RESEARCH ARTICLE

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Computational Insights into the Interaction of Pinostrobin with Bcl-2 Family Proteins: A Molecular Docking Analysis

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Abstract

Background: Cancer research has emphasized the Bcl-2 family of proteins because of their interaction in apoptosis process, a critical mechanism that regulates cellular survival and death. Recently small molecules from diverse sources have gained much attention in anticancer research due to their promising inhibitory action against Bcl-2 and Bcl-X_L that are pointedly known as the members of anti-apoptotic Bcl-2 family of proteins. Pinostrobin (PN) is a natural flavonoid with diverse pharmacological potential emerged as a molecule of interest as anticancer agent. The present study aims to screen the interaction of PN with anti-apoptotic protagonists Bcl-2 and Bcl- X_L at the molecular level through docking studies. **Method:** The molecular docking was performed using the Schrodinger software. The docking score of PN with the Bcl-2 (4IEH) and Bcl-X_L (3ZK6) and their molecular interactions was examined and analysed. **Results:** The result of the molecular docking analysis showed that PN and the anti-apoptotic proteins 4IEH and 3ZK6 had significant interactions and docking energy scores (ΔG) were found to be -5.112 kcal/mol and -7.822 kcal/mol respectively. The small molecule PN illustrated effective interaction with the active site amino acids of the Bcl-2 and Bcl-X_L proteins and has been associated through traditional hydrogen bond with 4IEH. Further, it was observed that PN and anti-apoptotic Bcl-2 proteins interaction was stabilized by other non-covalent interactions, such as π-alkyl or π-π interactions and van der Waals forces.**Conclusions:** This was the first study to reveal the inhibitory action of PN against anti-apoptotic Bcl-2 and Bcl-X_L proteins at the molecular level. The findings of this study concludes that PN ability to inhibit anti-apoptotic proteins, Bcl-2 and Bcl-X_L could be useful to induce intracellular apoptosis in tumorous cells.

Keywords: Pinostrobin- Apoptosis- Molecular Docking- Bcl-2 family- Anti-apoptotic proteins

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Introduction

Apoptosis is a natural process of programmed cell death that occurs in multicellular organisms and plays key role in regulating cell growth under physiological and pathological conditions [1]. Dysregulation of apoptosis has been reported to be associated with several diseases including, ischemia damage, cancer, cardiovascular diseases, autoimmune and neurological disorders [2]. The two basic pathways by which apoptosis is initiated include either the activation of death receptors (extrinsic pathway) or the mitochondria (intrinsic pathway) [3]. Bcl-2 family proteins are involved in the regulation of apoptosis and are involved in both pathways. The typical structure of Bcl-2 family proteins is a hydrophobic α-helix surrounded by amphipathic α-helices [4]. Anti-apoptotic Bcl-2 proteins have recently attracted attention as prospective cancer therapeutic targets. Small molecules that disrupt the interactions of these proteins could enhance cancer cells to be more susceptible to apoptosis [5].

Flavonoids, a class of polyphenolic substances, have been studied for possible interactions with the Bcl-2 family of proteins [6]. Some flavonoids may regulate the expression and activity of Bcl-2 family members, affecting the balance between cell survival and death [7, 8]. Pinostrobin (PN) is an isoflavone with the primary flavanone structure which is made up of a central phenyl ring connected to a benzopyran ring (Figure 1). Furthermore, it can interact with proteins because of the presence of hydroxyl groups and also possesses pharmacological properties such as anti-oxidant, anti-cancer, anti-ulcer, and anti-aromatase effects [9]. Scientific studies have shown that PN may have the ability to induce apoptosis in cancer cell lines [10].

PN appears to have qualities that would make it a successful small molecule therapy since it follows Lipinski's rule of five. The ability of PN for effective absorption, distribution, and focused action within the human body is indicated by its appropriate size, lipophilicity, and limited hydrogen bonding capabilities [11]. Because of its conformity to Lipinski's principles, PN is a potential candidate for further pharmaceutical

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research and development as a therapeutic agent.

