DNA Damaging Drugs

2

The classical anti-cancer agents comprise cytotoxic compounds. Mostly, these drugs act by exerting DNA damage. In essence, there are two major response phenotypes available to a cell upon DNA damage, such as a chemotherapeutic drug action,

- to arrest the cell cycle and repair the damage,
- to initiate a pathway to apoptosis (programmed cell death).

In either scenario, the uncontrolled growth of the tumor cells is curtailed. The major limitation of the cytotoxic anti-cancer drugs is the tumor non-specific action, and the suppression of all rapidly dividing cells[1].

DNA damaging drugs interfere with transcription and reduplication.
Affected cells respond with cell cycle arrest or programmed cell death.
DNA damaging drugs are mutagenic, teratogenic, and carcinogenic.
Adverse effects are exerted on rapidly proliferating cells (skin—hair loss, gastrointestinal tract—nausea and vomiting, bone marrow—anemia causing fatigue/leukopenia causing infections/thrombocytopenia causing bleeding).

2.1 Alkylating Agents

Alkylating agents[2] are electrophilic and bind covalently to electron-rich functional groups of various target molecules via first-order or second-order nucleophilic substitutions (nucleophiles are electron-rich molecules or ions, such as OH^-, H_2O, halogenides, alcohols, thiols and amines). The first order reactants include aromatic and aliphatic nitrogen and sulfur mustards. Second order reactants include ethylene imines and epoxides, alkylmethane sulfonates of the busulfan (Myleran) type, and α-halogenated acids, ketones, and their derivatives.

- For first-order reactions, the rate limiting step is the ionization of the alkylating agent to form a positively charged carbonium ion, which then rapidly reacts with water, or a negative center, or a nucleophilic center. Within DNA and RNA, the most reactive site is the *N*7 position of guanine (Fig. 2.1). In DNA, this is followed by *N*3 of adenine, *N*1 of adenine, *N*1 of cytosine, and *N*7 of adenine (Table 2.1). The rate limiting step of the reaction is the formation of positively charged cyclic immonium ions, whereas the rate of the reaction is essentially independent of the nature and concentration of the nucleophilic target being attacked.
- For second order nucleophilic substitutions, both reactants interact to form a transition complex. No carbonium ion is formed. The rate of the reaction depends on both concentrations, with bond strengths, electron affinity, and accessibility of both reagents being important (Knock 1967).

Alkylating agents exert cytotoxic effects by transferring alkyl groups to DNA, thereby damaging the DNA and interfering with DNA transcription and cell division. This class of drugs mainly works by three distinct mechanisms:

- The attachment of alkyl groups to DNA bases prevents DNA synthesis and RNA transcription, and it results in the DNA being fragmented by repair enzymes in a process to replace the altered bases.
- The two arms of mustard drugs can cross-link DNA strands. In this process, two bases are linked together. Bridges can be formed within a single molecule of DNA (intra-strand cross-links[3]) or a bridge may connect two DNA molecules (inter-strand cross-links). Cross-linking prevents DNA from being separated for reduplication or transcription. Although bifunctionality of alkylating

[1] Because the desired drug actions (damage to rapidly proliferating cancer cells) and the adverse effects (damage to rapidly proliferating healthy cells) are identical in targets and mechanisms, they are not separable.

[2] Alkyl designates a functional group (a "side chain") that consists solely of single-bonded carbon and hydrogen atoms. Alkylating anticancer agents attach alkyl groups to biomolecules.

[3] called limpet attachment of the drug molecule to the DNA.

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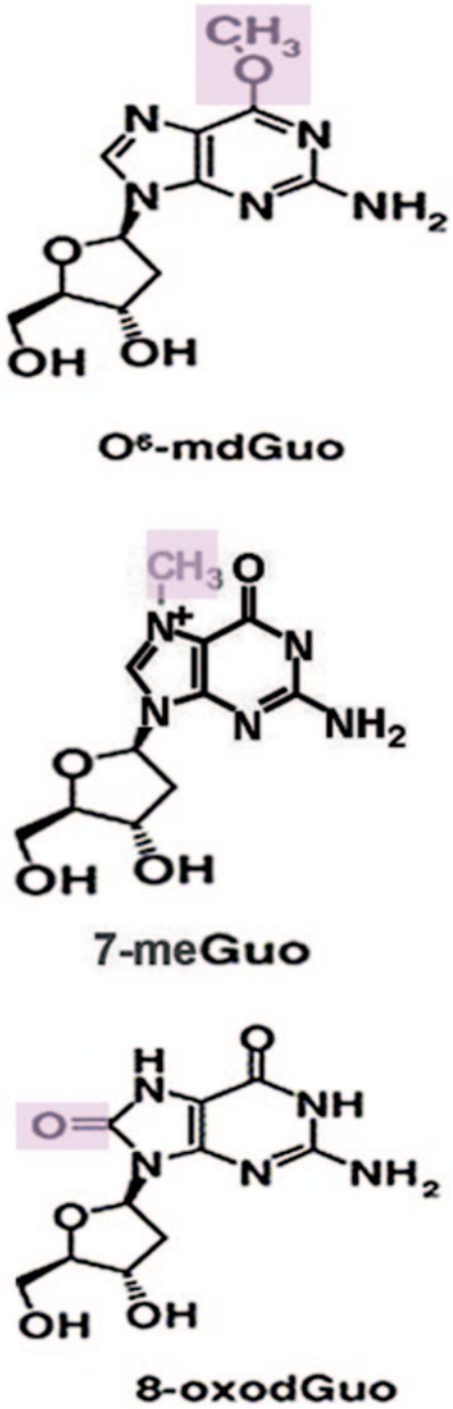


Fig. 2.1 Common DNA adducts of guanosine. DNA damaging anticancer drugs may alter guanine residues to mutagenize and kill proliferating cells. O^6-methyl-2′-deoxyguanosine (*O^6-mdGuo*), N^7-methyl-guanosine (*7meGuo*), and 8-oxo-7,8-dihydro-2′-deoxyguanosine (*8-oxodGuo*) represent such DNA lesions. The modifications are highlighted in *pink.* (Adapted from Brink et al. 2006; Nay 2013)

agents is not required for their mutagenic and carcinogenic properties, it is important for high anti-cancer activity.

- Alkylating agents can induce the mispairing of nucleotides, leading to mutations. Alkylated G bases may erroneously pair with Ts. If this altered pairing is not corrected by DNA repair it can result in permanent genetic change (Fig. 2.2).

Adverse Effects Due to the genetic damage exerted by the drug class, the treatment of cancer patients with alkylating agents is linked to an increased risk for secondary cancers. Alkylating agents can cause mutations not unlike those produced by radiation. These mutations occasionally lead to transformation. One form of cancer that may arise is a relatively rare type of acute non-lymphocytic leukemia (ANL). Highly treatment resistant cases appear as early as 2 years following initial therapy and peak around 5 years after exposure to alkylating agents. The risk of osteosarcomata may be elevated after the treatment of childhood cancers with alkylating agents.

Alkylating agents attach alkyl groups to DNA bases, leading to DNA fragmentation.
Alkylating agents cause intra- and inter-strand DNA cross-links.
Alkylating agents can induce mispairing of nucleotides, leading to mutations.
Alkylating agents exert cytotoxicity in all phases of the cell cycle.
Nitrogen mustards and chlorethylnitrosoureas have a preference for guanine-N7 alkylation.

2.1.1 Nitrogen Mustards

The beginning of the modern era of cancer chemotherapy is rooted directly in the discovery of nitrogen mustard. Mustard gas (sulfur mustard, H-gas) had been used as a chemical warfare agent during World War I, the first documented battlefield use being in Ypres, Belgium in 1917. The name assigned to the gas by the German military was Lost (referring to Lommel and Steinkopf, who in 1916 proposed the military use to the German Imperial General Staff). Exposure to mustard gas induces severe injuries to the eyes, skin, and respiratory tract. In 1917, Krumbhaar, a Captain in the U.S. Medical Corps, noted the development of profound leukopenia in individuals who survived a gas attack for several days (Krumbhaar 1919; Krumbhaar and Krumbhaar 1919). Following up on this observation, a group from the U.S. Office of Scientific Research and Development (OSRD) at Yale Medical School secretly studied the effects of nitrogen mustard on lymphomata. Milton Winternitz, who had worked on sulfur mustards in World War I, obtained the OSRD contract to study the chemistry of mustard compounds. He recruited the pharmacologists Louis S. Goodman and Alfred Gilman

Table 2.1 DNA target sites for alkylating anti-cancer agents

Base	Target	Drug	Class	Recognition sequence
Guanine	*N7*	Melphalan	Nitrogen mustard	
		Cyclophosphamide	Phosphoramide mustard	
		Temozolomide	Triazene	
		Cisplatin	Platinum drug	
		Fotemustine	Nitrosourea	3′ end of guanine tracts
Guanine	*N1*	Fotemustine	Nitrosourea	
Guanine	*N2*	Ecteinascidin-743	Minor groove binding antibiotic (G/C preference)	AGC, CGC, TGG
Guanine	*N3*	Duocarmycin A	Cyclopropylpyrroloindole antibiotic	5′-GCAATTGCG-CAATTGC-3′
Guanine	*O6*	Temozolomide	Triazene	
		Dacrbazine	Triazene	
		Laromustine	Hydrazine	
Guanine		Duocarmycin A	Cyclopropylpyrroloindole antibiotic	5′-CGCGTTGGGAG-3′
		Mithramycin A	Aureolic acid minor groove binder	
Adenine	*N3*	Duocarmycin A	Cyclopropylpyrroloindole antibiotic	
		CC-1065	Cyclopropylpyrroloindole antibiotic	5′-d(A/G)NTTA-3′
				5′-dAAAAA-3′
		Adozelesin, carzelesin, bizelesin	Minor groove binding antibiotic (A/T preference)	5′-(A/T)(A/T)A-3′
		Tallimustine	Minor groove binding antibiotic (A/T preference)	5′-TTTTGA-3′
Adenine	*N7*	Cisplatin	Platinum drug	
Adenine	*N1*	(Uncommon)		
Cytosine	*N3*	Fotemustine	Nitrosourea	
Cytosine	*N1*	(Uncommon)		

to perform the necessary animal experiments. Based on their successful research, Gustav Lindskog successfully treated a radio-resistant lymphosarcoma that compressed a patient's trachea with the injection of nitrogen mustard in December 1942. In 1943, Goodman and Gilman initiated the experimental treatment of Hodgkin disease and lymphosarcoma with nitrogen mustard. None of this was made public until 1946, when Goodman and Gilman reported their observation that exposure to mustard gas caused profound lymphoid and myeloid suppression, suggesting its utility for the treatment of lymphomata (Goodman et al. 1946; Gilman and Philips 1946). There was a parallel development: In World War II, General Eisenhower had ordered that a stockpile of mustard gas be kept near the front for possible use in a reprisal if the Nazis resorted to chemical warfare. During a military operation, allied ships in Bari Harbor, Italy[4], were sunk in an air assault by the German Luftwaffe on 2nd December 1943. At the center of the destruction was the vessel Liberty Ship S.S. John Harvey, laden with ammunition, supplies, and 2000 mustard gas bombs. A large number of military personnel were accidentally exposed to mustard gas and were later found by U.S. medical officer Lieutenant Colonel Stewart F. Alexander to have abnormally low white blood cell counts as a consequence of poisoning. It was implied that an agent, which damaged the rapidly growing white blood cells, might have a similar effect on cancer cells. Cornelius P. Rhoads served as chief of the medical division of the U.S. Army's chemical warfare unit during World War II. Based on his experience in the Bari incident, he investigated mustard gas as a tumor killing agent (Rhoads 1946). The research presaged classical chemotherapy[5]. Rhoads moved on to head one of the largest drug development programs at the Sloan-Kettering Institute in New York and pioneered the practice of contract research for pharmaceutical companies under confidentiality agreements.

Mustard gas is not derived from the mustard plant, it gets its name from its impurities. Impure mustard gas is yellow-brown and has an odor resembling mustard plants. Upon contact with the skin, it causes a burning sensation, which is similar to that caused by the oil from black mustard seeds. Sulfur mustards (bis(2-chloroethyl)-sulfide, 1,5-dichloro-3-thiapentane), which are related to mustard gas, are vesicants that have the ability to form large blisters on exposed skin. While sulfur mustards are too toxic for medical applications, nitrogen mustards[6] have found use as therapeutics.

[4] Allied ships were stationed in Bari under the assumption that the harbor was too far south to be reached by the Luftwaffe (the German airforce). The air raid was later called The Little Pearl Harbor.

[5] The recognition would have assured Rhoads' place in medical history had it not been revealed that he had likely committed serious ethics violations in unrelated assignments. In Puerto Rico, 1931, after Rhoads' car had been vandalized he wrote generally hateful comments on Puerto Ricans in a letter. He claimed that he had deliberately injected several Puerto Rican citizens with cancer cells.

[6] The characteristic bis(2-chloroethyl)amine- domain, which generates DNA cross-links, is contained in nitrogen mustards, phosphoramide mustards (ifosphamide is unique in its chain length that generates 7-membered cross-links), and selective minor groove DNA binding antibiotics (tallimustine and MEN 10710).

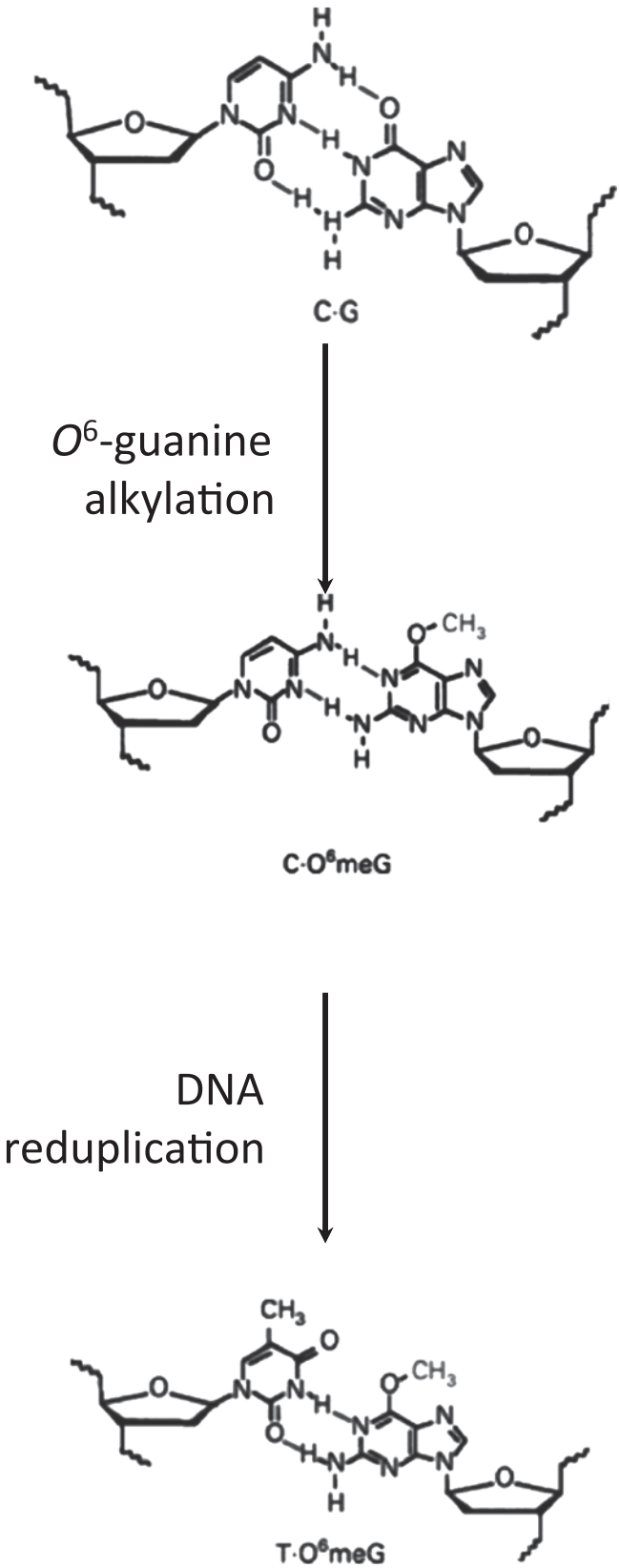


Fig. 2.2 DNA adducts caused by alkylating agents. The reaction scheme depicts the O^6 alkylation of guanine and the ensuing mispairing during DNA reduplication. (Adapted from Marra and Schär 1999)

After uptake, nitrogen mustard is metabolized to a highly reactive ethylene immonium derivative, which alkylates DNA (Papirmeister et al. 1985) and inhibits DNA reduplication (Fig. 2.3). In addition, increasing nitric oxide produced by Nitric Oxide Synthase may be responsible for some of the damage exerted by mustard drugs (Sawyer 1998). This toxicity probably comes from an over-production of reactive nitrogen species, in particular peroxynitrite ($ONOO^-$), by the reaction of nitric oxide and superoxide. There are several generations of nitrogen mustard drugs.

- In first generation nitrogen mustards, aliphatic radicals were attached to the mustard pharmacophore $–N(CH_2CH_2Cl)_2$.
- In second generation nitrogen mustards, electron withdrawing aromatic radicals were attached to reduce the reactivity and permit oral use.
- In third generation nitrogen mustards, the pyrimidine nucleus was chosen as a carrier for the mustard pharmacophore, which permits oral administration.
- More recent modifications include steroid-coupled nitrogen mustards and phosphoramide mustards

First generation nitrogen mustards The aliphatic alkylating agent mechlorethamine hydrochloride (2-chloro-*N*-(2-chloroethyl)-*N*-methyl-ethanamine, nitrogen mustard, chlormethine hydrochloride, mustine, chlorethazine hydrochloride, HN2 hydrochloride, N-Lost) (NSC-762) <Mustargen, Caryolysine, Cloramin, Erasol, Onco-Cloramin> is the salt of a synthetic nitrogen-containing sulfur mustard derivative (Fig. 2.4) with anti-neoplastic and lympholytic properties. Mechlorethamine hydrochloride is used because it induces a rapid response. It is primarily administered as part of the MOPP regimen. The agent may be indicated in treating Hodgkin disease (stages III and IV), lymphosarcoma, chronic myelocytic or chronic lymphocytic leukemia, polycythemia vera[7], mycosis fungoides[8], and bronchogenic carcinoma. It may also be included in the treatment of small cell lung cancer or medulloblastoma. The drug is not active in acute leukemias or chronic granulocytic leukemias (Knock 1967).

Mechlorethamine cannot be taken orally. It is given as an intravenous infusion over 20 min. The dosage varies with the clinical situation, the initial therapeutic response, and the magnitude of hematologic depression. Usually, a total dose of 0.4 mg/kg of body weight for each course is given either as a single dose or in divided doses of 0.1–0.2 mg/kg per day. Subsequent courses should not be given until the patient has recovered hematologically from the previous course. The drug administered intrapleurally, intraperitoneally, or

[7] A myeloproliferative disorder that results in the over-production of red blood cells.

[8] The most common form of cutaneous T-cell lymphoma.

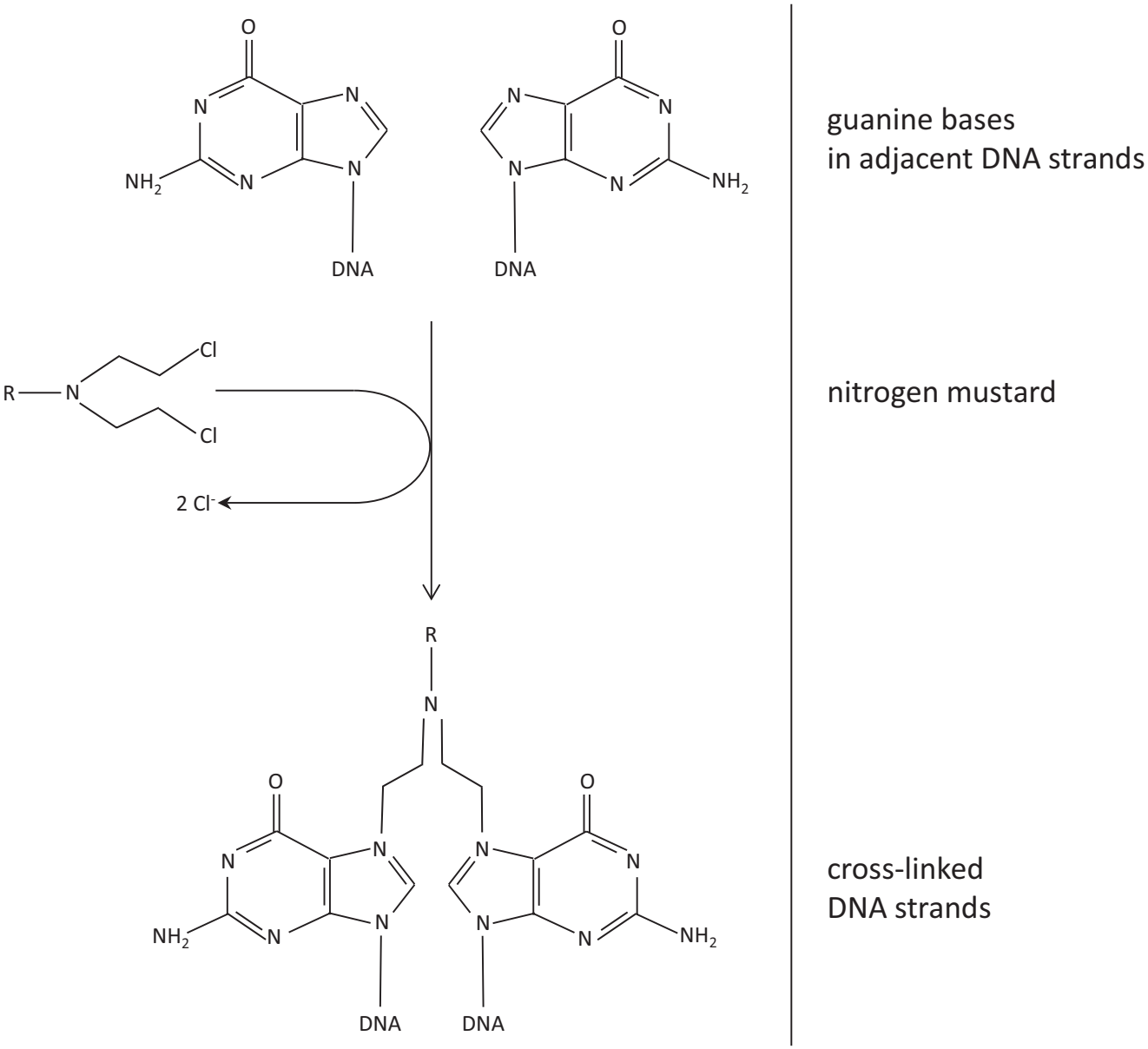


Fig. 2.3 Mechanism of action for nitrogen mustard. Alkylation of guanine bases in the DNA is partially responsible for the cytotoxic effect of nitrogen mustards. The alkylation of two guanines by the arms of the mustard leads to DNA cross-linking

intrapericardially is indicated for the palliative treatment of metastatic carcinoma resulting in effusion. Local therapy with nitrogen mustard is used only when malignant cells are present in the effusion. Intracavitary injection is not recommended when the accumulated fluid is chylous, because the results are likely to be poor. Paracentesis is first performed with most of the fluid being removed from the pleural or peritoneal cavity. The position of the patient should be changed every 5–10 min for an hour after injection to obtain more uniform distribution of the drug throughout the serous cavity.

- Mechlorethamine gel <Valchlor> is a topical gel for the second-line treatment of stage IA and IB mycosis fungoides cutaneous T-cell lymphoma

Pharmacokinetics Because of its extreme reactivity with water, nitrogen mustard is reconstituted immediately before use. In neutral or alkaline aqueous solution the drug is highly unstable and undergoes rapid chemical transformation. In body fluids, mechlorethamine combines with water or reactive compounds of cells within a few minutes after administration, so that the drug is no longer present in its active form.

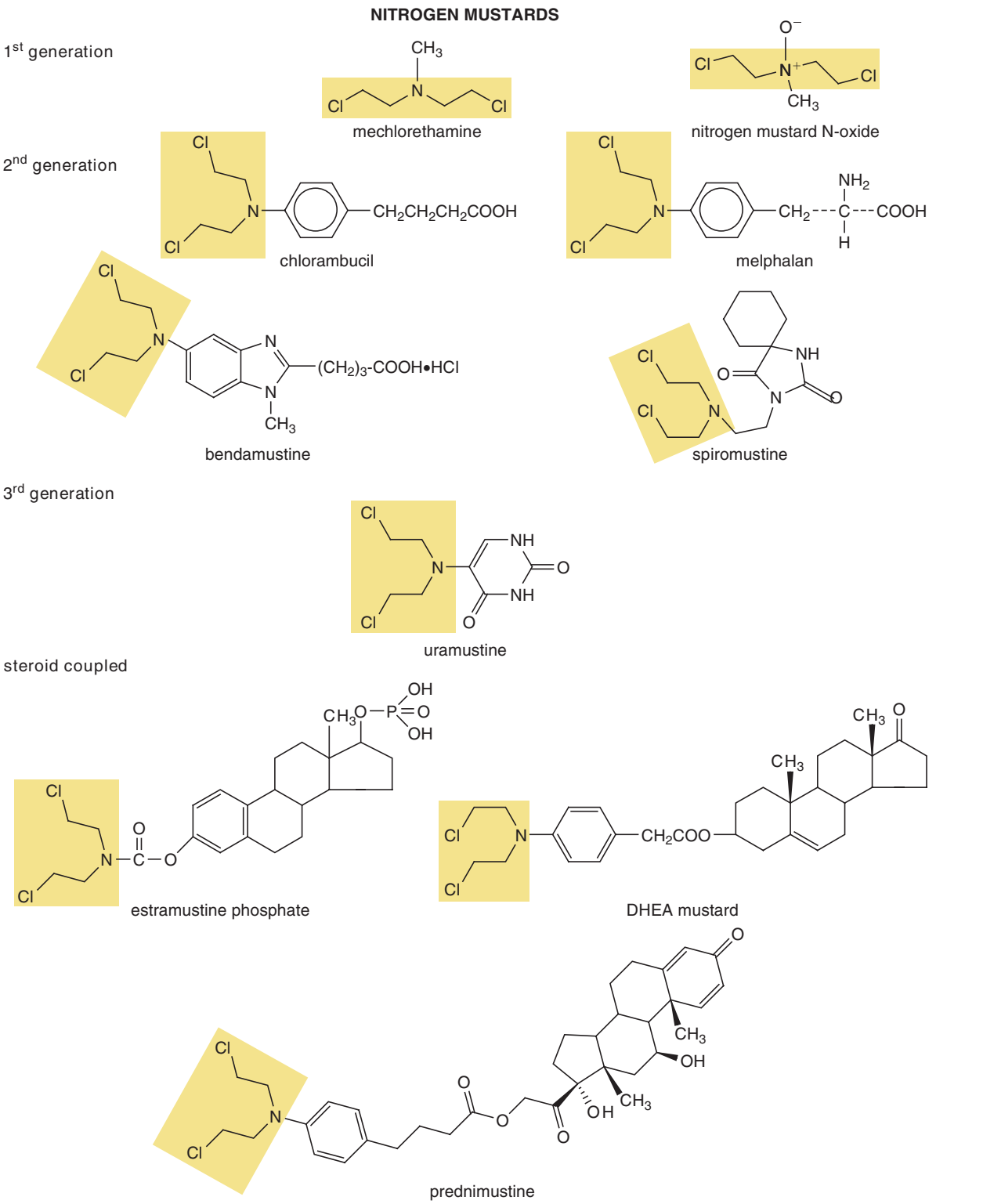


Fig. 2.4 Structures of nitrogen mustards. Three consecutive generations in this class of drugs have acquired increasing oral bioavailability. A 4th generation is coupled to steroids for bifunctionality. The common functional mustard moiety is highlighted in *yellow*

Adverse Effects For the comfort of the patient, nitrogen mustard may be given at bedtime, following administration of a barbiturate and an anti-emetic to minimize the adverse effects of nausea and vomiting, which usually occurs after 1–3 h. Emesis may disappear in the first 8 h, but nausea may persist for 24 h. Depression of the hematopoietic system may be present for up to 50 days (or more) after starting therapy. Mustard treatment generally produces lymphocytopenia

within 24 h after the first injection. Substantial granulocytopenia occurs within 6–8 days and lasts for 10–21 days. Thrombocytopenia is variable but the time course of the appearance and recovery from reduced platelet counts generally parallels the granulocyte levels. Rarely, hemolytic anemia associated with such diseases as lymphomata and chronic lymphocytic leukemia may be precipitated by treatment with alkylating agents including mechlorethamine. Serious adverse effects can include anaphylactic reactions or bleeding (bloody urine, tar stools, bleeding gums). Other common adverse effects include hyperuricemia, fatigue, hair loss, maculopapular skin eruptions, and herpes zoster. Jaundice, vertigo, tinnitus and hearing loss occur infrequently. As nitrogen mustard therapy can contribute to the extensive and rapid development of amyloidosis, it should be used only if foci of acute or chronic suppurative inflammation are absent.

Alkylating agents are carcinogenic, mutagenic and teratogenic. Oligomenorrhea or azoospermia can be induced by mechlorethamine and may not recover for years after termination of therapy. During preganancy, the agent can cause damage to the fetus. It is in the U.S. FDA Pregnancy Category D.

After intravenous injection, a local rash, pain, or burning may occur. Extravasation into the tissue surrounding the injection site is a potentially serious problem. It causes severe painful induration. The area can be infiltrated with an isotonic solution of 0.11 M sodium thiosulfate as an antidote. The mustard also poses a risk for venous thromboses at the site of injection from direct sclerosing action. Transient cardiac irregularities may occur with intrapericardial injection.

A contraindication is hypersensitivity to mechlorethamine or any component of the formulation. The presence of known infectious diseases may be an indication against immunosuppressive agents, such as mechlorethamine. Because of the bone marrow suppression, vaccinations during or shortly before or shortly after chemotherapy with mechlorethamine should be avoided.

Drug Interactions Turmeric may decrease the effect of mechlorethamine. The use of this spice in the diet should be avoided while receiving treatment. Precautions must be observed with the use of mechlorethamine and radiation therapy in alternating courses. Both depress hematopoietic function and neither regimen should follow the other until bone marrow function has recovered. In particular, irradiation of such areas as sternum, ribs, and vertebrae shortly after a course of nitrogen mustard may lead to hematologic complications. The decrease in platelet count can increase the risk of bleeding. Therefore, any aspirin or salicylate containing medicines are to be avoided.

Nitrogen mustard *N*-oxide hydrochloride (2-chloro-*N*-(2-chloroethyl)-*N*-methyl-ethanamine-*N*-oxide hydrochloride, NMNO) (NSC-10107, SK-598) <Nitromin> is the chloride salt of the oxide of nitrogen mustard. It is closely related to mechlorethamine. Nitrogen mustard *N*-oxide has been used especially in Japan[9]. Nitrogen mustard *N*-oxide is usually administered intravenously at 1 mg/kg/day for 10 days. Its therapeutic spectrum resembles that of nitrogen mustard.

Adverse Effects As a DNA damaging agent, nitrogen mustard *N*-oxide hydrochloride is potentially carcinogenic.

Second generation nitrogen mustards Chlorambucil (4-[bis(2-chlorethyl)amino]benzenebutanoic acid) <Leukeran> was developed in 1953 at the Chester Beatty Research Institute, England. It is an orally active, bifunctional, aromatic nitrogen mustard that alkylates and cross-links DNA during all phases of the cell cycle, resulting in a disruption of DNA function, cell cycle arrest, and apoptosis. The electron withdrawing properties of the aromatic ring lead to slower reactions with serum and cellular constituents compared to first generation nitrogen mustards. Therefore, chlorambucil can be given orally in tablet form. Chlorambucil is indicated in the palliative treatment of chronic lymphocytic leukemia, malignant lymphoma, lymphosarcoma, giant follicular lymphoma, and Hodgkin disease. It has been used in the treatment of Waldenström macroglobulinemia[10], polycythemia vera, oat cell and undifferentiated carcinomata of the lungs, trophoblastic neoplasms, and ovarian carcinoma.

Chlorambucil is well tolerated by most patients, although for initial treatment it has been largely replaced by fludarabine (Rai 2000).

- Chlorambucil is usually given at 0.1–0.2 mg/kg/day for 3–6 weeks. The entire daily dose may be administered at one time. The dose is reduced when leukocyte counts drop or there are signs of clinical improvement. When lymphocytic infiltration of the bone marrow is present, or when the bone marrow is hypoplastic, the dose should not exceed 0.1 mg/kg/day. The maintenance dose is usually about 2 mg/day and can be extended over months or years.
- Alternate schedules for the treatment of chronic lymphocytic leukemia employ intermittent, bi-weekly, or once-monthly pulse doses of chlorambucil. Intermittent schedules begin with an initial single dose of 0.4 mg/kg, which is increased by 0.1 mg/kg until control of lymphocytosis or toxicity is observed. Subsequent doses are modified to produce mild hematologic toxicity.
- Continuous maintenance therapy is considered less safe than short courses of treatment. If a maintenance dosage is used, it should not exceed 0.1 mg/kg/day and may be as low as 0.03 mg/kg/day. It may be desirable to withdraw the drug after maximal control has been achieved, since intermittent therapy—reinstituted at the time of relapse—may be as effective as continuous treatment.

[9] Of note, in the U.K. Nitromin is a brand name for the unrelated agent glyceryl trinitrate.

[10] Waldenström macroglobulinemia (lymphoplasmacytic lymphoma) is a lymphoproliferative disease of IgM secreting B-lymphocytes.

Pharmacokinetics Oral chlorambucil undergoes rapid and complete gastrointestinal absorption and blood clearance. After single oral doses of 0.6–1.2 mg/kg, peak chlorambucil levels in the blood are reached within 1 h and the terminal elimination half-life of the parent drug is roughly 1.5 h. The agent is extensively metabolized in the liver, primarily to phenylacetic acid mustard, which has anti-neoplastic activity. Chlorambucil and its major metabolite spontaneously degrade, forming monohydroxyl and dihydroxyl derivatives. Both chlorambucil and its metabolites are extensively bound to plasma and tissue proteins (99%), specifically to Albumin. Urinary excretion is below 1% in 24 h.

Adverse Effects A prominent adverse effect is myelosuppression (anemia, neutropenia, thrombocytopenia). Severe neutropenia usually occurs only in patients who have received a total dosage of 6.5 mg/kg or more in one course of therapy with continuous dosing. Upon withdrawal from the drug, this effect may be reversible after about 10 days from the last dose, but bone marrow failure can arise in rare cases. Less commonly occurring adverse effects are gastrointestinal distress (nausea, vomiting, diarrhea, oral ulcerations) and central nervous system damage (tremors, muscular twitching, confusion, agitation, ataxia, hallucinations; increased risk of seizures in children with nephrotic syndrome and patients receiving high pulse doses of chlorambucil) that often resolves upon discontinuation of the drug, skin reactions (urticaria, angioneurotic edema, rarely skin rash progressing to erythema multiforme, toxic epidermal necrolysis, and Stevens-Johnson syndrome), and infertility (sterility when administered to prepubertal and pubertal males, induction of amenorrhea in females). Uncommon adverse reactions include pulmonary fibrosis, hepatotoxicity and jaundice, drug fever, peripheral neuropathy, interstitial pneumonia, and sterile cystitis. As all DNA damaging agents, chlorambucil is itself carcinogenic and bears a risk for secondary malignancies (specifically acute leukemia) with increasing cumulative dose.

Radiation and cytotoxic drugs render the bone marrow more vulnerable to damage, and chlorambucil should be used with particular caution within 4 weeks of a full course of radiation therapy or chemotherapy. If bone marrow is infiltrated by the tumor, the daily dosage of chlorambucil should not exceed 0.1 mg/kg. The drug crosses the placenta and is contraindicated during pregnancy (Category D), but it is not known whether it is excreted into breast milk.

Chlorambucil should not be used in patients whose disease has shown a prior resistance or known hypersensitivity to the agent. There may be cross-hypersensitivity between chlorambucil and other alkylating agents.

Melphalan A (2-amino-3-[4-[bis(2-chloroethyl)amino] phenyl]-propanoic acid, L-phenylalanine mustard, L-PAM, L-sarcolysin) <Alkeran> is a bifunctional phenylalanine derivative of nitrogen mustard[11]. Melphalan alkylates DNA at the *N*7 position of guanine and induces DNA inter-strand cross-linkages. This results in the inhibition of DNA and RNA synthesis and cytotoxicity against both dividing and non-dividing tumor cells.

Melphalan is indicated for the palliative treatment of multiple myeloma and for the palliation of non-resectable epithelial ovarian carcinoma. It is also used to treat breast cancer, neuroblastoma, and rhabdomyosarcoma. While it is occasionally applied to malignant melanoma, melphalan is not enriched in melanocytes, despite the role of phenylalanine as a precursor in the synthesis of Melanin. Administration is oral or intravenous. Dosing varies by purpose, route of administration, and patient weight. The entire daily dose may be given at one time.

- Orally, melphalan is usually given at 6–10 mg per day for 2–3 weeks, followed by a rest period of several weeks before initiation of the next cycle. When the white blood cell and platelet counts are rising, a maintenance dose of 2 mg daily may be instituted. In multiple myeloma, melphalan shows clinical effectiveness in 50% of cases at 0.2 mg/kg/day orally.
- Alternatively, an initial course of 10 mg/day for 7–10 days is administered. The maximal suppression of the leukocyte and platelet counts occurs within 3–5 weeks and recovery within 4–8 weeks. Continuous maintenance therapy with 2 mg/day is instituted when the white blood cell count is greater than 4000 cells/μL and the platelet count is greater than 100,000 cells/μL. Dosage is adjusted to 1–3 mg/day depending upon the hematologic response. It is desirable to try to maintain a significant degree of bone marrow depression so as to keep the leukocyte count in the range of 3000–3500 cells/μL.
- A commonly employed regimen for the treatment of ovarian carcinoma is the administration of melphalan at a dose of 0.2 mg/kg daily for 5 days as a single course. Courses are repeated every 4–5 weeks, depending upon hematologic tolerance.

Melphalan has been used in combination with prednisone to boost effectiveness in the treatment of multiple myeloma. Early efforts to enhance chemotherapy with autologous bone marrow transplantation were performed with melphalan. For disseminated melanoma, bone marrow withdrawn from the sternum and stored cooled in citrate buffer plus dextrose was reinfused intravenously 6–8 h after administration of the chemotherapeutic (Ariel and Pack 1967).

[11] Melphalan is the active L-isomer of the D-isomer compound medphalan that was first synthesized in 1953 by Bergel and Stock. The L form is superior to the D form in anti-tumor activity.

Pharmacokinetics Blood melphalan levels are highly variable after oral dosing, possibly due to incomplete intestinal absorption, variable first-pass hepatic metabolism, or rapid hydrolysis. The time of the first appearance in the blood stream ranges approximately 0–6 h. The peak plasma concentration range is 70–4000 ng/mL, depending upon the dose. The extent of melphalan binding to plasma proteins ranges 60–90 %. Serum Albumin is the major binding protein, whileα_1-Acid Glycoprotein may account for about 20 % of the plasma protein binding. Approximately 30 % of melphalan is irreversibly bound to plasma proteins. Interactions with Immunoglobulins are negligible. Melphalan is eliminated from the circulation primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. The terminal elimination half-life of the parent drug in the blood is about 1.5 h; its 24-h urinary excretion is about 10 %, indicating that renal clearance is not a major route of elimination. Penetration into cerebrospinal fluid is low.

Adverse Effects Myelosuppression with reduced white blood cell count, increased risk of infection, and decreased platelet count (causing an elevated risk of bleeding) is common. Although bone marrow suppression frequently occurs, it is usually reversible if melphalan is withdrawn early enough. However, irreversible bone marrow failure has occurred. Common adverse effects include nausea and vomiting. Less frequent adverse effects comprise pulmonary fibrosis after prolonged use, interstitial pneumonitis, compromised ovarian or testicular functions, and hair loss. Allergic reactions, including urticaria, edema, skin rashes, and rare anaphylaxis, have arisen after multiple courses of treatment. Cardiac arrest can rarely result in association with such events.

Secondary malignancies, including acute non-lymphocytic leukemia, myeloproliferative syndrome, and carcinoma can occur in patients treated with alkylating agents. At cumulative doses above 700 mg, the 10-year cumulative risk increases from about 2 % to about 20 %.

Melphalan should be used with extreme caution in patients whose bone marrow reserve may have been compromised by prior irradiation or chemotherapy, or whose marrow function is recovering from previous cytotoxic therapy. During treatment with melphalan A, the intake of salicylic acid <Aspirin> should be avoided as it could intensify any bleeding problems. Interactions with cimetidine, steroids, and cyclosporine are possible. The drug is Pregnancy Category D; it is not known whether this agent is excreted into breast milk.

In the 1960s, bendamustine hydrochloride (4-[5-[bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid) (SDX-105) <Treanda, Ribomustin, Treakisym, Levact> was designed with the aim of creating a bifunctional anti-cancer agent that possesses DNA damaging properties (by virtue of an alkylating group) and also potential anti-metabolite properties (associated with a purine-like benzimidazole ring). It was first marketed in Germany in the early 1970s. In 2008, bendamustine was approved by the U.S. Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia (CLL). The drug also has therapeutic activity against multiple myeloma, and indolent B-cell non-Hodgkin lymphoma that has progressed within 6 months of treatment with a rituximab containing regimen. For these conditions, bendamustine has strong efficacy as well as low cross-resistance with other alkylating agents and fludarabine. The agent causes DNA damage that leads to cell death via several pathways, including apoptosis and mitotic catastrophe. A standard dose is 100–120 mg/m^2 body surface area, administered intravenously over a period of 30–60 min, for chronic lymphocytic leukemia on days 1 and 2 of a 28-day cycle up to six cycles, for non-Hodgkin lymphoma on days 1 and 2 of a 21-day cycle up to eight cycles.

Pharmacokinetics The binding of bendamustine to plasma proteins is largely concentration independent and ranges 94–96 %. The drug distributes freely in red blood cells, rendering the mean steady state volume of distribution approximately 25 L. Bendamustine is primarily hydrolyzed to metabolites with low cytotoxic activity. Two active minor metabolites, γ-hydroxy bendamustine (M3) and N-desmethyl-bendamustine (M4), are primarily formed via CYP1A2. The parent drug does not induce or inhibit Cytochrome P450 enzymes. While the intermediate half-life of the parent compound is approximately 40 min, the mean apparent terminal elimination of the metabolites γ-hydroxy bendamustine and N-desmethyl-bendamustine are approximately 3 h and 30 min, respectively. 90 % of the drug is eliminated unmetabolized, mostly in the feces. Due to pharmacogenetic predisposition, Japanese patients may have higher exposure than non-Japanese subjects to identical doses.

Adverse Effects The most common hematologic abnormalities for both indications (indolent B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia) are lymphopenia, anemia, leukopenia, thrombocytopenia, and neutropenia. Most common non-hematologic adverse reactions in chronic lymphocytic leukemia are pyrexia, nausea and vomiting. Most common non-hematologic adverse reactions for non-Hodgkin lymphoma are nausea and vomiting, diarrhea or constipation, anorexia, headache, fatigue, pyrexia, cough, dyspnea, rash, and stomatitis. Dose reduction or delayed administration is required if grade 3 or grade 4 toxicities occur. Dose re-escalation in subsequent cycles may be considered. Tumor lysis syndrome can arise within the first treatment cycle. Without intervention it may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of blood chemistry, particularly potassium and uric acid levels. Allopurinol may be used during the beginning of brendamustine therapy, however, it poses an increased risk of severe

skin toxicity (Stevens-Johnson syndrome or the more severe manifestation of toxic epidermal necrolysis).

Contraindications are hypersensitivities to bendamustine or mannitol (in rare instances severe anaphylactic and anaphylactoid reactions can occur, particularly in the second and subsequent cycles of therapy), renal impairment with creatinine clearance below 40 mL/min, or moderate to severe hepatic impairment. The agent is Pregnancy Category D.

Drug Interactions Because the active bendamustine metabolites, γ-hydroxy bendamustine and N-desmethyl-bendamustine, are formed via Cytochrome P450 CYP1A2, the concomitant intake of CYP1A2 inhibitors (such as fluvoxamine <Luvox> or ciprofloxacin <Cipro>) has the potential to increase the exposure to bendamustine and decrease the exposure to the active metabolites. Conversely, inducers of CYP1A2 (including cigarette smoke and omeprazole <Losec, Antra, Gastroloc, Mopral, Omepral, Prilosec>) have the potential to decrease the concentration of bendamustine and increase the concentrations of its active metabolites in the body.

Spiromustine (spirohydantoin mustard) (NSC 172112) is a nitrogen alkylating agent that contains a lipophilic hydantoin group, which serves as a carrier to cross the blood-brain barrier. This lipophilicity may also enhance alkylating activity against tumors outside the brain. Spiromustine forms covalent linkages with nucleophilic centers in DNA, causing depurination, base pair miscoding, strand scission, and DNA cross-linking, which may result in cytotoxicity. The agent acts in a cell cycle non-specific manner.

Pharmacokinetics In an aqueous environment, spiromustine is rapidly hydrolyzed. The drug has a biphasic plasma decay curve, with hepatic metabolism and excretion, enterohepatic circulation of metabolites, and approximately 50% renal excretion of the unmetabolized drug.

Third generation nitrogen mustards Uracil mustard (uramustine, 5-[bis(2-chloroethyl)amino]-1*H*-pyrimidine-2,4-dione) <Dopan> is a third generation nitrogen mustard that can be absorbed from the gastrointestinal tract. The drug is a bifunctional alkylating agent that acts in a cell cycle phase non-specific manner. Its activity is a result of the formation of an unstable ethylenimmonium ion. Uracil mustard has been administered to treat chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), Hodgkin lymphoma, non-Hodgkin lymphomata of the histiocytic or lymphocytic type, lymphosarcoma, breast cancer, and ovarian cancer. It has generated response rates of 65–75% in hematologic malignancies (Kennedy 1999), although its use has generally been replaced by that of other agents. The drug can be taken orally and is available as capsules. It is usually taken once a week for at least 4 weeks. The typical oral adult dose is 150 μg/kg; the typical oral pediatric dose is 300 μg/kg of body weight.

Pharmacokinetics The blood levels of uracil mustard drop to essentially undetectable levels within 2 h of administration. Less than 1% of the dose is excreted unchanged in the urine.

Adverse Effects Uracil mustard is relatively well tolerated and does not cause alopecia. Adverse effects comprise nausea and vomiting, diarrhea, and dermatitis. They can also include nervousness, irritability, and depression. The bone marrow depressant effects of uracil mustard may result in an increased incidence of microbial infections, delayed healing, and bleeding. Dental work should be completed prior to initiation of therapy or deferred until the blood counts have returned to normal. Uracil mustard may rarely cause stomatitis, associated with considerable discomfort.

Drug Interactions Uracil mustard can raise the concentration of blood uric acid. Drug interactions may arise with antigout agents, such as allopurinol, colchicines, probenecid, or sulfinpyrazone. Dosage adjustment may be necessary. Allopurinol may be preferred to prevent or reverse uracil mustard induced hyperuricemia and the risk of uric acid nephropathy.

Steroid-coupled nitrogen mustards Estramustine phosphate sodium (estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate) <Emcyt> is an orally available synthetic drug that combines estradiol and mechlorethamine through a carbamate link. The molecule was designed with the intent that its estradiol portion would facilitate uptake of the alkylating agent into hormone sensitive prostate cancer cells. It is phosphorylated for better water solubility. This agent exhibits anti-androgenic effects and is used for the palliative treatment of metastatic or progressive prostate cancer. In the mid-1980s, the classification of estramustine as an alkylating agent was called into doubt as it may act as an anti-microtubule agent. Estramustine and its major metabolite bind covalently to microtubule-associated proteins (MAPs) and Tubulin, thereby causing their separation from the microtubules, inhibiting microtubule assembly, and eventually causing their disassembly.

Estramustine is taken orally, at least 1 h before or 2 h after meals. The recommended daily dose is 14 mg/kg body weight, given in three or four doses. Patients should be treated for 30–90 days before a determination is made of the possible benefits of continued therapy. Therapy should be continued as long as the favorable response lasts, and may extend for years.

Pharmacokinetics Estramustine phosphate taken orally is readily dephosphorylated during absorption, and the major metabolites in the blood are estramustine, its estrone analog, and estradiol. Prolonged treatment produces elevated total blood concentrations of estradiol. The metabolic urinary patterns of the estradiol moiety of estramustine phosphate and estradiol itself are very similar, although the metabolites derived from estramustine phosphate are excreted at a slower rate. Estramustine may be poorly metabolized in patients with impaired liver function.

Adverse Effects Adverse effects caused by estramustine include allergic reactions, with hives, difficulty breathing, and blood clots. Accordingly, contraindications for this agent are hypersensitivity to estradiol or nitrogen mustard. Hypertension may be a consequence and blood pressure should be checked periodically. Gynecomastia and impotence are possible estrogenic effects. Careful monitoring of treatment is required in specific risk groups:

- There is an increased risk of thrombosis, including fatal or non-fatal myocardial infarction, in men receiving estrogens for prostatic cancer. Estramustine capsules should be used with caution in patients with a history of thrombophlebitis, thrombosis, or thromboembolic disorders, especially if these events were associated with prior estrogen therapy. Active thrombophlebitis is a contraindication. Caution should also be used in patients with cerebral vascular disease or coronary artery disease. However, estramustine may be given in cases where the actual tumor mass is the cause of the thromboembolic phenomenon and the benefits of therapy may outweigh the risks.
- Exacerbation of preexisting or incipient peripheral edema or congestive heart disease can occur in some patients. Conditions that might be influenced by fluid retention, such as epilepsy, migraine, or renal dysfunction, require careful observation.
- Because estramustine may influence the metabolism of calcium and phosphate, it should be used with caution in patients with renal insufficiency or in patients with metabolic bone diseases that are associated with hypercalcemia. Patients with prostate cancer and osteoblastic metastases are at risk for hypocalcemia and should have calcium levels closely monitored.
- Because glucose tolerance may be decreased, diabetic patients should be carefully observed while receiving estramustine.

Drug Interactions The simultaneous ingestion of calcium-rich food with estramustine needs to be avoided because it may impair the absorption of the drug. Vaccination with a live vaccine should be avoided in patients receiving estramustine. Killed or inactivated vaccines may be administered. However, the response to such vaccines may be diminished due to immune suppression.

DHEA mustard is a steroidal alkylating agent. The natural steroid hormone (produced from cholesterol by the adrenal glands, gonads, adipose tissue, brain, and skin) and dietary supplement dehydroepiandrosterone (DHEA) is a precursor to both estrogen and testosterone. While dehydroepiandrosterone may have an anti-proliferative or pro-apoptotic effect on cancer cell lines (Schulz et al. 1992; Yang et al. 2002), the clinical significance of these effects may be low.

Adverse Effects Due to the estrogenic effects of dehydroepiandrosterone, high doses are associated with an increased risk for developing breast cancer (Tworoger et al. 2006).

Various esters of progestins and alkylating agents have been produced and studied. Prednimustine (Leo-1031) is a steroidal alkylating agent, in which a corticosteroid (pregnadiene-dione-triol) is linked to chlorambucil. The drug has both alkylating and corticosteroid effects (Harrap et al. 1977). It exerts effects in chronic lymphoid leukemia, lymphosarcoma, acute myeloid leukemia, mammary carcinoma, and melanoma.

Phosphoramide mustards The adverse effects of nitrogen mustard drugs are substantial. Phosphoramide mustards are generated by conversion of the base nitrogen mustard into nontoxic prodrugs (Fig. 2.5), which are actively transported into the cancer cells. Once taken up, these agents are enzymatically converted into their active, cytotoxic forms. Phosphoramide

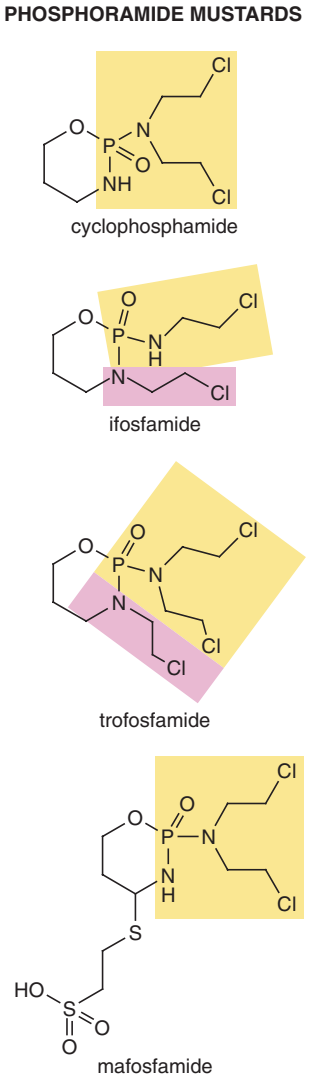


Fig. 2.5 Structures of phosphoramide mustards. The common phosphoramide mustard moiety is highlighted in *yellow*. In ifosfamide, one of the mustard chains is moved to a different nitrogen (shaded in *pink*); this leads to 7-member cross-links after DNA alkylation (the other phosphoramide mustards form 5-chain cross-links). Trofosfamide combines the two possible chain lengths

mustards form DNA inter-strand and intra-strand cross-links at guanine *N*7 positions. This leads to cell death.

Cyclophosphamide was first synthesized in 1958 by Arnold, Bourseaux, and Brock at ASTA Werke (Arnold et al. 1958). The related ifosfamide followed. For these discoveries, Norbert Brock and his team synthesized and screened more than 800 candidate oxazaphosphorine compounds (Brock 1996).

Cyclophosphamide (*N, N*-bis(2-chloroethyl)-2-oxo-1-oxa-3-aza-2u{5}-phosphacyclohexan-2-amine, CPA) <Cytoxan, Endoxan, Neosar, Procytox, Revimmune> is a synthetic alkylating agent. Although cyclophosphamide was intended to be an inactive carrier for the alkylating moiety to be activated in neoplasms that are rich in Phosphamidases, the drug is mainly activated by blood and liver. The predominant use of cyclophosphamide is in combination chemotherapy. Malignancies that are often susceptible to cyclophosphamide treatment include lymphomata (stage III-IV malignant lymphomata, Hodgkin disease, nodular or diffuse lymphocytic lymphoma, mixed-cell type lymphoma, histiocytic lymphoma, Burkitt lymphoma, multiple myeloma) and some leukemias (chronic lymphocytic leukemia, chronic granulocytic leukemia except in acute blastic crisis, acute myelogenous and monocytic leukemia, advanced mycosis fungoides). Unlike other common alkylating agents, cyclophosphamide can occasionally induce remission in acute childhood leukemias, and it can be used to prolong remission. Solid tumors that may respond to the agent include bladder cancer, bone cancer, lung cancer, cervical cancer, endometrial cancer, ovarian adenocarcinoma, breast carcinoma, prostate cancer, testicular cancer, melanoma, cancer of the adrenal cortex, disseminated neuroblastoma, and retinoblastoma.

Cyclophosphamide can be administered orally or intravenously. Many regimens of intravenous and oral cyclophosphamide have been applied.

- When used as the only oncolytic drug therapy, the initial course of cyclophosphamide for patients with no hematologic deficiency usually consists of 40–50 mg/kg given intravenously in divided doses over a period of 2–5 days.
- A cyclophosphamide regimen for adults gives 200 mg per day intravenously for 5 days, followed by oral doses of 50–200 mg per day thereafter, depending on the extent of marrow depression.
- In recent years, cyclophosphamide has been studied as an agent of metronomic chemotherapy, which can be administered at a low dose on an every 6-day schedule or continuously.

The dosages must be adjusted in accord with evidence of anti-tumor activity and tolerable myelosuppression (transient decreases in the total white blood cell count to 2000 cells/mm^3 or more persistent reduction to 3000 cells/mm^3 are tolerated without serious risk of infection if there is no marked granulocytopenia).

Pharmacokinetics The oral bioavailability of cyclophosphamide is over 75%. The blood concentrations of metabolites reach a maximum 2–3 h after an intravenous dose. Plasma protein binding of the unmodified drug is low, but some metabolites are bound to an extent greater than 60%. The unchanged drug has an elimination half-life of 3–12 h with 5–25% of the dose being excreted in the urine without having been altered.

Cyclophosphamide is metabolized in the liver by constitutive P450 enzymes of the CYP2C subfamily, and by drug-inducible enzymes belonging to the P450 subfamily CYP2B. CYP2B6 is the most active catalyst of 4-hydroxylation in the liver. The main active product is 4-hydroxy-cyclophosphamide, which exists in equilibrium with its tautomer, aldophosphamide. Most of the aldophosphamide is oxidized to the therapeutically inactive carboxyphosphamide by the enzyme Aldehyde Dehydrogenase (ALDH), while a small proportion is converted into phosphoramide mustard and acrolein. The active metabolites aldophosphamide and phosphoramide mustard bind to DNA. The mustard generates a highly electrophilic aziridinium species that forms DNA cross-links, which is the key cytotoxic lesion induced in tumors treated with oxazaphosphorines. The cross-links inhibit DNA reduplication and initiate cell death. There is no need for cyclophosphamide dosage modification in patients with renal function impairment.

Adverse Effects Leukopenia is common and increases the risk of infections. Thrombocytopenia or anemia develop occasionally, with high dosages causing pancytopenia. These hematologic effects usually can be reversed by reducing the drug dose or by interrupting treatment. Recovery from leukopenia usually begins 7–10 days after cessation of therapy.

Acrolein is toxic to the bladder epithelium and may lead to hemorrhagic cystitis, often diagnosed by the manifestation of hematuria. The cystitis can predispose to bladder cancer as a delayed adverse effect. It is preventable through the use of aggressive hydration or administration of mesna (sodium 2-sulfanylethanesulfonate) <Uromitexan>. When hemorrhagic cystitis occurs it requires the immediate discontinuation of cyclophosphamide.

Cyclophosphamide is itself carcinogenic, potentially causing transitional cell carcinoma of the bladder, myeloproliferative or lymphoproliferative disorders as long term complications. The agent also interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. Chemotherapy with cyclophosphamide, given to boys before or during puberty, results in a high rate of gonadal dysfunction. Amenorrhea associated with decreased estrogen and increased Gonadotropin secretion develops in a substantial proportion of women treated with cyclophosphamide. Affected patients generally resume regular menses within a

few months after cessation of therapy. The drug is Pregnancy Category D.

Nausea and vomiting commonly occur with cyclophosphamide therapy. Anorexia, abdominal discomfort or pain, and diarrhea may arise. There are isolated incidents of hemorrhagic colitis, oral mucosal ulceration, and jaundice. Cyclophosphamide is a medication that causes dry eye. Remedies can include artificial tears, punctual plugs, and nutritional therapy, as well as adjustments in the patient's environment. Alopecia caused by cyclophosphamide is common, but usually temporary despite continued administration of the drug. Skin rashes occur occasionally in patients receiving the drug. Pigmentation of the skin and changes in nails can arise. Interstitial pneumonitis or interstitial pulmonary fibrosis can be associated with high doses of cyclophosphamide over a prolonged period. SIADH (syndrome of inappropriate Anti-Diuretic Hormone secretion)[12] is a rare adverse effect with the use of cyclophosphamide.

Drug Interactions The rate of metabolism and the leukopenic activity of cyclophosphamide are increased by chronic administration of high doses of phenobarbital. Cyclophosphamide treatment, which causes a marked and persistent inhibition of Cholinesterase activity, potentiates the effect of succinylcholine chloride. This is relevant for patients who need to undergo general anesthesia.

Ifosfamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino] tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide, isophosphamide, IFA) <Mitoxana, Ifex> is isomeric to cyclophosphamide. Ifosfamide generates 7-atom DNA cross-links, whereas cyclophosphamide generates 5-atom cross-links. Ifosfamide is used mainly in the treatment of germ cell testicular cancer, but also for breast cancer, non-Hodgkin lymphoma, soft tissue sarcoma, osteogenic sarcoma, lung cancer, cervical cancer, and ovarian cancer. Ifosfamide is administered intravenously at a dose of 1.2 g/m^2 body surface area per day for five consecutive days. Treatment is repeated every 3 weeks or after recovery from hematologic toxicity, which is reflected in platelet counts of at least 100,000/μL and white blood cell counts of at least 4000/μL.

Pharmacokinetics Ifosfamide exhibits dose dependent pharmacokinetics, which may reflect the saturation of relevant metabolic pathways at high concentrations. At single doses of 3.8–5.0 g/m^2, the plasma concentrations decay biphasically and the mean terminal elimination half-life is about 15 h. At doses of 1.6–2.4 g/m^2/day, the plasma decay is mono-exponential and the terminal elimination half-life is about 7 h. 4-carboxyifosfamide, thiodiacetic acid, and cysteine conjugates of chloroacetic acid are the major urinary metabolites of ifosfamide, with only small amounts of 4-hydroxyifosfamide and acrolein.

Ifosfamide is a prodrug alkylating agent that undergoes metabolic activation by the hepatic Cytochrome P450 monooxygenase system. 4-hydroxylation is primarily catalyzed by CYP3A enzymes, with minor contributions made by other CYP enzymes, including CYP2B6. Hydroxylation at C4 forms the unstable intermediate 4-hydroxyifosfamide. It initiates the metabolic pathway that ultimately results in fragmentation of the oxazaphosphorine to yield 2-propenal (acrolein) and an electrophilic phosphoramide mustard (Fig. 2.6). While acrolein can exhibit cytotoxic effects by binding covalently to proteins, the phosphoramide mustard possesses DNA alkylating activity (Weber and Waxman 1993a). Whereas both cyclophosphamide and ifosfamide are activated by Cytochromes P450 2B1 and 2C6/2C11, only ifosfamide is also activated by Cytochrome P450 3A.

N-dechloroethylation of the parent drug yields monofunctional metabolites that have lost their DNA cross-linking activity and therapeutic efficacy. In contrast to the metabolism of cyclophosphamide, the *N*-dechloroethylation pathway may consume up to 50 % of the administered ifosfamide. It is associated with the production of the neurotoxic metabolite chloroacetaldehyde.

Adverse Effects Dose fractionation and hydration aid substantially in limiting the toxicities of ifosfamide. Alopecia (over 80 %), nausea and vomiting (60 %), and temporary ridging of the nails may occur under therapy. Ifosfamide produces less myelotoxicity (mainly leukopenia) than cyclophosphamide and also exhibits little cross-resistance. The metabolic ifosfamide product acrolein can contribute to the hemorrhagic cystitis associated with oxazaphosphorine therapy. Hemorrhagic cystitis may require discontinuation of the drug. The adverse effect may be preventable with extensive hydration and co-administration of mesna <Uromitexan>. Ifosfamide is Pregnancy Category D. The drug is excreted into the breast milk and nursing should be avoided during treatment.

Some patients treated with ifosfamide develop severe neurotoxicity and urotoxicity, which may both be associated with the formation of chloroacetaldehyde via N-dechloroethylation, an alternative Cytochrome P450 catalyzed metabolic pathway, which deactivates the drug through side chain oxidation. It yields the inactive, mono-functional alkylating metabolites 2- and 3-dechloroethyl-ifosfamide and produces the toxic byproduct chloroacetaldehyde. This pathway, catalyzed mainly by CYP3A4 and CYP2B6, may consume up to 50 % of the therapeutic dose of ifosfamide. Encephalopathy (manifested in somnolence, confusion, depressive psychosis, hallucinations, dizziness, disorientation, cranial nerve dysfunction, seizures, or coma) is a serious, sometimes fatal,

[12] The syndrome of inappropriate antidiuretic hormone secretion (SIADH, Schwartz-Bartter syndrome) is characterized by an excessive release of Anti-Diuretic Hormone, typically from the posterior pituitary gland. The result is often dilutional hyponatremia, in which the sodium levels remain normal but the total body fluid increases.

