LECTURE 9:

ENZYME INHIBITION AND APPLICATIONS

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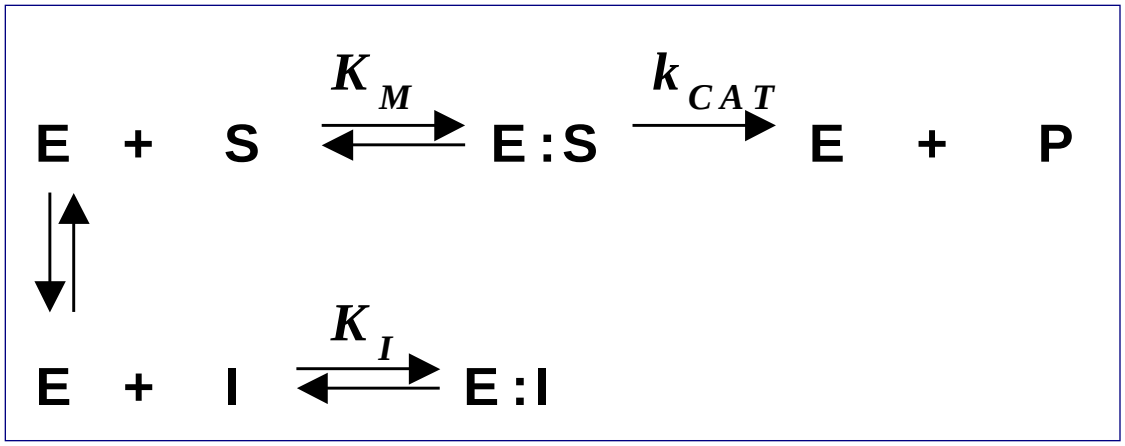
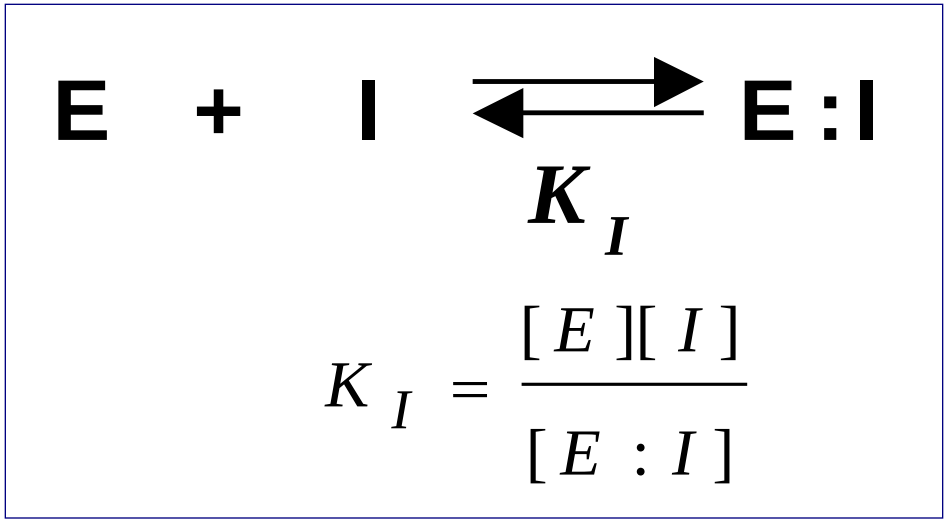




Units of K_I and IC_{50} = M

Why inhibit enzymes?

- Certain disease states may be caused by the product of an enzyme catalysis

- Drugs can target enzymes which are present in pathogens but not in the host

- Understanding the nature of enzyme catalysis AND the mechanism of a biochemical reaction can lead to the design of effective drugs

Enzymes as Drug Targets - a Closer Look

Transition state analogs and "suicide substrates"

Table 1: Database of Enzyme Targets for Marketed Drugs

entry	enzyme common name	EC no.[a]	example drug	indication	no. of drugs	organism[b]	ref[c]
1	1,3-β-glucan synthase	2.4.1.34	caspofungin	antifungal	1	F	*87*
2	3(or 17)-β-hydroxysteroid dehydrogenase	1.1.1.51	trilostane	breast cancer	1	H	*88*
3	3′,5′-cyclic-GMP phosphodiesterase	3.1.4.35	sildenafil	ED[d]	3	H	*89*
4	3′,5′-cyclic-nucleotide phosphodiesterase	3.1.4.17	theophylline	asthma	11	H	*90*
5	4-hydroxyphenylpyruvate dioxygenase	1.13.11.27	nitisinone	tyrosinemia	1	H	*91*
6	3-oxo-5-α-steroid 4-dehydrogenase	1.3.99.5	finasteride	BPH[e]	2	H	*92*
7	acetylcholinesterase	3.1.1.7	pyridostigmine	MG[f]	11	H	*93*
8	adenosine deaminase	3.5.4.4	pentostatin	HCL[g]	2	H	*94*
9	alanine racemase	5.1.1.1	cycloserine	tuberculosis	1	B	*95*
10	alcohol dehydrogenase	1.1.1.1	fomepizole	alcoholism	1	H	*96*
11	aldehyde dehydrogenase (NAD)	1.2.1.3	disulfiram	alcoholism	1	H	*97*
12	α-amylase	3.2.1.1	acarbose	diabetes	1	H	*98*
13	α-glucosidase	3.2.1.20	miglitol	diabetes	1	H	*99*
14	amine oxidase (flavin-containing)	1.4.3.4	tranylcypromine	depression	5	H	*100*
15	arabinosyltransferase	2.4.2.34	ethambutol	antibacterial	1	B	*101*
16	arachidonate 5-lipoxygenase	1.13.11.34	zileuton	inflammation	4	H	*102*
17	aromatic L-amino acid decarboxylase	4.1.1.28	carbidopa	Parkinson's	1	H	*103*
18	β-lactamase	3.5.2.6	tazobactam	antibacterial	3	B	*104*
19	carbonate dehydratase[m]	4.2.1.1	acetazolamide	glaucoma	6	H	*105*
20	catechol *O*-methyltransferase	2.1.1.6	entacapone	Parkinson's	2	H	*106*
21	ceramide glucosyltransferase	2.4.1.80	miglustat	Gaucher's	1	H	*107*
22	D-alanine-D-alanine ligase	6.3.2.4	cycloserine	tuberculosis	1	B	*108*
23	dihydrofolate reductase	1.5.1.3	methotrexate	leukemia	7	H	*109*
24	dihydroorotate dehydrogenase	1.3.99.11	leflunomide	inflammation	1	H	*110*
25	dihydropteroate synthase	2.5.1.15	dapsone	antifungal	17	F	*111*
26	DNA topoisomerase	5.99.1.2	topotecan	ovarian cancer	2	H	*112*
27	DNA topoisomerase (ATP-hydrolyzing)	5.99.1.3	ciprofloxacin	antibacterial	18	B	*113*
28	DNA-directed DNA polymerase	2.7.7.7	acyclovir	herpes	11	V	*114*
29	DNA-directed RNA polymerase	2.7.7.6	rifapentine	antibacterial	3	B	*115*
30	dolichyl phosphatase	3.1.3.51	bacitracin	antibacterial	1	B	*116*
31	enoyl-[acyl carrier protein] reductase	1.3.1.9	isoniazid	tuberculosis	1	B	*117*
32	exo-α-sialidase	3.2.1.18	oseltamivir	influenza	2	V	*118*
33	factor Xa	3.4.21.6	fondaparinux	thrombosis	2	H	*119*
34	farnesyl-diphosphate farnesyltransferase	2.5.1.21	alendronate	osteoporosis	6	H	*120*
35	fatty acid synthase	2.3.1.85	pyrazinamide	tuberculosis	1	B	*121*

Enzymes as Drug Targets - a Closer Look

Transition state analogs and "suicide substrates"

