Enzyme inhibition

Alter enzyme activity by combining with it
Influences binding of substrate and/or its turnover number

Reversible
Irreversible

Mechanisms for enzyme inhibition
A. Competition inhibition: substrate analogs

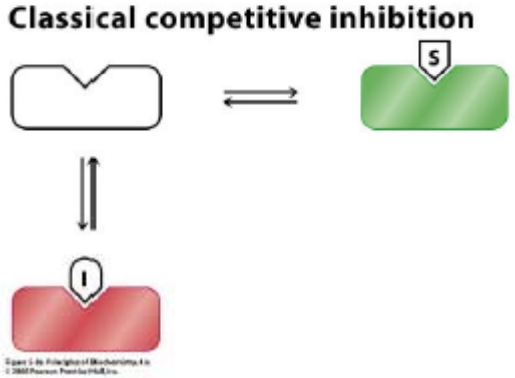
B. Uncompetitive inhibition: binds to the substrate-enzyme complex and presumably distorts the active site making the enzyme less active

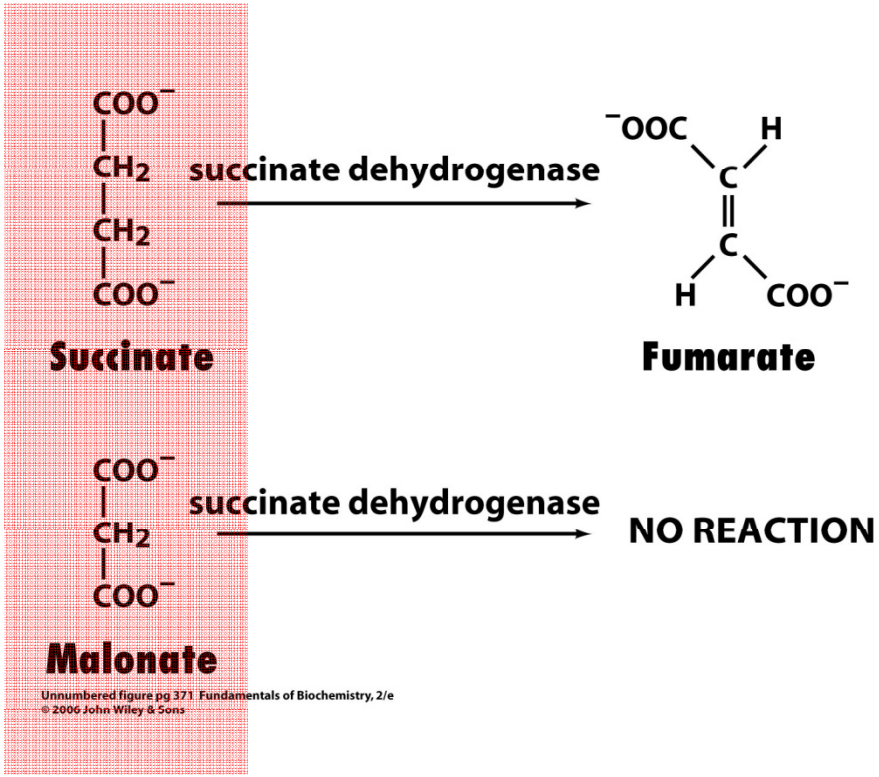
C. Mixed inhibition: combination of competitive and uncompetitive inhibition

D. Inactivator: irreversible reaction with enzyme

A. Competitive inhibition

- Compete with normal substrates
- Substrate analogs
- Responsible for product inhibition
- Transition state analogs



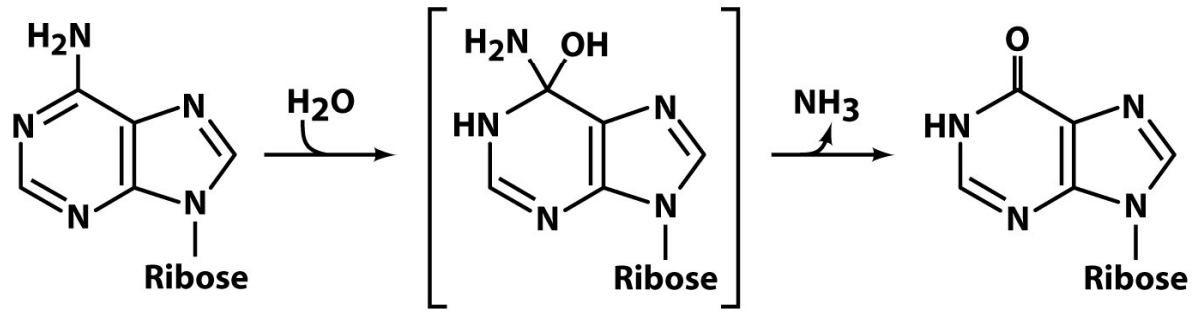


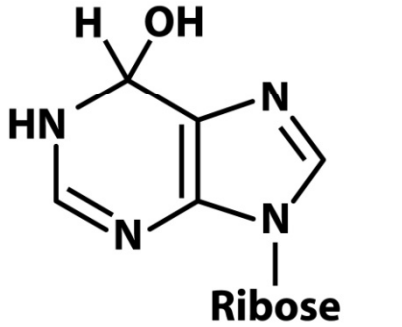
Adenosine deaminase

K_M for adenosine: 3 x 10^{-5} M

K_I of inosine: 3 x 10^{-4} M

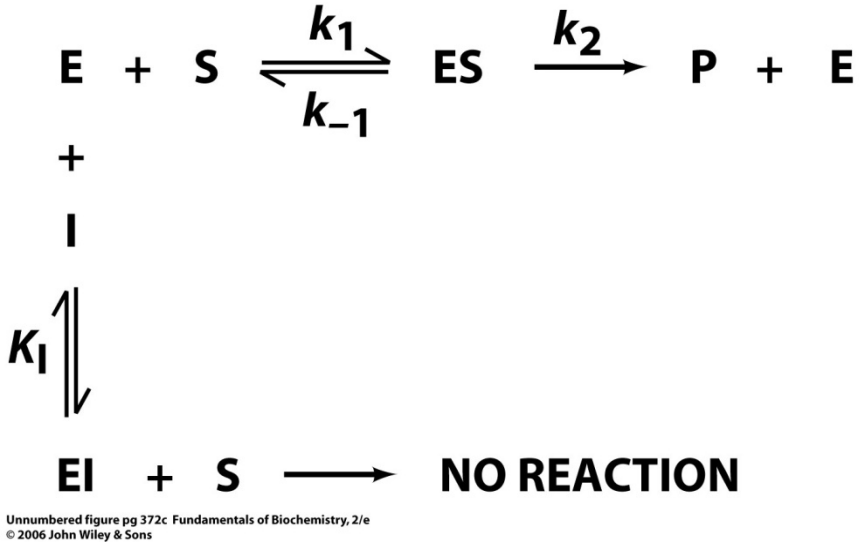
K_I of transition state analogs: 1.5 X 10^{-13} M





Reaction mechanism

K_I = [E][I]/[EI]
[EI]: enzyme-inhibitor complex
Inhibitor reduces the conc. of free enzymes



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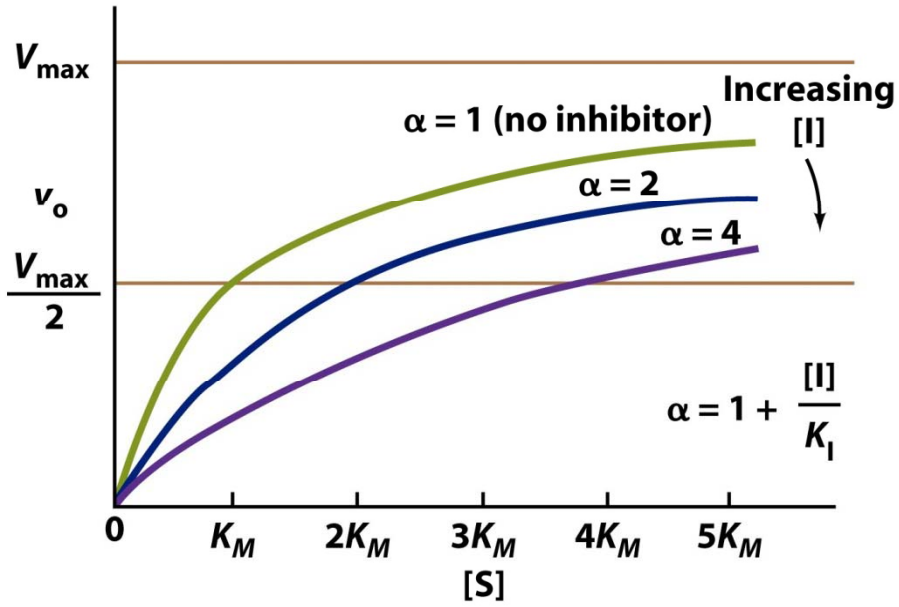
$$V_o = \frac{V_{max}[S]}{\alpha K_M + [S]} \qquad \alpha = 1 + \frac{[I]}{K_I}$$

