Medicinal Chemistry/ CHEM 458/658

Chapter 9- Enzymes

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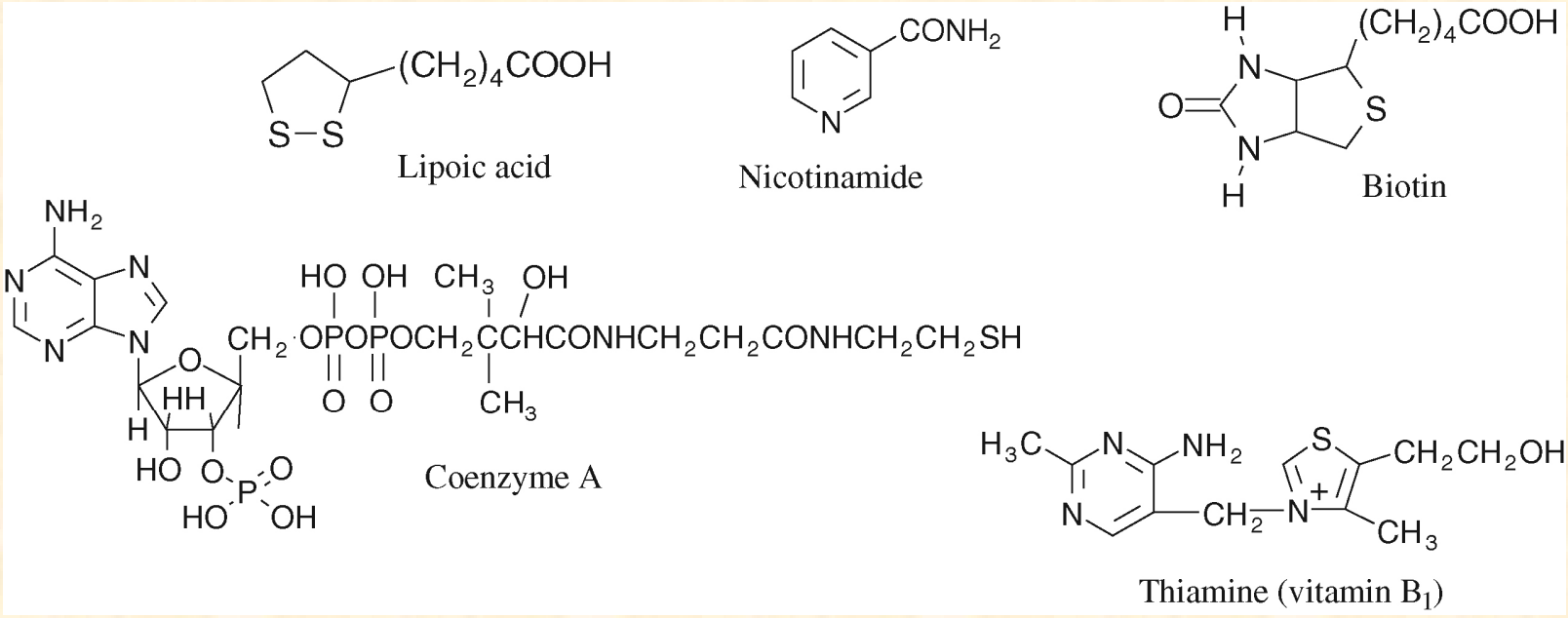
Introduction

- Enzymes – biocatalyst
  - usually large protein molecules (apoenzymes)
  - some RNA molecules (ribozymes)
  - sometimes metal is involved (metalloenzymes)

  some enzymes require coenzyme/metal ions

  Apoenzyme + Coenzyme/cofactor = Holoenzyme



Introduction

- Enzymes

  Enzymes can be produced from inactive proteins (proenzymes/ zymogens)

  Enzymes with different structures can catalyze the same reaction: isoenzymes or isozymes

Classification

- The International Union of Biochemistry - *ase* suffix

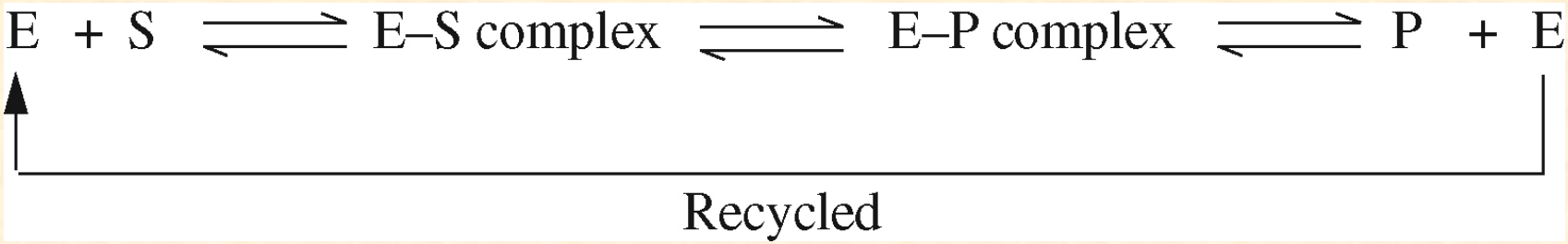
Enzyme Commission code : EC

lactate dehydrogenase - EC 1.1.1.27.

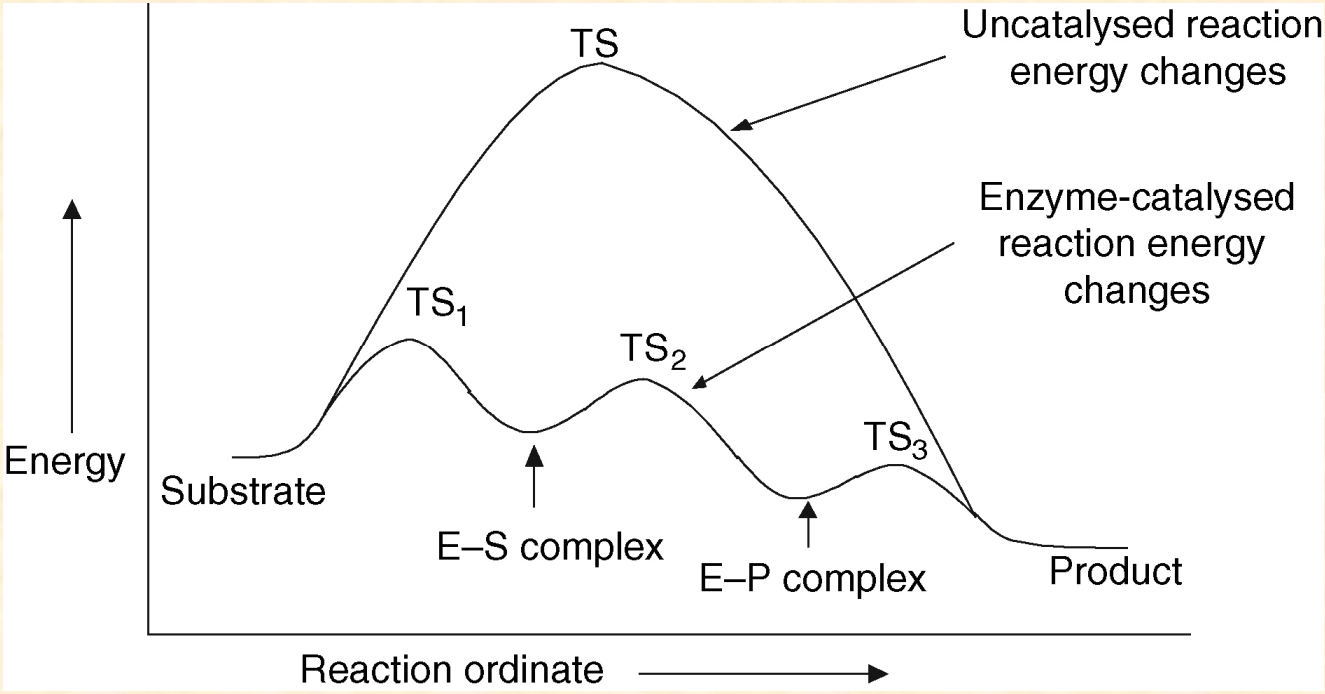
Code	Classification	Type of reaction catalyzed
1	Oxidoreductases	Oxidations and reductions
2	Transferases	Intermolecular transfer of groups
3	Hydrolases	Hydrolysis of various functional groups
4	Lyases	Cleaveage of bonds by non-oxidative or non-hydrolytic mechanism
5	Isomerases	Interconversion of isomers
6	Ligases (synthases)	The formation of bonds between molecules

Active Sites and Catalytic Action

- Active site - substrate



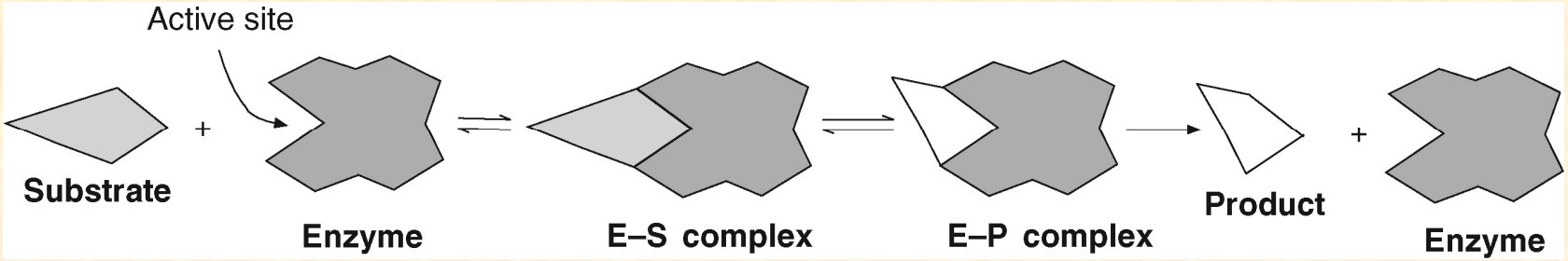
- Enzymes are catalysts - they reduce activation energy



