CHAPTER I

Introduction
**Introduction**

There has been considerable interest in the binding studies of small molecules with DNA [1-10] owing to their diverse applications viz., DNA-target chemotherapeutic agents [11-15], highly sensitive molecular probes [16-20], enantioselective catalysts [21] etc. Understanding the interaction of pharmaceutical agents to DNA is essential for underlyng their mode of action, site, sequence and structural specificity of their binding reactions. Interaction between small molecules and DNA provide a structural guideline in rational drug design regime for the synthesis of new, improved chemical drug entities with enhanced or more selective activity, thereby greater clinical efficacy and lower toxicity. These studies also lay a foundation platform for well defined models for bimolecular complex formation *in vivo* [22].

Metal complex-DNA interaction studies have attracted much attention after the serendipitous discovery of cisplatin [23], a well known anticancer drug clinically in use for treating solid tumors. The discovery of cisplatin has fostered a new discipline of inorganic chemistry–medicinal inorganic chemistry. It is an interdisciplinary field of research combining elements of inorganic chemistry, pharmacology, toxicology and biochemistry.

![Figure 1. Platinum(II) complexes in clinical use for cancer chemotherapy (a) cisplatin (b) carboplatin (c) oxaliplatin and (d) BBR3464.](image)
The clinical success of cisplatin and related platinum–based anticancer drugs [24-28] (Figure 1) provide an impetus for the development of new innovative strategies that meet the challenges of platinum based drugs viz., serious side effects, acquired drug resistance and general toxicity. Metal complexes were appealing for the design of chemotherapeutic agents, especially cancer chemotherapeutics as they display unique electronic and spectroscopic properties, allowing a diverse coordination environment that goes beyond the geometries of carbon and a mechanism of cytotoxic action related to DNA binding affinity [29]. Besides this, metal complexes play an important role in chaperoning and delivery of drugs away from the sites where they exert most toxicity and towards their site of action.

Metal ions exist as electron deficient cations (Lewis acids) in the biological system and hence are attracted to electron rich (Lewis bases) biological molecules such as protein and DNA. Since pharmacological target of the antitumor drugs is cellular DNA, N7 atom of the imidazole ring of guanine and N3 atom of adenine residues located in the major groove of the double helix, the most accessible and reactive nucleophilic sites for binding to DNA, therefore, search for the DNA binding non-platinum metal complexes has gained increased momentum. A novel DNA binding metal complex with good anticancer activity and clinical efficacy must fulfill the following key criteria i) good intrinsic properties, including saline solubility and enough stability to arrive intact at the cellular target ii) efficient DNA binding properties iii) efficient transport in blood and through membranes iv) the ability to differentiate between cancerous and normal cells v) activity against tumors that are, or have become resistant to cisplatin and its second generation derivatives [30].
Cancer is a class of disease in which a group of cells display uncontrolled growth (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body via lymph or blood). Most cancerous cells divide uncontrollably to form lumps or masses of tissue called *tumors* but some like leukemia (where cancer prohibits normal blood function by abnormal cell division in the blood stream) do not [31-33]. Systems that irreversibly bind to DNA such as cisplatin and its analogues have become some of the most important first-line treatment for solid tumors.

DNA, an inherently chiral molecule has a polymorphic structure with polyanionic nucleotide chains and sugar phosphate back bone [34]. The asymmetric D-ribose and D-2-deoxyribose units contain several stereogenic centers, whose configuration is important in overall DNA structure.

*Figure 2. Different conformational variations of DNA.*
It is well known that DNA does not exist in a single three-dimensional structure, but can adopt different conformations which are defined both locally and macroscopically by different structural parameters (Figure 2). Double stranded DNA commonly adopts a right-handed helical conformation that of B- and A-form, however, they differ in the conformation of sugar (C2’-endo for B-DNA and C3’-endo for A-DNA, and in helical parameters). It was discovered that certain sequences of DNA have the propensity to undergo conformational transitions to left-handed helical form termed as Z-DNA [35,36].

The DNA molecules are much susceptible to damage by interacting with metal complexes under various physiological conditions. This damage may lead to various pathological changes in living organisms. Thus, the interaction of metal complexes with DNA was regarded as deciding factor to ascertain the chemotherapeutic potential of these metal complexes. Based on these considerations, binding modes of metallomolecules can be classified broadly into covalent binding and non-covalent interactions [37-39]. Covalent binding involves the replacement of the labile ligand by nitrogenous base of DNA such as N7 of guanine. On the other hand, non-covalent interactions include intercalative, electrostatic and groove (surface) binding of the cationic metal complexes along outside of the DNA helix (Figure 3). Intercalative interaction involves stacking of the planar fused aromatic cations in between the adjacent base pairs of DNA helix.
Molecules that bind in the minor grooves comprise arc shaped aromatic architectures with terminal base functions while the electrostatic mode of binding is governed by charge of the molecule, ligand hydrophobicity and size of the ions. In the electrostatic mode of binding, complexes exhibit an effective binding with the phosphate backbone of DNA double helix.

