

PHARMACOLOGICAL PROFILE AND STRUCTURE-ACTIVITY RELATIONSHIP OF ALKYLATING AGENTS USED IN CANCER TREATMENT

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ABSTRACT

Cancer is a high prevalence disease treated with surgery, radiotherapy and chemotherapy. Among the chemotherapy therapies the class of alkylating agents stands out, which was the first to emerge. The objective of this study was to report pharmacokinetic and pharmacodynamic aspects and suggest a structure-activity relationship for the alkylating agents. The study was performed through literature review by searching Google Scholar, Scielo, PubMed and ScienceDirect databases. The 27 selected articles were analyzed and compared and the results were compiled in article form. Alkylating agents are electrophilic substances capable of alkylating DNA. The *bis*(2-chloroethyl) portion is essential for the cytotoxic activity in most of the structures, and all drugs in their metabolism or activation generate cations able to alkylate DNA in cancer cells. They are not analogs of any endogenous molecule or bind to a specific receptor; they are small and relatively lipophilic molecules capable of penetrating cells and forming reactive species that can react with DNA. In conclusion, the alkylating agents are widely used against various cancers, including refractory cases, however, there are still challenges in order to improve the properties of drugs and treatment for the patient.

Keywords: Pharmacokinetics, Alkylating agents, Pharmacodynamics.

INTRODUCTION

Cancer is a very prevalent disease, which affects millions of people each year. According to the predictions of National Cancer Institute José Alencar Gomes da Silva (INCA), in 2030 there will be 26 million new cases and 17 million deaths per year. In Brazil, the highest prevalence among men is prostate cancer and among women is breast and cervical cancer (INCA Estimativa, 2014).

This neoplasia is being treated with surgery, radiotherapy, and adjuvant chemotherapy. Chemotherapy treatment includes several compounds, for instance, antimetabolites, natural agents, alkylating agents, hormone, antibiotics, enzymes, various agents and platinum coordination complexes. (Almeida *et al.*, 2005; Puyo *et al.*, 2014). Alkylating agents emerged from the sulfur mustards used as chemical weapons since the First World War.

They are lethal vesicant agents in high doses and after exposure, they cause nausea and vomiting, respiratory toxicity, skin inflammation, eye irritation (blindness), burns, anemia, lymphoid hypoplasia, and myelosuppression. Moreover, they have a mutagenic and carcinogenic potential by reacting with proteins and RNA, and causing DNA alkylation by cytotoxic action. In 1942, Goodman and Gilman used these mustard properties in clinical trials with lymphoma patients. This enabled the approval of mechlorethamine by the FDA in 1949. It was the first alkylating agent, which was the first class of chemotherapy to emerge, revolutionizing cancer therapy and it is widely used today (Almeida *et al.*, 2005; Puyo *et al.*, 2014; Ghabili *et al.*, 2011; Brunton *et al.*, 2012).

Considering the great use of this class and its excellent response to different types of tumors, including refractory cases, the aim of this study is to discuss chemotherapy of cancer by reporting pharmacokinetic, pharmacodynamic aspects and suggesting a structure-activity relationship for alkylating agents.

MATERIAL AND METHODS

This study is a literature review on the alkylating agents used in cancer treatment. We aim to clarify what is the pharmacokinetic and pharmacodynamic behavior of these drugs, as well as if there is a relationship between the structure and activity of the same. We have searched articles and monographs on Google Scholar, Scielo, PubMed and ScienceDirect databases, comprising the months of February and March 2015. In order to refine our search, the following keywords were employed: antineoplastic, alkylating agents, pharmacodynamic, pharmacokinetic and structure-activity relationship. These words were searched alone or combined, in Portuguese or translated into English and Spanish.

RESULTS AND DISCUSSION

We have searched articles from 2005 to 2015. In each database and for each term/association between 70 and 120 articles were screened by title and abstract (except for Scielo due to lack of material). A total of 36 articles were pre-selected, of which 9 were excluded for not having information related to the aim of the study, resulting in 27 articles for the review.

In terms of material related to the study, the richest databases were PubMed and ScienceDirect. English was the predominant language. The vast majority of articles found were related to pharmacokinetic and pharmacodynamic studies of alkylating agents and development of new drugs.

Cancer

Cancer is a disease characterized by the uncontrolled growth of cells with genetic changes (malignant). It can invade several tissues and organs leading to impairment and loss of function. It can be triggered by genetic factors (mutations in genes), environmental exposure (sunlight), exposure to chemical compounds (benzene, chromium VI), virus infection (hepatitis B, Papillomavirus) and lifestyle (obesity, smoking, excessive consumption of alcohol). There is surgical treatment with adjuvant radiotherapy for solid tumors without metastasis. Chemotherapy is used for refractory cases, metastasis and

other tumors that require systemic action (Almeida *et al.*, 2005).

In chemotherapy, the class of alkylating agents stands out. It is classically subdivided into nitrogen mustards, oxazaphosphorines, ethylene imines and methylmelamines, nitrosoureas, triazines and hydrazines, alkylsulfonates and platinum coordination complexes (Almeida *et al.*, 2005; Puyo *et al.*, 2014; Brunton *et al.*, 2012).

The main drugs of each class with related information are shown in Table 1.

