



ICT PRAGUE

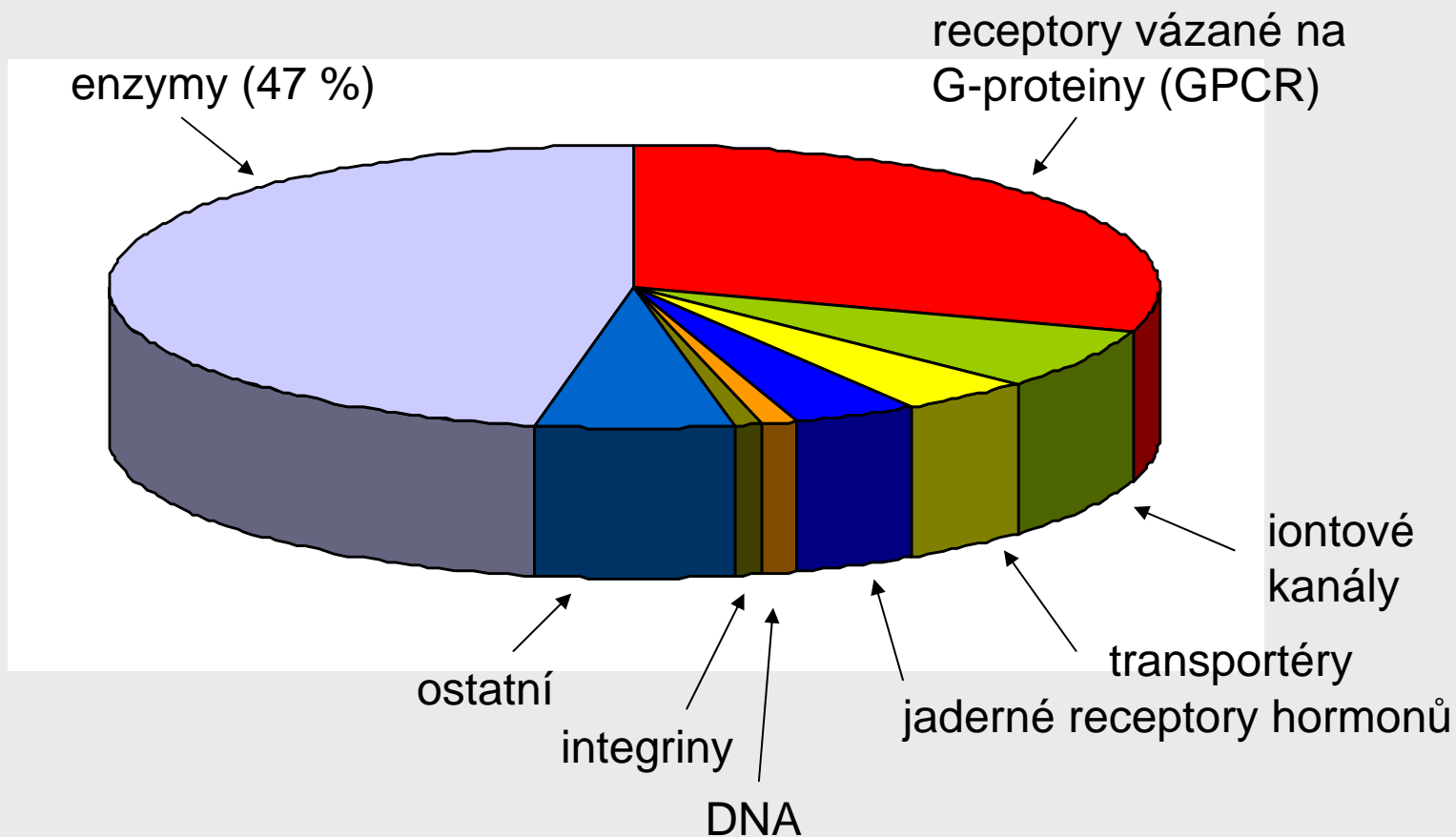
ENZYM Y

A

MEDICÍNA

Vývoj léčiv

Cíle léčiv (targets):



Vývoj léčiv

Cíle léčiv (targets):

Target finding, target validation:

- biologické studie
- genomika
- proteomika, transkriptomika ...

Léčiva:

- nízkomolekulární látky
syntetické nebo přírodní sloučeniny
 - biopolymery (biotechnologická léčiva)
protilátky, enzymy atd.
- a další

Vývoj léčiv

knihovny sloučenin

kombinatorická chemie
přírodní látky
virtuální knihovny
...
miliony sloučenin

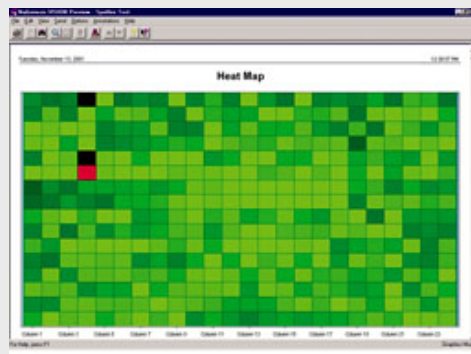


„lead compounds“

slabé ligandy/inhibitory

stovky sloučenin

high throughput screening



virtuální screening



Vývoj léčiv

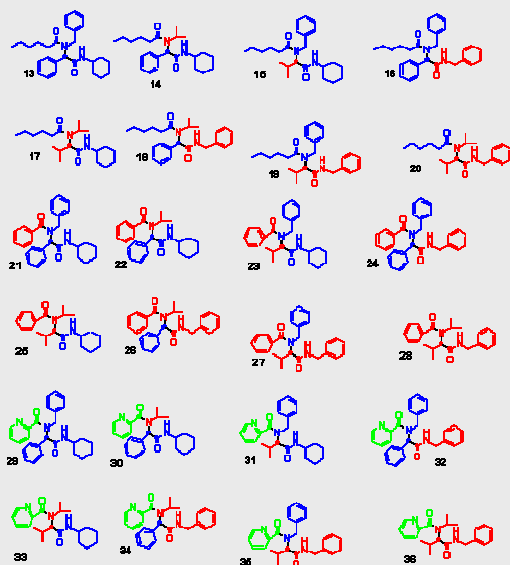
„lead compounds“
slabé ligandy/inhibitory

stovky sloučenin



„drug candidate“
silné ligandy/inhibitory

desítky sloučenin



lead optimization

chemická syntéza
kombinatorická chemie

R:	Ki (nM)
-H	6 300
-CH ₃	3 700
-CH ₂ CH ₃	2 000
-CH ₂ CH ₂ CH ₃	180
-CH ₂ CH ₂ CF ₃	225
-CH ₂ OCH ₃	2 000
-CH ₂ CH=CH ₂	2 200
-CH ₂ CH ₂ CH ₂ CH ₃	300
-CH ₂ CH(CH ₃) ₂	200
-CH(CH ₃)CH ₂ CH ₃	10
-CH(CH ₂ CH ₂ CH ₃) ₂	16
Cyclopentyl	22
Cyclohexyl	60
Phenyl	530
-CH(CH ₂ CH ₃) ₂	1

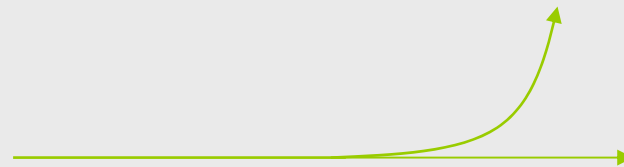
denotes bond formed in reaction

Vývoj léčiv

„drug candidate“

silné ligandy/inhibitory

desítky sloučenin

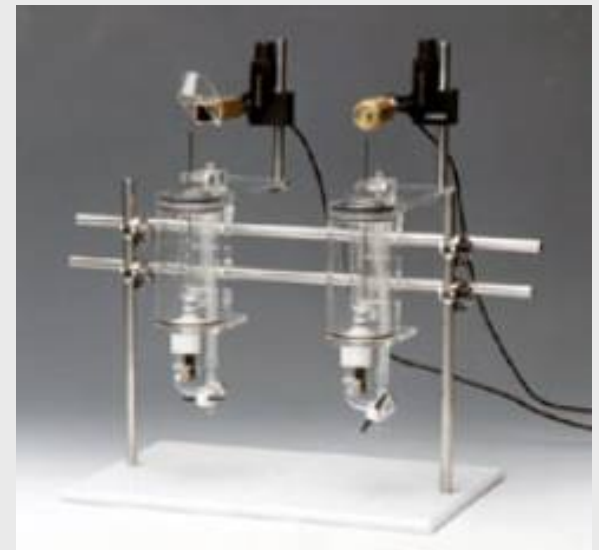


preklinické studie

testy na tkáňových kulturách
isolovaných orgánech
laboratorních zvířatech

drug

jedna sloučenina



Vývoj léčiv

klinické studie

1. fáze

podávání zdravým dobrovolníkům (20 – 100)
studium účinků na tělesné funkce, vedlejších účinků,
farmakokinetiky, určení dávkování

2. fáze

podávání vybraným pacientům (stovky)
„blind“/„double blind“
bezpečnost, účinnost, dávka, farmakokinetika

3. fáze

podávání větší skupině pacientů (stovky až tisíce)
srovnání se standardní terapií

povolovací řízení

4. fáze

zkoušení v praxi, dlouhodobé vyhodnocování

TOP 10 DRUGS (USA, 2004)

obchodní
název

terapie

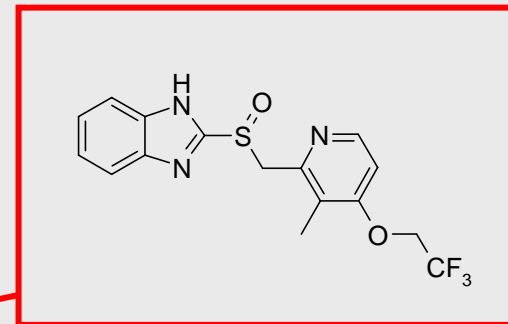
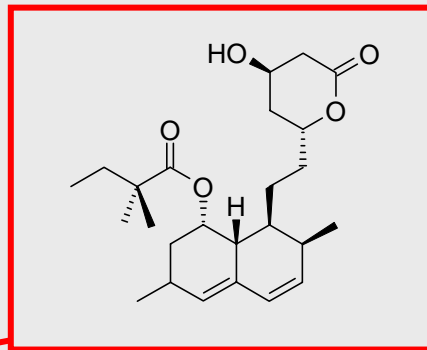
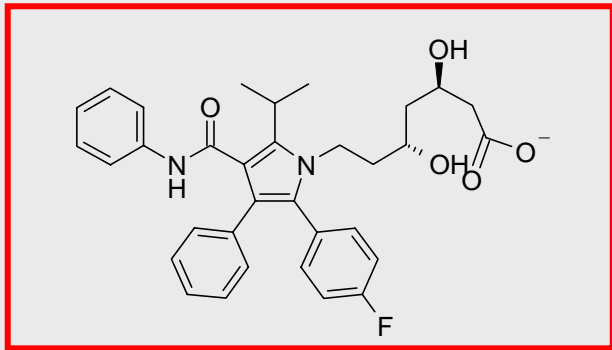
výrobce

1	LIPITOR	atorvastatin	tuky	HMG-CoA reductase	PFIZER, INC	7 100 000 000 USD
2	ZOCOR	simvastatin	tuky	HMG-CoA reductase	MERCK & CO.,INC	5 500 000 000 USD
3	PREVACID	lansoprazol	žaludek	H ⁺ /K ⁺ -ATPasa	TAP PHARMA	4 000 000 000 USD
4	NEXIUM	esomeprazol	žaludek	H ⁺ /K ⁺ -ATPasa	ASTRAZENECA PHARM. LP	3 600 000 000 USD
5	PROCRIT	erythropoetin	krvetvorba	EPOR	JOHNSON & JOHNSON	3 300 000 000 USD
6	ZOLOFT	sertralin	deprese, obezita	serotonin transporter	PFIZER, INC	3 000 000 000 USD
7	PLAVIX	clopidogrel	oběhový systém	ADP receptor	BRISTOL-MYERS SQUIBB CORP	2 900 000 000 USD
8	ADVAIR DISKUS	fluticason	astma	glucocorticoid receptor	GLAXOSMITHKLINE	2 800 000 000 USD
9	ZYPREXA	olanzapin	hlava	serotonin-dopamine-receptor	ELI LILLY	2 700 000 000 USD
10	CELEBREX	celecoxib	záněty	COX-2	PFIZER, INC	2 700 000 000 USD

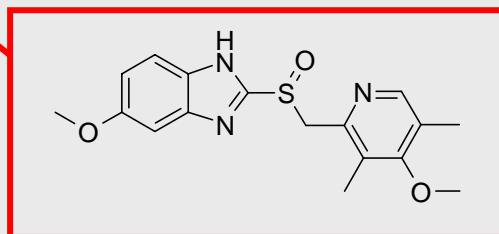
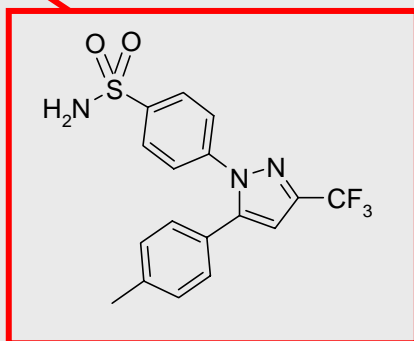
účinná
látka

cíl

roční
tržby



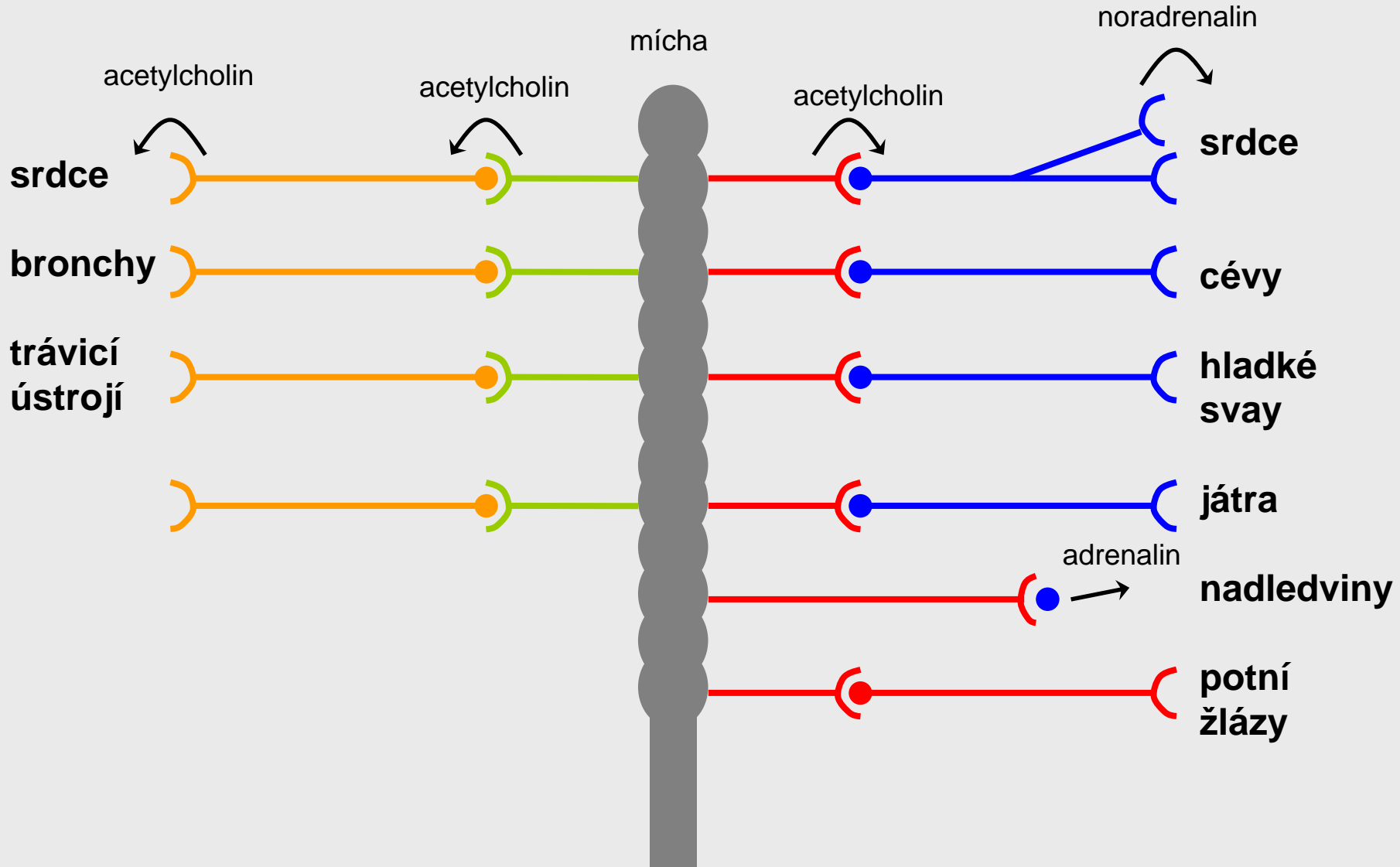
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vegetativní nervový systém

