G. A Study on phytochemical and antioxidant properties of the leaves of *Ficus hispida* Linn. *Res J Pharmacogn Phytochem*. 2023; 15: 203–208.
- Sadhu SP, Pragada RR, Konduri P, Yarla NS. Anticancer, Antioxidant Activity and Molecular Docking Studies of Saccharumside-B. *Res J Pharm Technol*. 2023; 16: 608–614.
- Huang RZ, Zhang B, Huang XC, Liang G Bin, Qin JM, Pan YM et al. Synthesis and biological evaluation of terminal functionalized thiourea-containing dipeptides as antitumor agents. *RSC Adv* 2017; 7: 8866–8878. doi: 10.1039/C6RA25590F
- Widiandani T, Siswandono, Meiyanto E, Sulistyowaty MI, Purwanto BT, Hardjono S. New N-allylthiourea derivatives: synthesis, molecular docking and in vitro cytotoxicity studies. *Trop J Pharm Res*. 2018; 17: 1607–1613.
- Liu S, Louie MC, Rajagopalan V, Zhou G, Ponce E, Nguyen T et al. Synthesis and evaluation of the diarylthiourea analogs as novel anti-cancer agents. *Bioorganic Med Chem Lett* 2015; 25: 1301–1305. doi: 10.1016/j.bmcl.2015.01.042
- Kesuma D, Nasyanka AL, Rudyanto M, Siswandono, Purwanto BT, Sumartha IGA. A prospective modification structure: the effect of lipophilic and electronic properties of N-(phenylcarbamothioyl)benzamide derivatives on cytotoxic activity by in silico and in vitro assay with T47D cells. *Rasayan J Chem* 2020; 13: 1914–1918. doi: 10.31788/RJC.2020.1335694
- Widiandani T, Siswandono, Meiyanto E. Anticancer evaluation of N-benzoyl-3-allylthiourea as potential anticancer cancer agent through enhances HER-2 expression. *J Adv Pharm Technol Res*. 2020; 11: 163–168. doi: 10.4103/japtr.JAPTR_77_20
- Ahmed A, Shafique I, Saeed A, Shabir G, Saleem A, Taslimi P et al. Nimesulide linked acyl thioureas potent carbonic anhydrase I, II and α -glucosidase inhibitors: Design, synthesis and molecular docking studies. *Eur J Med Chem Reports*. 2022; 6: 1–12. doi: 10.1016/j.ejmcr.2022.100082
- Maryoosh AA, Al-Jeilawi OHR. Synthesis, Identification, Antioxidant, Molecular Docking, and In Silico ADME Study for Some New Derivatives Containing Thiourea Moiety. *Russ J Bioorganic Chem*. 2024; 50: 170–180. doi: 10.1134/S1068162024010084
- Pingaw 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. doi: 10.1016/j.bioorg.2018.05.002
- Kirishnamaline G, Magdaline JD, Chithambarathanu T, Aruldas D, Anuf AR. Theoretical investigation of structure, anticancer activity and molecular docking of thiourea derivatives. *J Mol Struct*. 2021; 1225: 129118. doi:10.1016/j.molstruc.2020.129118
- Huong DQ, Bay M Van, Nam PC. Antioxidant activity of thiourea derivatives: An experimental and theoretical study. *J Mol Liq*. 2021; 340: 117149. doi: 10.1016/j.molliq.2021.117149
- Olewi AQ, Al-Jeilawi OHR, Dayl SA. Synthesis, Characterization of Some Thiourea Derivatives Based on 4-

- Methoxybenzoyl Chloride as Antioxidants and Study of Molecular Docking. Iraqi J Sci. 2023; 64: 1–12. doi: 10.24996/ijsc.2023.64.1.1
- 24 Sudzhaev AR, Rzaeva IA, Nadzhafova RA, Safarov YS, Allakhverdiev MA. Antioxidant properties of some thiourea derivatives. Russ J Appl Chem. 2011; 84: 1394–1397. doi: 10.1134/S1070427211080167
- 25 Haque A, Khan MWA, Alenezi KM, Soury R, Khan MS, Ahamad S et al. Synthesis, Characterization, Antilycation Evaluation, Molecular Docking, and ADMET Studies of 4-Thiazolidinone Derivatives. ACS Omega. 2024; 9: 1810–1820. doi: 10.1021/acsomega.3c08463
- 26 Megawati DS, Fasya AG, Pratiwi RA, Maghfiroh N. Pharmacology potency of thin layer chromatography steroid isolates of *Chlorella* sp. chloroform fraction. In: IOP Conference Series: Earth and Environmental Science. 2020, p 012012. doi: 10.1088/1755-1315/456/1/012012
- 27 Fasya AG, Baderos A, Madjid ADR, Amalia S, Megawati DS. Isolation, identification and bioactivity of steroids compounds from red algae *Eucheuma cottonii* petroleum ether fraction. In: AIP Conference Proceedings. 2019 doi:10.1063/1.5115629.
