Chemotherapy
Part 1

[DNA Alkylating agents]

Dr. Mai Ramadan

Introduction

Alkylating agents can be defined as compounds capable of **covalently** attaching an alkyl group to a biomolecule (DNA) under physiological conditions

Drugs always behave as **carbon electrophiles**

Two types:

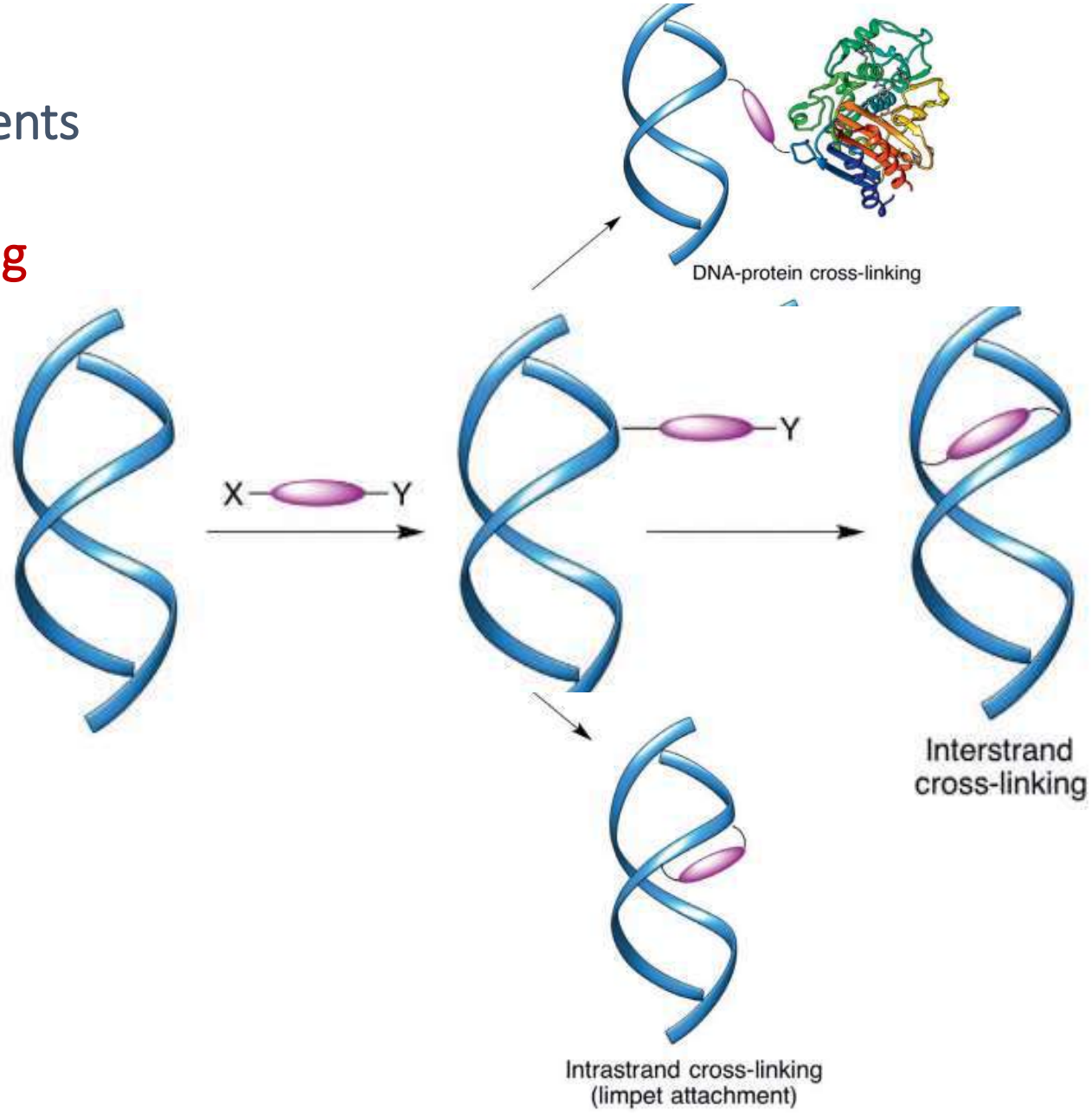
Monofunctional alkylating agents [Can form one covalent bond with DNA]

Bifunctional alkylating agents [Can form two covalent bonds with DNA Intrastrand-, interstrand- cross linkage, DNA and enzymes]

Introduction

Bifunctional alkylating agents

X,Y are leaving groups



Introduction

DNA Alkylating agents **[Mechanism of action]**:

Alkylation prevents DNA replication and RNA transcription

The fragmentation of DNA

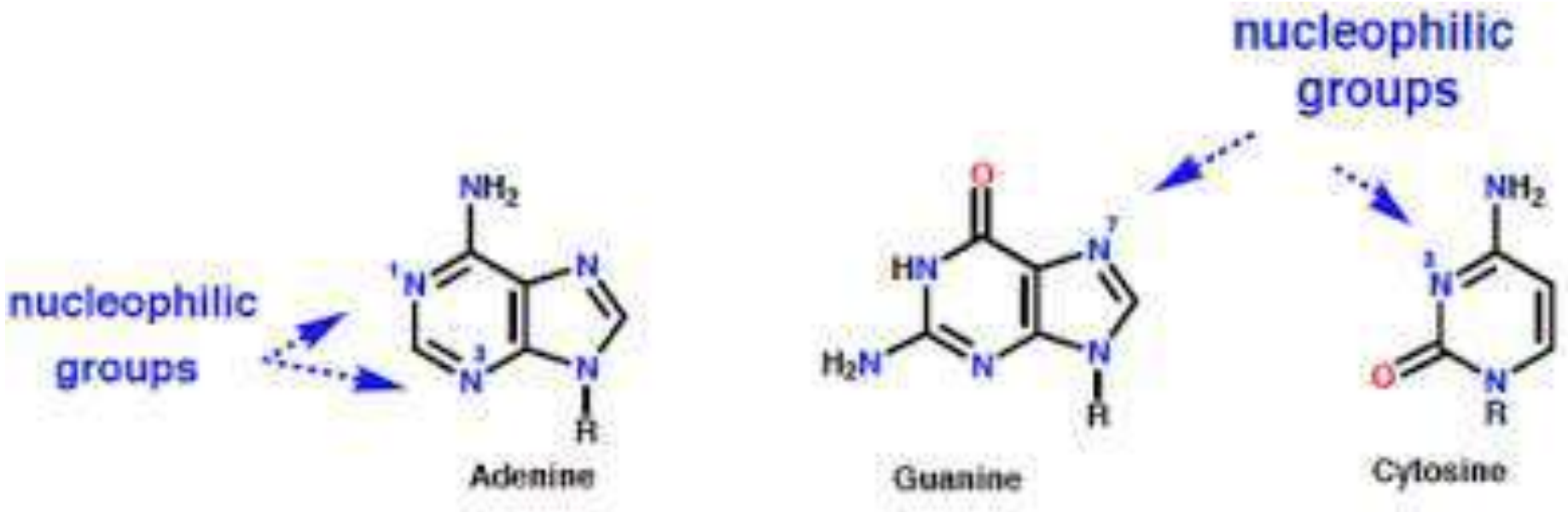
Alkylation induces the mispairing of the nucleotides by alteration of the normal hydrogen bonding between bases.

Bis-alkylation can form bridges within a single DNA strand (intrastrand cross linkage) or between two complementary DNA strands (interstrand cross-linkage), preventing their separation during DNA replication or transcription.

Introduction

DNA alkylating agents react at (**Nucleophilic sites**)

Nitrogen sites in the following order: N7 of guanine>N1 of adenine>N3 of cytosine>N3 of thymine.



Anticancer: DNA alkylating agents

N- Mustard: Chlormethamine, Chlorambucil, Melphalan, Cyclophosphamide, Ifosphamide, Estramustine

Nitrosurea : Carmustine

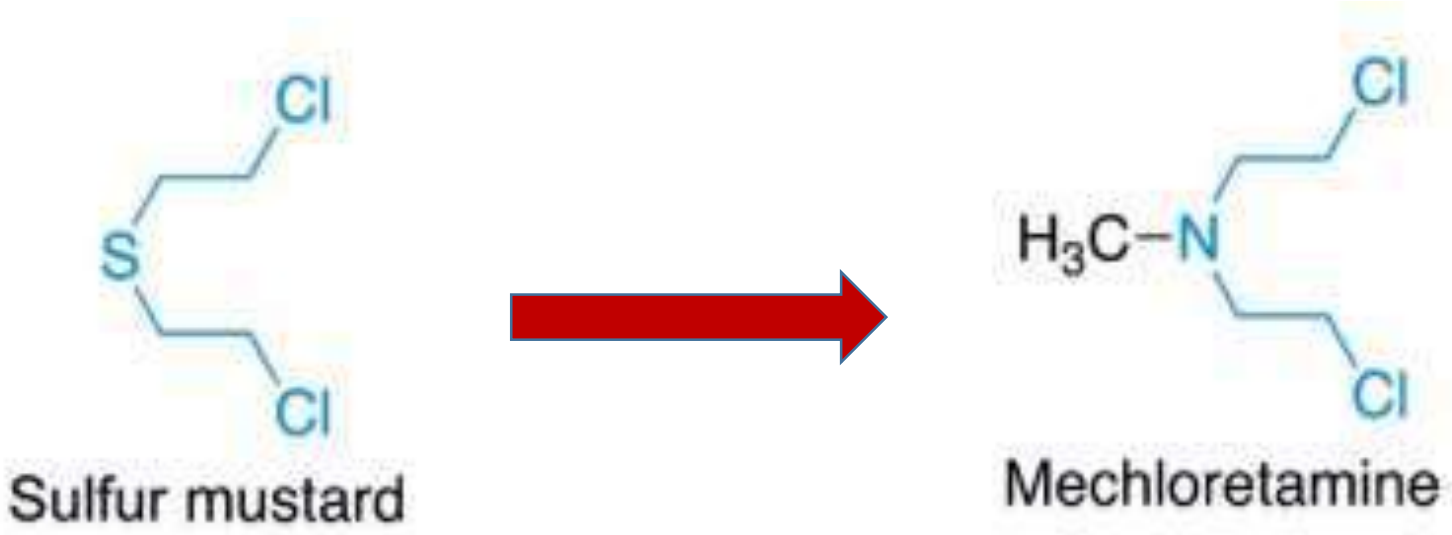
Thiotepa

Triazene: Dacarbazine, Timozolomide

Miscellaneous: Busulfan

Organoplatinium (II) complexes: Cisplatin, Carboplatin

DNA ALKYLATION BY NITROGEN MUSTARDS

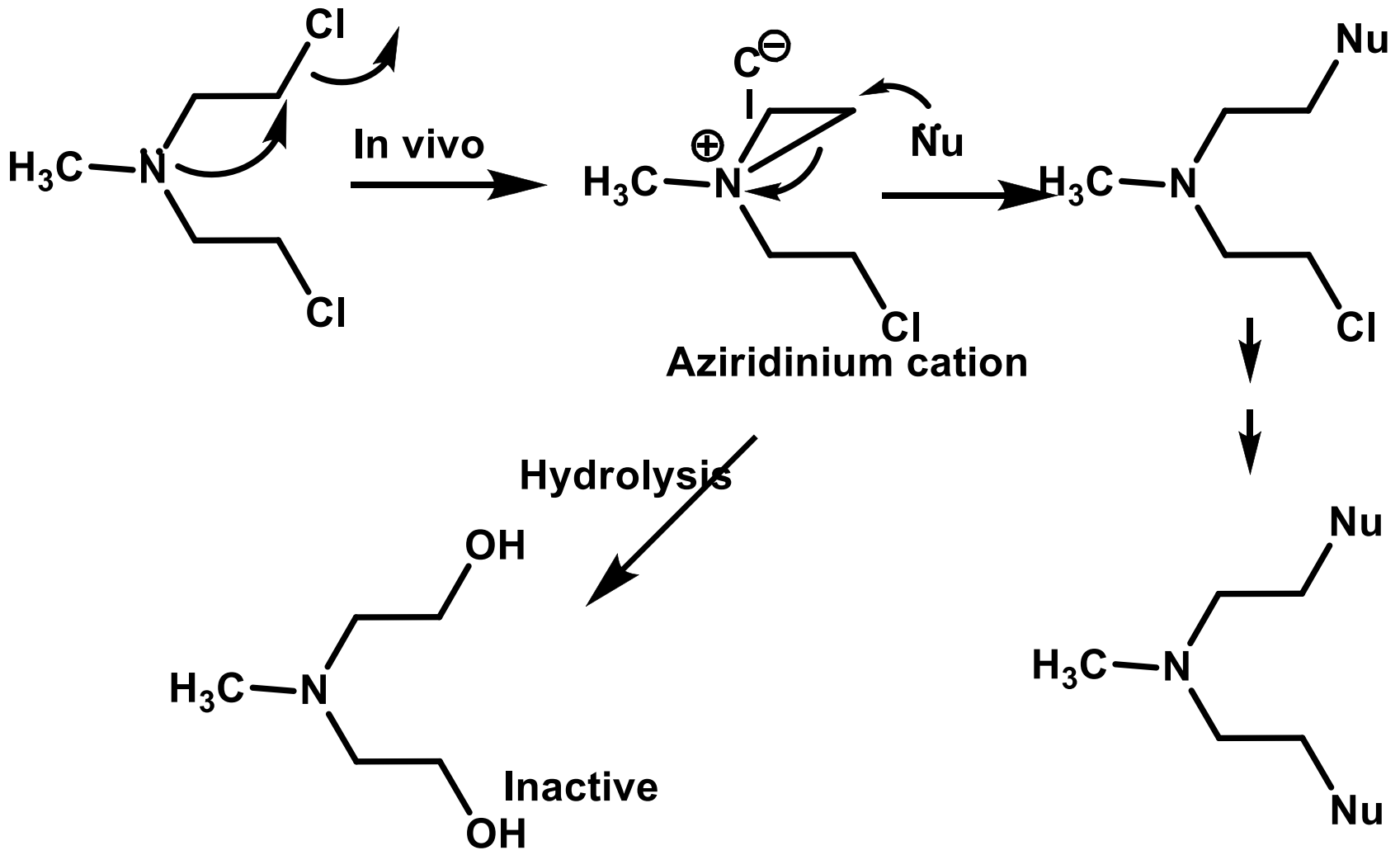


Sulfur mustard (mustard gas) was used in World War I
After the war, it was realized that it also caused systemic effects such as leukopenia, aplasia of the bone marrow, dissolution of lymphoid tissue.

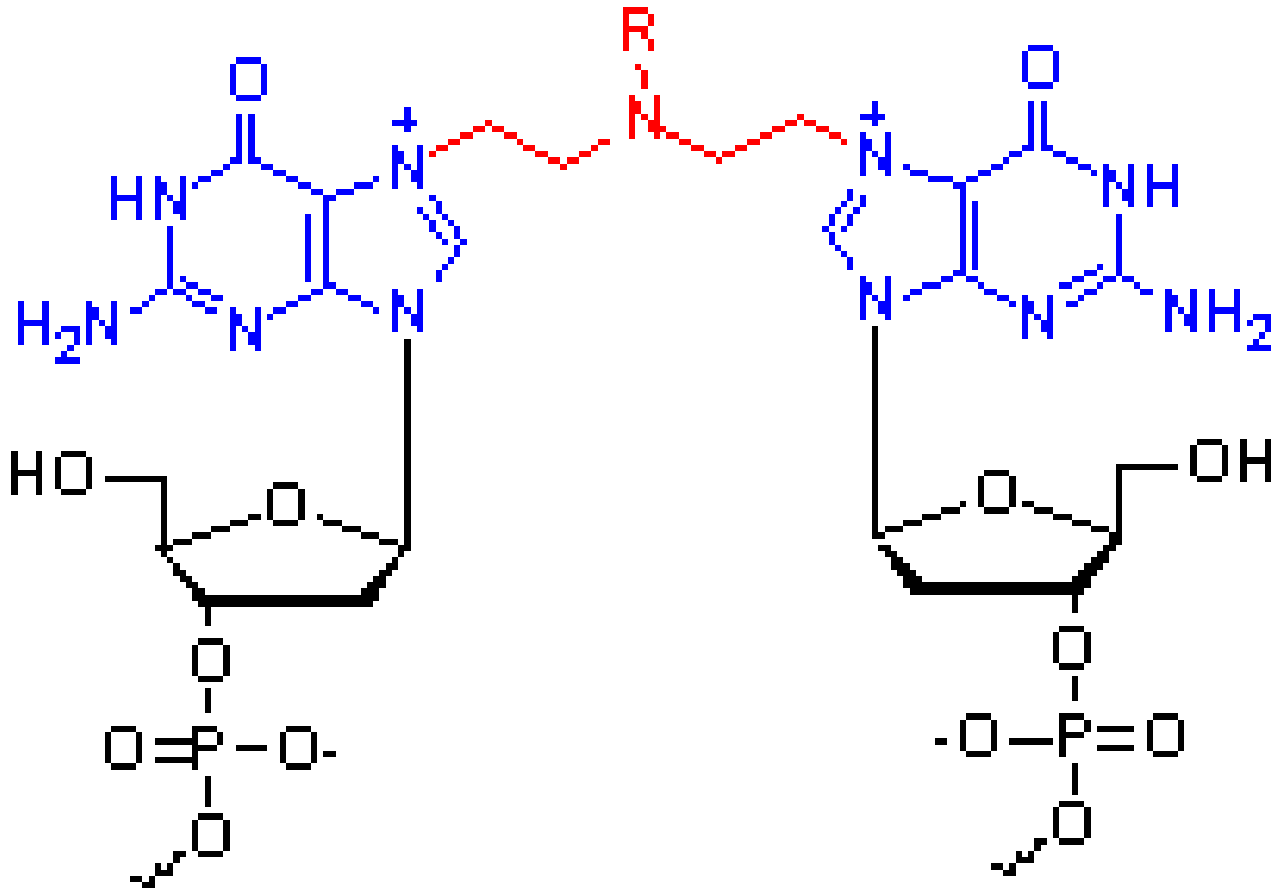
DNA Alkylation by N-mustard

Mechlormethamine Hydrochloride

2,2-Dichloro,N-methyl-diethyldiethylamine hydrochloride



DNA Alkylation by N-mustard



DNA crosslink formed from reaction at N-7 guanine on both strands (G-G crosslink)

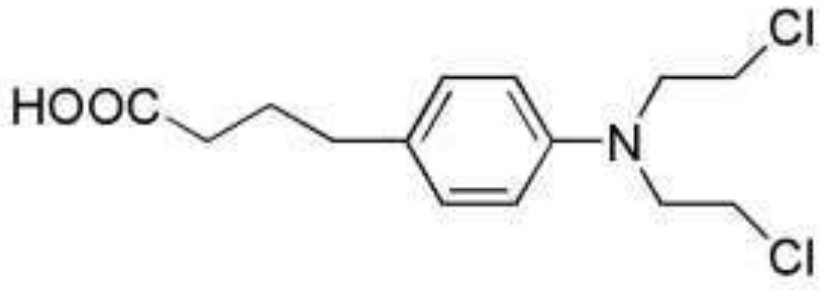
DNA Alkylation by N-mustard

Mechlormethamine is given IV

Aziridinum ion is very reactive deactivated by hydrolysis to inactive diol

Side effect: Intense local reactions at the site of injection. $S_2O_3^{-2}$ injection at site, reacts very rapidly with aziridinium ion and protect from side effects.

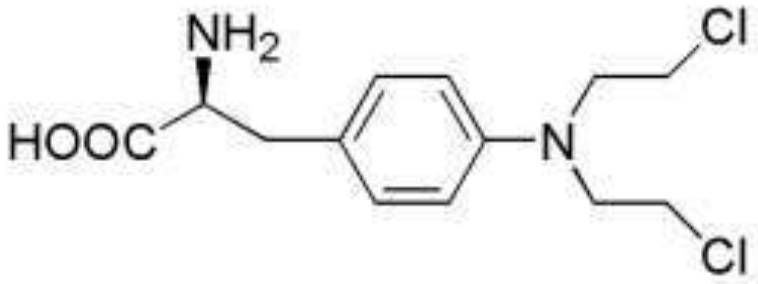
DNA Alkylation by N-mustard



Chlorambucil

Dichloroethyl residues as in mechlomethamine

Aromatic ring and butanoic acid



Melphalan

Aromatic ring is a part of L- phenylalanine

The aminoacid is used as it believed that it is transported into the cancer cells preferentially

DNA Alkylation by N-mustard

Aryl substituted nitrogen mustards like chlorambucil and melphalan

Aryl group in N-mustard lead to:

Stabilize electron pair of nitrogen by resonance [decrease nucleophilicity of N atom]

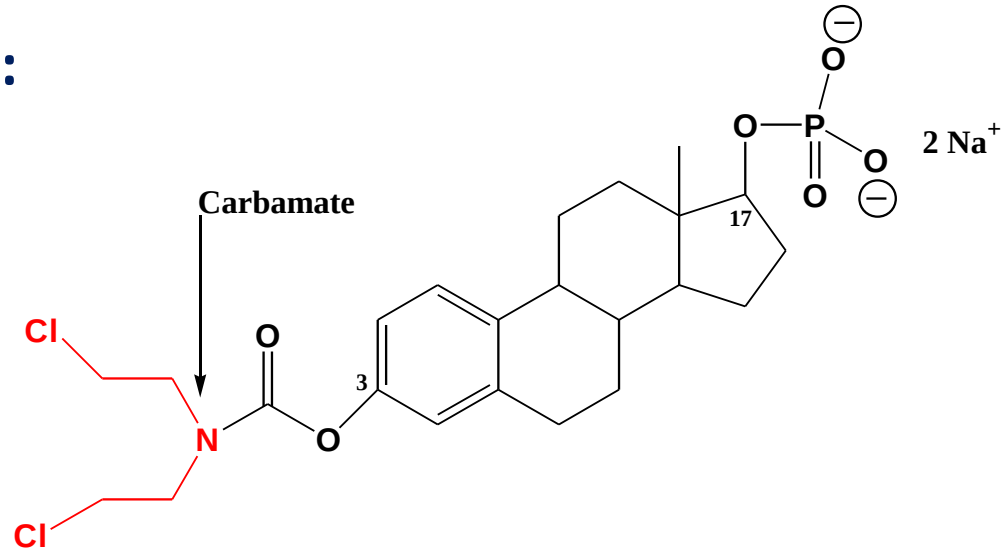
Slow the rate of aziridinum ion formation and DNA alkylation.

Orally active and Less side effect severity

DNA Alkylation by N-mustard

Estramustine phosphate sodium:

Estradiol is a carrier of the drug to selectively deliver drug to steroid dependent prostate.



