Chapter 1

Advances in Cancer Chemotherapy
List of Abbreviations

NMR : nuclear magnetic resonance
PBr₃ : phosphorus tribromide
PPh₃ : triphenylphosphene
POCl₃ : phosphorus oxychloride
RT : room temperature
q : quartet
s : singlet
t : triplet
TAE : triarylthelene
TFA : trifluoroacetic acid
TEA : triethylamine
Temp : temperature
TLC : thin layer chromatography
PTLC : preparative thin layer chromatography
µg : microgram
µM : micro mole
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1.1 Introduction

Cancer is not one disease, but in fact, it comprises of more than 200 different diseases together affecting different organs and systems of the body. It is one of the most dreaded disease of the 20th century and spreading further with continuance and incidence in 21st century. It is the second most common cause of death and accounts for 13% of all deaths in humans presently. Cancer prevalence in India is around 2.5 million with over 8 lakhs new cases and 5 lakhs deaths each year. Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age.

Healthy cells divide and proliferate under the influence of various growth stimulates and are subjected to arrest growth (senescence) and programmed cell death (apoptosis). In cancer these regulatory processes have gone away and uncontrolled growth of cell in the body is started due to stimuli, laid the foundation of cancer (Figure 1). Complete process of the cell multiplication is mediated by various enzymes some of which have been identified.

A tumor or the mass of cells formed of these abnormal cells may remain within the tissue of its origin (the condition is called as benign tumor), or it may begin to invade nearby the tissue (the condition is called invasive tumor).

Benign tumor is not cancerous, it can often be removed and in most cases does not spread to other parts of the body, whereas, an invasive tumor is known to be malignant. Cell in these tumors can invade nearby tissue and spread to other parts of the body. Formation of secondary tumor by the cells that have been released from initial or primary tumor and have reached to other sites through the blood vessels or lymphatic is termed as metastases.
Figure 1: Cell division in normal cell and cancer cell

1.2 Classification

Generally cancer can be classified as lymphatic, epithelial, nerve or connective tissue related and its nomenclature is based on tissue of origin as follows:

- **Carcinoma**: Cancer arising from epithelial cell. It is the most common group of cancer overall. Cancer of lung, breast, prostate, bladder and intestine fall in this category.

- **Sarcoma**: Cancer originates from bone, cartilage, fat muscles or other connective or supportive tissues.

- **Leukemia**: Cancer cells originate in blood-forming tissue such as bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

- **Lymphoma and myeloma**: Cancer that originates in the cells of the immune system.
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- **Glioma**: Cancer that originates in brain or spine. (Neural origin)

1.3 **Causes of cancer**

Cancer is caused by exogenous chemicals, physical and biological carcinogens. A carcinogen is something that helps to cause cancer. Endogenous process in human body also contributes to the development of cancer, either on their own or by interacting with exogenous chemicals. The mechanism of carcinogenesis in human is multifactorial and complex. Different factors may act by different mechanism at different stages of tumor development.

1.3.1 **Exogenous carcinogens**

It can be classified as chemical, physical and biological agents.

1.3.1.1 **Chemical carcinogens**

Nickel, cadmium, arsenic, nitrosamine, trichloroethylene, alylamine, benzopyran and many other are chemical carcinogens. Natural product from plant and mould can be highly carcinogenic like alfatoxin B1 and carcinogens in tobacco smoke.

1.3.1.2 **Physical carcinogens**

Any rich radiation can act as a carcinogen, depending on dose and absorption. UV radiation especially UVA (320-400nm) and UVB (280-320nm) are carcinogenic for the skin. Radioactive (α, β, γ) radiations also act as carcinogen. Radiowave, microwave and electromagnetic radiation are the subject of public debate as carcinogen.

1.3.1.3 **Biological carcinogens**

Certain viruses and bacteria act as biological carcinogen in human.

- **Viruses**: Viruses can help to cause some cancer by genetic alteration in the cell.

  Cervical cancer and other genital cancer caused by specific strain of Human
Papilloma Viruses (HPV16 and HPV18). Specific herpes virus can also act as carcinogen or co-carcinogen like Human Herpes Virus 8 (HHV 8) in Kaposi sarcoma or Epstein-Barr virus in lymphomas. Hepatitis B and C virus involved in causation of liver cancer.

- **Bacteria:** People having infection of *Helicobacter pylori* bacteria in their gastrointestinal system are at high risk of stomach cancer. *Schistosoma* also causes cancer in urinary bladder.

### 1.3.2 Endogenous Carcinogens

Endogenous process modulates the response of exogenous carcinogens. A cell becomes cancerous due to number of genetic mutations within it and endogenous process like DNA replication and metabolic reaction. Sometimes carcinogenic compounds such as nitrosamines, aromatic amines, reactive aldehydes and reactive oxygen species are generated by metabolic processes.

### 1.4 Treatments of cancer

The treatment of cancer\(^2\) depends on a number of factors including the type, location and amount of disease and the health status of the patient. The treatments may be divided into different categories based on their goal and mode of action as follows:

- **Surgery:** It is the first line of treatment for many solid tumors detected at an early stage. Benign tumors are often removed by surgery.

- **Radiation:** Radiation is used to kill the cancer cells directly by damaging them with high energy beams, in conjunction with surgery and/or drug treatments.
• **Chemotherapy**: The term chemotherapy is used for the treatment of cancerous cell with a wide range of drugs. These drugs work by damaging cancerous cells at different stages of cell cycle according to their mode of action. Cytotoxic drugs, hormone therapy and target therapy come in this category.

• **Antibodies**: Antibodies are naturally occurring proteins in our body, for cancer treatment they have been manufactured for use as drugs. They are specific in action and work by several different mechanisms, either depriving the necessary signals of cancer cells or causing the direct death of the cells.

• **Biological Response Modifiers (BRMs)**: These are compounds that are used to treat cancer by altering or augmenting naturally occurring processes within the body. Immunotherapy makes use of BRMs to enhance the activity of the immune system to increase the natural defense mechanisms of body against cancer.

• **Vaccine**: Cancer vaccines are used to stimulate the body's defenses against cancer. Vaccines usually contain proteins found on, or produced by cancer cells, and by administering these proteins, response of the body against the cancer cells can be increased. For cervical cancer, presently two vaccines are available that target human papilloma virus (HPV).

1.4.1 Chemotherapy

The use of folk remedies in the treatment of cancer and other tumor is recorded early in the history of medicine. However, the development of drugs for the treatment of cancer is not parallel to other diseases. Chemotherapy is used to kill the cancerous cell. This therapy can be used before and after the surgery and known as neoadjuvant and adjuvant therapy, respectively. Most commonly, chemotherapy acts on rapidly dividing
cancerous cells but also harms rapidly dividing normal cells in bone marrow and hair follicles. This is the most common side effect of chemotherapy. Drugs used in chemotherapy belongs to different categories:

- Cytotoxic drugs
- Hormone therapy
- Target therapy

1.4.1.1 Cytotoxic drugs

Cytotoxic drugs include following categories:

- Alkylating agents and related compounds
- Antimetabolites
- Cytotoxic antibiotics
- Plant derivatives

1.4.1.1.1 Alkylating agents

Alkylating agents are important class of antitumor agents.\(^3\) Their cytotoxic effect is exhibited through a covalent reaction with cellular macromolecules.\(^4\) They are also known as DNA cross linking agents. Most of the cytotoxic anticancer alkylating agents are bifunctional, that is one molecule of the drug can bind to two distinct DNA bases. It is reported that the cytotoxicity of many alkylating agents is the result of DNA mono adduct formation followed by inter or intra-strand cross-links in DNA.\(^5\) The nitrogen at position 7 of guanine (N-7), being strongly nucleophilic, is probably the main molecular target for alkylation in DNA. Excision of guanine caused due to cross linking with two
nucleophilic sites which leads to the cell death. These drugs are used singly, or in combination with other drugs. Few commonly used alkylating agents are discussed here.

1.4.1.1.1 Nitrogen mustards

Nitrogen mustards are bis (β-haloalkyl) amine (1, Figure 2). They are stable solid of low vapour pressure and highly soluble in water. Mechanism of nitrogen mustard is that it forms cyclic iminium ion (aziridinium ion) intermediate by the attack of nitrogen on the β-carbon of the mustard. DNA nucleophile then attacks on this intermediate, breaks the aziridine ring and thus DNA get alkylated. Second arm of the mustard involves second molecule of DNA by the same way. Finally, hydrolytic depurination cleaves the bound guanine residue from the DNA strand and releases the damaged and unstable DNA from this mustard trap. This leads to inevitable result of cell death. Reactivity of the nitrogen mustard depends on the third group (R) attached to the amino nitrogen. It can be aliphatic or aromatic. Aliphatic group increase the activity of nitrogen mustard by it’s +I effect whereas aromatic group stabilized the lone pair of the nitrogen through resonance. Resonance delocalization slows down the reactivity of aromatic nitrogen mustards. Aromatic mustards sufficiently control reactivity which permits oral administration and attenuate the selectivity of side effects. Some specific drugs of nitrogen mustard are given below.

- **Mechloretamine:** Mechloretamine [2-chloro-N-(2-chloroethyl)-N-methylethanamine, 2] is also known as chloromethane, mustine nitrogen mustard and sold under the brand name mustagen. It is used specially in the treatment of Hodgkin’s disease and certain leukemias. It is also used for carcinoma of breast and lung, lymphosarcoma and neuroblastoma. Side effects related to this drug are severe nausea, vomiting, myelosuppression and alopecia.
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- **Melphalan**: Melphalan {4-[bis(2-chloroethyl)amino]-L-phenyllalanine, 3} was prepared in England by Berjel and in Russia by Larionov. This phenyllalanine mustard has the same spectrum of action as other nitrogen mustards. However, they are less reactive than mechlorethamine and may be administered orally. It is safer and convenient in multidose therapy. Melphalan is used in the treatment of a wide variety of tumors. It has lower side incidence of nausea and vomiting as compared to mechlorethamine but the drug is also mutagenic and can induce leukemia.

- **Chlorambucil**: Chlorambucil (4) is used for the treatment of chronic lymphocytic leukemia, ovarian carcinoma and lymphomas. Its synthesis was reported in 1953. This drug is active intact and also undergoes β-oxidation to provide an active phenyl acetic acid mustard metabolite, responsible for some antineoplastic activities.

- **Cyclophosphamide**: Cyclophosphamide (5) is probably the most commonly used alkylating agent. It is an antineoplastic agent which is activated by metabolic and nonmetabolic processes in liver. It is used for the treatment of variety of human carcinomas and sarcomas as well as leukemias and lymphomas. It can be effectively administered in a number of ways and used in combination with a number of agents. Main side effects associated with this drug are leucopenia, alopecia and cystitis. It is also used as immunosuppressive agent. Cyclophosphamide was found definitely superior to other nitrogen mustards.

- **Ifosfamide**: Ifosfamide (6) is an analogue of cyclophosphamide having two arm of the mustard on different nitrogen atoms. Ifosfamide currently used against testicular cancer although it has also shown activity against number of solid
tumors and hematologic cancer. Ifosfamide commonly exhibit toxicity against central nervous system.

- **Aziridines**: Aziridines are slower alkylating agents then mechlorethamine. Triethylenemelamin [2,4,6-tris(1-aziridinyl)-1,3,5-triazine, TEM, 7] was the first alkylating agent found suitable for oral administration. Carbamoyl derivative of aziridine [5-(1-aziridinyl)-2,4-dinitrobenzamide, 8] showed exceptional activity against the Walker tumor, yet was inactive against number of other tumors.