parasymptický

sympatický



synapse

MAO

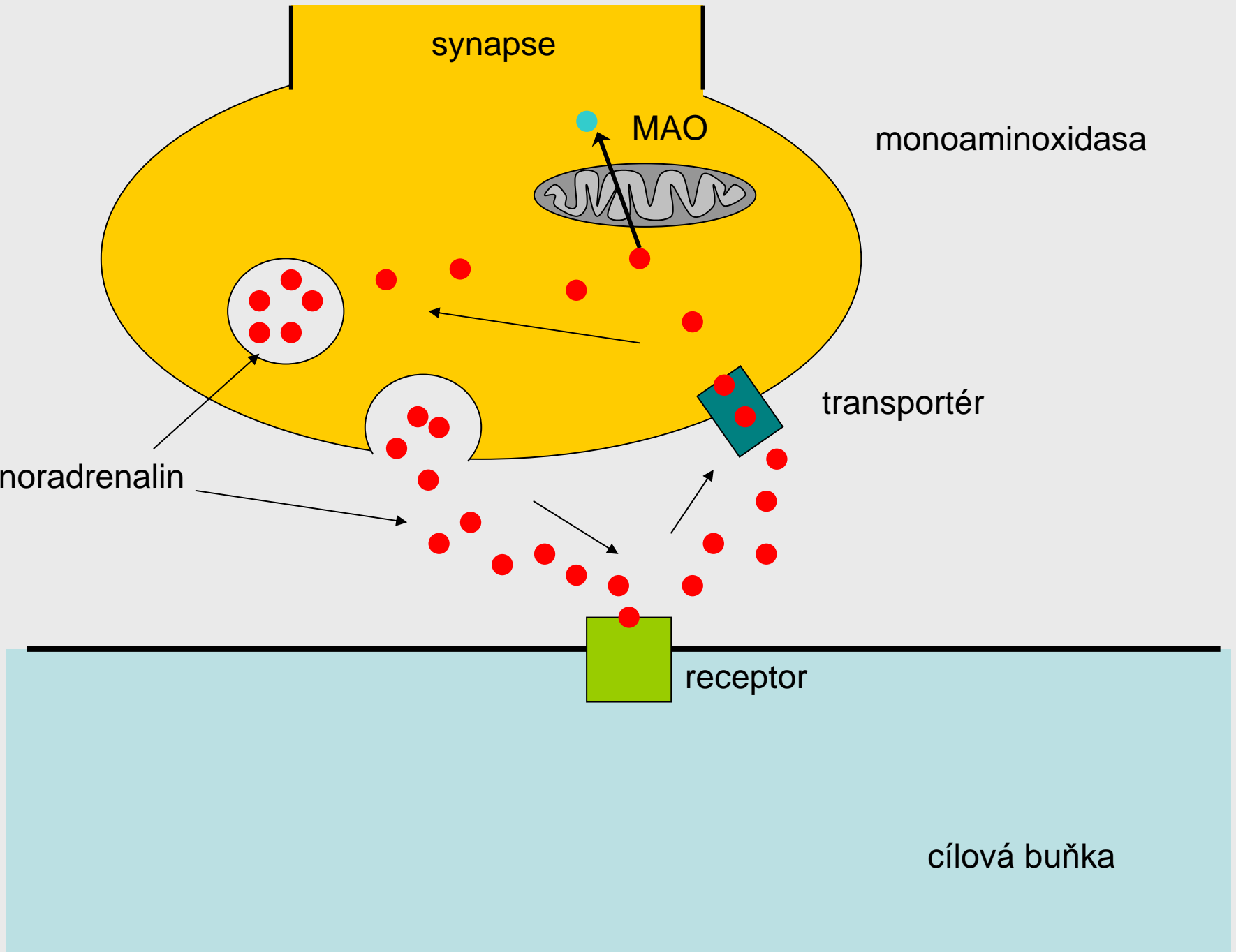
monoaminoxidasa

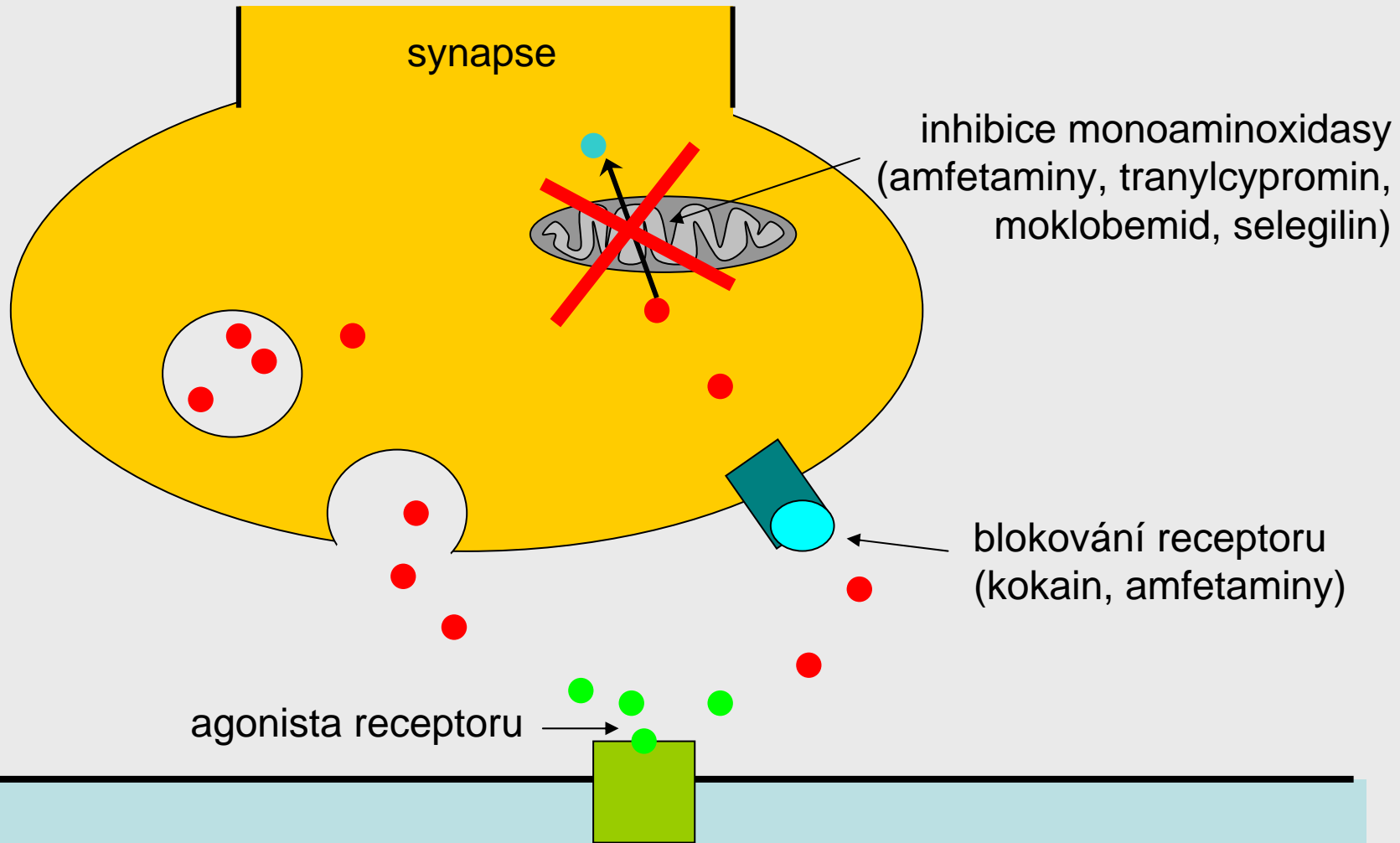
transportér

noradrenalin

receptor

cílová buňka





posilování sinálu (sympatomimetika)

léčba depresí, léčba parkinsonovy chroby,
nosní kapky, léčba hypotenze

cílová buňka

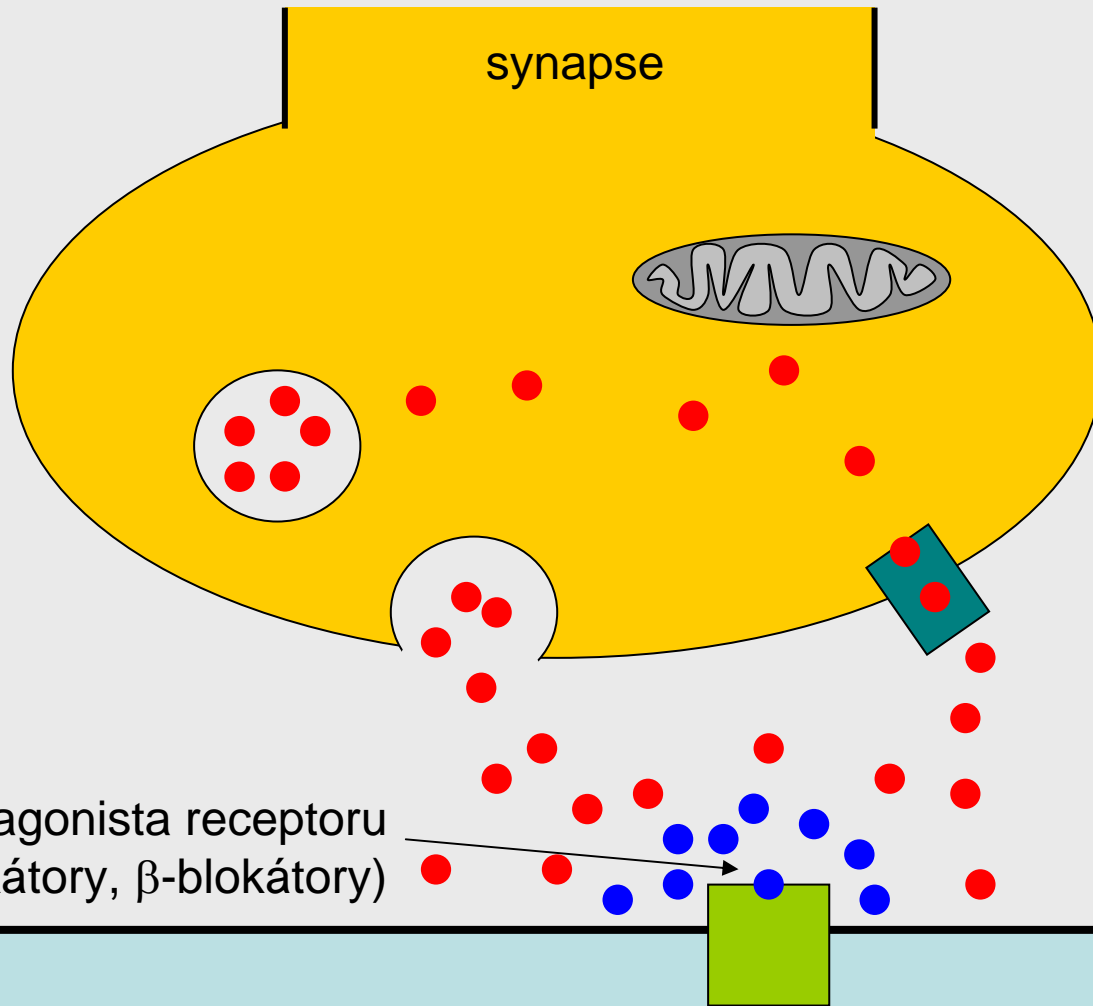
synapse

antagonista receptoru
(α -blokátory, β -blokátory)

zeslabování sinálu (sympatolytika)

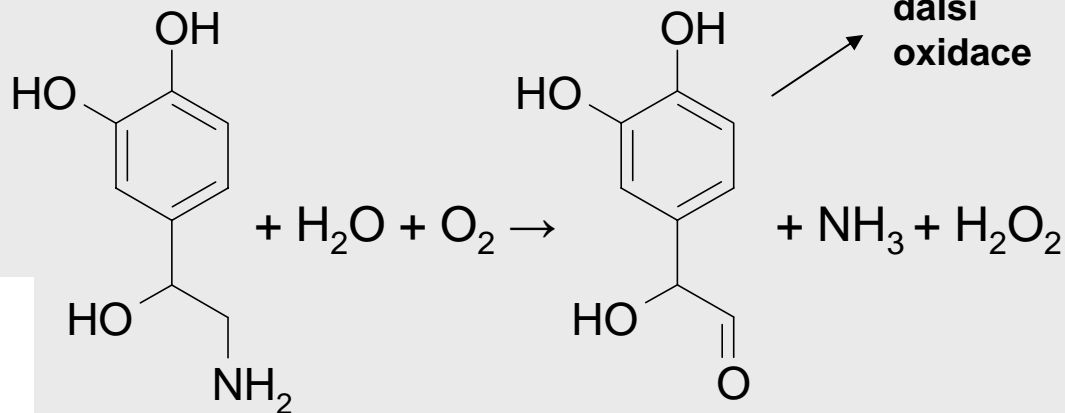
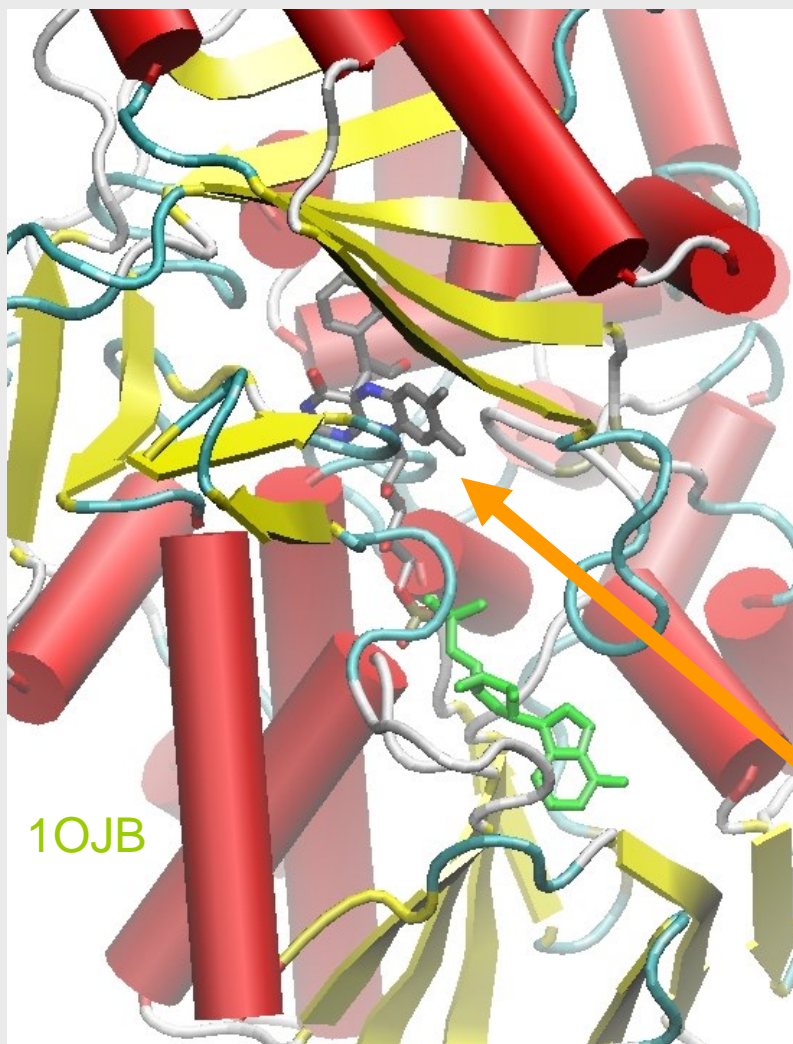
léčba hypertenze a arytmie, léky na uklidnění

