**Introduction**

Medicinal inorganic chemistry emerges from the interdisciplinary areas of bioinorganic chemistry (which is at the interface between medical biology and inorganic chemistry), organometallic chemistry which includes metal–based therapeutics derived from organic scaffold/ metal–containing diagnostic agents and supramolecular chemistry [1–5]. This field gained momentum after the serendipitous discovery of cisplatin (cis–diamminedichloroplatinum(II), \([\text{cis}–(\text{NH}_3)_2\text{PtCl}_2]\)) [6], which was known to prevent mitotic division and later developed and approved by FDA in 1979 as an antitumor drug for treating solid malignancies; metal–based drugs were traditionally undervalued by pharmaceutical manufacturers as they were dominated by organic chemistry. Many other coordination compounds attracted considerable attention which includes gadolinium complexes in magnetic resonance imaging (MRI) [7], for both therapy and diagnosis, titanocene dichloride [8] as a potent antitumor agent and ruthenium polypyridyl complexes –as bacteriostatic and an anticancer agents [9–11] (Figure 1).

The medicinal inorganic chemistry is not a new discipline as it can be traced back to 3000 BC when metal complexes were explored for their medicinal and pharmacological applications. Copper was used to sterilize water in Egypt and the Chinese and Arabs were using gold in number of medicines since antiquity in metal–based therapies. Mercurous chloride was used as a “diuretic” during renaissance period in Europe while Paul Ehrlich the so called “founder of chemotherapy” developed arsenical, Salvarsan, as a drug for the treatment of Syphilis in early twentieth century [12].
Coordination complexes offer a diverse arena and their applications in medicine encompass cancer chemotherapy [13–16], antimicrobial agents [17, 18], anti HIV drugs [19, 20], anti–hypertensive, etc [21].

![Coordination complexes](image)

Figure 1. (a) Cisplatin, (b) A dual–function \(\text{Gd}^{3+}\) –textaphyrin derivative for cancer imaging and therapy, (c) Dwyer Ru(II) complex

Though medicinal inorganic chemistry was strengthened by the discovery of anticancer properties of platinum ammine complexes namely cisplatin, (which was an important component in chemotherapeutic regimens for the treatment of ovarian, testicular, lung,
and bladder cancers); second generation platinum compounds *viz.* carboplatin, oxaliplatin, nedaplatin, and lobaplatin, yet there are well–known drawbacks associated with their use such as significant toxicity due to non–specific interactions (severe side effects and activity to act in a restricted spectrum of tumors) as well as inherent or acquired resistance; these effects limit their successful clinical use. Therefore, a plethora of alternative metal–based drugs with broader spectrum of activity and reduced toxic side effects were developed and some of these have entered pre–clinical testing and clinical trials [22].

Transition metals offer potential advantages over the common organic–based drugs, which include a wide range of coordination numbers and geometrics, accessible redox states, electron shuttling thermodynamic and kinetic stability [23]. Metal complexes are more appealing for design of new lead therapeutic drugs as metal plays an important role in the chaperoning and or delivery of drugs away from the sites where they exert most toxicity and toward their sites of action [24, 25]. Additionally, the formation of a metal complex will alter the solubility and lipophilicity of the drug, resulting in changes in pharmacokinetics, biodistribution and biotransformation [26]. However, platinum–based anticancer agents are non–specific resulting in significant toxicity; these effects limit their successful clinical use [27]. Therefore, alternative strategies based on non–platinum metals and ligand scaffold bearing functionalities are being developed with improved pharmacological properties and a broader range of antitumor activity, particularly, based on increased understanding of the biochemical differences between normal and cancerous tissues. It is now well established that the biological activity and mechanism of action of
metal complexes can be fine–tuned by an appropriate choice of metal, its oxidation state and the ligands [28, 29].

Metal ions exist as electron deficient cations (Lewis acid) 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 and N7 atom of the imidazole rings of guanine and N3 atom of adenine residues located in the major groove of the double helix [30], 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 have become resistant to cisplatin and its second generation derivatives [31].

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. 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 [32–36]. On the other hand, complexes that bind to DNA
reversibly have possible application as tools for probing the structure and function of DNA [37–39].

DNA, an inherently chiral molecule has a polymorphic structure with polyanionic nucleotide chains and sugar phosphate back bone [40]. 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 structure.

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 the 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 [41].

A large percentage of the chemotherapeutic anticancer drugs currently used fall into the category of DNA–binding drugs, which interact with DNA duplex by any of the three
general binding modes which have been thoroughly characterized from a structural point of view. DNA intercalators [42], groove binders [43], and covalent binders [44–47] (Figure 3), with some peculiarities within each general category, may be cytotoxic and thus therapeutic either by direct interaction with DNA or by inhibition of topoisomerase enzymes (preventing DNA relaxation) [48].

![Figure 3](image.jpg)

**Figure 3.** The three binding modes of metal complexes with DNA: (a) groove binding, (b) intercalation and (c) insertion.

Metal–based pharmaceuticals can be arranged into seven categories depending on the function of the metal and ligand moieties according to Hambley et al [49]: I) the metal complex is active in its inert form, II) the metal complex is active in its reactive form, III) the metal serves as a radiation enhancer, IV) the compound contains a radioactive metal, V) the metal or its biotransformation product is active, VI) ligand is biologically active, and VII) only a fragment of the complex is active. Investigations in this area were primarily focused on the use of biologically active complexes formed by essential metal ions, such as 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.

Copper is widely distributed in the biological system and it is the most familiar redox metal serving diverse biological functions [50–55]. Copper can be regarded as a “modern bio–element” as suggested by Kaim [56], Ochiai [57] and Williams [58]. It has been demonstrated that copper accumulates in tumors due to selective permeability of the cancer cell membranes [59–62]. Because of this, a number of copper complexes have been screened for anticancer activity and some of them were found active both in vitro and in vivo [63–66]. Copper(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 [67–70] as well as brain tumors [71, 72]. 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 [73]. The binding of copper ions to specific sites can modify the conformational structures of proteins, polynucleotides or DNA and bio–membranes [74]. 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 [75, 76]. 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 $d^9$ electronic configuration which promotes $d$–$d$ transitions resulting in intense colored species while Cu(I) complexes due to the closed–shell $d^{10}$ electronic configuration are usually colorless solids and strongly prefer ligands having soft donor atoms. Copper complexes are a class of the most frequently studied metallonucleases due to their biologically accessible redox states.