Competitive: K_M is increased, V_{max} unaffected



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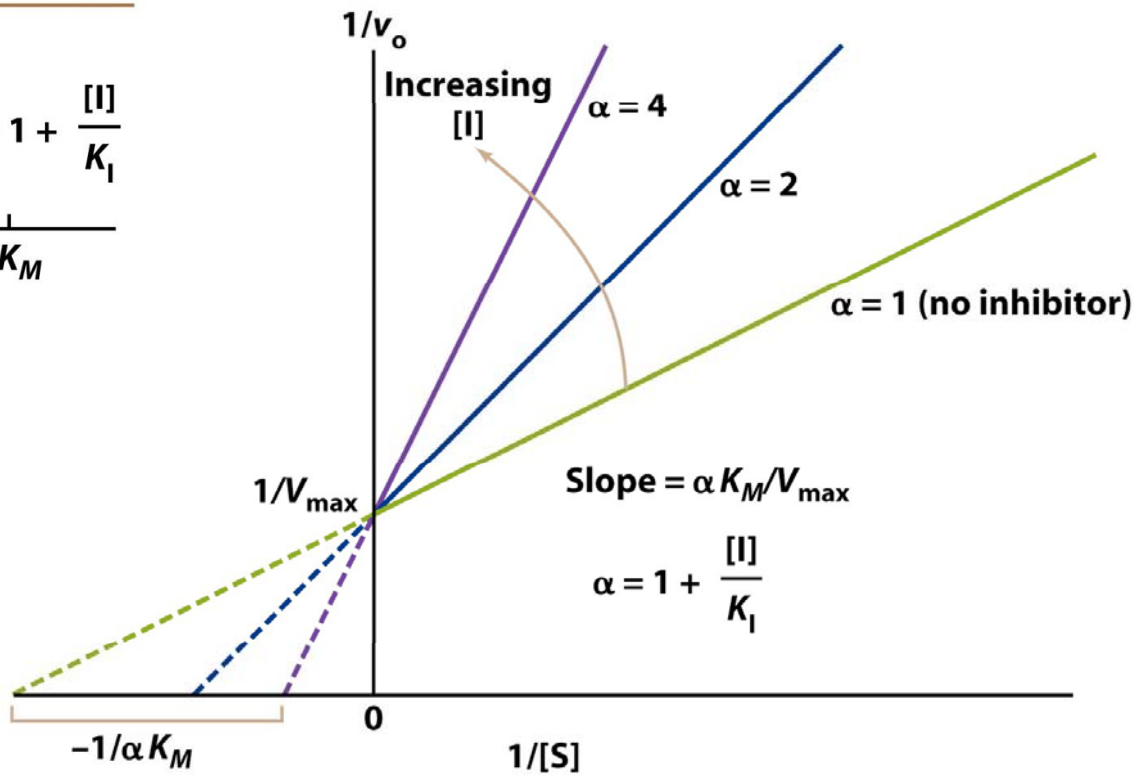

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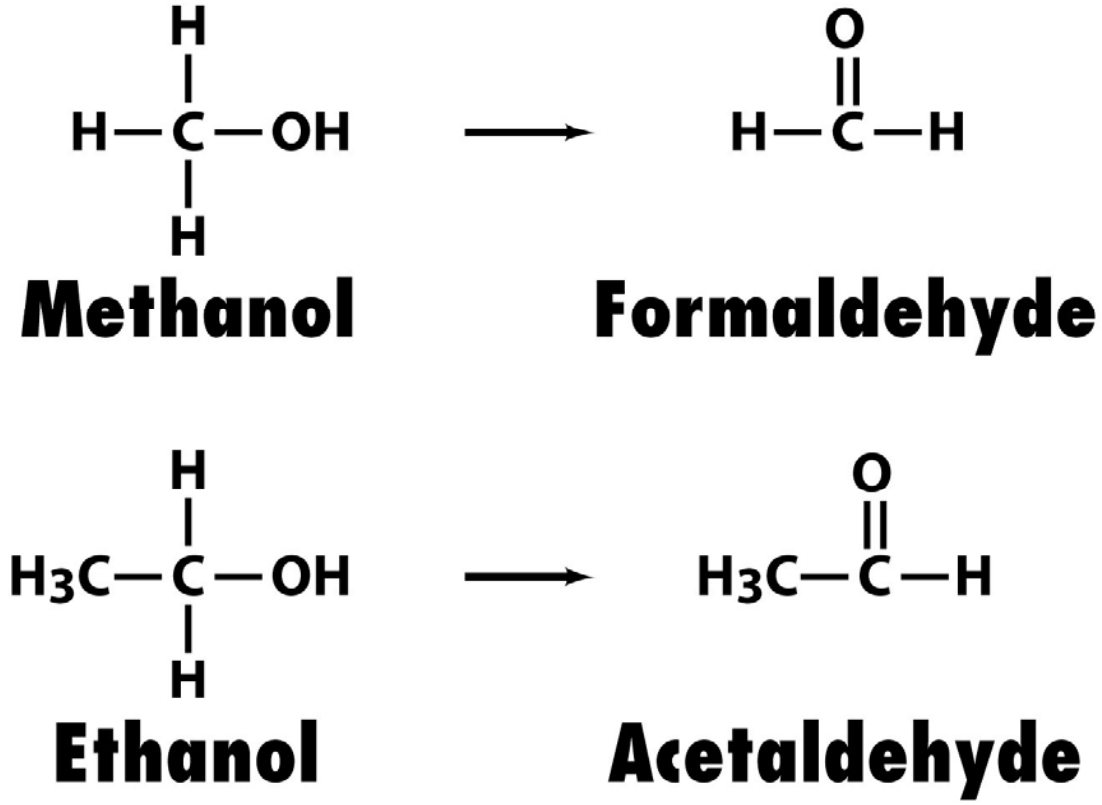
Methanol poisoning



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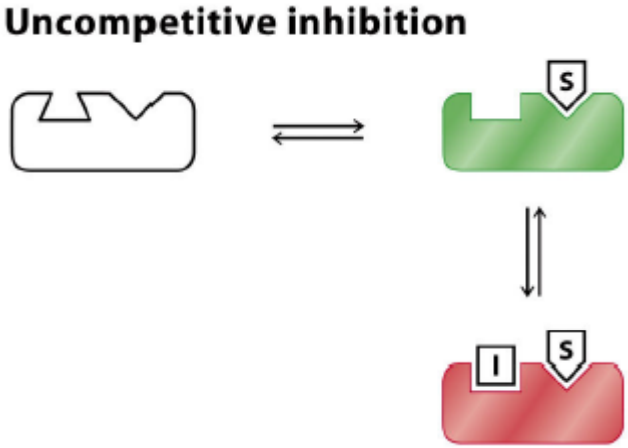
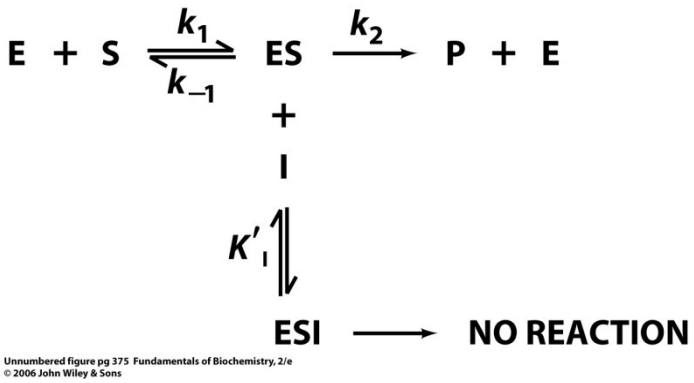
Uncompetitive Inhibition

K_m and V_{max} are both decreased



Binds only to the enzyme-substrate complex to form an enzyme-substrate-inhibitor complex that cannot catalyze the reaction (dead-end pathway). Rare among single substrate reactions, more common with multisubstrate reactions.



