Section A

Chapter 1. Introduction
1.0 Introduction

Clinically, cancer is a name given to the large family of diseases, maybe a hundred or more, that vary in age of onset, rate of growth, state of cellular differentiation, diagnostic detectability, invasiveness, metastatic potential, and response to treatment and prognosis.\(^1\) Cancer occurs when cells in a part of the body start to grow out of control or cells become abnormal and forming new cells without any control or order.\(^2\) All organs of the body are made up of cells. Normally, cells divide to form new cells only when the body needs them. If cells divide when new ones does not need, they form a mass of excess tissue, called a tumor. Tumors can be benign (not cancer) or malignant (cancer). The cells in the malignant tumors can damage and invade nearby tissues and organs. Cancer cells can also break away from the malignant tumor and travel through the bloodstream to form new tumors in other parts of the body. The spread of cancer is called metastasis.

Cancer is a disease that has tormented man throughout recorded history. Among the first to document cancer were the ancient Egyptians, whom 1600 B.C. years ago wrote detailed accounts of breast cancer.\(^3,4\) Continuing through the ages, cancer was researched and described by numerous historical figures including Greek physician Hippocrates (460–370 B.C.), considered the “Father of Medicine”, Galen, and Giovanni Morgagni. It was not until the second half of the 20th century, however, that cancer has been more fully researched and its molecular and cellular basis began to be understood. As a result, many effective treatment regimens have been developed. However, cancer continues to be a major killer.\(^5,6\)

In the world cancer is the second leading cause of death behind heart disease.\(^7,8\) Cancers in all forms are causing about 12 per cent of deaths throughout the world. While In India, Cancer has become one of the ten leading causes of death.\(^9\) It is estimated that there are nearly 1.5-2 million cancer cases at any given point of time. More than 7 lakh new cases of cancer and 3 lakh deaths occur annually due to cancer.\(^10\) Nearly 15 lakh patients require facilities for diagnosis, treatment and follow up at a given time. Data from the National Cancer Registry Programme indicate that the leading sites of cancer are oral cavity, lungs, esophageal, cervical and colic amongst men and cervix, and breast amongst women. Cancers namely those of oral
and lungs in males, and cervix and breast in females account for over 50% of all cancer deaths in India.

From the average diagnosis, most common types of cancer have seen at age 67. Although cancer is relatively rare in children, still it is a leading cause of death between ages 1 and 14. Millions of people alive today have had some type of cancer. Of these, about half are considered cured. The good news is that more and more people are now being cured of their cancers. This progress is due to better techniques of diagnosis and treatment.

In many cases, the causes of cancer are not clear, but both external and internal factors play a role. Due to the complex nature of cancer development, it is not possible to pinpoint one specific agent as the cause of cancer. Rather, it is most likely due to a number of contributing factors. These factors include exposure to certain chemicals, action of viruses, exposure to radiation, and heredity. Today we recognize and avoid many specific substances that cause cancer: coal tars and their derivatives (like benzene), some hydrocarbons, aniline (a substance used to make dyes), asbestos, and others. Radiation from variety of sources, including the sun, is known to cause cancer. To ensure the public safety, the government has set standards for many substances, including benzene, asbestos, hydrocarbons in the air, arsenic in drinking water, and radiation. Collectively, these are known as risk factors. Brief risk factors are summarized in Table 1.1

**Table 1.1** Risk factors for the development of cancer.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Risk Factor</th>
<th>Mechanism of Action</th>
<th>Example</th>
</tr>
</thead>
</table>
| 1       | Chemicals   | • Damage DNA, leading to mutations | • Encountered in the manufacture of dyes, chemicals, petroleum products  
• Associated with tobacco usage, general environmental pollution, diet, some |
| Chapter 1                                                                                                    Introduction… |
|---------------------------------------------------------------|-------------------------------------------------------------|
| 2 Radiation                                                   | medicines                                                   |
|                                                               | • Damage DNA, leading to mutations                           |
|                                                               | • UV, ionizing radiation, radioactive elements               |
| 3 Viruses                                                     | • Not thought to induce Cancer                               |
|                                                               | • Epstein-Barr virus, human papilloma Virus, hepatitis B virus, HIV |
|                                                               | • Participate in early stages leading to cancer               |
| 4 Heredity                                                    | • Transmission of a single gene increases likelihood of cancer |
|                                                               | • Inherited cancers are commonly diseases of childhood: retinoblastoma, Wilm’s tumor |
|                                                               | • Adult diseases: colon and breast carcinoma                 |
|                                                               | • Rare hereditary diseases increase likelihood of cancer     |
|                                                               | • Xenoderma Pigmentosum, Ataxia telangiectasia               |

