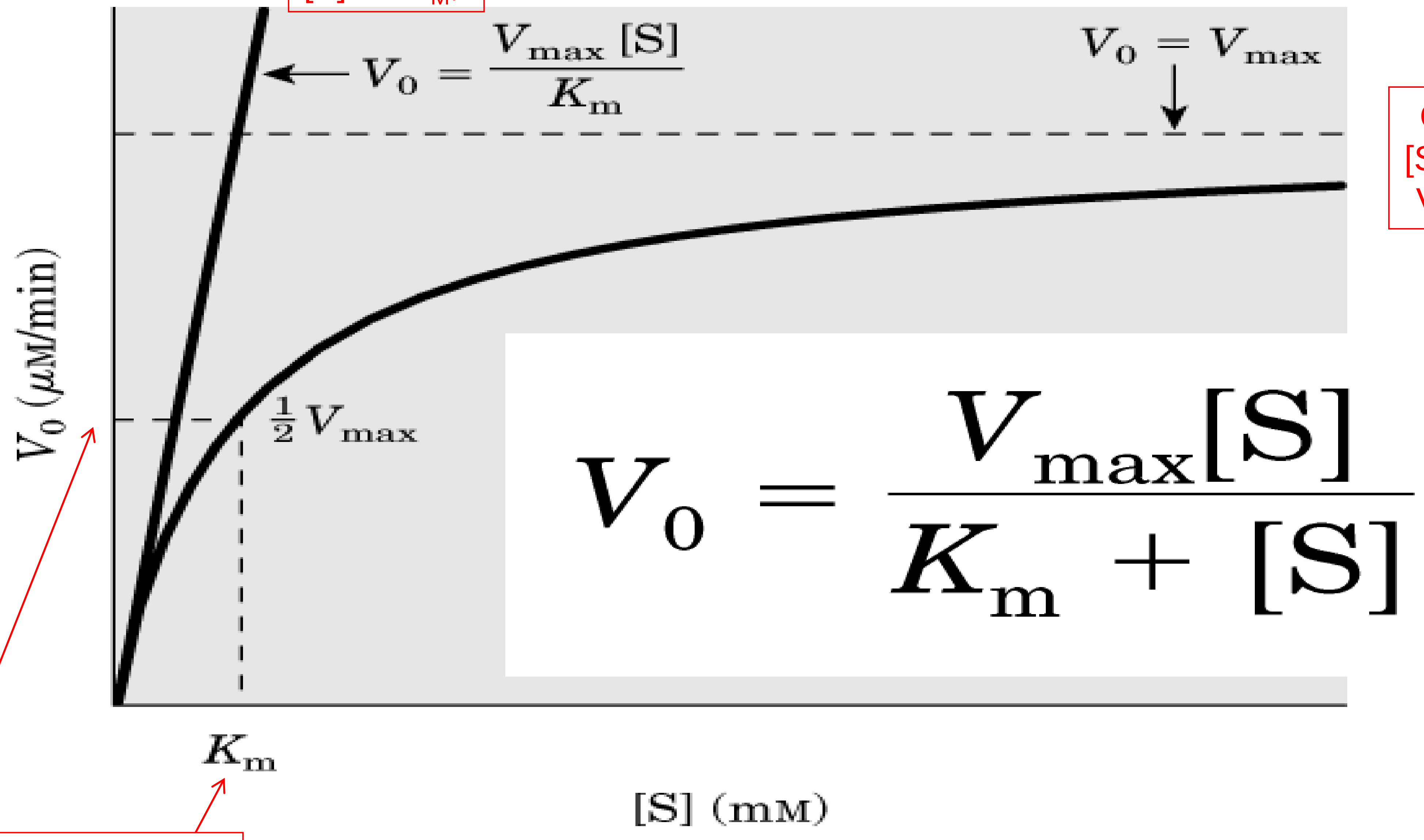
Quando
[S] << K_M,

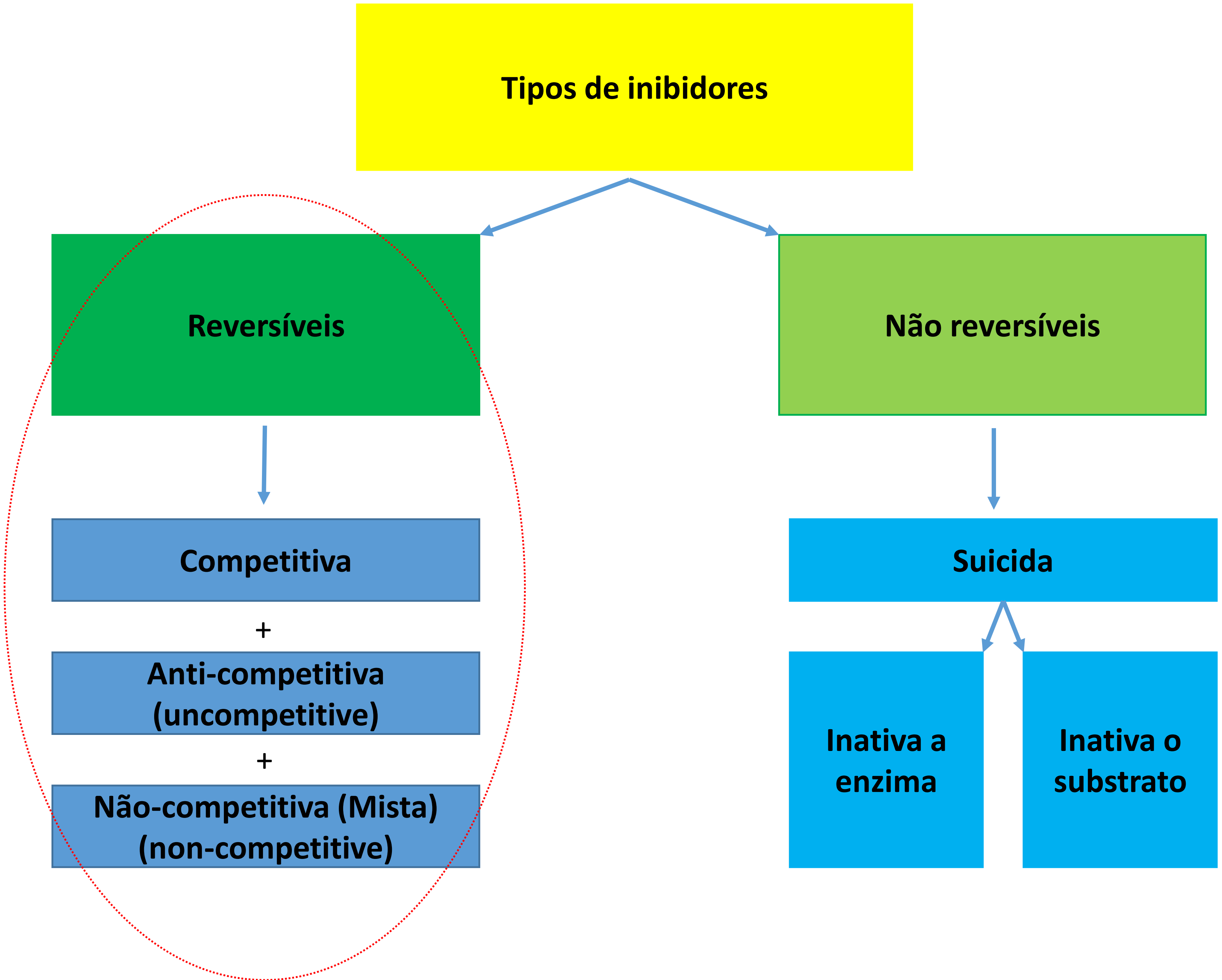


$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Quando
[S] >> K_M,
$V_o \sim V_{max}$

Quando [S] = K_M,
$V_o = V_{max}/2$

Mecanismos de Inibição de Enzimas


Tipos de inibidores
Reversíveis
Não reversíveis
Competitiva
+
Anti-competitiva
(uncompetitive)
+
Não-competitiva (Mista)
(non-competitive)
Suicida
Inativa a enzima
Inativa o substrato

INIBIÇÃO COMPETITIVA:
substrato e inibidor competem para o mesmo sítio

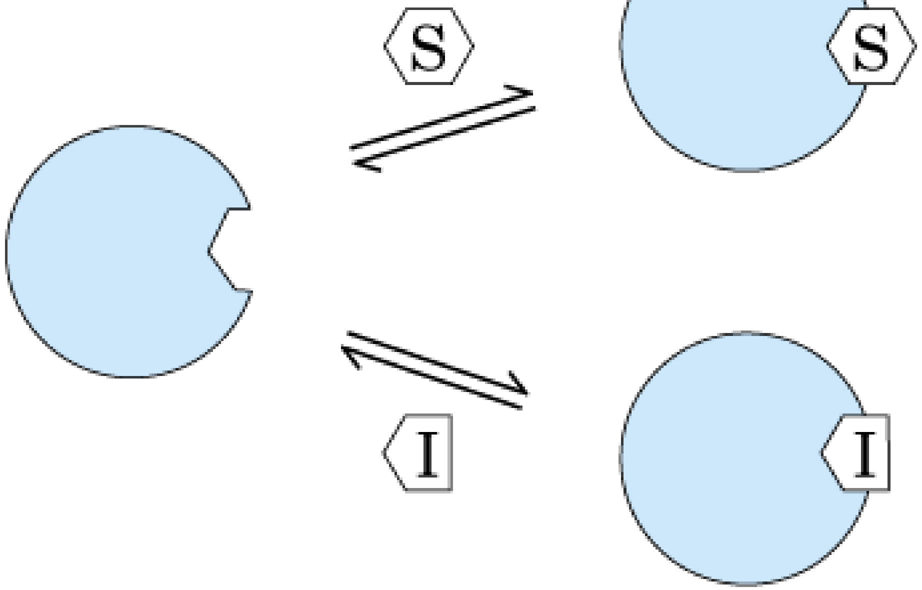
$$E + S \underset{Km}{\rightleftharpoons} ES \xrightarrow{kcat} E + P$$

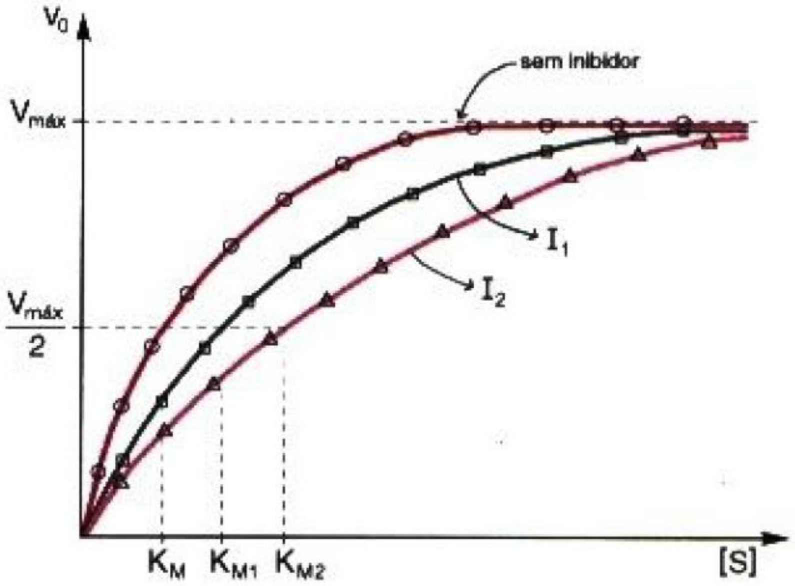
$$+$$

$$I$$

$$\Big\updownarrow K_I$$

$$EI$$

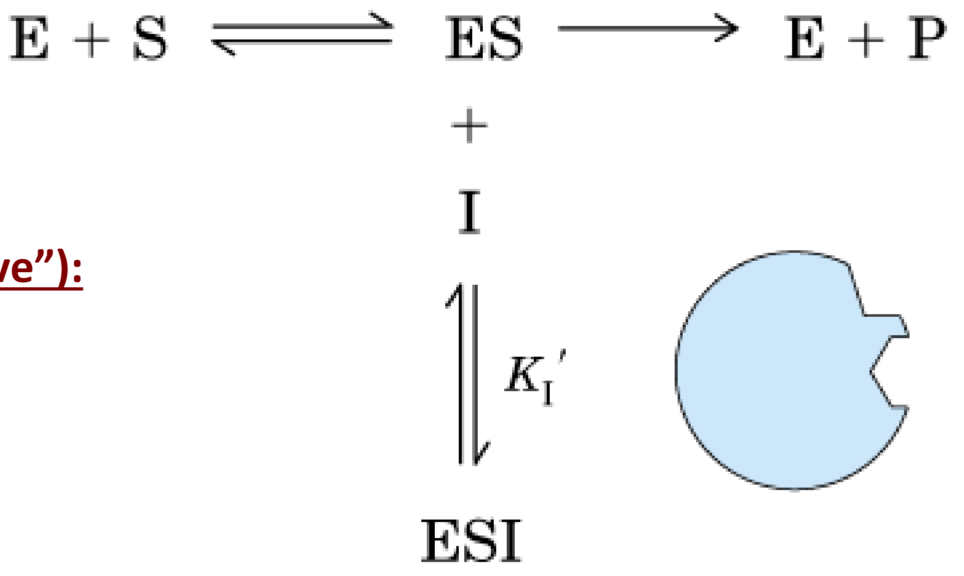




$$V_o = \frac{V_{max}[S]}{(\alpha K_m + [S])}$$

$$K_I = [E][I]/[EI]$$

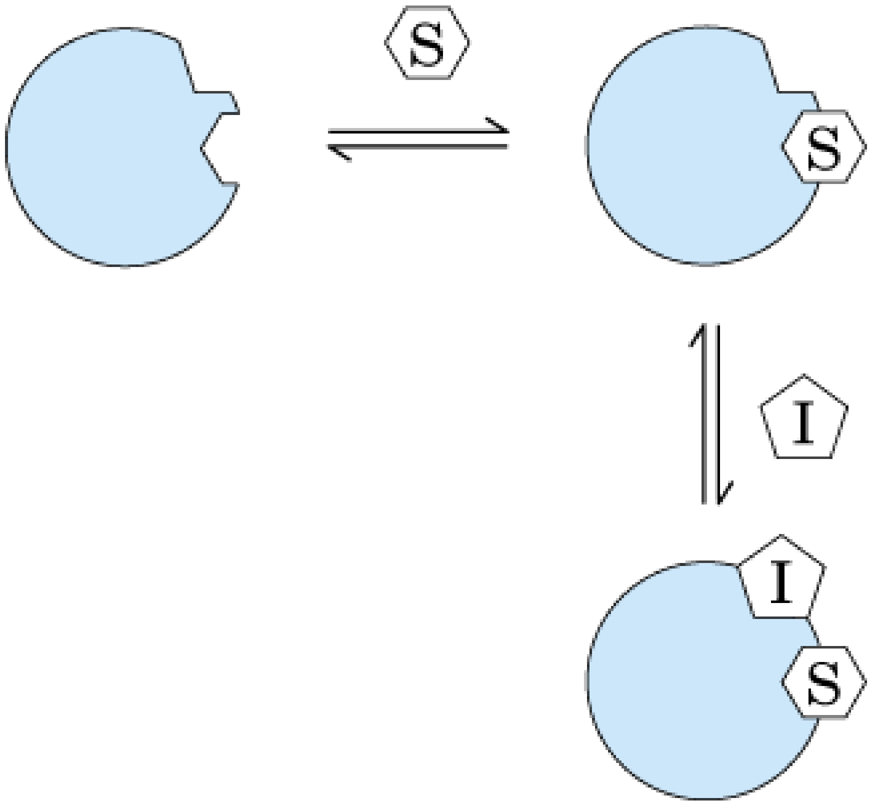
$$\alpha = 1 + [I]/K_I = 1 + [EI]/[E]$$



INIBIÇÃO ANTI-COMPETITIVA (ou "uncompetitive"):

