Chapter I
CHAPTER I

Introduction

1.1 General:

Cancer is the second most frequent cause of death in humans after cardiovascular diseases. It is a heterogeneous group of diseases representing the phenotypic expression of a series of genetic and epigenetic changes that accumulate over years in the somatic cells and which leads to the loss of growth controlling mechanisms resulting in a de-regulated growth having invasive characteristics [1].

Cancer is a disorder of cells and although it usually appears as a tumor or a swelling, made up of a mass of cells, it is the end result of a whole series of changes that have taken years to develop. An important characteristic of cancer cells is their uncontrolled proliferation, which does not respond to normal growth inhibition signals, which, limit the cell division. A second characteristic of cancer cells is that they appear to be less differentiated than the tissues from which they arise and behave more like embryonic cells. For example, many cancers produce 'ectopic proteins' which are inappropriate to the tissue involved and often identical to proteins synthesized by embryonic or fetal cells. A third characteristic of cancer cells is their tendency to →
How Cancer Spreads

1. Detachment from the Primary Site (often epithelial tissue)
2. Penetrate into basement membrane as well as endothelial lining
3. Freely circulates via blood stream
4. Cancer cell lodges in capillary and penetrates capillary wall leading to secondary tumor*

* Fewer than one in 10,000 cancer cells metastasise.

Figure 1.1: The process of metastatization canerous cell.

metastasize which is the detachment of cells from the site of tumor growth and their development in distant body parts. Cancer cells possess the ability to migrate from the site of origin and invade surrounding and distant tissues thereby forming masses at the distant sites in the body [2].

Cancer cells do not grow faster than normal cells but they continue to divide when normal cells would not. Hence, as cancer proliferates in the body its demand for nutrients can literally starve the host. Additionally cancer tends to weaken the immune system,
immune system, making the host susceptible to other infections. Cancer can also interfere with the normal functioning of various organs and may cause death in this manner.

1.2 Types of Cancers:

It is not possible to define a tumor cell in absolute terms and hence cancers can be broadly classified into three categories:

Benign tumors: These tumors arise in any tissue of the body, grow locally and can cause damage by local pressure or obstruction. The distinct feature of such tumors is that they do not spread to distant sites, i.e. they do not metastasize.

In situ tumors: These tumors develop in the epithelium and do not invade the basement membrane on the skin layer and supporting mesenchyme. Metastasis of the in situ tumors has not been observed.

Malignant tumors: These tumors have the capacity to invade and destroy the underlying mesenchyme layer and obtain nutrients via the blood stream from normal cells. Malignant tumors also produce proteins that stimulate the growth of blood vessels into tumor, which allows a continuous growth of tumor cells. This process of new blood vessel formation is known as ‘Angiogenesis’

1.3 Process of Carcinogenesis:

Carcinogenesis is a multistage process where a series of changes take place in normal cells after the initiation step producing factor commonly referred as carcinogen. Cancer seems to arise from the combined effects of two different kinds of carcinogens, one comprising of agents that damage genes involved in controlling cell
proliferation and migration while the other involving compounds which selectively enhance the growth of cancer cells. The process of carcinogenesis is initiated when a single cell accumulates several mutations, usually over many years and finally escapes from most of the cellular restraints on the processes of proliferation. These mutations allow the cell and its descendants to develop some additional alterations allowing them to accumulate in increasingly large numbers forming a “new growth” normally referred as a tumor that consists of these abnormal cells. Some of the important stages in the process of carcinogenesis are summarized in Figure 1.2.

**a. Cell proliferation**

Two important aspects of cellular metabolism include DNA synthesis and mitosis to produce new cells as well as cell differentiation, which produces specialized cells. A normal cell has mechanisms to control these two processes and hence it is referred as the non-transformed cell. Chemical signals from growth factors or growth inhibitors produced by the cell regulate cell growth and differentiation as well as its division processes. Consequently, many cells have a negative feed back loop to counter-balance the effects of growth factors. The growth factors and inhibitors exert their effects by binding to various cell surface receptors. In cancer cells, these regulatory processes are aberrant. The activation of growth factors or decreased expression of growth inhibitors leads to the loss of normal growth control, resulting in abnormal and increased proliferation. The root causes of these aberrations, at the cellular levels have not been completely understood. However, the general belief is that the proto-oncogenes, which control normal proliferation and differentiation, are transformed into oncogenes. In turn,
oncogenes alter the cellular control mechanisms thereby stimulating processes that support cellular proliferation.

**How Cancer Arises** (creation of a malignant tumor in epithelial tissue)

1. Genetic Mutation - suffered by some cells within normal population results in their rapid proliferation. (Hyperplasia)
2. After years one in a million of these cells suffers another mutation leading to uncontrolled cell growth.
3. New cell has abnormal shape and orientation (dysplasia). Another rare mutation leads to change in cell behavior.
4. Cells are more abnormal in growth and appearance. "In situ cancer" If localized some will undergo further mutations.
5. Tumor may remain dormant or invade underlying tissue & other tissues through blood or lymph (Metastases)

Figure 1.2: Development of normal cell into cancerous cell.
b. **Cell cycle and growth regulation**

In recent years some impressive evidences have been uncovered related to the destination of stimulatory and inhibitory pathways in the cell. These converge on a molecular apparatus in the cell nucleus that is referred to as “cell cycle clock”. The cell cycle is the progression of events that ensues during the generation of two daughter cells from one parental cell (Figure 1.3).

