Synopsis

Transition metal bis(thiosemicarbazone) complexes have been of great interest in the last five decades. One of the most striking features of these complexes is that they possess a wide range of biological properties including antimalarial, antibacterial and anticancer activity. Zinc and copper bis(thiosemicarbazone) complexes have recently attracted attention due to their intracellular fluorescence and anticancer activity, respectively. The present work "Targeting Cancer Cells and Live Cell Imaging Using Bis(thiosemicarbazone) Complexes of Copper and Zinc" is an effort to target cancer cells using folic acid or biotin linked anticancer active copper bis(thiosemicarbazone) complexes. Interestingly, bis(thiosemicarbazone) ligands form zinc complexes that could be used to image cancer cells and one of the ligands could be used for imaging zinc in the cells.

Chapter 1, provides a brief introduction to metal complexes in medicine. Different classes of metallodrugs and their mechanism of action are listed. A short discussion on different types of diagnostic drugs and transition metal complexes possessing anticancer activity is presented. An overview of the strategies available to target cancer cells is included. Furthermore, the use of thiosemicarbazone compounds for anticancer activity is reviewed in detail. Recent examples of bis(thiosemicarbazone) compounds in medicinal studies is briefly mentioned. This section ends with the scope of the present work which involves bis(thiosemicarbazone) complexes of zinc and copper.

Chapter 2, "Zinc bis(thiosemicarbazone) complexes for live cell imaging and anticancer activity" deals with the synthesis and characterization of a series of mononuclear and binuclear zinc bis(thiosemicarbazone) complexes by varying substituents at the diketone moiety or at the thiosemicarbazide fragment of the ligand. The crystal structures of mononuclear ligand benzil-bis(4-pyrrolidine-3-thiosemicarbazone) ($BTSCH_2$), zinc glyoxal-bis(4-methyl-4-phenyl-3-thiosemicarbazone) $[Zn(GTSC)]_3$ and [Zn(BTSC)(DMSO)] complexes were determined using single-crystal X-ray crystallography. Here, the mononuclear zinc complexes were utilized as live cell imaging agents whereas binuclear zinc complexes proved to be anticancer agents. Among the many mononuclear complexes prepared, the trimeric zinc complex derived from glyoxal- bis(4-methyl-4-phenyl-3-thiosemicarbazone) was found to be the most fluorescent complex owing to its unique structure. This permitted live cell imaging in a number of cancer cell lines. In comparison with the well studied zinc biacetyl-bis(4-methyl-3-thiosemicarbazone) Zn(ATSM) complex,

which was used as a reference, $[Zn(GTSC)]_3$ had a 2.5 fold higher fluorescence quantum yield in DMSO. The cellular fluorescence was measured in collaboration with Prof. K.Somasundaram's laboratory at MCBL using flow cytometry. It was observed that $[Zn(GTSC)]_3$ had 3 to 12 fold higher fluorescence than Zn(ATSM) in various cell lines (n = 9) of different tissue origin. Confocal fluorescence microscopy studies established that $[Zn(GTSC)]_3$ localizes in the nucleus of human breast cancer MCF-7 and MDA-MB-231 cells within 30 minutes of addition. Moreover, $[Zn(GTSC)]_3$ showed relatively less cytotoxicity compared to the Zn(ATSM) complex in all the cancer cell lines tested. DNA interaction studies such as binding and cleavage showed that $[Zn(GTSC)]_3$ was less harmful to DNA as well. All these features make $[Zn(GTSC)]_3$ a good fluorescent imaging agent for live cells. Binuclear zinc bis(thiosemicarbazone) complexes were also synthesized and their cytotoxicity was evaluated in different cancer cells. One of the ligands, 1,3-bis{biacetyl-2′-(4"-N-pyrrolidinethiosemicarbazide)-3′-(4"-N-thiosemicarbazide)} propane ($ProBATpyrH_4$), and its zinc complex were found to show excellent anticancer activity against human hepatocellular cancer (HepG2) cell line. However, the cellular uptake studies as followed by flow cytometry revealed that these compounds do not fluoresce inside the cells. However, the DNA interaction studies using ethidium bromide displacement assay revealed that these complexes have better binding ability to DNA than mononuclear zinc complexes and the viscometric titrations suggested the binding mode to DNA is through partial intercalation. Apparently, these complexes do not induce DNA cleavage as evident from the cleavage experiments performed on pBR322 DNA. It is likely that their anticancer activity is due to unique DNA binding properties.

