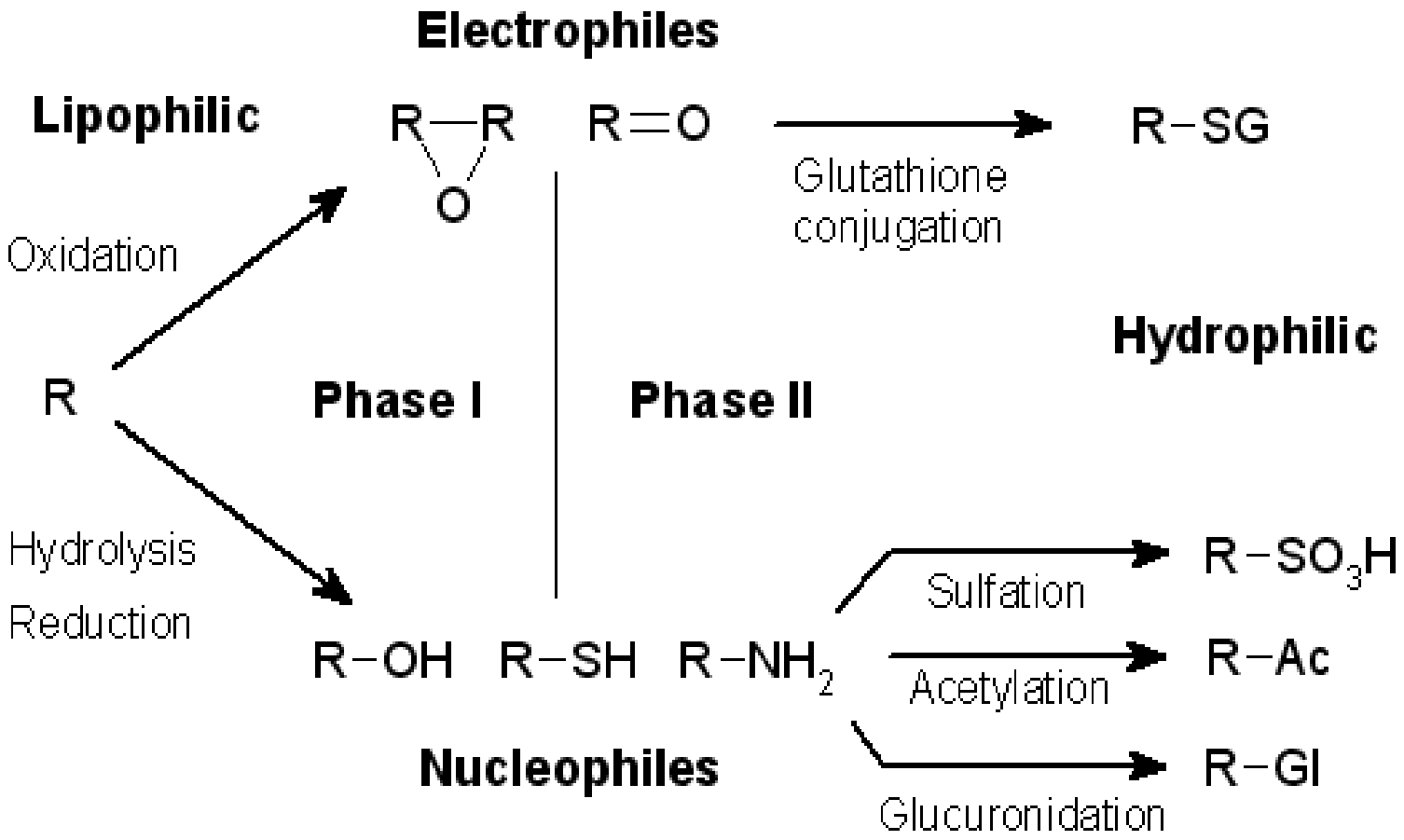
- Metabolism and toxicity of drugs, covalent drugs

ADMET
Metabolism of drugs

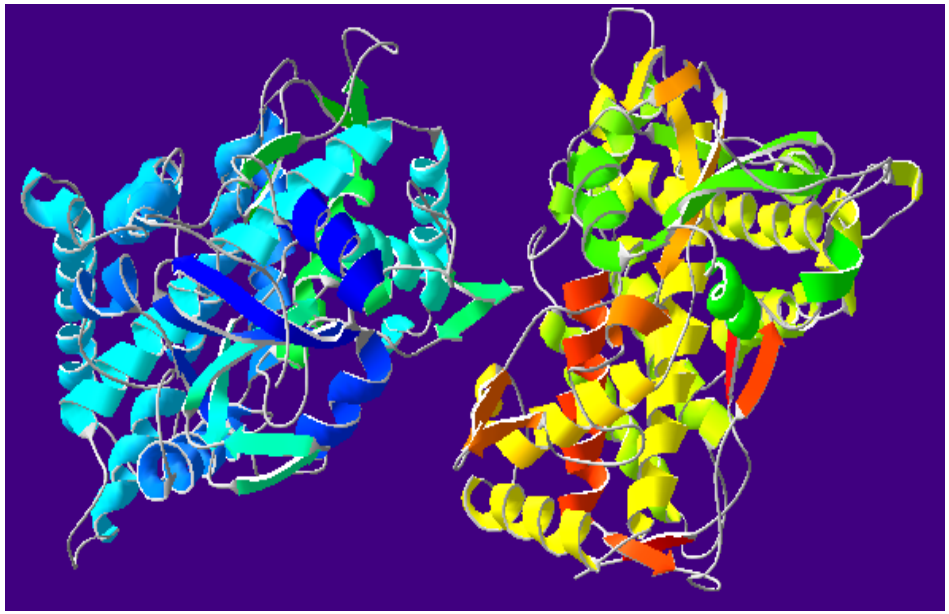
- Phase I, oxidative metabolism: oxidation by cytochrome P450 enzymes (CYP450), reductive metabolism, hydrolysis
- **THE MOLECULAR WEIGHT DECREASES (FRAGMENTATION)**
- Phase II, conjugative metabolism: conjugation with polar moieties as glutathione, glucuronic acid, amino acids, phosphates and sulfates
- **THE MW INCREASES (CONJUGATION)**
- Drug becomes progressively more polar and is easily excreted in the urinary tract

drug metabolism and detoxification



oxydation: the Cytochromes P450

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorized as P450-containing systems.



CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses.

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (RH) while the other oxygen atom is reduced to water:

$$RH + O_2 + NADPH + H+ \rightarrow ROH + H_2O + NADP+$$

Interaction of drugs with CyP450

- Many drugs may increase or decrease the activity of various CYP isozymes either by inducing the biosynthesis of an isozyme (enzyme induction) or by directly inhibiting the activity of the CYP (enzyme inhibition).
- This is a major source of adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs. For example, if one drug inhibits the CYP-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels.
- Hence, these drug interactions may necessitate dosage adjustments or choosing drugs that do not interact with the CYP system.

- Some natural molecules in food and beverages can interfere with the activity of cytochrome P450.
- For instance, some substances in the Bruxelles sprouts, and cigarette smoke accelerate P450 activity, while grapefruit juice slow P450 activity.
- In the preclinical phase of drug development the interaction with P450 is investigated: if the molecules interacts with P450 interfering with activity is immediately discarded.

- sp^3 carbons hydroxylations @ activated positions (allylic, benzylic, alpha to heteroatoms)
- **FRAGMENTATION**

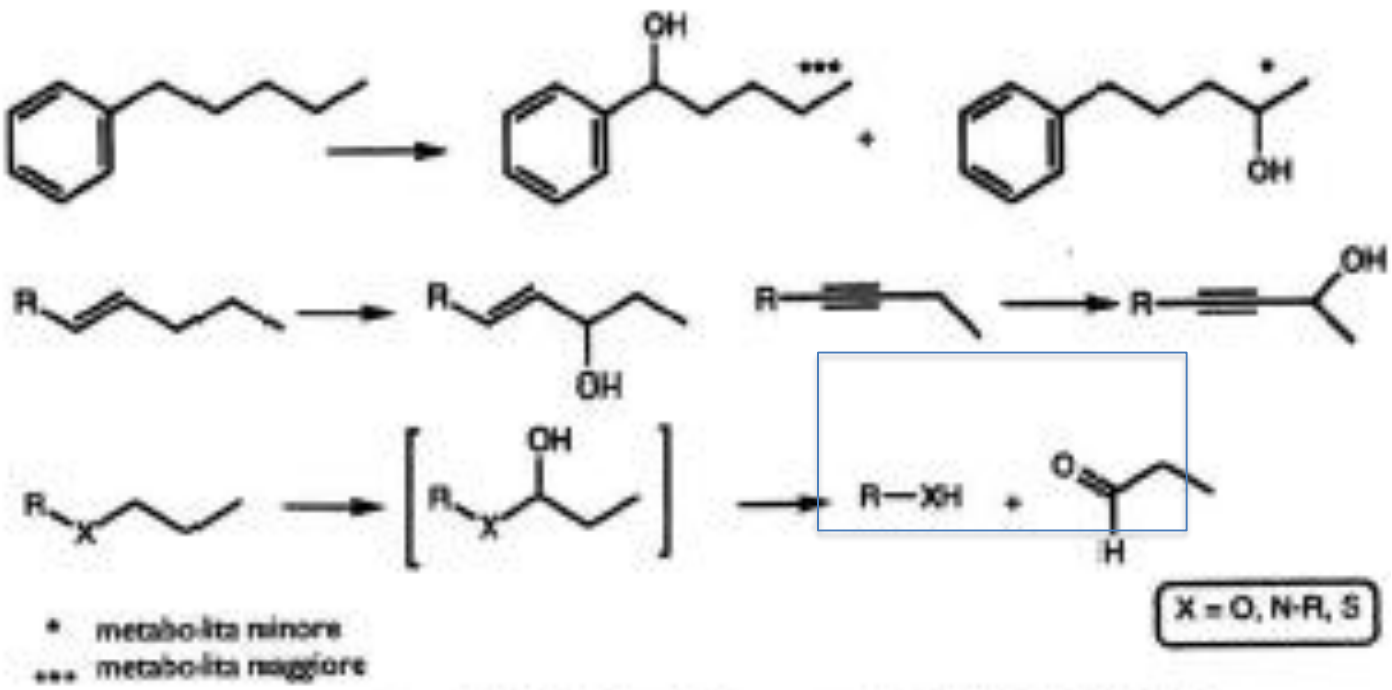


Fig. 29.7 Regioselettività dell'idrossilazione su carboni attivati di tipo sp^3.

oxidative formation of acetals and aminals: fragmentation

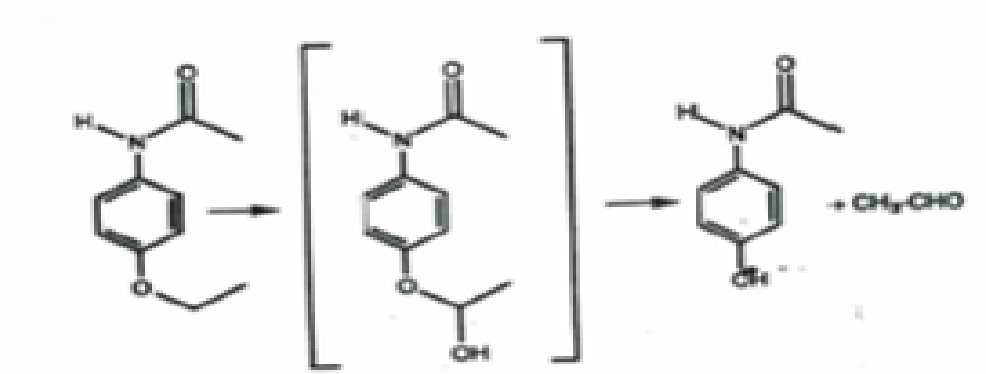


Fig. 29.9 La O-dealchilazione ossidativa della fenacetina produce paracetamolo.

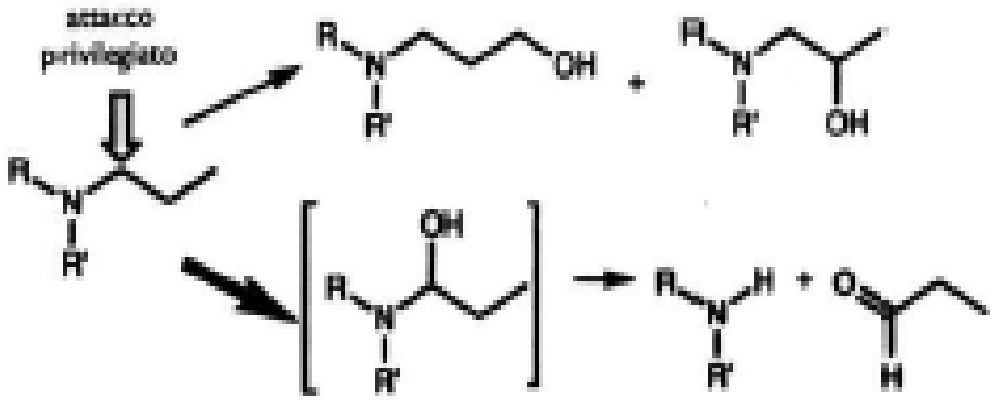
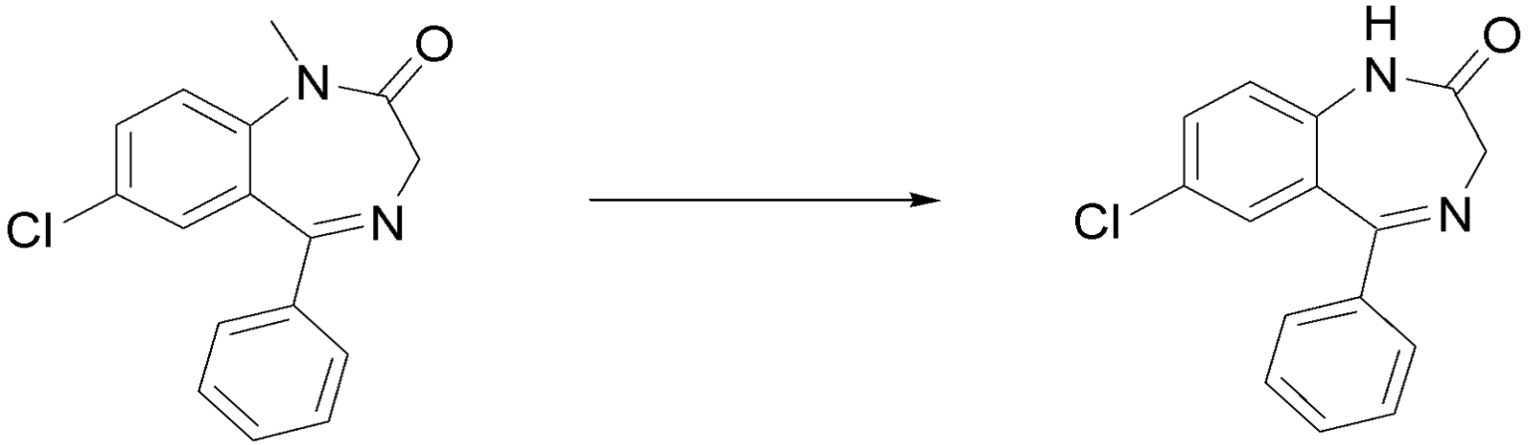


Fig. 29.8 Per ammine a lunga catena, possono essere coinvolte le posizioni α,ω e ω –1, ma la posizione α è la preferita.

Drug metabolism by CyP450

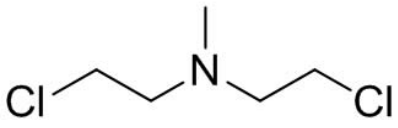
- CYPs are the major enzymes involved in drug metabolism, accounting for about 75% of the total metabolism. Most drugs undergo deactivation by CYPs, either directly or by facilitated excretion from the body.
- Also, many substances are bioactivated by CYPs to form their active compounds.