36	glucan 1,4-α-glucosidase	3.2.1.3	miglitol	diabetes	1	H	*122*
37	histone acetyltransferase	2.3.1.48	valproic	seizures	1	H	*123*
38	HIV-1 retropepsin	3.4.23.16	nelfinavir	AIDS[h]	8	V	*124*
39	hydrogen/potassium-exchanging ATPase	3.6.3.10	esomeprazole	GERD[i]	5	H	*125*
40	HMG-CoA reductase (NADPH2)	1.1.1.34	atorvastatin	hyperlipodemia	6	H	*126*
41	IMP dehydrogenase	1.1.1.205	mycophenolate	IS[j]	2	H	*127*
42	iodide peroxidase	1.11.1.8	propylthiouracil	hyperthyroid	2	H	*128*
43	isoleucine tRNA ligase	6.1.1.5	mupirocin	antibacterial	1	B	*129*
44	membrane dipeptidase	3.4.13.19	cilastatin	resistance	1	H	*130*
45	ornithine decarboxylase	4.1.1.17	eflornithine	trypanosomes	1	P	*131*
46	orotidine-5′-phosphate decarboxylase	4.1.1.23	allopurinol	gout	1	H	*132*
47	peptidyl-dipeptidase A	3.4.15.1	captopril	hypertension	12	H	*133*
48	phosphoribosylglycinamide formyltransferase	2.1.2.2	pemetrexed	cancer	1	H	*134*
49	plasma kallikrein	3.4.21.34	aprotinin	thrombosis	1	H	*135*
50	plasmin	3.4.21.7	aminocaproic	thrombosis	3	H	*136*
51	prostaglandin-endoperoxide synthase	1.14.99.1	etodolac	inflammation	30	H	*137*
52	proteasome endopeptidase complex	3.4.25.1	bortezomib	myeloma	1	H	*138*
53	protein-tyrosine kinase	2.7.1.112	imatinib	leukemia	3	H	*139*
54	ribonucleoside-diphosphate reductase	1.17.4.1	gemcitabine	cancer	4	H	*140*
55	RNA-directed RNA polymerase	2.7.7.48	ribavirin	pneumonia	1	V	*141*
56	RNA-directed DNA polymerase	2.7.7.49	abacavir	AIDS	13	V	*142*
57	serine-type D-Ala-D-Ala carboxypeptidase	3.4.16.4	cefonicid	antibacterial	52	B	*143*
58	sodium/potassium-exchanging ATPase	3.6.3.9	digitoxin	CHF[k]	3	H	*144*
59	squalene monooxygenase	1.14.99.7	butenafine	antifungal	3	F	*145*
60	sterol 14-demethylase	1.14.13.70	itraconazole	antifungal	11	F	*146*
61	sucrose α-glucosidase	3.2.1.48	miglitol	diabetes	1	H	*147*
62	thrombin	3.4.21.5	lepirudin	thrombosis	10	H	*148*
63	thymidylate synthase	2.1.1.45	floxuridine	cancer	6	H	*149*
64	thyroxine 5′-deiodinase	1.97.1.10	propylthiouracil	hyperthyroid	1	H	*150*
65	triacylglycerol lipase	3.1.1.3	orlistat	obesity	1	H	*151*
66	tyrosine 3-monooxygenase	1.14.16.2	metyrosine	PC[l]	1	H	*152*
67	UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase	2.5.1.7	fosfomycin	antibacterial	1	B	*153*
68	unspecific monooxygenase	1.14.14.1	aminoglutethimide	breast cancer	5	H	*154*
69	urease	3.5.1.5	acetohydroxamic	gastritis	1	B	*155*
70	vitamin-K-epoxide reductase (warfarin-sens.)	1.1.4.1	dicumarol	thrombosis	5	H	*156*
71	xanthine oxidase	1.17.3.2	allopurinol	gout	1	H	*157*

[a] EC numbers are from IUBMB Enzyme Nomenclature (www.chem.qmw.ac.uk/iubmb/enzyme). [b] Organism is the target organism: H, human; B, bacterial; V, viral; F, fungal; P, protozoal. [c] The reference is a general reference for the target. [d] Erectile dysfunction. [e] Benign prostatic hyperplasia. [f] Myasthenia gravis. [g] Hairy cell leukemia. [h] Acquired immunodeficiency syndrome. [i] Gastroesophageal reflux disease. [j] Immunosuppression. [k] Congestive heart failure. [l] Pheochromocytoma. [m] Several enzymes are better known by their more popular names: 3-oxo-5-α-steroid 4-dehydrogenase as 5-α-reductase, carbonate dehydratase as carbonic anhydrase, DNA topoisomerase as DNA gyrase, DNA-directed DNA polymerase as DNA polymerase, DNA-directed RNA polymerase as RNA polymerase, HIV-1 retropepsin as HIV protease, peptidyl-dipeptidase A as angiotensin-converting enzyme, RNA-directed DNA polymerase as reverse transcriptase, ribonucleoside-diphosphate reductase as ribonucleotide reductase, serine-type D-Ala-D-Ala carboxypeptidase as DD transpeptidase, and unspecific monooxygenase as microsomal P_{450} or aromatase.

Drugs that act as enzyme inhibitors:

A. Reversible inhibitors

- Competitive
- Non-competitive

B. Irreversible inhibitors

Poisonous snake bite, plant alkaloids, Nerve gas, Malathion, Parathion, etc…

Reversible Inhibitors

To design a reversible competitive inhibitor as a drug, design a mimic of the substrate or the transition state

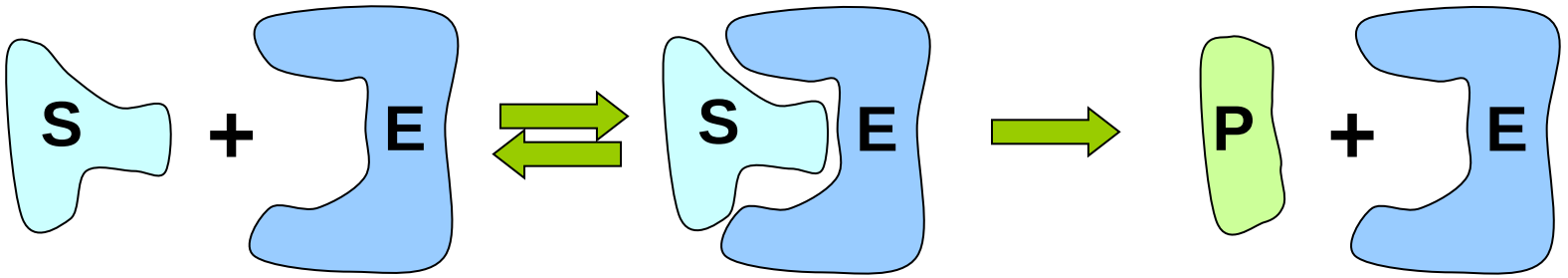
1. Transition state mimic for adenosine deaminase (enzyme which degrades anticancer drugs)

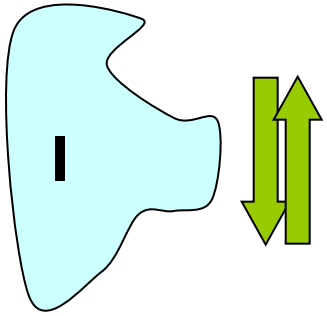
2. Substrate mimic for dihydropteroate synthase (dihydrofolate synthesis)

3. Transition state mimic for HMG-CoA reductase (cholesterol synthesis)

Substrate versus Transition-state analogs: Which approach should result in the highest affinity drug? Why?

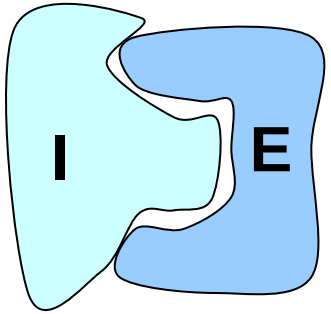
a) Competitive Inhibition





$$E + S \underset{K_M}{\rightleftharpoons} E{:}S \xrightarrow{k_{CAT}} P + E$$

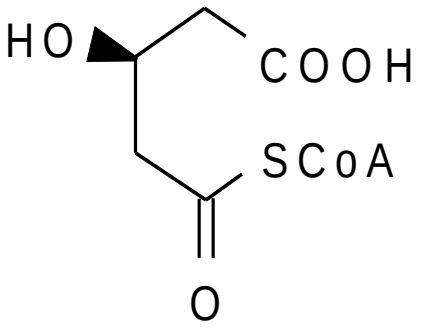
$$E + I \rightleftharpoons E{:}I \quad (K_I)$$

