

Lecture 9: DNA cross-linkers and alkylating agents – regarded as non-cell cycle specific

Objectives:

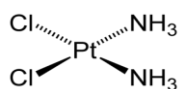
1. Be able to recognize the different DNA cross-linkers and alkylating agents
2. Understand the mechanism of action of several specific agents
3. Understand the important toxicities of several specific agents

DNA is “electron rich” as it contains lots of O and N.

The cross-linkers and alkylating agents are “electron poor”; hence, they react with DNA.

1. Platinum agents (we will discuss 3 of them)

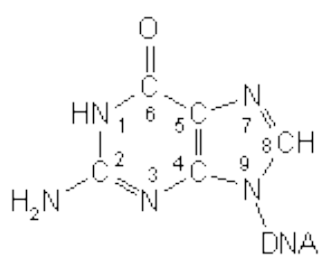
Cisplatin (Platinol): Among oldest, of all anti-cancer agents. Everyone should be able to draw cisplatin. Discovered prior to 1900; it's utility as an anticancer agent realized about 1960. Light sensitive.



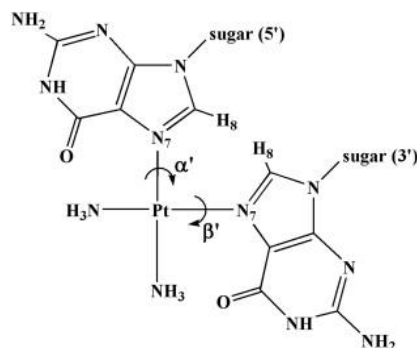
Square planar geometry, not tetrahedral; helps bind to DNA.

Uses: Lung cancer, testicular cancer, ovarian cancer, bladder cancer. Administration is IV only. Dose ranges 20-100 mg/m² (typical human is 1.5-1.7 m²) but dosing is dependent on type of cancer and renal function. All patients should be adequately hydrated prior to and after receiving cisplatin (see toxicities below).

Figure 1: Structure of guanine (left) and cisplatin adduct with two guanines in DNA (right)



Guanine (G)



Mechanism: Reacts with DNA bases, especially nitrogen 7 of guanine (see Figure 1 above). The cis geometry is critical to this molecule; the trans isomer (transplatin) is much less effective.

Toxicities: Quite toxic: nephrotoxicity, ototoxicity, myelosuppression, severe nausea and vomiting (N/V). Warning on vials not to exceed 100 mg/m² for any single cycle of cisplatin. Caution with other drugs that are also nephrotoxic (ifosfamide, cyclophosphamide). Sodium thiosulfate can be administered to reduce nephrotoxicity. Amifostine can be used to reduce ototoxicity.

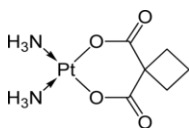
ADME: Plasma t_{1/2} is very short (less than 30 minutes). No real metabolism but “aquation” (see below). The high renal excretion of cisplatin predisposes to renal toxicity. Renal toxicity can impact other drugs that are excreted by the kidneys.

Cisplatin distribution: Outside cells, cisplatin remains largely intact due to high chloride concentration (~100 mM) in blood. Therefore the cisplatin molecule remains intact and neutral, and can penetrate cells. However, inside cells the chloride concentration is much lower (~10 mM). The equilibrium drives water molecules to replace the chloride atoms and the aquated forms are charged and are “locked” inside cells. The aquation steps within cells occur as follows:

1. $\text{Pt}^{\text{II}}(\text{NH}_3)_2\text{Cl}_2 + \text{H}_2\text{O} \rightarrow [\text{Pt}^{\text{II}}(\text{NH}_3)_2\text{Cl}(\text{H}_2\text{O})]^+ + \text{Cl}^-$
2. $[\text{Pt}^{\text{II}}(\text{NH}_3)_2\text{Cl}(\text{H}_2\text{O})]^+ + \text{H}_2\text{O} \rightarrow [\text{Pt}^{\text{II}}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+} + \text{Cl}^-$

Note(s): Aluminum reacts with cisplatin so take precautions with certain syringes or infusion equipment. Also, the “aquation equilibrium” can occur with cisplatin in a vial. Reconstitution prior to administration should not occur with D5W – diluent must include saline (NaCl). Pre-prepared solutions of cisplatin now exist but they contain NaCl.

Carboplatin (Paraplatin):



Square planar geometry just like cisplatin; retains cis orientation.

Uses: Mainly ovarian cancer (often with cyclophosphamide); also used in prostate cancer (with docetaxel), and lung cancer (often with paclitaxel).

Mechanism: Similar to cisplatin but chemically more stable and less active; also less toxic.

