Spectroscopic Studies and Molecular Docking Investigation of the Binding Interactions of Evans Blue dye with Calf Thymus DNA

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Abstract

Binding of toxic ligands to DNA could result in undesirable biological processes, such as carcinogenesis or mutagenesis. Evans Blue dye, a widely used synthetic diazo compound, has been extensively employed in various biomedical applications. This study investigated the interaction between Evans Blue dye (EB) and calf thymus DNA (ctDNA) using a combination of spectroscopic techniques and molecular docking, which could potentially lead to insights into the dye's mutagenic or carcinogenic risks. Molecular docking was performed to predict the binding mode and calculate binding energies.UV-visible spectroscopy and circular dichroism (CD) studies were employed to analyze the EB-ctDNA interactions in physiological buffer (pH 7.4). The study revealed that the interaction of Evans Blue dye with ctDNA induces structural and conformational changes in a concentration-dependent manner, leading to the destabilization of DNA. The results suggest an intercalation binding mode between the dye and DNA, leading to the compaction of the B-form of DNA and the formation of a stable dye-DNA complex. This research contributes to a deeper understanding of DNA-ligand interactions and paves the way for the development of novel therapeutic agents.

Introduction

- **Evans Blue dye, a synthetic diazo dye, used as biological dye and in clinical diagnostics, has demonstrated a capability to bind with biomolecules**
- **Due to its high water solubility and slow excretion, as well as its tight binding to serum albumin, Evans Blue has has been widely used in biomedicine, including its use in estimating blood volume and vascular permeability, detecting lymph nodes, and localizing the tumor lesions.**
- **Calf-thymus DNA (ctDNA) is used in biochemical and biophysical studies due to its ready availability and structural homology to human DNA.**
- **This study aims to elucidate the binding dynamics and interaction forces between Evans Blue dye and calf-thymus DNA (ctDNA), a commonly used proxy for human DNA, which could potentially lead to insights into the dye's mutagenic or carcinogenic risks.**

Materials Used

- **Evans Blue Dye**
- **ctDNA**
- **Tris Buffer**
- **Phosphate buffered saline**
- **Acetate Buffer**
- **Ethylenediaminetetraacetic acid (EDTA)**
- **Sodium Chloride (NaCl)**

Techniques Used

UV Vis Spectroscopy | Circular Dichroism | Molecular Docking

UV-Visible Studies

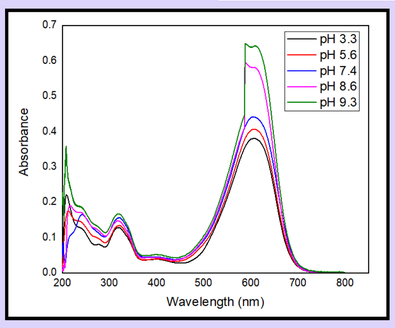


Fig 4: pH dependence of the absorption spectra of evans blue dye

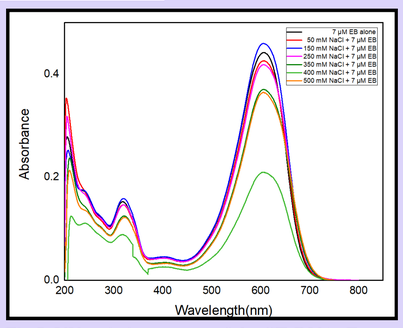


Fig 5: UV emission spectra of the CT-DNA- Evans Blue Dye in the presence of increasing amounts of NaCl (50 mM to 500 mM).

