Ad-Dawaa' Journal of Pharmaceutical Sciences
ISSN: 2654-7392, E-ISSN: 2654-6973
Vol. 7, No. 1, June 2024, p. 1 – 10
DOI: https://doi.org/10.24252/djps.v7i1.46796

Aaptamine Enhanced Doxorubicin Activity on B-Cell Lymphoma 2 (Bcl-2): A Multi-Structural Molecular Docking Study

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Article history:
Submited: 4-5-2024
Revised: 21-5-2024
Accepted: 5-6-2024

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Cite this aticle: Setiawansyah, A., Susanti, G., Alrayan, R., Hadi, I., Arsul, M. I., Luthfiana, D., Wismayanti, L., Hidayati, N. (2024). Aaptamine Enhanced Doxorubicin Activity on B-Cell Lymphoma 2 (Bcl-2): A Multi-Structural Molecular Docking Study. Ad-Dawaa' J. Pharm. Sci. 7(1): 1-10.

ABSTRACT

Doxorubicin, a widely used chemotherapeutic agent, targets Bcl-2, but its efficacy can be limited by drug resistance. Its combination with natural derived compound can be a therapeutic approach to overcome this problem. This study aimed to investigate the molecular interactions and binding affinities of aaptamine and doxorubicin with Bcl-2 using molecular docking simulations, and to evaluate the potential synergistic effects of their combination. Molecular docking studies were performed to predict the binding modes and affinities of aaptamine and doxorubicin along with their combination to Bcl-2. Molecular docking simulation results showed that aaptamine binds to the BH3 binding groove of Bcl-2, forming key interactions with residues like Asp70, Tyr67, Phe112 and Glu111. Aaptamine stabilized the binding of doxorubicin to Bcl-2 through hydrophobic bonding and van der Waals interactions, resulting in enhanced binding affinity. The combination of aaptamine and doxorubicin exhibits synergistic anticancer effects by enhancing the binding affinity of doxorubicin to Bcl-2 (from -6.809 ± 0.059 to -8.131 ± 0.391 kcal/mol) via hydrogen bounding, van der Waals forces, and hydrophobic interactions. Molecular docking simulations provided insights into the stabilizing interactions between aaptamine, doxorubicin, and Bcl-2, suggesting a potential strategy for overcoming Bcl-2-mediated drug resistance in cancer.

***KEYWORDS**: Aaptamine, doxorubicin, Bcl-2, molecular docking combination, multicompound docking.*

INTRODUCTION

B-Cell Lymphoma 2 (Bcl-2) is a critical anti-apoptotic protein that plays a crucial role in the development and progression of various cancers, including lymphoma, breast cancer, and lung cancer (Cory & Adams, 2002). Overexpression of Bcl-2 has been associated with resistance to chemotherapeutic agents, leading to poor treatment outcomes and increased mortality rates (Maji et al., 2018).

Consequently, targeting Bcl-2 has emerged as a promising therapeutic strategy for enhancing the efficacy of existing cancer treatments. Doxorubicin, a widely used chem-

otherapeutic agent, has been shown to induce apoptosis in cancer cells by decreasing Bcl-2 expression (Utami et al., 2023). However, its clinical application is often limited by the development of drug resistance and severe side effects, such as cardiotoxicity (Rawat et al., 2021). Therefore, there is a pressing need to explore strategies that can potentiate the anti-cancer effects of doxorubicin while reducing its adverse effects.

One promising approach is the use of combination therapy with anticancer drugs and bioactive compounds from natural sources. Aaptamine is an alkaloid compound initially isolated from marine sponge, *Aaptos sp*., known to have anticancer activity (Aoki et al., 2006; Jin et al., 2011). Previous studies have shown that aaptamine can inhibit cancer cell proliferation through apoptosis induction and angiogenesis inhibition (Dyshlovoy et al., 2014). Combining aaptamine with doxorubicin has the potential to provide synergistic effects in combating cancer cells, as well as reducing the side effects of doxorubicin through dose reduction, since doxorubicin exhibit different binding site to Bcl-2 (Nandana et al., 2023). Recently, the utilization of molecular docking to assess the synergistic effect of small molecule has been used by Wiraswati et al. (2024) that assess the synergistic effect of doxorubicin with pyrazoline B against DNA Topoisomerase I. Several studies have also demonstrated the potential of combining anticancer drugs with natural compounds in enhancing therapy effectiveness. For example, Ashrafizadeh et al. (2020) showed that combining curcumin with doxorubicin can enhance the anticancer activity of doxorubicin and reduce its cardiotoxicity. Additionally, Wang et al. (2016) revealed synergistic effects between the natural compound paclitaxel and doxorubicin in combating lung cancer.