cílová buňka

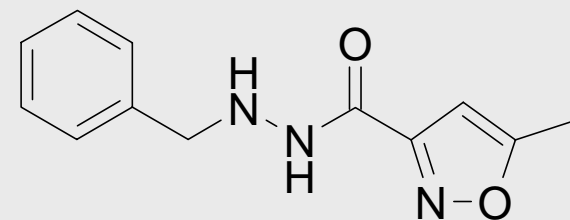


Monoaminoxidasa (MAO) EC 1.4.3.4

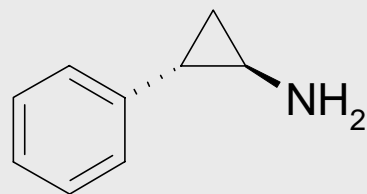
léčba depresí



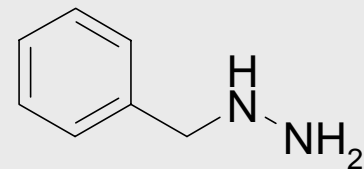
noradrenalin
a další monoaminy



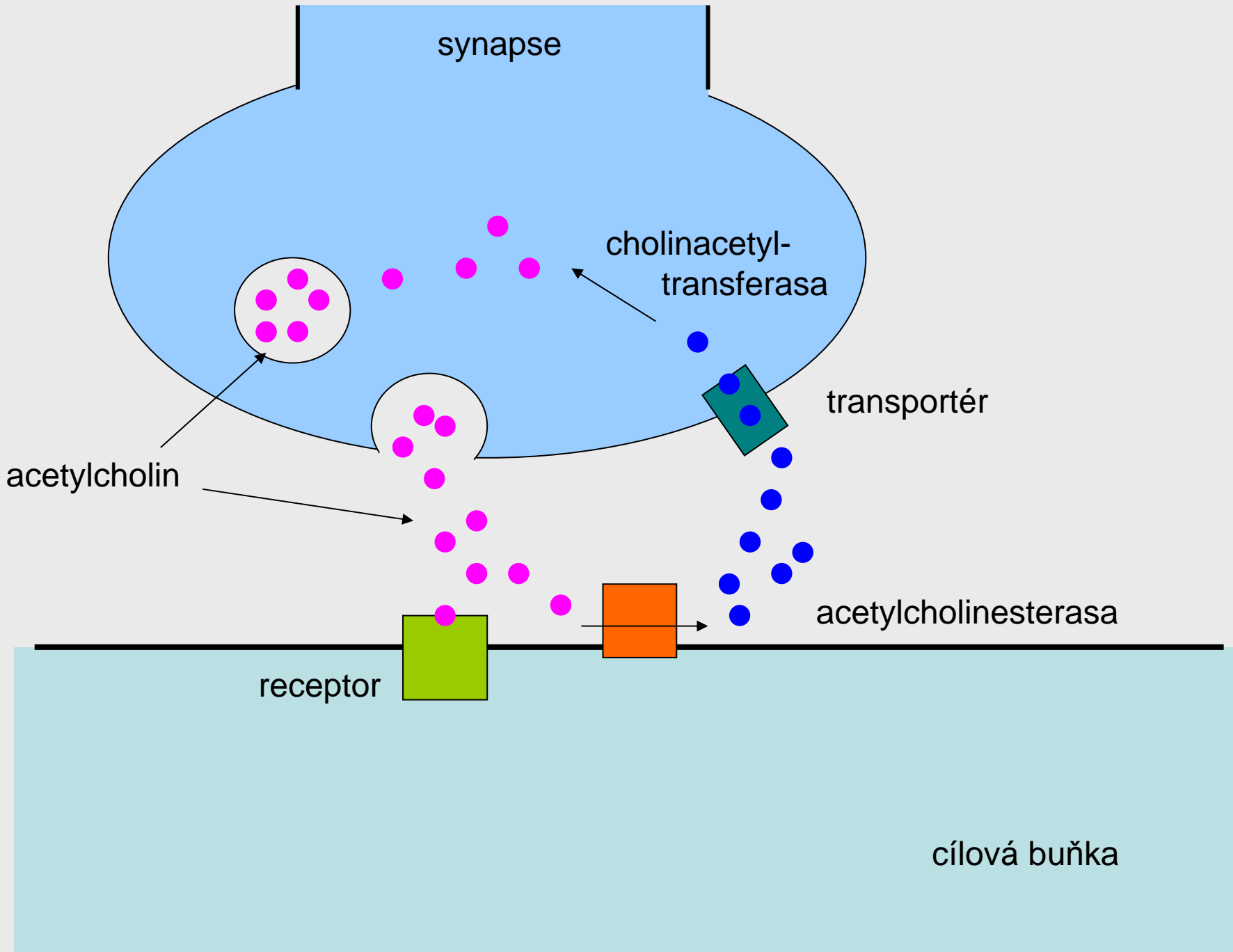
Isocarboxazid

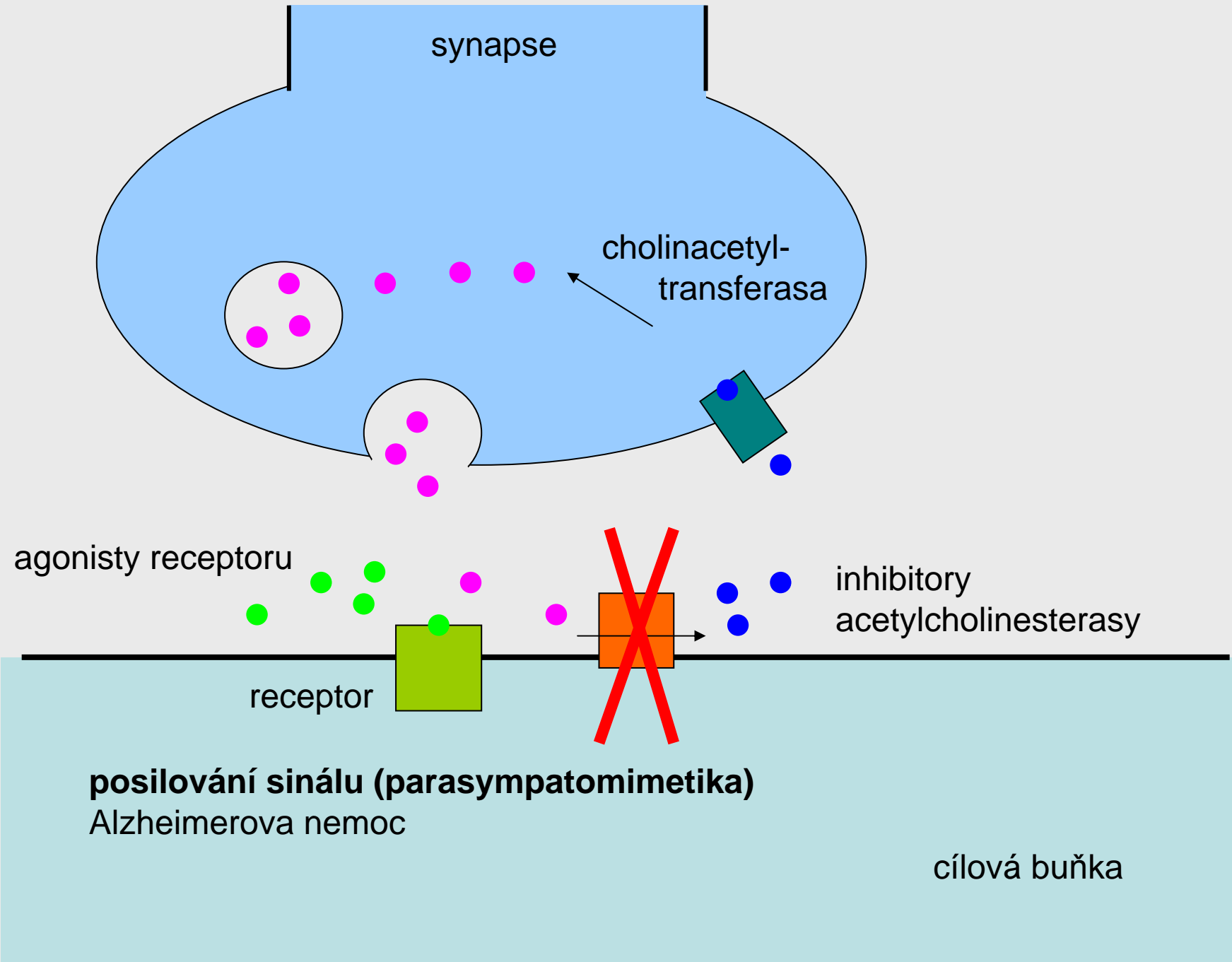


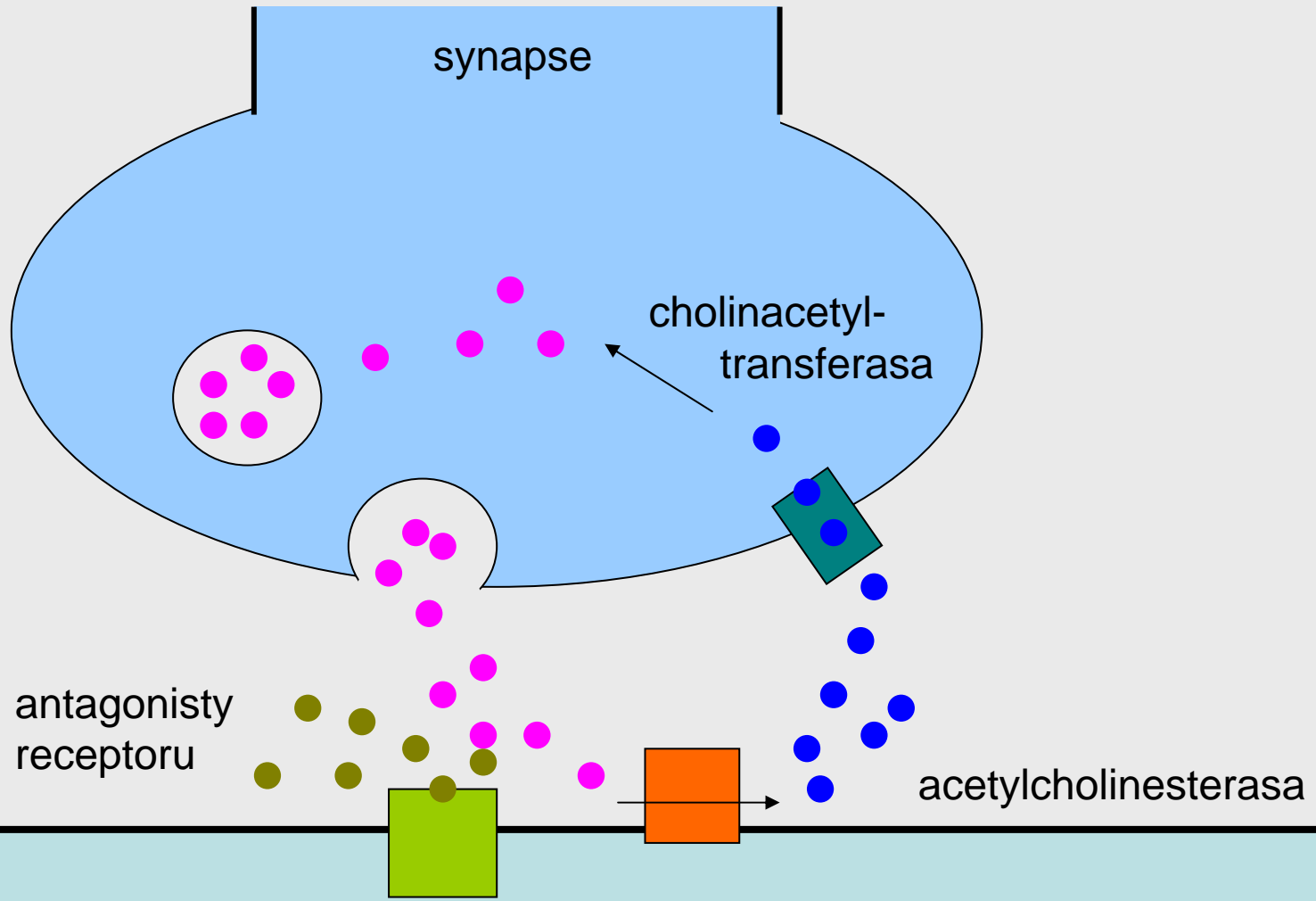
Tranlylcypromine



Phenzazine





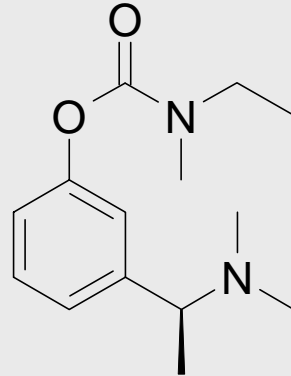
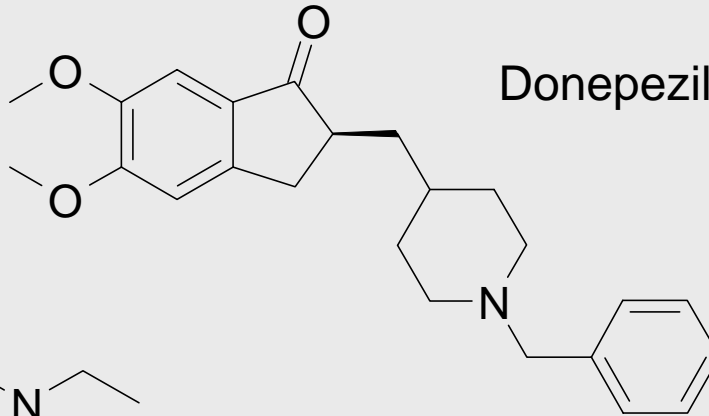


zeslabování sinálu (parasympatolytika)

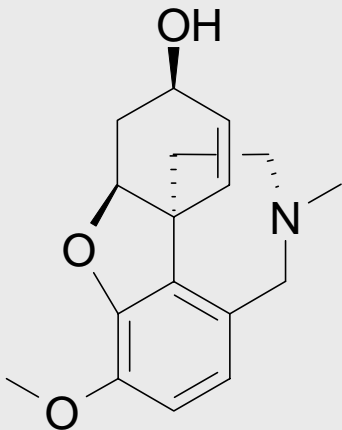
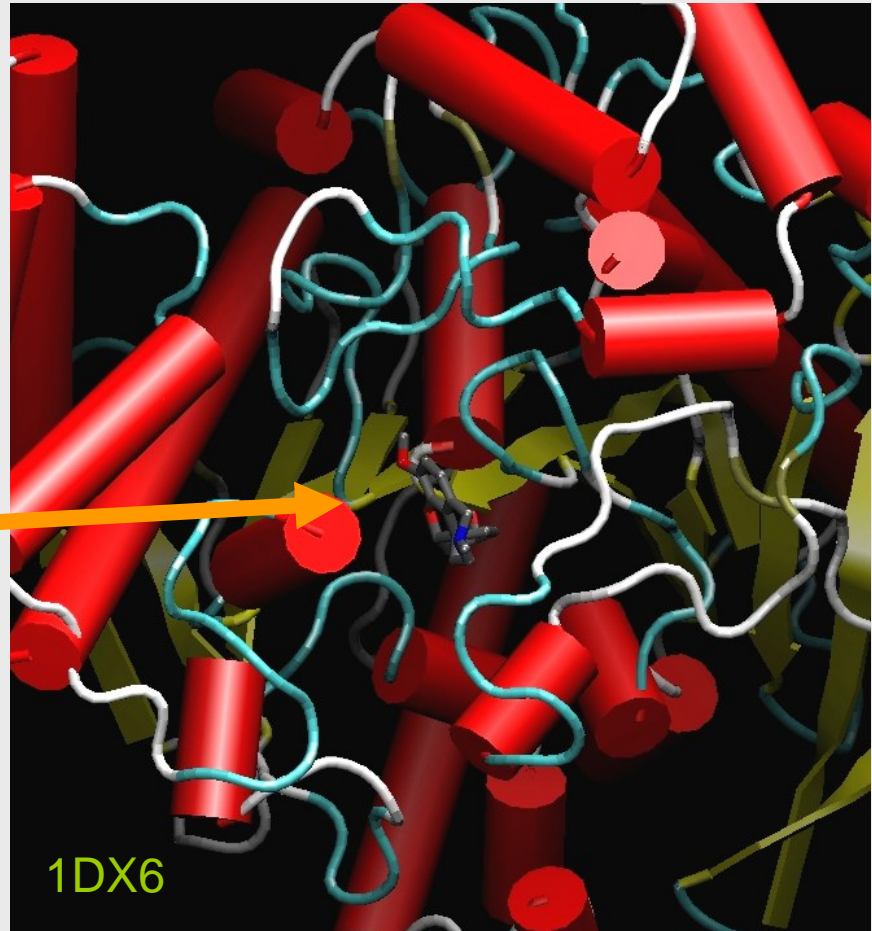
žaludeční vředy, vyšetření očí,
léčba bronchitidy, proti zvracení,
léky na uklidnění

cílová buňka

Acetylcholinesterasa EC 3.1.1.7



Rivastigmine



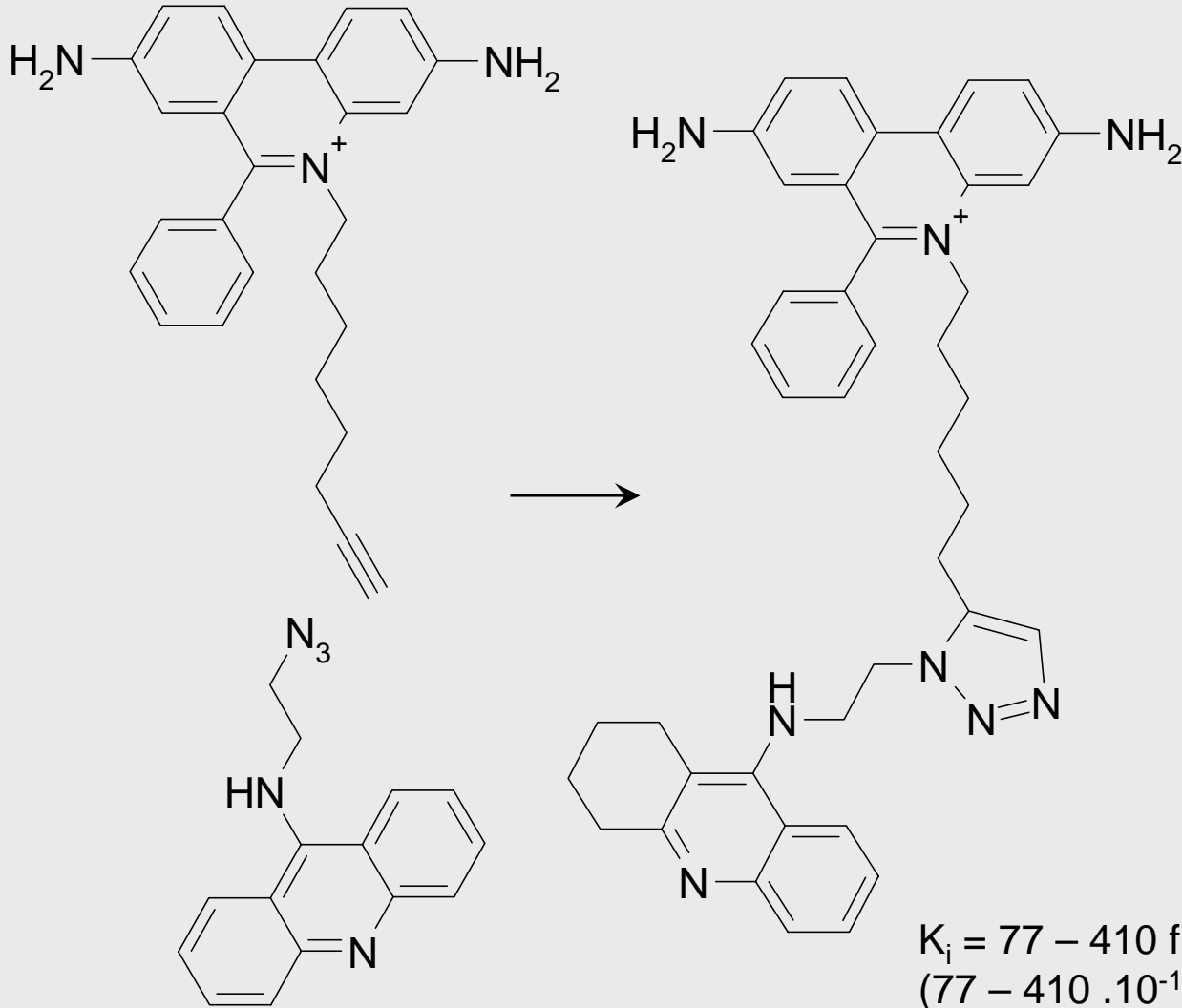
Galantamine



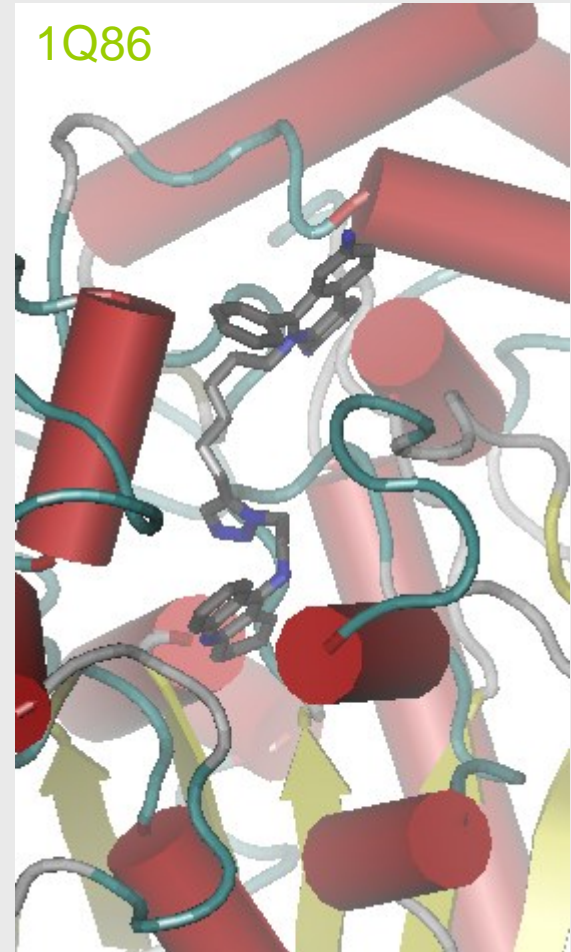
K. Barry Sharpless



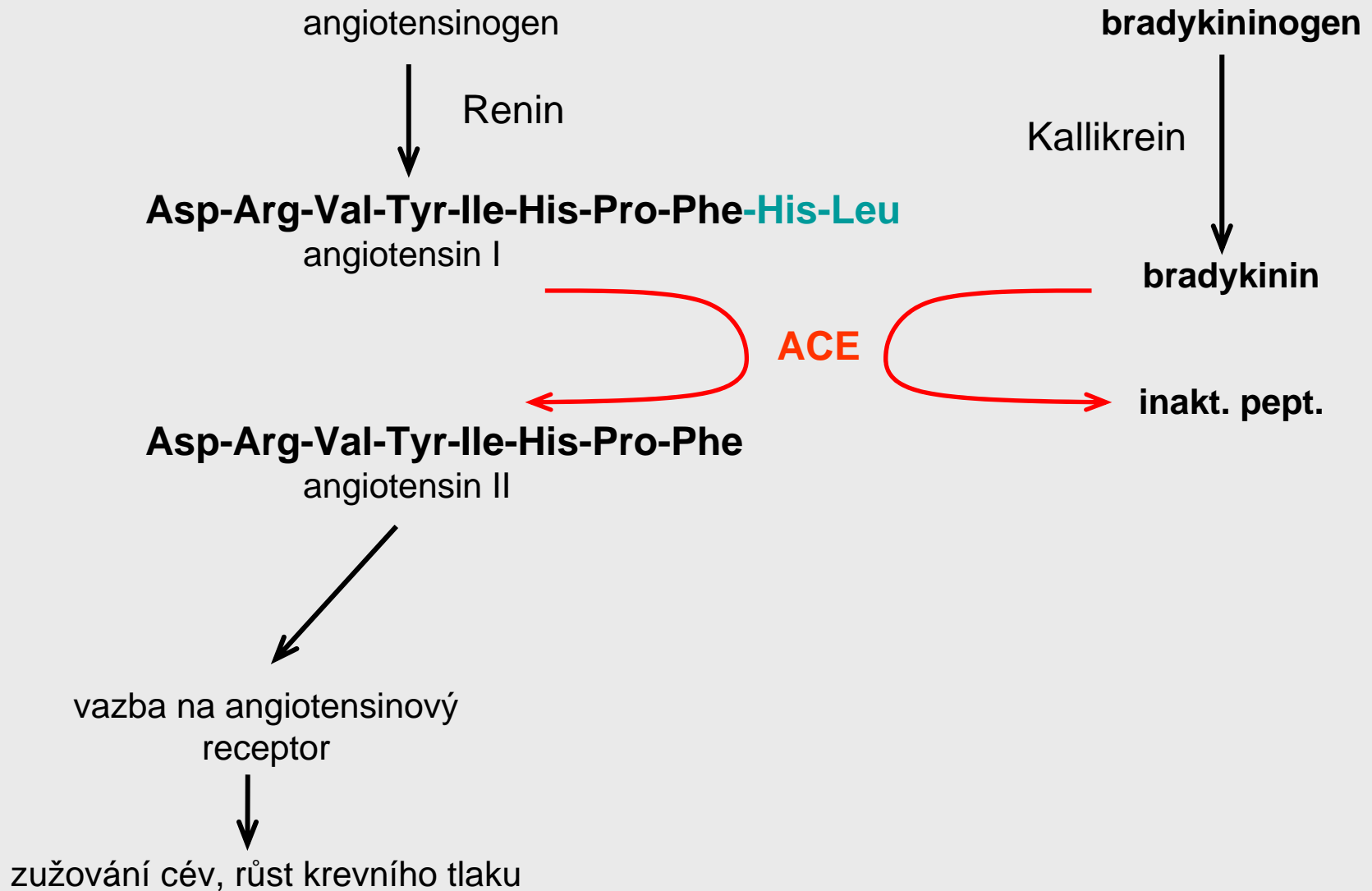
Acetylcholinesterasa



$K_i = 77 - 410 \text{ fM}$
($77 - 410 \cdot 10^{-15}$)

