A review of alkylating-like agents in reducing mortality of cancer patients: Structure & Synthesis

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
Brac University
July, 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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Approval

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Ethics Statement

The study involved no animal and human trial.

Abstract

Cancer is known as one of the world's deadliest diseases characterized by uncontrolled cellular proliferation. Alkylating agents are one class of anticancer drugs which cross-link DNA strands and induce cell death. The ultimate objective of alkylating agents is to enhance patient outcomes and lessen the impact of cancer on society. In this review article, a thorough overview of alkylating agents utilized in cancer treatment is covered. It will discuss mechanism of actions, structure-activity relationships (SARs), synthesis, and potential future direction of alkylating agents. Specifically, alkylating agents that are historically important, currently used or seem promising in clinical trials are mainly discussed here. The objective of the review is expected to aid pharmacologists, synthetic and medicinal chemists and other healthcare professionals who are working in the field of antineoplastic drugs to provide a state-of-the-art knowledge in making these drugs, mechanisms of actions and its synthetic procedures in the literature.

Keywords: Anticancer drug; Alkylating agents; Types; Mechanism of action; SAR; Synthesis.

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List of Acronym

BBB Blood brain barrier

DNA Deoxyribonucleic acid

MMR Mismatch repair

MOA Mechanism of action

SAR Structure activity relationship

TMZ Temozolomide

TSG Tumor suppressor gene

Chapter 1

Alkylating agent

1.1 Introduction and objectives of the review article

Clinically the most important actions of the alkylating agents are to inhibit DNA synthesis so that cell division cannot occur. It is mainly intended to treat cancer patients. The purpose is that the cell cycle of a particular site of the body is suppressed and so that it causes cell death.(Holland Frei et al., 2003). Moreover, the goal of this paper is to present a thorough overview of alkylating agents utilized in cancer treatment. This will discuss their mechanism of action, SAR (structure-activity relationship), synthesis and potential future direction for exploring. Though it is difficult to address all alkylating drugs utilized clinically as anticancer agents. This paper mostly discusses drugs that are historically significant, currently used or promising in clinical trials as alkylating agents (Holland Frei et al., 2003). In order to give an in-depth study of various classes of alkylating substances, this review article is going to focus on their modes of action, synthesis techniques, and structure-activity relationships (SAR). The purpose of the study is to fill the gap in knowledge in the areas of understanding and description of various alkylating agents, chemical modifications, and the effects of structural variations on their physiological functions. This work also aims to synthesize the current understanding of many groups of alkylating agents, such as nitrosoureas, platinum-based compounds, nitrogen mustards, and others. The review article will clarify how alkylating chemicals interact with cellular components like Proteins, DNA, and enzymes to produce either therapeutic or harmful effects by thoroughly assessing the mechanisms of action. The review will also look at how structural changes affect the effectiveness, selectivity, and mechanism of action of various classes of alkylating agents.

The main focus of this review will be the synthesis of alkylating agents. The goal of the study is to present an overview of the synthetic techniques used to create several types of alkylating agents, covering both conventional and cutting-edge methods. The review will evaluate the major intermediates, reaction conditions, and synthetic pathways used to prepare alkylating drugs, highlighting the benefits, drawbacks, and most recent developments in their synthesis.

Overall, the review article will advance our understanding of the structure-activity relationships (SAR) of alkylating agents by fusing the mechanisms of action with synthesis techniques. It will examine how structural elements including alkyl groups, leaving groups, and carrier ligands affect the pharmacological effects and biological functions of alkylating drugs. The review will highlight general SAR patterns among several alkylating agent classes as well as distinctive SAR properties particular to each class.

1.2 Methodology

i. The information came from academic papers, studies and articles that were kept in various digital libraries. Some examples of such resources are PubMed, Frontiers, MDPI, ScienceDirect, Google Scholar and different renowned pharmaceutical databases. Keywords like "anti-cancer medication," "mechanism of action", "alkylating agents", "synthesis of alkylating agents", "different classes of alkylating agents" and "SAR" among others, were used to locate relevant articles. Articles that were relevant to the topic were collected, and further context has been looked into. Creating a well-thought-out plan for searching the literature has made it possible to read all the necessary papers. Articles from reputable journals published within a certain time range were considered, whereas publications which are written in other non English languages were excluded. Relevant study on the SAR, synthesis techniques, and mechanisms of action of several kinds of

alkylating compounds were considered. After settling on a topic, an outline was written out with all the necessary headers and subheadings.

ii. Google Scholar helped a lot to find relevant articles on the topic. It is a very useful tool for searching a large database of scholarly articles.

iii. Mendeley Desktop comprised both the bibliography and the in-text citation. Complete paraphrasing and in-text citations were performed on the essay.

iv. PubMed is a free, user-friendly online search tool that works like a library of articles from the medicines, clinical, biosciences. The National Institutes of Health's National Library of Medicine manages and maintains this database as part of the Entrez database. It helps me to get access to many of the most recent articles.

v. ScienceDirect provides online access to a large bibliographic database of scholarly articles in the fields of science and medicine. I have got a lot of useful knowledge from this.

vi. All of MDPI's online journals are open accessible. So, relevant data was easily collected using this. The membership in Mendeley overall helped me to cite and appropriately give credit to all the article and paper writers from which I got numerous information. It has a resource management and scholarly social network.

1.3 Relation between Cancer and Alkylating agent

According to WHO, cancer is a generic term for an extensive range of diseases, which may affect any area of the body. It is a set of illnesses marked by irregular cellular growth and division that can result in the emergence of tumors. It is a primary and fatal cause of death worldwide, and it may be developed in almost any organ. Uncontrolled division of cells is the root cause of some of these world's deadliest diseases. Oncogenes, mutation of TSG

(tumor suppressor gene) and miRNA gene are among the primary causes of cancer development and progression.

In addition to nearly 6 million annual cases, it is considered as one of the most severe diseases of the 21st century. For several decades, alkylating drugs have been part of standard treatment for malignant tumors. Inhibiting cancer growth and killing cancer cells leads to tumors shrinking or disappearing which improves patient outcomes and ultimately increases long-term survival. However, there are risks associated with alkylating chemicals use, such as harm to healthy cells and tissues. Careful observation and supportive aids will help control this. The DNA of cancer cells is damaged by alkylating chemicals, which in turn slows their growth and reproduction. Due to their very rapid proliferation, tumor cells are particularly susceptible to alkylating agents. Since alkylating drugs are very selective, they kill rapidly proliferating cancer cells while trying not to destroy healthy tissues.

Surgery, radiation therapy, chemotherapy, and targeted therapy are only a few of the methods used to treat cancer clinically. In addition, numerous anticancer drugs have been identified and have proven effective in treating cancer patients. For instance:

1. Alkylating agents
2. Organoplatinum agents
3. Metabolic Inhibitors (antimetabolites)
4. Topoisomerase I & II inhibitors (antitumor antibiotics)
5. Microtubule inhibitors (cytotoxic alkaloids)

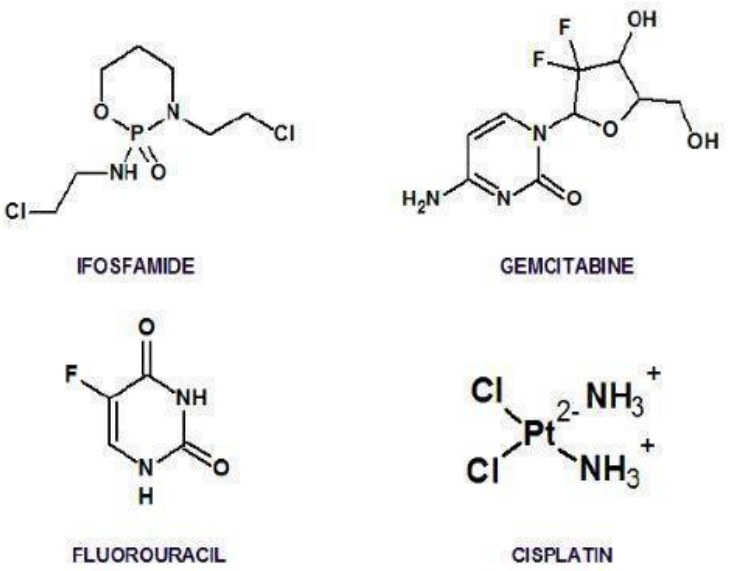


Figure 1: Example of different types of anticancer drugs (Ronald Bartzatt et al., 2003)

1.4 General Introduction of Alkylating agents

Alkylating agents are mainly taken intravenously or orally. These can be administered alone or in combination with other chemotherapy drugs. These drugs are clinically effective in treating cancer, including many forms of cancers like leukemia, lymphoma, multiple myeloma, and solid tumors such as breast and lung cancer (Bayat Mokhtari et al., 2017). The working process of alkylating DNA results in the destruction of cells, although the exact mechanism remains uncertain for all types of alkylating agents. The death of unwanted cells may occur by the initiation of apoptosis through the activation of p53 and interference with the template function of DNA. For instance, cisplatin and carboplatin which are platinum-containing medications are classified as one of the "alkylating-like" agents. These act just like alkylating drugs in that they degrade the DNA of cancer cells. These medications are used to treat a variety of cell malignancies, such as ovarian, lung, and testicular cancer.

Alkylating and alkylating-like agents have been proven to be beneficial when treating several types of malignant tumors, especially when combined with radiation therapy or other

chemotherapy medications. However, the effectiveness of these drugs varies not just with types of cancer and its stage but also with the patient's overall physical condition and age. There are serious adverse effects as well, including gastrointestinal abnormalities, exhaustion, bone marrow suppression, alopecia, nausea, vomiting, oral mucosal ulcers, a greater likelihood of infections, and many more. Alkylating agents, for instance, are chemotherapeutic medications that can be successful in treating cancer, but they can have substantial side effects and may not be suitable for all individuals. As a consequence, each individual situation should be considered before any decision is taken to utilize these medications, in cooperation with a licensed medical practitioner.