Zinc is second most abundant essential transition metal ion in human following Fe, 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 [77, 78]. Mechanistically, zinc has significant impact on DNA as a component of chromatin structure, DNA replication and transcription and DNA repair [79]. Zinc enzymes efficiently catalyse 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 zinc(II) on DNA suggest that this cation can bind to both the nucleobase and the phosphate group [80]. Zinc is vital for recovery of leukemic cells because Zn is required for proper functioning of genetics, immunity, formation of red blood cells, organ, muscle and bone function, cell membrane stability, cell growth, division, differentiation and genetics [81]. Zinc has beneficial interactions with several chemotherapy drugs. Zinc(II) complexes of p–isopropyl benzaldehyde and methyl 2–pyridyl ketone thiosemicarbazones show potent cytotoxic activity and induction of apoptosis in cells resistant to cisplatin [82].

A series of copper–based drugs registered with the name of Casiopeinas® (Cas) has been developed by Ruiz–Azuara et al [83]. These compounds are mixed chelate copper(II)
complexes with a general condensed formula \([Cu(N-N)(A-A)]\left[NO_3\right]\), 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.

![Structure of Casiopeina III–ia.](image)

**Figure 4. Structure of Casiopeina III–ia.**

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 [84]. Experiments in rats employing one of the most promising derivatives (Figure 4), showed a strong inhibition of cell proliferation against C6 glioma 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 [85].

The chemical, biological and pharmaceutical properties of tin(IV) and diorganotin(IV) complexes have been studied extensively [86, 87]. In the search for better chemotherapeutic drug alternatives, organotin(IV) dithiocarboxylates received utmost attention on account of their potential apoptotic inducing character [88]. Since 1980,
United states national cancer institute (NCI) has tested over one thousand tin compounds, and 170 of these were found to be active [89].

The binding ability of organotin compound towards DNA depends on the coordination number, nature of the groups bonded to the central tin atom [90]. The phosphate group of DNA sugar back bone usually acts as an anchoring site.

**Figure 5.** Irreversible binding to the peripheral phosphate groups of phosphoribose residues by Sn(IV).

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 (Figure 5) [91]. The presence of cyclic groups (aromatic or heterocyclic) in the tin–containing molecules was found to be important for anticancer activity [92, 93]. The structure–antitumor relationship of diorganotin(IV) derivatives has been well documented in literature [94].
Recently, a novel organotin complex 1–{(2–hydroxyethyl)amino}–2–amino–1,2–dideoxy–D–glucose triphenyltin(IV) (GATPT) (Figure 6), a sugar based apoptosis inducer was synthesized by our research group [95]. GATPT was tested for its cytotoxic properties against SY5Y, PC–12 and N2A neuronal tumor cell lines. GATPT induced significant apoptosis in the PC–12 cell line which was characterized by DNA fragmentation and chromosome condensation.

![Molecular structure of GATPT, (b) Molecular docked model with VHR, (c) Detailed hydrogen bonds between VHR and GATPT, (d) The LIGPLOT showing hydrogen bonds, and spiked residues form hydrophobic contacts with GATPT.](image)

Treatment of PC–12 cells with GATPT resulted in a dramatic up–regulation of Bax and Bak and down–regulation of the anti–apoptotic factor Bcl–2. Apoptotic induction by GATPT was shown to be mediated in a p53–dependent manner and loss of p53 impaired the release of cytochrome c from mitochondria to cytosol. Caspase–3 was found to be indispensable for the GATPT triggered apoptosis signaling pathway. Furthermore, in vivo studies using a nude mice model revealed that GATPT exhibits significant antiproliferative activity against tumor development with minimal cytotoxicity.
A novel class of heterobimetallic compound tri–phenyl tin benzimidazole thiol–copper chloride (TPT–CuCl₂) was reported by Hoti et al [96]. The effect of TPT–CuCl₂ on the regulation of apoptosis in HeLa cells, MCF–7 human cervical cancer cell in vitro and in vivo on Wistar rat model was evaluated which demonstrated significant anti proliferative activity against tumor development with minimal cytotoxicity toward normal physiological function of experimental rats. Treating confluent HeLa cells with the –TPT and chemotherapeutic drugs cisplatin alone, separately for different time intervals resulted in a significant differences in cell viability by MTT assay showing that –TPT is a stronger inducer of apoptosis than cisplatin. The TPT moiety can efficiently kill tumor cells at a very low dose (2 µg/mL) thus minimizing the toxic side effect in contrast to high dosage of cisplatin (16 µg/mL) [97]. Combining –TPT with –CuCl₂ yields more efficacious drug regime due to their preferential selectivity at the DNA helix (–TPT prefers vicinal phosphate oxygen atoms of phosphate sugar backbone, while –CuCl₂ component coordinates to N7 atom of guanine).

Understanding the concept of combining the metal centres with preferential selectivity towards DNA with a new critical feature of chirality was examined in trinuclear aminoacid derived complexes Cu–Sn₂/Cu–Zr₂ by our group [98] (Figure 7). Introduction of L–aminoacid additionally enhances / modulates the pharmacological behaviour of the metal complexes by adopting specific conformation and target selective binding affinity with DNA. The interaction with DNA was carried out in tris buffer by electronic absorption titration, luminiscence titration, cyclic voltammetry, circular dichroism and viscometric measurements. The emission quenching of these complexes by [Fe(CN)₆]⁴⁻ depressed greatly when bound to DNA. Observed changes in the circular dichoric spectra
of DNA in presence of Cu–Sn₂/Cu–Zr₂ supported the strong binding of complexes with DNA. The relative specific viscosity of DNA bound to Cu–Sn₂/Cu–Zr₂ decreased, indicating that the complexes bind to DNA via covalent binding. To evaluate the mechanistic pathway of DNA inhibition, counting experiments and MTT assay was employed to assess the induction of apoptosis by Cu–Sn₂.

![Figure 7. Chemical structures of heteronuclear Sn(IV)/Zr(IV) modulated Cu(II)/Zn(II) trinuclear complexes.](image)

Western blot analysis of whole cell lysates and mitochondrial fractions with Bcl–2 and p–53 family proteins and caspase–3 colorimetry assay was also carried out on a human neuroblastoma cell line SY5Y. Cu–Sn₂ complex exhibited selective antitumor activity and further studies pertaining to this complex are currently underway.

The design of heterobimetallic trinuclear architecture utilizes a unique building block strategy which involve combination of both the active metal centres exhibiting differential behaviour towards the cellular target site on DNA [99–104]. Such complexes have been prepared considering the intrinsic DNA–interaction characteristic of the two separate active metallic centres. For example, heterodinuclear bifunctional molecules containing copper and platinum active metal centres were designed as combination
agents, due to their preferential reactivity at site of interaction namely, the nitrogen atom N7 of guanine in the major groove for the paltinum component while the minor groove is preferred for copper unit \[105, 106\] (Figure 8). The minimum separating distance required to achieve such concomitant minor and major interaction was determined from crystal structure of DNA–cispaltin adduct \[107\].

