Enzymes are Subject to Inhibition

(Enzyme Inhibition)

1. **Pharmaceutical Importance of Enzyme Inhibition Study**

   Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reaction…… the most important pharmaceutical agents known.

   Example : Aspirin → prostaglandin inhibitor → Antiinflamation

   Anticancer agents → DNA, RNA, protein synthesis inhibitor (enzyme inhibitor)

2. **Classification**

   1) **Reversible Inhibition : Competitive Inhibition**

      Uncompetitive Inhibition

      Mixed Inhibition

   2) **Irreversible Inhibition**

Enzyme Inhibition

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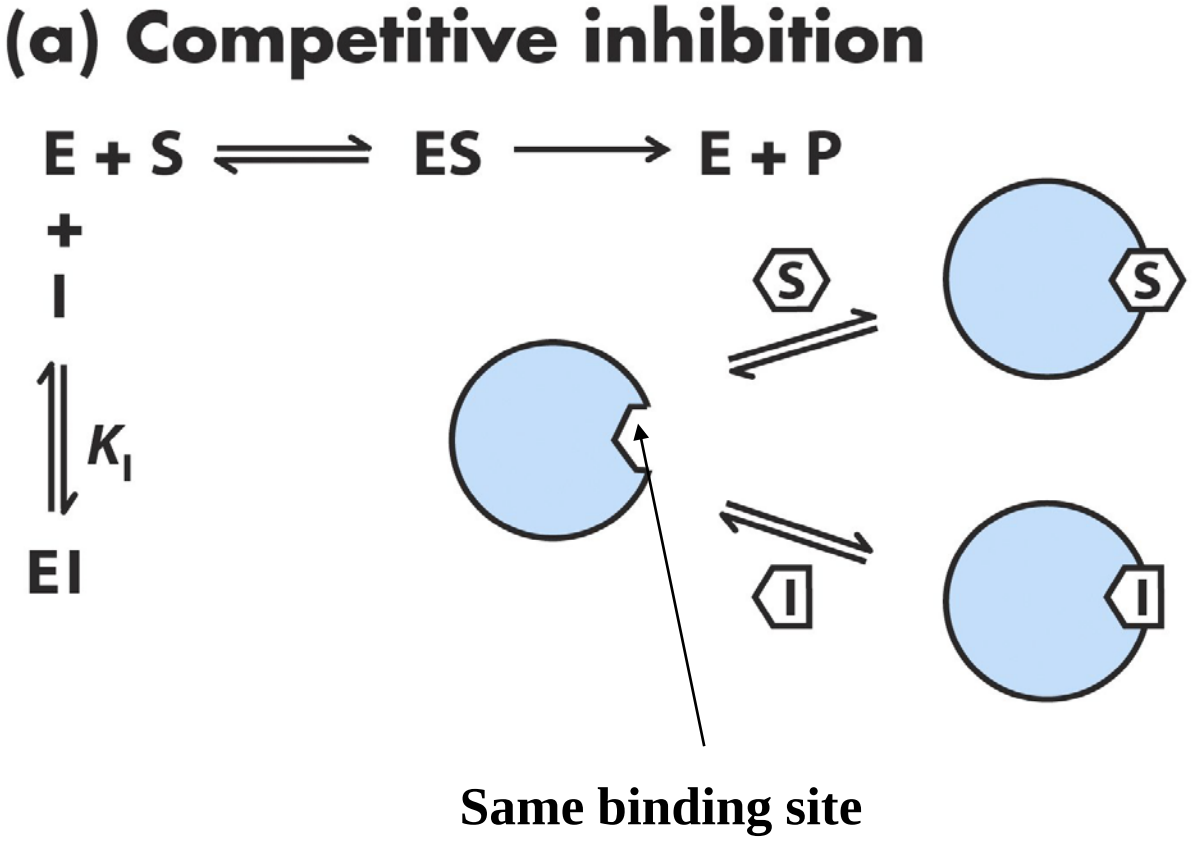
To describe inhibition: K_i