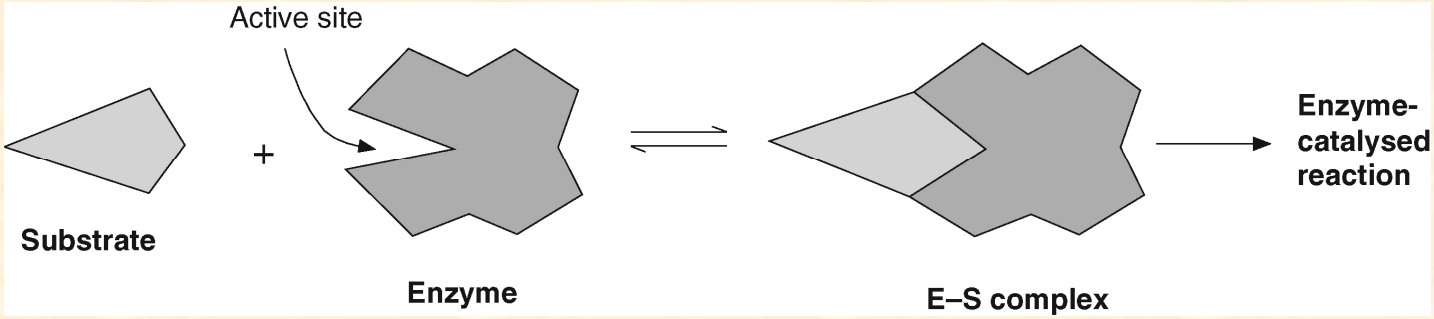
Active Sites and Catalytic Action

- Active site – substrate interaction

  - Lock and Key model

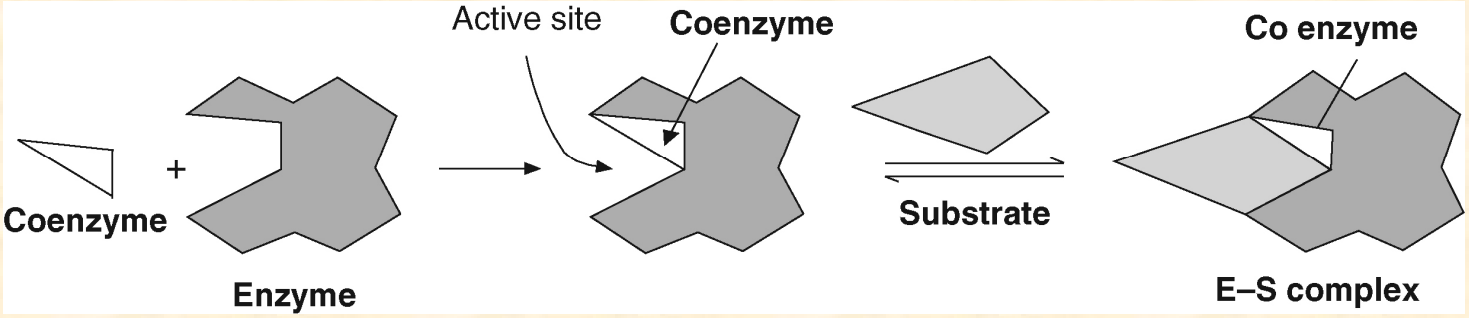


  - Induced fit model



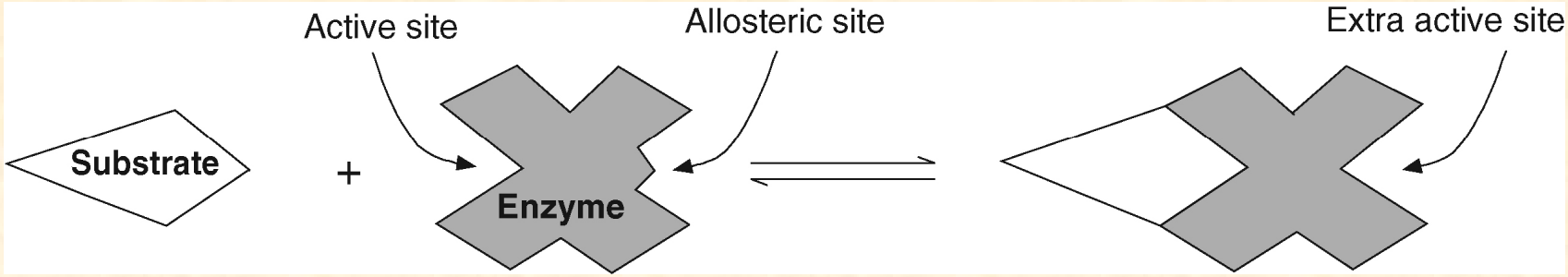
Daniel Koshland

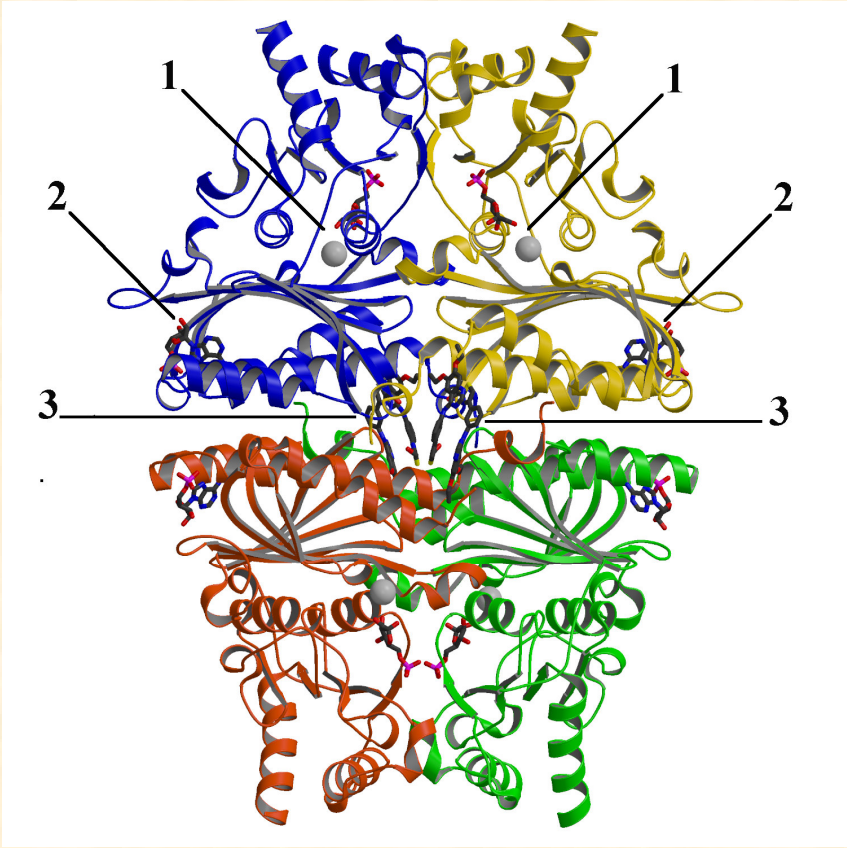
  - Coenzyme model



Active Sites and Catalytic Action

- Allosteric activation





FBPase

1 – active site

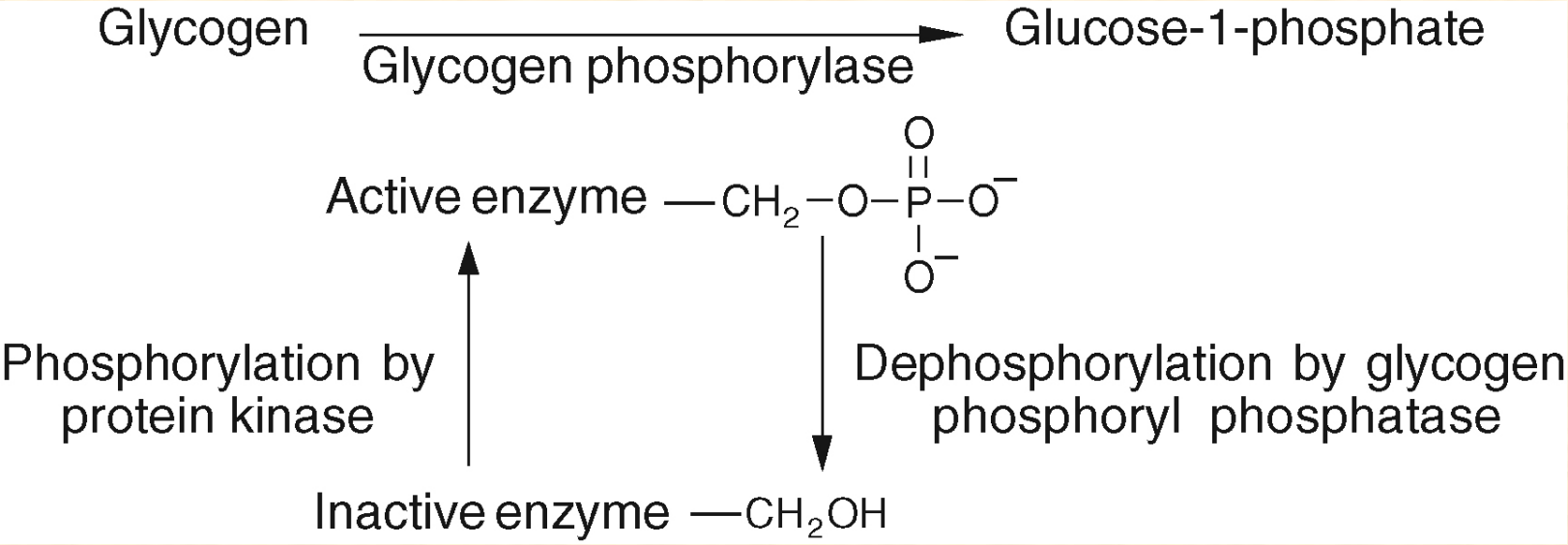
2 – allosteric site

3 – tetrameric allosteric site

Regulation of Enzyme Activity

- Covalent modification

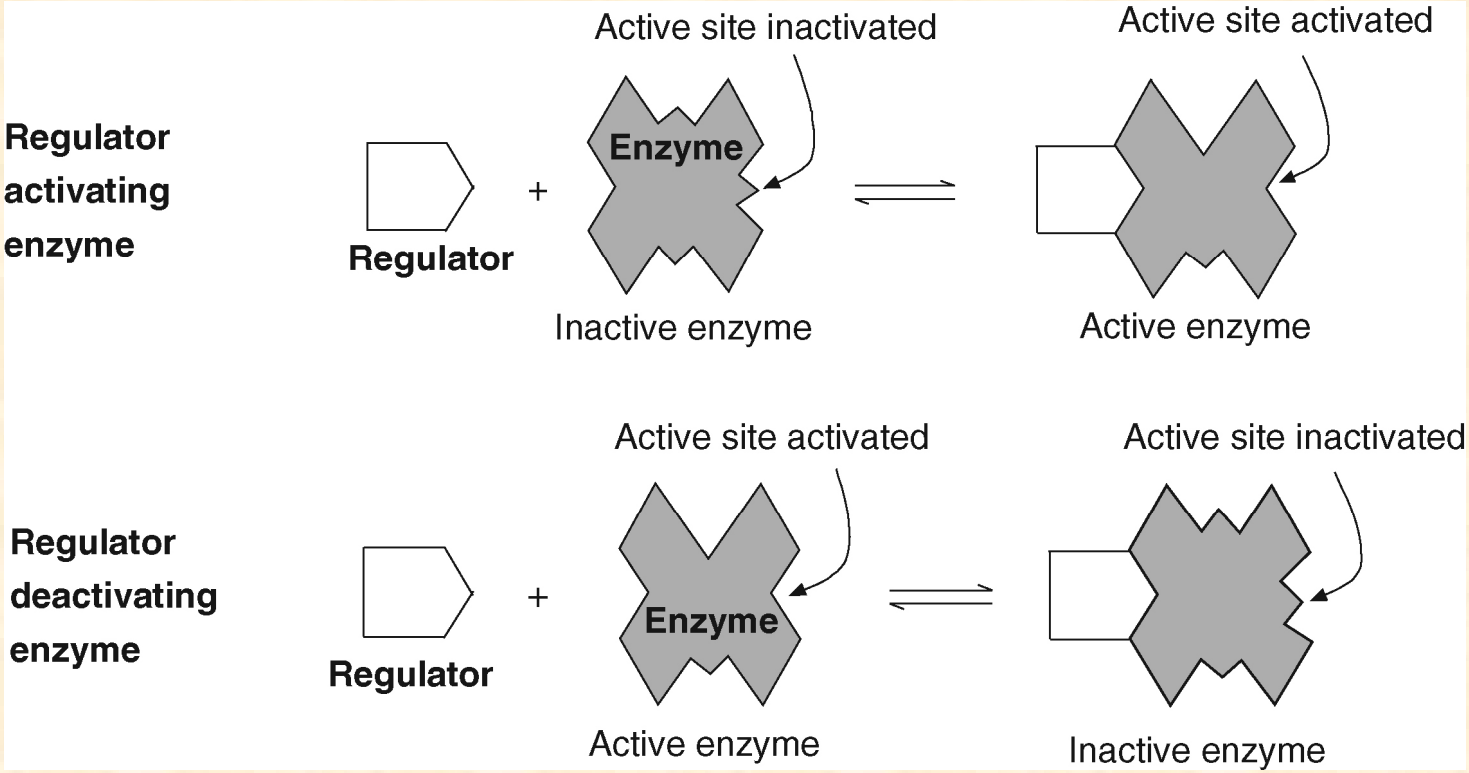
  - attachment of a chemical moiety by a covalent bond (regulators)
  - activate or inactivate (switch on/off)
  - modifying/converter enzymes



Regulation of Enzyme Activity

- Allosteric control

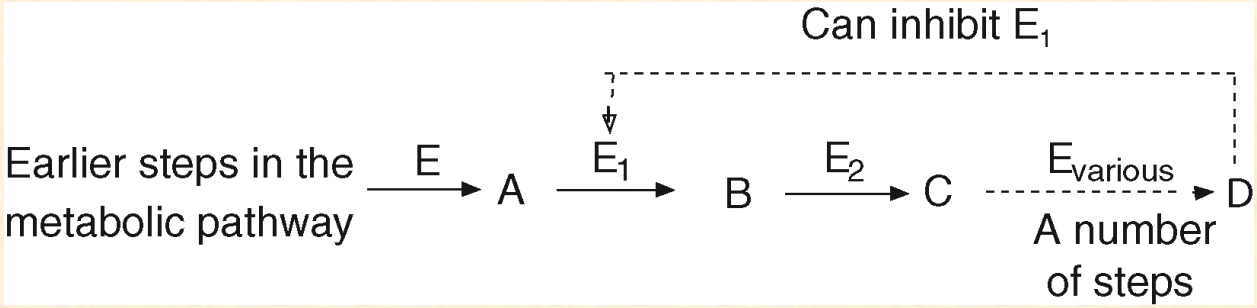
  - reversible binding to an allosteric site – activate/deactivate
  - compounds from metabolic pathway or others (effectors, modulators, regulators)
  - allosteric site – regulatory site



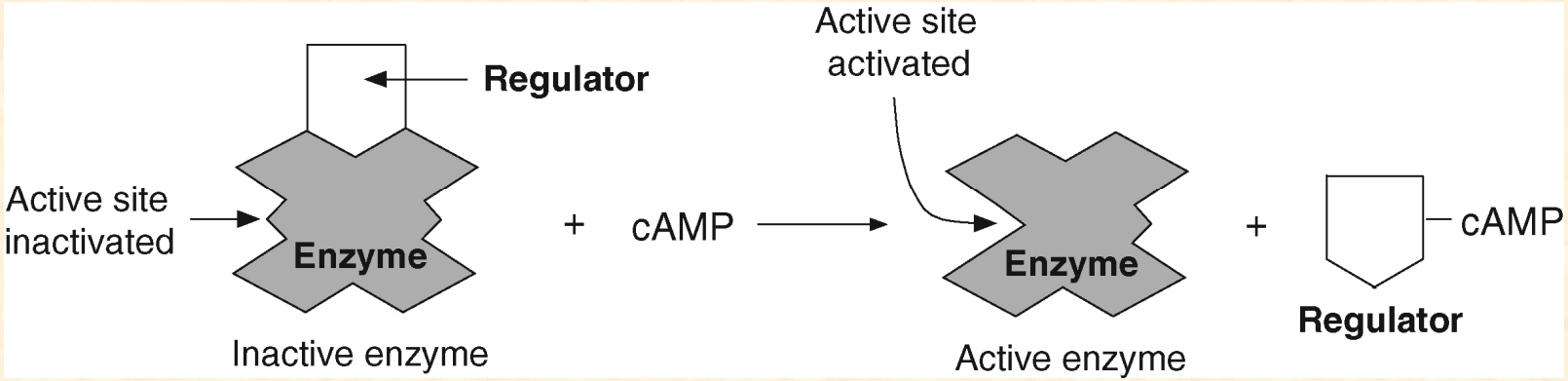
Regulation of Enzyme Activity

- Allosteric control

  - Feedback control



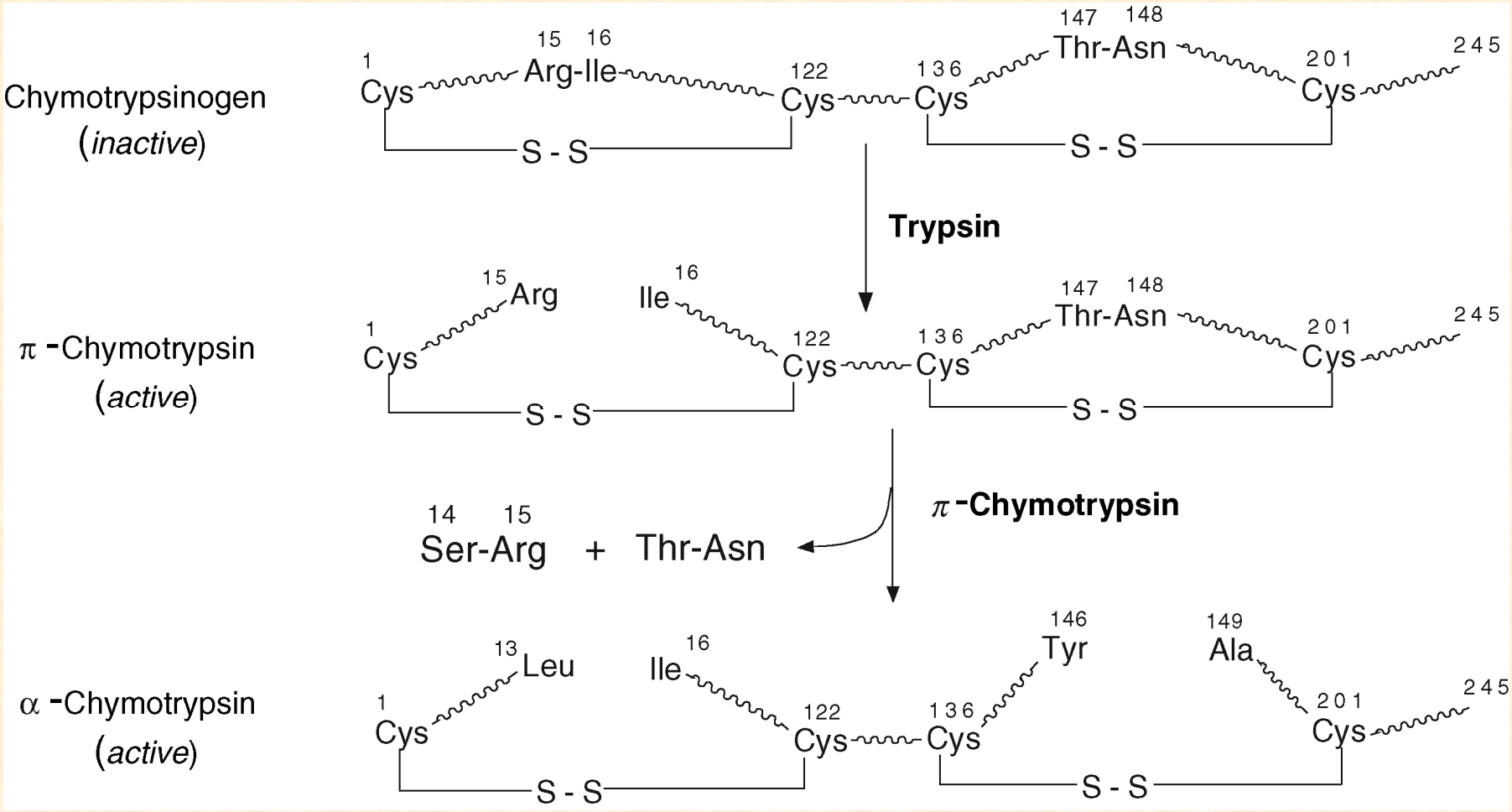
- Second modulator (positive modulators)