### 1.1 Cancer Prevention

The best way to reduce deaths from cancer is to prevent it. Medical doctors generally agree that about one-third of all human cancers are directly related to cigarette smoking. For smokers, the risk of cancer is much higher than that of the nonsmokers. Excluding the UV rays of sunlight which cause skin cancer, the next most common cited cancer-causing factor is diet. The National Cancer Institute and the American Cancer Society recommend a diet low in fat, high in natural fiber, and rich in fruits and vegetables. On the other hand Chemoprevention is simply prevention with drugs. The word “drugs” is used to include dietary supplements, hormones, and vitamins etc., as well as real drugs such as aspirin and other synthetic agents used for therapeutic purposes. The number of chemopreventive agents is increasing.
1.2 Cancer Treatments

There are three main strategies for cancer treatment: surgery, radiation and chemotherapy, which are used to attempt the damage cancer cell. The role of each depends upon types of tumor and the stage of its development.

1.2.1 Surgery

Surgery is the oldest and still the most common local treatment for cancer. Surgery is a procedure to remove or repair a part of the body. Surgery also can help to decrease the tumor bulk and, along with other treatment measures, may provide a cure for certain cancers. However, surgery is not always the best answer. It generally works best on slow-growing cancers.

1.2.2 Radiation therapy

Similar to surgical intervention, radiation therapy is a localized treatment. Radiation therapy is a use of high-energy radiation from x-rays, gamma rays, neutrons, protons, and other sources to treat cancer cells. Radiation may come from a machine outside the body (external-beam radiation therapy), or it may come from radioactive material placed in the body near cancer cells (internal radiation therapy). Systemic radiation therapy uses a radioactive substance, such as a radiolabeled monoclonal antibody, that travels in the blood to tissues throughout the body. Radiation therapy is used at some point in the treatment of more than half of all cancer cases.

1.2.3 Chemotherapy

Chemotherapy is the use of cancer-fighting medications to stop the growth of malignant cells. During Second World War, naval personnel who were exposed to mustard gas as a result of a military action were found to have toxic effects on the bone marrow cells that developed into blood cells. In the course of that observation, compound called nitrogen mustard was studied and found to work against a cancer of the lymph nodes called lymphoma. The development and use of chemotherapy drugs (chemo) have resulted in the successful treatment of many people with cancer. It works by either killing the cells or preventing them from dividing. Many of cancers can be controlled with chemotherapy for a long period of time, even if they are not cured.
1.2.4 Other therapies

**Targeted therapy** is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with rapidly dividing cells.¹⁸

**Immunotherapy** is the use of the immune system to reject cancer. The main premise is stimulating the patient’s immune system to attack the malignant tumor cells that are responsible for the disease. This can be either through immunization of patient (e.g., by administering a cancer vaccine) or through the administration of therapeutic antibodies as drugs.¹⁹

