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A Comparative Docking Analysis of Non-Carcinogenic DNA Staining Dyes to Propose the Best Alternative to Ethidium Bromide

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Abstract: ***Fluorescent dyes that stain a cell's DNA are used in live cell imaging as they allow for tracking of cell division, for the visualization and sizing of dsDNA restriction fragments, and for the examination of properties of the isolated DNA molecules. Conventionally, Ethidium bromide (EtBr) is the cationic dye used to visualize DNA after separating the fragments on Agarose Gel Electrophoresis. It is widely used due to the striking fluorescence enhancement it displays upon intercalation into the dsDNA at the minor groove. Although a highly sensitive stain, it is notoriously unsafe, not only is it a very strong mutagen, it may also be a carcinogen or teratogenic. Histopathological changes of Ethidium Bromide treated rats showed little degenerative changes characterized by glomerular and tubulointerstitial injury, nephrosis, synechia, necrotic changes, cirrhosis, and ischemia. Ethidium bromide revealed pronounced degenerative changes in ovarian histoarchitecture. The sequence of atretic changes involved nuclear degeneration, characterized by Chromatolysis, rupture, and dissolution of the nuclear membrane. Granulosa cells associated with degenerating follicle types (bilaminar, Multilaminar and graffian follicle) showed desquamation, cytolysis, and nuclear dissolution. Extensive vacuolation also occurred in these follicles. Only a few follicles reached the maturity. In most of the females, Graffian follicle failed to rupture, which led to failure in ovulation. This resulted in Sterility of the females.Hence need has arisen for now –carcinogenic and not- hazardous nucleic acid staining dyes.Our Present study has thus been aimed at finding out the non-carcinogenic DNA staining dyes and to compare their binding efficiency with DNA and find out which is the best DNA staining Dye which has least binding energy score and is non-carcinogenic as well. Hence nowadays safer alternatives to EtBr are being sought after. A comparative docking study of six of such The interaction of EtBr and the six other alternative dyes (Crystal violet, GelRed, Hoechst 33258, Methylene blue, SYBR Green and GelGreen) with the ds-B-DNA were studied, their chemical structures were drawn using ChemSketch and the potential ligands were optimized using ArgusLab. Further, the docking studies of these DNA stains on a dsDNA molecule (1D49) was performed using AutoDock tools and their interaction with the DNA was visualized using Discovery Studio. The binding energies of Hoechst 33258 with the DNA was found to be better (-11.96 Kcal/mol) as compared to the other stains, thus suggesting its use as both a non-carcinogenic and highly sensitive alternative to EtBr.***

Keywords: Ethidium Bromide, Intercalating Agents, Fluorescent Dyes, Non-Carcinogenic.

I. INTRODUCTION

Ethidium bromide is a heterocyclic aromatic compound commonly used to detect nucleic In the case of DNA, this is usually double-stranded DNA from PCRs, restriction digest, etc. Single-stranded RNA can also be detected since it usually folds back onto itself and thus provides local base pairing for the dye to intercalate. It has also been used extensively to reduce mitochondrial DNA copy number in proliferating cells. Effect of EtBr on mitochondrial DNA is used in veterinary medicine to treat trypanosomosis in cattle, as EtBr binds molecules of kinetoplastid DNA and changes their conformation to Z-DNA form. This form inhibits replication of kinetoplastid DNA which is lethal for trypanosomes.[1]. Histopathological changes of Ethidium Bromide treated rats showed little degenerative changes characterized by glomerular and tubulointerstitial injury, nephrosis, synechia, necrotic changes, cirrhosis, and ischemia. [11] Ethidium bromide revealed pronounced degenerative changes in ovarian

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histoarchitecture. The sequence of atretic changes involved nuclear degeneration, characterized by Chromatolysis, rupture, and dissolution of the nuclear membrane. Granulosa cells associated with degenerating follicle types (bilaminar, Multilaminar and graffian follicle) showed desquamation, cytolysis, and nuclear dissolution. Extensive vacuolation also occurred in these follicles. Only a few follicles reached the maturity. In most of the females, Graffian follicle failed to rupture, which led to failure in ovulation. This resulted in Sterility of the females[12]. Hence need has arisen for now –carcinogenic and not- hazardous dyes.Our Present study has thus been aimed at finding out the non-carcinogenic DNA staining dyes and to compare their binding efficiency with DNA and find out which is the best DNA staining Dye which has least binding energy score and is non-carcinogenic as well.