The term ‘chirality’ (originated from the Greek word “cheir meaning hand”) or handedness is used to describe the structural property of an object that is non-superimposable on its mirror image. Life is a typical chiral system, and chiral phenomena are ubiquitous in nature from the macroscopic to the molecular level, ordinarily, proteins and DNA wind in right handed helixes; left-handed versions are rare and true mirror image versions do not appear in nature. Right and left-handed amino acid molecule exists at different energy levels as a result of the asymmetric weak nuclear force; those in organisms are almost always left-handed. The elementary particle known as neutrino exists only as a left handed object [40]. Because of the chirality of its key molecules, human chemistry is highly sensitive to enantiomeric differences. Some examples of chiral structures are given in figure 4.
Figure 4. Depiction of enantiomeric pairs.

Chiral drugs are at forefront in pharmaceutical drug research; over one third of marketed drugs worldwide are chiral, and regulators will now only approve new chiral drugs in single enantiomer form, preferably with their in vivo profile. The introduction of chirality not only enforces stereoselective specific drug interaction but also promotes the formation of active compounds with therapeutic benefit as most of the targets of drugs at the molecular level (viz., DNA, RNA and proteins etc.) are chiral in nature. The growing use of enantiomeric drugs is closely related to structure-function relationship. The structure-function relationship in nature is so powerful that when a functional disorder manifests in the form of disease, it can be handled in many cases only by using a molecule of specific chiral structure. The preferential interaction of one enantiomer of a racemate with chiral macromolecules of the body leads to expressed pharmacokinetic and pharmacodynamic effect. The term “eutomer” has been used for more potent isomer and “distomer” for less potent one in terms of their pharmacological effect [41]. Enantiomers can be absorbed, distributed, metabolized and excreted differently. Further, disease states, route of administration, genetic variability and drug-interactions may be stereospecific [42].
Chiral molecules play a critical role in the exploitation of three-dimensional space at the target site and regulate stereoselectivity in a highly organised fashion. Furthermore, the use of stereochemistry can give clear insight into the mechanism of action allowing the discrimination between unspecific interaction which is common to both enantiomers and specific contacts that give rise to enantioselectivity [43]. Consequently, the appropriate designing of enantiomeric tumor inhibiting motifs or compounds is well-understood. Chirality in metal complexes can arise by the asymmetry of ligands or in an inherently achiral coordination mode (such as square planar complexes) or by coordination chirality such as Λ or Δ isomers of octahedral complexes of bidentate or terdentate ligands [44]. First example of asymmetric catalysis using a structurally well defined chiral transition metal complex was demonstrated in 1966 at H. Nozaki’s laboratory [45]. Later, BINAP (2,2′-bis(diphenylphosphanyl)-1,1′-binaphthyl) was discovered as an excellent chiral ligand in 1974 at Nogaya with the help of H. Takaya [46,47] (Scheme 1).

Scheme 1. Access to enantiomerically pure BINAP.
Chirality has a well known relevance in the field of medicine and has played important roles in the quest for new and more efficacious drugs. Both (S)-ibuprofen and (S)-ketoprofen are chiral switch drugs of popular racemates (Figure 5). The use of (S)-enantiomers of these drugs in therapy reduces total dose and toxicity that is associated with (R)-enantiomers. It has been observed that in case of ketoprofen, (S)-(+-)ketoprofen is several times more potent than the racemate. Similarly, omeprazole is a gastric antisecretory proton pump inhibitor. The chiral switch drug esomeprazole which is (S)-(−)-enantiomer of omeprazole has therapeutic benefit than its (R)-enantiomer. Amlodipine, an antihypertensive drug exhibits chirality and receptor binding studies has shown (S)-(−)-isomer of amlodipene has higher L-type calcium channel blocking activity than its (R)-(−)-isomer [48].

Figure 5. Some chiral switch drugs.
Cisplatin, due to undesirable side effects, inherent and acquired resistance has been modulated by inducing a novel feature of chirality. Oxaliplatin, (1R,2R-diaminocyclohexane)oxalatoplatinum(II)- a cisplatin analogue produces the same type of inter- and 1,2-GG intrastrand cross-links as cisplatin but has a spectrum of activity and mechanisms of action and resistance different from those of cisplatin and carboplatin. Lippard et al.; reported structural evidence for the importance of chirality in mediating the interaction between oxaliplatin and duplex DNA [49]. Oxaliplatin has a non-hydrolyzable diaminocyclohexane (DACH) carrier ligand which is maintained in the final cytotoxic metabolites of the drug. The intrinsic chemical and steric characteristics of the DACH-platinum adducts appear to contribute to the lack of cross-resistance with cisplatin [50,51]. Oxaliplatin has shown a wide antitumor effect both in vitro and in vivo, a better safety profile than cisplatin and a lack of cross-resistance with cisplatin and carboplatin.

The initial success of platinum chemotherapeutic metallopharmaceuticals shifted the attention of researchers to non-platinum chemotherapeutics starting from the basic cisplatin framework with the aim to optimize the efficiency of such drugs [52-57]. Non-platinum active compounds are likely to have mechanism of action, biodistribution and toxicity which are different from those of platinum drugs and might be effective against human cancers that are poor chemosensitive or have become resistant to conventional platinum drugs [58]. Investigations in this area were primarily focused on the use of biologically active complexes formed by essential metal ions, such as cobalt, copper and zinc. Since, any essential metal ion which escapes its normal metabolic pathway can be
very toxic to organism; complexes of such metals can serve as effective cytotoxic agents.