![Chemical structures](image)

**Figure 2**: Nitrogen mustards and its derivatives

### 1.4.1.1.2 Nitrosourea

Nitrosourea (Figure 3) are unstable structures that decompose readily in aqueous environment of the cell. They belong to a class of compound containing a nitroso (R-NO) group and a urea group. Loss of proton from the urea moiety stimulates the fragmentation, followed by the generation of cytotoxic electrophiles that are vinyl carbocation, acetaldehyde and 2-chloroethylamine. These electrophiles are capable of alkylating DNA in the standard manner.¹⁴ Carmustine, lomustine, streptozocin and ethylnitrosourea are some examples of this class.
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- **Carmustine and lomustine:** Carmustine or BCNU [bis-chloroethylnitrosourea, 9] and lomustine or CCNU [chloro-cyclohexylnitrosourea, 10] both are lipophilic chloroethylnitrosourea analogues. Lomustine is stable enough for oral use and is marketed in capsule form. These drugs are active against a variety of solid neoplasms as well as Hodgkin’s disease but appear most promising in the treatment of certain brain tumors\(^ {15}\) and for certain colon tumors in combination with 5-fluorouracil.\(^ {16}\) Both the drugs can induce thrombocytopenia and leucopenia, leading to hemorrhage and massive infection.

- **Streptozocin:** Streptozocin (11), a natural nitrosourea\(^ {17}\) and a broad spectrum antibiotic, has shown clinical activity against functional pancreatic islet cell cancer.\(^ {18}\) It is a glucosamine-nitrosourea compound. Like other alkylating agents in the nitrosourea class, it is also toxic to cells by causing damage to the DNA and through other mechanisms.

- **Procarbazine:** Procarbazine (12) exerts antineoplastic effect through the O\(_6\) methylation of the guanine nucleoside. O\(_6\)-methyl-guanine pairs preferentially with thymine and these ‘mispair’ promote point mutation during subsequent DNA replication cycle and so trigger cell destruction. It is used in the treatment of Hodgkin’s disease\(^ {10}\) and administered as a part of multidrug regimen. It is also used in the therapy of brain tumors in combination with other nitrosoureas.

- **Dacarbazine:** Dacarbazine (13) is also a methylating agent like procarbazine and is used as single agent for the treatment of malignant melanoma, and in combination with other agents in the treatment of metastatic melanoma. Leukopenia and thrombocytopenia are the most common side effects associated with the drug.
1.4.1.1.3 Busulphan

Chemically, busulphan (14, Figure 4) is classified as alkyl sulfonate. One or both of the methylsulfonate ester moieties can be displaced by the nucleophilic N-7 of guanine, leading to monoalkylated and cross-linked DNA. It has a selective effect on the bone marrow by depressing the formation of granulocytes and platelets in low dosage and red cells in higher dosage. It is used in chronic myelogenous leukemia.

1.4.1.1.4 Organoplatinum compounds

Organoplatinum antineoplastic agents (Figure 5) contain an electron-deficient metal atom that acts as a magnet for electron-rich DNA nucleophile. Being bifunctional they form cross linking between adjacent guanine residues or adjacent guanine and adenine residues. The DNA bases become coordinated with platinum and this structural change affects DNA replication leading to inhibition of tumor growth. DNA repair mechanisms
Inhibit the action of topoisomerase II enzyme, so prevent the relaxation of supercoiled DNA and thus block DNA transcription and replication.

- Generate cytotoxic free radicals that cause single-strand breaks in DNA.

Anthracyclines, daunomycin and adriamycin appear to be the most promising agents for clinical application. Example of anthracycline antibiotics are doxorubicin (adriamycin, 29), daunorubicin (cerubidine, 30), idarubicin (idamycin, 31), epirubicin (ellence, 32) etc.

![Chemical structures of anthracycline antibiotics](image)

**Figure 8:** Anthracycline antibiotics

### 1.4.1.1.3.2 The Actinomycins

Actinomycins are the class of polypeptide antibiotics isolated from soil bacteria. All actinomycins contain the same 2-amino-4,6-dimethylphenoxaz-3-one-4,5-dicarboxylic acid chromophore with a variety of polypeptide side chain attached by the way of the carboxylic functional group. Actinomycin D (33, Figure 9) is the most significant antibiotic of this class isolated from *Actinomycetes* in 1940, and since then a number of structural variants have been reported. Actinomycin D forms a complex with
are unable to correct the damage. Cisplatin, oxaplatin carboplatin, nedoplatin etc. are some organo platinum anticancer drugs.

- **Cisplatin and Carboplatin**

Cisplatin (CDDP, 15) and its less toxic derivative carboplatin (CBDCA, 16) are two mostly used drugs in cancer chemotherapy. Cisplatin is used for the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer. They are also used in combination with other anticancer drugs for cure of many cancers. Cisplatin forms interstand cross-linking, probably between two of adjacent guanine molecule which results in distorting of DNA. It is highly nephrotoxic and can cause significant damage to the renal tubules. It is low myelotoxic but causes very severe nausea and vomiting. The lower excretion rate of carboplatin shows that it is more retained in the body, hence its effects are longer lasting. It has less severe nausea and vomiting than cisplatin. Both the drugs are given by intravenous injection or infusion. Oxaliplatin (17) and satraplatin (18) are also used as anticancer drug.

![Organoplatinum compounds](image)

**Figure 5: Organoplatinum compounds**

1.4.1.1.2 Antimetabolites

The antimetabolites stop the *de novo* synthesis of DNA by inhibiting the formation of nucleotides. They serve as false substrate for the enzyme involved in nucleotide biosynthesis. They entice the enzyme to choose them over the endogenous substrates, and bind to it irreversibly, so that DNA synthesis is stopped leading to tumor growth arrest.
Antimetabolites are classified according to the class of nucleotide they inhibit:

- Purine antagonist
- Pyrimidine antagonist
- Folic acid antagonist

1.4.1.1.2.1 Purine and pyrimidine antagonists

Purine and pyrimidine are the chemicals used as building block for the synthesis of DNA and RNA. Purine and pyrimidine antagonists (Figure 6) inhibit the DNA and protein synthesis by inhibiting the production of purine and pyrimidine containing nucleotides and so in deficiency of nucleotides, DNA synthesis is halted and therefore cell division and growth of tumor.\(^{21}\) It is thought that these antagonists are incorporated in the DNA molecule and so interfere with further cell division. Their major effect is on rapidly growing cancerous cells than to normal cells.

Some purine antagonists are 6-mercaptopurine (19), dacarbazine (20), and fludarabine (21).\(^{22}\) 6-Mercaptopurine (6MP) is the most useful purine antimitabolite. It is used widely, specially in the treatment of Leukemia.

Some pyrimidine antagonists used in cancer therapy are 5 fluorouracil (22), capecitabine (23), gemcitabine (24), decitabine (25), arabinosylecytosin (cytarabine, 26) etc.\(^{23}\) 5-Fluorouracil has profound activity against different transplanted tumors. It is a thymidylate synthase inhibitor. By interrupting the action of this enzyme it blocks the synthesis of the pyrimidine and thymidine nucleotide required for DNA replication. Cytarabine is active against acute non-lymphocytic leukemia, chronic myelocytic leukemia and non-Hodgkin lymphoma.\(^{24}\) Cytarabine is approved by U.S. FDA and marketed in US under the trade name cytosar-U.
1.4.1.1.2.2 Folic acid antagonists

Folic acid antagonists (Figure 7) also known as antifoliate, inhibit dihydrofolate reductase (DHFR), an enzyme involved in the formation of nucleotides. Folic acid itself is not biologically active until it is chemically reduced. Folic acid firstly reduced into dihydrofolic acid and then to tetrahydrofolic acid by the same enzyme known as dihydrofolate reductase (DHFR). The inhibition of DHFR keeps the folic acid in an inactive state, so inhibits the synthesis of purine and pyrimidine, which leads to inhibited production of DNA, RNA and protein. Aminopterin (27) and methotrexate (28) are two potent antifoliate with a high degree of anticancer activity.25

Figure 7: Folic acid antagonists
1.4.1.1.3 Antibiotics

The antibiotic antineoplastics are a broad category of natural or semisynthetic compounds that block DNA transcription and replication processes. Some antibiotic antineoplastic agents interact with DNA, firstly by intercalating the double-stranded helix and then forming strong noncovalent interaction with DNA base. Many of the antineoplastic antibiotic compounds inhibit topoisomerase II, an enzyme responsible for maintaining proper DNA structure during replication and transcription of RNA. Topoisomerase II normally cleaves DNA during the replication and repairs its own damage after the replication process completed. Some common antibiotic antineoplastics are anthracyclines, actinomycins, bleomycin, mithramycin and mytomycins.

1.4.1.1.3.1 Anthracyclines

Structurally anthracycline antineoplastic\textsuperscript{26a} (Figure 8) antibiotics are glycoside and contain a sugar portion and a nonsugar organic portion. Nonsugar portion is known as aglycon, also called as anthracyclinone. All anthracyclines share quinine containing rigid planar aromatic ring structure (the chromophore), bound by an O-glycoside bond to an aminosugar.\textsuperscript{26b} This group of antibiotic is used to treat a wide range of cancer including leukemias, lymphomas, breast, uterine, ovarian and lung cancer. Anthracyclines has three mechanism of action:

- Inhibit DNA and RNA synthesis by intercalating between base pairs of the DNA or/and RNA strands, thus preventing the replication of rapidly growing cancer cells. In fact the anticancer activity of these drugs are related more to the proper positioning and stabilization of the drug at its binding site than actual affinity to the drug for DNA.\textsuperscript{27}
• Inhibit the action of topoisomerase II enzyme, so prevent the relaxation of supercoiled DNA and thus block DNA transcription and replication.

• Generate cytotoxic free radicals that cause single-strand breaks in DNA.

Anthracyclines, daunomycin and adriamycin appear to be the most promising agents for clinical application. Example of anthracycline antibiotics are doxorubicin (adriamycin, 29), daunorubicin (cerubidine, 30), idarubicin (idamycin, 31), epirubicin (ellence, 32) etc.

![Anthrac cycline antibiotics](image)

**Figure 8:** Anthracycline antibiotics

1.4.1.1.3.2 The Actinomycins

Actinomycins are the class of polypeptide antibiotics isolated from soil bacteria. All actinomycins contain the same 2-amino-4,6-dimethylphenoxaz-3-one-4,5-dicarboxylic acid chromophore with a variety of polypeptide side chain attached by the way of the carboxylic functional group. Actinomycin D (33, Figure 9) is the most significant antibiotic of this class isolated from *Actinomycetes* in 1940, and since then a number of structural variants have been reported. Actinomycin D forms a complex with
DNA and selectively inhibits RNA synthesis.\textsuperscript{31} The synthesis of ribosomal RNA being preferentially affected, in a model for the actinomycin-DNA complex based on X-ray data obtained from a crystalline complex of actinomycin and 2'-deoxyguanosine. The phenoxazine ring system intercalates between adjacent G-C base pair of DNA, where guanine moieties are on opposite DNA strands, and the 2 amino group of the guanine interact with bonds.\textsuperscript{10} Actinomycin D has proved to be of value against Wilm’s tumor, soft tissue sarcoma, trophoblastic malignancies and testicular tumor.\textsuperscript{32}

![Figure 9: Actinomycin D](image)

1.4.1.1.3.3 Bleomycin

Bleomycin (34, Figure 10) is a glycopeptide antibiotic isolated from \textit{Streptomyces verticillus}.\textsuperscript{33a} It is relatively of high molecular weight peptide antibiotic. Bleomycin A was found superior then bleomycin B in antitumor activity. Bleomycin binds to DNA and causes cleavage of the macromolecule.\textsuperscript{10} Some studies suggested that bleomycin affects
Figure 10: Bleomycin

thymidine-containing compounds such as DNA and polydeoxyribonucleotides by releasing free thymine and leaving aldehyde functions.\textsuperscript{33b} The drug is used in the treatment of Hodgkin's lymphoma, squamous cell carcinomas and testicular cancer. Most serious complications of this drug are pulmonary fibrosis and impaired lung function.

