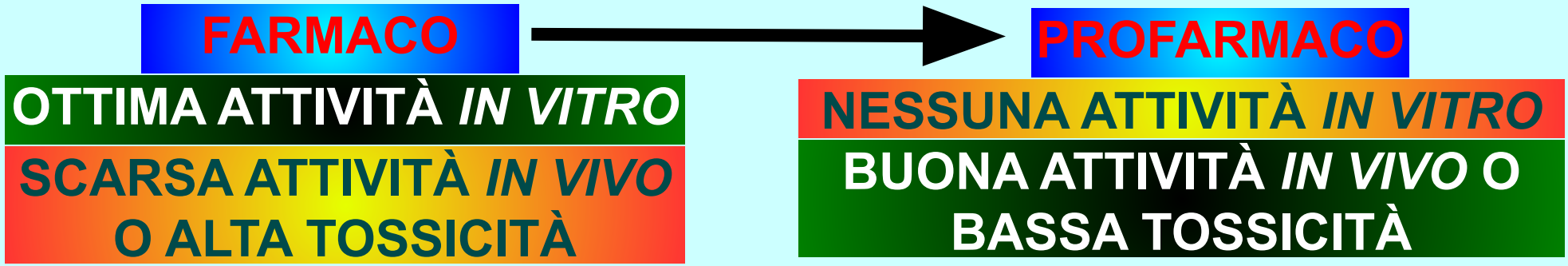
PROFARMACI

Il termine **PROFARMACO** è stato introdotto per la prima volta dal ricercatore A. Albert nel 1958 per descrivere derivati inattivati di principi attivi inattivi che potevano essere utilizzati per alterare temporaneamente le caratteristiche chimico-fisiche delle molecole aumentandone l'**EFFICACIA** e/o diminuendone la **TOSSICITÀ**.

Si usano quando la molecola da somministrare ha un'adeguata attività farmacologica ma un'inadeguata farmacocinetica (assorbimento, distribuzione, metabolismo, eliminazione), soprattutto in caso di scarso assorbimento nel tratto gastrointestinale o breve emivita.



PROFARMACI

Nel progettare un profarmaco bisogna tenere presente l'esigenza di bilanciare l'aumento della lipofilia, necessario per un ottimale assorbimento passivo, con una sufficiente solubilità in acqua, altrimenti la velocità dell'assorbimento orale sarà limitato dalla scarsa dissoluzione.

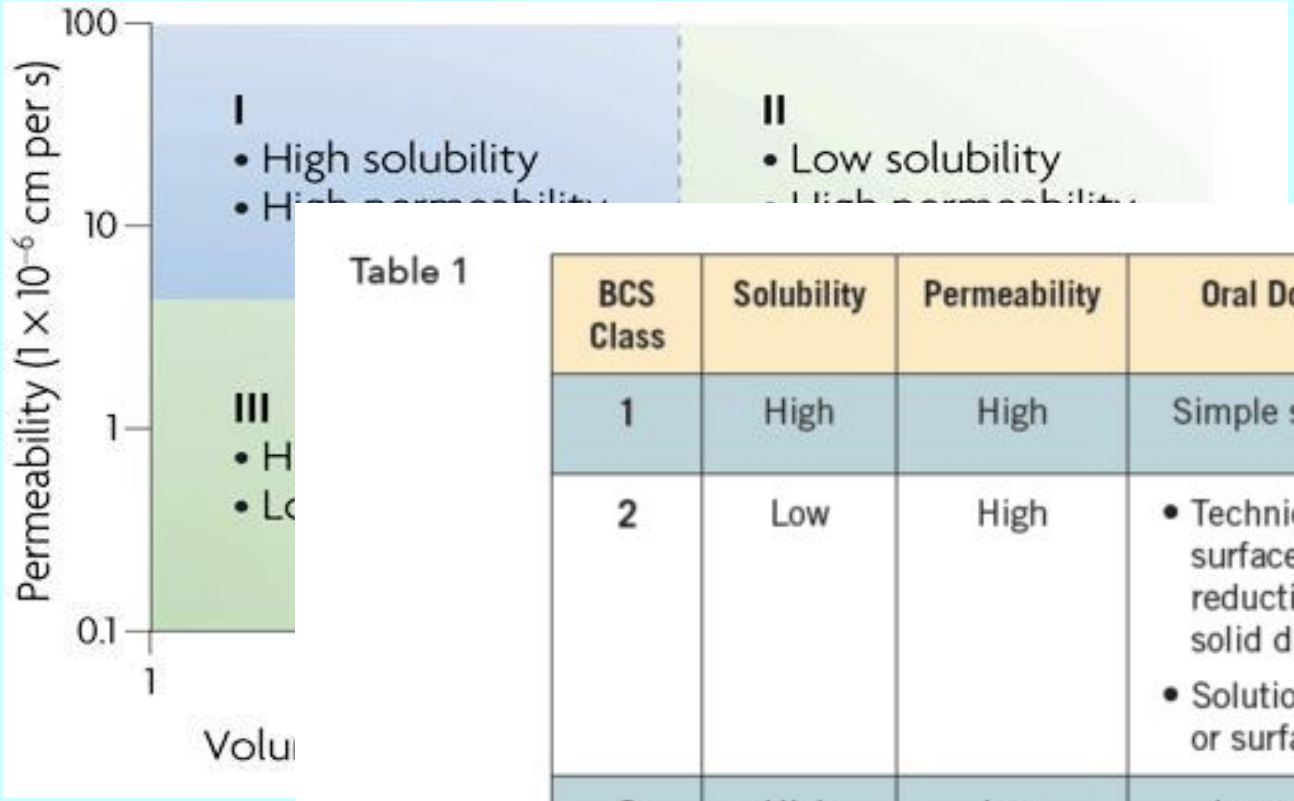


Table 1

BCS Class	Solubility	Permeability	Oral Dosage Form Approach	Chances of Non-oral Dosage Form being Required
1	High	High	Simple solid oral dosage form	Increasing
2	Low	High	• Techniques to increase surface area like particle size reduction, solid solution, solid dispersion • Solutions using solvents and/or surfactants	
3	High	Low	Incorporate permeability enhancers, maximize local lumenal concentration	
4	Low	Low	Combine 2 and 3	

Lubrizol Life Science

Biopharmaceuti

PROFARMACI

Il profarmaco deve avere un'adeguata riconversione in vivo, la riconversione può avvenire:

- prima dell'assorbimento (nel tratto GI)
- durante l'assorbimento (pareti del tratto GI o derma)
- dopo l'assorbimento
- sul sito specifico d'azione

La completezza della riconversione è importante ma anche la velocità può essere regolata.

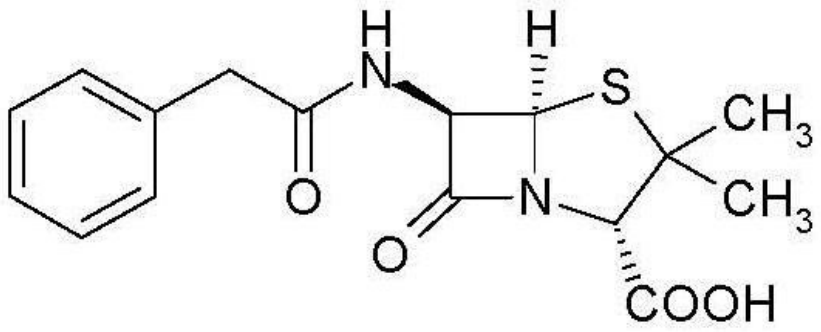
PROFARMACI

CONDIZIONI PER LA SINTESI DI PROFARMACI

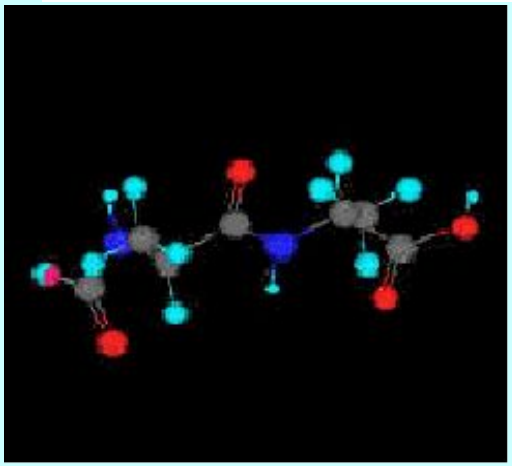
- Gli intermedi chimici devono essere disponibili con alto grado di purezza e a basso costo
- Non si possono utilizzare schemi di sintesi complicati
- Il profarmaco deve essere stabile
- La labilità in vivo deve essere efficiente e la velocità di rilascio (enzimatica o non enzimatica) adeguata
- Il profarmaco ed il “carrier” non devono essere tossici
- La farmacocinetica del principio attivo deve essere ben documentata
- Il profarmaco deve presentare rilevanti vantaggi clinici rispetto all’uso del principio attivo come tale.

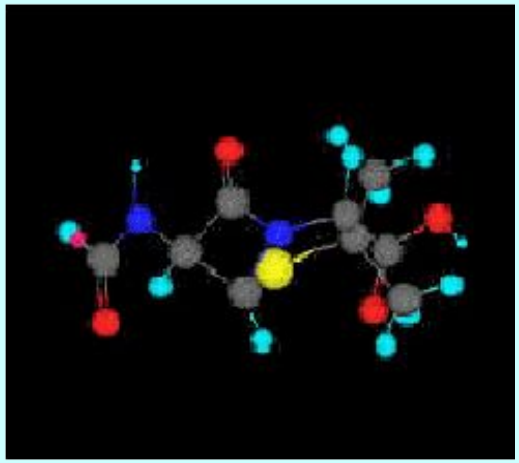
CONCETTO DI FARMACOFORO

Struttura molecolare con le caratteristiche minime indispensabili per ottenere una data attività farmacologica.



PENICILLINA G

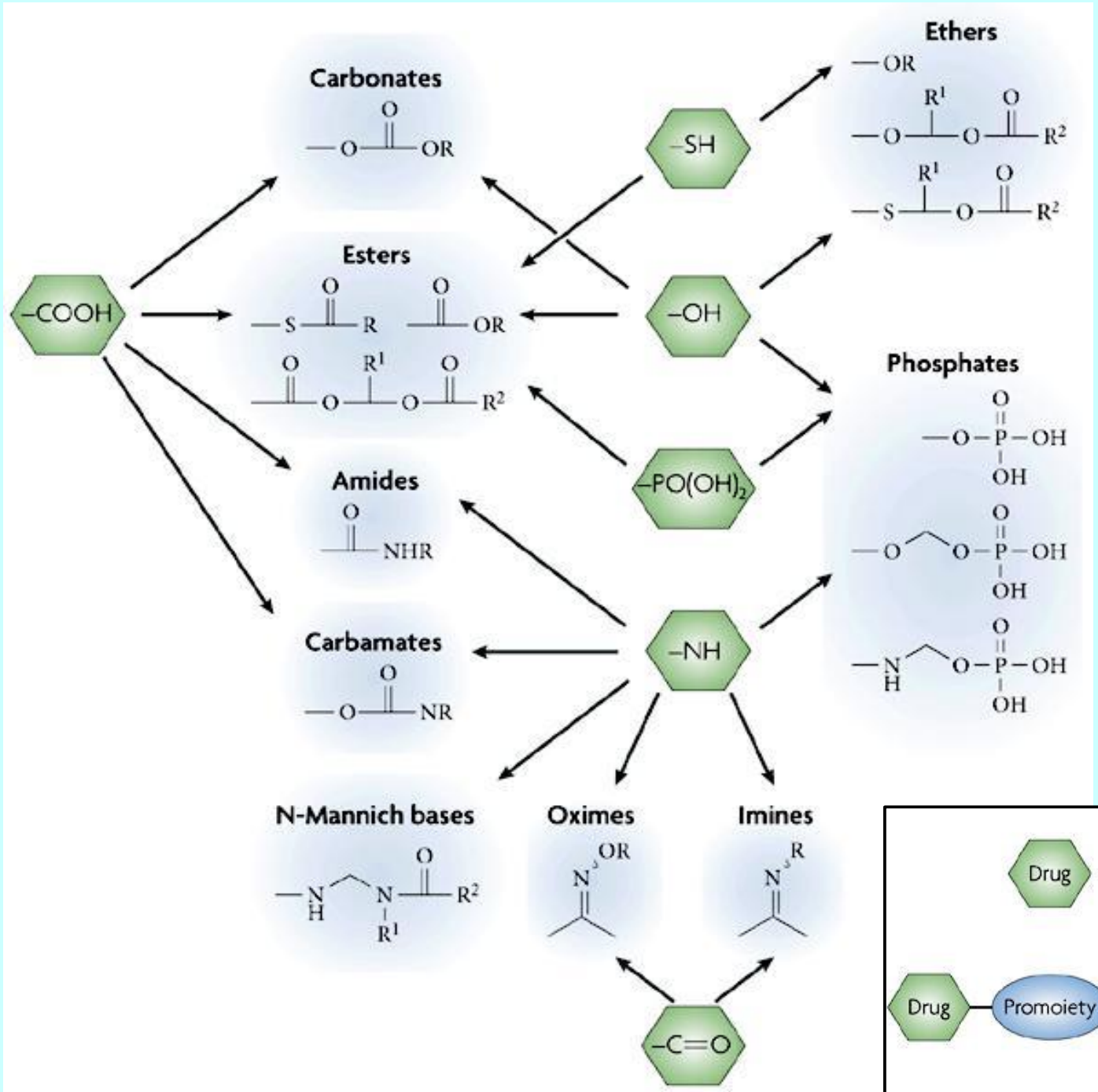


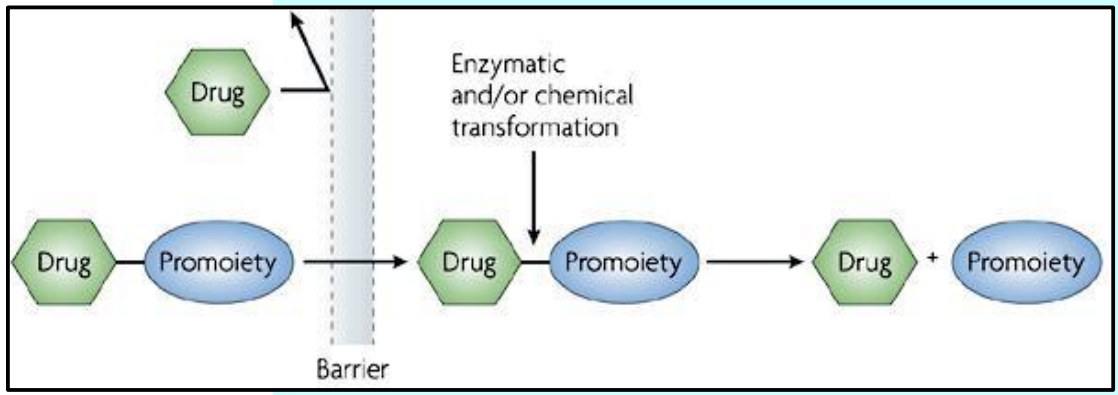


Modifiche della molecola per:

- Migliorare le caratteristiche **farmacodinamiche** (modifiche del farmacoforo)
- Migliorare le caratteristiche **farmacocinetiche**
  - Ottenere un pro-farmaco
- Altre problematiche (resistenza).