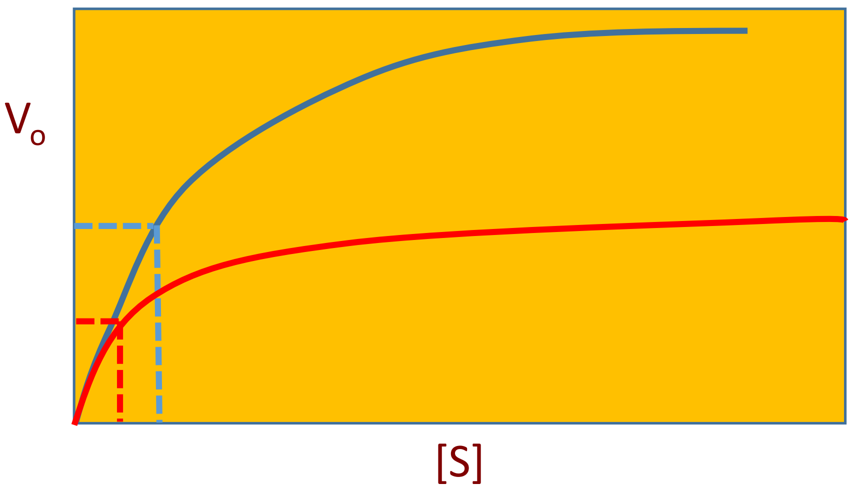
substrato e inibidor ligam em sítios diferentes;

inibidor somente liga ao complexo ES



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

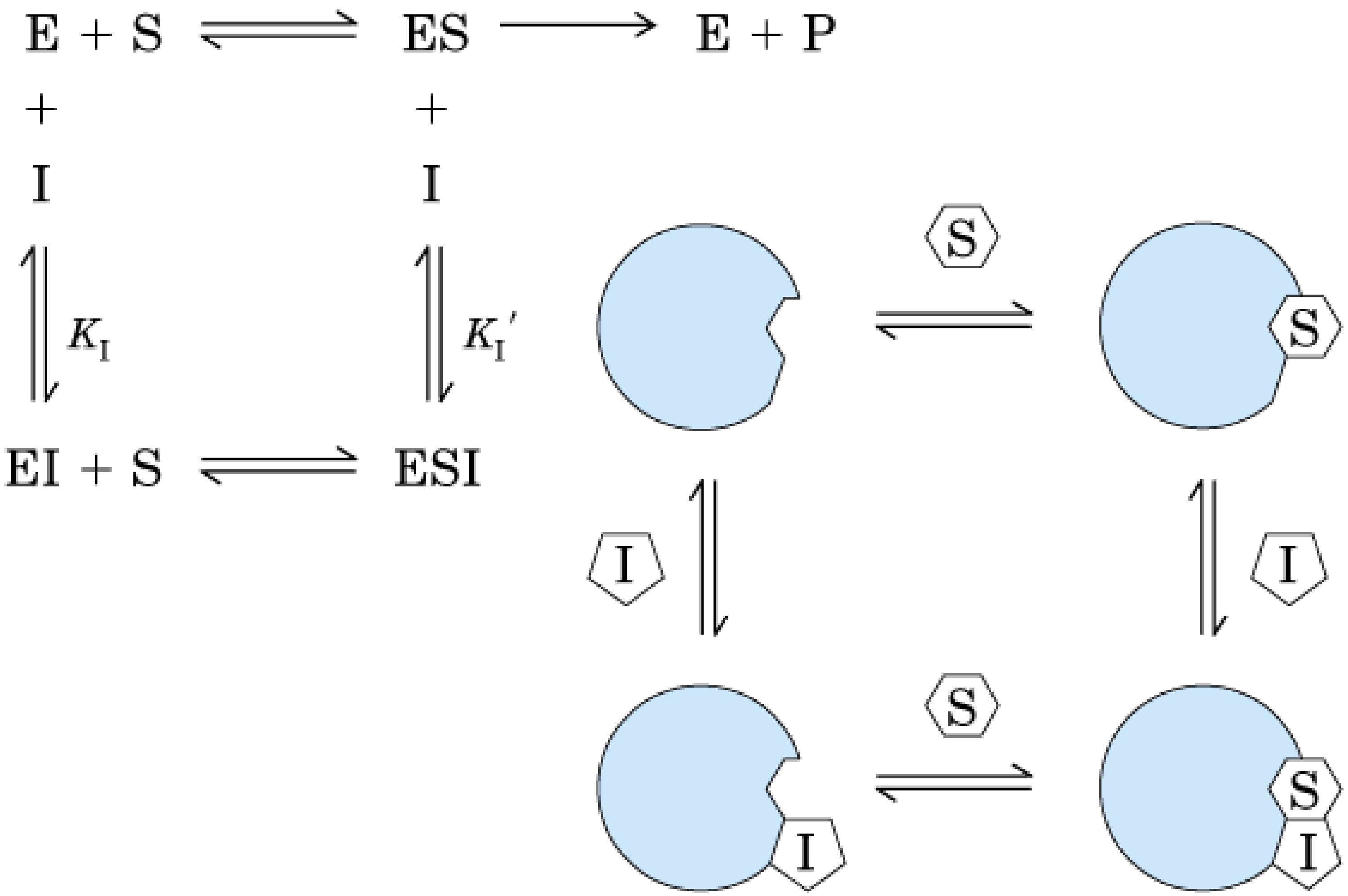
$$V_o = \frac{(V_{max}/\alpha')[S]}{K_m/\alpha' + [S]}$$



$$\alpha' = 1 + [I]/K'_I$$

$$K'_I = [ES][I]/[ESI]$$

INIBIÇÃO NÃO COMPETITIVA (“non-competitive”) ou MISTA: substrato e inibidor ligam em sítios diferentes; inibidor pode ligar à E livre e ao complexo ES



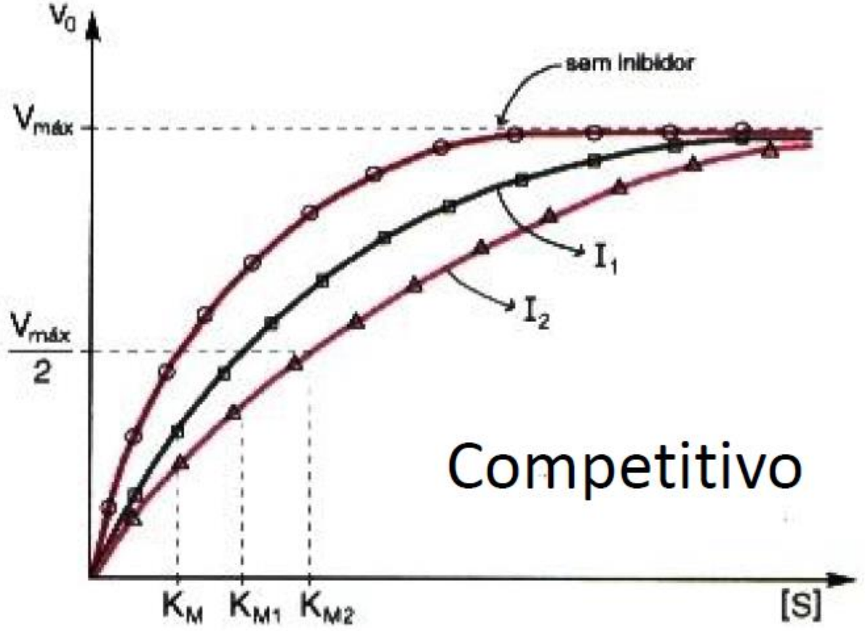
$$V_o = \frac{V_{max}[S]}{(\alpha K_m + \alpha'[S])}$$

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

(como na p79 do livro do Bayardo)

Resumo dos efeitos de diferentes tipos de inibidores nos parámetros K_M e k_{cat}

Tipo de inibidor	V_{max} aparente	K_M aparente
Nenhum	V_{max}	K_M
Competitivo	V_{max}	αK_M
Anti-Competitivo (ou "Uncompetitive")	V_{max}/α'	K_M/α'
Não-competitivo ("non-competitive" ou "misto")	V_{max}/α'	$\alpha K_M/\alpha'$



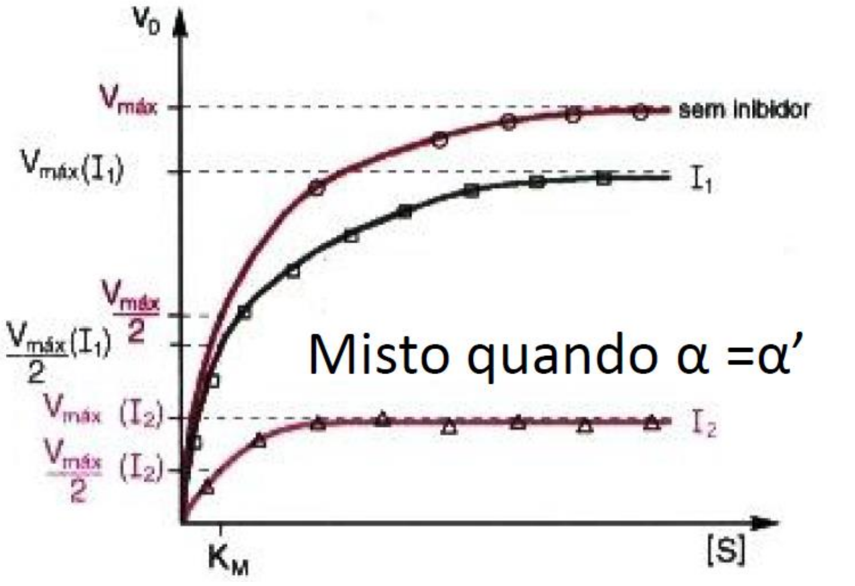
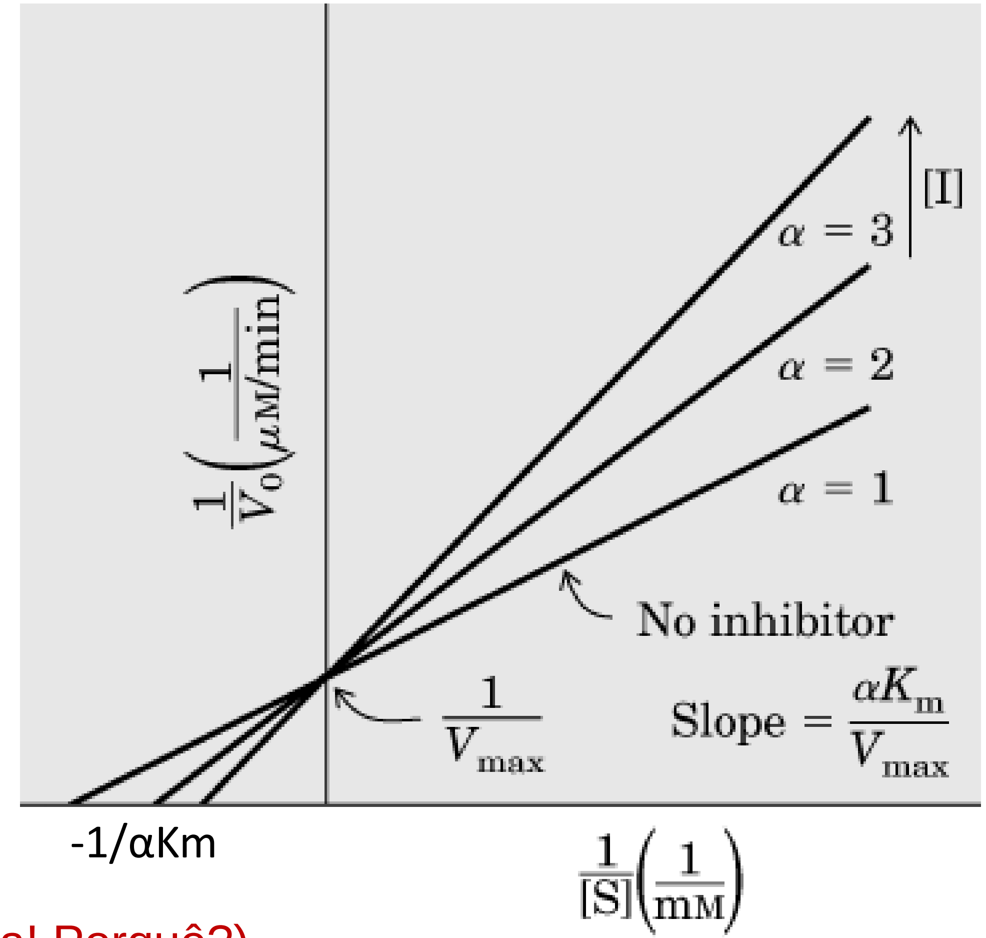


Diagrama Lineweaver-Burk para inibição competitiva

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

$K_m^{app} = \alpha K_M$

$\alpha = 1 + [I]/K_I$

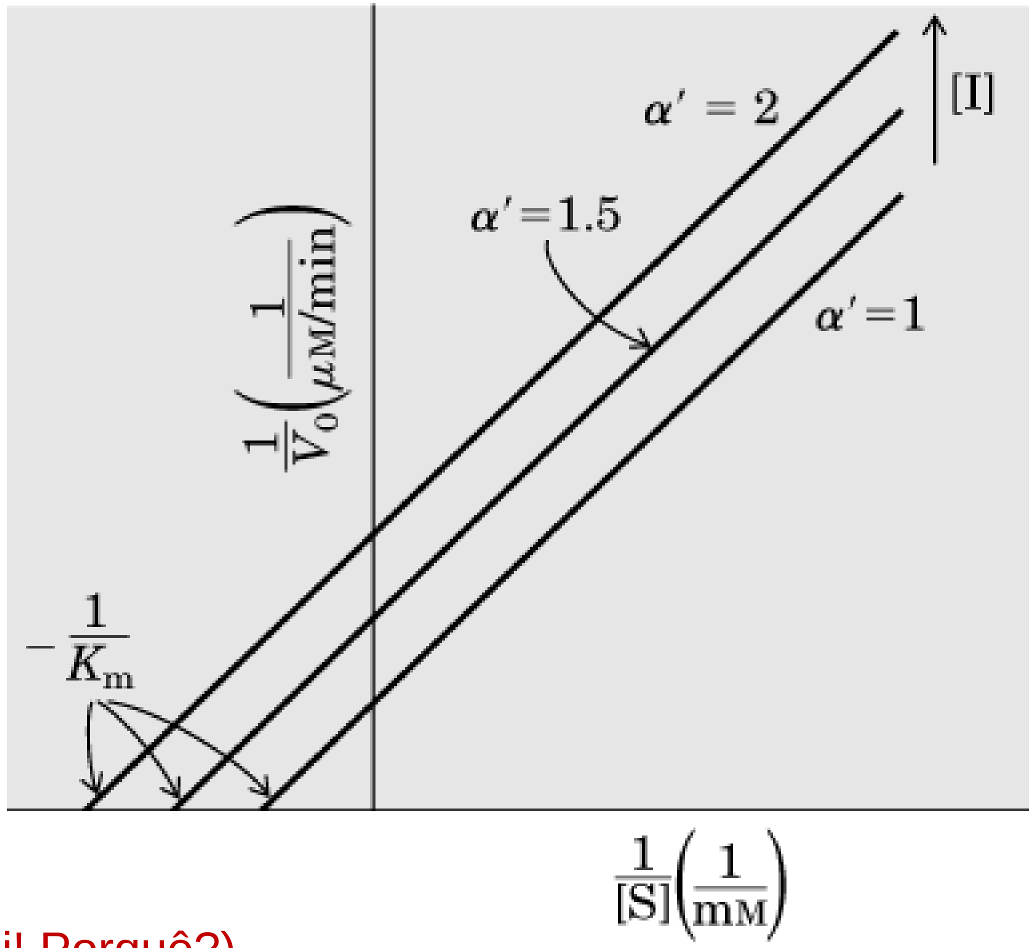


K_M muda (Aumenta! Porquê?)

