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Molecular Docking of Natural Alkaloids with Bcl-xL Protein in The Apoptosis Process

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Abstract*— Background: The anti-apoptotic protein Bcl-xL is a viable target for cancer therapy due to its critical role in cancer formation and resistance to chemotherapy. Insights into Bcl-xL's role have spurred the development of a new category of cancer drugs called Bcl-xL inhibitors, which mimic the BH3-only protein to cause apoptosis. It could be initiated using alkaloids, owing to their remarkable biological characteristics. Alkaloids, among the biggest obtainable from plants, possess a distinctive structure characterized by the presence of a nitrogen atom in various positions within the molecule. Objective: In the current study, the potential of various alkaloids as Bcl-xL inhibitors was investigated through a molecular docking study. Methods: AutoDock Vina software was used to perform docking simulations of the alkaloids. Results: Ten alkaloids/Bcl-xL protein complexes demonstrated strong binding affinities less than -8.0 kcal/mol^{-1}. These complexes include phaenthine (26), 4,5-Dioxoaporphine (1), anonaine (2), atherospermidine (4), limacine (14), liriodenine (16), monomargine (18), ouregidione (24), oxostephanine (25), and taliscanine (29) as the ligand of Bcl-xL protein. Notably, the complexes of Bcl-xL/dicentrinone (10), ouregidione (24), stepharine (28), and taliscanine (29) displayed three to four hydrogen bonds along with hydrophobic contacts. Conclusion: These results suggest that certain alkaloids could act as potential Bcl-xL inhibitors, mimicking BH3-only proteins and thereby potentially triggering apoptosis during cancer treatment.

***Keywords*— Bcl-xL protein; Alkaloids; Docking; AutoDock Vina; cancer chemotherapy**

I. INTRODUCTION

Natural Products (NPs) have long been a valuable source in searching for new drugs, particularly in treating infectious diseases and cancer [1]. Among many other examples include penicillin G, morphine, quinine, digoxin, paclitaxel, doxorubicin, and cyclosporine, which are NP-derived medications that are essential to contemporary pharmaceutical care [2]. NPs are a broad category of bioactive compounds derived from varying sources, including plants. Their bioactive compounds are known as secondary metabolites and can be classified into four main categories: sulfur-containing compounds, alkaloids, phenolic compounds, and terpenoids [3]. However, alkaloids are among the most significant bioactive compounds to study.

Alkaloids represent a diverse category of natural compounds originating from secondary metabolism, distinguished by the presence of a nitrogen atom in various positions within the molecule [4]. In nature, they are extensively dispersed; roughly 25% of plants contain these chemicals. Alkaloids' non-exclusive roles inside generating organisms have led to much research on their pharmacological characteristics [5]. Numerous alkaloids extracted from NP exhibit anti-metastatic, antibacterial, antiviral, insecticidal, and anti-proliferative properties against a range of cancer types in both in vitro and in vivo settings [6].

The alkaloids isolated from Malaysian plants in this study particularly target the anti-apoptotic protein Bcl-xL, which has not been described previously. Bcl-xL is the most prevalent Bcl-2 protein with an anti-apoptotic function. The hydrophobic surface groove in the Bcl-xL structure, which developed by the nine α-helices globular bundle-shaped, is mostly produced by the conserved BH1, BH2, and BH3 domains [7–9]. The anti-apoptotic action of Bcl-xL is mediated by its BH4 domain [10]. The BH4 domain of Bcl-xL has been discovered to play a significant role in regulating other cellular processes like angiogenesis, tumour growth, and cell migration [11]. Upregulation of the intracellular level of Bcl-xL has been associated with various tumours. Overexpression of Bcl-xL has also been reported in various viral infections, including hepatitis B and C, influenza A virus, and coronavirus [12].

Alkaloids are significant chemical substances that are valuable for the search for new drugs, particularly in finding Bcl-xL inhibitors for the induction of apoptosis in cancer cells [10] and overcoming multidrug resistance in malignancies [13]. These highlight the necessity of developing Bcl-xL inhibitors, which are facilitated by specific compounds, such as BH3 mimetics [11]. However, the investigation of natural substances is still limited. It is expected that alkaloids, which have long been investigated for their potential as therapeutic agents for a range of illnesses, will demonstrate proof of their capacity to function as potential Bcl-xL inhibitors. This research aims to increase the spectrum of alkaloid bioactivity, especially those that have not been studied as Bcl-xL inhibitors previously, which could lead to the development of a novel anticancer drug.