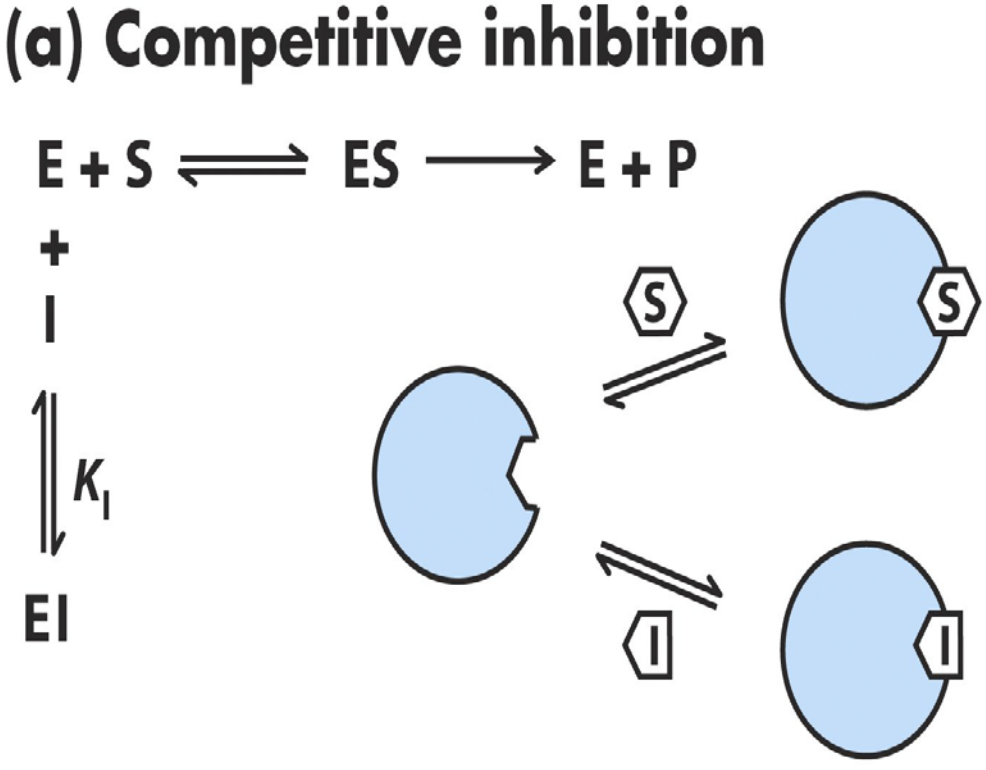
- Definition: K_i (analogous to K_m) is the concentration of inhibitor at which it occupies half of the sites on the enzyme
- It is conceptually equivalent to the *dissociation constant* of the enzyme, or enzyme-substrate complex, and the inhibitor
- Measure K_m and V_{max} in presence of I, get "apparent" values…

A. Reversible Inhibition



Competitive inhibition

- I binds to the same site as S, but can be competed away by adding more substrate, so
  - V_{max} is not affected
  - Apparent K_m (αK_m) will increase with inhibitor concentration.



$$V_o = \frac{V_{max}[S]}{\alpha K_m + [S]} \qquad \alpha = 1 + [I]/K_i$$

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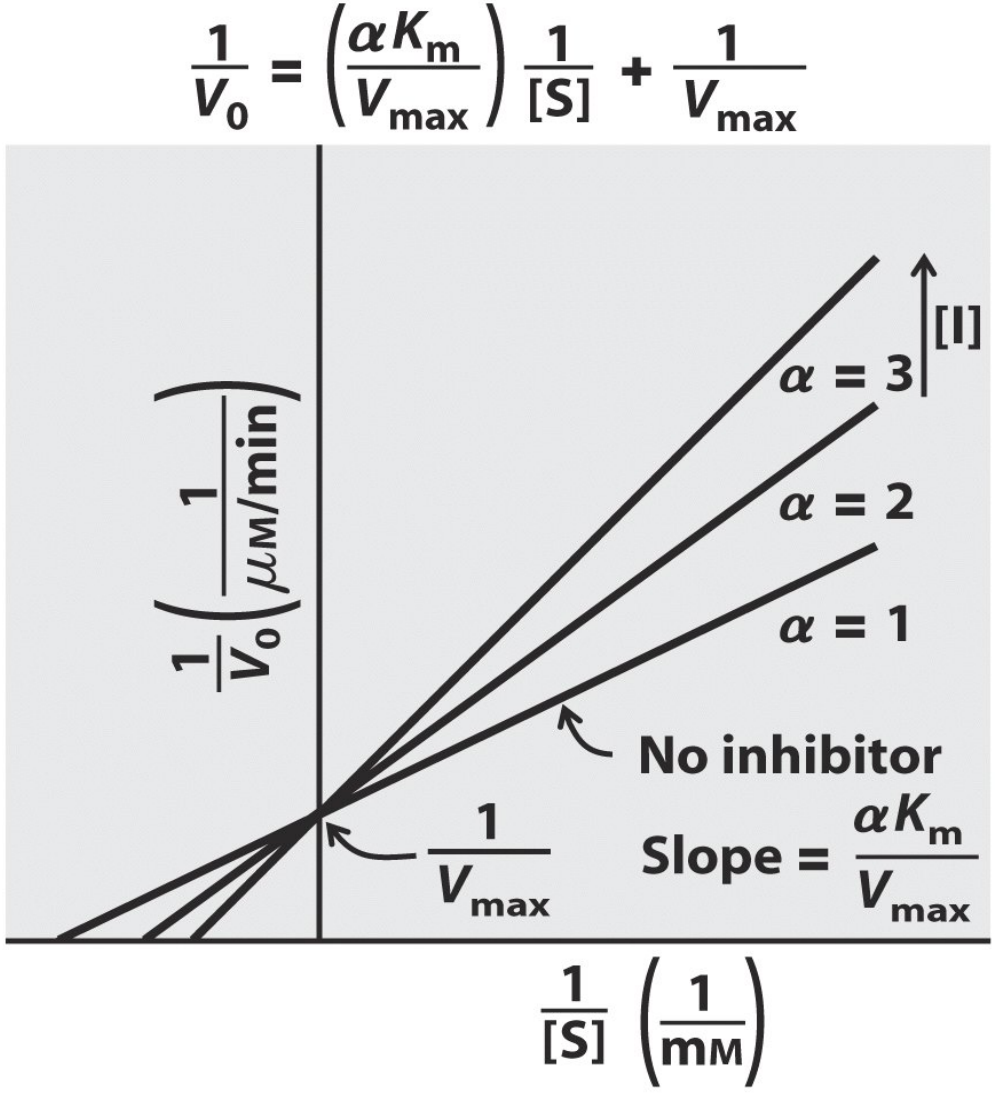
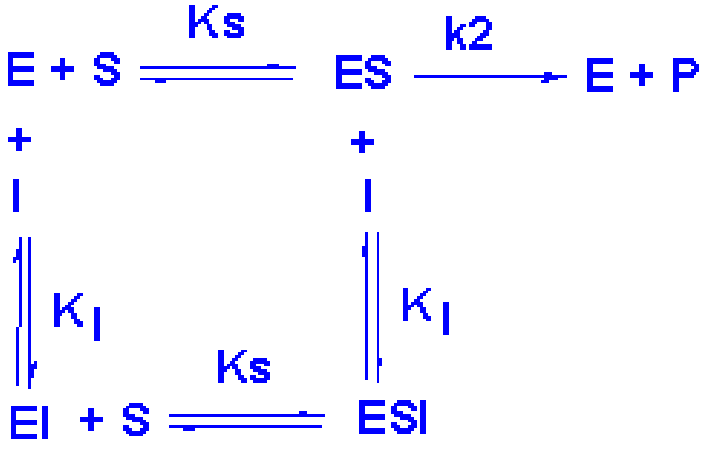


