Chapter 1

Introduction
1. Introduction

1.1. Physiological significance and medicinal applications of metals

Metals have played an important role in medicine for years, ever since humans have walked the planet. Many metals are essential in our diets in varying quantities, although people have realized their significance recently. Many metals and metallic elements play a crucial role in living systems by catalysing biological reactions (via hydrolysis, substrate transfer, electron transfer etc.), stabilising biomolecular structure (protein, DNA), charge balancing (osmotic balance and nerve function) and replication. Metals can easily lose electrons from the familiar elemental or metallic state to form positively charged ions, which tend to be soluble in biological fluids. Metals play their biological role in cationic form since most biological molecules such as proteins and DNA are electron rich and metals in cationic form are electron deficient. Such attraction of opposing charges leads to a general tendency for metal ions to interact with biological molecules. Same principle applies to the affinity of metal ions for many small molecules and ions crucial to life, such as oxygen. For the interaction of metals in biology, it is not surprising that natural evolution has incorporated many metals into essential biological functions. Metals perform a wide variety of tasks which are briefly shown in the Table 1.1 [1-3].

Medicinal inorganic chemistry as a discipline has emerged, since the discovery of antitumor activity of cisplatin. Platinum based combination chemotherapy is still the mainstay for the treatment of solid malignancies (especially testicular, ovarian and small cell lung cancers). The unique lesion made by platinum has not, to date, been mimicked by any organic drugs, which clearly shows that the metal-biomolecules interaction is critical to the antitumor activity of any metallodrug. Medicinal inorganic chemistry has been practiced for almost 5000 years. As far back as 3000BC, the Egyptians used copper to sterilize water. Gold was used in a variety of medicine in Arabia and China 3500 years ago. Various iron remedies were used in Egypt at about 1500BC. In Renaissance era Europe, mercurous chloride was used as a diuretic and nutritional essentiality of iron was discovered. The medicinal activity of inorganic compounds has slowly been developed in a rational manner for last 100 years. It started in the early 1900s with discovery of K[Au(CN)2] for tuberculosis, followed by various antimony compounds for leishmaniasis and the antibacterial activity of various gold salts.

When one thinks of drugs, one often thinks of organic compounds. The biochemical literature of the last 30 years chronicles the burgeoning understanding that many of the biological activities of proteins and enzymes can be ascribed to the metal centres, with the organic backbone acting as a scaffold to hold the metal ion in place for the requisite transformation. Because of this rapid growth of biological inorganic chemistry, it seems logical to explore in parallel the medicinal properties of the various metal ions. In the last 50 years, knowledge of the central importance
of inorganic elements in organisms has opened up the possibility for inorganic chemists to contribute to health and well-being of man and all other organisms.

**Table 1.1:** Metals function in biology and effects of metal deficiency in humans

<table>
<thead>
<tr>
<th>Metal</th>
<th>Function</th>
<th>Deficiency symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>Anti inflammatory; anti arthritis</td>
<td>Arthritis; gland and brain dysfunction</td>
</tr>
<tr>
<td>Copper</td>
<td>Oxidase; dioxygen transport; electron transfer</td>
<td>Artery weakness; liver disorders; secondary anemia</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Structure; hydrolase; isomerase</td>
<td>Muscle cramps</td>
</tr>
<tr>
<td>Calcium</td>
<td>Structure; trigger; charge carrier</td>
<td>Retarded skeletal growth</td>
</tr>
<tr>
<td>Chromium</td>
<td>Possible involvement in glucose tolerance</td>
<td>Diabetes symptoms</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Nitrogen fixation; oxidase; oxo transfer</td>
<td>Retardation of cellular growth; propensity for caries</td>
</tr>
<tr>
<td>Manganese</td>
<td>Photosynthesis; oxidase</td>
<td>Infertility; impaired skeletal growth</td>
</tr>
<tr>
<td>Iron</td>
<td>Oxidase; dioxygen transport and storage; electron transfer; nitrogen fixation</td>
<td>Anemia; disorders of the immune system</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Oxidase; alkyl group transfer</td>
<td>Pernicious anemia</td>
</tr>
<tr>
<td>Nickel</td>
<td>Hydrogenase; hydrolase</td>
<td>Growth depression; dermatitis</td>
</tr>
<tr>
<td>Zinc</td>
<td>Structure; hydrolase</td>
<td>Skin damage; stunted growth; retarded sexual maturation</td>
</tr>
</tbody>
</table>

Any metal ion or complex, or indeed any chemical compound, is subjected to the potential limitations in the Bertrand diagram [4], which is usually used in discussing the essentiality of elements. The area of optimum physiological response will vary greatly according to the element, its speciation and oxidation state and the biochemistry of the specific compound in which it is found. Therefore, the areas of deficiency, toxicity and optimum physiological response can be dramatically varied by considering a combination of these variables, as well as design features of the potential ligand which may be altered to tune the delivery of that metal ion into the biological system. This refinement of the biological properties of metal complexes by ligand modification along with the design of ligands to alter the homeostasis of endogenous metal ions, will provide many therapeutic and diagnostic agents over the coming years and will direct medicinal inorganic chemistry into discipline of central importance in medicine and science.

The complexes as drug work in one of the two ways: some use a process called redox chemistry to steal electrons from the bonds holding the target molecules together. Others use hydrolysis, meaning that they breakdown the target’s chemical waterproofing, so that the water that is naturally present in a cell dissolves the target. The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, metal-based drugs where central metal ion is usually the key
feature of the mechanism and secondly, ligands as drugs which target metal ions in some form, whether free or protein-bound.

1.2. Metal based drugs

The rich diversity of coordination chemistry provides exciting prospects for the design of novel therapeutic agents with unique mechanisms of action. Bioinorganic chemistry is an interdisciplinary field that draws on disciplines such as biochemistry, inorganic and theoretical chemistry, molecular biology, biophysics and spectroscopy, to address the key questions of how metals complement and mediate key biological processes [5]. Medicinal inorganic chemistry is a discipline of growing significance in both therapeutic and diagnostic medicine. The field of medicinal inorganic chemistry has evolved with three conceptual aims:

- the introduction of metal ions to the biological system,
- manipulation and redistribution of metal ions within the system and
- removal of metal ions from the system.

Inorganic compounds have been used in medicine for many centuries, but often only in an empirical way with little attempt to design the compounds with therapeutic uses and with little or no understanding of the molecular basis of their mechanism of action. In the late 1960s, the discovery and development of the antitumour compound cisplatin (cis-[PtCl$_2$(NH$_3$)$_2$]) played a profound role in establishing the field of medicinal inorganic chemistry [6].

