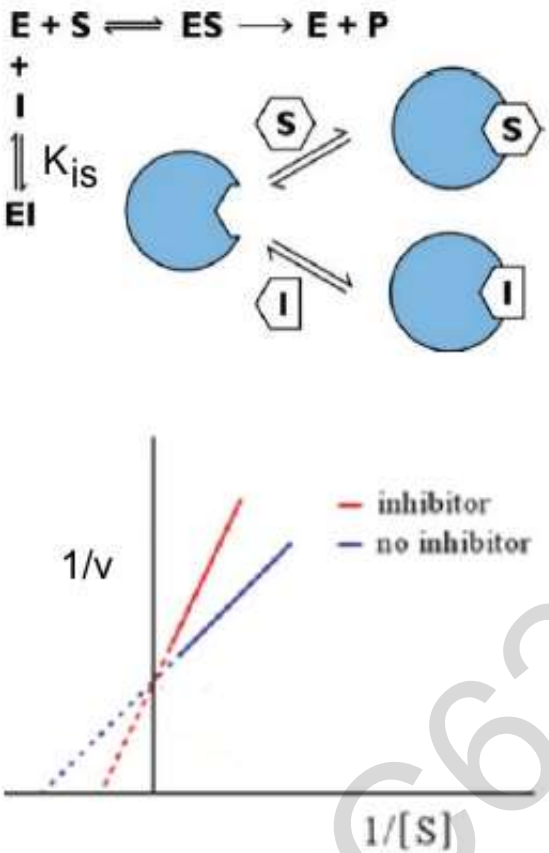
# Enzyme inhibitors

C6215 - Lecture 9

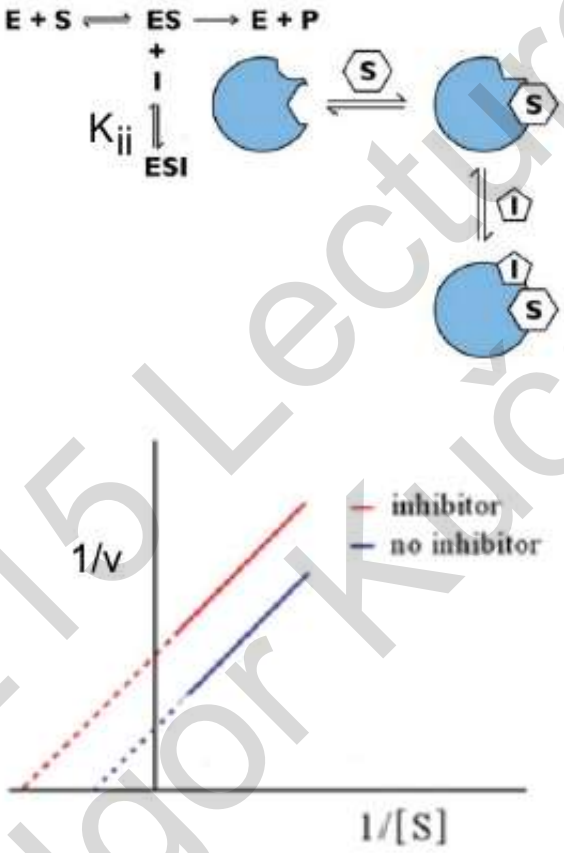
Igor Kučera

# Reversible inhibition

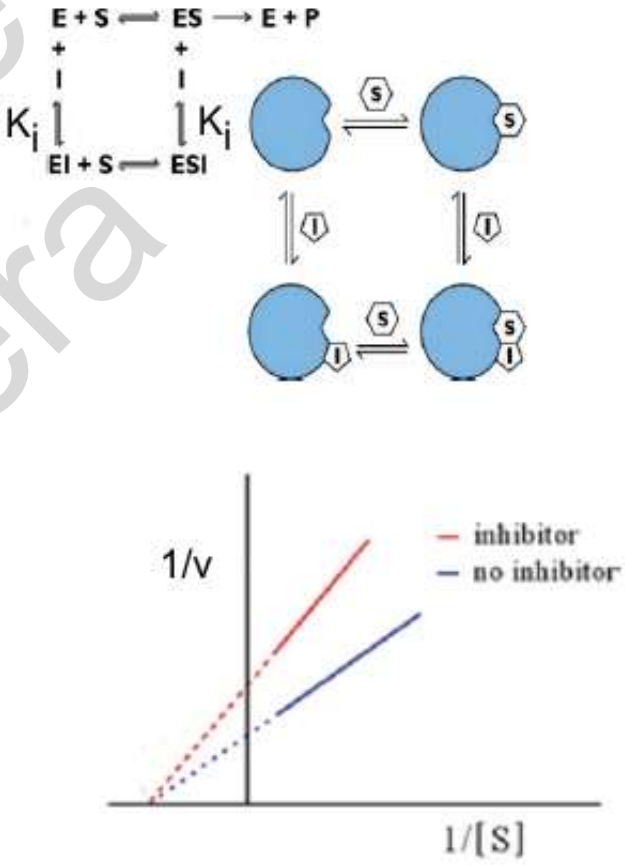
## Competitive inhibition




## Uncompetitive inhibition




## Non-competitive inhibition




$$\text{slope} = \frac{K_M}{v_{lim}} \left( 1 + \frac{[I]}{K_{is}} \right)$$

$$\text{intercept}_y = \frac{1}{v_{lim}} \left( 1 + \frac{[I]}{K_{ii}} \right)$$

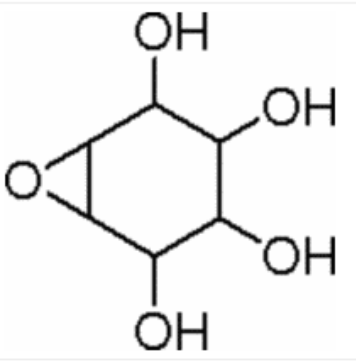
# The Taxonomy of Covalent Inhibitors

| Reversibility of Inhibition | Class of Inhibitor | Scheme | Major Selectivity Determinant |
|---|---|---|---|
| Reversible | Covalent Reversible | $E + I \rightleftharpoons E\bullet I \rightleftharpoons E\text{-}I$ | Thermodynamic equilibrium |
| | Slow Substrate | $E + I \rightleftharpoons E\bullet I \rightarrow E\text{-}I \rightarrow E + P$ | Enzymatic catalysis |
| Irreversible | Residue-Specific Reagent | $E + I \rightarrow E\text{-}I$ | Chemical reactivity |
| | Affinity Label: Classical | $E + I \rightleftharpoons E\bullet I \rightarrow E\text{-}I$ | Effective molarity & chemical reactivity |
| | Affinity label: Quiescent | $E + I \rightleftharpoons E\bullet I \xrightarrow[\text{"off pathway"}]{\text{catalyzed}} E\text{-}I$ | Effective molarity & "off pathway" cat. |
| | Affinity label: Photoaffinity | $E + I \rightleftharpoons E\bullet I \xrightarrow{h\nu} E\text{-}I$ | Effective molarity & light activation |
| | Mechanism-Based | $E + I \rightleftharpoons E\bullet I \xrightarrow[\text{"on pathway"}]{\text{catalyzed}} E\bullet I^* \rightarrow E\text{-}I$; $E\bullet I^* \rightarrow E + P$ | Enzymatic catalysis |



***Tuley and Fast, Biochemistry 2018, 57, 3326***

# An example of an affinity labeling experiment

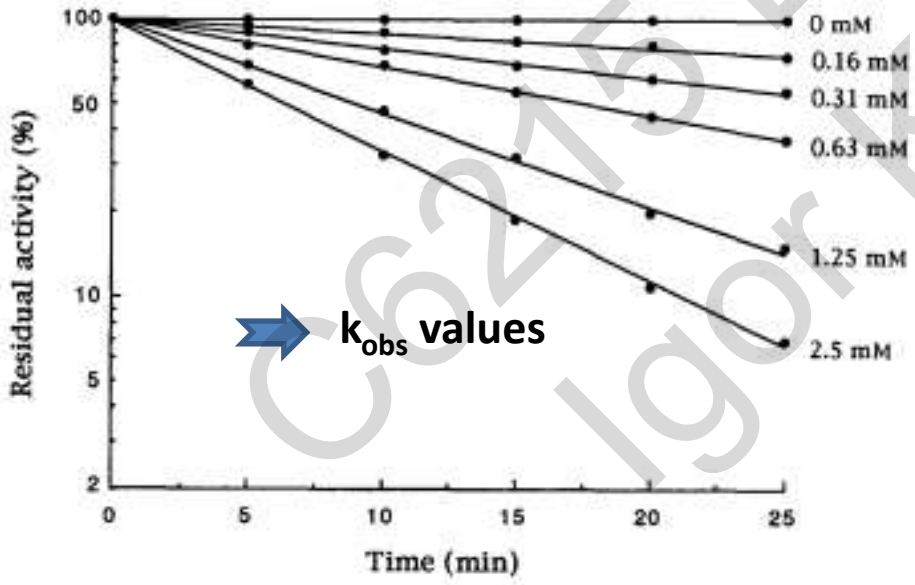



Conduritol B epoxide

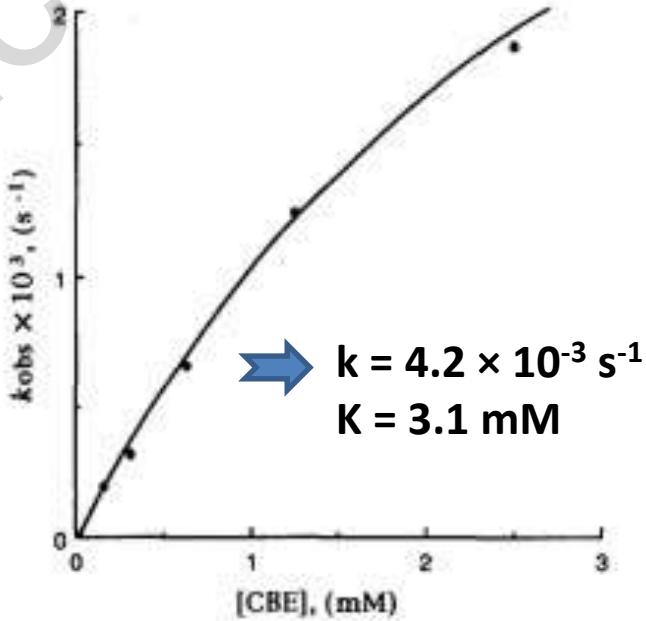
## Inactivation of Sugar Beet α-Glucosidase by CBE.

$$E + CBE \underset{}{\overset{K}{\rightleftharpoons}} E^*CBE \xrightarrow{k} E\text{-}CBE$$

α-Glucosidase (155 pmol) was treated with CBE (six different concentrations) in 80 μl of 100 mM sodium acetate buffer, pH 4.5, at 37°C, for 5 to 25 min.




$k_{obs}$ values




$k = 4.2 \times 10^{-3}\ s^{-1}$

$K = 3.1$ mM

*Iwanami et al., Biosci. Biotech. Biochem. 1995, 59, 459*

# Drug targets

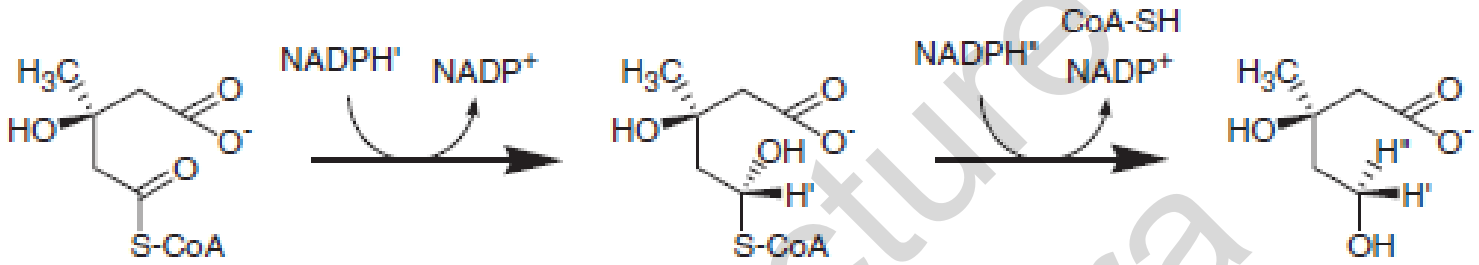
| Target class | Number of proteins | Most common therapeutic actions | Number of drugs |
|---|---|---|---|
| **Receptors** | **193** | **Antihypertensive, anti-allergic** | **563** |
| G protein-coupled receptors | 82 | Antihypertensive, anti-allergic | 357 |
| Ligand-gated ion channels | 39 | Hypnotic and sedative, anticonvulsant | 84 |
| Receptor tyrosine kinases | 22 | Antineoplastic, vasodilator | 22 |
| Immunoglobulin-like receptors | 21 | Immunomodulatory, antineoplastic | 28 |
| Other receptors | 12 | Immunomodulatory, platelet aggregation | 11 |
| Nuclear receptors | 17 | Antineoplastic, hormone replacement | 76 |
| **Enzymes** | **97(27)*** | **Anti-inflammatory, antineoplastic** | **234** |
| EC 1 Oxidoreductases | 22(11) | Anti-inflammatory, antineoplastic | 85 |
| EC 2 Transferases | 21(2) | Antineoplastic, bisphosphonate | 33 |
| EC 3 Hydrolases | 43(9) | Antihypertensive, vasodilator | 96 |
| EC 4 Lyases | 3(2) | Antihypertensive, diuretic | 11 |
| EC 5 Isomerases | 5(0) | Antineoplastic, immunosuppressive | 14 |
| EC 6 Ligases | 1(1) | Antineoplastic, antifibrinolytic | 4 |
| Multiple EC groups | 2(2) | Antineoplastic, antiadrenal | 3 |
| **Transporter proteins** | **67** | **Antihypertensive, anti-arrhythmia** | **181** |
| Voltage-gated ion channels | 29 | Anaesthetic, anti-arrhythmia | 83 |
| Other ion channels | 6 | Antihypertensive, diuretic | 4 |
| Solute carriers | 12 | Antihypertensive, diuretic | 63 |
| Active transporters | 7 | Antihypertensive, anti-ulcer | 19 |
| Other transporters | 3 | Hypnotic and sedative, anti-anxiety | 13 |
| Auxillary transport units | 10 | Antihypertensive, vasodilator | 21 |
| **Other** | **51** | **Anti-inflammatory, antineoplastic** | **84** |
| Enzyme-interacting proteins | 13 | Anti-inflammatory, glucocorticoid | 36 |
| Structural and adhesion proteins | 11 | Antineoplastic | 16 |
| Ligands | 12 | Antirheumatic | 15 |
| Other | 15 | Anti-inflammatory, antineoplastic | 24 |



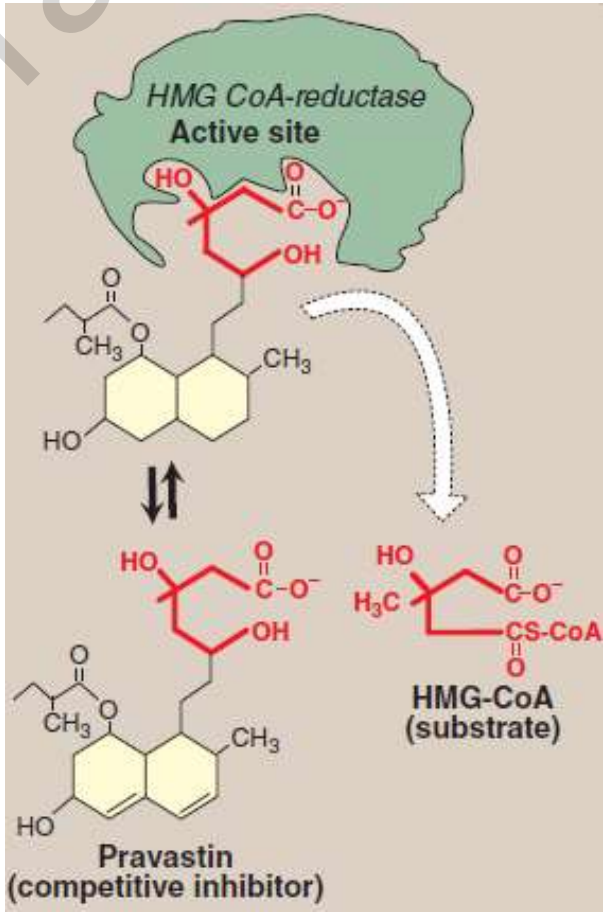
*Rask-Andersen et al., Nature Rev. 2011, 10, 579*

