Chapter – I

Introduction
1.1 INTRODUCTION

Society has come into existence on account of the realization among its members that collective action enables them to lead a much happier life. In course of its evolution, society in turn evolved various institutions to take care the needs, necessities and requirements of individuals either alone or in groups. Family, community, religion and state have been playing a pivotal role in providing the necessary welfare services for their members. With the growth of population and the development of complex socio-economic problems, the responsibility of undertaking social welfare services and economic development programmes fell largely on the institution of state in every society. The state primarily performed the duty of protection but subsequently started to provide the welfare services for its people. Thus, the "Welfare State" evolved itself from "Police State".

However, with further growth in population as well as the needs of the population, states experienced certain problems in carrying out their problems. Further, the problems of the society magnified to such an extent that it became impossible for the state to tackle these problems on its own. Under these circumstances, a new concept of 'Voluntary Action' emerged in the society in promoting the welfare of the individuals in the society.¹

India is predominantly rural. Rural areas are relatively backward and undeveloped. Rural people are poor. The development of the country cannot be surmised without developing rural areas and improving the economic status of the rural people. The rural backwardness and rural poverty led to several imbalances in regional development and income inequalities. But still rural areas are the backbone of our Indian economy. Hence, rural development has drawn much attention. The Government of India has accorded considerable importance to rural development, in all its five-year plans and allocated funds, liberally. With a view to develop the rural areas, the Government has undertaken many programmes. Poverty alleviation has become the primary concern.²

The Integrated Rural Development Programme (IRDP) was launched in 1978-79 and it highlighted the importance of area approach. IRDP was designed to help the poor by creating new assets, like sources of irrigation, providing implements, seeds, fertilizers, animals for dairy, tools and training for cottage industry and
handicrafts. The basic strategy was self-employment of the poor with the help of these assets so that they manage to earn enough to rise above the poverty line. IRDP has, now been restructured and renamed as Swarna Jayanthi Gram Swarozgar Yojana (SGSY) and certain allied programmes were merged into it. Sampoorana Grameena Rozgar Yojana (SGRY) was launched in September 2001, which aimed at providing wage employment and food security. National Rural Employment Guarantee Scheme (NREGS) was introduced in February 2006, which aimed to provide at least 100 days of guaranteed employment in a financial year to every household in the rural areas.3

The Government is committed to improve the economic standard of the rural people. But, the programmes of Governments are not yet so effective in poverty alleviation. The reasons may be many. The Government's tops down approach generally concentrates on providing food, services or assets, rather than enabling people to do more for themselves. Government policies and programmes are not very much favourable to the poor as they are still treated as passive recipients and humble beneficiaries even after the formulation of Swarnajayanthi Gram Swarozgar Yojana (SGSY). Politicians and bureaucrats, by and large, ignore the rural communities.4

Dr. Y.C. James Yen, Philippines has given the following approach to the rural construction movement.

- Go to the poor people
- Live among them
- Learn from them
- Plan with them
- Work with them
- Start with what they know
- Build on what they have
- Teach by showing, learn by doing
- Not a showcase, but a pattern
- Not odds and ends, but a system
- Not piecemeal, but integrated approach
- Not to confirm but to transform
- Not relief but release.5
The Voluntary organizations or Non-Government Organizations (NGOs) follow the above approach. NGOs have achieved a fair success, relative to Government efforts, in improving the rural livelihoods. NGOs have done spectacular works in the fields of rural development such as community health, agriculture, horticulture, social forestry, watershed development, capacity building, community organization, promotion of SHGs (Self- Help Groups) and education. NGOs are supplementing the efforts of government in the upliftment of rural livelihoods. Moreover NGOs are providing better and quality services when compared to Government agencies, to the rural poor. The increasing number of people’s organizations and NGOs is a clear demonstration of how people all over the world are demanding greater participation in civil society. People, who know more, earn more and can do more will be raising their voices even more loudly for greater participation in every process that affects their lives.6

In this context, it is pertinent to examine the impact of NGOs or voluntary organizations in improving the livelihoods of rural people.

1.2 EVALUATION OF VOLUNTARY MOVEMENT

The NGOs prefer to work in rural areas and with the rural people. NGOs have close access to the people and their efforts are very much benefitting the rural people. The people for their committed work identify them and so they have gained bastion of support of the people with whom they work. Their services are, undoubtedly, commendable in the betterment of the rural poor.

The Government of India recognized the importance of NGOs and is extending its considerable support. The NGOs works are quoted as Noble and Yeoman Services. The NGOs have emerged as a third sector in the country and the rest of the world, next to the Government and the corporate sector. The NGOs have taken up every activity in the field of rural development. “What the government could not do and what corporate sector did not do have been achieved by voluntary organizations”. NGOs may differ in their ideologies, be it Gandhian, Christian, Leftist, Ramakrishna’s, but in all contexts, NGOs keep people as their primary concern. The success of NGOs can be attributed to their commitment and bottom up approach. The success of NGOs made the government recognize their services in the field of rural development. So, the Government is encouraging the voluntary sector
and relying on it in implementing its programmes targeting rural poor. The number of activities allotted to voluntary sector is on the increase over the years. This itself indicates the very success of voluntary sector in our country.7

The remarkable achievements of some NGOs even prompted the foreign donors to extend their liberal financial assistance. NGOs have done spectacular works in the field of community health, agriculture, horticulture, social forestry, water shed development, animal husbandry, small and cottage industries, adult education, community organization, Sangam formation and promotion of SHGs. Many NGOs have joined debates of such issues as the debt crisis, international trade, structural adjustment, the environment, women in development and peace. They often employ economists to monitor such matters and produce detailed reports as well as campaigning materials.8

People in NGO supported areas are in a better position, economically, socially and politically than the people in NGO absent areas. This speaks of the success of NGOs in improving the socio, economic conditions of the rural poor. The corporate sector is also involved in voluntary work. Their assistance is extended to the needy people through the ‘Trusts’. Big industrialists have formed Trusts and are spending sizeable amounts on the welfare activities targeting the poor. Voluntary work is also done by rich and influential philanthropic individuals in the society. They have formed trusts and are contributing liberally to them. They are taking up many welfare activities in the interest of the poor. NGOs are deeply involved in the activity of micro finance. This financial assistance has helped many poor people in the rural areas. The activity of micro finance has helped many in the rural areas to improve their economic conditions.9

The activities of NGOs are ‘pro-poor’ and ‘pro-rural’. NGOs have become the social psychologists to look after the pains and sufferings of the disadvantaged. The capacity building activity of NGOs has helped in improving the capabilities of rural people. They have homogenously taken up hard tasks like tackling poverty and injustice. They, really, enabled the ordinary people to do extra ordinary things. They have adopted bottom up approach in their intervention, contrary to the top up approach of Government. They are working, truly for ‘gender quality’. They have become easily accessible to the most needy i.e. the rural poor. NGOs have reached
the unreached and are working for them and with them. They are active in promoting community based organizations in rural areas. They are successful in creating awareness among the people, in their working areas.10

Of late, the NGOs have certainly increased their outreach in recent years. Both the funds they spent and the numbers of people they deal with have been rising dramatically.

Any general assessment of the impact of NGOs can be made on the following:

- Tackling the poverty.
- Providing the credit to the poor.
- Reaching the poorest.
- Empowering marginal groups.
- Challenging gender discrimination.
- Delivering emergency relief.

NGOs receive funds from various sources like philanthropic individuals, government, foreign governments, international donors etc.