Estramustine phosphate sodium

Site directed nitrogen mustard

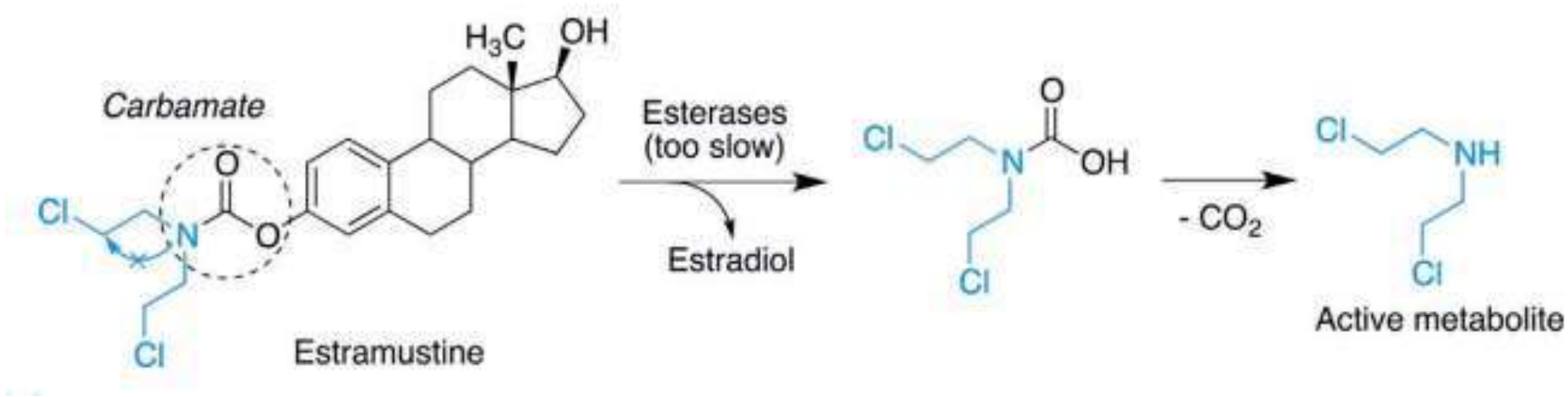
Phosphate ester water soluble

Hydrolyze rapidly to 17β-OH in blood [Estramustine]

Hydrolysis of carbamate ester from phenolic OH at position 3 is slowly.

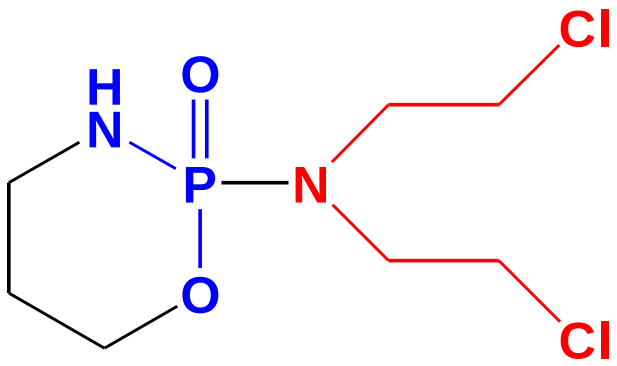
DNA Alkylation by N-mustard

Estramustine phosphate sodium:

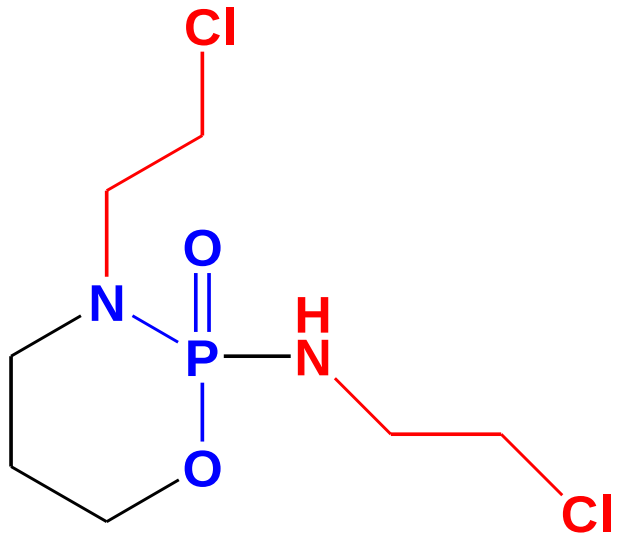


Estramustine phosphate sodium can be given orally

DNA Alkylation by N-mustard



Cyclophosphamide



Ifosfamide

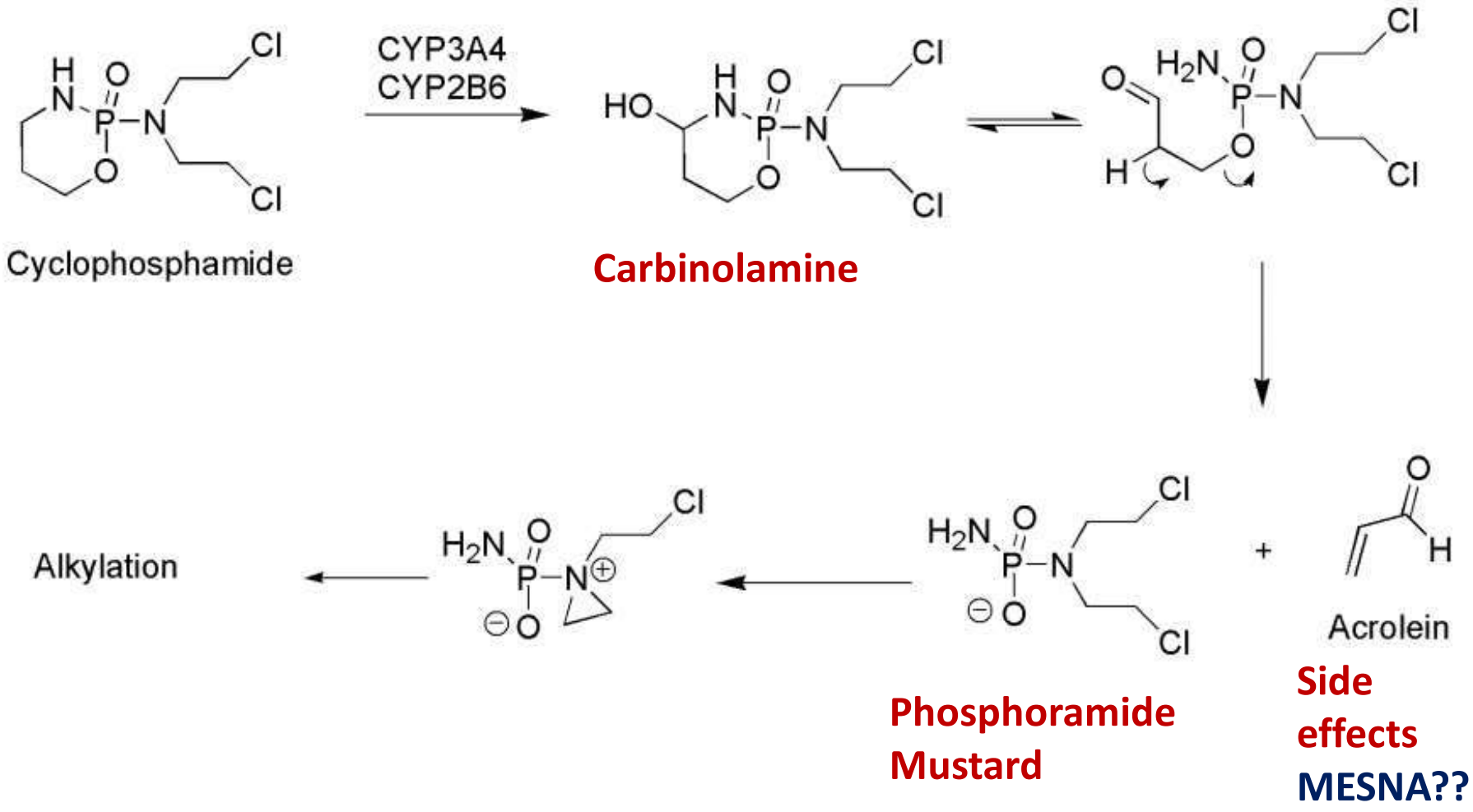
N atom is a part of phosphoramide [A highly electron –withdrawing]

Cyclophosphamide is **prodrug** activated in cancer cell

Activation begins by hydroxylation [produce carbinolamine by CYP] then non metabolic activation

Cyclophosphamide

Activation of cyclophosphamide



SAR of Nitrogen mustard

Summarize what you have studied
3-4 points

Look in Foy's principles of medicinal chemistry chapter 42

Chemotherapy
Part 2

[DNA Alkylating agents]

Dr. Mai Ramadan

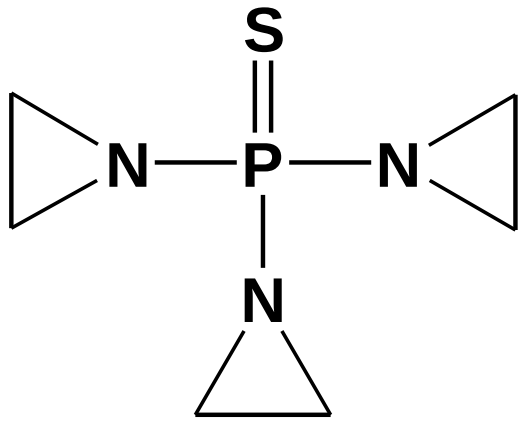
DNA Alkylation by Thiotepa

Thiotepa

Tertiary aziridine

Less reactive than aziridinium

pKa 6 [low ionization at 7.4]

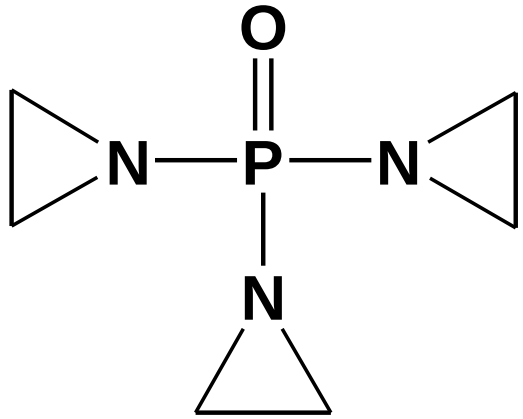


Thiotepa

Oxidative desulfuration to TEPA

[Triethylenephosphoramide]

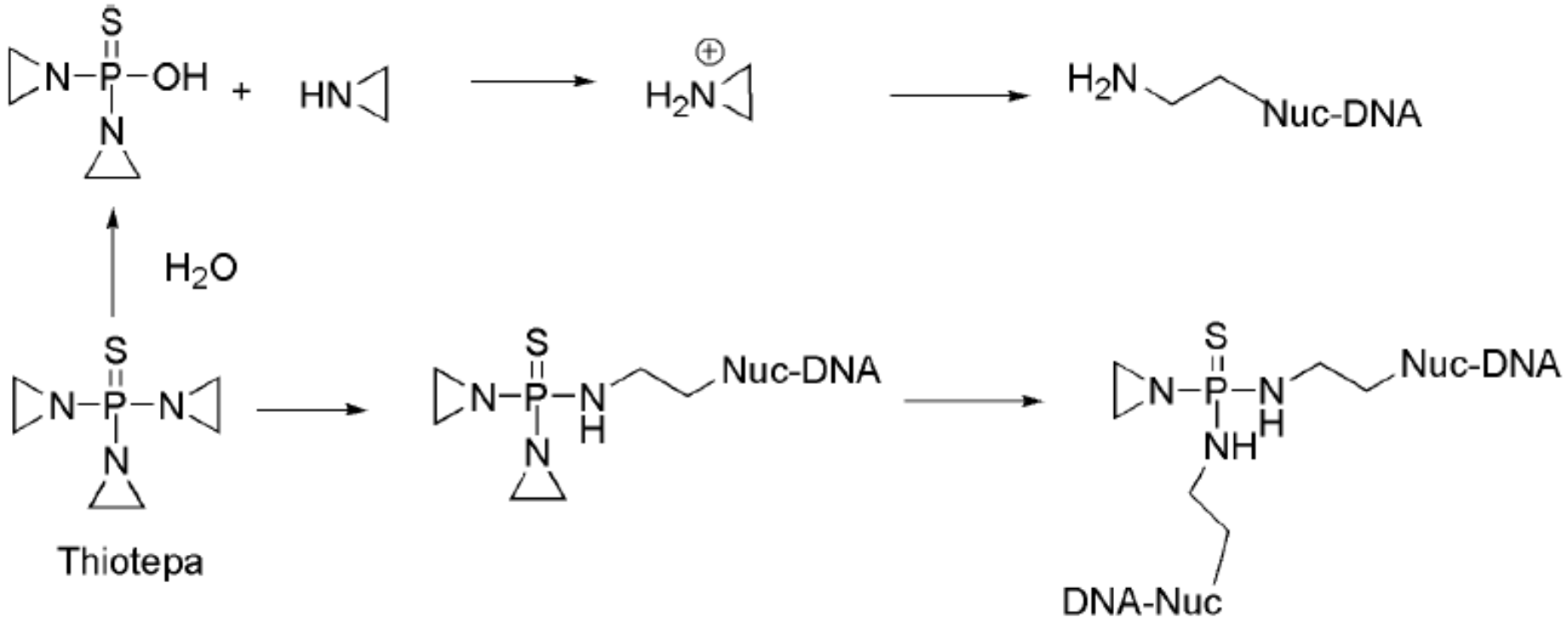
Active cytotoxic metabolite



TEPA

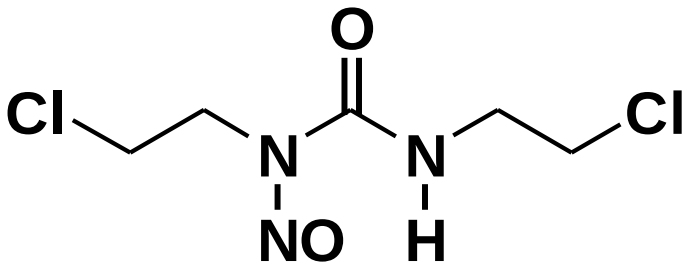
DNA Alkylation by Thiotepa

Mechanism of Thiotepa Study



DNA Alkylation by Nitrosurea

Carmustine



Carmustine

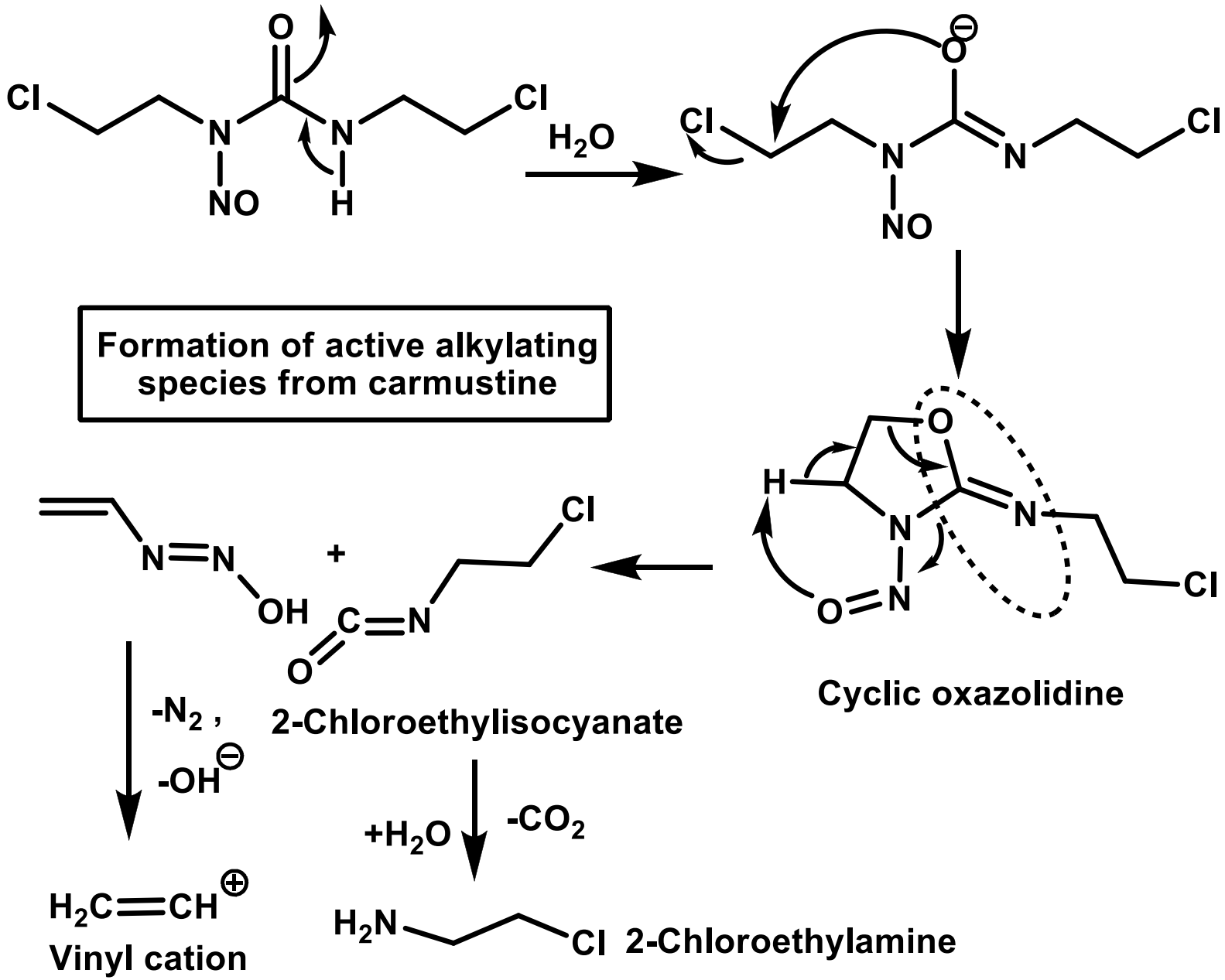
It is administered intravenously because of its rapid metabolism.

It is used against **brain tumors [cross BBB]**

Formation of alkylating agents begins with loss of proton from urea [nitro group is electron withdrawing group], forming oxazolidine intermediate

Alkylating agents are 2-chloroethylamine and vinyl cation

Mechanism of carmustine Study



DNA Methylation by Triazene

Dacarbazine [Triazene]

Dimethyl triazenyl imidazole carboxamide (DTIC)

IV route

Bioactivation: CYP 450 producing MTIC intermediate.

DNA Methylator **at O^6- and N^7- guanine nucleotide**

Timozolomide [Tetrazine]

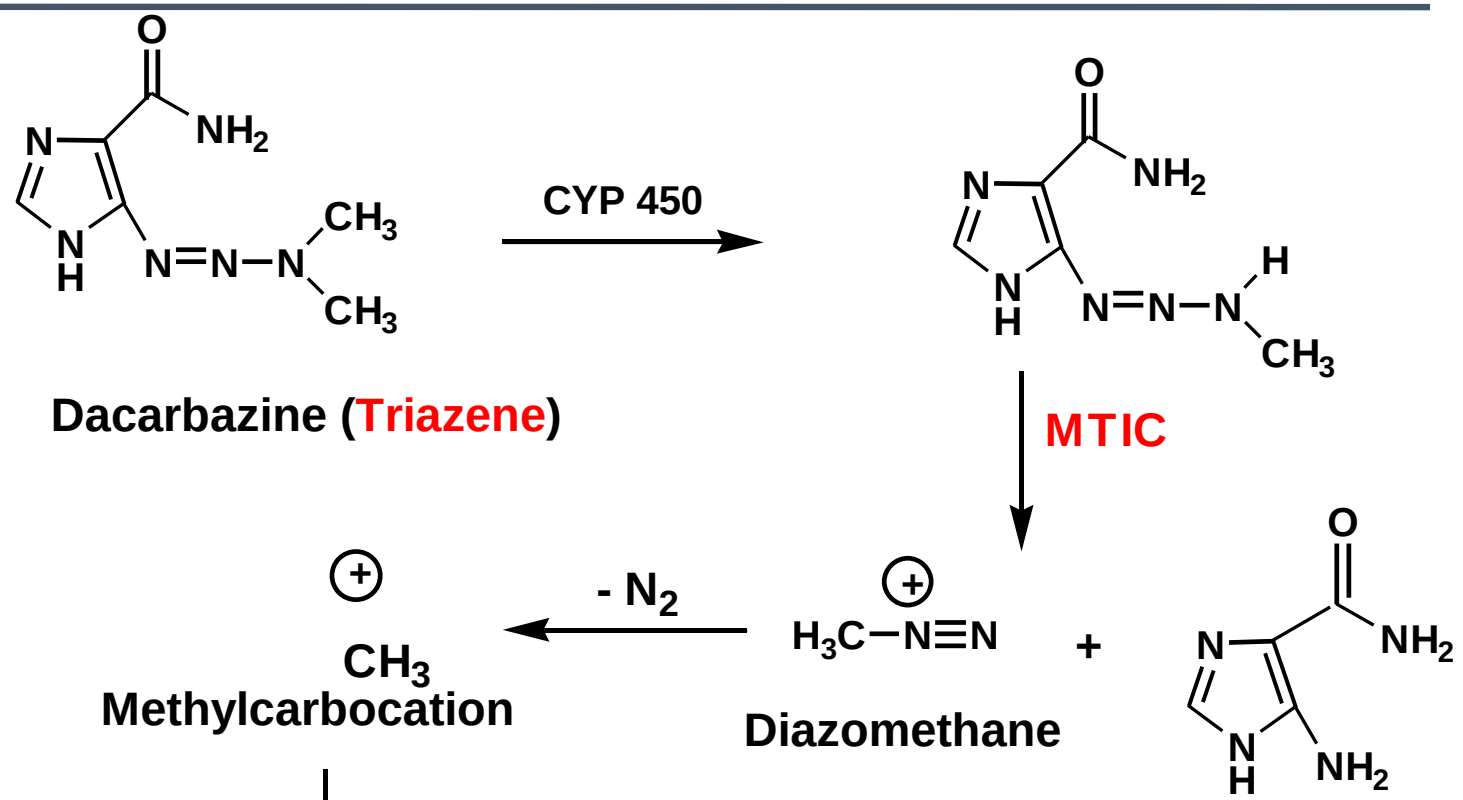
Similar mechanism of action and activation Formation of MTIC