II. MATERIALS AND METHODS

A. Preparation of Alkaloids

The alkaloids (1–30) were retrieved from the PubChem databases http://pubchem.ncbi.nlm.nih.gov/. These alkaloids were selected based on previously isolated alkaloids in Malaysian plants [14]. No inclusion criteria were made for molecular weight and types of studied alkaloids. Table 1 lists their corresponding IDs and compound structures. Prior to ligand preparation using the AutoDock tools, all alkaloid structures were downloaded from PubChem and converted into the .pdbqt format using the AutoDock Vina version 1.1.2 program [15]. During the ligand preparation, polar hydrogen atoms were incorporated into the alkaloids, and nonpolar hydrogen atoms were eliminated.

B. Preparation of Bcl-xL Protein

The Bcl-xL protein structure (PDB ID: 3ZK6) was acquired from the RCSB Protein Data Bank [16]. The BIOVIA Discovery Studio Visualizer 2017 R2 version 17.2 was employed to eliminate water molecules from the Bcl-xL. The hydrogen and nonpolar hydrogen atoms were incorporated into the Bcl-xL protein structure prior to performing the molecular docking procedure.

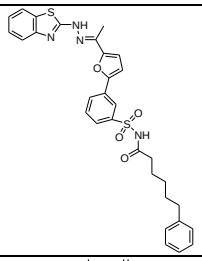
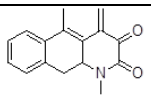
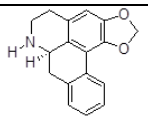
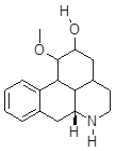
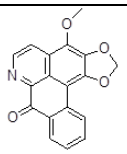
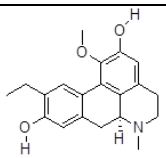
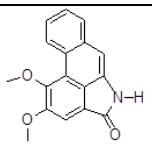
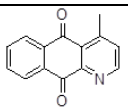
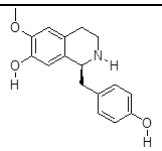
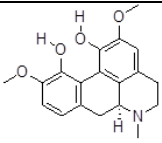
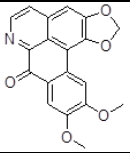
C. Molecular docking of alkaloid into Bcl-xL protein

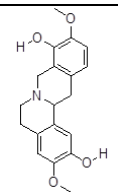
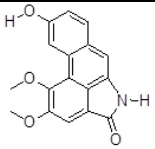
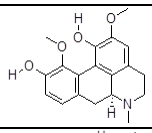
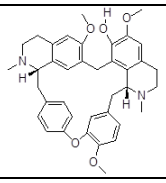
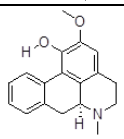
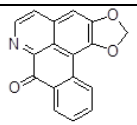
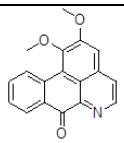
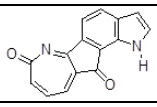
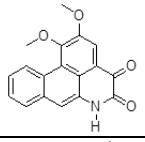
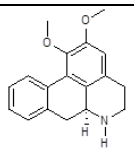
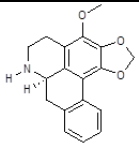
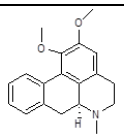
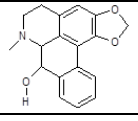
The AutoDock Vina software was used to perform molecular docking of thirty natural alkaloids into the active site of the Bcl-xL protein. The grid box was positioned in the active site pocket on the centre of x (21.07), y (49.64), and z (1.33) using the autogrid4 application. Accordingly, partial charges for Bcl-xL and the alkaloids were assigned based on Kollmann's charges and Gasteiger-Marsili's calculations. The repetition for the docking simulation was set to 100 exhaustiveness, while the software's default settings were applied to the other parameters. The best conformations were chosen based on the binding affinity and interactions with the essential amino acid residues. Lastly, the selected positions were studied to evaluate the molecule's interaction with one another, as well as the binding interactions, using the BIOVIA Discovery Studio Visualizer 2017 R2 version 17.2 [17].