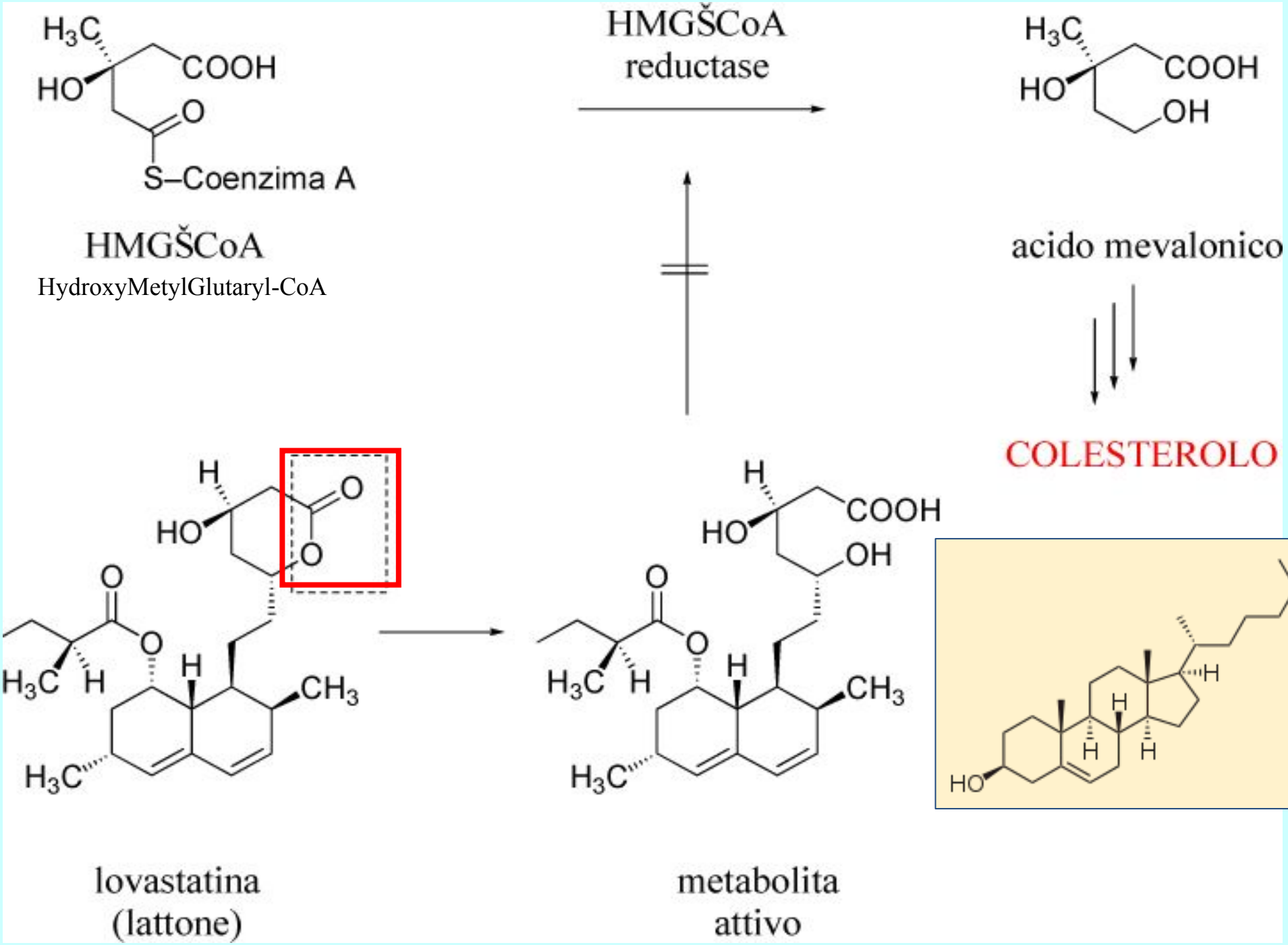
PROFARMACI


Carbonates
Esters
Amides
Carbamates
N-Mannich bases
Oximes
Imines
Ethers
Phosphates
–COOH
–SH
–OH
–PO(OH)2
–NH
–C=O

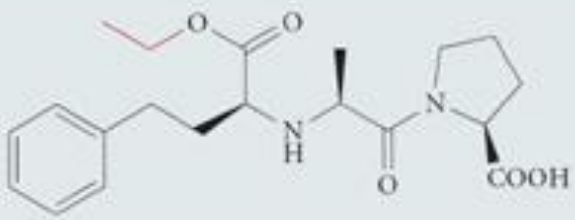
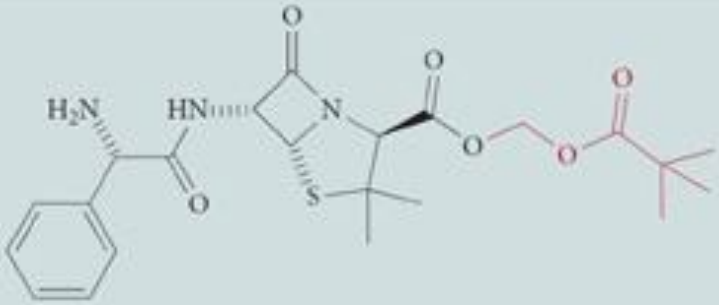
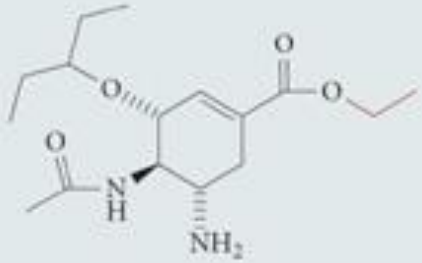
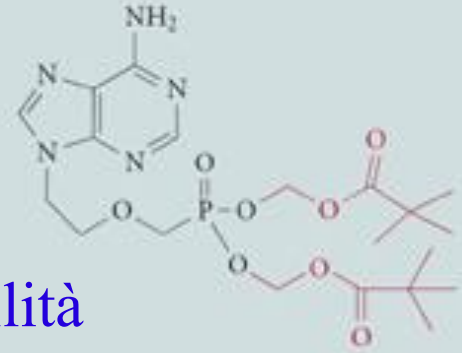

Drug
Enzymatic
and/or chemical
transformation
Drug
Promoiety
Drug
Promoiety
Drug
+
Promoiety
Barrier

PROFARMACI

Esteri

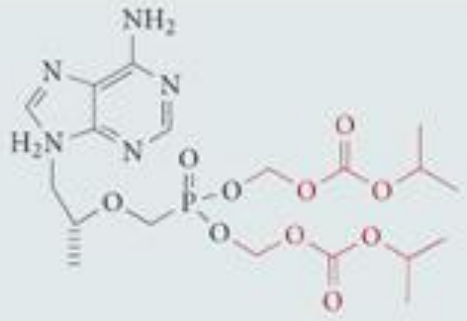
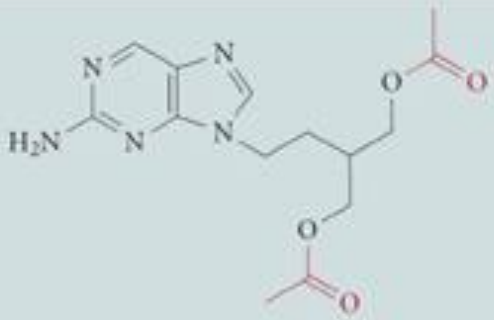
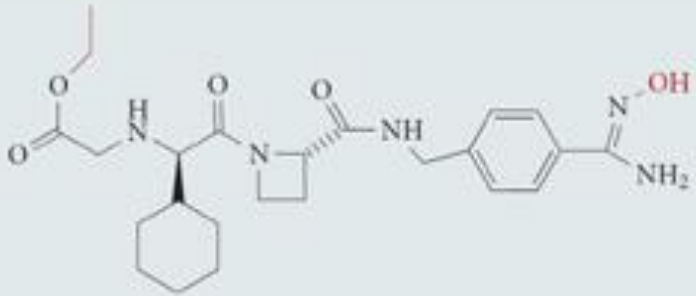
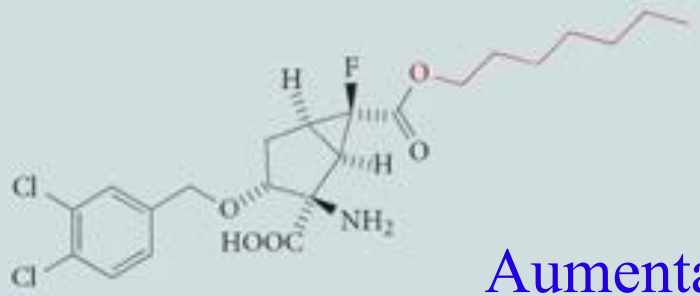


PROFARMACI

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Enalapril (angiotensin-converting enzyme inhibitor)	Monoethyl ester of enalaprilat		• Bioconversion by esterases • The oral bioavailability of enalaprilat in humans is 36–44% • 53–74% of the administered dose is absorbed[3,172]
Pivampicillin (β-lactam antibiotic)	Pivaloylmethyl ester of ampicillin		• Bioconversion by esterases • The oral bioavailability of 32–55% for ampicillin increased to 87–94% for pivampicillin[171,174]
Oseltamivir (anti-influenza) Tamiflu	Ethyl ester of oseltamivir carboxylate		• Bioconversion by esterases • The oral bioavailability of less than 5% in rat and marmoset for oseltamivir carboxylate increased to 80% for oseltamivir in humans[80–82]
Adefovir dipivoxil (antiviral)	Bis-(pivaloyloxy-methyl) ester of adefovir		• Bioconversion by esterases and phosphodiesterases • The oral bioavailability of ~10% for adefovir increased to 30–45% for adefovir dipivoxil[78,79]

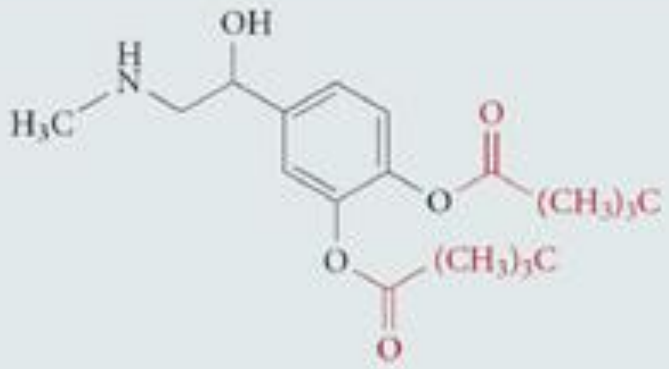
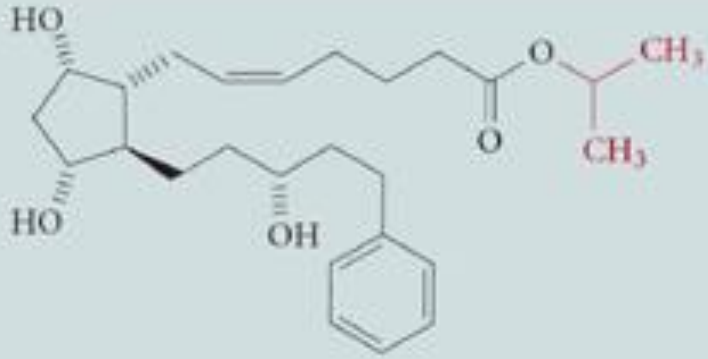
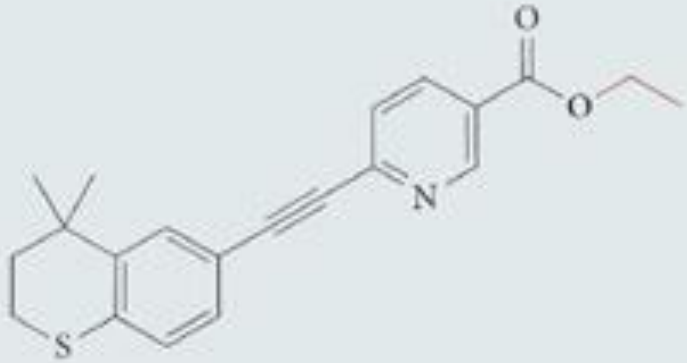
Aumentata lipofilicità o permeabilità

PROFARMACI

Tenofovir disoproxil (antiviral)	Bis-(isopropyloxy-carbonyloxymethyl) ester of tenofovir		• Bioconversion by esterases and phosphodiesterases • The oral bioavailability of tenofovir from tenofovir disoproxil is 39% in the fed state[74,76,77]
Famciclovir (antiviral)	Dimethyl ester of penciclovir		• Bioconversion by esterases and oxidation from purine to guanide • The oral bioavailability of 4% for penciclovir increased to 75% for famciclovir[175–177]
Ximelagatran (anticoagulant)	Hydroxyamidine and ethyl ester of melagatran		• Bioconversion by esterases and reductive enzymes • The oral bioavailability of 3–7% for melagatran increased to 20% for ximelagatran[84,86]
MGS0210 (glutamate receptor (MGLUR2) antagonist)	*n*-Heptyl ester of MGS0039		• Bioconversion by esterases • The oral bioavailability of less than 13% for MGS0039 in monkeys increased to 44% for MGS0210 in monkeys[41,50]

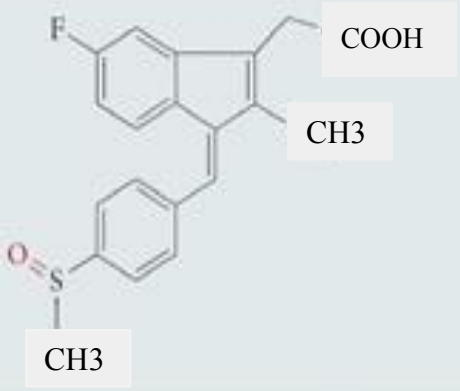
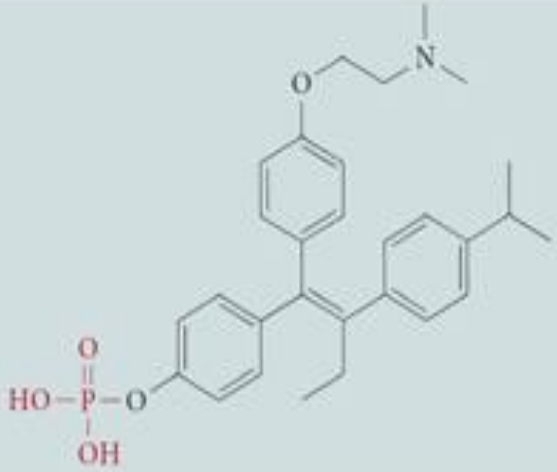
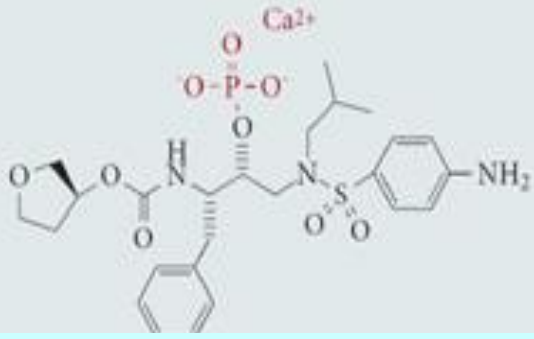
Aumentata lipofilicità o permeabilità

PROFARMACI

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Dipivefrin (glaucoma)	Dipivalic acid diester of adrenaline		• Bioconversion by esterases • More lipophilic (600-fold) dipivefrin is able to permeate the human cornea 17-times faster than adrenaline[119,120]
Latanoprost (glaucoma)	Isopropyl ester of latanoprost acid		• Bioconversion by esterases • Improved lipophilicity achieves better ocular absorption and safety[124,186]
Tazarotene (topical skin disorders, psoriasis, acne)	Ethyl ester of tazarotenic acid		• Bioconversion by esterases • Is both a prodrug and soft drug (undergoes oxidative deactivation) • Improved lipophilicity and maintained adequate aqueous solubility; resulted in better skin permeation[131,132]

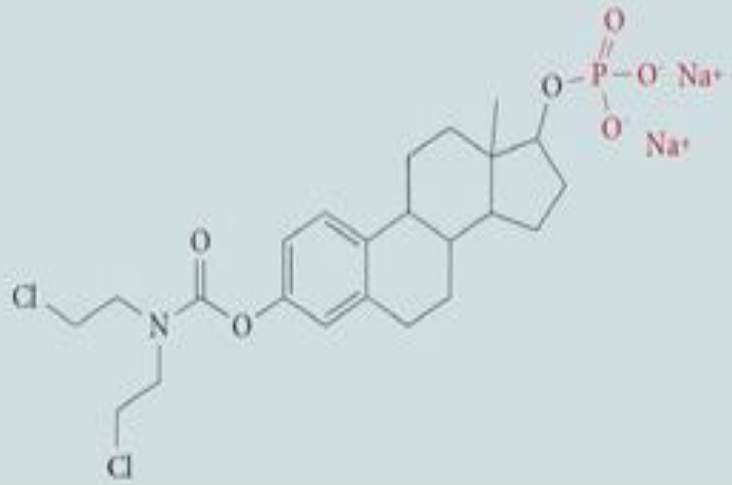
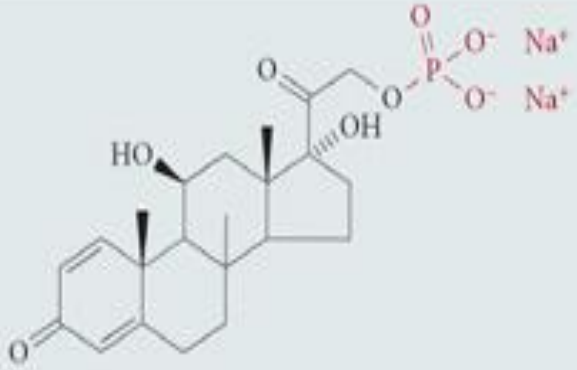
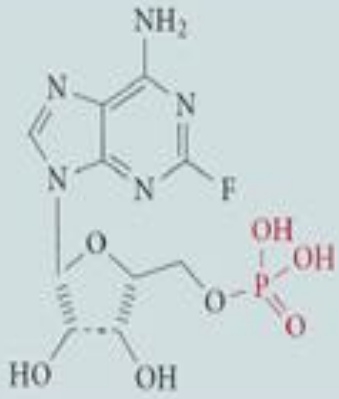
Somministrazione topica