It can be administered orally

DNA Methylation by Triazene

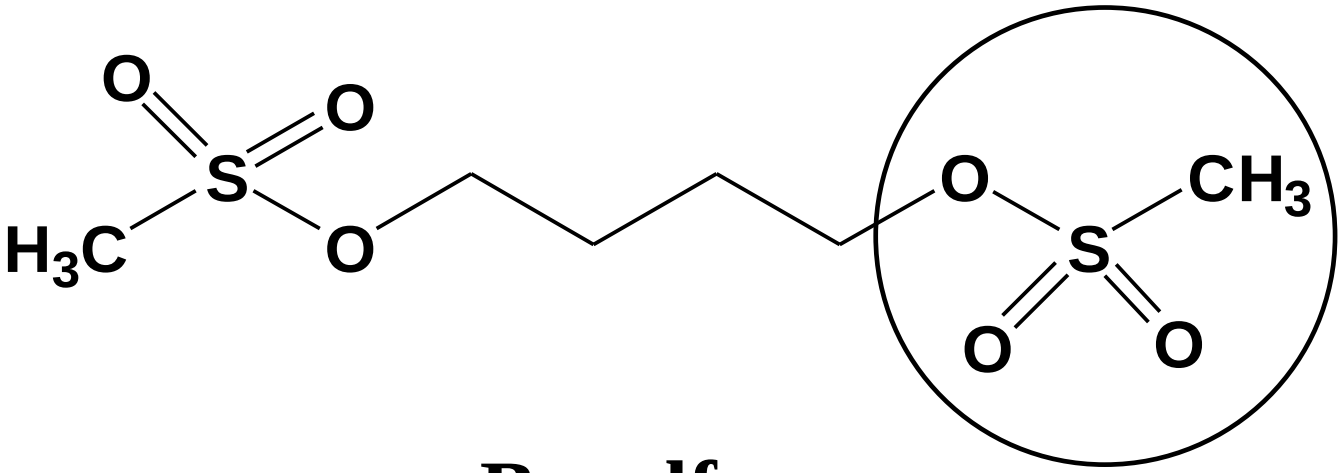
Mechanism of action

O^6- and N^7 Methylation of guanine nucleotide



DNA alkylation by alkylsulfonate

Busulfan

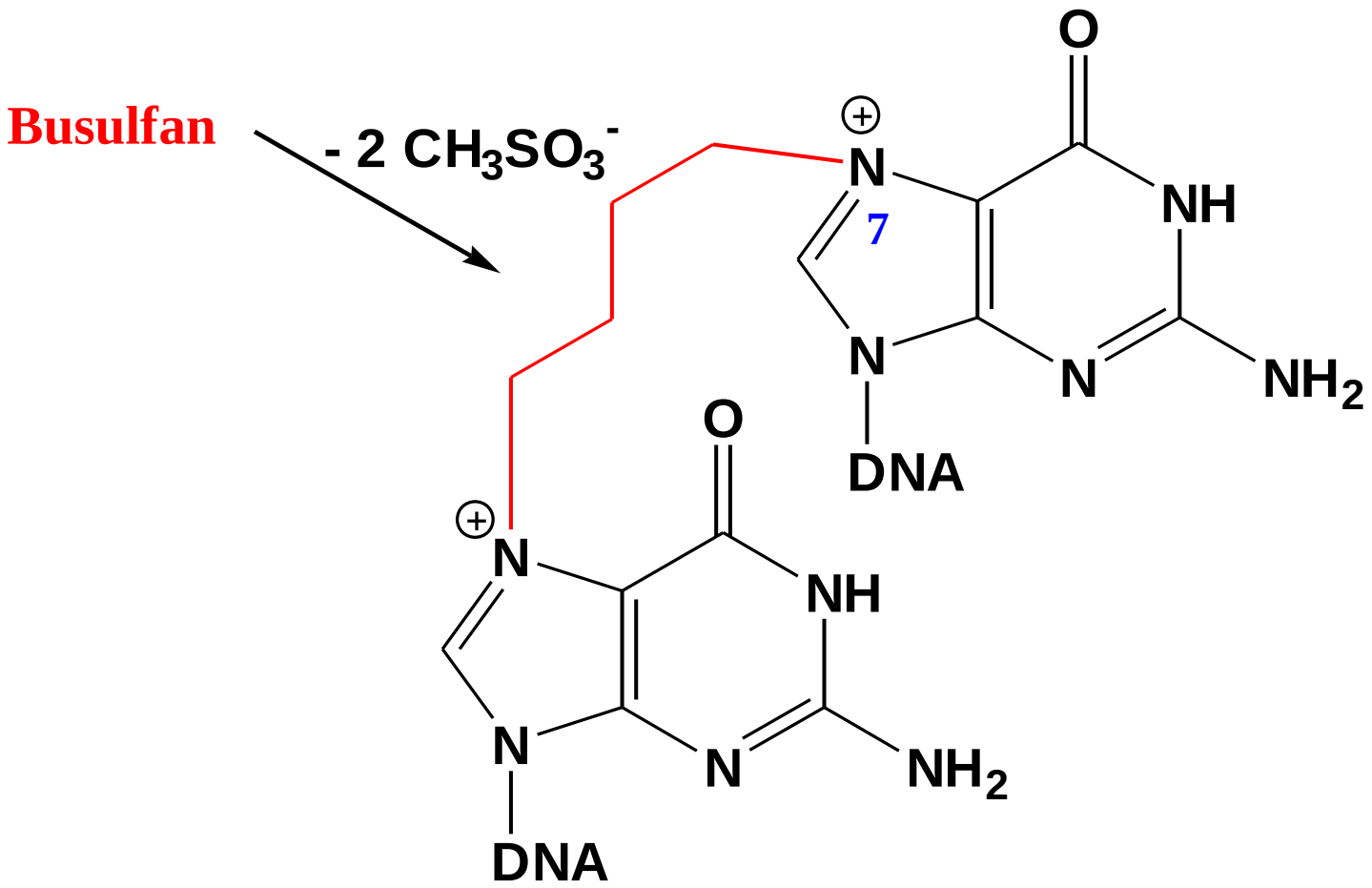


Busulfan

Methylsulfonate is a good leaving group

DNA alkylation by alkylsulfonate

Busulfan: Study

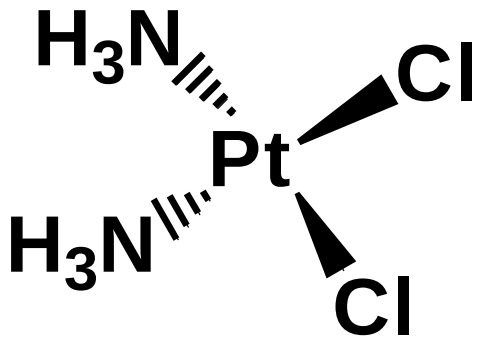


Organoplatinum (II) complexes

Pt(II) ion can accept electrons from two DNA nucleophile

Transplatin is an **inactive** isomer

Cispaltin is a square planner complex of Pt(II)



Cisplatin

Reaction with DNA occurs preferentially at the N-7 of guanine of two adjacent guanine residues resulting in primarily (95%) intra-strand cross-links.

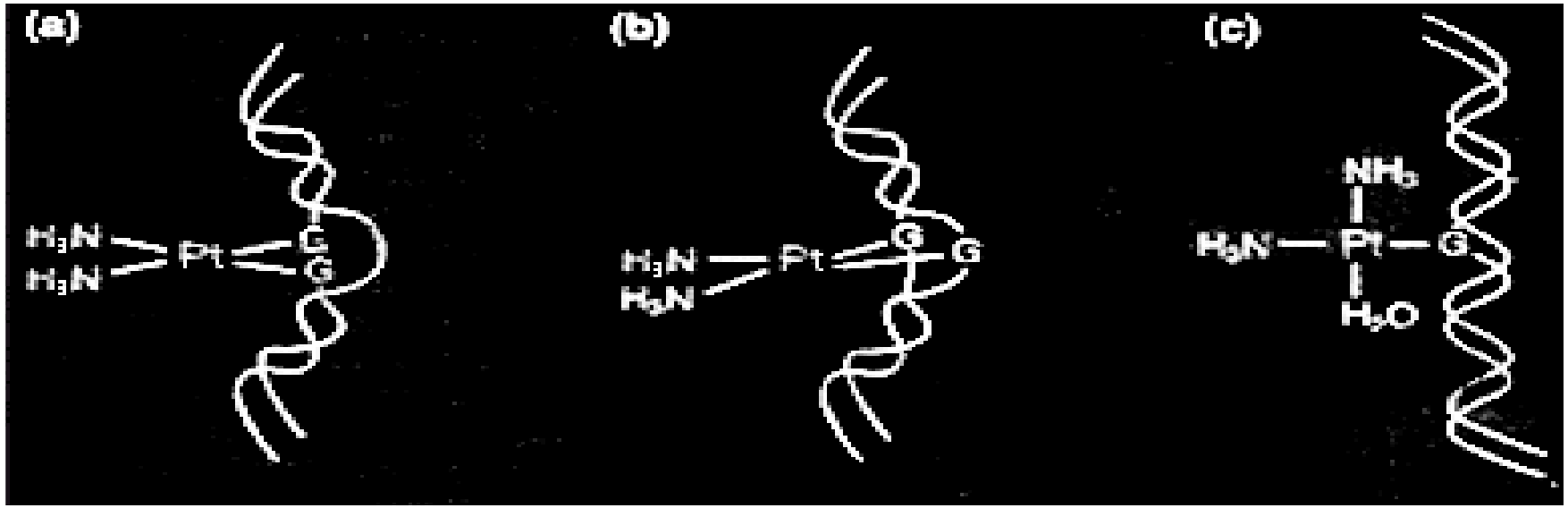
Organoplatinum (II) complexes

DNA adducts of cisplatin

Intrastrand crosslink ~ 90%

Interstrand crosslink ~ 5%

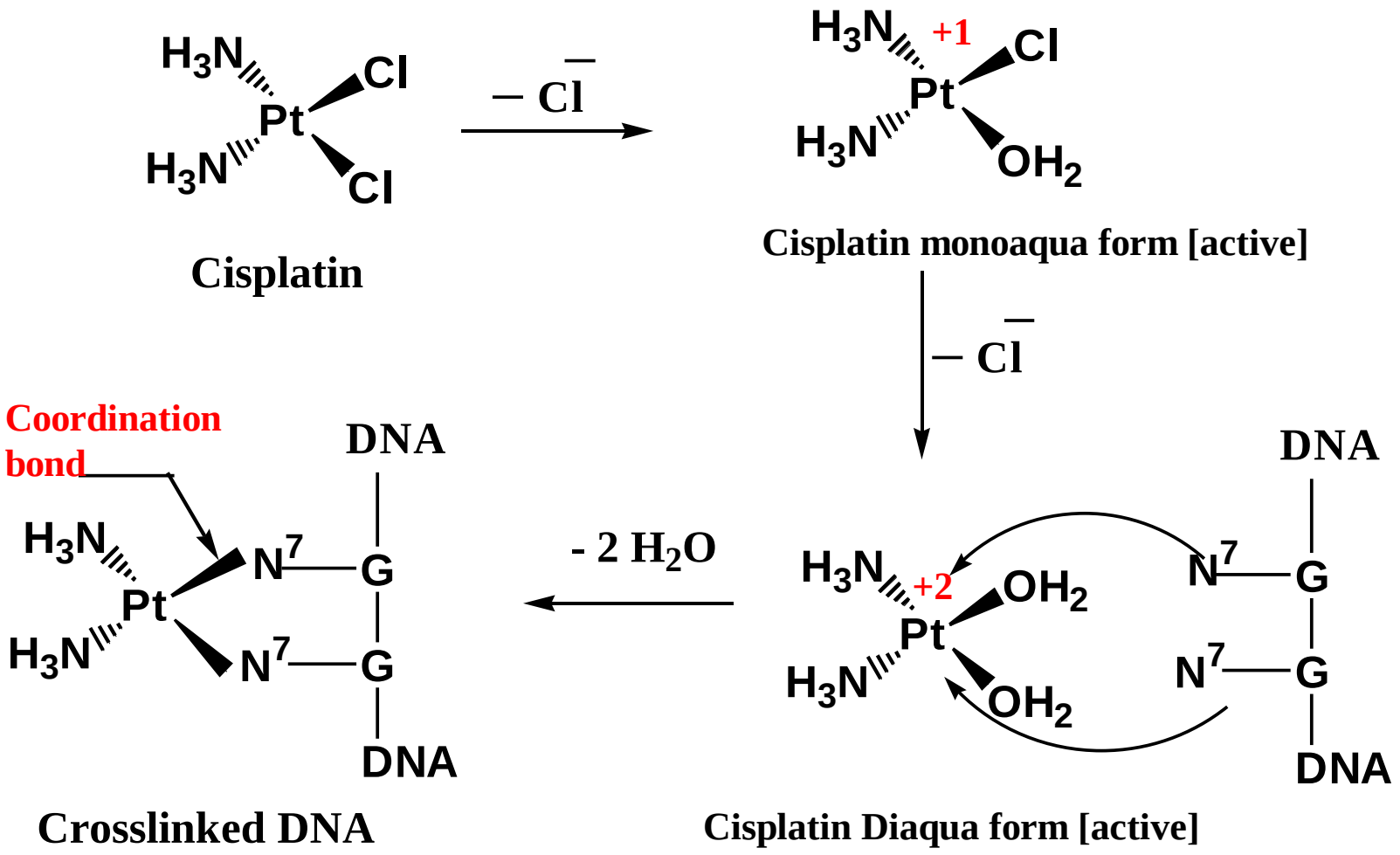
Monofunctional adducts ~ 2%



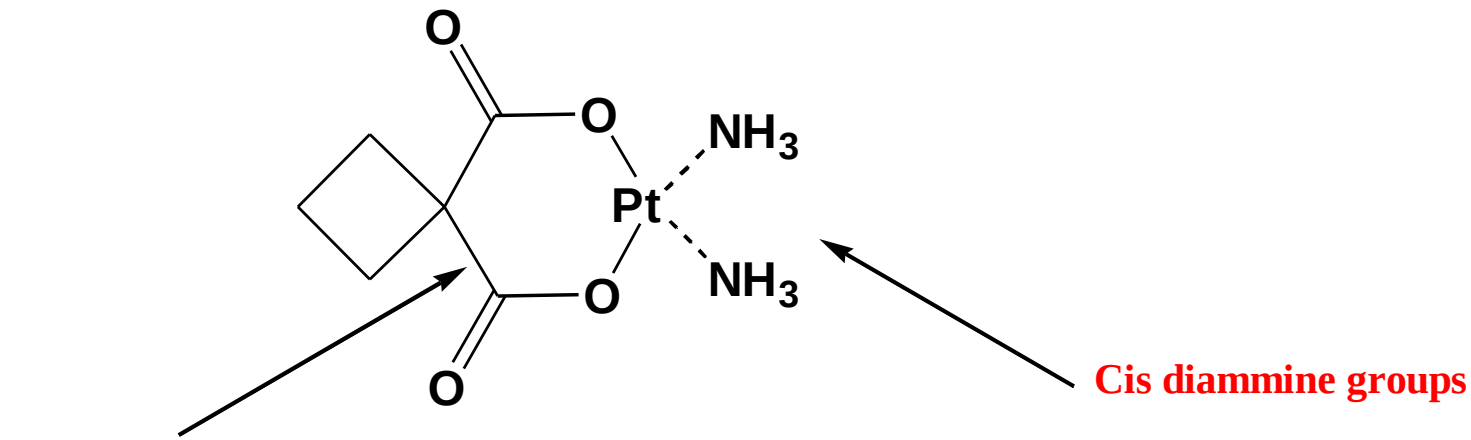
Two NH_3 ligands are irreversibly coordinated with Pt(II), and are not displaced by nucleophile (DNA).

They stabilize cross linked Pt-DNA complex by ion dipole bonds with ionic phosphate of DNA.

Organoplatinum (II) complexes



Organoplatinum (II) complexes



Carboplatin

Dicarboxylic acid groups hydrolyze at a slower rate than –Cl in cisplatin

Less side effects: Nephrotoxicity and neurotoxicity than cisplatin

Form the same cytotoxic hydrated intermediate as cisplatin

Organoplatinum (II) complexes

Read about
Oxaliplatin & Satraplatin

DNA alkylating agents

Monofunctional alkylating agents:

Nitrosourea

Dacarbazide (DNA Methylator)

Bifunctional alkylating agents:

N- Mustard

Thiotepa

Platinium (II) commplexes

Busulfan

Chemotherapy

Part 3

[Antimetabolite]

Dr. Mai Ramadan

Anticancer: Antimetabolite

DNA Polymerase inhibitor

Cytarabine

Fludarabine

Clofarabine

Pyrimidine antagonist:

Flourouracil, Floxuridine: [Direct inhibitor of thymidylate synthase]

Methotrexate and Pemetrexed: [Indirect inhibitor of thymidylate synthase, **Antifolate**]

Purine antagonist:

Mercaptopurine

DNA Polymerase inhibitor

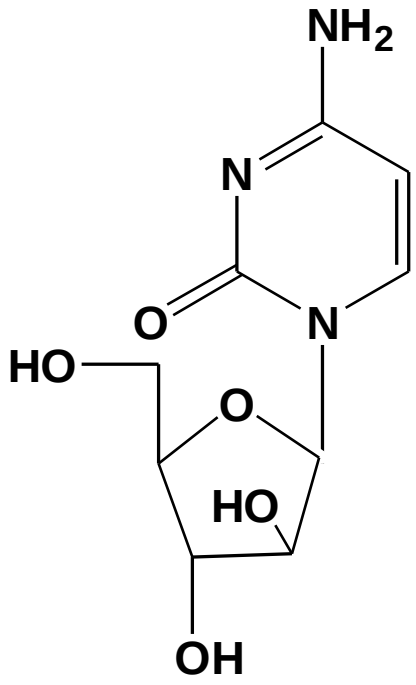
What is the function of this enzyme?

- ❑ Cytarabine
- ❑ Fludarabine
- ❑ Clofarabine

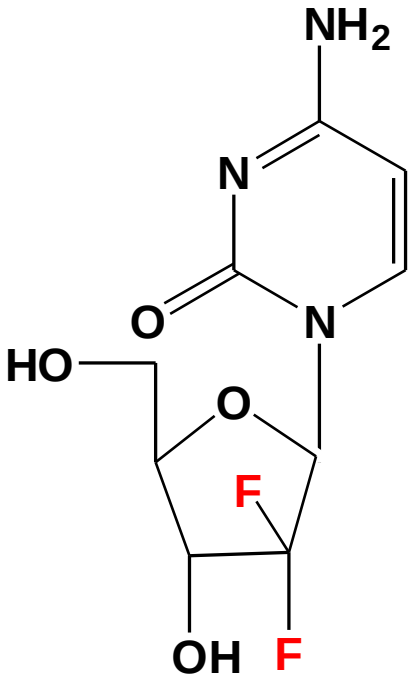
Anticancer: Antimetabolite

DNA Polymerase inhibitor

Cytarabine: (ara-C), Gemcitabine



Cytarabine: Arabinose sugar
Cysteine



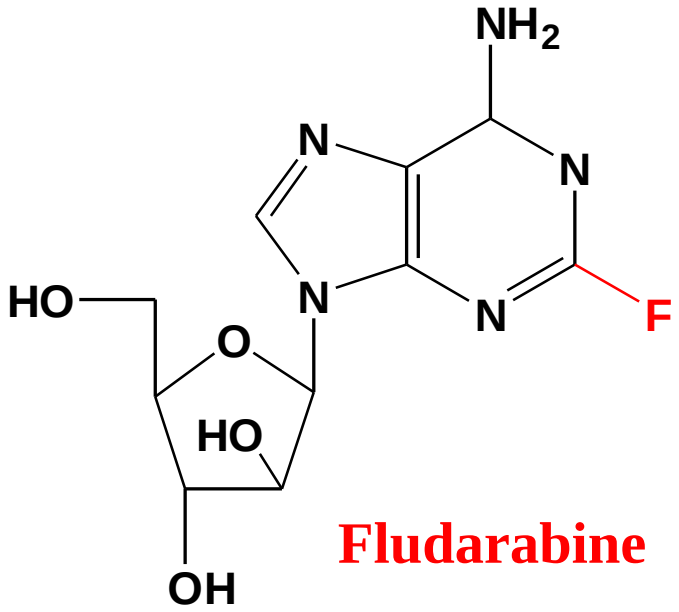
Gemcitabine Diflourodeoxycytidine (dFdC)
Cytidine based anticancer

$t_{1/2}$: 3.6 h → $t_{1/2}$: 19 h

Anticancer: Antimetabolite

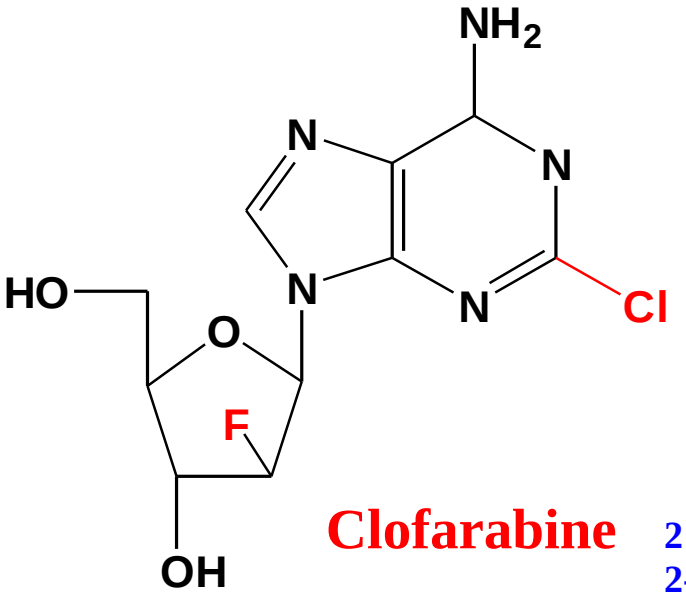
DNA Polymerase inhibitor

Fludarabine, Clofarabine



Fludarabine

Arabinose sugar
2-Flouroadenine



Clofarabine

2 flouroarabinose sugar
2-chloroadenine

Anticancer: Antimetabolite

DNA Polymerase/DNA Chain elongation inhibitor

Cytarabine, Gemcitabine, Fludarabine, Clofarabine

Ribose modified DNA nucleoside ⟶ arabinose or halogenated arabinose

Halogenated nucleobase: adenine

Prodrugs

Activation: Conversion to triphosphate nucleotides

Entry in cancer cells by active transport, then activation.