Pharmacodynamic of alkylating agents

Alkylating agents are considered non-cell-cycle specific because its activity is not restricted to a specific cell-cycle phase, although some cells are more susceptible to alkylation in post-mitotic (G1) and synthetic (S) phases. This unspecific activity increases their power of action (Almeida *et al.*, 2005; Brunton *et al.*, 2012).

These agents are electrophilic substances capable of reacting covalently with DNA and proteins by transferring alkyl groups. This reaction is a nucleophilic substitution (SN), which can happen in two steps, with the formation of a carbocation that is attacked by a nucleophile (SN1), as seen in Figure 1, or in a single step with the attack by a nucleophile and product generation (SN2). The following groups are susceptible to nucleophilic substitution: sulfhydryl, hydroxyl, phosphate, amine, imidazole and carboxyl (Puyo *et al.*, 2014; Brunton *et al.*, 2012; Kaina *et al.*, 2007). Alkylating agents are divided into monofunctional and bifunctional. The monofunctional binds to a protein or a base on double-stranded DNA. The bifunctional can bind to a protein and to the DNA, two bases of the same strand or different DNA strands (Puyo *et al.*, 2014; Fu *et al.*, 2012). The site of alkylation on DNA bases depends on the reaction mechanism (SN₁ or SN₂), the alkylating agent (monofunctional or bifunctional) and the substitution group (methyl or ethyl), but it occurs predominantly at the N7 position of guanine. Other sites are as follows: O6, N1 and N3 of guanine, N3, N4 and O2 of cytosine, N1, N3, N6 and N7 of adenine and N1, N3, O2 and O4 of thymine, as shown in Figure 2 (Puyo *et al.*, 2014; Brunton *et al.*, 2012; Fu *et al.*, 2012; Gates, 2009).

These bindings change gene expression, inhibit cell division and cause DNA damage leading to mutagenic and cytotoxic activity with consequent cell death. The damages are related to the severity of the injury and the difficulty/impairment to repair it. In this case, the bifunctional agents are more potent than the monofunctional, because crosslinks inflict

greater damage to DNA and there is greater difficulty in repair (Fu *et al.*, 2012; Gates, 2009; Bignold, 2006).

Despite having a different structure and not producing DNA alkylation itself, platinum coordination complexes have mechanisms of action and resistance very similar to those of alkylating agents, thus being discussed together with this class by many authors. They enter the cell by transporters and, once there, they react with water to form positively charged and very reactive species. This reaction is favored by the low concentrations of intracellular chloride. These species react covalently with nucleophilic sites in DNA or proteins, forming metal complexes. The main binding site is also the N7 position of guanine, with inter-strand or intra-strand crosslinks. These platinum-DNA complexes inhibit transcription and synthesis of DNA, due to strand breaks and coding errors that lead to mutations and apoptosis (Almeida *et al.*, 2005; Puyo *et al.*, 2014; Brunton *et al.*, 2012).

Because of their potent cytotoxic effects, the alkylating agents are indicated for several types of tumors. They are not as specific for tumor cells; instead, they act on cells with high proliferation rate (tumor, mucosa, blood cells), which ultimately affects normal cells of the organism, causing many side effects and toxicity. Overall, they cause myelosuppression (leukopenia, thrombocytopenia and anemia), nausea, vomiting, diarrhea, mucositis and stomatitis (pain and ulceration), rash, alopecia, neurotoxicity (coma, convulsions), azoospermia, amenorrhea, secondary malignancies and, they are contraindicated in pregnancy due to their mutagenic potential (Brunton *et al.*, 2012; Fernandini & Ferreira, 2009). For example, it is worth mentioning the nephrotoxicity of ifosfamide that may lead to renal failure (Lowenberg *et al.*, 2014) and hepatotoxicity of busulfan and treosulfan (Brink *et al.*, 2014). Given the potency and toxicity of these drugs, many of them are also used in myeloablative conditioning regimens of high doses, to destroy the bone marrow prior to transplant. For instance, busulfan, treosulfan, melphalan and bendamustine (Nath *et al.*, 2005; Cheson & Rummel, 2009; Brink *et al.*, 2014; Galaup & Paci, 2013).

Pharmacokinetic of alkylating agents

According to the class, these drugs have some peculiarities. Some are drugs and the others are prodrugs. The nitrogen mustards, nitrosoureas, platinum coordination complexes, ethylene imines and metilmelaninas and the alkylsulfonates are bifunctional drugs while the oxazaphosphorines, triazenes, and hydrazines

are prodrugs (Puyo *et al.*, 2014; Brunton *et al.*, 2012). These prodrugs are activated via enzymes of the cytochrome P450, except for temozolomide that undergoes nonenzymatic activation. The main enzymes involved are CYP2A6, CYP2B6, CYP3A4, CYP3A5, CYP2C9 and CYP2C18, which generate active and toxic metabolites. Genetic polymorphisms of these and other enzymes involved in the process lead to differences in drug metabolism, thus directly interfering with its action and toxicity (Lowenberg *et al.*, 2014).

