In silico analysis of molecular interactions between the anti-apoptotic protein survivin and dentatin, nordentatin, and quercetin

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Abstract. Survivin is a member of the inhibitor of apoptosis (IAP) family and is reportedly overexpressed in various types of human malignancies. Because the phytochemical compounds dentatin, nordentatin, and quercetin have demonstrated antiproliferative effects in various cancer cell lines, we compared their binding affinities for survivin in silico. Molecular docking analyses were performed using PyMol, Discovery Studio Biovia 2017, AutoDock Vina, and AutoDock Tools version 1.5.4. These computations indicated greater survivin binding affinity of quercetin (∆G = −7.0 kcal/mol) than nordentatin and dentatin (∆G = −6.5 and −5.5 kcal/mol, respectively), but suggest that all three compounds act as ligand inhibitors of survivin. The present data warrant validation using in vitro and in vivo assays.

1. Introduction
Apoptosis or programmed cell death plays important roles in the regulation of cell growth and tissue development [1]. Accordingly, defects in apoptotic mechanisms promote carcinogenesis and confer resistance of cancers to chemotherapeutic agents, which are designed to induce apoptotic cell death in cancer cells [2]. Hence, regulators of apoptosis offer a diverse range of targets for anticancer therapeutic approaches [3].

Survivin is a member of the inhibitor of apoptosis (IAP) protein family that regulates cell division and apoptosis [4]. Vertebrates and invertebrate homologues of survivin are highly conserved [5], and wild-type human survivin is a 142-amino acid protein encoded by the baculoviral IAP repeat-containing-5 (BIRC5) gene [4]. Many studies show high expression of survivin in various human cancers and have associated the protein with poor overall prognosis and chemoresistance [6]. Phytochemical compounds such as coumarins and flavonoids have a wide range of pharmacological effects, including anticancer activities [7]. Among coumarins, dentatin (C₂₀H₂₂O₄) and nordentatin (C₁₉H₂₆O₄) from Clausena excavata have demonstrated strong cytotoxicity against several cancer cell
lines [8–11]. Moreover, the flavonoid quercetin (3,3',4,5,7-pentahydroxy-flavone) is commonly found in fruits and vegetables and affects various biological functions, in part culminating in antineoplastic properties [12].

In silico computational protein–ligand docking has become an important tool for drug discovery and development and is generally initiated with structure identification of known target protein molecules of medical interest. Molecular docking is then used to predict protein interactions with candidate small molecules (ligands) according to conformations and binding free energies, which are expressed as ligand–protein binding forces in kilocalories per mole (kcal/mol) [13,14]. As a direct and rational approach for drug discovery, computational docking analyses establish virtual models of ligand–protein interactions at the atomic level and can be used to inform subsequent validation using traditional in vitro and in vivo assays, thus minimizing the time and cost of the drug discovery process [15]. Herein, we report in silico binding affinities of dentatin, nordentatin, and quercetin for survivin using a set of open-source molecular docking programs.

2. Materials and Methods
2.1 Software and program
PyMol (DeLano Scientific LLC, Palo Alto, California, USA) and Discovery Studio Biovia 2017 (Dassault Systèmes, San Diego, California, USA) were employed to visualize and modify receptor and ligand structures. AutoDock Vina (The Scripps Research Institute, La Jolla, San Diego, USA) was the primary docking program used in this study. The Survivin.PDBQT file format was prepared, and the grid box size was determined using AutoDock Tools version 1.5.4 (ADT; Scripps Research Institute, La Jolla, San Diego, USA).

2.2 Preparation of ligand structures for dentatin, nordentatin, and quercetin
Structures of dentatin, nordentatin, and quercetin were downloaded in the Spatial Data File (.SDF) file format from the PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/). Physicochemical properties of the ligands (Table 1) met the criteria of Lipinski's rule of five, otherwise known as Lipinski's rule of drug-likeness. These rules are a guideline for assessing the structural similarities of compounds with those of active oral drugs and are based on physicochemical profiles. Molecular weights and hydrogen-bonding interactions between donors and acceptors are crucial structural determinants of protein targets and ligand-binding sites [16,18]. In particular, compounds are more likely permeable and active as ligands when they have >5 hydrogen-bond donors, >10 hydrogen-bond acceptors, a molecular mass of >500, and calculated log P (CLog P) values of >5 [17,18].

Table 1. Physicochemical properties of the present ligands.

| Physicochemical properties | Dentatin | Nordentatin | Quercetin |
|---------------------------|----------|-------------|-----------|
| PubChem CID               | 342801   | 5320206     | 5280343   |
| Molecular Formula         | C_{20}H_{22}O_{4} | C_{19}H_{20}O_{4} | C_{15}H_{10}O_{7} |
| Molecular Weight (g mol^{-1}) | 326.392 | 312.365     | 302.238   |
| Hydrogen-binding donors   | 0        | 1           | 5         |
| Hydrogen-binding acceptors| 4        | 4           | 7         |
| Rotatable bond count      | 3        | 2           | 1         |
| XLogP3                    | 4.7      | 4.4         | 1.5       |
Chemical structures in the .SDF format were converted to the .PDB format using Discovery Studio Biovia 2017. ADT was then used to investigate ligand structures in terms of combinations with non-polar hydrogens, additions of Gasteiger charges, and rotatable bonds. Structures in the ligand .PDB format were then converted to the ligand .PDBQT format using ADT, enabling use with AutoDock4 (AD4) and AutoDock Vina [19].