![Diagram](image)

**Figure 1.1:** Some of the areas of medicinal inorganic chemistry

Cisplatin, and the second generation alternatives carboplatin and oxaliplatin, are still the most widely used chemotherapeutic agents for cancer, greatly improving the survival rates of patients worldwide. The success of cisplatin has aroused great interest in the development of new metal complexes to diagnose and/or treat diseases
including diabetes, Alzheimer and cancer. The history and basic concepts of medicinal inorganic chemistry have been widely reviewed [7-9]. As indicated in Fig. 1.1, key areas in the design of active compounds are the control of toxicity (side-effects) and targeting of the metal to specific tissues, organs or cells, where activity is needed.

Metal ions can be introduced into a biological system either for therapeutic effect or as diagnostic aids. Alternatively, metal ions can be removed from a biological system by judicious use of metal binding molecules (ligands) [10]. Thus, biomedical inorganic chemistry offers the potential for the design of novel therapeutic and diagnostic agents and for the treatment and understanding of diseases which are currently intractable [11]. It is seldom useful to describe elements as “toxic” or “non-toxic”. Even so-called “toxic compounds” can usually be tolerated in low doses, and may exhibit therapeutic effects within narrow concentration ranges and biologically-essential elements can become toxic at high doses (Fig. 1.2, the Bertrand diagram) [4].

Figure 1.2: A Bertrand diagram, showing that physiological and toxic effects are a continuum

Moreover, the same element may be beneficial or noxious depending on speciation (the nature of the molecule or ion that contains the element). About 24 elements are currently thought to be essential for mammalian life: H, C, N, O, F, Na, Mg, Si, P, S, Cl, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Sn, I [12] and 14 of them are metals or metalloids.

Inorganic elements play crucial roles in biological and biomedical processes [8], and it is evident that many organic compounds used in medicine do not have a purely organic mode of action; some are activated or biotransformed by metal ions including metalloenzymes [13], others have a direct or indirect effect on metal ion metabolism. Some of the metal compounds and their therapeutic uses are shown in Table 1.2.
### Table 1.2: Some metal compounds in clinical use

<table>
<thead>
<tr>
<th>Compound</th>
<th>Example (brand name)</th>
<th>Function</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active complexes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-[PtCl₂(NH₃)₂]²⁻ (Cisplatin)</td>
<td>Extracellular contrast agent for MRI</td>
<td>Trans-isomer is inactive</td>
<td></td>
</tr>
<tr>
<td>[Gd(DTPA)(H₂O)]²⁻ (Magnevist)</td>
<td>Myocardial imaging</td>
<td>Low toxicity</td>
<td></td>
</tr>
<tr>
<td>[99mTc(CNCH₂C(CH₃)₂OCH₃)₆]³⁺ (Cardiolite)</td>
<td>Myocardial imaging</td>
<td>Positively-charged complex taken by heart</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>Coenzyme</td>
<td>Deficiency causes pernicious anaemia</td>
<td></td>
</tr>
<tr>
<td><strong>Active metals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li₂CO₃</td>
<td>Prophylaxis for bipolar disorders</td>
<td>Li forms weak complexes, labile</td>
<td></td>
</tr>
<tr>
<td>[Au(thiomalate)]⁻ (Myocrisin)</td>
<td>Antirheumatoid arthritis</td>
<td>Facile thiol exchange on Au(I)</td>
<td></td>
</tr>
<tr>
<td>Ammonium potassium Bi(III) citrate (De-Nol)</td>
<td>Antibacterial, antiulcer</td>
<td>Strong binding of Bi to thiolsh, facile exchange</td>
<td></td>
</tr>
<tr>
<td>Na₂[Fe₂(CN)₆(NO)]·2H₂O (Nipride)</td>
<td>Hypotensive</td>
<td>Releases NO, relaxes vascular muscles</td>
<td></td>
</tr>
<tr>
<td>p-Xylyl-bicyclam-8HCl (AMD3100)</td>
<td>Anti-HIV, stem cell mobilization</td>
<td>May bind metals in vivo</td>
<td></td>
</tr>
<tr>
<td>CaCO₃, Mg(OH)₂</td>
<td>Antacid</td>
<td>Slow release of alkali</td>
<td></td>
</tr>
<tr>
<td>La₂(CO₃)₃ (Fosnol)</td>
<td>Chronic renal failure</td>
<td>Reduces phosphate absorption</td>
<td></td>
</tr>
</tbody>
</table>

### 1.3. Drug based metal complexes

The metallo-elements, which are present in trace and ultra-trace quantities, play vital roles at the molecular level in a living system. The transition metal ions are responsible for the proper functioning of different enzymes. Drugs play a vital role as bioligands in the biological systems. Nitrogen containing bases, such as derivatives of pyridine, pyrimidine, purine and pyrrole, amines such as histamines, carbohydrates such as pentose, glucose and different vitamins such as ascorbic acid are well recognized bioligands. It has been found that the activity of the biometals is attained through the formation of complexes with different bioligands and the thermodynamic and kinetic properties of the complexes govern the mode of biological action. Sometimes, the permeability i.e. lipophilicity of drugs is increased through the formation of chelates in vivo and the drug action is significantly increased due to much more effective penetration of the drug into the site of action. The knowledge of drug action in vivo is extremely important in designing more potential drugs.

It is worth-mentioning that many drug substances such as aspirin, thiosemicarbazides, anticancer drugs and antibiotics exhibit the drug action through complexation with the available biometals in vivo [14]. In absolutely metal free
condition they are inactive. Interaction of various metal ions with antibiotics may enhance or suppress their antimicrobial activity but usually in many cases, the pharmacological activity of antibiotics after complexation with metals is enhanced as compared to that of the free ligands [15,16]. Many of the well-known antibiotics like penicillin, streptomycin, bacitracin, tetracycline etc. are chelating agents and their action is improved by the presence of small amounts of metal ions [17]. Some of the chelates are model analogues of certain metalloenzymes [18,19]. Furthermore, some of the chelates develop considerable fungicidal and antimicrobial activity [20]. Chelate compounds obtained from Schiff bases are convenient for the study of change in structure and associated biological activity, since varying a substitute in the metal ring permits variation in the three dimensional structure of the molecule [21-25]. It has been demonstrated through several studies that the biological activity of chelating compounds is enhanced on chelation with a metal atom. Some of the inactive ligands develop such properties upon chelation. The antitumor and antibacterial activity of some Schiff bases [26-29] has been attributed to their ability to chelate with trace transition metals [30,31]. Several explanations have been suggested for this enhancement in activity of metal complexation [32,33]. Generally it has been observed that transition metal complexes have greater activity and less toxic effects [34]. Other examples where the antibacterial action of drugs is enhanced by metal ions are kojic acid and sodium dimethyldithiocarbamate [14].