# Inhibition of HMG-CoA reductase

**Reduction of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate, catalyzed by the HMG-CoA reductase, is the major point of regulation on the pathway to cholesterol**



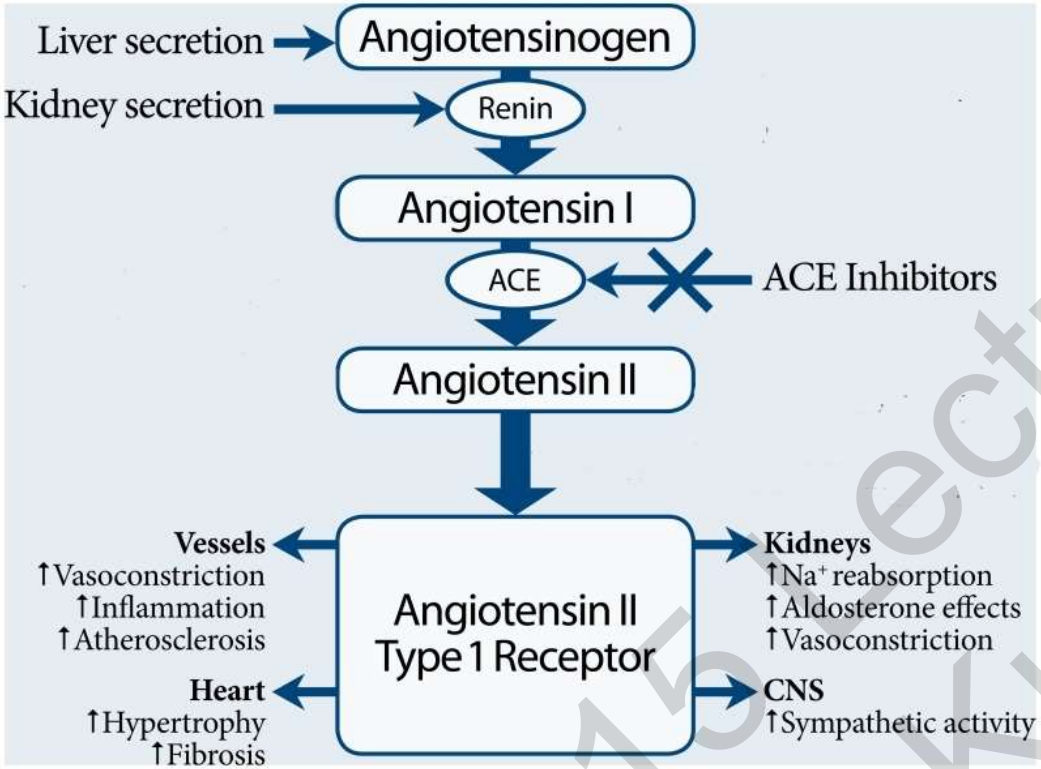

**Statins, originally discovered by a Japanese biochemist Akira Endo (*1933) in moulds, are effective in reducing the low-density cholesterol (LDL-C) by inhibiting HMG-CoA reductase. The first statin commercially marketed (Merck) was lovastatin isolated from the mould *Aspergillus terreus*. There are various other statins in the market.**




**Statin side effects include: muscle pain (myalgia), muscle disease (myopathy) and liver abnormalities. Since statins block production of farnesyl diphosphate, an intermediate in the biosynthesis of coenzyme $Q_{10}$, statin-induced $CoQ_{10}$ deficiency may be instrumental in the pathogenesis of myopathy.**


*Endo, Proc. Jpn. Acad., Ser. B, 2010, 86, 484*
*Ginter and Simko, Bratisl. Lek. Listy 2009, 110, 664*

# Inhibition of angiotensin-converting enzyme (ACE)



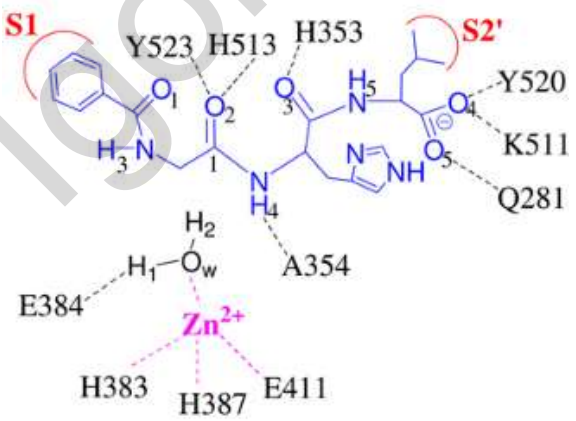

*https://www.ncbi.nlm.nih.gov/books/NBK82803*

**ACE is located in the capillaries of the lungs and in endothelial and kidney epithelial cells. It is a zinc metalloprotease that converts the inactive decapeptide angiotensin I to the octapeptide angiotensin II by removing the dipeptide His-Leu from the C-terminus. Angiotensin II binds to the type 1 angiotensin II receptor (AT1), which sets off a number of actions that result in vasoconstriction and increased blood pressure. ACE also inactivates bradykinin, a vasodilator nonapeptide.**

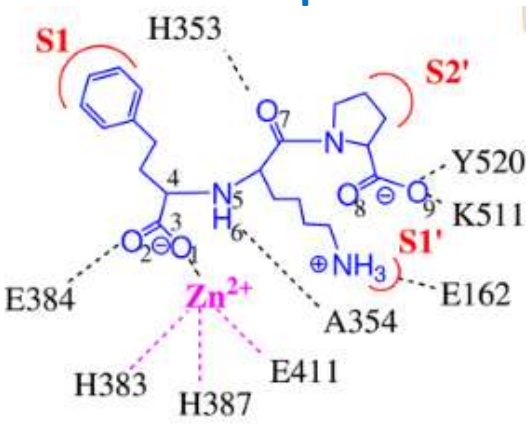
| inhibitor | Ki (nM) |
|---|---|
| captopril | 1.1 |
| enalaprilate | 0.78 |
| lisinopril | 0.27 |

*Wang et al., J. Chem. Inf. Model 2011, 51, 1074*

**Hippuryl-His-Leu**




**lisinopril**

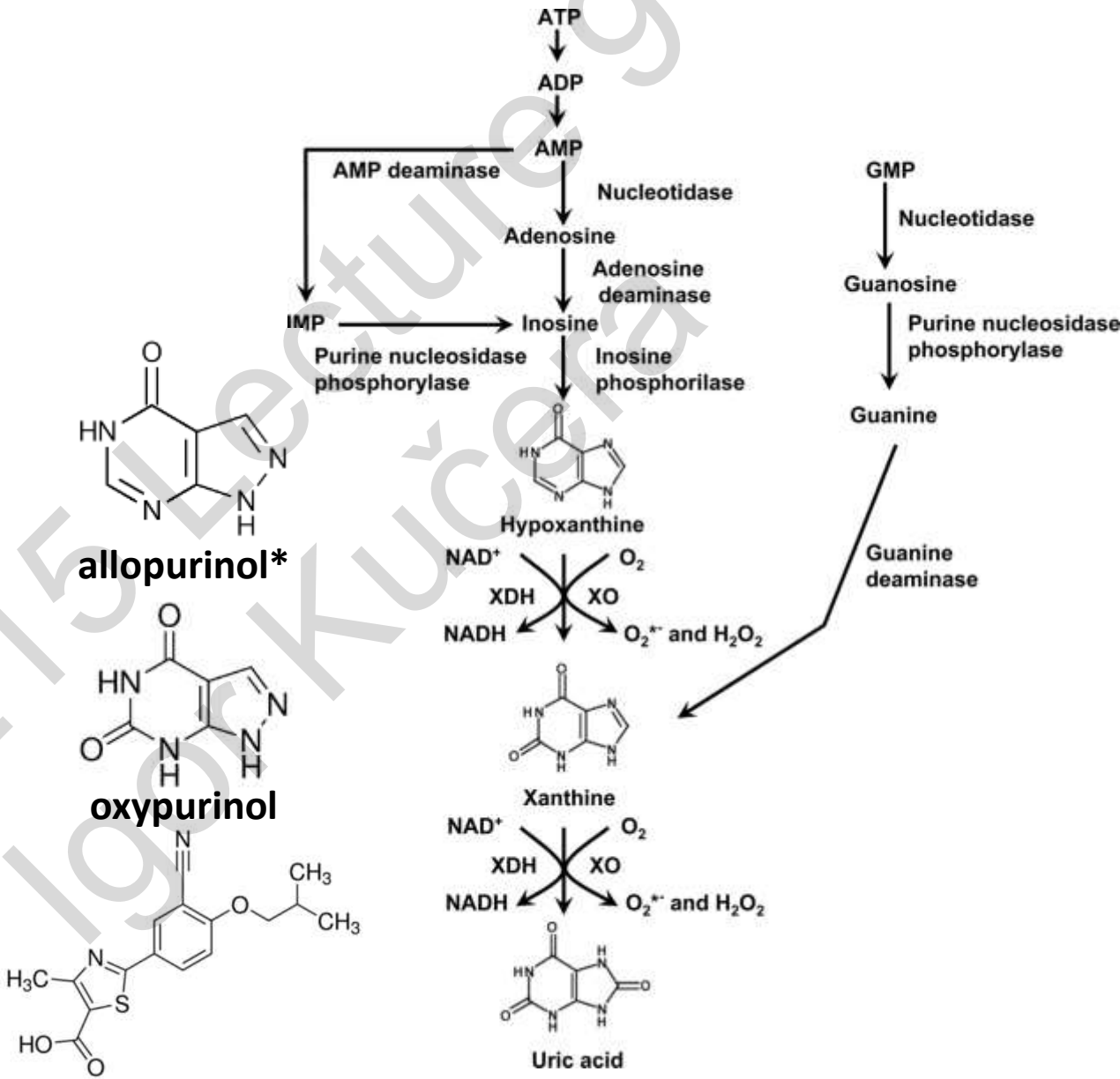


# Inhibition of xanthine oxidase/dehydrogenase

**Gout is a disorder of purine metabolism. The final metabolite, uric acid, crystallizes in the form of monosodium urate, precipitating and forming deposits (tophi) in joints, on tendons, and in the surrounding tissues. This is accompanied by recurrent attacks of acute arthritic joint inflammation.**

**Treatment with XO/XDH inhibitors results in an accumulation of xanthine and hypoxanthine – compounds more soluble than uric acid.**

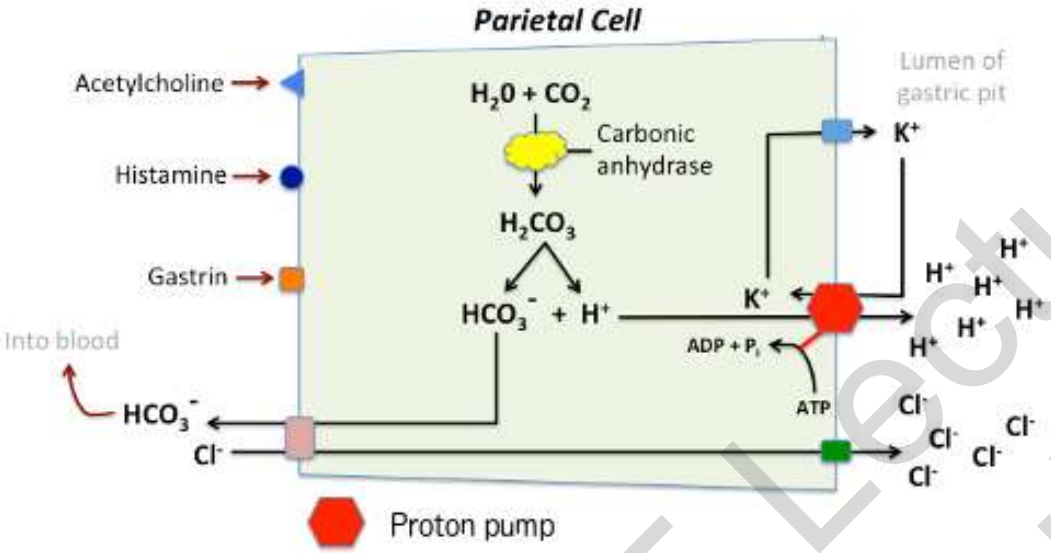
***Allopurinol is converted to oxypurinol (alloxanthin) which binds tightly to Mo(IV) of the molybdopterin cofactor.**

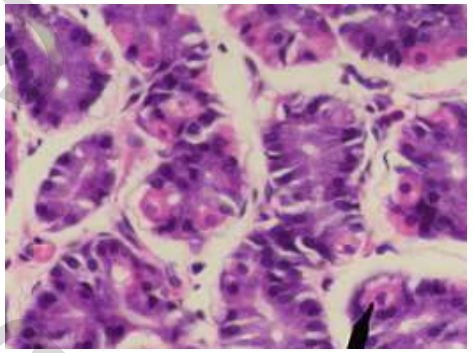



*Pacher et al., Pharmacol. Rev. 2006, 58, 87*

# Inhibition of gastric proton pump

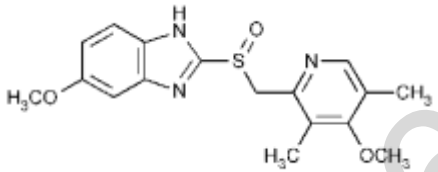
## Hydrochloric acid secretion by the parietal cells



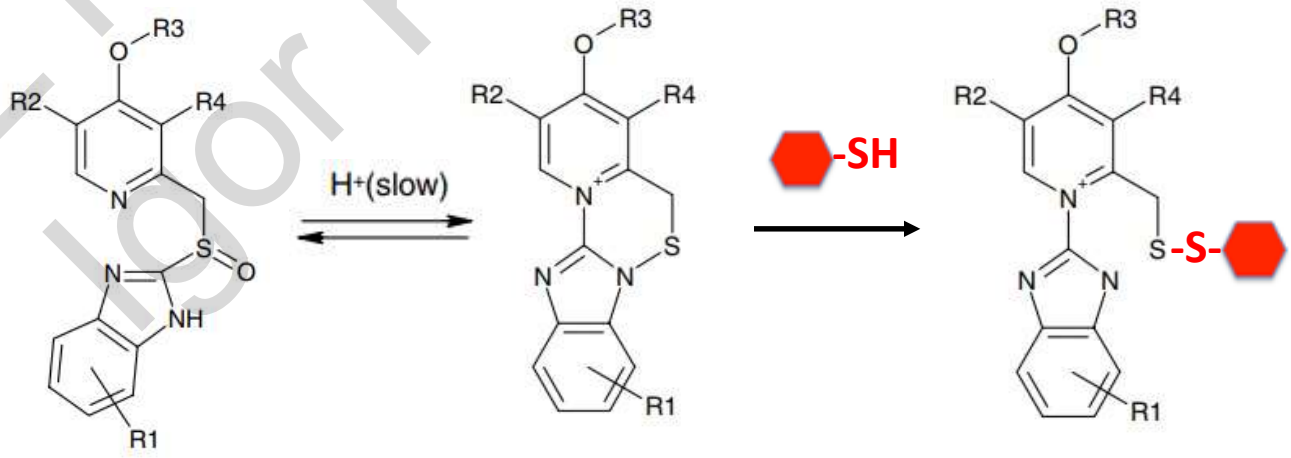



**Gastric juice:**
**155 mM HCl (pH 0.8)**
**15 mM KCl**

***http://www.vivo.colostate.edu/hbooks/pathphys/digestion/stomach/parietal.html***




**omeprazole**

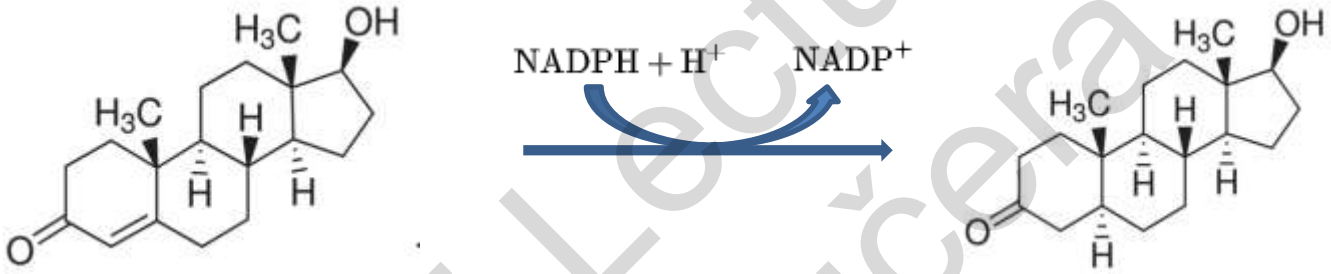



**After acid transformation to sulfenamides the drugs bind covalently to sulfhydryl groups of cysteines of the proton pump.**

