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Complex disruption effect of natural polyphenols on Bcl-2-Bax: molecular dynamics simulation and essential dynamics study

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Apoptosis (programmed cell death) is a process by which cells died after completing physiological function or after a severe genetic damage. Apoptosis is mainly regulated by the Bcl-2 family of proteins. Anti apoptotic protein Bcl-2 prevents the Bax activation/oligomerization to form heterodimer which is responsible for release of the cytochrome c from mitochondria to the cytosol in response to death signal. Quercetin and taxifolin (natural polyphenols) efficiently bound to hydrophobic groove of Bcl-2 and altered the structure by inducing conformational changes. Taxifolin was found more efficient when compared to quercetin in terms of interaction energy and collapse of hydrophobic groove. Taxifolin and quercetin were found to dissociate the Bcl-2-Bax complex during 12 ns MD simulation. The effect of taxifolin and quercetin was, further validated by the MD simulation of ligand-unbound Bcl-2-Bax which showed stability during the simulation. Obatoclax (an inhibitor of Bcl-2) had no significant dissociation effect on Bcl-2-Bax during simulation which favored the previous experimental results and disruption effect of taxifolin and quercetin.

Keywords: Bcl-2-Bax complex; taxifolin; quercetin; obatoclax; molecular dynamics simulation

1. Introduction

Apoptosis (programmed cell death) is a molecular process by which cells commit suicide after completing physiological function, or after a severe genetic damage. A common apoptotic mechanism appears to be preserved throughout evolution and regulated mainly by the Bcl-2 family of proteins (Igney & Kramer, 2002; Reed, 2002). Some of them, such as Bcl-2, Bcl-xL, Bcl-w, or Mcl-1 block apoptosis while others, such as Bad, Bak, Bax, Bid, Bim, or Hrk, induce it. It has been established that overexpression of Bcl-2 and Bcl-xL proteins is related to the initiation and development of different types of cancer, as well as resistance to chemotherapeutic treatments (Pinto, Perez, & Rubio-Martinez, 2004). Bcl-2 family proteins are characterized by containing up to four conserved sequences of amino acids which are known as Bcl-2 homology (BH) domains (Adams & Cory, 2001; Cao, Yap, & Newell-Rogers, 2013; Czabotar, Lessene, Strasser, & Adams, 2014; Hosseini et al., 2013; Opferman & Korsmeyer, 2003). They are usually grouped into three distinct subclasses: (1) Bax and Bak (contain the BH1 to BH3 domains) that mediate apoptosis by triggering destabilization of the outer mitochondrial membrane and releasing the cytochrome c from mitochondria to the cytosol (Antignani & Youle, 2006; Green & Kroemer, 2004), (2) Bim, Bad, Puma, and Noxa (the BH3-only proteins) that communicate pro-death signals and ultimately activate downstream Bax and Bak (Kim et al., 2006; Willis et al., 2007), (3) Bcl-xL, Bcl-w, Mcl-1, A1, and Bcl-B (contain all the four BH1 to BH4 domains) that suppress the activation of Bax/Bak (Ku, Liang, Jung, & Oh, 2011; Liu, Dai, Zhu, Marrack, & Kappler, 2003; Maity, Yadav, Verma, & Dastidar, 2013; Petros et al., 2000; Sattler et al., 1997).

Bcl-2 prevents the Bax activation/oligomerization by forming heterodimer (Bcl-2-Bax). Several mutagenesis studies showed that the BH1–3 regions of Bcl-2 and the BH3 region of Bax are critically required for their interaction (Dlugosz et al., 2006; Lin et al., 2004; Wang, Gross, Waksman, & Korsmeyer, 1998; Yin, Oltvai, & Korsmeyer, 1994; Zha, Aime′-Sempe, Sato, & Reed, 1996). In all of the complex structures determined so far, the BH3 peptide forms an amphipathic α-helix whose hydrophobic surface interacts with a hydrophobic groove on the Bcl-2-like protein formed by the BH1–3 regions (Ding et al., 2010; Suzuki, Youle, & Tjandra, 2000). The structures of antiapoptotic protein (Bcl-2, Bcl-xL, and Mcl-1) consist primarily of two central hydrophobic helices surrounded by amphipathic helices (Petros, Olejniczak, & Fesik, 2004; Day et al., 2005). The binding groove is formed mainly by the α2, α 3, α 4, and α 5 helices (Acoca, Cui, Shore, & Purisima, 2011) (Figure 1).

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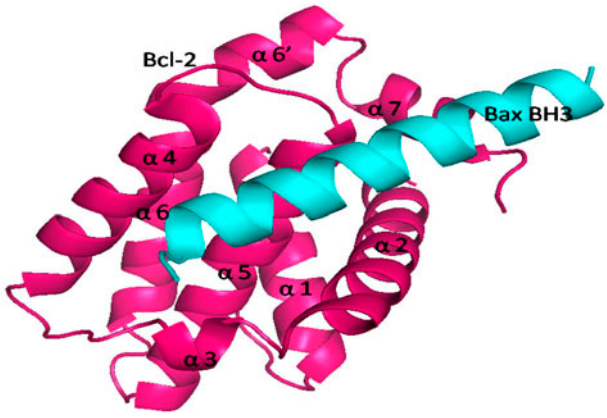

Figure 1. Cartoon presentation of Bcl-2-Bax complex.

Ku et al. (2011) found that the 31-mer Bax peptide (residues 52–82) and the 36-mer Bax peptide (residues 49–84) bind Bcl-2 nearly with equal potency. But a 26-mer Bax peptide (residues 52–77) has poor binding affinity for Bcl-2 when compared to the above-mentioned Bax peptides. Intermolecular interactions and increased helical propensity involving the last five residues (Arg78-Asp82) present in the 31-mer, but not in the 26-mer peptide, appear to be involved in difference in affinity. Arg78 is involved in the hydrophilic interaction with Bcl-2 and Ala81 in the van der Waals (vdW) interaction. While the rest three residues do not interact with Bcl-2 directly, but by increasing the helical propensity of the Bax peptide.

All the previous studies regarding the inhibition of Bcl-2 were mainly targeted to the Bax-unbound form of Bcl-2 (Acoca et al., 2011; Mohammadi et al., 2014; Saxena et al., 2013) in our knowledge. It is well defined that the interaction of bcl-2 and bax is mainly dependent on few residues of both proteins. We had selected quercetin and taxifolin (natural phytochemicals) for the present study as these polyphenolic compounds were found to have anticancerous property (Lee et al., 2007; Seufi et al., 2009). The molecular docking and molecular dynamics simulation were performed to elucidate the potential of quercetin and taxifolin in inhibition of the Bcl-2 and in the disruption of Bcl-2-Bax interaction. For further validation we performed MD simulation of Bcl-2-Bax with obatoclax (an inhibitor of Bcl-2) which has no significant disruption effect on Bcl-2-Bax complex as control (Samuel et al., 2010).

2. Computational methods

2.1. Method 1

AutoDock 4.0 suite was used as molecular-docking tool in order to carry out the docking simulations. PDB id: 2XA0, obtained from RCSB protein data bank, was used as initial structure for Bcl-2. *All the heteroatoms and Bax peptide were removed from the coordinate file, and Bcl-2 alone was used for docking study.* The structure of ligands (quercetin and taxifolin) was generated from smile strings followed by energy minimization. Hydrogen atoms were added to protein crystal structures using autodock program while all non-polar hydrogen atoms were merged. Lamarckian genetic algorithm was used as a search parameter which is based on adaptive local search. Short range vdW and electrostatic interactions, hydrogen bonding, entropy losses were included for energy-based autodock scoring function (Morris, Goodsell, & Halliday, 1998; Morris et al., 2009). The Lamarckian GA parameters used in the study were numbers of run, 30, population size, 150, maximum number of eval, 25,000,000, number of generation, 27,000, rate of gene mutation, 0.02, and rate of crossover, 0.8. Blind docking was carried out using grid size 126, 126, and 126 along the *X*, *Y*, and *Z* axes with 0.375 Å spacing. RMS cluster tolerance was set to 2.0 Å. Semi-flexible docking was performed which includes a flexible ligand and a rigid receptor. All the protein and ligand structural images were generated using PYMOL (DeLano, 2004).

