In Silico Molecular Docking of Anthraquinone identified from *Boerhavia diffusa linn* against Bax and Bcl-2 gene

ABSTRACT

In today's medical environment, natural products have made a substantial contribution to the therapeutic approach in the treatment of diseases ranging from the simple to the complex. The old or traditional approach of standardization in medicinal plant research is a time-consuming, costly, and to some extent antiquated process. As a result, a computational technique that includes an in silico molecular docking simulation study has become an important tool for drug development, standardisation, and screening of phytochemicals. To investigate the cardioprotective research and the interaction of the strong chemical against Bax and Bcl-2 cardiomyocyte gene, docking was conducted using multiple Protein Data Bank files (3EOO, 3D2U, 2I42, and 3D2Y). The Anthraquinone has shown more potent interaction with apoptotic regulators Bcl-2 and Bax genes by showing good binding energy. The study also evident that Anthraquinone (UBA) was an ideal drug agent with better drug likeliness. Further, the compound can be used as therapeutic molecule for myocardial infarction. However, the results are preliminary and experimental evaluation will be carried out in near future.

Keywords: Anthraquinone, Bax, BCL_2, Myocardial infarction, molecular docking, Drug discovery.

1. INTRODUCTION

A recent economic tendency is to emphasise natural resources, and anthraquinones are abundant in many plant species. The latter are natural substances with a variety of biological features and resulting positive and/or negative consequences, and have been used in industry as natural colours. Anthraquinones are structurally constructed from an anthracene ring (a tricyclic aromatic ring) with carbonyl groups at positions 9 and 10.

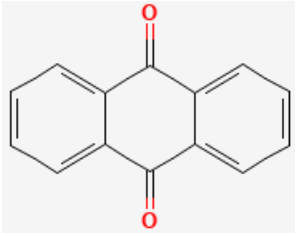


Figure 1. Structure of Anthraquinone

Anthraquinones' ability to inhibit bacterial and fungal expansion are two fascinating natural properties, but they also have antioxidant, anticancer, anti-inflammatory, laxative (Johanna Duvala et al., 2016), immunosuppressive, laxative, cathartic diuretic, vasorelaxing, and phytoestrogen properties (Chien et al., 2015; Reynolds,2004; Locatelli, 2011).

Bcl-2 family proteins have been found as apoptosis regulators, with Bcl-2 being the first to be acknowledged as an oncoprotein linked to B-cell lymphoma. They are divided into two groups, one containing anti-apoptotic proteins (Bcl-2) and the other containing pro-apoptotic proteins (Bax). Functions of these proteins, for example, controlling permeability of mitochondrial membrane, releasing mitochondrial apoptogenic factors into the cytoplasm, and regulating apoptosis, are dependent on I their ability to homo- or heterodimerize with BH3 domains, and (ii) the integration of the carboxy-terminal hydrophobic domain, or TM (transmembrane) domain, into specific cytoplasmic membranes **(Petros *et al*., 2004; Thomadaki *et al*., 2006).**

Bax and BCL2 – Antiapoptotic activity:

Apoptosis regulator BAX, also known as bcl-2-like protein 4, is a protein that in humans is encoded by the *BAX* gene. *BAX* is a member of the Bcl-2 gene family. BCL2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. This protein forms a heterodimer with BCL2, and functions as an apoptotic activator. The BCL-2 family of proteins controls cell death primarily by direct binding interactions that regulate mitochondrial outer membrane permeabilization (MOMP) leading to the irreversible release of intermembrane space proteins, subsequent caspase activation and apoptosis. (Justin Kale **et al.,** 2018).

Molecular docking is a computer tool used to investigate the structure of molecular drugs. It is a safe and simple to use technology that aids in the examination of molecular structures in three dimensions. Molecular docking prediction is a method for predicting the interactions of intermolecular complexes, such as ligands (drugs) and receptors (target proteins), and it has been effectively utilised in the pharmaceutical industry to find lead molecules (Ghosh et al., 2006). The iGEMDOCK suite was used to undertake docking

studies for natural molecules (ligands) from the plant Boerhavia diffusa with the Bax and BCl-2 genes.

iGEMDOCK has interactive interfaces for preparing the target protein's binding site as well as the screening chemical library. Using the in-house docking programme iGEMDOCK, each compound in the library is then docked into the active binding site **(Balavignesh *et al*., 2013).** The Anthraquinone has shown more potent interaction with apoptotic regulators Bcl-2 and Bax genes by showing good binding energy. The present research reveals that Anthraquinone used as promising medication applicant against different malignancies.