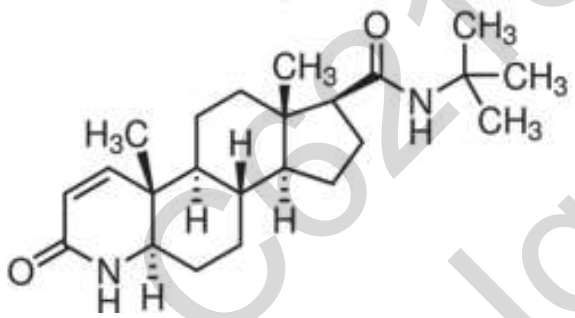
***Jain et al., Bioorg. Med. Chem. 2007, 15, 1181***

# Inhibition of 5 alpha-reductase

**The androgen hormone testosterone is secreted in the adrenal glands and ovaries in women and in the adrenal glands and testes in men. T is taken from circulation to cells and converted to dihydrotestosterone (DHT), the preferred ligand for androgen receptor (AR), by the enzyme 5 alpha-reductase (5αR).**




**testosterone** **dihydrotestosterone**

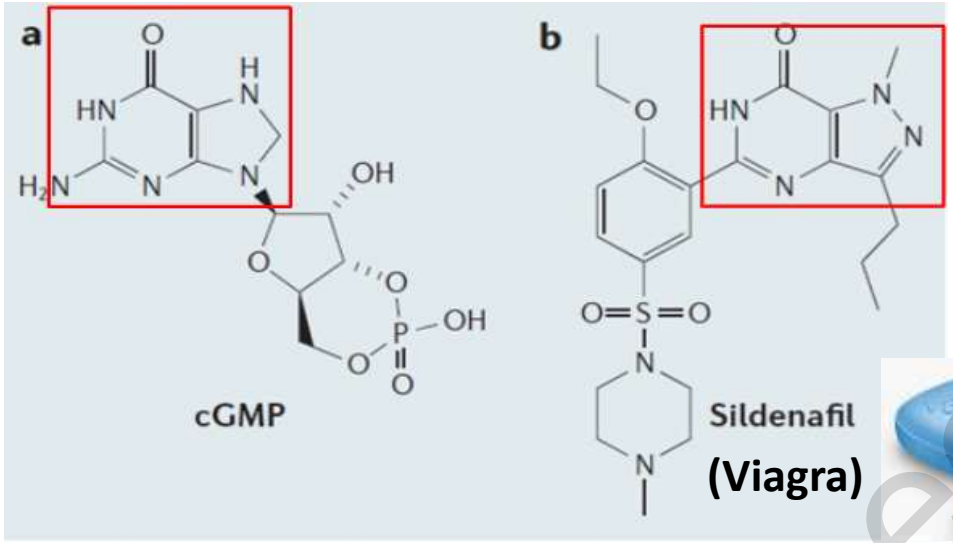


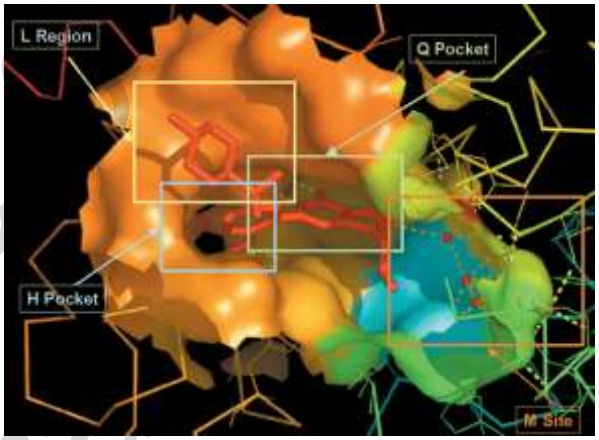

**Finasteride is a synthetic 4-azasteroid and is the first 5α-RI approved for treatment of benign prostatic enlargement (BPE) and subsequently androgenic alopecia.**



*Azzouni et al., Adv. Urol. 2012, 530121*

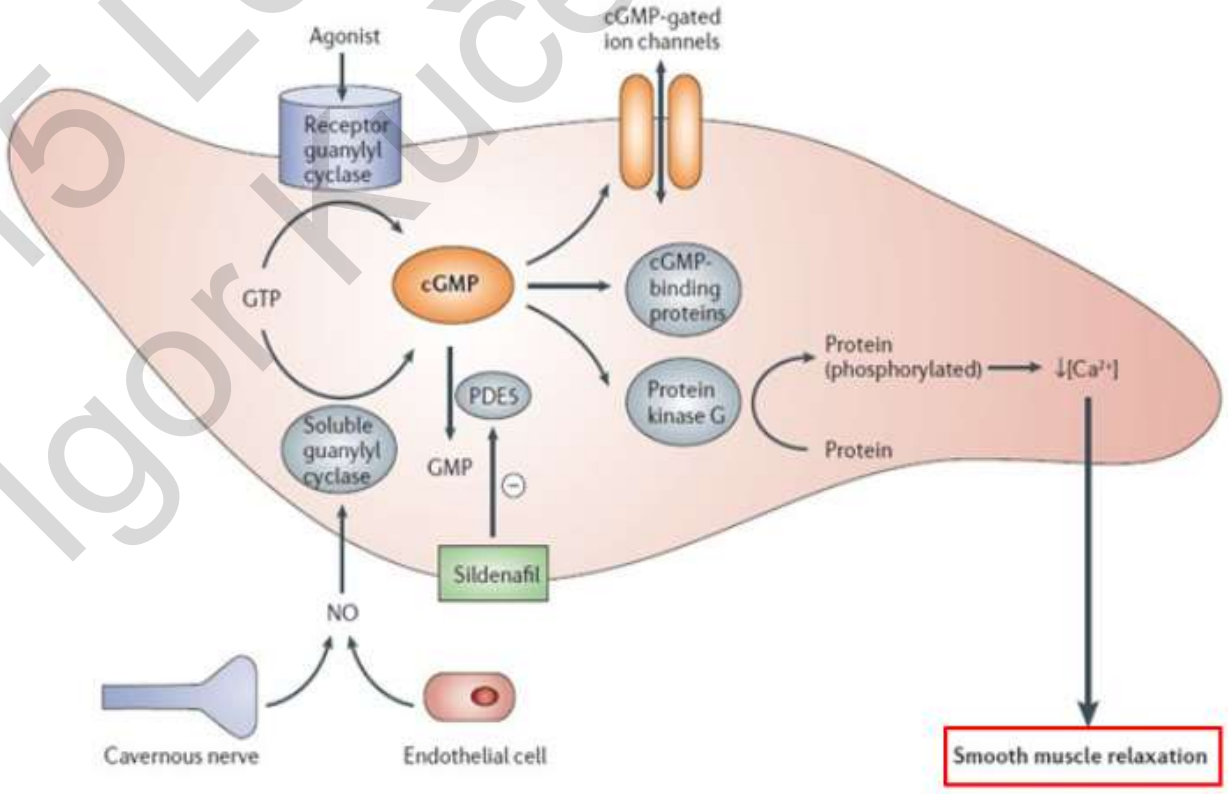
# Inhibition of phosphodiesterase-5



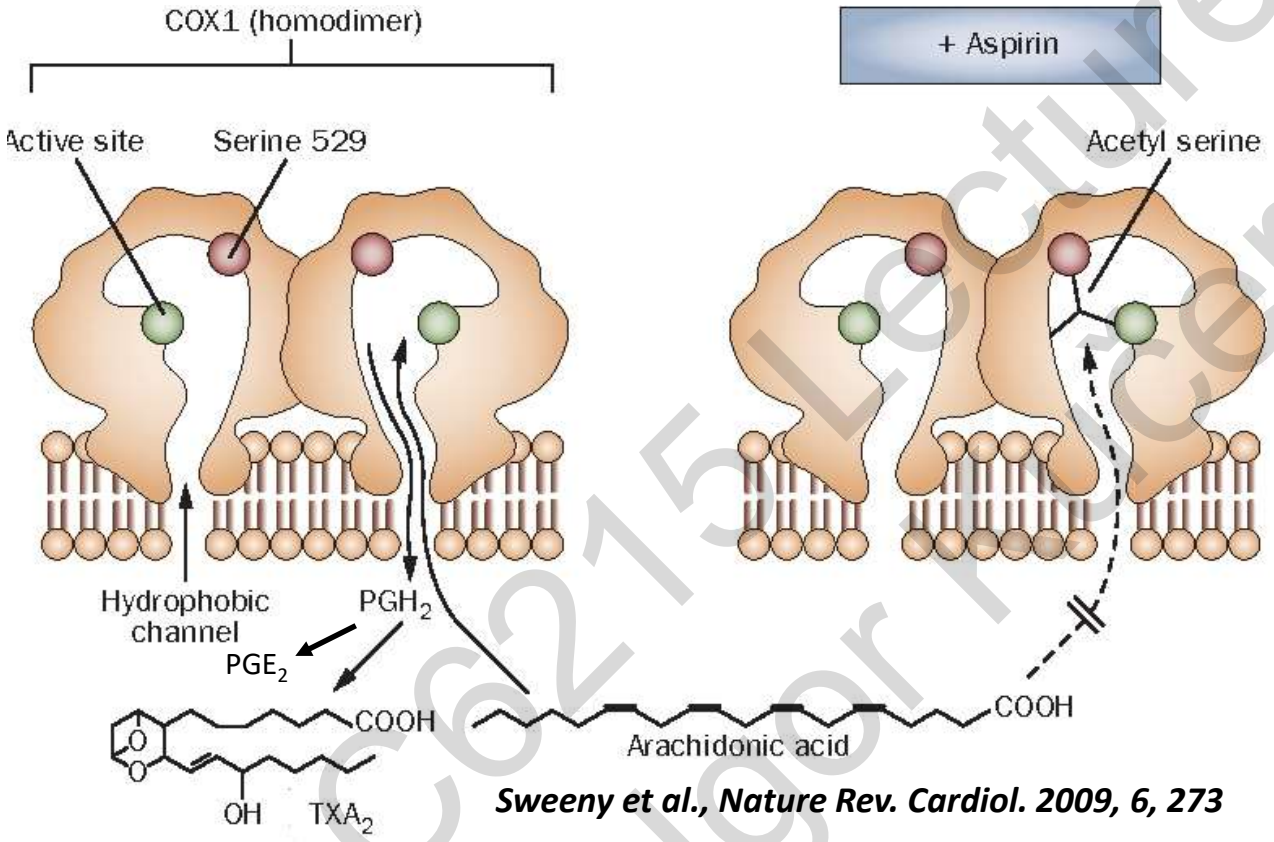

*Jeon et al., Cell. Mol. Life Sci. 2005, 62, 1198*

**Nitric oxide (NO) released from nerve endings in the penis and from endothelial cells activates soluble guanylate cyclase in smooth muscle cells. This results in increased levels of cGMP, activation of PKG, decreased $Ca^{2+}$, muscle relaxation and penile erection. PDE5 degrades cGMP. Sildenafil fits into the same site of PDE5 as cGMP, thus inhibiting the enzyme.**

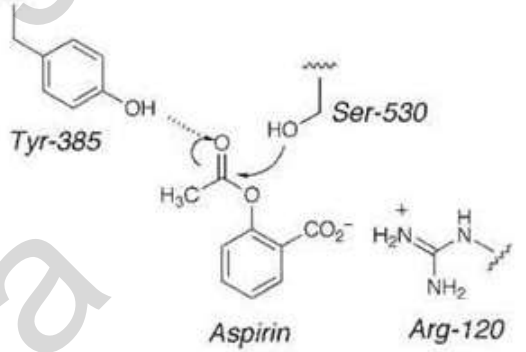


# Inhibition of cyclooxygenases

**Aspirin (acetylsalicylic acid ) is widely used to treat pain, fever, or inflammation and as a long term treatment to prevent heart attacks, ischaemic strokes, and blood clots in people at high risk.**



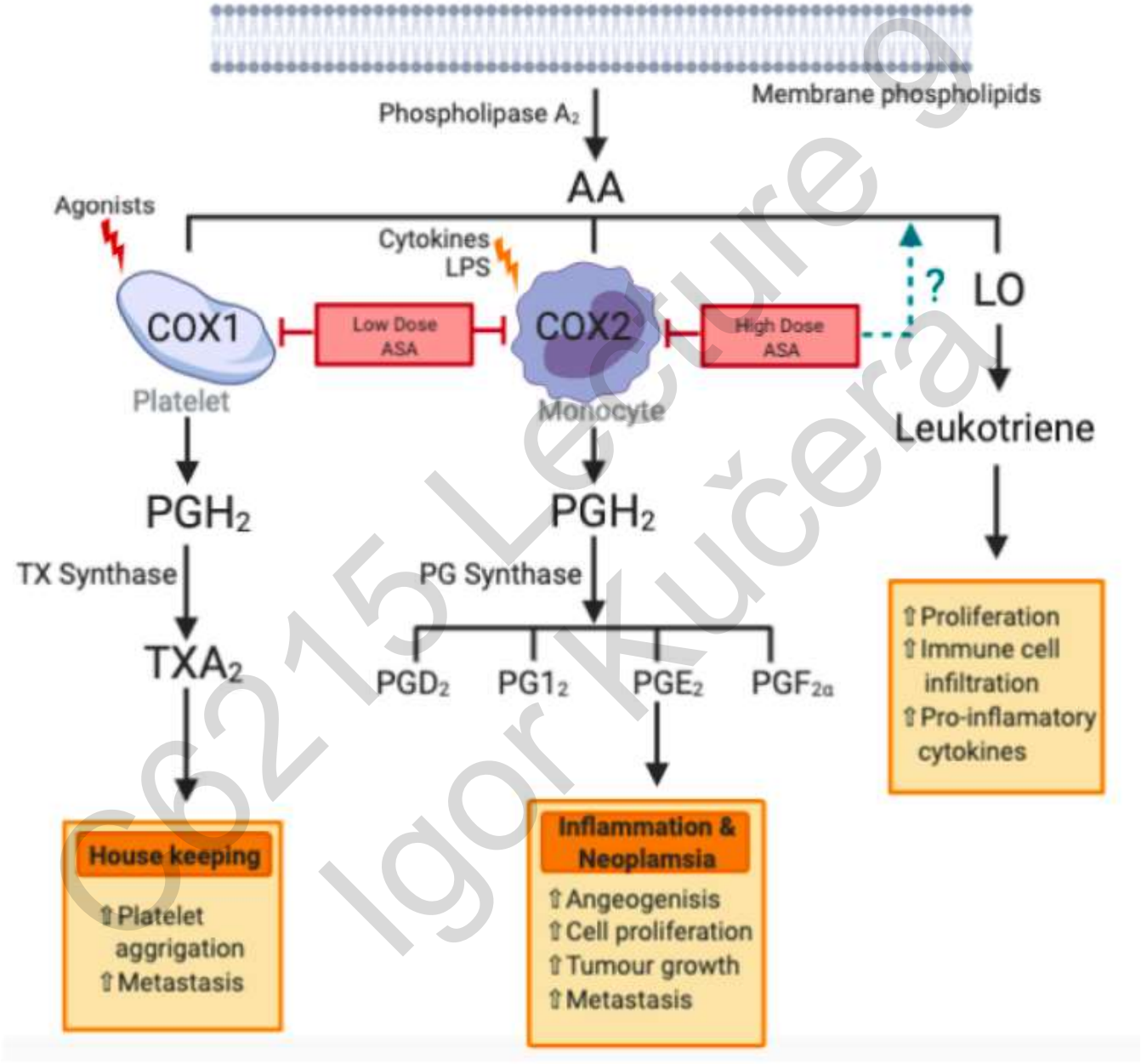

*Sweeny et al., Nature Rev. Cardiol. 2009, 6, 273*




*Hochgesang et al., J. Am. Chem. Soc. 2000, 122, 6514*

**Aspirin irreversibly acetylates a serine residue of cyclooxygenase, thereby blocking synthesis of prostaglandin $G_2$ and $H_2$, and consequently thromboxane $A_2$ generation.**