WEAKNESSES AND THREATS

Voluntary movement has certain weaknesses and threats. They are

- Many NGOs lack professionalism. They lack clear vision and strategies and hence, their interventions become a journey without a destination.
- In many cases it is a one man show.
- They are not directly answerable to the beneficiaries because the donors are different.
- Lack of co-ordination between field staff and office staff in some cases.
- Political and religious interference in some cases.
- Absence of accountability and transparency.
- Whims and fancies of promoters govern the organizations, while the written bylaws are held in safety lockers.
- To some, it has become money earning platform and investment less business.
- Corruption and mismanagement of funds.
- Many NGOs face fund-raising problem.
Mushrooming of NGOs leading to unhealthy competition among them (criticize one another)
Political interference in funds allotment.

The weaknesses and threats are not so difficult to overcome. If these weaknesses are removed, the voluntary movement (action) will be completely successful and will enable to achieve the desired objectives (elimination of poverty, social equality, gender equality, women development etc.)

Modifications to NGOs for Better Performance

- NGOs must think and critically analyze before doing things.
- NGOs must focus on building people, individually and collectively (Awareness building, knowledge building, confidence building, capacity building, morale building and gender friendly attitude building).
- They must plan to protect the vulnerable rural communities from the impact of ‘Nation’ i.e. (Globalization, liberalization, industrialization and commercialization).
- NGOs must think globally and act locally.
- Facilitation concept should be understood in its true sense.
- NGOs must reduce their dependence on foreign sources and have to explore funding sources locally.
- They must remember that people are the best judges of the NGOs.
- NGOs can collaborate with other actors of development, be it Government, banks, local bodies, resource agencies and other organization.
- NGOs must remember that their development work in not favour to the poor. It is the person’s legitimate right.
- They must work for the sustainability of the people and sustainability of organizations.
- They must be scrupulous and principled.
- People must be involved not only in planning and implementation, but also in the evaluation and impact assessment.
- They must minimize their own expenses to maximize their spending on the needy.
1.3 DEFINITION OF VOLUNTARY ORGANIZATION, VOLUNTARY ACTION AND VOLUNTARY WORKER

The term Voluntarism is derived from the Latin word 'Volontas' which means 'will or freedom'. The term voluntary action was primarily used to denote any individual action, which was done independent of the state control. The persons who take part in voluntary action without any remuneration for their service have been considered as 'voluntary workers'. A voluntary organization, properly speaking, is an organization which, whether its workers are paid or unpaid, is initiated and governed by its own members without any external control.

Voluntary organization is a group of persons organized on the basis of voluntary membership without state control for the furtherance of some common interests of its members. The term voluntary action, as used here, means private action, that is to say, action not under the direction of any authority wielding the power of the state. Lord Beveridge, who laid foundation for scientific voluntary action in Britain, considers all such private actions, outside the purview of the state, in the service of mankind as voluntary action. The persons who take part in voluntary action without any remuneration for their service have been considered as 'voluntary workers'. The institution or group formed by these voluntary workers is regarded as 'voluntary organization'.

In the recent past, there has been a shift in the meaning of voluntary organization. Now-a-days, many professional voluntary organizations are manned by trained and paid servants. A voluntary organization is a social service and development institution motivated to meet the needs of the most disadvantaged in society, either through direct service to the people or through facilitative / indirect services to other voluntary organizations or government, nonprofit making and not undertaken to be fully funded for its maintenance, directly or indirectly by the government.

Voluntary organizations are non-profit making agencies that are constituted with a vision by a group of like-minded people, committed for the uplift of the poor, marginalized, unprivileged, underprivileged, impoverished, downtrodden and the needy and they are closer and accessible to the target groups, flexible in administration, quicker in decision making, timely in action, facilitating the people
towards self-reliance ensuring their fullest participation in the whole process of development. A voluntary organization is the one, which, whether its workers are paid or unpaid, is initiated and governed by its own members without external control. Voluntary organizations are otherwise called Non-Governmental Organizations (NGOs). The word NGO has been much more used in all contexts by the U.N. Organizations, World Bank funding agencies abroad, the resource agencies within the country, writers and various government departments. 

1.4 TYPES OF NGOs

Basing on the activities of the NGOs, they may be classified into the following types. The functions of NGOs differ in accordance with the programmes they undertake.

1.4.1 Charity NGOs

Charity is the foremost function of the NGOs. Most of them believe that giving something to the poor is like giving the same to God, for example - offering food, dress to the orphans and the destitute.

1.4.2 Relief and Rehabilitation NGOs

NGOs involved in providing relief and rehabilitation programmes fall under this type. NGOs provide relief and rehabilitation services during natural calamities like floods, fire accidents or epidemic diseases or man-made catastrophes like war genocide. The services are provided by such NGOs directly or in collaboration with the Government authority.

1.4.3 Service Providing NGOs

These are welfare oriented. These provide services for the poor and work with welfare motive, for example - maintaining mobile clinics, hospitals, schools and training programmes.

Their services are provided with great sacrifice, high efficiency, low expenses, commitment and dedication. These services oriented NGOs operate in those areas, where government programmes are not adequate or non-existent.
Most of these conjugates were initially tested at a single dose higher concentration (10 μM) in the sixty-cell line panel of the National Cancer Institute. This panel is organized into subpanels representing leukaemia, melanoma, cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. Amongst these conjugates 3m, 3r and 3s were active in the preliminary test and progressed to the five-concentration (0.01, 0.1, 1.0, 10 and 100μM) assay. These conjugates showed potential cytotoxicity with GI50 values ranging from 0.32 - 7.1 μM in most of the human cancer cell lines panel of the NCI. Among them conjugate 3r in which methoxy groups present on both indole and oxindole rings showed better activity than remaining conjugates. The detailed biological studies are actively under progress in our laboratory.

**Scheme 1.** Reagents & conditions: (a) substituted oxindoles, EtOH, Piperidine (catalytic), reflux, 3-4h.
Active conjugates

MDA-MB-435 GI50 = 0.57μM
KM-12: GI50 = 0.32μM

HOP-92: GI50 = 0.57μM
KM-12: GI50 = 0.63μM

KM-12: GI50 = 0.35μM

XIV
CHAPTER-I

GENERAL INTRODUCTION
Cancer is a major worldwide health problem, representing the second leading cause of death worldwide.\(^1\) Improvements in treatment and prevention have led to a decrease in cancer deaths, but the number of new diagnoses continues to rise. According to World Health Organization (WHO) information, it is estimated that there will be 12 million deaths from cancer in 2030.\(^1,2\) Cancer, medically called a malignant neoplasm, is a term for a large group of different diseases. Normal cells in the body follow an orderly path of growth, division, and death; when this process breaks down, cancer begins to form. There are nearly 200 different types of cancers each named for the organ or type of cell from which it originates. Among which colon, stomach, lung, liver, and breast cancer cause the most cancer deaths each year.

