Chapter 1

INTRODUCTION
Hippocrates, 25 centuries ago, had named malignant tumors in the human body as
*karkinoi* (crab) because the swollen blood vessels around the tumor mass gives it the appearance of a crab. Cancer has been with man all along his existence. This disease, characterized by abnormal and uncontrolled division of cells, arises due to genetic instabilities that develop over the years in the DNA of these cells. Increased life expectancy, coupled with the increase of numerous environmental pollutants and mutagens has cumulated the genetic aberrations, which increased the incidence of cancer. Inheritance of these genetic aberrations has also turned it into a potential inheritable disease.

**Cancer in the world:**

Cancer is the second common cause of death in the developed countries next to cardiovascular diseases. In Europe and North America, approximately one in five people die of cancer. Such is the widespread mortality of cancer that according to a WHO estimate, out of 50 million deaths annually in the world, more than 5 million are attributed to cancer, and this number is rapidly increasing.

**Cancer in India:**

Increase of human life span in India over the last 5 decades has also increased the incidence of cancer. Approximately, 500,000 new cases of cancer are reported every year and according to a survey conducted by the Indian Cancer Society, 1.5 million people suffer from this disease at any given point of time. Oral and Cervical cancer are the major cancers in this country.
Cancer Therapy:

Though great strides are being made in unraveling the molecular basis of carcinogenesis, this knowledge has not yet been translated into a complete and effective cure for cancer. Whatever the treatment procedure adopted, there is always a doubt of an incomplete cure or a relapse. Presently, cancers are treated by the following methods depending upon the site and stage of the disease.

1. Surgery
2. Radiation therapy
3. Chemotherapy
4. Hormonal therapy

Chemotherapy is the treatment of choice because of its effectiveness on various types of cancers. It is used as a combination regimen of different drugs or as an adjunct to surgery and radiation.

Development of Chemotherapeutics:

Traditionally, cancer chemotherapeutics were discovered through random large-scale screening of synthetic chemicals and natural products against tumor systems, primarily murine leukemias. Advances in molecular cell biology and knowledge of the macromolecules involved in cellular functions has lead to the identification of target sites and mechanisms of action of these drugs. An overview of different chemotherapeutic drugs and their sites of action is presented in panel 1. The identification of specific
intracellular targets for various drugs has paved the way for the development of drugs targeted at these sites of action.

**PANEL 1**

![Diagram of intracellular targets for various drugs]
**Anticancer targets:**

A large number of anticancer drugs target DNA at various stages, causing lethal genetic aberrations, which results in death of the cancer cells. Unfortunately, this generalized action takes a huge toll on normal cells too, which narrows the therapeutic index of these agents. In the light of this drawback, it would be more meaningful to develop drugs which target a specific molecule involved in cancer progression, without whose function, the division of cancer cells, but not normal cells will be grossly affected. Numerous such cellular targets have been identified and have become the basis for the development of cancer therapeutics. Some of these anticancer targets are-

- Adenosine deaminase
- Ribonucleotide reductase
- Dihydrofolate reductase
- Farnesyl transferase
- Topoisomerases
TOPOISOMERASES

DNA is a very dynamic molecule and during the lifetime of a cell, it constantly undergoes various topological changes without affecting its genetic makeup. Numerous topological problems like negative/positive supercoiling and catenation arise in DNA during replication and transcription. This causes intertwining of DNA that has to be resolved in order to maintain normal functioning of the genome. Topoisomerases resolve this intertwining and thus maintain genome integrity (Wang, 1985, 1991, 1996; Prus and Drlica, 1986; Watt and Hickson, 1994). The enzymes are also involved in decatenation of DNA in the G2 phase of cell division for separation of newly replicated chromatids (Downes et. al., 1994). In the M phase, they help in chromosome condensation and segregation (Adachi et. al, 1991).