Cobalt is an element of biological interest and its role is mainly focused on its presence in the active center of vitamin B\textsubscript{12}, which regulates indirectly the synthesis of DNA [59]. Co(III) complexes have been extensively studied and reviewed in recent years due to their applications in bioinorganic chemistry. Since the first report on the biological activity of cobalt complexes in 1952 [60], diverse structurally characterized cobalt complexes have been studied as hydrolytic agents for DNA cleavage [61] and others showing antitumor-antiproliferative [62-64], antimicrobial [65-67], antifungal [68,69], antiviral [70,71] and antioxidant [72,73] activity have been reported.

Copper is widely distributed in the biological system and it is the most familiar redox metal serving diverse biological functions [74-79]. Copper can be regarded as a “modern” bioelement as suggested by Kaim [80], Ochiai [81] and Williams [82]. It has been demonstrated that copper accumulates in tumors due to selective permeability of the cancer cell membranes [83-86]. Because of this, a number of copper complexes have been screened for anticancer activity and some of them were found active both \textit{in vitro} and \textit{in vivo} [87-90]. Cu(II) complexes are regarded as the most promising alternatives to cisplatin as anticancer drugs. Serum copper levels correlate with tumor incidence, tumor weight, malignant progression, and recurrence in a variety of human cancers: Hodgkin’s lymphoma, sarcoma, leukemia, and cancer of the cervix, breast, liver, and lung [91-94] as well as brain tumors [95,96]. Consistently, the high serum and tissue levels of copper found in many types of human cancers support the idea that copper could be used as a potential tumor-specific target.
Copper binds to DNA with high affinity than any other divalent cation, thus promoting DNA oxidation [97]. The binding of copper ions to specific sites can modify the conformational structures of proteins, polynucleotides or DNA and biomembranes [98]. This binding is dependent on size, charge, electron affinity and geometry of the formed adduct. The properties of copper-coordinated compounds, whether in classical inorganic coordination complexes, in organometallic compounds, or in bioinorganic model compounds are largely determined by the nature of ligands and donor atoms bound to the metal ion [99,100]. Three oxidation states of the metal can be stabilized: Cu(III), Cu(II) and Cu(I). However, copper is dominated by Cu(II) oxidation state with d⁹ electronic configuration which promotes d-d transitions resulting in intense colored species while Cu(I) complexes due to the closed-shell d¹⁰ electronic configuration are usually colorless solids and strongly prefer ligands having soft donor atoms. The biologically accessible oxidative/reductive potential made copper complexes a class of the most frequently studied metallonucleases.

Zinc is second most abundant essential transition metal ion in humans following iron, divalent zinc is an integral part of all biological systems. Zn ions possess nutritional features important to human health and health care. Zinc plays important role in genetic stability and function [101,102]. Mechanistically, zinc has significant impact on DNA as a component of chromatin structure, DNA replication and transcription and DNA repair [103]. Zinc enzymes efficiently catalyze the hydrolysis of nucleic acid under physiological condition in the living system. Many proteins possess a Zn-containing motif that serves to bind the DNA embedded in their structure. Structural changes induced by Zn(II) on DNA suggest that this cation can bind to both the nucleobase and
the phosphate group [104]. Zinc is vital for recovery of leukemic cells because zinc is required for proper functioning of genetics, immunity, formation of red blood cells, organ, muscle and bone function, cell membrane stability, cell growth, division and differentiation [105]. Zinc has beneficial interactions with several chemotherapy drugs. Zn(II) complexes of p-isopropylbenzaldehyde and methyl 2-pyridyl ketone thiosemicarbazones show potent cytotoxic activity and induction of apoptosis in cells resistant to cisplatin [106].

Ruiz-Azuara et al.; developed a series of copper-based drugs registered with the name of Casiopeinas® (Cas) [107]. These compounds are mixed chelate Cu(II) complexes with a general condensed formula [Cu(N–N)(A–A)][NO₃], where N–N represents neutral diimine donors, either phen or bipy, A–A stands for uninegative N–O or O–O donors, either aminoacidates or acetylacetonate. Cas were designed as a chemotherapeutic alternative for cancer treatment and according to some preliminary experiments, some of them have indeed shown antineoplastic activity both in vitro and in vivo and were able to induce apoptosis in murine cancer cell lines, such as L1210 and CH1 [108]. Experiments in rats employing one of the most promising derivatives (Figure 6), showed a strong inhibition of cell proliferation against glioma C6 cells. It was observed that the drug promoted an increment in ROS which in turn caused subsequent damage to mitochondria followed by apoptosis elicited through both, caspase-dependent and caspase-independent pathways [109].
Previously, our research group has synthesized new chiral enantiomeric L- and D-tryptophan derived Co(II), Cu(II) and Zn(II) complexes of 1,2-DACH (Figure 7) [110]. The comparative DNA binding profile of the enantiomeric complexes revealed the maximal potential of L-enantiomeric form of copper complex to bind DNA. *In vitro* anticancer activity of complexes screened against different human carcinoma cell lines of different histological origin revealed that the L-enantiomer of copper complex showed significant antitumor activity in comparison with other metal ions and is particularly selective for MIA PaCa-2 (pancreatic cancer cell line).

![Proposed structure of the complexes.](image-url)
Recent advances in ligand design have resulted in potent antitumor compounds that are active in cisplatin resistant cell lines, and also include additional features to allow for an increased understanding of the mechanism of action of drug. Tailored multifunctional ligands for metal based medicinal drugs offer many exciting possibilities including targeting specific tissues, membrane receptors, or endogenous molecules and can play an integral role in modulating the potential toxicity of a metallodrug [111].