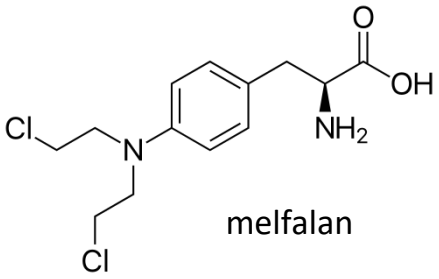
VALIUM (DIAZEPAM)

NORDIAZEPAM

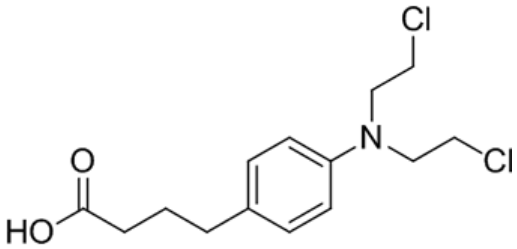
Metabolically activated antitumor drugs



Mecloretammina

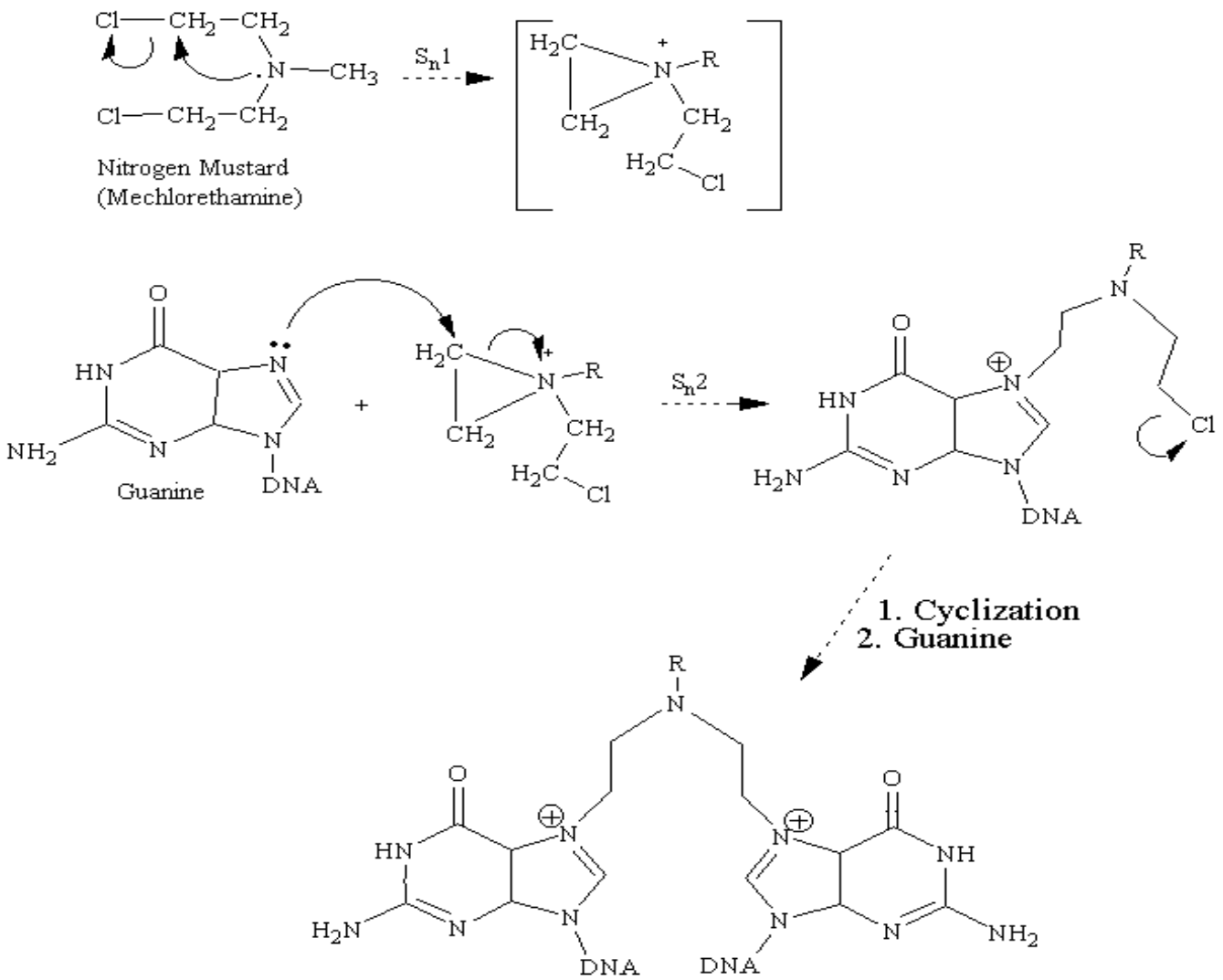


melfalan

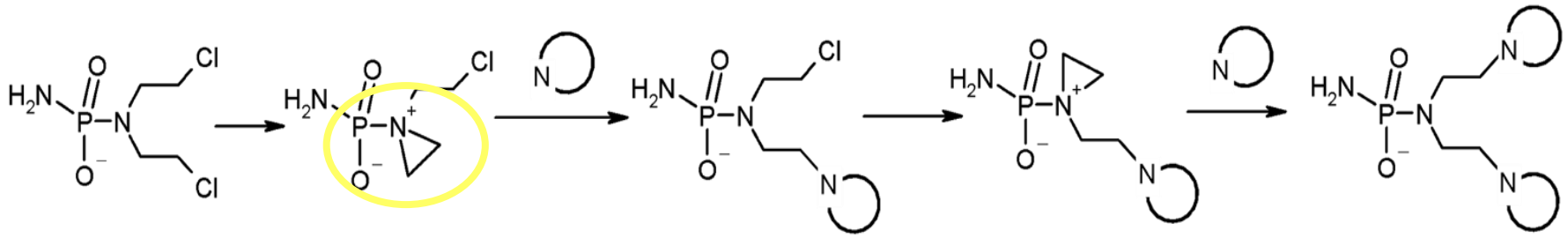


chlorambucil

mechanism of action

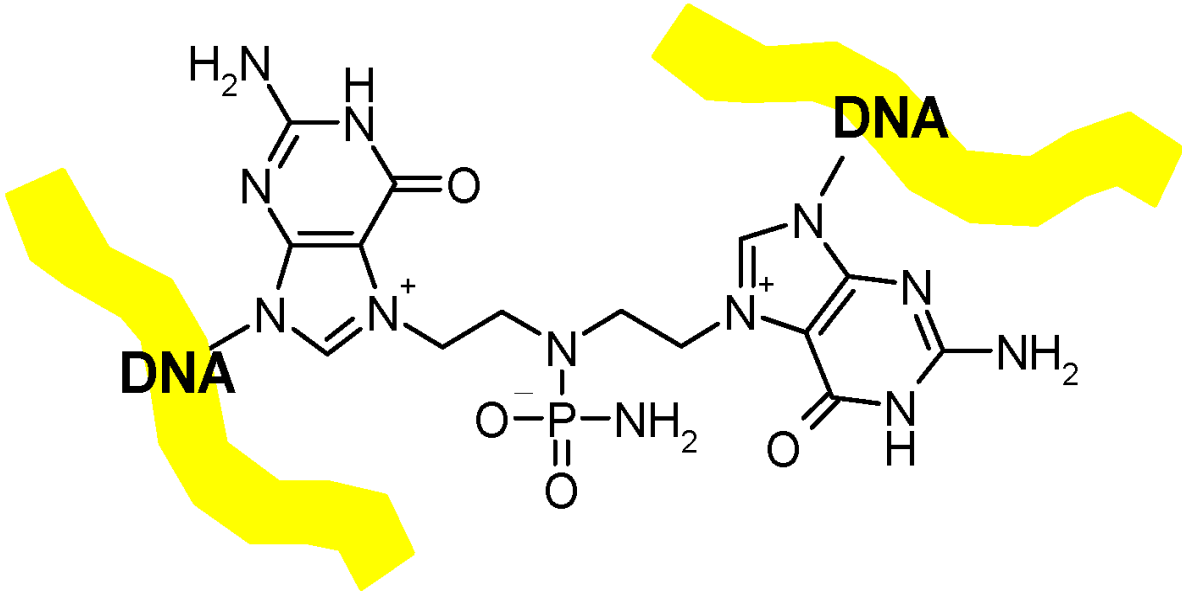


Nitrogen mustards: ANTICANCER AGENTS
DNA CROSSLINKING AGENTS

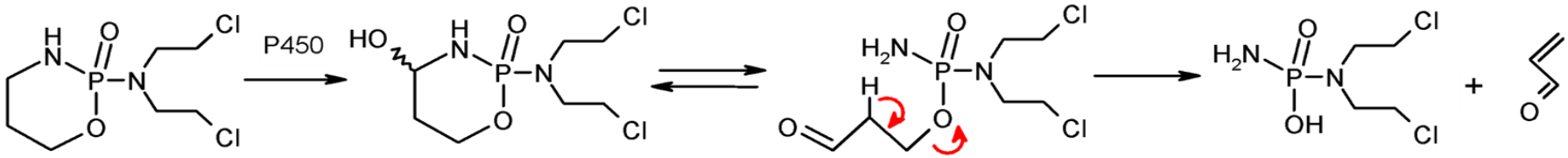


IN CLINIC:

IN COMBINATION WITH OTHER CHEMOTHERAPICS



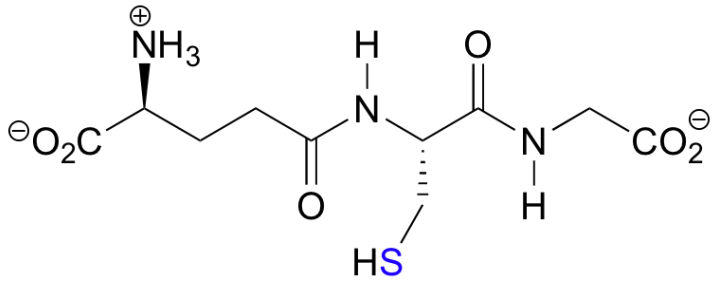
The antitumor agent cyclophosphamide



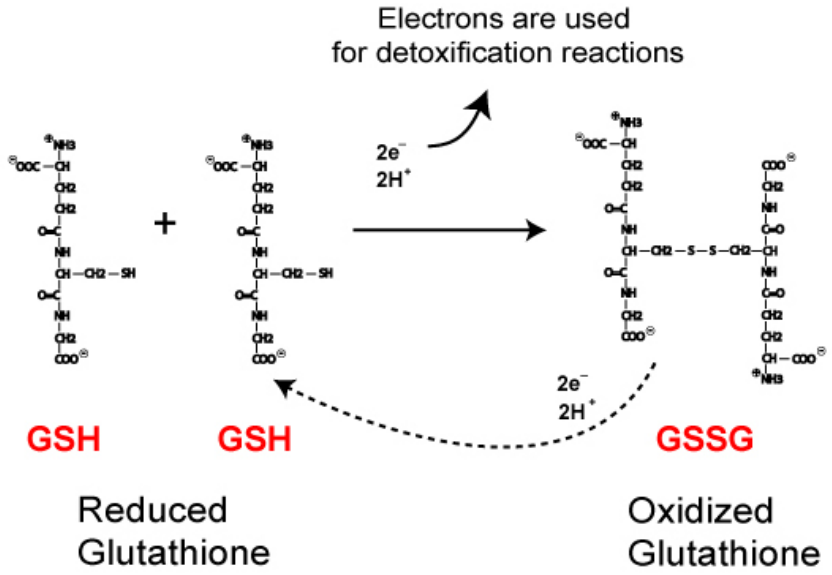
- Cyclophopsphamide is a pro-drug of a nitrogen mustard that reacts with DNA and RNA.
- The cyclophopshamide is transformed into the active drug by a reaction initiated by CyP450 oxidation at the carbon alpha to the nitrogen

Phase I: reductions

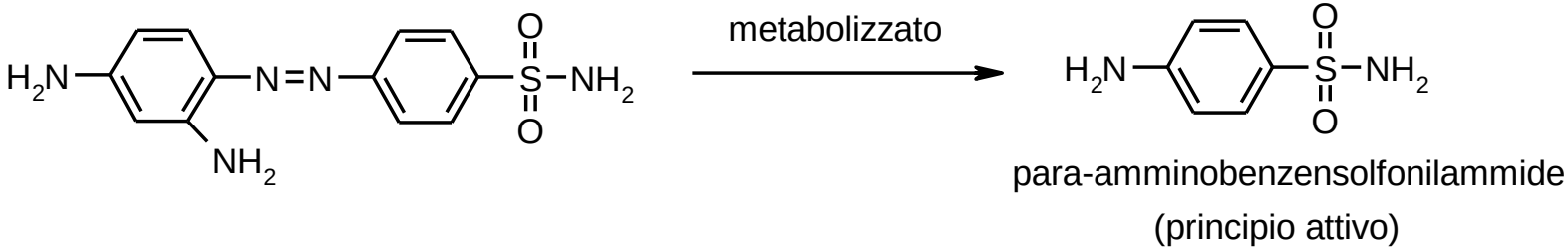
- GSH can act as:
- a reducing agent (phase 1)
- A nucleophile (phase 2)



Glutathione (GSH)



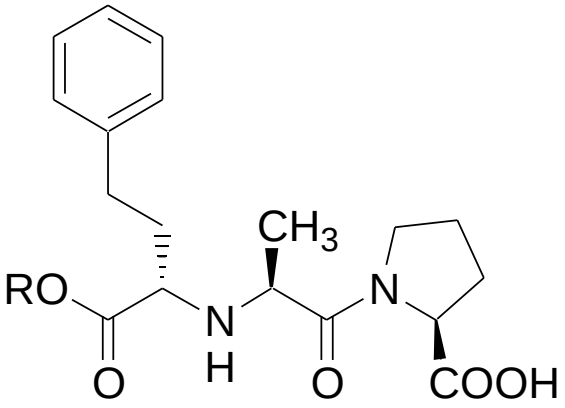
reductive metabolism
remember sulfonamides



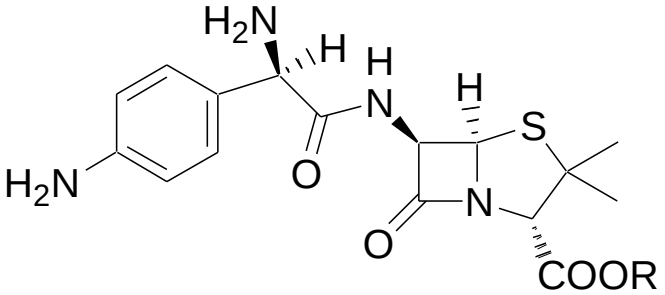
prontosil red (dye)

Phase I: hydrolysis

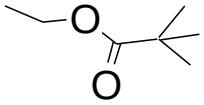
- Esters, amides
- Remember ester-based prodrugs
- Peptide-based drugs are NOT stable



