Anticancer agents

THE ALKYLATING AGENTS

The alkylating agents are the oldest group of cytostatic drugs.
The first of them – mechlorethamine was introduced into therapy in 1949.

The alkylating agents can contain one or two alkylating groups.
In the body the alkylating agents may react with DNA, RNA and proteins, although their reaction with DNA is believed to be the most important.

- The primary target of DNA coross-linking agents is the actively dividing DNA molecule.

- The DNA cross-linkers are all extremly reactive nucleophilic structures (δ^+).

- When encountered, the nucleophilic groups on various DNA bases (particularly, but not exclusively, the N^7 of guaninę) readily attack the electrophilic drud, resulting in irreversible alkylation or complexation of the DNA base.

- Some DNA alkylating agents, such as the nitrogen mustards and nitrosoureas, are bifunctional, meaning that one molecule of the drug can bind two distinct DNA bases.

- Most commonly, the alkylated bases are on different DNA molecules, and intrastrand DNA cross-linking through two guaninę N^7 atoms results.

- The DNA alkylating antineoplastics are not cel cycle specific, but they are more toxic to cells in the late G_1 or S phases of the cycle.

- This is a time when DNA unwinding and exposing its nucleotides, increasing the Chance that vulnerable DNA functional groups will encounter the nucleophilic antineoplastic drug and launch the nucleophilic attack that leads to its own destruction.

- The DNA alkylators have a great capacity for inducing toth mutagenesis and carcinogenesis, they can promote caner in addition to treating it.

- Organometallic antineoplastics – Platinum coordination complexes, also cross-link DNA, and many do so by binding to adjacent guanine nucleotides, called disguanosine dinucleotides, on the single strand of DNA. This leads to intrastrand DNA cross-linking.

- The anionic phosphate group on a second strand of DNA stabilizes the drug DNA complex and makes the damage to DNA replication irreversible.

- Some organometallic agents also damage DNA through interstrand cross-linking.

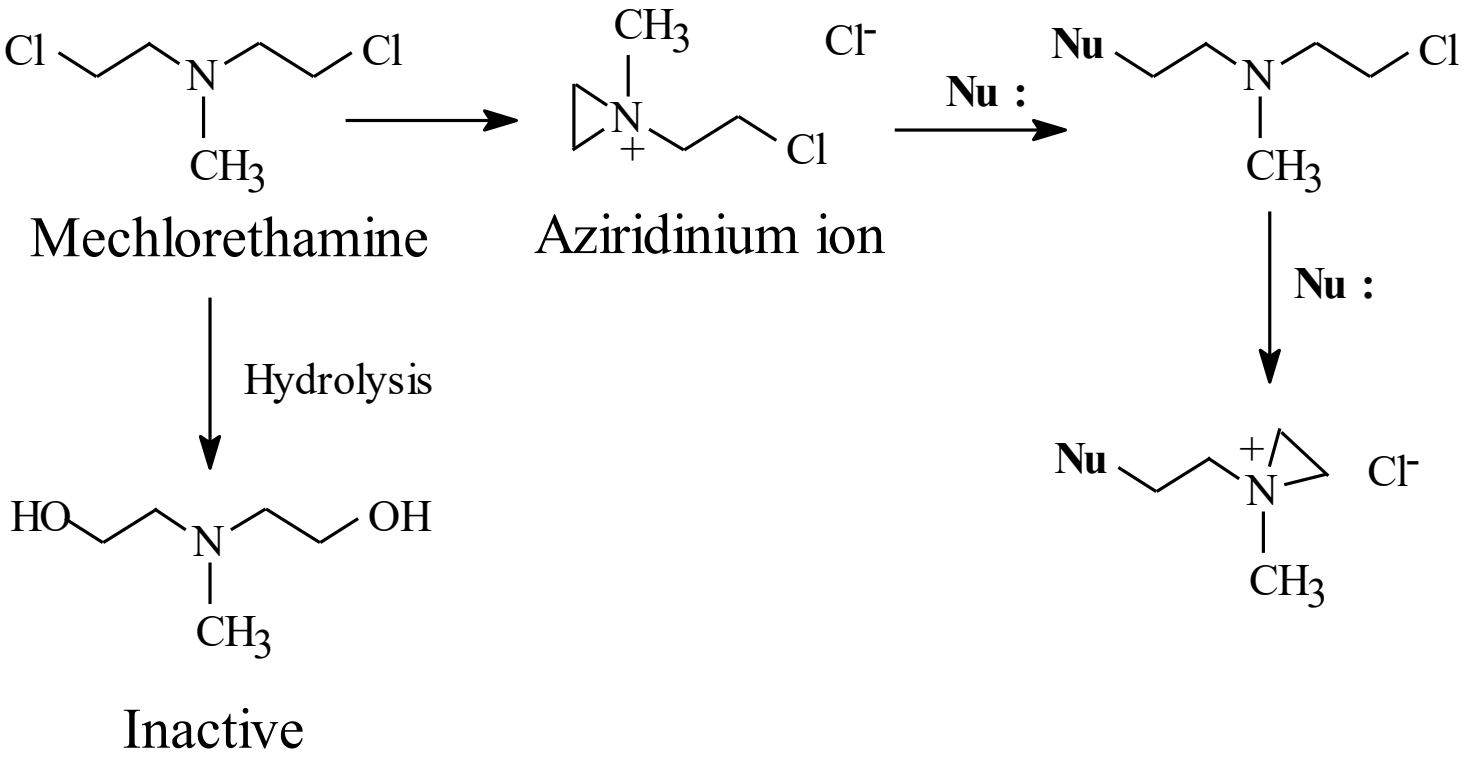
The most common site of alkylation is the N-7 position of guanine.

Other sites on the DNA bases (guanine, adenine, thymine or cytosine) or the phosphate oxygens of the DNA backbone may also be alkylated.

Bifunctional alkylating agents can produce inter- and intra-strand cross-links.

Inter-strand links, which form from mechlorethamine aziridinium ions, prevent DNA separation and are cytotoxic.

Bifunctional alkylating agents do not necessarily produce inter-strand cross-links. Alkylation of the N-7 position of guanine can result in the formation of an apurinic site.

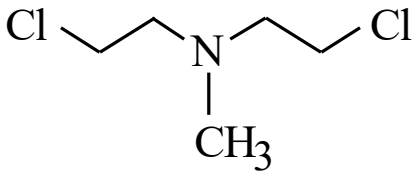


The alkylating agents appear to be the most effective in the G1 or S phase.

The alkylatig agents are classified as follows:

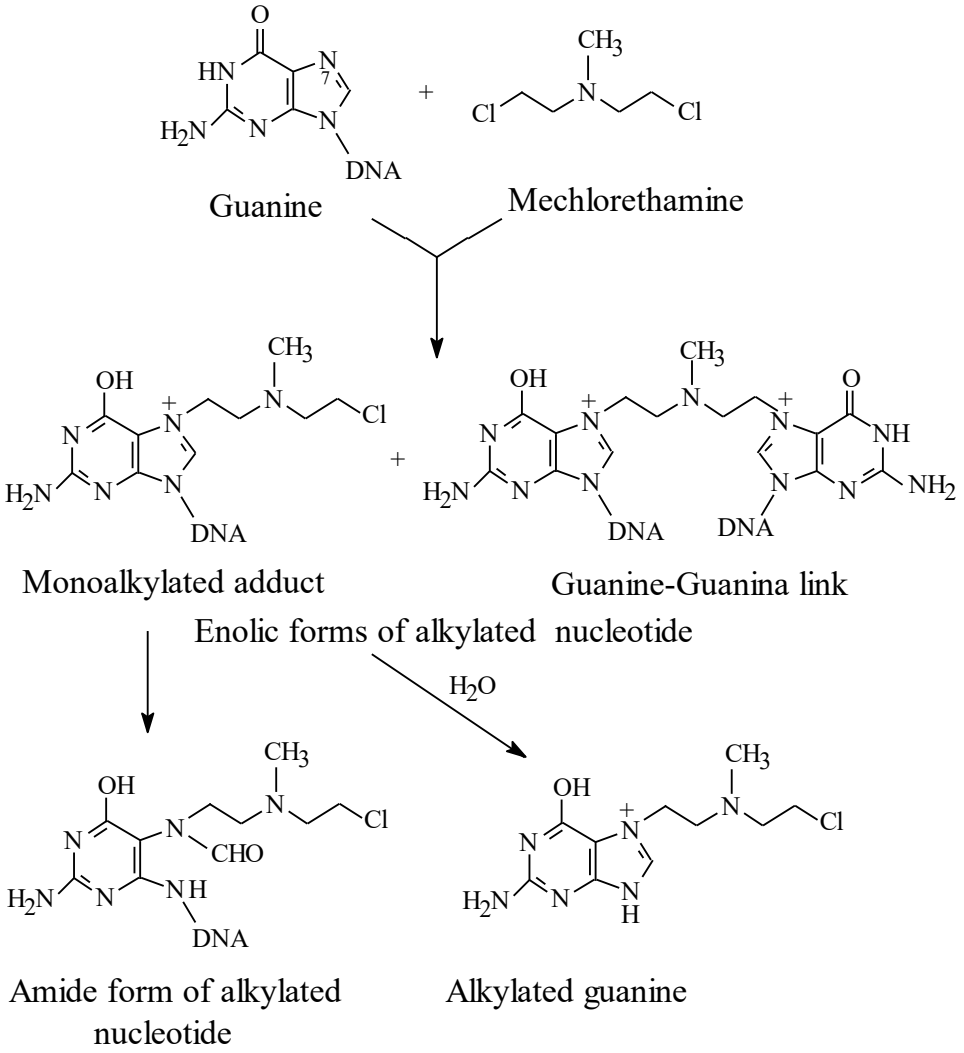
- β-Chlorethylamine derivatives
- Ethyleneimine derivatives (aziridines)
- Methanesulfonic esters of aliphatic diols
- Nitrosourea derivatives
- Other alkylating agents.

