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Scholars Research Library

**Der Pharma Chemica, 2010, 2(6):67-72**
*(http://derpharmachemica.com/archive.html)*

**ISSN 0975-413X**

# Computer aided drug studies of BH3 derivatives as anti-cancer agents against Bcl-xL receptor

**Daisy. P and Suveena.S**

*Bioinformatics Facility (BIF), Department of Biotechnology & Bioinformatics, Holy Cross College, Teppakulam, Tiruchirapalli, India*

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## ABSTRACT

*Informatics and computational design methods were used to create new molecules that could potentially bind antiapoptotic proteins, thus promoting death of cancer cells. Apoptosis is a cellular process that leads to the death of damaged cells. Its malfunction can cause cancer and poor response to conventional chemotherapy. After being activated by cellular stress signals, proapoptotic proteins bind antiapoptotic proteins, thus allowing apoptosis to go forward. Molecular docking is routinely used for understanding the drug-receptor interactions in modern drug design. Here we described the docking of BH3 derived peptides from BID as inhibitors to Bcl-xL, which is over expressed in cancerous cells. The inhibitory activities against Bcl-xL were investigated by molecular docking using Hex docking software. All the designed peptides were showed good binding energy, among which GDGVQ & VGDGV are showed moderate binding energy (-277.94 & -258.24 respectively) and satisfied reasonably the Lipinski Rule of Five and ADME/T properties. Further modifications are needed for these designed compounds to increase its docking score as well as properties and finally we planned to synthesis and also screen for in-vitro anti cancerous effect.*

**Keywords:** Apoptosis, Cancer, BH3 domain, Docking, Hex.

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## INTRODUCTION

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target).

Bcl-2 family of proteins is critical regulators of apoptosis [1]. It is also thought that some Bcl-2 family proteins can induce (pro-apoptotic members) or inhibit (anti-apoptotic members) the release of cytochrome c into cytosol which, once there activates caspase-9 and caspase-3, leading

to apoptosis.Although Zamzami et al. suggest that the release of cytochrome c is indirectly mediated by the PT pore on the inner mitochondrial membrane (2). Overexpression of Bcl-2 or Bcl-xL potentially inhibits apoptosis in response to many cytotoxic insults (13). The anti apoptotic proteins inhibits cytochrome c release by blocking BH3-only proteins. The members of the Bcl-2 family share one or more of the four characteristic domains of homolog entitled the Bcl-2 homology (BH) domains (named BH1, BH2, BH3 and BH4).The anti-apoptotic Bcl-2 proteins,(Bcl-2 and Bcl-xL) conserve all four BH domains. The BH domain also serves to subdivide the pro-apoptotic Bcl-2 proteins into those with several BH domains (eg.Bax and Bak) or those proteins that have only the BH3 domain (eg.Bid, Bim and Bad). Amongst the members of the Bcl-2 family, the BH3-only proteins have now been recognized as essential initiators of programmed cell death and stress-induced apoptosis (3).As BH3 only proteins are suggested to kill cells by interacting with the BH3 receptors (4), in this study we designed some BH3 derivatives from BID as targeted anticancer agents based on molecular docking between designed peptides and Bcl-xL using Hex docking software.

## MATERIALS AND METHODS

For our present study we used bioinformatics tools, biological databases like KEGG, PDB and softwares like Hex,ACD Chemsketch,Accord for Excel and SwissPDB Viewer.

The PDB(protein Data Bank) is the single worldwide archive of Structural data of biological macromolecules, established in Brookhhaven National Laboratories (BNL) (5).It contains structural information of the macromolecules determined by the X-ray crystallographic,NMR methods etc. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of online databases dealing with genomes, enzymatic pathways and biological chemicals. Hex is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes [6].It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result [7].

To study the interaction we have used Swiss pdb viewer. Deep View - Swiss-PdbViewer is an application which provides a user friendly interface allowing analyzing several proteins at the same time. H-bonds, angles and distances between atoms are easy to obtain.

ACD/ChemSketch software is an integrated software package from Advanced Chemistry Development Inc. for drawing chemical structures, 3D optimization algorithm allows the planar (2D) structure from ChemSketch to be rapidly translated into a realistic 3-dimensional structure. It is based on the modifed molecular mechanics which take into account, bond stretching, angle bending, internal rotation and Van der Waals nonbonded interactions. The 3D optimization algorithm is a proprietary version of molecular mechanics with the force field initially based on CHARMM parameterization [8, 9]. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. By the help of chemsketch software we have generated the molecular properties of the peptide.