The body is made up of various types of cells. These cells grow and divide in a controlled way to produce more cells (Figure 1a). As they are necessary to keep the body healthy. When cells are turn into old or injured, they die and replaced with new cells. However, sometimes this systematic process goes erroneous. The genetic material (DNA) of a cell can turn into damaged or altered, producing mutations that affect the normal cell growth and division (Figure 1b). If cells divide when new cells not needed, they form a mass of excess tissue called a tumor that could be benign or malignant. Benign tumors are not cancer and do not spread to the other parts of the body and they are seldom a threat to life. Malignant tumors are cancerous and they have inclination to spread, occupy and destroy nearby tissues as well as organs. Cancer cells can also smash away from a malignant tumor and travel through the bloodstream (leukemia) or the lymphatic system (lymphoma) to form new tumors in other parts of the body is called metastasis. Diagnosis of cancer has been improved greatly in modern times owing to treatment advances and early detection programs. However, even though survival rates have improved, cancer remains the second top cause of death in the United States and major public health problem in the other developed countries. The rate of its induction all over the world is increasing every year and making it a challenging area for major focus for both physicians as well as scientists.
As cancer is a complex set of diseases each cancer is unique in the way it grows and develops, its chances of spreading, the way it affects the body. Several factors, including location and by the type of cell that the tumor resembles and is therefore presumed to be the origin of the tumor. These types include:

- **Carcinoma** - Carcinoma is a malignant neoplasm of epithelial origin. It is a tumor that arises in the tissues that line the body’s organs like the nose, the colon, the penis, breasts, prostate, urinary bladder, and the ureter. About 80% of all cancer cases are carcinomas.

- **Sarcoma** - Sarcomas are tumors that originate in bone, muscle, cartilage fibrous tissue or fat (connective tissues). Ewing sarcoma (Family of tumors) and Kaposi’s sarcoma are the common types of sarcomas.

- **Lymphoma and leukemia** - These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. When leukemia develops, the body produces a large number of abnormal blood cells. In most types of leukemia, the abnormal cells are white blood cells.

- **Myeloma** - Cancers that begin in the cells of the immune system.
Central nervous system cancers- Cancers that begin in the tissues of the brain and spinal cord.

Types of Treatment

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. The choice of therapy depends upon the location and grade of the tumour and the stage of the disease, as well as the general state of the patient. A number of experimental cancer treatments are also under development. Complete removal of the cancer without damage to the rest of the body is the goal of treatment.

Chemotherapy

It is a treatment in which chemotherapeutic agents are used to kill fast growing cancer cells. But chemotherapy can affect healthy cells that can grow. Therefore, treatment should be planned to minimize side effects. It is the most effective method of treating leukemia.

Surgery

In theory, cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other parts in the body prior to surgery, complete surgical excision is usually impossible. Examples of surgical procedures for cancer include mastectomy for breast cancer and prostatectomy for prostate cancer.

Monoclonal Antibody Therapy

Immunotherapy is the use of immune mechanisms against tumors. These are used in various forms of cancer, such as breast cancer (trastuzumab/Herceptin) and leukemia (gemtuzumab ozogamicin/Mylotarg). The agents are monoclonal antibodies directed against proteins that are characteristic to the cells of the cancer in question, or cytokines that modulate the immune system’s response.

Biological Therapy (Immunotherapy)

Living organisms and substances derived from living organisms are used to treat cancer in this therapy. Some biological therapies, such as vaccine or bacteria do not kill cancer cells directly, whereas other biological therapies, like antibodies or segment of genetic material, target cancer cells directly.

Radiation Therapy

Radiation therapy also called as radiotherapy, X-ray therapy, or irradiation is the use of ionizing radiation to kill cancer cells and shrink tumours. Radiation therapy can be
administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy. Radiation therapy injures or destroys cells in the area being treated by damaging their genetic material, and enables to grow and divide.

**Hormonal Therapy**

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumours include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment.

**Targeted Therapy**

Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with exact molecules occupied in tumor growth and progression. Targeted therapy may be more effective than other types of treatment and less damaging to normal cells.

**Photodynamic Therapy**

Combines a drug called a photosensitizer or photosensitizing agent with a specific type of light to kill cancer cells. It may be used with other therapies, such as surgery, radiation, or chemotherapy.

**Chemotherapy**

Chemotherapy is a type of cancer treatment in which drugs are used to kill cancer cells. It works by preventing or slowing the growth of cancer cells which divide and grow quickly and harms healthy cells also which divide quickly. The choice of chemotherapy depends on the type of cancer and its stage. Chemotherapy can be used to kill cancer cells that are spreading to others parts of body, to slow cancer growth and to shrink tumors that are causing pain.

Although chemotherapeutic drugs attack reproducing cells, they cannot differentiate between reproducing cells of normal tissues and cancer cells. The damage to normal cells can result in side effects. These cells usually repair themselves after chemotherapy. However the toxicity of chemotherapeutic agents to normal cells is the cause of unpleasant side effects such as hair loss, decreased blood cell count, mouth sores, fatigue, diarrhea, anemia, pain and nausea. However new drugs, new combinations of chemotherapy drugs and new delivery techniques are the expected advances in the coming years for curing or controlling cancer with limited side effects and improving the quality of life for people with cancer.
CLASSIFICATION OF CHEMOTHERAPEUTIC AGENTS

Chemotherapeutic drugs are classified into several categories based on how they affect specific chemical substances within the cancer cells, which cellular activities or processes the drug interferes with, and which specific phases of the cell cycle the drug affects. These include DNA interactive agents, DNA topoisomerase I and II inhibitors, carbonic anhydrase (CA) inhibitors, CDK inhibitors, tubulin polymerization inhibitors, antimitotic agents, antimetabolites, and miscellaneous agents.

DNA INTERACTIVE AGENTS

DNA AS A CELLULAR TARGET FOR CHEMOTHERAPEUTIC AGENTS

DNA has long been considered a favoured target for cancer chemotherapeutic agents. In fact, many of the most effective clinical agents, such as alkylating and interactive agents, are DNA interactive. Achieving the desired sequence specificity with DNA-interactive agents is considered to be one of the most formidable hurdles in the development of new agents to achieve therapeutic invention. The double helical structure of deoxyribonucleic acid (DNA) represents the richest source of information within a living organism. Importantly, its sequence codes not only for protein/enzyme synthesis via the process of translation, but it also codes for RNA synthesis. DNA forms the famous alpha helix structure discovered by Watson and Crick in the 1950s.

![Figure 3. Hydrogen bonding between adenine/thymine and guanine/cytosine base pairs of DNA.](image)

In the alpha helix, two strands of DNA run in opposite directions twisting about themselves, held together by sets of complimentary hydrogen bonds between either adenine (A) and thymine (T), or guanine (G) and cytosine (C) (Figure 4). Replication of DNA
treatments. The bases form unique hydrogen bonded pairs (purine with pyrimidine), AT and GC. Besides the hydrogen bonds between the bases, the double helix is stabilized by π-π stacking, Vander Waals interactions and hydrophobic interactions. The particular order of the bases that are arranged along the sugar-phosphate backbone is called the DNA sequence; the sequence specifies the exact genetic instructions required to create a particular organism with its own unique traits. There are different conformational forms of DNA, these are known as A, B, D, and Z-DNA (the latter is a left handed helix), although B-DNA is the most common form. The B-form is more stable under high humidity conditions because water molecules stabilize the structure by forming a spine of hydration in the minor groove.

Certain structural features of the alpha helix are particularly important when considering drug-DNA interaction. Double helical DNA is not a uniform structure, there are places where the strands are more apart, and where they are closer together. These are known as major and minor grooves. In B-DNA, the major groove is wider (12 versus 6 Å) and deeper (8.5 versus 7.5 Å) than the minor groove, creating more accessible to interacting molecules (Figure 3). The base pair arrangement for each groove is very specific, each containing certain hydrogen bond donors and acceptors. In addition, the major groove also contains the methyl group of thymine. Molecules such as drugs and proteins bind at these sites. Of great importance is the difference between the donor and acceptor groups in each groove, this makes it feasible for drug molecules to distinguish selectively between the different bases and sequences of bases.

