INTRODUCTION

Cancer is one of the major problems of modern medicine. Cancer can be characterized by an uncontrolled accelerated cellular growth accumulating genetic alterations as they progress to a more malignant phenotype.¹ It is believed that cancer is a consequence of heredity, lifestyle and environment.² Cancer is causing numerous fatalities worldwide. In 2004, it accounted for 7.4 million deaths, around 13% of all deaths. In the developed countries, one in three individuals will develop cancer during his lifetime.³ To cure this disease, several therapies have been employed over the years. In combination with surgery and radiotherapy, chemotherapy has been effectively employed for the treatment of cancer.

There is a growing awareness in the last 50 years about natural products, which offer a promising effective cure of potentially lethal diseases with minimal toxicity. The natural products include a variety of chemical substances derived from plants and animals, and are consumed by human population. Among various kinds of drugs used in cancer chemotherapy, two types are the most important drugs that act through binding with (a) DNA and (b) other parts of the cellular organelles. Nucleic Acids are most common targets for anti-viral, anticancer and antibiotic drugs.⁴ The research groups of G. S. Kumar⁵ at IICB, Kolkata and Surat Kumar⁶ at Dayalbagh have furnished drug-Nucleic acid complexation studies, where they have shown intercalation and minor groove binding mode of drug – DNA interactions.

DRUG-DNA INTERACTIONS.

The earliest research on DNA structure used photospectroscopic methods that are still used for studying drug-DNA interactions. Franklin and Gosling⁷ carried out a study on DNA structure in 1950, using X-ray diffraction and obtained the first diffraction image of DNA fibers. In 1951, Wilkins⁸ obtained better X-ray images of DNA duplex. These images clearly showed the helical structure of DNA, and enabled the identification of the location of the phosphate sugars. The study of physico-chemical basis of the interaction of drugs with DNA began early with the quest for the structure of DNA itself. Later it encompassed the expression of gene and the genetic inheritance of biological characteristics.
Until early 1980s, the X-ray crystallographic and NMR techniques were employed to investigate detailed structure of DNA at molecular level. The X-ray studies provided a plenty of information about the conformational variability and flexibility of DNA. DNA is highly hydrated, and water molecules bind within the minor groove forming a spine of hydration. The geometry of double helix, including the depth and width of the minor and major grooves varies from one conformation to another. According to Kennard (1993), interactions between the drug and DNA generally involve hydrogen bonds between the functional groups of the two components i.e. ligand and DNA.

There are two principal modes for non-covalent binding to DNA (a) intercalation and (b) minor groove binding. Intercalating drugs have planar, heteroaromatic ring systems in which drug inserts between two adjacent base pairs in a helix. The drug-DNA complex is stabilized by π−π hydrophobic and van der Waal’s interactions between the DNA bases and the drug molecule. Intercalating drugs induce structural perturbations in the DNA structure to accommodate the drug binding, such as the unwinding of the helix, in turn lengthening of the DNA. Known intercalators are ethidium bromide (Figure 1), daunorubicin and actinomycin.

![Figure 1: Structure of Ethidium Bromide](image)

The second mode of drug binding to DNA is minor groove binding. These drugs consist of several aromatic rings viz. pyrrole, imidazol, indole etc. These rings are connected by amide bonds which have torsional freedom. Such ligands typically have a crescent- shape, which allows sterically favorable fit in the minor groove binding of drugs and the drug aligns itself on the binding site in the minor groove of the DNA. Drug binding is stabilized by hydrophobic interactions, as well as van der Waal’s interactions and
hydrogen bonding. A majority of drug candidates binds preferentially to the A-T base pairs. Minor groove binders do not induce significant structural changes to the DNA. Drugs in this category include netropsin, distamycin and Hoechst 33258 etc.

![Distamycin-H binding](image1)

![Netropsin-H binding](image2)

Figure 2: Hydrogen-bonding scheme of distamycin and netropsin with dodecamer duplex.