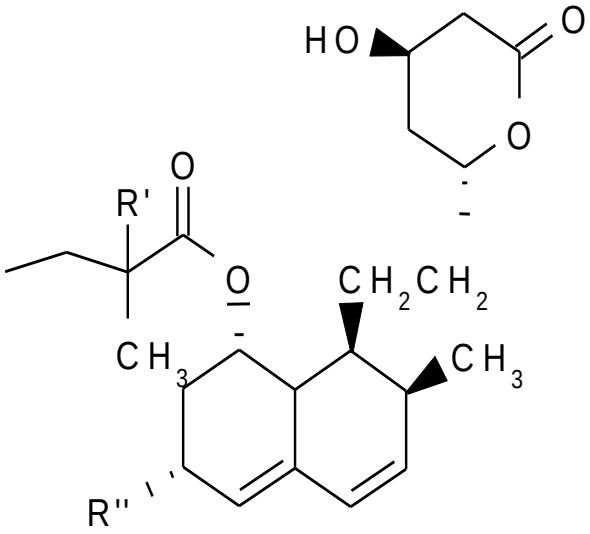


$$Vi = \frac{k_{CAT}\,[E]_O\,[S]}{K_M\,(1 + \frac{[I]_O}{K_I}) + [S]}$$

❑ Competitive Inhibition …. Example

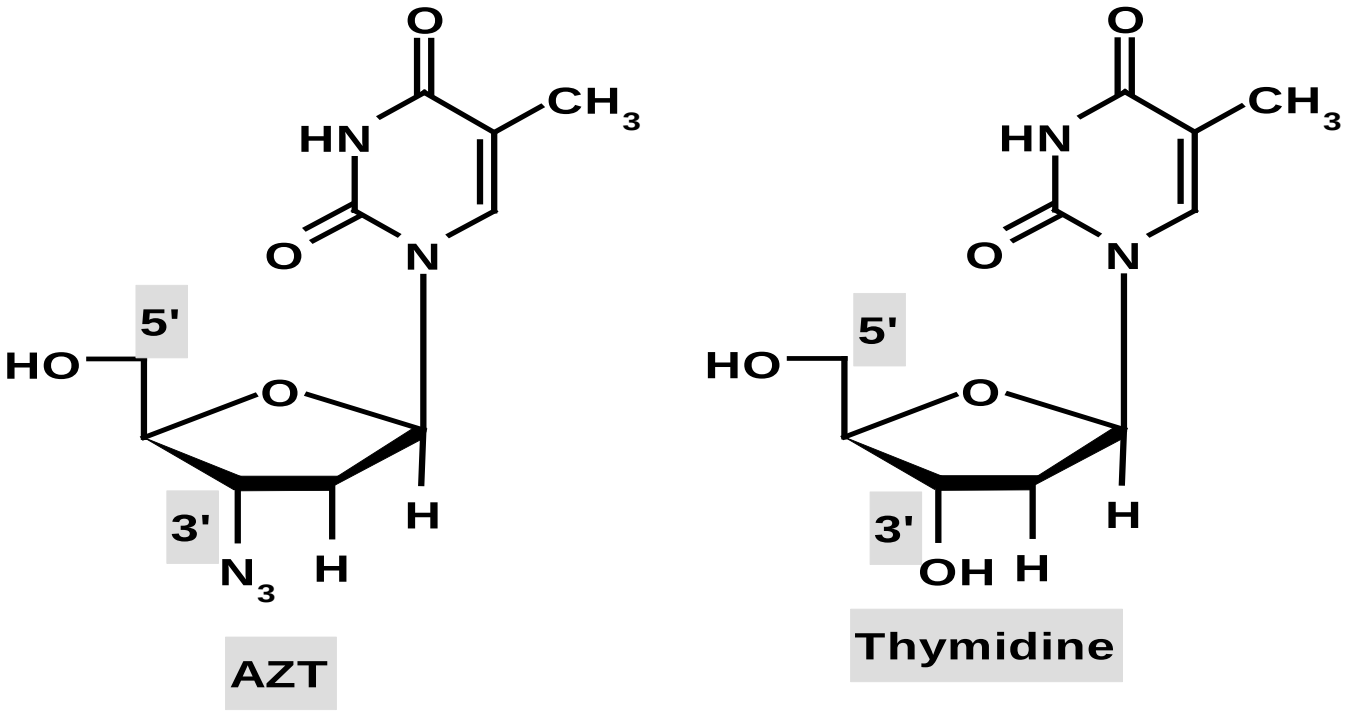
Design of Statins





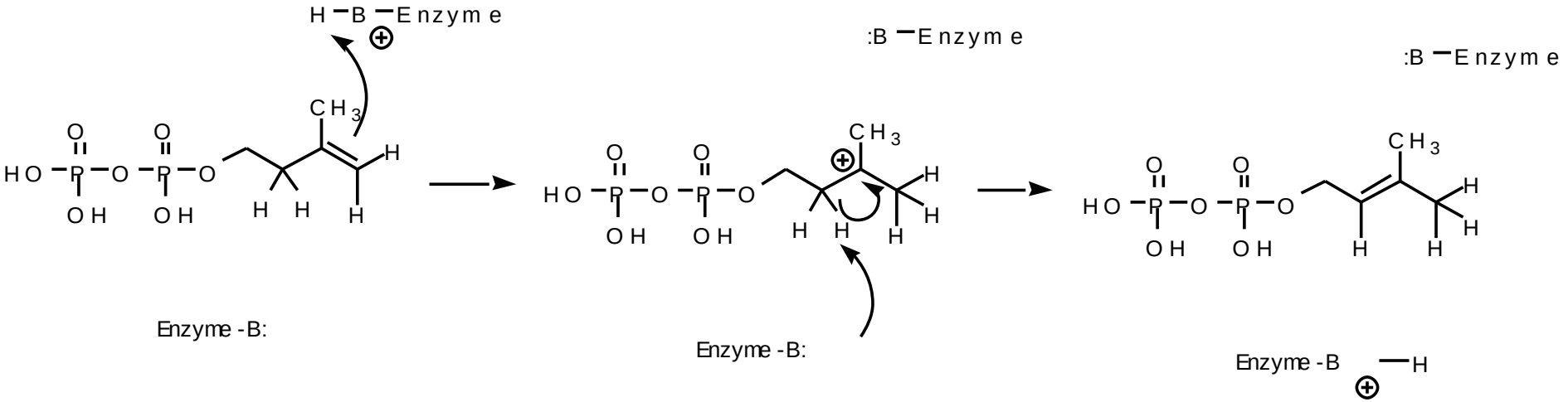
Substrate **Inhibitor**

❑ Competitive Inhibition …. Example

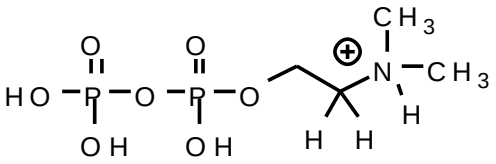


AZT → potent inhibitor of HIV-RT, the retroviral polymerase which catalyzes the formation of proviral DNA from viral RNA

The transition state is stabilized *more than* the substrate



Carbocationic Intermediate
Resembles transition state



Transition-state analog inhibitor

Example 1: Isopentyl Diphosphate isomerase, a key enzyme in isoprenoids synthesis

Example 2: Purine nucleoside phosphorylase. Lower activity causes T-cell immunodeficiency. Potential therapy for T-cell cancer and T-cell autoimmune disorders

Transition state structure was determined with analogs of substrates:

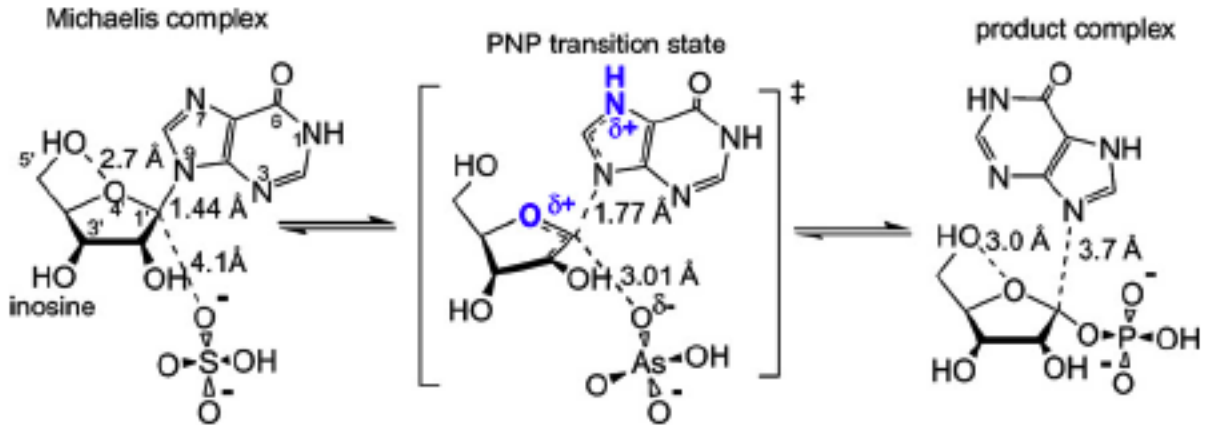
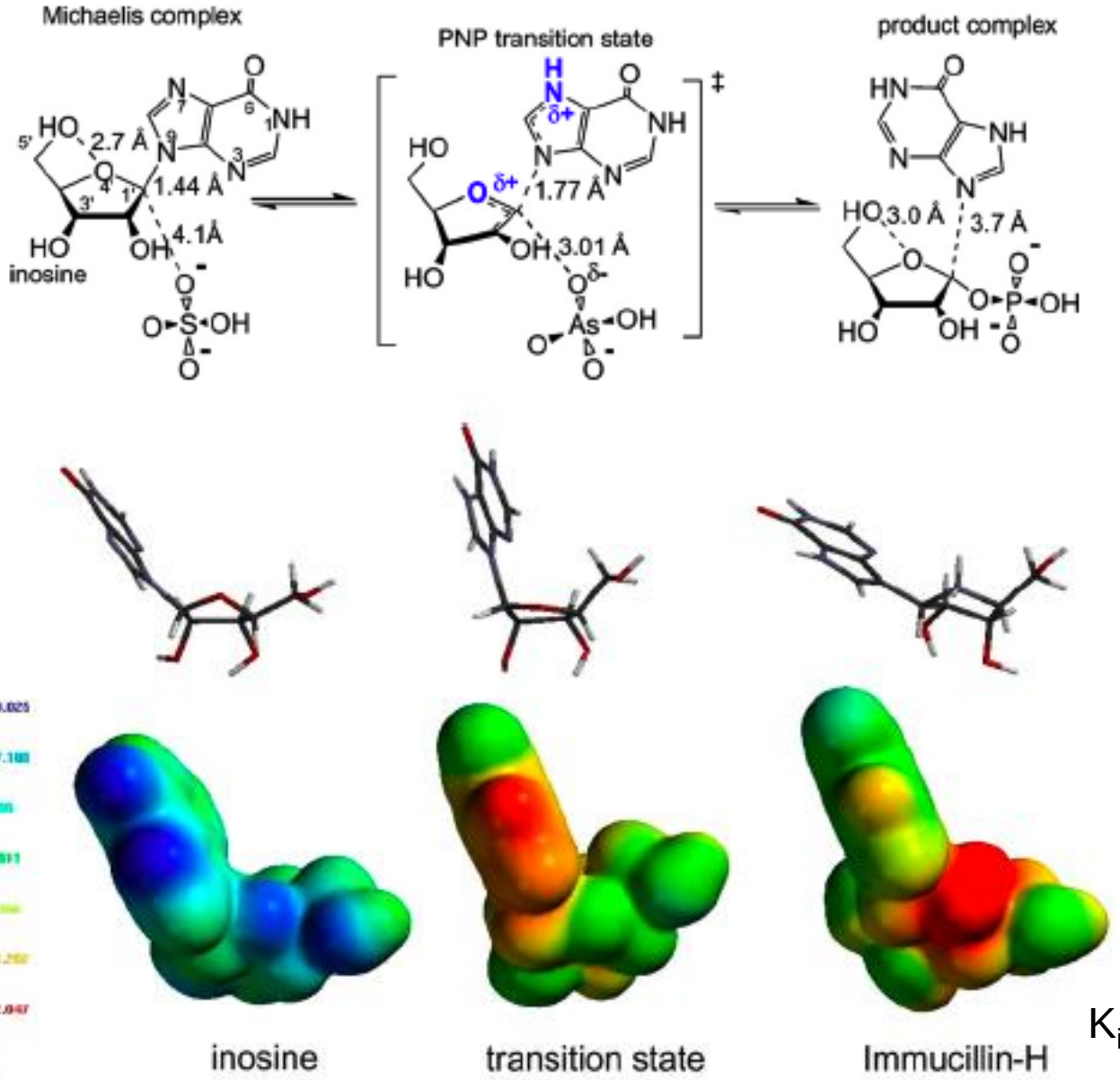


Fig. 2. Crystal structure distances of complexes representing the reaction catalyzed by PNP. The transition state parameters are for arsenolysis of inosine by bovine PNP. Distances in substrate and product complexes are from crystallography studies with the complexes shown in the figure [25,59].

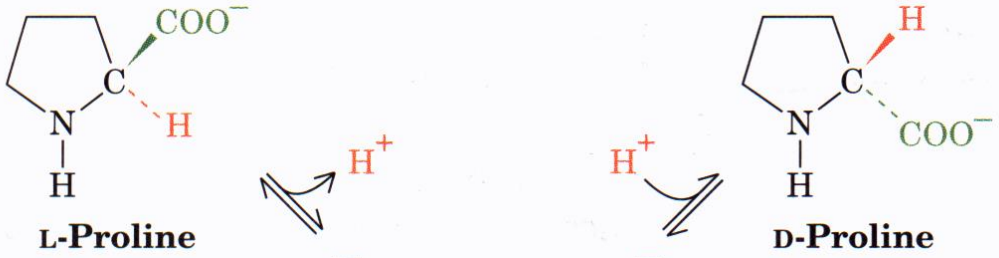
(Ex. 2 continued)….



K_i = 23pM

Fig. 3. Molecular electrostatic potential surfaces of inosine, transition state and Immucillin-H as the cation. The transition state is for bovine PNP. The figure is from [34] with permission of the publisher.