1.4.1.3.4 Mithramycin

It is also known as plicamycin (35, Figure 11) and trade name is Mithracin. Mithramycin is another streptomycetes antibiotic having antineoplastic activity. The mechanism of action of this antibiotic has been investigated and it has been shown that the antibiotic binds to DNA and inhibits DNA dependent RNA synthesis. It has been used in the treatment of testicular cancer.\textsuperscript{34}
1.4.1.1.3.5 Mitomycins

The mitomycins are a family of aziridine containing natural products isolated from *Streptomyces caespitosus* or *Streptomyces lavendureus*. Mitomycin C (36, Figure 12) has antitumor antibiotic activity and is administered IV in the treatment of disseminated adenocarcinoma of stomach or pancreas and it has been used intravenously in superficial bladder cancer. Myelosuppression is the major use limiting side effect of this drug, which is slow to manifest but quite prolong in duration.
1.4.1.1.4 Plant based anticancer molecules

1.4.1.1.4.1 Vinca alkaloids (Figure 13)

1.4.1.1.4.1.1 Vinblastine and Vincristine

Bisindole alkaloids vinblastine (37) and vincristine (38), the first natural products to enter in clinical use, were isolated from the Madagascar periwinkle Catharanthus roseus (L.) G. Don (previously known as Vinca rosea L.) in the late 1950s and early 1960s by two independent groups. It was discovered serendipitously when Robert Noble and Charles Beer at the University of Western Ontario, were actually looking for substances that could affect blood glucose levels, but observed that extract reduced white blood cell counts and found to be active against lymphocytic leukemia. These observations led to the isolation of vinblastine (VLB). Vincristine (VCR) was isolated by Gordon Svoboda and his colleagues at Eli Lilly.

The vinca alkaloids are dimeric asymmetrical compounds consisting of two multi-ringed subunits, vindoline and catharanthine, linked by a carbon-carbon bridge. Structurally, vinblastine and vincristine differ only in the nature of the substituent, a methyl group or a formyl group respectively, on the indole nitrogen atom, in the lower vindoline portion of the molecules. Despite this single difference in structure their clinical and toxicological profile has significant difference. Vinblastine is used in combination chemotherapy to treat bladder and breast cancers and in Hodgkin’s disease whereas vincristine is used in combination chemotherapy of acute lymphoblastic leukemias and lymphomas. Bone marrow suppression is dose limiting factor in VLB whereas in VCR it is peripheral neuropathy.

Vinca alkaloids inhibit the cell division during early mitosis. They bind to the β tubulin at a different site from the taxane drugs and colchicines, and prevent the
formation of dimer with α tubulin and so block its ability to polymerize into microtubulin. This leads to the death of actively dividing cancerous cell. These drugs also affect cell division in normal cells, explaining many of the side effects like nausea and vomiting alopecia (loss of hair), swelling of feet or lower legs, headache, constipation etc.

1.4.1.1.4.1.2 Vinorelbine

Vinorelbine (39) the first new second generation vinca alkaloid, is a semisynthetic derivative of vinblastine, in which two carbon chain bridging the indole to the piperidine nitrogen has been shortened by one carbon and one water molecule has been eliminated from the piperidine ring. Pierre Fabre company launched vinorelbine for the treatment of non-metastatic breast cancer and non-small cell lung cancer (NSCLC) in 1989 and is available for both IV and oral formulation.\(^{39}\) However, the side effect and toxicity of vinorelbine are milder than other vinca alkaloids but some common side effects are myelosuppression which results in a reduced number of platelets, red blood cells, white blood cells, shortness of breath, constipation or loose bowels and alopecia (hair loss).

1.4.1.1.4.1.3 Vindesine

Vindesine (40) was the first semisynthetic analogue of vinblastine to enter in clinical use. Like other vinca alkaloids, vindesine also binds to the tubulin building blocks, used to make microtubules. It differs from vinblastine in lacking an acetyl group on vindoline ring system and in having an amide function in place of methyl ester on the same ring. Vindesine has been incorporated into several effective combination regimens for treatment of leukemia, lymphoma, and non-small cell lung cancer (NSCLC).\(^{40}\) Dose-limiting granulocytopenia is the major side effect of the drug.
1.4.1.4.1.4 Vinflunine

Vinflunine (41) a dihydrofluoro derivative of vinorelbine, has been synthesized by superacid chemistry.\textsuperscript{41} Vinflunine inhibits tubulin assembly without any stabilization of assembled microtubules at concentrations comparable to those of other vinca alkaloids such as vincristine, vinblastine and vinorelbine. This effect on microtubule dynamics results in cell cycle arrest in mitosis finally leading to apoptosis. It is in phase III clinical trials at Pierre Fabre for the treatment of bladder cancer and NSCLC.\textsuperscript{42} It is also being evaluated for second-line chemotherapy in hormone refractory prostate cancer (HRPC), HER2-overexpressing metastatic breast cancer (with trastuzumab) and ovarian cancer.\textsuperscript{43}

37. Vinblastin: $R=\text{CH}_3$

38. Vincristine: $R=\text{CHO}$

40. Vindesine

41. Vinflunine

Figure 13: Vinca alkaloids
1.4.1.4.2 Camptothecin

1.4.1.4.2.1 Introduction

Camptothecin (CPT, 42, Figure 14) a naturally occurring molecule, has been attracting the academic community and the pharmaceutical industries for many years. It was isolated by Monroe E. Wall and Mansukh C. Wani, during an extensive screening programme in a search of steroids from the extract of *Camptotheca acuminata*, a tree native to China and Tibet, which has been extensively used in traditional Chinese medicine in 1958. The structure of this naturally occurring alkaloid (CPT) was firstly reported in 1966.\(^{44}\) Camptothecin (as its sodium salt) was advanced to clinical trials by National Cancer Institute (NCI), U.S. in 1970s. Despite promising preclinical and clinical antitumor activity, the use of the first camptothecin formulation was hindered by severe and unpredictable toxicity associated with treatment, including hemorrhagic cystitis and myelotoxicity.\(^{45}\) After years of intense research in 1996 two semisynthetic camptothecin analogues, irinotecan (Camptosar) and topotecan (Hycamtin) entered the clinics for the treatment of colorectal and ovarian cancer, respectively.\(^{46}\)

Camptothecin a pyrrolo[3,4-b]quinoline alkaloid, has also been isolated from *Ophiorphiza pumila* and *Mapia foetida*. It occurs in different parts of the plant like roots, twigs and leaves. CPT has a basic five ring (A-E)\(^{47}\) structure with a chiral centre located at position 20 in the terminal lactone (E) ring with (S) configuration.

![Figure 14: Camptothecin](image)

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1.4.1.1.4.2.2 Structure activity relationship (SAR)

Numerous studies exploring the structure activity relationships (SAR) of CPT have provided novel insights and contributed to the clinical successes. Conjugation and planarity of the A, B, C and D rings are required for \textit{in vitro} and \textit{in vivo} activity of the CPT. It has been thought that the E-ring lactone is a necessary structural feature, but few reports\(^{48}\) suggested that there is a requirement of a reevaluation of the belief that the E-ring lactone is essential. The A and B rings of CPT have proven to be the most amenable to modifications with the maintenance of moderate to good activity. Modifications of the C and D rings of CPT have generally resulted in loss of activity whereas, pyridone carbonyl is essential for antitumor activity. The stereochemistry at carbon 20 of CPT is apparently crucial, as the analogue having a 20(R)-OH group is inactive.\(^{49}\) Replacement of oxygen at 20 with sulfur or nitrogen abolishes the activity of Camptothecin.

1.4.1.1.4.2.3 Mode of action

CPT shows anticancer activity mainly against colon and pancreatic cancer cells, but its analogues showed anticancer activity against breast, liver and prostate cancer. Camptothecin inhibits DNA topoisomerase I.\(^{50}\) It is reported that CPT can cause cell death by inhibiting DNA synthesis via strand scission during the S phase of the cell cycle.\(^{51}\)

Topoisomerase I and topoisomerase II catalyze the relaxation of super coiled chromosomal DNA during DNA replication.\(^{52}\) DNA relaxation by topoisomerase I involves the transient single-strand cleavage of duplex DNA, unwinding and relegation.\(^{53}\) Topoisomerase I mediated DNA strand scission involves formation of a 'covalent binary complex' intermediate between enzyme and DNA followed by DNA relaxation.
Camptothecin inhibits topoisomerase I by stabilizing cleavable covalent binary complexes thus diminishing relegation and ultimately DNA synthesis and cell viability.

The exact mechanism by which CPT stabilizes the DNA-topoisomerase I covalent binary complex is not fully understood. CPT has been shown not to exhibit a significant degree of binding to either DNA or topoisomerase I alone. Despite the apparent lack of affinity of CPT for DNA or topoisomerase I alone, the binding of CPT to the covalent binary complex is suggested to be responsible for the observed stabilization. Camptothecin was approved against colon cancer in 1970, by the US Food and Drug Administration and thus it was evaluated as a possible drug for the treatment of human cancers in phase I and phase II studies.

1.4.1.1.4.2.4 Synthetic studies

Topotecan (43) and irinotecan (44) are first-generation water soluble analogues of camptothecin, which were approved for use by the U.S. Food and Drug Administration (FDA) in 1996. There are nearly one dozen second-generation analogues of the camptothecin (Figure 15) in different phases of clinical trials. Some water soluble analogues 9-amino-CPT (IDEC-132, 45), 9-nitro-CPT (rubitecan, 46) and 10,11-methylenedioxy-CPT (10,11-MDC) have shown sufficiently good in vivo antitumor activity. IDEC-132 did not perform well in phase II and III clinical studies and so was dropped out afterwards. Rubitecan is currently in phase III clinical studies for the treatment of pancreatic cancer. Substitutions at 11 and 12 position are generally unfavorable to biological activity. But the analogues containing 10,11-(methylenedioxy), 11-fluoro and 11-cyano functional group have shown better topoisomerase I inhibition. Hydroxy-CPT was much more active than CPT in a number of assays.
1.4.1.4.2.5 Topotecan

Topotecane (Hycamtin, 43) also known as hycamptamine, is a derivative of camptothecin with a N,N-dimethylaminomethylene functional group at C-9 and a hydroxyl group at C-10 of ring A. These polar groups increase the water solubility of the molecule. Mode of action of hycamptin is same as of parent compound CPT. Topotecan was approved by FDA for ovarian cancer in 1996, and for cervical cancer in 2006. It is marketed by Glaxo-SmithKline and is the first topoisomerase inhibitor for oral use. Diarrhoea, low blood counts and susceptibility to infection are its common side effects.

