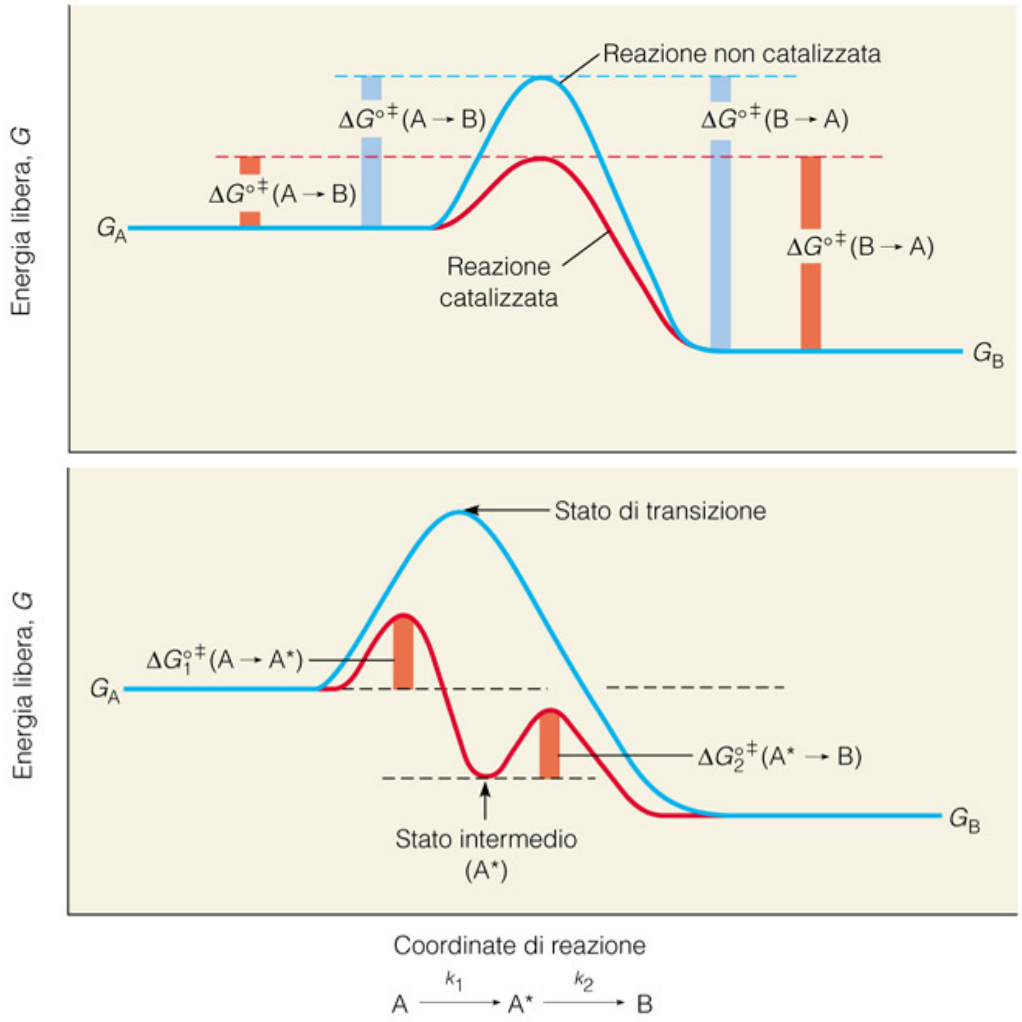
Enzymes and enzymatic inhibition.

Enzymatic catalysis



The rate of a reaction is described by the Arrhenius equation:

$$k = Ae^{-Ea/RT}$$

where
A is the pre-exponential factor
R is the gas constant
T is the temperature
E_a is the activation energy (ΔG^+)

$$\Delta G^+ = \Delta H^+ - T\Delta S^+$$

CATALYTIC MECHANISMS EMPLOYED BY ENZYMES

Enzymes decrease the activation energy by

- decreasing the energy of the transition state
- increasing the energy of the starting state

This is possible because an enzyme-substrate complex is formed

1. Preferential binding of the transition state
2. Proximity and orientation effects
3. Acid-base catalysis
4. Covalent catalysis
5. Metal ion-mediated catalysis
6. Electrostatic catalysis

CLASSIFICATION OF ENZYMES

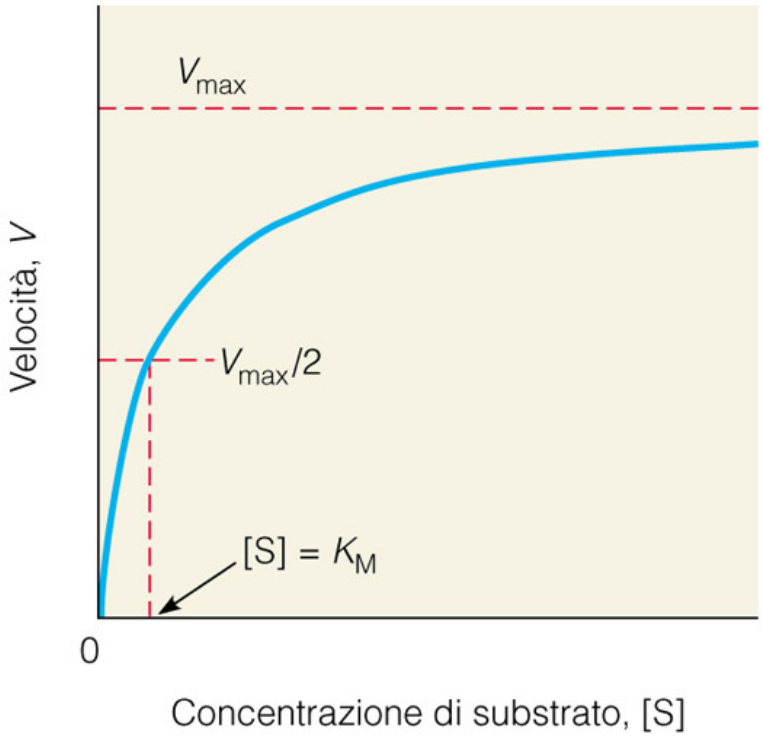
- Oxidoreductases
  - Electron transfer reactions
- Transferases
  - Transfer of groups from a molecule to another
- Hydrolases
  - Hydrolysis reactions (bond cleavage using a water molecule)
- Lyases
  - Addition or removal of groups by non-hydrolytic reactions
- Isomerases
  - Transfer of groups within a molecule
- Ligases
  - Synthesis of bonds using ATP
- *Translocases*
  - *Translocation across a membrane*

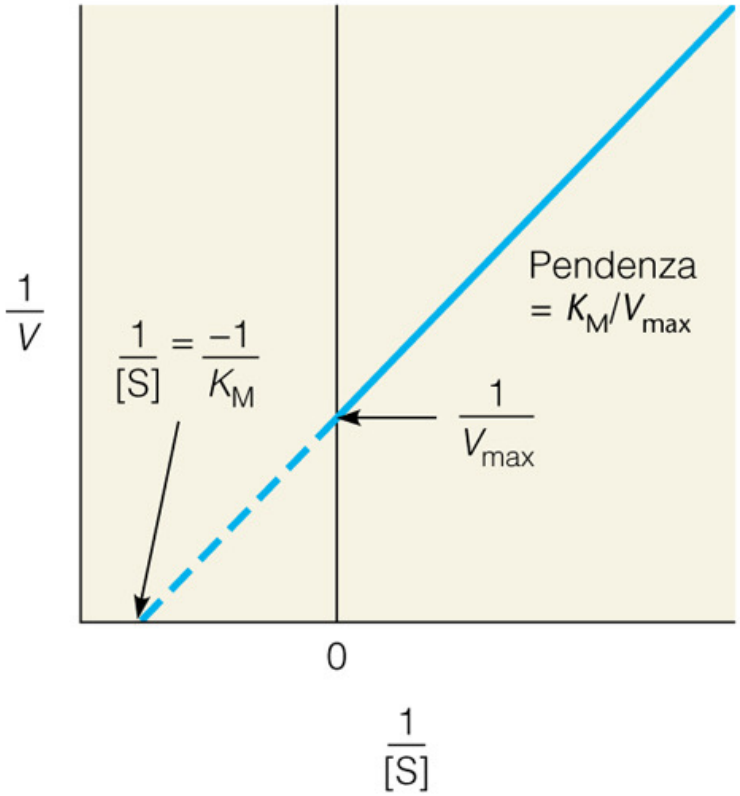
Enzyme kinetics

Michaelis-Menten equation

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

Kinetic parameters: K_M, V_{max}, k_{cat} and k_{cat}/K_M





Kinetic Parameters

- K_M
  - Substrate concentration when the reaction velocity is half V_{max}.
  - Comparable to the equilibrium K_D of $E + S \leftrightarrow ES$
- V_{max}
  - Maximal Velocity that is reached when the enzyme is saturated (all the enzyme molecules are in complex ES)
  - Depends on the concentration of enzyme
- k_{cat}
  - *turnover* number: number of substrate molecules transformed for time unit
  - It is defined by $V_{max}/[E]$ so it is independent of enzyme concentration
- k_{cat}/K_M
  - Ratio of catalytic frequency and substrate affinity, it defines the specificity constant of the enzyme
  - It expresses the catalytic efficiency of the enzyme

SELECTIVITY OF ENZYMES

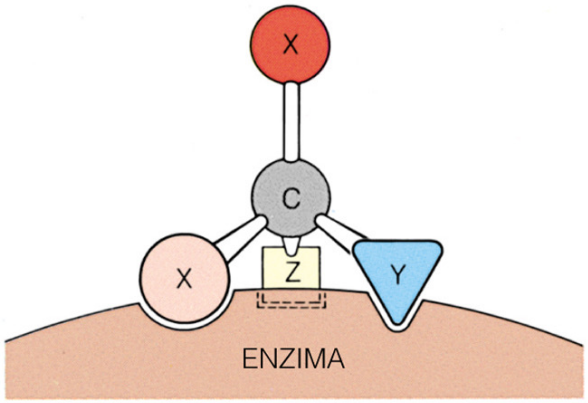
- **Chemioselectivity**: specificity for the substrate, activity towards a specific type of chemical compound (group)
- **Regioselectivity**: capacity to distinguish identical functional groups within a molecule
- **Enantioselectivity**: capacity to distinguish between enantiomers or functional groups bound to a prochiral center.