Amino acids, which are naturally occurring chiral compounds, have been extensively used as chiral auxiliaries to create/induce a quaternary stereogenic carbon center. The relevance of amino acids lies in their biological importance, as they form the basic constituents of living organisms. They take part in important metabolic reactions, including the biosynthesis of polypeptides and proteins and the synthesis of nucleotides. High biological importance, chirality and amphiphilicity combined with a low molecular weight and relative simplicity of molecular structures make amino acids the most suitable candidates for drug scaffold representing typical features of natural bioactive substances [112,113]. Different configurations of amino acids have different roles in living system. L-amino acids are used in the synthesis of proteins, while some D-amino acids are not participated in protein synthesis. The introduction of chirality due to amino acid ligands could enhance the pharmacological behavior of the complexes by adopting a specific conformation and may also confer selective binding affinity for the chiral DNA [114].

Yang et al.; investigated several novel heterocyclic fused napthalimides having chiral amino side chains (Figure 8); effects of chirality of amino side chains on DNA binding ability, photo damage activity as well as antitumor cytotoxicity were evaluated [115].
Their side chain chiral configuration determined DNA binding activities in the order; (S)-enantiomer>(R)-enantiomer. The DNA photodamaging activities were in good agreement with their DNA binding constants. (S)-enantiomer cleaved plasmid pBR322 DNA from supercoiled form (Form I) to circular nicked form (Form II) completely at 50 µM. Furthermore, (S)-enantiomer demonstrated effective antitumor cytotoxicity against human lung cancer cells and murine leukemia cells while (R)-enantiomer exhibited different cytotoxicity.

![Heterocyclic fused naphthalimide chiral amino side chains.](image)

*Figure 8. Heterocyclic fused naphthalimide chiral amino side chains.*

Metal complexes of amino acids are involved in exchange and transport mechanism of trace metal ions in human body. Such systems provide models of protein-DNA and peptide derived antitumor agent-DNA interaction. Various side groups of amino acids are found to have a potential to recognize the specific base sequence through hydrogen bond formation with the nucleic acid bases in DNA [116,117]. The physiologically interesting mixed ligand complexes of amino acids are the most studied class of this field. Two copper complexes (Figure 9) of the type [Cu(L\(^1\))(L\(^2\))] (where L\(^1\) = tryptophanate or phenylalaninate, L\(^2\) = cysteinethiolate) were prepared, characterized and their spectrophotometric and voltammetric behavior was investigated [118].
As nucleic acids and proteins recognize each other by very specific and selective interactions through amino acid side chain and nucleic acid constituents [119,120], mixed ligand ternary metal complexes of amino acids and nucleic acid constituents play an important role in biochemical processes. Mixed ligand ternary complexes of nucleic acid bases and nucleotides such as thymine, cytidine, 2-thiouracil and amino acids viz., L-Alanine, L-Phenylalanine and L-Tryptophan (Figure 10) were synthesized and characterized by various spectroscopic techniques [121,122].
Palaniandavar et al.; have demonstrated ternary Cu(II) complexes of L-Tyrosine and diimines, where, diimine = 2,2'-bipyridine (bpy), 1,10-phenanthroline (phe), 5,6-dimethyl-1,10-phenanthroline (5,6-dmp) and dipyrido[3,2-d:2',3'-f]quinoxaline (dpq) (Figure 11). The 1,10-phenanthroline complex was characterized by X-ray Crystallography. The complexes bind to DNA non-covalently and they showed profound efficiency to cleave plasmid DNA oxidatively. The amino acid residue in the complexes promotes the cleavage of DNA. The complexes exhibited anticancer activities that are more or less equivalent to cisplatin. The 5,6-dmp complex was the first Cu(II) complex reported so far to exhibit a mitotic catastrophe mode of cell death [123].

Figure 11. (a) Copper(II) Complexes of L-Tyrosine and diimines (b) Ball-and-stick representation of the crystal structure of [Cu(L-tyr)(5,6-dmp)(H₂O)] (ClO₄).
The condensation of primary amines with carbonyl compounds was first reported by Schiff and since then condensation products are referred to as Schiff bases [124]. Several Schiff base complexes were found to inhibit tumor growth. A few structurally characterized amino acid derived Schiff bases and their metal complexes were reported [125,126]. Amino acid Schiff base complexes of first row transition metals such as Co(II), Ni(II), Cu(II) etc. were reported to exhibit fungicidal, bactericidal, antiviral and antitubercular activity [127,128].

Wang et al.; [68] have reported Cu(II) and Co(II) complexes of valine derived Schiff base (Scheme 2). These complexes were characterized by elemental analysis FTIR and X-ray diffraction. Biological studies of the complexes were carried out in vitro for antimicrobial activity against Gram-positive and Gram-negative bacteria and human pathogenic fungi. The in vitro cytotoxicity of this compound revealed that it was nontoxic to human erythrocytes even at 500 μg/mL concentration.

