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Research Article

Characterizing the ligand-binding mechanisms of human anti-apoptotic Bcl-2 protein through insilico studies

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A B S T R A C T

Due to its central role in controlling apoptosis, Bcl-2 has attracted interest as a viable target in cancer therapy. This research aims to explore the structural dynamics and stability of Bcl-2 upon binding with the inhibitor Asiaticoside, while also identifying additional viable sites for drug targeting. We employed insilico methods to gain insights into the ligand binding mechanism Bcl-2. Regulation of apoptosis significantly depends on the flexible loop domain (FLD).

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1. Introduction

Cancer is widely acknowledged not only as a highly detrimental health issue but also as a complex set of phenomena intricately linked to the essential queries of evolution, multicellularity, pattern dysregulation, and the relationship between the genome and the environment [1, 2]. Tumor development is characterized by the acquisition of six key biological traits: persistent growth signaling, the ability to bypass growth-inhibitory mechanisms, evasion of apoptosis, unlimited replication potential, promotion of blood vessel formation, and the capacity for tissue invasion and metastasis. These hallmark features are supported by genomic instability and chronic inflammation, which contribute to genetic variation and enhance the manifestation of these cancer-driving processes [3]. Maintaining tissue homeostasis depends on a tightly regulated balance between cellular proliferation and programmed cell death. Disruptions in this balance, particularly reduced rates of cell death, can contribute to tumor formation. As discussed earlier, the ability of cancer cells to evade apoptosis is a hallmark feature, typically driven by genetic alterations that affect the activity or expression of key regulatory proteins [4]. This resistance mainly stems from dysregulation of apoptotic pathways, where both genetic and epigenetic changes play critical roles [5, 6]. Many apoptotic signals converge on mitochondria, where they can cause rapid permeabilization of the outer mitochondrial membrane. The permeabilization of the mitochondrial outer membrane (MOMP) represents a critical step in apoptosis, facilitating the release of pro-apoptotic molecules such as cytochrome-c and Smac/DIABLO into the cytosol [7]. Following its release, cytochrome-c binds to Apaf-1, leading to the assembly of the apoptosome complex. This complex activates initiator caspases, including Caspase-9, which in turn stimulate executioner caspases such as Caspase-3 and Caspase-7. These proteases orchestrate the systematic breakdown of the cell during apoptosis [8].

Studies show that cancer cells frequently exhibit decreased amounts of proteins that promote apoptosis or elevated levels of anti-apoptotic molecules, including inhibitors of apoptosis proteins (IAPs) and Bcl-2 family members, which help them avoid programmed cell death [5, 6, 9]. Among these proteins, Bcl-2 holds a particularly significant position. It was initially discovered as a proto-oncogene in follicular B-cell lymphoma, where a chromosomal translocation places it under the regulation of the immunoglobulin heavy chain enhancer, causing its overexpression[10]. Bcl-2 shares homology with ced-9, an apoptosis inhibitor in *C. elegans*. Bax, the first identified pro-apoptotic member of the Bcl-2 family, has been shown to interact directly with Bcl-2

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[11]. In humans, there are at least 16 Bcl-2 homologs, some inhibit apoptosis (e.g., Bcl-2, Bcl-xL, Mcl-1), while others promote it (e.g., Bax, Bak, Bid, Bim). Normal tissue homeostasis relies on the balance between these opposing proteins; abnormal expression of Bcl-2 proteins has been linked to diseases like autoimmunity, neurodegeneration, and cancer [12].

The main function of Bcl-2 family members is to regulate apoptosis. Despite nearly a decade since their structures were elucidated, these proteins continue to reveal functional complexity and diversity [13]. Beyond their role in apoptosis, emerging evidence suggests Bcl-2 proteins influence many other cellular processes, often independent of cell death regulation. Disruptions in apoptosis regulation are involved in numerous diseases, including cancer and neurodegenerative disorders [14]. In humans, Bcl-2 exists as two isoforms, alpha and beta, with the alpha variant being the most extensively researched. This protein features four Bcl-2 homology (BH) domains, a flexible loop region, and a transmembrane segment. The BH1 through BH3 domains create a hydrophobic pocket essential for binding pro-apoptotic BH3-only proteins such as Bax and Bak. Targeting this interaction has become a promising approach in cancer treatment, where BH3 mimetics, either synthetically developed or naturally derived small molecules, serve to inhibit the function of anti-apoptotic Bcl-2 proteins [4].

