Original Article

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Cross Docking Study Directed Towards Virtual Screening and Molecular Docking Study of Phenanthrene 1,2,4-triazine Derivatives As Novel Bcl-2 Inhibitors

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Abstract

Apoptosis is critical for tissue homeostasis and also for the physiological removal of abnormal cells. Bcl-2 family proteins are important regulators of apoptosis. It is observed that antiapototic Bcl-2 family members are generally overexpressed in many cancer cells. As a result, it has motivated a growing interest in the discovery of small molecules targeting such proteins as potential anticancer therapeutics. With the aim of designing new phenanthrene based Bcl-2 inhibitors, we performed a cross-docking study. This study followed by virtual screening is conducted for different available Bcl-2 X-ray crystal structures in order to find the most appropriate PDB code of this enzyme. After analytical analysis, the selected crystal structure was used to screen the library of phenanthrene triazine based structures containing different substitutions attached to the hydrazone moiety. The ligand which interacts with the target with the lowest binding energy was determined. The lowest binding energy was -10.19 kcal/mol. As a conclusion, cross docking study could be a validated strategy for finding the most appropriate crystal structure for docking study and the virtual screening of the designed library indicates the best ligand for the specified target. Our designed library of phenanthrene triazine-based derivatives containing hydrazone pendant, could be served as potential candidates for Bcl-2 inhibition.

Keywords: Apoptosis, Bcl-2 inhibitors, Cross docking, Phenanthrene triazine, Virtual screening.

1. Introduction

Apoptosis has introduced itself as an important target for cancer therapy (1). The role of recently found proteins in the apoptosis mechanism, and insufficient response to cancer chemotherapeutic agents, has enforced scientists to identify novel chemicals as anti-tumor drugs (2, 3).

Apoptosis is an important process which plays a critical role in the maintenance of tissue homeostasis and leads the damaged cells to death (1, 4). Its induction is one of the most potent mechanisms against cancer progression (5). Two pathways have been identified for apoptosis: extrinsic and intrinsic pathways, which each are controlled by different classes of proteins (6, 7). In this regard, Bcl-2 family plays a major role. Bcl-2 pro-apoptotic proteins like Bak and Bax induce apoptosis which causes the death of the cell, but anti-apoptotic ones like Bcl-2 and Bcl-xL prevent cell death by prohibiting the apoptosis procedure (8, 9).

Apoptosis can be regulated by small molecules (10). Therefore, exploring and identifying novel chemicals to inhibit anti-apoptotic proteins can be of interest, helping towards the induction of apoptosis and removing cancer cells. To reach this

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goal, computational methods can be useful for discovering novel anti-apoptotic agents (11). Today, computational studies are widely used in drug design, and virtual screening is one of the best computational methods to find the hit compounds with favorite biological effects (12, 13).

Structure-based virtual screening serves as a fast and reliable approach to screen the large libraries of chemicals in order to identify the hits. That's why in the recent years, it has become a mandatory part of novel drug discovery. Identifying the hit compounds is a starting point in the drug discovery procedure (14, 15, 16).

Docking is one of the most-used methods for virtual screening, and is performed to find the appropriate ligands for a defined protein. Before this process, we performed a cross-docking study to find the best PDB entry for the docking procedure. We chose 18 PDB codes for Bcl-2 protein and then we cross-docked them to find the model which best describes the relationship between pIC50 and free binding energies of the ligand-protein complexes. At the end, docking was performed for the library of 130 different phenanthrene 1,2,4-triazine derivatives, to identify the ligands with the most affinities to the Bcl-2 protein. These ligands can be used as potential anti-apoptotic agents in the future.

2. Materials & methods

2.1. Cross-docking

The ligand-flexible docking studies were carried out using the common molecular docking software namely AutoDock4. To find the best conformation of the Bcl-2 protein which interacts with different ligands, a cross-docking procedure was performed. 72 different X-ray crystallographic structures of Bcl-2 protein with their cognate ligands were retrieved from the protein data bank (http://www.rcsb.org). 18 entries were selected, having the resolution below 2 Å. The RMSD parameter for each target and its cognate ligand were further evaluated. The RMSD value was calculated by extracting the structure of the cognate ligand from the crystallographic complex and re-docking it to its target protein. This process is called "self-docking". It is used to validate the docking procedure. (If the RMSD value is less than 2 Å, the method is validated). After validating the method, each target protein molecule was docked to all 18 ligands. To prepare the protein, crystallographic water molecules were removed and the Kollman charges and polar hydrogens were added. For the ligand, Gasteiger charges were added. The maximum number of rotatable bonds in ligands were seated to 6. The grid for docking process was calculated using AutoGrid and its size was set to 60×60×60 points in x, y and z directions. The grid box covers the active site of the protein and also its surrounding, allowing a good fitting for the ligand. For this flexible ligand rigid protein docking procedure, a Lamarckian Genetic Algorithm (LGA) was employed. Docking parameters depicted in