β-Chlorethylamine derivatives



Chlormethine,
Mechlorethamine,
NITROGRANULOGEN
N-Methyl-bis(2-chloroethyl)amine

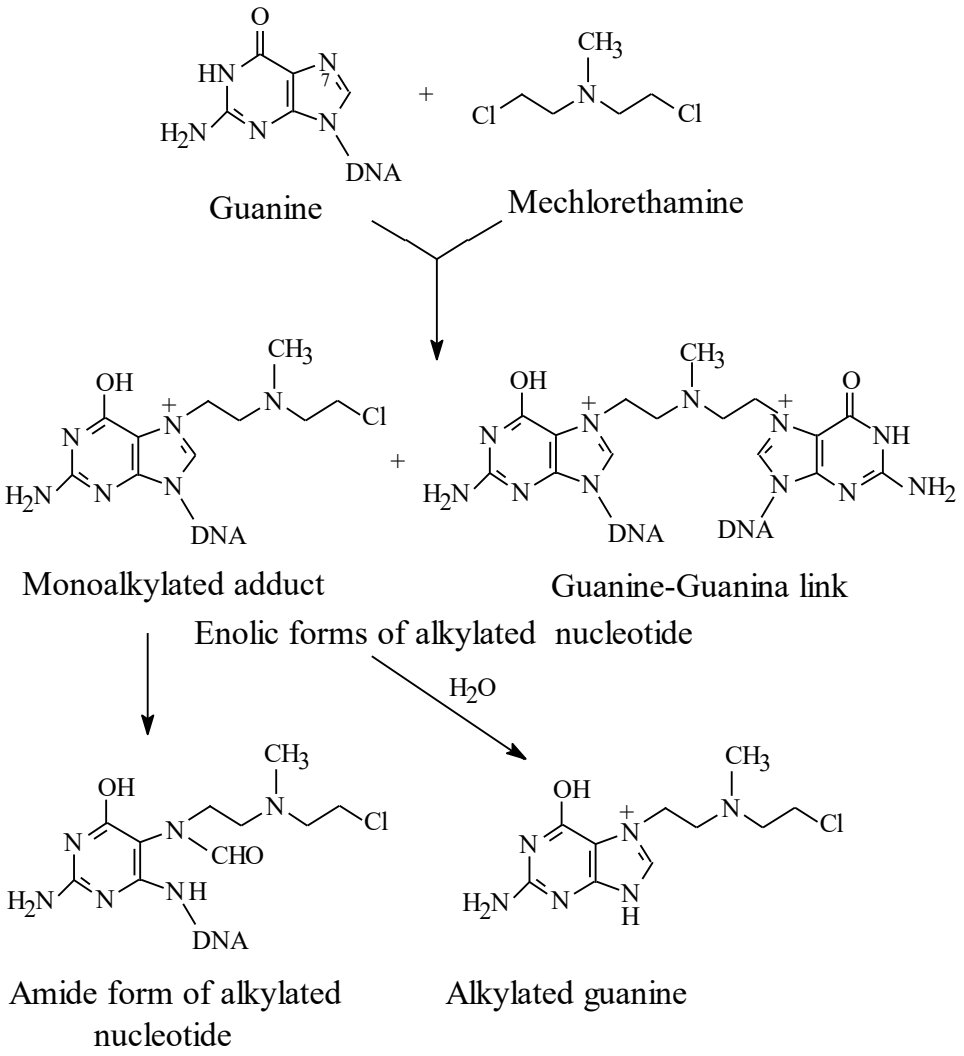
Chlormethine is a bifunctional alkylating agent which forms intra-strand cross-links.



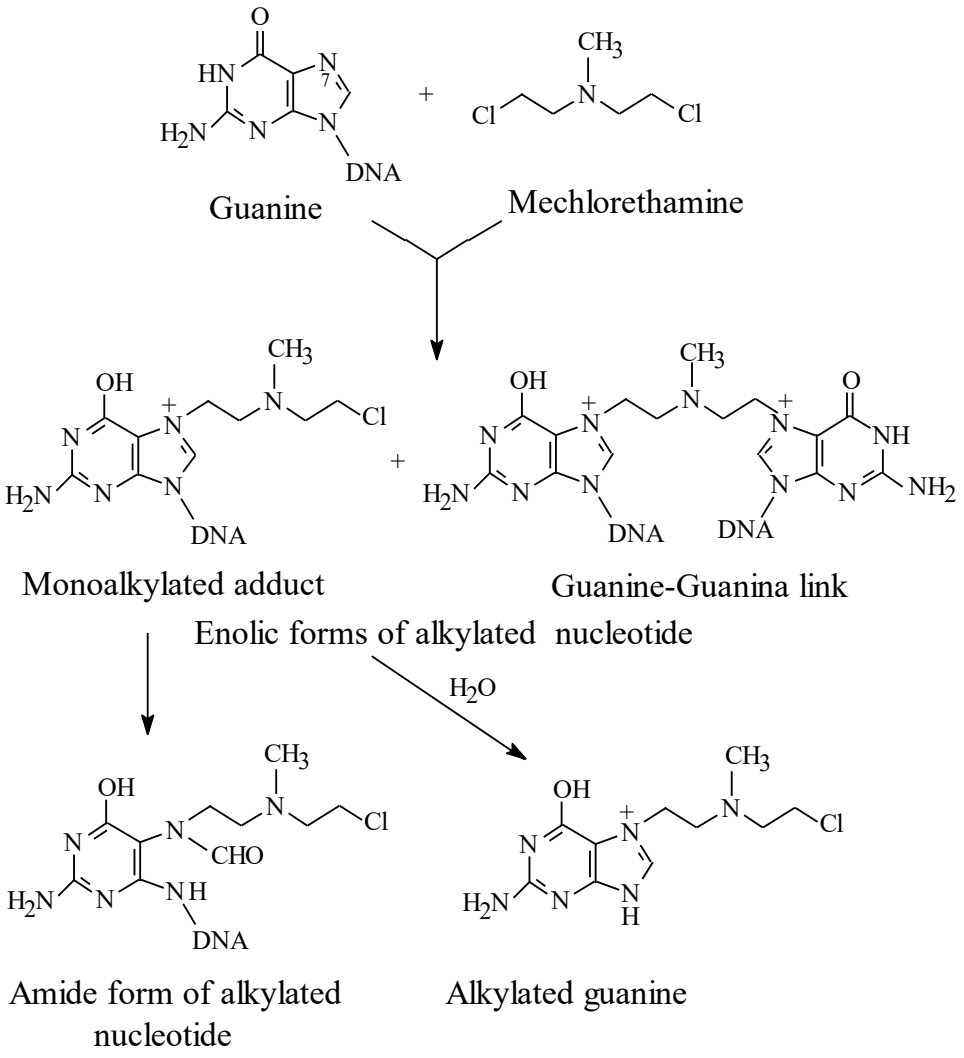
One of the characteristic properties of neoplastic cells is that they develop faster than healthy cells. This results in increased glycolysis, an elevated concentration of lactic acid and decreased pH in neoplastic cells.

The elevated concentration of hydrogen ions in neoplastic cells facilitates nucleophilic substitution and conversion of chlormethine to a very active aziridinium ion which alkylates guanine. Guanine bases, in keton form, are connected with cytosine by the hydrogen bridge.