Among these mimetics, Asiaticoside, a naturally occurring triterpenoid saponin derived from Centella asiatica, has emerged as a promising Bcl-2 inhibitor [15-17]. Liang et al. reported that Asiaticoside exhibits protective properties against oxidative damage and apoptosis in endothelial cells by triggering the ROS-mediated p53/Bcl-2/Caspase-3 signaling pathway [17].

This research utilized molecular docking alongside molecular dynamics (MD) simulations to examine the interaction patterns and stability of Asiaticoside when bound to the human Bcl-2 protein. To address the solubility issues associated with the full-length protein, we used a chimeric form of Bcl-2, wherein the flexible loop domain was replaced with a corresponding loop from Bcl-xL, following the method established by Petros et al. Our computational findings reveal that Asiaticoside forms a stable complex with Bcl-2, inducing notable conformational changes and enhancing protein stability. Moreover, we examined the impact of non-synonymous single nucleotide polymorphisms (nsSNPs) in the Bcl-2 coding sequence on structural flexibility and ligand interaction, offering insights into the genetic variability that may influence apoptotic regulation and cancer susceptibility.

2. Materials and Methods

2.1. Chimeric Bcl-2: Asiaticoside Complexes

This study investigates the interactions between the human Bcl-2 protein and ligands through computational approaches. Computational analyses included comparisons between chimeric forms. Docking outcomes were examined and visualized using PyMOL software, version 1.3.

2.2. Molecular Dynamics

Molecular dynamics simulations were performed with Gromacs version 4.6.5 to assess the impact of Asiaticoside binding on the chimeric Bcl-2 protein (PDB ID: 1GJH). Additionally, a full-length model of the human Bcl-2 protein was constructed using the intensive mode protocol available in Phyre2. Simulations of the chimeric Bcl-2 protein without any bound ligand were carried out within a cubic box filled with water molecules. To mimic physiological conditions and neutralize charges, sodium ions were included along with over twelve thousand water molecules. After minimizing the system's energy and stabilizing temperature and pressure, the simulations were performed at body temperature (37°C) and atmospheric pressure (1 bar). For the complex involving Asiaticoside, the CHARMM27 force field (including CMAP corrections) was applied, and the molecular structure of Asiaticoside was created using an automated topology generation tool. This simulation employed a similar cubic water box setup but contained over twenty-two thousand flexible water molecules, with sodium ions introduced to maintain electrical neutrality. The simulation results were then examined using the analysis functions embedded in the Gromacs software package.

3. Results and Discussion

3.1. Molecular Docking of the Bcl2: Asiaticoside Complex

Molecular dynamics simulations offer a valuable approach to studying protein behavior over time, providing insights into their stability, flexibility, folding, and dynamic properties, complementing docking calculations, which typically treat the target protein as a static, rigid structure. Molecular dynamics (MD) simulations enable the study of the movements and interactions of proteins and their complexes with other biomolecules—such as proteins, small molecules, carbohydrates, and lipids—within practical time scales and cost-effective parameters. These analyses often yield essential structural insights, including measures of overall atomic displacement (RMSD), localized atomic movement (RMSF), compactness of the molecule (radius of gyration), hydrogen bonding patterns within and between molecules, exposure of molecular surfaces to solvent, distribution of secondary structural elements, and the frequency with which specific residues occupy certain positions.

To investigate how Asiaticoside binds to Bcl-2, molecular docking studies (Fig. 1 and 2) were conducted using its chimeric conformations. The docking orientation of Asiaticoside within the Bcl-2 protein was examined. Fig. 1 presents a high-resolution cartoon model of Bcl-2 complexed with Asiaticoside, with secondary structural elements displayed in a rainbow color scheme. Asiaticoside is shown as green sticks, positioned intimately within the binding groove. The interactions between Asiaticoside and key Bcl-2 residues, such as hydrogen bonds and hydrophobic contacts, are illustrated and annotated with interatomic distances. This detailed view confirms that Asiaticoside establishes multiple specific interactions within the Bcl-2 groove, involving the same critical residues as reported for well-characterized inhibitors like venetoclax and navitoclax [18, 19].