It is described in four major phases consisting of the First gap phase (G1), DNA synthesis stage (S), the Second gap phase (G2) and the Mitosis phase (M) respectively [3]. The time period that cell remains in the G1 phase depends on the tissue type and whether it is normal or tumor cell. If the cell is a proliferating cell it will quickly move into the synthesis phase (S). During this phase the DNA is replicated and at the end of the S phase, two copies of DNA are present in the cell. The next phase is the G2 phase where largely preparations are made for the final cell cycle phase, the M phase or the mitosis phase.

There are two major control points in the cell cycle. One of these is at the G1/S stage when cells commit to replicate while the second is at G0/M stage when cells commit to divide. Of these two major points in the cells cycle, the G1/S stage is of major importance in understanding cancer and chemotherapy. During the G1 phase cell can take one of the three possible routes. For example, the cell may enter the S phase or a cell in the G1 phase may enter into the G0 phase in which the cells remain in the quiescent state or the cell may terminally differentiate and die respectively.
Figure 1.3: The Cell Cycle shows four phases: G1, S, G2 and M phases where S is the DNA synthesis step and M is the Mitotic phase. G1 and G2 are gap phases.

A cell can be born into a proliferative state or a non-proliferative state. In tumors, the new cells that are produced in the hypoxic regions are typically near the center of the tumor and are poorly perfused, i.e have poor blood supply and hence may be born into the non-proliferative state. These are not sensitive to chemotherapeutic agents. Thus, when the tumor is exposed to chemotherapeutic agents the outer-most cells, which are mainly in the proliferative state, are susceptible to the action of the chemotherapeutic agent and are destroyed while those in the non-proliferative state
are not. It is thus logical to expect that in order to eradicate a tumor, it usually require several rounds of chemotherapeutic cycles.

c. Growth factors and Growth inhibitors

The cell cycle points G1/S and G2/M appear to be controlled by the water soluble proteins called as growth factors that are secreted by cells, which bind to the membrane of other cells at certain cell surface receptors (Figure 1.4). This binding then initiates a series of biochemical reactions that ultimately result in gene expression of the growth factors. Growth factor concentration is typically in the picomolar range. However, the response of a cell to the growth factor appears to be limited by the number of cell receptors and not by the growth factor concentration.

Presently three main types of growth factors are known which include the following:

a) **Endocrine growth factors**: These are hormones like insulin or thyroxin. They are produced at one site, are distributed throughout the body and act on other cells at distant sites.

b) **Paracrine type growth factors**: These are produced at multiple locations but due to short plasma half-life, only act on nearby cells.

c) **Autocrine growth factors**: They are produced by a somatic cell and stimulate the same cell.

Many cancer cells produce excessive levels of growth factors. For example, epithelial growth factor receptor is often over-expressed in squamous carcinoma.
Figure 1.4: Control of cell growth involves five types of proteins whose mutant forms lead to cancer

The over-expression of this autocrine growth factor by cancer cells offers new targets in cancer therapy because antibodies can be prepared to target these autocrine growth factors. The approach has been shown to produce cell death at least in the cultured tumor cells.

d. Initiation of Cancer

A carcinogen causes significant increase in the incidence of neoplasms or uncontrolled growth of cells when administered to a population of previously treated collection of cells or an organism. The carcinogens are classified into four major categories:

(i) Chemical agents:
These agents have a variety of molecular structures and include complex hydrocarbons, aromatic amines, certain metals, drugs and hormones as well as naturally occurring chemicals in plants and moulds. Hydrocarbons and nitrosamines are components of cigarette smoke and may contribute to lung cancers in smokers [4]. Certain aromatic amines like 2-naphthylamine used in the dye industries have also been shown to cause bladder cancers in the workers of these industries [5]. Another industrial chemical gas, viz. vinyl chloride, has been implicated as a causative agent for sarcoma of blood vessels in the livers of the exposed workers [6].

(ii) Biological agents:

Various forms of parasites have been associated with some animal and human cancers. For example, blood fluke Schistosomiasis, have been found to induce bladder cancer in Egyptian population [7]. The most established biological agents causing cancers are the oncogenic viruses, which are now linked strongly to human cancers. A number of Papilloma viruses and the Herpes virus called Epstein Barr have been linked to the malignancy called Burkitt’s lymphoma and cancer of the nose and throat [8]. Another human cancer that is related to a virus infection is a liver carcinoma that sometimes follows Hepatitis-B infection [9]. An established link between leukemia and a form of retrovirus called HTLK-1 is now well accepted [10].

(iii) Physical agents:

Ultraviolet and high energy radiations are regarded as causative agents for human and animal cancers. Over exposure to the high energy UV rays has been shown to lead to the development of the skin carcinomas [11]. Cancers caused by the harmful radiations include leukemia as well as cancers of the thyroid, breast, stomach, uterus
and bone. Hence, the routine diagnostic tools such as X-rays have to be used with care so that the person is not overexposed to these high energy radiations.

(iv) Genetic factors:

It has been established that various factors are responsible for causing mutations and alterations in the cellular DNA, which can potentially induce cancers. The cells in the tumor descend from a common ancestral cell that at one point gets initiated into a program of inappropriate reproduction through the action of the oncogenes. Such malignant transformation of the cell is brought about by the accumulation of mutations in specific classes of genes within the cell. These genes obviously provide the key to understanding the processes at the root of human cancers. The expression of oncogenes within cells is a crucial event in the early stages of tumor formation. Oncogenes can arise in cells via two mechanisms: infection of cells by tumor viruses and conversion of cellular proto-oncogenes to oncogenes. In 1911, Peyton Rous discovered that sarcomas (solid tumors) in chickens could be transmitted between animals using a “cell-free filtrate” [12]. The active agent in this “cell-free filtrate” was found to be a virus called Rous sarcoma virus (Ras) (Figure 1.5).
During the period 1970-1980 while studying Rous sarcoma virus, the virologists were able to show that a single viral gene among the half dozen others was responsible for tumorogenesis by these viruses. This oncogene was named as “src” (short for “sarcoma”). In 1981, Michael Bishop and Harold Varmus discovered that normal cells contained a gene that was related to the viral src gene [13]. This normal cellular gene, called cellular src (c-src), is a member of a family of genes called proto-oncogenes. Since then numerous other tumor viruses infecting various animals have been discovered. However, molecular epidemiological studies demonstrating the presence of a specific viral DNA in the same tumor type from many individuals provide circumstantial evidence that some human tumors may be arising from some viral infections. This type of correlation has, perhaps, been most clearly shown in
case of the human papilloma virus and cervical carcinoma as well as Epstein Barr virus and Burkett’s lymphoma [8].