**Figure 8.** Strategy adopted for the synthesis of heterodinuclear minor / major groove interacting complexes.

Heterobimetallic complexes exhibit cleavage activities and were able to perform direct double strand cuts in contrast to monometallic Cu(3–clip–Phen) alone which was capable of carrying out successive single strand cuts (Figure 9).

**Figure 9.** Comparison of the oxidative cleavage of ΦX174 plasmid DNA performed by \(C_{35}H_{37}Cl_4CuN_7O_2Pt\), \(C_{39}H_{45}Cl_4CuN_7O_2Pt\), and Cu(3–Clip–Phen) in the presence of 5 mM MPA. Lane 1: control DNA. Lane 2: 250 nM \(C_{35}H_{37}Cl_4CuN_7O_2Pt\) without MPA. Lane 3–5: 100–250 nM \(C_{35}H_{37}Cl_4CuN_7O_2Pt\). Lane 6: 250 nM \(C_{39}H_{45}Cl_4CuN_7O_2Pt\) without MPA. Lane 7–9: 100–250 nM \(C_{39}H_{45}Cl_4CuN_7O_2Pt\). Lane 10: 250 nM Cu(3–Clip–Phen) without MPA. Lane 11–13: 100–250 nM Cu(3–Clip–Phen).
The DNA binding of compounds such as BBR 3464 (Figure 10) was covalent, but a significant noncovalent component was observed by the presence of the central platinum (tetramine) unit which interacted with DNA only through electrostatic and hydrogen–bonding effects [108]. The highly charged compound induce B→A and B→Z conformational changes in canonical sequences of DNA.

![Figure 10. Structure of BBR 3464.](image)

Noncovalent concept was extended in this complex by use of dangling amines which increase the charge and charge dispersion along these linear cations, surprisingly resulted in significantly enhanced cellular accumulation. However, the complex with higher charge (8+) exhibited about 5 times greater cellular uptake than the parent BBR 3464 (4+) [109]. As a consequence of this accumulation, enhanced cytotoxicity was observed across a panel of ovarian tumor cell lines despite the reversible nature of DNA binding.

With the objective of lowering the systemic toxicity of metal–based drugs, successful modular synthesis of heterotrinuclear complexes has been carried out that combines the class of compounds with paltinum(II) centre, resembling cisplatin and two ruthenium(III) centres that resemble NAMI by bridging dinitrogen ligands [110]. Such design has been done to achieve both efficacy and selectivity of the drugs and studies pertaining to these complexes have demonstrated that these complexes are potent against both neoplastic tumors and metastatic cancer.
More recently, copper–based heteronuclear complexes were explored for displaying some intriguing nuclease properties [111]. The combination of a site–specific group and a scission moiety could enhance the regional selectivity and cleavage efficacy of such an artificial nuclease model. A number of natural nucleases require multinuclear metal centers to achieve a synergistic effect in the process of substrate recognition and scission [112]. Guo et al have demonstrated the synergistic DNA cleavage effect in multicopper(II) centres [113–115]. Two monometallic copper(II) complexes $C_{25}H_{24}Cl_2CuN_4O$, $C_{25}H_{24}Cl_2CuN_4O$ and heteronuclear complexes $C_{27}H_{30}Cl_4CuN_4O_2PtS$, $C_{27}H_{30}Cl_4CuN_4O_2PtS$. were synthesized using bifunctional ligands [116] (Figure 11).

![Chemical structures of complexes](image)

**Figure 11.** Chemical structures of complexes $C_{25}H_{24}Cl_2CuN_4O$ (1), $C_{25}H_{24}Cl_2CuN_4O$ (2), $C_{27}H_{30}Cl_4CuN_4O_2PtS$ (3), $C_{27}H_{30}Cl_4CuN_4O_2PtS$ (4).

The DNA binding ability of these complexes follows an order of $C_{25}H_{24}Cl_2CuN_4O < C_{25}H_{24}Cl_2CuN_4O < C_{27}H_{30}Cl_4CuN_4O_2PtS < C_{27}H_{30}Cl_4CuN_4O_2PtS$. as revealed by results of spectroscopic and agarose gel electrophoretic studies. The introduction of Pt(II) centre to the Cu(II) complexes induced a significant enhancement in DNA binding and cleaving ability, underlining the importance of heterobimetallic complexes in contrast to monometallic complexes in drug design regimes.
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. Ligands can modify the reactivity, lipophilicity, oral/systematic bioavailability of metal ions, stabilization of oxidation state, substitutional inertness depending on the requirements for chemotherapy [117, 118]. They ensure protection of tissues from toxic metal ion or in a contrasting strategy, enhance cellular uptake of pharmacological beneficial metal ion [119]. It has been well documented in literature that carrier amine ligands of cisplatin analogues appear to modulate the antitumor properties of this class of drug [120, 121]. The antitumor activity is usually lost or diminished if the primary or secondary amines on platinum are replaced by tertiary amines [122]. In addition, the carrier ligands may also affect bio–distribution and recognition of DNA adducts by repair enzymes, regulatory and DNA binding proteins.

Recently, Kellett and coworkers have synthesized two square–planar copper(II) complexes, [Cu(ph)(1,10–phen)].2H₂O (1) (ph = o–phthalate) and [Cu(ph)(2,2′–bipy)].2H₂O (2) (Figure 12) and investigated their in vitro DNA–binding profile, supercoiled plasmid pUC18 DNA cleavage in the presence of exogenous reductant, superoxide dismutase (SOD) and catalase (CAT), in addition to anticancer activity against human–derived breast (MCF–7), prostate (DU145), colon (HT29), and intrinsically cisplatin–resistant ovarian (SK–OV–3) cancer cells. Furthermore, the ability of complexes [Cu(ph)(1,10–phen)].2H₂O (ph = o–phthalate) and [Cu(ph)(2,2′–bipy)].2H₂O to induce cellular dsDNA breaks within SK–OV–3 cancer cells was
presented using immuno detection of γ–H2AX foci by confocal microscopy and flow cytometry [123].

1,10–phenanthroline (Phen) is well–known chelating bidentate ligand for transition metal ions which played an important role in many fields such as coordination chemistry. Metal complexes with phen based ligand have gained much importance in molecular recognition of DNA [124–126].