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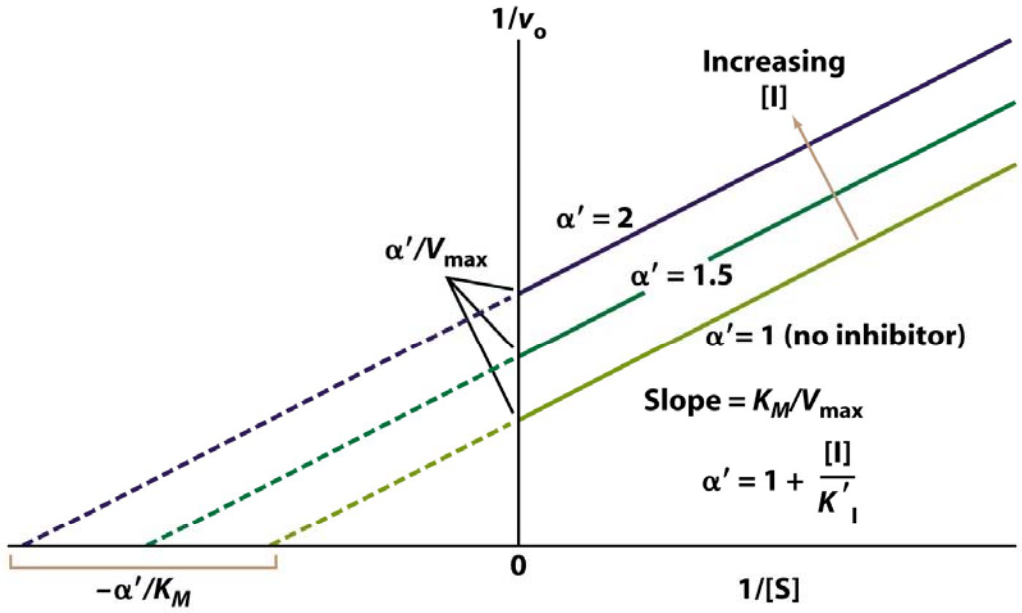

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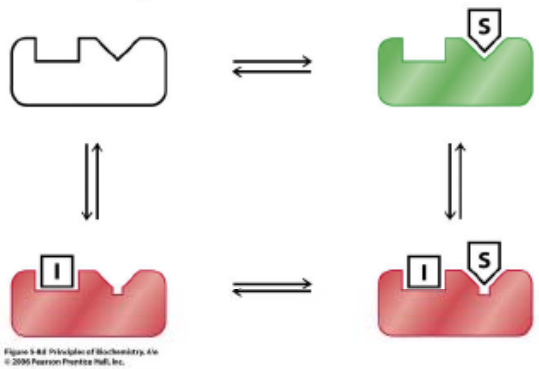
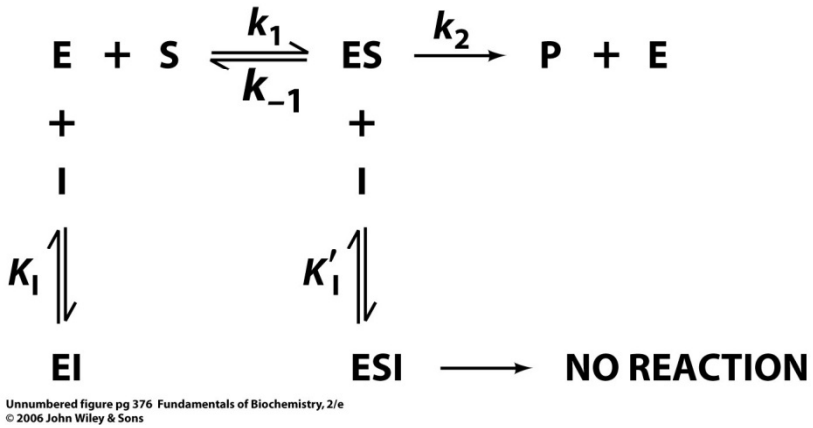
Mixed Inhibition (noncompetitive inhibition)

K_m is increased and V_{max} is decreased



Combines essential features of both competitive and uncompetitive inhibitors.
Mechanism looks similar to uncompetitive inhibitor, but decreases substrate binding by formation of complex with enzyme (EI).

Purely noncompetitive inhibition, if α = α' (Km is unchanged).



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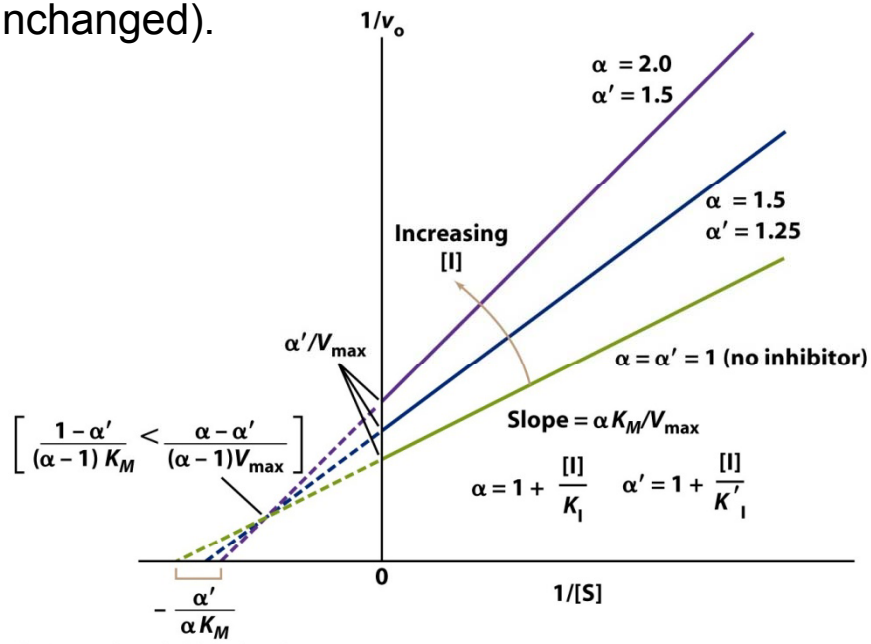

Figure 12-9 Fundamentals of Biochemistry, 2/e
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Table 12-2 Effects of Inhibitors on Michaelis–Menten Reactions[a]

Type of Inhibition	Michaelis–Menten Equation	Lineweaver–Burk Equation	Effect of Inhibitor
None	$v_o = \frac{V_{max}[S]}{K_M + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	None
Competitive	$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	Increases K_M^{app}
Uncompetitive	$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{K_M/\alpha' + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases V_{max}^{app}; may increase or decrease K_M^{app}

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K_I'}$

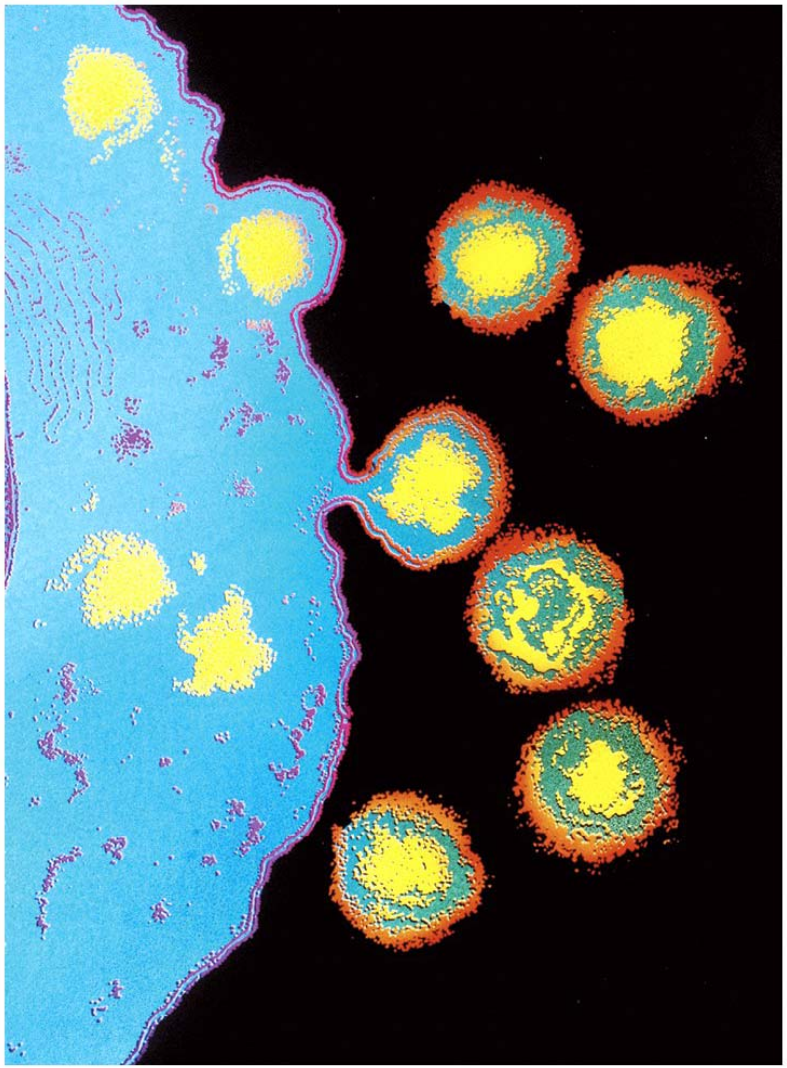
Table 12-2 Fundamentals of Biochemistry, 2/e