The catalytic activity of these enzymes typically involves breaking one strand (topoisomerase I) or both strands (topoisomerase II) of a duplex DNA segment and passing the other strand in case of a single strand break or a duplex DNA segment in case of a double strand break through a gate created by the broken DNA strand(s), and then resealing the broken strands. The strand passage reaction is central to the various functions of topoisomerasers.
CLASSIFICATION OF TOPOISOMERASES:

Topoisomerases in general, are of two types in both prokaryotes and eukaryotes. They are the type I topoisomerases and type II topoisomerases (Reviewed by Roca, 1995; Wang, 1996).

Type I topoisomerases: These are monomeric enzymes which do not require ATP for their activity. They change the linking number of DNA in steps of one \( \text{linking number is the number of right handed turns that one DNA strand makes around the other in a DNA duplex} \). The type I topoisomerases are of two types.

Topoisomerase I- 5': Molecular weight of this protein is \(~97\text{ kDa}\). This enzyme binds to a single strand of duplex DNA and forms an enzyme-DNA intermediate through a covalent bond between a tyrosine residue of the enzyme and the 5'-phosphate at the DNA break site.

**Functions:** Partial relaxation of negatively supercoiled DNA and knotting of single stranded DNA rings into a double stranded ring.

**Examples:** E. coli DNA topoisomerase I, III and eukaryotic topoisomerase III.

Topoisomerase I- 3': This is a 95-135 kDa protein. It is similar to the 5' enzyme but binds preferentially to double stranded DNA and cleaves a single strand of DNA. It forms a phosphorytyrosyl linkage between a tyrosine residue of the enzyme and the 3'-phosphate at the break site. The unbroken strand is passed through this break to release the twisting stress on the helix.
Functions: complete relaxation of both positive and negative supercoils in DNA.

Examples: eukaryotic topoisomerase I, vaccinia virus topoisomerase I and topoisomerase V of hyperthermophilic bacteria.

Type II topoisomerases: These are essential enzymes for the life of all organisms. They are dimeric molecules and are ATP dependent. These enzymes change the linking number of DNA in steps of 2. Depending on whether they are prokaryotic or eukaryotic, they are divided into two types.

DNA gyrase: This is the prokaryotic topoisomerase II. Its catalytic activity is the same as that of the eukaryotic enzyme as described below.

Functions: Preferential relaxation of positive supercoils and induction of negative supercoiling in the bacterial chromosome and extrachromosomal DNA (plasmids).

Examples: All the prokaryotic type II topoisomerases and topoisomerase IV fall under this category.

Topoisomerase II: This is the eukaryotic equivalent, which is a 160-180 kDa protein and is highly conserved in all organisms. It binds to duplex DNA and breaks both the strands, 4 base pairs apart. The 5' broken ends are covalently bonded to two tyrosine residues (one from each monomer) through phosphotyrosyl linkages. Additional interactions restrict free rotation of the free 3' ends at the break site. A second duplex segment is transported through this break.
Functions: If the gated and transported segments reside in the same DNA segment, this enzyme catalyzes relaxation of supercoils or knotting/unknotting of DNA. If they are in different DNA segments, the enzyme catalyzes their catenation or decatenation.

Examples: All the eukaryotic topoisomerase II enzymes.

Human topoisomerase II: In humans and also most mammals, two genetically distinct isoforms of topoisomerase II have been detected (Drake et. al., 1987). The first type is the topoisomerase IIα, which is a ~170 kDa protein, similar to the topoisomerase II of all eukaryotes and performs all the functions of the typical type II enzymes (Woessner et. al., 1990). The second is topoisomerase IIβ, which shares a sequence homology of 68% with the α isoform and has a molecular weight of ~180 kDa. The two isoforms appear to have arisen from a recent gene duplication event which included several flanking markers like the retinoic acid receptor α and β genes (Coutts et. al., 1993). The α isoform has been mapped to chromosome 17q21-22 (Tsai-pflugfelder et. al., 1988) while the β enzyme is on chromosome 3p24 (Tan et. al., 1992). The two isoforms show distinct patterns of expression during cell cycle and also during oncogenic transformation (Woessner et. al., 1990, 1991). While the α enzyme is over-expressed during the G2 and M phases of the cell cycle, the β enzyme is constant throughout. The functions of the β isoform are not clearly known, but it is believed that it may be required for housekeeping functions during the resting phase of the cell cycle, while the α enzyme is required for cell cycle progression and for fast growth and division of cancer cells.
STRUCTURE, CATALYTIC ACTIVITY, FUNCTIONS AND REGULATION OF TOPOISOMERASE II