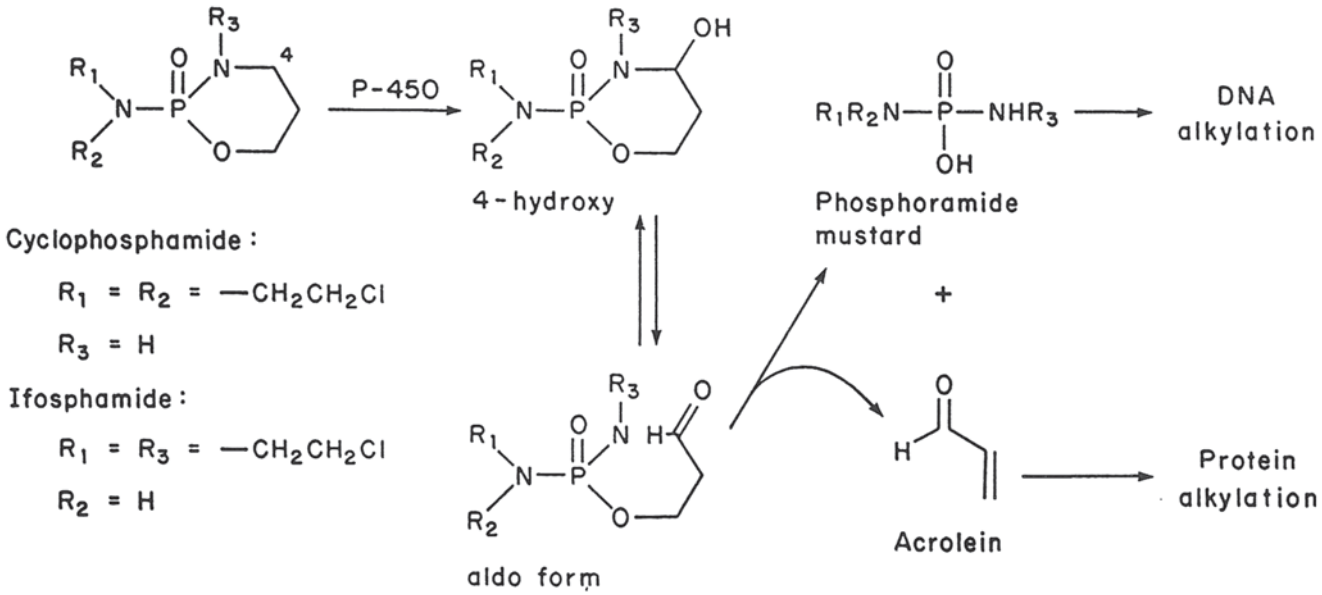


Fig. 2.6 CYP450 catalyzed metabolism of ifosfamide and cyclophosphamide via the 4-hydroxylation pathway. The prodrugs are converted by Cytochrome P450 enzymes into the active agents phosphoramide mustard and acrolein. (Weber and Waxman 1993a)

and limiting adverse effect. This adverse reaction is particularly associated with the oral administration of ifosfamide, where it may affect more than 30% of patients. It is caused by drug metabolites that agonize AMPA/Kainate Receptors and induce cellular acidification in cortical neurons. The metabolite chloroacetaldehyde may lead to the formation of chloroacetic acid and then to S-carboxymethylcysteine (SCMC) after conjugation with the amino acid cysteine. S-carboxymethylcysteine can account for about 80% of the administered dose of ifosfamide (Küpfer et al. 1996), which is then further degraded metabolically to thiodiglycolic acid (TDGA) (Hofmann et al. 1991). The S-carboxymethylcysteine chemical structure shares a close similarity with the excitatory neurotransmitter glutamic acid and may therefore affect glutamatergic neurons (Chatton et al. 2001).

Trofosfamide (*N, N*,3-tris(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amide 2-oxide, TFF, TRO) (ASTA Z 4828) <Ixoten, Genoxal Trofosfamida> is an orally bioavailable oxazaphosphorine prodrug. The activation product, isophosphoramide mustard alkylates DNA to form DNA-DNA cross-links, which may cause inhibition of DNA, RNA, and protein synthesis, thus resulting in tumor cell death. Trofosfamide is used in first and second-line treatments of soft tissue sarcomata, and it may be effective as palliative treatment in non-Hodgkin lymphoma. Trofosfamide therapy has an overall response rate of 50–80% at oral daily doses of 150 mg. In alternative regimens, remissions have been accomplished by administering trofosfamide metronomically at low dose.

Pharmacokinetics Trofosfamide is a substrate for Cytochrome P450s. After oral administration, the prodrug is mainly metabolized in the liver to its active 4-hydroxy derivative ifosfamide, which is then converted via hydroxylation at the cyclic C-4 position to the active isophosphoramide mustard. Compared with ifosfamide, trofosfamide is much more strongly hydroxylated to the tumoristatically active 4-OH derivatives with a 20–30 times higher mean area under the concentration-time curve[13]. Based on the lipid solubility, small molecular size, and minimal binding to plasma and tissue proteins, there is penetration by trofosfamide through the blood-brain barrier.

Adverse Effects Trofosfamide therapy is rather non-toxic. The maximum tolerated dose is around 125 mg/m^2. Adverse effects include dose dependent hematotoxicity and, rarely, hemorrhagic cystitis, nausea and vomiting.

Mafosfamide (2-{(2-[bis(2-chloroethyl)amino]-2-oxido-1,3,2-oxazaphosphinan-4-yl}thio)ethanesulfonic acid) is a synthetic oxazaphosphorine derivative that alkylates DNA, forming DNA cross-links and inhibiting DNA synthesis. Although closely related to cyclophosphamide, mafosfamide

[13] The area under the drug (plasma/serum/blood) concentration-time curve (AUC) reflects the actual body exposure to the drug after administration of one dose. It is typically visualized as a graph of the plasma drug concentration (on the y-axis) over time (on the x-axis) and is expressed in mg*h/L. The AUC is inversely proportional to the clearance of the drug. When a drug follows linear kinetics, the AUC is directly proportional to the dose.

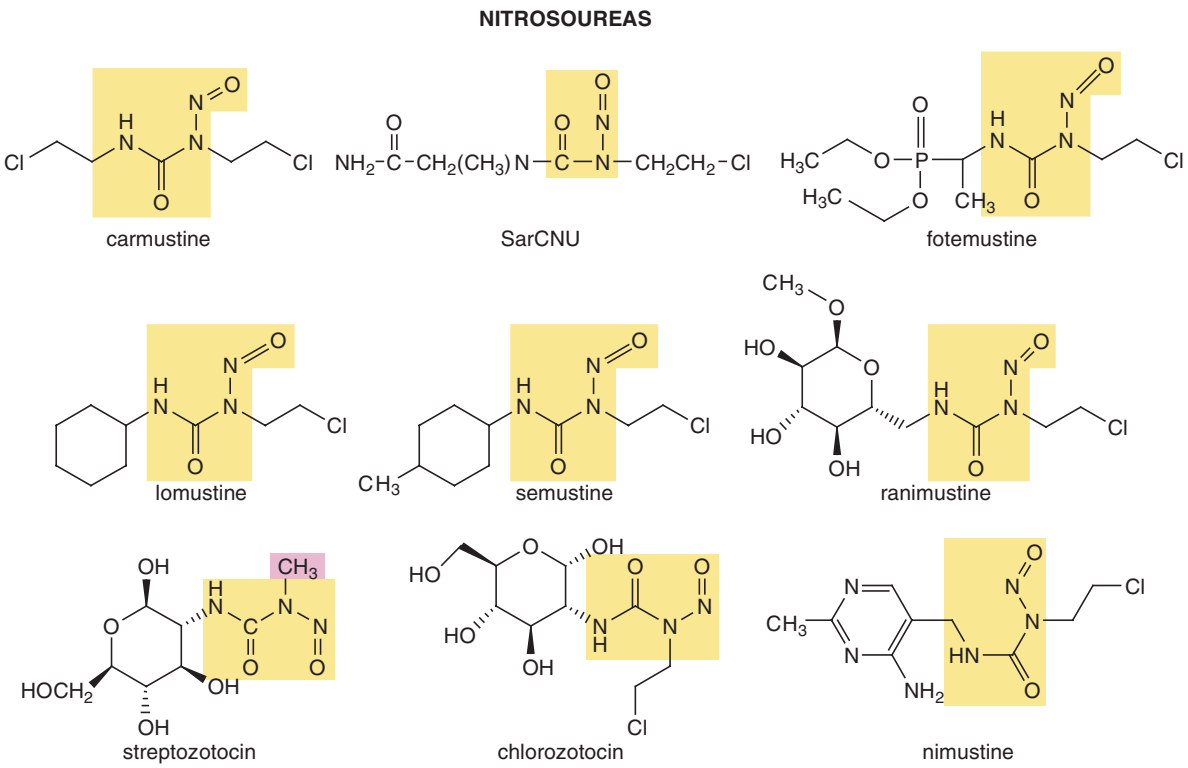


Fig. 2.7 Structures of nitrosoureas. All drugs in this class contain the nitrosoureea moiety (highlighted in *yellow*). With the exception of streptozotozin (*pink box*), all nitrosoureas have a chloroethyl side chain attached to the nitrogen adjacent to the N=O group

does not require hepatic activation to generate its active metabolite 4-hydroxy-cyclophosphamide. Accordingly, it is potentially useful in the intrathecal treatment of neoplastic meningitis.

The mustard pharmacophore is –N(CH_2CH_2Cl)$_2$.
The two arms of mustard drugs can cross-link DNA strands.
Nitrogen mustard also damages DNA through the production of reactive nitrogen species.

First generation nitrogen mustards cannot be taken orally.
In second generation nitrogen mustards, electron withdrawing aromatic radicals reduce the reactivity and permit oral use.
In third generation nitrogen mustards, the pyrimidine nucleus carrier for the mustard pharmacophore permits oral administration.
Steroid-coupled nitrogen mustards combine the effect of the alkylating agent with a steroid for selective uptake or combined anti-cancer action.
In phosphoramide mustards, the base nitrogen mustard is administered as non-toxic prodrug that is actively transported into the cancer cells and enzymatically converted into its cytotoxic forms.

2.1.2 Nitrosoureas

Under a contract with the U.S. National Cancer Institute (NCI), a group led by John Montgomery at the Southern Research Institute synthesized nitrosoureas, which contain a nitroso group and urea (Fig. 2.7). These are alkylating agents that cross-link DNA. Due to their lipophilicity, they cross the blood-brain barrier and can be applied to the treatment of brain tumors.

Adverse Effects Pulmonary toxicity is a common problem associated with nitrosoureas. Acute leukemia or bone marrow dysplasia can be complications in patients following long term nitrosourea therapy. Representatives of this drug class may affect the vision.

The alkylating agent carmustine (BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea) <BiCNU> cross-links DNA during all phases of the cell cycle, causing a disruption of DNA function, and resulting in cell cycle arrest and apoptosis. This agent also carbamoylates proteins, including DNA repair enzymes, which leads to an enhanced cytotoxic effect. As an irreversible Glutathione Reductase inhibitor, carmustine renders cells susceptible to oxidative stress[14]. Being highly lipophilic, carmustine is particularly useful for the treatment of brain tumors, owing to its ability to cross the blood-brain barrier. The drug is used as a single agent or in combination therapy in the palliative treatment of several types of brain cancer (including glioma and glioblastoma, medulloblastoma, astrocytoma, ependymoma, and metastatic brain

[14] Many alkylating agents readily react with thiol containing small molecules, such as cysteine and Glutathione. The resulting reduced cellular Glutathione levels can contribute to cytotoxicity.

cancers), multiple myeloma (in combination with prednisone), Hodgkin and non-Hodgkin lymphomata (as secondary combination chemotherapy in patients, who fail to respond to or relapse under primary therapy). The recommended dose of carmustine as a single agent in previously untreated patients is 150–200 mg/m^2 intravenously every 6 weeks. This may be given as a single dose or divided into daily injections of 75–100 mg/m^2 on two successive days. When administered in combination with other myelosuppressive drugs or in patients whose bone marrow reserve is depleted, dose reduction may be required. Doses subsequent to the initial one should be adjusted according to the hematologic response. A repeat course should not be given until the blood cell counts have recovered, which is usually after 6 weeks because the hematologic toxicity is delayed and cumulative.

Pharmacokinetics Intravenously administered carmustine is degraded within 15 min. About half of the drug given enters the cerebrospinal fluid. Its metabolism occurs in the liver prior to its excretion in the urine (60–70 %) and breath (about 10 %).

Adverse Effects Pulmonary and bone marrow toxicities are functions of lifetime cumulative dose. Pulmonary toxicity, characterized by infiltrates or fibrosis, occurs 1 week to 3.5 years after treatment. Risk factors include prolonged therapy with total doses reaching 1400 mg/m^2 and a past history of lung disease. Delayed onset progressive pulmonary fibrosis can occur up to 17 years after the treatment of adolescents with cumulative doses of 770–1800 mg/m^2 combined with cranial radio-therapy for intracranial tumors. Thrombocytopenia arises at about 4 weeks post administration and persists for 1–2 weeks. Leukopenia occurs at 5–6 weeks after a dose of carmustine and persists for 1–2 weeks. The thrombocytopenia is generally more severe than the leukopenia. Weekly monitoring of platelet and white blood cell counts is recommended as a basis for patient-specific adjustments to the dosage regimens.

Nausea and vomiting, appearing within 2 h and lasting 4–6 h, are frequent after intravenous infusion of carmustine. The prior administration of anti-emetics can alleviate, and sometimes prevent this adverse effect. Renal abnormalities (progressive azotemia, decrease in kidney size, renal failure) may arise in patients who have received large cumulative doses after prolonged therapy. Rapid infusion of carmustine may produce intensive flushing of the skin and suffusion of the conjunctiva within 2 h, lasting about 4 h. It is also associated with burning at the site of injection. Accidental contact of reconstituted carmustine with the skin can cause transient hyper-pigmentation of the affected areas. Rare adverse effects comprise neuroretinitis, chest pain, headache, allergic reaction, hypotension, and tachycardia. The drug is Pregnancy Category D.

Drug Interactions Drug interaction with cimetidine, amphotericin B, digoxin, or phenytoin may arise and require adjustment. There is no cross-resistance of carmustine with other alkylating agents.

Drug Resistance The emergence of carmustine resistant tumor cell populations is a common problem under treatment (Weber and Waxman 1993b). Resistance may be due to increased expression of Glutathione S-Transferase, activation of DNA repair mechanisms, or altered cellular transport of the agent. Specifically, carmustine anti-tumor efficacy is inversely correlated to the activity of the DNA repair enzyme MGMT (O^6-Methylguanine-DNA Methyltransferase).

SarCNU (sarcosinamide chloroethylnitrosourea, 2-chloroethyl-3-sarcosinamide-1-nitrosourea) (NSC364432) is an alkylating chloroethylnitrosourea that forms covalent linkages with nucleophilic centers in DNA, causing depurination, base pair miscoding, strand scission, and DNA cross-linking. It does not form an organic isocyanate because the *N*3 position is blocked with a methyl group. The carrier group of SarCNU is the amino acid derivative methylglycinamide. The compound was originally synthesized under the hypothesis that the carrier group would allow for uptake of the drug through amino acid transporters. SarCNU enters cells via transport through the Extraneuronal Transporter for Monoamine Transmitters (EMT, Extra-Neuronal Catecholamine Uptake-2 Carrier). Its cytotoxicity correlates positively with the expression levels of EMT as well as inversely with the DNA repair enzymes MGMT and ERCC2 (Chen et al. 1999). SarCNU is effective against gliomata.

Adverse Effects SarCNU has pulmonary toxicity (Webster et al. 2005). HIV patients have an increased risk of severe SarCNU toxicity.

Fotemustine (diethyl (1-{[(2-chloroethyl)(nitroso)carbamoyl]amino}ethyl)phosphonate) (S10036) <Muphoran> is a chloroethylating nitrosourea. It includes a bioisostere of alanine (1-amino ethylphosphonic acid) in order to facilitate cellular penetration and passage across the blood-brain barrier. Fotemustine alkylates guanine by forming chloroethyl adducts at the 6 position, resulting in *N*1 guanine-*N*3 cytosine cross-linkages. This leads to an inhibition of DNA synthesis, cell cycle arrest, and finally cell death. As a result of its alkylating and carbamoylating effect, the drug exerts a potent cytostatic activity on cells in cycle, inducing an accumulation of cells in G_2/M phase. Fotemustine is indicated for disseminated malignant melanoma. An interval of 8 weeks after the start of induction treatment and 3 weeks after each cycle of maintenance treatment is recommended. The agent should not be administered to patients who received chemotherapy in the previous 4 weeks (6 weeks if the treatment included nitrosoureas).

Pharmacokinetics Fotemustine is lipophilic and crosses the blood-brain barrier. After infusion, the blood levels decline to 0 within 3 h. The binding to plasma proteins is 25–30 %,

mostly to Acid α-1-Glycoprotein and Albumin. 50–60 % of the dose administered is excreted in the urine, 5 % in the feces.

Adverse Effects Fotemustine may cause retinal atrophy or retinal detachment. Ophthalmoscopic examinations should be carried out routinely during treatment. Delayed hematologic toxicity is characterized by thrombocytopenia (nadir[15] 4–5 weeks after the first administration) and leukopenia (nadir 5–6 weeks). Moderate nausea and vomiting may occur during 2 h following the injection. The drug is Pregnancy Category D.

Lomustine (CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-urea) <CeeNU> is a nitrosourea that carbamoylates, alkylates and cross-links DNA, thereby inhibiting DNA and RNA synthesis. This agent also carbamoylates amino acids in proteins, resulting in the disruption of RNA processing and protein function. Lomustine is lipophilic and crosses the blood-brain barrier. Malignancies for which lomustine is used include primary and metastatic brain tumors, Hodgkin lymphoma (as secondary combination chemotherapy in patients who fail to respond to or relapse under primary therapy), multiple myeloma, breast cancer, ovarian cancer, pancreatic cancer, lung cancer, and melanoma. The drug can be taken orally.

- As a single agent in previously untreated patients, the recommended oral dose every 6 weeks for adults and children is 100–150 mg/m^2.
- In individuals with compromised bone marrow function, the dose may need to be reduced to 100 mg/m^2 every 6 weeks.
- When lomustine is used in combination with other myelosuppressive drugs, dose reduction may be required.

Doses subsequent to the initial dose need to be adjusted according to the hematologic response of the patient to the preceding dose. A repeat course of lomustine should not be given until blood cell counts have returned to acceptable levels.

Pharmacokinetics The serum half-lives of the metabolites range from 16 h to 2 days. The tissue levels are comparable to the blood levels at 15 min after intravenous administration. About half of the drug given is excreted in the urine in the form of degradation products within 24 h.

Adverse Effects The most common and severe toxic effect of lomustine is bone marrow suppression 4–6 weeks after drug administration, notably thrombocytopenia (occurs at about 4 weeks post-administration and persists for 1–2 weeks) and leucopenia (occurs at 5–6 weeks and persists for 1–2 weeks), which may contribute to bleeding and overwhelming infections in already compromised patients. Anemia also occurs, but is less frequent and less severe than thrombocytopenia or leukopenia. The occurrence of acute leukemia and bone marrow dysplasia is possible in patients following long term nitrosourea therapy.

Pulmonary toxicity, characterized by pulmonary infiltrates or fibrosis can arise after an interval of 6 months or longer (up to 17 years) from the start of therapy, usually consecutive to cumulative doses greater than 1100 mg/m^2. Renal abnormalities consisting of progressive azotemia (elevated blood urea nitrogen), decrease in kidney size, and renal failure can occur in patients consecutive to large cumulative doses after prolonged therapy.

Nausea and vomiting may constitute acute adverse effects 3–6 h after an oral dose that usually last less than 24 h. Prior administration of anti-emetics is effective in diminishing and sometimes preventing this toxicity. Nausea and vomiting can also be reduced if lomustine is administered to fasting patients. Stomatitis, alopecia, optic atrophy, visual disturbances (possibly blindness), lethargy, ataxia, or dysarthria may arise infrequently. Lomustine is Pregnancy Category D.

Drug Resistance Lomustine does not encounter cross-resistance with other alkylators.

Semustine (methyl-CCNU, *N*-(2-chloroethyl)-*N*′-(4-methylcyclohexyl)-*N*-nitrosourea) is a methylated derivative of carmustine. As an alkylating agent, semustine forms covalent linkages with nucleophilic centers in DNA, causing depurination, base pair miscoding, strand scission, and DNA cross-linking. Together, these effects result in cytotoxicity. Semustine has been applied to the treatment of brain tumors, lymphomata, colorectal cancer, and stomach cancer. It is not widely in use because it has not proven clearly superior to other treatments for these diseases. Semustine is taken orally as a capsule at bedtime, 3–4 h after a meal.

Adverse Effects A common adverse effect of semustine is prolonged myelosuppression, which may result in infection and bleeding. Therefore, the required interval between courses of semustine is longer than with other agents. The drug causes nausea and vomiting. Sometimes anorexia or loss of appetite persists after nausea and vomiting have subsided. There is an increased risk for the development of secondary leukemia. Fetal abnormalities are likely if pregnancy occurs while taking this drug. Rare adverse effects include sores in the mouth or on the lips, hepatotoxicity, renal toxicity, disorientation, difficulty walking, blurred vision, and pulmonary fibrosis.

Ranimustine (methyl 6-({[(2-chloroethyl)(nitroso)amino] carbonyl}amino)-6-deoxy-α-D-glucopyranoside, MCNU)

[15] The nadir (from the Arabic term for opposite) is the point of the celestial sphere that is directly opposite the zenith and vertically downward from the observer. Its use has been broadened to indicate the lowest point of a state or a relationship. In chemotherapy, the nadir describes the lowest number in blood cell counts that occurs as a consequence of bone marrow suppression.

<Cymerin> is a chloroethylnitrosourea derivative that inhibits proliferation and growth of tumor cells by alkylation and cross-linkage of DNA strands within tumor cells. It is approved in Japan for the treatment of chronic myelogenous leukemia and polycythemia vera.

Streptozotocin was discovered in the late 1950s as an antibiotic in a strain of the soil microbe Streptomyces achromogenes (Vavra et al. 1959). Streptozotocin (Streptozocin, STZ, 1-methyl-1-nitroso-3-[2,4,5-trihydroxy-6- (hydroxymethyl) oxan-3-yl]-urea) <Zanosar, Sicor, Teva> is a glucosamine-nitrosourea compound, which is sufficiently similar to glucose to be transported into the cell by the glucose transport protein GLUT2, but it is not recognized by other glucose transporters. This underlies its preferential toxicity to pancreatic β-cells, as these have relatively high levels of GLUT2. Accordingly, streptozotozin is used for the treatment of pancreatic islet cell cancer, but is generally limited to patients whose cancer cannot be resected surgically. Streptozotocin can reduce the tumor burden and ameliorate symptoms, such as hypoglycemia due to excessive Insulin secretion by insulinomata.

- A typical dose is 500 mg/m^2/day by intravenous injection or infusion for 5 days, repeated every 4–6 weeks. Dose escalation with this schedule is not recommended.
- An initial intravenous dose of 1000 mg/m^2 of body surface area can be given at weekly intervals for the first two courses. There is an option for dose escalation in subsequent courses for patients who have not achieved a therapeutic response and who have not experienced significant toxicity. On this schedule, the median time to onset of response is about 17 days (median total dose about 2000 mg/m^2) and the median time to maximum response is about 35 days (median total dose about 4000 mg/m^2).

Adverse Effects Because of its inherent renal toxicity, therapy with streptozotocin should be limited to patients with symptomatic or progressive metastatic disease. Many patients experience dose dependent renal toxicity, as manifested in azotemia, anuria, hypophosphatemia, glycosuria, and renal tubular acidosis. Such toxicity is cumulative and may be severe or fatal. Therefore, a single dose of 1500 mg/m^2 body surface area should not be exceeded.

Most patients experience severe nausea and vomiting, occasionally requiring discontinuation of the drug therapy. Some patients encounter diarrhea. Streptozotocin is generally non-myelosuppressive. However, rare cases of fatal hematologic toxicity are possible. Mild to moderate abnormalities of glucose tolerance arise in some patients. Local inflammation (edema, erythema, burning, tenderness) can be a consequence of extravasation of the product, possibly leading to tissue necrosis. The drug is Pregnancy Category D.

Drug Interactions Streptozotocin may demonstrate additive toxicity when used in combination with other cytotoxic agents. This drug should not be used concomitantly with other potential nephrotoxins. Streptozocin prolongs the elimination half-life of doxorubicin and thus can lead to severe bone marrow suppression. A reduction of the doxorubicin dosage may be required.

Chlorozotocin (1-(2-Chloroethyl)-1-nitroso-3-[(2R,3R,4S,5R)-3,4,5,6-tetrahydroxy-1-oxohexan-2-yl]urea, CZT) is a glucose linked chloroethylnitrosourea (Johnston et al. 1975). The glucose is intended to diminish bone marrow toxicity. The agent alkylates DNA and proteins, induces the formation of inter-strand DNA and DNA-protein cross-links, and causes DNA strand breakage, thereby damaging DNA and resulting in cell death.

Adverse Effects Chlorozotocin is a mutagen, but it is less myelotoxic than most other nitrosoureas.

Nimustine (ACNU) (CS 439) <Nidran> alkylates and cross-links DNA, thereby causing DNA fragmentation, inhibition of protein synthesis, and cell death. The drug is in use in China and Japan.

Nitrosoureas are alkylating agents that cross-link DNA. Due to their lipophilicity, they cross the blood-brain barrier. Pulmonary toxicity is a common adverse effect of nitrosoureas. Acute leukemia or bone marrow dysplasia can be long term complications.

2.1.3 Triazenes and Hydrazines

Triazenes [3,3-bis(2-chloro ethyl)-l-triazeno] imidazole-carboxamides[16] constitute a series of prodrugs synthesized in the 1960s (Shealy et al. 1962; Shealy and Krauth 1966). They arose empirically out of the synthetic program at the U.S. National Cancer Institute for agents potentially useful in the inhibition of purine metabolism. Although these compounds had no such activity, they displayed anti-tumor effects in animal experiments prompting their further development. Triazene molecules have three adjacent nitrogen atoms, two of which are joined by a double bond, and the 3rd is affixed by a single bond (Fig. 2.8). Their activation occurs with the release of the single-bonded nitrogen, leaving the highly reactive double-bonded diazonium fragment exposed. Triazenes are anti-proliferative agents. When activated, they

[16] Carboxamide moieties are essential to the structures of triazenes, various small molecule kinase inhibitors (sunitinib, sorafenib, tandutinib, lonafarnib, veliparnib), the Topoisomerase inhibitor asculariine isethionate, the purine antagonist tiazofurin, the HSP90 inhibitor AUY922, and the hydroxamate MMP inhibitor prinomastat.

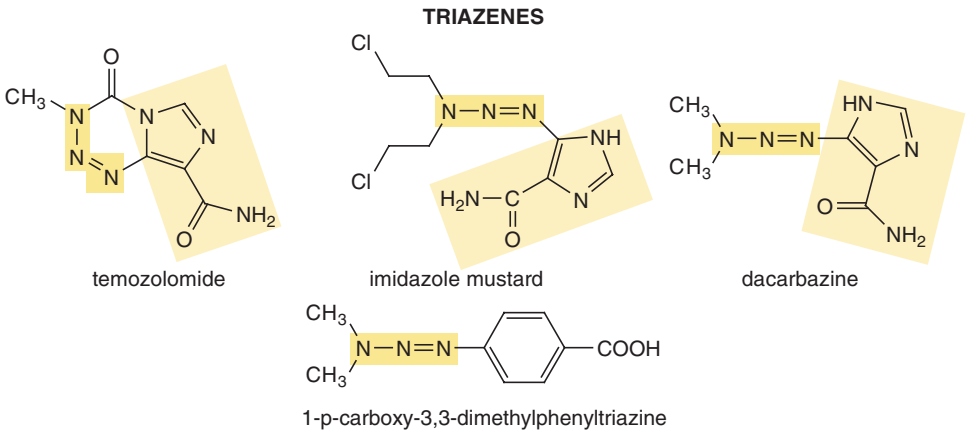


Fig. 2.8 Structures of triazene anti-cancer drugs. The common functional triazene moiety is highlighted in *yellow*. The *lighter shade* of *yellow* indicates the imidazole-4-carboxamide moiety that is common to three of the four representatives in this drug class

bind to cellular DNA and damage it. Both temozolomide and dacarbazine are prodrugs of the active monomethyl triazeno imidazole carboxamide (MTIC).

Temozolomide (methazolastone) (SCHS2.365, NSC 362856) <Temodar, Temodal> was first synthesized by Malcolm Stevens in Birmingham, Great Britain in the early 1980s and was approved for use in the U.S. in 1999. Temozolomide is an imidazotetrazine derivative of dacarbazine. Its indications are newly diagnosed glioblastoma multiforme in adult patients (concomitantly with radio-therapy and then as maintenance treatment) and refractory anaplastic astrocytoma (in patients who have experienced disease progression on a drug regimen containing nitrosourea and procarbazine). The drug may also be used in the treatment of metastatic melanoma and is effective against mycosis fungoides. Temozolomide is available in capsules and is taken orally. The initial dosage is determined based on body height and weight. The typical dose for the first treatment cycle is 150 mg per day taken for five consecutive days, with each treatment cycle lasting 28 days. The number of treatment cycles depends on patient tolerane for the drug and its effectiveness in treating the cancer. The maximum tolerated dose is 1000 mg/m^2/cycle in both children and adults. Dose adjustments may be required based on the nadir neutrophil and platelet counts in the previous cycle as well as the neutrophil and platelet counts at the time of initiating the next cycle.

Pharmacokinetics Temozolomide is nearly 100% bioavailable when given on a completely empty stomach. Its absorption is affected by food. Peak plasma concentration is reached 1 h after intake. Bioequivalence exists between identical doses of the oral route and infusion over 90 min (infusion over a shorter or longer period of time may result in suboptimal dosing). The agent penetrates well into the central nervous system, and is therefore used primarily to treat refractory astrocytomata in adult patients. Temozolomide is metabolized by CYP450 enzymes to monomethyl triazeno imidazole carboxamide not only in the liver, but at all sites. The conversion is spontaneous, but pH dependent. The metabolic conversion yields the short-lived active compound monomethyl triazeno imidazole carboxamide (MTIC), the cytotoxicity of which is exerted primarily via DNA methylation at the O^6 and N^7 positions of guanine (Fig. 2.9). Inactive metabolites of temozolomide are amino imidazole carboxamide and temozolomide acid metabolite. Renal and hepatic excretions play a minor role in drug elimination.

Adverse Effects Women and patients over 70 years of age have about 5% lower clearance than men under 70, resulting in a higher incidence of grade 4 neutropenia and thrombocytopenia in the first cycle of therapy. Patients may experience myelosuppression, including prolonged pancytopenia, which can result in aplastic anemia. Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, can occur after temozolomide treatment. Prophylaxis against Pneumocystis carinii pneumonia is required for all newly diagnosed glioblastoma multiforme patients receiving concomitant temozolomide and radio-therapy for the 42 day regimen. Temozolomide is Pregnancy Category D. Although there is fetal risk, it may be acceptable in view of the benefits from temozolomide use in pregnant women.

Drug Interactions In astrocytoma therapy, temozolomide may be given in conjunction with irinotecan without pharmacokinetic interaction. Temozolomide clearance is not affected by CYP450 inducing drugs, nor does administration of temozolomide competitively inhibit the metabolism of those drugs. Temozolomide dosing may require interruption or discontinuation during concomitant radio-therapy (Table 2.2).

Imidazole mustard (5-[3,3-bis(2-chloroethyl)-1-triazeno] imidazole-4-carboxamide) (NSC 82196) alkylates DNA preferentially at guanine residues resulting in DNA interstrand cross-links, inhibition of DNA reduplication, and suppression of RNA and protein synthesis.

Pharmacokinetics Gastrointestinal absorption after oral administration is poor. The plasma half-life after intravenous administration is about 2 h. The drug has minimal penetration of the blood-brain barrier. The primary route of elimination is

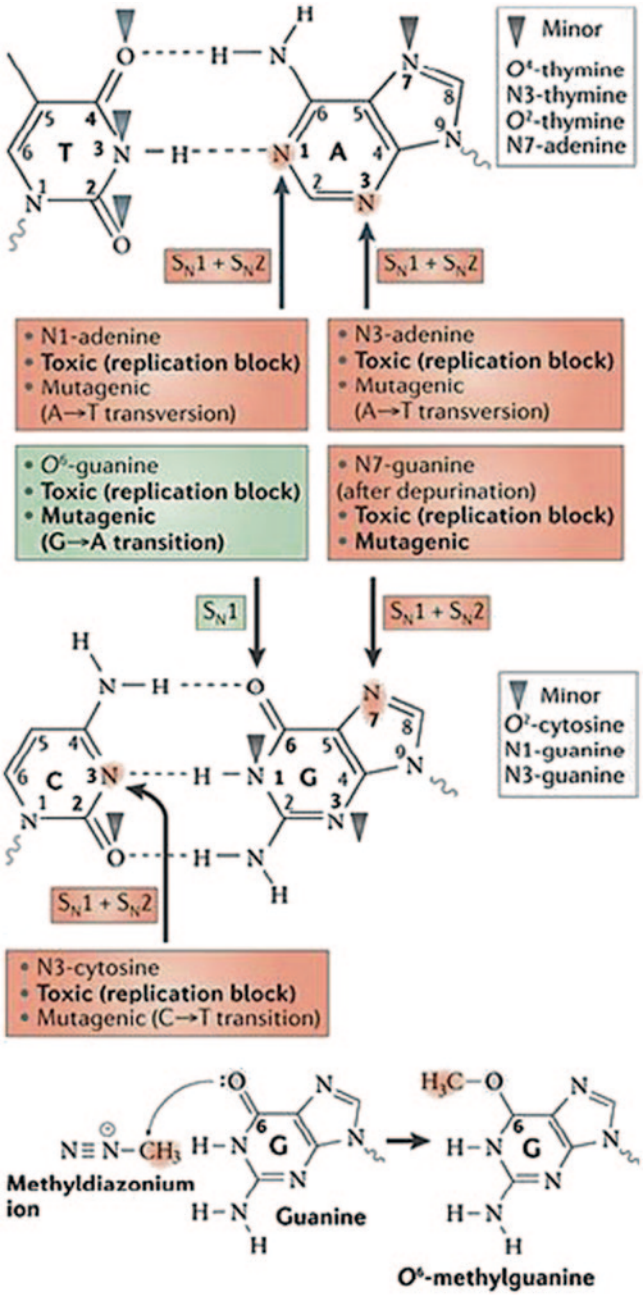


Fig. 2.9 Alkylation sites in DNA. **a** Alkylating agents react with the nitrogen and oxygen atoms of DNA bases to form covalent alkyl lesions. The major sites of alkylation on the DNA bases and biological effects of alkylation are shown in *red* and *green*, with minor lesions denoted by *grey arrow heads*. **b** An example of a DNA alkylation reaction between the methyldiazonium ion of the chemotherapeutic alkylating agent temozolomide with the O^6-position of guanine to form the O^6-methylguanine DNA lesion. (Fu et al. 2012)

renal, with greater than 60 % excreted in the urine within 6 h after i.v. administration. Approximately 2/3 of the excreted drug is metabolized to ionic transformation products.

Dacarbazine (5-(3,3-dimethyl-1-triazenyl)imidazole-4-carboxamide) (NSC 45388) <DTIC, DTIC-Dome, Imidazole Carboxamide> is a triazene derivative that alkylates and cross-links DNA during all phases of the cell cycle, resulting in a disruption of DNA function, cell cycle arrest, and apoptosis. Dacarbazine gained U.S. FDA approval in 1975. It is the first-line chemotherapy for metastatic malignant melanoma without cerebral metastasis and has a 15 % response rate. The drug is used in the ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) regimen for Hodgkin lymphoma. It is administered by injection or intravenous infusion under immediate medical supervision.

- For malignant melanoma, the recommended dosage is 2–4.5 mg/kg/day for 10 days, which may be repeated at 4 week intervals.
- An alternative regimen uses 250 mg/m^2 for 5 days, which may be repeated every 3 weeks.
- For Hodgkin disease, the recommended dose is 150–375 mg/m^2 per day for 5 days, in combination with other effective drugs. Treatment may be repeated every 4 weeks.

Pharmacokinetics At therapeutic concentrations, dacarbazine is not appreciably bound to plasma proteins. Following injection, its clearance from the blood is biphasic with an initial half-life of 20 min and a terminal half-life of 5 h. In patients with renal and hepatic dysfunctions, these half-lives are prolonged. Dacarbazine is metabolized by CYP450 enzymes to monomethyl triazeno imidazole carboxamide (MTIC) only in the liver. The *N*-demethylation involved in MTIC formation is catalyzed by CYP1A1, CYP1A2, and CYP2E1 and is required for anti-tumor activity. The hydroxymethyl and the monomethyl intermediates derived from MTIC are unstable. The monomethyl species, or the methyl carbonium ion formed from it, is capable of methylating DNA and alkylating the O^6 position of guanine. The agent poorly penetrates the central nervous system. About 40 % of the unchanged drug is excreted into the urine.

Adverse Effects Symptoms of anorexia, nausea, and vomiting affect over 90 % of patients with the initial few doses. The vomiting lasts 1–12 h and is incompletely and unpredictably palliated with phenobarbital or prochlorperazine. Hematopoietic depression is one of the most common toxicities and involves primarily the leukocytes and platelets. Leukopenia and thrombocytopenia may be severe enough to cause death. Anemia can sometimes occur. Other serious adverse effects are sterility, which is possibly permanent, and anaphylactic reactions. Liver problems, headaches, fatigue, and diarrhea may arise. Dacarbazine is Pregnancy Category C (it is unknown whether the drug can harm a fetus). It is not known whether dacarbazine passes into breast milk.

Drug Resistance The principal mechanism of resistance arises via the DNA repair protein O^6-Alkylguanine DNA Alkyl Transferase (ATase). In an auto-inactivating reaction,

Table 2.2 Temozolomide dosing interruption or discontinuation during concomitant radiotherapy. (adapted from [http://www.temodar.com/temodar/application?origin=index.jsp...refresh&pageid=hp&__event=goto_support_pharmacyinformation])

Toxicity	TMZ interruption[a]	TMZ discontinuation
Absolute neutrophil count	≥ 0.5 and $< 1.5 \times 10^9$/L	$< 0.5 \times 10^9$/L
Platelet count	≥ 10 and $< 100 \times 10^9$/L	$< 10 \times 10^9$/L
CTC non-hematologic toxicity (except for alopecia, nausea, vomiting)	CTC grade 2	CTC grade 3 or 4

TMZ temozolomide, *CTC* Common Toxicity Criteria

[a] Treatment with concomitant temozolomide may be continued when all of the following conditions are met: absolute neutrophil count $\geq 1.5 \times 10^9$ /L; platelet count $\geq 100 \times 10^9$ /L; common toxicity criteria = non-hematologic toxicity grade 1 (except for alopecia, nausea, vomiting)

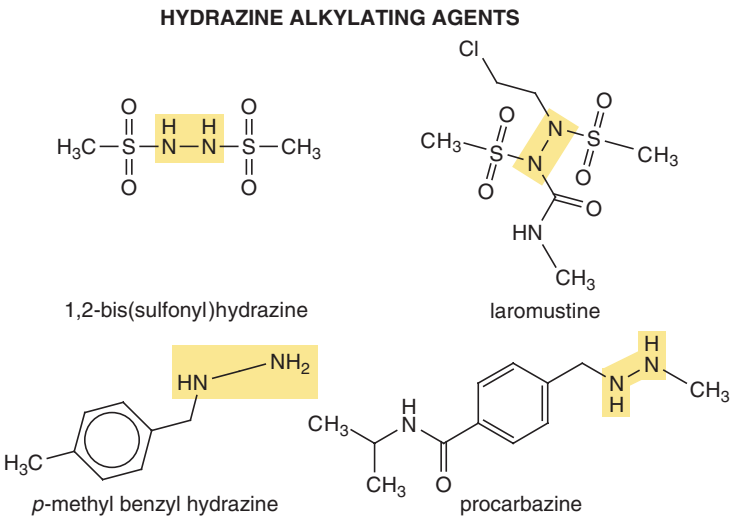


Fig. 2.10 Structures of hydrazine anti-cancer drugs. The basic structures of representatives in the sub-class are shown on the *left* (*top*: 1,2-bis(sulfonyl) hydrazine, *bottom*: methyl benzyl hydrazine), the major drug representatives of the sub-class are on the *right*. The common functional hydrazine moiety is highlighted in *yellow*

this enzyme mediates the stoichiometric transfer of the methyl group from the O^6 position of guanine in alkylated DNA to a cysteine residue in the protein itself.

1-*p*-carboxy-3,3-dimethylphenyltriazine (CB10–277) is a synthetic triazine derivative. It is soluble and stable in aqueous solutions at physiological pH. As a prodrug, 1-*p*-carboxy-3,3-dimethylphenyltriazine is metabolically converted to a monomethyl triazene form that alkylates DNA, resulting in the inhibition of DNA reduplication and repair. This agent may also act as a purine analog, thus causing the inhibition of DNA synthesis, and may interact with protein sulfhydryl groups.

Adverse Effects The dose limiting toxicity is nausea and vomiting which occurs in 80 % of patients. A flushing or warm sensation occurs in over 75 % of courses.

Hydrazines Hydrazine is an inorganic chemical compound with the formula N_2H_4 (Fig. 2.10).

- The 1,2-bis(sulfonyl)hydrazines are a class of alkylating agents that produce a chloroethylating species. Unlike other chloroethylating agents[17] they do not lead to vinylation or hydroxyethylation, which can produce toxicity in untransformed cells and damage healthy organs. The therapeutic index of bis(sulfonyl)hydrazine compounds may therefore be more favorable.
- Methyl benzyl hydrazine derivatives can suppress mitosis by prolonging interphase. These compounds act through slow auto-oxidation with the formation of hydrogen peroxide. The methyl group is important for biological activity.

The bifunctional sulfonyl hydrazine prodrug laromustine (1,2-bis(methylsulfonyl)-1-(2-choloroethyl)-2-(methylamino)carbonylhydrazine, 101M) (VNP40101M) <Cloretazine, Onrigin> releases the chloroethylating agent 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (90CE), which alkylates the O^6 position of guanine, resulting in DNA cross-linking, strand breaks, chromosomal aberrations, and disruption of DNA synthesis. The parent compound also releases a thiophilic carbamoylating methyl isocyanate, which inhibits the DNA repair enzyme O^6-Alkyl-Guanine Transferase. The agent is under investigation for the treatment of relapsed or refractory acute myelogenous leukemia. Laromustine may be a suitable second-line treatment for cancers that have acquired resistance to melphalan, cytoxan, or BCNU. It is administered as an intravenous infusion at a dose of 600 mg/m^2.

[17] Chloroethylating agents include nitrogen mustards, phosphoramide mustards, nitrosoureas, many triazenes, and some antibiotics (MEN 10710).

Pharmacokinetics The cellular metabolism of laromustine releases the two short lived active species, 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine and methyl isocyanate.

The anti-cancer effects of the methyl benzyl hydrazine procarbazine were discovered by DeVita, Serpick, and Carbone (DeVita et al. 1966). Procarbazine hydrochloride (MBH, 1-methyl-2-(*p*-isopropylcarbamoyl) benzylhydrazine hydrochloride, ibenzmethyzin) (NSC 77213) <Matulane, Natulan, Indicarb> has shown most promise in patients with Hodgkin lymphoma, chronic granulocytic leukemia, glioblastoma, and polycythemia. The metabolism of procarbazine can result in the formation of formaldehyde, which acts as an alkylating agent. The common dosage is 2–4 mg/kg/day either in a single dose or divided, given for 7 days, then increased to 4–6 mg/kg/day until response is obtained or myelosuppression occurs. Procarbazine gained U.S. FDA approval in 1969.

Pharmacokinetics The drug is metabolized and activated in the liver.

Drug Interactions Procarbazine inhibits MAO thus increasing the effects of sympathomimetics, tricyclic anti-depressants, and tyramine (hypertensive crisis can be caused by the ingestion of tyramine-rich food). It also inhibits the hepatic CYP450 microsomal system, leading to an increased effect of barbiturates, phenothiazenes, and narcotics, which are normally inactivated by the CYP450 system. When combined with ethanol, procarbazine can cause adverse drug reactions in some patients. The dose may need to be adjusted for renal or hepatic disease.

Triazenes are prodrugs of their active monomethyl forms. Their activation occurs with the release of the single-bonded nitrogen, leaving the highly reactive double-bonded diazonium fragment exposed.

Oral availability and penetration of the blood-brain barrier vary among triazenes.
The bis(sulfonyl)hydrazines are alkylating agents that produce chloroethylating species.
Methyl benzyl hydrazine derivatives can suppress mitosis by prolonging interphase.

2.1.4 Ethylene Imines and Methylmelamines

ThioTEPA (*N, N*′,*N*′-triethylenethiophosphoramide) <Thioplex> was first developed by American Cyanamid in the early 1950s. As a synthetic agent related to nitrogen mustard, thioTEPA (Fig. 2.11) alkylates and cross-links DNA. It is mostly administered

- in the palliation of breast adenocarcinoma and ovarian adenocarcinoma,
- for controlling intracavitary effusions secondary to diffuse or localized neoplastic diseases of serosal cavities,
- for the treatment of superficial papillary carcinoma of the urinary bladder.
- ThioTEPA has been effective against lymphomata, such as lymphosarcoma and Hodgkin disease (it is now largely superseded by other treatments).
- ThioTEPA may be used as conditioning treatment for bone marrow transplantation.

ThioTEPA is typically given by rapid intravenous administration in doses of 0.3–0.4 mg/kg. These doses must be carefully individualized to minimize toxicity. After maximum benefit is obtained by initial therapy, it is necessary to continue the patient on maintenance therapy in 1–4 week intervals. ThioTEPA can be used for direct intra-tumor injection of accessible tumors to maximize the concentration within

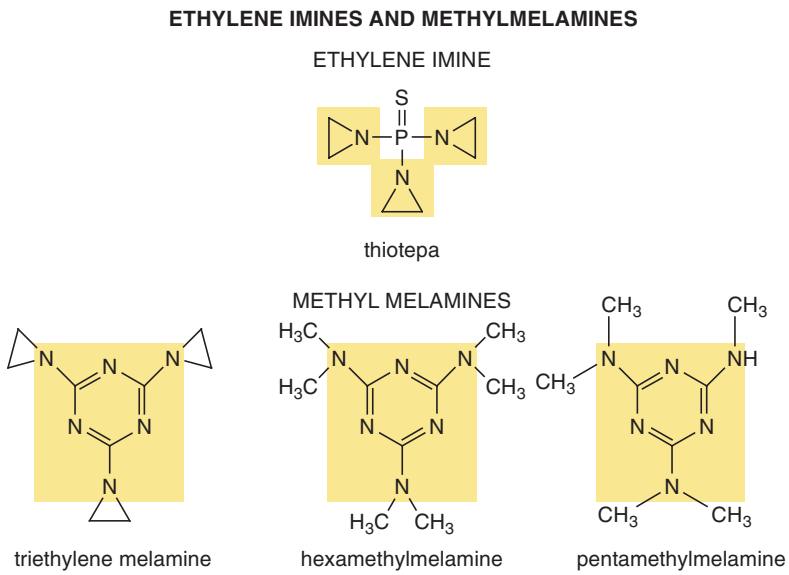


Fig. 2.11 Structures of ethylene imine and methylmelamine anti-cancer drugs. The functional moieties are highlighted in *yellow*

the tumor cells. This allows the application of about twice the dose compared to intravenous injection before comparable extents of bone marrow depression occur. For patients in good condition, 60 mg can be administered into the tumor. The maintenance weekly drug doses are adjusted to the leukocyte counts.

Pharmacokinetics Because absorption from the gastrointestinal tract is variable, thioTEPA should not be administered orally. After intravenous injection, the blood concentrations decline biexponentially with half-lives of 8 and 125 min. thioTEPA is activated by CYP3A4 and CYP2D6. TEPA, which possesses cytotoxic activity, is the major metabolite in the blood and urine.

Adverse Effects ThioTEPA is highly toxic to the hematopoietic system. The main adverse effect is myelosuppression, which can be fatal after intravesical administration. Dysuria and urinary retention are possible adverse effects. In rare cases, chemical cystitis or hemorrhagic cystitis may develop following intravesical, but not parenteral administration. Weight loss, fatigue, pain at the injection site, and tightness of the throat may occur. The drug is excreted onto the skin surface in the sweat, accumulates in covered areas (e.g. beneath adhesive bandages), and exerts a local toxic effect resulting in hyper-pigmentation. ThioTEPA causes amenorrhea and interferes with spermatogenesis. It is Pregnancy Category D.

Drug Interactions Following the combined use of thioTEPA with other anti-cancer agents, prolonged apnea can occur when succinylcholine is administered prior to surgery. This may be due to a decrease in Pseudocholinesterase activity induced by the anti-cancer treatment. It is generally not advisable to combine (simultaneously or sequentially) cancer chemotherapeutic modalities that have the same mechanism of action. Therefore, thioTEPA combined with other alkylating agents, such as nitrogen mustard or cyclophosphamide, or thioTEPA combined with irradiation would serve to intensify toxicity rather than to enhance therapeutic response.

Drug Resistance The aziridine moieties in thioTEPA are substrates for Glutathione S-Transferase (GST). The Glutathione conjugation may be a factor in the development of drug resistance.

The cytotoxic action of triethylenemelamine (TEM, trisethylene-imino-S-triazine) derives from the ethylene imine groups. The drug interacts primarily with phosphorylated DNA precursors, particularly with pyrimidines. Triethylenemelamine can be given orally and it rapidly distributes out of the blood stream. Because the reactivity of triethylenemelamine increases at low pH, its reliable resorption can be facilitated by formulation with a basic agent. However, its use has been restricted almost entirely to

- intraarterial infusion for the potentiation of radiation therapy of retinoblastoma
- treating diabetics with skin involvement by lymphosarcoma.

Due to the greater stability and shelf life of thioTEPA, triethylenemelamine has been largely replaced in clinical use.

Hexamethylmelamine (altretamine, HMM, N2,N2,N4,N4,N6,N6-hexamethyl-1,3,5-triazine-2,4,6-triamine) (ENT-50852, RB-1515, WR-95704) <Hexalen, Hexastat, Hexinawas> is a prodrug alkylating agent that may damage tumor cells through the production of the weakly alkylating species formaldehyde, which is a product of Cytochrome P450 mediated *N*-demethylation. The carbinolamine (methylol) intermediates of CYP450 metabolism also can generate electrophilic iminium species that are capable of reacting covalently with protein as well as guanine and cytosine residues in DNA. Hexamethylmelamine is not a first-line treatment but can be useful as salvage therapy. It is indicated for use as a single agent in the palliation of persistent or recurrent ovarian cancer following first-line therapy with a combination based on cisplatin or alkylating agents. It also has the advantage of being less toxic than other drugs used for treating refractory ovarian cancer. Hexamethylmelamine is active against various solid tumors including lung cancer and breast cancer. The drug was approved by the U.S. FDA in 1990. It is given orally as its sparing water solubility makes it unsuitable for parenteral administration. Capsules may be taken either for 14 or 21 consecutive days in a 28 day cycle at a dose of 260 mg/m^2/day. The total daily amount should be divided into four doses after meals and at bedtime.

Pharmacokinetics Administered orally, hexamethylmelamine is extensively metabolized on first pass, producing primarily mono- and di-demethylated metabolites. Additional demethylation reactions occur in the tumor cells, releasing formaldehyde. Excretion is mostly in the urine.

Adverse Effects Adverse effects include nausea, vomiting, diarrhea, renal toxicity, severe orthostatic hypotension, and neurotoxicity. The neurotoxicity can be reduced by intake of pyridoxine (vitamin B6), however, it adversely affects the response duration. Because of bone marrow suppression, peripheral blood counts should be monitored at least monthly, prior to the initiation of each course (temporary discontinuation of the drug is required if the white blood cell counts drop below 2000/mm^3, granulocyte counts drop below 1000/mm^3, or platelet counts drop below 5000/mm^3). The drug is Pregnancy Category D.

Drug Interactions Concurrent administration of hexamethylmelamine and antidepressants of the MAO inhibitor class can cause severe orthostatic hypotension. Cimetidine is an

inhibitor of microsomal drug metabolism that may increase the half-life and toxicity of hexamethylmelamine.

Water soluble analogs of hexamethylmelamine have been produced because of gastrointestinal adverse effects associated with the oral administration of the water insoluble preparation. Derivatives with greater intrinsic water solubility include pentamethylmelamine, N-methylether derivative, and N2,N4,N6-trimethylol-N2,N4,N6-trimethylmelamine.

Pentamethylmelamine is a principal metabolite of hexamethylmelamine. It alkylates DNA and other macro-molecules to form DNA intra-strand and DNA-protein cross-links, thereby preventing DNA reduplication.

Pharmacokinetics After administration, pentamethylmelamine is extensively demethylated by hepatic mixed-function oxidases. This leads to the formation of an intermediate hydroxymethyl product, which is cytotoxic, and can spontaneously degrade to result in the demethylated melamine and formaldehyde.

In patients with normal liver function, pentamethylmelamine is rapidly cleared from the plasma with a terminal half-life of 2.2 h. Compromised liver function correlates with an increased half-life and reduced total clearance.

Ethylene imines and methylmelamines are prodrugs that require metabolic activation.
Methylmelamines release the weakly alkylating formaldehyde upon demethylation.
Methylated melamines are extensively demethylated by hepatic mixed-function oxidases. Their anti-tumor activity decreases with a decreasing number of methyl groups.

2.1.5 Benzoquinone Containing Alkylating Agents

Quinones have traditionally represented a major source of anti-cancer compounds[18]. These agents are unique among cancer therapeutics insofar as they require enzyme catalyzed bioreduction of the quinine moiety before conversion to intermediates that can

- generate toxic free radical species
- bind to DNA and form covalent adducts.

3-membered ring compounds like aziridine are highly strained molecules because of the small bond angles[19]. This type of strain in an organic molecule is termed angle strain (Baeyer strain). Since the reaction of a nucleophile with an aziridine usually leads to an acyclic product, the ring strain is released, which highly favors the reaction. Chemical reduction facilitates the protonation of the aziridine (ethylene imine) ring[20], leading to the formation of the aziridinium ion, which is the required species for alkylation. Many one- and two-electron Reductases are capable of activating bioreductive drugs, including NADH:Cytochrome b_5 Reductase, Cytochrome P450 Reductase, and NAD(P)H:Quinone Oxidoreductase (NQO1). NQO1 is of specific importance in enzyme directed drug activation, because its activity and gene expression levels are elevated in various intrinsically drug resistant solid malignancies, including lung, colon, and liver cancer. Nevertheless, significant NQO1 expression is also present in untransformed tissues including the respiratory tract epithelium, kidney podocytes, and reproductive system, which may give rise to a potential mechanism of drug toxicity.

Triaziquone (2,3,5-tris-ethyleneimino-l,4-benzoquinone, 2,3,5-tris(1-aziridinyl)-2,5-cyclohexadiene-1,4-dione, tris-ethyleneiminoquinone) <Trenimon> (Fig. 2.12) was one of the first aziridinylbenzoquinone anti-cancer drugs synthesized at the Bayer scientific laboratories in Leverkusen, Germany (Gauss 1958). Triaziquone contains three ethylene imine rings linked to a quinone nucleus. This drug is a substrate for both one-electron and two-electron reducing agents and its cytotoxic activity may result from protein alkylation and oxidative stress. It is a polyfunctional compound, which combines the properties of a quinone, an α,β-unsaturated ketone, an enamine, and an aziridine (ethylene imine). Triaziquone exerts its anti-cancer effects through macromolecule alkylation of functional groups like –SH, –OH, and $–NH_2$. Since triaziquone has three aziridine groups with slightly different reactivity, it can react successively three times as an akylating reagent. By this mechanism, different parts of the DNA (even from different strands) can be connected. Cross-links may also be formed between DNA and other molecules, such as proteins (Rademacher 1995). Both the rate of reaction and the number of aziridine groups per molecule of trenimon that react are dependent on the acidity of the micro-environment. Therefore, the reactivity is increased in the acidic milieu of tumors. Triaziquone is especially efficacious for the treatment of polycythemias and carcinomatous effusions. The therapeutic dose is about 1 mg/kg body mass.

[18] Quinone is contained in the anti-cancer drug classes benzoquinones, anthracyclins, dynemicins, and anthracenediones. The photosensitizer perylenequinone also contains this functional group. The aminoquinone moiety is contained in the classical aminoquinones (mitomycin C, porfiromycin, KW-2149, streptonigrin), ansamycins (geldanamycin and derivatives, rafamycins, and maytansinoid agents), and actinomycin D.

[19] Alkylating anti-cancer drugs with 3-membered rings include ethylene imines, some methylmelamines (triethylenemelamine), benzoquinones, illudins and acylfulvenes, dianhydrogalactitol, and teroxirone.

[20] Aziridines (ethylene imines) include benzoquinones and thio-TEPA. Phosphoramide mustards and mitomycins may be metabolized to highly reactive aziridine derivatives. The Caspase activator imexon (see Sect. 3.3.4.) is a cyanoaziridine.

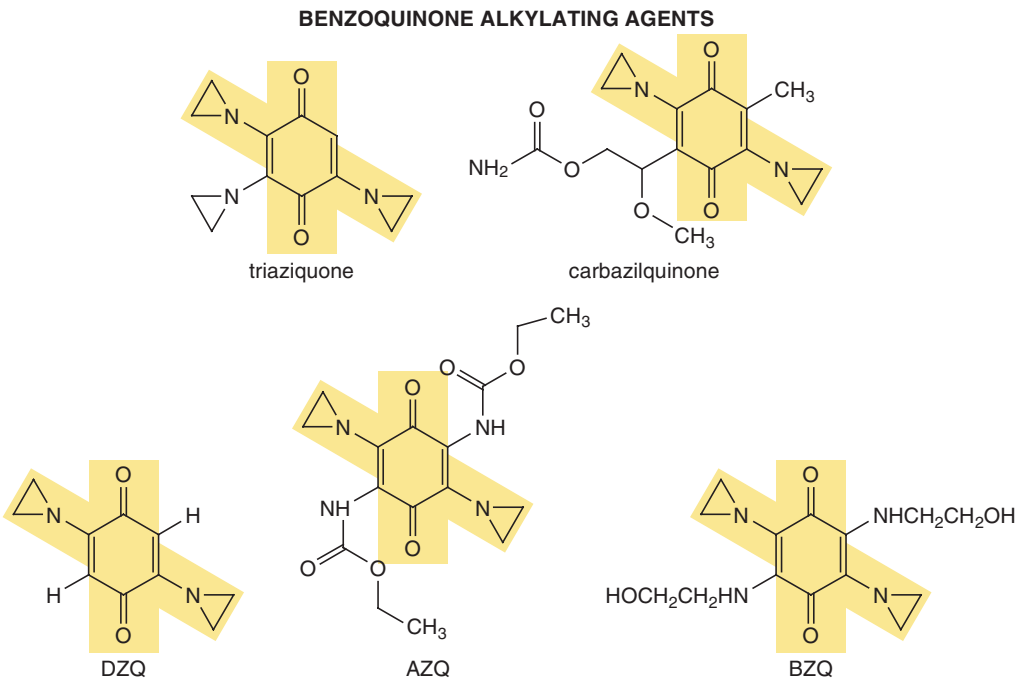


Fig. 2.12 Structures of benzoquinone alkylating anti-cancer drugs. The common aziridinyl benzoquinone moieties, shared by all compounds in this class, are highlighted in *yellow*

The agent was in more widespread use in Europe than in the United States. Because of its toxicity it has been replaced by other agents.

Pharmacokinetics Upon intravenous injection, triazoquinone is rapidly bound to blood proteins. The agent crosses the blood-brain-barrier. Detoxification of the drug involves its reduction by DT-Diaphorase (NAD(P)H:(Quinone Acceptor) Oxidoreductase).

Adverse Effects Triaziquone produces myelosclerotic reactions with low frequency.

P53 is a critical tumor suppressor protein that may arrest cell cycle progression or induce apoptosis. The cell cycle arresting function is exerted in part through $P21^{CIP1/WAF1}$. The P53→P21 signaling pathway may play a central role in mediating the gene regulatory and cytotoxic effects of aziridinylbenzoquinones (Wu et al. 1998). 3,6-diaziridinyl-1,4-benzoquinone (DZQ) causes the increased expression of $P21^{CIP1/WAF1}$, an inhibitor of Cyclin-Dependent Kinases. This induction is regulated at the transcriptional level and requires the activation of P53. In cancer cells that lack functional P53, DZQ mediated *p21* induction is greatly diminished. The drug is less effective.

Carbazilquinone ([2,5-bis(1-aziridine)-3-(1-methoxy-2-amino-formyl) ethyl]-6-methyl-1,4-benzoquinone) was first synthesized in 1970 as a mitomycin C analog having a quinone group and aziridine and carbamate alkylating groups (Arakawa et al. 1970). Carbazilquinone has been extensively studied in the clinic, primarily in Japan. It has activity as a single agent in gastric, ovarian and hematologic cancers.

Adverse Effects Leukopenia, thrombocytopenia, and anorexia are the major toxic effects of the drug.

Diaziquone (AZQ, 2,5-bis-(carboethoxyamino)-3,6-diaziridinyl-1,4-benzoquinone) (NSC 182986) is a synthetic bifunctional quinone derivative that alkylates and cross-links DNA during all phases of the cell cycle, resulting in a disruption of DNA function, cell cycle arrest, and apoptosis. This agent can also form free radicals, thereby initiating DNA damage via strand breaks. Due to its lipophilicity, diaziquone readily crosses the blood-brain barrier. It was first tested as a potential central nervous system anti-tumor agent in the early 1960s and displayed substantial activity in both intracerebral and intraperitoneal leukemias, as well as activity in solid tumors.

Adverse Effects Diaziquone is myelosuppressive with little non-hematopoietic toxicity. Its maximal tolerated dose is 30 mg/m^2 daily for 5 days.

2,5-bis-(2-hydroxyethylamino)-3,6-diaziridinyl-1,4-benzoquinone (BZQ) was first synthesized in 1976 (Chou 1976). The drug is not a substrate for DT-Diaphorase and does not produce DNA strand breaks. However, it produces DNA cross-links without reduction. The cytotoxicity of this agent may be due to its alkylating activity.

Quinones require enzymatic bioreduction of the quinine moiety to intermediates that generate free radicals and form covalent DNA adducts.

