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Molecular docking studies of Brucein D as a potential inhibitor of the Bcl-2 anti-apoptotic protein

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ABSTRACT

Background: Brucein D (BrD), a quassinoid isolated from *Brucea javanica* fruit, reportedly demonstrates anti-tumor activity. Bcl-2 is an anti-apoptotic protein that inhibits apoptosis and leads to cancer progression. This study aims to evaluate the inhibitory effect of BrD against the anti-apoptotic protein Bcl-2 by molecular docking study.

Methods: We investigated the activity of BrD against Bcl-2 through molecular docking *in silico* compared to the chemotherapeutic drugs doxorubicin and docetaxel. Molecular docking analysis was conducted using the Lipinski rule of five drug-likeness analyses, PASS Online prediction, PyRx v0.8, Discovery Studio DS BIOVIA 2016 v16, and PyMol v2.4.

Results: Current results show that BrD is qualified as a drug and can pass cell membranes without toxicity. The PASS prediction also shows that BrD can be activated as an anti-neoplastic inside the human body with Pa higher than Pi. BrD can make a constant and stable bond to Bcl-2 with a binding energy of -8.3 kcal/mol, almost equal to doxorubicin and docetaxel. BrD can form six types of hydrogen bonds that show stability to temperature and pressure.

Conclusion: BrD has shown to be a promising alternative for an anti-cancer drug by inhibiting the Bcl-2 anti-apoptotic protein with low toxicity in normal cells.

Keywords: Anti-Cancer, Bcl-2, Brucein D, Molecular Docking.
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INTRODUCTION

Cancer is a non-communicable disease with an increasing yearly death rate in almost all countries. Globocan 2020 noted around 19 million cancer cases worldwide, with a death rate of nearly 10 million people. In Indonesia, the data shows almost 400 thousand new cases with almost 250 thousand deaths, mainly breast and cervical cancer in females and lung and colorectal cancer in males.[1] Chemotherapy is still one of the main options for cancer treatment besides surgery, radiation, hormonal therapy, immunotherapy, and targeted therapy.[2] The success rate of chemotherapy in cancer treatment is not always satisfying; it has abundant side effects and affects normal cells. That is why recent studies focused on the natural compound that has a cytotoxic effect on cancer cells without affecting normal cells.[3]

Kerr, in 1972, concluded that apoptosis is closely related to cancer progression through the failure of the elimination of potentially malignant cells.[4] There are two main apoptosis pathways: the intrinsic or mitochondrial and extrinsic pathways. Bcl-2 family protein strictly regulates the intrinsic pathway of apoptosis. It consists of two types of proteins: pro-apoptosis and anti-apoptosis. The balance of pro- and anti-apoptotic proteins is essential in determining normal live and dead cells and preventing pathological cell development.[5] Bcl-2 is an anti-apoptosis protein and has recently been investigated for the failure of apoptosis in many cancer diseases and the progression of cancer cells.[6]

Brucea javanica, from the *Simaroubaceae* family, is an old traditional Chinese medicinal herb used for hundreds of years to treat many diseases. It spreads from Africa to Asia. In Indonesia, it is known as "Buah Makassar" or "Buah Wali". It is mainly found as shrubs or bushes, and the rest grows in herbal plant industries.[7] Since it has a bitter and cold taste, some say this fruit is poisonous. However, studies found this herb has many special functions, such as anti-inflammation, anti-diabetes, anti-malaria, and anti-cancer.[8-11] Brucein D (BrD), one of the most abundant active quassinoid compounds in Brucea javanica, shows cytotoxic activity in cancer cells. It has antiproliferative and apoptogenic effects, thus inhibiting cancer cell

growth, primarily in pancreatic, lung, and mammary cancer.[12-14]

This study aims to assess the interaction between BrD and the Bcl-2 protein receptor by molecular docking (in silico). The primary basis of this research is to show the drug-likeness of BrD, the biological activity and pharmacological effects of BrD, and the binding interaction of BrD to protein receptor Bcl-2 as an anti-apoptosis protein. Eventually, this study will determine BrD's ability to inhibit Bcl-2 protein activation and ensure a constant and proper apoptosis mechanism.