The significance of this research lies in its potential to improve the effectiveness and safety of doxorubicin-based anticancer therapy. By analyzing the synergistic effects between aaptamine and doxorubicin, valuable information can be obtained for the development of more effective and safer combination therapy strategies. Furthermore, this research can provide insights into the mechanism of action of aaptamine and its interaction with doxorubicin at the molecular level. It is hoped that new approaches can be found to enhance the effectiveness of doxorubicin-based anticancer therapy and reduce adverse side effects. This will benefit cancer patients by improving treatment success and their quality of life.

MATERIAL AND METHODS

Materials

The materials used in this study were an Asus personal computer equipped with Intel® core™-i3 with 10Gb of Random Access Memory, 2Gb VGA Intel® HD Graphic Family, and Windows 10-Pro operating system; Autodock 4.2 software, Autodock Tools (ADT) interface (MGL Tools 1.5.7

version), Biovia Discovery Studio 2024 Edition, and Notepad^{++} 8.6.5 version.

Molecular Docking Study

The molecular docking study was carried out in Autodock 4.2 supported by ADT human interface. Bcl-2 was subjected as the target macromolecule in which the x-ray diffraction structure was retrieved from the Protein Data Bank (PDB ID: 4IEH) and 3D structure of aaptamine (PubChem CID: 122826) and doxorubicin (PubChem CID: 31703) as test ligands were downloaded from PubChem database. Prior to molecular docking, the macromolecule was initially prepared in Biovia Discovery Studio to eliminate water, ligands, and heteroatoms. When it is ready, the molecular docking was deployed as follows:

Single compound docking

To prepare for docking studies, the crystallographic structure of Bcl-2 was further processed in Autodock by adding hydrogen atoms. The ligands were defined by setting the center node and the number of rotatable bonds. The docking process was carried out targeting the active site of Bcl-2 at coordinates x: 12.153, y: 25.794, z: 11.85. The grid-box size was adjusted to 50 x 50 x 50 with a spacing of 0.375 Å. A flexible-rigid docking approach was employed using the Genetic Algorithm with 100 runs, default crossover, and mutation rates. The Lamarckian GA was used as the output method. The molecular docking process was repeated ten times to ensure consistency.

Multi-compound docking

A multi-docking process was implemented by attaching the docked compound obtained from single docking technique to Bcl-2 crystallographic structure in Biovia Discovery Studio (see Figure 3). The docked ligand-Bcl2 combined structure was then saved in PDB format. The two version of docked ligand-Bcl2 complex was created including aaptamine-Bcl2 and doxorubicin-Bcl2 complexes. The molecular docking of doxorubicin to aaptamine-Bcl2 and aaptamine to doxorubicin-Bcl2 structures were employed using the same process and parameters in the similar active site coordinate (x: 12.153, y: 25.794, z: 11.85; xyz 50 x 50 x 50) as single compound docking protocol.

Data Analysis

The docking scores were generated computationally in a notepad^{++} and presented as ± SD (n=10) then statistically analyzed using one sample independent t-Test in GraphPad Prism version 9.5.1.

RESULTS AND DISCUSSION

In computational drug discovery, molecular docking is a widely used technique to predict the binding modes and affinities of small molecules (ligands) with target proteins. Validating the docking protocol is a crucial step to ensure the reliability and accuracy of the docking results. One of the key metrics used for validation is the root-mean-square

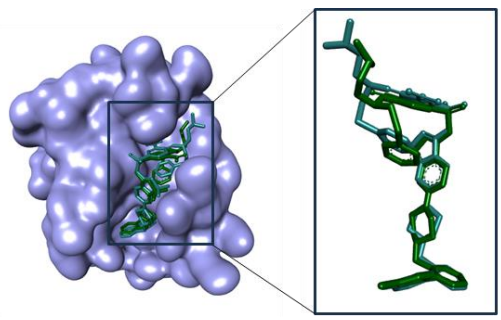

Figure 1. Superimposed of the best re-docking model to Bcl-2 with RMSD value of 1.559 Å (Original: green; Re-docked: blue).

deviation (RMSD) between the predicted and experimentally determined binding poses (Setiawansyah et al., 2022). To validate a docking protocol, a set of protein-ligand complexes with known crystal structures is typically used as a benchmark. The ligands are first extracted from the complexes, and the docking protocol is applied to predict their binding modes. The predicted binding poses are then compared with the experimental structures, and the RMSD values are calculated.