1.4.1.4.2.6 Irinotecan

Irinotecan (Camptosar, 44) is a derivative of camptothecin with a large 4-(1-piperidino)-1-piperidinocarbamate group at C-10 and an ethyl group at C-7 of ring A and B respectively. It does not stabilize the topoisomerase I-DNA binary complex, yet exhibits good clinical activity. Irinotecan hydrochloride is approved by the FDA to be used with other drugs to treat colorectal cancer that has metastasized. It is also approved to treat metastatic colorectal cancer that has recurred or gotten worse after earlier
chemotherapy. During development it was known as CPT-11. It is marketed by Pfizer (Pharmacia earlier) and mainly used in colon cancer treatment, particularly in combination with other chemotherapy agents. The most significant adverse effects of irinotecan are severe diarrhoea and extreme suppression of the immune system.

Different studies on modification of C and D (47-49, Figure 16) rings have generally resulted in the loss of activity. The reports on these two ring modifications are limited because the only sites available for substituents are C-5 of the C ring and C-14 of the D ring. Synthesis of 5-ethylidene-CPT (50) having activity equivalent to CPT against P388 cells suggests that CPT can accept additions upon the C ring when planarity is unperturbed.

Modification of E ring is not acceptable. Analogs with modified E ring are less or inactive as compared to camptothecin. Analogue having stable lactam ring in place of lactone was ineffective as a topoisomerase I inhibitor. Other reported analogues having carbinol lactam (51), imide (52) and thiolactone (53) were also found to be inactive.

![Figure 16: Modified camptothecin analogues](image-url)
1.4.1.1.4.3 Podophyllotoxin

1.4.1.1.4.3.1 Introduction

Podophyllotoxin (54, PDT, Figure 17) a naturally occurring aryltetralin lignan found in the roots of the North American Podophyllum peltatum Linnaeus (also referred to as American mandrake, or May apple) was firstly isolated by Podowyssotzki in 1880. Other source of podophyllotoxin is Indian podophyllum, *Podophyllum emodi* grows over most of the Himalayan range. Plant containing podophyllotoxin analogues have been used as folk remedies in traditional medicinal in the Chinese, Japanese and the Eastern world for gout, tuberculosis, gonorrhoea, syphilis, menstrual disorders, dropsy, cough, psoriasis, venereal warts and certain tumours. The correct empirical formula for podophyllotoxin was first advanced by Borsche and Niemann and later confirmed by Gensler et al. Extensive programme of structural modifications of Podophyllotoxin (about 600 derivative from 1950s to 1960s) escorted to discovery of the clinically important anticancer drugs etoposide and teniposide.

1.4.1.1.4.3.2 Chronology of Podophyllotoxin and its Drug Development

- Isolation and chemically investigated Podophyllotoxin 1880
- King and Sullivan reported the mechanism of action of Podophyllotoxin 1946
- Synthesis and biological evaluation of etoposide 1966
- Start of clinical trials of teniposide 1967
- Start of clinical trials of etoposide 1971
- FDA approved etoposide as VePesid for testicular cancer 1983
- Studies on mechanism of action: inhibition of topoisomerase II by stabilization of cleavable complex 1984
- The Institute of Microbial Chemistry and Nippon-Kayaku 1986
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identified NK-611

- US launched of the prodrug etopophos 1996

1.4.1.1.4.3.3 Structure activity relationship (SAR)

Podophyllotoxin is a cyclolignan having five ring system classified as A, B, C, D and E.

**Ring A:** A ring (methylenedioxy cycle) is crucial for activity.

**Ring B:** Modification at B ring reduces the activity of the molecule, as oxygenation at C-5 of B-ring led to the decreased activity.

**Ring C:** Modification of the C ring by aromatization or expansion gave compounds less potent than podophyllotoxin. But modification at C-4 position in this ring is acceptable and bulky group at this position enhanced biological activities. Epimerization at 4 position increases topoisomerase II activity, analogues of epi-series are more potent than the normal series.

**Ring D:** D ring (*trans* lactone) is important for the biological activity. Replacement of the lactone by a tetrahydrofuran ring gives rise to less active compounds.

**Ring E:** E ring is essential for activity. It is observed that free rotation of the E ring is necessary for antitumor activity. Demethylation at 4' position seems to be necessary for DNA breakage activity.

1.4.1.1.4.3.4 Mode of action

Podophyllotoxin is an effective and comparatively safe drug for the treatment of genital warts. It is an outstanding antitumor agent and effective against Wilms tumor, different type of genital tumor, lung cancer and in non-Hodgkin and other lymphomas. Podophyllotoxin as such is not used as drug due to complicated side effects such as nausea, vomiting, damage of normal tissue etc. Some of the semisynthetic analogues of
(62) was synthesised. Etopophos, a water soluble phosphate ester of etoposide is launched by Bristol-Myers Squib in 1996. Etoposide, teniposide and etopophos are widely used as drug against several types of neoplasm including testicular and small-cell lung cancers, lymphoma, leukaemia, Kaposi’s sarcoma etc.\textsuperscript{74}

**Figure 17:** Podophyllotoxin and its analogues

Several modifications have been done on A-ring. A-ring open compounds were less active than parent compound. Several isoxazole derivatives of podophyllotoxin with A-ring opened or with different functionalisation in the A-ring of the podophyllotoxin skeleton have been synthesized, their cytotoxicity level was two to three times lower than
PDT i.e. etoposide and teniposide are more potent and less toxic than PDT and possess different mode of action. Podophyllotoxin is a microtubulin inhibitor, it binds to the tubulin and disrupts the equilibrium assembly and disassembly. PDT binds to tubulin more rapidly than colchicine at the colchicine binding site.\textsuperscript{68} Podophyllotoxin completely inhibits tubulin polymerization at very low concentration (5µM).

Etoposide (55) and Teniposide (56) have shown no effect on microtubulin structure at concentration used in clinical studies.\textsuperscript{69} These two analogues have been established as DNA topoisomerase II inhibitors.\textsuperscript{70} Topoisomerase II alters DNA tertiary structure by creating transient double-stranded breakage of the DNA backbone, thus allowing subsequent passage of a second intact DNA duplex through the break.\textsuperscript{71} Etoposide and teniposide form a ternary complex with DNA and topoisomerase II, and prevent resealing of the DNA break leading to cell death. Common side effects associated with the PDT and its analogues are myelosuppression, development of drug resistance and cytotoxicity towards normal cells.

1.4.1.4.3.5 Synthetic studies

In order to achieve better antitumor activity with less toxicity, numerous structural modifications have been performed on the cyclolignane skeleton. Some derivatives (Figure 17) entered in phase I and phase II studies as antitumor agents are GP-11 (57), NK-611 (58), TOP-53 (59), NPF (60) and GL-331(61).\textsuperscript{72} Analogues with 4'-demethylation and β-glycoside moiety at 4-position (etoposide, teniposide and etopophos) are potent irreversible inhibitor of DNA topoisomerase II. Etoposide presents several limitations such as moderate potency, poor water solubility, development of drug resistance, metabolic inactivation and toxic effects.\textsuperscript{73} To obtain better therapeutic agents and to overcome with limitations associated with etoposide, a prodrug of it, etopophos
(62) was synthesised. Etopophos, a water soluble phosphate ester of etoposide is launched by Bristol-Myers Squib in 1996. Etoposide, teniposide and etopophos are widely used as drug against several types of neoplasm including testicular and small-cell lung cancers, lymphoma, leukaemia, Kaposi’s sarcoma etc.\textsuperscript{74}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig17.png}
\caption{Podophyllotoxin and its analogues}
\end{figure}

Several modifications have been done on A-ring. A-ring open compounds were less active than parent compound. Several isoxazole derivatives of podophyllotoxin with A-ring opened or with different functionalisation in the A-ring of the podophyllotoxin skeleton have been synthesized, their cytotoxicity level was two to three times lower than
those of the parent compound of podophyllotoxin.\textsuperscript{75} Extensive structure activity relationship study shows that C-4 position is most tolerable to significant structural diversification and resulted in a number of promising drug candidates.

Several 4-alkylamino and 4-arylamino epipodophyllotoxin analogues (63-66, Figure 18) have been synthesized.\textsuperscript{76} Variously substituted arylamino analogues showed comparable or greater % inhibition of DNA topoisomerase II activity. Analogue with p-nitroanilino moiety at the 4β position of etoposide (GL331) is a lead compound. GL331 is in phase II clinical studies. It has activity against many multidrug-resistant cancer cell lines (KB/VP-16, KB/VCR, P388/ADR, MCF-7/ADR, L1210/ADR, HL60/ADR, and HL60/VCR).

\textbf{Figure 18:} Different analogues of Podophyllotoxin

Several 4β-substituted sulfonamide derivatives of 4β-O-demethyl-4-deoxypodophyllotoxin (Figure 19, Table 1) have been synthesized. These analogues have similar or better DNA topoisomerase inhibition activity than etoposide.\textsuperscript{77}
Table 1: Biological evaluation of 4β-substituted sulfonamide derivatives of 4'-O-demethyl-4-deoxypodophyllotoxin

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>Topo II % linear DNA</th>
<th>IC₅₀(µM)ᵇ</th>
<th>Cell cycle effectᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide</td>
<td>-</td>
<td>50</td>
<td>0.83</td>
<td>80% (2.5 µM)</td>
</tr>
<tr>
<td>67</td>
<td>CH₃</td>
<td>50</td>
<td>0.07</td>
<td>77% (0.5 µM)</td>
</tr>
<tr>
<td>68</td>
<td>(CH₂)₇-CH₃</td>
<td>35</td>
<td>0.25</td>
<td>76% (1 µM)</td>
</tr>
<tr>
<td>69</td>
<td>(CH₂)₃N₃</td>
<td>32</td>
<td>0.035</td>
<td>65% (0.2 µM)</td>
</tr>
<tr>
<td>70</td>
<td>SO₂Me</td>
<td>10</td>
<td>0.55</td>
<td>75% (2.5 µM)</td>
</tr>
<tr>
<td>71</td>
<td>-N(Me)₂</td>
<td>44</td>
<td>0.37</td>
<td>66% (0.25 µM)</td>
</tr>
<tr>
<td>72</td>
<td></td>
<td>34</td>
<td>0.12</td>
<td>77% (0.5 µM)</td>
</tr>
<tr>
<td>73</td>
<td></td>
<td>45</td>
<td>0.048</td>
<td>84% (0.1 µM)</td>
</tr>
</tbody>
</table>

ᵃ Each value reported here is a medium value of three independent experiments in the presence of the drug at 50µM. ᵇ IC₅₀: concentration of drug required to reduce by 50% L1210 cell growth. ᶜ Percent of L1210 cells in the G2+M phases at the indicated concentration.