The study aimed to identify the precise conformation of the ligand in the protein's active site and determine its binding affinity by in silico docking analysis. The Schrodinger Grid-based Ligand Docking with Energetics (Glide) was considered the most precise tool for conformational investigations among the most frequently used docking programs [12, 13]. This could investigate whether PN could interact with the anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-X_L through Glide docking analysis.

Materials and Methods

A computational method known as "Molecular Docking" is employed in the drug development process to forecast and examine the binding interactions between a small molecule and a target molecule, such as a protein or nucleic acid [14]. The widely used software program for molecular docking, the Schrodinger Maestro Suite (Schrodinger, LLC), contains different modules such as LigPrep, SiteMap, Protein Preparation Wizard, Grid Generation, Glide Docking. Glide Docking is a docking tool that was employed for the ligand-target docking investigations [15]. This docking software offers a strong and flexible platform for molecular docking research. For its high level of precision and dependability, the Schrodinger software has established a strong reputation in the scientific community.

In silico Validation

Preparation of Protein Structure

The 3D crystal structure of Bcl-2 (4IEH) and Bcl-X_L (3ZK6) proteins were retrieved from the Research Collaboratory for Structural Bioinformatics, Protein Data Bank for Docking analysis (Table 1). The receptor protein structures were pre-processed for the docking analysis by the Protein Prep Wizard module [16]. The ions, ligand atoms, and crystal water molecules that were attached to the proteins are removed. The protein structure was then minimized with caution using the Optimized Potential for Liquid Simulations (OPLS) - 2005 force field to reposition side-chain hydroxyl groups and prevent potential steric conflicts [17].

Preparation of Ligand Structure

The ligand is a small molecule, important in the molecular docking process because its binding to the target can have an impact on the creation of novel therapies or provide information about molecular recognition [2]. The chemical structure of ligand, PN was obtained from the Public Chemical compound database, PubChem CID: 4101463 (Figure 2). The structural optimization and conformer generation for the ligand PN are carried out using Ligprep, a computer program included in Schrodinger's computational software package (User Manual). Using the Epik module, the ionization and tautomeric states were produced between pH 6.8 and 7.2 (Figure 2). Utilizing the Impact package of the OPLS-2005 force field, chemicals were minimized in the LigPrep's final stage [18].

Molecular Docking

The prepared ligand and protein structures were saved as files. Molecular docking between PN and antiapoptotic proteins (Bcl-2 and Bcl-X_L) was performed using the Schrodinger Maestro Suite [19]. For ligand interaction, the grid box was constructed at the centroid of the active site using specified Cartesian coordinates. The Sitemap tool's binding site residue identification was used to generate the receptor grid in the Maestro Glide application [20]. This allowed for the creation of a highly specialized receptor grid that aided in precise ligand docking. The OPLS-2005 force field was used to refine the docking, which includes the torsional and rigid body movements of the ligands. The ligands were subsequently docked to the protein using the Glide docking methodology, also referred to as Grid-based Ligand Docking with Energetics (Glide XP). This mode, which is renowned for its remarkable precision, allowed for a thorough investigation of the ligand-protein interactions and offers insightful information that may be used in future drug development and design projects. Using the Glide (G) Score, the docked conformers were assessed [13].

Results

In the present study, the interaction between the ligand (PN) and the anti-apoptotic target proteins, 4IEH and 3ZK6 were examined using the Schrodinger's GLIDE docking (Figure 3, 4). Based on the binding affinity and non-covalent interactions that existed between PN and 4IEH and 3ZK6. The docking score and Glide Emodel energy values were obtained and presented in Table 2.