2. MATERIALS AND METHODS

2.1. Preparation of the protein structure

The protein needed for the docking research was downloaded from the Protein Data Bank with a resolution of 1.3 A root mean square deviations (RMSD), representing the three-dimensional structure of target Bax and Bcl-2.

2.2. Preparation of Ligands

For the docking process, the chemicals isolated from B.diffusa were used to make the ligand molecules. The chemicals were found in the PubChem database. The compounds' structures were downloaded in (.sdf) layout and renewed to (.mol) format with the help of open babel software, which searched for tautomers and steric isomers as well as ligand geometry minimization.

2.3. Module for docking

Software for docking the cardiac proteins Bax and Bcl2 were docked with medicinal molecules using iGEM dock. iGEMDOCK is a virtual screening (VS) environment that includes everything from preparation through post-screening examination, including pharmaceutical associations.

iGEMDOCK creates hydrogen-bonding (H), electrostatic (E), and Van der Waal's (V) interaction profiles for protein compounds. iGEMDOCK suggests pharmacological associations and groups of the screening compounds for post-screening research depending on these profiles and compound structures.

2.4. Mechanism of docking

- The iGEMDOCK molecular docking software was used to do the docking. During

docking, the molecules are initially formed by designating bonds, bond ordering, specified hydrogens, charges, and flexible torsions to both the ligands and the protein. Wizard ligands were chosen from the Docking, and the scoring function utilised was iGEMDOCK score.

- If hydrogen bonding is available, a penalty is forced to the hydrogen bond energy addition to the Docking score depending on deviations from the ideal bonding angle. Internal hydrogen bond sp2-sp2 torsions are estimated from the pose by permitting the ligand assessment terms, which can dramatically minimise the amount of improbable hydrogen bonds and internal electrostatic interaction.
- The search algorithm is iGEMDOCK, and the number of runs is ten, with a maximum interaction size of 2000 and an energy threshold of 100. At each step, the least'min' torsions/translations/rotations are examined, and the one with the lowest energy is picked.
- The binding affinities in kcal/mol and the docking run time were calculated using docking between Protein and Inhibitor.
- The best inhibitor was chosen as the one with the lowest binding energy. When compared to other software, iGEMDOCK performed better overall in docking simulations.

Visualization: By integrating iGEMDOCK's pharmacological interactions and energy-based scoring system, iGEMDOCK ranks and visualises the screening compounds.

3. RESULTS AND DISCUSSION

Docking analysis of compound anthraquinone with Bax and Bcl-2 gene was performed using **iGEMDOCK** docking server. The protein-ligand complex is a reversible non-covalent interaction between two biological (macro)molecules. In non-covalent interactions there is no sharing of electrons like in covalent interactions or bonds. Non-covalent binding may depend on hydrogen bonds, hydrophobic forces, van der Waals forces, π-π interactions, electrostatic interactions in which no electrons are shared between the two or more involved molecules. The molecules (protein and ligand) recognize each other also by stereospecificity (Bongrand P,1999).

The drug's fit to the target molecules is aided by the maximum binding energy of receptor ligand interactions. The binding energy change (OG) has a negative value, indicating that the process of binding is spontaneous. The drug's fit to the target molecules is supported by the binding energy with the highest value. The lower the negative binding energy, the more likely the molecule will be taken as a medication **(Balavignesh, 2013).**

To investigate the cardioprotective research and the interaction of the potent chemical against Bax and Bcl-2 cardiomyocyte gene, docking was completed using multiple Protein Data Bank files (3EOO, 3D2U, 2I42, and 3D2Y). Docking can execute the result and provide structural theories of how the ligand represses the target, which is crucial in lead optimization (Rohit Babu et al., 2016). The Protein Data Bank files for Structure of Human Bax Gene and Human Bcl-2 are PDB ID: 1F16 and PDB ID: 4LVT, respectively. Bax and Bcl-2 are apoptotic regulators.