Another aspect often evaluated for risk/benefit to the patient are antineoplastic drug interactions among themselves and with drugs of other classes that are frequently used. These interactions directly influence the pharmacokinetic of drugs. For instance, cisplatin interacts with antibiotics by increasing toxicity and with anticonvulsants by reducing plasma levels. Busulfan interacts with acetaminophen, and with phenytoin and itraconazole, which are respectively an inducer and an inhibitor of cytochrome P450 enzymes that metabolize this drug. Therefore, the concomitant use of busulfan and phenytoin reduces its effects and with itraconazole increases its effects with a higher risk of toxicity. In order to avoid these interactions, alternative drugs are sought whenever possible, for example, clonazepam instead of phenytoin (Brink *et al.*, 2014; Romani, 2012).

The differences are also on the route of administration, which may be oral or intravenous injection according to the availability and stability/solubility of the drugs. Busulfan is poorly soluble in water, thus having an oral presentation (in 2000 intravenous formulation was released), while treosulfan is well soluble in water, which facilitates the intravenous and oral presentations (Brink *et al.*, 2014; Galaup & Paci, 2013). The form of action is usually systemic, but there are already drugs with local action or that are being developed for this purpose. Melphalan administered locally on retinoblastomas shows a better action due to its good ability of drug penetration, lower systemic toxicity and lower risk of inducing secondary leukemia (Schaiquevich *et al.*, 2012).

These drugs are well distributed throughout the body, only restricted in some specific cases, for instance, testicular cancer and central nervous system cancer due to lower irrigation and difficult access (brain-blood barrier – BBB), respectively. Temozolomide is highly lipophilic and can cross the BBB; therefore, it is a good alternative to treat gliomas (Brunton *et al.*, 2012; Diez *et al.*, 2010). Pharmacokinetic of alkylating agents is considered multicompartmental, generally

biphasic or three-phase. The half-life ($T_{1/2}$) of most drugs is short, from minutes to hours and, as seen in Table 1, drugs have shorter half-life compared to prodrugs that require prior activation.

Doses vary according to the regimen and length of the treatment cycle (combination therapy), administration route, type of cancer, age and physical condition of the patient (white and red blood cell counts before each cycle due to myelosuppression). For example, dacarbazine is administered intravenously in malignant melanoma treatment (2-4.5 mg/kg daily for 10 days), repeating the regimen every 28 days or 250 mg/m² daily for 5 days repeated every 3 weeks. In combination therapy for Hodgkin lymphoma treatment, dacarbazine can be administered in doses of 150 mg/m² for 5 days repeated every 4 weeks or in a single dose of 375 mg/m² repeated every 15 days (Brunton *et al.*, 2012).

The metabolism of drugs occurs primarily by liver enzymes with excretion usually through the urine, but also via feces. Some studies have noted differences in the clearance between adults and children; they have greater renal clearance and can tolerate higher doses of drugs compared to adults. This is due to the large size of the liver in proportion to their body weight and growth of kidneys with greater glomerular filtration rate (Nath *et al.*, 2005; Brink *et al.*, 2014). The liver and its enzymes are essential for drug activation and metabolism and kidneys are the main responsible for its excretion. As a result, changes in the functioning of these organs (failure, tumors) hinder treatment and health of the patient, with switching medications, increased risk of toxicity and need for dose adjustment.

Structure-activity relationship

The class of alkylating agents is divided into subclasses with structural peculiarities, as observed in the structures of the main drugs shown in Figure 3. These differences are due to the development of molecules with lower toxicity, higher cytotoxic activity, greater specificity to the site of action, BBB-penetrating ability, oral route availability and other characteristics. Thus, the relationship between structure and activity of drugs changes according to the subclass.

For nitrogen mustards, which emerged from the sulfur mustards with the replacement of sulfur by nitrogen due to the lower toxicity, the bis (2-chloroethyl) group is essential for the activity. It is responsible for the formation of the aziridine cation (heterocyclic with 2 methylene group attached to a positively charged nitrogen) that alkylates DNA. After

mechlorethamine (1st mustard), which is an unstable molecule, it was created melphalan and chlorambucil that have phenylalanine and aminophenyl butyric acid in the structure, respectively. The binding to amino acids and substituted phenyl group allowed the oral route availability of these drugs. In addition, the presence of the aromatic ring maintains the molecule more stable, enabling a better distribution of the drug throughout the body before forming the cation (Puyo *et al.*, 2014; Brunton *et al.*, 2012; Scutaru *et al.*, 2011). Bendamustine, which is another mustard, has a benzimidazole ring that provides stability to the molecule and a local and faster action, observed by the shorter half-life (Table 1) of the drug in relation to the other of its class (Cheson & Rummel, 2009; Scutaru *et al.*, 2011).

Similar to the prodrugs cyclophosphamide and ifosfamide, oxazaphosphorines is inactive when administered by the presence of oxazaphosphorine ring. They need to be activated via CYP, with hydroxylation that allows the disruption of the ring and release of the bis(2-chloroethyl) group, which is responsible for generating the active cation, as shown in Figure 4 (Puyo *et al.*, 2014; Wang D & Wang H, 2012; Lowenberg *et al.*, 2014).

The ethylene imines, for example, thiotepa, have in their structure three ethyleneimine-aziridine groups stabilized by a tiosforil base. They are essential for cytotoxic activity that generates active cation (aziridine) from the protonation of the nitrogen on the ring (Brunton *et al.*, 2012). The altretamine is a methylmelanine and, despite many studies, its alkylation mechanism remains unknown. They have higher half-life compared to thiotepa due to the greater complexity of the molecule (Figure 3).