Toxicities: Less toxic than cisplatin: myelosuppression, but less than cisplatin. Nephrotoxicity and ototoxicity can occur but rare. N/V but less than cisplatin. Main route of excretion is still renal, dosage adjustments are needed for patients with renal impairment. See below.*

ADME: Plasma t_{1/2} longer (about 3 hrs) than for cisplatin; although more lipophilic than cisplatin, it is still excreted renally.

*For patients with renal impairment, dosing of carboplatin can be guided by creatinine clearance as follows:

Normal dose of 300 mg/m² if creatinine clearance is >60 mL/min.

Reduce dose to 250 mg/m² if creatinine clearance is 41-59 mL/min.

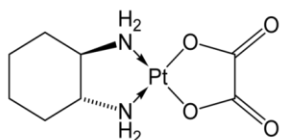
Reduce dose to 200 mg/m² if creatinine clearance is 16-40 mL/min.

Carboplatin can also be dosed to target AUC value of 4 -6 mg/mL x min. In this case, a formula (Calvert formula) is used to calculate the appropriate dose (in mg) as a function of the glomerular filtrate rate (GFR):

Total Dose (mg) = (target AUC) x (GFR + 25), and GFR can be measured by Cr-EDTA excretion.

Note(s): Aluminum reacts with carboplatin so take precautions with certain syringes or infusion equipment. Unlike cisplatin, carboplatin can be diluted with D5W or normal saline.

Oxaliplatin (Eloxatin):



The structure deviates slightly from square planar geometry; retains cis orientation.

Uses: Mainly colorectal cancer; part of the FOLFOX regimen (folinic acid/fluorouracil/**oxaliplatin**); was evaluated in a clinical trial for pancreatic cancer as component in the FOLFIRINOX regimen (folinic acid/fluorouracil/irinotecan/oxaliplatin) – found to be effective but highly toxic regimen.

Mechanism: Similar to cisplatin and carboplatin, but forms conformationally different adducts with DNA; also gets aquated in solution to active form; more lipophilic so more penetration into tissues. The need for prehydration and evaluating renal function is less critical.

Toxicities: Peripheral neuropathy is major toxicity; myelosuppression but less than cisplatin. The mechanism of the neuropathy is unknown but might be caused (at least in part) by chelation of Ca⁺² ions in sensory nerves by the oxalate moiety that is released from oxaliplatin upon aquation.

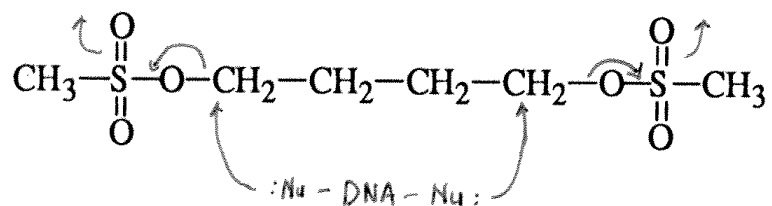
ADME: Tri-phasic elimination with elimination t_{1/2} of about 17 hrs; elimination still largely renal and clearance still correlates with GFR.

Note(s): Aluminum reacts with carboplatin so take precautions with certain syringes or infusion equipment. Final dilution of oxaliplatin should not include any saline (chloride ions) or agents that make the solution alkaline. The clinical trials were done this way.

Picoplatin and **Satraplatin** have been studied in recent clinical trials but have failed to show superior activity and/or decreased toxicity.

2. Methyl sulfates, aziridines, and hydrazines (we will discuss 4 of them)

Busulfan (Busulfex or Myleran):



Uses: Mainly IV for myeloablation prior to bone marrow or stem cell transplantation for CML; has been used in combination with cyclophosphamide for this purpose (called BuCy regimen). Can be administered IV or PO. Administered PO for palliative treatment of CML.

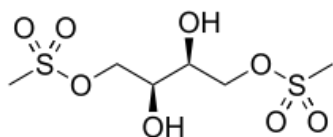
Mechanism: bifunctional and direct alkylator of DNA (shown above).

Toxicities: Extreme myelosuppression, hepatotoxicity (veno-occlusive disease), and seizures.

ADME: Busulfan is lipophilic and can penetrate the CNS (although the brain has a blood-brain-barrier it is lipophilic, and lipophilic drugs can enter more easily). Metabolism occurs mainly by conjugation with glutathione; elimination $t_{1/2}$ from plasma about 2 hrs; about 30% of the dose excreted renally with much of the drug bound to cellular components. Because busulfan can cross the blood-brain-barrier and induce seizures, patients can be pretreated with phenytoin to prevent seizures. But phenytoin reduces the plasma AUC of busulfan; other anticonvulsants can be used but they can increase the AUC of busulfan. Therefore, pharmacokinetic (PK) monitoring of busulfan level is common and is the best way to deliver the most precise dose to patients.