PROFARMACI

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Sulindac (non-steroidal anti-inflammatory)	Oxide prodrug of sulindac sulphide		• Bioprecursor prodrug that is reduced to the active sulphide form after oral absorption • ~ 100-fold increase in aqueous solubility[62,65]
Miproxifene phosphate, TAT-59 (anticancer)	Phosphate ester of miproxifene/DP-TAT-59		• Bioconversion by alkaline phosphatases • Aqueous solubility at pH 7.4 increased by ~1,000-fold[69] • Enhanced bioavailability to 28.8% in rats and 23.8% in the dog[66] • Dose-linear pharmacokinetics in humans[69]
Fosamprenavir (antiviral)	Phosphate ester of amprenavir		• Bioconversion by alkaline phosphatases • 10-fold increased aqueous solubility • More simplified and patient compliant dosage regimen • Prolonged exclusive patent[70-72]

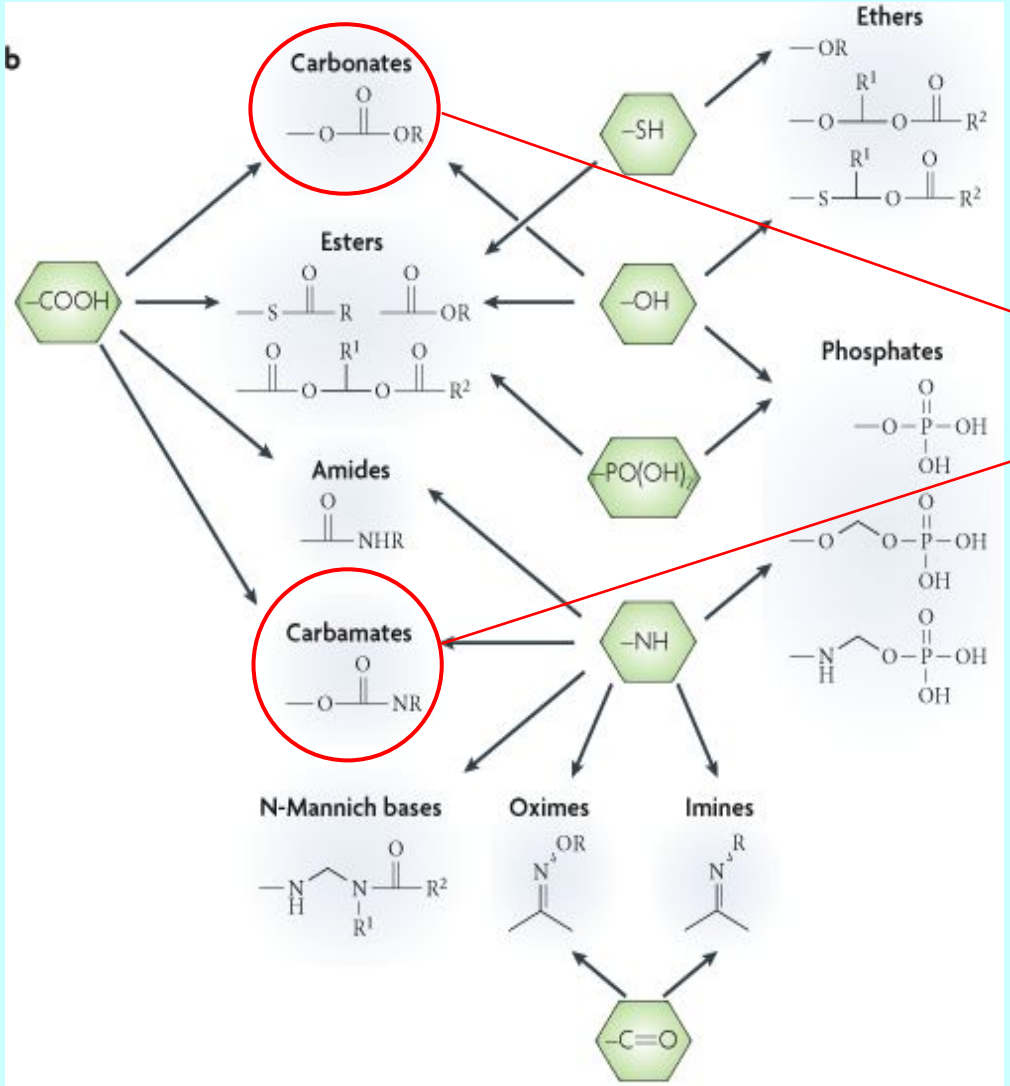
Aumentata solubilità

PROFARMACI

Estramustine phosphate (anticancer)	Phosphate ester of estramustine		• Bioconversion by alkaline phosphatases • Marketed both as injectable and oral formulations for the treatment of prostate carcinoma since the mid-1970s[178,179]
Prednisolone phosphate (glucocorticoid)	Phosphate ester of prednisolone		• Bioconversion by alkaline phosphatases • The prodrug enabled the development of a liquid formulation, and thus, improved childrens' compliance to prednisolone treatment[28,180]
Fludarabine phosphate (antiviral)	Phosphate ester of fludarabine		• Bioconversion by alkaline phosphatases • Until recently, fludarabine phosphate was marketed only for parenteral use[181] • Based on a modest advantage over the parent drug, development of an oral prodrug of fludaribine may have only been as a consequence of the prior existence of a commercial parenteral prodrug[28,181]

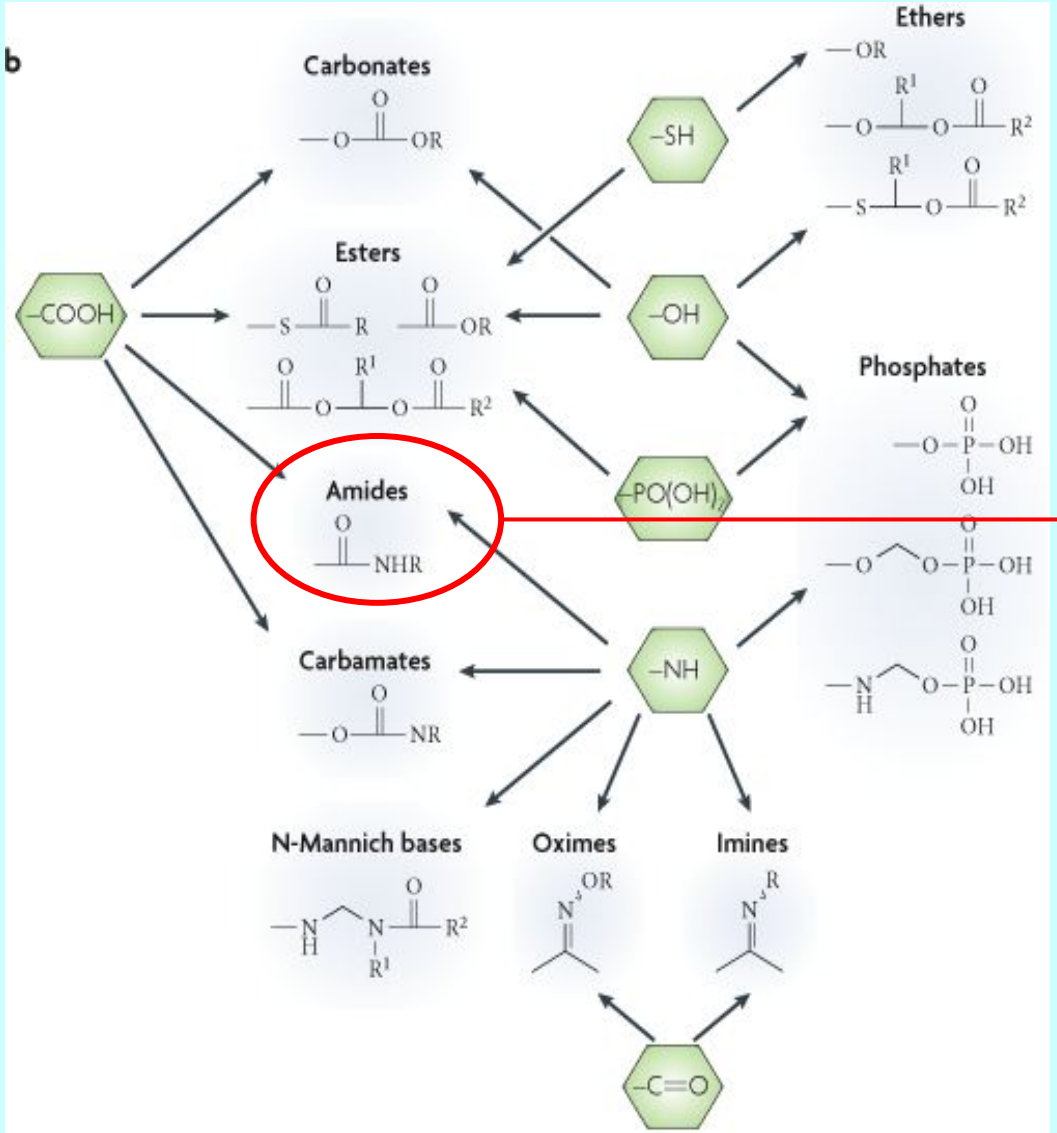
Aumentata solubilità

PROFARMACI



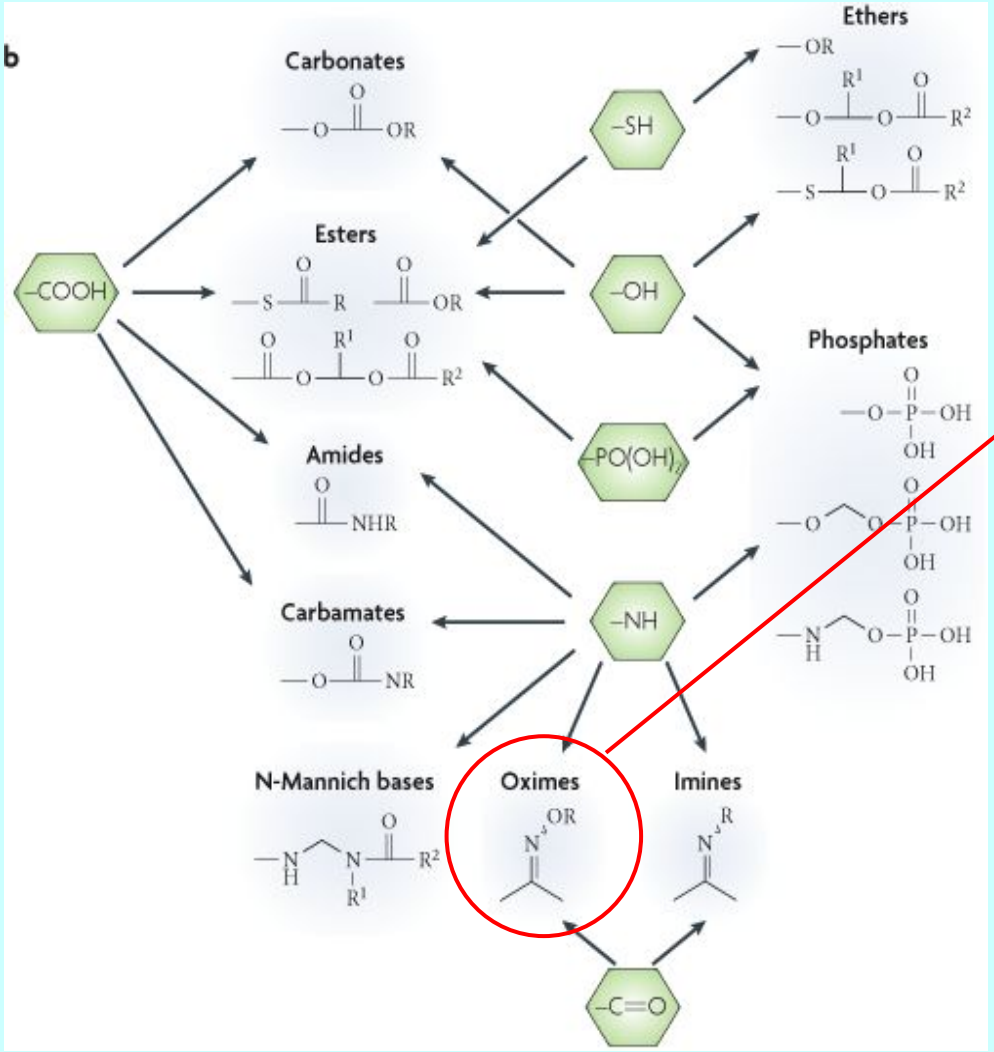
- Sono più stabili degli esteri ma più suscettibili all'idrolisi rispetto alle ammidi.
- I carbonati derivano da acidi carbossilici ed alcoli; i carbammati da isocianati ed alcoli.
- La biotrasformazione di carbonati e carbammati richiede le esterasi per la formazione del *parent drug*

PROFARMACI



- Le ammidi sono derivati di gruppi funzionali amminici e carbossilici
- Poco usate a causa della loro elevata instabilità enzimatica *in vivo*.
- Un legame ammidico è idrolizzato da carbossilesterasi, proteasi o peptidasi.
- Sono usate di solito per migliorare l'assorbimento orale

PROFARMACI



- Derivati di chetoni, ammidine e guanidine
- Utili per molecole che mancano di gruppi ossidrilici, amminici o carbossilici
- Ossidati dal CYP450
- Usati per migliorare la permeabilità attraverso le membrane e l'assorbimento del *parent drug*

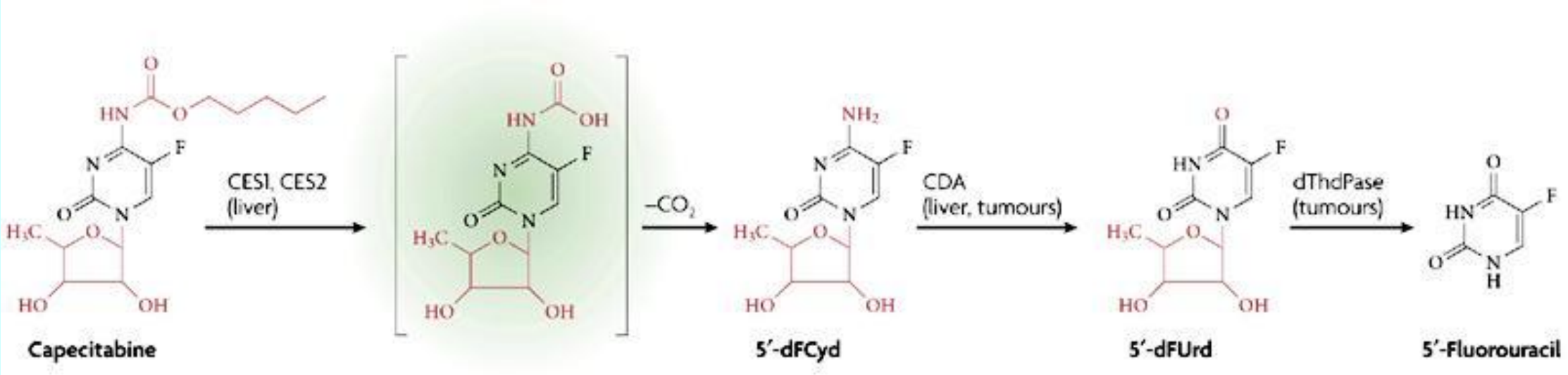
ENZIMI INDUCIBILI

Geni *housekeeping* o costitutivi: sono trascritti in tutte le cellule in ogni momento della loro vita.

Geni tessuto-specifici: guidano le cellule nella specializzazione verso diversi tipi cellulari, quindi sono espressi **solo** in alcuni tessuti.