MD simulation of the complex was carried out with the GROMACS4.5.4 package using the GROMOS96 43a1 force field (Berendsen, Van der Spoel, & Van Drunen, 1995; Lindah, Hess, & Van der Spoel, 2001). The lowest binding energy (most negative) docking conformation generated by Autodock was taken as initial conformation for MD simulation. The topology parameters of proteins were created using the Gromacs program. The topology parameters of taxifolin, quercetin, and obatoclax were built by the Dundee PRODRG server (Schuttelkopf & Van Aalten, 2004). The complex was immersed in an octahedron box of simple point charge (SPC) water molecules (Van Gunsteren et al., 1996; Van Gunsteren, Daura, & Mark, 1998). The solvated system (Bcl2, ligand and water) was neutralized by adding four Na ions in all the simulations. To release conflicting contacts, energy minimization was performed using the steepest descent method of 10,000 steps followed by the conjugate gradient method for 10,000 steps. MD simulation studies consist of equilibration and production phases. To equilibrate the system, the solute (protein, counterions, and ligand) were subjected to the position-restrained dynamics simulation (NVT and NPT) at 300 K for 300 ps. Finally, the full system was subjected to MD production run at 300 K temperature and 1 bar pressure for 10,000 ps. For analysis, the atom coordinates were recorded at every 0.5 ps during the MD simulation.

2.2. Method 2

In method 2, we used Bcl2-Bax complex as initial structure (PDB id:2XA0) for performing molecular docking

and molecular dynamics simulation. Methodology was the same as used in method 1. Obatoclax was also used along with quercetin and taxifolin as control. The lowest binding energy (most negative) docking conformation generated by Autodock was taken as initial conformation for MD simulation. The solvated system (Bcl2-Bax, ligand, and water) was neutralized by adding 5 Na ions in all simulation. The full system was subjected to MD production run at 300 K temperature and 1 bar pressure for 12,000 ps.

2.3. *Essential dynamics (principle component analysis)*

The essential degrees of freedom (essential subspace) of Bcl-2 in ligand-bound form were extracted from the trajectories according to the essential dynamics method used (principal component analysis) (Amadei, Ceruso, & Di Nola, 1999; Amadei, Linssen, & Berendsen, 1993; Garcia, 1992; Kitao, Hirata, & Go, 1991). The ED method involves constructing the covariance matrix to observe the fluctuations in the coordinates of Bcl-2. Correlated motions were observed during the MD trajectories through the eigenvectors of the non-mass-weighted covariance matrix for atomic position fluctuations. Before constructing covariance matrix, the overall rotation and translation was eliminated to allow the visualization of internal motion. This was achieved by performing least squares fitting to the average structure based on the protein backbone coordinates. After the fitting procedure, the internal motions described by the trajectory $x(t)$ and the covariance matrix were constructed from the coordinates of the positions of the protein backbone atoms:

$$C_{ij} = 1/S\Sigma_t\{x_i(t) - \langle x_i\rangle\}\{x_j(t) - \langle x_j\rangle\}$$

where S is the total number of configurations, $t = 1, 2, \ldots, S$; $x_i(t)$ are the position coordinates, with $i = 1, 2, \ldots .3N$, N is the number of atoms from which covariance matrix is constructed, and $<x_i>$ is the average for coordinate i over all configurations (Amadei et al., 1993; Raghav, Verma, & Gangenahalli, 2011).

3. Result and discussion

3.1. *Method 1*

Molecular docking results revealed that quercetin and taxifolin bound to hydrophobic groove of Bcl-2 with binding energy −28.56 and −30.66 kJ/Mol, respectively (Figure 2). Quercetin showed hydrogen bonding with

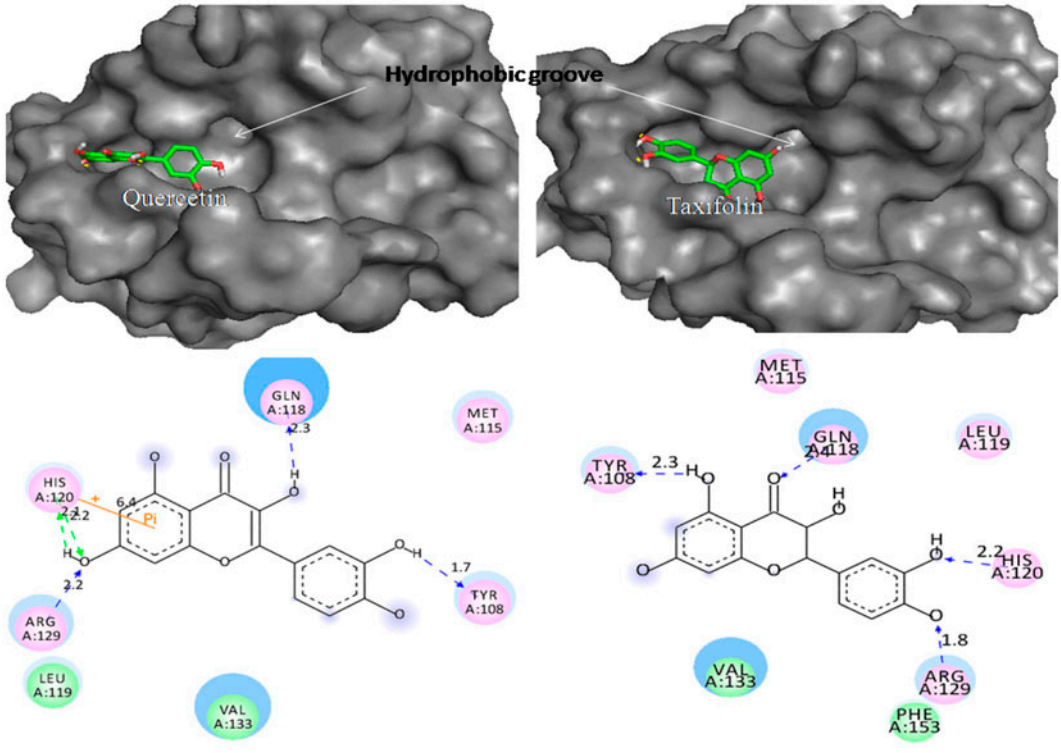

Figure 2. Ligand-docked Bcl-2: left; quercetin (upper surface presentation of Bcl-2-quercetin complex, lower 2D plot of interaction of quercetin with Bcl-2), right; taxifoin (upper surface presentation of Bcl-2-taxifoin complex, lower 2D plot of interaction of taxifolin with Bcl-2). All 2D plots were generated by Discovery Studio 3.1 [40].

Arg129, Tyr108, Gln118, and His120 along with π-cation interaction with His120. Taxifolin showed similar interaction as quercetin and showed hydrogen bonding with Arg129, Tyr108, Gln118, and His120.

The lowest binding energy (most negative) docking conformation generated by Autodock was taken as initial conformation for MD simulation. We have analyzed the time-dependent behavior of MD trajectories for Bcl-2-ligand complex including root mean square deviation (RMSD) for all backbone atoms, short range (SR) vdW and electrostatic energies along with essential dynamics. Figure 3(A) showed that the RMSD profiles were always less than 0.30 nm for both quercetin and taxifolin-bound Bcl-2 backbone during the entire simulation suggesting the stability of Bcl-2 in ligand-bound state and suitability for post analysis. Figure 3(B) showed the RMSD profile of ligands bound to Bcl-2 pocket. Quercetin showed increase in RMSD after ~3 ns for very short time interval while taxifolin showed more stable profile when compared to quercetin throughout the simulation. These results showed the stable binding of ligands in the hydrophobic pocket of Bcl-2.

To explore the interaction between the ligands and the Bcl-2, the energy contributions of SR vdW and electrostatic interaction energies were calculated. Quercetin interaction with Bcl-2 was dominated by SR vdW energies with average value −151.13 kJ/mol while the average value of SR electrostatic energies was −8.90 kJ/mol. The vdW energy between taxifolin and Bcl-2 during the simulation was approximately −161.31 kJ/mol on average. However, the average SR electrostatic energy was approximately −18.23 kJ/mol (Figure 4(A) and (B)). It was observed that SR vdW energy plays main role in the interaction with Bcl-2 and ligand binding.

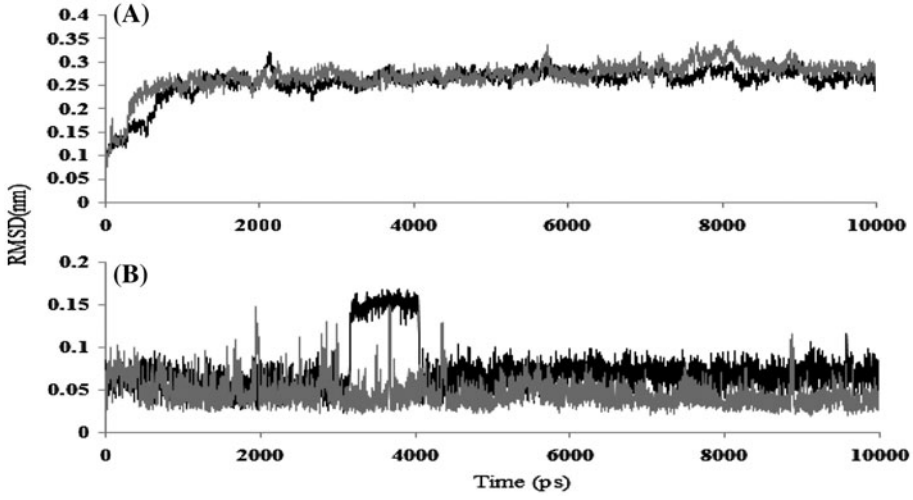

Figure 3. (A) Plot of RMSD of backbone of Bcl-2 bound to quercetin (black) and taxifolin complex (gray). (B) Plot of RMSD of quercetin (black) and taxifolin (gray) in hydrophobic groove of Bcl-2.