An RMSD value below 2 Angstroms (Å) is generally considered acceptable for a successful docking protocol validation (Huang et al., 2010; Mukherjee et al., 2010). This threshold indicates that the predicted binding poses are reasonably close to the experimental structures (Figure 1), considering the inherent flexibility of proteins and the approximations made in docking algorithms. In the present discussion, the obtained RMSD values for the docking protocol are below 2 Å (1.559 Å), suggesting that the docking method is valid and can reliably predict the binding modes of the ligands with the target protein. This result provides confidence in the docking protocol and its applicability for molecular docking of the test ligands.

Molecular docking studies have provided insights into the potential synergistic effects of combining aaptamine and doxorubicin in targeting the anti-apoptotic protein Bcl-2. As summarized in Table 1, the combination of aaptamine with doxorubicin has demonstrated promising results in enhancing the binding affinity of doxorubicin to Bcl-2. The potential mechanism of this synergistic effect might be caused by aaptamine possibly bind to the BH3 binding groove of Bcl-2 (Figure 2), a critical site for inhibiting its anti-apoptotic function, that potentially sensitizes cancer cells to doxorubicin by enhancing its interaction with Bcl-2 (Anantram et al., 2019). Moreover, molecular docking simulations demonstrated that the doxorubicin-aaptamine-Bcl-2 complex exhibited increased stability compared to the doxorubicin-Bcl-2 complex alone. This stability can be attributed to the additional interactions and conformational changes induced by aaptamine, further strengthening the binding of doxorubicin.

Table 1. Docking score of aaptamine, doxorubicin and their combination to Bcl-2

Ligands	Free binding energy (ΔG) (kcal/mol)/Macromolecule		
	Bcl-2	Aaptamine-Bcl-2	Doxorubicin-Bcl-2
Aaptamine	-5.3 ± 0.056[a]	-	-5.907 ± 0.037[a]
Doxorubicin	-6.809 ± 0.059[b]	-8.131 ± 0.391[b]	-

[a,b] Significant different ($p<0.05$; n = 10)