**Evaluation of Drug-DNA Interactions**

Over the past 20 years, spectacular advances have been made in our understanding of the interactions of small non-peptide molecules with DNA. Assays are available to evaluate the extent of DNA binding and to determine the sequences to which ligands bind. Calf thymus DNA is often used for physical studies of this type because it is readily available in a purified form and contains a relatively even distribution of AT and GC base pairs, which is preferable when examining new compounds of unknown selectivity. If a radio labelled version of the ligand is available, simple incubation with DNA can establish whether binding or bonding takes place. If the ligand contains a chromospheres or fluorescent groups, UV spectroscopy or Fluorescence spectroscopy can be used. Circular dichroism (CD), optical rotatory dispersion (ORD), IR, Raman spectroscopy and viscometer measurements have also been used. Thermal denaturation studies on DNA are common and involve measuring the
'melting point' of DNA alone and in the presence of a ligand (drug). Binding will often stabilise the helix and elevate the melting temperature.

Other powerful techniques for studying DNA binding with short lengths of DNA include NMR spectroscopy and X-ray crystallography,\(^7\) that can provide precise structural information about the types of interactions occurring and the functional groups involved. Ligands can be interacted with oligomers ranging from 6 to 15 base pairs or more, and two-dimensional \(^1\)H or \(^31\)P-NMR experiments such as NOSEY or COSY can be used to locate precisely the ligand on the strand. Data from X-ray crystallography and NMR can be used in conjunction with computational methods to generate useful three-dimensional models of ligand-DNA complexes.\(^8\) All the physical methods are helpful to know whether the DNA binding with ligands take place or not, but cannot give the knowledge about the exact location of binding on a DNA strand to be determined. To do this two types of assays are used, namely strand cleavage assay and affinity cleavage assay.\(^9\) DNA 'foot printing' is an alternative approach that can be used for covalent and non-covalent binders, intercalators and other types of adducts such as co-ordination complexes and triple helices.\(^10\)

**Figure 4.** The structure of part of a DNA double helix.
TYPES OF DRUGS THAT INTERACT WITH DNA

The major groups of clinically important DNA reactive agents are covalent and non-covalent binders.

NON-COVALENT BINDERS

INTERCALATORS

Intercalators are the ligands (molecules or complexes with metal cations) are inserted between two adjacent base pairs in DNA-double helix (Figure 5). This occurs only when the properties of ligands are suitable for this kind of process. Some of the important aspects for better intercalation are the size and the shape of the molecule, as well as a polarity, electronic nature, and overall ability to interact noncovalently with the DNA. Polycyclic planar systems are good candidates for intercalation. The only recognized forces that maintain the stability of the DNA–intercalators complex are Vander Waals hydrogen bonding, hydrophobic or charge transfer forces.\textsuperscript{11}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{Intercalation of molecule between the base pairs of DNA}
\end{figure}

In the early 1960s, Lerman\textsuperscript{12} conducted a number of physical studies on the interactions of DNA with planar aromatic cations, and concluded that planar aromatic molecules could bind to DNA by a process, which is termed as intercalation. This mode of binding has now been established for a large number of polycyclic aromatic systems which include amonafide (I)\textsuperscript{13} and amsacrine(II) (Figure 6).\textsuperscript{14} There are also bis-intercalators like bis-phenazines, which consist of two intercalating moieties joined by a linker, capable of intercalation at two sites separated by a distance defined by the linker length. Other class of intercalators includes ethidium bromide (III), daunorubicin (XXVIII) and mitoxantrone
(XXXIV) which is a simplified analogue of the anthracyclines that is easily synthesized and
has less toxic side effects, which display antitumor activity by this mechanism.

\[
\text{H}_3\text{C} - \text{N} - \text{CH}_3
\]

\[
\text{O} \text{N} \text{C} \text{O}
\]

\[
\text{NH}_2
\]

Amanafide (I)

\[
\text{H}_3\text{C} - \text{N} - \text{CH}_3
\]

\[
\text{H}_2\text{N} - \text{N} \text{NH}_2
\]

Ethidium bromide (III)

\[
\text{H}_2\text{N} - \text{N} \text{SO}_2\text{CH}_3
\]

Amsacrine (II)

**Figure 6.** The structure of DNA intercalators.

**COVALENT BINDERS**

**ALKYLATORS**

The alkylating agents are highly reactive compounds they damage DNA and prevent
the cancer cell from reproducing. They produce their effects by covalently linking an alkyl
group to cell nucleophiles in nucleic acids or proteins. The majority alkylating agents are
bipolar, and they contain two alkyl groups capable of reacting with DNA. Alkylating agents
substitute alkyl groups for hydrogen atoms of bases on DNA, resulting in the formation of
cross-linked bridges within the DNA and prevent replication of DNA and the transcription of
RNA. These agents are not phase-specific and work in all phases of the cell cycle. Alkylating
agents are used to treat various cancers, including multiple myeloma, lymphoma, leukemia,
ovarian, breast, sarcoma and non small cell lung cancers.

The important classes of alkylating agents (Figure 7), utilized in cancer chemotherapy
are nitrogen mustards (chlorambucil (IV), cyclophosphamide (V), mechlorethamine (VI)
and ifosfamide (VII), ethylenimines altretamine, thiotepa (VIII), methanesulfonates
(busulfan IX), nitrosoureas (carmustine (X), triazenes (dacarbazine, procarbazine,
temozolomide (XI) and platinum complexes (e.g., cisplatin (XII).
Groove binding can be via either major or minor groove by covalently (irreversible) or non-covalently (reversible). It is believed that groove binders with increased selectivity will produce a greater biological response for a given dose (and hence cause fewer toxic side effects) than non-selective groove binders. Molecules that target particular DNA sites also have the potential to be used for the selective suppression of transcription from particular gene sequences.

Most of the DNA interactive proteins bind in the major groove, while small molecules of less than 1000 Da, including many antibiotics bind in the minor groove e.g. distamycin (XIII), pyrrolo[2,1-c][1,4]benzodiazepines (anthramycin (XIV), tomaymycin (XV)), mitomycin C (XVI) and netropsin (Figure 8).
Antimetabolites are compounds similar in structure to naturally occurring molecules purines, pyrimidines and folic acid used in the nucleic acid synthesis (DNA or RNA). Because of their structural similarity with metabolites or enzymatic substrate these readily incorporated into either DNA and RNA (purine and pyrimidinenucleotides) induce cell death during the S phase of cell cycle or inhibit enzymes needed for nucleic acid production. Further, they divided into three classes such as purine analogues such as 6-mercaptopurine (XVII) and pyrimidine analogues such as 5-fluorouracil (XVIII) and antifolates methotrexate (XIX) (Figure 9).

Figure 8 Structure of DNA groove binders.