Pyrimidine Antagonist:

Direct inhibitor of thymidylate synthase

Flourouracil

Floxuridine

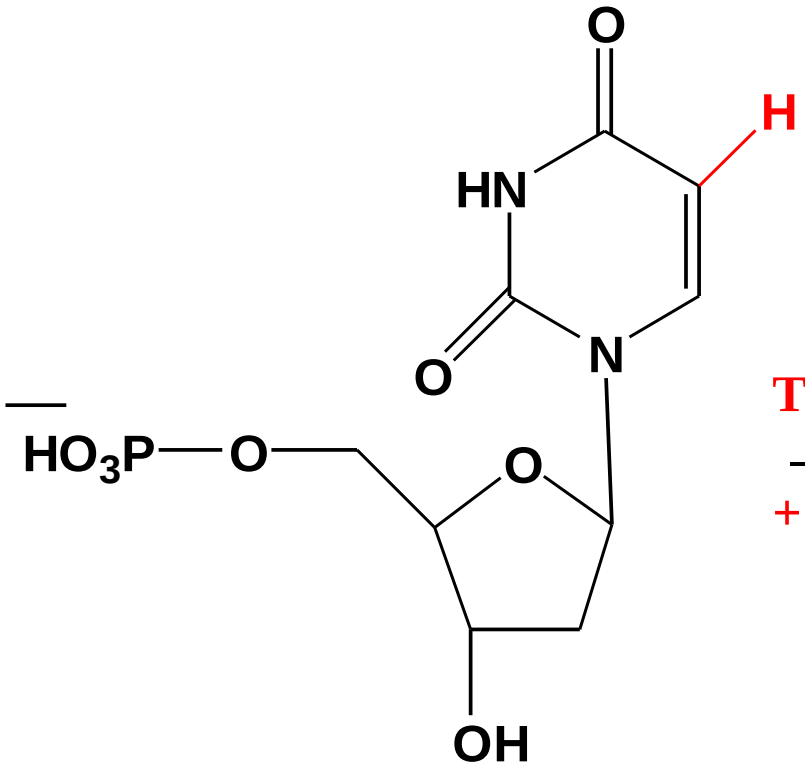
Capecitabine

Indirect inhibitor of thymidylate synthase

DHFR inhibitor (Antifolate)

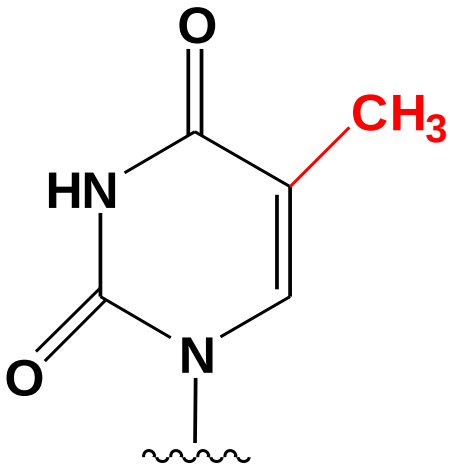
Anticancer: Antimetabolite

Pyrimidine Antagonist:



Thymidylate synthase

+ 5,10-methyleneTHF



The natural substrate for enzyme is dUMP

dTMP

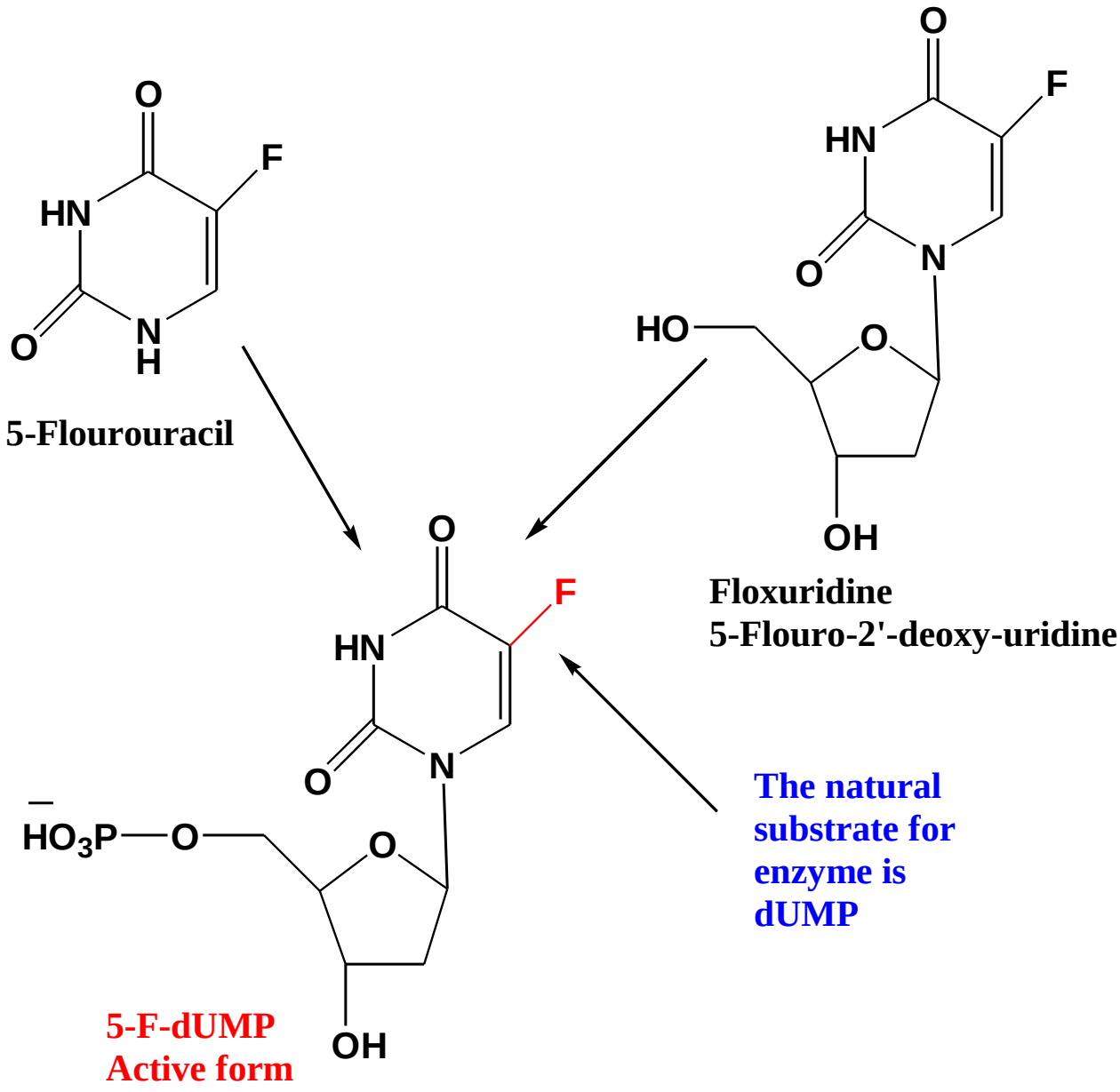
Anticancer: Antimetabolite

Pyrimidine Antagonist:

Direct inhibitor of thymidylate synthase

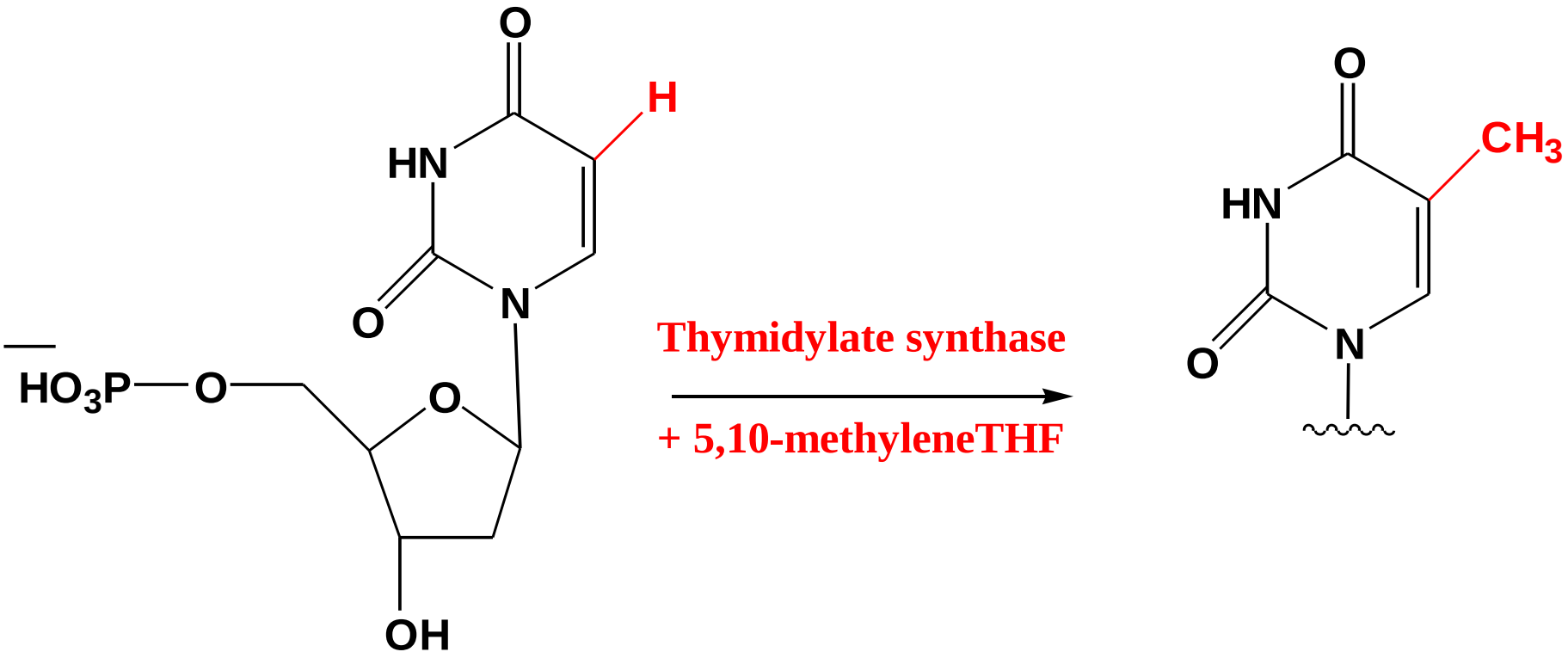
Flourouracil

Floxuridine



Anticancer: Antimetabolite

Pyrimidine Antagonist:



The natural substrate for enzyme is dUMP

dTMP

Anticancer: Antimetabolite

Direct inhibitor of thymidylate synthase

Flourouracil and Floxuridine are prodrugs activated to 5-flouro-2'-deoxyuridine monophosphate (5-F-dUMP)

The active metabolite forms a ternary complex with 5,10-methylenetetrahydrofolate [Cofactor] and HS-Cys-Thymidylate synthase enzyme

The natural substrate for enzyme is dUMP which should be methylated [methyl group is carried by tetrahydrofolate] at position 5 to produce dTMP. [Thymidine is a nucleotide in DNA]

See biosynthesis of deoxythymidine monophosphate Fig 42.24 in your book

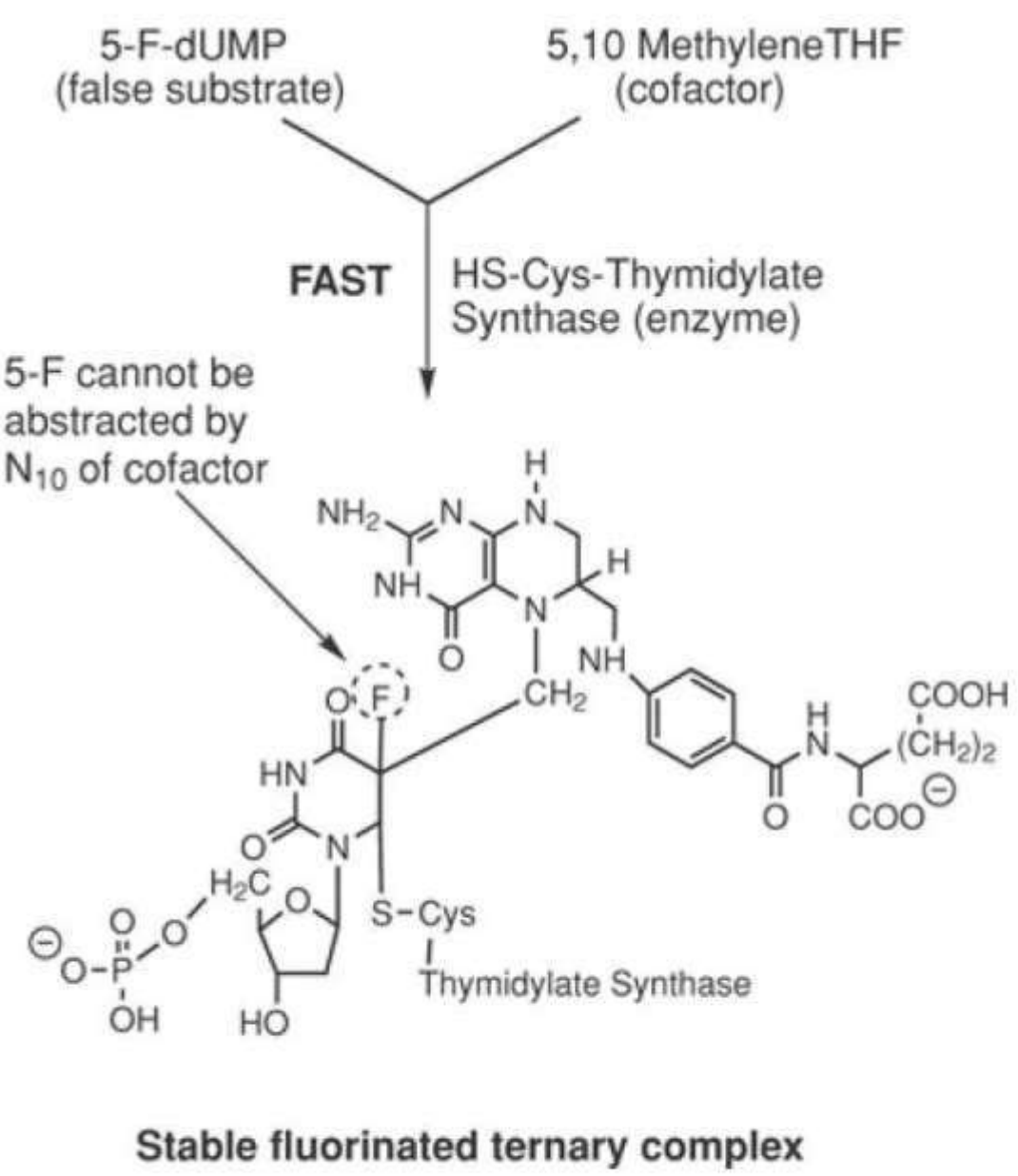
5- FU ⟶ 5-F-dUMP
active metabolite

C6 of 5-F-dUMP is significantly more electrophilic than normal F is strong electron withdrawal

Cys195 of thymidylate synthase attack C-6 to form **stable** fluorinated ternary complex
Small size of F no steric hindrance to formation of this false complex.

C5-F is stable to cleavage

Complex is not cleavable



Stable fluorinated ternary complex

Fig. 42.27. Mechanism of action of fluorouracil.

Capecitabine

Capecitabine is a carbamylated analogue of cytidine

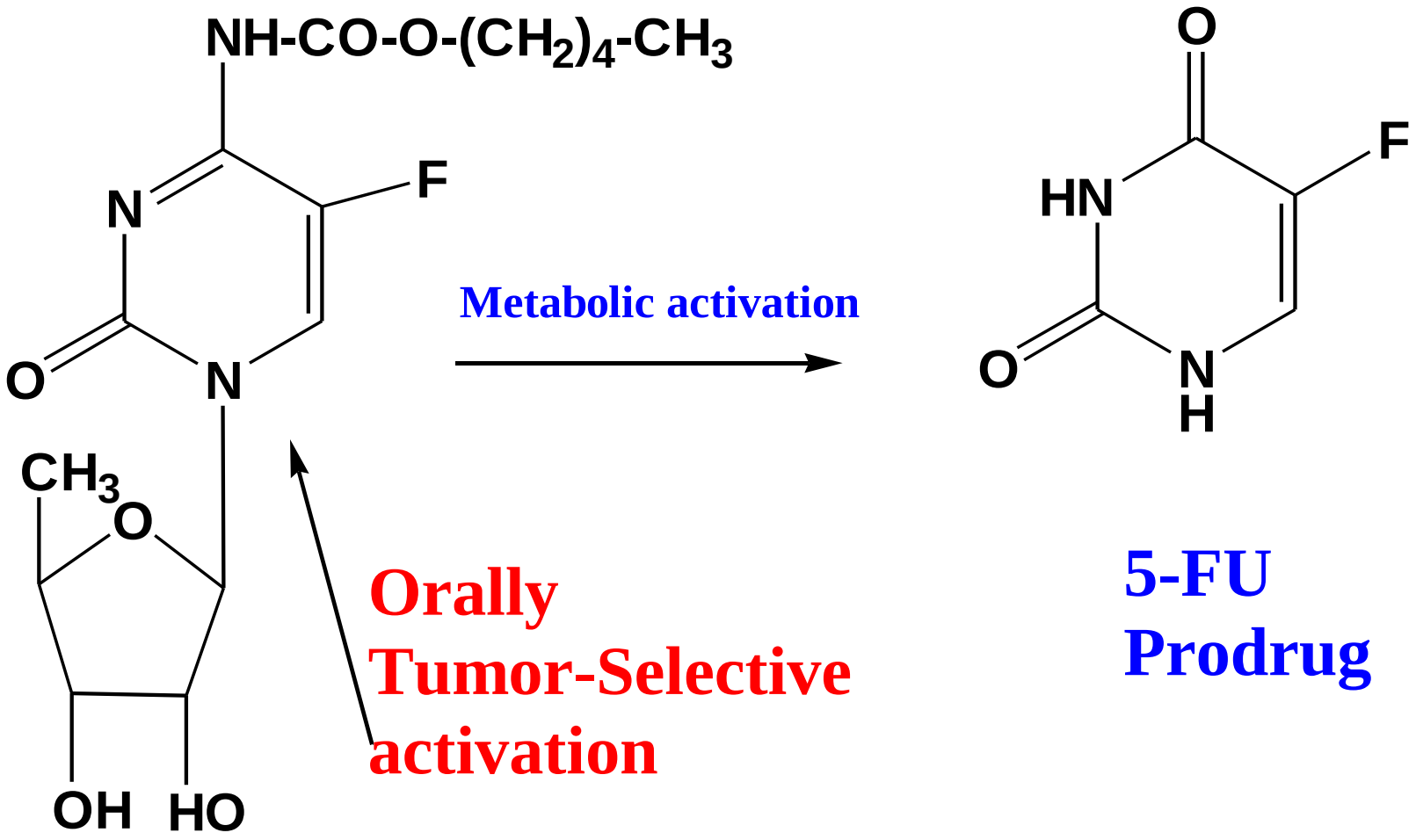
Prodrug of 5-F-dU → 5-F-dUMP.

Given orally

It is extensively metabolized to fluorouracil, which is then converted to the active fluorinated deoxyribonucleotide

Tumor selective generation of 5-FU:Thymidine phosphorylase, an enzyme involved in activation, is much more active in tumors than in normal tissue
Levels of active drug in the tumor can be up to 3.5-fold higher than in surrounding tissue. Lower incidence of side effects compared to 5-FU.

Capecitabine



Orally
Tumor-Selective
activation

5-FU
Prodrug

Capecitabine
Prodrug

Chemotherapy

Part 4

[Antimetabolite]

Dr. Mai Ramadan

Pyrimidine Antagonist:

Direct inhibitor of thymidylate synthase

Flourouracil

Floxuridine

Capecitabine

Indirect inhibitor of thymidylate synthase

DHFR inhibitor (Antifolate)

Anticancer: Antimetabolite

Pyrimidine Antagonist:

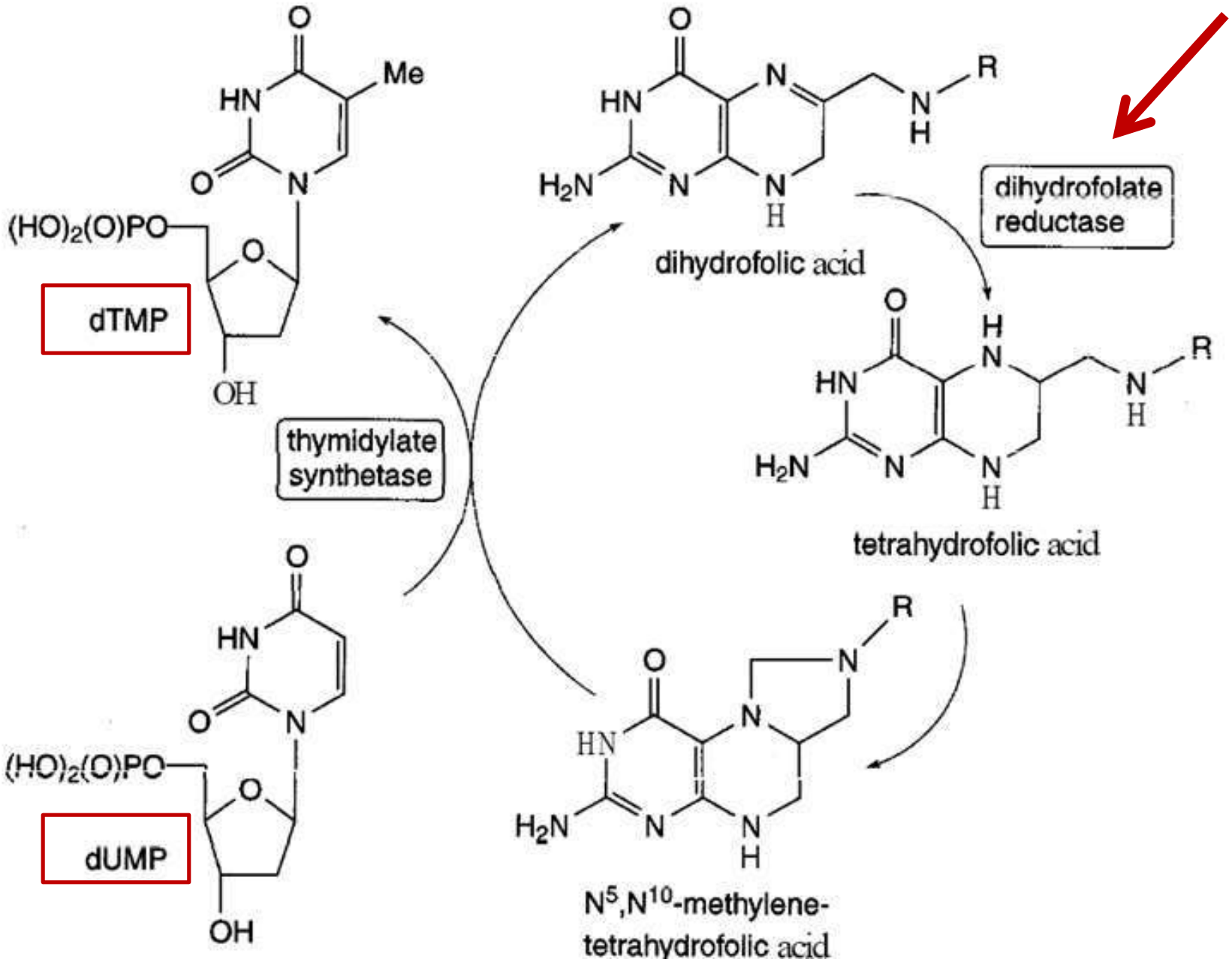
Indirect inhibitor of thymidylate synthase

Methotrexate
Folic acid antagonist

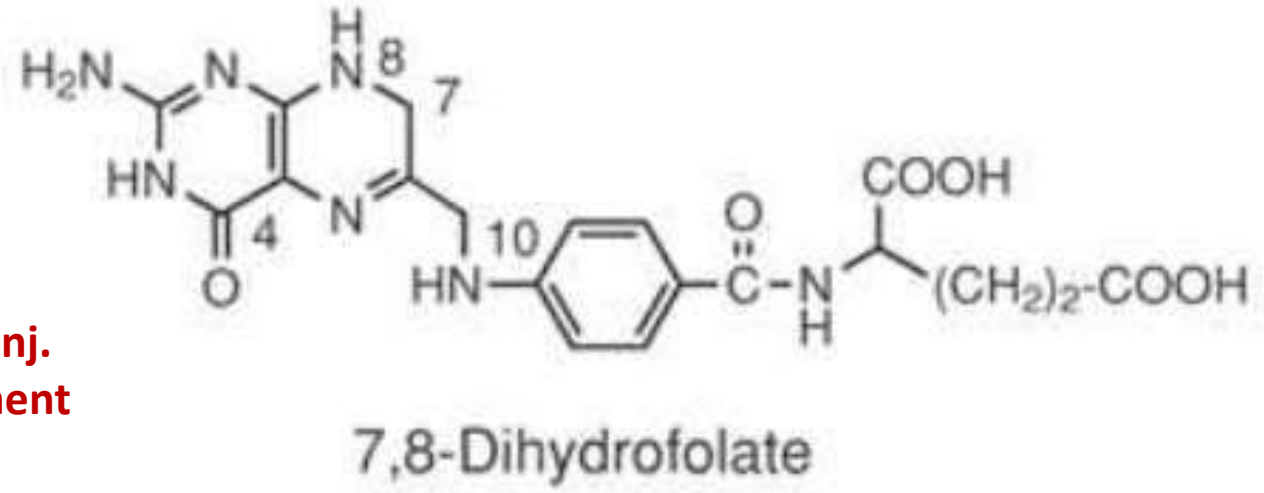
Compete with the natural substrate [7,8-DHF, Dihydrofolate] for the DHFR Dihydrofolate reductase enzyme.