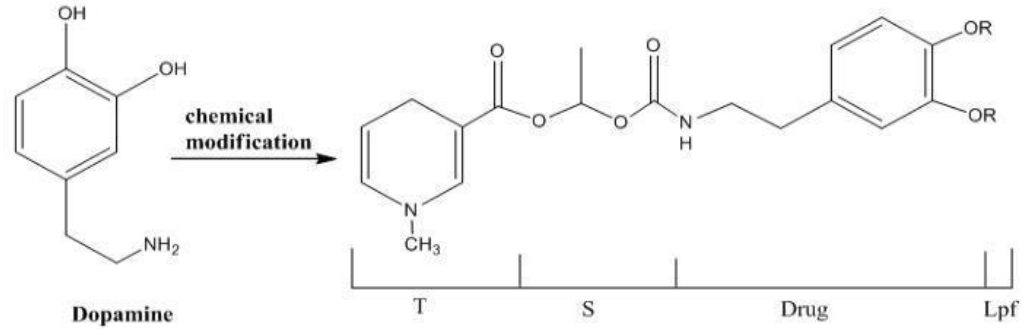
Geni inducibili: la loro espressione è regolata in modo da attivarsi esclusivamente a seguito di specifici stimoli

PROFARMACI



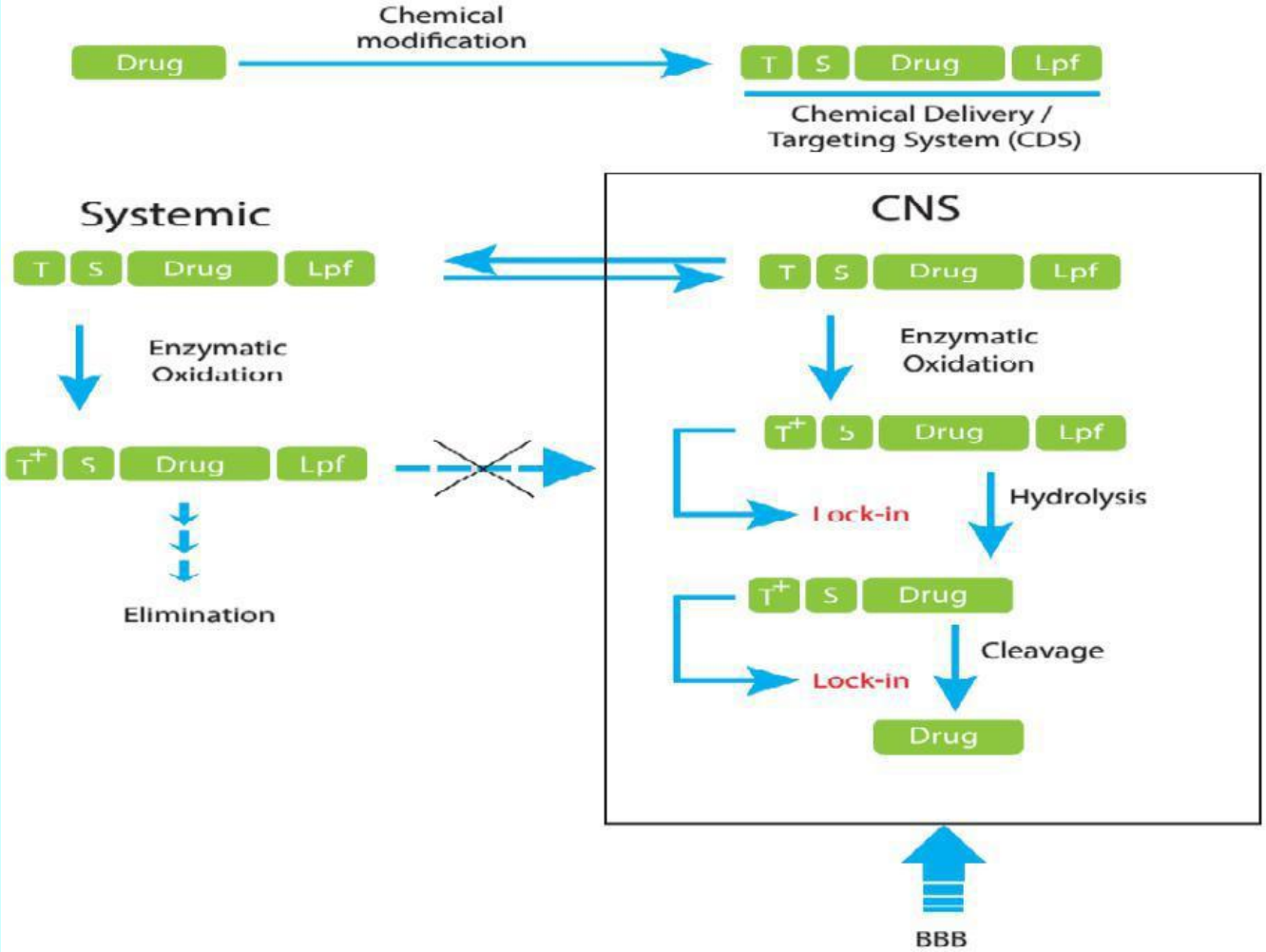
La capecitabina (Xeloda) è un profarmaco che ha ridotta tossicità gastrointestinale e alta selettività tumorale.
Le vie metaboliche enzimatiche iniziano nel fegato, dove le carbossilesterasi 1 e 2 (CES1 e CES2) scindono il legame estereo del carbammato. Successivamente avviene velocemente la reazione spontanea di decarbossilazione che porta alla 5'-deossi-5-fluorocitidina (5'-dFCyd). La rigenerazione verso il principio attivo continua nel fegato, e in buona parte anche nei tumori, ad opera della citidina deaminase (CDA), che trasforma 5'-dFCyd a 5'-deossi-5-fluorouridine (5'-dFUrd). Infine, la timidina fosforilase (dThdPase o ECGF1) libera il principio attivo 5'-fluorouracile nelle cellule tumorali.

PROFARMACI



Dopamine

Carbamate- type dopamine CDS

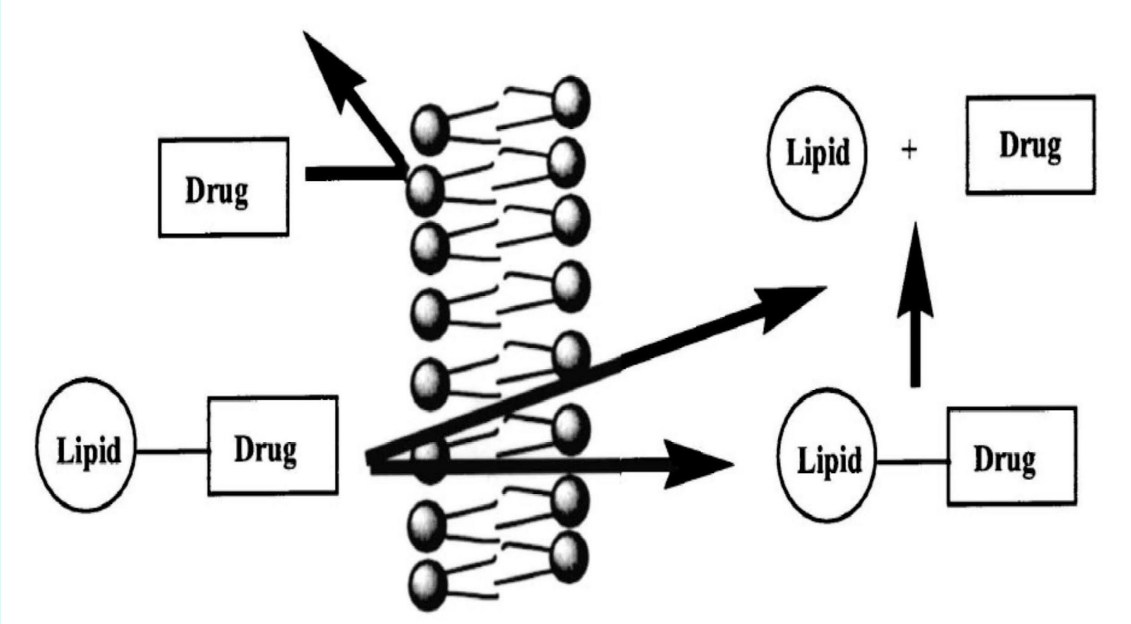


CHEMICAL DELIVERY TARGETING SYSTEM

Lpf = gruppi funzionali lipofilici
"Targetor" (T) = 1, 4-diidropiridina
Ossidazione enzimatica a ione piridinio **(T+)**
Funzione spaziatrice (S) controlla la velocità di rilascio enzimatica del principio attivo.

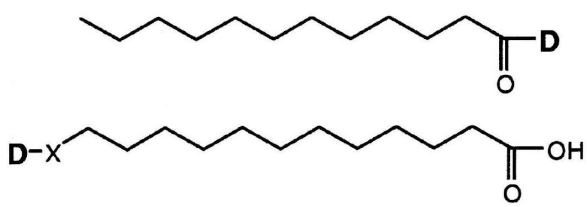
Dopo la distribuzione tra corpo e CNS con passaggio della BBB, il CDS viene trasformato in un composto ionico trattenuto dal tessuto cerebrale, mentre i metaboliti ionici prodotti nel resto del corpo vengono facilmente eliminati. I metaboliti impermeabili alle membrane che sono "bloccati" nel cervello subiscono un metabolismo sequenziale e liberano il principio attivo nel CNS.