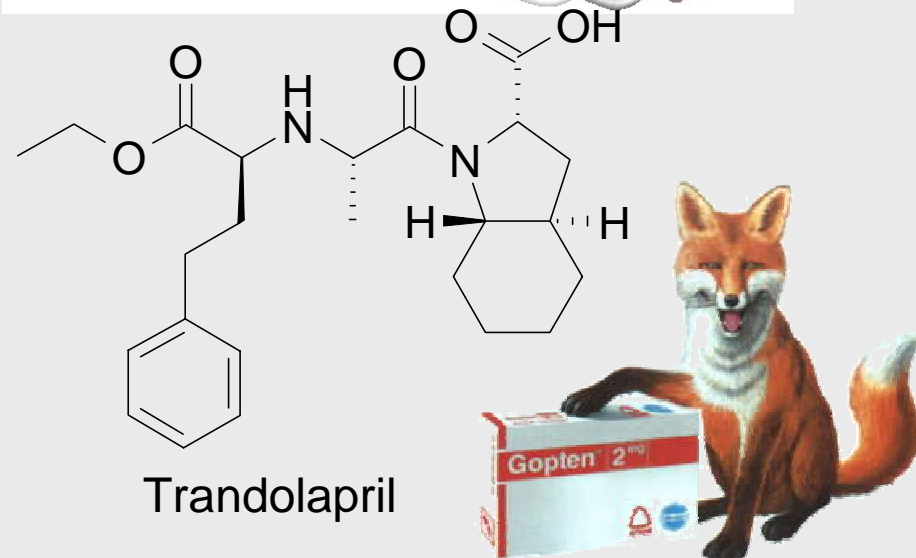
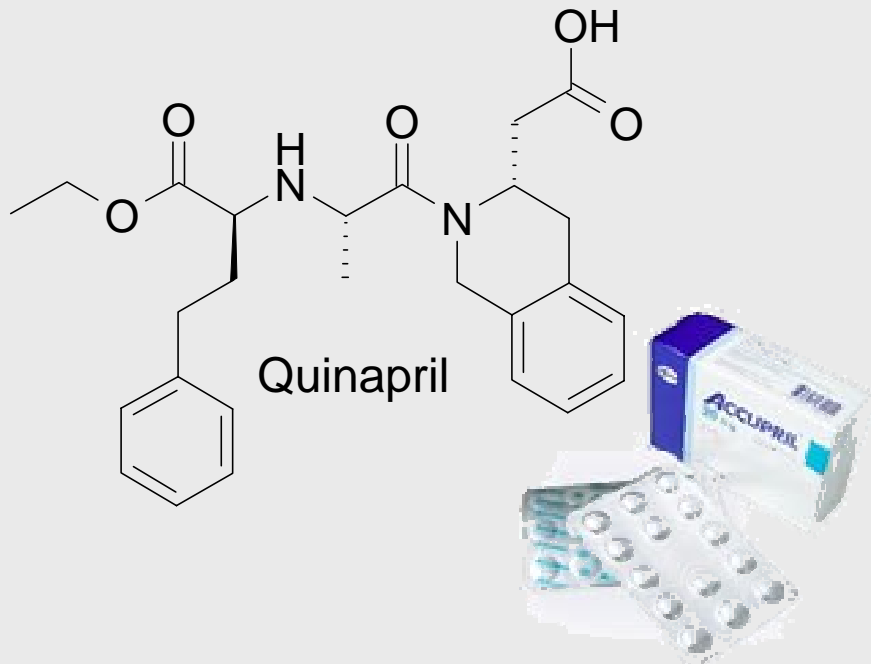
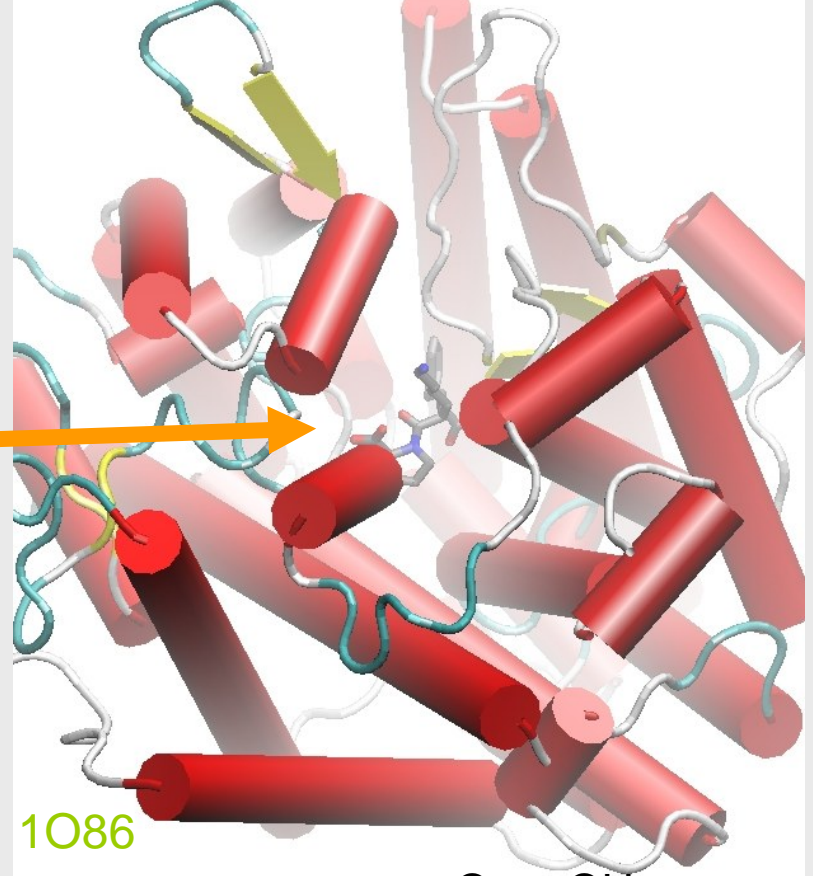
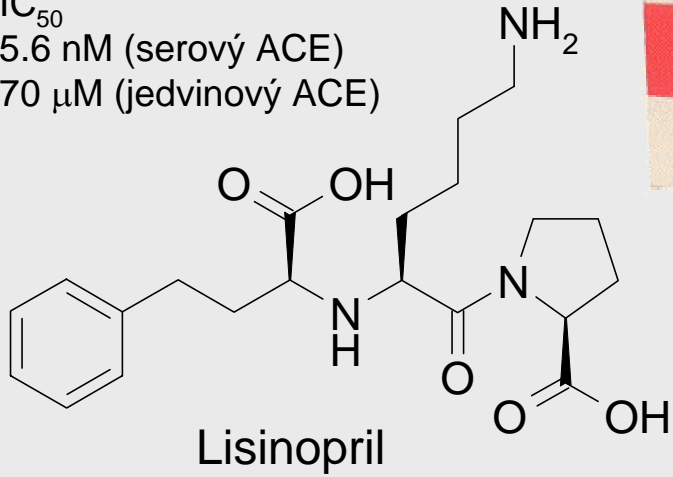
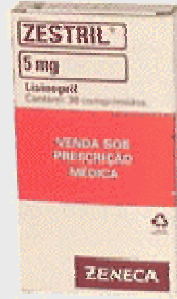


Angiotensin converting enzyme (ACE) EC 3.4.15.1

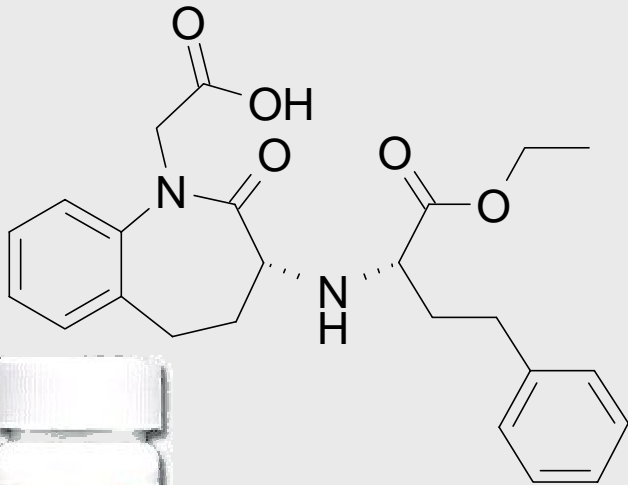


ACE inhibitory

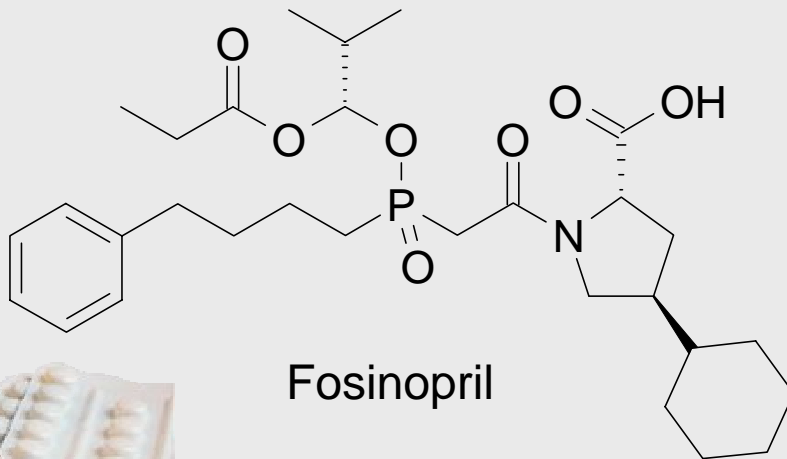
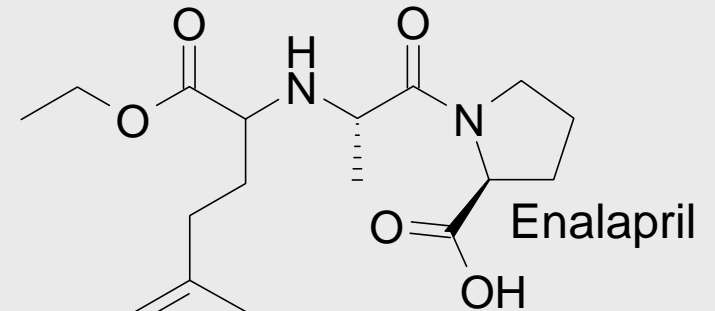
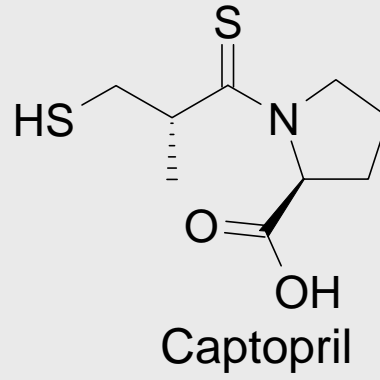
IC_{50}
5.6 nM (serový ACE)
70 μ M (jedvinový ACE)



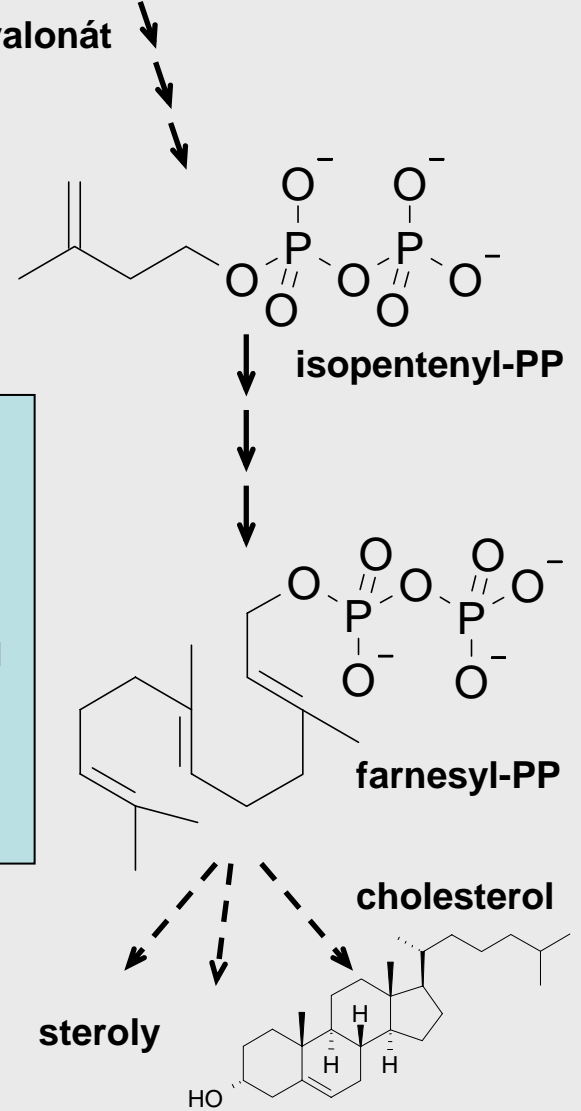
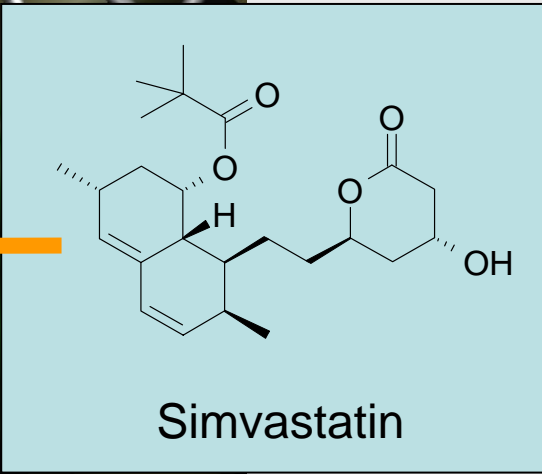
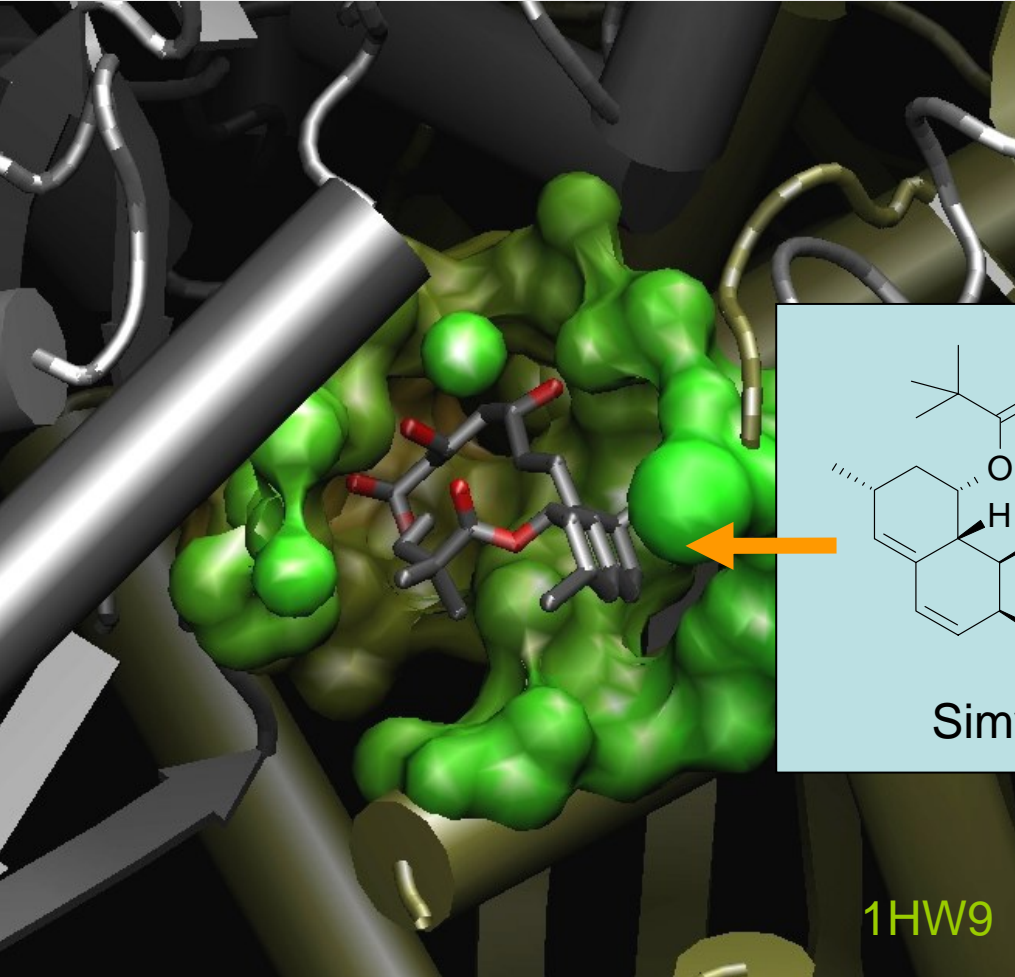
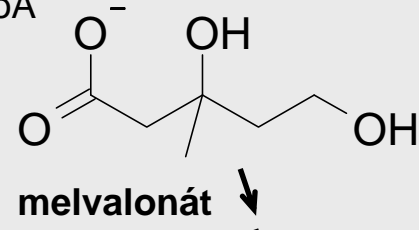
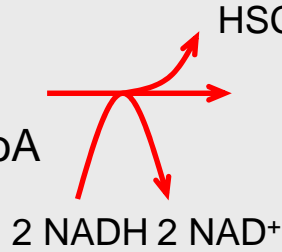
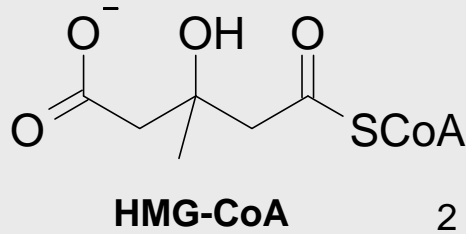
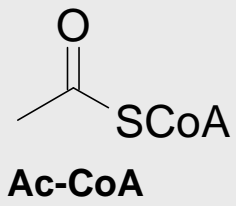
ACE inhibitory



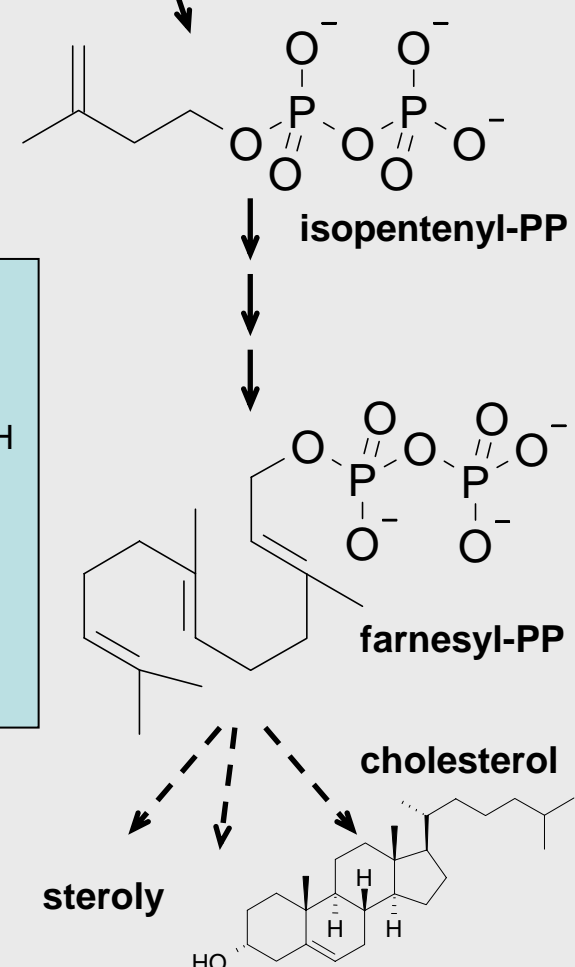
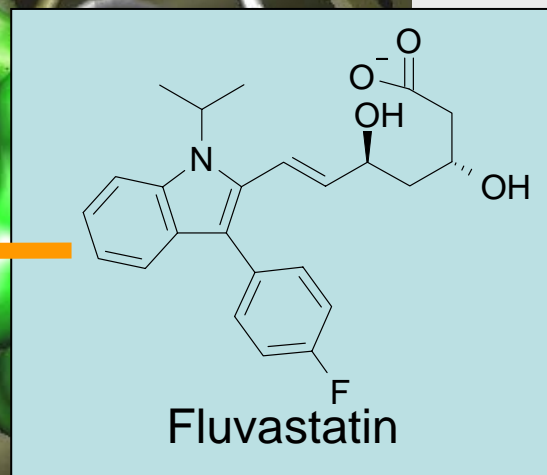
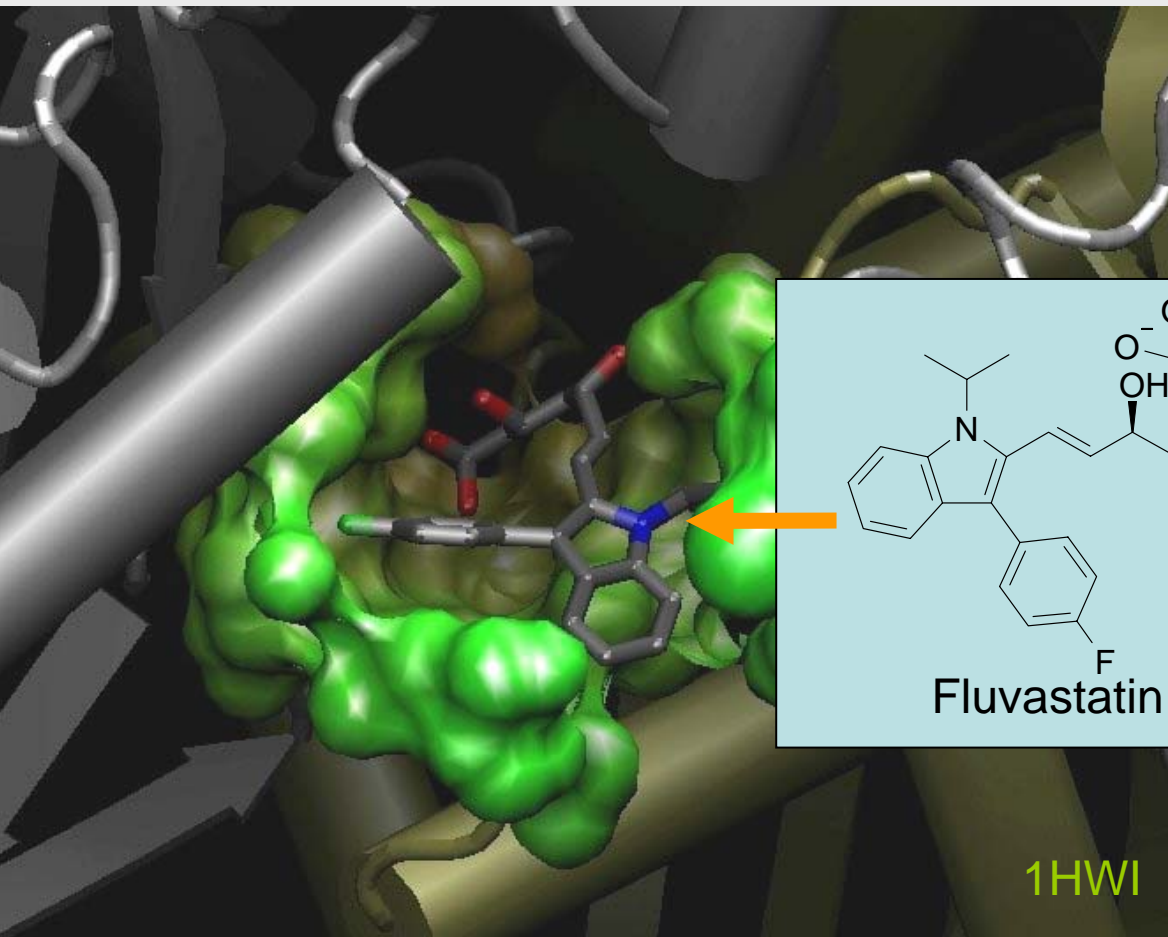
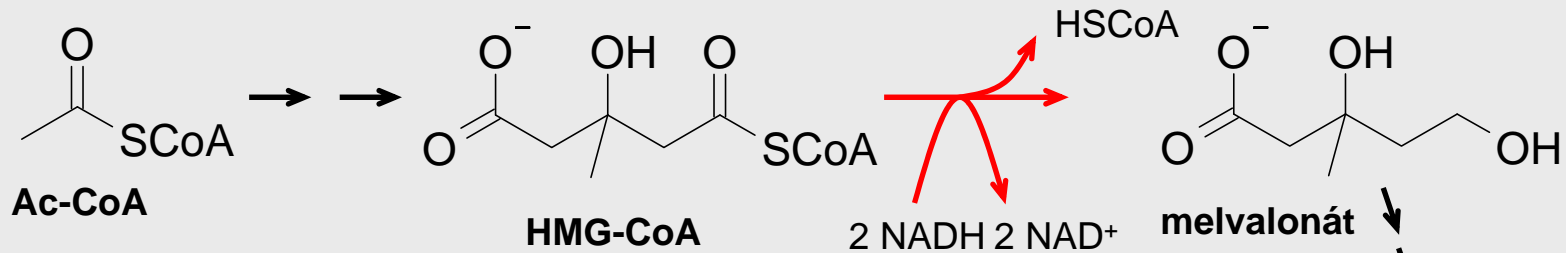
Benazepril



