INTERACTION STUDIES OF METAL-BASED POTENTIAL CANCER CHEMOTHERAPEUTIC AGENTS WITH BIOMOLECULES

ABSTRACT

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Abstract

Medicinal inorganic chemistry is an interdisciplinary area of research, which involves interface areas in chemistry, biology and medicine. The clinical success of cisplatin and other second generation platinum–based anticancer agents is severely affected by the toxic side effects manifested during administration, intrinsic resistance and narrow spectrum of activity for different phenotypes of cancer. This has spurred research in the development of non–platinum metal–based anticancer agents which are more efficacious, less toxic and can overcome resistance. Much of the attention has been laid towards the design of transition metal–based cancer chemotherapeutics, predominantly Cu(II), Co(II) complexes (endogenously biocompatible metal ion which are present as integral part of the active site of metalloprotein and thus familiarize their coordination with human body’s function) with active ligand pharmacophore. In particular, these metal complexes could efficiently bind and cleave DNA, thereby exert artificial nuclease activity. The specific delivery of a drug to their target cells could be achieved by the use of targeting groups or by tuning the chemical and physical characteristics of the drug or drug carrier, such as hydrophobicity and molecular size. Therefore, alternate strategies based on different metal ions, shape or geometric requirement and ligand functionality were opted to design these new cancer drug chemotherapeutic agents.

Different types of macromolecules viz., liposomes, dendrimers, polymers, nanoparticles, and protein biomolecules have been used as carrier molecules. In particular, HSA is the most versatile multifunctional transport protein which plays an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood. HSA is known to accumulate in tumors (uptake of HSA by tumor cells is more than normal cells) and thus has been considered as the carrier conjugate of various organic anticancer drugs such as doxorubicin and paclitaxel etc. The interactions of drugs with protein result in the formation of a stable drug–protein complex, which can exert an important effect on the distribution, free concentration and metabolism of the drug in the blood stream. Drug distribution is mainly controlled by HSA, because most drugs circulate in plasma and reach the target tissues by binding to HSA. Therefore, drug binding to proteins such as HSA has become an important determinant of pharmacokinetics, restricting the unbound concentration and affecting distribution and elimination.
The thesis embodies the six chapters which include Chapter I "Introduction" giving a detailed overview of literature relevant to this field and also helps to justify the objectives of the work done. Chapter II "Experimental" which describes detailed methodology and physical measurements employed in this work. Chapter III–VI embodies the work done i.e. molecular design, synthesis of novel metal–based chemotherapeutic agents derived from well–designed ligand motifs viz., L–phenylalanine, L–tryptophan, dipeptides (glycylglycine) and metal binding domains 1,2–diaminocyclohexane, o–phenylenediamine and piperazine with metal ions viz., Co(II), Cu(II), Ni(II), Sn(IV) and Zr(IV) ion, and exploring their in vitro binding propensity towards the specific target DNA and HSA at the molecular level. These studies reveal that these complexes can act as potential antitumor chemotherapeutic agents, and also show promise to overcome inherent drug resistance and may exhibit fewer side effects.