III. RESULTS AND DISCUSSION

Molecular docking allows the prediction of binding affinities and complex interactions between proteins and ligands. Additionally, it is possible to ascertain the most likely orientation and conformation of a ligand attached to the target protein [18]. A total of thirty alkaloids have been chosen from Malaysian plants for the screening of the potential Bcl-xL inhibitors through an *in silico* study from the docking results (Table 2, Figures 1 and 2). Table 2 summarises the binding affinities of all observed alkaloids ranging from -10.0 to -6.8 kcal/mol^{-1}, which is relatively good to moderate binding affinity. Among them, phaenthine **(26)** was reported to have the strongest binding energy of -10.0 kcal/mol^{-1}, which was comparable with 3ZK6's inhibitor (-10.2 kcal/mol^{-1}). Meanwhile, nine alkaloids, including 4,5-Dioxoaporphine **(1)**, anonaine **(2)**, atherospermidine **(4)**, limacine **(14)**, liriode nine **(16)**, monomargine **(18)**, ouregidione **(24)**, oxostephanine **(25)**, and taliscanine **(29)** exhibited good binding affinity, ranging from -8.5 to -8.0 kcal/mol^{-1}. A study conducted by Nordin et al. (2021) mentioned that the group of acetogenins has a much lower binding affinity (< 9.0 kcal/mol^{-1}) compared to studied alkaloids [19]. The difference between acetogenins from a previously reported study and alkaloids in this current study is that the molecular weight of acetogenins is bigger. Furthermore, the chemical structures of acetogenins are longer with the presence of long chains in all acetogenins, resulting in them occupying the hydrophobic groove of the Bcl-xL protein. Both conditions are predictable to cause low binding affinity compared to alkaloids.

TABLE 1. Molecular Data and Structure of Alkaloids

Compound	Molecular weight ($gmol^{-1}$)	Compound ID	Structure
Inhibitor, N-(3-(5-(1-(2-(benzo[d]thiazol-2-yl)hydrazono)ethyl)furan-2-yl)phenylsulfonyl)-6-phenylhexanamide	586.72	H1I	
4,5-Dioxoaporphine **(1)**	263.29	53849099	
Anonaine **(2)**	265.31	160597	
Asimilobine **(3)**	267.32	160875	
Atherospermidine **(4)**	305.3	77514	
Boldine **(5)**	327.4	10154	
Cepharanone B **(6)**	279.29	162739	
Cleistopholine **(7)**	223.23	457732	
Coclaurine **(8)**	285.34	160487	
Corytuberine **(9)**	327.4	160500	
Dicentrinone **(10)**	335.3	177744	

Discretamine **(11)**	327.4	22955	
Goniothalactam **(12)**	295.29	10447198	
Isocorytuberine **(13)**	327.4	12310875	
Limacine **(14)**	608.7	100257	
Lirinidine **(15)**	281.3	31069	
Liriodenine **(16)**	275.26	10144	
Lysicamine **(17)**	291.3	122691	
Monomargine **(18)**	248.24	135427687	
Norcepharadione B **(19)**	307.3	189168	
Nornuciferine **(20)**	281.3	41169	
Norstephalagine **(21)**	295.3	133381	
Nuciferine **(22)**	295.4	10146	
Oliveroline **(23)**	331.8	3085243	

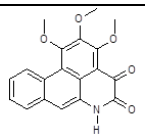
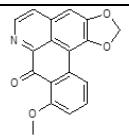
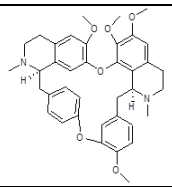
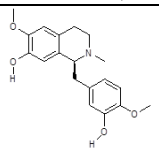
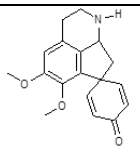
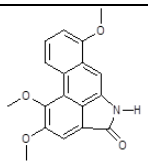
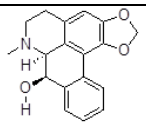
Ouregidione **(24)**	337.3	11958181	
Oxostephanine **(25)**	305.3	343547	
Phaenthine **(26)**	622.7	73664	
Reticuline **(27)**	329.4	439653	
Stepharine **(28)**	297.3	98455	
Taliscanine **(29)**	309.3	10859838	
Ushinsunine **(30)**	295.3	197018	

TABLE 2. Docking Binding Affinities and Interaction Profile of Ligands with Bcl-xL Protein (3ZK6).

