Biochemical pharmacology lecture slides

These lecture slides have been developed for undergraduate and graduate level university courses. They are free for everyone to use.

Please see `mpalmer.heresy.is/webnotes/Pharmacology` for updates, PowerPoint versions of these slides, and lecture notes.

Chapter 1

Introduction

What is biochemical pharmacology?

What is it?

- pharmacology, but with a focus on how drugs work, not on whether we should take them before or after dinner
- fascinating—you will love it, or double your money back

What is it not?

- just *molecular* pharmacology—physiological context is important, too
- a claim that we completely understand the biochemical action modes of all practically useful drugs—we don't

On drugs and poisons: Paracelsus' maxim

"Alle Ding' sind Gift und nichts ohn' Gift; allein die Dosis macht, dass ein Ding kein Gift ist."

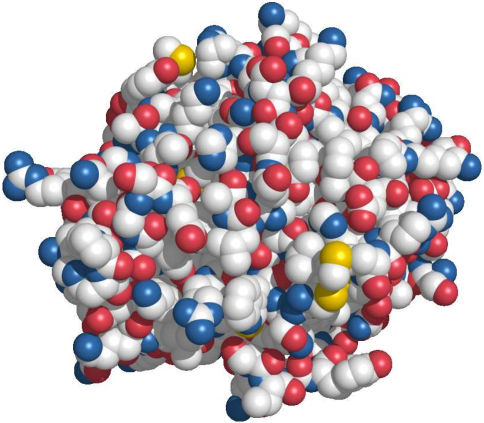
"All things are poison and nothing is without poison; only the dosage makes it so that something is not a poison."

"Dosis sola facit velenum."

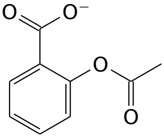
Image credit: Wikimedia

Picture from wikimedia

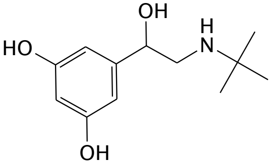
A very small drug particle and a very large one



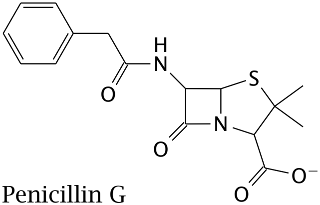
Some drug molecules of more typical size



Acetylsalicylic acid



Terbutaline



Penicillin G

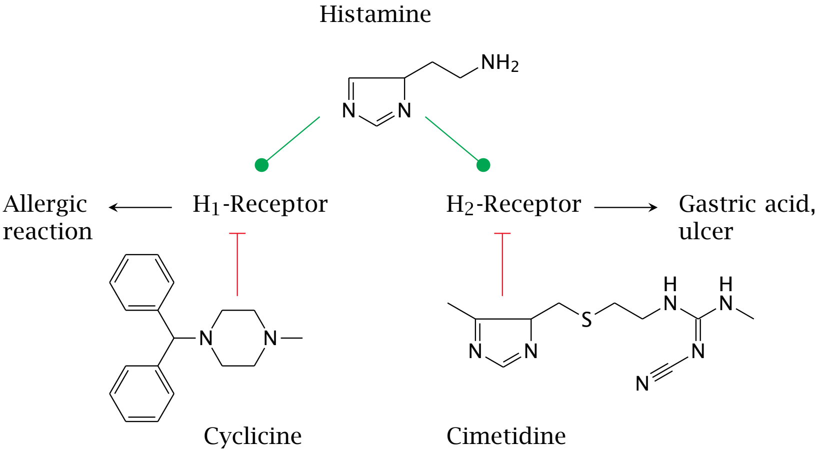
Functional classes of protein drug targets

1. Enzymes
2. Hormone and neurotransmitter receptors
3. Ion channels
4. Membrane transporters
5. Cytoskeletal proteins

Non-protein drug targets

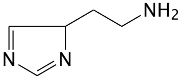
1. DNA: alkylating anti-tumor drugs
2. RNA: anti-ribosomal antibiotics, antisense oligonucleotides
3. Lipid membranes: antibiotics (amphotericin B, polymyxin); gaseous narcotics, alcohol?
4. Free space, or rather no target at all: osmolytes

Histamine receptor antagonists

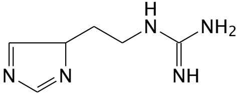


The development of H_2-receptor blockers

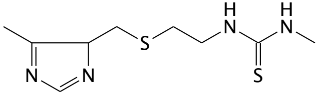
Histamine—physiological agonist



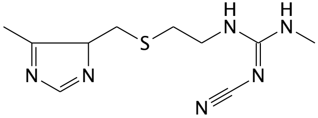
Guanylhistamine—weak antagonist



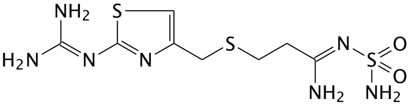
Methiamide—stronger antagonist



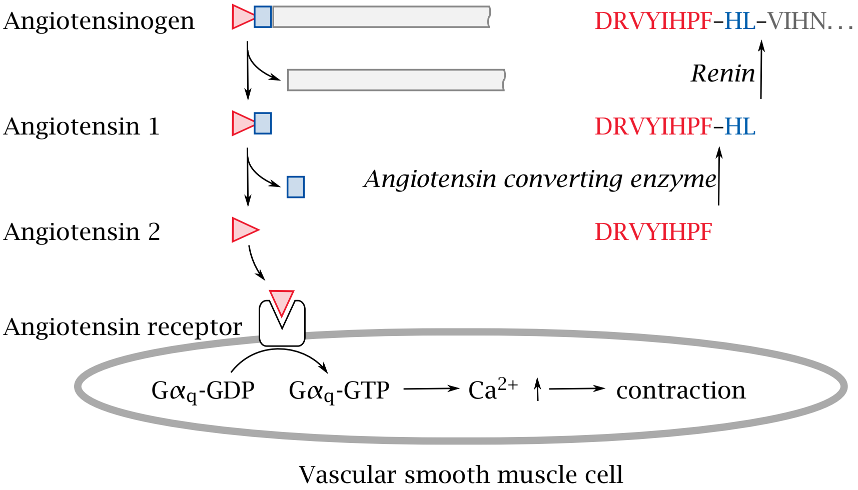
Cimetidine—first clinical antagonist



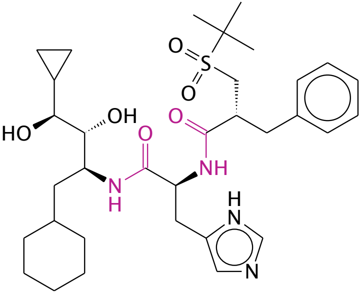
Famotidine—stronger clinical antagonist



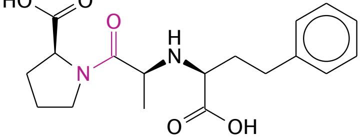
Angiotensin: Proteolytic release from angiotensinogen, and mode of action



Two inhibitors of proteolytic angiotensin release



Remikiren

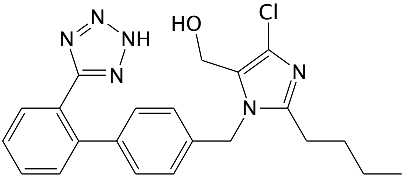


Enalaprilate

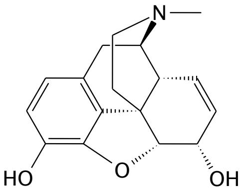
Sequence of saralasin, a peptide inhibitor of the angiotensin 2 receptor

Angiotensin	`Asp-Arg-Val-Tyr-Ile-His-Pro-Phe`
Saralasin	**Sar**-Arg-Val-Tyr-**Val**-His-Pro-**Ala**

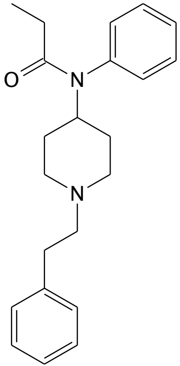
Non-peptide ligands of peptide receptors



Losartan



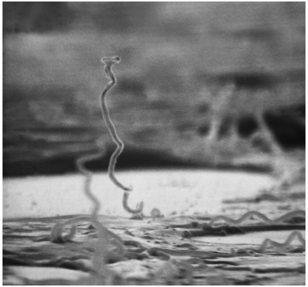
Morphine



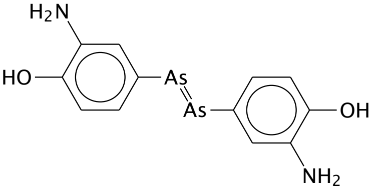
Fentanyl

Arsphenamine, the first modern antibacterial drug

EM photo credit: CDC image library



Treponema pallidum



Arsphenamine

Paul Ehrlich, the discoverer of arsphenamine and originator of the receptor concept

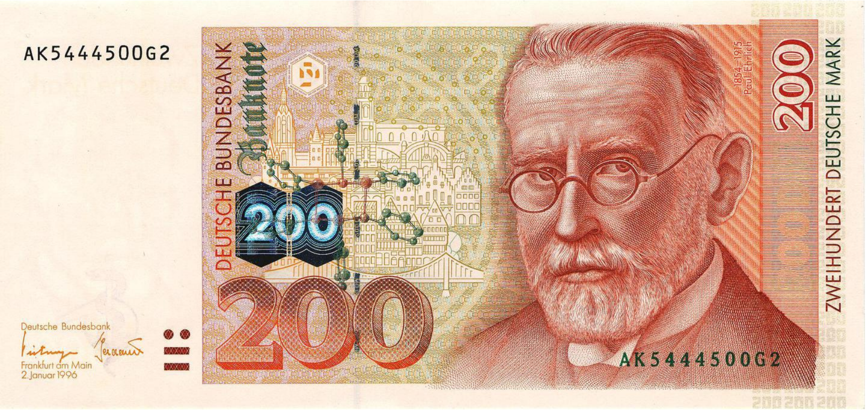
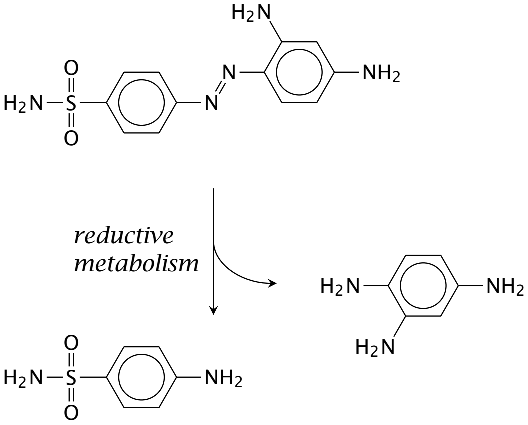


Image credit: wikimedia

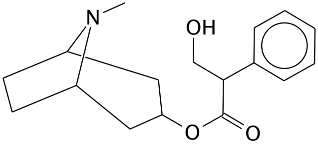
Drug discovery by brute force: sulfamidochrysoidine

Sulfamidochrysoidine

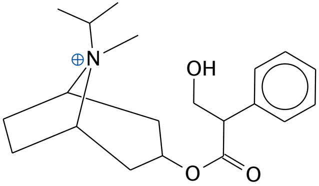
Sulfanilamide



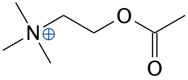
Natural compounds and semisynthetic derivatives



Atropine

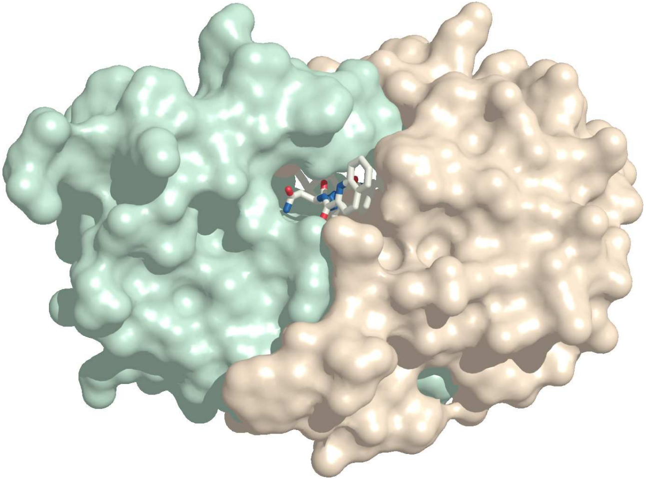


Ipratropium

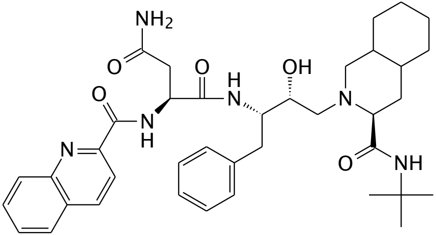


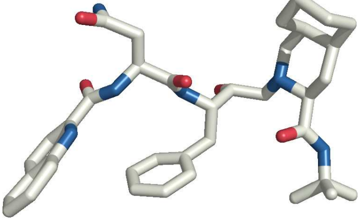
Acetylcholine

Protein structure-based drug discovery: HIV protease bound to its inhibitor saquinavir



Structure of saquinavir, and its conformation in the active site of HIV protease





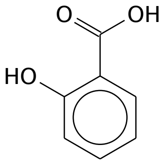
Drug discovery by accident (1): From a letter by Reverend Edmund Stone to the Royal Society, 1763

Among the many useful discoveries, which this age hath made, there are very few which, better deserve the attention of the public than what I am going to lay before your Lordship.

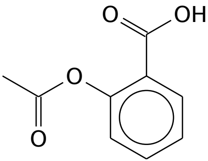
*There is a **bark of an English tree**, which I have found by experience to be a powerful adstringent, and very efficacious in curing anguish and intermitting disorders.*

*About six years ago, **I accidentally tasted it**, and was surprised at its extraordinary bitterness ... As this tree delights in a moist or wet soil, where agues chiefly abound, **the general maxim, that many natural maladies carry their cures along with them**, or that their remedies lie not far from their causes, was so apposite to this particular case, that I could not help applying it; and that this might be the intention of Providence here, I must own had some little weight with me ...*

The active ingredient of willow bark, and its more widely known derivative

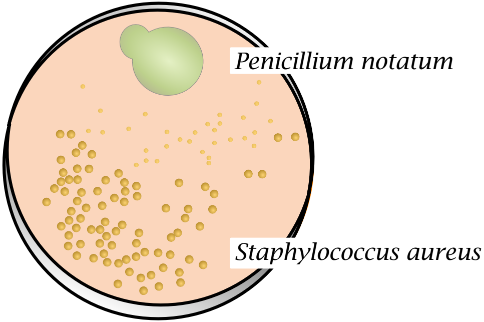


Salicylic acid

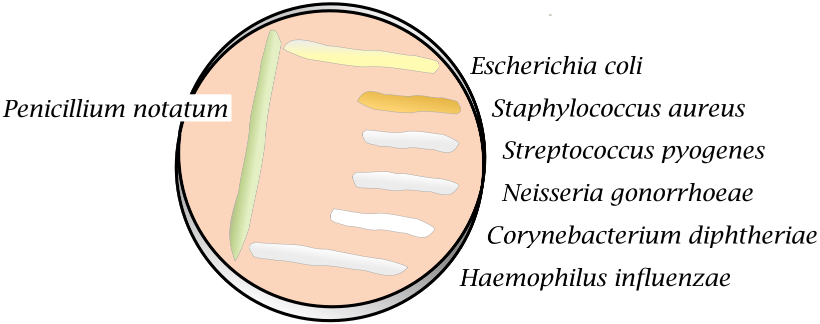


Acetylsalicylic acid

Drug discovery by accident (2): The discovery of penicillin



Not all bacteria are susceptible to penicillin



Drug development and approval

- preclinical, in-house: synthesis, *in vitro* and preliminary animal testing
- investigational drug application to Food and Drug Administration (FDA)—must be approved before clinical testing
- clinical trials in three phases:
  1. Healthy volunteers; focus on pharmacokinetics, toxicity
  2. Small number of patients with targeted disease
  3. Larger patient collective (several hundred to several thousand), comparison to established reference therapies
- new drug application—review by FDA
- post-introduction market surveillance

Pharmacodynamics

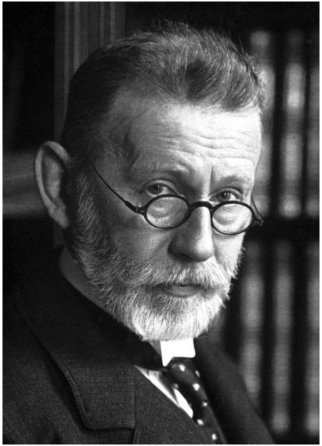
Pharmacodynamics: General principles of drug action

- Theory of drug-receptor interaction
- The two-state model of receptor activation
- Dose-effect relationships and their modulation by signaling cascades
- Potency, efficacy, and therapeutic index

The invention of the receptor concept

… I therefore assumed that the tetanus toxin must unite with certain chemical groupings in the protoplasm of cells … As these receptors, which may be regarded as lateral chains of the protoplasm … become occupied by the toxin, the relevant normal function of this group is eliminated …

Paul Ehrlich, from his Nobel Lecture, 1908

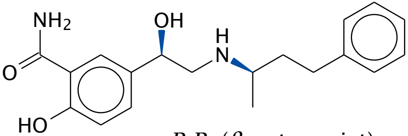


How do drugs affect their receptors?

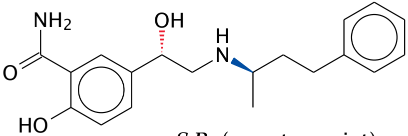
- Mode of binding: reversible *vs.* irreversible
- Binding site: orthosteric *vs.* allosteric
- Functional effect: activation *vs.* inhibition

▸ Histamine receptor antagonists

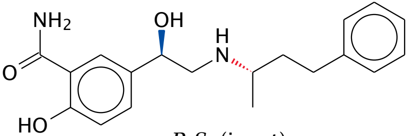
Labetalol as an example of stereoselective drug action



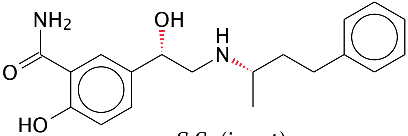
R,R (β-antagonist)



S,R (α-antagonist)

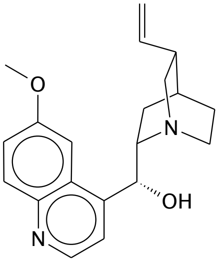


R,S (inert)

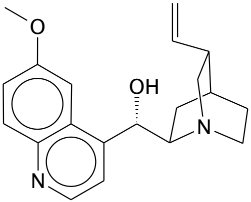


S,S (inert)

Two natural stereoisomers with separate therapeutic uses



Quinine



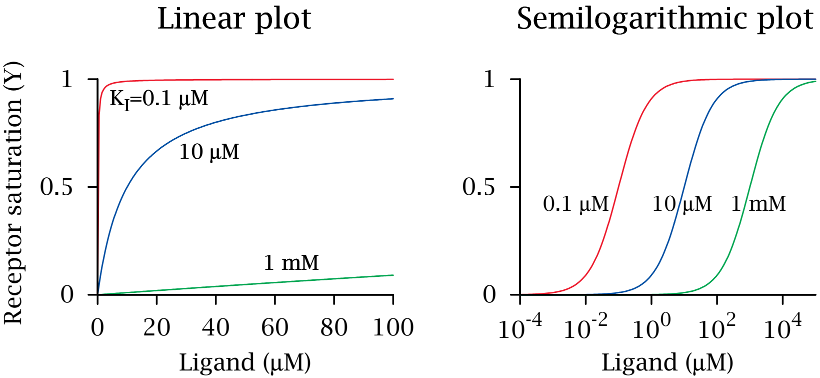
Quinidine

Mass action kinetics and receptor occupancy

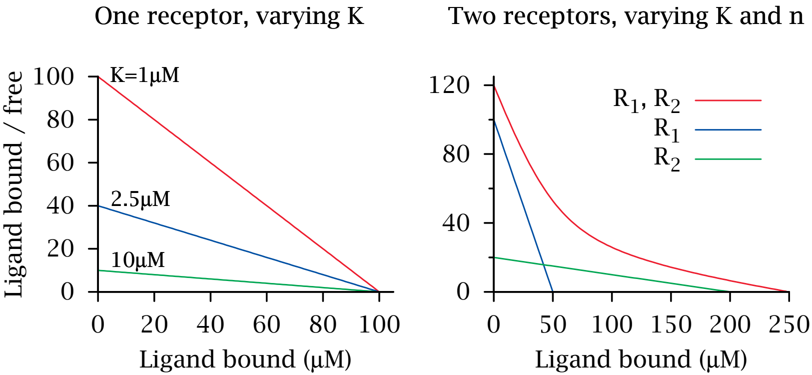
$$K = \frac{[\mathrm{L}][\mathrm{R}_{\mathrm{free}}]}{[\mathrm{LR}]}$$

$$\text{Receptor occupancy} = Y = \frac{[\mathrm{LR}]}{[\mathrm{R}_{\mathrm{total}}]} = \frac{[\mathrm{L}]}{[\mathrm{L}]+K}$$

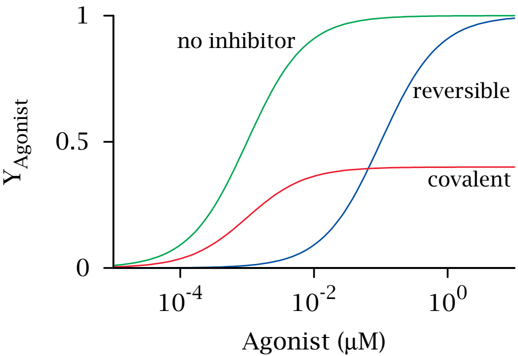
Linear and semi-logarithmic plots of receptor occupancy



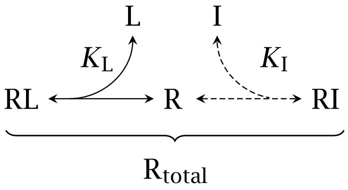
The Scatchard plot



Reversible and covalent receptor inhibition

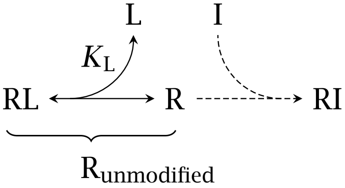


Theory of competitive inhibition



$$Y = \frac{[\mathrm{RL}]}{[\mathrm{R}_\mathrm{total}]} = \frac{[\mathrm{L}]}{[\mathrm{L}] + K_\mathrm{L}\left(1 + \frac{[\mathrm{I}]}{K_\mathrm{I}}\right)} = \frac{[\mathrm{L}]}{[\mathrm{L}] + K'}$$

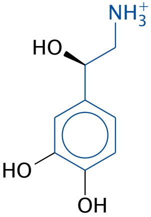
Theory of irreversible or covalent inhibition



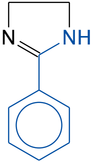
$$Y_u = \frac{[RL]}{[R]_{unmodified}} = \frac{[L]}{[L] + K_L}$$

$$Y_t = \frac{[RL]}{[R]_{total}} = \frac{[L]}{[L] + K_L} \frac{[R]_{unmodified}}{[R]_{total}}$$

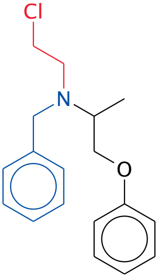
Two inhibitors of α-adrenergic receptors



Norepinephrine

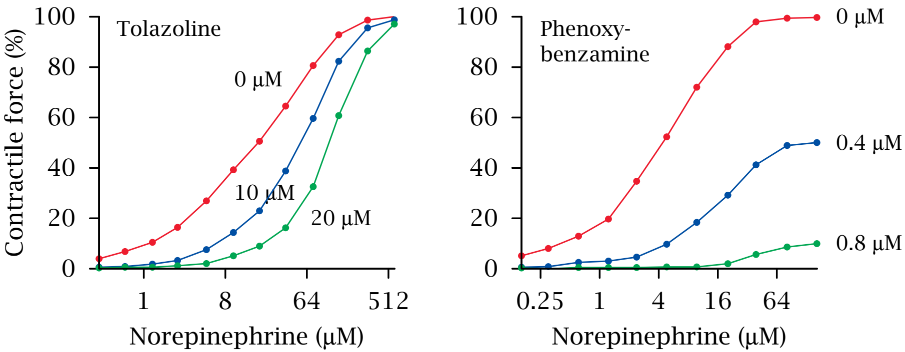


Tolazoline

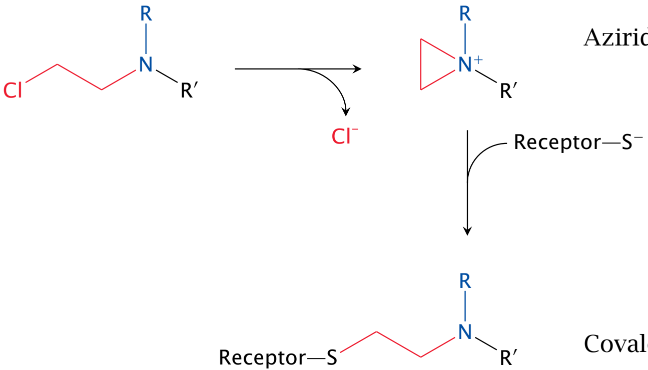


Phenoxybenzamine

Inhibition of spleen strip contraction by tolazoline and phenoxybenzamine



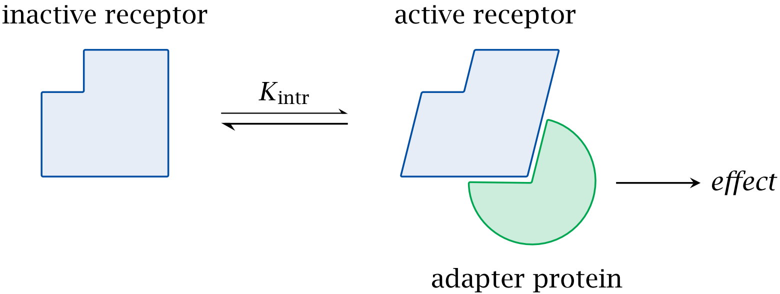
Mechanism of covalent receptor blockade by phenoxybenzamine



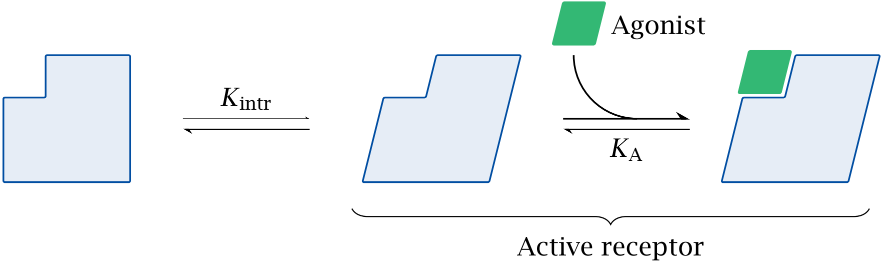
Aziridinium intermediate

Covalent receptor adduct

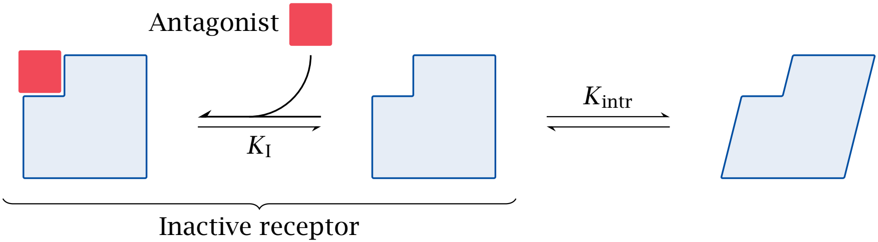
The two-state model of receptor activation

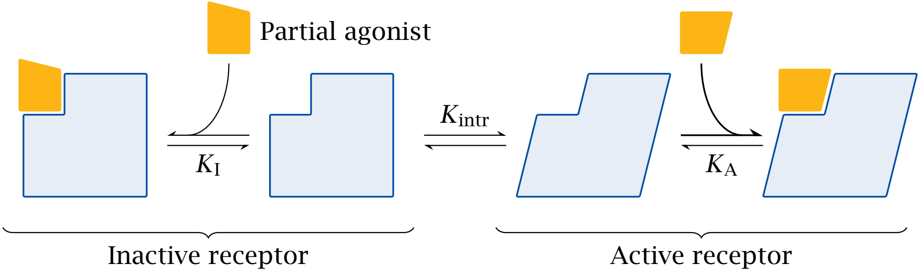


Agonist behavior in the two-state model

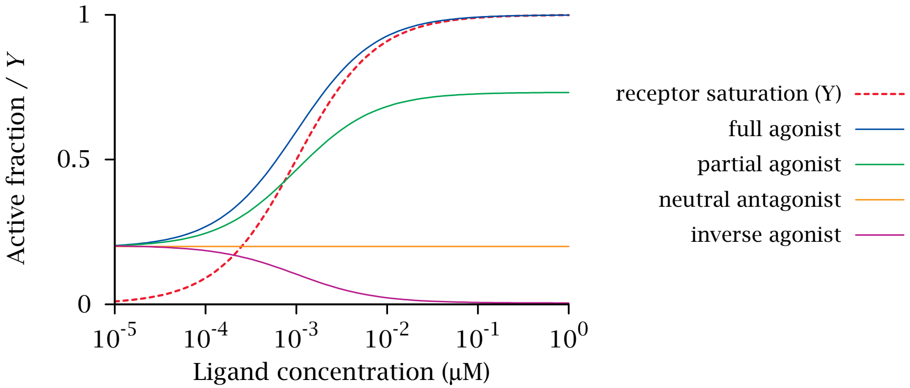


Antagonists and partial agonists

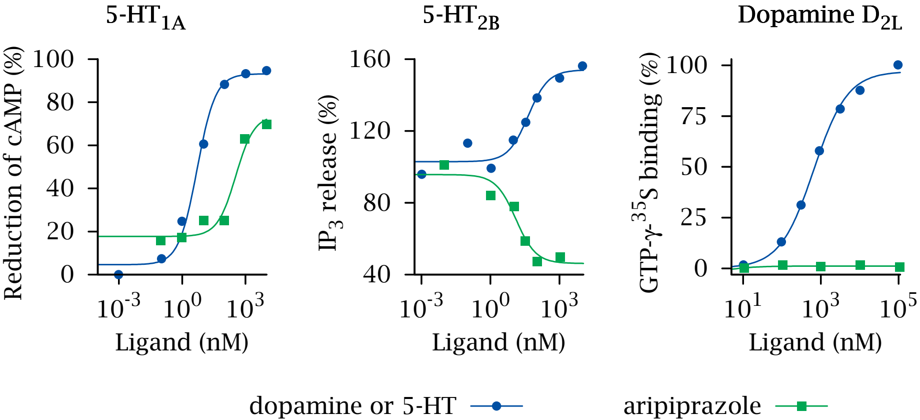




Dose-effect curves in the two-state model



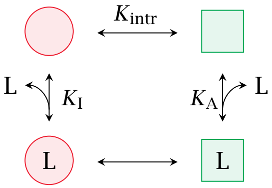
Application of the two-state model: Effects of aripiprazole on serotonin and dopamine receptors

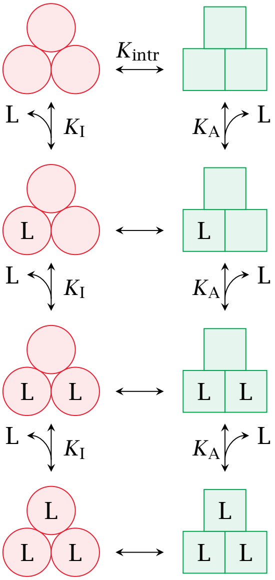


Receptor behaviour not explained by the two-state model

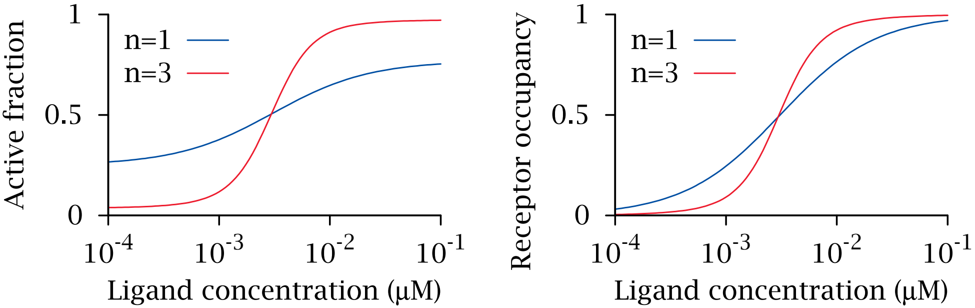
- cooperativity of oligomeric receptors
- agonist-specific coupling
- β-arrestin-biased ligands
- refractory receptor states

Cooperative behavior of oligomeric receptors

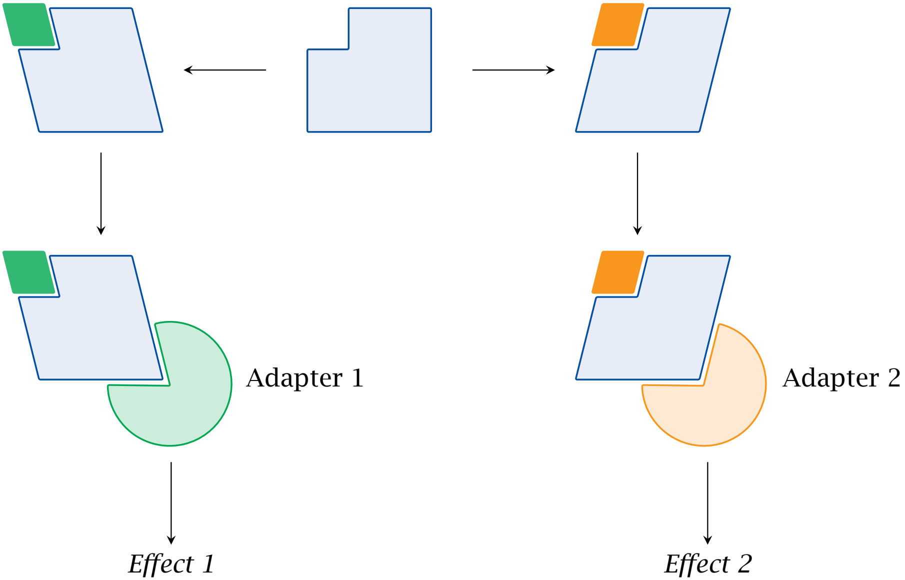




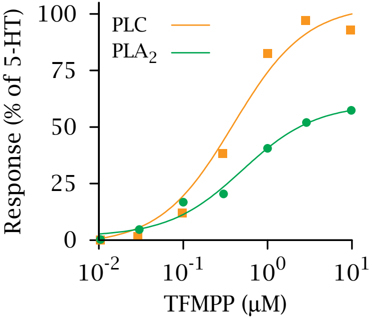
Effect of cooperativity on receptor activity

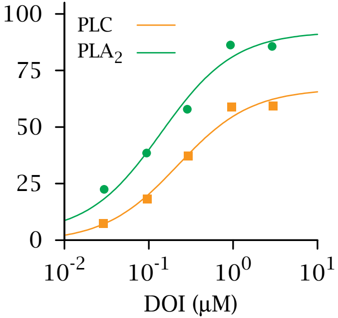


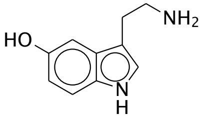
Agonist-specific coupling



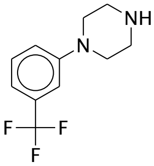
Experimental example: 5-HT$_2$ receptors



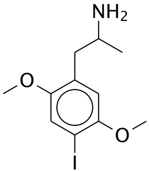




Serotonin (5-HT)



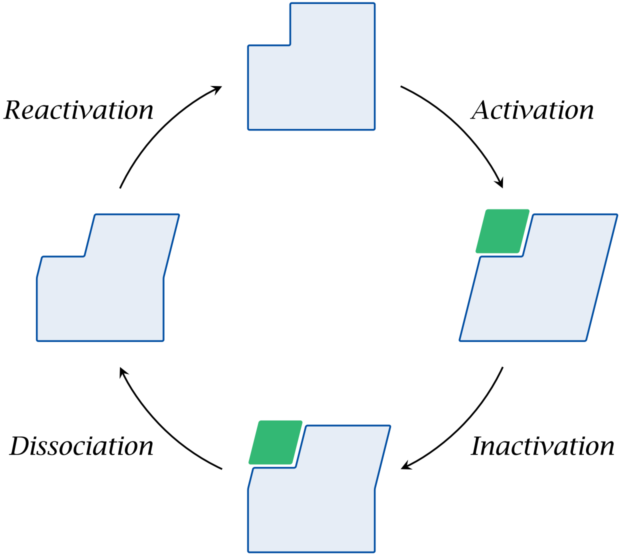
TFMPP



DOI

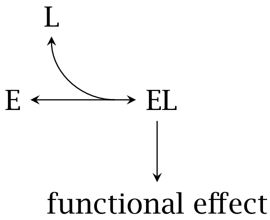
vayssiere

Some receptors have refractory states

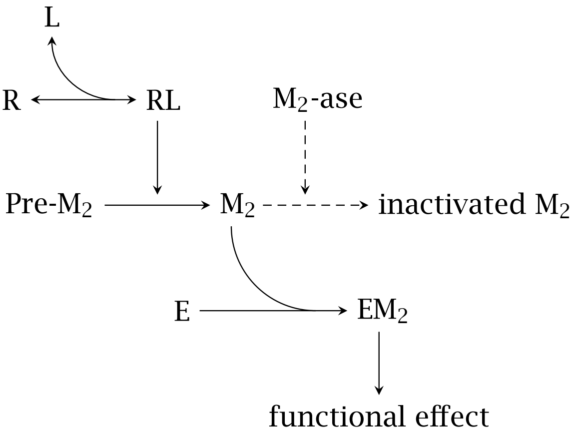


Dose-effect relationships in biochemical cascades

Receptor = effector

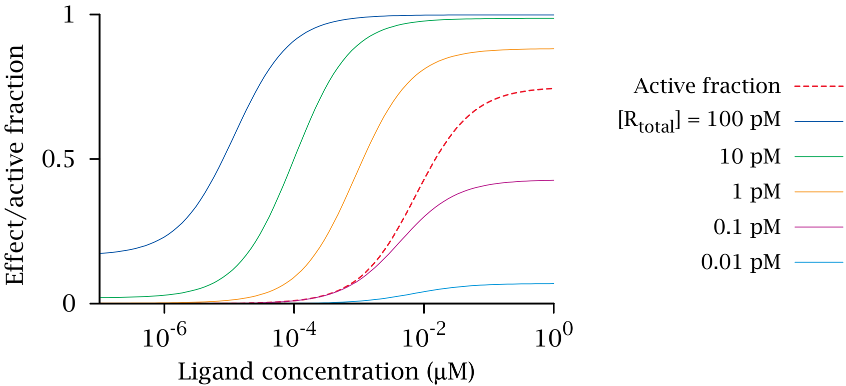


Receptor ⟶ second messenger ⟶ effector



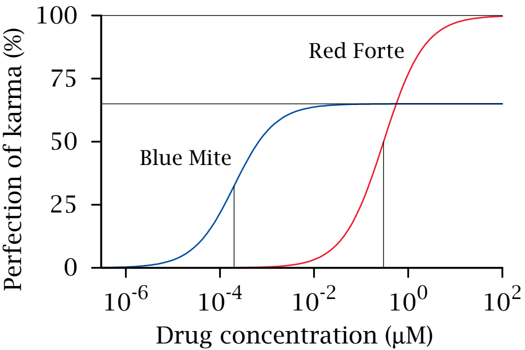
▸ angiotensin action mechanism

The response of a biochemical cascade depends on receptor density



- phenoxybenzamine

Potency and efficacy



Therapeutic and toxic drug effects

$$
\begin{tikzcd}
 & & & \text{benefit} \\
\text{Drug} \arrow[r] & \text{receptor} \arrow[r] & \text{effector} \arrow[ur] \arrow[dr] & \\
 & & & \text{toxicity}
\end{tikzcd}
$$

$$
\begin{tikzcd}
 & & \text{effector}_1 \arrow[r] & \text{benefit} \\
\text{Drug} \arrow[r] & \text{receptor} \arrow[ur] \arrow[dr] & & \\
 & & \text{effector}_2 \arrow[r] & \text{toxicity}
\end{tikzcd}
$$

$$
\begin{tikzcd}
 & \text{receptor}_1 \arrow[r] & \text{effector}_1 \arrow[r] & \text{benefit} \\
\text{Drug} \arrow[ur] \arrow[dr] & & & \\
 & \text{receptor}_2 \arrow[r] & \text{effector}_2 \arrow[r] & \text{toxicity}
\end{tikzcd}
$$

Pharmacokinetics

The scope of pharmacokinetics

Pharmacokinetics deals with the following questions:

- Will the drug reach its intended site of action? If not, can we improve the drug's uptake and distribution to help it reach its target?
- After uptake, how long will the drug stay in the system? How is it eliminated from the system?

Stages of drug transport

- Absorption: Uptake of the drug from the compartment of application into the blood plasma
- Distribution: Equilibration of the drug between the blood plasma and the rest of the organism
- Elimination: Excretion or metabolic inactivation of the drug

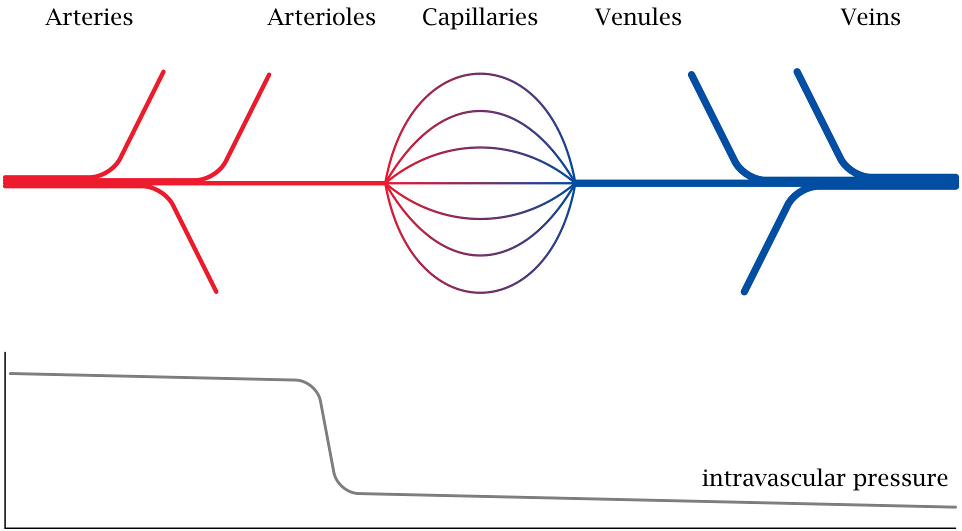
Absorption depends on the route of application

Route	Advantage	Disadvantage
Oral	Convenience—route of choice where possible	Multiple barriers between intestine and circulation
Intra-venous	No barriers to absorption	Involved; risk of infection; allergic reactions more severe
Pulmonary	Fast, quantitative uptake	Limited to gases (mostly narcotics)

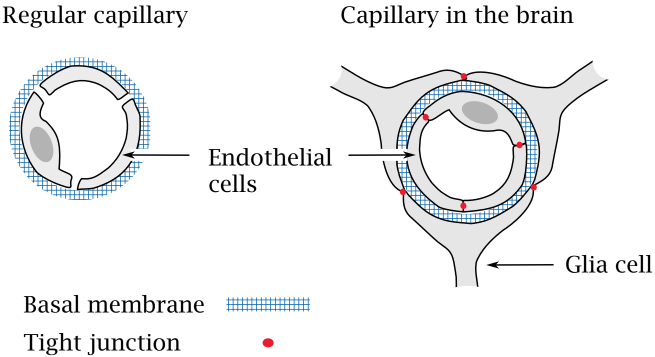
The need for distribution varies with the location of the drug target

Location of target	Example
Inside circulation, outside cells	Proteases in blood coagulation and fibrinolysis
Inside circulation, inside cells	Chemotherapy of malaria parasites
Outside circulation, on cell surfaces	Histamine receptors
Outside circulation, inside cells	Cyclooxygenase

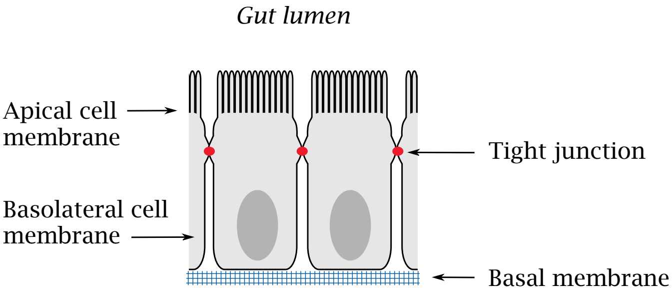
Sections of the blood circulation



Capillaries as barriers to drug transport



The intestinal epithelium as a barrier to drug absorption

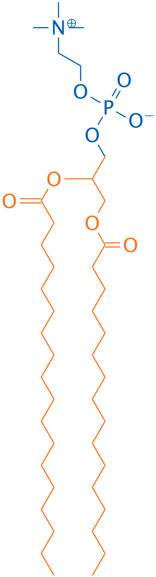


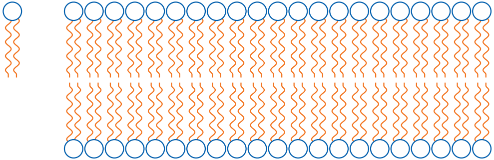
Mechanisms of solute transport across cell membranes

1. Active transport
   a) Primary: ATP-coupled
   b) Secondary: driven by ion gradients
2. Passive transport
   a) Facilitated diffusion: protein-mediated transport, not coupled to ATP or ion gradients
   b) Non-facilitated diffusion of lipophilic compounds; non-ionic diffusion

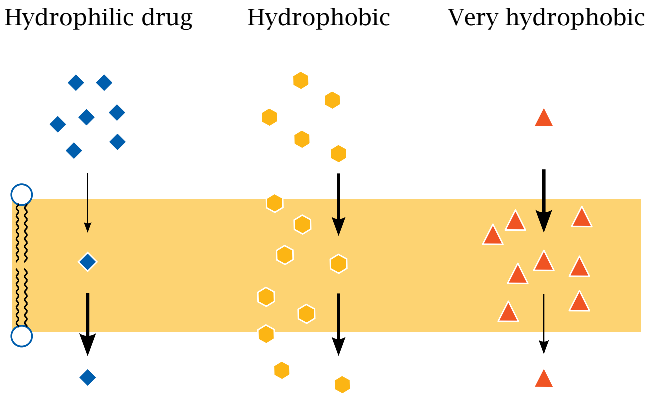
‣ araC structure

Structure of a phosphatidylcholine bilayer



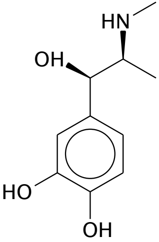


The polarity of drug molecules affects their rate of diffusion across lipid bilayers

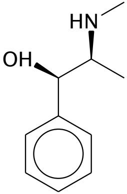


The membrane permeability of drugs can be improved by removing polar functional groups

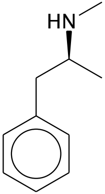
Epinephrine



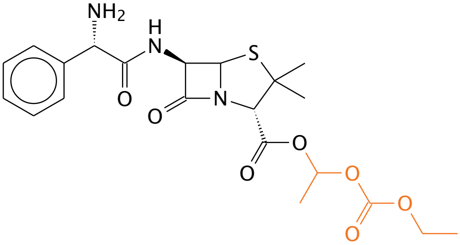
Ephedrine



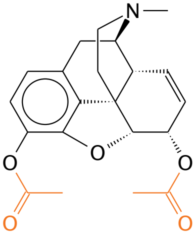
Metamphetamine



Resorption esters can improve the diffusion of drugs across membranes



Bacampicillin

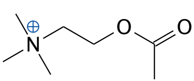


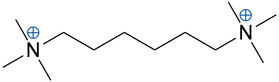
Heroin

History of heroin

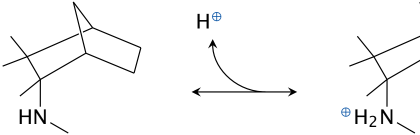
Ionizable drug molecules may cross bilayers by non-ionic diffusion

Acetylcholine





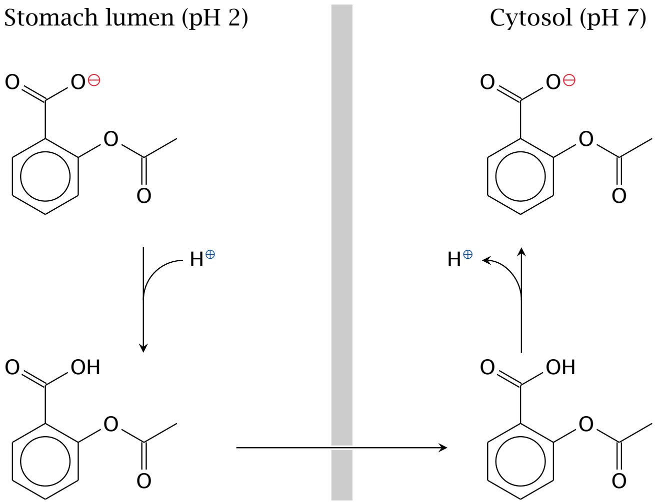
Hexamethonium



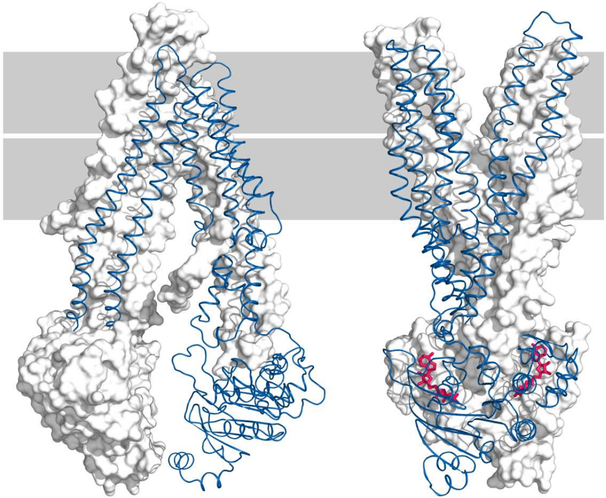
Mecamylamine

- ipratropium

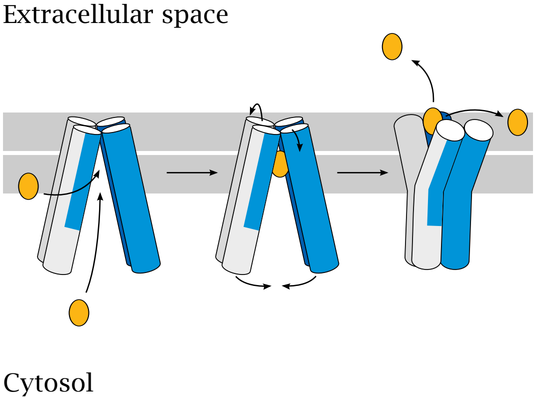
Gastric acid promotes accumulation of acetylsalicylic acid in the cells of the mucous membrane



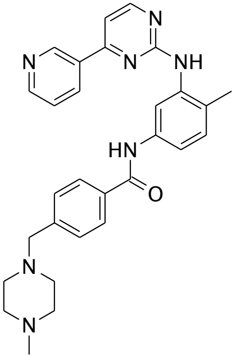
Inward- and outward-facing conformations of ABC transporters



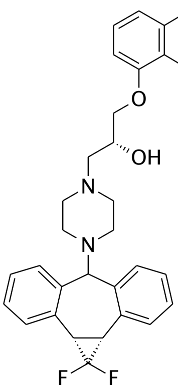
The functional cycle of ABC transporters



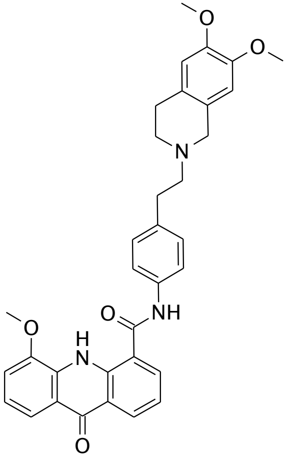
ABC transporters and the blood brain barrier (1)



Imatinib

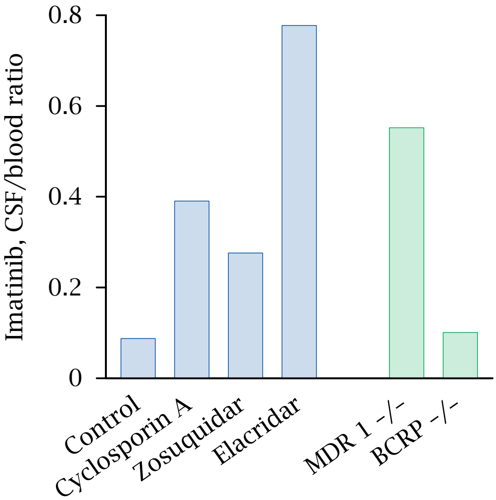


Zosuquidar

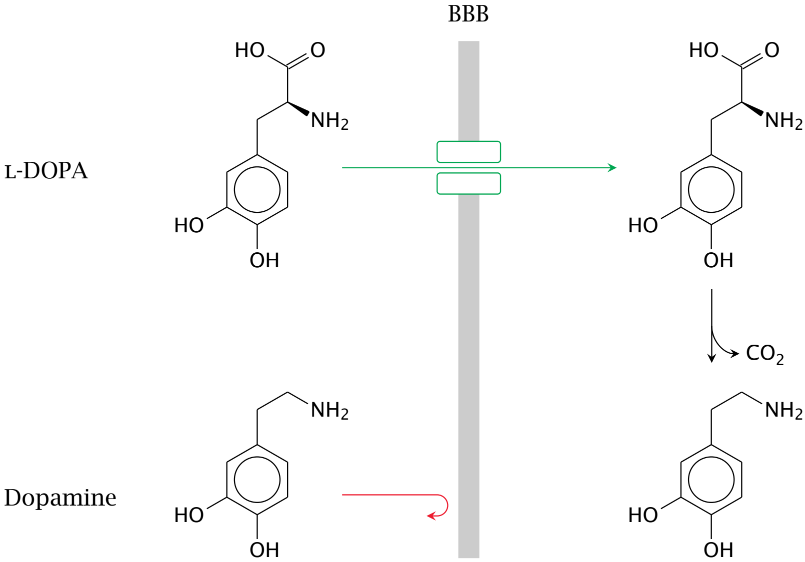


Elacridar

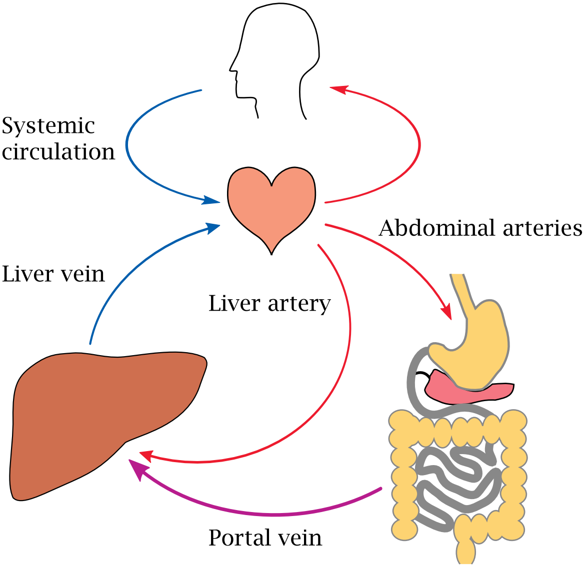
ABC transporters and the blood brain barrier (2)



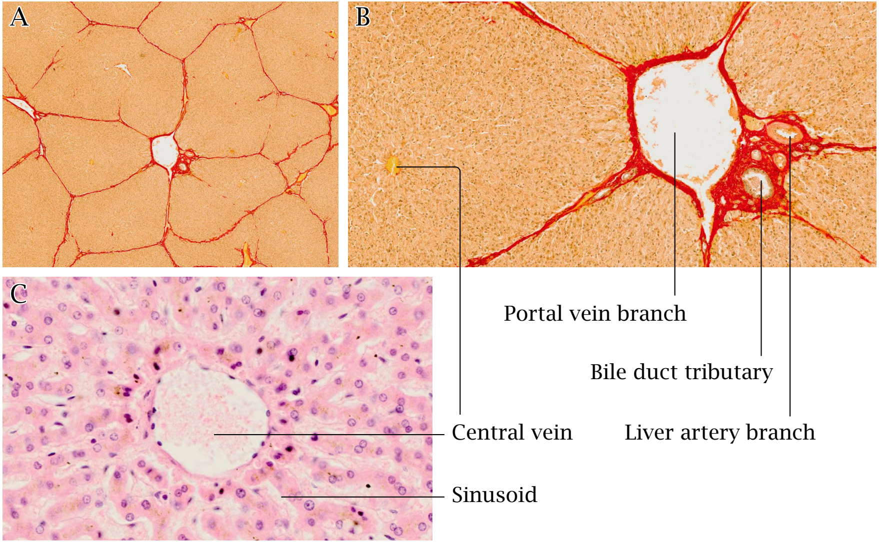
L-DOPA reaches the brain by specific transport



The portal circulation



Tissue structure of the liver



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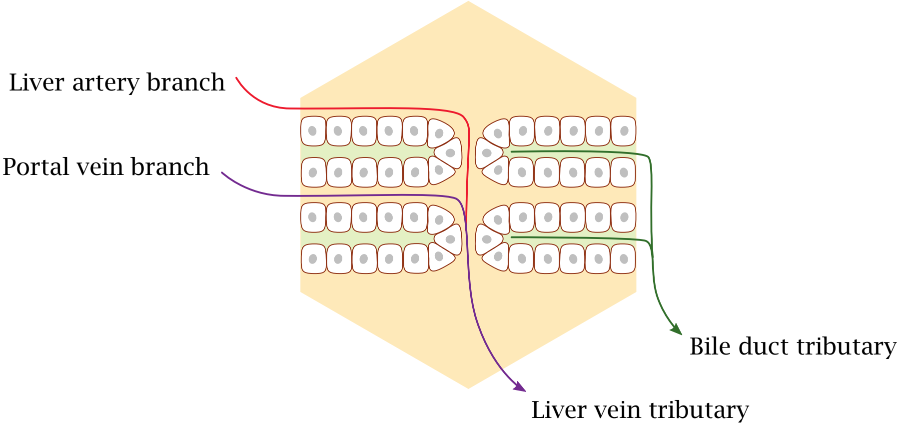
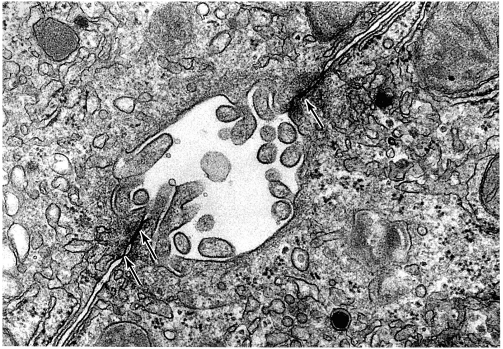
Blood flow and bile flow in the liver lobule