Hydrogen-bonds play a crucial role in determining the specificity of ligand binding. Their important contribution is explicitly incorporated into a computational method, called GRID, which has been designed to detect energetically favourable ligand binding sites on a chosen target molecule of known structure. Weak hydrogen bonds of 1-5 kcal/mole (4 - 21 kjoule/mole), sometimes formed with carbon as the proton donor, are no stronger than conventional dipole-dipole interactions**.** The bonds which are in electrostatic in nature, they are considered as weak hydrogen bonds (R C Wade, P J Goodford., 1989).

H-bonding serves to alter binding affinity and therapeutic efficacy by stabilising the ligand and the target site. The findings reveal a novel in silico method for designing pharmacological leads at the hydrophobic centre of the ligand-protein interphase. Based on the chemical environment at the ligand, target, and target-ligand interphase, weak hydrogen bonds can be broken and substituted for another type of bond **(Parul *et al*., 2018).**

The molecular docking server was used to calculate chemical information for docking computations. The total of the electrostatic, van der Waals, and ligand flexibility was used to compute the docking energy values. *The total energy of a specified pose in the binding site is called fitness. iGEMDOCK's empirical scoring function is calculated as*:

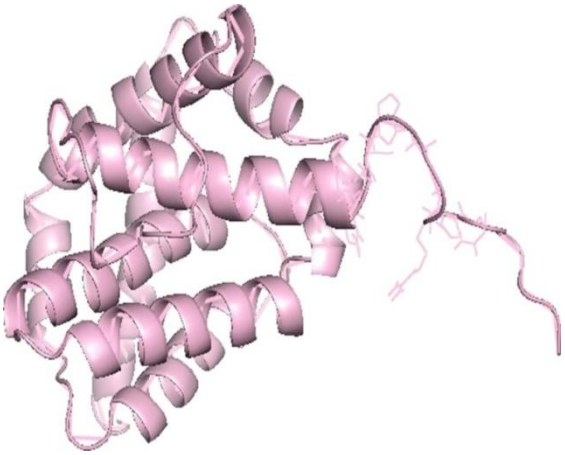
Fitness **= vdW + Hbond + Elec.**

Here, the vdW term is van der Waal energy. H bond and Elect terms are

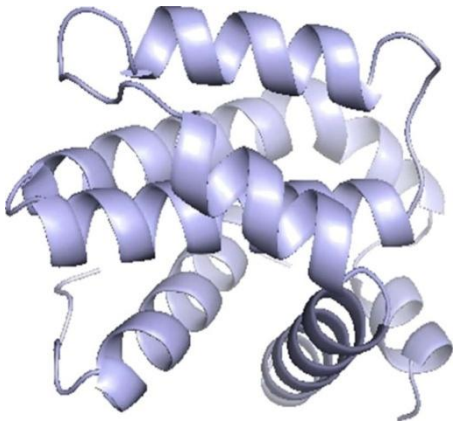
hydrogen bonding energy and electro statistic energy, respectively.

Figure 1 represent structure of Bax, Bcl-2 and Anthraquinone. ***Figure 2*** explains the promising binding pose of the selected ligand with proteins Bax and Bcl-2 gene. ***Figure 3 and 4*** determine the Bonding interactions for the proteins Bax, and Bcl_2 with ligand anthraquinone (UBA)

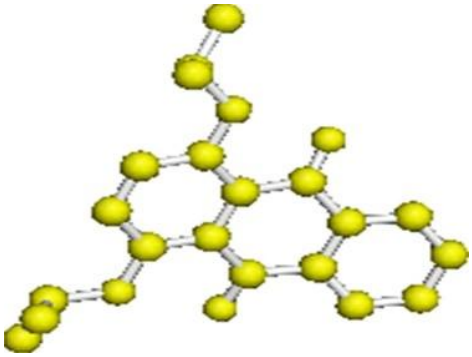
MOLECULAR DOCKING OF ANTHRAQUINONE WITH BAX AND BCL-2



Human Bax Gene (PDB ID: 1F16)



Human Bcl2 (PDB ID: 4LVT)



Anthraquinone or Unisol blue A

(Pubchem ID:

Structure3D_CID_61719)

Figure 1a: represents the structure of Bax, Bcl-2 and anthraquinone.

