

An In-Silico Study on Allicin Compound in Garlic (*Allium Sativum*) as A Potential Inhibitor of Human Epidermal Growth Factor Receptor (Her)-2 Positive Breast Cancer

Ardyarini Dyah Savitri¹, Hanik Badriyah Hidayati^{2,*}, Lysa Veterini³, Meidyta Sinantryana Widyaswari⁴, Akbar Reza Muhammad⁵, Ainiyah Fairus⁵, Mohammad Qoimam Bilqisthi Zulfikar⁵, Mega Astri⁵, Nosa Abdellah Ramasima⁵, Dini Putri Anggraeni⁵, Reghina Salsabila Ayuantia Nainatika⁵, Juliana⁶

¹Lecturer, Department of Internal Medicine, Faculty of Medicine - Universitas Nahdlatul Ulama, Surabaya; ²Lecturer, Department of Neurology, Faculty of Medicine - Universitas Airlangga, Surabaya; ³Lecturer, Department of Pathology and Anatomy, Faculty of Medicine - Universitas Nahdlatul Ulama, Surabaya; ⁴Lecturer, Department Dermatology and Venereology, Faculty of Medicine - Universitas Nahdlatul Ulama, Surabaya; ⁵Medical Student, Faculty of Medicine - Universitas Nahdlatul Ulama, Surabaya; ⁶Medical Student, Faculty of Medicine - Universitas Airlangga, Surabaya

Abstract

Allicin is the major sulfur-containing compounds in a single garlic (*Allium Sativum*) that play an important protective part in the oxidative processes. Because of its less toxicity and its potency in preventing and attenuating outcomes of several cancer types, it makes Allicin as a potential natural therapy for breast cancer. Moreover, because of the similar structure of N-Acetylcysteine (NAC) and Allicin, it predicts that Allicin may give the same therapeutic effects with NAC, which NAC is able to show the anti-tumor effect on Human Epidermal Growth Factor Receptor (HER-2) protein of breast cancer. Breast cancer as the most common cancer in women and the second-highest prevalence of cancer in the world, might provide benefits using Allicin in order to prevent cancer progression with a better safety profile. However, little study has been carried out on the functional role of Allicin in HER-2 positive breast cancer. In this study, we calculated and compared the binding energy, pharmacokinetic properties, and toxicity of Allicin and NAC with HER-2 receptor using in-silico study in order to evaluate the potency as HER-2 positive breast cancer alternative therapy. By using Molegro Virtual Docker, the affinity between Allicin and NAC to HER-2 receptor showed an equal result, -62,1239 kcal/mol for Allicin, and -65,8084 kcal/mol for NAC. Meanwhile, study on their pharmacokinetic properties and toxicity using pkCSM online tool showed that Allicin has a safer profile than NAC. Thus, it can be concluded that the Allicin compound has a relatively same anti-tumor potency in HER-2 positive breast cancer compared to NAC. Moreover, Allicin also has a fairly good pharmacokinetic profile and more tolerable toxicity properties, rather than NAC.

Keywords : Allicin, in silico, HER-2 positive breast cancer

1. Introduction

Breast cancer is the most common cancer in women. It is the second-highest prevalence of cancer in the world, which was found in 2.1 million people (11.6%) with 626,679 deaths (6.6%), based on the International Agency for Research on Cancer data in GLOBOCAN (Global Cancer Statistics) in 2018 (Bray *et al.*, 2018). About 1 of 5 women with breast cancer have the Human Epidermal Growth Factor Receptor (HER)-2 protein on the surface of their cancer cells. It is known that HER-2-positive breast cancer has more aggressive tumor phenotypes. Unfortunately potential therapeutics including trastuzumab, lately developed therapeutic resistance which lead to therapeutic failure.