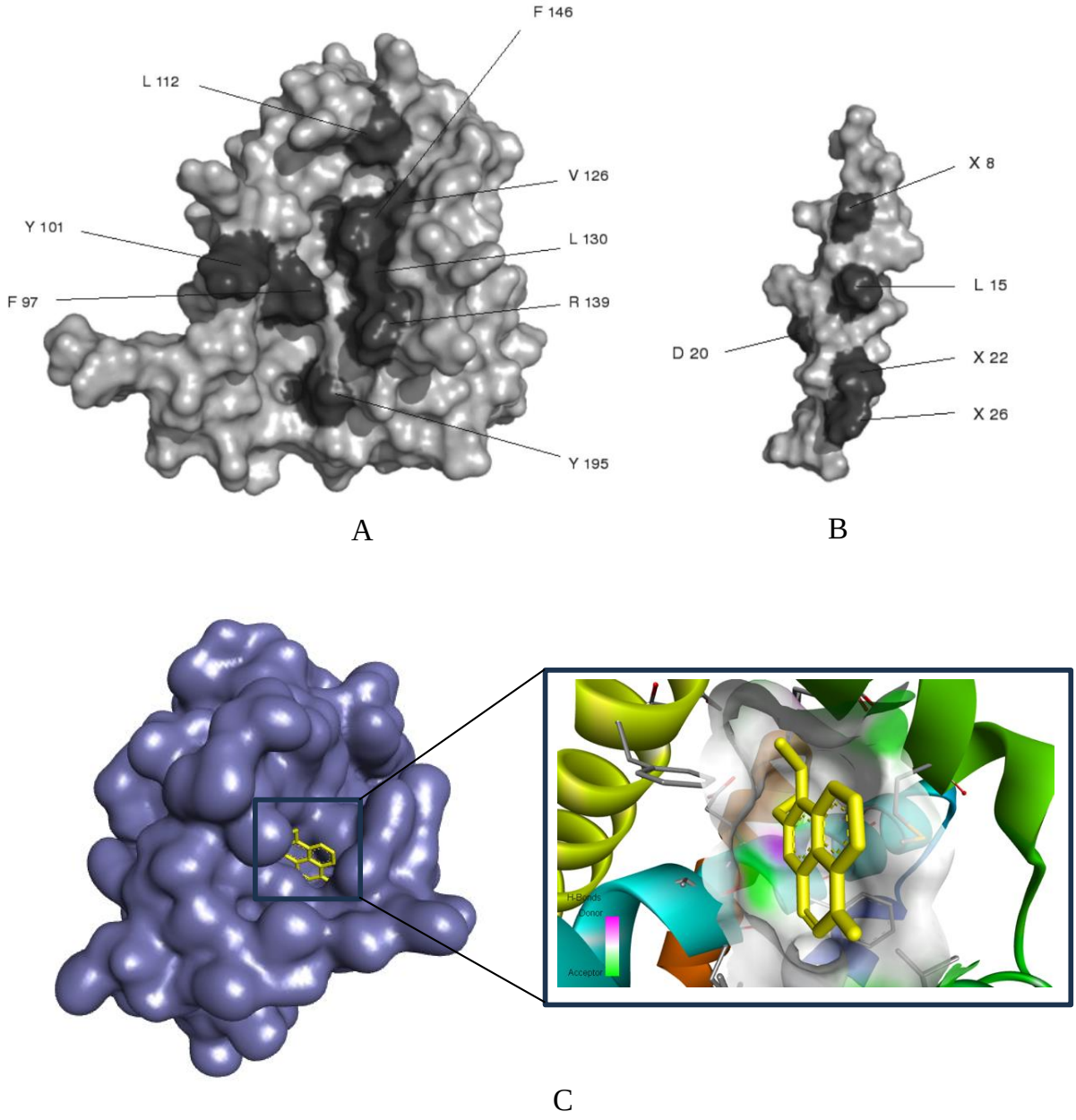

Figure 2. Representation of Bcl-2 surface (A); Bcl-2 homology 3 (BH3) domain (B); Aaptamine fits in BH3 binding groove (C). Picture A and B were taken from Moroy et al. (2009) with direct citation.

The enhancement of doxorubicin's affinity to the anti-apoptotic protein Bcl-2 in the presence of aaptamine can be attributed to the stabilization of the doxorubicin-Bcl-2 complex through specific interactions with key amino acid residues. Molecular docking simulations have provided insights into these interactions, which contribute to the increased binding affinity and potential synergistic effects. Specifically, as depicted in Figure 4A, aaptamine forms hydrophobic bonds (pi-alkyl, alkyl, and pi-sigma) within the Bcl-2 binding groove. Additionally, the authors observed that aaptamine stabilizes the binding of doxorubicin to Bcl-2 through van der Waals interactions with residues such as Asp70, Tyr67, Phe112 and Glu111.

Interestingly, the binding of aaptamine to

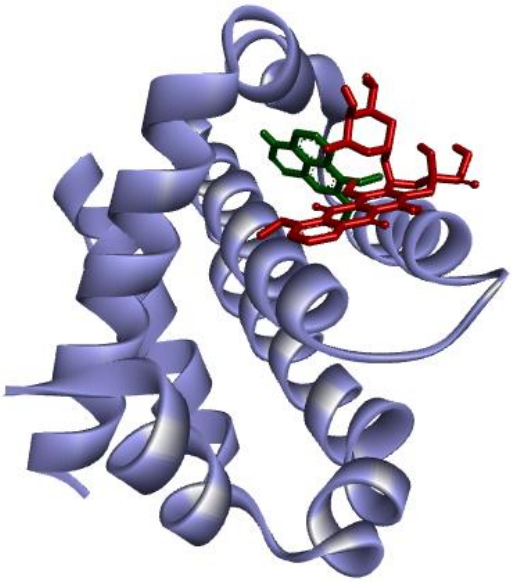

Figure 3. Complex of Aaptamine-Doxorubicin-Bcl2 (Grey: Bcl-2; Green: Aaptamine; Red: Doxorubicin)

the Bcl-2 groove appears to influence the molecular interactions between doxorubicin and Bcl-2 (Figure 3 and 4D). Docking simulations revealed that in the presence of aaptamine, doxorubicin forms stronger hydrogen bonding and van der Waals interactions with key residues such as Arg105, Glu95, Asp70, Leu96, Unk0, Gly104, Asn102, and Asp99. These residues, particularly Asn102 and Gly104, are known to play a significant role in the binding of Bcl-2 inhibitors and are involved in stabilizing interactions with doxorubicin (Acoca et al., 2011). The enhanced interactions between doxorubicin and these essential amino acid residues suggest that aaptamine induces conformational changes in Bcl-2 that facilitate a more favorable binding mode for doxorubicin. Asn and Gly are nonessential amino acids that play a role in cell development. The ability of Asn to inhibit apoptosis is demonstrated by its capacity to downregulate endoplasmic reticulum stress and apoptosis reliant on translation (Tabe et al., 2019). Inhibition of Asn holds potential for inducing apoptosis effectively.

