IN SILICO MOLECULAR DOCKING STUDIES OF OVARIAN EXTRACT SINGKARAK LAKE PUFFERFISH (TETRAODON LEIURUS) AGAINST BREAST CANCER

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ARTICLE INFO

INFORMATION

ABSTRACT

Targets the development of breast cancer chemoprevention by blocking the expression of Na\textsubscript{v} 1.5 channels and NHE, decreasing the expression of Bcl-2 and Bcl-X\textsubscript{L}, and increasing the expression of Bax. The ovarian extract Singkarak Lake Pufferfish (Tetraodon leiurus) has the potential as chemoprevention in breast cancer cell lines (MCF-7). This study aimed to analyze the interaction of STX, neoSTX, and dcSTX ligands against Na\textsubscript{v} 1.5, NHE, Bcl-2, Bcl-X\textsubscript{L}, and Bax receptors. This study used the molecular docking method using STX, neoSTX, and dcSTX ligands against Na\textsubscript{v} 1.5, NHE, Bcl-2, Bcl-X\textsubscript{L}, and Bax receptors. The result showed that the STX ligand has a more stable interaction with Na\textsubscript{v} 1.5, Bcl-2, Bcl-X\textsubscript{L}, and Bax receptors with ΔG values of -8.72, -7.32, and -8.86, and -6.31 kcal/mol compared to neoSTX and dcSTX ligands. Furthermore, the neoSTX ligand has a more stable interaction with the NHE receptor with an ΔG value of -7.5 kcal/mol compared to the STX and dcSTX ligands. This study shows that the ovarian extract of Singkarak Lake Pufferfish (Tetraodon leiurus) has the potential to be developed as cancer chemoprevention.

Keywords: Autodock, Breast cancer, In silico, Saxitoxin, Pufferfish

INTRODUCTION

Cancer cells have the characteristics of invasion and metastasis throughout the body and grow uncontrollably (Shan et al., 2019). Invasion and metastasis are caused by the expression of Na\textsubscript{v} 1.5 channels which increase the intracellular Na\textsuperscript{+} concentration, thereby activating NHE (sodium hydrogen exchanger) and NCX (sodium-calcium exchanger) in cancer cells (Angus and Ruben, 2019). Breast cancer cells specifically express Na\textsubscript{v} 1.5 channels (Gradek et al., 2019), so inhibition of Na\textsubscript{v} 1.5 channel expression is a target in the treatment of breast cancer (Luo et al., 2020).

In addition to the characteristics of invasion and metastasis, cancer cells also obtain immortality by escaping programmed cell death (apoptosis) (Chen et al., 2018) caused by overexpressing anti-apoptotic protein such as Bcl-2 (B-cell lymphoma 2) (Li et al., 2017) and Bcl-X\textsubscript{L} (B-cell lymphoma-extralarge) (Trisicuoglio et al., 2017), and also underexpression proapoptotic protein such as Bax in cancer cells (Bcl-2 associated X-protein) (Wang et al., 2019). Therefore, decreased expression of anti-apoptotic proteins and increased expression of proapoptotic proteins (Pistritto et al., 2016) are targeted in the development of cancer chemoprevention (Jagadeeshan et al., 2018).

Cancer chemoprevention is the process of using chemicals, either natural or synthetic, to prevent cancer, which is a complex interplay of a multitude of biological processes (Nahar and Sarkar, 2020). Cancer chemoprevention using natural compounds has minimal side effects and toxicity compared to synthetic (Ko and Moon, 2015). A natural compound such as toxin Pufferfish (Tetrodotoxin (TTX)/saxitoxin (STX)) can be used as cancer chemoprevention. Pufferfish in Lake Singkarak is a toxic fish, with the scientific name is Tetraodon leiurus. Kungsuwan et al. (1997) reported that the ovaries of Tetraodon leiurus from Thailand contained the toxins STX, neoSTX (neosaxitoxin), and dcSTX (decarbamoyl saxitoxin). Hanif et al. (2021), ovarian extract Singkarak Lake Pufferfish (Tetraodon leiurus) has potential as cancer chemoprevention in MCF-7 cells.