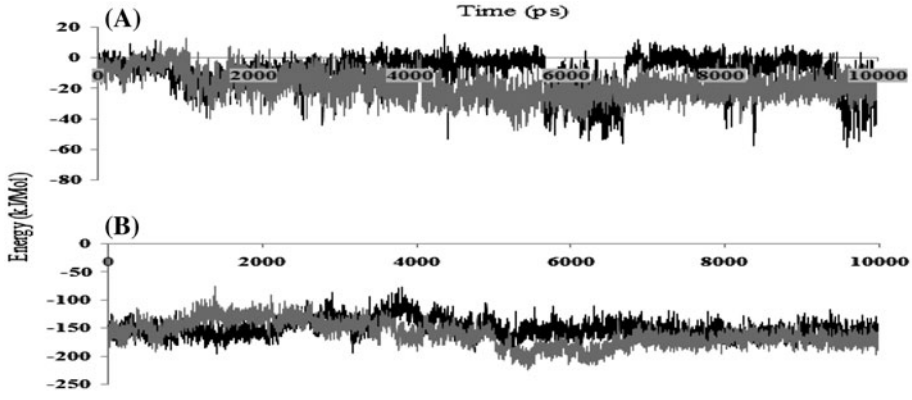

Figure 4. Profile of (A) Coulombic interaction energy and (B) vdW interaction energy between Bcl-2-quercetin (black) and Bcl-2-taxifolin (gray).

To elucidate the residues of hydrophobic groove of Bcl-2 which were involved in a stable interaction with ligands, 2D plots of interaction were generated using Discovery studio 3.1. (Accelrys Software Inc., 2011) Quercetin showed strong π–π and π–sigma interactions with Tyr108 and π–π with His120 along with h-bonding with Met115, Gln118, and Arg 139 (Figure 5). Taxifolin showed strong π–sigma interaction with Val133 and π–cation with Arg129 along with h-bonding with His120 and Thr 122 (Figure 6).

Comparative analysis of final pose of Bcl-2-ligand complex after 10 ns molecular dynamics simulation with crystal structure of Bcl-2 revealed that binding of the quercetin and taxifolin brings significant conformational changes in the Bcl-2 structure. Figure 7 clearly indicates the collapse of hydrophobic groove in the ligand-bound Bcl-2. However, taxifolin showed higher effect on the groove and found to completely disappear in taxifolin-bound Bcl-2. All these results indicated that quercetin and taxifolin have potential to inhibit the Bcl-2 by binding and distorting structure of hydrophobic groove.

3.2. *Essential dynamics (principle component analysis)*

The covariance matrix captured the degree of collinearity in atomic motions for each pair among backbone atoms of Bcl-2. Cross-correlations between residue fluctuations helped us to identify highly correlated, moderately correlated, and anticorrelated regions. In Bcl-2 backbone complexed with quercetin (Figure 8(A)), a highly anticorrelated motion was observed compared to taxifolin-bound Bcl-2 (Figure 8(B)). These very less anticorrelated motions in taxifolin-bound Bcl-2 was found to be responsible for the collapse of hydrophobic groove by making helices close together which participate in groove formation.

It is clear that antiapoptotic Bcl-2 proteins effectively inhibit apoptosis, at least in part by directly binding to proapoptotic Bcl-2 proteins such as Bim, Bax, Bid, and Bad. Experimentally determined three-dimensional structures of Bcl-2 showed that these proteins contain a well-defined hydrophobic surface-binding groove, known as the BH3 binding groove, into which Bid, Bax, Bim, Bad, or Noxa binds. 9–11 small molecules designed to bind to the BH3 binding groove of these antiapoptotic Bcl-2 proteins and to block their interactions with proapoptotic Bcl-2 members were predicted to promote apoptosis in cancer cells and represented a promising new cancer therapeutic strategy. There are several modeling studies which showed the binding of inhibitors to hydrophobic groove (Petros et al., 2000, 2001; Sattler et al., 1997; Tang et al., 2008; Zhou et al., 2012) but the collapse of hydrophobic groove on ligand binding was first time reported in the present study. These results may lead to the conclusion that quercetin and taxifolin

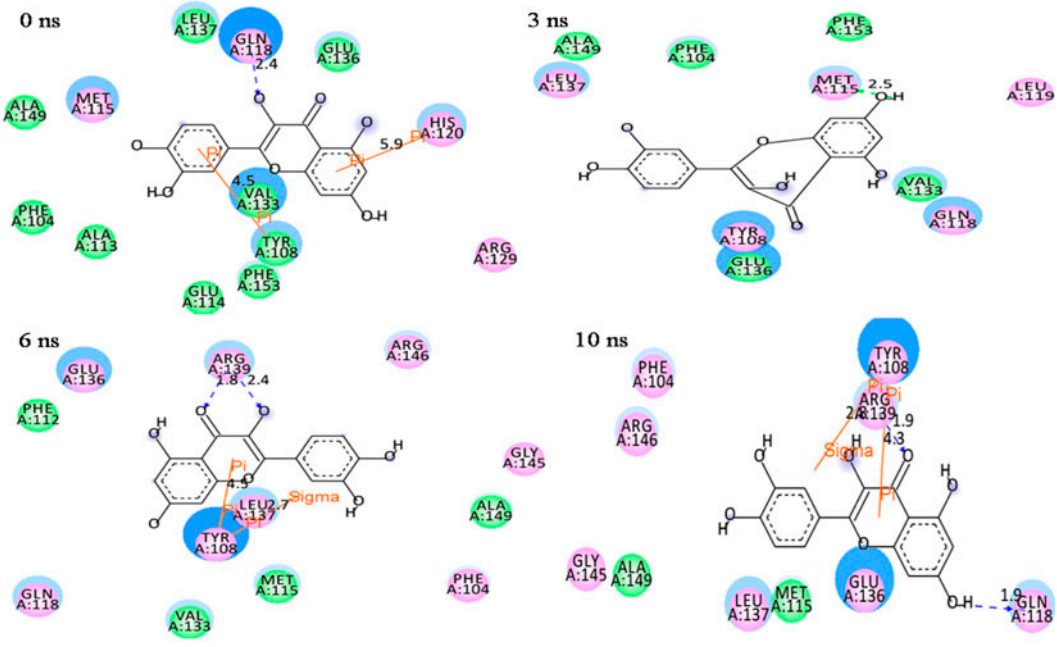

Figure 5. 2D presentation of interaction of quercetin with Bcl-2 residues at different times of MD simulation.

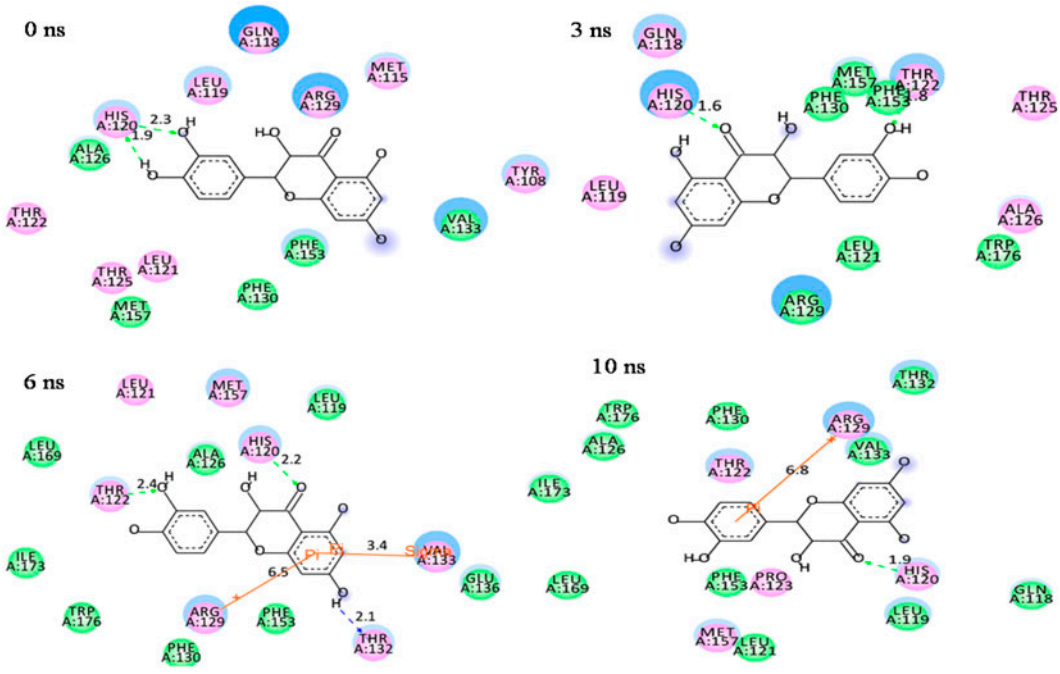

Figure 6. 2D presentation of interaction of taxifolin with Bcl-2 residues at different times of MD simulation.

efficiently bind to hydrophobic groove and alter the structure by inducing conformational changes. Taxifolin was found more efficient when compared to quercetin in terms of interaction energy and collapse of hydrophobic groove.