V_{max} não muda (Porquê?)

Diagrama Lineweaver-Burk para inibição anti-competitiva

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

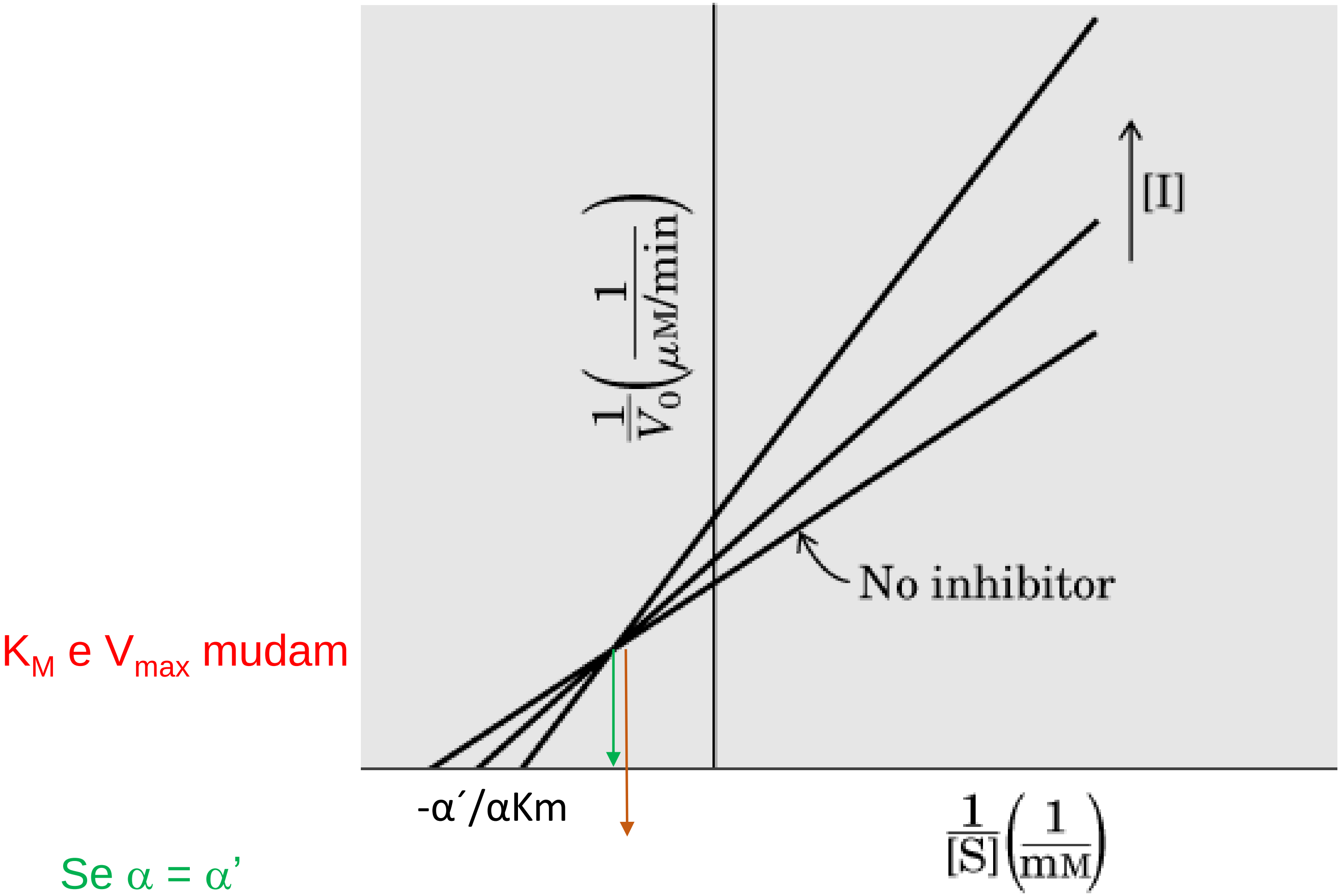


K_M muda (Diminui! Porquê?)

V_{max} muda

Diagrama Lineweaver-Burk para inibição não-competitiva (mista)

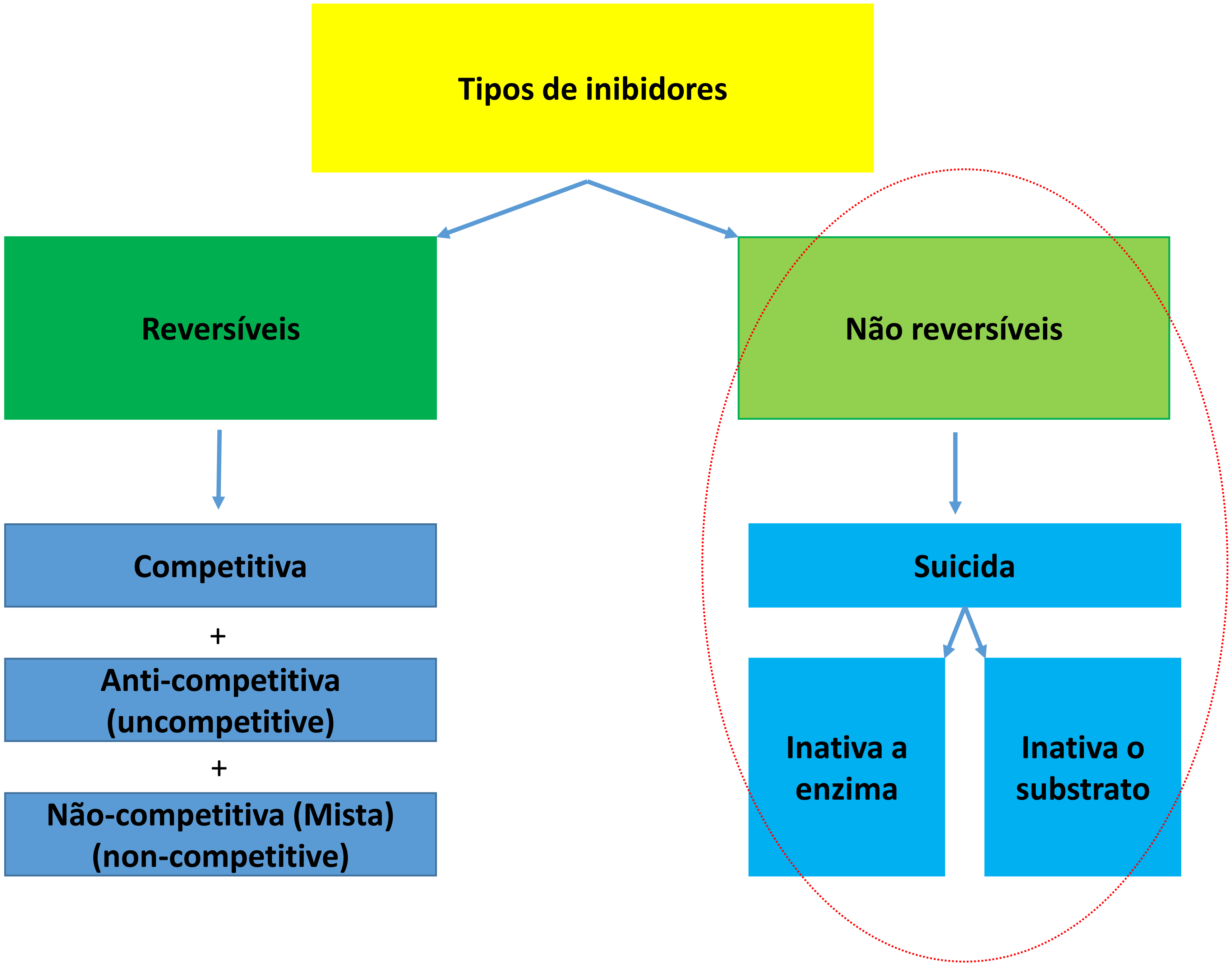
$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



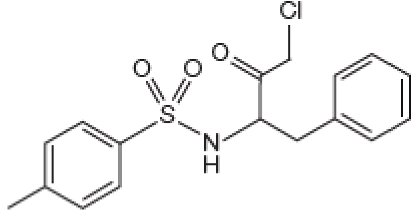
Se $\alpha = \alpha'$

$V_o = V_{max}[S]/(\alpha'(K_m + [S]))$

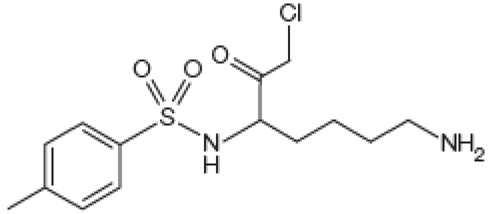
(como na p79 e fig 5.16 do livro do Bayardo) e Km não muda


Tipos de inibidores
Reversíveis
Não reversíveis
Competitiva
+
Anti-competitiva (uncompetitive)
+
Não-competitiva (Mista) (non-competitive)
Suicida
Inativa a enzima
Inativa o substrato

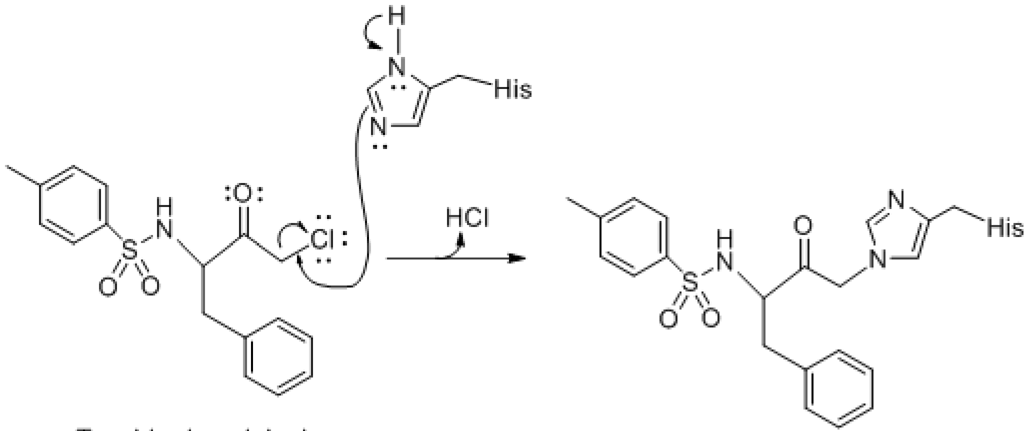
1) **Inibidores suicidas de serina proteases**
TPCK – inibe quimiotripsina
TLCK – inibe tripsina