**Prostaglandins have diverse effects, including the transmission of pain information to the brain, modulation of the hypothalamic thermostat, and inflammation. $TXA_2$, which is formed from $PGH_2$ via the enzyme thromboxane synthase, acts as a potent vasoconstrictor and inducer of platelet aggregation.**

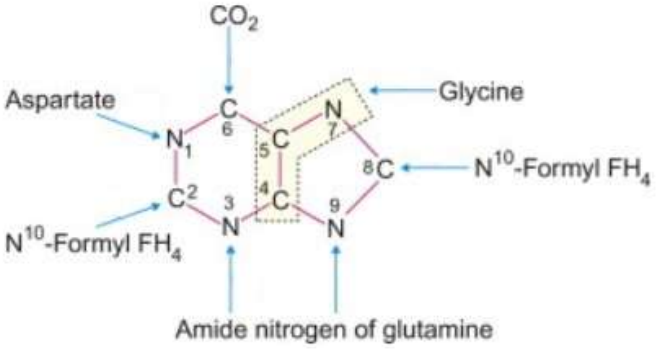


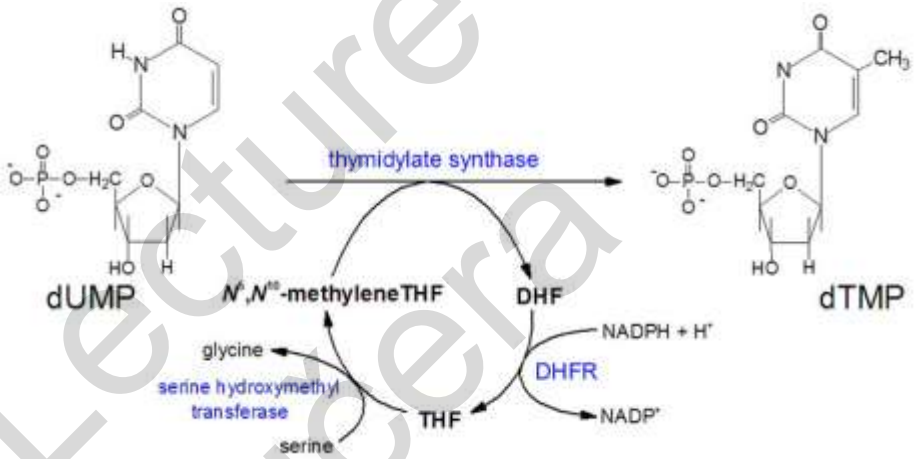


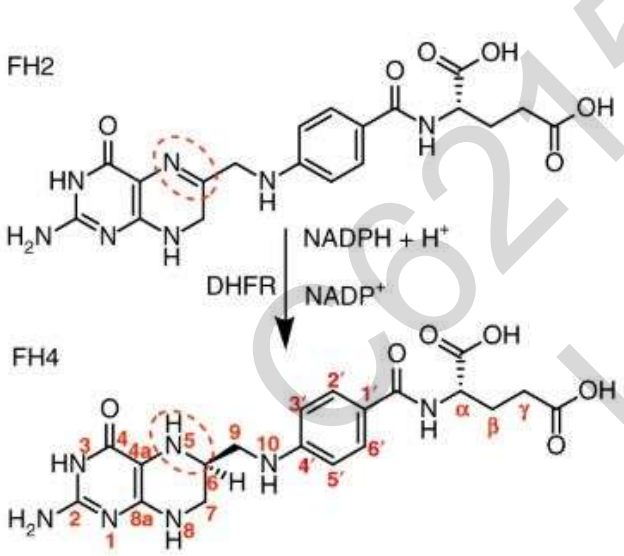

*Joshi S.N. et al. Cancer Treat. Res. Commun. 2021, 26, 100267*

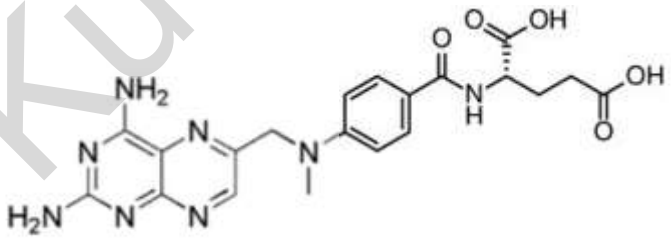
# Inhibition of dihydrofolate reductase

**Tetrahydrofolate, a metabolically active form of the vitamin folic acid, is involved in one-carbon metabolism, which includes the synthesis of purines and thymidylate for DNA synthesis.**



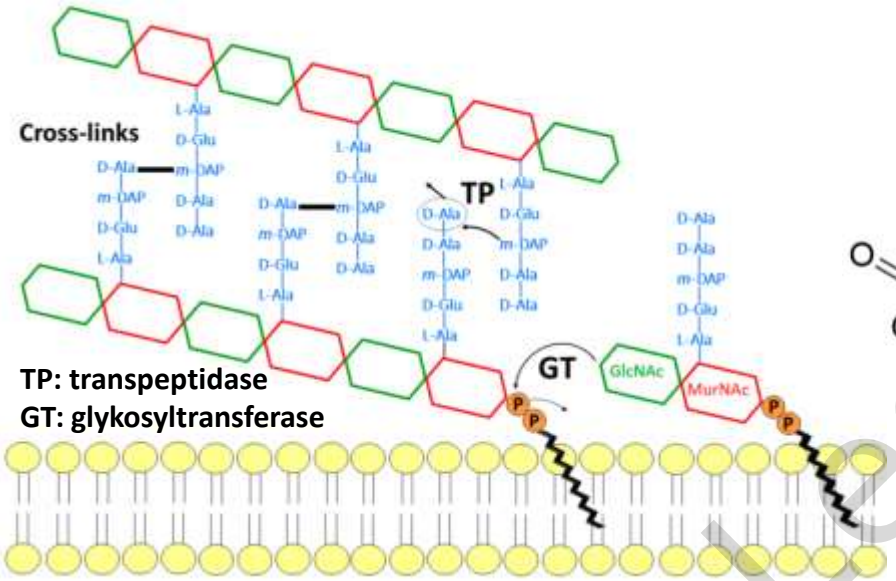



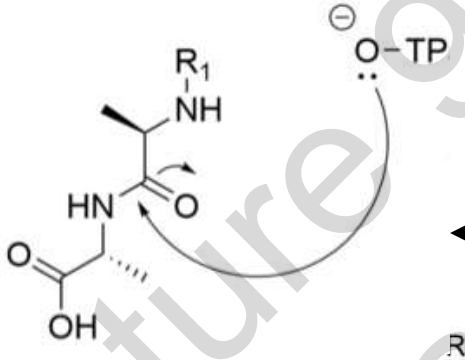



**Methotrexate is a slow and tight binding competitive inhibitor of dihydrofolate reductase (DHFR) ($K_i$=5 pM). Prevention of THF regeneration causes a toxic response on cells performing DNA replication, especially on rapidly proliferating cells. The drug is used in anticancer antimetabolite chemotherapy particularly for pediatric leukemia.**

# Inhibition of bacterial transpeptidase by beta-lactam antibiotics

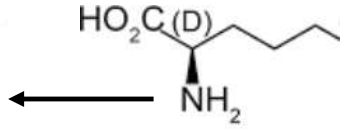


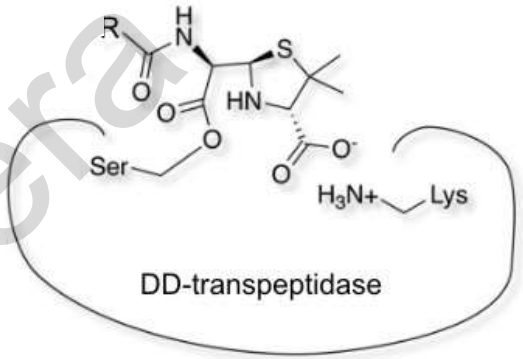

*Sauvage and Terrak, Antibiotics 2016, 5, 12*



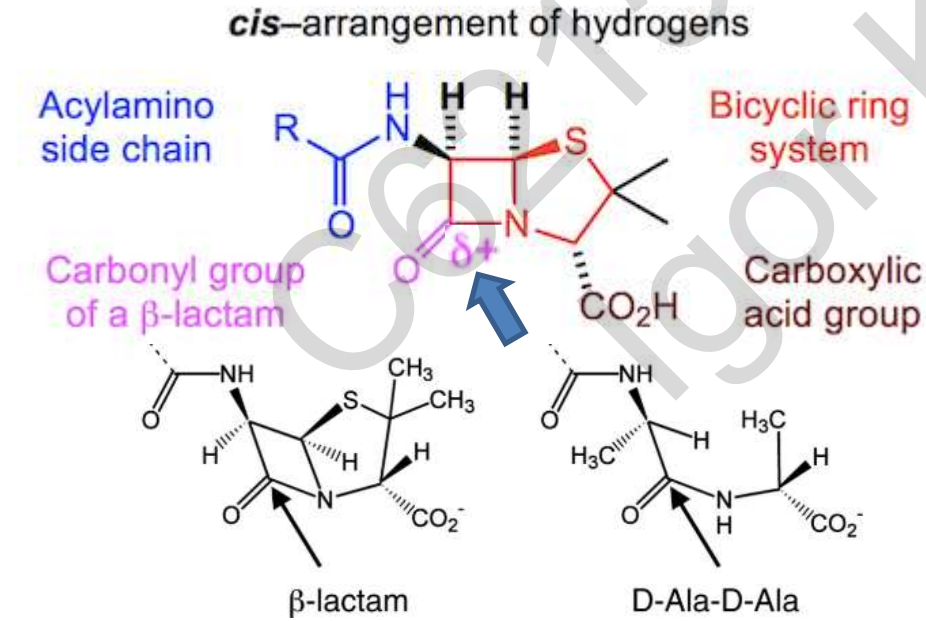

D-Ala

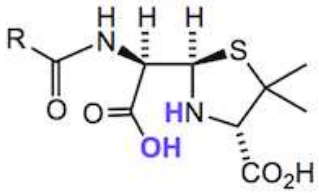
***meso*-diaminopimelate side chain**




**irreversible inactivation of transpeptidase**



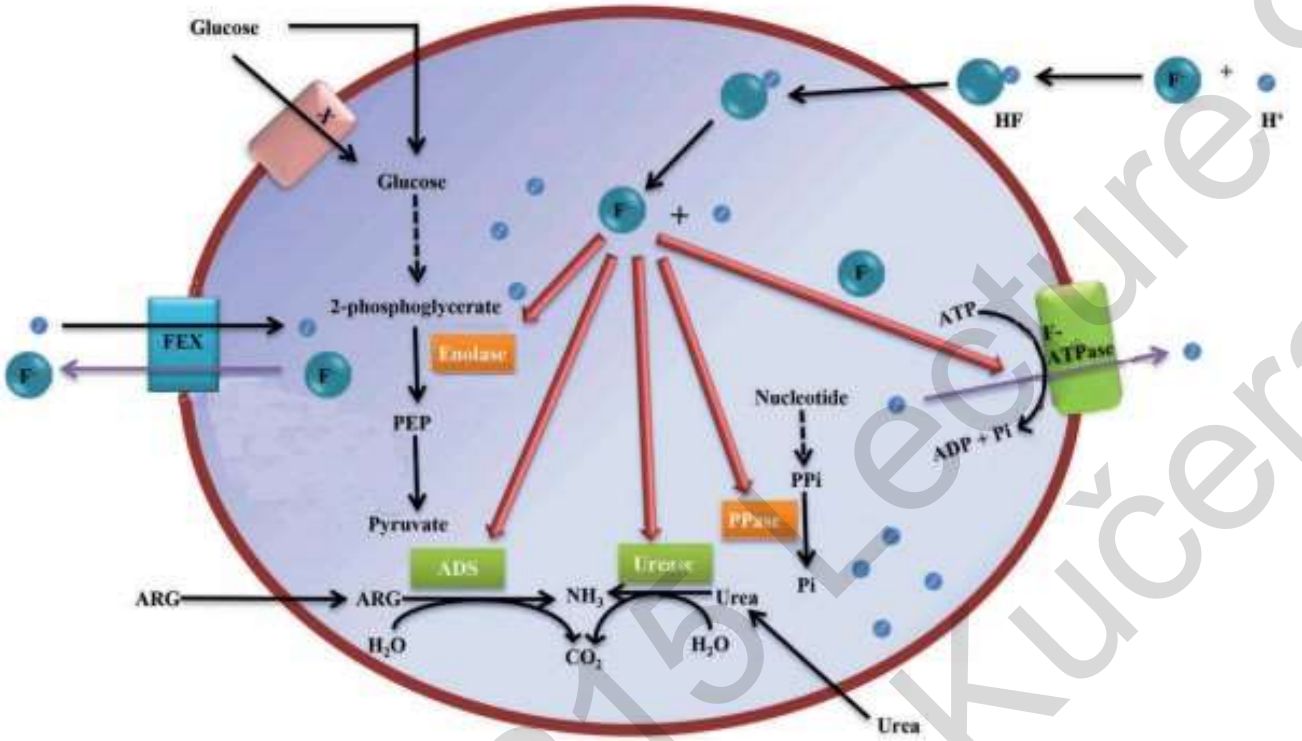




**hydrolysis**


**self-destruction (minimized by attaching a suitable substituent R)**

***https://www.futurelearn.com/courses/everyday-chemistry/0/steps/22314***

# Antimicrobial action of fluoride



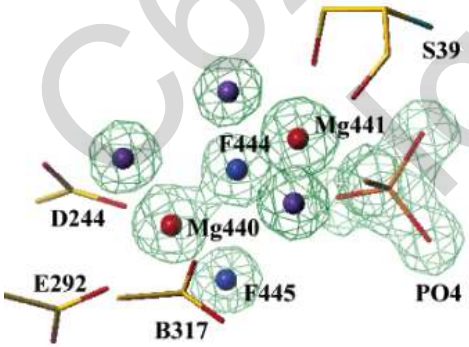

*Liao et al., J. Oral Microbiol. 2017, 9*

**HF diffusion into cells and dissociation to $H^+$ and $F^-$**

**Inhibition of enzymes:**

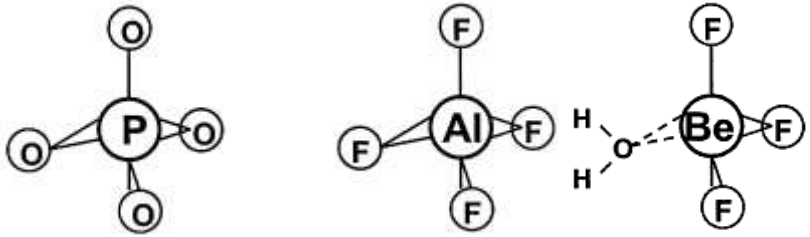
- **enolase**
- **F-ATPase**
- **PPase**
- **urease**
- **arginine deiminase**

**Enolase - $Mg_2F_2P_i$ inhibitory complex**

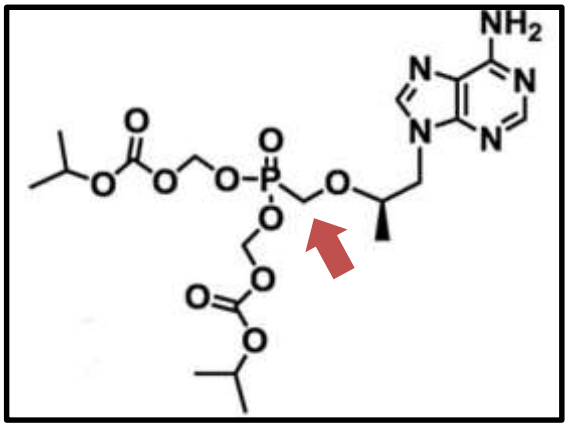



*Qin et al., Biochemistry 2006, 45, 793*

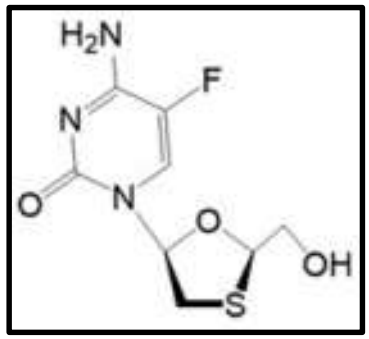
**Fluoride metal complexes as phosphate mimics**



