In silico molecular docking studies , ADMET and MD analysis of DOXORUBUCIN the anticancer potential against breast cancer

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Abstract

Background :Doxorubicin is a widely used anthracycline antibiotic in cancer chemotherapy, particularly effective against a broad range of solid and hematological malignancies. Its primary mechanism of action involves intercalation into DNA and inhibition of Topoisomerase II alpha (Top2A), a key enzyme responsible for managing DNA topology during replication and transcription. Dysregulation or overexpression of Top2A has been associated with tumor progression and resistance to therapy, making it a critical target in anticancer drug design. Molecular docking studies offer valuable insights into the binding interactions and affinity of Doxorubicin towards Top2A, facilitating the optimization of drug efficacy and minimizing off-target effects.

results :The molecular docking analysis of Doxorubicin with Topoisomerase II alpha revealed a strong binding affinity, with the most stable pose scoring -8.8 kcal/mol. Detailed interaction profiling indicated the formation of **7 hydrogen bonds** involving key amino acid residues such as **Glu837**, **Ser717**, **Arg673**, **Lys728**, and **Glu712**. Notably, **Arg673** exhibited dual hydrogen bond interactions, suggesting a crucial role in stabilizing the ligand. Visualization confirmed spatial proximity and favorable interaction distances (2.1–2.5 Å), particularly between the ligand and Glu837, while some residues (e.g., Lys728) showed indirect participation through polar contacts.

Conclusion : The docking results demonstrate that Doxorubicin exhibits high-affinity binding to Topoisomerase II alpha, forming multiple stable hydrogen bonds. The identified interactions, especially with Glu837 and Arg673, support its established anticancer mechanism via DNA intercalation and enzyme inhibition. These findings provide a foundation for further optimization or derivatization of Doxorubicin, as well as future **MD simulations** and **liposomal encapsulation studies** to enhance its therapeutic delivery.

Keywords : Doxorubicin , Molecular Docking , Topoisomerase II Alpha, Hydrogen Bonding Protein–Ligand Interactions, Drug Design Anticancer Agents ,Binding Affinity

1 Background

Doxorubicin is widely used as a chemotherapeutic agent in the treatment of various malignancies, most notably breast cancer. Its mechanism of action involves DNA intercalation, topoisomerase II inhibition, and the generation of free radicals, leading to DNA damage and apoptosis in rapidly dividing cells. Despite its effectiveness, Doxorubicin is associated with significant dose-limiting cardiotoxicity which has driven ongoing research toward optimizing its delivery and improving selectivity Breast cancer, being one of the most prevalent cancers among women worldwide, often involves complex genetic mutations, including BRCA1/2, making Doxorubicin a cornerstone in combination regimens, particularly in HER2-positive and triple-negative subtypes. Understanding its molecular interactions with target proteins such as Topoisomerase II alpha is crucial for improving efficacy and minimizing side effects.

2 Methodology

2.1 Selection of proteins for docking

Multiple crystallographic structures of the human DNA topoisomerase II alpha (Top2A) enzyme are available in the RCSB PDB database, such as 1ZXM, 1ZXN**,** 4FM9**,** 4R1F, and 8W50, all determined via X-ray diffraction methods. After careful comparison, the structure with PDB ID 4FM9, was selected for molecular docking studies. This selection was based on its high-resolution (2.51 Å) crystallography data, absence of mutations, and origin from Homo sapiens, ensuring biological relevance and structural accuracy. The 4FM9 structure provided a reliable template for understanding the binding interactions between Doxorubicin and Top2A, essential for subsequent docking and simulation analyses. **Table 1** presents these results.

Table 1 Different versions of DNA topoisomerase 2-alpha (protein) and their respective PDB IDs

PDB ID	protein
1ZXM	Human Topo IIa ATPase/AMP-PNP
1ZXN	Human DNA topoisomerase IIa ATPase/ADP
4FM9	Human topoisomerase II alpha bound to DNA
4R1F	Re-refined Human DNA topoisomerase IIa (ATPase and transducer domains) in complex with ADP and SO4
8W50	Crystal structure of DNA binding and cleavage core of human topoisomerase 2-alpha in a DNA binding-competent conformation

2.2 Preparation of proteins for docking

1- **Structure Cleaning**
Using **AutoDock Tools (ADT)** and **UCSF Chimera**, all **non-standard atoms** and molecules were removed, including water molecules, ions, and DNA chains B and C. However, three water molecules (HOH) in proximity to the binding site were retained due to their potential role in coordination and stabilization of the ligand. This step was essential to prevent non-specific interactions and focus on the relevant protein-ligand binding region.