Gensler et al have synthesised many modified D-ring analogues having D-ring cyclopentane, cyclopentanone, ether, sulfide, sulfone and sulfoxide analogues, these delactonised derivatives were found to be less active than their parent compounds in mitotic inhibition and cytotoxic assay.⁷⁸

E-Ring is crucial for the activity. E-ring with the trimethoxy group shows antimicrotubulin activity, while 3,5-dimethoxy,4-hydroxy aryl group shows DNA topoisomerase inhibition activity. Many esters of 4'-demethyl-4-deoxypodophyllotoxin
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(DDPT, 74, Figure 20) with alkanoic acids and alkanedioic acids have been synthesized and they showed better antitumor activity than the parent compound.\textsuperscript{79} Some analogues of podophyllotoxin in which one, two or all three methoxy groups on the phenyl ring were replaced by hydrogen atoms or an alky group were almost as potent as the parent compound. These finding suggests that oxygenated functions in the E-ring of podophyllotoxin is not a strong determinant of cytotoxicity.\textsuperscript{80}

![Podophyllotoxin Structure](image)

**Figure 20: 4'-Demethyl-4-deoxypodophyllotoxin**

1.4.1.1.4.4 Taxol

1.4.1.1.4.4.1 Introduction

Paclitaxel (trade name taxol, 75, Figure 21) a billion dollar molecule, is an addition to the armamentarium of plant derived chemotherapeutic agents, initially isolated from the Pacific Yew, *Taxus brevifolia*, as a part of random collection programme for the NCI by the U.S. Department of Agriculture (USDA), by the group of Dr. Wall and Dr. Wani, in 1967 and characterized in 1971.\textsuperscript{81} The compound was subsequently shown to posses excellent *in vivo* antitumor activity in a number of human tumor models in nude mice, which resulted in the initiation of preclinical formulation and toxicity studies by the NCI. Taxol is an anti-microtubule agent and has shown significant antineoplastic activity in patients with advanced ovarian cancer, with response rates ranging from 20% to 50%. Paclitaxel, along with several key precursors, occur in the
leaves of various *Taxus* species, including *Taxus Wallichiana*. Over 350 taxane diterpenoids are reported with their structure classification and plant source.\(^{52}\)

**Figure 21**: Taxol

**1.4.1.1.4.4.2 Chronology of Taxol Development**\(^{59}\)

- Isolation and structure 1971
- Preclinical development 1977
- Mode of action 1979
- Toxicological studies in animal 1982
- Phase I clinical trials 1983–1984
- Phase II clinical trials (activity in ovarian cancer) 1985–1986
- Side chain synthesis 1986
- Semisynthesis of taxol 1988
- Bristol–Myers Squibb receives CRADA from NCI 1991
- Received first FDA approval 1992
- Total synthesis of taxol (Holton–Nicolaou) 1994
- US FDA approval of nab-P (nanoparticle albumin-based paclitaxel) 2004
1.4.1.1.4.4.3 Structure activity relationship (SAR)

Taxol is a complex polyoxygenated diterpenoid molecule, having basic skeleton of a pentamethyl [9.3.1.0\(^3\)^1] tricyclopentadecane along with N-benzoyl-\(\beta\)-phenylisoserine side chain attached as an ester linkage at the C-13 hydroxyl. There are total 11 stereo centers in the molecule.

Study of SAR\(^{83}\) of taxol will be much easier if we divide the molecule in three parts, northern hemisphere, southern hemisphere and side chain.

**Northern hemisphere:** Modification in this part of molecule is suitable. Removal or derivatization of C-7 hydroxyl has no significant effect on the activity of taxol. Modification or removal of C-10 acetyl group is acceptable. Replacement of this group with some acyl groups has shown improvement in activity. Reduction of C-9 keto group improves the activity of taxol slightly.

**Southern hemisphere:** Modification in this part of taxol is not tolerable at all. C-1 hydroxyl is important but not essential for activity. C-2 acyloxy group is essential for activity. However, if it is replaced by some aromatic substituents, molecule gives better activity. Replacement of C-4 acetyl group by other group can increase the activity, but removal of this group reduces the activity. The 4,5,20-oxetane ring is vital for activity. Hydrogen bond acceptor properties and the rigidification of the taxol ring system by the oxetane ring play an important role in stabilizing the taxol–tubulin complex.

**Side chain:** The role of side chain in biological activity of taxol is not fully understood, however, the SAR revealed several key features about the mechanism of action of the molecule. The side chain is itself inactive but plays crucial role for the activity of taxol. It is thought that side chain may fit into a hydrophobic cleft on taxol binding site, which stabilize the drug-tubulin interaction. Free C-2' hydroxyl group is required for biological
activity of taxol, as protection of this group results in major loss of activity. But
derivatives having labile group at the same position can serve as prodrug of taxol. C-3’
aryl group is important for activity, replacement of this group with methyl group reduces
the activity by several folds. C-3’ N-acyl group is essential, although aromatic ring may
be substituted by other acyl or alkyl group.

Two stereo centers C-2’ and C-3’ of the side chain have significant effect on the
activity of the molecule. Removal or interchange of both of these centers causes great
loss of activity. 2’ S, 3’ R isomer is significantly less active than the natural 2’ R, 3’ S
isomer, but the 2’ S, 3’ S and 2’ R, 3’ R isomer show comparable activity to that of the
natural molecule.

1.4.1.1.4.4 Mode of action

Paclitaxel is used in the treatment of breast, ovarian, lung and also shown efficacy
against Kaposi sarcoma. Paclitaxel has also attracted attention in the potential treatment
of multiple sclerosis, psoriasis and rheumatoid arthritis.

Mode of action of taxol reported by Susan Horwitz in 1979, was completely new.
It promotes the assembly of the proteins α and β-tubulin into microtubules. Actually
microtubules are primarily composed of two subunits: α- and β- tubulin. Firstly α- and β-
tubulin form dimer and then polymerization of these tubulin dimer produces the
protofilament which organizes to form a microtubule. Microtubules are labile structures,
the equilibrium between the microtubule and tubulin results from continuous assembly
and disassembly at both ends of the microtubule (Figure 22).

Taxol stabilizes microtubules and inhibits its depolymerization back to tubulin,
this is opposite to the other antimitotic agents like vincristine, vinblastine, colchicine,
podophyllotoxin and others, which all bind to soluble tubulin and inhibit the
polymerization of tubulin to form microtubules. Normal microtubules are of 13 protofilaments and 24nm in diameter but in case of taxol, stabilized microtubules are of 12 protofilaments and 22nm in diameter. Due to the formation of these unnatural bundles of microtubules there is no mitotic spindle formation. This is because taxol some time also known as ‘spindle poison’. As tumor cells are often deficient in checkpoint, they can not recognize this change in cell and this leads to death.

Figure 22: Tubulin polymerization and microtubule assembly in normal cell and in case of taxol

Common side effects associated with the drug are vomiting, loss of appetite, pain in the joints, tingling in the hands or toes, hair loss and reduction in white blood cells.

1.4.1.4.4.5 Synthetic studies

By the early 2000s, taxol had become the best-selling anticancer drug of all time, but its development was in doubt because of its very poor yield. Approximately 0.5g of taxol was isolated starting with 12kg of air dried stem and bark of Taxus brevifolia, the yield was about 0.004%. The solution of this problem is obtained by semisynthesis from
10-deacetylbbaccatin III, which is extracted from the needles of the European yew tree, *Taxus baccata*.

![Figure 23: Synthetic analogues of taxol: taxotere and isotaxel](image)

### 1.4.1.4.4.6 Docetaxel

Docetaxel (Taxotere, 76, Figure 23) is primarily used in the treatment of breast cancer and NSCLC (non small cell lung cancer). It is a semisynthetic drug derived from 10-deacetylbbaccatin III, a precursor extracted from the needles of *Taxus baccata*. Structures, docetaxel differs from paclitaxel in two positions, at C-10 and C-3’ nitrogen, which are, hydroxyl and tertiary butyl ester respectively. Docetaxel uses the same tubulin-binding site as paclitaxel, but it has 1.9-fold higher affinity for the binding site than paclitaxel. Docetaxel showed tumor growth inhibition and water solubility better than paclitaxel. Side effects associated with this drug are bone marrow suppression, vomiting, hair loss etc.

To increase the tolerability and to reduce the clinical resistance of taxanes many efforts have been made to find new taxane formulations like albumin, nanoparticle, emulsion and liposome. Numerous new taxoids synthesized with improved properties are in clinical trials. Many research groups have synthesized water soluble prodrugs through the introduction of hydrophilic moieties to C2’ or/and C7 positions to increase bioavailability.
Isotaxel (77) is a prodrug of paclitaxel having much better water solubility (0.45 mg/mL) than taxol (0.00025 mg/mL). Isotaxel converts into paclitaxel through a simple chemical reaction via the O–N intramolecular acyl migration of the benzoyl group under physiological pH (7.4) with no side reaction.\textsuperscript{91}

1.4.1.1.4.5 Combretastatin A-4

1.4.1.1.4.5.1 Introduction

Combretastatins (Figure 24) are a group of anti-mitotic agents isolated from the South African tree, *Combretum caffrum*, collected in Southern Africa in 1970s as a part of random collection program for NCI by the USFDA. The combretastatins are a family of stilbenes, which cause vascular shutdown in the tumors and so tumor necrosis occurs, therefore, they are also known as anti-angiogenic agents.\textsuperscript{92} Initially, combretastatin A-1 (79)\textsuperscript{93} and A-4 (80) were isolated and found to be potent tubulin polymerization and cell growth inhibitors. Isolation and structure of the combretastatin (78) was reported by Pettit \textit{et al}. in 1982.\textsuperscript{94} Later, combretastatin A-5, A-6, B-1, A-2, A-3 and B-2 were also isolated. \textit{In vitro} evaluations of the growth inhibitory activity of combretastatins (CA-1 to CA-6) have been done against 60 human tumor cell lines.\textsuperscript{95} Combretastatin A-4 (GI$_{50}$=3.2nM) is the most potent member of the family. Combretastatin A-4 (CA-4) is a simple stilbene derivative having two substituted phenyl rings separated by a double bond. One ring has trimethoxy group and other ring has one methoxy and one hydroxyl group. It is a tubulin polymerization inhibitor and displays its cytotoxicity and antivascular activity by binding at colchicine site. It is now in phase III clinical studies.\textsuperscript{96} Because of its simple structure and potent cytotoxicity, CA-4 has been studied exhaustively and during last five years, more than 250 publications and 170 patents concerned combretastatins.\textsuperscript{97}
1.4.1.4.5.2 Structure activity relationship (SAR)

CA-4 displays a 1,2-diarylethene scaffold. Ring A having 3,4,5-trimethoxy group is separated with ring B having 3-hydroxy and 4-methoxy groups by a double bond. Many research groups have worked on SAR studies of CA-4. From different SAR studies it has been concluded that cis-configuration of the stilbene moiety is a very important factor for biological activity. Trans (E) stilbene is significantly less active as compared to cis (Z) stilbene.

A-Ring: 3,4,5-Trimethoxyphenyl ring is essential to obtain relevant cytotoxic and antitubulin responses. As significant loss of potency is reported when the trimethoxy phenyl ring is replaced by a simple phenyl ring, or the meta or para positioned methoxy groups were removed.

B-Ring: $p$-Methoxy group is fundamental for cytotoxicity but not pivotal for antitubulin action while the meta hydroxyl group is not essential for the biological activity. Modifications at C-3 position with other polar groups are tolerable. Oshumi et al. reported that $m$-OH-NH$_2$ isosteric substitution leads to a compound with a slight increase in potency compared to CA-4. Resulting compound (81) and its prodrug (AC7700 and
AVE8062) are currently in clinical trials. Additional hydroxyl group at 2-position decreased the activity of the molecule as in CA-1.