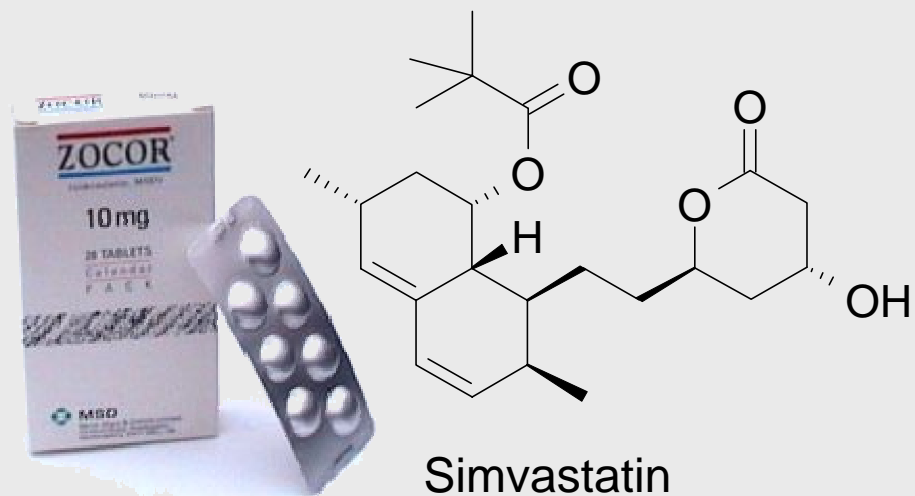
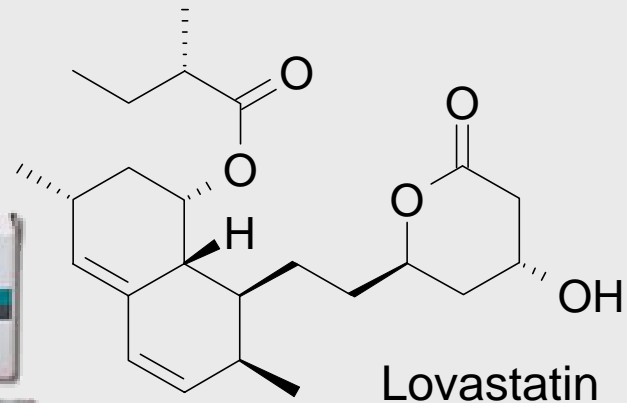
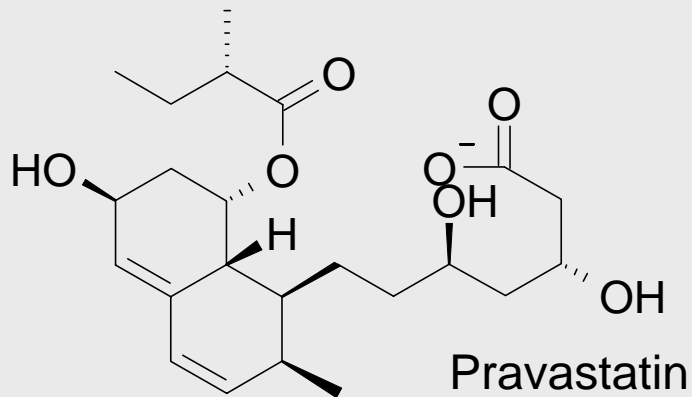
HMG-CoA reduktasa EC 1.1.1.34



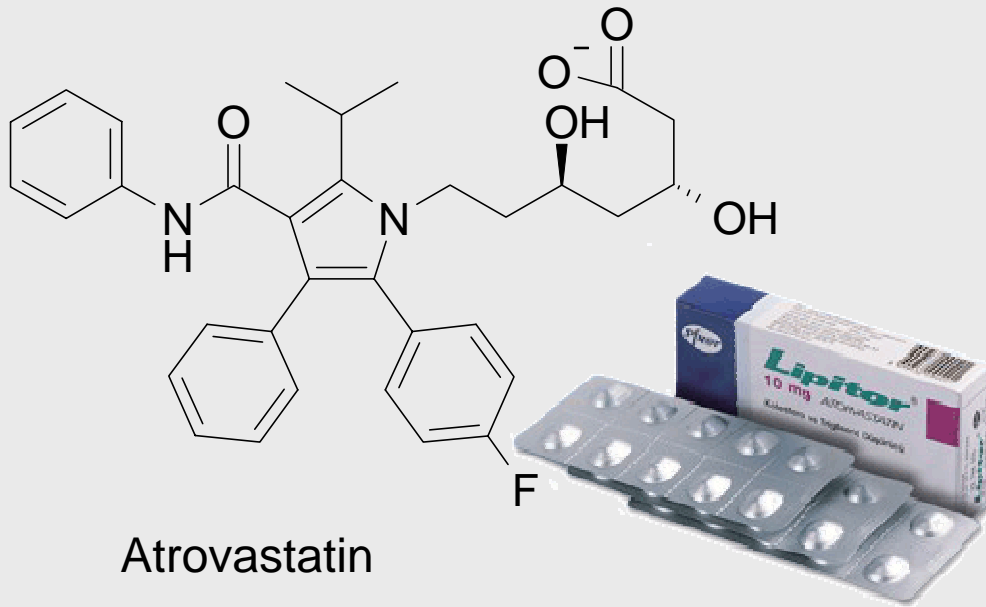
HMG-CoA reduktasa



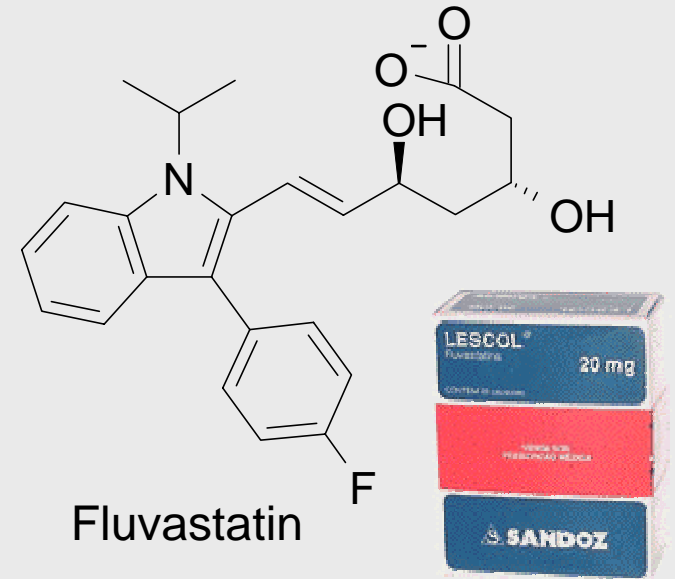
HMG-CoA reduktasa



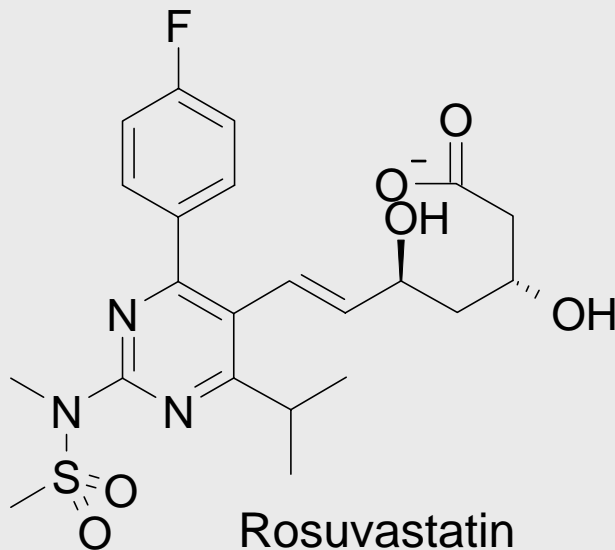
HMG-CoA reduktasa



Atrovastatin



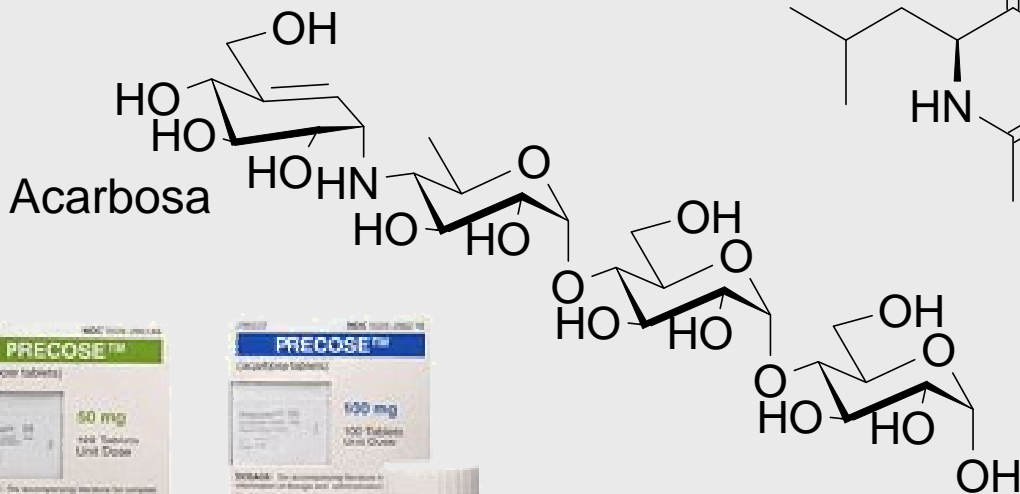
Fluvastatin



Rosuvastatin

Enzymy trávicího traktu

Glykosidasy – diabetes II. typu



Orlistat
Lipasy - obezita



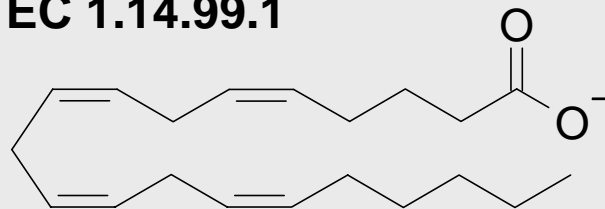
Cykloxygenasa (COX) EC 1.14.99.1

Prostaglandin-
endoperoxidsynthasa

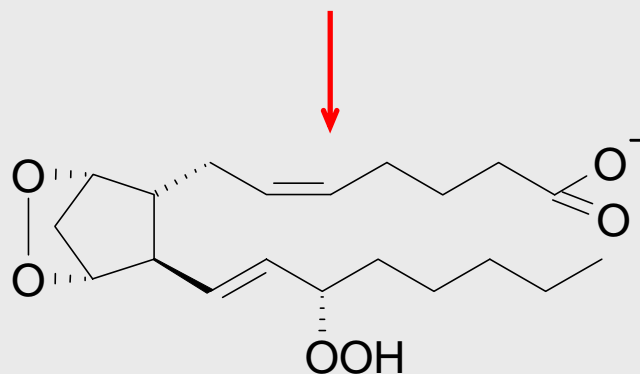
dioxygenasa a peroxidasa
zároveň

Isoenzymy

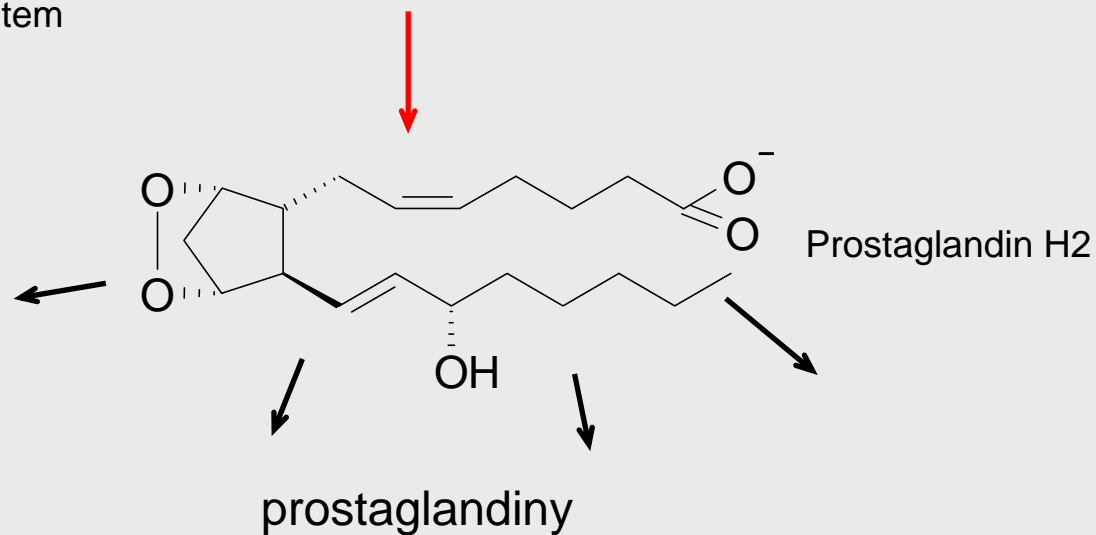
- COX-1 – žaludek, ledviny
- COX-2 – tkáň se zánětem



Arachidonová kyselina



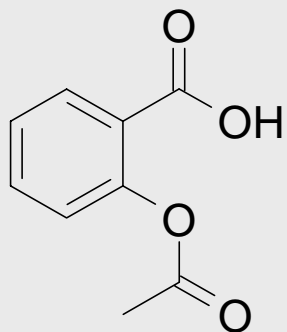
Prostaglandin G2



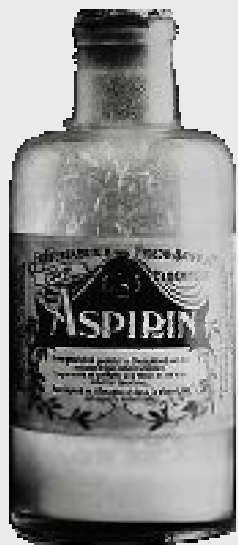
Prostaglandin H2

Cyklooxygenasa

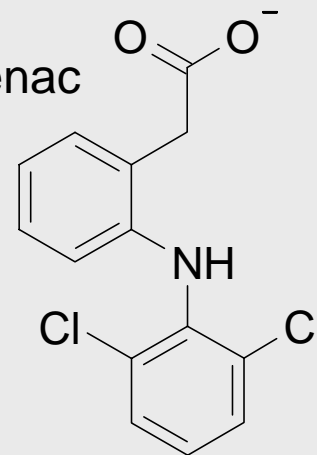
neselektivní (COX-1 & COX-2)



kyselina
acetylsalicylová



Diclofenac

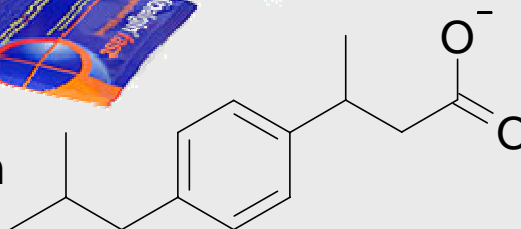


IC₅₀
300 nM (COX-1)
18 nM (COX-2)



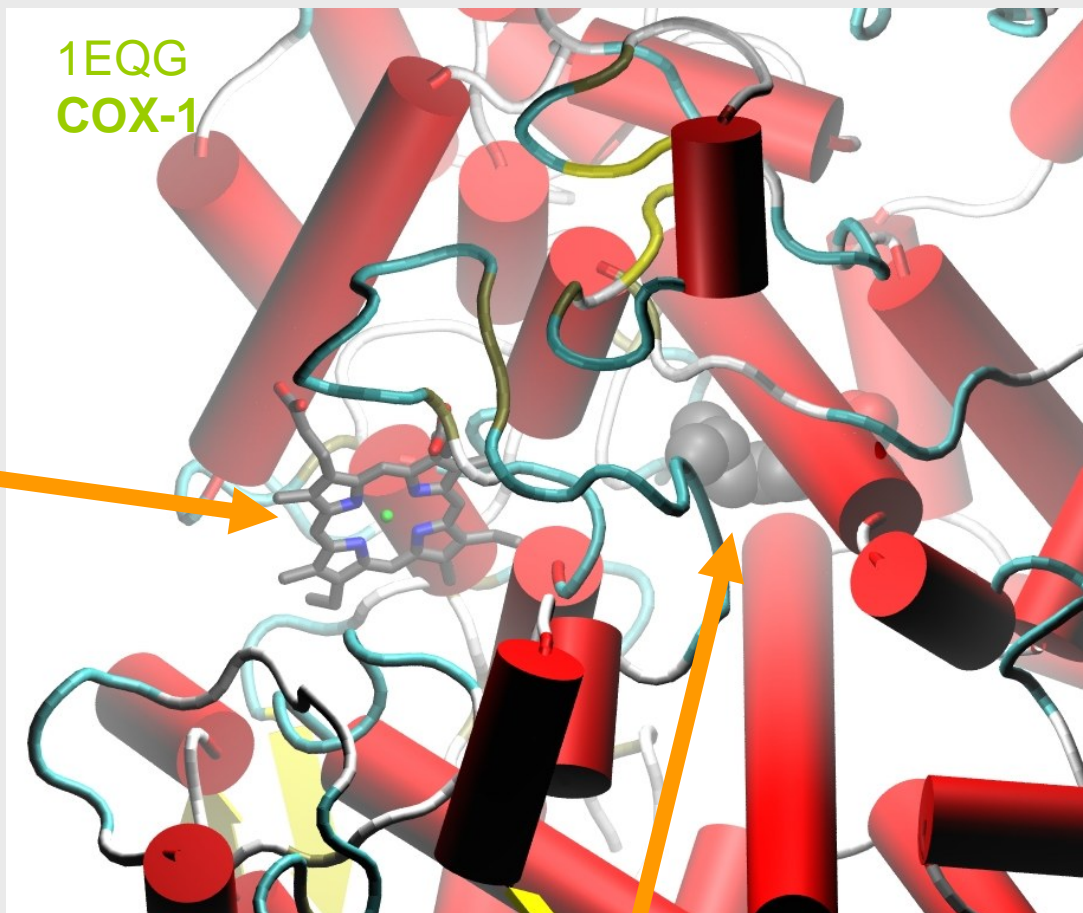
IC₅₀
2.6 μM (COX-1)
1.5 μM (COX-2)

Ibuprofen



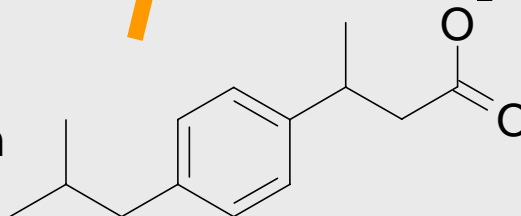
Cykloxygenasa

neselektivní (COX-1 & COX-2)



IC_{50}
2.6 μM (COX-1)
1.5 μM (COX-2)

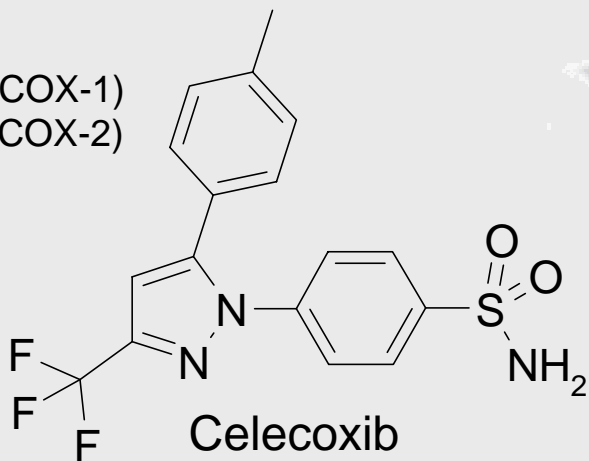
Ibuprofen



Cyklooxygenasa

selektivní (pouze COX-2)