**Hormone therapy** inhibited the growth of some cancers by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers.²⁰

1.3 Type of different cytotoxic agents

Agents for cancer chemotherapy are often organized into groups according to their origin or mechanism of action. The six major classes of agents include the alkylating agents, antimetabolites and nucleoside analogs, antitumor antibiotics, antimitotic, hormonal, and miscellaneous agents. The mechanism of action, drug group, and relevant examples of each class are shown in Table 1.2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Class of Anticancer</th>
<th>Mechanism of Action</th>
<th>Drug Group</th>
<th>Example Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkylating Agents</td>
<td>React with DNA</td>
<td>Aliphatic</td>
<td>Mechlorethamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrogen Mustard</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aromatic Nitrogen Mustard</td>
<td>Melphalan, Chlorambucil</td>
</tr>
<tr>
<td></td>
<td>Phosphoramidip</td>
<td></td>
<td>Phosphoramidip Cyclophosphamide, Ifosfamide</td>
<td></td>
</tr>
<tr>
<td>Class</td>
<td>Mechanism</td>
<td>Antimetabolites</td>
<td></td>
<td></td>
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<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Antimetabolites &amp; Nucleoside Analogs</td>
<td>Interact with cellular Enzymes</td>
<td>Nitrosourea&lt;br&gt;Pyrimidine Antimetabolites&lt;br&gt;Purine Antimetabolites&lt;br&gt;5-Fluorouracil, Cytarabine, Gemcitabine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Antitumor Antibiotics</td>
<td>Intercalate DNA, formation of free radicals</td>
<td>Antitumor Antibiotics&lt;br&gt;Streptomyces&lt;br&gt;Doxorubicin, Mitomycin C, E0-9, Bleomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Antimitotic</td>
<td>Interfere with microtubule function</td>
<td>Antimitotic&lt;br&gt;Vinca Alkaloids&lt;br&gt;Vincristine, Vinblastine, Vinorelbine&lt;br&gt;Taxanes&lt;br&gt;Paclitaxel, Docetaxel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Hormonal</td>
<td>Bind to receptors</td>
<td>Hormonal&lt;br&gt;Bind to receptors&lt;br&gt;Antiestrogens&lt;br&gt;Antiestrogens&lt;br&gt;Tamoxifen, Estramustine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Miscellaneous</td>
<td>Various interactions with cellular DNA, inhibiting replication, transcription</td>
<td>Miscellaneous&lt;br&gt;Miscellaneous&lt;br&gt;Platinum Complex&lt;br&gt;Other class&lt;br&gt;Enzymes&lt;br&gt;Platinum Complex&lt;br&gt;Cisplatin, Carboplatin, Tetraplatin&lt;br&gt;L-asparagin&lt;br&gt;Other class&lt;br&gt;Hydroxyurea, Mitotane, Camptothecins, Topotecan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.4 Alkylating Agents

Alkylating agents are the oldest class of anticancer drugs which are still commonly used; they play a vital role in the treatment of varieties of cancers. Alkylating agents that induce permanent DNA damage are often exhibit potent antitumor activity. A range of DNA alkylating agents is known including monofunctional alkylating (reacts only one DNA strand to form genotoxic monoadducts) and bifunctional alkylating drugs. The latter were found to intrastrand cross-links, interstrand crosslinks, inter-helix or DNA-protein cross-links (ICLs) on DNA, which resulted in more potent and efficacious agents. Currently, a variety of bifunctional alkylating agents are widely used for the treatment of patients with malignant diseases.

1.5 Naturally Occurring DNA Bifunctional Alkylating agents

1.5.1 Mitomycine C and its analogues

The existence of bioactive compounds in plants and other natural sources has played an important role in the development of new drugs. For e.g., In the 1950s, the Mitomycines were identified as a new and potent class of antibiotics agents. The mitomycines displays a wide array of substitution patterns inherent to core structure of pyrrolo[1,2-a]indole. (Figure 1) Originally isolated from Streptomyces caespitosus in Japan, one of the members of the family, Mitomycine C (MMC, 1) has been use for antitumor antibiotic activity. They have obtained a prominent place in cancer chemotherapy as a DNA bifunctional alkylating agents and used to treat a verity of tumors.
Chapter 1

Introduction…

Figure 1. Examples of naturally occurring Mitomycine derivatives.

The synthetic analogue of the antitumor antibiotic Mitomycin C, EO-9 (7) is a bioreductive alkylating agent indoloquinone which serves as a target for nucleophilic DNA and capable to cross-linked with DNA. The aziridinylquinone is a derivative of banzoquinone, represented perhaps the simplest of the mitomycine C like prodrugs. Most studied aziridinylquinone derivative are the simple unsubstituted diaziridinylquinone (DZQ, 8) and the more clinically promising quinone derivative is AZQ (9), active against human brain tumors.