2.1.6 Alkyl Sulfonates

The sulfonic acid ester busulfan (1,4-butanediol dimethanesulfonate) <Busulfex, Myleran> is a dialkylating agent (Fig. 2.13), from which the methane sulfonate can be eliminated as a leaving group, resulting in a butylene cross-link

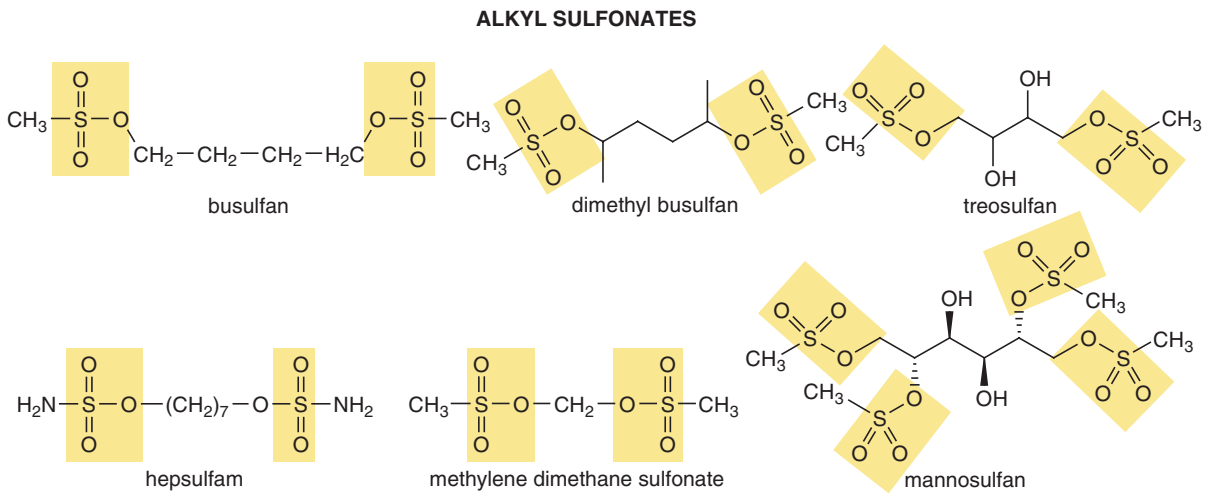


Fig. 2.13 Structures of alkyl sulfonates. The common pairs of sulfonate moieties are highlighted in *yellow*. In busufan, dimethyl busulfan, and treosulfan they are connected by a 4-member chain. The chain length varies in hepsulfam and methylene dimethane sulfonate. Mannosulfan has 4 sulfonate moieties

between biomolecule target groups. Busulfan attacks almost exclusively protein-sulfhydryl groups in the nucleus. In contrast to mustard drugs, it has little effect on DNA or RNA synthesis.

- Busulfan is relatively unique among conventional chemotherapy compounds in its ability to deplete non-cycling primitive stem cells in the host and consequently to allow for high levels of long-term, donor-type engraftment after bone marrow transplantation. It is therefore indicated in combination with cyclophosphamide as a conditioning regimen prior to allogeneic hematopoietic progenitor cell transplantation for chronic myelogenous leukemia (BuCy2 conditioning regimen).
- Chronic myelogenous leukemia (CML) frequently progresses through two phases. The chronic phase can be controlled for a number of years by busulfan. However, ultimately the majority of patients undergo blastic transformation (acute phase) and no longer respond to the antitumor effects of this drug.

Although busulfan is poorly soluble in water it is readily resorbed from the gastrointestinal tract and is therefore usually administered as an oral tablet. Initial doses of 4–12 mg are used and adjusted according to the response. Maintenance chemotherapy at 1–3 mg/day may be of benefit. Busulfan can also be administered as intravenous injection. The usual adult dose is 0.8 mg/kg of ideal body weight or actual body weight (whichever is lower) administered every 6 h for 4 days (at a total of 16 doses).

Pharmacokinetics Consistent with the reactive electrophilic properties of busulfan, the irreversible binding to plasma proteins (primarily Albumin) is around 30 %. The drug achieves concentrations in the cerebrospinal fluid approximately equal to those in the blood. It is predominantly metabolized by conjugation with Glutathione, both spontaneously and by Glutathione S-Transferase catalysis. The resulting conjugate undergoes further extensive oxidative metabolism in the liver. Approximately 30 % of the administered dose is excreted into the urine over 48 h, while negligible amounts are excreted in the feces.

Adverse Effects The most common adverse effect of busulfan is bone marrow depression, which requires frequent monitoring. Among the alkylating agents, busulfan is unique in affecting mainly the granulocytes (this has led to its clinical use for the treatment of chronic granulocytic leukemia. In chronic granulocytic leukemia, busulfan can induce remission in 90 % of patients after the initial course of therapy. It is not effective, however, in the blast crisis of chronic granulocytic leukemia or in acute leukemias). The rapid destruction of granulocytes can yield a dangerous rise in serum uric acid and consecutive kidney damage from urate stones. Preventive treatment with allopurine <Zyloprim> is available.

Neurological symptoms may comprise hallucinations (onset at 1 day post completion of administration, associated with EEG changes), somnolence, lethargy, and confusion. As with other alkylating agents, nausea and vomiting, diarrhea, impotence or sterility, and teratogenic effects can occur. Unusual complications from busulfan include skin pigmentation (especially in the creases of the hands), pulmonary fibrosis, gynecomastia, anhidrosis, cheilosis, and glossitis. Mild or moderate tachycardia or dyspnea are possible. Because busulfan is poorly water soluble the solvent used in the Busulfex preparation is dimethylacetamide (DMA), which has a maximum tolerated dose of 14.8 g/m^2/d for 4 days. 16 doses of intravenous busulfan contain about 40 % of

the maximum tolerated dose of dimethylacetamide. The drug is Pregnancy Category D.

Busulfan may be used in preconditioning prior to bone marrow transplantation. Hepatic veno-occlusive disease is a potential complication of this conditioning therapy. It may arise in 8% of patients treated with busulfan in the setting of allogeneic transplantation. Graft-versus-host disease can develop.

Busulfan is contraindicated in patients in whom a definitive diagnosis of chronic myelogenous leukemia has not been firmly established, and in patients who have previously suffered a hypersensitivity reaction to busulfan or any component of its preparation.

Drug Interactions Busulfan may cause additive myelosuppression when used with other myelosuppressive drugs. The anti-fungal itraconazole <Sporanox, Onmel> decreases busulfan clearance by up to 25%. The anti-convulsant phenytoin <Dilantin, Phenytek> increases the clearance of busulfan by 15% or more, possibly due to the induction of Glutathione-S-Transferase. A fraction of patients receiving continuous busulfan and thioguanine therapy for chronic myelogenous leukemia develop portal hypertension and esophageal varices associated with abnormal liver function. This condition is due to nodular regenerative hyperplasia.

Dimethylbusulfan (2,5-hexanediol dimethanesulfonate, 1,4-Bis(methylsulfonyloxy)-1,4-dimethylbutane) is an aliphatic analog of busulfan. It has been under investigation as a second-line treatment for various cancers.

Adverse Effects As an alkylating agent, dimethylbusulfan induces neutropenia.

Treosulfan (2,3-dihydroxybutane-1,4-diyl dimethanesulfonate) (NSC 39069) is the prodrug of a bifunctional sulfonate alkylating agent, which converts non-enzymatically via a monoepoxide intermediate to L-diepoxybutane. Both the monoepoxide intermediate and L-diepoxybutane can alkylate DNA at guanine residues. L-diepoxybutane can also produce DNA inter-strand cross-links. These DNA modifications lead to DNA fragmentation and cell death. Treosulfan also has myeloablative and immunosuppressive effects. Therefore, this agent may have applications in allogeneic stem cell transplantation conditioning regimens.

Hepsulfam (1,7-heptanediol disulfamate, sulfamic acid 1,7-heptanediyl ester) (NSC 329680) is a bisulfamic ester, which is similar in structure to busulfan but is more hydrophilic. Hepsulfam forms covalent linkages with nucleophilic centers in DNA, resulting in depurination, base miscoding, strand scission, DNA-DNA and DNA-protein cross-linking, and cytotoxicity. It has efficacy for the treatment of leukemias.

Pharmacokinetics Hepsulfam enters the cerebrospinal fluid following intravenous administration. The cerebrospinal fluid/plasma ratios are lower than those obtained following oral busulfan administration.

Adverse Effects Encephalopathy is the dose limiting toxicity of intravenous hepsulfam in patients with refractory leukemias. In patients with refractory solid tumors, the dose limiting toxicity is cumulative myelosuppression, resulting in prolonged leukopenia and thrombocytopenia. The symptoms are reversible upon discontinuation of therapy (because there is only minor non-hematologic toxicity, substantial dose escalation may be possible with hematopoietic stem cell support). Uncomfortable paresthesias can be avoided by slowing the infusion and administering it over 2 h.

Methylene dimethane sulfonate is a member of the homologous series of dimethane sulfonic acid esters with alkylating properties. Its anti-cancer properties are under study.

Mannosulfan ([(2R,3S,4S,5R)-3,4-dihydroxy-2,5,6-tris(methylsulfonyloxy)hexyl] methanesulfonate) <Zitostop> has been used, mostly in Hungary, in the treatment of chronic myeloid leukemia and polycythemia vera.

Alkyl sulfonates attack protein-sulfhydryl groups in the nucleus with little effect on DNA or RNA synthesis.
Busulfan is indicated in conditioning prior to allogeneic hematopoietic progenitor cell transplantation, because it depletes non-cycling stem cells.

2.1.7 Illudins

The illudins (Fig. 2.14) are a family of sesquiterpenes (terpenes that consist of three isoprene units and have the molecular formula $C_{15}H_{24}$), produced as antibiotic toxins by some mushrooms. They exert relatively selective cytotoxicity against some myelocytic leukemia cells and carcinoma cells. To the anti-cancer effects of this class of drugs may contribute

- their alkylating activity (Fig. 2.15),
- inhibition of DNA synthesis from thymidine,
- the depletion of the Glutathione and Thioredoxin systems.

At low pH, illudins act as bifunctional alkylating agents, but at physiological pH they do not react with oxygen or nitrogen nucleophiles. Rather, they react spontaneously with sulfur nucleotides. Acylfulvenes were derived from illudins to reduce the reactivity with thiol containing small molecules, and thus the non-specific toxicity.

Irofulven (6-hydroxymethylacylfulvene, HMAF) (MGI 114) is a semi-synthetic derivative of illudin S, which is synthesized by the mushroom Omphalotus illudens. While irofulven is less cytotoxic than illudin S, it exhibits much greater selectivity in damaging malignant cells compared to healthy cells. Besides alkylating DNA, the drug interferes with transcription-coupled DNA repair. Irofulven may undergo

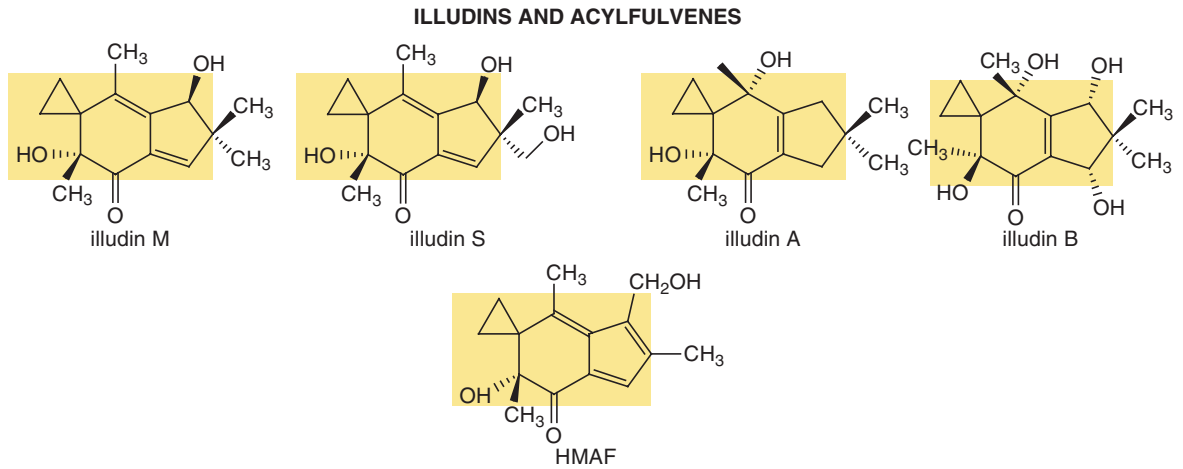


Fig. 2.14 Structures of illudins. The shared structural motif is highlighted in *yellow*. The molecule on the *bottom* represents the investigational drug 6-hydroxymethylacylfulvene (HMAF)

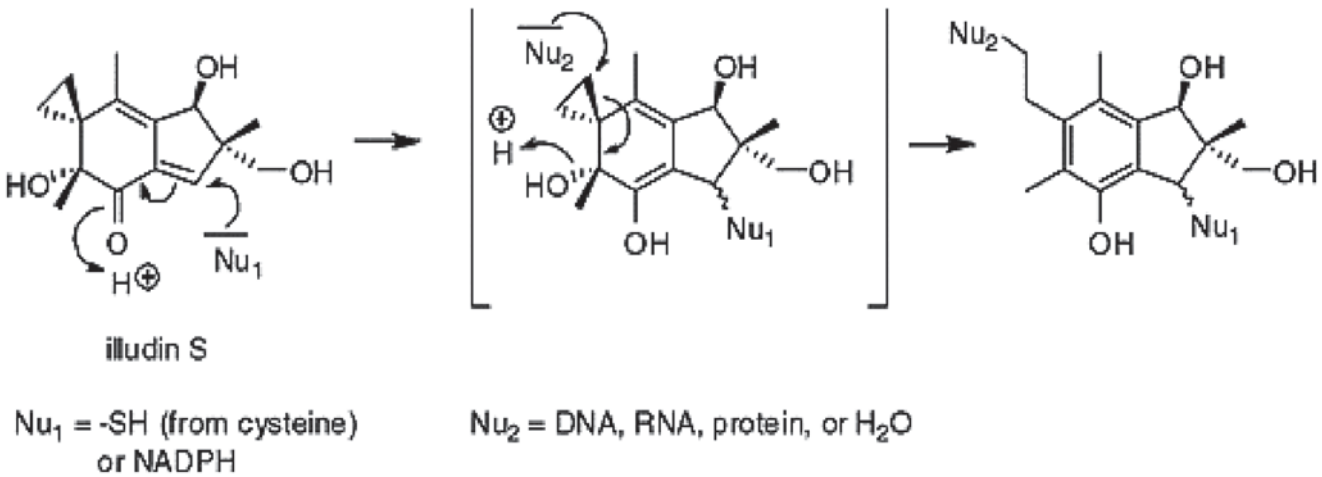


Fig. 2.15 Alkylation by illudins. The reaction scheme depicts the alkylation reaction of illudin S with the cellular nucleophiles Nu1 and Nu2. (Greve 2010)

bioreductive activation. The drug is under investigation as mono-therapy or in combination therapies for the treatment of refractory and relapsed ovarian, prostate, hepatocellular, pancreatic, breast, lung or colon cancers.

Adverse Effects Toxicity in the form of myelosuppression and fatigue are schedule dependent, with reduced toxicity under intermittent administration compared to continuous regimens.

Drug Interactions There is synergy between irofulven and Topoisomerase inhibitors. The repair of the irofulven induced DNA damage is dependent on functioning DNA Helicases (the double helical configuration, in which DNA strands naturally reside, requires their separation by Helicases for transcription or reduplication). Hence the drug efficacy can potentially be enhanced by Helicase inhibitors.

Illudins are sesquiterpenes.
Illudins and acylfulvenes alkylate DNA, inhibit DNA synthesis, and deplete thiol anti-oxidant defenses.

2.1.8 Platinum Drugs

In 1965, cisplatin was discovered by Barnett Rosenberg, who explored the effects of electric fields on the growth of Escherichia coli bacteria (Rosenberg 1965). He observed that the bacteria unexpectedly ceased to divide due to the exposure to an electrolysis product of the platinum electrodes. The discovery soon initiated studies into the effects of platinum compounds on cell division. Various platinum salts were synthesized, and cis-diamminetetrachloroplatinum, but not its trans-isomer, was identified to be the most effective inhibitor of tumor cell proliferation. Subsequently, Eve Wiltshaw and others at the Institute of Cancer Research, United Kingdom extended the clinical usefulness of the platinum compounds with the development of carboplatin, a cisplatin derivative with broad anti-tumor activity and comparatively

less nephrotoxicity. Cisplatin proved pivotal in achieving full remission of testicular cancer, and most ovarian cancers initially respond well to platinum anti-cancer drugs.

There are two structural groups of platinum compounds,

- *cis*-[Pt(amine)$_2$X$_2$], including cisplatin <Platinol>, carboplatin <Paraplatin>, and oxaliplatin <Eloxatine>, for the treatment of testicular, ovarian, and colon cancers
- polynuclear Pt^{IV} species, including triplatin (BBR3464), satraplatin, and picoplatin, which are much more inert to ligand substitution reactions than their Pt^{II} counterparts. It is likely that Pt^{IV} complexes are reduced to Pt^{II} by extracellular and intracellular biomolecules prior to their reaction with DNA.

The compositions of platinum anti-tumor drugs mostly adhere to a set of consistent structure-activity relationships (Cleare and Hoeschele 1973). These entail that the Pt^{II} or Pt^{IV} complex should have a cis geometry with the general formulas of cis-[PtX$_2$(Am)$_2$] or cis-[PtX$_2$Y$_2$(Am)$_2$], where X is the leaving group[21] and Am is an inert amine with at least one N–H moiety. The leaving group should be an anion with intermediate binding strength to platinum and have a weak trans-effect to avoid labilizing the amine. Complexes with labile leaving groups such as ClO_4^- or NO_3^- are highly toxic, while complexes with inert leaving groups are generally inactive. Upon administration of platinum drugs, an integral ligand undergoes slow displacement with water molecules in a process termed aquation. The aqua ligand is highly reactive, allowing the platinum compounds to coordinate a base in DNA, forming a subsequent cross-link after loss of the second chlorine ligand. These linkages to DNA occur at the positions where the chloride ion (cisplatin, picoplatin), cyclobutane-1,1-dicarboxylate (carboplatin), or oxalate (oxaliplatin) ligands reside in the original platinum compound. The leaving group of carboplatin confers good aqueous solubility and stability because it forms a 6-membered ring with the platinum atom.

For some tumors (germ cell tumors, head and neck cancers, bladder cancers), cisplatin may be therapeutically more effective than carboplatin, whereas for lung cancer and ovarian cancer the effectiveness is comparable. The choice of the most appropriate analog is a function of the cancer being treated, the treatment goal (palliative or curative), and the other component drugs used in combination. The treatment of Estrogen Receptor-positive (ER$^+$) cells with estradiol sensitizes them to cisplatin. This has led to the design and synthesis of a series of estrogen tethered Pt^{IV} compounds (Barnes 2004).

[21] A leaving group is an atom or a group of atoms that is displaced as a stable species in heterolytic bond cleavage, taking with it the bonding electrons.

First generation platinum compound Cisplatin (cis-diamminedichloroplatinum^{II}, cis-DDP) (CS-310, NSC-134679) <Abiplatin, Biocisplatinum, Briplatin, Carboquone, Esquinon> is the prototypical first generation platinum anti-cancer drug (Fig. 2.16). It is an inorganic compound that forms highly reactive charged platinum complexes, which bind to nucleophilic groups, such as GC rich sites in the DNA. The intra-strand and inter-strand DNA cross-links, as well as DNA-protein cross-links caused in this manner result in the inhibition of cell growth and induction of apoptosis (Fig. 2.17). Although cisplatin often fails to cause G_1 arrest, the induction of G_2 arrest is essential to the process of engaging cell death following treatment (Sorenson and Eastman 1988). Cisplatin entered clinical trials in 1971 and proved effective against various cancers. It received approval for the treatment of testicular and ovarian cancers in 1978. The use of cisplatin, usually as a principal component of combination regimens, has rendered testicular cancer curable with a success rate in excess of 90 %, and is important in the treatment of bladder and ovarian cancers. Ovarian cancer initially responds well to platinum drugs[22]. Cisplatin is indicated in two regimens,

- in the initial treatment of advanced ovarian carcinoma, cisplatin is used in established combination chemotherapy; one established combination consists of cisplatin and cyclophosphamide.
- in the palliative treatment of patients with ovarian carcinoma recurrent after prior chemotherapy, including patients who have been previously treated with cisplatin.

The drug is also given to treat small cell lung cancers, sarcomata, lymphomata, germ cell tumors, and gestational trophoblastic tumors. Further, it is applied to the palliation of bladder, cervical, nasopharyngeal, esophageal, and head and neck cancers. Typical doses range 20–120 mg/m^2, usually for up to five consecutive days. The compound needs to be administered intravenously.

- Synthetic formulations, in which the anti-neoplastic agent cisplatin is encapsulated in lipids, generate cisplatin liposomal (liposome-encapsulated Carboquone) <CQ-liposome, Lipoplatin>. They consists of small aggregates of cisplatin (generated through the formation of reverse micelles between cisplatin and dipalmitoyl-phosphatidyl-glycerol under certain conditions) covered by a single lipid bilayer. The encasement in liposomes improves the tumor bioavailability and toxicity profile of cisplatin, while it does not directly affect the pharmacological properties of

[22] Although women with mutations in *brca1* or *brca2* have an increased lifetime risk of contracting ovarian cancer, mutations in *brca2* are associated with an increased response to platinum based drug treatment—and increased survival, because homologous recombination pathway deficiencies compromise DNA repair in response to chemotherapy.

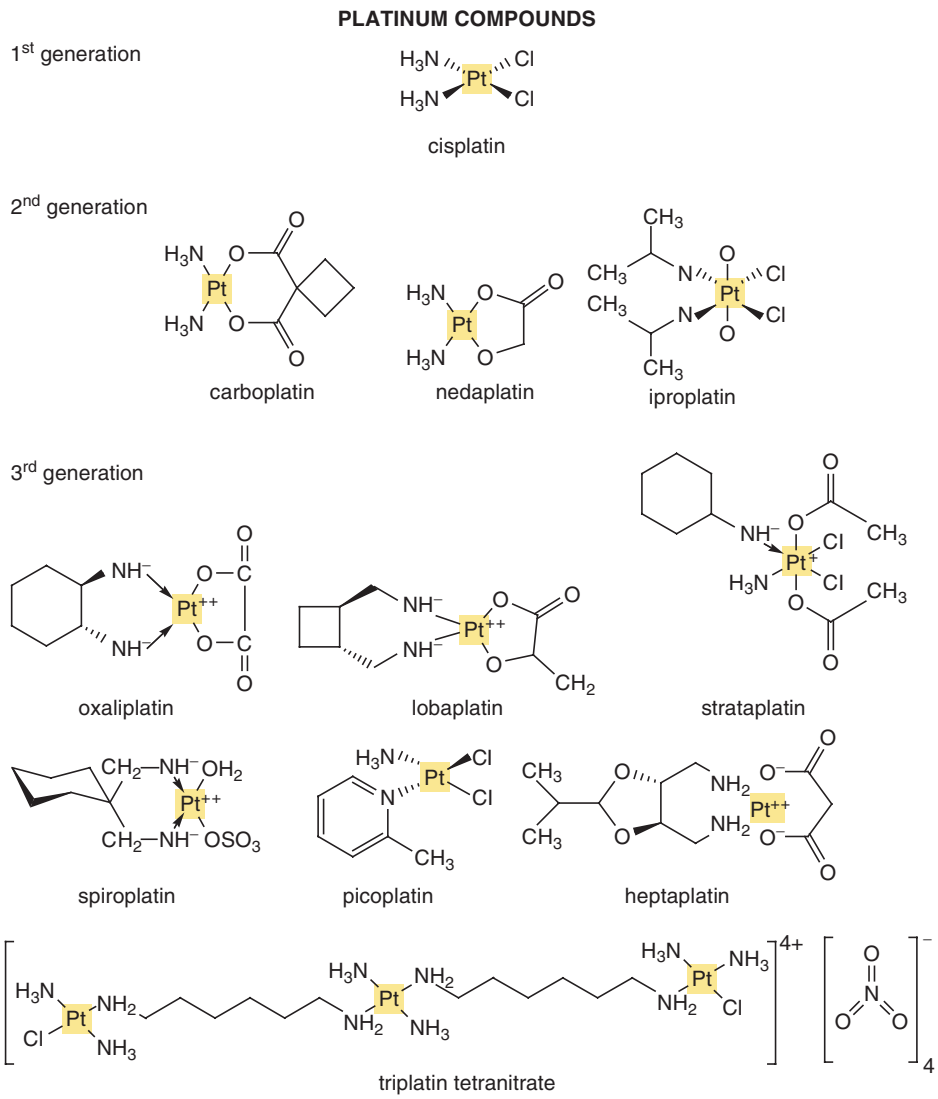


Fig. 2.16 Structures of platinum anti-cancer drugs. The agents are grouped by generations one through three. The essential platinum entity is highlighted in *yellow*

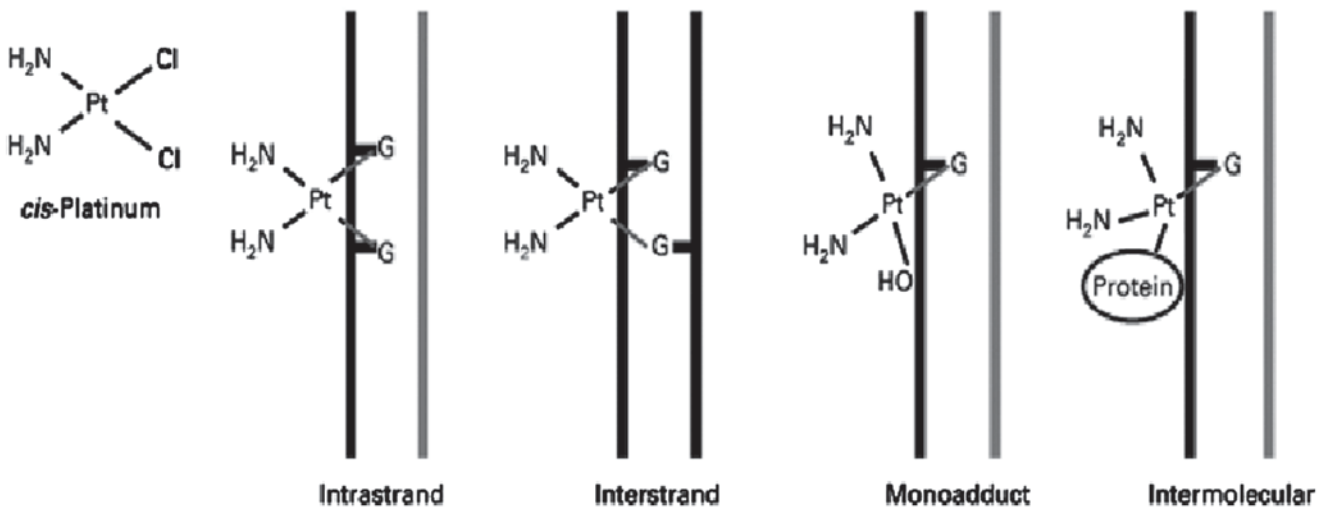


Fig. 2.17 DNA adducts generated by cisplatinum. Cisplatin produces inter-strand, intra-strand and mono-functional adduct cross-linking in DNA. The most prevalent form is the 1,2-intra-strand cross-link, where the platinum drug is covalently bound to the *N*7 position of adjacent purine bases (the inactive isomer of cisplatin, trans-DDP, cannot form this ubiquitous 1,2-intra-strand crosslink). In the 1,2-intra-strand cross-link, the DNA is unwound and bent towards the major grove. Other platinum-DNA adducts, including the mono-functional and the 1,3- and longer range intra-strand, inter-strand and protein-DNA cross-links, each form a distinct structural element that uniquely interferes with DNA function. ((http://bioweb.wku.edu/courses/Biol588/Bishopl.html; Marra and Schär 1999) with permission)

cisplatin. Cationic liposomes (cationic lipoplexes) have favorable interactions with negatively charged DNA and cell membranes. Further the encapsulation alters biodistribution, tumor uptake, and toxic exposure to healthy tissues. The negatively charged dipalmitoyl-phosphatidyl-glycerol (DPPG) on the particle surface conveys fusogenic properties for cell entry.

Pharmacokinetics Cisplatin enters the cells mainly by passive diffusion. Once inside the cell, it undergoes aquation to form $[Pt(NH_3)_2Cl(OH_2)]^+$ and $[Pt(NH_3)_2(OH_2)_2]^{2+}$. The aquated forms are more reactive to their cellular targets. Although many cellular components interact with cisplatin, DNA is the primary biological target of the drug. Most notable among the DNA changes are the 1,2 intra-strand cross-links with purine bases. These include 1,2 intra-strand d(GpG) adducts, which form nearly 90% of the adducts, and the less common 1,2 intra-strand d(ApG) adducts. 1,3 intra-strand d(GpXpG) adducts occur but are readily eliminated by the nucleotide excision repair mechanism (Farrell 2003). Cisplatin modifications distort the structure of the DNA duplex. The intra-strand 1,2 cross-links bend the DNA significantly towards the major groove, exposing a wide, shallow minor groove surface, to which HMG box proteins, repair proteins, transcription factors, and Histone H1 bind. The affinity of the high mobility group protein HMGB1 for cisplatin-damaged DNA is enhanced by P53. This can lead to cell cycle arrest or apoptosis. Further, HMG box proteins, such as HMGB1 (HMG Box Protein 1) and tsHMG (testes specific HMG Box Protein) shield the Pt-1,2-d(GpG) cross-link from nucleotide excision repair proteins.

Adverse Effects The adverse effects of cisplatin are severe. Its gastrointestinal and renal toxicity nearly led to the discontinuation of its use in the 1970s, until it was realized that they can be managed. Gastrointestinal events (nausea, vomiting, and diarrhea) can be softened by anti-emetic drugs (Serotonin Receptor antagonists). Although the kidney damage is cumulative and irreversible, the nephrotoxicity can be substantially managed by proactive hydration combined with the use of diuretics.

Angina pectoris and myocardial infarction have occurred during or shortly after cisplatin treatment, possibly due to direct endothelial damage or vasospasm. The vasospasm could be a consequence of magnesium wasting. Thrombembolic phenomena including arterial occlusion, deep venous thrombosis, pulmonary emboli, transient ischemic attack, stroke, and renal artery occlusion are possible with cisplatin treatment. Microalbuminuria, a marker of generalized endothelial dysfunction, is present in up to 20% of long-term testicular cancer survivors treated with cisplatin. Other indicators of endothelial cell activation (von Willebrandt Factor, Plasminogen Activator Inhibitor 1, carotid artery thickness) may also increase with cisplatin therapy, and could aid in monitoring the vascular risks of patients.

Peripheral neuropathies and hypomagnesemia with resulting tetany may be encountered (Von Hoff 1979). Ototoxicity (tinnitus and hearing loss) can lead to deafness. In rare cases, the administration of cisplatin is associated with anaphylactic reactions, myelosuppression, and occasional transient liver dysfunction. Due to the affinity of Pt^{II} for sulfur binding, sulfur compounds may be used as rescue agents in cisplatin toxicity (Reedijk 1999).

Drug Resistance If ovarian cancer recurs within less than 6 months after the completion of chemotherapy, the tumor is considered to be platinum resistant. Resistance to cisplatin can be caused by several mechanisms (Farrell 2003; Wang and Lippard 2005). It may be due to a combination of

- increased efflux of platinum from the cell. The transporter ATP7B (Copper Transporting P-Type Adenosine Triphosphate) has an important physiological role in regulating copper levels in the cell. It is associated with cisplatin resistance in various cancers. ABCC2 (ATP-Binding Cassette sub-family C 2, MRP2, cMOAT) also has a role in cisplatin resistance, presumably by promoting drug efflux.
- increased cytoplasmic detoxification through cellular thiols such as Glutathione (GSH). Due to the affinity of Pt^{II} for sulfur binding, platinum drugs can bind to Glutathione, Metallothionein, and serum Albumin. Therefore, the cellular thiol levels are associated with drug resistance.
- increased resistance to apoptosis. The kinase PKB (Protein Kinase B, AKT) promotes cell survival and downregulates apoptosis. An elevated activity of PKB associated pathways protects cells from apoptotic death induced by cisplatin. c-ABL is a member of the SRC family of non-receptor tyrosine kinases, which also carries a DNA binding motif. Nuclear c-ABL activity can be stimulated by cisplatin and acts to transmit DNA damage signals. Because c-ABL tyrosine kinase has a role in cisplatin mediated activation of apoptosis, a reduction in its activity can confer cisplatin resistance. The P53 pathway can partially mediate cisplatin cytotoxicity. Many tumors harbor defective P53 and are deficient in this pathway.
- enhanced repair of platinum-DNA adducts. Cisplatin-DNA adducts are removed primarily by the nucleotide excision repair mechanism. High levels of nucleotide repair proteins in cancer cells can mediate resistance. The favorable clinical response of testicular cancer to cisplatin reflects, in part, its intrinsically low levels of the nucleotide excision repair proteins XPA and ERCC1/XPF. DNA Polymerase η is a lesion bypass polymerase that aids tumor cells to gain resistance against cisplatin based chemotherapy. It allows cells to reduplicate DNA across cross-link lesions such as 1,2-d(GpG) cisplatin adducts (Pt-GG).

Second generation platinum compounds The continuously improving understanding of the chemical properties

and pharmacological actions of cisplatin guided the development of analogs that also have cis geometry but differ from cisplatin either in the ammine[23] carrier ligands or in the leaving chlorides. In general, modification of the chloride leaving groups of cisplatin results in compounds with different pharmacokinetic properties, whereas modification of the carrier ligands alters the efficacy or the spectrum of activity of the resulting complex (Coluccia and Natile 2007). The second generation compounds were developed in attempts to reduce toxicity or to expand the range of useful anti-cancer activity.

Carboplatin (cis-diammine(cyclobutane-1,1-dicarboxylate-*O, O′*)platinum^{II}) <Paraplatin, Blastocarb, Carbosin, Carbosol, Displata, Ercar, Nealorin, Novoplatinum, Ribocarbo> replaces the unidentate chloride ligands of cisplatin with a chelating cyclobutanedicarboxylate ligand. It has a lower rate of aquation and increased water solubility. Carboplatin entered the clinic in 1989 (U.S. FDA approval), principally in response to the necessity for reducing the toxic effects of the parent drug cisplatin. Carboplatin is essentially active in the same set of tumors as cisplatin, with no broader spectrum of activity indicated. The main uses include ovarian carcinoma, lung cancers, and head and neck cancers. The agent possesses tumoricidal activity similar to that of its parent compound, but is more stable and less toxic. Carboplatin is less effective than cisplatin to treat non-seminomatous germ cell tumors. It is not recommended for use except during a stem cell transplant.

Carboplatin contains a platinum atom complexed with two ammonia groups and a cyclobutane-dicarboxyl residue. The agent is activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups, such as GC rich sites, in DNA, thereby inducing intra-strand and inter-strand DNA cross-links, as well as DNA-protein cross-links. These carboplatin induced DNA and protein effects result in apoptosis and cell growth inhibition. In keeping with the kinetic inertness of carboplatin, significantly higher doses, compared to cisplatin, are required for equivalent levels of DNA platination and to generate equitoxic and equivalent anti-tumor effects. The clinical doses are 800–900 mg/m^2. Carboplatin is usually given by intravenous infusion. It can also be administered intra-peritoneally.

- Experimental carboplatin formulations include nano-capsules and liposomes.

Pharmacokinetics The blood levels of intact carboplatin decay in a biphasic manner after a 30-minute intravenous infusion of carboplatin with an initial half-life of 1–2 h and a post-distribution half-life of 2.5–6 h. Carboplatin exhibits linear pharmacokinetics over a therapeutic dose range. The drug is not bound to plasma proteins. However, the platinum from carboplatin becomes irreversibly bound to plasma proteins and is slowly eliminated with a minimum half-life of 5 days. The major route of elimination for carboplatin is renal excretion.

Adverse Effects The benefit of carboplatin, compared to cisplatin, is reduced nephrotoxicity as the drug can be excreted essentially intact. Carboplatin is also substantially less emetogenic than cisplatin. Carboplatin causes thrombocytopenia with a nadir of 10–14 days. Neutropenia with granulocyte counts below 1000/mm^3 occurs in 15% of patients; leukopenia with white blood cell counts below 2000/mm^3 arises in 15%. Marrow suppression is usually more severe in patients with impaired kidney function. Patients with poor performance status also experience a higher incidence of severe leukopenia and thrombocytopenia.

Nausea and vomiting occur in 65% of the patients (80% of previously treated ovarian cancer patients) within 24 h of treatment, and in about 1/3 of these patients it is severe. Carboplatin also leads to alopecia, fatigue, and abnormal blood electrolyte levels (magnesium, sodium, potassium, calcium). With a frequency below 30%, carboplatin injection can cause a burning sensation at the injection site, abdominal pain, diarrhea or constipation, mouth sores, taste changes, peripheral neuropathy (decreased sensation and paresthesia of the extremities), central neurotoxicity (patients over age 65 are at increased risk for dizziness, confusion, visual changes, tinnitus), ototoxicity (hearing loss of high pitched sounds), abnormal blood liver function, cardiovascular events (heart failure, blood clots, strokes), or allergic reaction. The adverse effects of carboplatin are almost always reversible. The drug is Pregnancy Category D.

Drug Interactions Aluminum reacts with carboplatin causing precipitate formation and loss of potency. Therefore, intravenous sets containing aluminum parts that may come in contact with the drug must not be employed. Very serious drug interactions may occur with nalidixic acid, a medication that is commonly used for the treatment of urinary tract infections. Drugs that may interact with carboplatin include, aminoglycoside antibiotics (gentamicin, neomycin), amphotericin B, hydantoin anti-seizure medications, loop diuretics (furosemide, bumetanide, ethacrynic acid).

Nedaplatin (cis-diammine-glycoloato-*O, O′*-platinum^{II}) <Aqupla> is a second generation cisplatin analog that initially received approval for use in Japan. Containing a ring structure, in which glycolate is bound to the platinum by a bidentate ligand, nedaplatin forms reactive complexes that bind to nucelophilic groups in the DNA. The agent is administered at 100 mg/m^2.

Adverse Effects Nedaplatin is less nephrotoxic and neurotoxic compared to both cisplatin and carboplatin. It causes myelosuppression with a late recovery of 6 weeks.

Iproplatin is a synthetic second generation platinum compound that is based on a Pt^{IV} complex. The agent binds to

[23] Metal ammine complexes contain one or more ammonia (NH_3) ligands.

and forms DNA cross-links and platinum-DNA adducts, resulting in failure of DNA reduplication and cell death.

Drug Resistance Although less prone to Glutathione inactivation compared to cisplatin, resistance to iproplatin may arise due to the repair of platination damage by tumor cells.

Third generation platinum compounds Oxaliplatin (ethanedioate platinum (2-aminocyclohexyl)amine, trans-L-diaminocyclohexane)oxalatoplatinum^{II}) <Eloxatin> was first synthesized in 1976. This third generation platinum analog received U.S. FDA approval in 2002. In this organoplatinum agent, the platinum atom is complexed with 1,2-diaminocyclohexane (DACH) and with an oxalate ligand as a leaving group. After displacement of the labile oxalate leaving group, active oxaliplatin derivatives (such as monoaquo- and diaquo- 1,2-diaminocyclohexane platinum) alkylate macro-molecules, forming both inter- and intra-strand platinum-DNA cross-links, which result in the inhibition of DNA reduplication and transcription, and in cell cycle non-specific cytotoxicity. The 1,2-diaminocyclohexane side chain may counteract alkylating agent resistance. Oxaliplatin is typically administered by infusion every 2 weeks in combination with fluorouracil and leucovorin for the treatment of advanced colorectal cancer (until the disease progresses or unacceptable toxicity sets in) or for the adjuvant treatment of stage III colon cancer in patients who have undergone complete resection of the primary tumor (12 cycles over 6 months). For this malignancy, oxaliplatin in combination with 5-fluorouracil/folinic acid (5-FU/FA) has increased the response rates from 15% for 5-fluorouracil alone to over 40%, although the increases in overall survival rates are modest. The FOLFOX regimen is the most common combination therapy use for oxaliplatin.

- Liposomal encapsulation of oxaliplatin generates lipoxal. Adverse effects are ameliorated to moderate myelotoxicity, nausea, peripheral neuropathy, and asthenia. Nausea can be controlled with ondansetron administration.
- Liposomally entrapped cis-bis-neodecanoato-trans-R, R-1,2-diaminocyclohexane platinum^{II} (L-NDDP) <Aroplatin> is a liposomal formulation of the structural oxaliplatinum analog cis-bis-neodecanoato-trans-R, R-1,2-diamino cyclohexane platinum^{II} (NDDP). This lipophilic, non-cross-resistant platinum compound is formulated in multi-lamellar liposomes of 1–3 mm diameter. The maximum tolerated dose after intravenous administration is 300 mg/m^2 with myelosuppression being dose limiting.

Pharmacokinetics At the end of a 2-h infusion, approximately 15% of the administered platinum is present in the systemic circulation. The remaining 85% is rapidly distributed into tissues or eliminated in the urine. The irreversible plasma protein binding of platinum is greater than 90%, the main binding proteins being Albumin and γ-Globulins. The major route of platinum elimination is renal excretion, whereas only about 2% of the drug are eliminated in the feces.

Adverse Effects Patients experience nausea and vomiting. Premedication with anti-emetics, including 5-HT$_3$ blockers (see Sect. 17.1.) with or without dexamethasone, is recommended. Other adverse effects may include diarrhea, neutropenia, or fatigue. Some patients experience an allergic reaction to platinum. Prolongation of the infusion time for oxaliplatin from 2–6 h may mitigate acute toxicities. Oxaliplatin has lower ototoxicity and nephrotoxicity than cisplatin and carboplatin. However, neuropathy (abnormal sensations around the mouth, possibly leading to an inability to swallow) may be dose limiting. Severe symptoms regress in most patients within 4–6 months. The drug is Pregnancy Category D.

Drug Resistance Oxaliplatin circumvents mismatch repair and reduplicative bypass (the ability of a cell to synthesize DNA past a site of DNA damage) (Fink et al. 1996; Raymond et al. 1998). In many types of tumor cells, it is not subject to cross-resistance with cisplatin. Although cisplatin and oxaliplatin form the same types of adducts at identical sites on DNA, their structures are distinct and are differentially recognized by mismatch repair proteins as well as some damage recognition proteins. They include HMGB1 (High Mobility Group Box Protein 1), TBP (TATA Box Binding Protein) and UBF (Upstream Binding Factor). DNA Polymerase η has a greater efficiency of error-free bypass of oxaliplatin adducts compared with cisplatin.

Lobaplatin (1,2-diammino-methyl-cyclobutaneplatinum^{II}-lactate) (D-19466) was developed by ASTA Medica (Degussa) for the treatment of cancer. ASTA Medica discontinued the development, and it subsequently became the responsibility of Zentaris AG, which had been formed in 2001 from the biopharmaceutical, inhalation technology and gene therapy activities of ASTA Medica. In 2002, Zentaris was acquired by AEterna Laboratories. Lobaplatin consists of a nearly equal mixture of two diastereoisomers, comprising the SSS configuration and the RRS configuration. Lobaplatin is approved in Japan and China for the treatment of chronic myelogenous leukemia (CML) and inoperable metastatic breast and small cell lung cancers. It is given at 50–70 mg/m^2 by intravenous bolus every 3–4 weeks.

Adverse Effects The limiting toxicity of lobaplatin is thrombocytopenia, with a nadir at approximately 2 weeks after drug administration. Leukopenia is less severe, and the drug does not induce nephrotoxicity, neurotoxicity, or ototoxicity. The pharmacokinetics and adverse effects of lobaplatin are strongly affected by the renal function, which determines total body clearance.

Drug Resistance Lobaplatin is not subject to cross-resistance with cisplatin.

Satraplatin is an orally administered third generation compound that forms highly reactive, charged platinum

complexes, which bind to nucleophilic groups in the DNA. This binding induces intra-strand and inter-strand cross-links, as well as DNA-protein cross-links, resulting in cell growth inhibition and apoptosis. Satraplatin may be indicated for non-small cell lung cancer and for hormone refractory prostate cancer.

Spiroplatin (aqua-1,1-bis(aminomethyl)-cyclohexane-sulfatoplatinum^{II}) is a synthetic derivative of cyclohexane sulfatoplatinum that induces DNA cross-links. It is under study for the treatment of solid tumors. The recommended dose is 30 mg/m^2 by 4 h infusion every 3 weeks.

Adverse Effects Similar to other platinum compounds, this agent itself is mutagenic and carcinogenic. Myelosuppression and renal toxicity (glomerular and tubular damage) are dose limiting.

Picoplatin (cis-amminedichloro[2-methylpyridine]platinum^{II}) (ZD-0473, JM-473, AMD-473) is a cisplatin analog designed to sterically hinder the approach of Glutathione and other cellular thiols, which intercept and sequester platinum drugs before they can reach the genome, thus generating drug resistance. Compared to cisplatin, this third generation platinum drug has slower rates of hydrolysis, and the reactivities toward thiourea, pyridine, methionine, and GMP are lower. Picoplatin forms unique platinum-DNA adducts that differ from those of cisplatin or carboplatin. It displays improved safety and efficacy over earlier generations of platinum drugs. The agent is in clinical trials for mono-therapy as well as for combination with gemcitabine, navelbine, doxorubicin, docetaxel, paclitaxel, or topotecan. The compound is orally active.

Drug Resistance The drug contains a bulky methylpyridine ligand at its platinum center, which is responsible for its ability to overcome platinum resistance.

Cisplatin resistance is a major obstacle in the treatment of gastric cancer. Heptaplatin (cis-malonatol) (SKI-2053R) <Sunpla> is approved for the treatment of gastric cancers in South Korea.

Metallothioneins constitute a family of stress induced proteins that contain a substantial portion of cysteine residues. They have high affinity for heavy metals and free radical scavenging ability. Whereas the cellular Metallothionein levels may confer resistance to cisplatin, they have a lower involvement in heptaplatin resistance.

Multi-nuclear platinum complexes with bridging linkers have been designed to circumvent platinum resistance in tumor cells. Triplatin (trans-{bis[trans-diamminechloroplatinum^{II} (μ-1,6-hexanediamine)]} diammineplatinum^{II} tetranitrate) (BBR3463) is a trinuclear compound of the third generation. The agent contains two reactive platinum centers, each containing a single labile chloride.

Adverse Effects Adverse effects include nausea and vomiting, and neutropenia.

Drug Resistance Cisplatin resistant cells are not cross-resistant to BBR3463.

Emerging platinum drug derivatives According to the original empirical structure-activity relationships, the trans-platinum complexes were considered to be inactive due to

- the kinetic instability promoting their deactivation; trans-chloro species are more reactive than the corresponding cis-isomers, therefore undesired reactions could contribute to the lack of pharmacological activity
- the formation of DNA adducts characterized by a regioselectivity and a stereochemistry different from those of cisplatin; the major cytotoxic DNA lesion formed by cisplatin, the 1,2-intra-strand cross-link between adjacent purines, is stereochemically inaccessible to trans-isomers.

However, some newer trans compounds are under investigation for anti-tumor activity. Most of the active trans-platinum compounds fall into four classes. One class includes platinum^{IV} complexes of the general formula trans-[$PtCl_2X_2(L)(L')$] (X = hydroxide, carbamate, or carboxylate; L, L′ = ammine or amine). The remaining three classes, all containing platinum^{II} species, have the general formula trans-[$PtCl_2(L)(L')$] (with a) L = N-donor aromatic heterocycle and L′ = ammine, sulphoxide, or a second aromatic N-donor ligand; (b) L = sterically hindered aliphatic amine, including piperazine and L′ = ammine, a second aliphatic amine, or a pyridine-type ligand;(c) L = imino ligand and L′ = ammine or a second molecule of imino ligand) (Coluccia and Natile 2007). Both cisplatin and transplatin react preferentially at the *N*7 position of guanine and adenine residues of DNA to form a variety of mono-functional and bi-functional adducts. However, transplatin is characterized by a reduced capability to form bifunctional adducts in double helical DNA, and cannot produce DNA 1,2-intra-strand cross-links. Trans-platinum compounds may have value in the treatment of tumors that have developed resistance to cis-platinum compounds.

In the early development of analogs to the platinum compounds, complexes with windows of reactivity similar to the platinum complexes were examined extensively. Whereas direct Ni and Pd analogs of Pt complexes are too kinetically reactive to be of use as drugs, Ir and Os ammine compounds are in general too inert. Ruthenium and rhodium have produced compounds with the greatest promise, although no direct analogs have yet advanced to the clinic.

Upon aquation of a ligand, platinum drugs become highly reactive, allowing them to coordinate DNA bases.
Complexes with labile leaving groups are toxic, compounds with stable leaving groups are inactive. Modifications in the

chloride leaving group affect pharmacokinetics; modifications in the ligand affect efficacy and spectrum of activity.
Most platinum compounds cannot be taken orally.
Early generation platinum drugs have severe adverse effects.
Recent generation platinum drugs reduce toxicity and avoid cross-resistance.
Drug resistance may be caused by platinum efflux, detoxification through thiols, apoptosis resistance, or enhanced DNA repair.

2.1.9 Others

Ecteinascidins are isolated from marine organisms, ecteinascidin-743 (trabectedin, ET-743) <Yondelis> is produced by the Caribbean tunicate Ecteinascidia turbinate. The alkaloid is composed of three tetrahydroisoquinoline moieties, eight rings, and seven chiral centers (Fig. 2.18). It contains three fused tetrahydroisoquinoline subunits (A, B, and C). The A and B subunits provide the scaffold for DNA recognition and covalent bonding. The C subunit protrudes out and interacts with adjacent nuclear proteins. Ecteinascidin-743 binds to the minor groove of DNA, effects guanine *N*2 alkylation (via an intermediate iminium generated from an active carbinolamin functional group), and bends the duplex toward the major groove. This may sequester specific transcription factors, inhibit inducible gene transcription, and result in cytotoxicity. The alkylation at guanine 2–NH_2 positions is sequence selective and region specific to DNA triplets containing a central guanine (AGC, CGC, TGG). Ecteinascidin-743 also binds tightly to Topoisomerase 1, with which it produces cleavage complexes. The mechanism of action may also involve the production of superoxide via reduction of molecular oxygen near the DNA strand (through an unusual auto-redox reaction on a hydroxyquinone moiety), resulting in DNA backbone cleavage and cell apoptosis. The compound has activity against a variety of soft tissue sarcomata and is in clinical trials for breast, ovarian, and prostate cancers.

The pyrrolizidine alkaloids are distributed widely in nature as the tertiary bases of *N*-oxides and are typically generated in species of Compositae, Leguminosae, and Boraginaceae. Indicine-*N*-oxide (NSC 132319) is a natural pyrrolizidine alkaloid produced by Heliotropium indicum Linn. (family Boraginaceae). Indicine-*N*-oxide alkylates and cross-links DNA. It is under study as anti-cancer agent for use at 3.0 g/m^2 daily for 5 days, repeated every 5 weeks.

Pharmacokinetics The intravenous injection of indicine-*N*-oxide produces a dose dependent increase in blood levels. The blood concentrations then exhibit a biphasic decline (consistent with a 2-compartment open model). The gut flora as well as the liver are capable of reducing indicine-*N*-oxide to form indicine. Approximately 40 % of the administered dose is eliminated in the urine within 24 h as unmetabolized drug and below 5 % as the free base indicine.

Adverse Effects Dose limiting toxicities of indicine-*N*-oxide are reversible leukopenia and thrombocytopenia. Repetitive courses have a cumulative effect on the severity of myelosuppression. Other adverse effects include mild

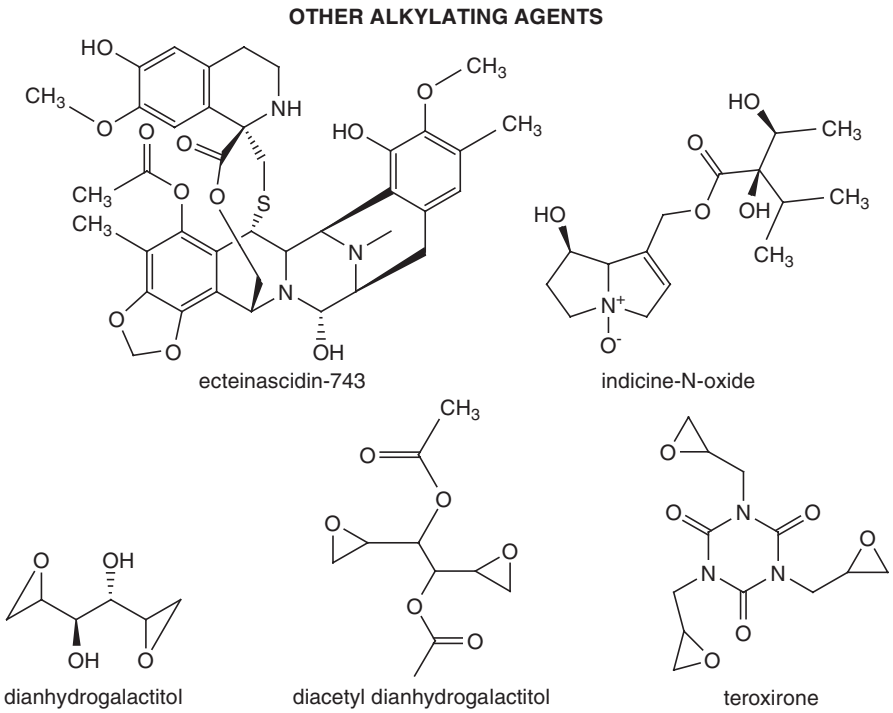


Fig. 2.18 Structures of various alkylating agents

nausea and vomiting during treatment, bone marrow hypoplasia, nephrosis, emesis, and bloody diarrhea.

Drug Interactions Prior treatment with a nitrosourea enhances the hematopoietic toxicity of indicine-*N*-oxide.

Dianhydrogalactitol (1,2:5,6-diepoxyhexane-3,4-diol, 1,2-di(oxiranyl)ethylene glycol, dulcitol diepoxide, DAG) (NSC 132313) is a bifunctional hexitol diepoxide that was developed in Budapest (Nemeth et al. 1972). It alkylates and cross-links DNA via an epoxide group during all phases of the cell cycle, resulting in a disruption of DNA function and cell cycle arrest. The drug has good penetration into the brain and can be applied to the treatment of brain tumors. It has been investigated in combination with radiation therapy.

- Diacetyl dianhydrogalactitol (DADAG) is a derivative of dianhydrogalactitol.

Pharmacokinetics The mean residence time for pharmacologically active dianhydrogalactitol in the body is 2 h. The drug is rapidly consumed by intracellular alkylation and degradation.

Adverse Effects Adverse effects comprise nausea and vomiting, bone marrow depression, immunosuppression, psychiatric and neurologic disturbances, and allergic eczema. With lesser incidence, kidney and liver damage or ulcerative stomatitis may arise.

Teroxirone (1,3,5-triglycidyl isocyanurate, Henkel's Compound) is a triazine triepoxide that alkylates and cross-links DNA, thereby inhibiting DNA reduplication (Atassi et al. 1980). An experimental regimen gives the drug intravenously on five consecutive days every 4 weeks.

Pharmacokinetics Following rapid intravenous administration, teroxirone has a rapid blood elimination half-life below 5 min, and total body clearance over 5 L/min. The drug is metabolized in the liver, and 70% of the metabolites are excreted in the urine.

Adverse Effects Myelosuppression is the dose limiting toxicity. Local phlebitis and cutaneous flare reactions commonly arise at high doses. The maximal tolerated dose is 340 mg/m^2/day.

Treatment of Testicular Cancer

In the mid-1970s, following the work on acute leukemia, lymphoma and breast cancer, Lawrence Einhorn and his group, building on the initial work of M.C. Li at Memorial Hospital (Li 1969), began a series of studies that resulted in an improvement of the cure rate of metastatic testicular cancer. A landmark trial, reported in 1977, used a regimen of cisplatin, vinblastine, and bleomycin for four cycles, followed by 21 months of maintenance vinblastine to increase the complete response rates from 25 to 80% (Einhorn and Donohue 1977). Today, chemotherapy is used for all stages of this tumor and testicular cancer is curable in most patients with rates of nearly 80%, survival being primarily due to effective chemotherapy. Patients may initially present to a variety of practitioners, and delays in therapy are associated with more extensive disease, resulting in more intensive treatment and lower cure rates.

1. **Non-seminomatous cancers**

Testicular non-seminomatous cancers often respond well to cisplatin. By contrast, the substitution of the less toxic carboplatin for cisplatin results in poor relapse-free survival rates. Treatment decisions are made based on risk stratification into good, intermediate, and poor prognostic groups according to the universal classification scheme developed by the International Germ Cell Cancer Collaborative Group (IGCCG).

- Approximately 90% of patients classified as having a good prognosis (primary site not mediastinum, no extrapulmonary visceral metastases, hCG below 5000 international units/L, AFP below 1000 units/L, LDH below 1.5-fold upper limits of normal) achieve a durable complete remission with either four cycles of etoposide and cisplatin (EP) or three cycles of bleomycin, etoposide, and cisplatin (BEP). The survival benefits of both regimens are comparable. Neutropenia is more common with EP, while neuropathy and adverse dermatologic effects occur more frequently with BEP.
- Complete responses are achieved less frequently for patients with intermediate and poor risk germ cell tumors, in whom four cycles of bleomycin, etoposide, and cisplatin (BEP) remains the standard of care. Attempts to improve outcomes by intensifying the BEP regimen (increased cisplatin dose, replacement of bleomycin with ifosfamide, high-dose chemotherapy with autologous stem cell support) have not demonstrated any advantage but have been associated with more intense toxicities.
- Fewer than 10% of patients suffer relapses. For those, second- and third-line programs also have curative potential. They include standard doses of 3-drug combinations based on ifosfamide and cisplatin (plus either vinblastine or etoposide or paclitaxel). High-dose salvage chemotherapy has been used since the late 1980s, but was initially limited by high mortality rates. This prompted the addition of growth factor support or autologous stem cell support. At least two high dose cycles are con-

sidered necessary to achieve a benefit. Alternative options to provide disease control and palliation include the combination of gemcitabine with oxaliplatin or paclitaxel and the combination of cisplatin with epirubicin.

Adverse Effects The commonly used chemotherapeutic agents can all cause myelosuppression leading to anemia, bleeding, and febrile neutropenia (5–25% risk with BEP or EP). Growth factor support and prophylactic fluoroquinolone may lower this risk. Acute cardiovascular and thromboembolic toxicities may be linked to germ cell tumor chemotherapy. Nephrotoxicity, ototoxicity, and neuropathy can occur and may persist in 20–40% or patients. Azoospermia always arises during cisplatin therapy. However, approximately 50% of patients regain normal sperm counts within 2 years from treatment, and this proportion increases to 80% within 5 years. Testicular cancer survivors are at a 2–3-fold increased risk of secondary malignancies following chemotherapy, radiation, or a combination of these modalities. They include tumors of the pleura, pancreas, stomach, bladder, and connective tissue. Myelodysplastic syndrome and secondary leukemia (3–7% risk) are also potential consequences of combination chemotherapy, in particular of etoposide.

Surgery may follow chemotherapy. Tumor marker normalization is a prerequisite for post-chemotherapy surgery because elevated markers predict a high likelihood of incomplete resection (Feldman et al. 2008).

Testicular cancer survivors have an elevated risk of serious co-morbidities. Of patients who survive at least a year from their initial diagnosis, more than 40% of deaths are from non-malignant causes. These include gastrointestinal disorders (intestinal vascular lesions, hepatobiliary disease, ulcers), cardiovascular disease (angina pectoris, myocardial infarction), infections, and possibly respiratory illness. The metabolic syndrome, a constellation of three or more characteristics (abdominal obesity, hypertriglycerinemia, low levels of high-density lipoprotein, hypertension, Insulin resistance), may occur in 25–40% of testicular cancer survivors compared to 3–4% of the general population. Sarcoidosis is also more common in patients with germ cell tumors, although the causes underlying this association are unknown. Most relapses occur within the first 2 years after completion of treatment, but late relapses may also occur with an incidence of 2–5%. Because of the generally poor outcome for late relapse testicular cancer with chemotherapy, surgery is the mainstay of management.

2. **Seminomatous cancers**

Almost all seminomata are curable with orchiectomy with or without radiation therapy. They rarely require chemotherapy. When chemotherapy is necessary, the same regimens are used as for other testicular germ cell tumors. Post-chemotherapy surgical resection of a seminoma carries a higher morbidity than other germ cell tumors due to the desmoplastic reaction frequently induced by the treatment.

Treatment of Astrocytoma

Chemotherapy The chemotherapy of brain cancers requires the administration of drugs that can penetrate the blood-brain barrier. Agents that commonly have efficacy in patients with high-grade gliomas include procarbazine, platinum analogs (cisplatin, carboplatin), the nitrosoureas, and temozolomide <Temodar>.

The oral alkylating agent temozolomide <Temodar> has become the standard therapy for glioblastoma. It is indicated for the treatment of

- adult patients with newly diagnosed glioblastoma multiforme concomitantly with radiotherapy and then as maintenance treatment
- adult patients with refractory anaplastic astrocytoma. These are patients who have experienced disease progression on a drug regimen containing nitrosourea and procarbazine.

The daily dose is calculated based on the body surface area and rounded off to the nearest 5 mg. The dosage must be adjusted according to nadir neutrophil and platelet counts in the previous cycle and neutrophil and platelet counts at the time of initiating the next cycle. Patients should take each day's dose with a full glass of water at the same time each day. Taking the medication on an empty stomach or at bedtime may help ease nausea.

Patients with newly diagnosed high grade glioma in the concomitant phase are treated with temozolomide at 75 mg/m^2 daily for 42 days concomitant with focal radiotherapy (60 Gy administered in 30 fractions). This is followed by maintenance temozolomide for six cycles. Although dose interruption or discontinuation may be inevitable if toxicity becomes substantial, no dose reductions are recommended during the concomitant phase. The dose should be continued throughout the 42-day concomitant period, if

- the absolute neutrophil count is at least 1500/μL
- the absolute platelet count is at least 100,000/μL
- common toxicity criteria do not exceed grade 1 (except for alopecia, nausea, and vomiting).

In the maintenance phase, temozolomide is administered for an additional six cycles of treatment. Dosage in cycle 1 is 150 mg/m^2 daily for 5 days followed by 23 days without treatment. At the start of cycle 2, the dose can be escalated to 200 mg/m^2 if the toxicity for cycle 1 is below grade 2 and the absolute neutrophil count and platelet count are in the acceptable range. The dose remains at 200 mg/m^2 per day for the first 5 days of each subsequent cycle except if toxicity occurs.

In 2009, the U.S. FDA approved an intravenous form of temozolomide. It is given by infusion over 90 min. Various dosing schedules are in use.

Adverse Effects The most common adverse effects are nausea and vomiting, constipation, anorexia, alopecia, headache, convulsions, fatigue, and thrombocytopenia. Patients may experience dose limiting myelosuppression, with grade 3–4 neutropenia occurring in 10% and grade 3–4 thrombocytopenia in 15% of cases. Prophylaxis against Pneumocystis carinii pneumonia is required for all patients receiving concomitant temozolomide and radiotherapy for the 42-day regimen. All patients receiving temozolomide, particularly patients who also receive steroids, should be observed closely for the development of Pneumocystis carinii pneumonia, regardless of the regimen applied. Temozolomide is contraindicated in patients who have hypersensitivity to any of its components or to dacarbazine (DTIC). The drug may cause fetal harm when administered to a pregnant woman; nursing should be discontinued in women during treatment. As temozolomide may affect testicular function, male patients should exercise adequate birth control measures.

Drug Resistance The DNA repair protein O^6-Alkylguanine DNA Alkyl Transferase (AGAT, MGMT), which removes methyl groups from O^6-methyl-guanine lesions that arise from temozolomide treatment, may confer dug resistance. MGMT is consumed stoichiometrically during the DNA repair reaction (it does not act as an enzyme). As DNA repair by MGMT is the primary mechanism of drug resistance to temozolomide, its expression level in the cancer cells determines the extent of the drug response.