Among the eukaryotic topoisomerases, topoisomerase II is the major target for some of the most important anticancer drugs. This is comprehensible because this enzyme is absolutely essential for viability of cells; though the function of topoisomerase I can be replaced by topoisomerase II, the latter enzyme's function cannot be replaced in most cases (DiNardo et al., 1984; Uemura and Yanagida, 1986). A closer look at the structure and functions of topoisomerase II will explain its importance in the growth and division of living cells.

Structure of topoisomerase II:

The crystal structure of topoisomerase II was worked out in detail by Berger et al. in 1996. The study shows that topoisomerase II in its active form is a heart shaped homodimer with a large central hole (panel 2). The monomer is a flat crescent shaped fragment which can be distinguished into three discrete domains. The first is the ATP binding domain in the N-terminal region (B' region). It has a consensus sequence for ATP binding and has the capacity to hydrolyze ATP. This domain dimerizes with the other monomer upon binding of ATP, and imposes a conformational change all over the enzyme, required for catalytic activity. The second is the DNA binding domain or the DNA breakage/reunion domain, present in the A' region. The active site tyrosines which associate with the broken ends of DNA during the catalytic cycle are present in this domain. The third is the primary dimer interface in the C-terminal region which forms the
dimer interface of the enzyme by associating with the other monomer. Apart from forming the dimer interface, this region is also implicated in regulation of enzyme activity and nuclear localization.

**PANEL 2**

**CRYSTAL STRUCTURE OF TOPQISOMERASE II**

(reproduced from Berger et al., 1996)
Catalytic Activity:

In its catalytic cycle shown in panel 3 (described by Berger et al, 1996), the topoisomerase II dimer first binds to a duplex DNA segment termed as the ‘G’ (gated) segment and undergoes a conformational change. It then binds to ATP with the ATP binding domain and also binds to a second DNA segment called the ‘T’ (transported) segment. This binding causes a series of conformational changes in the enzyme, which causes the $A'$ regions to be pulled apart from each other, leading to cleavage of the G-segment in both the strands, four base pairs apart. The active site tyrosines in the DNA binding domains then form covalent bonds with the nicked DNA strands through a trans-esterification reaction between the phenolic hydroxyl groups of the tyrosines and the 5-phosphoryl ends of the nicked DNA. Concomitantly, the ATP domains dimerize and the T-segment is transported through the gate formed by the nicked DNA into the central hole. Following this transport, the G-segment is rejoined by a second trans-esterification reaction and the T-segment is transported out of the enzyme through the opening formed in the dimer interface. The monomers immediately dimerize at the interface and the ATP is hydrolyzed and released. This regenerates the starting state and the enzyme is ready to begin a fresh catalytic cycle.

The DNA cleavage/religation reactions do not require energy from a high energy co-factor (like ATP) because the phosphate bond energy is conserved in the two successive trans-esterification reactions (Roca, 1995). The ATP binding and hydrolysis is involved in introducing conformational changes in the enzyme for carrying out its catalytic functions and not for DNA nicking and resealing.
Topo II binds to the ‘G’ segment of DNA and undergoes a conformational change. Upon binding of a second segment (‘T’ segment) and ATP, the enzyme further undergoes conformational changes leading to generation of double strand breaks in the ‘G’ segment and passage of the ‘T’ segment through the gate formed by the broken strand into the large central hole in the enzyme. Following this transport, the ‘G’ segment is rejoined and the ‘T’ segment is released out of the enzyme through an opening of the primary dimer interface. At this stage, ATP is hydrolyzed and the enzyme starts a fresh reaction cycle.
Functions of topoisomerase II:

Resolving the need for a *Molecular Swivel* : The local unwinding of the DNA helix during DNA replication and transcription leads to positive supercoiling ahead of the advancing fork and negative supercoiling in the region behind the fork (Lockshon and Morris, 1983, Liu and Wang, 1987). This causes torsional stress on the DNA, which is removed either by topoisomerase I or II by relaxing the positive and negative superhelices (Kim and Wang, 1989).