Figure 2. Examples of DNA alkylating Quinone derivatives

Mitomycine C itself inactive, which is activated by chemical (Na₂S₂O₄, NaBH₄, H₂, NO/HCO₂Na/hv), electrochemical or enzymatic (old yellow enzyme, NADPH-cytochrome c reductase/NADPH, xanthine oxidase/NADH, DT-diaphorase) reduction of the quinone moiety to
semiquinone or hydroquinone to initiate alkylation. Iyer and Szybalski49 proposed a mechanism for the activation and alkylation reactions, their mechanism was purely based on structural considerations and chemical precedent shown in Scheme 1. While its structure exhibits similarities to other quinone natural products, Initial One or two-electron reduction of the quinone ring 1 to either the semiquinone or the hydroquinone (10).58-60 Generation of hydroquinone facilitates the loss of the methoxy group, leading to the formation of the hydroquinone intermediate (11). Tautomerization followed by the reaction with the N2-amino group of guanine produces monoadduct (14). Elimination of the carbamoyl group produces the highly reactive vinylogous hydroquinone methide intermediate (15), which alkylates the guanine on the opposite strand of DNA to produce (16), after oxidation, an interstrand cross-link (17).

**Scheme 1.** Proposed mechanism of DNA cross-linking by two electron reductive activation of Mitomycin C.
1.5.2 Pyrrolizidine alkaloids

Other natural products, Pyrrolizidine alkaloids (PAs) are constituents of over 6000 species of plants, most notably Boraginaceae, Graminae, Leguminosae and Compositae, insects including various species of butterflies and even animals such as amphibians. They elicit hepatotoxic, carcinogenic, antineoplastic and genotoxic activity, primarily via DNA cross-linking.\(^6^1\) Their ubiquitous presence, as well as their interesting biological activity is through the formation of DNA-protein cross-links.\(^6^2\)

The common structural feature of all these alkaloids is the presence of a functionalized pyrrolizidine moiety – the necine base.\(^6^1\) Although a large variety of necine bases have been isolated (Figure 3), most of the alkaloids contain retronecine (20) as their pyrrolizidine portion.

![Diagram of Pyrrolizidine alkaloids](image)

\textbf{Figure 3.} Example of naturally occurring common necine bases.

The mechanism of DNA cross-linking by pyrrolizidine alkaloids (e.g. retrorsine, 23) has been extensively investigated. Inhibition of DNA synthesis in pyrrolizidine alkaloids treated liver-slices, corroborated with the lack of a similar effect in lung tissue cultures.\(^6^3\) It is well established mechanism of action involving oxidative activation of the pyrrolizidine moiety by chemical oxidation in vitro or hepatic oxidation in vivo.\(^6^4\) Further studies demonstrated that the retronecine portion of (23) is oxidized by liver cytochrome P450 to a highly reactive pyrrole (26)\(^6^5\) (Scheme 2).
Conjugation with the pyrrole nitrogen lone pair makes the C-7 and C-9 positions of (26) highly reactive toward nucleophilic attack. Thus, such species is extremely reactive was reported by Niwa et al.\(^{65}\)

Along with mitomycin C (1) and pyrrolizidine alkaloids (23), several other naturally occurring or synthetic antitumor agents contains at least one, often two or three, reactive electrophilic centers in the molecule through which they exert their anticancer activity. For examples (Figure 4), aflatoxin B1 (27),\(^{66}\) 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) (28),\(^{67}\) naphthoquinone β-lapachone (29),\(^{68}\) anthramycin (30),\(^{69}\) azinomycin B (31),\(^{70}\) anthracycline derivatives (i.e. doxorubicin, 32),\(^{71}\) cyclopropylpyrroloindoles (i.e. duocarmycin, 33).\(^{72}\)
Studies involving the structure-activity relationship among pyrrolizidine alkaloids suggest that although the presence of the unsaturated pyrrolizidine base (retronecine) is the most important element for the cross-linking capacity of these compounds, there are other, more subtle structural features modulating both the potency and preference of various alkaloids for different types of DNA lesions. The patterns of proteins crosslinked to DNA are similar to those induced by standard bifunctional alkylating agents such as mitomycin C.73