The development of cancer chemoprevention compounds can be carried out by several tests, namely in vitro, in vivo (Singh et al., 2014), and in silico (Chen et al., 2012). In vitro tests aim to evaluate various biological phenomena in certain cells in a controlled environment and free from systemic variations (Arango et al., 2013). In vivo tests are used to evaluate the biological response of living organisms to given chemoprevention (Haas et al., 2012). In silico test with molecular docking to predict the interaction between compounds (ligands) and protein receptors with computational procedures (Meng et al., 2011). Molecular docking has been shown to contribute to cancer progression (Edelman et al., 2009).

Research on the Na\textsubscript{v} 1.5, NHE, Bcl-2, Bcl-X\textsubscript{L}, and Bax proteins induced by the ovarian extract Singkarak Lake Pufferfish (Tetraodon leiurus) in breast cancer cells has not been carried out. In this study, in silico test with molecular docking using the STX, neoSTX, and dcSTX ligands against Na\textsubscript{v} 1.5, NHE, Bcl-2, Bcl-X\textsubscript{L}, and Bax receptors. Therefore, it is necessary to conduct this research to utilize toxins from the ovarian extract Singkarak Lake Pufferfish (Tetraodon leiurus) as cancer chemoprevention.

MATERIAL AND METHODS

Ligand preparation

The ligands used in this study were Pufferfish toxin compounds obtained from the PubChem database, namely STX (CID: 56947150), neoSTX (CID: 135562690), and dcSTX (CID: 21117969) (Figure 1). The SDF ligand file format was converted into a PDB file using Discovery Studio Visualizer 2021.

Receptor preparation

The receptors used in this study were obtained from the Protein Data Bank database, namely Na\textsubscript{v} 1.5 channel (PDB ID: 4DJC), NHE (PDB ID: 2E30), Bcl-2 (PDB ID: 4IEH), Bcl-X\textsubscript{L} (PDB ID: 4QVF), and Bax (PDB ID: 1F16). The macromolecular crystal structure obtained was prepared using Discovery Studio Visualizer 2021 and MGLTools 1.5.6 equipped with AutoDock 4.2.6 by removing water molecules, native ligands, and adding polar hydrogen atoms and Kollman partial charge.

![Figure 1 Ligands 2D Interaction. A. STX; B. neoSTX; C. dcSTX](image-url)
Molecular docking method validation

Docking studies using the docking tool, MGL Tools 1.5.6 equipped AutoDock 4.2.6 with the re-docking method and RMSD (Root Mean Square Deviation) value < 2Å. The distance between the surface of the receptor and the ligand is limited by a maximum radius of 0.375Å, a grid box size of 126Å x 126Å in the x, y, and z dimensions, and the Lamarckian Genetic Algorithm method with 100 conformations.

Molecular docking results visualization

The molecular docking results were visualized using PyMOL for molecular surface and BIOVIA Discovery Studio Visualizer for docked complex, 3D interaction, and 2D interaction.

RESULTS AND DISCUSSION

In silico test with molecular docking, results are displayed based on the interaction of STX, neoSTX, and dcSTX ligands against Na1.5, NHE, Bcl-2, Bcl-XL, and Bax receptors.

Na1.5 receptor

The results of the molecular docking of the test ligands to the Na1.5 receptor with several parameters (Table 1) and the interaction between the test ligand to the Na1.5 receptor (Fig. 3).

Table 1 Molecular docking of STX, neoSTX and dcSTX ligands with Na1.5 receptor

| Ligands   | ΔG (kcal/mol) | KI (µM) | Interacting Residues                  |
|-----------|---------------|---------|---------------------------------------|
| STX       | -8.72         | 408.57  | Glu121; 124; 128                       |
| neoSTX    | -8.42         | 673.3   | Glu124; 128, Met145                   |
| dcSTX     | -7.71         | 2.22    | Glu121; 124; 128                       |

Figure 2 Crystal structure of the receptor macromolecule. A. Na1.5; B. NHE; C. Bcl-2; D. Bcl-XL; E. Bax

Figure 3 STX ligand interactions with Na1,5 receptor. A. Docked complex; B. Molecular Surface; C. 3D Interaction; D. 2D Interaction

Values of ΔG, KI, and interacting residues of STX, neoSTX, and dcSTX ligands with Na1,5 receptor (Table 1). The lowest ΔG value for STX ligand was -8.72 kcal/mol. The smaller value of ΔG, the more stable the interaction of the ligand with the receptor (Pantsar and Poso, 2018). Based on the ΔG value, the interaction of the STX ligand with the Na1,5 receptor was more stable than the interaction of the neoSTX and dcSTX ligands. The lowest KI value for the dcSTX ligand was 2.22 µM. The smaller value of KI indicates the smaller concentration of the ligand required to inhibit the target receptor (Kim et al., 2021). Based on the KI value, the interaction of the dcSTX ligand was more effective in inhibiting the Na1,5 receptor in a small concentration than the STX and neoSTX ligands.