Scheme 2. Synthetic route of Cu(II) and Co(II) complexes of valine derived Schiff base.
Peptides are attracting much attention due to their high biological activity associated with low toxicity and high specificity which renders them attractive for therapeutic use. Peptides play important roles as hormones, enzyme inhibitors, neurotransmitters, and immunomodulators in living systems and therefore, are expected to have significant role in the treatment of many diseases viz., cancer, AIDS, Alzheimer’s disease, malaria and as antimicrobials [129,130]. They can surmount the hurdles of present cancer chemotherapeutic drugs including, little unspecific binding to molecular structures other than the desired target, minimization of drug-drug interactions and less accumulation in tissues reducing risks of complications due to intermediate metabolites. Recently, it has been reported that some peptide derivatives show antitumor activity with little toxicity against non-malignant cells either by triggering apoptosis [131] or by forming ion channels/pores [132]. Furthermore, some peptides were found to be cytotoxic against MDR cancer cells [133]. The fact that peptides affect the tumor cells rapidly and that their secondary metabolites are free amino acids prove that the peptides have minimized side effects when compared to other chemotherapeutic agents that are available currently.

Peptides can be very effective ligands for a range of metal ions since they contain a variety of potential donor centers. Metals may be directly bound into peptides and have been used for DNA selective recognition and/or cleavage. It was reported that peptide functionalisation of polypyridyl ruthenium(II) [134,135] or rhodium(III) [136,137] metallo-intercalators improved the selectivity of the parent intercalators, with similar effects observed by conjugating minor groove binders with short peptides that mimic natural protein motifs [138]. Recently, Lippard et al.; reported a Pt(IV) complex
conjugated to the cancer-targeting peptide chlorotoxin which delivers cisplatin selectively to cancer cells [139]. The investigation of Cu(II)/Zn(II)-peptide complexes is of scientific and technological importance, since such systems may be regarded as models for both protein-DNA and antitumor agent-DNA interactions. The development of peptide complexes that retain the initial biological activity of the lead peptide but with improved pharmacokinetic profile can be achieved whereby optimal physical, chemical and pharmacological properties are engineered through application of medicinal chemistry to transform a given lead peptide into a viable drug candidate.

Bleomycin is an important naturally occurring glycopeptide displaying distinct anticancer activity [140] (Figure 12). It consists of four distinct regions; i) an N-terminal domain responsible for metal binding and site selective DNA cleavage; ii) methyl valerate threonine linker peptide iii) a C-terminal domain containing a bithiazole moiety having DNA binding affinity and; iv) a disaccharide moiety consisting of glucose and mannose sugars connected to metal binding domain.

![Figure 12. Structure of bleomycin, naturally occurring glycopeptide possessing anticancer activity.](image_url)
Manessi-Zoupa et al.; carried out a systematic study of the coordination chemistry of
dipeptides and tripeptides containing \(\alpha\)-aminoisobutyric residue. Cu(II) complexes of
H-Aib-X-OH, (where Aib = \(\alpha\)-aminoisobutyric residue, X = Aib, Gly, L-Ala, L-Leu, L-
Phe) were synthesized and structurally characterized by crystallography (Figure 13)
[141,142]. The coordination chemistry of the complexes revealed that “fine tuning” of
the synthetic and crystallization conditions can bring remarkable changes in the
coordination mode of the dipeptides and in the structure of the resulting Cu(II)
complexes.

![Figure 13](image_url)

**Figure 13.** A portion of the polymeric structure of (a) \([\text{Cu(II)}\text{-Aib-Gly-OH}]_n\) \(n\text{H}_2\text{O}\), (b)
\([\text{Cu(II)}\text{-}(\text{Aib-L-Leu-OH})(\text{MeOH})]_n\) \(n\text{MeOH}\) and (c) \([\text{Cu(II)}\text{-}(\text{Aib-L-Phe-OH})]_n\).

Reddy et al.; demonstrated the interaction of Zn(II) and Co(II) with cysteinylglycine and
histidylglycine. The DNA binding studies of the complexes revealed that only zinc
complexes bind to DNA [143]. The DNA cleavage ability of \([\text{Zn}^{\text{II}}(\text{CysGlyc})(\text{HisSer}])\]
and \([\text{Zn}^{\text{II}}(\text{CysGlyc})(\text{HisPhe})]\) was investigated in another attempt [144]. The presence of
Zn\(^{\text{II}}\) in the active sites of hydrolytic enzymes such as alkaline phosphatases,
phospholipase C, etc. which are responsible for the hydrolytic cleavage of
phosphodiester DNA backbone revealed hydrolytic cleavage of pBR322 DNA by the complexes.

Schiff bases, regarded as privileged ligands are under vigorous scrutiny as they show diverse pharmacological applications [145,146] including inhibition of several animal tumors [147]. Schiff bases with donors (N, O, etc.) have structure similarities with neutral biological systems and due to the presence of imine group are utilized in elucidating the mechanism of transformation of rasemination reaction in biological system [148,149].

Schiff bases derived from chromones (1-benzopyran-4-one) are biologically significant, with superior chelating ability exhibiting efficient DNA binding. Chromones are ubiquitous in nature, especially in plants which were exploited since long as potential therapeutic agents due to the high anti-tumor activity and low toxicity. Their biological and physiological activities also include antimycobacterial, antifungal, anticonvulsant, antimicrobial, mushroom tyrosinase inhibition activities [150,151]. Flavonoid-related chromone and its derivatives {Hormothamnione and 6-desmethoxyhormothamnione} (Figure 14) are well known naturally occurring heterocyclic compounds with oxygen as heteroatom [152].