TPCK



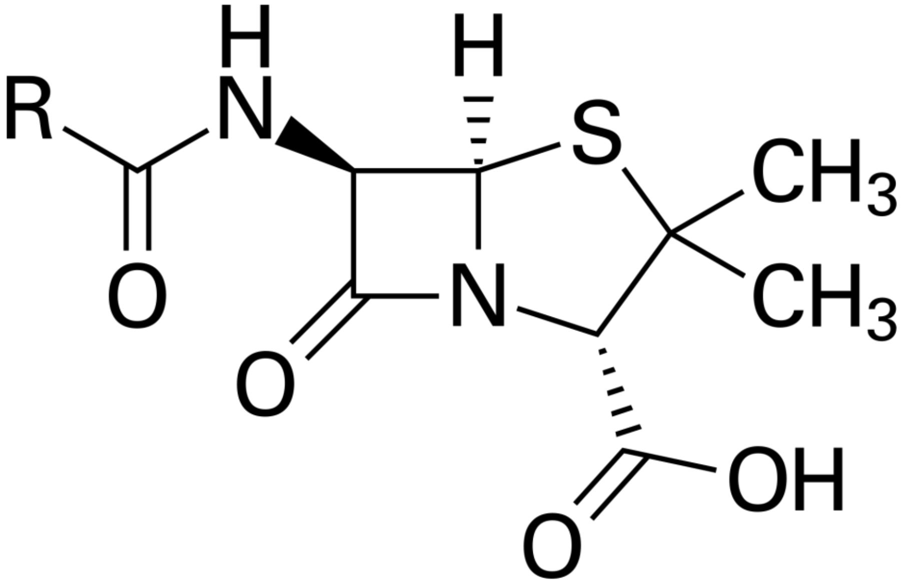
TLCK

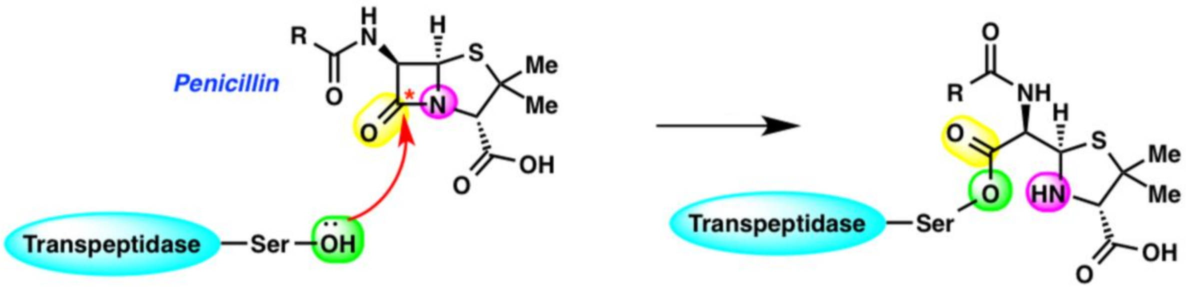


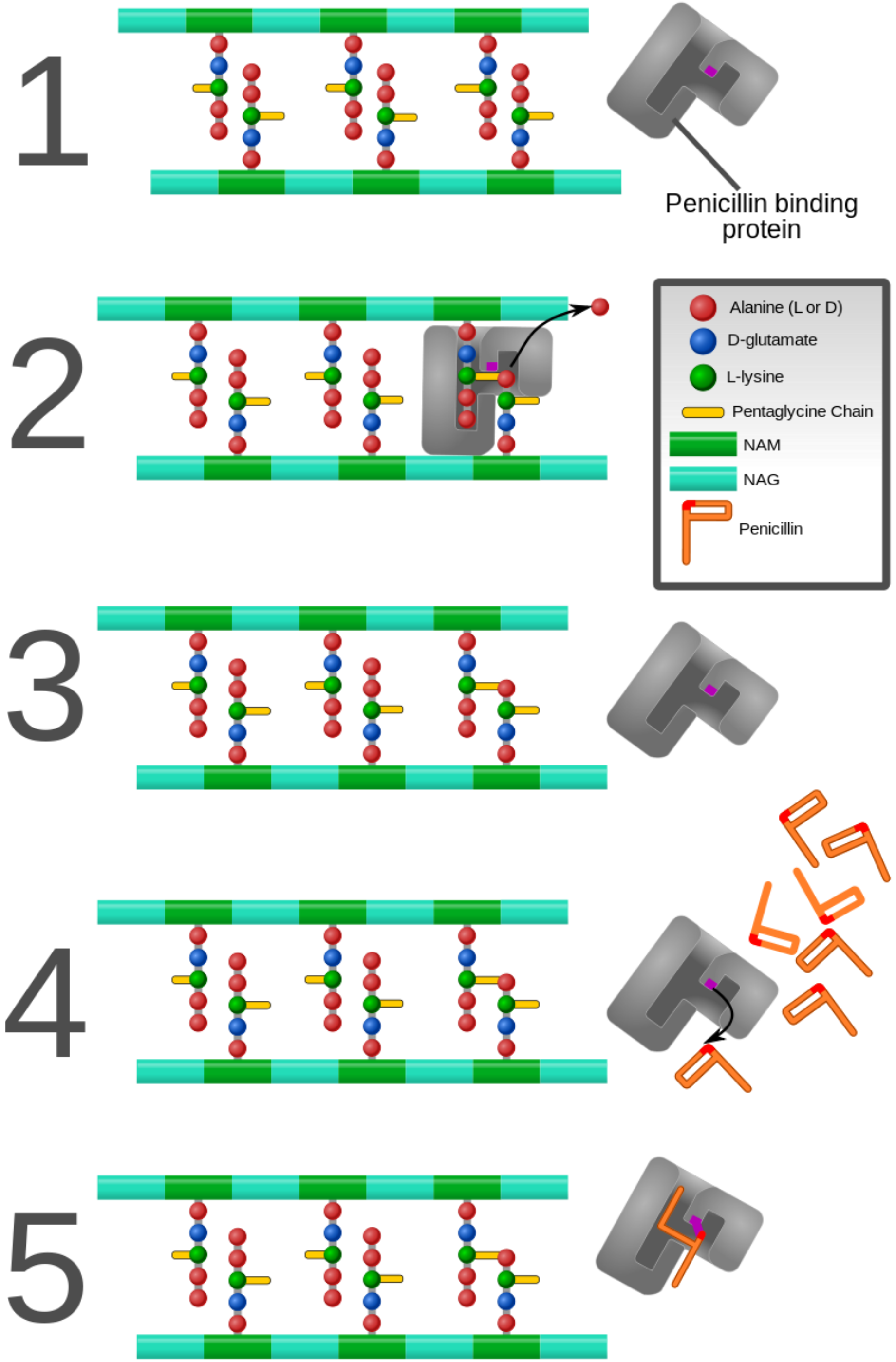
Tosyl-L-phenylalanine chloromethylketone (TPCK)

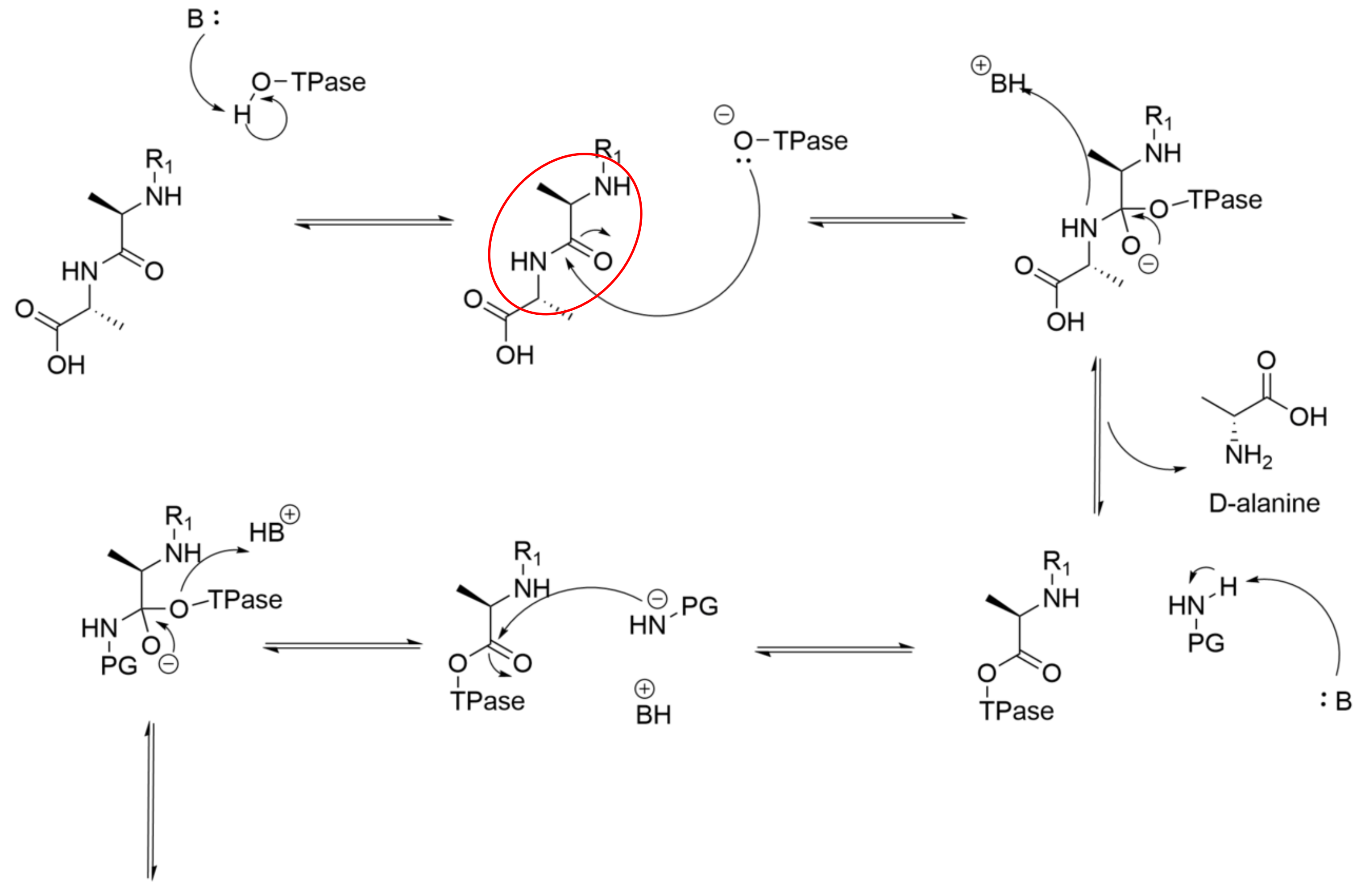
Product(s)

2) Penicilina
-inibidor suicida de
DD-transpeptidase

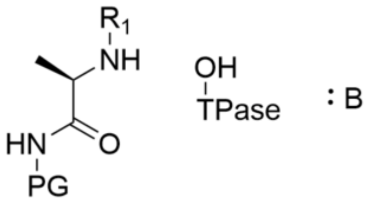


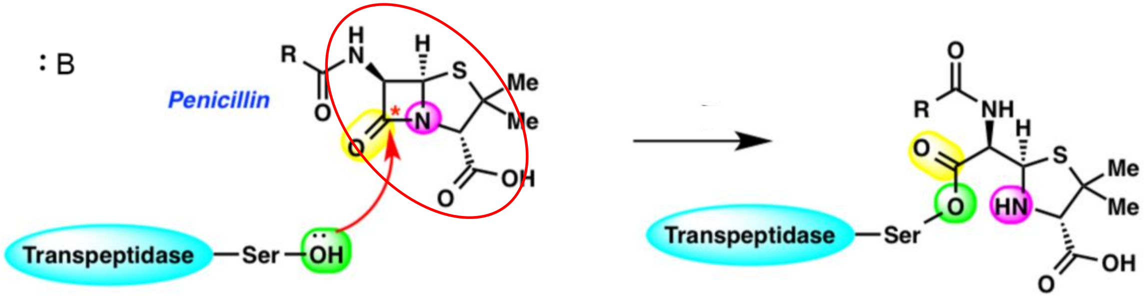




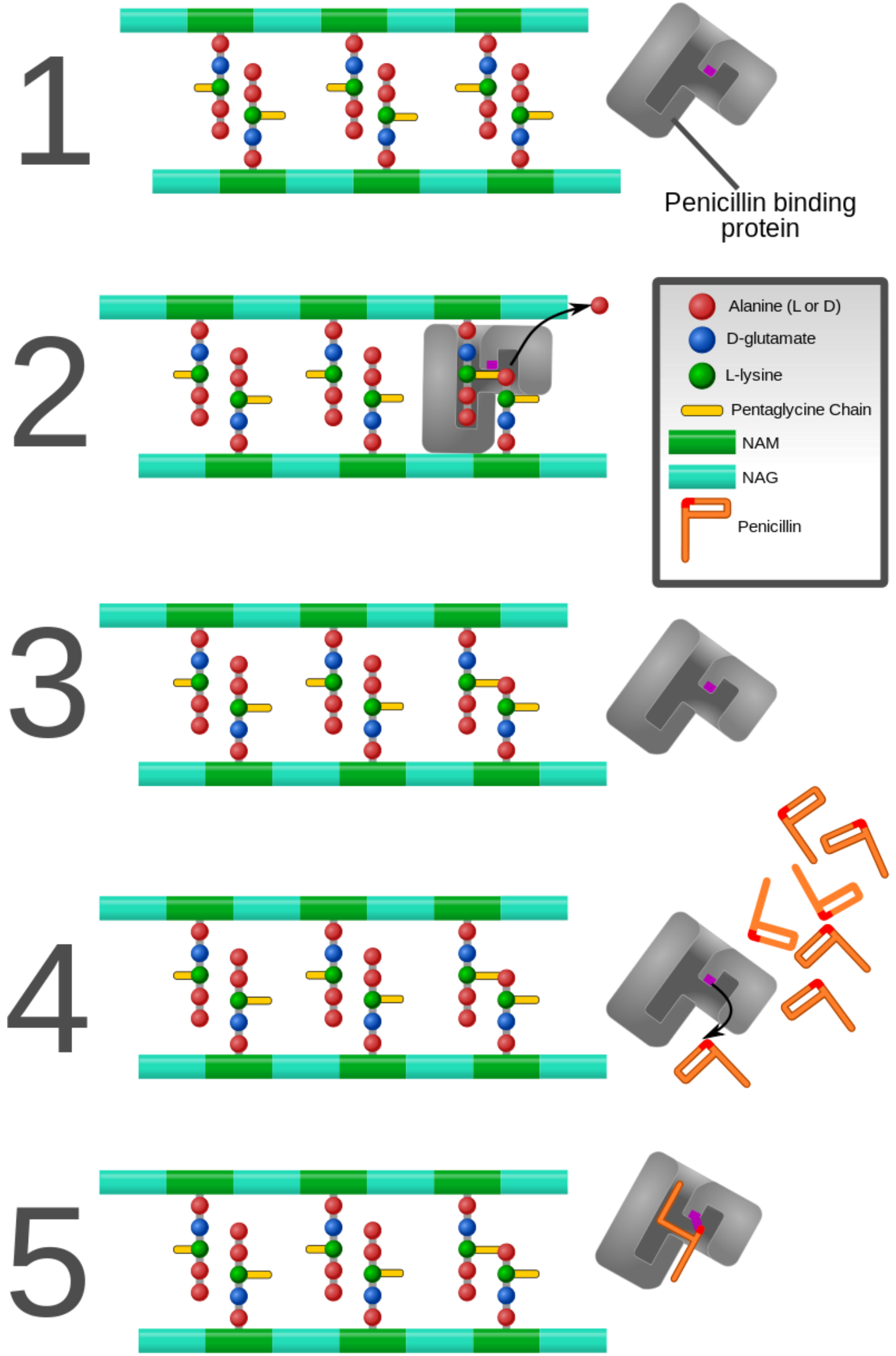


B :
O−TPase
H
R1
NH
HN
O
OH
⊖ O−TPase
⊕ BH
D-alanine
NH2
HB⊕
PG
TPase
:B





Penicillin
R
H
N
S
Me
Me
O
OH
Transpeptidase
Ser
OH
NH
HN



1
2
3
4
5
Penicillin binding protein
Alanine (L or D)
D-glutamate
L-lysine
Pentaglycine Chain
NAM
NAG
Penicillin

