Synopsis

Metal-nucleic acid interaction studies have been gaining attention due to their biological and chemical importance. Nucleic acids are negatively charged bio-polymers and neutralization of their negative charge is essential for the stability and function. In the cells, organic positive ions (positively charged amino acids and polyamines) and some of the metal ions (e.g. Na⁺, K⁺, Mg²⁺...etc) neutralize the charge of nucleic acids. Whereas, interactions of some metal ions (e.g. Cd²⁺, Hg²⁺...etc) with nucleic acids destabilize the structure. The stability and conformation of nucleic acids alter due to metal interactions. Further, metal interactions with nucleic acids can bring changes in conformation of ribose, H-bonding and π-π stacking interactions. To understand the metal interactions with nucleic acids, various spectroscopic techniques are being used. However, X-ray crystallographic technique is advantageous over all other spectroscopic techniques since it gives thorough detail of coordination mode and structure. However, crystallization of large molecules like nucleic acids with metals is associated with great difficulty. In order to simplify the problem, nucleic acid constituents and derivatives have been used as model systems for metal-nucleic acid interactions. Nucleic acid constituents and derivatives are multidentate ligands. Moreover, binding mode of metal with nucleic acid constituents and derivatives depends on various factors include pH, temperature, type of metal...etc. Further, understanding of metal nucleic acid interactions can aid to develop new anticancer drugs targeting nucleic acids. For example, cisplatin is a platinum based anticancer drug, which coordinates to N(7) of guanine in DNA brings cell death. There have been several reports in literature on the complexes of metal nucleic acid constituents. However, much more research is warranted for thorough understanding of metal-nucleic acid interactions.

On the other hand, nucleic acid constituents and derivatives are used extensively in anticancer drug development. Some of nucleic acid constituent derivatives, 5-Fluorouracil and 6-Mercaptopurine, are currently in use for the treatment of colorectal cancer and leukemia, respectively. Moreover, cisplatin is a platinum based anticancer drug used in the treatment of various types of cancers. However, use of these drugs for long time poses severe side effects and drug resistance. Most of the side effects are due to non bio-compatibility of drugs. To overcome problems associated with present anticancer drugs, bio-compatible metal based anticancer drug development could be an attractive and alternative strategy.

To address this, in this study, we report synthesis of a number of new metal complexes of nucleic acid constituents and their derivatives and characterization by various spectroscopic techniques. Also, the interactions of Ni, Cu and Zn ions with various nucleic acid constituents and their derivatives have been elucidated by single crystal X-ray crystallography. Interestingly, Ni, Cu and Zn ions showed various coordination modes to nucleic acid constituents and their derivatives. Further, anticancer studies were carried out for all these complexes in various cancer cell lines. Several complexes showed better cytotoxicity than the well-known drug cisplatin. My thesis work is divided into five parts based on the nature of molecules.
I. Synthesis, X-ray crystallographic and anticancer studies on metal (Zn/Ni) complexes of guanine (G) based nucleic acid constituents