1.4.1.1.4.5.3. Mode of action

CA-4 is tubulin polymerization inhibitor and binds to the tubulin protein at colchicine’s binding site.\(^{102}\) This binding inhibits the polymerization of \(\alpha-\beta\) tubulin dimers into microtubulin. It is thought that trimethoxy phenyl ring plays a crucial role in this activity, probably it provides a favorable binding site for tubulin.\(^ {103}\) Colchicine binding to the tubulin is irreversible but it is not so in case of CA-4.\(^ {92}\) It induces irreversible vascular shutdown within solid tumors while leaving normal vasculature intact.\(^ {104}\) Results of several \textit{in vivo} experiments suggested that more bioavailability of disodium phosphate analogue of CA-4, that is combretastatin A-4,3-O phosphate (CA-4P, 82) significantly reduces blood supply to the tumor cells in a dose-dependent manner and so causes tumor necrosis. CA-4P induced a complete vascular shutdown within metastatic tumors at doses one-tenth of the maximum tolerated dose,\(^ {105}\) while the reduction in blood flow by CA-4 is up to 70%.

1.4.1.1.4.5.4 Synthetic studies

**Ring A:** Results of different studies show that modifications on A-ring are not much acceptable. Gaukroger \textit{et al} reported that replacement of ring-A with trimethylbenzene ring resulted in significant loss of cytotoxicity but inhibition of tubulin was maintained.\(^ {106}\) When \(m\)-methoxy is replaced by fluorine group the resulting compound showed antitubulin activity comparable to that of CA-4 with slight loss of potency.\(^ {107}\) Substitution of methoxy group with bulkier group reduced the activity of the molecule.

**Ring B:** This ring is most amenable to modifications and so received greater attention from medicinal chemists. Isosteric substitution of hydroxyl group with fluorine (83)
displayed significant cytotoxic activity. Replacement of this group with NH$_2$ slightly increases the potency of the compound. When hydroxyl group is replaced by boronic acid (84) moiety, it displays high cytotoxic and antitubulin action. From different studies it is concluded that $p$-methoxy group is important for cytotoxic activity but not pivotal for antitubulin action. B-ring is replaced by heterocyclic and other aromatic ring but the resulting derivatives were not active as compare to CA-4 (85-87, Figure 25).

![Chemical structures](image)

**Figure 25**: B Ring modified analogues of combretastatin A-4

**Double bond**: From different studies it is concluded that restricted rotation of both the rings of CA-4 is necessary. Presence of cis-double bond is not so important. Investigational studies of length of bridge revealed that two carbon linkers were most active. If the alkene bridge is replaced by the carbonyl group the resulting molecule, phenstatin (88) shows antitubulin activity similar to that of CA-4 with a modest loss of potency. Some benzil derivatives (89, 90) were found to inhibit cell growth at the nanomolar level (20–50nM) on four human tumor cell lines. Structure bearing α,β unsaturated carbonyl linker (chalcone, 91), is a potent cytotoxic inhibitor of tubulin polymerization. Synthesis of sulfide, sulfoxide and sulphone spacers between two aromatic rings have been done and results suggested that sulfide can be a lead compound. Introduction of sulfonate group (92) as a linker between both the rings gave product having cytotoxicity and antitubulin action in nanomolar range. Number of
anallogues were prepared where the olefinic group is replaced by three to six membered rings. It can be inferred that five membered rings are best suited at this position. Among the five membered rings, imidazoles (93), oxazole (94), furazan (95) and arylcoumarin (96) have been synthesized (Figure 26).\textsuperscript{114}

![Chemical structures](image)

**Figure 26:** Double bond modified analogues of combretastatin A-4

From various studies it is clear that the two phenyl rings must have 1,2 relationship to show maximum potency. Many macrocyclic analogues of CA-4 have been synthesized which were found to be less potent than the CA-4.
1.4.1.1.4.6 Homoharringtonine

1.4.1.1.4.6.1 Introduction

Homoharringtonine (97, Figure 27) [cephalotaxine, 4-methyl-2-hydroxy-4-methylpentyl butanedioate (ester) (HHT)] is a natural alkaloid isolated from alcoholic extraction of the fastigiate (entire plant) of the evergreen Chinese tree, Cephalotaxus harringtonia var. drupacea. It is an ester of cephalotaxine (98), an alkaloid isolated from the same plant. It can also be obtained from various species of Cephalotaxus, which are coniferous shrubs with yew-like leaves indigenous not only to China but also to India and Japan. These shrubs are used in Chinese medicine for the treatment of cancer. Isolation of HHT and related alkaloids [cephalotaxine (98), harringtonine (99), isoharringtonine (100)] was reported by Paudler in 1963,\textsuperscript{115} and its structure was firstly described in 1970.\textsuperscript{116} A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). In 1990s, HHT was proved to be significantly active as salvage therapy for patients with CML, after failure of interferon-\(\alpha\) therapy. HHT is effective against various leukaemias and currently is in phase II clinical studies.

\begin{align*}
\text{Figure 27: Alkaloids isolated from Cephalotaxus harringtonia}
\end{align*}
1.4.1.1.4.6.2 Structure

Homoharringtonine is an ester of cephalotaxine. It is a polycyclic alkaloid having an embedded 1-azaspiro[4,4]nonane ring system. It is made up of 5 ring system with a side chain, out of which three rings are five membered, one is six membered aromatic ring and one ring is seven membered.

1.4.1.1.4.6.3 Mechanism of action

HHT exerts its antitumor activity through inhibition of protein synthesis and promotion of apoptosis. It inhibits chain elongation during translation by suppressing the substrate binding to the receptor site on the 60S ribosome subunit and so blocks aminoacyl-tRNA binding and peptide bond formation.\textsuperscript{117} It is found to be a selective inhibitor of transpeptidation during the elongation cycle. Cytotoxicity exerts by HHT is cell-cycle specific, affecting mostly cells in the G1 and G2 phases, as expected for a drug that inhibits primarily protein synthesis.\textsuperscript{118}

\textit{In vitro} and \textit{in vivo} studies of HHT against leukemia sublines demonstrated a proportional relation between cytotoxicity and protein synthesis inhibition. It has moderate activity against CD8F1 mammary carcinoma and has marginal effect against B16 interaperitoneal melanoma.\textsuperscript{119} \textit{In vitro} investigation of HHT with other cytotoxic agents showed that it has consistent significant synergistic effect with cytarabine and modest synergy with 5-fluorouracil and hexamethylene bisacetamide.\textsuperscript{120} Common side effects associated with HHT are myelosuppression, hypotension, diarrhoea, mild nausea, vomiting and intravenous infusion often provokes cardiovascular disturbances.\textsuperscript{121}

1.4.1.1.4.6.4. Synthetic studies

Synthesis of semisynthetic HHT (sHHT) (2'R,3S,4S,5R-[-]-Homoharringtonine) from its abundant biosynthetic precursor cephalotaxine, extracted from the dry leaves of
Cephalotaxus, was reported by Robin et al in 1999.\textsuperscript{122} shHHT is presently known as omacetaxine mepesuccinate (ceftazolin) and is being developed by ChemGenex Pharmaceutical Ltd. in collaboration with Stragen Pharma (Geneva, Switzerland). shHHT has subcutaneous bioavailability and activity against imatinib-resistant CML. Initial studies support the use of shHHT for patients who have imatinib-resistant CML, including those who carry the tyrosine kinase inhibitor-insensitive mutation that exchanges the amino acids threonine and isoleucine at position 315 (the T315I mutation).\textsuperscript{123}

1.4.1.1.4.7 Resveratrol

1.4.1.1.4.7.1 Introduction

Resveratrol (3,5,4′-trihydroxystilbene, \textbf{101}, Figure 28) was first isolated from the roots of Veratrum grandiflorum O. Loes. (White hellebore) in 1940. Resveratrol is found in at least 72 plant species\textsuperscript{124} and is synthesized through condensation reaction between 3 molecules of malonyl-CoA and one molecule of 4-coumaroyl-CoA. It is present in a variety of dietary substances including grapes, plum, peanuts and mulberries.\textsuperscript{125} In plant world, it is regarded as antibiotic and plays an important role in plant defense mechanism. It is a phytoalexin produced by the plant in response to infection by pathogens and variety of stress condition.\textsuperscript{126} It is also present in skin of red grapes and is a constituent of red wine and has been suggested to be linked with “French paradox”.\textsuperscript{127} Different studies have shown that resveratrol can slow the progression of variety of disease including cancer, cardiovascular disease and ischemic injuries, and has also enhanced the stress resistance and life span of various organisms. Mechanism by which resveratrol exerts such a range of biological activities is not clear yet. Anticarcinogenic effects of resveratrol are thought to be closely associated to its antioxidant properties as free radical mediated oxidative damage of DNA might play a causative role in cancer.\textsuperscript{128} It may
reduce oxidant-induced apoptosis and low-density lipoprotein (LDL) oxidation. Different studies suggested that resveratrol activates various signal pathways to inhibit tumor cells growth and directly induces apoptosis by the activation of caspases, p53, and Bax and inhibition of Bcl2 and NF-κB. Resveratrol induces phase I drug-metabolizing enzymes and also could act as prooxidant. This prooxidant action is believed to play an important role in its cancer chemoprevention and apoptosis inducing properties.

Figure 28: Resveratrol

1.4.1.1.4.7.2 Chemistry and analogues

Resveratrol is a trans stilbene molecule having three hydroxyl groups. Two rings are separated by a trans double bond. The presence of more than one phenol in the molecule is classified as polyphenol. Polyphenols generally possess antioxidant properties and can react with free radicals to form stable and less toxic molecules than the starting free radicals. Some analogues of resveratrol having α-diphenoxyl group are found to be more active in inhibiting ROS-induced DNA damage, accelerating DNA damage in the presence of cupric ions, and inducing apoptosis of HL-60 cells. Some analogues studied for SAR are 3,4-dihydroxy-trans-stilbene (3,4-DHS, 102), 3,4,4'- trihydroxy-trans-stilbene (3,4,4'-THS, 103), 3,4,5-trihydroxy-trans-stilbene (3,4,5-THS, 104), 2,4-dihydroxy-trans-stilbene (2,4-DHS 105), 3,5-dihydroxy-trans-stilbene (3,5-DHS, 106) and 3,5,4'-trimethoxy-trans-stilbene (3,5,4'-TMS, 107). Piceatannol (3,4,3',5'-tetrahydroxy-trans-stilbene, 108) is a naturally occurring analogue of resveratrol found in
red wine (Figure 29). Piceatannol has been observed to inhibit the proliferation of cancer cells via apoptosis and cell cycle arrest.\textsuperscript{131}

![Chemical structures](image)

**Figure 29:** Different analogues of resveratrol

### 1.4.1.1.4.7.3 Biological activities and mode of action

Many studies in cell culture systems as well as animal models have shown the cancer chemoprevention and cancer therapeutic effects of resveratrol. Resveratrol has been found to inhibit tumor initiation, promotion and progression \textit{in vitro} and inhibited tumorigenesis in mouse skin model. Topical application of resveratrol to SKH-1 hairless mice inhibited skin hyperplasia induced by multiple exposures to UVB radiation. Resveratrol inhibits tumor-induced neovascularization at a dose of 2.5-100 mg/Kg (body weight).\textsuperscript{132} The antiproliferate effect of resveratrol was shown to be modulated by cell cycle regulatory proteins. It decreases the expression of D1 and D2, Cdk2, 4 and 6, and proliferate cell nuclear antigen (PCNA), whereas, P21WAF1/C1P1 was increased. Resveratrol has shown pro-apoptotic activity comparable to etoposide and also had an inhibitory effect on endothelial cell migration.\textsuperscript{133} Resveratrol exhibits antitumor activity on murine hepatoma-22. This activity might involve the inhibition of cell cycle progression by decreasing the expression of cyclin B1 and P34cdc2.\textsuperscript{134} Resveratrol can
combat tumor formation by induction of cell cycle arrest and apoptosis. It induces caspase independent apoptosis through Bcl-2 down regulation.