3) Aspirina: Inibidor suicida de ciclooxigenases 1 e 2

COX-1: prostaglandin G/H synthase 1

COX-2: prostaglandin-endoperoxide synthase 2

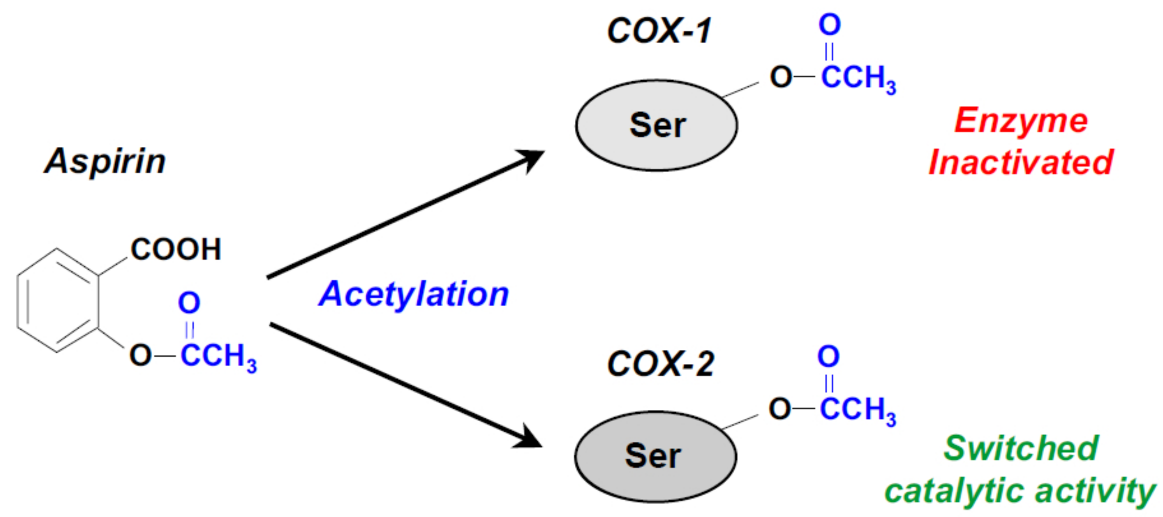
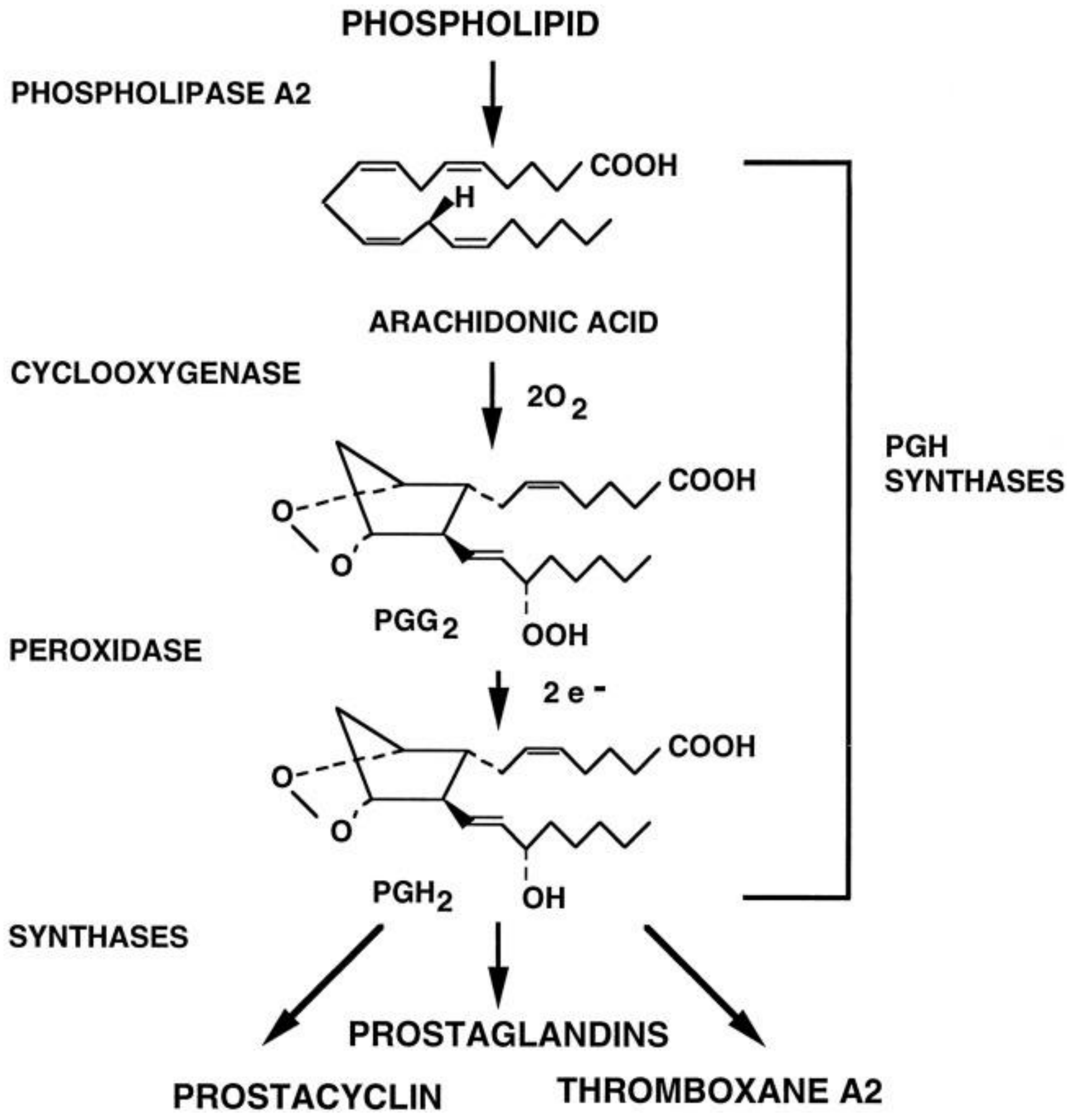
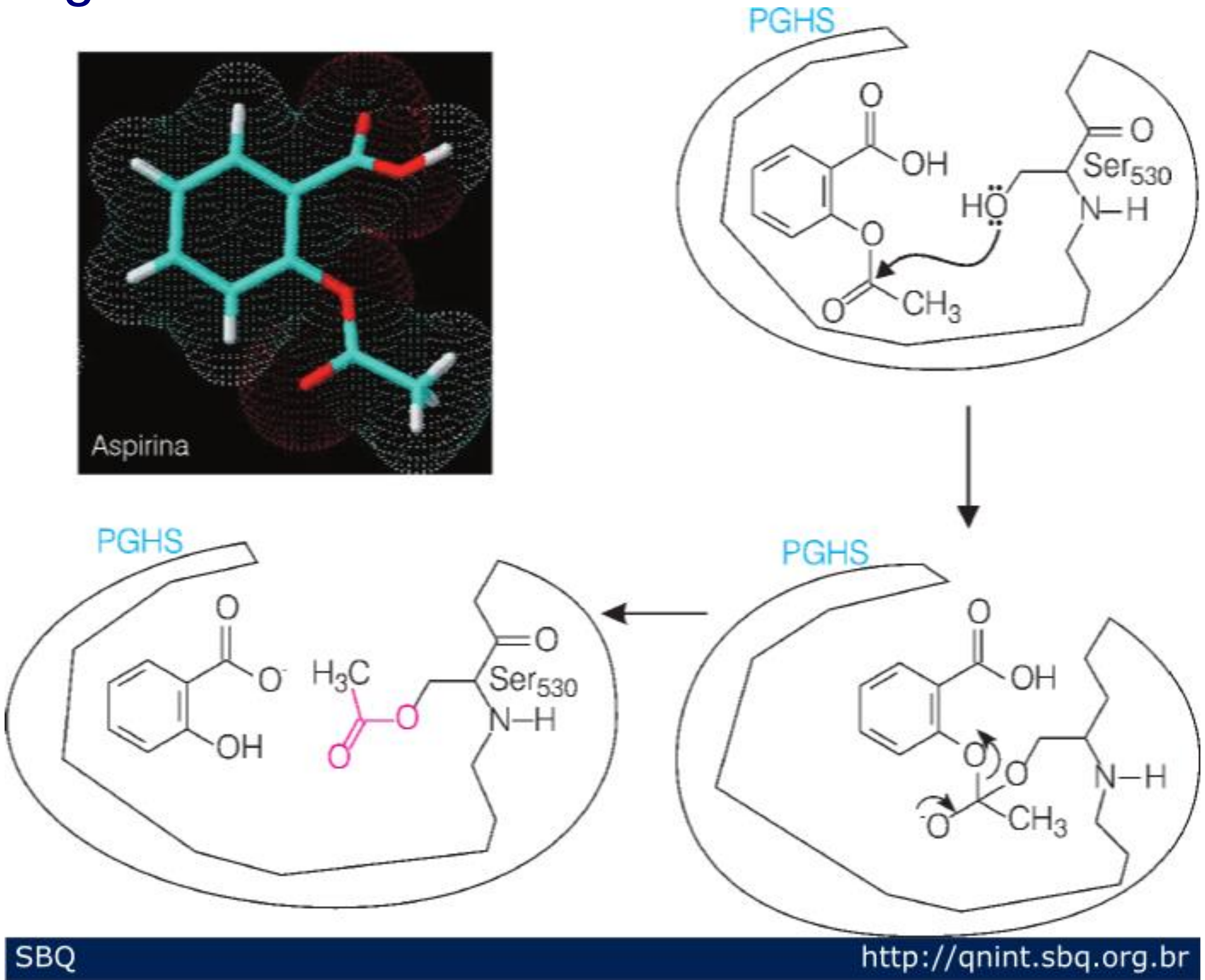


Figure 1. Aspirin mechanism of action -- acetylation of cyclooxygenase (COX). Aspirin acetylates a serine (Ser) residue of COX and irreversibly inactivates COX-1. In the case of COX-2, aspirin "turns off" its ability to generate prostaglandins, but "switches on" its capacity to produce novel protective lipid mediators.



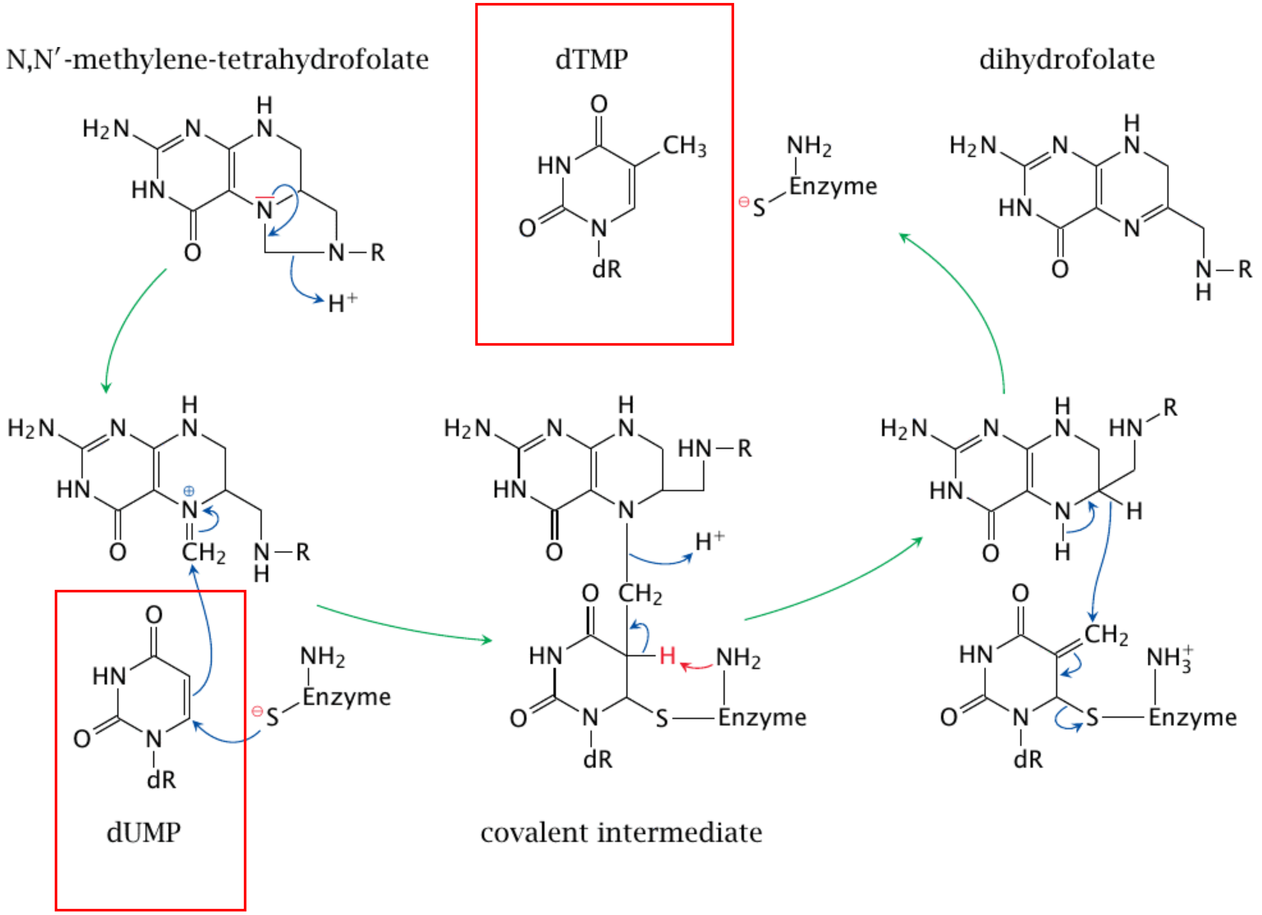
Inibição Sucicida/Irreversível

Mecanismo de inibição da aspirina sobre a prostanglandina H sintase



4) **5-fluorouracila: um precursor de 5-fluoro-desoxi-UMP (5-F-dUMP), um inibidor suicida de timidilato sinthase**