Crystal Violet, GelRed, Hoechst 33258, Methylene Blue, SYBR Green,etc.are the alternative dyes to stain DNA.Crystal violet or gentian violet (also known as methyl violet 10B or hexamethyl pararosaniline chloride) is a triaryl methane dye used in DNA gel electrophoresis as a nontoxic DNA stain which can be used as an alternative to fluorescent, intercalating dye-ethidium bromide. Sedimentation and viscosity experiments reveal that when Crystal violet binds to closed circular DNA the helix becomes unbound. It preferentially binds to a helical form of DNA[2],[3].GelRed is a new generation of fluorescent nucleic acid gel stains designed to replace the highly toxic ethidium bromide (EtBr). GelRed these dyes use a novel yet very simple concept to reduce genotoxicity by preventing the dyes from entering living cells. A DNA-binding dye can be made non-mutagenic or substantially less mutagenic by denying contact with genomic DNA in living cells. Thus, GelRed is engineered such that they are incapable of crossing cell membranes.

Hoechst 33258 nucleic acid stain is a popular nuclear counterstain that emits blue fluorescence when bound to dsDNA. The interaction of this bis benzimidazole dye with DNA and chromatin is characterized by binding at A-T rich DNA sequences enhance both dye binding and fluorescence quantum yield, while chromosomal proteins apparently exclude the dye from approximately half of the sites available with DNA[3]. Methylene Blue is the most widely used alternatives to ethidium bromide. ACMA (9-amino-6-chloro-2-methoxyacridine) is a DNA intercalator that selectively binds to poly(d(A-T)) with a binding affinity constant of 2 × 105 M-1 at pH 7.4.)[4],) Excitation of the ACMA–DNA complex (excitation/emission maxima ~419/483 nm) is possible with most UV-light sources, making it compatible for use with both shorter- and longer-wavelength dyes. ACMA also apparently binds to membranes in the energized state and becomes quenched if a pH gradient forms.[5]. It has been extensively employed to follow cation and anion movement across membranes (Schapendonk et.al, 1980) and to study the proton-pumping activity of various membrane-bound ATPases [6].The bisbenzimide dyes Hoechst 33342 is a cell membrane permeant, minor groove–binding DNA stains that fluoresce bright blue upon binding to DNA. Hoechst 33342 has slightly higher membrane permeability than Hoechst 33258 [7] but both dyes are quite soluble in water (up to 2% solutions can be prepared) and relatively nontoxic. These Hoechst dyes, which can be excited with the UV spectral lines of the argon-ion laser and by most conventional fluorescence excitation sources, exhibit relatively large Stokes shifts (excitation/emission maxima ~350/460 nm), making them suitable for multicolor labeling experiments. Their fluorescence is also enhanced by surfactants such as sodium dodecyl sulfate (SDS) [8]. These dyes appear to show a wide spectrum of sequence-dependent DNA affinities and bind with sufficient strength to poly(d(A-T)) sequences that they can displace several known DNA intercalators [9]. They also exhibit multiple binding modes and distinct fluorescence emission spectra that are dependent on dye: base pair ratios (J Histochem) Hoechst dyes are used in many cellular applications, including in cell-cycle and apoptosis studies and they are common nuclear counterstains.

This project aims to predict alternative dyes of EtBr as a DNA staining dye, along with interaction using docking approach. The alternative dyes of EtBr will be retrieved from PubChem database which can be of natural or synthetic origin. Further, they will be evaluated based on their best binding energy values.

Docking is a computational procedure of searching for an appropriate ligand that fits both energetically and geometrically the protein's binding site.[10]. Docking involves is the formation of noncovalent protein-ligand complexes in silico. Given the structure of a protein and a ligand, the task is to predict the structure of the complex. A docking method estimates the forces involved in the protein-ligand recognition viz. electrostatic, van der walls and hydrogen bonding and places the ligand appropriately in the active site[9],[10]. It allows virtually screening a database of compounds and calculating the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme, fit together and dock to each other well. The molecule may bind to the receptor and modify their function. The interaction of drug and receptor complex can be identified via docking and their relative stabilities can be evaluated using molecular dynamics. It is also an effective and competent tool for in silico screening that plays an important and ever-increasing role in rational drug design.

Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. [7][9]. Knowledge of the preferred orientation, in turn, may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs [8].Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site. In general, there are two aims of docking studies: accurate structural modeling and correct prediction of activity. It is generally devised as a multi-step process in which each step introduces one or more additional degrees of complexity. The process begins with the application of docking algorithms that pose small molecules in the active site. Algorithms are complemented by scoring functions that are designed to predict the biological activity through the evaluation of interactions between compounds and potential targets.

ChemSketch is such an integrated graphical package for 2D-structure drawing and text manipulations,2D/3D conversion with optimization, and export/import for a variety of file formats. It is a free download for educational use. It can be used to produce structures of organic molecules, names of organic molecules as well as Lewis structures, 3D structures, space filling models or

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ball and stick models, among other things. The Ethidium bromide analogs generated using ChemSketch were optimized using ArgusLab software 4.0 which has fast become a favourite introductory molecular modeling package with academics mainly because of its user-friendly interface and intuitive calculation menus.

Computer-aided docking is an important tool for gaining understanding of the binding interactions between a ligand (small molecule) and its target receptor (Anderson, 2003; Schneider, [10] and has emerged as a reliable, cost-effective and time-saving technique for the discovery of lead compounds [3][5]. In recent years, the virtual screening approach for docking small molecules into a known protein structure is a powerful tool for drug design and has become an integral part of the drug discovery process.

II.OBJECTIVES

(a)To perform a literature survey to find out possible non-carcinogenic alternatives to Ethidium bromide which can be used as a DNA staining dye.

(b) To study the interactions of non-carcinogenic DNA staining dyes like Crystal violet, GelRed, Hoechst 33258, Methylene blue, SYBR Green, etc. with the DNA.

(c)To draw the structures of the various Ethidium bromide alternatives using ChemSketch.

(d) To optimize the ligands using ArgusLab software and

(e) To dock them on the DNA structure using AutoDock Tool.

(f) To validate the docked structures using Discovery Studio.

(g) To evaluate the docking efficiencies of these non-carcinogenic DNA staining dyes and to propose the best alternative to Ethidium bromide.

III.MATERIAL AND METHODS

1. Protein Data Bank (PDB)

The Protein Data Bank (http://www.pdb.org) is the single worldwide archive of structural data of biological macromolecules. The main function of this database is to organize 3-D structural data of large biological molecules including proteins and nucleic acids of all the organisms including bacteria, yeast, plants, flies, other animals, and humans. The three-dimensional structures of the biological macromolecules data available with PDB are determined by experimental methods such as X-ray crystallography, Nuclear magnetic resonance (NMR) spectroscopy, electron microscopy.

2. PubChem Database

The PubChem Substance database contains chemical information deposited by individual data contributors to PubChem, and the Compound database stores unique chemical structures extracted from the Substance database. Biological activity data of chemical substances tested in assay experiments are contained in the Bioassay database. It is a searchable database containing descriptions of chemical samples, from a variety of sources, and links to PubMed citations, protein 3D structures, and biological screening results available in PubChem.

3. ACD/ChemSketch

ACD/ChemSketch which is a powerful all purpose chemical drawing and graphics package from ACD/Labs developed to help chemists quickly and easily draw molecules, reactions, and schematic diagrams, calculate chemical properties and design professional reports and presentation

4. ArgusLab

ArgusLab 4.0 has fast become a favorite introductory molecular modeling package with academics mainly because of its user-friendly interface and intuitive calculation menus (Thompson, 2004). It is the electronic structure program that is based on the quantum mechanics, it predicts the potential energies, molecular structure , geometry, optimization of structure, vibration frequencies of coordinates of atoms, bond length bond angle and reactions pathway (Peng et al., 1995)

5. AutoDock

Computational tools like Auto Dock offer the advantage of delivering new drug candidates more quickly and at a lower cost (Gilbert, 2004; Warren et al., 2006). It is an excellent non-commercial docking program that is widely used.