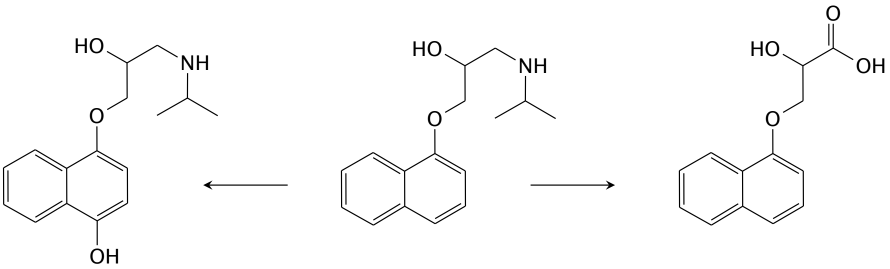
Ultrastructure of liver tissue

Sinusoid

Bile canaliculus

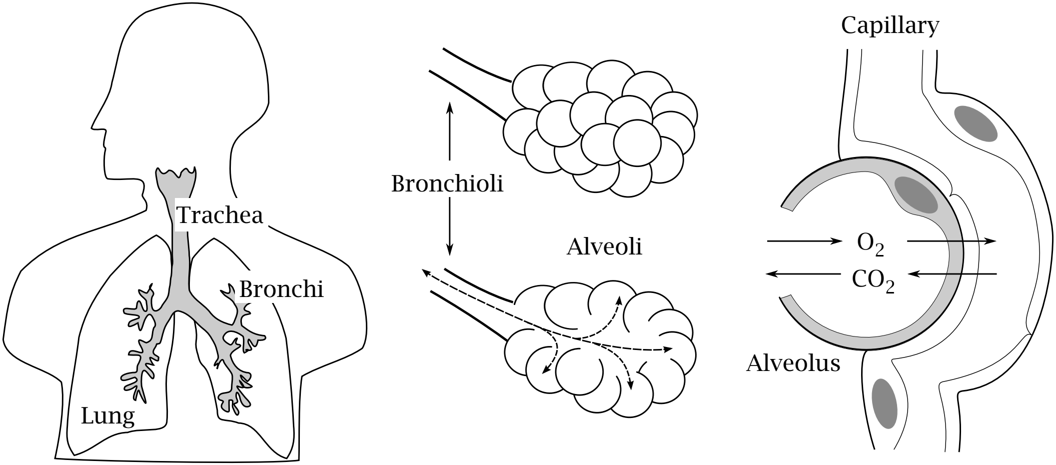


Propranolol and the first-pass effect

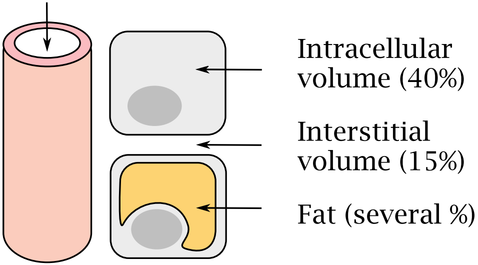


Active metabolite　　Propranolol　　Inactive metabolite

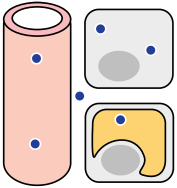
Outline of lung anatomy

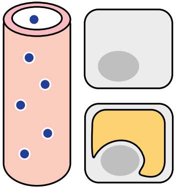


Major compartments of drug distribution

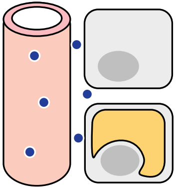


Drug evenly distributed (uncommon)

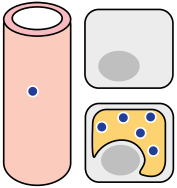




Drug confined to circulation (very large drug molecules)

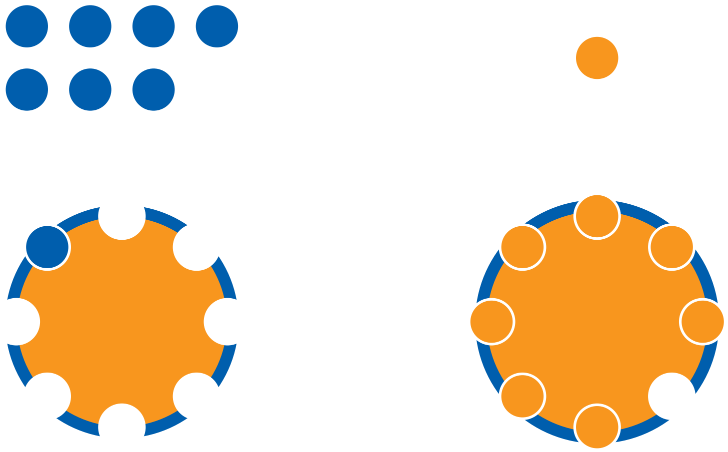


Drug excluded by cell membranes (very polar drug molecules)



Drug enriched in fat (lipophilic drugs)

Hydrophobic drugs tend to bind to proteins

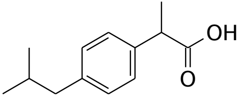
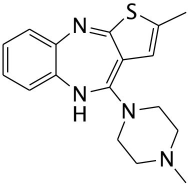
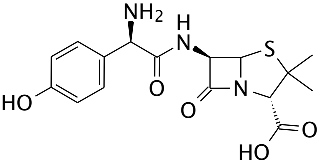


The volume of distribution: two alternate definitions

$$V_d = \frac{n}{[\text{Drug}]_{\text{plasma}}} \text{ (in absolute terms and in liters)}$$

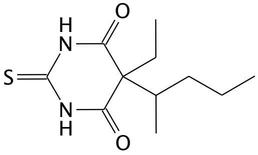
$$V_d = \frac{n}{[\text{Drug}]_{\text{plasma}} \times \text{body weight}} \text{ (relative to body weight, } {}^{\text{l}}\!/_{\text{kg}}\text{)}$$

Example drugs and their uptake and distribution parameters

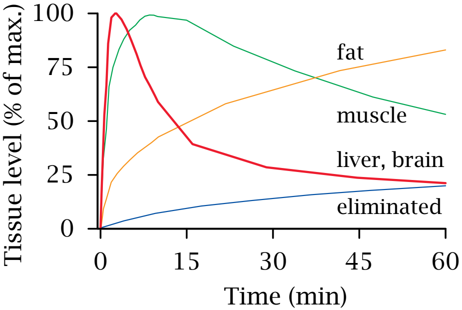
Drug	Structure	V_d (l/kg)	Protein-bound	Oral availability
Ibuprofen		0.15	99%	80%
Olanzapine		16.4	93%	60%
Amoxicillin		0.21	18%	93%

▸ resorption esters

Kinetics of thiopental distribution



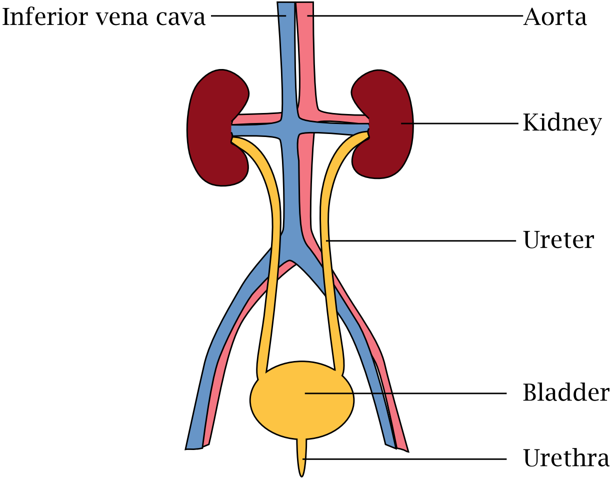
Thiopental



Overview of drug elimination

$$
\begin{tikzcd}
\text{Hydrophilic drug molecule} \arrow[dd] & \text{Hydrophobic drug molecule} \arrow[d] \\
 & \text{Liver} \arrow[d] \\
\text{Kidney} \arrow[d] & \text{Hydrophilic drug metabolite} \arrow[l] \arrow[d] \\
\text{Urine} & \text{Bile}
\end{tikzcd}
$$

Location and perfusion of the kidneys

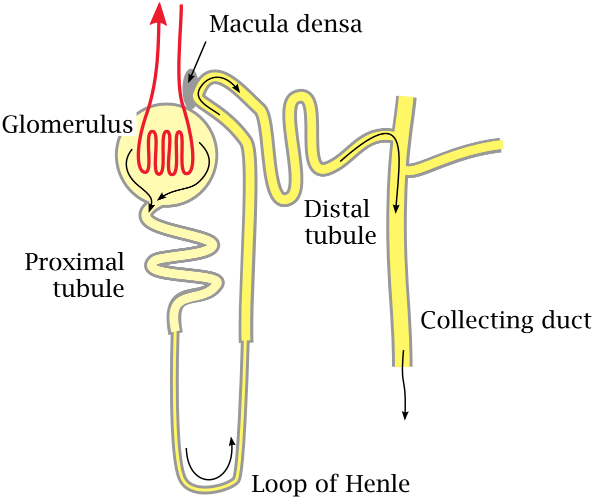


Overview of kidney function

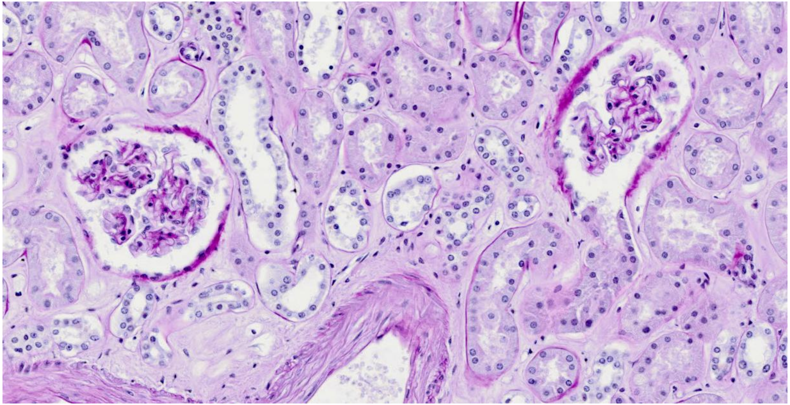
Urine is "distilled" from blood plasma in several stages:

1. Ultrafiltration: 10-20% of the blood plasma volume flow is squeezed out; macromolecules are retained
2. Solute reuptake: glucose, salts, amino acids etc. are recovered from the primary filtrate by active transport
3. Water reuptake: driven by osmotic gradient
4. Solute secretion: some substrates are actively secreted into the nascent urine

The nephron


Macula densa
Glomerulus
Distal tubule
Proximal tubule
Collecting duct
Loop of Henle

Cross sections of glomeruli and tubules in kidney



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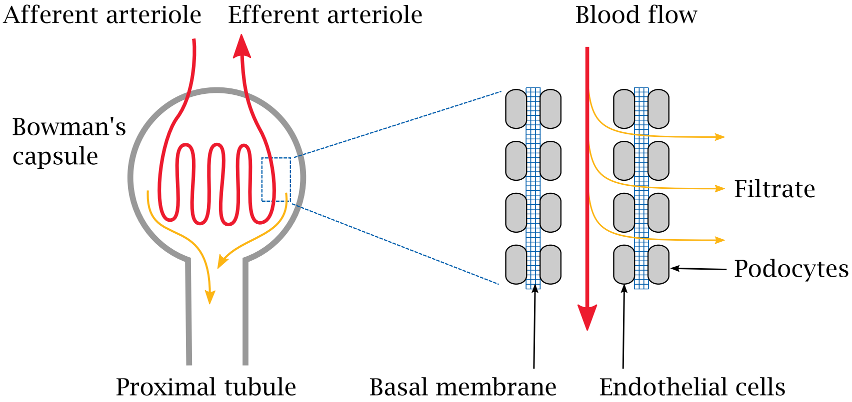
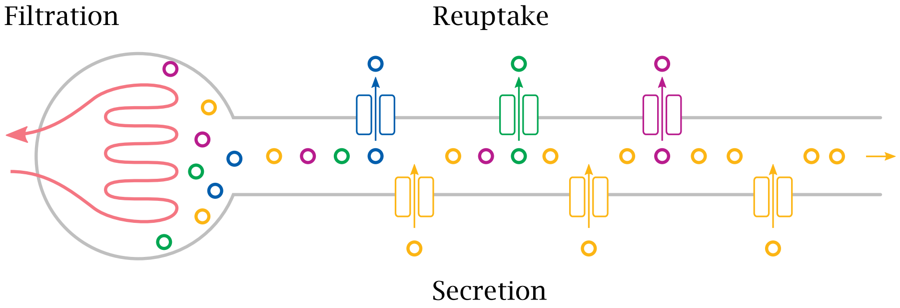
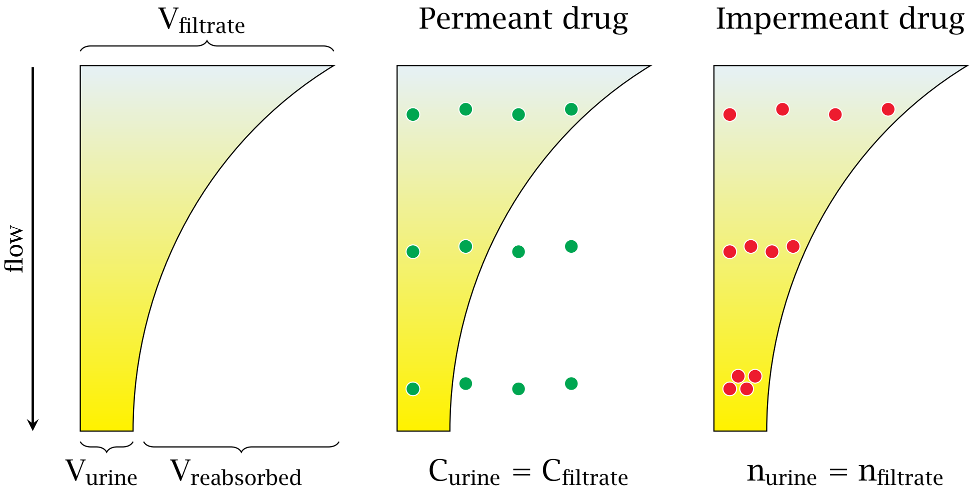
Plasma ultrafiltration in the glomerulus



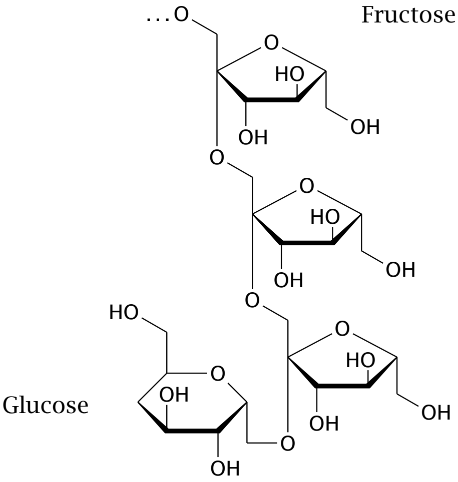
Filtration, reuptake, and active secretion



A drug's rate of urinary excretion depends on its membrane permeability



Inulin, a model compound that is quantitatively filtrated and retained in the urine

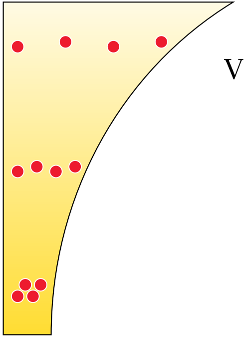


Definition of the renal clearance

$$\text{clearance}_{\text{Drug}} = \frac{dV_{\text{urine}}}{\text{dt}} \times \frac{[\text{Drug}]_{\text{urine}}}{[\text{Drug}]_{\text{plasma}}}$$

Intuitive meaning: what volume of plasma is being "cleared completely" of the drug per unit of time?

The volume flow of glomerular filtration can be measured from the renal clearance of inulin



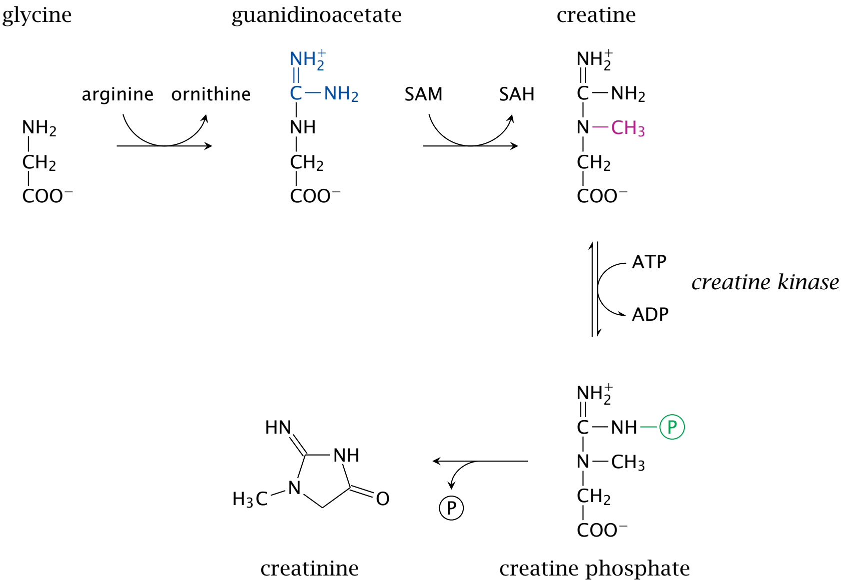
$$V_{filtrate} \times [\mathrm{Inulin}]_{filtrate} = V_{urine} \times [\mathrm{Inulin}]_{urine}$$

$$V_{filtrate} = V_{urine} \times \frac{[\mathrm{Inulin}]_{urine}}{[\mathrm{Inulin}]_{filtrate}}$$

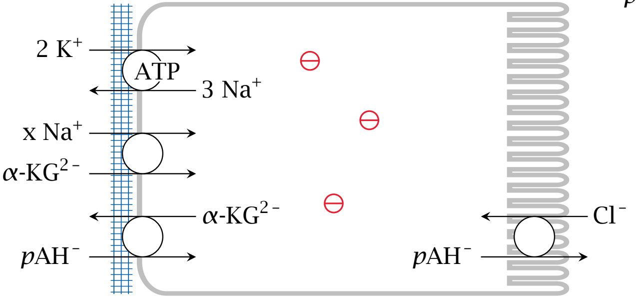
$$\frac{dV_{filtrate}}{dt} = \frac{dV_{urine}}{dt} \times \frac{[\mathrm{Inulin}]_{urine}}{[\mathrm{Inulin}]_{filtrate}}$$

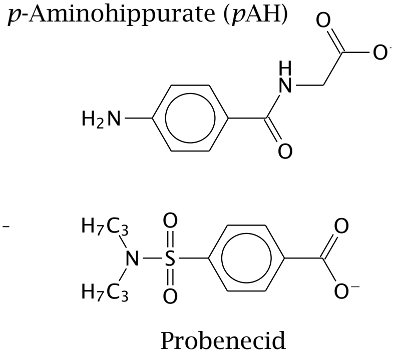
$$= \frac{dV_{urine}}{dt} \times \frac{[\mathrm{Inulin}]_{urine}}{[\mathrm{Inulin}]_{\mathbf{plasma}}}$$

The GFR can be approximately determined using the creatinine clearance

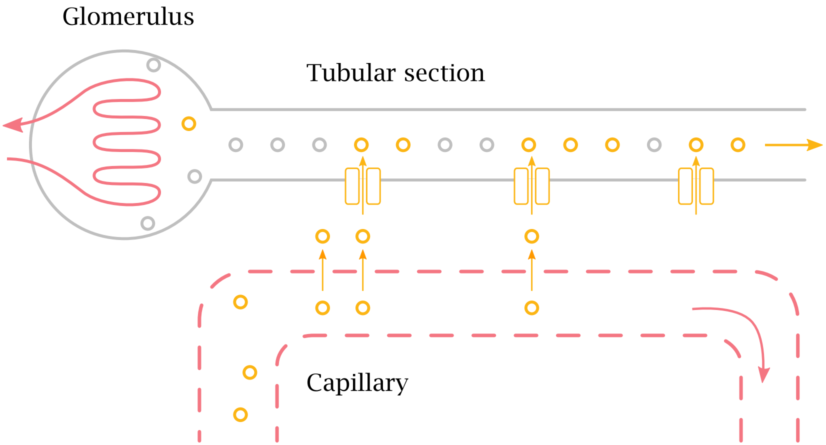


Tubular secretion of *p*-aminohippurate





Tubular secretion of *p*-aminohippurate is almost quantitative



The *p*-aminohippurate clearance measures the renal plasma flow

$$\frac{\mathrm{dn}_{p\text{-AH, plasma}}}{\mathrm{dt}} \approx \frac{\mathrm{dn}_{p\text{-AH, urine}}}{\mathrm{dt}}$$

$$\mathrm{n}_{\text{p-AH}} = [\text{p-AH}] \times \mathrm{V}$$

$$\frac{\mathrm{dV}_{\text{plasma}}}{\mathrm{dt}} \times [p\text{-AH}]_{\text{plasma}} \approx \frac{\mathrm{dV}_{\text{urine}}}{\mathrm{dt}} \times [p\text{-AH}]_{\text{urine}}$$

$$\frac{\mathrm{dV}_{\text{plasma}}}{\mathrm{dt}} \approx \frac{\mathrm{dV}_{\text{urine}}}{\mathrm{dt}} \times \frac{[p\text{-AH}]_{\text{urine}}}{[p\text{-AH}]_{\text{plasma}}}$$

Non-equilibrium kinetics of drug elimination

$$\mathrm{n} = [\mathrm{D}]_{\mathrm{plasma}} \times V_{\mathrm{d}} \times \text{body weight}$$

$$\frac{\mathrm{dn}}{\mathrm{dt}} = -k \times [\mathrm{D}]_{\mathrm{plasma}}$$

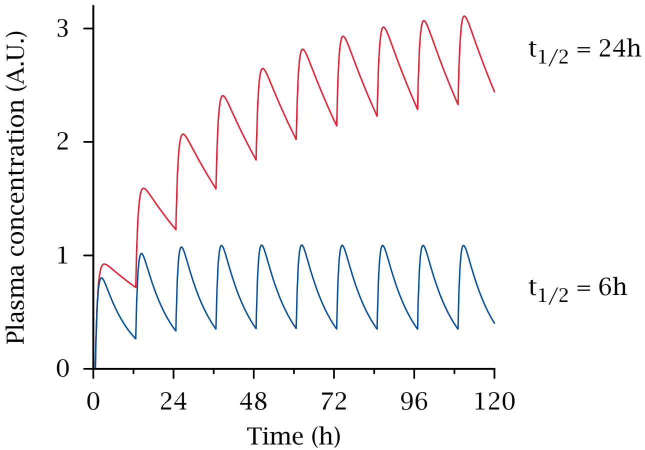
$$\frac{[\mathrm{D}]_{\mathrm{plasma,t}}}{[\mathrm{D}]_{\mathrm{plasma,0}}} = e^{-\frac{k}{V_{\mathrm{d}} \times \text{body weight}} t}$$

$$0.5 = e^{-\frac{k}{V_{\mathrm{d}} \times \text{body weight}} t_{1/2}}$$

$$t_{1/2} = \ln 2 \times \frac{V_{\mathrm{d}} \times \text{body weight}}{k}$$

▸ Vd definition

Repeated drug application can result in accumulation



Drug metabolism

The place of metabolism in drug elimination

$$
\begin{tikzcd}
\text{Hydrophilic drug molecule} \arrow[dd] & \text{Hydrophobic drug molecule} \arrow[d] \\
 & \text{Liver} \arrow[d] \\
\text{Kidney} \arrow[d] & \text{Hydrophilic drug metabolite} \arrow[l] \arrow[d] \\
\text{Urine} & \text{Bile}
\end{tikzcd}
$$

Types of reactions in drug metabolism

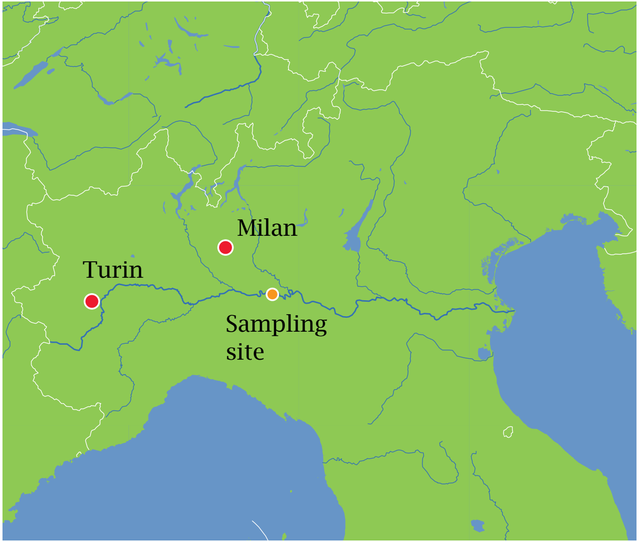
1. Oxidation
2. Conjugation
3. Reduction
4. Hydrolysis

▸ resorption esters ▸ sulfamidochrysoidine

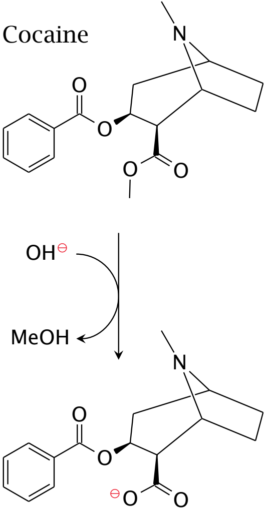
Functional outcomes of drug metabolism

1. Inactivation and accelerated elimination of drugs
2. Activation of prodrugs
3. Formation of active metabolites with similar or novel activity
4. Detoxification of toxic xenobiotics
5. *Toxification* of non-toxic xenobiotics

A hydrolytic metabolite of cocaine can be detected in wastewater

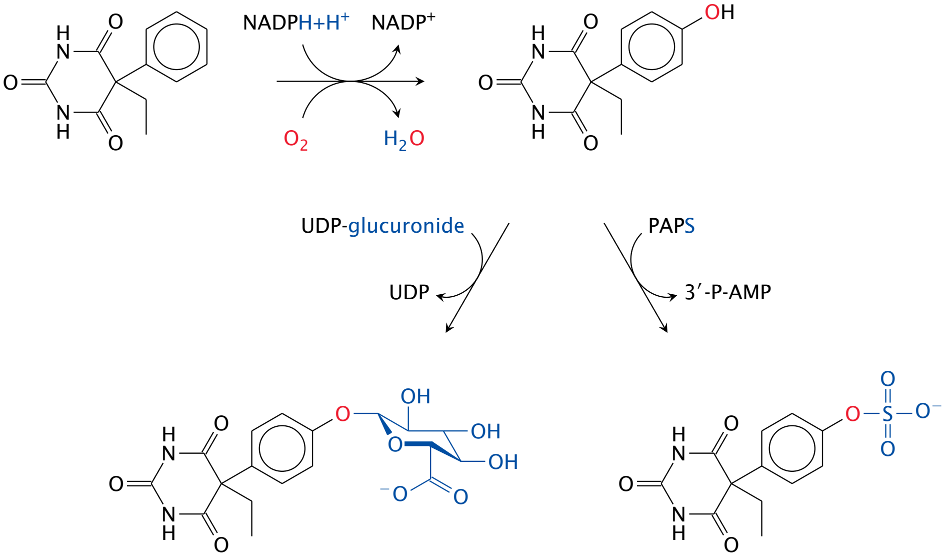


Cocaine

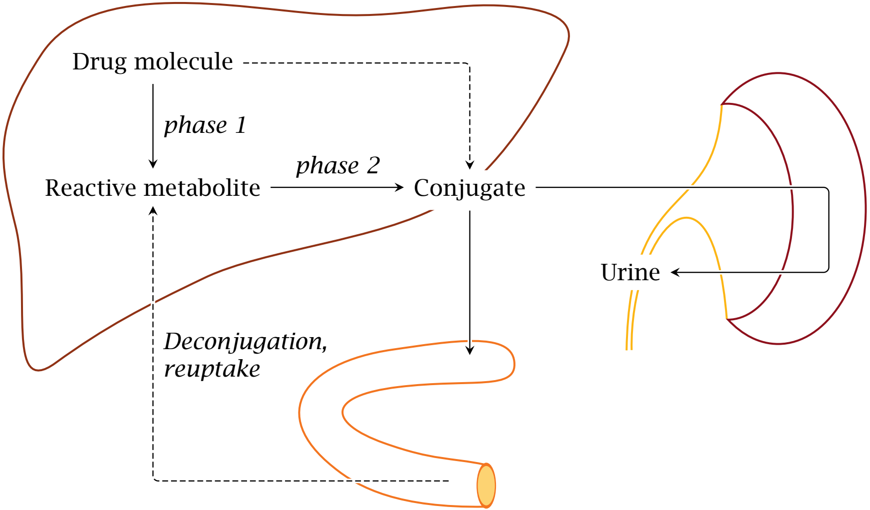


Benzoylecgonine

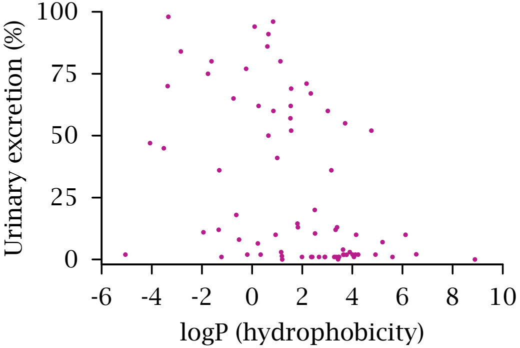
Metabolism of phenobarbital



With many drugs, metabolic transformation facilitates excretion



Hydrophobicity does *not* strongly predict the extent of metabolism



Major types of drug-metabolizing enzymes

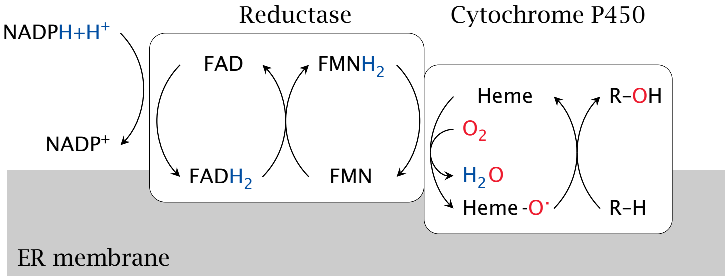
Phase I

- Cytochrome P450 enzymes
- Diaphorase (NADH:quinone oxidoreductase)

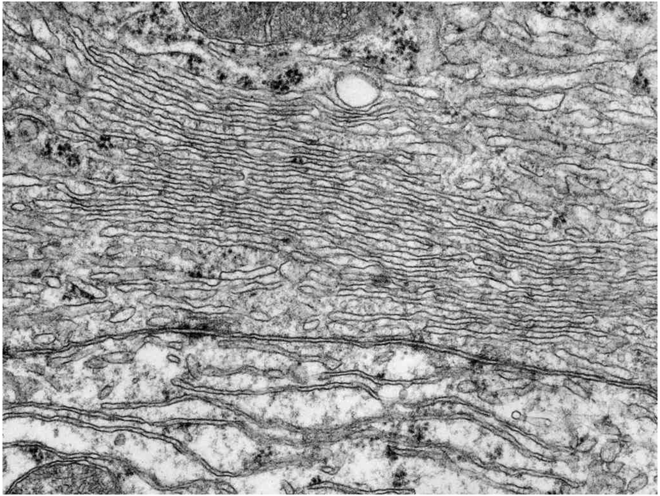
Phase II

- UDP-glucuronosyltransferases
- Sulfotransferases
- Glutathione-*S*-transferases
- *N*- and *O*-acetyltransferases

Mode of action of cytochrome P450 enzymes



Drug metabolism occurs to a large degree in the smooth endoplasmic reticulum



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Reactions catalyzed by cytochrome P450

Carbon oxidation

$$R-H \xrightarrow{[O]} R-OH$$

$$RCH_2-OH \xrightarrow{[O]} RCH{=}O + H_2O$$

$$RCH{=}O \xrightarrow{[O]} RCOOH$$

Heteroatom oxidation

$$R_2N-H \xrightarrow{[O]} R_2N-OH$$

$$R_3N \xrightarrow{[O]} R_3N \rightarrow O$$

$$R_2S \xrightarrow{[O]} R_2S{=}O$$

Dealkylation

$$RO-CH_2R \xrightarrow{[O]} ROH + O{=}CHR$$

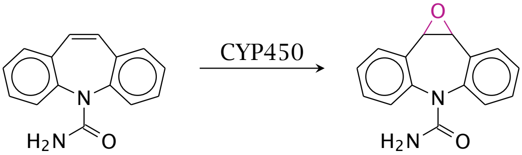
$$R_2N-CH_2R \xrightarrow{[O]} R_2NH + O{=}CHR$$

Epoxide formation

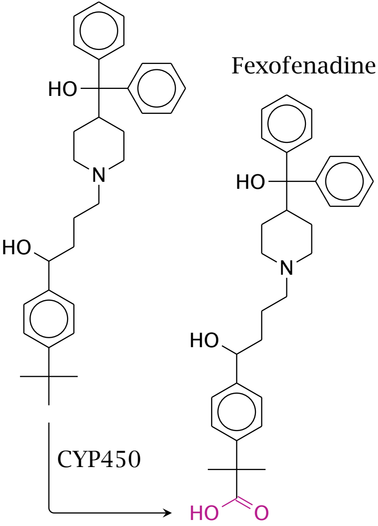
$$R-HC{=}CH-R \xrightarrow{[O]} R-HC\overset{\overset{\displaystyle O}{\diagup\;\diagdown}}{-}CH-R$$

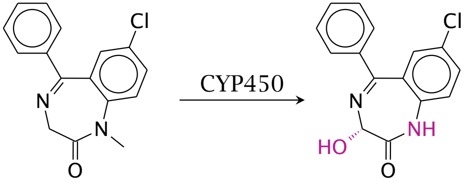
Formation of active metabolites by CYP450 enzymes

Carbamazepine → Carbamazepine-10,11-epoxide



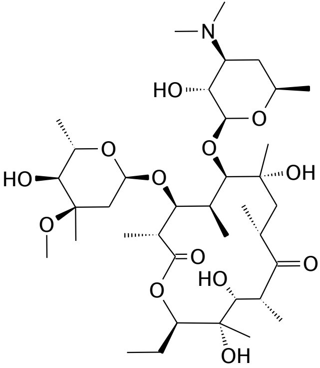
Terfenadine → Fexofenadine

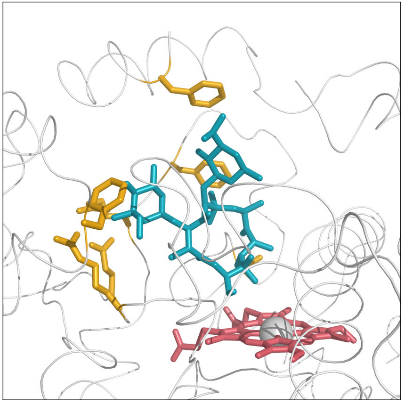




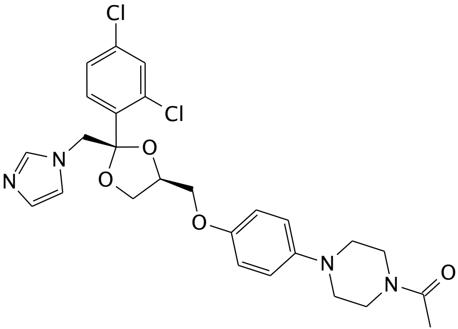
Diazepam → Oxazepam

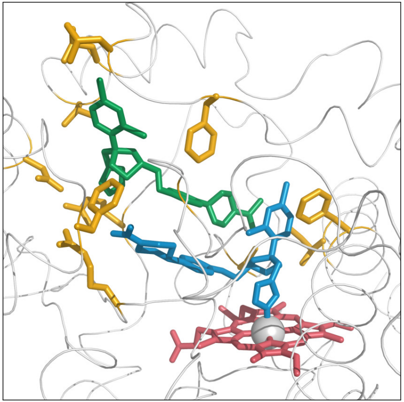
Erythromycin bound to the active site of cytochrome P450





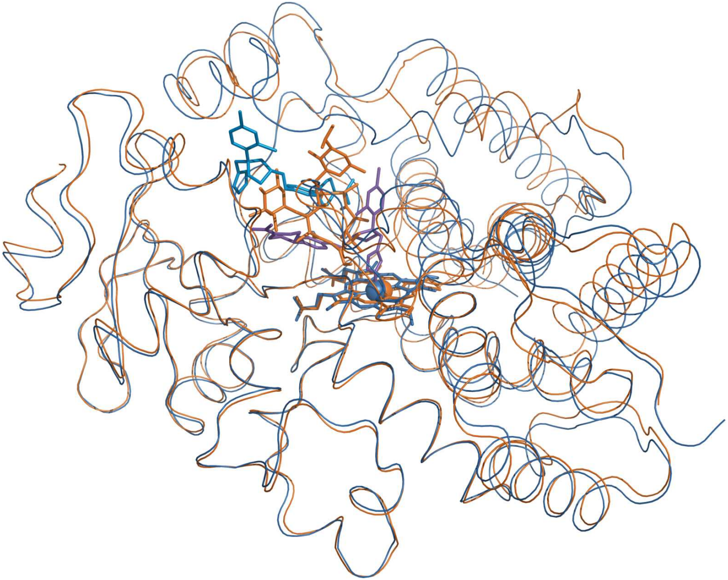
Ketoconazole bound to the active site of cytochrome P450



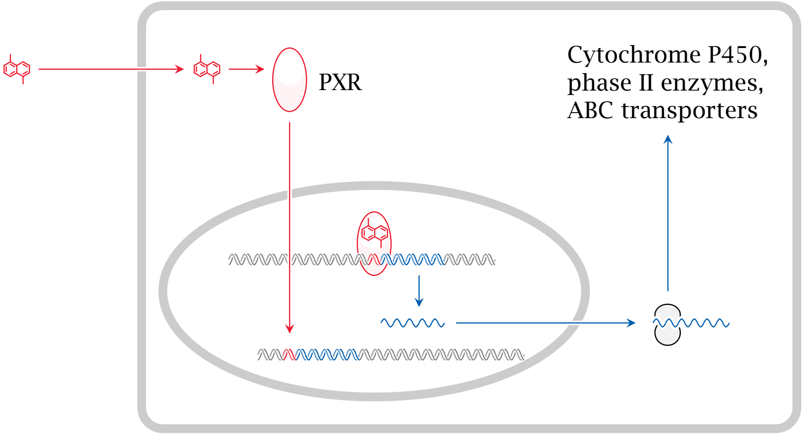


- active metabolites

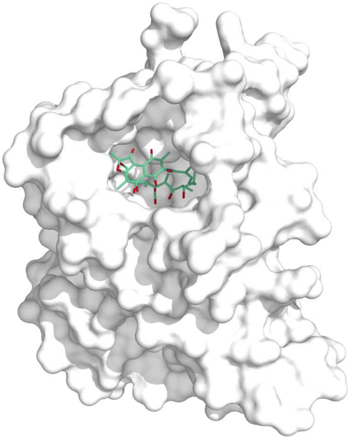
Superposition of the erythromycin- and the ketoconazole-bound CYP3A4 structures

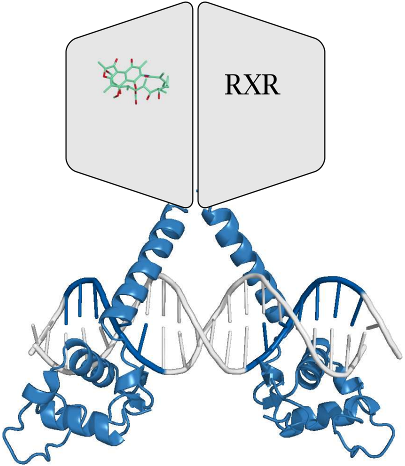


Transcriptional induction of drug metabolism

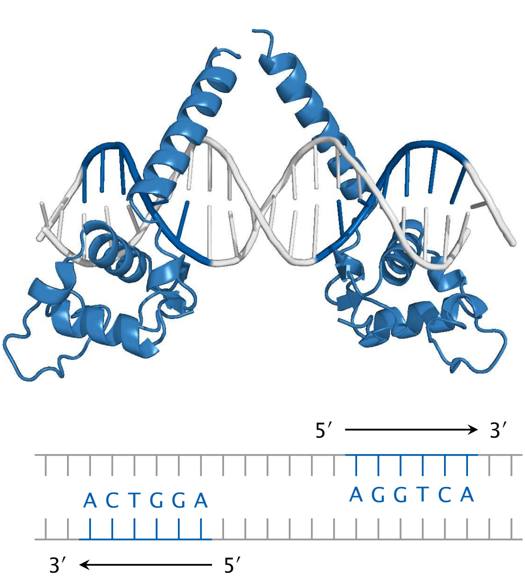


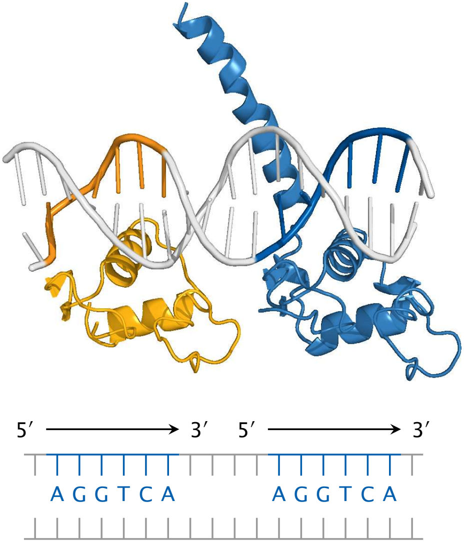
Transcriptional induction of cytochrome P450: Rifampicin bound to the pregnane X receptor





Interaction of nuclear hormone receptors with DNA

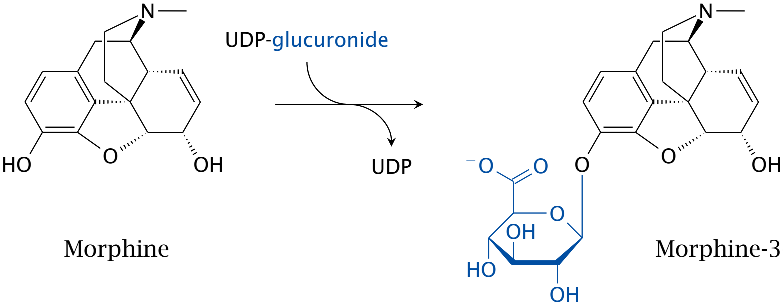




Conjugation reactions

Functional group	Enzyme	Cosubstrate
Glucuronic acid	UDP-glucuronosyl-transferases	UDP-glucuronide
Sulfate	Sulfotransferases	3′-Phosphoadenosine-5′-phosphosulfate (PAPS)
Glutathione	Glutathione-*S*-transferases / spontaneous	Free glutathione
Acetate	*N*-acetyltransferases	Acetyl-CoA
Methyl	*N*-, *S*-, and *O*-methyl-transferases	*S*-adenosylmethionine (SAM)
Amino acids	Amino acid transferases	Free amino acids/ATP

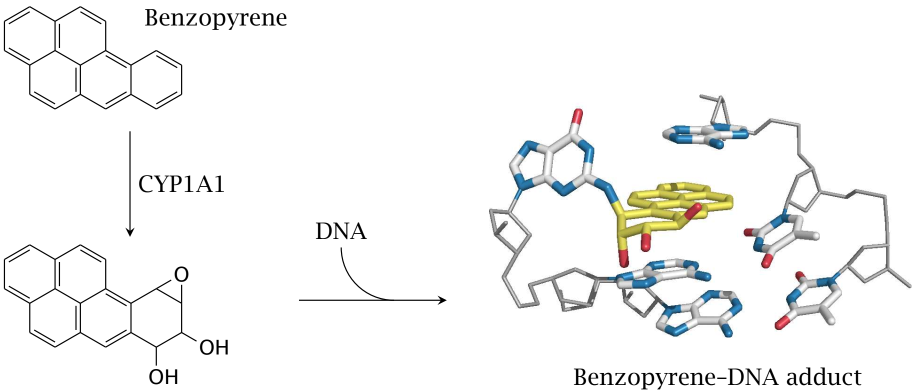
Morphine skips phase I and is conjugated directly



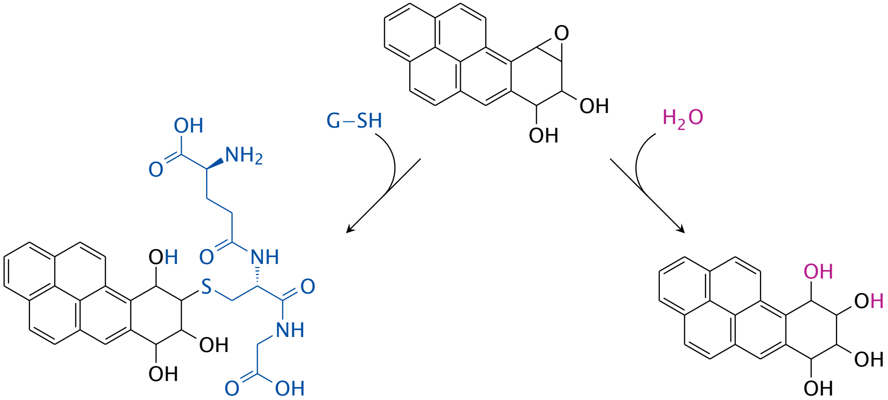
Morphine

Morphine-3-glucuronide

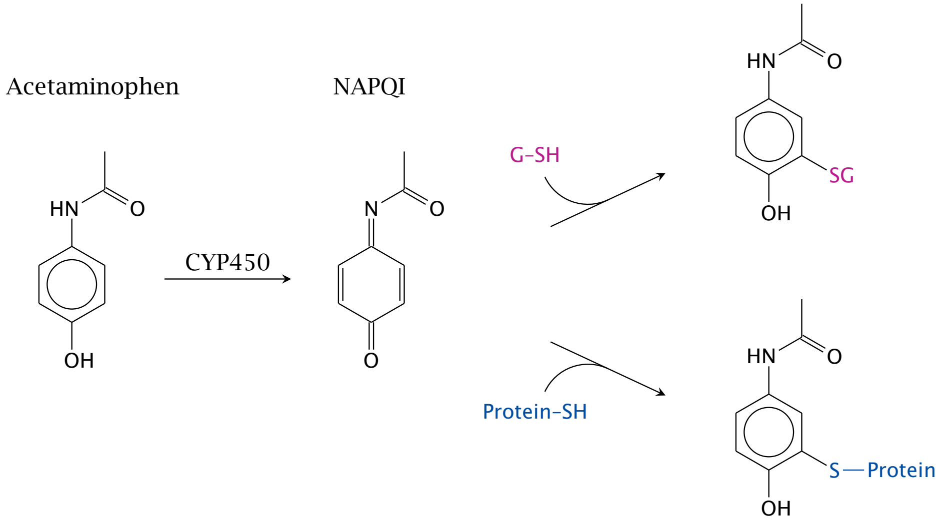
Epoxides of aromatic hydrocarbons can intercalate and covalently react with DNA



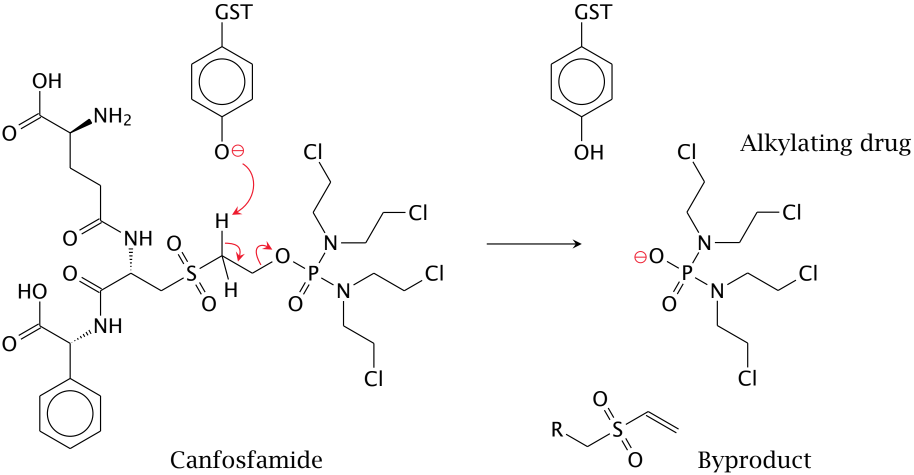
Enzymatic detoxification of benzopyrene epoxy-derivatives



Hepatic metabolism of acetaminophen

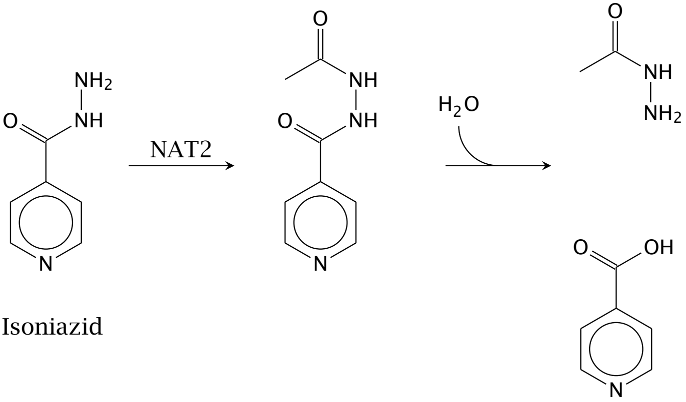


Activation of canfosfamide by glutathione-*S*-transferase



‣ alkylants

Acetylation of INH by N-acetyltransferase 2 (NAT 2)

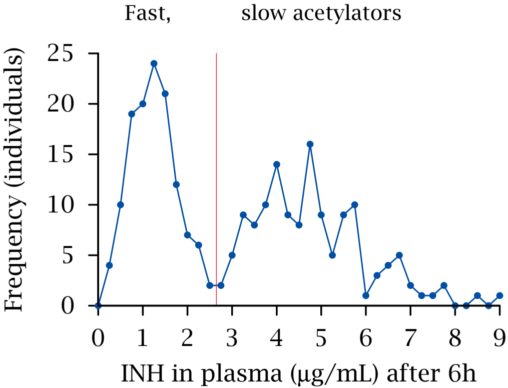


Isoniazid

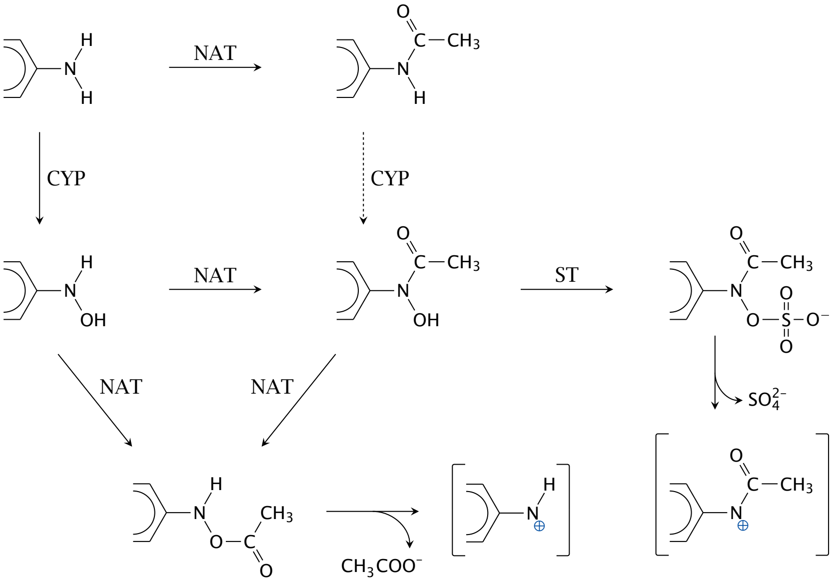
Acetylhydrazine

Isonicotinic acid

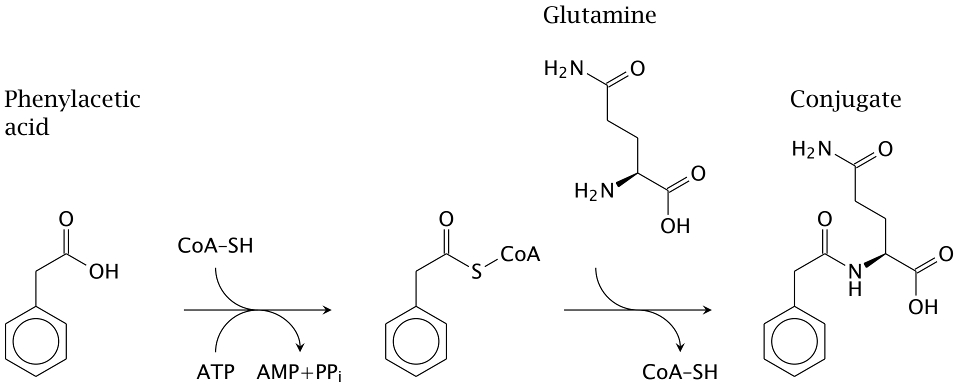
Bimodal distribution of INH acetylation speed



Metabolic activation of arylamine carcinogens



Glutamine conjugation of phenylacetate

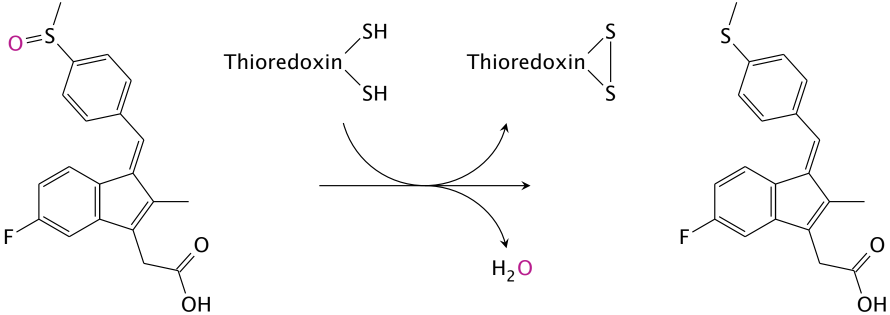


‣ urea cycle

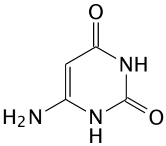
Reductive drug metabolism

- important functional groups in substrates: nitro, azo, sulfoxide, quinones
- diverse enzymology
- “incidental”—most enzymes that cause reductive drug metabolism primarily serve other roles in metabolism
- some reductive reactions can occur without enzyme catalysis

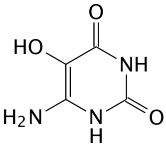
Reductive activation of sulindac by thioredoxin



Redox-active ingredients of *Vicia faba*

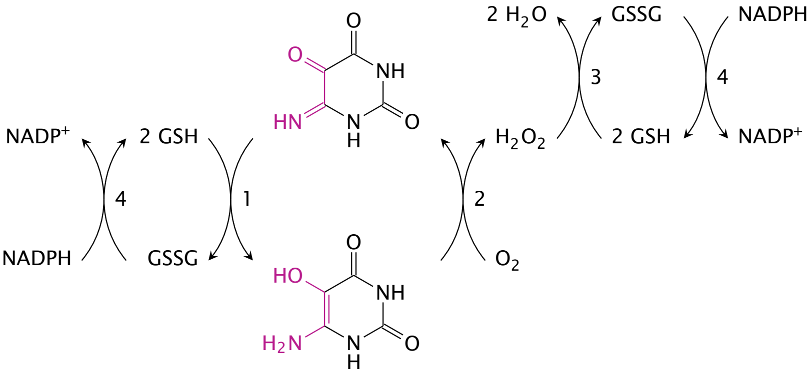


Divicine



Isouramil

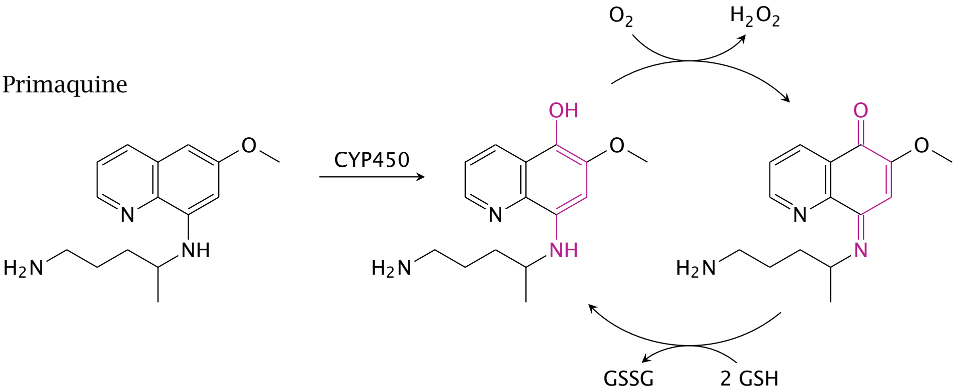
Redox cycling of isouramil



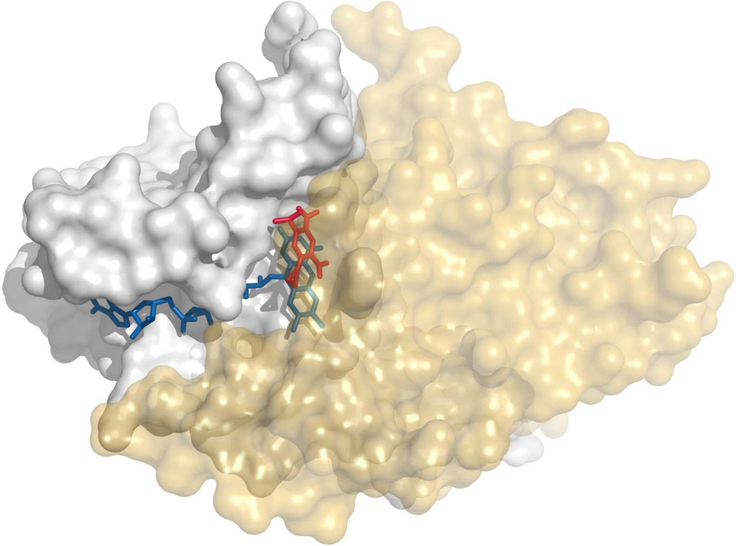
Glucose-6-phosphate dehydrogenase deficiency leads to favism

- Most patients are healthy most of the time—hemolytic crises triggered by drugs or food ingredients that cause redox cycling
- Manifest in red blood cells because these cells lack protein synthesis—no replacement of deficient enzyme molecules during the lifetime of the cell
- Affords partial protection against malaria—similar to sickle cell anemia and other hemoglobinopathias

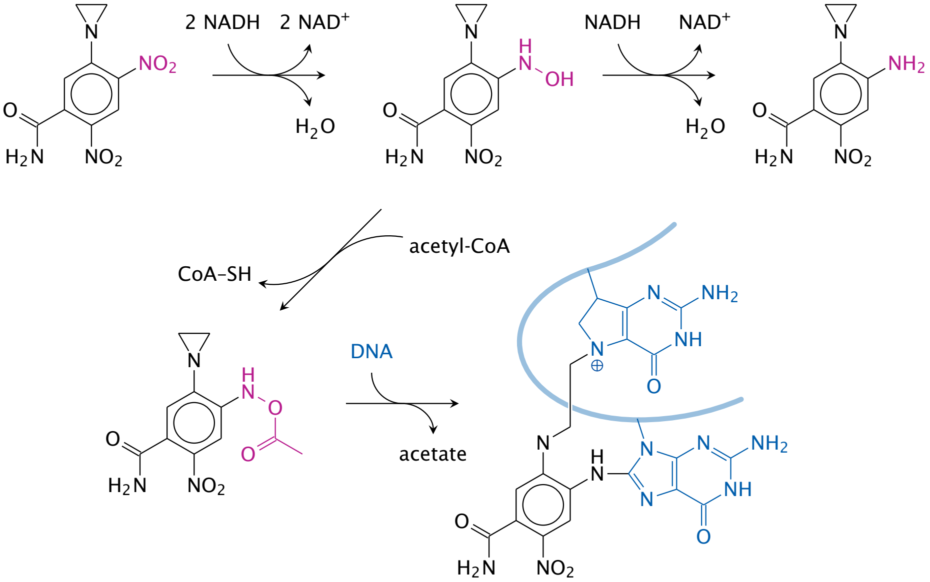
Redox cycling of 5-hydroxyprimaquine



The anticancer prodrug CB1954 bound to quinone reductase 2



Two-step activation of the anticancer prodrug CB1954



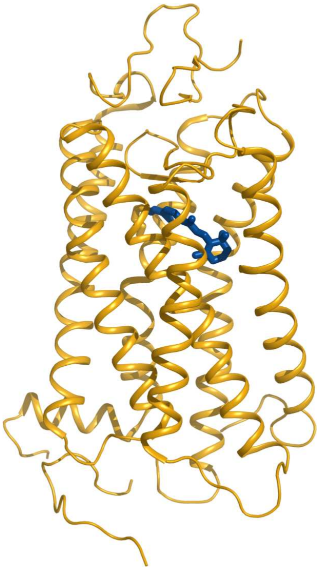
‣ Arylamine activation

G protein-coupled receptors

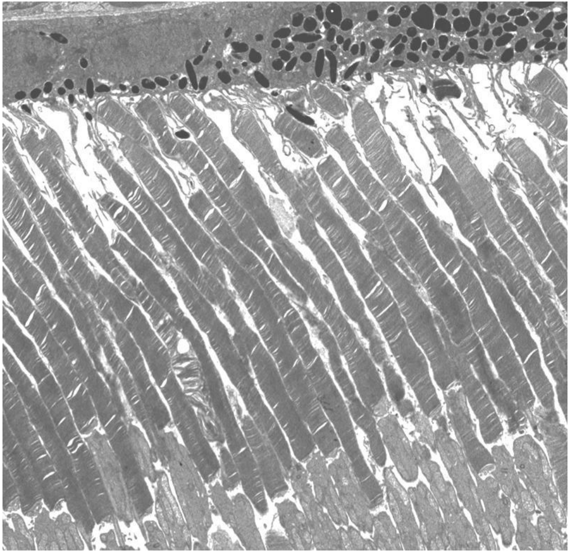
Drugs that act on G protein-coupled receptors: Some examples