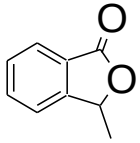
R = Et ENALAPRIL
R = H ENALAPRILAT



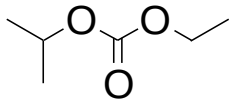
Pivampicillina: R =



Talampicillina: R =

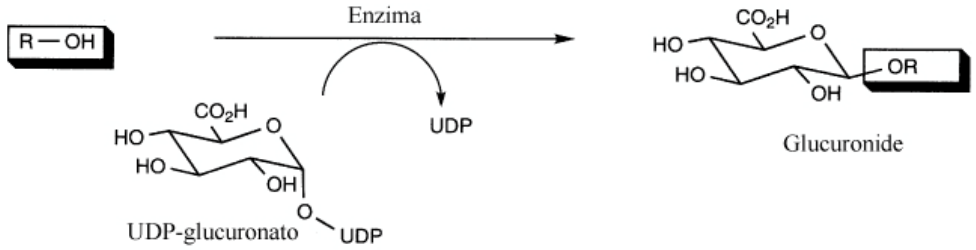


Bacampicillina: R =

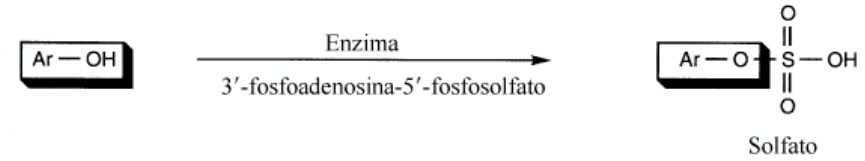


PHASE II DRUG METABOLISM: Glucuronidation and Sulfation

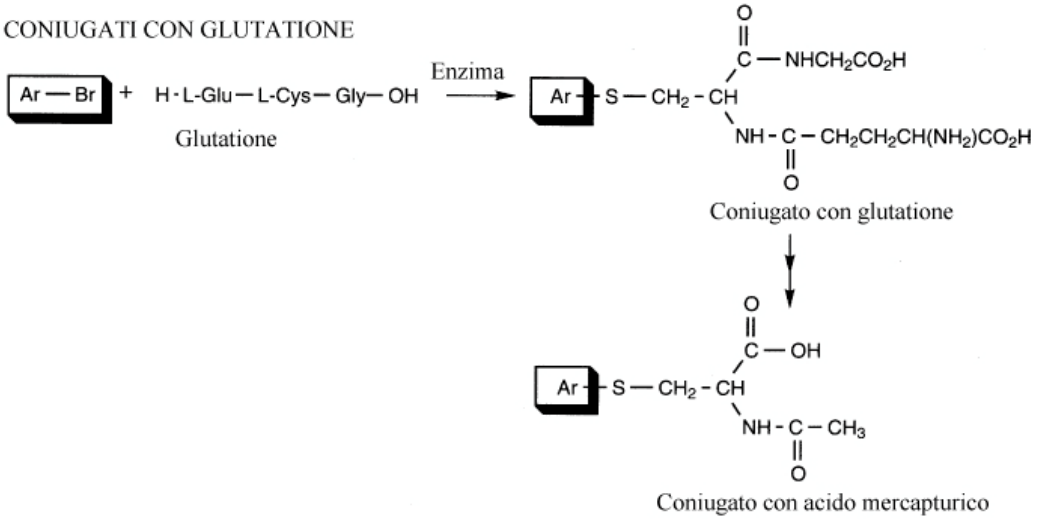
CONIUGATI CON ACIDO GLUCURONICO



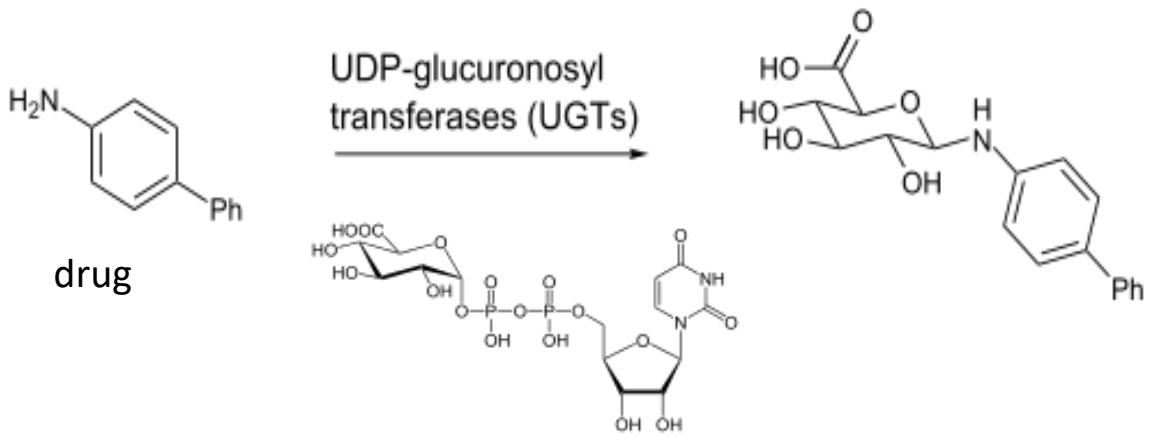
CONIUGATI CON ACIDO SOLFORICO



CONIUGATI CON GLUTATIONE



glucuronidation



Glucuronidation is an important metabolism pathway for many drugs and endogenous substances. Liver, gastrointestinal tracts, and kidney are the major organs where glucuronidation occurs. Mechanistically, the glucuronosyl group is enzymatically transferred from the cofactor uridine 5′-diphosphoglucuronic acid (UDPGA) to the substrates with the nucleophilic function groups of −OH, −NH2, −SH, −COOH, or others, which are catalyzed by the enzyme family of uridine 5′-diphosphoglucuronosyltransferases (UGTs).

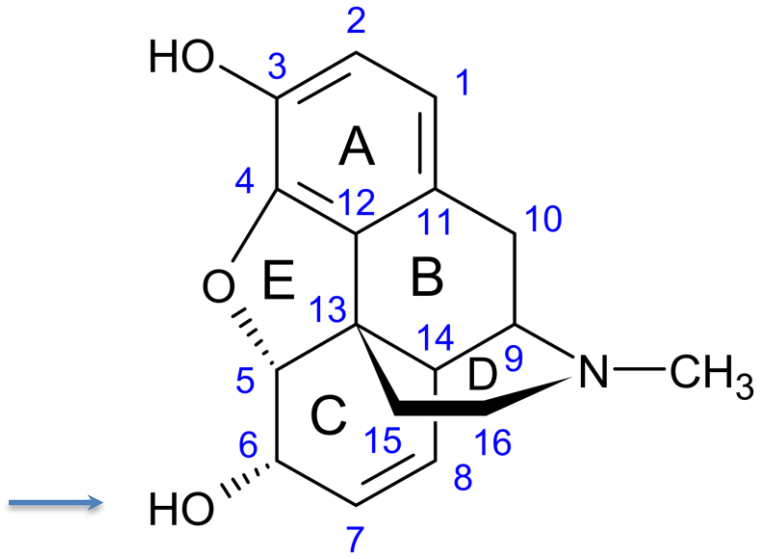
Conjugation does not always result in less toxicity or inactivation,

Morphine-6-glucuronide is pharmacologically active

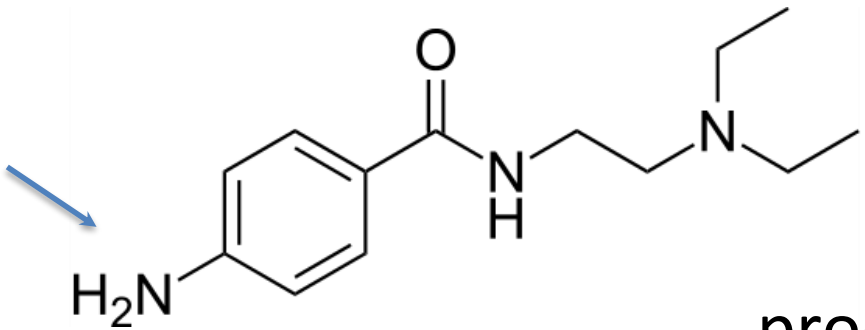
N-acetyl procainamide is pharmacologically active

GSH conjugates of haloalkenes are nephrotoxic via β-lyase

Acyl glucuronides are reactive, binding covalently to proteins



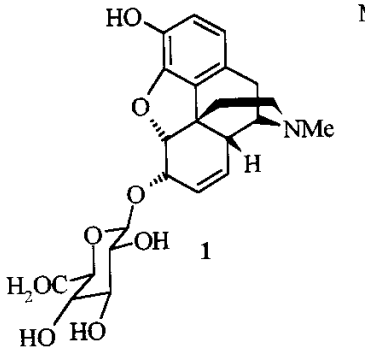
morphine



procainamide

Mophine 6-glucuronide was discovered in 1969 to have analgesic activity, then more fully evaluated in the late 1980s and 1990s. Subsequent studies were conducted in humans for its use as a potential drug.

morphine - 6 - glucuronide



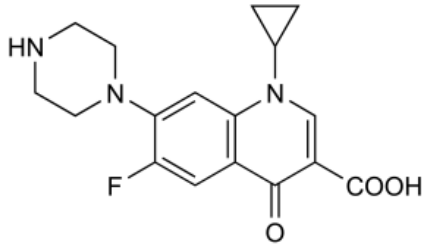
1

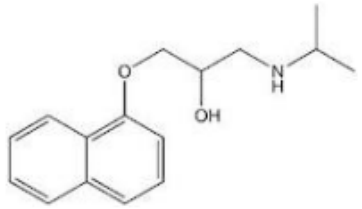
Questions to consider:

- Physical properties of M6G relative to M?
- Would M6G be expected to have good oral bioavailability?
- Would M6G likely cross the blood brain barrrier, BBB, passively?

glucuronidation

cyprofloxacin



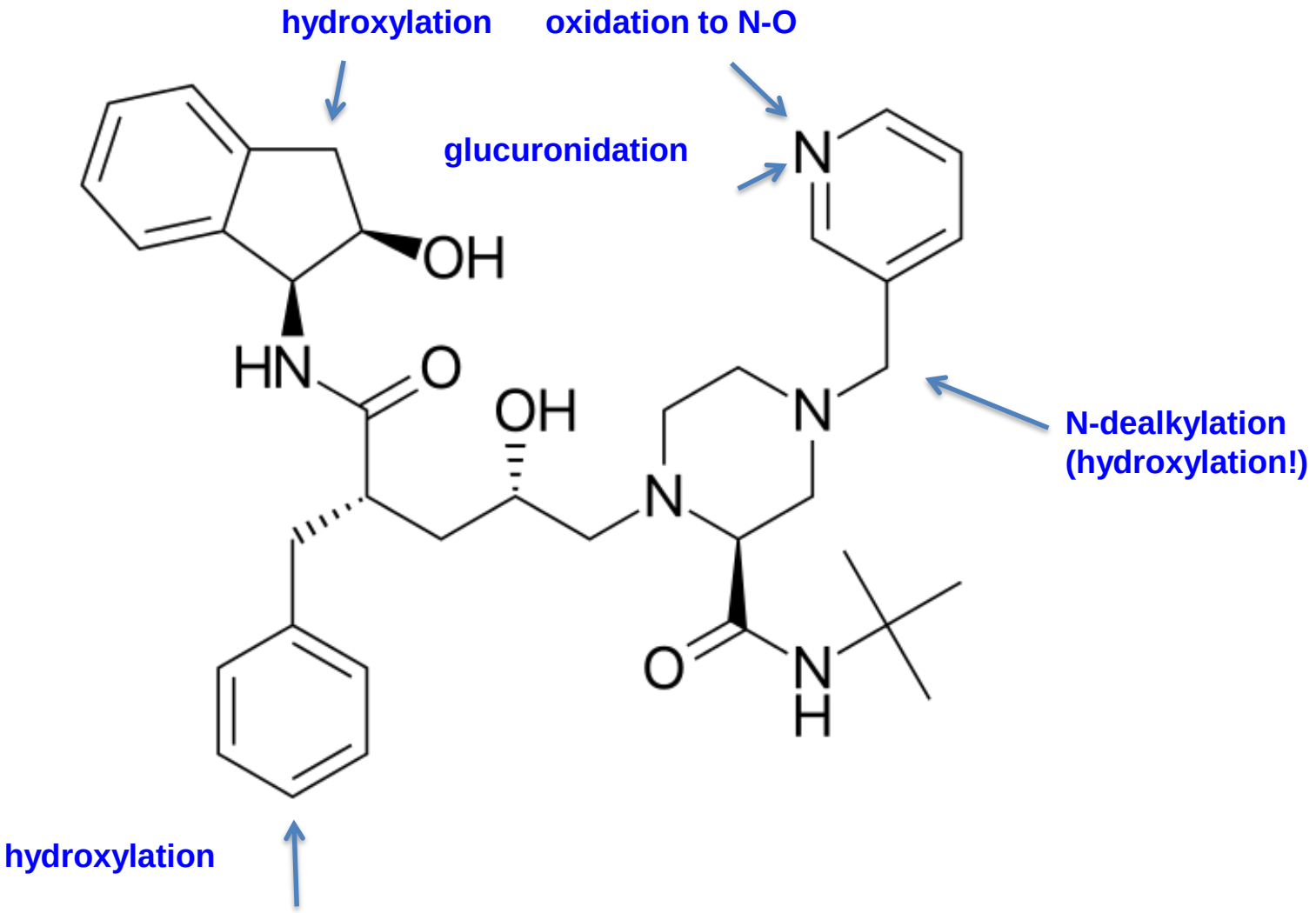


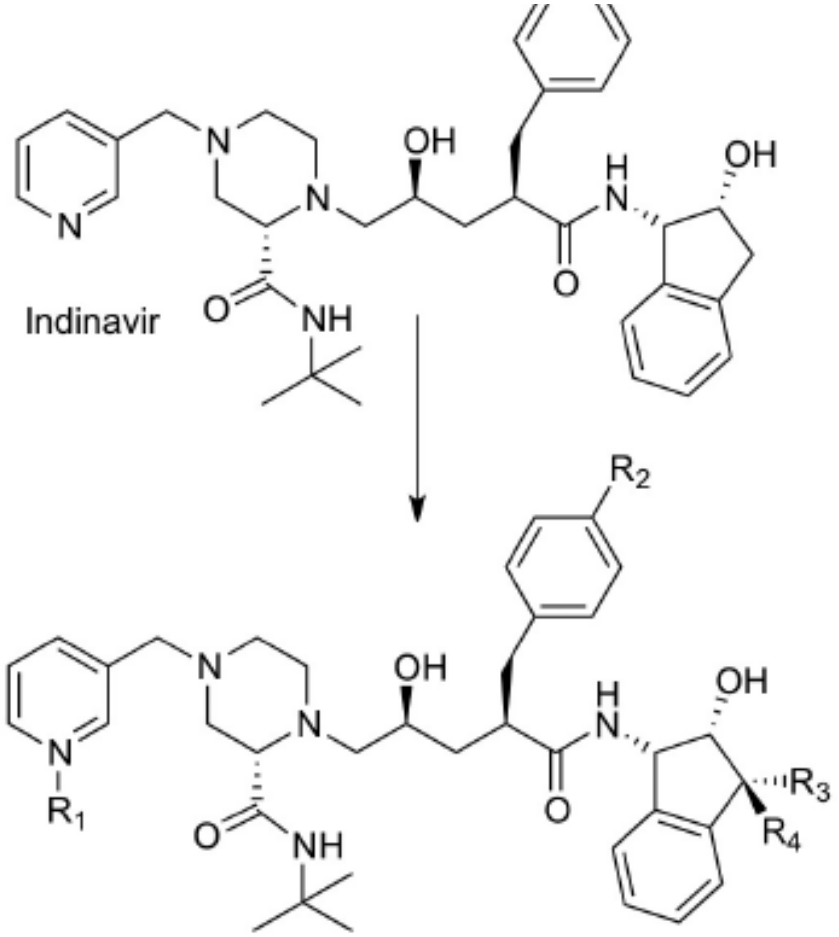
propranolol

Many compounds or drugs contain functional groups that can be directly conjugated and thus do not require Phase I metabolism to create “handles” (nucleophiles) for conjugation.

Which groups in these molecules will react with glucuronic-UDP?

an example: indinavir
antiviral, anti-HIV





(**55**) R_1 = *N*-glucuronide, R_2 = H, R_3 = H, R_4 = H

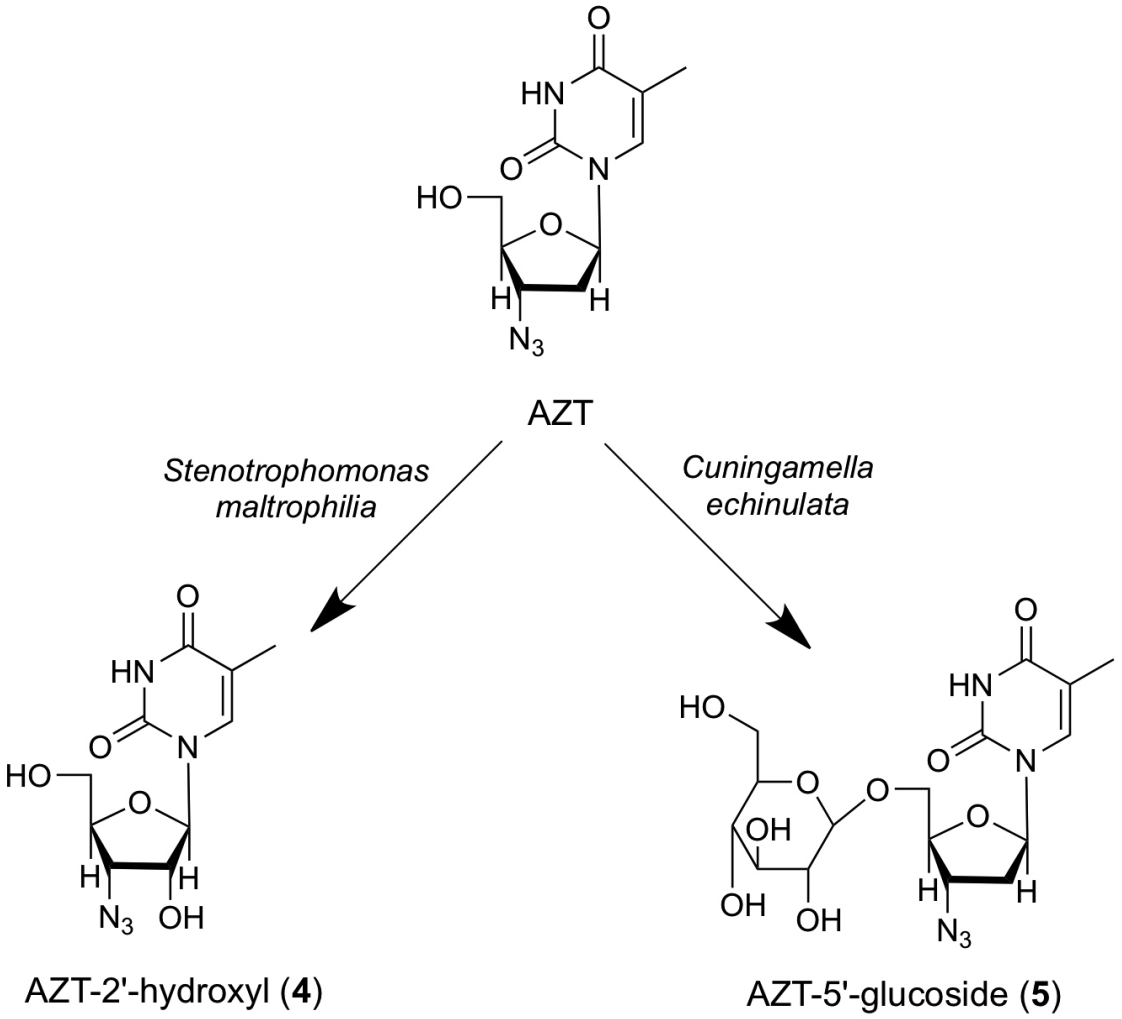
(**56**) R_1 = *N*-oxidation, R_2 = H, R_3 = H, R_4 = H

(**57**) R_1 = H, R_2 = OH, R_3 = H, R_4 = H

(**58**) R_1 = H, R_2 = H, R_3 = OH, R_4 = H

(**59**) R_1 = H, R_2 = H, R_3 = H, R_4 = OH

Troncatori di catena….