6. Discovery Studio

Discovery Studio is a comprehensive software suite for analyzing and modeling molecular structures, sequences, and other data of relevance to life science researchers. The product includes functionality for viewing and editing data along with tools for performing basic data analysis. It is a free viewer that can be used to open data generated by other software in the Discovery Studio product line. It is designed to offer an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, scripts, and other data. It also provides a rich set of viewers for displaying plots and other graphical representations of data.

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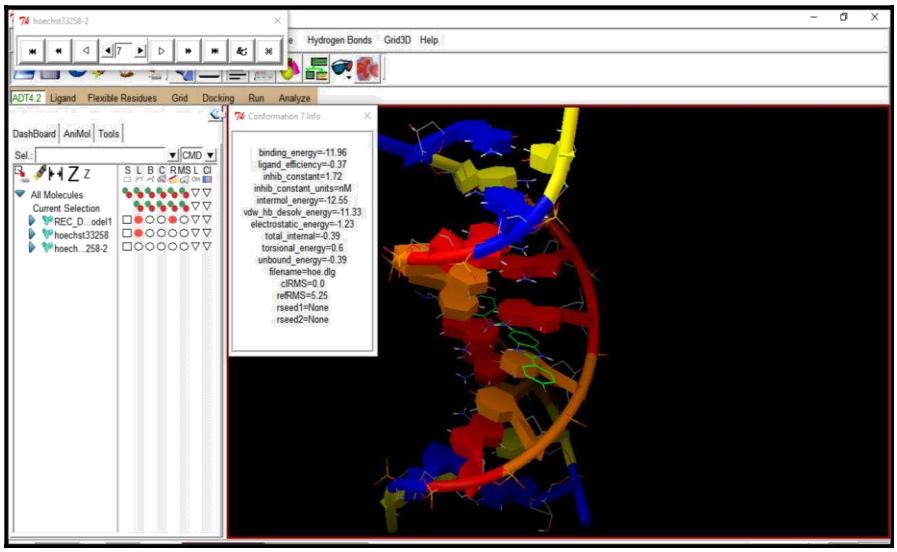
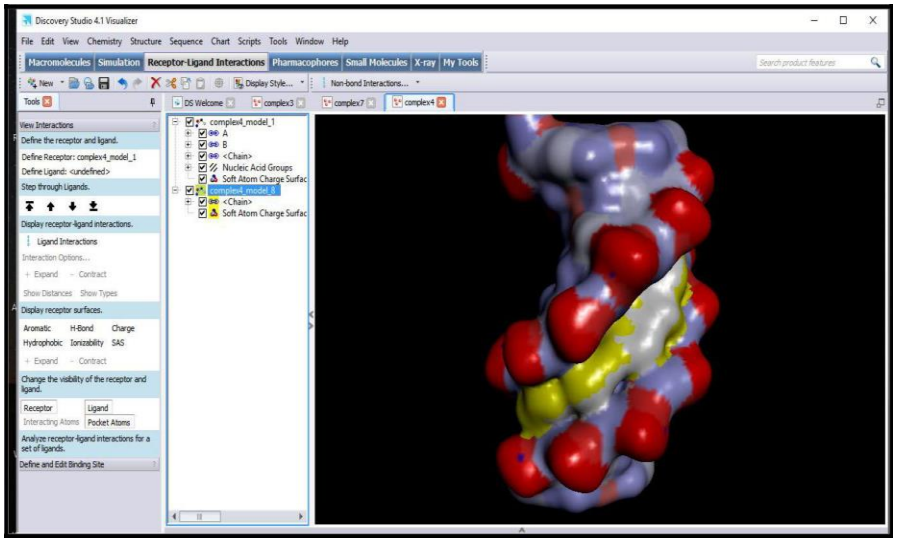
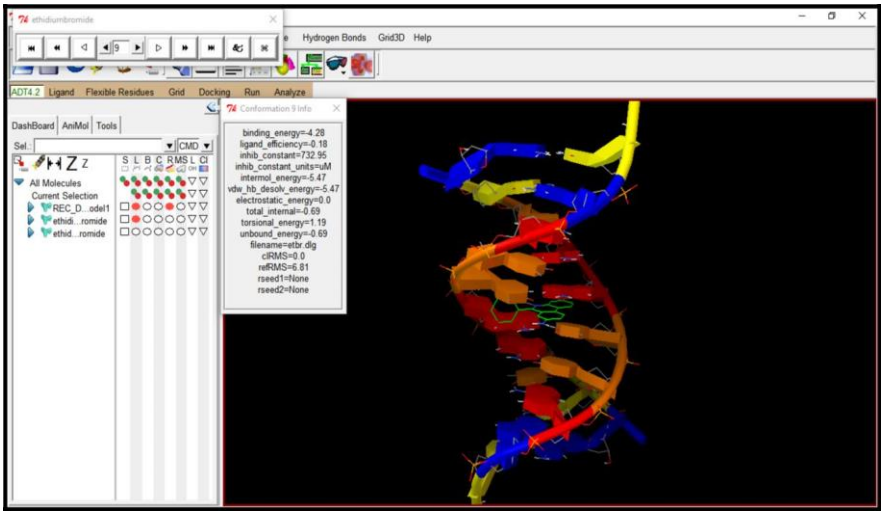
III.RESULTS AND DISCUSSIONS