Competitive: substrate binding

Uncompetitive: catalytic activity

Mixed: substrate binding + catalytic activity

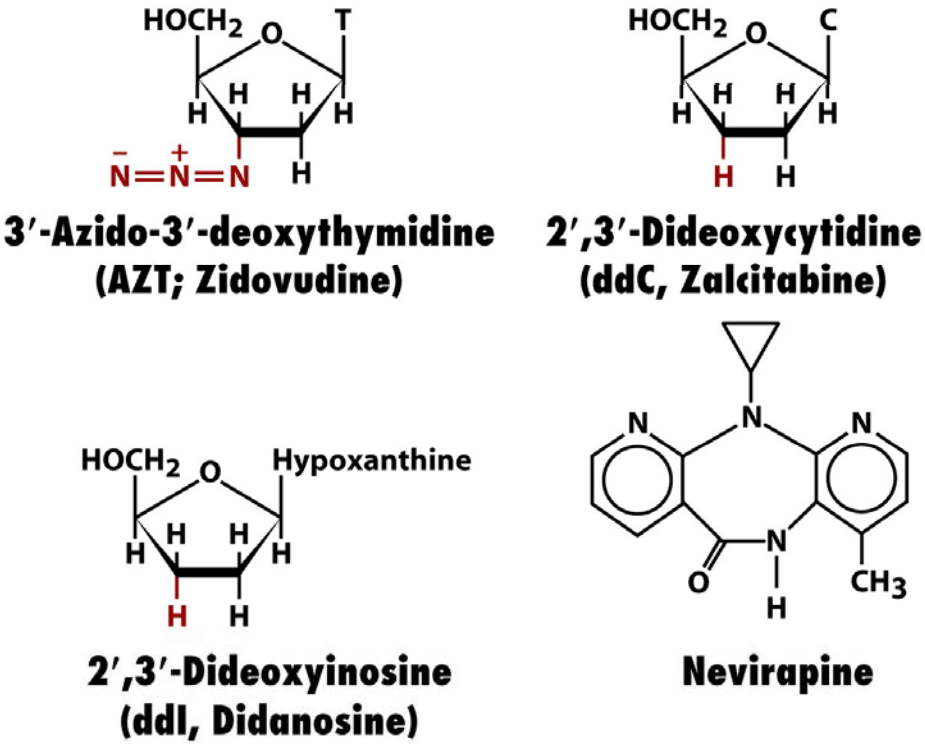
HIV enzyme inhibitors



Box 12-4 figure 1 Fundamentals of Biochemistry, 2/e

HIV particles budding from a lymphocyte

Several inhibitors
targeting reverse transcriptase

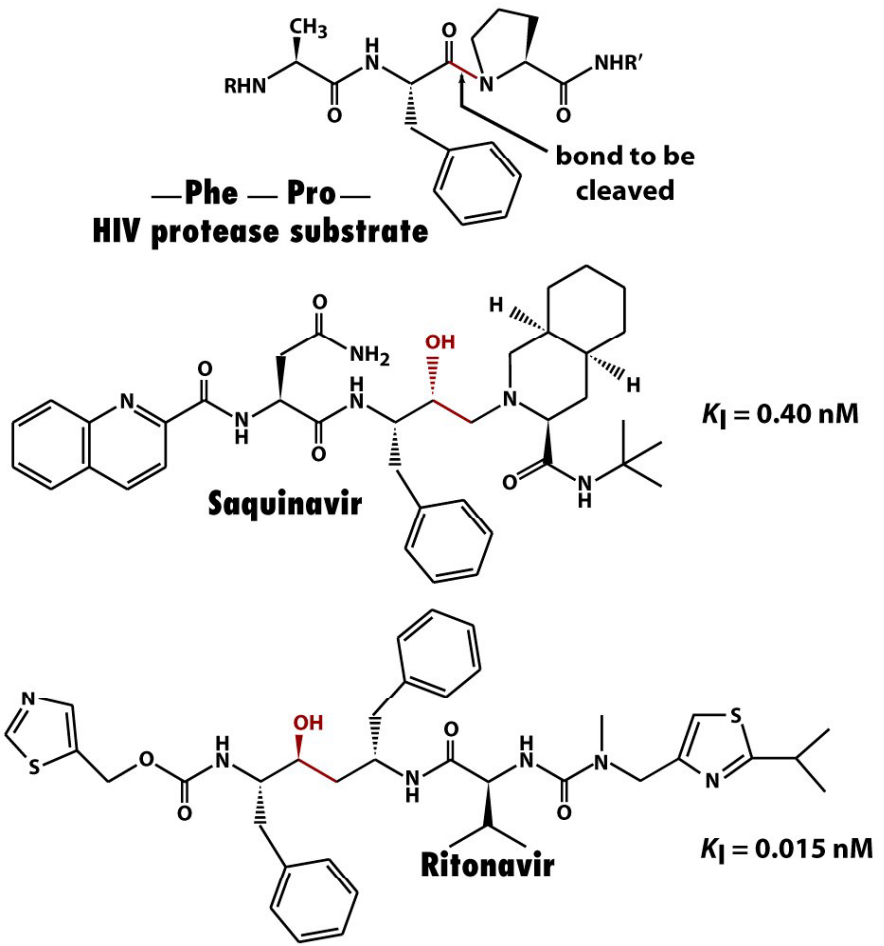


Box 12-4 figure 2 Fundamentals of Biochemistry, 2/e

Several inhibitors targeting protease

Aspartic protease: specific to Phe-Pro and Tyr-Pro

Peptidomimetic (peptide-imitating) drugs: tetrahedral transition state analog



Box 12-4 figure 3 Fundamentals of Biochemistry, 2/e

Control of Enzyme Activity

Mechanisms of control

Long term control: *indirectly* through turnover of the enzyme synthesis and degradation

Short term control: *Direct* control of enzyme activity is achieved by

- *Allosteric* or regulatory enzymes
- *Isozymes*
- *Covalent modification* of enzymes: zymogen, phosphorylation

Allosteric enzymes

Catalytic activities change in response to changes in concentrations of substrates, activators and inhibitors.

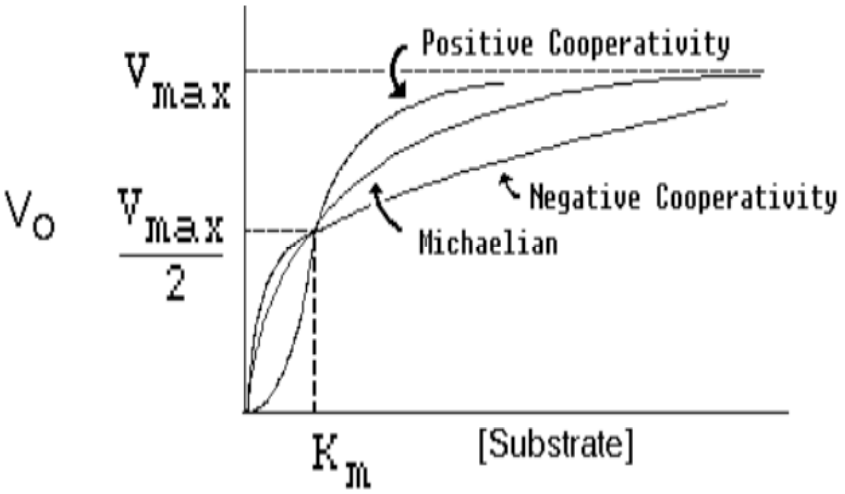
Velocity vs substrate concentration curves: sigmoidal

Positive cooperativity- *sharp increase* in rate with increase in [S].

Negative cooperativity- rate *increases more slowly* with increased [S].

Activators and inhibitors bind at *(allosteric)* sites other than active site.

Enzyme	Substrate bound cooperatively	Activator	Inhibitor
aspartate transcarbamoylase	aspartate	ATP	CTP
phosphofructokinase	fructose 6-phosphate	AMP,ADP,Pi, fructose-2,6-bisphosphate	ATP, citrate
pyruvate kinase	PEP	fructose 1,6-bisphosphate	alanine
fructose bisphosphatase	fructose 1,6-bisphosphate		AMP



Isozymes

- *Isozymes* are enzymes that catalyze the same reaction,
  but have different primary structures (amino acid sequences).
- Role in controlling metabolism to meet the needs of a
  particular tissue or developmental stage of an organism.
- Encoded by different genes.
- Different kinetic and regulatory properties.

Lactate dehydrogenase:

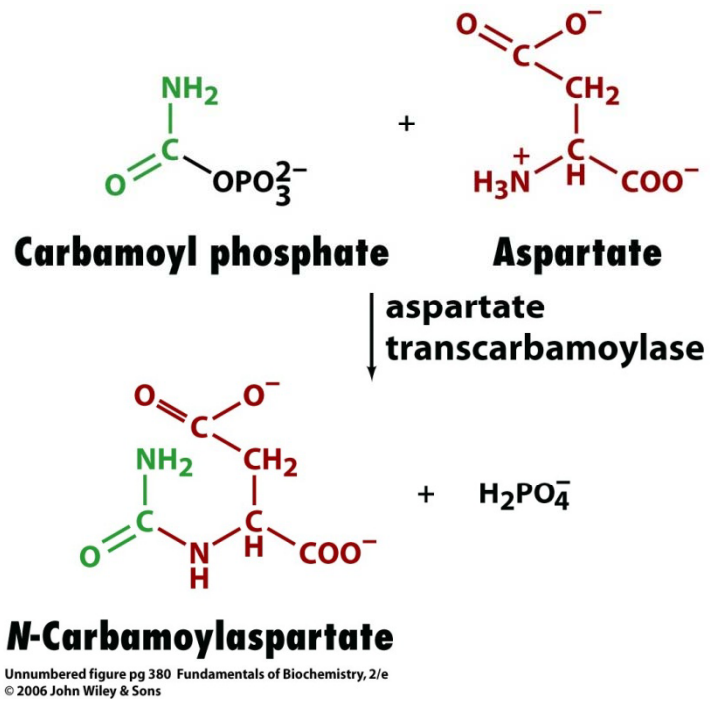
H_4- heart, kidney, red blood cells
aerobic metabolism, oxidation of lactate to pyruvate