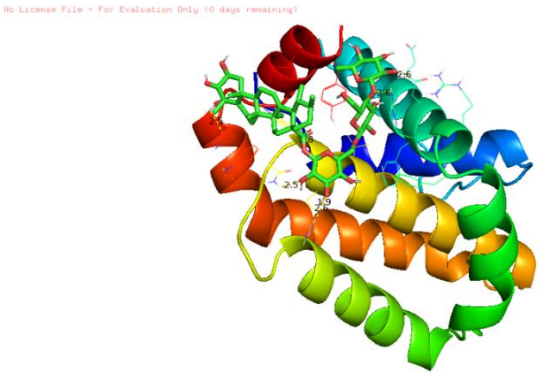

Fig. 1. Docked conformation of Asiaticoside within the Bcl-2 binding site. The Bcl-2 protein is represented as a rainbow-colored cartoon, and Asiaticoside is depicted in green stick format. Key interactions and distances between Asiaticoside and neighboring Bcl-2 residues are indicated. Visualization generated using PyMOL.

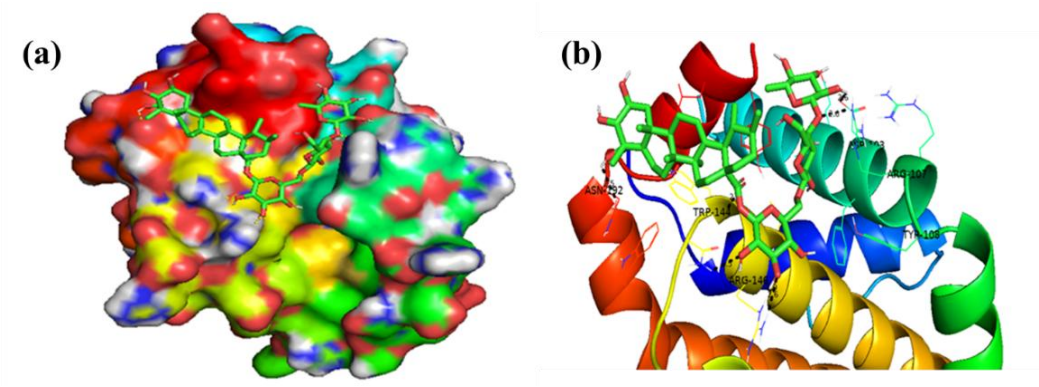

Fig. 2. (a) Surface representation of the Bcl-2 protein displaying Asiaticoside (green sticks) bound within its binding pocket. The protein's surface is visually represented by mapping electrostatic charges, where areas of negative charge appear in red and regions of positive charge are shown in blue. (b) Cartoon representation of Bcl-2 (rainbow-colored ribbon) complexed with Asiaticoside (green sticks), showing key interacting residues as sticks and labeled. Visualization generated using PyMOL. Stability and structural shape are two fundamental characteristics influencing how ligands bind to proteins.

The close proximity and specific hydrogen bond networks suggest that Asiaticoside fits tightly and selectively, supporting a stable binding mode. Such molecular docking outcomes are consistent with previous computational and crystallographic studies showing natural triterpenoids or glycosides engaging Bcl-2 through similar key interactions [20]. Therefore, these results provide computational validation that Asiaticoside can serve as a promising Bcl-2 modulator, encouraging further biophysical and cellular studies to confirm its functional inhibition properties.

Fig. 1 illustrates the ribbon model of the Bcl-2 protein in complex with Asiaticoside, emphasizing the spatial orientation of key residues and their direct interactions with the ligand. The protein is color-coded by secondary structure, progressing through the spectrum (red to violet) to reflect the orientation of α-helices and connecting loops. Asiaticoside is displayed in green stick representation, docked into the hydrophobic groove of Bcl-2, an area corresponding to the BH3-binding domain responsible for interactions with pro-apoptotic proteins.

Several critical residues are observed forming direct hydrogen bonds or non-covalent interactions with Asiaticoside. Notably, the hydrogen bond distances are labeled (e.g., 2.5–2.6 Å), confirming strong and favorable binding. Such proximity is indicative of energetically stable interactions, reinforcing the proposed inhibitory role of Asiaticoside on Bcl-2 function. The alignment of Asiaticoside within this groove suggests it effectively mimics the BH3-domain binding motif, thus potentially displacing or preventing binding of native pro-apoptotic partners.