Regulation of Enzyme Activity

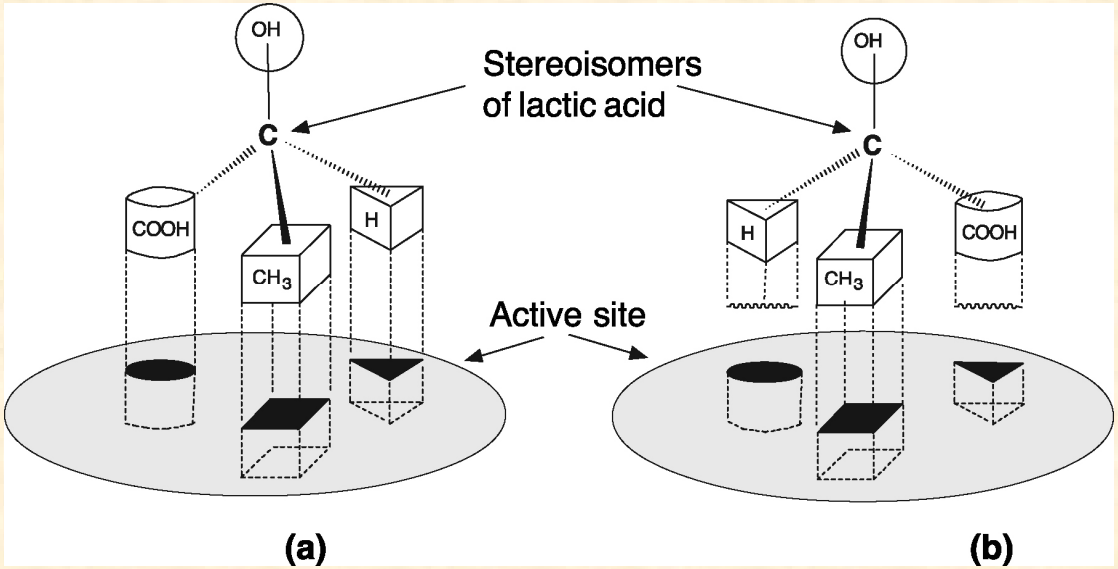
- Allosteric control

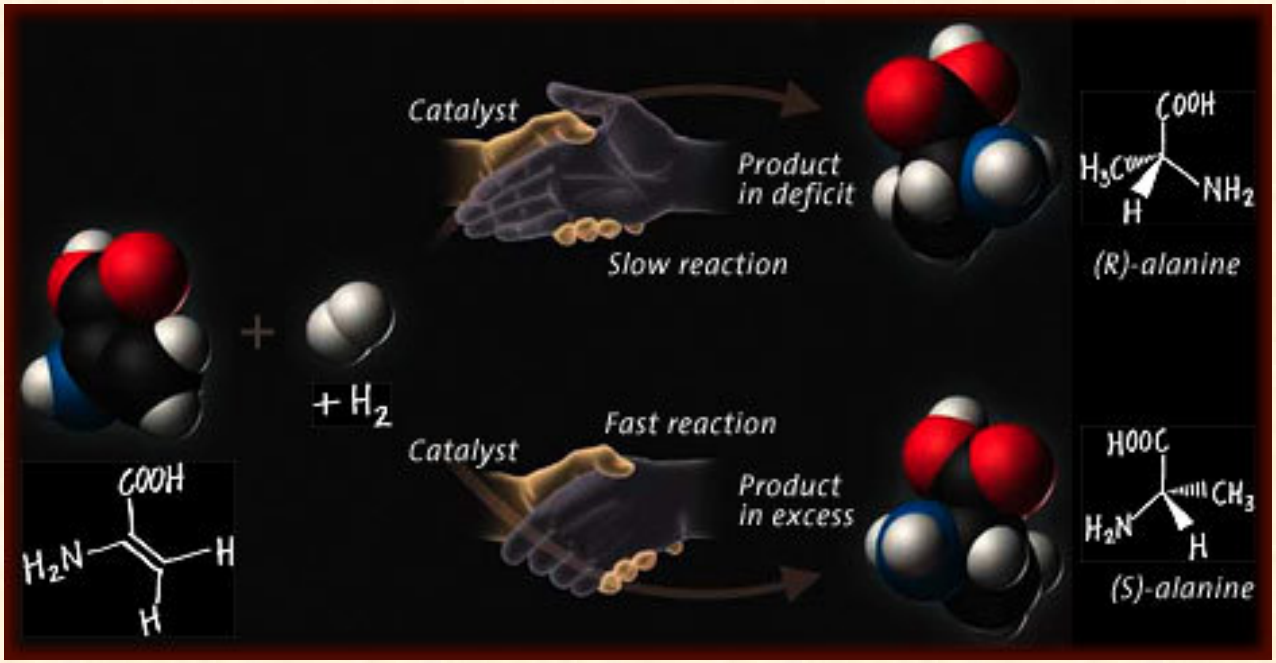
- Proenzyme control



Specific Nature of Enzyme Action

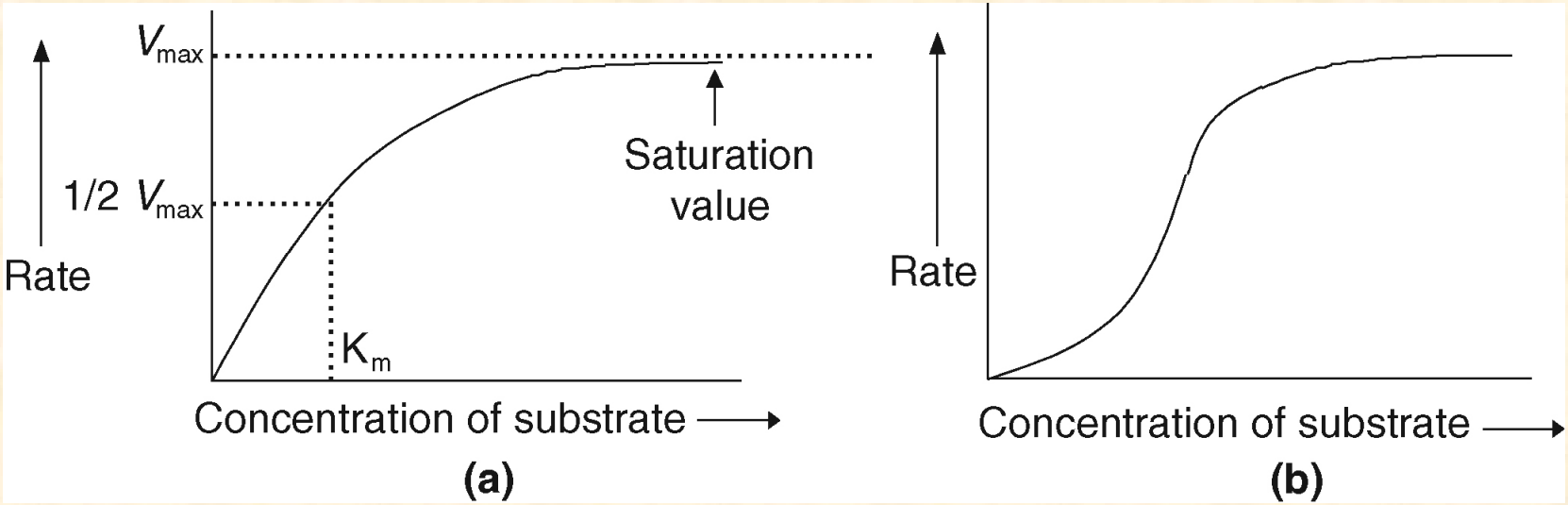
- Role of chirality



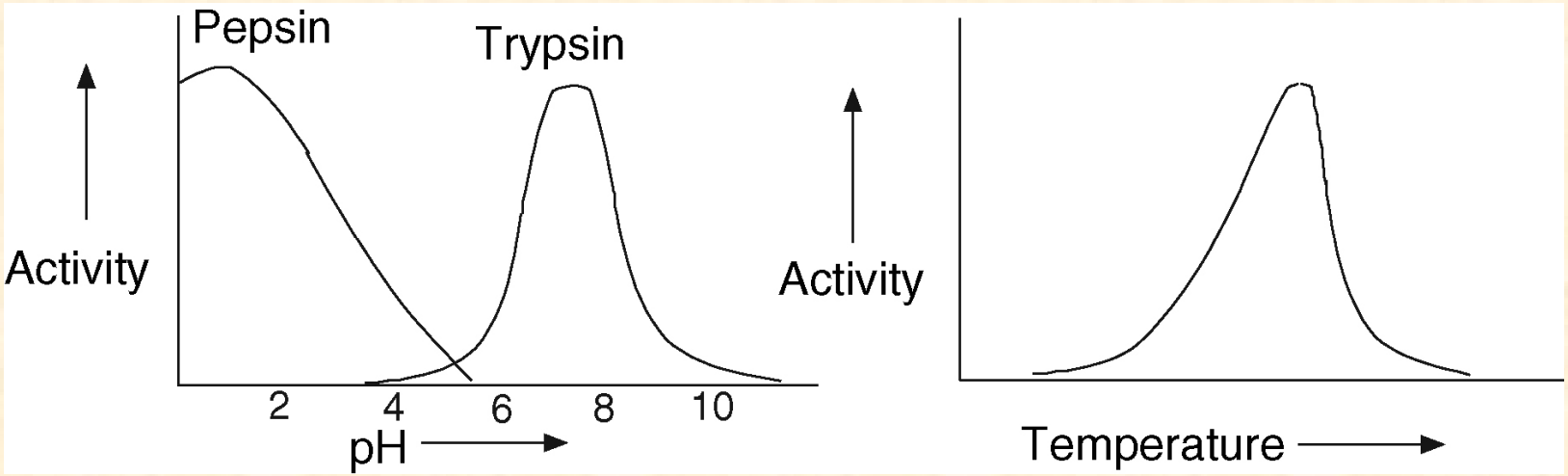


Physical Factors of Enzyme Action

- Allosteric vs. feedback regulated enzyme catalysis



- pH, T



Enzyme Kinetics

- Single substrate reactions

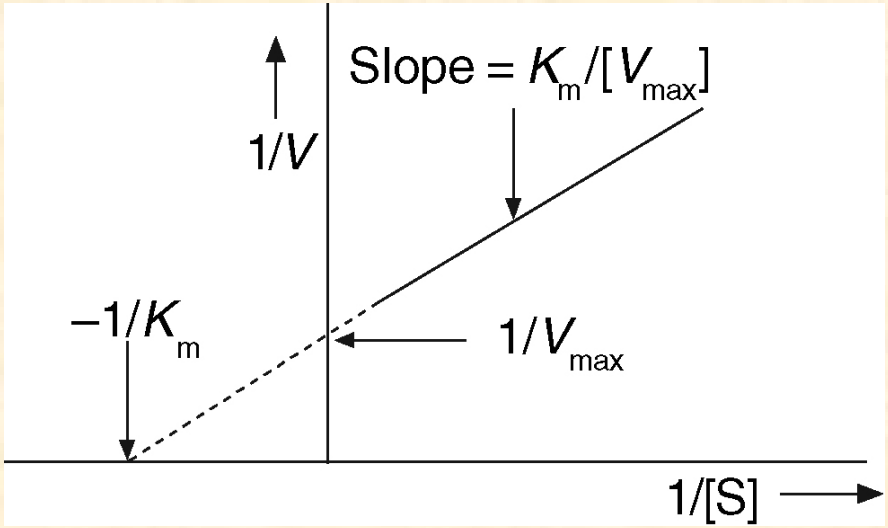
$$E + S \rightleftharpoons E\text{—}S \rightleftharpoons E\text{—}P \longrightarrow E + P$$

- Michaelis – Menten equation

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

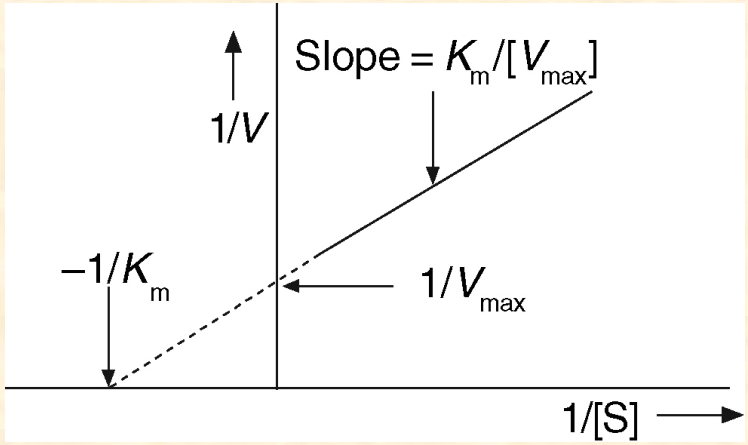
- Lineweaver – Burk equation

$$\frac{1}{V} = \frac{K_m}{V_{max}\,[S]} + \frac{1}{V_{max}}$$

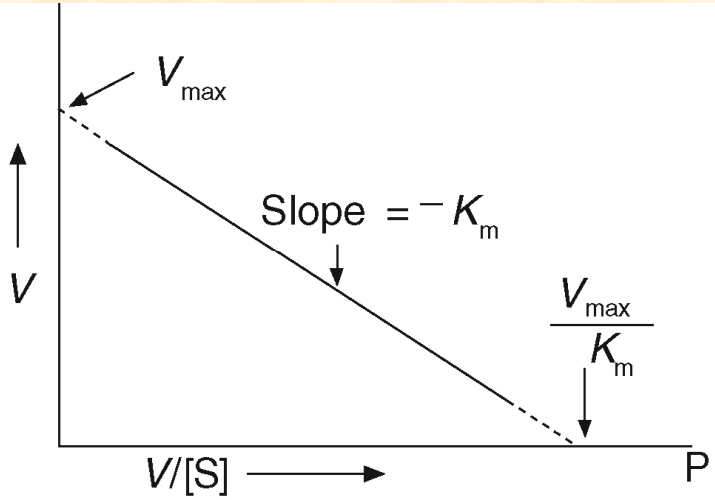


Enzyme Kinetics

Lineweaver – Burk plot



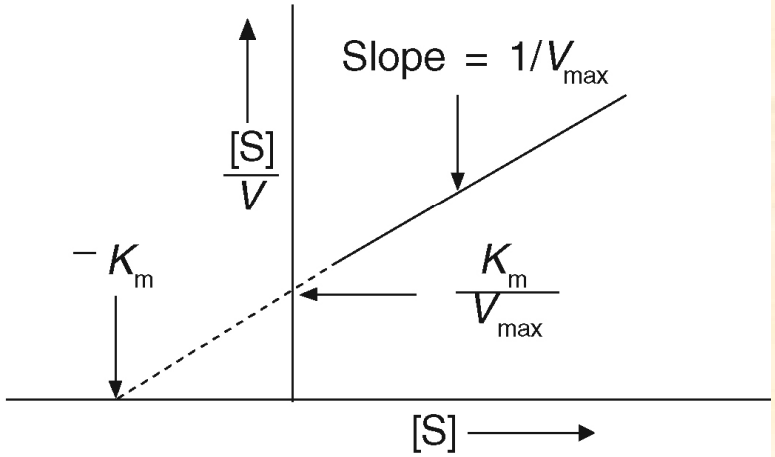
Eadie – Hofstee plot



$$V = -K_m \frac{V}{[S]} + V_{max}$$

(a)

Hanes – Wolf plot



$$\frac{[S]}{V} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}}$$

(b)

Enzyme Kinetics

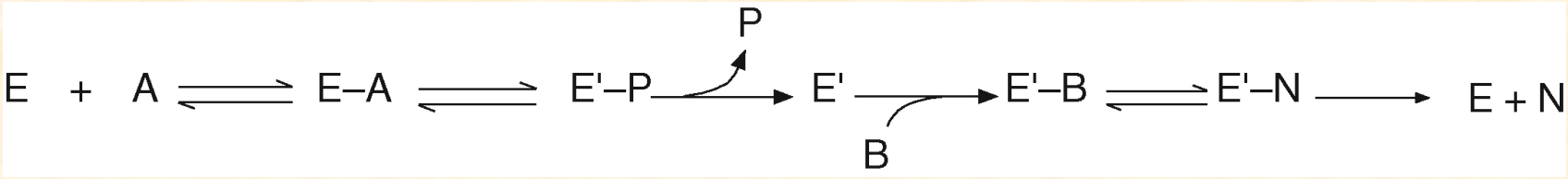
- Multiple substrate reactions

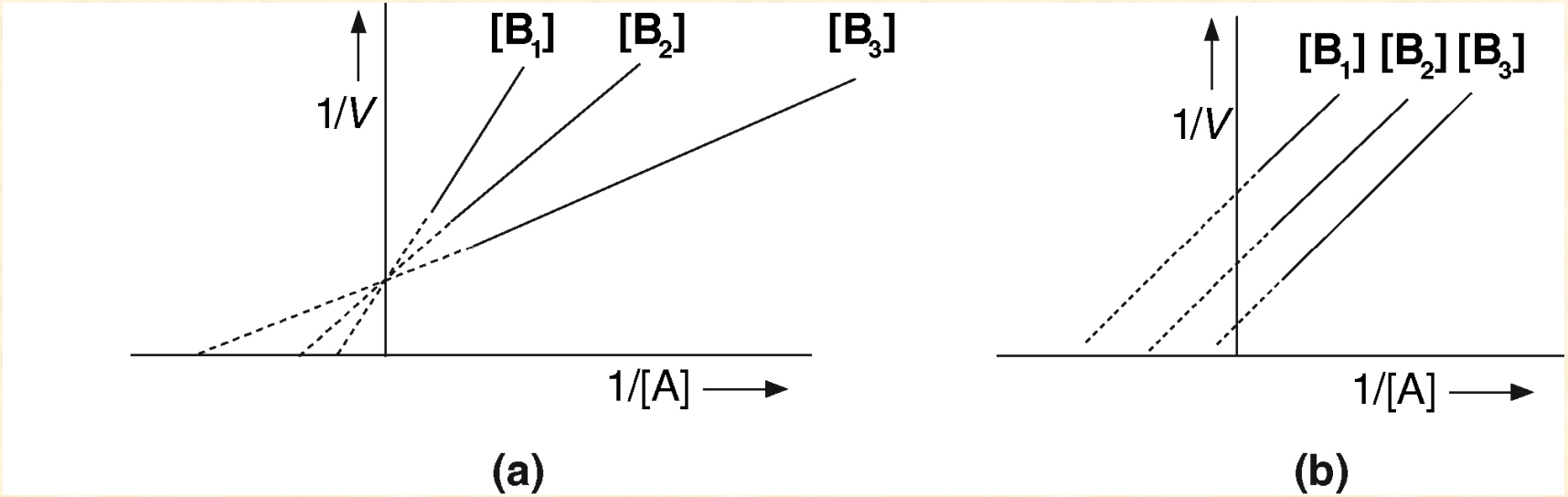
  - The sequential or single displacement reactions

$$E + A + B \rightleftharpoons A—E—B \rightleftharpoons N—E—P \longrightarrow E + P + N$$

$$E + A \rightleftharpoons A—E + B \rightleftharpoons A—E—B \rightleftharpoons N—E—P \longrightarrow E + P + N$$