Note(s): Syringes or syringe filters that contain polycarbonate are not to be used as they react with busulfan.

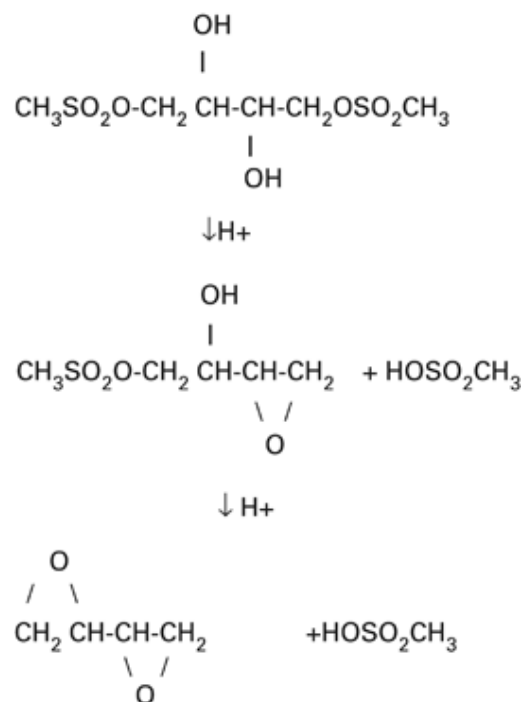
Treosulfan (approved in Europe but not in the U.S.; being studied in many clinical trials):



Uses: Historically for ovarian cancer in EU but now being studied for myeloablation prior to allogeneic stem cell transplantation, in place of busulfan. Potentially, no PK monitoring will be necessary.

Mechanism: Powerful myelosuppression; alkylator of DNA as for busulfan, however it forms epoxides intramolecularly prior to reacting with DNA. Also, more potent immunosuppressant than busulfan so potential replacement for busulfan in allogeneic transplant procedures.

Figure 2. Formation of epoxides (intramolecularly) in treosulfan

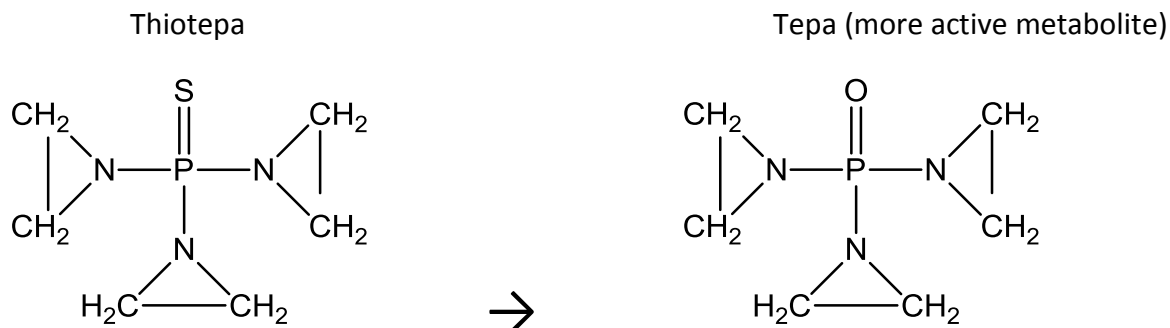


Toxicities: Similar to busulfan but less penetration and toxicity to CNS.

ADME: Elimination t_{1/2} similar to busulfan (1-2 hrs); more polar than busulfan so less penetration into CNS.

Note(s): Because less toxic than busulfan, decreased need (or perhaps no need) for pharmacokinetic monitoring.

Thio-triethylenephosphoramidate (Thiotepa):



Uses: Breast cancer, ovarian cancer; also myeloablative agent (like busulfan) prior to allogeneic and autologous stem cell transplantation; Administered IV. Also used for bladder cancer but administration is intravesical (directly into the bladder).

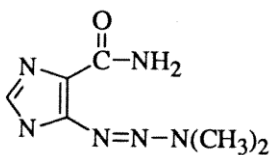
Mechanism: A direct alkylator of DNA but more active following conversion to Tepa (see above); active parent and metabolite. So Thiotepa is not a true prodrug.

Toxicities: When given IV, severe myelosuppression, infections, and N/V.

ADME: Elimination $t_{1/2}$ of thiotepa is about 2 hrs, that of Tepa is nearly 20 hrs. Both molecules can penetrate the CNS and cause dizziness, headaches, etc. Most excretion is renal.

Note(s): Administered as initial dose, followed by maintenance doses every 1-4 weeks. A delayed or slow response does not necessarily indicate a lack of effect. Increasing the frequency of dosing may only increase toxicity. After maximum benefit is obtained by initial therapy, maintenance doses should not be administered more frequently than weekly in order to preserve correlation between dose and blood counts.