Noncompetitive inhibition

- Since noncompetitive inhibitors do not interfere in the binding of the substrate (the dissociation constant of ES and ESI have the same value Ks)
  - ➢ Km is not affected



- However, increasing [S] can not abolish the inhibition → (ESI) complex are formed and these are incapable of progressing to reaction products
- The effect of a noncompetitive inhibitor is to reduce [ES] that can advance to product
- Since Vmax = k_2[Et], and the concentration of competent Et is diminished by the amount of ESI formed
  - ➢ Vmax is decreased

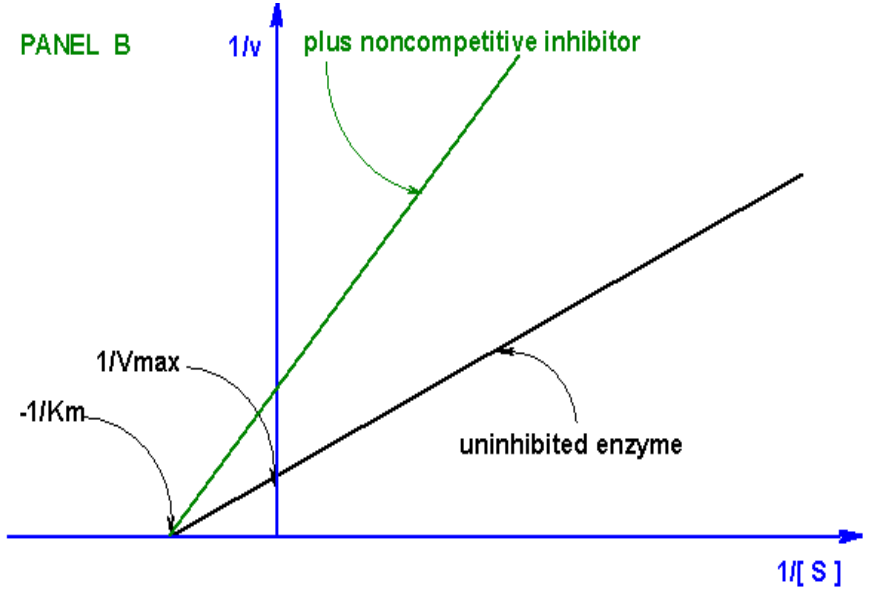
Noncompetitive inhibition

$$v = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]_0}{K_i}\right)+[S]\left(1+\frac{[I]_0}{K_i'}\right)}$$