Selectivity depends on:

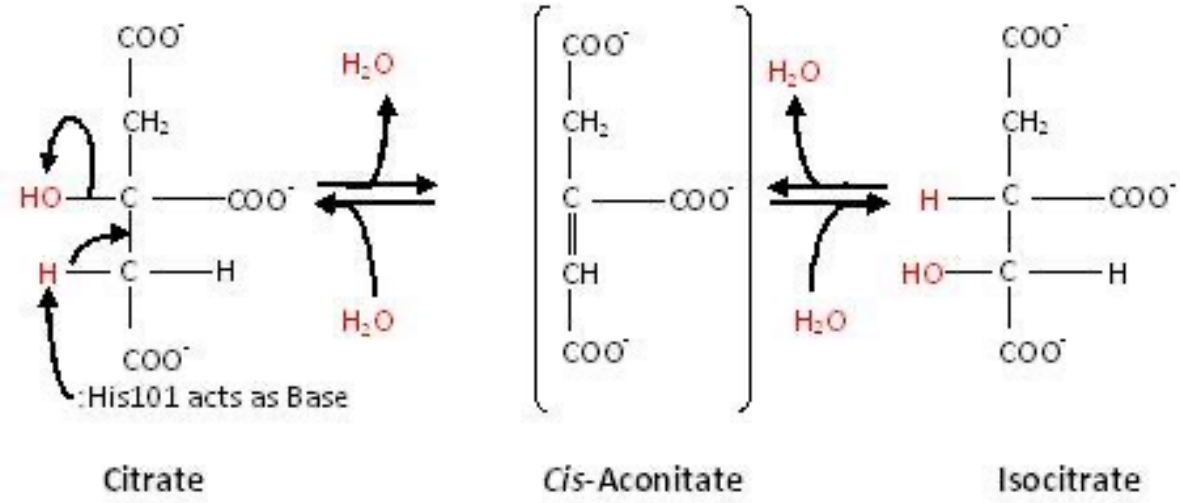
the enzyme
the substrate
the reaction conditions

Molecular basis of the capacity of an enzyme to distinguish identical functional groups bound to a prochiral center

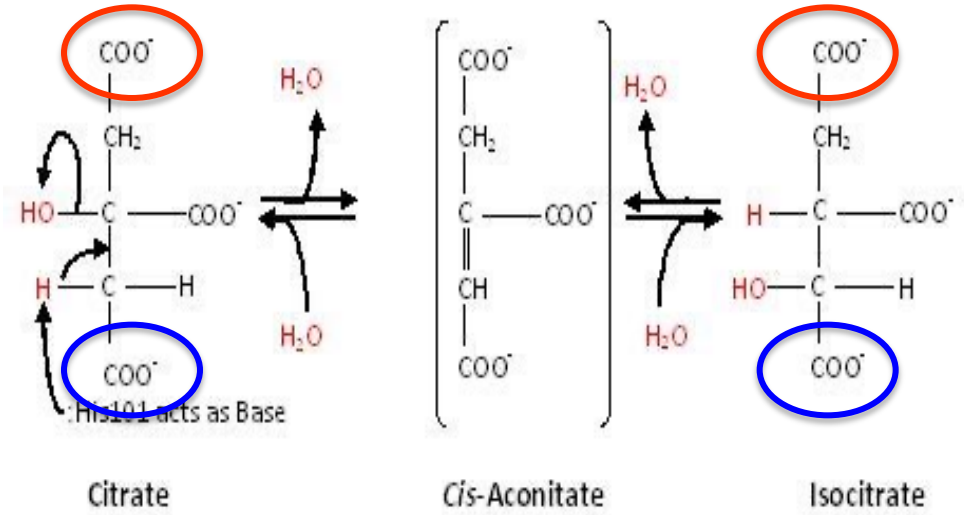
If the substrate molecule interacts in three sites with specific complementary groups on the asymmetric surface of the enzyme, then the two X atoms/groups are no longer equivalent.

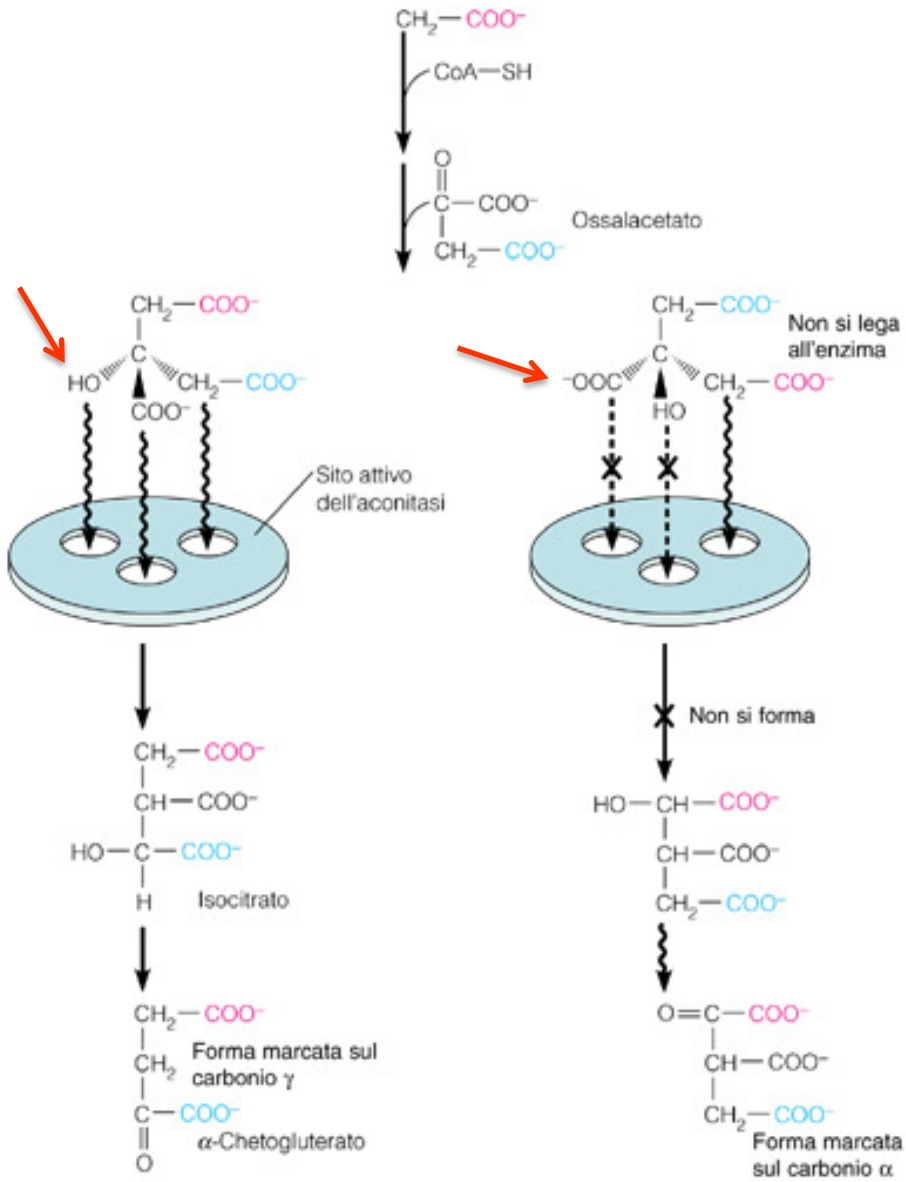


Reaction catalysed by aconitase

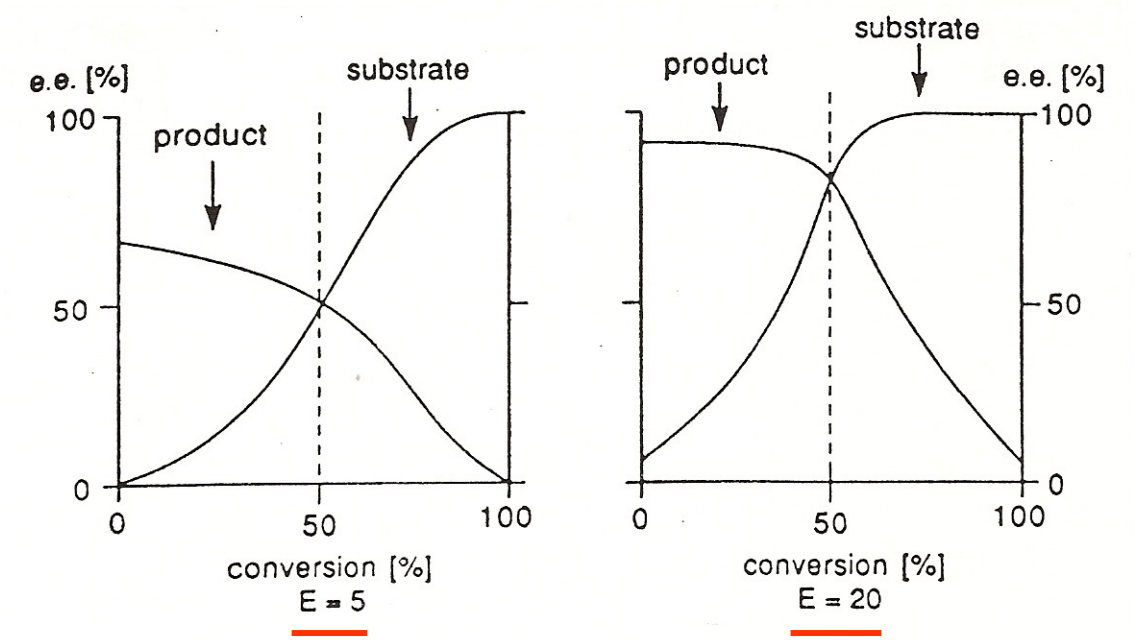


The enzyme aconitase distinguishes the two CH_2COO^- groups





Evaluation of the chiral purity of products and substrates in a reaction catalysed by an enantioselective enzyme



Enantioselectivity is evaluated by measuring:

- The enantiomeric ratio **E** = $(k_{cat}/K_M)S/(k_{cat}/K_M)R$
- The enantiomeric excess e.e. (e.e.% = (S-R)/(S+R)*100)

Enzymes are able to retain catalytic activity in organic solvents

Enzymes retain catalytic activity in organic solvents because they retain a thin shell of water molecules.

Enzymes maintain the pre-existing ionization state before transfer in the organic solvent

→ pH memory.