In order to understand (Zn/Ni) interactions with guanine based nucleic acid constituents and their anticancer activity, several (Zn/Ni) complexes of 5′-GMP, 5′-IMP and hypoxanthine complexes were prepared. The synthesized complexes are (1) \([\text{Zn (5′-GMP)}]_{n}.11\text{H}_2\text{O}\), (2) \([\text{Ni (5′-GMP)}]_{2}\text{Na}_2(\mu-\text{OH})_3(\text{H}_2\text{O})_8.2\text{H}_2\text{O}\), (3) \([\text{Ni (5′-IMP)}]_{2}\text{Na}_2(\text{H}_2\text{O})_{12}\) and (4) \([\text{Ni (hx)}]_{2}\text{Cl}_2\) [Here 5′-GMP = 5′-Guanosine Mono Phosphate, 5′-IMP = 5′-Inosine Mono Phosphate and hx = Hypoxanthine]. These complexes were characterized by various spectroscopic and X-ray crystallography techniques. Complex 1: The X-ray structure revealed that zinc is coordinated to 5′-GMP through N(7) position of purine and phosphate moieties, the uncoordinated water molecules are making interesting complicated network of hydrogen bonds in the unit cell. The geometry of zinc coordination centre is distorted tetrahedral. Fascinatingly, zinc exhibited two different coordination environments. In one case, all phosphate oxygens participated in coordination with zinc. In second case, N(7) position of purine and phosphate oxygens participated in coordination with zinc. Moreover, zinc formed a coordination polymer with 5′-GMP. The conformation of ribose changed upon zinc interaction with 5′-GMP from C(3′)-endo to C(2′)-endo, these results suggest that zinc interaction with nucleic acids may change their conformation. Complex 1 is stabilized in solid state by H-bonding and π-π stacking interactions. Complex 2: In complex 2, 5′-GMP is coordinated to nickel through N(7) position of purine but phosphate moiety did not take place in coordination. Two molecules of 5′-GMP and four water molecules coordinated to nickel and formed distorted octahedral geometry. The charge of complex 2 is balanced by sodium coordination to sugar hydroxyl groups and nickel coordinated water molecules. The geometry of sodium coordination centre is distorted octahedral. The conformation of 5′-GMP is altered due to nickel interaction. Moreover, complex 2 is stabilized in solid state by H-bonding and π-π stacking interactions. Complex 3: Nucleotide 5′-IMP also showed similar coordination modes like 5′-GMP towards nickel, where N(7) position of purine participated in coordination with nickel and phosphate moieties did not coordinate to nickel. Two molecules of 5′-IMP and four water molecules participated in coordination with nickel and formed distorted octahedral geometry. Interestingly, the charge of complex 3 is balanced by sodium coordination to sugar hydroxyl moieties. The geometry of sodium coordination centre is distorted octahedral. Moreover, nickel is forming coordination polymer with 5′-IMP. Further, nickel interactions with 5′-IMP brought changes in the conformation of ribose moiety. These results suggest that nickel interactions with nucleic acids may bring changes in their conformation. Interestingly, right hand helical structure formation is observed for complex 3 in crystal structure. Further, the chirality of complex 3 was confirmed by circular dichroism studies. Complex 3 is stabilized by both H-bonding and π-π stacking interactions in solid state. Complex 4: Surprisingly, nickel is coordinated to hypoxanthine through N(9) position of purine in acidic conditions and not through N(7) or N(3). The coordination mode of nickel with hypoxanthine is different from complexes 2 and 3. Two hypoxanthine moieties are coordinated to nickel in axial manner. The geometry of nickel coordination centre is distorted...
octahedral. Further, complex 4 is stabilized by H-bonding and π-π stacking interactions in solid state. Cytotoxicity studies of complexes 1-4 on various cancer cell lines revealed that complex 1 is better cytotoxic than complexes 2-4. Moreover, complex 1 exhibited comparable cytotoxicity with cisplatin on various cells lines and induced apoptotic cell death.