From the class of triazenes and hydrazines, dacarbazine and temozolomide stand out since they have the triazene group. In the temozolomide structure, triazene is inserted in a cyclic structure, which combined with the imidazole ring forms a relatively small lipophilic molecule capable of penetrating the BBB. The imidazole ring in both molecules confers a more rapid and located onset of action. Both prodrugs originate the active metabolite 5-(3-methyltriazene-1-yl)-imidazole-4-carboxamide (MTIC), dacarbazine via CYP (N-demethylation reaction) and temozolomide by spontaneous hydrolysis at physiological pH. This metabolite forms the methyl diazonium cation, which is responsible for DNA binding (Figure 5) (Puyo *et al.*, 2014; Brunton *et al.*, 2014; Diez *et al.*, 2010).

Carmustine and lomustine present the nitrosourea group that gives the name to the

class and have the 2-chloroethyl as the essential group for activity. In the structure (Figure 3) is noted that carmustine is bifunctional, having two 2-chloroethyl groups, while lomustine is monofunctional and has as a second substituent a cyclohexyl that does not contribute to its cytotoxic activity but generates an increase in its half-time. Spontaneous degradation of molecules releases diazo-hydroxide, which decomposes by releasing the aziridine cation responsible for the cytotoxic activity, as seen in Figure 6 (Puyo *et al.*, 2014; Brunton *et al.*, 2014).

The alkylsulfonates, such as busulfan and treosulfan, present in their structure sulfonates that are good output group in a reaction. In busulfan, removal of one of these groups generates a cation that alkylates DNA. The treosulfan, in turn, has two hydroxyl groups in its structure, which makes it a more hydrophilic molecule and confers water solubility and oral availability. In a pH-dependent reaction, treosulfan is converted into mono and diepoxides that have alkylating activity (Puyo *et al.*, 2014; Brink *et al.*, 2014; Galaup & Paci, 2013). The platinum coordination complexes require it in their structure and substituents to stabilize the metal, as the hydrochloride and oxalate capable of being displaced by water in a reaction that generates a cation which alkylates the DNA (Puyo *et al.*, 2014; Brunton *et al.*, 2014). It is observed the greatest half-time of carboplatin (Table 1) in relation to cisplatin by the larger substituent (Figure 3), which will take longer to be metabolized.

In general, the effects of the alkylating agents are not related to specific receptors or similarity to endogenous molecules, but to the ability to penetrate cells and form reactive species that bind and cause damage to DNA. Alkylating agents require an electronegative group in their structure, for instance, chloride, to induce poles in molecules and electron acceptor group, as nitrogen, so that the formation of ions able to alkylate DNA and proteins occur.

Perspectives

Despite the extensive use of alkylating agents and of their good results in cancer treatment, there are still challenges ahead. Most of these drugs have many adverse effects, are unspecific regarding the type of cell and action site, and cause mainly hematologic, renal and neurologic toxicity. Furthermore, both normal cells and many types of tumor cells have a DNA-repair system. In normal tissues, this is a defense and a way of preserving the genetic information, however, in tumor cells, this is a resistance mechanism to chemotherapy treatment. Thus, the tendency is the

development of drugs with release systems or specific carriers to the action site, less toxic analogs and strategies to dribble the DNA repair systems.

DNA repair pathways involve mainly the O⁶-methylguanine-DNA methyltransferase (MGMT), which directly removes the alkyl group from the O6 positions and chloroethyl group between the DNA chains. Other pathways, ABH2 and ABH3, directly remove methyl from N1, N7 and N3 positions of double chains of DNA and single chains of RNA, respectively. Base excision repair (BER) repairs other N-alkylation sites and nucleotide excision repair (NER) assists in alkylation repair. The DNA mismatch repair (MMR) corrects incorrectly paired nucleotides and the homologous recombination (HR) and non-homologous end-joining (NHEJ) enable the resynthesis of damaged DNA. There are other repair mechanisms and if this system fails, the cell can activate death by apoptosis via p53 gene (malignant cells with altered p53 do not respond to control and do not undergo apoptosis) (Puyo *et al.*, 2014; Brunton *et al.*, 2014; Kaina *et al.*, 2007; Fu *et al.*, 2012).

An example of repair enzyme inhibitor is O⁶-benzilguanine. This is an inhibitor of O⁶-alkylguanine-DNA alkyltransferase and as a result of an experiment the addition of a *meta*-aminomethyl group improved the solubility of the compound and increased the capacity of inactivating the enzyme by about 20 times (Pauly *et al.*, 2008). Aiming local action to increase the effect and lower toxicity, drugs for cerebral delivery involve redox systems wherein the hydrophilic prodrug is linked to a lipophilic carrier (1,4-dihydropyridine) allowing penetration of the BBB and undergoing oxidation in the brain, forming polar pyridinium salt that releases the active drug (Singh *et al.*, 2015). Analogs of ifosfamide were developed with the intention of reducing toxicity, for instance, glufosfamide and mafosfamide, which do not require activation by CYP^(9,24) and C7, C9-dimethyl-ifosfamide that by steric hindrance prevents the formation of toxic metabolites production (Storme *et al.*, 2009).