Allicin as the main active compound of single garlic contains quite high sulfur. Some studies revealed that Allicin exhibits not only as an antioxidant but also as

antibacterial and anticarcinogenic (Nikolic *et al.*, 2004). Several sulfur-containing antioxidants are known to provide benefits in inhibiting cancer progression, one of which is N-Acetylcysteine (NAC). It is said that NAC has an anti-tumor effect on Human Epidermal Growth Factor Receptor (HER-2) protein of breast cancer (Wang and Xu, 2019; Wimana *et al.*, 2017). Based on this fact, we predict that Allicin has the same potential effect in inhibiting HER-2 positive breast cancer as NAC. Unfortunately, there is still no study about this recently.

This study aimed to compare the potential of Allicin and NAC in inhibiting HER-2 positive breast cancer. In addition to their potency, NAC and Allicin compounds will be tested for their pharmacokinetic properties consisting of absorption, distribution, metabolism, and excretion (ADME) along with the toxicity properties using the pkCSM online tool.

This study began with downloading HER-2 receptor from Protein Data Bank with ID code: 3PP0 and native

* Corresponding author. e-mail: hanikhidayati@fk.unair.ac.id.

ligand 03Q. Prediction using Molegro Virtual Docker was carried out by re-docking between the HER-2 receptor and the native ligand. The docking method is valid if Root Mean Square Deviation (RMSD) is equal to or less than 2 Å. Allicin and NAC were docked alternately to target protein using Molegro Virtual Docker. The results achieved were in the form of Rerank score, which explains the energy required for binding of the compound (ligand) to the receptor. A comparison of scores between Allicin and NAC was carried out, in which a higher Rerank score indicated that the required binding energy between ligand and receptor was greater, which means that the interaction was less stable than the lower score (Kufareva and Abagyan, 2012).

Before the docking process is commenced, it is necessary to check the properties of the ligands using the pkCSM online tool. In addition, Allicin and NAC compounds will also be tested for their pharmacokinetic properties consisting of absorption, distribution, metabolism, and excretion (ADME) along with compounds' toxicity properties using the pkCSM online tool (Pires *et al.*, 2015).

2. Materials and Methods

2.1. Tools

This study uses a set of computers with Windows 10 specifications, 64 bit. The programs used in this study are ChemDraw Professional 16.0, Chem 3D 16.0, and Molegro Virtual Docker 5.

2.2. Ligand and Receptor Preparation

Allicin was used as the ligand tested in this study, and its potency will be compared with N-Acetylcysteine (NAC). The structures of Allicin and NAC were downloaded from <https://pubchem.ncbi.nlm.nih.gov/>. The 2-dimensional structure of both ligands was redrawn using ChemDraw Professional 16.0 and converted to a 3-dimensional structure using Chem3D 16.0. The structure obtained in Chem3D determined the most stable conformation and the minimum energy. Data is stored in the form mol2 {SYBYL2(*.mol2)}.

The receptor used in this study is HER-2 and its 3-dimensional structure obtained from <https://www.rcsb.org/structure/3PP0> with ID code: 3PP0. The downloaded results show that HER-2 binds to the native ligand 03Q. Data is saved in PDB format. By using Molegro Virtual Docker 5, cavity detection is carried out on the receptor. The cavity in which there is an active native ligand 03Q is used as a docking location between the receptor and the ligand.

2.3. Molecular Docking and Compound Potential Prediction

The first step that needs to be done in predicting the potential of a compound is to validate the molecular docking method by re-docking between the receptor and the native ligand. It is indicated by the Root Mean Square Deviation (RMSD) value. If the RMSD is equal to or less than 2 Å, then it can be continued with the docking process between the receptor and both ligands (Purnomo, 2013; Nauli, 2014). Prediction of Allicin and NAC potential activity on HER-2 protein is in the form of Rerank score. A lower score indicates that the energy required for ligand

and receptor interaction is smaller, so that the bond is more stable, and vice versa.

The amino acid residues was formed and observed in the interaction between the receptor and the ligand. These bond interactions can be in the form of hydrogen bonds, steric interactions, and electrostatics.