The amino acid residues involved in these interactions have hydrogen bonds, hydrophobic interactions, and electrostatic interactions. The STX ligand has seven conventional hydrogen bonds (Glu121; 124; 128) (Fig. 3). The neoSTX ligand has three conventional hydrogen bonds (Glu124; 128), one alkyl hydrophobic interaction (Met145), and one attractive charge electrostatic interaction (Glu128). The dcSTX ligand has five hydrogen bonds consisting of four conventional hydrogen bonds (Glu121; 124; 128) and one carbon-hydrogen bond (Glu124). Based on the interaction of the ligand with the receptor, the STX ligand has a more stable binding to the Na1,5 receptor than the neoSTX and dcSTX ligands. Hydrogen bonding is the main bond that provides stability to the protein structure, the more hydrogen bonds, the more stable the ligand bond with the receptor (Hubbard and Kamran, 2010). Based on the value of ΔG, KI and molecular interactions on STX, neoSTX, and dcSTX ligands have the potential as inhibitors of Na1,5 activity to suppress invasion in cancer cells.

NHE receptor

The results of the molecular docking of the test ligands to the NHE receptor with several parameters (Table 2) and the interaction between the test ligand to the NHE receptor (Fig. 4).

Table 2 Molecular docking of STX, neoSTX and dcSTX ligands with NHE receptor

| Ligands   | ΔG (kcal/mol) | KI (µM) | Interacting Residues                  |
|-----------|---------------|---------|---------------------------------------|
| STX       | -7.16         | 5.6     | Arg132, Asp127; 168, Glu128, Ser131   |
| neoSTX    | -7.5          | 3.19    | Asp39; 50; 76, Glu77, Glu49, Lys40, Thr45 |
| dcSTX     | -6.87         | 9.21    | Asp39; 49; 50; 76, Lys40, Thr45        |

Figure 4 neoSTX ligand interactions with NHE receptor. A. Docked complex; B. Molecular Surface; C. 3D Interaction; D. 2D Interaction

Molecular interactions at NHE receptor, the lowest ΔG and KI values for the neoSTX ligand were -7.5 kcal/mol and 3.19 µM (Table 2). Based on ΔG and KI values, the neoSTX ligand interaction was more stable and more effective in inhibiting NHE activity compared to STX and dcSTX ligands. The amino acid residues involved in these interactions have hydrogen bonds and hydrophobic interactions. The STX ligand has ten hydrogen bond interactions consisting of eight conventional hydrogen bonds (Arg132, Asp127, 168, Glu128, and Ser131) and two carbon-hydrogen bonds (Asp168 and Ser131). The neoSTX ligand has twelve hydrogen bond interactions consisting of nine conventional bonds (Asp39; 50; 76, Glu77, Glu49, Thr45) and two carbon-hydrogen bonds (Asp39; 50), one alkyl hydrophobic interaction (Lys40) and there is an unfavorable donor-donor bond (Asp76 and Thr45) (Fig. 4). Unfavorable donor-donor bonds affect the ligand-receptor complex and reduce the stability of the complex because this type of bond exhibits repulsive forces that occur between two molecules and atoms (Dhorajiwala et al., 2019). The dcSTX ligand has seven hydrogen bonds is six conventional hydrogen bonds (Asp39; 49; 50; 76 and Thr45) and one carbon-hydrogen bond (Asp50), and one alkyl hydrophobic interaction (Lys40). Based on the interaction of the ligand with the receptor, the neoSTX ligand has a more stable...
binding to the NHE receptor than the STX and dcSTX ligands. Based on the value of ΔG, KI, and molecular interactions on STX ligands, neoSTX, and dcSTX have the potential as inhibitors of NHE activity.

Bcl-2 receptor

The results of the molecular docking of the test ligands to the Bcl-2 receptor with several parameters (Table 3) and the interaction between the test ligand to the Bcl-2 receptor (Fig. 5).