# Inhibition of viral reverse transcription

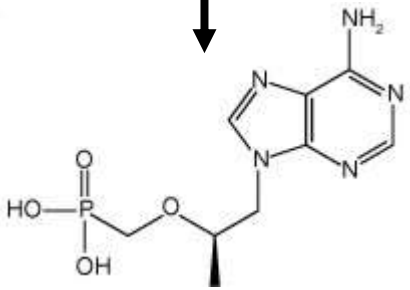


tenofovir disoproxil

emtricitabine

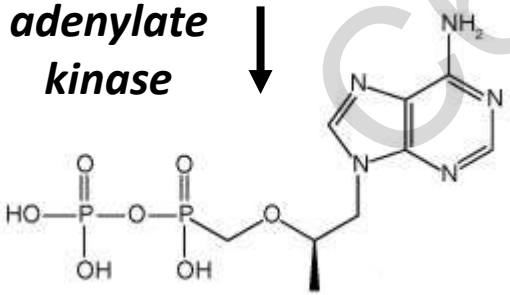
a **nucleoside**



tenofovir

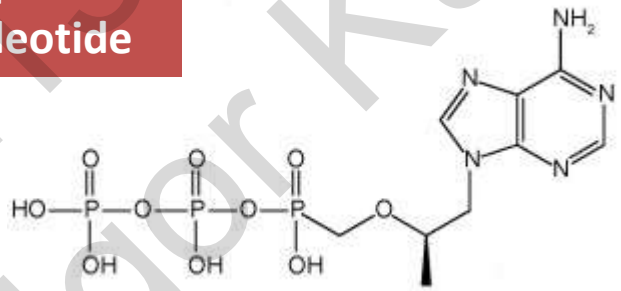
**an acyclic phosphonate nucleotide**

*adenylate kinase*

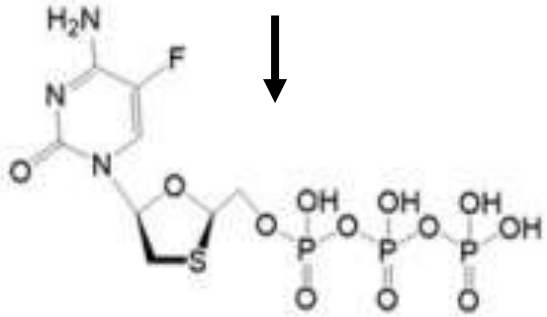


tenofovir phosphate

*NDP kinase*



tenofovir diphosphate

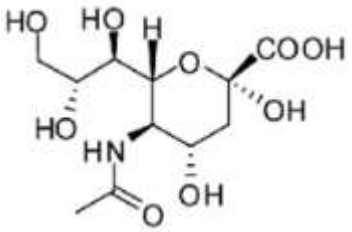


emtricitabine triphosphate

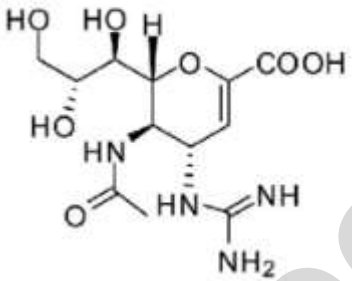
termination of DNA elongation

# Inhibition of viral neuraminidase (sialidase)

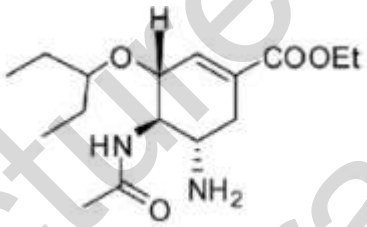
**Sialidase is the influenza virus surface enzyme recognizing the sialic acid N-acetylneuraminic acid (Neu5Ac) moiety, which is typically associated as α-linked terminal saccharidic unit of mammalian glycoconjugates. Its inhibition decreases the release of virus from infected cells.**



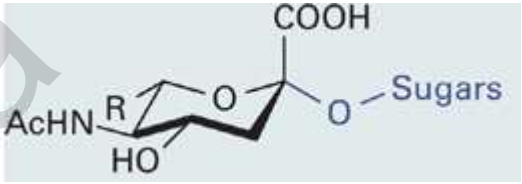
**Neu5Ac**



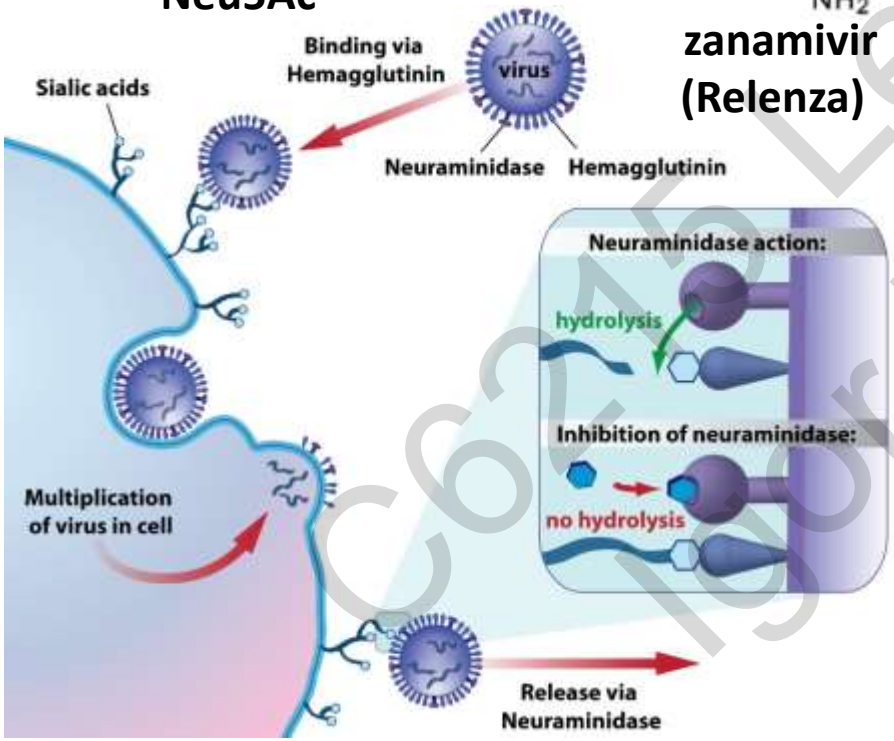
**zanamivir (Relenza)**

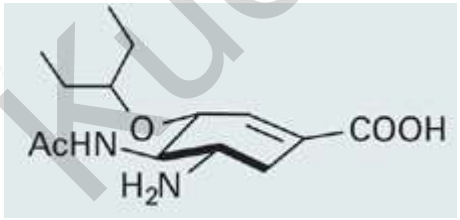


**oseltamivir (Tamiflu)**

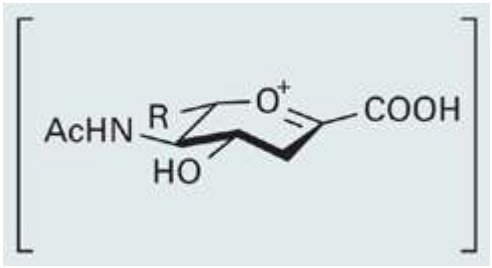


**sialic acid glycan**

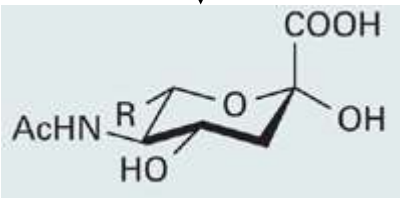


**oseltamivir transition state mimic**



**transition state**



**sialic acid**

*https://medicalxpress.com/news/2013-02-flu-drug-virus-tracks.html*

*https://basicmedicalkey.com/study-neuraminidase-inhibitors-and-the-influenza-virus/*

# Pesticides

**Pesticides are substances that are meant to control pests, including weeds.**

**Target organism:**

- herbicides
- insecticides
- rodenticides
- …

**Chemical structure:**

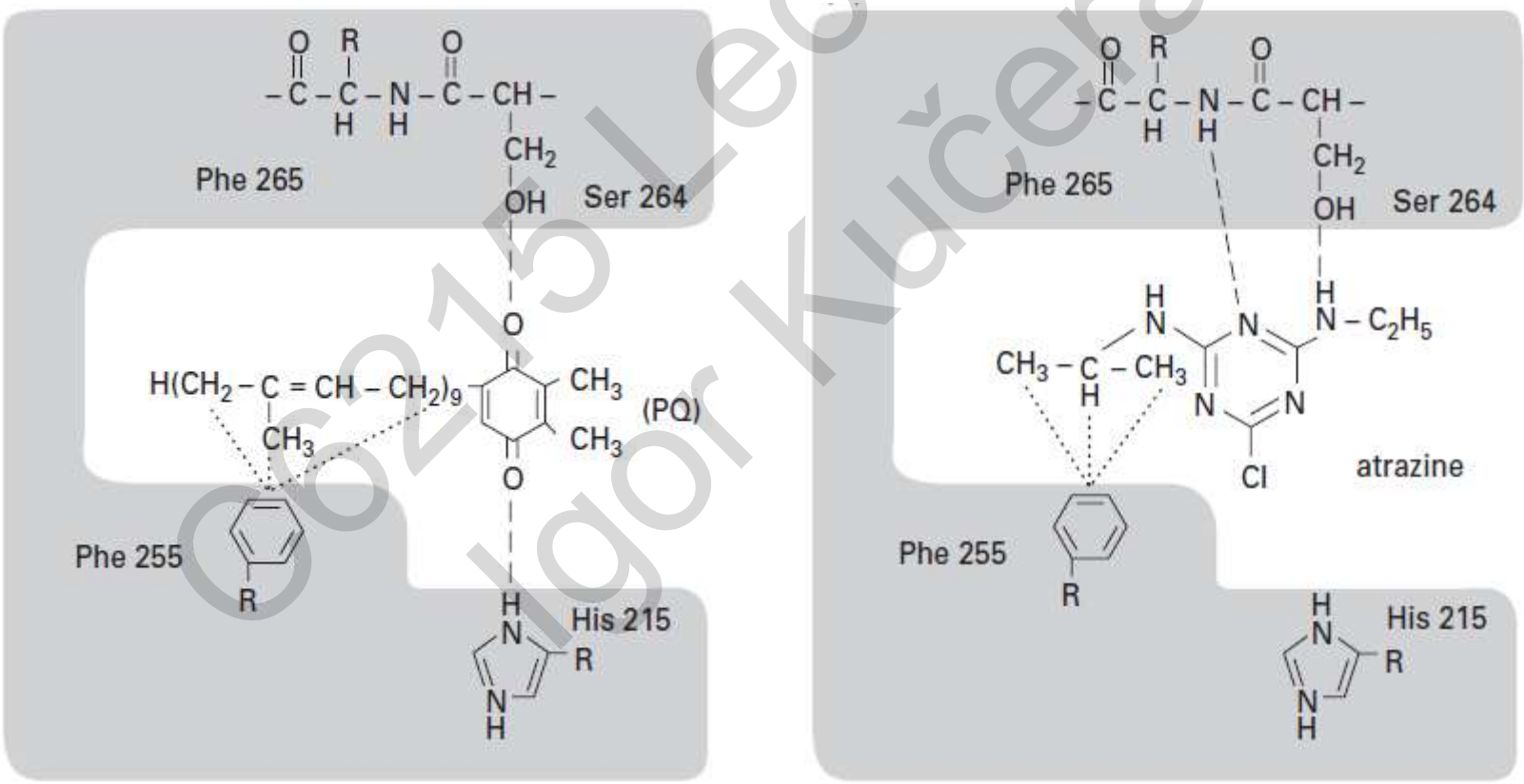
- organic
- inorganic
- biological
- …

**Target site of herbicides:**

- photosynthesis
- pigment biosynthesis
  - protoporphyrinogen oxidase
  - 4-hydroxyphenylpyruvate dioxygenase
- lipid biosynthesis
- amino acid biosynthesis
  - glutamine synthetase
  - EPSP synthase
  - acetolactate synthase
- …

# Inhibition of photosystem II

**In the photosynthetic electron transport chain, plastoquinone (PQ) binds to the D1 protein in the PS II reaction center, accepts two electrons and two protons, and is released as plastoquinol ($PQH_2$). Triazine (e.g., atrazine, simazine) and substituted urea (e.g., diuron, monuron) herbicides bind to the plastoquinone (PQ)-binding site on the D1 protein and prevents the binding of PQ. Excitation energy cannot be dissipated by normal electron flow and activated oxygen species are generated.**

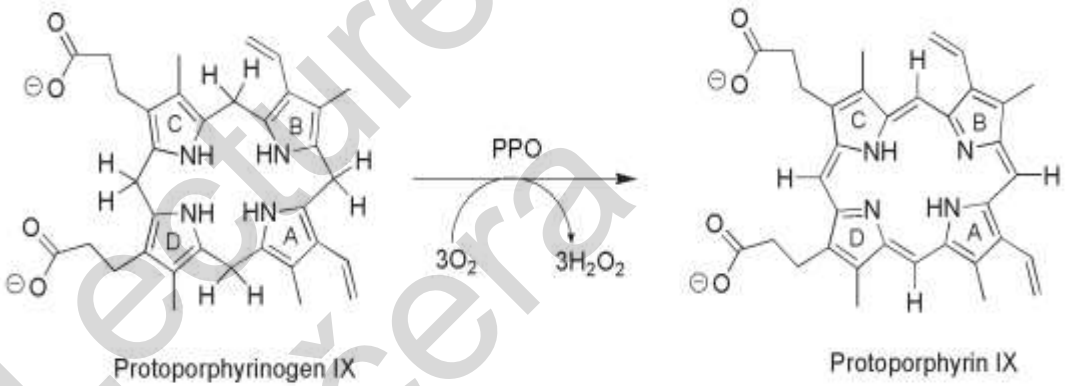



*Cobb and Reade, Herbicides and Plant Physiology 2010*

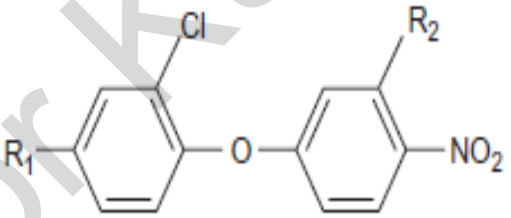
# Inhibition of protoporphyrinogen oxidase

**PPO is the last common enzyme in the biosynthetic pathway leading to heme and chlorophyll. In plants, there are two isoforms of PPO; the plastidic PPO1 and the mitochondrial PPO2. PPO1 is located in the thylakoid and in the envelope membranes of chloroplasts; PPO2 is situated on the outer surface of the inner mitochondrial membrane. Inhibition of PPO causes accumulation of the protoporphyrinogen-IX substrate in the cytoplasm where it is nonenzymatically oxidized to the photosensitive protoporphyrin IX. With exposure to light, this protoporphyrin IX generates singlet oxygen molecules that cause lipid peroxidation and cell death. Therefore, PPO-inhibiting herbicides are also known as light-dependent bleaching herbicides.**

**PPO catalyzes the six electron oxidation of proto-porphyrinogen IX to the fully conjugated macrocyclic protoporphyrin IX.**




**diphenylethers**



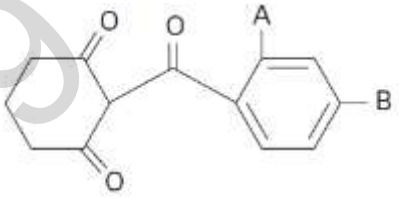

| $R_1$ | $R_2$ | |
|---|---|---|
| $CF_3$ | COOH | acifluorfen |
| $CF_3$ | $OC_2H_5$ | oxyfluorfen |
| Cl | H | nitrofen |
| Cl | COOH | bifenox |

**phenylpyrazoles, oxadiazoles, triazolinones, thiadiazoles, pyrimidindiones, oxazolidinedione, N-phenylphthalimides, and others**