2.4. Prediction of Pharmacokinetic and Physicochemical Properties and Compounds' Toxicity

The physicochemical properties of Allicin and NAC were predicted using the pkCSM online tool. These properties include molecular weight (BM), Log P, number of rotating atomic bonds (Torsion), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), and Polar Surface Activity (PSA). Compliance to Lipinski's rule consisting of a molecular weight of fewer than 500 Daltons, hydrogen bond donors less than 5, log P value of less than 5, and hydrogen bond acceptor number of less than 10 is required for docking preparation (Lipinski *et al.*, 2001).

Prediction of pharmacokinetic parameters and toxicity properties were performed using the pkCSM online tool. Using ChemDraw Professional 16.0 program, the 2-dimensional structure of the Allicin Compound and NAC was drawn. Then it converted into a 3-dimensional structure using Chem3D 16.0 program. The 3-dimensional image was then saved in the form of *.sdf file. The structure obtained was translated into a SMILES structure with the help of the Online SMILES Translator (<https://cactus.nci.nih.gov/translate>). After that, the compounds were processed with the pkCSM online tool (<http://biosig.unimelb.edu.au/pkcsm/prediction>) for predicting the pharmacokinetic properties and toxicity tests by inputting the compound's SMILES structure into the program (Pires *et al.*, 2015).

3. Results

3.1. Ligand and Receptor Preparation

The 2-dimensional structures of the ligands are shown in Figure 1. The image was converted into 3-dimensional structure using Chem3D 16.0. The results obtained conform to the most stable form with the least energy for optimization of the compound structure. The data obtained is kept in the form mol2 {SYBYL2(*.mol2)}. The results of optimization of both compounds can be seen in Figure 2.

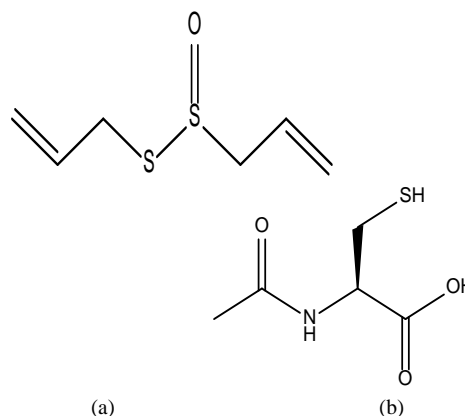


Figure 1. 2D Structure (a) Allicin; (b) N-Acetylcysteine (NAC)

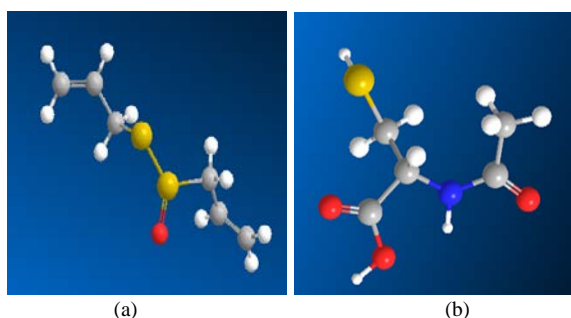


Figure 2. 3D Structure with Optimization (a) Allicin (b) *N*-Acetylcysteine

The HER-2 protein was downloaded through the Protein Data Bank with ID code: 3PP0, which has 03Q as the native ligand. After downloading the receptor, the cavity was detected using Molegro Virtual Docker. The compound-complex in the cavity is shown in Figure 3.