![Figure 14. Structures of (a) chromone (4H-benzopyran-4-one), (b) hormothamnione and (c) 6-desmethoxyhormothamnione.](image-url)
Flavonoids are effective metal ion chelators and play a key role in the initiation of free radical processes by acting on two antioxidant pathways: 1) direct reaction with free radicals and 2) chelating of metal ions involved in production of reactive oxygen pathways. Recently Budzisz et al. [153] described some coumarin complexes with excellent cytotoxic activity obtained from the reaction of chromone derivatives. Starting from 2-methyl-4-oxo-chromone-3-carboxylic acid, new coumarin derivatives 3-[1-(alkylamino)-ethylidene]-chroman-2,4-dione were synthesized which displayed excellent cytotoxic activity against HL-60, NALM-6 cell lines. Another chromone derivative namely 2-methyl-4-oxo-4H-chromone-3-carboxylate also exhibited \textit{in vitro} activity against human cancer cell lines A549, K562 and HeLa.

The DNA-binding properties of chromone complexes were also exploited by Yang et al. [154]. They have synthesized and characterized novel glycine Schiff base sodium salt, 6-hydroxychromone-3-methylidyneiminoacetate (LNa), derived from chromone and their complexes [CuL·(H$_2$O)$_3$]NO$_3$·H$_2$O and [NiL·H$_2$O]NO$_3$·2H$_2$O (Figure 15).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image15.png}
\caption{Speculated structures of (a) [CuL·(H$_2$O)$_3$]NO$_3$·H$_2$O and (b) [NiL·H$_2$O]NO$_3$·2H$_2$O.}
\end{figure}

The DNA-binding properties of the complexes have been elucidated by means of UV-visible spectroscopy, fluorescence spectroscopy, and viscosity measurements revealing an intercalative mode of interaction of these complexes. The interaction with DNA
revealed 50% hypochromism in the absorption band with a bathochromic shift of 3-10 nm for $[\text{CuL} \cdot (\text{H}_2\text{O})_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ while $[\text{NiL} \cdot \text{H}_2\text{O}]\text{NO}_3 \cdot 2\text{H}_2\text{O}$ exhibited 42% hypochromism with a bathochromic shift of 6 nm (Figure 16).

![Figure 16](image)

**Figure 16.** (a) Electronic spectra of $[\text{CuL} \cdot (\text{H}_2\text{O})_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ (10 μM) and (b) Electronic spectra of $[\text{NiL} \cdot \text{H}_2\text{O}]\text{NO}_3 \cdot 2\text{H}_2\text{O}$ (10 μM) in presence of increasing amounts of DNA; [DNA] = 0-35 μM. The arrow indicates the absorbance changes upon increasing DNA concentration.

Yang et al.; [155] have described the synthesis, characterization and DNA binding properties of novel fluorescent Zn(II) and Ni(II) complexes of the ligand, 6-ethoxy chromone-3-carbaldehyde benzoyl hydrazone (L) (Figure 17). The comparative interaction of the ligand and $[\text{ZnLNO}_3]\text{NO}_3$ with DNA were carried out using UV-visible, fluorescence, circular dichroic methods and viscosity measurements. It was found that the Zn(II) complex strongly binds to DNA presumably by an intercalative mode.

![Figure 17](image)

**Figure 17.** Proposed structure of complexes (a) $[\text{ZnLNO}_3]\text{NO}_3$ complex and (b) $[\text{NiLNO}_3]\text{NO}_3 \cdot \text{H}_2\text{O}$ complex.
The chemical, biological and pharmaceutical properties of tin(IV) and diorganotin(IV) complexes have been studied extensively [156-158]. Organotin derivatives are promising as new lead chemotherapeutic agents, which have been demonstrated by their significant \textit{in vitro} cytotoxicity and antiproliferative \textit{in vivo} activity. During the last 30 years a large number of organotin complexes has been synthesized and tested for their antitumor profile [52,159,160].

Organotin(IV) compounds are characterized by the presence of at least one covalent C-Sn bond. Depending on the number of organic moieties, organotins are classified as mono, di, tri and tetraorganotin (RSnX₃, R₂SnX₂, R₃SnX, R₄Sn), where X is an anionic species (halide, oxide, hydroxide, carboxylate or thiolate). Tin and organotin compounds containing electronegative atoms such as halogen atoms show Lewis acid character; tin atom increases its coordination number from four to seven upon addition of neutral organic donor ligands [161,162]. The binding ability of organotin compound towards DNA depends on the coordination number and nature of the groups bonded to the central tin atom. The phosphate group of DNA sugar back bone usually acts as an anchoring site. Nitrogen of DNA base binding is extremely effective, stabilizing the tin centre as an octahedral stable species. However, researches indicate that there is negligible interaction of tin complexes with nucleotide bases, but rather strong and irreversible binding to the vicinal phosphate groups of phosphoribose residues (Scheme 3) [163]. The presence of cyclic groups (aromatic or heterocyclic) in the tin-containing molecules was found to be important for anticancer activity [164,165]. The structure-antitumor relationship of diorganotin(IV) derivatives are well documented in literature [166].
Scheme 3. Irreversible binding to the peripheral phosphate groups of phosphoribose residues by tin(IV) metal ion.