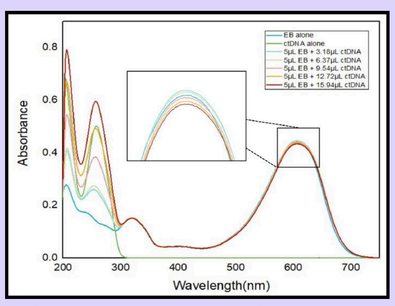


Fig 6: UV emission spectra of the ct-DNA- Evans Blue Dye in the presence of increasing amounts of ct-DNA

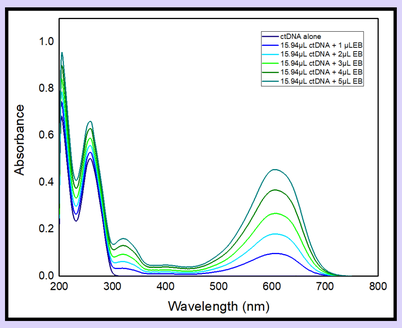


Fig 7: UV emission spectra of the CT-DNA- Evans Blue Dye in the presence of increasing amounts of EB

Circular Dichroism Study

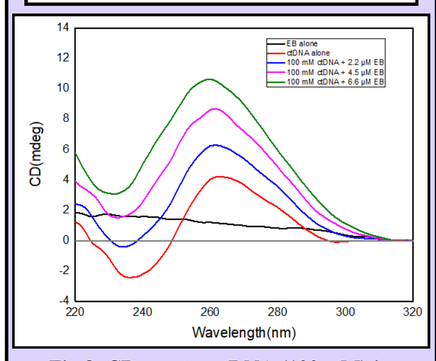


Fig 8: CD spectra ctDNA (100 mM) in presence of increasing concentrations of Evans Blue Dye)

Future Work

We aim to extend our investigation into the interactions between Evans Blue dye and calf thymus DNA by employing molecular dynamics simulations using GROMACS. This will enable us to analyze the dynamic behavior and stability of dye-DNA complexes in detail.

The project will focus on fluorescence spectroscopy for competitive binding and thermodynamic studies to further understand the binding affinity and specificity of Evans Blue dye.

Molecular Docking

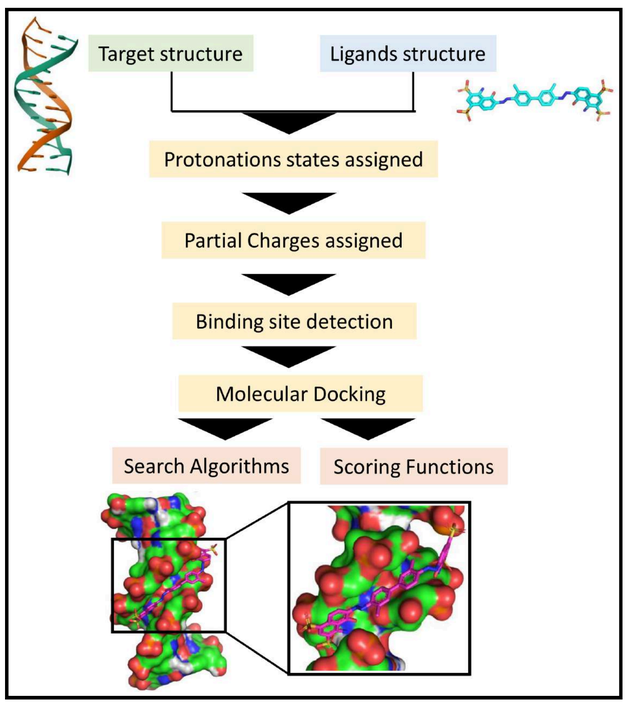


Figure 1: General workflow of molecular docking.

DRUG	BINDING AFFINITY
Evans Blue	**-8.9**
Colistin Sulphate	-8.6
Imatinib mesylate	-7.8
Congo Red	-7.4
Coumarin6	-7.3
Riboflavin	-7.1
Methylene Blue	-7
Disperse Blue14	-6.7
Azithromycin	-6.5
Orange G	-6
Acridine yellow	-5.7
Amoxicillin trihydrate	-5.5
Ciprofloxacin hydrochloride	-5.4
1,3 - Diphenylisobenzofuran	-5.2
Cotinine	-4.4

Figure 2: Binding Affinity Values for Various Drugs with a Biological Target (measured in kcal/mol)