Drug	Major receptor	Drug action	Clinical use
salbutamol	β_2-adrenergic	partial agonist	bronchodilation
fexofenadine	histamine H_1	inhibitor	antiallergic
atropine	muscarinic	inhibitor	pupil dilation
haloperidol	dopamine	inhibitor	antipsychotic
morphine	opioid	agonist	pain killer
losartan	angiotensin	inhibitor	antihypertensive
clopidogrel	adenosine	inhibitor	anticoagulation

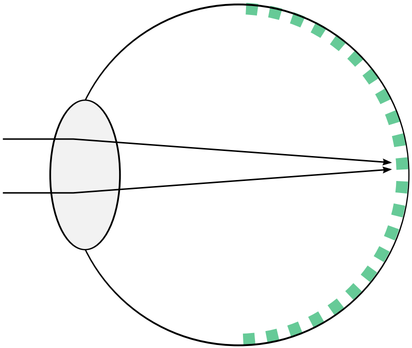
Rhodopsin as a model system of GPCR structure and function

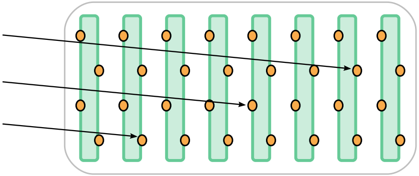


Membrane disks in the outer segments of retinal photoreceptors

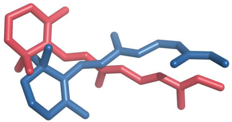


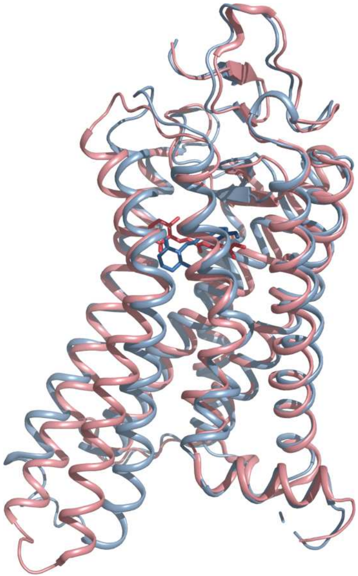
Light harvesting by stacked disks in photoreceptors



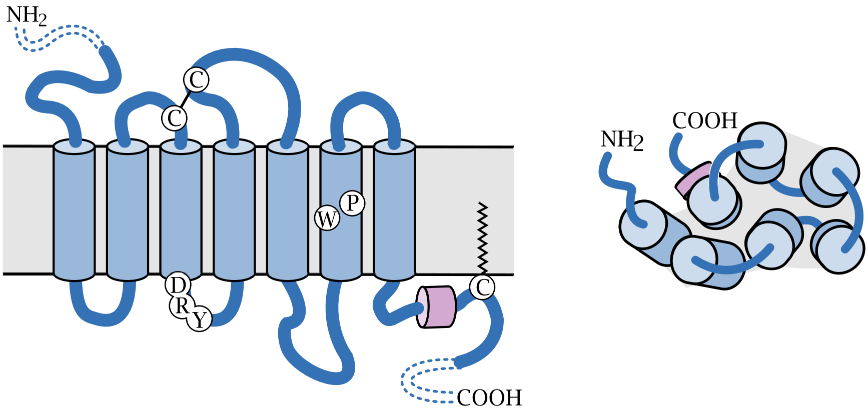


Rhodopsin in the ground state and the activated state

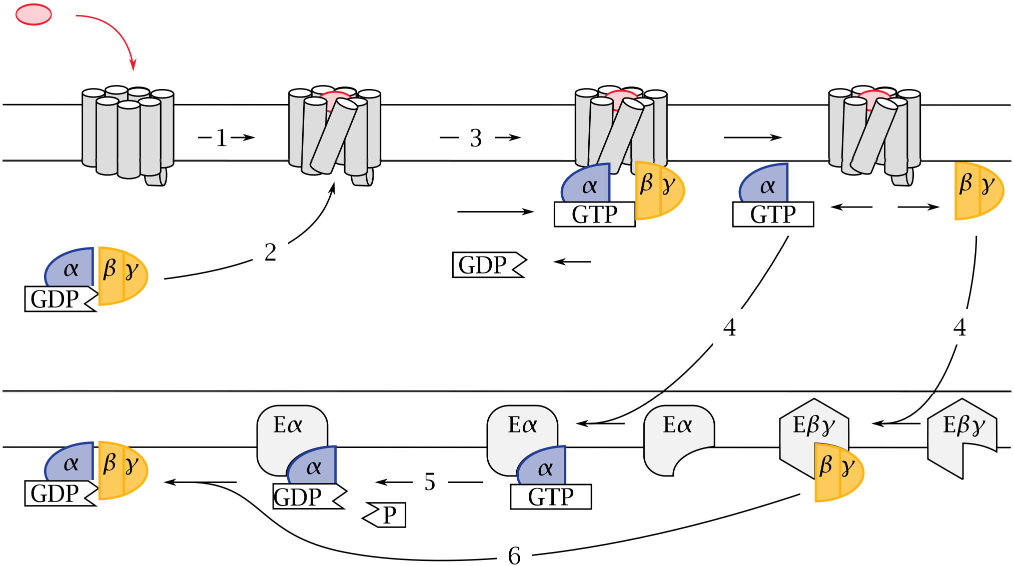




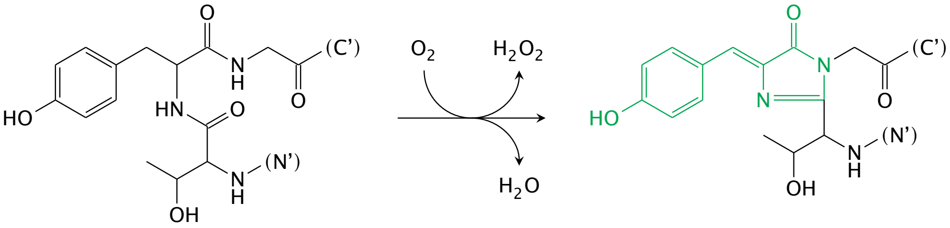
GPCR structure



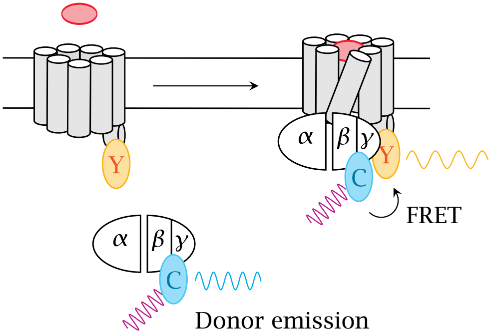
The G protein cycle

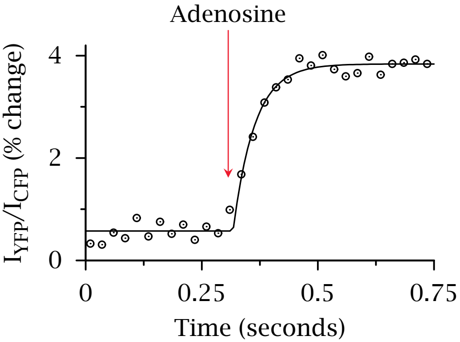


The fluorophore in green-fluorescent protein forms autocatalytically

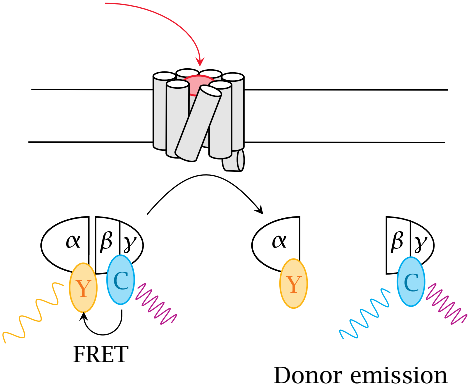


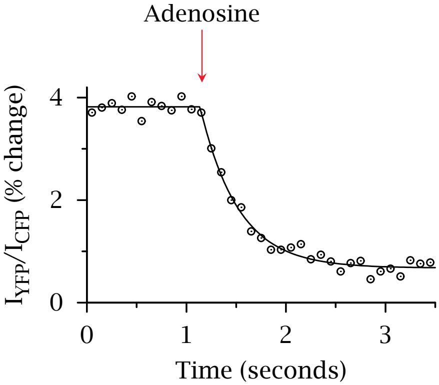
FRET detection of G protein binding to adenosine receptors



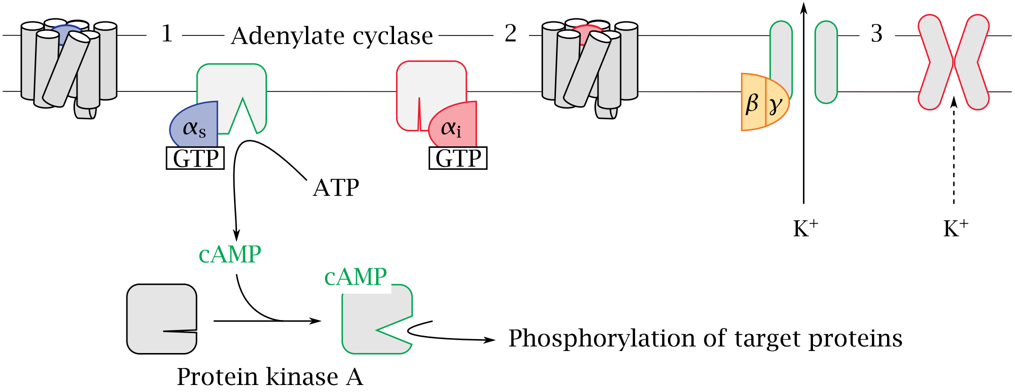


FRET detection of G protein dissociation

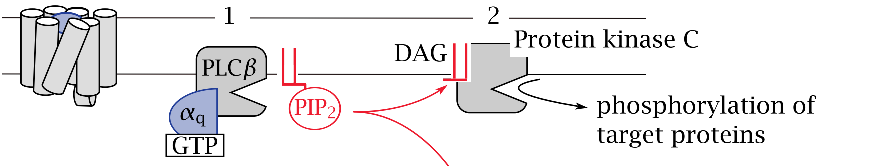


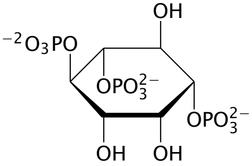


G protein effector mechanisms: adenylate cyclase

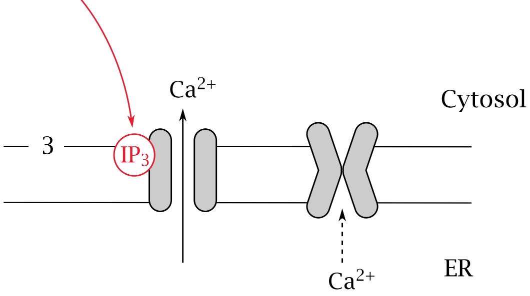


G protein effector mechanisms: the phospholipase C cascade





Inositol triphosphate (IP_3)

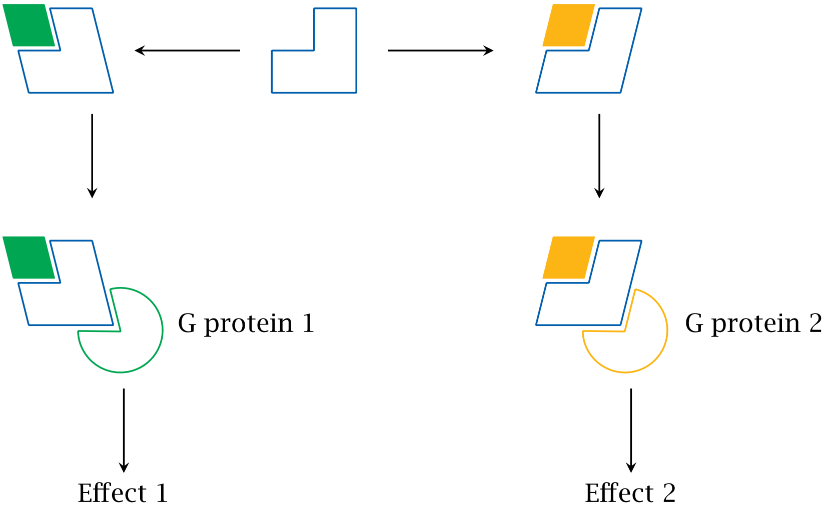


- angiotensin

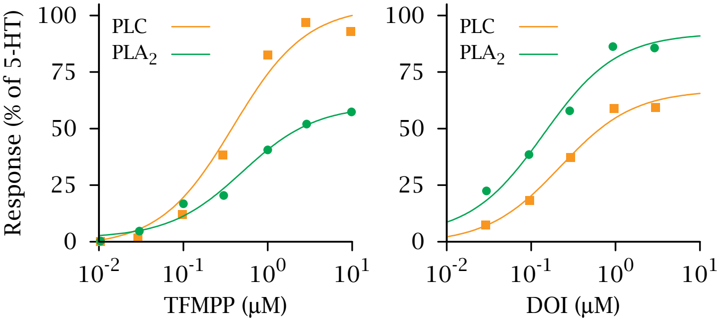
Summary of G protein effector mechanisms

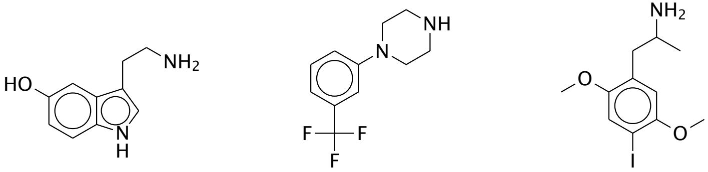
Class	Effectors and Effects	Some activating GPCRs
$G\alpha_s$	stimulation of adenylate cyclase (various types)	β-adrenergic, 5-HT$_4$, 5-HT$_6$, 5-HT$_7$, D$_1$, D$_5$; ACTH
$G\alpha_{i/o}$	inhibition of adenylate cyclase; activation of extracellular signal-regulated kinase (ERK)	α_2-adrenergic, 5-HT$_1$, D$_2$, D$_3$, D$_4$
$G\alpha_{q/11}$	stimulation of Phospholipase Cβ (various subtypes)	α-adrenergic, 5-HT$_2$, H$_1$, GABA$_B$
$G\alpha_{12/13}$	indirect activation of RhoA GTPase and of phospholipase A$_2$	5-HT$_4$, AT$_1$, protease- activated receptors

Agonist-specific coupling with GPCRs



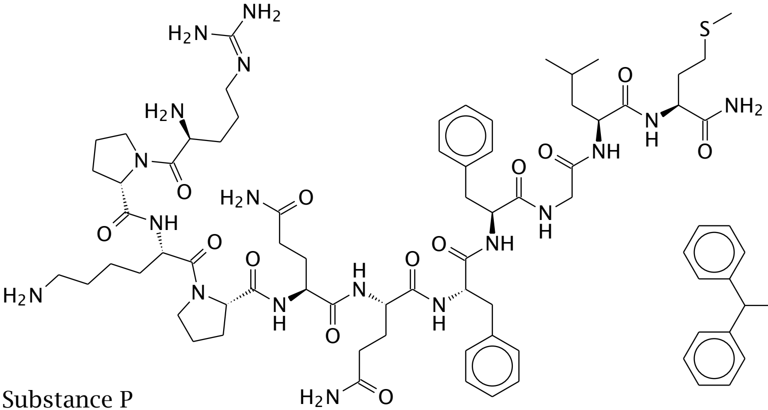
Agonist-specific coupling of 5-HT$_2$ receptors





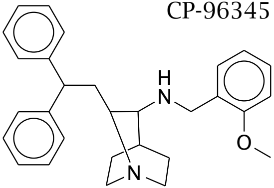
Serotonin (5-HT) TFMPP DOI

Substance P and its competitive antagonist CP-96345

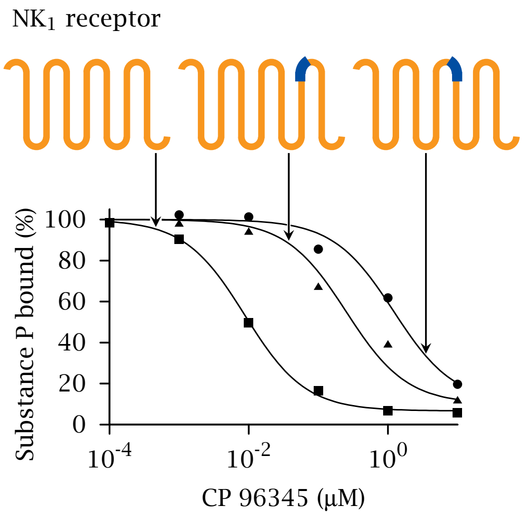


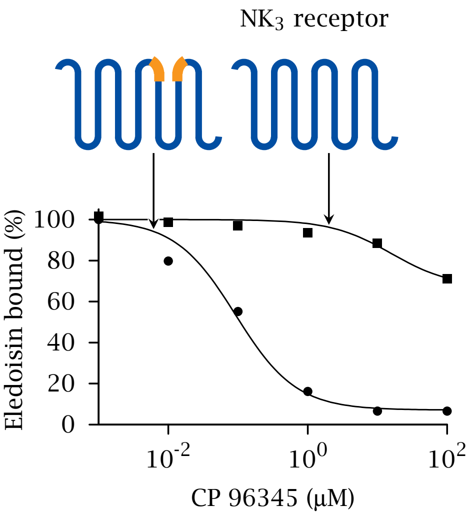
Substance P

CP-96345

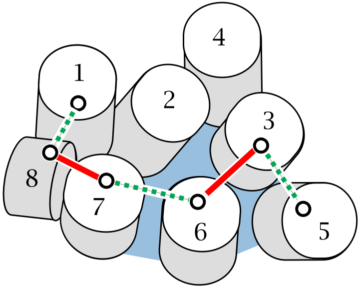


Using receptor chimeras to locate the ligand binding sites of NK receptors



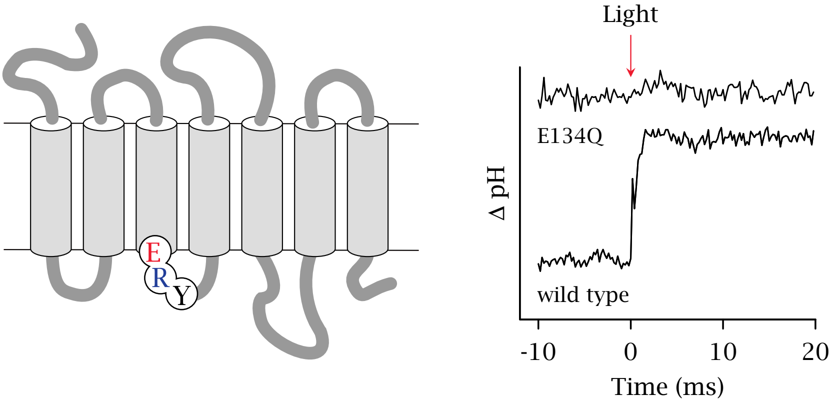


Engineered disulfide bonds pinpoint helix movements involved in GPCR function

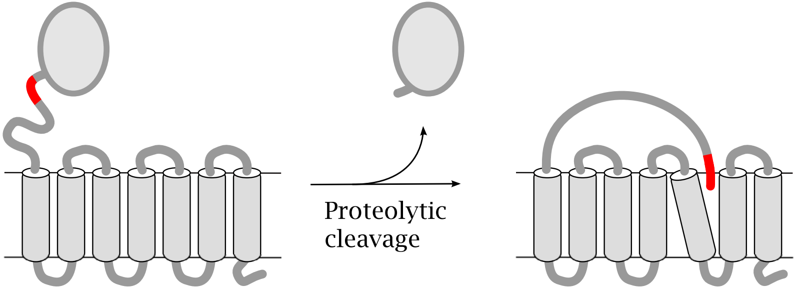


Rhodopsin conformations

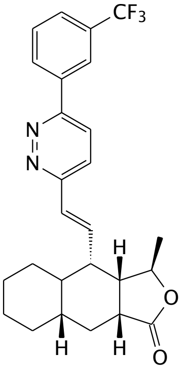
Protonation of residue E134 of rhodopsin in response to light stimulation

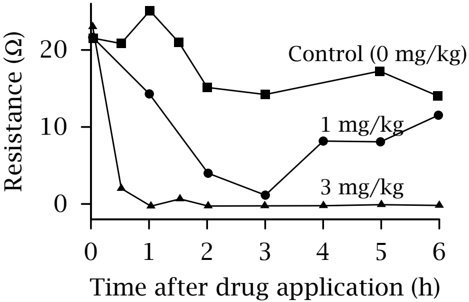


Protease-activated GPCRs



Pharmacological inhibition of protease-activated receptors



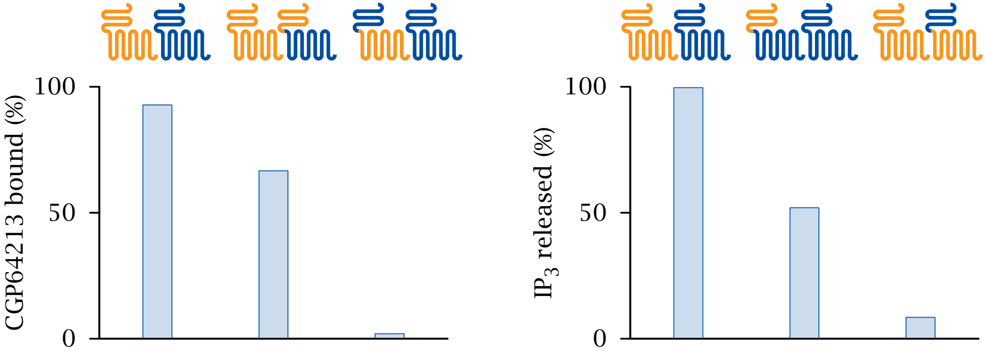


- blood coagulation

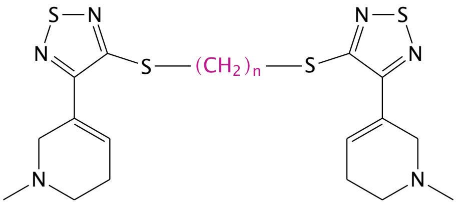
GPCR oligomerization

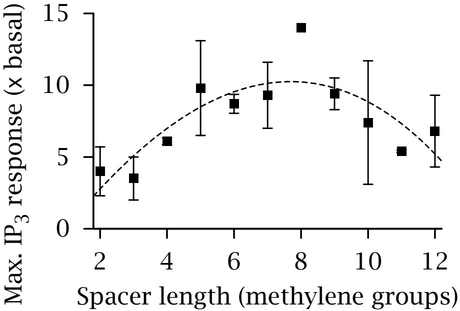
- Oligomers can comprise identical or different subunits
- Potential for cooperativity
- Potential for novel ligand specificity
- When receptors for antagonistic mediators form heterodimers, these mediators can “duke it out” already at the cell surface, reducing noise inside the cell

Functional specialization in GABA$_B$ receptor heterodimers

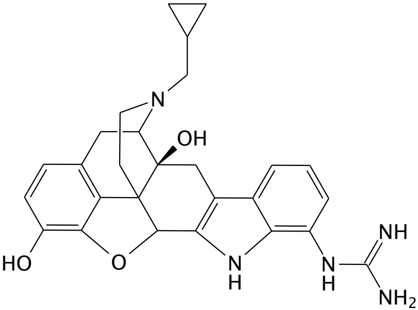


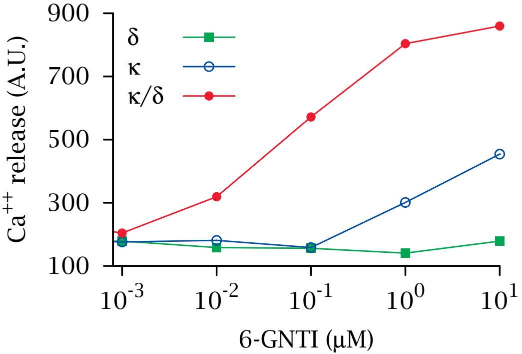
Bivalent agonists of muscarinic acetylcholine receptors



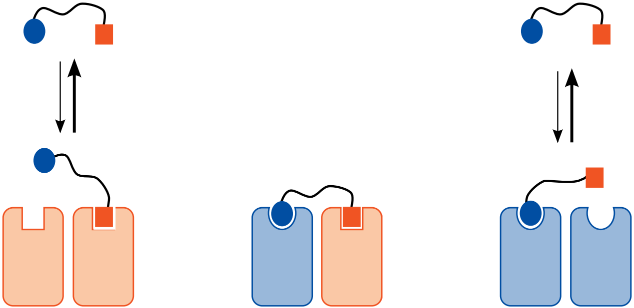


Novel receptor specificity: selective activation of $\kappa\delta$ opioid receptor hetero-oligomers by a monovalent ligand

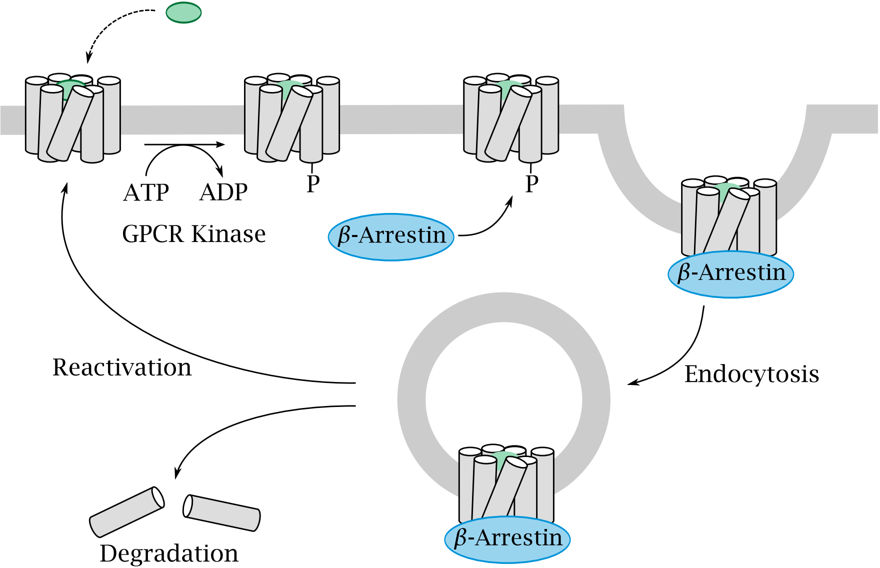




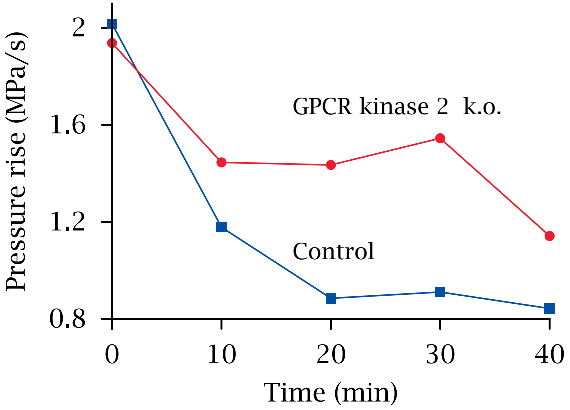
Could receptor heterodimers be targeted with heterodimeric drugs?



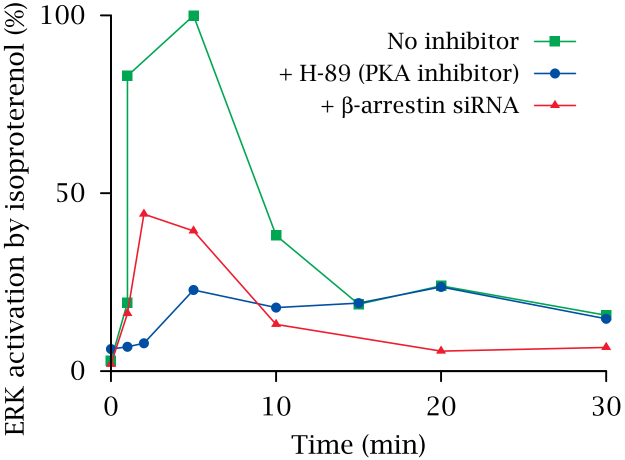
GPCR deactivation by phosphorylation and endocytosis



GPCR kinase 2 knockout attenuates tachyphylaxis of cardial β-receptors



Knock-down of arrestin may *reduce* GPCR-mediated signals



Pharmacology of cell excitation

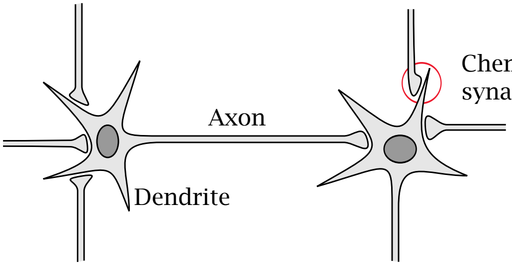
Clinical applications of drugs that influence excitable cell function

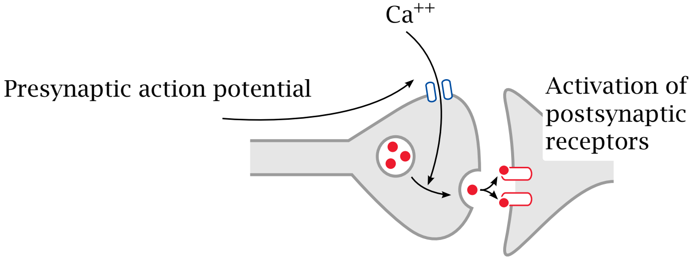
- blockade of nerve conduction for local anesthesia
- reduction of nerve cell excitability in the brain in epilepsy
- stabilization of mood in the treatment of bipolar disorder
- reduction of vascular smooth muscle tone to reduce blood pressure
- suppression of aberrant excitation in cardiac arrhythmia

The nature of cell excitation

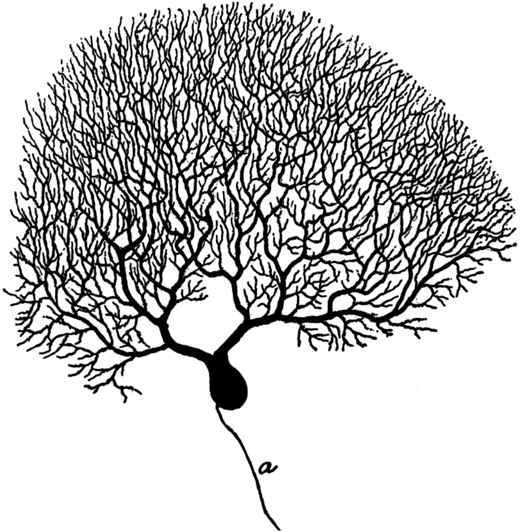
- all cells have an electrical potential across the cytoplasmic membrane, such that the cell interior is electrically negative relative to the outside ($\sim -70\,\mathrm{mV}$)
- in non-excitable cells, this membrane potential is stable; in excitable cells, it forms the *resting potential*
- cell excitation consists in transient reversals of the membrane potential, called *action potentials*, which spread rapidly across the entire cell membrane
- action potentials are spontaneously generated by some cells and transmitted between cells through chemical or electrical synapses

Neurons and synapses



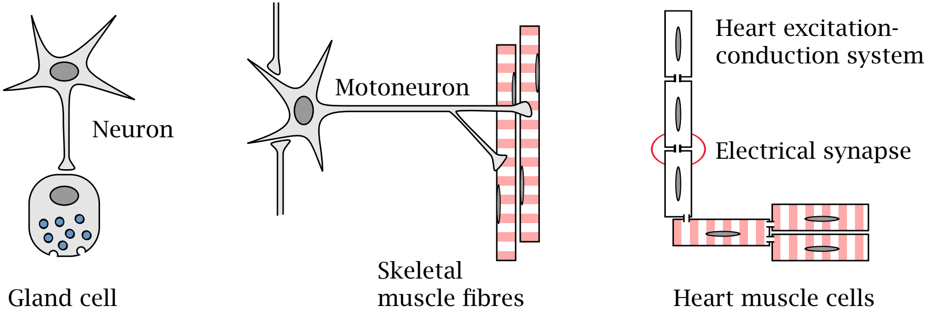


Some nerve cells have huge dendrites and axons

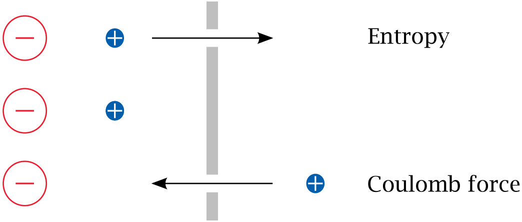


from Piersol, G.A., "Human Anatomy", Lippincott, 1908

Other types of excitable cells



The two driving forces that generate diffusion potentials across membranes

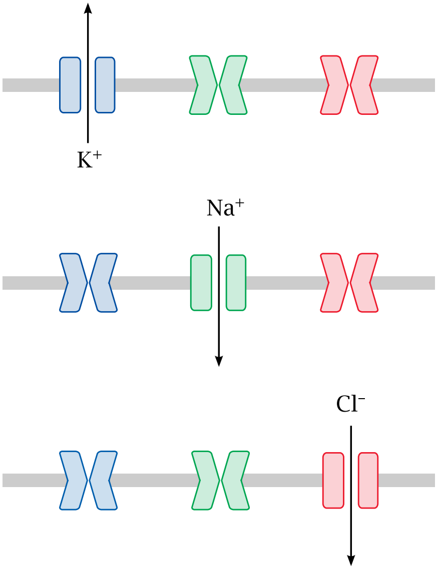


$$\Delta E = \frac{RT}{zF} \ln \frac{[\text{cation}]_{\text{left}}}{[\text{cation}]_{\text{right}}}$$

Equilibrium potentials for the major salt ions

Ion	Cytosolic	Extracellular	E_0 at 37°C
K^+	150 mM	6 mM	– 86 mV
Na^+	15 mM	150 mM	+ 62 mV
Ca^{++}	100 **n**M	1.2 mM	+ 126 mV
Cl^-	9 mM	150 mM	– 70 mV

Specific channels control ion permeabilities



Potassium channels open:
$P_K > P_{Na}, P_{Cl}$

Sodium channels open:
$P_{Na} > P_K, P_{Cl}$

Chloride channels open:
$P_{Cl} > P_K, P_{Na}$

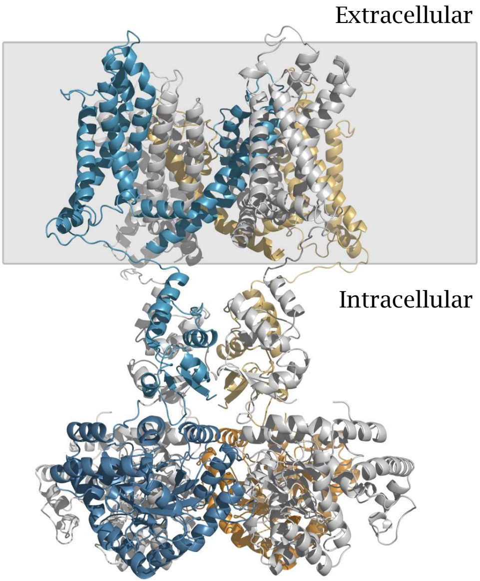
Diffusion potentials with multiple ions: the Goldman equation

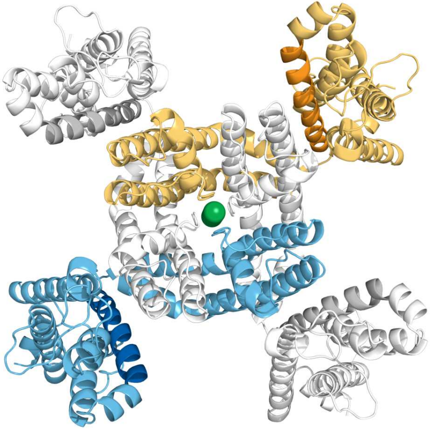
Permeabilities change as ion channels open and close

$$\Delta E = \frac{RT}{zF} \ln \frac{P_{\mathrm{K}}\,[\mathrm{K}^+]_{\text{outside}} + P_{\mathrm{Na}}\,[\mathrm{Na}^+]_{\text{outside}} + P_{\mathrm{Cl}}\,[\mathrm{Cl}^-]_{\text{inside}}}{P_{\mathrm{K}}\,[\mathrm{K}^+]_{\text{inside}} + P_{\mathrm{Na}}\,[\mathrm{Na}^+]_{\text{inside}} + P_{\mathrm{Cl}}\,[\mathrm{Cl}^-]_{\text{outside}}}$$

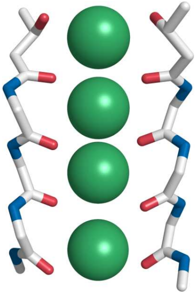
Concentrations do *not* change significantly

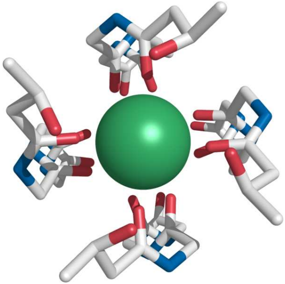
Structure of a voltage-gated K^+ channel



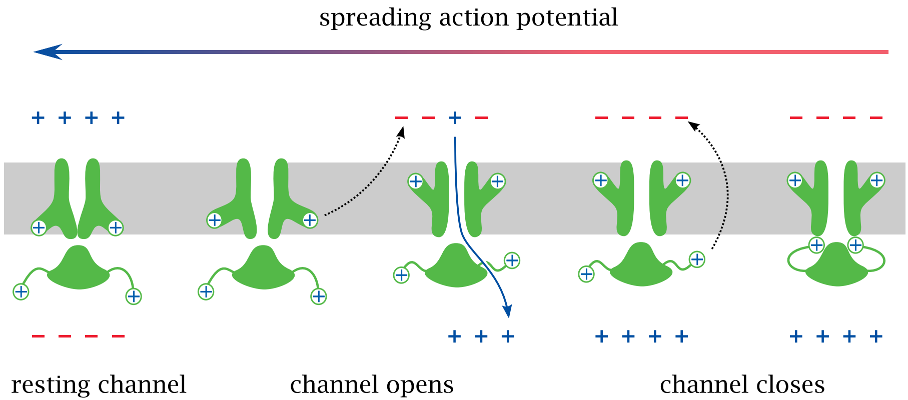


Structure of the K^+ selectivity filter

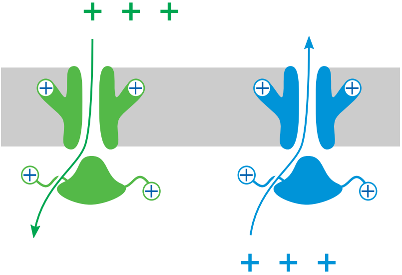


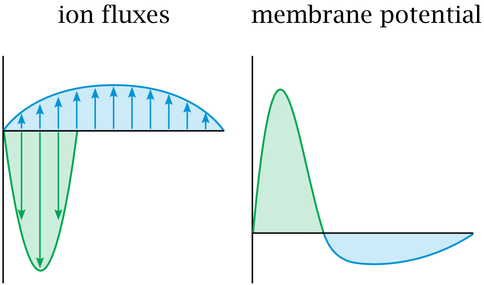


Voltage-gated sodium channels sustain and spread the action potential



Voltage-gated potassium channels extinguish the action potential



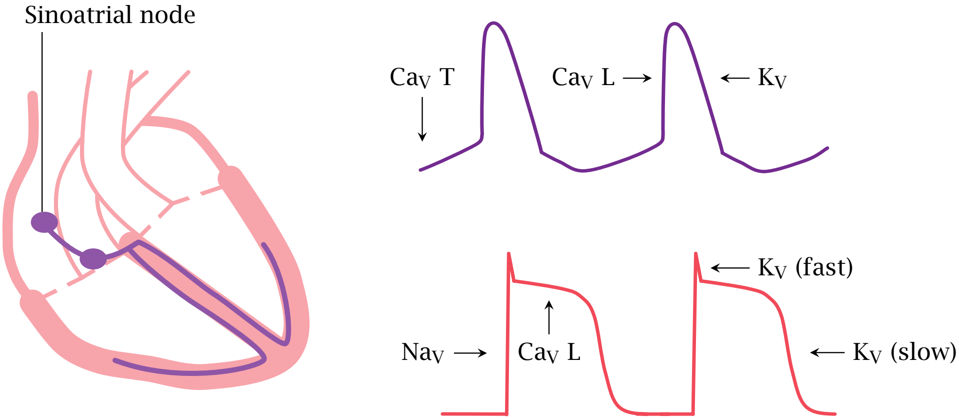


sodium channels
let sodium in

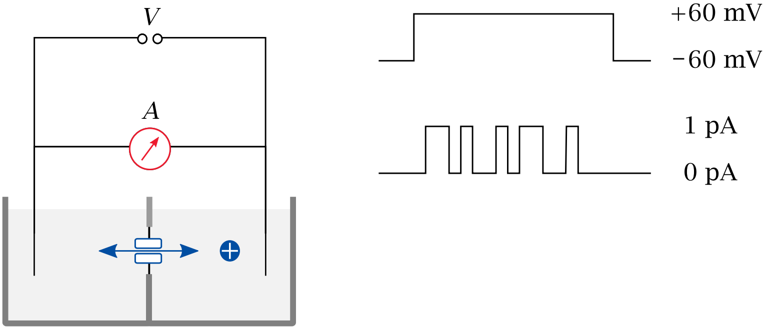
potassium channels
let potassium out

sodium channels respond fast,
potassium channels more slowly

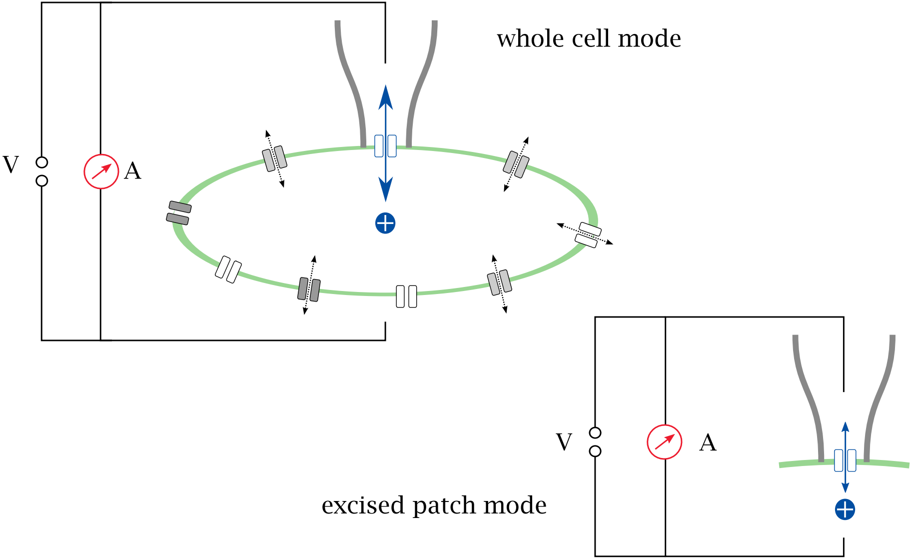
Voltage-gated channels and action potentials in the heart



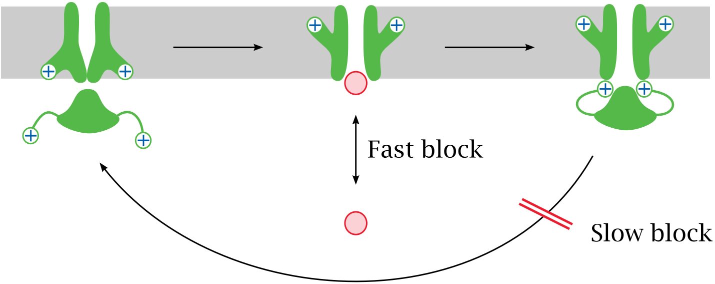
Measuring ion fluxes across single channels using planar lipid bilayers



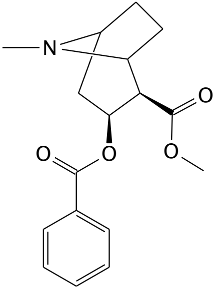
The patch clamp technique



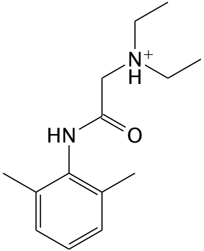
Fast and slow channel block



Diethylamine and phenol resemble parts of the lidocaine molecule

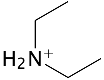


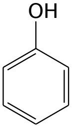
Cocaine



Lidocaine

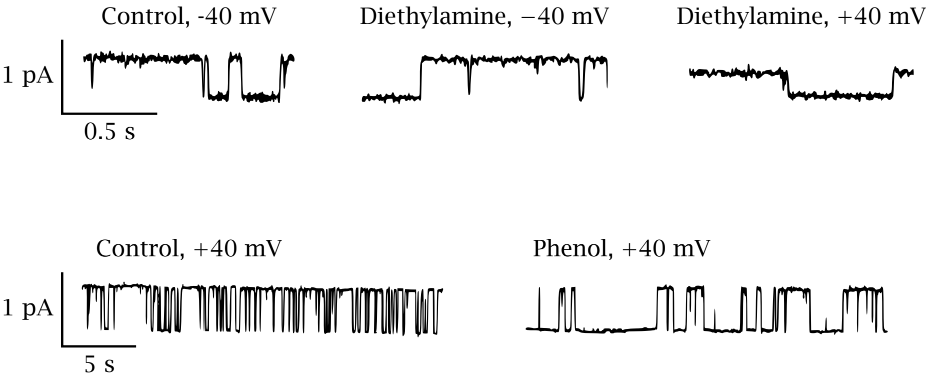
Diethylamine



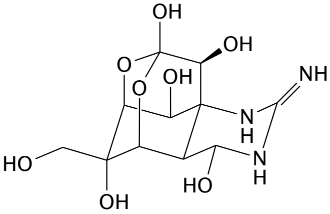


Phenol

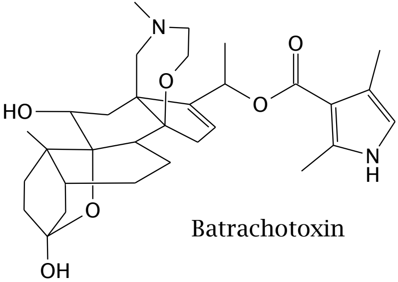
Effects of diethylamine and of phenol on Na_V channel conductance



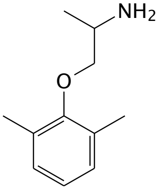
Drugs and poisons that act on Na_V channels



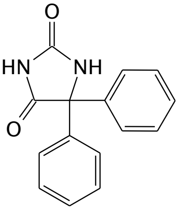
Tetrodotoxin



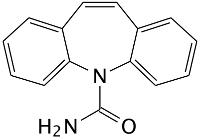
Batrachotoxin



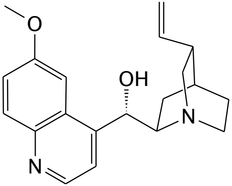
Mexiletine



Phenytoin

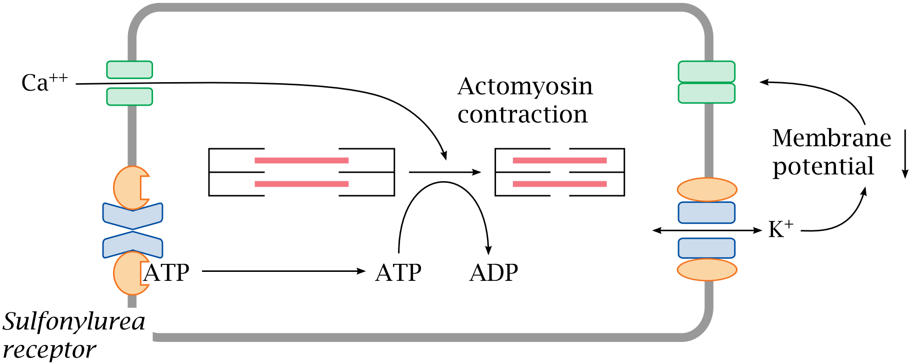


Carbamazepine

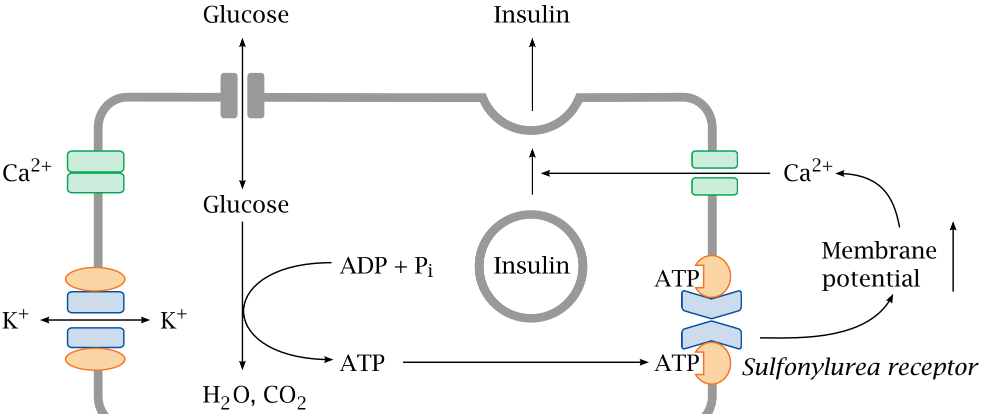


Quinidine

K_{ATP} channels regulate the tone of smooth muscle cells

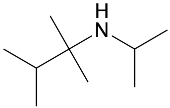


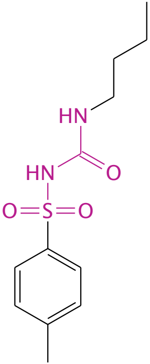
K_{ATP} channels in pancreatic β cells regulate insulin secretion



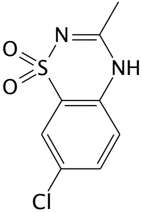
Drugs that act on K^+ channels

Iptakalim

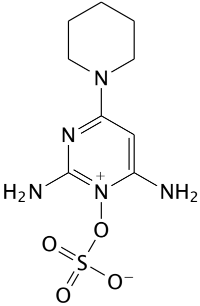




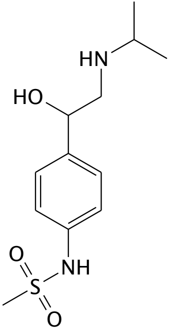
Tolbutamide



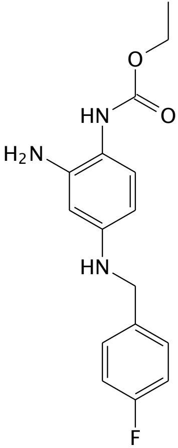
Diazoxide



Minoxidil sulfate

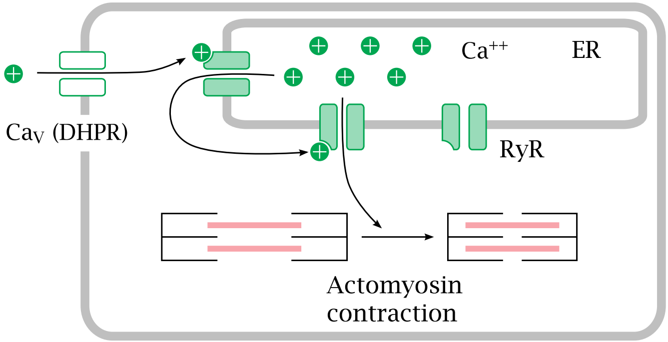


Sotalol



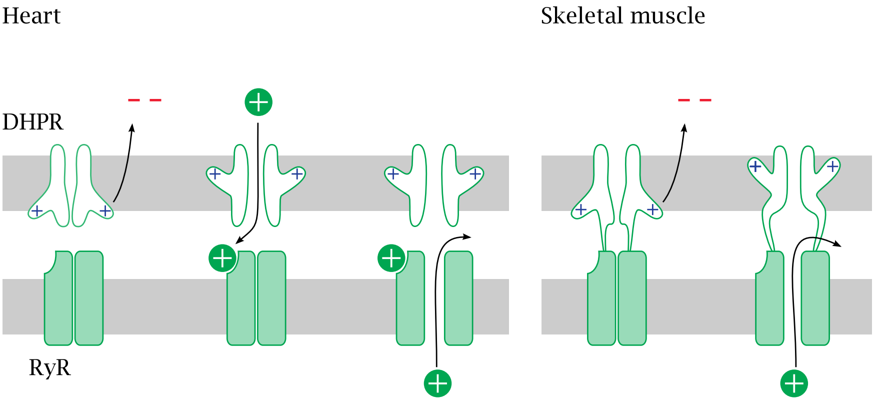
Retigabine

Two calcium channels control the contraction of striated muscle cells



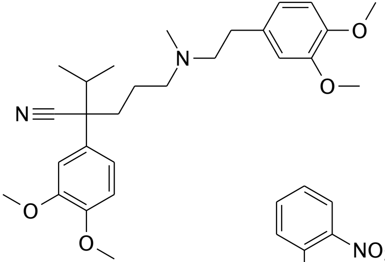
- ion channels in the heart

Entry of Ca^{++} through the DHPR is necessary in the heart, but not in skeletal muscle cells

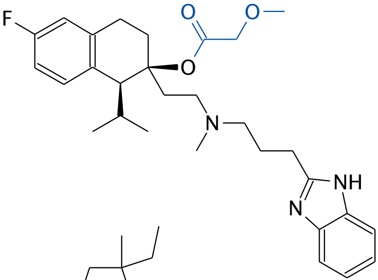


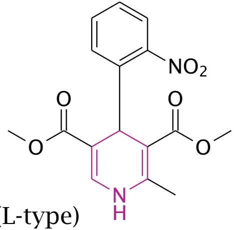
Inhibitors of voltage-gated calcium channels

Verapamil (L-type)

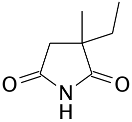


Mibefradil (L,T-type)





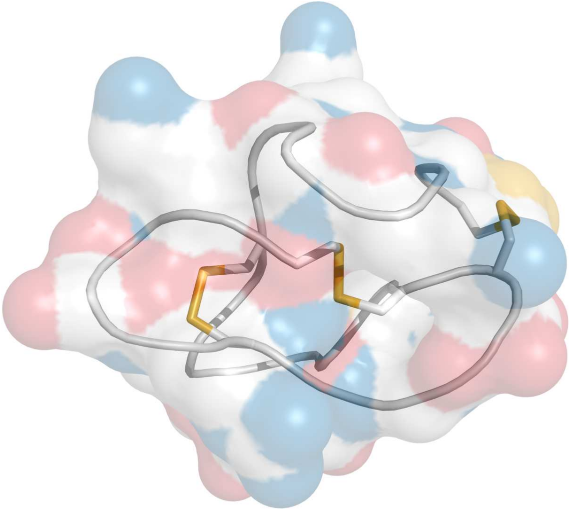
Nifedipine (L-type)



Ethosuximide (T-type)

- heart Ca channels

Structure of ω-conotoxin, an inhibitor of N-type Ca_V channels

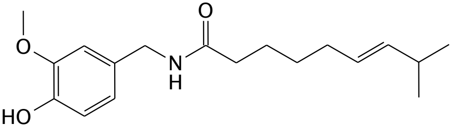


‣ synapse sketch

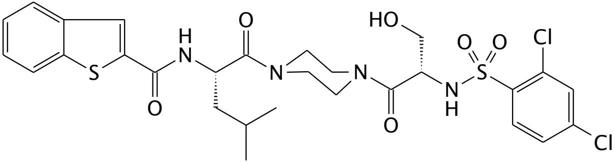
Transient receptor potential channels

- activated by physical stimuli such as heat and mechanical tension
- conduct multiple cations (in hydrated form)
- function in various modes of sensory perception
- may be activated by ligands also

Agonists of transient receptor potential (TRP) channels

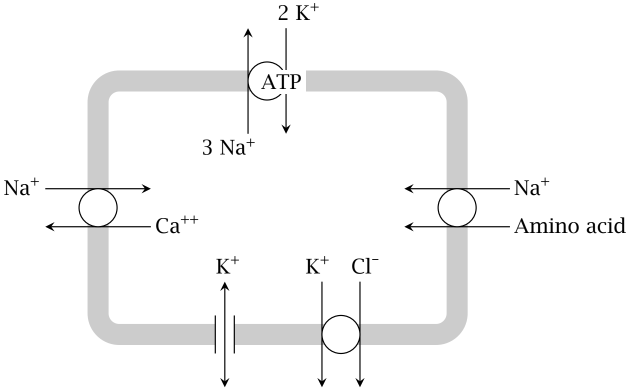


Capsaicin

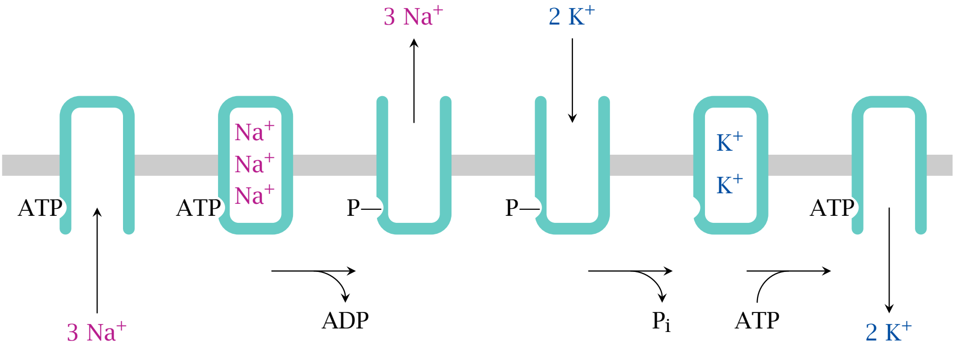


GSK1016790A

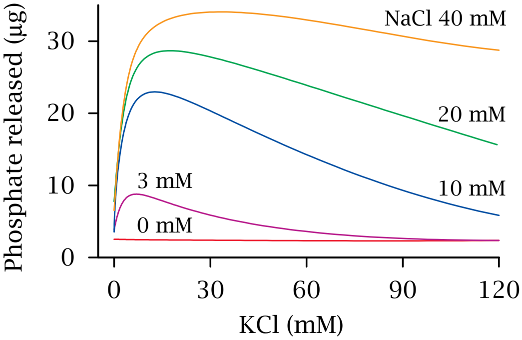
Na^+/K^+-ATPase maintains the ion gradients at the plasma membrane