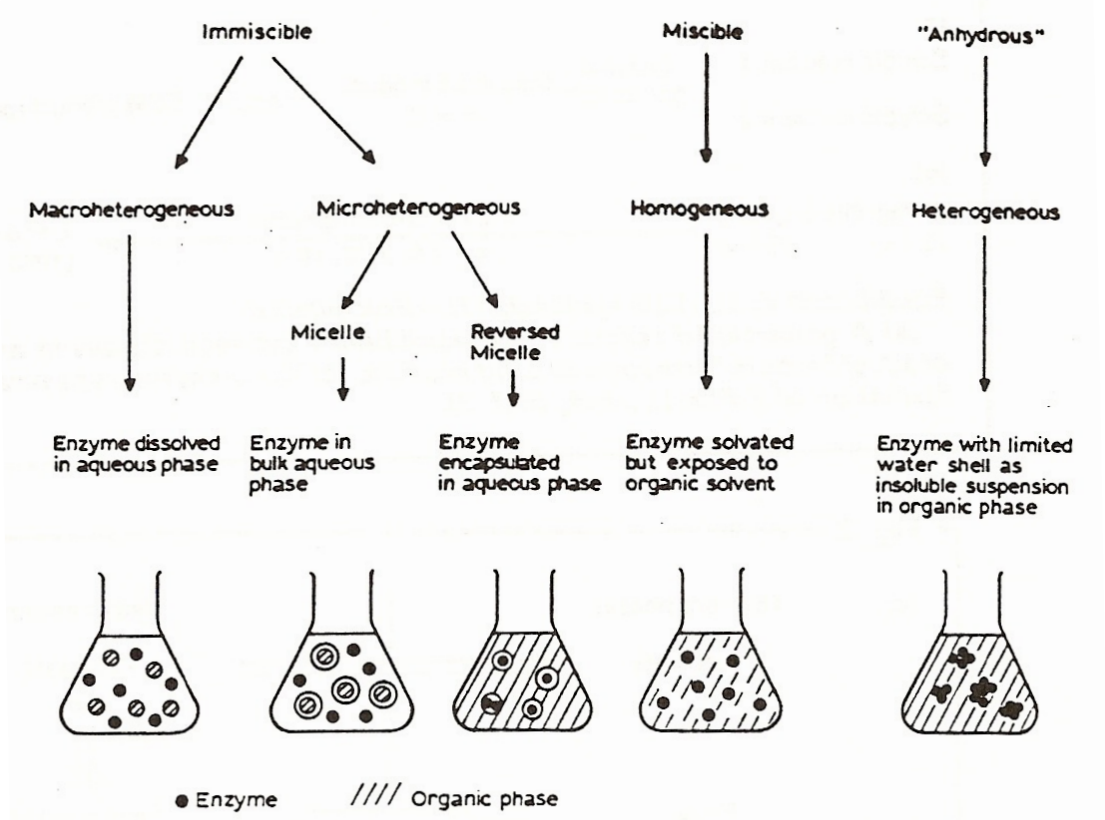
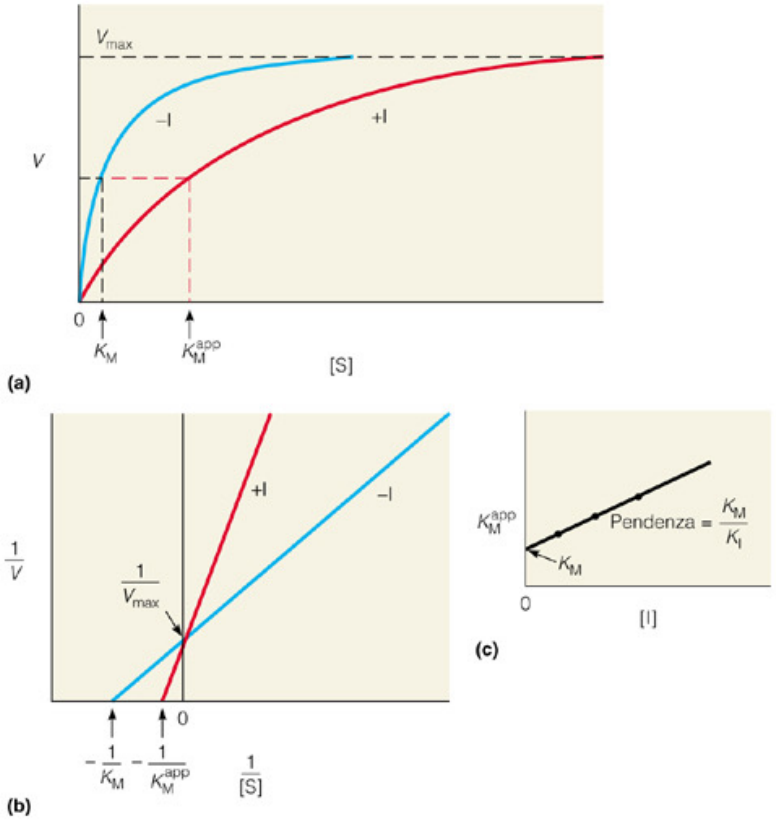


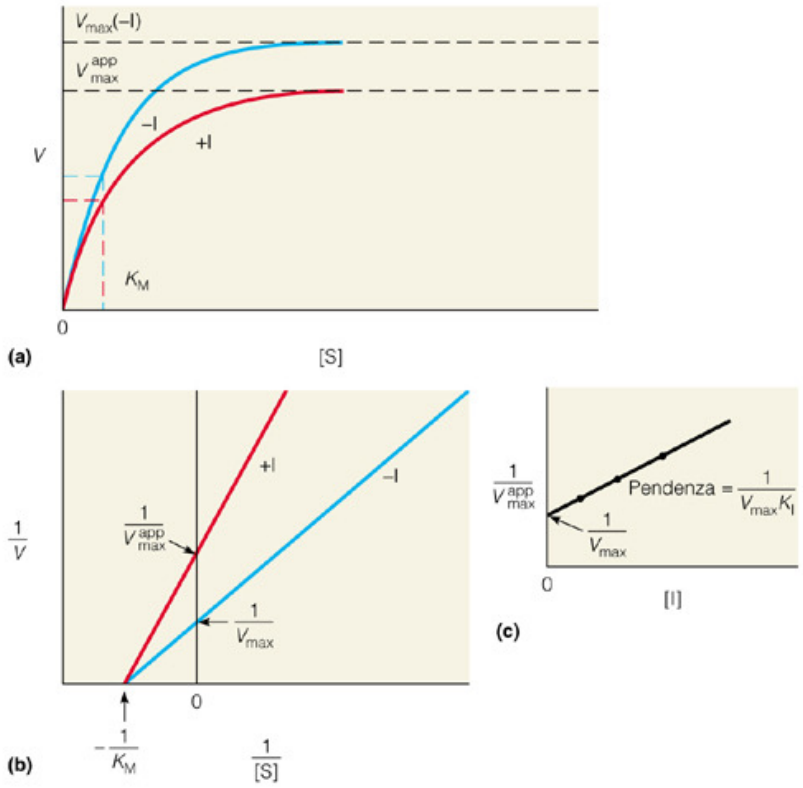
Figure 25. Schematic representation of the alternative miscible and immiscible organic: aqueous solvent systems.

Reversible inhibition

competitive



non competitive

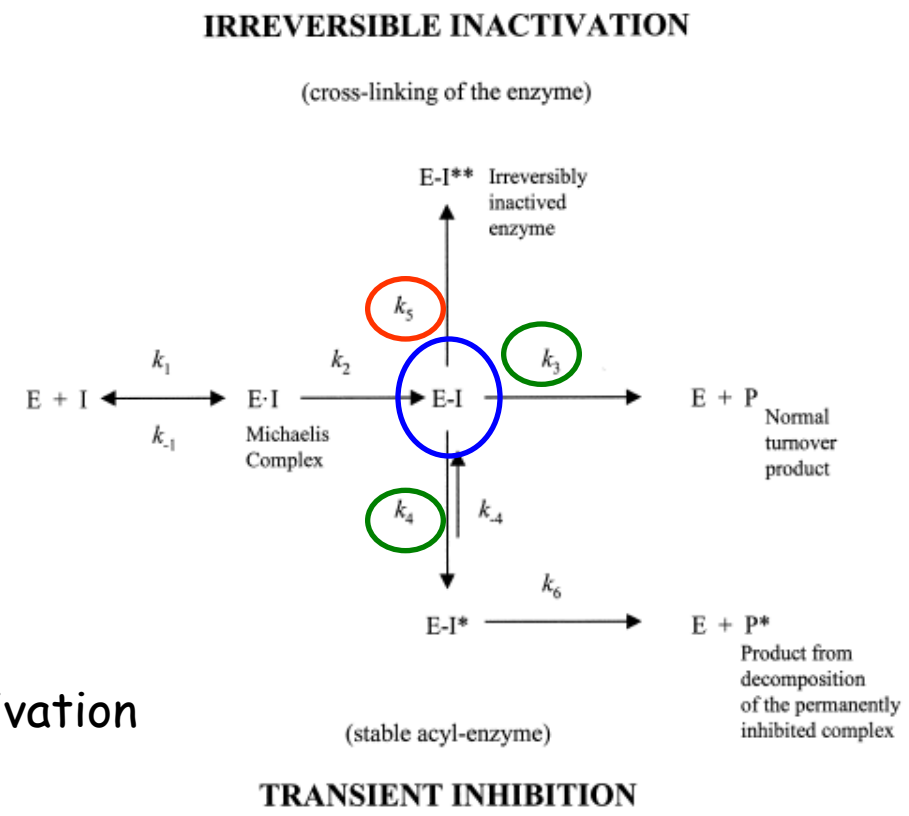


Irreversible inihbition: suicide or mechanism-based inhibitors

Suicide inhibitors are molecules that are 'activated' by the enzyme during the catalytic cycle and become able to inhibit the enzyme.
The E-I intermediate can partition in two ways that lead to transient or irreversible inhibition.
To evaluate the efficiency of a suicide inhibitor we use the ratio

molecules of product/events of inactivation

$$\frac{K_3 + K_4}{K_5}$$



How can you detect irreversible inhibitors?

The activity of the enzyme incubated with the inhibitor decreases with time: the enzymatic activity assay is carried out after having incubated the enzyme with the inhibitor for increasing time.