BCNU (carmustine) <BiCNU> is a prodrug that has displayed efficacy against astrocytoma. It may be given systemically or topically. By applying it directly to the diseased area of the brain, adverse effects are limited and the drug has a more beneficial effect.

- The regimen BCNU-DAG entails 90 mg/m^2 carmustine and 70 mg/m^2 dianhydrogalactitol intravenously on day 1. Repetition of the cycle at 5 weeks, followed by subsequent cycles at 5–7 week intervals during the first year and 10–12 week intervals during the second year. It is used to treat primary brain tumor recurrence after irradiation.
- The regimen BCNU-FU entails 180 mg/m^2 carmustine intravenously on day 1 plus intravenous infusion of 1000 mg/m^2/day fluorouracil for 72 h beginning on day 15. The cycle is repeated every 6 weeks until there is evidence of tumor progression. The approach is a combination therapy for recurrent malignant brain tumors.
- A biodegradable polifeprosan 20 wafer impregnated with carmustine <Gliadel> may be used for topical postsurgical treatment preceding ionizing radiation. The wafers produce high local concentrations of carmustine for several weeks directly into the tumor bed after surgery when the tumor burden is low.

Molecularly targeted therapy The cells of glioblastomata and anaplastic gliomatas secrete glutamate and also express AMPA Glutamate Receptors, which contribute to proliferation, migration and neurotoxicity. Talampanel (GYKI 537773, LY300164) is a synthetic derivative of dioxolo-benzodiazepine, which non-competitively binds to the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of glutamate excitatory amino acid receptors and may inhibit the growth of gliomata by blocking a growth factor signal.

Adverse Effects The long-term effects of benzodiazepines include drug dependence as well as the possibility of adverse effects on cognitive function, physical health, and mental health. There is evidence that benzodiazepine use may lead to an increased risk for cancer.

Glioblastoma is a highly aggressive cancer, in which the PI 3-Kinase pathway is excessively activated via receptor tyrosine kinases. Activation of ERBB1 (EGFR) is a critical pathogenetic event. Other receptor tyrosine kinases, including PDGFR and MET may also be altered. Because these receptors can be co-activated, monotherapy with ERBB1 inhibitors or PDGFR inhibitors has a low rate of success. However, combined

regimens that target multiple receptor tyrosine kinases may effectively suppress the PI 3-Kinase pathway, inhibit tumor growth, and lead to improvement (Stommel et al. 2007).

Supportive treatment Patients with malignant glioma may require anti-epileptic medicine. Often, their anticancer chemotherapy contains irinotecan. The plasma concentration of irinotecan and its active metabolite SN-38 is reduced and the clearance is increased in patients receiving anticonvulsants. This is likely based on the induction of hepatic Cytochrome P450 enzymes (CYPs) by anticonvulsant agents and needs to be considered in irinotecan dosing.

2.2 Antibiotics

Among the research programs related to World War II was a large scale screening of bacterial and fungal fermentation products by pharmaceutical companies to isolate and produce antibiotics suitable for treating wound infections. Some of the agents under investigation were also examined for their anti-tumor effects. The program was largely based on observations with penicillin, which had initially been thought to have tumor suppressing properties. In particular, Streptomyces species are a genus of soil bacteria, from which numerous antibiotic and antifungal compounds have been derived. The antibiotic actinomycin D, extracted from Streptomyces, was an early product of the large scale screening endeavor. Introduced by Sidney Farber into the clinic in 1954, actinomycin D[24] had substantial anti-cancer effects and therefore found considerable use in the treatment of pediatric tumors through the 1960s. This drug established feasibility and led to a continued search for more active anti-tumor antibiotics[25]. The effort has yielded a series of compounds that are in common use today (DeVita and Chu 2008).

Anti-neoplastic antibiotics fall into two broad functional classes. Cyclopropylpyrroloindoles, minor groove DNA binding antibiotics, and aminoquinones alkylate DNA. Polycyclic aromatic antibiotics (anthracyclins, anthracenediones, anthrapyrazoles) and enediynes generate free radicals through redox cycling by a mechanism that depends in part on cellular iron; the generated reactive oxygen species damage the DNA of cancer cells. In addition, polycyclic aromatic antibiotics inhibit Topoisomerase 2.

Adverse Effects The production of reactive oxygen species is in some measure responsible for the cardiotoxicity exerted by drugs in the class of redox cycling antibiotics. Specifically, the prolonged treatment with anthracyclins can lead to a distinctive and life threatening form of cardiomyopathy.

Cyclopropylpyrroloindoles, minor groove DNA binding antibiotics, and aminoquinones alkylate DNA.
Polycyclic aromatic antibiotics (anthracyclins, anthracenediones, anthrapyrazoles) and enediynes damage DNA through redox cycling.
Polycyclic aromatic antibiotics inhibit Topoisomerase 2.
Cardiotoxicity of redox cycling antibiotics is an adverse class effect.

2.2.1 Cyclopropylpyrroloindole Antibiotics

The cyclopropylpyrroloindole analogs (drug names ending on -zelesin) contain a cyclopropyl group, which mediates sequence selective *N*3 adenine covalent adduct formation. These DNA minor groove binding compounds do not react with single stranded DNA, RNA, or protein

Adverse Effects Myelosuppression, arising in a biphasic time course, is the dose limiting toxicity.

The prototypical cyclopropylpyrroloindole anti-cancer antibiotic CC-1065 (NSC 298223) (Fig. 2.19) was isolated from the fermentation products of the soil organism Streptomyces zelensis in the 1970s (Hanka et al. 1978). The drug had high potency and a novel mechanism of action. CC-1065 binds covalently, non-intercalatively to double stranded DNA within the minor groove with a sequence preference for 5′-d(A/G)NTTA-3′ and 5′-dAAAAA-3′ (N denotes any base). It alkylates the *N*3 position of the 3′-adenine with its left-hand cyclopropylpyrroloindole segment. It also inhibits gene transcription by interfering with the binding of TATA Box Binding Protein (TBP) to its target DNA.

Adverse Effects Despite the high potency and broad spectrum of anti-tumor activity, the clinical development of CC-1065 was precluded by irreversible hepatic and renal toxicities and delayed lethality.

CC-1065 analogs have been synthesized and tested to pursue compounds that retain the potent anti-cancer activity, but are devoid of the toxic effects of the parent compound. (+)-duocarmycin A and (+)-duocarmycin SA are derived from Streptomyces species. They exert their biological

[24] The suffix -mycin is conventionally used to describe a substance derived from a bacterium in the order Actinomycetales.

[25] The term antibiotic was coined by Selman Waksman, the discoverer of actinomycin D.

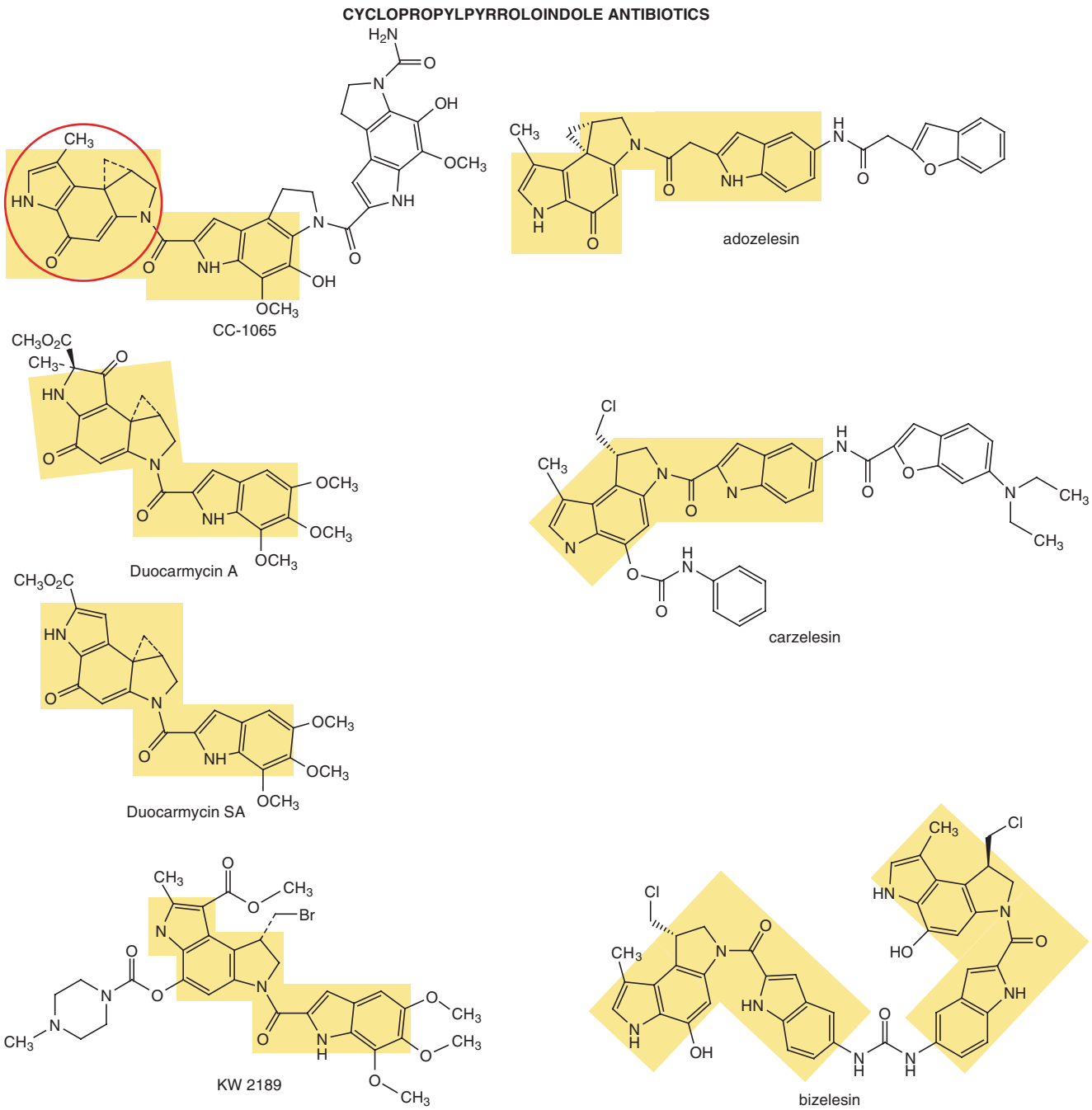


Fig. 2.19 Structures of cyclopropylpyrroloindole antibiotics. The common chemical components are highlighted in *yellow*. The alkylating unit of CC-1065 is circled in *red*.

effects through participation in a stereoelectronically controlled minor groove adenine *N*3 alkylation of duplex DNA. Duocarmycin A also alkylates a guanine in the recognition sequence 5′-CGCGTTGGGAG-3′.

KW 2189 (Methyl(1*S*)-1-bromomethyl-7-methyl-5-[(4-methylpiperazinyl)-carbonyloxy]-3-[(5,6,7-trimethoxy-2-indolyl)-carbonyl]-1,2-dihydro-3H-pyrrolo[3,2-e]indole-8-carboxylate 3 hydrobromide) (Kobayashi et al. 1994) is a semi-synthetic, water soluble derivative of duocarmycin B2. After activation by Carboxyl Esterase, KW 2189 alkylates DNA by binding to A/T-rich sequences in the minor groove of DNA, thereby inhibiting DNA reduplication and inducing apoptosis.

Adverse Effects Severe and prolonged hematologic toxicity is dose limiting and may prevent the further development of this agent for clinical use.

Adozelesin, carzelesin, and bizelesin were originally isolated from Streptomyces zelensis. They bind genomic DNA

at the sequence motif 5′-(A/T)(A/T)A-3′ and alkylate the *N*3 position of adenine at the 3′ end of their binding sites. Adozelesin induces single strand DNA lesions, whereas bizelesin induces both single strand lesions and double strand DNA cross-links. At equivalent cytotoxic concentrations, these agents induce distinct biological responses. However, in cells lacking the tumor suppressor protein P21, bizelesin, as well as adozelesin, trigger apoptosis, reflecting a crucial role for P21 in sustained bizelesin induced G_2/M arrest (Cao 2003).

Adozelesin (U-73,975, NSC-615284) causes genomic lesions by alkylating DNA and introducing single strand breaks.

- The agent interacts with the minor groove of DNA and forms covalent adducts with the *N*3 of adenines. This results in the formation of adozelesin-DNA adducts, which leads to an inhibition of DNA reduplication. The S phase specific induction of DNA damage responses by adozelesin depends on active reduplication forks.
- The inhibition of DNA reduplication by adozelesin occurs in part through inactivation of the hetero-trimeric, trans-acting factor RPA (Replication Protein A). RPA is the major single strand DNA binding protein. It is essential for reduplication and plays critical roles in repair and recombination.
- Low adozelesin concentrations induce a transient S phase block and cell cycle arrest in G_2/M, as well as the induction of P53 and P21, whereas high drug concentrations cause apoptosis but no P21 induction.

Carzelesin (U-80,244, NSC-619029) is a prodrug cyclopropylpyrroloindole analog that contains a relatively non-reactive chloromethyl precursor to the cyclopropyl function. Activation of carzelesin requires two steps, the hydrolysis of a phenylurethane substituent followed by ring closure to form the cyclopropyl containing DNA reactive drug.

Adverse Effects Bone marrow suppression is the primary adverse effect. Fatal hepatotoxicity is possible.

Bizelesin (U-77,779, NSC-615291) is a cyclopropylpyrroloindole dimer with two reactive chloromethyl moieties that can form DNA adducts with adenines in either one or both DNA strands.

- Bizelesin binds to A/T-rich sequences in the minor groove of DNA and induces inter-strand cross-linking of DNA, thereby inhibiting DNA reduplication and RNA synthesis. The canonical 5′-T$(A/T)_4$A-3′ target motifs are on average scarce and scattered. However, they have higher local motif densities in distinct mini-satellite regions. These A/T islands exhibit the sequence attributes of matrix attachment regions, domains that organize DNA loops on the nuclear matrix. Bizelesin may interfere with that organization.
- Bizelesin enhances P53 and P21 induction and triggers G_2/M cell-cycle arrest, resulting in cell senescence without apoptosis.

Bizelesin has activity against colorectal cancer.

The cyclopropyl groups in cyclopropylpyrroloindole antibiotics mediate N3 adenine covalent adduct formation in a sequence selective fashion.
Myelosuppression is the dose limiting toxicity.
Adozelesin, bizelesin, and carzelesin alkylate the N3 of adenines at the 3′ end of the DNA sequence motif 5′-(A/T)(A/T)A-3′.
Low doses of adozelesin, bizelesin, or carzelesin induce cell cycle arrest, high doses induce apoptosis.
P21 is crucial to sustained bizelesin induced G2/M arrest.

2.2.2 Minor Groove DNA Binding Antibiotics

The minor groove of DNA represents a vulnerable site for attack that is normally unoccupied because most DNA interactive proteins bind to the major groove. Certain sequences in the minor groove are sites of highly specific, non-covalent, and reversible interactions with some antibiotics.

Binders to A/T-rich sites Minor groove binders to A/T-rich sequences are derived from distamycin A (Fig. 2.20), which is produced by Streptomyces distallicus. Distamycin A alters DNA conformation by forming strong reversible complexes preferentially at nucleotide sequences that consist of 4–5 adjacent A/T base pairs. Thus, the drug displaces essential transcription factors and interferes with gene expression. While distamycin A has anti-viral properties it exerts weak cytotoxicity and does not possess sufficient efficacy against cancer. Further, distamycin is cardiotoxic.

The pyrrole-amide backbone of distamycin A has been used as a sequence selective vehicle for the delivery of DNA alkylating functions, leading to a sharp gain in cytotoxicity. Several such derivatives are in testing for anti-cancer treatment.

Tallimustine (FCE-24517, PNU-152241) is a benzoyl nitrogen mustard derivative of distamycin. Tallimustine alkylates *N*3 adenines in the target sequence 5′-TTTTGA-3′. This may lead to an accumulation of cells in the G_2 phase of the cell cycle. The therapeutic dose is 750 μg/m^2, the maximally tolerable dose is 1250 μg/m^2. The agent is under study for anti-leukemia treatment.

Adverse Effects The dose limiting toxic effect is neutropenia, which may become febrile. Platelet counts may drop.

Drug Resistance Treatment with verapamil may reduce resistance to tallimustine.

MEN 10710 is a synthetic distamycin derivative that possesses four methyl-pyrrole rings and a bis-(2-chloroethyl) aminophenyl moiety linked to the oligopyrrole backbone by a flexible butanamido chain.

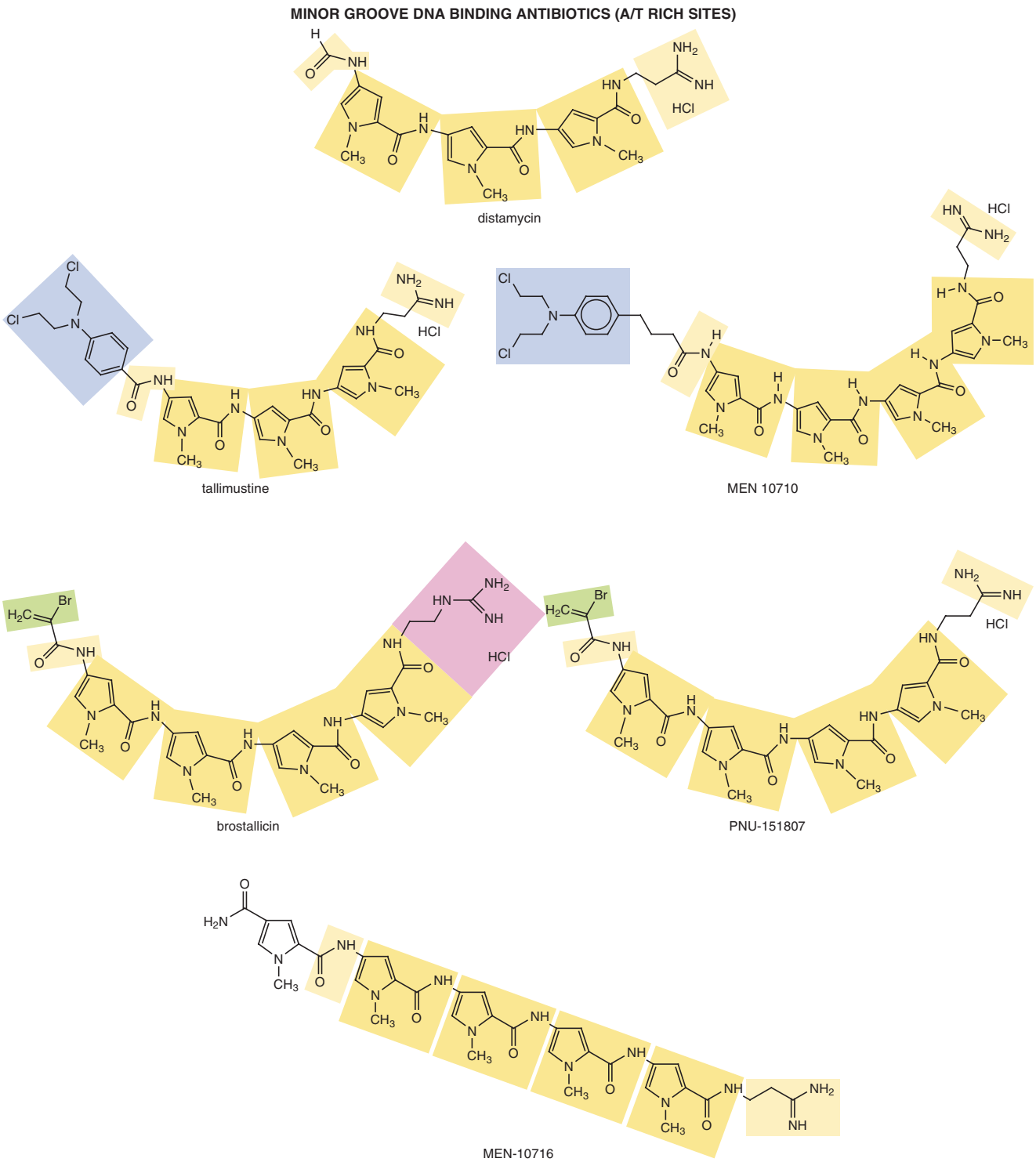


Fig. 2.20 Structures of minor groove DNA binding antibiotics with a preference for A/T rich sites. Bright *yellow* indicates the common methyl-pyrrole-carboxamide motif that repeats 3 (distamycin, tallimustine) or 4 (MEN 10710, brostallicin, PNU-151807, MEN 10716) times. The units are flanked by identical carboxamide and 3-amino-3-iminopropyl groups (light *yellow*), with only one modification within the drug class in brostallicin (pink). Tallimustine and MEN 10710 also have a bis-(2-chloroethyl)aminophenyl group (*blue*) that is similar to nitrogen mustards. Brostallicin and PNU-151807 contain a bromoacrylate functional group (*green*).

Brostallicin (PNU-166196) is a bromo-acrylamido tetrapyrrole distamycin derivative with high cytotoxic potency. The action of the drug involves its activation upon binding to Glutathione, catalyzed by Glutathione-S-Transferase (GST). Therefore, cells with high GST and Glutathione levels (which is commonly the case in tumors) are susceptible to

treatment with brostallicin. Activated brostallicin is capable of DNA minor groove attack, leading to alkylation of DNA nucleophilic functions. The agent is in clinical trials for the treatment of breast cancer, ovarian cancer, and soft tissue sarcomata.

Adverse Effects Brostallicin has reduced myelotoxicity compared with other minor groove DNA binding agents.

Drug Resistance Glutathione conjugation is a prevalent mechanism of resistance to various anti-cancer drugs. Therefore, brostallicin may be used to treat cells that are resistant to other forms of chemotherapy.

PNU-151807 is a synthetic α-bromoacryloyl derivative of distamycin A (Marchini et al. 1999) that interacts non-covalently with the same A/T-rich DNA regions. Unlike other drugs in this class, however, there is no evidence that it produces DNA alkylation. PNU-151807 abolishes the kinase activities of CDK2/Cyclin A, CDK2/Cyclin E, and CDC2/Cyclin B complexes. PNU-151807 triggers apoptosis via activation of Caspases. While it may induce the activation of P53, a disruption of P53 function does not substantially affect the cytotoxic activity of the drug. This may make it useful for treating tumors with inactivating *p53* mutations.

Drug Resistance Mismatch DNA repair deficiency is associated with resistance to certain anti-cancer drugs. It may occur as a consequence of loss of MLH1 expression. By contrast, loss of mismatch repair does not mediate resistance to PNU-151807.

The distamycin derivative MEN 10716 is a minor groove DNA binding antibiotic. After prolonged exposure, the drug also acts as a Telomerase inhibitor.

Binders to G/C-rich sites The aureolic acid anti-cancer antibiotics contain an identical tricyclic poly-ketide core moiety, with a unique dihydroxy-methoxy-oxo-pentyl side chain attached at *C*3 (cyclized only in chromocyclomycin). These members are glycosylated compounds with 2 oligosaccharide chains of variable 2,6-dideoxy-sugar residue lengths. The *C*7 residue can be either a H atom or a small alkyl side chain. Aureolic acid antibiotics non-intercalatively bind to the DNA minor groove in high-GC-content regions as dimers that form in the presence of divalent cations, such as Mg^{2+}. Upon binding to DNA, the chromophores form hydrogen bonds with NH_2 residues of guanines, thus determining the selectivity for C/G-rich sequences. A result is partial unwinding of the DNA.

These antibiotics are produced by various Streptomycete species. A common step in their biosynthesis is the formation of the tetracyclic intermediate pre-mithramycinone. Further steps (glycosylation, methylations, acylations) proceed through tetracyclic intermediates, which in the end are converted into tricyclic compounds by the action of a Monooxygenase, a key event for the biological activity.

Mithramycin A (MTA, aureolic acid, plicamycin) (LA-7017, PA-144) <Mithracin> (Fig. 2.21)is an anti-neoplastic antibiotic, produced by Streptomyces plicatus, that was first tested in cancer therapy in the 1960s. The drug binds to C/G-rich DNA tracts in the minor groove. Because of this sequence selectivity, mithramycin A blocks the binding of the SP-1 family of transcription factors to C/G-rich sequences in gene promoters and inhibits gene transcription, which in turn alters the regulation of cell proliferation and differentiation. Mithramycin A has been under study for the treatment of testicular cancer, Ewing sarcoma, and chronic myeloid leukemia. Mithramycin A also inhibits bone degradation, which in turn lowers blood calcium. Thus, it is used to treat hyper-calcemia and hyper-calciuria associated with a variety of advanced cancers. Plicamycin is given through intravenous infusion over a period of 4–6 h.

- Mithramycin SK is structurally related to mithramycin A.

Adverse Effects Mithramycin A has myelotoxicity, leading to leukopenia, neutropenia, anemia, and thrombocytopenia. Other adverse effects include nausea and vomiting, diarrhea, loss of appetite, mouth sores, and electrolyte imbalance.

Olivomycin A, a compound produced by Streptomyces olivoreticuli, causes apoptosis in transformed cells of the hematopoietic system, breast, colon, ovaries, and skin (melanoma). The drug suppresses the expression of genes coding for transcription factors, heat shock proteins, and DNA repair proteins. Among the transcription factors down-regulated by olivomycin A is c-MYC, a major regulator of DNA synthesis and cell survival.

Drug Resistance The drug effect is not dependent on P53, which implies efficacy in cancers with loss of function in P53.

Chromomycin A3 (toyomycin, aburamycin) is a fermentation product of Streptomyces griseus. Chromomycin binds to G/C sequences, usually at least three consecutive G/C, and inhibits transcription. The clinical use of chromomycin is limited because of its immunosuppressive properties and high cytotoxicity.

- Chromocyclomycin is a metabolite of Streptomyces LA-7017.

UCH9 is produced by Streptomyces species. It consists of one aglycone, three D-olivoses, one 4-*O*-methyl-D-olivose, and one D-oliose. In contrast to other aureolic acid derivatives, which have di- and trisaccharide moieties and a methyl or a hydrogen attached to the aglycone, in UCH9 there are mono- and tetra-saccharide moieties and a long hydrophobic side chain. UCH9 binds as a dimer to the minor groove of $d(TTGGCCAA)_2$. The agent exhibits cytotoxic activity against transformed cells.

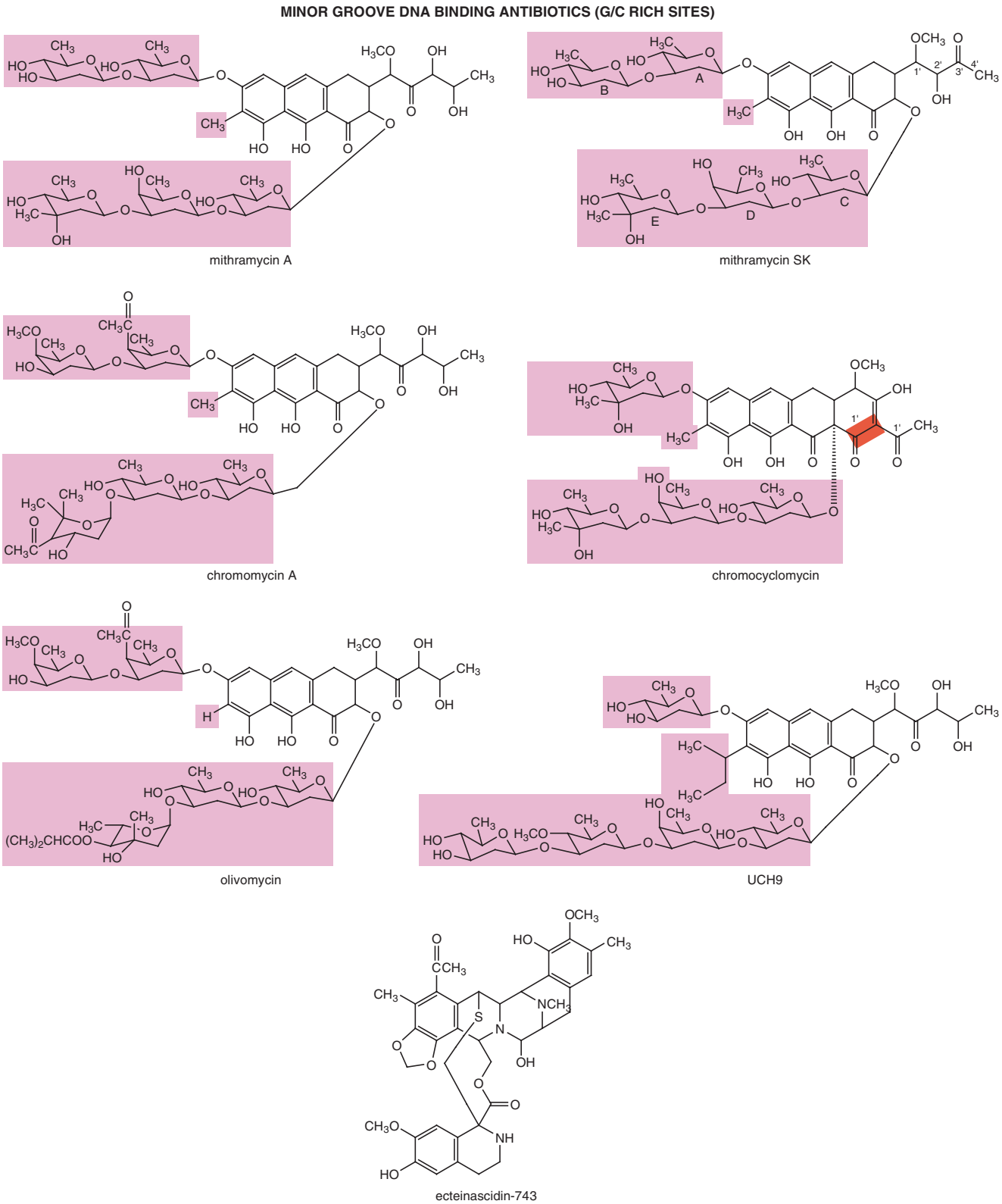


Fig. 2.21 Structures of minor groove DNA binding antibiotics with a preference for G/C rich sites. The aureolic acid antibiotics share a common tricyclic poly-ketide core motif but differ in their side chains in 3 positions (*pink*). Only chromocyclomycin also has a ring closure in the core structure (*red*). Ecteinascidin-743 is a non-aureolic acid minor groove binder.

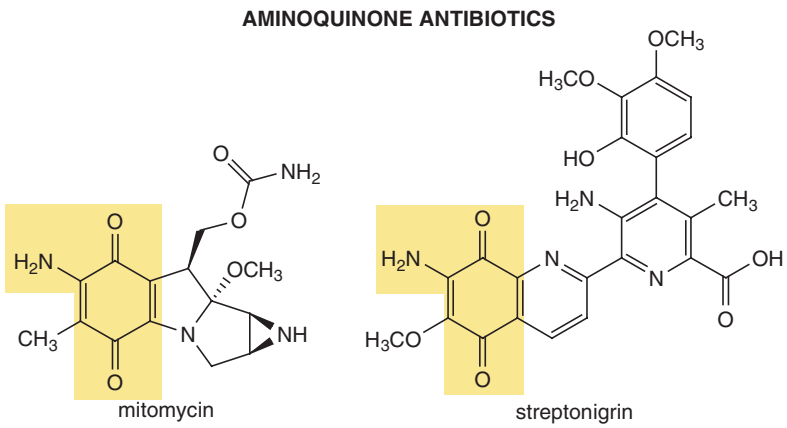


Fig. 2.22 Structures of aminoquinone antibiotics. The common aminoquinone functional unit is highlighted in *yellow*.

Ecteinascidins are isolated from marine organisms. Ecteinascidin-743 (ET-743) is produced by the Caribbean tunicate Ecteinascidia turbinate. The alkaloid is composed of three fused tetrahydroisoquinoline subunits (A, B, and C). The A subunit and the B subunit provide the scaffold for DNA recognition and covalent bonding. The C subunit protrudes out and interacts with adjacent nuclear proteins. Ecteinascidin-743 binds to the minor groove of DNA, effects guanine *N*2 alkylation (via an intermediate iminium generated from an active carbinolamin functional group), and bends the duplex toward the major groove. This may sequester specific transcription factors, inhibit inducible gene transcription, and result in cytotoxicity. The alkylation at guanine 2–NH_2 positions is sequence selective and region specific to DNA triplets containing a central guanine (AGC, CGC, TGG). Ecteinascidin-743 also binds tightly to Topoisomerase 1, with which it produces cleavage complexes. The compound has activity against a variety of soft tissue sarcomata and is in clinical trials for breast cancer.

Adverse Effects Prolonged infusion benefits efficacy, while hepatic toxicity and myelosuppression are ameliorated by low peak blood concentrations.

Drug Resistance Ecteinascidin-743 decreases the induced expression of ABCB1, the over-expression of which may account for multi-drug resistance in cancer cells.

The minor groove of DNA is frequently unoccupied and vulnerable for chemical attack.
Some minor groove binding antibiotics prefer A/T-rich sequences, others prefer G/C rich sequences.
Antibiotic binding may displace essential transcription factors and interfere with gene expression.
Antibiotics that bind sequence specifically, non-covalently, and reversibly to the minor groove may serve as carriers for alkylating moieties.

The pyrrole-amide backbone of distamycin A, which binds 4–5 adjacent A/T base pairs, serves as a sequence selective vehicle for the delivery of DNA alkylating functions.
Tallimustine alkylates N3 adenines in the target sequence 5'-TTTTGA-3'.
Brostallicin is activated upon binding to Glutathione, catalyzed by Glutathione-S-Transferase (GST). It may thus treat cancers that are resistant to other forms of chemotherapy.

PNU-151807 abolishes Cyclin/CDK kinase activity. Its cytotoxicity is not affected by disruption of P53 function or loss of DNA repair.

In the presence of divalent cations, aureolic acid antibiotics form dimers that non-intercalatively bind to the DNA minor groove in high-GC-content regions.
Upon binding to DNA, the aureolic acid chromophores form hydrogen bonds with NH2 residues of guanines.
Mithramycin A binds to C/G-rich tracts as a dimer and blocks SP-1 family transcription factors.
Ecteinascidin-743 effects guanine N2 alkylation.

2.2.3 Aminoquinone Antibiotics

Quinone containing alkylating drugs (Holzer 1958) have a quinone moiety, which can be reduced, and an alkylating group, which can form covalent bonds with a variety of cellular components (Fig. 2.22). The oxidation state of the quinone moiety modulates the activity of the alkylating element. In many of these agents, reduction of the quinone is required for induction of the alkylating activity. The quinone element may also contribute directly to cytotoxicity through the formation of reactive oxygen species during redox cycling.

The mitomycins were originally discovered by Hata and coworkers (Hata et al. 1956). As prodrugs, they require intracellular activation by 1-electron reducing enzymes like NADPH:Cytochrome P450 Reductase or NADH:Cytochrome b5 Reductase, or by 2-electron reducing enzymes like DT-Diaphorase or Xanthine Dehydrogenase. Reduction of the mitomycins activates the aziridine and carbamate alkylating groups and results in the formation of DNA cross-links, which is the primary mechanism for their anti-tumor effects. In the presence of sufficient levels of oxygen, the reduced mitomycin products can also redox cycle to form reactive oxygen species, which damage cellular components, including DNA.

The natural product mitomycin C <Mutamycin, Mitozytrex>(Wakaki et al. 1958) is a methylazirinopyrroloindoledione

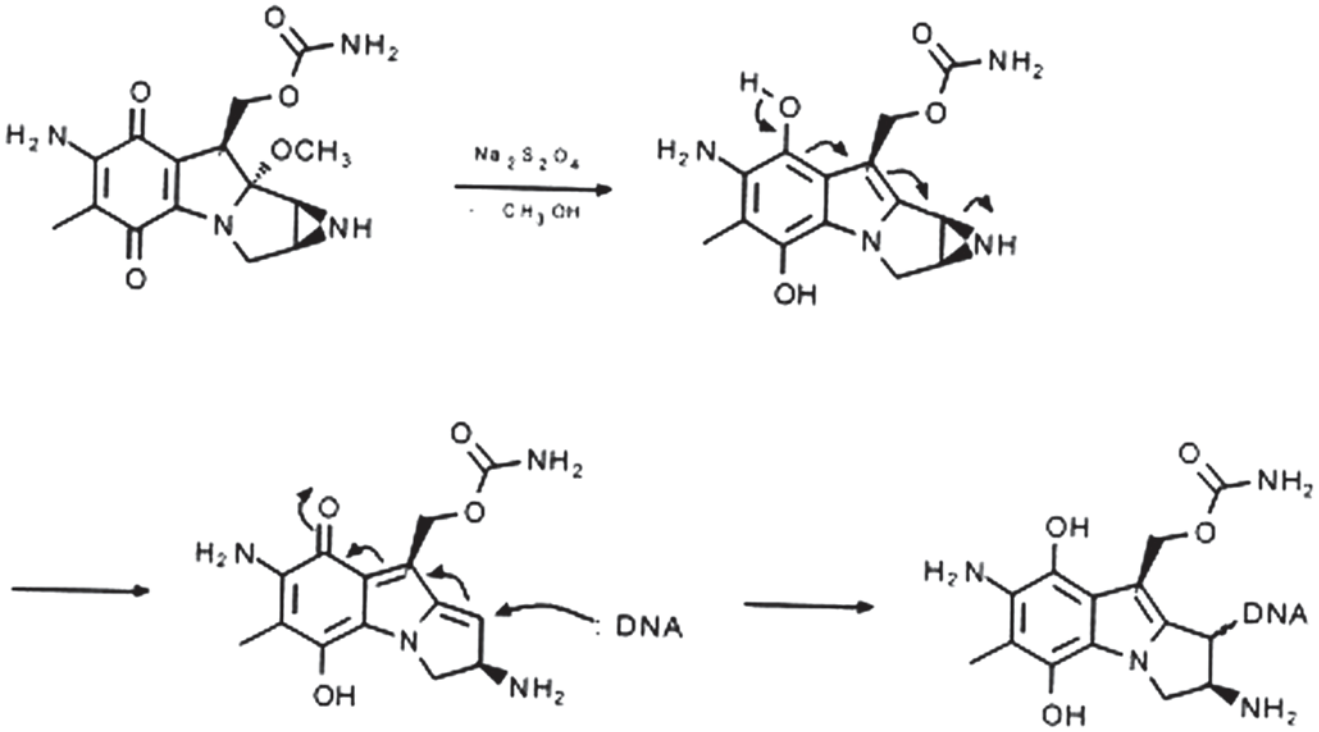


Fig. 2.23 DNA alkylation by mitomycin C. The quinone of mitomycin C is reduced, altering the azinidine group to be opened and set up a conjugated system to a susceptible carbon. Immediately, the nucleophilic DNA attacks the reactive carbon, and the DNA is successfully alkylated. [http://chemistry.creighton.edu/Opportunities/Baumann/WaetzigReport.html]

antibiotic[26] isolated from the bacterium Streptomyces caespitosus. It has been one of the most widely used quinine containing alkylating agents in the clinic (Begleiter 2000). Bioreduced mitomycin C alkylates DNA, produces inter-strand DNA cross-links, and generates oxygen radicals, thereby inhibiting DNA reduplication (Fig. 2.23). Mitomycin C at high concentrations also inhibits RNA and protein synthesis. There is effectiveness against hypoxic, radioresistant cells. The drug is most effective in the front line treatment of select solid tumors, including superficial bladder cancer, and gastric, pancreatic, anal, and esophageal carcinomata. Mitomycin C has been used extensively as a single agent in the treatment of gastric cancer, especially in Japan, with an overall objective response rate of 30%. More recently, the drug has also been extensively applied in combination chemotherapy regimens. It is used in the palliative treatment of cancers that are advanced or have become resistant to other forms of therapy, generally in combination regimens with doxorubicin and 5-fluorouracil or with bleomycin and vincristine. Mitomycin C is normally administered intravenously at a single dose of 20 mg/m^2 or at 2 mg/m^2/day over 12 days. Other forms of drug delivery are used for specific tumors. Cycles are repeated every 6–8 weeks, provided the leucocyte and platelet counts have recovered sufficiently. The dose may need to be reduced if mitomycin C is combined with other myelosuppressive agents.

- Porfiromycin (methylmitomycin C) is a methyl derivative of mitomycin C, with the methyl group on the nitrogen of the aziridine ring. Porfiromycin was first isolated from Streptomyces ardus in 1960 (DeBoer et al. 1960). The agent is less toxic to tumor cells than mitomycin C under aerobic conditions, but shows similar or greater activity under hypoxic conditions. Thus, porfiromycin has a greater hypoxic:oxic ratio and has greater preferential toxicity to hypoxic cells than mitomycin C. This is likely due to poorer activation of porfiromycin by DT-Diaphorase and a greater dependence of this drug on activation by NADPH:Cytochrome P450 Reductase compared with mitomycin C (Begleiter 2000).
- KW-2149 (7-N-(2-((2-(γ-glutamylamino)ethyl)dithio) ethyl)mitomycin C) (KT6149) is a semi-synthetic, water soluble disulfide derivative of mitomycin C. Activated by blood components and Glutathione, KW-2149 causes inter-strand DNA cross-links and DNA-protein

[26] The pyrroloindole group is common between the mitomycins and cyclopropylpyrroloindole antibiotics.

cross-links, resulting in single strand DNA breaks and inhibition of DNA synthesis.

Pharmacokinetics After intravenous administration, mitomycin C is rapidly cleared from the blood with a half-life of about 15 min. Clearance occurs mostly through hepatic metabolism. Approximately 10% of the drug is excreted unchanged in the urine.

Adverse Effects The utility of mitomycin C is limited by substantial adverse effects. The dose limiting toxicity is myelosuppression, which occurs over 3–4 weeks after drug administration, and is followed by recovery within 8 weeks. This toxicity increases with doses in excess of 10–20 mg/m^2, but the effects are cumulative. Generally, thrombocytopenia or leukopenia are most severe, but anemia is also common. The appearance of thrombocytopenia after mitomycin C treatment is frequently delayed. Approximately 5% of patients receiving the drug develop pulmonary toxicity, including non-cardiogenic pulmonary edema, interstitial pneumonitis and pleural effusions, leading to progressive respiratory insufficiency and death. Corticosteroid therapy can improve the pulmonary symptoms. The risk for this toxicity may increase with exposure to high oxygen concentrations or when mitomycin C is combined with other anticancer drugs that cause pulmonary toxicity (like bleomycin). 5–15% of patients treated with mitomycin C develop cancer associated hemolytic-uremic syndrome, which comprises a complex of micro-angiopathic hemolytic anemia, thrombocytopenia, and renal failure that may lead to death. The syndrome is most common in patients who receive 6–12 months of mitomycin therapy or total doses over 60 mg. Other common toxicities include anorexia, nausea, vomiting and diarrhea.

Mitomycin C extravasation during administration can cause painful skin ulcerations. An important dermatological problem associated with the drug is the necrosis and consequent sloughing of tissue that results if the drug is extravasated during injection. Extravasation is not always immediately symptomatic through accompanying stinging or burning sensations.

Drug Interactions In some cases, severe congestive heart failure arises in patients previously treated with doxorubicin.

Streptonigrin (rufochromomycin, bruneomycin) (BA-163, PC-501) is a metal dependent aminoquinone antibiotic isolated from the bacterium Streptomyces flocculus. Streptonigrin forms complexes with DNA and Topoisomerase 2, resulting in DNA cleavage. This leads to inhibition of DNA reduplication and transcription. This agent also causes free radical mediated cellular damage. Metal-streptonigrin complexes can be reduced to their semiquinone forms by NADH to induce the cleavage of DNA in a manner that depends on superoxide and peroxides. At the chromosome level, this antibiotic causes chromosome damage and increases the frequency of sister chromatid exchanges. The clastogenic action of this compound is partially mediated by free radicals. Streptonigrin has been studied in the treatment of leukemias and lymphomata, breast and cervix carcinomata, gastrointestinal cancers, and head and neck cancers. Oral doses vary 0.2–0.4 mg/day, intravenous doses are 5–7 mg/kg/day for 6 days.

Adverse Effects The clinical potential has been limited by serious adverse effects.

The antibiotics in the class of ansamycins (ansa macrolides[27]), comprising geldanamycin and derivatives, rafamycins, and maytansinoid anti-tumor agents, contain aminoquinone moieties. However, their main mode of action is the inhibition of Heat Shock Protein 90 (HSP90) (see Sect. 4.2.2.).

Mitomycins and streptonigrins comprise the group of anticancer aminoquinone antibiotics.
In quinone containing alkylating drugs, the quinone moiety is subject to redox cycling, while the alkylating group can form covalent bonds with cellular components.
The mitomycins require intracellular activation of their alkylating groups by reducing enzymes (NADPH:Cytochrome P450 Reductase, NADH:Cytochrome b5 Reductase, DT-Diaphorase, or Xanthine Dehydrogenase).
Mitomycin C has effectiveness against hypoxic cells, which are resistant to radiation.
Streptonigrin forms complexes with DNA and Topoisomerase 2 and undergoes redox cycling, resulting in DNA damage.

2.2.4 Polycyclic Aromatic Antibiotics

The anti-tumor activity of polycyclic aromatic compounds is associated with their ability to intercalate into DNA. The planar 3-ring structure accounts for the biological characteristics of these molecules. Anthracyclines, anthracenediones and anthrapyrazoles are groups of agents in this class of drugs.

Classical anthracyclines The first anthracyclines were isolated from the pigment producing Streptomyces peucetius early in the 1960s and were named doxorubicin and daunorubicin. The only difference between these compounds is that the side chain of doxorubicin terminates with a primary alcohol, whereas that of daunorubicin terminates with a methyl group. While doxorubicin is an established component in the treatments of breast cancer, childhood solid tumors, soft tissue sarcomata, and aggressive lymphomata, daunorubicin shows activity in acute lymphoblastic or myeloblastic leu-

[27] The macrolides comprise drugs (typically antibiotics), whose activity stems from the presence of a large macrocyclic lactone ring (usually 14-, 15-, or 16-membered), to which one or more deoxy sugars (usually cladinose and desosamine) may be attached.

kemias. The anthracyclines (drug names end on -rubicin, except for the oligosaccharide anthracyclines which end on -mycin) share aglyconic and sugar moieties. Their two main modes of action comprise Topoisomerase inhibition and the generation of reactive oxygen species.

Topoisomerases modify the topology of DNA without altering the deoxynucleotide structure and sequence. Anthracyclines (Fig. 2.24) intercalate into the DNA. They act by stabilizing a reaction intermediate, in which DNA strands are cut and covalently linked to specific tyrosine residues of Topoisomerase 2, eventually impeding DNA resealing. The formation and stability of a ternary anthracycline-DNA-Topoisomerase 2 complex rely on defined structural determinants (Minotti et al. 2004):

- The planar ring system is important for the intercalation into DNA, as rings B and C overlap with adjacent base pairs and ring D passes through the intercalation site.
- The sugar residue and the cyclohexane ring A are non-intercalating moieties that may play an important role in the formation and stabilization of the ternary complex.
- The sugar moiety, located in the minor groove, is a critical determinant of the activity of anthracyclines as Topoisomerase 2 inhibitors, because inhibition increases in the absence of amino-substituents at C-3′ in the sugar or of the methoxy group at C-4 in ring D.

DNA damage through Topoisomerase 2 inhibition is followed by growth arrest in G_1 and G_2, suppression of RNA and protein synthesis, and ensuing programmed cell death.

Anthracyclines also produce toxic free radical intermediates (Fig. 2.25). A one-electron addition to the quinone moiety in ring C of anthracyclines results in the formation of a semiquinone that rapidly regenerates its parent quinone by reducing oxygen to superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). This redox cycle is supported by a number of NAD(P)H-Oxidoreductases.

- The redox cycling is accompanied by a release of iron from intracellular stores. Ligand binding interactions of anthracyclines with the released iron then result in the formation of 3:1 drug-iron complexes that convert O_2^- and H_2O_2 into more reactive hydroxyl radicals (OH). Iron mediated free radical reactions enable anthracyclines to produce formaldehyde (HCHO) from carbon cellular sources like spermine and lipids. The anthracy-

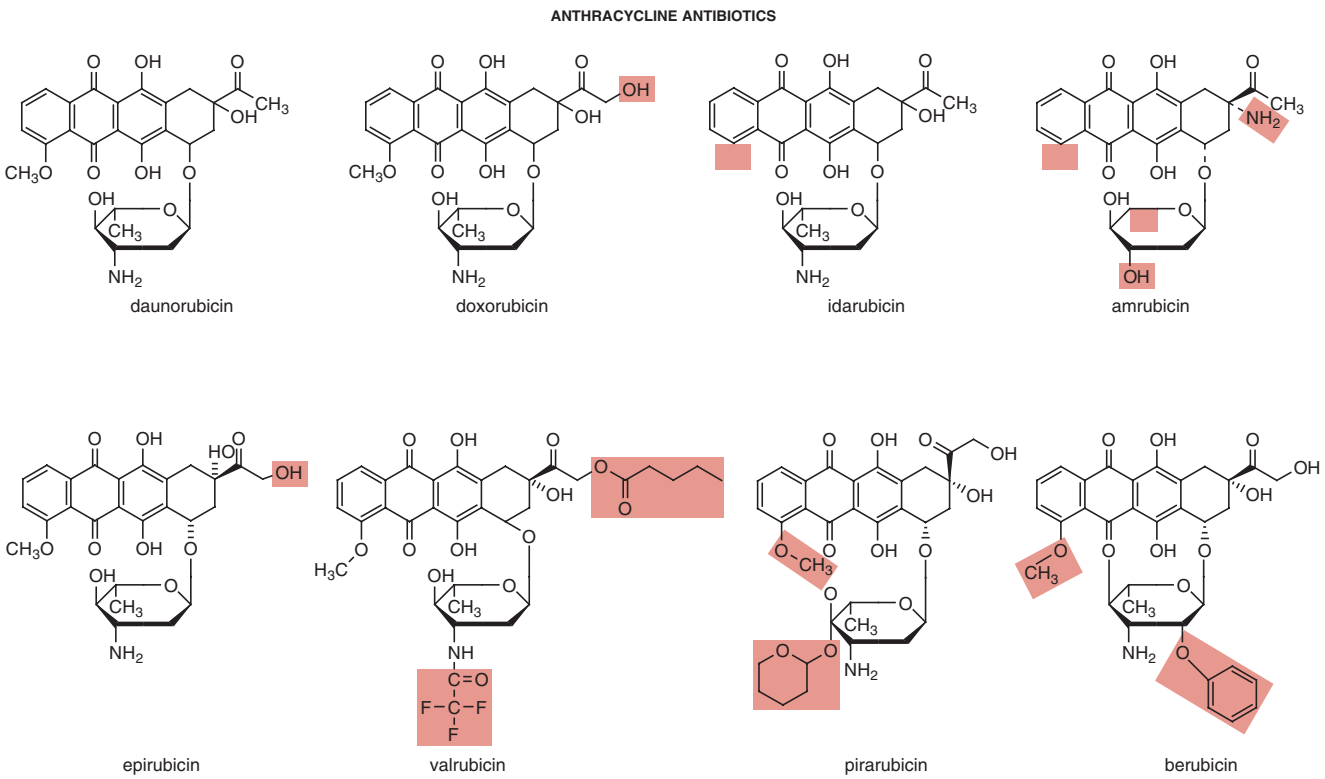


Fig. 2.24 Structures of anthracycline antibiotics. Functional groups that differ from the parent compound, daunorubicin, are highlighted in *pink*

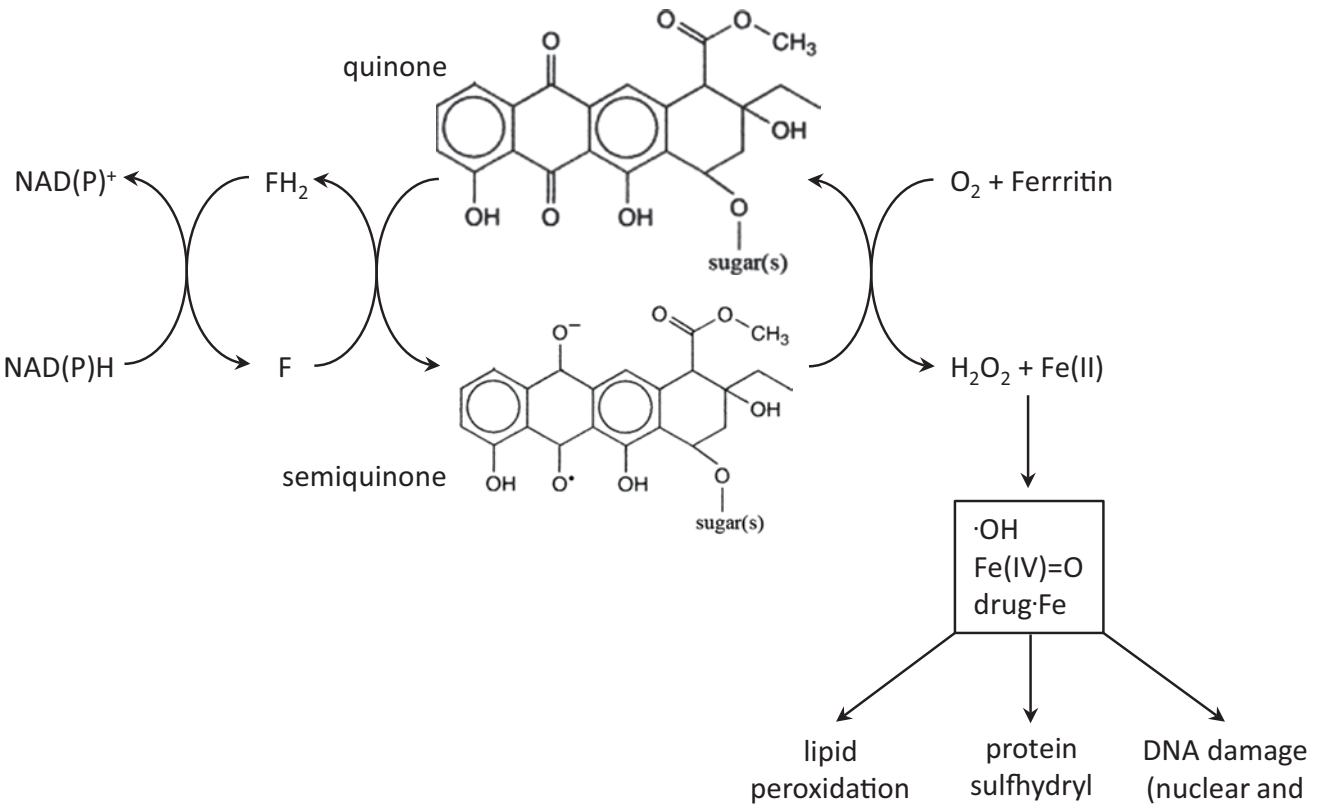


Fig. 2.25 The iron and free radical mechanism of anthracycline cytotoxicity. F/FH_2 represents oxidized/reduced flavoproteins (e.g. NADH Dehydrogenase, NADPH Cytochrome P450 Reductase); Fe(II) indicates low molecular weight Fe^{II}, OH stands for hydroxyl radical, Fe(IV)=O is ferryl ion; drug·Fe is a drug-iron complex. (Redrawn from Minotti et al. 1999)

cline and HCHO then react to form a conjugate, in which two anthracycline molecules bind together with three methylene groups, two forming oxazolidine rings and one binding the oxazolidines together at their 3′-amino nitrogens. The conjugate eventually hydrolyzes to give an active monomeric metabolite, in which the carbon of HCHO is recovered in the form of a Schiff base. Anthracycline-formaldehyde conjugates intercalate into DNA by covalent bonding of the Schiff base with the 2-amino group of a G in the minor groove of the DNA (Taatjes et al. 1997).

- Ceramide formation occurs after reactive oxygen activation of Neutral Sphingomyelinase. Anthracyclines interact with cell membrane lipids, causing lipid peroxidation. At clinically relevant concentrations, they trigger a cyclical cascade of sphingomyelin hydrolysis and formation of ceramide, which activates downstream cell death pathways.
- Anthracyclines can directly release Cytochrome *c* from the mitochondria, thereby inducing apoptosis regardless of DNA damage, active signaling pathways, or P53 status.

Like most anti-cancer drugs, the anthracyclines are used in combination chemotherapy. Because telomeres accumulate single strand breaks and shorten more rapidly after exposure to agents that induce oxidative stress and DNA damage, combining Telomerase inhibition with anthracycline treatment is under consideration as an option for cancer treatment (Minotti et al. 2004).

Pharmacokinetics The intracellular transport of anthracyclines follows a multi-step mechanism,

- they enter cancer cells by diffusion
- they bind with high affinity to the 20S proteasomal subunit in the cytoplasm, forming a complex that translocates into the nucleus via nuclear pores (an ATP-dependent process, facilitated by nuclear localization signals)
- due to their higher affinity for DNA, the anthracyclins dissociate from the proteasome and bind to DNA (Kiyomiya et al. 2001).

In an inactivation step, Carbonyl Reductase (AKR1B10) catalyzes the reduction of daunorubicin to the alcohol daunorubicinol.

Adverse Effects The clinical use of anthracycline antibiotics is hampered by toxicity in healthy tissues. Secondary acute myelogenous leukemia (AML) may arise in cancer patients after treatment. The occurrence of refractory secondary leukemia is more common when anthracyclines are given in combination with DNA damaging anti-neoplastic agents, when patients have been heavily pretreated with cytotoxic drugs, or when doses of anthracyclines have been escalated. Anthracyclines are Pregnancy Category D.

Anthracyclines can cause acute cardiotoxicity that consists of arrhythmias, hypotension, and a mild depression of contractile function—usually within a week of initiation of a regimen. With current treatment protocols, acute toxicity is infrequent, afflicting no more than 1 % of patients, and it is usually reversible. An even more rare acute complication may consist of myocarditis and pericardial effusion, occurring a few weeks after anthracycline administration (Zucchi and Danesi 2003). Chronic cardiac toxicity may develop any time after completion of anthracycline regimens. The prolonged administration induces cardiomyopathy—usually within a year, but very late forms of cardiac dysfunction can arise. Children may develop cardiotoxicity at longer intervals after treatment completion. Chronic cardiomyopathy can evolve into congestive heart failure, which is refractory to standard inotropic medications. The risk for cardiotoxicity is high in patients who have been given cumulative anthracycline doses above 550 mg/m^2. It may occur at lower cumulative doses if anthracyclines are given in combination with agents like taxanes or trastuzumab (anti-ERBB2), and possibly also in conjunction with COX-2 inhibitors. The adverse effects include the loss of myofibrils, dilation of the sarcoplasmic reticulum, cytoplasmic vacuolization, swelling of the mitochondria, and increased numbers of lysosomes. The main mechanism of anthracycline dependent cardiac toxicity is the induction of apoptosis. Cardiomyocytes are more susceptible than other tissues because they exhibit low levels of Catalase and readily undergo inactivation of GSH-PX1 (Selenium-Dependent GSH-Peroxidase-1) after drug exposure. Protection from the cardiotoxicity of anthracyclines can be achieved by slow infusion (48–96 h), provision of antioxidants (flavonoids), and administration of iron chelators such as dexrazoxane (ICRF-187, ADR 529) <Zinecard, Totect>. According to the American Society for Clinical Oncology (ASCO), the use of dexrazoxane is recommended in

- patients who have received more than 300 mg/m^2 for metastatic breast cancer and who may benefit from continued doxorubicin treatment
- patients who have received more than 300 mg/m^2 doxorubicin for the treatment of malignancies other than breast cancer
- patients who responded to previous anthracycline based chemotherapy for advanced breast cancer and who may benefit from continued therapy with epirubicin (Schuchter et al. 2002).

Dexrazoxane does not affect the pharmacokinetics of anthracyclines. Unrelated to cardiac protection, dexrazoxane may also be indicated as an emergency treatment of extravasation resulting from intravenous anthracycline chemotherapy[28].

Drug Resistance The clinical use of anthracycline antibiotics is hampered by the development of resistance in tumor cells, which may be due to an over-expression of ABCB1 (MDR1, PGP), increased levels of enzymes that protect form reactive oxygen damage, activation of the transcription factor NF-κB that exerts crucial functions in cellular resistance to oxidants, altered *topoisomerase 2* gene expression or activity, or enzymatic drug inactivation.

The anthracycline daunorubicin was originally isolated from bacteria in soil samples taken from the Italian castle Castel del Monte. A French research group also discovered the compound at about the same time. Jointly, the teams named it daunorubicin, combining the name Dauni (an ancient Roman tribe that once occupied the Italian area where the compound was isolated) with rubis (the French word for ruby describing the color of the compound). Based on the risk for adverse cardiac effects, the maximum recommended cumulative dose of daunorubicin is tentatively set at 500 mg/m^2.

- Daunorubicin hydrochloride <Cerubidine> for infusion is indicated in combination chemotherapy for remission induction in acute non-lymphocytic (myelogenous, monocytic, or erythroid) leukemia in adults and for remission induction in acute lymphocytic leukemia in children and adults. Profound myelosuppression is inevitable when used in therapeutic doses. Extravasation during administration results in severe local tissue necrosis.
- The use of liposomes as carriers is an approach to decrease anthracycline related cardiac toxicity. Liposomal PEGylated forms of daunorubicin have been designed to increase the amount of drug delivered to tumors and decrease the peak distribution to the heart and gastrointestinal mucosa. This formulation preferentially accumulates in the tumor vasculature, resulting in a sustained targeted release of free daunorubicin in the tumor micro-environment. Peglyation increases the half-life of the liposome through inhibiting its phagocytosis by the reticuloendothelial system. However, the polyethylene

[28] Dexrazoxane is administered over 3 days (1000 mg/m^2 within the first 6 h, 1000 mg/m^2 around 24 h, 500 mg/m^2 around 48 h). The dosages are reduced by half in patients with creatinine clearance values below 40 mL/min. Possible adverse effects include nausea and vomiting, diarrhea, sore mouth, decreased white blood cell count, decreased platelet count, and infusion site burning. Dexrazoxane may also compromise the anti-tumor effects of athracyclines.

glycol (PEG) in the formulation may cause hand-foot syndrome as an adverse effect[29].

- Liposomal daunorubicin <DaunoXome> is a liposome encapsulated preparation with a diameter of 45 nm that is free of polyethylene glycol. A bilayer of distearoylphosphatidylcholine and cholesterol encapsulates daunorubicin citrate (10:5:1 molar ratio). The half-life is shorter than that of PEGylated liposomes. Liposomal daunorubicin is under investigation for the treatment of AIDS-related Kaposi sarcoma, acute myeloblastic leukemia, multiple myeloma, non-Hodgkin lymphoma, and breast cancer.

Pharmacokinetics Carbonyl Reductase (CBR) catalyzes the reduction of daunorubicin to its corresponding alcohol, daunorubicinol, which changes the pharmacological properties of this cancer chemotherapeutic drug. The ratio of area under the concentration-time curve (AUC) for metabolite to parent drug is lower for liposomal daunorubicin than for free daunorubicin, mainly due to higher concentrations of the parent drug in the blood.