Glucuronides as Potential Anionic Substrates of Human Cytochrome P450 2C8 (CYP2C8)

Miniperspective

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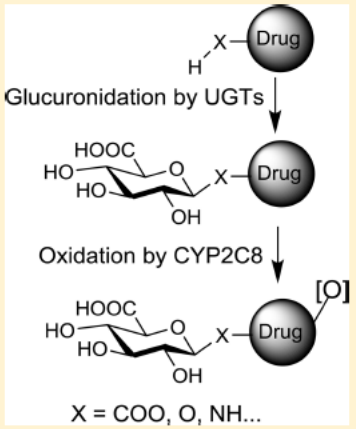
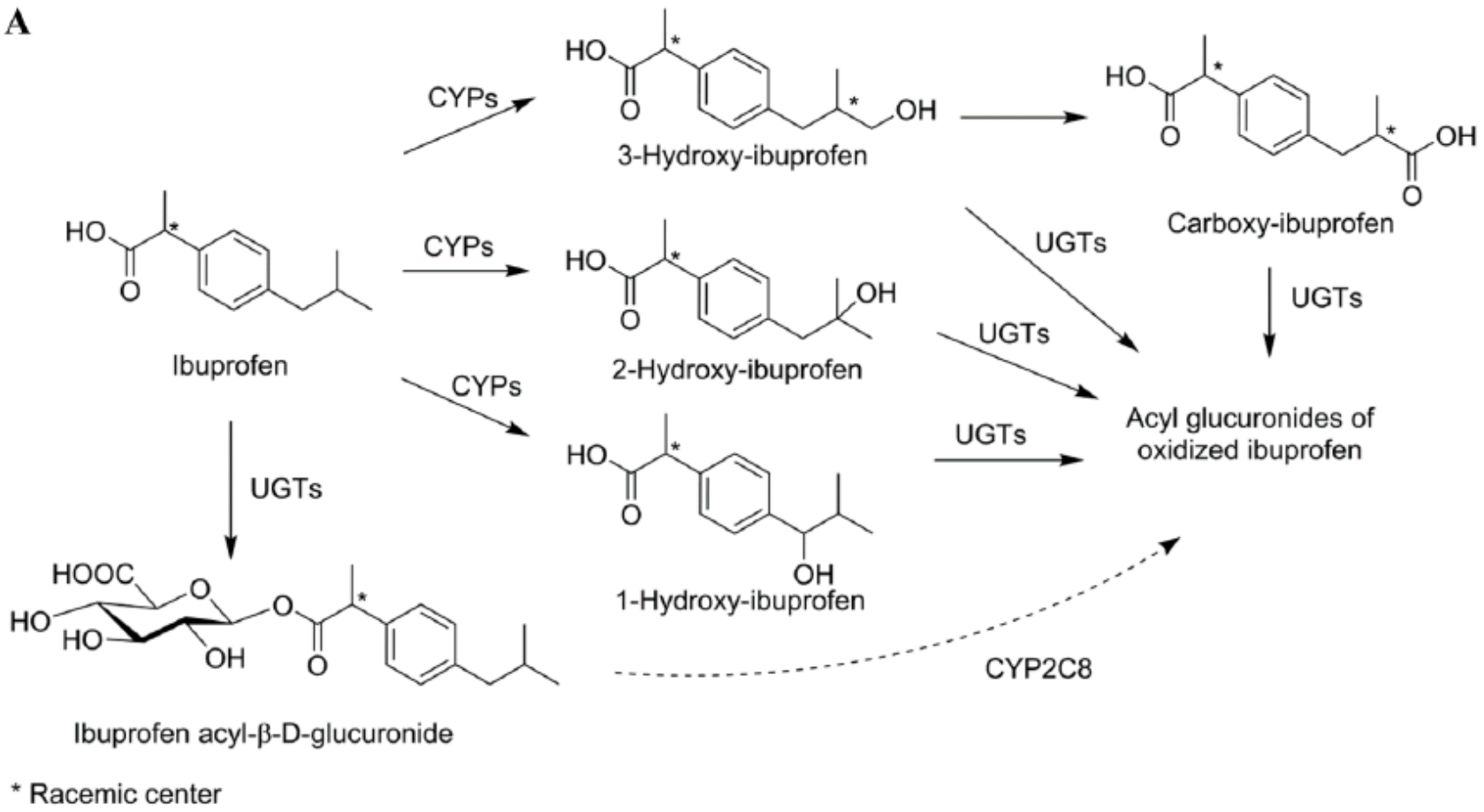
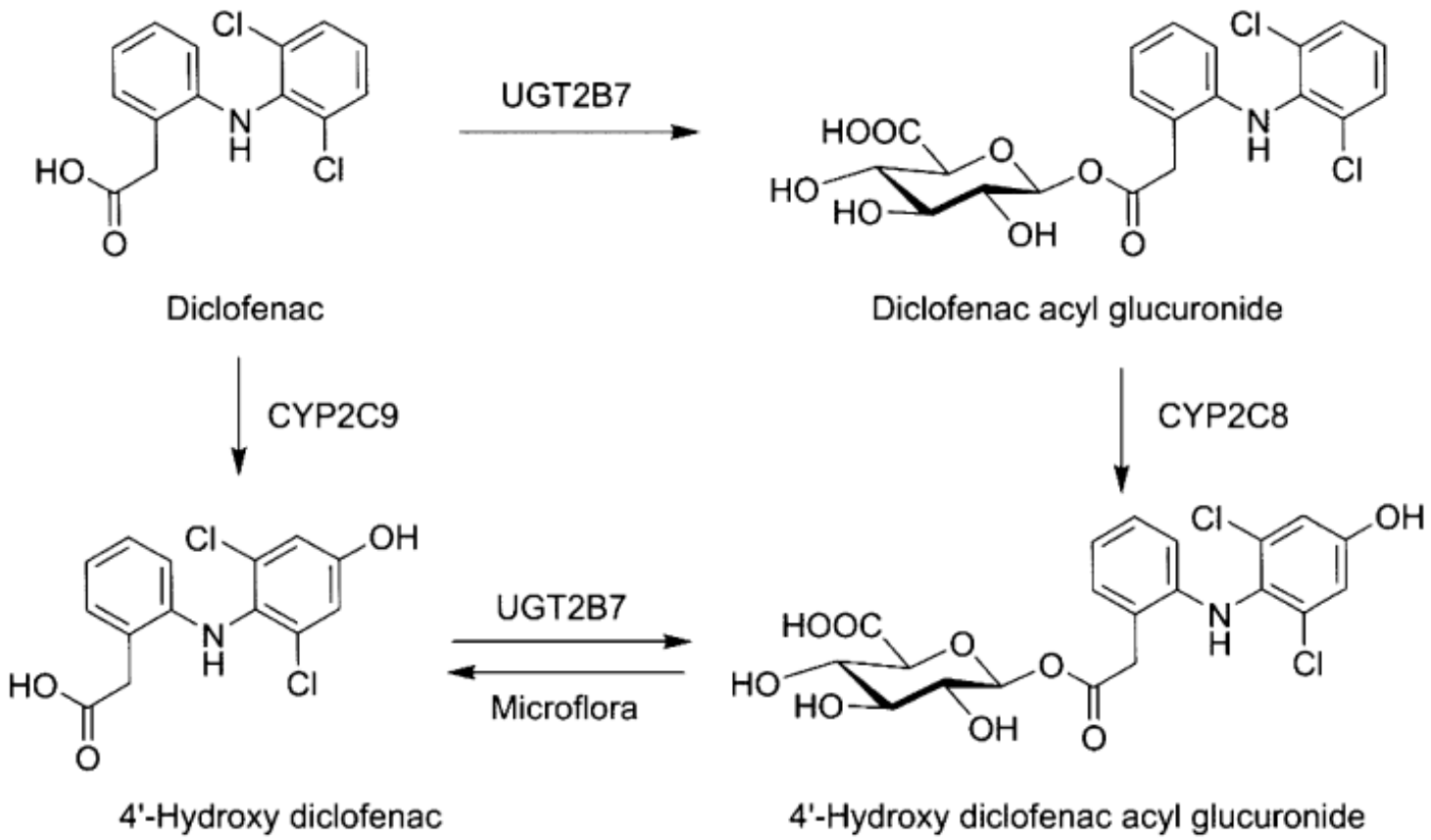
ABSTRACT: Glucuronidation is in general considered as a terminal metabolic step that leads to direct elimination of drugs and generally abolishes their biological activity. However, there is growing evidence to suggest that glucuronides can be ligands of human CYP2C8, making CYP2C8 distinct from the other CYP isoforms. Several classes of glucuronide conjugates, which include acyl glucuronides, ether glucuronides, *N*-glucuronides, and carbamoyl glucuronides, have been shown to be substrates or time-dependent inhibitors of CYP2C8. Although the structures of CYP2C8-glucuronide complexes have not been determined, the structural features of CYP2C8 active site support its binding to anionic and bulky ligands like glucuronides. As interaction perpetrators with CYP2C8, glucuronides of gemfibrozil and clopidogrel showed marked clinical drug−drug interactions (e.g., with cerivastatin and repaglinide), which are more than expected from the parent drug. This review summarizes glucuronides as CYP2C8 ligands and the active-site structural features of CYP2C8 that allow potential binding to glucuronides.

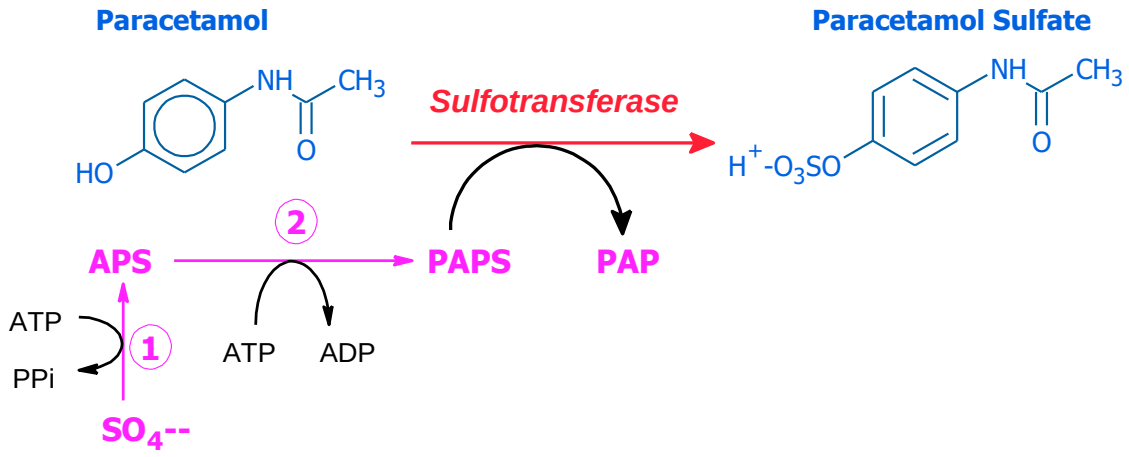


The significant contribution of CYP2C8 in drug metabolism has been recognized. The important drugs that are metabolized by CYP2C8 include rosiglitazone, cerivastatin, amodiaquine, and paclitaxel


A
CYPs
3-Hydroxy-ibuprofen
Carboxy-ibuprofen
UGTs
CYPs
UGTs
Ibuprofen
2-Hydroxy-ibuprofen
UGTs
CYPs
UGTs
Acyl glucuronides of oxidized ibuprofen
UGTs
1-Hydroxy-ibuprofen
CYP2C8
Ibuprofen acyl-β-D-glucuronide
* Racemic center


Cl
N
H
Cl
HO
O
Diclofenac
UGT2B7
HOOC
O
HO
HO
OH
O
O
Cl
N
H
Cl
Diclofenac acyl glucuronide
CYP2C9
CYP2C8
Cl
OH
N
H
Cl
HO
O
4'-Hydroxy diclofenac
UGT2B7
Microflora
HOOC
O
HO
HO
OH
O
O
Cl
OH
N
H
Cl
4'-Hydroxy diclofenac acyl glucuronide

Sulfotransferases (SULTs)



① = ATP Sulfurylase, ② = APS Kinase
Bifunctional Enzyme in Mammals – PAPS Synthetase (2 isoforms) M.Coughtrie, U. Dundee

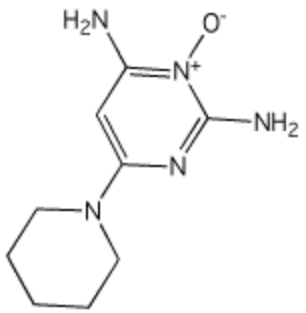
Co-factor is PAPS (3' -phosphoadenosine 5' -phosphosulfate) – a high energy intermediate. Low levels in vivo, but synthesized quickly from inorganic sulfate or catabolism of cysteine and methionine.

Cosubstrate Depletion. Reduced inorganic sulfate or cysteine (secondary to GSH depletion) can cause co-substrate-dependent decrease in sulfation rates.

e.g. high doses of acetaminophen or harmol (Levy and Morris, Pang)

Substrates for Sulfotransferases (SULTs)

SULT1A1	SULT1A3	SULT1E1
Paracetamol	Salbutamol	Ethinylestradiol
Minoxidil	L-Dopa	(4-OH Tamoxifen)
Troglitazone	Dopamine	Raloxifene
Apomorphine	Dobutamine	
4-OH Tamoxifen	Carbidopa	



minoxidil

- Phenols and analine type amines are sulfated
- Frequently phenols have both glucuronidation and sulfation as competing conjugation rxn.
- Sulfation is often a high affinity, low capacity pathway, while glucuronidation is frequently low affinity, high capacity.
  Thus, at low dose, sulfation may dominate, but as dose increases, glucuronidation can become the major route.
- Sulfates are frequently excreted in urine, though some bile acid sulfates are excreted into bile.

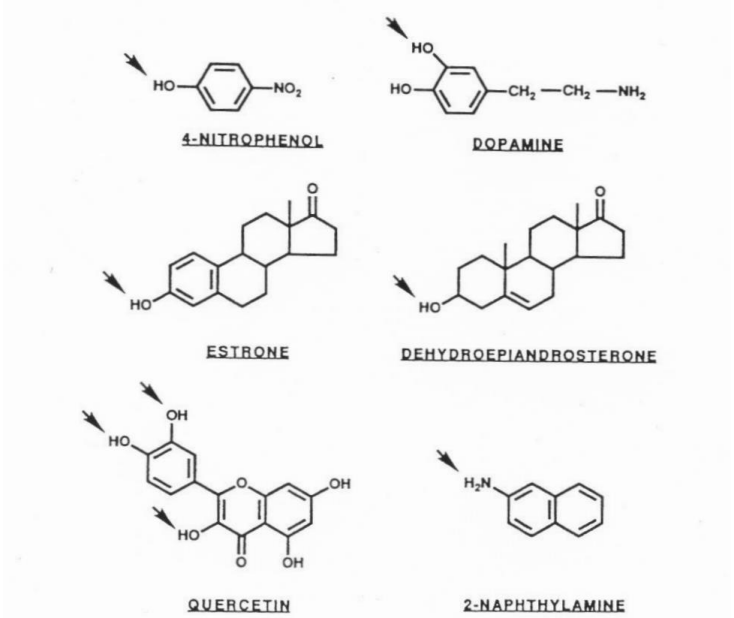


Fig. 1. Selected cytoplasmic sulfotransferase enzyme sulfate acceptor substrates. *Arrows* indicate sites of sulfate conjugation

Species Differences in UGTs

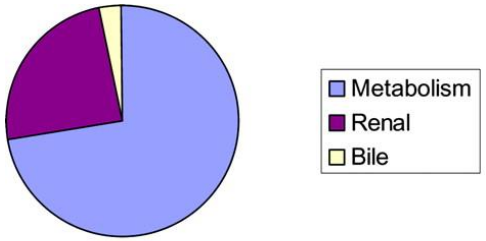
Cats of all types appear to lack Ugt1A6 (pseudozyme) which may be due to cats being "requisite carnivores", thus evolution removed an enzyme that was not needed.