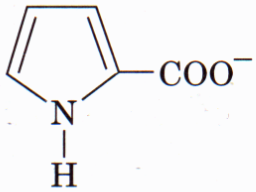
Example 3: Transition state analogues……



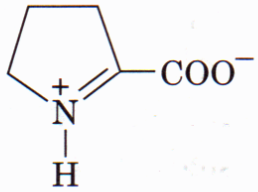
L-Proline

D-Proline

Planar transition state

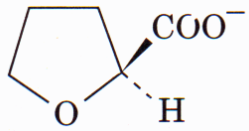


Pyrrole-2-carboxylate



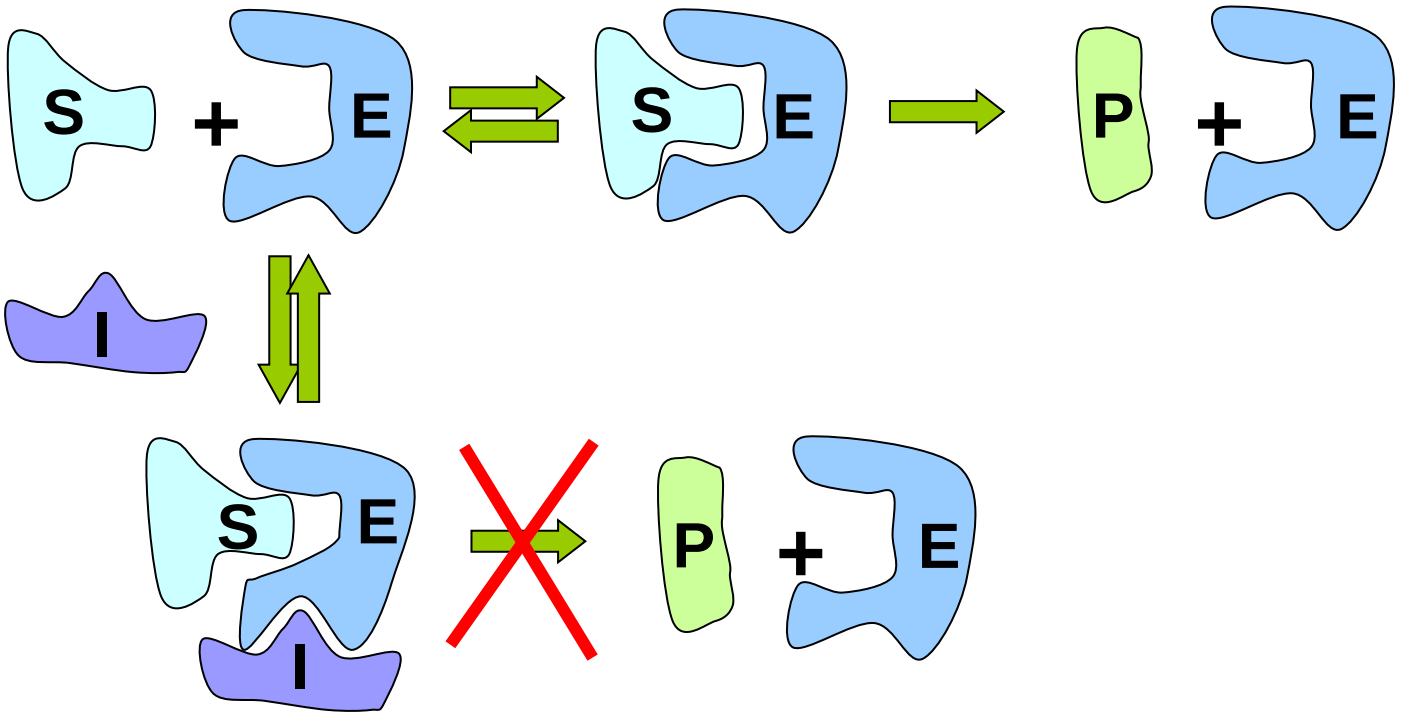
Δ-1-Pyrroline-2-carboxylate

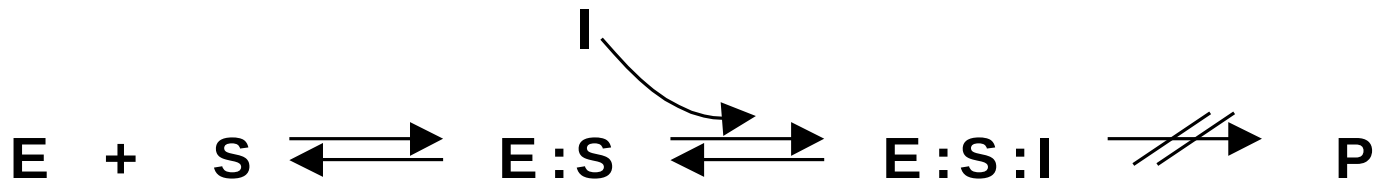
both of which bind to the enzyme with 160-fold greater affinity than does proline. These compounds are therefore thought to be analogs of the transition state in the proline racemase reaction. In contrast, **tetrahydrofuran-2-carboxylate,**



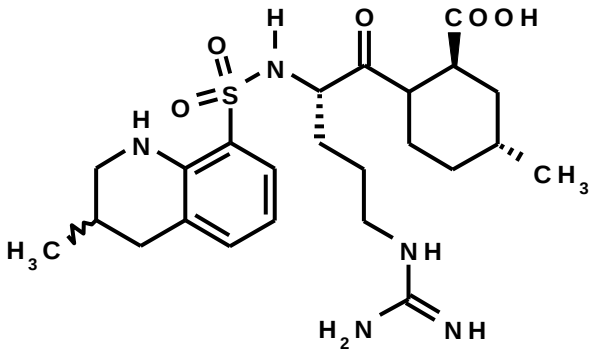
Tetrahydrofuran-2-carboxylate

b) Non-Competitive Inhibition





❑ Non-Competitive Inhibition … Example



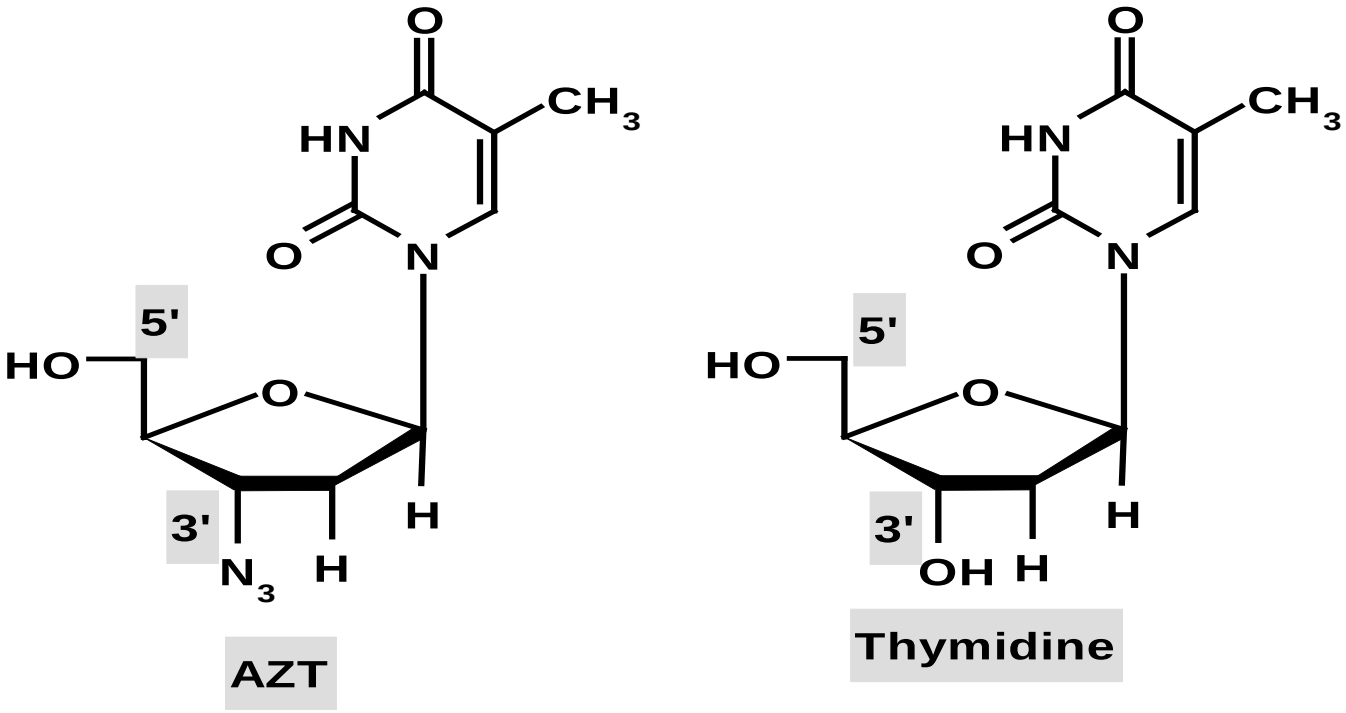
Argatroban

H_2N-D-Phe-Pro-Arg-Pro-Gly-Gly-Gly-Gly-Asn-Gly

HOOC-Leu-Tyr-Glu-Glu-Pro-Ile-Glu-Glu-Phe-Asp

Bivalirudin

❑ Competitive Inhibition …. Example



AZT → potent inhibitor of HIV-RT, the retroviral polymerase which catalyzes the formation of proviral DNA from viral RNA

Recap:

- Reversible enzyme inhibitors bind *reversibly*!
- Competitive inhibitors' structure should be more similar to that of the transition state for stronger binding
- Non-competitive and uncompetitive inhibitors can't be "designed", because they don't resemble the substrate or transition state

Irreversible Inhibition

Can be grouped as:

(a) coenzyme inhibitors

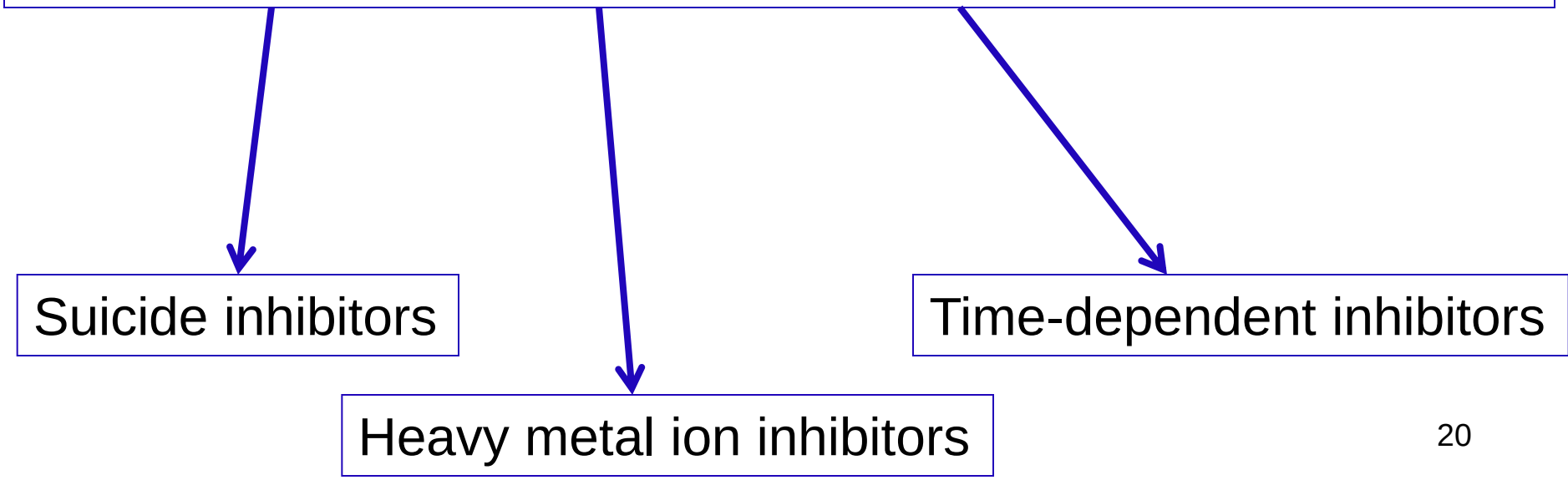
(b) inhibitors of specific ion cofactor

(c) prosthetic group inhibitors

(d) apoenzyme inhibitors

(e) Physiological modulators of the reaction, such as the pH and temperature that denature the enzyme catalytic site