Antibiotics like streptomycin, cycloserine, tetracycline, arapicillin, isoniazid and others are known to have chelating properties. Some antibiotics are delicately balanced so as to be able to compete successfully with the metal binding agents of bacteria while not disturbing the metal processing by the host. The chelating properties of antibiotics may be used in metal transport across membrane or to attach the antibiotics to specific site from which it can interfere with the growth of bacteria [35]. Tetracycline forms an important group of antibiotics. Their activity appears to result from their ability to form metal chelates. The extent of antibacterial activity parallels the ability to form stable chelates. In a study it has been shown that tetracycline and cycloserine bindings to metal ions suppress their antimicrobial activity because the associated pH changes alter the intra- and inter-molecular interactions. A similar correlation has been drawn between active tetracycline and the ability to form 2:1 complexes with Cu(II), Ni(II) and Zn(II) [14]. Qualitative and quantitative differences in biological activities have been observed among metal chelates, differing in the metal ion or in the ligand. Metal chelates during chemical synthesis can be varied in size, charge distribution, stereochemistry, redox potentials and other physical properties [36].

### 1.3.1 Fluoroquinolones: Revolution in antibiotic medicinal field

The term fluoroquinolones refers to the potent synthetic chemotherapeutic antibacterials family, quinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position.
The first generation of the quinolones begins with the introduction of nalidixic acid in 1962 for treatment of urinary tract infections in humans [37]. Nalidixic acid was discovered by George Lesher and coworkers in a distillate during an attempt at chloroquine synthesis [38]. Nalidixic acid is considered to be the predecessor of all members of the quinolone family, including the second, third and fourth generations commonly known as fluoroquinolones. Fluoroquinolones have many medical uses in curing of pneumonia, respiratory disorders, acute sinusitis, bronchitis, pyelonephritis or bacterial prostatitis and many bacterial infections.

**Mechanism of action:** The basic pharmacophore, or active structure, of the fluoroquinolone class is based upon the quinoline ring system [39]. The addition of the fluorine atom at C6 is what distinguishes the successive-generation fluoroquinolones from the first-generation quinolones. The addition of the C6 fluorine atom has since been demonstrated to not be required for the antibacterial
activity of this class [40]. Various substitutions made to the quinoline ring resulted in the development of numerous fluoroquinolone drugs available today. Each substitution is associated with a number of specific adverse reactions, as well as increased activity against bacterial infections [41], whereas the quinoline ring, in and of itself, has been associated with severe and even fatal adverse reactions [42].

Compounds with small substituents such as C-5 amino group enhances absorption and/or tissue distribution. 5-Amino group counteracts the effect of the 8-fluoro substituent. Replacement of 4-keto group with other groups has generally produced inactive or weakly active compounds.

Positions 3 and 4, having a link between the carboxylic acid group and the keto group, are generally considered necessary for binding of quinolones to DNA gyrase.

Fluoroquinolones are the only class of antimicrobial agents in clinical use that are direct inhibitors of bacterial DNA synthesis. Fluoroquinolones inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV, which have essential and distinct roles in DNA replication [43]. The enzymes are referred as topoisomerases because they are able to change the topology or three-dimensional geometry of DNA molecules without changing the underlying chemical structure of the DNA. All type II topoisomerases require ATP. The quinolones bind to the complex of each of these enzymes with DNA; the resulting complexes, including the drug, block progress of the DNA replication enzyme complex [44]. Ultimately, this action results in damage to bacterial DNA and bacterial cell death. Thus, fluoroquinolones are bactericidal agents.

Figure 1.3: **Pharmacokinetics of fluoroquinolones**

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Figure 1.4: Antibacterial mechanism of action of fluoroquinolone drugs

Topoisomerase II functions as a molecular clamp (Fig. 1.4). The model indicated is based on the crystallographic structures of bacterial gyrase and yeast topo II as well as on the kinetic analysis of yeast topo II. Both the prokaryotic and eukaryotic type II topoisomerase show sequence homology. The bacterial topo II is a heterotetramer. For bacterial gyrase, the arms of the clamp represent the gyr B subunits and they are open and ready to capture the DNA segment (T segment) that will be transported through the enzyme catalyzed DNA break. The DNA segment which will be cleaved by the enzyme is referred as the gate segment (G segment) and it is bound by the gyr A subunits which contain the active site tyrosine. After binding ATP, the gyr B arms clamp around the T segment. The gyr B subunit contains the ATPase activity. The first ATP is hydrolyzed as the G segment is broken and the T segment is passed through the break. Then the first ADP is released. At some later point in the reaction, the DNA strands are released from the enzyme and the second ATP is hydrolyzed. The precise point in the reaction where the second ADP is released is not entirely clear and this fact is indicated by the question mark. The enzyme is then ready to begin another round of catalysis. The eukaryotic enzyme apparently arose from gene fusion of an earlier ancestor so that the ATPase activity and the active site tyrosines are not on separate polypeptides as in the bacterial enzyme, but reside on the same subunit. The mechanism of catalysis is probably quite similar.
1.4. Gold

Gold is a chemical element with the symbol Au (from Latin: *aurum* "gold") and an atomic number of 79. Gold is a dense, soft, shiny, malleable and ductile metal. Pure gold has a bright yellow color and luster traditionally considered attractive, which it maintains without oxidizing in air or water. Chemically, gold is a transition metal and a group 11 element. It is one of the least reactive solid chemical elements. The metal therefore occurs often in free elemental (native) form, as nuggets or grains in rocks, in veins and in alluvial deposits. Less commonly, it occurs in minerals as gold compounds, usually with tellurium.

1.4.1 Chemistry

Gold resists attacks by individual acids, but it can be dissolved by the aqua regia (nitro-hydrochloric acid), so named because it dissolves gold. Gold also dissolves in alkaline solutions of cyanide, which have been used in mining. Gold dissolves in mercury, forming amalgam alloys. Gold is insoluble in nitric acid, which dissolves silver and base metals, a property that has long been used to confirm the presence of gold in items, giving rise to the term the acid test. Gold forms many and diverse compounds. The oxidation states of gold in its compounds ranges from −1 to +5, but Au(I) and Au(III) dominate. Au(I), referred as the aurous ion, is the most common oxidation state in the gold complexes with soft ligands such as thioethers, thiolates and tertiary phosphines. Au(III) (auric) is a common oxidation state, and is illustrated by gold(III) chloride, Au2Cl6. The Au(III) complexes are typically square planar, with chemical bonds that have both covalent and ionic character.

1.4.2 Toxicity

Pure metallic (elemental) gold is non-toxic and non-irritating when ingested [45] and is sometimes used as a food decoration in the form of gold leaf. Metallic gold is also a component of the alcoholic drinks Goldschläger, Gold Strike and Goldwasser. Metallic gold is approved as a food additive in the European Union (EU) (E175 in the Codex Alimentarius). The acceptance of metallic gold as a food additive is due to its relative chemical inertness and resistance to being corroded or transformed into soluble salts (gold compounds) by any known chemical process which would be encountered in the human body. Soluble compounds (gold salts) such as gold chloride are toxic to the liver and kidneys. Common cyanide salts of gold such as potassium gold cyanide, used in gold electroplating, are toxic by virtue of both their cyanide and gold content. There are rare cases of lethal gold poisoning from potassium gold cyanide [46,47]. Gold toxicity can be ameliorated with chelation therapy with an agent such as Dimercaprol. Gold metal was voted Allergen of the year in 2001 by the American Contact Dermatitis Society. Gold contact
allergies affect mostly women [48]. Despite this, gold is a relatively non-potent contact allergen, in comparison with metals like nickel [49].