In this study, despite several recognized amino acid residues, an unknown amino acid was also observed as Ukn0. Based on the molecular docking results of doxorubicin in the presence of aaptamine in the binding groove of Bcl-2, it is possible that unknown amino acid residues may be involved in the binding interactions. The involvement of the unknown amino acid residues in the multi-ligand binding could potentially enhance the affinity of doxorubicin towards Bcl-2. These residues may contribute to additional favorable interactions, such as hydrogen bonding, which could stabilize the doxorubicin-Bcl-2 complex. Furthermore, the conformational changes induced by the pre-docked aaptamine may expose or create new binding pockets that better accommodate doxorubicin, leading to an improved fit and higher binding affinity (Moreira et al., 2009). The initial docking of aaptamine to the Bcl-2 structure may have induced conformational changes or created new binding pockets that could accommodate the subsequent docking of doxorubicin with increased affinity. These ligand-induced structural rearrangements within the Bcl-2 binding groove could potentially expose or occlude certain amino acid residues that were previously not involved in the binding interactions. Conversely, the

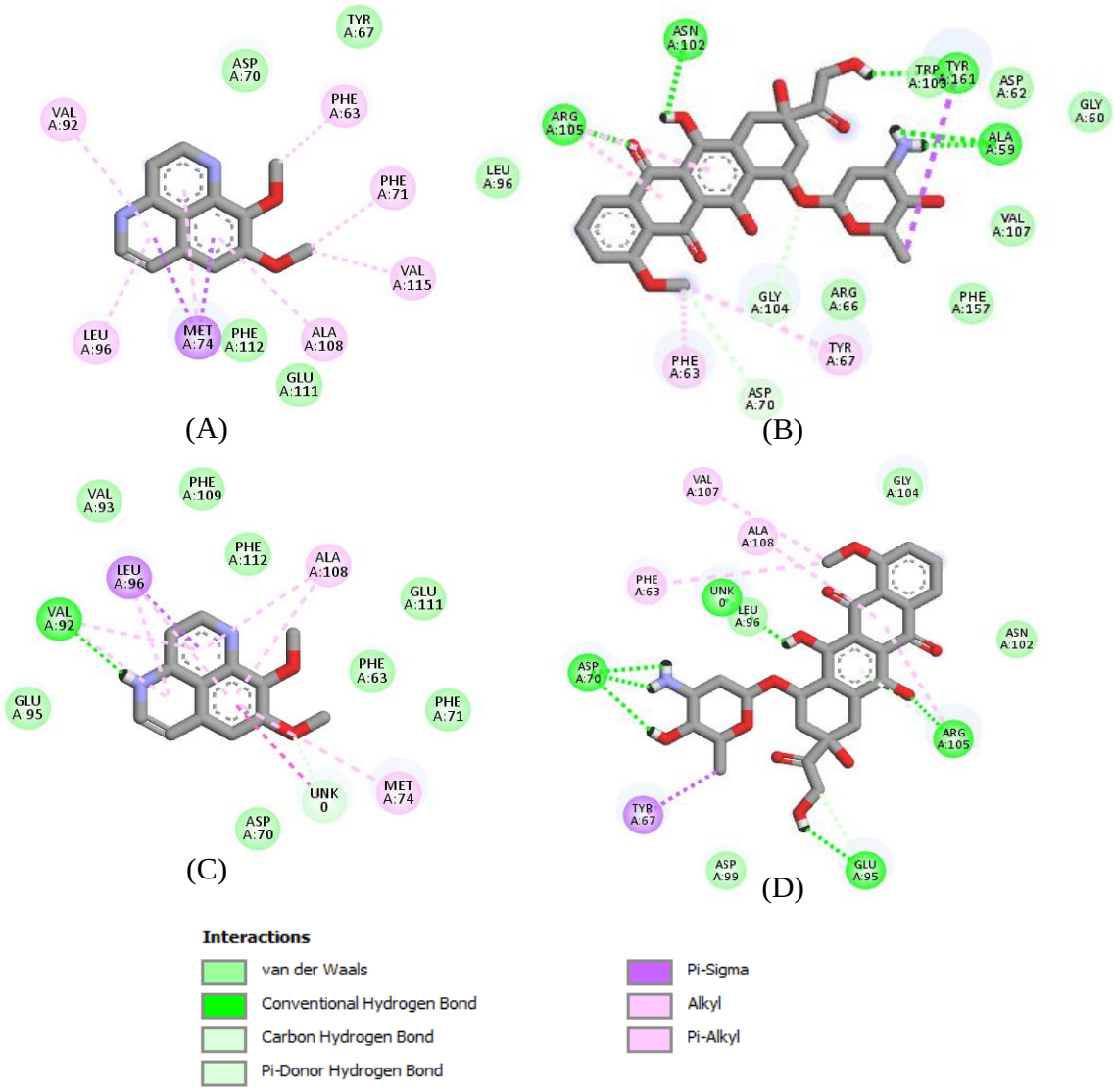

Figure 4. Two-dimensional molecular interaction of test ligands with essential amino acid residues of Bcl-2. (A) Aaptamine; (B) Doxorubicin; (C) Aaptamine in the presence of doxorubicin; (D) Doxorubicin in the presence of aaptamine.

pre-docked doxorubicin may have altered the binding environment, facilitating the binding of aaptamine in a manner that enhances the overall affinity of doxorubicin for Bcl-2. Additionally, the presence of both ligands simultaneously may lead to novel interactions, involving amino acid residues that were not observed in the single-ligand docking studies.

The stabilization of the doxorubicin-Bcl-2 complex by aaptamine is crucial because it can potentially overcome drug resistance mechanisms mediated by Bcl-2 overexpression in cancer cells. By enhancing the binding of doxorubicin to Bcl-2, aaptamine could sensitize cancer cells to the cytotoxic effects of doxorubicin and promote apoptosis. These findings highlight the importance of specific amino acid residue interactions in mediating the synergistic effects between aaptamine and doxorubicin. The stabilization of the doxorubicin-Bcl-2 complex by aaptamine through hydrogen bonding, van der