METHODS

This in silico study was performed using Intel® Core™ i5-DDR4-3200 MHz RAM with PyMOL v.2.4.1 (Schrodinger Inc, USA) for protein preparation and visualization of molecular docking results, PyRx v.0.8 (Scripps Research, USA) for molecular docking study, and DS BIOVIA Discovery Studio 2016 v16.10.0 x 64 (Dassault Systemes, France) for visualization the interaction between ligand and target protein.

Analysis of drug-likeness for BrD was done by Lipinski Rule of Five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp), and the prediction of biological activity of target ligand was made by PASS Online Prediction (http://www.way2drug.com/passonline/predict.php). BrD as a ligand was collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and receptor protein Bcl-2 was collected from Protein Data Bank (https://www.rcsb.org/) (PDB ID: 6QGG) then prepared by PyMOL v.2.4.1. There are no inclusions or exclusions criteria in this study since we are not performed any ligand or protein selection, this study simulates one specific ligand to a particular protein, which has previously downloaded from the PubChem and PDB. Therefore, there is only BrD as a ligand and Bcl-2 as the target protein included in this study.

The BrD data that had been downloaded previously were analyzed for its molecular weight, the number of hydrogen acceptors and donors, lipophilicity, or the coefficient of solubility in water, which is marked with log P, and Molar Refractivity, which is a measure of the overall polarization of the molecule. If it meets the drug-like requirements, then the data for the BrD compound is stored in .pdb format as a ligand and continues the preparation for molecular binding[15]. Open the protein Bcl-2 data downloaded previously in pdb format with PyMol ver. 2.4.1 analyze the protein structure in the form of lines and water molecules in dotted red lines. Usually, the protein collected from PDB is still contaminated with unwanted molecules such as water, native ligands, or foreign proteins. However, in this study, according to references from the PDB, the structure only consists of one chain (chain A), so only water contaminants and native ligands are sterilized.[15]

After qualifying for the drug-likeness criteria, the BrD ligand was then analyzed for its biological activity, pharmacological effects, and mechanism of action by PASS (Prediction of Activity Spectra for Substances) Online prediction; the software virtually determined the Pa (Probability of activation) and Pi (Probability of inactivation) of the compound in accordance to the database before any biological testing.[16]

The next step is to determine the grid box and docking ligand BrD to the protein Bcl-2 receptor by PyRx ver 0.8 to measure the energy required to form a strong and stable bond; the result will show whether BrD can inhibit the activation of Bcl-2 from ensuring the continuity of the apoptosis process. As a comparison, in this study, we use two chemotherapy drugs, doxorubicin, and docetaxel, as a positive control.[17]

Construction and visualization of molecular docking complexes were carried out with the PyMol ver 2.4.1 software and followed by visualization of the bond between the ligand and the target protein with the DS BIOVIA Discovery Studio software to predict the number of bonds, types of bonds, the strength of each bond, and the amino acid residue generated by protein receptor.[18]

RESULTS

Analysis of drug-likeness

Analysis of drug-likeness was done using the Lipinski Rule of Five. The rule defines the molecular properties required for a chemical compound with a specific pharmacological and biological activity to act as a drug in humans with no harmful effects (Figure 1). In order to meet the drug criteria, the compound must meet at least two of Lipinski's rules.[19] BrD can be categorized as a drug because its molecular weight is less than 500.000 D so that it can penetrate the cell membrane, the number of hydrogen acceptors is less than 5, and donors are less than 10, so it can build a stable bond with Bcl-2, and Lipophilicity or the coefficient of solubility in fat (logP) is in between -0.4 – 5, the more negative the number means that the compound is non-permeable (Table 1). The higher number means that the compound is more hydrophobic, connected longer to the lipid bilayer membrane, and widely distributed to other tissue. Hence, it tends to have higher toxicity and is less effective in targeting protein.[16] With that result, BrD is qualified as a drug and can pass cell membranes without toxicity.