Table 3 Molecular docking of STX, neoSTX and dcSTX ligands with Bcl-2 receptor

| Ligands | ΔG (kcal/mol) | KI (µM) | Interacting Residues |
|---------|---------------|---------|----------------------|
| STX     | -7.32         | 4.32    | Ala59, Asp62, Gin58, Gly162, Leu160, Tyr161 |
| neoSTX  | -6.76         | 11.12   | Asp62, Gin58, Gly162, Thr55, Tyr161 |
| dcSTX   | -6.22         | 27.8    | Asp62, Gin58, Gly162, Thr55, Tyr161 |

At the Bcl-2 receptor, the lowest ΔG and KI values for the STX ligand were -7.32 kcal/mol and 4.32 µM (Table 3). Based on the values of ΔG and KI, STX ligand interaction was more stable and effective in inhibiting Bcl-2 activity than neoSTX and dcSTX ligands. The amino acid residues involved in these interactions have hydrogen bonding, hydrophobic interactions, and electrostatic interactions. The STX ligand has nine conventional hydrogen bonds (Asp62, Gin58, Gly162, Leu160, and Tyr161) and one alkyl hydrophobic interaction (Ala59) (Fig. 5). The neoSTX and dcSTX ligands have seven conventional hydrogen bonds (Asp62, Gin58, Gly162, and Thr55) and one alkyl hydrophobic interaction (Tyr161). Based on the interaction of the ligand with the receptor, the STX ligand has a more stable binding to the Bcl-2 receptor than the neoSTX and dcSTX ligands. Based on the value of ΔG, KI, and molecular interactions on STX ligands, neoSTX, and dcSTX have potential as inhibitors of Bcl-2 activity.

Bcl-XL receptor

The results of the molecular docking of the test ligands to the Bcl-XL receptor with several parameters (Table 4) and the interaction between the test ligand to the Bcl-XL receptor (Fig. 6).

Table 4 Molecular docking of STX, neoSTX and dcSTX ligands with Bcl-XL receptor

| Ligands | ΔG (kcal/mol) | KI (µM) | Interacting Residues |
|---------|---------------|---------|----------------------|
| STX     | -6.86         | 9.33    | Asn128, Asp176, Glu124, Trp169, Tyr120 |
| neoSTX  | -6.16         | 30.33   | Glu124, His177, Trp169 |
| dcSTX   | -6.23         | 27.09   | Asp176, Glu124, Trp169, Tyr120 |

Interactions with Bcl-XL receptor, the lowest ΔG and KI values for STX ligands were -6.86 kcal/mol and 9.33 µM. Based on the values of ΔG and KI, the interaction of the STX ligand was more stable and more effective in inhibiting Bcl-XL activity than neoSTX and dcSTX ligands. The amino acid residues involved in these interactions have hydrogen bonding, hydrophobic interactions, and electrostatic interactions. The STX ligand has four conventional hydrogen bonds (Asp176, Glu124, Trp169, and Tyr120) (Fig. 6). The neoSTX ligand has five conventional hydrogen bonds (Asp176, Glu124, Trp169, and Tyr120). Based on the interaction of the ligand with the receptor, the STX ligand has a more stable binding to the Bcl-XL receptor than the neoSTX and dcSTX ligands. Based on the value of ΔG, KI, and molecular interactions on STX ligands, neoSTX, and dcSTX have potential as inhibitors of Bcl-XL activity.

Bax receptor

The results of the molecular docking of the test ligands to the Bax receptor with several parameters (Table 5) and the interaction between the test ligand to the Bax receptor (Fig. 7).

Table 5 Molecular docking of STX, neoSTX and dcSTX ligands with Bax receptor

| Ligands | ΔG (kcal/mol) | KI (µM) | Interacting Residues |
|---------|---------------|---------|----------------------|
| STX     | -6.31         | 23.64   | Ala46, Asp48, Gly39, Leu45 |
| neoSTX  | -5.6          | 78.98   | Ala183, Asp98, Ser184, Val180 |
| dcSTX   | -5.71         | 65.66   | Ala183, Asp98, 102, 101, Val180 |

Figure 5 STX ligand interactions with Bcl-2 receptor. A. Docked complex; B. Molecular Surface; C. 3D Interaction; D. 2D Interaction

Figure 6 STX ligand interactions with Bcl-XL receptor. A. Docked complex; B. Molecular Surface; C. 3D Interaction; D. 2D Interaction

