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Molecular Docking Studies of Selected Natural Compounds as Caspase-3 Enzyme Inhibitors

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ABSTRACT

Caspase-3 plays a role in necrosis, apoptosis, and inflammation activities. Many studies have shown that caspase-3 expression and cancer are closely related. The regulatory mechanism performs their catalytic activities to promote their aggregation into dimers or macromolecular complexes. Thus, the mechanism's loss of function and insufficient caspase activation may result in tumour formation due to apoptosis malfunction. Therefore, it is essential to recognise small molecules that modulate caspase to facilitate treatment for apoptosis-related and inflammatory diseases. This work aimed to study the molecular interaction of selected natural compounds with inhibitory activities towards caspase-3 by molecular docking approaches. The docking was performed onto caspase-3 with the potential compounds using Autodock Vina. Our results demonstrated that the 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl) - 1H-indole-2, 3-dione (PDB ID: 1GFW), and B92 (PDB ID: 3KJF) were found to be the best potent inhibitors of caspase-3 with the binding energy of -8.4 Kcal/mol and -8.3 Kcal/mol, respectively. Hydrogen bond formations in the caspase-3 complex were found through T62, H121, G122, S205, R207, N208, and W214 residues. The potential inhibitory activities of these compounds may be further validated through in vitro and in vivo studies in future works.

Keywords: *Caspase-3; inhibitor; molecular docking; natural compounds*

1. INTRODUCTION

Caspases are well-classified as a special class of cysteine proteases and are responsible for persistent homeostasis by cell death and inflammation controls [1]. The enzymes were classified based on their roles in apoptosis and inflammation activities. The apoptotic caspases include caspase-2, -3, -6, -7, -8, and -9 in mammals and they are further subclassified as initiator/apical caspases (caspase-2, -8, -9, and -10) and effector/executioner caspases (caspase-3, -6, and -7) [2]. Human caspase-1, -4, -5, -12 and mice caspase-1, -11, and -12 are involved in inflammation [3]. Initially, these enzymes were inactive monomeric procaspases that formed dimers prior to proteolytic activation. Various adapter proteins bind to specific regions in the prodomain of the procaspase and contribute to the dimerization mechanisms. Each caspase has its protein-protein interaction domains in its prodomain to form complexes with various adapters [4].

Irregular regulation of caspase-3 activation may cause dire consequences. In cancer patients, activated caspase-3 regulates prostaglandin E2 (PGE2), potentially stimulating surviving tumour cell growth [5], thus causing relapse in cancer patients. Moreover, caspase-3 activates

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the critical stage of Alzheimer's synaptic dysfunction, which causes synaptic loss and cognitive dysfunction by a caspase-3-dependent mechanism [6]. Excessive neuronal apoptosis involving caspase-3 also leads to various diseases such as stroke, Huntington′s and Parkinson′s disease [7]. These examples show that the deactivation of caspase-3 could halt the problems as discussed. Therefore, targeting caspase-3 is an interesting approach for developing a drug that could deactivate the triggered caspase-3.

Until now, intense efforts have been made to find drugs that suit the above purposes. A previous experimental study on Dillenia suffruticosa root dichloromethane extract (DCM-DS) showed significant cytotoxicity to human caspase-3 breast cancer cells [8]. Caspase-3 treatment in human lymphoid leukaemia cells by the bioactive compounds from carrots (*Daucus carota* L.), polyacetylenes, beta-carotene, and lutein showed cellular proliferation inhibition [9]. Moreover, there was also a report concerning the potential caspase ligand as a potent inhibitor in the enzyme activity with chemically reactive compounds [9]. Regardless of these findings, none of these compounds remains in the drug development pipeline due to several factors, and the search for an effective caspase inhibitor remains a crucial and challenging task.

Most of the known caspase inhibitors show the presence of the electrophilic group as a significant feature [10]. The formation of the reversible or irreversible bond with the cysteine active site has played a vital role in enzyme inactivation. These current marks on reported compounds could be one of the starts for our computational approaches to identify the potential ligands. Therefore, our main objective was to identify the potential binding of previously reported caspase inhibitors against caspase-3, a key therapeutic target involved in apoptosis and cell functionality disorders, using molecular docking approaches. We have explored the binding of selected natural compounds against caspase-3 that could explain the inhibition activities reported in the database. Molecular docking simulation was used to determine the interaction between the protein and the selected compounds that lead to the *in vitro* inhibitory activity.