Researchers at the Italian pharmaceutical company Farmitalia mutated a strain of Streptomyces (Streptococcus peucetius var. caesius) using *N*-nitroso-*N*-methyl urethane to produce a red colored antibiotic different from daunorubicin. They originally named it adriamycin, after the Adriatic Sea. Doxorubicin (hydroxydaunorubicin, adriamycin) became available in 1968. The drug is an intercalator and a Topoisomerase 2 inhibitor that prevents DNA reduplication and ultimately inhibits protein synthesis. It also generates oxygen free radicals, resulting in the cytotoxic peroxidation of cell membrane lipids. Like many other genotoxic agents, doxorubicin activates the binding of P53 to DNA, likely inducing apoptosis in this manner. P53 activation also contributes to the induction of the $p21^{CIP1/WAF1}$ gene product, which is a strong inhibitor of Cyclin-Dependent Kinases (CDKs) involved in the G_1/S transition of the cell cycle. The maximum recommended cumulative dose of doxorubicin is tentatively set at 450–600 mg/m^2.

Pharmacokinetics Steady state plasma concentrations are 25–250 nM, with peak concentrations reaching 5 μM. Tissue uptake of doxorubicin is rapid, while its slow elimination from tissues is reflected by a terminal half-life of 20–48 h. Clearance occurs mostly through metabolism (mainly to doxorubicinol) and biliary excretion. The systemic clearance of doxorubicin is substantially reduced in obese women. The drug does not cross the blood brain barrier.

Formulations of doxorubicin include the salt, PEGylated liposomes, liposomes, coupling to HPMA, coupling to targeting peptides, conjugation to magnetic carriers, derivatization.

- The hydrochloride salt of doxorubicin <Adriamycin PFS, Adriamycin RDF, Rubex> is indicated for inducing regression in disseminated neoplastic conditions such as acute lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, Wilms tumor, neuroblastoma, soft tissue and bone sarcomata, ovarian carcinoma, transitional cell bladder carcinoma, thyroid carcinoma, gastric carcinoma, and small-cell bronchogenic carcinoma. Doxorubicin is also indicated as a component in the adjuvant therapy for women with axillary lymph node involvement of breast cancer.
- Liposomes are multi-lamellar lipid particles suitable for drug delivery. They improve doxorubicin penetration into tumors and decrease drug clearance, thereby increasing the duration of therapeutic drug effects. This form may exhibit an improved toxicity profile due to the lower concentrations of free doxorubicin to which non-transformed tissues are exposed. Liposomal PEGylated doxorubicin hydrochloride <Doxil, Dox-SL, Evacet, LipoDox, Caelyx, Doxilen> is a formulation, in which a doxorubicin containing liposome is surrounded by a layer of polyethylene glycol (PEG). Liposomal PEGylated forms of doxorubicin may be indicated for the second-line treatment of ovarian cancer (disease progression or recurrence after platinum based chemotherapy), multiple myeloma (in combination with bortezomib), AIDS-related Kaposi sarcoma (after failure of prior systemic chemotherapy), high grade glioma or glioblastoma or secondary brain tumors, and head and neck squamous cell carcinoma. An intermittent 3-weekly schedule is appropriate, and the maximum tolerated dose is 60–90 mg/m^2. Hypersensitivity may occur in up to 10 % of patients.
- Liposomal doxorubicin citrate (TLC D-99) <Myocet> is a liposome encapsulated form of the doxorubicin citrate salt that does not contain polyethylene glycol. The disposition of doxorubicin when administered as Myocet reflects that of the liposome and differs substantially from plain doxorubicin. The formulation improves the drug penetration into tumors and decreases drug clearance, thereby increasing the duration of therapeutic drug effects. It may have benefits in the treatment of breast cancer.
- Doxorubicin-HPMA conjugate is a co-polymer conjugate of doxorubicin and the water soluble polymer N-(2-hydroxypropyl) methacrylamide (HPMA). Conjugation with HPMA improves the therapeutic effects of doxorubicin, by promoting drug accumulation inside tumors, while decreasing systemic toxicity.

[29] Hand-foot syndrome (palmar-plantar erythrodysesthesia, acrauding erythema, Burgdof syndrome) manifests in painful bilateral swelling, erythema, and tenderness over the palms and soles that evolves into desquamation. The long-term circulation of the PEGylated daunorubicin allows protracted extravasation and underlies this form of hand-foot syndrome. Interactions between daunorubicin and metallic Cu^{II} ions abundant in skin tissue generate reactive oxygen species, which stimulate Chemokine secretion from keratinocytes and result in inflammation.

- An extracellularly tumor-activated prodrug (ETAP) incorporates both a tumor specific recognition site and a tumor selective enzymatic activation sequence. It is unable to enter healthy cells but is proteolytically activated by Peptidases that are rather selectively secreted by cancer cells. Doxocycline delivery to tumors can be improved by coupling it to ETAPs. The bicyclic peptide CDCRG-DCFC (RGD-4C) selectively binds to Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$, which are highly over-expressed in invading tumor endothelial cells. A distinct tumor specific sequence is the tripeptide D-Ala-Phe-Lys that is recognized by the tumor associated protease Plasmin, an important factor of tumor invasion and metastasis. An aminocaproyl residue is incorporated as a spacer between the two peptide sequences, whereas a self-eliminating 4-aminobenzyl alcohol spacer is inserted between the Plasmin substrate and doxorubicin. This prodrug is designed to have Plasmin dependent cytotoxicity for endothelial cells and some tumor cells, but it requires improvements in water solubility and bioavailability (de Groot et al. 2002).
- L-377,202 is an extracellularly tumor-activated prodrug characterized by a covalent linkage of doxorubicin to N-glutaryl-[4-hydroxyprolyl]-Ala-Ser-cyclohexaglycyl -Glu-Ser-Leu. In the presence of prostate cancer cells that secrete the serine protease PSA (Prostate Specific Antigen), the peptide moiety of L-377,202 is hydrolyzed to release doxorubicin or Leu-doxorubicin, the latter being freely diffusible and activated to doxorubicin inside the target cells. L-377,202 is considerably more toxic to PSA expressing tumor cells than to PSA negative normal or tumor cells (Denmeade et al. 1998).
- The extracellularly tumor-activated prodrug N-succinyl-Ala-L-Leu-L-Ala-L-Leu- doxorubicin (CPI-0004Na) is a tetrapeptidic derivative of doxorubicin. CPI-0004Na does not undergo hydrolysis in the blood nor does it enter cells, but it is activated by tumor Peptidases to give N-(L-Leu- doxorubicin), which eventually diffuses inside the cells. CPI-0004Na is substantially less toxic than doxorubicin due to its lower accumulation in the heart and other untransformed tissues (Trouet et al. 2001).
- Doxorubicin-magnetic targeted carrier complex consists of doxorubicin bound to microscopic beads of activated carbon and iron (magnetic targeted carriers). With the placement of a magnet on the body surface overlying a tumor site, this carrier complex delivers doxorubicin preferentially to the tumor, thereby prolonging the duration of the therapeutic effects while decreasing its systemic toxicity.

Adverse Effects The occurrence of doxorubicin dependent cardiac toxicity is related to the total lifetime dose, and increases sharply in people who receive a cumulative dose of more than 400 mg/m^2. The combination of doxorubicin with cyclophosphamide causes a dramatic increase in the risk of secondary malignancies, most often acute myelomonocytic leukemia.

Second generation anthracyclines Idarubicin <Idamycin, Idamycin PFS> is a semi-synthetic anthracycline derived from daunorubicin. Due to its high lipophilicity (caused by the absence of a methoxyl group in position four), idarubicin penetrates cell membranes better than other anthracycline antibiotics. The hydrochloride salt is the therapeutic form of this drug, which is active against acute myelogenous leukemia (FAB classifications M1 through M7), multiple myeloma, non-Hodgkin lymphoma, and breast cancer (Borchmann et al. 1997). For adult AML, idarubicin is given at 12 mg/m^2 daily for 3 days by slow intravenous injection in combination with cytarabine for 12 days. A second course may be administered.

Pharmacokinetics Idarubicin may be administered orally with 10–30 % bioavailability. The tissue distribution of idarubicin is extensive. The elimination rate of idarubicin from the blood is slow with a mean terminal half-life around 22 h. The elimination of the metabolite idarubicinol is slower than that of the parent drug with a mean terminal half-life that exceeds 45 h. There is substantial hepatic metabolism, with idarubicinol being the primary active metabolite being formed. As idarubicinol has cytotoxic activity, it contributes to the effects of idarubicin. The drug is eliminated predominately by biliary excretion and to a lesser extent by renal excretion.

Adverse Effects Idarubicin is given slowly by intravenous infusion over 10–15 min. Local tissue necrosis can occur if there is extravasation. The agent is associated with a reduction in the left ventricular ejection fraction (LVEF) that may progress to congestive heart failure. The risk is increased in patients over 50 years of age. Severe myelosuppression occurs when idarubicin is used at effective therapeutic doses. Administration of the 2nd course should be delayed in patients who experience severe mucositis, until recovery from this toxicity has occurred, and a dose reduction of 25 % is recommended. Other adverse effects comprise infection, nausea and vomiting, hair loss, abdominal cramps and diarrhea, hemorrhage, alopecia, generalized rash, urticaria, and a bullous erythrodermatous rash of the palms and soles. The drug is Pregnancy Category D.

Amrubicin ((7S,9S)-9-acetyl-9-amino-7-[(2S,4S,5R)-4,5-dihydroxyoxan-2-yl]oxy-6,11-dihydroxy-8,10-dihydro-7*H*-tetracene-5,12-dione) (SM-5887) <Calced> is a synthetic anthracycline that exerts stronger anti-cancer activity than conventional anthracyclines, with reduced cumulative cardiotoxicity. Developed in Japan, it has been marketed there since 2002. Testing and use of this drug in the U.S. for small cell lung cancer and potentially for breast cancer has been expedited under the U.S. FDA orphan drug designation.

Adverse Effects Adverse events of grade 3 or worse occur in the majority of patients, with neutropenia (65%) and thrombocytopenia (40%) being the most common. The incidence is highest during the 1st or 2nd amrubicin cycle, and then decreases during subsequent cycles. There is no indication of cardiotoxicity.

Epirubicin hydrochloride <Pharmorubicin, Ellence> is a hydrochloride salt that intercalates into DNA and interacts with Topoisomerase 2, thereby inhibiting DNA reduplication and repair, as well as RNA and protein synthesis. This agent also produces toxic free radical intermediates and interacts with cell membrane lipids causing lipid peroxidation. The drug is a semi-synthetic derivative of doxorubicin obtained by an axial-to-equatorial epimerization of the hydroxyl group at C-4′ in daunosamine. This positional change has little effect on the spectrum of activity, but it introduces pharmacokinetic and metabolic effects, including an increased volume of distribution, 4-*O*-glucuronidation, and consequently enhanced total body clearance. It can therefore be used at high cumulative doses, resulting in anti-tumor activity without increased cardiotoxicity. Epirubicin is indicated as a component of adjuvant therapy following the resection of primary breast cancer with evidence of axillary node involvement. The recommended starting dose of epirubicin is 100–120 mg/m^2. The recommended maximum cumulative dose is 900 mg/m^2.

Pharmacokinetics Plasma clearance is not affected by the duration of infusion or administration schedule. The drug is rapidly and widely distributed into the tissues. About 75% of the drug binds to plasma proteins. The blood concentration declines in a triphasic manner with mean half-lives for the α-, β-, and γ- phases of about 3 min, 3 h, and 33 h. Epirubicin is extensively and rapidly metabolized by the liver and by red blood cells. Four main metabolic inactivation pathways include

- reduction of the C-13 keto group to form the 13(S)-dihydro derivative epirubicinol
- conjugation of both the parent drug and epirubicinol with glucuronate
- hydrolytic removal of the amino sugar moiety to form epirubicin and epirubicinol aglycones
- loss of the amino sugar moiety through a redox process with the formation of the 7-deoxy-epirubicin aglycone and 7-deoxy-epirubicinol aglycone.

Epirubicin and its major metabolites are eliminated primarily through biliary excretion and, to a lesser extent, by urinary excretion. The dosage should be reduced in patients with impaired hepatic function. The clearance of epirubicin from the blood declines in older women.

Adverse Effects Substantial myelosuppression (predominantly leukopenia and neutropenia) is a common adverse effect. Generally, the white blood cell counts return to normal within 3 weeks after the end of treatment. Myocardial toxicity, potentially leading to fatal congestive heart failure, may occur either during therapy with epirubicin or months to years after the termination of therapy. Amenorrhea and hot flashes (hot flushes) can arise in female patients. Lethargy, nausea and vomiting (can be limited with prophylactic antiemetics), and alopecia are common. Mucositis of the mouth or esophagus is possible. Extravasation may cause severe local tissue necrosis. Contraindications are

- baseline neutrophil count below 1500 cells/mm^3
- severe myocardial insufficiency, recent myocardial infarction, severe arrhythmias
- previous treatment with anthracyclines up to the maximum cumulative dose
- hypersensitivity to epirubicin, other anthracyclines, or anthracenediones
- severe hepatic dysfunction.

The drug is Pregnancy Category D.

Drug Interactions The concomitant use of epirubicin with compounds that can cause heart failure requires close constant monitoring of cardiac function. The administration of paclitaxel prior to epirubicin or cimetidine with epirubicin results in an increase in the epirubicin drug exposure.

Valrubicin (*N*-trifluoroacetyladriamycin-14-valerate) <Valstar> is a semi-synthetic analog of the anthracycline doxorubicin. Valrubicin causes DNA damage, inhibits Topoisomerase 2, and leads to cell cycle arrest in G_2. It is administered by infusion directly into the bladder to treat BCG-refractory bladder cancer[30]. It penetrates into the bladder wall. The drug is given at a dose of 800 mg, administered intravesically once a week for 6 weeks. The success rate of valrubicin for remission of bladder carcinoma in situ is about 20%. If there is not a complete response to treatment within 3 months or if there is a recurrence, cystectomy must be considered to avoid the development of metastatic bladder cancer.

Pharmacokinetics During a 2 h dose-retention period in the bladder wall, conversion to the major metabolites *N*-trifluoroacetyladriamycin and *N*-trifluoroacetyladriamycinol is negligible. The drug is essentially completely excreted by voiding the instillate.

Adverse Effects Over 80% of patients experience local adverse events. They are typically reversible within 1 week.

[30] Immunotherapy in the form of Bacillus Calmette-Guerin (BCG) instillation is used to treat and prevent the recurrence of superficial bladder tumors. Patients at highest risk for recurrence after transurethral resection may receive adjuvant intravesical immunotherapy with BCG. It is effective in up to 2/3 of the cases at this stage.

Urinary tract infections can arise. Most systemic adverse events associated with the use of valrubicin are mild, resolving within 24 h after drug administration. To avoid systemic exposure, valrubicin should not be administered if the integrity of the bladder mucosa is compromised. It is contraindicated in patients with known hypersensitivity to anthracyclines or polyoxyl castor oil. Valrubicin is Pregnancy Category C, the drug is secreted into the milk.

Pirarubicin (4′-O-tetrahydropyranyl doxorubicin) <Pinorubicin, Theprubicine, Therarubicin, THP-Doxorubicin> is a 4-tetrahydropyranyl doxorubicin.

Pharmacokinetics Pirarubicin has a triphasic elimination α, β, and γ half-lives of 0.1, 1.4, and 34 h, respectively. The cumulative 24-h urinary excretion is less than 10% of the administered dose. Pirarubicin has a higher hepatic extraction than doxorubicin. It may therefore be suited for intraportal injection to treat liver metastases.

Adverse Effects Pirarubicin may cause a significant decrease in the left ventricular ejection fraction at cumulative doses over 460 mg/m^2. In elderly patients with non-Hodgkin lymphoma, pirarubicin may cause severe cardiac dysfunction at cumulative doses as low as 360 mg/m^2 (Dhingra et al. 1995; Niitsu et al. 1998).

Berubicin hydrochloride (RTA 744) is an anthracycline derivative that intercalates into DNA and interrupts Topoisomerase 2 activity. It has U.S. FDA approval as an orphan drug for the treatment of malignant gliomata.

Pharmacokinetics Unlike other anthracycline derivatives, berubicin crosses the blood-brain barrier and reaches therapeutic levels in the brain. It is therefore used for the treatment of primary and secondary (breast or lung cancer metastases) brain cancers.

Drug Resistance The drug is not subject to efflux via ABC transporters.

Carubicin (carminomycin) is an anthracycline cytostatic antibiotic, initially isolated from Actinomadura carminata and developed in the former USSR. Carubicin intercalates into DNA and interacts with Topoisomerase 2, thereby inhibiting DNA reduplication and repair, as well as RNA and protein synthesis. The agent is used to treat patients with advanced breast cancer or leukemia. Carubicin may be given at 20 mg/m^2 every 3 weeks.

Adverse Effects There is no substantial risk for cardiotoxicity at a cumulative dose of up to 150 mg/kg. The drug has similar hematotoxicity to doxorubicin. Other adverse effects include nausea, vomiting, anorexia, and diarrhea.

Esorubicin (4′-deoxydoxorubicin) (NSC 267469) is a synthetic derivative of doxorubicin which differs by the lack of a hydroxyl group in the 4′ position of the amino sugar. The recommended dose is 30 mg/m^2 intravenously every 3 weeks

Adverse Effects The mucosal injury, which has deterred advocacy for doxorubicin in the treatment of acute leukemia, is not induced by esorubicin. However, the drug causes dose limiting bone marrow suppression in solid tumor patients, leading to a higher incidence of neutropenia (and resulting risk of sepsis). Esorubicin may offer slightly less cardiotoxicity than doxorubicin. A 5% drop in the left ventricular ejection fraction (LVEF) arises following approximately 240 mg/m^2, a 10% drop arises after approximately 480 mg/m^2. Nausea and vomiting are severe in 15% of patients, total alopecia arises in about 5% of all cases.

Detorubicin (14-Diethoxyacetoxydaunorubicin) is the semi-synthetic 14-diethoxyacetoxy ester of daunorubicin. Detorubicin remains stable in acidic environments while it is very quickly hydrolyzed into doxorubicin under neutral pH conditions, and therefore acts as a prodrug. Although hydrolysis occurs within minutes after injection in the blood stream the tissue distribution of detorubicin is unique. The drug is administered in a dose range of 120–180 mg/m^2 and repeated at 3-week intervals.

Adverse Effects The cardiotoxicity is similar to daunorubicin. The general myelosuppressive and gastrointestinal toxicities were acceptable and short in duration.

Duborimycin (daunorubicinol, 13-dihydrodaunorubicin) (KST-1A3492, AC1Q6JIZ, CID83845, LS-94072) is an anthracycline that represents the main metabolite of daunorubicin.

Adverse Effects In rare cases, grade 3–4 mucositis can arise. Cardiac toxicity may arise in close to 20% of patients and can be serious.

Zorubicin (rubidazone, benzoyl hydrazone daunorubicin) (RP-22050) is a benzoyl-hydrazone derivative of daunorubicin. It has limited activity against breast cancer and hairy cell leukemia.

New generation anthracyclines New generation anthracyclines include

- anthracyclines with morpholinyl or alkyl substituents at the amino group at C-3 and disaccharide anthracyclines, in which the amino group is displaced to the second sugar. Like the first generation anthracyclines, these are nuclear targeted compounds that intercalate into DNA
- anthracyclines that combine modifications at C-14 with modifications of the aminosugar. These non-nuclear targeted analogs no longer intercalate into DNA, but potentiate apoptotic events modulated by Protein Kinase C (PKC)

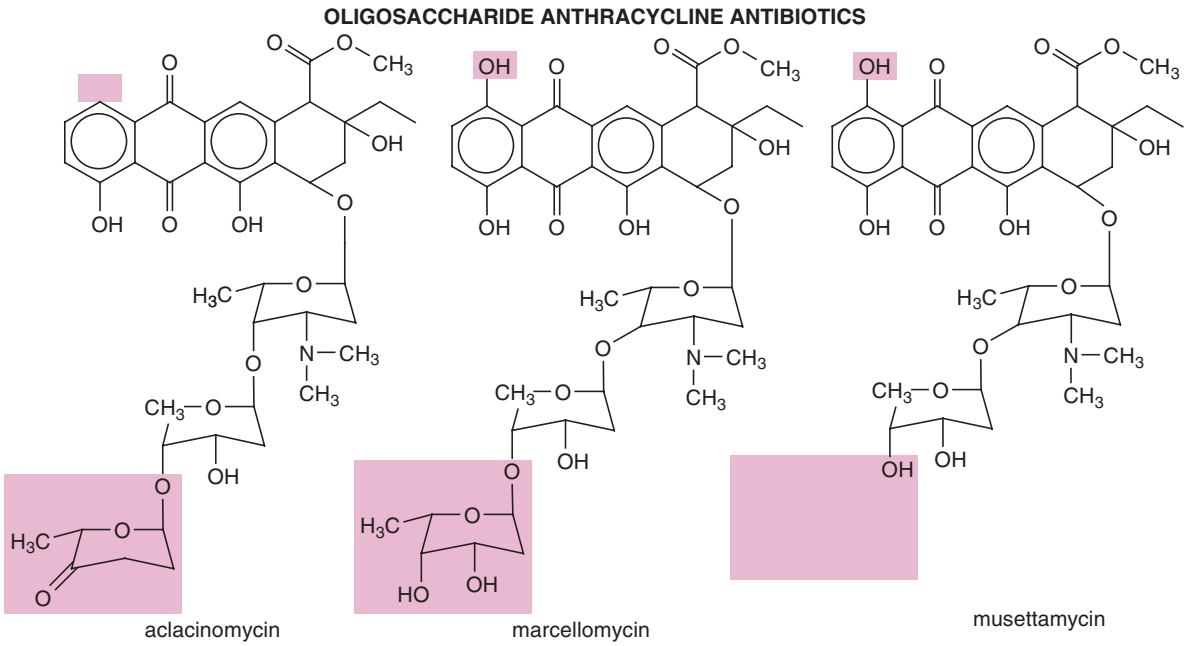


Fig. 2.26 Structures of oligosaccharide anthracycline antibiotics. The agents in this sub-group differ predominantly in the lengths of the sugar chains. Domains of structural variation are highlighted in *pink*

- Oligosaccharide anthracyclines constitute a class of inducers of hematopoietic differentiation.

Aclarubicin (aclacinomycin A) (NSC-208734, MA144-A1) <Aclacin, Aclacinomycine, Aclacinon, Aclaplastin, Jaclacin, Klasinomycin> is a trisaccharide anthracycline isolated form the bacterium Streptomyces galilaeus (Fig. 2.26). It is active in adult patients with acute myeloblastic leukemia. However, it is inactive in women with metastatic breast cancer (Natale et al. 1993). The drug is administered by intravenous infusion. In adults, the initial dose is 175–300 mg/m^2, divided over 3–7 consecutive days, followed by maintenance at 25–100 mg/m^2 every 3–4 weeks.

Adverse Effects Aclarubicin is contraindicated in patients with severe heart disease, bone marrow depression, or hypersensitivity. It is generally cardially tolerable but may induce late cardiac events in adult patients with refractory acute myelogenous or lymphoblastic leukemia (Dabich et al. 1986). The agent has no known mutagenicity or carcinogenicity. The drug is Pregnancy Category D.

Drug Resistance Aclarubicin is non-cross-resistant to the first line anthracyclines daunorubicin and doxorubicin.

The oligosaccharide anthracyclines marcellomycin, musettamycin, and rudolfomycin were isolated from the bacterium Actinosporangium bohemicum and are poetically named after characters in Puccini's opera La Boheme.

The trisaccharide marcellomycin intercalates into DNA and induces DNA cross-links. This agent may induce differentiation in promyelocytic leukemia cells by interfering with glycoprotein synthesis. Marcellomycin induces little myelosuppression and is less cardiotoxic than doxorubicin.

Musettamycin, collinemycin, and schaunardimycin are disaccharides.

Anthracenedione antibiotics The anthracenediones (anthraquinones, dioxoanthracenes) (drug names end on -antrone) were developed to reduce the frequency and severity of anthracycline induced adverse effects on the heart by substituting the cardiotoxic aminoglycone with aminoalkylamino side chains (Fig. 2.27). The diaminoalkyl groups are crucial for the biological activity of these compounds. However, while the anthracenedione mitoxantrone is less cardiotoxic than the anthracyclines, it is also somewhat less effective. Ametantrone and mitoxantrone are structurally similar members of the class. While both drugs are strong intercalators, their cytostatic, cytotoxic, and anti-tumor activities differ, with mitoxantrone being 10–100 times more potent on a molar basis. There is a correlation between their potency and their ability to condense nucleic acids inasmuch as mitoxantrone condenses nucleic acids at concentrations that are 5–40-fold lower than those of ametantrone. The condensation is base- and sugar-specific, with the long purine sequences of single stranded RNA being the most sensitive

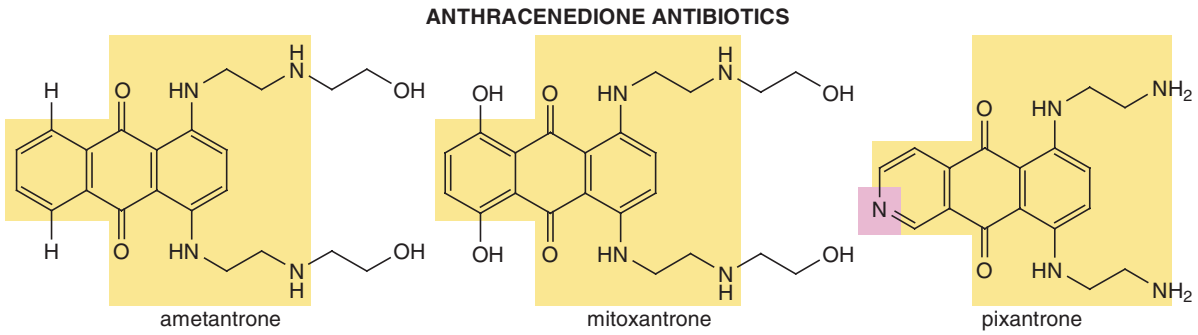


Fig. 2.27 Structures of anthracenedione antibiotics. The common functional unit is highlighted in *yellow*. The divergent nitrogen in the triple ring structure of pixantrone is highlighted in *pink*

(Kapuscinski and Darzynkiewicz 1986). Anthracenediones may also act as anti-oxidants. These agents concentration-dependently inhibit conjugated diene formation from linoleic acid.

Pharmacokinetics Anthracenediones may require metabolic activation before effectively intercalating into DNA. They are substrates for UDP-Glucuronosyl Transferase 1–8 (UGT1A8).

Random screening at the U.S. National Cancer Institute of a large number of compounds provided by the Allied Chemical Company identified the anti-cancer activity of ametantrone acetate (1,4-bis((2-((2-hydroxyethyl)amino)ethyl) amino)-9,10-anthracenedione diacetate) (NSC 287513, NSC-196473).

Pharmacokinetics The blood levels of ametantrone decline in a triexponential fashion, with a mean terminal half-life of 25 h. The steady state volume of distribution is large. About 5 % of the total dose is excreted unchanged in the urine, indicating that there is another major route of elimination.

Adverse Effects Reversible leukopenia is the dose limiting toxic effect. Non-hematologic toxicity includes a marked cumulative blue discoloration of the skin that occurs in patients who have received more than three courses of the drug. The agent is teratogenic.

Mitoxantrone was synthesized in an attempt to develop agents with comparable anti-tumor activity to doxorubicin, but with an improved safety profile. It was designed as a deliberate modification of the parent 1,4-dehydroxyl analog, ametantrone. Mitoxantrone hydrochloride (DHAD, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl) amino]ethyl] amino]-9,10-anthracenedione dihydrochloride) (NSC-29919) <Novantrone> intercalates into and cross-links DNA, thereby disrupting DNA reduplication and RNA synthesis. This agent also binds to Topoisomerase 2, resulting in DNA strand breaks and inhibition of DNA repair. The mechanism of action of mitoxanthrone is similar to that of the anthracyclines.

- Mitoxantrone in combination chemotherapy is indicated in the initial treatment of adult acute non-lymphocytic (myelogenous, promyelocytic, monocytic, and erythroid) leukemias.
- Mitoxantrone in combination with corticosteroids is indicated as a palliative treatment for advanced hormone refractory prostate cancer.
- Because mitoxantrone is somewhat less effective than the anthracyclines it has not received U.S. FDA approval for the treatment of breast cancer.

The recommended individual dose is 12–14 mg/m^2 with the timing being determined by the combination therapy regimen used.

Pharmacokinetics The pharmacokinetics of mitoxantrone is characterized by a three compartment distribution, with the mean α half-life of mitoxantrone being 5–15 min, the mean β half-life 1–3 h, and the mean γ (terminal or elimination) half-life 1–10 days (median approximately 75 h). The distribution to tissues is extensive with the steady-state volume of distribution exceeding 1,000 L/m^2. In patients administered 15–90 mg/m^2 of mitoxantrone intravenously, there is a linear relationship between dose and the area under the concentration-time curve. In the concentration range of 25–450 ng/mL, mitoxantrone is 80 % bound to plasma proteins. The metabolites are composed of monocarboxylic and dicarboxylic acid derivatives and their glucuronide conjugates. The drug is excreted in urine and feces, either unchanged or in the form of inactive metabolites.

Adverse Effects Mitoxantrone is administered slowly by infusion. Severe local tissue damage can occur if there is extravasation during this process. Except for the treatment of acute non-lymphocytic leukemia, mitoxantrone therapy generally should not be given to patients with baseline neutrophil counts of fewer than 1500 cells/mm^3. The occurrence of bone marrow suppression can cause neutropenia, which may lead to infections. Although mitoxantrone is less cardiotoxic than doxorubicin, the monitoring of the left ventricular ejection fraction is important during therapy. Secondary acute

myelogenous leukemia (AML) may arise in cancer patients treated with mitoxantrone.

Pixantrone was originally synthesized by Miles P. Hacker and Paul A. Krapcho. It was developed to reduce the heart damage resulting from anthracycline treatment while retaining efficacy. The drug was structurally designed so that it cannot bind iron and perpetuate oxygen radical production or form a long-lived hydroxyl metabolite, both of which are mechanisms for anthracycline induced acute and chronic cardiotoxicity.

Pixantrone dimaleate (6,9-bis[(2-aminoethyl)amino] benzo[*g*]isoquinoline-5,10-dione) (BBR 2778) <Pixuvri> acts as a Topoisomerase 2 inhibitor and intercalating agent. It is under study for the treatment of various solid and hematologic malignancies. It received fast track designation by the U.S. FDA for patients who have previously been treated two or more times for relapsed or refractory aggressive non-Hodgkin lymphoma. In Europe it is available on a Named-Patient basis (a compassionate use designation). The drug is administered through intravenous infusion.

Anthrapyrazole antibiotics Chromophore modification of the anthracenediones in an attempt to provide agents with diminished or no cardiotoxicity has resulted in the anthrapyrazole class of DNA binders. The anthrapyrazoles maintain the planar conformation and cationic nature of the anthracyclines, essential for DNA intercalation. Anthrapyrazole analogs show a broad range of activity for inhibiting Topoisomerase 2. Several anthrapyrazoles have been developed (Fig. 2.28). A tertiary amine in the basic side chain at N2 increases the cytotoxic activity compared with a secondary amine in this side chain (unless there is a basic side chain at the C5 position). A chlorine substituent on the basic side chain at N2 does not have a consistent effect on activity, neither does moving a chlorine substituent from C5 to C7 or introducing a basic side chain at C5 (Begleiter et al. 2006).

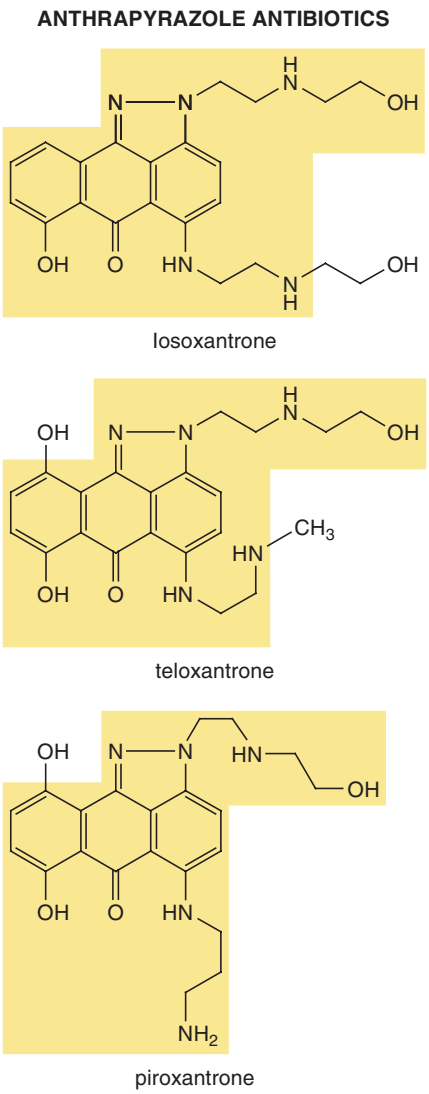


Fig. 2.28 Structures of anthrapyrazole antibiotics. The major portion of the molecule is common to all members of the class (highlighted in *yellow*)

Losoxantrone (biantrazole, 7-hydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]-5-[[2-[(2-hydroxyethyl)amino]ethyl] amino]anthra[1,9-cd]pyrazol-6(2H)-one dihydrochloride) (DuP-941, Cl-941) is an anthrapyrazole that induces both single and double strand breaks in DNA and is a potent inhibitor of DNA synthesis. The drug is in clinical trials for the treatment of breast cancer and of prostate cancer that is refractory to androgen ablation. It is used at a total dose of 50 mg/m^2 per course of losoxantrone therapy.

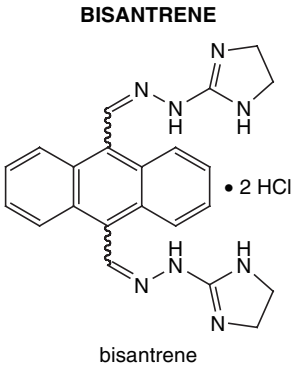
Pharmacokinetics 60 % of the administered dose is excreted in the feces unmetabolized, presumably via biliary excretion. 10 % is excreted in the urine, primarily during the first 24 h. The remainder is metabolized,

- mono-hydroxylation of the phenolic sub-structure of the chromophore occurs via Cytochrome P450 catalysis, resulting in metabolites that have ortho- and para-hydroquinonoid sub-structures, respectively
- the ortho-hydroxylated losoxantrone metabolite undergoes Glutathione conjugation or forms covalent bonds with intracellular nucleophilic targets.

Adverse Effects Acute toxicity is negligible. Losoxantrone may be less cardiotoxic than doxorubicin. When the drug is administered in an intermittent single-dose schedule, the maximum tolerated dose is 55 mg/m^2, the dose limiting toxicities being leukopenia and neutropenia. Up to 40 % of patients encounter alopecia. 3 % of patients develop congestive heart failure.

Teloxantrone hydrochloride (moxantrazole hydrochloride) (CI-937, DuP-937) is the salt of an anthrapyrazole. The

Fig. 2.29 Structure of bisantrene



drug intercalates into DNA and interacts with Topoisomerase 2, thereby inhibiting DNA reduplication and repair, as well as RNA and protein synthesis.

Adverse Effects The pattern, frequency, and severity of toxicity are similar to those of losoxantrone.

Piroxantrone (oxanthrazole, oxantrazole) (CI-942, PD-111815) is an anthrapyrazole that intercalates into DNA and interacts with Topoisomerase 2.

Pharmacokinetics The elimination from the blood is biexponential with an α half-life of around 3 min and a β half-life of around 80 min.

Adverse Effects While being less cardiotoxic than doxorubicin, this agent exhibits a narrow spectrum of anti-neoplastic activity. The dose limiting toxicity is myelosuppression.

Related compounds Bisantrene (NSC-33776) (Fig. 2.29) is an anthracenyl bishydrazone that intercalates with and disrupts the configuration of DNA, resulting in DNA single strand breaks, DNA-protein cross-linking, and inhibition of DNA reduplication. This agent is similar to doxorubicin in activity, but unlike doxorubicin, does not exhibit cardiotoxicity. Bisantrene has low anti-tumor activity and needs to be taken at a high dose to be effective.

Anthracyclines, anthracenediones and anthrapyrazoles are related anti-neoplastic antibiotics. They share a polycyclic aromatic core structure.

The 2 main modes of anthracycline action comprise Topoisomerase inhibition and generation of reactive oxygen species.
Because first generation anthracyclines are associated with myocardial dysfunction and alopecia, second generation drugs attempt to reduce these adverse effects.
Because of enhanced total body clearance, epirubicin can be used at high cumulative doses without increased cardiotoxicity.
Oligosaccharide anthracyclines induce hematopoietic differentiation.

The diaminoalkyl groups are crucial for the biological activity of anthracenediones. Anthracenediones may require metabolic activation before effectively intercalating into DNA.
Anthracenediones may act as anti-oxidants by inhibiting conjugated diene formation from linoleic acid.

2.2.5 Enediyne Antibiotics

Enediyne antibiotics are a class of anti-tumor antibiotics, which are produced by Actinomadura verrucosospora, Micromonospora echinospora, and Streptomyces species. The structures of the calicheamicins and esperamicins were described in 1987, the dynemicins in 1989, kedarcidin in 1992, and lidamycin and maduropeptin in 1993. The unique structures of antibiotics in this class (Fig. 2.30) contribute to their very high potency as anti-tumor agents (Sugiura et al. 1989). The heart of these molecules is a bicyclo [7.3.1] tridecane system that combines a methyl trisulfide and double and triple bonds in a 3-ene-1,5-diyne formation. The enediyne group readily undergoes cyclo-aromatization via a diradical intermediate. The highly reactive radical is capable of abstracting hydrogen atoms from the DNA backbone, resulting in DNA damage at specific sites (Table 2.3) and giving rise to a powerful anti-proliferative activity. Enediyenes bind at specific sites to the minor groove of double-helical DNA and inflict primarily sequence specific DNA double-strand breaks. These drugs have three important functional domains,

- an assemblage that consists of an enediyne moiety
- a delivery system that conveys the enediyne moiety to its DNA target
- a triggering device that, when activated, initiates a cascade of reactions that generate the reactive chemical species (Shao and Zhen 2008).

Enediynes are characterized by a 9- or 10-membered ring containing two triple bonds separated by a double bond (called the warhead). The sub-family of 10-membered cyclic enediynes includes calicheamicins, esperamicins, dynemicins, and shishijimicins A-C. The subfamily of 9-membered cyclic enediynes includes neocarzinostatin, kedarcidin, lidamycin, maduropeptin, and N1999A2.

A major challenge to clinical applications of the enediyne antibiotics is their inadequate selectivity. This has stimulated efforts to conjugate members of this class of drugs to tumor targeting entities, such as monoclonal antibodies.

10-membered cyclic enediynes The enediyne core is essential for biological activity. Calicheamicins, esperamicins, and dynemicins have potent DNA breakage activity in the presence of thiol compounds. They cleave DNA via free radical damage after activation by Cytochrome P450 Reductase and NADPH[31]. The 10-membered cyclic enediynes (drug names

[31] Cytochrome P450 Reductase and NADPH can generate superoxide anion (O_2^-) from quinone containing anti-tumor agents, heterocyclic amines, or enediynes.

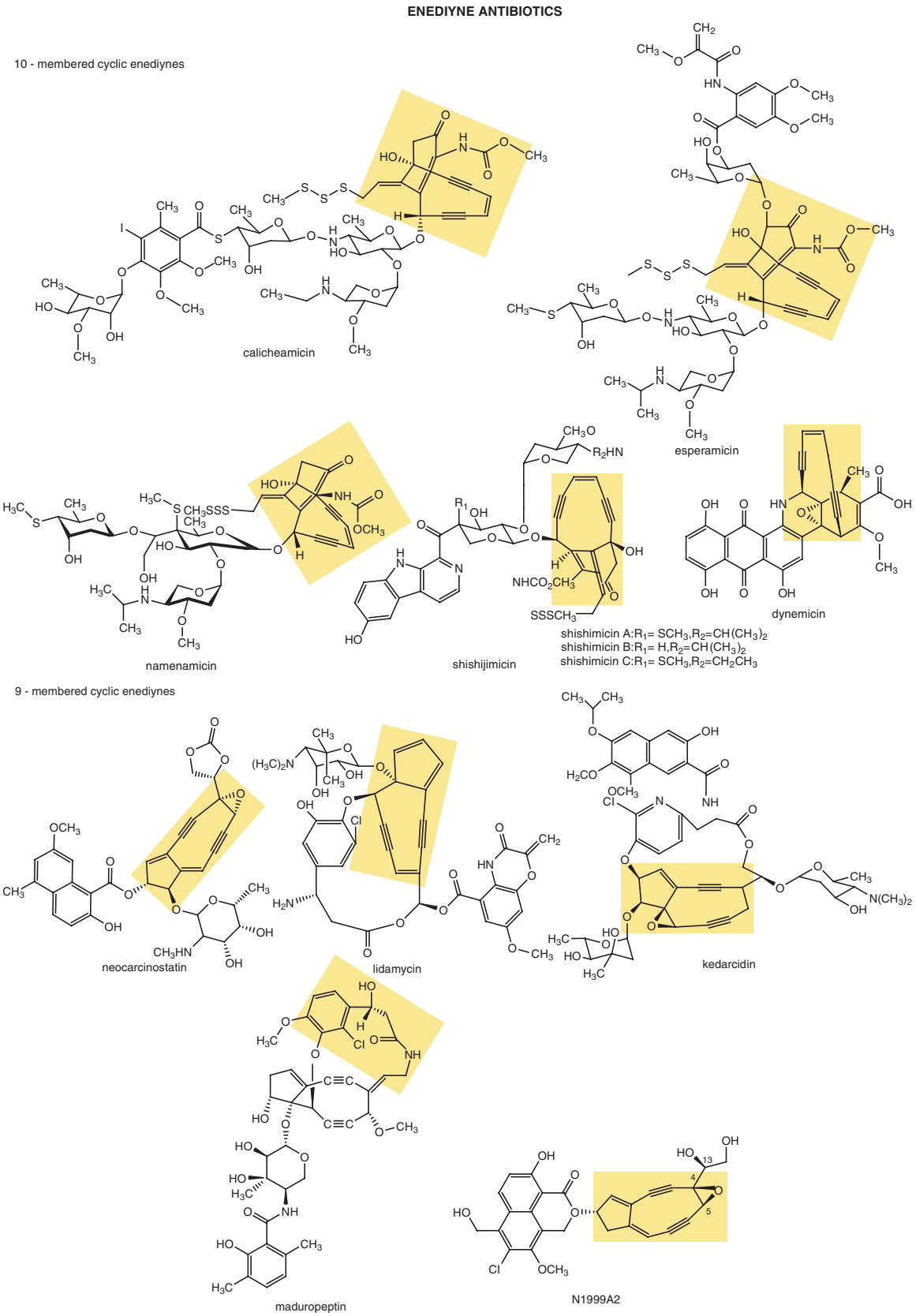


Fig. 2.30 Structures of cyclic enediyne antibiotics. For each group, 9-membered and 10-membered enediynes, the shared portion of the molecule is highlighted in *yellow*. In addition to the chromophor, the 9-membered enediynes also contain an apoprotein portion, which is not shown

Table 2.3 Preferred DNA binding sites for endiyne antibiotics

Drug	Recognition sequence	DNA domain
Chalicheamicins	5′-TCCT-3′, 5′-TTGT-3′, 5′-ATCT-3′	Minor groove
Esperamicins	5′-CTC-3′, 5′-TTC-3′, 5′-TTT-3′	Minor groove
Dynemicins	5′-CTACTACTGG-3′, 5′-AG-3′, 5′-AT-3′,5′-GC-3′	Minor groove
Neocarcinostatin	5′-GGAGCGC-3′	Minor groove
Lidamycin	5′-CTTTT-3′, 5′-ATAAT-3′, 5′-CTTTA-3′, 5′-CTCTT-3′, 5′-GTTAT-3′	Minor groove
Kedarcidin	5′-TCCT-3′	Minor groove
N1999A2	5′-GGT-3′	Minor groove

ending on -micin) do not contain apoproteins. They tend to be more stable than the 9-membered cyclic enediynes.

The chalicheamicins were discovered at the Medical Research Division of American Cynamid in the 1980s through the use of a biochemical induction assay. They were isolated from the fermentation broth of the bacterium Mocromonospora echinospora species calichensis. Several components of the calicheamicins (at least 15 are known) are assigned Greek letters based on their HPLC retention times. Calicheamicin is one of the most complex natural products to have been developed into an anti-cancer drug. After binding to the minor groove of DNA, preferentially to the sequences 5′-TCCT-3′, 5′-TTGT-3′, or 5′-ATCT-3′, calicheamicin is reduced by cellular thiols. The resultant product undergoes a rearrangement to generate 1,4-dehydrobenzene diradical that abstracts hydrogen atoms from DNA, thereby initiating double strand breaks.

- Calicheamicin exhibits a very narrow therapeutic window and delayed toxicities. The agent can be modified by reaction with a thiol hydrazide to contain a hydrazine disulfide linker, which can be reacted with the oxidized carbohydrates of a monoclonal antibody. Thus, the drug has been improved by conjugation to tumor specific antibodies, such as gemtuzumab (anti-CD33) for the treatment of AML. This combination gemtuzumab ozogamicin (CMA-676) <Mylotarg> was approved by the U.S. FDA in 2000 for patients 60 years and older in first relapse with CD33-positive acute myeloid leukemia (AML), who are not candidates for cytotoxic chemotherapy. After infusion, near complete saturation of CD33 antigenic sites is reached for AML blasts, monocytes, and granulocytes. Gemtuzumab ozogamicin does not bind to lymphocytes. The mechanism of action involves the drug binding to CD33 antigen on the AML cell membrane, then being internalized. Calicheamicin (ozogamicin) is released from the antibody inside the lysosomes of cells. It enters the cell nucleus and binds to DNA, causing breaks in the double helix resulting in cell death.

Adverse Effects Gemtuzumab ozogamicin therapy does not result in hair loss, severe oral mucositis, or damage to the intestinal mucosa. An acute infusion related symptom complex of fever and chills, and less commonly hypotension and dyspnea, may occur within 24 h of treatment. Hypertension, hyperglycemia, and hypoxia may also arise. The drug was withdrawn in 2010, when a clinical trial indicated that it increased patient death and added no benefit over conventional cancer therapies.

The esperamicins were discovered in the 1980s at Bristol-Myers Squibb. Esperamicin A1 is a bacterial chromoprotein enediyne antibiotic produced by Actinomadura verrucosospora. The esperamicins (A, B, C, and D) are extremely toxic DNA splicing compounds. The favored DNA binding sites are 5′-CTC-3′, 5′-TTC-3′, and 5′-TTT-3′ sequences. The preferential cutting sites of esperamicin are adjacent to thymidylate residues.

Shishijimicins A-C were isolated from the tunicate Didemnum proliferum Kott (Oku et al. 2003). They encompass a unique sugar component, which is a conjugation product of a hexose and a β-carboline, attached to the calicheamicinone aglycone. Shishijimicins A-C are highly cytotoxic.

Namenamicin was isolated from the tunicate Polysynchraton lithostrotum off the coast of the Fiji island of Namenalala (McDonald et al. 1996). It is the first enediyne natural product of marine origin. The trisaccharide domain contains an isopropyl aminosugar (C ring) and two unusual 6-deoxysugars (A and B rings), with an unusual linkage between the A and B rings, in which C-4 of the A ring is disubstituted with a methyl thioether and a two carbon fragment to which the B ring is appended at the C-7 oxygen.

Dynemicins are synthesized by Micromonospora chersina M956-1. Dynemicin A binds DNA at 5′-CTACTACTGG-3′, 5′-AG-3′, 5′-AT-3′, or 5′-GC-3′ sequences. It intercalates into the DNA base stack with its anthraquinone functional group, while the (*Z*)-enediyne bridge is positioned in the minor groove. Dynemicin A cleaves double stranded DNA in the presence of reducing cofactors, such as GSH or NADPH. The ID_{50} is around 30–60 μg/kg.

9-membered cyclic enediynes 9-membered cyclic enediynes are composed of non-covalently bound chromophore and apoprotein. The anti-cancer activity lies in the chromophore, while the apoprotein has a stabilizing function. Major binding interactions between the apoprotein and the chromophore are the hydrophobic contacts between the core of the chromophore and the hydrophobic side chains of the pocket forming residues.

Neocarzinostatin (zinostatin, vinostatin), discovered in 1964, was the first enediyne antibiotic used clinically and has

become a prototypical anti-cancer agent for the treatments of leukemia, gastric carcinoma and pancreatic adenocarcinoma. The agent is produced by the bacterium Streptomyces carzinostaticus. It is an enediyne antibiotic hybrid containing an aminoglycoside chromophore. Neocarcinostatin consists of a non-protein chromophor, which is complexed with a protein carrier and stabilizer. The aminoglycoside component intercalates into DNA, and the benzene diradical intermediate of the enediyne core binds to the minor groove of double-helical DNA at 5′-GGAGCGC-3′ sites. Neocarzinostatin requires sulfhydryl activation. It is converted to a diradical that directly attacks the deoxyribose of residues in DNA, resulting in single and double strand breaks in DNA and consecutive apoptosis.

- Styrene maleic acid neocarzinostatin (SMANCS) is a chemical conjugate of a synthetic co-polymer of styrene maleic acid (SMA) and neocarzinostatin (NCS). The drug is soluble in organic solvents, such as pyridine, acetone, and Lipiodol[32]. The coupling to a styrene maleic acid based polymer reduces the immunogenicity of neocarzinostatin. A principal advantage is the tumor targeting mechanism through an enhanced permeability and retention effect, and the potential for a reduction of toxicity, such as a marked reduction in bone marrow toxicity. SMANCS was approved in Japan in 1993 for the treatment of hepatoma, gastric carcinoma, and lung cancer. SMANCS may also be beneficial in the treatment of renal cell carcinoma. Hepatic arterial infusion of a SMANCS/Lipiodol emulsion has been used as a treatment for advanced or recurrent hepatocellular carcinoma. SMANCS is used most successfully when it is administered as a treatment of dose per tumor size and follow-up treatments are given on an as needed basis (Duncan 2006).

Adverse Effects Anorexia, nausea, and vomiting are the most frequent adverse effects of neocarcinostatin. Dose limiting toxicity is myelosuppression, which tends to occur late. Myelosuppression is more pronounced in patients who have received prior chemotherapy. Acute administration of neocarcinostatin is associated with fever, chills, and cyanosis (rigors) in approximately half of the patients. Severe allergic reactions are possible.

In 1988, Otani and coworkers isolated and characterized lidamycin (C-1027) from the broth filtrate of Streptomyces globisporus C-1027 (the strain was isolated from a soil sample collected in the Qianjiang area of China to serve antibiotic production) (Hu et al. 1988). Lidamycin is water soluble and has a molecular weight of 15 kD. The apoprotein consists of a single polypeptide chain cross-linked by two disulfide bonds and contains a total of 110 amino acid residues. The chromophore possesses a highly strained 9-membered 1,5-diyn-3-ene core structure together with a 16-membered macrocyclic ring. Functionally important domains of the chromophore include the benzoxazine moiety that is necessary for DNA intercalation and the 9-membered enediyne moiety that is necessary for DNA cleavage. Lidamycin acts through

- inducing DNA double strand breaks at target sequences 5′-CTTTT-3′, 5′-ATAAT-3′, 5′-CTTTA-3′, 5′-CTCTT-3′, and especially 5′-GTTAT-3′
- directly causing DNA single strand breaks without the need for reducing agents
- inhibiting DNA and RNA synthesis and subsequently delaying the progression through the cell cycle (lidamycin induces G_1 arrest in cells with wild-type P53 and G_2/M arrest in cells with mutant P53)
- inducing apoptosis and mitotic cell death (lidamycin induced apoptosis is likely more effective in cancers with wild type P53 than those with mutant or deleted P53) (Shao and Zhen 2008).

Besides inhibiting tumor growth, lidamycin suppresses angiogenesis by blocking the binding of FGF-2 to its receptor and inhibiting the formation of the FGF-2/Receptor immune complex (Zhen et al. 2005).

- Efforts are in progress to generate conjugates with targeting moieties, such as monoclonal antibodies. This is more difficult for 9-membered cyclic enediynes than for 10-membered cyclic enediynes because the apoprotein is important for function. Instead of linking the chromophor directly to the antibody, an assembled conjugate is synthesized, which links the apoprotein to the antibody and then reconstitutes the complex by adding the chromophore.

Kedarcidin is an enediyene natural product produced by Actinomycete strain L585-6. It is water-soluble and acidic with a molecular weight of 12.4 kD. The holoprotein is 10-fold more potent than the chromophore itself. It binds the minor groove of DNA at 5′-TCCT-3′ sequences. The apoprotein has activity as a Peptidase and may cleave Histones.

Maduropeptin, produced by Actinomadura madurae, has the ability to cleave DNA by undergoing a Bergman cyclization and forming a *p*-benzyne biradical, which abstracts hydrogens from DNA and initiates a cascade of DNA breakage, ultimately leading to cell death. Beside the enediyne core, maduropeptin also contains a 3,6-dimethylsalicylic acid moiety, an aminosugar (previously named madurose),

[32] Lidipol is an ethyl ester of iodinated poppy seed oil, in which most of the unsaturated double bonds in oleic, linoleic and linolenic acid are almost completely iodinated.

and an (S)-3-(2-chloro-3-hydroxy-4-methoxyphenyl)-3-hydroxypropionic acid moiety.

Only N1999A2 is a non-protein 9-membered cyclic enediyne antibiotic that lacks an aminoglycoside residue (which is present in neocarcinostatin). It is epimeric with the neocarzinostatin chromophore at positions 4, 5, and 13. N1999A2 binds DNA at sites containing 5′-GGT-3′.

Enediyenes bind at specific sites to the minor groove of double-helical DNA and inflict primarily sequence specific DNA double strand breaks.
The 9-membered, but not the 10-membered cyclic enediynes contain apoproteins. The anti-cancer activity of the 9-membered cyclic enediynes lies in the chromophore, while the apoprotein has a stabilizing function.
The 10-membered cyclic enediynes tend to be more stable than the 9-membered cyclic enediynes.
The 10-membered cyclic enediynes cause DNA double strand breaks in the presence of thiol compounds, the characteristic of DNA cleavage is the induction by NADPH.
The 9-membered cyclic enediynes may also cause DNA single strand breaks, cell cycle arrest, or apoptosis.

2.2.6 Others

Actinomycins were first described by Selman Waksman and H.B. Woodruff in 1940 (Waksman and Woodruff 1940). Actinomycin D <Dactinomycin, Cosmegen, Oncostatin K, Actinomycin IV, Actinomycin C1> was isolated from the soil bacterium Streptomyces parvulus. It is a polypeptide antibiotic containing an aminoquinone chromophore.

- Actinomycin D specifically intercalates to GC-rich sites of DNA. It inhibits transcription by binding to the DNA at transcription initiation complexes and preventing elongation by RNA Polymerase.
- As actinomycin D can bind DNA duplexes, it can interfere with DNA reduplication. The agent acts in a cell cycle non-specific manner.
- Actinomycin D causes single strand DNA breaks, possibly via a free radical intermediate or an interaction with Topoisomerase 2.

The potential of Actinomycin D to act as a radio-sensitizer is under debate.

Actinomycin D (Fig. 2.31) was approved by the U.S. FDA in 1964. The drug is administered intravenously for the treatment of gestational trophoblastic neoplasia, germ cell tumor, choriocarcinoma, testicular cancer, melanoma, neuroblastoma, retinoblastoma, Wilms tumor, rhabdomyosarcoma, Ewing sarcoma, uterine sarcoma, Kaposi sarcoma, sarcoma botryoides, or soft tissue sarcoma. It may be a component of regional perfusion for the palliative or adjunctive treatment of locally recurrent or locoregional solid malignancies. While actinomycin D can produce a response, the duration of remission varies among patients. The usual dose is 15 mg/kg/day, repeated for 5 days as a course. A 2nd course can be started after 2–4 weeks. Dosage reduction may be necessary when additional chemotherapy or radiation therapy is used concomitantly or has been applied previously.

Pharmacokinetics Actinomycin D is minimally metabolized. The drug does not cross the blood-brain barrier. The terminal plasma half-life is 35 h. Approximately 30% of the dose is excreted in urine and feces within 1 week.

Adverse Effects The drug solution is extremely irritating and extravasation produces severe tissue damage (intermittent application of ice to the site for 15 min four times per day for 3 days may be useful). Blistering, ulceration, or persistent pain are indications for wide excision surgery, followed by split-thickness skin grafting. Nausea and vomiting may occur several hours after administration of actinomycin D and can be alleviated through anti-emetics. Toxic effects on bone marrow, kidneys, and liver may not become manifest until several days after completion of the course of treatment, and reach their maximum for 1–2 weeks. Liver toxicity may manifest in ascites, hepatomegaly, hepatitis, or hepatic veno-occlusive disease associated with intravascular clotting disorder and the risk for multi-organ failure. Other adverse reactions include glossitis, cheilitis, ulcerations of the oral mucosa, and proctitis. Actinomycin is Pregnancy Category D.

Bleomycin was discovered in 1962 in Japan, when Hamao Umezawa found anti-cancer activity while screening culture filtrates of Streptomyces verticullus. The findings were published in 1966. Bleomycin refers to a family of structurally related, glycosylated linear peptide antibiotics produced by Streptomyces verticillus. When used as anti-cancer agents, the chemotherapeutic forms are primarily bleomycin A_2 and B_2. Bleomycin is toxic to cells in the G_2 and early M phase of the cell cycle. The main damaging action is DNA strand scission. It depends on binding of a bleomycin/iron complex to DNA, which then reduces molecular oxygen to free oxygen radicals that cause primarily single strand breaks.

Bleomycin sulfate <Blenoxane, Teva> is used in the treatment of Hodgkin and non-Hodgkin lymphoma as a component of the ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) regimen, of squamous cell carcinoma, and of testicular cancer. The drug may be given by intramuscular, intravenous, subcutaneous, or intrapleural routes. It is effective as a sclerosing agent for the treatment of malignant pleural effusions and for the prevention of recurrent pleural effusions (60 units administered as a single dose, intrapleural bolus injection). Bleomycin was launched in Japan in 1969. It gained U.S. FDA approval in 1973.

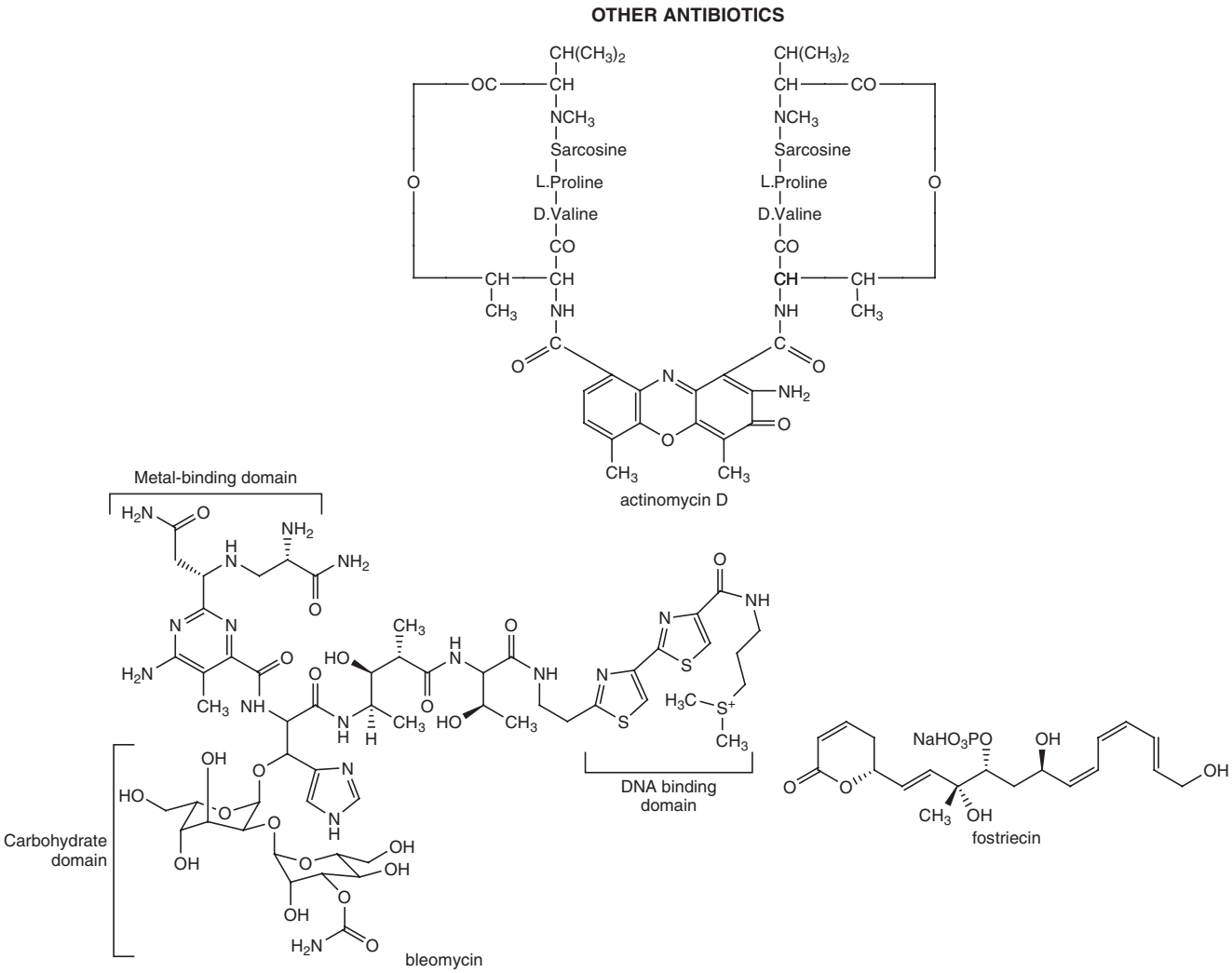


Fig. 2.31 Structures of other antibiotics. Antibiotics with very diverse chemical structures may exert anti-cancer effects

Pharmacokinetics Bleomycin is rapidly absorbed following intravenous (100 % systemic bioavailability), intramuscular (100 % bioavailability), subcutaneous (70 % bioavailability), intraperitoneal (45 % bioavailability), or intrapleural (45 % bioavailability) administration, reaching peak blood concentrations in 30–60 min. As the main route of elimination is through the kidneys, dose reduction may be necessary for patients with renal insufficiency. Systemic elimination of the drug by enzymatic degradation is probably only important in patients with severely compromised renal function. Bleomycin is inactivated by the cytosolic Aminohydrolase (Bleomycin Hydrolase). The enzyme is widely distributed in healthy tissues with the exception of the low expression in skin and lungs, both of which are susceptible to bleomycin toxicity.

Adverse Effects Toxic pulmonary effects may manifest as interstitial pneumonitis or as bronchiolitis obliterans with organizing pneumonia. There is a risk of pulmonary fibrosis at large doses. The incidence of pulmonary toxicity is elevated in patients over 70 years of age and is proportionate to the cumulative bleomycin dose, occurring in about 10 % of patients treated with more than 300 units, and causing death in up to 3 %. Cases of pulmonary toxicity may resolve with the administration of corticosteroids. Adverse reactions of the skin and mucous membranes consist of erythema, rash, striae, vesiculation, hyper-pigmentation, hyperkeratosis, nail changes, alopecia, pruritus, and stomatitis. They arise in 50 % of patients, usually in the 2nd–3rd week of treatment, after 150–200 units have been administered. The incidence of the Raynaud phenomenon (transient vasoconstriction of the digital arteries causing pallor or cyanosis) may reach 35 %, occurring most commonly 4–12 months after completion of chemotherapy. In 25 % of afflicted patients, the symptoms may persist for 10–20 years after treatment. Other adverse effects include fever, alopecia, vascular toxicities,

and ototoxicity. However, no significant myelosuppression is associated with the drug. The drug is Pregnancy Category D.