Thus, cats are sensitive to aspirin and acetaminophen that rely on glucuronidation for elimination.

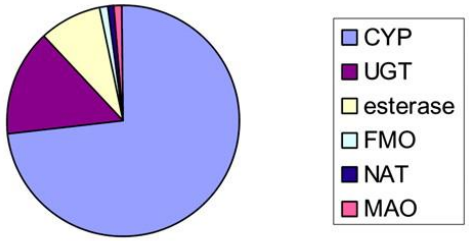
Cats **would not** be a good model for drug development.

Another requisite carnivore that lacks Ugt1A6 is the hyena. Also the Northern Elephant Seal

Clearance mechanisms for the top 200 drugs prescribed in the United States in 2002

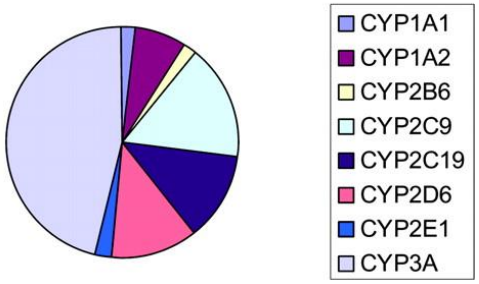


•Metabolism most important. CLr of unchanged drug is significant. Bile is more likely larger than the sliver shown due to difficulty of measuring CLbile and many newer drugs are quite high MW.



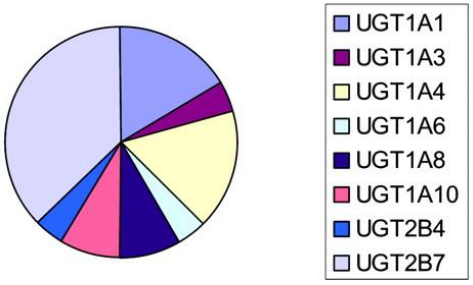
•CYPs dominate, but UGTs are dominant for Phase II. Glucuronidation is also very important for sequential metabolism, after oxidation.

•UGT pie is likely not so accurate and all of the active UGTs are not listed.



•Establishing which UGT is dominant for a drug glucuronide formed is difficult, since tissue specific expression is not well know (certainly not in 2002).

From: Williams JA, et al. Drug Metab Dispos. 2004, 32:1201-1208

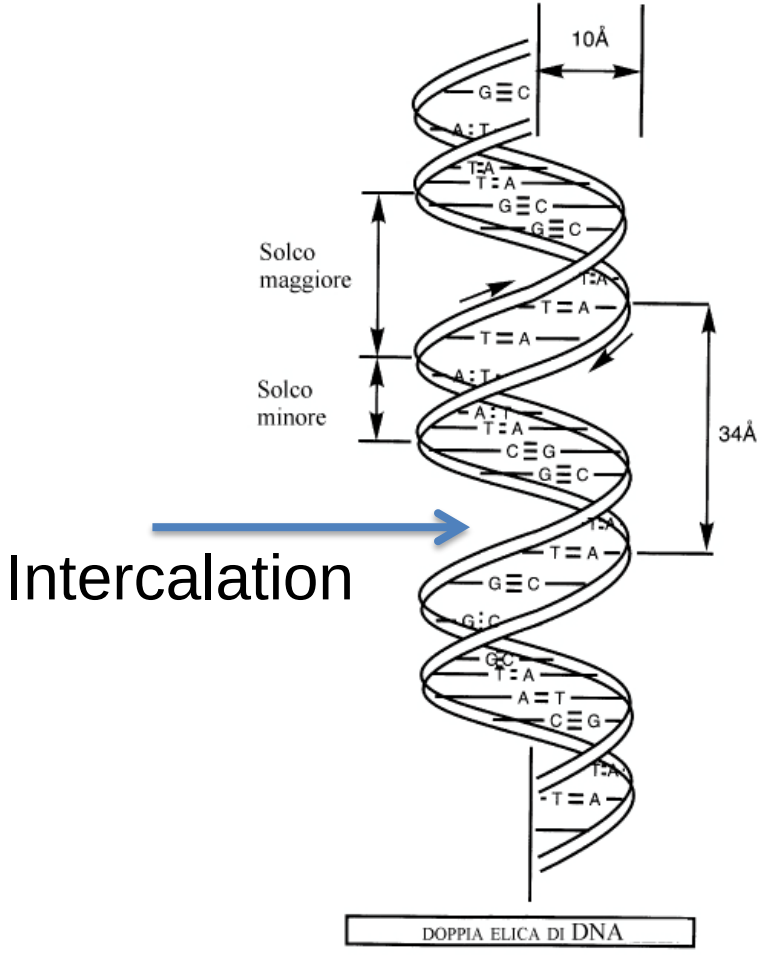


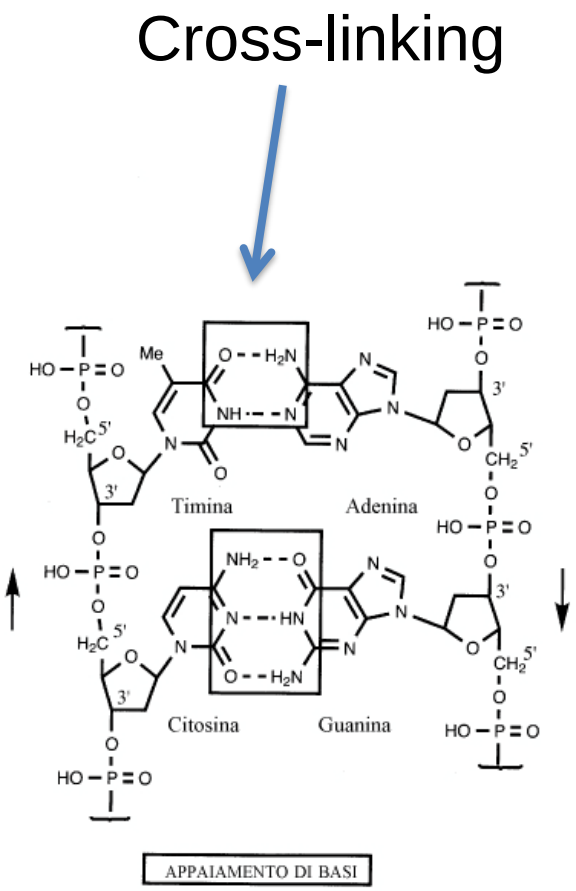
Covalent drugs

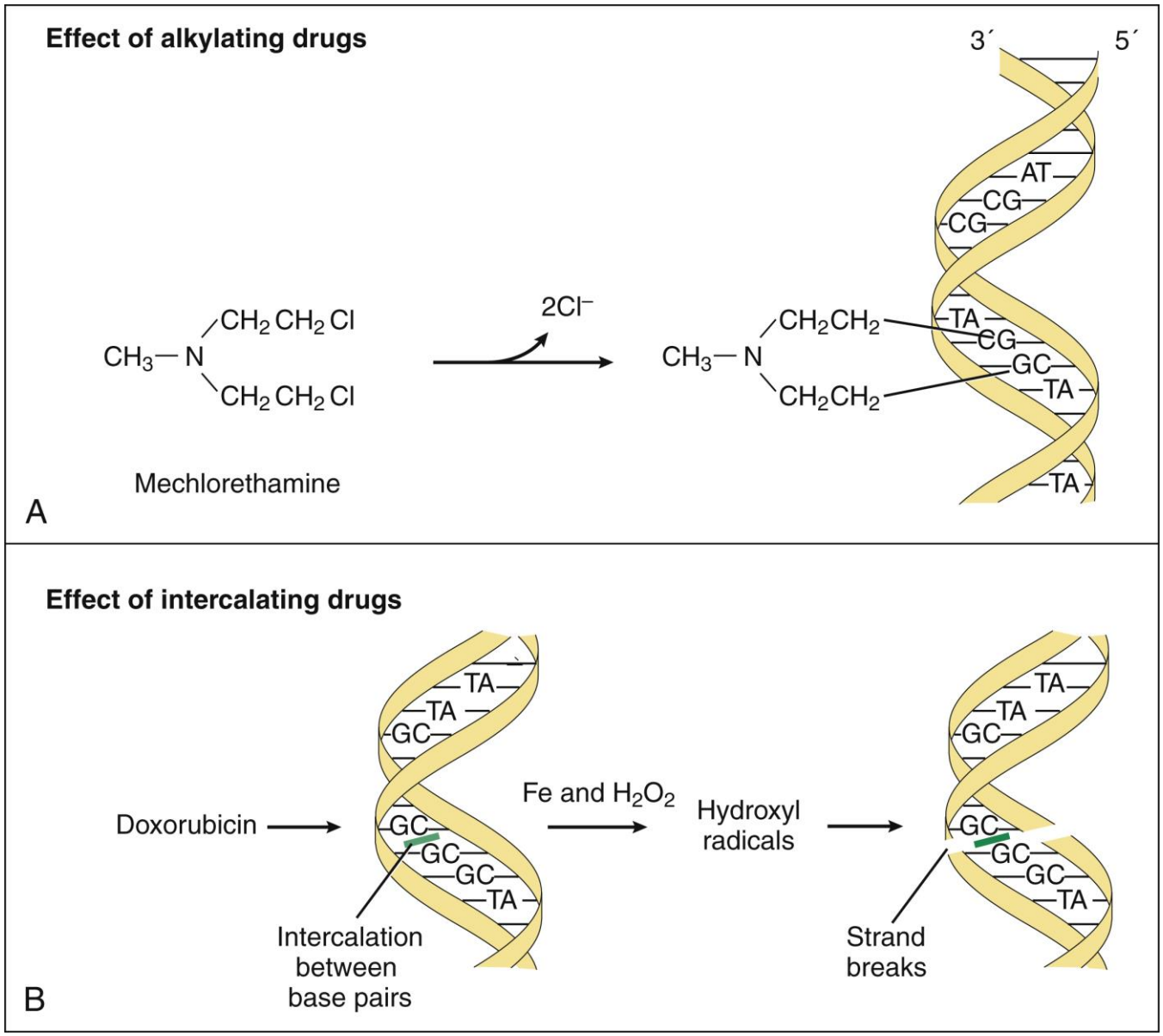
- Normally drugs form non-covalent complexes with targets.
- The affinity of the drug for its target is a measure of drug potency (at least in vitro) and the potency increases when the IC_{50} or AC_{50} pass from mM to μM to nM to pM.
- Some antibacterial and antitumor drugs can form a covalent bond with the target, this is associated to high toxicity of the drug.

DNA as target

chemical drugs are intercalators or binders (non-covalent) or cross-linking agents (covalent bond forming)

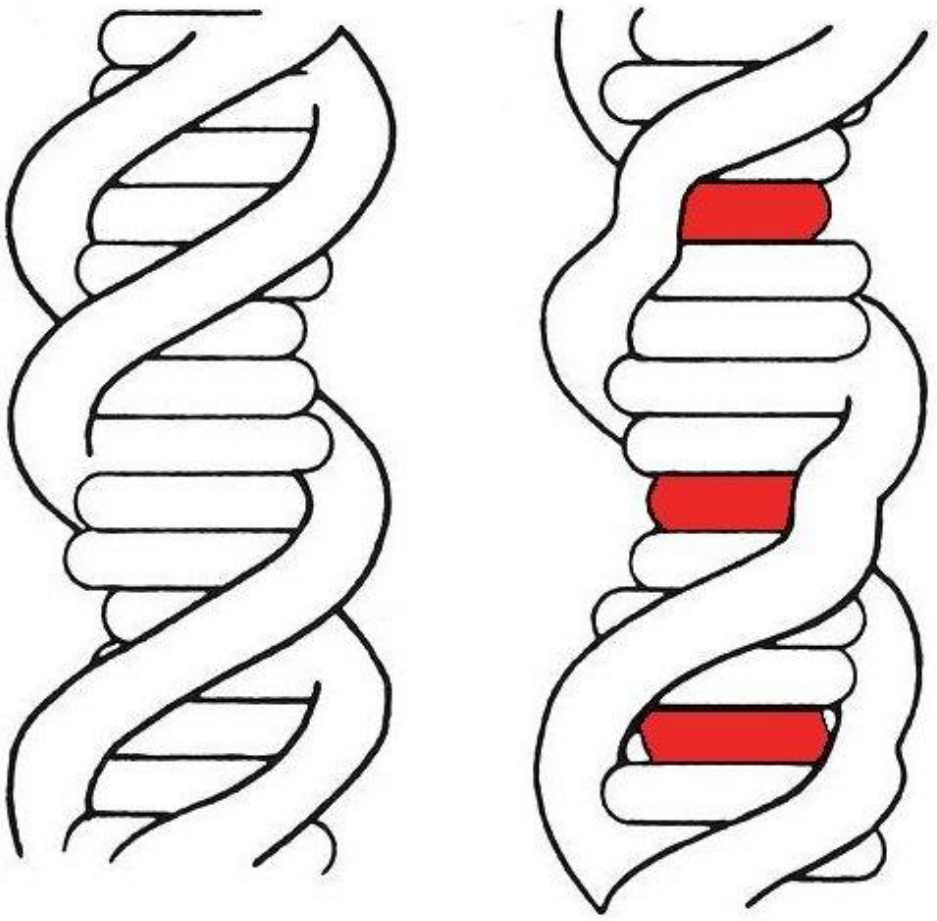




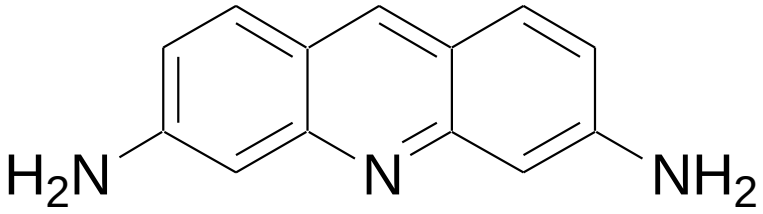

Effect of alkylating drugs
3′
5′
AT
CG
CG
TA
CG
GC
TA
TA
CH_3-N
CH_2CH_2Cl
CH_2CH_2Cl
$2Cl^-$
CH_3-N
CH_2CH_2
CH_2CH_2
Mechlorethamine
A
Effect of intercalating drugs
TA
TA
GC
GC
GC
GC
TA
Doxorubicin
Intercalation between base pairs
Fe and H_2O_2
Hydroxyl radicals
Strand breaks
B

DNA intercalation (non-covalent interaction)

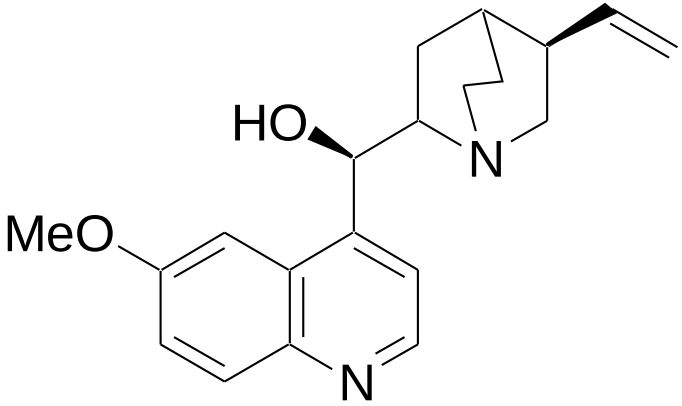
- Some molecules insert between adjacent couples of bases through stacking interactions and charge interactions (these are generally positively charged molecules that interact with the negative charge of phosphates of DNA backbone)