Figure 9. Antimetabolites
CARBONIC ANHYDRASE (CA)

The carbonic anhydrase (CA) family of Zn (II) metalloenzymes catalyzes the reversible hydration of CO$_2$ to HCO$_3$. These are involved in various physiological processes associated with pH control, respiration and transport of CO$_2$/HCO$_3$ between metabolizing tissues and the lungs, fluid secretion, biosynthetic reactions, such as the lipogenesis, gluconeogenesis and ureagenesis.$^{34}$

There are fourteen different carbonic anhydrase isoforms have been identified in mammals. It has been known for some time that several of these isozymes are cytosolic (CAI, CAII, CA III, CA VII), CA IV is membrane-bound. CA V is present only in mitochondria, and CA VI is secreted in saliva. The inhibition of CAs has been exploited clinically for several decades for the treatment of a variety of conditions including glaucoma, epilepsy and gastric ulcers. More recently, CA inhibition has been implicated as playing an important role in cancer progression.$^{35}$ Generally, an aromatic or heteroaromatic sulfonamide moiety (ArSO$_2$NH$_2$) is the primary recognition element necessary for small molecules to bind the active site of CA. Coordination of the nitrogen atom of the ionized sulfonamide anion (ArSO$_2$NH-) to the active site Zn (II) of CA facilitates this protein-small molecule interaction. Several classical clinical agents from this class of CA inhibitors include acetazolamide (AAZ (XX)), methazolamide (MZA (XXI)), ethoxazolamide (EZA (XXII)), dichlorophenamide (DCP (XXIII)), brinzolamide (BRZ (XXIV)), dorzolamide (DZA (XXV)) and indisulam (IND (XXVI)) are in phase II clinical trials as anticancer agent to treat solid tumours (Figure 10).$^{36}$

![Figure 10. Sulfonamides inhibiting carbonic anhydrase.](image-url)
ANTITUMOR ANTIBIOTICS

Several antibiotics are widely used as anticancer agents. Which include a group of anticancer antibiotics known as the anthracyclines, dactinomycin, mitomycin-C and bleomycin antibiotics (Figure 11). They are isolated from soil bacteria of the genus
**Streptomyces.** Anthracyclines are antitumor antibiotics that interfere with enzymes involved in DNA replication. These drugs work in all phases of the cell cycle and major drugs in this class are daunorubicin (XXVII), doxorubicin (XXVIII), epirubicin and idarubicin.

Anthracyclines attack cancer cells by multiple mechanisms the major pathways are (i) DNA intercalation: inhibits DNA and RNA synthesis through intercalation between base pairs of the DNA/RNA strand; (ii) DNA topoisomerase II inhibition: prevents the relaxation of supercoiled DNA into circular/linear form and thus blocking DNA transcription and replication; (iii) Free radical generation: generates iron mediated oxygen free radicals that damage the DNA and cell membranes leads to cell death; (iv) induce aggregation of chromatin.

**Topoisomerase Inhibitors**

DNA is normally a supercoiled double helix of two strands and is periodically relaxed into circular/linear form in the process of replication during cell division or in the process of reading the code in protein synthesis. There are two enzymes topoisomerase I and II play an important role in the uncoiling and recoiling process. In addition they play a key role in
Figure 12. Mechanism of action of DNA topoisomerase inhibitors fixing DNA damage that takes place on exposure to harmful chemicals or UV rays. Topoisomerase I cuts a single strand of the DNA double helix while topoisomerase II cuts both strands of DNA leading to relaxation of the supercoiled DNA during the replication or repair after the completion of the process the strands are paired back together and reform a supercoiled DNA (Figure 12).

In general cancer cells divide much more rapidly than normal cells, when these enzymes are inhibited in a cell, it stops the DNA replication, reading of the DNA for protein synthesis and repair of DNA damage, leading to cell death. The topoisomerase I inhibitors are camptothecin (XXXI) and its derivatives Topoisomerase II inhibitors include doxorubicin (XXVIII), etoposides (XXXII), ellipticine (XXXIII) and mitoxantrone (XXXIV) (Figure 13).
Tyrosine kinase inhibitors (TKIs) are a class of chemotherapeutic drugs that inhibit, or block the tyrosine kinase enzyme. TKIs are used for targeted treatment of specific cancers, which decrease the risk of damage to healthy cells and receptor tyrosine kinases (RTKs) are a family of tyrosine protein kinases. In general, these signalling binders are growth factors and involved in the initialization and regulation of cell cycles. There are three primary growth factors relating to tyrosine kinase. Epidermal growth factors (EGF) help regulate cell growth and differentiation, platelet-derived growth factor (PDGF) regulates cell growth and development and vascular endothelial growth factors (VEGFR) are involved in the creation of blood vessels.

Figure 13. Topoisomerase inhibitors

**Kinase Inhibitors**

Tyrosine kinase inhibitors (TKIs) are a class of chemotherapeutic drugs that inhibit, or block the tyrosine kinase enzyme. TKIs are used for targeted treatment of specific cancers, which decrease the risk of damage to healthy cells and receptor tyrosine kinases (RTKs) are a family of tyrosine protein kinases. In general, these signalling binders are growth factors and involved in the initialization and regulation of cell cycles. There are three primary growth factors relating to tyrosine kinase. Epidermal growth factors (EGF) help regulate cell growth and differentiation, platelet-derived growth factor (PDGF) regulates cell growth and development and vascular endothelial growth factors (VEGFR) are involved in the creation of blood vessels.
Figure 14. Kinase inhibitors

The growth factors, and the kinesis, act as an "on/off" switch process wherein removal of a phosphate group changes the shape and actions of the protein. This essentially "turns on" the cellular action and after removal of the phosphate group, the protein is "turned off". If this "on/off" process is disrupted, it leads to a mutated kinase and these actions can become unregulated. If an unregulated or mutated RTK bound to EGF, an uncontrolled growth and division occurs in the cell, this rapid cell growth leads to cancer. Mutations in the RTKs often lead to oncogenes, the genes that help to revolve a healthy cell into a cancerous cell. Tyrosine kinase inhibitors treat cancer by correcting this deregulation. The major TKIs are imatinib (XXXV), gefinitib (XXXVI), Erlotinib (XXXVII), Lapatinib (XXXVIII), Sunitinib (XXXIX).

Tyrosine kinase inhibitors treat cancer by correcting this deregulation. The major TKIs are imatinib (XXXV), gefinitib (XXXVI), Erlotinib (XXXVII), Lapatinib (XXXVIII), Sunitinib (XXXIX).
TUBULIN AND MICROTUBULES

MICROTUBULES

Microtubules are the key components of the cytoskeleton of eukaryotic cells. They are essential in development and maintenance of cell shape, transport of vesicles, mitochondria, cell signaling, cell division and mitosis. They play a critical role in cell division by involving in the movement and attachment of the chromosomes during various stages of mitosis. Therefore microtubule dynamics is an important target for the developing anti-cancer drugs.49

STRUCTURE

Microtubules are composed of two globular protein subunits, α- and β-tubulin, and these two subunits combine to form an α, β-heterodimer which then assembles in a filamentous tube-shaped structure. The tubulin heterodimers arrange themselves in a head to tail manner with the α-subunit of one dimer coming in contact with the β-subunit of the other. This arrangement results in the formation of long protein fibres called protofilaments. These protofilaments form the backbone of the hollow, cylindrical microtubule which is about 25 nanometers in diameter and varies from 200 nanometers to 25 micrometers in length. About 12-13 protofilaments arrange themselves in parallel to form a C-shaped protein sheet, which then curls around to give a pipe-like structure called the microtubule. The head to tail
arrangement of the hetero dimers gives polarity to the resulting microtubule, which has α-subunit at one end and a β-subunit at the other end. The α-tubulin end has negative (-) charges while the β-tubulin end has positive (+) charges (Figure 15). \(^5^0\)

The functional diversity of microtubules is achieved through the binding of various regulatory proteins, including microtubule associated proteins (MAPs). Two molecules of GTP (guanosine triphosphate) are also important components of the microtubule structure. One molecule of GTP is tightly bound to the α-tubulin and is not hydrolyzed whereas the other GTP molecule is bound to β-tubulin and can be hydrolyzed to GDP (guanosine diphosphate). The stability of the microtubule will depend on whether the β-end is occupied by GTP or GDP. A microtubule having a GTP molecule at the β-end will be stable and continue to grow whereas a microtubule having a GDP molecule at the β-end will be unstable and will depolymerise rapidly. \(^5^1\)