- 28 Salama ZA, Aboul-Enein AM, Gaafar AA, Asker MS, Aly HF, Ahmed HA. In-vitro antioxidant, antimicrobial and anticancer activities of Banana leaves (*Musa acuminata*) and olive leaves (*olea europaea* l.) as by-products. Res J Pharm Technol. 2020; 13: 687–696. doi: 10.5958/0974-360X.2020.00132.8
- 29 Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem 2009; 112: 654–658. doi: 10.1016/j.foodchem.2008.06.026
- 30 Vasić SM, Stefanovic OD, Ličina BZ, Radojević ID, Comic LR. Biological activities of extracts from cultivated *granadilla* *Passiflora alata*. EXCLI J 2012; 11: 208–218. PMID: 27385958
- 31 Megawati DS, Ekowati J, Siswandono S. Quantitative Structure-Activity Relationship (QSAR) of N-Benzoyl-N'-Naphthylthiourea Derivative Compounds by in Silico as Anticancer Through Inhibition of VEGFR2 Receptors. In: Proceedings of the 12th International Conference on Green Technology (ICGT 2022). Atlantis Press International BV, 2023, pp 137–148. doi: 10.2991/978-94-6463-148-7_15
- 32 Indratmoko S, Nurani LH, Wahyuningsih I, Murti TD, Setiyabudi L. Effectiveness of Active Compounds of Herbal Plants as Aphrodisiacs Through Molecular Docking Against Human Phosphodiesterase-5 Receptors. J Penelit Pendidik IPA. 2023; 9: 2047–2054. doi: 10.29303/jppipa.v9i4.3137
- 33 Megawati DS, Ekowati J, Siswodihardjo S, Fasya AG. In Silico study of the effect of substituents on the structure of N-benzoyl-N'-naphthylthiourea as anti-breast cancer HER-2 positive candidates. J Med Pharm Chem Res. 2023; 5: 1077–1088. doi: 10.48309/jmpcr.2023.181527
- 34 Fasya AG, Millati N, Rahmawati LM, Iyani R, Hanapi A, Ningsih R et al. Isolation and bioactivity of steroids isolates from petroleum ether fraction of *Chlorella* sp. In: AIP Conference Proceedings. 2020 doi:10.1063/5.0001490.
- 35 Legiawati L, Fadilah F, Bramono K, Pratama AI. In Silico Study of *Centella asiatica* Derivatives as Antioxidant: Enhancer of Superoxide Dismutase and Glutathione Peroxidase Activity. Res J Pharm Technol. 2023; 16: 399–403. doi: 10.52711/0974-360X.2023.00068
- 36 Pratama MRF, Praditapuspa EN, Kesuma D, Poerwono H, Widiandani T, Siswodihardjo S. Boesenbergia Pandurata as an Anti-Breast Cancer Agent: Molecular Docking and ADMET Study. Lett Drug Des Discov. 2022; 19: 606–626. doi: 10.2174/1570180819666211220111245
- 37 Pratama MRF, Poerwono H, Siswodihardjo S. Molecular docking of novel 5- O -benzoylpinostrobin derivatives as wild type and L858R/T790M/ V948R mutant EGFR inhibitor. J Basic Clin Physiol Pharmacol. 2019; : 1–11. doi: 10.1515/jbcpp-2019-0301
- 38 Dubey SK, Batra A. Study of anti oxidant and anti-inflammatory activity from ethanol fraction of *Thuja occidentalis* Linn. Res J Sci Technol. 2009; 1: 39–42.
- 39 Vaghela JS, Sisodia SS. In vitro antioxidant activity of *Terminalia chebula* fruit extracts. Res J Pharm Technol. 2011; 4: 1835–1843. PID=2011-4-12-2
- 40 Roy A, Bhounik D, Sahu RK, Dwivedi J. Phytochemical screening and antioxidant activity of *Sesbania grandiflora* Leaves Extracts. Asian J Res Pharm Sci. 2014; 4: 16–21.
- 41 Amirah S, Mohd Zain HH, Husni I, Kassim NK, Amin I. In vitro antioxidant capacity of *peperomia pellucida* (L.) kunth plant from two different locations in malaysia using different solvents extraction. Res J Pharm Technol. 2020; 13: 1767–1773. doi: 10.5958/0974-360X.2020.00319.4
- 42 Sudhamani H, Syam Prasad G, Venkataramaiah C, Raju CN, Rajendra W. In silico and in vitro antioxidant activity profiles of urea and thiourea derivatives of 5-hydroxytryptophan. J Recept Signal Transduct. 2019; 39: 373–381. doi: 10.1080/10799893.2019.1683864
- 43 Fasya AG, Warsito, Ifitah ED, Rollando. Molecular Docking of Selected Phytosterol from *Hydrilla verticillata* on Estrogen Receptors as Candidates of Anti-breast Cancer. In: Proceedings of the 12th International Conference on Green Technology. (ICGT 2022). Atlantis Press International BV, 2023, pp 304–311. doi: 10.2991/978-94-6463-148-7_30