Numerous reports in literature are available for cytotoxicity of tin(IV) and organotin(IV) complexes. Nath et al.; synthesized di- and tri-organotin(IV) derivatives of the general formula $R_2Sn(L/HL')$ and $Ph_3Sn(HL/H_2L')$, where $R$ is n-Bu and Ph, and $L/HL$ is dianion/monoanion of D-penicillamine ($H_2L-1$) and L-carnosine ($H_2L-2$), and $HL'/H_2L'$ is dianion/monoanion of triglycine and described a comparative study of structure–activity relationship (Figure 18). All the diorganotin derivatives possessed a distorted trigonal bipyramidal structure in which the amino acid/peptides are tridentate coordinating through $N_{\text{amino}}, C(O)_{\text{carboxyl}}$ and $S_{\text{thio}}/N_{\text{peptide}}$. Some of the complexes exhibited good anti-inflammatory activity. The triorganotin(IV) derivatives exhibited significantly better activities than the diorganotin derivatives [167].

Figure 18. Structures of diorganotin(IV) complexes of (a) L-carnosine and (b) triglycine.
The *in vitro* antiproliferative activity of the organotin compounds \([n\text{-C}_4\text{H}_9\text{Sn(cys)}],\) \([\text{(C}_6\text{H}_5\text{Sn(cys)}],\) \([\text{(CH}_3\text{Sn(Hcys)}\cdot\text{H}_2\text{O)}],\) \([[(\text{CH}_3\text{Sn(Kcys)}\cdot\text{H}_2\text{O)}],\) \([[(\text{n-C}_4\text{H}_9\text{Sn(Kcys)}\cdot\text{H}_2\text{O})],\) and \([[(\text{C}_6\text{H}_5\text{Sn(Kcys)}\cdot\text{H}_2\text{O})] \) against various human cell lines were evaluated by Hadjiliadis et al.; [168]. The complexes exhibited high cell toxicity against Hela (Human cervix epithelial human carcinoma) and BGC (Human stomach).

Diorganotin(IV) complexes with dianionic dipeptides containing \(\alpha\)-aminoisobutyryl residue (Aib) and L-Leucine/L-Alanine (Figure 19) were prepared and structurally characterized by Manessi-Zoupa et al.; In the complexes the five coordinated metal has distorted trigonal bipyramidal geometry. A different network of intermolecular hydrogen bonds in the compounds resulted in a very dissimilar supramolecular feature. The complexes are active against Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*. The IC\(_{50}\) values expressed promising *in vitro* cytotoxic activity against a series of human cancer cell lines [169].

*Figure 19.* (a) Structure of \((\text{n-Bu})\text{Sn(Aib-Ala)}\cdot\text{MeOH}\) (b) partially labelled ORTEP schematic representation of the adamantoid network.
Present work

The design and synthesis of anticancer chemotherapeutics which prove to be efficacious for treating solid malignancies, and are target specific require innovative strategies for combining organic building blocks, which are usually biologically significant ligands possessing bioactive pharmacophore viz., amino acids, peptides, Schiff bases derived from heterocycles such as chromones etc. In this work, our interest stems in particular for metal-based drugs, therefore metal binding domain with potential donors is an essential criterion and in addition to this, chiral ligand scaffolds were explored for complexation to synthesize asymmetric single enantiomeric complexes employing de novo synthetic strategy as the enantiomers are therapeutically more beneficial than racemic mixtures. The metal ions used are essential 3d metal ions-Cu(II) and Zn(II), Sn(IV) and Zr(IV) in contrast to platinum drugs which exhibit severe systemic toxicity and other side effects. These complexes were then screened for their ability to carry out specific pharmacological function viz., anticancer activity for exploring their cancer chemotherapeutic potential. Since DNA is the primary cellular target for numerous antitumor drugs, therefore in vitro DNA binding profile of the complexes was carried out employing various important biophysical techniques.

Chirality plays a profound role in different pharmacological effects exhibited by enantiomeric drug molecules revealing a preferential binding of one conformation over another, which is termed as enantioselectivity. The introduction of chirality enhances the pharmacological behavior of the metal complexes by adopting specific conformation and target selective binding with DNA. Those complexes that best fit against the helical structure of DNA display the highest binding affinity for DNA. We have designed and
synthesized enantiomeric tin(IV) complexes of valine derived peptides. The complexes were thoroughly characterized by elemental analysis, IR, NMR, ESI-MS and UV spectroscopy. The comparative DNA binding studies of enantiomeric forms (R)/(S)-[C_{18}H_{30}N_{2}O_{6}SnCl_{2}] and (R)/(S)-[C_{20}H_{36}N_{2}O_{6}Sn] were carried out using various biophysical methods including UV–vis, fluorescence, CD and viscosity. Furthermore, specific mode of binding of the complexes to mononucleotides 5′-GMP and 5′-TMP using electronic absorption and NMR interaction was carried out. The cleavage studies of (R)-form of complexes were carried out using gel electrophoresis. In vitro anticancer activity of (R)-[C_{20}H_{36}N_{2}O_{6}Sn] and (S)-[C_{20}H_{36}N_{2}O_{6}Sn] in terms of GI_{50}, TGI and LC_{50} values were screened against seven different human carcinoma cell lines of different histological origin: A498 (Renal Cell), A549 (Lung), Zr-75-1 (Breast), HT29 (Colon adenocarcinoma grade II cell line), A2780 (Ovary), SiHa (Uterine Cervix) and MCF7 (Human breast).