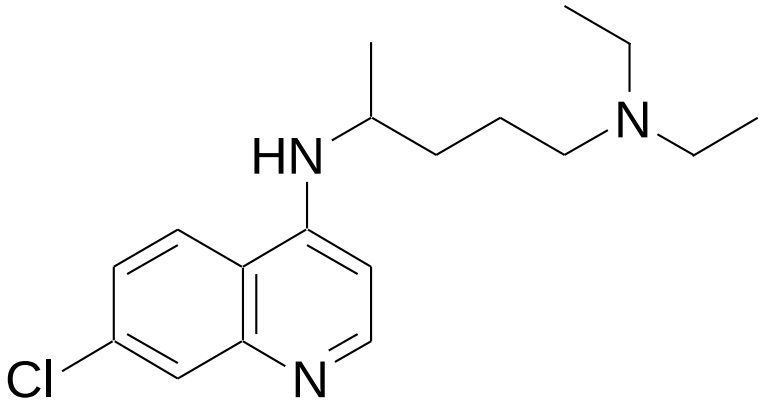
- Aminoacridines, proflavin, antibacterial for topic use
- Quinine and chloroquine, antimalaria drugs



PROFLAVINA

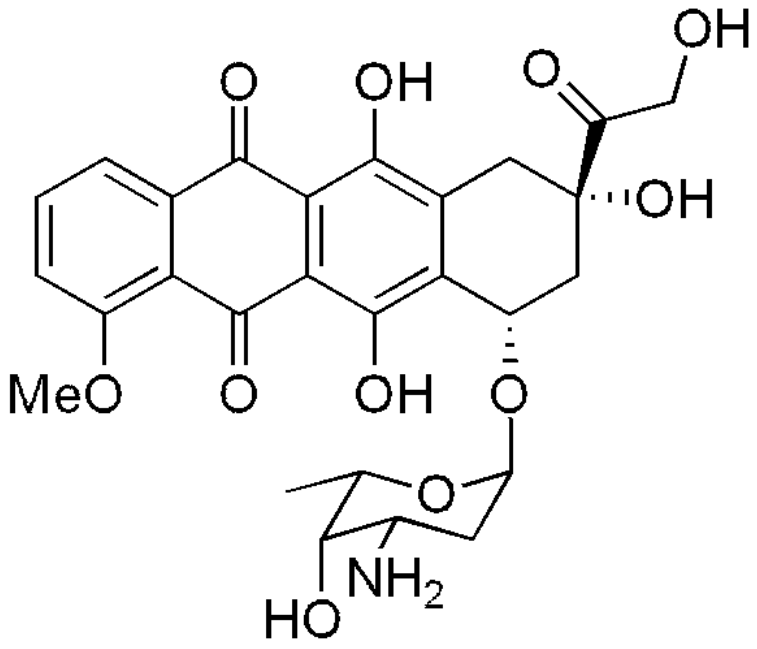


CHININA

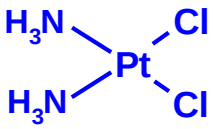


CLOROCHINA

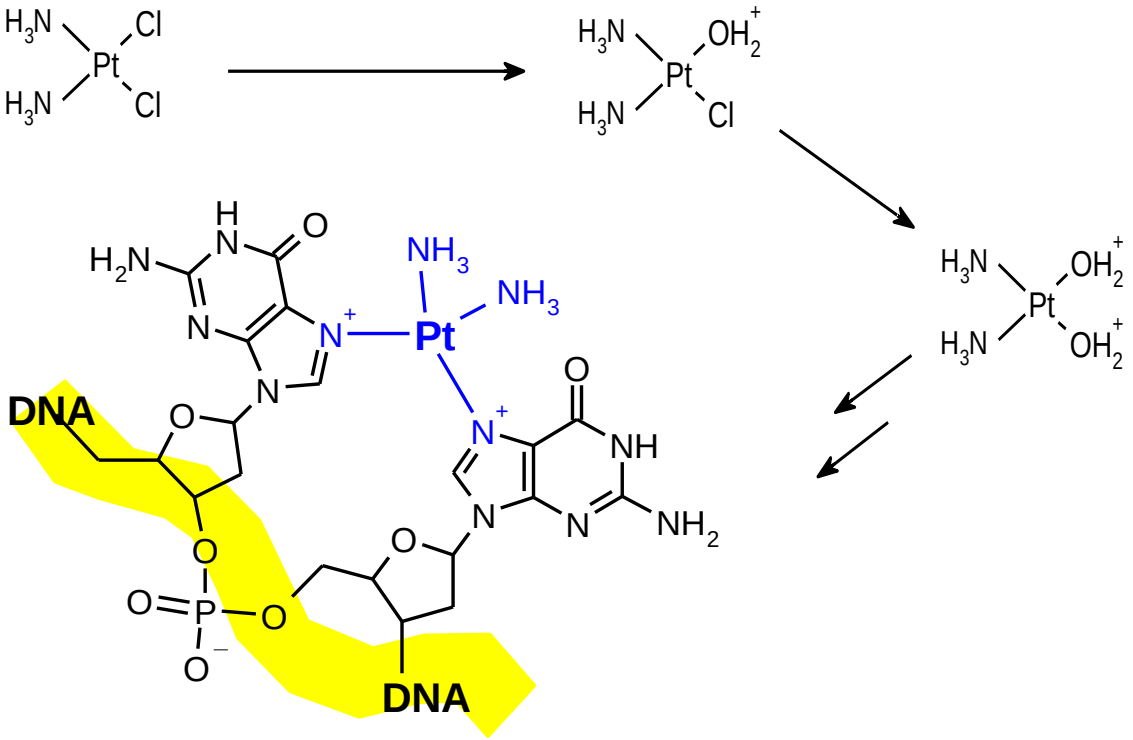
- The antibiotic doxorubicin (adriamicin) is an INTERCALATOR has a tricyclic aromatic ring that intercalates and an amino-glycoside moiety that forms ionic bonds with DNA phosphates



Drugs forming covalent bonds with the target: **cysplatin and nitrogen mustards** (chemotherapeutics)

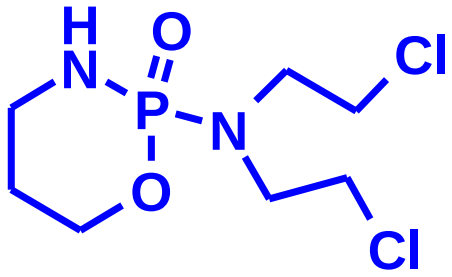


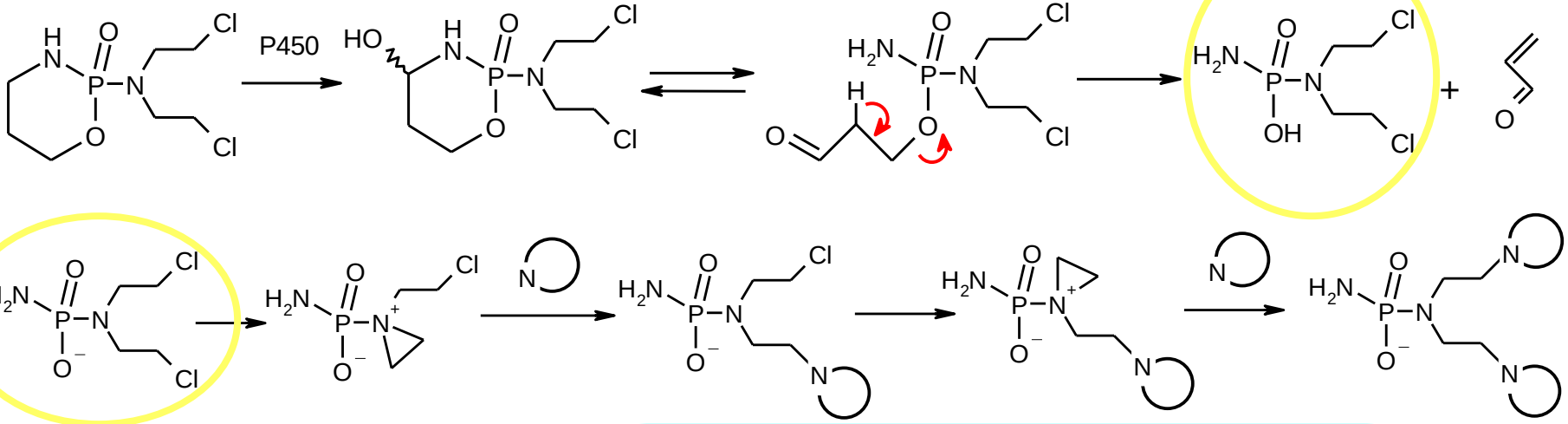
cysplatin



- Mechanism of action: irreversible covalent bond formation with guanine N-7

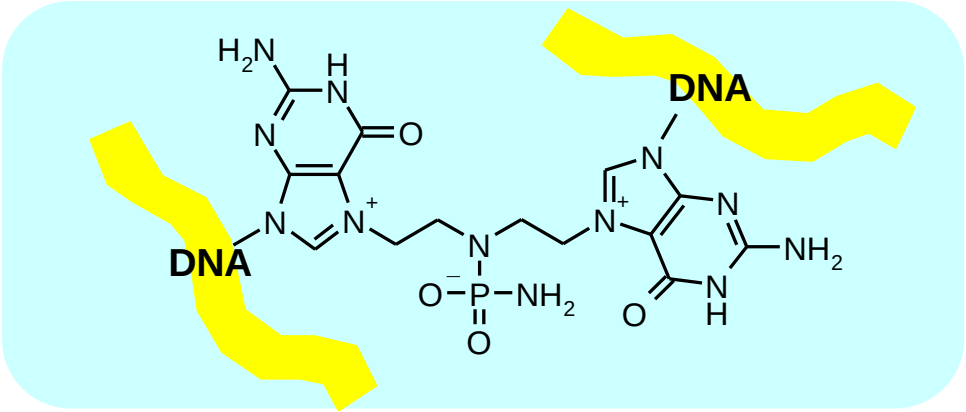
Cyclophosphamide (prodrug)



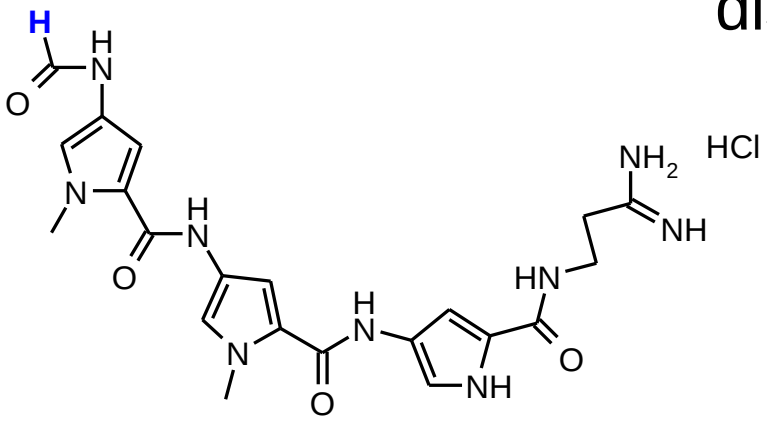


Clinical use:

Orally active also in combination with other chemotherapeutics Leukaemia, adenocarcinomas.

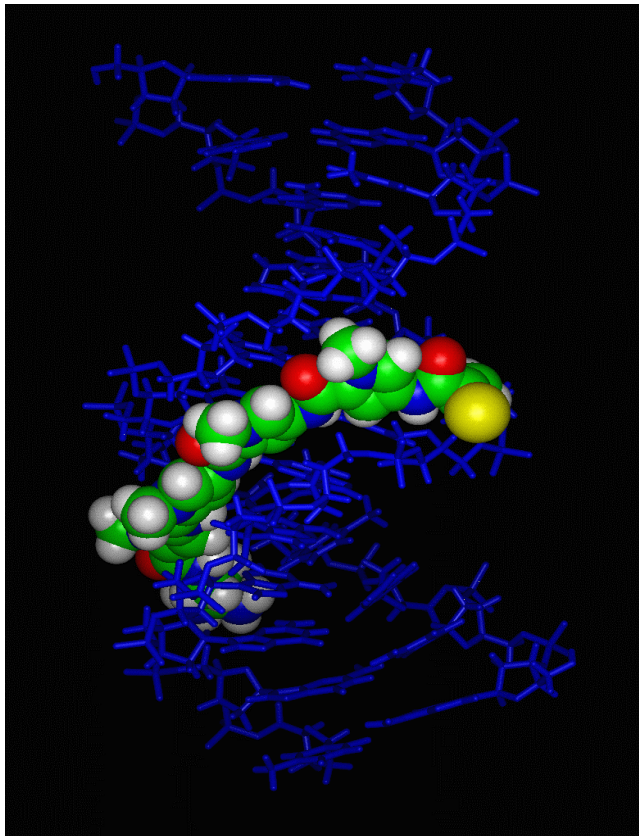


Covalent bond formed after target binding: distamycins

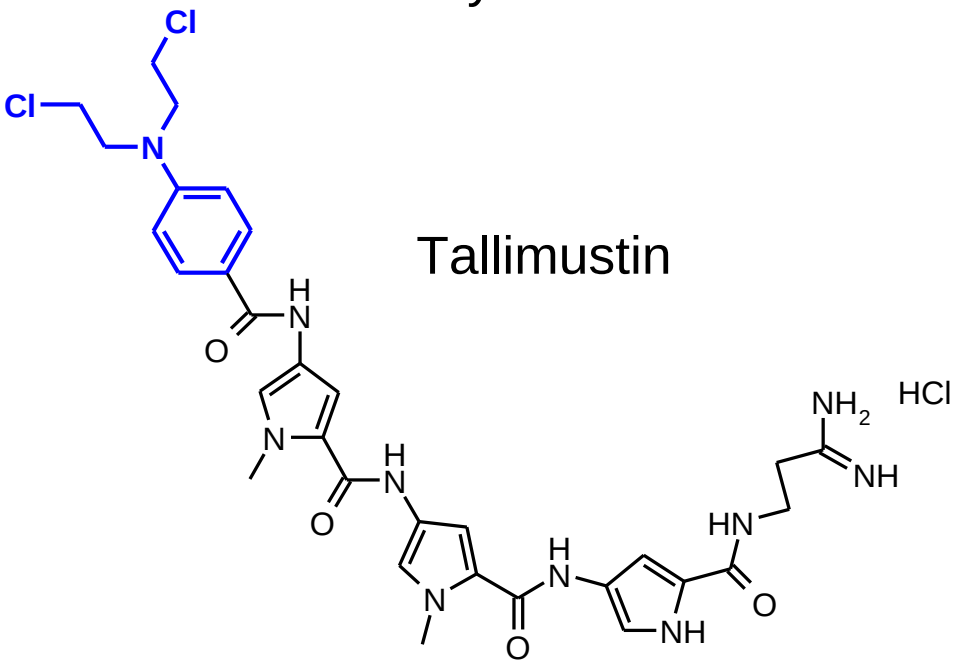


Distamycin A

DNA minor groove binder



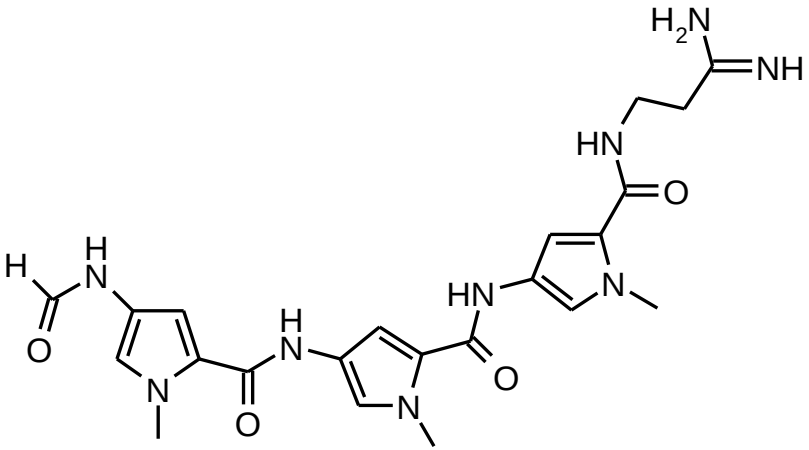
Interaction of PNU 166196 with DNA duplex tridecamer OBN5758 : 5'-d(ACCTTTTTGATGT)-3'