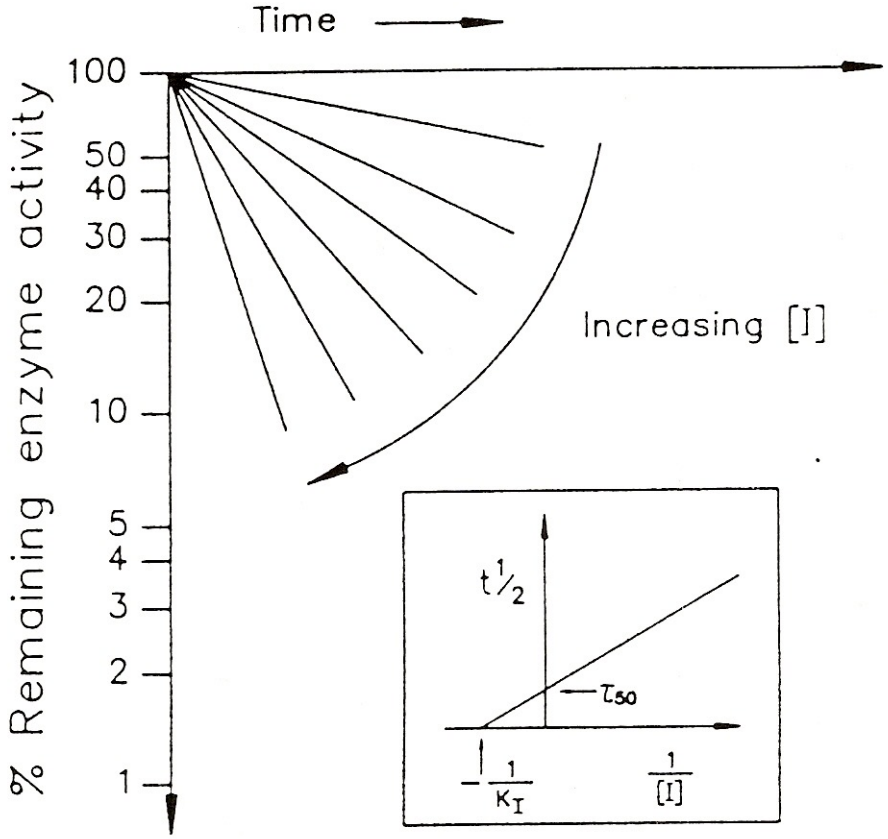
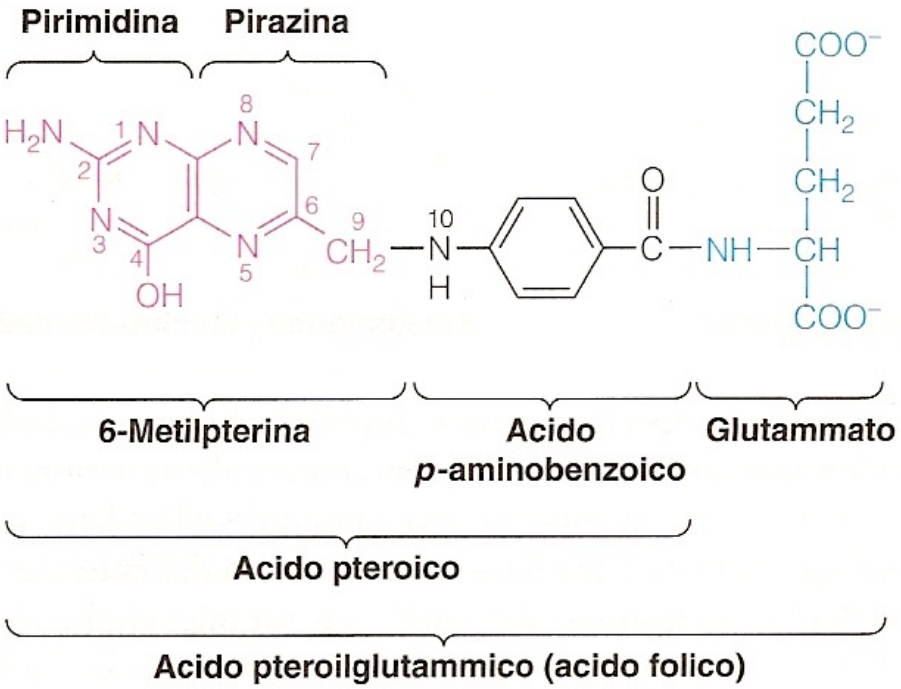
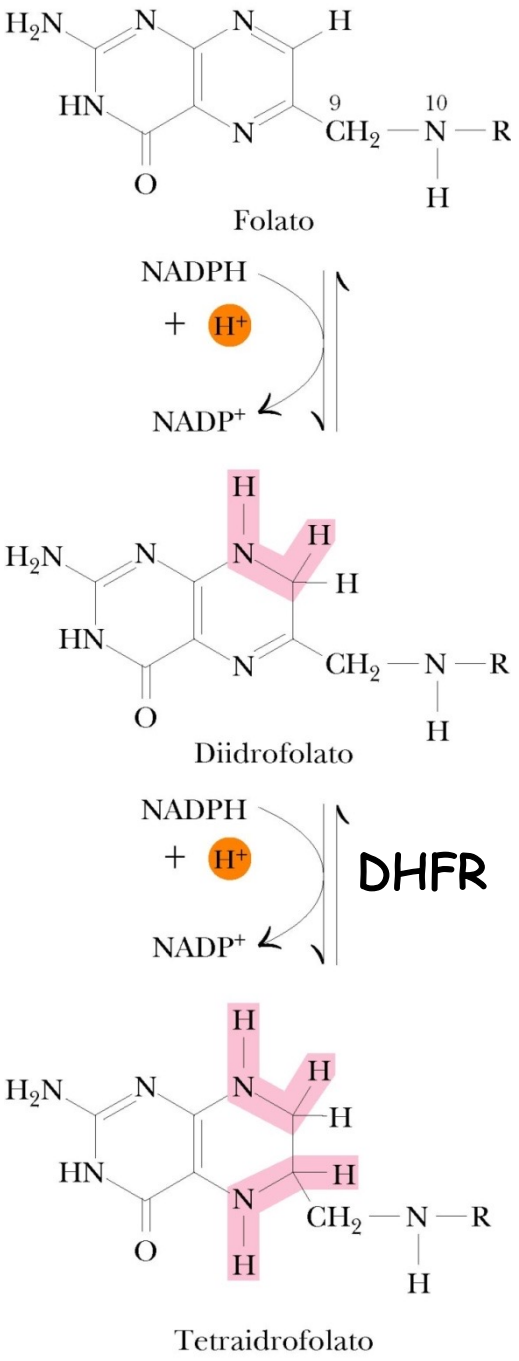


Fig. 1. Semilog plot of remaining enzyme activity against time as a function of various inhibitor concentrations.

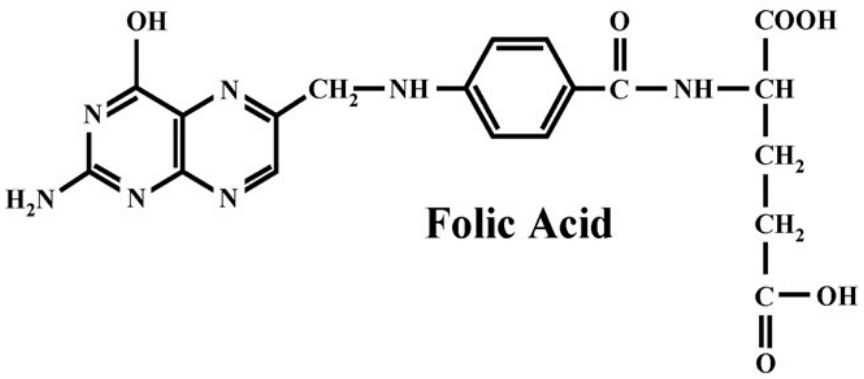
A target for enzyme inhibitors: enzymes involved in the synthesis and metabolism of tetrahydrofolate (THF)



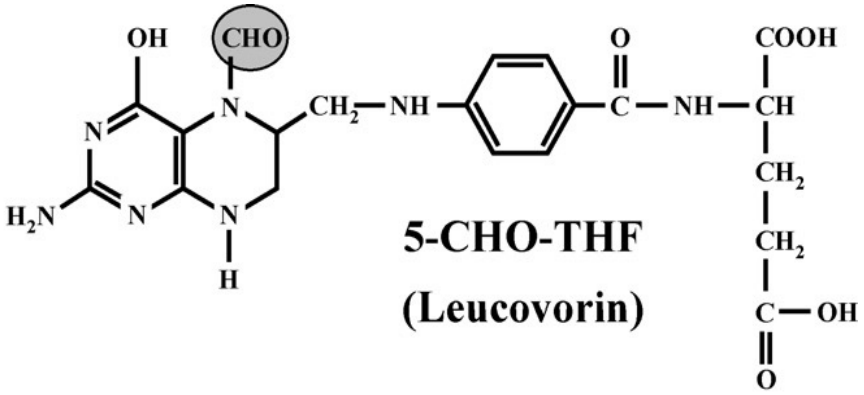
Vitamin B9



Main one-carbon atom adducts of tetrahydrofolate

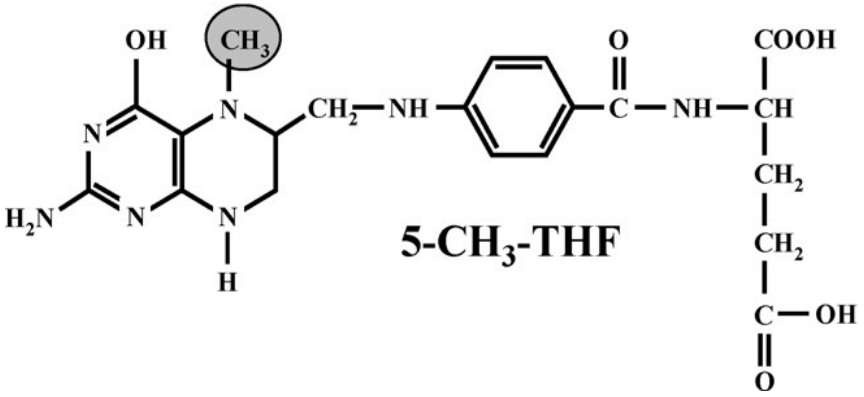


Folic Acid

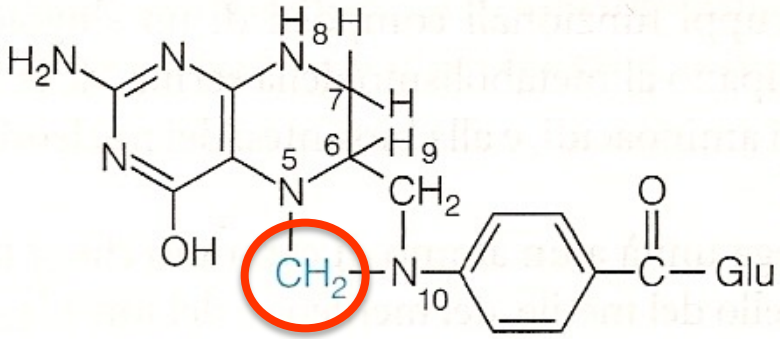


5-CHO-THF

(Leucovorin)