2.3 Preparation of macromolecule structures of the protein Survivin
The crystal structure of the human scaffold protein survivin was downloaded from the RCSB protein data bank (http://www.rcsb.org) and was encoded with the PDB code 2QFA. Protein structure inhibitors were separated by releasing atomic coordinates of the .PDB file. All water molecules were removed, and ADT software was used to prepare the required files for AutoDock Vina by assigning hydrogen polarities, calculating Gasteiger charges to protein structures, and converting protein structures from the .PDB file format to .PDBQT format [20–22].

2.4 Docking Methodology
Molecular docking was performed using the AutoDock Vina program. Ligands were docked individually to the receptor with grid coordinates (grid center) and grid boxes of certain sizes for each receptor. The ligand was in a flexible condition when interacting with macromolecules under rigid conditions. The configuration file was engaged by opening notepad to run AutoDock Vina. ADT was required to prepare the input. PDBQT file for survivin and to set the size and the center of the grid box. Kollman charges and polar hydrogen atoms were included in the survivin structure. The grid size was set at 14 × 14 × 14 (x, y, and z) points, and the grid center was designated at x, y, and z dimensions of 43.762, 8.472, and 42.921, respectively, with a grid spacing of 1000 Å. The prepared file was saved in the .PDBQT format. Ligand-binding affinities were predicted as negative Gibbs free energy (ΔG) scores (kcal/mol), which were calculated on the basis of the AutoDock Vina scoring function [19]. Post-docking analyses were visualized using PyMOL and Discovery Studio Biovia 2017, which showed the sizes and locations of binding sites, hydrogen-bond interactions, hydrophobic interactions, and bonding distances as interaction radii of <5 Å from the position of the docked ligand. Compounds were docked to the active site of survivin. Subsequently, binding poses of each ligand were observed and their interactions with the protein were characterized, and the best and most energetically favorable conformations of each ligand were selected.

3. Results
Docking simulations of candidate ligands with survivin using ADT showed that quercetin has a ΔG score of −7.0 kcal/mol at the lowest (1st) conformation, whereas ΔG values of nordentatin and dentatin were −6.5 and −5.5 kcal/mol, respectively (Table 2). These results indicate that, of the three ligands, quercetin possesses the greatest binding affinity for survivin.

Table 2. Binding affinities of dentatin, nordentatin, and quercetin at the active site of surviving.

| Ligands   | Highest (9th) to lowest (1st) modes of conformation with corresponding RMS (root mean square) binding affinities in | ΔG (kcal/mol) |
|-----------|---------------------------------------------------------------------------------------------------------------|--------------|
|           |                                                                                                              | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th |
| Dentatin  |                                                                                                              | −5.5 | −5.4 | −5.4 | −5.3 | −5.1 | −5.1 | −4.9 | −4.8 | −4.7 |
| Nordentatin|                                                                                                              | −6.5 | −6.4 | −6.3 | −5.9 | −5.8 | −5.7 | −5.7 | −5.6 | −5.6 |
| Quercetin |                                                                                                              | −7.0 | −6.7 | −6.4 | −6.4 | −6.2 | −6.0 | −6.0 | −5.9 | −5.7 |
In simultaneous analyses, we mapped survivin amino acid residues that are involved in hydrogen bond, hydrophobic, and electrostatic interactions with the ligands using *AutoDock Vina* (Figure 1 and Table 3).

**Figure 1.** Binding conformation of the compounds in the survivin binding site. (A) Dentatin is shown with green carbon atoms. (B) Nordentatin is shown with cyan carbon atoms. (C) Quercetin is shown with magenta carbon atoms. (D) Superimposition of dentatin, nordentatin, and quercetin. (E) Ligand-binding interactions between dentatin and the survivin. (F) Interaction between nordentatin ligand and survivin receptor. (G) Interaction between quercetin and the survivin receptor.
Dentatin formed six hydrophobic interactions with survivin, involving the amino acid residues Val89 (4.89), Lys90 (4.75), Ile74 (4.89), Ile74 (4.95), Phe86 (4.17), and Phe93 (5.24). An electrostatic interaction was also identified with Lys78 (4.62). No hydrogen bonds were imputed between dentatin and the survivin binding site, whereas nordentatin interacted with survivin via a hydrogen bond with Gln92 (2.80) by forming a hydrogen bond and hydrophobic interactions with the residues Ile74 (4.39), Val89 (4.44), Ile74 (4.93), Phe86 (4.04), Phe86 (4.82), and Phe93 (4.56). Quercetin–survivin interactions were supported by three hydrogen bonds at residues Phe86 (2.21), Arg18 (2.80), and Phe93 (3.11) and by three hydrophobic interactions at residues Phe86 (5.48), Phe93 (5.19), and Ile74 (4.63). No electrostatic interactions were detected in nordentatin–survivin or quercetin–survivin interactions (Table 3).