1.4.3 Medicine

In medieval times, gold was often seen as beneficial for the health. Even some modern esotericists and forms of alternative medicine proposed that metallic gold has healing power [50]. Some gold salts have anti-inflammatory properties and are used as pharmaceuticals in the treatment of arthritis and other similar conditions [51]. However, only salts and radioisotopes of gold are of pharmacological value, as elemental (metallic) gold is inert to all chemicals, encounters inside the body. In modern times, injectable gold has been proven to reduce the pain and swelling of rheumatoid arthritis and tuberculosis [52].

Gold alloys are used in restorative dentistry, especially in tooth restorations, such as crowns and permanent bridges. The gold alloys’ slight malleability facilitates the creation of a superior molar mating surface with other teeth and produces results that are generally more satisfactory than those produced by the creation of porcelain crowns. The use of gold crowns in more prominent teeth such as incisors is favoured in some cultures and discouraged in others.

Colloidal gold is used in research applications in medicine, biology and materials science. The technique of immunogold labelling exploits the ability of the gold particles to adsorb protein molecules onto their surfaces. Colloidal gold particles coated with specific antibodies can be used as probes for the presence and position of antigens on the surfaces of cells [53]. In ultrathin sections of tissues viewed by electron microscopy, the immunogold labels appear as extremely dense round spots at the position of the antigen [54]. Gold, or alloys of gold and palladium, are applied as conductive coating to biological specimens. The isotope gold-198, (half-life 2.7 days) is used in some cancer treatments and for treating other diseases [55].

Gold compounds are used in tumor therapy and several studies have been carried out to evaluate their anticancer activity [56,57]. Today still symptoms of rheumatoid arthritis are treated with various gold drugs (Fig. 1.5) including aurothioglucose (solganol), aurothiomalate (myocrisin), aurothiosulfate (sanocrysin), aurothiopropanol sulfonate (allocrysin) and triethylphosphinegold(I)tetraacetylthio glucose (auranofin). Rheumatoid arthritis is a painful and disabling chronic autoimmune disease causing inflammatory conditions and progressive joint erosion [58]. Gold compounds are also used as antiinfective and antiparasitic agents [59,60].

Mechanism of action of auranofin and related gold complexes: The mechanism of action of antirheumatic or antiproliferative gold complexes had been under question for a long time. Concerning its antirheumatic properties various enzymes such as cyclooxygenases had been considered as possible targets and the suppression of the activities of these enzymes as well as others has been reported.
Similarly, for auranofin and aurothioglucone, the inhibition of selenium glutathione peroxidase had been reported [61,62]. However, reports that auranofin and other gold(I) complexes potently inhibited the enzyme TrxR (Thioredoxin reductase) have most probably answered the question on the biological main target of gold complexes [63]. Auranofin inhibited TrxR with high potency and approximately 1000 fold selectivity compared to other related enzymes (glutathione reductase and glutathione peroxidase) [64]. TrxR has been identified in different species, such as the malaria parasite *Plasmodium falciparum*, *Drosophila melanogaster* and humans. The enzyme shows broad substrate specificity and is involved in numerous metabolic pathways (e.g. antioxidative network, nucleotide synthesis) and pathophysiological conditions (tumors, infectious diseases, rheumatoid arthritis etc. [65]). The active site of TrxR contains a selenocysteine (Sec) containing GlyCysSecGly motif involved in the catalytic mode of action of the enzyme. Based on the high affinity of the electrophilic gold centre of gold(I) complexes to the nucleophilic sulfur and selenium containing residues, a covalent interaction seemed likely as a mode of drug action. The preferred binding of auranofin to the selenocysteine residue was suggested based on the fact that the agent inhibited glutathione reductase, an enzyme which is structurally and functionally closely related to TrxR but lacks the Sec residue in the active site, with significantly lower affinity [63,66-70].

The cellular uptake of auranofin can in general be rated as high if compared to other metallodrugs and could be correlated with the triggered cytotoxic effects [71]. Thus, in HT29 colon carcinoma cells the intracellular gold concentration exceeded the exposure level approximately 10–50 fold (for comparison with the platinum anticancer drugs cisplatin and carboplatin, only up to 6 fold accumulation can be reached) and the gold uptake increased with increasing exposure concentrations of auranofin [40].
1.5. Copper

Copper is a chemical element with the symbol Cu (from Latin: cuprum) and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Its compounds are commonly encountered as copper(II) salts, which often impart blue or green colors to minerals. Copper is in group 11 of the periodic table, and have one s-orbital electron on top of a filled d-electron shell, which is responsible for their high ductility and electrical conductivity.

1.5.1 Chemistry

Copper forms a rich variety of compounds with oxidation states +1 and +2, which are often called cuprous and cupric, respectively [72]. It does not react with water, but it slowly reacts with atmospheric oxygen forming a layer of brown-black copper oxide. Sulfides react with copper to form various copper sulfides on the surface. Copper(II) ions are water-soluble and exist as [Cu(H$_2$O)$_6$]$^{2+}$. This complex exhibits the fastest water exchange rate (speed of water ligands attaching and detaching) for any transition metal aquo complex.

1.5.2 Toxicity

Copper toxicity refers to the consequences of an excess of copper in the body. Copper toxicity can occur from eating acid food that has been cooked in uncoated copper cookware, or from exposure to excess copper in drinking water or other environmental sources. Copper in the blood exist in two forms: bound to ceruloplasmin (85–95%) and the rest "free" loosely bound to albumin and small molecules. Free copper causes toxicity as it generates reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radical. These damage proteins, lipids and DNA [73]. Increased ceruloplasmin and copper levels in various tissues have been linked to cancer progression [74], inflammation [75], Alzheimer’s disease [76], Menkes disease [77], Wilson’s disease [78], liver and kidney damage, anemia and immunotoxicity [79]. Many of these effects are consistent with oxidative damage to membranes or macromolecules.

Acute symptoms of copper poisoning by ingestion include vomiting, hematemesis (vomiting of blood), hypotension (low blood pressure), melena (black "tarry" feces), coma, jaundice (yellowish pigmentation of the skin) and gastrointestinal distress [80]. Individuals with glucose-6-phosphate deficiency may be at increased risk of hematologic effects of copper [80]. Chronic (long-term exposure) effects of copper exposure can damage the liver and kidneys [81]. Other target organs include bone, central nervous and immune systems [82]. Mammals have efficient mechanisms to regulate copper stores such that they are generally protected from excess dietary copper levels [83]. Copper deficiency can also results
in osteoporosis, osteoarthritis, rheumatoid arthritis, cardiovascular disease, colon cancer and chronic conditions involving bone, connective tissue, heart and blood vessels [83-85].