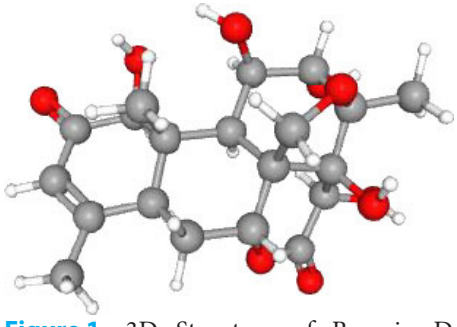

Figure 1. 3D Structure of Brucein D (CID: 441788).

Table 1. Result of Lipinski Rule of Five

Compound	Lipinski Rule of Five				
	MW (Dalton)	HBD	HBA	LogP	MR (g/mol)
Brucein D (PubChem CID: 441788)	410.400	5	9	2.1097	106.5129
	YES	YES	YES	YES	YES

Note: MW, Molecular Weight; HBD, Hydrogen Bond Donors; HBA, Hydrogen Bond Acceptors; LogP, High Lipophilicity; MR, Molar Refractivity

Table 2. Result of Lipinski Rule of Five

Compound	Activity	Pa	Pi
Brucein D (PubChem CID: 441788)	Antineoplastic	0,927	0,005

Table 3. Result of Molecular Docking Study

Compound	PubChem CID	Binding Energy (kcal/mol)
Brucein D	441788	-8.3

Table 4. Result of Molecular Docking Study for Positive Control

Compound	PubChem CID	Binding Energy (kcal/mol)
Doxorubicin	443939	-9.0
Docetaxel	148124	-9.0

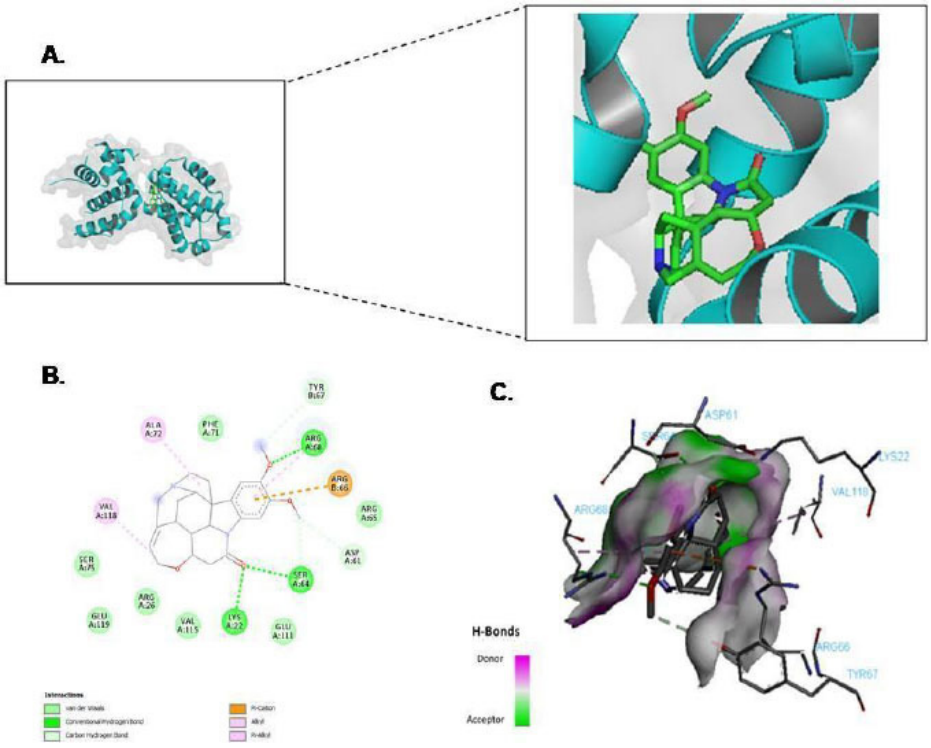

Figure 2. (A) Molecular Visualization of the binding complex of BrD and Bcl-2 protein receptor. (B) Chemical interaction of the bond formed by BrD to amino acid residue produced by Bcl-2 receptor protein. (C) 3D models of chemical interaction between BrD and Bcl-2 protein receptor binding complex.