II. Synthesis, structure elucidation and anticancer activity of copper-adeninyl complexes

In order to understand copper-adenine interactions and anticancer activity, several copper complexes of adenine derivatives were prepared. Here, most of adenine derivatives used in complex preparation is known as cycline dependent kinase inhibitors. Prepared copper complexes are 1) [Cu (N⁶-benzyl adenineH)₂Cl₃].Cl₂H₂O, 2) [Cu (2-amino-N⁶-benzyladenineH)₂Cl₃].(2-amino-N⁶-benzyl adenineH)₂.3Cl.5H₂O, 3) [Cu (α-(Purin-6-ylamino)-p-toluenesulfonamide H₂Cl₄], 4) [Cu (kinetinH)₂ Cl₃].Cl₂H₂O, 5) [Cu (N-1H-purine-6-yl-alanineH) (H₂O) Cl₃].H₂O, 6) [(Cu (N-1H-purine-6-yl-alanineH)Cl)₂(μ-Cl)₂].Cl₄H₂O. All these complexes were characterized by X-ray crystallography and various spectroscopic techniques. Complex 1: Synthesis and X-ray structures of complex 1 were reported in literature. However, anticancer activity of complex 1 is not known. Therefore, it was prepared based on the reported lines to assess the anticancer activity. The anticancer activity of complex 1 was studied on various cell lines. Interestingly, complex 1 exhibited better cytotoxicity than cisplatin in MCF-7 and MDA-MB-231 cell lines. Complex 2: Ligand 2-amino-N⁶-benzyl adenine is coordinated to copper through N(9) of purine. In addition, two uncoordinated 2-amino-N⁶-benzyl adenine, three chloride and five water molecules are making it as a co-complex with uncoordinated ligands. The copper coordination centre adopted distorted trigonal bipyramidal geometry [3+2] with τ = 0.671 (α-β/60, where α, β are two greatest valence angles of coordination centre). Further, complex 2 is stabilized in solid state by both H-bonding and π-π stacking interactions. H-bonding is observed between N-H⋯Cl. Uncoordinated water molecules formed six-member rings with H-bonding network. The π-π stacking interactions are observed between phenyl and purine moieties. Complex 2 exhibited better cytotoxicity than 2-amino-N⁶-benzyl adenine and copper salt. Complex 3: Ligand α-(2-Amino purin-6-ylamino)-p-toluene sulfonamide is coordinated to copper through N(9) position and protonation is observed at N(3) position. Two molecules of α-(2-Amino purin-6-ylamino)-p-toluene sulfonamide and four chloride ions are forming a distorted octahedral geometry with copper. Complex 3 is stabilized by N-H⋯Cl and N-H⋯O H-bonding. Further, complex 3 exhibited better cytotoxicity than cisplatin in U251 cells. Complex 4: Kinetin is coordinated to copper through N(9) position of purine. Protonation is observed on N(3) position and balanced the charge of complex 4. Two molecules of kinetin and three chloride moieties are coordinated to copper and forming distorted trigonal bipyramidal geometry [3+2] with τ = 0.431. Moreover, complex 4 is stabilized by both H-bonding interactions and π-π stacking interactions. The H-bonding of complex 4 is observed between N-H⋯Cl and C-H⋯Cl. The π-π stacking interactions are observed between furanyl aromatic ring and imidazole ring of purine. Complex 4 exhibited...
better cytotoxicity than kinetin and copper salt. Complex 5: The N-1H-purine-6-yl-alanine is coordinated to copper through N(9) position of purine. Complex 5 crystallizes in the monoclinic space group P2₁ with Z=4. One molecule of N-1H-purine-6-yl-alanine, two chloride ions and one water molecule coordinated to copper. The geometry of copper coordination centre is distorted trigonal bipyramidal [3+2] with Cu(1) τ₁ = 0.613 and Cu(2) τ₂= 0.671. Protonation is observed on N(3) position. Complex 5 is stabilized by both H-bonding and π-π stacking interactions. The H-bonding of complex 5 is observed between N-H···Cl and C-H···Cl. The π-π stacking interactions are observed between imidazole moieties. Moreover, complex 5 exhibited better cytotoxicity than N-1H-purine-6-yl-alanine and copper salt. Complex 6: Complex 6 is a co-complex, where two different complexes are co-crystallized. The crystal structure of complex 6 indicate that geometry of Cu(1) and Cu(2) coordination centre are distorted trigonal bipyramidal [3+2] with τ₁ = 0.3261 and τ₂ = 0.8, respectively. Two molecules of N-1H-purine-6-yl-alanineH are coordinated to Cu(2) through N(9) position of purine. The N-1H-purine-6-yl-alanineH ligands are arranged in geometry in trans manner with respect to axis passing through the N(9) atom and copper. Whereas, in second co-complex two N-1H-purine-6-yl-alanineH are coordinated to Cu(1) through N(9) and N(3) position of purine. Both Cl(1) and Cl(3) coordinated to copper are forming a bridge between copper. In addition, one uncoordinated chloride and two water molecules are present in the unit cell. Complex 6 is stabilized in crystalline state by both H-bonding and π-π stacking interactions. Complex 6 exhibited better cytotoxicity than complex 5, N-1H-purine-6-yl-alanine and copper salt on various cell lines.