As a natural product, bleomycin may be the cause of anaphylactic reactions, immediate or delayed for several hours. Such adverse events occur in approximately 1 % of lymphoma patients, usually after the 1st or 2nd dose. They manifest in hypotension, confusion, fever, chills, wheezing, shortness of breath, or low blood pressure. They require the drug to be stopped for treatment with dexamethasone, diphenhydramine, and if necessary epinephrine. Therefore, lymphomata should be treated with ≤2 units for the first two doses; if no acute reaction occurs, regular dosage schedule is followed.

Drug Resistance The presence of Hydrolases in tumor cells is the primary mechanism of resistance to bleomycin. Cells can also become resistant by repairing the DNA breaks produced by bleomycin.

Drug Interactions The response to bleomycin is compromised in patients with previously irradiated head and neck cancer. Because bleomycin is eliminated predominantly through renal excretion, the administration of nephrotoxic drugs with bleomycin may affect its renal clearance.

Fostriecin (2H-Pyran-2-one,5,6-dihydro-6-[3,6,13-trihydroxy-3-methyl-4-(phosphonooxy)-1,7,9,11-tridecatetraenyl) (CI-920, NSC 339638, PD 110 161) was isolated from the bacterium Streptomyces pulveraceus subspecies fostreus. It is a phosphate monoester anti-tumor antibiotic containing an unsaturated lactone and a conjugated triene system.

- Fostriecin inhibits Topoisomerase 2 catalytic activity, resulting in protein associated strand breaks and impaired DNA and RNA synthesis in various malignant cell types.
- This agent inhibits serine/threonine Protein Phosphatase type 2A (and to a lesser extent type 1) and Protein Phosphatase 4 (PP4), which leads to phosphorylation and reorganization of Vimentin filaments, thereby interfering with cellular proliferation.
- Fostriecin activates the mitotic entry checkpoint, leading to G_2/M arrest.

Adverse Effects Renal tubular damage, reflected in a rise in serum creatinine after 1–2 doses, is reversible and not dose limiting.

Actinomycin D suppresses transcription, interferes with DNA reduplication, and inhibits Topoisomerase 2.
Bleomycin is inactivated by Aminohydrolase (widely expressed except in skin and lungs, both of which are targets of bleomycin toxicity). The presence of Hydrolases in tumor cells is the primary mechanism of resistance to bleomycin.
Fostriecin is a Topoisomerase 2 inhibitor and acts as an inhibitor of several Protein Phosphatases.

2.3 Topoisomerase Inhibitors

Otherwise identical loops of DNA having different numbers of twists are topoisomers (Gr. τόπος= place, ἰσομερής= equal parts). They cannot be interconverted by any process that does not involve the breaking of DNA strands. Topoisomerases (Gyrases) (type 1: EC 5.99.1.2, type 2: EC 5.99.1.3) are enzymes that catalyze and guide the unknotting of DNA. They can cause transient single strand (Topoisomerase 1) or double strand (Topoisomerase 2) DNA breaks that are resealed after changing the twisting status of the double helix. Topoisomerases wrap around the DNA and make cuts permitting the helix to spin. Once the DNA is relaxed, the enzymes reconnect the broken strands. Thus, Topoisomerase activity releases the tension generated by the winding of DNA and facilitates transcription and reduplication. Topoisomerase inhibitors interfere with this process.

2.3.1 Topoisomerase 1 Inhibitors

Topoisomerase 1 is a 100 kD monomeric protein that makes a single cut in the DNA duplex and relieves transcription-associated torsional strain. No energy cofactor is required to carry out this reaction. Drugs may stabilize the Topoisomerase 1/DNA complex (referred to as the "cleavable complex"), thus blocking DNA relegation and converting the enzyme into a DNA damaging agent. Topoisomerase 1 inhibitors exhibit S phase cytotoxicity and G_2/M cell cycle arrest.

In 1956, C. Gordon Zubrod, who had formerly led the development of anti-malarial agents for the United States Army, took over the Division of Cancer Treatment of the National Cancer Institute and guided the development of new drugs. In the two decades that followed the establishment of the NCCSC (National Cancer Chemotherapy Service Center), a large network of cooperative clinical trial groups evolved under its auspices to test anti-cancer agents. Zubrod had a particular interest in natural products, and established a broad program for collecting and testing plant and marine sources. Although this was a controversial project, it led to the discovery of the camptothecins in 1966.

Camptothecins Camptothecin (4-ethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-b]quinoline-3,14-(4*H*,12*H*)-dione) is a cytotoxic, quinoline based, non-terpenoid alkaloid that was extracted from the Asian tree Camptotheca acuminata in 1966 (Wall et al. 1966) (Fig. 2.32). The drug was isolated and characterized by the laboratory of Monroe Wall at the Research Triangle Institute in the U.S. During the S phase of the cell cycle, camptothecin inhibits Topoisomerase 1 activity by stabilizing the cleavable complex between Topoisomerase 1 and DNA, resulting in DNA T-G/A single

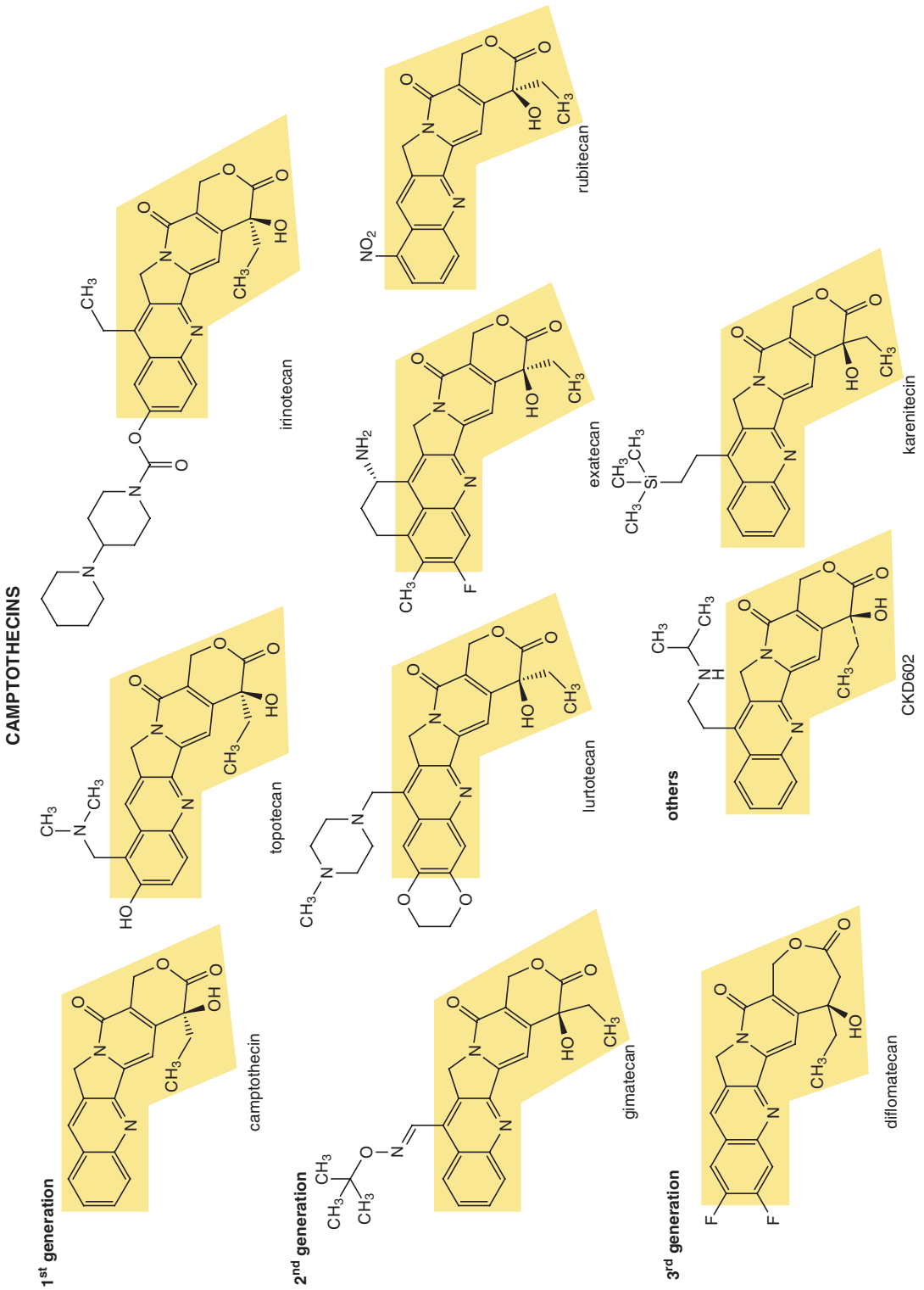


Fig. 2.32 Structures of camptothecins. The core structure, common to all camptothecins in all generations is highlighted in *yellow*

strand breaks that cannot be religated. Camptothecin/Topoisomerase 1/DNA cleavable complexes are rapidly conjugated with SUMO by SUMO-Protein Ligase (UBE2I, UBC9). This inhibits reduplication and triggers apoptotic cell death. Camptothecin also blocks RNA synthesis through Topoisomerase 1 mediated effects.

Pharmacokinetics Because camptothecin exists in equilibrium between the lactone and the ring-opened conformations its drug action is limited. The E ring-opened carboxylate form has less than 10% potency of the lactone form. The

stability of the lactone ring at physiological pH is a determinant of activity for all camptothecin analogs.

Adverse Effects Despite showing promise in preclinical studies, camptothecin had poor bioavailability and little antitumor activity in early clinical trials. Dosing was limited by severe diarrhea and urinary tract toxicity (the unstable lactone ring at neutral pH is activated in the acidic environment of the kidneys, exerting damage to the renal tubules). Because Camptothecin manifests a life threatening adverse reaction in the form of severe cystitis, chemically related analogs with less toxicity and enhanced therapeutic potency have been developed.

Drug Resistance Although camptothecin and its analogs can freely enter cells via passive diffusion, their intracellular concentrations are greatly reduced by efflux pumps. All camptothecins are substrates for the efflux pump ABCG2 (Breast Cancer Resistance Protein, BCRP, MRX). Leukemia cells may develop the Topoisomerase 1 mutations Asn722Ser or Arg364His and become resistant to camptothecins.

Topotecan hydrochloride ((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7] indolizino[1,2-b]quinoline-3,14(4*H*,12*H*)-dione monohydrochloride) <Hycamptin> is the water soluble salt of a semi-synthetic derivative of camptothecin. Approved in 1996 for treating ovarian cancer, topotecan was the first Topoisomerase inhibitor in clinical use. During the S phase of the cell cycle, the agent selectively stabilizes Topoisomerase 1/DNA covalent complexes, thereby inhibiting the religation of the single strand DNA breaks mediated by Topoisomerase 1. This constellation may produce potentially lethal double strand DNA breaks when encountered by the DNA reduplication machinery. Topotecan is indicated for small cell lung cancer after the failure of first-line chemotherapy, metastatic carcinoma of the ovary following failure of initial or subsequent chemotherapy, and stage IV-B, recurrent, or persistent cervical carcinoma which is not amenable to surgery or radiation therapy. The drug is sometimes used to treat acute myelogenous leukemia (AML) and renal cell carcinoma (RCC). Topotecan is given at 0.75–1.5 mg/m^2 by infusion daily for 5 days at the beginning of a 3 week cycle. Topotecan capsules can be taken orally over five dosings a day.

- ^{11}C-topotecan is a semi-synthetic derivative, radio-labeled with carbon 11. It has therapeutic and radio-tracer properties. Quantitation of ^{11}C-topotecan accumulated in tumor tissues by positron emission tomography (PET) may help predict the responses to topotecan therapy.

Pharmacokinetics Topotecan exhibits multi-exponential pharmacokinetics with a terminal half-life of 2–3 h. Binding to plasma proteins is about 35%. Like camptothecin, topotecan undergoes a reversible, pH dependent hydrolysis of its pharmacologically active lactone moiety. Renal clearance is an important determinant of topotecan elimination (50% of the administered dose as parent drug, 3% as desmethyl topotecan, 2% as topotecan-*O*glucuronide and *N*-desmethyl topotecan-*O*-glucuronide). Fecal elimination accounts for 15% of the parent drug and 1.5% of *N*-desmethyl topotecan. Dose adjustments may be required in patients with renal or hepatic impairment.

Adverse Effects The dose limiting toxicity of topotecan is leucopenia, which results in lowered resistance to infections. Adverse effects include bruising or bleeding, anemia, nausea or vomiting (70%), hair loss (60%), asthenia or fatigue, sore mouth and ulcers (30%), diarrhea (40%), loss of appetite (20%), and a reversible change in taste. Topotecan is contraindicated in patients who have a history of severe hypersensitivity reactions to the drug or to any of its ingredients. The drug is Pregnancy Category D, it is secreted into the milk.

Drug Interactions Myelosuppression is more severe when topotecan is given in combination with cisplatin or other cytotoxic agents, thereby necessitating a dose reduction. Concomitant treatment with G-CSF and topotecan can prolong the duration of neutropenia. Therefore, G-CSF should not be initiated until day 6, 24 h after completion of the administration of topotecan.

Drug Resistance Multi-drug resistance can result from drug efflux via transporters. Topotecan is a substrate for ABCB1 (MDR1, P-Glycoprotein) and ABCG2.

Irinotecan hydrochloride ((*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano[3′,4′:6,7]-indol izino[1,2-b]quinolin-9-yl-[1,4′bipiperidine]-1′-carboxylate) (CPT-11) <Campto, Camptosar> is the water soluble salt of a semi-synthetic camptothecin derivative. Because ongoing DNA synthesis is necessary for irinotecan to exert its cytotoxic effects, it is classified as S phase specific agent. The drug was approved by the U.S. FDA in 1994. It is indicated for colorectal cancers, where it is usually a component of combination chemotherapy, such as in FOLFIRI, and confers a survival benefit. This agent is also used to treat (small cell and non-small cell) lung cancers, ovarian cancers, leukemias, and lymphomata.

Pharmacokinetics As a prodrug, irinotecan is converted to the 1000-fold more active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) by a Carboxylesterase, primarily in the liver. 95% of SN-38 is bound to plasma proteins, compared to approximately 50% of irinotecan. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. Acidic pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form. Irinotecan blood concentrations decline in a multi-exponential manner, with a mean terminal elimination half-life of 6–12 h. The mean terminal elimination half-life of the active metabolite SN-38 is 10–20 h. SN-38 is inactivated

via glucuronidation by UGT1A1 (Uridine Diphosphate Glucoronosyltransferase 1A1) into the non-toxic SN-38-glucuronide (SN-38G) and is excreted into the gastrointestinal tract.

Adverse Effects The most substantial adverse effects of irinotecan are grade 2 or higher nausea and vomiting, alopecia, asthenia, fever, and abdominal pain. Extensive suppression of the immune system due to neutropenia is likely. A new cycle of therapy should not begin until the granulocyte count has recovered to at least 1500/mm^3 and the platelet count has recovered to at least 100,000/mm^3. Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions may arise. Interstitial pulmonary disease-like events, including fatalities, are possible. The drug is Pregnancy Category D. It is secreted in the milk.

Irinotecan can induce both early and late forms of diarrhea, which may be severe.

- Early diarrhea can be accompanied by cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. It may be prevented or ameliorated by 0.25–1.0 mg atropine (i.v. or s.c.).
- β-Glucuronidases expressed by intestinal commensal bacteria can hydrolyze SN-38-glucuronide to generate SN-38. SN-38 amounts in the intestinal lumen play an essential role in the delayed severe diarrhea that prevents dose intensification and efficacy in up to 40% of treated patients. Late diarrhea (generally occurring more than 24 h after administration) can be life threatening as it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide. Before the next ensuing chemotherapy administration, patients should return to pre-treatment bowel function without requiring antidiarrhea medications for at least 24 h. Intestinal biota play essential roles in carbohydrate metabolism, vitamin production, and the processing of bile acids, sterols, and xenobiotics. Thus, the removal of gastrointerstinal bacteria is not recommended for patients already challenged by neoplastic growths and chemotherapy. In addition, elimination of symbiotic gastrointestinal flora increases the chances of infections by pathogenic bacteria, including enterohemorrhagic Escherichia coli and Clostridium difficile. Inhibitors of β-Glucuronidases prevent this conversion and diminish irinotecan gut toxicity.

The Glucuronyltransferase UGT1A1, which conjugates bilirubin and renders it water-soluble, is largely responsible for the metabolism of irinotecan. The allele TA_7 (*28 variant) is expressed at lower levels than other alleles. Gilbert syndrome (Gilbert-Meulengracht syndrome), is the most common hereditary form of increased unconjugated blood bilirubin, based on the autosomal recessive inheritance of the reduced activity allele of UGT1A1. During chemotherapy, homozygous patients effectively receive a larger than expected dose of irinotecan because the drug is not cleared as fast as in non-carriers of this allele. Hence, Gilbert syndrome is associated with a high incidence of severe diarrhea and neutropenia in patients who are treated with irinotecan. Genetic testing can predict this toxicity before chemotherapy administration and can allow dose adjustment. Irinotecan is one of the first widely used chemotherapy agents to be dosed for each patient according to the underlying genotype.

Drug Interactions Ketoconazole (a strong inhibitor of CYP3A4 enzymes), CYP3A4 enzyme-inducing anticonvulsants (including phenytoin, phenobarbital, carbamazepine), and St. John's Wort (an inducer of CYP3A4 enzymes) have drug-drug interactions with irinotecan.

Drug Resistance Multi-drug resistance results from drug efflux via ABCB1 (MDR1, P-Glycoprotein). Irinotecan is a substrate for ABCB1.

Gimatecan (7-t-butoxyiminomethylcamptothecin) (ST1481) is a second generation, 7-position modified lipophilic camptothecin that was developed to provide rapid uptake and accumulation in cells and to achieve a stable Topoisomerase 1/DNA/drug ternary complex.

Pharmacokinetics Gimatecan is orally rapidly absorbed. Its plasma levels are related to the plasma concentrations of α_1-Acid Glycoprotein (AGP). Gimatecan has a long elimination phase (mean half-life of 90 h) with a clearance of 0.6 L/h. Fecal excretion is the main elimination pathway. Gimatecan is not a substrate for ABCB1 or ABCG2.

Adverse Effects Bone marrow suppression is the main dose limiting toxicity. Diarrhea is less severe than with camptothecin.

The second generation camptothecin derivative lurtotecan is a semi-synthetic analog of camptothecin, which selectively stabilizes the Topoisomerase 1/DNA covalent complex and forms an enzyme/drug/DNA ternary complex. As a consequence of the formation of this complex, both the initial cleavage reaction and the religation step are inhibited. Subsequently, collision of the reduplication fork with the cleaved strand of DNA results in inhibition of DNA reduplication, double strand DNA breakage, and triggering of apoptosis. Through additional mechanisms of action, lurtotecan also inhibits RNA synthesis, multi-ubiquitination and degradation of Topoisomerase 1, and chromatin reorganization.

The second generation camptothecin derivative exatecan mesylate[33] is a semi-synthetic water soluble drug. It inhibits

[33] Mesylate is a salt or ester of methanesulfonic acid, which may be used to to obtain crystalline salts of amine containing drug substances. Mesylate is a suitable leaving group in nucleophilic substitution reactions.

Topoisomerase 1 activity by stabilizing the cleavable complex between Topoisomerase 1 and DNA, thus blocking the religation of DNA breaks. Thereby exatecan inhibits DNA reduplication and triggers apoptotic cell death. This agent does not require enzymatic activation and exhibits greater potency than other camptothecin analogs.

The second generation camptothecin derivative rubitecan (9-NC, 9-Nitro-20(S)-Camptothecin, 9-Nitro-Camptothecin, 9-Nitrocamptothecin) (RFS 2000, RFS2000) <Orathecin> is a semi-synthetic agent that binds to and inhibits Topoisomerase 1. It is extracted from the bark and leaves of the Camptotheca acuminata tree, which is native to China. Rubitecan is under investigation for the treatment of advanced pancreatic cancer, as well as for prostate, breast, lung, and ovarian cancers.

Pharmacokinetics Rubitecan is an orally available camptothecin analog that also has potential for delivery transdermally or by inhalation. The drug exists in equilibrium between 9-nitro-camptothecin (9-NC) and 9-amino-camptothecin (9-AC). Both contain a lactone ring that is required for activity. The open ring, carboxylic acid forms are substantially less active or inactive. An acidic environment favors the lactone ring structure, whereas neutral or basic conditions favor the conversion to the carboxylic acid structure.

Homocamptothecins, in which the metabolically labile 6-membered α-hydroxylactone of camptothecin is replaced with a more stable 7-membered β-hydroxylactone, are potent anti-cancer agents. Diflomotecan (10,11-difluoro-homocamptothecin) (BN80915) is a third generation camptothecin derivative. The drug may be given intravenously or orally (Kroep and Gelderblom 2009). The recommended dose is 4 mg/m^2 for intravenous administration.

Pharmacokinetics Diflomotecan has enhanced plasma stability and superior anti-tumor activity as compared to the established camptothecins, irinotecan and topotecan. It has an oral bioavailability of 70–95 %.

Adverse Effects In contrast to other Topoisomerase 1 inhibitors, there is no severe gastrointestinal toxicity. The adverse effects are primarily hematologic, with severe neutropenia occurring in some patients.

Drug Resistance The homocamptothecins, like the camptothecins, are substrates for cellular efflux pumps, such as ABCG2. Cancer cells that are resistant to camptothecins due to over-expression of multi-drug resistance efflux pumps or mutations in Topoisomerase 1 are also resistant to homocamptothecins.

Derivatives of camptothecins have been synthesized to improve the pharmacokinetic properties. S-CKD602 (STEALTH-CKD602) is a sterically stabilized, polyethylene glycol coated (PEGylated) liposomal formulation containing CKD602, a semi-synthetic analog of campthotecin. The agent stabilizes the Topoisomerase 1/DNA complex, thereby preventing religation of DNA breaks. This leads to an inhibition of DNA reduplication and triggers apoptosis.

Pharmacokinetics The polyethylene glycol coating of S-CKD602 allows for greater plasma circulation time, thus enhancing the concentration of CKD602 at the tumor site. Encapsulation of CKD602 also preserves the active lactone form, leading to an increased cytotoxic effect of CKD602.

Karenitecin is a synthetic silicon containing agent related to camptothecin. It stabilizes the cleavable complex between Topoisomerase 1 and DNA, resulting in DNA breaks, and consequently triggering apoptosis.

Pharmacokinetics Because it is lipophilic, karenitecin exhibits enhanced tissue penetration and bio-availability compared to water soluble camptothecins.

Indolocarbazoles The indolocarbazole family of natural products was discovered in 1977. Its members are defined by their characteristic structure containing either indolo[2,3-a]pyrrolo[3,4-c]carbazole, indolo[2,3-a]carbazole, or bis-indolyl-maleimide[34]. The chemical structures (Fig. 2.33), pharmacokinetics, and pharmacodynamics of indolocarbazoles differ from many other Topoisomerase 1 inhibitors, including camptothecin derived agents.

Rebeccamycin (Bush et al. 1987), the first anti-cancer active indolocarbazole, was described in 1987 by Bristol-Myers scientists and named after the daughter of the group leader. Rebeccamycin is a secondary metabolite originally isolated from cultures of the actinobacterium Lechevalieria aerocolonigenes in 1985. The compound is halogenated and possesses a maleimide indolo[2,3-a]carbazole framework with a carbohydrate moiety attached to one of the indole nitrogens. As a sugar derivative, it has increased (albeit still poor) water solubility compared to the parent indolocarbazole. Rebeccamycin acts as a potent stabilizer of the Topoisomerase 1/DNA complex.

NB-506 (L-753 000) is a synthetic Topoisomerase 1 inhibitor with an indolocarbazole structure. Edotecarin (J-107088, ED-749, PHA-782615) is a derivative of NB-506 that more effectively induces DNA cleavage. It also targets

[34] Indolocarbazole antibiotics with a single glycosidic linkage are often strong inhibitors of Topoisomerases, whereas indolocarbazoles with two bonds between the glycoside and indolocarbazole heterocycle act as inhibitors of Protein Kinases. The straurosporine sub-class is characterized by two bonds between the glycoside and indolocarbazole heterocycle. These fused structures exhibit potent inhibition of protein kinases, such as Protein Kinase C (PKC), FLT3, and RET (see Sect. 4.1.3., 4.1.5., and 4.1.7.).

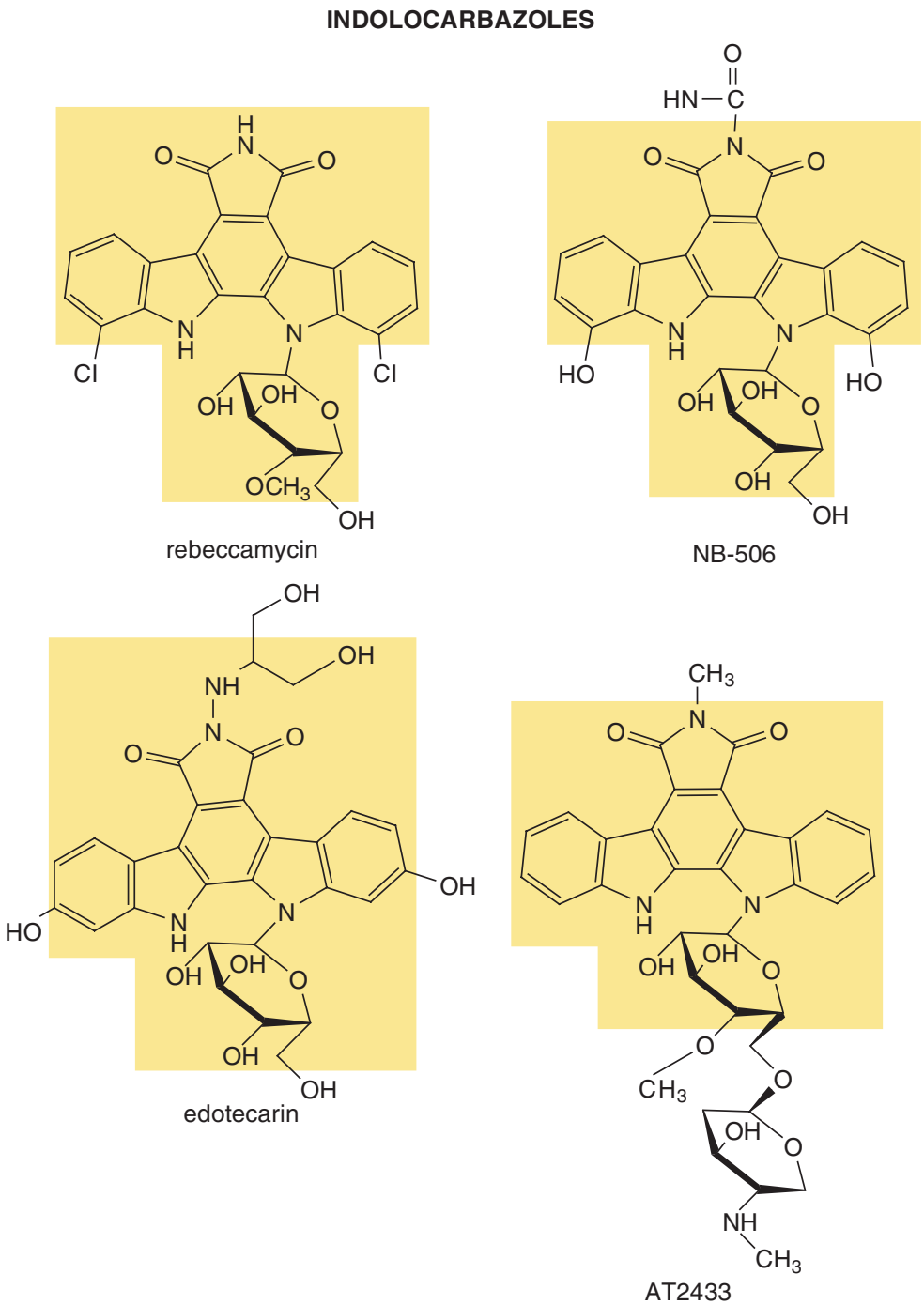


Fig. 2.33 Structures of indolocarbazoles. The common core structure, comprising the bulk of the drug molecules in this class, is highlighted in *yellow*

different DNA sequences than camptothecin or NB-506. The drug stabilizes the enzyme/DNA complex and enhances single strand DNA cleavage at C/T-G sites, resulting in inhibition of DNA reduplication and suppression of tumor cell proliferation. Endotecarin may have potent anti-tumor activity in the mono-therapy of colorectal cancer, and it is under study for the treatment of locally advanced breast cancer and glioblastoma. A recommended dose is 13 mg/m^2 intravenously every 3 weeks.

Pharmacokinetics Edotecarin has a moderate plasma clearance (45–53 L/h/m^2) and a large volume of distribution (650–850 L/m^2). It is largely eliminated as unchanged parent molecule via biliary excretion (Yin et al. 2005).

Adverse Effects The dose limiting toxicity is hematologic, comprising neutropenia, leukopenia and anemia.

Drug Resistance Edotecarin is subject to efflux from cells via ATP-binding cassette transporters (ABC transporters). Drug resistance may be acquired by over-expression of

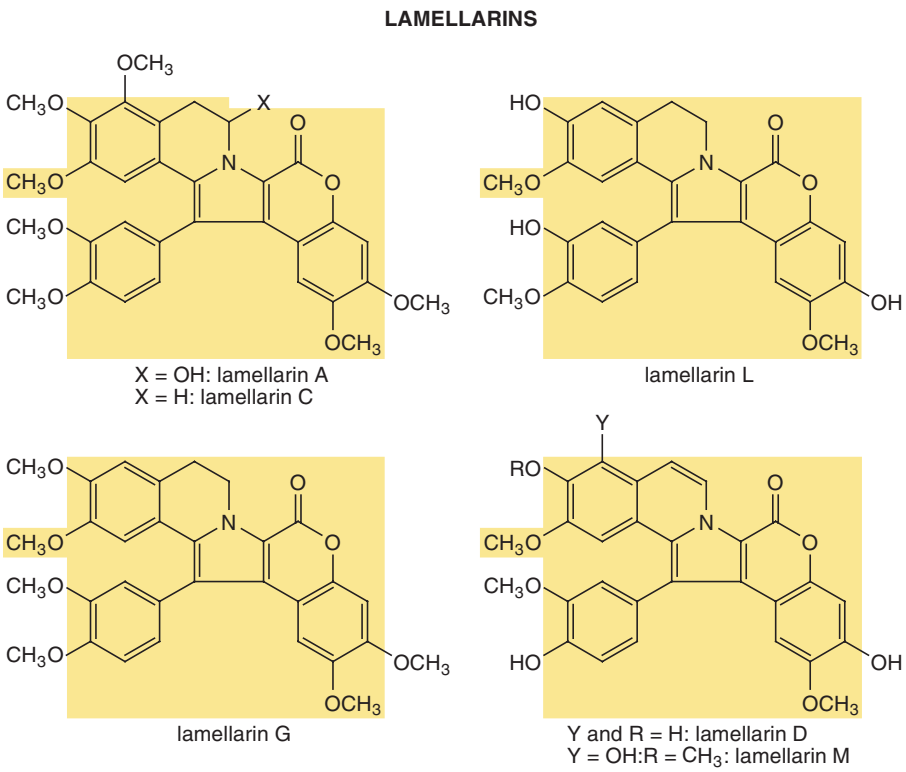


Fig. 2.34 Structures of lamellarins. The common core structure, highlighted in *yellow*, comprises the bulk of the molecules

ABCP (BCRP/MXR), which pumps the drug out of the cancer cells.

The indolocarbazole AT2433 was originally isolated from Actinomadura melliaura. There are five forms, AT2433-A1, AT2433-A2, AT2433-B1, its diastereoisomer iso-AT2433-B1, and AT2433-B2. It contains a unique disaccharide (aminodideoxypentose disaccharide, consisting of a methoxyglucose and the amino sugar subunit 2,4-dideoxy-4-methylamino-l-xylose) and an asymmetrically halogenated *N*-methylated aglycon.

Lamellarins Lamellarins were isolated from the marine mollusk Lamellaria in 1985, and have subsequently been identified in various ascidians (Fig. 2.34). More than 30 lamellarins have been produced since, some of which display equally potent cytotoxic activities against both multidrug resistant tumor cells and their corresponding non-resistant cells.

Lamellarin-D (LAM-D) is a hexacyclic marine alkaloid initially isolated from a prosobranch mollusk of the genus Lamellaria (Facompré et al. 2003). It bears a 6H-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-one pentacyclic planar chromophore. Lamellarin-D recognizes structural elements of the Topoisomerase 1/DNA covalent complex different from those recognized by camptothecin.

LAM-501 is a synthetic 5,6-dehydro analog of lamellarin-D. It has a non-planar structure. LAM-501 does not bind to DNA and fails to inhibit Topoisomerase 1.

Indenoisoquinolines The indenoisoquinolines selectively trap Topoisomerase 1/DNA cleavage complexes (Cushman and Cheng 1978) in a manner that is more persistent than the complexes induced by camptothecins. The indenoisoquinolines target different genomic sites (break DNA at different sequences) than camptothecins and therefore may potentially exhibit a different spectrum of anti-cancer activity. Unlike camptothecins, indenoisoquinolines are not subject to inactivation by lactone hydrolysis at physiological pH. They are seldom substrates for the ABCG2 multi-drug efflux pump that confers resistance to irinotecan and topotecan.

The indenoisoquinoline NSC314622 (Fig. 2.35) was identified in 1978 as a potential Topoisomerase 1 inhibitor by analysis from 48-h cytotoxicity screening of the NCI panel of 60 cell lines, where it had moderate activity. The second lead compound, MJIII-65 (NSC 706744) reversibly traps Topoisomerase 1/DNA cleavage complexes. It is not a DNA intercalator by itself, as it does not unwind DNA in the absence of Topoisomerase 1. MJ-III-65 remains active against the camptothecin resistant Topoisomerase 1 mutants Asn722Ser and Arg364His. However, the poor solubility of MJ-III-65 impaired its further development.

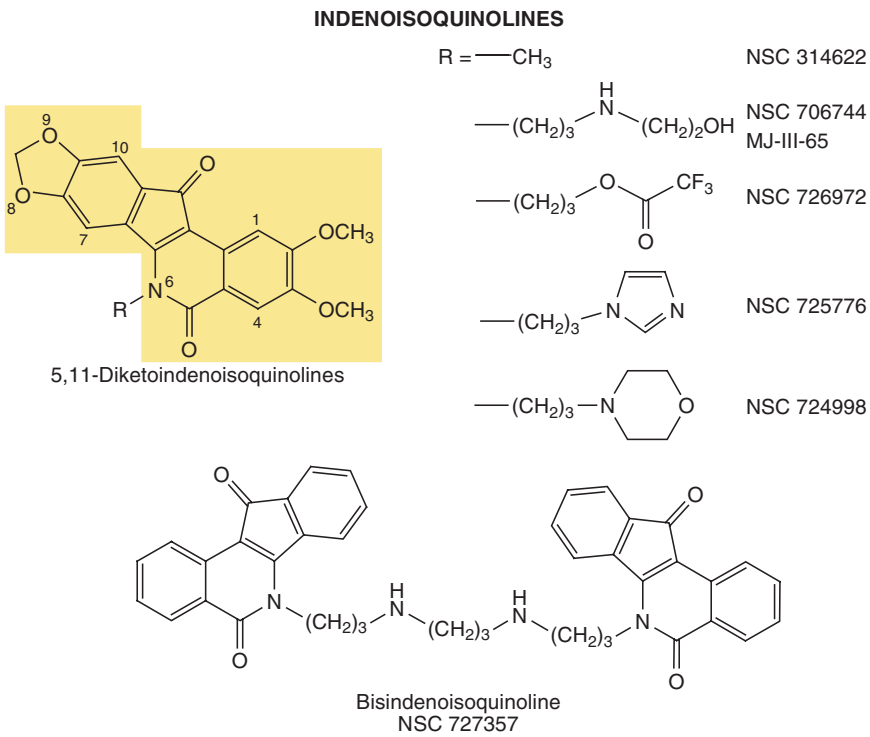


Fig. 2.35 Structures of indenoisoquinolines. The common core structure for various lead compounds is highlighted in *yellow*. Bisindenoisoquinoline comprises two indenoisoquinoline moieties bridged by a linker

Lead compounds under investigation are indimitecan (NSC 725776) and indotecan (NSC 724998). The agents achieve concentration dependent foci formation of the Histone γ-H2A.X, which is a biochemical indicator of DNA double strand breaks, at pharmacologically relevant doses.

The dimeric indenoisoquinoline NSC 727357 induces Topoisomerase 1 cleavage complexes at specific DNA sites. NSC 727357 also induces a limited number of Topoisomerase 2/DNA cleavage complexes. In contrast to the effect of other Topoisomerase 1 inhibitors, cells treated with NSC 727357 display arrest of cell cycle progression in G_1 with no significant inhibition of DNA synthesis following a short exposure to the drug. The cytotoxicity of NSC 727357 is only partially dependent on Topoisomerase 1 and P53, indicating that this drug has additional targets.

Others Ecteinascidin-743 (trabectedin, ET-743) <Yondelis> is produced by the Caribbean tunicate Ecteinascidia turbinate. The drug binds tightly to Topoisomerase 1, with which it produces cleavage complexes. It also alkylates DNA and may induce the production of superoxide near the DNA strand, resulting in DNA backbone cleavage and cell apoptosis. The compound has activity against a variety of soft tissue sarcomata and is in clinical trials for breast, ovarian, and prostate cancers.

Camptothecin/Topoisomerase 1/DNA complexes inhibit reduplication and trigger apoptosis. Camptothecin also inhibits RNA synthesis.

Irinotecan is an S phase specific agent.
Camptothecins exist in a pH dependent equilibrium between their active lactone and inactive ring-opened conformations.
The stability of the lactone ring at physiological pH is a determinant of activity for all camptothecin analogs.
Topotecan and irinotecan are substrates for the efflux pump ABCB1. Camptothecins are substrates for ABCG2.
Indolocarbazoles with one glycosidic linkage are Topoisomerase inhibitors, indolocarbazoles with two glycosidic bonds are Protein Kinase inhibitors.
The sugar moieties of indolocarbazoles increase their water solubility.
Indolocarbazoles are substrates for efflux pumps, which may constitute a mechanism of drug resistance.

Lamellarins may be second-line-of-defense drugs for multidrug resistant tumor cells.

Indenoisoquinolines trap Topoisomerase 1-DNA cleavage complexes in unique genomic sites.
Indenoisoquinolines are seldom substrates for the multi-drug resistance efflux pumps.
Bisindenoisoquinolines induce Topoisomerase 1-DNA cleavage complexes and Topoisomerase 2-DNA cleavage complexes. They lead to cell cycle arrest in G1.

2.3.2 Topoisomerase 2 Inhibitors

Type 2 Topoisomerases are enzymes that change the topology of DNA by introducing transient double strand breaks to from a cleavage complex, through which other DNA strands are passed. These 2-fold symmetric enzymes cleave a pair of opposing phosphodiester bonds four base pairs apart.

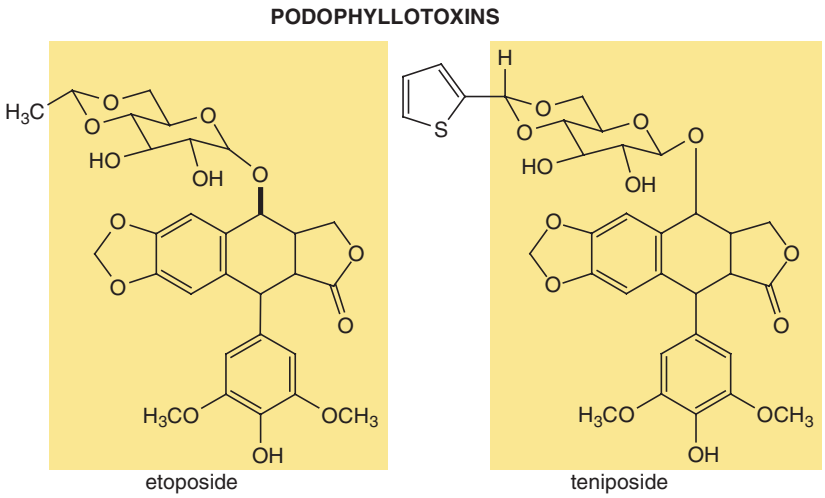


Fig. 2.36 Structures of podophyllotoxins. The common core is highlighted in *yellow*. The drug structures differ in one position

Topoisomerase 2 inhibitors promote the formation of DNA lesions by increasing the steady state level of the cleavage complex.

Podophyllotoxins Podophyllotoxin (5,8,8a,9-Tetrahydro-9-hydroxy-5-(3,4,5-trimethoxylphenyl) furo[3′,4′:6,7] naphtho[2,3,d]-1,3-dioxol-6(5aH)-one, podofilox) is a plant derived lignin that is present in the mandrake roots of the American mayapple (podophyllum peltatum) and in the Indian podophyllum hexandrum royle. Podophyllum plants were used as cathartics for centuries. Podophyllotoxin <Oclassen's Condylox, Condyline, Warticon> is used for the treatment of genital warts[35]. It constitutes the parent molecule for the cytostatic drugs etoposide and teniposide (Fig. 2.36).

Etoposide (4′-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-D-glucopyranoside]) <Etopophos, Vepesid, VP-16> is a semi-synthetic derivative of podophyllotoxin. The drug exerts its anti-neoplastic properties by binding to and inhibiting Topoisomerase 2, which prevents the religation of cleaved DNA molecules, resulting in the accumulation of strand breaks, the inhibition of DNA reduplication, and apoptotic cell death. It acts primarily in the G_2 and M phases of the cell cycle. Etoposide is used in combination chemotherapy, mainly to treat refractory testicular cancer following unsuccessful standard treatment and in first-line treatment for small cell lung cancers. It also has use in the treatment of chorionic carcinoma, Kaposi sarcoma, malignant melanoma, and lymphomata. A phosphate salt improves the drug properties because the water solubility of etoposide phosphate lessens the potential for precipitation following dilution and during intravenous administration. Etoposide is administered intravenously or orally as liquid capsules. Standard dosing covers the range of 5–100 mg/m^2/day.

Pharmacokinetics On intravenous administration, the disposition of etoposide follows a biphasic process with a distribution half-life of about 1.5 h and a terminal elimination half-life of 4–11 h. In the blood, etoposide is 97% protein bound. The cytotoxicity of the prodrug etoposide phosphate is lower than that of etoposide, which may be due to the necessity for conversion to the active moiety by dephosphorylation. *O*-demethylation of the etoposide dimethoxyphenol ring occurs through the CYP450 3A4 pathway to produce the corresponding catechol. The hydroxy acid metabolite 4′-demethylepipodophyllic acid-9-(4,6-*O*-(R)-ethylidene-β-D-gluco-pyranoside) is formed by opening of the lactone ring. It enters the blood, likely as the trans isomer. Within 5 days, 55% of the injected dose is excreted in the urine and 45% in the feces. The hydroxy acid metabolite, as well as glucuronide or sulfate conjugates of etoposide are excreted renally.

Individuals with low Albumin have an elevated fraction of free drug, which may represent an increased risk for etoposide associated toxicities or may lead to accelerated renal excretion. In patients with impaired renal function, initial dose modification should be considered based on creatinine clearance.

Adverse Effects The most frequent adverse effects is leukopenia. The occurrence of a platelet count below 50,000/mm^3 or an absolute neutrophil count below 500/mm^3 is an indication to withhold further therapy until the blood counts have sufficiently recovered. Nausea and vomiting are the major gastrointestinal toxicities. Their severity is generally mild to moderate with treatment discontinuation required in 1% of patients. Nausea and vomiting can usually be controlled with standard anti-emetic therapy. Major additional adverse effects include reversible hair loss (45% of patients), anorexia, and diarrhea. Due to its mechanisms of action through genetic damage, etoposide may increase the

[35] Genital warts, which are caused by the human papillomavirus (HPV), are associated with an increased risk for squamous cell carcinomata of the genitals.

risk of developing leukemia. The drug causes fetal damage and birth defects, and hence should not be used by pregnant or nursing women.

Hypersensitivity reactions can occur in 0.5–2% of patients and manifest as chills, fever, shortness of breath, wheezing, or low blood pressure. In such cases, the drug needs be stopped and the reaction treated with dexamethasone, diphenhydramine, and if necessary epinephrine. For mild reactions, the infusion may be restarted at a slower rate along with intravenous fluids and gradually increased with blood pressure monitoring.

Drug Interactions In combination regimens, the etoposide dosage needs to be modified to take into account the myelosuppressive effects of other drugs. Similarly, the effects of prior radiation or chemotherapy, which may have compromised the bone marrow reserve, can mandate dose reduction. Caution must be exercised when administering etoposide phosphate with drugs that inhibit Phosphatase activities (such as levamisole hydrochloride). High-dose cyclosporin A, resulting in concentrations above 2000 ng/mL, administered with oral etoposide leads to an 80% increase in etoposide exposure with a 40% decrease in total body clearance compared to etoposide alone. Phenylbutazone, sodium salicylate, and aspirin can displace Albumin bound etoposide. The use of cisplatin therapy is associated with reduced total body clearance of etoposide. Prior use of cisplatin may also result in a decrease of etoposide total body clearance in children.

Teniposide <Vumon, VM-26, EPT> is a semi-synthetic derivative of podophyllotoxin. It forms a ternary complex with Topoisomerase 2 and DNA, resulting in dose dependent single and double stranded breaks, as well as DNA/protein cross-links. This agent acts in the late S or early G_2 phase of the cell cycle. Teniposide is used to treat lymphomata and leukemias, mainly childhood acute lymphocytic leukemia.

Adverse Effects Common adverse effects include nausea, vomiting, diarrhea, and thinned or brittle hair. A major problem with teniposide is the lowered count of white blood cells 1–2 weeks after treatment. Although they mostly return to normal after 3–4 weeks, the decreased count puts the patient at risk for infections. The time course for platelets is similar. When given as an intravenous infusion over 30–60 min, teniposide causes a burning sensation if it leaks under the skin.

Quinoxalines Chloroquinoxaline sulfonamide (chlorosulfaquinoxaline, CQS) (NSC 339004) is a chlorinated heterocyclic sulfanilamide (Fig. 2.37). It inhibits Topoisomerase 2α and Topoisomerase 2β, thereby causing DNA double strand breaks and accumulation of unrepaired DNA, which result in apoptosis (Gao et al. 2000).

Adverse Effects This agent exerts lymphotoxicity by inhibiting lymphocyte activation in a cell cycle specific manner.

The synthetic quinoxaline phenoxypropionic acid derivative XK469 has structural similarity to chloroquinoxaline sulfonamide. The agent selectively inhibits Topoisomerase 2β (Gao et al. 1999) by stabilizing the enzyme/DNA intermediates, in which Topoisomerase subunits are covalently linked to DNA through 5-phospho-tyrosyl linkages. The formed complexes interfere with DNA repair and reduplication, as well as with RNA synthesis. XK469 possesses solid tumor selectivity and activity against multi-drug resistant cancer cells. The racemic form XK469R is more water soluble and active than either of the pure isomers R(+)XK469 and S(−)XK469.

Antibiotics Some of the anti-neoplastic antibiotics also inhibit Topoisomerase 2. They include several anthracyclines (daunorubicin, doxorubicin, epirubicin, detorubicin, carubicin, idarubicin, RTA 744), anthracenediones (mitoxantrone), anthrapyrazoles (teloxantrone, piroxantrone), dactinomycin, fostriecin, and streptonigrin (see Sect. 2.2.).

Others Asulacrine isethionate (amsalog, 9-[2-Methoxy-4-(methylsulfonylamino)phenylamino]-N,5-dimethyl-4-acridinecarboxamide 2-hydroxyethanesulfonate) (NSC-343499, CI-921) (Fig. 2.38)is a synthetic derivative of acridine carboxamide and an amphiphilic analog of amsacrine. It inhibits Topoisomerase 2, thereby blocking DNA reduplication and RNA synthesis.

Razoxane (ICRF 159, ICI 59118) <Razoxin> is a bisdioxopiperazine and a derivative of the chelating agent EDTA. It constitutes a racemic mixture, of which dexrazoxane (used for cardioprotection in anthracyclin treatment)

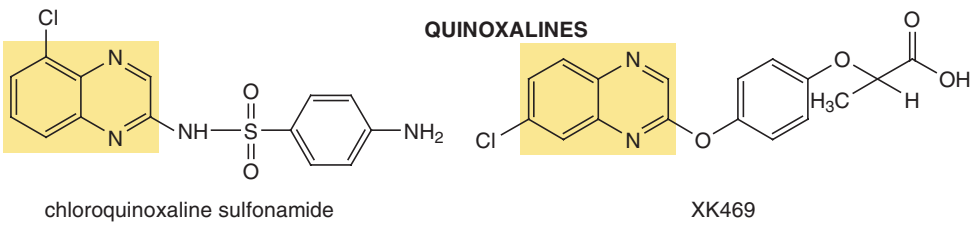


Fig. 2.37 Structures of quinoxalines. The quinoxaline structure is highlighted in *yellow*

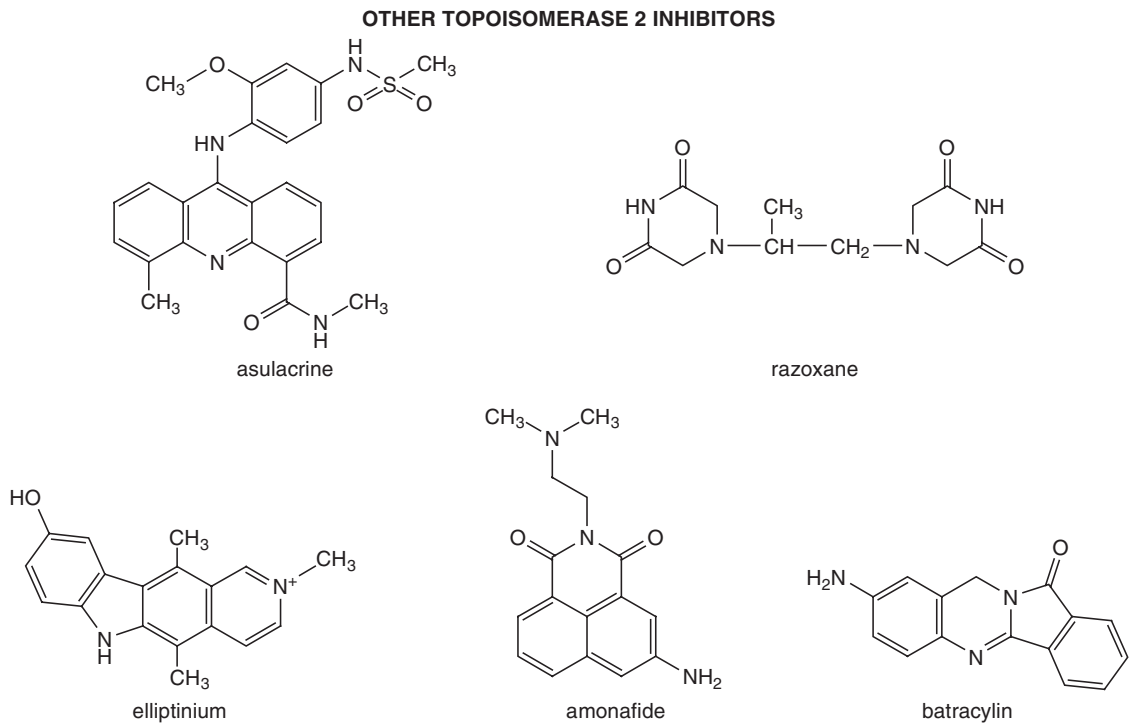


Fig. 2.38 Structures of other Topoisomerase 2 inhibitors

is the S(+)-enantiomer. Razoxane inhibits Topoisomerase 2 without inducing DNA strand breaks, thereby inhibiting DNA synthesis and inducing cytotoxicity. The drug also exhibits anti-angiogenic activity.

Adverse Effects Secondary malignancies, primarily acute myeloid leukemia, may arise in patients treated chronically with oral razoxane. The risk is high in patients, whose total cumulative dose of razoxane ranges 25–500 g over 40–320 weeks.

Drug Interactions The use of dexrazoxane concurrently with the initiation of fluorouracil, doxorubicin, and cyclophosohamide (FAC) therapy of breast cancers interferes with the anti-tumor efficacy of the regimen. Dexrazoxane should only be used in those patients who have received a cumulative doxorubicin dose of 300 mg/m^2 and are continuing with doxorubicin therapy, not at the onset of FAC treatment.

Elliptinium acetate (9-hydroxy-2,5,11-trimethyl-6H-pyrido[4,3-b]carbazolium acetate, 9-HME) <Celiptium> was developed in the 1970s. It is a derivative of the alkaloid ellipticine, isolated from species of the plant family Apocynaceae, including Bleekeria vitensis. As a Topoisomerase 2 inhibitor and intercalating agent, elliptinium stabilizes the cleavable complex of Topoisomerase 2 and induces DNA breakages, thereby inhibiting DNA reduplication and RNA synthesis. The agent has anti-tumor activity against breast, kidney, and other cancers (Buzdar et al. 1990).

Adverse Effects Although the drug lacks myelosuppressive toxicity, other adverse effects, including hemolytic anemia and renal failure, limit its clinical use. Hence, semisynthetic analogs with molecular modifications have been developed to reduce the severe dose limiting toxicities of the parent compound.

Amonafide dihydrochloride (benzisoquinolinedione, nafidimide) (AS1413) <Quinamed> , an imide derivative of naphthalic acid, is a Topoisomerase 2 inhibitor and intercalating agent that is in clinical trials for the treatment of cancer. It also affects a number of targets in the EGFR pathway. The drug has modest anti-tumor activity against prostate and small cell lung cancer.

Pharmacokinetics Amonafide is acetylated to N-acetylamonafide. Therefore, the rate of metabolism of the drug is strongly determined by the genotype of NAT2. Fast acetylators have a much higher overall response rate than slow acetylators. Dosing should account for the genotype of the patient.

Adverse Effects The dose limiting toxicity is reversible myelosuppression. Non-hematologic toxicity is mild, consisting mostly of nausea and vomiting, which is easily controlled with anti-emetics.

Batracylin (isoindolo[1,2-b]quinazolin-12(10*H*)-one) (NSC 320846) was developed at the U.S. National Cancer Institute as an anti-cancer agent, but was dropped in 1989 and resumed much later. Topoisomerase 2 inhibition by the

compound is not dependent on ATP, which implies a favorable characteristic for treating hypoxic tumors.

Pharmacokinetics Oral absorption is variable. Drug activation to the highly toxic acetylated form, *N*-acetyl-batracylin is catalyzed by *N*-Acetyl Transferase 2 (NAT2). In patients with the fast-metabolizing NAT2 allele dose reduction may be required to avoid toxicity.

Drug Resistance Batracylin is not a substrate for ABCB1, thus eliminating this mechanism for resistance.

Topoisomerase 2 inhibitors promote the formation of DNA lesions by increasing the steady state level of the cleavage complex.
Podophyllotoxins (etoposide and teniposide) arrest cells in G2/M phase. They may cause DNA single and double strand breaks.
Quinoxalines may be active in multi-drug resistant cancers.
Other Topoisomerase 2 inhibitors include asulacrine isethionate, razoxane, elliptinium, amonafide, and batracyclin.

Treatment of Embryonic Tumors

1. **Seminomatous tumors**

The therapy regimen depends on the stage of the tumor. External beam radiation is used for stage I and non-bulky stage 2 disease. Over a 3-week period, 2500 cGy are administered in a "hockey-stick field", including the para-aortic, para-caval, bilateral common iliac, and external iliac nodal regions. In some cases, the radiation field can be reduced only to the para-aortic area. As an alternative to radiotherapy or as a follow-up to radiation, single agent carboplatin protocols are under study. The long-term success of carboplatin therapy is unknown and should be considered experimental (Oliver et al. 2005).

For stage 2 bulky or stage 3 disease, radical orchiectomy and metastatic workup, are followed by administration of four cycles of chemotherapy without radiation therapy. The combination of bleomycin, etoposide, and cisplatin (BEP) is standard. Ongoing clinical trials are evaluating the omission of the 4th cycle, or of bleomycin, in patients with favorable prognosis. For high risk and salvage cases, alternative regimens may use ifosfamide and vinblastine with dose escalation.

2. **Non-seminomatous tumors**

Metastatic non-seminomatous germ cell tumors (NSGCT) are highly sensitive to cisplatin based chemotherapy, with cure rates of approximately 80% for advanced disease and nearly 100% for early stage disease. Risk-adapted protocols are available to tailor treatment regimens toward patients with good, moderate, or poor risk factors.

Despite a high cure rate in men with testicular cancer, some in the poor prognosis group require aggressive treatment. Poor prognosis non-seminomatous germ cell tumors are defined as those with high tumor markers, non-pulmonary visceral metastases, or a mediastinal primary site at presentation. When treated with standard chemotherapy regimens, such as bleomycin, etoposide and cisplatin (BEP), cure rates below 50% are achieved. Some strategies aimed at improving results include the use of multi-agent regimens (e.g. POMB/ACE: cisplatin, vincristine <Oncovin>, methotrexate, bleomycin, actinomycin D, cyclophosphamide, etoposide) and intensive-induction chemotherapy (e.g. CBOP/BEP: carboplatin, bleomycin, vincristine <Oncovin>, cisplatin followed by BEP). Other drugs, such as ifosfamide, gemcitabine, oxaliplatin, and paclitaxel have been added to therapeutic strategies in first-line and salvage treatment of poor-prognosis non-seminomatous germ cell tumors (Sirohi and Huddart 2005).

Choriocarcinoma Historically, methotrexate <Trexall> was the first drug treatment to force a cancer into remission when it was used for treating choriocarcinoma.

Choriocarcinoma is highly sensitive to chemotherapy, which is therefore the main type of treatment. The cure rate, even for metastatic choriocarcinoma, is around 90–95%. However metastatic disease to the kidneys or the brain is usually fatal. Hysterectomy and radiation therapy are rarely needed. In the case of a molar pregnancy (gestational trophoblastic disease, hydatiform mole), prophylactic chemotherapy (methotrexate and actinomycin D) at the time of molar evacuation reduces the frequency of postmolar tumors.

- Single agent chemotherapy with either methotrexate (MTX-FA: intramuscular methotrexate 1.0 mg/kg days 1, 3, 5, and 7, intramuscular or oral folinic acid 0.1 mg/kg on days 2, 4, 6, and 8; 5-day MTX: intravenous or intramuscular methotrexate 0.4 mg/kg/day for 5 days; Pulse MTX: intramuscular methotrexate 50 mg/m^2 weekly) or actinomycin D (5-Day Act-D: intravenous actinomycin D 12 μg/kg/day for 5 days; Pulse Act-D: intravenous actinomycin D 1.25 mg/m^2 every 2 weeks) is the preferred treatment in patients with stage 1 gestational trophoblastic tumors, who desire to retain fertility.
- Patients with resistance to single agent chemotherapy are treated with combination chemotherapy of methotrexate, actinomycin D, and cyclophosphamide (MAC), or etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine <Oncovin> (EMA-CO), or surgical therapy. MAC is pre-

ferred as the initial combination chemotherapy in these patients because etoposide is associated with an increased risk for secondary tumors.
- While patients with low-risk stages 2 and 3 are treated with primary single agent chemotherapy with methotrexate or actinomycin D, patients with high-risk disease are managed with primary combination chemotherapy with EMA-CO. Patients with disease resistant to single agent chemotherapy are then treated with EMA-CO. Patients with disease resistant to EMA-CO may be treated by modifying that regimen by substituting cisplatin and etoposide on day 8, and escalating the dose of methotrexate infusion to 1 g/m^2 (EMA-CE).
- All patients with stage 4 disease are managed with primary EMA-CO combination chemotherapy. In the presence of cerebral metastases, the methotrexate dosage in the infusion is increased to 1 g/m^2. Patients with disease resistant to EMA-CO may then be treated with EMA-CE (Kufe et al. 2003).
- Choriocarcinoma in primary locations other than the placenta is rare. Very rarely it arises in testicles. These tumors are aggressive and highly resistant to chemotherapy. The same is true of choriocarcinoma of the ovaries.

Yolk sac tumor Primary chemotherapy may be initiated with VAC (vincristine, actinomycin D, cyclophosphamide), PVB (cisplatin, vinblastine, bleomycin), or BEP (bleomycin, etoposide, cisplatin). Therapeutic effect can be monitored with the levels of α-Fetoprotein (AFP) in the blood.

Teratoma The treatment of choice is complete resection. Teratomata are normally well encapsulated and non-invasive, hence they are relatively easy to remove from surrounding tissues. For malignant teratomata, surgery is followed by chemotherapy.

Desmoplastic small round cell tumor is a form of teratoma. Although there is no standard protocol for the disease, some patients respond to high dose chemotherapy (P6 Protocol) (Donadio et al. 2003), maintenance chemotherapy, surgery, and radiation therapy. Other treatment options include hematopoietic stem cell transplantation or intraperitoneal hyperthermic chemoperfusion. The tumor may be responsive to irinotecan.

2.4 Anti-Metabolites

Folic acid (folacin, vitamin B9) was discovered and synthesized by Lucy Wills in 1937. Previously, nutritional research in the early part of the twentieth century had identified a factor present in green leafy vegetables that was important for bone marrow function. It turned out to be folic acid, a vitamin crucial for DNA metabolism. However, the same compound administered to children with acute lymphoblastic leukemia (ALL) seemed to exacerbate their cancer (Farber 1949). Sidney Farber (1903–1973) in Boston recognized that folic acid stimulated the proliferation of leukemia cells. In one of the first examples of rational anti-cancer drug design, he collaborated with Harriett Kilte and Lederle Laboratories of the American Cyanamid Company to devise folate analogs. In November 1947, when a sufficient amount of aminopterin became available[36], Farber administered it to 16 children with acute leukemia and achieved remissions in ten of them. He demonstrated that aminopterin blocked a critical chemical reaction needed for DNA reduplication (Farber et al. 1948). Aminopterin was the predecessor of methotrexate (developed by Seeger and colleagues at Lederle Laboratories in 1948 and called amethopterin), which in 1956 became the first compound cure of metastatic cancer, when it was used by Roy Hertz (1909–2002) and Min Chiu Li (-1980) to treat two cases of choriocarcinoma. The principal architect of the treatment, using methotrexate in an unusual way for the time, was Min Chiu Li. As a sign of the times, after the first two patients went into remission, they were presented at National Cancer Institute Grand Rounds under the title “the spontaneous regression of cancer” with the speaker being Gordon Zubrod (Li’s superior and at that time a detractor of chemotherapy). Li was told if he persisted in using his radical treatment, he would have to forfeit his position at the newly opened clinical center. He continued and was asked to leave. After his initial success with methotrexate, Li went on to develop the first effective combination chemotherapy programs for metastatic testicular cancer (DeVita and Chu 2008). In 1972, when the Lasker Prize was awarded to investigators who had contributed to studies of

[36] Aminopterin was synthesized by Yellapragada Subbarao at Lederle Laboratories. Subbarao had been denied tenure at Harvard, despite his discovery of the roles of phosphocreatine and adenosine triphosphate (ATP) in muscular activity. He joined Lederle, where he developed a method to synthesize folic acid, and with considerable input from Farber, he produced anti-folates. Subbarao remained obscure because he did not actively publicize his work or himself. It was said that some of the nucleotides isolated by him at Harvard Medical School had to be rediscovered years later by other workers because his superior, Cyrus Fiske, apparently out of jealousy, had not let Subbarao’s contributions see the light of the day.

the cure of gestational choriocarcinoma, Li had to share his part of the prize with the person who had discharged him.37

The development of anti-purines was based on the hypothesis that nucleic acid synthesis in cells might be stopped by nucleic acid base analogs. In the early 1940s, very few chemists were interested synthesizing nucleic acid bases, so that relevant references dated back to the manuscripts of Emil Fischer (1852–1919) in the old German literature. Gertrude Belle Elion (1918–1999) was a medicinal chemist interested in the pharmacology of anti-purines. She had enrolled in City College of New York at the age of 15 (importantly, it was tuition-free as her father had been bankrupted by the stock market crash), and earned her chemistry degree from Hunter College at age 19. Unable to obtain a graduate research position due to gender discrimination during the great depression, she worked as a laboratory assistant and a high school chemistry teacher. During that period, her fiancé died from endocarditis. Elion never married and it seems that her love was entirely devoted to medicinal chemistry. She finally took a position as an assistant to George H. Hitchings (1905–1998) at the pharmaceutical company Burroughs Wellcome & Company. From 1942 on, research by Hitchings and Elion tested many purine analogs and corroborated that it was possible to treat cancer with anti-metabolite compounds. By 1951, they had synthesized and tested over 100 purine analogs. Using one of them, 6-mercaptopurine, Joseph N. Burchenal (1912–2006) achieved a high percentage of complete remissions in childhood leukemias. Later, Sullivan and co-workers improved on the efficacy by infusing methotrexate intra-arterially into solid tumors with systemic protection by intramuscular citrovorum factor.