2- **Chain Selection**
From the multiple chains available in the crystal structure, a single monomeric chain representing the active enzyme was retained. This simplification reduces computational complexity and ensures biologically relevant interactions are preserved.

3- **Addition of Hydrogens**
As hydrogen atoms are typically not resolved in X-ray crystallography, polar hydrogens were added to the protein model. This step is crucial for accurately modeling hydrogen bonding and electrostatic interactions during docking.

4- **Charge Assignment**
Kollman charges were assigned to the protein atoms, as required for AutoDock-based docking protocols. These charges help simulate realistic electrostatic interactions between the ligand and the receptor.

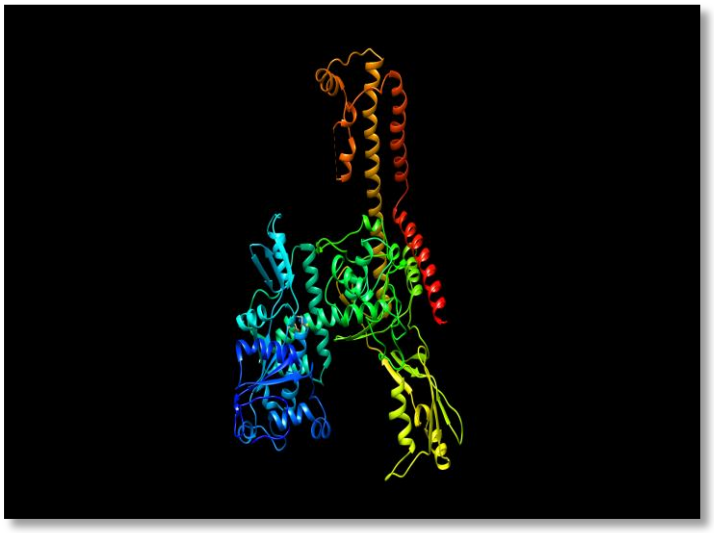


Fig.1 Topoisomerase II enzyme following its pre - docking preparation

2.3 Preparation of ligands

The three-dimensional structure of Doxorubicin was retrieved from the PubChem database (Compound CID: 31703, Molecular Formula: $C_{27}H_{29}NO_{11}$, Molecular Weight: 543.5 g/mol). The compound was downloaded in SDF format and subsequently converted to PDB format using OpenBabel. Since molecular structures obtained from chemical databases often contain unfavorable torsional, steric, or angular strain, energy minimization was performed using Avogadro to optimize the ligand geometry. The MMFF94 (Merck Molecular Force Field 94) was selected for the minimization, as it is well-suited for small organic molecules and provides reliable geometry refinement. This step ensures that the ligand adopts a more realistic conformation prior to the docking process, thereby enhancing the accuracy of binding predictions.

Energy Minimization Results

The energy of the Doxorubicin molecule prior to minimization was **796,501 kcal/mol**. Following successive rounds of energy minimization using the MMFF94 force field in Avogadro, the energy levels were progressively reduced as follows:

- **First minimization attempt**: 603,135 kcal/mol
- **Second minimization attempt**: 586,135 kcal/mol
- **Third minimization attempt**: 585,589 kcal/mol
- **Final minimized energy**: **583,698 kcal/mol**

This progressive energy reduction indicates successful geometry optimization and elimination of steric and torsional strain, resulting in a more stable and realistic molecular conformation suitable for docking analysis.

Finally, Gasteiger charges were computed using AutoDock, resulting in a total charge of +1.0 added to the molecule**.**