A detailed study of resveratrol with MCF-7 cells showed its antiestrogenic effect at a concentration of $10^{-6}$M and above, at both the cellular and molecular levels. It antagonizes the growth promoting effect of 17β-estradiol (E2) in a dose-dependent manner. The molecular mechanisms associated with the anti-proliferative effects in cancer cells involve the activation of p53, and suppression of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). Growth inhibitory effect of resveratrol has been shown in various cultured prostate cancer cells, both in hormone-sensitive and hormone refractory. Resveratrol is shown to have a synergistic effect in vitro with quercetin and ellagic acid for apoptosis. Some studies showed adverse effects of resveratrol at high doses (100mg/Kg) in such a manner that it enhanced the MNU-induced mammary carcinogenesis in an estrogen free environment.

1.4.1.1.4.8 Indole-3-carbinol

1.4.1.1.4.8.1 Introduction

Indole-3-carbinol (I3C, 109, Figure 30) is present in cruciferae family particularly in genus brassica including broccoli, cauliflower, brussels and cabbage. I3C is one of the most extensively examined bioactive dietary supplement for the prevention and treatment of breast and other types of cancers. It may also have beneficial effects in the management of Herpes Simplex Virus (HSV) and Human Papilloma Virus (HPV). Several mechanisms have been reported for the anticancer properties of I3C and its analogues including apoptosis, antiproliferation, modulation of gene expression, cell cycle arrest etc. Initial clinical studies show that I3C is a potential anticancer agent
against breast and cervical cancer. Some reports show that I3C has immunomodulatory effects\textsuperscript{139} and also exhibits hepatoprotective activity induced by various carcinogens.\textsuperscript{140}

![Indole-3-carbinol](image)

**Figure 30:** Indole-3-carbinol

### 1.4.1.1.4.8.2 Chemistry and analogues

Indole-3-carbinol is produced by the hydrolysis of glucosinolates by the plant enzyme myrosinase. Glycoboressicin is the precursor of indole-3-carbinol.\textsuperscript{141} Under acidic condition, starting with I3C number of polyaromatic indolic compounds (110-120, Figure 31) are produced involving few reaction steps, among these 3,3'-diindolylmethane (DIM, 110) is the main one.\textsuperscript{142} I3C is converted to a series of oligomeric compounds that are thought to be responsible for \textit{in vivo} effect of I3C. 5,6,11,12,17,18-Hexahydrocyclonona[1,2-b:4,5-b':7,8-b’”] triindole (CTR, 111) is the major trimeric product of this category. Methylsubstituted DIMs include 1,1'-dimethyl- DIM (112), 5,5'-dimethyl-DIM (113), 2,2'-dimethyl-DIM (114), 6,6'-dimethyl-DIM (115), and 7,7'-dimethyl-DIM (116) and 1,1',2,2'-tetramethyl-DIM (117).\textsuperscript{143} 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1, 118) is reported as a major antiproliferative component that can suppress the proliferation of both estrogen receptor (ER) positive and ER negative cells.\textsuperscript{144} CTET (119) and LTET (120) are two tetrameric products cyclic and linear respectively.
1.4.1.1.4.8.3 Biological activity and mode of action

Indole-3-carbinol induces G1 cell cycle arrest\textsuperscript{145} and this arrest correlates with the inhibition of the expression of cyclin-dependent kinase 6 (CDK6), inhibition of the endogenous retinoblastoma (Rb) protein phosphorylation and increase of the p21 and p27
CDK inhibitors. It induces cell cycle arrest in estrogen receptors (ER) positive and estrogen receptor (ER) negative breast cancer cells, but yet, it is not clear that the biological activity observed is attributed to I3C or its polymeric products.

According to a study tetrameric derivative of I3C inhibits cyclin-dependent kinase-6 expression and induces G1 cell cycle arrest in both estrogen-dependent and estrogen-independent breast cancer cell lines.\textsuperscript{146} It is five times more potent then I3C against human breast cancer cells. I3C binds at the ER and modulate the metabolism of estrogen.\textsuperscript{147} It binds to aryl hydrocarbon receptor (AhR) and induces cytochrome P450 (cyp) A1/A2 gene expression in both \textit{in vivo} and \textit{in vitro} models. It also inhibits \(E_2\) induced cell proliferation by inhibiting the transcription or ER responsive gene by competing for coactivators or increasing ER degradation.\textsuperscript{148} DIM exhibits potent antiproliferative and antiandrogenic properties in androgen-dependent human prostrate cancer cells.

I3C upregulates the expression of gene related to phase I and phase II enzymes and so increases the elimination of potential carcinogens and toxins.\textsuperscript{149} It also reduced the risk of breast cancer by inducing the expression of CPY1A1, which shifts the estrogen metabolic pathway in favour of 2-hydroxy estrone (2OHE1 estrogen antagonist) and away from 16-\(\alpha\) hydroxyl estrone (16\(\alpha\)OHE1 estrogen agonist). In clinical trials urinary 2OHE1:16\(\alpha\)OHE1 ratio has constantly increased in women when I3C is given as dietary supplement,\textsuperscript{150} although the relation between urinary 2OHE1: 16\(\alpha\)OHE1 ratio and breast cancer is not clear.

I3C induces apoptosis through downregulation of antiapoptotic gene products, including Bcl-2, Bcl-xL, inhibitor-of-apoptosis protein (IAP) and up regulation of proapoptotic protein Bax. DIM was reported to increase the Bax/Bcl-2 ratio in both
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estrogen dependent and estrogen independent human breast cancer cells. It also induces
apoptosis in various cell types including myeloid leukemia and breast cancer cell through
nuclear factor kappa-B (NF-κB) pathway. Along with a variety of anticarcinogenic
activity some deleterious effects have also been reported in some studies including tumor
promotion over prolonged exposure.\textsuperscript{151}

1.5 Breast cancer

1.5.1 Introduction

Breast cancer is the fifth most common cause of cancer deaths. The incidence of
breast cancer in India is on the rise and is rapidly becoming the number one cancer in
females pushing the cervical cancer to the second spot. It is reported that one in 22
women in India and one in eight in America are likely to suffer from breast cancer during
their lifetime. The problem with preventing breast cancer is that there is no single cause
that can be pinpointed as the culprit. Breast cancer is about 100 times as frequent among
women as compared to men, but survival rates are equal in both sexes. The risk of breast
cancer increases with age, eight in ten cases are diagnosed in women aged 50 and above.
About 90\% of the breast cancers are initially hormone dependent, where estradiol plays a
significant role in there development and progression.\textsuperscript{152}

1.5.2 Types of breast cancer

Breast cancer can begin in different areas of the breast; the ducts, the lobules or
in some cases the tissue in between. Majority of breast cancer cases are classified as
either \textit{in situ} or invasive as follows.

- **Ductal Carcinoma \textit{In situ} (DCIS):** It is the most common type of non-invasive
  breast cancer, starts inside the milk ducts. The most common treatment plan for
  DCIS is breast-conserving surgery with or without radiation.
• **Lobular Carcinoma In Situ** (LCIS): It is an invasive breast cancer, starts in lobules, the milk-producing glands at the end of breast ducts. LCIS are usually HER2 negative (-) and ER/PR positive (+) and therefore may be treated with hormonal therapy (tamoxifen).

• **Invasive Ductal Carcinoma** (IDC): It is the most common type of breast cancer. About 80% of all breast cancers are invasive ductal carcinoma; it starts in the milk ducts and invades the surrounding tissues.

• **Invasive Lobular Carcinoma** (ILC): It is the second most common type of breast cancer after invasive ductal carcinoma. ILC develops in the milk-producing glands (lobules) of the breast and spread to other parts of the body.

### 1.5.3 Stages of breast cancer

The most common system used to describe the stages of breast cancer is the American Joint Committee on Cancer (AJCC) TNM system. The TNM staging system classifies cancers based on their T, N, and M stages:

- **T** stands for tumor (its size and how far it has spread within the breast and to nearby organs).

- **N** stands for spread to lymph nodes (bean-shaped collections of immune system cells).

- **M** is for metastasis (spread to distant organs).

Additional letters or numbers appear after T, N, and M to give more details about the tumor, lymph nodes and metastasis.
1.5.4 Treatments of breast cancer

Treatments can be classified into broad groups, based on how they work and when they are used.

- **Local therapy**

  Local therapy is used to treat a tumor at the site without affecting the other parts of the body. Surgery and radiation therapy fall in this category.

- **Systemic therapy**

  Systemic therapy refers to the drugs which can be given orally or intravenously. Chemotherapy, hormone therapy and targeted therapy are systemic therapies.

1.5.4.1 Chemotherapy

Chemotherapy is the treatment of cancer in which cancer cells are killed with cytotoxic drugs (discussed previously in this chapter) that may be given intravenously or orally. Chemotherapy is given in cycle of treatment periods and recovery periods. It is recommended in different situation.

- **Adjuvant chemotherapy:** Chemotherapy therapy given to patients after surgery is called adjuvant therapy. It reduces the recurrence of cancer and kills any cancer cells that may have been left behind.

- **Neoadjuvant chemotherapy:** Chemotherapy given before surgery is called neoadjuvant therapy. It is used to reduce the size of cancer so that they are small enough to be removed by lumpectomy instead of mastectomy.

- **Chemotherapy for advanced breast cancer:** Chemotherapy can be used as main treatment for women having cancer in advance stages, when cancer has already spread outside the breast.
Chemotherapy is best effective when it used in combination but the best known is still not clear. Some common combinations are:

- CMF: cyclophosphamide (cytoxan), methotrexate (amethopterin, mexate, folex) and 5-fluorouracil (fluorouracil, 5-FU, adrucil)
- CAF (FAC): cyclophosphamide, doxorubicin (adriamycin) and 5-fluorouracil
- AC: doxorubicin (adriamycin) and cyclophosphamide
- EC: epirubicin (ellence) and cyclophosphamide
- TAC: docetaxel (taxotere), doxorubicin (adriamycin) and cyclophosphamide
- AC→T: doxorubicin (adriamycin) and cyclophosphamide followed by paclitaxel (taxol) or docetaxel (taxotere) (herceptin may be given with the paclitaxel or docetaxel for HER2/neu positive tumors.)
- A→CMF: doxorubicin (adriamycin) followed by CMF
- CEF (FEC): cyclophosphamide, epirubicin and 5-fluorouracil (this may be followed by docetaxel)
- TC: docetaxel (taxotere) and cyclophosphamide
- TCH: docetaxel, carboplatin and herceptin for HER2/neu positive tumors

1.5.4.2 Hormone therapy

Hormone therapy is used to treat estrogen receptor (ER) positive breast cancer. It is also used adjuvant therapy. Female hormones estrogen and progesterone are naturally produced by the ovaries before menopause. Estrogen primarily controls the feminine character and the reproductive cycle of female. Estradiol and its metabolites estrone and estriol are the major endogenous estrogen. After menopause adrenal gland produces androgen which is converted into estrogen by the enzyme called aromatase. Estrogen
helps in cell proliferation, so that it can stimulate the growth of estrogen receptor positive and progesterone receptor (PR) positive breast cancer. By reducing the amount of estrogen or blocking its action, the growth of ER and PR positive breast cancer can be reduced. Hormone therapy can be classified into following category.