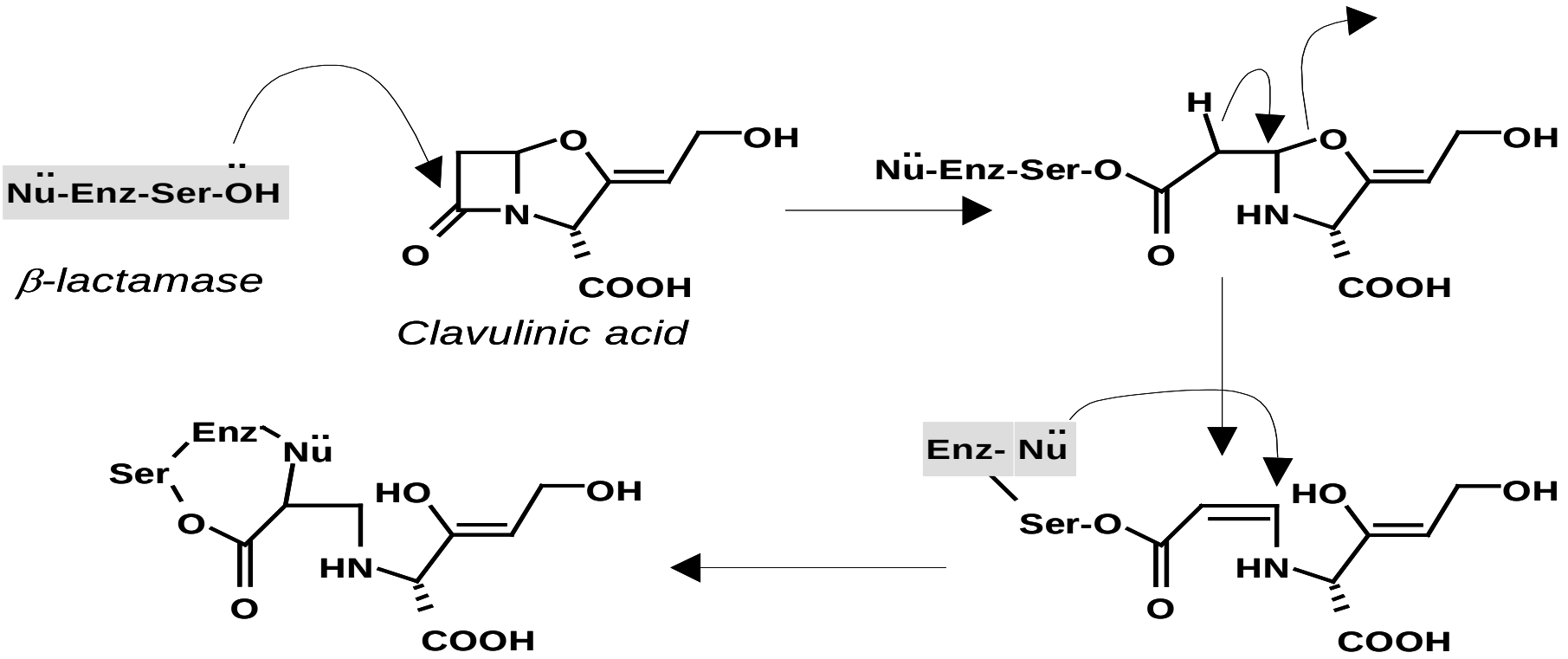
- Most irreversible inhibitors interact with functional groups on the enzyme and destroy enzyme activity
- These interactions are covalent in nature
- These inhibitors are highly useful in studying enzyme reaction mechanisms



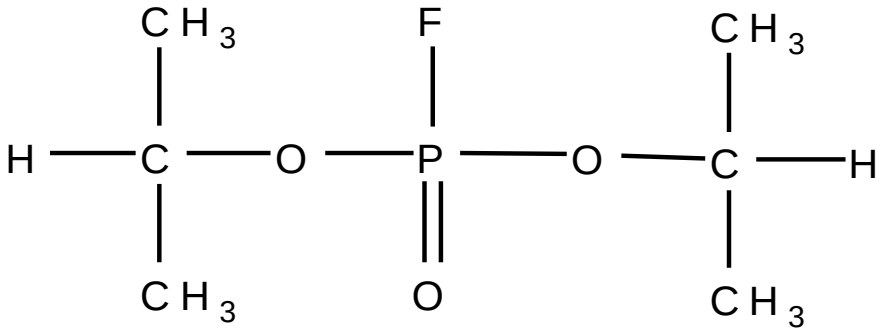
Irreversible Inhibition

E + S ⇌ E:S ⟶ E-S ⟶ No further rxn.

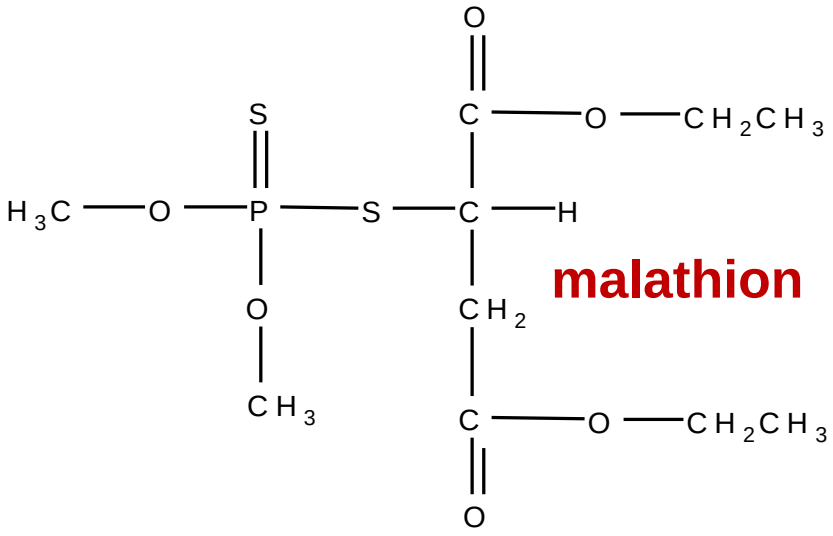
Substrate behaves as an inhibitor!
aka …. Suicide substrate inhibitors or Mouse trap inhibitors or Trojan horse inhibitors



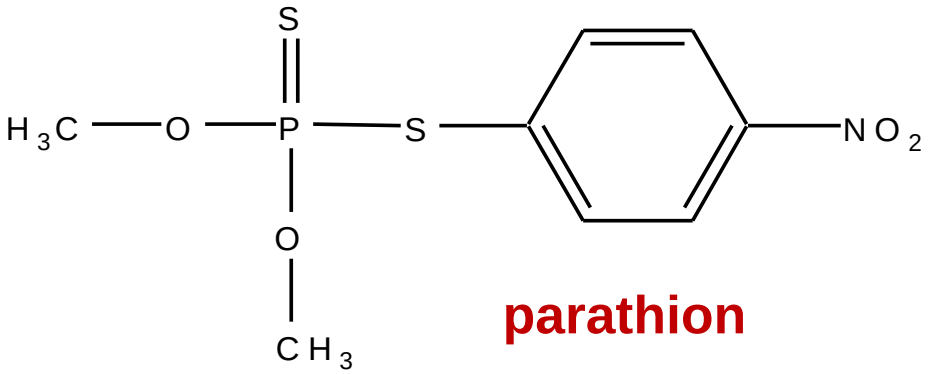
Irreversible Inhibitors



Diisopropyl fluorophosphate (nerve gas)