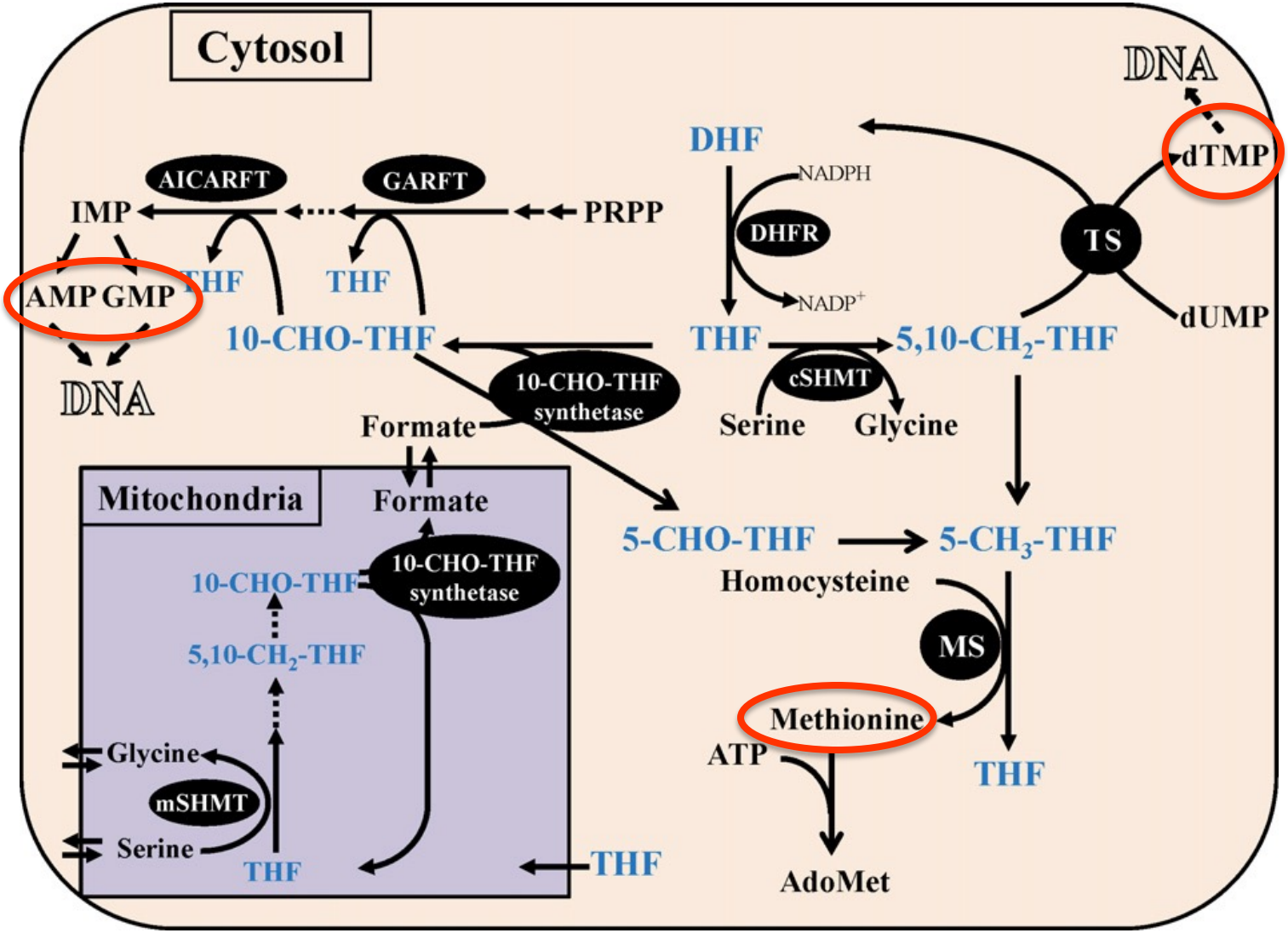


5-CH_3-THF

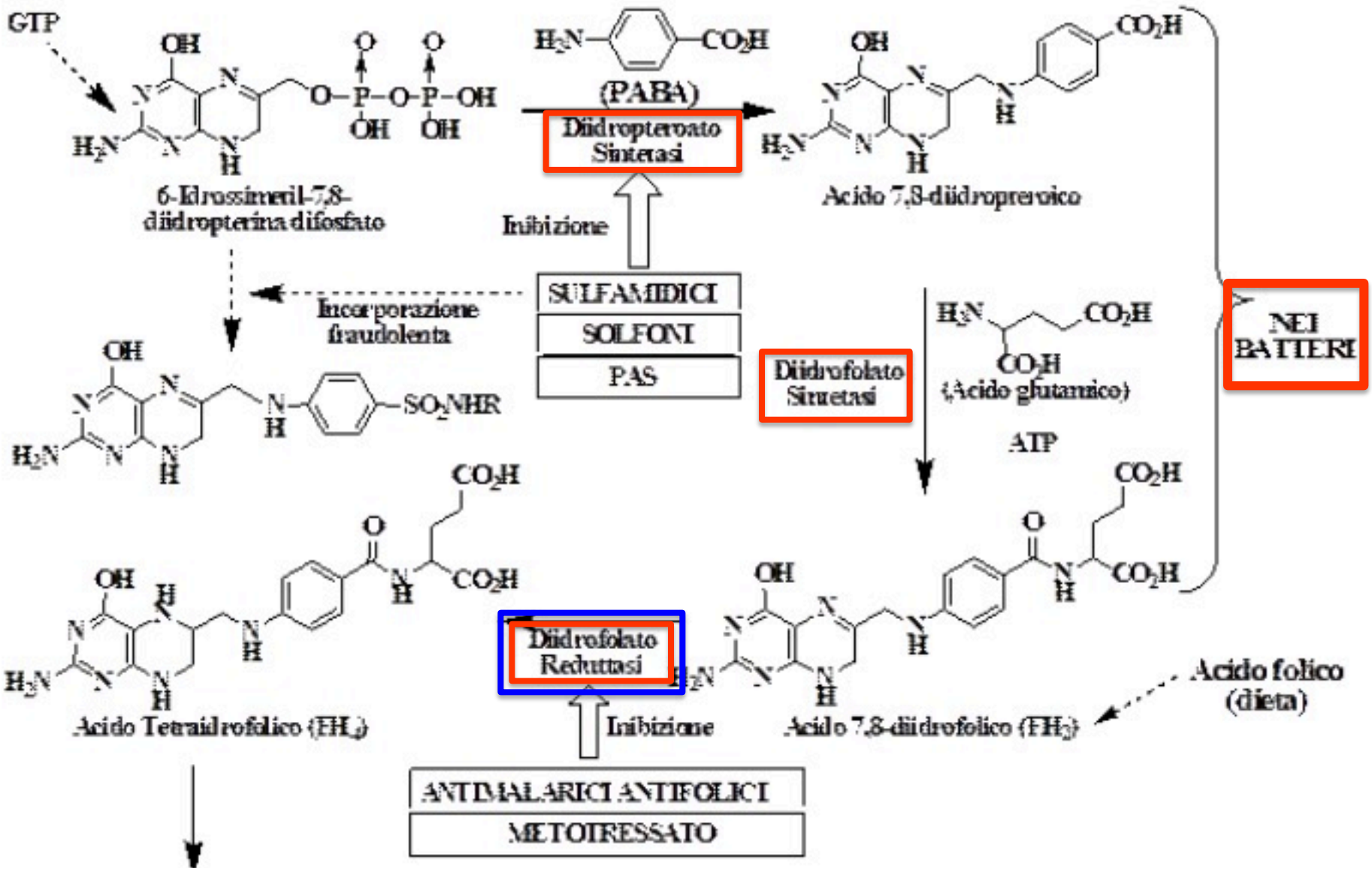


5,10-Metilentetraidrofolato

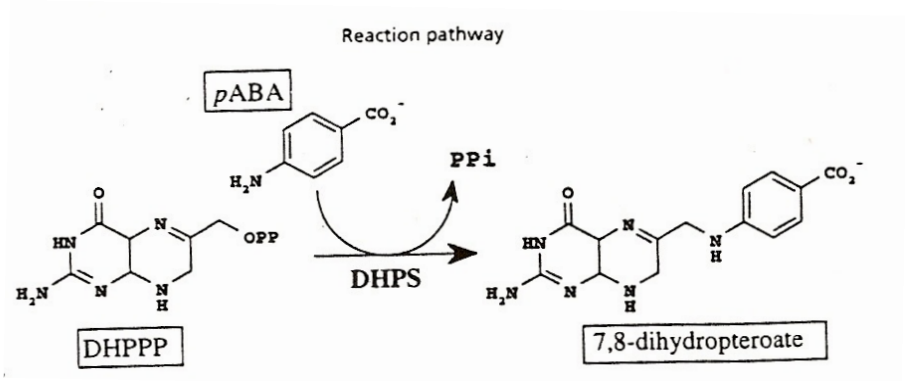
Cellular metabolism of THF: synthesis of nucleotides and amino acids

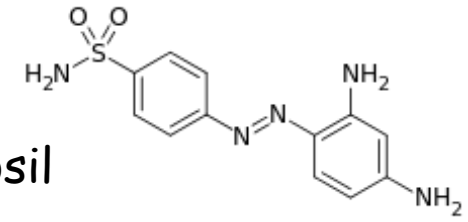


Biosynthesis of tetrahydrofolate

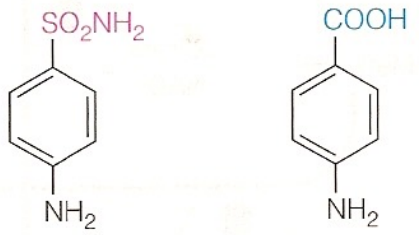


SULFAMIDES
Competitive inhibitors of folic acid synthesis





Prontosil

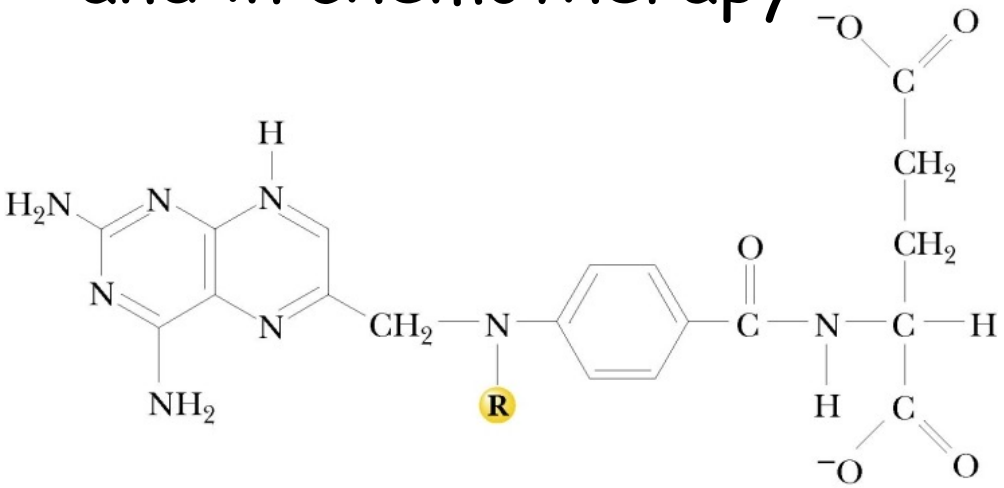


Sulfanilammide Acido *p*-aminobenzoico (PABA)

- Competition with *p*-aminobenzoic acid (*p*-ABA) for 7,8-dihydropterin in the active site of Dihydropteroate synthase (DHPS)
- Demostration of competitive inhibition from kinetic assays.
- Demostration of formation of the product of the inhibition reaction: the products of the reaction pteroate + inhibitor were isolated from E. coli cells grown in the presence of inhibitor labeled with ^{35}S

The inhibitor can be incorporated in place of the substrate and it forms an inactive product.
The product inhibits Dihydrofolate synthase (competitive inhibition)

Competitive inhibitors of Dihydrofolate reductase (DHFR) are used as antibacterials and in chemotherapy

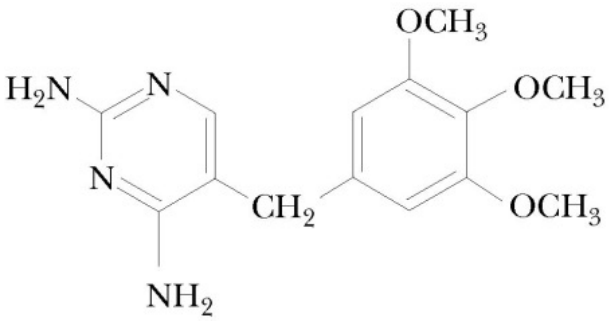


2-Ammino, 4-ammino analoghi dell'acido folico

R = H **Amminopterina**

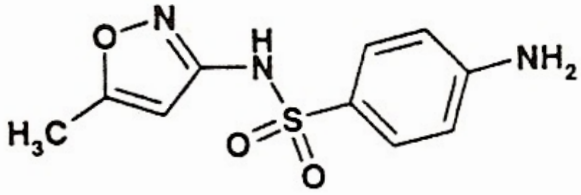
R = CH_3 **Ametopterina (metotressato)**