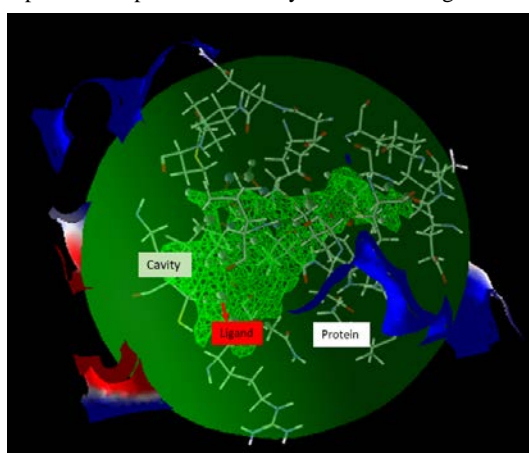


Figure 3. HER-2 complex (3PP0) with native ligand 03Q in the cavity

Before docking process, it is necessary to undergo re-docking process between the receptor and the native ligand in order to know the validation docking method. The re-docking process showed 1,695Å for RMSD result (Lipinski *et al.*, 2001). On the other hand, the re-docking process between native ligand 03Q and HER-2 receptor showed quite low energy, with rerank score of -165,051 Kcal/mol. The interaction of the HER-2 receptor with the native ligand 03Q is shown in Figure 4. Figure 6 and Table 1 showed the amino acid residues formed during the interaction, and those are:

1. Hydrogen bond: Asp863, Thr862, Met801, Ser728
2. Steric bond: Asp863, Met774, Met801, Ala751

Since molecular docking has shown valid results, docking was then carried out between the receptor with the test and comparison ligands. The interaction between HER-2 receptor and the Allicin and NAC ligands is shown in Figures 5(a) and 5(b).

1. Interaction between HER-2 receptor and Allicin

The prediction of the required binding energy in the interaction between the HER-2 receptor and the Allicin is shown in Table 2. It is predicted that the affinity between Allicin and HER-2 receptor is -62.1239 Kcal/mol. The amino acid residues formed during the interaction process between Allicin and the receptor are shown in Figure 6 and Table 1, and the residues are Thr798 and Ser783 for hydrogen bond and Leu785 for steric bond.

2. Interaction between HER-2 receptor and NAC

The prediction of the required binding energy in the interaction between the HER-2 receptor and the NAC is shown in Table 2. It is predicted that the affinity between NAC and HER-2 receptor is -65.8084 Kcal/mol. The amino acid residues formed during the interaction process between NAC and the receptor are shown in Figure 6 and Table 1, and the residues are hydrogen bonds only and those are Asp863, Thr862, Thr798, and Ser783.

Table 1. Amino Acid Residues formed in the interaction between the HER-2. Receptor and the Ligand

Ligand	Hydrogen Bond	Steric Bond	Electrostatic Bond
03Q	Asp863, Thr862, Met801, Ser728	Asp863, Met774, Met801, Ala751	-
Allicin	Thr798, Ser783	Leu785	-
NAC	Asp863, Thr862, Thr798, Ser783	-	-