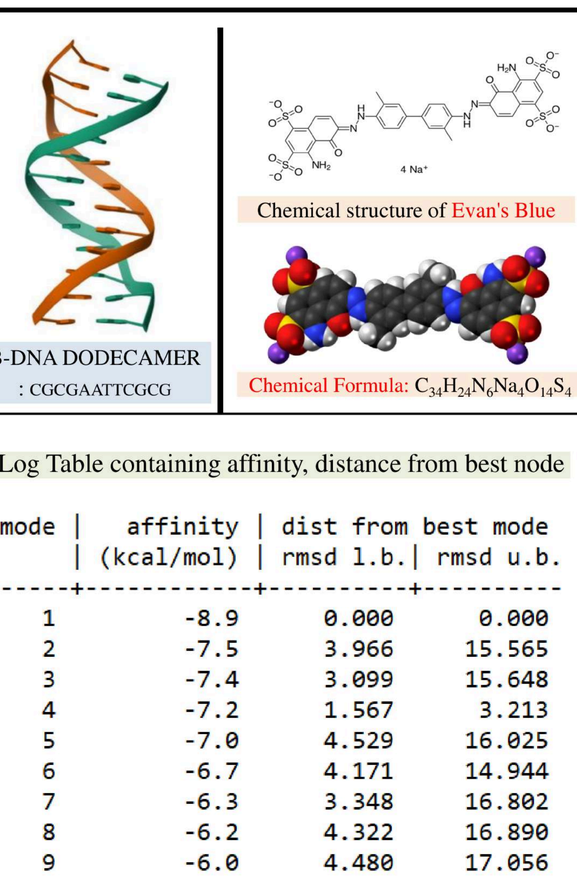
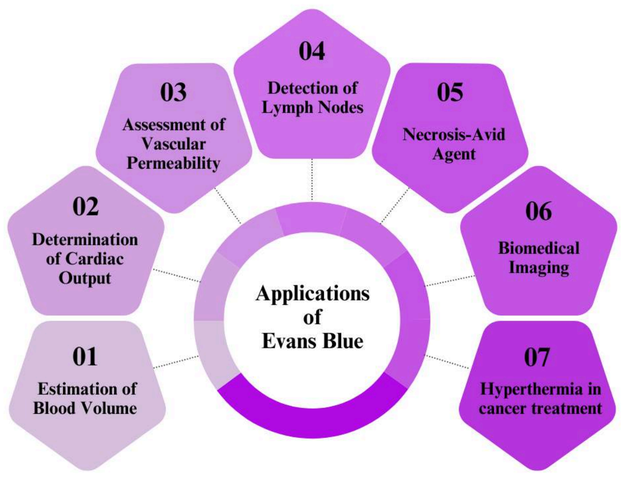


Figure 3: In this study, molecular docking was employed to screen the interaction of 20 different drugs with B-DNA using AutoDock Vina, where Evans Blue dye demonstrated the highest binding affinity with a score of -8.9 kcal/mol, suggesting a strong potential for significant interaction with DNA.

Applications of Evans Blue



Results

- **The absorption peak at 608 nm is characteristic of Evans Blue dye and indicates the wavelength at which the dye absorbs light most efficiently.**
- **In the UV vis spectra analysis of the interaction between Evans Blue dye and calf-thymus DNA (ctDNA), it was observed that the absorbance of ctDNA increased progressively with the incremental addition of Evans Blue dye. As the concentration of calf thymus DNA is increased, the absorbance of evans blue starts decreasing.**
- **In CD, an increase in the positive peak is observed in addition of dye to ctDNA, which is in turn the result of intercalation of dye into the DNA base pairs.**
- **In CD results Evans Blue dye interaction with calf thymus DNA (ctDNA) not only involves intercalation but leads to significant DNA damage due to agglomeration as evidenced by a shift of the characteristic negative CD peak to positive values**

Acknowledgment

Authors are thankful to the research scholars (Amit Sir, Pankaj sir, Nikita mam and Aishwarya mam) of Prof. Kaushik's Laboratory for their constant support and encouragement during the whole project.

References

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