Table 3. Interactions of survivin amino acid residues with ligands at receptor sites.

| Ligands      | Binding Affinity, ΔG (kcal/mol) | Amino acids involved and distance (Å) | Hydrogen-Binding Interaction | Hydrophobic Interaction | Electrostatic Interaction |
|--------------|---------------------------------|--------------------------------------|------------------------------|-------------------------|----------------------------|
| Dentatin     | −5.5                            | Val89 (4.89), Lys90 (4.75), Ile74 (4.89), Ile74 (4.95), Phe86 (4.17), Phe93 (5.24) | Lys78 (4.62)                |                         |                            |
| Nordentatin  | −6.5                            | Gln92 (2.80) | Val89 (4.39), Ile74 (4.44), Ile74 (4.93), Phe86 (4.04), Phe86 (4.82), Phe93 (4.56) |                         |                            |
| Quercetin    | −7.0                            | Phe86 (2.21), Arg18 (2.80), Phe93 (3.11) | Phe86 (5.48), Phe93 (5.19), Ile74 (4.63) |                         |                            |

4. Discussion

Virtual screening using molecular docking programs has become an increasingly popular approach to the development of new drugs, in part because of the favorable time and pecuniary costs of in silico drug screening compared with traditional laboratory experiments. In this study, we applied a computational protein–ligand docking technique using only open-source software and virtualized interactions of the candidate ligands dentatin, nordentatin, and quercetin with the anti-apoptotic protein survivin.

Protein–ligand binding only occurs spontaneously when the free energy change is negative, and the difference in ΔG levels of complexed and unbound free states is proportional to the stability of the protein–ligand interaction. It follows that both protein folding and protein–ligand binding occur when ΔG is low in the system [23,24]. Hence, negative ΔG scores indicate the stability of the resulting complexes with receptor molecules, and this is an essential characteristic of efficacious drugs [14]. In the present work, quercetin–survivin interactions had the largest negative ΔG values, yet considerable negative changes in ΔG levels indicated high survivin binding affinity of dentatin and nordentatin. Thus, we suggest that all tested ligands have potential to establish strong and stable complexes with the survivin protein.

Although ΔG values are informative of ligand docking in the active pocket of a protein active pocket, types of molecular interactions, such as hydrogen bond, hydrophobic, and electrostatic interactions, with essential amino acid residues are indicative of ligand docking in favorable conformations [25]. Hydrophobic interactions are the predominant contributors to the stability of proteins. Hydrogen bonding also supports protein stability, but to a lower degree than hydrophobic
interactions, even in the smallest globular proteins. Accordingly, hydrophobic binding is the main determinant of folding configuration equilibria in many native proteins [26].

Our results show that hydrogen bond, hydrophobic, and electrostatic interactions are mediated by different amino acid residues in each ligand–protein interaction. Specifically, quercetin formed hydrophobic interactions with only three residues, but these were the same residues as those involved in binding to dentatin and nordenatin (Phe86, Phe93, Ile74). In particular, Phe86 was identified in all hydrophobic interactions of all ligands with survivin. Electrostatic interactions are generally associated with binding affinity, structure, chemical characteristics, and stability, and with the biological reactivity of proteins and nucleic acids [27]. Although we identified an amino acid that was involved in electrostatic interactions between dentatin and survivin (Lys78), no electrostatic interactions were present in nordenatin–survivin and quercetin–survivin complexes.

Increasing evidence indicates that survivin is a unique member of IAP protein family and is highly expressed in numerous human cancers [6]. Hence, restoring apoptotic activities by targeting survivin is widely considered as a viable strategy for inhibiting cancer cell growth. Survivin overexpression in cancer cells was also inactivated or blocked in previous studies of small molecules such as siRNA, monoclonal antibodies, and synthetic or natural compounds. In keeping with these findings, our in silico analyses show that the natural compounds dentatin, nordenatin, and quercetin have potential as ligands that inhibit the anti-apoptotic survivin protein.

5. Conclusion
The present molecular docking experiments suggest that dentatin, nordenatin, and quercetin are candidate ligands for restoring apoptotic activities in cancer cells and act through interactions with survivin. Further in vitro and in vivo experiments are required to validate these in silico results.

Acknowledgements
This study was supported by a grant from the Ministry of Higher Education Indonesia to DA and FRGS 15-251-0492 grant from MOHE Malaysia to SJAI.

Conflict of interest
There are no conflicts of interest to declare.

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