Anti-metabolites have affinity to the enzymes of nucleic acid biosynthesis. They may be incorporated into cellular polymers and act as false building blocks. Alternatively, they may compete with the normal monomeric components of nucleic acids for essential synthetic enzymes and inhibit them. Because this group of drugs compromises the synthesis of DNA and RNA in healthy and transformed cells alike, they are more effectively used in localized therapy than in systemic therapy. However, only a few types of cancer are accessibly for this treatment modality.

Anti-metabolites may act as false building blocks for nucleic acids.
Anti-metabolites may inhibit essential synthetic enzymes for nucleic acids.

[37] The clinical use of the anti-metabolite parent drug aminopterin discontinued in favor of the successor drug methotrexate. In some parts of the world, aminopterin is still used as a rodent poison in farming. It is a suspect in a 2007 pet poisoning incident that led to a massive recall of pet food by Menu Foods, U.S. The wheat gluten used as a base for the preparation of these foods had been imported from China. It was likely contaminated.

Due to their mechanism of action, anti-metabolites inhibit cell proliferation in S phase.

2.4.1 Anti-Folates

Folic acid (pteroylglutamic acid) dependent oxidation or reduction reactions of single carbons are important in biosynthetic pathways leading to the production of DNA, RNA, and membrane lipids. Dietary folates must be chemically reduced to their tetrahydro-forms, bearing four hydrogens on the pteridine ring, to be active. The enzyme responsible for this reduction is Dihydrofolate Reductase (DHFR). Folic acid in its fully reduced form serves as a carbon carrier for transfer reactions that are required in purine and thymidylate synthesis, and consecutively in the formation of DNA and cell division. The folic acid cycle also supports the synthesis of certain amino acids, such as methionine (Fig. 2.39). Natural folates circulating in the blood have a single glutamic acid group, but within cells they are converted to poly-glutamates, which are more efficient cofactors and are preferentially retained inside the cells. Hence, folate is an important biosynthetic component for proliferating cells.

The cytotoxic activity of anti-folates is mainly due to their ability to inhibit several folate dependent enzymes involved in DNA synthesis. Old generation anti-folates comprise Dihydrofolate Reductase inhibitors and Thymidylate Synthase inhibitors. The new generation of anti-folates includes Glycinamide Ribonucleotide Formyl Transferase inhibitors and Dihydropteroate Synthase inhibitors (Fig. 2.40).

The intracellular antifolate concentration is important for the inhibition of specific target enzymes. It depends on

- uptake: antifolate membrane transport into cells, typically via the Reduced Folate Carrier (RFC)
- retention: the conversion to poly-L-γ-glutamate forms catalyzed by Folylpoly-γ-Glutamate Synthetase (FPGS)
- elimination: cellular hydrolysis of antifolate poly- L-γ-glutamates to mono-glutamates and the extrusion of the mono-glutamates through the cell membrane.

Recent synthetic efforts have focused on developing antifolates that are selectively delivered to cancer cells, but not to normal proliferating cells, exploiting differences in the properties of their folate transporters. In another approach, drugs structurally and mechanistically unrelated to folates are linked to and use folic acid as a carrier to be endocytosed by folate receptors, which are abundant on cancer cells, and then released to inhibit their cellular targets (Goldman et al. 2010).

Dihydrofolate Reductase inhibitors Dihydrofolates must be reduced to tetrahydrofolates by Dihydrofolate Reductase

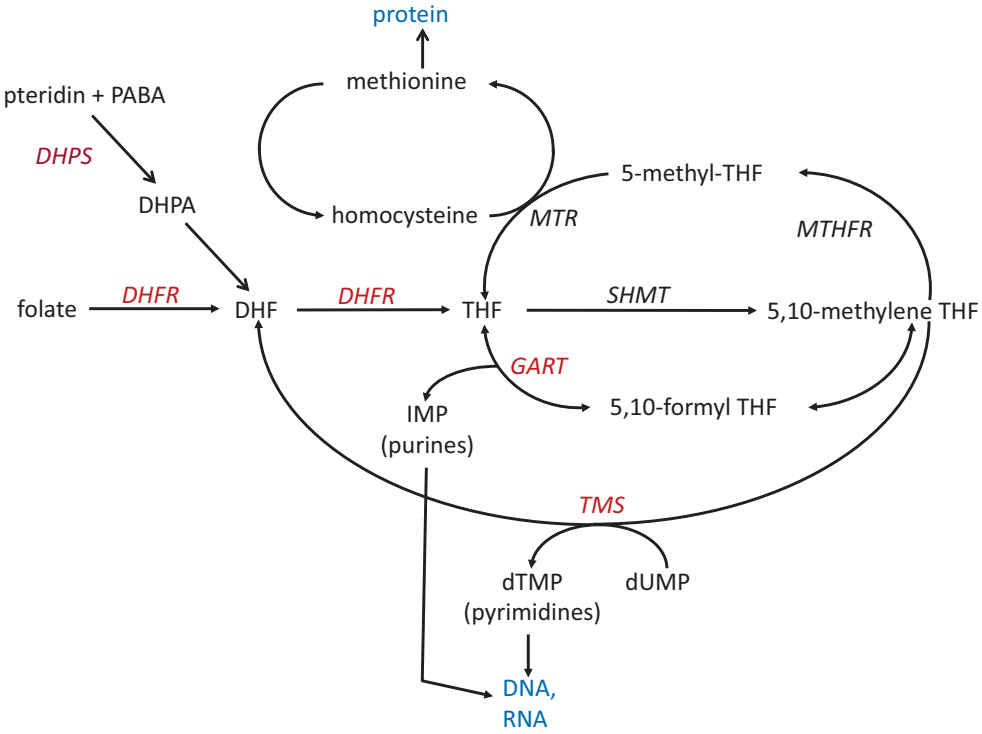


Fig. 2.39 The folate pathway. The synthetic end products DNA, RNA, and protein are shown in *blue*. The enzymes that serve as drug targets for anti-folates are depicted in *dark red*. Enzyme names are written in italics. *PABA* para-aminobenzoate, *DHPS* Dihydropteroate Synthase, *DHPA* dihydropteroate, *DHFR* Dihydrofolate Reductase, *DHF* dihydrofolate, *THF* tetrahydrofolate, *TMS* Thymidylate Synthase, *GART* Glycinamide Ribonucleotide Formyl Transferase, *SHMT* Serine Hydroxyl Methyl Transferase, *MTR* 5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase (Methionine Synthase), *MTHFR* Methylene Tetrahydrofolate Reductase

before they can be utilized as carriers of one-carbon groups in the synthesis of purine nucleotides and thymidylate.

Aminopterin, the first antifolate drug, was used in the United States from 1953 through 1964 for pediatric leukemia. Due to manufacturing difficulties it was discontinued in favor of methotrexate. Aminopterin (4-aminofolic acid, 4-aminopteroylglutamic acid) is a synthetic derivative of pterin. The agent acts as an enzyme inhibitor by competing for the folate binding site of Dihydrofolate Reductase. Its affinity for this enzyme effectively blocks tetrahydrofolate synthesis, resulting in the depletion of nucleotide precursors and inhibition of DNA, RNA, and protein synthesis.

Adverse Effects Adverse effects include nausea and vomiting, weight loss, chills or fever, stomatitis (inflammation of the oral mucosa), pharyngitis, erythematous rashes, gastrointestinal hemorrhage, and renal failure. Exposure to aminopterin during pregnancy is associated with congenital malformations, collectively described as the fetal aminopterin syndrome. It includes short stature, skull anomalies (delayed calvarial ossification, craniosynostosis, clover-leaf skull), hydrocephalus, abnormal auricles, ocular hypertelorism, micrognathia, and cleft palate. The risk of malformation associated with aminopterin exposure is estimated at 50%.

Methotrexate ((S)-2-(4-(((2,4-diaminopteridin-6-yl) methyl)methylamino)benzamido) pentanedioic acid, mtx, amethopterin, MTX) <Trexall> binds to, and competitively and reversibly inhibits the enzyme Dihydrofolate Reductase. The affinity of methotrexate for Dihydrofolate Reductase is about 1000-fold that of folate. This results in the suppression of purine nucleotide and thymidylate synthesis, and subsequently in the inhibition of DNA, RNA, and protein synthesis (Fig. 2.41). The drug is cytotoxic during the S phase of the cell cycle.

- Methotrexate is used for the treatment of acute leukemia in children. In adult leukemias, it is applied in the VAMP (vincristine, amethopterine, 6-mercaptopurine, prednisone) combination. Methotrexate is also administered in combination with other chemotherapeutic agents in the treatment of advanced stage non-Hodgkin lymphomata and advanced mycosis fungoides (cutaneous T-cell lymphoma).
- Regression of solid tumors can be achieved with methotrexate. Indications include gestational choriocarcinoma, chorioadenoma destruens, and hydatidiform mole. This drug is used alone or in combination with other anti-cancer agents in the treatment of breast cancer, epidermoid cancers of the head and neck, and lung cancer (particularly the squamous cell and small cell types).

The agent gained U.S. FDA approval as an oncology drug in 1953. Various preparations and modifications of methotrexate are in use.

- Methotrexate-e therapeutic implant (methotrexate/epi, MTX/epi gel) is an injectable Collagen matrix gel containing the anti-metabolite methotrexate and the

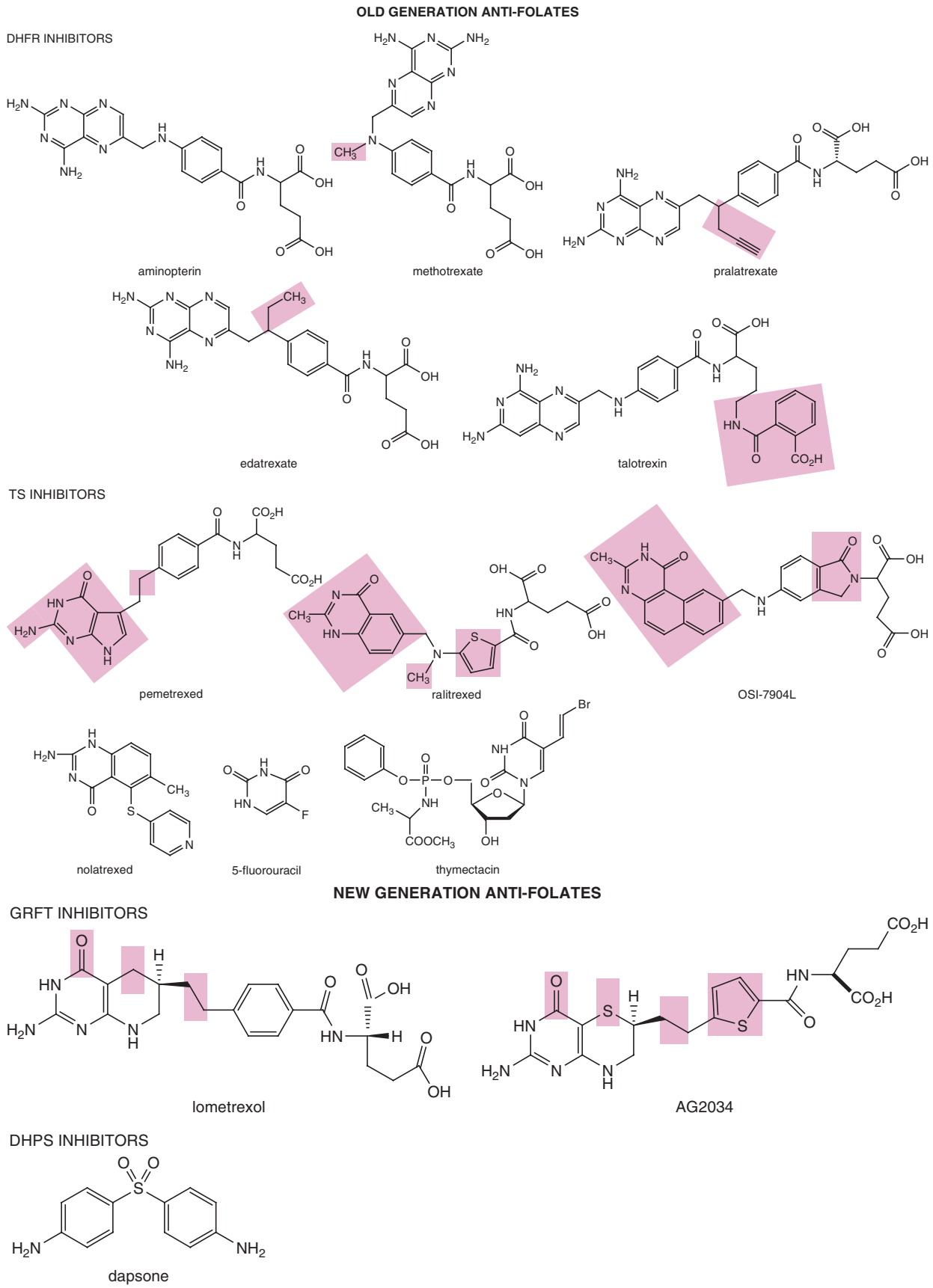


Fig. 2.40 Structures of anti-folates. Old generation drugs inhibit Dihydrofolate Reductase (*DHFR*) or Thymidylate Synthase (*TS*). New generation agents comprise inhibitors of Glycinamide Ribonucleotide Formy7ltransferase (*GRFT*) and Dihydropteroate Synthase (*DHPS*). Many anti-folate drugs are analogs of folate. Within this subgroup, differences in structure from the parent compound aminopterin are shaded in *pink*

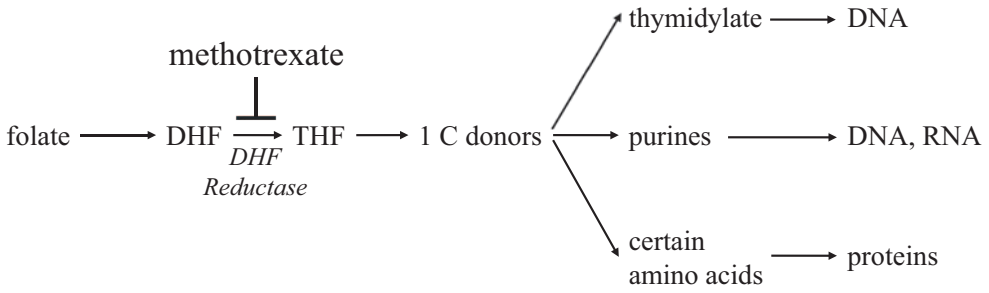


Fig. 2.41 Mechanism of action of methotrexate. The drug (*large font*) inhibits the enzyme Dihydrofolate Reductase (*DHF* Reductase, italics). Consequently, downstream steps of the synthetic pathway are blocked, comprising the synthesis of tetrahydrofolate (*THF*), 1-carbon donors (1 C donors), and the production of essential molecules for cell proliferation

sympathicomimetic agent epinephrine. After intra-tumoral injection, methotrexate inhibits DNA and RNA syntheses. The vasoconstrictor epinephrine in the gel enhances the penetration of methotrexate into the tumor tissue and reduces the dispersion to the surrounding tissues, thereby increasing the local concentration of methotrexate and improving its anti-tumor activity. Intra-tumoral injection of methotrexate combined with epinephrine may increase the chemotherapeutic efficacy and reduce systemic adverse effects compared to systemic administration.

- 3′,5′-Dichloromethotrexate (DCM, *N*-[3,5-dichloro-4-[(2,4-diamino-6-pteridinylmethyl)methylamino]benzoyl] glutamic acid) (NSC 29630) is a chlorinated methotrexate derivative that is inactivated by the liver to the primary metabolic product 4-deamino-4,7-dihydroxy dichloromethotrexate. Most tumors do not metabolize dichloromethotrexate thus allowing the active drug to accumulate. Frequently occurring toxicities include leukopenia, thrombocytopenia, and mucositis. Nausea, vomiting, diarrhea, and an elevation of hepatic enzymes and bilirubin occur less often.
- Trimetrexate glucuronate (TMQ, 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl] quinazoline mono-D-glucuronate) <Neutrexin> is a lipid soluble methotrexate derivative that inhibits Dihydrofolate Reductase. Trimetrexate glucuronate is administered by injection, and must be given with concurrent leucovorin protection to avoid potentially serious toxicities.
- Triazinate is a synthetic dihydrotriazine derivative of methotrexate. It inhibits Dihydrofolate Reductase as well as the transport of folates. Unlike methotrexate, this agent is not converted to polyglutamate forms and may be selectively toxic to methotrexate resistant tumor cells.

Methotrexate can be taken orally or administered by injection (subcutaneous, intramuscular, intravenous, or intrathecal). Although daily preparations are occasionally used, most patients take weekly doses. Numerous treatment protocols involving methotrexate have been applied. For choriocarcinoma and similar trophoblastic diseases, methotrexate is administered orally or intramuscularly in doses of 15–30 mg daily for a 5-day course. Courses are usually repeated 3–5 times as required, with rest periods of 1 or more weeks interposed between courses, until any manifesting toxic symptoms subside. In Burkitt lymphoma, a recommended dosage for stage I-II disease is 10–25 mg/day orally for 4–8 days; for stage III-IV disease, methotrexate is commonly given concomitantly with other anti-tumor agents with methotrexate given in doses of 0.625–2.5 mg/kg daily.

Pharmacokinetics The oral absorption of methotrexate is dose dependent. At doses below 30 mg/m^2, methotrexate is generally well absorbed with a mean bioavailability of about 60 %. The absorption of doses greater than 80 mg/m^2 is significantly less, possibly due to a saturation effect. Peak blood levels are reached within 1–2 h. In leukemic pediatric patients, there is substantial interindividual variability in both, oral bioavailability and time to peak concentration. Factors that decrease the absorption of the drug and reduce its peak concentration include food, oral non-absorbable antibiotics, and rapid transit through the gastrointestinal tract as it occurs in diarrhea, while slower transit time in the gastrointestinal tract from constipation will increase the absorption. Oral methotrexate is metabolized by intestinal bacteria to the inactive 4-amino-4-deoxy-*N*-methylpteroic acid (DAMPA), which accounts for less than 5 % loss of the dose. In contrast, the drug is generally completely absorbed from parenteral routes of administration. After intramuscular injection, peak blood concentrations occur in 30–60 min. In pediatric patients receiving methotrexate for acute lymphocytic leukemia, the terminal half-life ranges 0.5–6 h. Methotrexate does not penetrate the blood-brain barrier in therapeutic amounts, however, high cerebrospinal fluid concentrations of the agent may be attained by intrathecal administration.

At low concentrations, methotrexate competes with reduced folates for the active transport across cell membranes by means of a single carrier mediated process. At concentrations greater than 100 μM, passive diffusion becomes a major pathway, by which effective intracellular concentrations can be achieved. After uptake, methotrexate undergoes hepatic and cancer cell metabolism to polyglutamated forms.

The formation of poly-γ-glutamyl metabolites, catalyzed by Folylpolyglutamate Synthase (FPGS), allows the prolonged retention of methotrexate in various tissues. These metabolites also have very high affinity to Thymidylate Synthase and potentiated inhibitory activity, and they are likely to exert a substantial portion of the drug action. The polyglutamated forms can be converted back to methotrexate by Hydrolases. At therapeutic doses, a small fraction of the drug may be metabolized to 7-hydroxymethotrexate. The aqueous solubility of 7-hydroxymethotrexate is 3–5 fold lower than the parent compound.

Methotrexate clearance rates vary widely and are generally decreased at higher doses. The terminal half-life for methotrexate is approximately 3–10 h for patients receiving low dose anti-neoplastic therapy (less than 30 mg/m^2). For patients receiving high doses of methotrexate, the terminal half-life is 8–15 h. Renal excretion by glomerular filtration and active tubular secretion is the primary mode of elimination, and is dependent on dosage and route of administration (with intravenous administration, 80–90 % of the dose is excreted unchanged in the urine within 24 h). There is limited biliary excretion, amounting to 10 % or less of the administered dose. Enterohepatic recirculation of methotrexate may occur. Delayed drug clearance is one of the major factors responsible for methotrexate toxicity. When a patient has delayed drug elimination due to compromised renal function, third-space effusion, or other causes, the methotrexate blood concentrations may remain elevated for prolonged periods. The extent of adverse effects exerted on healthy tissues may be more dependent on the duration of exposure to the drug than on the peak level achieved.

Adverse Effects In general, the incidence and severity of acute adverse effects are related to dose and frequency of methotrexate administration. There are assays available for the measurement of serum methotrexate levels that allow therapeutic drug monitoring, which also enables individualized dosing based on target levels. The threshold for cytotoxic effects of the agent is approximately 50 nM. Toxicity and efficacy are determined by the time span for which the concentrations remain above this threshold level.

The high doses of methotrexate often used in cancer chemotherapy can cause toxic effects to the rapidly dividing cells of the bone marrow and the gastrointestinal mucosa, resulting in myelosuppression (leukopenia, anemia) and mucositis (gingivitis, pharyngitis, ulcerative stomatitis). The myelosuppression can lead to potentially fatal opportunistic infections (Pneumocystis carinii pneumonia, nocardiosis, histoplasmosis, cryptococcosis, Herpes zoster, Herpes simplex hepatitis, disseminated Herpes simplex). Pericarditis, pericardial effusion, hypotension, and thromboembolic events are possible. Respiratory fibrosis, interstitial pneumonitis, or chronic interstitial obstructive pulmonary disease can occasionally occur. There have been occurrences of central nervous system reactions to methotrexate, including myelopathies and leukoencephalopathies, especially when the agent was given via the intrathecal route. Methotrexate causes ocular adverse effects in up to 25 % of patients[38]. They may include blepharitis, periorbital edema, conjunctival hyperemia, increased tearing, or photophobia. Methotrexate is secreted into breast milk. Methotrexate exposure during pregnancy can lead to congenital malformations. It is Pregnancy Category X.

Anorexia, nausea and vomiting, diarrhea, hematemesis, melena, or pancreatitis can arise. These symptoms may be preventable by rescue therapy with folinic acid (leukovorin) supplements. High doses of methotrexate (generally doses greater than 1000 mg/m^2) require leucovorin rescue. Leucovorin must be administered until the levels fall below 50 nM.

Although methotrexate competes with folate for Dihydrofolate Reductase, the enzyme inhibition caused by methotrexate is sometimes referred to as pseudo-irreversible because it cannot be displaced substantially by any physiologically attainable concentration of folate. Thus, an overdose of methotrexate cannot be corrected by administering folate. It requires folinic acid as an antidote.

Drug Interactions Vitamin preparations containing folic acid or its derivatives decrease the responses to systemically administered methotrexate. Vitamin C supplements can increase the adverse effects of methotrexate. Oral antibiotics, such as tetracycline, chloramphenicol, and non-absorbable broad spectrum antibiotics, may decrease the intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing the metabolism of the drug by bacteria. There is a risk of a severe adverse reaction if penicillin is prescribed alongside methotrexate. Methotrexate in the blood is approximately 50 % protein bound. It may be displaced by various compounds including sulfonamides, salicylates, phenylbutazone, tetracyclines, chloramphenicol, and phenytoin. The concurrent use of drugs that also undergo tubular secretion can markedly increase the methotrexate blood levels. Non-steroidal anti-inflammatory drugs (NSAIDs) and probenecid reduce the tubular secretion of methotrexate. Concomitant administration of some NSAIDs with high dose methotrexate therapy can elevate and prolong the blood methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity.

Drug Resistance The efflux transporter ABCG2 (Breast Cancer Resistance Protein, BCRP) exports polyglutamates out of cells, and mutations within the *abcg2* gene may confer resistance to various antifolates. Polymorphisms of the gene *slc19A1* (*reduced folate carrier*, *rfc*), such as A80G, which encodes the uptake transporter for methotrexate, or its

[38] The drug is excreted in the tears and may cause ocular irritation, thus interfering with corneal and conjunctival epithelial metabolism.

regulatory elements are associated with clinical outcome in the treatment of osteosarcoma or childhood acute lymphoblastic leukemia (ALL). In high doses, passive diffusion of the drug can to some degree overcome tumor cell resistance caused by saturated active transport systems. Cancers can become resistant to antifolates by amplification of *dihydrofolate reductase* or decreased affinity of Dihydrofolate Reductase for methotrexate. Other potential causes of resistance are slow rates of thymidylate synthesis and lack of polyglutamation within tumor cells.

Pralatrexate (PDX, *N*-(4-{1-[(2,4-diaminopteridin-6-yl) methyl]but-3-yn-1-yl}benzoyl)-L-glutamic acid) <Folotyn> is a folate analog metabolic inhibitor. It is the first drug to have been approved (in 2009 by the U.S. FDA) as a treatment for patients with relapsed or refractory peripheral T-cell lymphoma (PTCL), a biologically diverse group of aggressive blood cancers with a poor prognosis.

Pharmacokinetics Pralatrexate preferentially enters cancer cells through the transporter SLC19A1 (Reduced Folate Carrier type 1, RFC-1).

Adverse Effects Common adverse effects include mucositis (70%, grade 2 or higher requires dose adjustment or discontinuation), myelosuppression (40% thrombocytopenia), nausea (40%), fatigue (35%), skin reactions, vomiting, and diarrhea. Other adverse effects may include dizziness or fainting spells, rash or itching, loss of appetite and weight loss, joint and muscle pain. Patients should be instructed to take folic acid and receive vitamin B12 to potentially reduce treatment related hematologic toxicity and mucositis. The drug is Pregnancy Category D.

Drug Interactions The co-administration of drugs subject to renal elimination may result in delayed renal clearance of pralatrexate.

Edatrexate (10-ethyl-10-deaza-aminopterin, 10-EDAM, EDX) (CGP-30694) is a water soluble, polyglutamatable analog of methotrexate. Edatrexate inhibits Thymidylate Synthase and Glycinamide Ribonucleotide Formyl Transferase, impairing the synthesis of purine nucleotides and amino acids, and resulting in tumor cell death. Advantages over methotrexate include better transport across cancer cell membranes and increased selectivity for tumor cells, resulting in greater anti-tumor effect. Edatrexate may be suitable for treating lung cancer, head and neck cancer, breast cancer, and non-Hodgkin lymphoma. The dosage is 40–80 mg/m^2 by infusion once a week.

Adverse Effects The adverse effects have a profile similar to methotrexate, but are tolerable. Mouth ulcers are the dose limiting toxicity, followed by myelosuppression with decreased formation of all blood elements, diarrhea, skin rash, nausea and vomiting, pneumonitis, and a mild impact on liver function.

Drug Resistance Edatrexate may overcome tumor resistance to methotrexate. Unlike methotrexate, edatrexate has additive activity when used with cisplatin.

Talotrexin ammonium (*N*(α)-(4-amino-4-deoxypteroyl)-*N*(δ)-hemiphthaloyl-L-ornithine) (PT523) <Talvesta> is a non-polyglutamable anti-metabolite analog of aminopterin that binds to and inhibits the function of Dihydrofolate Reductase. Talotrexin is water soluble and is actively transported into cells by SLC19A1 (RFC, Reduced Folate Carrier). In 2006, the U.S. FDA granted orphan drug designation for talotrexin use in patients with ALL. The agent is also under study for the treatment of solid tumors, including non-small cell lung cancer.

As folylpolyglutamates are essential for cell survival and proliferation, the synthesis of poly(γ-glutamate) metabolites of natural folates is a critical process. Polyglutamates of antifolates are formed as long half-life metabolites (Baugh et al. 1973; Nair and Baugh 1973), and they are often critical for their cytotoxic action. The poly-glutamylation of anti-folates has been attempted to stabilize the compounds.

Thymidylate Synthase inhibitors Thymidylate Synthase catalyzes the methylation of 2′-deoxyuridine-5′-monophosphate (dUMP) to 2′-deoxythymidine-5′-monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate, an essential precursor in DNA synthesis (Fig. 2.42). Over-expression of Thymidylate Synthase is associated with many solid tumors.

Pemetrexed disodium (*N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid disodium salt) <Alimta> is a synthetic pyrimidine based antifolate that binds to and inhibits Thymidylate Synthase. It also is an inhibitor of Dihydrofolate Reductase (DHFR), and Glycinamide Ribonucleotide Formyl Transferase (GARFT). The agent is taken up into cells by membrane carriers, such as SLC19A1 (Reduced Folate Carrier) and membraneous folate binding protein transport systems. Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme Folylpolyglutamate Synthase. The drug is indicated

- as a single agent for the treatment of patients with locally advanced or metastatic non-squamous, non-small cell lung cancer after prior chemotherapy
- for the maintenance treatment of patients with locally advanced or metastatic non-squamous, non-small cell lung cancer whose disease has not progressed after four cycles of platinum based first-line chemotherapy

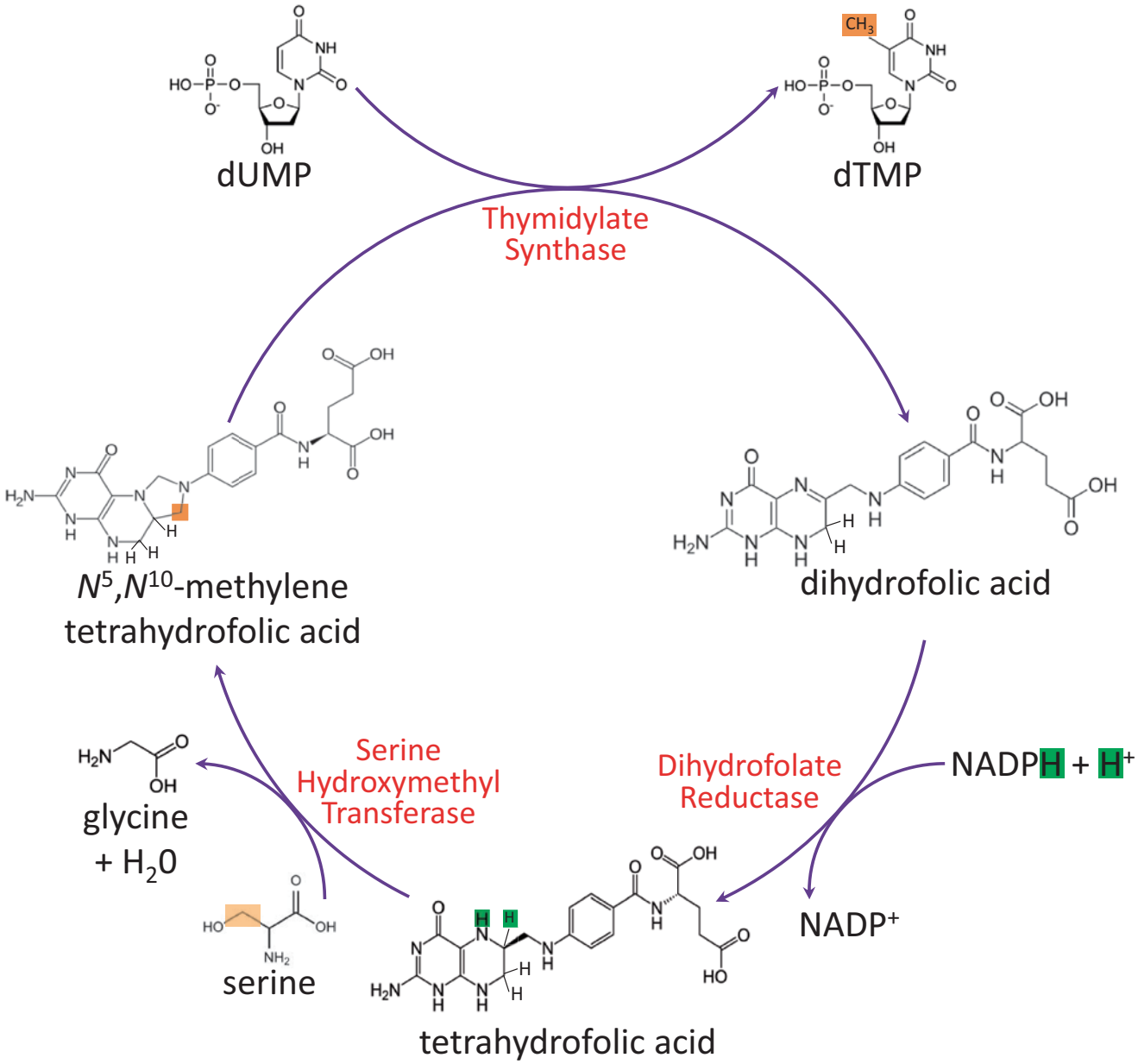


Fig. 2.42 The Thymidylate Synthase cycle. Thymidylate Synthase is embedded in a cycle that supplies the co-substrate methylene-tetrahydrofolate and yields dihydrofolate, which then regenerates methylene-tetrahydrofolate via tetrahydrofolate. In the process, glycine is synthesized from serine and *dTMP* (deoxythymidine monophosphate, thymidylate) is synthesized from *dUMP* (deoxyuridine monophosphate). Transferred functional groups are tracked by color (*green, orange*)

- in combination with cisplatin therapy for the initial treatment of patients with locally advanced or metastatic non-squamous, non-small cell lung cancer
- in combination with cisplatin for the treatment of patients with malignant pleural mesothelioma, whose disease is unresectable or who are otherwise not candidates for curative surgery.

The recommended dose for single agent use is 500 mg/m^2 administered as an intravenous infusion over 10 min on day 1 of each 21-day cycle. If used in combination, the recommended dose of cisplatin is 75 mg/m^2 infused over 2 h beginning approximately 30 min after the end of the pemetrexed administration (appropriate hydration of patients under cisplatin treatment is important).

Pharmacokinetics Pemetrexed total systemic exposure and maximum blood concentration increase proportionally with the dose. Approximately 80% of taken up drug is bound to plasma proteins (this binding is not affected by the degree of renal impairment). Pemetrexed is not metabolized to any

appreciable extent and does not affect Cytochrome P450 enzymes. The agent is primarily eliminated in the urine, with 70–90 % of the dose excreted unchanged within the first 24 h following administration. The drug should not be given to patients with a creatinine clearance below 45 mL/min. While the effect of third-space fluid, such as pleural effusion or ascites, is unknown, in affected patients consideration should be given to draining the effusion prior to pemetrexed administration. The pharmacokinetics does not change over multiple treatment cycles.

Adverse Effects In single-agent therapy, the most common adverse effects are fatigue, nausea, and anorexia. Myelosuppression is usually the dose limiting toxicity of pemetrexed. Complete blood cell counts, including platelets, and periodic chemistry tests should be performed on all patients receiving the drug. Dose adjustments at the start of a subsequent cycle are to be based on nadir hematologic counts or maximum non-hematologic toxicity from the preceding cycle of therapy. Pemetrexed therapy should be discontinued if a patient experiences any hematologic or non-hematologic grade 3–4 toxicity after two dose reductions or immediately if grade 3–4 neurotoxicity is observed. The drug is contraindicated in patients who have a history of severe hypersensitivity reactions to pemetrexed or to any ingredient used in the formulation. Pemetrexed is Pregnancy Category D.

Patients must be instructed to take folic acid and vitamin B_{12} during treatment as a prophylaxis to reduce hematologic and gastrointestinal adverse effects. The toxicity of pemetrexed is reduced by daily intake of a low dose oral folic acid preparation (commonly 400 μg) or multi-vitamin with folic acid. Supplementation should continue for 21 days after the last dose of pemetrexed. Patients must also receive one intra-muscular injection of vitamin B_{12} during the week preceding the first dose of pemetrexed and every three cycles thereafter[39].

Drug Interactions In combination with cisplatin, common adverse reactions include vomiting, neutropenia, leukopenia, anemia, stomatitis or pharyngitis, thrombocytopenia, and constipation. Patients with mild to moderate renal insufficiency should avoid taking non-steroidal anti-inflammatory drugs (NSAIDs) with short elimination half-lives for a period of 2-days-before through 2-days-following the administration of pemetrexed. Although ibuprofen (400 mg four times a day) can decrease the clearance of pemetrexed, it may be given to patients with normal renal function (creatinine clearance 80 mL/min or higher). The concomitant administration of nephrotoxic drugs or substances that are tubularly secreted could result in delayed clearance of pemetrexed.

Raltitrexed (*N*-(5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl)-L-glutamic acid) <Tomudex> is a quinazoline folate analog. It has been used in the treatment of colorectal cancer since 1998. After transport into cells via SLC19A1 (Reduced Folate Carrier 1, Folate Transporter), raltitrexed undergoes intracellular polyglutamation and blocks the folate binding site of Thymidylate Synthase. Thereby it inhibits tetrahydrofolate activity and DNA reduplication and repair, thus resulting in cytotoxicity.

OSI-7904 ((S)-2-(5-(((3-methyl-1-oxo-1,2-dihydrobenzo[f]quinazolin-9-yl)methyl)amino)-1-oxoisoindolin-2-yl) pentanedioic acid) (GW1843, OVI-237, GS7904L) is a 3-methyl substituted benzoquinazoline folate analog. It noncompetitively binds to Thymidylate Synthase and acts as an inhibitor, resulting in the suppression of thymine nucleotide synthesis and DNA reduplication.

- OSI-7904L (GS7904L) is a liposome encapsulated formulation of the drug. Liposome encapsulation improves the efficacy and increases the half-life of OSI-7904.

Nolatrexed dihydrochloride (3,4-dihydro-2-amino-6-methyl-4-oxy-5-(4-pyridylthio)-quinazoline dihydrochloride) (AG337) <Thymitaq> is a quinazoline folate analog that occupies the folate binding site of Thymidylate Synthase, resulting in the inhibition of enzymatic activity and thymine nucleotide synthesis with subsequent blocking of DNA reduplication. This leads to DNA damage, S phase cell cycle arrest, and Caspase mediated apoptosis. Nolatrexed also exhibits radio-sensitizing activity.

Originally synthesized in the late 1950s, 5-fluorouracil (5-FU) is a fluorinated analog of the naturally occurring pyrimidine uracil. It is a prodrug and must be metabolized to the nucleotide form, fluorodeoxyuridine monophosphate, to be active. In the presence of folates, fluorodeoxyuridine monophosphate binds tightly to and interferes with the function of Thymidylate Synthase, resulting in decreased thymidine synthesis and consecutively reduced DNA synthesis. Another metabolite of 5-fluorouracil, the triphosphate nucleotide, is incorporated into RNA as a false base, and interferes with RNA function. Various forms of 5-fluorouracil are in use (see Sect. 2.4.2. pyrimidine analogs).

- 5-(trifluoromethyl)-2′-deoxyuridine (F_3TDR) was synthesized by Heidelberger as a trifluoromethyl analog of 5-fluorouridine deoxyribose (5-FUDR, floxuridine). The

[39] The pretreatment causes skin rashes, more commonly in men than in women. Dexamethasone (4 mg orally twice daily the day before, the day of, and the day after pemetrexed administration) or its equivalent may reduce the incidence and severity of the skin rash.

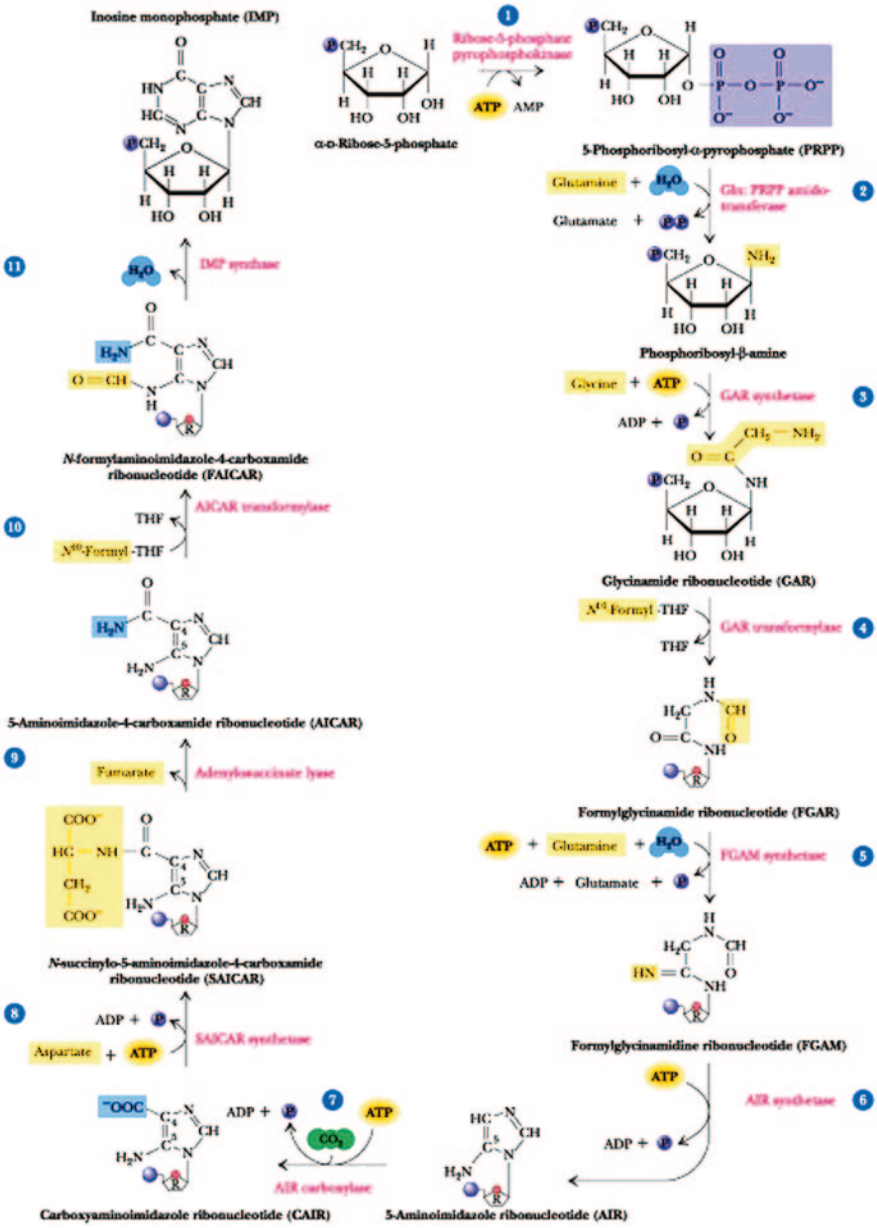


Fig. 2.43 De novo purine biosynthetic pathway. The pathway synthesizes inosine monophosphate from ribose-5-phosphate, the ribonucleotide of hypoxanthine and the first nucleotide formed during the synthesis of purine. Step 4 is catalyzed by Glycinamide Ribonucleotide Formyl Transferase. [http://images.google.com/imgres?imgurl=http://web.virginia.edu/Heidi/chapter27/Images/8883n27_29.jpg&imgrefurl= http://web.virginia.edu/Heidi/chapter27/chp27.htm&usg=__pQ-tPnCfgUt4p7CCSR1gLMpFd7k=&h=319&w=338&sz=21&hl=en&start=3&sig2=OqPAmXXgOx6sEqnhNQ35Bw&tbnid=fGhtr2vXFFlvSM:&tbnh=112&tbnw=119&prev=/images%3Fq%3Dthymidylate%2Bsynthase%26gbv%3D2%26hl%3Den&ei=cDqySu6OB4qo8AbGtd3FDQ] There are instances where we have been unable to trace or contact the copyright holder. If notified the publisher will be pleased to rectify any errors or omissions at the earliest opportunity

drug undergoes metabolic phosphorylation to become a Thymidylate Synthase inhibitor. It may also be incorporated into DNA in place of thymidine.

Thymectacin (NB1011) is a small molecule phosphoramidate derivative of (E)-5-(2-bromovinyl)-2′-deoxyuridine. It is selectively active against tumor cells that express high levels of Thymidylate Synthase, a critical enzyme in DNA biosynthesis, the over-expression of which is associated with many solid tumors. In contrast to other Thymidylate Synthase targeting drugs, which are effective through inhibition of the enzyme, the cytotoxicity of thymectacin depends on its activation by Thymidylate Synthase. While it is independent of the tumor suppressor P53, it may induce the expression of *bax* (encodes a pro-apoptotic BCL-2 family member), *p21* (encodes a cell cycle checkpoint protein), and *gadd45* (encodes a DNA damage response protein). The activation of these genes results in a deregulation of the cell cycle and leads to apoptosis (Neuteboom et al. 2002).

Glycinamide Ribonucleotide Formyl Transferase inhibitors Purine depletion causes slow cell death in cells that have passed the G_1 checkpoint (many cancer cells), but cytostasis in cells that reside in G_0 or are arrested at the G_1 checkpoint (most differentiated cells). Therefore, Glycinamide Ribonucleotide Formyl Transferase inhibitors, at physiological folate concentrations, cause selective cytotoxicity to cells lacking a functional G_1 checkpoint (Zhang et al. 1998).

In the late 1980s, Gerald B. Grindey demonstrated anti-tumor activity for lometrexol, the first selective inhibitor of Glycinamide Ribonucleotide Formyl Transferase (GARFT), the enzyme that catalyzes a key step of the de novo purine biosynthetic pathway (Fig. 2.43). The agent showed efficacy against tumors that were unresponsive to methotrexate, and it had enhanced adverse effects in conjunction with a folate depleted diet[40].

Lometrexol (6R-5,10-dideazatetrahydrofolate, (6R)-DDATHF, LMTX) is a folate analog anti-metabolite. As the 6R diastereomer of 5,10-dideazatetrahydrofolate, lometrexol inhibits Glycinamide Ribonucleotide Formyl Transferase, thereby inhibiting DNA synthesis, arresting cells in the S phase of the cell cycle, and inhibiting tumor cell proliferation.

Pharmacokinetics Since lometrexol is extensively polyglutamylated, it is cleared from cells slowly, and the extensive accumulation that occurs in low folate conditions causes delayed toxicity.

Adverse Effects Repetitive administration of the drug is compromised by cumulative myelosuppression and gastrointestinal toxicities. Weekly administration of lometrexol is feasible and well tolerated when co-administered with daily oral folic acid (Roberts et al. 2000).

Drug Resistance Lometrexol is active against tumors that are resistant to the folate antagonist methotrexate.

4-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4–6] [1,4]thiazin-6-yl)-(S)-ethyl]-2,5-thienoyl-L-glutamic acid (AG2034) is an inhibitor of Glycinamide Ribonucleotide Formyl Transferase (Boritzki et al. 1996). AG2034 also has a high affinity for the Folate Receptor, and is a substrate for Folylpolyglutamate Synthetase. This compound can enter cells by utilizing the Reduced Folate Carrier. The recommended dose is 5.0 mg/m^2.

Adverse Effects Dose limiting toxicities are anemia, thrombocytopenia, mucositis, diarrhea, hyperbilirubinemia, fatigue, and insomnia. They are modestly cumulative over three courses.

Dihydropteroate Synthase inhibitors Dihydropteroate Synthase (DHPS) catalyzes the condensation of *p*-aminobenzoate and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate to form 7,8-dihydropteroate, which is an essential precursor to the cofactor tetrahydrofolate. Sulfa drugs are blockers of folate synthesis, acting as competitive inhibitors of *p*-aminobenzoate.

Dapsone (4,4′-sulfonyldianiline) <Croysulfone, Diphenasone, Dumitone, Novophone, Sulfona-Mae, Sulphadione> is a synthetic derivative of diamino-sulfone. A structural analog of *p*-aminobenzoic acid (PABA), dapsone inhibits Dihydropteroate Synthase, resulting in a depletion of the folate pool and a reduction in the amount of thymidylate available for DNA synthesis. Dapsone has been used to treat Kaposi sarcoma.

Adverse Effects Adverse effects include hemolysis, which may lead to hemolytic anemia, and methemoglobinemia. Agranulocytosis occurs rarely in mono-therapy, but is more frequent in combination regimens. Abnormalities in white blood cell formation (including aplastic anemia) are rare, but are the major cause of death due to dapsone therapy. Toxic hepatitis and cholestatic jaundice may occur.

Dapsone reaction is a form of hypersensitivity that is more frequent in patients receiving multiple drug therapy. It always involves a rash and may also include fever, jaundice, and eosinophilia. These symptoms will generally occur within the first 6 weeks of therapy or not at all, and may be ameliorated by corticosteroid therapy.

Drug therapy supportive of anti-folates Agents are available to enhance the therapeutic effects or ameliorate the adverse effects caused by anti-folates.

Folinic acid (citrovorum factor, levoleucovorin, (6-S)-leucovorin) <Leucovorin> is the active l-isomer of the 5-formyl derivative of tetrahydrofolic acid. As an active metabolite of folic acid, it does not require activation by Dihydrofolate Reductase. Folinate calcium (leucovorin calcium) counteracts the toxic effects of anti-metabolite medications, rescuing the patient while permitting the anti-tumor activity of the folate antagonist. This agent may also enhance the effects of fluoropyrimidines by stabilizing their binding to the enzyme Thymidylate Synthase, thus prolonging drug activity. The drug is indicated

- after high dose methotrexate therapy in osteosarcoma,
- to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists,
- in combination with 5-fluorouracil to prolong survival in the palliative treatment of patients with advanced colorectal cancer.

The agent is given by intravenous injection. There are multiple distinct regimens that combine 5-fluorouracil and folinic acid. The de Gramont regimen is initiated by infusion of folinic acid over 2 h, followed by 5-fluorouracil over 22 h. This is repeated over day 2. A rest period of 12 days completes the cycle. In the modified de Gramont protocol, the infusions are administered through a portable pump, allowing outpatient treatment. In the case of treating a methotrexate overdose, folinic acid needs to be given within 4 h to avoid irreversible damage.

[40] Lometrexol is transported by Folic Acid Binding Protein (FBP), the expression of which is unregulated under folate restriction, with the result that some healthy tissues accumulate much greater amounts of lometrexol when dietary folate is limited.

Serum creatinine levels should be determined daily. In the case of high dose methotrexate therapy, hydration and urinary alkalinization need to be continued. Folinic acid can enhance the toxicity of 5-fluorouracil; in elderly patients, deaths from severe enterocolitis, diarrhea, and dehydration can result. The drug is Pregnancy Category C.

Folic acid in large amounts may counteract the anti-epileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible children. It is not known whether folinic acid has the same effects.

5-fluorouracil causes myelosuppression. Uridine is a nucleoside consisting of uracil and D-ribose, which is incorporated into RNA. Uridine may serve as a rescue agent to reduce the toxicities associated with 5-fluorouracil, thereby allowing the administration of higher doses of 5-fluorouracil in chemotherapy regimens.

Old generation anti-folates comprise Dihydrofolate Reductase inhibitors and Thymidylate Synthase inhibitors.
New generation anti-folates include Glycinamide Ribonucleotide Formyl Transferase inhibitors and Dihydropteroate Synthase inhibitors.
Within cells, anti-folates are converted to polyglutamates, which typically are more efficient enzyme inhibitors.
Agents are available to enhance the efficacy and ameliorate the toxicity of anti-folates (folinic acid and uridine).

2.4.2 Anti-Pyrimidines

Anti-pyrimidines were developed in attempts to synthesize analogs that inhibit uracil utilization by tumors (Fig. 2.44). In 1954, Abraham Cantarow and Karl Paschkis had found that liver tumors absorbed uracil more readily than healthy liver cells. Because tumors tend to use more uracil than orotic acid for nucleic acid synthesis, Charles Heidelberger reasoned that an anti-metabolite resembling uracil would show some selectivity for inhibiting tumors over normal tissues. He hypothesized "because acetic acid is vinegar and fluoroacetic acid is rat poison" a fluorine-substituted compound might be active. Such substitution in position five of the uracil ring was feasible and stable. The substitution also interfered with the synthesis of thymine nucleotides from uracil nucleotides. This rationale resulted in the anti-cancer drug 5-fluorouracil. It was synthesized in 1957 in a collaboration of Duschinsky, Pleven, and Heidelberger (Duschinsky et al. 1957; Heidelberger et al. 1957).

Uracil analogs Thymine (5-methyl uracil) is the base that distinguishes DNA from RNA (which uses uracil instead). The class of halogenated uracil drugs carries a halogen on uracil or its derivatives. The replacement of the 5-methyl group with a halogen interferes with DNA synthesis. As the van der Waals radii are similar for hydrogen and fluoride, 5-fluorouracil behaves like uracil with hydrogen in the five position. For the heavier halogenated analogs (containing chlorine, bromine, or iodine), the radius of the methyl group of thymine is more closely approximated.

5-fluorouracil (5-fluoro-1*H*-pyrimidine-2,4-dione, 5-FU) is a fluoropyrimidine nucleoside analog that was introduced into the clinic in 1958. This agent has broad activity against a range of solid tumors. Fluorouracil and its metabolites possess a various mechanisms of action that lead to S phase specific anti-proliferation:

- Fluoruracil is converted to the active metabolite 5-fluoroxyuridine monophosphate (F-UMP). FUMP is further metabolized to FUTP, which inhibits RNA and protein synthesis by competing with uridine triphosphate for incorporation into the RNA strand, thereby suppressing cell growth.
- The active metabolite 5-fluoro-2′-deoxyuridine-5′-*O*-monophosphate (F-dUMP), inhibits Thymidylate Synthase, resulting in the depletion of thymidine triphosphate (TTP), a necessary constituent of DNA. FdUMP inhibits DNA synthesis and cell division by reducing thymidine production.
- Several fluorouracil metabolites incorporate into both RNA and DNA. Incorporation into RNA results in major effects on both RNA processing and functions. When 5-fluorouracil is incorporated into DNA it is removed by Uracil N-Glycosylase, leaving an apyrimidinic sugar for the process of DNA repair. Errors in this process provide a basis for cytotoxicity.

Safety and efficacy of 5-fluorouracil administration are higher with continuous intravenous infusion than with a bolus injection. Intra-arterial infusion into the tumor may be beneficial. Tumors that have previously been irradiated respond poorly to fluorinated pyrimidines. 5-fluorouracil is not radiosensitizing, but is a potentially phototoxic drug.

Diverse preparations and various pro-forms of 5-fluorouracil are in use for cancer chemotherapy.

- 5-Fluorouracil <Adrucil, Fluoroplex, Fluorouracil> has its principal use is in colorectal cancer and pancreatic cancer, in which it is the established form of chemotherapy. For good-risk patients, 5-fluorouracil is usually given intravenously (infusion is superior to bolus injection) at 15 mg/kg for 5 days. After rest for 1 day, 7.5 mg/kg are then given every other day for 4–5 doses. A new course of treatment is repeated after an interval of 4 weeks. For poor-risk patients, the dose of 5-fluorouracil is reduced or 4 doses of 15 mg/kg/day are given as a course, whereafter treatment is stopped.
- Cream preparations of 5-fluorouracil <Efudex, Fluoroplex, Carac> are used for the topical treatment of multiple

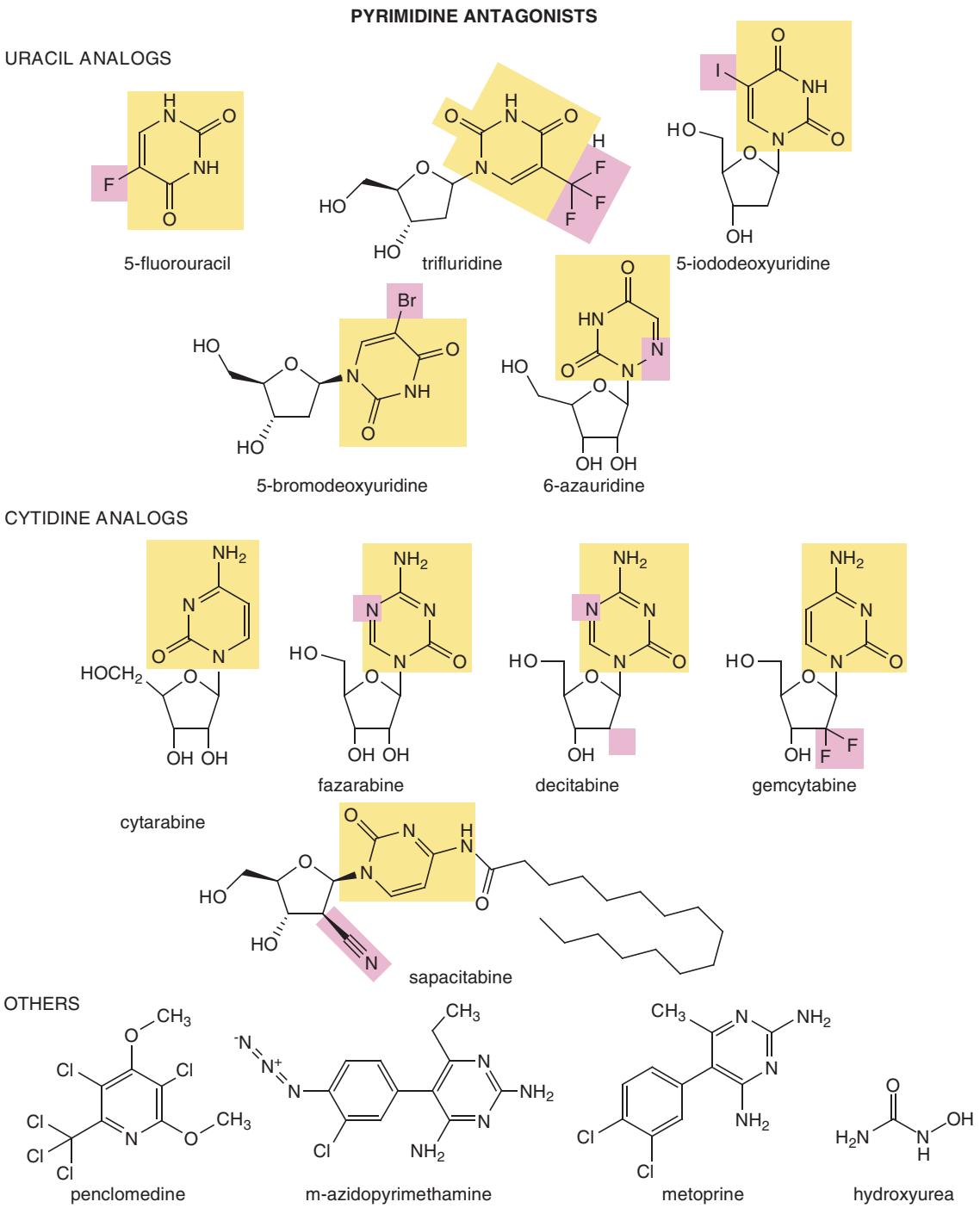


Fig. 2.44 Structures of anti-pyrimidines. Three groups comprise uracil analogs, cytidine analogs, and others. The common uracil-related or cytosine-related functional groups are highlighted in *yellow*. Differences among the uracil analogs are shaded in *pink*. They pertain mostly to various halogen substitutes (in one case to a nitrogen substitution in the ring structure). All cytidine analogs contain a sugar, analogous to the DNA building block. In this group, changes in the sugar moiety are shaded in *pink*. Also shaded in *pink* are the nitrogen substitutions of the base in fazarabine and decitabine

actinic or solar keratoses. Efudex 5% strength is administered to treat superficial basal cell carcinomata. The most common adverse effects include reversible pain, itching, burning, soreness, scaling, rash, irritation, dryness, swelling, inflammation, tenderness at the injection site. Less commonly (10–30% of patients), increased sensitivity to sunlight, discoloration of the skin, or scarring can arise.

- Floxuridine (5-fluoro-2′-deoxyuridine-5′-phosphate, 5-FUDR monophosphate) <FUDR, FUDR-MP> is a fluorinated pyrimidine monophosphate analog of 5-fluorouracil that was synthesized by Duschinsky. The goal was a reduction in the toxicity of 5-fluorouracil that arises when the drug is converted to a ribonucleotide. It competes with 2′-deoxyuridylate for Thymidylate Synthase and thereby

blocks thymidylate synthesis. The resulting lack of thymidylate consecutively impairs DNA synthesis. This agent is metabolized to fluorouracil and other derivatives that can be incorporated into RNA and inhibit the utilization of preformed uracil in RNA synthesis. 5-FUDR, but not 5-fluorouracil, is incorporated preferentially into colon carcinoma compared to normal colon (Mukherjee et al. 1963). Floxuridine is usually given rapidly at twice the dose of 5-fluorouracil. It first gained U.S. FDA approval in 1970.