3.3. Method 2

Molecular docking results revealed that quercetin, taxifolin, and obatoclax bound to Bcl-2-Bax complex. The interacting residues were Arg 65, Asp 68, Glu 69, and Ser 72 of Bax and Arg 110, and Asp 111 of Bcl-2 as

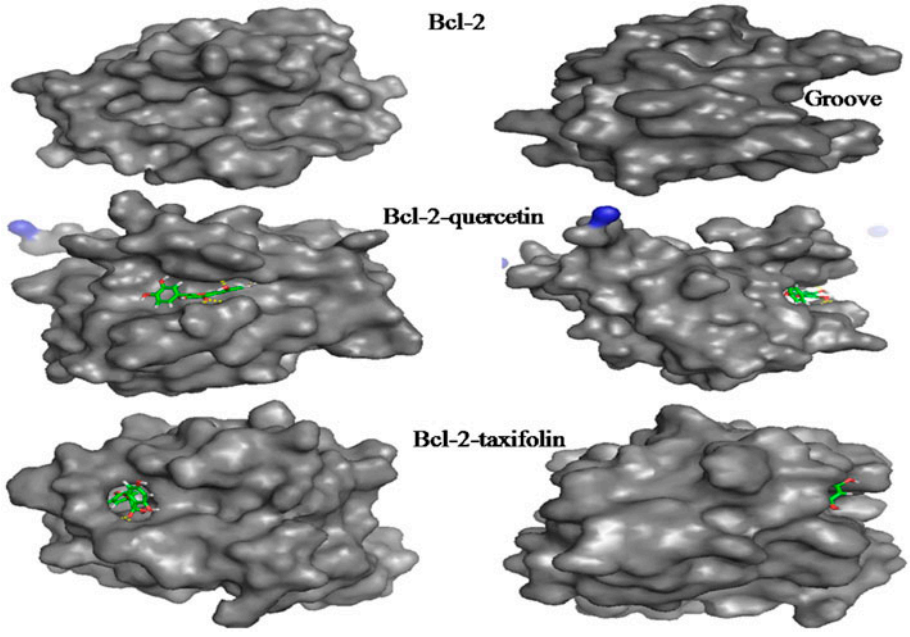

Figure 7. Comparison of surface structure of Bcl-2 crystal structure and ligand-bound Bcl-2 after 10 ns MD simulation.

shown in Figure 9. Previous studies showed that Asp 68 of Bax plays a crucial role in the interaction with Bcl-2. Mutation analysis of this residue to alanine revealed remarkable decrease in the interaction with Bcl-2. However, Lys 64, Arg 65 Glu 69, and Asp 71 are also important for this interaction but the Asp 68 regarded as key for Bcl-2-Bax interaction (Ku et al., 2011; Pinto et al., 2004). As the interaction of Bcl-2 and Bax is depend on a number of residues, on the basis of previous studies, we had selected those ligand docked complex to carry out molecular dynamic simulation in which ligands were found to interact with key residues Asp 68. Figure 10(A) showed that the RMSD profiles were always less than 0.25 nm for ligand-unbound Bcl-2-Bax complex during the entire simulation suggesting the stability of Bcl-2-Bax complex in ligand-unbound state. An initial steep rise in the RMSD for the first, ~1000 ps and subsequently a constant profile was observed for the Bcl-2-Bax complex. RMSD profile along with snapshots recorded at different time intervals (Figure 10(B)) of MD simulation revealed the stability of ligand-unbound Bcl-2-Bax complex. Figure 11(A) shows that the RMSD profile of taxifolin-bound Bcl-2-Bax was always less than 0.25 nm up to ~7000 ps afterward a high rise in the RMSD was observed subsequently a constant profile was observed with up and down for very small time intervals. This increase in RMSD was found to be in the good agreement with the snapshots recorded after 7000 ps which revealed the separation of Bax peptide from Bcl-2 (Figure 11(B)). In quercetin-bound Bcl-2-Bax complex, the RMSD profile of backbone showed high increase from the value of 0.25 nm after ~5000 ps and subsequently a more or less constant profile was observed followed by high increase in RMSD (Figure 12(A)). Similar to taxifolin, this increase of RMSD was found to be associated with the dissociation of Bcl-2-Bax complex as evidenced by recorded snapshots (Figure 12(B)). However, in case of obatoclax-complexed Bcl-2-Bax showed constant profile at 0.25 nm with increase at ~8000 ps for very short time interval (Figure 13(A)). Snapshots, further, confirmed that obatoclax did not show significant disruption effect on Bcl-2-Bax (Figure 13(B)) which favored the previous experimental results (Samuel et al., 2010). These results suggest that taxifolin and quercetin disrupted the Bcl-2-Bax stable interaction during 12 ns MD simulation, while obatoclax did not do so as reported by previous studies (Samuel et al., 2010).

To identify the flexible regions of the proteins, RMSF of backbone from its time-averaged position

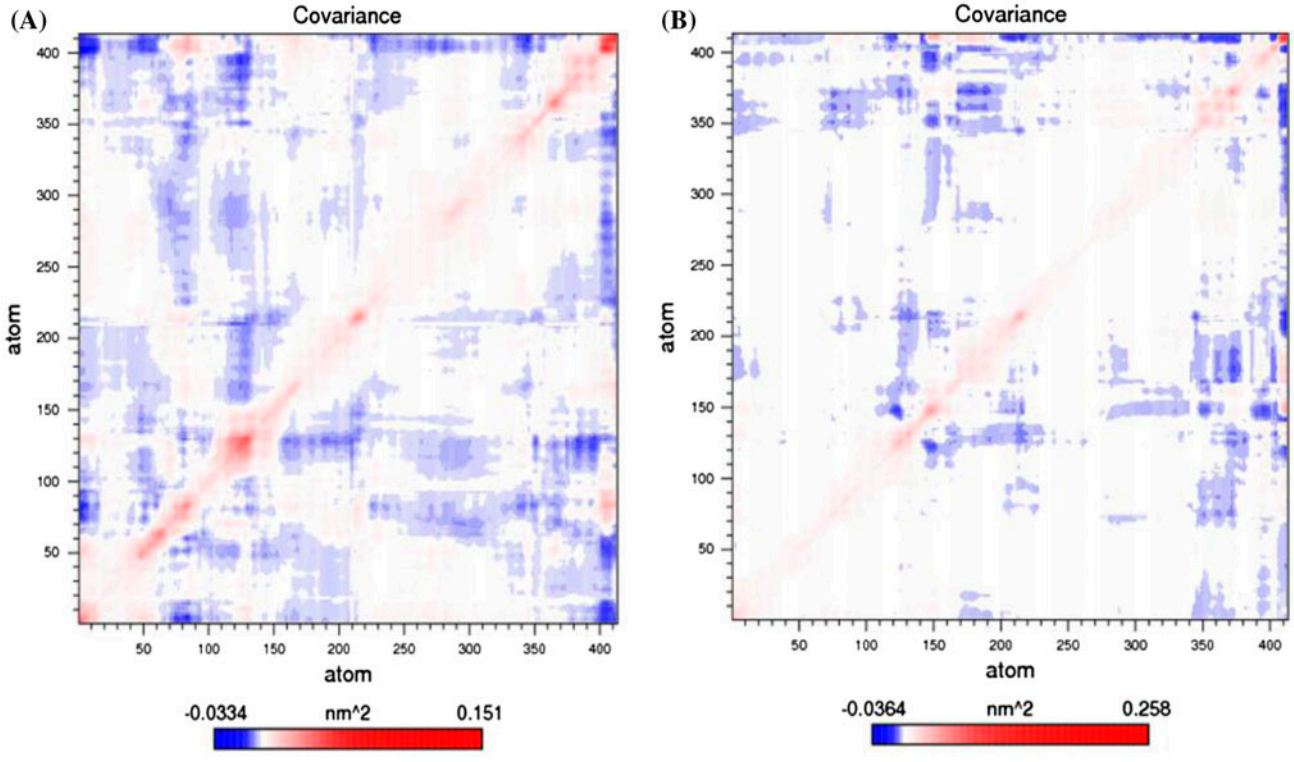

Figure 8. Covariance matrix of Bcl-2 backbone atom bound to (A) quercetin and (B) taxifolin.