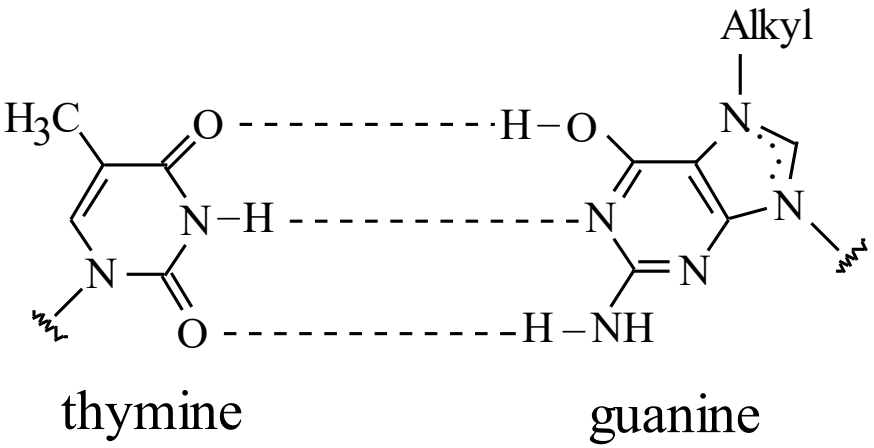
The alkylation of guanine at position N-7, in the imidazol ring, which is rich in π-electrons, increases its acidity and causes its transformation to enolic form.



The alkyl rest at the N-7 atom of guanine causes its quaternization resulting in the decreasing stability of the imidazol ring, its hydrolysis at position C-8 and transformation of the cyclic aziridinium ion to a formamide derivative. The hydrolysis of the nucleotide structure and separation of the alkylated guanine from DNA is also possible.



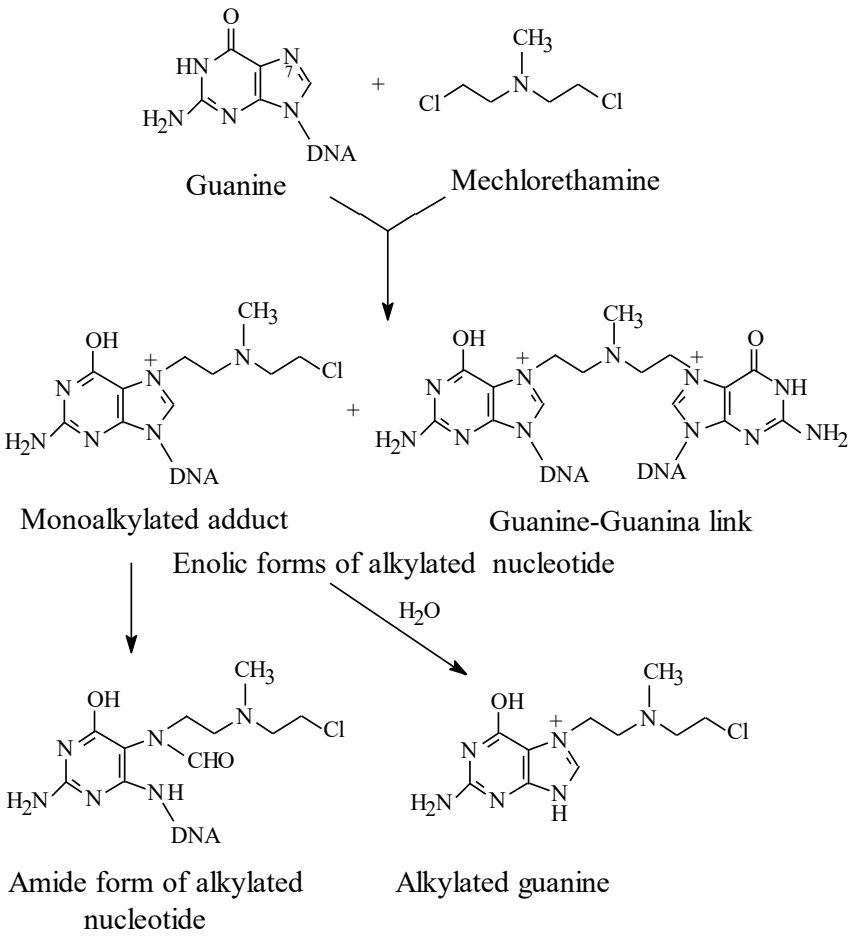
A repair process of DNA is more difficult when guanine is alkylated at position O-6. The O_6 and N_7 of the alkylated guanine link with thymine instead of cytosine during replication.



The elongated time of O_6-alkylguanine action can lead to the damage of the cell genome.

Bifunctional chlormethine can also alkylate the second molecule of guanine in DNA, which results in the covalent connection of two nucleophilic centers of the DNA helix.

Such a cross-link is responsible for the cytotoxicity of the bifunctional alkylating agents, because the replication of the cross-link DNA strands is impossible. It is caused by the inhibition of DNA-dependent DNA polymerase.



- The application of chlormethine in therapy is limited because of its high toxicity, which is caused by the strong basic character of this drug.

- Mechlorethamine is marketed in hydrochloride salt form to provide water solubility for intravenous or intracavitary administration.

- The strong electron-withdrawing effect of the two chlorine atoms reduces the pKa of mechlorethamine to 6.1, which gives a ration of un-ionized to ionized drug forms of approximately 20:1 at pH 7.4.

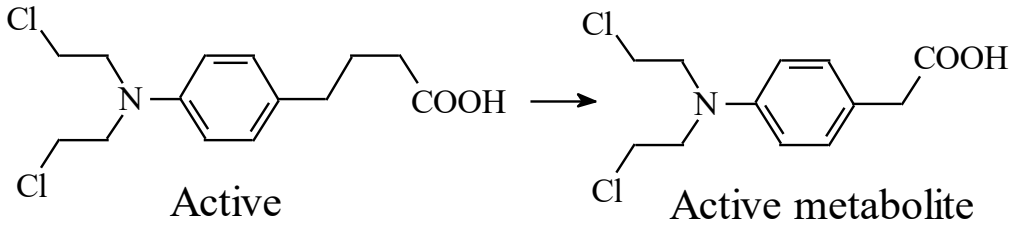
- This agent is too reactive for oral administration and too toxic to use alone. In addition to sever nausea and vomiting, myelosuppression, and alopecia, it can cause myelogenous leukemia with extend use due to its mutagenic/carcinogenic effects on bone marrow stem cells.

- Mechlorethamine is still used in regiments for cancers of the blood (Hodgkin’s disease, chronic myelocytic leukemia, chronić lymphocytic leukemia. It is also used in the treatment of microcellular pulmonary carcinoma.

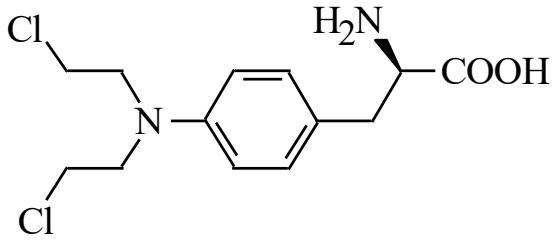
- A correlation between the alkalinity of the alkylating agents and their toxicity is observed.

Chlorambucil and melphalan are weaker bases, so they demonstrate lower toxicity.

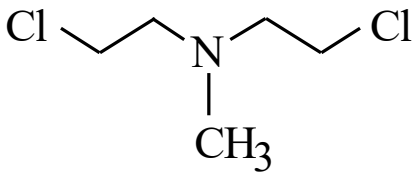
Chlorambucil is metabolised to 4-(bis-2-chloroethyl)amino]-phenylacetic acid (its main metabolite), which is active.



Chlorambucil, LEUKERAN

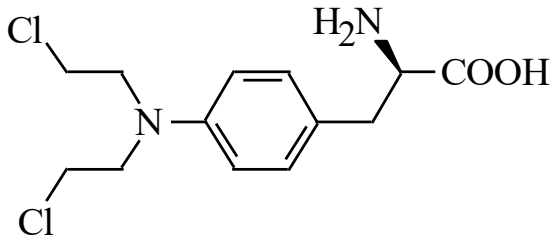


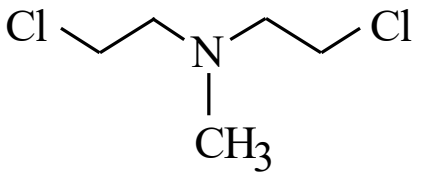
Melphalan, ALKERAN



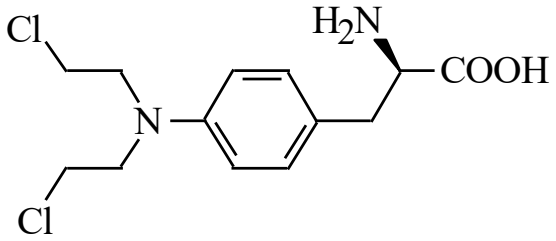
Chlormethine

A decrease in alkalinity is possible as a result of the connection of the alkylating group with a benzene or oxazophosphorine ring, which are rich in π-electrons.





Melphalan



- This aromatic mustard, used primarily in the treatment of multiple myeloma, is able to stabilize the lone pair of electorns on the mustard nitrogen through resonance with the conjugated phenyl ring, slowing the formation of the reactive aziridinium ion.