- Dihydropyrimidine Dehydrogenase represents the initial rate-limiting step in the catabolism of the pyrimidines uracil, thymine, and 5-fluorouracil. Doxifluridine (5′-deoxy-5-fluorouridine) is a fluoropyrimidine derivative and oral prodrug of 5-fluorouracil, designed to circumvent the rapid degradation of 5-fluorouracil by Dihydropyrimidine Dehydrogenase in the gut wall and liver. The agent is converted into 5-fluorouracil by Pyrimidine Nucleoside Phosphorylase.
- 5,10-methylenetetrahydrofolate (MTHF) is a folate based biomodulator that stabilizes the covalent binding of the fluorouracil metabolite 5–5-fluoro-2′-deoxyuridine-5′-O-monophosphate (FdUMP) to its target enzyme, Thymidylate Synthase. The binding results in enzyme inhibition and depletion of the essential DNA constituent thymidine triphosphate (TTP). Tumor cell death ensues. 5,10-methylenetetrahydrofolate, as the active form of folate, does not require metabolic activation and may exceed the chemotherapeutic effects of fluorouracil with lower toxicity.
- Tegafur (5-fluoro-1-(oxolan-2-yl)pyrimidine-2,4-dione, tetrahydrofuranyl-5-fluorouracil) is a prodrug that is gradually converted to fluorouracil by Cytochrome P450 in the liver. Tegafur competes with uridine triphosphate, thus inhibiting RNA and protein synthesis.
- Tegafur-uracil (UFT) <Ftorafur, Tefudex, Ufur, Uftoral> is a formulated oral agent consisting of a combination of the 5-fluorouracil prodrug, tetrahydrofuranyl-5-fluorouracil, and uracil (1:4). Because of its higher concentration in the combination, uracil saturates the uracil reducing enzymatic activity of Ddihydropyrimidine Ddehydrogenase, thereby inhibiting first pass hepatic metabolism of 5-fluorouracil and permitting its administration as the orally bioavailable prodrug tetrahydrofuranyl-5-fluorouracil. Tegafur-uracil was developed in Japan during the 1980s. The drug has been administered on a continuous daily basis for 6–65 months at 300–400 mg per day in divided doses (metronomic dosing), in cycles of 5 days dosed at 300 mg/m^2 per day followed by the weekend off, or in cycles of 28 days dosed at 300–600 mg/m^2 per day followed by a week off.
- S-1 was developed in Japan. It is an anti-pyrimidine preparation composed of tegafur combined with two modulators of 5-flurouracil activity, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate, in a molar ratio of 1:0.4:1. 5-chloro-2,4-dihydroxypyridine is a reversible inhibitor of Dihydropyrimidine Dehydrogenase, the liver enzyme responsible for rapid catabolism of 5-fluorouracil into inactive metabolites. Potassium oxonate preferentially localizes in the gut and inhibits the enzyme Orotate Phosphoribosyl Transferase, which is the major enzyme responsible for 5-fluorouracil activation. Thus it decreases the activation of 5-fluorouracil in the gut and the associated gastrointestinal toxicity. S-1 is orally more active than 5- fluorouracil.
- Capecitabine (5′-deoxy-5-fluoro-*N*-[(pentyloxy) carbonyl]-cytidine) <Xeloda> is a fluoropyrimidine carbamate with nearly 100% oral bioavailability. As a prodrug, capecitabine is metabolized by the liver to 5′-deoxy-5-fluorocytidine and subsequently to 5′-deoxy-5-fluorouridine (5′-dFUR). 5′-deoxy-5-fluorouridine is converted to 5-fluorouracil by Thymidine Phosphorylase, which is present in amounts 3–10-fold higher in neoplastic tissue compared with untransformed tissue. A typical daily oral dose is 1250–2000 mg/m^2. It is split into morning and evening intake (within 30 min after a meal) for 2 weeks, followed by 1 week of rest to complete a cycle. Capecitabine may be used in mono-therapy or combination chemotherapy. It is indicated for the adjuvant treatment of Dukes C colon cancer[41] following the complete resection of the primary tumor, as first-line treatment of metastatic colorectal carcinoma, for metastatic breast cancer after failure of prior anthracycline containing chemotherapy (combination with docetaxel), for metastatic breast cancer resistant to both paclitaxel and anthracycline containing chemotherapy or resistant to paclitaxel and contraindicated for anthracycline therapy. The safety profile of capecitabine is superior to that of 5-fluorouracil, with less diarrhea, stomatitis, nausea, alopecia, and grade 3–4 neutropenia, but a higher incidence of hand and foot syndrome and hyperbilirubinemia (typically unconjugated bilirubin). Care needs to be exercised when capecitabine is co-administered with CYP2C9 substrates. Patients receiving concomitant oral coumarin derivatives should have their anticoagulant response closely monitored. The phenytoin dose may need to be reduced when given simultaneously with capecitabine.

Pharmacokinetics 5-fluorouracil is limited by poor oral absorption, its administration therefore requires a permanent venous access and a portable pump. The drug has a volume of distribution of 0.20–0.25 L/kg, which reflects distribution

[41] Dukes classification of colorectal cancer: Dukes A means the cancer is only in the lining of the colon or rectum. Dukes B means the cancer has grown through the muscle layer of the colon or rectum. Dukes C means the cancer has spread to at least one lymph node in the area close to the bowel. Dukes D means the cancer has spread to distant sites, such as the liver or lung.

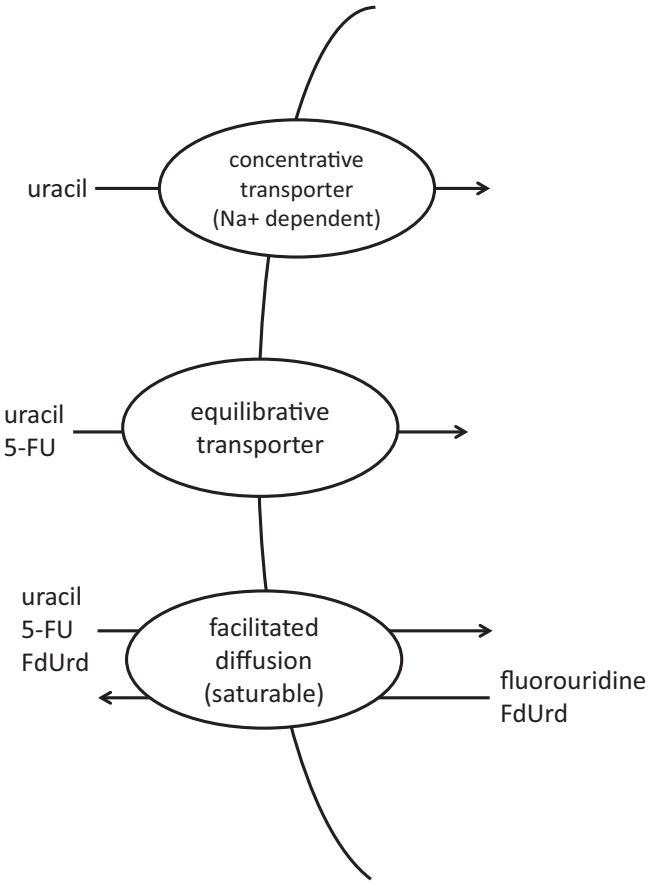


Fig. 2.45 Pyrimidine uptake and excretion. Three transport mechanisms differentially shuttle pyrimidines between the extracellular space (*left*) and the intracellular space (*right*). *5-FU* 5-fluorouracil, *FUdR* fluorouridine deoxyribose

into the extracellular space. There is good penetration into the cerebrospinal fluid, lymph, and neoplastic effusions. The rate of blood clearance generally is 1st order with a half-life of 10–20 min and ranges between 500 and 1500 mL/min. 5-fluorouracil enters cells by a carrier mediated transport mechanism and by facilitated diffusion (however, alterations of 5-fluorouracil entry into cells are not responsible for either natural or acquired resistance). In contrast to 5-fluorouracil, the entry of fluorodeoxyuridine (FdUrd) into most neoplastic cells involves the saturable but non-concentrative mechanism for the facilitated diffusion of nucleosides. Fluorouridine and FdUrd, released from 5-fluorouridylic acid and 5-fluorodeoxyuridylate by Phosphatase action, exit the cells via this same facilitated diffusion transporter (Fig. 2.45). Thus, agents that affect this transporter may affect 5-fluorouracil cytotoxicity[42].

The route of administration of anti-pyrimidine drugs influences the mechanism of action, with thymidylate synthesis inhibition playing a greater role in continuous infusion regimens, and incorporation into RNA being more important for intermittent bolus schedules. Intraarterial infusion of 5-fluorouracil can be used in patients with isolated hepatic metastases. 5-fluorouracil is metabolized to the 2 active moieties 5-fluoro-2-deoxyuridine mono-phosphate (FdUMP) and 5-fluorouridine mono-phosphate (FUMP) by both tumor cells and untransformed cells. The anabolic path, in which 5-fluorouracil is incorporated into nucleotides, is the mechanism of carcinostatic action. Catabolism via the degradation pathway for uracil is the immediate fate of the bulk of an administered dose of 5-fluorouracil and leads to drug inactivation (Fig. 2.46). α-fluoro-β-alanine is the major urinary excretion product of 5-fluorouracil. In patients with cancer, it is conjugated with bile acids and constitutes the primary biliary secretion product. There are marked circadian variations in the metabolism of 5-fluorouracil related to 24-h cyclic fluctuations in Dihydrouracil Dehydrogenase activity, which are reflected in inverse variations of 5-fluorouracil blood concentrations during infusions.

Adverse Effects Gastrointestinal toxicity and myelosuppression are the most common adverse reactions to 5-fluorouracil. Specifically, they include anemia, thrombocytopenia, leukopenia, neutropenia, nausea and vomiting, and diarrhea (4–5 liquid stools per day). Also, stomatitis, mucositis, and hand-foot syndrome (palmar-plantar erythrodysesthesia; drug leakage through capillaries results in redness, tenderness, and possibly peeling of the palms and soles) may arise. Cardiotoxicity can occur due to supraventricular arrhythmia.

Dihydropyrimidine Dehydrogenase, involved in the metabolism of uracil and thymine, is responsible for much of the intra-individual and inter-individual variability in clinical pharmacokinetics of 5-fluorouracil. An autosomal recessive deficiency of Dihydropyrimidine Dehydrogenase constitutes a metabolic disorder that affects over 5% of the population. Individuals with this condition may develop life threatening toxicity following exposure to 5-fluorouracil or capecitabine.

Drug Resistance Reduced anabolism of 5-fluorouracil to the nucleotide form can lead to drug resistance. This may reflect altered condensation with pyrophosphorylribose-5-phosphate (PRPP) or activation via the salvage pathway that involves ribose-1-phosphate or deoxyribose-1-phosphate and the appropriate Nucleoside Phosphorylase, with subsequent phosphorylation of the resultant nucleoside by Uridine Kinase or Thymidine Kinase. Alternatively, lack of drug sensitivity may be correlated with enhanced Nucleotide Phosphatase activity. Increases in the catabolism of 5-fluorouracil, mainly dependent on the rate limiting enzyme

[42] The facilitated diffusion mechanism may play a secondary role in the modulation of 5-fluorouracil action by uridine because this natural nucleoside, but not 5-fluorouridine (5-FUrd) or fluorodeoxyuridine, is actively concentrated by a Na^+dependent system. Neoplastic cells are less capable of this transport and are not protected by the added uridine.

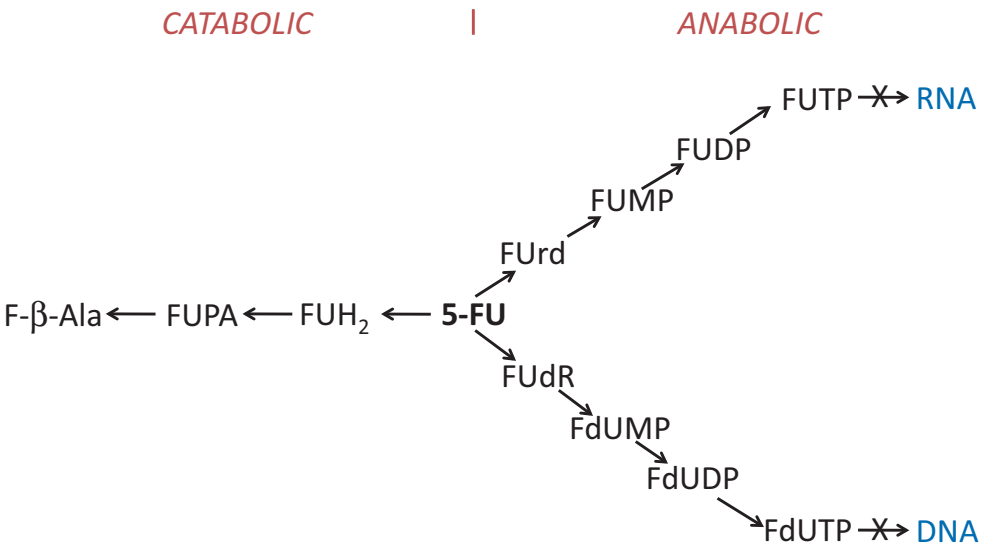


Fig. 2.46 Pathways of 5-fluorouracil metabolism. Two anabolic pathways convert the drug 5-fluorouracil (*5-FU*) into false building blocks for RNA or DNA. Their incorporation terminates the synthesis of these biopolymers. A catabolic pathway incorporates the drug into alanine (*Ala*) to inactivate it. *FUdR* fluorouridine deoxyribose, *F(d)UMP* flouro-(deoxy)uridine-monophosphate, *F(d)UDP* flouro-(deoxy)uridine-diphosphate, *F(d)UTP* flouro-(deoxy) uridine-trisphosphate, FUH_2 5,6-dihydro-5-fluorouracil, *FUPA* α-fluoro-β-ureidopropionic acid

Dihydropyrimidine Dehydrogenase, can cause resistance to fluoropyrimidine based chemotherapy. Another mechanism of resistance includes changes in Thymidylate Synthase, with reduced affinity for FdUMP, or increases in the rate of synthesis and activity of the enzyme, possibly associated with gene amplification or altered enzyme turnover rates.

Trifluridine (5-(trifluoromethyl)-2′-deoxyuridine, trifluorothymine deoxyriboside, 1-[4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-5-(trifluoromethyl) pyrimidine-2,4-dione) was synthesized by Heidelberger as a trifluoromethyl derivative of 5-FUDR[43]. It represents a fluorinated thymidine analog that undergoes metabolic phosphorylation to become a Thymidylate Synthase inhibitor. It may also be incorporated into DNA in place of thymidine. These effects result in the suppression of DNA and protein synthesis, and in the induction of apoptosis.

5-Iododeoxyuridine (IUDR) <Idoxuridine> is an analog of deoxyuridine, which has an iodine atom substituted for the fluorine atom of 5-fluorodeoxyuridine (FUDR). The agent can undergo metabolic phosphorylation and incorporation of the resulting nucleotide into DNA instead of thymidylate, because the van der Waals radius of iodine is similar to that of the methyl group in thymine. In contrast to the generally immunosuppressive actions of most conventional anti-cancer drugs, iododeoxyuridine does not interfere with some components of the immune system. The incorporation of iodine conveys radio-sensitizing properties to the drug that depend on the incorporation of this molecule into DNA during S phase. When this agent contains a radioactive isomer of iodine, the radioactive drug kills cancer cells directly after preferential incorporation into the DNA of rapidly dividing tumor cells.

Pharmacokinetics Iododeoxyuridine differs from fluorodeoxyuridine in metabolic disposition.

5-Bromodeoxyuridine (BrUDR) substitutes a bromine for fluorine in 5-FUDR. After incorporation into DNA, it causes increased susceptibility to breakage of the phospho-ester bond to the adjacent nucleotides. The drug also acts as an immunosuppressive agent and has radio-sensitizing properties.

6-Azauridine (AzUR) is a triazine analog of uridine. It acts at an essential step along the de novo path of pyrimidine biosynthesis by inhibiting Orotidylate Decarboxylase, which suppresses the formation of uridylic acid from its carboxylated precursor, orotidylic acid. Although the drug can produce remissions in some acute adult leukemias, results are mostly only temporary. The triacetylated derivative 6-azauridine 2′,3′,5′-triacetate (6-AzUrd-TA) can be absorbed from the gastrointestinal tract.

Adverse Effects Anemia is a common and moderate adverse effect. 6-Azauridine causes a metabolic disturbance similar to congenital orotic aciduria. Large amounts of orotic acid and orotidine are excreted by patients. Hypercholesterolemia may arise (Knock 1967).

Cytidine analogs Cytarabine (cytosine arabinoside, 1-β-D-arabinofuranosylcytosine, ara-C) <Cytosar-U> was discovered in Europe in the 1960s; it was approved by the U.S. FDA in 1969. The compound of arabinose and cytosine is metabolized by Deoxycytidine Kinase and other nucleotide kinases to a nucleotide triphosphate, which is an effective inhibitor of DNA Polymerase A and competes with

[43] While used as an antiviral drug mainly in the treatment of primary keratoconjunctivitis and recurrent epithelial keratitis due to herpes simplex virus <Trifluridine, Viroptic>, trifluridine has also been tested as an anti-cancer agent in clinical trials.

thymidine for incorporation into DNA. The agent also interferes with DNA synthesis by inhibiting the conversion of cytidylic acid to deoxycytidylic acid, leading to a deficiency of deoxycytidine. Cytarabine therefore kills cells primarily during S phase. Best results are achieved when contact of the drug with the tumor is ensured while the largest possible fraction of cells is in the susceptible S phase.

The introduction of this drug constituted a major advance in the treatment of leukemia, as it achieved 25–40% complete remission in adults with acute myelogenous leukemia (AML) or acute lymphocytic leukemia (ALL). The agent is also useful for remission induction in acute nonlymphocytic leukemia of pediatric patients and the treatment of acute lymphocytic leukemia and the blast phase of chronic myelocytic leukemia. Intrathecal administration is indicated in the prophylaxis and treatment of meningeal leukemia. Cytarabine can effect some improvement of reticulum cell sarcoma, melanoma, and metastasizing cancers of bladder or testicles. Total doses of 20–100 mg/kg per course can be used. The pharmacological effect is dependent on the treatment schedule. Courses of therapy need to be separated by intervals sufficient to permit adequate host recovery. The drug is not effective orally as only 20% of the administered dose is absorbed from the gastrointestinal tract.

- Liposomal cytarabine is an intrathecal formulation of cytarabine. The liposomal composition allows more effective delivery to the brain.

Pharmacokinetics The therapeutic efficacy and toxicity of cytarabine are sensitively dependent on the schedule of administration. Following intravenous injection, clearance from the blood is biphasic with an initial distributive phase of 10 min half-life, followed by an elimination phase of 1–3 h half-life. By the end of the distributive phase, more than 80% of the drug in circulation is converted by a Pyrimidine Nucleoside Deaminase to the inactive metabolite 1-β-D-arabinofuranosyluracil (ara-U). After intrathecal administration, the levels of cytarabine in the cerebrospinal fluid decline with a first order half-life of about 2 h. Because cerebrospinal fluid levels of Deaminase are low, there is little conversion to ara-U. Within 24 h, about 80% of the administered drug is excreted into the urine.

Adverse Effects Cytarabine is contraindicated in patients who have hypersensitivity to the drug or any of its components. Adverse effects include leukopenia, thrombocytopenia, megaloblastosis, nausea, and vomiting. The cytarabine syndrome is characterized by fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, conjunctivitis and malaise, which usually occurs 6–12 h following drug administration. Corticosteroids are beneficial in treating or preventing this syndrome, and cytarabine therapy may be continued (Castleberry et al. 1981).

The toxicity of cytarabine is dose dependent. Experimental high dose regimens of cytarabine may induce toxicities not commonly associated with standard cytarabine therapies. They include reversible corneal toxicity and hemorrhagic conjunctivitis (preventable or treatable with corticosteroid eye drops), cerebral dysfunctions (personality changes, somnolence, coma), severe gastrointestinal ulceration (pneumatosis cystoides intestinalis, leading to peritonitis, sepsis, liver abscess) or bowel necrosis and necrotizing colitis, pulmonary edema, rare skin rashes leading to desquamation, and sudden respiratory distress (rapidly progressing to pulmonary edema, cardiomegaly). The most characteristic toxicity of high-dose (more than 1 g/m^2 per dose) cytarabine regimens is a cerebellar syndrome of dysarthria, nystagmus, and ataxia. The risk is strongly correlated with advanced age and renal dysfunction. Hepatic dysfunction, high cumulative doses, and bolus dosing may also increase the risks of neurotoxicity. The drug is Pregnancy Category D.

Drug Interactions Acute pancreatitis can occur in patients who have had prior treatment with L-asparaginase. The risk for cardiomyopathy is high when cytarabine is used in combination with cyclophosphamide for bone marrow transplant preparation.

Fazarabine (arabinosyl-5-azacytidine, Ara-AC) is an orally active pyrimidine analog of an aza-substituted cytidine, in which the ribose moiety is replaced by an arabinose sugar. Similar in action to cytarabine, fazarabine is phosphorylated by Deoxycytidine Kinase to a triphosphate form, which competes with thymidine for incorporation into the DNA. Once incorporated, it inhibits DNA synthesis, resulting in tumor cell death.

Drug Resistance The presence of Deoxycytidine Kinase in a tumor is a determinant of its sensitivity to fazarabine.

5,6-dihydro-5-azacytidine (NSC-264880) is a synthetic nucleoside analog of deoxycytidine. Dihydro-5-azacytidine inhibits DNA Methyl Transferase, thereby interfering with the DNA methylation patterns that are associated with genetic instability in some tumor cells. Inhibition of this enzyme may restore the expression of tumor suppressor genes and result in anti-tumor activity.

Decitabine (5-aza-2′-deoxycytidine; 5-AzaC) (NSC-127716) <Dacogen> is a cytidine anti-metabolite that incorporates into DNA, resulting in intra-S phase arrest of DNA reduplication. The agent also inhibits DNA Methyltransferase and exerts anti-cancer activity via the hypo-methylation of DNA (see Sect. 3.2.5.). Decitabine is used for the treatment of myelodysplastic syndrome, acute myelogenous leukemia (AML), and chronic myelogenous leukemia (CML). It was approved by the U.S. FDA in 2004.

Adverse Effects The dose limiting toxicity is bone marrow suppression. Neutropenia occurs in 90% of patients.

It requires a recovery phase of 30–50 days and may need growth factor support. While worsening neutropenia is common in the first two treatment cycles, it may not correlate with progression of an underlying myelodysplastic syndrome. Febrile neutropenia arises in 30 % of patients. Also common are thrombocytopenia (90 %), anemia (80 %), leukopenia (30 %), and lymphadenopathy (10 %).

Gemcitabine hydrochloride (2′-deoxy-2′,2′-difluorocytidine hydrochloride, 4-amino-1-[3,3-difluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl]-1H-pyrimidin-2-one hydrochloride) <Gemzar> is an analog of the anti-metabolite nucleoside deoxycytidine. Gemcitabine exhibits cell cycle specificity, primarily killing cells in S phase and blocking their progression through the G_1/S boundary. The agent was approved by the U.S. FDA in 1998 after a clinical trial reported improvements in the quality of life in patients with advanced prostate cancer. This marked the first U.S. FDA approval of a chemotherapy drug for a non-survival endpoint. Gemcitabine is indicated

- in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (stage IIIA or IIIB), or metastatic (stage IV) non-small cell lung cancer,
- in combination with paclitaxel for the first-line treatment of patients with metastatic breast cancer after failure of prior anthracycline containing chemotherapy, unless anthracyclines were contraindicated.
- as first-line treatment for patients with locally advanced (non-resectable stage II or stage III) or metastatic (stage IV) pancreas adenocarcinoma, also for patients previously treated with 5-fluorouracil,
- in combination with carboplatin for the treatment of patients with advanced ovarian cancer that has relapsed at least 6 months after completion of platinum based therapy.

Gemcitabine is administered by injection over a period of 30 min. The dosage and number of administrations depend on a variety of factors, including the type of cancer, body size, gender, and other concurrent treatments. A common regimen is 1000 mg/m^2 on days 1 and 8 of a 21-day cycle. Dosage adjustment is based on the degree of hematologic toxicity experienced by the patient.

Pharmacokinetics Gemcitabine plasma protein binding is negligible. Compared with cytarabine, gemcitabine achieves intracellular concentrations about 20-fold higher, secondary to an increased penetration of cell membranes via equilibrative and concentrative nucleoside transporters, and a greater affinity for the activating enzyme Deoxycytidine Kinase. The drug is converted intracellularly to the active metabolites difluorodeoxycytidine di- and triphosphate (dFdCDP and dFdCTP).

- dFdCDP inhibits Ribonucleotide Reductase[44], thereby decreasing the deoxynucleotide pool available for DNA synthesis
- dFdCTP is incorporated into DNA, resulting in DNA strand termination and apoptosis.

After incorporation into DNA, gemcitabine has a prolonged intracellular half-life. Drug inactivation to 2′-deoxy-2′,2′-difluorouridine (dFdU) is catalyzed by Cytidine Deaminase. Polymorphisms in this enzyme, in particular homozygous Ala70Thr (CDA*3), are major determinants of drug clearance. The administered dose is almost entirely excreted in the urine as the parent drug (10 %) and its inactive uracil metabolite 2′-deoxy-2′,2′-difluorouridine (90 %). Gemcitabine clearance in women and the elderly is compromised. Therefore, women tend to be somewhat less able to progress to subsequent cycles.

Adverse Effects Gemcitabine may temporarily reduce the number of white blood cells, particularly during the first 10–14 days after administration. Treatment can cause chicken pox or shingles (herpes zoster) to become very severe and spread to various parts of the body. Immunizations or contact with individuals who have recently taken oral polio vaccines should be avoided during or after gemcitabine administration. Flu-like symptoms are common following the first treatment cycle. The drug can also lower the blood platelet count. The risk of bleeding may be reduced by using caution when cleaning teeth, avoiding dental work, and avoiding cuts, bruises, or other injuries.

Non-hematologic adverse effects of gemcitabine may include nausea, vomiting, chills and fever. Gemcitabine induced severe pulmonary toxicity (GISPT) is an inflammatory reaction in the lungs following treatment with gemcitabine. The incidence ranges 0.5–5 % of patients. A potentially fatal adverse event is vasculitis. The hemolytic uremic syndrome is very rare but potentially fatal. The drug is Pregnancy Category D.

Drug Resistance Failure of gemcitabine therapy in pancreatic cancer is often due to chemoresistance after initial response. Resistance is typically mediated by the down-regulation of Equilibrative Nucleoside Transporter 1 (hENT1) and Concentrative Nucleoside Transporter 1 (hCNT1), which are required for the cellular entry of gemcitabine.

Sapacitabine (CYC682) is an orally available 2′-deoxycytidine analog. It synergizes with inhibitors of Histone Deacetylase or DNA Methyl Transferases. The agent can be effectively combined with other nucleoside analogs, particularly clofarabine and gemcitabine, which inhibit Ribonucleotide Reductase.

[44] Inhibitors of Ribonucleotide Reductase include gemcitabine (via its metabolite dFdCDP), clofarabine, fludarabine, inosine dialdehyde, triapine, 5-azacytidine, zebularine, and GTI-2040 (a phosphorothioate oligonucleotide that suppresses gene expression).

Other pyrimidine antagonists Penclomedine (3,5-dichloro -2,4-dimethoxy-6 trichloromethyl pyridine) (NSC338720) is a synthetic derivative of pyrimidine that alkylates and cross-links DNA, resulting in DNA strand breaks and inhibition of DNA and RNA synthesis. This agent is more active against tumor cells that are defective in P53 function than in cells with intact P53. Neurotoxicity (ataxia, vertigo, nystagmus, motor aphasia) may be dose limiting.

m-Azidopyrimethamine is an anti-metabolite derived from diaminopyrimidine. With a mechanism of action similar to that of antifolates, m-azidopyrimethamine blocks tetrahydrofolate synthesis, resulting in the depletion of nucleotide precursors and inhibition of DNA, RNA and protein synthesis. The agent is lipophilic.

Metoprine (DDMP, 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine) (BW 197U) is a diaminopyrimidine folate antagonist that inhibits Dihydrofolate Reductase, resulting in decreased cellular folate metabolism and cell growth. It also inhibits Histamine-N-Methyltransferase, resulting in decreased histamine catabolism. Due to its lipid solubility, metoprine is capable of crossing the blood-brain barrier.

Adverse Effects The drug has a long duration of action, leading to adverse effects related to its blood levels. The usual dose limiting toxicity is thrombocytopenia. Other possible adverse effects include skin rashes, headaches, nausea, and dyspepsia.

Hydroxyurea (hydroxycarbamide, carbamyl hydroxamate) <Hydrea, Droxia, Biosupressin, Litalir, Myelostat> was first synthesized in 1869 by W.F.C. Dresler and R. Stein in Heidelberg, Germany, and was investigated as an anti-neoplastic agent in the 1960s. It is a monohydroxyl-substituted urea anti-metabolite. Hydroxyurea selectively inhibits Ribonucleoside Diphosphate Reductase, an enzyme required to convert ribonucleoside diphosphates into deoxyribonucleoside diphosphates[45]. Thereby the drug prevents cells from leaving the G_1/S phase of the cell cycle. Hydroxyurea also exhibits radio-sensitizing activity by maintaining cells in the radiation sensitive G_1 phase and interfering with DNA repair. The agent is used to treat hematologic malignancies, such as chronic myelogenous leukemia (CML) and myeloproliferative disease, specifically polycythemia vera, essential thrombocytosis, and hypereosinophilic syndrome. It also has had positive results in the treatment of chronic granulocytic leukemia. Responses by melanomata, epidermoid carcinomata, teratocarcinomata, or cervical carcinomata can be achieved.

Hydroxyurea is readily absorbed from the gastrointestinal tract. It can be given orally or intravenously. Numerous dosing schedules exist and depend on underlying disease, initial response, and concomitant therapy. For treating solid tumors in adults, intermittent therapy may comprise 80 mg/kg administered orally as a single dose every 3rd day, while continuous therapy may be 20–30 mg/kg as a single dose daily. Accumulation occurs with daily dosing. For hematologic malignancies, continuous treatment is typically given. The dosage may be reduced or delayed in patients with bone marrow depression due to prior myelotoxic therapy.

Pharmacokinetics Peak blood levels are reached in 1–4 h after an oral dose. The drug is distributed throughout the body and crosses the blood-brain barrier. It concentrates in leukocytes and erythrocytes. Hydroxyurea is converted to the free radical nitroxide (NO) and transported into cells by diffusion. 50–60 % of the agent is metabolized in the liver, resulting in the inactive products carbon dioxide and urea. The major route of elimination with first order kinetics is renal (55 %), the CO_2 metabolite is exhaled. A minor pathway of breakdown may be the degradation by Urease in intestinal bacteria.

Adverse Effects There are marked adverse effects. They include a rapid fall in white cell and platelet counts, nausea and vomiting, diarrhea, stomatitis, alopecia, and impaired renal function[46]. If taken for the long term, hydroxyurea is potentially leukemogenic. Patients who have received irradiation therapy prior to hydroxyurea may have an exacerbation of post-irradiation erythema. Additional, possibly fatal toxicities, including pancreatitis, hepatic failure, or peripheral neuropathy may occur in HIV infected patients during combination therapy that includes hydroxyurea. Hydroxyurea is Pregnancy Category D, the drug is secreted in the milk.

Drug Resistance Resistance to hydroxyurea can arise. It may be caused by elevated synthesis of the Ribonucleoside Diphosphate Reductase component R_2 or by elevated levels of Ferritin.

Anti-pyrimidines inhibit uracil utilization by tumors.
Anti-pyrimidines may radio-sensitize by maintaining cells in the radiation sensitive G1 phase and interfering with DNA repair. However, tumors that have previously been irradiated respond poorly to fluorinated pyrimidines.
Halogenated uracil drugs carry a halogen on uracil or its derivatives.
Cytidine drugs interfere with DNA synthesis by inhibiting the conversion of cytidylic acid to deoxycytidylic acid.
Other anti-pyrimidine drugs have various modes of action.

2.4.3 Anti-Purines

In 1948, Gertrude Elion and George Hitchings discovered diaminopurine, an adenine antagonist, which inhibited

[45] Ribonucleoside Diphosphate Reductase consists of two proteins, R_1 and R_2. Hydroxyurea destroys a tyrosyl free radical that is formed in the catalytic center of the enzyme. This results in the destabilization of the iron center in protein R_2, rendering the enzyme inactive.

[46] Due to the effect of hydroxyurea on the bone marrow, regular monitoring of the full blood count is vital, as well as swift responses to possible infections. In addition, renal function, uric acid and electrolytes, as well as liver enzymes, are commonly checked.

experimentally induced leukemia. Clinical trials in patients were initially promising but had to be terminated due to toxic effects. By 1951, Elion and Hitchings had developed 6-thioguanine and 6-mercaptopurine as a part of a comprehensive chemical program to generate analogs for the purine constituents of nucleic acids. The substitution of sulfur for oxygen in position six of guanine and hypoxanthine produces compounds with substantial anti-tumor activity (Fig. 2.47). These drugs inhibited adenine metabolism and later played an important role in the treatment of acute leukemia. In collaboration with the Sloan-Kettering Institute, 6-mercaptopurine was tested in leukemic patients who were resistant to methotrexate. About 1/3 of the patients responded with complete remission. In part for this work, Elion and Hitchings received the Nobel Prize in Physiology or Medicine in 1988.

One limitation of anti-purines is a relative lack of specificity because purines are required for multiple biochemical pathways in addition to nucleic acid synthesis. These pathways are vital to tumor cells and host cells alike.

First generation purine antagonists 6-Mercaptopurine (6-MP,3,7-dihydropurine-6-thione)<Purinethol>isaprodrug, the free base form of which is converted by sensitive tumor cells via 6-thioinosine 5′-monophosphate (timp) to the ribonucleotide 6-mercaptopurin-9-yl (mprp). 6-Mercaptopurine competes with hypoxanthine and guanine for the enzyme Hypoxanthine-Guanine Phosphoribosyltransferase (HGPRT), and is converted by it to thioinosinate. Further, 6-methylthioinosinate is formed by the methylation of thioinosinate. The intracellular nucleotide thioinosinate inhibits the conversion of inosinate to xanthylate and the conversion of inosinate to adenylate. Both thioinosinate and 6-methylthioinosinate inhibit Glutamine-5-Phosphoribosylpyrophosphate Amidotransferase, the first enzyme unique to the de novo pathway for purine ribonucleotide synthesis.

6-Mercaptopurine is indicated in combination regimens for the maintenance therapy of acute lymphatic (lymphocytic, lymphoblastic) leukemia. However, the response depends on the particular sub-classification of the disease and the age of the patient. The drug is also used for treating pediatric non-Hodgkin lymphomata and sometimes choriocarcinomata. 6-Mercaptopurine may be beneficial for treating the pre-cancerous condition polycythemia vera. The drug is not effective for prophylaxis or treatment of central nervous system leukemia, or for treatment of acute myelogenous leukemia, chronic lymphatic leukemia, and solid tumors.

Derivatives of 6-mercaptopurine have been synthesized with the goal to improve its pharmacological properties:

- The guanosine analog 6-thioguanine (6-TG, 2-amino-6-mercaptopurine) <Tabloid> was synthesized and developed by Hitchings, Elion and associates at the Wellcome Research Laboratories. It is one of a large series of purine analogs, which interfere with nucleic acid biosynthesis. After phosphorylation by Hypoxanthine-Guanine Phosphoribosyltransferase, thioguanine incorporates into DNA and RNA, resulting in the inhibition of DNA and RNA synthesis and cell death. This agent also inhibits Glutamine-5-Phosphoribosylpyrophosphate Amidotransferase, thereby suppressing purine synthesis. Thioguanine is used for the treatment of acute non-lymphocytic leukemia.

  Adverse Effects Serious adverse effects resulting from the exposure to thioguanine include allergic reactions (difficulty breathing, closing of the throat, swelling of the lips, tongue, or face, hives), decreased bone marrow function (extreme fatigue, easy bruising or bleeding, tarry stools, fever or chills, sore throat), impaired liver function (jaundice, abdominal pain), severe nausea, vomiting, diarrhea, loss of appetite, or sores in the mouth. Symptoms of a thioguanine overdose tend to be similar to the adverse effects caused by the medication, although often more severe. Thioguanine causes birth defects if taken during pregnancy.

  Drug Interactions Administration of a live vaccine can be dangerous during treatment with thioguanine. Dose adjustments may be required for patients on balsalazide <Colazal> , mesalamine <Asacol, Pentasa, Rowasa>, olsalazine <Dipentum>, or sulfasalazine <Azulfidine>.

- Azathioprine (6-[(1-methyl-4-nitro-γ-imidazolyl)thio] purine) (BW57–322) <Azasan, Imuran> was synthesized by Ellion and coworkers to provide a 6-mercaptopurine derivative, in which the 6-mercapto group is substituted to limit oxidative degradation. Azathioprine has become one of the most commonly used anti-purine drugs. It is non-enzymatically cleaved to 6-mercaptopurine, the active agent that acts as a purine analog and an inhibitor of DNA synthesis.

  Adverse Effects The principal and potentially serious toxic effects are hematologic and gastrointestinal. There are also risks of secondary infection and neoplasia. The frequency and severity of adverse reactions depend on the dose and duration of azathioprine treatment as well as on the underlying disease or concomitant therapies. Adverse effects of low frequency include skin rashes, alopecia, fever, arthralgias, diarrhea, steatorrhea, and reversible interstitial pneumonitis. Patients with low or absent Thiopurine S-Methyltransferase (TPMT) activity are at an increased risk of developing severe, life threatening myelotoxicity. The drug is Pregnancy Category D.

  Pharmacokinetics 6-Mercaptopurine is given orally at 2.5 mg/kg. However, the absorption of an oral dose is incomplete and variable, averaging approximately 50%. Plasma protein binding is around 20%. Variability in 6-mercaptopurine metabolism is one of the major causes of

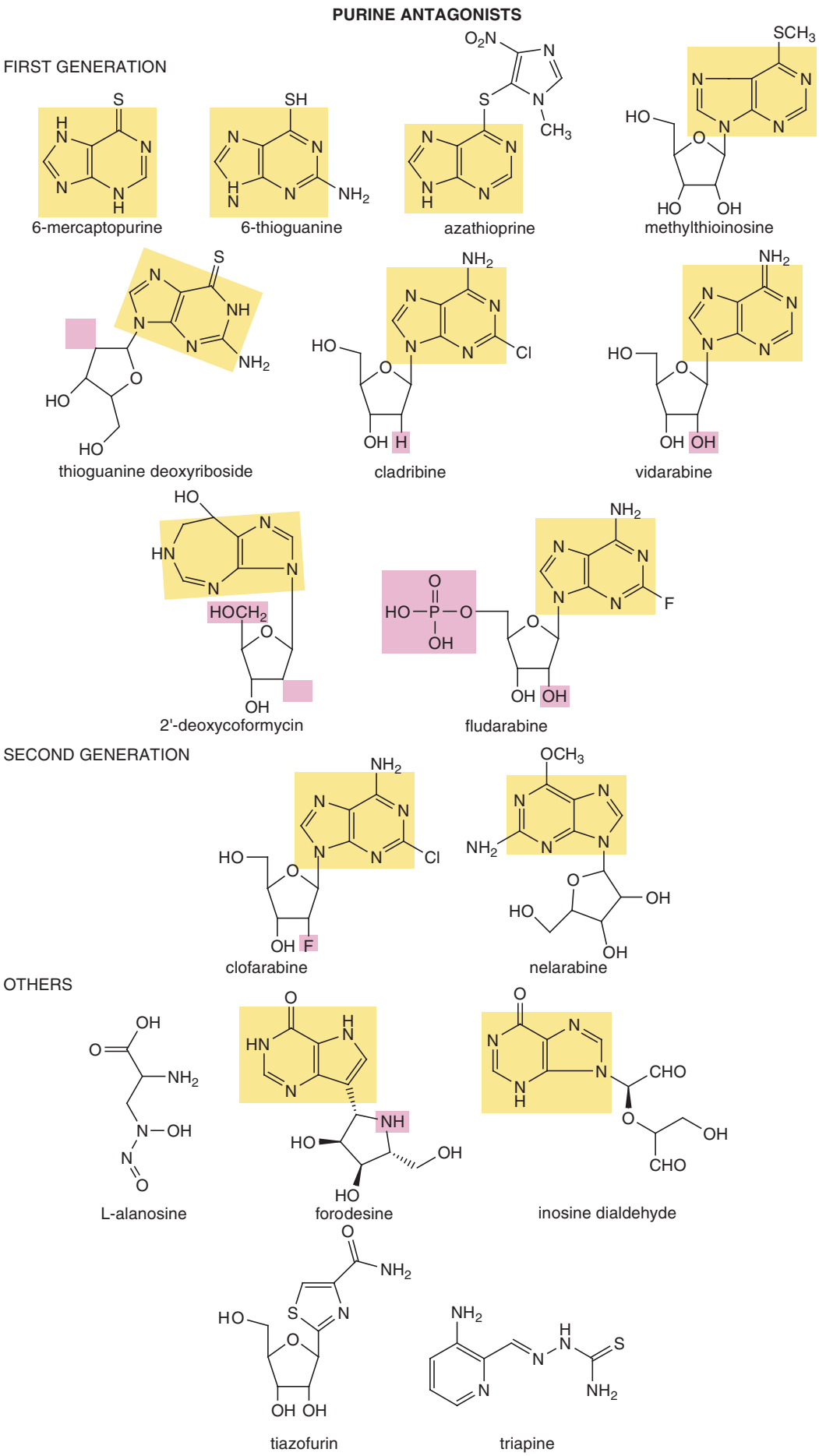


Fig. 2.47 Structures of anti-purines. The common purine moiety is highlighted in *yellow*. Among the first generation anti-purines, the *top row* contains drugs without a sugar moiety, the *second row* depicts ribose containing drugs, and the *third row* shows drugs with arabinose as the sugar. Variations in the structure of the nucleoside sugar are depicted in *pink*

interindividual differences in systemic exposure to the drug and its active metabolites. The half-life of 6-mercaptopurine in the blood is shorter in pediatric patients than in adult patients. It ranges about 0.75–1.5 h, reflecting a combination of cellular uptake and degradation. 6-Mercaptopurine is inactivated via two major pathways,

- thiol methylation is catalyzed by the highly polymorphic enzyme Thiopurine S-Methyltransferase (TPMT), to form methyl-6-mercaptopurine.
- oxidation is catalyzed by Xanthine Oxidase and forms 6-thiouric acid.

The metabolic products are rapidly eliminated by the kidneys, with close to 50% of the administered dose being excreted in the first 24 h.

Adverse Effects Bone marrow depression is the main toxic effect. The induction of complete remission of acute lymphatic leukemia is frequently associated with marrow hypoplasia. This may be manifest as anemia, leukopenia, thrombocytopenia, or any combination of these. The marrow depression is usually reversible upon termination of the drug treatment. Other adverse effects include anorexia, nausea, vomiting, and less frequently alopecia, stomatitis, diarrhea, intestinal ulceration, and dermatologic disorders (skin rashes, hyper-pigmentation). Rare cases of drug fever can arise, but differential diagnosis needs to exclude more common causes of pyrexia in acute leukemia patients, such as sepsis. The drug is Pregnancy Category D.

As a consequence of the rapid death of neoplastic cells, hyperuricemia or hyperuricosuria may occur. The ensuing renal adverse effects can be limited by hydration, urine alkalinization, and the prophylactic administration of a Xanthine Oxidase inhibitor, such as allopurinol. Allopurinol may diminish the rate of 6-mercaptopurine degradation by competing for the oxidizing enzymes that are part of the inactivating pathway, specifically the Xanthine Oxidase mediated degradation to thiouric acid. Therefore, when allopurinol and 6-mercaptopurine are administered concomitantly, the dose of mercaptopurine must be reduced to 1/4–1/3 of the usual dose to avoid severe toxicity. Patients with inherited low Thiopurine S-Methyltransferase activity are at increased risk for severe toxicity from conventional doses of 6-mercaptopurine and generally require substantial dose reduction. In patients with impaired renal or hepatic function the starting doses of 6-mercaptopurine should be reduced. Clinically detectable jaundice can be an adverse effect. It usually appears within the first 1–2 months in the course of treatment. Hepatic injury can occur with any dosage, but seems have higher frequency when doses of 2.5 mg/kg/day are exceeded. In rare cases, fatal hepatic necrosis may be a consequence of 6-mercaptopurine therapy. Hepatic encephalopathy has occurred.

Drug Resistance 6-Mercaptopurine should not be used in patients whose disease has demonstrated prior resistance to this drug. There is usually complete cross-resistance between mercaptopurine and thioguanine. Drug resistance can arise through several mechanisms. Some tumors that are resistant have lost the ability to convert 6-mercaptopurine to 6-thioinosine 5′-monophosphate. A loss of 5-Phosphoribosyl Transferase in the tumor cells may lead to the inability to convert the free base form of 6-mercaptopurine to the active ribonucleotide 6-mercaptopurin-9-yl.

Drug Interactions Enhanced marrow suppression may occur in some patients who also receive trimethoprim-sulfamethoxazole. 6-Mercaptopurine can inhibit the anti-coagulant effect of warfarin, when given simultaneously. Because aminosalicylate derivatives may inhibit the enzyme Thiopurine S-Methyltransferase (TPMT) they should be administered with caution to patients on 6-mercaptopurine therapy.

The mercaptopurine derivative methylthioinosine (6-(methylmercapto)purine riboside, 6-MMPR) inhibits Amidophosphoribosyl Transferase, the first committed step in de novo purine synthesis, and inhibits cell proliferation induced by Fibroblast Growth Factor-2 (FGF-2).

β-Thioguanine deoxyriboside is a thiopurine nucleoside derivative. After conversion to a triphosphate, β-thioguanine deoxyriboside is incorporated into DNA, resulting in inhibition of DNA reduplication. This agent is cytotoxic against leukemia cells. It demonstrates anti-neoplastic activity against 6-thioguanine resistant tumor cells.

Cladribine (2-chlorodeoxyadenosine, 2-CDA) <Leustatin, Litak> is an adenosine analog. Cladribine triphosphate, a phosphorylated metabolite of cladribine, incorporates into DNA, resulting in single strand breaks, and depletion of nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP). These events lead to apoptosis. Because the agent is resistant to Adenosine Deaminase (ADA), an enzyme that inactivates some anti-neoplastic agents, it is selectively toxic to lymphocytes and monocytes, which exhibit little Deoxynucleotide Deaminase activity. Cladribine is mostly used for the treatment of hairy cell leukemia. It is also a second-line treatment for patients with chronic lymphocytic leukemia (CLL) who have not responded to alkylating agents. The drug is administered by injection or infusion. The recommended dose and schedule for active hairy cell leukemia is as a single course given by continuous infusion for seven consecutive days at 0.09 mg/kg/day.

Adverse Effects Treatment should be delayed or discontinued if renal toxicity or neurotoxicity occurs. Common adverse effects include fatigue, bone marrow depression (neutropenia predisposing to infections), and fever. Less common adverse effects comprise dizziness, insomnia,

abdominal cramps and constipation, diarrhea, skin reaction at the infusion side (swelling, pain, redness), tachycardia, swollen ankles (arthralgia), cough, or loss of fertility. The drug is Pregnancy Category D.

The two nucleosides spongothymidine and spongouridine, isolated in the 1950s from the Caribbean sponge Tethya crypta, provided the structural basis for the exploration of the sugar-modified nucleoside analog vidarabine (2-amino-2-deoxy-β-arabinofuranosyladenine, adenine arabinoside, Ara-A). The drug was first synthesized in 1960 by Bernard Randall Baker at the Stanford Research Institute. In 1969, it was isolated from the fermentation beers of Streptomyces antibioticus.

Vidarabine contains an arabinoside sugar rather than a ribose. The agent inhibits DNA Polymerase, resulting in the inhibition of DNA reduplication. Due to this mechanism, cytotoxicity is S phase specific. Vidarabine has been administered as an anti-leukemic drug. However, it has limited clinical usefulness because of its rapid inactivation by Adenosine Deaminase.

A product of the fermentation by Streptomyces antibioticus is 2′-deoxycoformycin (co-vidarabine, dCF) (NSC-218321, CL-825) <Premarin, Pentostatin, Nipent>. Co-vidarabine is a 6-thiopurine analog of the naturally occurring purine bases hypoxanthine and guanine. The agent is an inhibitor of Adenosine Deaminase (ADA), an enzyme that is critical in purine base metabolism and has its highest activity in cells of the lymphoid system. T-lymphocytes have higher Adenosine Deaminase activity than B-lymphocytes, and T-cell malignancies have higher activity than B-cell malignancies. The inhibition of Adenosine Deaminase by deoxycoformycin leads to an intracellular accumulation of deoxy-ATP, which causes apoptosis. The drug is effective in the treatment of several lymphoproliferative conditions, particularly acute non-lymphocytic leukemia, hairy-cell leukemia that is refractory to Interferon-α, and relapsed chronic lymphocytic leukemia (CLL). It is not suitable for the treatment of acute lymphocytic leukemia because the transformed cells contain such high levels of Adenosine Deaminase that their suppression would require toxic doses of 2′-deoxycoformycin. Deoxycoformycin is given by intravenous bolus injection or diluted in a larger volume for infusion over 20–30 min once every 2 weeks for 3–6 months until a complete response is achieved, then two additional cycles are administered. The standard dosage is 4 mg/m^2.

Pharmacokinetics Deoxycoformycin is not absorbed orally. Plasma protein binding after injection is 5% and the half life is 5–6 h. The agent crosses the blood-brain barrier. Biotransformation occurs primarily in the liver, but only small amounts are metabolized. 90% of the drug is eliminated in the urine unchanged or as metabolites. Patients should receive hydration with 0.5–1 L of 5% dextrose in saline before administration. An additional 0.5 L should be administered after deoxycoformycin is given.

Adverse Effects Common adverse effects (occurring in more than 30% of patients) include nausea and vomiting, low blood counts, skin rashes, fever, and fatigue. Less common adverse effects comprise itching, muscle aches, chills, headache, diarrhea, abdominal pain, anorexia, weakness, upper respiratory infections, mouth sores, cough, and shortness of breath. Patients with severe pre-existing infections should not receive deoxycoformycin. It is contraindicated in patients with severe renal impairment. The drug is Pregnancy Category D.

Drug Interactions Simultaneous treatment of deoxycoformycin with clozapine or carbamazepine is to be avoided. The combined use with fludarabine may lead to severe, even fatal, pulmonary toxicity.

Fludarabine phosphate ([(2R,3R,4S,5R)-5-(6-amino-2-fluoro-purin-9-yl)-3,4-dihydroxy-oxolan-2-yl]methoxy-phosphonic acid, 2-fluoro-ara-AMP) <Fludara, Oforta> is a fluorinated analog of vidarabine.

- Fludarabine phosphate is susceptible to glycosidic bond cleavage, which results in the formation of 2-fluoro-adenine, and is further converted to the highly toxic 2-fluoro-ATP.
- Fludarabine phosphate may be rapidly dephosphorylated by Phosphorylase cleavage to 2-fluoro-arabinosyladenine and then phosphorylated intracellularly by Deoxycytidine Kinase to the active triphosphate, 2-fluoro-arabinosyl-ATP. This metabolite inhibits DNA Polymerase α, Ribonucleotide Reductase, and DNA Primase, thereby interrupting DNA synthesis and inhibiting tumor cell growth.

Fludarabine is used in the treatment of hematologic malignancies. Specifically, it is indicated for patients with B-cell chronic lymphocytic leukemia after an initial treatment with alkylating agents has failed. The recommended adult dose of the intravenous drug is 25 mg/m^2, administered over a period of approximately 30 min daily for five consecutive days. The recommended adult dose of the tablet form (to be swallowed with water) is 40 mg/m^2 daily for five consecutive days. Cycles should commence every 28 days. The dosage may be decreased or delayed based on hematologic, neurologic, or other toxicity.

Adverse Effects Fludarabine causes anemia, thrombocytopenia and neutropenia. Severe pancytopenia can arise and sometimes lead to death. The association with profound lymphopenia is a substantial risk factor for opportunistic infections. Patients on fludarabine are often treated with cotrimoxazole or monthly nebulized pentamidine to prevent Pneumocystis jiroveci pneumonia. Ractivations of latent

viral infections, such as varicella zoster virus (Herpes zoster), Epstein-Barr virus (lymphoproliferative disorders), or JC virus (progressive multifocal leukoencephalopathy) have occurred. Because the lymphopenia also renders patients susceptible to potentially fatal transfusion associated graft-versus-host disease, all patients who have ever received fludarabine should only be given irradiated blood components.

Fludarabine is associated with the development of severe autoimmune hemolytic anemia or autoimmune thrombocytopenia in a proportion of patients. Other common adverse events include fever and chills, infection, nausea and vomiting, malaise, fatigue, and anorexia. Less common are confusion, seizures, optic neuritis or optic neuropathy, adult respiratory distress syndrome (ARDS), pulmonary fibrosis, and skin rashes. Advanced age, renal insufficiency, or bone marrow impairment constitute predispositions to increased fludarabine toxicity. The drug is Pregnancy Category D.

Drug Interactions The use of fludarabine in combination with deoxycoformycin is not recommended due to the risk of severe pulmonary toxicity.

Second generation purine antagonists Clofarabine (2-Chloro-9-(2-deoxy-2-fluoroarabinofuranosyl)adenine) <Clolar, Clofarex, Evoltra> is a second generation nucleoside analog, which was developed to reduce the glycosidic cleavage that its predecessor drugs are susceptible to. The agent is activated to its triphosphate inside the tumor cells.

- Clofarabine acts by terminating DNA chain elongation and inhibiting DNA repair through incorporation into the DNA. When the ratio of clofarabine triphosphate to dATP is > 1, clofarabine monophosphate is preferentially inserted into the end of the DNA chain, resulting in the termination of chain elongation. A ratio of clofarabine triphosphate to dATP < 1 results in the insertion of clofarabine monophosphate into the middle of the DNA structure and inhibits DNA repair.
- Clofarabine triphosphate inhibits Ribonucleotide Reductase, leading to a depletion of the deoxyribonucleotide triphosphate (dNTP) pools.
- Clofarabine induces apoptosis through direct and indirect action on mitochondria by releasing Cytochrome *c* and other pro-apoptotic factors, including AIF (Apoptosis Inducing Factor), APAF-1 (Apoptotic Protease Activating Factor-1), and Caspase-9.

The agent received accelerated approval from the U.S. FDA in 2004. Clofarabine is used for the treatment of pediatric patients (up to 21 years), with relapsed or refractory acute lymphoblastic leukemia, consecutive to at least two prior regimens.

Pharmacokinetics Except for tissues with specialized barriers, such as eyes, brain, and testes, clofarabine distribution is fairly widespread, with the highest concentrations occurring in the metabolic and excretory organs (kidneys and liver) and in the gastrointestinal tract (small and large intestine). Clofarabine is a prodrug that gains entry into cells primarily by nucleoside transporter mechanisms. Nucleoside transporters are specialized proteins located in the cell membrane that mediate the uptake and release of nucleosides and nucleoside analogs. Clofarabine is efficiently taken up via the equilibrative transporters hENT1 and hENT2, and the concentrative transporter hCNT247. The abundance of nucleoside transporters on a tumor or host cell may be a major determinant of the amount of nucleoside or nucleoside analog that is taken up into a particular tissue type. At higher concentrations and longer exposure times, the slightly lipophilic agent clofarabine also enters cells by passive diffusion across lipid membranes.

Clofarabine is phosphorylated by cytosolic kinases, including Deoxycytidine Kinase (DCK) and a Purine Nucleoside Monophosphate Kinase, to yield the active form clofarabine triphosphate. Transformed cells have higher DCK concentrations than their healthy counterparts.

Because of the natural lipophilicity of the parent drug and the need to de-phosphorylate the active clofarabine nucleotides prior to their export out of the cell, the tissue-to-blood concentration ratios in organs without special barriers are almost always larger than two (Bonate et al. 2006). Removal of the phosphates by 5′-Nucleotidase is a prerequisite for the cellular export of clofarabine. Hence, the ratio of DCK to 5′-Nucleotidase activities may be an indicator of tumor cell sensitivity to this drug. Even though clofarabine has a short half-life of 4–6 h its active metabolite remains in the cells much longer, having a half-life of at least 24 h (Gandhi et al. 2003).

Adverse Effects The adverse effects of the drug, involving bone marrow, lymphoid tissue, heart, and liver, are not the result of unique tissue uptake. The predominant toxicities are reversible liver dysfunction (reflected in elevated Transaminases and hyperbilirubinemia), skin rashes, palmoplantar erythrodysesthesia (hand-foot syndrome), and mucositis. Clofarabine can produce systemic inflammatory response syndrome (SIRS) and capillary leak syndrome, manifested by the rapid development of tachypnea, tachycardia, hypo-

[47] The ENT transporters work bi-directionally, driven by the nucleoside concentration gradient between the inside and the outside of the cell membrane. The CNT channels transport purine nucleosides inward, coupled to the inward movement of sodium along its concentration gradient.

tension, shock, and multi-organ failure. Cardiac toxicity may cause tachycardia or left ventricular systolic dysfunction.

Nelarabine (506U78) <Arranon, Atriance> is an arabinonucleoside anti-metabolite prodrug. After uptake, it is *O*-demethylated by Adenosine Deaminase to ara-G, monophosphorylated by Deoxyguanosine Kinase and Deoxycytidine Kinase, and subsequently converted to the biologically active 9-β-D-arabinosylguanine 5′-triphosphate (ara-GTP). Arabinosylguanine incorporates into DNA, thereby inhibiting DNA synthesis and inducing the apoptosis of tumor cells during S phase. Nelarabine is indicated as a chemotherapy drug for T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma that has not responded to or has relapsed following treatment with at least two chemotherapy regimens. It was approved in the U.S. in October 2005 and in the European Union in 2007. The recommended adult dose is 1500 mg/m^2 (650 mg/m^2 for pediatric patients) administered intravenously over 2 h on days 1, 3, and 5. This cycle is repeated every 21 days. Treatment is generally continued until there is evidence of disease progression, until the patient experiences unacceptable toxicity, until the patient becomes a candidate for bone marrow transplantation, or until the patient no longer benefits from treatment.

Pharmacokinetics Conversion of nelarabine to arabinosylguanine occurs rapidly and extensively after injection. Less than 25% of nelarabine or its metabolite are bound to plasma proteins. Both are partially eliminated by the kidneys (5–25% over 24 h). Age and gender have no effect on the pharmacokinetics of nelarabine or arabinosylguanine.

Adverse Effects Adverse events associated with the use of nelarabine may include

- in the pediatric population, hematologic toxicities (anemia, neutropenia, leukopenia, thrombocyopenia), infections, asthenia, liver damage (elevation of hepatic enzymes), vomiting, deficiencies of potassium, calcium, or magnesium
- in the adult population, hematologic toxicities (anemia, neutropenia, thrombocyopenia), febrile neutropenia, fatigue, asthenia, nausea, cough, dyspnea, edema, and petechiae.

Neurological adverse events arise in over 60% of subjects. Most are mild to moderate (grade 1–2), including headache, somnolence, neuropathy, hypoesthesia. Grade 4 and grade 5 (fatal) adverse neurological events (including 3rd and 6th nerve paralysis, progressive multifocal leukoencephalopathy, demyelination similar to Guillain-Barré syndrome, cerebral and intracranial hemorrhage, coma, and metabolic encephalopathy) are possible. Close monitoring of such events is recommended. The appearance of grade 2 or higher neurological toxicities may require the discontinuation of treatment. Appropriate measures must be taken to prevent hyperuricemia. They may include hydration, urine alkalinization, and prophylaxis with allopurinol. The drug is Pregnancy Category D.

Drug Interactions 2′-deoxycoformycin is an inhibitor of Adenosine Deaminase, which may cause a reduction in the activation of the prodrug nelarabine. Co-administration can lead to a reduction in nelarabine efficacy or changes in the adverse reaction profile of either drug.

Other purine antagonists Purine Nucleoside Phosphorylase (PNP) is an enzyme in the purine salvage pathway that mediates the phosphorolysis of (deoxy)nucleoside analogs to their respective bases and (deoxy)ribose phosphate. Forodesine hydrochloride (BCX-1777) <Immucillin-H> is a salt of the synthetic high affinity transition state analog forodesine[48]. Forodesine binds preferentially to and inhibits Purine Nucleotide Phosphorylase, resulting in the accumulation of deoxyguanosine triphosphate, the subsequent inhibition of the enzyme Ribonucleoside Diphosphate Reductase[49], and the abrogation of DNA synthesis. This agent selectively causes apoptosis in stimulated or transformed T-lymphocytes. It is orally available. Forodesine has been granted orphan drug status by the U.S. FDA for T-cell non-Hodgkin lymphoma, chronic lymphocytic leukemia (CLL) and related leukemias, and for treatment of B-cell acute lymphocytic leukemia (ALL).

Inosine dialdehyde (NSC 118994) <Inox> is a toxic purine analog that inhibits Ribonucleotide Reductase, resulting in the decreased synthesis of DNA, RNA, and proteins, and leading to cell cycle arrest at the G_2/M transition. This agent also forms stable covalent cross-links in proteins, thereby inhibiting the activity of enzymes involved in nucleic acid synthesis. Thus, the effects of inosine dialdehyde are different from those caused by typical inhibitors of Ribonucleotide Reductase, which rapidly suppress DNA synthesis and cause arrest of the cells in G_1, with minimal effects on RNA and protein synthesis.

In salvage pathways, nucleotides are synthesized from intermediates of canonical degradative pathways. These salvage pathways recover nucleosides (nucleobases bound to sugars) and nucleotides (composed of nucleobase, sugar, and at least one phosphate) that are formed during the degradation of RNA and DNA. They are particularly important in tissues that cannot undergo de novo synthesis. Most healthy cells utilize both de novo purine synthesis and the adenine

[48] A transition state analog is a substrate designed to mimic the properties or the geometry of the transition state of a reaction.

[49] Ribonucleoside Diphosphate Reductase is also inhibited by hydroxyurea.

salvage pathway to produce ATP. Methylthioadenosine Phosphorylase (MTAP) plays a major role in polyamine metabolism and is important for the salvage of both adenine and methionine. Its encoding gene is located in the same 9p21 chromosomal region as the gene for the tumor suppressor $P16^{INK4A}$. *mtap* and *p16*, along with other genes in that region, are frequently co-deleted in select cancers. Tumors deficient in Methylthioadenosine Phosphorylase are dependent on the de novo purine synthesis pathway, making them potential targets for selective chemotherapy with inhibitors of adenine synthesis.

L-alanosine (L-2-amino-3-(N-hydroxy-N-nitrosamino) propionic acid) (NSC153353, SDX-102) is an amino acid analog derived from Streptomyces alanosinicus, a purine antagonist, and an Adenylosuccinate Synthetase inhibitor. L-alanosine deprives Methylthioadenosine Phosphorylase deficient tumor cells of de novo synthesized adenosine and is responsible for diminished DNA synthesis.

Adverse Effects The clinical use of L-alanosine may be narrow due to its toxicity profile. Mucositis, fatigue, nausea, or renal failure can be dose limiting. In several clinical trials, tolerable doses of L-alanosine have not shown efficacy.

Tiazofurin (2-β-D-ribofuranosyl-4-thiazolecarboxamide) (NSC 286193) <Riboxamide> is a synthetic nucleoside analog. It is anabolized intracellularly to tiazole-4-carboxamide adenine dinucleotide (TAD), an analog of nicotinamide adenine dinucleotide (NAD) and a potent inhibitor of Inositol Monophosphate Dehydrogenase (IMPDH). Inositol Monophosphate Dehydrogenase is the rate limiting enzyme for de novo purine synthesis, the inhibition of which leads to reduced levels of guanylates, resulting in the suppression of tumor cell growth.

Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) (OCX-191) is a synthetic heterocyclic carboxaldehyde thiosemicarbazone. Triapine acts as an iron chelator and suppresses the enzyme Ribonucleotide Reductase. This inhibits the conversion of ribonucleoside diphosphates to deoxyribonucleotides necessary for DNA synthesis. Triapine is administered at a dose of 95 mg/m^2 by intravenous infusion daily for 5 days, on a biweekly schedule. It displays an acceptable safety profile.

Sensitive tumor cells convert the prodrug 6-mercaptopurine to thioinosinate and 6-methylthioinosinate, which inhibit Glutamine-5-Phosphoribosylpyrophosphate Amidotransferase, the first enzyme unique to the de novo synthesis pathway for purine ribonucleotides.

The Adenosine Deaminase (ADA) inhibitor co-vidarabine is effective against several lymphoproliferative conditions, but not acute lymphocytic leukemia because the transformed cells contain too high levels of Adenosine Deaminase.

After uptake, nelarabine is O-demethylated by Adenosine Deaminase to ara-G, mono-phosphorylated by Deoxyguanosine Kinase and Deoxycytidine Kinase, and subsequently converted to the biologically active 9-β-D-arabinosylguanine 5′-triphosphate (ara-GTP).

Because cladribine is resistant to Adenosine Deaminase it is selectively toxic to lymphocytes and monocytes, which exhibit little Deoxynucleotide Deaminase activity.

Methylthioadenosine Phosphorylase-deficient tumors depend on the de novo purine synthesis pathway, making them targets for inhibitors of de novo adenine synthesis, such as L-alanosine.

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