2. METHODOLOGY

2.1 Protein and Ligand Preparation

The protein database, Protein Data Bank (PDB) (https://www.rcsb.org/), has been used to retrieve the crystal structure of caspase-3 (PDB ID: 4EHA). The structure was removed with the heteroatoms and water molecules. The minimisation and conjugate gradient method used a CHARMm force field [11] and dependent dielectric implicit solvent models. The 3D structure from previous studies on natural compounds known as the caspase enzyme inhibitor was extracted from the protein data bank [12]. These compounds include 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2, 3-dione (PDB ID: 1GFW), B92 (PDB ID: 3KJF), ACE-1MH-ASP-B3L-HLX-1U8 (PDB ID:4JJE), 4-oxo-3-[(6-{[4-(quinoxalin-2-ylamino)-benzoylamino]-methyl}-pyridine-3-carbo-nyl)-ami -no]-butyric acid (PDB ID: 1RWW), NA4 (PDB ID: 1RHM), PZN (PDB ID: 1RHJ), 0ZZ (PDB ID: 1RHQ) and 3-(2-mercapto-acetylamino)-4-oxo-pentanoic acid (PDB ID: 1RWK).

2.2 Protein and Ligand Preparation

Re-docking was executed to validate the method used throughout the molecular docking process. The enzyme inhibitor found at the active site of the crystal structure of caspase-3 was detached. Autodock Vina was used during the re-docking. Re-docking results, and structural

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orientation was checked by comparing the results with the performance from the Protein Data Bank (PDB ID: 4HEA).

2.3 Protein and Ligand Preparation

Autodock Vina and AutoDockTools (ADT) suite program [13] were used during our docking studies. The potential inhibitor compounds were assigned with hydrogen atoms, Gasteiger charges and torsion angles. Before preparing enzyme structure input files, all water molecules and ligands were eliminated. The affinity maps of the active site were emphasized using the software Autogrid, and 60 x 60 x 60 Å grid spaces with a distance of 0.375 Å were generated. The docking simulations were performed in 100 runs for every docking trial using the Lamarckian genetics algorithm (LGA). The parameters of population size, energy evaluations, mutation and crossover rates and local search probability were defaulted. Cluster number and hydrogen bonding compared with the native ligand of the target were evaluated in the molecular docking performance. The best coordinates of enzyme compounds and their mode of interaction with binding site residues were analyzed with PyMOL.

3. RESULTS AND DISCUSSION

Caspases are a family of aspartate cysteine proteases, which have essential roles in apoptosis and several processes in physiology and disease. The active caspases initiate apoptosis and/or inflammatory reactions by cleavage particular substrates. The effector induces the cell damage induces cell damage (caspase-3, -6 and -7). The initiator/apical caspase (caspase-2, -8, -9 and -10) is stimulated proteolytically, which leads to caspase cascades. After activation, the effector caspases transmit the death signal by cleavage their particular substrates. It stimulates other destructive enzymes, such as the DNases, structural and signal protein degradation inside the cell. Therefore, deregulation of caspase activity may contribute to nearly every phase of tumorigeneses, such as overproliferation, cell death avoidance, immune destruction, inflammation that promotes tumour, and metastatic invasion.

Caspase-3 was identified in comprehensive studies as the critical effector of caspase in most mammalian cells, including cancer cells [14]. This enzyme has been the target caspases in cancer therapeutics [15] and other therapy, including Alzheimer's disease [16]. The findings of Alzheimer-like phenotypes were surprisingly rescued by pharmacological inhibition of caspase-3 activity. As most apoptotic events involve activation of effector caspase-3, molecules aimed directly at the effectors would be ideal for drug use.

Molecular docking has become a powerful tool for drug discovery. Here, we applied the rigid body docking method, where the ligand was flexible, and the enzyme was kept rigid. Re-docking steps were performed to ensure the exact conformation of ligands and the binding pocket. The size and the grid box midpoint coordinate were evaluated during this step. The presence of enzyme inhibitors within the caspase-3 active site was re-docked into its particular binding site. Based on the results, similar interactions occurred between the inhibitor and the amino acids at the active site of the crystal structure (Figure 1). The conformations showed the RSMD value of 0.09Å accuracy and represented a high degree of similarity to the crystallographic complex.

Later, molecular docking simulations were performed to predict the interactions between the potential inhibitors and enzymes. Interactions of the binding modes were identified and rated

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based on their binding energy (Table 1). The binding energy is the sum of the intermolecular forces acting upon the receptor-ligand complex. The conformation with the lowest energy shows the complex's strongest binding and better stability [17]. The results revealed that 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2, 3-dione (PDB ID: 1GFW), and B92 (PDB ID: 3KJF) were able to bind within the potential binding site of caspase-3 with the lowest binding energy of -8.4 Kcal/mol and -8.3 Kcal/mol, respectively, as compared to other compounds. We found that two amino acids of 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1- sulfonyl)-1H-indole-2,3-dione, G122, and R207 were involved in the hydrogen bond formation.