Tallimustin

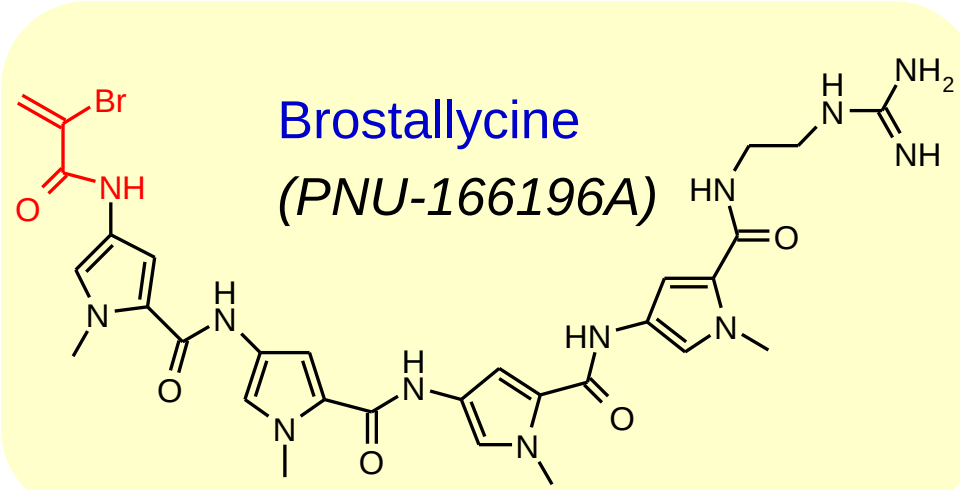
Distamycin

Streptomyces distallicus



Brostallycine

(PNU-166196A)



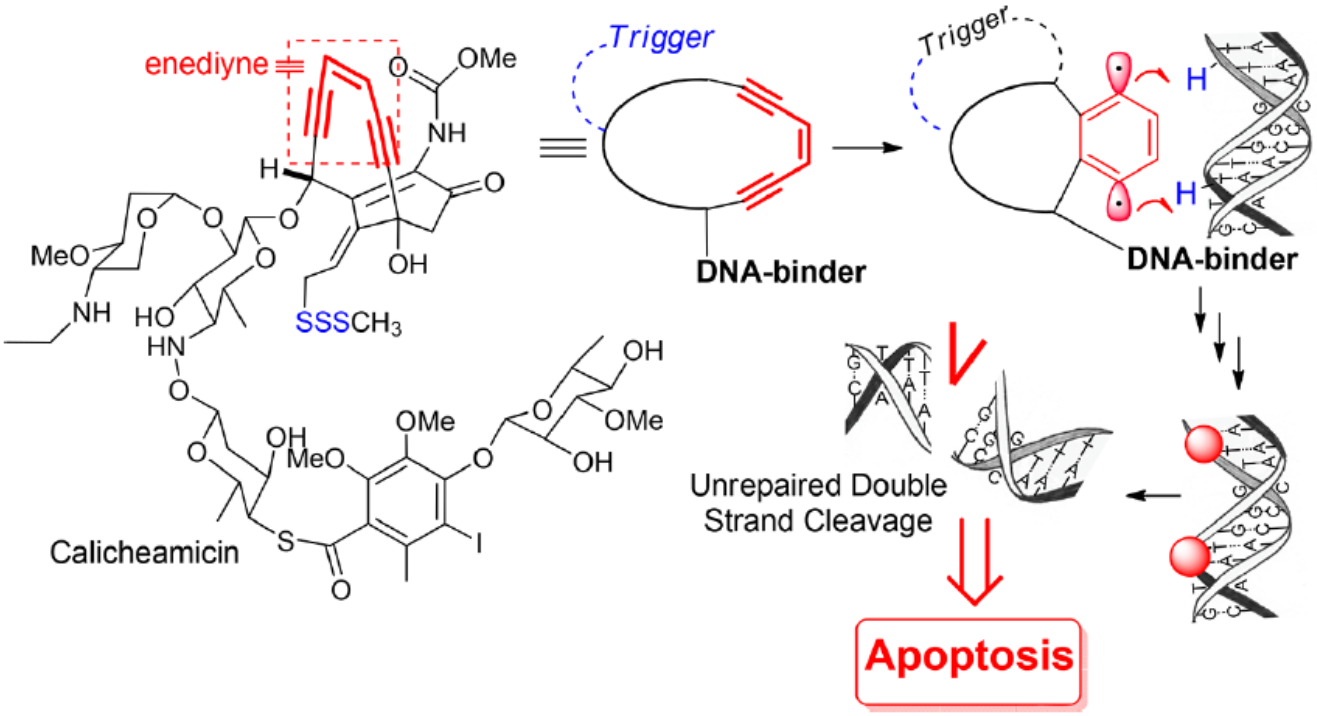
New DNA binder

Mechanism of action:

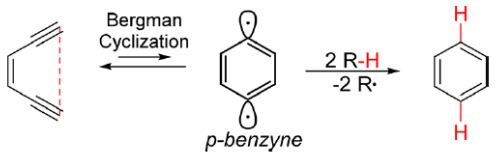
1) *Binding to DNA*

2) Michael addition with a DNA nucleophile

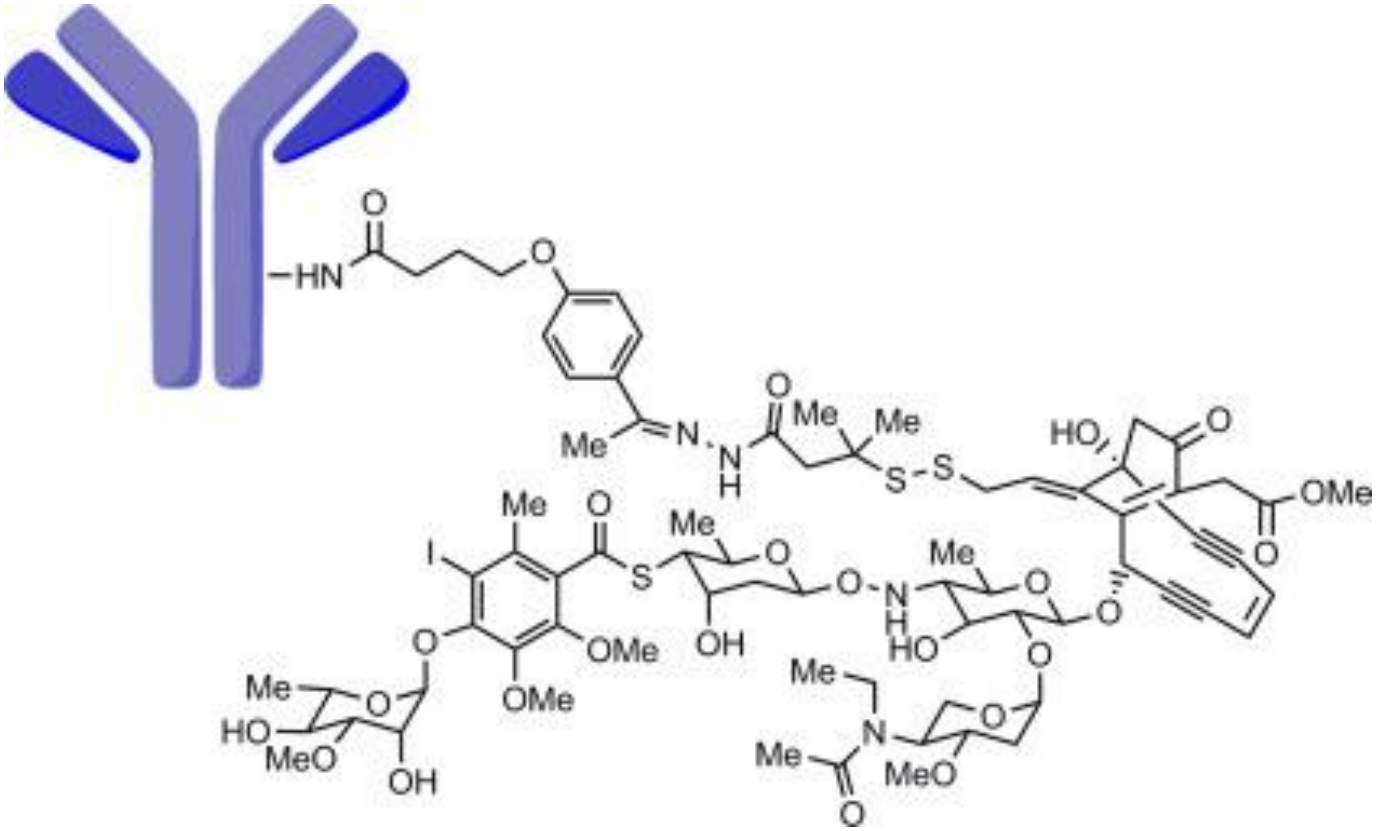
Calicheamicin cleaves double strand DNA



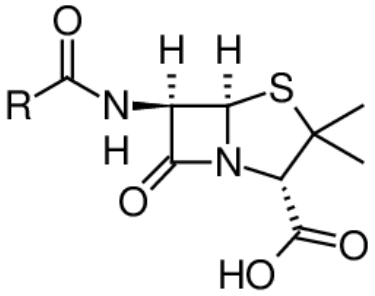
Scheme 1. Bergman Cyclization Leading to the Formation of 1,4-Diradical



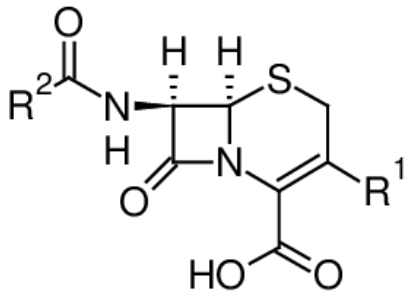
Calichemicin-antibody conjugates



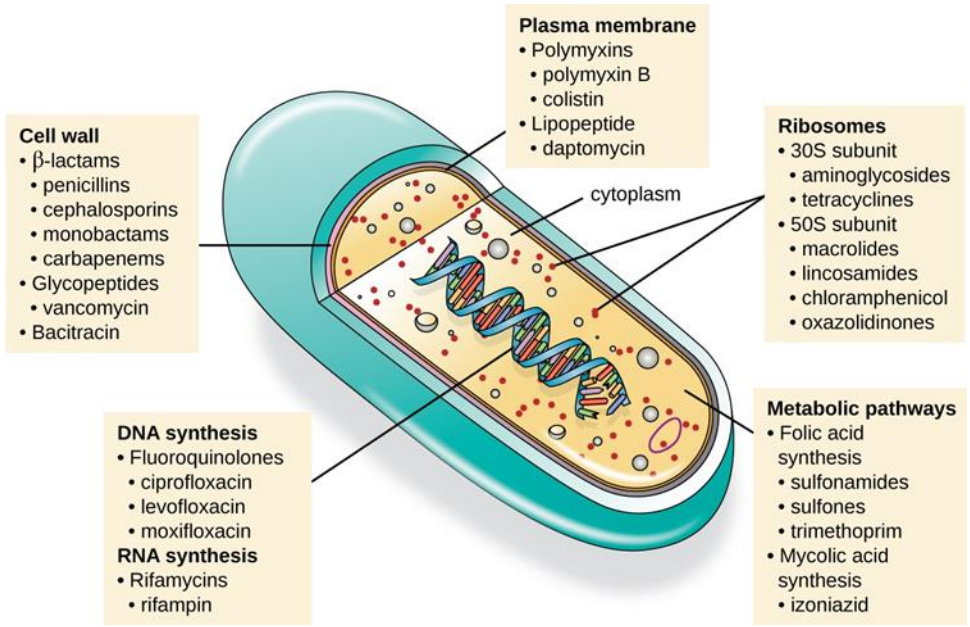
Mechanism of action of penicillins and cefalosporins (beta lactams antibiotics): covalent inhibition of baterial DD-transpeptidase



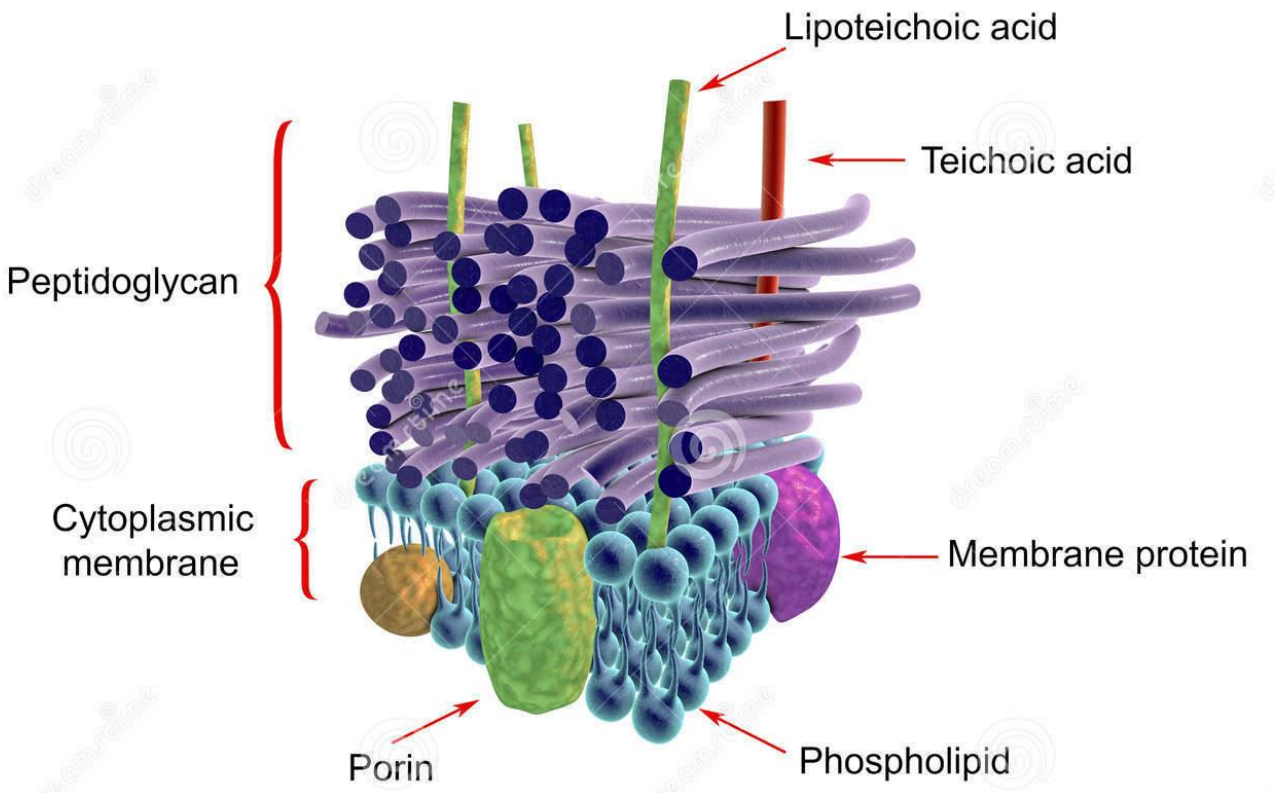
Penicillins



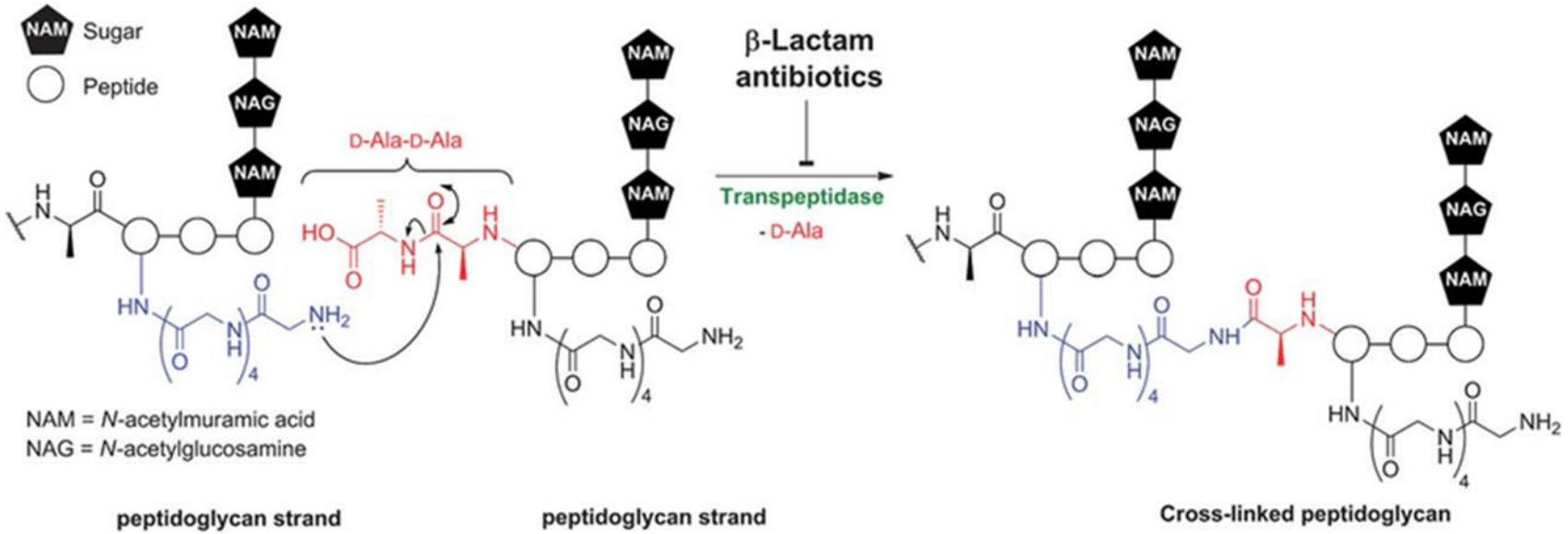
Cephalosporins



The bacterial cell wall is a crosslinked 3D polymer that protects bacteria from external dangers