Dacarbazine (DTIC):



Uses: Hodgkin's lymphoma (part of ABVD regimen: adriamycin, bleomycin, vinblastine, **dacarbazine**); also used for melanoma. Administered IV only.

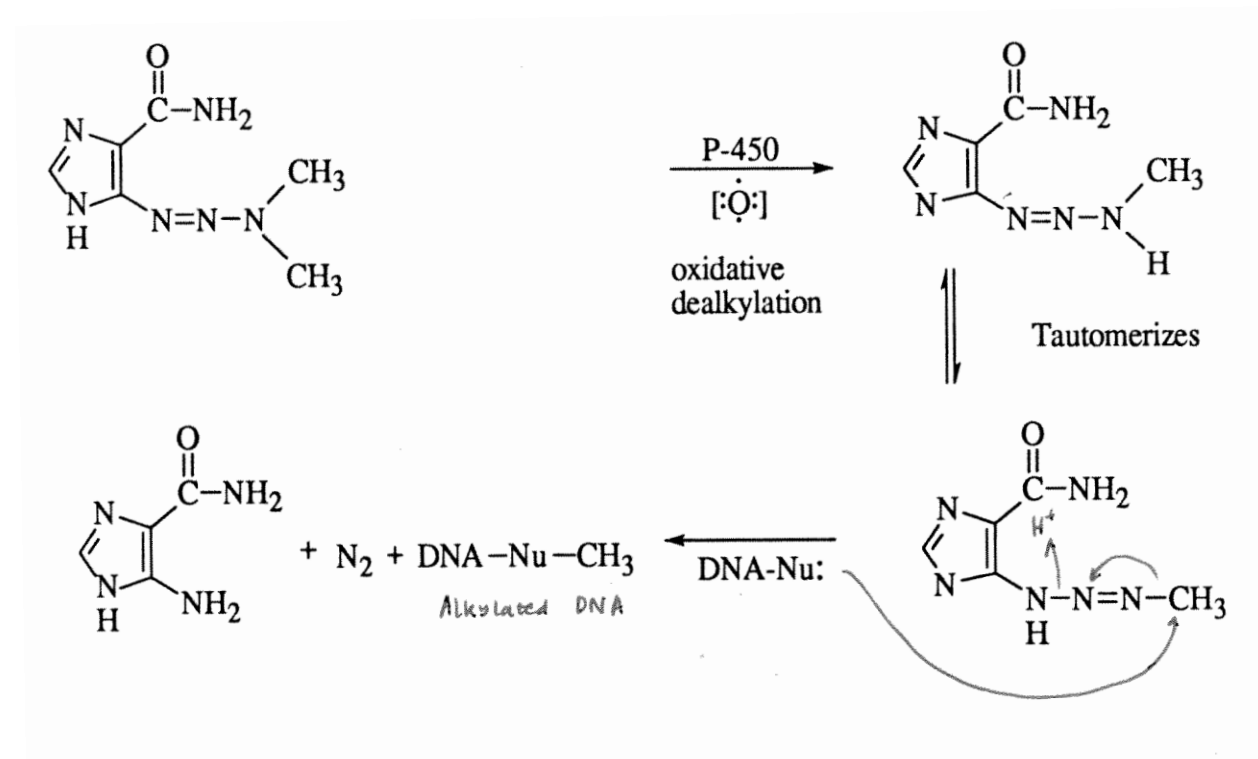
Mechanism: A prodrug; following N-dealkylation by cytochrome P450, the molecule tautomerizes to more reactive form which reacts with DNA, specifically the O-6 position on guanine (see Figure 3 below).

Toxicities: Myelosuppression, hepatotoxicity, N/V.

ADME: Biphasic elimination from plasma; terminal elimination t_{1/2} of about 5 hrs; metabolized by the liver, especially CYP1A, but nearly half the drug excreted unchanged.

Note(s): The hepatotoxicity of DTIC can be extreme and has caused some deaths.