Analysis of the ligand biological activity, pharmacological effects, and mechanism of action

PASS Online prediction measured the Pa (probability of activation) and Pi (probability of inactivation). The Pa value measurement has to be higher than Pi to say that the compound can penetrate a semipermeable membrane as an active form[14]. The result shows that BrD has a Pa value of 0.927 and a Pi value of 0.005 (Table 2). Based on the PASS Online Prediction database (http://www.way2drug.com/passonline/predict.php), BrD has shown biological activity as an anti-neoplastic and can be activated inside the human body.

Prediction of the Binding Energy value of ligand and Bcl-2 receptor

The Molecular docking study was performed using PyRx ver 0.8 with grid docking center X: 35.6902 Y: 33.6798 Z:2.6833, and dimension (Å) X: 61.7967 Y: 42.7430 Z: 56.1228. This study was performed by blind docking because the functional protein domain is unknown.[20] The binding energy result for BrD to Protein Bcl-2 was -8.3 kcal/mol, which means that BrD can perform as a Bcl-2 inhibitor (Table 3). The lower the binding energy between ligand and protein receptor, the more constant and stable the bond complex formation against temperature and pressure.[17] For comparison, the binding energy result for doxorubicin and docetaxel are -9.0 kcal/mol, so the BrD binding energy to Bcl-2 protein is almost the same as chemotherapy drugs (Table 4).

Molecular docking complex visualization

The molecular docking complex visualization was constructed using PyMol v.2.4. Then, it analyzed the ligand and protein interaction and the type of chemical bond and interaction position prediction when BrD binds to the Bcl-2 protein receptor using DS BIOVIA (Figure 2).

Analysis of molecular docking interaction between BrD and Bcl-2 protein receptor shows that six bond types are formed; Van der Waals is the strongest, followed by Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Cation, Alkyl, and Pi-Alkyl Hydrogen Bond. The more Hydrogen bonds formed, the more robust and stable the bond complex between ligand and complex (Table 5).[18]

DISCUSSION

BrD, a bioactive quassinoid compound isolated from Brucea javanica fruit, has been previously known to have an anti-cancer effect, especially in pancreatic, breast, and lung cancer.[12-14] The primary goal of anti-cancer treatments is to eliminate the cancer cells without affecting the normal cells, which can be very difficult to obtain by chemotherapy or radiotherapy, Chemotherapy drugs such as doxorubicin have several adverse effects like bone marrow suppression, nausea or vomiting, hair loss, and cardiotoxicity.[19-21] Docetaxcel is also used as intravenous second-line chemotherapy for several cancers (non-platinum-based chemotherapy), has an acceptable efficacy, and is well-tolerated. However, it still has moderate adverse effects like gastrointestinal disturbance, dermatologic and neuropathy, and myelosuppression.[22]

Bcl-2 family protein strictly regulated

Table 5. The Summary of Amino Acid Residues from Brucein D[18]

Compound	Bond Types	Amino Acid Residues
Brucein D (PubChem CID: 441788)	van der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Cation, Alkyl, and Pi-Alkyl	SER A:75, GLU A: 119, ARG A:26, VAL A:115, LYS A:22, GLU A: 111, SER A:64, ASP A: 61, ARG A:65, ARG B:66, ARG A:68, TYR B: 67, PHE A:71, ALA A:72, VAL A: 118

the intrinsic pathway of apoptosis. Bcl-2 shows an anti-apoptotic effect. In contrast to other family members, Bax and Bak have a pro-apoptotic effect. The balance of pro and anti-apoptotic proteins is essential in determining the normal live and dead cells and preventing pathological cell development.[5] Studies show that overexpression of the Bcl-2 protein is noticed in many cancer cells; it is the leading cause of cancer cells avoiding apoptosis, initiating cancer progression, and gaining resistance to chemotherapy drugs.[6,23] Many in silico studies found that some natural products like Curcuma longa, Coumarin, or Brucea javanica have an inhibitory effect on Bcl-2, thus preventing the activation of anti-apoptosis protein and keeping the apoptosis process working properly.[24-27]