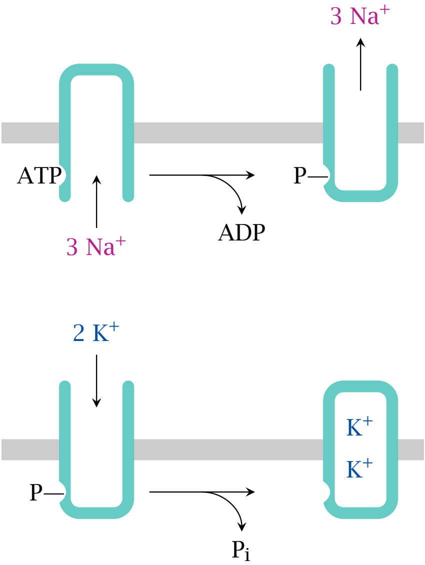
Functional cycle of Na^+/K^+-ATPase

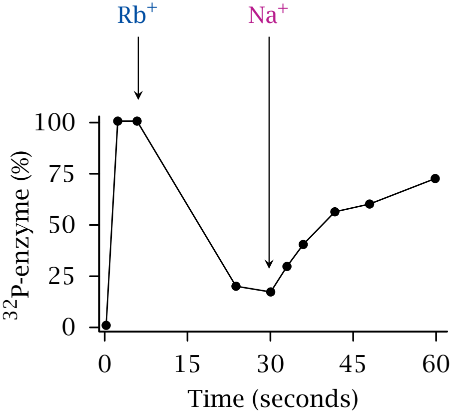


Na^+/K^+-ATPase activity as a function of KCl and NaCl concentrations

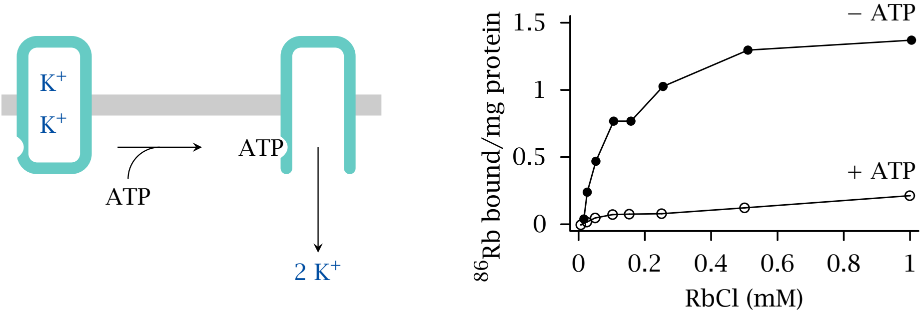


Effects of Rb^+ and of Na^+ on the phosphorylation state of Na^+/K^+-ATPase



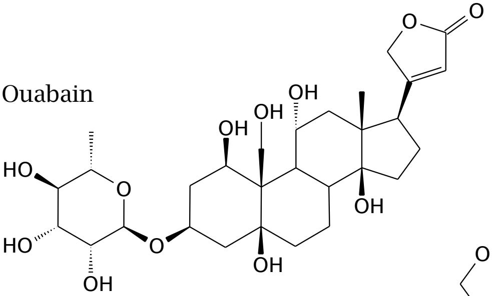


ATP is required to release Rb^+ from tight binding to Na^+/K^+-ATPase

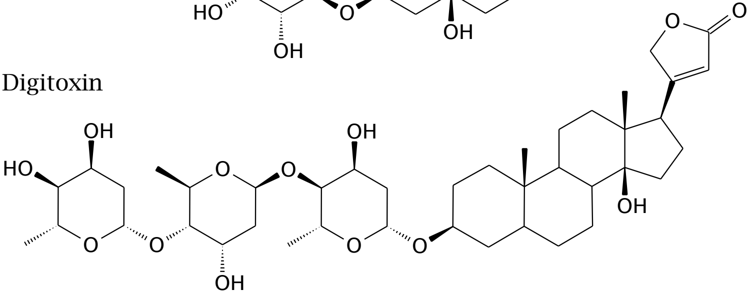


Structures of the Na^+/K^+-ATPase inhibitors ouabain and digitoxin

Ouabain

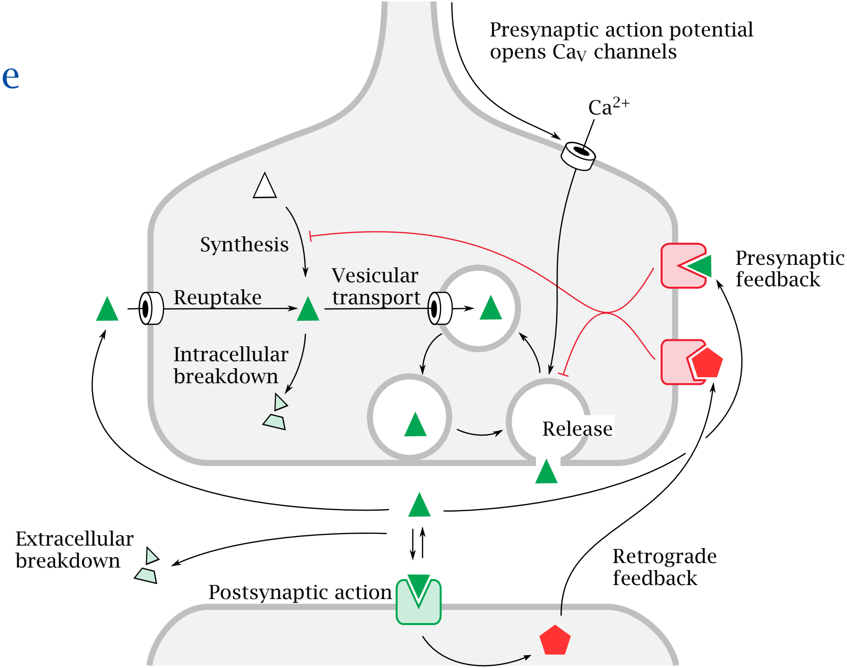


Digitoxin

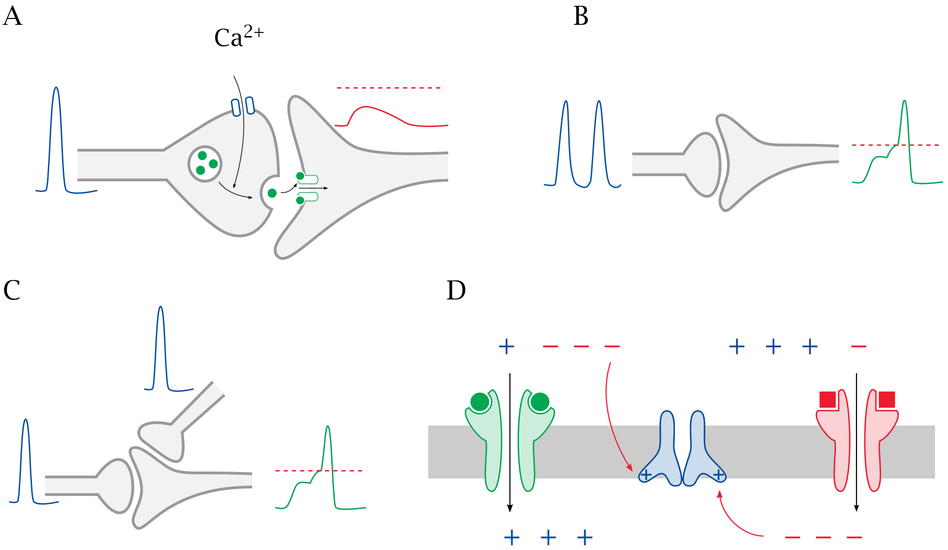


▸ NaKATPase function ▸ drug toxicity

Function of a chemical synapse



Summation of postsynaptic potentials

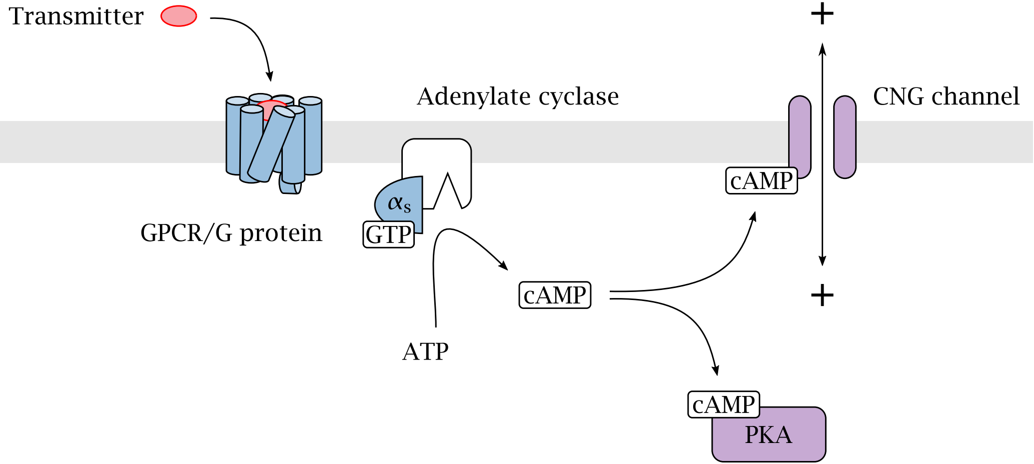


‣ Purkinje cell

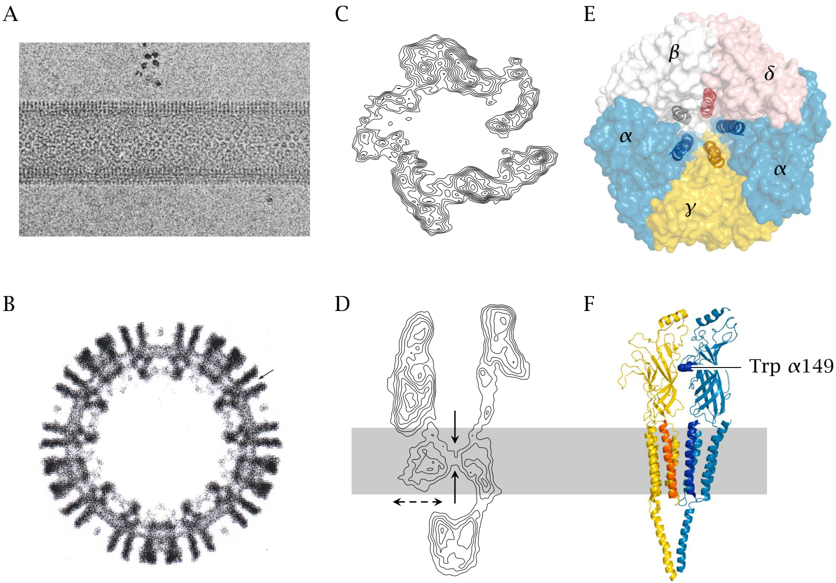
Neurotransmitter receptor families

1. Ligand-gated channels
   a) Cys-loop family
   b) Glutamate receptors
   c) Purine P2X receptors
2. G protein-coupled receptors

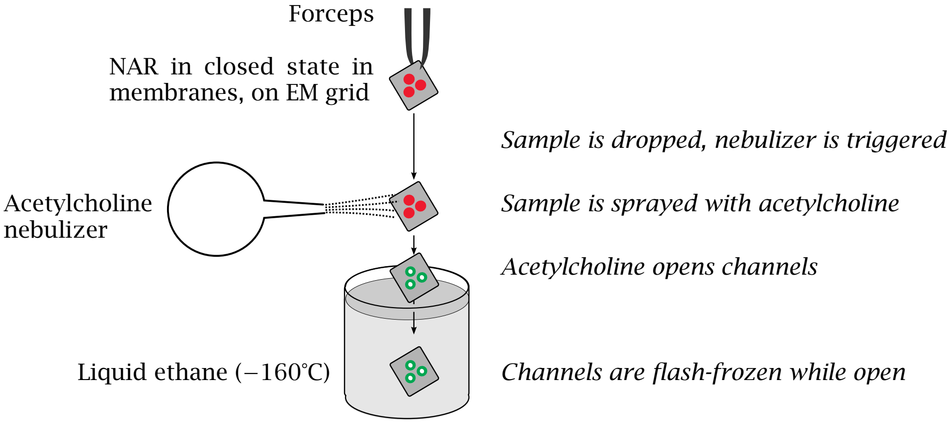
Postsynaptic GPCRs can signal through cyclic nucleotide-gated (CNG) channels



Structure of the nicotinic acetylcholine receptor



Trapping the nicotinic acetylcholine receptor in the open state

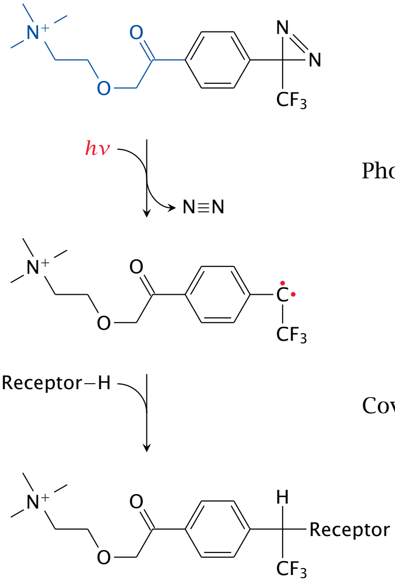


Photoaffinity labeling of the acetylcholine binding site

Photactivatable acetylcholine analog (TDBzcholine)

Carbene radical

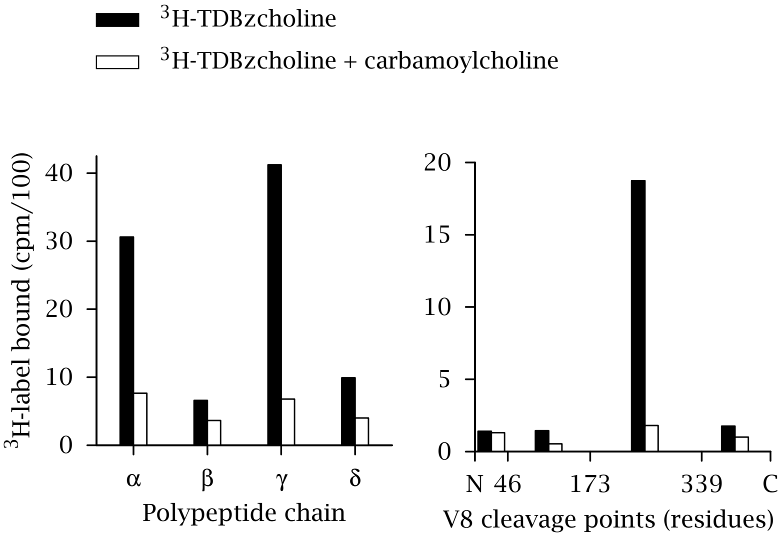
Receptor adduct



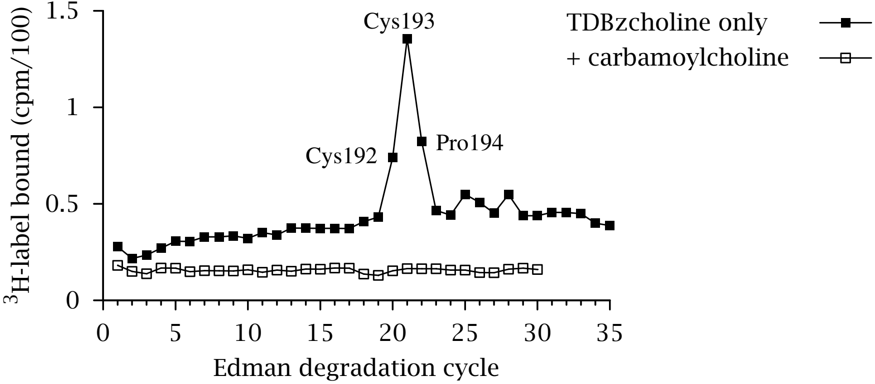
Photoactivation

Covalent reaction

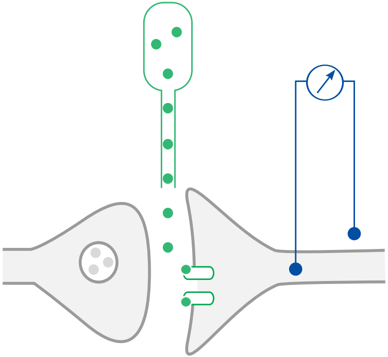
Isolation of affinity-labeled polypeptide chains and fragments



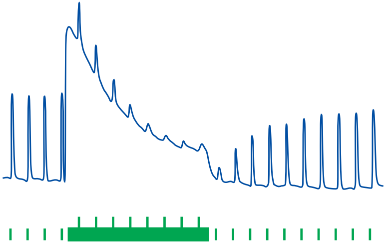
Identification of affinity-labeled amino acid residues



Desensitization of the nicotinic acetylcholine receptor

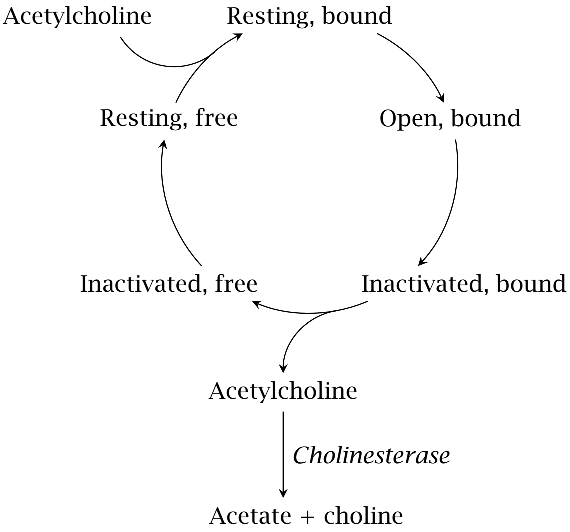


Resulting postsynaptic potentials



Pulsed or continuous application of acetylcholine

Functional states of the nicotinic acetylcholine receptor

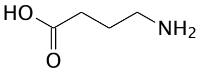


Ionotropic receptors in the cys-loop family

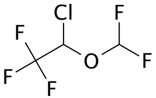
Receptor	Ion selectivity	Effect	Comments
nicotinic acetyl-choline	cations	excitatory	pharmacologically distinct subtypes, various applications
5-HT_3 serotonin	cations	excitatory	inhibitors are used to treat emesis
$GABA_A$	chloride	inhibitory	major drug target in narcosis, epilepsy, psychoses
glycine	chloride	inhibitory	regulates motor activity

▸ postsynaptic potentials

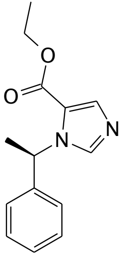
Drugs that interact with GABA receptors and transporters



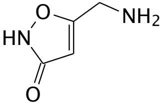
GABA



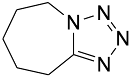
Isoflurane



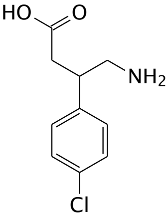
Etomidate



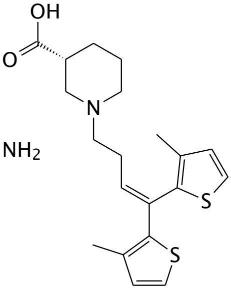
Muscimol



Pentylenetetrazole



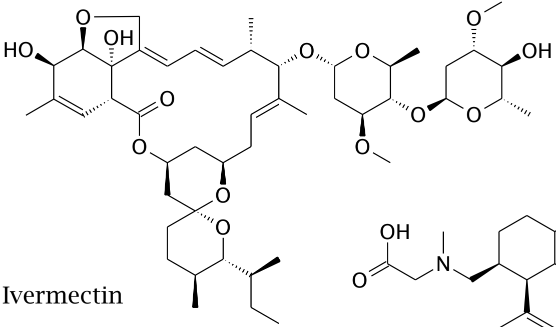
Baclofen



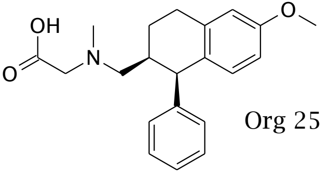
Tiagabine

▸ phenobarbital ▸ diazepam

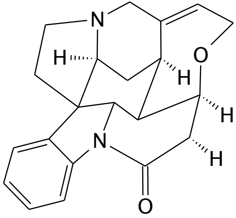
Drugs that interact with glycine receptors and transporters



Ivermectin

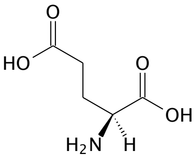


Org 25935

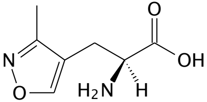


Strychnine

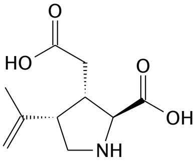
Chemical structures of subtype-selective glutamate receptor ligands



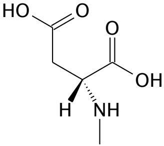
L-Glutamate



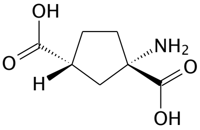
AMPA



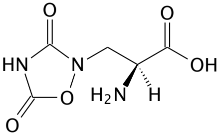
Kainate



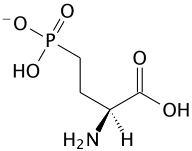
NMDA



ACPD



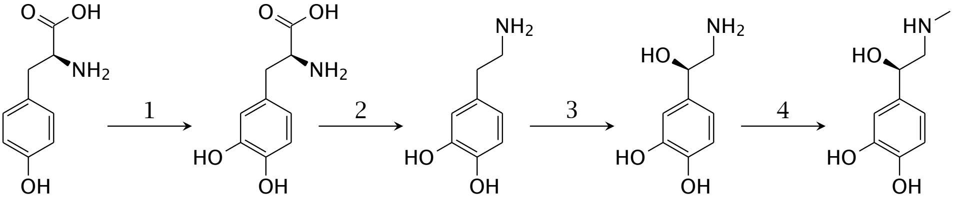
Quisqualate



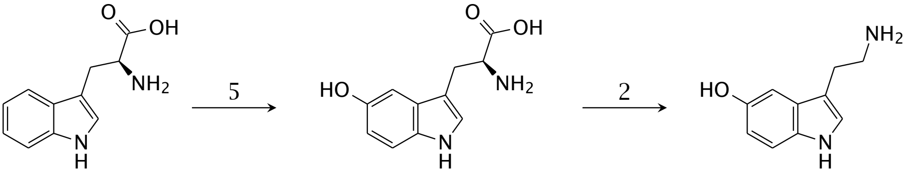
L-AP4

▸ Receptor families

Biosynthesis of the catecholamines and of serotonin



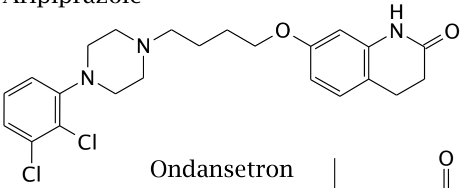
L-tyrosine L-DOPA Dopamine Norepinephrine Epinephrine



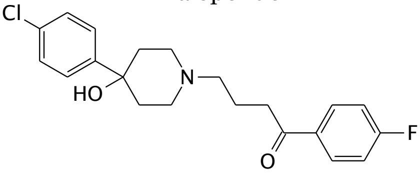
Tryptophan 5-Hydroxytryptophan Serotonin

Drugs that interact with dopaminergic and serotoninergic synapses

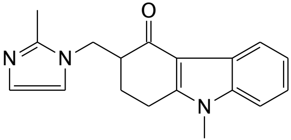
Aripiprazole

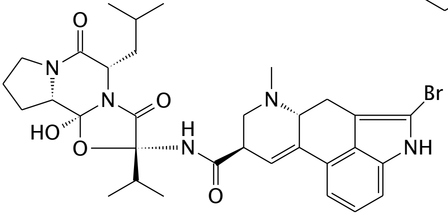


Haloperidol

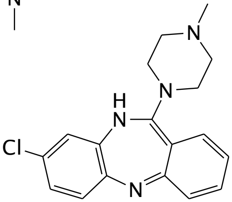


Ondansetron

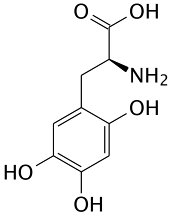




Bromocriptine

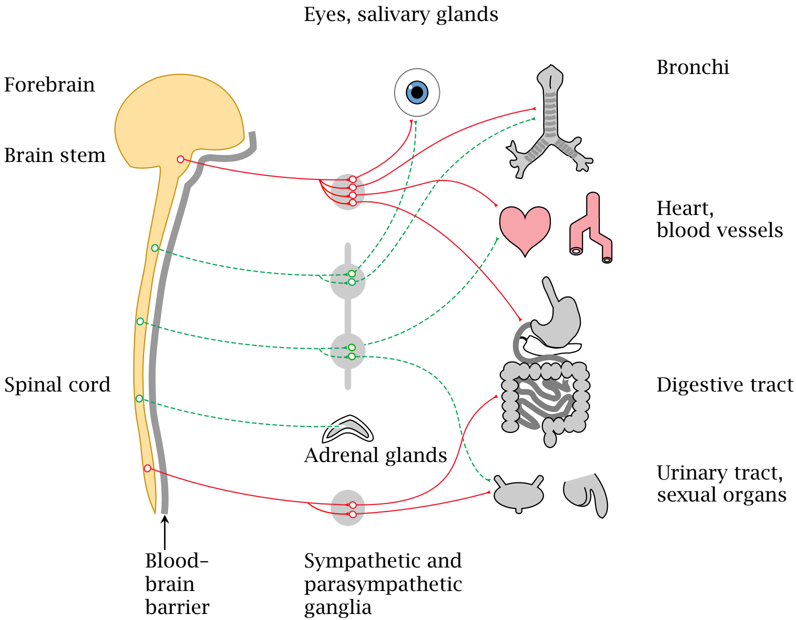


Clozapine



6-Hydroxy-L-DOPA

Organization of the autonomic nervous system



Transmitter receptors in the peripheral autonomic nervous system

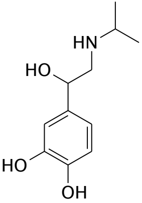
Subsystem	1^{st} Synapse	2^{nd} Synapse
sympathetic	nicotinic	α- or β-adrenergic
parasympathetic	nicotinic	muscarinic

▸ Therapeutic and toxic drug effects

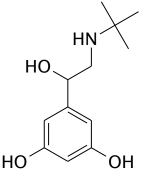
Receptor agonists or antagonists and false transmitters at adrenergic synapses

▸ synapse

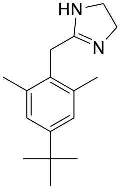
Isoproterenol (β ↑)



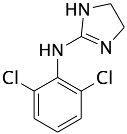
Terbutaline (β_2 ↑)

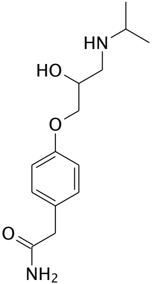


Xylometazoline (α ↑)

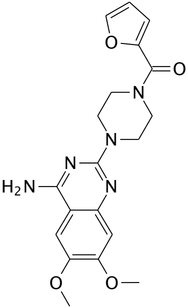


Clonidine (α_2 ↑)

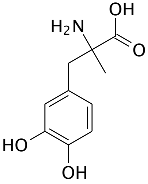




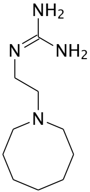
Atenolol (β_1 ↓)



Prazosine (α_1 ↓)

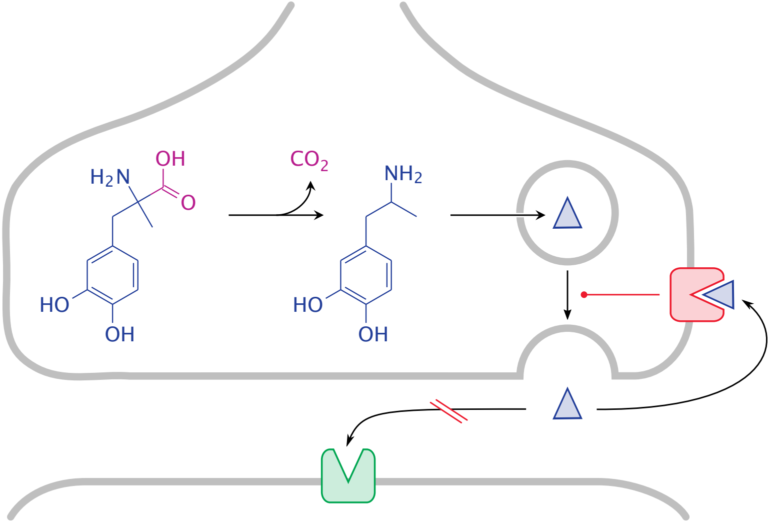


α-Methyldopa



Guanethidine

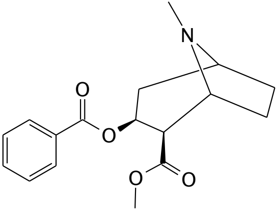
Methyldopa is a false transmitter in noradrenergic synapses



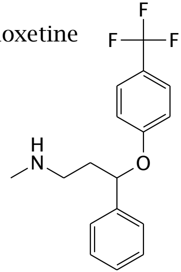
- DOPA transport

Drugs that act on the membrane transport of monoamine transmitters

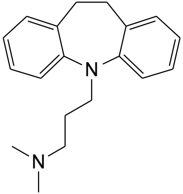
Cocaine



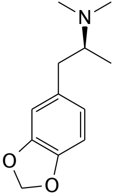
Fluoxetine

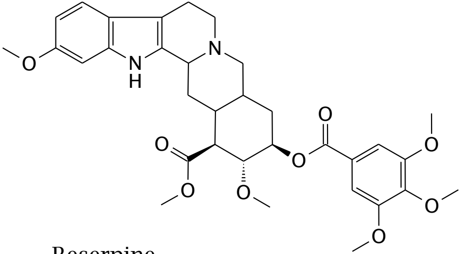


Imipramine

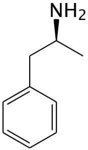


MDMA

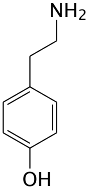




Reserpine

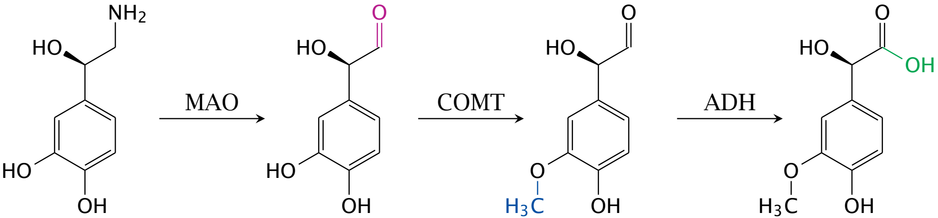


Amphetamine



Tyramine

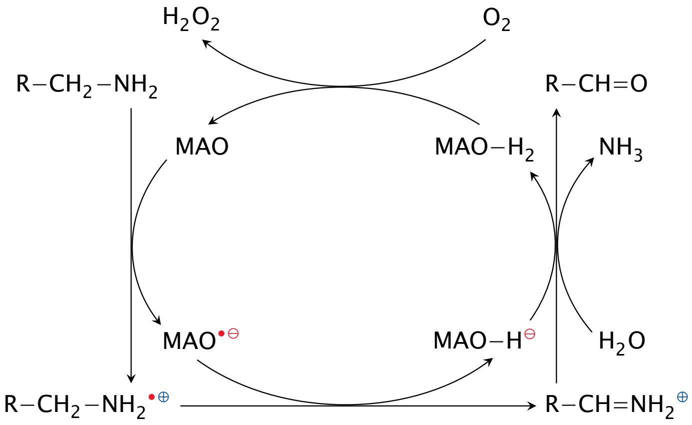
Degradation of norepinephrine



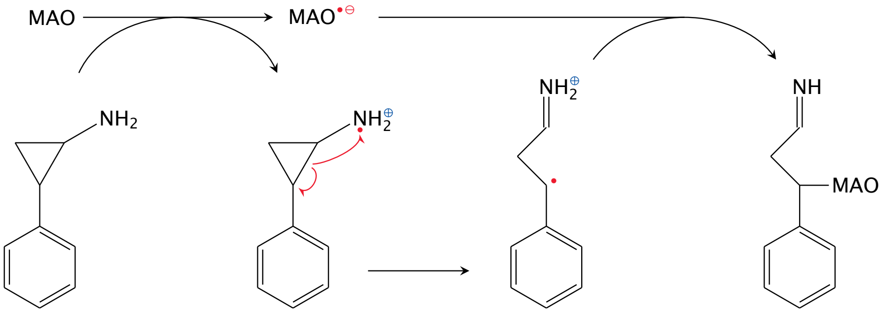
Norepinephrine

Vanillylmandelate

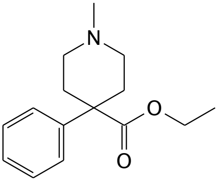
Reaction mechanism of monoamine oxidase (MAO)



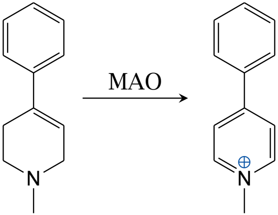
Mechanism-based inhibition of MAO by tranylcypromine



MAO-induced toxicity of MPTP (N-methyl-4-phenyl-tetrahydropyridine)

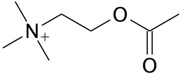


Meperidine

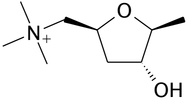


MPTP $\quad$ MPP^+

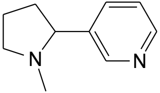
Structures of cholinergic receptor agonists



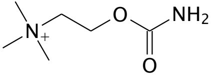
Acetylcholine



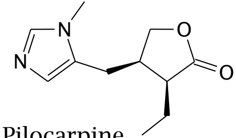
Muscarine



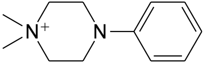
Nicotine



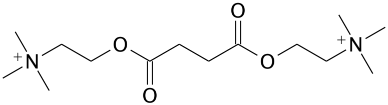
Carbamoylcholine



Pilocarpine



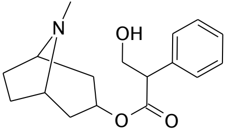
Dimethylphenylpiperazinium



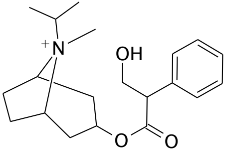
Succinylcholine

▸ NAR desensitization

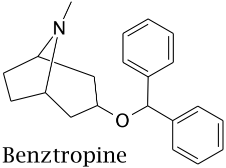
Structures of cholinergic receptor antagonists



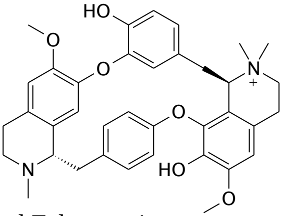
Atropine



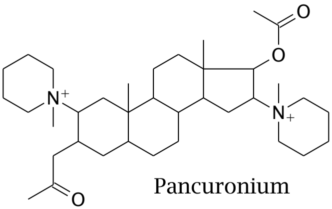
Ipratropium



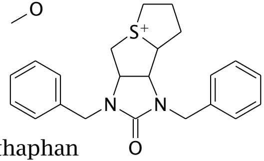
Benztropine



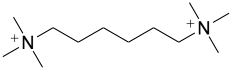
d-Tubocurarine



Pancuronium



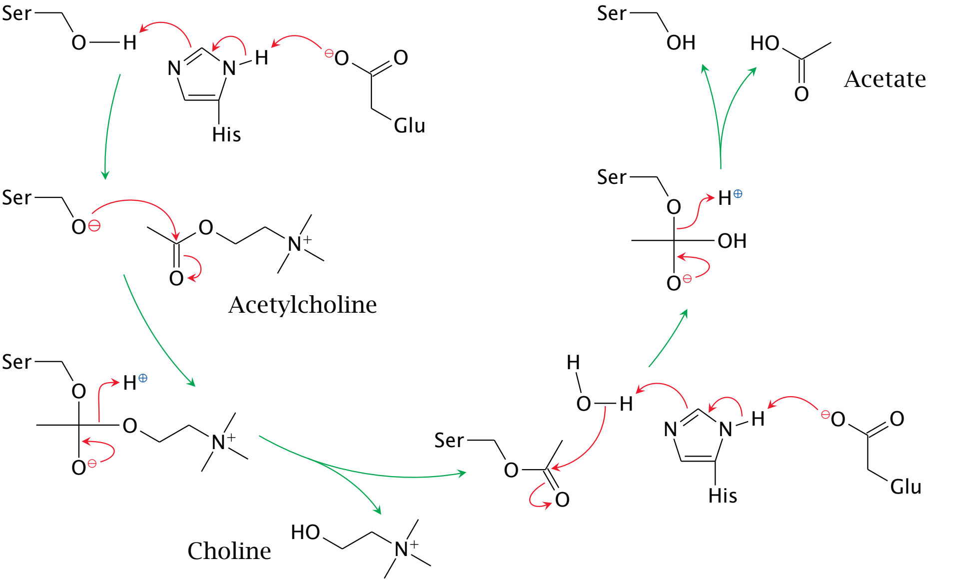
Trimethaphan



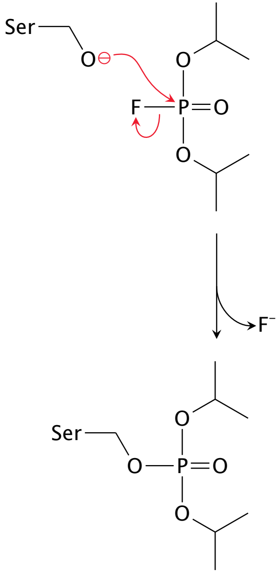
Hexamethonium

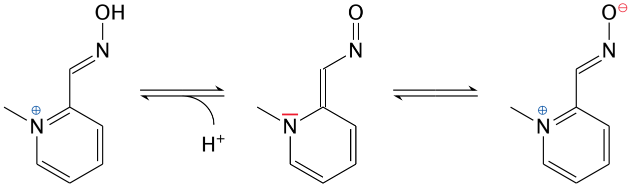
▸ autonomic NS

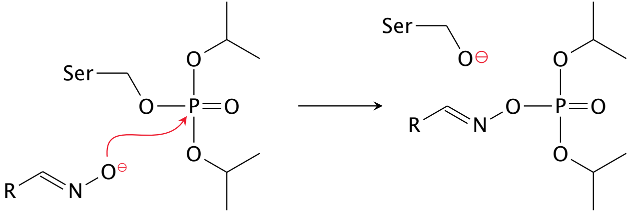
The catalytic mechanism of cholinesterase



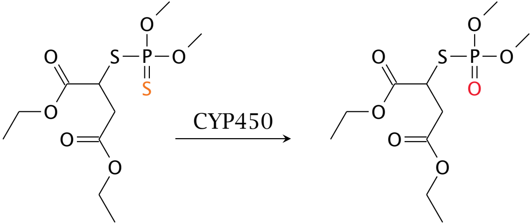
Covalent inactivation of cholinesterase by DFP, and its reactivation by pralidoxime



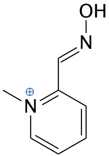




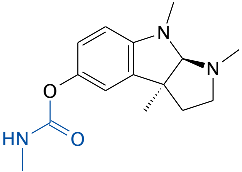
Structures of cholinesterase inhibitors and of a reactivator



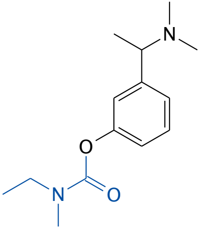
Malathion Malaoxon



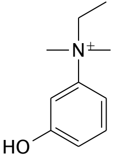
Pralidoxime



Physostigmine



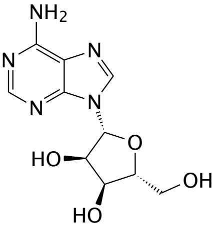
Rivastigmine



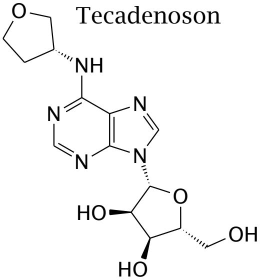
Edrophonium

Purine receptor agonists and antagonists

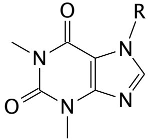
Adenosine

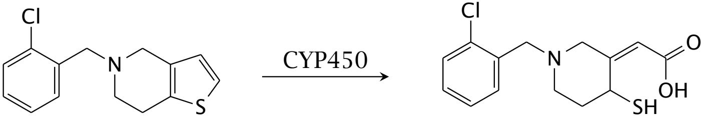


Tecadenoson



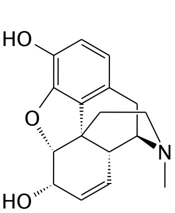
R = CH_3: Caffeine
R = H: Theophylline



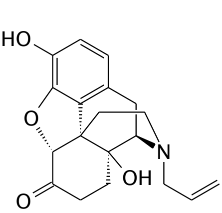


Ticlopidine

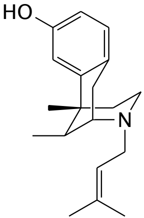
Opioid receptor agonists and antagonists



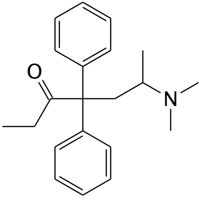
Morphine



Naloxone



Pentazocine



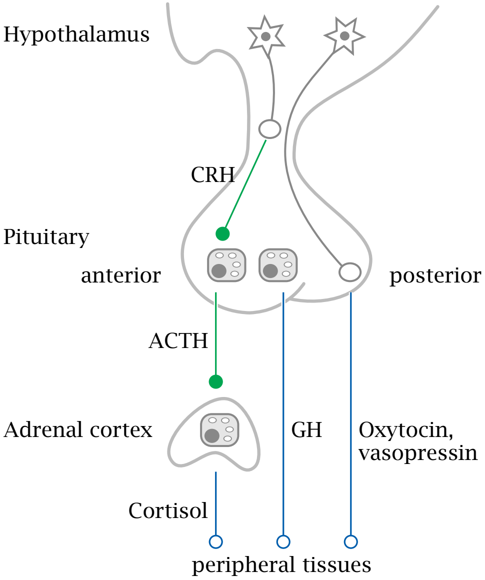
Methadone

Hormones

Major types of hormone receptors

- G protein-coupled receptors
  - Many peptide hormones: glucagon, hypothalamic and hypophyseal hormones
  - Epinephrine, norepinephrine
- Receptor tyrosine kinases
  - Insulin
  - Growth hormone and growth factors
- Nuclear hormone receptors
  - Steroid hormones
  - Thyroid hormones

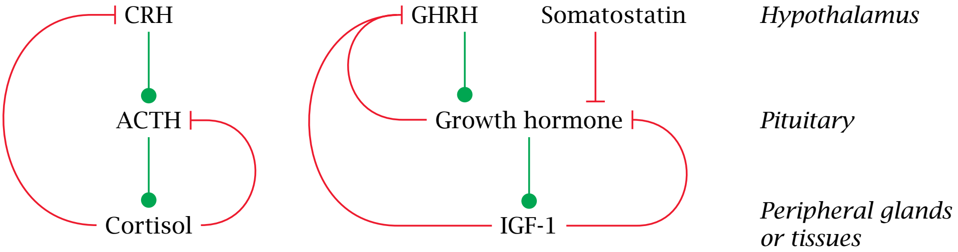
The hypothalamic-pituitary axis



Peripheral glands and hormones controlled by the anterior pituitary

Gland	Stimulated by	Hormones produced
adrenal glands (cortex)	adrenocorticotropic hormone (ACTH)	gluco-, mineralocorticoids; androgens
thyroid gland	thyroid-stimulating hormone (TSH)	tri-, tetraiodothyronine (T_3, T_4)
testicles / ovaries	follicle-stimulating hormone (FSH), luteinizing hormone (LH)	androgens, estrogens, progestins
diffuse	growth hormone	growth factors
mammary gland	prolactin	—

Regulatory patterns in the hypothalamic-pituitary axis



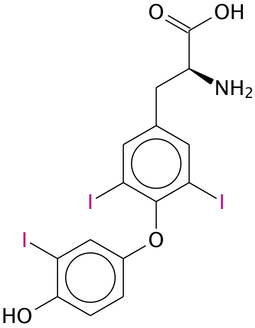
The posterior lobe of the hypophyseal gland produces oxytocin and vasopressin

Oxytocin H_2N-Cys—Tyr—Ile—Gln—Asn—Cys—Pro—L-Leu—Gly-CO-NH_2

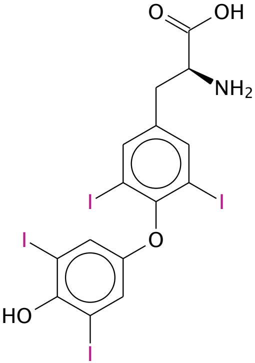
Vasopressin H_2N-Cys—Tyr—Phe—Gln—Asn—Cys—Pro—L-Arg—Gly-CO-NH_2

Desmopressin H-Cys—Tyr—Phe—Gln—Asn—Cys—Pro—D-Arg—Gly-CO-NH_2

Thyroid hormones

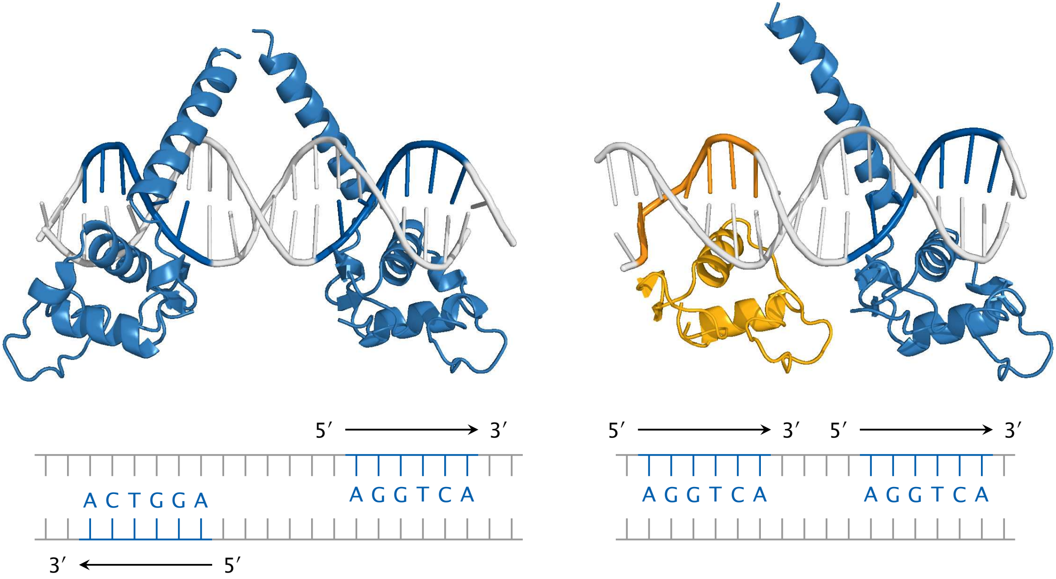


Triiodothyronine (T_3)

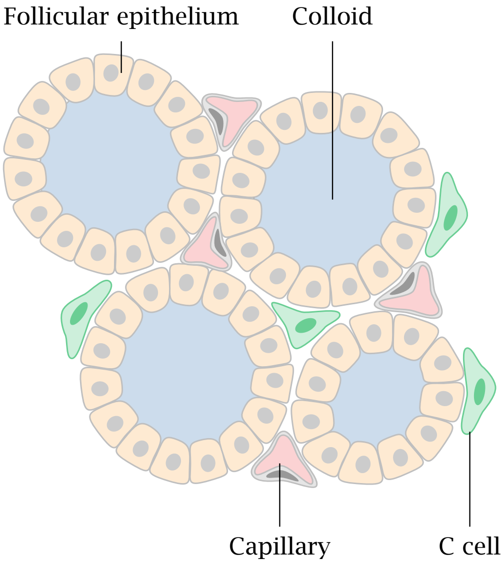


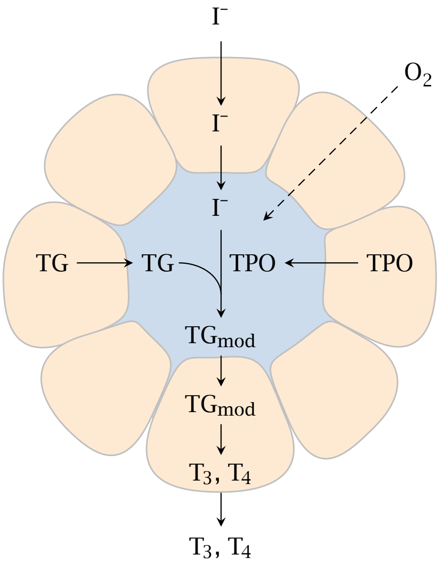
Thyroxine (T_4)

Thyroid hormones activate cognate nuclear hormone receptors

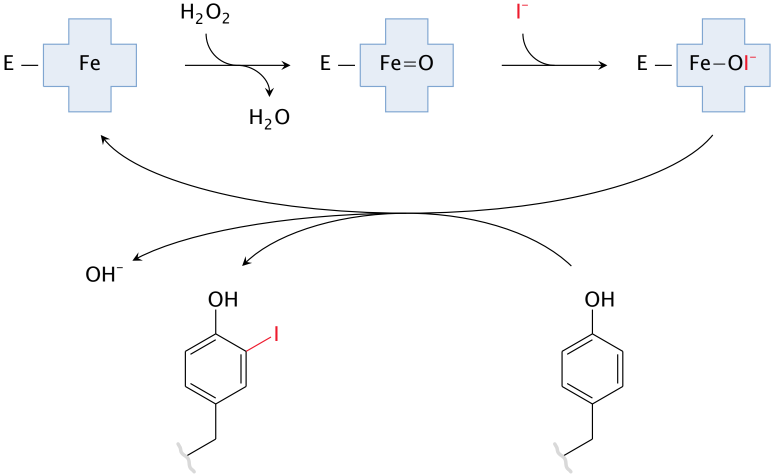


Tissue structure of the thyroid gland, and localization of hormone synthesis

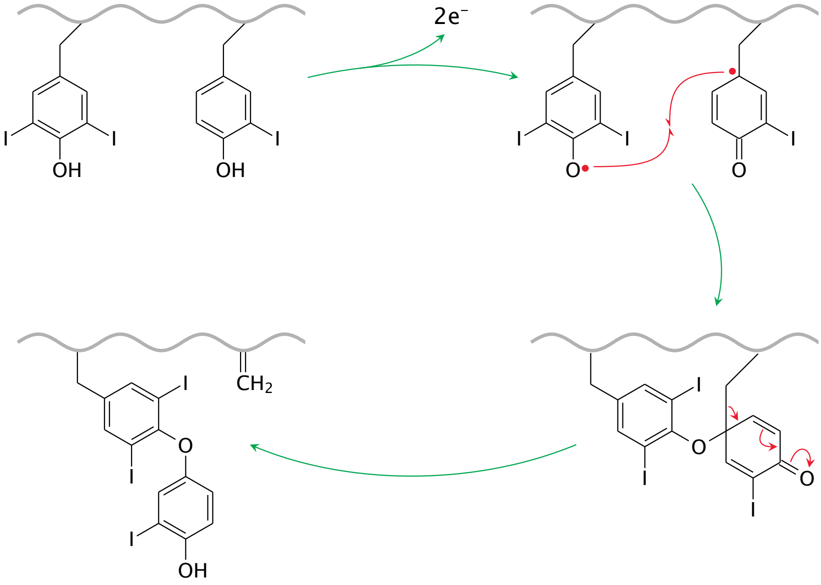




Tyrosine side chain iodination by thyroid peroxidase



Coupling of two iodinated tyrosine side chains

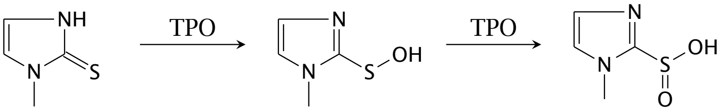


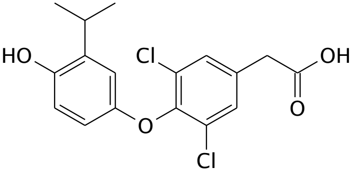
Thyroid hormones and pharmacotherapy

- Goiter: iodide supplementation
- Hyperthyroidism due to anti-TSH-receptor autoantibodies or hormone-producing tumors:
  - thyroid peroxidase inhibitors
  - radioiodine (^{131}I)
- Lowering blood lipid levels: TR-β-selective agonists (KB-141)
- Interference with thyroid hormone release or conversion: lithium and amiodarone

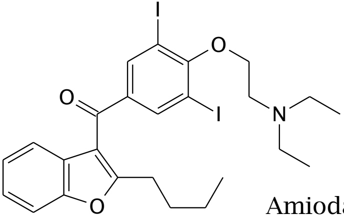
Drugs that influence thyroid hormone function

Methimazole Sulfenic acid Sulfinic acid





KB-141



Amiodarone

- T_3, T_4

Steroid hormones

Class	Major members	Glands
Glucocorticoids	Cortisol, cortisone	Adrenal glands
Mineralocorticoids	Aldosterone	Adrenal glands
Androgens	Testosterone, dihydrotestosterone	Testicles
Estrogens	Estradiol, estriol	Ovary
Progestins	Progesterone	Ovary, placenta

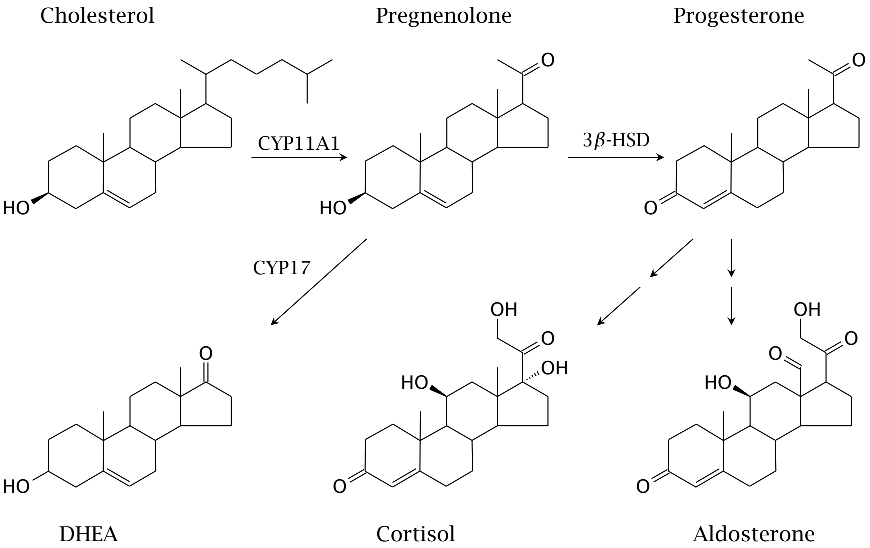
Effector mechanisms

Mechanism/target	Receptor binds to	Example
Transcriptional induction (transactivation)	DNA directly	Induction of enzymes of gluconeogenesis by cortisol
Transrepression	Other proteins that regulate transcription	Inhibition of transcription factors AP-1 and NF-κB by cortisol

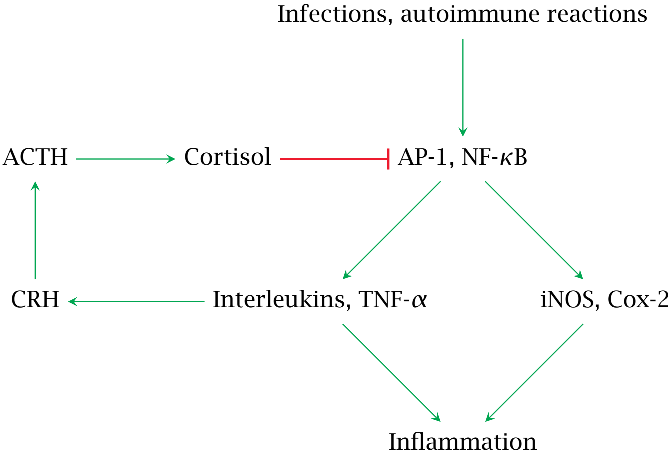
Adrenal steroid hormones: functional classes

Class	Physiological function
Glucocorticoids	Metabolic regulation, strong anti-inflammatory action
Mineralocorticoids	Control of sodium and potassium elimination in the kidneys
Androgens	Precursors for gonadal androgen and estrogen synthesis

Synthesis of adrenal steroids

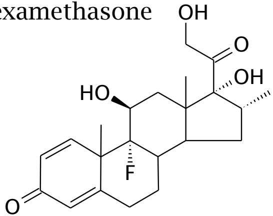


Glucocorticoids control inflammation via transrepression

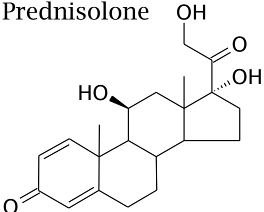


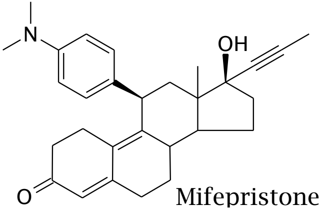
Glucocorticoid receptor agonists and antagonists

Dexamethasone

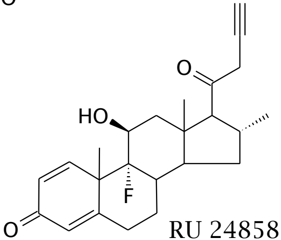


Prednisolone

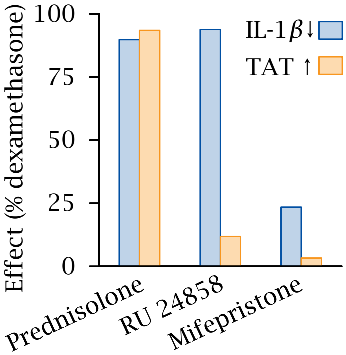




Mifepristone

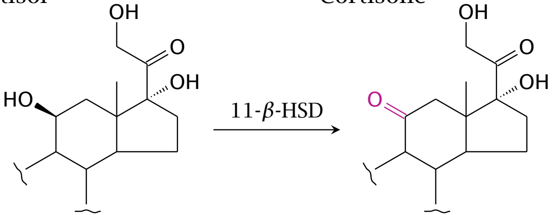


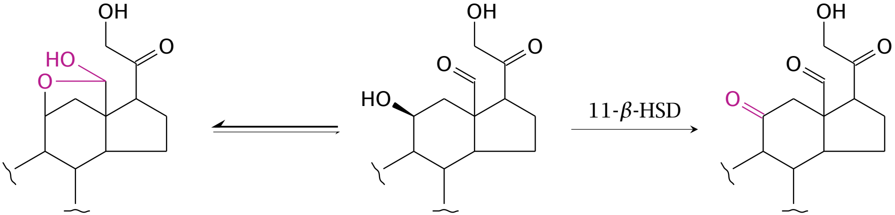
RU 24858



11-β-Hydroxysteroid dehydrogenase prevents non-specific mineralocorticoid receptor activation by cortisol