Hopkins et al. have demonstrated that pyrrolizidine alkaloids metabolites target the same 5’GpC3’ DNA sequence as antitumor antibiotic mitomycin C (1) albeit with less selectivity.74 These findings can be explained by the structural similarities between the alkylating moiety of active mitomycin metabolite 34 and activated pyrrolizidine alkaloids the added sequence promiscuity. In the case of pyrrolizidine alkaloids suggest the lack of some molecular recognition constituents inherent to the mitomycins (Figure 5).
1.6 Synthetic DNA Bifunctional Alkylation agents

Structure-activity relationship studies with many of these natural products are often limited by the small quantities of material available and by the relatively limited number of modifications which actually can be performed on these complex molecules. On the other hand, to synthesize this simple molecules for SAR study; one can use biological active natural products as the base template.\textsuperscript{75-77} Based on structural characteristics of the mitomycin C and pyrrolizidine alkaloids, Hopkins et al. have demonstrated that dehydroretrorsine (36), and dehydromonocrotaline (37), dehydroretronecine diacetate (38), 2,3-bis(acetoxymethyl)-1-methylpyrrole (39), and 3,4-bis(acetoxymethyl)-1-methylpyrrole (40) also cross-linked with DNA in same manner like MMC targets, with a high degree of selectivity for 5-CpG.\textsuperscript{78,79}
In additionally, Anderson et al.\textsuperscript{80,81} have designed and synthesized several “acylated vinylogous carbinolamine” tumor inhibitors including (Figure 6); l-Phenyl-2,5-dimethyl-1-3,4-bis(hydroxymethyl)-pyrrole-bis(N-methylcarbamate) (41a-j)\textsuperscript{80} and 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrol bis(N-methylcarbamate) (42a-r)\textsuperscript{81} also demonstrated to have DNA interstrand cross-linking activity as same as MMCs.

The rationale design of these agents is based on the concept that these agents can act as bifunctional electrophiles in which [(carbamoy1)oxyl]methyl groups serve as reactive electrophilic centers. The SAR studies of these agents against P338 lymphocytic leukemia revealed that the compounds having 1-substituted derivatives 41, compounds possessed significant reproducible activity against the P-388 lymphocytic leukemia (PS) assay with the lowest dose of 12.5 mg/kg. Activity at this dose ranged from %T/C = 190-132 for compounds 41e and 41c, respectively. In the series of C-2 substituted derivatives 42 the electronic and lipophilic properties of the substituent X can be varied rather extensively without loss of significant antileukemic activity.

In the course of exploring the structure-biological activity relationships of pyrrole-derived bifunctional alkylating agents, a series of 6,7-disubstituted pyrrolizine diesters have been synthesized and evaluated for antitumor studies.\textsuperscript{82}
The potential electrophilic reactivity of the allylic esters in 43 (via O-alkyl cleavage) will be enhanced by participation of the ring nitrogen similar to that of MMC and PAs. All of these compounds exhibited significant antileukemic activity against P-388 in animal model. Of these derivatives, bis(methylcarbamate) derivative 44 showed potent activity at lowest dose tested, 0.78 mg/kg and 45, afforded “cures” at dose levels as low as 12.5 mg/kg. Several other compounds showed high activity against P-388 over a four-fold dose range without appeared acute toxicity. The most potent compound of this class was 5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizidine-6-7-bis(isopropylcarbamate) (46), which had significant reproducible antitumor activity against a broad range of experimental murine neoplasias and hormone tumor xenografts in nude mice. This compound was selected for more extensive preclinical studies, but there was no further information has been published whether this agent was in clinical trials.