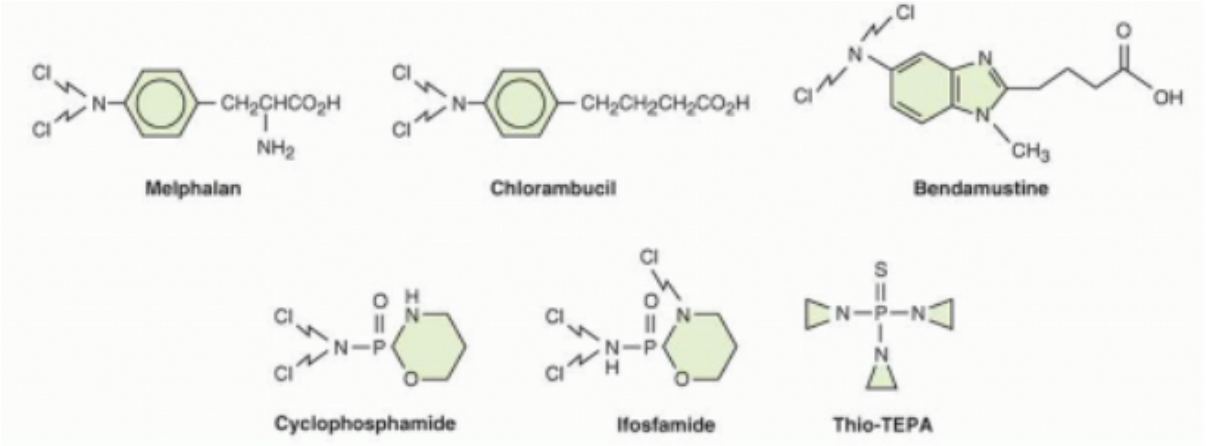


Figure 2: Example of Alkylating agents (Stanton L.et al., 2019)

More than sixty years have passed since the first use of alkylating compounds in cancer treatment and that number continues to grow. These chemicals destroy unusual base pairing of DNA strands and prevent cell division. It eventually kills cells by directly cross linking N-7-guanine residues of DNA strands throughout the cell cycle. However, systemic toxicity and drug resistance limit their therapeutic efficacy. So, to deal with this problem, scientists are still exploring the potential of combining alkylating drugs with other anticancer medications as well blockers of multidrug resistance proteins, DNA repair enzymes, topoisomerase enzymes, COX-2, p34cdc2 kinase, phosphatases and antivascular agents (Ralhan & Kaur,

2007). To make cancer cells more susceptible to alkylating anticancer drugs, researchers are creating alternative modulators including naphthalimides and selective androgen receptor modulators.

1.5 History

Alkylating agents are one of the most initial discoveries of researchers to treat anticancer effectively (Gate & Tew, 2011). This famous class of anticancer drugs were used in WWI. After the discovery and early success of alkylating agents like nitrogen mustard, researchers started to explore other alkylating compounds with a preexisting aziridine ring, thiotepa and busulfan were products of those researches.

Back to the early 1940s alkylating agents have been widely used as an early form of chemotherapy to treat cancer. Although they can cause significant damage, they also have great potential for healing. One example of a class of agent with several applications is nitrogen mustard, which was first developed and used by the military during World War II but was later researched by Dr. Cornelius Packard Rhoads for its potency as an effective treatment for cancer.

1.6 Site for alkylating agent in DNA base

The N-2, N-3, and N-7 of guanine, the N-1, N-3, and N-7 of adenine, the 0-6 of thymine, and the N-3 of cytosine are all possible nucleophilic sites on DNA that can be attacked by an alkylating agent which act as an electrophilic compound. For many alkylating compounds, the N-7 position of guanine is crucial because its nucleophilicity is potentially increased by neighboring guanine residues (Stanton L. Gerson Alina D. et al., 2019).

An alkylating compound can generates intermediates that are reactive and interacts to a wide range of biological components, particularly proteins, amino acids, nucleotides, and nucleic acids. An example of a selective nucleophile is nitrogen mustard, which preferentially attacks, (i) protein amino groups, (ii) base oxygens, (iii) purine amino groups, (iv) phosphate oxygens, (v) methionine sulfur atoms and (vi) glutathione cysteinyl thiol groups. In most cases, steric and hydrophilic/hydrophobic barriers prevent tissue nucleophiles from reacting. In addition, the presence of GSTs, which provide catalysis, frequently favors glutathione conjugation. As a result, making generalizations regarding the targets of alkylating agents is challenging. Although DNA is usually a preferred target, it is possible that other metabolic targets of alkylating agents could also lead to cytotoxicity. The activity of DNA repair enzyme, as shown by benzyl-C-nitro-urocanic acid (BCNU) and alkylguanine alkyltransferase (AGT) repair, (a) modifications to the network of genetic and epigenetic events, (b) determined and evaluated using gene expression arrays and (c) the presence of particular DNA adducts as identified by mass spectrometric assessment, may provide evidence for this claim. Alternative hazardous routes not involving DNA must be rejected due to the strictness of such investigations, which is a challenging criterion to achieve. Because of this, it is necessary to consider the fundamental understanding of alkylating agent activity as incomplete.

The phosphoryl oxygens of the sugar phosphate backbone in the DNA molecule are the most evident electron-rich targets for alkylation. Phosphate groups are alkylated, and the hydrolysis of the phosphotriesterase produced as a result can cause strand breaks. Although the biological significance of the phosphate alkylation-induced strand breaking is yet unknown, the process is so slow that it doesn't seem probable to play a significant role in cytotoxicity, even for monofunctional drugs. Studies have proven that almost all of the oxygen and nitrogen atoms of the purine and pyrimidine bases of DNA can be alkylated to variable degrees by carcinogenic alkylating chemicals such methyl methanesulfonate. It is still

uncertain how important these specific DNA sites and bases' alkylation sites are in determining cytotoxicity, organ-specific toxicities, or carcinogenesis. Guanine's extra cyclic nitrogen and O-6 atoms appear to be particularly significant for carcinogenesis by alkylation. (Stanton L., Gerson Alina D. et al., 2019)

For example, Alkylation of guanine at the N-7 position is observed in busulfan and mechlorethamine. Guanine cross-links (two guanine molecules shortened at the N-7 position by an alkylating agent) have been separated from the acid hydrolysates of the reaction mixtures. Nitrogen mustard reacts with native DNA, alkylating not only the N-7 position of guanine but also the N-1 position of adenine. Base stacking and charge transfer might lead to increased nucleophilicity at guanine's N-7 position. Guanine N-7 and adenine N-3 are the preferred targets for melphalan alkylation. The alkylating reaction is dependent on the base sequence. When surrounded by guanines on both the 3' and 5' sides, the aziridinium cation intermediate of nitrogen mustards is most likely to attack the N-7 position of guanine because it is the most electronegative. Nitrosoureas and non-classic methylating agents like procarbazine and dacarbazine appear to primarily target the O-6 methyl group of guanine. A higher rate of site repair is related with resistance to treatment. Thus, the optimal sites for alkylation differ according to the nature of the alkylating agent and the chemical environment of the DNA base (Stanton L., Gerson Alina D. et al, 2019).

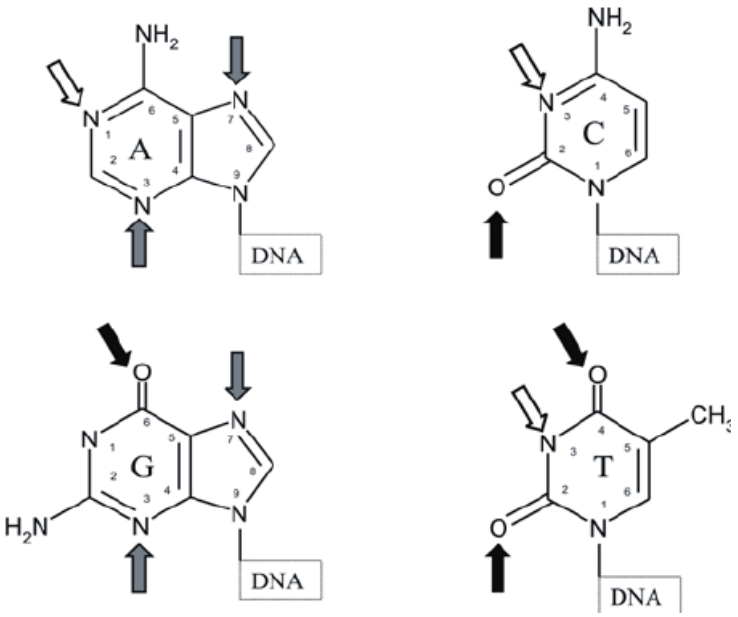


Figure 3: Site of alkylating agent on DNA bases (Nieminuszczy & Grzesiuk, 2007)

Chapter 2

SAR, Synthesis, Mechanism

2.1 Chemistry of common Alkylating Agent

Mostly in biological molecules with the help of covalent bonds alkylating agents form bonds with electron-rich atoms. They are commonly classified into two types based on their mechanism of action.

The first mechanism is direct reaction with biological molecules which is also known as SN1 reaction mechanism. SN1 reactions are limited by the formation of a carbonium ion that reacts fast with a nucleophile. This reaction has a constant pace that is proportional to the alkylating agent concentration (first-order kinetics). Moreover, the production of a reactive intermediate that subsequently reacts with the biological molecules is a second process. It is categorized as SN2. The rates of these reactions are referred to as SN1 and SN2 respectively. The rate of an SN1 reaction is independent of any other factors, including the concentration of the reactive intermediate, as we learn from our study of medicinal chemistry.

In contrast, an SN2 reaction relies on the concentrations of both the alkylating agent and the molecule it reacts with. In order to properly understand the cellular and molecular pharmacology of particular alkylating drugs, this difference is essential (Holland Frei et al., 2003). SN2 reactions are governed by the principles of second-order kinetics and are sensitive to the nucleophile and alkylating agent concentrations. In such reactions, the combined action of the reactants results in the formation of a transition-state entity, which then divides thereby giving rise to the alkylated cellular component.