Cortisol Cortisone

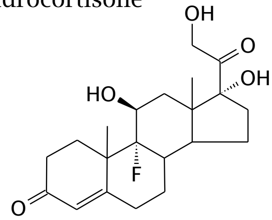




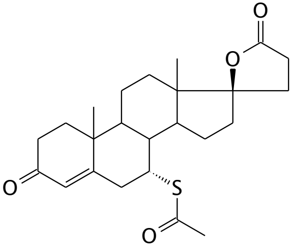
Aldosterone hemiacetal Aldosterone

Mineralocorticoid receptor ligands

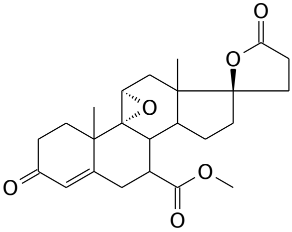
Fludrocortisone



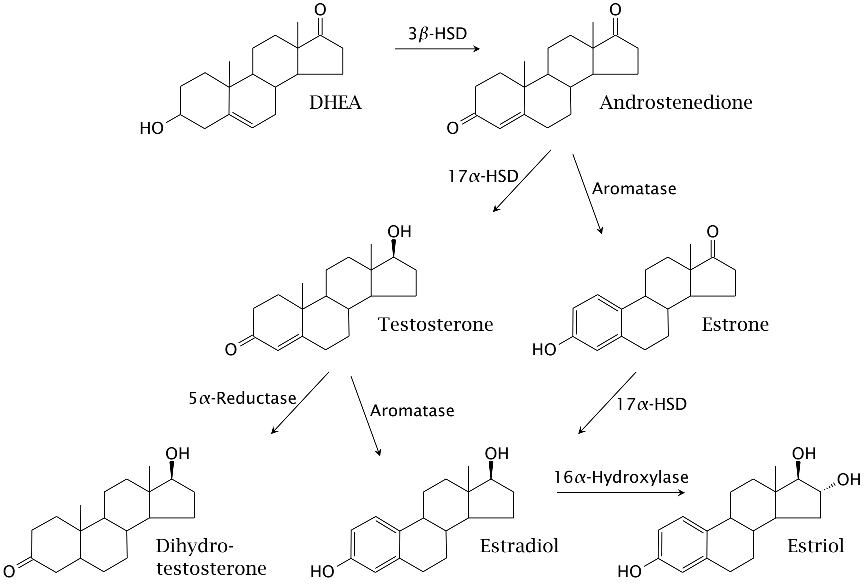
Spironolactone



Eplerenone

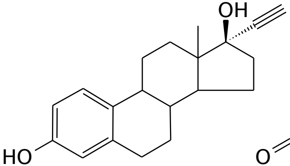


Gonadal biosynthesis of androgens and estrogens

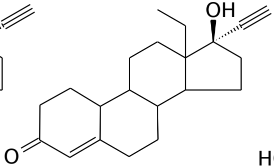


Synthetic analogues of gonadal steroids

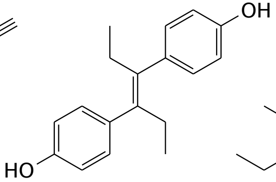
Ethinylestradiol



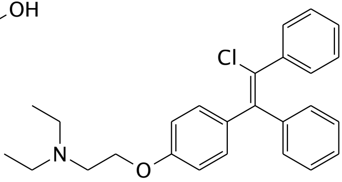
Norgestrel

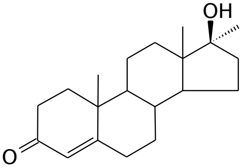


Diethylstilbestrol

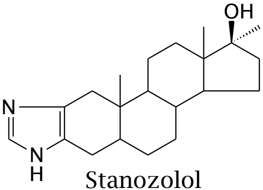


Clomiphene

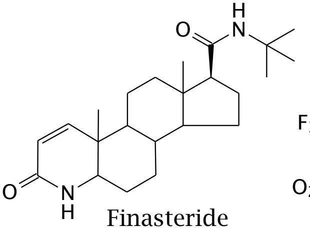




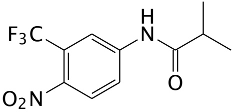
Methyltestosterone



Stanozolol

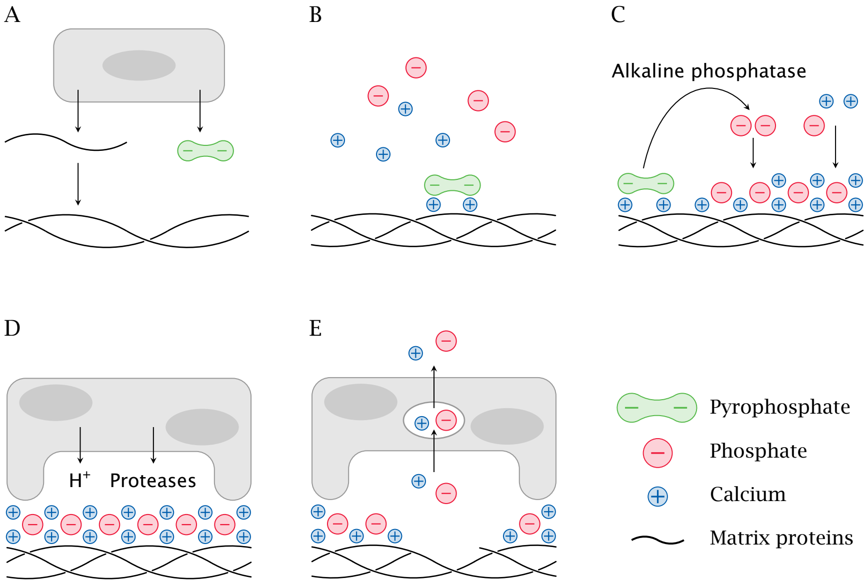


Finasteride



Flutamide

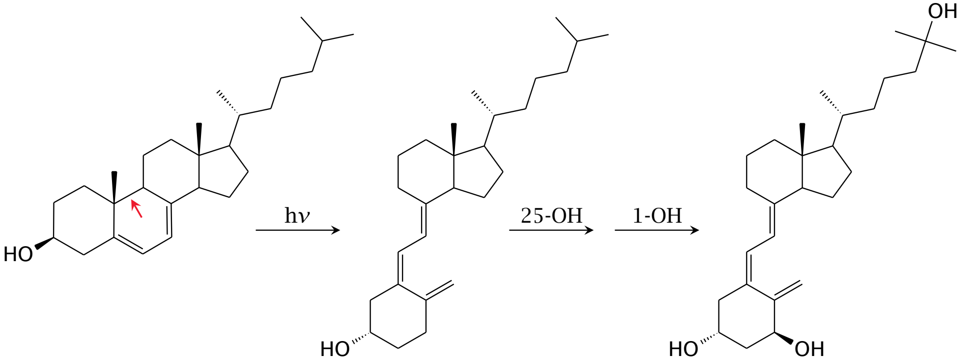
Formation and resorption of bone matrix



Hormones that affect bone mineralization and bone matrix

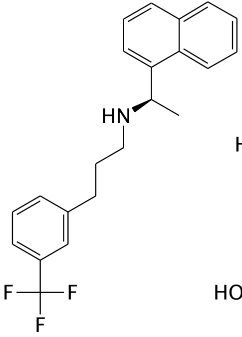
- Parathyroid hormone (PTH)
  - mobilizes calcium and phosphate from the bone
  - promotes calcium retention and phosphate elimination in the kidneys
  - promotes activation of vitamin D by hydroxylation
- Calcitonin: promotes deposition of calcium and phosphate in the bone
- Calcitriol (activated vitamin D)
  - promotes intestinal calcium and phosphate uptake
  - inhibits PTH secretion
- Estrogens: sustain bone matrix

Endogenous biosynthesis of calcitriol



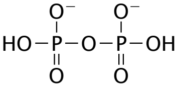
7-Dehydrocholesterol Cholecalciferol 1,25-Dihydroxycholecalciferol

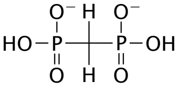
Drugs that influence calcium balance and bone mineralization



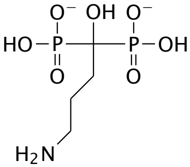
Cinacalcet

Pyrophosphate

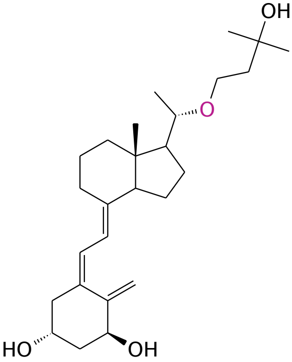




Medronate



Alendronate



22-Oxacalcitriol

‣ bone matrix cartoon

Sites of action of statins and bisphosphonates

$$
\begin{tikzcd}
\text{Acetyl-CoA} \arrow[r] & \text{HMG-CoA} \arrow[r, "\textit{Statins}"] & \text{Mevalonate} \arrow[r] & C_5\text{-},C_{10}\text{-prenoids} \arrow[d, "\textit{Bisphosphonates}"] \\
\text{Cholesterol} & \text{7-Hydroxycholesterol} \arrow[l] \arrow[d, "h\nu"'] & & \text{Farnesyl-PP} \arrow[ll] \arrow[d] \\
& \text{Cholecalciferol} & & \text{Farnesylated proteins (Ras, Rho \ldots)}
\end{tikzcd}
$$

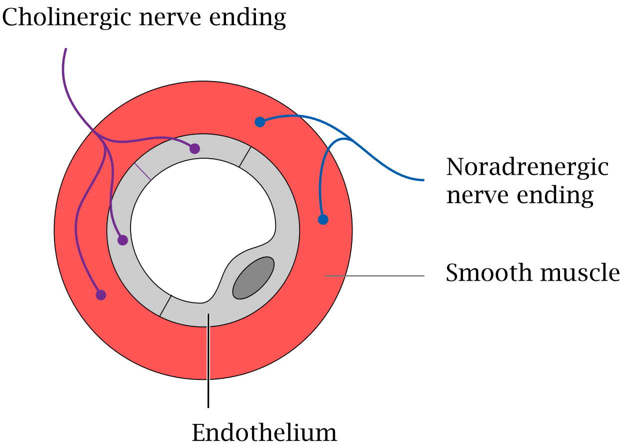
‣ bone matrix cartoon

Pharmacology of nitric oxide

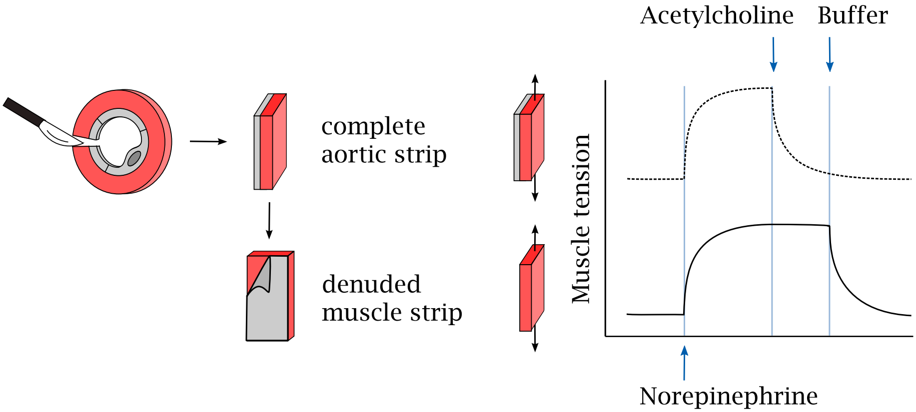
Physiological significance of nitric oxide (NO)

- Powerful vasodilator—NO-releasing drugs are used in the treatment of cardiovascular disease
- Neurotransmitter—signaling in the CNS and the autonomic nervous system
- Inflammatory mediator—inhibition of NO synthesis is of interest as a therapeutic strategy in infection and chronic inflammation

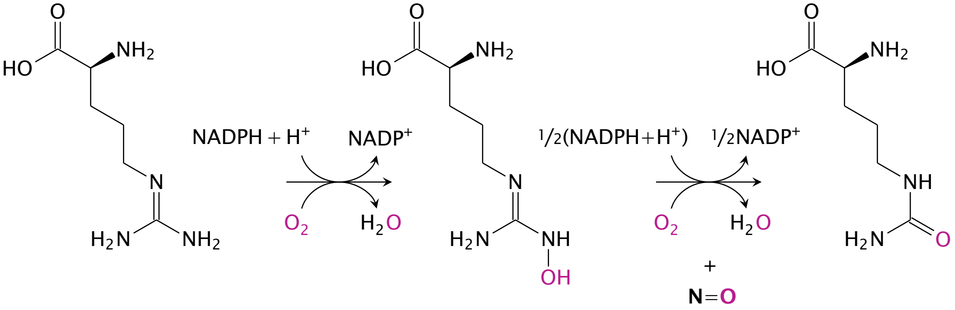
Cholinergic and adrenergic nerve endings in a blood vessel wall



The endothelium is required for vascular relaxation in response to acetylcholine

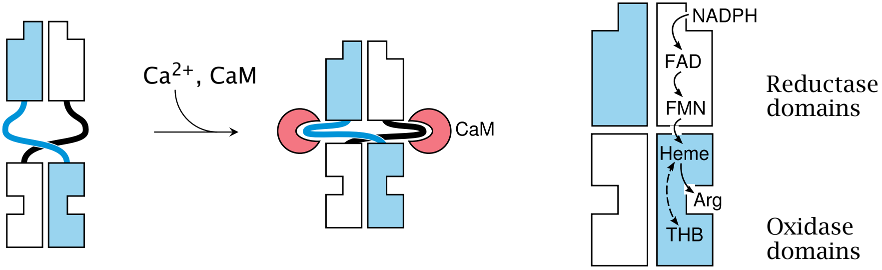


The nitric oxide synthase reaction

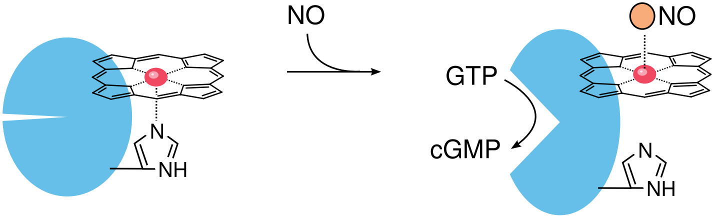


arginine N-hydroxyarginine citrulline

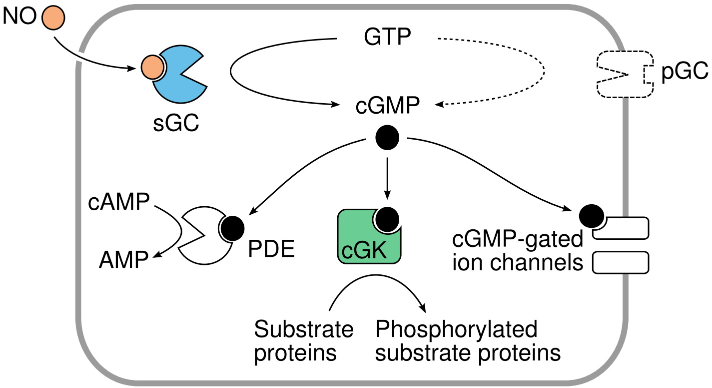
Activation of NOS by calcium and calmodulin



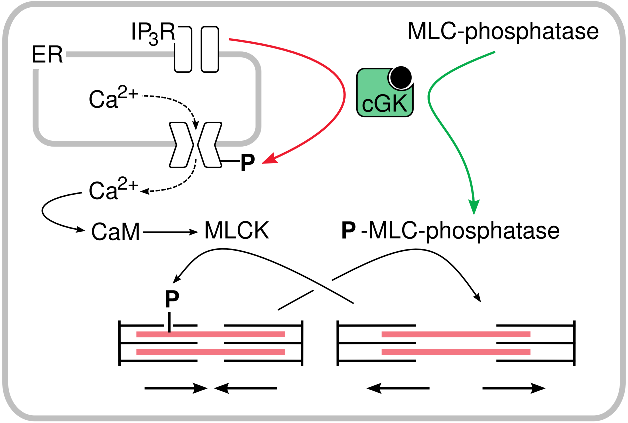
NO activates soluble guanylate cyclase (sGC)



Signaling effects of cGMP

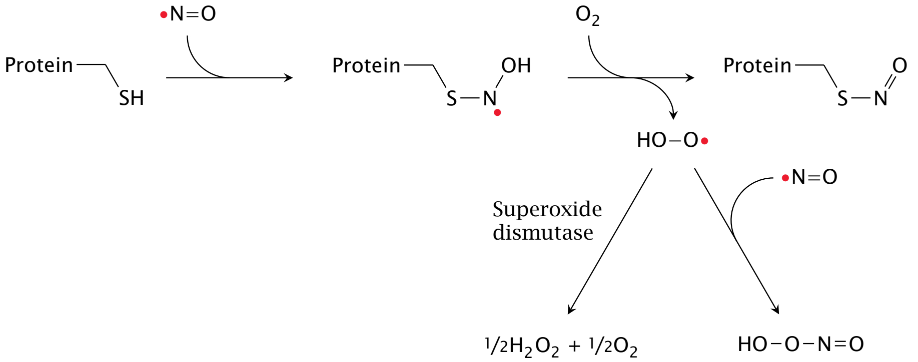


NO-induced relaxation of smooth muscle cells is mediated by cGK

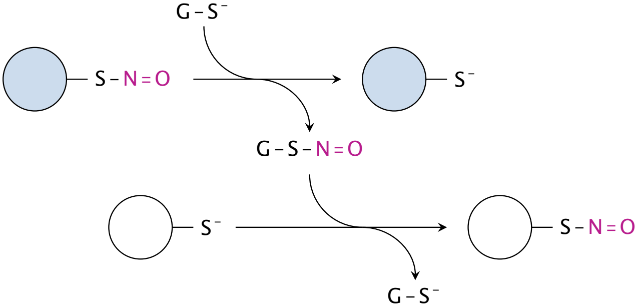


▸ PLC cascade

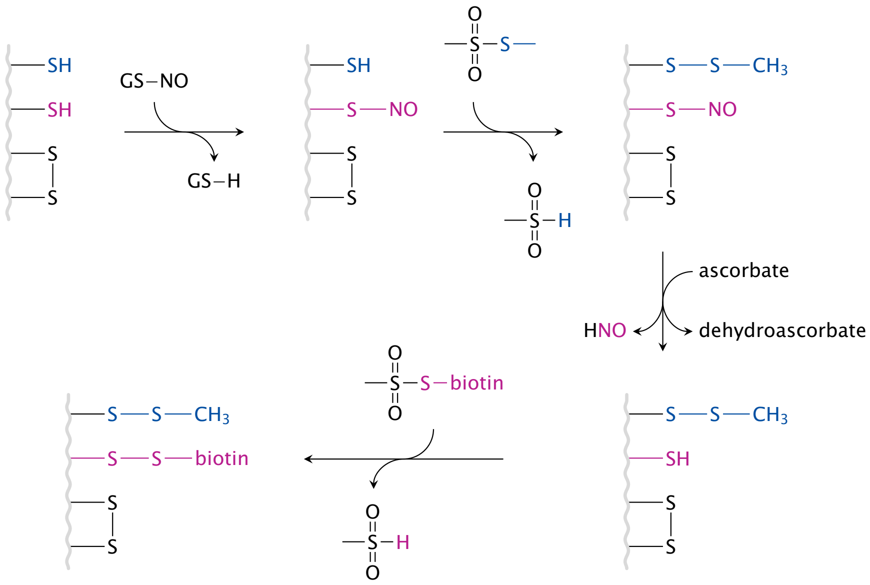
S-nitrosylation of cysteine residues in proteins by NO



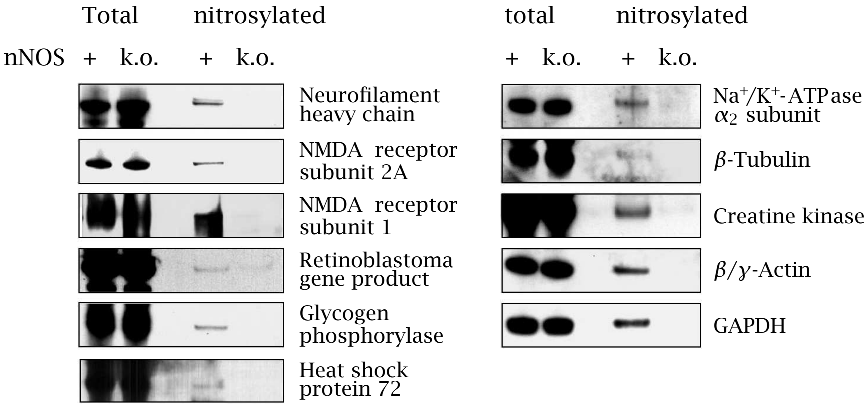
Transfer of nitrosyl groups between proteins by glutathione



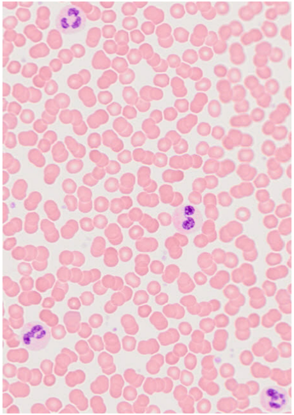
Identification of cysteines affected by S-nitrosylation: The biotin switch method

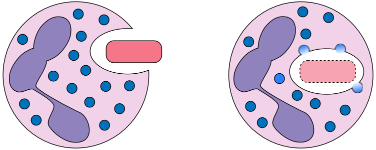


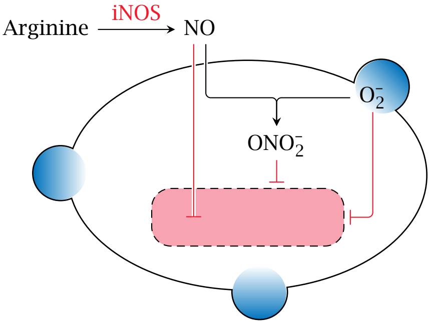
Identification of proteins subject to nNOS-dependent S-nitrosylation



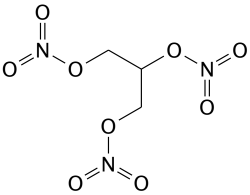
Role of NO and iNOS in the killing of microbes by phagocytes



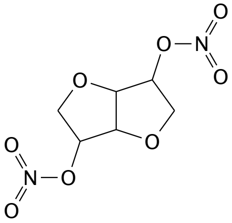




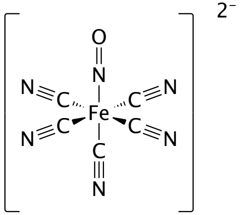
NO-releasing drugs



Nitroglycerin

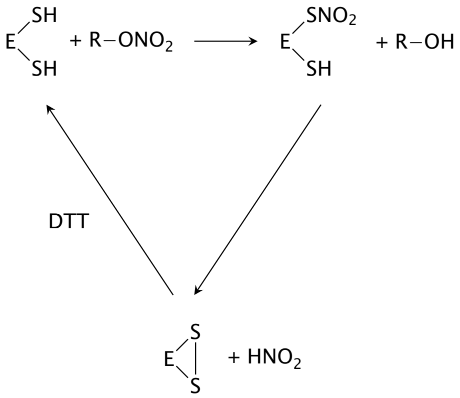


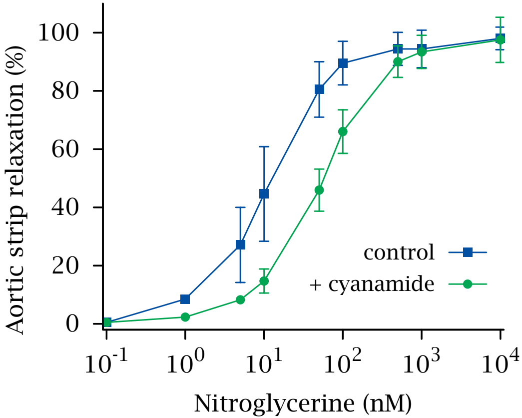
Isosorbide dinitrate



Nitroprusside

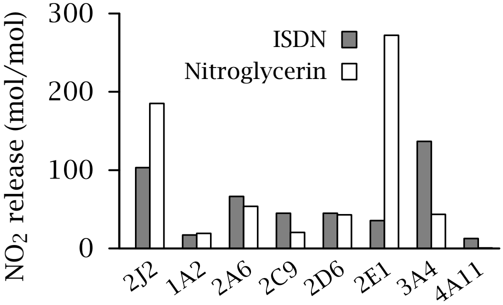
Bioactivation of nitroglycerin by mitochondrial aldehyde dehydrogenase



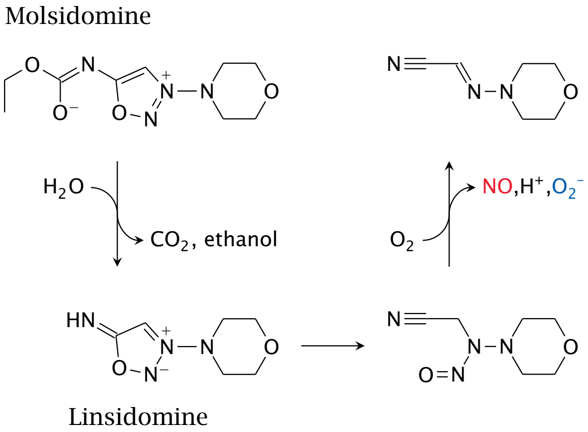


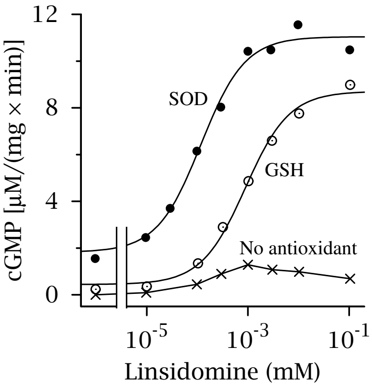
- aortic strips

Bioactivation of nitroglycerin and ISDN by human cytochrome P450 isoforms

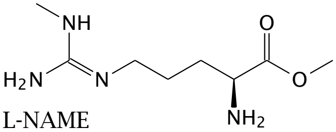


NO release by molsidomine

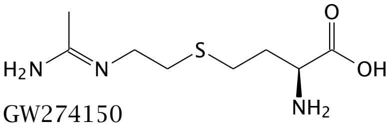




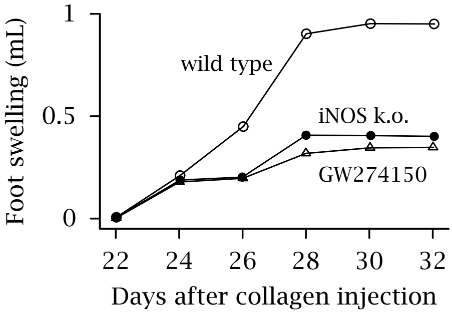
Antiinflammatory effect of iNOS inhibition



L-NAME

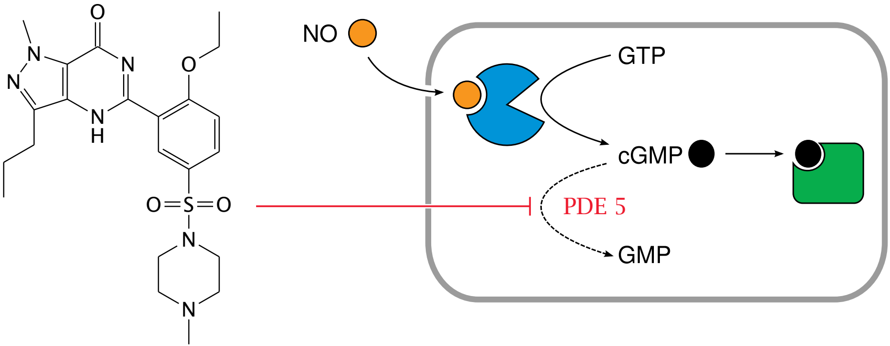


GW274150



‣ NOS reaction

Structure and mode of action of sildenafil (Viagra™)



Eicosanoids and related drugs

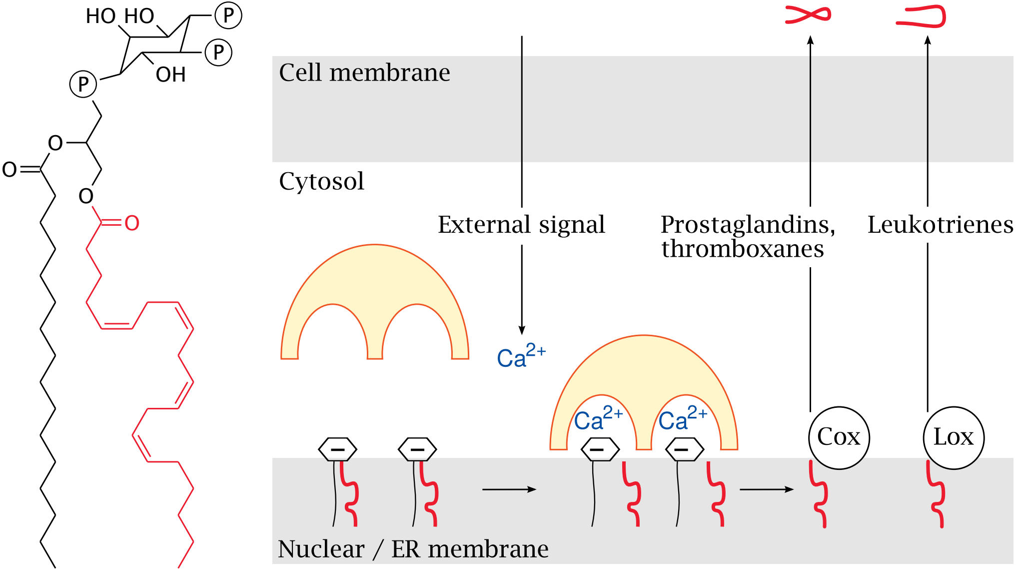
Eicosanoids

- "Local hormones"—sphere of action often limited to same anatomical site or tissue
- Involved in control of inflammation, fever, blood coagulation, pain perception, labor
- Derived from arachidonic acid and other polyunsaturated fatty acids, which occur in membrane phospholipids
- Most effects mediated by G protein-coupled receptors
- Some effects mediated by ion channels and nuclear hormone receptors

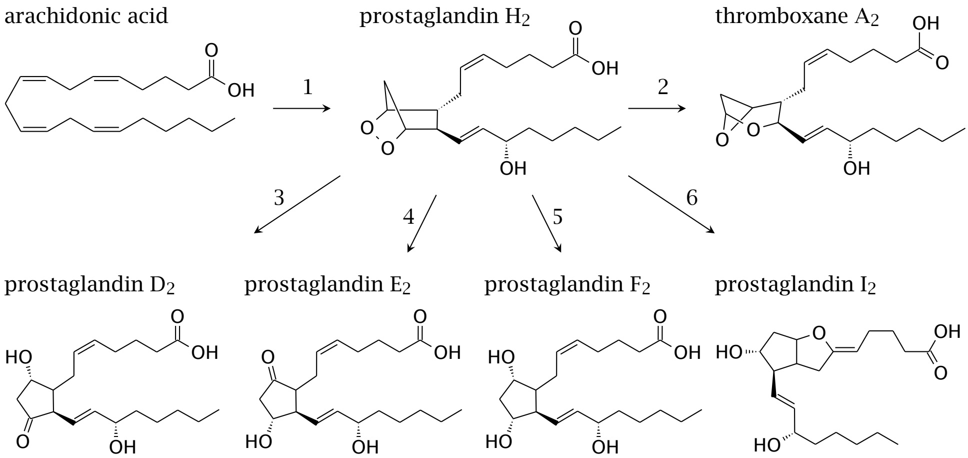
Pathways and key enzymes in eicosanoid synthesis

$$
\begin{tikzcd}
 & \text{Endocannabinoids} \arrow[r, "\text{Cox-2}"] \arrow[dr, "\text{FAAH}"] & {} \arrow[rr] & & \text{Prostamides} & & \text{Prostaglandin } D_2 \\
\text{Phospholipids} \arrow[ur] \arrow[rr, "cPLA_2"] & & \text{Arachidonic acid} \arrow[rrr, "\text{Cox-1, Cox-2}"] \arrow[dl, "\text{Lox}"'] \arrow[dd, "\text{Lox}"] \arrow[dr, "P_{450}"] & & & \text{Prostaglandin } H_2 \arrow[ur] \arrow[r] \arrow[dl] \arrow[dd] \arrow[dr] & \text{Prostaglandin } E_2 \\
 & \text{Leukotrienes} & & \text{EET} & \text{Prostaglandin } F_2 & & \text{Thromboxane } A_2 \\
 & & \text{Lipoxin } A_4 & & & \text{Prostaglandin } I_2 &
\end{tikzcd}
$$

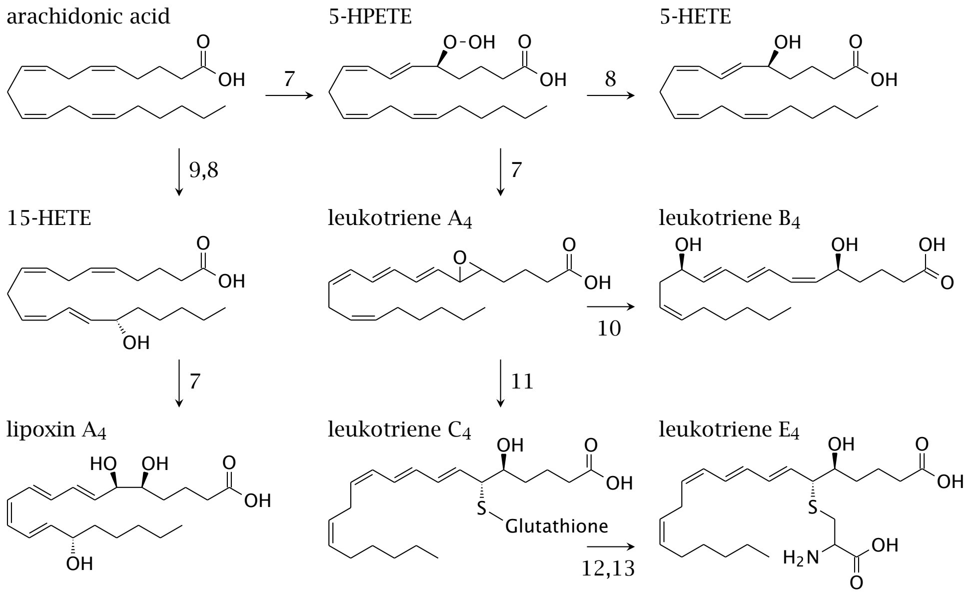
Calcium signals activate cPLA$_2$ and initiate the synthesis of prostaglandins and leukotrienes



Biosynthetic pathways of prostaglandins and thromboxanes



Eicosanoids synthesized by lipoxygenases

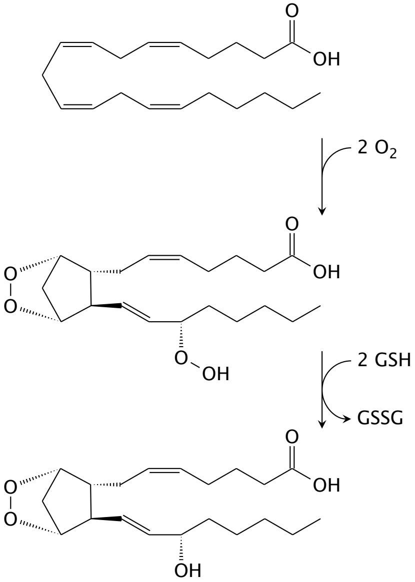


The two steps of the cyclooxygenase reaction

Arachidonic acid

Prostaglandin G_2

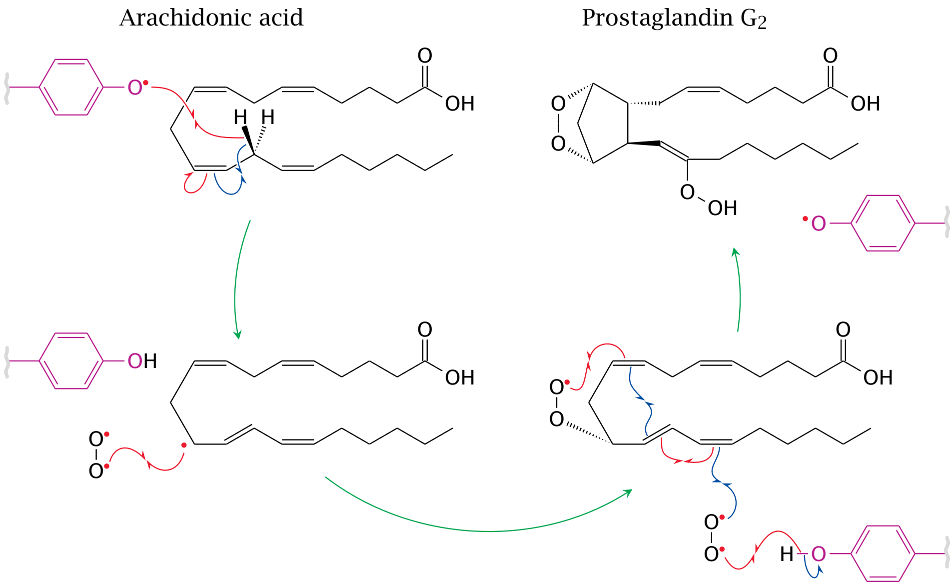
Prostaglandin H_2



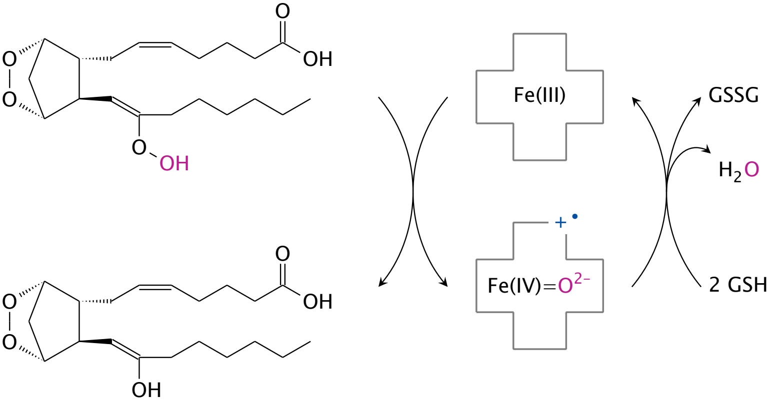
Cyclooxygenase site

Peroxidase site

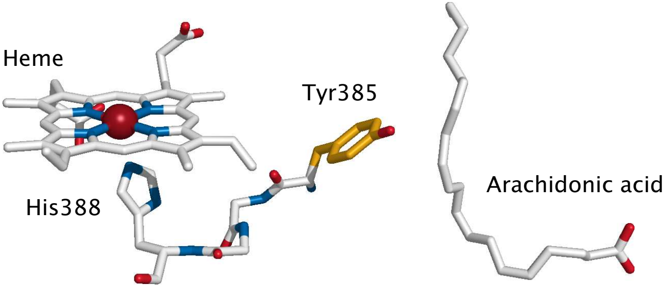
Reactions in the cyclooxygenase site



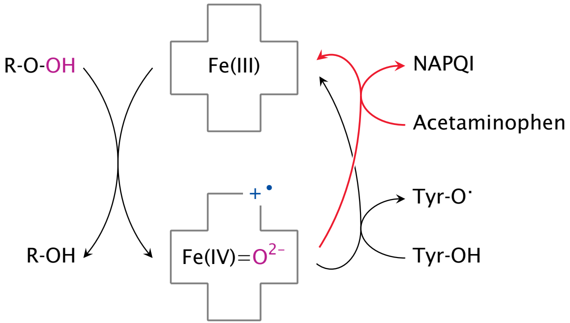
Reduction of prostaglandin G_2 to prostaglandin H_2

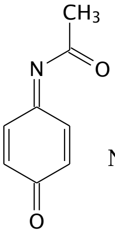


Interaction between the cyclooxygenase and peroxidase sites

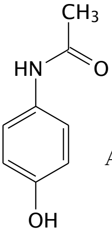


Priming of the active site tyrosine radical, and the action mode of acetaminophen



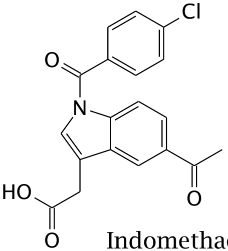


NAPQI

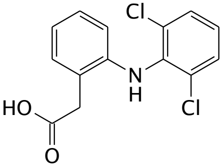


Acetaminophen

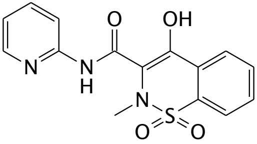
Noncovalent cyclooxygenase inhibitors



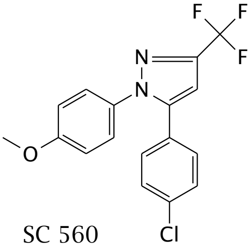
Indomethacin



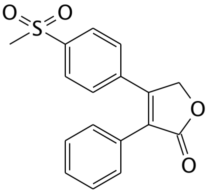
Diclofenac



Piroxicam

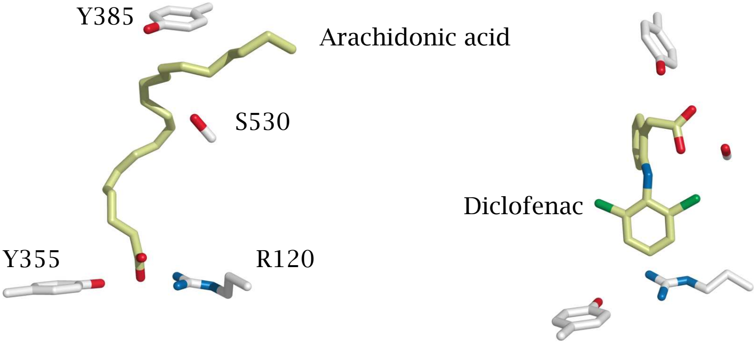


SC 560



Rofecoxib

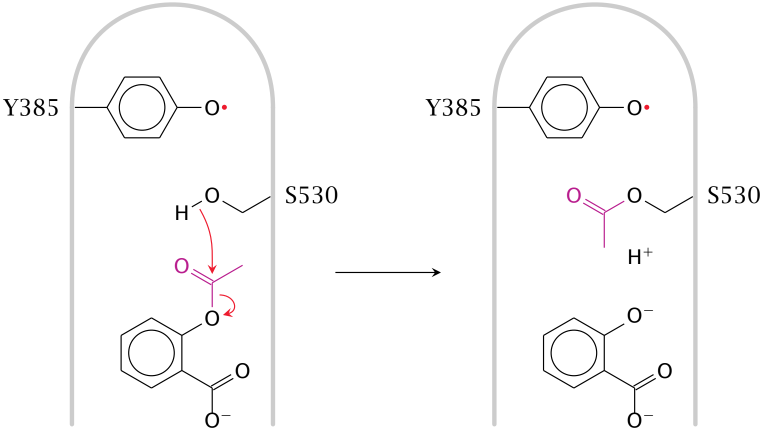
Conformation of arachidonic acid and of diclofenac in the active site



Cox inhibitors and Cox mutants

Inhibitor	Relative IC_{50}		
	R120A	Y355F	S530A
Indomethacin	>240	>240	1.1
Diclofenac	3.3	1.8	>650
Piroxicam	>109	>109	>109

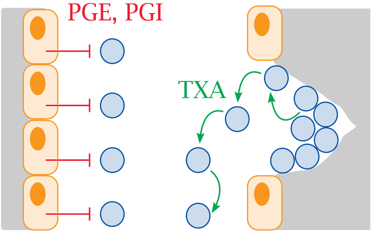
Acetylsalicylic acid is a covalent Cox inhibitor

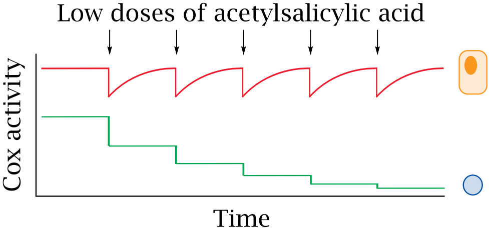


Acetylsalicylic acid Salicylic acid

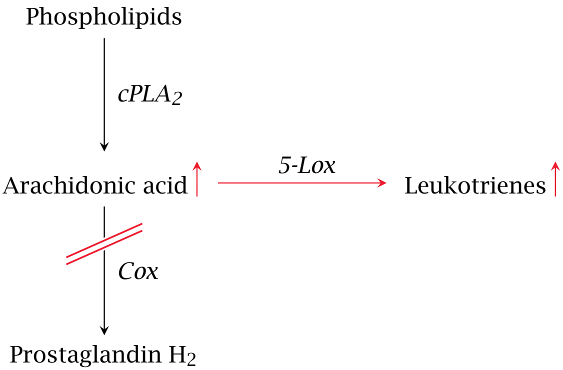
▸ mediators overview

Rationale of low-dose acetylsalicylic acid treatment

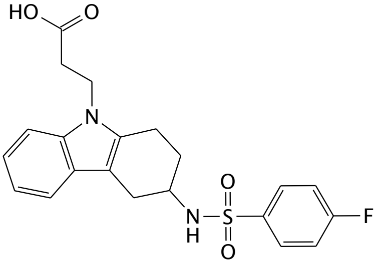




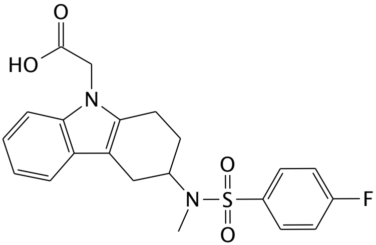
Cox inhibition can promote the synthesis of leukotrienes



Inhibitors that act downstream of Prostaglandin H_2



Ramatroban

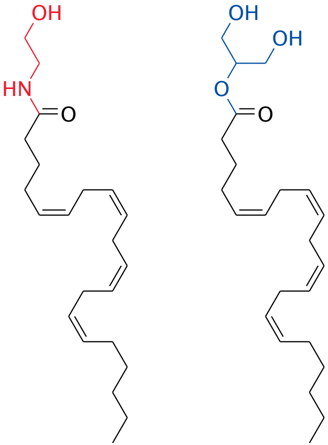


Cay10471

Endocannabinoids

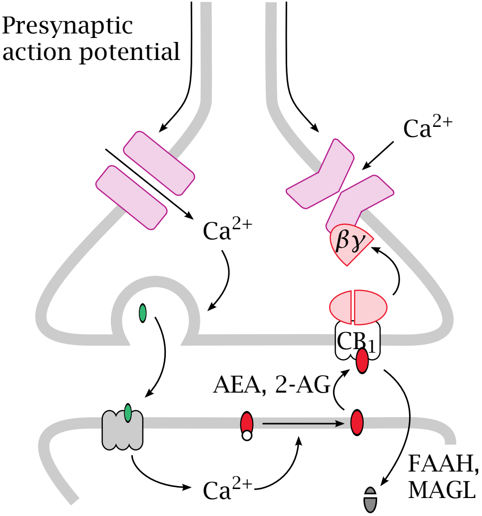
- Arachidonate-containing, membrane-derived mediators
- Synthesis on demand, activated by calcium
- Mediate negative synaptic feedback
- CB_1 receptors involved in pain perception both in the peripheral and the central nervous system
- CB_2 receptors found on immune cells

Feedback inhibition of synaptic transmission by endocannabinoids



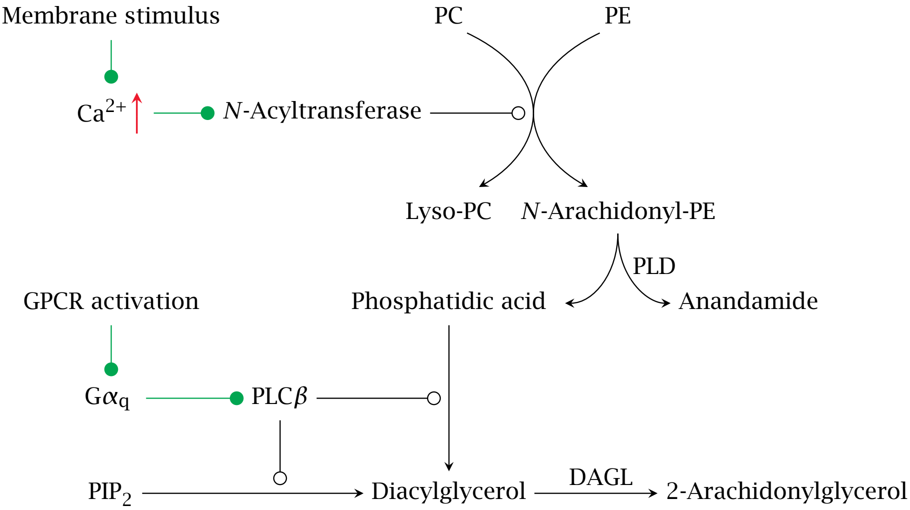
Anandamide (AEA)

2-Arachidonyl-glycerol (2-AG)



‣ conotoxin

Biosynthesis of endocannabinoids

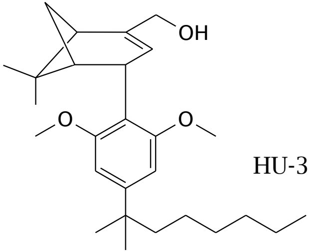


Degradation of endocannabinoids

$$\begin{tikzcd}
& \text{Anandamide} \arrow[dl, "\text{Cox-2}"'] \arrow[dr, "\text{FAAH}"'] & & \text{2-Arachidonylglycerol} \arrow[dl, "\text{MAGL}"] \arrow[dr, "\text{Cox-2}"] & \\
\text{PGH}_2\text{-ethanolamide} & & \text{Arachidonic acid} & & \text{PGH}_2\text{-glyceryl ester}
\end{tikzcd}$$

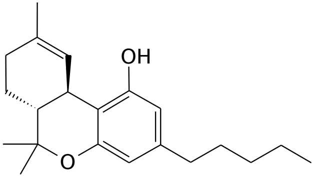
▸ synthetic pathways

Drugs that interact with the endocannabinoid system

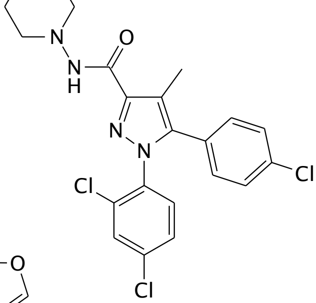


HU-308

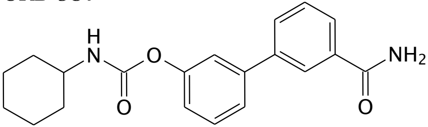
Δ^9-Tetrahydrocannabinol (THC)



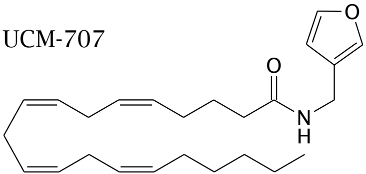
Rimonabant



URB-597



UCM-707



Intermediate metabolism, diabetes, and atherosclerosis

Intermediate metabolism, diabetes, and atherosclerosis

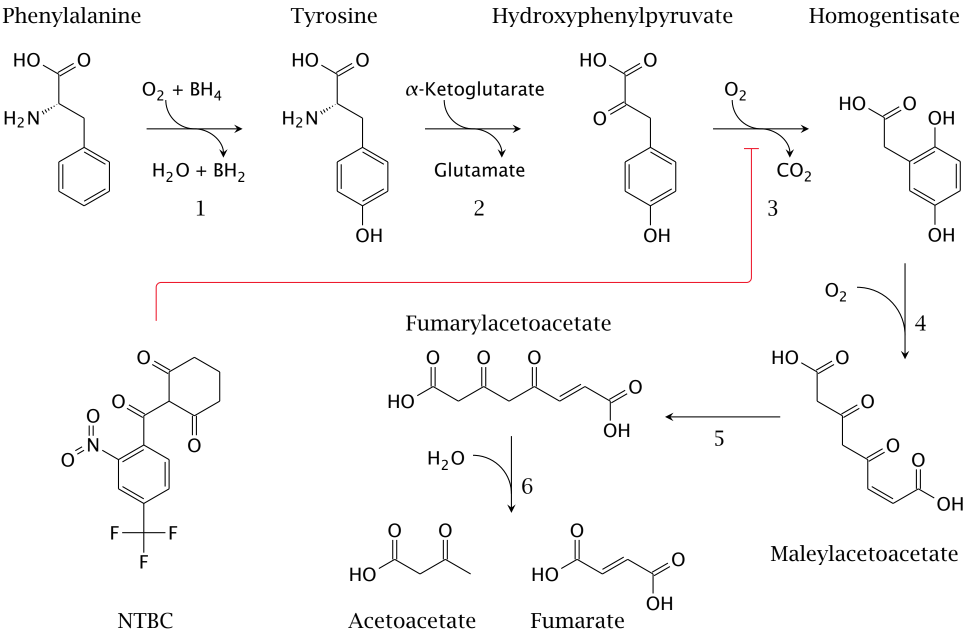
Genetic enzyme defects rare; comparatively little effort spent on targeted drug development; only a few defects can be treated with drugs

Gout more common; multiple causes, similar manifestation and treatment

Diabetes mellitus very common; treatment with insulin injections (types 1 and 2) and oral antidiabetics (type 2)

Atherosclerosis exceedingly common; drug therapy targets underlying metabolic conditions, other risk factors, and consequences of advanced disease

Degradation of phenylalanine and tyrosine

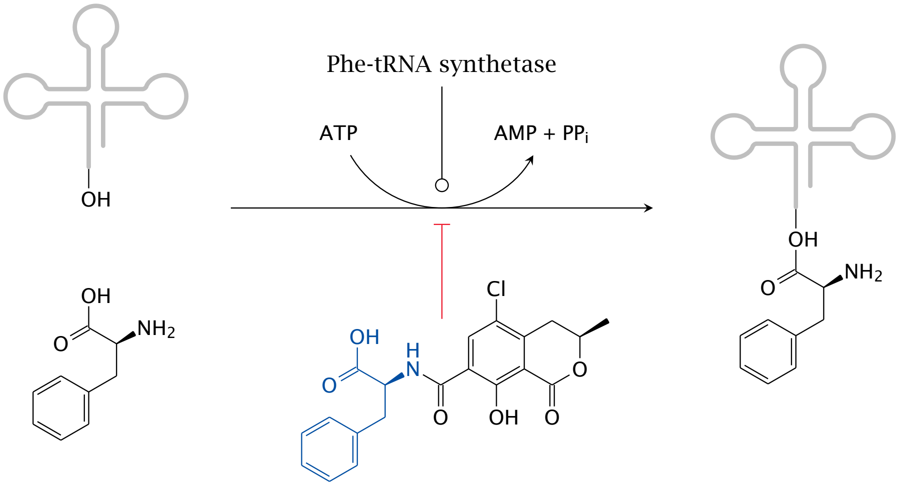


Phenylketonuria (PKU)

- Homozygous defect of phenylalanine hydroxylase
- Frequency: 1 newborn among 10,000 in Caucasians; lower frequency in other races
- Possible *heterozygote advantage*: reduced fetal susceptibility to ochratoxin A
- Symptomatic excess of phenylalanine manifest only after birth; intrauterine development normal
- Cognitive and neurological deficits, probably due to cerebral serotonin deficit
- Treated with phenylalanine-restricted diet
- Some cases due to reduced affinity of enzyme for cofactor tetrahydrobiopterin (BH_4), can be treated with high dosages of BH_4

▸ DOPA transport

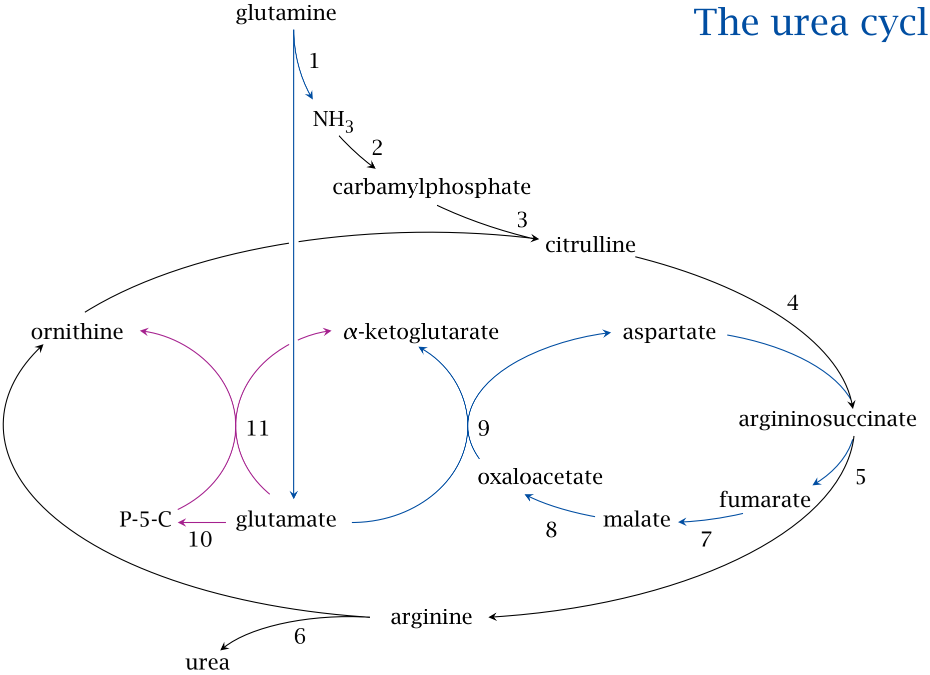
Ochratoxin A inhibits phenylalanyl-tRNA synthetase



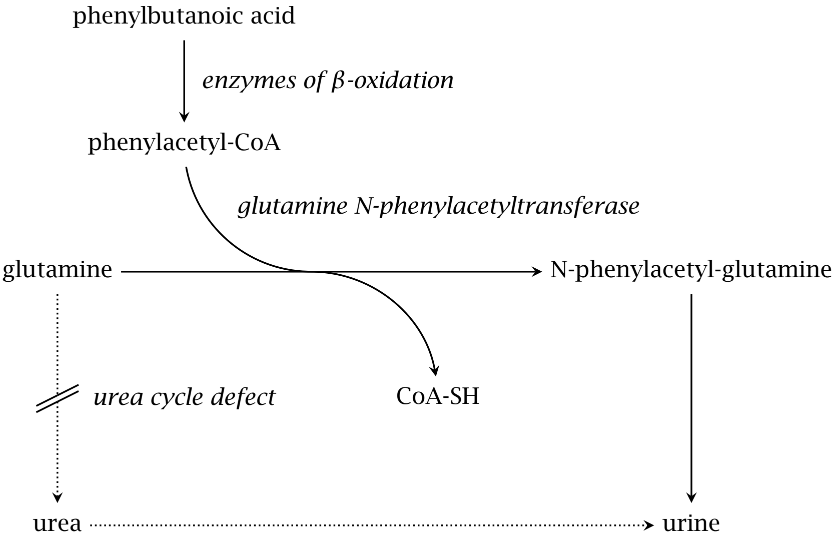
Tyrosinemia

- Homozygous defect of fumarylacetoacetate hydrolase
- Prevalence: 1 in 100,000 people worldwide; 1 in 1,850 in the Saguenay region (Quebec)
- Fumarylacetoacetate and preceding metabolites back up
- Fumaryl- and maleylacetoacetate react with glutathione and other cellular nucleophiles, causing liver toxicity, cirrhosis, carcinoma
- The drug NTBC inhibits *p*-hydroxyphenylpyruvate dioxygenase, intercepting the degradative pathway upstream of the toxic metabolites ▸ pathway

The urea cycle in context

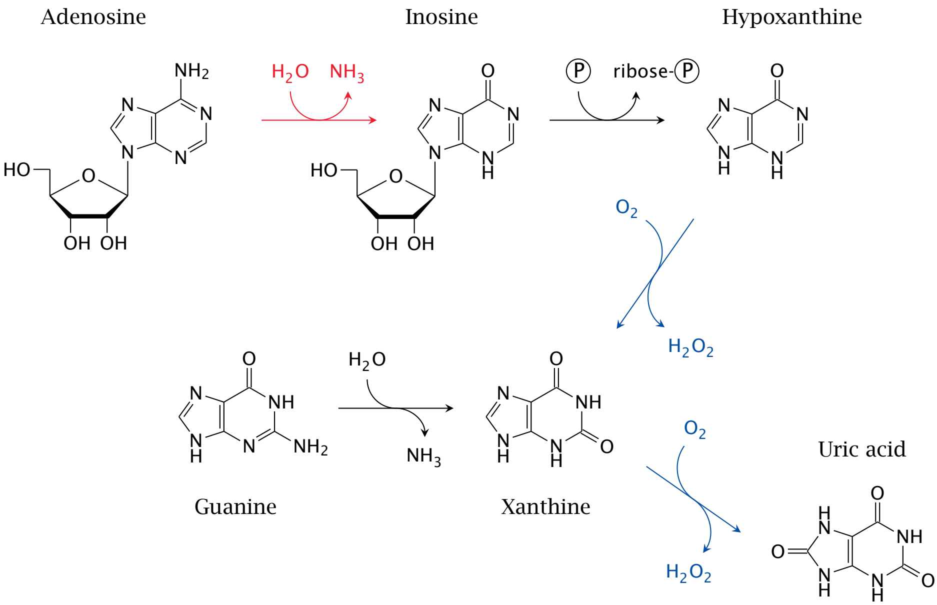


Alternate pathway therapy in urea cycle enzyme defects

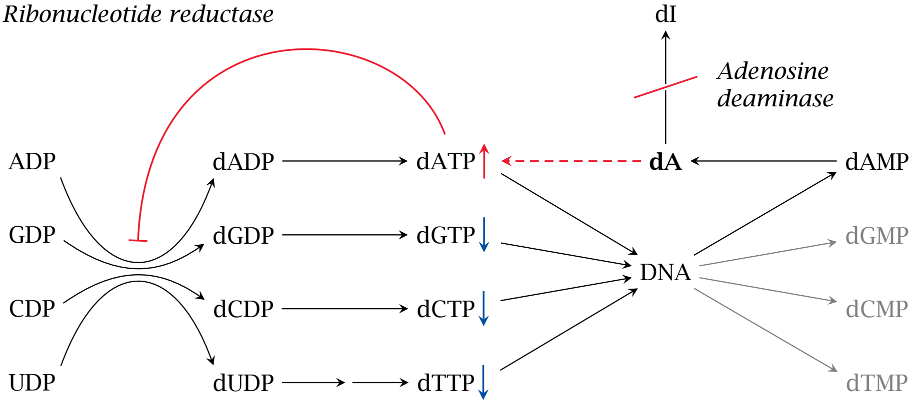


‣ Gln conjugation

Overview of purine degradation



Adenosine deaminase deficiency causes dysregulation of DNA synthesis



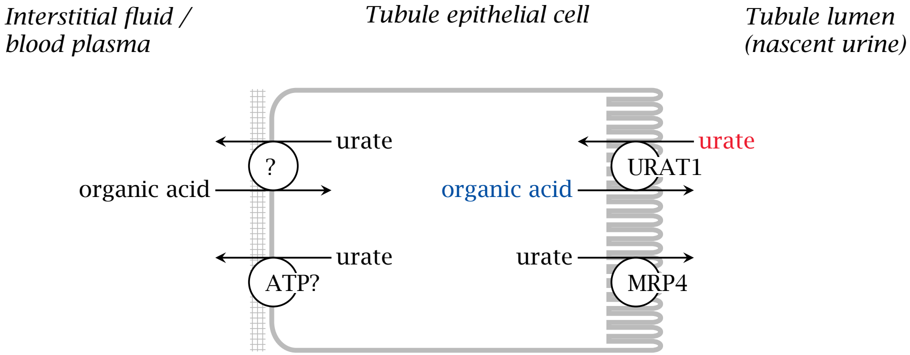
Therapy of adenosine deaminase deficiency