![Diagram of microtubule structure and dynamics](image)

**Figure 16.** Structure and dynamics of microtubules. (Nature Reviews Cancer, reference 43, copyright © 2004)

The polymerization of microtubules occurs through nucleation and elongation mechanism in which the relatively slow formation of a short microtubule 'nucleus'is followed by rapid elongation of the microtubule at its ends by the reversible and non-covalent addition of tubulin dimers (Figure 16). They show complex polymerization dynamics that use
energy provided by the hydrolysis of GTP bound with β-tubulin, these dynamics are crucial to their cellular functions. The correct movements of the chromosomes and their proper segregation to daughter cells require extremely rapid dynamics, making mitosis exquisitely sensitive to microtubule targeted drugs. The biological functions of microtubules in all cells are determined and regulated mainly by their polymerization dynamics. Microtubules show two kinds of non-equilibrium dynamics, one is dynamic instability and the other is treadmilling. Dynamic instability is a highly prominent process in which the individual microtubule ends switch between phases of growth and shortening. The two ends of a microtubule are not equivalent, at one end (positive/plus) grows and shortens more rapidly and more extensively than the other end (negative/minus). The microtubules undergo relatively long periods of slow lengthening, brief periods of rapid shortening and periods of attenuated dynamics or pause in which there is neither growth nor shortening. Dynamic instability is characterized by four main variables the rate of microtubule growth, the rate of shortening, the frequency of transition from the growth or paused state to shortening called 'catastrophe' and the frequency of transition from shortening to growth or pause called 'rescue'.

The second dynamic behaviour is called 'treadmilling' the net growth at one microtubule end and balanced net shortening at the opposite end (Figure 16). It involves the intrinsic flow of tubulin subunits from the plus end of the microtubule to the minus end of the microtubule. This behaviour occurs in cells as well as in vitro, and might be particularly important in mitosis. Molecules or drugs, which act as inhibitors of tubulin also act as inhibitors of cell division. Microtubule exists in a constant dynamic position of growing and shortening by reversible association and dissociation of α/β-tubulin heterodimers at both the ends. This dynamic behaviour and resulting control over the length of the microtubule is vital to the proper functioning of the mitotic spindle in mitosis of the cell division. The drugs, which repress the microtubule dynamics are adequate to block the cell cycle and result in the death of the cells by apoptosis.
**Figure 17. Tread milling microtubule.** Tubulin hetero dimers are added at the plus end of the microtubule at time 0, treadmill through the microtubule and are lost from the minus end of the microtubule at time 3. The length of the microtubule is unchanged. Tread milling is brought about by the different tubulin critical concentrations at the opposite ends (Nature Reviews Cancer, reference 43, copyright © 2004)

**Classification of Tubulin Binding Drugs**

Large number of chemically diverse substances binds to soluble tubulin and/or directly to tubulin in the microtubules. They differ from the other anticancer drugs in their mode of action because most of these compounds are antimitotic agents and inhibit cell proliferation by acting on the polymerization dynamics of spindle microtubules. Tubulin binding drugs have been classified based on their mode of action and binding site. Microtubule-targeted antimitotic drugs are generally classified into two main groups:

i). Tubulin polymerization inhibitors (microtubule destabilizing agents).

ii). Tubulin depolymerization inhibitors (microtubule stabilizing agents).

Tubulin inhibitors act by interfering with the dynamics of the microtubule, one group of compounds reduce the formation of microtubules by inhibiting the tubulin polymerization and are called tubulin polymerization inhibitors like the colchicine analogues and the vinca alkaloids. They reduce the microtubule polymer mass in the cells at high concentration and act as microtubule-destabilizing agents. The other group of compounds inhibit the depolymerization of polymerized tubulin and increase the microtubule polymer mass in the cells, act as microtubule-stabilizing agents and are called depolymerization inhibitors like the paclitaxel analogues. Natural products have been the basis and the most reliable source for new and effective anticancer agents. Most of the tubulin-interactive agents are natural products or semi-synthetic derivatives of natural products, and this must be due in part by the fact that most natural products (tubulin-interactive natural products) are complex compounds with significant stereochemistry. Hence, they are well adapted to binding at the complex
three-dimensional surface of a protein such as tubulin. Among the various mechanisms of action of natural products, interaction with the cellular protein tubulin/microtubule is one of the most important, and over 25% of the new clinical candidates listed by Butler operate by this general mechanism.

**Tubulindepolymerization Inhibitors**

**Taxanes**

Taxanes are remarkable cytotoxic diterpenes derived from natural products. Taxanes include paclitaxel (XL) and docetaxel (XLI) Figure 19. Paclitaxel identified as the first member of a novel group of anti-cancer drugs, isolated it from the bark of the pacific yew tree, *Taxus brevifolia* and named as taxol. Paclitaxel (XL) binds β-tubulin and promote the assembly of microtubules, inhibits microtubule depolymerization. Taxol inhibits chromosome transport in a dividing cell by binding in a pocket of β-tubulin above the β-sheet. Paclitaxel is used as a first line drug for the treatment for ovarian, breast, lung, and colon cancer and the second line drug for the treatment of AIDS-related Kaposi's sarcoma.

Docetaxel (XLI) is a semi-synthetic analogue of paclitaxel and extracted from the bark of pacific yew tree, *Taxus brevifolia*. Docetaxel differs from paclitaxel at C10 and C13 positions in its chemical structure. It has a hydroxyl functional group on C10, whereas paclitaxel has an acetate ester, and a tert-butyl carbamate ester exists on C13 instead of the benzyl amide in paclitaxel. The presence of hydroxyl group on C10 causes docetaxel to be more water soluble than paclitaxel.

The antiproliferative activity of docetaxel is exerted by promoting and stabilising microtubule assembly, while preventing microtubule depolymerisation/disassembly in the absence of GTP. This leads to inhibition of mitotic cell division between metaphase and anaphase, preventing further cancer cell progeny. The main mode of mechanism action is suppression of microtubule dynamic assembly and disassembly, rather than microtubule bundling leading to apoptosis, or the blocking of bcl-2 oncoprotein. Docetaxel mainly used for the treatment of breast, ovarian, prostate and non-small cell lung cancer (NSCLC).
Figure 18. Binding of Paclitaxel along the interior surface of the microtubule suppressing its dynamics (Nature Reviews Cancer, reference 43, copyright © 2004)

EPOTHILONES

Epothilones A and B (XLII, XLIII, Figure 19) are naturally occurring 16-membered macrolides that were isolated in 1993 from the myxobacterium Sorangium cellulorum. They are more water soluble than paclitaxel analogues and also show efficacy in taxol resistant cells. Among the synthetic derivative ixabepilone (XLIII) amide-NH in place of lactone oxygen) was approved for clinical use by FDA in 2007 for the treatment of metastatic or locally advanced breast cancer in patients whose tumors are resistant or refractory to anthracyclines, taxanes and capecitabine.

Figure 19. Taxol domain-binding agents

TUBULIN POLYMERIZATION INHIBITORS

Tubulin polymerization inhibitors are two types according to their binding sites. They include:
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a) Vinca alkaloids binding site: includes vinblastine, vinflunine, vinorelbine, vincristine, dolastatins, halichondrins, hemiasterlins, cryptophycin etc.

b) Colchicine binding site: includes the colchicine, combretastatin, methoxy benzene sulfonamides (E7010), 2-methoxy estradiol, etc.