Decatenation of replicated chromatids and segregation : DNA replication in the dense chromatin results in catenation of the replicated chromatids. The separation of these intertwined daughter strands requires decatenation, which is performed by topoisomerase II in the G2 phase of the cell cycle (Ishida et al., 1994). This helps in segregation of the newly replicated daughter strands at mitosis and meiosis. Cells lacking topoisomerase II accumulate multiply-intertwined, catenated dimers (DiNardo et al, 1984, Uemura and Yanagida, 1986). The failure to segregate intertwined DNA molecules eventually leads to cell death as the cells attempt to divide (Holm et al., 1985).

Maintaining Genome Stability : Both the type I and type II topoisomerasases, through relaxation of supercoils and unlinking of inappropriately paired intertwined DNA strands, greatly reduce recombination frequency, especially in the rDNA clusters and help in maintenance of genome stability (Christman et al., 1988). Topoisomerase II plays an important role in recombination suppression during meiosis (Holm et al., 1989). Absence
of any of these enzymes inadvertently results in a hyper-recombination phenotype, in which the rDNA gene clusters tend to get excised as extrachromosomal DNA rings (Kim and Wang, 1989b).

**Chromosome Structure**: Topoisomerase II is associated with interphase chromatin as well as the cell division stage chromosomes (Swedlow et al., 1993, Earnshaw and Heck, 1985). In fact, topoisomerase II is the major component of the chromosome scaffold and is concentrated at the base of the chromosomal loops, called the scaffold attachment regions (Gasser and Laemmli, 1987). Though the detailed structural interaction of topoisomerase II with chromosomes is yet to be worked out, it is believed that topoisomerase II gives structural alignment to chromosomes prior to mitosis but is not required for maintaining the chromosomal scaffold through mitosis (Hirano and Mitchison, 1993).

**Chromosome condensation/decondensation**: Topoisomerase II is required for chromosome assembly and condensation prior to cell division (Adachi et al., 1991; Wood and Earnshaw, 1990). The enzyme is believed to interact with other proteins of the chromosomal scaffold like the SMC proteins (SCII, XCAP-C and XCAP-E) during chromosome condensation (Ma et al., 1993; Saitoh et al., 1994). Removal of topoisomerase II activity either through immuno-depletion or antibody blocking completely inhibits chromosome assembly and condensation (Hirano and Mitchison, 1993). Similarly, the enzyme is also required for chromosome decondensation after cell division.
Regulation of topoisomerase II activity:

Topoisomerase II expression and activity is tightly regulated in cells. In the G1 and S phases of the cell cycle, topoisomerase II activity is largely confined to the relaxation of supercoils generated during the processes of transcription and replication (Cardenas and Gasser, 1993). This requires low expression levels and the enzyme activity is also very less, which is regulated through phosphorylation. As the cell passes on to the G2 and M phases, the phosphorylation status of enzyme is very high, consistent with its high activity (Cardenas and Gasser, 1993). The functions of topoisomerase II and its regulation in the cell cycle are schematically shown in panel 4.

Fast growing cancer cells, unlike the normal cells show very high expression of topoisomerase II in all the phases of the cell cycle (Hsiang et al., 1988). The enzyme is also highly phosphorylated in these fast dividing cells, without which the cells cannot accomplish their high turnover.