In order to increase the cytotoxic activity, analogs of melphalan were created, for example, mel-flufen that not only has a better activity but it is also independent of p52 gene and reduces angiogenesis (Chauhan *et al.*, 2013) and bivalent analogs of melphalan and steric hindered bendamustine with maleimide and linked by diamines (Scutaru *et al.*, 2011). Moreover, new molecules are being developed such as quinolones (quinoline-N-phenylhydrazinecarboxamide mustard or urea as a binder), and pyrrolo [1,2-b] isoquinoline (Kakadiya *et al.*, 2010; Chaniyara *et al.*, 2011).

Most alkylating agents bind to the major DNA groove. Currently, minor groove binders that are more specific, less toxic and have a different mechanism are sought. They may also connect to proteins, being related to inhibition of gene transcription linked to cancer cells. These drugs have a higher affinity for linking themselves to adenine and thymine bases and for having a bigger structure with fused aromatic rings that are related to the cytotoxic action. For instance, illudins (irufulven) tallimustine, brostallicin, the tetrahydroquinolines and other derivatives that are still under study (Almeida *et al.*, 2005; Puyo *et al.*, 2014; Iyer *et al.*, 2013).

CONCLUSION

The class of alkylating agents was a pioneer in chemotherapy treatment and the bibliographic research evidenced the extensive use and knowledge regarding this class. The aim was achieved, we discussed pharmacokinetic and pharmacodynamics parameters, we also demonstrated that there is a relationship

between drug structure and activity. They are largely used against leukemia, lymphomas, melanomas, in refractory cases, brain tumors and other solid tumors, for example, breast, testicle, head and neck, lung and bladder. Concerning toxicity, the most striking characteristic is myelosuppression that makes the treatment difficult.

Despite the widespread use and the good results of these drugs across the different types of cancer, further improvement is necessary. Studies on the development of new drugs with lower toxicity, greater effectiveness, and specificity as to the site of action are being performed. The constant improvement and innovation provide a more effective, safe and less toxic treatment to the patient. This improves their adherence to treatment, quality of life and chances of survival. Thus, this study serves as a means to disseminate knowledge regarding cancer treatment in order to assist and encourage studies concerning the treatment and cure of this disease.