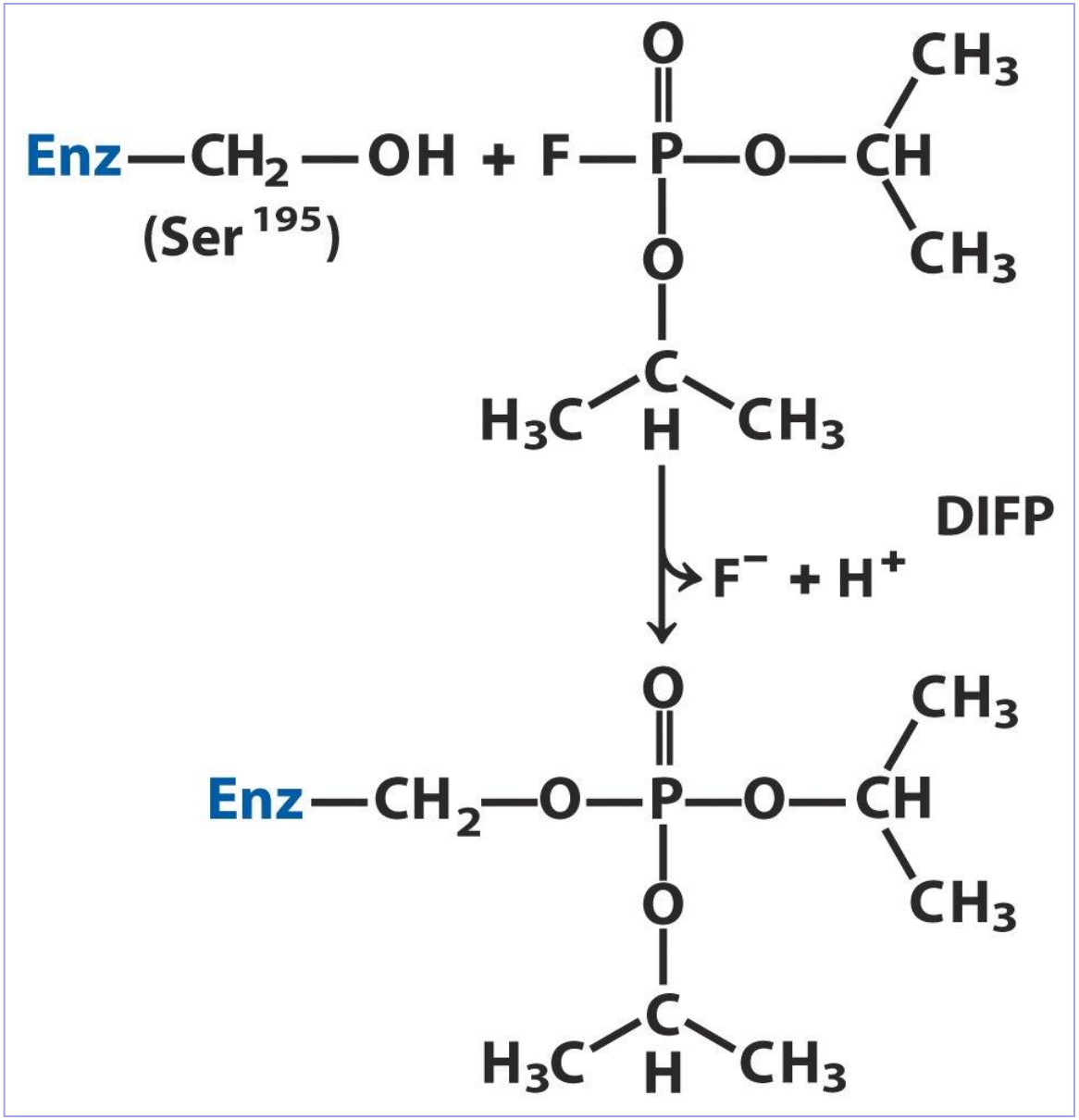


malathion



parathion

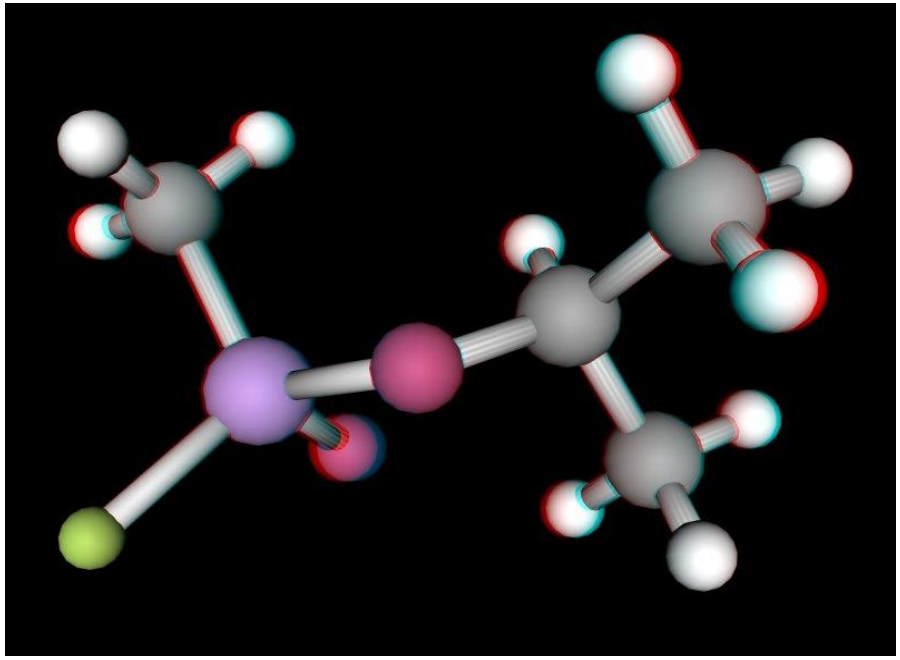
- Organophosphates
- Inhibit serine hydrolases
- Acetylcholinesterase inhibitors



The organophosphofluoride DIFP inactivates the enzyme by forming a permanent **P-O** covalent bond (suicide inhibitor)

More examples...

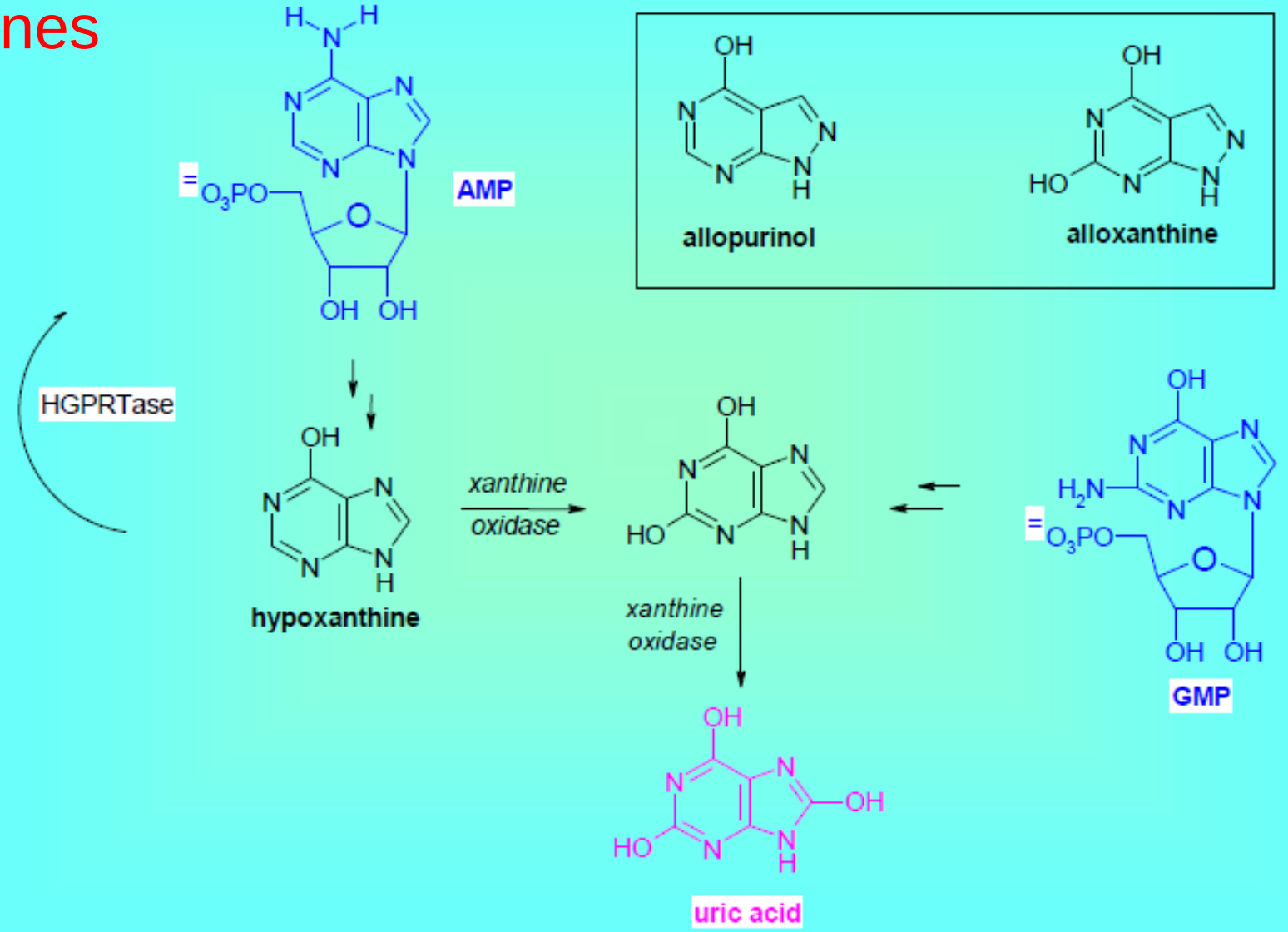
- Poisonous snake bite
- Plant alkaloids – milkweed, more examples?