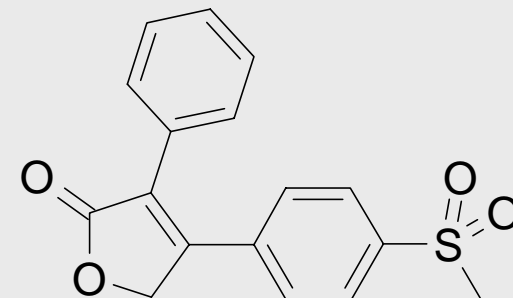
IC₅₀
15 μM (COX-1)
40 nM (COX-2)



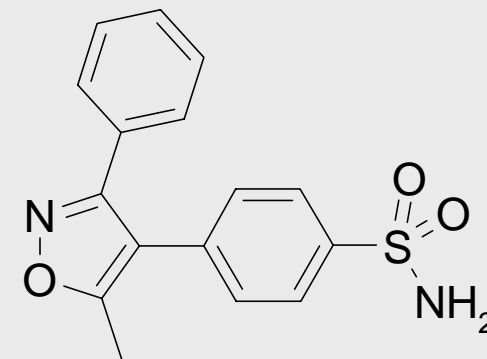
přísnější regulace



Od podzimu 2004 staženo z prodeje (dobrovolně)



Rofecoxib

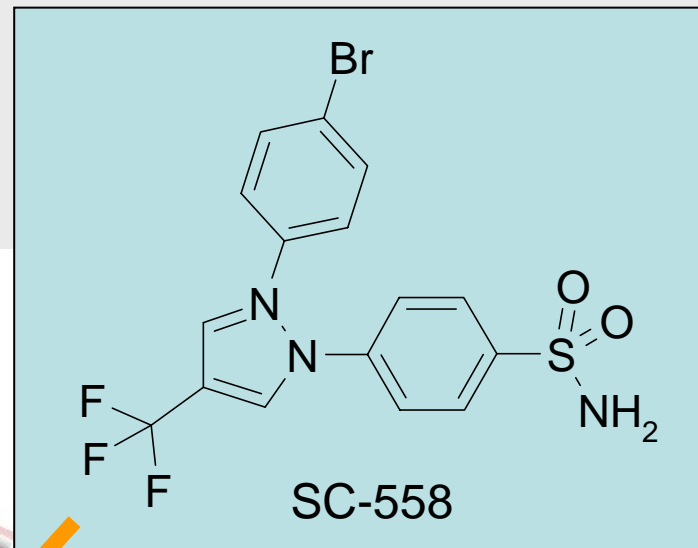
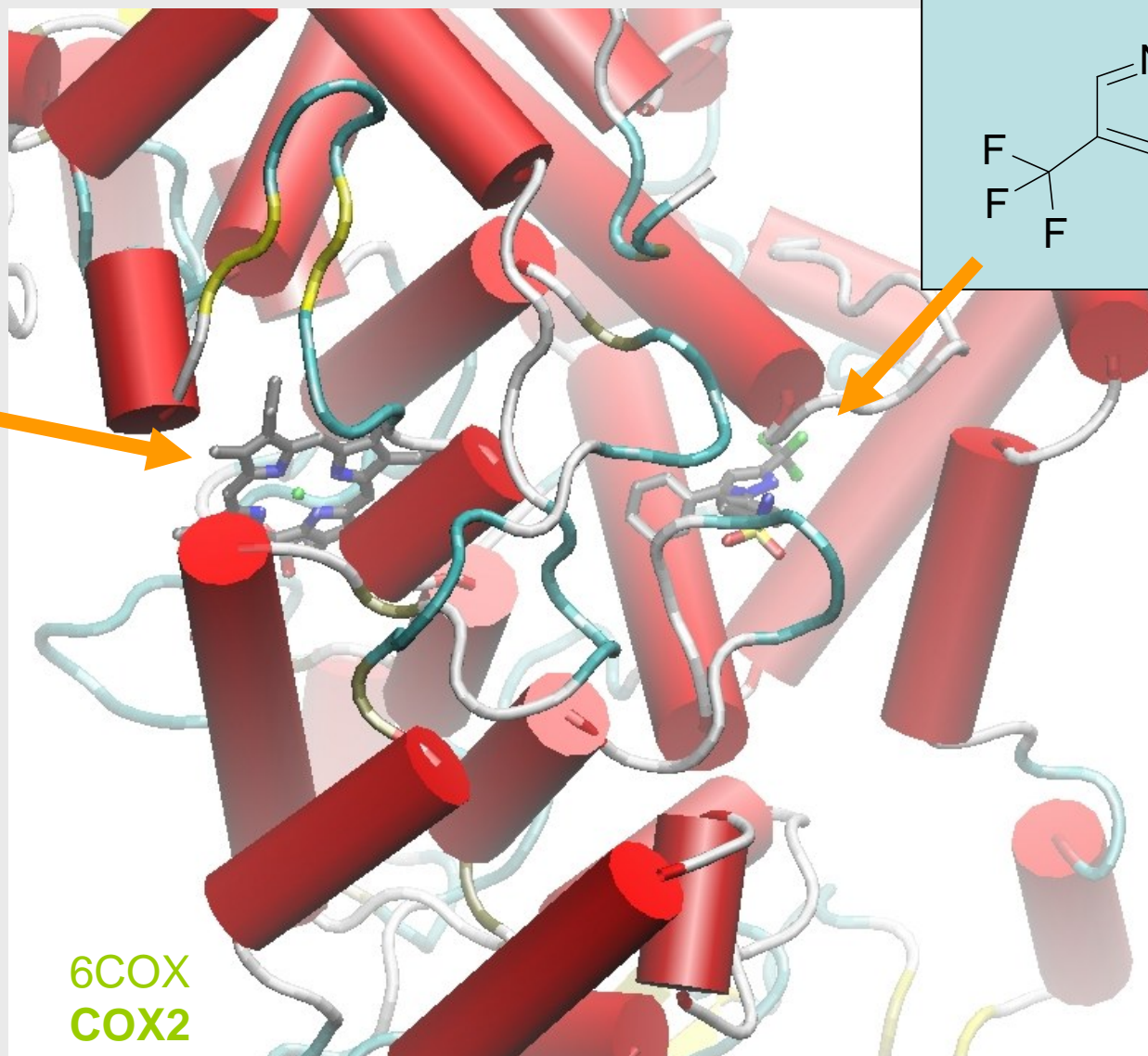


Valdecoxib

na jaře 2005 dobrovolně staženo z prodeje

Cykloxygenasa

selektivní (pouze COX-2)



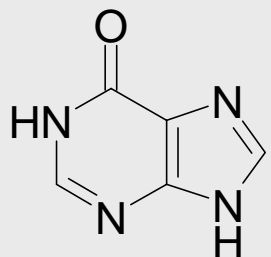
6COX
COX2

Xanthinoxidasa

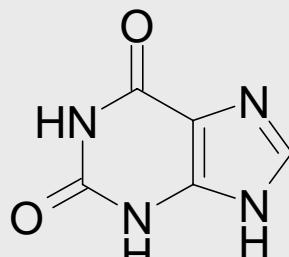
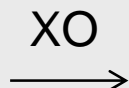
oxidasas - EC 1.1.17.32 (dříve EC 1.1.3.22)

dehydrogenasas - EC 1.1.1.4 (dříve EC 1.1.1.204)

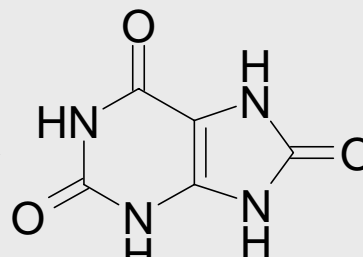
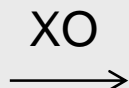
dna



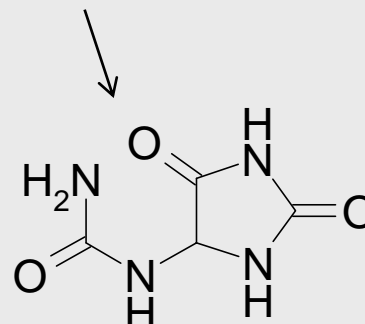
hypoxanthin



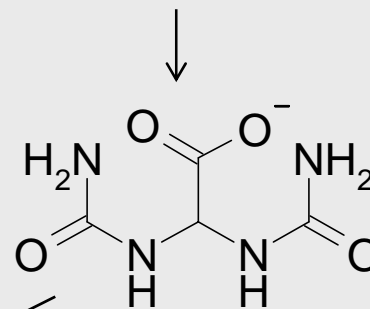
xanthin



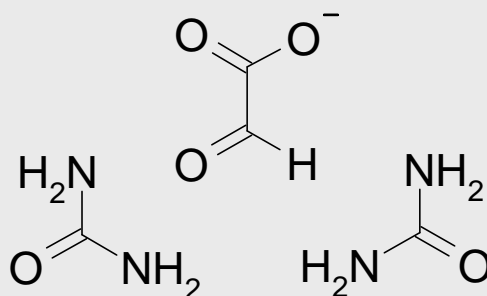
k. močová



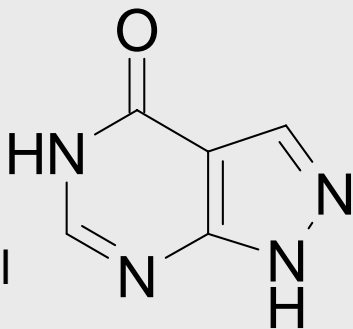
allantoin



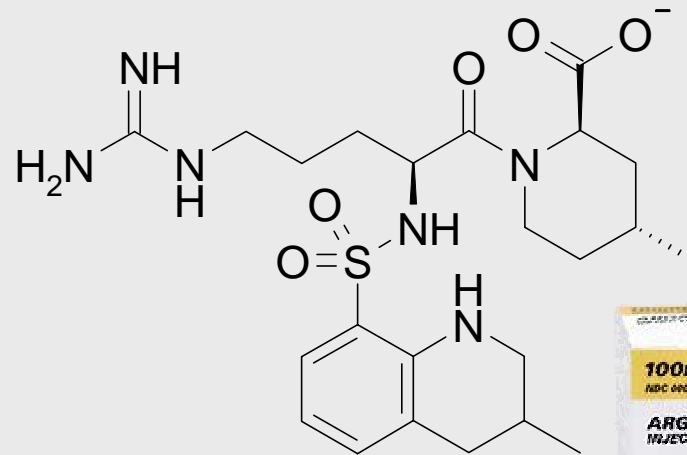
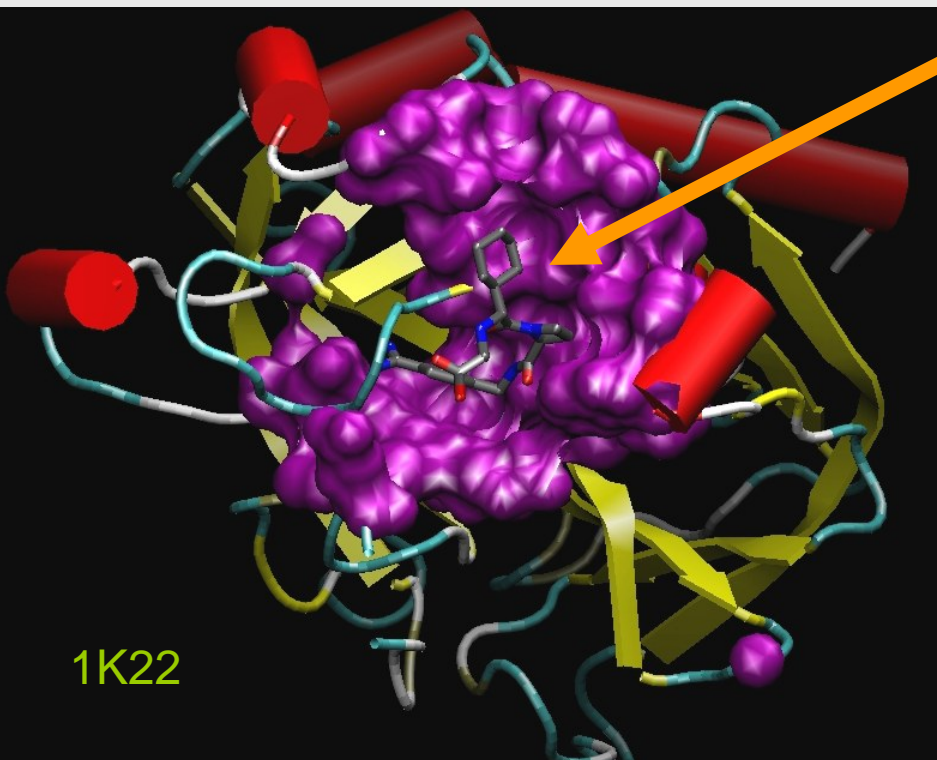
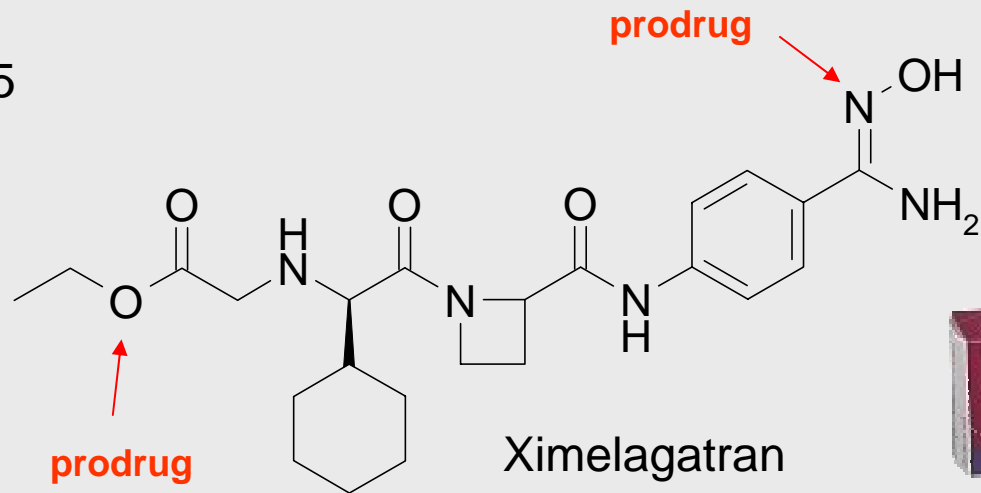
allantoát



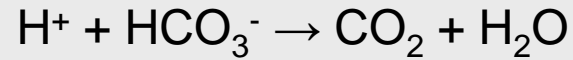
allopurinol



Thrombin EC 3.4.21.5



Karbonáthydrolyasa EC 4.2.1.1

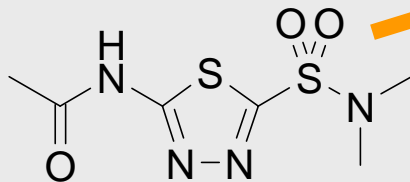


Role

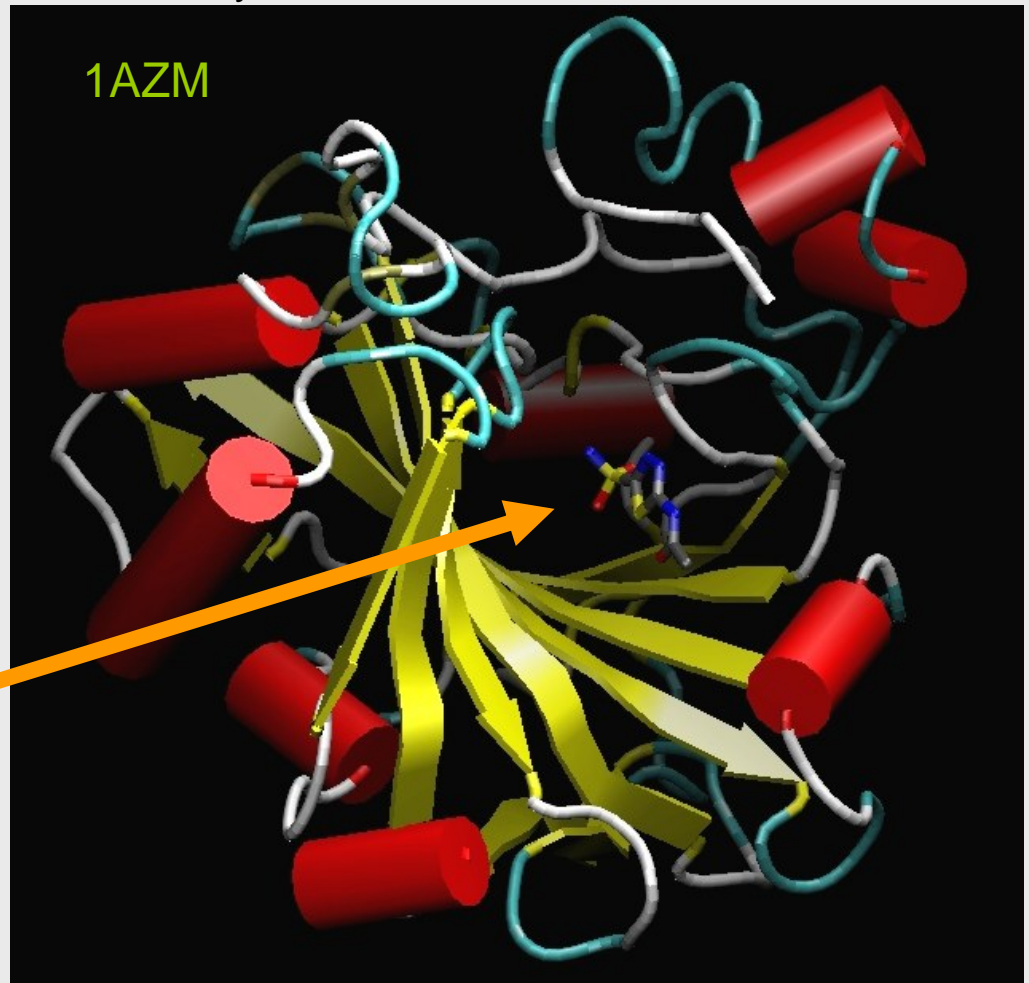
udržování acidobasické rovnováhy
spoluúčast na transportu iontů přes membrány
regulace osmotického tlaku atd.