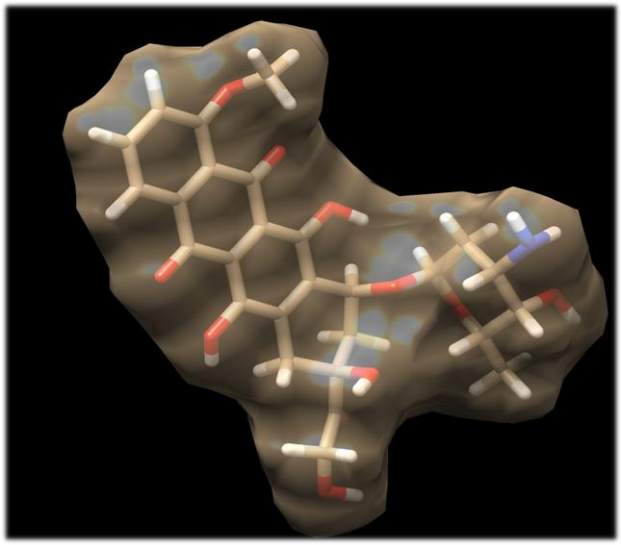
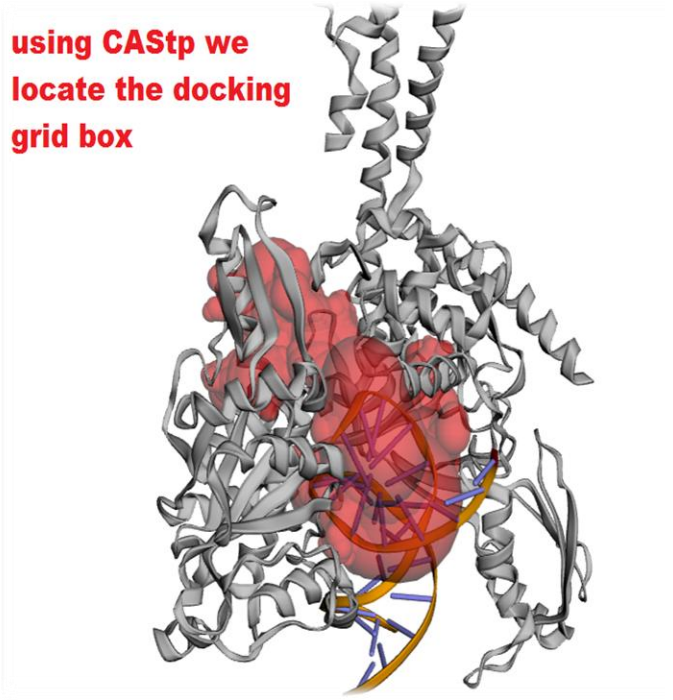


fig.2 Electrostatic potential surface of Doxorubicin was visualized using Chimera with a transparency of 30%

Docking Process

1. **Initially**, the Topoisomerase II Alpha (Top2A) protein was analyzed using the **CASTp server** to gain insight into the active site and potential binding hotspots. The largest pocket was identified with the following parameters:

- **Pocket ID:** 1
- **Surface Area (SA):** 3377.341 $Å^2$
- **Volume (SA):** 5245.667 $Å^3$



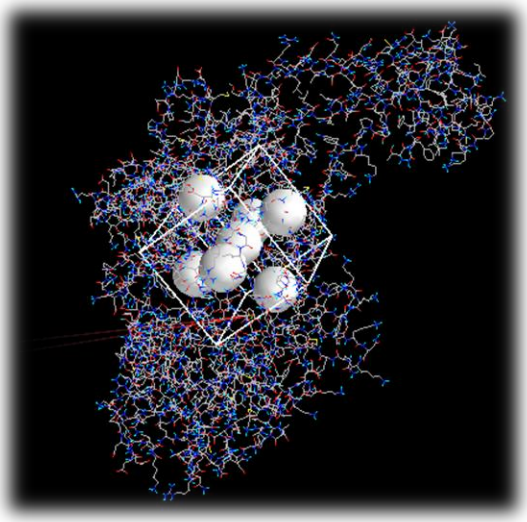


fig.3 Grid box for the active site (hot spot) of Topoisomerase II

In molecular docking, defining a precise grid box is a critical step that determines the accuracy and efficiency of the ligand-receptor binding prediction. For this study, the grid box was centered around the active site (hot spot) of the Topoisomerase II alpha (Top2A) enzyme, as identified using the CASTp server. CASTp provided detailed information on surface pockets, and the largest pocket (Pocket ID 1) was selected based on its surface area (3377.341 $Å^2$) and volume (5245.667 $Å^3$), indicating its potential as a primary binding site. By restricting the docking search space to this biologically relevant region, the grid box ensures that the ligand — in this case, Doxorubicin — is only sampled in the most probable binding area. This focused approach not only increases the computational efficiency but also enhances the reliability of the docking results by mimicking the actual interaction environment of the enzyme's active site.

3 - **Docking Procedure**

Molecular docking was carried out using PyRx integrated with AutoDock Vina. The docking was targeted specifically to the coordinates of the major binding pocket identified by CASTp. An exhaustiveness value of 5 was applied to balance between computational efficiency and docking accuracy.