- The L-isomer of the amino acid phenylalanine (L-Phe) was purposefully incorporated into this antineoplastic agent because naturally occuring L-amino acids are preferentially transported into cells by the action of specific amino acid carrier proteins.

- L-Phe would act as homig device and actively transport the toxic mustard inside the tumor cells, but some studies indicate that melphalan enters cells through facilitated diffusion rather than by active transport.

Melphalan

- Is also mutagenic and can induce leukemia
- Is orally active but absorption can be erratic (absorption is decreased with food)
- The hydrochloride salt is available for intravenous administration but the risk of serious side effects is higher
- Is distributes into body water, toxicity can be pronounced in hehydrated patients or in those with renal dysfunction

Chlorambucil

- Has good oral bioavailability and the potential to induce nonlymphocystic leukemia
- Undergoes beta-oxidation to an active phenylacetic mustard metabolite, which is responsible for some of the observed antineoplastic activity

- Is used in the palliative treatment of chronić lymphocytic leukemia, malignant lymphoma and Hodgkin disease.

Bendamustine hydrochloride

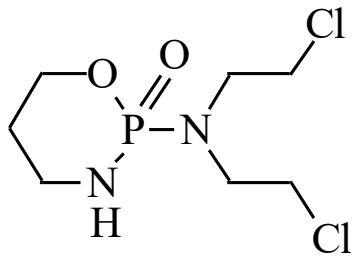
- Is the N-methylbenzimidazole analog of chlorambucil and the substitution of this purine-like aromatic ring was purposefully done to promote an antimetabolite mechanism along with DNA alkylation

- DNA damage is more extensive and less repairable than that induced by other alkylating agents

- Is given only intravenously on days 1 and 2 of a 21-day or 28-day cycle

- It may cause myelosuppresion, hypersensitivity/anaphylaxis and skin reactions
- Pretreatment with antihistamines and corticosteroids can help minimize infusion reactions, a major cause of drug discontinuation.

Cyclophosphamide, iphosphamide, trophosphamide and maphosphamide are tetrahydro-2*H*-1,2,3-oxazaphosphorine-2-oxide derivatives.



Cyclophosphamide, ENDOXAN

2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide

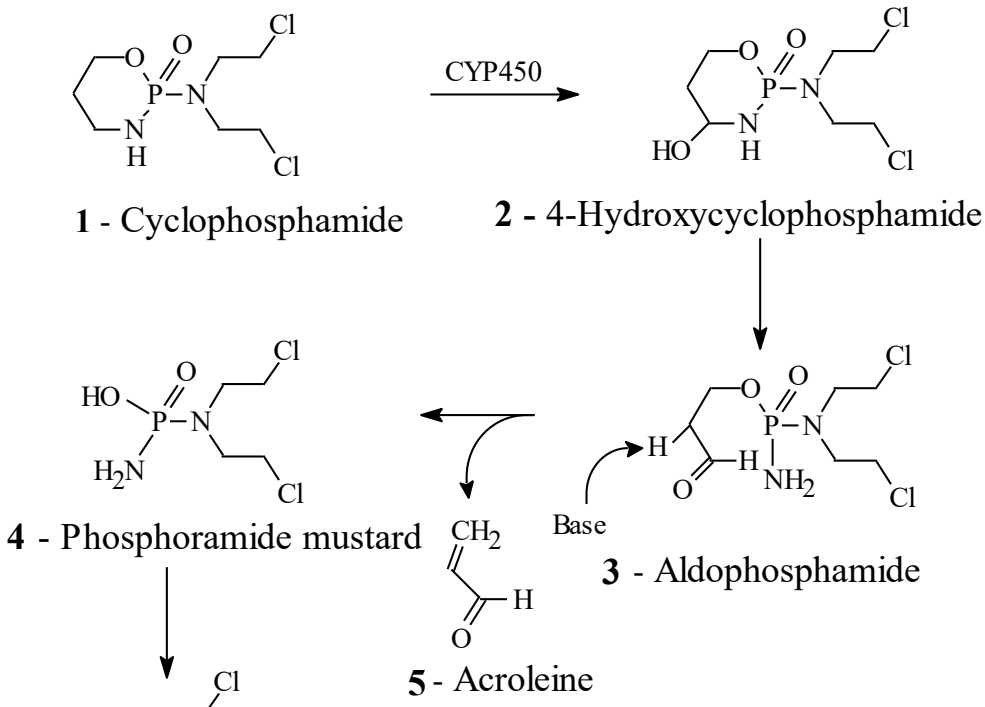
Cyclophosphamide shows a cytostatic and immunosupressive activity.

It is a bioprecursor with latent alkylating activity. Chlorambucil is metabolized to an active metabolite.

The metabolism of cyclophosphamide occurs in the microsomes of the liver under the influence of oxygenases.

Cyclophosphamide (**1**) is oxidised to 4-hydroxy-cyclophosphamide (**2**), which is in equilibrium with aldophosphamide (**3**).

This unstable compound transforms to toxic phosphoramide mustard (**4**) and acroleine (**5**).



Compound **6** has strong nucleophilic properties and forms an aziridinium ion (**7**), which alkylates DNA.
Acroleine, formed during the biotransformation of cyclophosphamide, is also toxic and active and plays some role in the inhibition of tumor development.

- Acrolein generated during the formation of phosphoamide mustard, is a very electrophilic and highly reactive species and causes extensive damage to cells of the kidney and bladder.
- Bladder damage from acrolein results from attack by Cys SH group at δ^+ terminal carbon of the acrolein sturucture, resulting in renal cell alkylation and cell death. Physiological results can include severe hemorrhage, and on occasion, induction of bladder cancer.
- Acrolein also damages the nephron, particuraly when used in high doses, in children, in patients with one kidney, or when coadministered with other nephrotoxic agents.

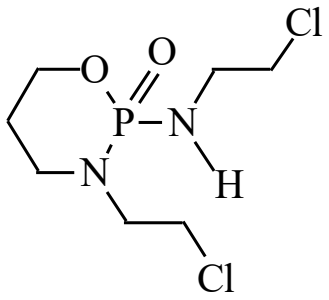
Mesna - its SH group competes with cysresidues for the alkylating acrolein.

Mesna concentrates in the bladder and will prevent damage to those cell.

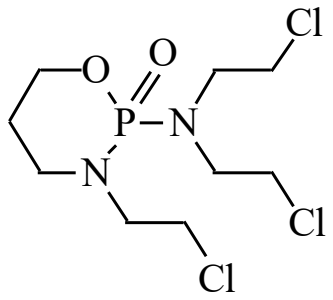
It does not concentrate to any appreciable extent in the nephron and, therefore, is not good protection against cyclophosphamide-induced nephrotoxicity.

Cyclophosphamide

- Is used in combination with other antineoplastic agents to treat a wide range of neoplasms, including leukemias and malignant lymphomas, multiple myeloma, ovarian adenocarcinoma and brest cancer

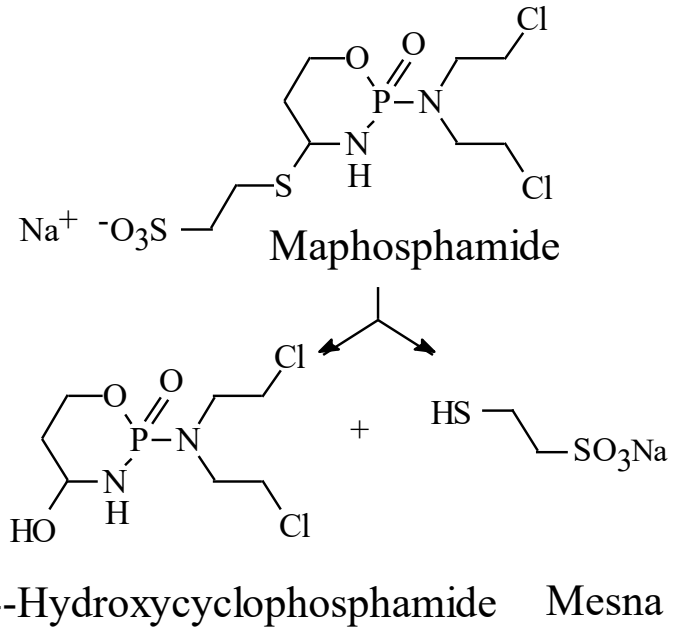


Ifosphamide, HOLOXAN



Trophosphamide, IXOTEN a new chiral oxazaphosphorine

Maphosphamide is metabolised to active *cis*-4-hydroxy-cyclophosphamide and mercaptoethylsulfonate sodium (mesna).