Bactrim



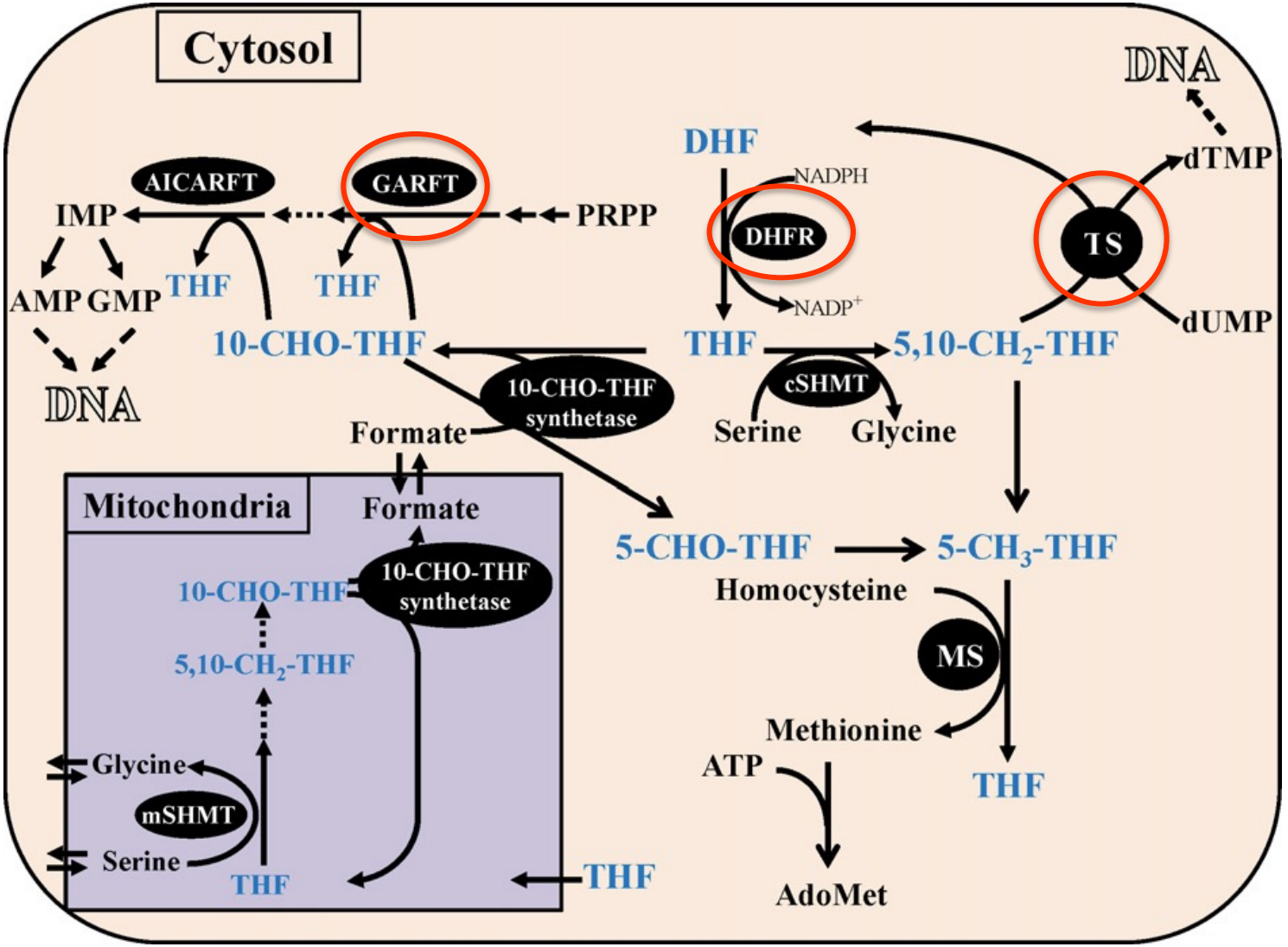
Trimetoprim

+



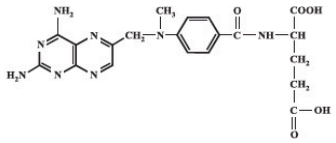
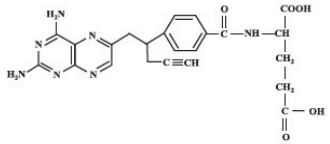
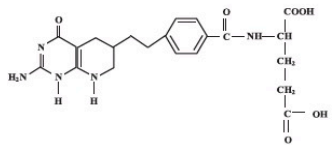
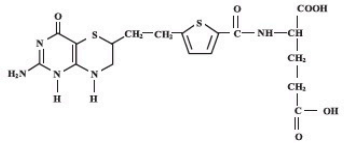
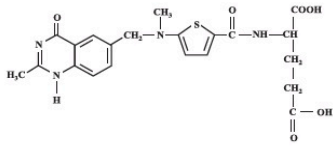
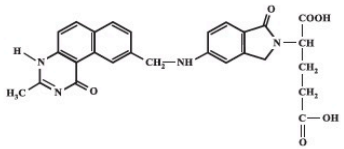
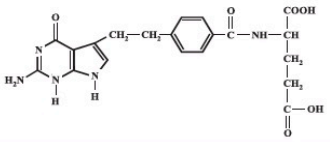
Sulfamethoxazole

Cellular metabolism of THF: synthesis of nucleotides and amino acids

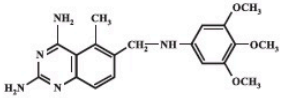
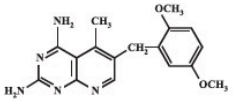
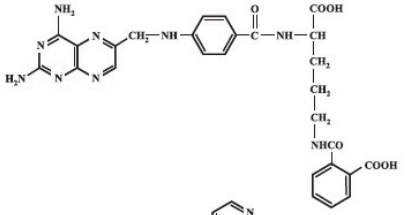
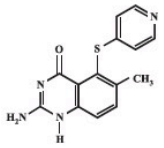
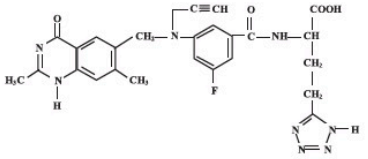
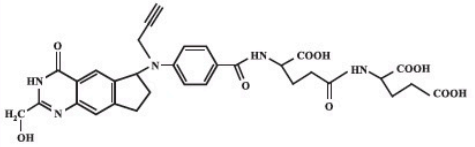


Anti-folates.
A class of drugs used in anti-cancer therapy

Polyglutamatable Antifolate

Antifolate	Chemical Structure	Target Enzyme
Methotrexate		DHFR
Pralatrexate		DHFR
Lometrexol		GARFT
AG2034		GARFT
Raltitrexed		TS
GW1843		TS
Pemetrexed		TS DHFR GARFT

Non-Polyglutamatable Antifolate

Antifolate	Chemical Structure	Target Enzyme
Trimetrexate		DHFR
Piritrexim		DHFR
Talotrexin		DHFR
Nolatrexed		TS
Plevitrexed		TS
BGC 945		TS

Anti-folates: a class of drugs used in anti-cancer therapy

Table 1
Summary of transport, polyglutamylation and target enzyme properties of various antifolates.

Antifolate	Synonyms	Target enzyme	Polyglutamylation	Transport system	Approved for treatment
Polyglutamatable					
Methotrexate	MTX	DHFR	+	RFC	+
Pralatrexate	Folotyn®	DHFR	+	RFC	+
Lometrexol	DDATHF	GARFT	+	RFC/FRα	–
AG2034		GARFT	+	RFC/FRα	–
Pemetrexed	Alimta®/PMX/MTA/LY231514	TS/DHFR/GARFT	+	PCFT/RFC	+
Raltitrexed	Tomudex®/ZD1694	TS	+	RFC/FRα	+
GW1843	GSL7904L/BW1843/1843U89/OSI-7904	TS	+	RFC	–
Non-polyglutamatable					
Trimetrexate	TMQ/Neutrexin®	DHFR	–	PD*	–
Piritrexim	PTX/BW3014	DHFR	–	PD	–
Talotrexin	PT523	DHFR	–	RFC	+
Nolatrexed	AG337/Thymitaq®	TS	–	PD	+
Plevitrexed	ZD9331/BGC9331	TS	–	RFC/FRα	–
BGC 945	ONX-0801	TS	–	FRα	–

*PD—Passive diffusion.

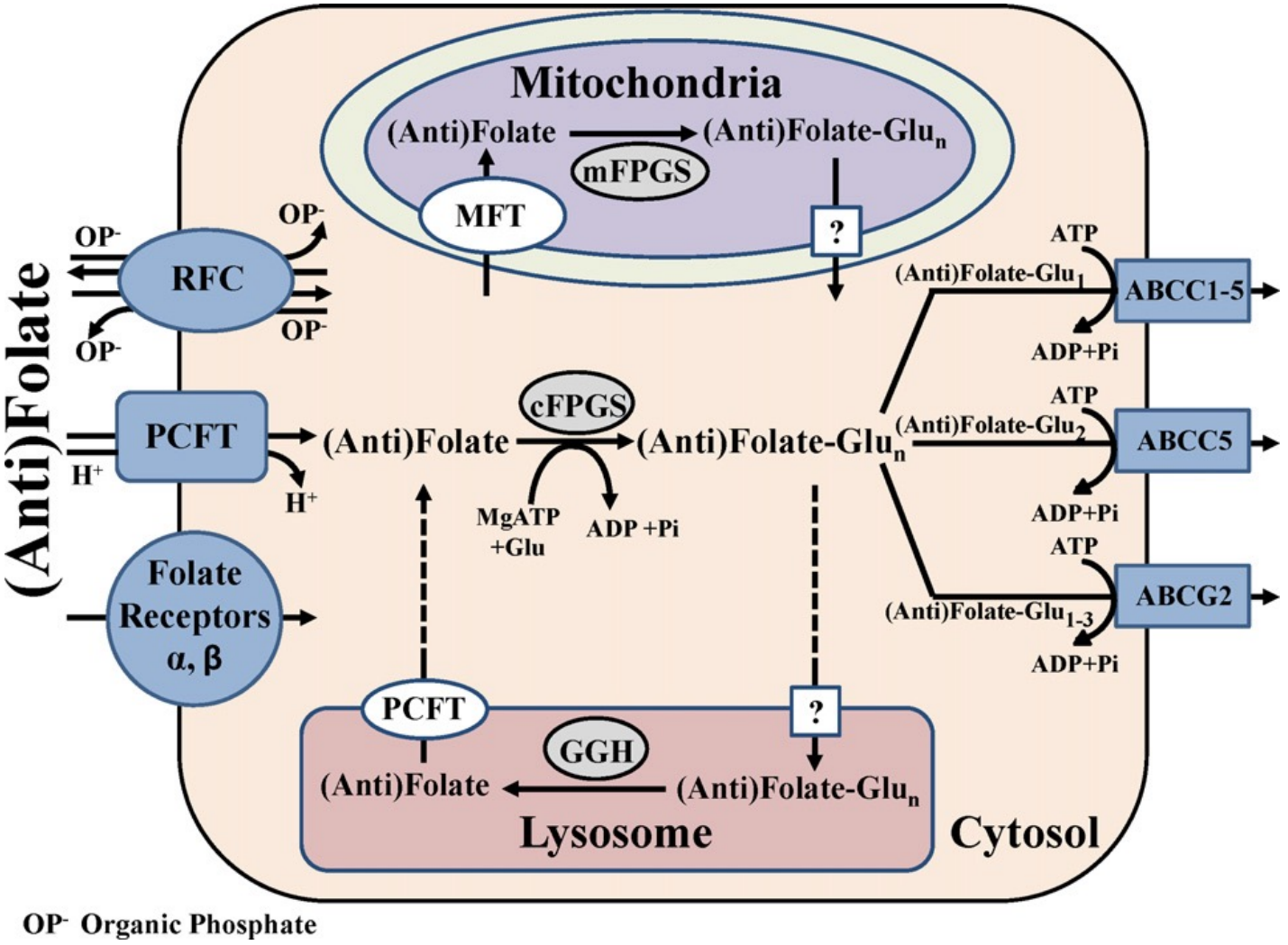
Anti-folates are competitive inhibitors of different enzymes