Depending on the requirement, phosphorylation of the enzyme can lead to increase in its activity by 2 to 15 fold (Corbett et al., 1992, 1993b; Takano et al. 1991). Casein Kinase II and protein kinase C are the major enzymes that phosphorylate topoisomerase II (Ackerman et al., 1985, 1988; DeVore et al., 1992). In addition, other ‘mitotic kinases’ like the mitogen-activated protein kinase (MAP kinase) phosphorylate the enzyme during the G2 and M phases (Kuang and Ashorn, 1993). The phosphorylation of topoisomerase II by these kinases may be regulated by the master controller of mitotic events, the p34^cdk kinase (Cardenas et al., 1992).
In the G1 and S phases of the cell cycle, topo II expression and activity is very less. Phosphorylation of the enzyme is also very minimal (shown as ‘P’). In these cell cycle phases, the enzyme is involved mostly in resolving the supercoiling generated during transcription. As the cell cycle passes on to the G2 and M phases, topo II expression and activity through phosphorylation is drastically increased. The enzyme in these phases helps in replication, segregation of daughter chromosomes (shown in red), condensation of chromosomes through precondensation complex (PCx) formation and during decondensation. The enzymes that phosphorylate topo II are Casein Kinase II, Protein Kinase C, etc. Cancer cells generally over-express topo II throughout the cell cycle because of the heavy requirement for this enzyme during the various DNA topological transformation processes.
ANTAGONISM OF TOPOISOMERASE II ACTIVITY

In contrast to the limited number of drug classes that act on topoisomerase I or DNA gyrase, topoisomerase II is a target for a number of structurally disparate compounds (Chen and Liu, 1994). The present topoisomerase II drugs can be classified into four groups (Drlica and Franco, 1988; D’Arpa and Liu, 1989, Liu, 1989). They are -

*DNA intercalating topoisomerase II poisons*: These molecules possess a domain for intercalation with DNA and a domain for enzyme interaction. Through this bi-directional interaction, they form an Enzyme-Drug-DNA ternary complex called the 'cleavage complex'. The formation and importance of this 'cleavage complex' has been described in the next section. Examples of this class of drugs are amsacrine (m-AMSA), adriamycin, ellipticine.

*DNA non-intercalating topoisomerase II poisons*: These molecules also form the ternary cleavage complex, but do not intercalate with DNA. They interact with the enzyme and may or may not interact with DNA, but without intercalation. Examples: Etoposide and Teniposide
Drugs that interfere with the ATP hydrolysis reaction of topoisomerase II:

ATP hydrolysis causes a conformational change in the enzyme which opens a molecular clamp for passing a double stranded DNA segment through the distal (C-terminal) end of the enzyme. Drugs which inhibit ATP hydrolysis by the enzyme do not allow the passage of the DNA segment through this clamp. Examples are Novobiocin, Coumeromycin and Amonafide.

Topoisomerase II inhibitors:

These class of drugs bind to the enzyme and inhibit the catalytic activity of the enzyme without forming the cleavage complex or interfering with the ATP hydrolysis. Example of this class is Fostrieicin.

These four classes of topoisomerase II drugs are important antineoplastic agents because topoisomerase II antagonism brings about anticancer action. Among these classes of drugs, the DNA intercalating and non-intercalating poisons are the most effective anticancer agents for the specific reasons which are described in the next heading.
TOPOISOMERASE II IS AN ANTICANCER DRUG TARGET

The strand passage event in the catalytic cycle of topoisomerases comes with a heavy price, which is, generation of double stranded breaks in the DNA. Under normal circumstances, these DNA breaks are fleeting intermediates between the DNA cleavage and religation action of the enzymes (Reece and Maxwell, 1991; Gupta et al., 1995). However, conditions that significantly increase the lifetime and physiological concentrations of these DNA breaks unleash a myriad of deleterious effects on the genetic material (Corbett and Osheroff, 1993; Anderson and Berger, 1994; Ferguson and Baguley, 1994).