In 1978, Robert Weinberg and coworkers demonstrated the presence of oncogenes even in tumors of non-viral origin [14] by a simple, elegant gene transfer experiment in which DNA isolated from tumor cells was used to convert oncogenically transformed normal cells in culture. This experiment clearly demonstrated the presence of oncogenes in the large majority of tumors. Currently it is understood that tumor formation is initiated by the expression of an oncogene within the cell, which can be brought about in two ways. However, infection by a tumor virus and subsequent expression of the viral genes, including the viral oncogene, is the cause of relatively few tumors. Most tumors are caused by the conversion (via mutation) of a subset of normal cellular genes, the protooncogenes, into oncogenes.

1.4 Progress in Anticancer Drug development:

The field of anticancer drug development has undergone profound changes in the last two decades with emergence of the critical roles of bioinformatics and computational power, explosive growth of molecular biology, success of genome project, and combinatorial design for rapid methods of chemical synthesis, new drug delivery systems and pre-clinical models. This has led to the decline of empirical approaches to the new drug discoveries in the past with more focus on defined disease related targets [15].

There is also an upward trend in delegation of more financial resources for drug development from both governmental agencies and pharmaceutical industries which has been prompted by the emergence of cancer as the major cause of morbidity and
mortality among the ageing population in the industrialized countries. As a result, the top ten pharmaceutical companies spent a total of 27.9 billion dollars in research in 2002 employing of about 241,000 people. Consequently, a single laboratory or a small team of researchers can no longer successfully accomplish anticancer drug development. The process has become rather a complex interplay between the drug discovery staff and pre-clinical formulation group, toxicology and metabolism group, combinatorial synthesis and purification group, the analytical group, the clinical group and the people dealing with regulatory affairs. The chances of uncertainty prevail at each step where the lead candidate may fail despite enormous effects. As a consequence, target validation, focused drug development and well designed clinical trials have become critical for the rapid development of new active anticancer compounds. Thus, the process has become an exercise in partnership between academia, government and industry.

Presently about 10000 candidate targets are postulated to exist for therapeutic intervention of cancer on the basis of recent discoveries in molecular biology. Hence, target validation has assumed a critical role in drug development and algorithms developed in silico or by pre-clinical model systems have become essential to transform potential leads into attractive drugs. Drug discovery thus remains a labor-intensive area of investigation with many leads not necessarily resulting in clinically useful drugs. Studies of Phase I trials of many anticancer drugs for cancer have indicated that approximately 1 out of 40 compounds reaching the clinical trials finally results in a commercially viable drug.
At the present time, animal and human testing are still required to fully evaluate the new drug entity and strict guidelines of safety established by the regulatory agencies also need to be followed in this exercise. Apart from these considerations a new perspective is emerging from the knowledge of different populations, such as children, the elderly or the patients with polymorphic enzymes involved in the drugs activation or metabolism. It demonstrates existence of differences in pharmacology or pharmacodynamics of individuals, which complicated drug development. These parameters are areas of active investigation in order to identify surrogate models that eventually will be able to identify lead compounds and exclude compounds with potential adverse properties.

Finally, in the field of anticancer drug development, approximately 500 compounds are now in development with potential of thousands of entities. To select appropriate compounds requires well-integrated processes from the initial synthesis through clinical trials. In the following discussion we have provided a brief account of predictive designs for anticancer drug development both chemically and biologically. For the purpose of convenience we have categorized the existing anticancer drugs and recent exploratory agents into a few broad categories depending upon their site of action or mechanism so that proper perspective for the work described in the present dissertation can be provided.

1.5 Chemical Approaches to Cancer Therapy:

A. DNA-RNA interactive anticancer agents
(i) **Synthetic alkylating agents:** Nitrogen Mustards [16] (1) were used in World War I as the chemical warfare agents due to their toxic and vesicant properties on the skin, lungs and eyes respectively. They were noted to produce atrophy of lymphoid and myeloid tissues, which lead to their exploratory use for treating lymphomas and leukemias. Later analogs such as melphalan (2), clorambucil (3), cyclophosphamide endoxan (4) were found to be useful in the treatment of number of cancers including those of breast, ovary, uterine, leukemia, multiple myeloma, Hodgkin and NonHodgkin cancers [17]. To overcome the severe toxicity of nitrogen mustards, the N-nitrosourea drugs were developed during the 1950s. The presence of nitroxyl species in N-nitrosoureas was found to greatly increase their cytotoxicites while reducing their general toxicities and hence enhancing their therapeutic index [18-24]. A comprehensive review on nitrosourea anticancer drug is presently available [5]. According to these review four drugs, viz. carmustine (5), lomustine (6), semustine (7), and streptozotocin (8) respectively have been used in the clinical oncology.