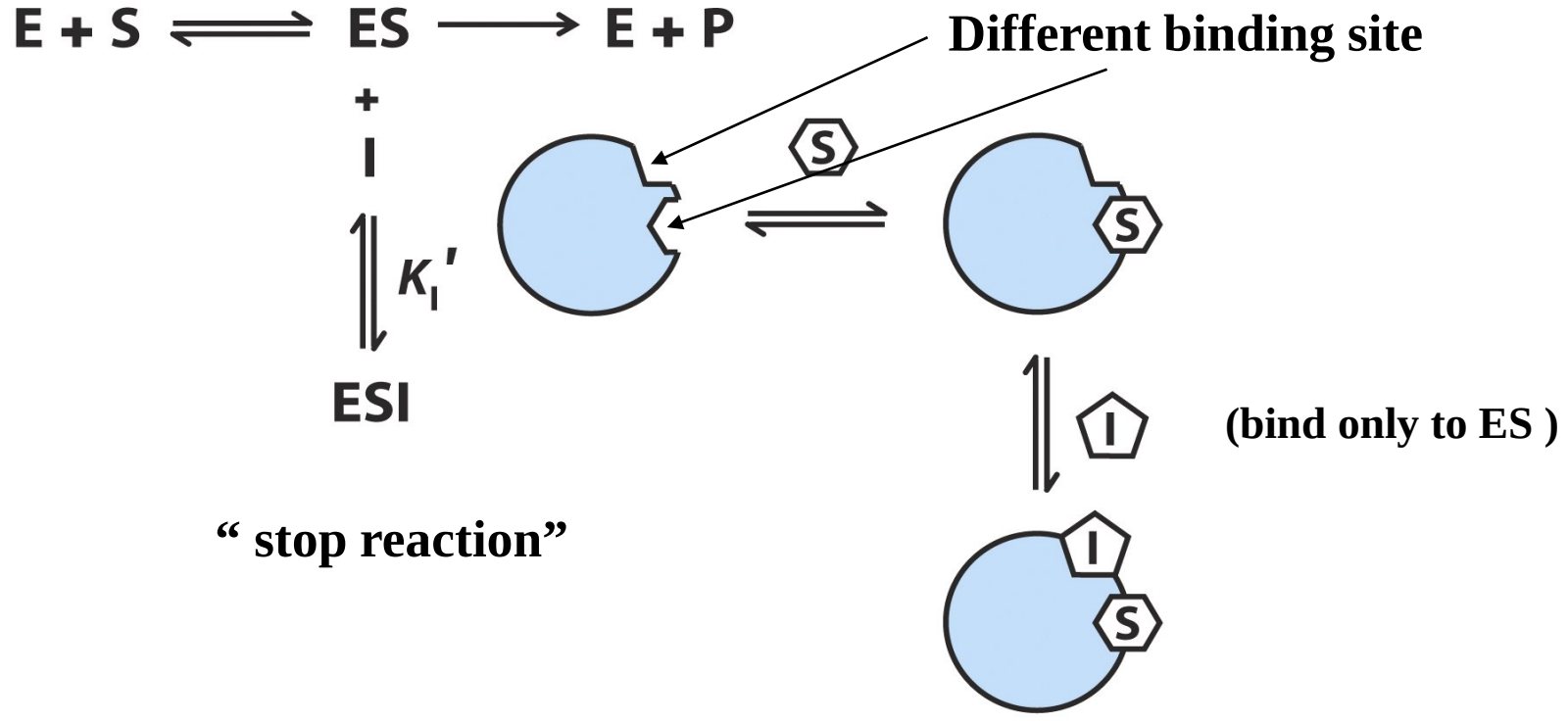
When Ki ≈ Ki'

$$v = \frac{V_{max}[S]}{([S]+K_m)\left(1+\frac{[I]}{K_i}\right)}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]}\left(1+\frac{[I]}{K_i}\right)+\frac{1}{V_{max}}\left(1+\frac{[I]}{K_i}\right)$$



(b) Uncompetitive inhibition

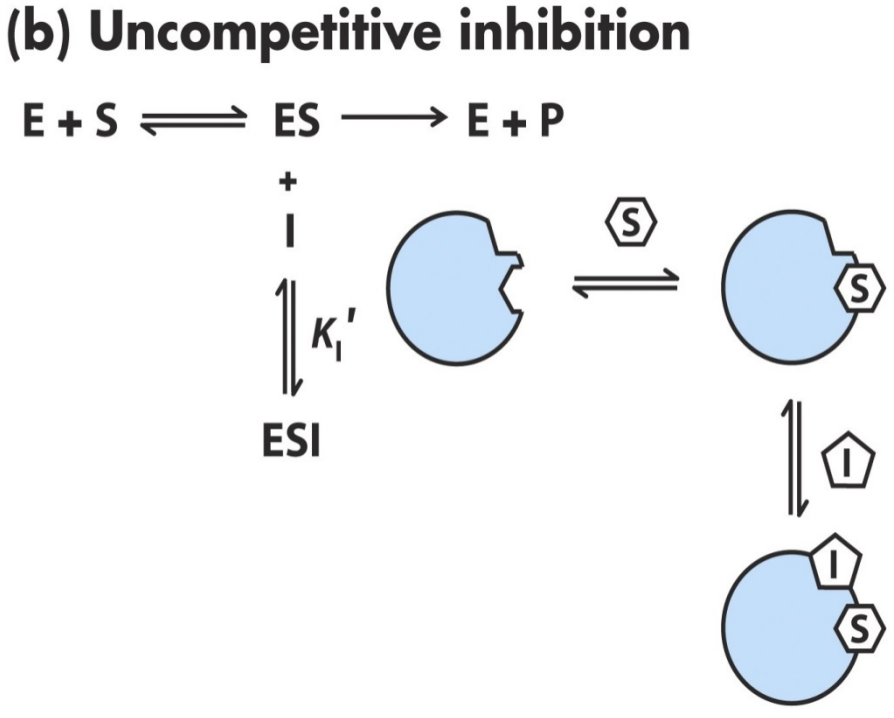


“ stop reaction”