Table 2. Prediction of bond energy between receptor and ligand lig

Ligand	Rerank score
03Q	-165,051
Allicin	-62,1239
NAC	-65,8084

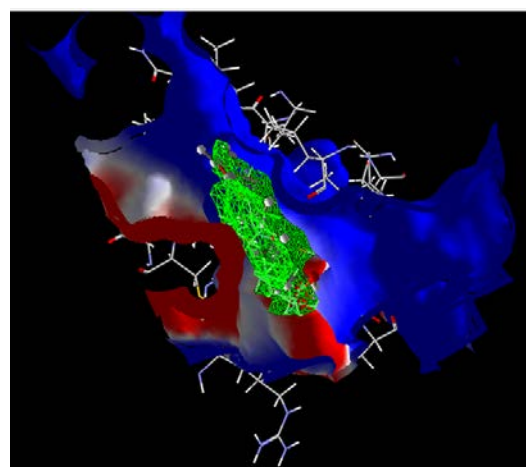


Figure 4. HER-2 Receptor Interaction with native ligand 03Q

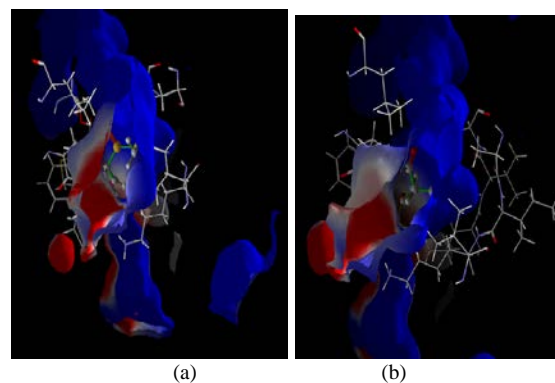


Figure 5. HER-2 Receptor Interaction with ligand (a) Allicin (b) NAC

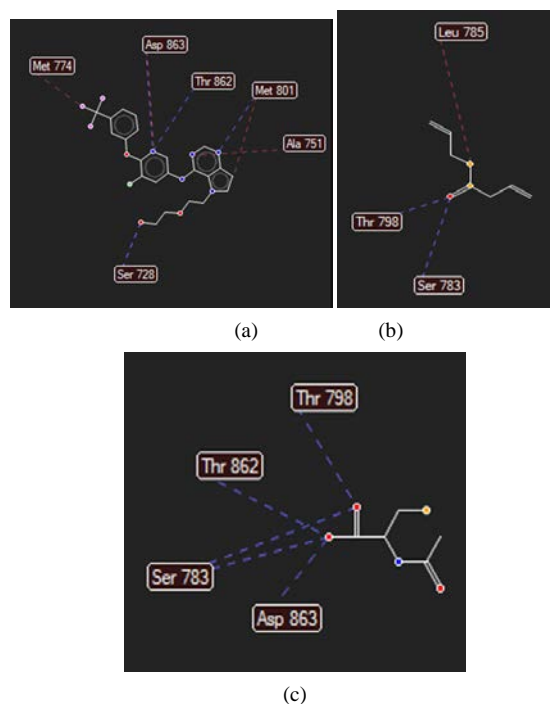


Figure 6. Receptor Interaction with Ligand and Amino Acid Residue formed (a) 03Q (b) Allicin (c) NAC

3.2. Prediction of Pharmacokinetic, Physicochemical, and Toxicity of Compounds

1. Allicin

Compliance with Lipinski's rule for Allicin was shown in Table 3. The analysis shows that Allicin has low molecular weight, hydrogen bond donors less than 5, log P value of less than 5, and hydrogen bond acceptor number of less than 10.

Prediction of pharmacokinetic properties and toxicity of Allicin is shown in Table 4. The intestinal absorption of Allicin is 96,299% and the skin permeability is -1,877 log Kp. The volume distribution of Allicin is -0,045 log L/kg, and its blood-brain barrier penetration properties are 0,506 logBB. Allicin is also neither a substrate nor inhibitor of CYP3A4.

The ability of Allicin to be excreted from the body, either through the liver or kidneys, was shown by the total the clearance of Allicin, which is 0,714 log ml/min/ kg. According to the results, Allicin is not renal Organic Cation Transporter (OCT)-2.

Table 3. Prediction test results of physicochemical properties of Allicin and NAC. compounds

	Allicin	NAC
BM	162,279	163,198
Log P	1,7553	-0,4945
Torsion	5	3
HBA	2	3
HBD	0	3
PSA (Å ²)	62,082	64,021

BM = Molecular Weight; Log P = Octanol logarithm / water partition coefficient; HBD = hydrogen bond donor; PSA = Polar Surface Area; HBA = hydrogen bond acceptor

Another safety profile that must be considered is the mutagenic ability of the drug candidate. This can be seen from the AMES toxicity test (Mortelmans and Zeiger, 2000). Table 4 shows that Allicin is not mutagenic.

The safety of drug administration in experimental animals is known from the LD50. LD50 is the dose given to animals that produce 50% of animal death in the population within a certain time (Chan *et al.*, 1989). Table 4 shows that the LD50 of Allicin is 2.366 mol/kg

Table 4. Pharmacokinetic prediction test results (ADME) of Allicin and NAC

ADME & Toxicity	Allicin	NAC
Intestinal Absorption (human) (%)	96,229	77,922
VDss (human) (log L/kg)	-0,045	-1,355
Skin Permeability (log Kp)	-1,877	-2,735
Blood Brain Barrier Permeability (log BB)	0,506	-0,355
CYP3A4 substrate (Yes/ No)	No	No
CYP3A4 inhibitor (Yes/ No)	No	No
Renal OCT2 substrate (Yes/ No)	No	No
Total Clearance (log ml/ min/ kg)	0,714	0,309
AMES Toxicity (Yes/ No)	No	Yes
LD50 (mol/ kg)	2,366	1,626