(ii) **Platinum compounds:**

The anticancer properties of platinum complexes were discovered serendipitously during studies on the effects of electrical current on the bacterial growth using platinum Electrodes [25]. The parent compound “Cisplatin” (9) has been found to be active against many malignancies such as non-small-cell lung, small-cell lung, breast, penile, head and neck, gastric, uterine, ovarian, testicular and osteosarcomas respectively [26]. It has been well established that the major target of cisplatin drugs
is the DNA molecule and the interaction results in the formation of intrastrand cross-links causing a bent formation and a local distortion of the DNA helix [27-28]. Although introduction of cisplatin in the clinical oncology had a major impact on cancer chemotherapy, the drug has been found to exhibit various toxicities towards normal cells even at low doses. As a result several new cisplatin analogs have

![Chemical Structures and Synonyms]

**Synthetic Alkylating agents**
been synthesized. However, only two among these, viz. carboplatin (10) and oxaloplatin (11) have been cleared for clinical use.

(iii) Minor groove binding agents:

The anticancer antibiotic duocarmycin (12) [29-30] and congeners represent a group of DNA minor groove binding agents. These compounds contain a cyclopropane pyrrole-indole (CPI) moiety, which is connected by the amide linkages to either one or several repeat pyrrole-indole subunits. The DNA-reactive cyclopropane ring interacts with the N3 position of the adenine in the minor groove of the DNA. However, these antibiotics produce severe liver and kidney toxicities and hence new therapeutic agents such as adozelesin (13), bizelesin and carzelesin have been developed for small-cell lung, melanoma and gastric cancers, which are in phase II clinical trials [31-33]. The DNA minor groove binding antibiotic, Bleomycin [8] has also been used extensively in clinical oncology against squamous cell carcinoma, reticulum cell sarcoma, lymphosarcoma and testicular carcinoma respectively [34-35].
(iv) Topoisomerase inhibitors:

DNA topoisomerases I and II and their is enzyme α and β forms are nuclear enzymes which control, maintain, and modify the structure and topology of DNA during the replication and translation processes of genetic materials [36-38]. The topoisomerases induce transient cuts in DNA, which enable the strands to pass through the nicks and then rejoin the nicked strands to DNA. During this process covalent links are formed between topoisomerases and DNA, which are called as “cleavable complexes”. Drugs, which stabilize such complexes, tend to inhibit topoisomerases and result in strand scission and inhibition of DNA synthesis. Different drugs induce site-specific DNA damages because they bind DNA at different sites. The well-known topo I inhibitor is a plant alkaloid called Camptothecin (14) [39-40] and its synthetic analogs topotecan (15) and irinotecan (16) [41]. All these compounds contain a lactone ring, which is critical for their activity. Topotecan has been found to possess activity against small-cell lung cancers and ovarian cancers while irinotecan was shown to have promising anticancer activity against metastatic colon and cervical cancers.

The other class of potent topoisomerase II inhibitors includes the anthracyclin antibiotics such as adriamycin (17), daunomycin (18), idarubicin (19) and epirubicin (20) [42-43]. However, their anticancer activity has been attributed to several other factors including their free radical generating abilities through redox cycling. Most agents belonging to this class of compounds were discovered accidentally and suffer from a lack of selectivity and specificity in general. They are also less effective against solid cancers.
B. Antimetabolites

(i) Folate antagonists and thymidylate synthase inhibitors:

Having a structure closely resembling that of folic acid, methotrexate (21) [16,31,34] is an inhibitor of enzyme dihydrofolate reductase which is responsible for converting folic acid to reduced folate cofactors which are necessary for the transfer of one carbon unit during the biosynthesis of thymidine and in turn, DNA. It is used widely in the treatment of leukemias, sarcomas, breast cancers, head and neck tumors.
and bladder cancers. Unfortunately the compound possesses many side effects, which are being improved by designing inhibitors of another enzyme, viz: thymidylate synthase. These include compounds such as tomulde (22) [31,44], which is a water soluble quinazoline derivative that has a longer residence time inside the cell and thus greater \textit{in vivo} activity.

(ii) \textbf{Pyrimidine and purine antagonists:}

These compounds were designed to exploit differences in metabolism between neoplastic and normal cells. For example, 6-mercaptopurine (23) is an analog of the purine base hypoxanthine, which inhibits \textit{de novo} purine biosynthesis and is prescribed in the treatment of leukemia [45]. Similarly, 5-fluorouracil is metabolised to 5-fluoro-2 deoxyuridine-5 monophosphate (FdUMP), which binds to and inhibits the enzyme thymidylate synthetase [31,46].

The drug is usually given in combination therapy rather than as monotherapy in the treatment of breast, colon, gastric and pancreatic cancers. More recent addition to this class of drugs includes gemcitabine (24) [16,46], which is a synthetic pyrimidine antimetabolite that inhibits the enzyme ribonucleotide reductase, an obligatory enzyme for DNA
Different classes of antimetabolites and chromosome shortening inhibitors
synthesis. This class of compounds includes deliberately designed drug molecules to interfere with specific natural entities. Although the present drugs exhibit some undesirable toxicities the synthetic modifications are expected to yield more specific inhibitors without toxic side effects.

c. Chromosome shortening inhibitors:

During DNA replication, the DNA polymerase cannot fully replicate the ends of the linear DNA molecule [47,48]. To overcome the end replication problem, the 5' end of the newly synthesized DNA in each duplex is shortened following each round of DNA replication ultimately leading to cell death. The protective caps at the ends of the chromosomes are called as “telomeres” which consist of short guanine rich repeat sequences such as TTAGGG that protect from aberrant recombinations [49]. In normal somatic cells progressive telomere shortening is observed. However, mutations or viral oncogenes expressions can overcome this limitation wherein nearly all the telomeres with TTAGGG repeats are lost leading to abnormal division of cells. Usually, the telomers of cancer cells are short because of the late activation of the telomerase during their life cycle. The telomerase is the most widely expressed and specific marker presently known and almost 90 % of the telomerase activity has been detected in cancer samples using polymerase chain reaction (PCR). Hence, the telomerase inhibitors are a logical target for anticancer drug design and number of studies has been carried out in vitro using telomerase inhibitors [50-54].