Dickerson and coworkers\textsuperscript{22, 23} determined X-ray crystal structure of the 1:1 complex of netropsin and DNA dodecamer duplex which was similar to the binding of distamycin to DNA.\textsuperscript{24} The X-ray studies confirmed that drug molecule bound to the central 5’- AATT region in the minor groove by replacing the water molecules of the spine of hydration. Pelton and Wemmer\textsuperscript{19} determined the structure of distamycin complexed to d(CGCGAATTCGCG)\textsubscript{2} by 2D NMR spectroscopy; whereas Patel and coworker\textsuperscript{25} have determined the structure of Netropsin-DNA complex by 2D-NMR spectroscopy. The NMR data suggested that the distamycin molecules stacked on each other with their charged groups arranged in antiparallel directions and netropsin binds as a single molecule per binding site because binding of two molecules of netropsin was inhibited due to drug–drug electrostatic repulsion. (Figure 2)
Hoechst 33258 is a fluorescent dye often used as a chromosome stain. The crystal structures of Hoechst 33258 and its derivatives bound to oligonucleotides have been determined by several workers. The crystal structure of Hoechst 33258 complexed to d(CGCGAATTCGCG)2 was determined, and its binding to the central 5’-AATT region in the minor groove was confirmed. Hoechst 33258 binds to AT rich sequences preferentially, similar to other minor groove binding drugs.

ANTI-CANCER ALKALOIDS

Plants and trees have been an invaluable source of medicinal compounds throughout the civilization of mankind. In the modern times, the Vinca alkaloids and the taxanes have demonstrated their chemically proven anticancer profile. Vinca alkaloids belong to the family of indole–indoline dimeric compounds derived from the genus Apocynaceae, and they represent one of the most important classes of anticancer agents, widely used in cancer clinics. They are produced naturally by plants: Vinblastine and Vincristine from Madagascar periwinkle (Catharanthus roseus) and Vincamine from leaves of Vinca minor. Vinblastine is often used in combination to treat bladder and breast cancers and is an integral part of the curative treatment regime for Hodgkin’s disease. Vincristine is used in combination therapy to treat acute leukemias and lymphomas. It constitutes an important component of the regime that has been so successful in treating childhood leukemias. Both Vinblastine and Vincristine possess the identical Velbanamine upper subunit and nearly identical Vindoline derived lower subunits differing only in the dihydroindole N-substituent. Despite this small structural difference, Vinblastine and Vincristine differ in their antitumor properties and dose-limiting toxicities.

Vinblastine was found to be a cell cycle–dependent antimitotic agent that interacts with tubulin, a ubiquitous heterodimeric protein present in all eukaryotic cells. Tubulin and its polymerized form, microtubules, play crucial roles in the maintenance of cellular morphology and intracellular transport and in the construction of the mitotic spindle during cell division. Despite their many biochemical actions, the anti-tumor activity of Vinca alkaloids is usually attributed to their ability to disrupt microtubules, causing dissolution of mitotic spindle tubules and metaphase arrest in dividing cells. (Figure 3)
Figure 3: Mechanism of action of Vinca and taxanes.

Vincristine is also known to bind to tubulin with higher overall affinity than Vinblastine and at substoichiometric concentrations, induces the formation of spiral polymers similar to those found in the presence of Vinblastine. It has also been observed that at neutral pH, Vincristine more readily induced a “denatured” state of tubulin than Vinblastine. In human, the best established pharmacological property of Vincamine is the cerebroprotective activity, caused by the dilation of brain arteries, improving the global cerebral blood flow, as cerebro-vasodilating agent. Catharanthine, an indole alkaloid derived from leaves of *Vinca rosea*, has similar structure to the indole moiety of the dimeric oncolytic Vinca alkaloids.