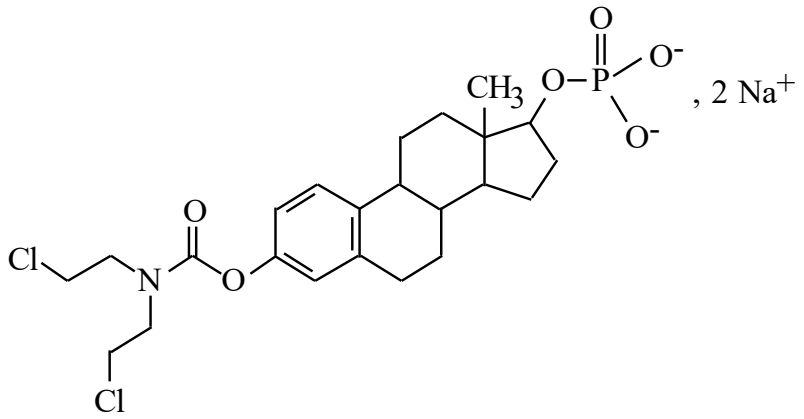


Mesna is used as a complementary agent during therapy with cytostatic drugs.

Ifosfamide -cyclophosphamide

- During the bioactyvation – acrolein is generated the same precautions must be taken
- Is currently used as third-line therapy in testucular cancer

- Patients on ifosfamide commonly exhibit cerebral neuropathy atrributed to the significantly higher levels of chloracetaldehyde generated by this drug



Estramustine phosphate, ESTRACYT

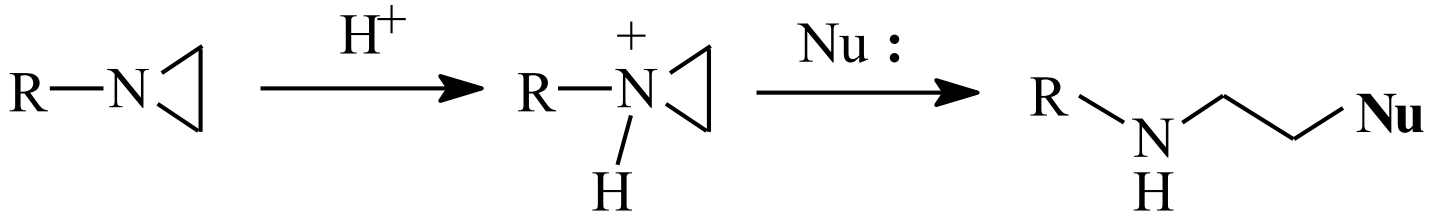
The preparation ESTRACYT is used in advanced prostate carcinoma.

Estramustine phosphate, after oral administration, is rapidly dephosphorylated during absorption.

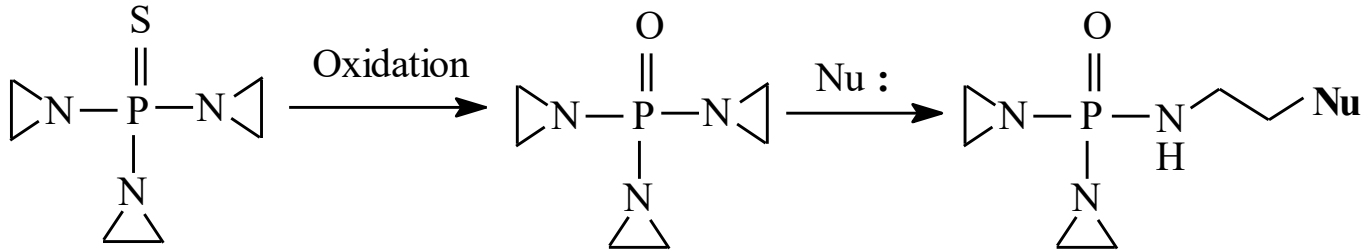
The major metabolites of estramustine phosphate in plasma are estramustine, an estrone analogue of estramustine, estradiol and its oxidation product, estrone.

Ethylenimine derivatives (aziridines)

Aziridine is a three-membered nitrogen heterocycle that reacts with nucleophiles in order to relieve ring strain.
In acidic pH, the aziridine group is protonated to provide a reactive aziridin ion that is known to alkylate DNA.
At physiologic pH, aziridine with a pKa of approximately 6 is primarily in the free base form that is less reactive as an alkylating species.



Thiotepa is a pro-drug with latent alkylating properties. Metabolic desulfurylation of thiotepa produces a toxic metabolite, TEPA (triethelenephosphoramide).



Thio-Tepa, *Thiophosphamid*

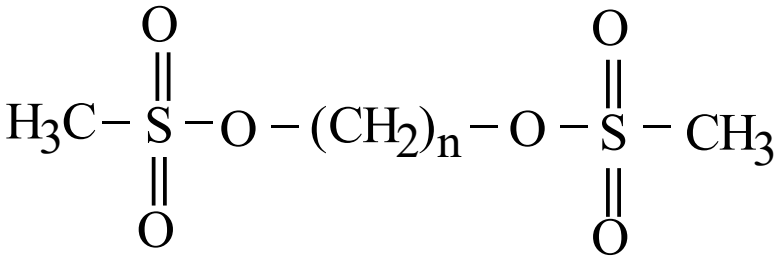
Tepa

Thiotepa is highly effective in multidrug chemotherapy of various tumors. At present, it is sometimes used in the form of infusion into body cavities for decreasing neoplastic exudates.

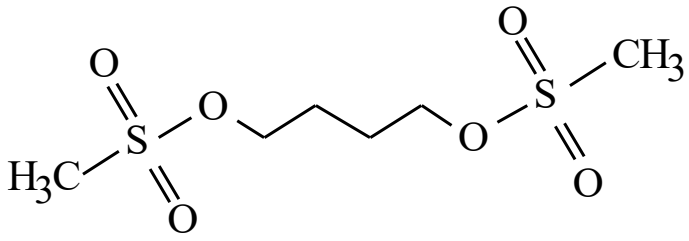
Aziridinequinone derivatives such as inproquone, diaziquone and triaziquone are rarely used in therapy now.

Methanosulfonic esters of aliphatic diols

Bifunctional alkylating properties are demonstrated by compounds represented by the following general formula:

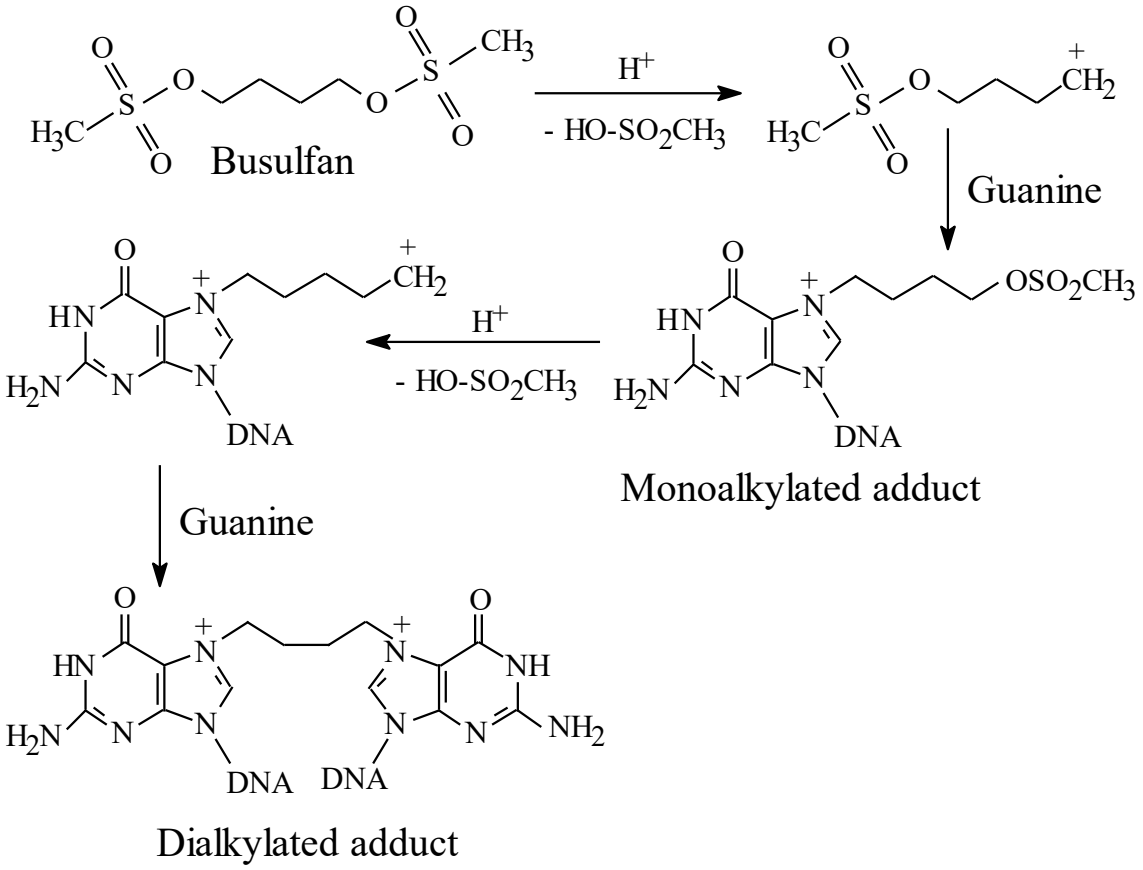


Compounds where n = 4 show the greatest activity.
Busulfan and treosulfan are examples of such drugs.