Another approach to the inhibition of telomerasases is the substitution of abnormal nucleotides during the elongation of the telomere repeat TTAGGG ending. Thus, the analogs 7-deaza-dGTP (25) and 7-deaza-dATP (26) have been shown to
inhibit telomerase in a cell-free assay [55]. In the past 10 years it was discovered that the repeats of guanine bases in DNA can align, in the presence of monovalent cations, in planar G-tetrad arrangements, stabilized through Hoogsteen base pairing i.e. by the parallel-strand alignment rather than the usual Watson-Crick antiparallel alignment. Hence, much attention has been focused on the inhibitors that will stabilize the G-quartet structures and act as anticancer agents [56-58]. A newer approach to the G-quadruplex factor is to develop small molecules such as N, N’ bis[2-(piperidino)ethyl]-3,4,9,10-perylenetetracarboxyl-ic diimde (PIPER), which will catalyze the folding of DNA single strands containing the repeat sequence of 5’-d(TTAGGG) to a G-quadruplex structure, thus making the repeat sequence unavailable for a telomerase elongation by the telomerase [57-59].

d. Signal transduction inhibitors:

Extensive scientific evidence has been gathered about the importance of cell signals, which determine the various functions of normal, and cancer cells. Signalling pathways include both extracellular and intracellular events where receptors for various growth factors such as epidermal growth factor (EDGF), platelet –derived growth factor (PDGF), macrophage colony stimulatory factor (M-CSF) for antigens and cytokines and the G protein-coupled receptors, located on the cell surface play a major role in the relaying of stimulus to the interior of the cell. The growth factor receptors are composed of transmembrane proteins with cytoplasmic protein-tyrosine kinase (PTK) domains which become excellent cellular targets for design of anticancer compounds. Some of the well-known inhibitors are mentioned below.
Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Ser-Cys

Somatostatin (28)

Quercetin (29)

Genistein (30)

Lavendustin B (31)

Erbstatin (32)

Herbimycin A (33)

Lovastatin (34)

Limonene (35)

Signal transduction inhibitors
(i) **Tyrosine kinase inhibitors:**

The tyrosine kinases are dominant signalling molecules in cells since they are present on the membrane as a part of growth factor receptors, i.e. receptor tyrosine kinases (RTKs) and could be a target in cancer therapy [60]. Monoclonal antibodies for tyrosine kinase proteins have been developed which down-regulate the expression of this protein and cause inhibition of cancer cell proliferation both *in vitro* and *in vivo* [61-62]. The octapeptide somatostatin (28) and its analogs were found to decrease the growth of cancer cells by activating the tyrosine phosphatases, which dephosphorylates and halts the growth signal.

Natural products such as quercetin (29), genistein (30), lavendustin A (31), erbstatin (32), herbimycin A (33) and many others also exhibit a broad but non-specific inhibition of tyrosine kinases by binding site in the protein domain [63]. Attempts have been made to design inhibitors, which would bind to the substrate binding site and not the ATP-binding site. Newer selective inhibitors of RTKs include dianilinopthalamide(DHAP-1),4-anilinoquinazoline,4-ar(alk)ylamino]\]

pyriidopyrimidine derivatives [64], which act as selective inhibitors of EGFR tyrosine kinase.

(ii) **Farnesyl kinase inhibitors:**

Another cell signaling transduction pathway involves the important protein ras, p21 [65-66] encoded by three ras genes H, K, and N, which is found mutated mainly in pancreatic, lung and colorectal carcinomas. Activation of p21 protein involves a series of lipid modifications of which farnesylation reactions are required for the sufficient activation of the ras protein. The enzyme farnesyl transferase
(FTase) mediates the transfer of the 15-carbon farnesyl moiety from farnesyl pyrophosphate to a cysteine at the carboxy terminal of proteins that end with CAAX sequences. Using combinatorial chemistry, synthesis of various farnesyl transferase inhibitors have been achieved by using the knowledge of the structure of the CAAX carbonyl terminal of the enzyme, the structure of the farnesyl moiety and the structure of farnesyl pyrophosphate [67-68]. These compounds include lovastatin (34), limonene (35) and many others. This compound was also found to inhibit the growth of human cancers with multiple genetic alterations, including ras mutations and p53 deletions. However, it should be noted that no correlation has been found between the ras mutations in human and their sensitivity to these inhibitors.

e. Steroid hormone receptor inhibitors:

The cytosolic fluid in normal and cancerous tissues contains specific receptors, for hormonal agents [69-74]. Consequently, the hormones and receptors bind to form hormone receptor complexes that are then translocated to the nucleus of the cells where they effect DNA, RNA and protein synthesis and hence the cell division. As a result the compounds designed to interfere with the binding processes between the hormone and the target estrogen receptor show regression (figure 1.6) of hormone dependent cancers. Such compounds are called as antiestrogens and include clinically used compounds such as Tamoxifen (36) [70]. It blocks the access of natural estrogens to the estrogen receptor and is effective against metastatic breast cancers in pre- and post-menopausal women. The related antiestrogens include toremifene (37), idoxifene (38) and raloxifene (39) [71].
Natural male hormone testosterone belongs to the class of hormones known as androgens, which are mainly produced by testicular cells. These compounds are
active against breast cancers in patients with either demonstrated progesterone receptors or those who respond favorably to steroids. The inhibition is attributable to the down regulation of gonadotrotin-releasing hormone receptor (GnRH) which ultimately results in blocking the synthesis of estrogen, progesterone and androgen in these organs.