III. Synthesis, structure and anticancer activity of zinc complexes of adenine derivatives

In order to understand zinc interaction with adenine and their anticancer activity, several zinc complexes of adenine derivatives were prepared. The prepared complexes are (1) [Zn(N⁶-benzyladenineH).Cl₃].2H₂O, (2) [Zn₂(μ-N⁶-benzyladenine)₂(H₂O)₂](OTf)₂.H₂O, (3) [N⁶-benzyl adenineH₂][ZnCl₄].2H₂O, (4) [Zn(2-Amino-N⁶-Benzylpurine)Cl₃].2-Amino-N⁶-BenzylpurineH).EtOH, (5) [2-Amino-N⁶-(3-picoyl)purineH₂][ZnCl₄].H₂O, (6) [2-Amino-N⁶-(3-picoyl)purineH₂][ZnCl₄].HCl, (7) [2-Chloro-N⁶-(3-picoyl) purineH₂][ZnCl₄].H₂O, (8) [(α-Purine-6-ylamino)-p-toluene sulfonamide H]₂[ZnCl₄].2HCl.2H₂O. Complex 1: The N⁶-benzyl adenine is coordinated to zinc through nitrogen atom N(7) of purine. One molecule of N⁶-benzyl adenine and three chloride ions are coordinated to zinc and forming distorted tetrahedral geometry. Interestingly, the nitrogen atom N(1) of purine is protonated. Complex 1 exhibited strong H-bonding interactions between N-H···O, N-H···Cl and N-H···N. The complex 1 showed better cytotoxicity than N⁶-benzyl adenine and ZnCl₂. Complex 2: The N⁶-benzyl adenine formed a dimeric complex with zinc at neutral pH. Complex 2 crystallizes in the triclinic space group P-1 with Z=1. Two Zn metal centres are bridged by two molecules of N⁶-benzyl adenine through nitrogen atoms N(3) and N(9) of purine forming a di-nuclear complex, further two zinc centres is bridged by two water molecules and other two water molecules on the other side completing the octahedral coordination for the Zn. Complex 2 is stabilized in crystalline state by H-bonding interactions. The H-bonding of complex 2 is
observed between O-H···O and N-H···O. Complex 2 exhibited better cytotoxicity than N\textsuperscript{6}-benzyl adenine and ZnCl\textsubscript{2} on various cell lines. Complex 3: The N\textsuperscript{6}-benzyladenine is not coordinated to the Zn metal at acidic pH and forms an ion-pair complex. Ion-pair complex 3 crystallizes in the monoclinic space group Cc with Z=4. The protonation is observed at N(1) and N(9) atoms of N\textsuperscript{6}-benzyl adenine. The positive charges on N\textsuperscript{6}-benzyl adenine is neutralized by the presence of two chloride ions in [ZnCl\textsubscript{4}]\textsuperscript{2-}. Alternative arrangement of cation and anion arrangement is observed in complex 3. Water channel formation is observed between cation and anion arrangement. Moreover, complex 3 is stabilized by H-bonding and π-π stacking interactions. H-bonding is observed in complex 3 between N-H···Cl, O-H···Cl and N-H···O. The π-π stacking interactions in complex 3 are observed between benzyl six-membered aromatic ring and purine six-membered rings. Complex 3 exhibited better cytotoxicity than N\textsuperscript{6}-benzyl adenine and ZnCl\textsubscript{2} in various cell lines. Complex 4: Ligand 2-amino-N\textsuperscript{6}-benzyl adenine resulted in a different structure from N\textsuperscript{6}-benzyl adenine with zinc. One molecule of 2-amino-N\textsuperscript{6}-benzyl purine is coordinated to zinc through nitrogen atom N(7) of purine. Surprisingly, one uncoordinated positively charged 2-amino-N\textsuperscript{6}-benzyl purineH is present in the asymmetric unit, which is balancing the charge of zinc complex 4. Protonation is observed on N(3A) atom. Interestingly, tautomeric proton is located on coordinated purine of N(9) atom and uncoordinated purine of N(7A) atom. Geometry of ‘Zn coordination