Terapie

- zelený oční zákal
- epilepsie (určité formy)
- vysokohorská nemoc
a další

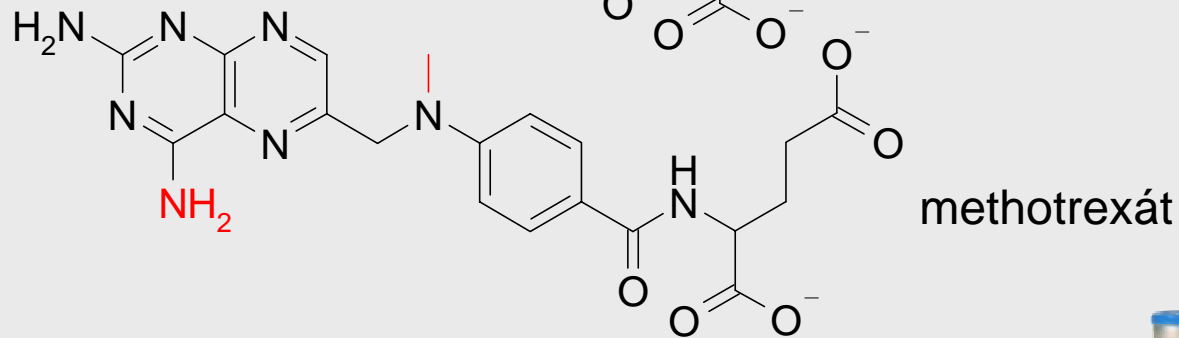
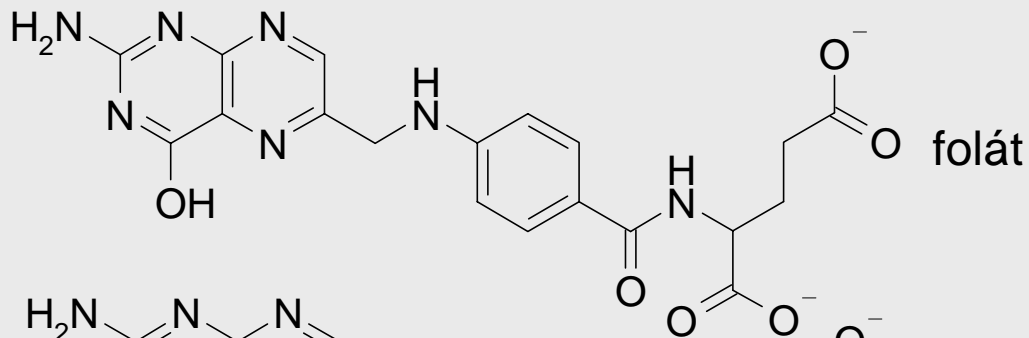
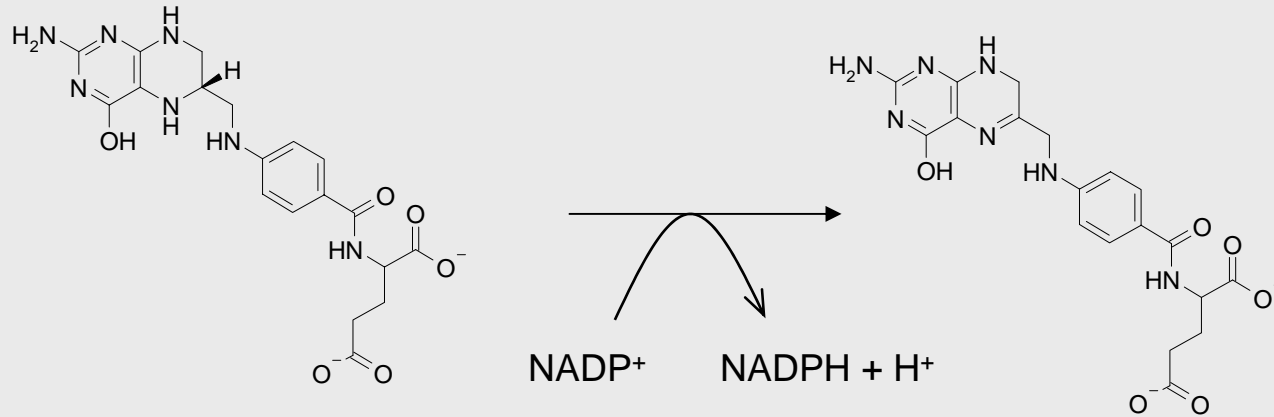


Acetazolamid



Dihydrofolát reduktasa

EC 1.5.1.3



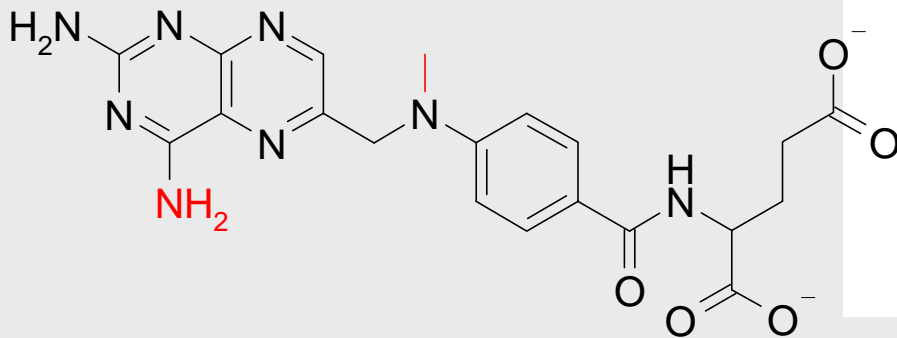
účinky: protinádorové (acute lymphoblastic leukemia a další)
imunosupresivní (revmatismus a jiná autoimunní onemocnění)



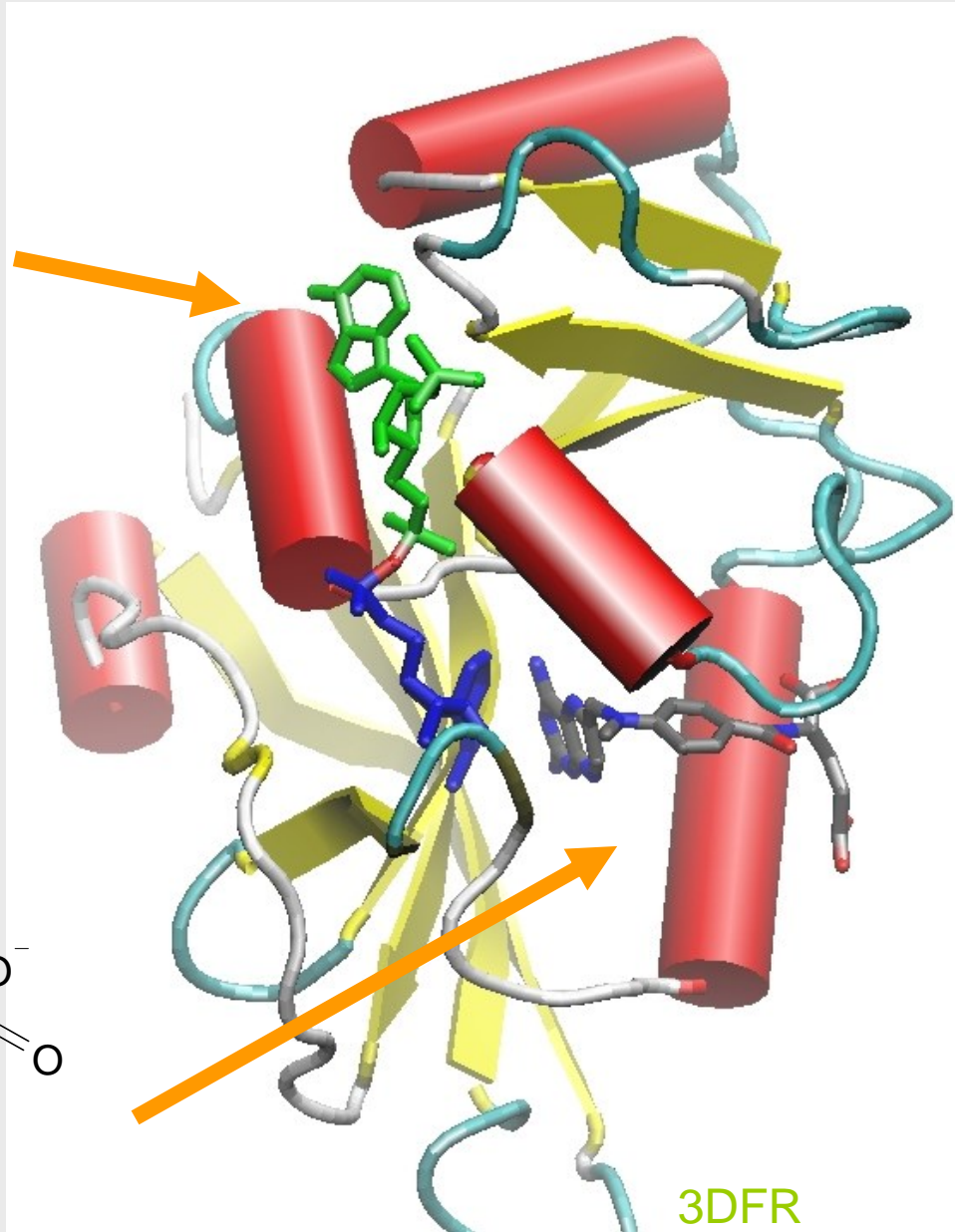
Dihydrofolát reduktasa



methotrexát



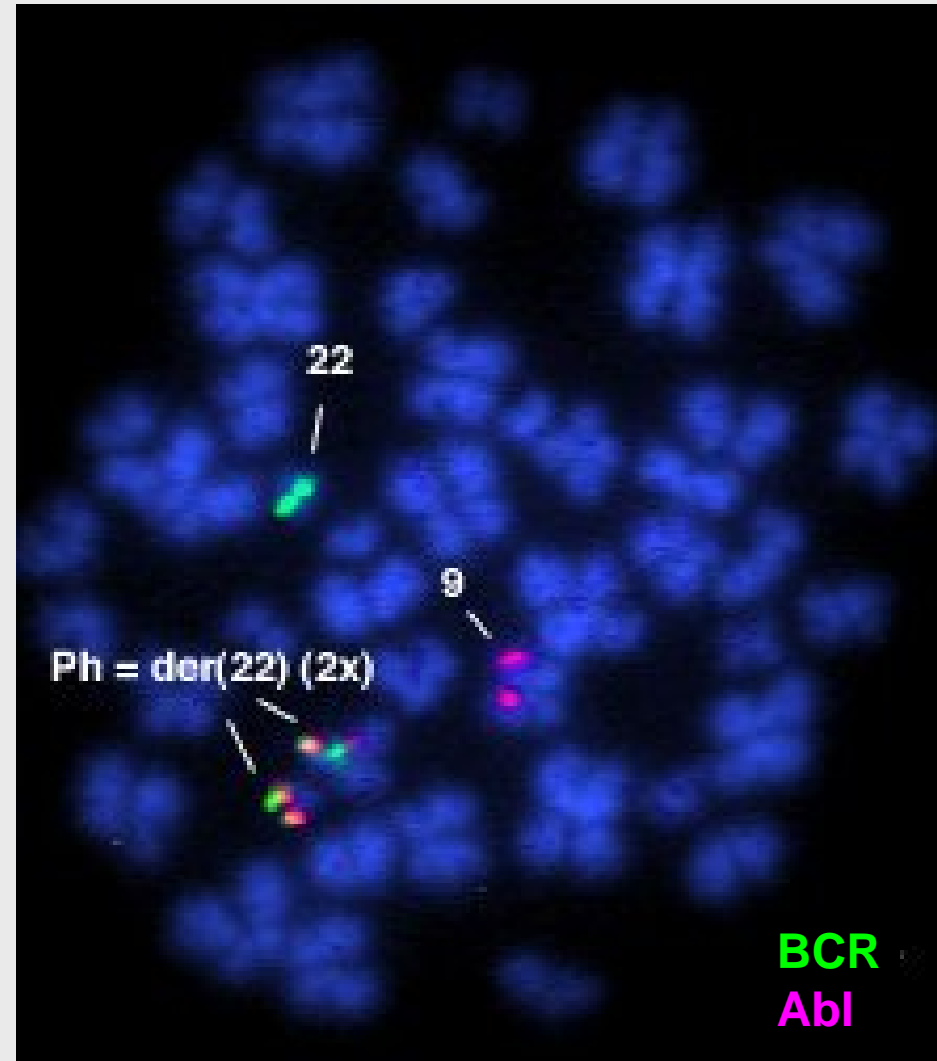
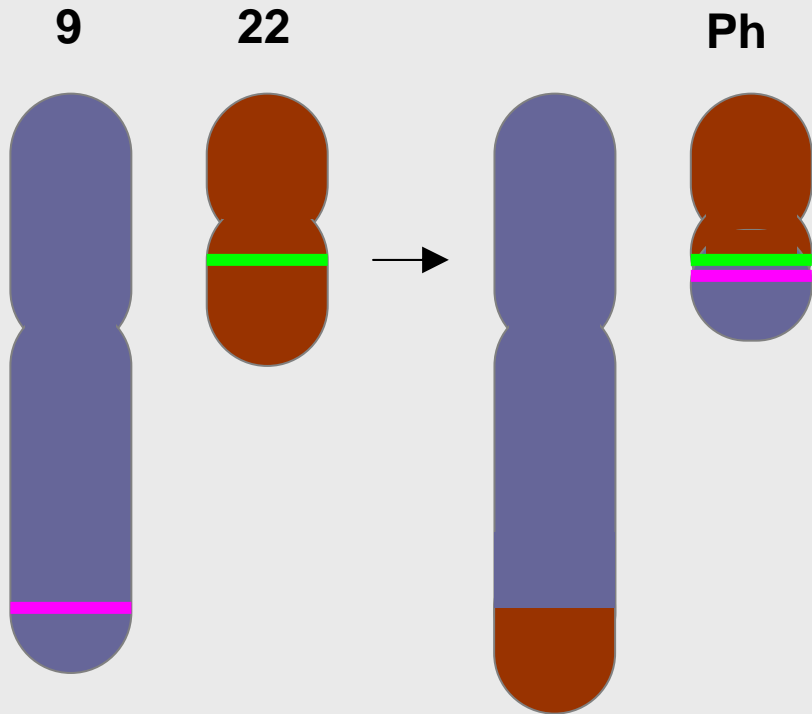
NADP⁺



3DFR

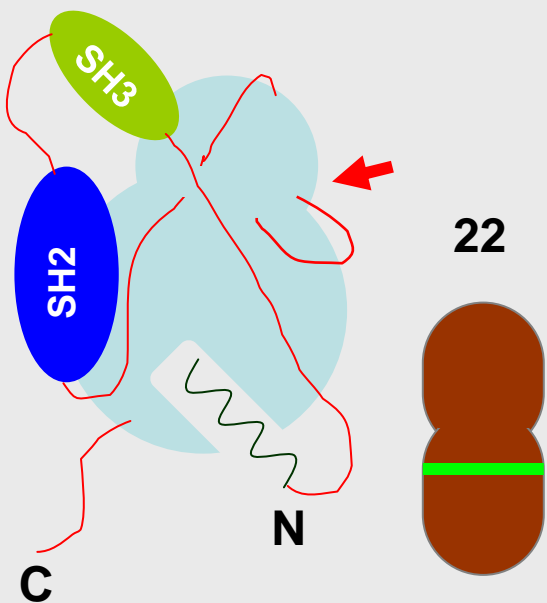
Chronická myeloidní leukémie (CML)

Philadelphia chromosom



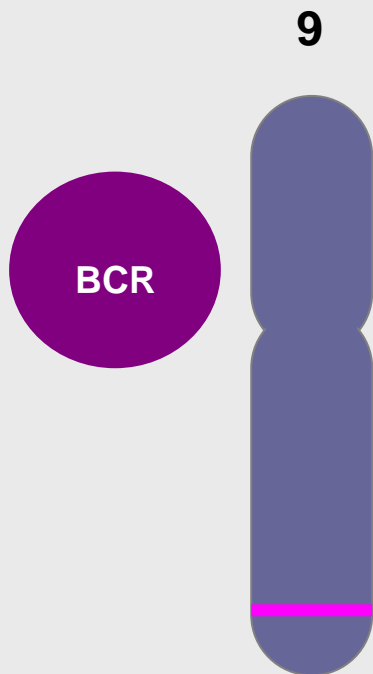
Chronická myeloidní leukémie (CML) Abelsonova kinasa

c-Abl

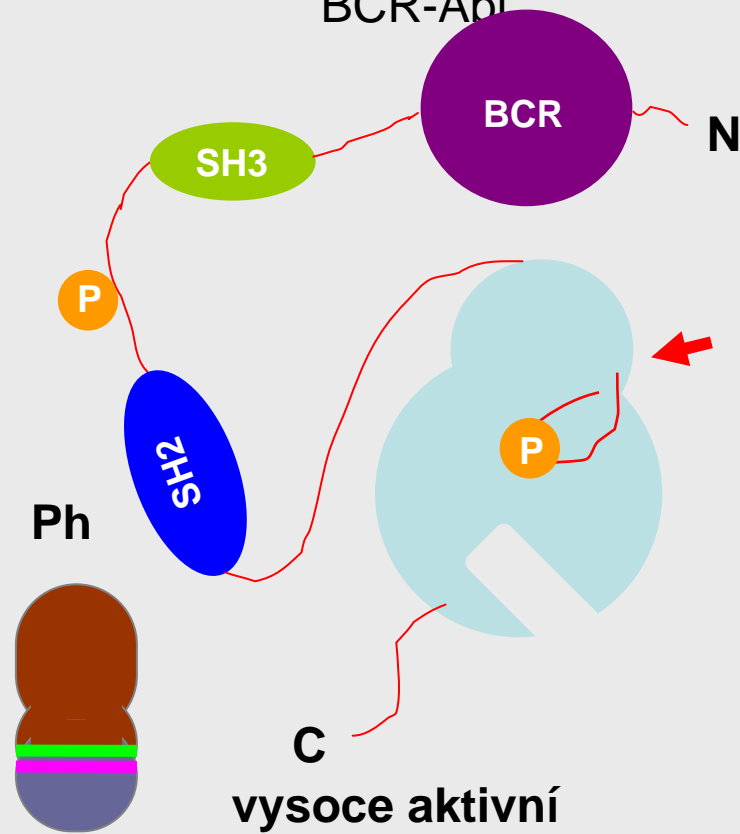


málo aktivní

BCR



BCR-Abl

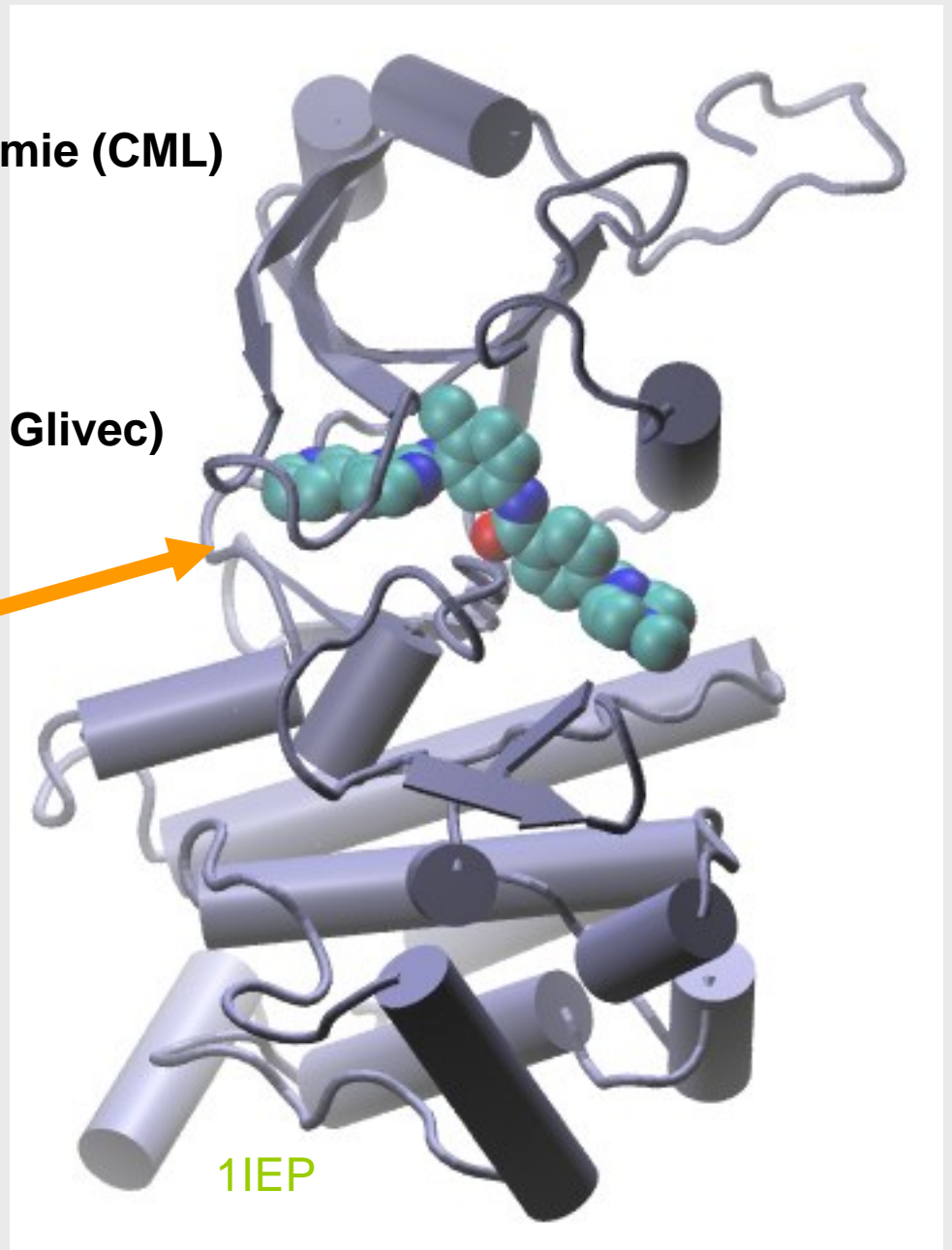
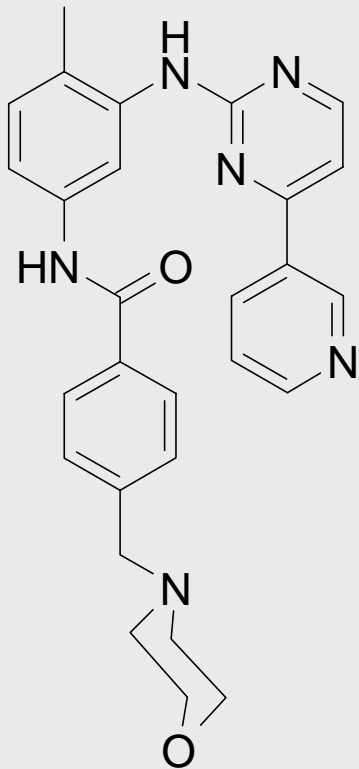


vysoce aktivní

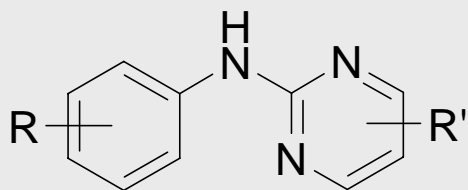
Chronická myeloidní leukémie (CML)