PROFARMACI



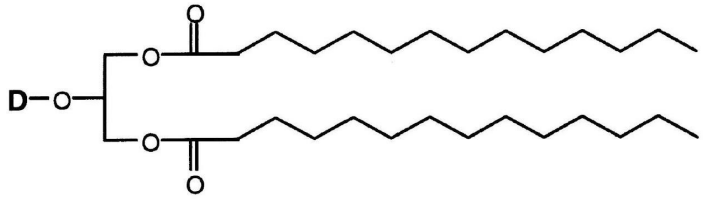
Lipidic prodrugs

1. **Drug attached to a fatty acid.**

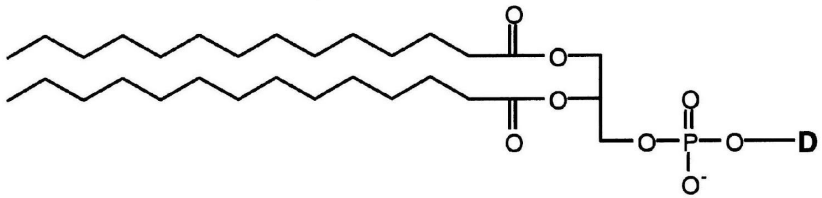


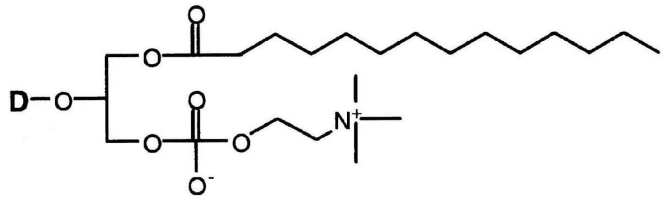
with X= O, NH

2. **Drug attached to a glyceride.**

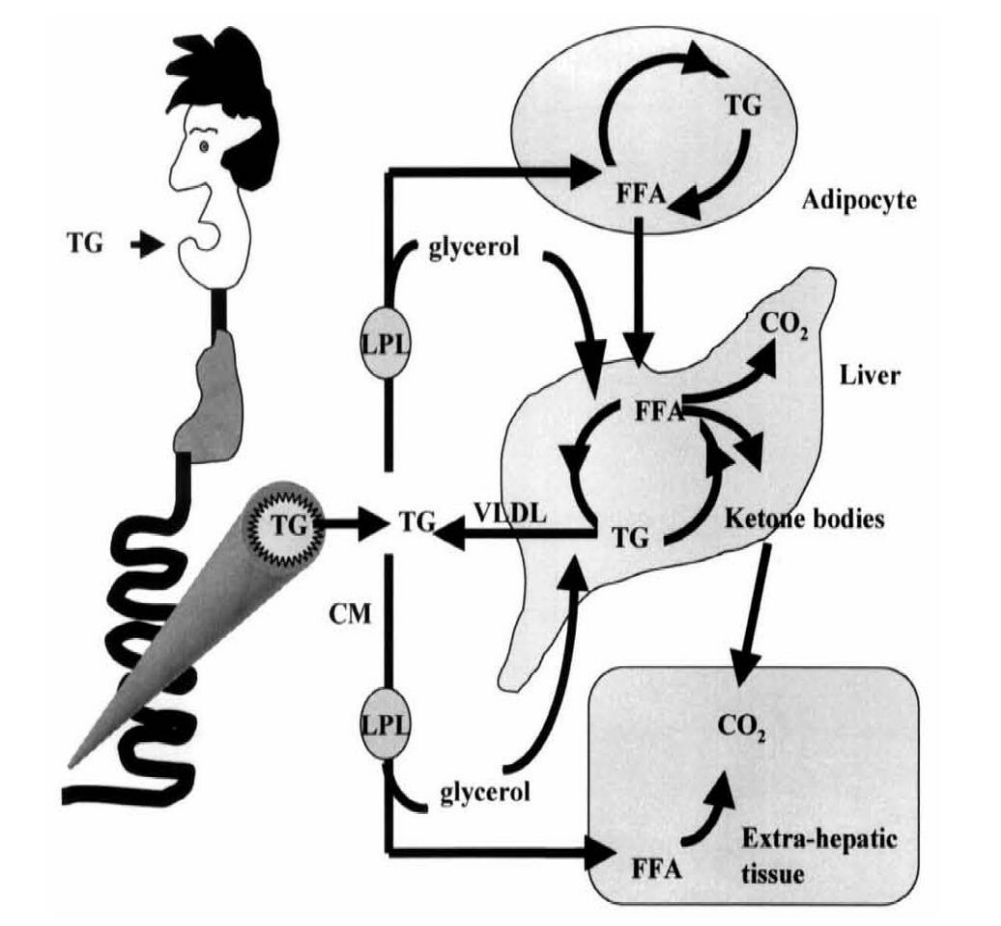


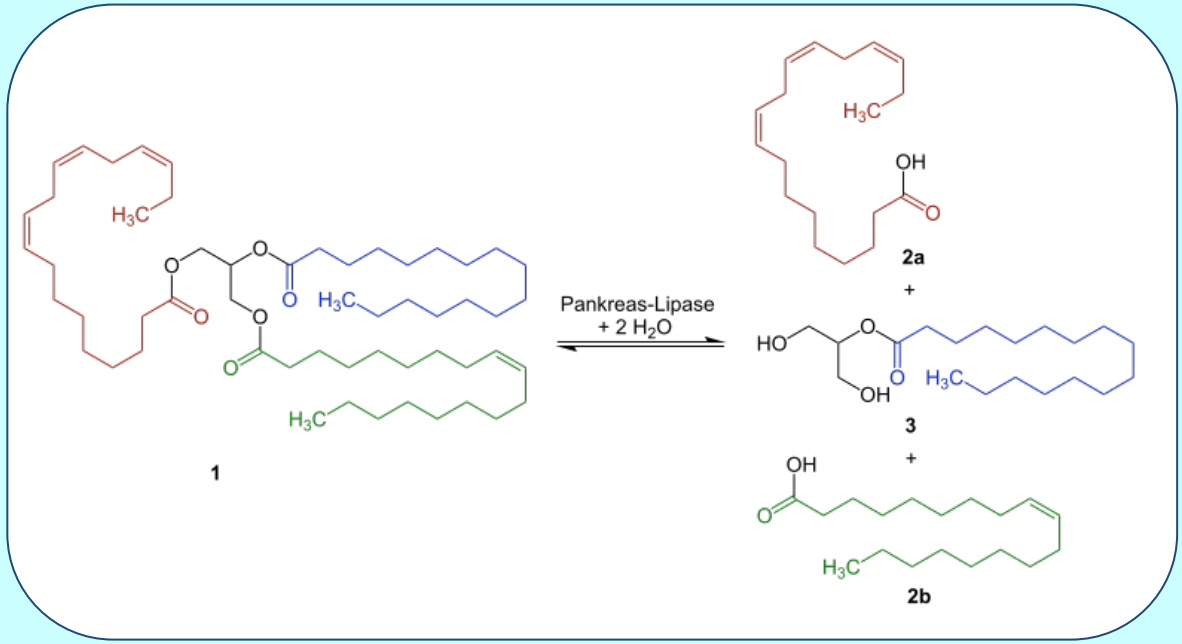
3. **Drug attached to a phospholipid.**





PROFARMACI

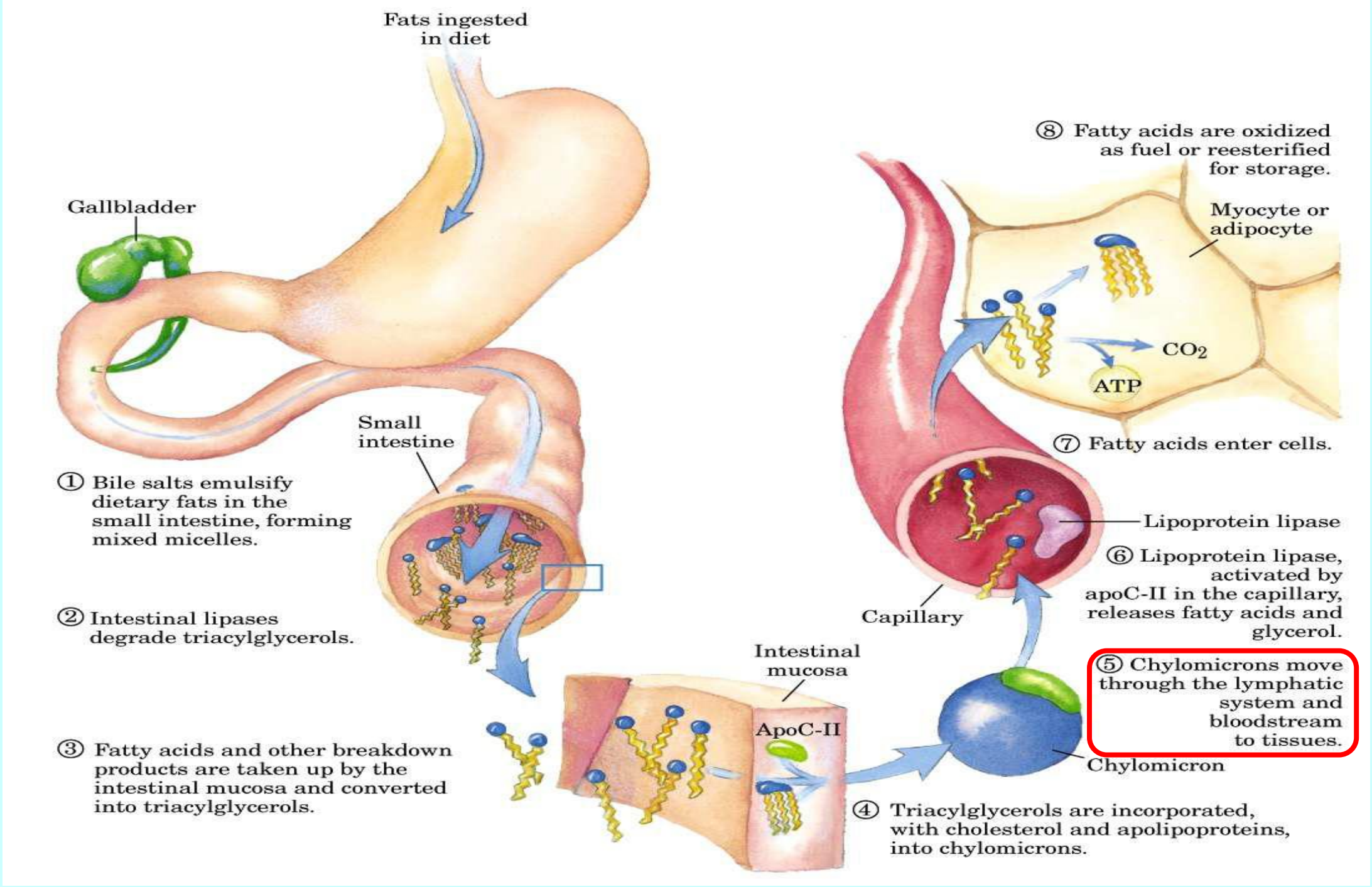




TG = Trigliceridi CM = Chilomicroni
LPL = Lipase lipoproteica
FFA = Acidi grassi liberi
VLDL = Very Low Density Lipoprtein

La lipase pancreatica genera 2-monogliceridi che penetrano negli enterociti. Una volta assunti dalla mucosa intestinale, gli acidi grassi e gli altri prodotti vengono riconvertiti in triacilgliceroli che legandosi a specifiche proteine di trasporto, chiamate apolipoproteine, formano aggregati lipoproteici detti chilomicroni. Queste lipoproteine sono trasferite dalla mucosa al sistema linfatico, entrano nel sangue e vengono trasportati al muscolo e al tessuto adiposo.

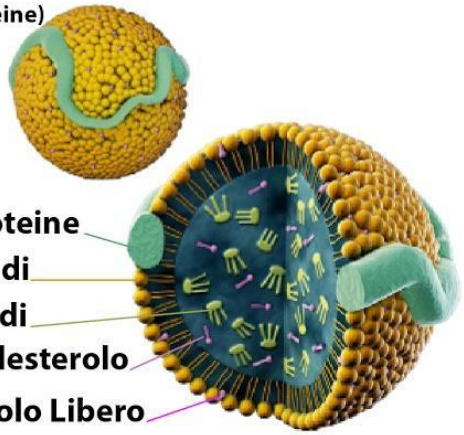
METABOLISMO DEI LIPIDI



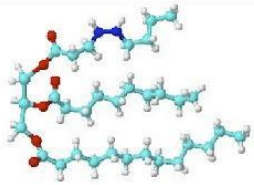
METABOLISMO DEI LIPIDI

" Colesterolo" totale (sanitario) = HDL + LDL + VLDL
" Trigliceridi" (sanitario) = Chilomicroni

Molecola del Chilomicrone (Lipoproteine)



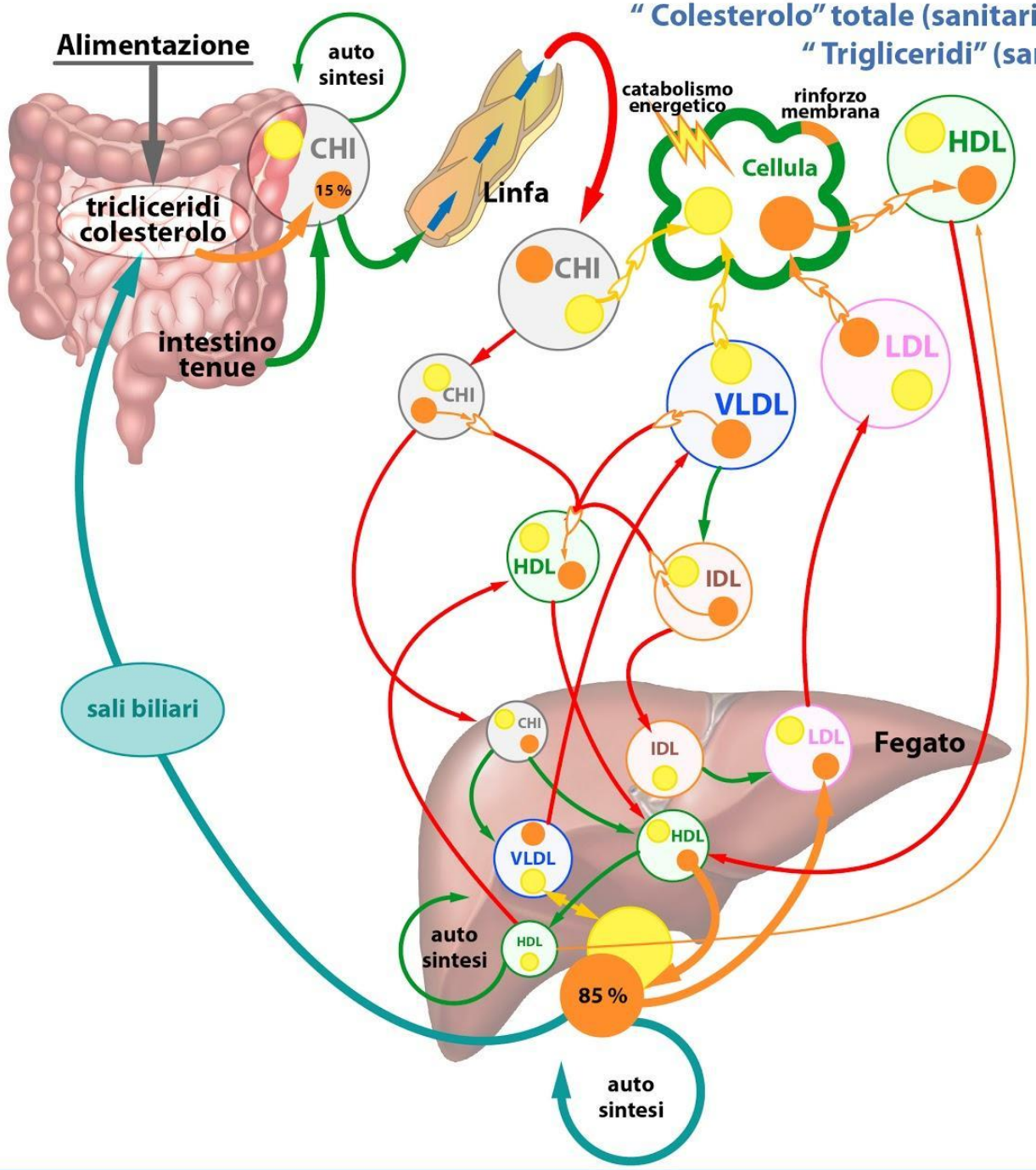

Molecole Colesterolo


Molecole Trigliceridi

sali biliari | tricliceridi | colesterolo

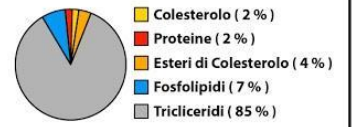
CHI: **Chilomicroni**	H (hight): **alto**
V (very): **molto**	D (density): **densità**
L (low): **basso**	L : **lipoproteine**

Trasporto plasmatico
Trasporto sanguigno
Trasporto biliare
vari recettori proteici

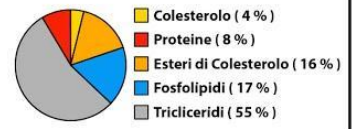


PERCENTUALI DI TRASPORTO

CHILOMICRONI



VLDL



IDL

LDL e Lp(a) **CATTIVO**

La lipoproteina (a) *[Lp(a)]* è uguale alle ***LDL*** ma contiene in più una proteina detta apoliproteina (a) *[Apo(a)]*. La *[Lp(a)]* è stata associata allo sviluppo dell'arterosclerosi.

HDL **BUONO**

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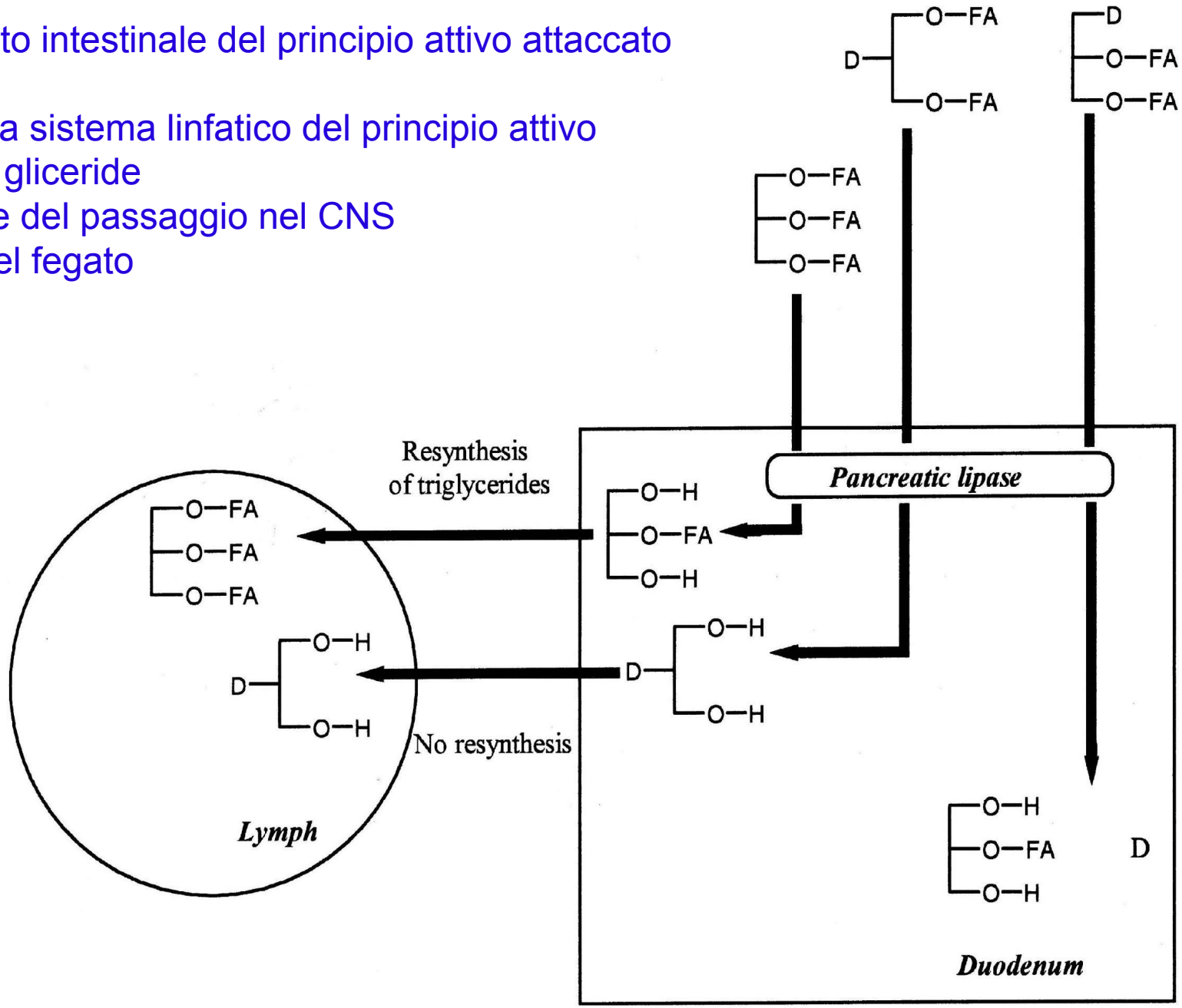
(1) Assorbimento intestinale del principio attivo attaccato al gliceride
(2) Trasporto via sistema linfatico del principio attivo attaccato al gliceride
(3) Facilitazione del passaggio nel CNS
(4) Trasporto nel fegato