The progress in the structural biology has permitted the determination of the tubulin structure at high resolution, allowing a better investigation of the interactions with Vinca alkaloids at the molecular level. Although the cellular target of the Vinca alkaloids has been known, the precise location of the binding site on tubulin has been reported. There are, however, only a few studies furnished on the synthesis of the Vinca alkaloids, their structural activity and DNA binding nature of these drugs. Mathew Voss et al. synthesized the iodo intermediates of Vinca alkaloids (12’-iodovinblastine, 12’-iodovincristine and 11’-iodovinorelbine) and showed the Structure Activity Relationship (SAR) assay based upon Hela and MCF-7 cell lines reporting good contrast between the analogs of Vinca alkaloids to its parents Vinca alkaloids.
Berberine, an isoquinoline plant alkaloid, belongs to the structural class of Protoberberine analogs. Berberine has been subsequently screened for anticancer activity following evidence of antineoplastic properties. Berberine was found to produce aggregation of DNA. Aggregated parts had a length of 1700-4000Å and a width of 1400-4000Å indicating that this alkaloid could aggregate thousand of molecules of DNA. Intercalation of Berberine in the DNA duplex has been studied by physico-chemical methods to suggest that Berberine interacted more with AT than with GC sequences. Recently, the complexation of Berberine with DNA in the leukemia HL-60 cell has been detected. Recent study from our research group in 2008 revealed the cooperative binding of Berberine, Palmatine and Ethidium on t-RNA molecule by fluorescence quenching method and concluded that Berberine and Palmatine are partially intercalated, while Ethidium was fully intercalated into the t-RNA molecule.

Taxol (paclitaxel) is a diterpenoid alkaloid isolated from the bark of Taxus brevifolia. It shows a significant cytotoxic and antitumor activity. In clinics, anticancer diterpene Taxol suppressed dynamic changes in microtubules, since anti-tumor activity of Taxol is related to tubules-polymerization activity. At low concentrations, Taxol binds stoichiometrically to β-tubulin in the assembled tubulin heterodimers in microtubules, causing conformational perturbations. These changes alter the structure of the microtubule and lead to microtubule stabilization (Figure 4). This alteration of the normal microtubule dynamics in cells leads to mitotic arrest and interferes with the formation of the mitotic spindle, which prevents segregation of the chromosomes.

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**Figure 4: Tubulin Polymeraization by Taxol**

![Figure 4: Tubulin Polymeraization by Taxol](image)
In 2004, Riahi and coworkers\textsuperscript{51} examined the binding of Taxol with nucleic acids (DNA and RNA) in aqueous solution at physiological pH, and observed the binding constant for Taxol-DNA complexes with $K_1 = 1.3 \times 10^4$ M$^{-1}$ and $K_2 = 3.5 \times 10^3$ M$^{-1}$. Whereas taxol-RNA complexes showed one type of binding with $K = 1.3 \times 10^4$ M$^{-1}$. This value of binding constant showed the partial helical stabilization of Taxol-polynucleotide complexes. Synder and coworkers\textsuperscript{52} furnished the NMR analysis of molecular flexibility of analogs of Taxol in solution, which showed high tubulin binding with cytotoxic profile comparable to Taxol. This study demonstrated that the configurational and steric factors are responsible for binding ability of Taxol.

Krishna and coworkers\textsuperscript{53} studied Taxol-DNA interactions by using fluorescence spectroscopy, estimated an affinity constant ($K_a$) of $1.08 \times 10^7$ M$^{-1}$ for the Taxol-DNA complex. The results pointed to an interaction of Taxol with its core eight-membered ring in the DNA minor groove. Most analogues synthesized to date are modified at C-13 side chain of Taxol and studied \textit{in vitro}. Georg and Zacharia\textsuperscript{54} (1992) first synthesized the substituted phenyl rings at C-13 ring and checked the biological evaluation of Taxol derivatives with tubulin assembly assay and also evaluated their cytotoxicity by comparing with that of Taxol. Potier and his group\textsuperscript{55} (1992) compared the \textit{in vitro} structure-activity of the various taxol analogues on tubulin with their conformation, by NMR experiments and molecular modeling techniques.