- Selective estrogen receptor modulators (SERM)
- Aromatase inhibitors
- Estrogen receptor downregulators (ERDs)

1.5.4.2.1 Selective Estrogen Receptor Modulators (SERMs)

Role of estrogen in the growth and development of breast cancer have been established during twentieth century. The mechanism by which estrogen increases the growth of breast cancer was unknown until the discovery of estrogen receptor (ER). The discovery of ER rationalized the target site-specific effect of estrogen around a woman’s body and has proved valuable to target breast cancer treatment and prevention. ERα was discovered first in mid 1960s and almost after three decades of its discovery, ERβ was identified in rat, human and mouse. Antiestrogens are antagonists of estrogen action as they block the binding of estradiol to the ER, and so prevent the proliferation of cancerous cells.

During the development of antiestrogen, it was observed that some of these antiestrogens show both estrogenic and antiestrogenic properties (partial agonist/ partial antagonist). They block the action of estrogen in certain tissues while mimicking estrogenic action in other tissues. Such selectivity of the antiestrogen with estrogen receptor of different tissues is made possible by the fact that the estrogen receptor of the different target tissues vary in their chemical structure. On the basis of their selectivity to
stimulate or inhibit the action of estrogen receptor in different target tissues, these antiestrogens are called as selective estrogen receptor modulators (SERMs). The existence of ER\(\alpha\) and ER\(\beta\) subtypes provide a possible explanation for this tissue selectivity of SERMs. The variation in the ratio of the two activities (estrogenic/antiestrogenic) differentiates and identifies SERMs for typical pharmaceutical use. Most of the nonsteroidal antiestrogens such as tamoxifen, raloxifene, toremifene, clomiphene etc. belong to this category.

**Figure 32:** A subcellular model of estradiol (E\(_2\)) action in a target tissue\(^{160}\)

For convenience estrogen receptor is subdivided into six functional domains (A-E Figure 32).\(^{161}\) The binding of agonist to ER, dissociates it from heat shock protein, dimerise, bind to specific DNA sequence and stimulate transcription of responsive gene. Now it is clear that co-activator protein acts as intermediate between the ER and the general transcription machinery.\(^{162}\) Helix 12 is an essential site for AF-2 activation in the ligand binding domain (LBD) of ER\(\alpha\). A crystallographic study of the LBD of ER with estrogen and raloxifene suggested a molecular basis for agonism and antagonism in ER\(\alpha\). Estrogen and raloxifene bind at the same site within the core of LBD, but demonstrate
different binding modes. Binding of the estradiol cause helix 12 to seal the ligand inside the hydrophobic pocket of the ligand binding domain (E-region) and this activates the complex by allowing the binding of coactivators at the activating function-2 (AF-2) site. In contrast, binding of raloxifene prevents helix 12 from sealing the hydrophobic pocket and repositioning of helix 12 blocks the binding of coactivators, which prevents gene transcription. The aminoethoxy side chain is essential structural feature of nonsteroidal antiestrogen. The shape of the ligand with strategically placed alkylaminoethoxy side chain decides the estrogenic and antiestrogenic character of the molecule. Chemically, SERM can be classified in five groups: triphenylethylenes, benzothiophenes, tetrahydronaphthylens, indoles and benzopyrans.

1.5.4.2.1.1 Tamoxifen

Triarylethylenes are developed for the treatment of the estrogen dependent breast cancer and tamoxifen (ICI-46,474, 121, Figure 33) is the representative of this category. Tamoxifen was discovered by Imperial Chemical Industries (ICI) Pharmaceutical (AstraZeneca) and is sold under the trade names nolvadex, istubal and valodex. It is used as first line endocrine treatment for postmenopausal hormone positive advanced breast cancer for over twenty years. It is most widely used breast cancer drug. Tamoxifen was first approved in UK in 1973 and in USA in 1977 by FDA for the treatment of breast cancer. Tamoxifen is selective ER modulator (SERM) and inhibits competitively estradiol binding to the receptor site and so regulates proliferation of cancerous cell. Tamoxifen competes with estradiol for binding to ER with an RBA of 2.0. Tamoxifen is metabolized by liver microsome to various products, out of which, 4-hydroxy tamoxifen is an active metabolite that binds to the ER with an affinity similar to that of estradiol. Activity of tamoxifen depends on its stereo conformations; Z-isomer of
tamoxifen is potent estrogen antagonist with weak estrogenic activity whereas E-isomer is potent estrogen agonist in rat and mouse models.\textsuperscript{168} Tamoxifen is the first estrogen agonist/antagonist identified to inhibit bone loss in postmenopausal women with breast cancer.\textsuperscript{169} Due to some partial agonist action tamoxifen is associated with a number of adverse effects like higher rate of thromboembolic event\textsuperscript{170} and a greater risk for developing endometrial cancer in women of 50 years and above.\textsuperscript{171} Breast cancer cells develop resistance against tamoxifen after a certain period, so there is a need to develop new endocrine agents.

1.5.4.2.1.2 Raloxifene

Raloxifene (122, Figure 33) is the main molecule of the bezothiophene group of SERM. It is an oral, selective SERM that has estrogenic action on bone and antiestrogenic action on breast and uterus. Raloxifene blocks estrogen induced DNA transcription in the breast and endometrium but due to its poor bioavailability and a short biological half-life,\textsuperscript{172} its antitumor action against breast cancer is not as effective as that of tamoxifen. Raloxifene increases bone density and lowers LDL (Low-density lipoprotein) without stimulation of endometrium.\textsuperscript{173} It is manufactured for the treatment of osteoporosis by Eli Lilly and company under the trade name evista. In 2006, it was announced that raloxifene is as effective as tamoxifen in reducing the incidence of breast cancer in certain high risk groups of females, though with a reduced risk of thromboembolic events and cataracts in patients taking raloxifene versus those taking tamoxifen.\textsuperscript{174} The Multiple Outcome Raloxifene Evaluation (MORE) trial reported that raloxifene reduced the risk of invasive breast cancer by 72\% [Relative Risk (RR) 0.28] and the risk of ER positive breast cancer by 84\% (RR 0.16).\textsuperscript{175}
1.5.4.2.1.3 Toremifene

Toremifene (123, Figure 33) is a chlorinated derivative of tamoxifen that lacks the DNA adduct forming ability of tamoxifen and has lower genotoxicity than tamoxifen.\textsuperscript{176} It is a potent antiestrogen that is extensively metabolizes in liver, N-desmethyloremifene is the main metabolite. Toremifene has been used for breast cancer treatment in many countries for more than a decade.\textsuperscript{177} It is licensed in United States under the brand name fareston. Toremifene citrate is approved by US-FDA for use in advanced (metastatic) breast cancer. It has been marketed in Finland since 1988 as oral tablets and has clinical efficacy similar to tamoxifen.\textsuperscript{178} Like tamoxifen, toremifene also causes endometrial cancer. It is also being evaluated for the prevention of prostate cancer under the brand name acapodene.\textsuperscript{179}

![Chemical structures](image)

Figure 33: Tamoxifen, raloxifene and toremifene

1.5.4.2.2 Aromatase inhibitors

Aromatase is a member of cytochrome P450 family of enzyme, present in skin muscles and fat tissues. Aromatase carries out conversion of androgens (C19 steroids) into estrogen (C18 steroid). Aromatase inhibitors stop the production of estrogen in post-menopausal women by blocking the enzyme aromatase. This lowers the estrogen level
and slows the growth of ER positive breast cancers. Aromatase inhibitor can be classified into first-generation (aminogluthethimide, 124), second-generation (formestane, 125 and fadrozole, 126) and third-generation (anastrozole 127, letrozole 128, and exemestane 129) agents. Aromatase inhibitor can also be divided into type I and type II inhibitors.\textsuperscript{180}

1.5.4.2.2.1 Type I inhibitors

Type I inhibitors (Figure 34) have a steroidal framework similar to androgens and inactivate the enzyme in irreversible manner by blocking the substrate binding site. Due to the irreversible nature of inhibition, the enzyme remains inactive even after drug is cleared from the circulation, therefore these inhibitors are marketed as inactivaters or suicide inhibitors.\textsuperscript{181} Formestane and exemestane are examples of type I inhibitors.

1.5.4.2.2.2 Type II inhibitors

Type II inhibitors (Figure 34) are nonsteroidal and generally reversible. Estrogen block ade is dependent on continuous presence of drug.\textsuperscript{182} Anastrozole and letrozole are type II inhibitors.

1.5.4.2.3. Estrogen receptor downregulators (ERDs)

Estrogen receptor downregulators work in the same manner as SERMs, they block the ER so that estrogen can not bind on it. In addition they induce a rapid and quantitative down-regulation of ER\textsubscript{α}.\textsuperscript{183} Fulvestrant is presently available drug in this category.
1.5.4.2.3.1 Fulvestrant

Fulvestrant (Faslodex, ICI182,780, 130, Figure 35), a novel estrogen receptor (ER) downregulator, is a pure steroidal antiestrogen. It exhibits none of the negative side effects associated with the partial agonist activity of tamoxifen. FDA approved fulvestrant for the treatment of ER positive, metastatic breast cancer in postmenopausal women progressing on prior antiestrogen therapy.\textsuperscript{84} It has provided alternative therapeutic strategy following the development of tamoxifen resistance. Fulvestrant has unique mechanism of action; it binds to the ER, thereby inhibiting DNA binding and promotes the rapid degradation of the ER, resulting in a great loss at cellular levels.\textsuperscript{105} It reduces expression of estrogen receptor, progesterone receptor and so proliferation of cancerous cells.
1.5.4.3 Target therapy

Target therapy is also known as molecular target therapy. As per our knowledge breast cancer is the first type of solid tumor cancer to be treated with molecular-target therapeutic approach. The therapy focuses on proteins that signal cancer cell to grow and divide uncontrollably. Gefitinib, bevacizumab, trastuzumab etc. are drugs used in this therapy. Trastuzumab (herceptin, a monoclonal antibiotic) was designed to attack the protein known as HER2. HER2 is overexposed in about 20% of the breast tumor cells and became a therapeutic target for drug research. The success of trastuzumab led to the development of other drugs, which bind to HER2 at different site than trastuzumab, such as pertuzumab.

1.6 Conclusion

Many research groups have targeted this disease with different approaches leading to a number of natural products, their derivatives and synthetic compounds as possible anticancer drugs. But the main problem associated with most of these anticancer drugs is their inadequate selectivity which leads to adverse side effects and thereby limiting their therapeutic usefulness. Drug resistance is another problem arising in this field with temporary solution of combination therapy. Latest approach in cancer chemotherapy is molecular target therapy. Tumor targeting drug delivery bestows some
hope to minimize undesired side effects and improves the quality of life of cancer patients.

We sincerely hope that some breakthrough will come out in the form of target specific, economic and safe cancer therapeutic agents with novel mode of action.
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