**Figure 12.** Molecular structures of \([\text{Cu}(\text{ph})(1,10–\text{phen})].2\text{H}_2\text{O}\) (1) (\(\text{ph} = \text{o–phthalate}\)) and \([\text{Cu}(\text{ph})(2,2’–\text{bipy})].2\text{H}_2\text{O}\) (2)

**Figure 13.** ROS generation and DNA toxicity by the synthetic artificial metallonuclease \([\text{Cu}(1,10–\text{phen})_2]^{2+}\)
The copper(II) salts and 1,10-Phenanthroline form stable bis-phen complexes \([\text{Cu(Phen)}_2]^{2+}\) which showed nuclease activity in presence of both exogenous reductant (red; e.g., L-ascorbic acid) and molecular oxygen (e.g., \(\text{O}_2\) or \(\text{H}_2\text{O}_2\)) and result from a cascade of redox reactions that ultimately lead to the formation of hydroxo and metal–oxo (iv) radical species, which initiate DNA toxicity (Figure 13) [127–130]. Zhuo et al have reported that \([\text{Cu(phen)}_2]\) induced G1-phase specific apoptosis in liver carcinoma Bel–7402 cell line [131]. More recently, Cai et al [132] demonstrated that apoptosis pathway in Bel–7402 cells treated with \([\text{Cu(phen)}_2]\) might be initiated by the excessive copper in cells transported by the lipophilic phen ligand.

Carbohydrates have a wide spread occurrence in nature and in dispensable compounds for living organisms [133]. They are involved in various processes, such as storage, transport and transmission of genetic information, enzymatic reaction and regulation of the metabolism [134]. Carbohydrates and their derivatives are qualified as potentially attractive molecules for drug designing because they either; (I) may be taken up selectively into tumors due to the high metabolic rate of tumor cells vs normal cells [135], (II) their nucleoside may be intracellularly converted to the corresponding nucleotides through phosphorylation by appropriate enzymes, (III) they may potentially be incorporated into tumors DNA, thereby enhancing the cytotoxicity of the compounds [136].

S. Yano et al described metal complexes for the first time of N–glycoside derived from various carbohydrates and their derivatives with different amines. Their approach was to attach a chelating ligand to a carbohydrate, which subsequently binds to the metal center. This tactic offers advantage by providing a number of stable and various spectroscopic
techniques and the results suggested that the stereo structures of these sugar complexes (Figure 14) could be modified by varying the polyamines and the metal ion used [137–142].

Application of carbohydrate–based metal complexes is an example of a targeted approach exploiting the biochemistry and metabolic functions of diverse sugar in living organisms for transport and accumulation.

Figure 14. Structure of Nickel (II) complexes containing N–glycoside derived from ethylenediamine and (a) D–glucosamine, (b) D–galactosamine

Advantages of carbohydrate over other ligands are biocompatibility, non–toxicity, enantiomeric purity, and water solubility. In recent years, several examples of carbohydrate compounds have been developed for diverse medicinal applications ranging from antibiotic, antiviral, fungicidal activity and anticancer compounds [143–146].

Figure 15. Structure of Nickel (II) complexes containing N–glycoside derived from ethylenediamine and (a) D–glucosamine, (b) D–galactosamine
The consequent enzymatic enhancements of tumor cells over many other cell types can be exploited by using glucose as a targeting vector for diagnostic or therapeutic compounds [147] (Figure 15).

Recently [148], a different approach to metal–carbohydrate complexes was chosen with the aim of forming stable and well defined complexes i.e., attaching a carbohydrate moiety via a linker to a specifically designed metal binding domain (Figure 16).

![structure of nickel(II) bis(sugar) complexes](image1)

**Figure 16. Structure of nickel(II) bis(sugar) complexes, (b) Peroxo–bridged dinuclear cobalt(III) complexes containing N–glycoside**

Heterocyclic ligands and their metal chelates display a wide range of biological activity, such as antitumor, antibacterial, antifungal, and antiviral agents [149]. The majority of pharmaceutical products that mimic natural products with biological activity are heterocycles. Therefore, researchers are on a continuous pursuit to design and produce better pharmaceuticals, by following natural models. Imidazoline, imidazole and benzimidazole rings are important biological building blocks and are present in many existing drugs. Most significant is the entry of two octahedral Ru$^{III}$ complexes into clinical trials for cancer treatment. The first trans–[Him]–[Ru$^{III}$Cl$_4$(DMSO)(Im)] (Im = imidazole; NAMI–A), is relatively nontoxic to primary cancer cells but exhibits
antimetastatic activity, whereas the second, trans–[HIn][Ru\textsuperscript{III}Cl\textsubscript{4}(Ind)\textsubscript{2}] (Ind = indazole), appears to be active against primary tumors (Figure 17) [150–152].

Figure 17. **Representative ruthenium complexes with monodentate ligands tested as anticancer drugs**

The condensation of primary amines with carbonyl compounds was first reported by Schiff and since then condensation products are referred to as Schiff bases [153]. Several Schiff base complexes were found to inhibit tumor growth [154]. Schiff base are considered as ‘privileged ligands’ as they bind with different metal ion in various oxidation states [155] and can be considered one of the fundamental goals in medicinal chemistry.

Natarajan et al has synthesized sulfur bridged binuclear copper(II) complex derived from Schiff–base ligand; 2–oxo–1,2–dihydroquinoline–3–carbaldehyde 4(N,N)–dimethyl thiosemicarbazone (HL) (Figure 18), and their interaction studies with calf thymus DNA (CT DNA) were studied by using absorption and emission spectral studies [156].
Drug delivery in oncology is of particular interest owing to the narrow therapeutic window of anti–neoplastic agents. In the past, numerous research efforts were focused on conjugating anticancer drugs with a diverse spectrum of low– and high–molecular–weight carriers which include sugars, growth factors, vitamins, peptides, antibodies, polysaccharides, lectins, serum proteins, and synthetic polymers. In most prodrug systems, the drug is bound to the carrier through a spacer that incorporates a pre–determined breaking point that allows the bound drug to be released at the cellular target site. Designing truly tumor–specific carriers remains a challenge in modern drug development. In this regard, confocal laser scanning microscopy (CLSM) is among the most widely used approaches for the high–resolution, noninvasive imaging of proteins or nucleic acids in living cells [157]. Recently, Linjuan Huang reported a one–pot strategy for the fluorescent labeling of saccharides with fluorescein–5–thiosemicarbazide (FTSC), which introduces the thiosemicarbazide group of FTSC to the aldehyde group at the reducing end of saccharides to form stable amino derivatives via reductive amination (Figure 19), and evaluated the adaptation of the products to living cells.
Figure 19. Labeling reaction of reducing saccharides with FTSC and its application to living cells.

The results of fluorescent labeling procedure have led to the development of a convenient and efficient method to prepare fluorescent polysaccharide tags and its utilization for imaging target molecules transported in living cells, consequently imaging by confocal microscopy the polysaccharide–FTSC derivatives transported into living HepG2 cells (Figure 20) [158].