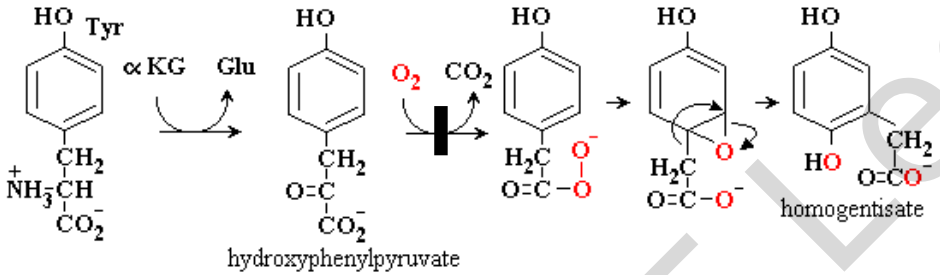
***Hao et al., Chimia 2011, 65, 961***

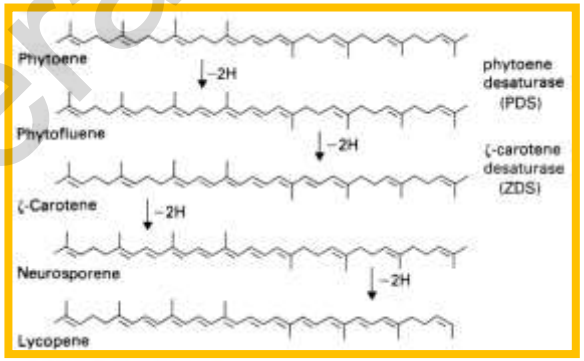
# Inhibition of 4-hydroxyphenylpyruvate dioxygenase

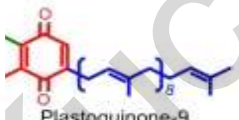
**Inhibition of the HPPD enzyme stops the catabolic degradation of tyrosine to plastoquinones and tocopherol (vitamin E). Plastoquinones are important for photosynthesis and carotenoid biosynthesis. Vitamin E protects biological membranes against oxidative stress and the photosynthetic apparatus against photo-inactivation.**



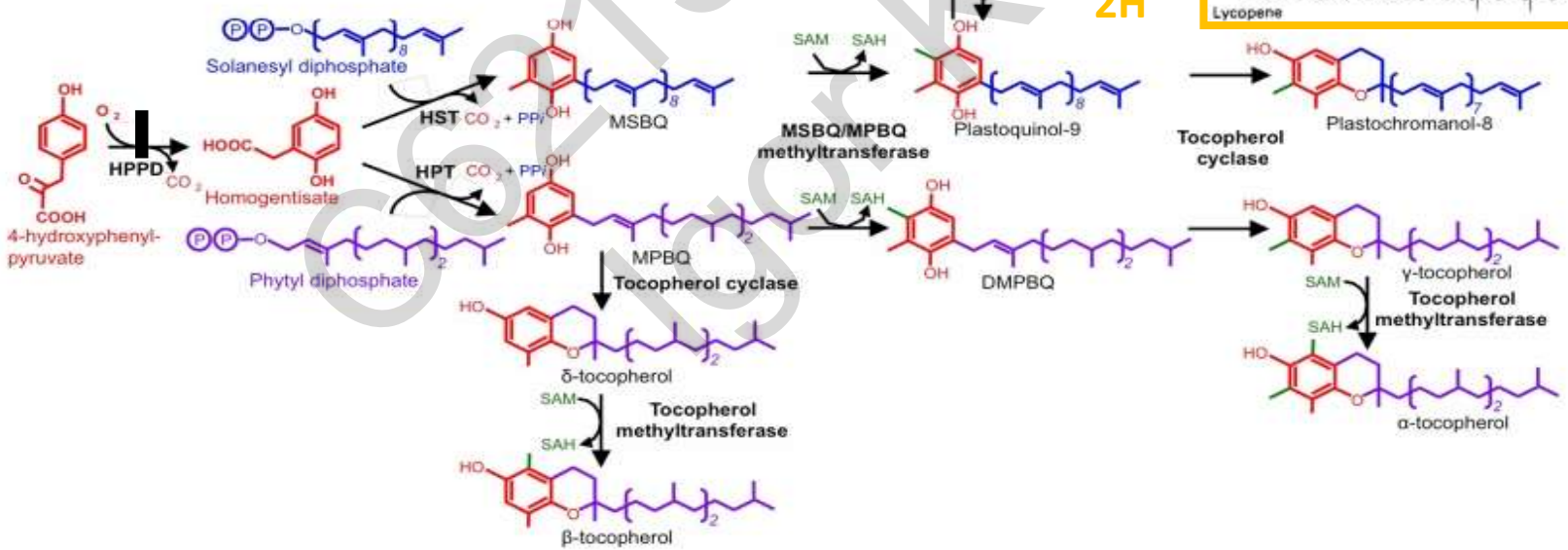

sulcotrione; A = Cl, B = $SO_2CH_3$
mesotrione; A = $NO_2$, B = $SO_2CH_3$
NTBC; A = $NO_2$, B = $CF_3$



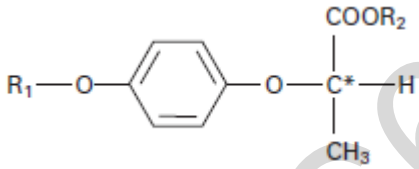




2H

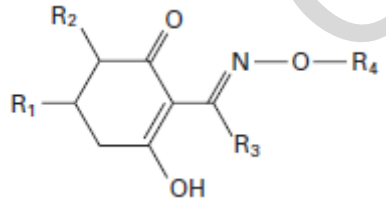


# Inhibition of acetyl coenzyme A carboxylase

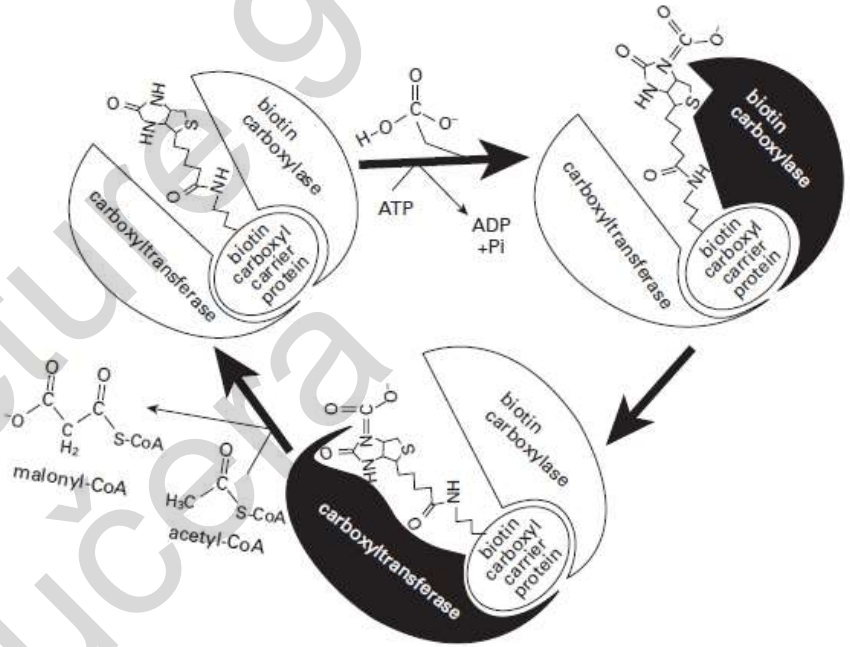
**ACCase is a biotin-containing enzyme that catalyzes the ATP dependent carboxylation of acetyl-CoA to form malonyl-CoA. This reaction is the first step in the synthesis of fatty acids. Two forms of ACCase are found in higher plants. In dicotyledons a heteromeric form (termed the prokaryotic form) of ACCase is located in plastids. This form of ACCase is absent from the monocotyledon grasses due to the absence of the accD gene that encodes the β-CT polypeptide. A homomeric form of ACCase is present in both dicotyledons and grasses. Its inhibitors selectively injure grasses.**

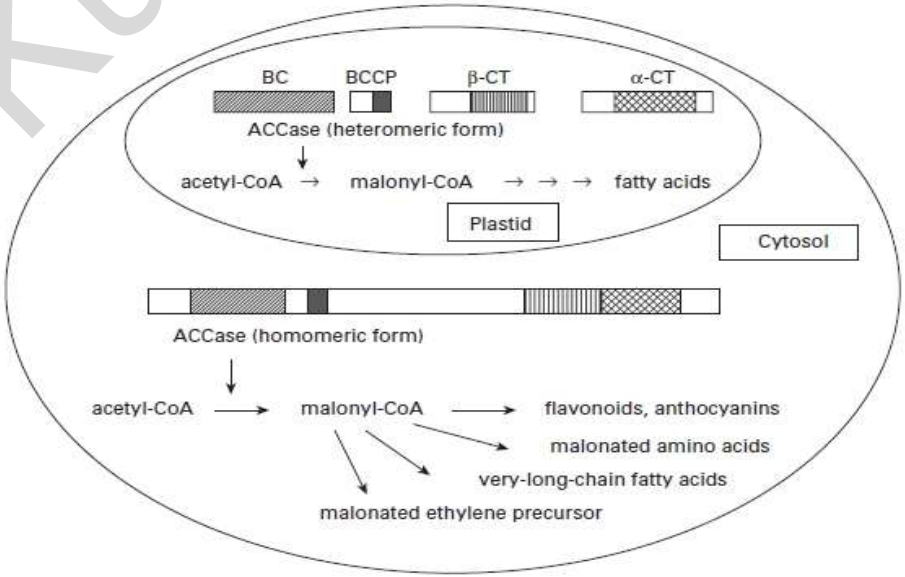



**aryloxyphenoxypropionates (Fops)**



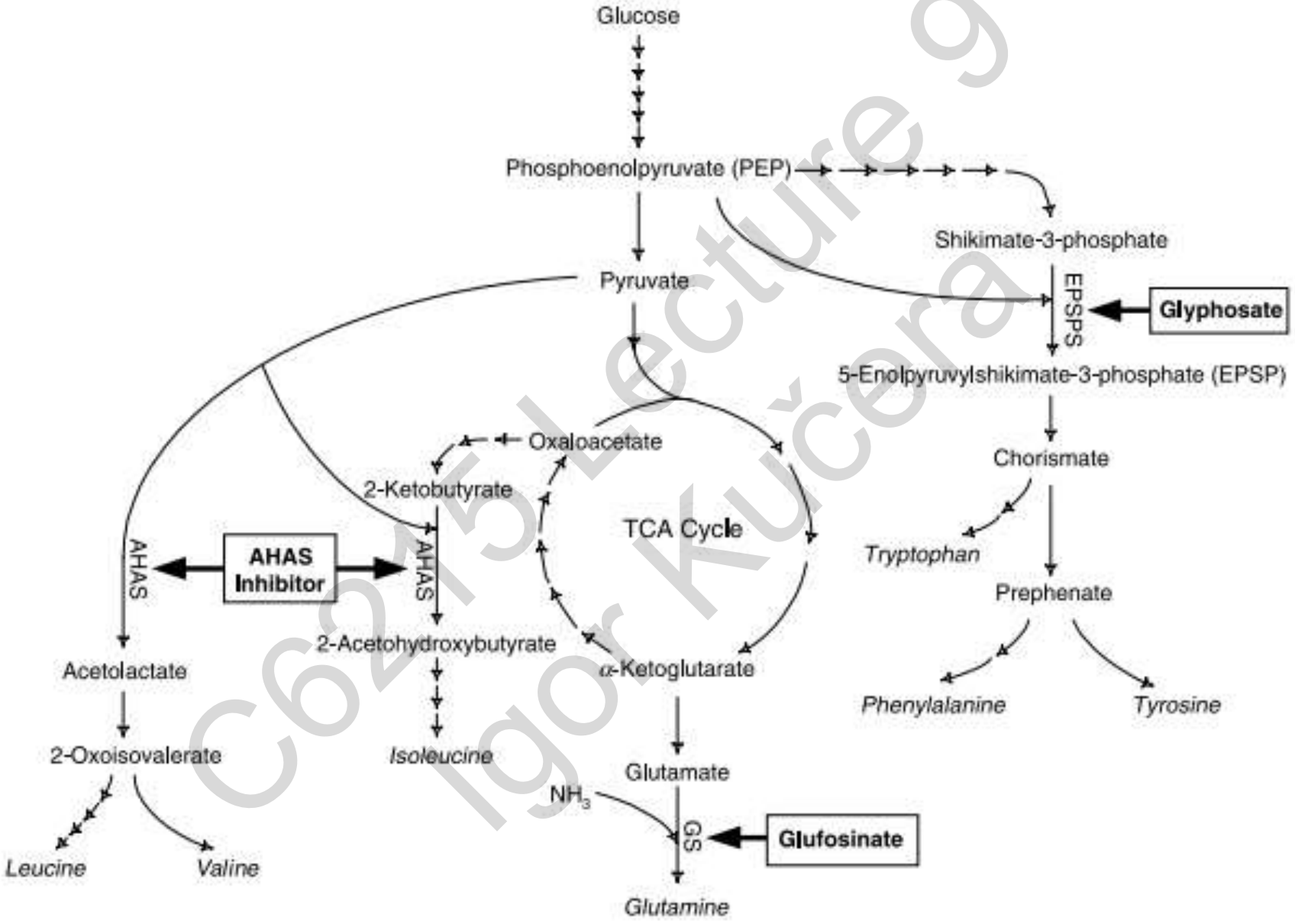

**cyclohexanediones (Dims)**




***Cobb and Reade, Herbicides and Plant Physiology 2010***

# Herbicidal inhibitors of amino acid biosynthesis

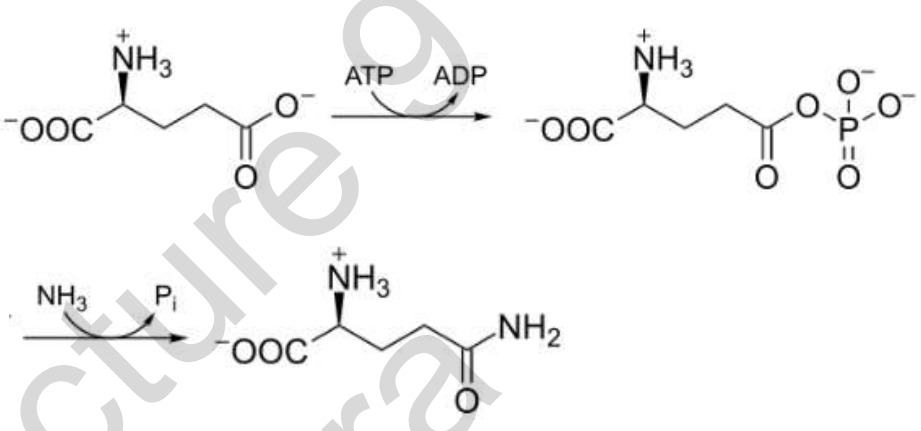


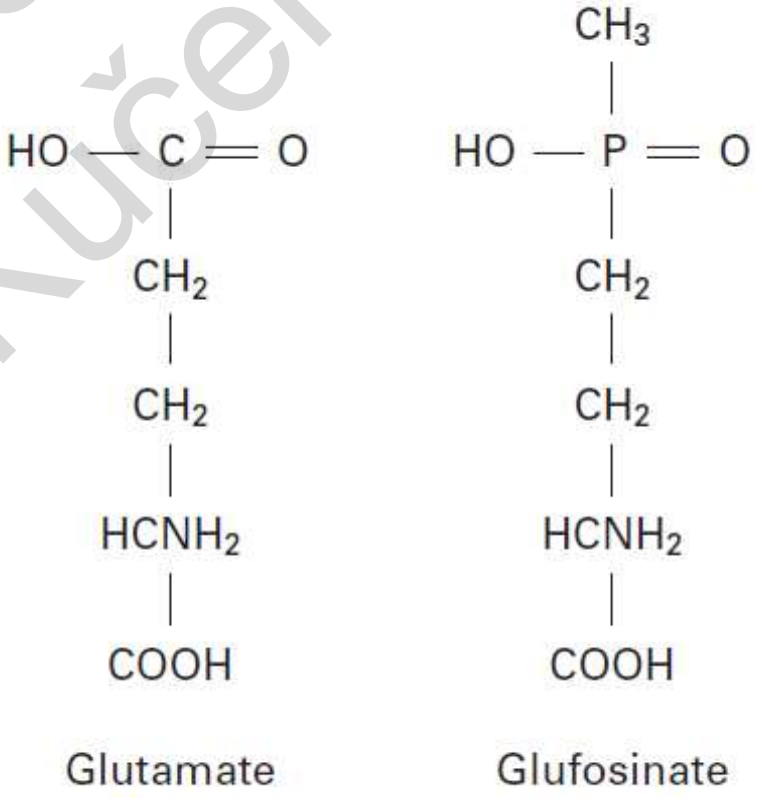

*Tan, Amino Acids 2006, 30, 195*

# Inhibition of glutamine synthetase

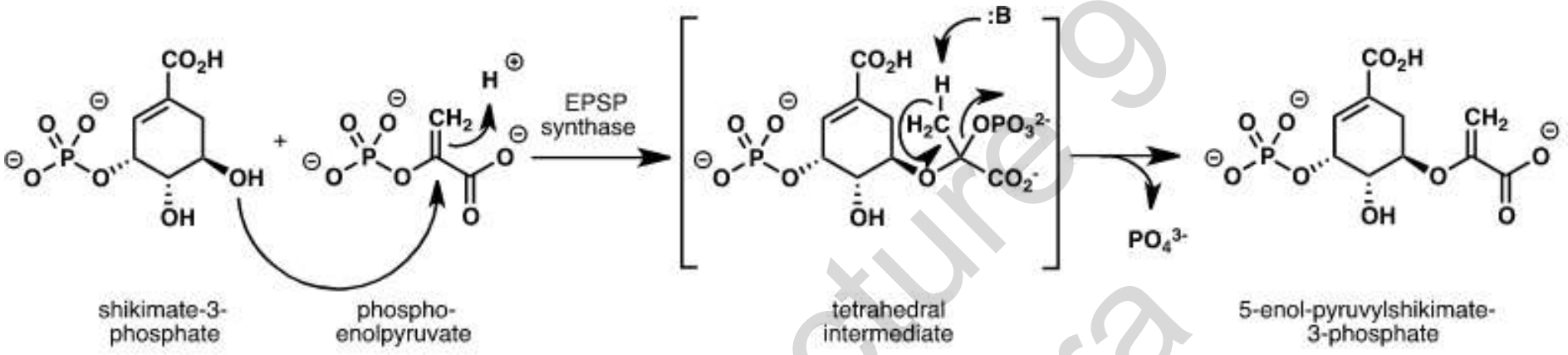
**Glutamine synthetase catalyzes the conversion of L-glutamate to L-glutamine in the presence of ATP and ammonia. γ - glutamyl phosphate is first produced from ATP and L-glutamate, and then ammonia reacts with this complex to release $P_i$ and L-glutamine. Glufosinate, as an L-glutamate analogue, can also be phosphorylated to produce an enzyme-glufosinate-phosphate complex to which ammonia cannot bind and the enzyme is irreversibly inhibited. The result is a rapid build-up of a high ammonia level, depletion of glutamine and several other amino acids and halting photosynthesis.**