M_4- muscle, liver
anaerobic metabolism, reduction of pyruvate to lactate

Others: hexokinase, alkaline phosphatase,
carbamoyl phosphate synthetase

E. coli ATCase (aspartate transcarbamoylase)

- Catalyzes the first step in pyrimidine synthesis
- cooperative binding of both substrates
- Allosteric inhibition by CTP
- Allosteric activation by ATP



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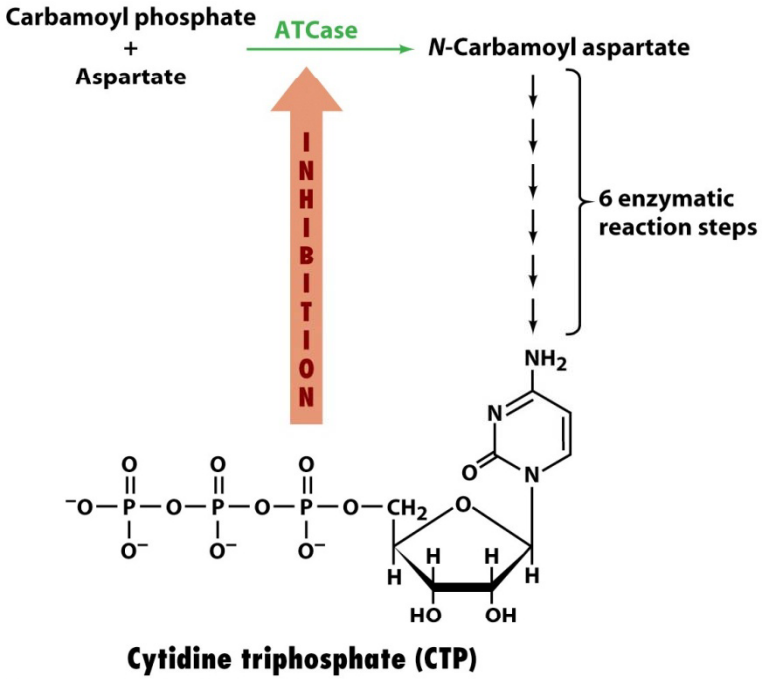

Figure 12-11 Fundamentals of Biochemistry, 2/e

Allosteric regulation by ATP & CTP

Coordinate purine and pyrimidine synthesis

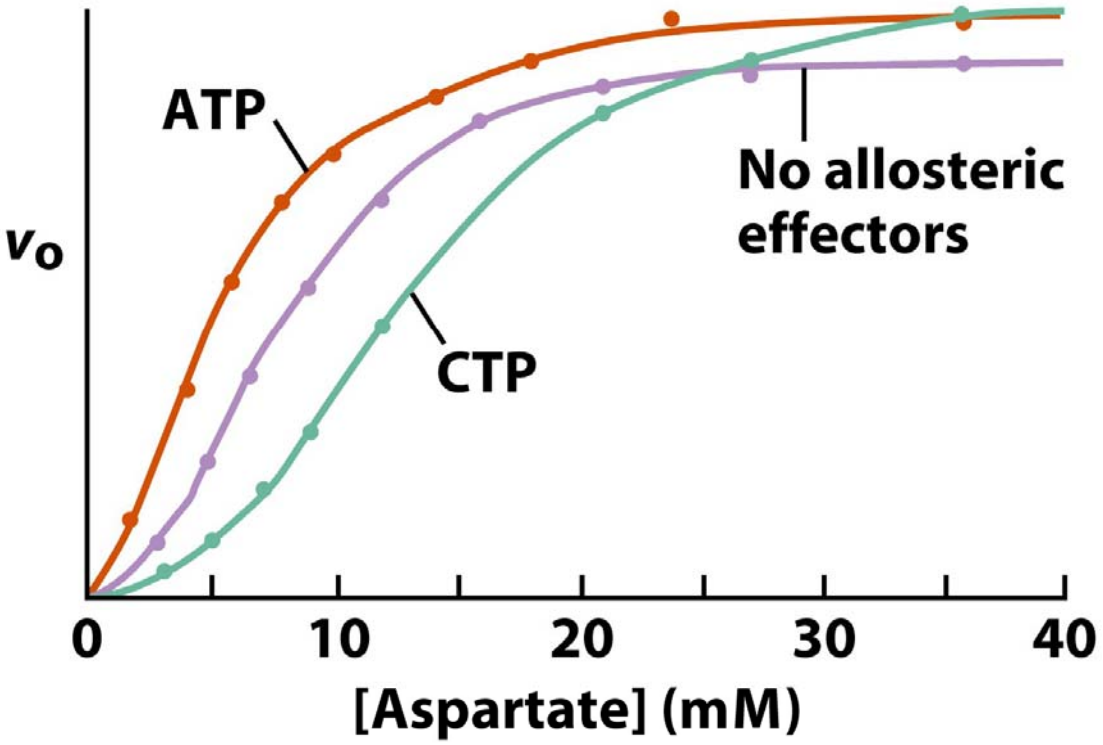

Figure 12-10 Fundamentals of Biochemistry, 2/e
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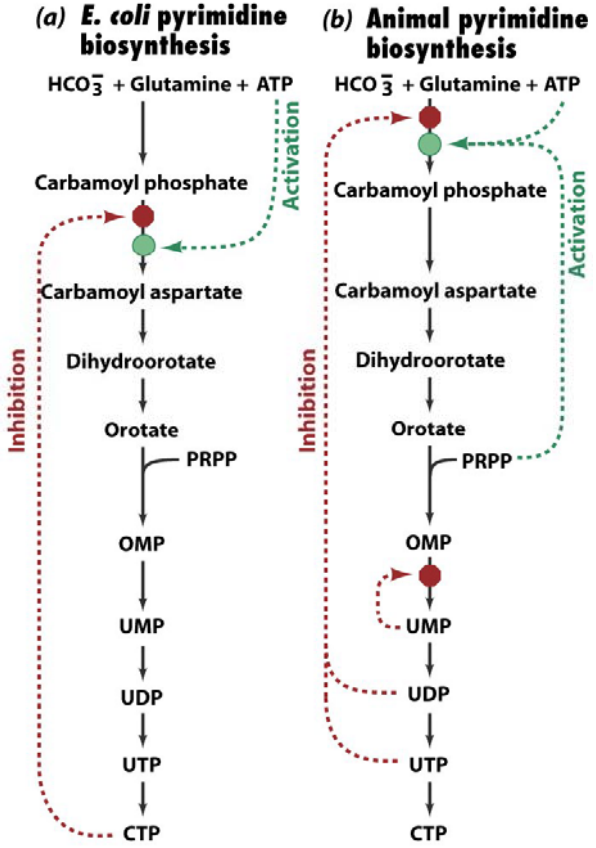

Figure 22-8 Fundamentals of Biochemistry, 2/e
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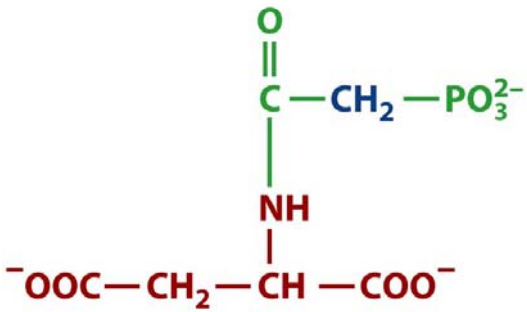
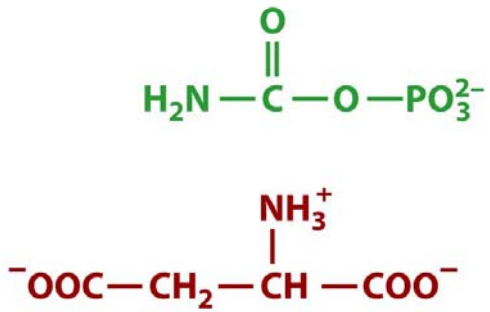
E. coli ATCase

300 kD
Subunit composition: c6r6
Catalytic trimers: active, hyperbolic catalysis, unaffected by ATP & CTP
PALA: bisubstrate analog: binding to R state