**HYPOTHESIS**

DNA binding studies of alkaloids are important because almost every cellular process is directly or indirectly controlled by this genetic material. It was revealed that both Vinca alkaloids and Taxol bind to Tubulin which was responsible for mitotic spindle formation at the time of cell division. As in the case of cancer, the uncontrolled cell growth can be managed by targeting the DNA. Most Nucleic acid (RNA and DNA) functions are carried out synergistically with proteins and other ligands; therefore, intervention in biochemical processes at gene level can significantly affect cellular processes. For the purpose of exploring interaction between DNA and alkaloids, we need to identify the structural information specifying host-drug stoichiometric ratio and size of the binding site on DNA.
duplex. Such structural information is the key factor in the rational design of drugs for potential use in the gene-targeted chemotherapy.

**OBJECTIVES**

Main objectives of the proposed study are:

(a) To investigate the control DNA oligomers (DNA-1, DNA-2, DNA-3, DNA-4 and DNA-5) for their detailed structural features.

(b) To study the interaction of control DNA sequences with the anticancer alkaloids by spectroscopic techniques.

(c) Comparative assessment of DNA binding affinities of proposed anticancer alkaloids with DNA decamer and dodecamer sequences.

**MATERIALS AND METHODS**

The proposed Control DNA Oligomers Sequence and Alkaloid agents are:

1. DNA 1: 5′-d(GATGGCCATC)₂
2. DNA 2: 5′-d(GATCCGGATC)₂
3. DNA 3: 5′-d(GGCAATTGCC)₂
4. DNA 4: 5′-d(GGCTTAAGCC)₂
5. DNA 5: 5′-d(CGCGAATTCGCG)₂ (Dickerson Dodecamer)

Anticancer Alkaloids:

1. Vinblastine
2. Vincristine
3. Vincamine
4. Vindoline
5. Catharanthine
6. Berberine
7. Taxol (Paclitaxel)

The DNA oligomer sequences proposed for carrying out our research work are self complementary. Thus by annealing, they form a double helix with the help of hydrogen bonds. As the first step, the DNA solution is prepared in the phosphate buffer at physiological pH of ~7.4. The concentration of DNA oligomers would be determined spectrophotometrically using the molar extinction coefficients. The concentration of
alkaloids would be calculated volumetrically. All experiments will be carried out at physiological pH.

Equilibrium Binding Titration technique has found universal application in Drug-DNA binding studies. Essentially, an alkaloid solution of fixed concentration is transferred to a thermostated cuvet and the progressive absorbance or fluorescence changes are recorded after an addition of serial aliquot of a DNA solution.
Optical changes are normally analyzed for the drug component in terms of free drug and the bound drug in the resulting complex. For binding study of the alkaloid-DNA complexes, the titration would be carried out by using fluorescence spectroscopy, as most of the alkaloids produced fluorescence. As the DNA binding profiles of these alkaloids are evaluated, it would be pertinent to study at least one of the complexes by more precise technique i.e. NMR to obtain the elaborated molecular structure.\(^{57}\) 1D- and 2D- \(^1\)H-NMR experiments would be conducted on DNA oligomers as reported.\(^{58, 59}\) Multiple 2D \(^1\)H-NOESY experiments (at different mixing time intervals) would be furnished in order to obtain the detailed spectra for extracting the spatial proximity information about various hydrogen atoms of alkaloids and DNA oligomers. After obtaining the structural details of DNA-alkaloid complex, Molecular Modeling and Molecular Dynamics experiments may be carried out using AMBER/XPLOR software in order to furnish an NMR refined structure of the DNA duplexes and the drug-DNA complex.

**FUTURE SCOPE OF THIS STUDY**

The latest approach in the area of drug design where natural products are explored for activity component encoded in their structure from their DNA binding profile. Developing more potent and less toxic compounds with anticancer activity using the structural leads taken from the natural products has triggered a new wave in drug chemistry. This approach has resulted in the explosive growth of Structural Biology of drug-receptor studies. Our objective is to obtain structural information, regarding the drug-DNA interaction. This comprehensive structural study of alkaloid-DNA complexes may lead to the generation of data base of structural information, which would be extremely helpful to design more therapeutic agents with similar or improved biological activity with least toxicity in the future.
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