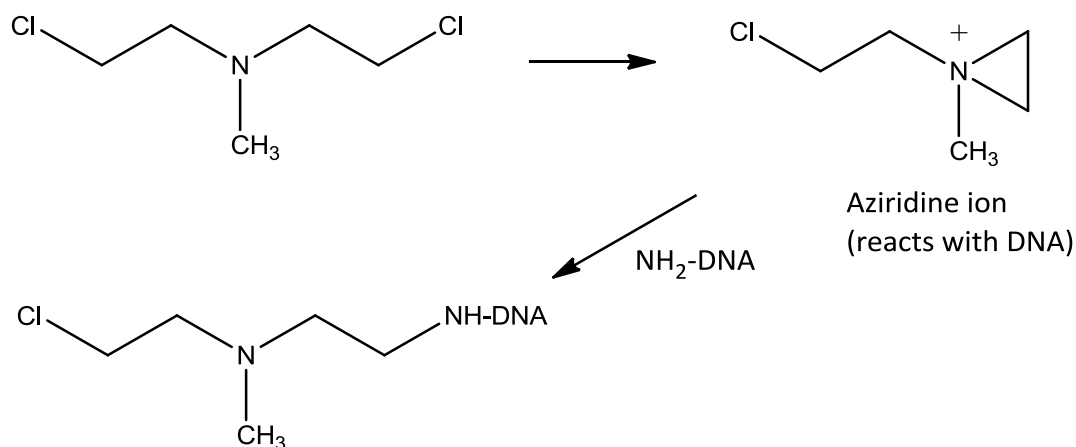
Figure 3. Dacarbazine activation by P450 (CYP1A)



Procarbazine and **Temozolomide** are similar. Again, methylation of O-6 guanine occurs. Patients who are able to repair this DNA damage respond less to these agents than patients who cannot readily repair.

3. Alkylating agents - Nitrogen mustards (we will discuss 5)

Mechlorethamine (Mustargen): Very old agent; originally a chemical warfare agent.



Uses: Formerly for various leukemias, less use today because of better agents; palliative treatment for late stage Hodgkin's lymphoma. The prototype of all alkylating agents. Formulated as the HCl salt. Administered IV, IP.

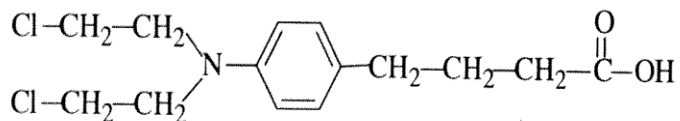
Mechanism: Binds to DNA after formation of reactive aziridine, which binds to guanine at N7 position.

Toxicities: Highly toxic; myelosuppression, severe N/V; severe vesicant and local toxicity if extravasation occurs; hepatotoxic; rarely but also secondary cases of hemolytic anemia or leukemia many years later.

ADME: Actually so reactive that it reacts with water and biomolecules very quickly after administration.

Note(s): Originally developed as a mustard agent for chemical warfare. Results of medical experiments were initially secret and released later. In most cases, better agents are available today.

Chlorambucil (Leukeran):



Uses: Chronic lymphocytic leukemia (CLL), lymphosarcoma, Hodgkin's lymphoma. Administered PO.

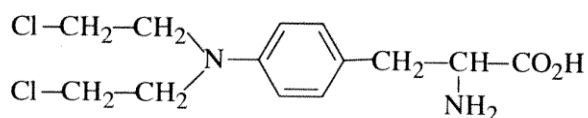
Mechanism: Active intact but less active than mechlorethamine (above). The electrons on the aromatic nitrogen are not as basic and the formation of the aziridinium ion is slower; can be activated by N-dealkylation and then formation of reactive aziridine ion.

Toxicities: Myelosuppression, hepatotoxicity (patients with impaired liver function should be closely monitored). CNS toxicities: seizures, tremors, hallucinations.

ADME: Oral absorption is good due to lack of charge; molecule is rapidly metabolized and the elimination t_{1/2} is a little over 1 hr; beta oxidation is important in the metabolism to form the chain shortened phenylacetic acid metabolite which retains antitumor activity.

Note(s): CNS toxicities can occur because the drug is non-polar (lipophilic) and can enter the CNS.

Melphalan (Alkeran):



Uses: Multiple myeloma, palliative treatment of non-resectable ovarian cancer. Administered PO and IV.

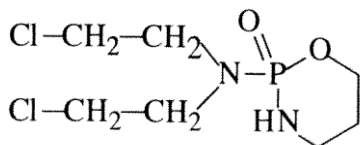
Mechanism: Active intact but less active relative to mechlorethamine (above). The electrons on the aromatic nitrogen are not as basic and the formation of the aziridinium ion is slower; can be activated by N-dealkylation and then formation of reactive aziridine. Note that the molecule contains an amino group on a chiral carbon. This was done intentionally to incorporate the L-phenylalanine to attempt to exploit specific amino acid transport into cells. It turns out that facilitated diffusion is more important for uptake of the agent.

Toxicities: Myelosuppression, hepatotoxicity, and N/V but certainly reduced; rare but serious pulmonary fibrosis and interstitial pneumonitis can occur.

ADME: Absorption can be variable when dosed orally; elimination t_{1/2} is 1-2 hrs; beta oxidation does not participate in metabolism, nor do CYP enzymes; modification of the parent molecule appears to be through chemical hydrolysis to mono and dihydroxy melphalan.