Ligands	**Binding Affinities (kcal/mol-1)**	**Hydrogen Bonding**	**Hydrophobic Interaction**
Inhibitor (H1I)	-10.2	Ser106 Asn136 Arg139	Arg102, Phe105, Leu108, Leu130, Val141, Ala142 Phe146, Ala149
4,5-Dioxoaporphine (1)	-8.3	Arg139	Phe105. Leu108, Glu129 Leu130, Arg139, Ala142
Anonaine (2)	-8.1	-	Phe105, leu108, Leu130, Gly138, Arg139, Ala142
Asimilobine (3)	-7.9	Glu129	Phe97, Phe105, Leu108, Leu130, Gly138, Arg139 Ala142
Atherospermidine (4)	-8.5	Glu129, Arg139	Phe105, Leu108, Leu130, Ala142
Boldine (5)	-6.9	Asn136, Arg139	Phe105, Ala142
Cepharanone B (6)	-7.4	Arg139	Phe105, Arg139, Ala142
Cleistopholine (7)	-7.3	-	Ala93, Tyr101, Tyr195
Coclaurine (8)	-7.6	-	Tyr101, Phe105, Leu130, Arg139, Ala142, Tyr195
Corytuberine (9)	-7.7	Arg139	Phe97, Phe105, Arg139, Ala142
Dicentrinone (10)	-7.6	Asp107, Leu108, Gly138	Phe97, Arg102, Phe105, Ser106, Leu130, Arg139, Ala142

Discretamine (11)	-7.7	Arg139	Ala93, Phe97, Tyr101, Phe105, Arg139, Val141, Ala142, Tyr195
Goniothalactam (12)	-7.2	Arg139	Phe105, Arg139, Ala142
Isocorytuberine (13)	-6.9	Arg139	Phe105, Arg139, Ala142
Limacine (14)	-9.4		Phe97, Tyr101, Phe105, Gly138, Arg139, Val141,
Lirinidine (15)	-7.1	Arg100, Tyr195	Ala93, Glu96, Val141
Liriodenine (16)	-8.2	Arg139	Phe105, Ala142
Lysicamine (17)	-7.2	Arg139	Phe105, Leu130, Arg139, Ala142
Monomargine (18)	-8.1	Arg139	Phe105, Leu130, Arg139, Ala142
Norcepharadione B (19)	-7.7	Arg139	Phe105, Leu130, Arg139, Ala142
Nornuciferine (20)	-7.1	Arg100	Glu96, Asn197
Norstephalagine (21)	-7.5	Gly138, Arg139	Phe105, Leu108, Leu130, Arg139, Ala142,
Nuciferine (22)	-6.8	Arg100, Tyr195	Ala93, Glu96, Val141
Oliveroline (23)	-7.5	Arg139	Phe105, Leu130, Arg139, Ala142,
Ouregidione (24)	-8.1	Glu129, Leu130, Arg139	Phe105, Leu108, Leu130, Arg139, Ala142
Oxostephanine (25)	-8.0	Arg139	Phe105, Leu108, Leu130, Arg139, Ala142
Phaenthine (26)	-10.0	Glu92, Ala93	Ala93, Phe97, Tyr101, Phe105, Gly138, Tyr195
Reticuline (27)	-7.7	-	Tyr101, Arg139, Try195
Stepharine (28)	-7.0	Gly138, Tyr195, Asn197	-
Taliscanine (29)	-8.0	Ala93, Gly138, Tyr195, Asn197	Ala93, Glu96, Tyr101, Val141, Tyr195
Ushinsunine (30)	-7.4	Ala93, Tyr101	Ala93, Tyr101, Tyr195