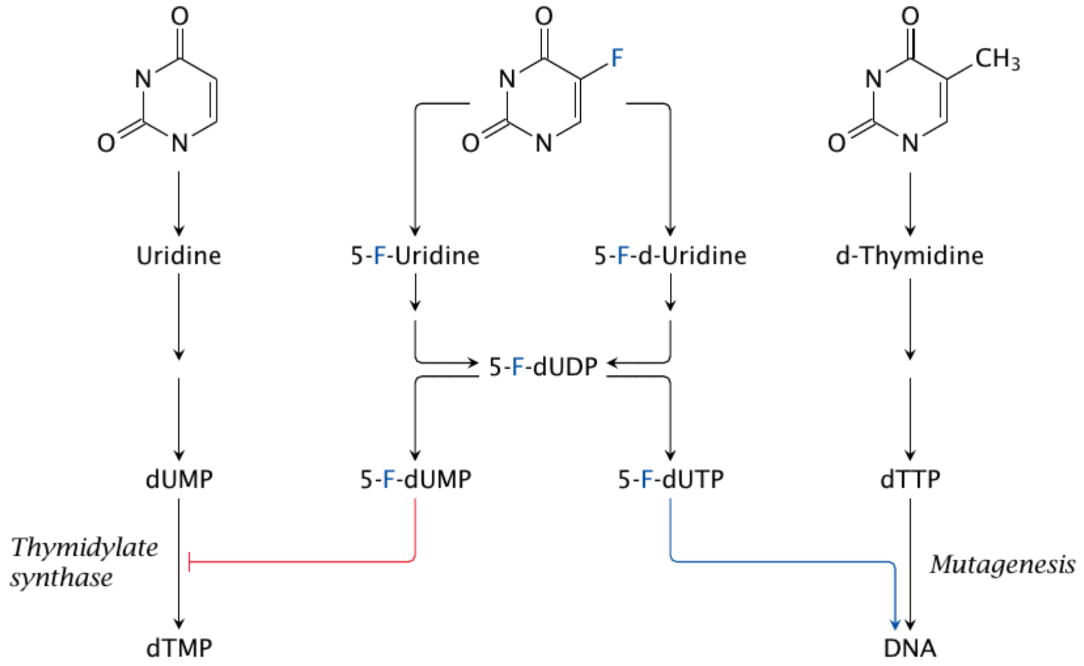
produto



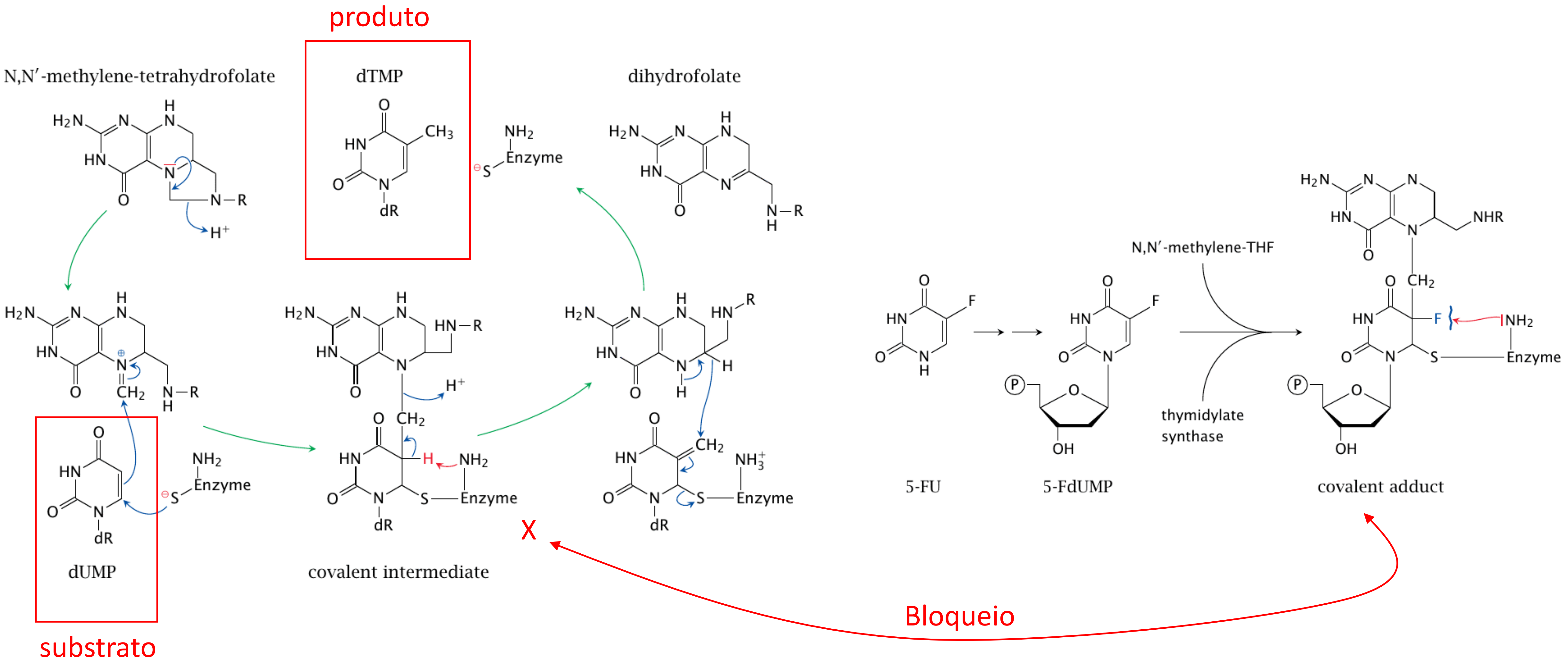
substrato

timidilato sinthase

5-fluorouracila

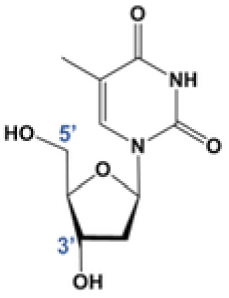


4) 5-fluorouracila: um precursor de 5-fluoro-desoxi-UMP (5-F-dUMP), um inibidor suicida de timidilato sinthase

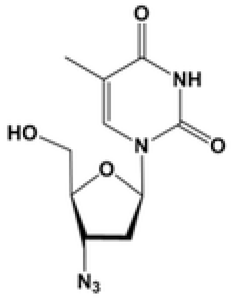


timidilato sinthase

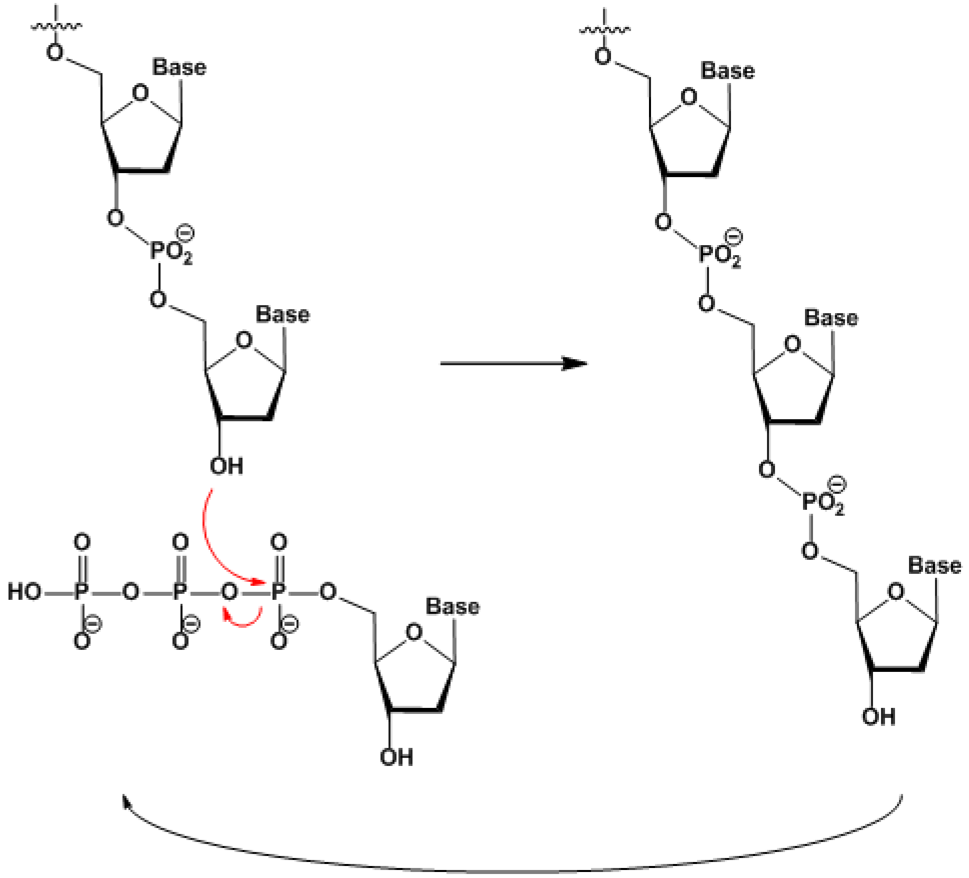
5) **AZT é um precursor do azidotimidina trifosfato,**
Um inibidor suicida da transcriptase reversa do HIV

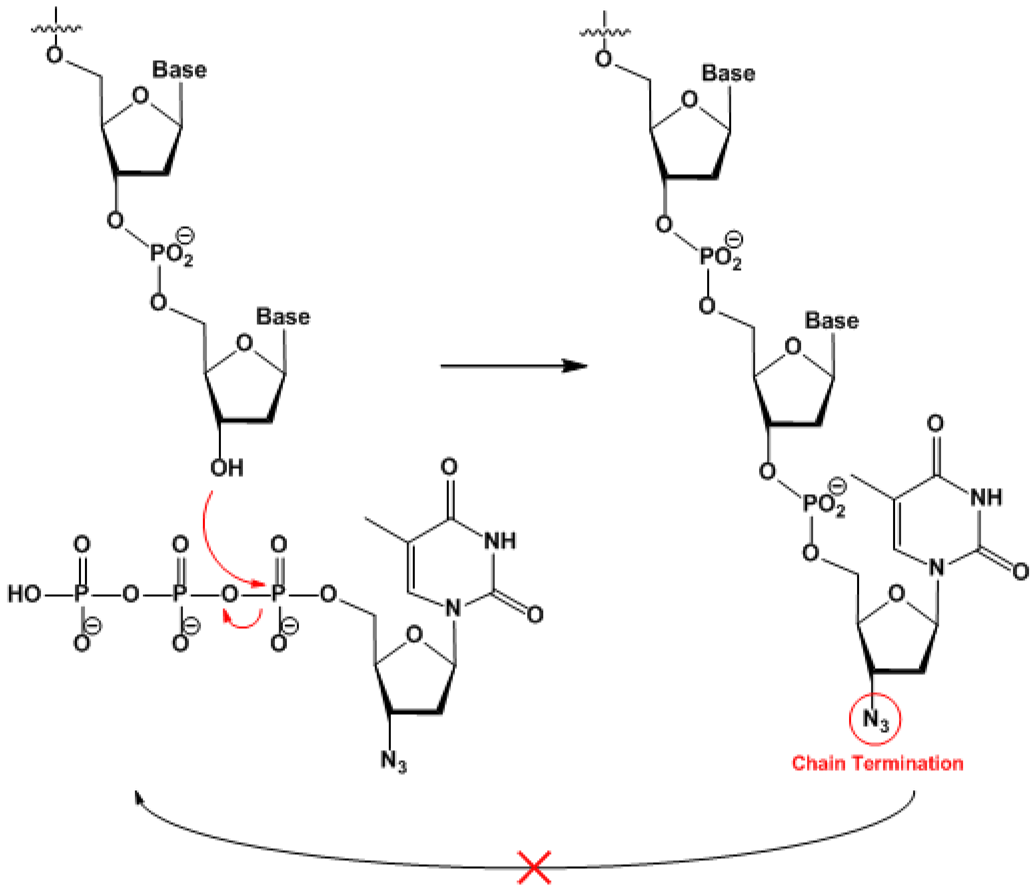


Thymidine

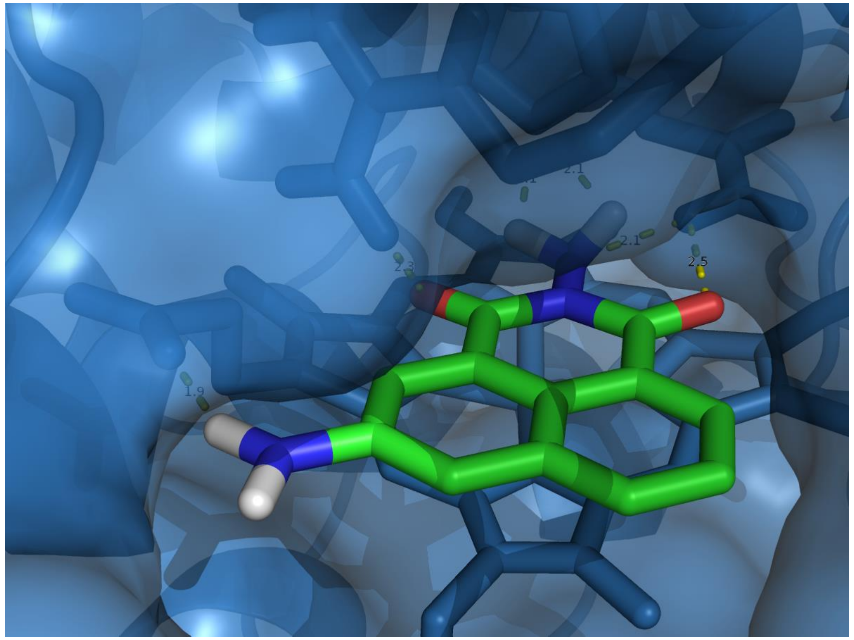


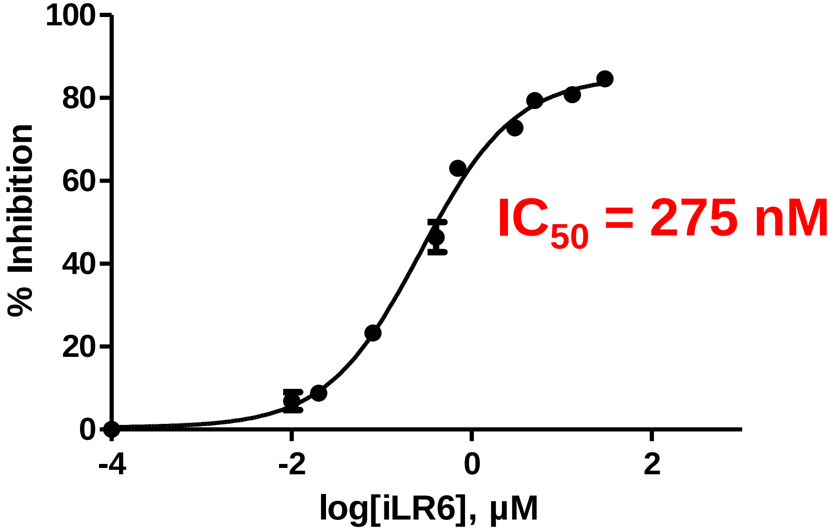
Azidothymidine (AZT)
Zidovudine





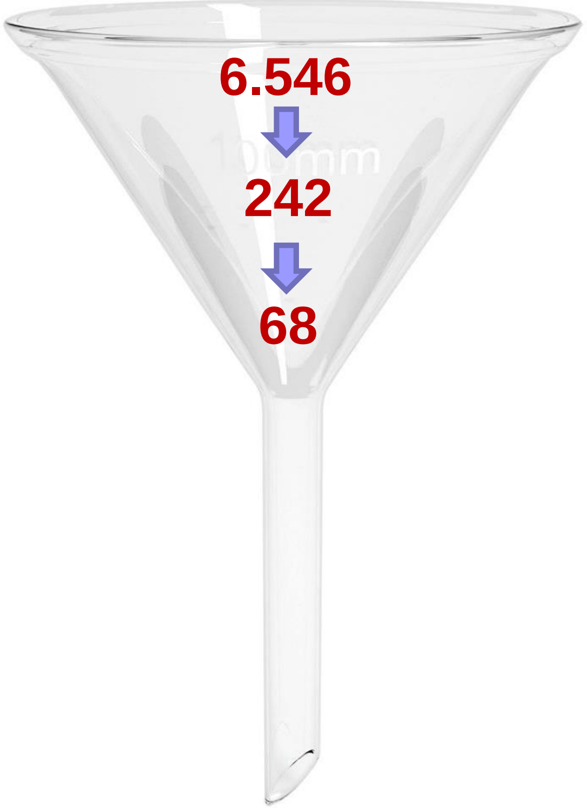
Desenho racional de fármacos inibidores de enzimas





ZINC 12

~35 milhões



Testes de inibição da enzima

30

Exercicios

ADENDOS

Inibidor Competitivo

$$[E_T] = [E] + [ES] + [EI]$$

Assumindo-se a cinética do estado estacionário:

$$\frac{d[ES]}{dt} = k_1 [E][S] - (k_{-1} + k_2) [ES] = 0$$

Resolvendo para [E]

$$[E] = \frac{(k_{-1} + k_2)[ES]}{k_1 [S]} = \frac{K_M [ES]}{[S]}$$

Encontra-se a [EI]

$$K_I = \frac{[E][I]}{[EI]} \Longrightarrow [EI] = \frac{[E][I]}{K_I} = \frac{K_M [ES] [I]}{[S] K_I}$$