Uncompetitive inhibition

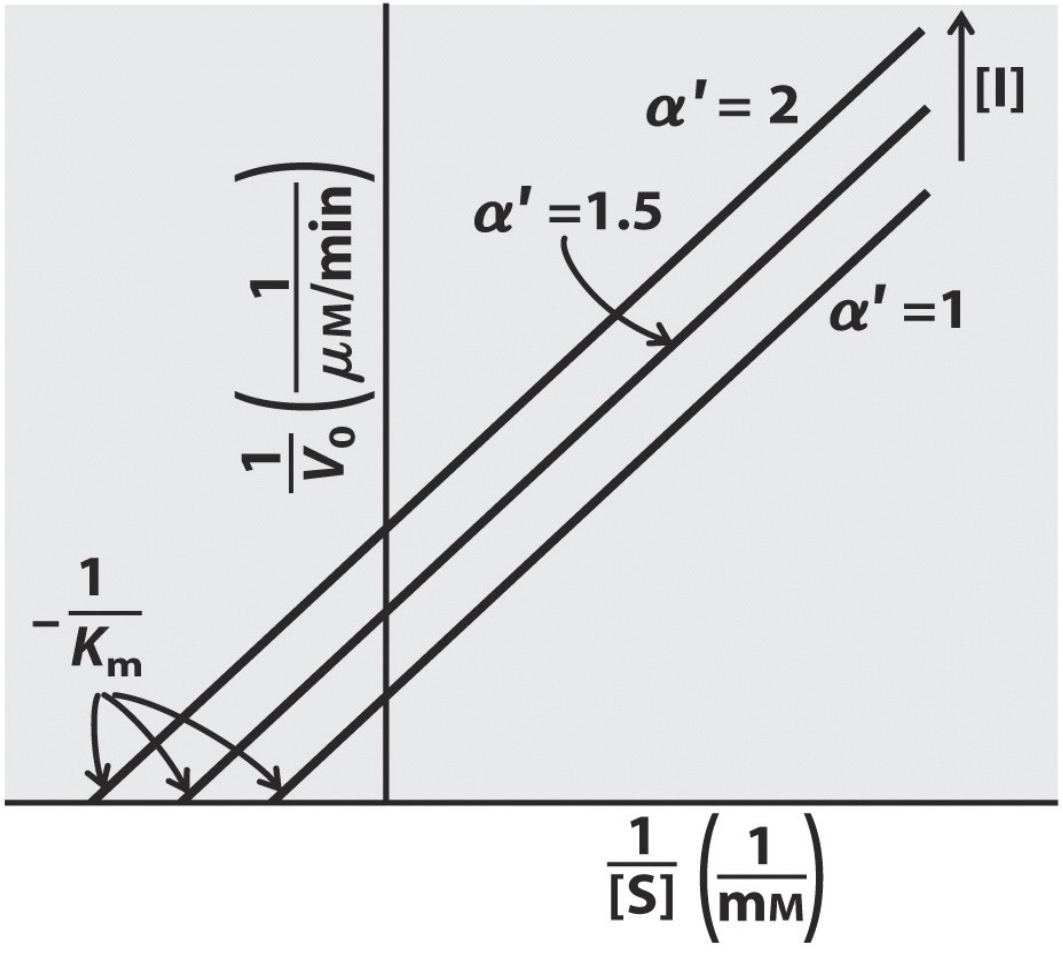
Inhibitor binds only to ES complex - increased [S] cannot overcome it and since I removes some fraction of ES from participation,

- V_{max} is decreased, and
- K_m is proportionally decreased
- K_i is fixed



$$V_o = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



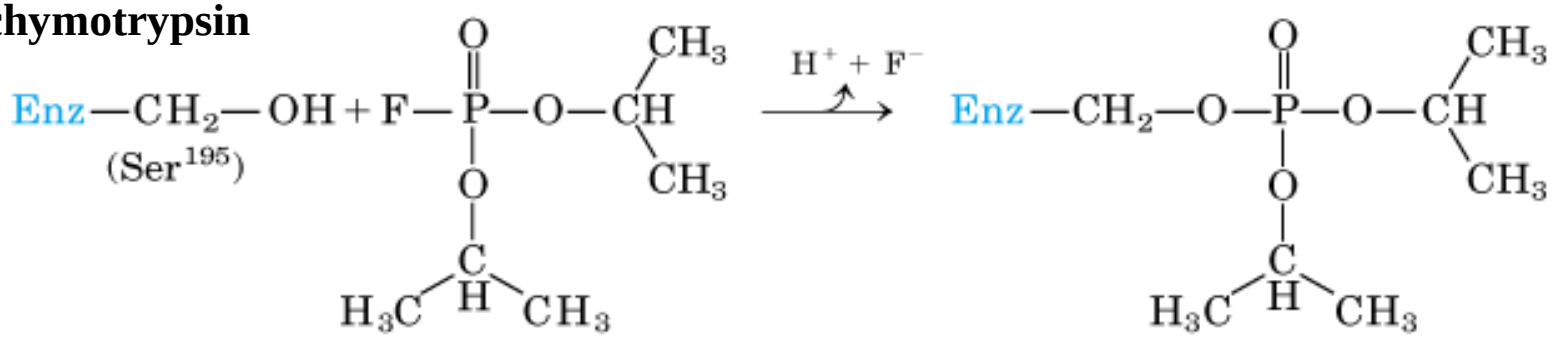
$$\alpha' = 1 + [I]/K_i'$$

B. Irreversible Inhibition

Irreversible inhibitors are those that combine with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or that form a particularly stable noncovalent association.