1. Bone marrow transplant
2. *Ex vivo* gene therapy
3. Enzyme replacement therapy—PEGylated bovine ADA
4. Experimental *in vitro* approach: Inhibition of salvage kinases

Gout

- Genetic or dietary factors promote increased production or retention of uric acid
- Uric acid has low solubility, and excess levels form crystalline deposits, preferentially in joints and soft tissue
- Urate crystals promote inflammation and lead to arthritis that is painful and destructive

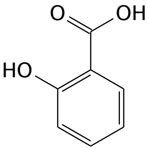
Transport of uric acid in the kidneys



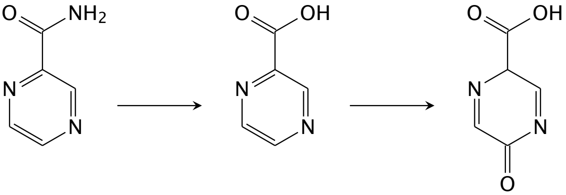
Dietary factors that promote gout

- Overly purine-rich food
- Drugs that contain purines: dideoxyadenosine
- Alcoholic beverages—but not all kinds: beer yes, wine no
- Anorexia nervosa (!)
- Drugs that interfere with uric acid excretion: pyrazinamide, salicylic acid
- Excessive fructose or sucrose

Drugs that may promote gout by promoting tubular reuptake of urate

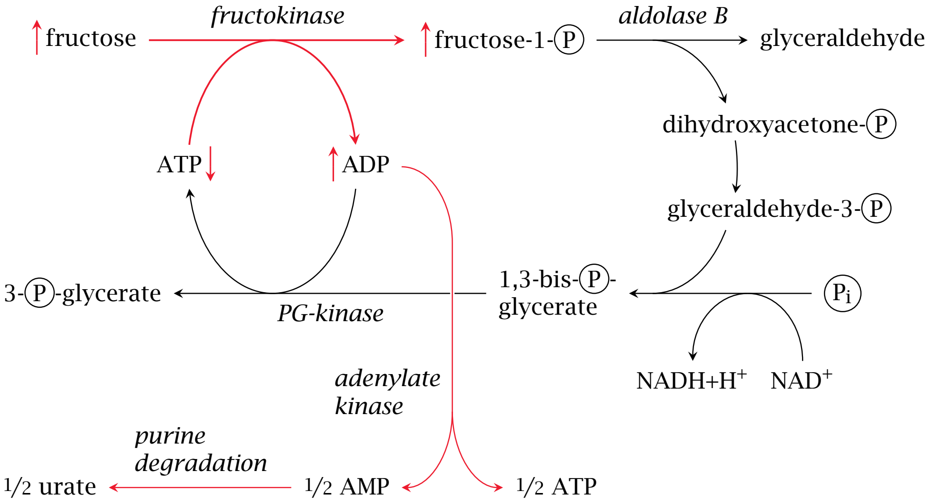


Salicylic acid

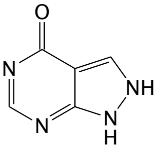


Pyrazinamide Pyrazinoate 5-Hydroxypyrazinoate

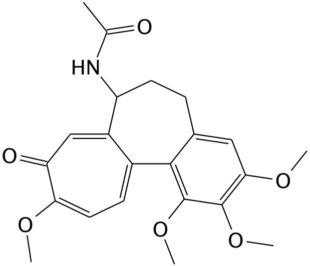
High dietary fructose promotes gout



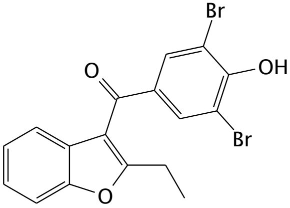
Drugs used in the treatment of gout



Allopurinol

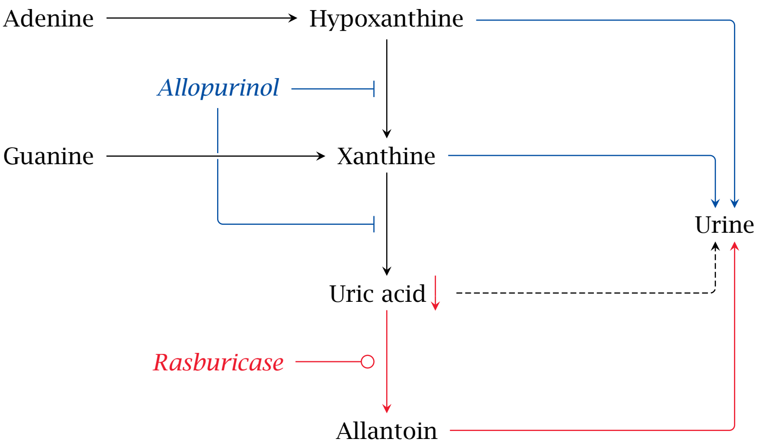


Colchicine



Benzbromarone

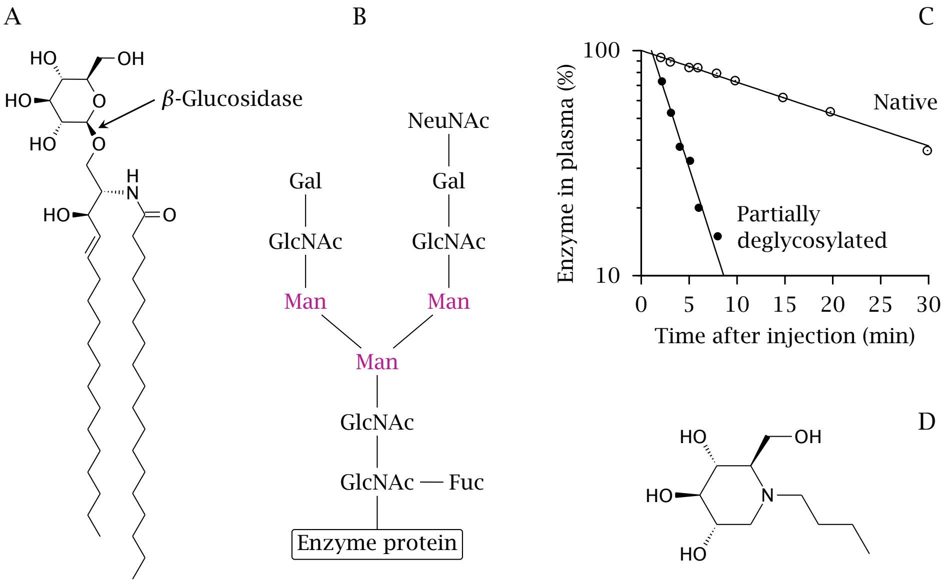
Complementary effects of allopurinol and “rasburicase”



Lysosomal storage diseases

- Acidic hydrolases in lysosomes break down many cellular macromolecules, including lipids and mucopolysaccharides
- Enzyme defects cause accumulation of undegraded substrates, often in liver, spleen, and bone marrow, leading to organ enlargement and loss of function
- Some enzyme defects can be treated with enzyme substitution therapy

Biochemistry of Gaucher disease

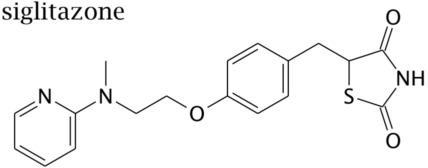


Diabetes mellitus

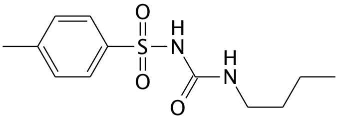
- Lack of insulin activity due to
  - destruction of pancreatic island β cell (type 1)
  - loss of insulin sensitivity in peripheral organs (type 2)
  - excessive levels of hormones antagonistic to insulin
- Blood glucose accumulates and causes acute and chronic pathology
- Treated with insulin substitution (type 1 and 2) and oral drugs (type 2)

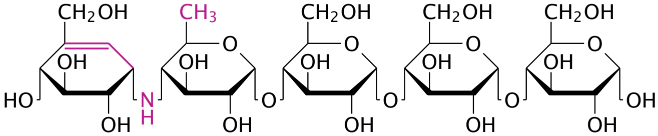
Oral antidiabetic drugs

Rosiglitazone

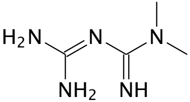


Tolbutamide





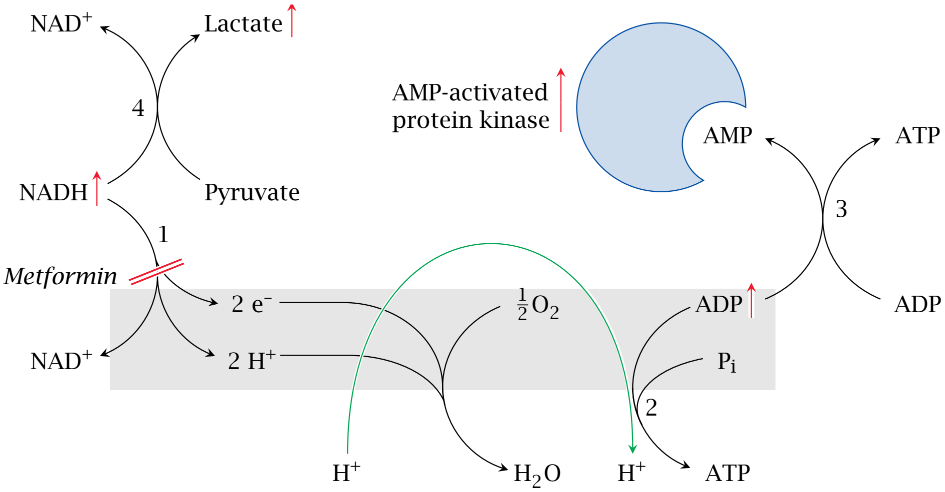
Acarbose



Metformin

- K_{ATP} channels

Hypothetical mode of action of metformin

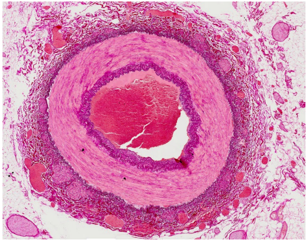


Atherosclerosis

- Degenerative and inflammatory disease of the arteries
- Promoted by hypercholesterolemia, hypertension, diabetes, smoking
- Damaged arteries subject to chronic or acute obstruction, hemorrhage
- Most common cause of death, ahead of all cancers and leukemias combined
- Treatment strategies address cholesterol levels, blood pressure, blood coagulation

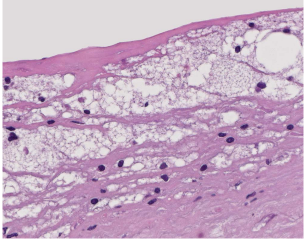
Appearance of atherosclerotic lesions

A



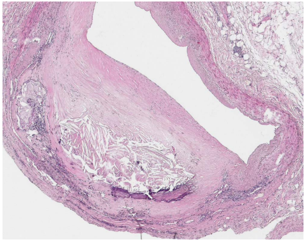
Normal artery

B



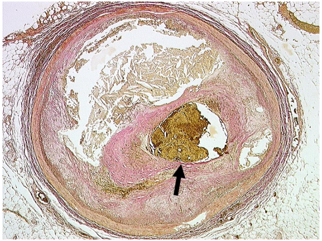
Foam cells in early lesion

C



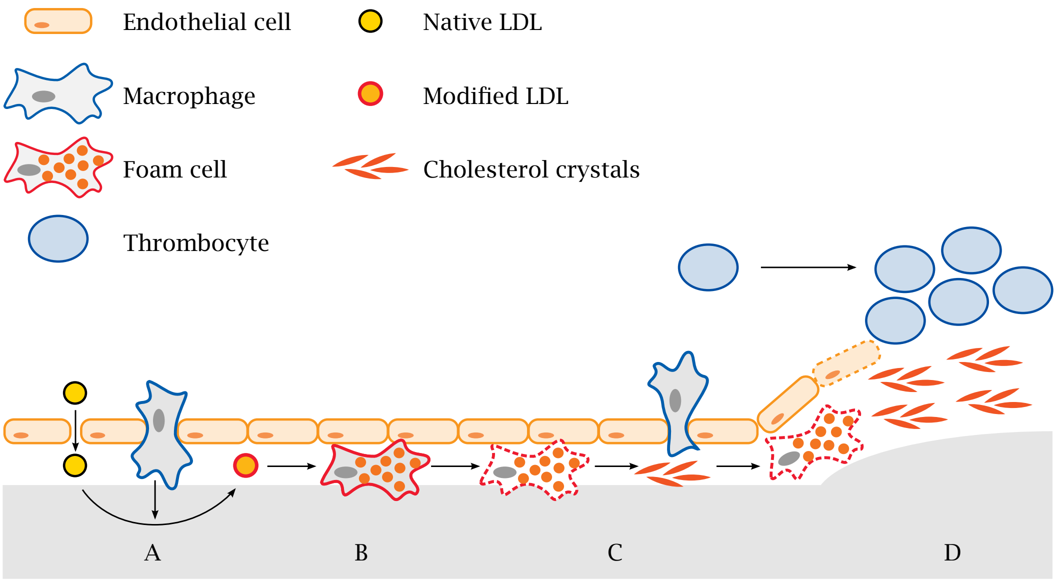
Detritus, fibrosis in advanced lesion

D

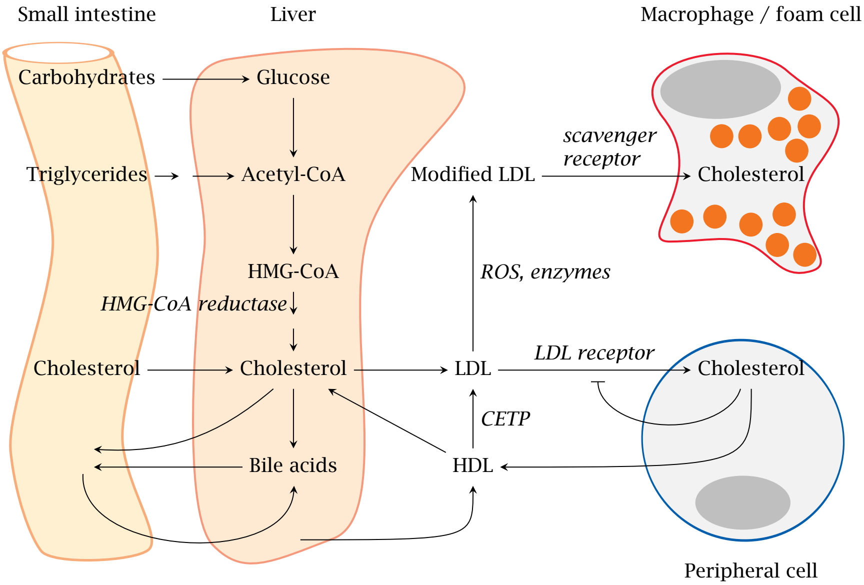


High-grade stenosis, thrombus

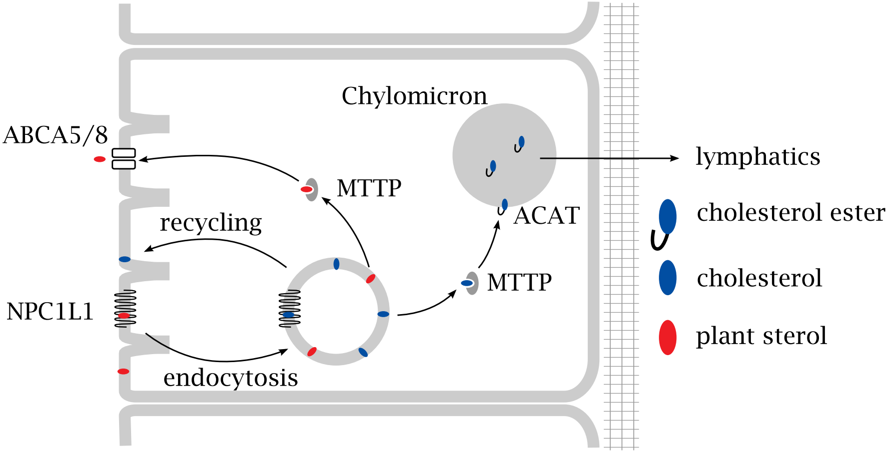
Development of an atherosclerotic lesion



Transport and metabolism of cholesterol

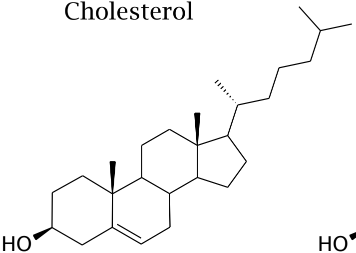


Intestinal cholesterol uptake

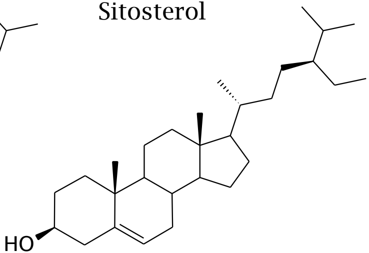


Inhibitors of intestinal cholesterol uptake

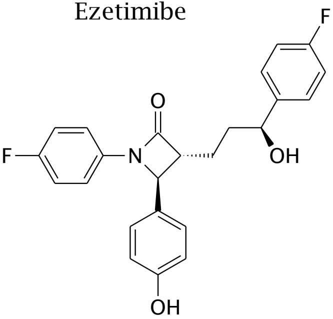
Cholesterol

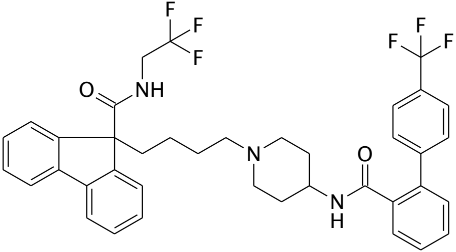


Sitosterol

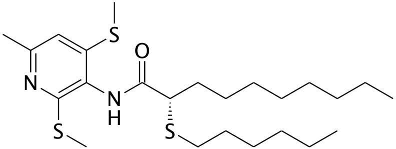


Ezetimibe





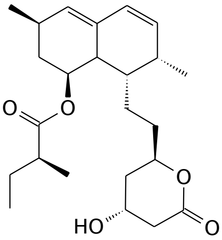
Lomitapide



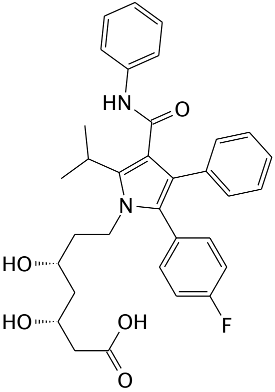
CP-113,818

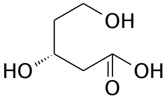
Inhibition of HMG-CoA reductase with statins

Mevastatin

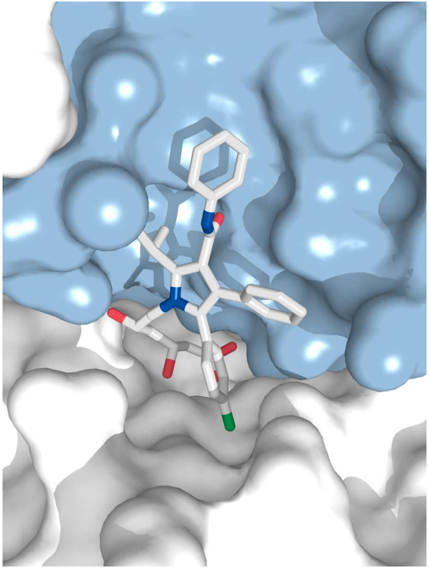


Atorvastatin

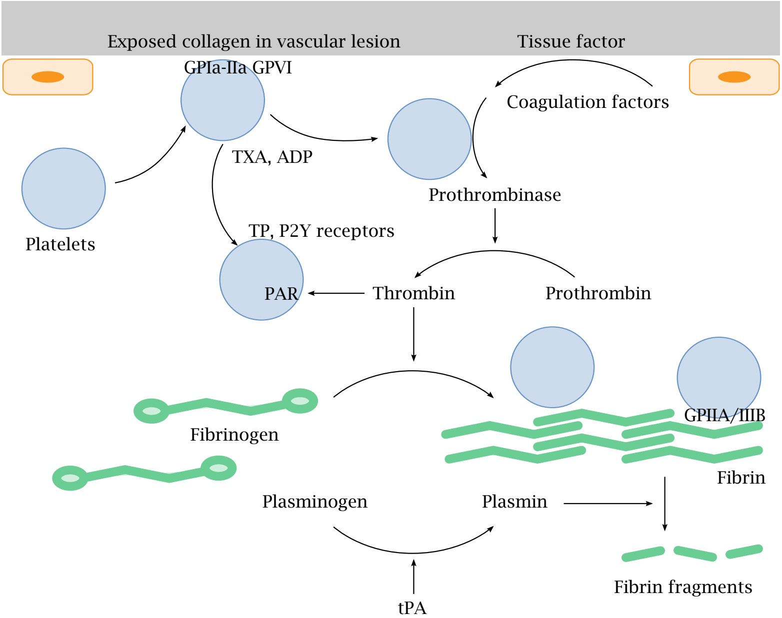




Mevalonate



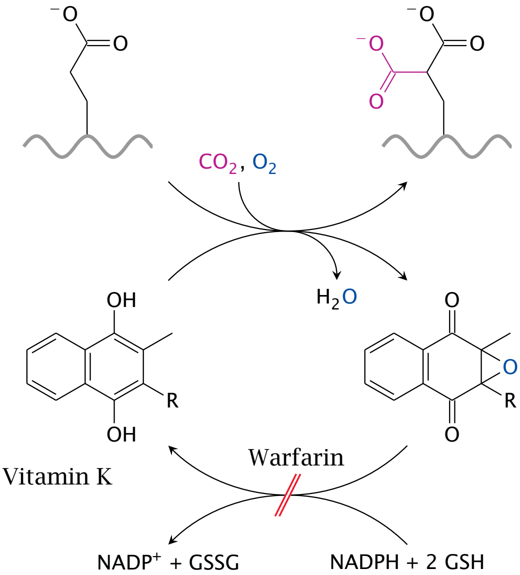
Overview of blood coagulation



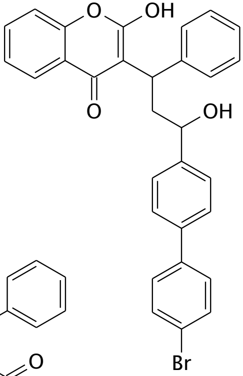
Strategies for inhibiting thrombocyte activity

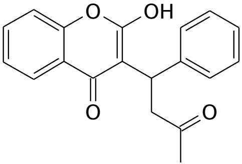
- Low-dose acetylsalicylic acid
- Inhibitors of P2Y receptors (e.g. ticlopidine)
- Inhibitors of thromboxane A synthase and thromboxane receptors (e.g. ramatroban)

Inhibition of plasmatic blood coagulation with warfarin



Bromadiolone





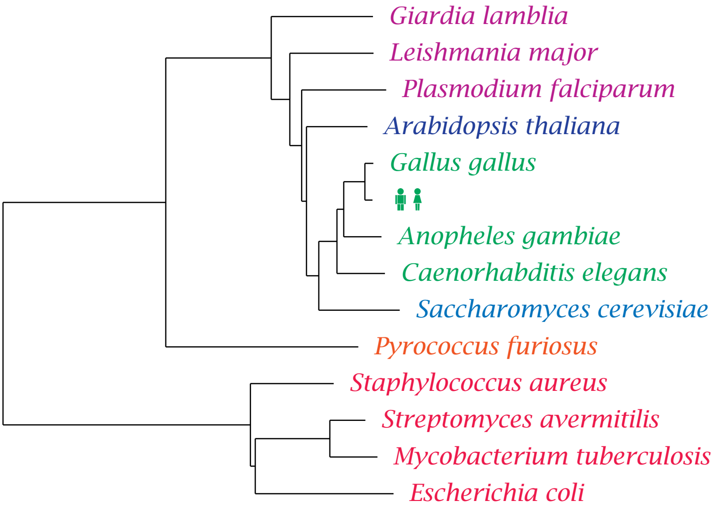
Warfarin

Chemotherapy of infectious diseases

Diversity of infectious pathogens

- Bacteria
- Fungi
- Parasites—eukaryotes other than fungi
  - Protozoa—unicellular
  - Metazoa—multicellular
- Viruses

The tree of life, slightly pruned

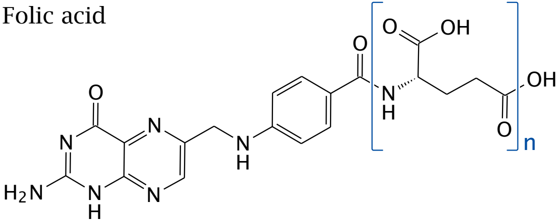


Drug targets for antimicrobial therapy

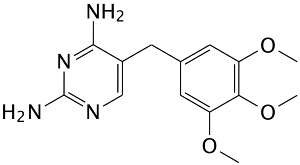
- Macromolecules that occur in the cells of the pathogen but not within the human host. Examples:
  - the bacterial cell wall (penicillin)
  - *de novo* synthesis of folic acid (sulfonamides)
- Macromolecules that occur in both humans and the pathogen but are structurally divergent. Examples:
  - ribosomes (chloramphenicol)
  - dihydrofolate reductase (trimethoprim)
  - DNA topoisomerase (ciprofloxacin)

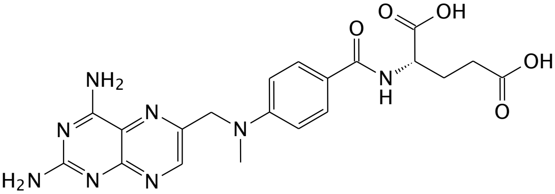
Structures of folic acid and of three inhibitors of dihydrofolate reductase

Folic acid

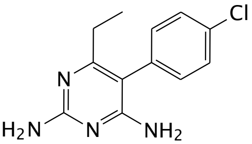


Trimethoprim





Methotrexate



Pyrimethamine

Microbial resistance mechanisms

- Mechanisms affecting the target:
  - Structural alteration / mutation
  - Compensatory overexpression
- Mechanisms affecting the drug:
  - Reduced uptake
  - Active extrusion
  - Enzymatic inactivation

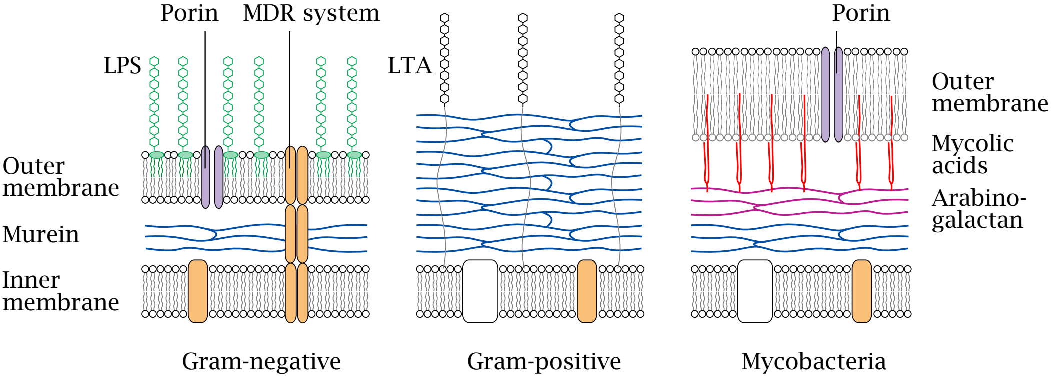
Overview of antibacterial chemotherapy

- Targets
  - Cell wall
  - Ribosomes
  - Enzymes related to cell division
  - Intermediate metabolism
- Antibiotic resistance
  - Bacteria have short generation times—fast *de novo* evolution of resistance
  - Resistance genes exist in producers of antibiotics—can spread to pathogenic bacteria by gene transfer

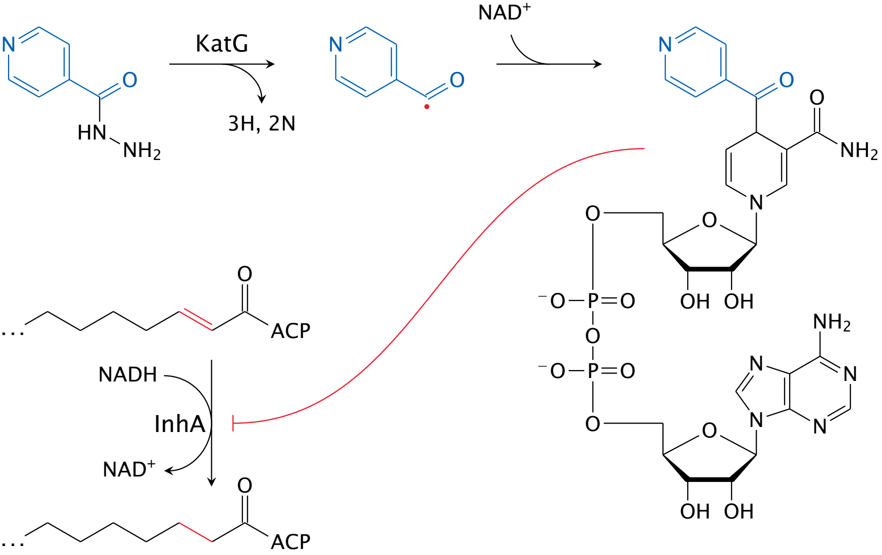
Gene transfer mechanisms in bacteria

- Transformation: cellular uptake of naked DNA
- Conjugation: plasmid-encoded active transfer between bacterial cells
- Transduction: gene transfer mediated by bacteriophages
- Transposons: transfer of genes between carrier DNA molecules (chromosomes, plasmids)

Bacterial cell wall structure

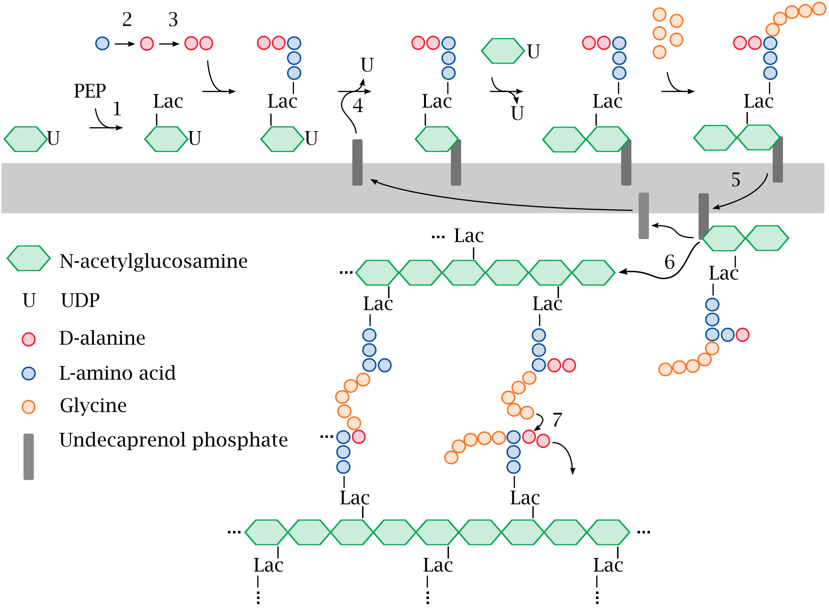


Action mechanism of isoniazid (INH)

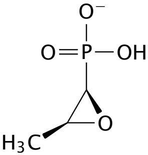


‣ Isoniazid metabolism

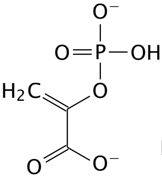
Outline of bacterial murein synthesis



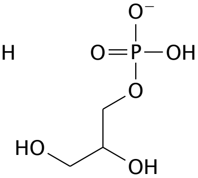
Fosfomycin mimics both phosphoenolpyruvate and glycerophosphate



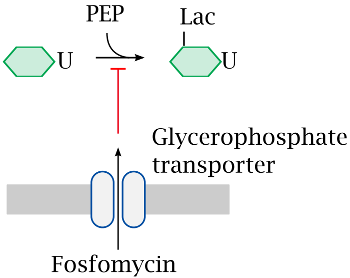
Fosfomycin



PEP



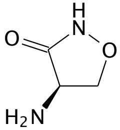
Glycerophosphate



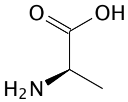
Cycloserine inhibits alanine racemase and D-alanine ligase

D-cycloserine

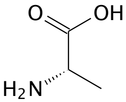
$$2\ \text{L-Ala} \longrightarrow 2\ \text{D-Ala} \longrightarrow \text{D-Ala-D-Ala}$$



D-cycloserine

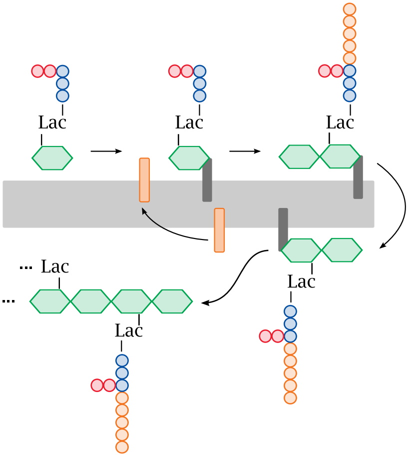


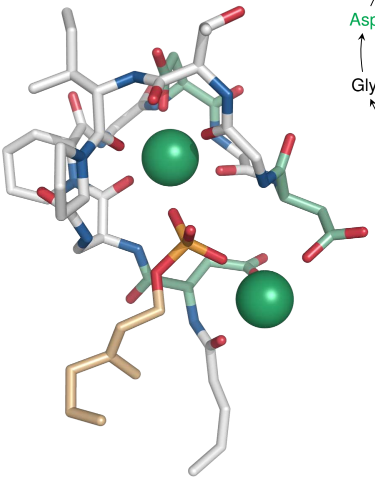
D-alanine

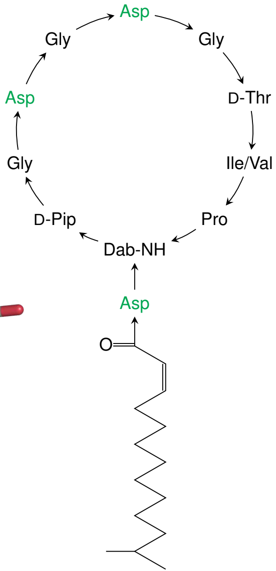


L-alanine

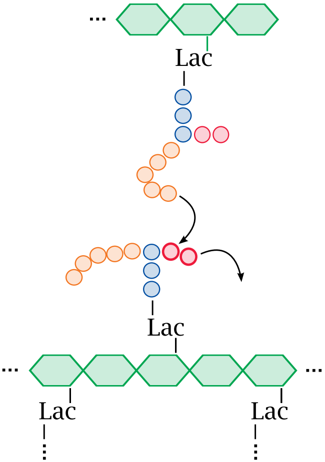
The lipopeptide laspartomycin sequesters undecaprenol phosphate

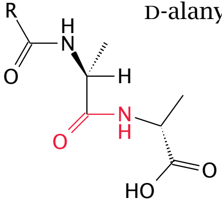




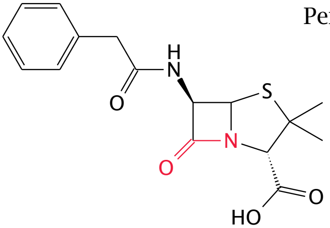


β-Lactam antibiotics resemble the substrate of the transpeptidase reaction



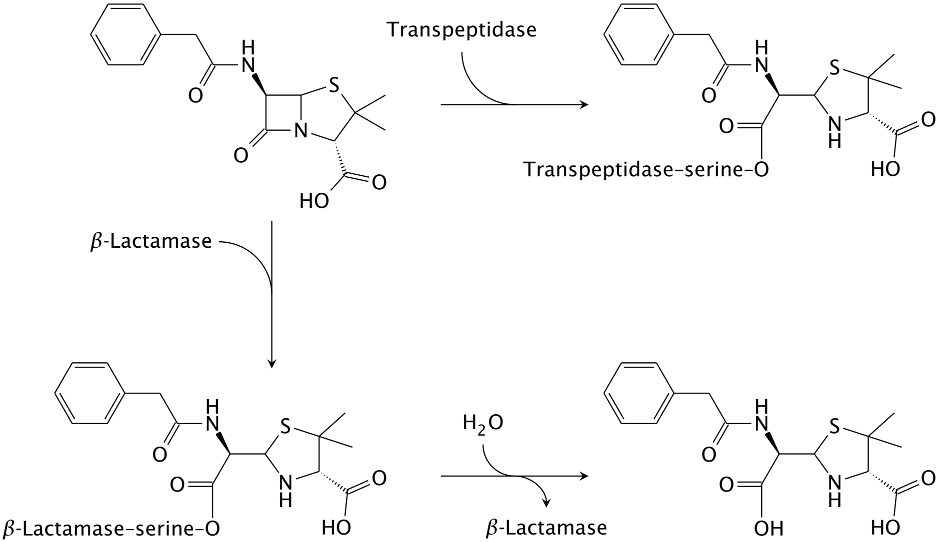


D-alanyl-D-alanine



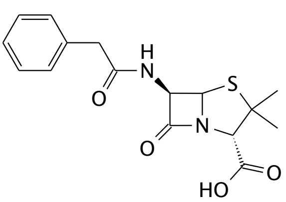
Penicillin G

Reactions of penicillin G with transpeptidase and β-lactamase

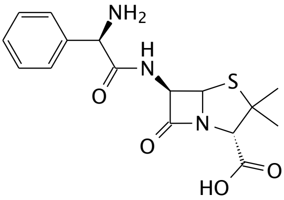


Structures of β-lactam antibiotics (1)

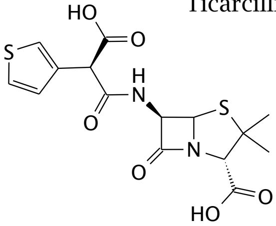
Penicillin G



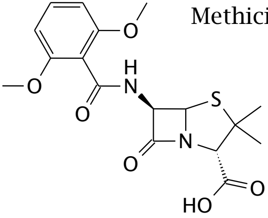
Ampicillin



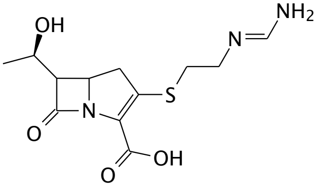
Ticarcillin



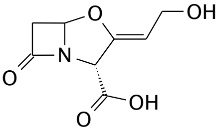
Methicillin



Imipenem



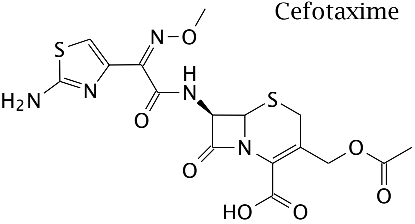
Clavulanic acid



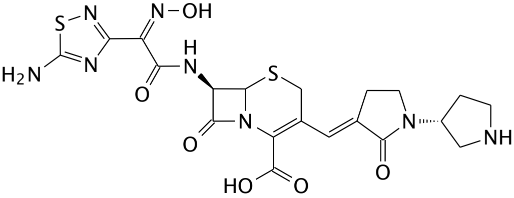
‣ penicillin G spectrum

Structures of β-lactam antibiotics (2)

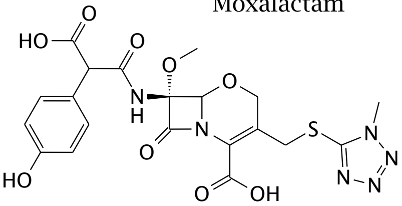
Cefotaxime



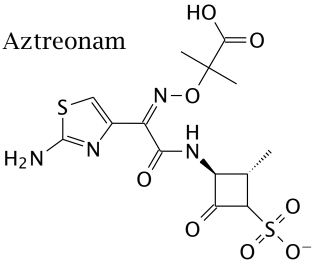
Ceftobiprole



Moxalactam

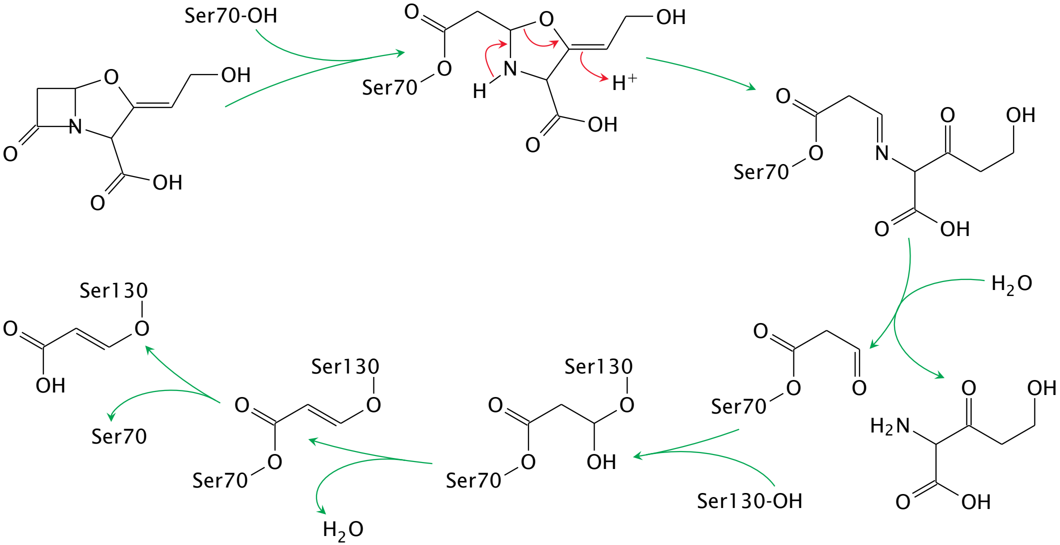


Aztreonam

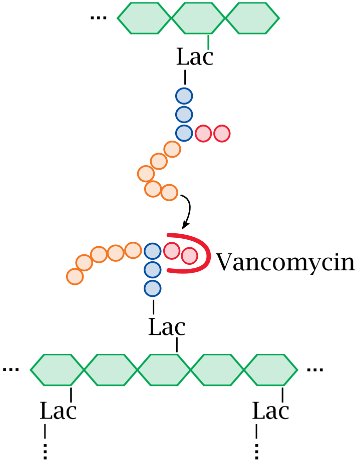


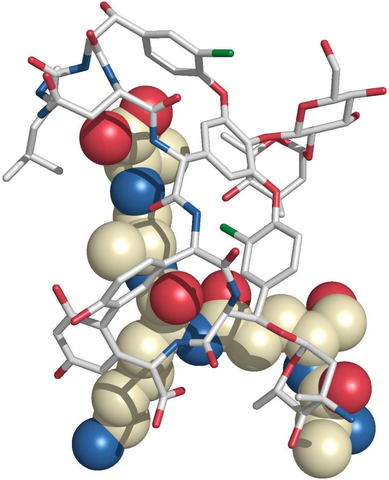
▸ warfarin

Inactivation of SHV-1 β-lactamase by clavulanic acid



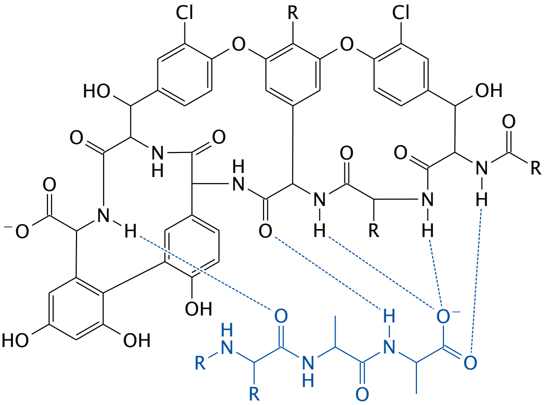
Vancomycin sequesters the substrate of the transpeptidase reaction





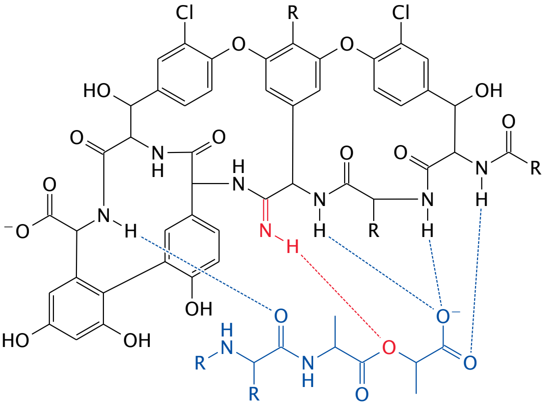
Vancomycin can be modified to overcome bacterial resistance

Vancomycin



D-Ala—D-Ala

Vancomycin amidine derivative

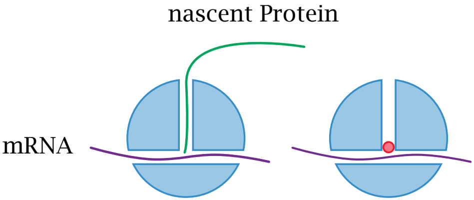


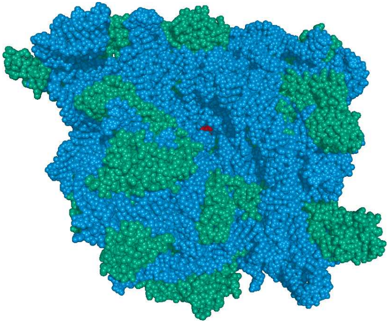
D-Ala—D-Lac

Antibiotics that inhibit ribosomal protein synthesis

- Aminoglycosides
- Tetracyclines
- Macrolides
- Chloramphenicol
- Puromycin
- ...

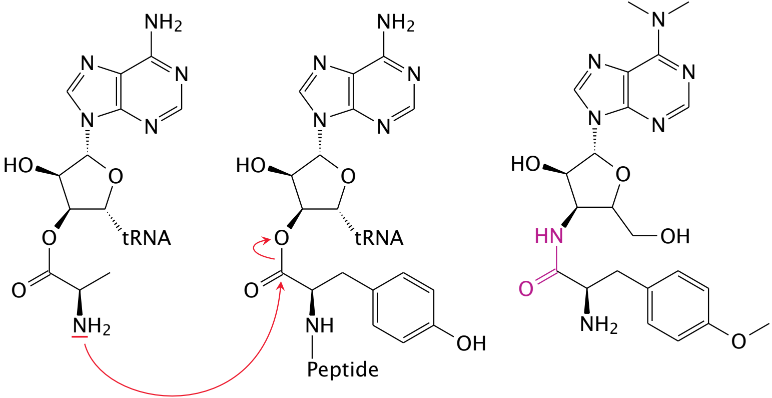
Chloramphenicol lodges into the peptidyl-transfer site of the ribosome



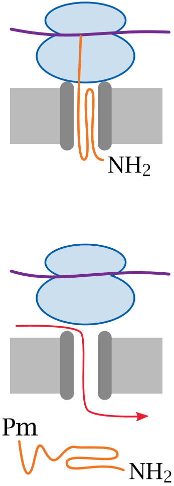


- chloramphenicol base interactions

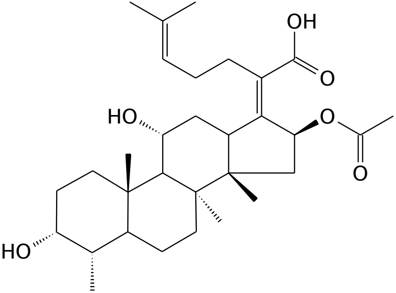
Structure and action mechanism of puromycin



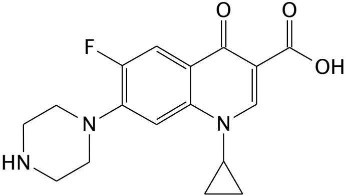
Aminoacyl-tRNA   Peptidyl-tRNA   Puromycin



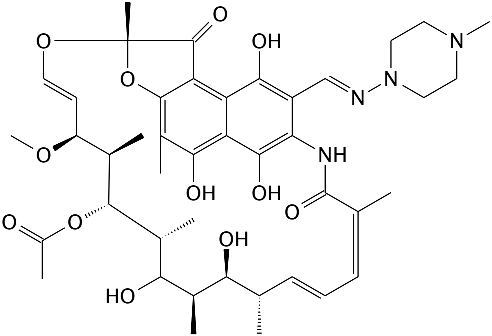
Diverse inhibitors of bacterial macromolecular synthesis



Fusidic acid (Elongation factor 2)

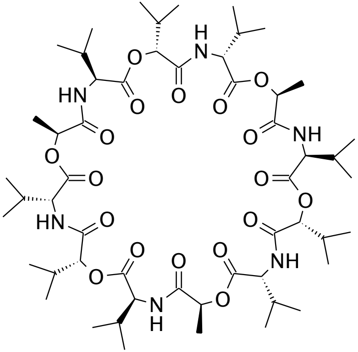


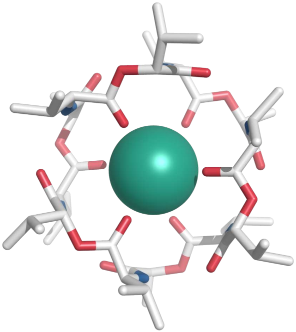
Ciprofloxacin (DNA gyrase)

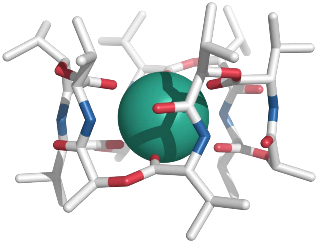


Rifampicin (mRNA transcription)

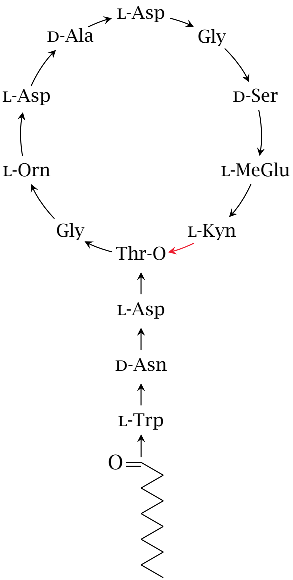
Structure of the potassium ionophore valinomycin

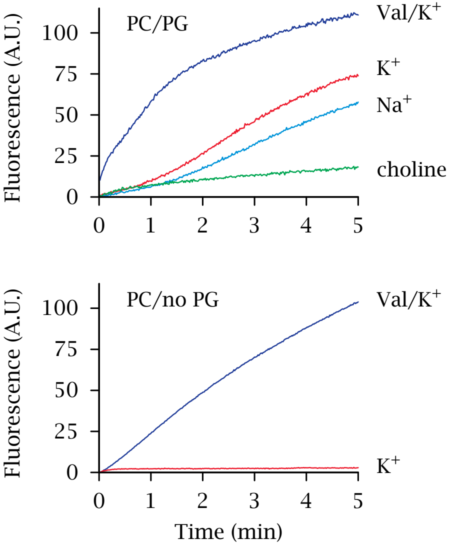






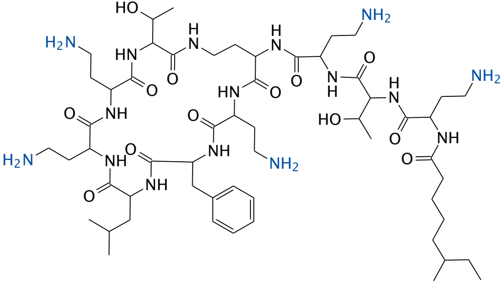
Daptomycin permeabilizes membranes containing phosphatidylglycerol



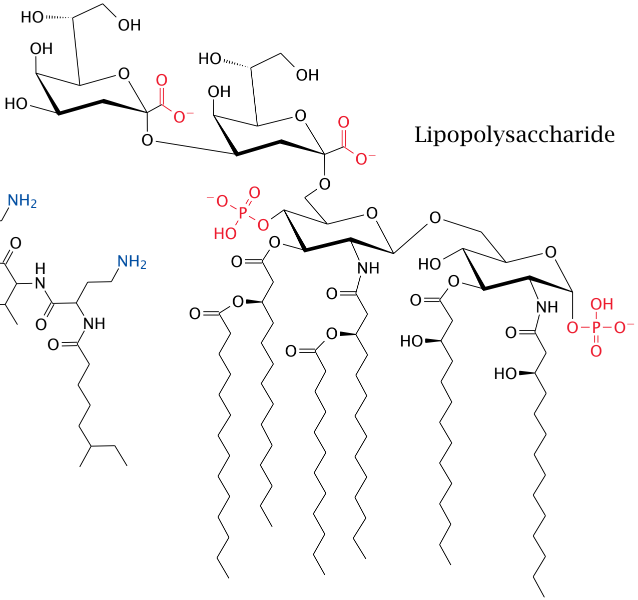


Structures of polymyxin B and lipopolysaccharide

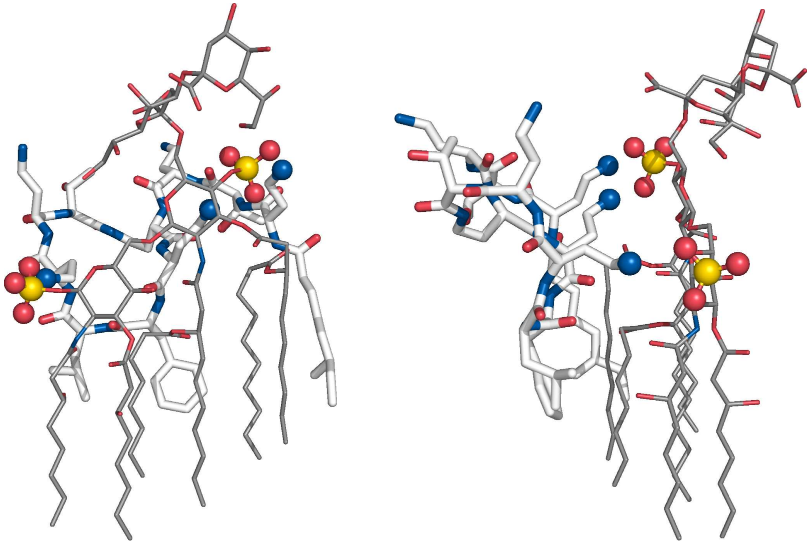
Polymyxin B



Lipopolysaccharide



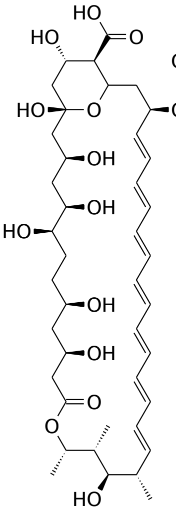
A model of the polymyxin B–LPS complex



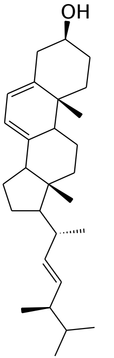
Drug targets in antifungal chemotherapy

- ergosterol in cell membranes (amphotericin B)
- ergosterol synthesis (ketoconazole; terbinafine)
- thymidylate synthase (5-fluorocytosine)
- 1,3-β-glucan synthesis (echinocandins)

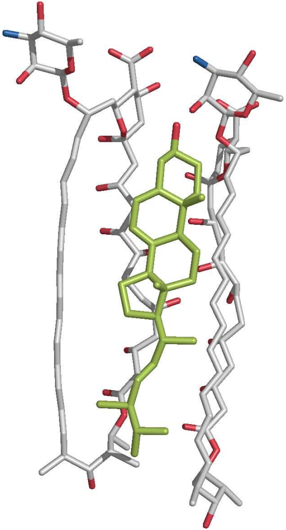
Structure and action mode of amphotericin B

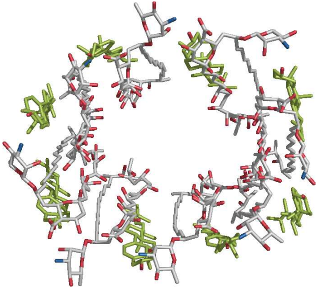


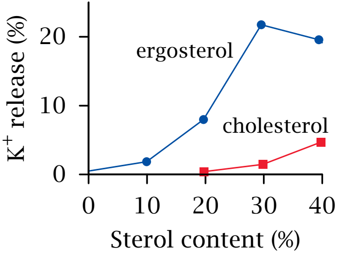
Amphotericin B



Ergosterol



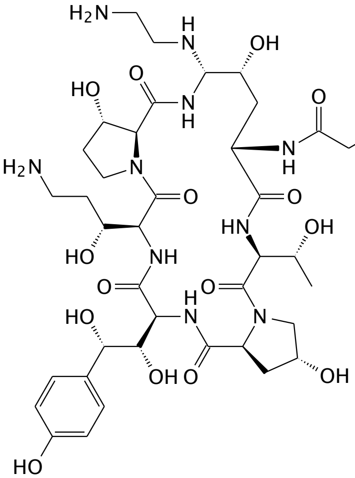


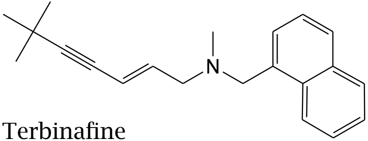


‣ drug delivery

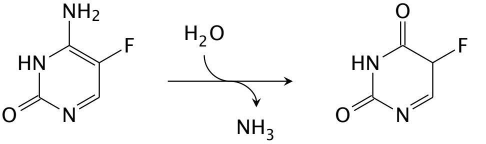
Other antifungal drugs

Caspofungin





Terbinafine

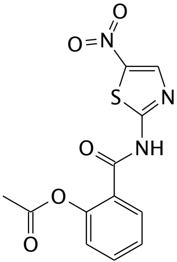


Flucytosine 5-Fluorouracil

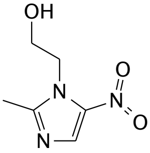
▸ ketoconazole

Antiprotozoal drugs

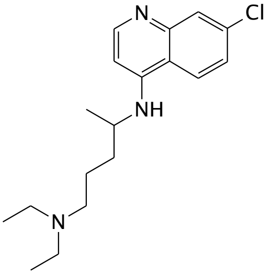
Nitazoxanide



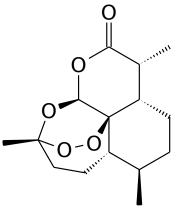
Metronidazole



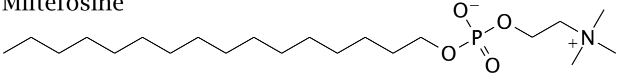
Chloroquine



Artemisinin

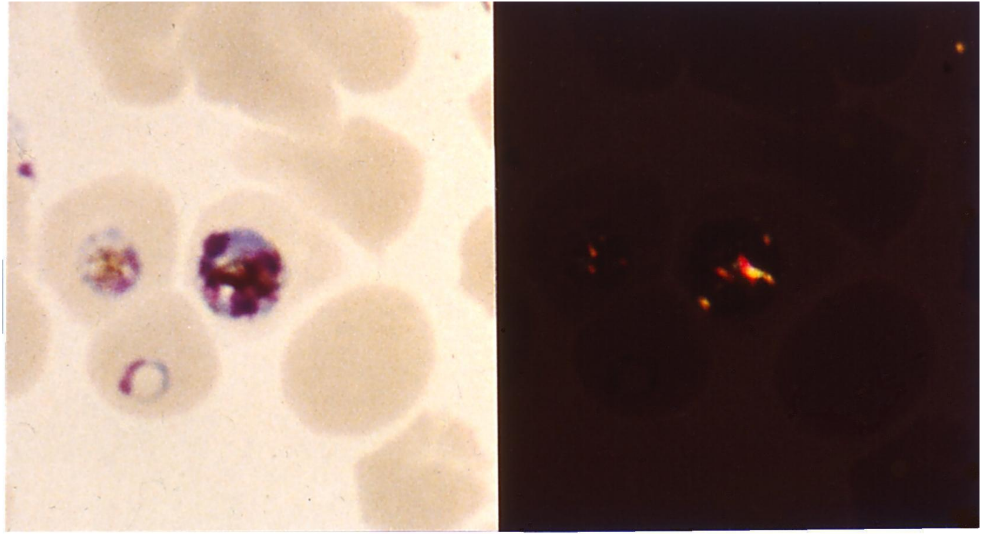


Miltefosine

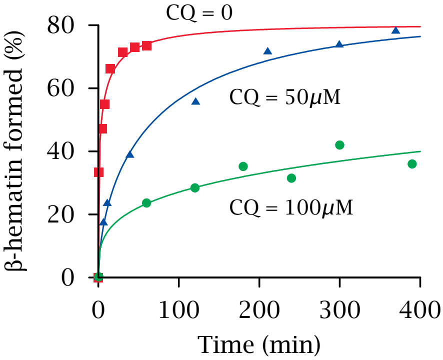


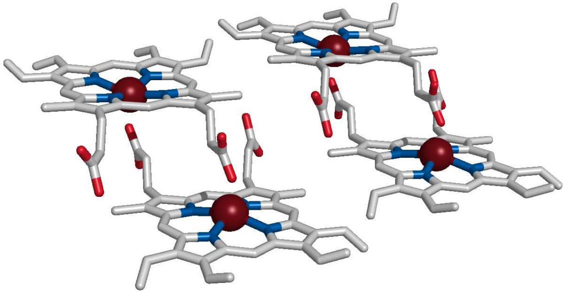
▸ tree of life

Hemozoin inside malaria parasites inside red blood cells

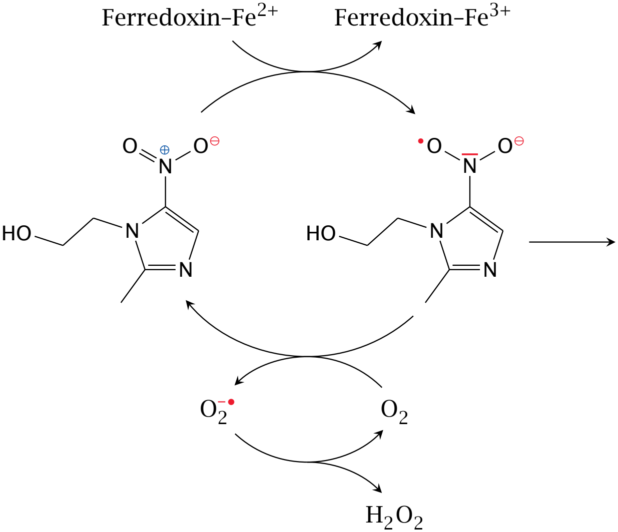


Chloroquine inhibits formation of β-hematin





Action mechanism and selective toxicity of nitroimidazoles

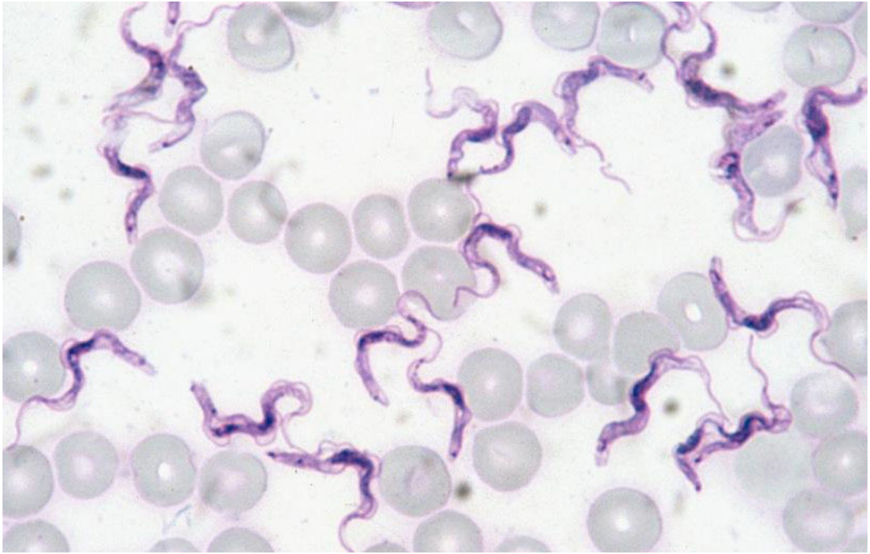


Reductive radical formation (aerobes and anaerobes)