Note(s): CNS toxicities are low because the drug is polar (ionized) and cannot enter the brain well.

Cyclophosphamide (Cytosan):



Uses: Lymphomas, leukemias, multiple myeloma, breast cancer, myeloablation prior to transplantation. Administered IV (PO occasionally).

Mechanism: A prodrug; parent molecule not active. Following dealkylation by cytochrome P450 (CYP2B6 mainly, CYP3A4 less), the 4-hydroxy metabolite tautomerizes and eliminates acrolein to form a phosphoramidate mustard which forms an aziridine which will react with DNA (see Figure 4). Metabolism by CYP enzymes is also important in the formation toxic metabolites, chloroacetaldehyde and acrolein.

Toxicities: Myelosuppression, hemorrhagic cystitis (bladder toxicity). CNS toxicities. Because of potential for bladder toxicity, all patients should be well hydrated prior to administration to cyclophosphamide.

ADME: CYP enzymes are extremely important in the metabolism and activation with more than 5% of the dose metabolized; the parent molecule has a terminal t_{1/2} of about 5-10 hrs; excretion of metabolites is mainly renal.

Note(s): Co-administration of an agent (MesNa), which concentrates in the urine, will bind to and inactivate the acrolein and protect from bladder toxicity. Conjugation with the endogenous thiol glutathione (GSH), helps reduce the nephrotoxicity of chloroacetaldehyde.

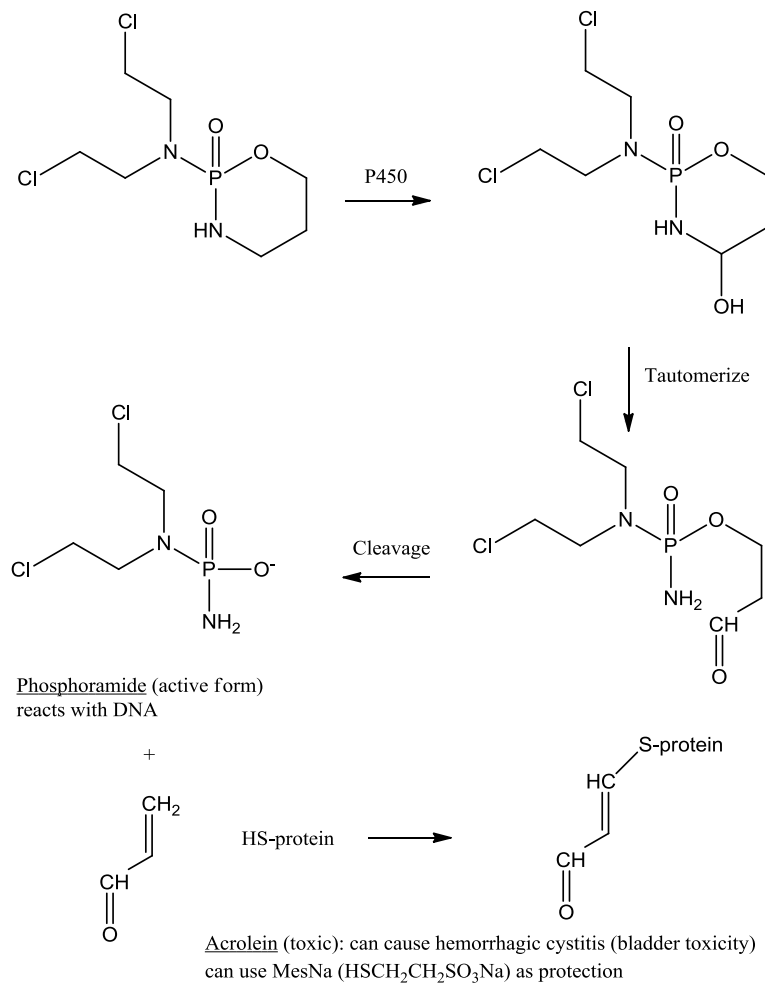
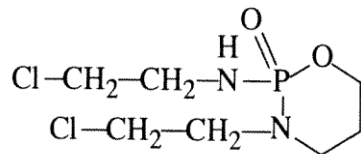


Figure 4. Activation of cyclophosphamide by P450

Ifosfamide (Ifex):



Uses: Mainly testicular cancer; experimental use in other cancers. Administered IV.

Mechanism: Like cyclophosphamide, ifosfamide is a prodrug that is converted to the active form by CYP450 (CYP3A4 mainly, CYP2B6 less). Note that the chloroethyl groups are on different nitrogen atoms and steric hindrance slows hydroxylation at C-4.