New valine derived chiral complexes [C_{10}H_{22}N_{2}O_{5}SnCl_{2}] and [C_{10}H_{22}N_{2}O_{5}ZrCl_{2}] were synthesized in a 2:1 ligand to metal ratio. Structure elucidation was done by elemental analysis and spectroscopic (IR, NMR and ESI-MS) data. Interaction of complexes with DNA was carried out using different techniques viz., electronic absorption titrations, fluorescence and viscometric studies. The effect of ionic strength and phosphate group on the binding of the complexes to DNA was also carried out. pBR322 DNA cleavage activity of [C_{10}H_{22}N_{2}O_{5}SnCl_{2}] was also carried out. In vitro anticancer activity of [C_{10}H_{22}N_{2}O_{5}SnCl_{2}] in terms of GI_{50}, TGI and LC_{50} values were screened against seven different human carcinoma cell lines of different histological origin: A498 (Renal Cell),...
A549 (Lung), Zr-75-1 (Breast), HT29 (Colon adenocarcinoma grade II cell line), A2780 (Ovary), SiHa (Uterine Cervix) and MCF7 (Human breast).

The ligand framework plays significant role in metal-based pharmaceuticals via alteration in the biological properties by modifying reactivity or substitution inertness. Metal complexes of amino acids and peptides are gaining importance as they are basic structural units of proteins capable of specific recognition of DNA base sequence. However, peptides can behave as carrier molecules for the delivery of drugs to the target. A series of dipeptide-Cu(II)/Zn(II) complexes $[\text{C}_{20}\text{H}_{34}\text{N}_{4}\text{O}_{6}\text{Cu}]$, $[\text{C}_{20}\text{H}_{34}\text{N}_{4}\text{O}_{6}\text{Zn}]$, $[\text{C}_{16}\text{H}_{26}\text{N}_{4}\text{O}_{6}\text{Cu}]$ and $[\text{C}_{16}\text{H}_{26}\text{N}_{4}\text{O}_{6}\text{Zn}]$, and tetrapeptide-Cu(II)/Zn(II) complexes, $[\text{C}_{16}\text{H}_{26}\text{N}_{4}\text{O}_{6}\text{Cu}]$ and $[\text{C}_{16}\text{H}_{26}\text{N}_{4}\text{O}_{6}\text{Zn}]$ were synthesized and thoroughly characterized by elemental and spectroscopic (IR, NMR, ESI-MS, EPR and UV) techniques. The solution stability studies were carried out by UV–vis absorption titration over a broad range of pH to determine the stability of the complexes in solution. The DNA binding properties of the complexes were elucidated by various biophysical techniques. Furthermore, specific mode of binding of the complexes to mononucleotides 5′-GMP and 5′-TMP using electronic absorption was carried out. The DNA cleavage activity of the complexes $[\text{C}_{20}\text{H}_{34}\text{N}_{4}\text{O}_{6}\text{Cu}]$ and $[\text{C}_{20}\text{H}_{34}\text{N}_{4}\text{O}_{6}\text{Zn}]$ were ascertained by gel electrophoresis assay. In vitro anticancer activity of $[\text{C}_{20}\text{H}_{34}\text{N}_{4}\text{O}_{6}\text{Cu}]$ in terms of GI$_{50}$, TGI and LC$_{50}$ values were screened against seven different human carcinoma cell lines of different histological origin: A498 (Renal Cell), A549 (Lung), Zr-75-1 (Breast), HT29 (Colon adenocarcinoma grade II cell line), A2780 (Ovary), SiHa (Uterine Cervix) and MCF7 (Human breast).
The selective binding of chiral molecules is regio-directional and in pharmaceuticals, one chiral form is more dominant than another. The chiral discrimination of DNA has been crucial for the determination of the binding mode of the complexes with DNA. In an attempt to unravel details of conformational differences, enantiomeric pairs of Schiff base ligands derived from natural chromone derivative, 2-amino-3-formylchromone and (R)/(S)-2-amino-1-propanol and their Cu(II)/Zn(II) complexes were synthesized. The complexes were thoroughly characterized by elemental analysis, IR, $^1$H NMR, $^{13}$C NMR, ESI-mass spectra and molar conductance measurements. The DNA binding studies of the complexes with CT-DNA was carried out by employing different biophysical methods. The absorption studies of (R)-form of complexes with mononucleotides (5'-GMP and 5'-TMP) were performed to assess the base specific interaction of the metal ions. The cleavage activity of (R)-[C$_{13}$H$_{18}$N$_4$O$_{11}$Cu] and (R)-[C$_{13}$H$_{18}$N$_4$O$_{11}$Zn] with pBR322 plasmid DNA was examined by gel electrophoresis. The comparative molecular docking studies of both enantiomers of complexes with DNA was also carried out to further predict the exact binding site available at the molecular target. *In vitro* anticancer activity of (R)-[C$_{13}$H$_{18}$N$_4$O$_{11}$Cu] and (S)-[C$_{13}$H$_{18}$N$_4$O$_{11}$Cu] in terms of GI$_{50}$, TGI and LC$_{50}$ values were screened against seven different human carcinoma cell lines of different histological origin.