Amino acids are a source of chiral stereogenic carbon center, which enhance the pharmacological behavior of the metal complex by adopting specific conformation and target selective binding affinity and therefore provide conformational complementarity to the DNA molecular target, as DNA itself exists in nature only in one chiral form. The essential amino acids that include L–phenylalanine and L–tryptophan, both contain phenyl and indole rings and are classified as nonpolar. They are necessary for many bio–essential processes and several spectroscopic studies of ternary complexes of transition metals with these amino acids and peptides have been carried out. In solution, the amino acids exist in zwitterionic form ("\(\text{NH}_3^–\text{CH}(\text{R})–\text{COO}^–\)" which can be stabilized by counter ion or water molecules. The interactions of ternary amino acid complexes are great importance for understanding the pathogenesis of various diseases and designing new chemotherapeutic agents. Literature reports reveal that many Cu(II) complexes of amino acids with diamine ligands exhibit good antitumor and remarkable cleavage activities. In the context of above rationale, Chapter III deals with the synthesis of the new transition metal–based \{Cu(II), Ni(II) and Co(II)\} molecular entities \([\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CuCl}_2]\), \([\text{C}_{13}\text{H}_{31}\text{N}_2\text{O}_2\text{NiCl}_2]\) and \([\text{C}_{15}\text{H}_{29}\text{N}_3\text{O}_4\text{CoCl}_2]\) derived from amino acid auxiliary ligand viz., L–phenylalanine and 1,2–diaminocyclohexane. These complexes were thoroughly characterized by using elemental analysis, IR, UV–vis, NMR, EPR and XRPD spectral studies. The crystalline nature of the complexes was authenticated by
X-ray powder diffraction (XRPD) measurements. The in vitro DNA binding studies of complexes [C_{15}H_{29}N_{3}O_{4}CuCl_{2}], [C_{15}H_{31}N_{3}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{3}O_{4}CoCl_{2}] with CT DNA were carried out by employing different methods viz., electronic absorption titrations, fluorescence, circular dichroism and thermal denaturation studies. Upon addition of CT DNA, complexes exhibited an increase in molar absorptivity (Δε), (hyperchromism; 45–54%), without any significant shifts in band position suggesting that complexes bind to CT DNA via electrostatic interaction with the exterior phosphates of DNA duplex. The intrinsic binding constant \( K_b \) values obtained for complexes were found to be \( 5.30 \times 10^4 \), \( 3.41 \times 10^4 \) and \( 2.74 \times 10^4 \) M\(^{-1} \), respectively revealing higher binding propensity of complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] as compared to [C_{15}H_{31}N_{3}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{3}O_{4}CoCl_{2}]. To gain further insight into the molecular recognition at the target site, interaction of complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] with 5'-GMP was carried out by UV–vis titration which showed hyperchromic effect in the absorption bands attributed to the specific recognition of complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] for guanine nucleobase. The cleavage activity of complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] with plasmid pBR322 DNA was carried out by electrophoretic mobility assay, which exhibited remarkable ability to affect DNA scission by an oxidative mechanism involving the generation of ROS. The cleavage patterns in presence of recognition elements demonstrated that complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] binds in the minor groove of DNA duplex. Molecular docking studies were done for all complexes [C_{15}H_{29}N_{3}O_{4}CuCl_{2}], [C_{15}H_{31}N_{3}O_{5}NiCl_{2}] and [C_{15}H_{29}N_{3}O_{4}CoCl_{2}] which further validate our experimental results those complexes binds in the DNA minor groove. Furthermore, topoisomerase-mediated DNA relaxation assay results showed that complex [C_{15}H_{29}N_{3}O_{4}CuCl_{2}] could remarkably inhibit the activity of Topo–I at a very low concentration ~20 µM.

Chapter IV describes the interaction studies of novel antitumor chemotherapeutic agents [C_{23}H_{31}N_{6}O_{6}CuSn_{2}Cl_{5}] and [C_{26}H_{32}N_{2}O_{5}Sn] (GATPT) {previously synthesized by our research group} with HSA by employing different spectroscopic (fluorescence, UV–vis, CD, FTIR, 3D fluorescence). Fluorescence quenching results indicated the binding of both complexes [C_{23}H_{31}N_{6}O_{6}CuSn_{2}Cl_{5}] and [C_{26}H_{32}N_{2}O_{5}Sn] with HSA in the vicinity of tryptophan residue in IIA subdomain following the static quenching mechanism. Binding constants (\( K_b \)) and the number of binding sites (n≈1) were calculated using modified Stern–Volmer equations. The 3D fluorescence
spectral studies also revealed strong fluorescence quenching of HSA in presence of complexes \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) attributed to micro-environmental and conformational changes near the tryptophan residues of HSA. However, for complex \([C_{26}H_{32}N_2O_5Sn]\) thermodynamic parameters \(\Delta G\) at different temperatures were calculated and subsequently the value of \(\Delta H\) and \(\Delta S\) was also calculated which revealed that the hydrophobic and hydrogen bonding interactions play a major role in HSA–complex \([C_{26}H_{32}N_2O_5Sn]\) association. The distance \(r\) between donor (HSA) and acceptor (complex) was obtained to be 3.58 nm according to fluorescence resonance energy transfer and the alterations of HSA secondary structure induced by complex \([C_{26}H_{32}N_2O_5Sn]\) were confirmed by FT–IR and CD measurements. Our results are well supported by molecular modeling experiments which further validated the binding of both complexes \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) in subdomain IIA of HSA. These studies provide valuable information to understand the mechanistic pathway of drug delivery and pharmacological behavior of these chemotherapeutic drug entities.