1.5.3 Biochemistry

Copper in food (organic copper) is processed by the liver and is transported and sequestered in a safe manner. Copper in drinking water and copper supplements, largely bypasses the liver and enters the free copper pool of the blood directly. This copper is potentially toxic because it may penetrate the blood/brain barrier [86].

Table 1.3: Key copper-containing enzymes and their functions

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine oxidases</td>
<td>Group of enzymes oxidizing primary amines (e.g., tyramine, histidine and polyamines)</td>
</tr>
<tr>
<td>Ceruloplasmin (ferroxidase I)</td>
<td>Multi-copper oxidase in plasma, essential for iron transport</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>Terminal oxidase enzyme in mitochondrial respiratory chain, involved in electron transport</td>
</tr>
<tr>
<td>Dopamine β-hydroxylase</td>
<td>Involved in catecholamine metabolism, catalyzes conversion of dopamine to norepinephrine</td>
</tr>
<tr>
<td>Hephaestin</td>
<td>Multi-copper ferroxidase, involved in iron transport across intestinal mucosa into portal circulation</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>Cross-linking of collagen and elastin</td>
</tr>
<tr>
<td>Peptidylglycine alpha-amidating mono-oxygenase (PAM)</td>
<td>Multifunction enzyme involved in maturation and modification of key neuropeptides (e.g., neurotransmitters, neuroendocrine peptides)</td>
</tr>
<tr>
<td>Superoxide dismutase (Cu, Zn)</td>
<td>Intracellular and extracellular enzyme involved in defense against reactive oxygen species (e.g., destruction of superoxide radicals)</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Enzyme catalyzing melanin and other pigment production</td>
</tr>
</tbody>
</table>

About 50% of the average daily dietary copper is absorbed from the stomach and the small intestine. Absorbed copper is transported to the liver in portal blood bound to albumin and is transmitted to peripheral tissues mainly bound to ceruloplasmin and, to a lesser extent, albumin. Excess copper is excreted in bile into the gut, and the faecal copper output is the sum of unabsorbed dietary copper and that reexcreted into the gut [87]. Copper is incorporated into a number of metalloenzymes involved in hemoglobin formation, drug/xenobiotic metabolism, carbohydrate metabolism, catecholamine biosynthesis and the cross-linking of collagen, elastin and hair keratin as well as in the antioxidant defence mechanism [86]. Moreover, copper-dependent enzymes such as cytochrome-c oxidase, superoxide dismutase, ferroxidases, monoamine oxidase and dopamine β-monooxygenase, function to reduce reactive oxygen species (ROS) or molecular oxygen [86].
1.5.4 Medicine

Copper(II) ions are water-soluble, where they function at low concentration as bacteriostatic substances, fungicides and wood preservatives. It is an essential trace nutrient to all higher plant and animal life at lower concentrations. Copper is believed to possess anti-inflammatory activity [89,90]. Copper-alloy touch surfaces have natural intrinsic properties to destroy a wide range of microorganisms (e.g., *E. coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus*, *Clostridium difficile*, influenza A virus, adenovirus and fungi). The use of SOD, a copper based enzyme, as a pharmaceutical has been proposed for treatment of a number of diseases including hyperoxia, reperfusion injury, auto-immune deficiency disease (AIDS), ulcerative colitis, bronchopulmonary dysplasia in premature neonates, as well as inflammation and inflammation-associated diseases, such as rheumatoid arthritis and osteoarthritis. [91] Copper compounds are believed to possess antiproliferative, anticancer, protease mimetics, antiamoebic, antitrypanosomal, proteosome inhibitor, apoptosis inducer and antibacterial activity.

1.5.5 Superoxide dismutase (SOD)

Superoxide dismutases, discovered by Irwin Fridovich and Joe McCord, are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen.

There are three major families of superoxide dismutase exist, they are proteins cofactored with copper and zinc, or manganese, iron, or nickel.

![Figure 1.6: Crystallographic structure of the human SOD1 enzyme (rainbow colored N-terminus = blue, C-terminus = red) complexed with copper (blue-green sphere) and zinc (grey spheres)](image1)

![Figure 1.7: Structure of the active site of human superoxide dismutase 2 (mitochondrial SOD 2)](image2)

![Figure 1.8: Crystallographic structure of the tetrameric human SOD3 enzyme (cartoon diagram) complexed with copper and zinc cations (orange and grey spheres respectively)](image3)
1. **Cu-Zn-SOD**: most commonly used by eukaryotes, located in cytoplasm, available commercially and is normally purified from the bovine erythrocytes.

2. **Fe-SOD or Mn-SOD**: is human mitochondrial SOD enzyme and is used by prokaryotes and protists.

3. **Ni-SOD**: is extracellular and is used by prokaryotes.

**Cu-Zn-SOD**: The Cu-Zn enzyme is a dimer of molecular weight 32,500. The bovine Cu-Zn protein (153 amino acid protein) was the first SOD structure solved in 1975. It is an 8-stranded "Greek key" beta-barrel, with the active site held between the barrel and two surface loops. The two subunits are tightly joined back-to-back, primarily by hydrophobic and some electrostatic interactions. The ligands of the copper and zinc are six histidine and one aspartate side-chains; one histidine is shared between the two metals.

ROS (Reactive Oxygen Species) is an atom or molecule that contains one or more unpaired electrons. ROS are highly reactive and are likely to take part in chemical reactions. Their reactivity can participate in unwanted side reactions resulting in cell damage. Because superoxide has an unpaired electron, it can scoop up a hydrogen or electron from any macromolecule (lipids, amino acids or proteins) to complete its outer shell. The atom robbed of an electron or hydrogen then gets one from a neighbour, starting off a chain reaction down the macromolecule and between macromolecules that disrupts their structure and function.

**Figure 1.9: SOD function**

$\text{O}_2^-$ is the most abundant free radical, easily convertible to other. It is also a direct byproduct of metabolism of glucose in mitochondria via Krebs cycle. Excess of ROS may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease like aging, heart disease, cancer, inflammatory-immune injuries, rheumatoid arthritis, diabetes, AIDS, lupus, Alzheimer’s disease, adult respiratory distress syndrome and more. Antioxidant compounds scavenge ROS and inhibit the oxidation of bio-molecules leading to degenerative diseases. Defect in antioxidant system of RBC leads to hemolytic anemia, in which RBC count in blood decreases.
1.6. Deoxyribonucleic acid (DNA): History, structure and properties

Deoxyribonucleic acid or DNA, is the hereditary material used in the development and functioning in humans and almost all other organisms. The DNA segments that carry this genetic information are called genes. Gene serve as instruction books for making functional molecules such as ribonucleic acid (RNA) and proteins, which perform the chemical reactions in our bodies. Nearly every cell in a person’s body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

![Schematic diagram of double helix DNA]

Figure 1.10: Schematic diagram of double helix DNA

The Swiss physician Friedrich Miescher first observed DNA in the late 1800s [92]. Rosalind Franklin, a physical chemist working with Maurice Wilkins at King's College in London, was among the first to use X-ray diffraction method to analyze genetic material [93]. Her experiments produced what were referred to at the time as "the most beautiful X-ray photographs of any substance ever taken."