Figure 2: Docking pose of Anthraquinone (UBA) with Bax Gene and Bcl2 gene

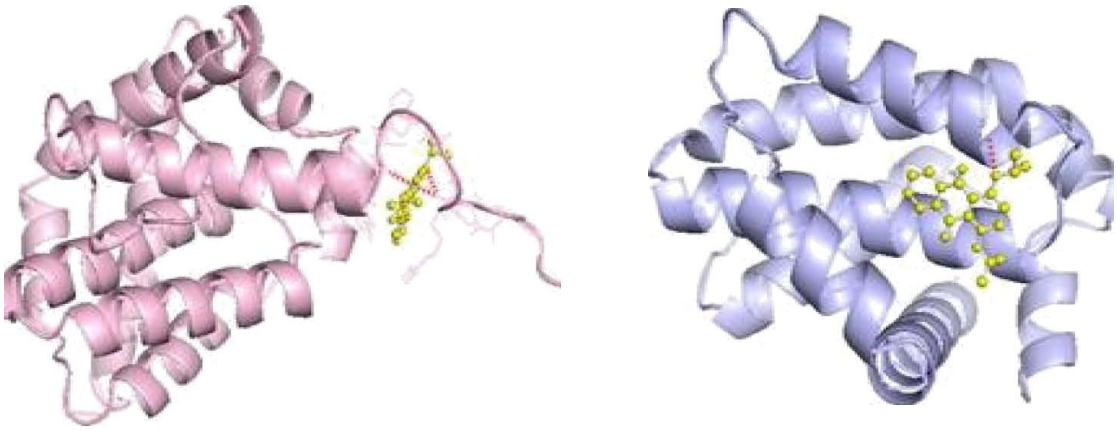


Figure 3: Bonding interactions for the protein Bax and ligand anthraquinone (UBA)