Figure 20. Imaging of polysaccharide AAG–FTSC derivatives transported in living HepG2 cells by confocal microscopy.
K. K. –W. Lo et al have designed and synthesized three luminescent rhenium(I) polypyridine complexes appended with an α-D-Glucose \([\text{Re}(N^N)(CO)\text{py}3\text{–glu}])\](PF\(_6\)) (py–3–glu = 3–(N–(6–(N’–(4–(α–D glucopyranosyl)phenyl)thioureidyl)hex–yl)thioureidyl)pyridine, N^N = 1,10–phenanthroline (phen) (1), 3,4,7,8–tetramethyl–1,10–phenanthroline (Me\(_4\)–phen) (2), 4,7–diphenyl–1,10–phenanthroline (Ph\(_2\)–phen) (3)) and their glucose–free counterparts \([\text{Re}(N^N)(CO)\text{py}3\text{–Et}])\text{–}(CF\(_3\)SO\(_3\)) (py–3–Et=3–(ethylthioureidyl)pyridine, N^N = phen (1a), Me\(_4\)–phen (2a), Ph\(_2\)–phen (3a)) to understand the incorporation of a glucose unit towards over–expression of glucose transporters (Figure 21). Furthermore, intracellular localization of complex \([\text{Re}(N^N)(CO)\text{py}3\text{–glu}])\](PF\(_6\)) (py–3–glu = 3–(N–(6–(N’–(4–(α–D glucopyranosyl)phenyl)thioureidyl)hex–yl)thioureidyl)pyridine, N^N = 4,7–diphenyl–1,10–phenanthroline (Ph\(_2\)–phen) upon internalization by HeLa cells was analyzed by laser–scanning confocal microscopy. The complex was diffusely distributed in the cytoplasm with punctate staining (Figure 22) [159].

![Figure 21. Structures of the rhenium(I) polypyridine complexes](image-url)
Figure 22. Laser–scanning confocal microscopy image of HeLa cells upon incubation with complex \([\text{Re}(\text{N}^\text{N})(\text{CO})_3(\text{py–3–glu})](\text{PF}_6)\) (100 μM) in a glucose–free medium at 37 °C for 5 min.

The nucleus gave much weaker or no emission, indicative of negligible nuclear uptake. In addition to the perinuclear region, the rhenium(I) polypyridine glucose complex was concentrated in specific compartments of the cells, which appeared to be the mitochondria. The fluorescence staining pattern showed that the mitochondria of a typical HeLa cell have been co–stained by the fluorescent dye and the rhenium(I) complex (Figure 23).

Figure 23. Laser–scanning confocal microscopy images of a HeLa cell upon incubation successively with MitoTracker Deep Red FM (100 nm, 20 min, \(\lambda_{\text{ex}} = 633\) nm) and complex \([\text{Re}(\text{N}^\text{N})(\text{CO})_3(\text{py–3–glu})](\text{PF}_6)\) (100 μM, 5 min, \(\lambda_{\text{ex}} = 405\) nm) in a glucose–free medium at 37 °C.
The intracellular localization of the rhenium(I) polypyridine glucose complex was different to that of its glucose–free complex, which did not show granular appearance, but a diffused staining of the whole cytoplasm [160].

DNA and enzymes involved in replication and transcription represent the most targeted bioreceptors for small molecules and a target for the control of gene expression. Most anticancer drug binds to DNA and proteins either in a reversible or irreversible manner suggesting a direct relationship between their interactions with macromolecules, hence, leading to their therapeutic effect. Numerous reports in literature are available for the binding studies using various biophysical techniques.

H. –L. Huang et al [161] has carried out DNA binding studies of two ruthenium(II) complexes \([\text{Ru(bpy)}_2(\text{APIP})](\text{ClO}_4)_2\) and \([\text{Ru(bpy)}_2(\text{HAPIP})](\text{ClO}_4)_2\) where bpy = 2,2′-bipyridine, APIP = 2–(2–aminophenyl)imidazo[4,5–f][1,10]phenanthroline) and HAPIP = 2–(2–hydroxyl–5–aminophenyl)–imidazo[4,5–f][1,10]phenanthroline (Figure 24) by employing absorption spectroscopy, fluorescence spectroscopy, viscosity measurements, DNA melting experiments and gel mobility shift assays.

\[
\text{Figure 24. Structure of the ruthenium(II) complexes.}
\]

Monitoring the effect of adding increasing amounts of DNA on the absorption spectrum of a metal complex is one of the most widely used methods for determining overall
binding constants. In general, hypochromism and red shifts are associated with the
binding of the complex to the helix because of the interaction between the aromatic
chromophore of the complex and the base pairs of the DNA through intercalation. The
magnitude of the hypochromism and red shifts depend on the strength of the interaction
between the DNA and the complex [162]. For metallo–intercalators, DNA–binding is
associated with hypochromism and a red shift in the MLCT and ligand bands [163]. The
absorption spectra of [Ru(bpy)$_2$(APIP)](ClO$_4$)$_2$ and [Ru(bpy)$_2$(HAPIP)](ClO$_4$)$_2$ in the
absence and presence of CT DNA (at a constant concentration of complexes, [Ru] = 20
µM) (Figure 25). Upon increasing concentration of DNA, both complexes exhibit
hypochromism, along with a modest bathochromic shift. These spectral characteristics
may suggest a mode of binding that involves a stacking interaction between the aromatic
chromophore and the DNA base pairs.

![Figure 25](image)

**Figure 25.** Absorption spectra of (a) [Ru(bpy)$_2$(APIP)](ClO$_4$)$_2$ and (b) [Ru(bpy)$_2$(HAPIP)](ClO$_4$)$_2$ in the presence of an increasing amount of CT DNA. [Ru] = 20 µM. Inset: $(ε_a−ε_f)/(ε_b−ε_f)$ vs. [DNA], and the nonlinear fit curve.

The intrinsic binding constants “$K_b$” derived for [Ru(bpy)$_2$(APIP)](ClO$_4$)$_2$ and
[Ru(bpy)$_2$(HAPIP)](ClO$_4$)$_2$ complexes were 6.08 ($±$0.29) x 10$^4$ and 1.78 ($±$0.29) x 10$^5$
M$^{-1}$, respectively.
The luminescence titration experiments were carried out in Tris–HCl buffer at ambient temperature with a maximum appearing at 599 and 600 nm. Upon addition of CT DNA, the emission intensities of complexes \([\text{Ru(bpy)}_2(\text{APIP})](\text{ClO}_4)_2\) and \([\text{Ru(bpy)}_2(\text{HAPIP})](\text{ClO}_4)_2\) increase about 1.63 and 1.88 times larger than the original and saturate at a ratio of \([\text{DNA}] / [\text{Ru}] = 15.4\) and 14.6, respectively. This implies that both complexes exhibited strong interaction with DNA, since the hydrophobic environment inside the DNA helix reduces the accessibility of water molecules to the complex and the complex mobility is restricted at the binding site, leading to decrease of the vibrational modes of relaxation (Figure 26).