Permanent inactive form

chymotrypsin



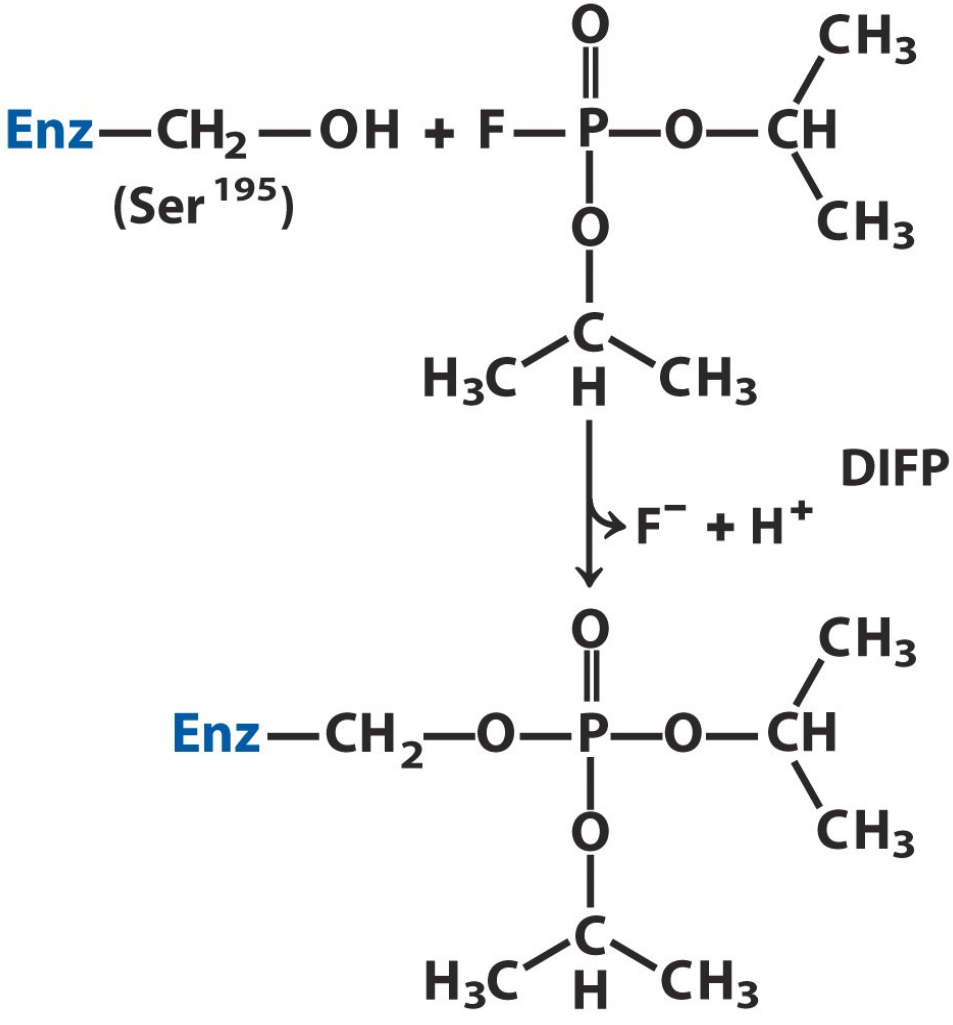
DIFP **Diisopropylfluorophosphate**

Irreversible Inhibitor(suicide inactivator)…… a modern approach to obtaining new pharmaceutical agents.

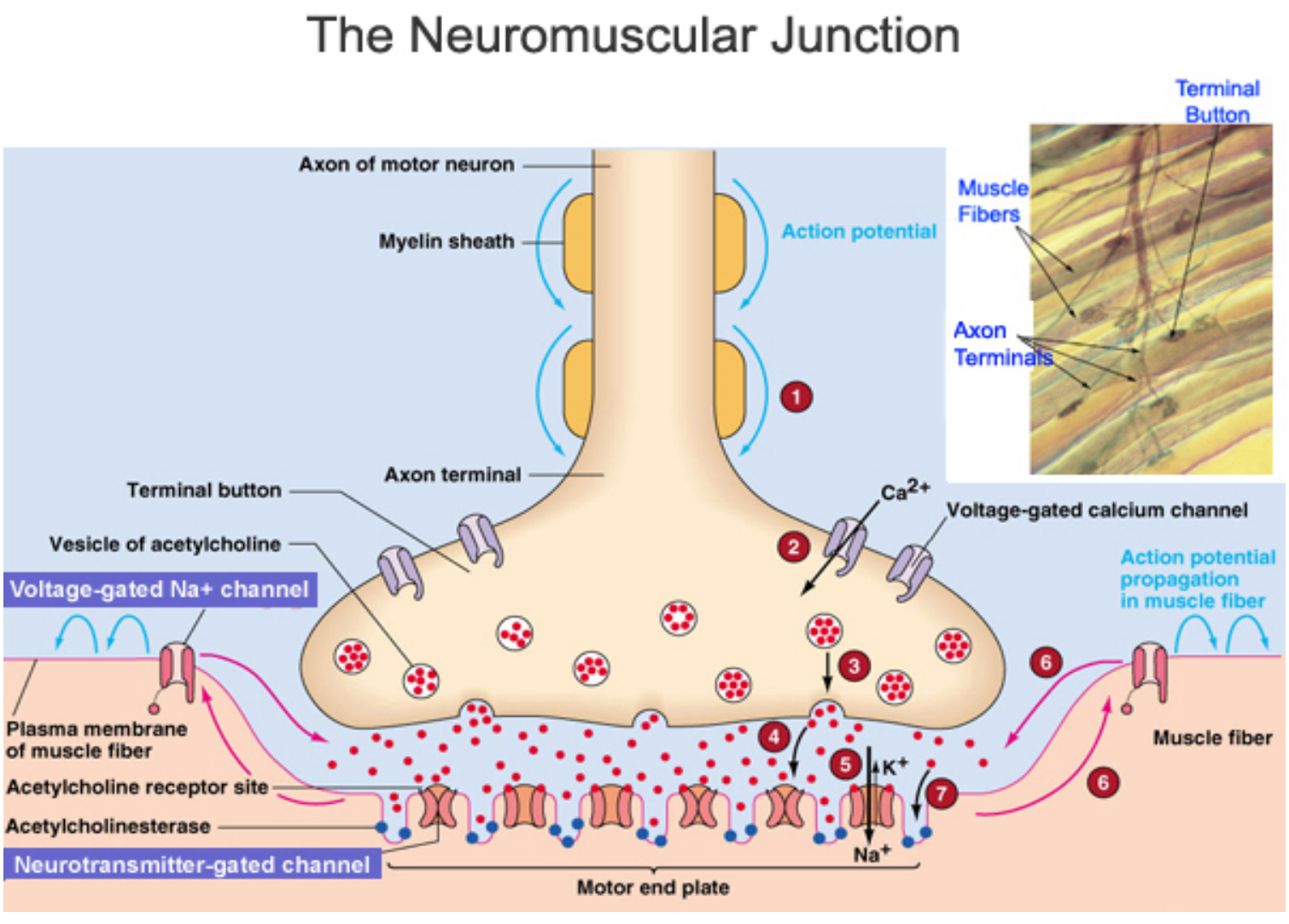
" New Drugs Design"