VDSS = Volume Distribution *Steady State*; CYP3A4 = Cytochrome P3A4; Renal OCT2 = Renal *Organic Cation Transporter 2*

2. N-Acetyl Cysteine (NAC)

As with Allicin, NAC shows good compliance with Lipinski's rule. As shown in Table 3, NAC has low molecular weight, hydrogen bond donors less than 5, log P value of less than 5, and hydrogen bond acceptor number of less than 10.

Prediction of pharmacokinetic properties and toxicity of NAC is shown in Table 4. Intestinal absorption of NAC is 77,992%, and the skin permeability is $-2,735 \log Kp$. Volume distribution of NAC is $-1,355 \log L/kg$ and the blood-brain barrier penetration properties is $-0,355 \log BB$. NAC is also neither a substrate nor inhibitor of CYP3A4.

The total clearance of NAC is $0,309 \log ml/min/kg$. According to the results, NAC is not renal Organic Cation Transporter (OCT)-2.

The mutagenic ability of NAC from AMES toxicity test shows positive results, which means NAC is a mutagenic drug (Mortelmans and Zeiger, 2000).

The safety of drug administration in experimental animals for NAC is $1,626 \text{ mol/kg}$ as shown in Table 4.

4. Discussion

HER-2 positive type breast cancer is a quite aggressive breast cancer type. Strong positive HER-2 expression also indicates a high risk of breast cancer relapse. HER-2 itself is a transmembrane protein encoded by the gene *c-ERBB2* (Her-2/neu) located on chromosome 17 and it is an ideal target for targeted therapy in breast cancer (Ferrero *et al.*, 2000).

Because of the same sulfur-containing structure, in this research, we used Allicin as the test ligand, and NAC as the comparison ligand to be docked to the HER-2 receptor. Both chemical structures are shown in Figures 1 and 2.

The predictive physicochemical properties test in Table 3 shows that both ligands meet Lipinski's rules. It means that both of them have met the criteria of drug-likeness and both ligands have high permeability properties and are easily absorbed by the body (Purnomo, 2013).

The result of re-docking process between native ligand 03Q and HER-2 receptor showed the lowest energy, with rerank score of $-165,051 \text{ Kcal/mol}$. On the other hand, Allicin showed the highest energy ($-62.1239 \text{ Kcal/mol}$). It is shown in Table 2. The result obtained by Allicin is less stable than other ligands. However, the energy required for the bond between Allicin and NAC on HER-2 is not significantly different.

In the ligand and receptor interaction, amino acid residues are formed, both in hydrogen bonds, steric and electrostatic interactions. This is as shown in Figure 6 and Table 1, which there are similarities in the amino acid residues formed in the hydrogen bonds of native ligand and NAC, namely Asp863 and Thr862. However, there was no similarity of amino acid residues formed between Allicin and the other two ligands. This indicates that NAC has more similar activity with the native ligand on the HER-2 receptor, compared to Allicin (Cosconati *et al.*, 2010).

The pharmacokinetic properties and toxicity of the two ligands are shown in Table 4. From these results, it can be concluded that Allicin has better intestinal absorption (96.2%) compared to NAC (77.9%). However, both compounds are generally well absorbed. A compound is said to have good intestinal absorption if its absorption

ability reaches $> 80\%$, and bad if its ability is $< 30\%$ (Chander *et al.*, 2017).

For absorption through the skin, Table 4 above shows that Allicin has lower skin permeability than NAC, where the log Kp value of Allicin is -1.877 and NAC is -2.735 . This is following what was stated by Pires *et al.* (2015), that the drug has low skin absorption if the log Kp value is > -2.5 (Pires *et al.*, 2015).