  - Double-displacement or ping-pong reactions

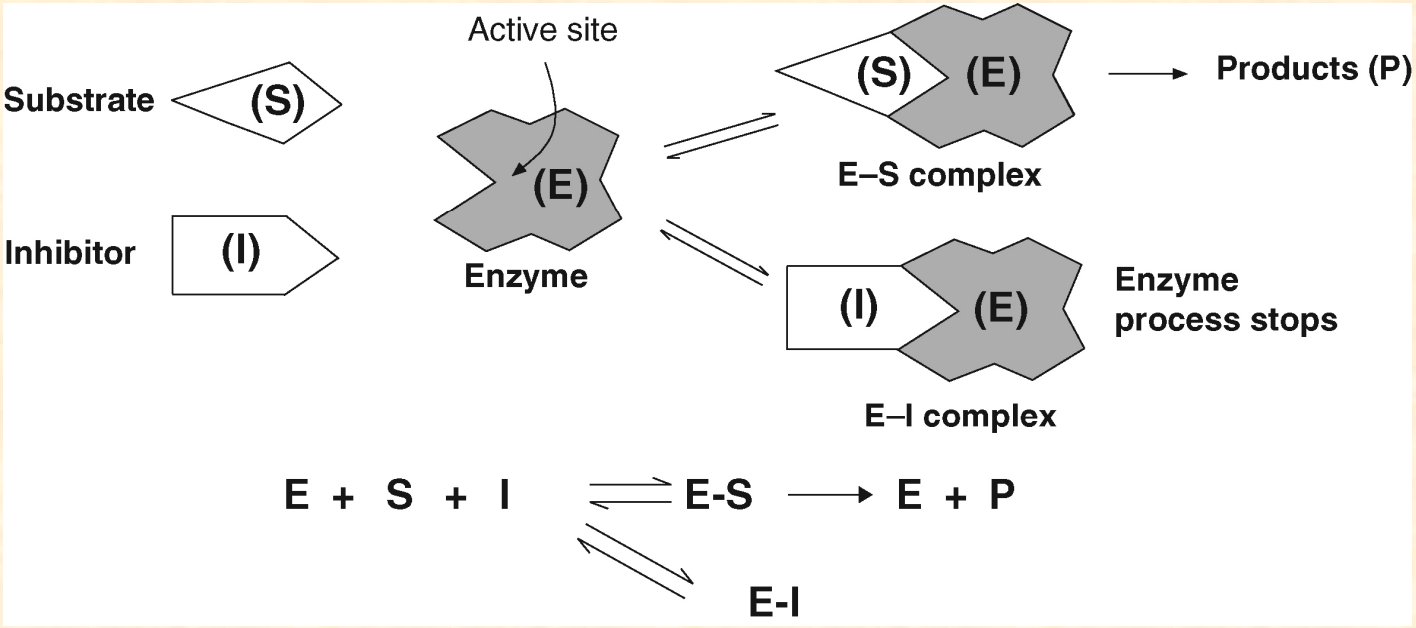


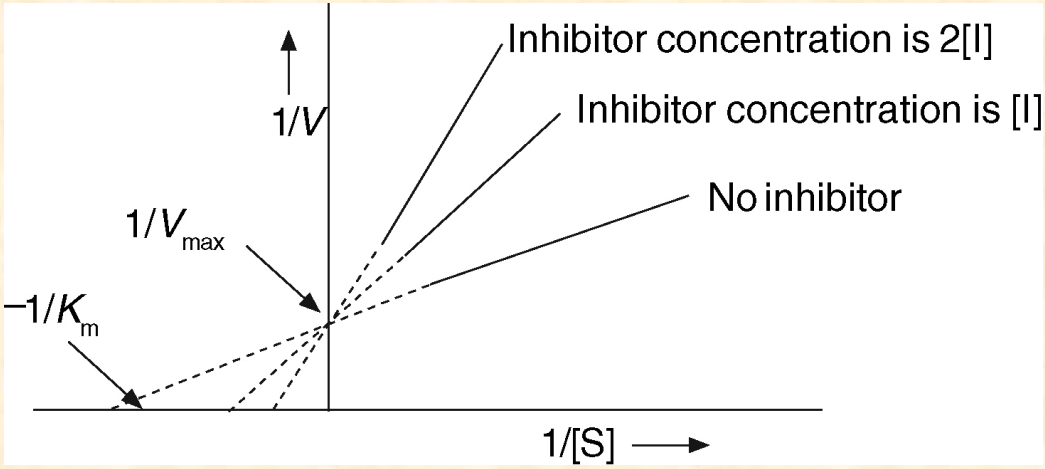


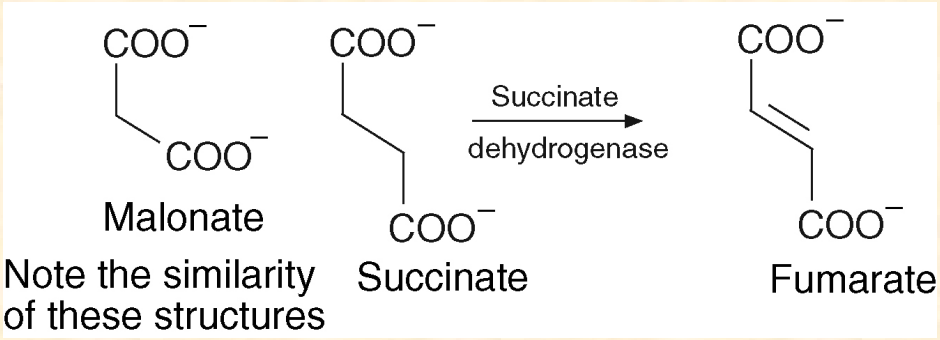
Enzyme Inhibitors

- Reversible Inhibitors

  - Competitive inhibition

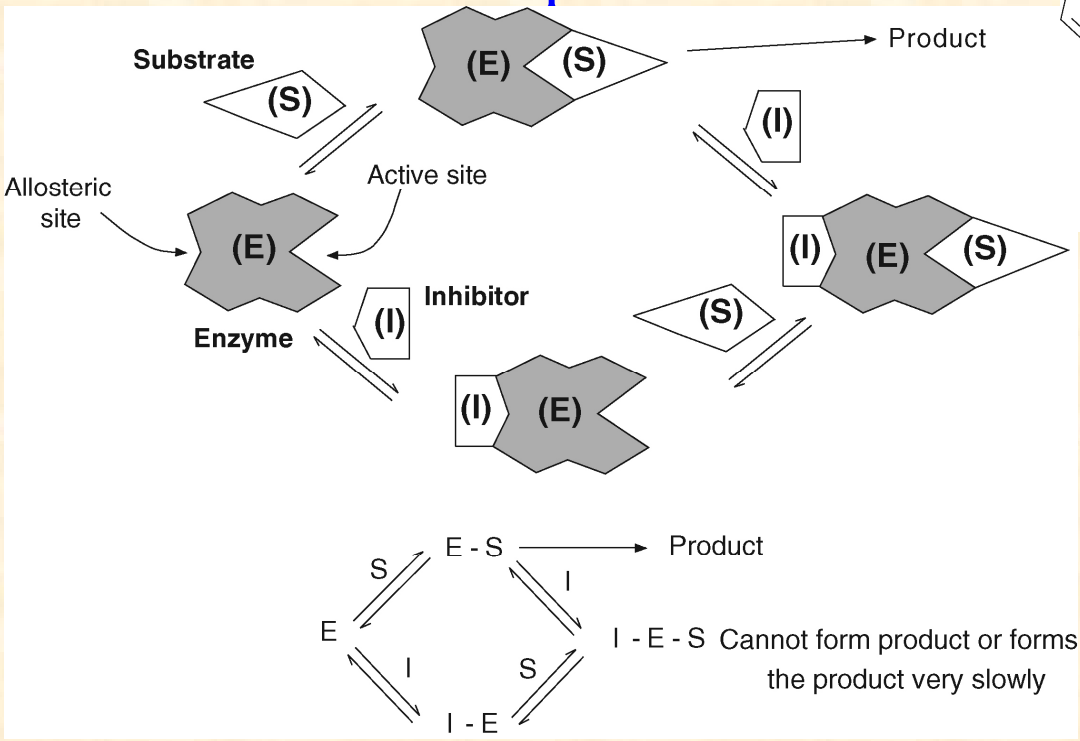


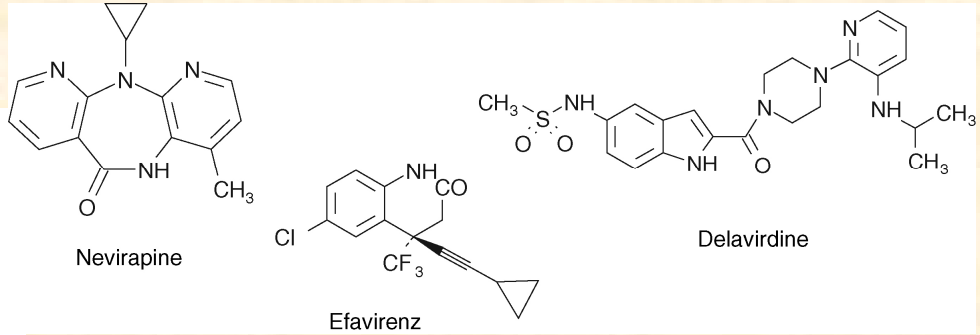


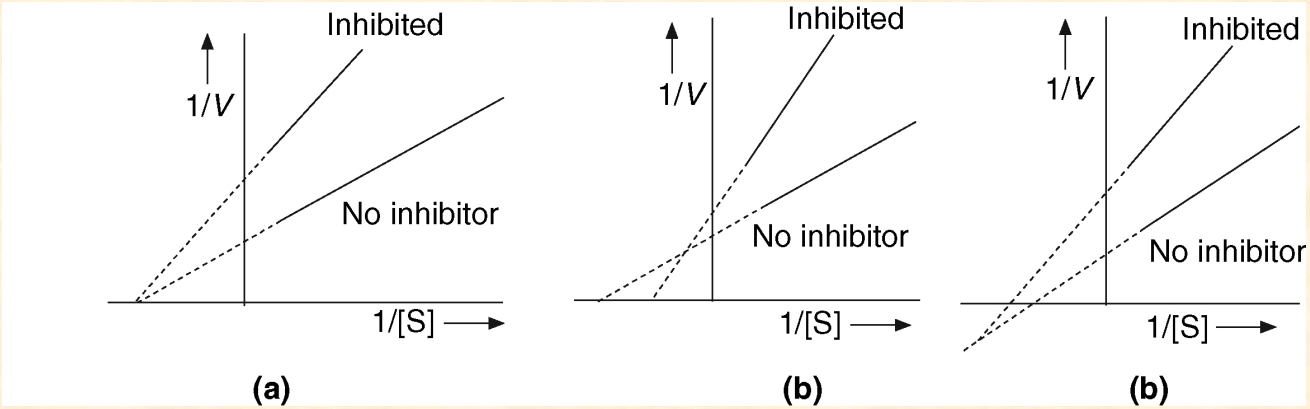


Enzyme Inhibitors

- Reversible Inhibitors
  - Non-competitive inhibition



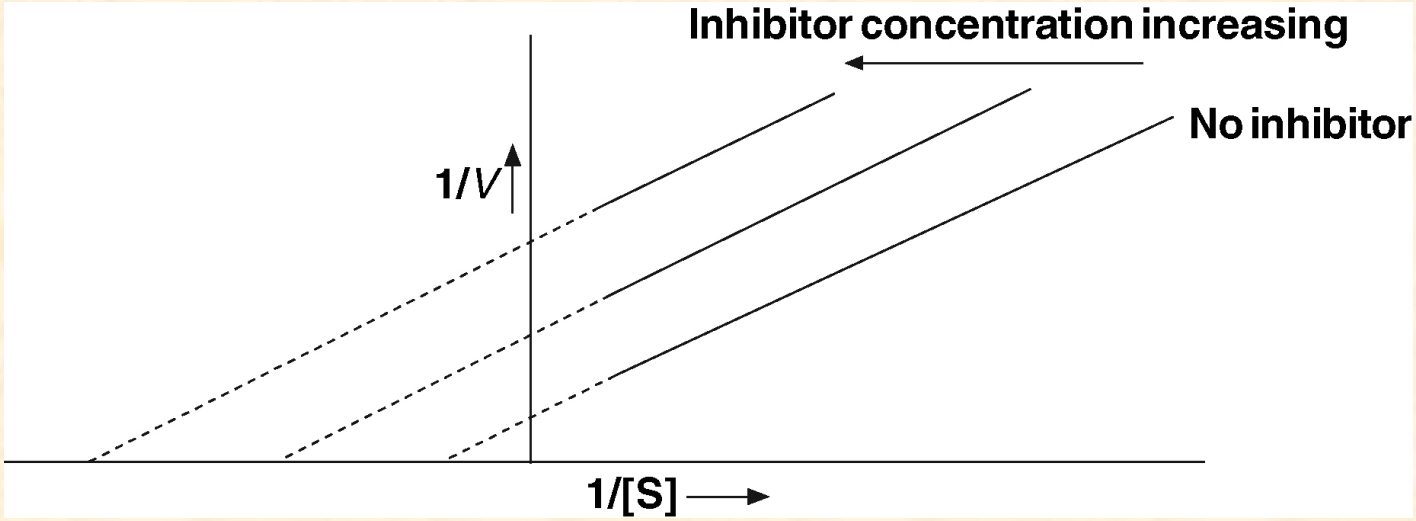




Enzyme Inhibitors

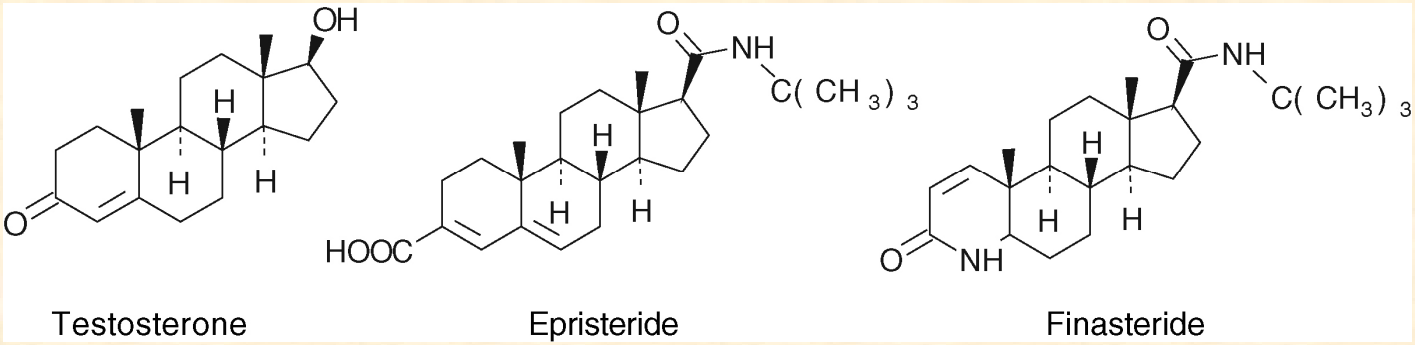
- Reversible Inhibitors
  - Uncompetitive inhibition

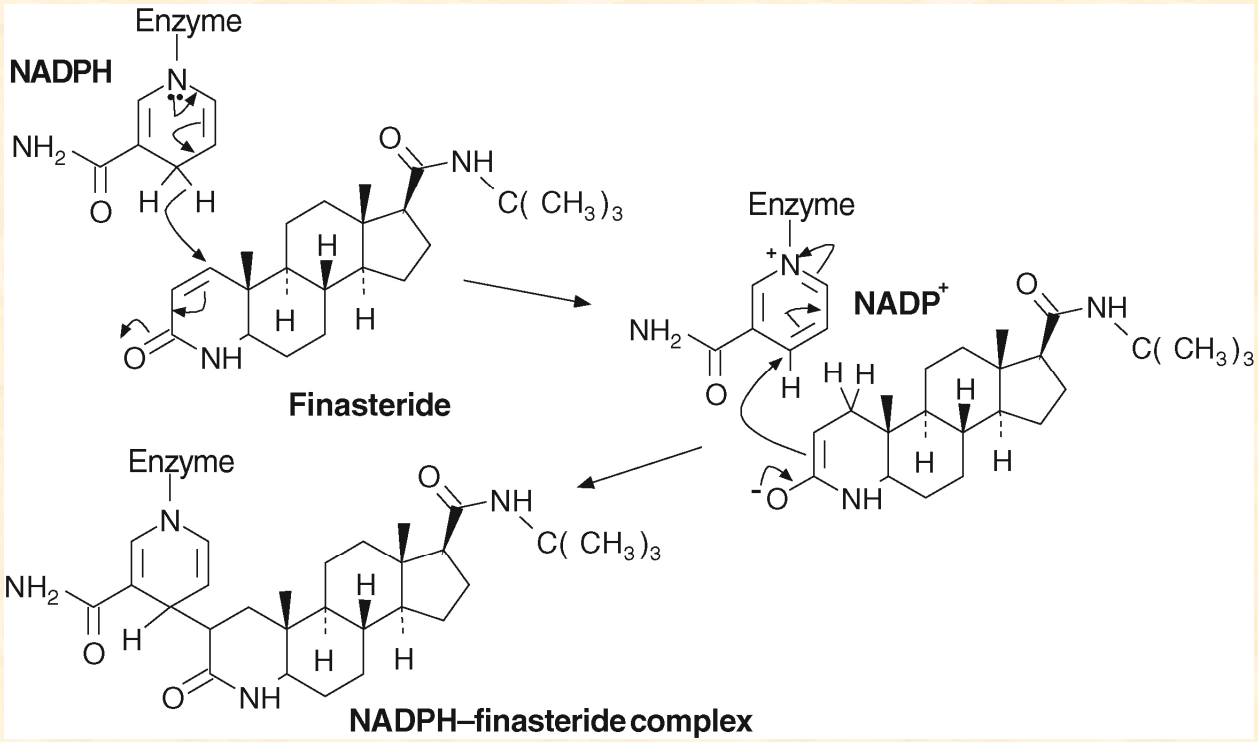
$$E + S \rightleftharpoons S\text{—}E + I \rightleftharpoons S\text{—}E\text{—}I$$