Examples of SN1 agents include nitrogen mustards and nitrosoureas that can create covalent adducts that bond with oxygen and nitrogen atoms in DNA via an SN1-type process while busulfan is categorized as an SN2 agent that ends up reacting more slowly and with less oxygen site alkylation. Often the elimination half-lives of parent compounds are less than 5 hours because alkylating agents are made to form reactive intermediates. (Stanton L. Gerson Alina D. Bulgar Lachelle D. Weeks Bruce A. Chabner, 2019)

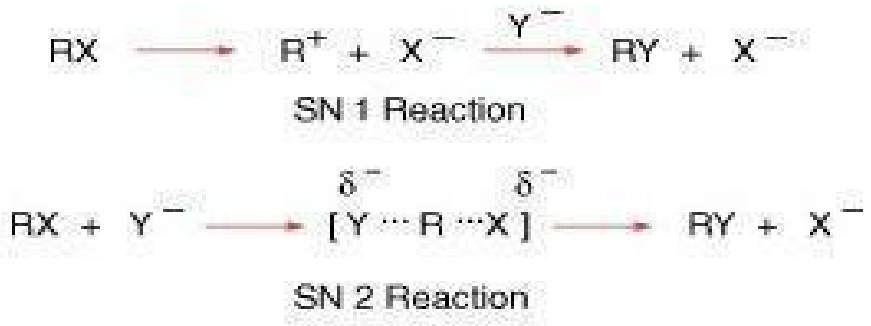


Figure 4: SN1 and SN2 reactions for alkylating agents (Holland Frei et al., 2003)

2.2 Classes of Alkylating agents for Anticarcinogenic effects

Based on their chemical structure and properties, alkylating agents are primarily divided into the six categories below. These groups include:

i) **Nitrogen mustards,** including cyclophosphamide, ifosfamide, mechlorethamine or mustine, uramustine or uracil mustard, chlorambucil, and melphalan;

ii) **Alkyl sulfonates**, which includes busulfan;

iii) **Ethyleneimines and methyl melamines,** such as hexamethylmelamine or altretamine;

iv) **Triazenes,** like dacarbazine and temozolomide;

v) **Piperazines**; and

vi) **Nitrosoureas**, such as carmustine and streptozocin (Ronald Bartzatt et al., 2003)

Then again, according to the different mechanisms of action of these alkylating agents which are also associated with their chemical structures, they are classified into several classes of alkylating agents used in cancer treatment. Here are some examples of alkylating agents, organized by their mechanism of actions:

1. The nitrogen mustards for example: [mechlorethamine, cyclophosphamide, isofamide, melphalan and chlorambucil]

2. Alkyl sulfonates for example: busulfan, Treosulfan

3. Nitrosoureas for example: carmustine, lomustine, Semustine

4. Triazenes for example: dacarbazine, procarbazine, temozolomide

5. The platinum-containing antineoplastic agents for example: cisplatin, carboplatin, oxaliplatin (*Role of Nutrigenomics in Modern-Day Healthcare and Drug Discovery*, 2023)

Mainly these are only a few examples of alkylating agents which are used as potent anticancer drugs. Also, there are numerous other medicines with similar ways to work that can be useful against different types of cancer.

2.3 Mechanism of action, SAR and Synthesis Alkylating Agents

The DNA of cancer cells are damaged by alkylating drugs, a type of chemotherapeutic drug. Specifically, they produce DNA crosslinks and strand breaks by adding an alkyl group to the DNA molecule. This damage prevents DNA from being replicated and prevents the cell from dividing, leading to the irreversible death of the cell. There is a considerable risk for toxicity with alkylating compounds because they can harm both malignant and healthy cells. The alkylating agents are a class of chemotherapeutic medications that cause cell death by damaging DNA by adding an alkyl group to it.

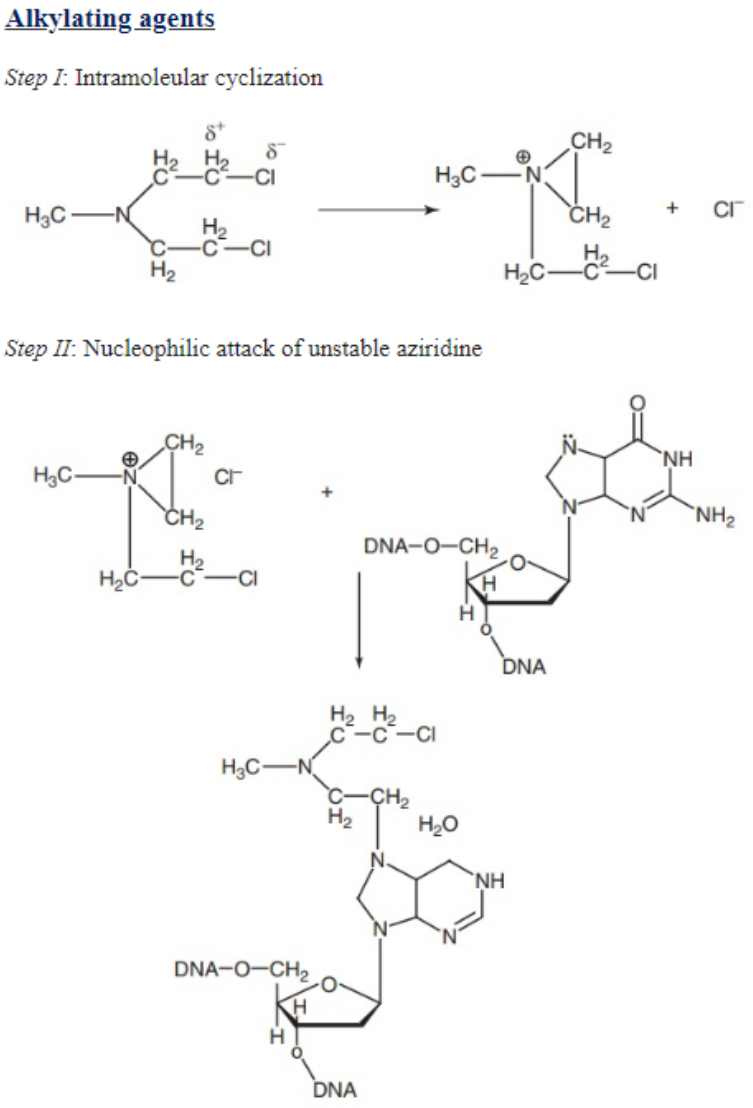


Figure 5: General mechanism of Alkylating agents (*Alkylating Agents*, 2018)

Different alkylating agents can have different mechanisms of action, SAR and Synthesis depending on their chemical structure and other factors. Here are some examples:

2.3.1 Nitrogen mustards with their MOA, SAR & Synthesis:

These drugs form highly reactive alkylating agents in the body, which can add alkyl groups to the nitrogen atoms in DNA bases. Cross-linking between DNA strands disrupts DNA replication and ultimately destroys cells. Nitrogen mustards are the original alkylating agents. Clinically, mechlorethamine (mustine) was the first nitrogen mustard. In the following figure nitrogen mustard alkylation has been shown. After losing chlorine, the β-carbon reacts with the nucleophilic nitrogen atom to generate extremely reactive aziridinium moiety which is a positively charged cyclic compound. The aziridinium ring reacts with a nucleophile (electron-rich atom) to produce the alkylated compound. The remaining chloroethyl group creates a second aziridinium, which alkylates again and cross-links two separate alkylated nucleophiles (Stanton L., Gerson Alina D.et al., 2019)

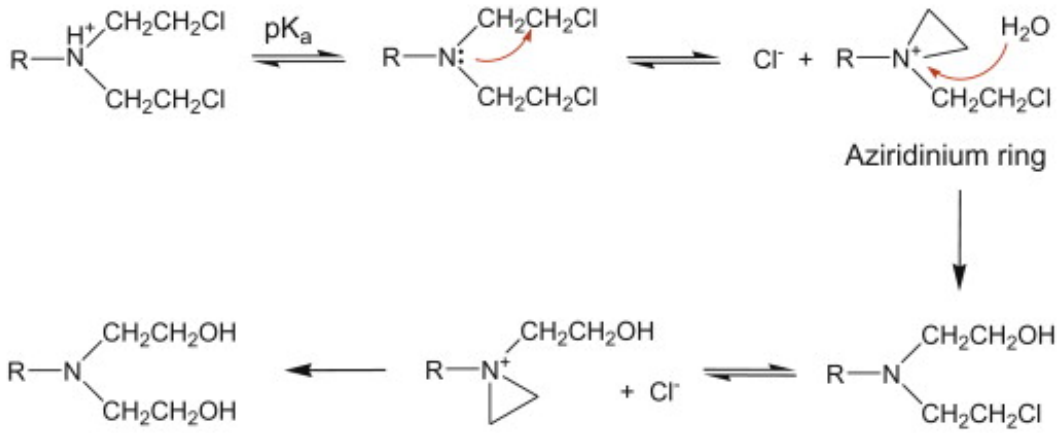


Figure 6: Alkylation of nucleophilic species by Nitrogen mustard (Loftsson et al., 2014)

- **One example of a nitrogen mustard is cyclophosphamide**

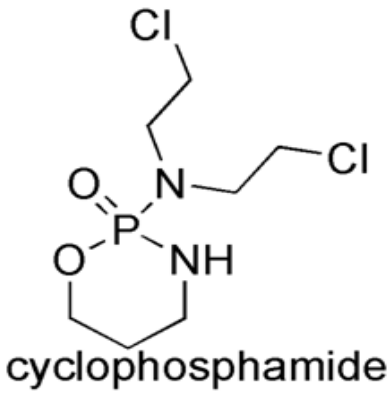


Figure 7: Cyclophosphamide (*Cyclophosphamide Synthesis, SAR, MCQ and Chemical Structure*, 2019)

Mechanism of Action

i. Cyclophosphamide gets metabolized to phosphoramide mustard in cells with low amounts of the enzyme aldehyde dehydrogenase (ALDH).

ii. At guanine N-7 locations, the metabolite will form cross-linkages between and within the DNA strands.

iii. Apoptosis, the final stage before cell death, will be the ultimate outcome (Tripathi KD, 2013).