BINDING OF HOECHST 33258 TO DNA (BEST CONFORMATION - 7TH MODEL, BINDING ENERGY= -11.96)



VISUALIZATION OF HOECHST 33258 IN DISCOVERY STUDIO



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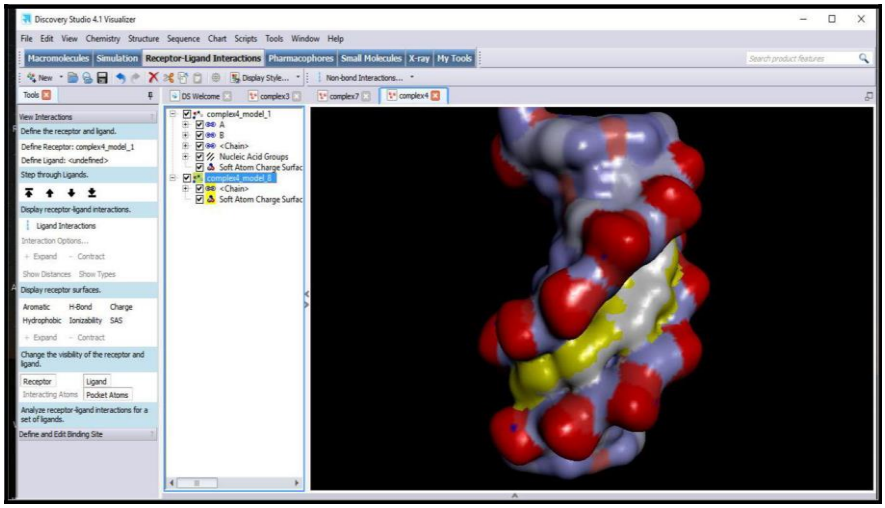
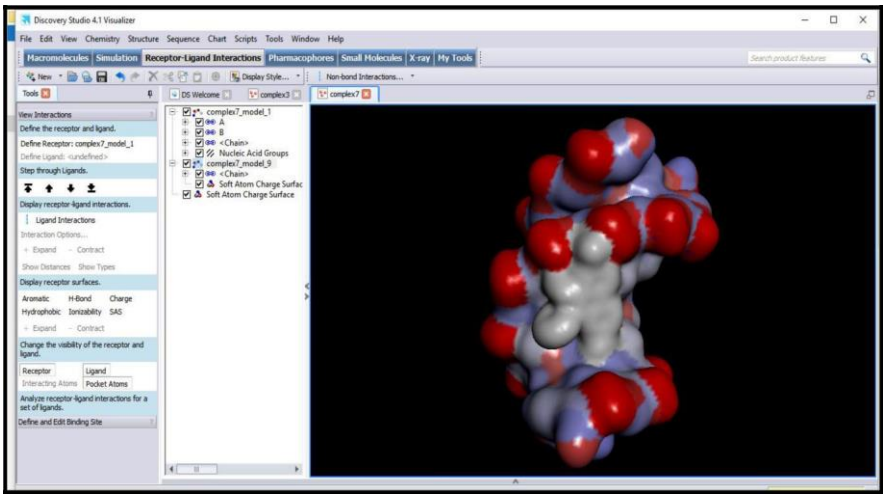