DHFR: dihydrofolate reductase — synthesis of THF

TS: thymidilate synthase — synthesis of thymine

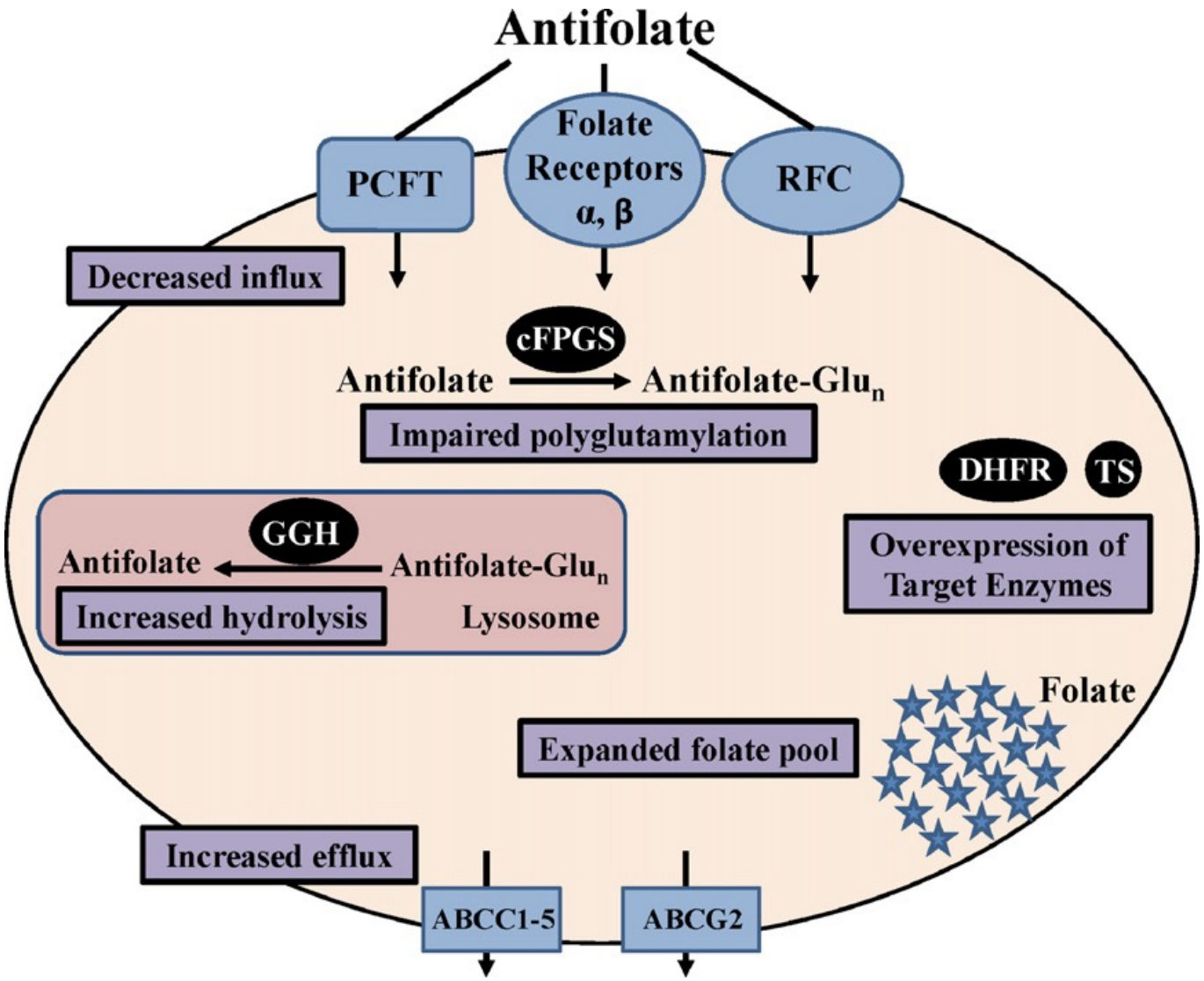
GARFT: glycinamide ribonucleotide formyltransferase — synthesis of purines

Cellular homeostasis of folates and anti-folates

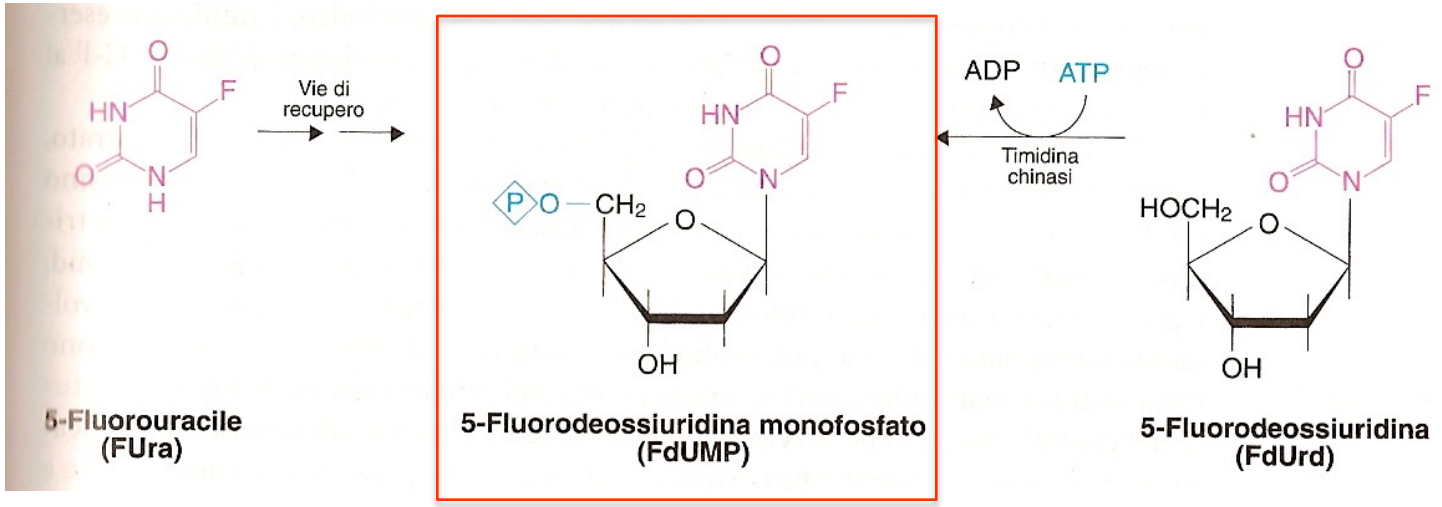


OP$^-$ Organic Phosphate

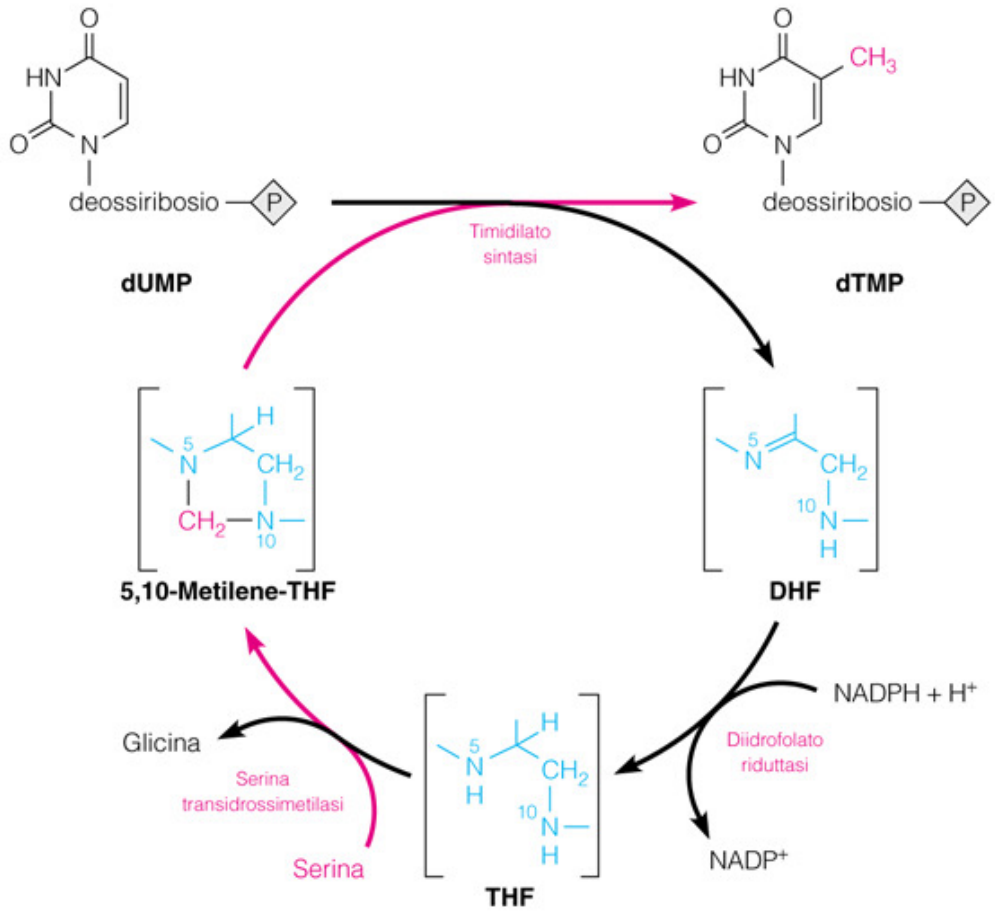
Mechanisms of resistance to anti-folates