VINCA ALKALOIDS

The vinca alkaloids, vincristine (XLIV) and vinblastine (XLV) isolated from the periwinkle plant Catharanthus roseus (vinca rosea) and vinorelbine (XLVII) is a semi synthetic vinca alkaloid, they stop the cell division and cause cell death. During cell division, vinca alkaloids molecules bind to tubulin protein, and thereby prevent tubulin assembly.66 In spite of their similarity in the structure and common mechanism of action, they have widely different toxicological properties and antitumor activities. Vinblastine (VBL) inhibits angiogenesis. Vinblastine regularly used to treat breast cancer, Hodgkin's disease, non-Hodgkin's lymphoma, and germ cell tumors.67 VBL is administered with bleomycin and cisplatin for the treatment of metastatic testicular carcinoma. Vinorelbine (VRB)) used for the treatment of breast cancer, osteosarcoma (bone tumor cells) and advanced non-small cell lung cancer either as a single agent or with cisplatin.68 Vincristine's inhibition of microtubule assembly is powerful. Vincristine (VCR) alkaloid has a high affinity for tubulin dimmers and the reaction between vincristine and the tubulin dimers is rapidly reversible. Vincristine is FDA approved drug to treat acute leukemia, neuroblastoma, Wilm's tumor,
Figure 20. Vinca domain-binding agents

Vincristine \( R_1 = \text{CHO} \quad R_2 = \text{CO}_2\text{Me} \quad R_3 = \text{OCOCH}_3 \) (XLIV)

Vinorelbine (XLVII)

Vinblastine \( R_1 = \text{CH}_3 \quad R_2 = \text{CO}_2\text{Me} \quad R_3 = \text{OCOCH}_3 \) (XLV)

Vindesine \( R_1 = \text{CH}_3 \quad R_2 = \text{CONH}_2 \quad R_3 = \text{OH} \) (XLVI)

Vinflunine (XLVIII)

Hemiasterlin \( R = 1\text{-methyl-3-indoly} \) (XLIX)

HTI-286 \( R = \text{Ph} \) (L)

Rhabdomyosarcoma, Hodgkin’s disease, and other lymphomas. Several synthetic analogs of these alkaloids are also in clinical use, particularly vindesine (XLVI) used to treat melanoma and lung carcinomas.\(^6^9\) The fluorinated analogue vinflunine (XLVIII) is in phase II clinical trials, and is used to treat advanced transitional cell bladder and urothelial tract cancer.\(^7^0\) Some of the drawbacks of vinca alkaloids and their analogues is their neurotoxicity and the easy development of resistance, normally mediated by the over expression of the Pgp-170 transport protein.

**HEMIASTERLINS**

Hemiasterlins are natural products isolated from the South African sponge *Hemiasterella minor*. hemiasterlins A, B and C criamides A and B and milnamide A are members of a small family of cytotoxic tri and tetra peptides that have been isolated from the marine sponges (*cymbastella sp, Auletta sp* and *Siphonochalina sp*).\(^7^1\) Hemiasterlins contain naturally occurring tripeptides with modified amino acids like tert-leucine, N-methylvinlogous valine residues and tri or tetra methylated tryptophan.\(^7^2\) Hemiasterlin is an effective inhibitor of cell growth that, inhibits polymerization of microtubules (XLIX, Figure 20). These agents bind to the tubulin assembly at the peptide binding site and are poor
substrates for p-glycoprotein and among the synthetic derivatives, HTI-286 (L) is under clinical use.  

**COLCHICINE BINDING SITE AGENTS**

**COLCHICINE**

Colchicine and demecolcine (LI and LII, Figure 21) are an alkaloids isolated from plant of the *Colchicum autumnale*, also known as "meadow saffron". These have been used in treatment of certain forms of leukemia and against solid tumors. Antimitotic activity and antigout activity of colchicine is usually the result of its interaction with tubulin protein. Colchicine inhibits tubulin polymerization by binding to tubulin protein, which is essential to mitosis and consequently colchicine effectively acts as a mitotic poison.

The colchicine molecule has at least three chemically diverse sites at which molecular changes are possible, without disturb the tricyclic arrangement. These includes, the three 1,2,3-tri methoxy groups in the aromatic ring A, the substituted amine or amide at the chiral center C7 of ring B, and the tropolone methyl ether moiety in ring C. Colchicine played an essential role in studies of mitosis. Colchicine binding site was characterized recently from a complex with colchicine and a stathmin-like domain. In recent years, a number of compounds with high potential being developed as anticancer drugs that were reported to interact with colchicine-binding site of tubulin, although, they apparently do not have structural similarity with colchicine. Some examples include combretastatin A-4, 2-arylo indoles and chalcones, indanocine etc. Among the all N-acylated analogues of colchicine and 3-demethylcolchicine showed significant effect in vitro and in vivo and it was less toxic than other colchicine analogues.
2-Methoxyestradiol (LIII)

2-Methoxyestradiol (LIII, Figure 21) is a natural metabolite of estrogenic hormone β-estradiol. 2-Methoxyestradiol exhibits potent apoptotic activity against rapidly growing tumor cells and it inhibits tubulin polymerization at the colchicine-binding site.76

Podophyllotoxin (LIV)

Podophyllotoxin (LIV, Figure 21) and several active analogues were isolated from Podophyllum peltatum and related species it is also a ligand of the colchicine binding site.77 Its tubulin activity is greatly reduced by epimerization at C-4 and completely abolished by the presence of sugar molecules as found in etoposide.
Combretastatins are a class of natural compounds and isolated from the bark of the South African tree *Combretum caffrum* in 1989 by Pettit and coworkers. Combretastatin A-1 (CA-1) and combretastatin A-4 (CA-4) are the most active of 17 compounds isolated from the natural source, in terms of their effects on tumour cells *in vitro* and their interaction with tubulin. The first isolated combretastatins from this family are combretastatin A-4 (LV) and A-1 (LVI, Figure 22) and exhibited potent cell growth and tubulin polymerization inhibition. Subsequently, CA-5 (LVII) and CA-6 (LVIII) were also isolated and evaluated their biological activity. Combretastatin B-series are reduced form of combretastatin A-series example combretastatin B-1 (LIX).

Among *Combretaceae* family, combretastatin A-4 (LV) showed to be the most effective at the same time as CA-1 was about one fifth and CA-2 was one tenth as potent as CA-4. CA-4 has been studied extensively because of its potent cytotoxicity. CA-4 has been shown to interact with tubulin at or near the colchicine binding site. Combretastatin A-4, which has cis-configuration at olefinic bridge, is more potent than its *trans*-isomer. Woods and coworkers in 1995 reported that the *cis* configuration along with other structural features to be important for biological activity of CA-4. Inspite of its simple structure and low molecular weight, CA-4 is one of the most potent anti-mitotic agent and powerful inhibitors of tubulin polymerization known to date from the combretastatin family. Combretastatin A-4 is a highly cytotoxic against a variety of cancer cell lines, including multidrug resistant cancer cell lines (daunorubicin resistant P-388 cell line). In addition, CA-4 disrupts the cell signaling pathways engaged in regulation and maintenance of the cytoskeleton of endothelial cells in tumor vasculature, thereby leading to selective shutdown of blood flow all the way through tumors. CA-4 inhibited cell growth by 50% at 7 nM and suppressed tubulin polymerization by 50% at 2.5 µM. CA-4 specifically binds to the colchicine binding site, which is known to be separate from the vinca binding site.