Inibidor Competitivo

$$[E_T] = [E] + [ES] + [EI]$$

$$[E_T] = \frac{K_M\,[ES]}{[S]} + [ES] + \frac{K_M\,[ES]\,[I]}{[S]K_I} = [ES]\left(\frac{K_M}{[S]} + 1 + \frac{K_M\,[I]}{[S]K_I}\right)$$

$$[E_T] = [ES]\left\{\frac{K_M}{[S]}\left(1 + \frac{[I]}{K_I}\right) + 1\right\}$$

$$[ES] = \frac{[E_T]}{\frac{K_M}{[S]}\left(1 + \frac{[I]}{K_I}\right) + 1} = \frac{[E_T]\,[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

$$v_0 = k_2[ES] \Rightarrow v_0 = \frac{k_2\;[E_T][S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

Inibidor Competitivo

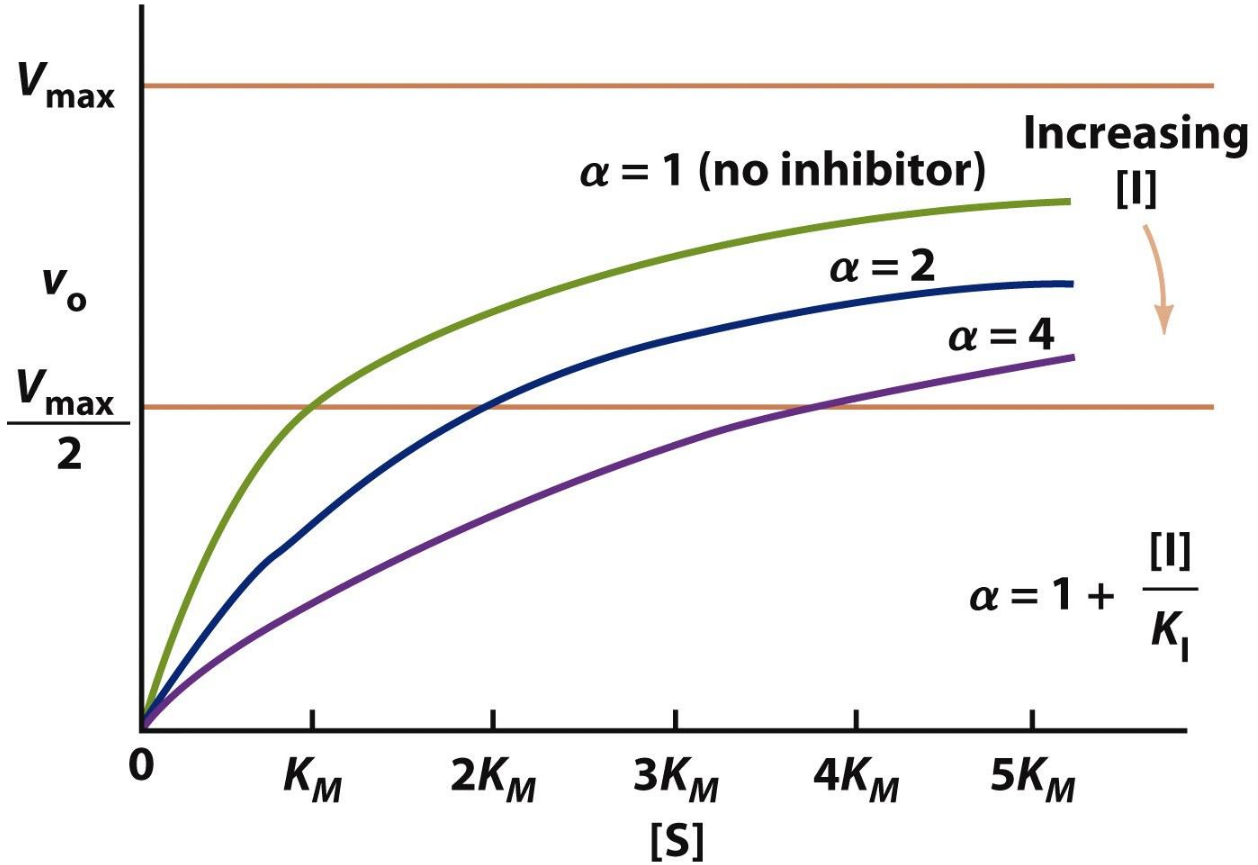
$$v_0 = \frac{k_2\ [E_T][S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

⇓

α

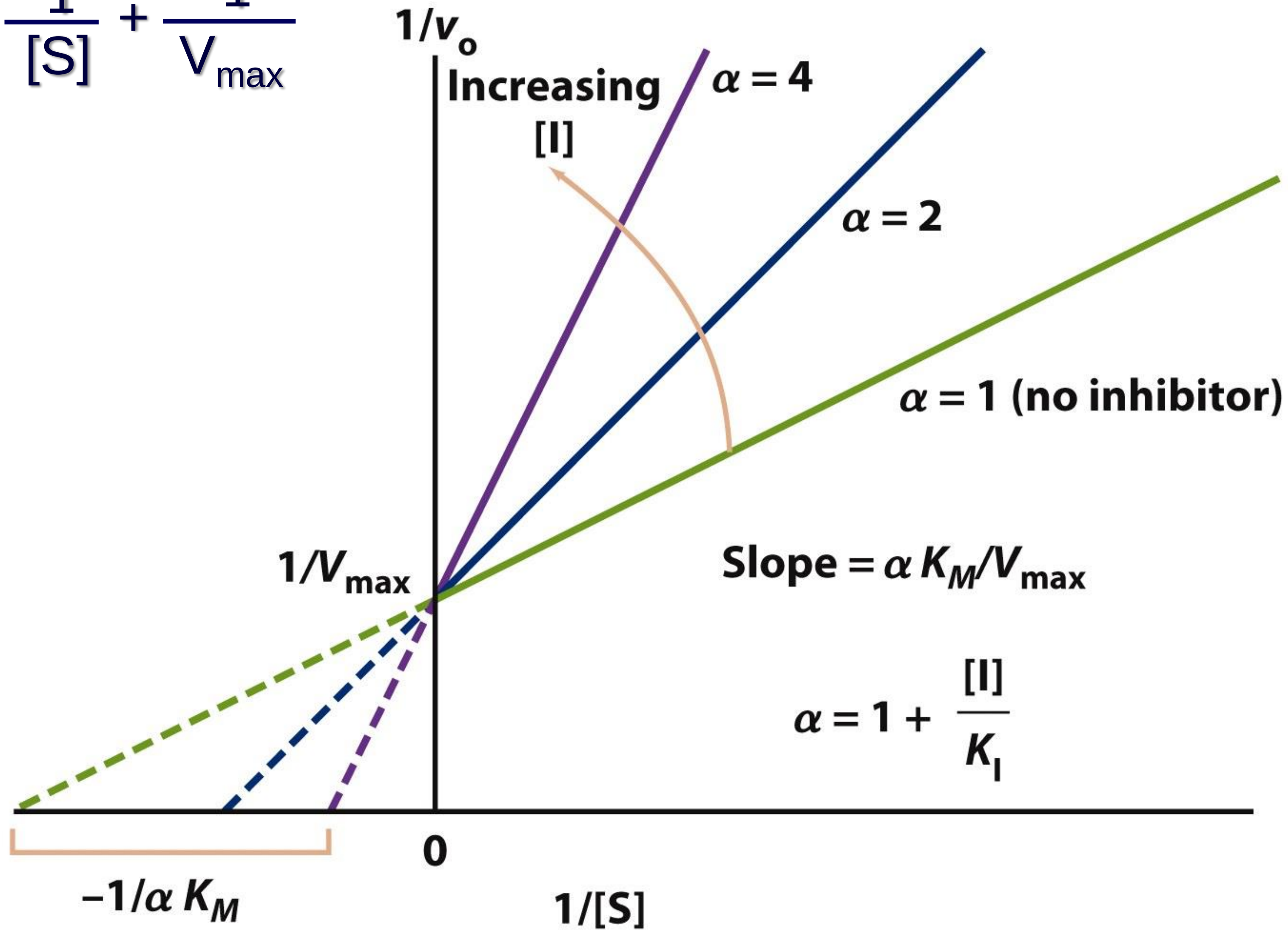
$$V_{max} = k_2[E_T]$$

$$v_0 = \frac{V_{max}\,[S]}{\alpha\, K_M + [S]}$$

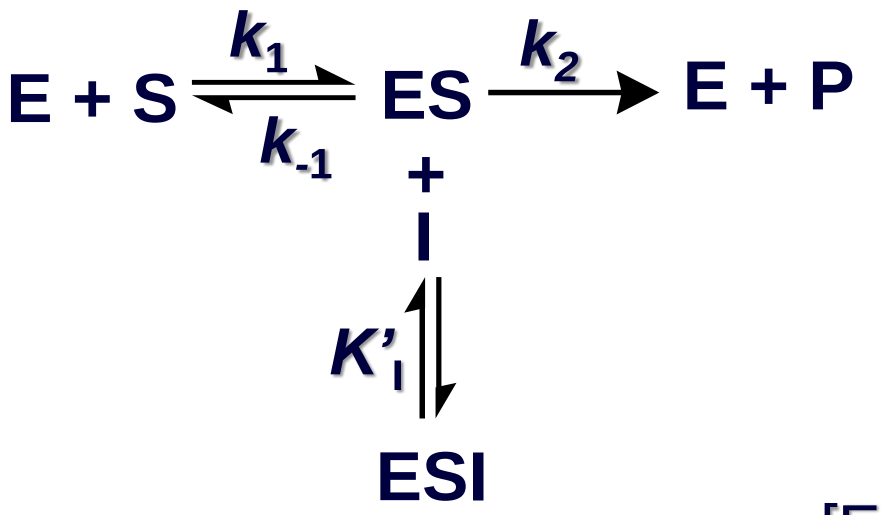


Transformando para o duplo-recíproco...

$$\frac{1}{v_0} = \frac{\alpha K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$



Inibidor Incompetitivo (Acompetitivo)



$$K'_I = \frac{[ES][I]}{[ESI]}$$

Inibidor Incompetitivo

$$[E_T] = [E] + [ES] + [ESI]$$

Assumindo-se a cinética do estado estacionário:

$$\frac{d[ES]}{dt} = k_1 [E][S] - (k_{-1} + k_2) [ES] = 0$$

Resolvendo para [E]

$$[E] = \frac{(k_{-1} + k_2)[ES]}{k_1 [S]} = \frac{K_M [ES]}{[S]}$$

Encontra-se a [ESI]

$$K'_I = \frac{[ES][I]}{[ESI]} \Longrightarrow [ESI] = \frac{[ES][I]}{K'_I}$$

Inibidor Incompetitivo

$$[E_T] = [E] + [ES] + [ESI]$$

$$[E_T] = \frac{K_M [ES]}{[S]} + [ES] + \frac{[ES][I]}{K'_I} = [ES]\left(\frac{K_M}{[S]} + 1 + \frac{[I]}{K'_I}\right)$$

$$[ES] = \frac{[E_T]}{\frac{K_M}{[S]} + 1 + \frac{[I]}{K'_I}} = \frac{[E_T]\,[S]}{K_M + [S]\left(1 + \frac{[I]}{K'_I}\right)}$$

$$v_0 = k_2[ES] \Rightarrow v_0 = \frac{k_2\,[E_T]\,[S]}{K_M + [S]\left(1 + \frac{[I]}{K'_I}\right)}$$

$$\left(1 + \frac{[I]}{K'_I}\right) \Rightarrow \alpha'$$

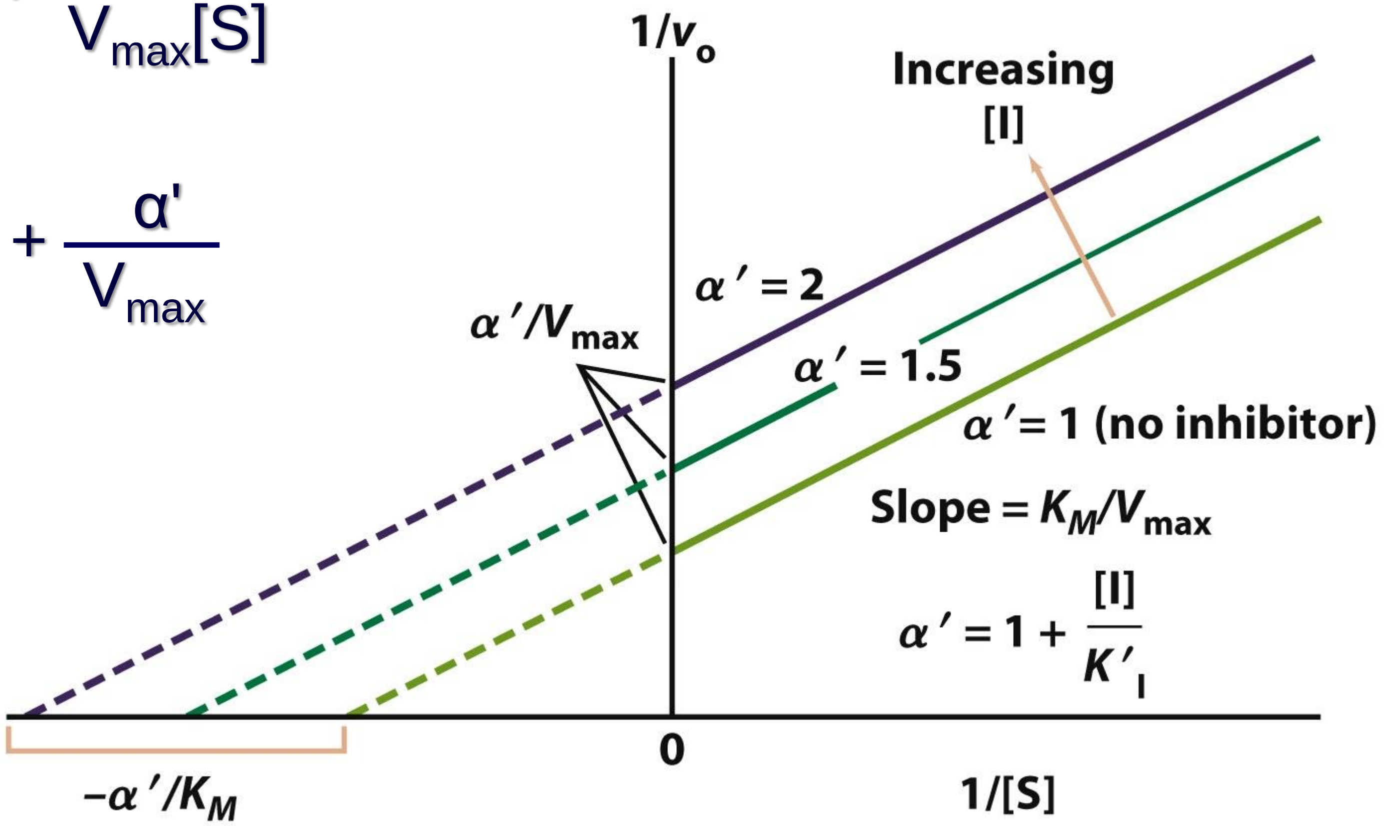
$$v_0 = \frac{V_{max}\,[S]}{K_M + \alpha'[S]}$$

Transformando para o duplo-recíproco...