Busulfan, BUSULFAN, MYLERAN
Butan-1,4-diol di(methansulphonate)

Busulfan, in the acidic pH of neoplastic cells, forms carbocation, which alkylates the N-7 atom of guanine, forming mono- and dialkylated adducts.

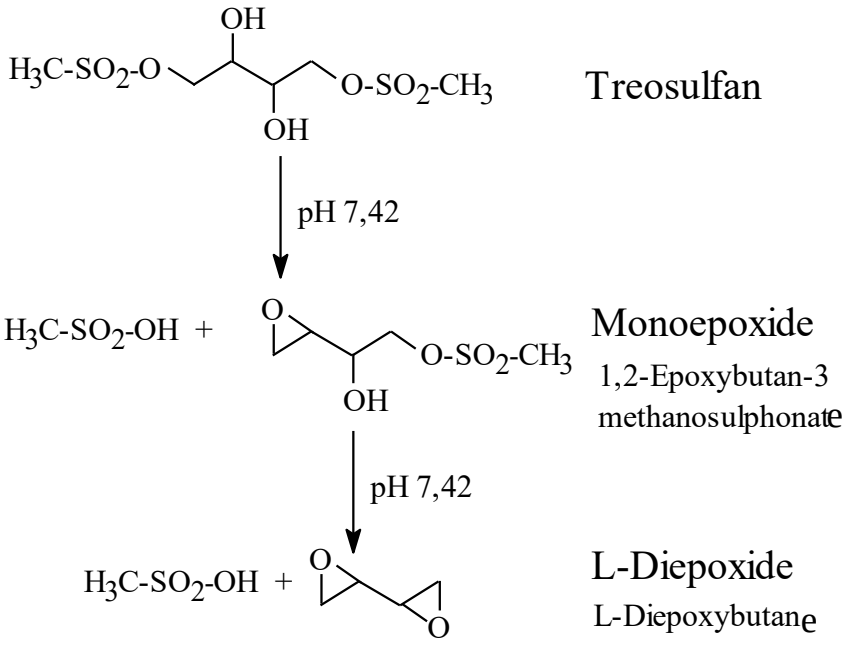


Busulfan is recommended in the treatment of chronic myelocytic leukemia.

Treosulfan is a dihydroxy derivative of busulfan and it is a bifunctional alkylating agent. Treosulfan is inactive.

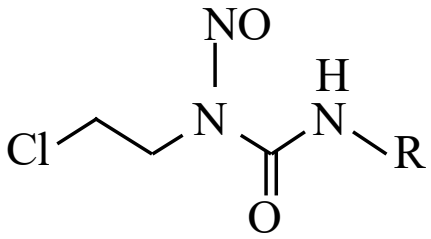
At physiologic pH (pH 7.4, 37 ^{0}C) active mono- and diepoxide derivatives (nonenzymatic reaction of nucleophilic substitution) are formed.

The alkylation of the N-7 atoms of guanine by mono- and diepoxide with formation of cross-link results in the antiproliferative and cytotoxic activity of treosulfan.



Treosulfan is recommended for the therapy of ovarian carcinoma, when the standard therapy with platinum compounds is ineffective.

Nitrosourea derivatives



Carmustine, R = -CH_2-CH_2-Cl

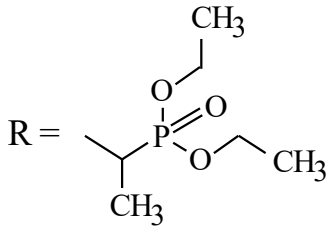
CARMUBRIS, NITRUMON
N,*N*-Bis(2-chloroethyl)-*N*-nitrosourea

Lomustine, R = cyclohexyl; BELUSTINE, LUCOSTINE

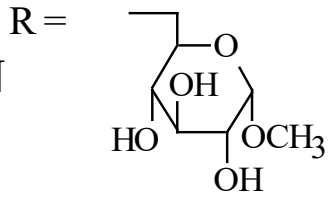
Semustine, R = 4-methylcyclohexyl

Tauromustine, R = -CH_2-CH_2-SO_2-$N(CH_3)_2$; TAURICYT

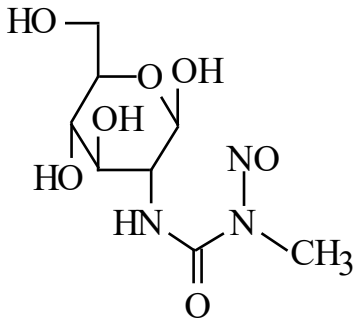
Fotemustine, MUSTOPHORAN



Ranimustine, THYMERIN



Streptozocin, ZANOSAR

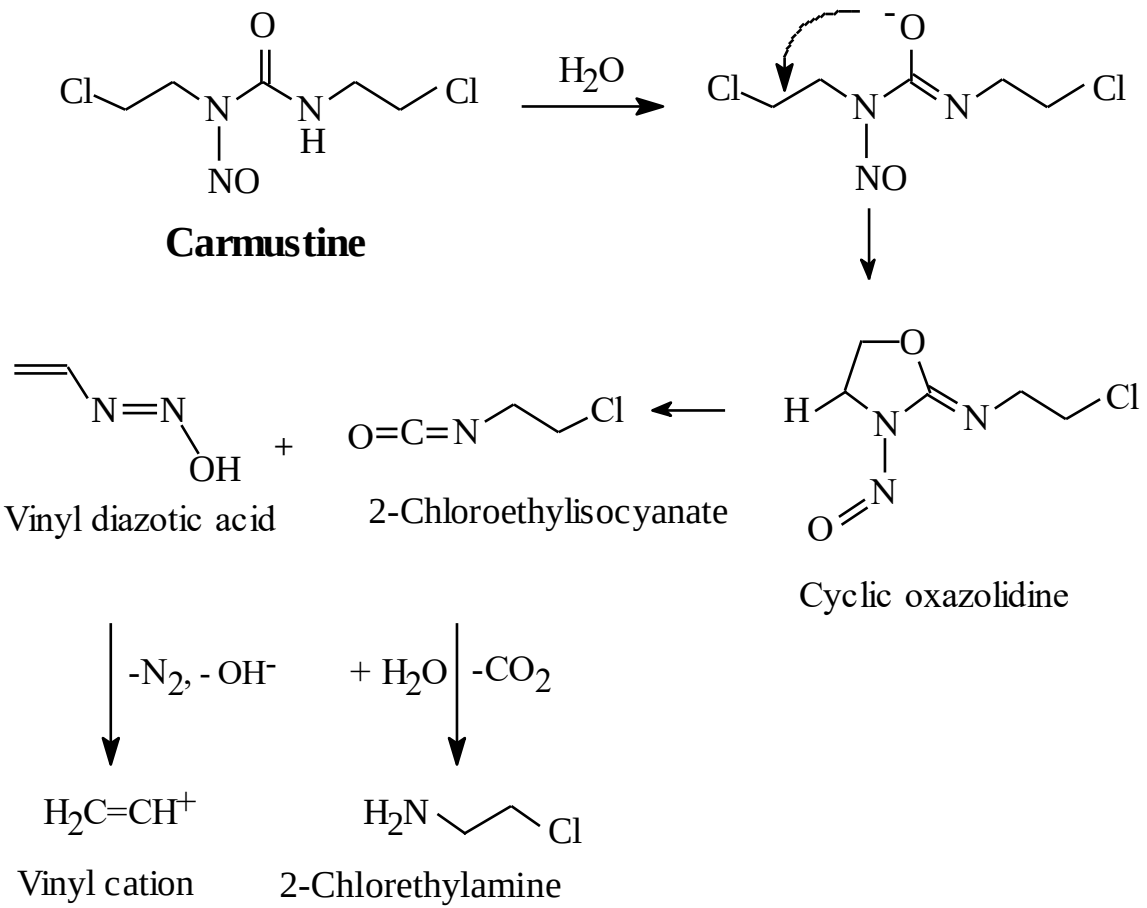


The mechanism of action

Nitrosourea derivatives are fairly reactive with water, similarly to the reactivity of the mustards and decompose as shown in Figure 75.6.

In water the urea NH is deprotonated and the negatively charged oxygen then displaces the chloride to give a cyclic oxazolidine.

This intermediate then fragments to a vinyl diazohydroxide and 2-chloroethylisocyanate.