Studies on the structure-activity relationships of derivatives of 5-phenylpyrrolizines 43 revealed that the nature of R and R¹ substituent significantly affected both chemical reactivity and biological activity of the system. The chemical reactivity of 43 toward nucleophiles was found that compounds having electron-donating substituent(s) (OMe) on the phenyl ring were generally less cytotoxic than those compounds bearing electron-withdrawing substituent(s) (halogen). While, the in vivo antitumor activities were comparable or slightly less potent in compounds having an electron-donating substituent. These studies also suggested that the lipophilicity of compound might affect its antitumor potency. The reactivity of this system depended upon the π–electron density in the pyrrole ring and the ability of the C-5 substituent
(R) to stabilize the formation of the positive charge on the heterocyclic nitrogen atom during the displacement of the ester moieties (via O-alkyl cleavage). In general, the electrophilic reactivity can be controlled by the electronic influence of the C-5 substituent. The size of the R1 substituent does not appear to have any significant effect upon potency, activity, or toxicity.

The water soluble analogues of (46, IPP) were also prepared for the oral availability, Anderson & co-workers have designed and synthesized several series of compounds to study their antitumor effect against P388 lymphocytic leukemia and B16 melanocarcinoma in mice. Among these agents, alcohol derivative 47a (where R = H) showed to have comparable activity to 46 with a dose of 50 mg/kg, while 47b (where R = COCH$_2$N(CH$_3$)$_2$) showed less active and 47d with very low activity. In order to prepared more lipophilic compounds, the same authors have synthesized hetero analogues of 43 with general formula 48 in which the C-2 methylene was replaced by S, SO or SO$_2$. However, these agents were found to be less cytotoxic as compare to (46, IPP). The water soluble derivatives 49 were also synthesized by using prodrug approach in which an α-halopyridinium moiety as the pyrrolizines C-5 substituent. The 4- and 5-pyrrolizinyl-2-halopyridinium iodides and the corresponding pyridones were evaluated against P388 lymphocytic leukemia in vivo. The compounds having small size (α-fluoropyridinium) were active but the α-chloro compounds were not. Compounds were active in the P388 screen, were evaluated in L1210 leukemia, M5076 carcinoma, and MX-1 mammary xenograft assays in mice.
From this observation, Lalezari et al. have prepared 1-Thia analogues of 46 (IPP).\(^8\) Of these derivatives, compounds having Ph, F and di-Cl were equipotent (IC\(_{50}\) = 1.5, 1.6, and 1.9 µM respectively) against the HL-60 (human leukemia) cells as compared to 46 (IC\(_{50}\) = 1.5 µM) while 4-Cl (51) derivative showed an increase potency of 75% (IC\(_{50}\) = 0.85 µM). All derivatives were also cytotoxic against HT-29 (human colon carcinoma) cells as similar inhibition of 46.

![Figure 9](image_url)

Further Anderson and group have also synthesized a different set of heterocyclic analogs (52-56)\(^9\) of pyrroles (41 and 42) to examine differences in several aspects—aromaticity, pKa, lipophilicity, dipole moments, charge-transfer donor/acceptor. All prepared derivatives were tested for antileukemic activity against murine P-338 lymphocytic leukemia. None of the compounds was active when compared to 41 and 42. It was shown that electrophilic reactivity is one requirement for antineoplastic activity in these bis(carbamates). It was also evident that the bis(carbamates) are not functioning as carbamoylating agents since so many of these nonpyrrole bis(carbamates) derivatives were inactive.
The rational used for the design of 46 (IPP) Anderson et al. have extended to imidazoles nucleus to prepared water soluble derivatives. Appropriate electron donating or electron withdrawing substituents were added to the imidazole ring to modulate the reactivity of the two electrophilic centers. The potent advantage of the imidazole is that it is sufficiently basic to allow for the preparation of salts to enhance water solubility. All synthesized imidazole derivatives were tested against murine P-388 lymphocytic leukemia. The structure activity relationship studies of this series of compounds were emerged that the electron-withdrawing substituents at either N-1 of C-2 gave rise to inactive compounds. However, the electron-donating substituents gave active compounds and the 2-(methylthio)-1-methyl derivatives 58 (carmethizole) was found to be most potent. The derivative 58, were also found to be active against the MX-1 mammary xenograft, the human amelanotic melanoma cell line LOX xenograft, the M-5076 sarcoma, and L-1210 lymphocytic leukemia. Finally, 58 was reached for clinical evaluation.