Other scientists, including zoologist James Watson and physicist Francis Crick, both working at Cambridge University in the United Kingdom, were trying to determine the shape of DNA too. Ultimately in 1953, this line of research revealed one of the most profound scientific discoveries of the century that DNA exists as a double helix [94]. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C) and thymine (T). Human DNA consists of about 3 billion bases and more than 99 percent of those bases are the same in all people. The order or sequence of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and
sentences. DNA bases pair up (complementary sequence) with each other, A with T and C with G, to form units called base pairs.

![Image of DNA structure](image)

**Figure 1.11: Double helix DNA with labelled specifications and base pair structures**

A nucleobase linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder’s rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder. An important property of DNA is that it can replicate or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell. A DNA chain, also called a strand, has a sense of direction, in which one end is chemically different than the other. The so-called 5' end terminates in a 5' phosphate group (\(-PO_4\)); the 3' end terminates in a 3' hydroxyl group (-OH). This is important because DNA strands are always synthesized in the 5' to 3' direction.

In spite of the enormous versatility of living creatures, and accordingly variability of genetic texts that DNA molecules in different organisms carry, they all have virtually identical physical and spatial structure. Seven major types of structures of DNA have been reported in the literature i.e. B-DNA, B'-DNA [95], A-DNA [96], Z-DNA [97], ps-DNA [98], triplexes [99-103] and quadruplexes [95,104]. Sequences of the two strands of the double helix obey the complementary principle.

### 1.7. DNA-drug interaction

The drugs (DNA binding species/ligands) can interact with DNA, primarily, in the following four ways:
1.7.1 Groove binding

Groove binding drugs, reviewed in detail by Zimmerman and Wahnert (1986), generally interact with the DNA minor groove. Groove binding involves the direct interactions of generally elongated crescent shaped molecules with the edges of the base pairs in either the major or minor grooves, and is dependent on the size, hydration and electrostatic potential of both DNA grooves. The exact location of binding is largely dependent on a favourable combination of these interactions, as well as the potential for hydrogen bonding. The organic anti-tumour drug netropsin has been shown (Fig. 1.12) to bind within the DNA minor groove [105]. The drug is held in place by amide hydrogen bonds to adenine N-3 and thymine O-2 atoms [105]. Once bound, it widens the groove slightly, but does not unwind or elongate the double helix.

1.7.2 Intercalation

It was first proposed by Lerman (1961) [106]. Intercalating molecules associate with DNA (from either the major or minor groove) through the insertion of their planar fused aromatic moiety between the base pairs of DNA. This interaction is stabilized by stacking between the aromatic moiety of the intercalator and the DNA base pairs. Optimal intercalating molecules are planar and contain three or four fused aromatic rings with a surface area of 28 Å² [106].

Intercalating molecules generally contain charged groups or metal ions, which make intercalation more favourable through electrostatic interactions between the intercalating molecule and the DNA [106]. The insertion of an intercalator forces the base pairs apart, increasing the base-to-base distance from 3.4 Å to 6.8 Å. The consequences of this increase in inter-base distance are unwinding and extension of the DNA backbone and loss of the regular helical structure. Unlike covalent binding, intercalation is reversible [107].
1.7.3 Electrostatic binding

Electrostatic or external binding occurs when cations or cationic molecules are attracted to the anionic surface of DNA. Ions and charged metal complexes such as Na\(^+\) associate electrostatically with DNA by forming ionic or hydrogen bonds along the outside of the DNA double helix [108,109]. An interesting example of the electrostatic DNA binding is \([\text{Co(NH}_3)_6\text{]}^{3+}\) [110].

1.7.4 Covalent and coordinate covalent binding

It occurs via replacement of the labile ligands by covalent binding to nitrogen base of the DNA. Such type of binding is observed for the case of complex where easily replaceable ligands like halides, aquo etc. are present. Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA processes and subsequently cell death. The widely used anticancer drug cisplatin obtains its cytotoxicity by forming coordinate covalent DNA intrastrand and interstrand cross-links as well as protein-DNA cross links in the cellular genome [111-115]. The complex \(\text{mer-}[\text{Ru(tpy)}\text{Cl}_3]\) \((\text{tpy} = 2,2':6',2''\text{-terpyridine})\) has been found to be active as a cytostatic drug in L1210 murine leukaemia cells, with an activity comparable to that of cisplatin [116]. \(\text{mer-}[\text{Ru(tpy)}\text{Cl}_3]\) is able to bind two sequential guanine residues in a trans-configuration, forming coordinate covalent interstrand cross-links with DNA.

Several techniques have been employed to measure the specific interaction between drugs and DNA i.e. X-ray diffraction [117], electron microscopy [118], NMR spectroscopy [119], Raman spectroscopy [120], circular dichroism [121], UV-vis. spectroscopy [122], fluorescence spectroscopy [123], thermal DNA denaturation [124], relative viscosity measurement [125], atomic force microscopy [126], gel electrophoresis [127] and surface plasmon resonance (biosensor technology) [128].

1.8. Literature Survey

In the field of medical chemistry, inorganic materials are gaining attention owing to their diverse molecular and unique spectral features [129–131]. Recent literature reveals that the metal based inorganic architecture displays a versatile utility from synthetic scaffolds for the preparation of small molecular therapeutics [132,133] to gene delivery vectors in the field of molecular biotechnology [134,135].
Hoti et al., reported that heterobimetallic complexes display efficient chemotherapeutic ability both in vitro (in different cell lines) [136] and in vivo. Weder et al., reviewed the biochemical action of copper complexes with non-steroidal anti-inflammatory drugs (NSAIDs) and showed enhanced anti-inflammatory and antiulcerogenic activity, as well as reduced gastrointestinal toxicity of complexes compared to the uncomplexed drug [137]. Tisato et al., reviewed the potential chemotherapeutic properties of copper based compounds [138]. Noyce et al., reported that infectivity of influenza A virus is reduced after exposure on copper surfaces [139]. The mechanism of this process is only partly understood, but it has been speculated that the degradation of the viral nucleic acid takes place after the intervention of copper ions. Lebon et al., reported that development of copper complexes could be helpful in design and production of antiviral and antibacterial materials, able to deactivate HIV or H1N1 viruses [140] and antibiotic resistant bacteria, respectively. Towards this direction, a method of producing copper impregnated materials that possess broad spectrum antimicrobial properties was reported by Borkow and Gabbay [141]. Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with bidentate Schiff bases derived using heterocyclic ketone and their antimicrobial study were reported by Singh et al [142]. The Fe(III), Ni(II), Co(II) and Cu(II) complexes with thiazoline and their fungicidal activity were evaluated by Sangal et al [143]. Eight salicylhydroxamic acids and their metal chelates with Cu(II), Ni(II), Co(II), Fe(III), Mn(II) and Zn(II) were screened for their antifungal activities by Khadikar et al [144]. Andrade et al., reported that neutral bimetallic complex [Cu2(ibuprofen)4] with the bridged ligand 2-(4-isobutylphenyl)propionate (ibuprofen) exhibits antiulcerogenic in vivo activity as estimated for gastric irritation in rats [145]. H. Elo reported that copper(II) chelates of salcylaldoxime and resorcyaldoxime [146] as potent antiproliferative agents, exhibit strong cytotoxic effects comparable to that of adriamycin, by inducing cell cycle arrest and apoptosis. Their action may involve the inhibition of the enzyme topoisomerase II activity, by preventing dimer formation of the enzyme and its interaction with DNA [147]. Binuclear copper(II) complexes of pyridyl-diamines as well as mixed-ligand acetylacetone/quinoxaline complexes exhibiting nuclease and apoptosis inducing activity have been reported recently [148,149]. In addition the antitumor activity of Schiff-base copper(II) complexes were investigated [150,151]. The evaluation of a new thiosemicarbazone copper(II) chelate was reported to induce tumor growth inhibition both in vitro and in vivo, through oxidative/endoplasmic reticulum stress [152].