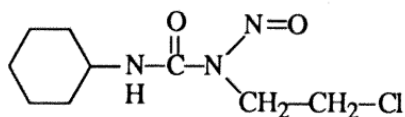
Toxicities: Myelosuppression, nephrotoxicity, hemorrhagic cystitis (bladder toxicity), CNS toxicities. Overall formation of chloroacetaldehyde is higher than from cyclophosphamide. All patients should be well hydrated prior to administration of ifosfamide. The enhanced CNS toxicity of the agent can induce a coma and even death.

ADME: Similar to cyclophosphamide but overall CYP metabolism is slower so higher doses needed; displays dose-dependent pharmacokinetics; elimination $t_{1/2}$ about 15 hrs, shorter at low doses; excretion is mainly renal.

Note(s): As a measure of protection from bladder toxicity, patients should be well hydrated and the protective agent (MesNa) should also be dosed prior to administration of ifosfamide. The sulfur agent N-acetylcysteine (NAC) may provide some protection from nephrotoxicity. No agent for protection against CNS toxicities.

4. Alkylating agents – Nitrosoureas (we will discuss 3 + 1)

Lomustine (CeeNU):



Uses: Brain cancer (glioblastoma), Hodgkin's lymphoma. Administered PO.

Mechanism: A prodrug and ultimately forms a diazonium ion that reacts with DNA (see Figure 5). Reacts with proteins and RNA in addition to DNA; might have some G1 or S phase specificity.

Toxicities: Myelosuppression, hepatotoxicity, pulmonary toxicity, seizures. Leukemias and myelodysplasias have occurred years later.

ADME: Rather unstable structure that begins to fragment in aqueous solution immediately. Metabolites (fragments appear in the urine) and can be eliminated over 2 days. The drug is non-polar (lipophilic) and therefore enters the CNS readily. This helps explain both its efficacy for brain cancer and CNS toxicities. Drug levels in CSF can reach 50x the levels in blood.

Note(s): Somewhat more stable than carmustine; can react with water

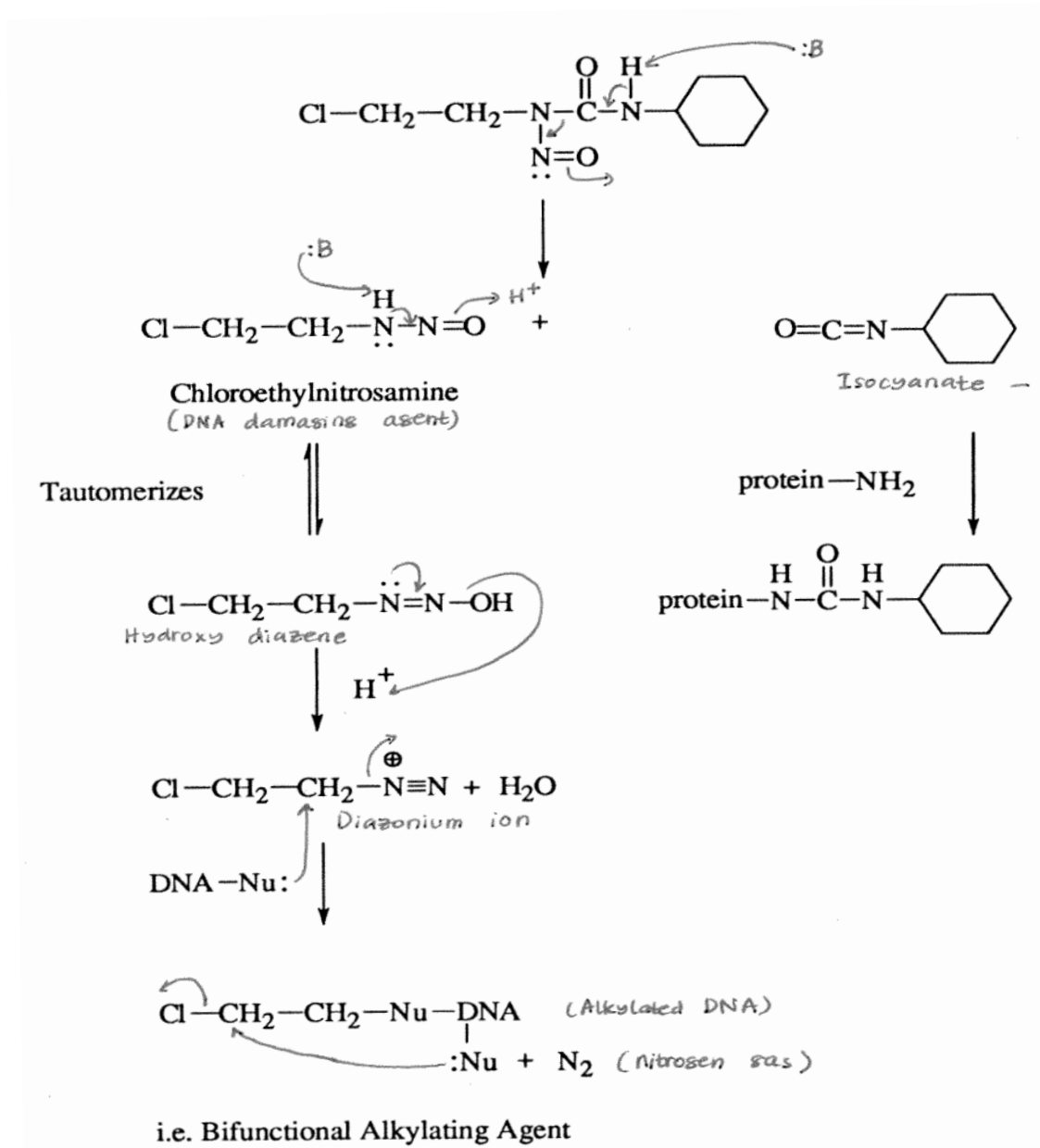
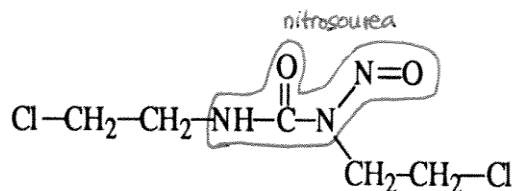