The former species is very reactive and loses nitrogen forming the even more reactive vinyl cation, the ultimate alkylating species.

The isocyanate is also reactive and in water yields 2-chlorethylamine, an additional alkylating agent.

- The alkylating properties of nitrosourea derivatives are very strong.

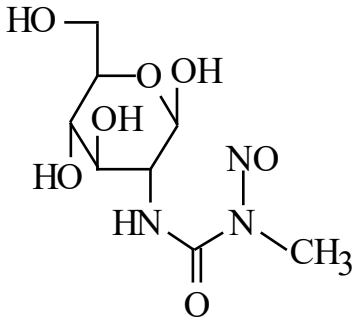
- They kill neoplastic cells in various phases, also in the G_0 phase. It is believed that they not only alkylate DNA and RNA but alternatively also inhibit some enzymes participating in the synthesis of nucleic acids as a result of the carbamoilating of proteins.

- Carcinomas resistant to other alkylating agents do not show cross-restinance to nitrosourea derivatives.

- Nitrosourea derivatives demonstrate highly lipophilic properties, so they easily permeate the blood-brain barrier and because of that they are used in primary and metastatic brain tumors.

- Nitrosourea derivatives are also used in Hodgkin's lymphoma and multiple myeloma.

Streptozocin is composed of a glucopyranose amino sugar and a nitrosourea. It is recommended for the therapy of pancreatic carcinoma.



Nitrosourea derivatives (carmustine, lomustine) cause bone marrow depression.

The use of **streptozocin** causes hepatotoxicity and nephrotoxicity.

Fotemustine demonstrates lower hepatoxicity. It is indicated in the treatment of such malignant neoplasms as primary brain tumor and melanoma (also with metastasis into the CNS).

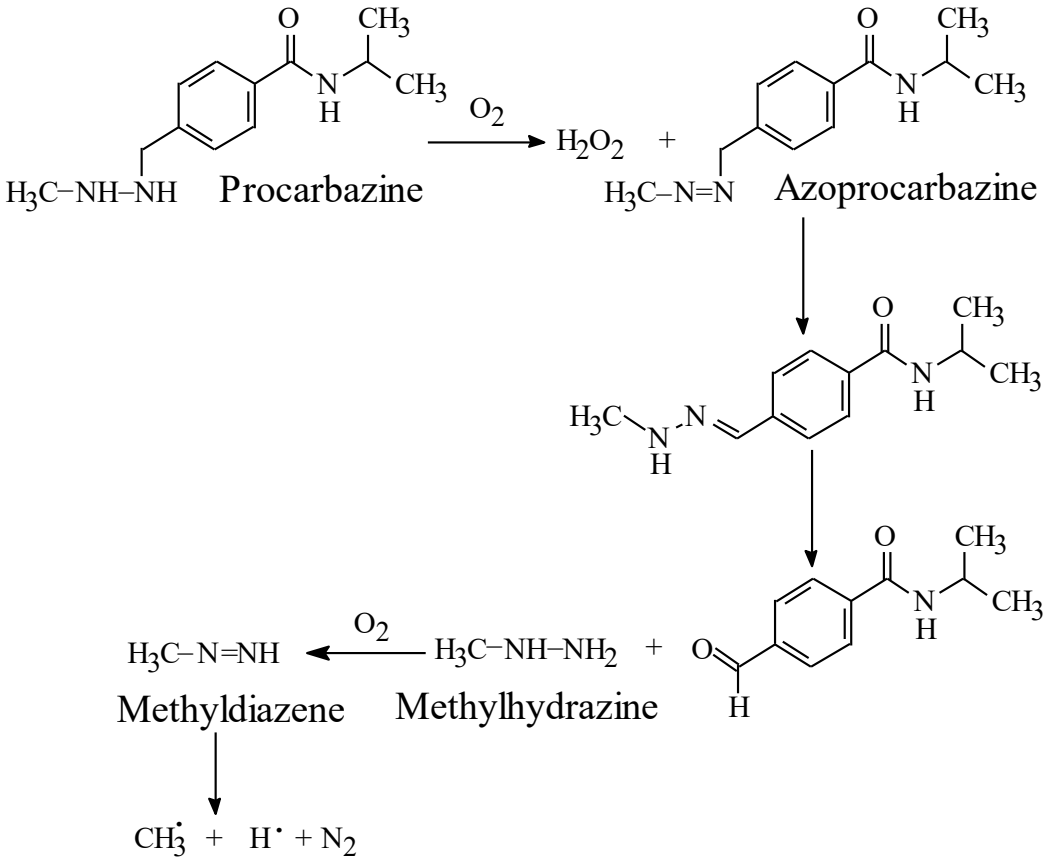
Many neoplastic cells have steroid hormone receptors. It is thought that a combination of nitrosourea derivatives with estrogens or androgens is better than the combination of bis-chlorethylamine with estradiol (estramustine) or prednisone (prednimustine).

Other alkylating agents

Alkylation of DNA can also occur by way of free radical intermediates.

As in the case of substituted hydrazines.

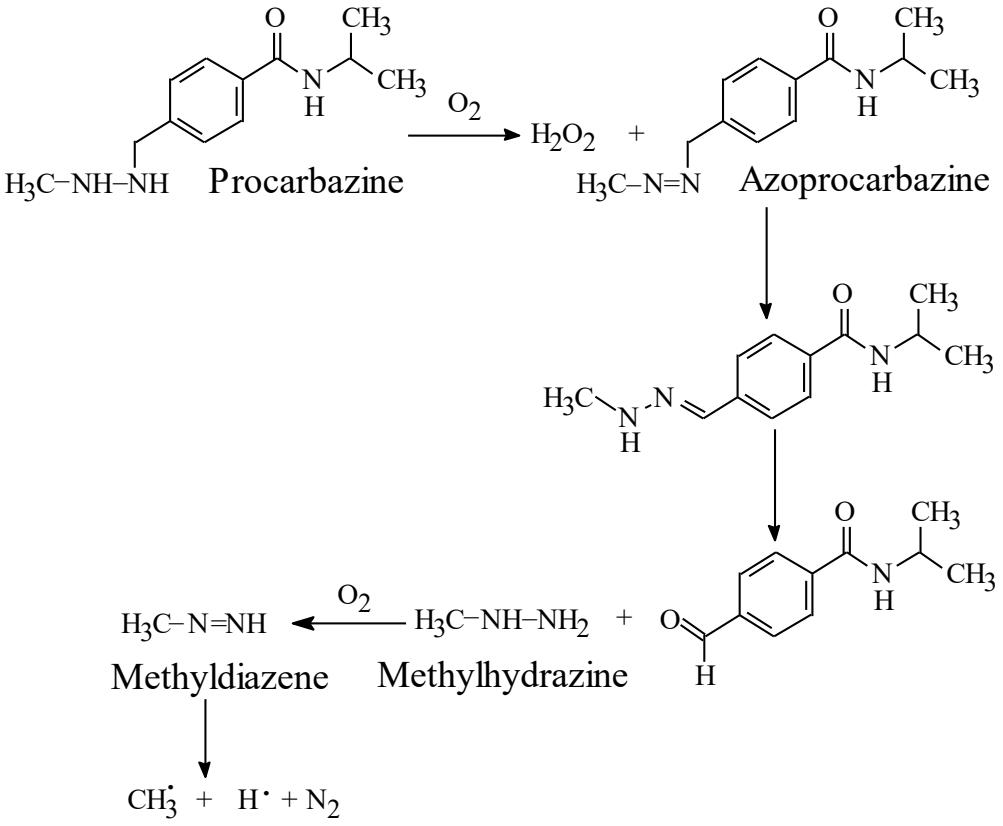
An example is **procarbazine** which is used to treat Hodgkin's disease.



At physiological pH and in the presence of oxygen, procarbazine decomposes by an autooxidation pathway with release of hydrogen peroxide.

The azo derivative formed during oxidation undergoes hydrolysis to yield a benzaldehyde derivative and methylhydrazine.

Methylhydrazine oxidation, which occurs both *in vitro* and *in vivo*, produces methyldiazene and then methyl radical.



Methyl hydrazine has been shown to methylate RNA and DNA.