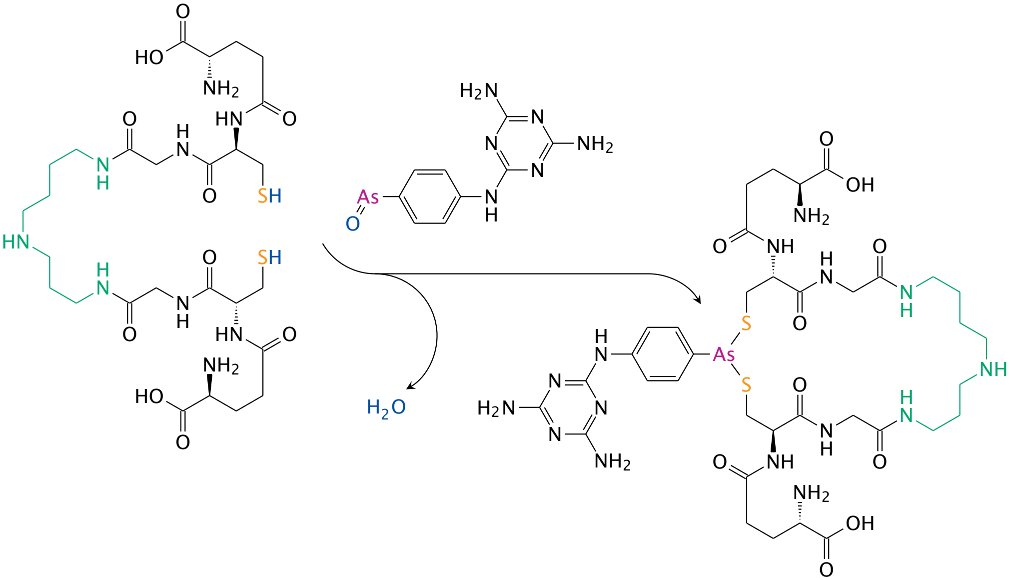
DNA damage (anaerobes)

Oxygen-mediated radical detoxification (aerobes)

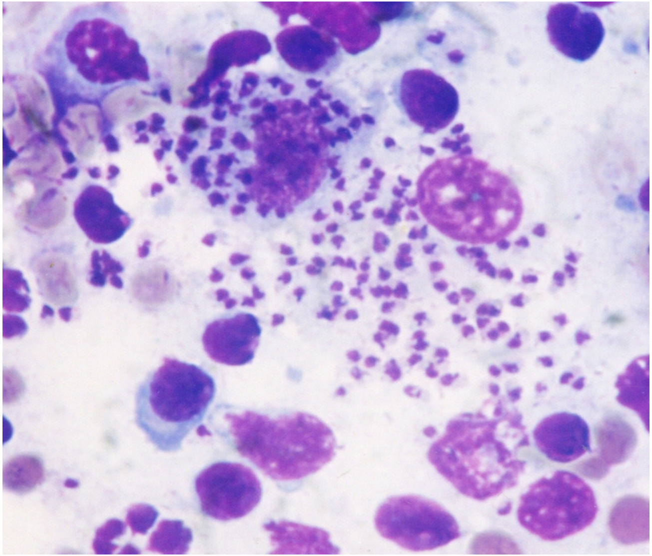
Trypanosomes in a blood smear



Melarsene oxide binds trypanothione



Leishmania in bone marrow

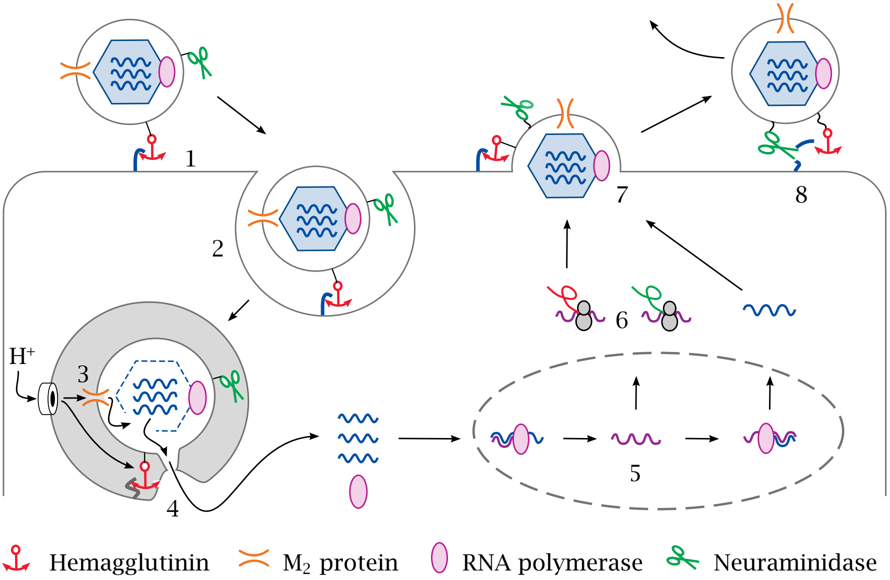


Antiviral chemotherapy

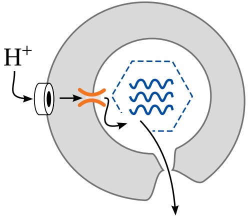
Viruses are diverse:

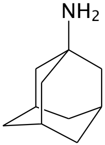
- The genome may be
  - small (5 kbp) or large (>300 kbp)
  - RNA or DNA
  - segmented or a single molecule
  - single-or double-stranded
  - if single-stranded, plus-or minus stranded
  - inserting itself into the host cell genome (retroviruses) or replicating independently (others)
- Proteins may be expressed separately or as a single polyprotein
- Propagation may be transient (hepatitis A) or persistent (hepatitis B)
- The virion may be naked or enveloped

The life cycle of influenza virus

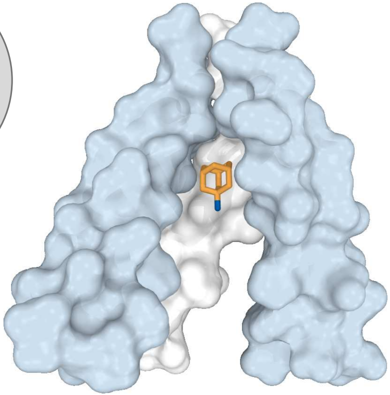


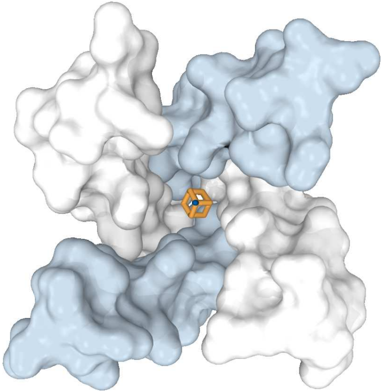
Amantadine blocks the M_2 proton channel



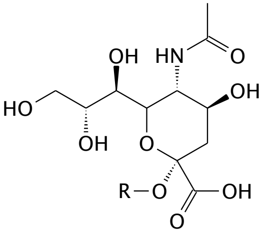


Amantadine

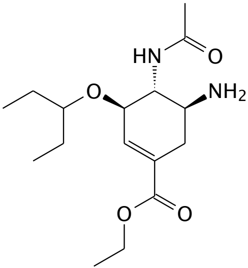




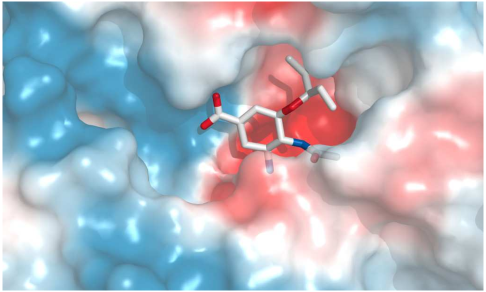
Oseltamivir inhibits influenzavirus neuraminidase



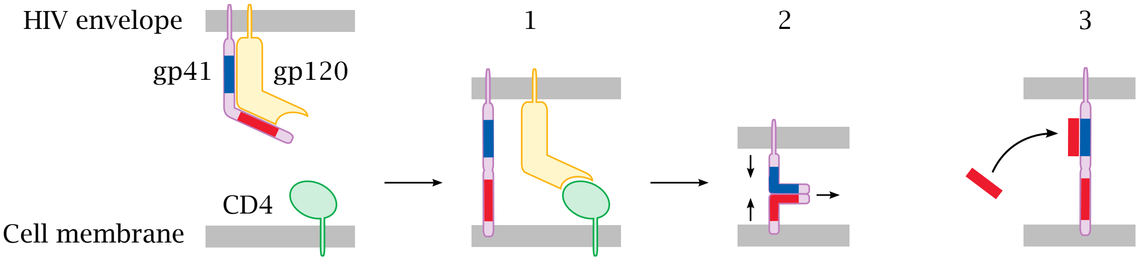
Neuraminic acid residue



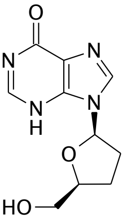
Oseltamivir



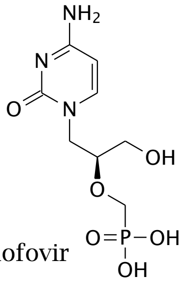
Inhibition of HIV fusion with target cells by the peptide enfuvirtide



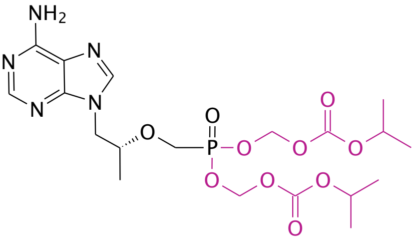
Inhibitors of virus genome replication



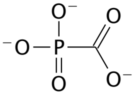
Didanosine



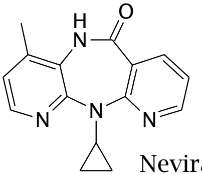
Cidofovir



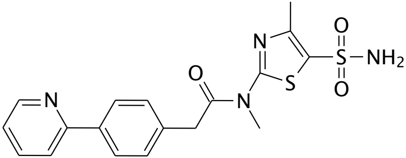
Tenofovir disoproxil



Foscarnet

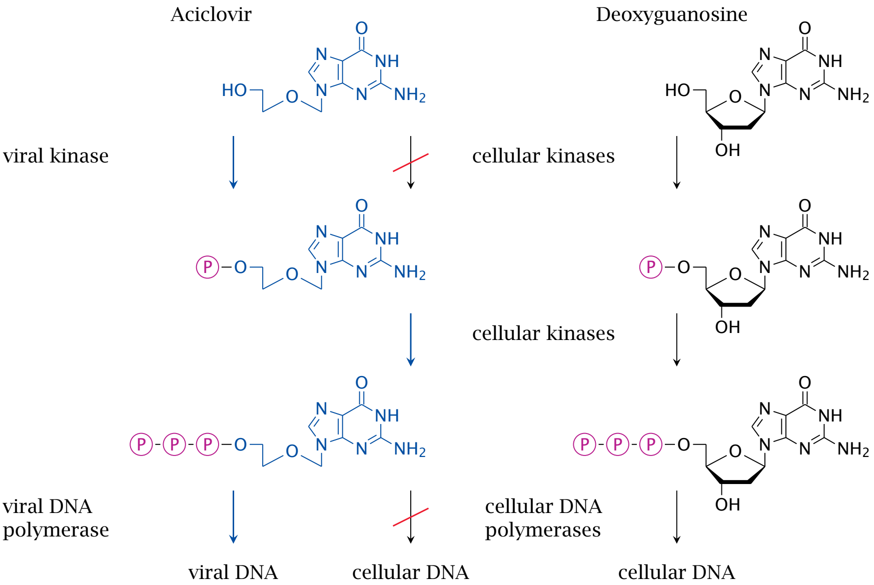


Nevirapine

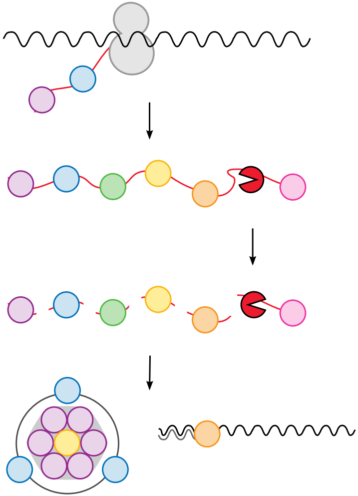


Bay 57-1293 (Herpes virus helicase/primase)

Activation of acyclovir



Function of virus proteases



- saquinavir

Cancer chemotherapy

Some terminology

- Malignant tumour / cancer: A tumour that
  - grows without regard for anatomical boundaries
  - typically also metastasizes
- Benign tumour: a tumour that does neither
- Carcinoma: a malignant tumour derived from an epithelial tissue
  - colon, lung, kidney carcinomas
  - hepatocellular carcinoma (liver)
- Sarcoma: a malignant tumour derived from a non-epithelial tissue
  - lipo-, myo-, fibro-, osteosarcoma
- Leukemia: a malignancy of white blood cell precursors
  - Lymphatic leukemia (related to lymphocyte precursors)
  - myeloic leukemia (related to granulocyte or monocyte precursos)
  - *Rare*: Erythremia/Erythroleukemia

Cancer therapy

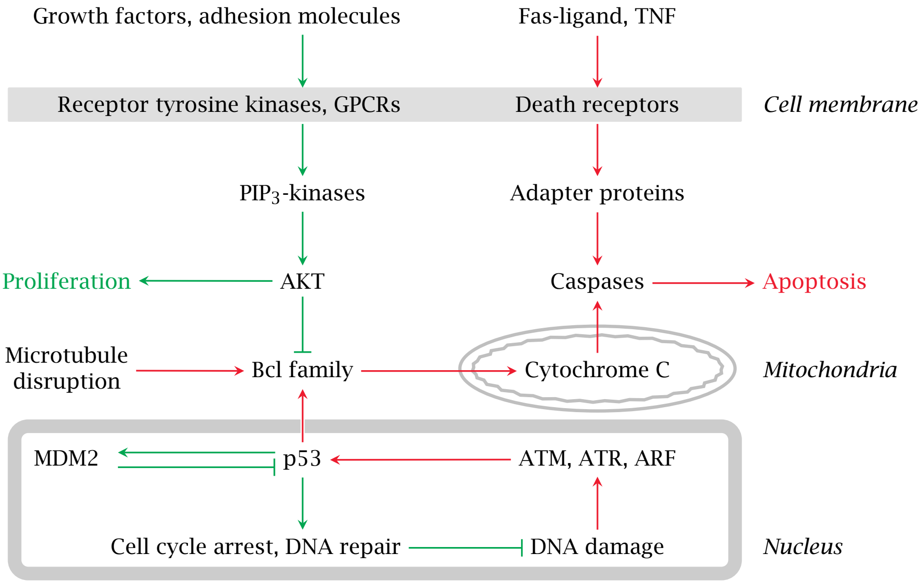
Forms of therapy

- Surgery
- Radiation
- Chemotherapy

Criteria for therapy selection

- Benign or malignant tumor
- Tissue of origin, histological variant of tumor
- Stage of tumor—early and localized *vs.* advanced and disseminated

Cellular pathways that control proliferation and apoptosis



Dysregulation of growth in tumor cells

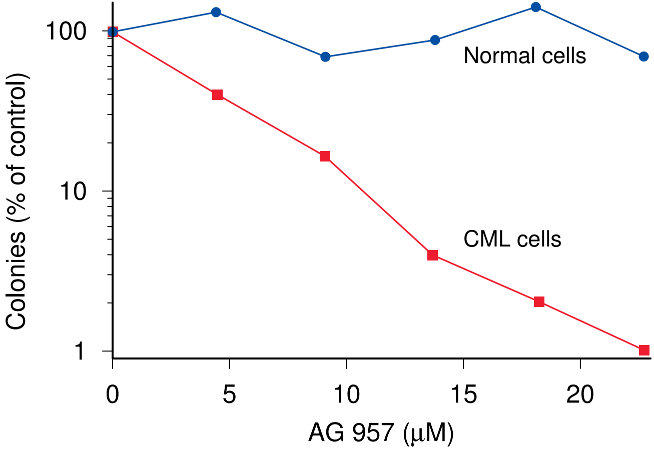
Normal body cells

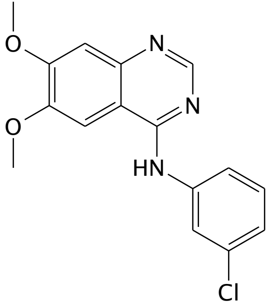
- grow or persist only when stimulated by growth factors
- undergo apoptosis when deprived of growth factor stimulation

Tumor cells contain mutations that

- create surrogate growth stimuli
  - constitutively active growth factor receptors
  - *autocrine* secretion of growth factors
- disrupt activation of apoptosis downstream of growth factor deprivation
- *but also* make tumor cells *more* susceptible to *some* apoptotic stimuli than normal cells

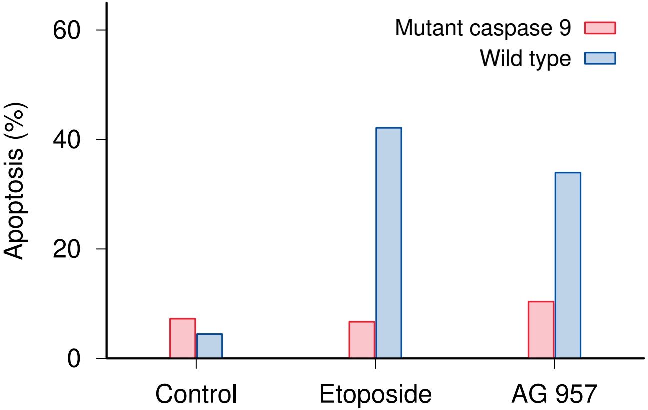
Leukemic (CML) cells are more susceptible to a tyrosine kinase inhibitor (AG 957)





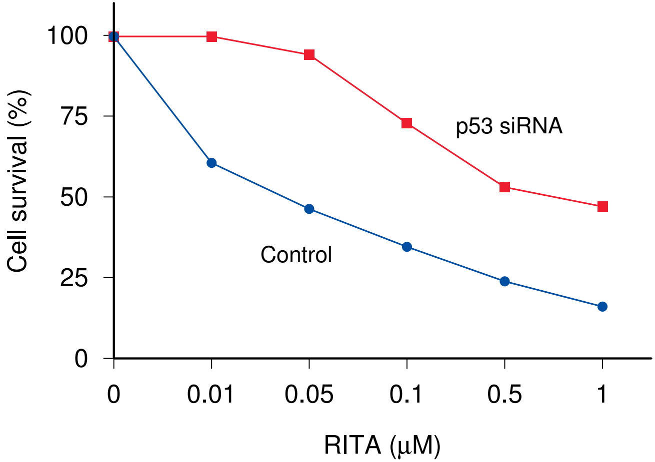
‣ apoptosis pathways

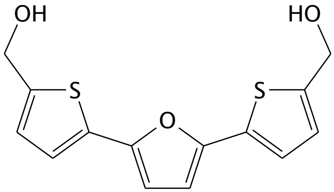
A mutant of caspase 9 inhibits apoptosis in response to anticancer drugs



▸ apoptosis pathways

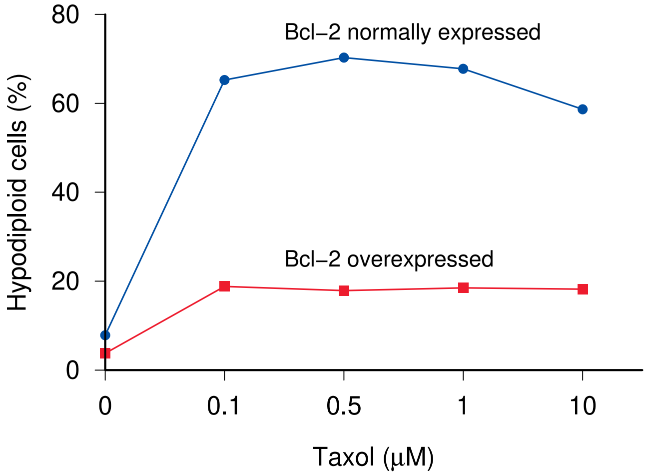
Knock-down of p53 promotes survival of cells treated with an inhibitor of MDM2

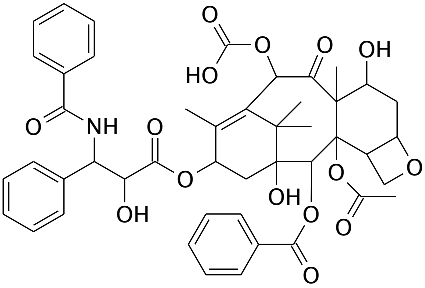




‣ apoptosis pathways

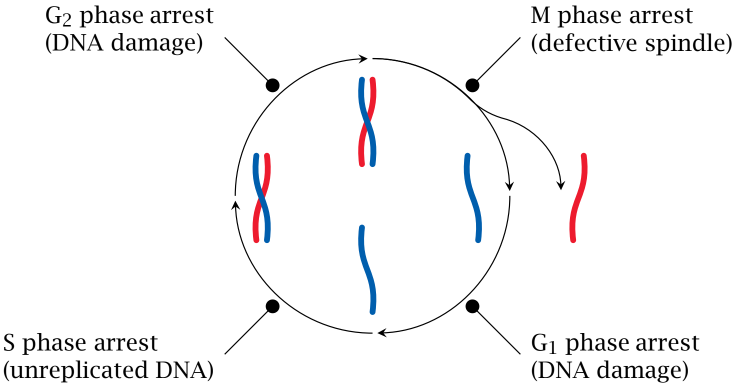
Disruption of microtubules with taxol induces apoptosis, which is inhibited by Bcl-2



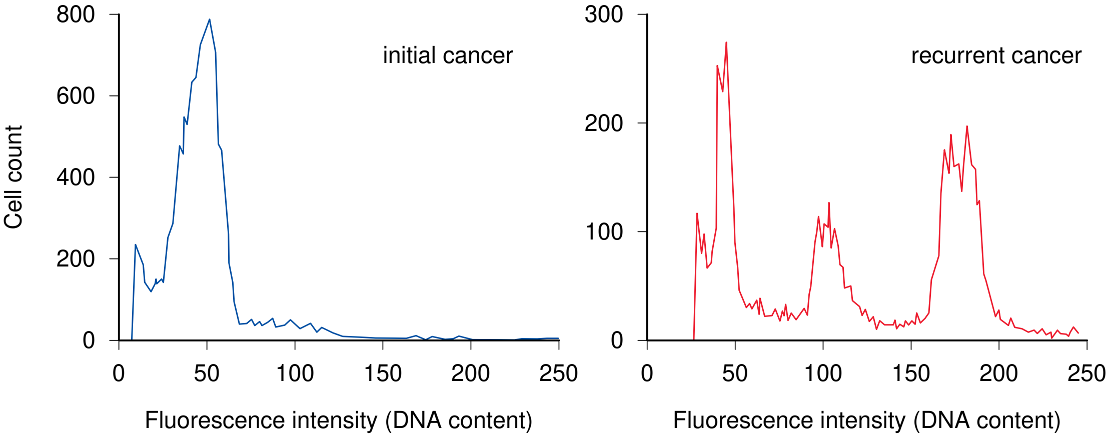


▸ apoptosis pathways

The cell cycle and its checkpoints



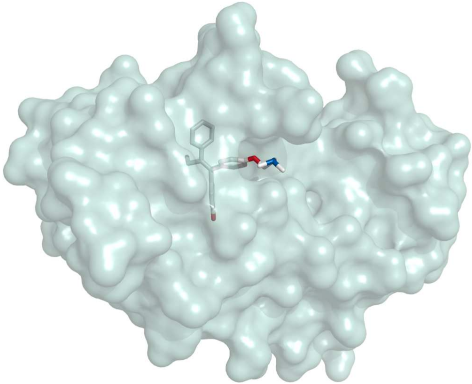
Progressive cell aneuploidy in a recurring tumor

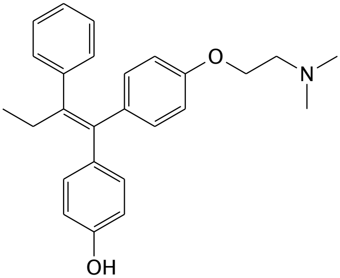


Cell type-specific anticancer drugs

- Hormones and growth factors: interferon-α in hairy cell leukemia
- Hormone antagonists: most significant with breast and prostate cancer
- Tissue-specific prodrug activation: mitotane in adrenal gland tumors
- Tissue-specific accumulation of radioactive iodine: thyroid cancer

Sexual hormones and receptor antagonists

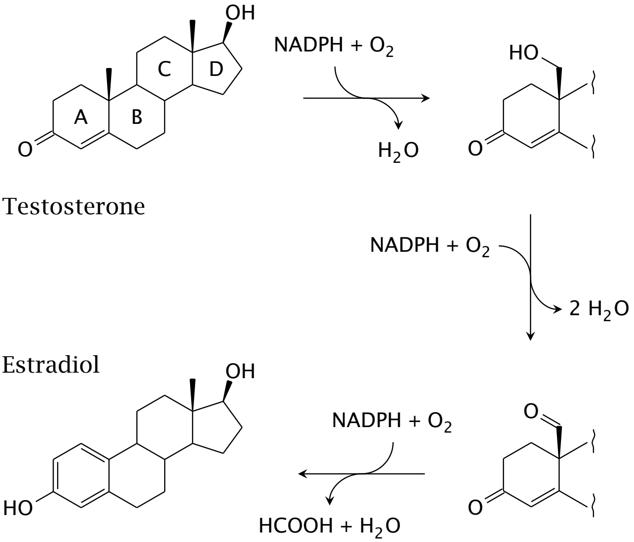




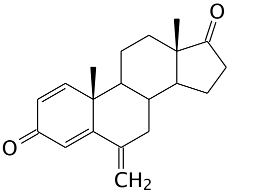
4-Hydroxytamoxifen

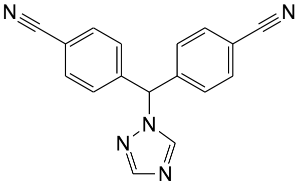
- mifepristone

Aromatase and two of its inhibitors



Exemestane

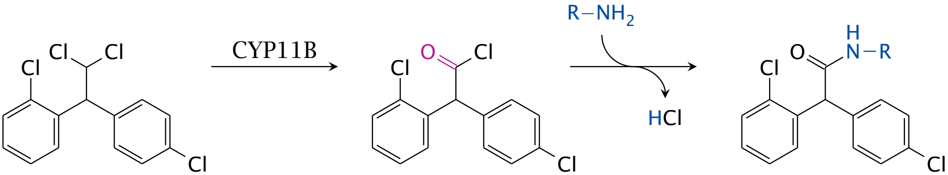




Letrozole

‣ estrogen biosynthesis

The anticancer prodrug mitotane is selectively activated in the adrenal cortex

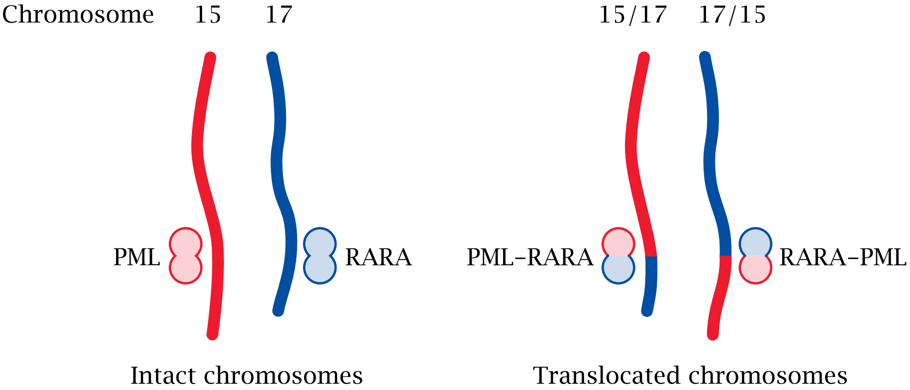


- adrenal steroid synthesis

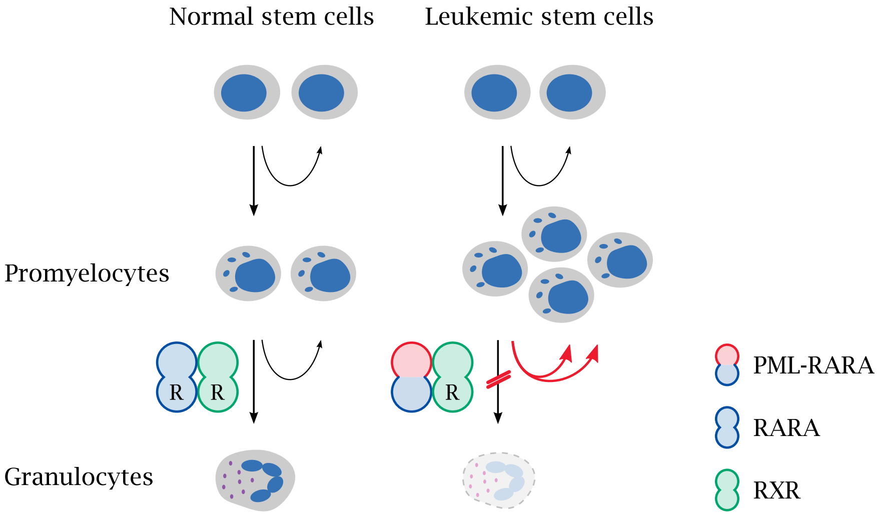
Anticancer drugs that target specific oncoproteins

- α-Retinoic acid in promyelocyte leukemia
- Imatinib in chronic myeloic leukemia; other tyrosine kinase inhibitors in various tumors
- Monoclonal antibodies against growth factor receptors on the cell surface, for example Her2/neu ("herceptin") in breast cancer

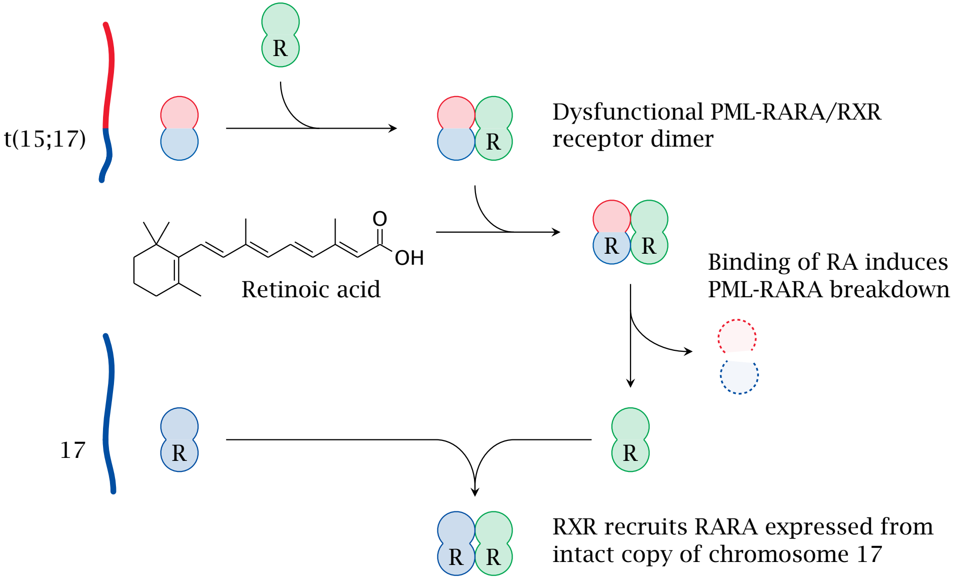
A chromosomal translocation causes promyelocytic leukemia



The mutant PML-RARA receptor blocks promyelocyte differentiation



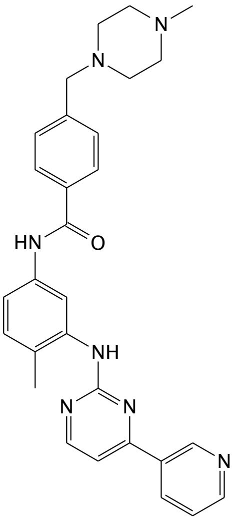
Retinoic acid therapy restores promyelocyte differentiation

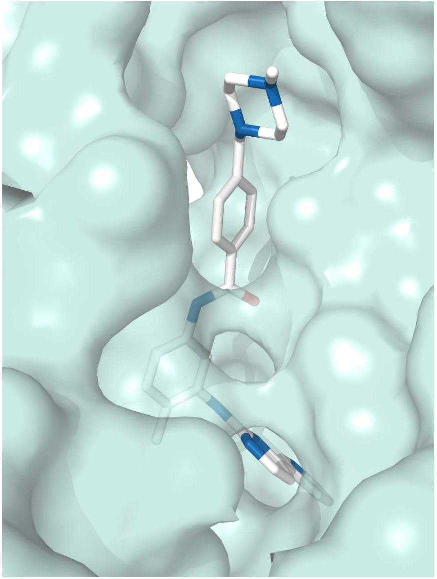


Chronic myeloid leukemia

- Caused by reciprocal translocation between chromosomes 9 and 22 (“Philadelphia chromosome”)
- Translocation produces a chimeric, constitutively active protein tyrosine kinase (*Bcr-Abl*) that drives the proliferation of myeloid precursor cells
- Treatment with imatinib or other tyrosine kinase inhibitors controls, but usually does not eradicate leukemic cells
- After several years, CML typically ends in a “blast crisis”, which resembles an acute myeloid leukemia

Imatinib bound to its target enzyme c-Abl kinase



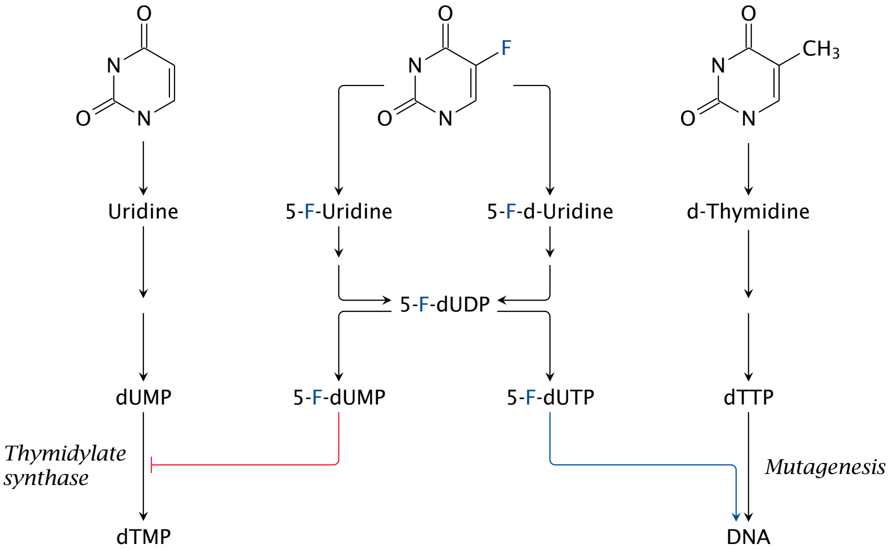


‣ Imatinib/BBB

Cytotoxic anticancer drugs

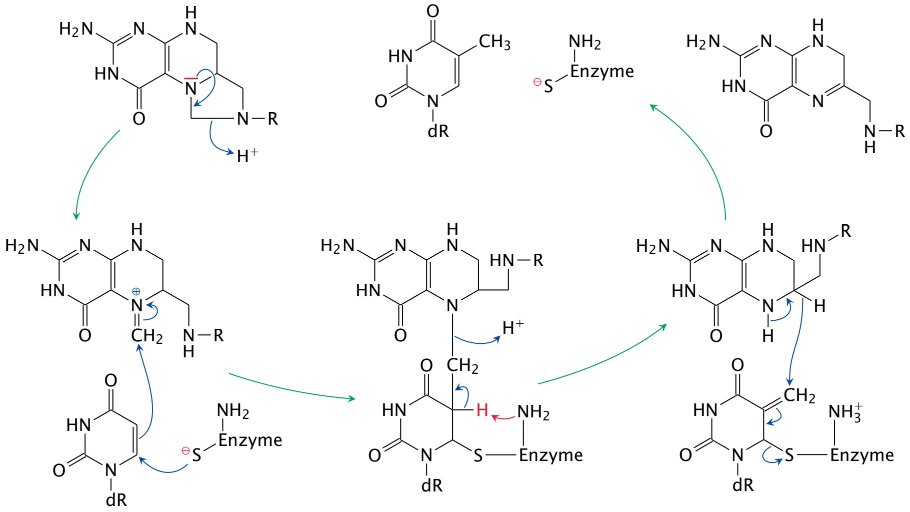
- Antimetabolites
- Inhibitors of DNA topoisomerase
- Proteasome inhibitors
- Inhibitors of mitosis
- DNA-alkylating and other DNA-damaging agents

Dual action mode of 5-fluorouracil



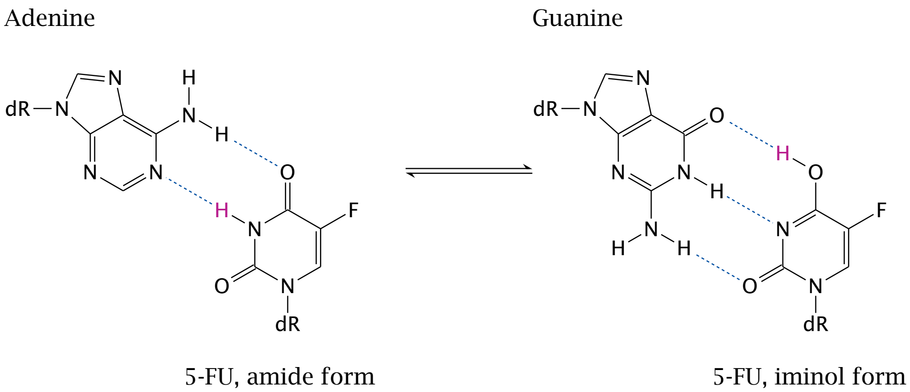
Catalytic mechanism of thymidylate synthase

N,N′-methylene-tetrahydrofolate dTMP dihydrofolate

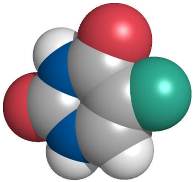


dUMP covalent intermediate

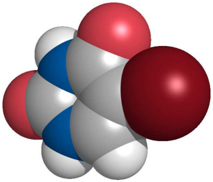
Mutagenesis through mispairing of the 5-FU iminol tautomer



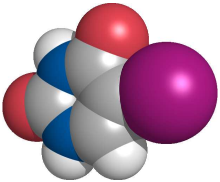
Thymine and various halogen analogues



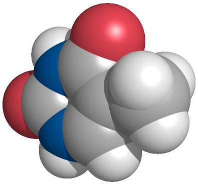
Fluorouracil



Bromouracil

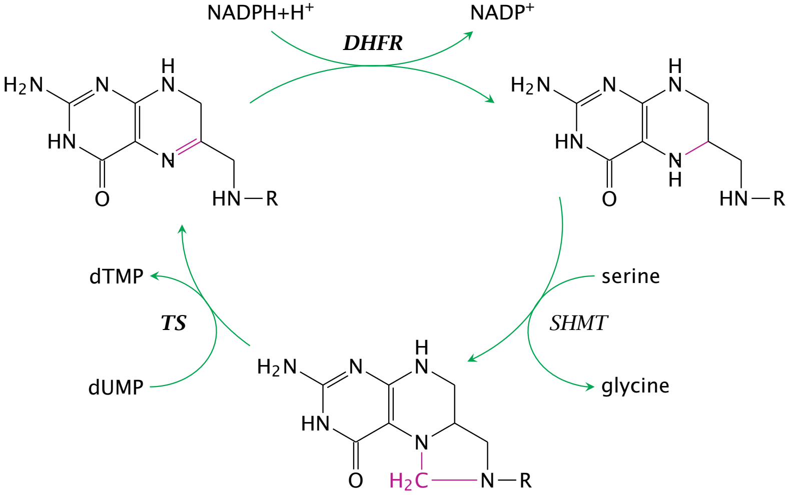


Iodouracil



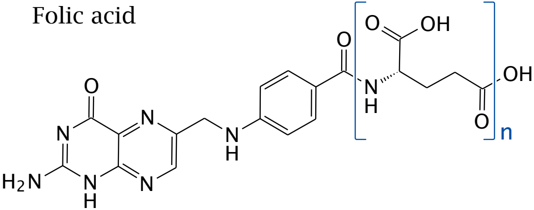
Thymine

Blockade of dihydrofolate reductase also inhibits thymidylate synthesis

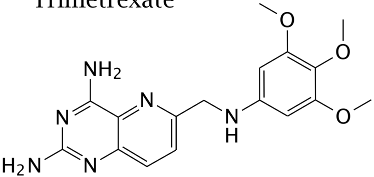


Inhibitors of dihydrofolate reductase

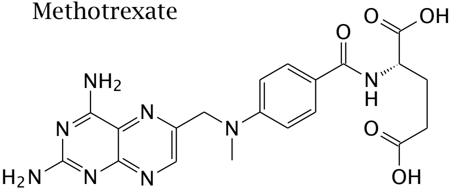
Folic acid



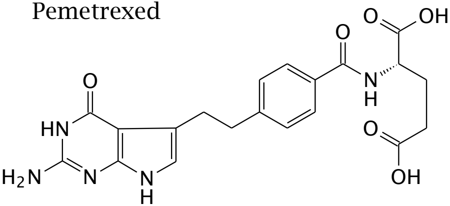
Trimetrexate



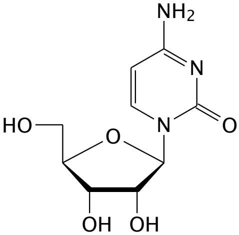
Methotrexate



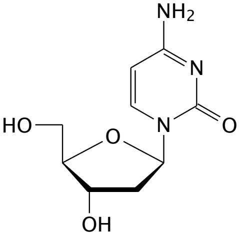
Pemetrexed



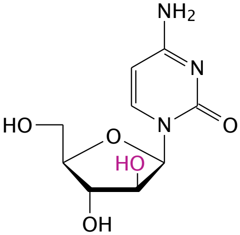
Structure of cytosine arabinoside (araC)



Cytidine

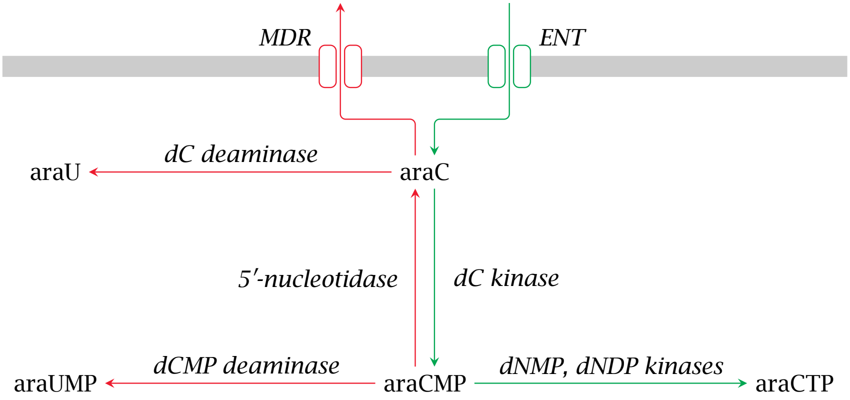


Deoxycytidine

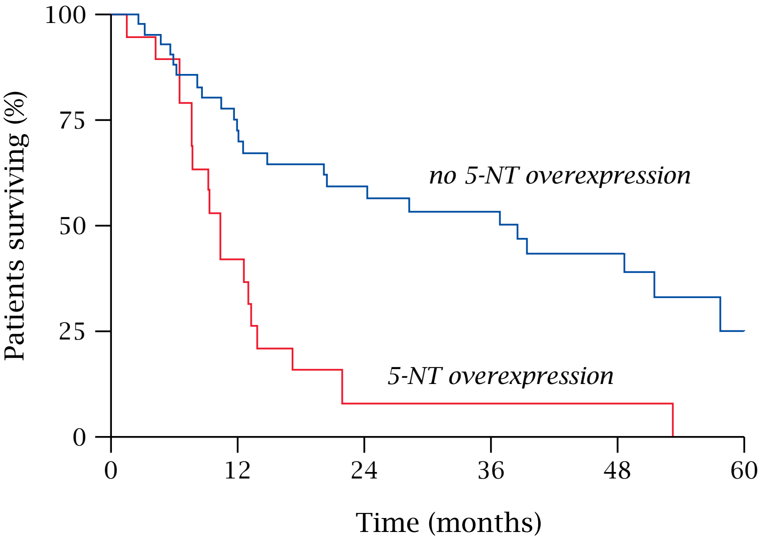


Cytosine arabinoside (araC)

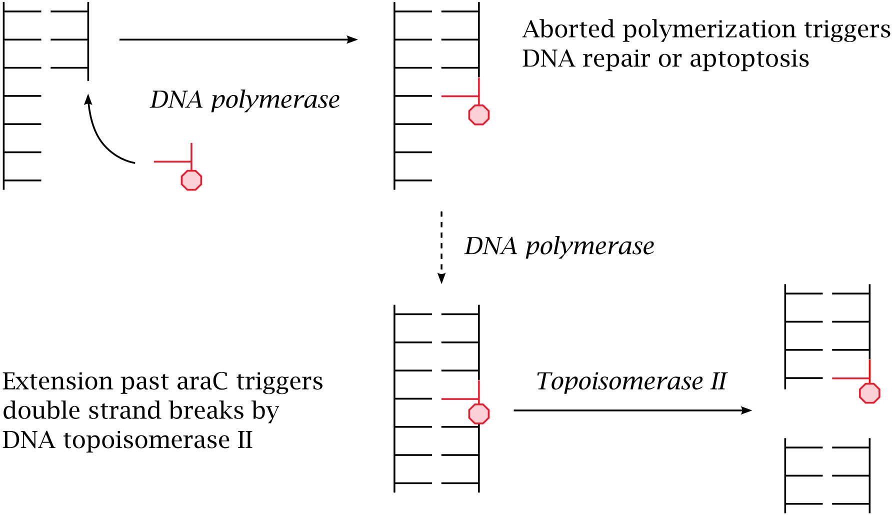
Metabolic activation and inactivation of araC



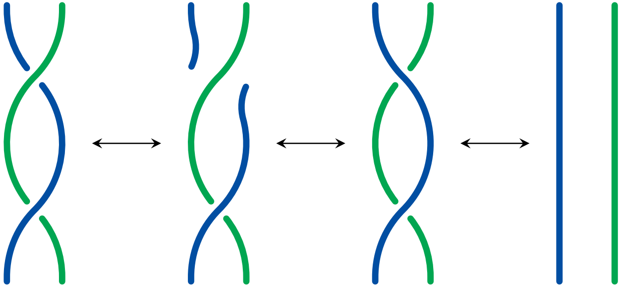
Overexpression of 5′-nucleotidase in leukemic cells correlates with reduced survival rates



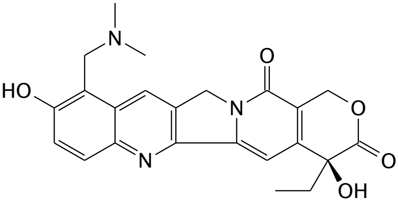
Action mode of araCTP



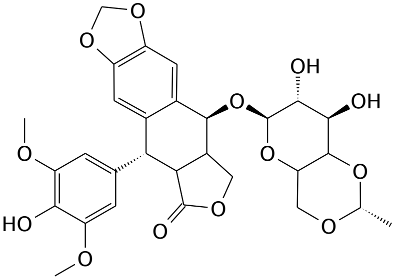
Function of DNA topoisomerases



DNA topoisomerase inhibitors

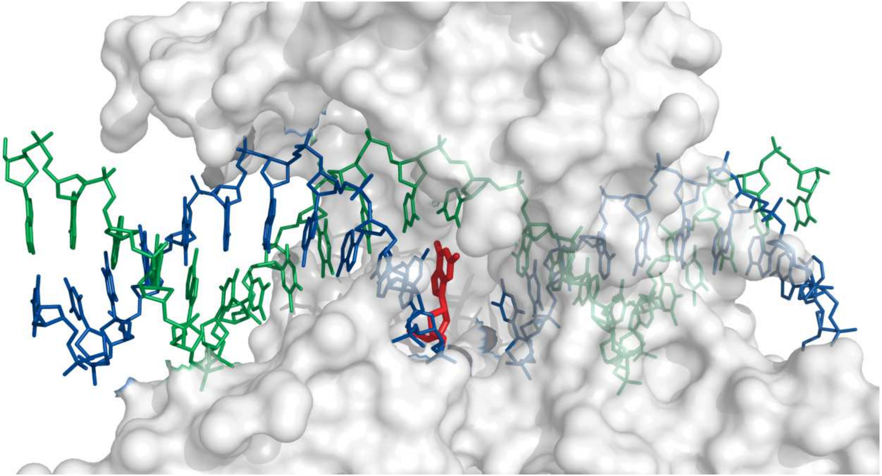


Topotecan (topo I)

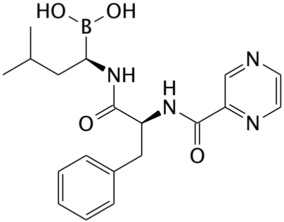


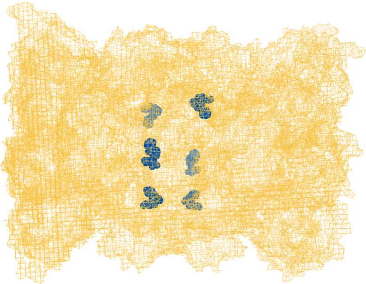
Etoposide (topo II)

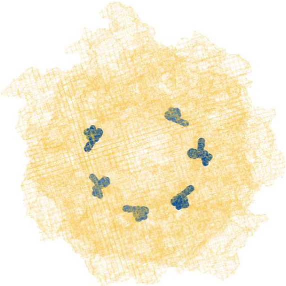
Topotecan bound to topoisomerase I and DNA



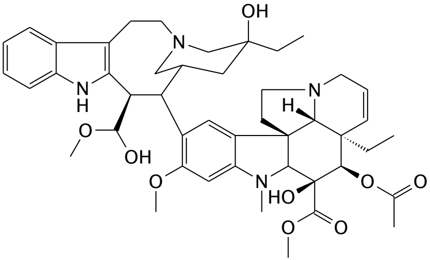
Bortezomib inhibits the proteasome

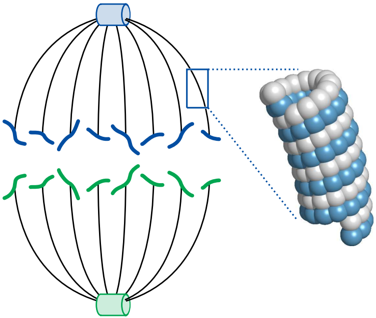


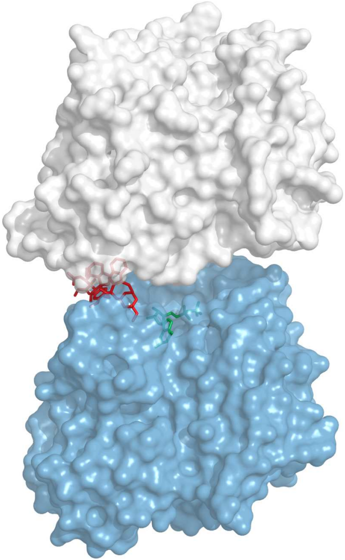




Vinblastine inhibits tubulin polymerization



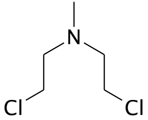




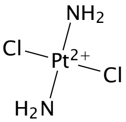
‣ apoptosis pathways

Alkylating anticancer drugs

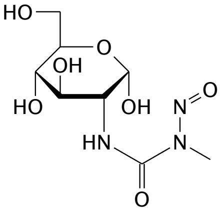
Mechlorethamine

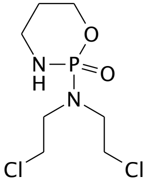


Cisplatin

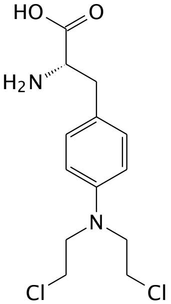


Streptozotocin

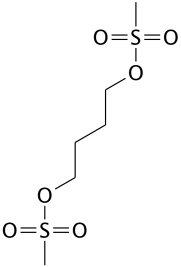




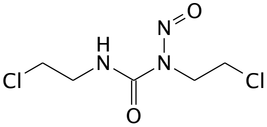
Cyclophosphamide



Melphalan

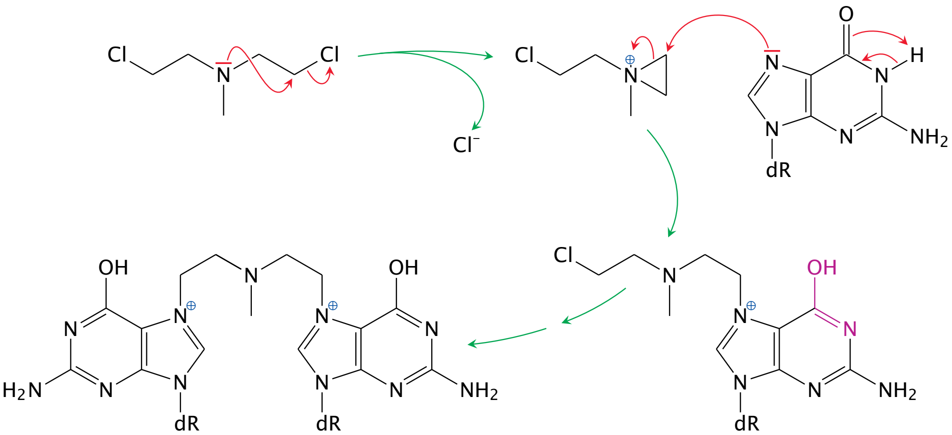


Busulfan

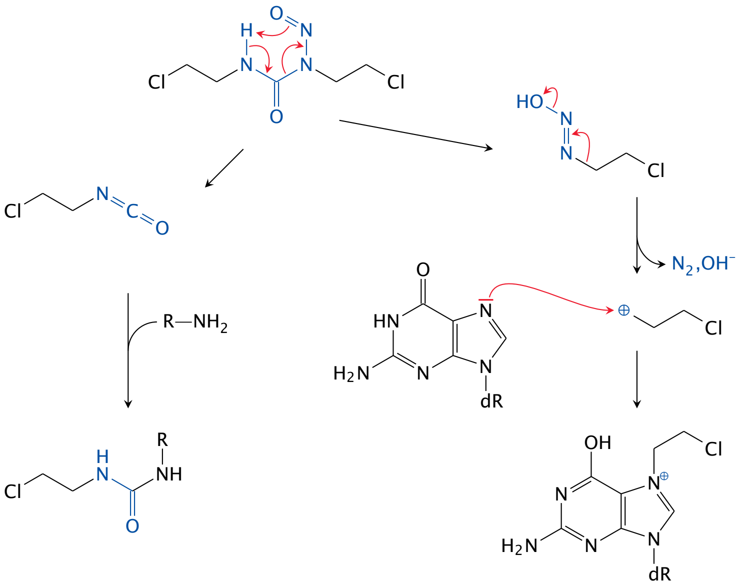


BCNU

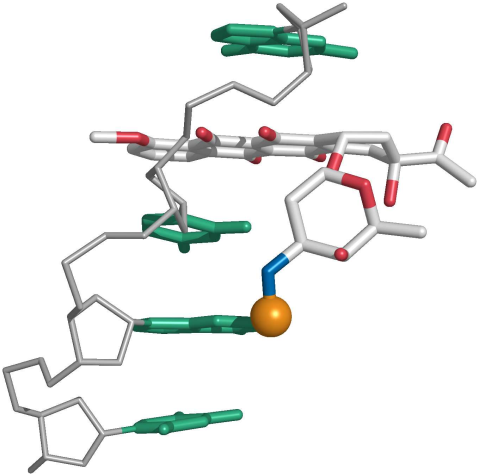
Reaction of mechlorethamine with DNA

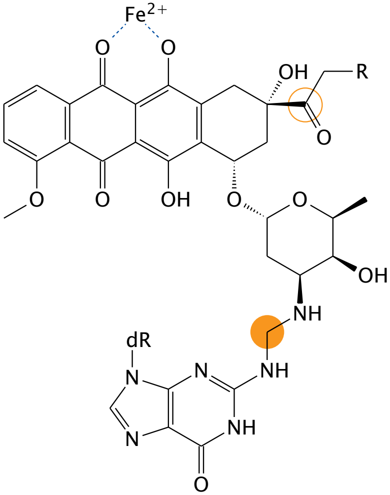


BCNU decay and adduct formation



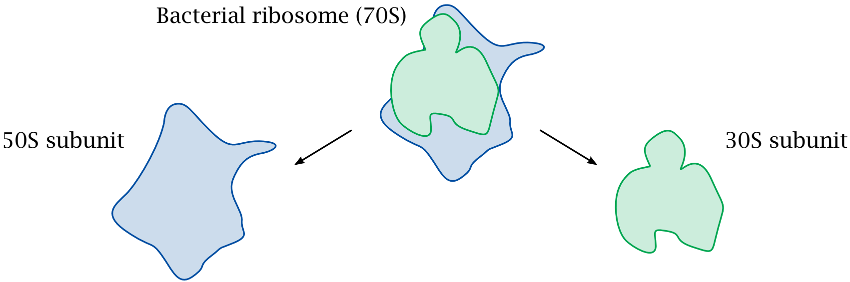
Reaction of daunorubicin with DNA





Ribonucleic acids as drugs and drug targets

Antibiotics that inhibit the bacterial ribosome



50S subunit

5S rRNA (120 nucleotides)
23S rRNA (2900 nucleotides)
34 proteins

Peptidyl transfer	Blasticidin S Chloramphenicol Tiamulin Virginiamycin M
Exit tunnel	Erythromycin