Drug distribution throughout the body is needed to increase the drug's effectiveness. This is expressed from VDss value, where a higher value of VDss means there is more drug distributed into the network. A compound is said to have a low VDss if the value is < -0.15 and high if it > 0.45 (Tedjamartono *et al.*, 2020). The results in Table 4 above show that Allicin has a better volume of distribution than NAC, where the Allicin VDss reaches -0.045 , while NAC is $-1,355$.

Breast cancer with HER-2 overexpression is a very aggressive cancer type with a higher risk for brain metastases (Zhou, 2008). Therefore, the most ideal HER-2 positive breast cancer drug is one that can penetrate the blood-brain barrier. According to Pires *et al.* (2015), a drug is said to have good penetration inside the brain if the log BB value reaches > 0.3 . On the contrary, it would have poor brain penetration if the BB log value reaches < -1 (Pires *et al.*, 2015). From Table 4 above, it can be concluded that both compounds have good blood-brain barrier penetration properties, but Allicin has a higher ability to penetrate the blood-brain barrier than NAC.

Drug metabolism process needs Cytochrome P450 (CYP450) super enzymes. CYP450 enzymes are essential for the metabolism of many medicines and endogenous compounds. There are seventeen CYP families recognized in humans, including CYP3A4 as the most abundant and important subfamily of the CYP isoforms in the liver. It contributes to bile acid detoxification, the termination of action of steroid hormones, and elimination of phytochemicals in food and the majority of medicines (Šrejber *et al.*, 2018). Drugs that can inhibit this enzyme are called CYP3A4 inhibitors. On the contrary, drugs that can induce cytochrome P450 are referred to as CYP3A4 substrates. Drugs that inhibit CYP3A4 will result in decreased drug metabolism and increased drug levels in the blood. However, drugs that can induce this enzyme will result in increased drug metabolism and consequently decrease the drug levels in the blood (Aslam *et al.*, 2003). Table 4 shows that both Allicin and NAC are neither inducers nor inhibitors CYP3A4, so they are safe when they were given together with other drugs that affect this enzyme activity.

Total clearance indicates the ability of the drug to be excreted from the body, either through the liver or kidneys (Belzer *et al.*, 2013). Table 4 shows that the clearance of Allicin is slower than that of NAC, so Allicin stays in the body longer than NAC.

Organic Cation Transporter (OCT)-2 is a transporter in the kidney that functions to increase drug uptake from the blood, then passes through the basal membrane of kidney, reaches the proximal tubular cells, and waste from the body. OCT-2 substrate has the capability to cause side effects when given along with OCT2 inhibitor (Zhou, 2008). According to predictive pharmacokinetic properties, it was found that both Allicin and NAC are not

renal OCT2 substrates, so they are safe when given together with OCT2 inhibitors.

Another safety profile that must be considered is the mutagenic ability of the drug. This can be seen from the AMES toxicity test (Mortelmans and Zeiger, 2000). Table 4 shows that Allicin is not mutagenic, while NAC is mutagenic.

The safety of drug administration in experimental animals is known from the LD50. LD50 is the dose given to animals that produce 50% of animal death in the population within a certain time (Chan *et al.*, 1989). Table 4 shows that the LD50 of Allicin is 2.366 mol/kg and NAC is 1.626 mol/kg. Thus, Allicin has a higher safe dose limit than NAC.

5. Conclusion

In-silico study showed that the Allicin compound of single garlic (*Allium Sativum*) relatively has the same ability to NAC as an anti-tumor in HER-2 positive breast cancer. Moreover, Allicin also has a fairly good pharmacokinetic profile, such as intestinal absorption, volume distribution, the ability to penetrate blood brain barrier, and the safety of combining drugs, especially drugs that affect CYP3A4 activity or when it is given together with OCT2 inhibitor drugs. Moreover, Allicin stays longer in the human body, with no mutagenic effect and lesser animal death in experimental studies. Nevertheless, in vitro and in vivo studies should be conducted to ensure the results of this study.