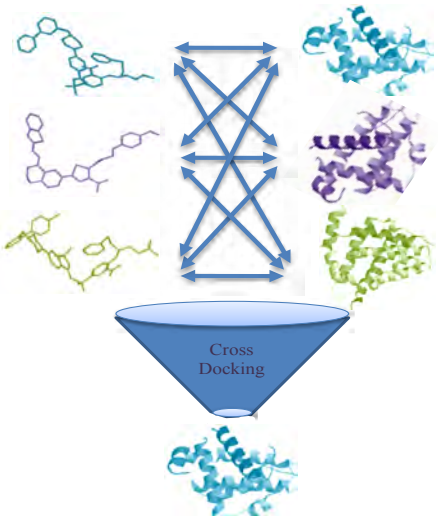

Figure 1. Schematic view of how cross-docking works.

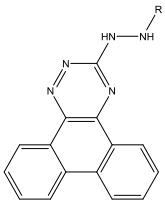

Figure 2. The scaffold of the library compounds.

table 1, and other parameters were preserved as the software's default values. Docking was done using DockFace software.

Cross-docking studies were performed to evaluate the binding affinities and binding free energies between different structures of Bcl-2 protein molecule and the ligands. It also helps determine which structure has the better conformation to interact with different ligands. The cross-docking procedure is depicted in Figure 1.

2.2. Multiple Linear Regression

After the cross-docking procedure, Multiple Linear Regression (MLR) analysis was utilized to establish the relationships between computed binding energies and pIC50 of the target in different structures (extracted from the www.rcsb.org). For this, the 21th version of SPSS software was employed. Based on the results, a model was built according to pIC50 as a function of the binding free energy. The efforts were made to find the best PDB code which showed high correlation between the pIC50 and binding energy. Finally, one PDB code was selected based on the MLR analysis and the correspondent R values.

2.3. Library Preparation

The core structure of the compounds was phenanthrene triazine with a hydrazone moiety on C3 position of the 1,2,4- triazine ring (Figure 2).

Different aryl and alkyl substitutions were attached to the hydrazone moiety (R) to construct different derivatives. The library was built using 130 different groups as substitutions.

2.4. Virtual Screening

The crystallography PDB code was used for virtual screening (PDB ID: 4IEH). A library containing 130 ligands with phenanthrene triazine scaffold bearing different aryl and alkyl hydrazone moieties was prepared. This process was performed using ChemBioDraw Ultra 14.0 and

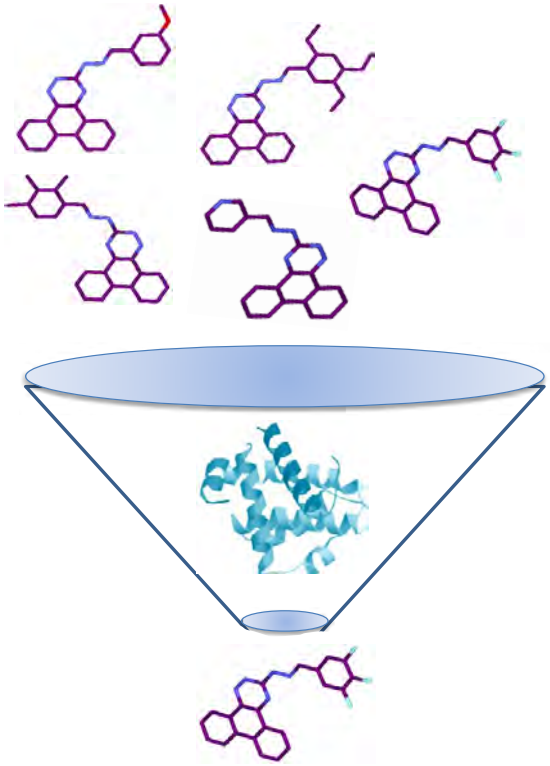

Figure 3. The concept of Virtual screening for a definite target.

HyperChem Professional 8.0.10 softwares. The ligands were spatially optimized by the HyperChem software. Target protein molecule and all the ligands were further processed using the AutoDock software. Cross-docking study encompasses the same docking parameters. Again, a Lamarckian Genetic Algorithm was used with 50 independent GA runs. The docking procedure was performed using DockFace interface for the selected PDB code (Figure 3).

Clustering and scoring the results were performed on the basis of the binding free energies calculated in the docking process. The best cluster was selected regarding its AutoDock score of energy and also the population of the cluster. For most ligands, the top-ranked cluster of DLG file was selected for further analyses.