Our in-silico study of BrD shows that the compound has met the drug-likeness criteria by Lipinski rule of five; it can pass the semipermeable cell membrane with no toxic effect and have an active anti-neoplastic effect with Pa higher than Pi. The drug-likeness study result is similar to others that said that BrD ligand is a drug candidate.[27] BrD also builds a strong and stable binding complex with the Bcl-2 protein receptor with binding energy -8.3 kcal/mol that causes an inhibitory effect on the activation of Bcl-2 as an anti-apoptosis protein. The energy binding result is comparable to doxorubicin and docetaxel as chemotherapy drugs with a binding energy of -9.0 kcal/mol. There is not much literature found to be compared to our findings; one study that assesses the interaction of BrD to Hsp70, Hsp90, NFκB, Bcl-W, and Bcl-XL shows that BrD has an average binding energy of -5 kcal/mol to those protein receptors and comparable to gemcitabine as a chemotherapy drug.[27] Hence, the result is not much different from ours. Another study found that the main compound from Brucea javanica fruit extract has a much lower binding energy compared to its native ligand, which means the main component of Brucea javanica has a potential anti-cancer effect; that is why our study uses BrD as the ligand, to assess the most abundant and active quassinoid component in Brucea javanica to have a more precise result.[24-28]

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The molecular docking interaction between BrD and Bcl-2 protein receptor shows that six bond types are formed. The more Hydrogen bonds formed, the more robust and stable the bond complex between ligand and complex. Another study showed that the number of hydrogen bonds formed by BrD binding to Hsp70, Hsp90, NFκB, Bcl-W, and Bcl-XL is between 2-5, with NFκB having the most bond formed. It is slightly lower than our study with Bcl-2, but their study also said that BrD is a high potential inhibitor of apoptotic resistance protein.[27-31]

Although the result was promising, this study still has limitations. This study just simulates how a drug candidate interacts with an apoptosis-related protein. It shows how BrD inhibits Bcl-2 from working as an anti-apoptosis protein. One of the main limitations is this study does not precisely describe the interaction of the ligand or drug molecules to the protein receptor inside human bodies. In this molecular docking study, the interaction is conducted in static conditions, but the interaction moves in dynamic conditions inside the human body. Another limitation is that prior to conducting this molecular docking study, the water molecules as a contaminant were sterilized from the protein receptor; in contrast to the drug's metabolism inside the human body, the water molecules are vastly involved in every process since the drugs enter the body. Both limitations can be overcome by performing a dynamic molecular docking study. The dynamic study was made to resemble conditions inside the human body more closely, performing dynamic interactions between ligand and protein receptors and involving water molecules. The result will also show how long bond formation can last in dynamic conditions.

Nevertheless, apoptosis is a complex process involving many proteins, pro- and anti-apoptosis, mediators, signals, genes, and pathways. We encourage further in silico, in vitro, and especially in vivo studies for this bioactive compound in various apoptosis signals, mediators, markers, pathways, and proteins. There are still many natural compounds that can be studied for cancer treatment; there are still many apoptotic pathways to be studied further.

CONCLUSION

Overall, our study provided a novel outlook on the potential medicinal use of BrD as an alternative therapy for cancers. The obtained result of this molecular docking study shows that BrD can perform as a promising drug candidate, has an anti-neoplastic effect, and can inhibit the activation of anti-apoptotic protein Bcl-2 with low toxicity and well tolerated. These results indicated that BrD is a promising plant-based bioactive compound in the fight against cancer.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL CONSIDERATION

This study was approved by the Institutional Review Board of the Faculty of Medicine at Universitas Hasanuddin (approval number: 371/UN.4.6.4.5.31/PP36/2021).

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AUTHOR CONTRIBUTIONS

Pandu Ishaq Nandana, Haerani Rasyid, Prihantono, Ika Yustisia, and Lukman Hakim were responsible for literature search, data analysis, statistical analysis, manuscript preparation, editing, and review. Pandu Ishaq Nandana, Haerani Rasyid, Prihantono, Ika Yustisia, and Lukman Hakim were accountable for concepts, design, the definition of intellectual content, clinical studies, data acquisition, and manuscript preparation. All authors read and approved the final manuscript.

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