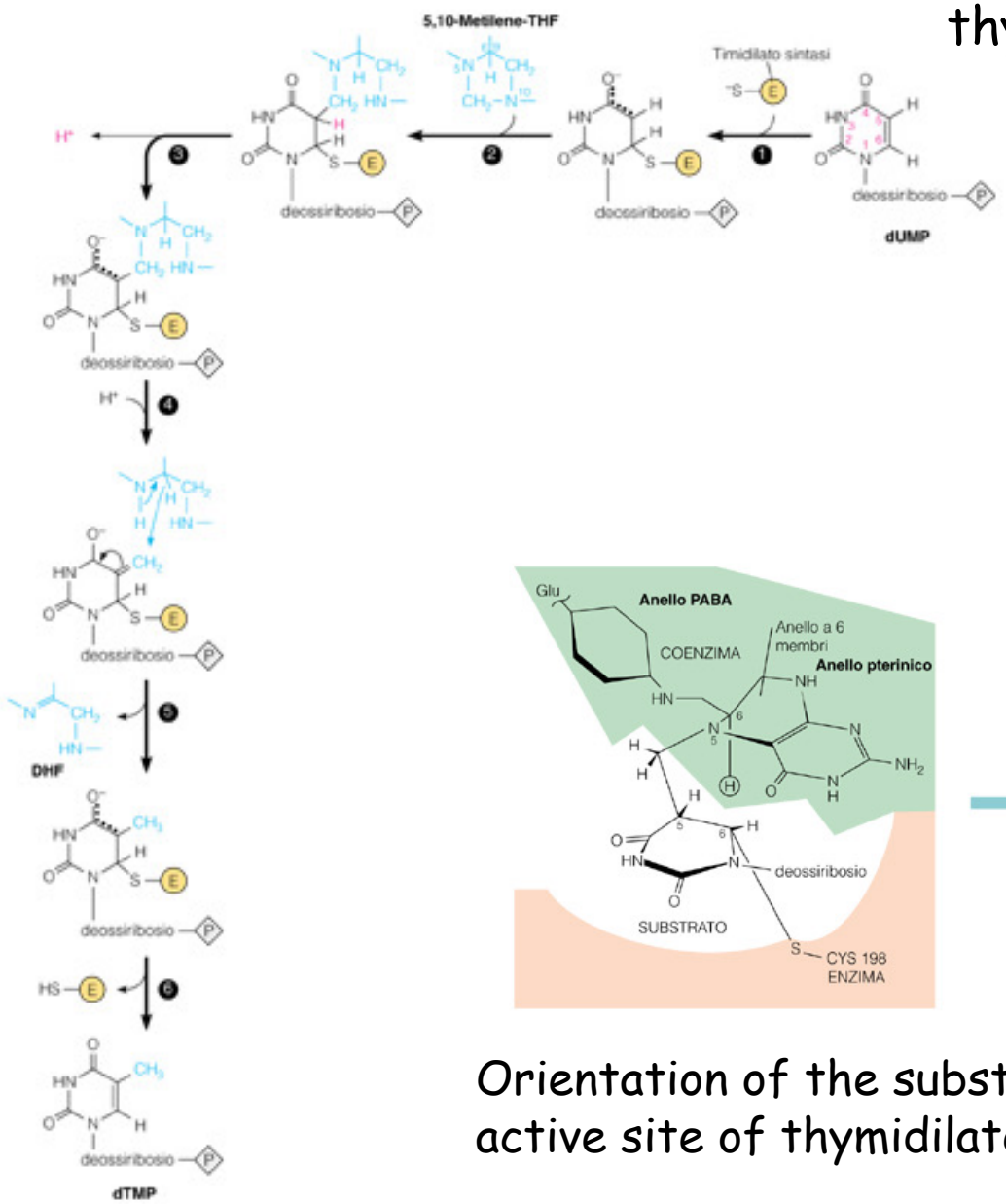
5-fluorouracil: a suicide inhibitor of thymidilate synthase

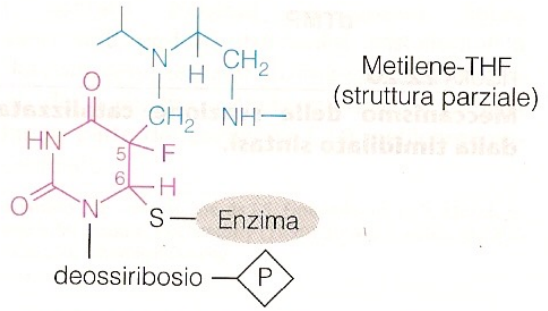


5-fluorouracil: a suicide inhibitor of thymidilate synthase

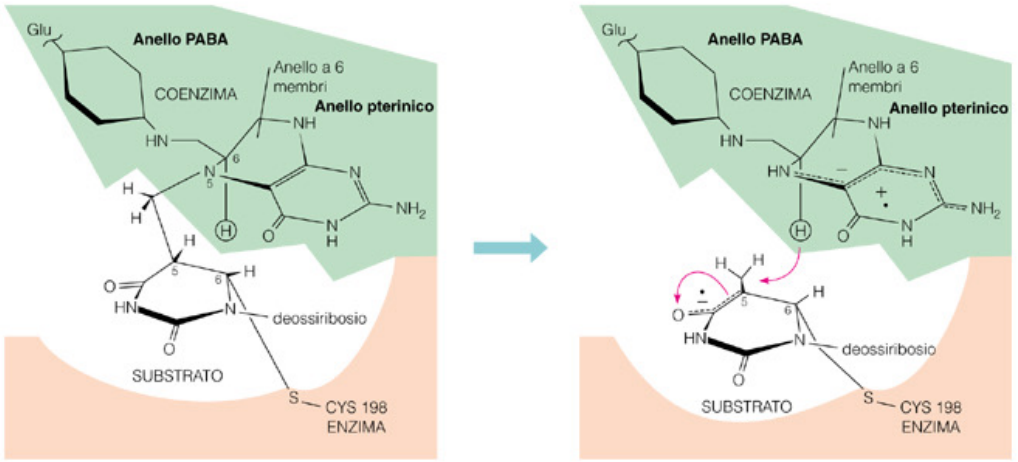


Reaction mechanism of thymidilate synthase





Complesso tra FdUMP legato all'enzima e il 5,10-metilene-THF



Orientation of the substrate and coenzyme in the active site of thymidilate synthase

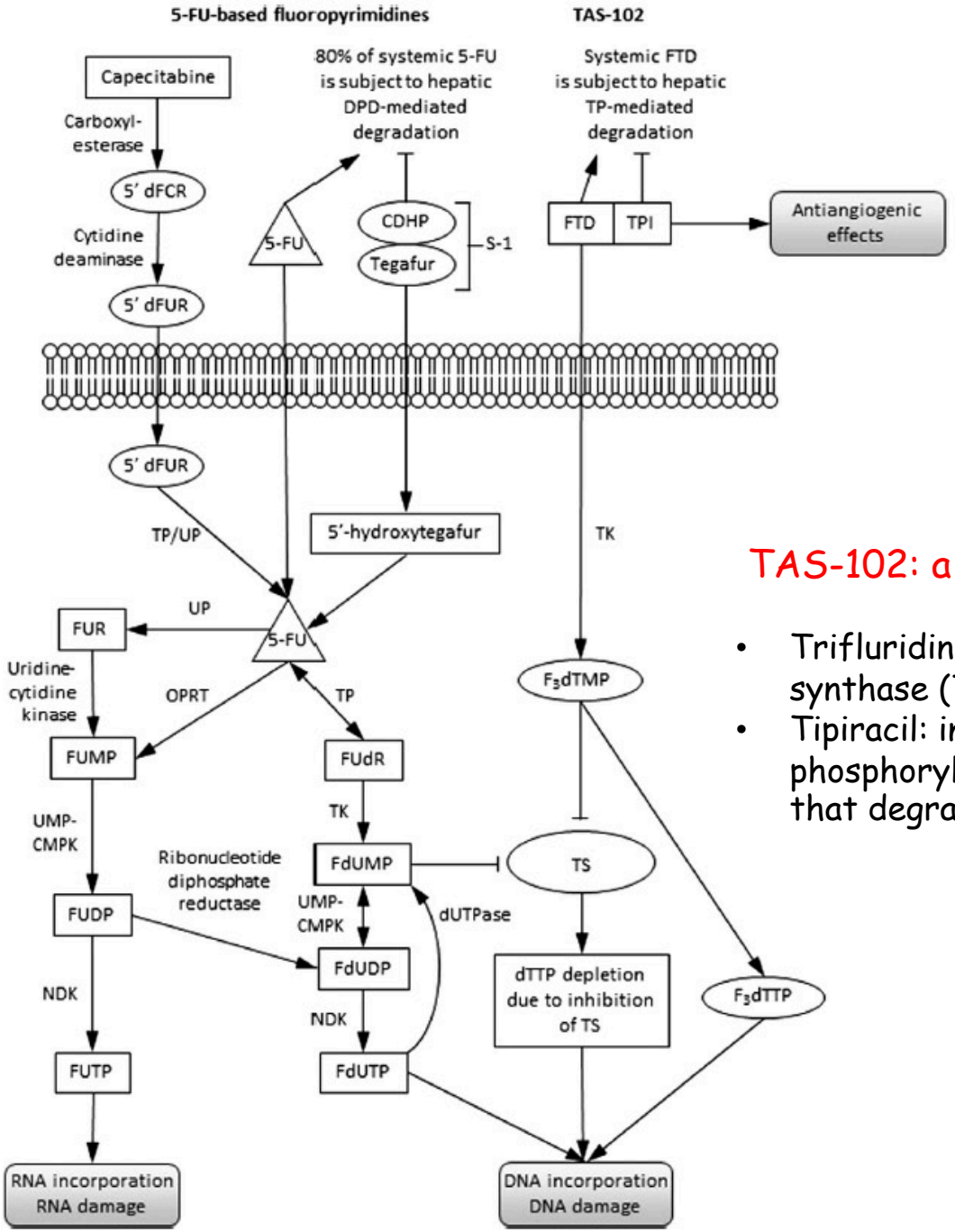


Table 1
Overview of TAS-102 and 5-FU-based chemotherapy agents [2].

Agent	Route of administration	Active metabolites and their functions
5-FU-based agents		
5-FU	IV	FdUMP: Irreversible inhibitor of TS
Capecitabine	Oral	FUTP: Incorporated into RNA
Tegafur–uracil	Oral	FdUTP: Incorporated into DNA
S-1	Oral	
FTD-based agents		
TAS-102 (FTD + TPI)	Oral	F_3dTMP: Reversible inhibitor of TS F_3dTTP: Incorporated into DNA TPI: TP inhibition

5-FU: 5-fluorouracil; F_3dTMP: trifluoromethyl deoxyuridine 5′-monophosphate; F_3dTTP: trifluoromethyl deoxyuridine 5′-triphosphate; FdUMP: fluorodeoxyuridine monophosphate; FdUTP: fluorodeoxyuridine triphosphate; FTD: α,α,α-trifluorothymidine (trifluridine); FUTP: fluorouridine triphosphate; IV: intravenous; TP: thymidine phosphorylase; TPI: tipiracil hydrochloride; TS: thymidylate synthase.

TAS-102: a new anti-tumor drug

- Trifluridine (FTD): thymidilate synthase (TS) inhibitor
- Tipiracil: inhibitor of thymidine phosphorylase (TP), the enzyme that degrades FTD