BCW is synthesized by bacterial DD-transpeptidase (penicillin binding protein)



Penicillins (and cefalosporins) are beta-lactam antibiotics that block transpeptidation mechanism

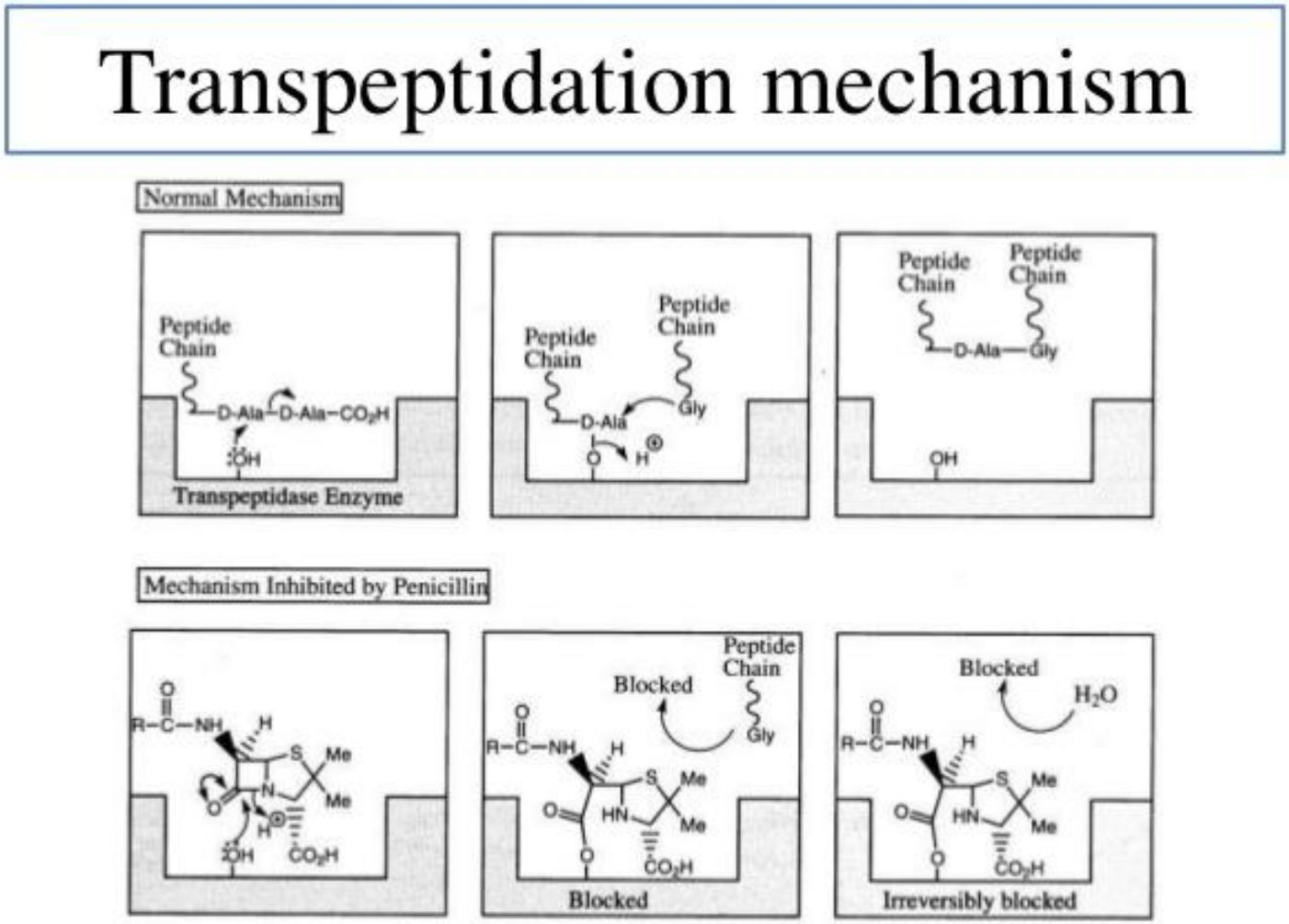
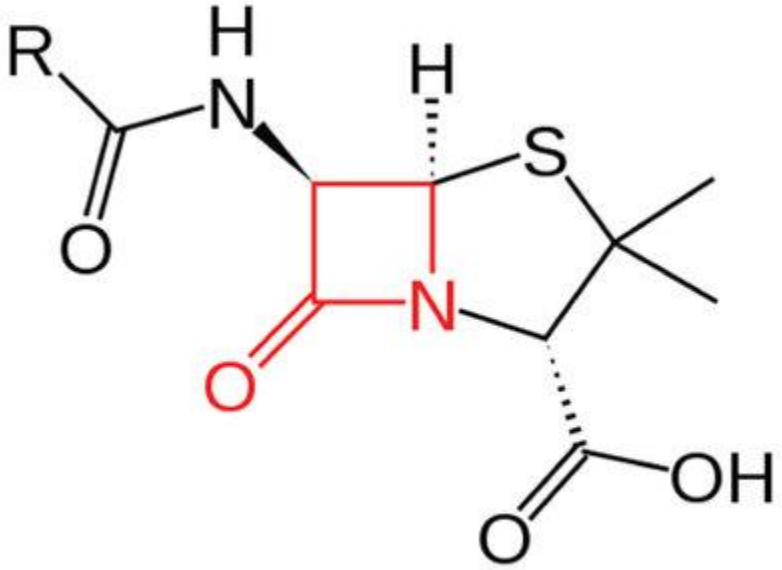


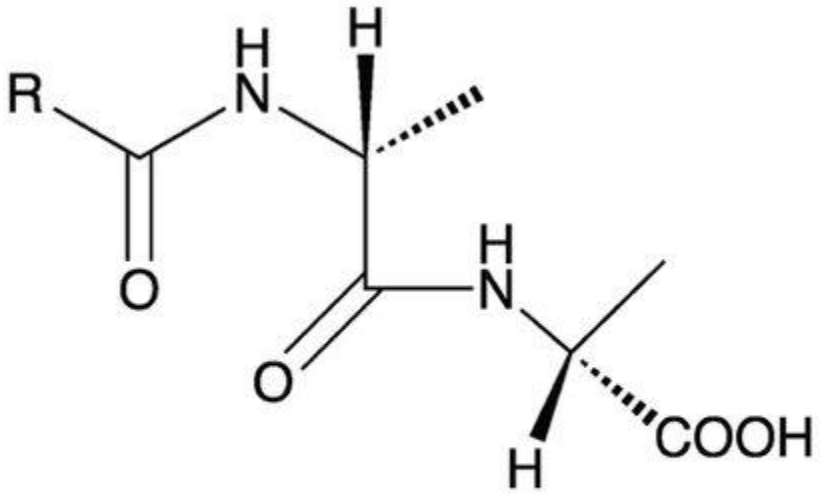
Fig. 14.63 Cross-linking mechanism by transpeptidase enzyme.

Source: Google Images

Penicillin is recognized by DD-transpeptidase because it mimics chemically the D-Ala-D-Ala dipeptide

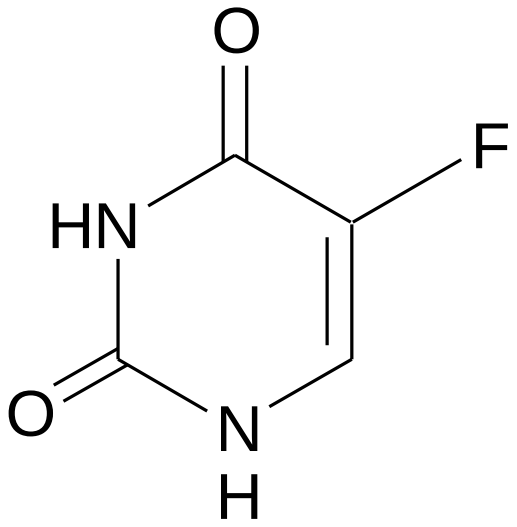


Penicillin



D-Ala-D-Ala

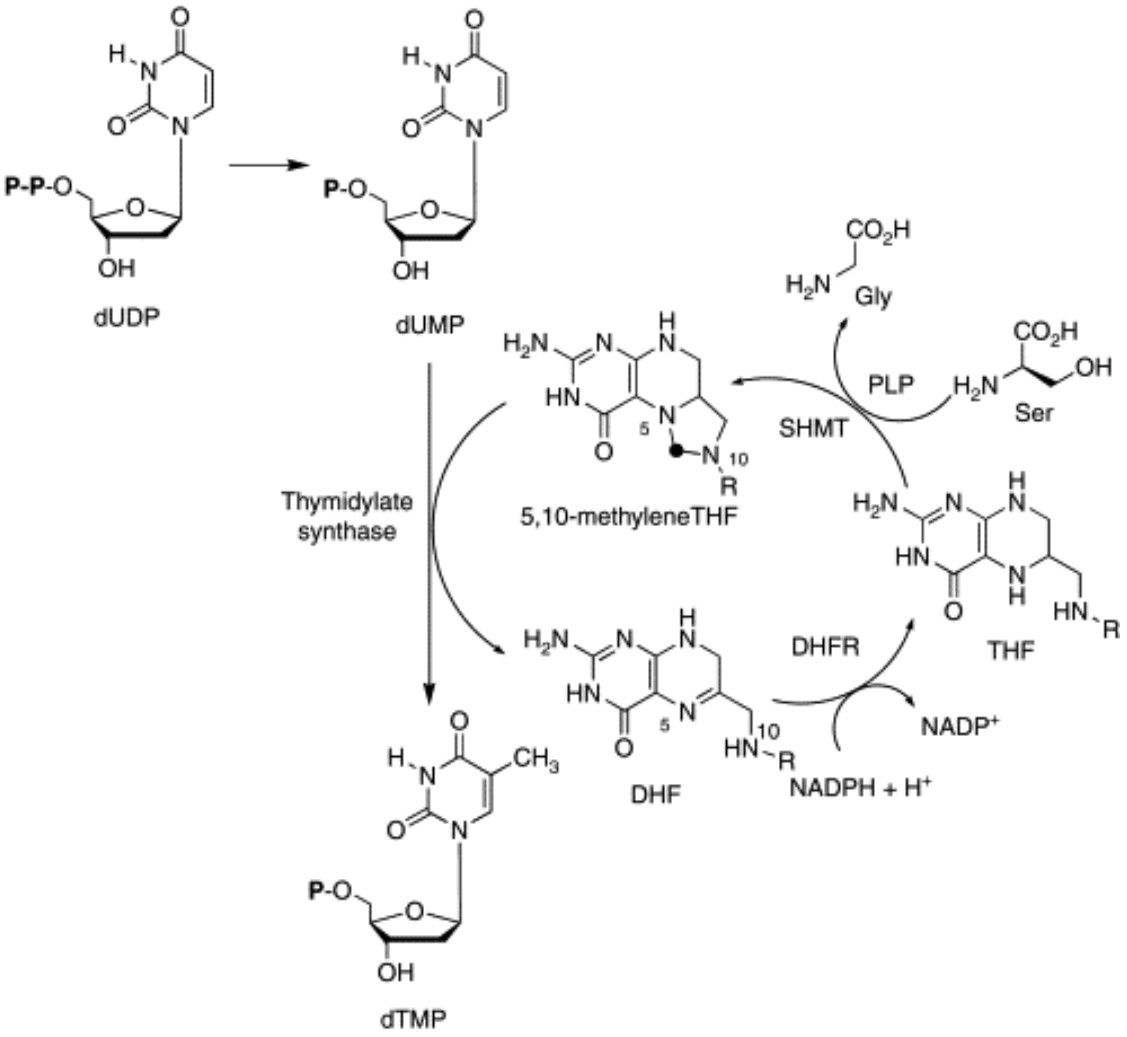
Another example of drug acting covalently:
The 5-fluorouracil is an ANTIMETABOLITE
Anti-tumor drug forming a covalent bond
with the coenzyme tetrahydrofolate



5-fluorouracile

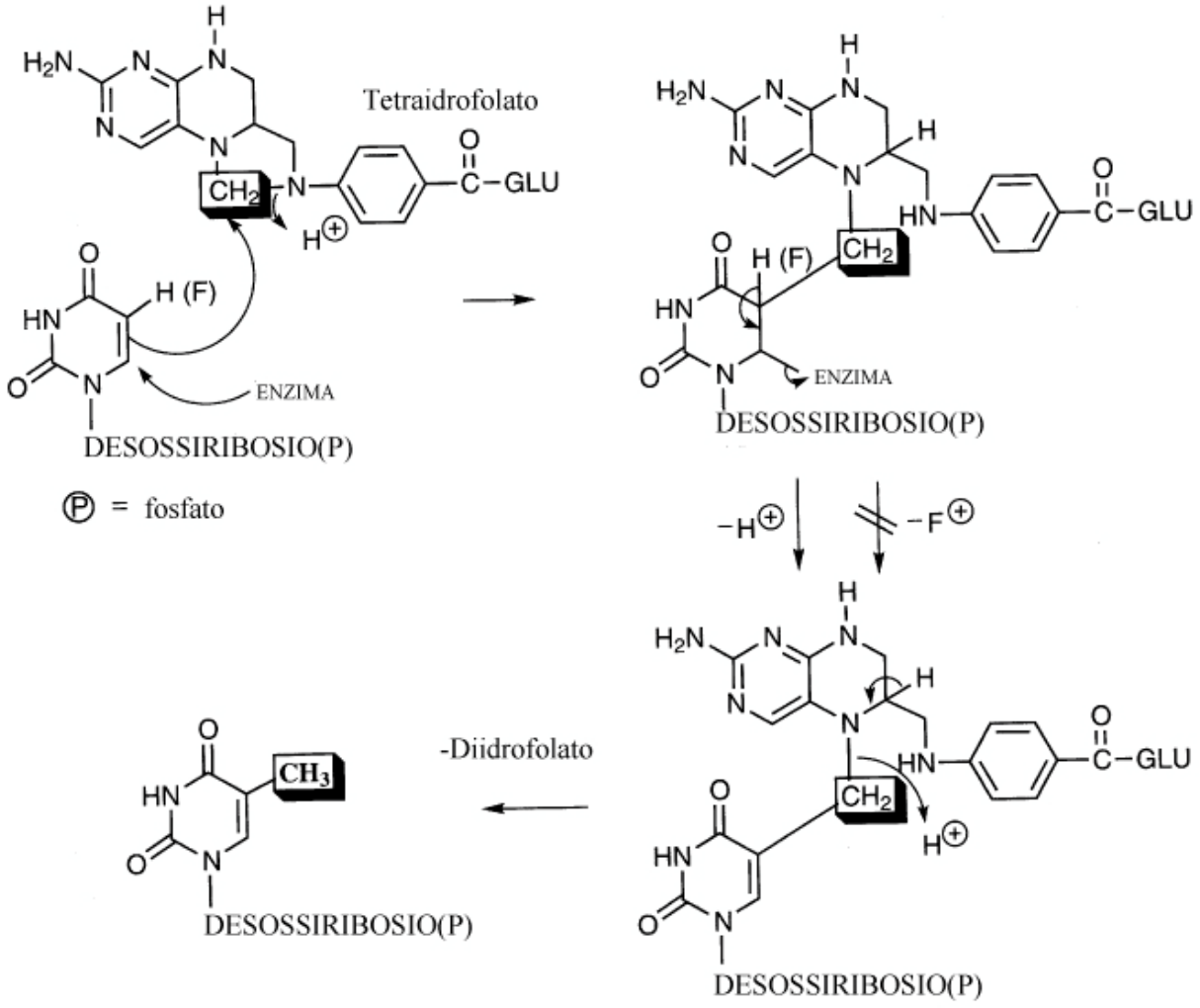
Used for solid cancers in combination with other drugs
(ovarian cancers)

The timidalate synthase is an enzyme that catalyses the methylation of uridine to give timidine using the methylene tetrahydrofolate as cofactor



The biosynthesis of timidine is very important for DNA replication: tumor cells replicate faster than other cells and are affected by inhbition of this process

The methylation of uracil is catalyzed by the enzyme timidilate synthase with the participation of tetrahydrofolate (coenzyme): 5-FU inhibits this process



Another possible mechanism