Molecular docking was used to reveal the binding mode and the main interactions between the designed ligands and the target molecule. The key residues involved in the interactions were identified. The strongest interactions between the ligands and the target were also determined regarding the free binding energies of different complexes.

Table 1. The docking parameters for cross-docking and virtual screening.

Docking Parameter	Value
Number of Independent GA Runs	30
Population Size	150
Maximum Number of Evaluations	2500000
Maximum Number of Generations	27000
Mutation Rate	0.02
Cross-over Rate	0.8

3. Results

3.1. Internal Validation

In order to validate the virtual screening method using the docking procedure, internal validation was performed by the RMSD value calculation. The RMSD value (<2 Å) was acceptable.

3.2. MLR analysis

Different plots were attained for each ligand-target complex. For each PDB code, the regression line equation was obtained. Based on the MLR analysis, 4IEH code was selected due to the relationship between its pIC50 values and the binding free energies. These values were obtained for target molecules docked with all 18 ligands of different crystallographic structures of Bcl-2 protein. The R Square value for the obtained model was 0.54 that implied satisfying relationship. Its plot is depicted in Figure 4.

3.3. Virtual Screening

The best docking results including free binding energies and inhibition constants are summarized in table 2. The important interactions between Bcl-2 protein and the ligands were obtained using PYMOL software. Principal target residues which are involved in the interactions with ligands are summarized in table 3.

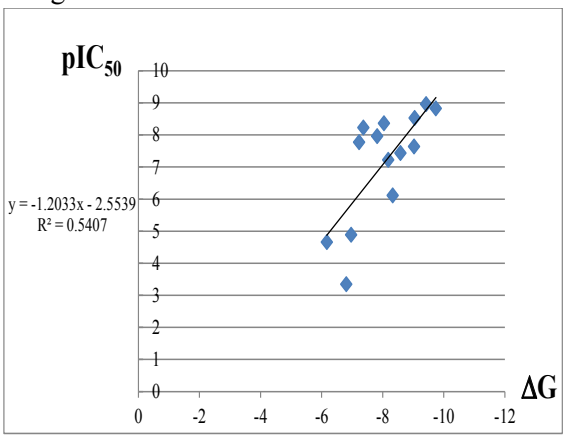

Figure 4. Plot of relationship between pIC_{50} values and free binding energies of 18 different ligands for 4IEH PDB code.

Table 2. Docking best results (free binding energies and inhibition constants).

	Binding Energy (Kcal/mol)	Inhibition Constant (nM)
1	-10.19	34.08
2	-9.99	47.73
3	-9.94	51.73
4	-9.88	57.40
5	-9.87	58.64
6	-9.8	66.08
7	-9.78	67.31
8	-9.77	69.10
9	-9.73	94.07
10	-9.71	75.90

4. Conclusion and Discussion

Virtual screening is a useful method for finding novel chemicals in large libraries of compounds which is necessary for drug discovery (17). Cross-docking and docking procedures generate a powerful tool for computational studies when combined with each other. It is useful for screening the crystallographic structures of a definite target to identify the most reliable entry. Cross-docking is briefly shown in figure 5. Then the best ligands for the specified protein could be found, using virtual screening of the library. Some examples of binding modes of the ligands with crystallographic structures of Bcl-2 protein are shown in figure 6. Based on binding energies, it's obvious that the interaction between target and ligand molecules is strong

Table 3. Principal target residues which are involved in the interactions with ligands.

PDB	H bond	Non-H bond
4IEH	hydrazone N: Gly 104, Asp 62 hydroxyl group: Arg 105, Tyr 67	phenanthrene: Arg 66, Tyr 161

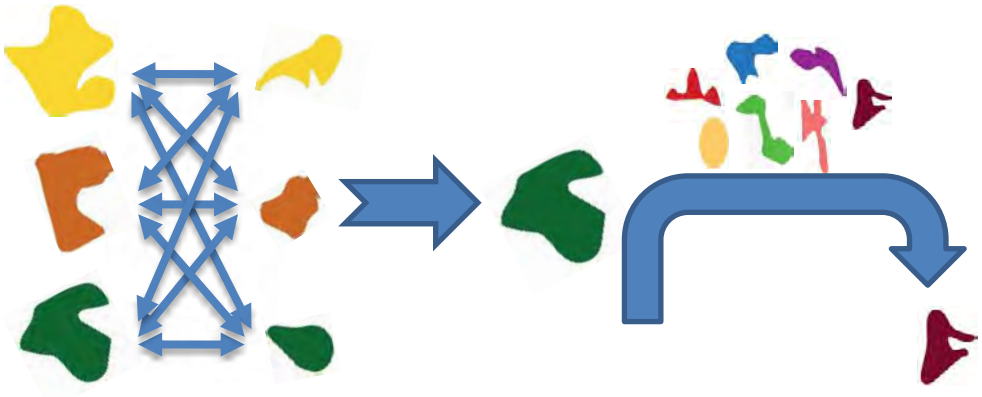

Figure 5. Cross-docking and docking procedures.

enough to have proposed pharmacological effects.