BINDING OF ETHIDIUM BROMIDE TO DNA (BEST CONFORMATION - 9TH MODEL, BINDING ENERGY= - 4.28)



VISUALIZATION OF ETHIDIUM BROMIDE IN DISCOVERY STUDIO

The 9th confirmation showed the best binding of Ethidium bromide to DNA with a binding energy of - 4.28 Kcal/mol.
The 9th confirmation showed the best binding of Hoechst 33342 to DNA with a binding energy of -11.42 Kcal/mol.
Ethidium bromide (EtBr) is the cationic dye used to visualize DNA after separating the fragments on Agarose Gel Electrophoresis. It is widely used due to the striking fluorescence enhancement it displays upon intercalation into the dsDNA at the minor groove. Although a highly sensitive stain, it is notoriously unsafe, not only is it a very strong mutagen, it may also be a carcinogen. A comparative docking study of Six non-carcinogenic DNA staining dyes was performed to propose the best possible alternative to Ethidium bromide. Chemical structures of the dyes were drawn using ChemSketch and the potential ligands were optimized using ArgusLab.The alternative DNA staining dyes like Crystal violet, GelRed, Hoechst 33258, Methylene blue, SYBR Green, Hoechst 33342,Acridine orange,DAPI,TOTO-1,Syntox,GelGreen etc. were docked on a ds-B-DNA molecule using the Autodock software to analyze their binding interactions with the DNA and their interaction with the DNA was visualized using Discovery Studio.After docking, the binding energy of Hoechst 33342 was found to be -11.42, but the best interaction was observed using Hoechst 33258 having a binding energy of -11.96 Kcal/mol. This suggests that Hoechst 33258 is the best among the different alternatives to Ethidium bromide, it is both a non-carcinogenic and a highly sensitive DNA staining dye.Further performing a Quantitative dye Structure-Toxicity Relationship (QSTR) on these dyes can confirm our findings and lead to the replacement of Ethidium bromide with safer alternatives like Hoechst 33258 on an industrial scale.

CONCLUSIONS

Various fluorescent polycationic homodimeric and heterodimeric dyes form complexes with double-stranded DNA (dsDNA) so stable that they could be separated by Agarose Gel Electrophoresis and detected with very high sensitivity, such complexes have been exploited for the visualization and sizing of dsDNA restriction fragments, to detect and quantitate nucleic acids in solution, for the analysis of PCR products and for the examination of the static and dynamic properties of isolated DNA molecules. Conventionally, ethidium bromide is used to visualize both DNA as well as RNA, it is a widely used stain in spectrofluorimetric studies because of the striking fluorescence enhancement it displays upon intercalation into the minor grooves of the DNA But following its mutagenicity which was proved by Ames test, other safer DNA staining dyes are being preferred. Therefore, docking analysis was performed to predict best possible alternative dyes to EtBr where comparative docking study of eleven such non-

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carcinogenic DNA staining dyes was performed propose the best possible alternative to Ethidium bromide. Alternative DNA staining dyes like Crystal violet, GelRed, Hoechst 33258, Methylene blue, SYBR Green, GelGreen, ACMA, Acridine Orange, DAPI, Hoechst 33342 and Sytox were docked on a ds-B-DNA molecule using the Autodock software to analyze their binding interactions with the DNA. After docking, the binding energies of Hoechst 33258 and Hoechst 33342 were observed to be -11.96Kcal/mol and -11.42Kcal/mol respectively. This predicts that Hoechst 33258 is the best alternative dye to Ethidium bromide, it is both a non-carcinogenic and a highly sensitive DNA staining dye. Further performing a Quantitative dye Structure-Toxicity Relationship (QSTR) on these dyes can predict the results and can lead to the replacement of Ethidium bromide with safer alternatives like Hoechst 33258 on an industrial scale which can be much safer to use.

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