In the early 1980’s, researchers had shown that some of the well known anticancer drugs like etoposide and amsacrine (m-AMSA) act on this aspect of the enzyme activity (Nelson et al., 1984; Chen et al., 1984). These drugs, which allow DNA cleavage by the enzyme but block the DNA religation event are known as 'topoisomerase II poisons', unlike the topoisomerase II inhibitors which basically interfere with enzyme turnover (Smith, 1990). Typically, these poisons interact bi-directionally with the enzyme and DNA or with the enzyme alone (when the enzyme is bound to DNA). The drug bound topoisomerase II, as per its normal catalytic cycle, cleaves the DNA. At this point, the transient intermediate of the covalently linked 'enzyme-cleaved DNA complex' is frozen by the drug. This ternary complex consisting of enzyme-drug-DNA is called the 'cleavage complex' (Liu, 1989; Smith, 1990). Formation of this complex disturbs the DNA cleavage/religation equilibrium, which shifts towards DNA cleavage and the enzyme is no
longer capable of resealing the breaks (Maxwell, 1992; Pommier, 1993). This results in permanent double strand breaks in DNA which are protected by the covalently linked topoisomerase II (Froelich-Ammon and Osheroff, 1995).

Cancer cells generally over-express topoisomerase II (Hsiang et al., 1988; Tricoli et al., 1985; Bodley et al., 1987), and these cells when treated with topoisomerase II poisons, tend to harbor numerous topoisomerase II induced DNA cleavage complexes (Potmesil and Kohn, 1991; Slichenmyer et al., 1993; Sinha, 1995). Traversal by replication or transcription complexes in the region of the breaks will apparently split up these cleavage complexes, which will expose the DNA breaks. Once exposed, these breaks will become targets for repair and recombination pathways. This in turn stimulates sister chromatid exchange, large insertions/deletions, translocations and large chromosomal aberrations (Corbett and Osheroff, 1993, Chen and Liu, 1994, Anderson and Berger, 1994; Ferguson and Baguley, 1994). When these genetic aberrations accumulate at high concentrations, they trigger a series of events which will ultimately lead to cell death through apoptosis or necrosis (Liu, 1994; Pommier et al., 1994; Beck et al., 1994). The impact of topoisomerase II poisons on enzyme activity and the subsequent effects are depicted schematically in panel 5.
In its normal catalytic cycle, topo II (shown in red) binds to DNA and generates double strand breaks. Binding of a topo II poison (green) freezes topo II and DNA in a 'cleavage complex', in which the enzyme cannot rejoin the broken DNA. When such a cleavage complex encounters unwinding stress due to an advancing DNA replication machinery or a transcription complex (shown in yellow), the cleavage complex dissociates, exposing the DNA double strand breaks. Accumulation of numerous such DNA breaks due to cleavage complex formation, eventually leads to cell death (described in the text).
METAL COMPLEXES AS CANCER THERAPEUTICS

The therapeutic use of metal containing compounds can be traced back to the ancient Chinese (between 2000 and 2500 B.C.), who used gold in metallotherapy for various diseases including cancer. Paracelsus (1493-1541), who is regarded as the father of metallotherapy, used alchemical mixtures of various heavy metals such as iron, cadmium, mercury, arsenic and antimony to treat patients with diseases like cancer. Ehrlich had used the arsenic compound, Salvarsan® to treat syphilis, until the discovery of penicillin (Ehrlich, 1910). In 1929, Bell had demonstrated the use of Lead phosphate and colloidal Lead for curing neoplastic diseases. The ancient Indian system of therapy called 'Siddha' also uses mixtures of metallic compounds with plant juices for treating numerous diseases.