Inhibition of **DHFR** will inhibit indirectly deoxythymidine monophosphate synthesis.

Pka of methotrexate is 3 pka more basic than folic acid. Higher binding to DHFR enzyme

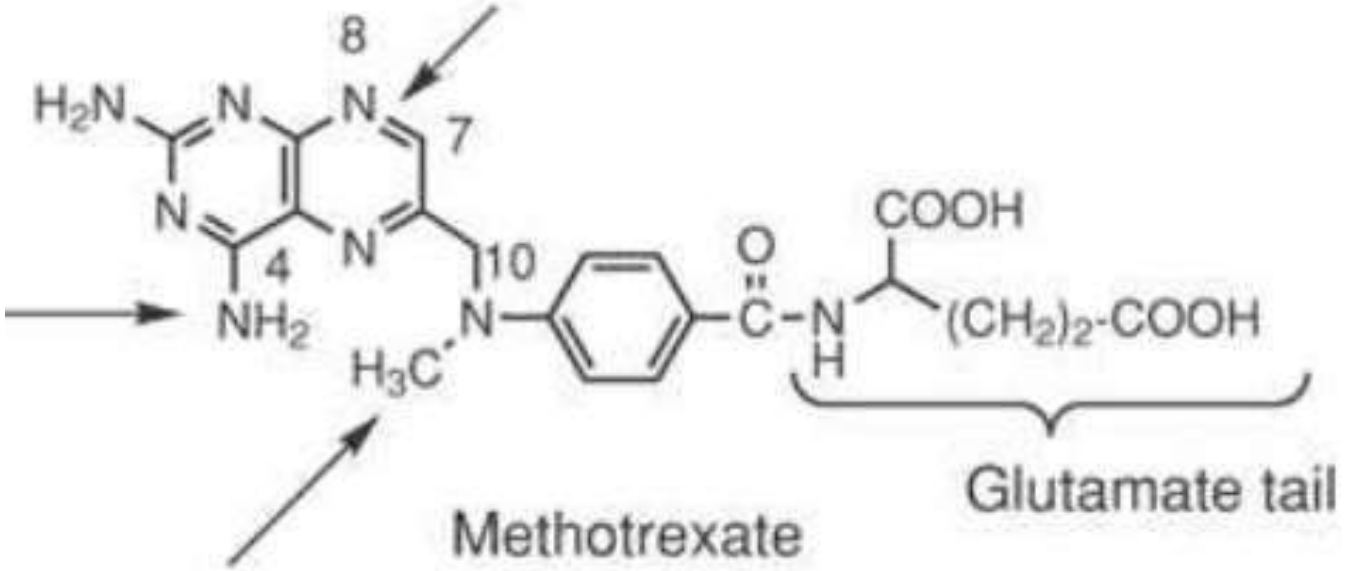

dihydrofolate reductase
dihydrofolic acid
tetrahydrofolic acid
thymidylate synthetase
dTMP
dUMP
N5,N10-methylene-tetrahydrofolic acid

Anticancer: Antimetabolite



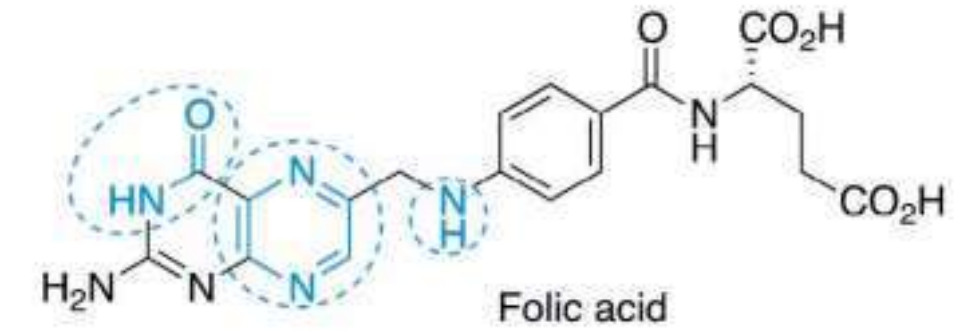
7,8-Dihydrofolate

-NH2 at C 4 is basic and conj. With basic guanidine fragment instead of an electron-withdrawing carbonyl

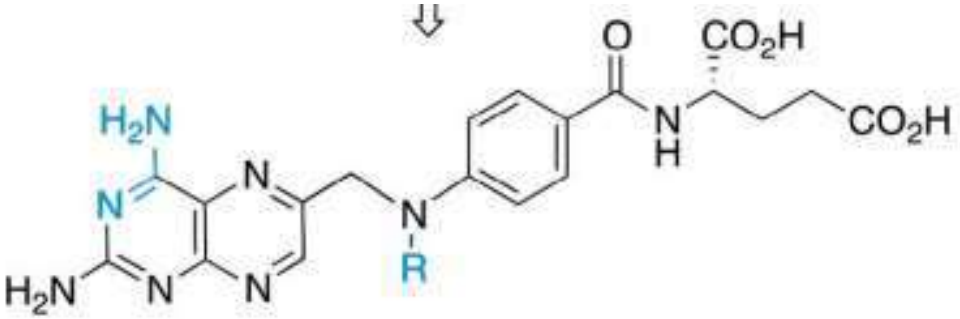


Glutamate tail

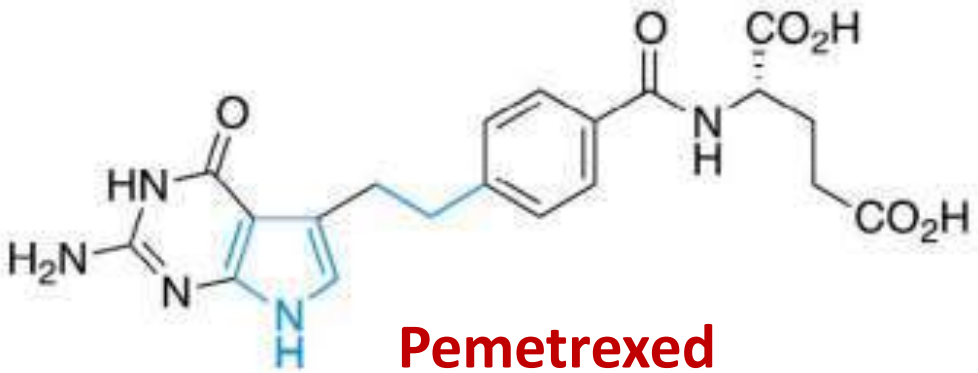
Methotrexate



Folic acid



R: CH_3 Methotrexate



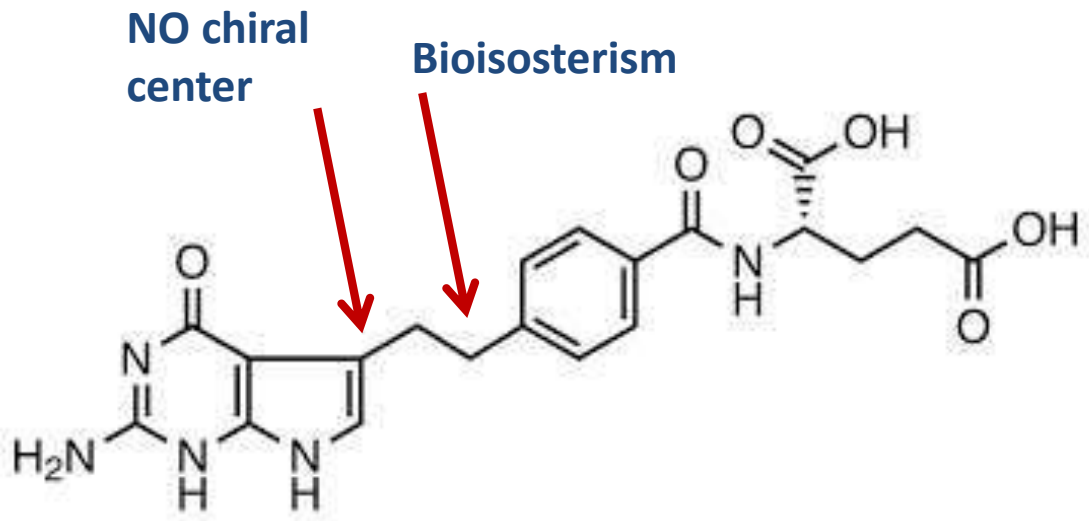
Pemetrexed

Anticancer: Antimetabolite

Pemetrexed

A novel multitarget antifolate used by the IV route

Pyrrolopyrimidine ring



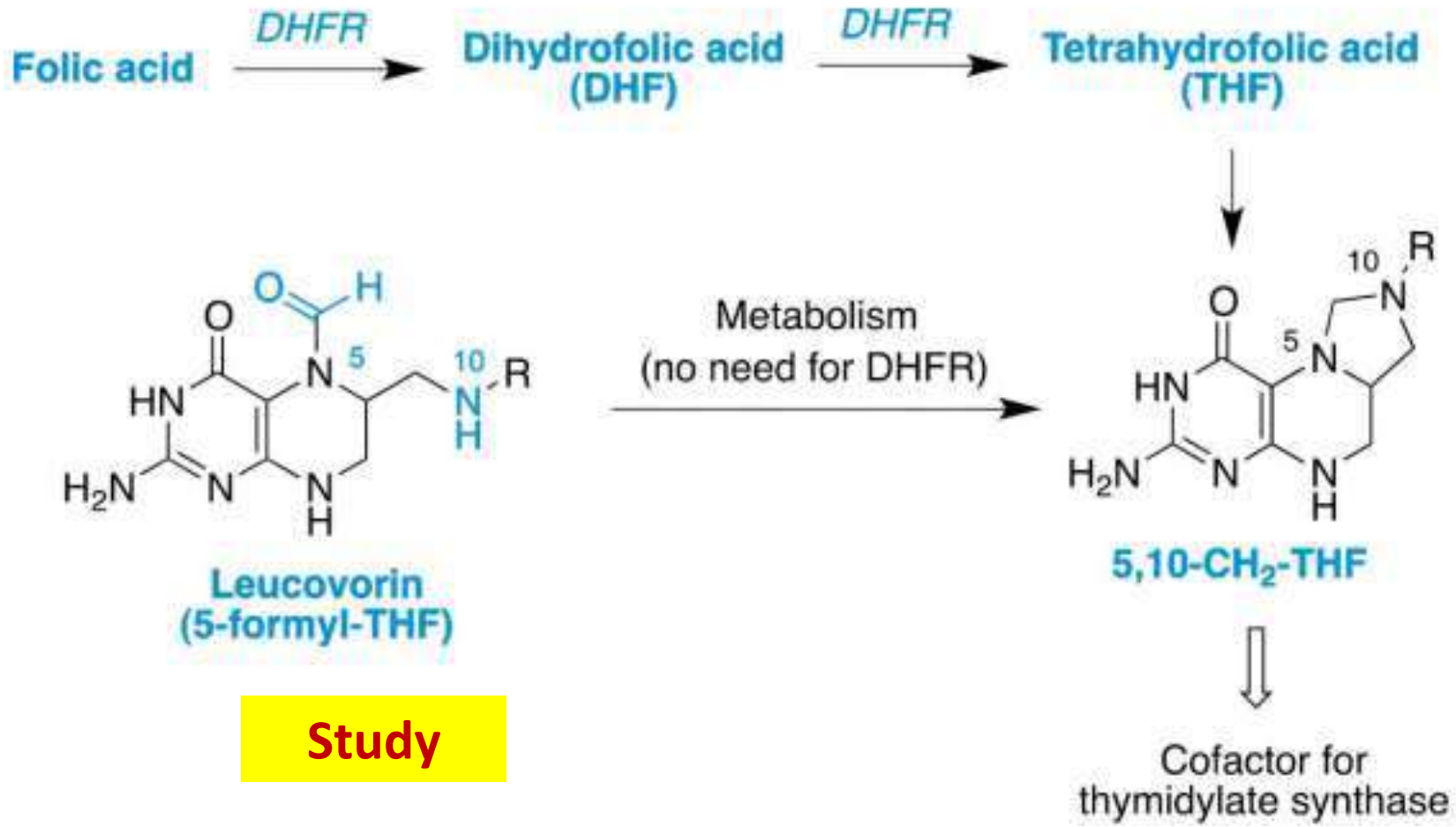
Inhibits the synthesis of pyrimidine and purine-based nucletotides by disrupting folate dependent metabolic processes

Inhibit DHFR , thymidylate synthase, GAR transformylase

Anticancer: Antimetabolite

Leucovorin: 5-Formyl-THF

Replace THF in cases of severe toxicity caused by DHFR inhibitor

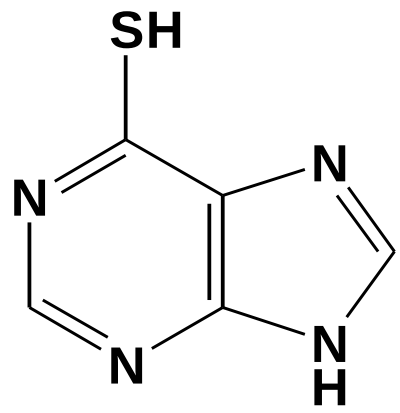


Study

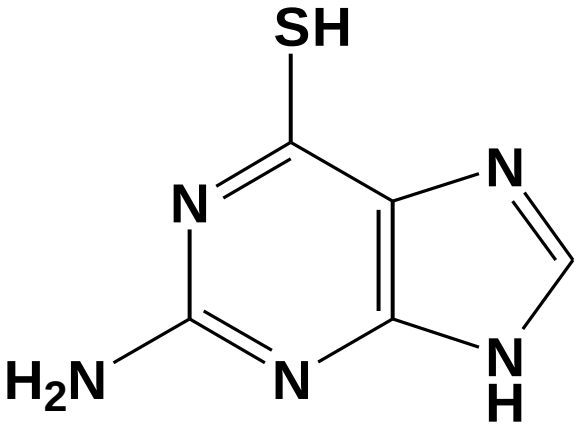
Anticancer: Antimetabolite

Purine antagonist:

Mercaptopurine and thioguanine [Prodrugs]



6-Mercaptopurine



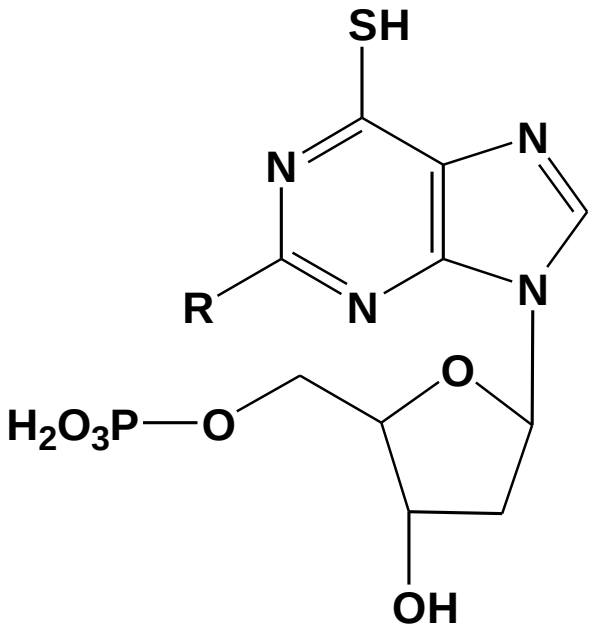
Thioguanine

Both compounds must be transformed into nucleotides by adding a **phosphoribosyl fragment**

Anticancer: Antimetabolite

Purine antagonist:
Mercaptopurine and thioguanine

A potent inhibitor of de novo purine synthesis
Inhibiting amidophosphoribosyl transferase [rate limiting enzyme]



Active thiopurine ribonucleotide metabolite

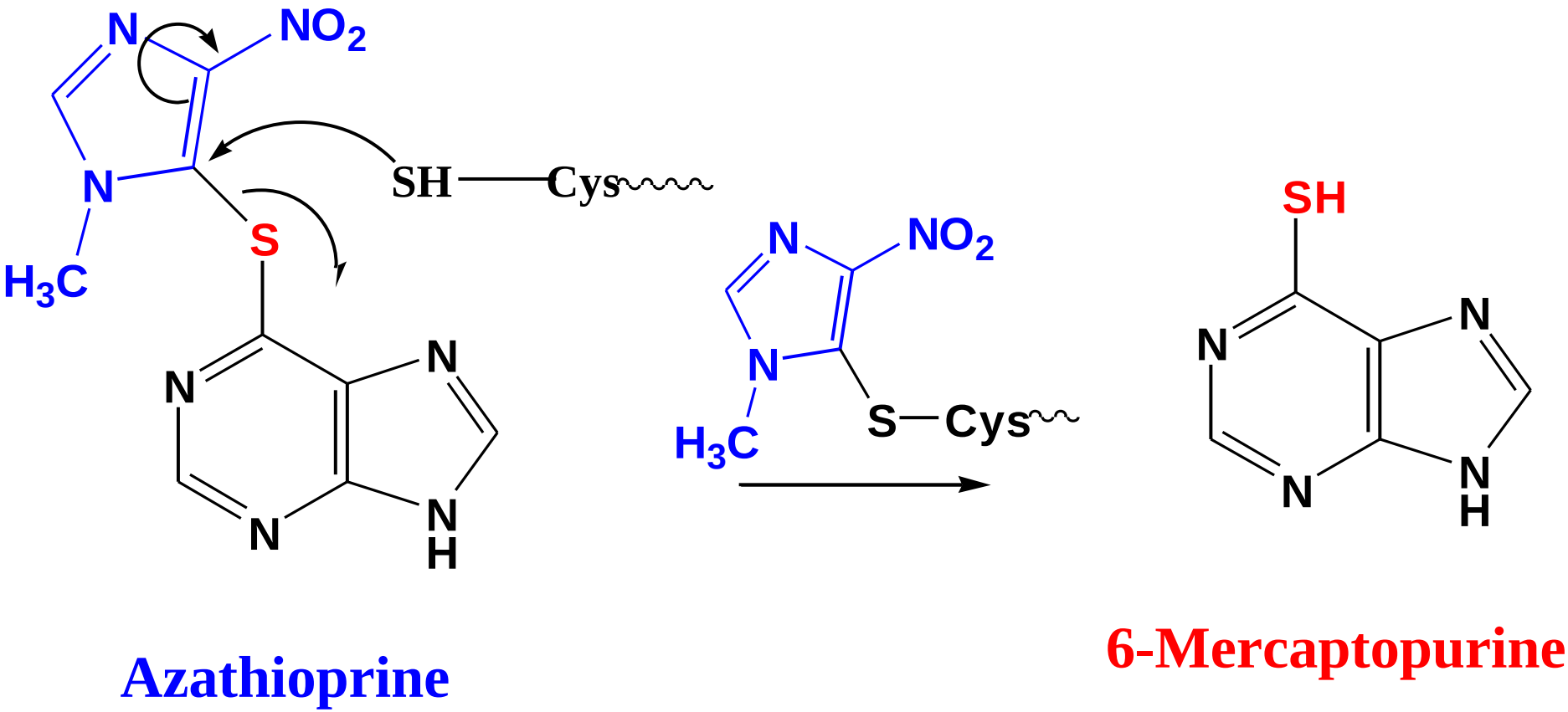
R: H 6-Thioinosinic acid
R: NH2 Thioguanylic acid

The ribose diphosphate and triphosphates of 6-mercaptopurine are active enzyme inhibitors

The triphosphate can be incorporated into DNA and RNA to inhibit chain elongation

Anticancer: Antimetabolite

Prodrug of 6-mercaptopurine: Azathioprine



Azathioprine

6-Mercaptopurine

Chemotherapy

Part 5

[Antibiotic]

Dr. Mai Ramadan

Introduction

Antibiotics

Anthracyclines:

Doxorubicin, Epirubicin, Daunorubicin, Idarubicin, Valrubicin

Mitoxantrone

Bleomycin

Actinomycin D

Mitomycin C

Introduction

Mechanism of action [Antibiotics]

❑ **Intercalate double stranded DNA:**

The drugs slide in the double stranded DNA and insert between the base pairs

Stabilization of Drug-DNA after insertion by van der Waals, hydrophobic, and H bond [Noncovalent interactions with DNA bases].

The highly stabilized complex **uncoils the DNA**, prohibiting proper replication.