Structural Activity Relationship

- Substituting the sulfur with a nitrogen atom lowers the toxicity.
- The 2-chloroethyl group is necessary for the formation of the aziridine cation. The aziridine cation will eventually bind to the alkylates in the DNA.
- After connecting with the amino group, the orally administered drug will be more readily absorbed. So, its bioavailability will be enhanced.

- In addition, its oral bioavailability is enhanced by the addition of a substituted phenyl group.
- Introducing an aromatic ring in the drug's structure increases its stability.
- Introducing aromatic rings in the drug's structure increases its bioavailability also.
- The benzimidazole ring allows for quick and locally targeted action.
- Benzimidazole ring also shorten the half-life of the molecule (Pires et al., 2018)

Methods of Synthesis

i. Bis(2-hydroxyethyl) amine is treated with $SOCl_2$ and chloroform.

ii. Then, $POCl_3$ is introduced to the newly generated product, bis(2-chloroethyl) amine, while pyridine is present.

iii. The last step is an interaction with 3-aminopropanol to create cyclophosphamide.

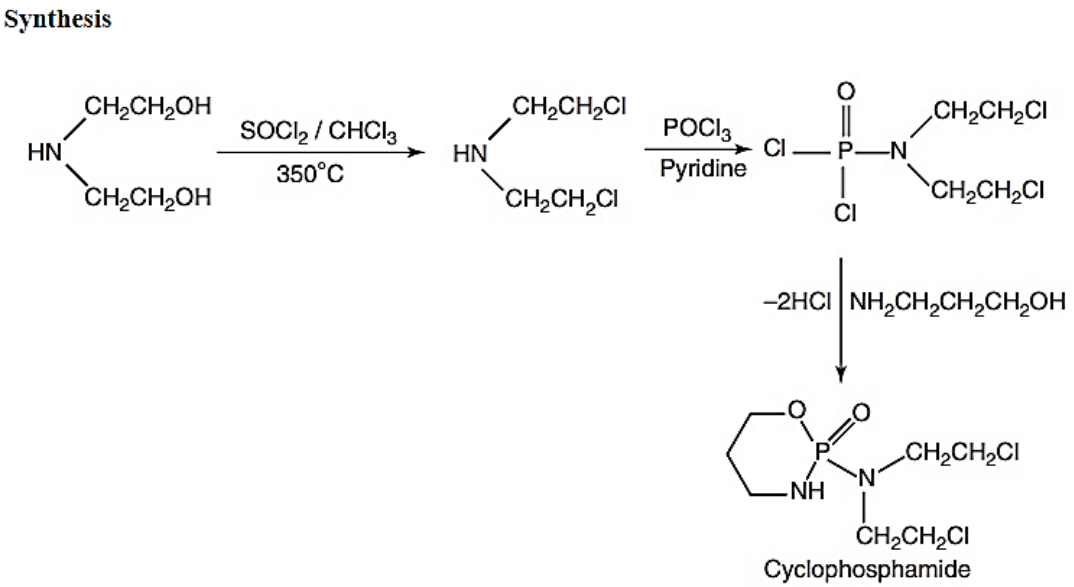


Figure 8: synthesis of Cyclophosphamide (*Alkylating Agents*, 2018)

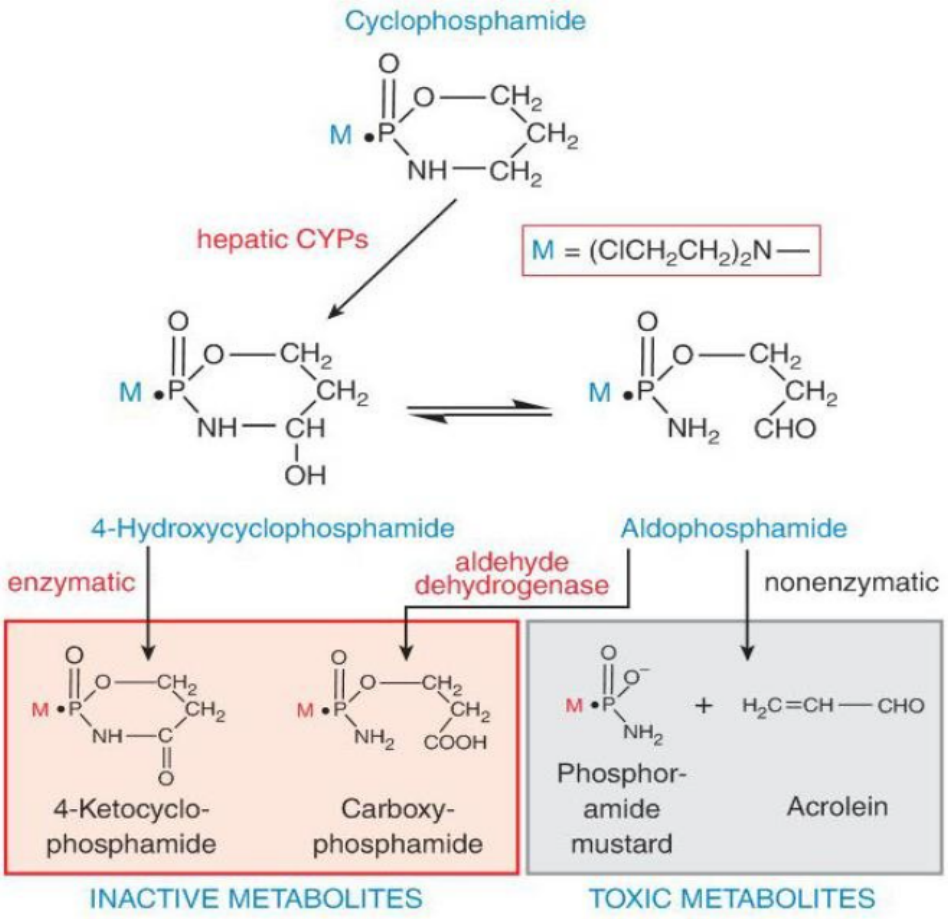


Figure 9: Metabolism and activation of cyclophosphamide. (uploaded by Delyan Ivanov)

Phosphoramide mustard is a highly reactive metabolite that is formed in the liver after administration. By attaching an alkyl group to the nitrogen atoms in DNA bases, this metabolite disrupts DNA replication and leads to cell death by generating cross-linking between DNA strands.

- **Another example of Nitrogen mustard is Mechlorethamine.**

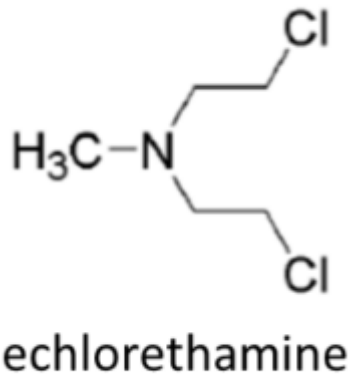


Figure 10: Mechlorethamine (*MECHLORETHAMINE Synthesis, SAR, MCQ and Chemical Structure*, 2019)

Mechanism of Action

The medicine causes DNA fragmentation by repairing enzymes when it binds with the guanine nitrogen base pair in the DNA. As a result, DNA synthesis is inhibited. The transcription of RNA within the cells is also affected by this. (Tripathi KD, 2013).

Structural Activity Relationship

- When the drug attaches to the guanine, repair enzymes are triggered to break apart the DNA.
- Like other classes of alkylating agents, substituting a nitrogen atom for a sulfur one decreases the toxicity.
- Since the aziridine cation can only be generated in the presence of the 2-chloroethyl group, its presence is crucial to the activity. Later, the aziridine cation will bind to the alkylates of DNA.
- The drug's oral bioavailability will improve after binding with the substituted phenyl group or amino group.

- An aromatic ring in the structure provides stability and bioavailability of the drug even further.
- The drug's benzimidazole ring allows for a more targeted and rapid response while reducing its half-life. (Dixit et al., 2011)

Method of synthesis

In order to neutralize ethylene oxide, methanamine is used. To get mechlorethamine, the resulting product is treated with SOCl2. Alkyl groups are added to the nitrogen atoms in DNA bases when it is administered, making a highly reactive alkylating agent. This can affect DNA replication and ultimately lead to cell death by causing cross-linking between DNA strands.

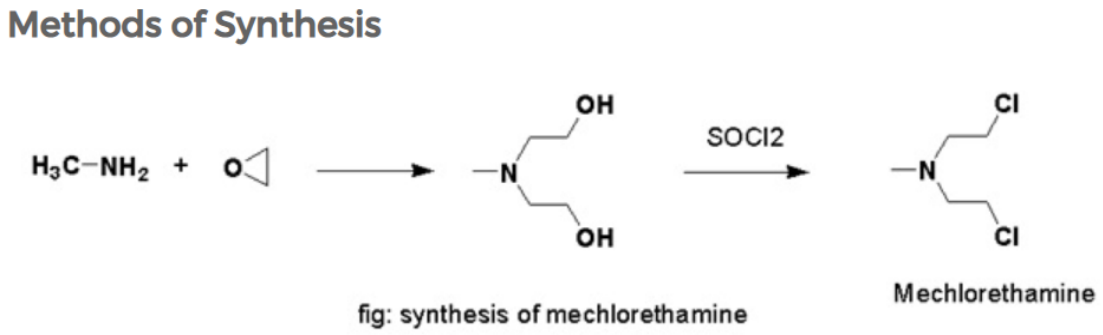
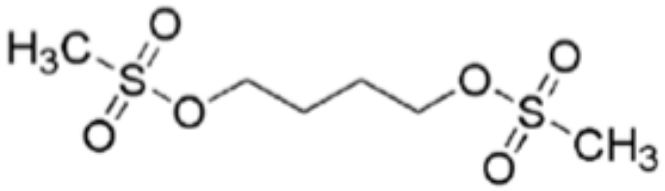


Figure 11: Synthesis of mechorethamine (*Mechlorethamine Synthesis, SAR, MCQ and Chemical Structure*, 2019)

2.3.2 Alkyl sulfonates with their MOA, SAR & Synthesis:

These drugs also form highly reactive alkylating agents in the body, which can add alkyl groups to DNA bases. This can cause DNA strand breakage, leading to cell death.