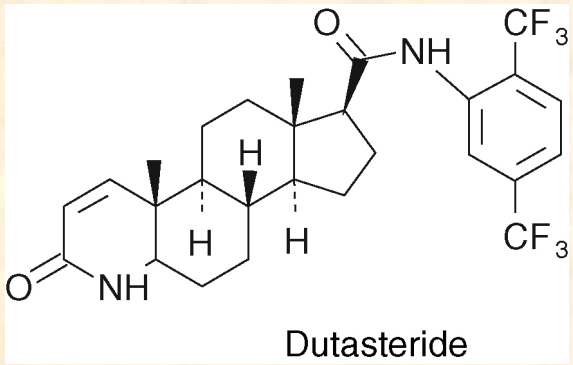


Enzyme Inhibitors

- Reversible Inhibitors
  - Uncompetitive inhibition of 5α-reductase

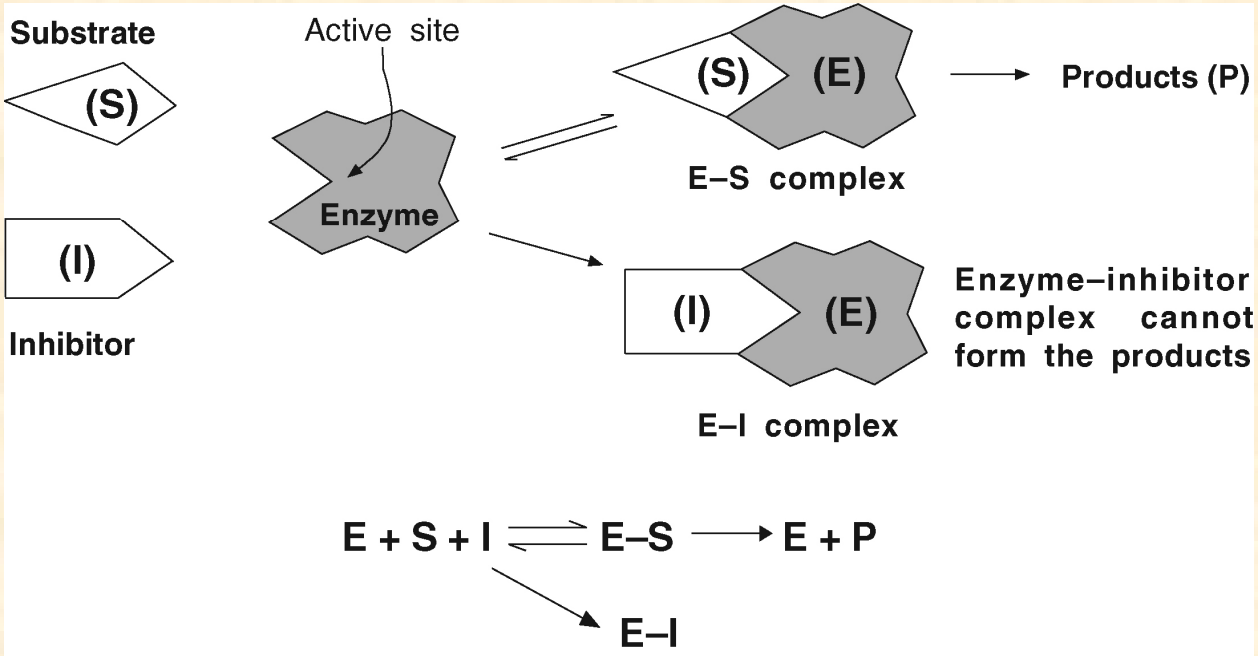






Enzyme Inhibitors

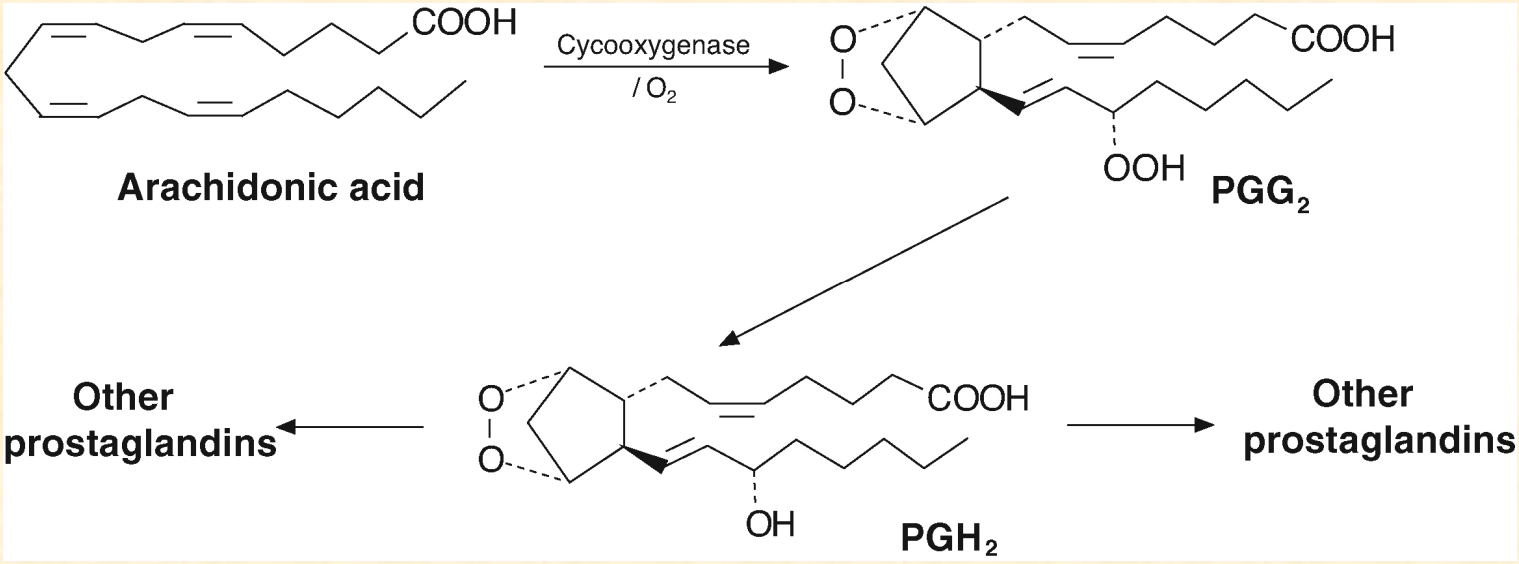
- Irreversible Inhibitors
  - Active site – directed inhibitors

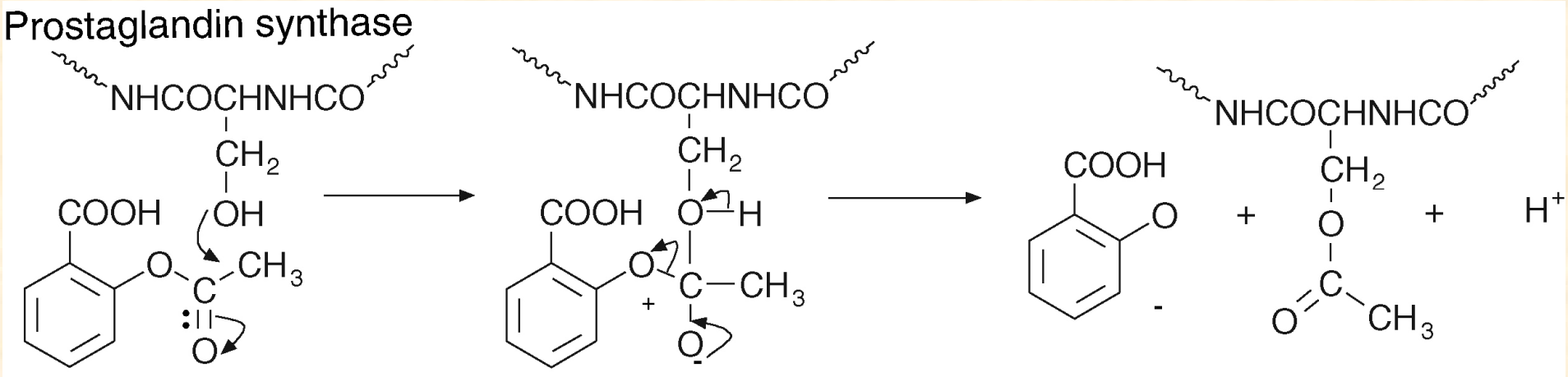


Most of them are too toxic – research use

Enzyme Inhibitors

- Anti-inflammatory drugs: Aspirin – a case study





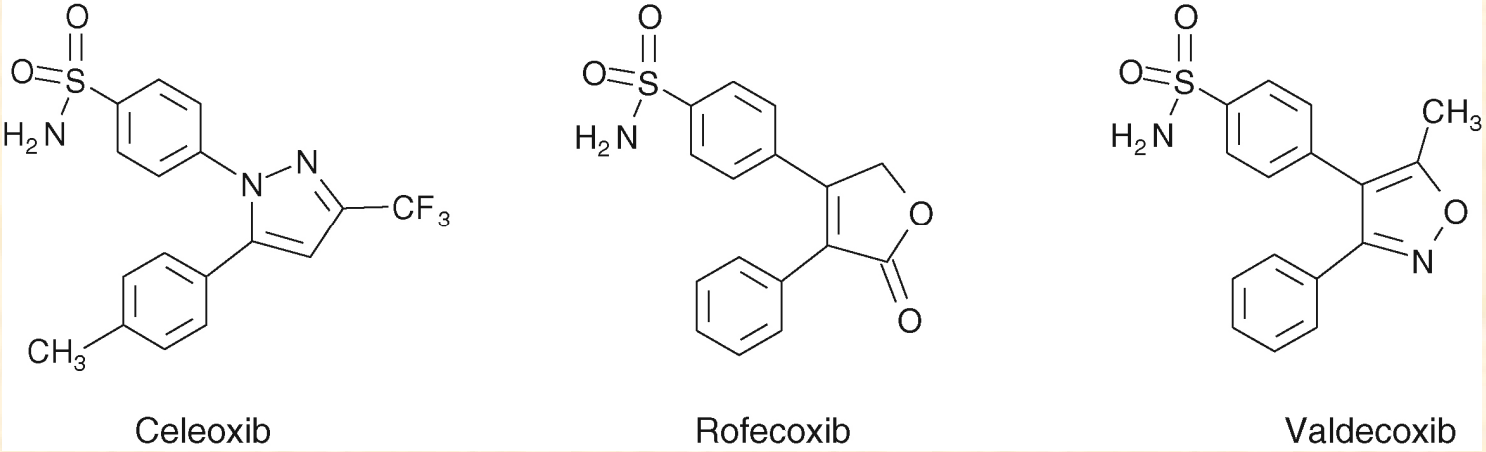
Enzyme Inhibitors

- Anti-inflammatory drugs: Aspirin – a case study

  aspirin, ibuprofen, naproxen (NSAIDs) – COX 1, 2 inhibition
  long term use : ulceration of both GI tract, and kidneys

  more selective inhibition of COX-2

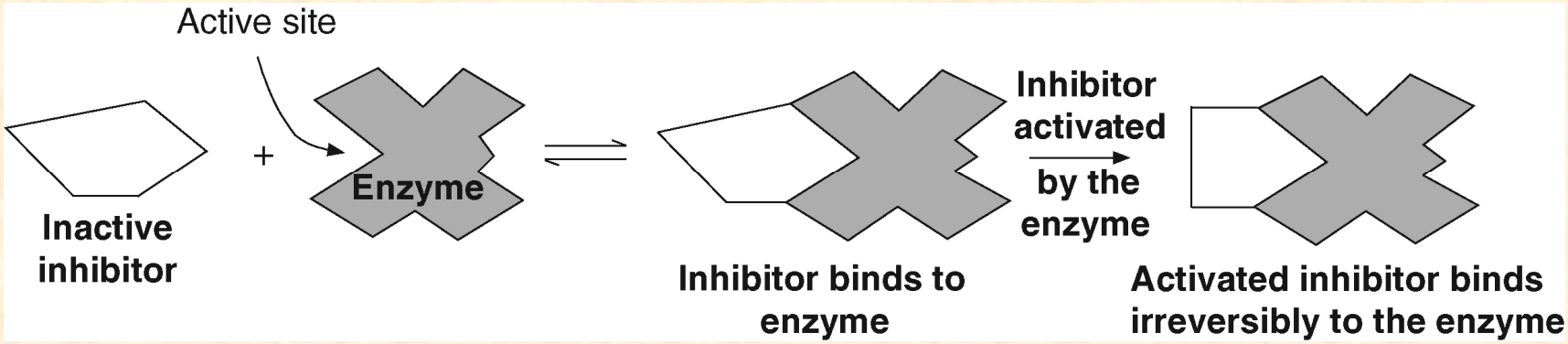
  COX-3 (CNS) target for drugs to decrease pain and fever



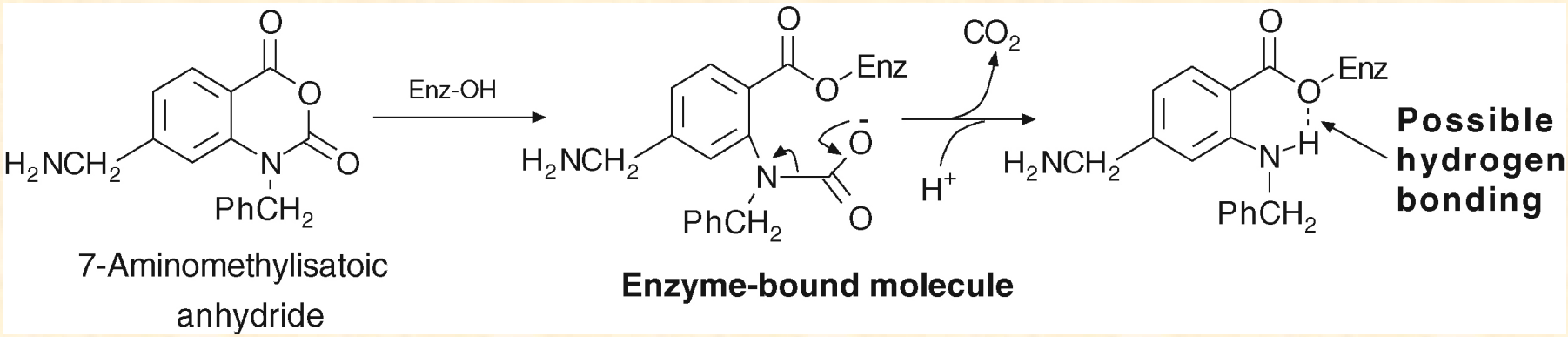
Enzyme Inhibitors

- Irreversible binding – suicide inhibitors (K_{cat} or IMBIs)