Waals forces, and hydrophobic interactions with key residues like Arg105, Glu95, Asp70, Leu96, Unk0, Gly104, Asn102, and Asp99 contributes to the enhanced binding affinity and potential therapeutic efficacy of this combination.

The docking simulations suggest that the presence of doxorubicin not only enhances its own binding affinity towards Bcl-2 but also increases the activity of aaptamine against Bcl-2. The formation of the doxorubicin-Bcl2 complex induces conformational changes in Bcl-2, altering the interactions between aaptamine and the amino acid residues of Bcl-2 (Figure 4A and 4C). These changes lead to an enhancement in the binding affinity of aaptamine for Bcl-2. In summary, the docking studies reveal a mutual synergistic effect, where aaptamine strengthens the binding of doxorubicin to Bcl-2, and reciprocally, the presence of the doxorubicin-Bcl2 complex increases the binding affinity of aaptamine towards Bcl-2. This synergistic interplay between the two molecules and their interactions with Bcl-2 contributes to the observed potentiation of their anticancer activities when used in combination.

CONCLUSION

The findings from this research provide valuable insights into the molecular mechanisms underlying the synergistic anticancer effects observed when combining aaptamine and doxorubicin. Molecular docking simulations revealed that aaptamine plays a crucial role in stabilizing the interaction between doxorubicin and Bcl-2. Specifically, aaptamine binds to the BH3 binding groove of Bcl-2, forming key van der Waals interactions with residues such as Asp70, Tyr67, Phe112 and Glu111. This binding induces conformational changes in Bcl-2 that favorably alter the molecular interactions between doxorubicin and essential amino acid residues like forming hydrogen bounding, van der Waals forces, and hydrophobic interactions with Arg105, Glu95, Asp70, Leu96, Unk0, Gly104, Asn102, and Asp99, leading to an increase doxorubicin affinity (ΔG from -6.809 ± 0.059 to -8.131 ± 0.391 kcal/mol). These residues are known to be critical for the binding of Bcl-2 inhibitors and doxorubicin. Additionally, molecular docking demonstrated increased stability of the aaptamine-doxorubicin-Bcl-2 complex compared to the doxorubicin-Bcl-2 complex alone.

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