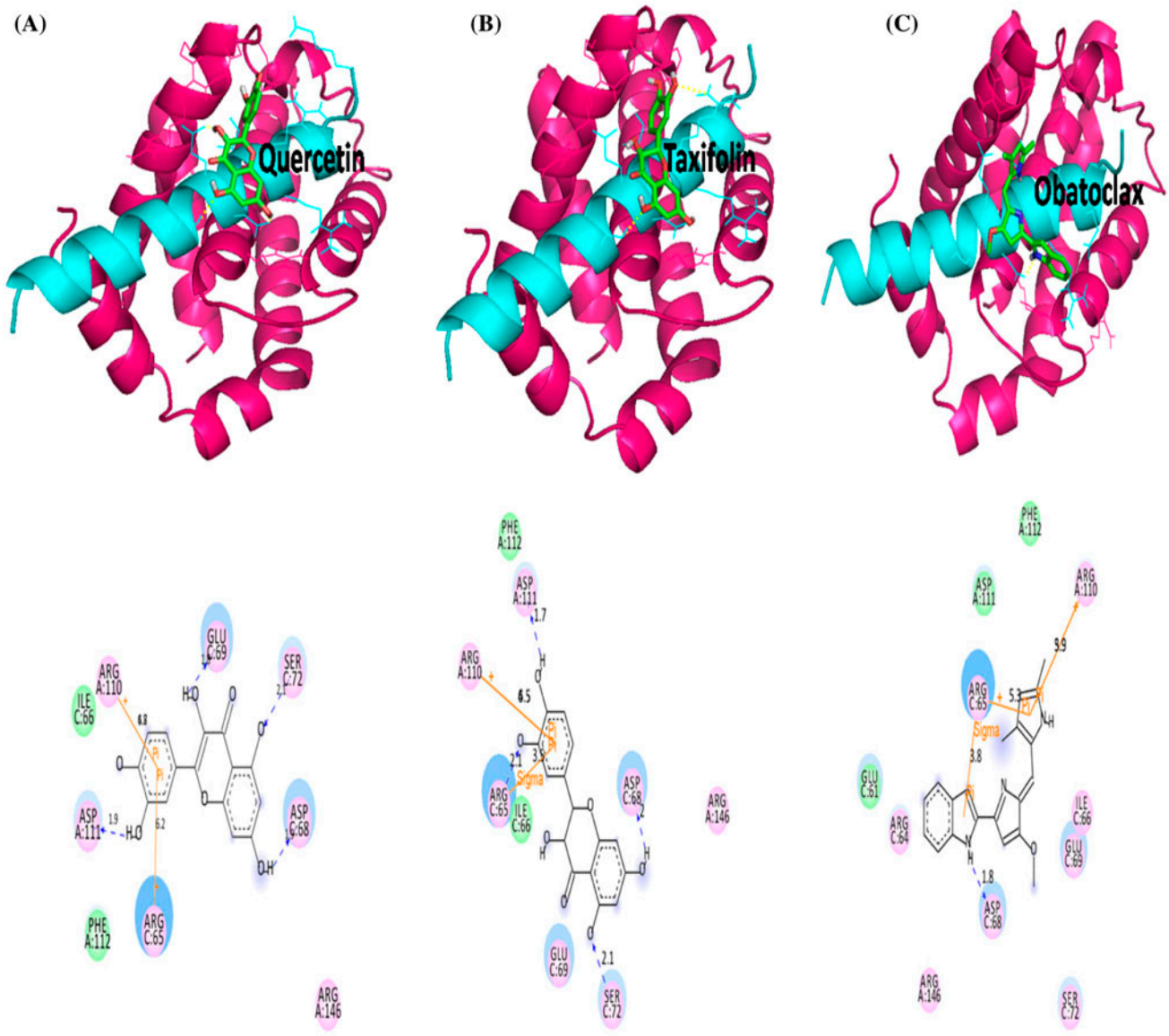

Figure 9. Ligand-docked Bcl-2-Bax complex (A) quercetin (upper cartoon presentation of Bcl-2-Bax-quercetin complex, lower 2D plot of interaction of quercetin with Bcl-2-Bax complex), (B) taxifoin (upper cartoon presentation of Bcl-2-Bax-taxifoin complex, lower 2D plot of interaction of taxifolin with Bcl-2-Bax complex), and (C) obatoclax (upper cartoon presentation of Bcl-2-Bax- obatoclax complex, lower 2D plot of interaction of obatoclax with Bcl-2-Bax complex).

was analyzed. The RMSF profile of protein backbone revealed higher fluctuation in taxifolin and quercetin-bound complex as compared to ligand-unbound and obatoclax-bound forms during the course of simulation. This suggests that binding of taxifolin and quercetin made backbone more flexible to move. Further, the flexibility of Bax peptide backbone was found more as compared to Bcl-2. This might be due to the restriction caused by interaction of taxifolin and quercetin to Bcl-2 (Figure 14). The RMSF profile of interface residue side chains also suggested higher fluctuation in taxifolin- and quercetin-bound complexes when compared to unbound and obatoclax-bound complexex (Figure 15).

These results suggested that binding of taxifolin and quercetin disrupted the stable interactions of residues at the Bcl-2-Bax interface which remained consistent in ligand-unbound form.

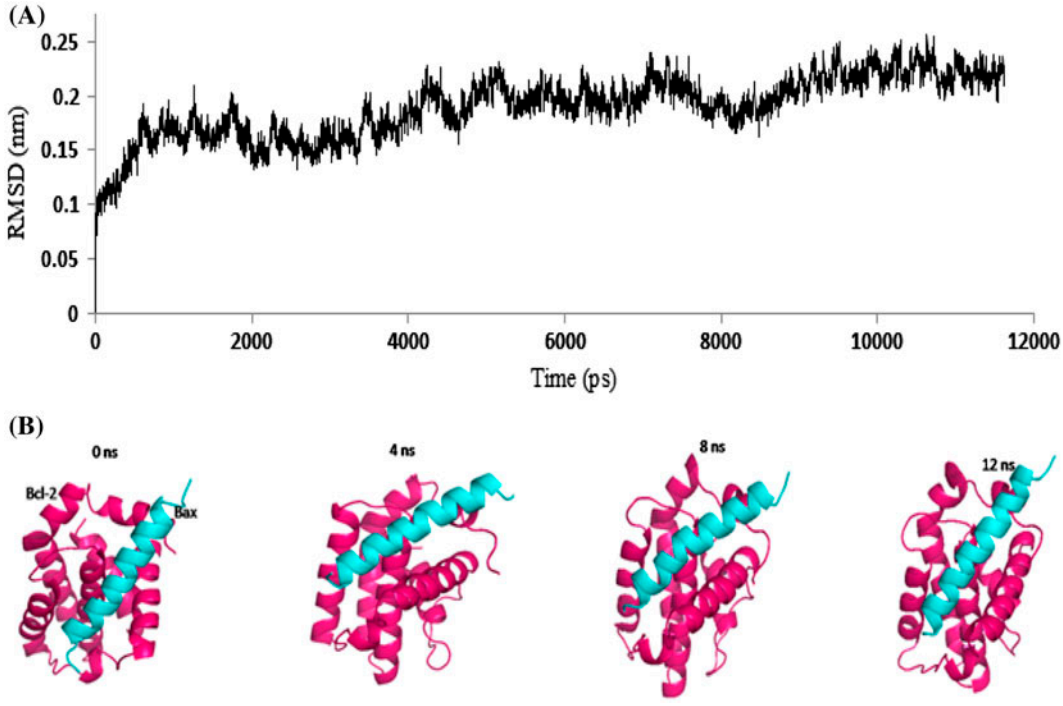

Figure 10. (A) RMSD profile of Bcl-2-Bax backbone and (B) conformations of Bcl-2-Bax complex during 12 ns MD simulation.