- **An example of an alkyl sulfonate is busulfan.**



Busulfan

Figure 12: Busulfan (*Busulfan Synthesis, SAR, MCQ,Chemical Structure and Therapeutic Uses*, 2019)

This drug also forms a highly reactive alkylating agent in the body by adding electrophilic alkyl groups to DNA bases. It results in destruction of DNA strands, which ultimately leads to cell death (Wu et al., 2015).

Mechanism of Action

i. The mesylate leaving group is attacked by the nucleophilic guanine N7 following the SN2 pathway.

ii. Guanine and adenine base pairs, as well as guanine-to-guanine base pairs, create DNA-DNA intrastrand crosslinks. The DNA replication is inhibited by this. Apoptotic cell death occurs.(Pacheco et al., 1989).

Structural Activity Relationship

- Like the nitrogen mustards, its toxicity can be decreased through substituting the sulfur atom with a nitrogen atom.
- If the medicine can bind to the amino group or substituted by phenyl group, it will be orally more bioavailable.

- The drug's stability can be improved because of the presence of an aromatic ring.
- This addition of aromatic ring boosts the drug's bioavailability even further.
- The drug's benzimidazole ring facilitates more targeted and rapid response. Besides, it reduces half-life of the drug (Pires et al., 2018).

Methods of Synthesis

Busulfan is obtained by reacting 1,4-butanediol with methansulphonyl chloride.

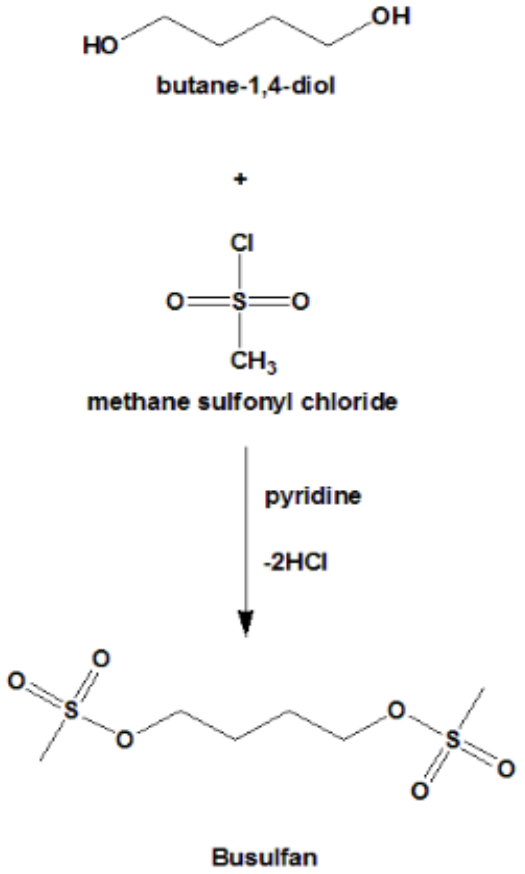


Figure 13: synthesis of Busulfan (*Busulfan Synthesis, SAR, MCQ,Chemical Structure and Therapeutic Uses*, 2019)

- **Another example of an alkyl sulfonate is Treosulfan.**

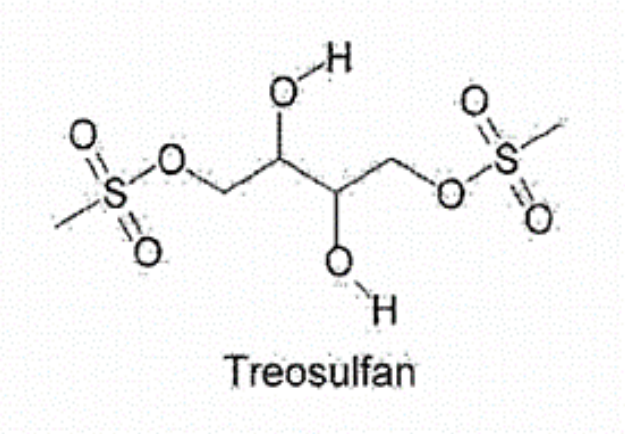


Figure 14: Treosulfan (Olesen et al., 2021)

When administered, it forms a reactive metabolite that adds alkyl groups to DNA bases, leading to DNA strand breakage and ultimately cell death.

2.3.3 Nitrosoureas with their MOA, SAR & Synthesis:

These unique medications are able to penetrate the blood brain barrier (brain's protective barrier) and target malignant growths. By attaching alkyl groups and DNA base pairs, they cause cross-linking between strands, which in turn ceases DNA replication and ultimately kills the cell.

- **Common example of a nitrosourea is Carmustine (also known as BCNU).**

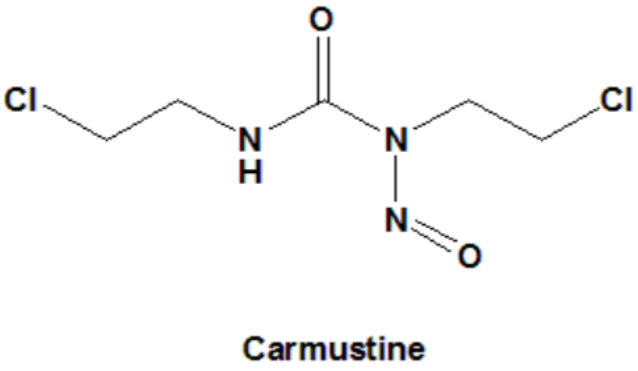


Figure 15: Carmustine ("Carmustine (BCNU) Synthesis, SAR, MCQ,Chemical Structure and Therapeutic Uses," 2019)

Mechanism of Action

i. Carmustine alkylates between DNA strands, creating a cross-link. This also causes cellular apoptosis by interfering with DNA activity.

ii. Enzymes like DNA repairing enzymes, those responsible for repairing DNA are among the proteins that are carbamylated by Carmustine.

iii. Because of its high lipophilicity, it is able to readily penetrate the BBB and reach the brain. (Weiss & Issell, 1982)

Structural Activity Relationship

- Oral bioavailability can be enhanced by interacting with amino groups.
- Also, the oral bioavailability of the medicine will rise as a result of the introduction of the substituted phenyl group.
- Adding an aromatic ring will improve the drug's stability.
- The additional aromatic rings enhance its distribution.
- Like many other alkylating agents, it can get more rapid and localized action because of the benzimidazole ring. Though its Half-life is already quite short, benzimidazol will make it even shorter. (Dixit et al., 2011)

Methods of Synthesis

Nitrosation of 1,3-bis(2-chloroethyl)urea with sodium nitrite provides an acidic and cold condition to produce and yield carmustine. (Dixit et al., 2011)

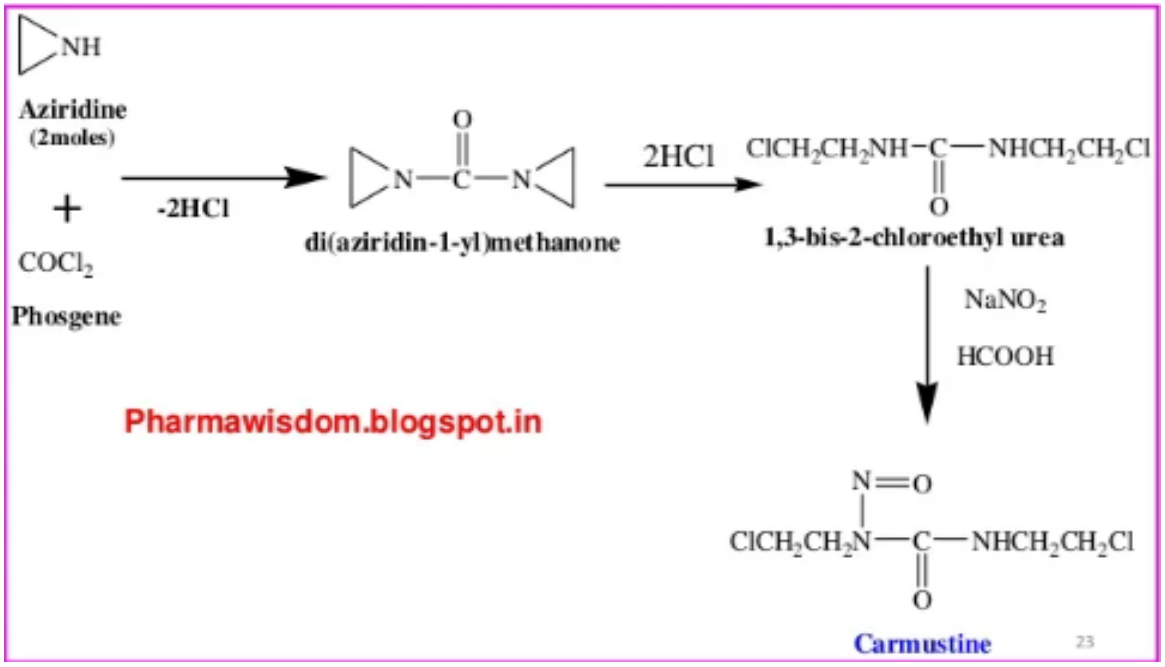


Figure 16: Synthesis of Carmustine (Celaries et al., 2006)

Alkyl groups on DNA bases cross-link DNA strands and disrupt DNA replication, ultimately leading to cell death.

- **Next example of a nitrosourea is Lomustine.**

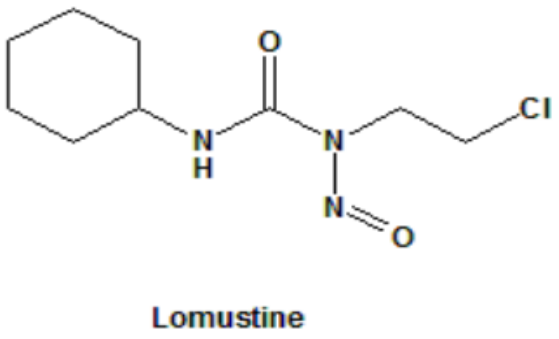


Figure 17: Lomustine (*Lomustine Synthesis, SAR, MCQ,Chemical Structure and Therapeutic Uses*, 2019)

Mechanism of Action

i. The in vivo hydrolysis of lomustine turns it into its metabolites.

ii. Guanine base pairs undergo alkylation and cross-linking at the C6 position due to metabolic products of lomustine.

iii. Thus, the cell will undergo cytotoxicity and then apoptosis as a result.

iv. In addition, it boosts cytotoxicity by blocking carbamylation, a process that modifies proteins. (Pfreundschuh et al., 1987)

Structural Activity Relationship

- Improvement of bioavailability:

The drug's oral bioavailability will improve after binding with the amino group or substituting phenyl group. The addition of aromatic rings will improve the drug's bioavailability even further.