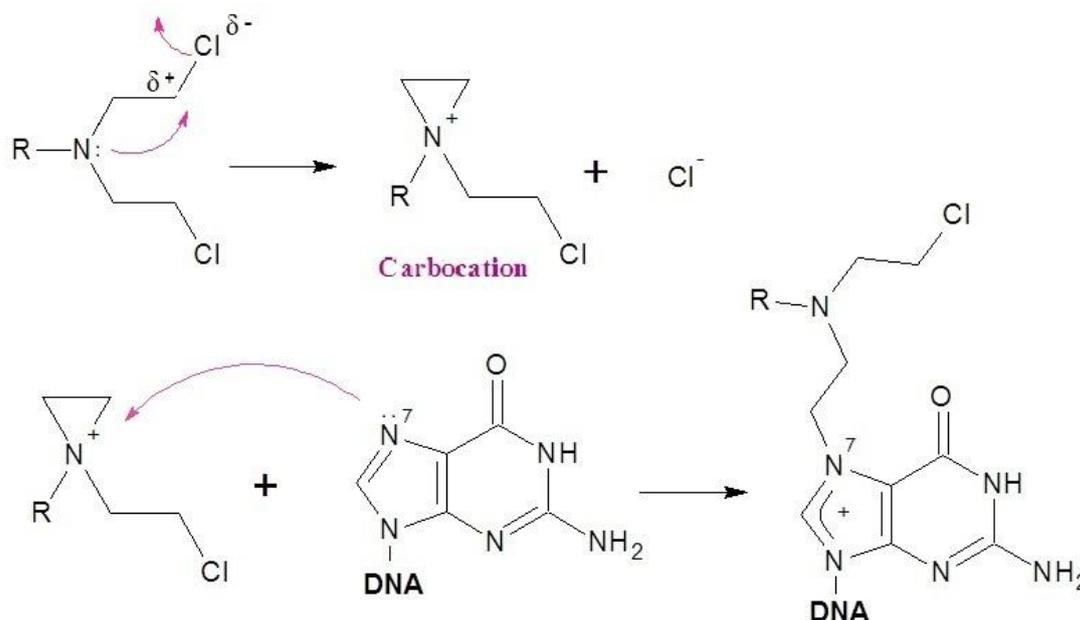


Fig. 1: Generic reaction mechanism of alkylating agents with DNA

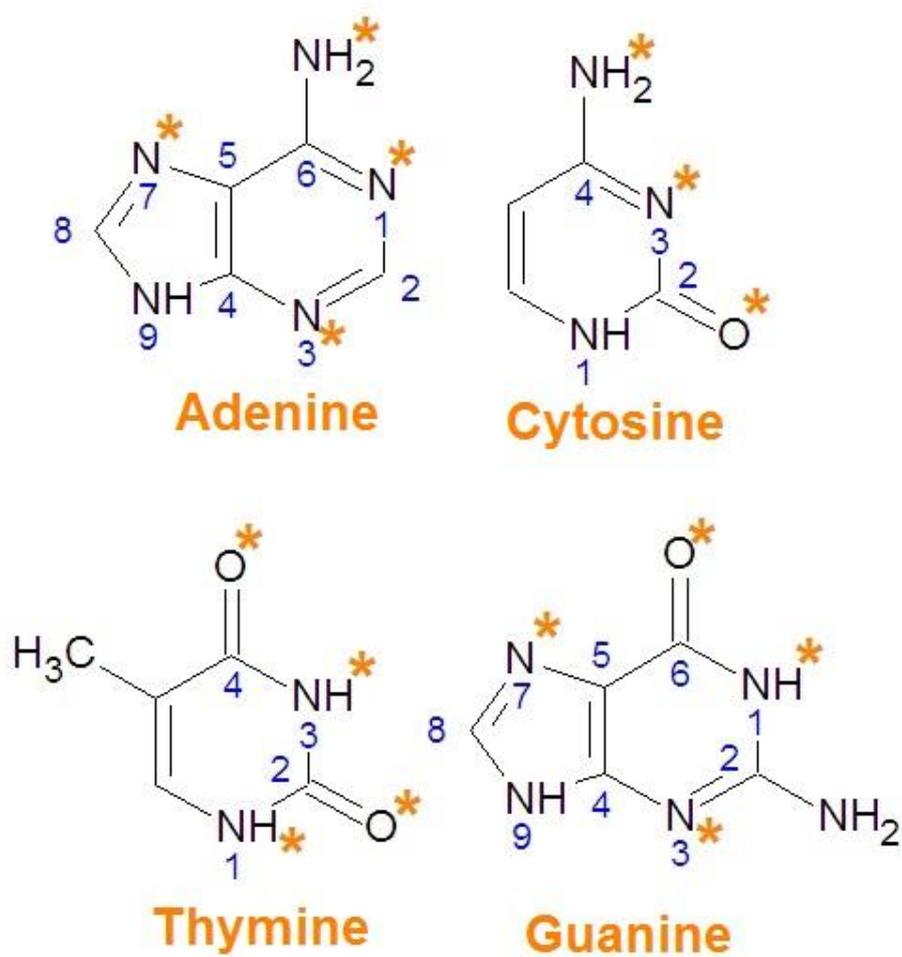


Fig. 2: Major DNA alkylating sites, indicated by an asterisk (*)

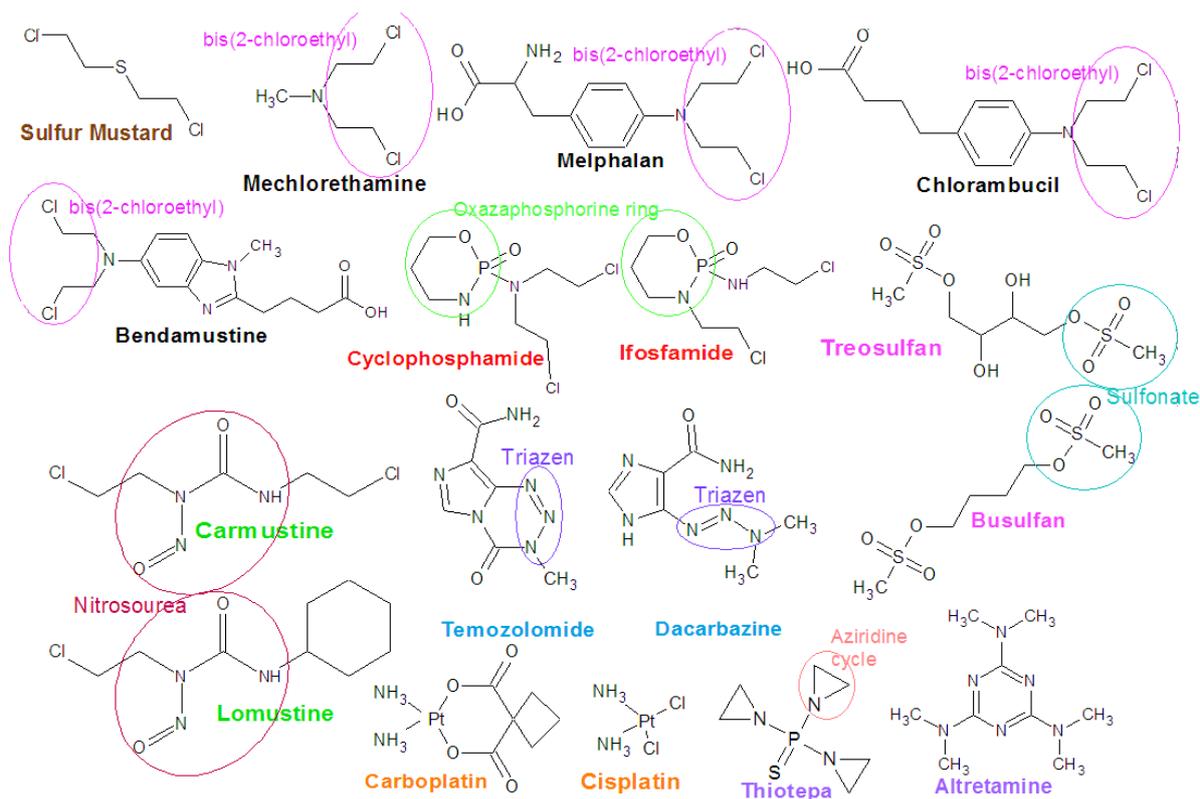


Fig. 3: Structure of the main drugs of the class of alkylating agents and its precursor, sulfur mustard