Figure 7 STX ligand interactions with Bax receptor. A. Docked complex; B. Molecular Surface; C. 3D Interaction; D. 2D Interaction
Molecular interactions at Bax receptor, the lowest AG and KI values for the STX ligand were -6.31 kcal/mol and 23.64 µM (Table 5). Based on the values of AG and KI, the STX ligand interaction was more stable and effective in activating Bax than neoSTX and dcSTX ligands. The amino acid residues involved in these interactions have hydrogen bonding, hydrophobic interactions, and electrostatic interactions. The STX ligand has eight conventional hydrogen bonds (Ala46, Asp48, Gly39, and Leu45) (Fig. 7). The neoSTX ligand has six hydrogen bonds: five conventional hydrogen bonds (Asp102, Ser184, and Val180) and one carbon-hydrogen bond (Ser184), one alky1 hydrophobic interaction (Ala183), and a salt bridge bond (Asp102) (Table 6). The salt bridge bond is a combination of two non-covalent interactions, namely hydrogen bonds and ionic bonds, these bonds contribute to protein stability (Kumar and Nussinov, 2002). The dcSTX ligand has six conventional hydrogen bonds (Asp98,102, Val180) and one alky1 hydrophobic interaction (Ala183). Based on the interaction of the ligand with the receptor, the STX ligand has a more stable binding to the Bax receptor than the neoSTX and dcSTX ligands. Based on the value of AG, KI and molecular interactions on STX ligands, neoSTX and dcSTX have potential as compounds that can activate Bax activity. Cytotoxic compounds can activate Bax which triggers the mechanism of apoptosis and is developed in the treatment of cancer (Jensen et al., 2019).

**Comparison of AG and KI values between ligands and receptors**

| Receptors | STX | neoSTX | dcSTX |
|-----------|-----|-------|-------|
| NaV1.5    | -8.72 | -8.42 | -7.67 |
| NHE       | -7.16 | -7.5 | -5.67 |
| Bc1-2     | -7.32 | -6.56 | -6.12 |
| Bc1-X     | -6.86 | -6.16 | -6.23 |
| Bax       | -6.31 | -5.6 | -5.71 |

Comparison of AG and KI values between ligands and receptors

Molecular docking results based on AG and KI values are shown in Table 6. Comparing the AG and KI values between STX, neoSTX, and dcSTX ligands with the receptors (Table 6), it was found that the STX ligand with the NaV1.5 receptor had the lowest AG value of -8.72 kcal/mol. Based on the AG value, the interaction of STX ligand with the NaV1.5 receptor has a more stable interaction than the interaction of the neoSTX and dcSTX ligands with the NaV1.5 receptor and also the interaction between the STX, neoSTX, and dcSTX ligands with the NHE, Bc1-2, Bc1-XL, and Bax receptors. On the KI value, the interaction of dcSTX ligand with the NaV1.5 receptor had the lowest KI value of 2.22 µM. Based on the KI value, the interaction of the dcSTX ligand was more effective in inhibiting the NaV1.5 receptor in a small concentration than the STX and neoSTX ligands and also the interaction between the STX, neoSTX, and dcSTX ligands with the NHE, Bc1-2, Bc1-XL, and Bax receptors. The mechanism of action of STX and STX derivative compounds that inhibit and block the action of NaV channels (Walker et al., 2012).

The main component of Pufferfish toxin (TTX/STX) is the guanineine structure that interacts with the carboxylate group on the NaV channel so that it can block the NaV channel (Mahdavi and Kucuyak, 2015). The STX binding site is located in the α subunit, site 1 is formed by four P-loop (Ruiz and Kraus, 2015), the subunit has four homologous domains (DI-DIV) arranged to form a symmetrical channel (Sheets et al., 2015). STX binds to selectivity filter residues in the DEKA ring (Asp-Glu-Lys-Ala) which plays an important role in the selectivity of NaV channels (Yen et al., 2019).

**CONCLUSION**

Molecular docking using STX, neoSTX, and dcSTX ligands against NaV1.5, NHE, Bc1-2, Bc1-XL, and Bax receptors has low AG and KI values so that the ligand and receptor interactions have a stable and effective interaction in inhibiting and activating the protein receptor. Based on the results of this molecular docking, the ovarian extract of Singkarak Lake Pufferfish (Tetraodon leiuus) has the potential as alternative cancer chemoprevention.

**Acknowledgments:** We want to thank the Directorate General of Learning and Student Affairs who has provided a student research grant. Our thanks also expressed to the Biology Department, Genetic and Biomolecular Laboratory, Faculty of Mathematics and Sciences, Andalus University, Padang, Indonesia.

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