centre’ is distorted tetrahedral. Complex 4 is stabilized by H-bonding and π-π stacking interactions. The H-bonding interaction in complex 4 is observed between N-H···O and N-H···Cl. The π-π stacking interactions are observed between five-member aromatic rings and six-membered aromatic rings. Complex 4 exhibited better cytotoxicity than 2-amino-N\textsuperscript{6}-benzyl purine and ZnCl\textsubscript{2} in various cell lines. Complex 5: 2-Amino-N\textsuperscript{6}-(3-picycol) purine forms an ion-paired complex with zinc at acidic pH. The protonation in 2-Amino-N\textsuperscript{6}-(3-picycol) purine is observed at N(3) of the purine and picycol N(14). The positive charge of 2-Amino-N\textsuperscript{6}-(3-picycol) purine is neutralized by the presence of two chloride ions in [ZnCl\textsubscript{4}]\textsuperscript{2-}. Moreover, complex 5 exhibited both H-bonding interactions and π-π stacking interactions. The H-bonding interactions are observed between N-H···Cl, N-H···N, O-H···Cl, N-H···O and C-H···N. One uncoordinated water molecule is present in unit cell, which is involved in H-bonding with both ions. The π-π stacking interactions are observed between purine five-membered rings and purine six-membered ring. Complex 5 exhibited better cytotoxicity than cisplatin in HeLa and MDA-MD-231 cells. Complex 6: 2-Amino-N\textsuperscript{6}-(3-picycol) purine formed similar structure of complex 5 in strong acidic conditions. Complex 6 exhibited both H-bonding and π-π stacking interactions. The H-bonding in complex 6 is observed between N-H···Cl and N-H···N. In complex 6, the π-π stacking interactions are observed between pyridyl six-membered rings and purine six-membered rings. Purine-Purine stacking interactions are observed between purine six-membered ring and five-membered rings. Complex 6 exhibited better cytotoxicity than cisplatin in HeLa, MCF-7, MDA-MB-231 and HeLa-Dox cells. Interestingly, complex 6 arrested (G2/M phase) cell cycle in HeLa and MCF-7 at higher concentration and induced apoptosis. Complex 7: 2-chloro-N\textsuperscript{6}-(3-picycol) purine formed ion-pair complex with zinc. The protonation in 2-chloro-N\textsuperscript{6}-(3-picycol) purine is observed on N(9)
of purine and N(14) of picoly1 atoms. The positive charge of 2-chloro-N6-(3-picolyl) purine is neutralized by the presence of two chloride ions in \([\text{ZnCl}_4]^2-\). Complex 7 is stabilized by both H-bonding and π-π stacking interactions. The H-bonding is observed between N-H···Cl, O-H···Cl and N-H···O in complex 7. The π-π stacking interactions are observed between pyridyl six-membered ring and six-membered ring of purine. Complex 7 exhibited better cytotoxicity than cisplatin in HeLa, MCF-7, U251 and HeLa-Dox cells. Complex 8: (α-Purine-6-ylamino)-p-toluene sulphonamide formed ion-pair complex with zinc. Ion-pair complex 8, crystallizes in the triclinic space group P-1 with Z=4. The protonation on (α-Purine-6-ylamino)-p-toluene sulphonamide is observed at N(9) and N(1) atoms of purine. The positive charge of the ligand is neutralized by two chloride ions present in \([\text{ZnCl}_4]^2-\). The H-bonding is observed between N-H···Cl, O-H···N, N-H···O and O-H···Cl. The π-π stacking interactions are observed between benzyl rings of benzene sulphonamide moieties. Complex 8 exhibited better cytotoxicity than cisplatin in HeLa, MCF-7 and HeLa-Dox cells. Moreover, these complexes induced apoptotic cell death as revealed by Annexin V/PI assay, FACS and microscopy analysis.