Synthetic antiestrogens include flutamide (40) and cyproterone acetate (41)[72-74]. Due to predictive response against specific cancers the steroid hormone receptor inhibitors are the most effective anticancer compounds. On the basis of prior experience and innovative approaches it should be possible to design improved drugs of this class, which in fact is attempted in Chapter 3 of the present thesis.

Figure 1.12: Steroid hormone inhibitors
f. Cell cycle regulators:

Cell cycle regulation is important in the maintainence of normal cells, whereas abnormal regulation of the cycle can result in neoplastic transformation. A series of protein kinase complexes and cyclin dependent kinases (cdks) are positive regulators of the cell cycle [75]. In normal non-cancerous cells there is a mechanism to ensure certain stoppages in the cell cycle to allow the repair of DNA damaged by radiation, carcinogens and cytotoxic drugs. In the cancerous cells these inhibitory controls are lost and hence the mutated DNA can proceed through the cell cycle. As a result the cancerous cells can multiply without corrections [76-77]. One of the cell arrest mechanism involves the transforming growth factor (TGF-β) which blocks the synthesis of cdk-4 in the G1 phase thereby, preventing the formation of the Cyclin d/CDK complex and the phosphorylation of Rb (retinoblastoma). This effective growth arrest leads to the accumulation of the cells in the G1 phase.

Based on this mechanism, inhibitor compounds of the cyclins or cdk enzymes have been prepared, most of which bind to the ATP pocket of the kinases [78]. Among these Flavopyridol (42), which is a polyhydroxylated, flavone, is an efficient inhibitor of protein kinases [79]. It is competitive with ATP, non-competitive with the peptide substrate, cytostatic to a variety of cancer cell lines and cytotoxic to certain cell lines [80]. The compound is in clinical evaluations. However, there is a need for more improved synthetic compounds with growth inhibitory activities but without the toxicities observed with such compounds.
Flavopiridol (42)

Staurosporine (43)

Olomucine (44)

Roscovitine (45)

Cell Cycle regulators
Other inhibitory compound includes the alkaloid, staurosporine (43) [81] and purine derivatives such as olomucine (44) [79-80] and roscovitine (45) [82] respectively. The cdk inhibitors can act as cytostatic and cytotoxic agents or can enhance the anticancer activity of the cytotoxic agents by inducing cancer cells to either arrest or delay progress through the cell cycle phases in which they are more sensitive than the cytotoxic drugs.

**g. Apoptotic inhibitors:**

Apoptosis or programmed cell death is another process for the elimination of cancer cells. In normal cells process of mitosis and apoptosis balance each other under steady state conditions. A large number of diverse natural and synthetic inhibitors of apoptosis have been discovered, such as phorbol esters, growth factors, granulocyte stimulating factors (GM-CSF), serum factors, cytokines, certain viruses, RNA protein synthesis inhibitors, PKC tyrosine phosphorylation inhibitors, endonucleases inhibitors of transglutaminases and proteases [83]. Additional important inducers and inhibitors of apoptosis include certain proto-oncogenes and the p53 cancer suppressor gene [84-85]. The c-myc and c-fos proto-oncogenes have also been implicated in the induction of apoptosis [86].

It has been pointed out that the relationship between some of the apoptosis inducing proteins like p53 and Bcl gene family is important for understanding apoptosis in cancer cells [87-88]. It is becoming increasingly clear that the anti-apoptotic effects of bcl-2 protein can be easily reversed by p53 protein contributing to the success of the treatment in patients with some types of cancers. It has been further observed that activation of another gene, viz. bax-2, can result in restoring
apoptosis and thereby limiting extent of cancer cell malignancy [89]. Thus, work in this area is making it obvious that drug target interactions alone are not important and stimulation of cell signals that lead to apoptosis are equally crucial.

h. Photodynamic therapy:

Photodynamic therapy (PDT) essentially involves selective incorporation of photosensitive compounds into cancer cells followed by an excitation of the photosensitizer with light of a suitable wavelength thereby generating reactive species which cause the destruction of biomolecules within the cancer cells. The selected photosensitizer is administered to the patient either orally or intravenously, and allowed to equilibrate with cells over a period of 3-96 h, depending upon the type of sensitizer. There is some selectivity of sensitizer absorption by cancer cells compared to normal cells, ranging from 8% for the extracranial cancers and up to 50% for the brain cancers.

The first photosensitizer to gain widespread clinical application was a hematoporphyrin derivative, photosan (46) [90-91], which is an empirically synthesized mixture of compounds. It has been used in clinics against esophageal, lung, bladder, head and neck and superficial bladder cancers. Extensive efforts have been devoted to overcome the shortcomings of this compound and several new well-defined sensitizers have been prepared which include macrocycles such as phthalocyanines, texaphyrins, porphycenes and the chlorin derivatives [90]. Synthetic attempts have also been directed towards preparing compounds that absorb light at longer wavelengths and to incorporate diamagnetic cations such as aluminium, zinc and tin [90].
Besides the synthetic efforts much attention has also been devoted to the localization of PDT sensitizers in biological tissues. This has been attempted by adjusting the degree of their lipophilicity, artificially lowering the pH in cancer cells by the administration of glucose and designing delivery systems such as liposome associated photosensitizers [91-92]. These developments perhaps would offer more promising anticancer agents in future.