The observed alkaloids were docked directly within the Bcl-xL protein's active binding region (Figure 2). The phaeanthine/Bcl-xL complex exhibited the strongest binding energies of all the complexes, followed by the limacine/Bcl-xL complex (-9.4 kcal/mol^{-1}). Interestingly, both ligands possessed the same basic skeleton of bisbenzylisoquinoline and were reported previously in *Phaeanthus crassipetalus* Becc. [20]. The molecular weight of both ligands, phaeanthine (622.7 gmol^{-1}) and limacine (608.7 gmol^{-1}) were also among the highest values, anticipating their contribution of strongest binding affinities compared to other alkaloids. Likewise, a bigger molecular weight was also detected for ABT-737 (813.43 gmol^{-1}) and acetogenins [19], resulting in the lowest binding affinity.

Meanwhile, alkaloids (**1, 2, 4, 16, 18, 24, 25,** and **29**) displayed less than -8.0 kcal/mol^{-1} of binding affinities, indicating good binding energy (Table 2). The chemical structure of **2**, **4**, **16**, **24,** and **25** demonstrated a similar basic skeleton of aporphine alkaloids comprising of an aporphine nucleus, which is the 4H-dibenzo[de,g]quinoline structure or its 3-methyl derivative [20]. These alkaloids are produced by the intramolecular C-C bond creation of (S)-reticuline, which results in the synthesis of the tetracyclic aporphine backbone. These structures may have hydroxyl, methoxy, or methylenedioxy moieties spread over all four rings as substituents [21]. The difference in their derivatives could be the reason for the binding affinity of these observed alkaloids.

One of the most important residues, Arg139, has been reported in almost all studied alkaloids (Table 2) through hydrogen bonding or hydrophobic contact. An Arg139 residue is the conserved region in the Bcl-xL groove [22], which can interact with inhibitors to mediate proapoptotic interactions. According to the current study, alkaloids exhibit good interaction with the Bcl-xL protein. They could inhibit its natural ligand (BH3 only protein) to bind with Bcl-xL and, therefore, induce apoptosis in cancer cells. The P2 and P4 of the Bcl-xL protein's hydrophobic groove pockets have been proven in earlier research to play a crucial part in the high-affinity binding of proapoptotic and BH3-only proteins [22-23]. It has also been proposed by earlier studies that conserved arginine (Arg) and aspartic acid (Asp) residues electrostatically interact to mediate proapoptotic interactions. The residues Glu96, Tyr101, Ser106, Asp107, Leu108, Arg139, and Tyr195 are among those implicated in the P2 and P4 pockets [23]. In contrast to the P4 pocket, the P2 hydrophobic pocket has a deep, deformable cavity that demonstrates greater ligand binding [7].

Additionally, a hydrogen bond (H-bond) is frequently formed inside proteins after interaction with strongly electronegative atoms such as N, O, or F [24]. It has been proven that most alkaloids/Bcl-xL complexes formed the H-bond with the N or O atom of the ligands (Figure 1). Furthermore, the number of H-bond formations could significantly influence the target protein's binding affinity. Four H-bonds are the highest number formed during this molecular docking simulation of taliscanine with Bcl-xL's active site, which is composed of the residues Ala93, Gly138, Tyr195, and Asn197. In contrast, limacine **(14)** was discovered to have a somewhat lower binding affinity (-9.4 kcal/mol^{-1}). It did not have any H-bond but two crucial residues from Tyr101 and Arg139 in the P4 pocket of Bcl-xL, which interacted with hydrophobically.

Hydrophobic contact is another interaction in this docking study that can contribute to the ligand-receptor recognition binding [25-26]. Most of the hydrophobic contact between alkaloid and Bcl-xL protein occurred at residues Tyr101, Phe105, Leu108, Leu130, and Ala142, which were also found in ABT-737 [19]. In the crystallized Bcl-xL template, these are a few reported amino acids coupled with inhibitors inside chain A's hydrophobic groove [27]. The docking results demonstrated that every tested alkaloid that had not previously undergone molecular docking analysis exhibited a potential BH3-only protein mimetic characteristic that might function as a Bcl-xL inhibitor in developing an anticancer agent.