Studies on the mechanism of action showed that the bis(carbamate)pyrroles (41 or 42) or pyrrolizines derivatives (43-51) exert their antitumor effect was
probably via an \( S_{N1} \) electrophilic reaction (Scheme 3).\(^79,82\) These derivatives are capable of forming DNA interstrand cross-link with the short oligonucleotide 5'-ACGT at the 5'-CG residues at the minor groove region.\(^79\)

\[
\begin{array}{c}
\text{Scheme 3. The proposed mechanism of DNA bis-alkylation by pyrrolizidine derivatives}
\end{array}
\]

These agents are not carbamoylating agents; instead the carbamate moieties are leaving groups in an alkyl-oxygen cleavage mechanism (60). The reactions take place on methylenic carbons bonded directly to a heteroaromatic pyrrole nucleus (61). The role of the heteroaromatic system is to stabilize reaction transition states, and this provides a means to control the reactivity of the two electrophilic centers. Control may be achieved through the alteration of the heteroaromatic system.

The degree of conjugation observed in any biaryl system is related to the capability of the system to adopt a coplanar conformation. Studies on comparison of nonbonding interactions in C-5-phenyl pyrroles (42a-r) and C-5-phenyl pyrrolizines (43-51) revealed that two significant nonbonded interactions exist in the 5-phenylpyrrole derivatives (65, Figure 12) between the ortho hydrogens of the phenyl substituent and the hydrogens on the N-1 methyl group and the C-4 methylene.\(^94\) While, one of these nonbonded interactions is absent in the pyrrolizine compounds (66, Figure 12): the pyrrolizine C-3 methylene hydrogens lie outside the van der
Waals radii of the ortho-hydrogen atoms of the C-5 phenyl ring and demonstrated that the phenyl and pyrrole rings in \textbf{43} are coplanar (or very nearly so).\textsuperscript{94}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure12.png}
\caption{A comparison of nonbonded interactions in pyrrole and pyrrolizine derivatives.}
\end{figure}

On the basis of this observation, several new set of analogues that possess angular tricyclic structures to limit the deviation from coplanarity of the phenyl and pyrrole rings. Specifically, tricyclic derivative of pyrrolo[2,1-\textit{a}]isoquinolines was first designed and evaluated for antitumor activity against P388 lymphocytic leukemia.\textsuperscript{94} It was demonstrated that the pyrrolo[2,1-\textit{a}]isoquinolin-bis(isopropylcarbamate) \textbf{67a} exhibited the best activity of the compounds tested. The carbamate derivative \textbf{67a} were also found broad spectrum of activity against the various human tumors xenograft (MX-1 breast, CX-1 colon and LX-1 lung) and murine tumors (B16 melanocarcinoma, L1210 lymphoid leukemia, CD8F\textsubscript{1} mammary and lewis lung carcinoma colon 38). However, the tricyclic compounds revealed to be more potent than pyrrole and pyrrolizine derivatives.
On the potency of 67, several other angular tricyclic pyrrolo[1,2-a]quinolines (68a-b), pyrrolo[1,2-a]benzazepines (69a-b) and pyrrolo[2,1-a]isobenzazepines (70) were also synthesized and evaluated for antileukemic activity (Figure 13). The bis(carbamates) (68-70) were tested in vivo against P388 lymphocytic leukemia. In the pyrrolo[2,1-a]isquinoline series (67a-b), the C-3 methyl group had more pronounced effect on activity and toxicity than the C-7 methoxy group whereas pyrrolo[1,2-a]quinolines were less active. The pyrrolo[1,2-a]benzazepine-bis(carbamates) 69a and 69b showed approximately equivalent activity to the comparable pyrrolo[1,2-a]quinolines. The fused benzazepines 70 were less potent and more toxic than the corresponding pyrrolo[2,1-a]isoquinoline (67a). It was concluded that ring fusion and ring substitution do alter the potency, activity and toxicity of the bis(carbamates) derivatives. It was also indicated that compounds with the phenyl ring attached directly to the pyrrole nitrogen will have different structure-activity requirements from the compounds in which the phenyl ring is attached to the pyrrole α-carbon.