Imaging zinc is important in the field of metallomics as alteration of zinc concentration in cells is associated with, or attributed to various diseases. In this regard, bis(thiosemicarbazone) ligands are useful. **Chapter 3**, **"**Imaging intracellular zinc using glyoxal-bis(4-methyl-4-phenyl-3-thiosemicarbazone) ligand" deals with imaging zinc in live cells using the bis(thiosemicarbazone) ligand, $GTSCH_2$. Since the trimeric zinc complex is fluorescent, the corresponding ligand, $GTSCH_2$, was utilized to visualize the zinc present within cells. The ligand $GTSCH_2$ is found to be a selective fluorescence "turn-on" sensor for zinc. This sensor exhibited excellent sensitivity and selectivity towards zinc over other physiologically relevant cations. The binding affinity of $GTSCH_2$ to zinc was estimated to be 0.59 nM in an aqueous MOPS (50 mM, NaCl; 100 mM; pH 7.3) buffer containing 30% DMSO, from competitive binding experiments carried out with ethylene glycol tetraacetic acid (EGTA). The sensor displayed maximal fluorescence response to zinc ion when present

in the ratio of 1:1 and displayed stable fluoresence in the pH range 5.0 to 7.8, which suggests that the probe may be suitable for imaging zinc in both normal and cancer cells. The potential of $GTSCH_2$ to image zinc inside the cell has been demonstrated in two human breast cancer cell lines using confocal fluorescence microscopy.

Unlike mononuclear zinc complexes, the mononuclear copper bis(thiosemicarbazone) complexes are cytotoxic. **Chapter 4**, "Anticancer activity of copper bis(thiosemicarbazone) complexes" deals with the synthesis, characterization and anticancer activity of mononuclear copper bis(thiosemicarbazone) complexes. All of them were characterized by spectroscopic methods and in three cases by single crystal X-ray diffraction. The redox properties, studied by cyclic voltammetry, showed reversible one electron- reduction process that varied from –0.53 V to –0.18 V *vs* SCE. Anticancer activity for the synthesized complexes and their ligands were tested against many human cancer cell lines where the complexes Cu(GTSC) and Cu(GTSCHCl) derived from glyoxal-bis(4-methyl-4-phenyl-3-thiosemicarbazone) are found to be most cytotoxic (GI_{50} <0.1 μM to 2.1 μM) in five cancer cell lines tested. Moreover, the cytotoxicity is similar to that of adriamycin, a known anticancer drug, in all cell lines. However, it is less potent than a copper bis(thiosemicarbazone) analog, copper biacetyl-bis(4-methyl-3-thiosemicarbazone) Cu(ATSM), a well studied anticancer agent in many cell lines. Cellular studies were carried out for the selected complexes Cu(GTSC) and Cu(GTSCHCl) along with Cu(ATSM) on HCT116 colon cancer cells. The order of lipophilicity and cellular uptake as studied by ICP-OES are correlated with their cytotoxicity. Based on the interaction of these complexes with DNA using the ethidium bromide displacement assay, DNA -melting, -viscosity and -cleavage studies, it is suggested that these complexes intercalate partially with DNA. DNA cleavage studies using pBR322 DNA revealed that only Cu(GTSCHCl) complex cleaves DNA. Mechanistic discrimination studies suggest that the complex cleaves DNA through the hydrolytic pathway. Since the topoisomerase IIα (Topo IIα), a nuclear enzyme resolving topological problems of DNA, is considered as one of the possible molecular targets for a number of anticancer drugs including some of the copper thiosemicarbazone complexes, Topo IIα inhibition studies were carried out in human Topo IIα. Interestingly, many copper bis(thiosemicarbazone) complexes are able to inhibit Topo IIα activity by acting as Topo IIα poison. Cu(GTSCHCl) complex was found to be the most active in this series of complexes (90 % inhibition at 100 μM) and it inhibits the enzyme in a dose dependant manner. Based on the results, it was concluded that the cell death may be mediated, at least in part, through DNA cleavage and Topo IIα inhibition.

Severe side effects, poor distribution profiles and or organ specific toxicity make the conventional chemotherapy of limited value with metal based drugs. Therefore, developing cancer-specific drug delivery systems is an urgent need in cancer therapy. Among the many strategies available to target cancer, targeting the receptors that are overexpressed in the cancer cell membrane is a novel strategy being used in recent studies. Therefore the last part my work, "Copper bis(thiosemicarbazone) complexes linked to poly(ethylene glycol) and multiwalled carbon nanotubes for targeted delivery to cancer cells " was designed to target cancer cells. Here, copper complexes (therapeutic molecule) were attached with PEG and MWCNT (carrier) along with folic acid or biotin (targeting molecule). First, CuATSM–A was functionalized with a disulfide linker and connected with folic acid *via* a poly(ethylene glycol) (PEG_{600}) linker. This was synthesized to target KB (human nasopharyngeal carcinoma) cells, a cell line that overexpresses the folate receptor on the cell surface. In order to investigate the targeting efficacy, the corresponding fluorescent labeled analogs and non-targeted PEG conjugates were synthesized. Flow cytometry studies with fluorescent marker (fluorescein isothiocyanate) labeled PEG analogs showed the targeting efficacy on KB cells. The copper complex, CuATSM–A, attached with biotin–PEG_{2000} also was synthesized to target high-biotin-using HeLa (human cervical carcinoma) cells. Multiwalled carbon nanotubes (MWCNT) were also used as nanocarriers. Here, the MWCNT was decorated with PEG_{600} diamine and then functionalized with the copper complex (therapeutic molecule), folic acid (targeting molecule), and FITC (fluorescent molecule). The conjugation of all the molecules with MWCNT is characterized by various spectroscopic techniques.