- Improving drug stability: By adding an aromatic ring.
- Presence of benzimidazole ring: It provides the drug's local and rapid effect. Also, it will shorten the half-life even more. (Pfreundschuh et al., 1987)

Methods of Synthesis

Synthesis:

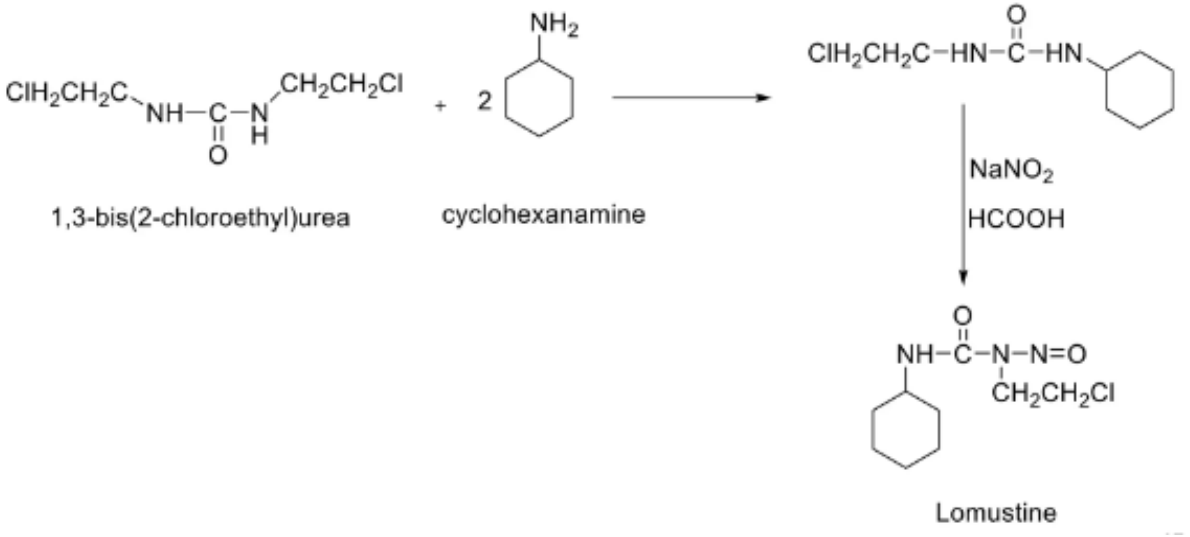


Figure 18: Synthesis of Lomustine (Pfreundschuh et al., 1987)

It also works by adding alkyl groups to DNA bases, making cross-linking between DNA strands. As a result, DNA replication is ceased leading to cell death.

2.3.4 Triazines with their MOA, SAR & Synthesis:

In order to destroy cancer cells, these medications add alkyl groups to DNA and produce extremely reactive intermediates to inhibit DNA strands.

- **The example of a Triazine is Temozolomide.**

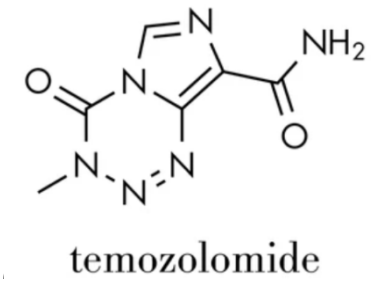


Figure 19: Temozolomide ("Temozolomide," 2014)

Mechanism of action

Glioblastoma (glioblastoma multiforme) is the most common and lethal primary brain tumor in adults, allowing approximately 45.6% of all primary malignant brain tumors. Histopathologically, glioblastomas are most easily identified by necrosis and microvascular development (WHO grade IV classification), making radiotherapy and alkylation-based chemotherapy using temozolomide the standard for treatment. (Zhang et al., 2012)

The imidazole tetrazine medication like temozolomide (TMZ) is a small (194 Da) lipophilic alkylating agent that is stable at acidic pH, suitable for both oral and intravenous administration and can cross the blood-brain barrier to affect CNS tumors. Soon after

absorption at physiological pH, TMZ is metabolized into 5-(3-methyl triazole-1-yl) imidazole-4-carboxamide (MTIC) and water, generating 5-aminoimidazole-4-carboxamide (AIC) and a highly reactive methyl diazonium cation. Because glioblastoma, a type of brain tumor, has a more alkaline pH than healthy tissue, TMZ is more likely to activate it. The extremely reactive methyl diazonium cation methylates DNA at the guanine N7 position (N7-MeG, 70%), the adenine N3 position (N3-MeA, 9%), and the guanine O6 position (O6-MeG, 6%). Although N7-MeG and N3-MeA lesions are not significant mediators of temozolomide toxicity, they are lethal if not modified via the base excision repair pathway. (Zhang et al., 2012)

However, the methylguanine-DNA methyltransferase (MGMT) which is a suicide enzyme must remove the methyl group to restore guanine after O6-MeG methylation. The DNA mismatch repair (MMR) pathway is activated when O6-MeG mispairs with thymine. This mechanism removes the thymine but leaves the O6-MeG, starting a vicious cycle of repair that can only end in broken DNA strands and apoptosis if MGMT has failed to fix the mismatch.

Temozolomide's cytotoxicity is maximized in cells with diminished or absent MGMT function and an intact mismatch repair (MMR) pathway. In glioblastomas, TMZ resistance develops when either MGMT or MMR is altered (or both). (“Temozolomide,” 2012)

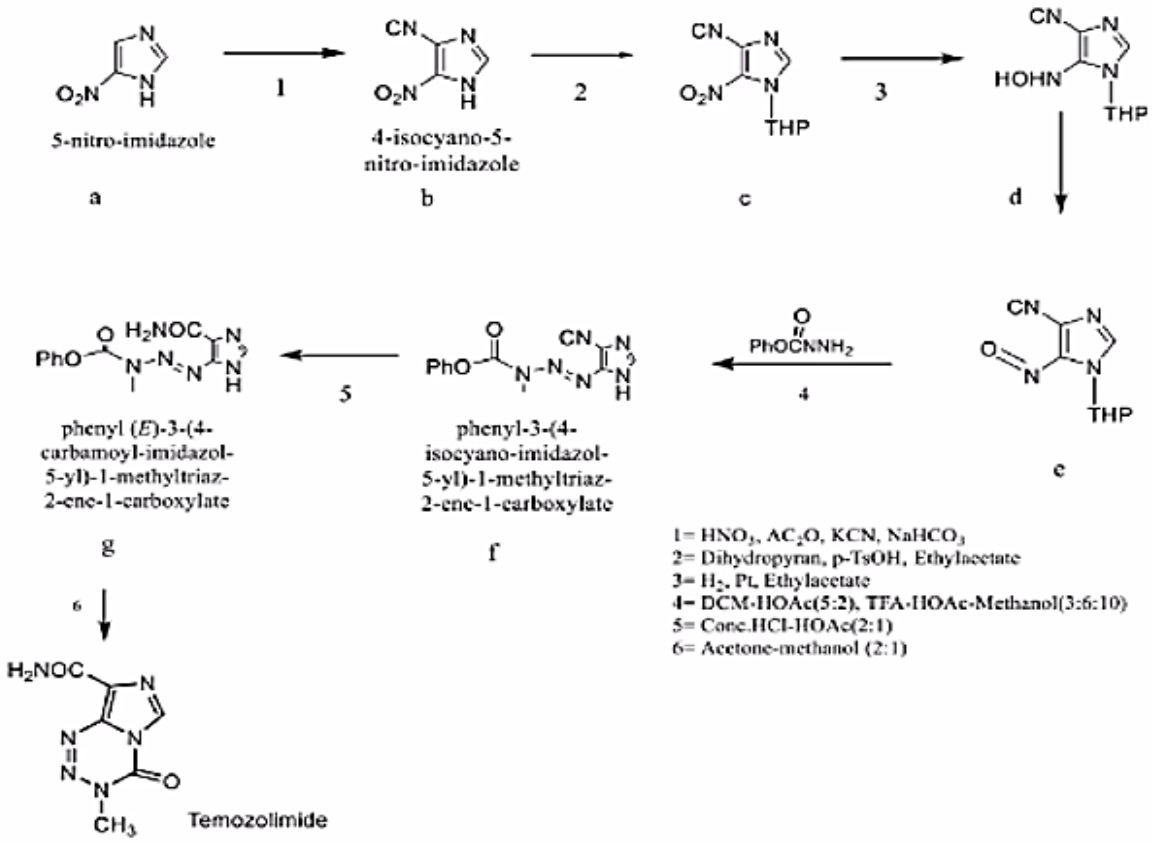
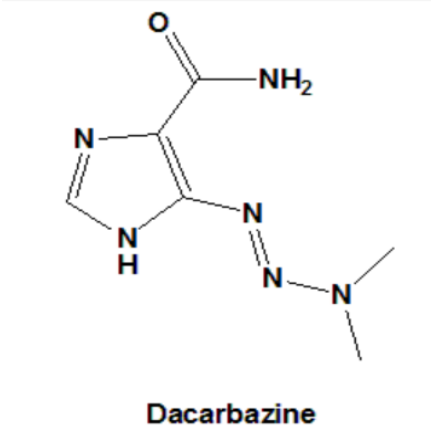


Figure 20: Synthesis of Temozolomide ("Temozolomide," 2014)

When administered, it is converted in the body to a reactive metabolite that adds alkyl groups to the DNA molecule. This leads to the formation of highly reactive intermediates that cause cell apoptosis.