![Figure 26. Luminescence spectra of complexes \([\text{Ru(bpy)}_2(\text{APIP})](\text{ClO}_4)_2\) and \([\text{Ru(bpy)}_2(\text{HAPIP})](\text{ClO}_4)_2\) in Tris–HCl buffer upon addition of CT DNA. \([\text{Ru}] = 5 \mu\text{M}\).](image)

To further explore the interaction with DNA, the specific relative viscosities of the DNA were measured by adding increasing concentrations of complexes \([\text{Ru(bpy)}_2(\text{APIP})](\text{ClO}_4)_2\) and \([\text{Ru(bpy)}_2(\text{HAPIP})](\text{ClO}_4)_2\) and the known DNA intercalator ethidium bromide (EB) for a comparison purpose. The viscosity of CT–DNA increases upon successive addition of both complexes, close to the proven DNA intercalators EB and previously reported mononuclear complexes [164].
There has been considerable interest in DNA endonucleolytic cleavage reactions that are activated by metal complexes thus, DNA mobility shift assay were carried out to investigate the ability of complexes to interact with plasmid DNA. Change in the electrophoretic mobility of plasmid DNA on agarose gel is commonly taken as evidence for direct DNA–metal interactions. When a circular plasmid DNA is subjected to agarose gel electrophoresis, the fastest migration will be observed for supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower moving open circular form (Form II). If both strands cleaved, a linear form (Form III) will be generated that migrates in between. One of the illustrative examples of concentration dependent pBR322 DNA cleavage of novel binuclear copper(II) complex \([\text{Cu}_2 \text{L} (\mu–\text{SO}_4)](\text{PF}_6)_2\) (L = 3,5–bis (bis(pyridine–2–ylmethyl)ami–no)methyl)–4H–1,2,4–triazol–4–amine) was reported by Yan et al [165], in which the enhancement of DNA cleavage activity was observed due to variation of complex concentration, Form I plasmid DNA was gradually converted into Form II (lanes 2–5). When the concentration of complex1 added to 0.75 mM, the Form I completely disappeared and Form III remained up to 20% (Figure 27).

![Figure 27](image)

**Figure 27.** Gel electrophoresis diagrams showing the cleavage of pBR322 DNA (0.1μg/μL) at different complex concentrations in Tris–HCl/NaCl buffer (pH = 7.2) at 37 °C for 3 h. Lane 1: DNA control (3 h); lane 2–5: DNA + \([\text{Cu}_2 \text{L} (\mu–\text{SO}_4)](\text{PF}_6)_2\) (0.15, 0.35, 0.5, 0.75 mM), respectively.

In order to obtain information about the active oxygen species which was responsible for the DNA damage, the DNA cleavage in the presence of hydroxyl radical scavengers
(DMSO), singlet oxygen quenchers (L–histidine), superoxide scavenger (SOD), hydrogen peroxide scavenger (catalase) and chelating agent (EDTA) was performed. The results revealed that, no inhibitions was observed in the presence of DMSO (Figure 28, lane 3), L–histidine (lane 4) and catalase (Figure 28, lane 11), ruling out the possibility of DNA cleavage by hydroxyl radical, singlet oxygen and hydrogen peroxide. The SOD enzyme showed little influence on the DNA cleavage suggesting that the superoxide anion was hardly involved in the process. The EDTA, a Cu(II)–specific chelating agent, could efficiently inhibit DNA cleavage, indicating Cu(II) complexes play the key role in the cleavage. The addition of SYBR Green, which is known to interact to DNA at minor groove, effectively inhibited DNA cleavage, indicating that the complex [Cu₂L(μ–SO₄)](PF₆)₂ mainly interact through DNA small groove.

Figure 28. Agarose gel showing cleavage of pBR322 DNA (0.1μg/μl) incubated with [Cu₂L(μ–SO₄)](PF₆)₂ (0.75 mM) in Tris–HCl/NaCl buffer (pH = 7.2) at 37 °C for 3 h. Lane 1: DNA control; lane 2: DNA +[Cu₂L(μ–SO₄)](PF₆)₂; lane 3: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 20 mM DMSO; lane 4: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 20 mM L–histidine; lane 5: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 20 U/mL SOD; lane 6: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 5 mM EDTA; lane 7: DNA control; lane 8: DNA +[Cu₂L(μ–SO₄)](PF₆)₂; lane 9: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 1 mM methyl green; lane 10: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 10 U/mL catalase; lane 11: DNA +[Cu₂L(μ–SO₄)](PF₆)₂ + 20 U/mL SYBR Green.

Molecular modeling allows the rapid screening of a large library of compounds against a nucleic acid target, in order to identify candidate compounds with favorable binding interactions which can be further enhanced through structure–based modification and development. G. Bifulco et al [166] have designed and synthesized a series of
bisnaphthalimide derivatives as bisintercalating agents. In order to gain new detailed molecular insights in their DNA binding events, a molecular modeling study has been carried out for complexes 1–6 (Figure. 29). The analysis of docking results reveals that all ligands share a similar binding mode. The linker of two naphthalimides also plays a crucial role in the affinity to nucleic acids, contributing to the complex stability interacting with the minor groove of the DNA.

![3D models showing interactions of bisnaphthalimide derivatives (1–6) with DNA (a, b, c, d, e and f).](image)

The common planar moieties of the ligands intercalate between base pairs establishing π–π interactions with DNA aromatic rings, with nitro groups extending these intermolecular interactions or forming hydrogen bonds with nucleotides.

Most of the drugs exert their cytotoxic effect and thereby therapeutic effect by direct interaction with DNA or by inhibition of topoisomerases (preventing DNA relaxation). Thus, new light has been shed on the discovery of metal–based drugs that inhibit enzymatic activities or even target protein directly. There are many reviews which
highlight some exciting results published recently on the development of metal as enzyme inhibitors for potential therapeutic applications [167]. DNA topoisomerases are crucial nuclear enzymes that control the topological state of DNA during replication, transcription, recombination and chromosome segregation at mitosis. There are two types of DNA topoisomerases: type–I enzymes change the DNA linking number by transiently breaking one strand of duplex DNA, and type–II enzymes transiently break both strands [168]. Many studies have shown that topoisomerases targeting agents are considered as attractive target for design of cancer chemotherapeutic, because they can cause permanent DNA damage that triggers a series of cellular events, inducing apoptosis and finally causing cell death [169]. To determine whether the complexes interfere with the DNA relaxation reaction by inhibiting Topoisomerase inhibition catalysis or by altering the apparent topological state of DNA, the DNA strand passage assay was performed.