PROFARMACI

FANS

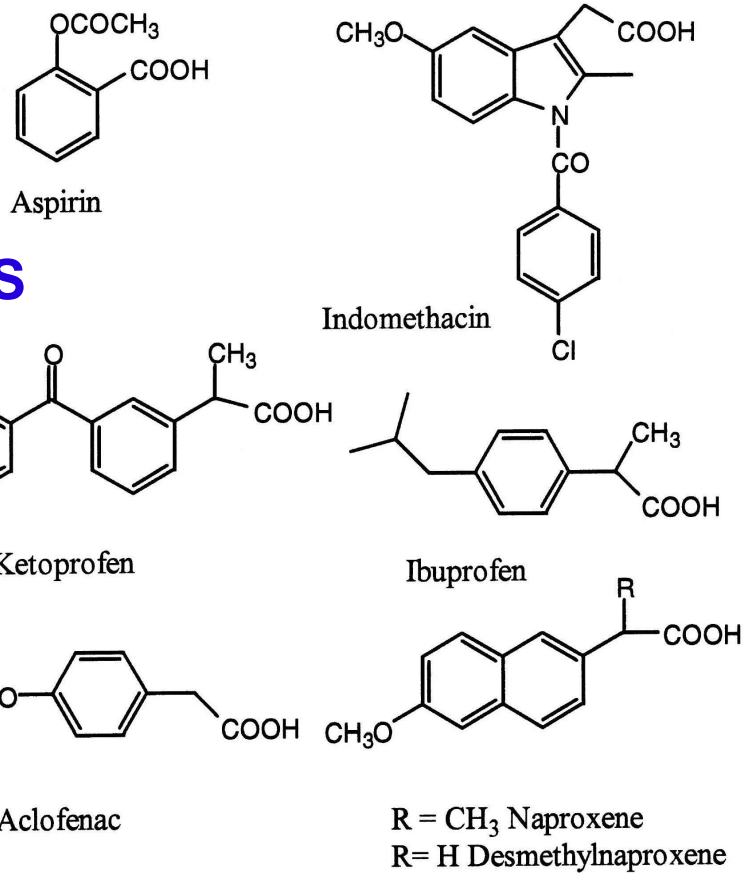
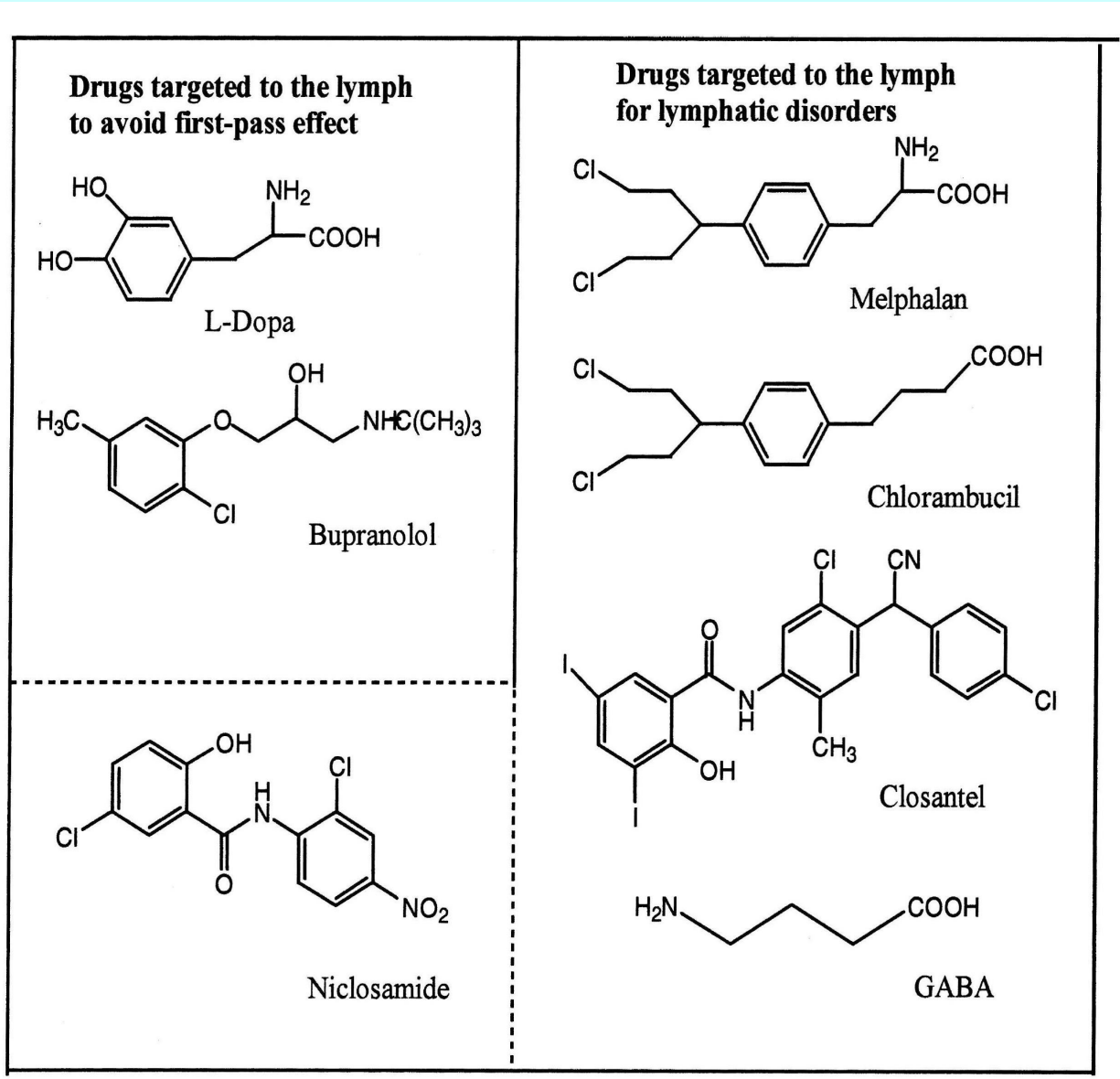


Fig. 6. Examples of drugs conjugated to diglycerides for enteral delivery of the drugs.

Indice terapeutico espresso come rapporto tra dose ulcerogenica (UD_{50}) e dose efficace (ED_{50}). L'indice terapeutico dei gliceridi modificati confrontati ai farmaci originali aumenta del triplo per l'indometacina e fino a 80 volte per l'aspirina.

Derivati mirati al sistema linfatico



PROFARMACI

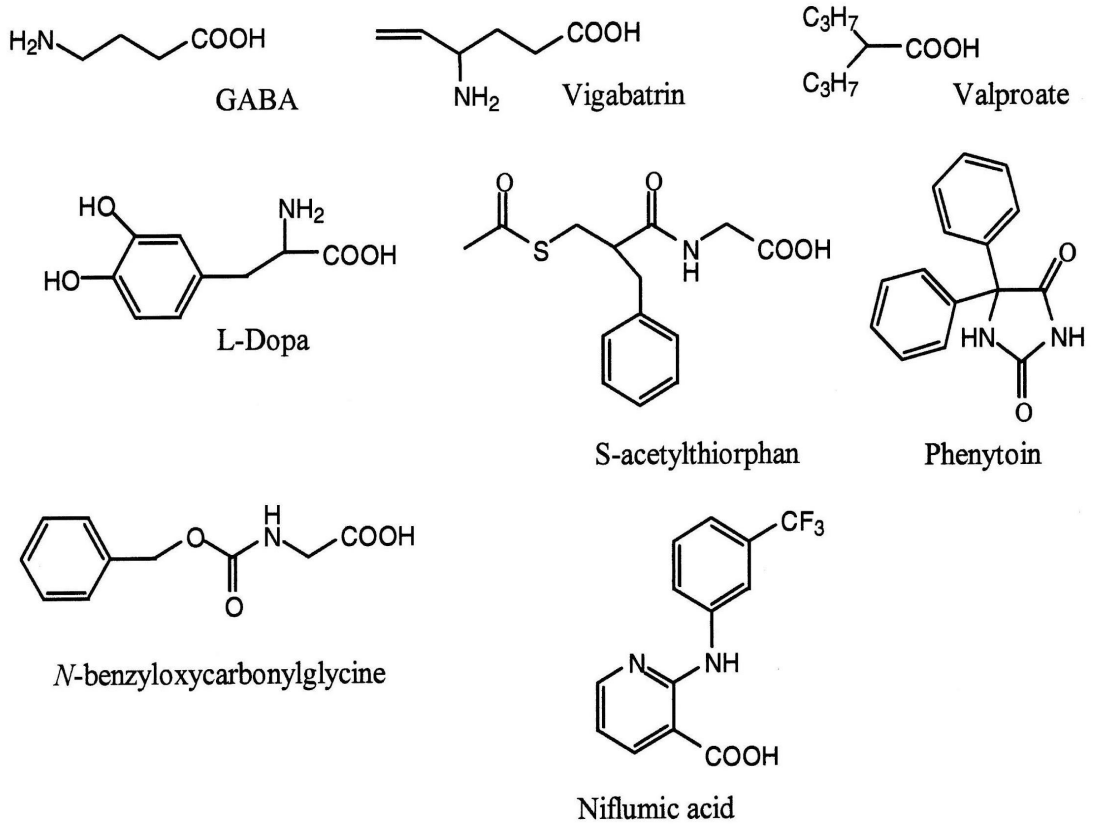


Fig. 8. Central nervous system-targeting of drugs coupled to diglycerides or modified diglycerides.

Uno pseudogliceride disostituito asimmetricamente sintetizzato con le tre funzioni ossidriliche esterificate con acido linolenoico, GABA e γ-vinil-GABA, porta ad un doppio profarmaco. L'attività questo pseudogliceride è circa 300 volte maggiore di quella dell'inibitore della GABA transaminase (γ-vinil-GABA).

Brain Penetration Index - BPI

Rapporto tra concentrazioni cerebrale e epatica x100
Nel digliceride è 127 volte più alta che nella molecola originale.

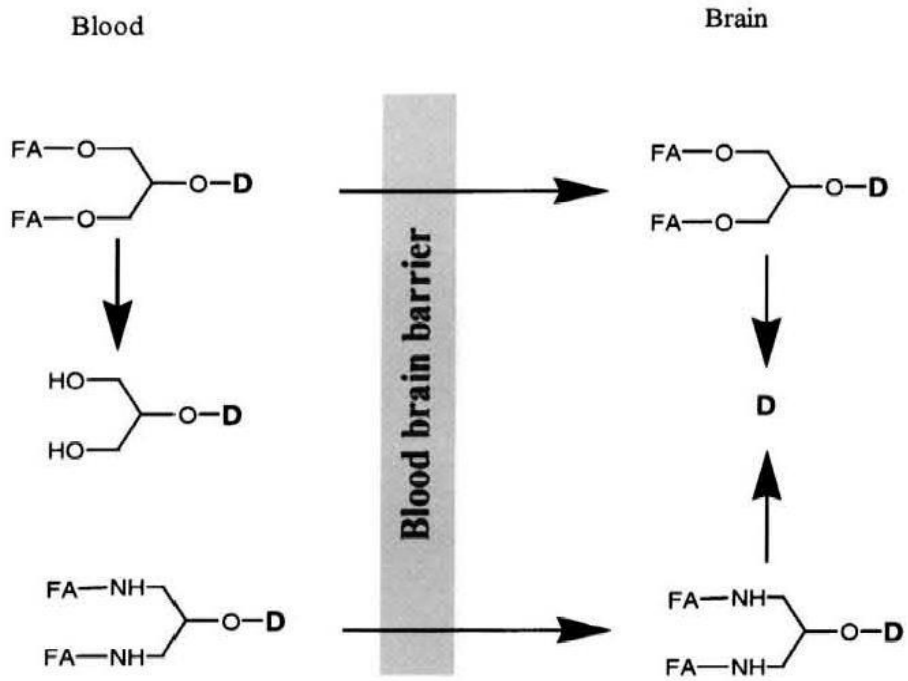
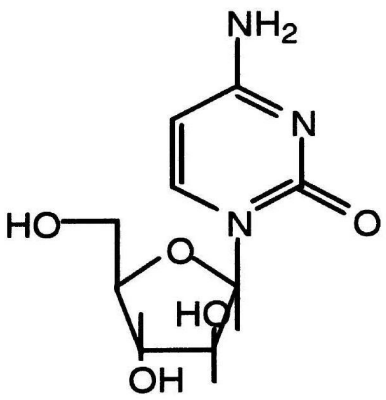
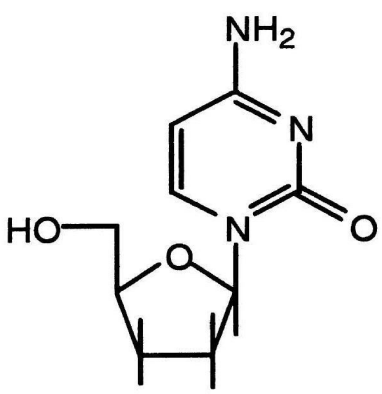


Fig. 9. Amide bio-isosteres of diglycerides as a central nervous system-targeting system.

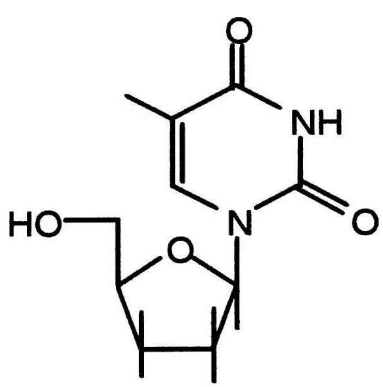
PROFARMACI



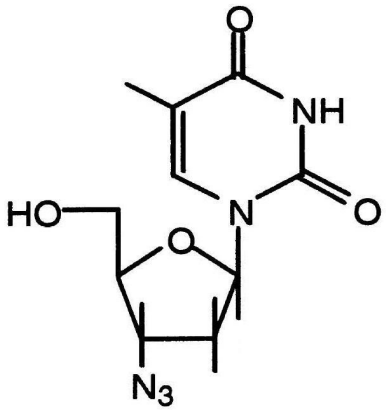
Ara-C



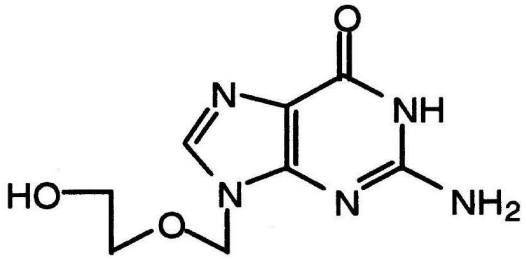
2',3'-Dideoxycytidine



3'-Deoxythymidine



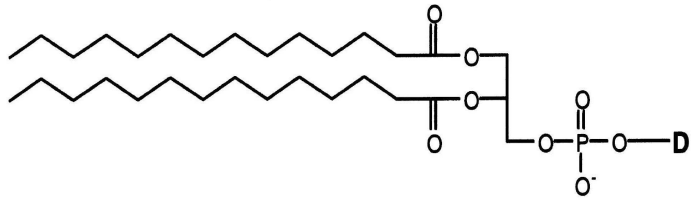
Zidovudine (AZT)

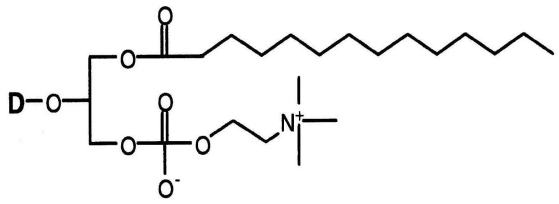


Acyclovir

Fig. 10. Nucleosides and nucleosides analogues conjugated to phospholipids.

3. Drug attached to a phospholipid.





Gli analoghi dei nucleosidi (analoghi di acidi nucleici e zuccheri) sono comunemente e largamente utilizzati per trattare infezioni virali e tumori. Molti analoghi di nucleosidi hanno però bassa biodisponibilità orale (alta polarità e bassa permeabilità)

PROFARMACI

PEPTIDE TRANSPORTER ASSOCIATED PRODRUG THERAPY

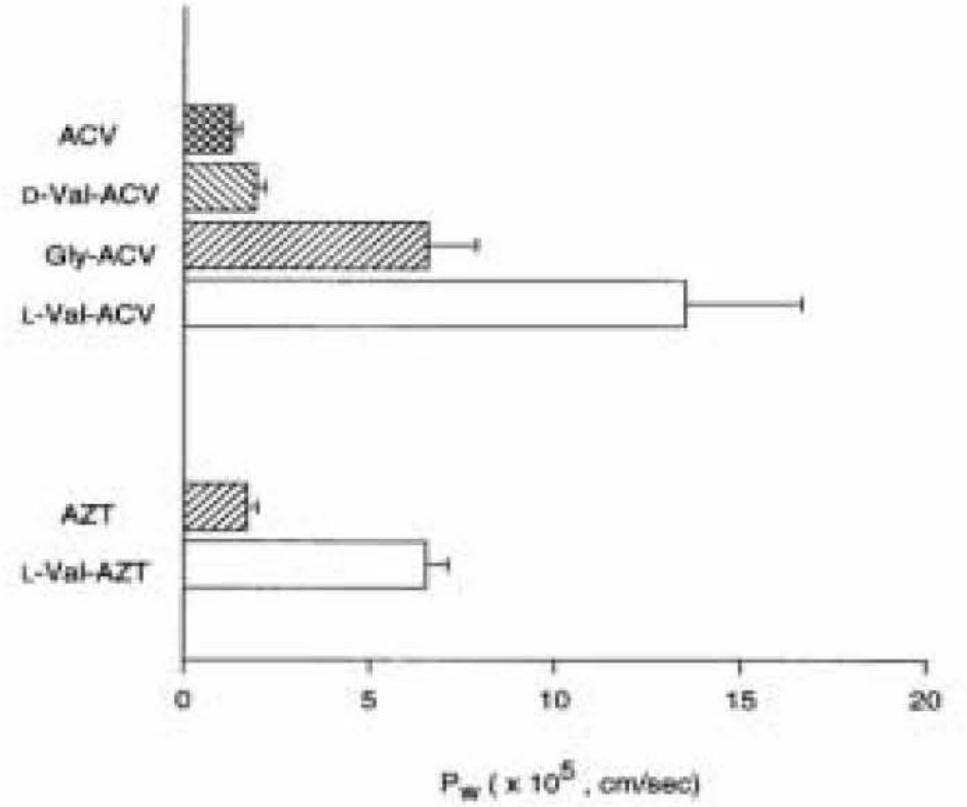


Figure 4. Intestinal membrane permeabilities of amino acid ester prodrugs and their parent drugs in rats (0.01 mM, Mean + SE). ACV and AZT: n = 6; the others: n = 4 (adapted from Han et al. [ref. 71], with permission).