usually analogs of natural substrate

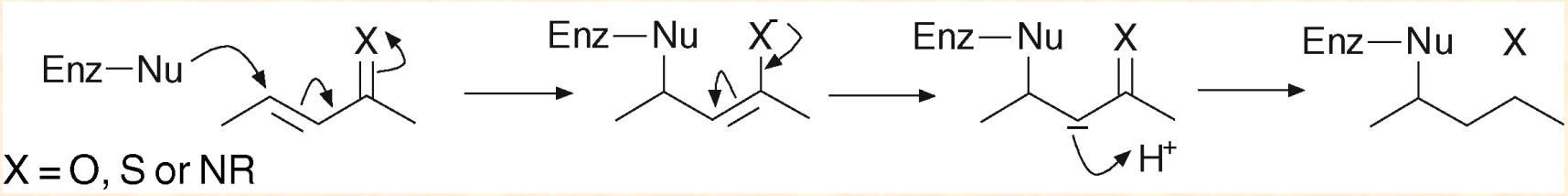


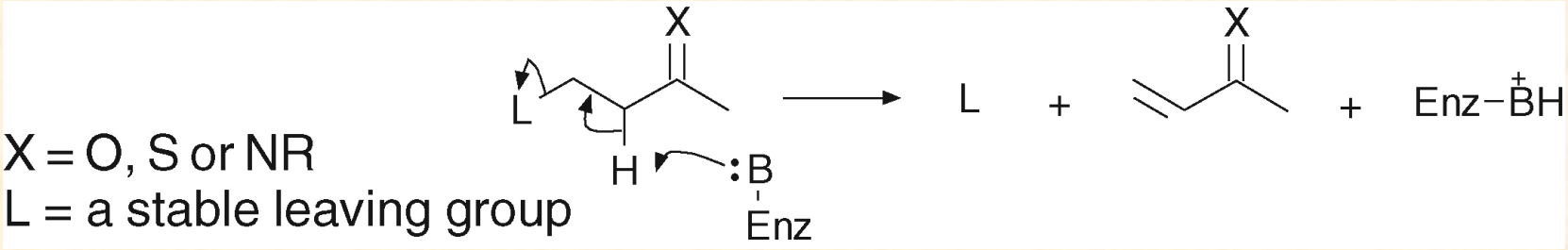
serine protease thrombin



Enzyme Inhibitors

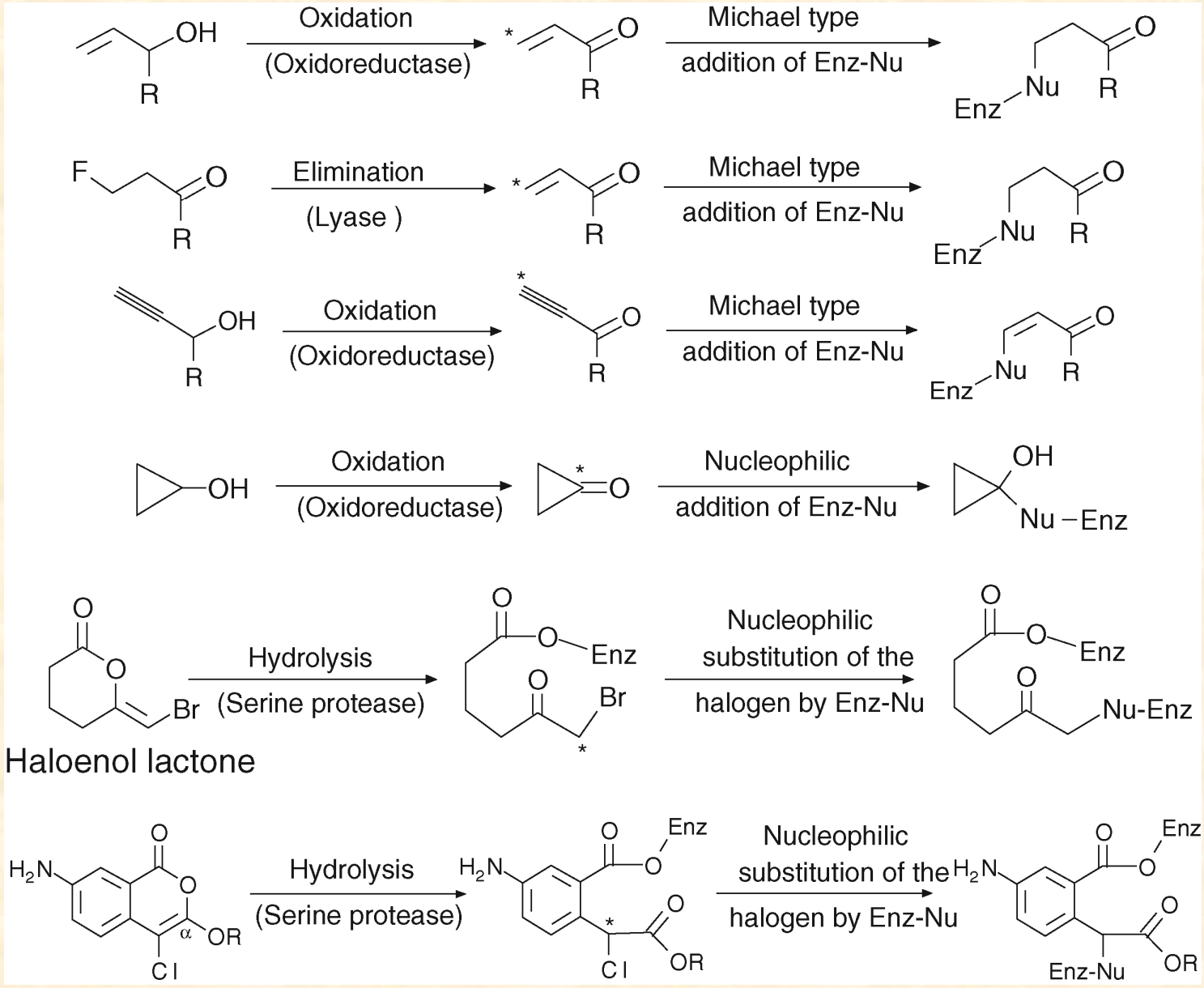
- Irreversible binding – suicide inhibitors (K_{cat} or IMBIs)





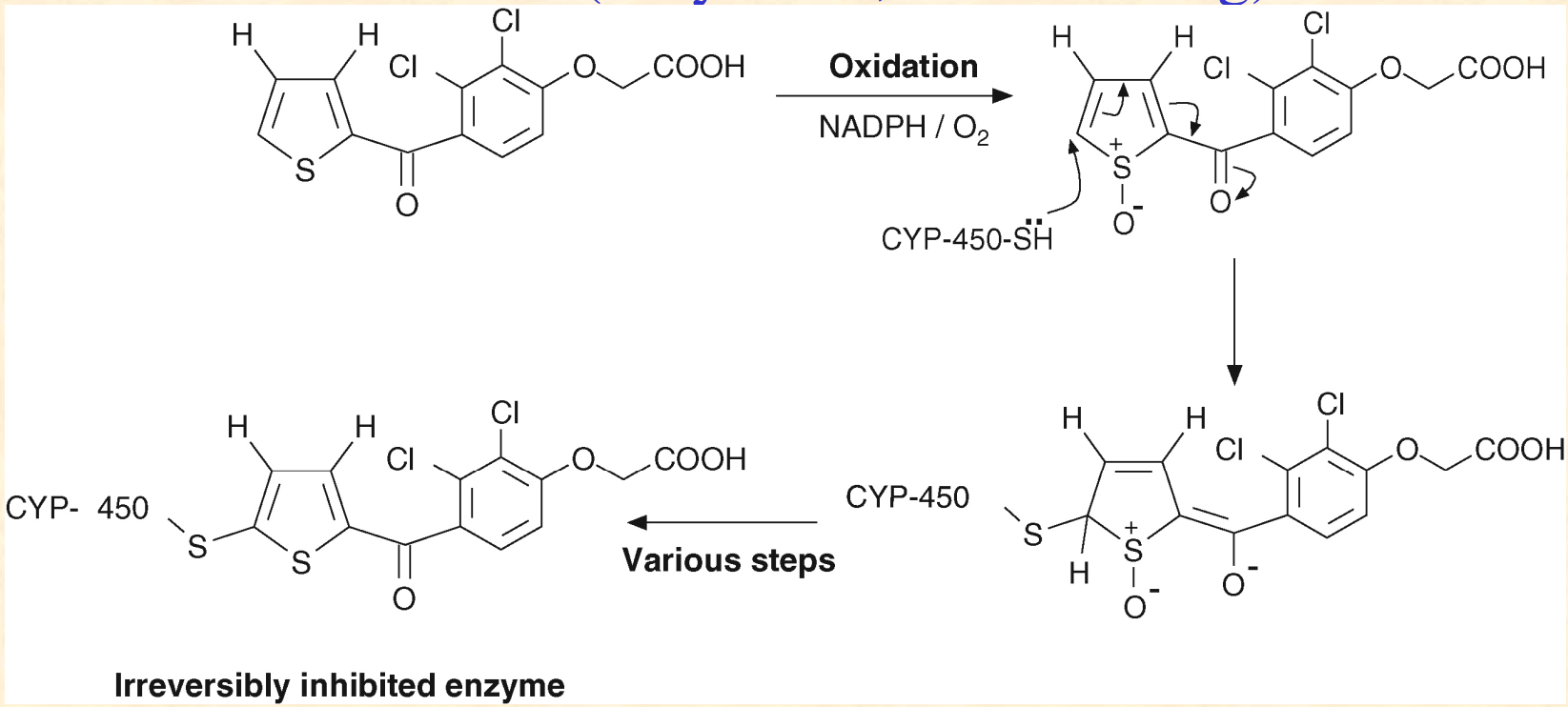
Enzyme Inhibitors

- Irreversible binding – suicide inhibitors (K_{cat} or IMBIs)

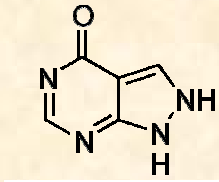


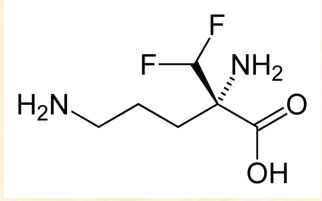
Enzyme Inhibitors

- Irreversible binding – suicide inhibitors (K_{cat} or IMBIs)
  - tienilic acid (ticrynafen, diuretic drug)



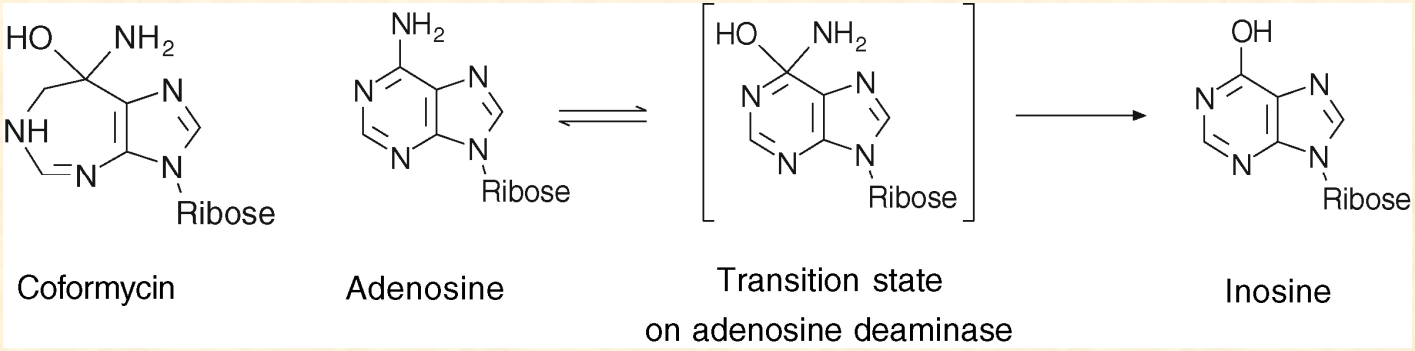
- penicillin (transpeptidase)
- allopurinol (xanthine oxidase – gout)
- eflornithine (ornithine decarboxylase sleeping sickness, hair growth)

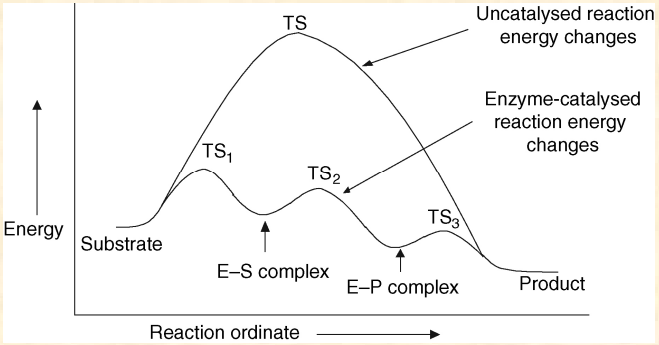


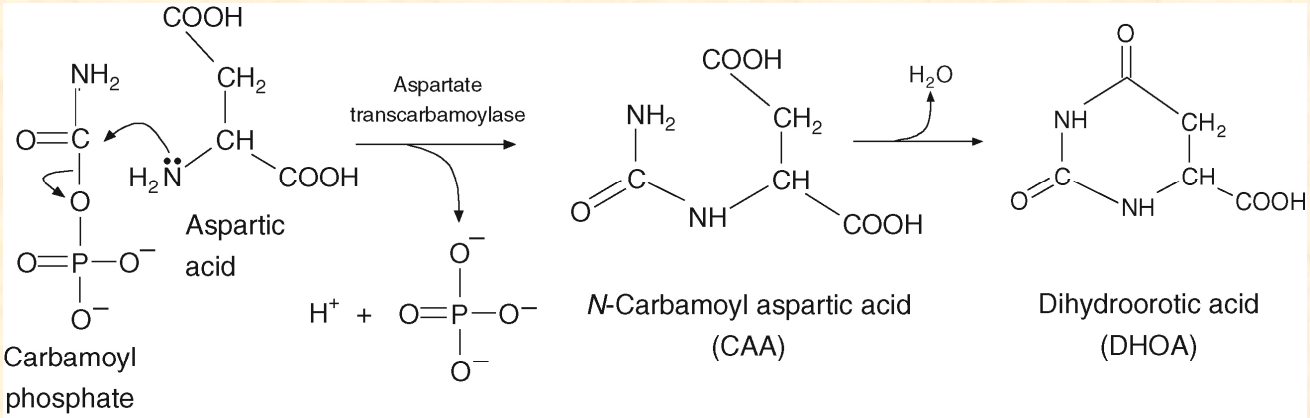


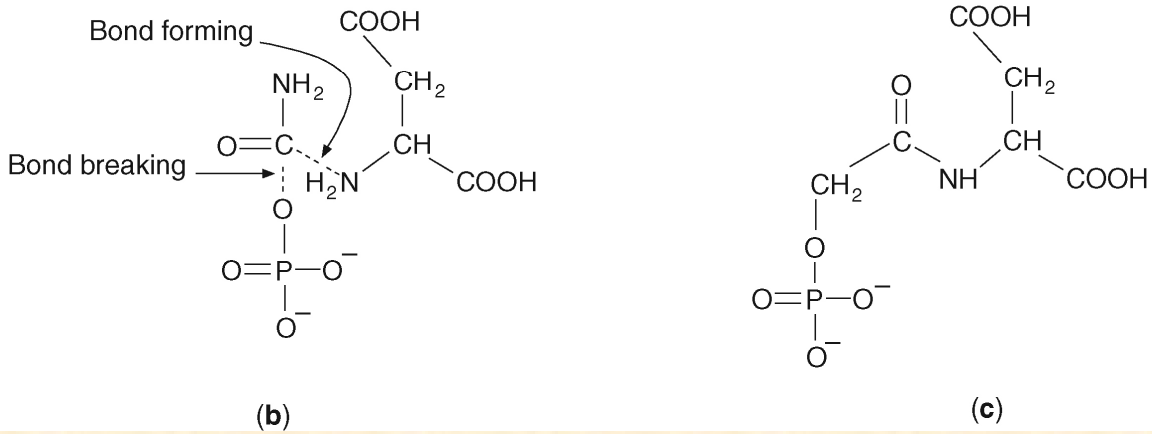
Enzyme Inhibitors

- Transition state inhibitors









N-phosphoacetyl-L-aspartate
PALA

Enzyme Inhibitors

- Enzymes and drug design

  - preventing or regulating cell growth

Advantage:

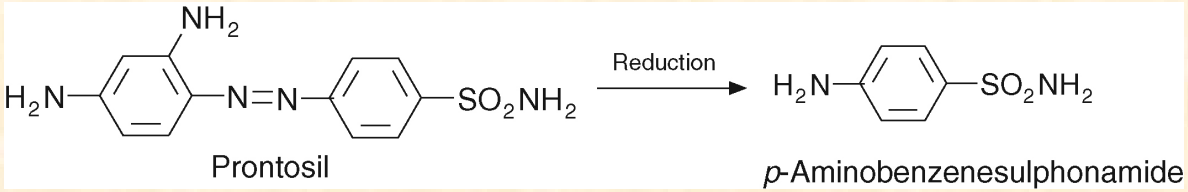
  - diversity of enzymes (activity in pathogens vs humans

Disadvantage:

  - specificity of the inhibitor
  - reversible vs. irreversible

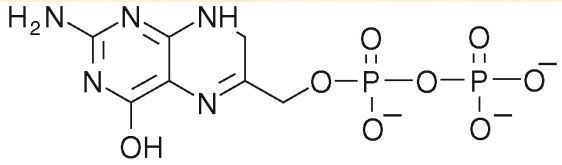
Examples of Enzyme Inhibitors

- Sulfonamides (bacteriostatic agents)

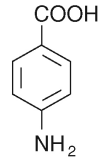


Prontosil

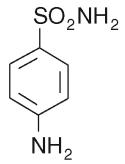
p-Aminobenzenesulphonamide



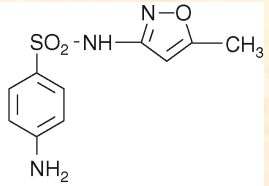
2-Amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine diphosphate



Para-aminobenzoic acid (PABA)



p-Aminobenzenesulphonamide



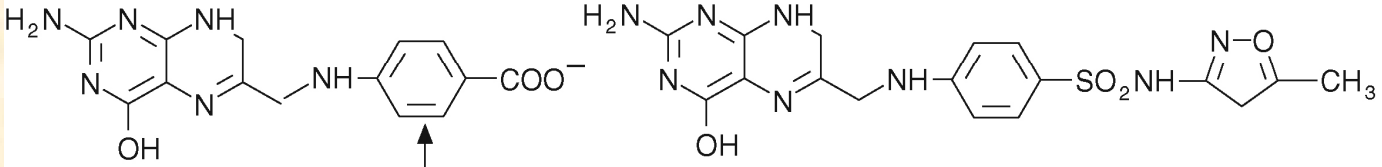
Sulphamethoxazole

Normal metabolism route

Dihydropteroate synthetase
PABA

Sulphonamide inhibition route

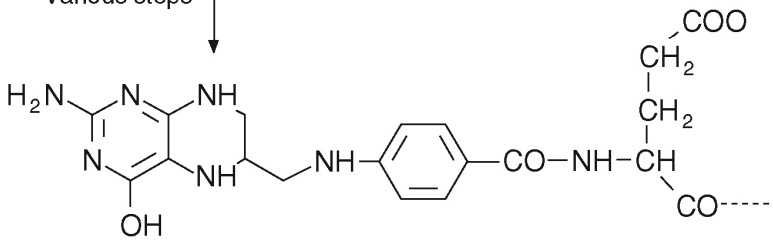
Treatment with sul-
phamethoxazole



Dihydrofolate

PABA residue

Various steps



Tetrahydrofolate (THF)

Cannot react to form the peptide chain since no suitable functional groups are present

Up to seven glutamate residues can be incorporated into the peptide chain

Examples of Enzyme Inhibitors

- Angiotensin inhibitors (captoril and related drugs)

Human angiotensinogen —Renin→ **Asp-Arg-Val-Try-Ile-His-Pro-Phe-His-Leu**
Angiotensin I

Angiotensin-converting enzyme (ACE)