![Figure 30. An ORTEP drawing of (a) [Ru(bpy)$_2$(bfip)]$^{2+}$ and (b) [Ru(bpy)$_2$(bfip)]$^{2+}$](image)

Chao et al [170] have investigated the concentrations dependent Topo I and Topo II inhibitory activity of DNA–intercalating Ru(II) polypyridyl complexes derived from [Ru(bpy)$_2$(bfipH)]$^{2+}$ (1) and [Ru(phen)$_2$(bfipH)]$^{2+}$ (2) (bfipH = 2–(benzofuran 2yl)imidazo[4,5–f], bpy = bipyridyl and phen = 1,10 phenanthroline) (Figure 30) by gel electrophoresis. Both complexes inhibited the ability of Topo I to relax negatively
supercoiled plasmid DNA (IC$_{50}$ is $\sim$17 μM for complex [Ru(bpy)$_2$(bfipH)](ClO$_4$)$_2$ and $\sim$8 μM for complex [Ru(phen)$_2$(bfipH)](ClO$_4$)$_2$) (Figure 31).

**Figure 31.** Effects of different concentrations of complexes [Ru(bpy)$_2$(bfipH)](ClO$_4$)$_2$ and [Ru(phen)$_2$(bfipH)](ClO$_4$)$_2$ on the activity of DNA topoisomerase–I (Topo I) activity.

The effects of the complexes on enzyme–catalyzed DNA strand passage were assessed by comparing the rate of relaxation of negatively supercoiled plasmid in the absence of drug to the rate of supercoiling of relaxed plasmid in the presence of EB. The rate of Topo–I–catalyzed DNA supercoiling in the presence of the Ru(II) complexes was lower than the rate of EB, which was identical to the rate of Topo I–catalyzed DNA relaxation in the absence of drug (Figure 32). These findings suggested that Ru(bpy)$_2$(bfipH)](ClO$_4$)$_2$ and [Ru(phen)$_2$(bfipH)](ClO$_4$)$_2$ were catalytic inhibitors of Topo I.

**Figure 32.** The time dependence of Topo I DNA strand passage assays in the presence of ethidium bromide (EB) and complexes [Ru(bpy)$_2$(bfipH)](ClO$_4$)$_2$ and [Ru(phen)$_2$(bfipH)](ClO$_4$)$_2$. 
The results of concentration–dependent Topo II inhibition assay of Ru(bpy)$_2$(bfipH)$_2^{2+}$ and [Ru(phen)$_2$(bfipH)]$_2^{2+}$ which showed that both Ru(II) complexes inhibited the activity of Topo II at a low concentration (IC$_{50}$ < 20 μM) (Figure 33).

Figure 33. Effects of different concentrations of complexes [Ru(bpy)$_2$(bfipH)](ClO$_4$)$_2$ and [Ru(phen)$_2$(bfipH)](ClO$_4$)$_2$ on the activity of DNA topoisomerase IIa (Topo II).

Similar to that described above for Topo I, DNA strand passage assay was also used to distinguish the effects of Ru(II) complexes on Topo II function from theirs effects on DNA topology. The religation rate of the relaxed plasmid in the presence of Ru(II) complexes are lower than that of EB. These findings suggest that Ru(bpy)$_2$(bfipH)$_2^{2+}$ and [Ru(phen)$_2$(bfipH)]$_2^{2+}$ are a catalytic inhibitor (or poison) of human Topo II (Figure 34).

Figure 34. The time dependence of Topo II DNA strand passage assays in the presence of ethidium bromide (EB) and complexes Ru(bpy)$_2$(bfipH)$_2^{2+}$ and [Ru(phen)$_2$(bfipH)]$_2^{2+}$.

Merfort et al [171], have analysed the molecular docking of diterpenes and compared to the docking pose of camptothecin with topoisomerase I.
Figure 35. (a) The predicted binding mode of camptothecin with topoisomerase I (PDB: 1T8I). (b) Diterpene docked inside the topoisomerase I–DNA covalent complex. (c) Diterpene docked in the binding pocket. (d) Diterpenes preventing the building of the topoisomerase I–DNA complex.

In contrast to camptothecin, the non–planar conformation of the diterpenes makes intercalation difficult within the cleaved site of the DNA, as observed with camptothecin (Figure 35a and b). Subsequently, alternative docking modes were evaluated using the DNA–unbound form of Topo–I. These results suggest a direct interaction of the diterpenes with topoisomerase I in a new described binding mode (binding pocket: Gly 490, Asn, 491, Thr 501, Lys 532, Figure 35c).

Youngjoo Kwon et al have performed the molecular docking studies of 1–Hydroxy–3–(2–hydroxy–3–(propylamino)propoxy)–9H–xanthen–9–one with ATP–binding domain of human topo IIα to examine their mode of action. An analysis of the docked pose to the
ATP–binding domain showed that the compound fits into the ATP–binding site (Figure. 36a–c) [172].

![Molecular docking](image)

**Figure 36.** Molecular docking between 1–Hydroxy–3–(2–hydroxy–3–(propylamino)propoxy)–9H–xanthen–9–one and ATP–binding domain of human topo IIa. (a) The ATP–binding domain topo IIa is shown as a ribbon diagram. (b) The residues of the ATP–binding site of topo IIa showing van der Waals contacts. (c) The ATP–binding domain is represented as a molecular surface (gray).

The position of the xanthone ring overlaps well with that of the purine ring of ATP. The xanthone ring has hydrophobic interaction with Asn91, Asn95, Asn120, Phe142, Thr215, and Ile217. The NH group in the side chain of the xanthone ring forms a hydrogen bond with Ser148. In addition, the hydroxyl group from the side chain forms hydrogen bonds with Ser149. The residues involved in the hydrophobic interactions, such as Ile141, Phe142 and Ala167, were observed. However, the Ser149 and Asn150 involved in the hydrogen bond interaction were also conserved. The molecular docking study showed that 1–Hydroxy–3–(2–hydroxy–3–(propylamino)propoxy)–9H–xanthen–9–one formed a stable binding complex with the ATP–binding domain in topo IIa.
**Present work**

The development of metal–based chemotherapeutic drugs has gained much emphasis owing to their superior binding ability and specific recognition to the molecular target DNA and enzymes. Metal–based therapeutic agents are “chemical entities” which involve inorganic architecture utilizing a combination of two or more metal ions, organic scaffold or ligand and sometimes an additional targeting moiety bound to the ligand viz. glycosylated appendage for better uptake and specific targeting at the molecular level. The ligands can fine tune or modulate the physico–chemical properties of the metal ions; I) by increasing the uptake of core metal ions, II) alter biodistribution among body tissues, III) target enzymes, biomolecules, or tissues and IV) predispose towards reduction of the core metal ions, (inert to active). The ligand framework is therefore a decisive factor, to control potency or efficacy of chemical entities particularly for cytotoxic agents.