The modern era of cancer chemotherapy started with the accidental discovery of the cytostatic properties of a platinum coordination complex, cis-diamine dichloro platinum (II) (cisplatin) (Rosenberg et al., 1969). Cisplatin therapy produced such dramatic results on testicular, ovarian, head and neck carcinomas that it spurred an extensive search for other platinum containing complexes. Subsequently, the derivatives of cisplatin- carboplatin, iproplatin and spiroplatin were synthesized, which showed increased efficacy (DeVita et al., 1985; Nicolini, 1988). But surprisingly, compounds like transplatin (trans isomer of cisplatin, whose ligands are in the trans-conformation) did not show any anticancer action. Detailed analysis revealed that cisplatin and its analogues interact with DNA by predominantly (>90%) forming the 1,2-intrastrand cross-links with adjacent purine bases (especially guanine) (Lippard, 1993).
Transplatin shows very less cross-linking with adjacent bases. This explains the difference in the anticancer action between cisplatin and transplatin. But most importantly, this suggests that the ligand orientation and possibly the type of ligand attached to the metal atom may influence the anticancer activity of such metal complexes.

Cisplatin discovery also sparked off an extensive search for anticancer metal complexes which contain a metal atom other than platinum. A very few among these complexes actually matched the cytostatic efficacy of cisplatin, but more importantly, some of these non-platinum metal complexes were active against tumor types which were unresponsive to cisplatin and other existing anticancer drugs, eg., gastrointestinal carcinomas insensitive to cisplatin and other chemotherapeutic treatment are very responsive to treatment with antitumor titanium compounds (Kopf-Maier, 1989). This is a promising aspect for development of non-platinum anticancer metal complexes. The noteworthy point here is that, apart from the type and orientation of the ligands, the central metal atom may also be an important determinant for anticancer action.

To date, the most effective non-platinum cytostatic agents are spirogermanium, a germanium complex (Rice et al., 1977), gallium nitrate (Adamson et al., 1975), titanocene dichloride and budotitane, which are titanium complexes (Kopf-Maier, 1989, Keppler et al., 1991) and trans-indazolium (bis indazole) tetrachloro ruthenate, a ruthenium complex (Keppler et al., 1989). The purported mechanism of cytostatic action by these complexes is through inhibition of DNA, RNA and protein synthesis (Waalkes et al., 1974; Hill et al., 1982, Kopf-Maier and Kopf, 1988; Fruhauf and Zeller, 1991). But, as mentioned earlier, this generalized action also causes enormous toxicity on the body.
A more viable approach for the development of anticancer metal complexes would be through a rational drug design. This concept stresses on the need to recognize specific targets of action, and then design antagonistic molecules that bind to the targets with a very high affinity to bring about specificity of action. This would not only increase the potency of anticancer action, but also decrease toxic side effects to a great extent. The work presented in this thesis is an attempt to develop anticancer organometallic complexes of iron and ruthenium which target topoisomerase II, and which can be safely delivered to cancer cells by using a natural delivery approach.
OBJECTIVES OF THE WORK

For developing potent anticancer metal complexes with minimal associated toxicity, a three pronged approach was adopted.

1. Metal induced toxicity of anticancer metal complexes can be reduced if the metal atom is strongly bonded with one or more ligands. The strongly bonded metal atom would, at least in part be inert towards biological molecules, which may otherwise easily interact with a metal which has lost its ligands. Organometallic compounds are excellent candidates for this purpose because they possess a strong bond (organometallic bond) between the metal atom and the \( \pi \)-electron cloud of an aromatic ring of the ligand. This linkage would prevent non-specific association of the metal atom with biological molecules.

2. Toxicity can be further reduced if the compounds are designed to specifically interact with a single molecular target in cancer cells, in the present case, topoisomerase II.

3. Targeted delivery of the drugs in a bound or encapsulated form to achieve selective localization in cancer cells will further reduce non-specific toxicity.

In the present study, organometallic compounds of iron and ruthenium have been designed to specifically poison the activity of topoisomerase II. Detailed analysis on their molecular interaction with topoisomerase II and DNA have been carried out to determine their mechanism of action. Anticancer activity of these compounds was tested using \(^3\text{H}\)-
thymidine incorporation assays. Lastly, a delivery approach was attempted for the ruthenium compounds to improve their potency of anticancer activity.