1.6 Biological Approaches for cancer therapy:

The pioneering of combination chemotherapy in the 1970s provided an impetus for oncological advances in combinatorial chemistry and techniques of parallel synthesis which brought about a major breakthrough in the 1990s in terms of synthesis of a large number of potential anticancer compounds. Increasing frustration with the incurable nature of the vast majority of solid tumors, coupled with the commercial presence of biotechnology companies to produce effective compounds
has resulted in a dramatic increase in the development of biologicals for the cancer therapy. This was in turn benefitted tremendously by the exponential expansion in recombinant DNA technology facilitating availability of large quantities of highly purified biological substances for use in clinical trials. The first biological modifiers to be used clinically were the interferons which are the secreted proteins having similar biological properties, described by Issacs and Lindemann in 1957. Initially the compounds were known only for their ability to induce an antiviral state but more recently they have been found to have potent immunomodulatory and antiproliferative actions. Many studies suggest that interferons play a major role in the host defence.

The most important aspect of biological therapy for cancer is the increased understanding of the molecular mechanism of malignancies it has unfolded so that biological manipulations can be tailored to an individual tumor. In the following discussion we have summarized the high lights of present biological approaches to cancer therapy.

a) Antisense constructs:

Several compounds that block the flow of information from cancer genes, such as oncogenes and cancer suppressor genes, either via the transcription process of DNA to RNA or the translation process of RNA to protein have been designed. Such inhibitors are called as antisense constructs because they bind to DNA and RNA sequences, blocking the synthesis of cellular proteins. There are 3 major classes of antisense agents: antisense oligonucleotides (ASOs), antigene sequence and ribozymes respectively.
(i) Antisense oligonucleotides:
Antisense oligonucleotides (ASOs) are the single stranded DNA or RNA that can block the transfer of information from the genetic template to the exposed protein by interfering with the expression of mRNA. Several theories have been advanced on the mechanism of action of ASOs, the most prevalent being the binding of the ASOs to the mRNA leading to the degradation of the adducts mediated by the RNAs. The ASOs have been used to target the mRNAs from a variety of oncogenes including the myc family, that is the myc and N-myc in leukemias and Burkett's lymphoma as the N-ras, K-ras and H-ras in many cancers. ASO permeability through cellular membranes is generally low and hence various carrier systems have been devised. The antisence therapy of cancers and the issue of ASO delivery, their pharmocakinetics and non-specificity are discussed in a number of reviews [93].

(ii) Peptide-nucleic acid adducts (PNA):
These materials form strong and sequence specific complexes with the target DNA by displacing one of the DNA strand and forming a triplex with two such PNA molecule. The DNA antisense activity occurs via steric blockage of the ribosome complex. However, a major disadvantage of PNAs is their poor cell membrane permeability, which is partly overcome by conjugation of the PNA to ligands for cellular receptors.

(iii) Ribozymes:
Ribozymes are small RNA molecules, which are capable of inhibiting the translation process from mRNA to protein by catalytic activity. These enzymes bind to the RNA with antisense base pairing and subsequently cleave the RNAs into smaller inactive fragments, due to their catalytic nature, one ribozyme can inactivate many RNA molecules.

Ribozymes have been designed against the mRNA of H-ras oncogenes capable of discriminating the mutated GUC sequence from GGC in codon 12. In the past decade substantial progress has been made in the development of antisense constructs for cancer chemotherapy by recognizing specific targets in cancerous cells and number of antisense drugs have undergone clinical trials.

b) Antibody directed enzyme pro-drug therapy:
One of the major problems in cancer chemotherapy is that the cytotoxic drug can be readily inactivated by the enzymes in the plasma and converted to unusable metabolites. As a result large quantities of total drug dosage are required to be administered to cancer cells without harming the normal cells. This task is achieved by employing pro-drugs, which are the inactive forms of drugs, which are converted to the active forms by the respective enzymes in vivo. Antibody directed enzyme pro-drug therapy (ADEPT) is a method where an enzyme, normally not found within the human body is attached to monoclonal antibody (mAB) which is specific for the cancer antigens. This complex recognizes the cancer cell antigens and the unreacted antigen which is free is eliminated from the body by natural clearance. The non-toxic pro-drug is then activated at the site of the cancer cells by the bound enzyme-antibody
conjugate. The active drug crosses the cell membrane resulting in the destruction of the cancer cells.

c) Angiogenesis inhibitors:

Angiogenesis is a process whereby endothelial cells divide and migrate to form new capillaries. This process can be observed under normal conditions such as wound healing, and in disease states such as arthritis, infantile hemangiomas, psoriasis, duodenal ulcers, certain disorders of female reproductive system and cancers. In the cancerous state, the new capillaries are thin-walled and leaky and provide access to the circulation for group of cancer cells, which can metastasize and also acquire the angiogenic phenotype. Among various angiogenic agents two proteins appear to be most important for sustaining cancer growth, viz. the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). When released from the cancer cells, these can trigger the growth of new capillary vessels. Additionally, migrating endothelial cells also produce matrix metalloproteinases (MMPs), which are thought to be responsible for the breakdown of basement membranes and extracellular matrices.

Since cancer cells require an additional blood supply for growth, inhibition of angiogenesis is a logical approach to control of the cancers. Angiogenesis inhibitors are grouped according to their mechanism of action. One class of compounds such as tecogalan, pentosan sulfate (PPS) and fumagillin, block the production of angiogenic growth factors. Another class of angiogenic compounds includes the signal transduction inhibitors, which inhibit angiogenesis and mitosis by interfering with the transduction of growth stimulus signals across cellular membranes. MMP inhibitors
such as batimastat (47) and marimastat (47) have been introduced into clinical trials in order to inhibit growth and metastatic spread of human xenografts and mouse cancers.