***N*-(Phosphonacetyl)-L-aspartate (PALA)**



Carbamoyl phosphate
+
Aspartate

X-ray structure of ATCase

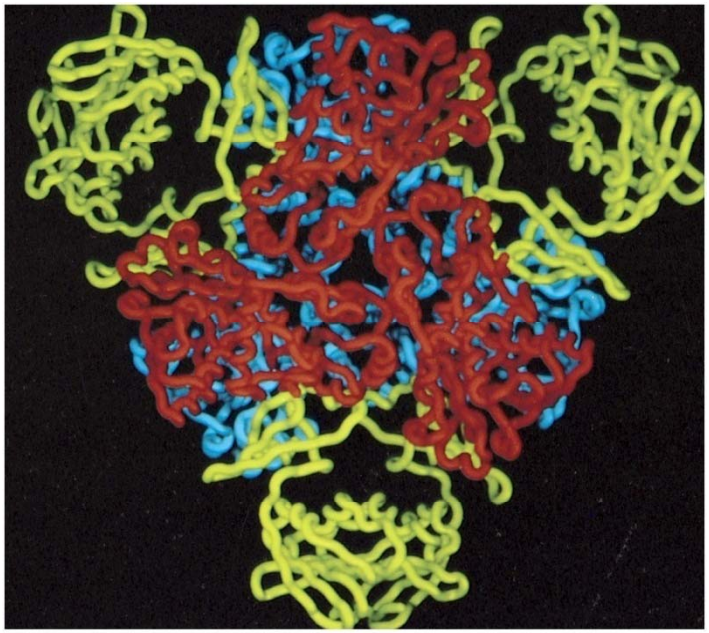


Figure 12-12a Fundamentals of Biochemistry, 2/e

Rotation of
regulatory dimers

T state

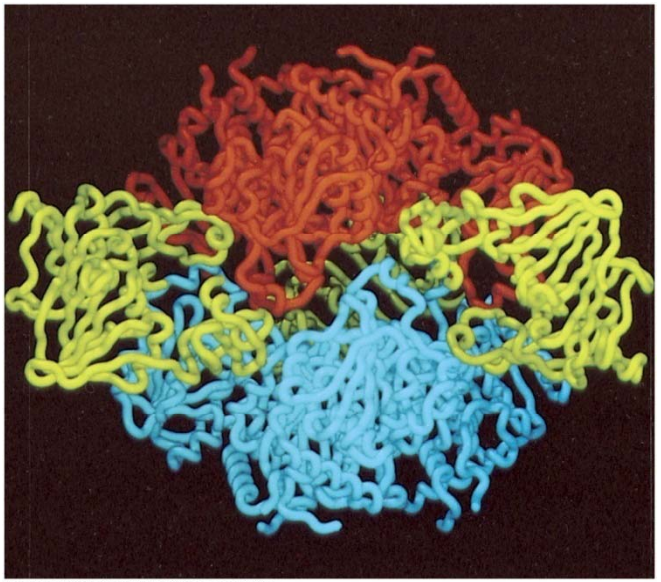


Figure 12-12b Fundamentals of Biochemistry, 2/e

R state

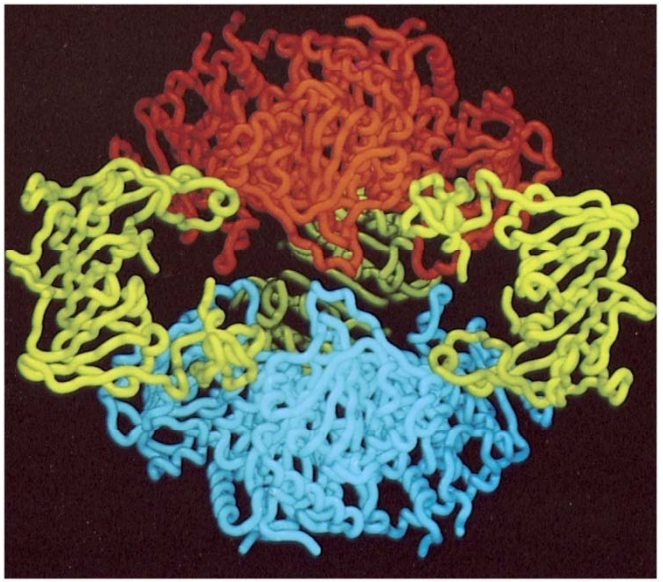
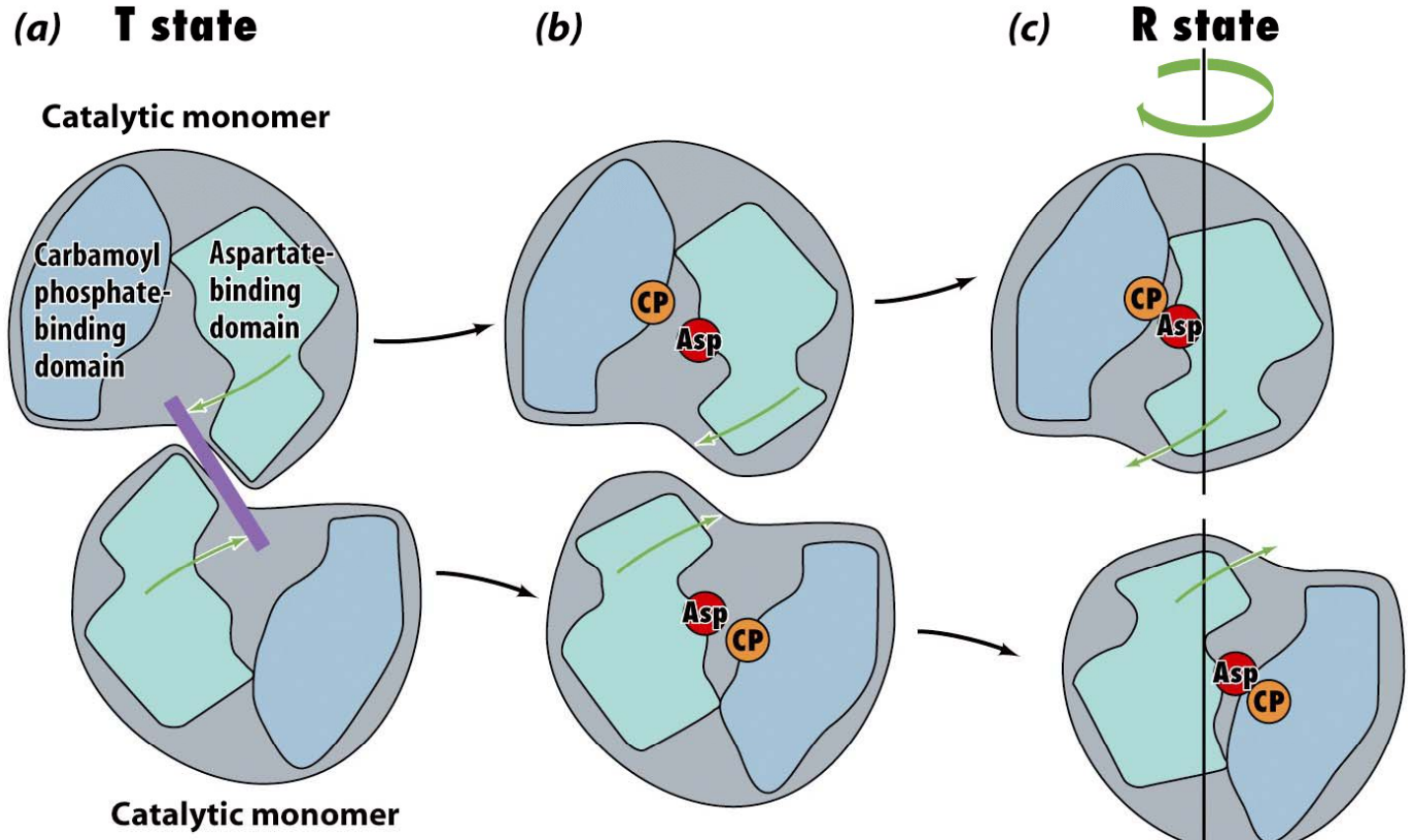


Figure 12-12c Fundamentals of Biochemistry, 2/e

CTP & ATP: binding to the same site of catalytic subunits
CTP: preferentially to T state & increase its stability
ATP: preferentially to R state & increase its stability

Tertiary and quaternary conformational changes in ATCase



CP: carbamoyl phosphate
Asp: aspartic acid

Figure 12-13 Fundamentals of Biochemistry, 2/e

Drug design

Almost all drugs in use today were discovered and developed in the past 3 decades