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References

- Aslam M, Tan CK, and Prayitno A. 2003. **Farmasi Klinis (Clinical Pharmacy) Menuju Pengobatan Rasional Dan Penghargaan Pilihan Pasien**. Elex Media Komputindo, Jakarta.
- Belzer M, Morales M, Jagadish B, Mash E, and Wright S. 2013. Substrate-Dependent Ligand Inhibition of the Human Organic Cation Transporter OCT2. *J Pharmacol Exp Ther.*, **346(2)**: 300-310.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. 2018. Global Cancer Statistics 2018: GLOBOCAN estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.*, **68(6)**: 394- 424.
- Chan PK and Hayes AW. 1989. **Principles and Methods of Acute Toxicity and Eye Irritancy**. In: Hayes AW (Eds), Principles and Methods of Toxicology. Raven Press, New York.
- Chander S, Tang CR, Al-Maqtari HM, Jamalis J, Penta A, Hadda TB, Sirat HM, Zheng YT, and Sankaranarayanan M. 2017. 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.*, **72**: 74-79.
- Cosconati S, Forli S, Perryman A, Harris R, Goodsell D, and Olson A. 2010. Virtual screening with AutoDock: theory and practice. *Expert Opin Drug Discov.*, **5(6)**: 597-607.
- Ferrero-Pous M, Hacene K, Bouchet C, Le Doussal V, Tubiana-Hulin M, and Spyrtos F. 2000. Relationship between c-erbB2/HER2 and other tumor Characteristics in Breast Cancer Prognosis. *Clin Cancer Res.*, **6(12)**: 4745-4754.
- Kufareva I and Abagyan R. 2012. Methods of protein structure comparison. *Methods Mol Biol.*, **857**: 231-257.
- Lipinski CA, Lombardo F, Dominy BW, and Feeney PJ. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.*, **46(1-3)**: 3-26.
- Mortelmans K and Zeiger E. 2000. The Ames Salmonella/microsome mutagenicity assay. *Mutat Res-Fund Mol M.*, **455(1-2)**: 29-60.
- Nauli T. 2014. Penentuan sisi aktif Selulase *Aspegillus Niger* dengan Docking Ligand. *J Kim Terap Indones.*, **16(2)**: 94-100.
- Nikolic V, Stankovic M, Nikolic Lj and Cvetkovic D. 2004. Mechanism and kinetics of synthesis of allicin. *Pharmazie.*, **59(1)**: 10-14.
- Pires D, Blundell T, and Ascher D. 2015. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem.*, **58(9)**: 4066-4072.
- Purnomo H. 2013. **Kimia Komputasi Uji In Silico Senyawa Anti Kanker**. Pustaka Pelajar, Yogyakarta.
- Šrejšber M, Navrátilová V, Paloncýová M, Bazgier V, Berka K, Anzenbacher P, and Otyepka M. 2018. Membrane-attached mammalian cytochromes P450: an overview of the membrane's effects on structure, drug binding, and interactions with redox partners. *J Inorg Biochem.*, **183**:117-136.
- Tedjamariono TD, Sudarsa IW, Winata A and Wetan NGAAMY. 2020. Overekspresi Her-2 Sebagai Faktor Risiko Terjadinya Metastasis Otak Pada Pasien Kanker Payudara. *E-J Medika Udayana.*, **9(9)**: 58-64.
- Wang J, and Xu B. 2019. Targeted therapeutic options and future perspectives for HER2-positive breast cancer. *Sig Transduct Target Ther.*, **4**:34.
- Wimana Z, Gebhart G, Guiot T, Vanderlinden B, Larsimont D, and Doumont G, Van Simaey G, Goldman S, Flamen P and Ghanem G. 2017. N-Acetylcysteine breaks resistance to trastuzumab caused by MUC4 overexpression in human HER2 positive BC-bearing nude mice monitored by 89Zr-Trastuzumab and 18F-FDG PET imaging. *Oncotarget.*, **8(34)**: 56185-56198.
- Zhou S. 2008. Potential Strategies for Minimizing Mechanism-Based Inhibition of Cytochrome P450 3A4. *Curr Pharm Des.*, **14(10)**: 990-1000.