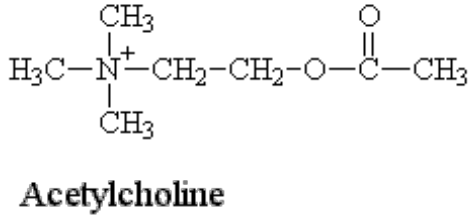
a suicide inhibitor

- Diisopropylfluorophosphate (DIFP) forms a covalent bond with an active -site residue (Ser) of the enzyme chymotrypsin.
- Every molecule that reacts is inactivated irreversibly.
- Here, a key active site Ser is bound irreversibly to the inhibitor, preventing it from doing its "normal" job.

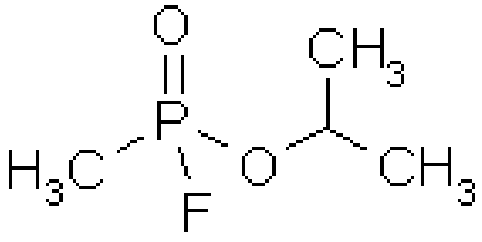


Irreversible Inhibition at the NM Junction

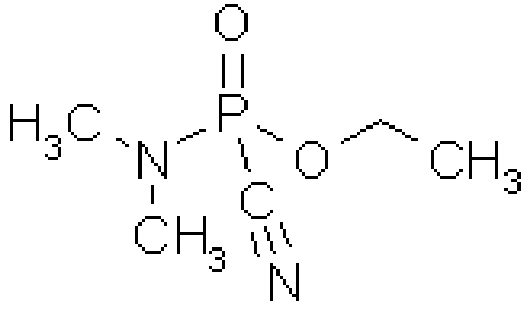




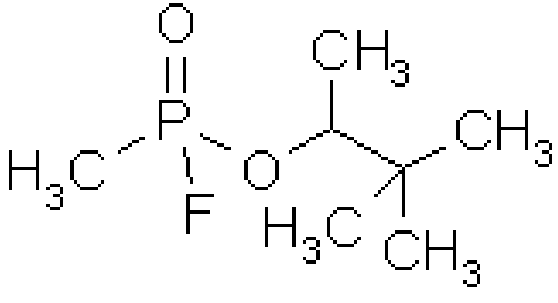
Nerve Gases



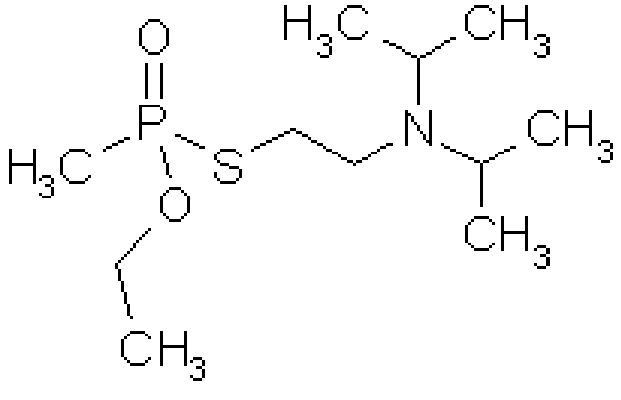
Sarin



Tabun



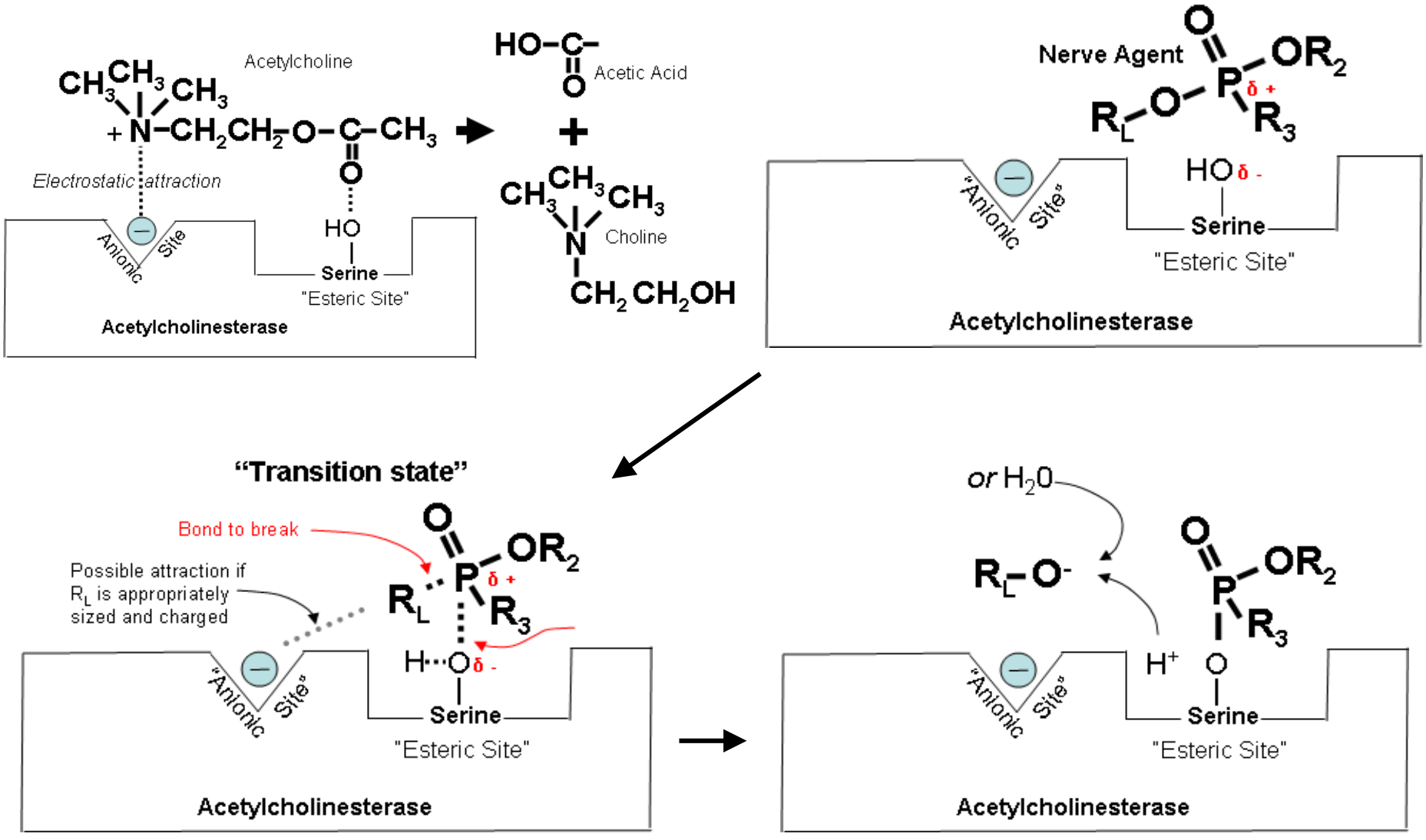
Soman



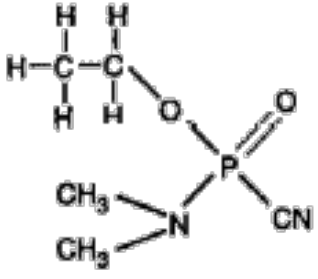
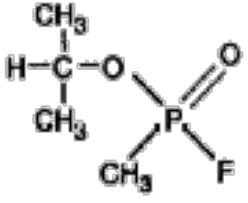