A. Drug discovery

B. Bioavailability and Toxicity

C. Clinical Trials

D. Cytochrome P450 and Adverse Drug Reactions

Antimalarial drugs

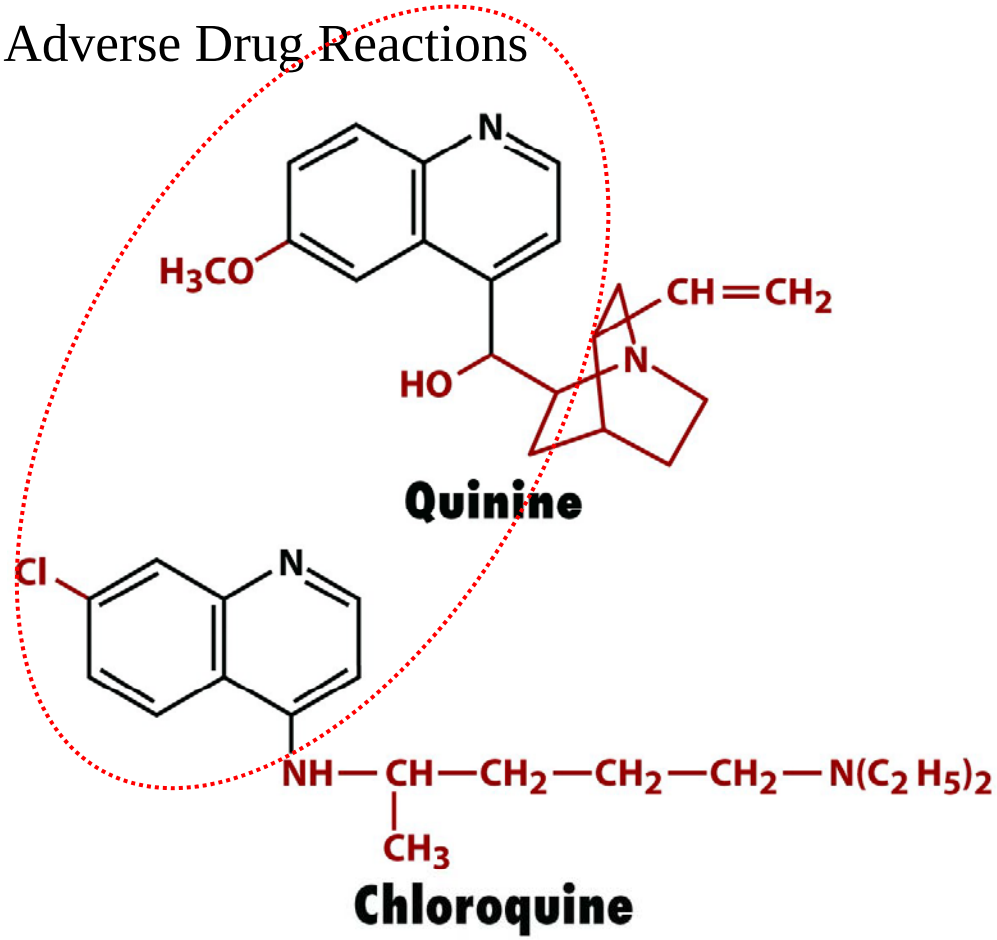
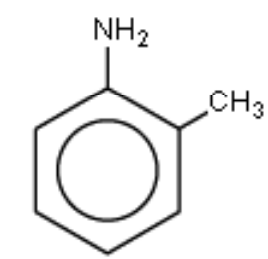
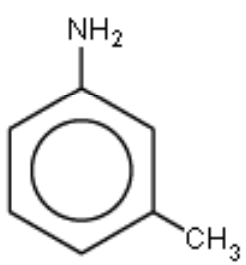


Figure 12-14 Fundamentals of Biochemistry, 2/e



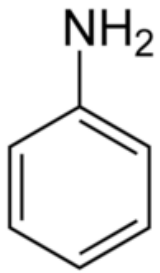
o-toluidine
(*o*-methylaniline)



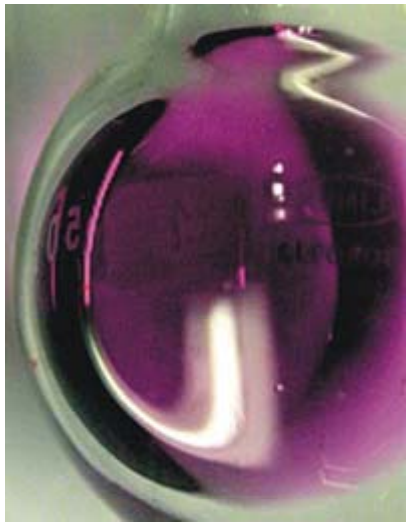
m-toluidine
(*m*-methylaniline)

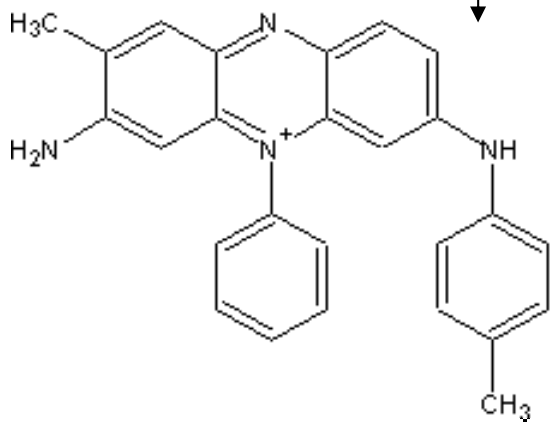
p-toluidine
(*p*-methylaniline)

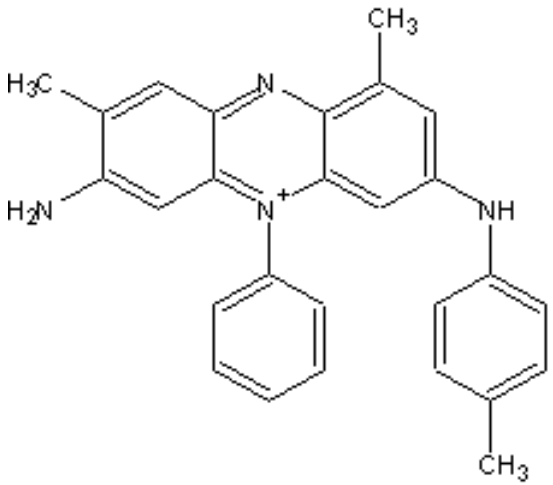
+



aniline







mauveine

A. Drug discovery

Drug screening: synthetic or natural

In vitro screening: ex. enzyme inhibitors: determine K_I values

In vivo test

Lead compound: K_I of less than 1 μM, meaning nonspecific binding to others

Design more efficient compounds: substitution of side chains

Structure-based drug design (rational drug design)

- direct visualization techniques
- molecular modeling tools
- organic synthesis

Combinatorial chemistry and high-throughput screening

rapid and inexpensive synthesis of large numbers of related compounds

make-many-compounds-and-see-what-they-do

automatic substitution of each modules

solid-phase synthesis

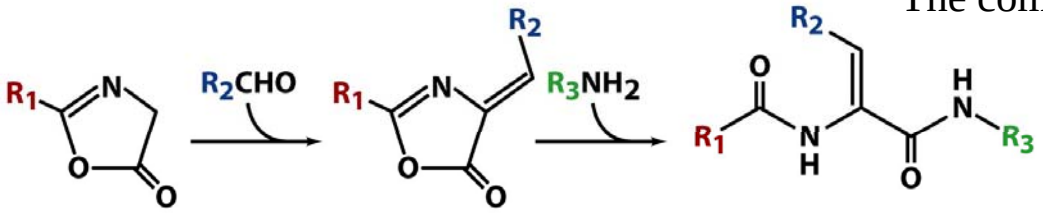


Figure 12-15 Fundamentals of Biochemistry, 2/e
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The combinatorial synthesis of arylidene diamides using different R1 groups

Principle of Combinatorial Chemistry

- large numbers of compounds generated quickly
- eg. to make ABC below

10 variations of A
10 variations of B → **1000 different compounds, each with different binding groups**
10 variations of C

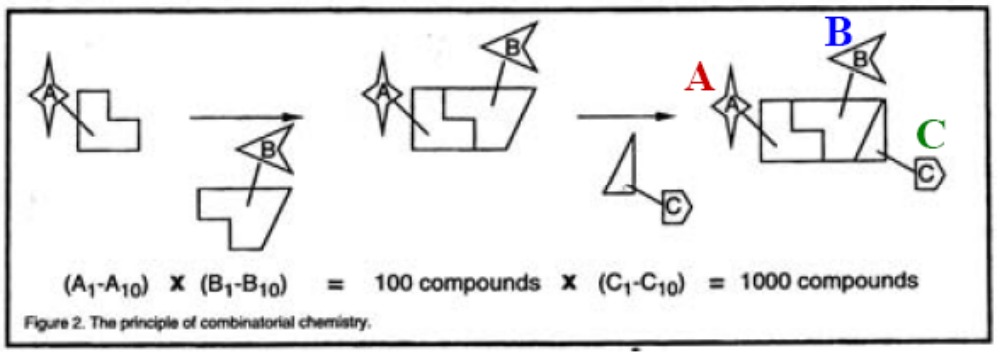
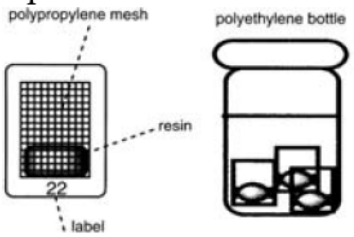


Figure 2. The principle of combinatorial chemistry.

Methods of parallel synthesis

Simplest - single reaction product in each vessel



- Houghton's tea bag procedure

- Automated parallel synthesis

Fig. 12.9 Automated synthesis.