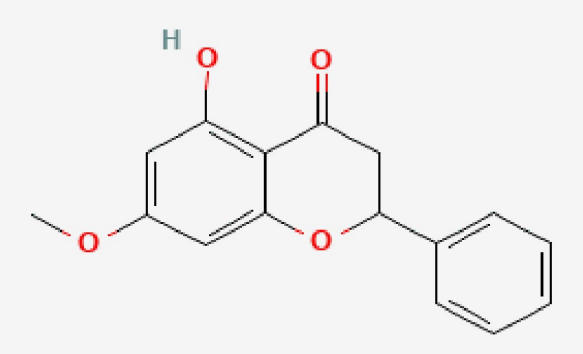

Figure 1. Two Dimensional Structure of Pinostrobin

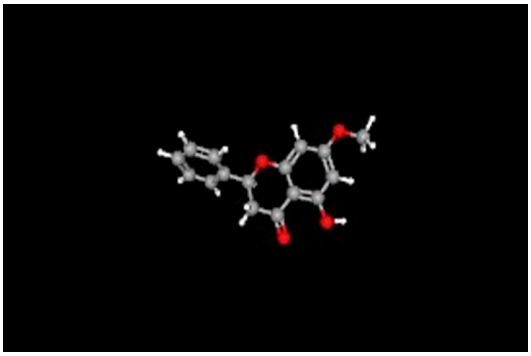

Figure 2.Three-Dimensional Structure of Pinostrobin

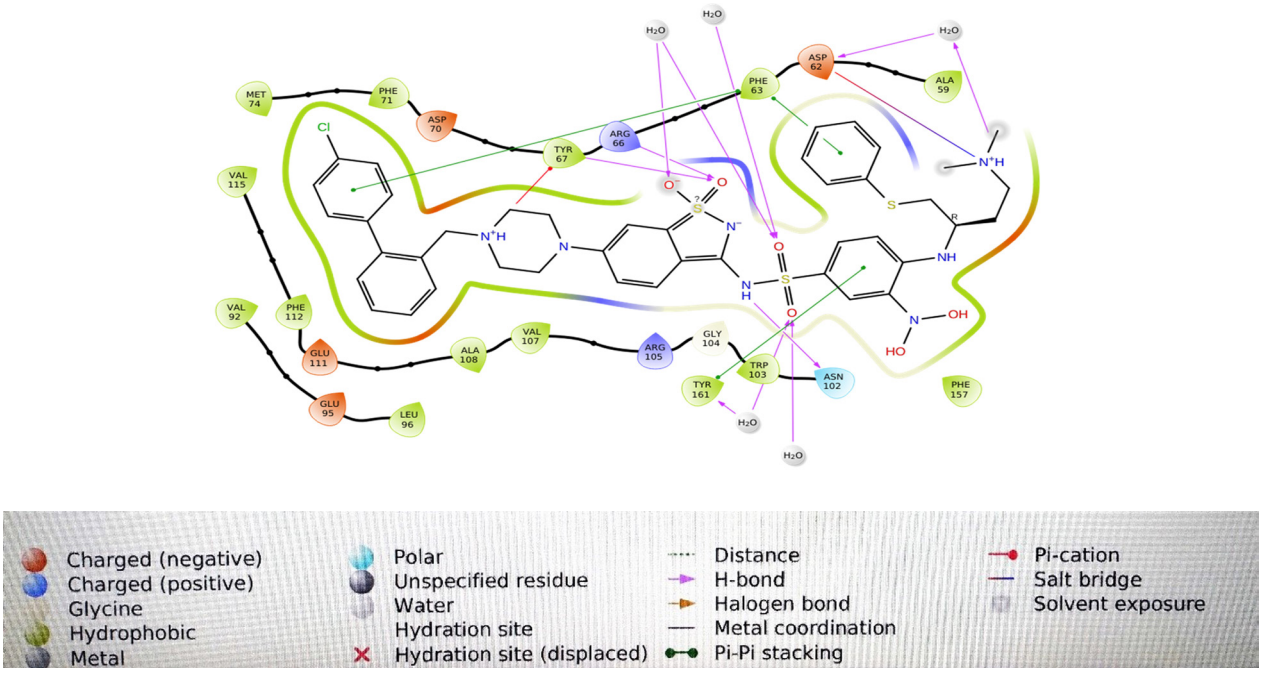

Figure 3. The 2D Ligand Interaction Diagram of Pinostrobin with 4IEH

Docked with Bcl-2 protein (41EH)

The docking score represented that PN and 4IEH interacted with a binding affinity of -5.112 kcal/mol (Table 2). Hydrogen bond interactions were formed when PN interacted with 4IEH specifically with the amino acid residues of ASN 102, TRP 103, and TYR 161. π-π stacking was observed with PHE 63 and TYR 161 residues. Additionally, π cation interaction was noticed with TYR 67 residue during docking with PN (Figure 3).