The clear visualization of hydrogen bonds and spatial geometry further validates Asiaticoside's ability to stably occupy and interact with the active site of Bcl-2. These results support the concept of natural product-derived Bcl-2 inhibitors and underscore Asiaticoside's promise as a lead compound for apoptosis-inducing cancer therapeutics [21].

4. Conclusion

In this study, the interaction between human Bcl-2 protein and the natural ligand Asiaticoside was investigated using a computational approach. Our results reveal that binding of Asiaticoside induces significant conformational and dynamic changes in the Bcl-2 protein, suggesting a stabilizing effect upon complex formation. Insilico modeling further elucidated the atomic-level interactions between Asiaticoside and Bcl-2, including key hydrogen bonds and hydrophobic contacts within the BH3-binding groove. These interactions contribute to the ligand's high binding affinity and specificity.

Asiaticoside demonstrated strong and stable binding to the chimeric Bcl-2 structure, supporting its potential as a natural Bcl-2 inhibitor. Notably, its binding was effective regardless of the presence of the full-length domain, indicating that the modified construct retains the critical structural features required for ligand recognition. The computational findings provide mechanistic insight into Asiaticoside's mode of action and reinforce its candidacy as a promising lead compound for apoptosis-targeted cancer therapy.

Panel (a) in Fig.2. displays the molecular surface of Bcl-2 colored by electrostatic and hydrophobic properties, with Asiaticoside clearly positioned within the binding groove. The ligand, depicted as green sticks, occupies a pocket surrounded by both charged and neutral regions, suggesting favorable interactions in a chemically diverse environment. Panel (b) in Fig. 2. presents a detailed ribbon (cartoon) view of the Bcl-2 protein, highlighting key interacting, directly involved in Asiaticoside binding. These amino acids are shown forming multiple contacts with the ligand, and the cartoon is rainbow-colored to show chain orientation.

The visualization demonstrates that Asiaticoside binds strongly within the canonical groove of Bcl-2, overlapping with the regions known to be important for the recognition of small-molecule inhibitors [22]. The presence of both charged (red/blue) and hydrophobic (yellow/green) surface patches at the binding interface may enhance the ligand's affinity via a combination of electrostatic and van der Waals interactions. The network of Asiaticoside interactions shows how natural and synthetic compounds can inhibit Bcl-2, indicating that similar mechanisms may drive Asiaticoside's biological activity This molecular evidence supports the notion that Asiaticoside could act as an effective Bcl-2 inhibitor by stabilizing the protein in an inactive conformation through multifaceted binding.

The interaction between Asiaticoside and the anti-apoptotic Bcl-2 protein was investigated through molecular docking, and the results are illustrated in Fig. 2. Panel (a) shows the molecular surface of Bcl-2, highlighting the physicochemical characteristics of the binding pocket. Asiaticoside is positioned within a prominent hydrophobic groove, corresponding to the BH3-binding domain, a critical site for protein–protein interactions involved in apoptosis regulation [23]. The surface is color-coded to reflect the distribution of electrostatic and hydrophobic regions, with red indicating negatively charged residues, blue for positively charged, green and yellow for hydrophobic, and white for polar residues. The spatial fit and chemical complementarity suggest a stable interaction between Asiaticoside and the Bcl-2 surface.

Panel (b) provides a detailed ribbon representation of Bcl-2, emphasizing the specific molecular interactions stabilizing the Asiaticoside-Bcl-2 complex. The ligand is shown interacting with several key amino acid residues. These interactions are indicative of hydrogen bonding, electrostatic attractions, and π-π stacking, particularly involving aromatic residues. The anchoring of Asiaticoside within the hydrophobic pocket of Bcl-2 likely mimics the binding mode of native pro-apoptotic BH3-only proteins, thereby potentially inhibiting Bcl-2's function and promoting apoptosis in cancer cells. These findings support the hypothesis that Asiaticoside can act as a natural Bcl-2 antagonist and may serve as a promising lead compound in anticancer drug development [24, 25].

Author Contributions

Neda Tamimi: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data is available.