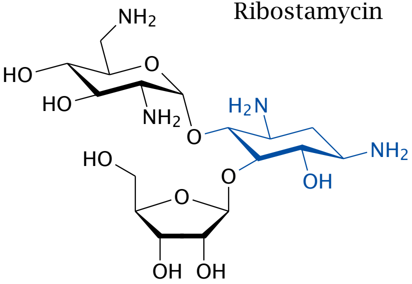
30S subunit

16S rRNA (1540 nucleotides)
21 proteins

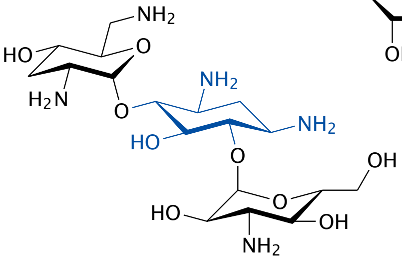
Aminoacyl site	Paromomycin Tetracycline Tobramycin Streptomycin Viomycin

Some aminoglycoside antibiotics

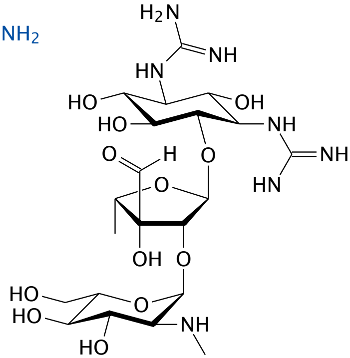
Ribostamycin



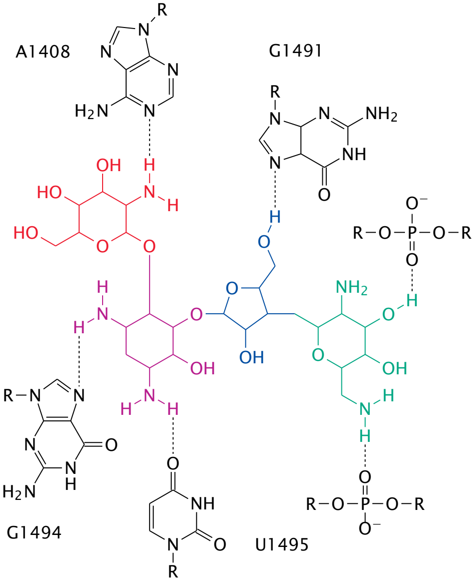
Tobramycin

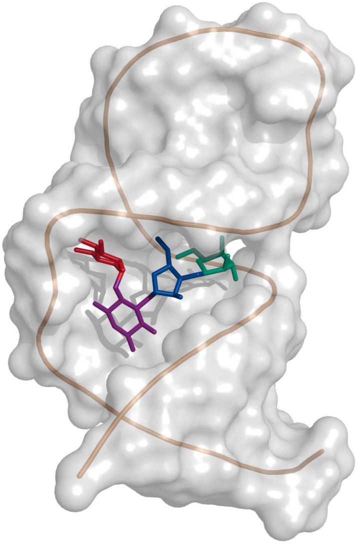


Streptomycin

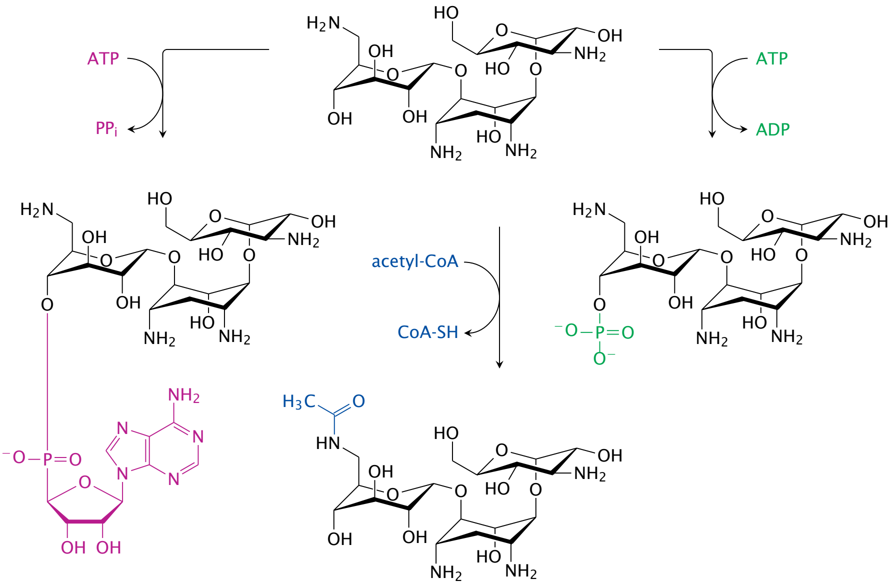


Paromomycin in the ribosomal aminoacyl acceptor site

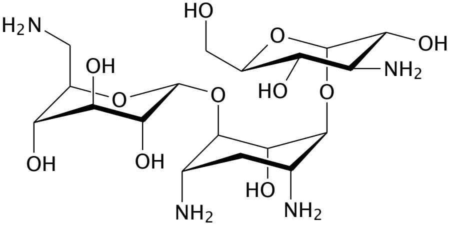




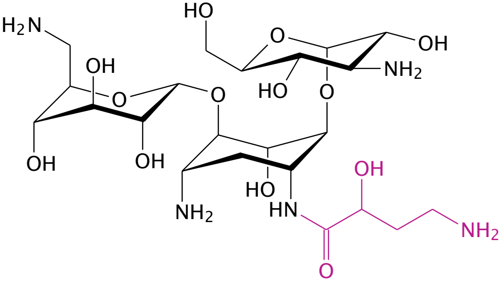
Inactivation of kanamycin by resistance enzymes



Amikacin, a semisynthetic derivative of kanamycin



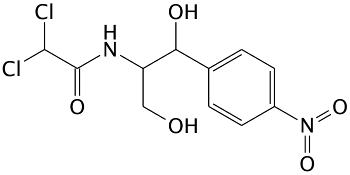
kanamycin

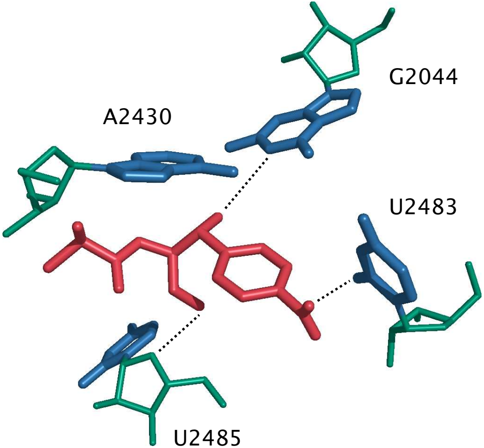


amikacin

Interactions of chloramphenicol with RNA in the peptidyl transferase site of the ribosome

Chloramphenicol



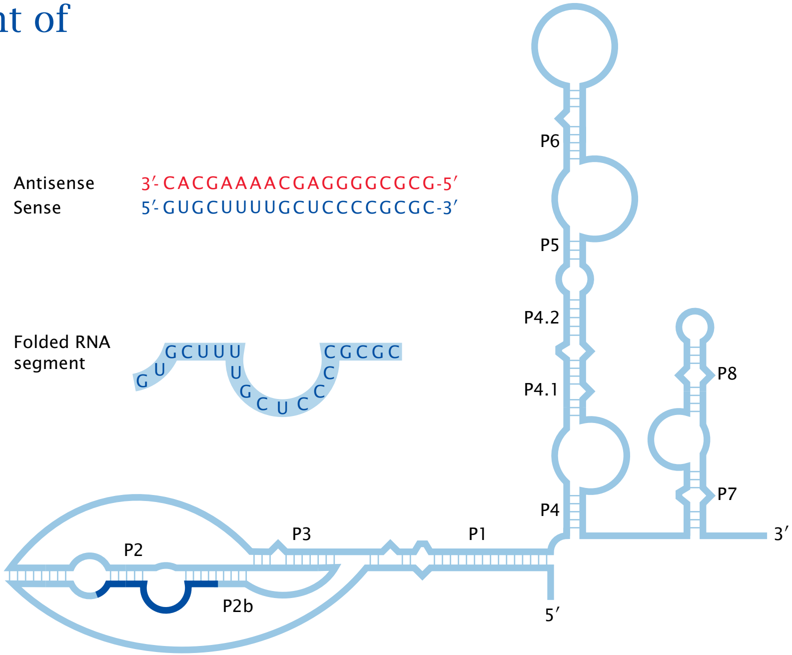


- Chloramphenicol in the ribosome

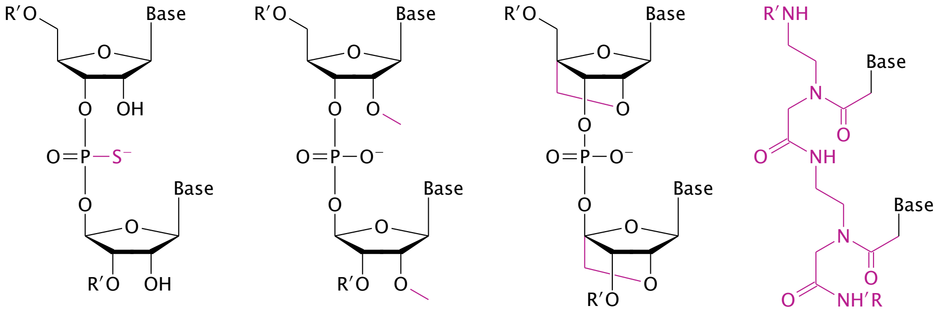
Telomerase as a target for cancer therapy

- in non-cancerous cells, number of successive cell divisions is limited by progressive shortening of the ends (telomeres) of chromosomes
- telomerase extends/restores the telomeres
- makes DNA from an RNA template (reverse transcriptase)
- required in germ line cells
- tumour cells ‘immortalize’ themselves by expressing telomerase

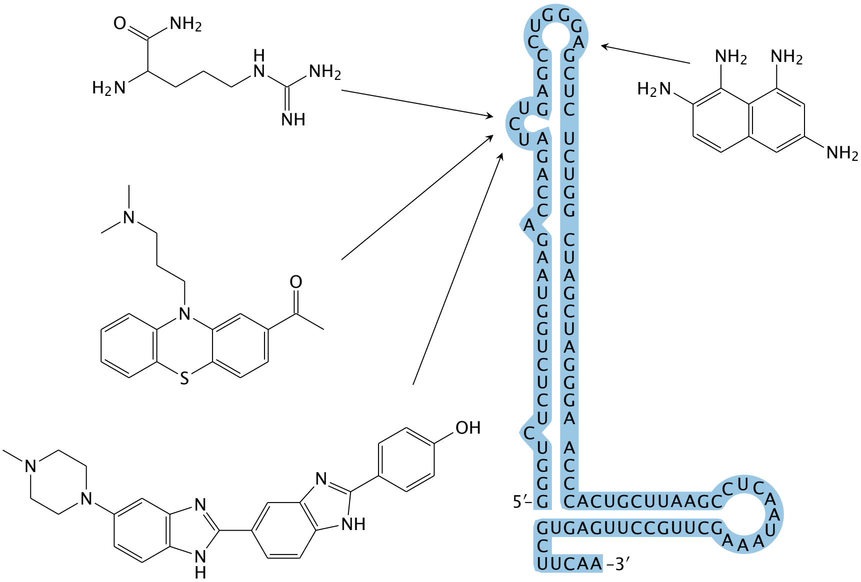
The RNA component of human telomerase



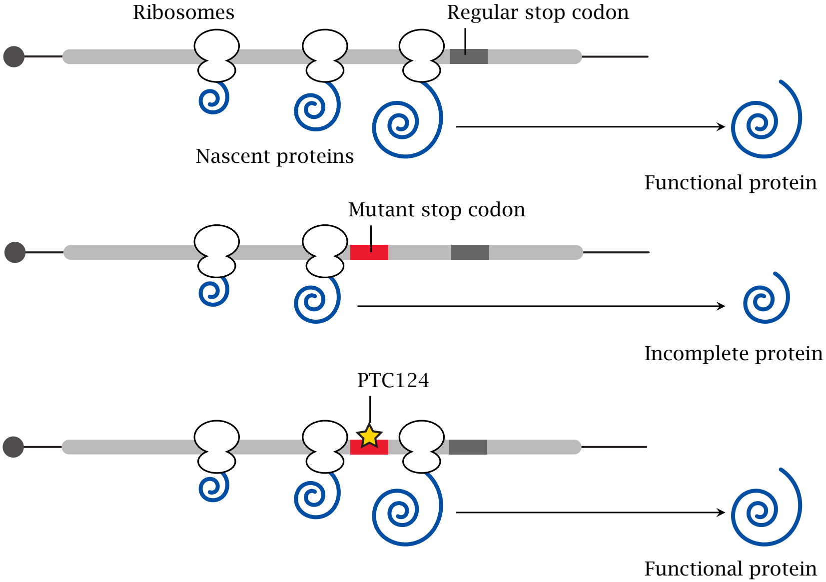
Unusual nucleotide linkages in synthetic oligonucleotides



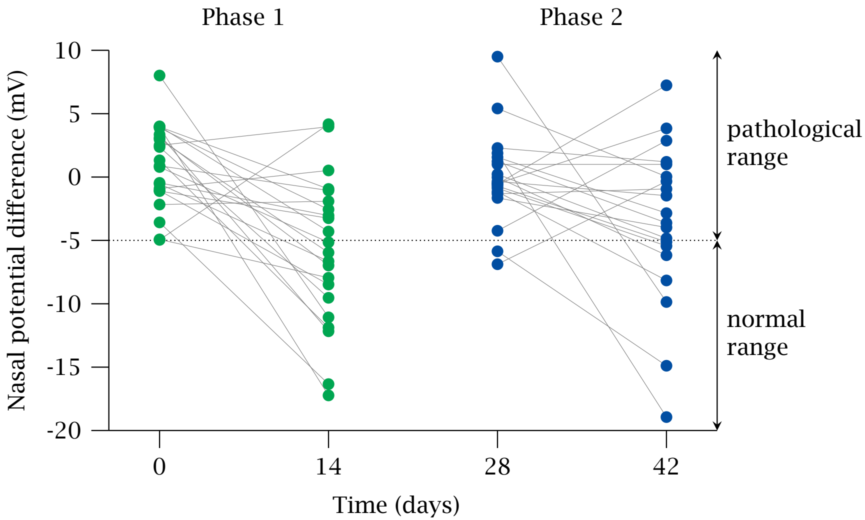
The HIV transactivation-responsive region (TAR)



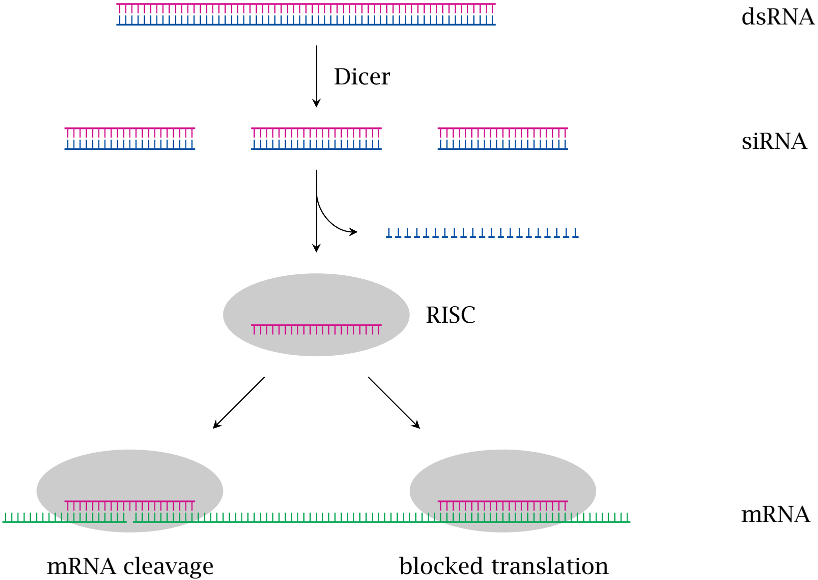
Blocking premature translational termination with PTC124 (ataluren)



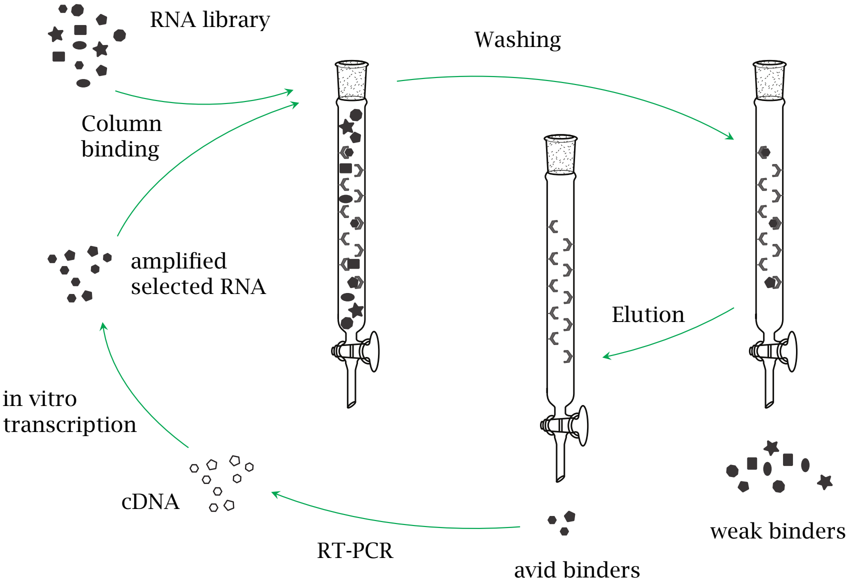
Ataluren in cystic fibrosis



RNA interference

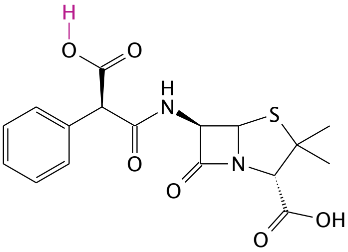


The SELEX process for generating RNA aptamers

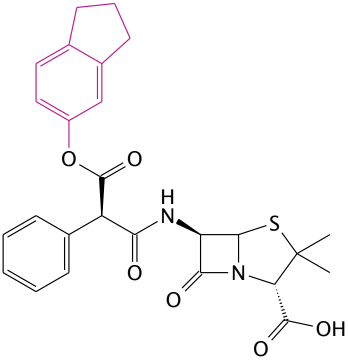


Drug delivery

Protecting drugs from gastric acid through prodrug formation



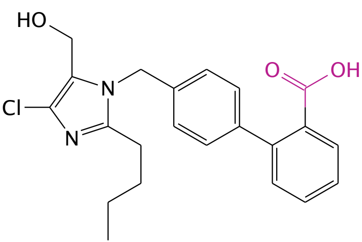
Carbenicillin



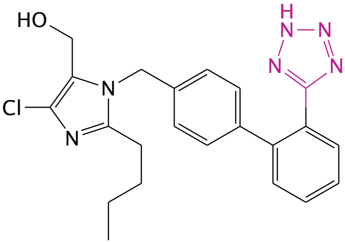
Carindacillin

- Bacampicillin

Optimizing a drug structure for bilayer permeation



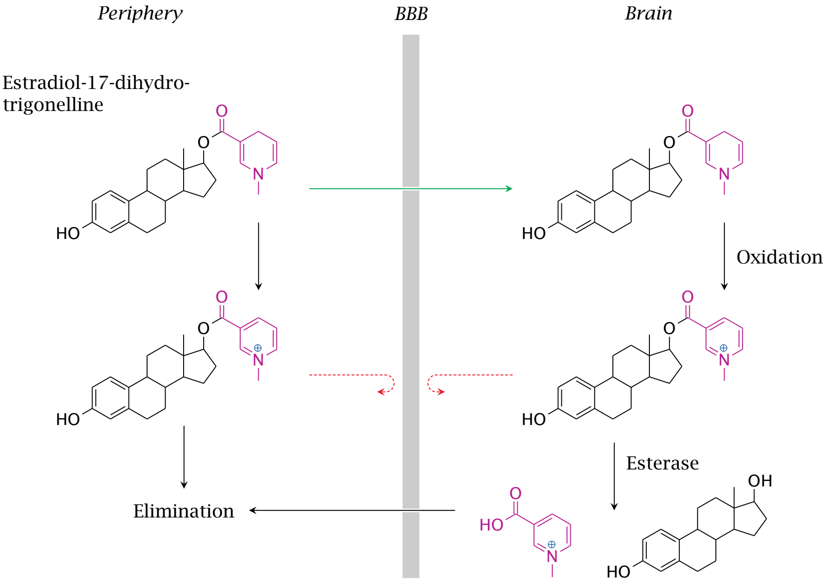
EXP7711



Losartan

▸ Angiotensin action mode

Trapping an estradiol prodrug inside the brain

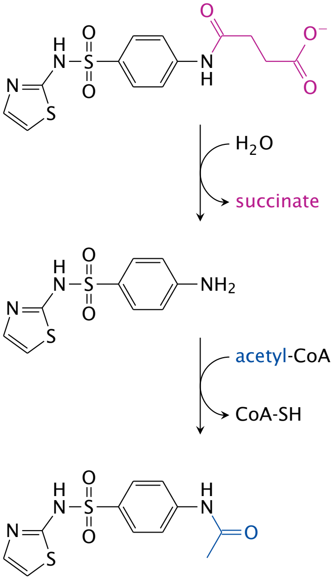


Succinylsulfathiazole, a prodrug designed for *reduced* absorption

Succinyl-sulfathiazole

Sulfathiazole

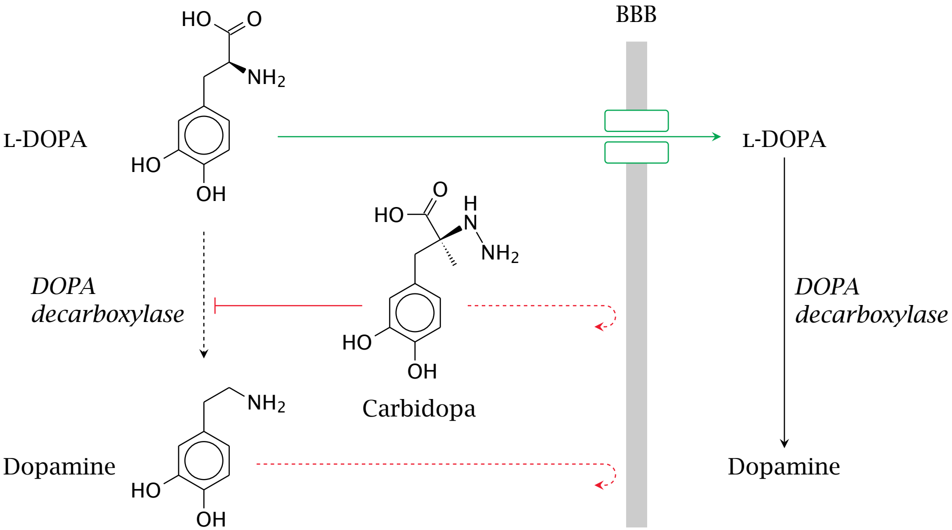
Acetyl-sulfathiazole
(poorly soluble)



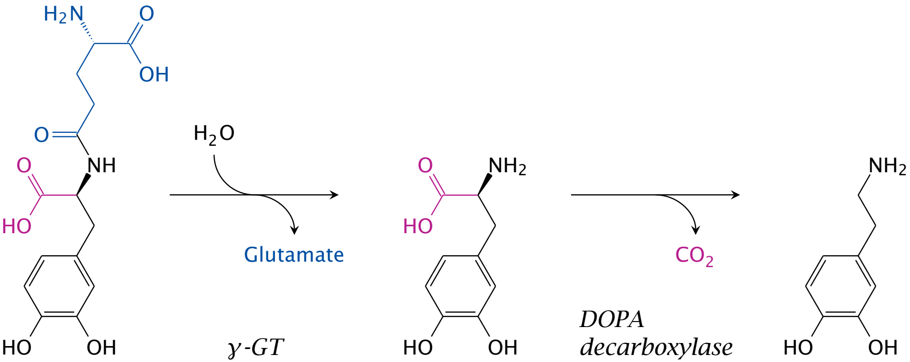
Bacterial esterase
(colon, slow)

N-Acetyltransferase
(liver, fast)

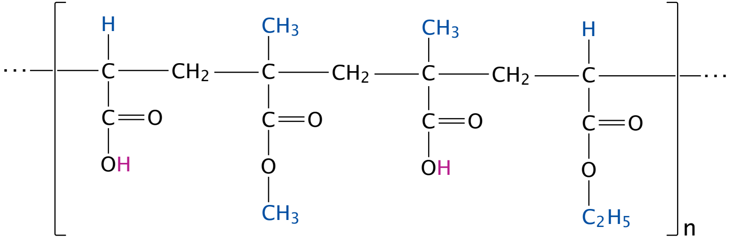
Inhibition of DOPA decarboxylase in the periphery improves L-DOPA uptake into the brain



Gludopa, a prodrug for selective release of dopamine in the kidneys



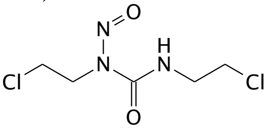
Protecting drugs from gastric acid through acrylate copolymer coating



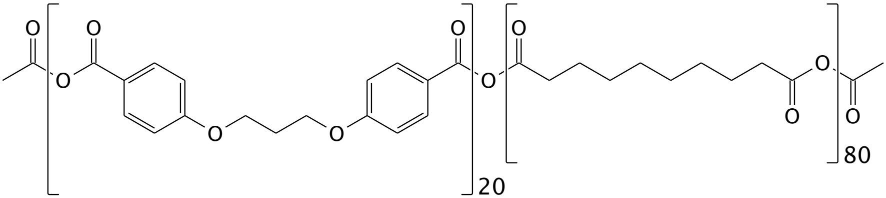
- aspirin gastric toxicity

Site-selective delivery of BCNU

Carmustine (BCNU)



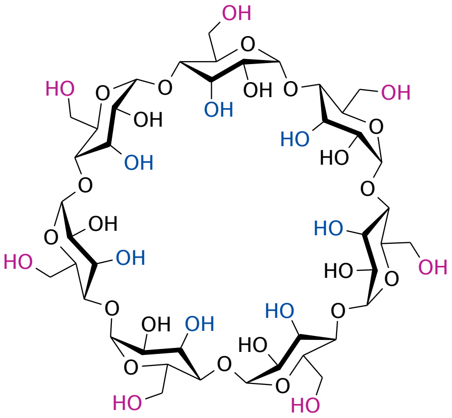
Gliadel®

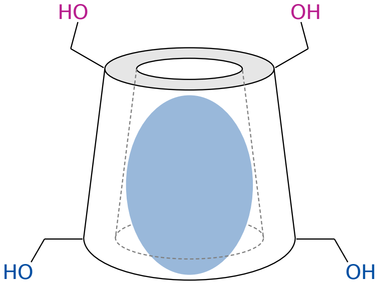


1,3-Bis(carboxyphenoxy)propane Decanedioic acid

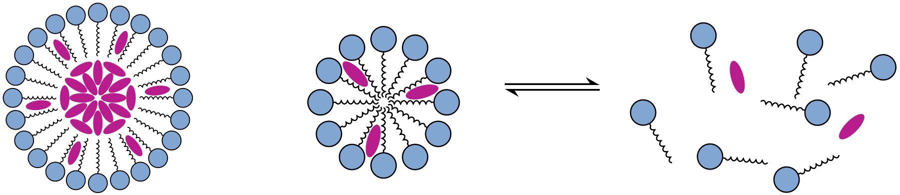
▸ BCNU reactions

Cyclodextrins: Structure and use in drug delivery

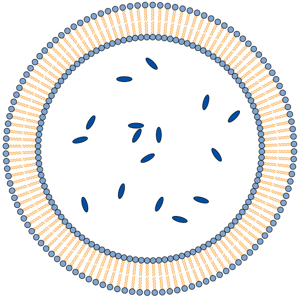




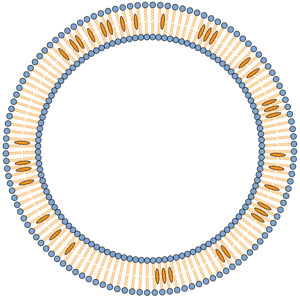
Solubilization of hydrophobic drugs with surfactants



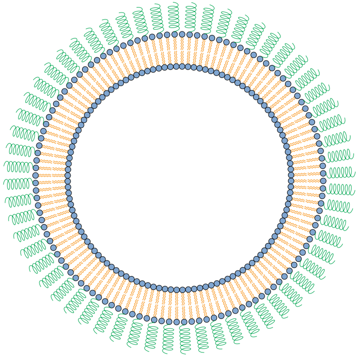
Liposomes as drug delivery vehicles



Hydrophilic cargo drug

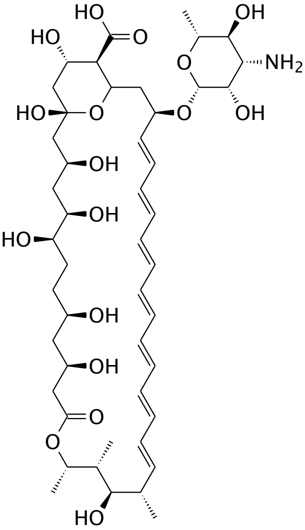


Hydrophobic cargo drug

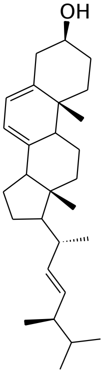


PEG surface modification

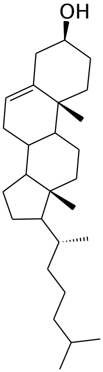
Amphotericin B, ergosterol, and cholesterol



Amphotericin B

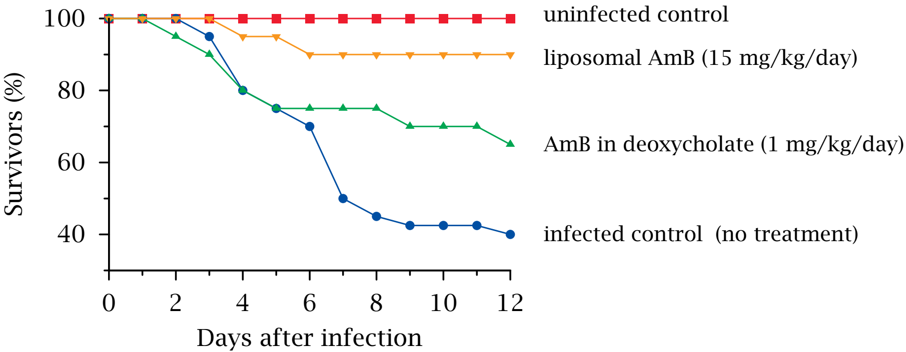


Ergosterol

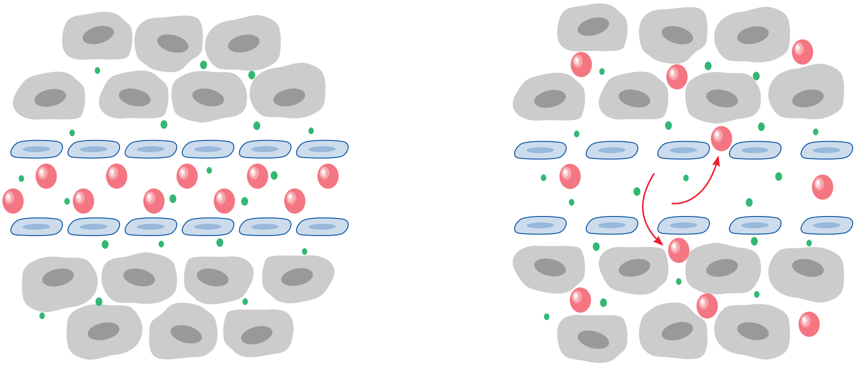


Cholesterol

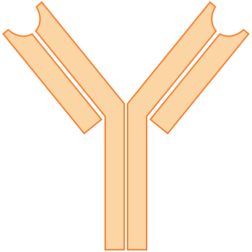
Liposomal vs. deoxycholate-solubilized amphotericin B in a mouse infection model



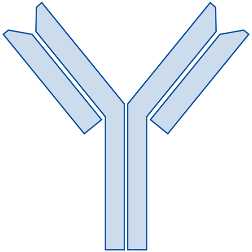
Liposomes and the Enhanced Permeability and Retention (EPR) effect



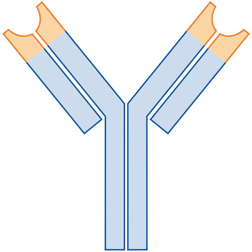
Humanized antibodies



Monoclonal mouse
antitumor antibody

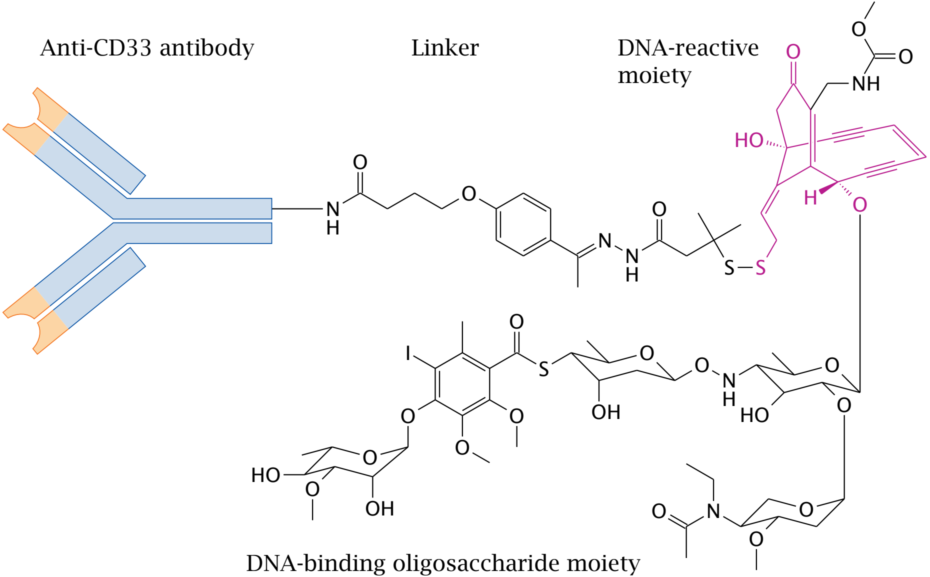


Human antibody without
antitumor specificity

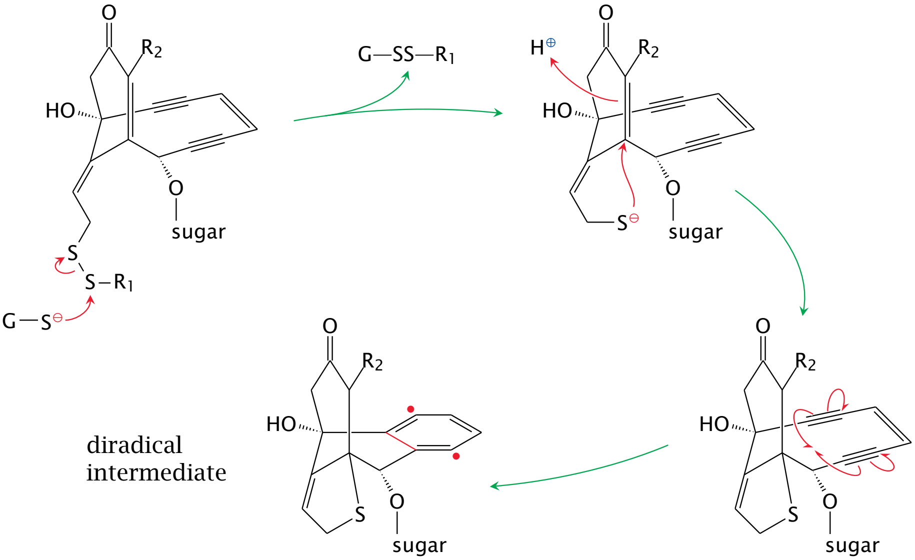


“Humanized” hybrid
antitumor antibody

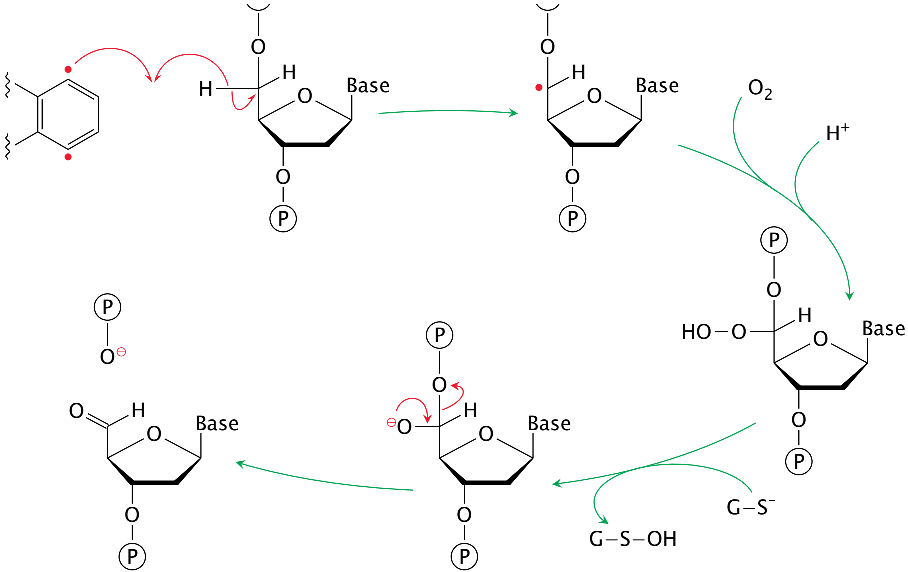
A calicheamicin-antibody conjugate



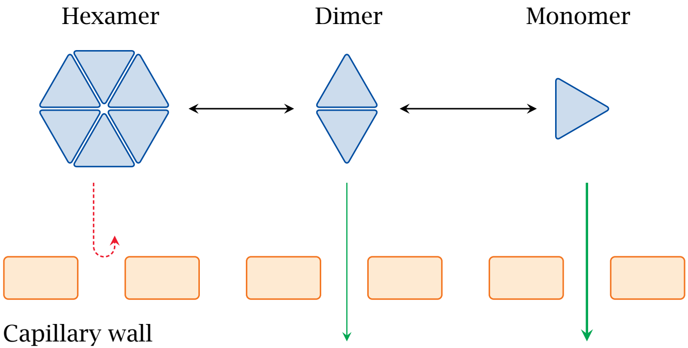
Activation of calicheamicins



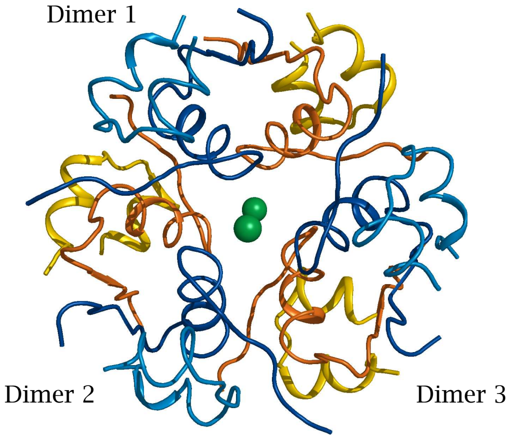
DNA cleavage by activated calicheamicins

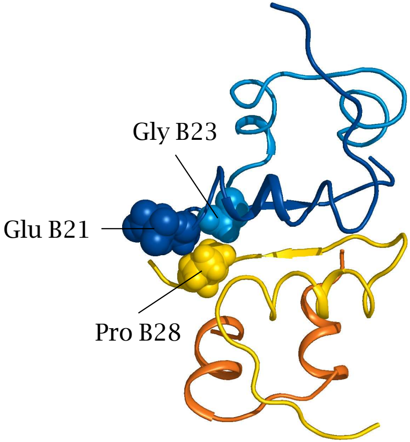


Aggregation of insulin delays its uptake into the circulation

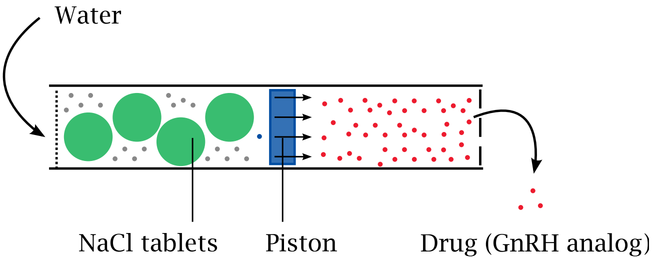


Structure of the insulin hexamer





The Viadur® implant

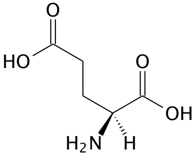


Drug discovery

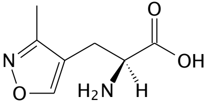
Stages of drug discovery

- Target molecule
  - selection
  - validation
- Candidate compounds
  - acquisition
  - screening

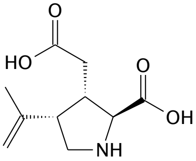
Chemical structures of subtype-selective glutamate receptor ligands



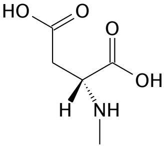
L-Glutamate



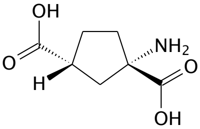
AMPA



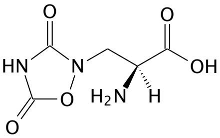
Kainate



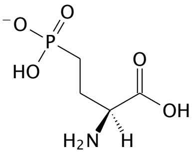
NMDA



ACPD



Quisqualate

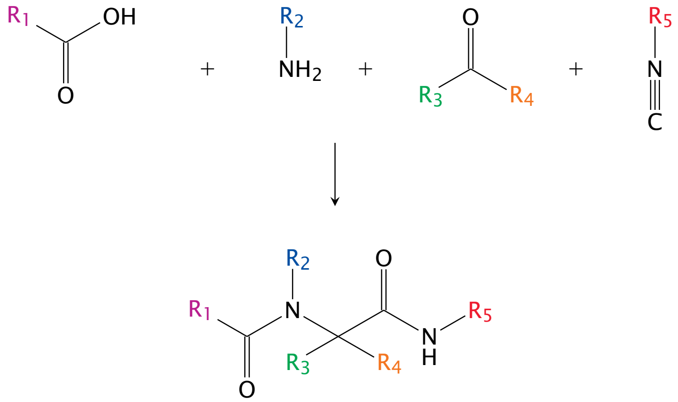


L-AP4

Sources of candidate compounds

- Synthetic libraries
- Natural compounds
- Semisynthesis
- Gene technology

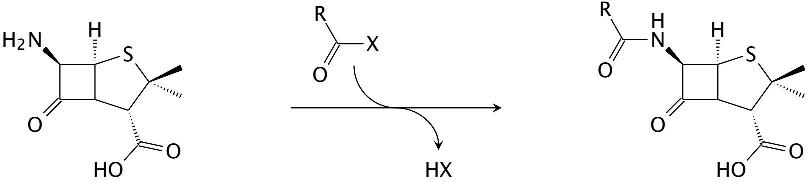
Combinatorial synthesis: the Ugi reaction



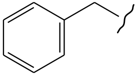
Semisynthesis of penicillins

6-Aminopenicillanic acid

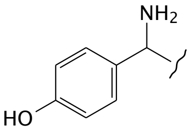
Semisynthetic penicillin



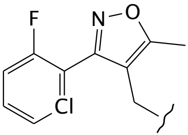
R =



Penicillin G

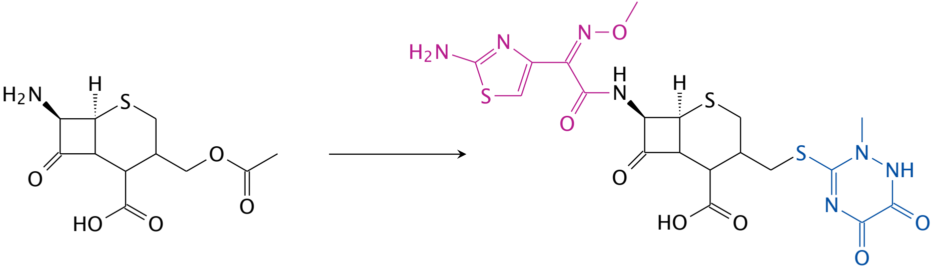


Amoxicillin



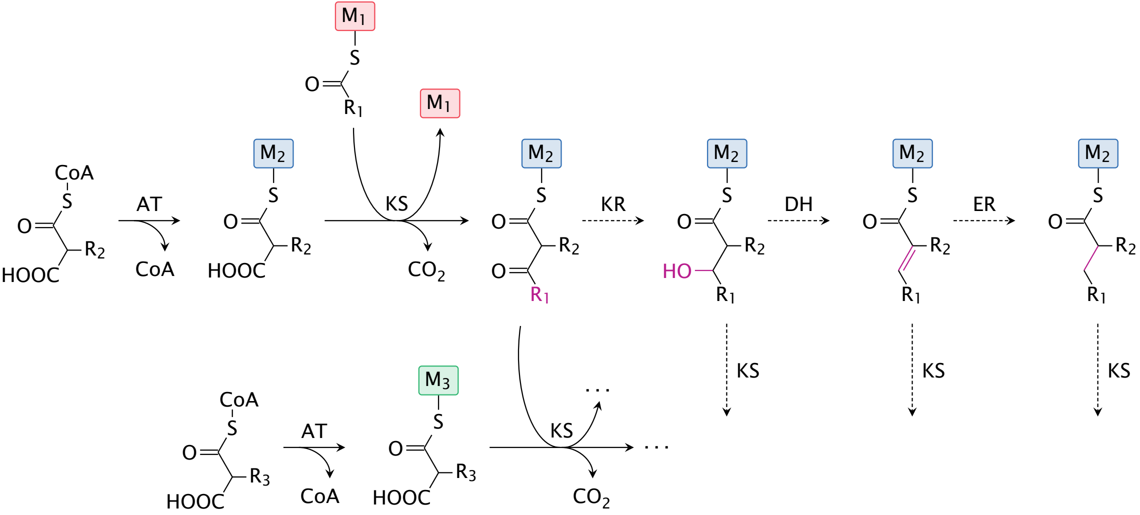
Flucloxacillin

Semisynthesis of cephalosporins

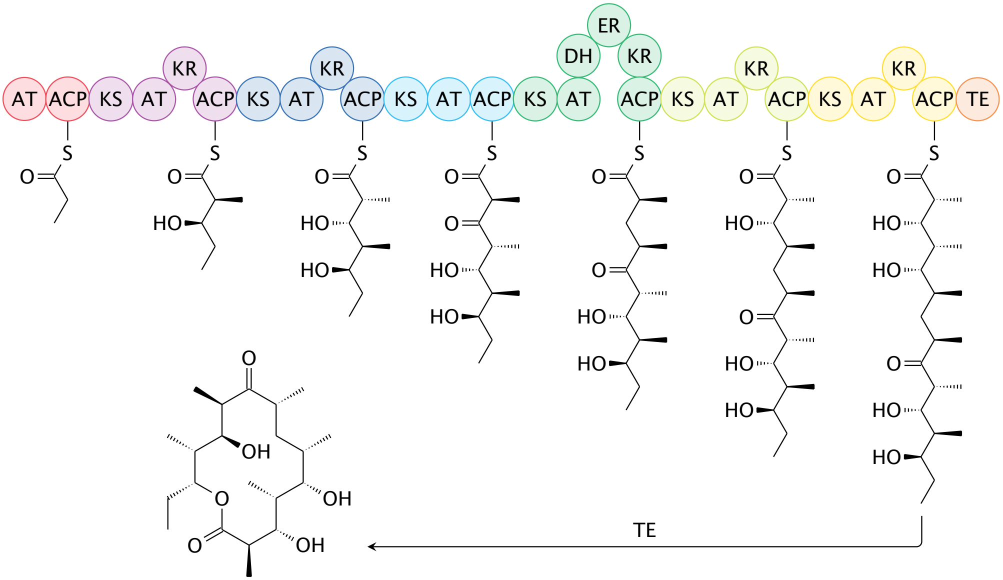


7-Aminocephalosporanic acid   Ceftriaxone

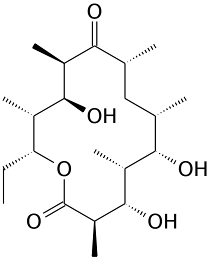
Biosynthesis of polyketides



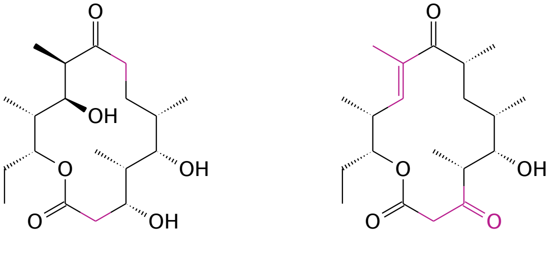
Structure of native 6-deoxyerythronolide B synthase



Two compounds produced by engineered variants of 6-deoxyerythronolide B synthase



6-Deoxyerythronolide B

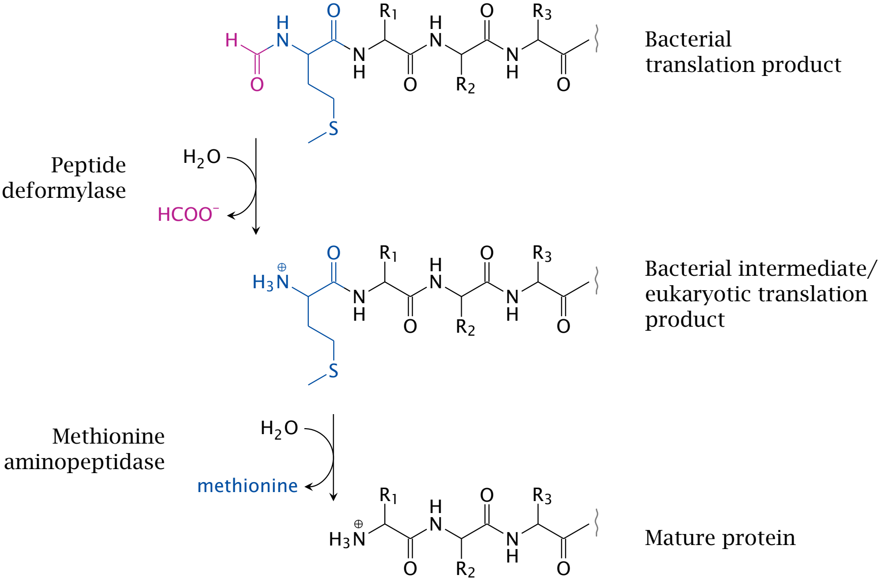


Engineered 6-deoxyerythronolide B analogues

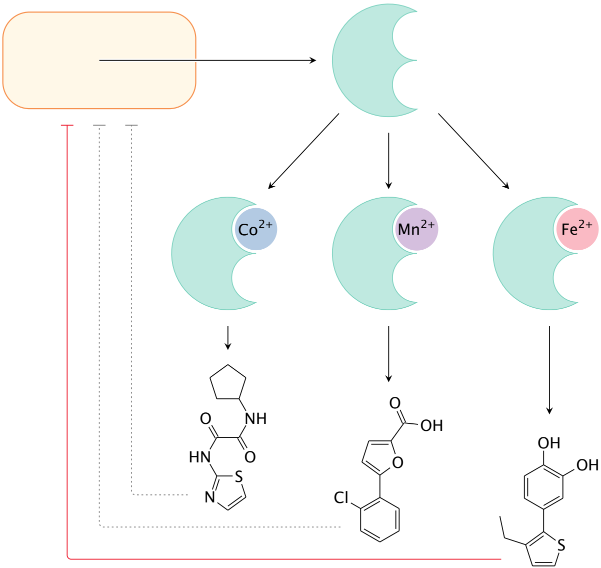
Compound screening

Experimental approach	Typical applications
Activity assays on purified target proteins	Enzymes
Cell-based activity assays	GPCRs, ion channels
Computational screening	Targets with available 3D structure
Phenotypic screening	Cytotoxic activity

Peptide deformylase and Met aminopeptidase



In vitro screening of Met aminopeptidase inhibitors



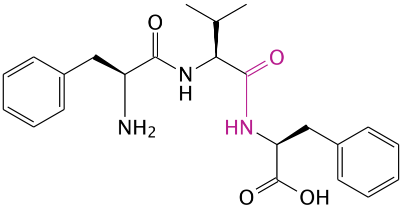
Apoenzyme purified from cells is inactive

Enzyme is activated by various metals

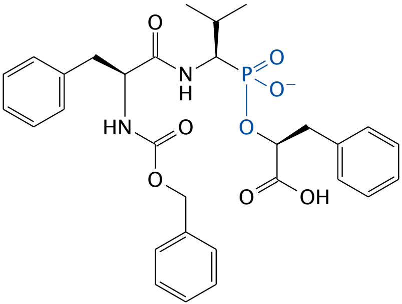
Inhibitors screened on enzymes *in vitro*

Measurement of antibacterial activity

A non-covalent yet irreversible enzyme inhibitor

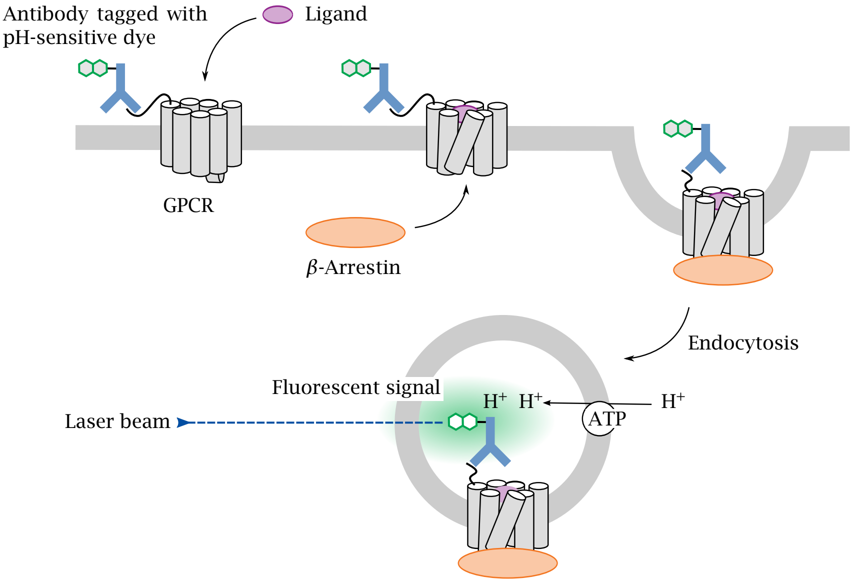


Carboxypeptidase A peptide
substrate (Phe–Val–Phe)

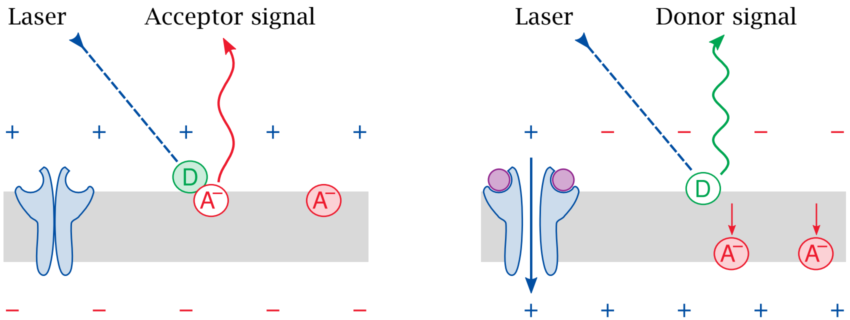


Cbz–Phe–Val–Phe
phosphonate analog

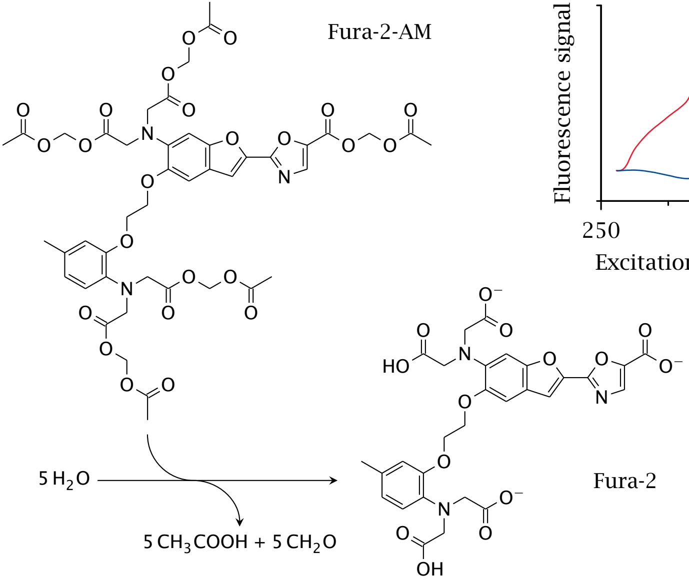
A generic assay for GPCR activation

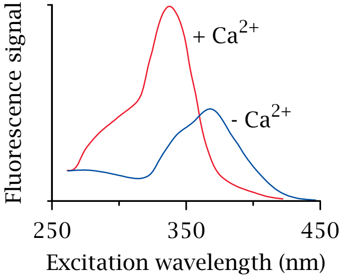


A cell-based fluorescence assay of membrane depolarization

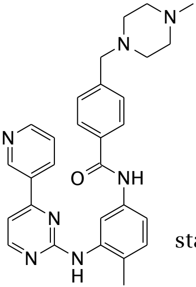


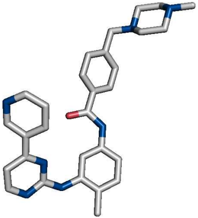
A fluorescence assay of Ca^{++} influx



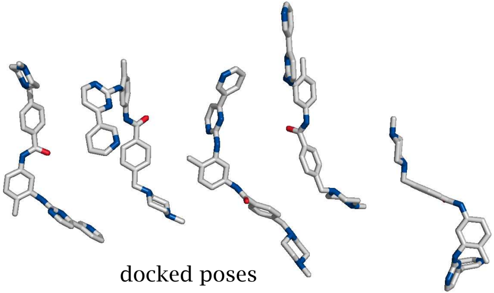


An *in silico* docking experiment

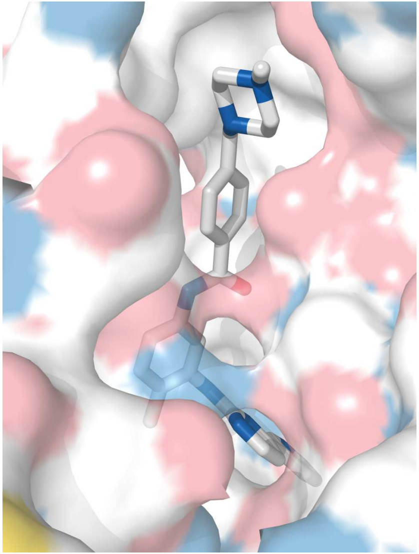




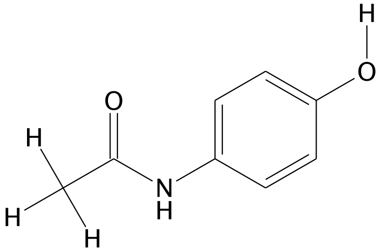
starting conformation

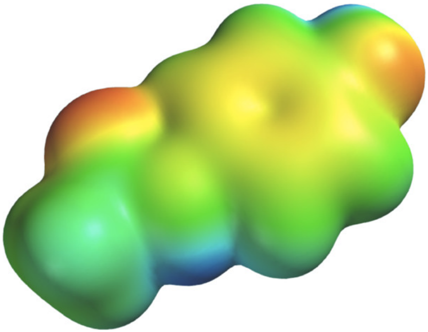


docked poses



Electrostatic potential mapped onto the electronic density for acetaminophen





Hypothetical pharmacophore for inhibitors of ATP:L-Methionine S-Adenosyltransferase