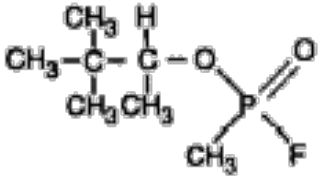
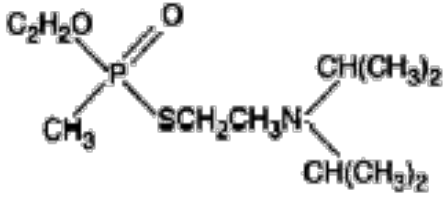
VX

Symptoms: Contraction of pupils, profuse salivation, convulsions, involuntary urination and defecation and eventual death by asphyxiation as control is lost over respiratory muscles.

Action of nerve agents



Nerve gases - properties

Property	Tabun (GA)	Sarin (GB)	Soman (GD)	VX
Appearance	Colourless to brown liquid giving off colourless vapour	Colourless liquid giving off a colorless vapour	Colourless liquid giving off a colourless vapour	Amber coloured liquid
Chemical formula				

Agent	***LD50***	***LCt50***
Tabun (GA)	1000 mg	400 mg/min-m^3
Sarin (GB)	1700 mg	100 mg/min-m^3
Soman (GD)	50 mg	70 mg/min-m^3
VX	10 mg	50 mg/min-m^3