Quinolone are antimicrobial agents with a broad range of activity against both Gram(–ve) and Gram(+)ve bacteria [153,154]. The first member of the quinolone carboxylic acid family of antimicrobials introduced into clinical practice was nalidixic acid, used in the treatment of urinary tract infections. The ciprofloxacin and norfloxacin have been used to treat resistant microbial infections [155]. The participation of intracellular metals in the activity of quinolones is strongly
influenced by the metal environment, which can give rise to dramatic effects on their physicochemical properties [156]. Dreveňsek et al., proposed that copper(II) is not directly involved into quinolone binding to DNA via phosphate groups [157]. Song et al., pointed out that quinolone–copper(II) binary complex can interact with DNA by intercalative binding mode, and copper(II) plays an intermediary role [158]. From theoretical and experimental studies on the structural activity of certain quinolones, and the interaction of its copper(II) complexes on a DNA model, it was suggested that the intercalation of the quinolone complexed to a metal is an important step in these processes [159]. Synthesis of fluoroquinolone metal complexes were carried out as an attempt to clarify their physico-chemical properties where as some antibacterial activity studies showed that the complexes allowed the alteration of the potency and specificity of fluoroquinolones [160-163]. Low molecular weight copper(II) complexes were proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers and cancers [164-166]. The role of transition metals, how they exert their action on biological systems and the coordinative behavior of quinolone drugs in complexes of the type [Cu(phen)(antib)]⁺ (where antib is a quinolone or fluoroquinolone) was explored [167,168]. Ramirez-Ramirez et al., demonstrated that [Cu(phen)(nal)]⁺ interacts in vivo with bacterial DNA and maintains its structural integrity [169]. Complexation of ciprofloxacin with the metal cations and how this complexation affects ciprofloxacin bioavailability and antimicrobial activity were extensively studied by Turel et al [170]. Ma et al., reported that the presence of metal ions results in a higher ciprofloxacin uptake by bacterial cells compared to that of the drug alone [171] and it was also found that different metal cations reduce its in vitro antibacterial activity to varying extents [172]. Lomaestro and Bailie reported that reduction in bioavailability is a consequence of metal complex formation in the gastric system using carboxylate functional groups of the molecule [173]. Also it was reported by Polk et al., that zinc reduced bioavailability by an average of 24% [174]. Mendoza et al., designed and synthesized series of ternary complexes of quinolone as an attempt to understand how coordination affects the activity of quinolone [175]. Method for the determination of fluoroquinolone (levofloxacin, ciprofloxacin, norfloxacin) by ion pair complex formation with cobalt(II) tetrathiocyanate was reported by El-Brashy et al [176]. First nanosized neutral cavity of vanadium based copolymer of norfloxacin was reported by Chen et al [177]. Perchlorate complexes of ciprofloxacin and norfloxacin with magnesium, calcium and barium were studied by Al-Mustafa [178]. Toxicological studies of some iron(III) complexes with ciprofloxacin were studied by Obaleye et al., and concluded that the toxic effect of drugs like ciprofloxacin can be reduced by complexation [179]. The process of complexation completely inhibited the crystallinity of ciprofloxacin which is helpful in control release therapy, was studied by Pisal et al [180]. Effect of pH on complexation of ciprofloxacin and Zn(II) under aqueous condition was studied by Zupancic et al [181]. Son et al.,
reported that the binding mode of quinolone to DNA was just like a classical intercalator [182].

Medical properties of gold and gold based compounds have been explored throughout the history of civilization. The earliest therapeutic application of gold can be tracked back to 2500 B.C. in China where gold was used to treat smallpox, skin ulcers, and measles [183]. The first gold complex used for medicinal purpose was gold cyanide, employed by bacteriologist Robert Koch to kill the mycobacteria, the causative agent of tuberculosis [184]. In the early 1930s, the French physician Jacque Forestier introduced the gold thiolate complexes for the treatment of rheumatoid arthritis, a condition which he believed was related to tuberculosis [185]. Champion et al., reported first gold(I) thiolates (aurothiomalate (ATM) and aurothioglucose (ATG)), used to treat rheumatoid arthritis, administered by an intramuscular injection [186]. Although the gold complexes were rapidly cleared from plasma, much of the drug was still distributed to organs throughout the body. The highest concentration of gold accumulates in kidneys, resulting in nephrotoxicity [183]. Many gold complexes with triethylphosphine have optimal pharmacological activity in models of rheumatoid arthritis and one of the complexes, named auranoﬁn, [tetra-o-acetyl-β-d-(glucopyranosyl)thiol(triethylphosphine)gold(I) was developed as a new drug for the rheumatoid arthritis treatment [187]. Additionally, current research has described promising results using gold complexes to treat malaria [188], Chagas disease [189] and cancer [190]. Mirabelli et al., reported in vitro cytotoxic potency and in vivo antitumor activity of auranoﬁn analogs of gold(I) coordination complexes [191]. However, the complexes were completely inactive against solid tumors [192]. The main observations from the experiments were that (i) lack of potency in vitro correlated well with lack of antitumor activity; (ii) potent cytotoxicity in vitro did not necessarily translate into antitumor activity in vivo; and (iii) in vivo anti-tumor activity optimized by ligation of Au(I) with a substituted phosphine. Based on that, a series of gold(I) complexes with 1,2-bis(diphenylphosphine)ethane (DPPE), were synthesized and found to confer in vitro cytotoxic activity especially in some cisplatin resistant cell lines by Sadler and Sue [193]. Surprisingly, mechanistic studies suggested that, in contrast to cisplatin, DNA was not the primary target for the gold(I) complexes and that their cytotoxicity was mediated by their ability to alter mitochondrial function and inhibit protein synthesis by interfering with DNA–protein interactions. A series of gold(III) complexes with 2-[(dimethylamino)methyl][phenyl] (damp) ligand were synthesized by Fricker and evaluated their cytotoxic activity against several human cancer cell lines [194]. Another class of gold(III) complexes with N,N-dimethylbenzylamine, was synthesized by Dinger and Henderson [195,196]. In vitro activities of a series of gold(III) complexes such as [Au(en)2]Cl3, [Au(dien)Cl]Cl2, [Au(cyclam)](ClO4)Cl2, [Au(terpy)Cl]Cl2 and [Au(phen)Cl2]Cl, against the A2780 ovarian cancer cell line and a cisplatin-resistant variant were described [197,198]. The relative order of cytotoxicity was: Au(terpy)> >Au(phen)>Au(en), Au(dien) >>Au(cyclam). Calamai
et al., tested a group of square planar gold(III) complexes containing at least two gold–chlorine bonds in cis-position [199], while Bruni et al., synthesized four gold(III) complexes: trichloro(2-pyridylmethanol)gold(III), dichloro(N-ethylsalicylaldiminato)gold(III), trichlorodiethylendiaminegold(III) and trichlorobisethylenediaminegold(III) [200]. The complexes showed significant cytotoxic effects against the A2780 human ovarian cancer cell line, comparable to or even greater than cisplatin, and were able to overcome resistance to cisplatin to a large extent [200]. Buckley et al., synthesized gold(III) complexes with damp ligand and tested against a panel of cancer cell lines in vitro and in vivo [201]. Kim et al., suggested that the anti-inflammatory effect of auranofin was associated with inhibition of Janus family of tyrosine kinase/signal transducer and activator of transcription (JAK1/STAT3) signalling pathway [202]. Wang et al., demonstrated the role of mitochondria in apoptotic cell death induced by gold(III) porphyrin complexes in human nasopharyngeal carcinoma cell lines [203]. Saggioro et al., confirmed that gold(III) dithiocarbamates exert their cytotoxic effects, at least partly, by stimulating production of ROS [204]. Ronconi et al., synthesized new gold(III) dithiocarbamate derivatives with defined coordination geometry [205].