- **Another example of a triazine is Dacarbazine.**



Dacarbazine

Figure 21: Dacarbazine (*Dacarbazine Synthesis, SAR, MCQ,Structure and Therapeutic Uses*, 2019)

Mechanism of Action

i. Through alkylation and subsequent suppression of DNA synthesis, dacarbazine causes cytotoxicity in the cell.

ii. A purine analog that interacts with -SH groups which block DNA synthesis.

iii. Additionally, it is important to highlight that the activation of dacarbazine takes place in the liver, and that it primarily inhibits RNA and protein synthesis (Erichsen & Jönsson, 1984).

Structural Activity Relationship

- In order to produce the aziridine cation, the 2-chloroethyl group must be present. Later the alkylated DNA will bond to the aziridine cation.
- Improving the drug's oral bioavailability as a result of:

Binding with the amino group.

Addition of the substituted phenyl group.

- Adding an aromatic ring: Improving the drug's stability and drug distribution in the body.

- Adding the benzimidazole ring: provides a local and rapid effect that reduces the half-life of this drug. (Ma & Armstrong, 2014)

Methods of Synthesis

i) 20% of excess sodium nitrite was used to convert 5-aminoimidazole-4-carboxamide into 5-diazoimidazole-4-carboxamide.

ii) 5-Diazoimidazole-4-carboxamide is converted to dacarbazine by treating it with anhydrous dimethylamine in methanol.

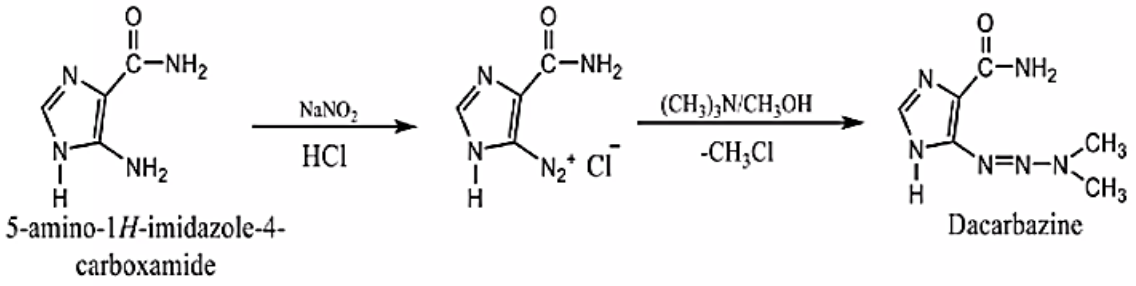


Figure 22: Synthesis of Dacarbazine (Ma & Armstrong, 2014)

It adds alkyl groups to the DNA that lead to the synthesis of highly reactive intermediates causing cell death.

2.3.5. Platinum compounds with their MOA, SAR & Synthesis:

The platinum compounds work by attaching to the DNA and producing DNA adducts, which interrupts with DNA replication. Thus, the cell is destroyed.

- **One example of a platinum compound is cisplatin.**

It works by following the general mechanism of platinum compounds as just described.

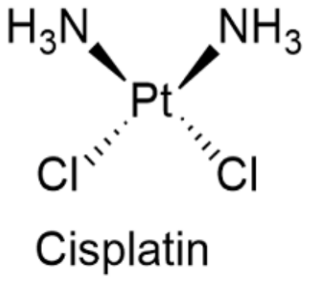


Figure 23: Cisplatin (*Cisplatin Synthesis, SAR, MCQ,Structure,Chemical Properties and Therapeutic Uses*, 2020)

Mechanism of Action

i) It is responsible for incorporating the DNA's alkyl groups. DNA ruptures when repair enzymes extract the alkylated fragments.

ii) It forms cross-links in DNA by binding to the molecule.

iii) It promotes cellular mutations due to DNA mispairing. (Dasari & Tchounwou, 2014; Fuertes et al., 2003)

Structural Activity Relationship

- Oxiplatin is more cytotoxic than cisplatin.
- Selective absorption by OTCs depends heavily on the organic functions of nonleaving groups which are connected to platinum. (Reedijk & Lohman, 1985)

Methods of Synthesis

i) An overabundance of potassium iodide is used to react with potassium tetrachloroplatinate.

ii) After reacting with ammonia, the resulting tetraiodide forms the yellow chemical $K_2[PtI_2(NH_3)_2]$.

iii) When this yellow substance is exposed to silver nitrate in water, the insoluble silver iodide precipitates out.

iv) The fourth and last step is the addition of potassium chloride. Thus, the drug is produced. (Reedijk & Lohman, 1985)

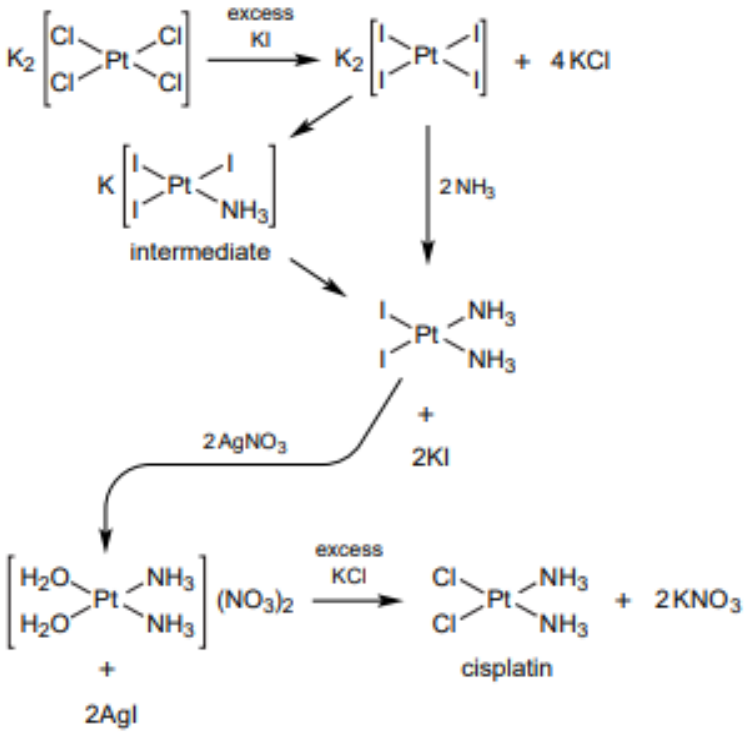


Figure 24: Synthesis of Cisplatin (Rebecca A. Alderden, 2006)

- **Next example of a platinum compound is carboplatin.**

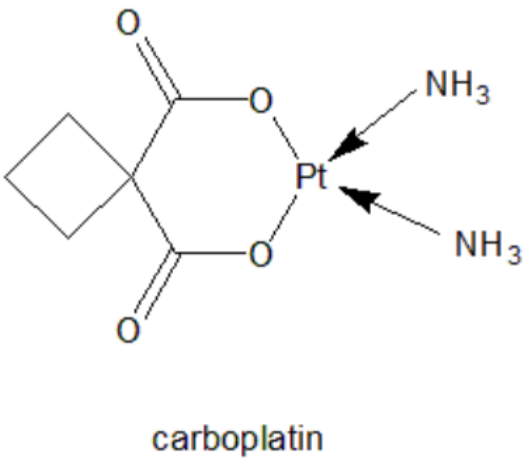


Figure 25: Carboplatin (*Carboplatin Synthesis, SAR, MCQ,Structure,Chemical Properties and Therapeutic Uses*, 2020)

Mechanism of Action

It works like cisplatin.

i) It damages DNA by incorporating alkyl groups. Repair enzymes cleave the strands to remove alkylated fragments.

ii) Cross-links are formed when it binds to DNA.

iii) It promotes DNA mispairing, which results in cellular mutations. (*Carboplatin Synthesis, SAR, MCQ,Structure,Chemical Properties and Therapeutic Uses*, 2020)

Structural Activity Relationship

- The activity can't proceed without a leaving group.
- Oxiplatin is more cytotoxic than carboplatin.
- Like cisplatin, its selective absorption by OTCs depends heavily on the organic functions of nonleaving groups which are connected to platinum. (Ralhan & Kaur, 2007)

Methods of Synthesis

i. Cisplatin is converted into cis-diamino-(1,1-cyclobutanedicarboxylate) platinum(II) by reaction with silver nitrate.

ii) This substance leads to the production of carboplatin by reacting with cyclobutane-1,1-dicarboxylic acid.

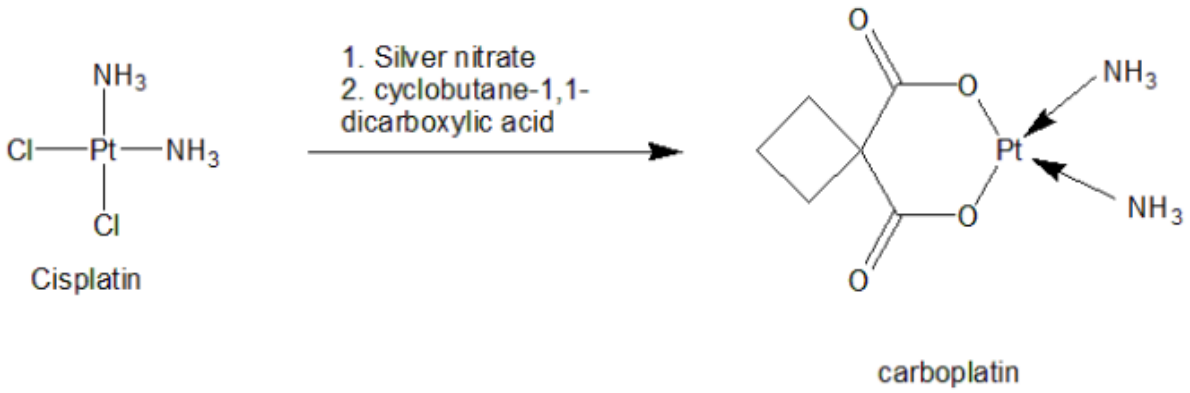


Figure 26: Synthesis of Carboplatin (*Carboplatin Synthesis, SAR, MCQ,Structure,Chemical Properties and Therapeutic Uses*, 2020)

2.3.6 Ethyleneimine and methyl melamine

- **Example Thiopeta**

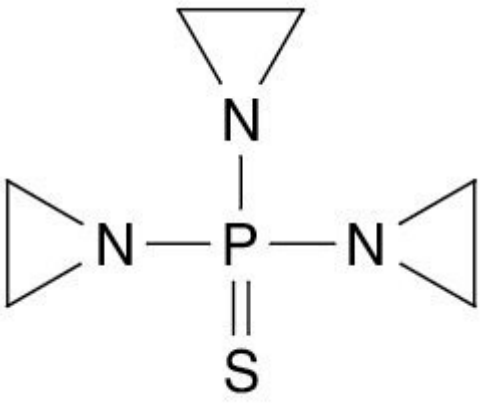


Figure 27: Thiotepa (https://doi.org/10.22427/NTP-OTHER-1003, 2021)

DNA's guanine base attached to an alkyl group in the seventh nitrogen base of the imidazole ring. It can prevent cancer growth by targeting DNA directly by creating crosslinks between guanine nucleobases in the strands of DNA. This prevents the strands from unwinding and breaking apart. This prevents cell division since essential DNA replication is inhibited. But one of its limitations is that these medications have no specified target. So, it damages healthy cells also.