IV. Synthesis, structure and cytotoxicity studies of zinc complexes of uracil-1-acetic acid and N6-adeninebutyric acid

To understand the zinc interactions with nucleic acid constituent derivatives and their anticancer activity, zinc complexes of uracil-1-acetic acid and N6-adeninebutyric acids were prepared. (1) [Zn (uracil-1-acetato)]_2 (H_2O)_4 and complex (2) [Zn (N6-adeninebutyric acid)]_2 (H_2O)_2] were characterized by X-ray crystallography and various spectroscopic techniques. The X-ray structures showed acetate moiety coordination to zinc rather than purine and pyrimidine moieties. The geometry of zinc coordination centre is distorted octahedral. Complexes 1 and 2 are stabilized by non-covalent interactions. Anticancer studies of these complexes showed better cytotoxicity than cisplatin in MDA-MB-231 cells.

V. Copper (II) complexes of 6-mercaptopurine, hypoxanthine and uracil-1-acetic acid: Synthesis, structures, antioxidant and potent anticancer activity

To delineate copper interactions with purine and pyrimidine derivatives and anticancer activity, several copper complexes of 6-mercaptopurine, hypoxanthine and uracil-1-acetic acid were prepared. The prepared complexes are (1) [Cu (6-MP) (bpy) Cl_2], (2) [Cu (hx) (phen) Cl_2] H_2O and (3) [Cu (bpy) (uracil-1-acetato)]_6H_2O] (bpy = 2, 2′-bipyridine, phen = 1, 10-phenanthroline, 6-MP = 6-Mercapto Purine and hx = hypoxanthine). All these complexes were characterized by various spectroscopic and X-ray diffraction techniques. Complexes 1 and 2 crystallize in the monoclinic space groups Cc and C2/c, respectively with eight molecules in the unit cell. All the complexes 1-3 adopt distorted trigonal bipyramidal geometry. Surprisingly, most potent coordination sites of sulfur in 6-MP and acetato in uracil-1-acetato did not participate in coordination with copper. In complexes 1 and 2, the N(7) position of purine and the N(3) position of pyrimidine in complex 3 are coordinated with copper. All these complexes 1-3 are stabilized by non-covalent interactions in solid
state. Anticancer studies showed better cytotoxicity for copper complexes than cisplatin, 6-meracptopurine and temozolomide in various cell lines. Interestingly, copper complexes of 6-MP and hypoxanthine showed antioxidant activity and reduced ROS level in cells. In contrast, copper complex of uracil-1-acetic acid produced ROS in cells. In contrast, copper hypoxanthine showed better cytotoxicity than cisplatin in HeLa-Dox cells. All these complexes induced apoptotic cell death.

In summary, we studied the interaction of metal-nucleic acid constituents and derivatives by X-ray crystallography. We found new coordination modes for Ni, Cu and Zn towards various nucleic acid constituents and derivatives. Some of these complexes showed better cytotoxicity than well known anticancer drugs cisplatin, 6-meracptopurine and temozolomide. Complex [Cu (hx) (phen) Cl2].H2O showed better cytotoxicity than cisplatin in doxorubicin resistant (HeLa-Dox) cells. These complexes induced apoptotic cell death in various cancer cells. All in all, the results of present studies/findings could form a potential lead for the development of newer anticancer therapeutics.