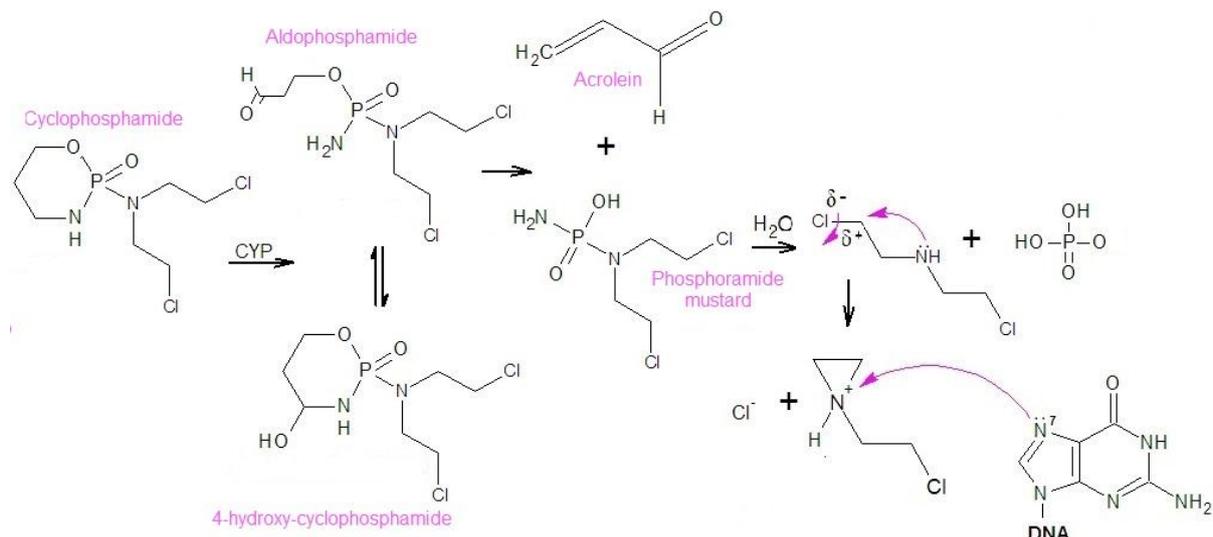


Fig. 4: Cyclophosphamide activation and reaction with DNA

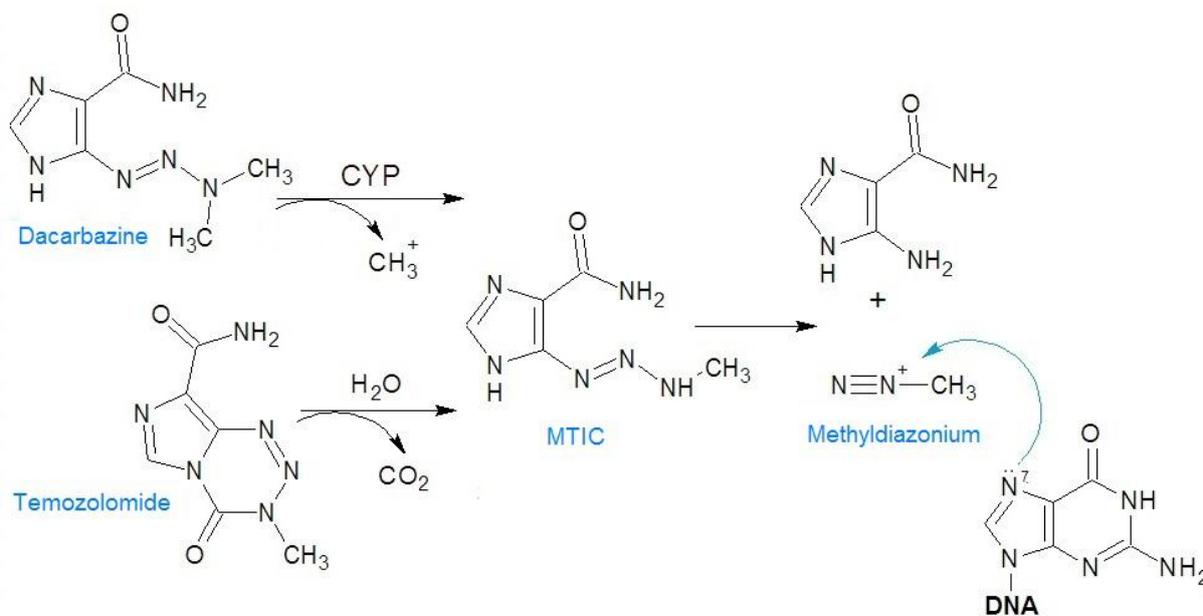


Fig. 5: Reaction mechanism of dacarbazine and temozolomide

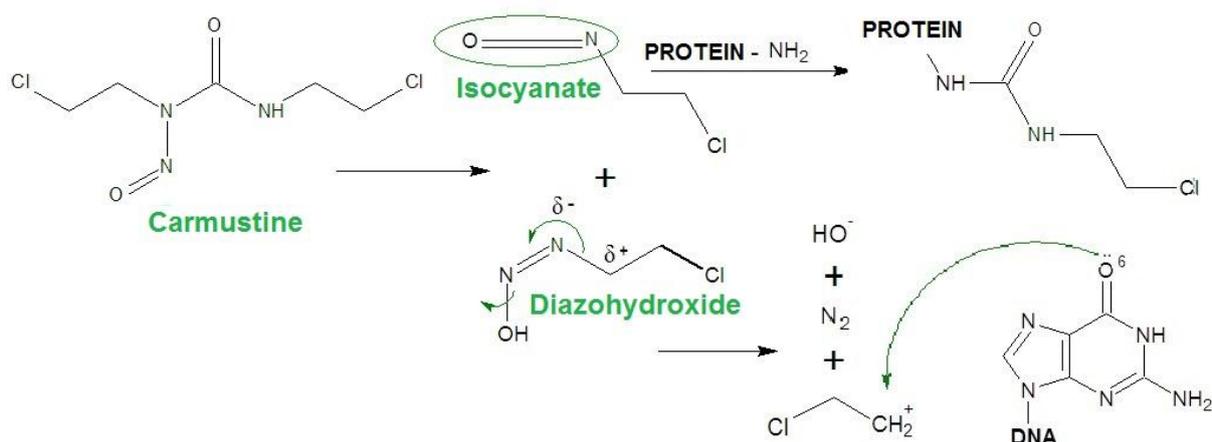


Fig. 6: Action mechanism of carmustine with the generation of alkylating intermediate compounds (DNA) and carbamoylation (protein)

Table 1: Drugs from the class of alkylating agents and their main pharmacodynamics and pharmacokinetic characteristics

Class	Drugs	Route of Adm.	Metabolites	T _{1/2}	Toxicity	Therapeutic indications
Nitrogen mustards	Mechlorethamine ^(1,3)	IV	-	-	Myelosuppression	Lymphoma and Hodgkin's disease
	Melphalan ^(3,4)	PO and IV	Mono and dihydroxy inactive	45-90 min.	Hematologic	Multiple myeloma and leukemia
	Chlorambucil ⁽³⁾	PO	Hydrolysis inactive products	90 min.	Hypoplasia	Chronic Lymphoid Leukemia
	Bendamustine ^(3,5,6)	IV	Mono and dihydroxy inactive and γ -hydroxy and N-desmethyl active	30-40 min.	Myelosuppression	Chronic Lymphoid Leukemia and non-Hodgkin's lymphoma
Oxazaphosphorines	*Cyclophosphamide ^(1,2,7)	PO and IV	4-hydroxy-CPA, aldophosphamide and mustard active phosphoramidate and toxic acrolein	420 min.	Neurotoxicity, nephrotoxicity	Leukemia, lymphoma, sarcomas, breast and ovarian cancer and tumors in children
	*Ifosfamide ^(1,3,8)	IV	4-hydroxy-IFO, aldophosphamide active and ifosfamide mustard and acrolein toxic	90 min.	Neurotoxicity, nephrotoxicity	Testicular and germ cell cancer and sarcomas in children and adults