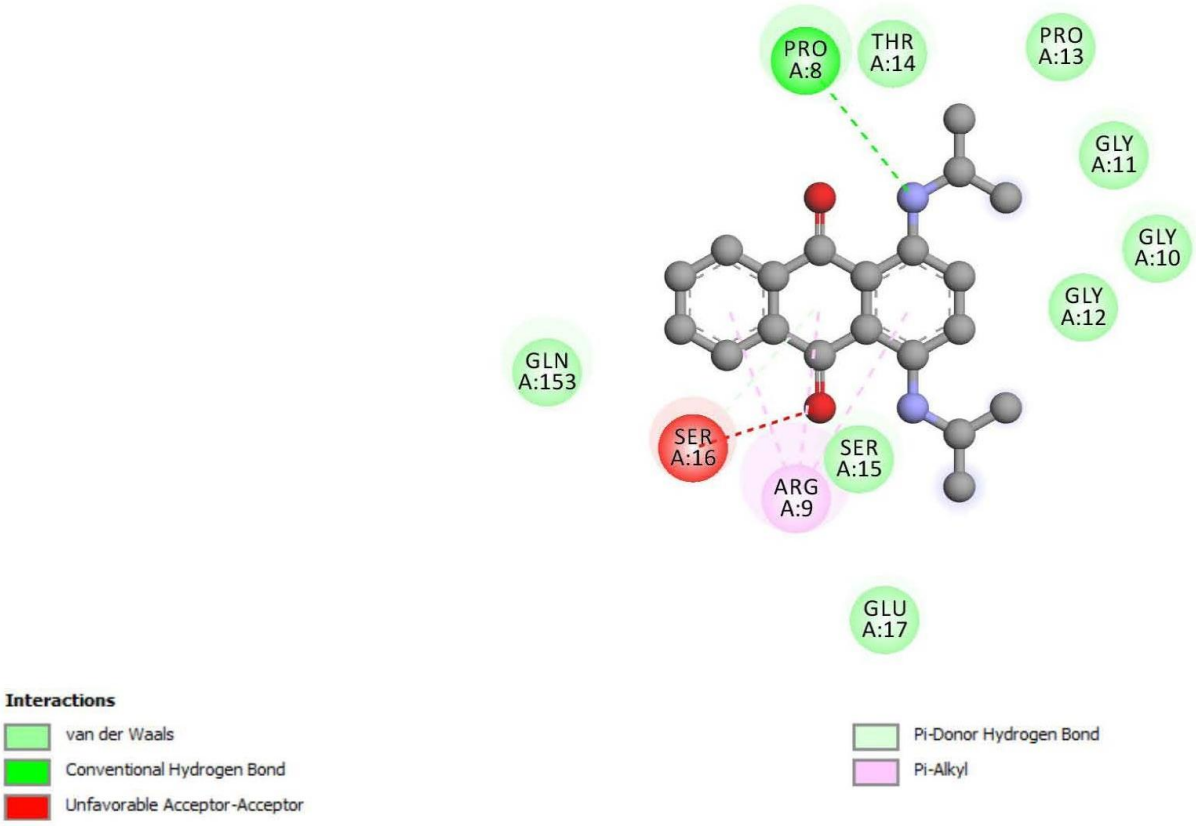
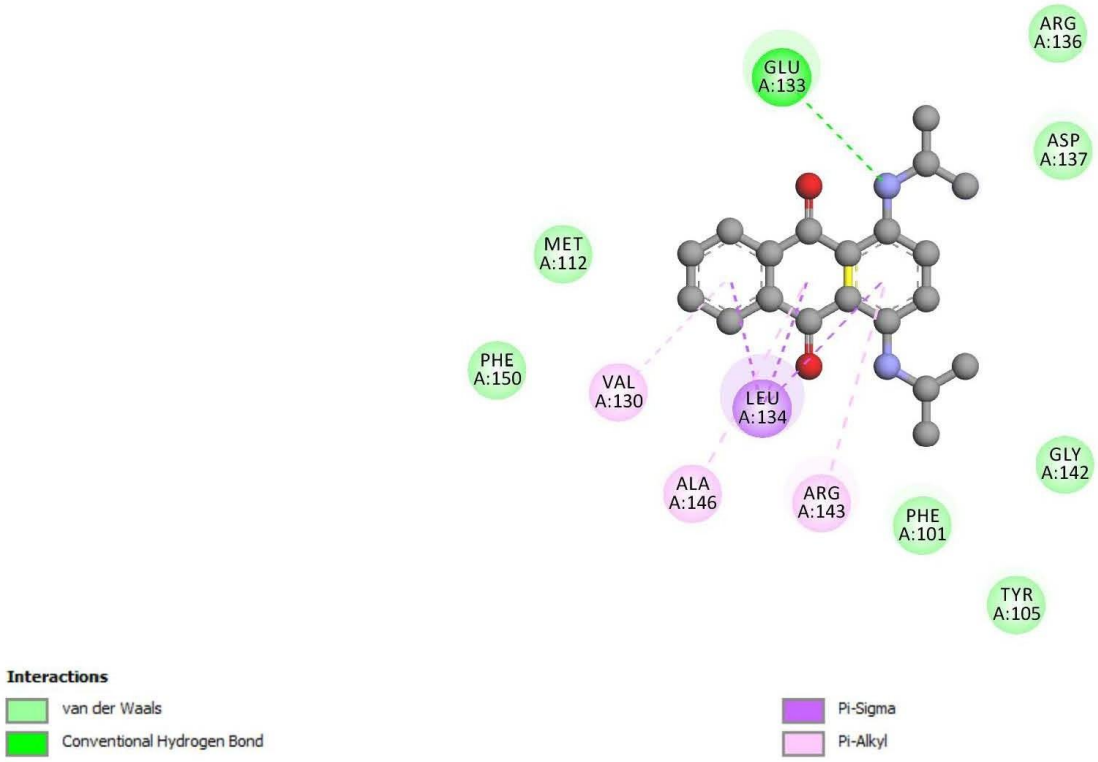


Table 1. Summarized bonding interactions for the proteins and ligand

S.No	Protein Name	Ligand	Interaction	Aminoacid Involved in Interaction
1	Anthraquinone (UBA)	Bax	Vanderwaal	LEU76, ILE80, VAL83, ASP84, THR85, PRO88, VAL 91, PHE92, VAL95, TYR115, PHE116, LYS119, LEU120, LYS123, ALA124, THR127, VAL129, LEU132 ILE136, TRP139, LEU181, LEU18, RP188
			Hydrogen Bond	
		Bcl-2	Vanderwaal	PHE101, ASP108, PHE109, MET 112, VAL 130, LEU134. ALA146, GLU149, PHE150. VAL153
			Hydrogen Bond	

Figure 4: Bonding interactions for the protein Bcl-2 and ligand anthraquinone (UBA)



Numerous interactions were observed between the proteins and the ligands docked. Bonding interaction and total energy score of compound interactions with the ligands are shown in Table 1 & 2. After comparative analysis, it was understood that hydrogen bond and other interactions namely van der Waals, Pi – Sigma and Pi – Alkyl are involved in binding of the ligands to the proteins.