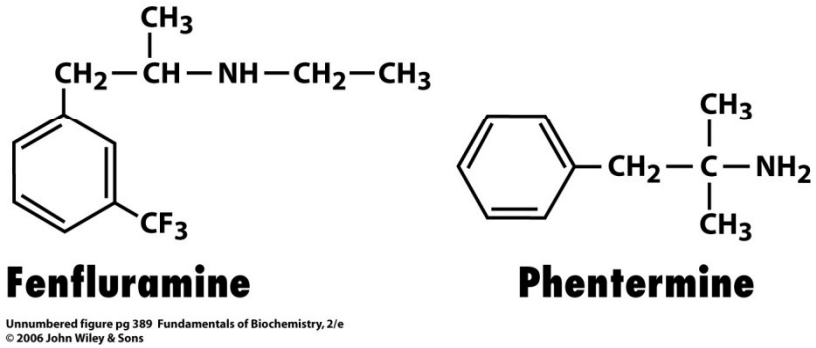
B. Bioavailability and toxicity

Pharmacokinetics: the ways in which a drug interacts with the below barriers

1) Chemical stability
    acidic environment of stomach
    degradation by digestive enzymes
2) Absorption
    from gastrointestinal tract into the bloodstream
3) Avoid nonspecific binding
    little interaction with other substances in the body
4) Survive derivatization by enzymes (especially liver)
    detoxification by liver enzymes (xenobiotics)
5) Avoid rapid excretion by the kidneys
6) Pass from the capillaries to its target tissue
7) Pass through blood-barriers
8) Pass through plasma membranes

Bioavailability: dose & pharmacokinetics:

C. Clinical trials



<u>Safety & efficacy</u>

Animal test

Human test: clinical trials

appetite suppressant/heart valve damage

Phase I: safety test

dosage range & optimal dosage method (oral vs injected)

small number (20-100) of normal healthy volunteers or volunteer patients

Phase II: efficacy test

100-500 volunteer patients

refines dosage range

check side effects

single blind tests (patients), placebo

Phase III:

monitors adverse reactions from long-term use

confirming efficacy in 1000-5000 patients

double blind tests (both patients & doctors)

Phase IV: post-marketing surveillance

D. Cytochrome P450 and adverse drug reactions

Differences in reactions to drugs (problems of adverse drug reactions)

- genetic differences among individuals
- differences in their disease states
- other drugs they are taking, age, sex, and environmental factors

Cytochrome P450: detoxify xenobiotics

Typical reaction: $NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + R\text{-}OH$

RH indicates a variety of lipophilic compounds

- polycyclic aromatic hydrocarbons, polycyclic biphenols, steroids, etc

Converted to water-soluble forms

Aids in excretion by the kidney

Further conjugation with glucuronic acid, glycine, etc

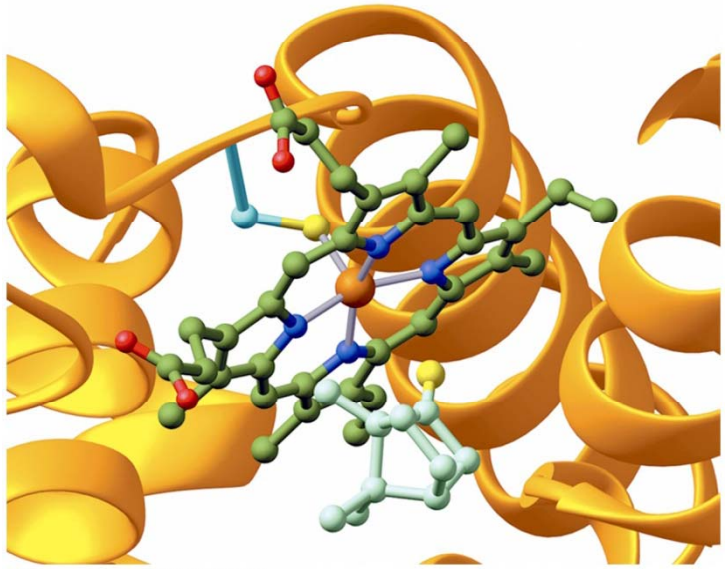


Figure 12-16 Fundamentals of Biochemistry, 2/e

Cytochrome P450 Isoforms (>60 in human)
http://drnelson.utmem.edu/CytochromeP450.html

- CYP1A2
- CYP3A
- CYP2C9
- CYP2C19
- CYP2D6

CYP = cytochrome P450

2 = genetic family

D = genetic sub-family

6 = specific gene

Catalyzed reactions (at least 20 more)

- Aromatic hydroxylation
- Aromatic epoxidation
- Aliphatic hydroxylation
- Alkene epoxidation
- N-dealkylation
- O-dealkylation
- S-dealkylation
- N-oxidation
- N-hydroxylation
- S-oxidation
- Aldehyde oxidation
- Androgen aromatization
- Halothane oxidation
- Halothane reduction
- Arginine oxidation
- Cholesterol side-chain cleavage
- Dehydrogenation
- Dehalogenation
- Azoreduction
- Deamination
- Desulphuration
- Amide hydrolysis
- Ester hydrolysis
- Peroxidation
- Denitration

Drug-drug interactions by CYP

Coadministration & induced expression of CYP isozymes

Increased toxicity

From innocuous to toxic:

ex. Acetaminophen (tylenol) in large doses

Pharmacogenomics

CYP are polymorphic

Individual's genetic makeup

Age & gender

Tailor drug treatment

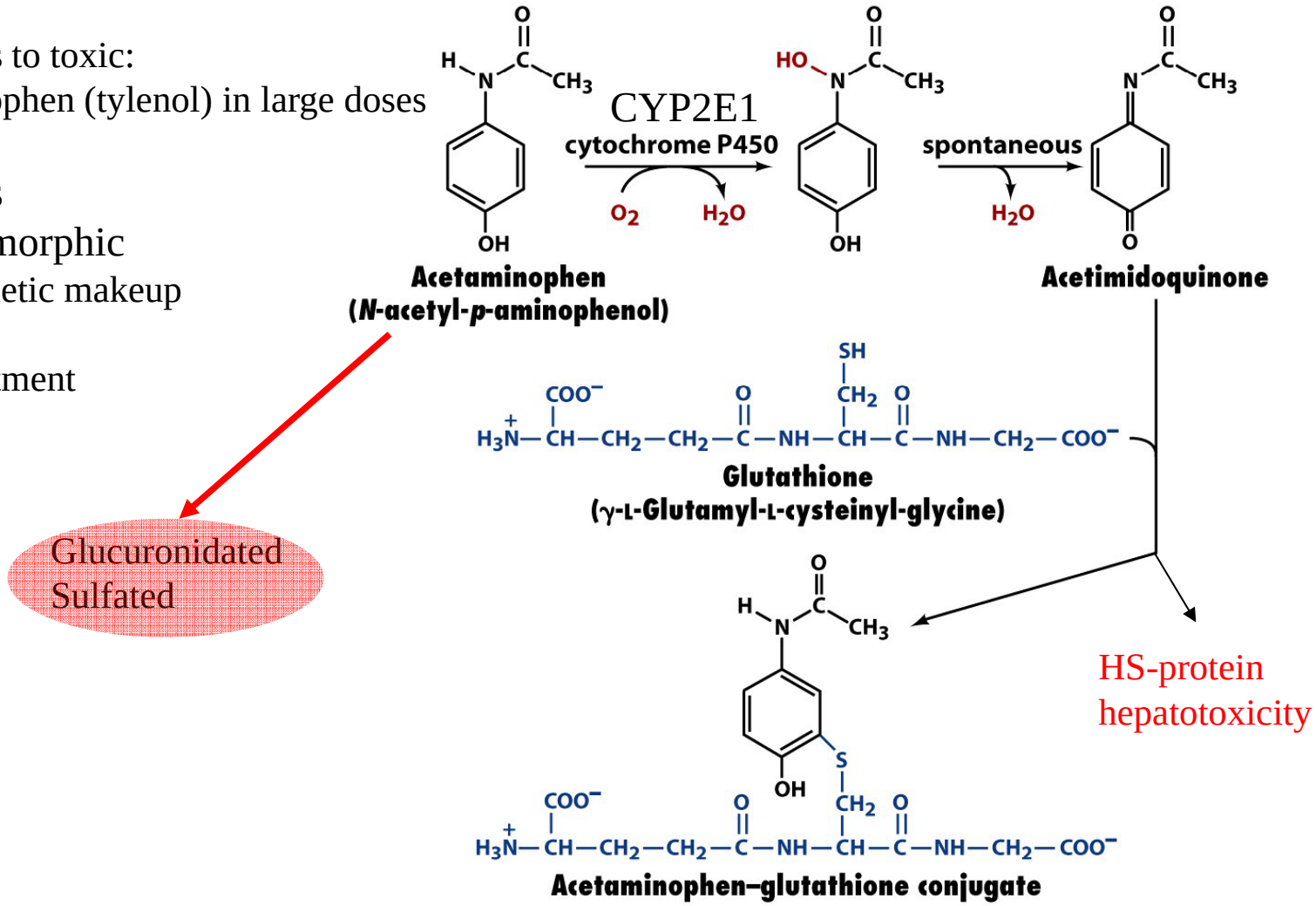


Figure 12-17 Fundamentals of Biochemistry, 2/e