Recently, Su et al. have designed and synthesized a series of DNA bifunctional alkylating agents (Figure 14), bis(hydroxymethyl)-8H-3a-azacyclopenta[a]indene-1-yl derivatives and their bis(methylcarbamate) derivatives (71). These agents can be considered as “benzologues” of bis(hydroxymethyl)pyrrolizines and were able to cross-link to DNA double strands. It was demonstrated that these analogues exhibited potent antitumor activity against human lymphoblastic leukemia and various solid tumor cell growths in vitro and potent antitumor efficacy in vivo with a relatively low toxicity. Detailed SAR studies
were demonstrated that the size and electron properties of the substituent at C-3 affected the cytotoxicity of these agents. Remarkably, complete tumor remission (CR) in nude mice bearing human breast carcinoma MX-1 xenograft by bis(hydroxymethyl) derivatives (72 and 73, Figure 14) and bis(methylcarbamate) derivatives (74 and 75) were achieved. Interestingly, compound 73 was able to significantly suppress against prostate adenocarcinoma PC3 xenograft in nude mice. Studies on the DNA interstrand cross-linking suggested that these derivatives were potent bifunctional DNA cross-linking agents. Furthermore, 3a-aza-cyclopenta[a]indene derivatives were able to induce substantial G2/M phase arrest of the cell cycle.

In addition, they also demonstrated that the effect of combining alkylating agents 74 and arsenic trioxide (ATO, DNA repair inhibitor) significantly suppressed human large cell lung carcinoma H460 xenograft (>82%) and cisplatin-resistant NTUB1/P human bladder carcinoma xenografts (>92%) in nude mice. From this observation, it was concluded that a combination of bifunctional alkylating agents and ATO may be a rational approach for treating cancers with inherited or acquired drug resistance. These exciting results provoked to continue designing and synthesizing new bis(hydroxymethyl)pyrrolizine analogues for antitumor studies.

### 1.7 Rational Drug Design of Bis(hydroxymethyl) and their Bis(alkylcarbamates) Derivatives.

As mentioned previously, pyrrolo[2,1-α]isoquinolines (67, Figure 13) bear a angular tricyclic ring system and it showed best activity of the other angular tricyclic (68-70, Figure 13). Moreover, tricyclic derivatives do appear to be most cytotoxic.
than pyrrole and pyrrolizine derivatives. Based on the potent antitumor activities and mechanism of action of MMC (1) and pyrrolo[2,1-a]isoquinolines (67), to investigate whether analogues of 67 with a linear tricyclic ring system also possess potent antitumor activity. In medicinal chemistry term, the molecule differing one from another by only a methylene group is called homologues. By using ring enlargement approach in 3a-azacyclopenta[a]indene (71) derivatives to design and manipulation of the original B ring systems, it is great interest to synthesize a series of new linear bis(hydroxymethyl) of 5,10-dihydropyrrolo[1,2-b]isoquinolines and their bis(alkylcarbamates) (76) derivatives having structure in Figure 15. One can expect that the newly synthesized compounds might be able to cross-link to the macromolecular DNA via a similar mechanism of action as that of pyrrolizines derivatives. (Scheme 4)

![Figure 15](image)

**Scheme 4.** Proposed mechanism of action of 5,10-dihydro-pyrrolo[1,2-b]isoquinolin-1-yl derivatives

The chemical synthesis of bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-b]isoquinolines and their bis(alkylcarbamates) derivatives are described in the
Chapter 2. The antitumor activities, *in vitro, in vivo* evaluation and mechanism of action of these agents are described in the Chapter 3.