Mechanism of Action

i. In the body Thiotepa is metabolized into ethylenimine groups.

ii. Ethyleneimine groups bind to the N7 site of the guanine in a DNA base pair.

iii. This will cause the ds-DNA to pair up with itself.

iv. This will have further adverse impacts on the processes of DNA replication and transcription.

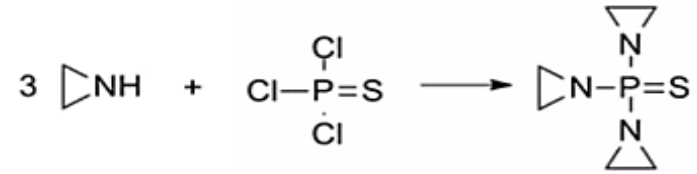
v. This will cause the cells to stop proliferating and eventually die off through a process called apoptosis. (Maanen et al., 2000)

Structural Activity Relationship

- Its SAR is like other common general SAR of alkylating agents (Pires et al., 2018).

Methods of Synthesis

Ethyleneimine(aziridine) reacts with thiophosphoryl chloride (tricholorophosphine sulfide) and also with triethylamine in dry benzene to create thiotepa.(Maanen et al., 2000)



Aziridine Thiophosphoryl chloride. Thiotepa

Figure 28: Synthesis of Thiotepa (Maanen et al., 2000)

Alkylating drugs generally work by altering cancer cells' DNA, resulting in the cells to die. The particular method of action, which may involve various forms of DNA damage such cross-linking, strand breaking, and adduct formation, can vary according to the chemical makeup of the drug.

Indications

Leukemia, lymphoma, breast, ovarian, lung, and brain tumors are just a few of the cancers that are commonly treated with alkylating drugs. They are often used in combination with other chemotherapies depending on the cancer's stage, the patient's health condition and other factors.

2.4 Side Effects

Intestinal toxicity and dose-limiting toxicity to bone marrow are common with most of the alkylating drugs. Also, most alkylating drugs reduce granulocyte production in the bone marrow as an immediate side effect. All blood components, stem cells in particular, are suppressed by busulfan. Some autoimmune diseases are treated with alkylating drugs because they suppress both cellular and humoral immunity. Because of their toxicity, alkylating agents not only affect the bone marrow, but also the oral mucosa and the intestinal mucosa. The lungs (pulmonary fibrosis) and the liver are also damaged by several alkylating drugs. The metabolite acrolein is responsible for the severe hemorrhagic cystitis brought on by cyclophosphamide and ifosfamide. The use of thiol flushing agents allows for control of this problem. The absorption of the more unstable alkylating chemicals, like nitrogen mustard and the nitrosoureas, can induce ulceration due to their high vesicant properties. Alopecia is one of the adverse effects of the immunosuppressive effects of alkylating drugs (Scholar, 2007). The severe cytotoxicity of alkylating chemicals means that they can have a wide range of unwanted effects. Different agents, different doses, and different lengths of treatment can cause different degrees and types of side effects. Nausea, vomiting, hair loss, fatigue, and anemia are only some of the usual undesirable responses to alkylating drugs. Additionally can cause, lowered white and platelet counts, raised risks of infection, harmed organs including the kidneys and liver and a heightened potential for secondary malignancies.

Chapter 3

3.1 Alkylating agent in cancer mortality rate

For many decades, cancer chemotherapy has relied on alkylating agents. The use of alkylating substances has been shown to reduce cancer-related mortality by slowing cancer progression and killing off cancer cells (Holland Frei et al., 2003). Therefore, this may cause malignant growths to shrink or eliminate, enhancing long-term survival. However, alkylating chemicals use may cause unwanted consequences as secondary effects, such as damage to normal cells and tissues that require careful monitoring and caring support for recovery.

The DNA damage induced by alkylating chemicals in cancer cells may hamper the cells' capacity for mitosis and the division of cells. This may cause cancer cells to die off or slow their growth. Furthermore, cancer cells are especially sensitive to the actions of alkylating agents since they are rapidly proliferating cells. Therefore, alkylating chemicals can be used to selectively attack the uncontrollable growth of cancer cells while causing minimum harm to the surrounding healthy cells.

3.2 Future Direction

The significant potential for toxicity and the evolution of drug resistance are just two of the problems that might arise when using alkylating drugs, regardless of their efficacy. That's why researchers are constantly looking for ways to make these medicines even more potent and secure. Designing prodrugs that are metabolized into active medications in the body is one strategy. This strategy has the potential to enhance the drug's selectivity and decrease its toxicity. Combination therapy is another option; these include the use of various medications

that work in distinct ways. This method has the potential to improve therapeutic efficacy while decreasing the risk of drug resistance.

Chapter 4

4.1 How to choose better alkylating agents

Chemotherapy medications like alkylating agents, which class of drugs are effective against cancer in many instances. First, they perform by causing mutations in cancer cells' DNA, which in turn delays the spreading of the disease. Due to their mechanism of action, they are useful in curing numerous cancers. In addition, alkylating medicines can be used in combination with other chemotherapeutic treatments to boost the efficacy of both drugs. Due to the fact that they are able to pass via the blood-brain barrier, alkylating drugs are also effective against malignancies that have reached the central nervous system. Last but not least, some patients may find alkylating medicines like temozolomide more acceptable than other types of chemotherapy treatments since they have fewer severe adverse effects.(Dinnes J et al., 2002). It's crucial to remember, though, that no single category of anticancer drugs is always better than all the others. Chemotherapy medications are used to treat cancer, yet the specific drugs utilized for each patient will vary according to their specific diagnosis, health status and other circumstances. Sometimes in order to achieve the best potential result for any cancer patient, administering a combination of medications with alkylating agent is a very effective solution. (Holland Frei et al., 2003)

4.2 How to meet patient demand

A patient's health, the drug, and the dosage all play a role in determining whether or not an alkylating agent is safe and effective to use. Chlorambucil and cyclophosphamide are two examples of older alkylating medicines that have a higher incidence of side effects than newer ones like temozolomide. One example of a recently formulated alkylating agent with an

improved safety profile and efficacy is treosulfan, which is very well tolerated by patients with certain types of cancer. (Burroughs LM et al., 2014). The patient's medical history and some risk factors are taken into consideration while deciding whether or not to administer any alkylating drugs in cancer treatment. In the field of cancer treatment, certain modern alkylating agents with improved safety profiles are currently considered to be the most effective till now. Temozolomide, bendamustine and treosulfan are three examples of such drugs. The best treatment plan for a patient's individual health concerns can only be determined after discussion with a qualified and experienced medical professional.

Chapter 5

5.1 Impact of this review paper

It is a detailed review of the available literature on different classes of alkylating agents. It can provide a valuable contribution to the scientific community as a review article on alkylating agents to get an overall idea. Alkylating drugs' mechanisms of action, therapeutic applications, structure activity relationship and synthesis have been summarized here. This review article provides researchers and clinicians with a clear understanding of alkylating drugs and their consequences for several fields of medicine, including oncology and immunology, by integrating and evaluating the current information. This review paper can also be used as a resource for new research and to guide the design of improved therapeutic approaches on oncology. To find opportunities for future research as we look over the existing works on alkylating agents, we might find some spaces, questions, or areas that need more research. These are promising areas in which to direct future study. This review article can be a valuable resource for future researchers to fill these knowledge gaps by pointing them out and discussing them in their paper. To fill in the gaps and learn more about alkylating drugs, this overview may aid the beginning of more research, experiments, or clinical trials. This particular work might have the potential to inspire further study in the topic and contribute to overall advancement of the treatment of cancer. More research into alkylating agents may lead us to learn new information, spot new tendencies, or perhaps come up with some novel ideas. New theories, therapeutic uses, alternative treatment techniques, and recommendations to enhance the safety profile of alkylating agents all qualify as contributions. By sharing some of these original findings in this review paper, we hope to help shape the future of this field of study by inspiring discussion and drawing attention to new ideas.

5.2 Conclusion

Alkylating agents have treated cancer and tumors for several decades. Due to their potent pharmacologic effects against tumor cells, alkylating compounds have been widely recognized as potentially beneficial products in an era when cancer has become a devastating global health concern. The way they work involves destruction of the DNA of cancerous cells and resulting in cell death. However, the high potential for toxicity of these agents can lead to various adverse reactions. Nowadays due to their toxicity and the possibility of development of drug resistance, the usage of these agents is often restricted. Nevertheless, scientists are currently exploring more novel strategies to enhance the effectiveness and safety of these drugs. These approaches include the development of prodrugs and combination therapies to ensure their safe therapeutic effects on cancer patients. The ultimate objective of alkylating agents is to enhance patient outcomes and lessen the impact of cancer on society. This review article provide an overview of alkylating agents, structures, mechanisms of action and general synthetic routes to access these agents to aid shape the future alkylating agents by inspiring discussion and drawing attention to new ideas.

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