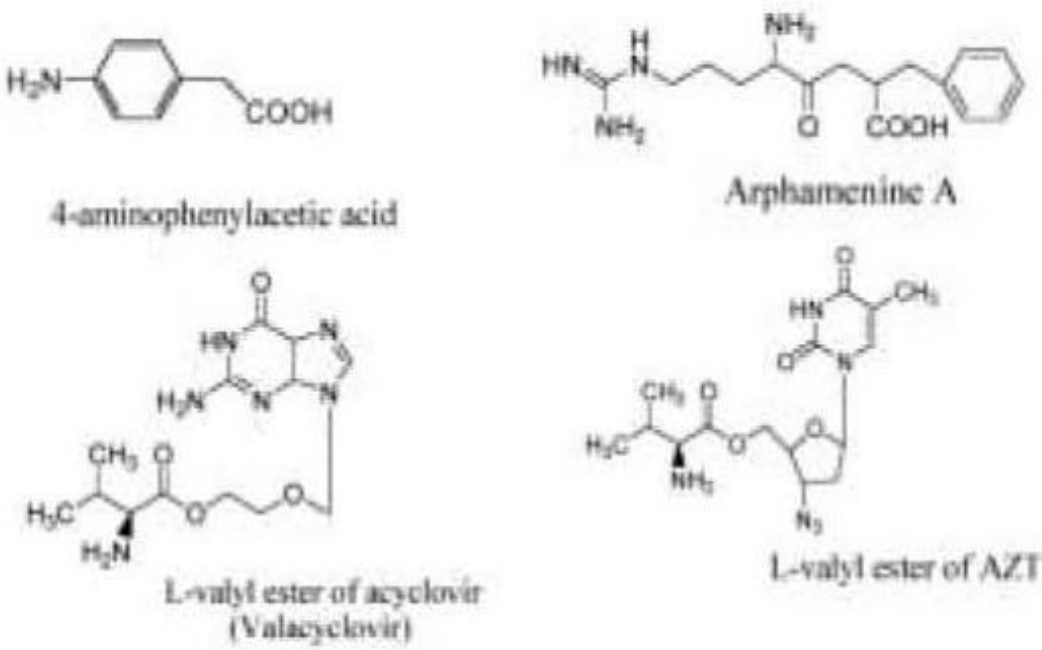
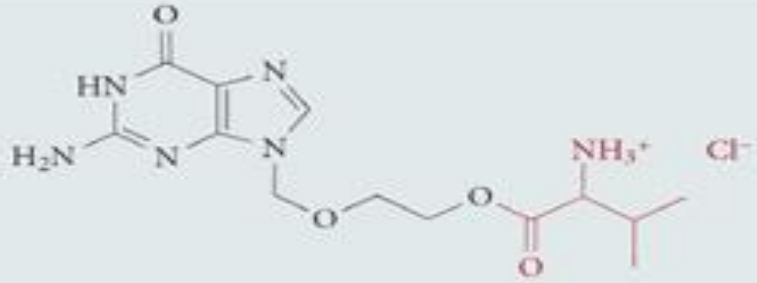
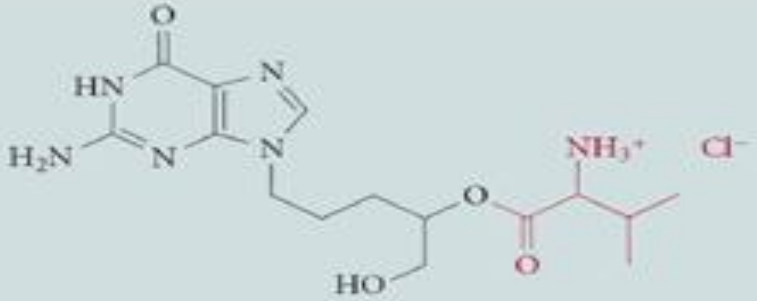
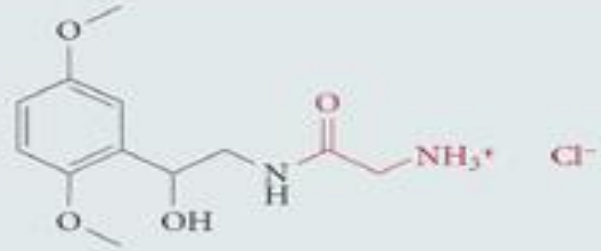
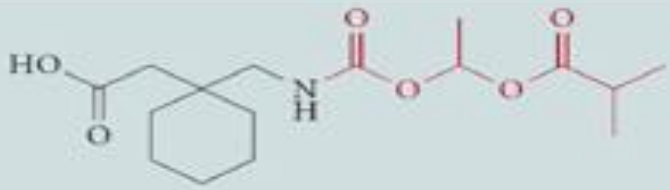


Figure 2. Substrates of peptide transporters.

PROFARMACI

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Valacyclovir (antiviral)	L-Valyl ester of acyclovir		• Bioconversion by valacyclovir hydrolase (valacyclovirase) • Transported predominantly by hPEPT1 • Oral bioavailability improved from 12–20% (acyclovir) to 54% (valacyclovir)[90–92,182]
Valganciclovir (antiviral)	L-Valyl ester of ganciclovir		• Bioconversion by intestinal and hepatic esterases • Transported predominantly by hPEPT1 • Oral bioavailability improved from 6% (ganciclovir) to 61% (valganciclovir)[183,184]
Midodrine (vasopressor)	Glycyl amide of desglymidodrine		• Bioconversion by unknown peptidase • Transported by hPEPT1 • Oral bioavailability improved from 50% (desglymidodrine) to 93% (midodrine)[94]
XP13512 (restless leg syndrome, neuropathic pain)	Isobutanoyloxy-ethoxy carbamate of gabapentin		• Bioconversion by esterases • Transported by both MCT1 and SMVT • Oral bioavailability improved from 25% (gabapentin) to 84% (XP13512) in monkeys[98,99]

hPEPT1, human peptide transporter 1 (also known as SLC15A1); MCT1, monocarboxylic acid transporter 1 (also known as SLC16A1); SMVT, sodium-dependent vitamin transporter (also known as SLC5A6).

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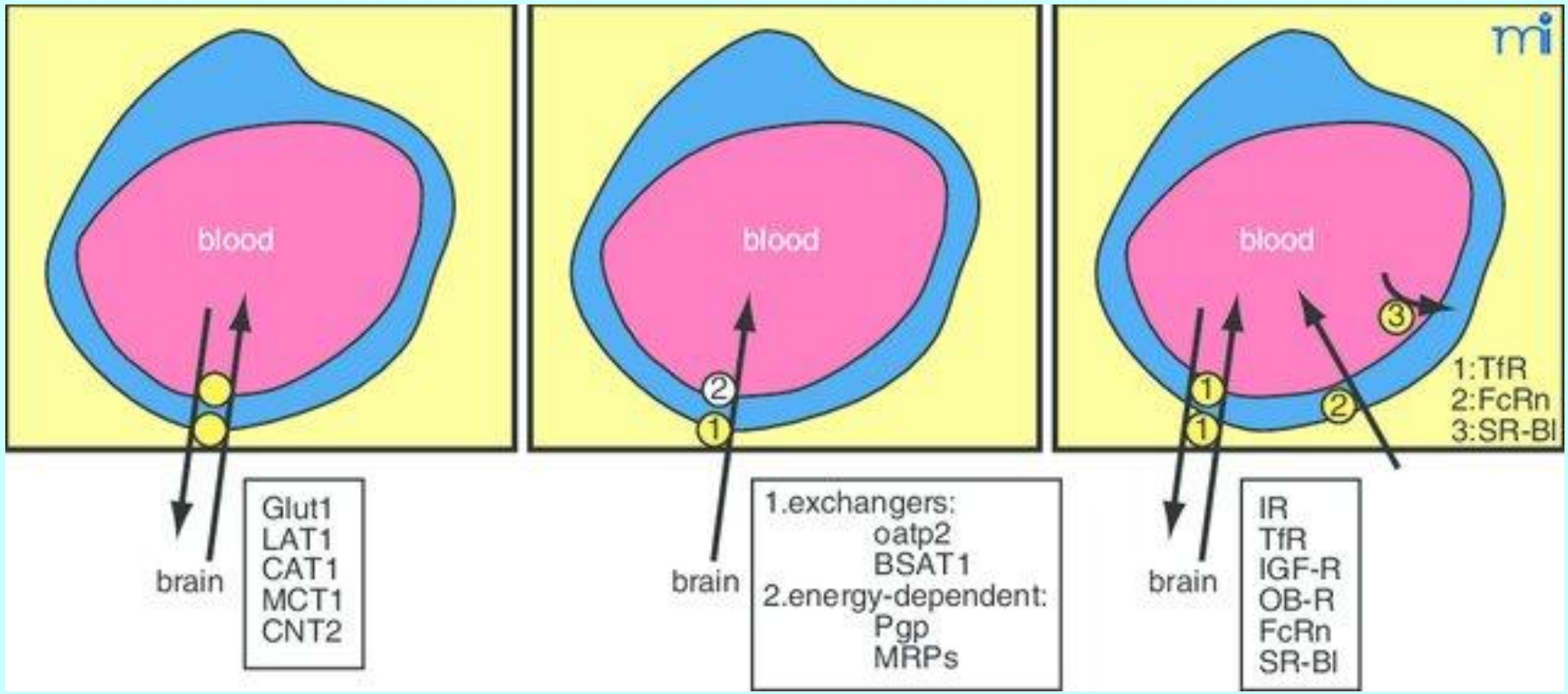
Table 1. CMT systems involved in the transport of nutrients into the CNS

Carrier	Type	Representative substrate	Main expression in blood/ CNS barriers
Neutral amino acid	LAT 1	Phenylalanine	BBB
Hexose	GLUT 1	Glucose	BBB
Monocarboxylic acid	MCT 1	Lactic acid	BBB
Cationic amino acid	CAT 1	Arginine	BBB
Nucleoside	CNT 2	Adenosine	BBB
Ascorbic acid	SVCT 2	Vitamin C	Choroid Plexus



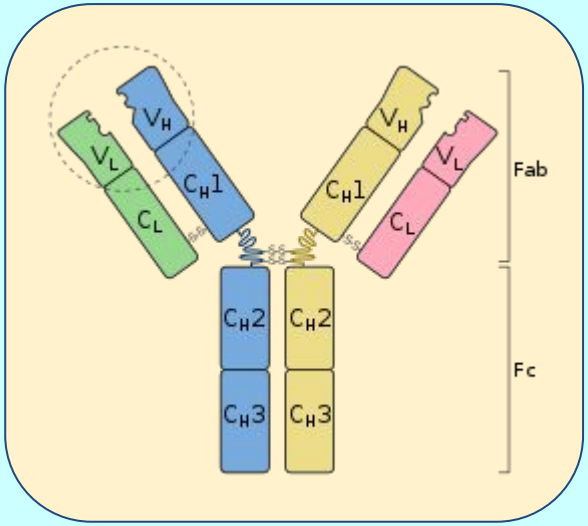
Carrier-Mediated Transport (CMT)

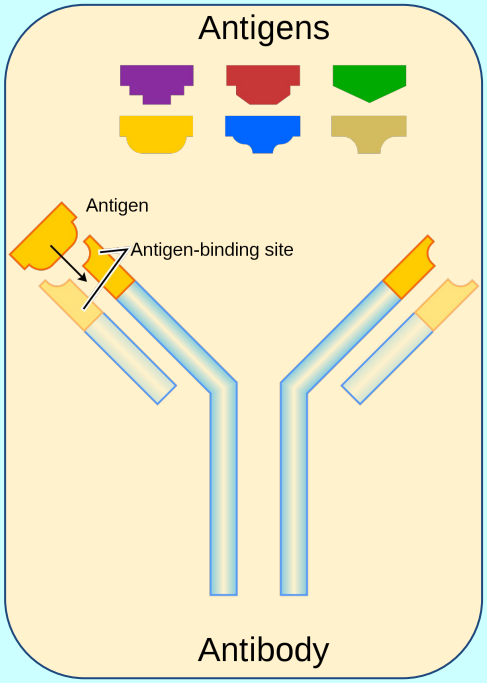
Active Efflux Transport (AET)

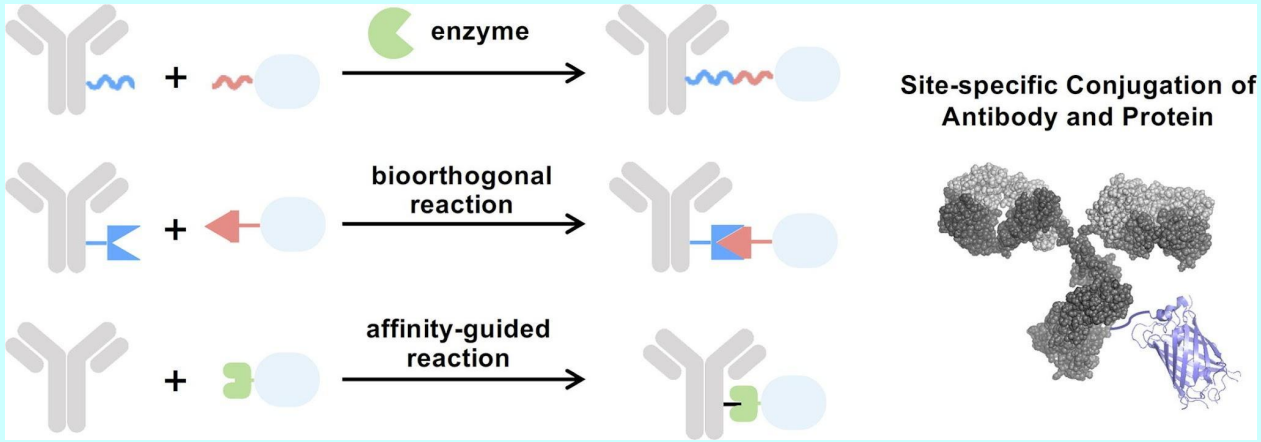
Receptor-Mediated Transport (RMT)