To be inserted between the bonded DNA strands, a segment of the antibiotic must have **flat ring system** guaranteed by aromaticity.

Introduction

Mechanism of action [Antibiotics]

❑ Topoisomerase II inhibitor:

Topoisomerase II normally cleaves DNA during the replication phase but repairs its own damage after replication is complete.

Topoisomerase II inhibitors act to stimulate the cleavage reaction but **inhibit the DNA resealing activity of the enzyme, leaving the DNA irreversibly damaged** and unable to replicate.

Introduction

Mechanism of action [Antibiotics]

❑ Generation of cytotoxic free radicals:

Cytotoxic free radicals [$O_2^{\cdot -}$, $OH^{\cdot}$] that cause single-strand breaks in DNA.

❑ Alkylating DNA

Mitomycin

Like nitrogen mustard antineoplastics

Note that : Antibiotics antineoplastics are natural or semisynthetic

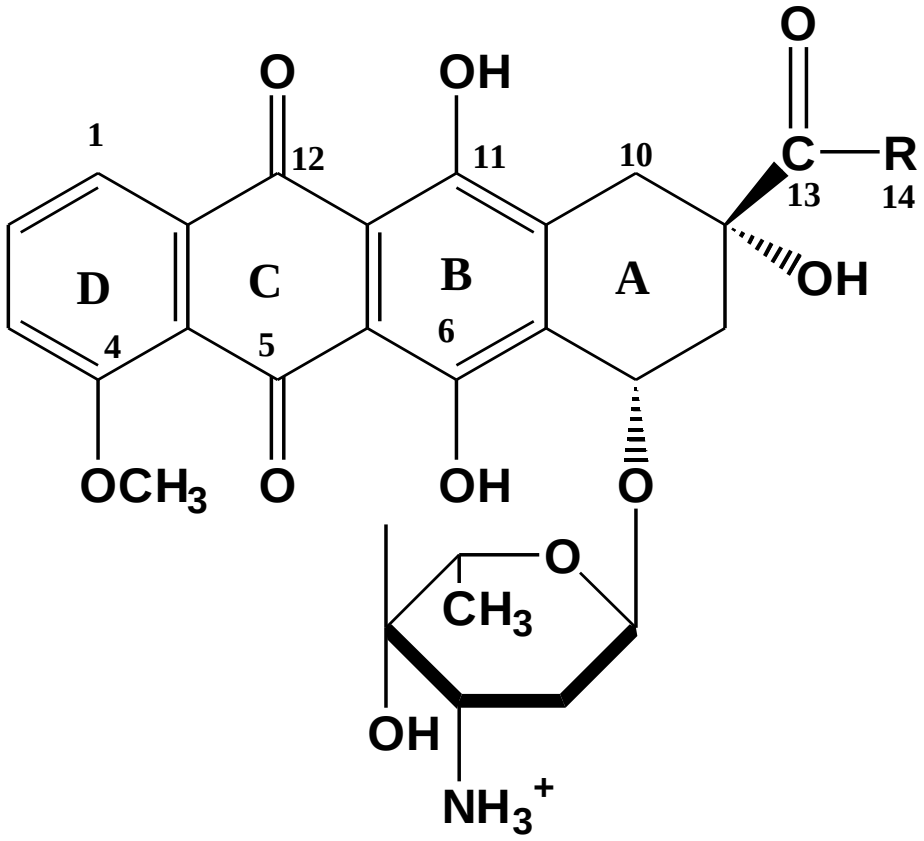
Anthracyclines

Aglycone:

Anthroquinone, or
Anthracyclinone

Sugar:

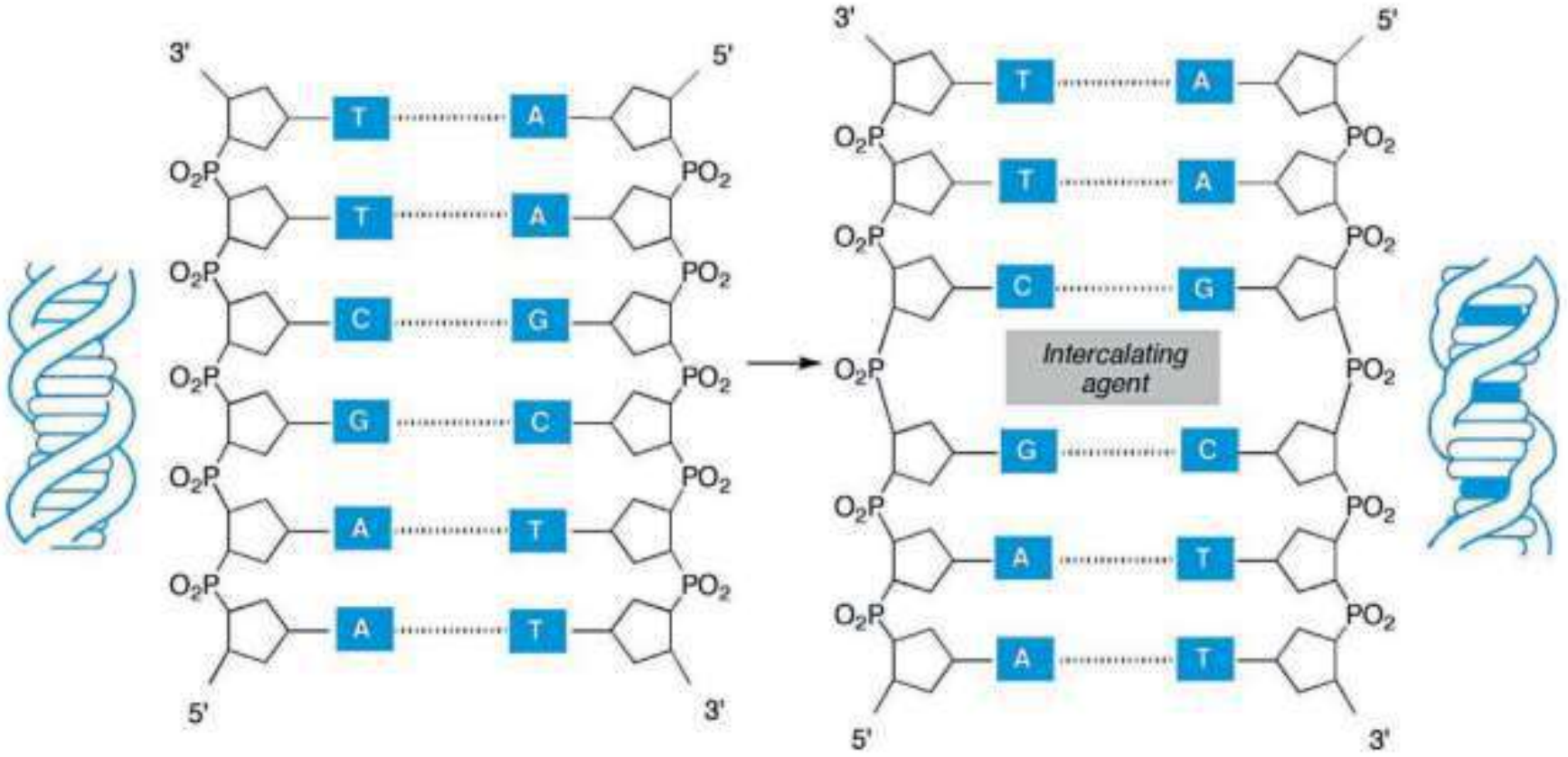
L-Daunosamine



Anthracyclines

Anthracyclines

Intercalation between base pairs of double strand DNA



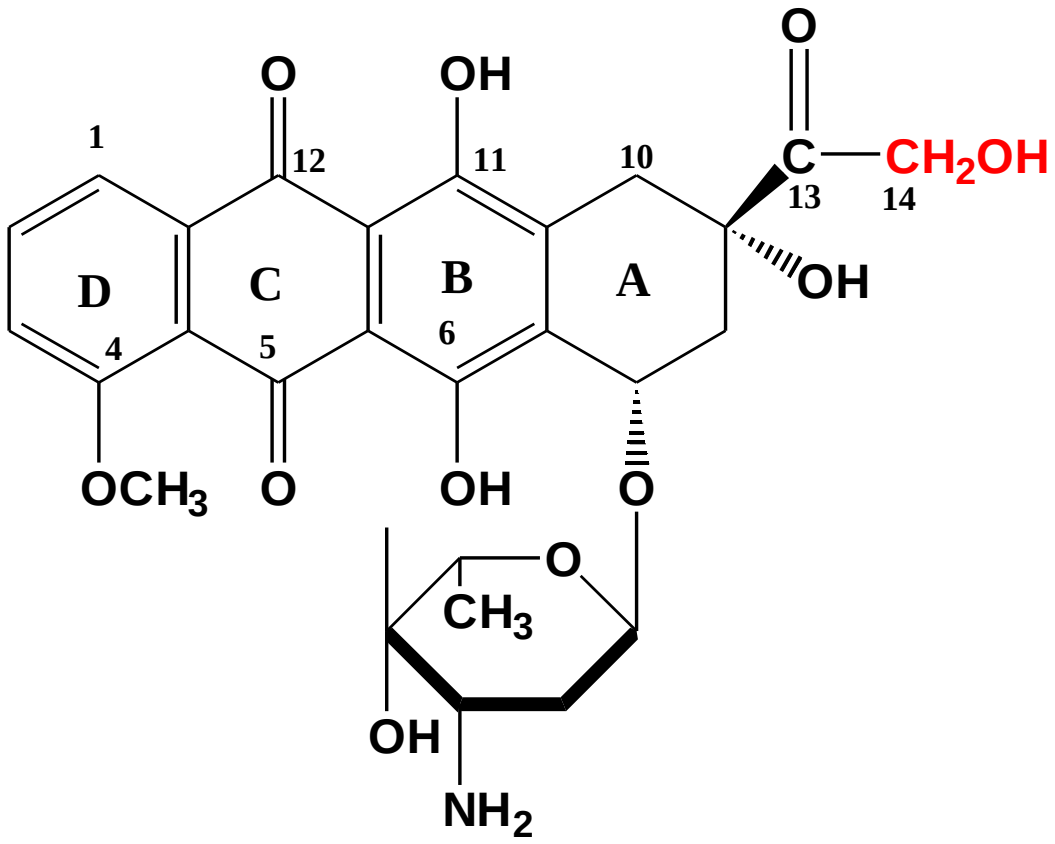
Planar aromatic or heteroaromatic ring systems are inserted between adjacent base pairs perpendicularly to the axis of the helix

Anthracyclines

Doxorubicin

Reduction of CO at 13 by ketoaldoreductase produces doxorubicinol which is a chronically cardiotoxic

t ½: 40 h



Doxorubicin

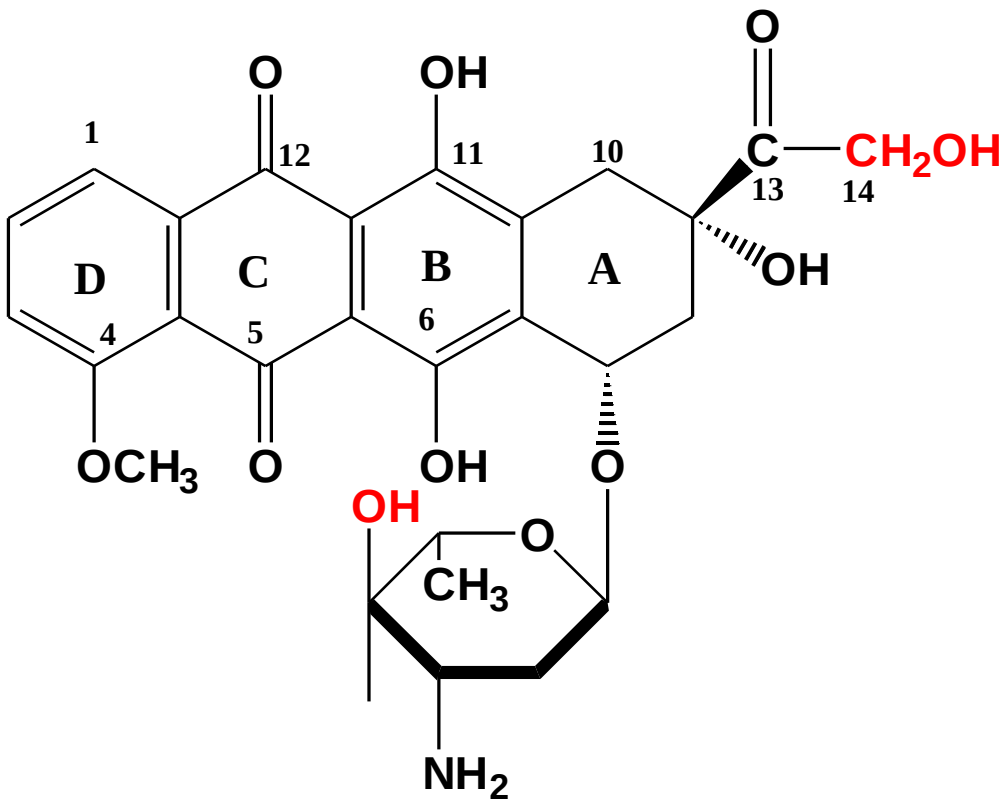
Anthracyclines

Epirubicin

Reduced cardiotoxicity

Epimerization of 4`-OH, which places this –OH function in β position resulting in increased glucuronidation, faster clearance.

Reduced metabolic reduction to epirubicinol, the C-13 alcohol (compared with doxorubicin)



Epirubicin

Anthacyclines

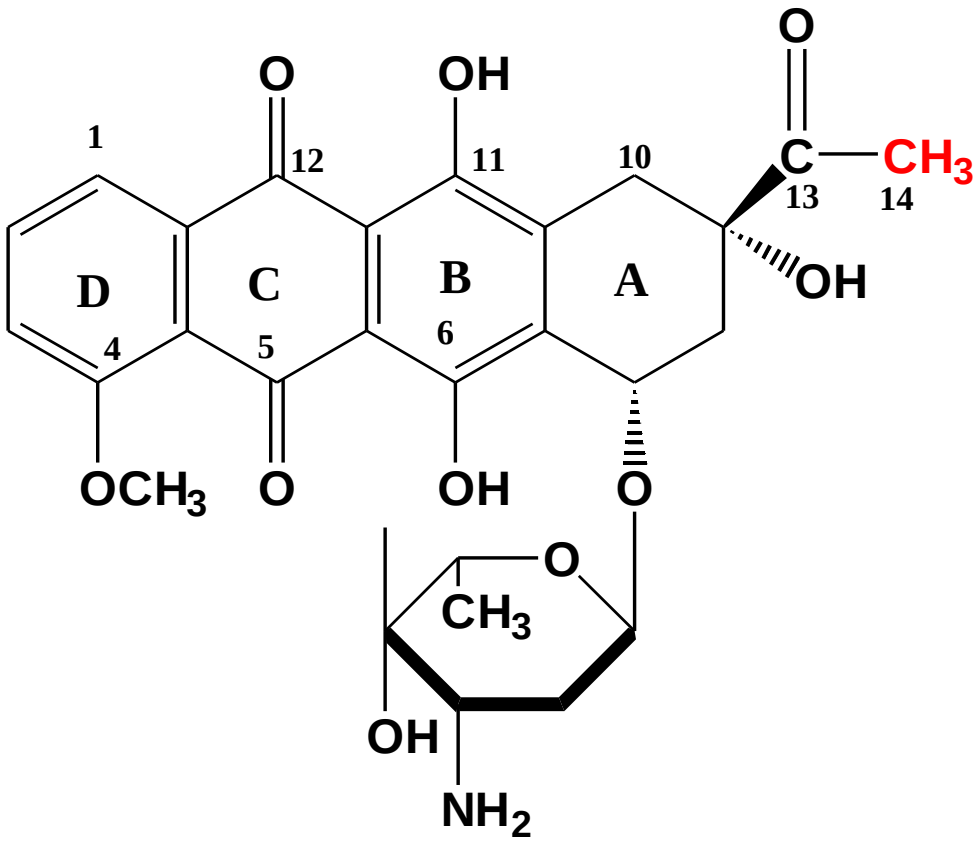
Daunorubicin

Reduced cardiotoxicity

R at 14: CH_3

Rapid conversion to C 13 –ol metabolite daunorubicinol compared to doxorubicin

t ½: 18 h



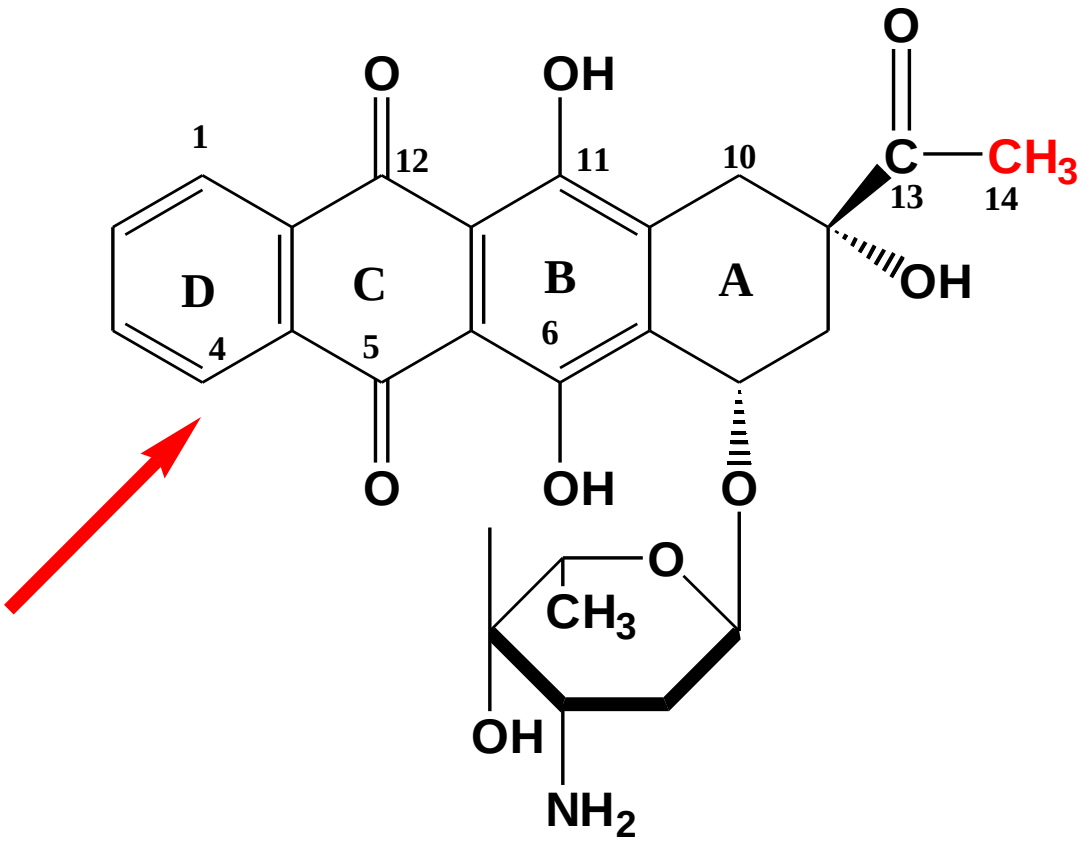
Daunorubicin

Anthracyclines

Daunorubicin

More lipophilic than daunorubicin

Loss of –OCH3 flattens the ring D enhances intercalation between DNA base pairs



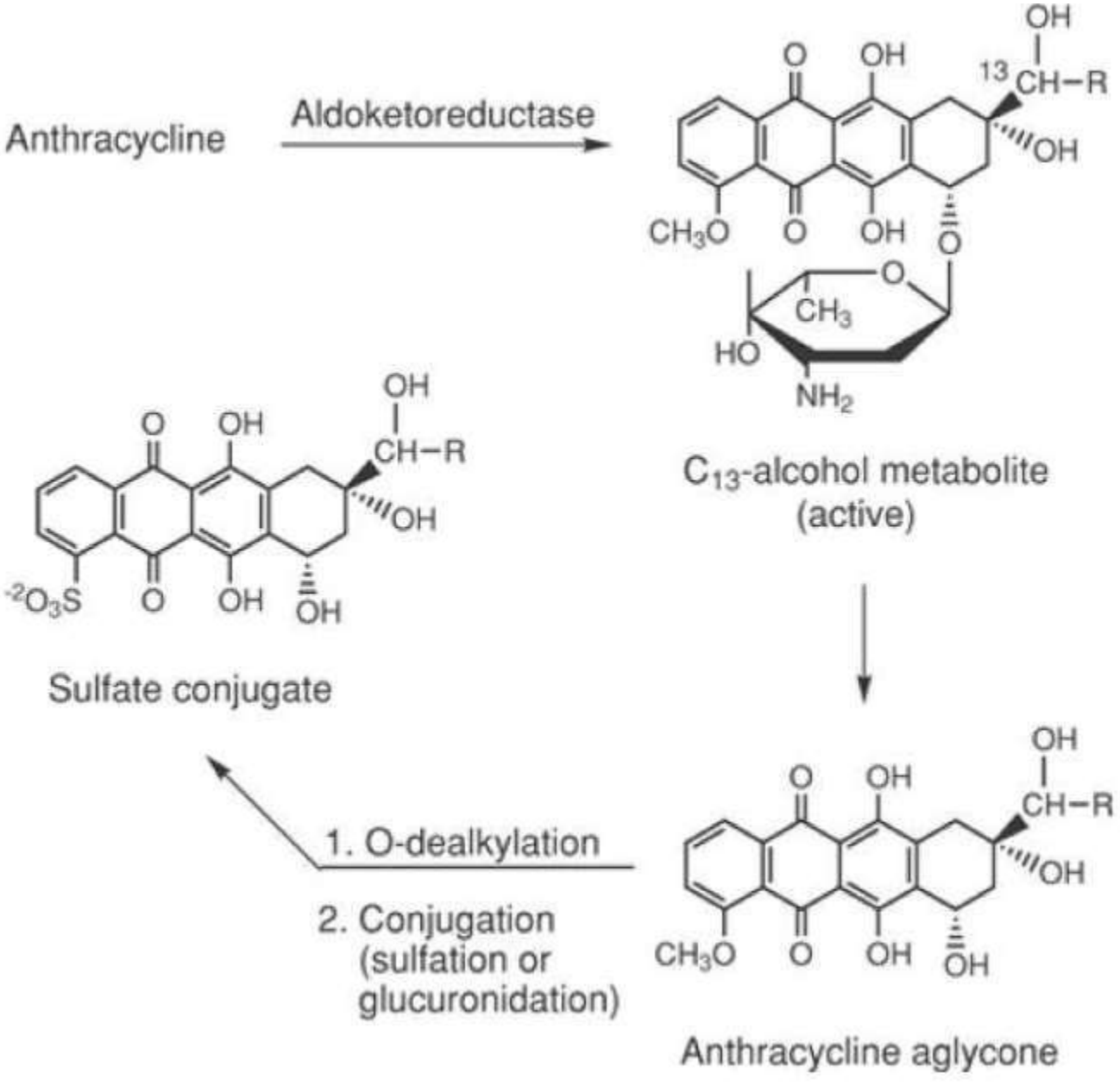
Idarubicin

Anthracyclines : Metabolism

C13- alcohol active metabolite

Chronic cardiotoxicity

Opens a selective ca ion channel leading to increased cytosolic levels of Ca2+ in the sarcoplasmic reticulum



Anthracyclines

Mechanism of actions:

DNA intercalation

Topoisomerase II inhibition

Free radical generation

Main side effect:
Cardiotoxicity limiting its' application.