Accord for Excel's ADME add-on enables scientists to compute and predict absorption, distribution, metabolism, and excretion (ADME) properties for chemical libraries, screening collections, and synthesis candidates (12).

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Computer-Aided Drug Design(CADD) is a specialized discipline that uses computational methods are heavily dependent on bioinformatics tools, applications and databases(10).The structure of Bcl-xL antiapoptotic receptor was retrieved from PDB(3IHC).Using Chemsketch the structures of peptide drugs were sketched. The docking analysis of these compounds with 3IHC was carried by using Hex docking software.

Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an target fit together and dock to each other well, like pieces of a three-dimensional jigsaw puzzle. The molecules binding to a receptor, inhibit its function, and thus act as drug. The BH3 derived peptide and receptor complexes were identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations.

**The parameters used for the docking process were**
Correlation type – Shape only, Grid Dimension – 0.6, Receptor range – 180, Ligand Range – 180, Twist range – 360, Distance Range–40. The drug was docked with the receptor using the above parameters.

**Lipinski Rule of Five**
Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules (11).It predicts high probability of success or failure due to drug likeness for molecules complying with 3 or more of the following rules:

- Molecular mass less than 500Da
- High lipophilicity(expressed as Log P less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors

These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures. In this study, we also calculated these 5 parameters for all designed compounds.Also verified its ADME/Toxicity properties by Accord Excel.

## RESULTS AND DISCUSSION

**Table 1.Docking Results of 3IHC receptor with BH3 derivative peptides**

| S.I | BH3 Derivatives | Docking score | In/out of BH3 or BH2 region of receptor |
|---|---|---|---|
| 1 | ALETL | -327.46 | In BH2 |
| 2 | LETLR | -337.77 | Just in BH3 |
| 3 | **ETLRR** | **-389.73** | **In BH3** |
| 4 | TLRRV | -298.40 | In BH3 |
| 5 | LRRVG | -334.49 | Out of BH3 & BH2 |
| 6 | RRVGD | -310.46 | In BH2 |
| 7 | RVGDG | -289.63 | Out of BH3 & BH2 |
| 8 | **VGDGV** | **-258.24** | **Just in BH3** |
| 9 | **GDGVQ** | **-277.94** | **Just in BH3** |
| 10 | DGVQR | -284.90 | Just in BH3 |
| 11 | GVQRN | -318.97 | In BH2 |

**Figure 1. Compound ETLRR docked exactly on the BH3 domain of the receptor. Green zone represents the BH3 region, Yellow the BH2 and Red stick indicates the compound.**