5- Results and observations

The docking process yielded **9 possible binding poses** for the interaction between Doxorubicin and Topoisomerase IIα, with binding affinities ranging from **−8.8 kcal/mol to −7.3 kcal/mol**. Among these, **Pose 6 and Pose 4** appear to be the most promising based on their **RMSD values (UB/IB)**, which are **2.077 / 1.593 Å** and **4.820 / 2.289 Å**, respectively. These values indicate that the binding conformations are consistent and structurally reliable, with Pose 6 demonstrating particularly favorable alignment. Lower RMSD values suggest that the docked pose closely resembles the predicted ideal interaction, enhancing confidence in the docking prediction.

Ligand	Binding Affinity (kcal/mol)	Mode	RMSD UB	RMSD IB
Dox-Top IIα	−8,8	0	0,0	0,0
*	−8,6	1	17,151	20,289
*	−8,5	2	2,784	8,423
*	−8,2	3	2,289	4,82
*	−8,2	4	16,635	19,822
*	−7,9	5	2,612	5,096
*	−7,7	6	1,593	2,077
*	−7,6	7	14,536	17,983
*	−7,3	8	13,589	17,061

Pose 0:
The reference binding mode is identified as *Pose 0*, which exhibited a binding energy of **-8.8 kcal/mol**, considered excellent in molecular docking, indicating strong and favorable interactions. This result is further supported by detailed analysis using PyMOL.

Interaction Analysis:
The docking data revealed that **Doxorubicin** interacts with **Topoisomerase IIα** through **seven hydrogen bonds**, involving the following amino acid residues:

- **Glu837** – 2.3 Å
- **Arg673** – 2.0 Å
- **Ser717** – 2.7 Å
- **Trp840** – 3.6 Å
- **Lys728** – 2.3 Å
- **Arg727** – **2.4 Å and 2.0 Å** (two separate interactions)

A particularly interesting observation is that **Arg727** forms *two hydrogen bonds* with the ligand. This is due to the presence of a **guanidinium group**, which contains multiple hydrogen donors. The **difference in bond lengths** (2.4 Å vs. 2.0 Å) is attributed to the **spatial orientation** of the side chain relative to the ligand's electron-rich regions.

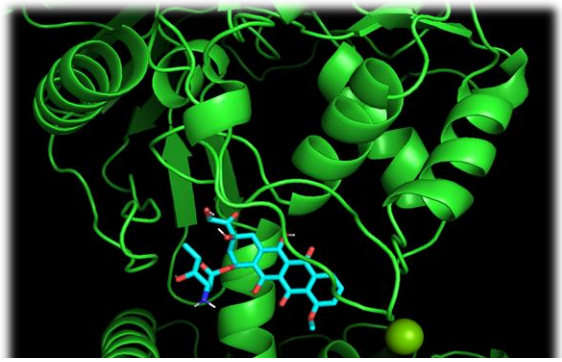


Fig .4 The optimal binding pose (Pose 0) of Doxorubicin docked onto Topoisomerase IIα is shown, highlighting key interaction sites within the binding pocket

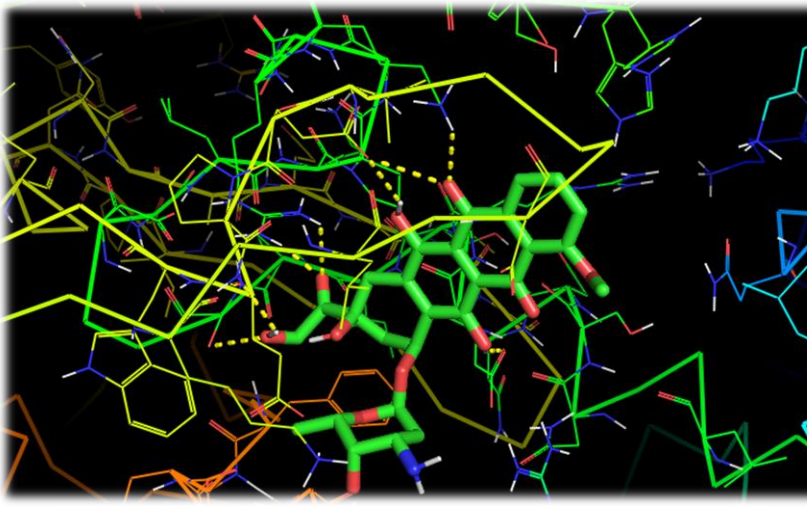


Fig .5 The figure illustrates how Pose 0 of Doxorubicin binds with Topoisomerase IIα.