1.9. Rational

To know specific interactions between therapeutic agent and nucleic acids (i.e. covalent bonding, groove binding, intercalation and electrostatic interactions) is the basic requirement for drug design. The concept of gene targeting could not be formulated unambiguously, until the realizations that DNA could provide fine receptors for drugs. Gene targeting, similar to the molecular recognition is the key in receptor–ligand, antigen–antibody and DNA–protein binding study. The employments of functionalized chromophore/ building blocks with complementary motif analogous to nucleic acid base pair are being exploited to study the binding between small molecules and biomolecules.

Metal complexes with vacant coordination sites have been frequently used for research on molecular recognition and in the development of chemosensors. Coordination of ligand to metal ion can be exploited effectively for binding and signalling purposes in chemosensors. Metal complexes have a role to play in the world of medicine because approximately one third proteins and enzymes use metal ion for their proper functioning. Inorganic compounds have been used in medicine for many centuries, but only little attempts are made to design the compound to be used with little or no understanding of their mechanism of action. Metal ions are often classed as toxic or nontoxic, however their biological activity depends very much on speciation and it is now widely accepted that, with carefully controlled coordination chemistry, even toxic metals can exhibit therapeutic properties. Among all the mechanism of detoxification, chelation by various ligands or proteins like metallothioneins or phytochelatins provides greater resistance to the toxic effects of the heavy metals. Metal ions and coordination compounds are known to affect the
cellular processes dramatically, not only by affecting the natural processes like cell division, gene expression etc., but also by some non–natural processes like toxicity, carcinogenicity, antitumour effect etc.

Copper is an essential nutrient to all higher plant and animal at lower concentration. In animals, including humans, it is found widely in tissues like liver, muscle, bone etc and also act as a co–factor in functioning of various enzymes and copper based pigments. Copper found in blood stream is either in erythrocytes or associated with superoxide dismutase (SODs: the metalloenzymes present in living organism involving oxygen as a part of metabolism). It eliminates the superoxide radical (O$_2^-$) generated during metabolism which can cause significant cellular damage. Copper and its compounds are also known for their antibacterial activity. Gold complexes are being studied intensively for their anticancer properties. Gold is seen as a promising metal after platinum due to its kinetics and timescales comparison to cellular division processes. Therefore, it can bind to biomolecules such as serum–albumin and transferring.

In accumulation of above facts and with aim to design artificial metallonucleases and therapeutic applicable molecules (antibacterial and antitumor), we planned to synthesize metal complexes of gold and copper metal ions and tested for their diverse biological activity like DNA interaction, SOD mimic, cytotoxic and antibacterial activity.

1.10. Objectives

- In order to check coordination effect of biologically active copper(II) and gold(III) metal ions, mononuclear drug based copper(II) complexes with bidentate ligands and gold(III) complexes with bidentate/tridentate ligands have been synthesized.
- The ligands and complexes have been characterized using various analytical and spectroscopic techniques.
- Determination of antibacterial activity (MIC) of metal complexes in vitro against Gram$^{-ve}$ and Gram$^{+ve}$ bacteria.
- To access binding behaviour of complexes with DNA.
- Study of artificial metallonucleases for proper understanding of DNA cleavage mechanism.
- Study of superoxide dismutase (SOD) like activity of copper(II) complexes.
- Evaluation of cytotoxic properties of metal complexes.
1.11. Outline of thesis

Synthesis, characterization and biological activities of copper(II) and gold(III) complexes

Ciprofloxacin
Bipyridines (A₁⁻A₁²)
CuCl₂·2H₂O

Elemental analysis, magnetic measurement, TGA, reflectance, IR and FAB-mass

Copper(II) complexes

Nature: Neutral
Geometry: Square pyramidal

Bidentate/ tridentate
N-donor ligands (A₃³⁻A₄¹)
HAuCl₄·3H₂O

Elemental analysis, IR, LC-MS, ¹H and ¹³C-NMR spectroscopy

Gold(III) complexes

Nature: Ionic
Geometry: Square planar

Synthesis
Characterization

Applications

DNA binding
Nucleolytic
Antibacterial
Cytotoxicity
SOD mimic

Findings

Intercalation
Kₘ = 1.1 - 1.9 x 10⁴ M⁻¹

Potent nuclease
MIC = 0.3 – 2.3 µM
LC₅₀ = 4.9 – 12.0 µM
IC₅₀ = 0.6 – 1.5 µM

Applications

DNA binding
Nucleolytic
Cytotoxicity

Findings

Covalent binding
Kₘ = 1.3 - 4.4 x 10⁴ M⁻¹

Potential nuclease
% Cleavage = 77 - 87

LC₅₀ = 6.8 – 19.5 µM
1.12. References


[48] Henna tattoo ingredient is Allergen of the Year, Clinical Rounds (2009).