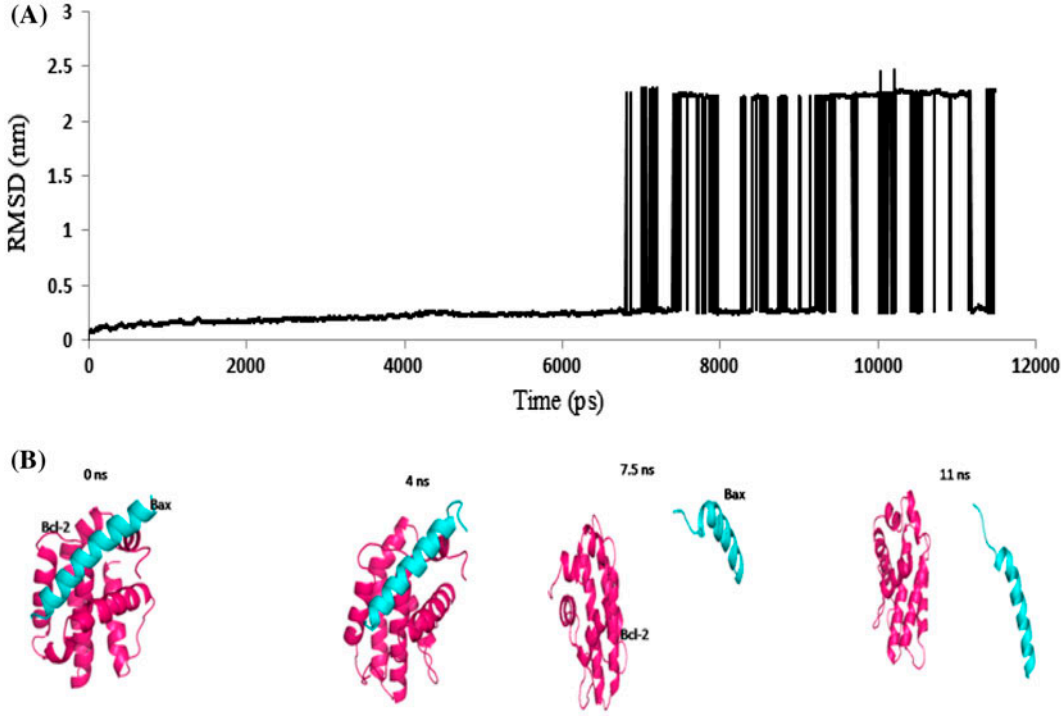

Figure 11. (A) RMSD profile of taxifolin-bound Bcl-2-Bax complex backbone and (B) conformations of taxifolin-bound Bcl-2-Bax complex during 12 ns MD simulation showed dissociation of complex.

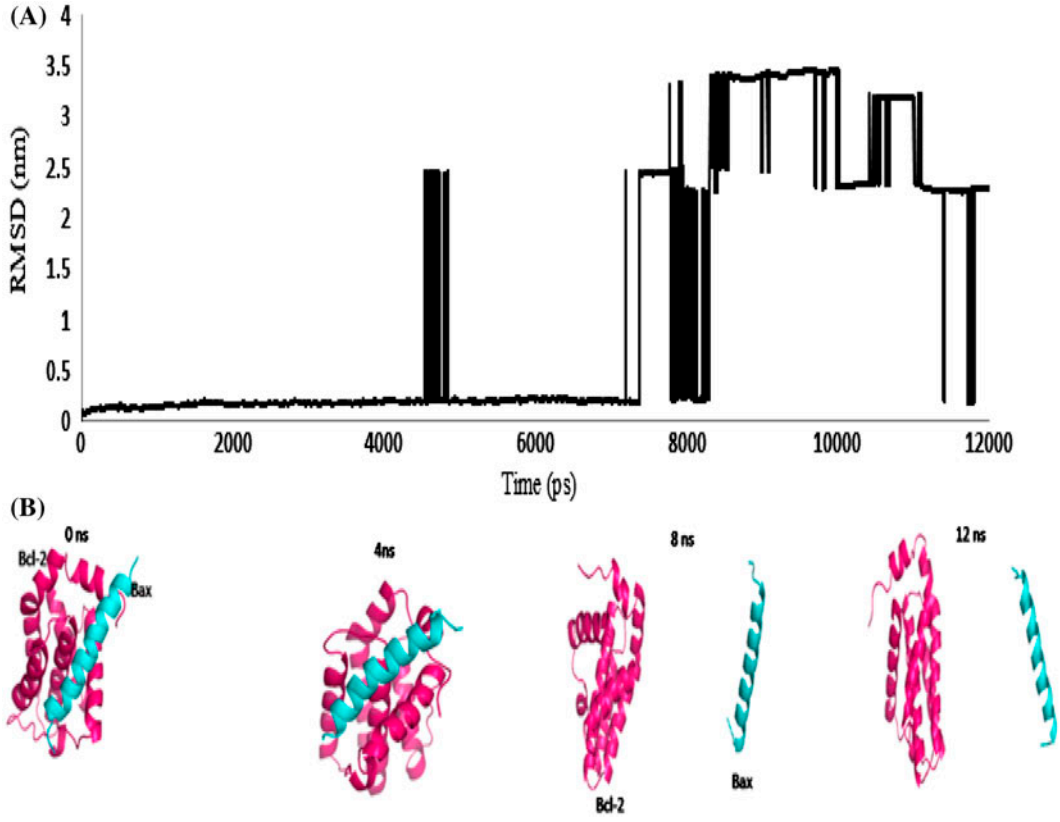

Figure 12. (A) RMSD profile of quercetin-bound Bcl-2-Bax complex backbone and (B) conformations of quercetin-bound Bcl-2-Bax complex during 12 ns MD simulation showed dissociation of complex.

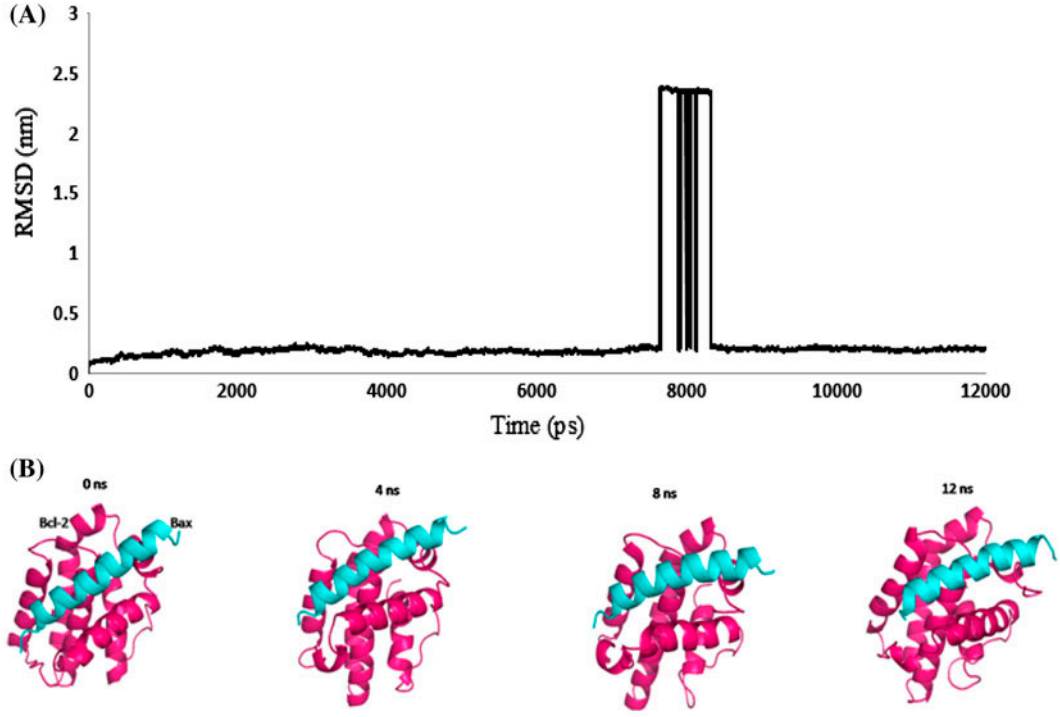

Figure 13. (A) RMSD profile of obatoclax-bound Bcl-2-Bax complex backbone and (B) conformations of obatoclax-bound Bcl-2-Bax complex during 12 ns MD simulation showed insignificant dissociation effect.