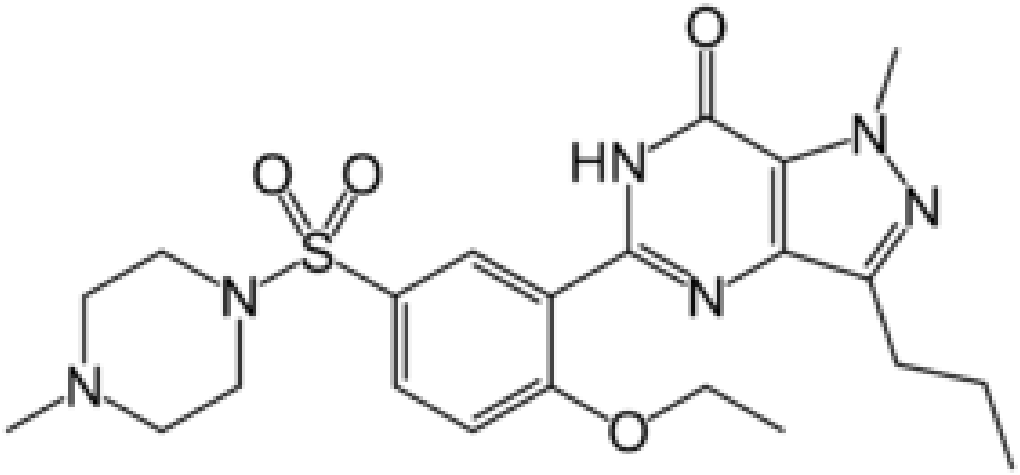
- Nerve gas

Sarin $C_4H_{10}FO_2P$

Allopurinol is an suicide inhibitor of xanthine oxidase; prevents the formation of uric acid from precursorial purines



The molecule below inhibits the enzyme PDE-5



Identify the drug and describe its mechanism of inhibition

Case Study 1

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RESEARCH ARTICLE

An AMPK–caspase-6 axis controls liver damage in nonalcoholic steatohepatitis

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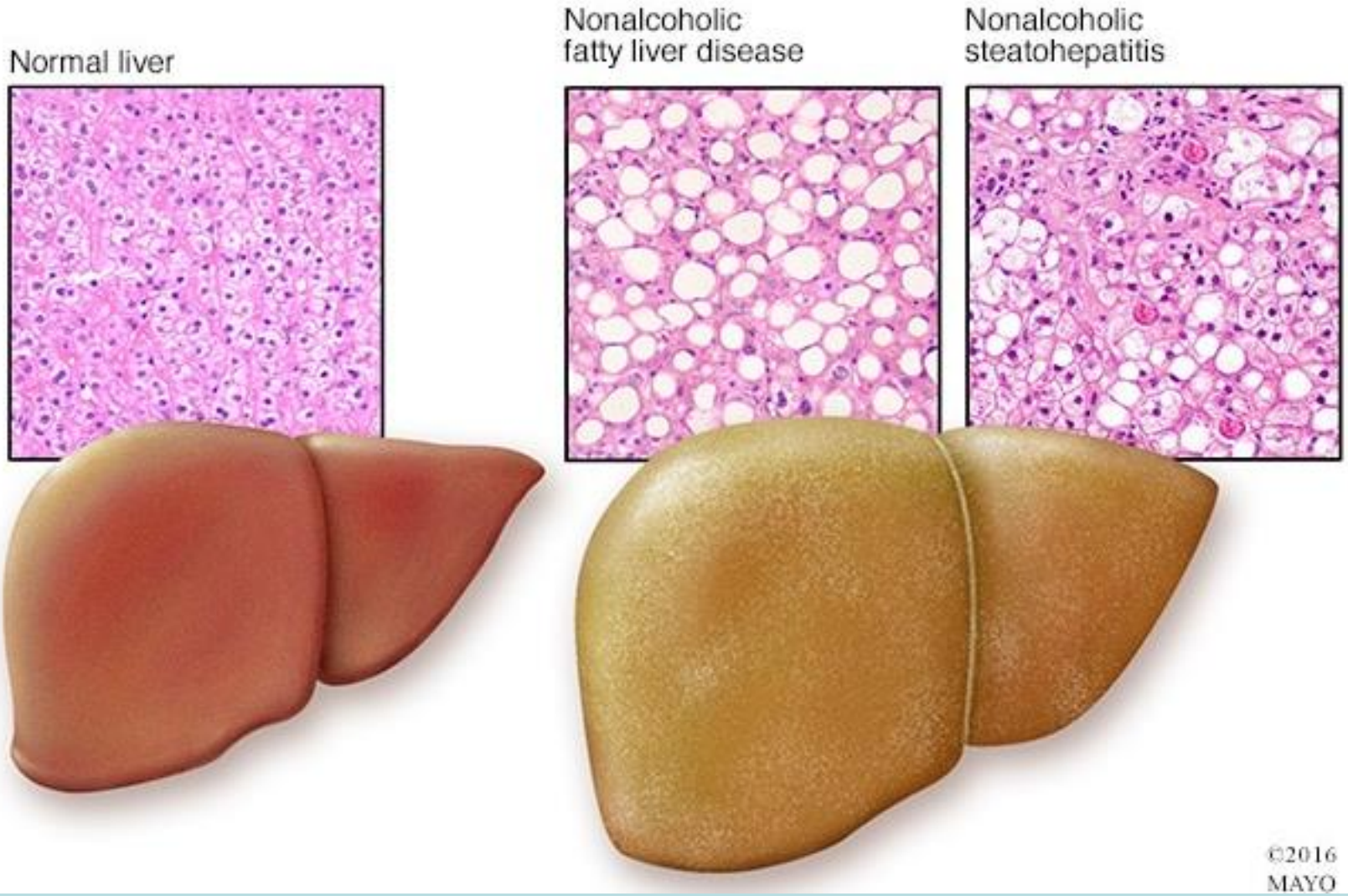
Science 07 Feb 2020:
Vol. 367, Issue 6478, pp. 652-660
DOI: 10.1126/science.aay0542



NASH- is liver inflammation and damage caused by a buildup of fat in the liver. It is part of a group of conditions called nonalcoholic fatty liver disease.

https://www.mayoclinic.org/diseases-conditions/nonalcoholic-fatty-liver-disease/symptoms-causes/syc-20354567

Abstract

Liver cell death has an essential role in nonalcoholic steatohepatitis (NASH). The activity of the energy sensor adenosine monophosphate (AMP)–activated protein kinase (AMPK) is repressed in NASH. Liver-specific AMPK knockout aggravated liver damage in mouse NASH models. AMPK phosphorylated proapoptotic caspase-6 protein to inhibit its activation, keeping hepatocyte apoptosis in check. Suppression of AMPK activity relieved this inhibition, rendering caspase-6 activated in human and mouse NASH. AMPK activation or caspase-6 inhibition, even after the onset of NASH, improved liver damage and fibrosis. Once phosphorylation was decreased, caspase-6 was activated by caspase-3 or -7. Active caspase-6 cleaved Bid to induce cytochrome c release, generating a feedforward loop that leads to hepatocyte death. Thus, the AMPK–caspase-6 axis regulates liver damage in NASH, implicating AMPK and caspase-6 as therapeutic targets.

5. Self-directed study

Next lecture

Clinical applications of enzymes