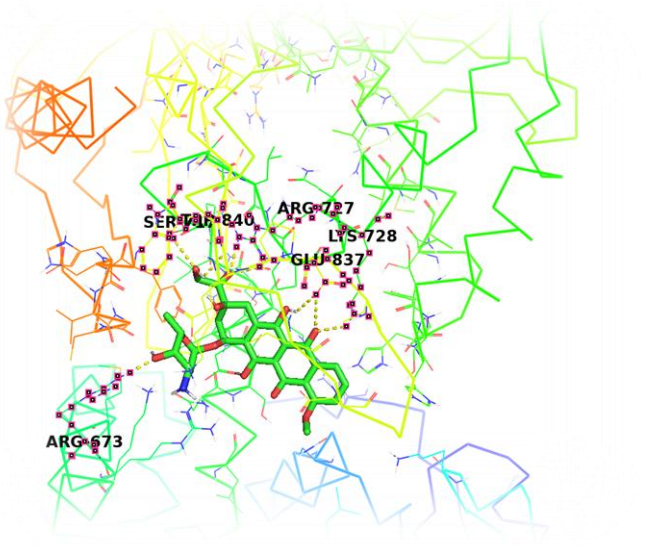


Fig .6 The figure illustrates amino acid residues

Analysis of Interaction Types in Pose 0 between Doxorubicin and Topoisomerase IIα

In the optimal docking pose (Pose 0), doxorubicin interacts with Topoisomerase IIα (Top2A) through a variety of non-covalent interactions, mainly hydrogen bonds and electrostatic interactions. The specific interactions observed are as follows:

1. **GLU 837 – Ligand (2.3 Å):**
   A classical **hydrogen bond** is likely formed between the carboxylate group of Glu837 and a hydroxyl or amino group on the ligand, due to the short distance and polar nature of both participants.
2. **ARG 673 – Ligand (2.0 Å):**
   This distance suggests a **strong hydrogen bond** or an **electrostatic interaction**, with the positively charged guanidinium group of Arg673 interacting with an electron-rich atom on the ligand.
3. **SER 717 – Ligand (2.7 Å):**
   A **moderate hydrogen bond** is suggested here, involving the hydroxyl group of Ser717 as a hydrogen donor or acceptor.
4. **TRP 840 – Ligand (3.6 Å):**
   The relatively long distance implies **π–π stacking** or **van der Waals interactions**, likely between the aromatic indole ring of Trp840 and the planar aromatic system of doxorubicin.
5. **LYS 728 – Ligand (2.3 Å):**
   A **hydrogen bond or electrostatic interaction** is plausible, involving the protonated amino group of Lys728 and an electronegative atom on the ligand.
6. **ARG 727 – Ligand (2.4 Å and 2.0 Å):**
   Two **strong hydrogen bonds** or **electrostatic interactions** are formed from the same residue (Arg727), which can be attributed to the guanidinium group's ability to participate in multiple hydrogen bonding events. The variation in bond length reflects differences in **spatial orientation** toward the ligand rather than interaction strength.

6 - Conclusion

In this study, the interaction between the anticancer agent Doxorubicin and the human Topoisomerase IIα (Top2A) enzyme was successfully investigated using molecular docking techniques. The protein was carefully prepared to ensure structural integrity, and the ligand was optimized through energy minimization and charge assignment to enhance docking accuracy. A focused docking approach targeting the largest and most accessible active site, identified via CASTp, revealed nine potential binding poses. Among them, Pose 0 emerged as the most promising, exhibiting the strongest binding affinity (−8.8 kcal/mol).

Detailed analysis of Pose 0 demonstrated that Doxorubicin forms multiple stabilizing interactions with key active site residues, including hydrogen bonds and electrostatic interactions with Glu837, Arg673, Ser717, Lys728, and a dual interaction with Arg727. Additionally, potential π–π stacking with Trp840 further contributed to ligand anchoring. These findings not only support the strong binding potential of Doxorubicin to Top2A but also provide molecular insights into its mechanism of action at the structural level.

The results highlight the utility of molecular docking in predicting drug–target interactions and pave the way for further studies involving dynamic simulations, structure–activity relationship (SAR) analysis, and potential drug optimization strategies targeting Topoisomerase IIα.