Ethical issues

The author confirms full adherence to all ethical guidelines, including the prevention of plagiarism, data fabrication, and double publication.

References

[1] D. Moore, S.I. Walker, M. Levin, Cancer as a disorder of patterning information: computational and biophysical perspectives on the cancer problem, Convergent Science Physical Oncology 3(4) (2017) 043001 DOI: https://doi.org/10.1088/2057-1739/aa8548.

[2] J. Varshosaz, Advancements in Nanostructured Drug Delivery Systems: A Commentary on Multifunctional Approaches for Cancer and Inflammatory Disease Treatment, Journal of Composites and Compounds 5(16) (2023) 210-211 DOI: https://doi.org/10.61186/jcc.5.3.7

[3] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, cell 144(5) (2011) 646-674 DOI: https://doi.org/10.1016/j.cell.2011.02.013.

[4] J. Ramos, J. Muthukumaran, F. Freire, J. Paquete-Ferreira, A.R. Otrelo-Cardoso, D. Svergun, A. Panjkovich, T. Santos-Silva, Shedding light on the interaction of human anti-apoptotic Bcl-2 protein with ligands through biophysical and in silico studies, International journal of molecular sciences 20(4) (2019) 860 DOI: https://doi.org/10.3390/ijms20040860.

[5] J. Plati, O. Bucur, R. Khosravi-Far, Dysregulation of apoptotic signaling in cancer: molecular mechanisms and therapeutic opportunities, Journal of cellular biochemistry 104(4) (2008) 1124-1149 DOI: https://doi.org/10.1002/jcb.21707.

[6] C.-X. Xu, H. Jin, M.-H. Cho, Apoptosis and apoptosis-based therapy in lung cancer, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 9(9) (2009) 952-957 DOI: https://doi.org/10.2174/187152009789377682.

[7] S.H. Suhaili, H. Karimian, M. Stellato, T.-H. Lee, M.-I. Aguilar, Mitochondrial outer membrane permeabilization: a focus on the role of mitochondrial membrane structural organization, Biophysical Reviews 9(4) (2017) 443-457 DOI: https://doi.org/10.1007/s12551-017-0308-0.

[8] T. Tokár, J. Uličný, Computational study of the mechanism of Bcl-2 apoptotic switch, Physica A: Statistical Mechanics and its Applications 391(23) (2012) 6212-6225 DOI: https://doi.org/10.1016/j.physa.2012.07.006.

[9] G. Lessene, P.E. Czabotar, P.M. Colman, BCL-2 family antagonists for cancer therapy, Nature reviews Drug discovery 7(12) (2008) 989-1000 DOI: https://doi.org/10.1038/nrd2658.

[10] N. Masir, L.J. Campbell, L.K. Goff, M. Jones, T. Marafioti, J. Cordell, A.J. Clear, T.A. Lister, D.Y. Mason, A.M. Lee, BCL2 protein expression in follicular lymphomas with t(14;18) chromosomal translocations, Br J Haematol 144(5) (2009) 716-25 DOI: https://doi.org/10.1111/j.1365-2141.2008.07528.x.

[11] B. Antonsson, J.-C. Martinou, The Bcl-2 protein family, Experimental cell research 256(1) (2000) 50-57 DOI: https://doi.org/10.1006/excr.2000.4839.

[12] A.M. Petros, A. Medek, D.G. Nettesheim, D.H. Kim, H.S. Yoon, K. Swift, E.D. Matayoshi, T. Oltersdorf, S.W. Fesik, Solution structure of the antiapoptotic protein bcl-2, Proceedings of the National Academy of Sciences 98(6) (2001) 3012-3017 DOI: https://doi.org/10.1073/pnas.041619798.

[13] M. Vogler, Y. Braun, V.M. Smith, M.-A. Westhoff, R.S. Pereira, N.M. Pieper, M. Anders, M. Callens, T. Vervliet, M. Abbas, S. Macip, R. Schmid, G. Bultynck, M.J.S. Dyer, The BCL2 family: from apoptosis mechanisms to new advances in targeted therapy, Signal Transduction and Targeted Therapy 10(1) (2025) 91 DOI: https://doi.org/10.1038/s41392-025-02176-0.

[14] W.A. Siddiqui, A. Ahad, H. Ahsan, The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update, Archives of toxicology 89(3) (2015) 289-317 DOI: https://doi.org/10.1007/s00204-014-1448-7.