Figure 5. Scheme of lomustine activation

Carmustine (BiCNU or BCNU):



Uses: Brain cancer (glioblastoma), multiple myeloma (often in combination with other agents); also NHL. Administered IV. Formulated as lyophilized powder that is reconstituted prior to use.

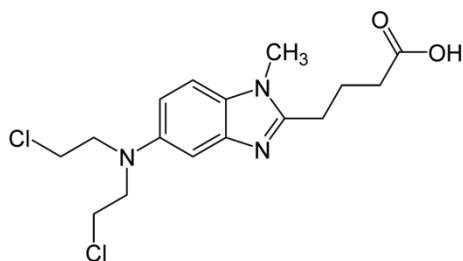
Mechanism: Like lomustine (see Figure 5) but can lead to additional electrophiles.

Toxicities: Similar to lomustine; myelosuppression, hepatotoxicity, acute pulmonary toxicity (pulmonary fibrosis), seizures. Leukemias and myelodysplasias have occurred years later. Also pulmonary fibrosis can appear many years later.

ADME: Unstable structure that begins to fragment in aqueous solution immediately; no intact molecule in plasma after 15 minutes; metabolites (fragments) mainly appear in the urine. The drug is non-polar (lipophilic) and therefore enters the CNS readily. This helps explain both its efficacy for brain cancer and CNS toxicities. Drug levels in CSF can reach 50x the levels in blood.

Note(s): Discard if contents of the vial appear as an oil - this indicates decomposition; can react with water

Bendamustine (Treanda)



Uses: CLL, NHL. Administered IV.

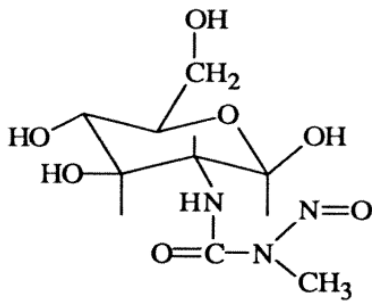
Mechanism: An alkylating agent of the nitrogen mustard type, not a nitrosourea. Causes inter- and intra-strand cross links with DNA bases.

Toxicities: Nausea, fatigue, vomiting, diarrhea, fever, constipation, loss of appetite, cough, headache, unintentional weight loss, difficulty breathing, rashes, and stomatitis, as well as immunosuppression, anemia; notably, this drug has a low incidence of alopecia unlike most other chemotherapy drugs.

ADME: Highly protein bound and extensively metabolized by cytochrome P450 enzymes. Excreted by the kidneys.

Note(s): Being studied for treatment of sarcomas – which are rare.

Streptozocin (Zanosar):



Uses: Pancreatic cancer (islet cell). Administered IV.

Mechanism: A prodrug that ultimately forms a diazonium ion that reacts with DNA; the glucopyranose moiety helps confer specificity to islet cells.

Toxicities: N/V; only mild myelosuppression; use of the agent commonly leads to some level of renal toxicity; rarely but has been associated with diabetes insipidus. Local irritation if extravasation occurs.

ADME: Agent degrades rapidly and distributes to both the liver and kidney; elimination from the plasma occurs within minutes; substantial excretion by the kidneys.

Note(s): A drug interaction with doxorubicin can occur which can increase the t_{1/2} and toxicity of doxorubicin. Drug is polar and does not penetrate the CNS very well.