Asp-Arg-Val-Try-Ile-His-Pro-Phe + His-Leu
Angiotensin II

Aminopeptidase / angiotensinases

Asp + Arg-Val-Try-Ile-His-Pro-Phe
Angiotensin III

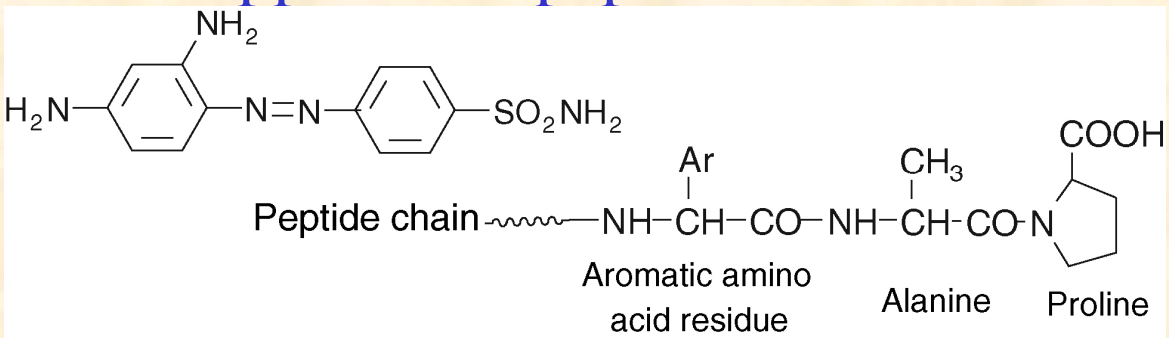
Inactive metabolites

inactive

vaso-constrictor

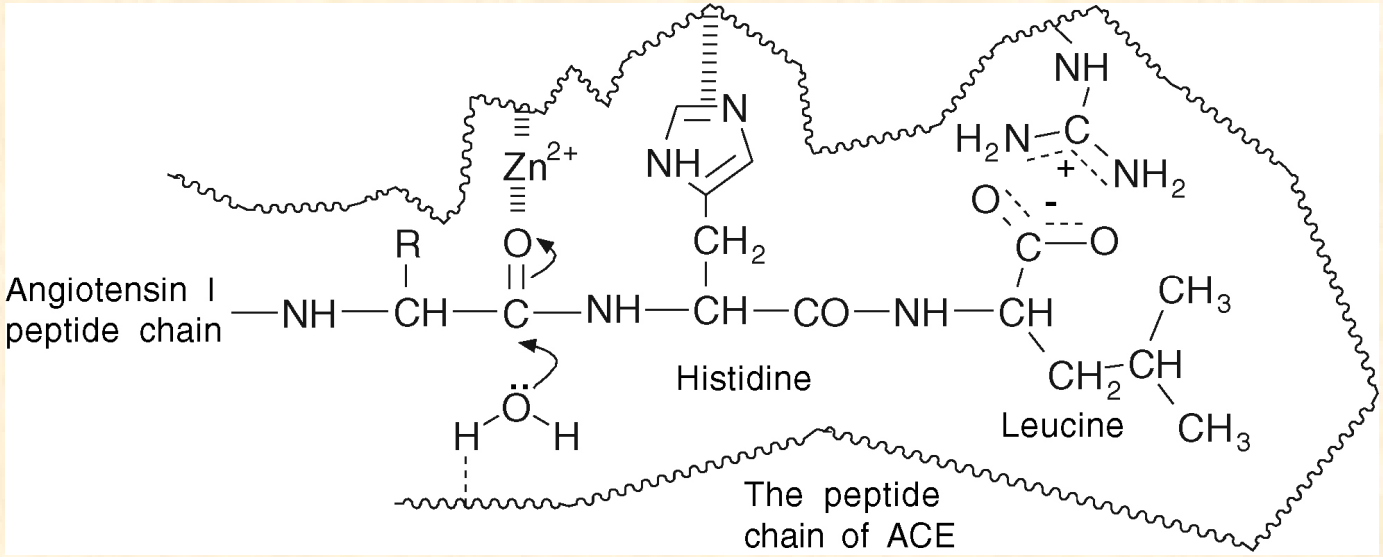
Na+ release from kidney

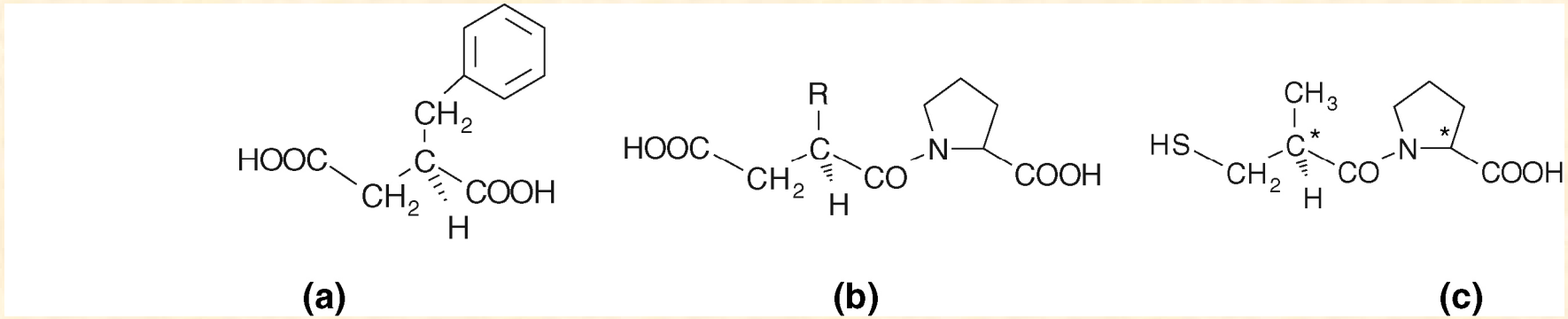
initial approach – peptide inhibitors



Examples of Enzyme Inhibitors

- Angiotensin inhibitors (captoril and related drugs)





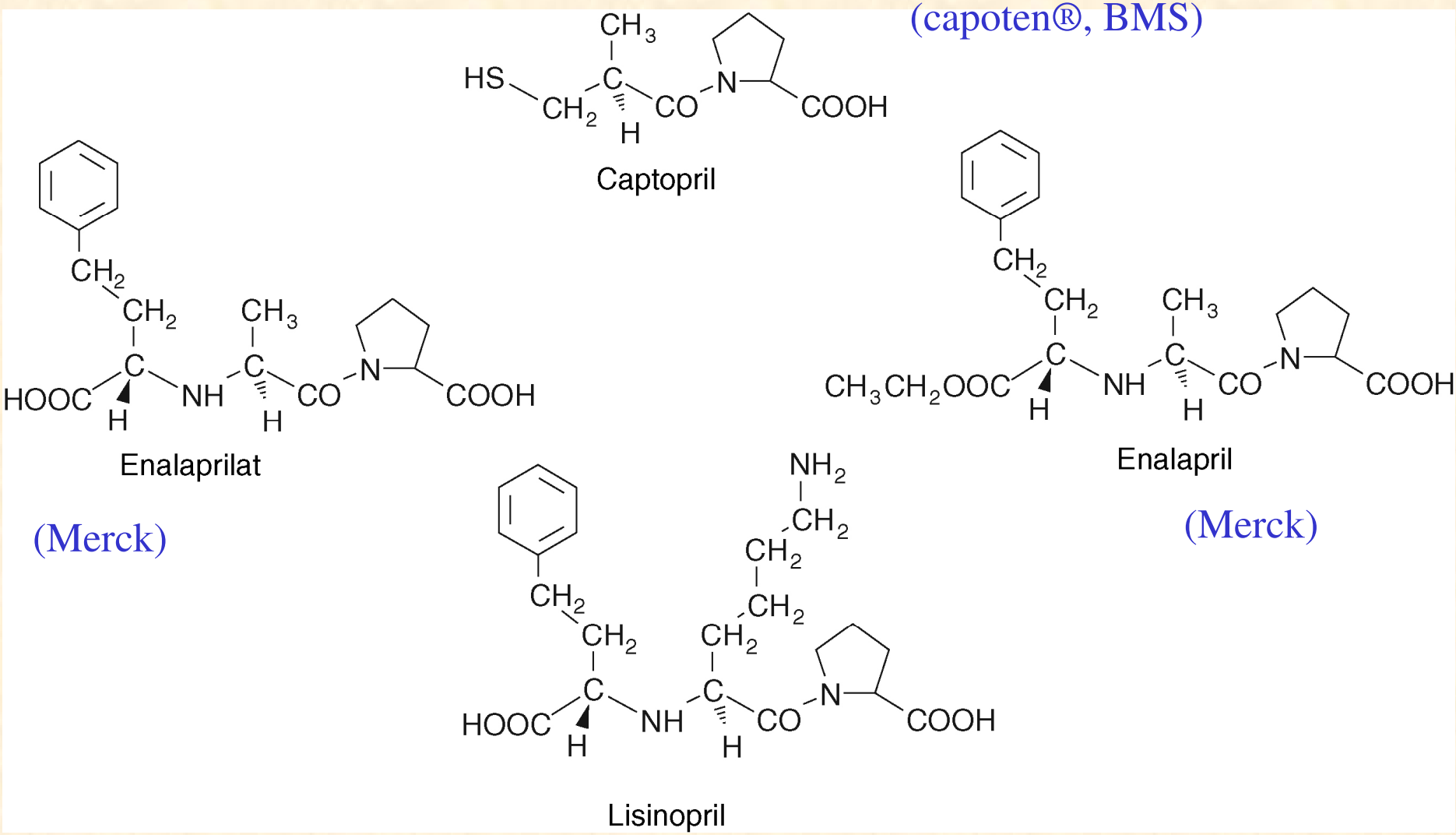
(*R*)-benzylsuccinic acid

Carboxyacylproline Derivatives (ACE inh.)

Captopril (capoten®, BMS)

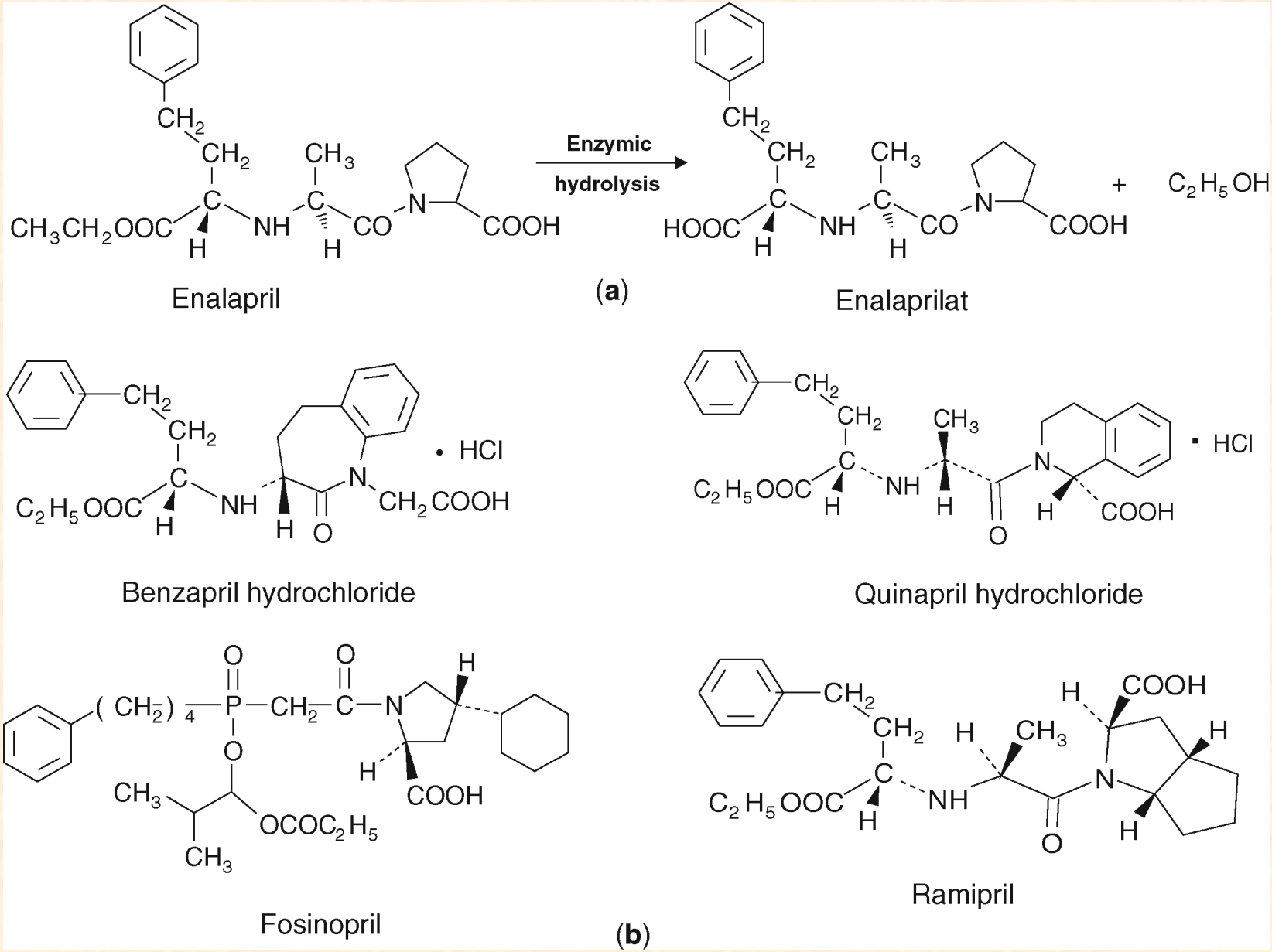
Examples of Enzyme Inhibitors

- Angiotensin inhibitors (captoril and related drugs)



Examples of Enzyme Inhibitors

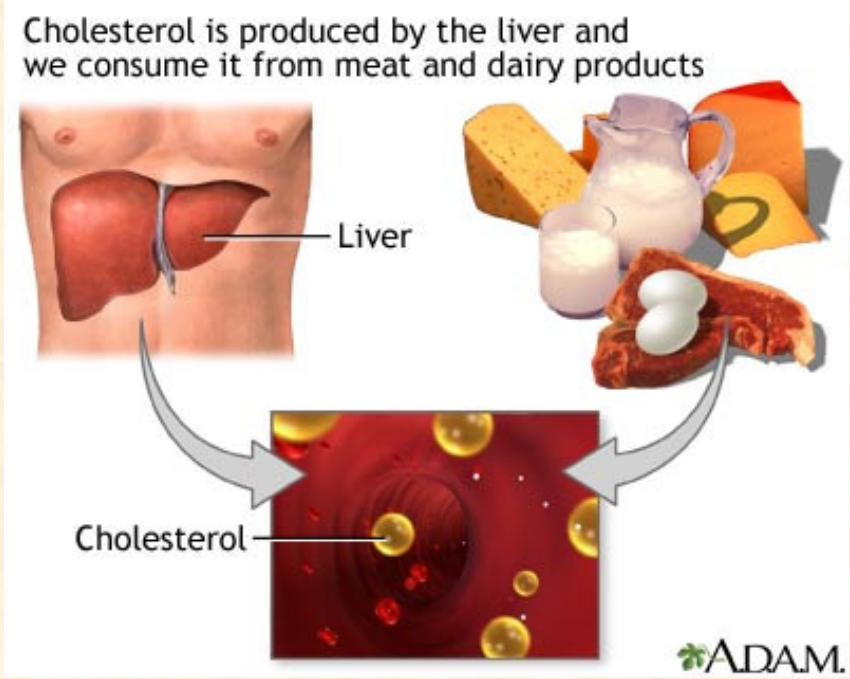
- Angiotensin inhibitors (captoril and related drugs)