Docked with Bcl-X_L (3ZK6)

Docking analyses of PN with 3ZK6 demonstrated good interaction with a binding affinity of -7.822 kcal/mol when compared to 4IEH (Table 2). In molecule interaction the classic hydrogen bonds were observed in the active site pocket of Bcl-X_L protein with ASN 136, ARG 139, SER 106, and LEU 108 residues. Moreover, one π-π stacking was observed with the PHE 105 residue during the binding of ligand (Figure 4).

Discussion

This study demonstrates the critical role played by molecular docking in anticipating the molecular interactions of the small molecule PN with the targeted protein. The pharmaceutical industry makes extensive

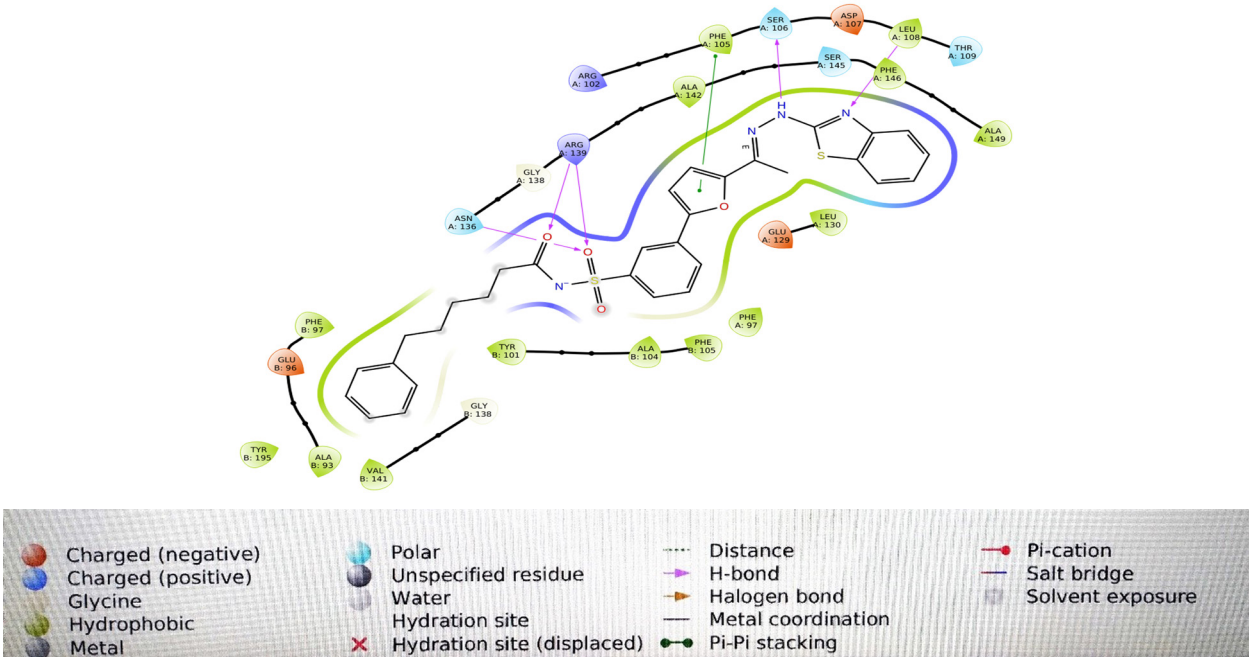

Figure 4. The 2D Ligand Interaction Diagram of Pinostrobin with 3ZK6

Table 1. Details of Anti-Apoptotic Proteins Docked with Pinostrobin

S. No.	Protein	PDB ID	Resolution	Description
1	Bcl-2	4IEH	2.10Å	Crystal structure of human Bcl-2 in complex with a small molecule
2	Bcl-X_L	3ZK6	2.48Å	Crystal structure of Bcl-XL in complex with inhibitor 2

Table 2. Docking Scores and Energy Values of Pinostrobin with Bcl-2 and Bcl- X_L Proteins