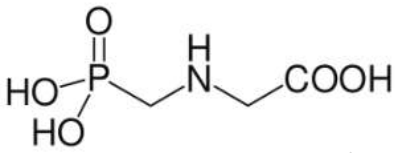
**Glufosinate can inhibit bacterial, plant and mammalian glutamine synthetase in vitro. It is only moderately toxic to mammals, apparently because of its rapid clearance by the kidneys.**



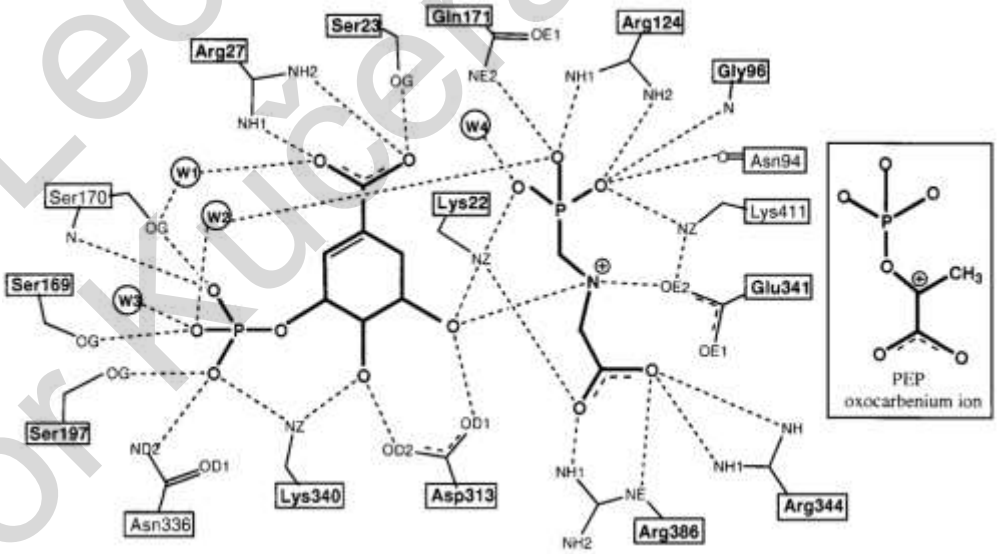



# Inhibition of EPSP synthase



**glyphosate**

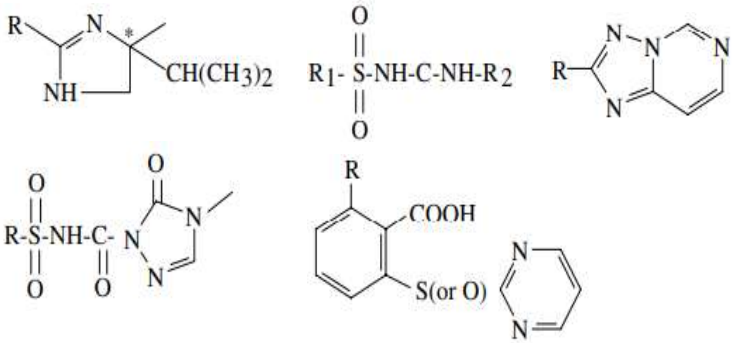



**Glyphosate (Roundup) is a non-selective total herbicide. Its target enzyme, EPSP synthase, is involved in the biosynthesis of the aromatic amino acids tryptophan, phenylalanine and tyrosine, and numerous secondary plant products (vitamins, lignins, alkaloids, and a wide array of phenolic compounds such as flavonoids).**

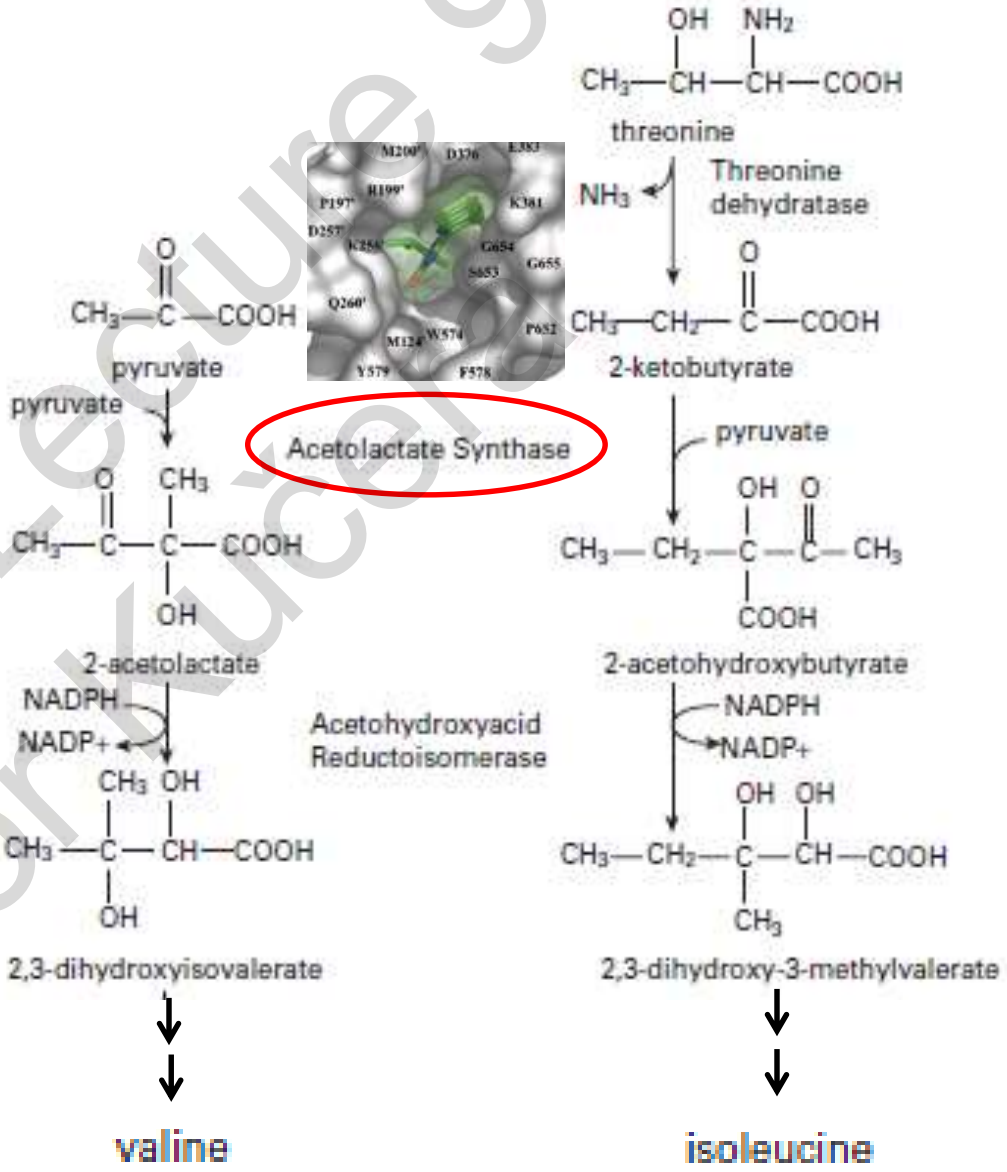
**X-ray crystallographic studies show that glyphosate functions by occupying the binding site of the phosphoenolpyruvate, mimicking an intermediate state of the ternary enzyme–substrate complex.**

***Schonbrunn et al., PNAS 2001, 98,1376***

# Inhibition of acetolactate synthase

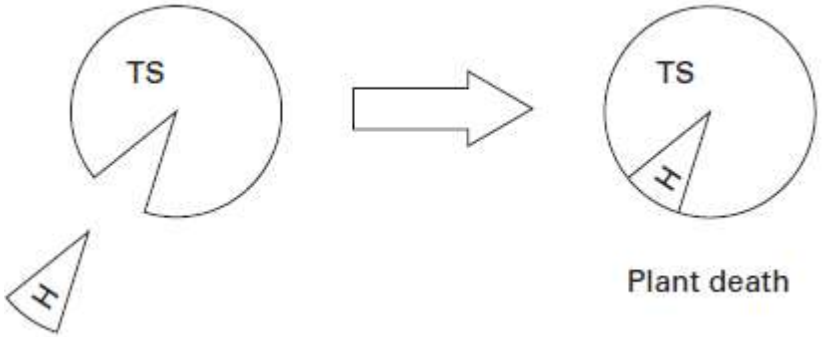


**The imidazolinones, sulphonylureas triazolopyrimidines, sulfonylamino-carbonyltriazolines and pyrimidinyl-oxy-benzoates are chemically different, yet all share the same site of action, namely acetolactate synthase, a key enzyme in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine. IMs and SUs were shown to block a channel through which access to the active site is gained.**
**The diminution of the branched-chain amino acid pool contributes to a cessation of protein synthesis and inhibition of cell division.**

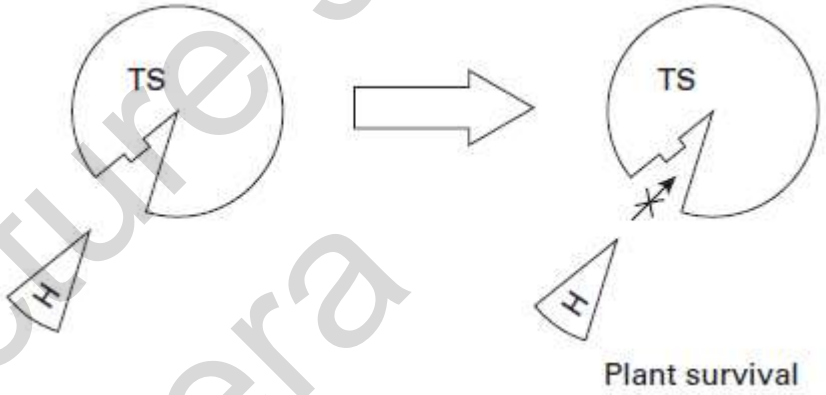


# Mechanisms of herbicide resistance

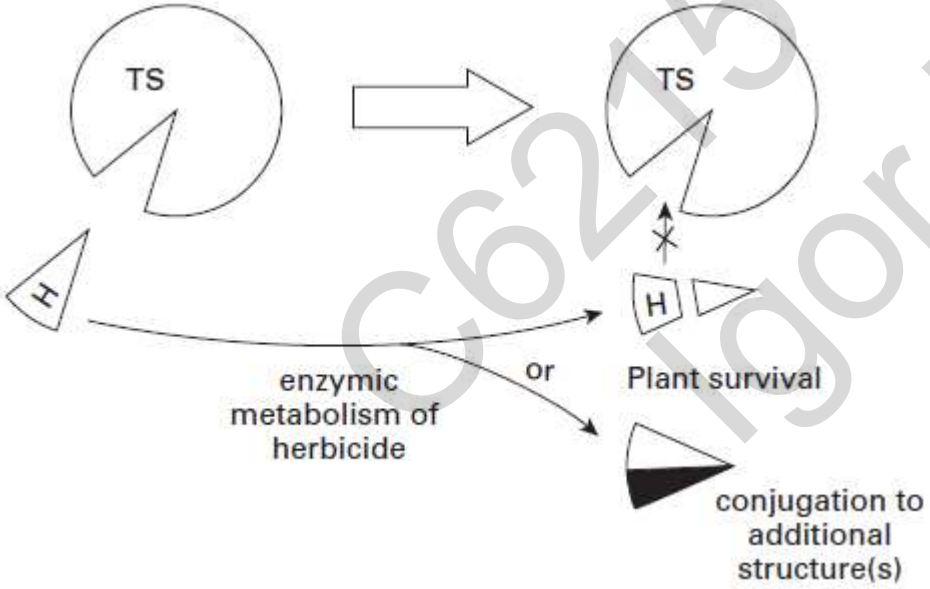
## Susceptible (TS = Target Site)



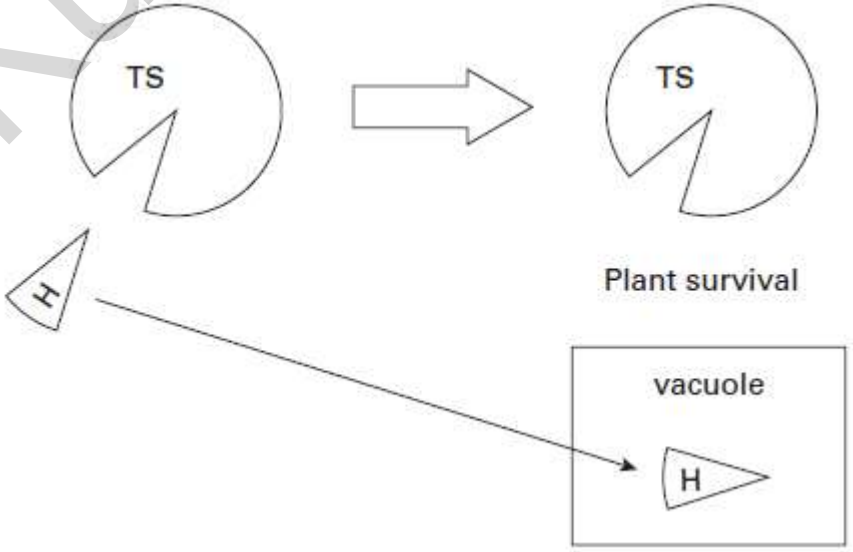

## Target Site Resistance




## Enhanced Metabolism Resistance




## Enhanced Compartmentalisation Resistance






*Cobb and Reade, Herbicides and Plant Physiology 2010*

# Genetically modified herbicide-tolerant crops

**The term Genetically Modified (GM) is used to denote the transfer of genes from one organism to another or the alteration of gene expression using genetic engineering techniques.**