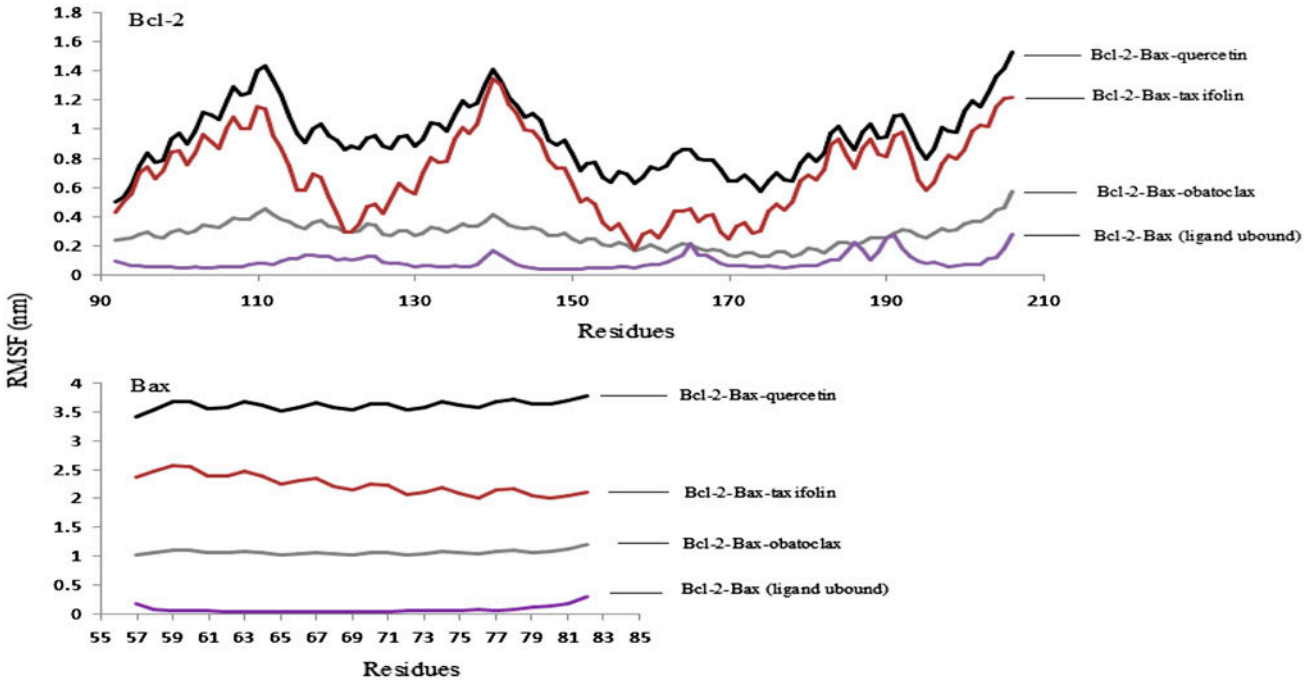

Figure 14. RMSF of Bcl-2 residues backbone (upper) and Bax residues backbone (lower) during MD simulation.

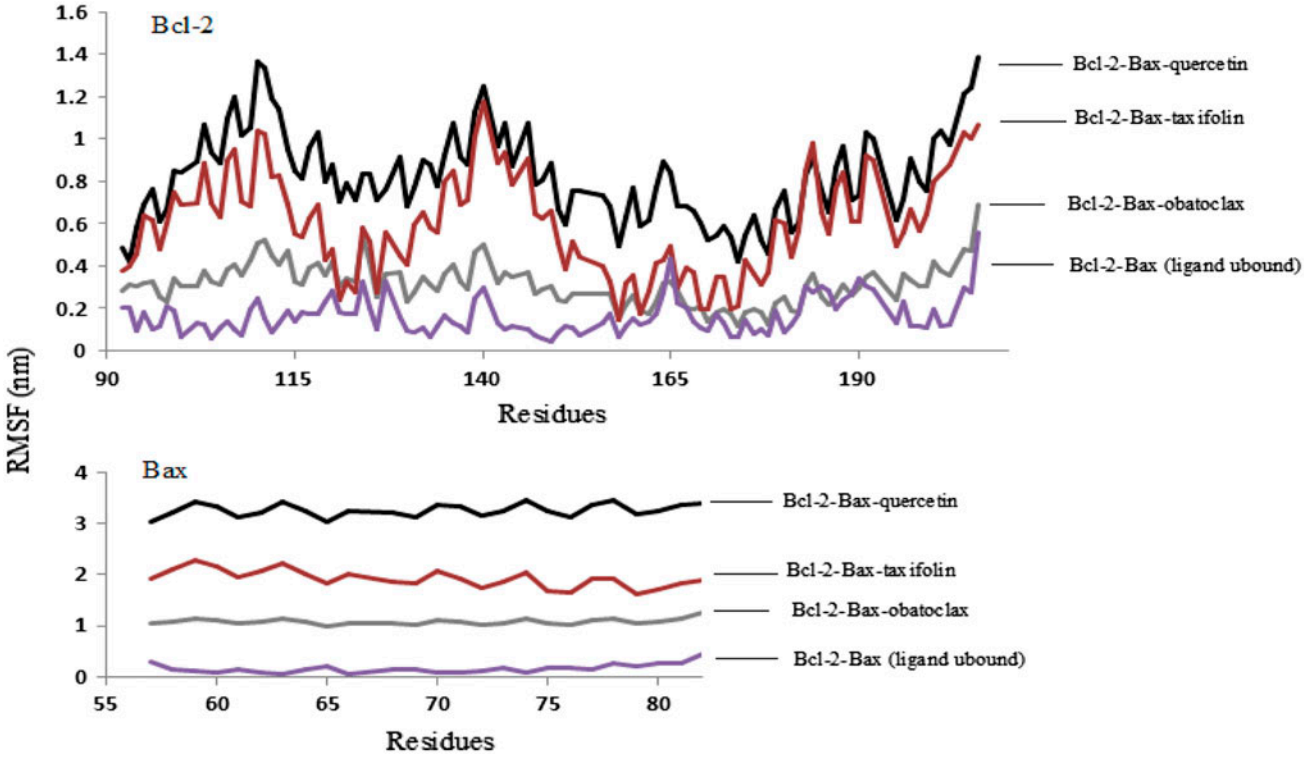

Figure 15. RMSF of Bcl-2 residues side chain (upper) and Bax residues side chain (lower) during MD simulation.

4. Conclusion

Anti apoptotic protein Bcl-2 prevents the Bax activation/oligomerization by forming heterodiner and *halts* the apoptosis. Quercetin and taxifolin efficiently bound to hydrophobic groove and alter the structure of by inducing conformational changes. Taxifolin was found more efficient when compared to quercetin in terms of interaction energy and collapse of hydrophobic groove. *Apart from this*, it was found that taxifolin and quercetin disrupted the Bcl-2-Bax heterodimer during 12 ns MD simulation. Taxifolin and quercetin that interacted with residues were Arg 65, Asp 68, Glu 69, and Ser 72 of Bax, and Arg 110 and Asp 111 of Bcl-2 which are known to be responsible for interaction between Bcl-2 and Bax. Taxifolin and quercetin binding to complex induced the mobility in protein by disrupting stable interaction of interface residues. Further, validation by the MD simulation of ligand-unbound Bcl-2-Bax and obatoclax-bound Bcl-2-Bax showed stability during simulation which favored the previous experimental results and disruption effect of taxifolin and quercetin on Bcl-2-Bax complex.

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References

Accelrys Software Inc. (2011). *Discovery studio modeling environment, Release 3, 1*. San Diego, CA: Author.

Acoca, S., Cui, Q., Shore, G. C., & Purisima, E. O. (2011). Molecular dynamics study of small molecule inhibitors of the Bcl-2 family. *Proteins: Structure, Function, and Bioinformatics, 79*, 2624–2636.

Adams, J. M., & Cory, S. (2001). Life-or-death decisions by the Bcl-2 protein family. *Trends in Biochemical Sciences, 26*, 61–66.

Amadei, A., Ceruso, M. A., & Di Nola, A. (1999). On the convergence of the conformational coordinates basis set obtained by the essential dynamics analysis of proteins' molecular dynamics simulations. *Proteins: Structure, Function, and Genetics, 36*, 419–424.

Amadei, A., Linssen, A. B., & Berendsen, H. J. C. (1993). Essential dynamics of proteins. *Proteins: Structure, Function, and Genetics, 17*, 412–425.

Antignani, A., & Youle, R. J. (2006). How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Current Opinion in Cell Biology, 18*, 685–689.

Berendsen, H. J. C., Van der Spoel, D., & Van Drunen, R. (1995). GROMACS: A message-passing parallel molecular dynamics implementation. *Computer Physics Communications, 91*, 43–56.

Cao, X., Yap, J. L., & Newell-Rogers, M. K. (2013). The novel BH3 α-helix mimetic JY-1-106 induces apoptosis in a subset of cancer cells (lung cancer, colon cancer and mesothelioma) by disrupting Bcl-xL and Mcl-1 protein–protein interactions with Bak. *Molecular Cancer, 12*, 42.

Czabotar, P. E., Lessene, G., Strasser, A., & Adams, J. M. (2014). Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. *Nature Reviews Molecular Cell Biology, 15*, 49–63.

Day, C. L., Chen, L., Richardson, S. J., Harrison, P. J., Huang, D. C. S., & Hinds, M. G. (2005). Solution structure of prosurvival Mcl-1 and characterization of its binding by proapoptotic BH3-only ligands. *Journal of Biological Chemistry, 280*, 4738–4744.