Ligand	Protein	PDB ID	Docking Score (kcal/mol)	Glide Emodel Energy (kcal/mol)
Pinostrobin	Bcl-2	4IEH	-5.112	-39.548
Pinostrobin	Bcl-X_L	3ZK6	-7.822	-53.529

use of this application as a potent tool, notably in the investigation of structure-activity relationships [7]. The capacity to precisely predict how small molecule ligands will bind to their specified target binding sites is provided by molecular docking [21]. The hydrogen bonding interaction between a small molecule and a protein determines the effectiveness of the molecule in inhibiting the protein [22]. We used the Glide docking program to examine the probable binding patterns and interaction processes of PN. The evaluation of molecular docking results, including binding affinity is used to identify promising ligands [23, 24]. Glide score and binding energy were used to assess the interaction. The Glide score evaluates how efficiently a ligand fits into the target protein's active site. The interaction of PN with active sites of Bcl-2 and Bcl-X_L proteins was the focus of our work. The ability of apoptosis to prevent and treat cancer is frequently seen as a beneficial mechanism. Bcl-2 and Bcl-X_L, the targeted antiapoptotic proteins are important regulators of the intrinsic apoptotic process. In comparison to other Bcl-2 protein family members, these two proteins are more crucial for apoptotic signalling [25]. The intrinsic mechanism of mitochondrial apoptosis, which is under the primary control of the Bcl-2 family, is a form of cell death. Inducing mitochondrial outer membrane permeabilization and starting the production of apoptogenic factors, which results in caspase activation and apoptosis, can be done by proapoptotic members in response to external or internal stimuli [26]. In addition to the mitochondrial pathway, the extrinsic pathway, which depends on cell surface death receptors including Fas and tumor necrosis factor receptors, can also cause apoptosis. These death receptors when activated cause the recruitment and activation of caspases, which results in programmed cell death [27]. It's interesting to note that by interacting with these death receptors, the Bcl-2 family of proteins can modify the cell's reaction to death signals and affect the extrinsic apoptosis pathway [28]. The binding interactions of several flavonoids with Bcl-2 family proteins were thoroughly investigated in a similar work [29]. The results of their research, despite not being directly focused on PN, highlight the great potential of flavonoids in altering the complex apoptotic pathways directed by Bcl-2 protein family members. Also on many cancer cell lines, pinostrobin chalcone and pinocembrin have antiproliferative properties which have structural similarity with PN [30]. Together, these findings point to the possibility that PN, a small molecule, can affect cellular death by interacting with Bcl-2 family proteins, supporting the growing body of research on the potential therapeutic value of small molecules in this area. The molecular docking analysis of several small molecules showed a binding affinity with the Bcl-2 family [31]. The similarities between the outcomes of the research highlight the important role Bcl-2 plays in modulating cellular functions. This common evidence highlights the positive direction for future studies into the therapeutic potential of these substances in the context of cancer treatment. This study further advances our understanding of the complex interplay between cell survival and apoptosis regulation by shedding light on how small molecules have a potential effect on important cellular pathways.

In conclusion, this was the first study to reveal the interaction of PN with the context of Bcl-2 family proteins through molecular docking analysis and proposed to document its antitumorigenic potential. The findings of the in-silico results conclude the effectiveness of PN as an inhibitor of antiapoptotic protein and could be a promising lead molecule with anticancer property. However, further validation by in vitro studies is warranted to support its cytotoxic effects in malignant cells.

Author Contribution Statement

The author(s) have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Conflict of interest

The authors declare that there is no conflict of interest.

Study Limitations

The major focus of this study is to identify the molecular targets of PN in cancer by in-silico analysis. To support the current hypothesis, the data should be assessed with in vitro and in vivo experiments to strengthen the findings of this study and their further application.

Future Directions

The current findings provide an essential foundation to identify the anticancer potential and to understand the therapeutic action of PN in malignancy. The present computational discoveries with experimental findings help to identify novel therapeutic strategies that could have a substantial impact in the field of cancer treatment. Further in vitro and in vivo studies should be performed to measure the targeted anticancer effect of PN. By bridging the gap between in silico predictions and biological reality, these additional experimental studies are needed to validate the therapeutic efficacy of PN.

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