Heterodinuclear metal complexes have advantages over monometallic complexes, they can be explored as design of DNA/RNA specific cleavage agents, and in chemotherapeutic specific targeted drug design. The binding of the heterobimetallic complexes with DNA increases manifold as compared to monometallic complexes. The discriminating synergistic power of essential transition Cu(II), Ni(II), Zn(II) and non–transition metal ions, Sn(IV) has been utilized in the synthetic strategy for the construction of heterobimetallic complexes to explore their chemotherapeutic potential to act as drug candidates. Previous literature reports reveal that copper has a tendency to bind covalently to N7 of guanine nucleobase of DNA double helix while Sn(IV) prefers to bind to negatively charged oxygen of phosphate backbone of DNA helix *via*
electrostatic binding mode. In this context, new metal–based entities \([C_{28}H_{28}N_8CuSn_2Cl_6]\) and \([C_{28}H_{28}N_8NiSn_2Cl_6]\) bearing bioactive pharmacophore 1,10–phenanthroline and ethylenediamine as a metal coordinating domain have been developed as antitumor agents that show promise to overcome inherent resistance and exhibit fewer side effects. These chemical entities bearing Cu(II)–Sn(IV) heterobimetallic core were thoroughly characterized by spectroscopic (IR, UV–vis, NMR, ESI–MS) and analytical methods. The interesting chemistry which these complexes display towards DNA disposition is due to the presence of two different metal ions, Copper an essential biometal has a predisposed tendency to bind covalently to N7 of guanine DNA base while tin atoms– a strongly apoptotic director prefers oxygen of phosphate diester linkage of DNA phosphate backbone. Tin complexes within a narrow concentration range have exhibited remarkable ‘therapeutic effects’ and are strongly cytotoxic agents. The \textit{in vitro} DNA binding studies of \([C_{28}H_{28}N_8CuSn_2Cl_6]\) and \([C_{28}H_{28}N_8NiSn_2Cl_6]\) with CT–DNA were carried out by employing various biophysical methods which reveal strong electrostatic binding via phosphate backbone of DNA helix, in addition to partial intercalation in the minor groove and stabilized by intramolecular hydrogen bonding. To gain further insight into the molecular recognition at the target site, UV–vis titrations of \([C_{28}H_{28}N_8CuSn_2Cl_6]\) with 5′–GMP was carried out and validated by \(^1\text{H}\) and \(^{31}\text{P}\) NMR. Complex \([C_{28}H_{28}N_8CuSn_2Cl_6]\) cleaved pBR322 DNA via oxidative pathway and exhibited high inhibition activity against Topo–I at 20 μM. Furthermore, the cytotoxicity of \([C_{28}H_{28}N_8CuSn_2Cl_6]\) was examined on a panel of human tumor cell lines of different histological origins showing promising antitumor activity.
In an another attempt, carbohydrate–conjugate heterobimetallic complexes \([C_{22}H_{46}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{54}N_6O_{17}NiSnCl_2]\) derived from N–glycoside ligand \([C_{14}H_{28}N_2O_{10}]\) (L) were synthesized and characterized by various spectroscopic and analytical techniques. To validate their potential to act as chemotherapeutics, in vitro DNA binding studies of the \([C_{14}H_{28}N_2O_{10}], [C_{22}H_{46}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{54}N_6O_{17}NiSnCl_2]\) were studied by various biophysical and molecular docking techniques. Complex \([C_{22}H_{46}N_6O_{13}CuSnCl_2]\) cleaves pBR322 DNA via hydrolytic pathway which was authenticated by T4 DNA ligase assay. The complex \([C_{22}H_{46}N_6O_{13}CuSnCl_2]\) exhibited remarkably good anti–tumor activity on a panel of human cancer cell lines (GI\(_{50}\) values < 10 μg/ml), and to elucidate the mechanism of tumor inhibition, topoisomerase I enzymatic activity was carried out. Finally, confocal microscopy using HeLa cells revealed intracellular localization of the complex \([C_{22}H_{46}N_6O_{13}CuSnCl_2]\) which supports our claim that these complexes can be used as photo probed for nucleic acid structures. Subsequently, a new modulated series of carbohydrate–conjugate monometallic complexes \([C_{22}H_{52}N_6O_{13}Cu], [C_{22}H_{60}N_6O_{17}Ni]\) and heterobimetallic complexes \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\) were designed and synthesized from previously synthesized N–glycoside ligand. The proposed structure of the complexes was formulated on the basis of elemental analysis, and other spectroscopic data including \(^1H\), \(^{13}C\) and \(^{119}Sn\) NMR (in case of \([C_{22}H_{60}N_6O_{17}Ni]\) and \([C_{22}H_{58}N_6O_{17}NiSnCl_2]\)). In vitro DNA binding studies were carried out employing absorbance and fluorescence spectroscopy to examine their DNA binding propensity as quantified by \(K_b\) and \(K_{sv}\) values. Cleavage studies were carried out by gel electrophoresis which demonstrated that the complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) are specific groove binder.
and cleave supercoiled pBR322 DNA via hydrolytic pathway, which was further confirmed by T4 DNA ligase assay. Complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) inhibited Topo–II mediated relaxation activity in a dose–dependent manner that appeared in accordance with remarkably good anti–tumor activity against MIAPACA2, A498 and HCT15 tumor cell lines. Finally, complex \([C_{22}H_{50}N_6O_{13}CuSnCl_2]\) was docked into the ATPase domain of human–Topo–II in order to probe the possible mechanism of inhibition. An important finding of this work is that these metal–based chemical entities bearing glycosylated appendage, show good promise as antiproliferative agents, artificial nuclease–hydrolytic cleaving agents and Topo II inhibitors.

New heterobimetallic complexes \([C_{16}H_{16}N_3O_2SCuSnCl]\) and \([C_{16}H_{16}N_3O_2SZnSnCl]\) derived from Schiff base ligand of salicylaldehyde and 5–amino–2–mercaptobenzimidazole were synthesized and thoroughly characterized by spectroscopic (IR, UV, NMR and ESI–MS) and analytical techniques. The \textit{in vitro} DNA binding studies were carried out by employing different biophysical and optical methods which reveal strong electrostatic binding \textit{via} phosphate backbone of DNA helix, in addition to other binding modes \textit{viz.} coordinate covalent and partial intercalation. Cleavage studies employing gel electrophoresis demonstrate both the complexes are efficient cleaving agents and are specific groove binders; binding events were further validated by molecular docking studies. \([C_{16}H_{16}N_3O_2SCuSnCl]\) complex cleaved pBR322 DNA \textit{via} oxidative mechanism while \([C_{16}H_{16}N_3O_2SZnSnCl]\) followed hydrolytic pathway. Furthermore, the cytotoxicity of \([C_{16}H_{16}N_3O_2SCuSnCl]\) was examined on a panel of human tumor cell lines of different histological origins showing promising antitumor activity.