Although computational studies cannot ensure us of their results, they can certainly eliminate a large portion of compounds and so conserve our time and expenses. In this manner, we can focus on a more limited group of chemicals which can lead to higher precision in hit finding.

First, we identified the appropriate PDB code of Bcl-2 protein by the cross-docking procedure. Then, we screened our designed library of chemicals to find the best ligand-protein complexes, based on binding free energies. The resulted ligands can be subjected to further experiments to prove their effects on the Bcl-2 protein as anti-apoptotic agents.

Conflict of Interest

None declared.

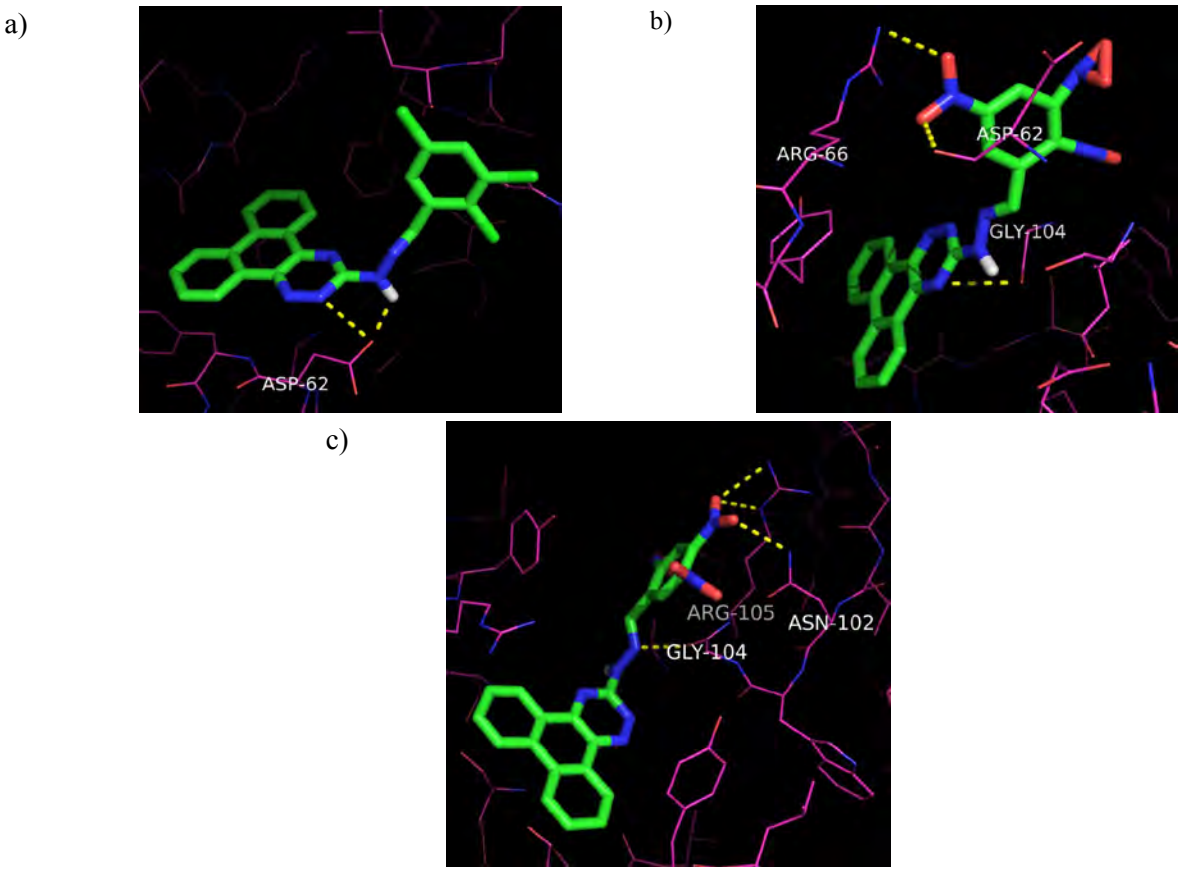

Figure 6. The binding mode of three phenanthrene 1,2,4-triazine derivatives with Bcl-2 protein (PDB: 4IEH).

5. References

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