Chapter V describes the new tin(IV) and zirconium(IV) complexes \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\), respectively derived from the dipeptide (glygly) and o–phenylenediamine ligand scaffold. The structure elucidation of \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\) was done by analytical techniques and spectroscopic methods viz., IR, UV–vis, \(^1\)H, \(^{13}\)C, \(^{119}\)Sn NMR, ESI–Mass techniques. The in vitro DNA binding studies of these complexes \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\) were carried out by using various biophysical and spectroscopic techniques. On the addition of increasing amount of CT DNA to complexes \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\), there was a sharp increase in absorbance (hyperchromism; 44–57%) with a significant blue shift of ~ 3–5 nm in the absorption band attributed to the electrostatic binding mode. The intrinsic binding constant, \(K_b\) of complexes \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\) were found to be \(5.17 \times 10^4\) and \(2.64 \times 10^4\) M\(^{-1}\). The \(K_b\) values revealed that complex \([C_{10}H_{22}N_4O_7SnCl_2]\) exhibited greater propensity towards CT DNA than complex \([C_{10}H_{18}N_4O_5ZrCl_2]\). The cleavage efficiency of \([C_{10}H_{22}N_4O_7SnCl_2]\) and \([C_{10}H_{18}N_4O_5ZrCl_2]\) were demonstrated with pBR322 plasmid DNA gel electrophoretic method and it was observed that both the complexes undergo prominent double–strand DNA cleavage in a concentration dependent manner by the hydrolytic pathway. These studies were validated by...
computational docking technique with biomolecule DNA to examine its mode of binding. The studies supported electrostatic interaction, selectively binding in the minor groove of DNA double helix.

In Chapter VI, new dinuclear transition metal–based molecular entities \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Cu}_{2}\text{Cl}_4}\), \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Co}_{2}\text{Cl}_4}\) and \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{6}\text{Ni}_{2}\text{Cl}_4}\) derived from amino acid auxiliary ligand L–phenylalanine and bridged by piperazine were designed and synthesized. The structure of the complexes was proposed on the basis of elemental analysis, IR, UV–vis, NMR and EPR spectral studies which revealed pentacoordinate environment of the central metal ion. The complexes exhibit novelty in structure and fulfill all the pre–requirements for chemotherapeutic drug design (i) a well defined complex shape and metal binding domain, (ii) cleaving DNA oxidatively in a fashion reminiscent of the bleomycin group which is clinically employed antitumor agent, (iii) increased bio–activity due to multifaceted binding modes (hydrogen bonding interaction, groove binding and electrostatic interaction). In vitro DNA binding studies of complexes \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Cu}_{2}\text{Cl}_4}\) and \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Co}_{2}\text{Cl}_4}\) were carried out by employing various biophysical techniques viz., electronic absorption and fluorescence spectroscopy and CD spectral studies. Upon addition of incremental amount of CT DNA \((0.00–0.23 \times 10^{-4} \text{ M})\), to a fixed concentration of complexes \((1 \times 10^{-4} \text{ M})\), an increase in absorption intensity 'hyperchromism' of 40–53% at the intraligand absorption bands without significant shifts in band position was observed. The results revealed that complexes interact with DNA through electrostatic binding mode via phosphate backbone of DNA helix, additionally augmented by covalent bonding to the N7 atom of guanine nucleobase. The intrinsic binding constant, \(K_b\) values for complexes \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Cu}_{2}\text{Cl}_4}\) and \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Co}_{2}\text{Cl}_4}\) were found to be \(6.2 \times 10^4\) and \(3.5 \times 10^4 \text{ M}^{-1}\), respectively. The spectroscopic binding titrations showed that complex \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Cu}_{2}\text{Cl}_4}\) exhibited higher binding propensity as compared to \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Co}_{2}\text{Cl}_4}\). To determine the specific interaction of both complexes towards DNA nucleobases interaction studies with guanosine mononucleotide 5'–GMP was also performed by employing UV–vis titration method. The results showed that both complexes \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Cu}_{2}\text{Cl}_4}\) and \([\text{C}_{22}\text{H}_{32}\text{N}_{4}\text{O}_{4}\text{Co}_{2}\text{Cl}_4}\) interact electrostatically with phosphate oxygen atoms in addition to the preferential coordinate covalent linkage to guanine nucleobase. Furthermore, the computational docking technique was carried out to correlate and
rationalize the observed binding affinities with the molecular target DNA and these studies validate the non-covalent electrostatic interaction with the minor groove of DNA in G/C rich sequences. Therefore, it is concluded that drugs that bind to the DNA minor groove represent an important class of anticancer drugs.