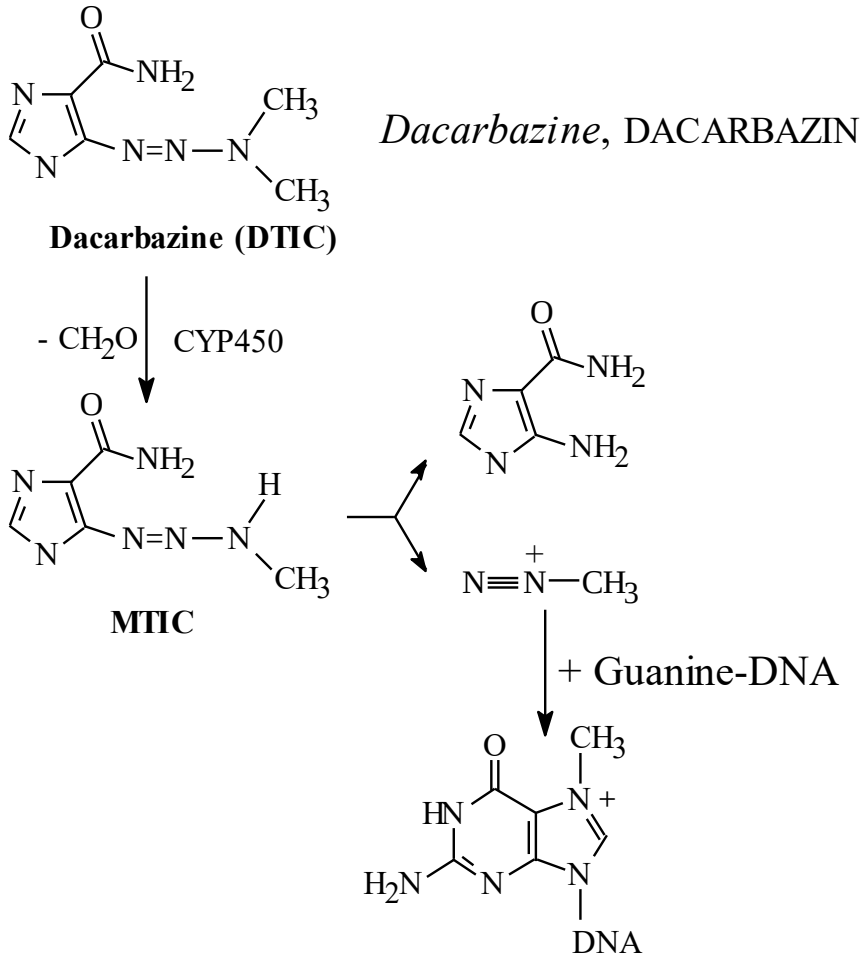
In DNA, methylation occurs on the C-8 position of guanine.

Procarbazine inhibits enzymes involved in alcohol metabolism (disulfiram-like reaction) and catecholamine metabolism (inhibits MAO).

Drug interactions with procarbazine and sympathomimetic drugs, tricyclic antidepressant agents, and other drugs and food rich in tyramine are possible.

Procarbazine demonstrates many adverse effects – GI disturbance, hepatotoxicity, nephrotoxicity and neurotoxic disturbance.

Dacarbazine is a dimethyl triazenyl imidazole carboxamide (DTIC).



DTIC is a pro-drug which is metabolically bioactivated through a series of reactions involving CYP450.

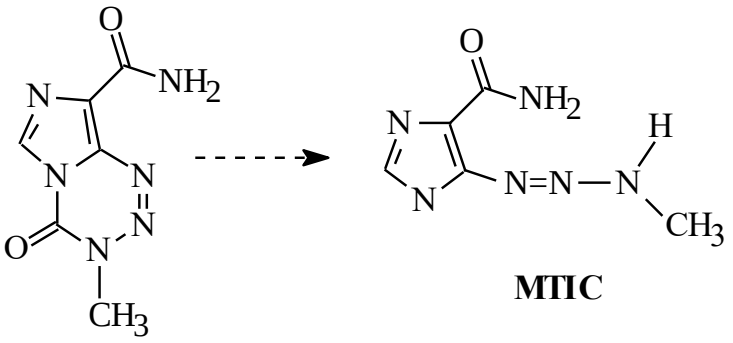
Initial demethylation to MTIC is followed by formation of diazomethane, a potent methylating agent.

Diazomethane in turn is capable of methylating the N-7 position of guanine (Fig. 75.8).

Indications for using dacarbazine are Hodgkin's lymphoma and metastatic malignant melanoma.

Proposed metabolism and mechanism of action of DTIC

Temozolomide is a pro-drug which is nonenzymatically converted into MTIC which then alkylates DNA in a manner similar to that of DTIC.



MTIC

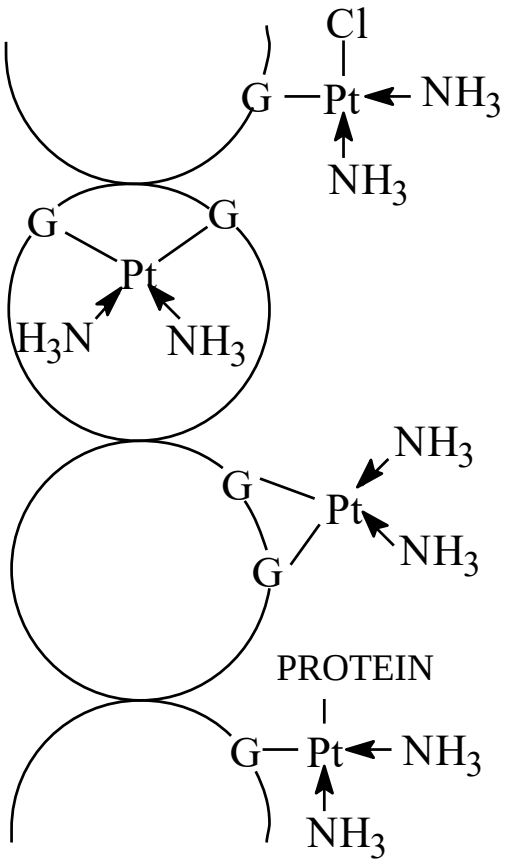
Temozolomide, TEMODAL

A major advantage of temozolomide over DTIC is the fact this drug is administered orally and is rapidly absorbed via this route.

Temozolomide crosses the blood-brain barrier and it has been approved for the treatment of brain tumors.

Platinum complex compounds

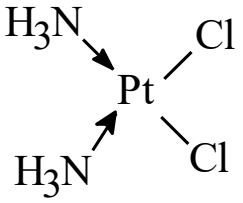
The action of platinum complex compounds, similarly to other alkylating agents, appears to be associated with the ability of these drugs to alkylate the N-7 position of guanine forming intrastrand and interstrand cross-links.



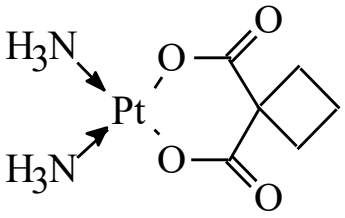
Binding of platinum complex compounds with DNA.

Platinum complex compounds, which demonstrate cytostatic and immunosupressive action, have the following chemical structure: two of the four ligands must be chemically active and have a *cis* configuration; the other two ligands (mostly amino groups) have to be neutral and inactive.

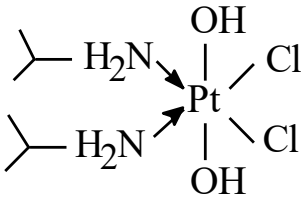
The general formula is:
(amine)$_2$PtX$_2$, in which
X = active functional groups, for example Cl^-, SO_4^{2-}, oxalate, malate.



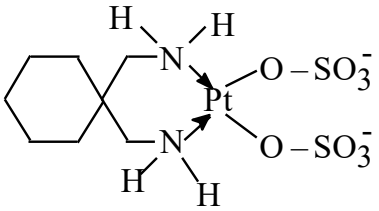
Cisplatin, PLATIDIAM



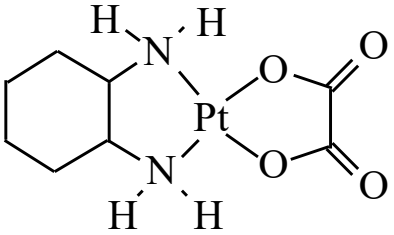
Carboplatin, CYCLOPLATIN



Iproplatin



Spiroplatin



Oxaliplatine, ELOXATINE

The first drug of this group, cisplatin, was introduced into therapy in the early 1970s.

Platinum complex compounds demonstrate activity against neoplastic cells in the G_0 phase.

Cisplatin shows high potency, but it has a low chemotherapeutic index. The *trans* isomer does not form a cross-link with DNA and does not act cytostatically.

A new generation of platinum complex compounds – carboplatin, iproplatin, spiroplatin - demonstrate lower nephrotoxicity and are better soluble in water.

The following are indications to use platinum complex compounds:

- cisplatin and carboplatin – carcinoma of testes, ovaries and bladder, Wilms' tumor, osteogenic sarcoma, carcinomas of the head and neck, especially in combination with other chemotherapeutics

- oxaliplatin – colorectal carcinoma, especially advanced colon and rectal carcinoma, breast and ovary carcinoma, malignant neoplasms of the head and neck.

Platinum complex compounds demonstrate many adverse effects. Their intensity varies between derivatives.

They show nephrotoxicity, neurotoxicity, myelotoxicity, ototoxicity and cause vomiting (cisplatin more strongly than carboplatin and oxaliplatin).