To Prevent cardiotoxicity: Dexrazoxane

Anthracyclines: Cardiotoxicity

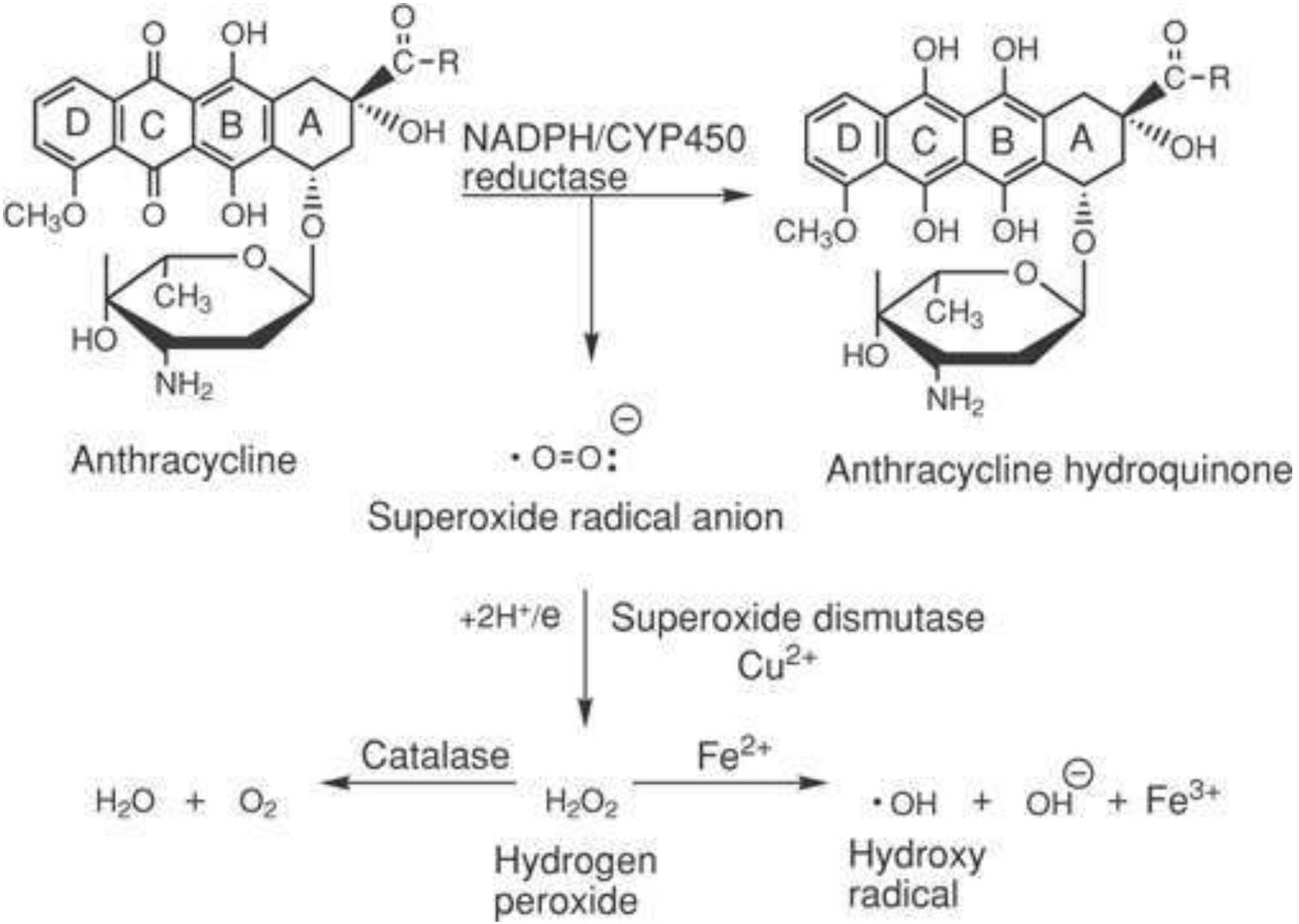
Anthracyclines causes cardiotoxicity which limits the dose and duration of therapy.

It is connected with quinone moiety of the aromatic system which after reduction to semiquinone radical spieces which leads to lipids peroxidation by free radical mechanism.

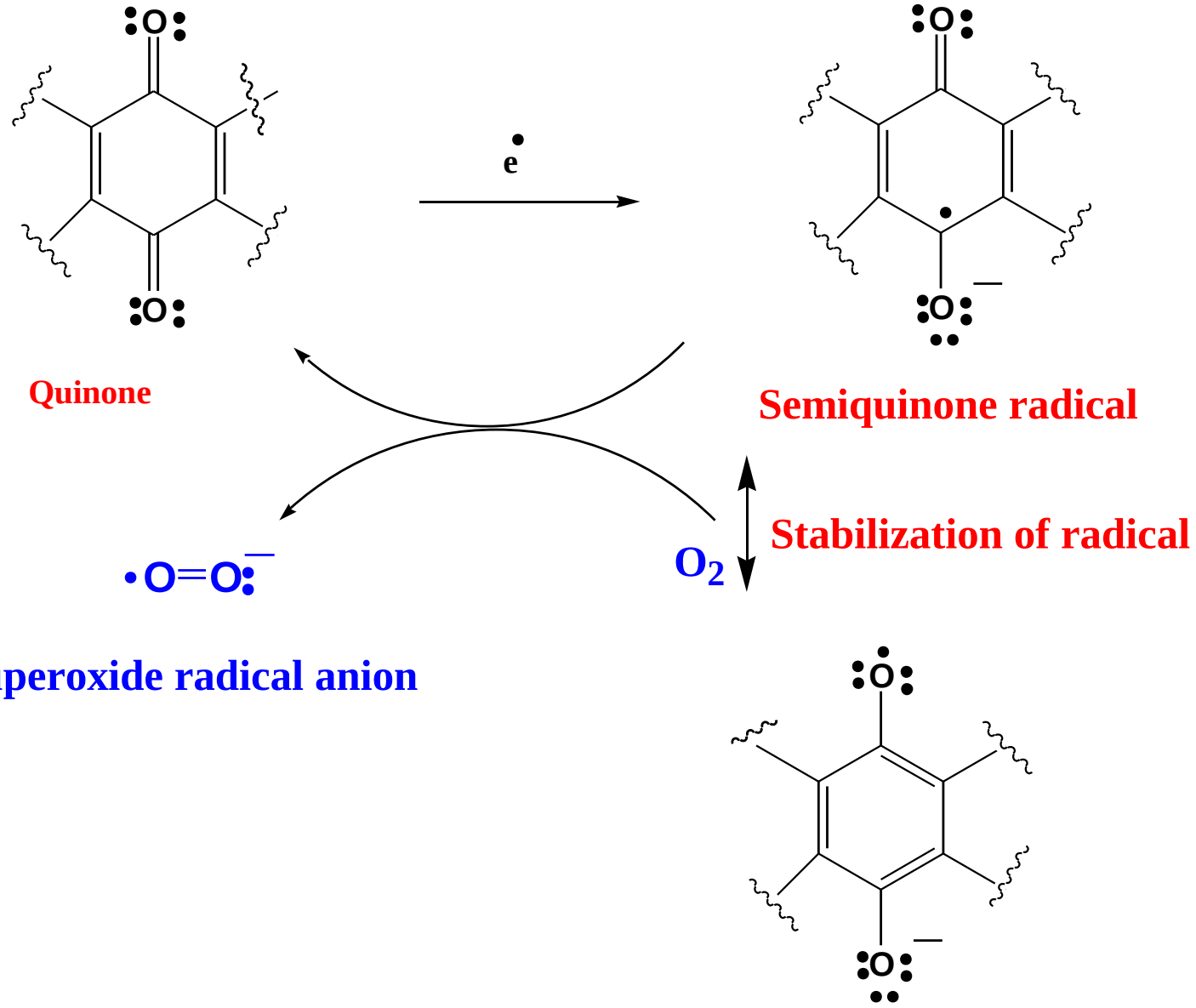
Free-radical formation in the heart Leads to forms of congestive heart failure (Due to low levels of catalase enzyme in heart)

Iron (II) is involved in the process.

Anthracyclines: Cardiotoxicity

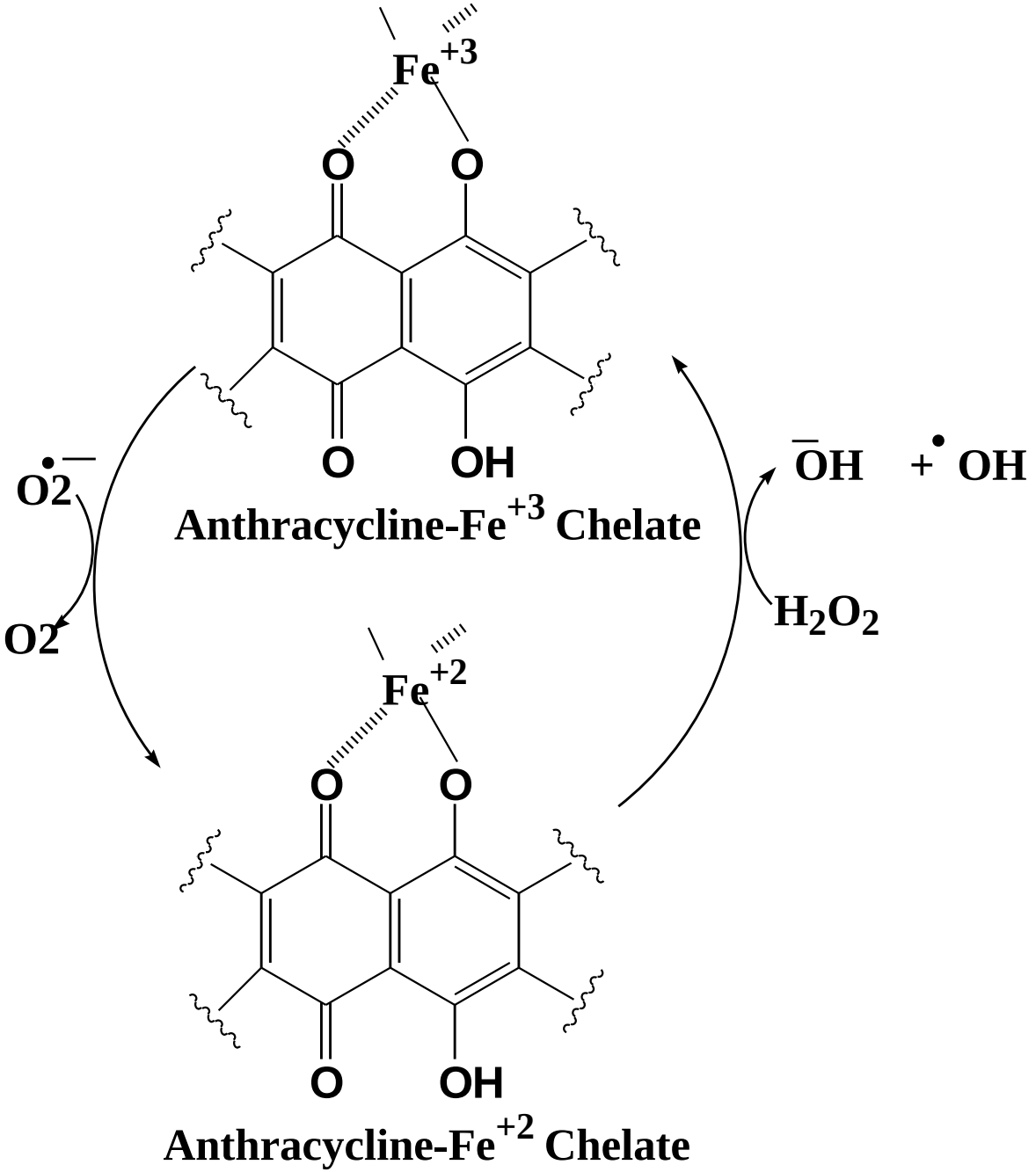


Anthracyclines: Cardiotoxicity

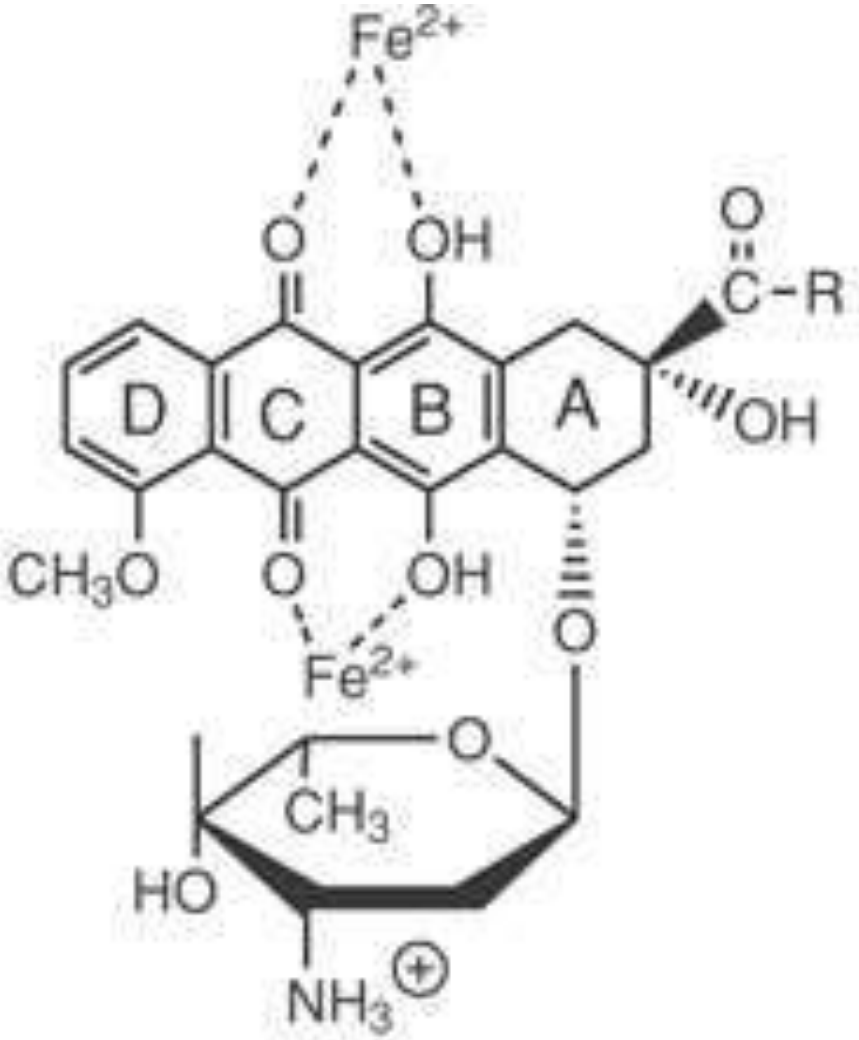


Anthracyclines: Cardiotoxicity

- Chelation of Fe+3 with anthracycline
- Chelate binds more strongly to DNA than anthracycline

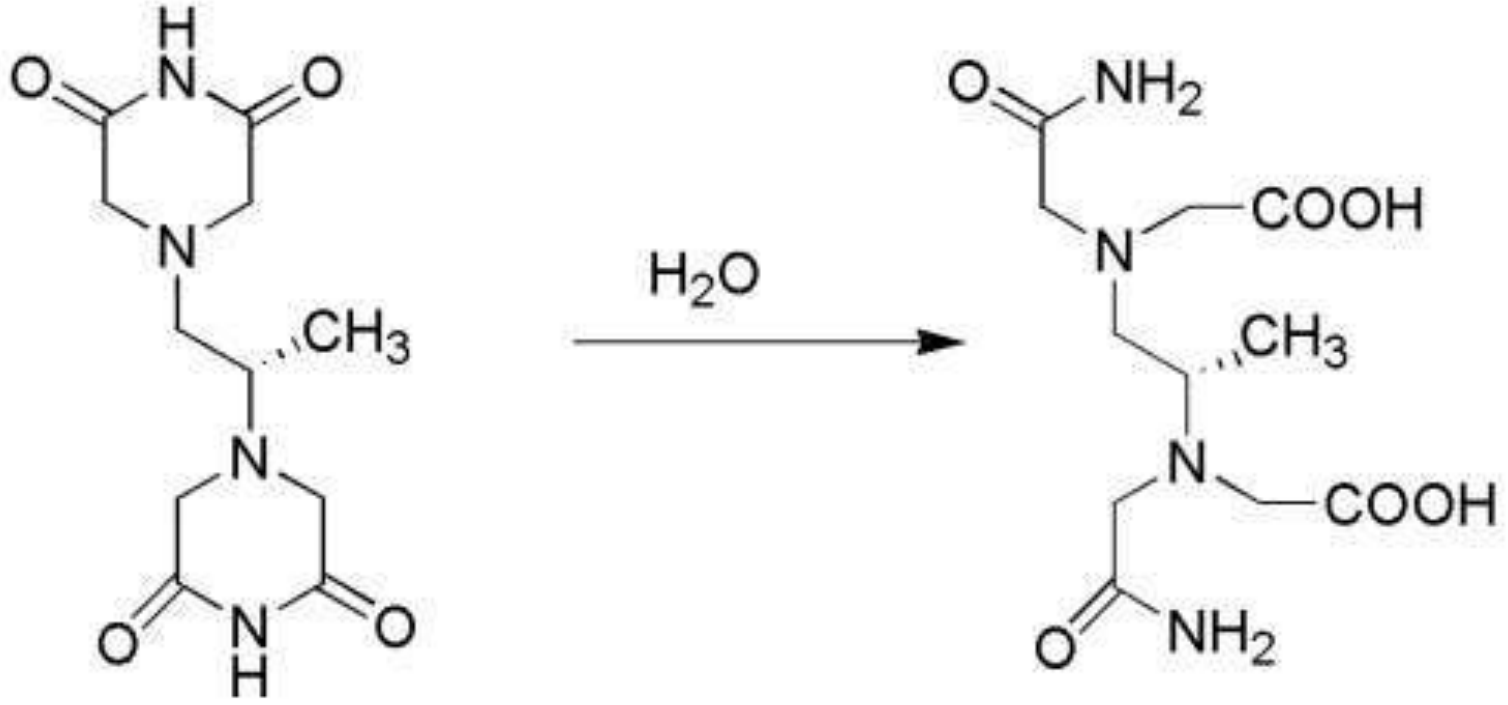


Anthracyclines: Cardiotoxicity



Chelation of ferrous ion by anthracyclines

Anthracyclines: Cardiotoxicity



Dexrazoxane

To counteract cardiotoxicity: Dexrazoxane
It is a chelating agents [resemble EDTA] of Fe(II)
Only (+) enantiomer is active

Chemotherapy

Part 6

[Antibiotic]

Dr. Mai Ramadan

Introduction

Antibiotics

Anthracyclines:

Doxorubicin, Epirubicin, Daunorubicin, Idarubicin, Valrubicin

Mitoxantrone

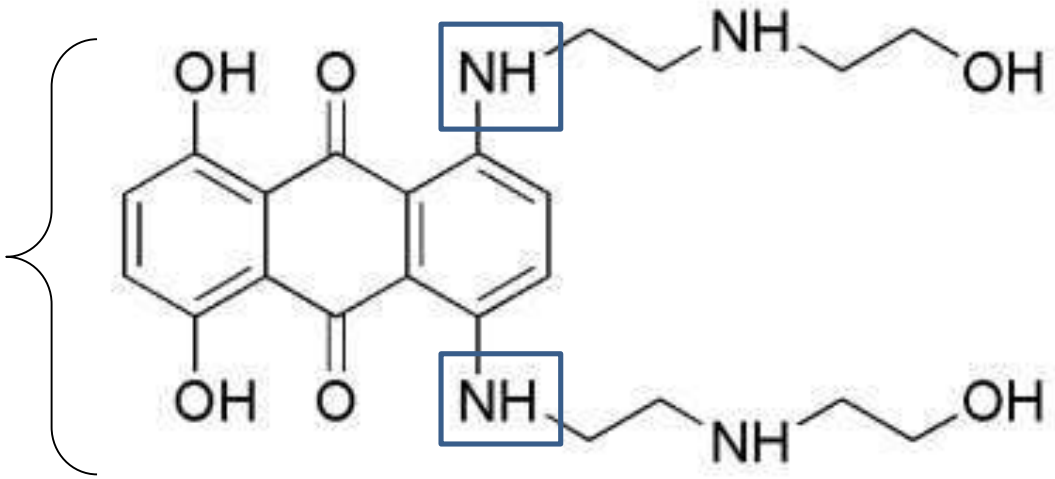
Bleomycin

Actinomycin D

Mitomycin C

Mitoxantrone

Anthracenedione ring



Mitoxantrone

like L-Daunosamine sugar is protonated Ionic interaction of the protonated amines with the phosphate backbone of DNA.

Mitoxantrone

Mitoxantrone:

A synthetic compound related to natural anthracyclines

Aminoalkyl anthraquinone derivative

The aminosugar and ring A are replaced by a side chain with possible H-bond and protonation possibilites

Drug has less cardiotoxic side effects [No active cardiotoxic metabolite, reduced free radical formation]

Mitoxantrone

Resemble anthracyline in mechanism of action.

- Intercalate between DNA base pairs. Flat ring system.