DeLano, W. L. (2004). *The PyMOL molecular graphics system*. San Carlos, CA: DeLano Scientific LLC. Retrieved from http://www.pymol.org

Ding, J., Zhang, Z., Roberts, G. J., Falcone, M., Miao, Y., Shao, Y., … Andrews, J. (2010). Bcl-2 and Bax interact via the BH1-3 groove-BH3 motif interface and a novel interface involving the BH4 motif. *Journal of Biological Chemistry, 285*, 28749–28763.

Dlugosz, P. J., Billen, L. P., Annis, M. G., Zhu, W., Zhang, Z., Lin, J., … Andrews, D. W. (2006). Bcl-2 changes conformation to inhibit Bax oligomerization. *The EMBO Journal, 25*, 2287–2296.

Garcia, A. E. (1992). Large-amplitude nonlinear motions in proteins. *Physical Review Letters, 68*, 2696–2699.

Green, D. R., & Kroemer, G. (2004). The pathophysiology of mitochondrial cell death. *Science, 305*, 626–629.

Hosseini, A., Espona-Fiedler, M., Soto-Cerrato, V., Quesada, R., Pérez-Tomás, R., & Guallar, V. (2013). Molecular interactions of prodiginines with the BH3 domain of anti-apoptotic Bcl-2 family members. *PLOS One, 8*, e57562.

Igney, F. H., & Kramer, P. H. (2002). Death and anti-death: Tumour resistance to apoptosis. *Nature Review, 2*, 277–288.

Kim, H., Rafiuddin-Shah, M., Tu, H. C., Jeffers, J. R., Zambetti, G. P., Hsieh, J. J. D., & Cheng, E. H. Y. (2006). Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. *Nature Cell Biology, 8*, 1348–1358.

Kitao, A., Hirata, F., & Go, N. (1991). The effects of solvent on the conformation and the collective motions of protein: Normal mode analysis and molecular dynamics simulations of melittin in water and in vacuum. *Journal of Chemical Physics, 158*, 447–472.

Ku, B., Liang, C., Jung, J. U., & Oh, B. (2011). Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. *Cell Research, 21*, 627–641.

Lee, S. B., Cha, K. H., Selenge, D., Solongo, A., & Nho, C. W. (2007). The chemopreventive effect of taxifolin is exerted through ARE-dependent gene regulation. *Biological & Pharmaceutical Bulletin, 30*, 1074–1079.

Lin, B., Kolluri, S. K., Lin, F., Liu, W., Han, Y. H., Cao, X., … Reed, X. K. (2004). Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3. *Cell, 116*, 527–540.

Lindah, E., Hess, B., & Van der Spoel, D. (2001). Gromacs 3.0: A package for molecular simulation and trajectory analysis. *Journal of Molecular Modeling, 7*, 306–317.

Liu, X., Dai, S., Zhu, Y., Marrack, P., & Kappler, J. W. (2003). The structure of a Bcl-xL/Bim fragment complex implications for Bim function. *Immunity, 19*, 341–352.

Maity, A., Yadav, S., Verma, C. S., & Dastidar, S. G. (2013). Dynamics of Bcl-xL in water and membrane: Molecular simulations. *PLOS One, 8*, e76837.

Mohammadi, M. K., Firuzi, O., Khoshneviszadeh, M., Razzaghi-Asl, N., Sepehri, S., & Miri, R. (2014). Novel 9-(alkylthio)-acenaphtho[1,2-e]-1,2,4-triazine derivatives: Synthesis, cytotoxic activity and molecular docking studies on B-cell lymphoma 2 (Bcl-2). *DARU Journal of Pharmaceutical Sciences, 22*, 2. doi:10.1186/2008-2231-22-2

Morris, G. M., Goodsell, D. S., & Halliday, R. S. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry, 19*, 1639–1662.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry, 30*, 2785–2791.

Opferman, J. T., & Korsmeyer, S. J. (2003). Apoptosis in the development and maintenance of the immune system. *Nature Immunology, 4*, 410–415.

Petros, A. M., Medek, A., Nettesheim, D. G., Kim, D. H., Yoon, H. S., Swift, K., … Oltersdorf, S. W. (2001). Solution structure of the antiapoptotic protein Bcl-2. *Proceedings of the National Academy of Sciences, 98*, 3012–3017.

Petros, A. M., Nettesheim, D. G., Wang, Y., Olejniczak, E. T., Meadows, R. P., Mack, J., … Matayoshi, S. W. (2000). Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. *Protein Science, 9*, 2528–2534.

Petros, A. M., Olejniczak, E. T., & Fesik, S. W. (2004). Structural biology of the Bcl-2 family of proteins. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research, 1644*, 83–94.

Pinto, M., Perez, J. J., & Rubio-Martinez, J. (2004). Molecular dynamics study of peptide segments of the BH3 domain of the proapoptotic proteins Bak, Bax, Bid and Hrk bound to the Bcl-xL and Bcl-2 proteins. *Journal of Computer-Aided Molecular Design, 18*, 13–22.

Raghav, P. K., Verma, Y. K., & Gangenahalli, G. U. (2011). Molecular dynamics simulations of the Bcl-2 protein to predict the structure of its unordered flexible loop domain. *Journal of Molecular Modeling, 18*, 1885–1906.

Reed, J. C. (2002). Apoptosis-based therapies. *Nature Review, 1*, 111–121.

Samuel, S., Tumilasci, V. F., Oliere, S., Liên-Anh Nguyên, T., Shamy, A., Bell, J., & Hiscott, J. (2010). VSV oncolysis in combination with the Bcl-2 inhibitor obatoclax overcomes apoptosis resistance in chronic lymphocytic leukemia. *Molecular Therapeutics, 12*, 2094–2103.

Sattler, M., Liang, H., Nettesheim, D., Meadows, R. P., Harlan, J. E., Eberstadt, M., … Fesik, S. W. (1997). Structure of Bcl-xL-Bak peptide complex: Recognition between regulators of apoptosis. *Science, 275*, 983–986.

Saxena, N., Katiyar, S. P., Liu, Y., Grover, A., Gao, R., Sundar, D., … Wadhwa, R. (2013). Molecular interactions of Bcl-2 and Bcl-xL with mortalin: Identification and functional characterization. *Bioscience Reports, 33*, e00073.

Schuttelkopf, A. W., & Van Aalten, D. M. F. (2004). PRODRG: A tool for high-throughput crystallography of protein–ligand complexes. *Acta Crystallographica, 60*, 1355–1363.

Seufi, A. M., Ibrahim, S. S., Elmaghraby, T. K., et al. (2009). Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: Molecular and histological evidences. *Journal of Experimental & Clinical Cancer Research, 28*, 80. doi:10.1186/1756-9966-28-80

Suzuki, M., Youle, R. J., & Tjandra, N. (2000). Structure of Bax. *Cell, 103*, 645–654.

Tang, G., Nikolovska-Coleska, Z., Qiu, S., Yang, C., Guo, J., & Wang, S. (2008). Acylpyrogallols as inhibitors of antiapoptotic Bcl-2 proteins. *Journal of Medicinal Chemistry, 51*, 717–720.

Van Gunsteren, W. F., Billeter, S. R., Eising, A. A., Hünenberger, P. H., Krüger, P., Mark, A. E., … Tironi, I. G. (1996). *Biomolecular simulation: The GROMOS96 manual and user guide*. Zurich: Vdf Hochschulverlag AG.

Van Gunsteren, W. F., Daura, X., & Mark, A. E. (1998). The GROMOS force field. In P. Von Rague Schleyer (Ed.), *Encyclopedia of Computational Chemistry* (Vol. 2, pp. 1211–1216). Chichester: Wiley and Sons.

Wang, K., Gross, A., Waksman, G., & Korsmeyer, S. J. (1998). Mutagenesis of the BH3 domain of BAX identifies residues critical for dimerization and killing. *Molecular Cell Biology, 18*, 6083–6089.

Willis, S. N., Fletcher, J. I., Kaufmann, T., van Delft, M. F., Chen, L., Czabotar, P. E., … Huang, D. C. (2007). Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science, 315*, 856–859.

Yin, X. M., Oltvai, Z. N., & Korsmeyer, S. J. (1994). BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature, 369*, 321–323.

Zha, H., Aime′-Sempe, C., Sato, T., & Reed, J. C. (1996). Proapoptotic protein Bax heterodimerizes with Bcl-2 and homodimerizes with Bax via a novel domain (BH3) distinct from BH1 and BH2. *Journal of Biological Chemistry, 271*, 7440–7444.

Zhou, H., Chen, J., Meagher, J. L., Yang, C., Aguilar, A., Liu, L., … Cong, S. (2012). Design of Bcl-2 and Bcl-xL inhibitors with subnanomolar binding affinities based upon a new scaffold. *Journal of Medicinal Chemistry, 55*, 4664–4682.