Ethylene imines and Methylmelamines	Thiotepa ^(1,3)	IV	Triethylene Phosphoramidate (TEPA) active	70-120 min.	Myelosuppression	Bladder, breast, and ovarian cancer
	Altretamine ^(1,3)	PO	Penta and tetra methyl melamine	240-600 min.	Neurotoxicity	Ovarian cancer
Triazines and Hydrazines	*Temozolomide ^(1,3,9)	PO and IV	5-(3-methyl-1-triazenyl)imidazole-4-carboxamide (MTIC)	60-120 min.	Nausea and vomit, myelosuppression	Glioma e malign melanoma
	*Dacarbazine ^(1,3,9)	IV	Active MTIC	20 min. e 300 min.	Nausea and vomit, myelosuppression	Melanoma, lymphoma, and sarcomas
Nitrosoureas	Carmustine ⁽¹⁻⁵⁾	IV	Active diazohydroxide	15-90 min.	Myelosuppression	Brain tumors, melanoma
	Lomustine ^(1,5)	PO	Active diazohydroxide	90 min.	Myelosuppression	Brain tumors, melanomas
Alkylsulfonates	Busulfan ^(3,10,11)	PO and IV	Glutathione conjugate	120-180 min.	Hepatic (veno-occlusive disease)	Chronic Myeloid Leukemia and myeloablative regimens
	*Treosulfan ^(10,11)	PO and IV	Active mono- and diepoxide	100-120 min.	Myelosuppression	Ovarian cancer
Platinum coordination complexes	Cisplatin ^(1,3)	IV	-	25-50 min. e 1440 min.	Ototoxicity, nephrotoxicity	Ovarian, testicular, head and neck and bladder cancer
	Carboplatin ^(3,5)	IV	-	120 min.	Myelosuppression	Ovarian and lung cancer

PO: oral route
IV: intravenous
*: prodrugs

1. Puyo et al., 2014
2. Ghabili et al., 2011
3. Brunton et al., 2012

4. Nath et al., 2014
5. Dubbelman et al., 2013
6. Cheson e & Rummel, 2009

7. Wang D & Wang H, 2012
8. Lowenberg et al., 2014
9. Diez et al., 201

10. Brink et al., 2014
11. Galaup & Paci, 2013

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