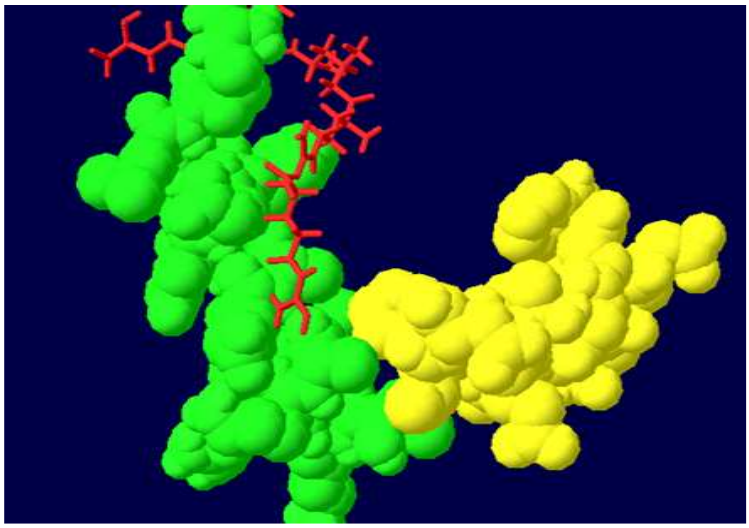

**Table 2. Lipinski properties of docked Compounds**

| S.I | Derivative | Hacc | Hdon | LogP | Mol.wgt | Lip.Viol. |
|---|---|---|---|---|---|---|
| 1 | **ALETL** | 13 | 10 | False | 529.62 | 3 |
| 2 | **LETLR** | 16 | 14 | False | 614.73 | 3 |
| 3 | **ETLRR** | 19 | 17 | False | 657.76 | 3 |
| 4 | **TLRRV** | 17 | 16 | False | 627 | 3 |
| 5 | **LRRVG** | 16 | 16 | False | 568 | 3 |
| 6 | **RRVGD** | 20 | 18 | False | 555 | 3 |
| 7 | **RVGDG** | 15 | 12 | False | 486 | 2 |
| 8 | **VGDGV** | 11 | 6 | False | 414 | 2 |
| 9 | **GDGVQ** | 14 | 10 | False | 458 | 2 |
| 10 | **DGVQR** | 17 | 14 | False | 557 | 3 |
| 11 | **GVQRN** | 17 | 15 | False | 556 | 3 |

**Table 3. ADME/T properties of docked Compounds**

| S.I | Derivative | ADME/ FPSA | ADME/ AQ.SOL. | CYP 2D6 | HEPAT TOX | HIA | BB penetration |
|---|---|---|---|---|---|---|---|
| 1 | **ALETL** | Low absorption | Good soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 2 | **LETLR** | Low absorption | Very low Soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 3 | **ETLRR** | Low absorption | Extremely Low soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 4 | **TLRRV** | Low absorption | Very low soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 5 | **LRRVG** | Low absorption | Very low soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 6 | **RRVGD** | Low absorption | Extremely Low soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 7 | **RVGDG** | Low absorption | Low Soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 8 | **VGDGV** | Low absorption | Optimal Solubility | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 9 | **GDGVQ** | Low absorption | Good Soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 10 | **DGVQR** | Low absorption | Very low Soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |
| 11 | **GVQRN** | Low absorption | Low soluble | Non-inhibitor | Non-toxic | Low absorption | Very low |

*If LogP is less than 4.15 it returns as false, otherwise true.*

BH3 only proteins are suggested to kill cells by interacting with the BH3 receptor (4), which is a hydrophobic groove, formed by the apposition of the BH1, BH2 and BH3 domains. The so-called "BH3 mimetics"pharmacological compounds that bind to BH3 receptors, can induce apoptosis or facilitate apoptosis induction in cancer cells(18).From Table 1 it is clear that the 3rd compound ETLRR was exactly binding to the BH3 region of the receptor with decrease in energy values (-389.73) which means it was more compatible with the receptor. Lipinski Rule of 5 was calculated for all the designed compounds. Out of 11 compounds violation of rule of five was showed by 8 compounds. Out of these 8 compounds surprisingly the compound **ETLRR** showed good docking score and exactly binding to the receptor. ADME/Toxicity properties of the compounds were also verified. From Table 2, it is clear that all the 11 compounds were non-toxic as well as non-inhibitor to CYP2D6 protein. Blood brain penetration and human intestinal absorption was very low for all the compounds. Only three compounds that is, VGDGV, GDGVQ & RVGDG were found fairly eligible as drug-likeness. But after docking these three compounds showed moderate interaction energies with the receptor when compared with other derived compounds and their docking scores were -258.24,-277 & -289.63 respectively. Among the three VGDGV and GDGVQ are exactly binding to the BH3 domain of the receptor where as RVGDG was not in the accurate site. As per Table 3, the aqueous solubility of VGDGV is optimal and that of GDGVQ is good. Other compounds predicted to have poor adsorption or permeation ability because of one or more following features: more than 5 H –bond donors, more than 10 H-bond acceptors, and the molecular weight is greater than 500 and the calculated Log P value greater than 5.So the compounds VGDGV & GDGVQ were satisfied the rule for potent inhibitors (Table 2 & Table 3).

## CONCLUSION

Virtual HTS is cost effective and reliable technique that can be applied to identify potential leads and avoid undesirable compounds that would otherwise result in expensive and time consuming experimental methods. However, virtual HTS often requires careful preparation of both target and compound library, use of optimal parameters as well as careful analysis of the results. We have covered some of the essential considerations in designing virtual HTS experiment. Hence we conclude that the BH3 derived compounds VGDGV & GDGVQ can be potent anti-cancer agent. Future studies are needed to rectify the errors for all the other compounds and finally screen for their in-vitro anti-cancer effect.

**Acknowledgement**
This project is funded by BTIS (Biotechnology information system), DBT (Department of biotechnology), Ministry of Science and technology, Government of India, India.

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