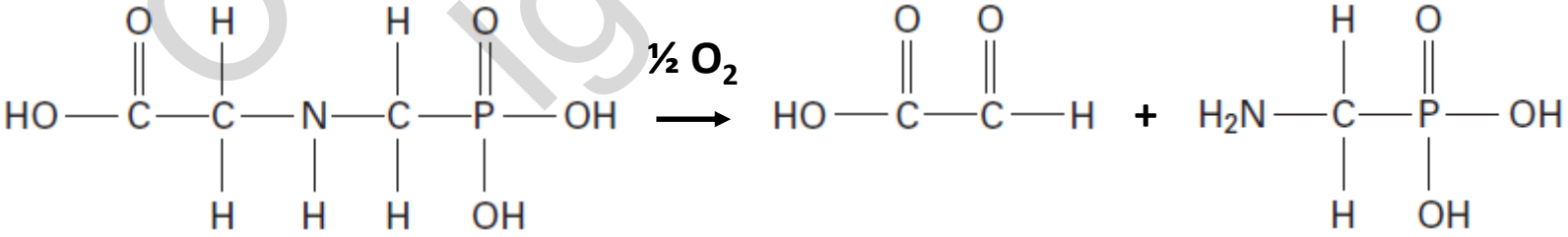
**Introduction of new genes into plants: *Agrobacterium tumefaciens*, particle bombardment, electroporation, microinjection …**

- **Glyphosate-tolerant crops**

**Insertion of the EPSP synthase CP4 gene (crops with the trade name Roundup-Ready)**

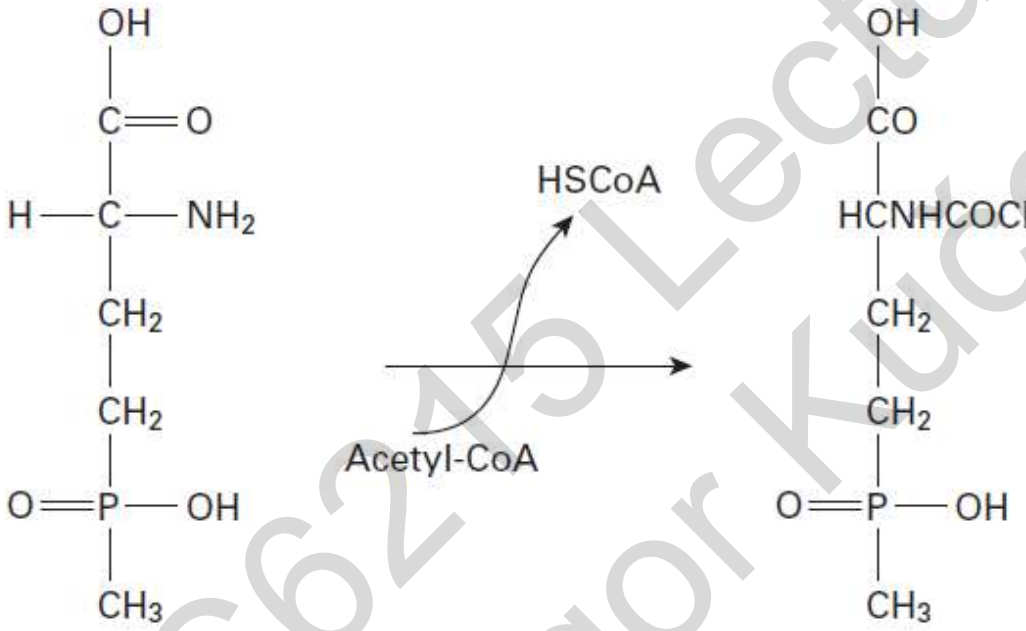
| Enzyme source | $K_m$ (PEP) (μM) | $K_i$ (glyphosate) (μM) | $K_i$ / $K_m$ |
|---|---|---|---|
| Petunia (wild type) | 5.0 | 0.4 | 0.08 |
| Agrobacterium sp. CP4 | 12 | 2720 | 227 |

**Insertion of the glyphosate oxidoreductase (*gox*) gene (z *Ochrobactrum anthropi*)**



- **Glufosinate-tolerant crops**

**Created by insertion of the *bar* or *pat* genes from streptomyces. These genes encode the enzyme acetyl transferase that detoxifies glufosinate by acetylation.**

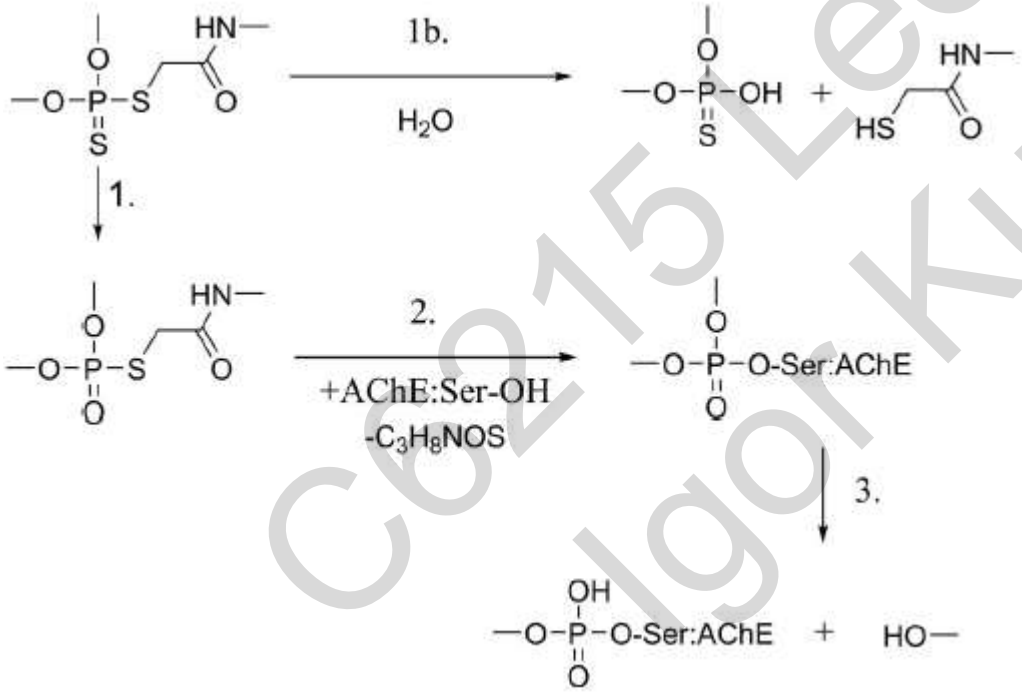



LIBERTY LINK®

**In 1995 the first glufosinate-resistant crop, canola, was brought to market, and it was followed by corn in 1997, cotton in 2004, and soybeans in 2011.**

# Organophosphorous insecticides

**Neurotoxic compounds, the mode of action of which is the inhibition of acetylcholinesterase; this inhibition blocks the hydrolysis of the AChE substrate acetylcholine, a neurotransmitter. The toxic action is a result of excess stimulation at the neuromuscular junction by an accumulation of acetylcholine. The active form is the "oxon" (P=O), the "thion" (P=S) being converted in vivo to the oxon by the cytochrome P450 monooxygenase system; the oxon then phosphorylates the serine residue in the active center of the enzyme.**




**1. Dimethoate activation by cyt P450**

**1b. Hydrolysis (preferred in vertebrates)**

**2. Phosphorylation of the serine hydroxyl group in the active site of acetylcholinesterase**

**3. Spontaneous dealkylation ("aging"), conversion of the inhibited enzyme into a non-reactivable form**

*Vlček and Pohanka, Chem. Listy 2011, 105, 908*

# SZPI zjistila pekingské zelí s nadlimitním obsahem pesticidu dimethoat

31. 05. 2019

V rámci cílených kontrol cizorodých látek v sortimentu čerstvého ovoce a zeleniny, které Státní zemědělská a potravinářská inspekce (SZPI) provádí v maloobchodní síti, velkoobchodě i v rámci kontrol dovozu, zjistila SZPI nevyhovující šarži potraviny „pekingské zelí" ve velkoskladu provozovatele Jilemnická obchodní společnost, spol. s r.o., Komenského 70, Jilemnice 51401.

Jednalo se o čerstvé pekingské zelí volně ložené v přepravkách, země původu: Polsko, u které laboratorní rozbor SZPI potvrdil kontaminaci pesticidem dimethoat v množství 0,038 miligramů na kilogram (mg/kg). Příslušný právní předpis stanovuje maximální povolený limit pro přítomnost látky dimethoat na 0,01 mg/kg. Dimethoat je insekticid, který se používá jako ochrana rostlin proti hmyzím škůdcům.

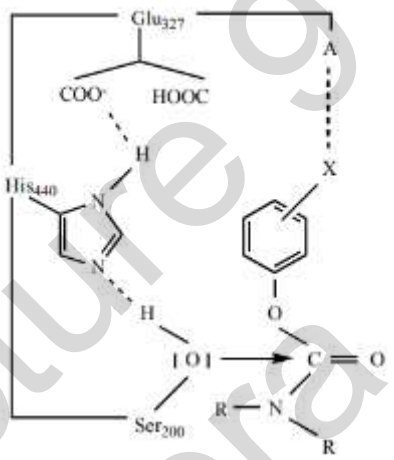
Z uvedených skutečností vyplývá, že provozovatel porušil Nařízení (ES) č. 396/2005 o maximálních limitech reziduí pesticidů v potravinách a krmivech a SZPI zahájí správní řízení o uložení pokuty.

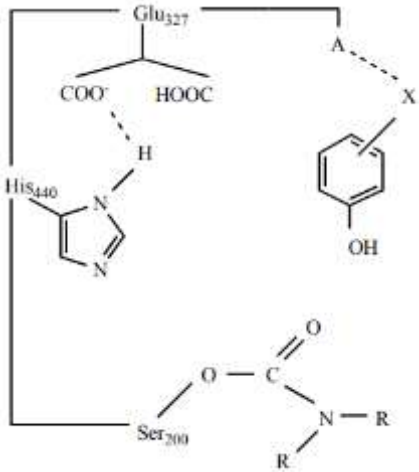
O dalších případech zjištění nadlimitního množství pesticidů v potravinách SZPI informovala v tiskových zprávách.

**Zpracoval: Mgr. Pavel Kopřiva - tiskový mluvčí, tel.: +420 542 426 633**

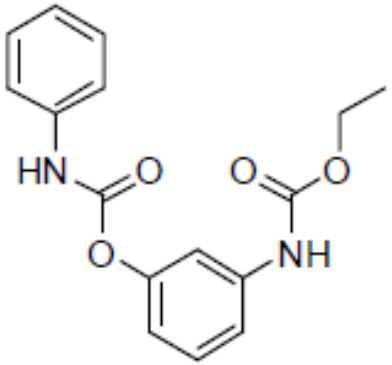
# Carbamate insecticides

**Kill insects by reversibly inactivating the enzyme acetylcholinesterase. An attack of the carbonyl function of the carbamate through Ser generates a temporary (in the order of min to hr) carbamylated complex that is incapable of further substrate catalysis.**

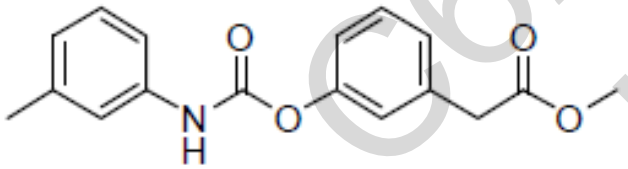



***Štěpánková and Komers, Curr. Enzyme Inhib. 2008, 4, 160***




desmedipham

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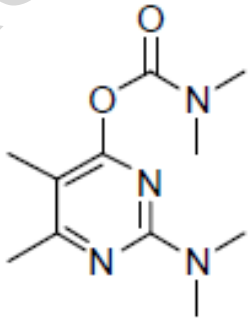
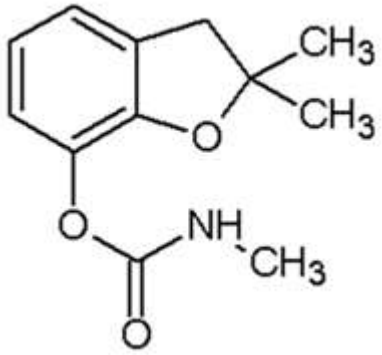



pirimicarb

# Carbofuran: illegal killing of animals and suicides




**Many animals - mostly raptors and other carnivores - have been poisoned by carbofuran in the Czech Republic. The poisoning substance is now banned in the EU, however, it had been widely used in agriculture as insecticide and large supplies are still available.**

**Suicidal ingestion cases are also known, e.g. Madalina Manole (1967-2010), a Romanian pop star**



*https://www.iucnosgbull.org/Volume27/Polednikova_et_al_2010.html*

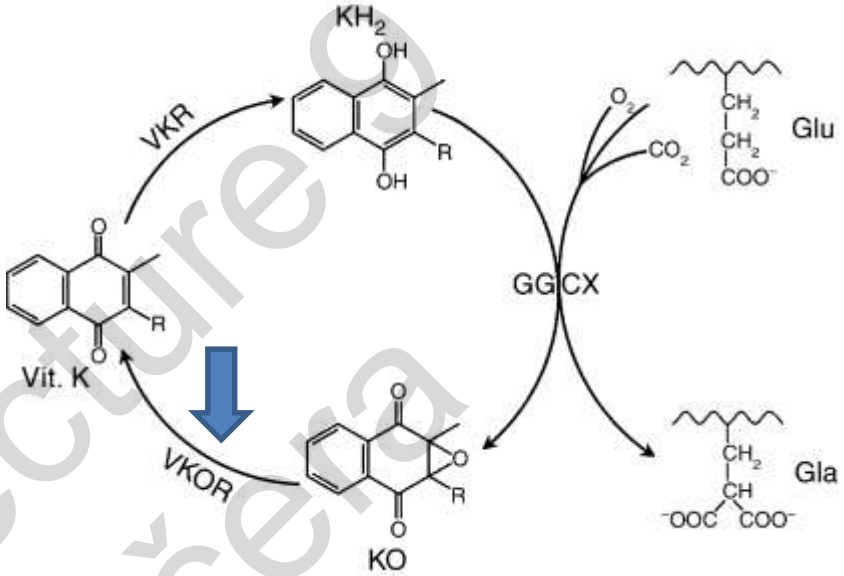
*http://www.karbofuran.cz/index.php?m*

*https://en.wikipedia.org/wiki/M%C4%83d%C4%83lina_Manole*

# Rodenticides

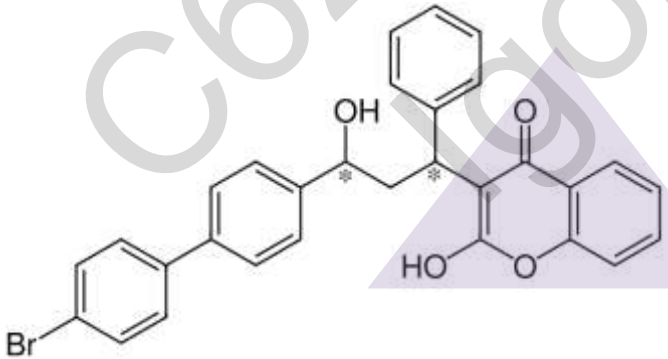
- **Anticoagulants**

**Act by blocking of the vitamin K cycle, resulting in inability to produce essential blood-clotting factors—mainly coagulation factors II (prothrombin) and VII (proconvertin). Designed to have high potency and long residence times in the body.**
**Death occurs after a period of several days to two weeks, usually from internal hemorrhaging.**



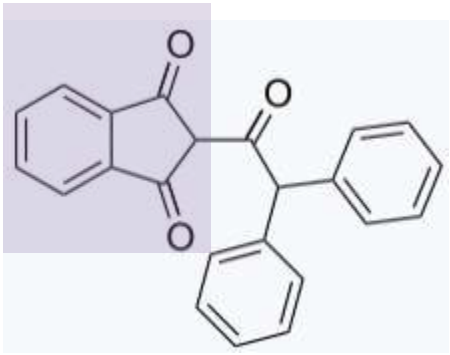

**GGCX: γ-glutamyl carboxylase**
**VKOR: vitamin K epoxide reductase**
**VKR: vitamin K reductase**

**4-hydroxycoumarins**




**bromadiolone**

**1,3-indandiones**




**diphenadione**

- **Metal phosphides**

**React with the acid in the digestive system to generate the phosphine gas:**

**$Zn_3P_2 + 6\ H^+ \rightarrow 3\ Zn^{2+} + 2\ PH_3$**

**$PH_3$ slows down mitochondrial respiration by inhibiting Complex IV (cytochrome c oxidase). Partial inhibition of mitochondrial respiration enhances electron leakage from the transport chain, leading to increased reactive oxygen species (ROS) generation.**
**Death occurs commonly within several hours after bait ingestion. The garlic-like odor of baits attracts rodents, but repels other mammals. Birds are not sensitive to the smell and can be poisoned.**


**STUTOX**
**5 % $Zn_3P_2$**
**LD50 (rat): 250 mg**
**LD50 (mouse): 20 mg**