4) Cancer Immunotherapy:

Biological response modifiers (BRMs) are a large group of immuno modifiers that have the ability to modify the host’s biological response to cancers. Specific BRMs like monoclonal antibodies, \textit{in-vivo} stimulated lymphocytes and cancer derived vaccines react with specific cancer antigens. In contrast, non-specific BRMs mediate an immune response without interacting with specific antigens. These include the cytokines, bacillus Calmette-Guerin (BCG) and the newly developed anticancer vaccines.

5) Gene therapy:

It has been well established that a number of cancers are caused either by defective genes, absent genes or overexpression of genes respectively. In gene
therapy, three strategies have been developed, viz. namely mutation compensation, molecular chemotherapy, and genetic immunopotentiation. Mutation compensation involves either suppression of the dominant oncogenes or augmentation of cancer suppressor gene expression by ribozymes and single chain antibodies. Molecular immunotherapy involves the delivery of a toxin to cancer cells for their elimination. Here a gene is used that encodes for a toxin whereas in the indirect approach, the delivery of pro-drug is used that requires activation for its toxicity. Genetic immunopotentiation involves an increase in the host immune response against cancer-associated antigens by the delivery of immune stimulatory molecules.

There have been several drawbacks to gene therapy including the inaccurate delivery of the gene to the desired cellular location, the inaccurate transposition of the gene and the lack of activity against metastasized cancer cells. Gene therapy should have much fewer side effects than conventional chemotherapy because it is focused on a specific target in the cancer cell since these genes can be administered directly into solid tumors avoiding toxicity resulting from systemic administration.

1.7 Natures and Scope of the Present Dissertation:

The present thesis deals with the drug design strategies for hormone responsive cancers using steroid-linked cytotoxic conjugates with or without metals. The cytotoxic groups are linked to the ring A of the steroid moiety with due consideration for the ligand binding domains. These compounds exhibit good cellular uptake and very potent antiproliferative activities leading to programmed cell death (apoptosis). The other part of the thesis deals with design of colon cancer drugs targeting
cyclooxygenase enzymes using metal conjugates of a non-steroidal anti-inflammatory drug (NSAID). The compounds show excellent inhibition of COX-positive tumors while having non-selective activity against COX-negative tumors.

**Chapter I** gives a brief account of cancer biology and the novel chemotherapeutic and immunotherapeutic agents evolved during the past decade through the joint efforts of molecular biologists, synthetic chemists, pharmacologists and bioinformatists based on the discoveries of the target proteins and enzymes that play crucial role in the carcinogenesis process. It also explains the advantages of targeted drug design strategies, which constitutes the basis of present work.

**Chapter II** includes all the experimental methods and protocols used in present work.

**Chapter III** describes the synthesis and characterization of copper, nickel and platinum complexes of a novel ligand, progesterone thiosemicarbazone, which is prepared by modifying the C-3 ketonic function in the estrogenic scaffold with the thiosemicarbazone side chain. The synthesized metal conjugates are examined for their antiproliferative properties against hormone dependent (MCF-7) and progesterone receptor rich (T47D) breast cancer cell lines as well as hormone independent (MDA-MB 231 and BT20) breast cancer cells alongwith the parent ligand. The phase contrast studies carried out using Hoescht and Propidium iodide dyes reveal morphological changes characteristics of apoptotic cell death.

**Chapter IV** of the thesis presents an account of our investigations on the progesterone ethylenediamine conjugates appended with the redox active quinonoidal ligands, viz. juglone and plumbagin. Their antiproliferative properties are evaluated
against B16F10 melanoma cell line since several biochemical and histochemical studies have indicated presence of the hormone receptors in melanoma cells. In view of the increasing incidence of melanoma in recent years the effectiveness of these compounds suggests further investigations are needed on these compounds.

Chapter V presents an account of synthesis and characterization of the copper complexes of the hydrazone derivatives of one of the mixed cycloxygenase targeting non-steroidal anti-inflammatory (NSAIDs) compounds, viz. Ketoprofen. This is followed by the evaluation of its antiproliferative activities against the colon cancer cell lines with and without COX receptors. It has been well established that the cycloxygenase enzymes are highly induced in colon cancers and are responsible for promoting the development of colon polyps into colorectal cancer. NSAIDs have been employed for reducing inflammation in colon cancers although without much selectivity. In the present work we have appended ketoprofen with the hydrazonic side chain and have prepared the copper conjugates of such ligands. There antiproliferative activities are examined on both COX + colon cancer cells (HT-29, BXPC3) as well as COX - colon cancer cells (MiaP, HCT 116, BT-20, PC3) respectively.

In general the studies carried out in the present dissertation have laid the foundation for evolving some selective metal based anti-cancer compounds targeting certain receptor proteins which are induced in higher concentrations in some cancers and thereby providing selectivity in their therapeutic action. It has also shown that these metalloantitumor derivatives are more potent by several orders of magnitude than their parent organic ligands.
References:

6. Agency for Toxic Substances and Diseases Registry (ATSDR), Atalanta GA.,
   US Dept. of Health and Human Services, USA, (2000)
   Development ( Ed: 1), Lippincott Williams and Wilkins, USA (2003) 9
   Lange, Norwalk, (1994) 3.
17. S. K. Carter, M. T. Bakowski, K. Hellmann, Chemotherapy of Cancer, (Ed:


(1994) 982.


385.


31. C. Britten, L. Hammond, M. Hidalgo, M. L. Rothenberg, A. Sharma, S.


34. R. B. Cameron, Practical Oncology (Ed: 1) Appleton and Lange, Norwalk (1994) 9.


60. A. Levitzki, FASEB, 6 (1992) 3275.