Examples of Enzyme Inhibitors

- Cardiovascular diseases – Cholesterol problem

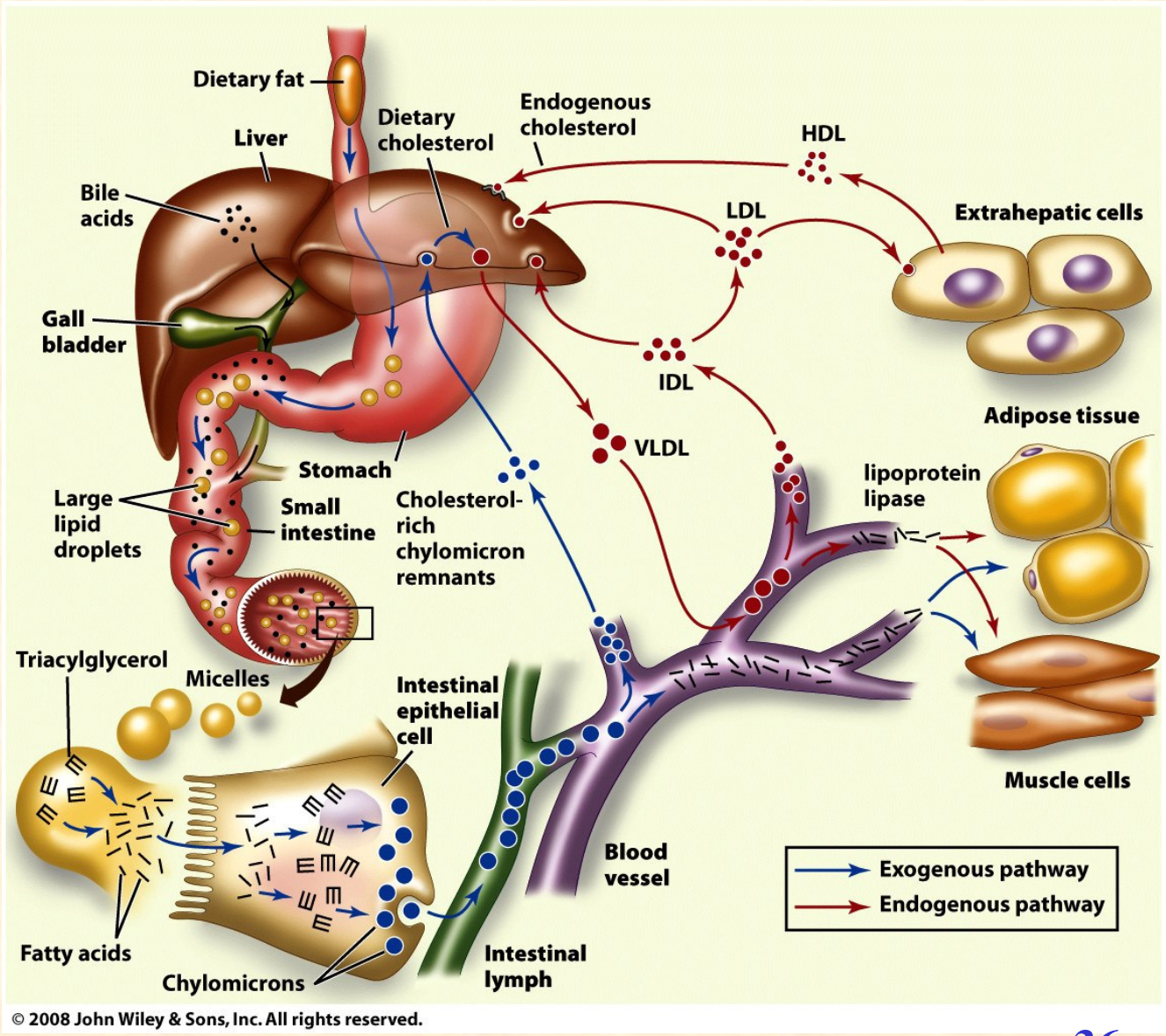
sources of cholesterol



Examples of Enzyme Inhibitors

- Cardiovascular diseases – Cholesterol problem

pathways of cholesterol



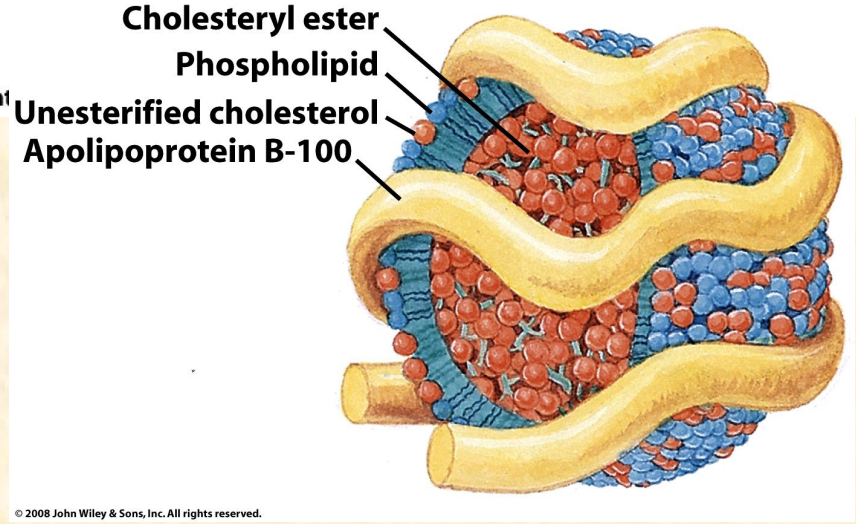
Examples of Enzyme Inhibitors

- Lipoproteins

Table 20-1 Characteristics of the Major Classes of Lipoproteins in Human Plasma

	Chylomicrons	VLDL	IDL	LDL	HDL
Density ($g \cdot cm^{-3}$)	<0.95	<1.006	1.006–1.019	1.019–1.063	1.063–1.210
Particle diameter (Å)	750–12,000	300–800	250–350	180–250	50–120
Particle mass (kD)	400,000	10,000–80,000	5000–10,000	2300	175–360
% Protein[a]	1.5–2.5	5–10	15–20	20–25	40–55
% Phospholipids[a]	7–9	15–20	22	15–20	20–35
% Free cholesterol[a]	1–3	5–10	8	7–10	3–4
% Triacylglycerols[b]	84–89	50–65	22	7–10	3–5
% Cholesteryl esters[b]	3–5	10–15	30	35–40	12
Major apolipoproteins	A-I, A-II, B-48, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100	A-I, A-II, C-I, C-II, C-III, D, E

[a]Surface components
[b]Core lipids.



Examples of Enzyme Inhibitors

- Solutions for the cholesterol problem

  - cholesterol uptake inhibitors

    ion-exchange resins – bile salts – excretion

  - cholesterol synthesis inhibitors

  - both

Examples of Enzyme Inhibitors

- Statins – inhibition of cholesterol biosynthesis

Konrad Bloch

Nobel Prize in Medicine 1964

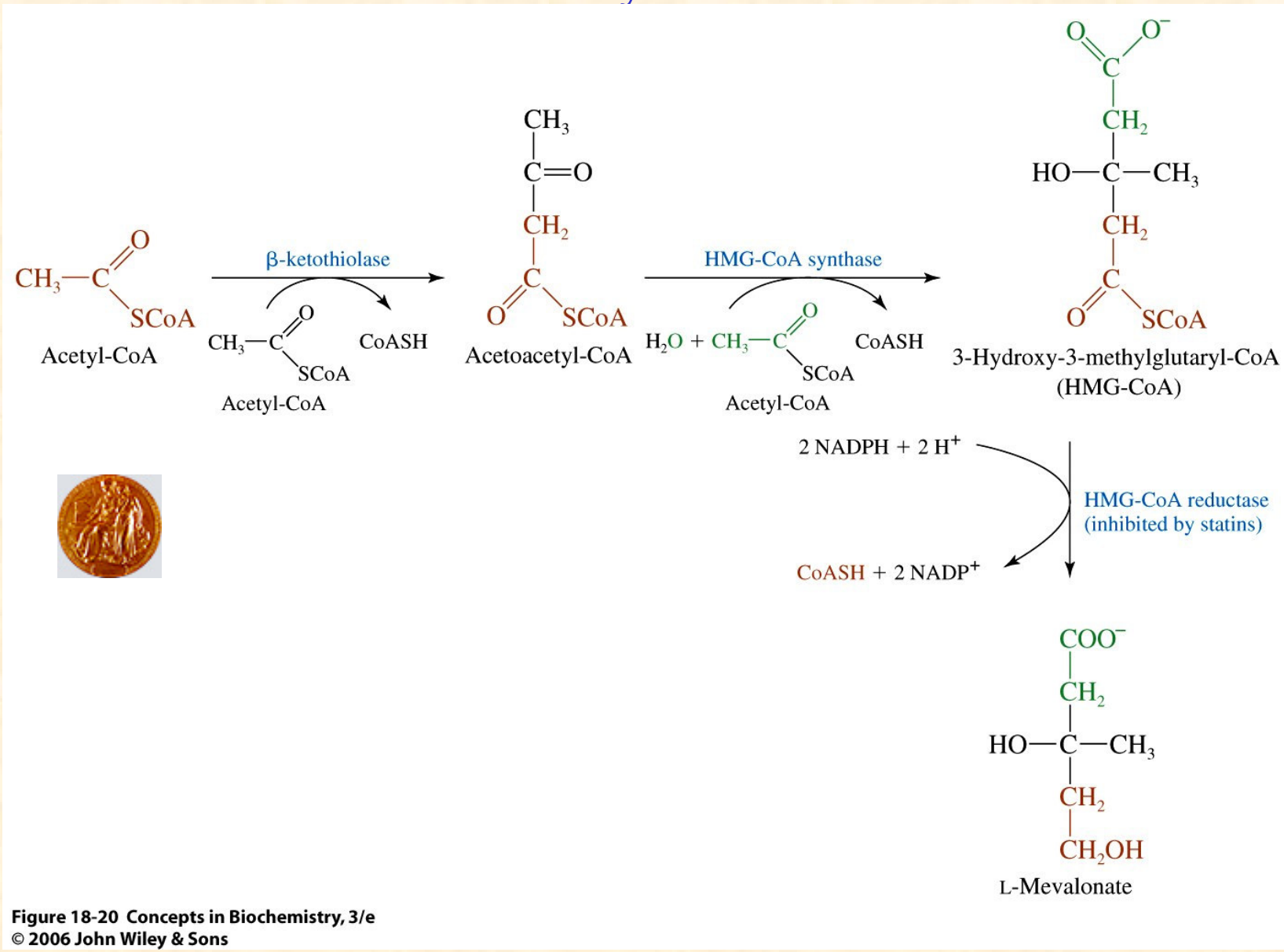
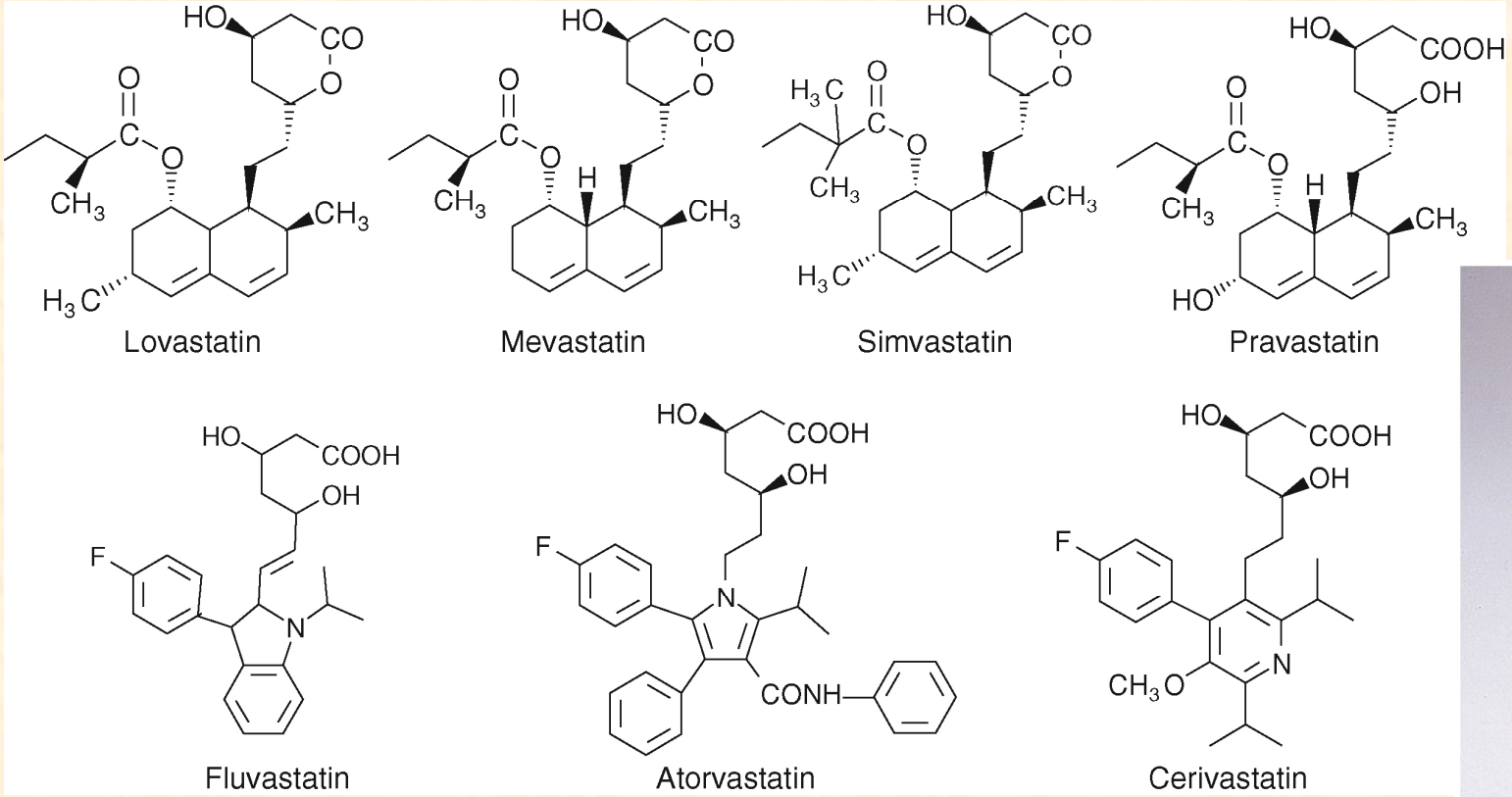
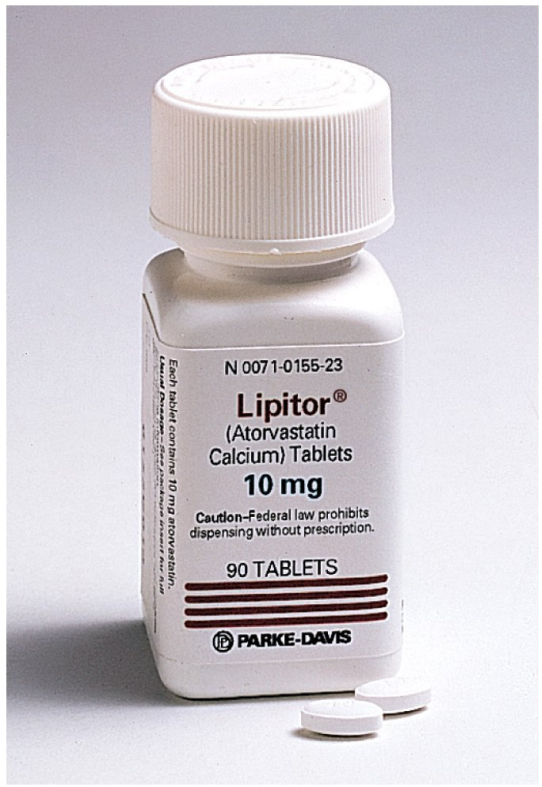


Figure 18-20 Concepts in Biochemistry, 3/e

Examples of Enzyme Inhibitors

- Statins





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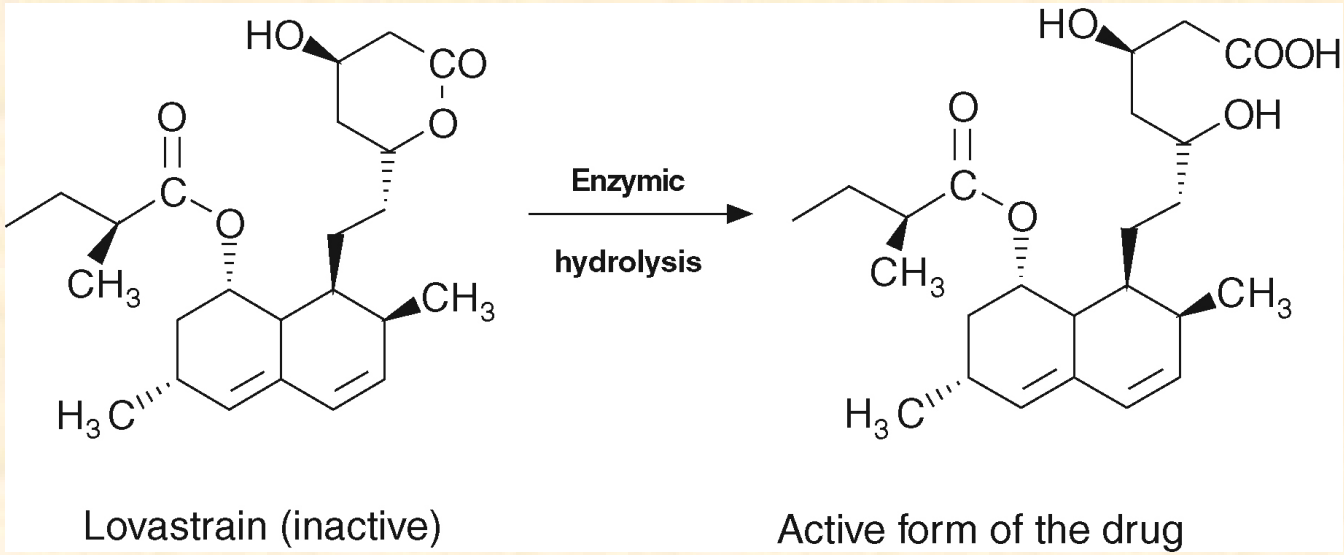
Examples of Enzyme Inhibitors

- Statins



Mevacor

Enzymes and Drug Resistance

- Changes in enzyme concentration

  - excess enzyme is produced
  - increased production of metabolic enzymes

- An increase in the production of the substrate

- Changes in the structure of the enzyme

- Alternative metabolic pathway