Table 2: Ligand and docked proteins score along with their van der Waals value and Hydrogen bond value.

S.No	Complex	Total Energy	VDW	HBond
1	Bax -anthraquinone	-133.272	-119.115	-14.1572
2	Bcl-2 - anthraquinone	-77.9126	-70.7134	-7.19921

Anthraquinone showed the lowest binding energy of -77.9126kcal/mol with Bcl-2 and highest binding energy -133.272 kcal/mol with Bax. The best-docked conformation of Anthraquinone (UBA) with Bcl-2 showed hydrogen-binding interactions and Vander wall forces with the active residues Polar aminoacids ASP 108, GLU 149, Non polar aromatic aminoacids PHE 101, PHE 109, PHE 150, non-polar aromatic amino acids MET 112, VAL 130, VAL 153, LEU 134, ALA 146 with a binding free energy -77.9126kcal/mol.

When Anthraquinone (UBA) was docked with protein Bax, hydrogen bonds are formed with, Polar aminoacids ASP84, THR85, THR127, LYS119, LYS123, non ploar aliphatic amino acids LEU18, LEU76, , LEU120, LEU132, LEU181, ILE80, ILE136, VAL83, VAL95, VAL91, VAL129, ALA124, PRO88 non-polar aromatic aminoacids PHE92, PHE116, TYR115, TRP139 RP188 with a binding free energy -133.272 kcal/mol

In Anthraquinone - Bax Interaction, Pi donor Hydrogen bond interaction with PRO 8, THR 14, GLN 153 and Pi Alkyl bond interaction with SER 16 and in ARG 9. In Anthraquinone - Bacl-2 Interaction, Pi sigma bond interaction with LEU 130, Pi alkyl Bond interaction with VAL 130, ALA 146, ARG 143 in Bcl-2 gene. The large number of Pi-sigma interactions (Pi-alkyl and Pi-Sulphur) which largely involves charge transfer helps in intercalating the drug in the binding site of the receptor. Finally the result conclude that Anthraquinone (UBA) have more binding interaction with Bcl-2 in active site residues than

Bax.

The results of binding energy reveal that the ligand molecules were more potent in binding to Bax gene than Bcl-2 gene. The variation in binding energies of the ligand molecules may be attributed to the intermolecular interaction energy between Bax, Bcl-2 and ligand molecule. The binding energy is nothing but the binding strength of the ligand which not only helps predicting the stable conformation of ligand protein complex but also optimizes the newly formed bonds **(Patil *et al*., 2010).**

Finally from the result it was concluded that binding energy will be high for Bax than Bcl-2, but more aminoacids bind with active site highly in Bcl-2, than Bax. So Bcl-2 has potent cardioprotective activity.

Natarajan *et al*., (2012) revealed that docking simulation and molecular dynamics results confirmed that ginsenosides compounds are potential ligands for anti-apoptotic proteins such as BCL-2, BCL-XL, and MCL-1 which support our report. **Govindappa *et al*., (2018)** had reported that docking simulations of 10-Hydroxycamptothecine involve interactions between ligand and GLY 10, 11, 12, THR 14, PRO13, SER15,16 and ARG9/ TRY 195, 196, GLN190, GLY 193,194, TYR 9 suggesting these aminoacids to be important residues in binding of compound with protein.

Bharathi *et a*l., (2014) had reported in their studies that the docking calculations showed that van der Waals, electrostatic and desolvation energies play a key role in binding and these factors are considered for designing new drug for myocardial infarction.

4. CONCLUSION

Every day the Heart problem is increasing in world due to various factors and the synthetic chemicals have lot of adverse effects. The natural products may not show their activity immediately but they are potential and have no side effects. The Anthraquinone (UBA) is isolated from *B. diffusa* showing many biological activities. Based on *in silico* virtual screening using computer software, the Anthraquinone has shown more potent interaction with apoptotic regulators Bcl-2 and Bax genes by showing good binding energy. The study also evident that Anthraquinone (UBA) was an ideal drug agent with better drug likeliness. Further, the compound can be used as therapeutic molecule for

myocardial infarction. The present research reveals that Anthraquinone used as promising medication applicant against different malignancies.

5. REFERENCES

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