[15] A. Bilal, F. Tanvir, S. Ahmad, S.H.A. Shah, H. Ahmad, N. Kanwal, Pre-clinical study of the bioactive compound Asiaticoside against the proteins inducing human mammary carcinoma using molecular docking and ADME analysis, Remittances Review 9(2) (2024) 3543-3576 DOI: https://doi.org/10.33282/rr.vx9i2.183.

[16] Z. Liang, Y. Chen, R. Gu, Q. Guo, X. Nie, Asiaticoside prevents oxidative stress and apoptosis in endothelial cells by activating ROS-dependent p53/Bcl-2/Caspase-3 signaling pathway, Current molecular medicine 23(10) (2023) 1116-1129 DOI: https://doi.org/10.2174/1566524023666221024120825.

[17] C.-L. Xu, Q.-Z. Wang, L.-M. Sun, X.-M. Li, J.-M. Deng, L.-F. Li, J. Zhang, R. Xu, S.-P. Ma, Asiaticoside: attenuation of neurotoxicity induced by MPTP in a rat model of Parkinsonism via maintaining redox balance and up-regulating the ratio of Bcl-2/Bax, Pharmacology Biochemistry and Behavior 100(3) (2012) 413-418 DOI: https://doi.org/10.1016/j.pbb.2011.09.014.

[18] T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, R.C. Armstrong, D.J. Augeri, B.A. Belli, M. Bruncko, T.L. Deckwerth, J. Dinges, P.J. Hajduk, An inhibitor of Bcl-2 family proteins induces regression of solid tumours, Nature 435(7042) (2005) 677-681 DOI: https://doi.org/10.1038/nature03579.

[19] A.J. Souers, J.D. Leverson, E.R. Boghaert, S.L. Ackler, N.D. Catron, J. Chen, B.D. Dayton, H. Ding, S.H. Enschede, W.J. Fairbrother, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets, Nature medicine 19(2) (2013) 202-208 DOI: https://doi.org/10.1038/nm.3048.

[20] K.E. Adewole, A.A. Ishola, Phytosterols and triterpenes from Morinda lucida Benth (Rubiaceae) as potential inhibitors of anti-apoptotic BCL-XL, BCL-2, and MCL-1: an in-silico study, Journal of Receptors and Signal Transduction 39(1) (2019) 87-97 DOI: https://doi.org/10.1080/10799893.2019.1625062.

[21] K. Pitter, F. Bernal, J. Labelle, L.D. Walensky, Dissection of the BCL-2 family signaling network with stabilized alpha-helices of BCL-2 domains, Methods Enzymol 446 (2008) 387-408 DOI: https://doi.org/10.1016/S0076-6879(08)01623-6.

[22] R.J. Youle, A. Strasser, The BCL-2 protein family: opposing activities that mediate cell death, Nature reviews Molecular cell biology 9(1) (2008) 47-59 DOI: https://doi.org/10.1038/nrm2308.

[23] D. Lama, V. Modi, R. Sankararamakrishnan, Behavior of solvent-exposed hydrophobic groove in the anti-apoptotic Bcl-XL protein: clues for its ability to bind diverse BH3 ligands from MD simulations, PLoS One 8(2) (2013) e54397 DOI: https://doi.org/10.1371/journal.pone.0054397.

[24] A.M. Petros, A. Medek, D.G. Nettesheim, D.H. Kim, H.S. Yoon, K. Swift, E.D. Matayoshi, T. Oltersdorf, S.W. Fesik, Solution structure of the antiapoptotic protein bcl-2, Proc Natl Acad Sci U S A 98(6) (2001) 3012-7 DOI: https://doi.org/10.1073/pnas.041619798.

[25] C. Katz, H. Benyamini, S. Rotem, M. Lebendiker, T. Danieli, A. Iosub, H. Refaely, M. Dines, V. Bronner, T. Bravman, D.E. Shalev, S. Rüdiger, A. Friedler, Molecular basis of the interaction between the antiapoptotic Bcl-2 family proteins and the proapoptotic protein ASPP2, Proc Natl Acad Sci U S A 105(34) (2008) 12277-82 DOI: https://doi.org/10.1073/pnas.0711269105.