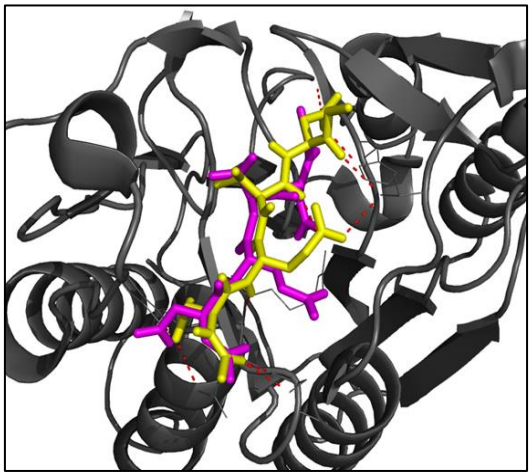

Figure 1: Binding mode of crystallized inhibitor (magenta) and re-docked (yellow) peptide-like inhibitor within the active site of caspase-3 (red line). The RMSD of all atoms between these two conformations was 0.09Å

Table 1: Interactions and binding energies of selected compounds into the binding site of caspase-3

Rank	PDB ID:	Name of compound	Residues involved in hydrogen bond formation	Binding energy (Kcal/mol)
1	1GFW	1-methyl-5-(2-phenoxymethyl-pyrrolidine-1- sulfonyl)-1H-indole-2,3-dione	G122, R207	-8.4
2	3KJF	B92	T62, H121, S205, R207, N208, W214	-8.3
3	4JJE	ACE-1MH-ASP-B3L-HLX-1U8	T62, H121, Y204, R207, W214	-7.9
4	1RWW	4-oxo-3-[(6-{[4-(quinoxalin-2-ylamino)-benzoylamino]-methyl}-pyridine-3-carbonyl)-amino]-butyric acid	F55, M61, S63, S65, R207	-7.7
5	1RHM	NA4	R64, S120, H121, Q161, S205, R207, S209, W214	-7.4
6	1RHJ	PZN	R64, H121, R207, W214	-7.2
7	1RHQ	0ZZ	R207, N208, S209	-7.0
8	1RWK	3-(2-mercapto-acetylamino)-4-oxo-pentanoic acid	R64, H121, R207	-4.9

The 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole- 2, 3-dione compound belonged to an organic compound known as isatin sulfonamide [18]. A recent report on the molecular dynamics of the caspase-3-isatin sulfonamide complex revealed that G122 and R207

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were involved in the carbonyl oxygen-hydrogen bond partner in the bicyclic aromatic scaffold residue, and its consistency was observed throughout the 20 ns simulation [19]. We anticipate that these amino acid contacts can provide clues about its potential role in inhibiting the caspase-3 enzyme. Moreover, this isatin sulfonamide was the earlier reported example of potent and selective inhibitors of caspases-3 and -7 [18].

Several studies have identified analogues of isatin sulfonamide as potent and selective, non-peptide small-molecule caspase-3 inhibitors and have shown their potential as in vivo apoptosis therapy drugs and radiotracers [20]. They were represented as novel strategies for developing active agents that block apoptosis and maintain cell functionality. These compounds suggest an initial study of effector caspase inhibition activity in cell-based models of apoptosis. Apart from caspase-9, the inhibitor showed a 100-fold or excellent selectivity for highly homologous caspase-3 and -7 against all other family members. The isatin sulfonamide can also inhibit the human chondrocyte apoptosis by its anti-apoptotic property [21].

The B92 is the novel β-strand irreversible peptidomimetic inhibitor synthesized by Wang and his co-workers [22]. This compound belongs to urazole ring-containing irreversible peptidomimetics inhibitors. It can bind covalently by cleaving an internal peptide bond and trapping the enzyme molecule. This was called a trapping mechanism that will change the compound conformation. This will not allow the inhibitor or the enzyme to participate in further reactions. The saturated carbocyclic fusion in this compound offers an excellent opportunity for the enzyme active site binding and provides opportunities for further elaboration in future clinical trials. The report claimed that this compound could bind to caspase-3 binding pocket to inhibit the enzyme activities. It was reported as the potential inhibitor of apoptosis, where it bound and inhibited the caspase-3 and caspase-8 enzymes and stopped the proteolysis cascade, as well as protecting Fas [22].