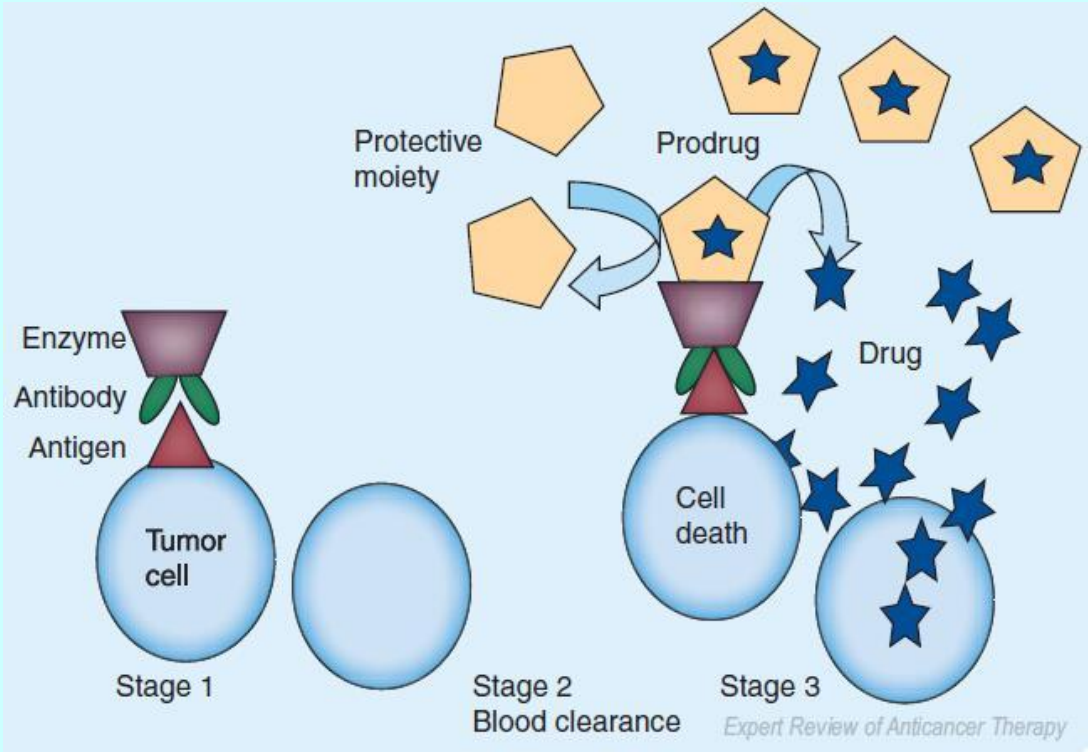
PROFARMACI

ANTIBODY DIRECTED ENZYME PRODRUG THERAPY

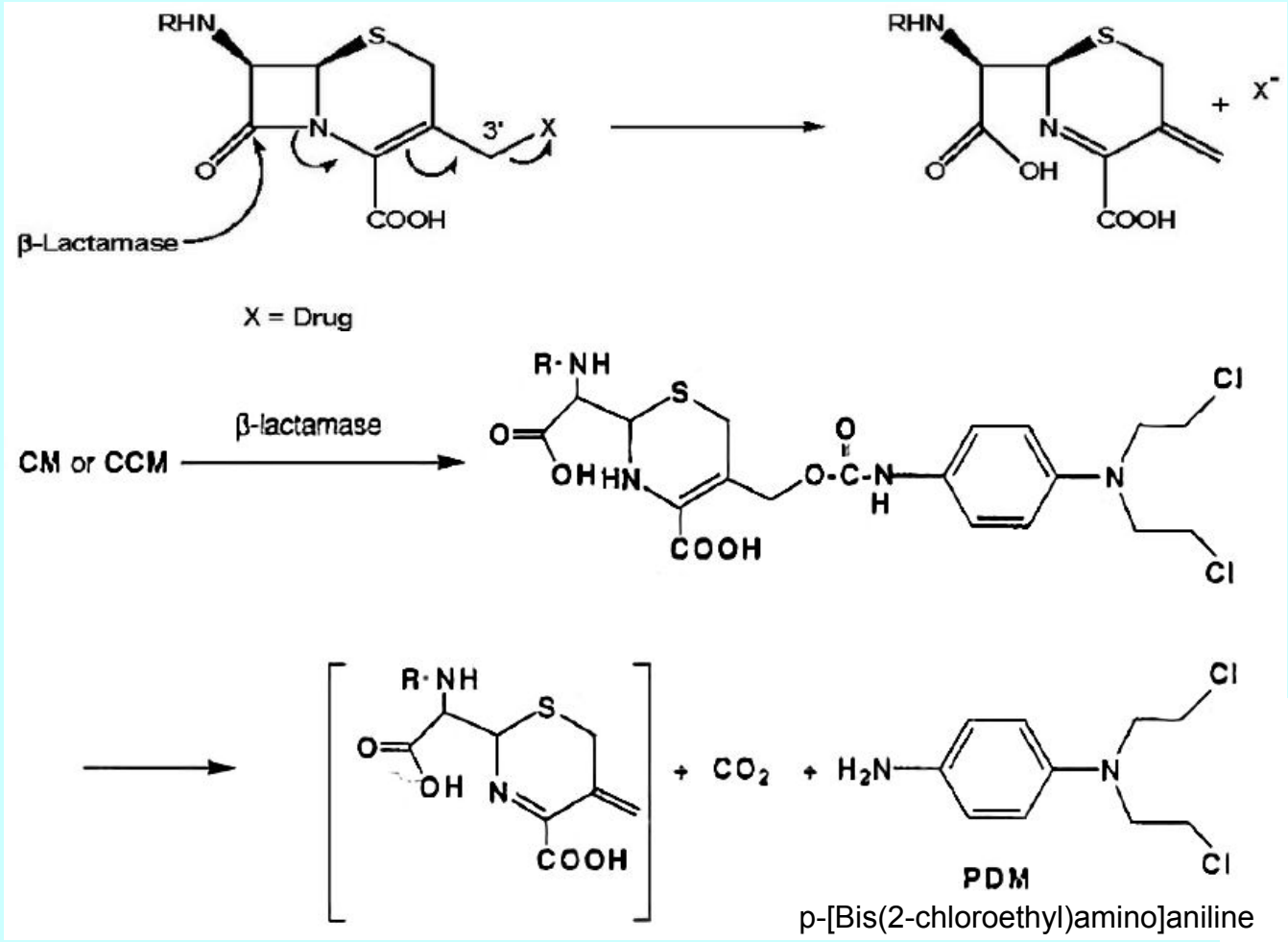




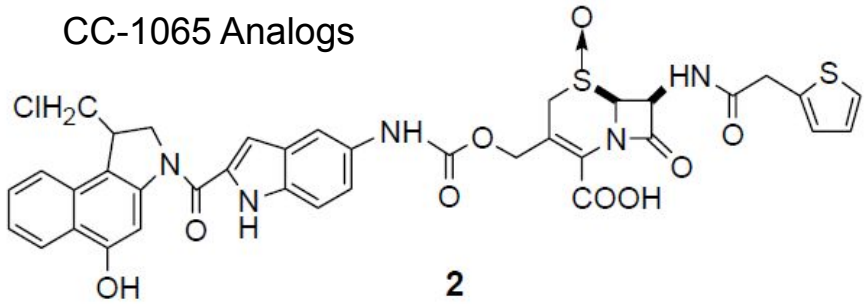




PROFARMACI



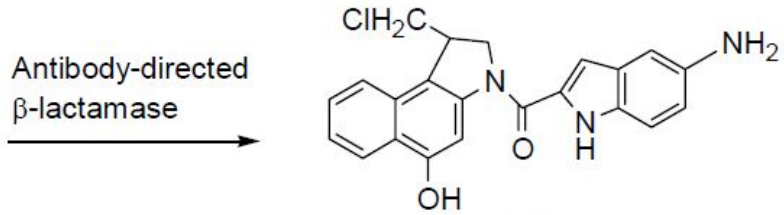
CC-1065 Analogs



2

ANTIBODY DIRECTED ENZYME PRODRUG THERAPY

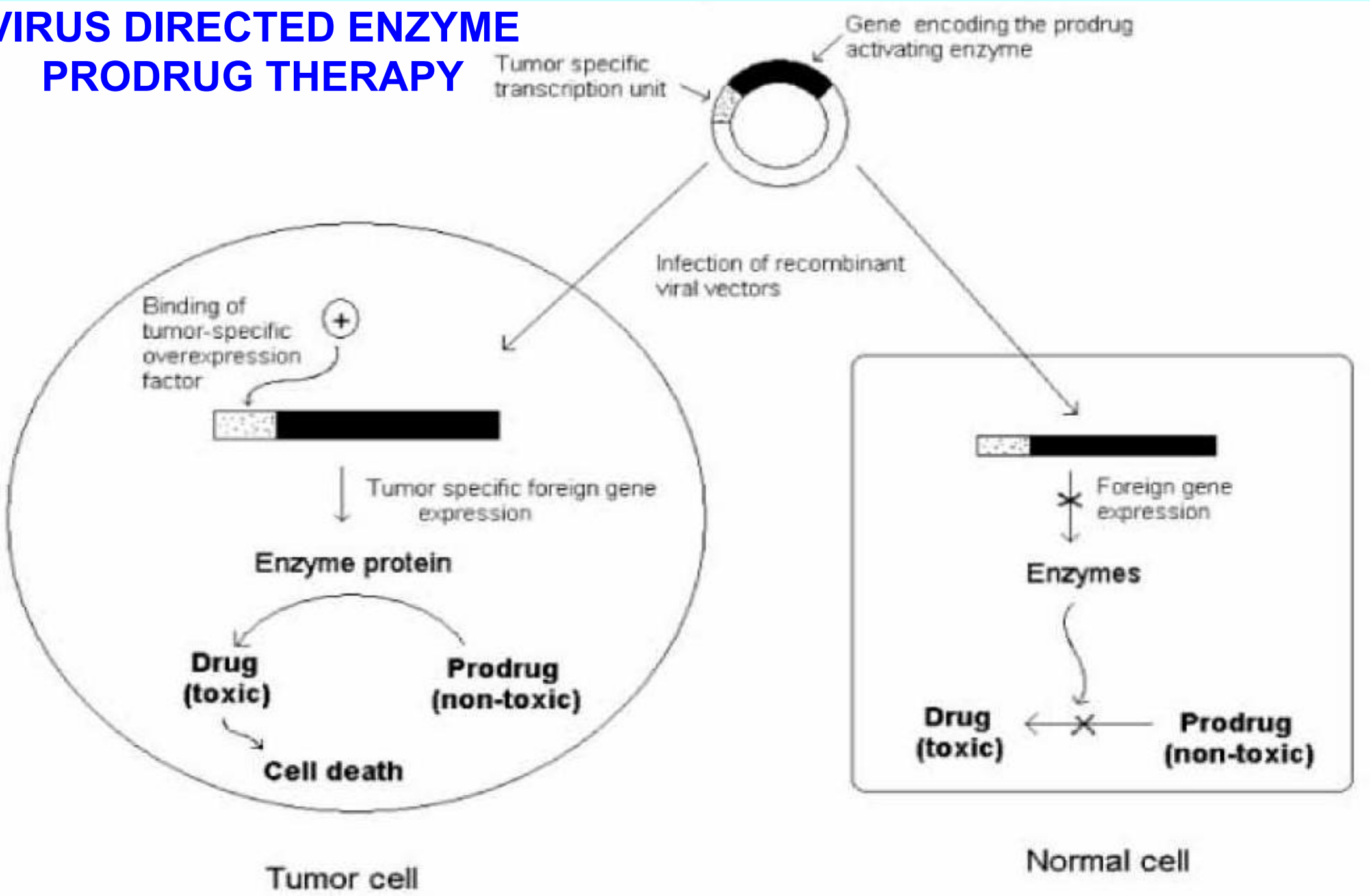
Antibody-directed
β-lactamase



1

PROFARMACI

VIRUS DIRECTED ENZYME PRODRUG THERAPY



PROFARMACI VDEPT

Virus-Directed Enzyme Prodrug Therapy

Strategy	Target tumour characteristics	Type of vector	Refs
*Transductional targeting**			
Vector affinity for a tumour cell surface antigen	FGFR	AdV5 adenoviral vector with bifunctional conjugate retargeting fibre knob domain	104
	ERBB2	AdV5 adenoviral vector with genetically modified fibre protein	105
	ERBB2	AdV adenoviral vector with bispecific adaptor protein	106
Inherent tropism enhanced by genetic modification of the vector or by artificial selection	Actively dividing cells	Replication competent	107
	Active DNA synthesis by proliferating cell nuclear anti		
	Anaerobic conditions		
	Leaky vasculature, as in tumou be necessary to allow large vi escape vessels		
	Availability of nutrients and environmental conditions wit tumour		
Transcriptional targeting‡			
Promoter driving vector replication	hTERT expression		
	Human melanoma inhibitory (hMIA) expression		
Promoter driving suicide-gene transcription	Secretory leukoprotease inhil (SLPI) expression		
	Carcinoembryonic antigen		
	Human hexokinase II (hHK2) expression		
Translational targeting§			
	eIF4E overexpression		
	eIF4E overexpression		

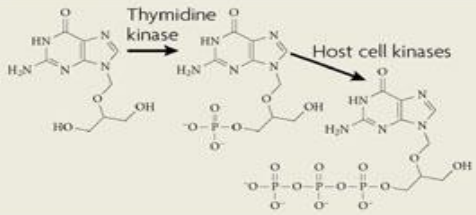
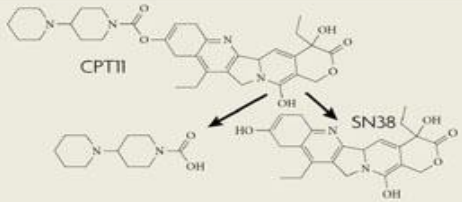
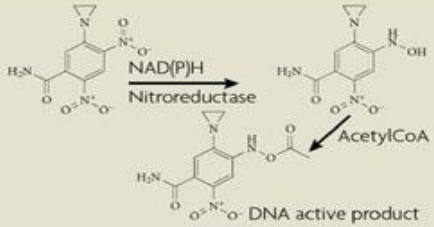
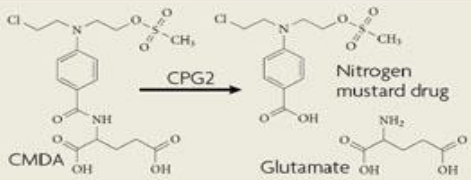
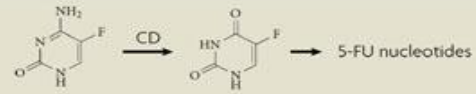
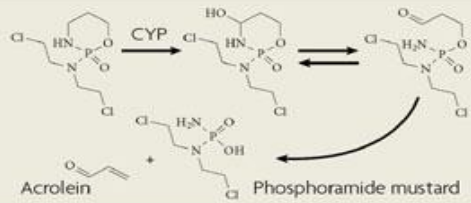
Transductional and transcriptional strategies are not mutu selectivity[105]. In some cases targeting has been shown with product such as a GDEPT enzyme suicide gene. *In transd delivers a suicide gene to tumour cells rather than normal delivered to any cell but promoters selectively switch it on ‡In translational targeting, suicide gene mRNA is translat present corresponding to an upstream sequence. There and gene therapy in general, which are covered in depth associated virus; eIF4E, eukaryotic translational initiatio

Enzyme	Prodrug	Tumour	Phase	Refs
Adenovirus				
HSV-TK	Ganciclovir	Hepatocellular carcinoma	I	117
		Glioma	I	118
		Ovarian	I	119
		Prostate	I	120–124
		Colorectal	I	125
CD with HSV-TK	Ganciclovir and 5-FC	Prostate	I	126–128
Nitroreductase	CB1954	Primary and metastatic hepatocellular carcinoma	I	129
Retrovirus				
CYP2B6	Cyclophosphamide	Breast, melanoma	I	130
		Thyroid	I	131
HSV-TK	Ganciclovir	Melanoma, breast, non-small-cell lung and osteogenic sarcoma	I	132
		Glioma	I/II; III	61,62, 133

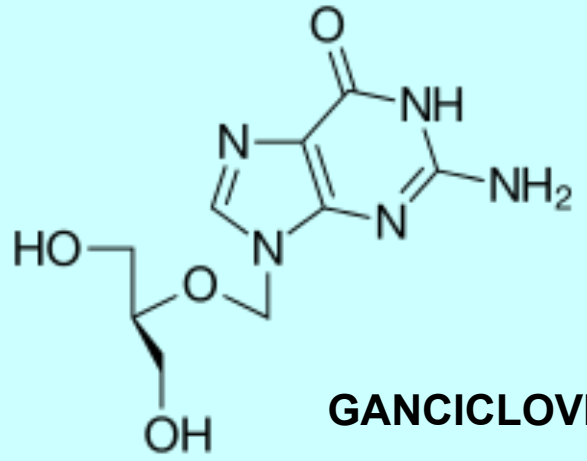
CD, cytosine deaminase; CYP2B6, a member of the cytochrome P450 group of enzymes; 5-FC, 5-fluorocytosine; GDEPT, gene-directed enzyme–prodrug therapy; HSV-TK, Herpes simplex virus-thymidine kinase.

PROFARMACI GDEPT

Gene-Directed Enzyme Prodrug Therapy

Enzyme	Prodrug	Drug	Structures	Action	Refs
HSV-TK	Ganciclovir	Ganciclovir monophosphate		Intracellular metabolism to triphosphate nucleotide which competes with dGTP for DNA polymerases	4
Purine nucleoside phospho-rylase (PNP)	6-methylpurine deoxyriboside	6-methylpurine		Inhibition of RNA, protein and DNA synthesis	135
Carboxy-esterases	Irinotecan (CPT 11)	SN38 (Camptothecin)		Binding to nuclear enzyme topoisomerase I-DNA adducts leads to single strand breaks	136
Nitrore-ductase	CB1954	5-(azirdin 1-yl) 4-hydroxyl-amino 2-nitro benzamide		DNA interstrand crosslinking	22,81
Carboxy-peptidase G2 (CPG2)	Nitrogen mustard L glutamates, such as CMDA (4 [(2-chloroethyl)(2-mes yloxyethyl)amino] benzoyl L-glutamic acid)	Nitrogen mustards 4 [(2-chloroethyl)(2-mesyloxyethyl)amino] benzoic acid		DNA interstrand crosslinking	137
Cytosine-deaminase (CD)	5-fluorocytosine	5-fluorouracil (5-FU)		Intracellular metabolism to 5-fluorodeoxy-uridylate, which inhibits thymidylate synthetase and thereby DNA synthesis; other metabolites inhibit DNA and RNA synthesis	138
Cytochrome P450 (CYP450)	Cyclophosphamide	4-hydroxycyclo-phosphamide		Spontaneously disintegrates to acrolein (potential cytotoxin) and a bifunctional alkylating oxazaphosphorine mustard drug (DNA crosslinker)	80

HSV-TK, Herpes simplex virus thymidine kinase.



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