Figure 1. Two-dimensional illustrations of alkaloids bind into Bcl-xL protein. **(1)** 4,5-Dioxoaporphine, **(2)** Anonaine, **(3)** Asimilobine, **(4)** Atherospermidine, **(5)** Boldine, **(6)** Cepharanone B, **(7)** Cleistopholine, **(8)** Coclaurine, **(9)** Corytuberine, **(10)** Dicentrinone, **(11)** Discretamine, **(12)** Goniothalactam, **(13)** Isocorytuberine, **(14)** Limacine, **(15)** Lirinidine, **(16)** Liriodenine, **(17)** Lysicamine, **(18)** Monomargine, **(19)** Norcepharadione B, **(20)** Nornuciferine, **(21)** Norstephalagine, **(22)** Nuciferine, **(23)** Oliveroline, **(24)** Ouregidione, **(25)** Oxostephanine, **(26)** Phaenthine, **(27)** Reticuline, **(28)** Stepharine, **(29)** Taliscanine, **(30)** Ushinsunine. Different interactions are depicted in dashed lines between ligands and protein residues: green (hydrogen bond), purple (Pi-sigma), pink (Pi-alkyl), and red (unfavourable donor-donor).

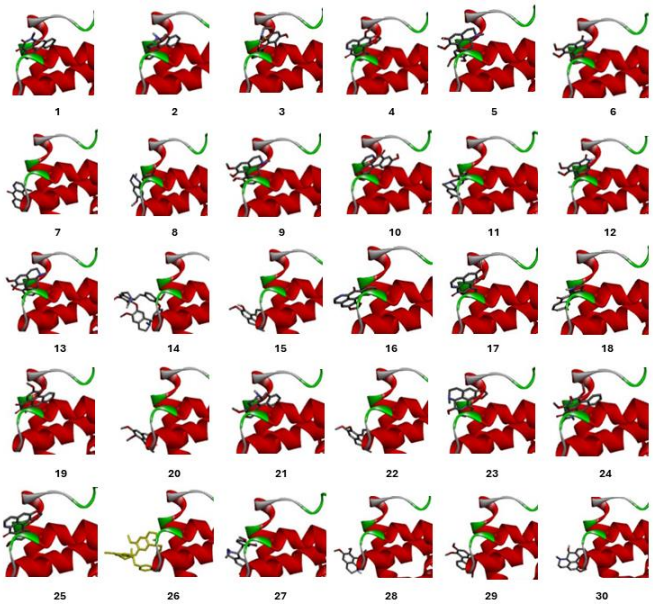


Figure 2. Illustration of three-dimensional binding interactions of thirty alkaloids into the active site of Bcl-xL protein. **(1)** 4,5-Dioxoaporphine, **(2)** Anonaine, **(3)** Asimilobine, **(4)** Atherospermidine, **(5)** Boldine, **(6)** Cepharanone B, **(7)** Cleistopholine, **(8)** Coclaurine, **(9)** Corytuberine, **(10)** Dicentrinone, **(11)** Discretamine, **(12)** Goniothalactam, **(13)** Isocorytuberine, **(14)** Limacine, **(15)** Lirinidine, **(16)** Liriodenine, **(17)** Lysicamine, **(18)** Monomargine, **(19)** Norcepharadione B, **(20)** Nornuciferine, **(21)** Norstephalagine, **(22)** Nuciferine, **(23)** Oliveroline, **(24)** Ouregidione, **(25)** Oxostephanine, **(26)** Phaenthine, **(27)** Reticuline, **(28)** Stepharine, **(29)** Taliscanine, **(30)** Ushinsunine.

IV. CONCLUSIONS

The current study suggests that alkaloids may provide promising insights for finding new drugs to treat cancer. The docking study predicts that the interaction of Bcl-xL with eight alkaloids **(1**, **14**, **16**, **18**, **24**, **25**, **26**, and **29)** is noticeably good binding affinities with several hydrogen bonds. Their hydrogen bond interactions with Arg139 residue indicated strong inhibition of Bcl-xL function. Detailed validation of molecular dynamics is required to observe the stability of these active complexes as well as further experimental research to confirm our understanding, especially regarding the mechanism of apoptosis action. Consequently, these alkaloids have the potential as selective Bcl-xL inhibitors, causing cancer cells to undergo apoptosis, resembling the action of BH3-only protein.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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