The discovery of the irreversible peptidomimetic inhibitor in caspase-3 is a great deal of effort to enhance retention in apoptotic versus healthy cells, caused by slow dissociation kinetics [23]. The compound structure and the binding mode of those two ligands in the caspase-3 active site are shown in Figure 2 and Figure 3, respectively. According to our analysis, all the compounds interact with 16 amino acids; F55, M61, T62, S63, R64, S65, S120, H121, G122, Q161, Y204, S205, R207, N208, S209, and W214 (Table 1). The Arg207 was significantly involved in the compounds positioning at the active site. This observation also can be seen in the previous study on caspase-8, where the Arg258 was the key role that points out in the top-middle of the active site cleft. The residue also clearly contributed toto the ligands' localisation within the active site [23].

(a) (b)

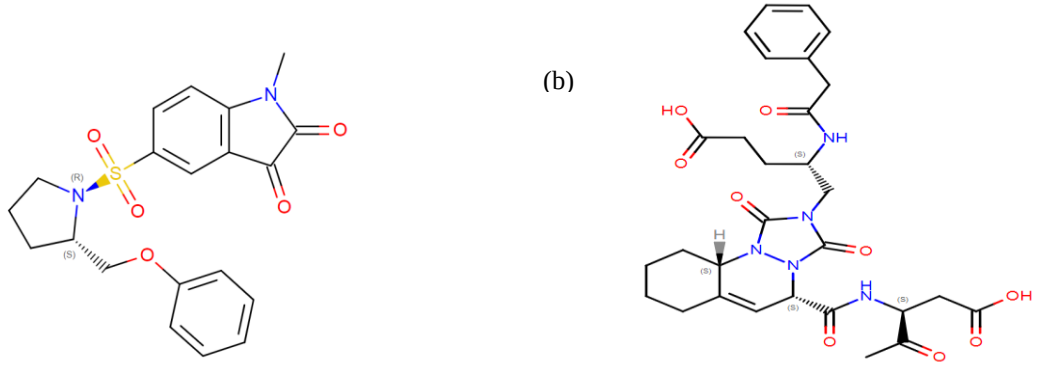

Figure 2: Two-dimensional (2D)-structure of compounds (a) 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2,3-dione and (b) B92

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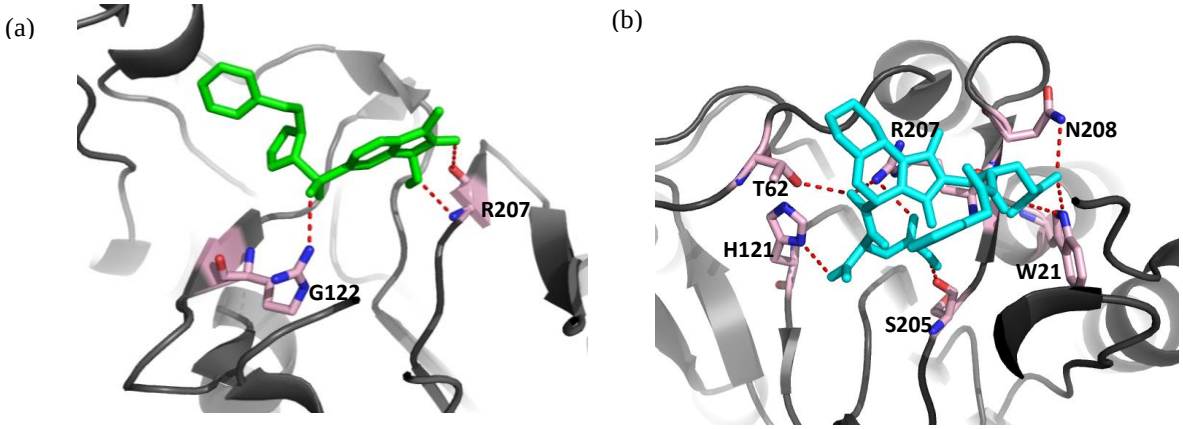

Figure 3: Binding mode of compounds (a) 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1- sulfonyl)-1H-indole-2,3-dione (green) and (b) B92 (cyan) located in the active site of caspase-3 (grey)

3. CONCLUSION

We studied the molecular interactions of the selected compounds with caspase-3 reported earlier by previous research. Based on the docking analysis, the hydrogen bond formations were found to play essential roles in the interactions between the potential compounds and caspase-3. Out of all the selected compounds, 1-methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1H-indole-2, 3-dione (PDB ID: 1GFW) were demonstrated to be the best potent inhibitors of caspase-3 in terms of binding energy, followed by the B92 (PDB ID: 3KJF), the novel β-strand irreversible peptidomimetic inhibitor. In the future, *in vitro* and *in vivo* experiments could be performed to verify the *in silico* observations. This discovery could provide a new perspective in the search for drug candidates against caspase-3 for the treatment of various diseases.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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