The main drawbacks of combretastatin A-4 as a drug are low water solubility and isomerisation to the less active *trans*-form. Initial efforts to overcome water solubility led to the design and synthesis of several water-soluble sugar derivatives of CA-4. However, these sugar-combretastatins experienced large decrease in cytotoxicity compared to CA-4. Consequently, CA-4 disodium phosphate (CA-4-DP), a water-soluble prodrug of CA-4 was developed. This pro-drug is cleaved to the active CA-4 by endogenous nonspecific
phosphatases, which are then taken up into cells. CA-4-DP is a vascular-disruptive agent that causes rapid decrease in tumor blood flow. The direct achievements of CA-4-DP include increases in vascular permeability and the endothelial cytoskeleton destabilization, which lead to occlusion of the tumor vasculature, as demonstrated that it caused no or little damage to normal liver tissue and vasculature but induced extensive blood flow shutdown in the tumor compared to normal tissues. In addition, several studies demonstrated that CA-4P act as a potent antimetastatic agent.

**BENZENE SULFONAMIDE DERIVATIVES**

Sulfonamides have a variety of diverse biological activities for example antibacterial, antidiabetic, antiinflammatory, carbonic anhydrase inhibitory, antithyroid and anticancer activity. Sulfonamide based molecules include E7010, E7070 (LX and LXI, Figure 23).
Figure 23. Benzene Sulfonamide Derivatives

**E-7010 (ABT-751)**

E7010 (LX) was designed and synthesized by Hiroshi Yoshino in 1992. It causes apoptosis and cell cycle arrest in M phase, it inhibits tubulin polymerization due to its reversible binding at colchicine binding site on tubulin. E7010 exhibits potent *in vivo* antitumor activity against tumor xenografts and various rodent tumors, and it is in phase II clinical trials.

**Miscellaneous Anticancer Drugs Acting on Tubulin Site**

Estramustine phosphate (a derivative of estradiol) is used in the treatment of advanced metastatic cancer, alone or in combination with other tubulin inhibiting agents. Benzoylphenylurea (NSC-639829) is another tubulin inhibiting agent, which was developed initially as insecticide but showed antitumor activity in random screening and is being evaluated in clinical trials in patients with refractory metastatic cancer. It is also a potent inhibitor of DNA polymerase. Discodermolide (LXII, Figure 24) is another marine natural product isolated from the sponge *Discodermia dissolute*, entered into clinical trials by Novartis but it proved to be toxic during its clinical trials and has been dropped. Eleutherobin (LXIII), isolated from a marine soft coral was shown to have similar microtubule-stabilizing properties to paclitaxel. Recently Reddy and coworkers reported a small molecule lead, aryl-3-arylamino-2-propen-1-ones as potential tubulin polymerization promoters and stabilizes microtubules. These synthetic small molecules showed cytotoxicity in the nano molar range and particularly (LXIV) was found to be highly active against a wide variety of human tumor cell lines including those that are resistant to the activity of many of the currently used chemotherapeutic agents. Romagnoli and coworkers in 2012, described a new series of tubulin polymerization inhibitors based on the 2aryl/heteroaryl-4-amino-5-(3',4',5'-trimethoxybenzoyl)thiazole scaffold, with a thienyl group at the 2-position of the thiazole skeleton (LXV) and it induced apoptosis in HeLa cells through the mitochondrial pathway with activation of caspase-3, as well as potential antivascular activity and significantly reduced the growth of the HT-29 xenograft in a nude mouse model, suggesting that it is a promising new anti-mitotic agent with clinical potential. More importantly, (LXVI) also showed significant *in vivo* activity in a colon cancer xenograft model.
**Figure 24. Some miscellaneous natural and synthetic tubulin interactive agents**

**INDIBULINE (D-24851)**

Indibulin (D-24851 (LXVII)) (N-(pyridine-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxyl-amid) is a orally applied, synthetic, small molecule with antitumor activity based upon destabilization of microtubules. It's anticancer activity was first reported by Bacher and coworkers, indibulin induces accumulation of cells with condensed nuclei and abnormal mitotic spindles, and arrests cells at metaphase. This antimitotic drug is active against a wide range of human tumor cell lines and xenografts, including multidrug resistant tumor cells and taxane refractory tumors. In preclinical studies indibulin lacks neurotoxicity typically associated with other tubulin binding drugs such as the taxanes and vinca alkaloids. Furthermore, good bioavailability after oral application and curative treatment at almost non-toxic doses were shown. Thus, the novel tubulin binding agent indibulin was predicted to have a significantly improved therapeutic index compared to other microtubule inhibiting compounds, such as paclitaxel and vincristine. Indibulin is currently undergoing early stage clinical trials for the treatment of solid tumors.
OBJECTIVES OF THE PRESENT WORK

In recent years, combination chemotherapy with different mechanisms of action is one of the methods that are being adopted for treatment of cancer. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of cancer. Recent studies have proved that DNA intercalation potentiality of β-carbolines. This DNA intercalation leading to altered DNA replication fidelity or to an influence on enzymatic activities in DNA repair process. On the other hand, oxindoles are versatile moieties that display diverse biological activities, including anticancer activity. They exhibit antitumor activity by inhibiting tyrosine kinase receptors such as PDGF-R, VEGF-R or CDK. In this context, we have synthesized a series of β-carboline-oxindole conjugates and evaluated for their biological activity. The detailed biological studies revealed that some of the compounds of the series have emerged as potent cytotoxic agents (Chapter II).

Microtubule is an important component of the cytoskeleton formed by α and β tubulin heterodimers, which has been demonstrated to be critical in many cellular processes such as cell shape maintenance, intracellular transport, cell division, cell growth and mitosis. As the key structural basis of microtubule, tubulin is considered as a highly attractive target for anticancer therapy. Several tubulin inhibitors have been approved as first line chemotherapeutic agents for different types of human cancer. However they have certain limitations such as high neurotoxicity, drug resistance and complex syntheses. This has encouraged developing new tubulin targeting agents with simple structure. Keeping this in mind, an attempt has been made to address this problem and the following new research investigations have been carried out.

Benzimidazole is a bicyclic heteroaromatic molecule which is structural isostere of naturally occurring nucleotides hence, it has been extensively utilized as a useful scaffold in
the medicinal chemistry. Benzimidazole derivatives have shown different therapeutic properties including antitumor activity. They were found to exert their antitumor activity by acting various mechanistic ways including tubulin polymerization inhibition. On the other hand, oxindoles are versatile moieties that display diverse biological activities, including anticancer activity. Recent investigations proved the tubulin polymerization inhibition potentiality of the oxindole moieties. In continuous efforts towards the development of novel anticancer agents, we have synthesized a series of benzimidazole linked oxindole conjugates and further their anticancer activity and tubulin polymerization inhibition ability was evaluated (Chapter III).

Small molecules such as combretastatin A-4 (CA-4) and indibulin (D-24851) which affect the tubulin polymerization have drawn considerable interest in the development of novel anticancer agents. Indibulin (D-24851) is a synthetic, small molecule with antitumor activity targeting the tubulin system. D-24851 destabilizes microtubules by direct interaction with tubulin at binding site different from those known destabilizing tubulin agents. In preclinical studies indibulin lacks neurotoxicity typically associated with other tubulin binding drugs such as the taxanes and vinca alkaloids. On the other hand sulfonamides known for various therapeutic properties including antitumor activity. E7010, a sulfonamide that exhibits antitumor activity by inhibiting tubulin polymerization. In this context, we have synthesized a series of N-sulfonyl indolyl glyoxylamide conjugates and further their biological activity was evaluated (Chapter IV Section-A).

In continuation of our research on microtubule targetting anticancer agents with small and simple structures we have synthesized a series of indole-indolinone conjugates and evaluated their antitumor activity activity (Chapter IV Section-B)
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