- No sugar part. The amino group in the side chain is responsible for ionic interacton of phosphate in DNA

- Inhibit topoisomerase II

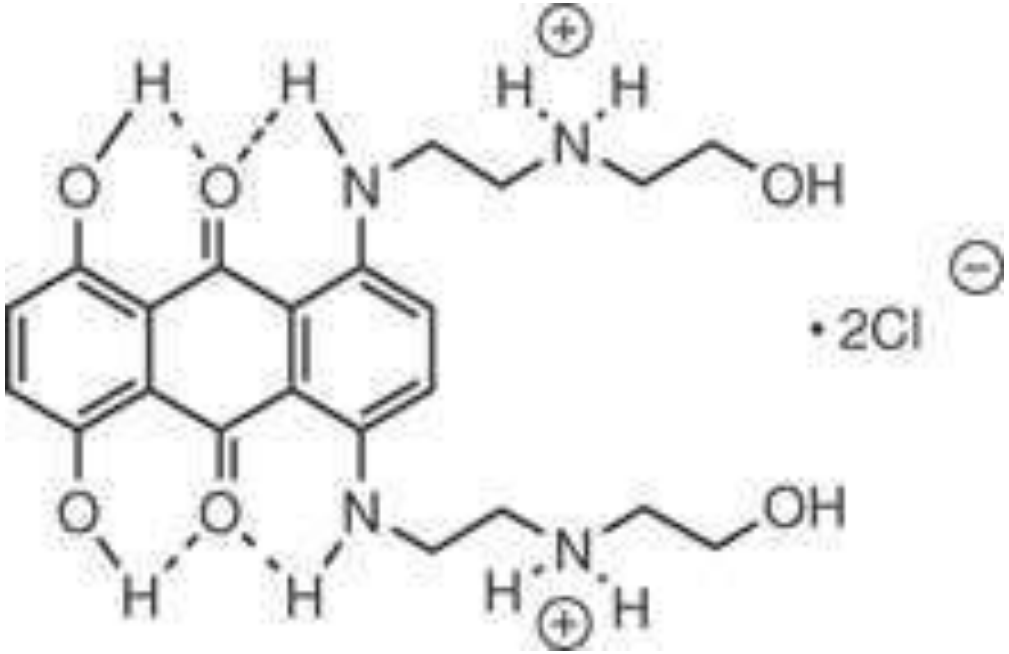
- Not reduced to semiquinone [**No reactive oxygen species ROS, Free radical formation]**

- Covalently bonded to DNA though HCOH.

Mitoxantrone

Mitoxantrone is not a substrate for NADPH/CYP reductase enzyme

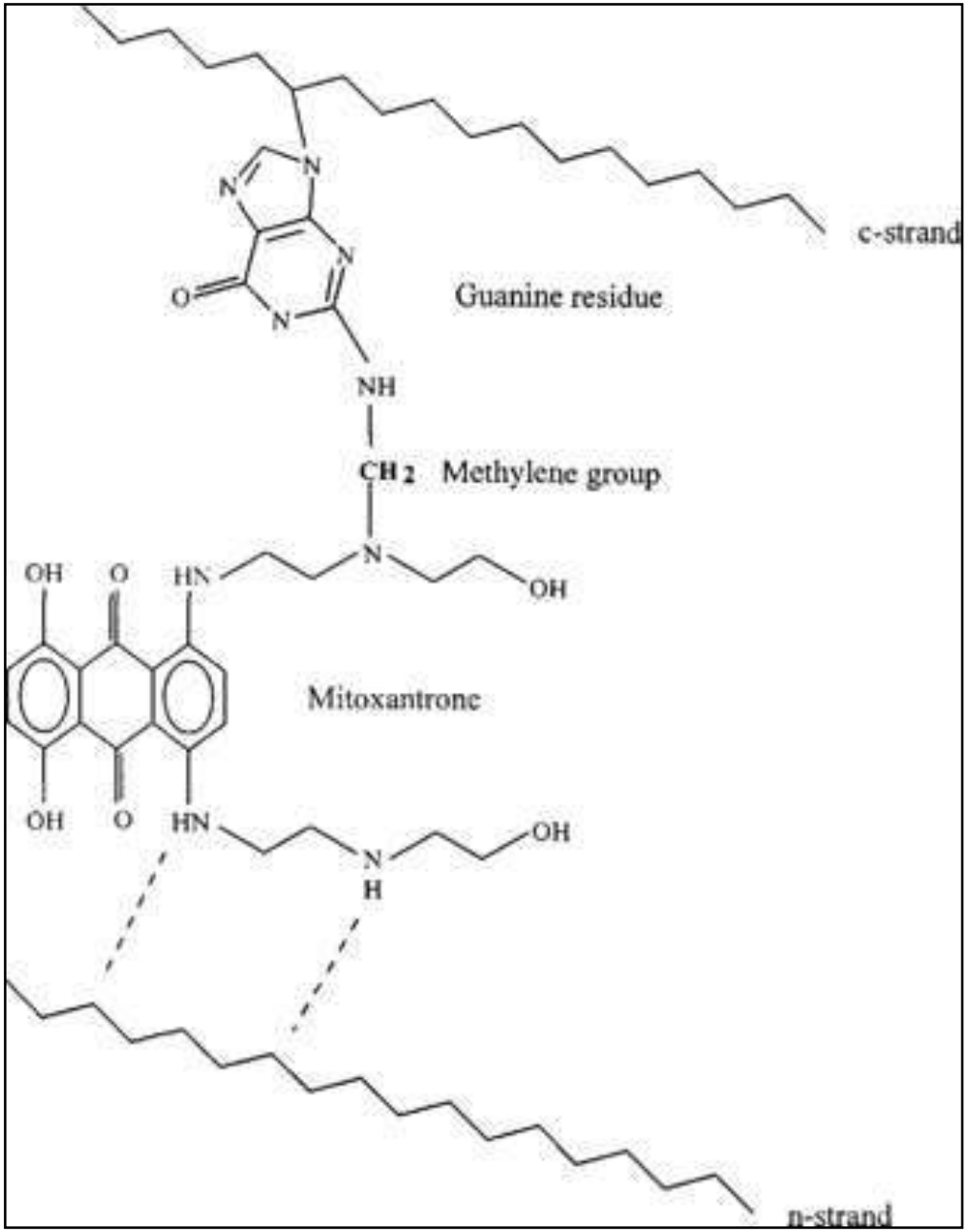
Quinone ring in mitoxantrone is stabilized by intramolecular H-Bond thus will not be reduced to semiquinone----------



Mitoxantrone

Covalent bonding with DNA using HCHO [High concentration in cancer cells]

The binding though –NH2 group at position 2 of G



Complex Glycopeptide

Bleomycin (BLM)

Natural glycopeptidic antibiotics produced by *Streptomyces verticillus*

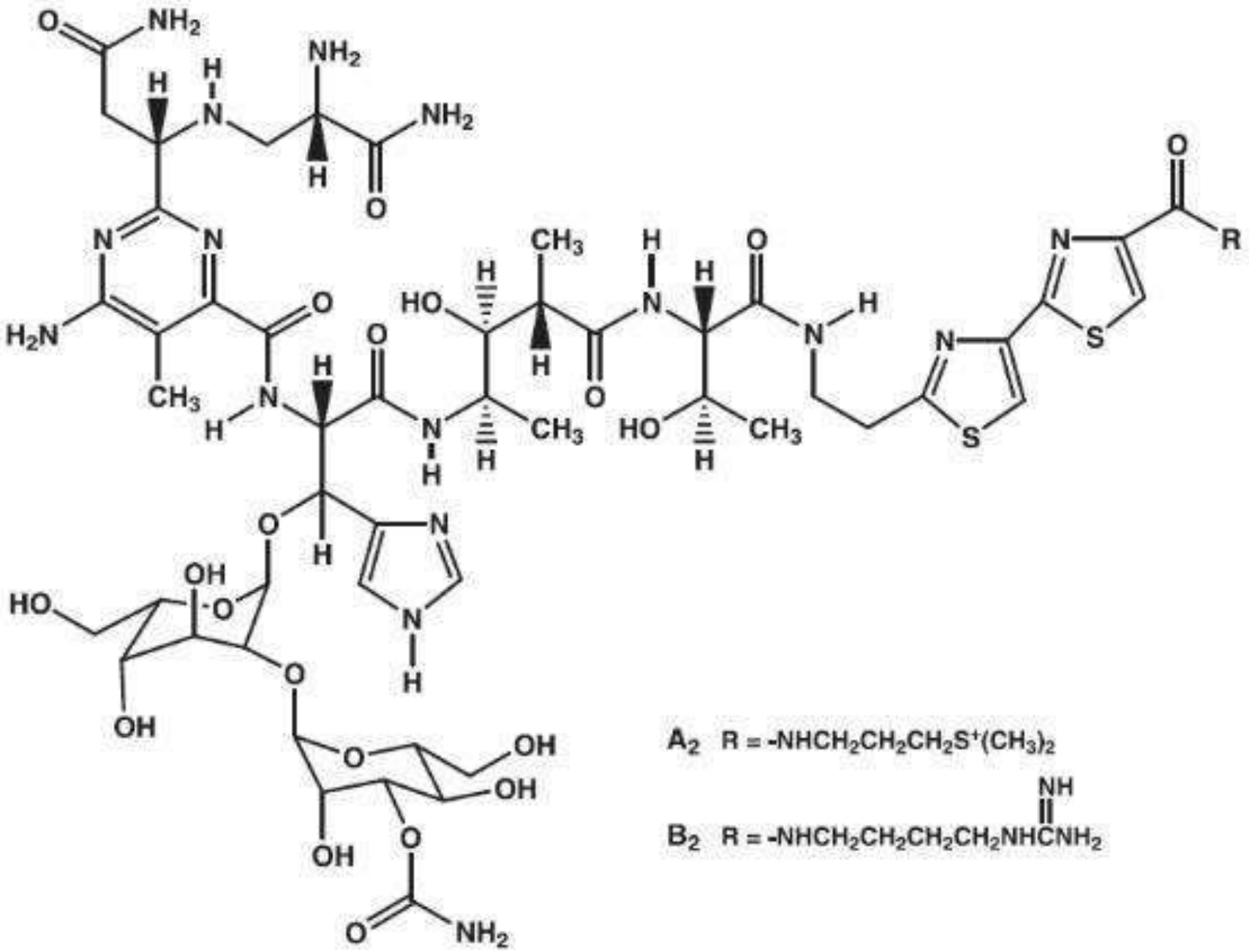
It consists of three regions:

Metal binding domain stable complex with Fe(II). Ternary complex [BLM-Fe-O2] responsible for the DNA cleavage activity

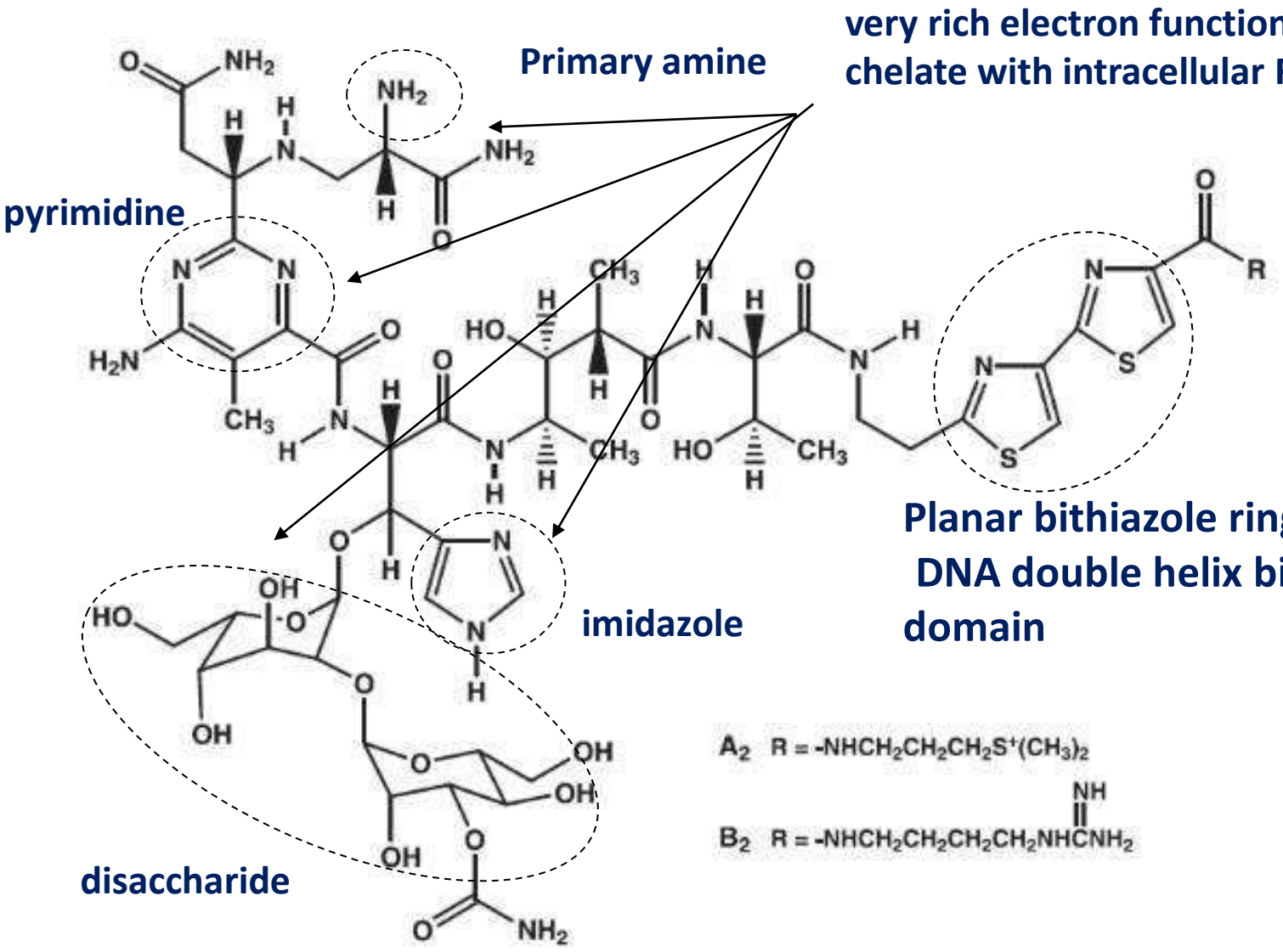
DNA binding domain intercalates into the double helix

Carbohydrate domain selected accumulation of bleomycin in some cancer cells

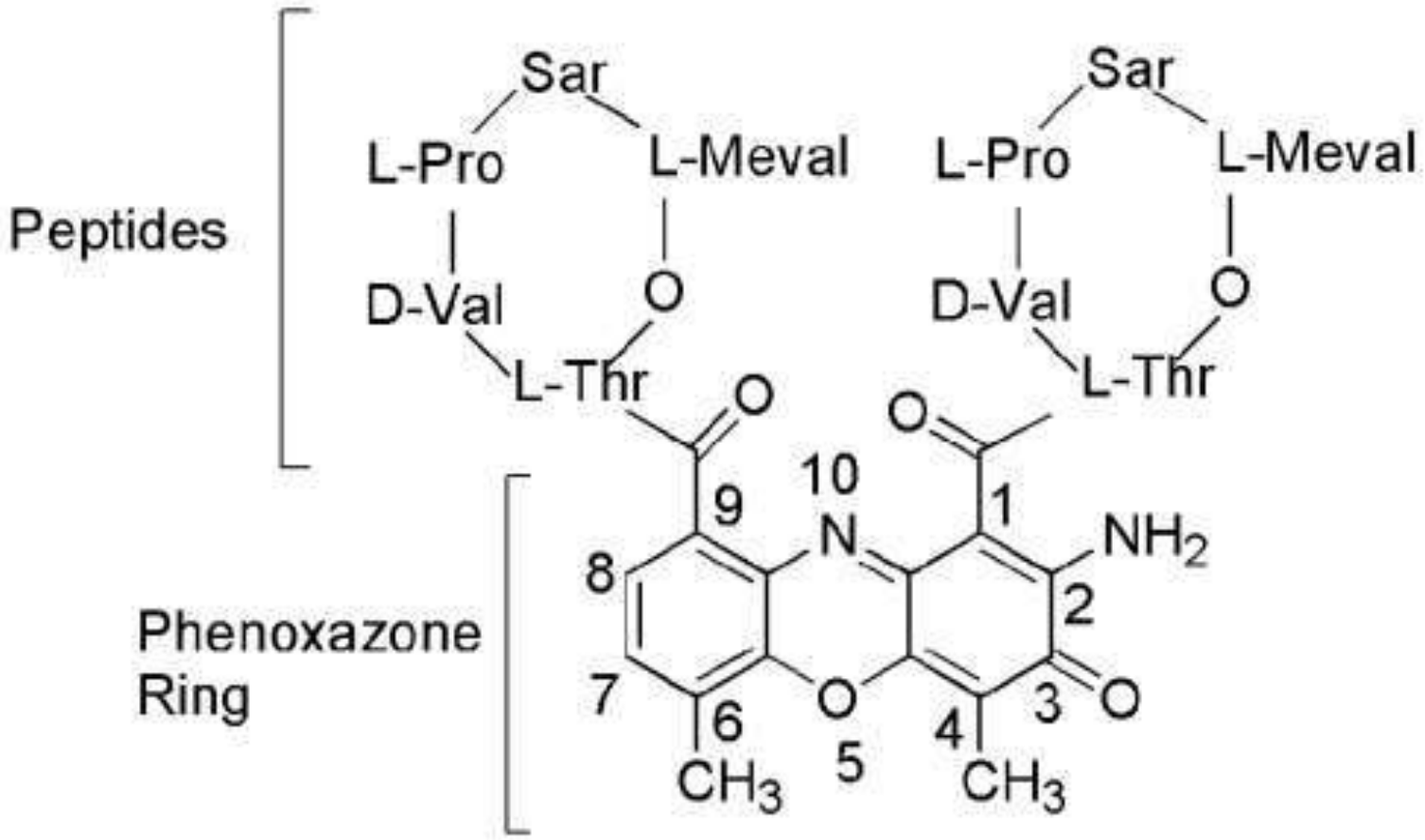
Complex Glycopeptide



Bleomycin


Primary amine
very rich electron functional group;
chelate with intracellular Fe^{2+}
pyrimidine
Planar bithiazole ring system:
DNA double helix binding
domain
imidazole
disaccharide
A_2 R = $-NHCH_2CH_2CH_2S^+(CH_3)_2$
B_2 R = $-NHCH_2CH_2CH_2CH_2NHC(=NH)NH_2$

Actinomycin D, (Dactinomycin)



Actinomycin D (Dactinomycin)

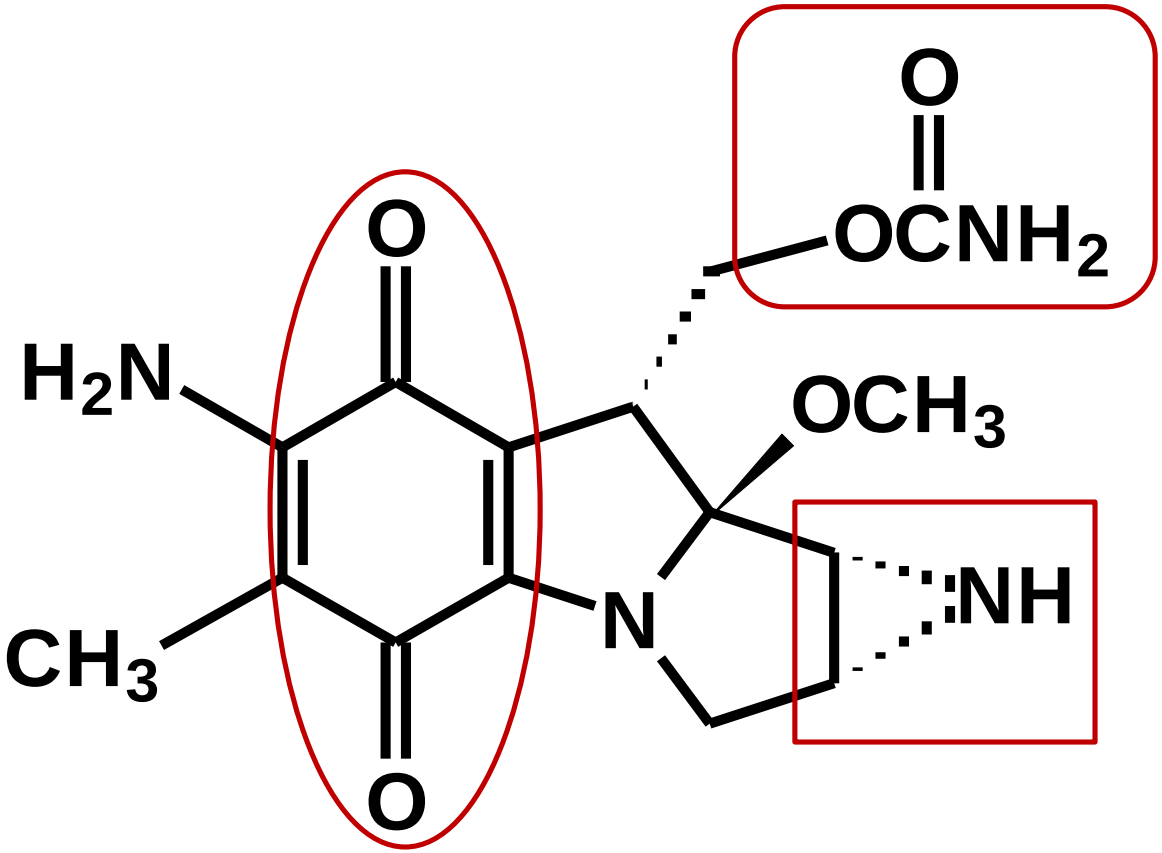
The planar phenoxazone ring, which facilitates intercalation between DNA base pairs.

The peptide loops form a lactone cycle, pentapeptide
Provide additional interactions with DNA after intercalation

Topoisomerase II inhibitor

Free radical formation

Mitomycin C



Three groups

Quinone: Free radical formation

Aziridine: Alkylating agent

Carbamate: Alkylating agent

Streptozocin

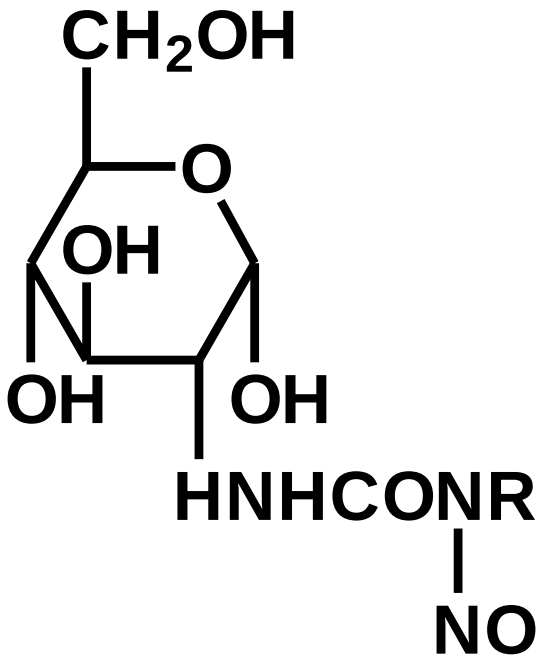
Antibiotic

Methylnitrosourea (MNU) moiety attached to C2 of glucose.

Glucopyranose moitey
Selectivity to Islet cell carcinoma

Water soluble: IV

Lack of 2-chloroethyl substituent of carmustine is less reactive DNA alkylating agents



Streptozocin

R: -CH_3