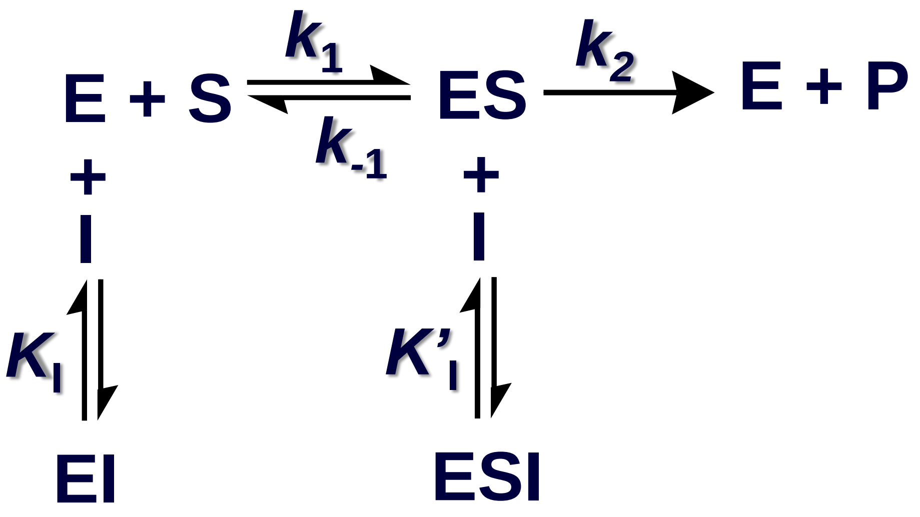
$$v_0 = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{\alpha'[S]}{V_{max}[S]}$$

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



Inibidor Misto



$$K_I = \frac{[E][I]}{[EI]} \quad \text{e} \quad K'_I = \frac{[ES][I]}{[ESI]}$$

Inibidor Misto

$$[E_T] = [E] + [EI] + [ES] + [ESI]$$

$$[E_T] = [E] + \frac{[E][I]}{K_I} + [ES] + \frac{[ES][I]}{K'_I}$$

$$[E_T] = [E]\left(1 + \frac{[I]}{K_I}\right) + [ES]\left(1 + \frac{[I]}{K'_I}\right) = [E]\,\alpha + [ES]\,\alpha'$$

$$[E_T] = \frac{K_M\,[ES]\,\alpha}{[S]} + [ES]\,\alpha' = [ES]\left(\frac{\alpha K_M}{[S]} + \alpha'\right)$$

$$[ES] = \frac{[E_T]}{\left(\frac{\alpha K_M}{[S]} + \alpha'\right)} = \frac{[E_T]\,[S]}{\alpha K_M + \alpha'\,[S]} \Longrightarrow v_0 = k_2[ES]$$

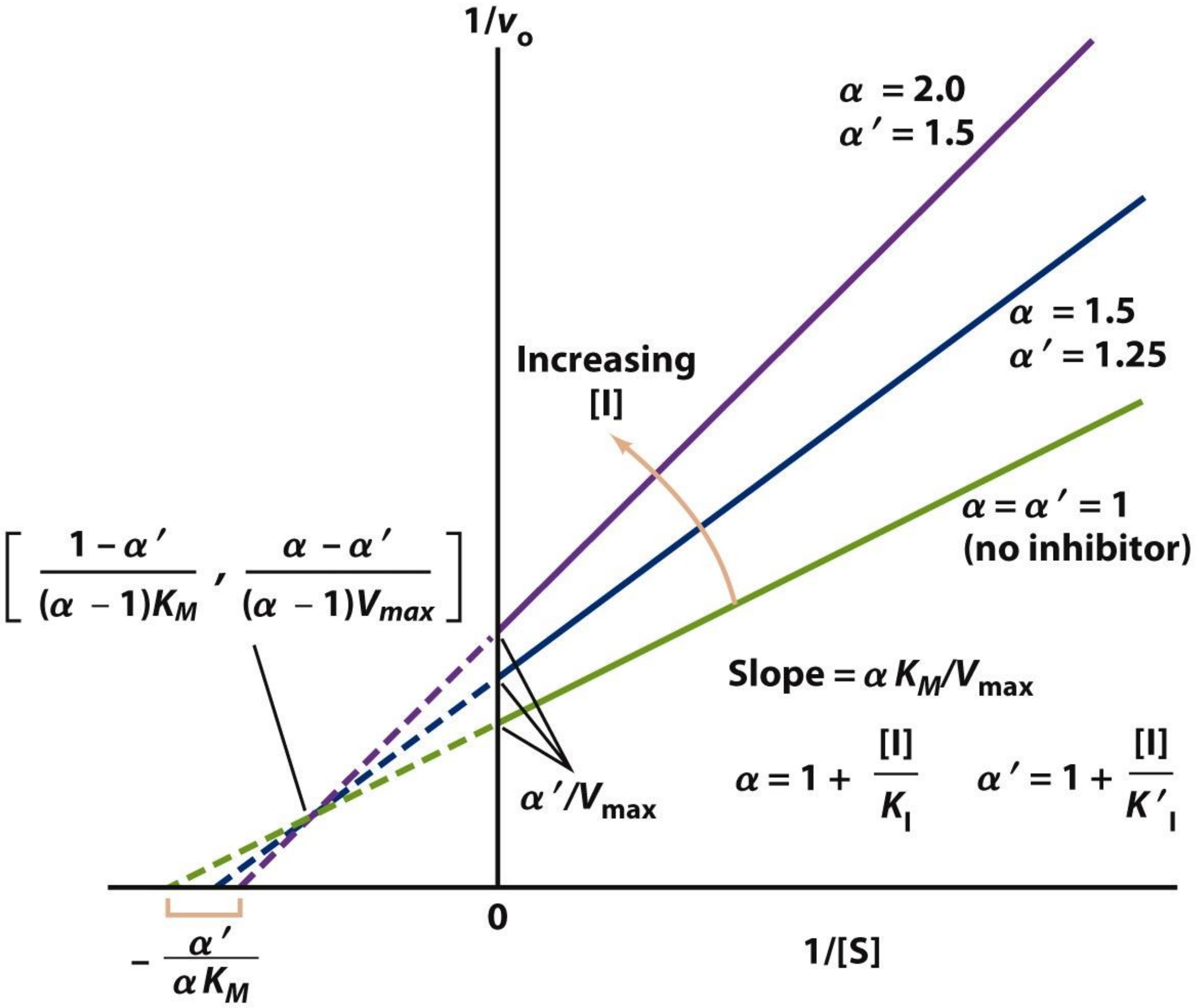
Inibidor Misto

$$[ES] = \frac{[E_T][S]}{\alpha K_M + \alpha' [S]} \Longrightarrow v_0 = k_2[ES]$$

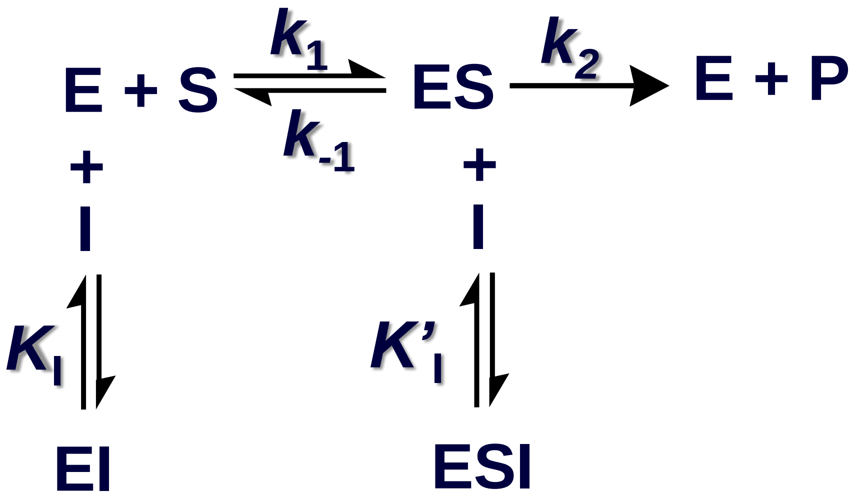
$$v_0 = \frac{k_2 \; [E_T] \; [S]}{\alpha K_M + \alpha' [S]}$$

$$v_0 = \frac{V_{max} \; [S]}{\alpha K_M + \alpha' [S]}$$

$$\frac{1}{v_0} = \frac{\alpha K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

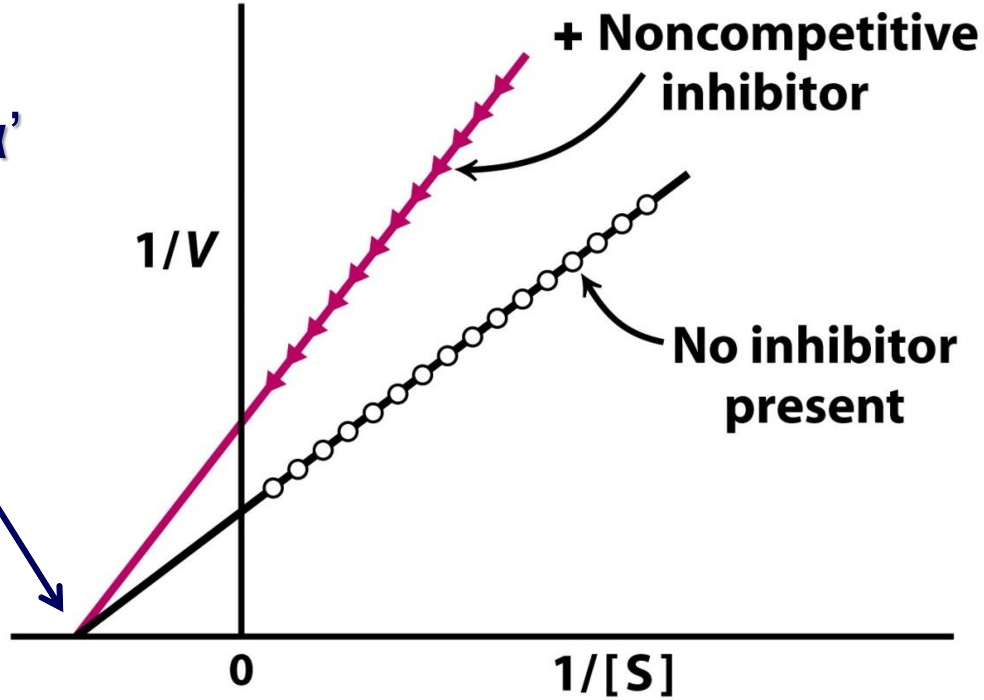


Inibidor Misto (Não-competitivo)

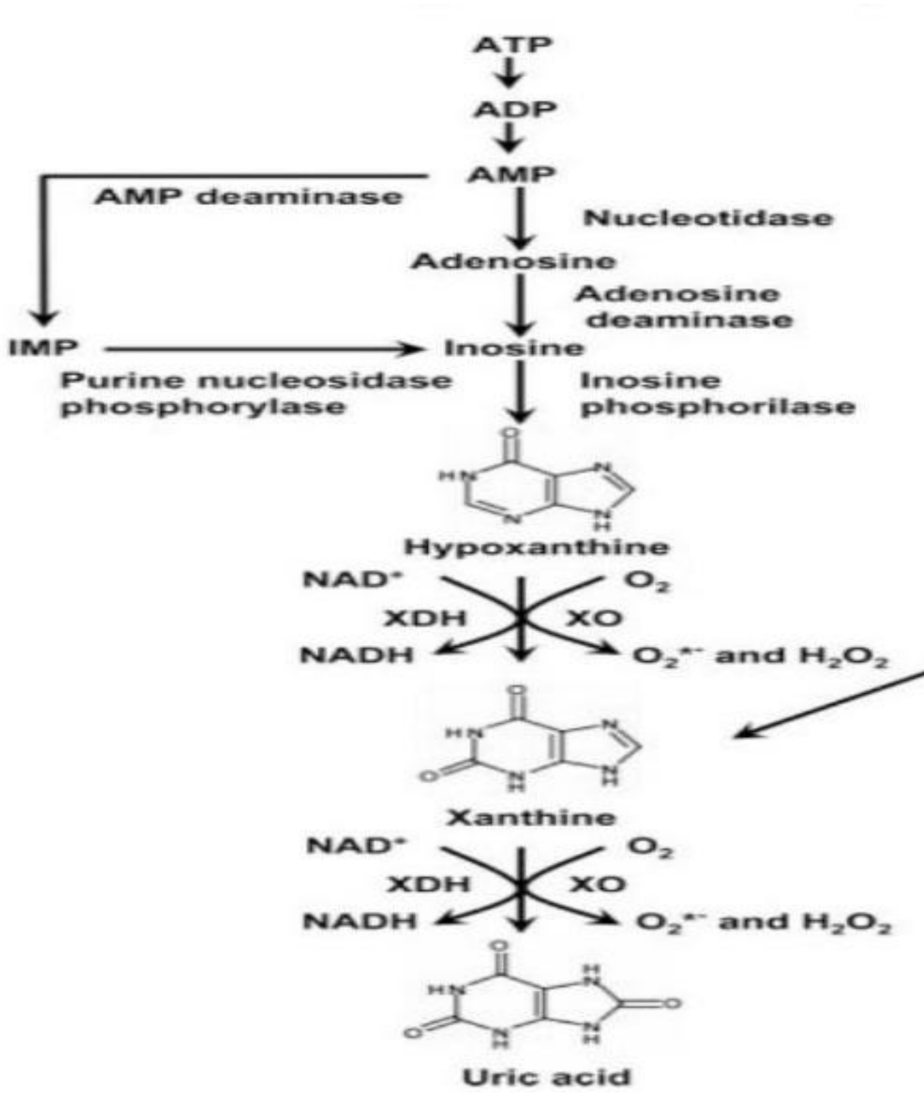


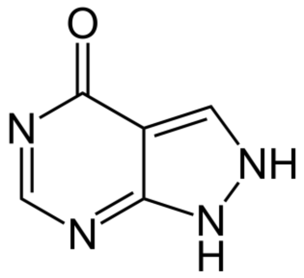
Quando $K_I = K'_I$, ou seja $\alpha = \alpha'$

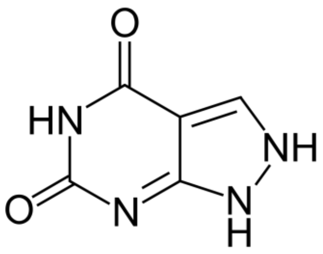
Neste caso, $\frac{-\alpha'}{\alpha K_M} = \frac{-1}{K_M}$



Allopurinol → Oxypurinol x xanthine oxidase







Vigabatrin x GABA transaminase

Sarin x acetilcolinesterase

Sulbactum x beta lactamase

N,N-dimethylpropargilamine x monoamine oxidase