Abelsonova kinasa
(protein-tyrosin kinasa)

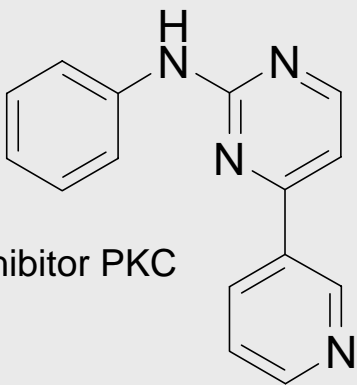
Imatinib (STI 571, Gleevec, Glivec)



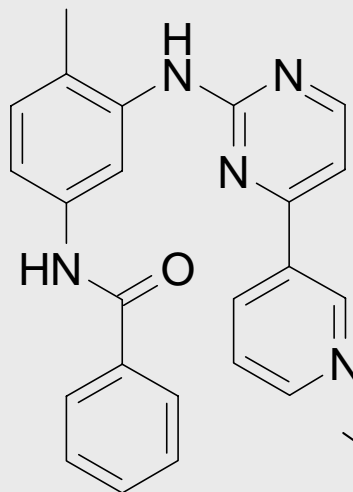
High throughput
screening



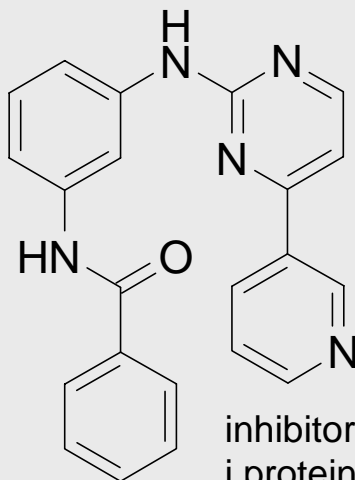
„lead compound“, inhibitor
proteinkinasy C



silný inhibitor PKC

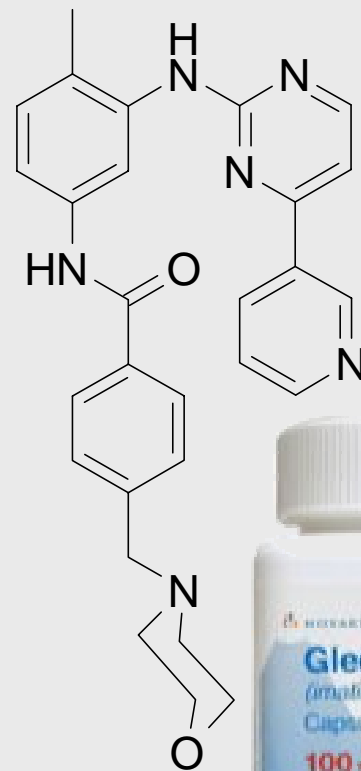


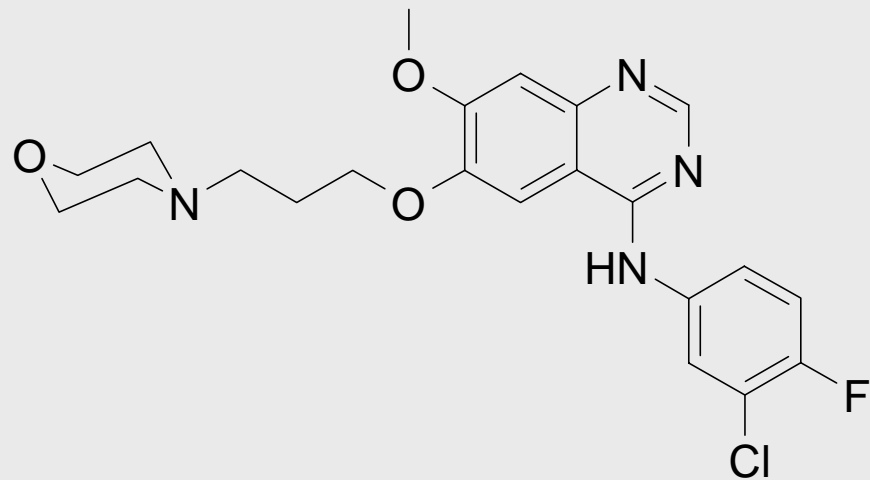
neinhibuje PKC
selektivní inh. c-Abl



inhibitor PKC
i proteintyrosinkinas
včetně Abl

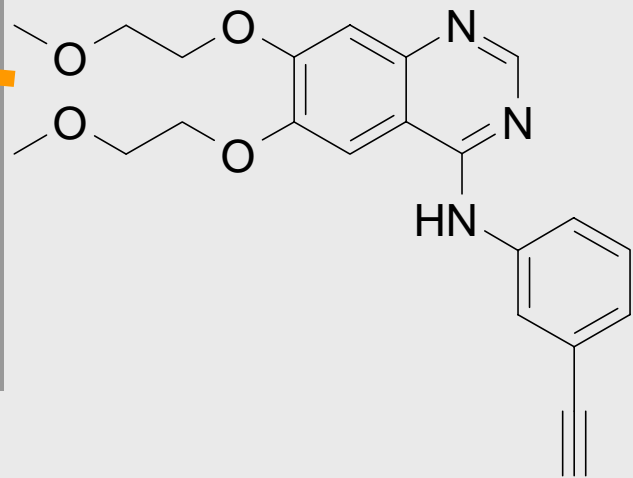
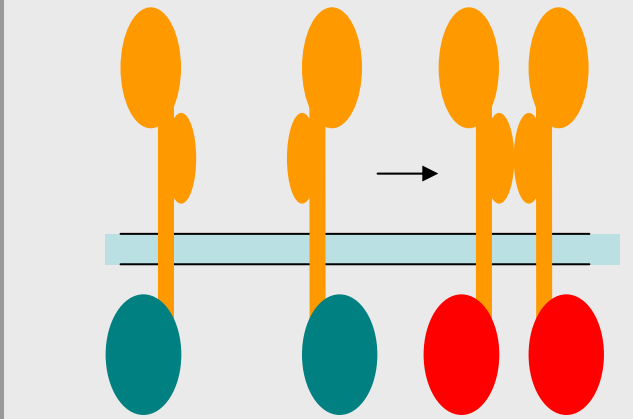
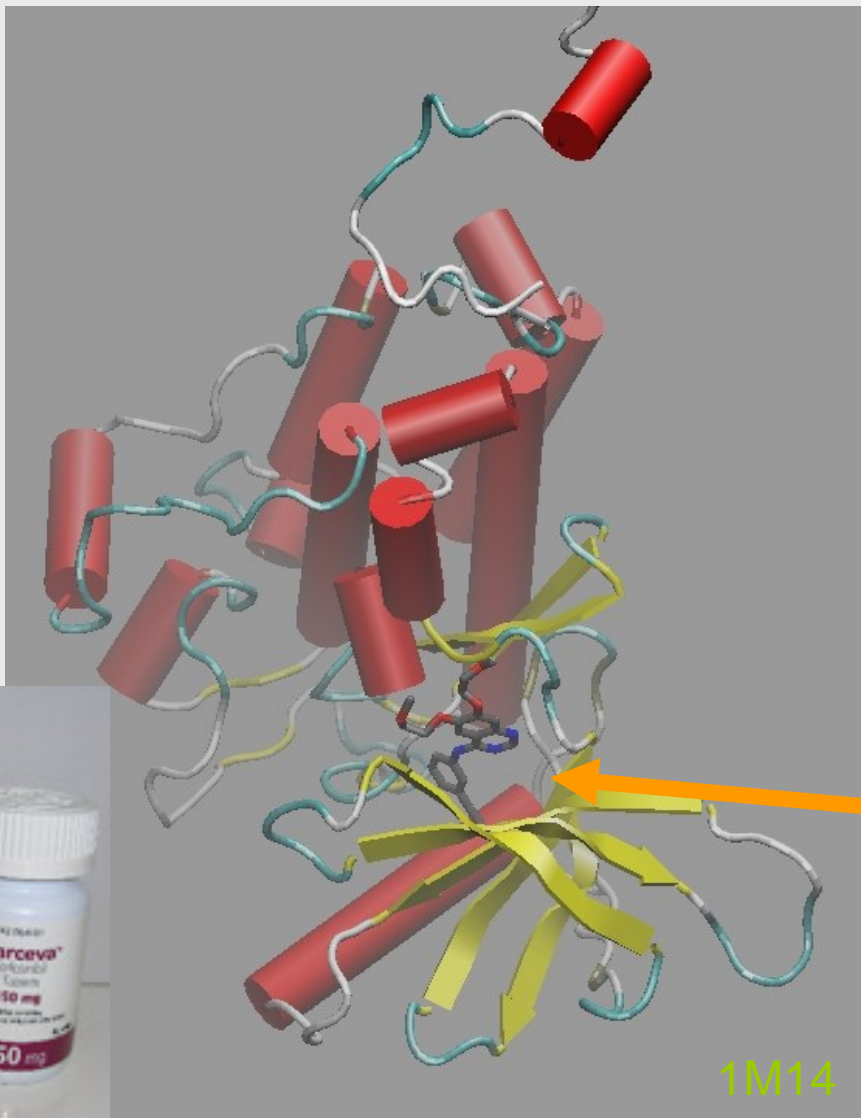
STI-571, Imatinib
 $IC_{50} = 25 \text{ nM}$





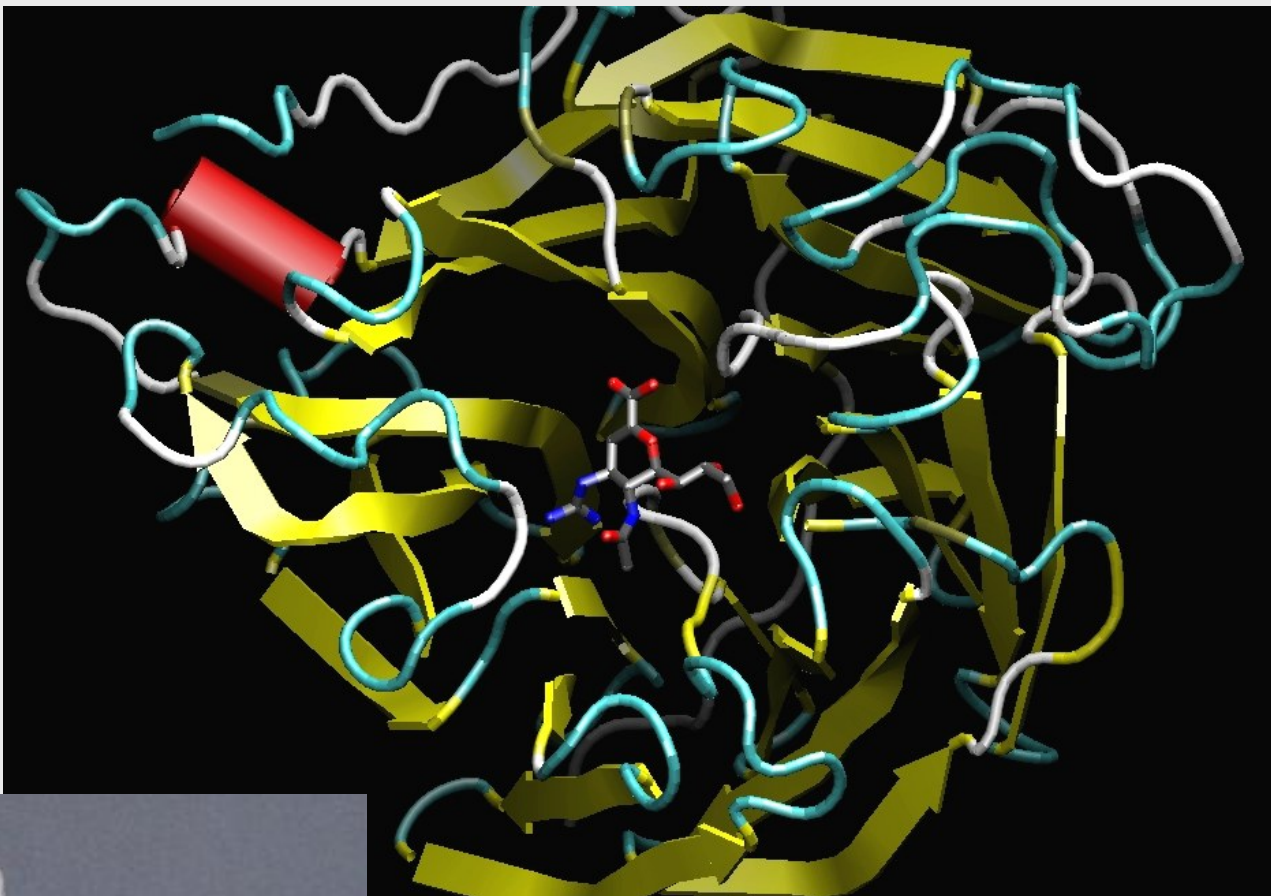
Gefitinib ('Iressa', ZD1839)

epidermal growth factor receptor-tyrosine kinase
rakovina plíc



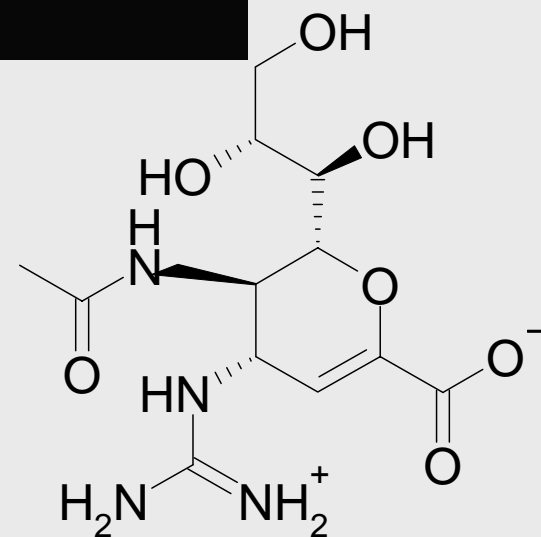
Tarceva, OSI-774, Erlotinib, CP 358 77
epidermal growth factor receptor-tyrosine kinase
rakovina plic

Neuraminidasa
EC 3.2.1.18

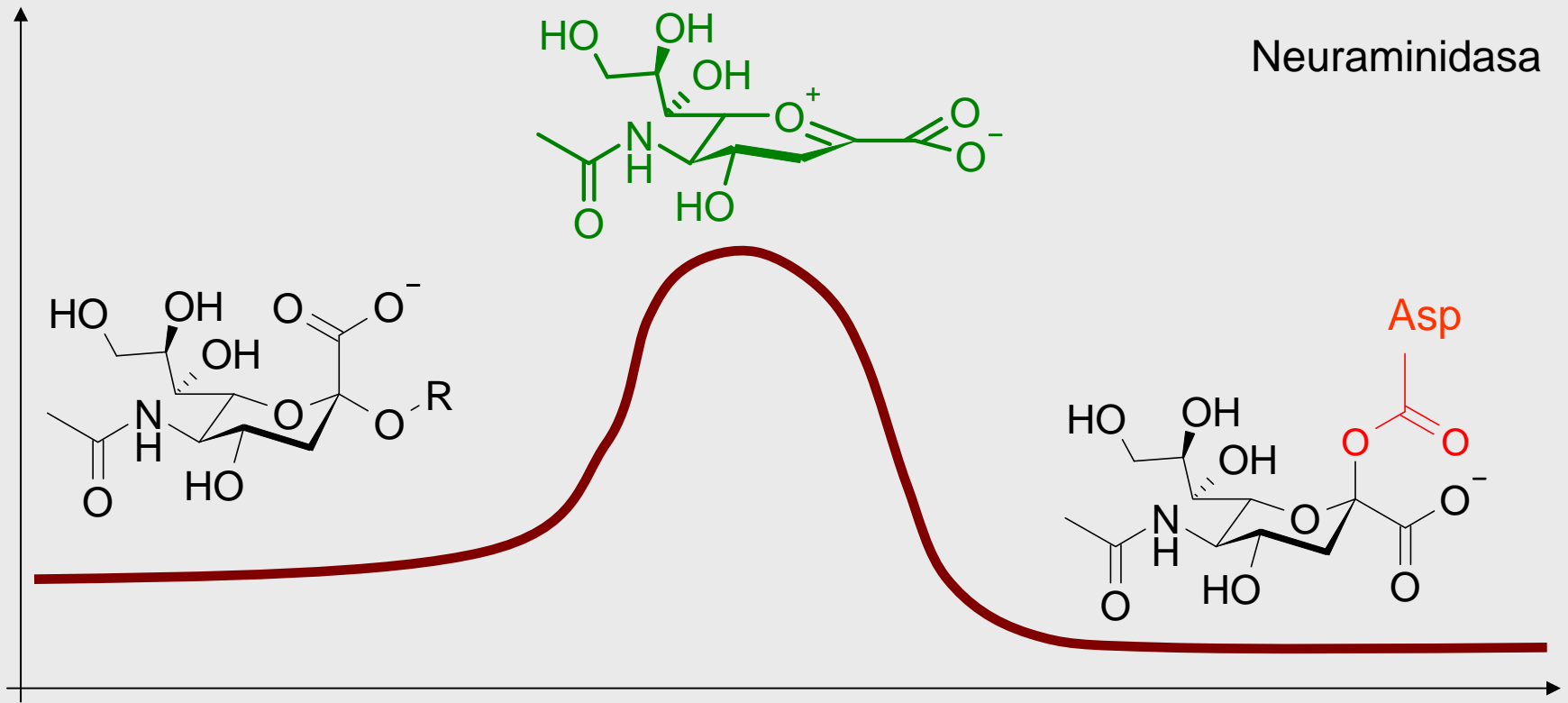


Relenza

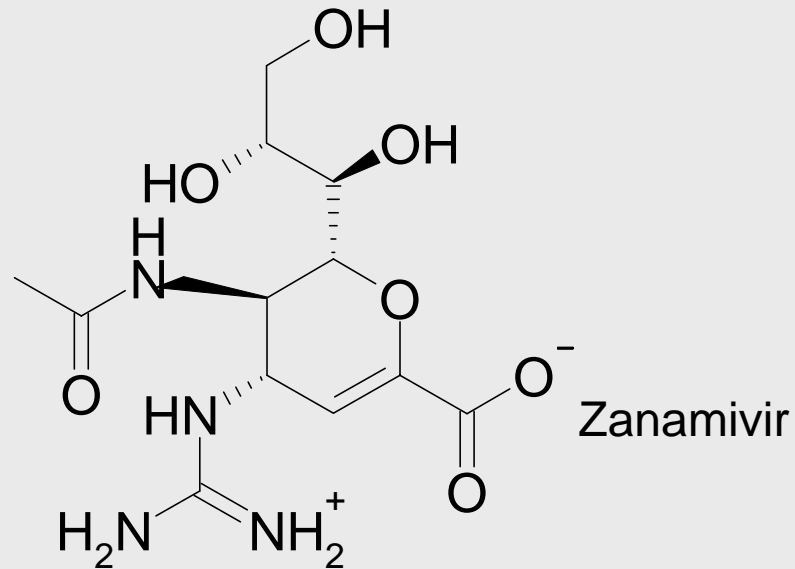
Zanamivir



Neuraminidasa

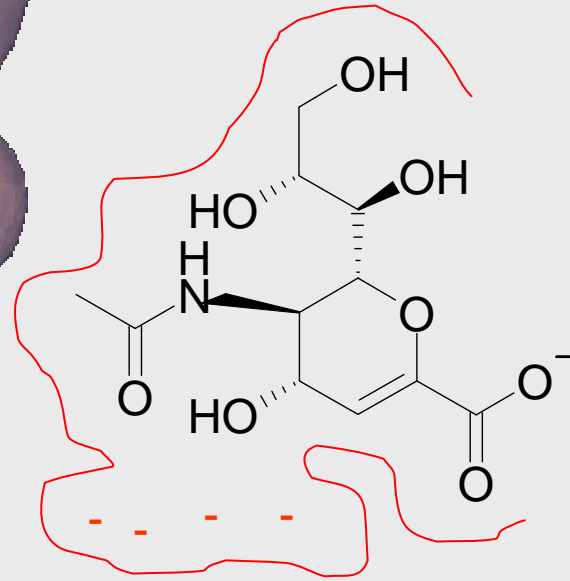
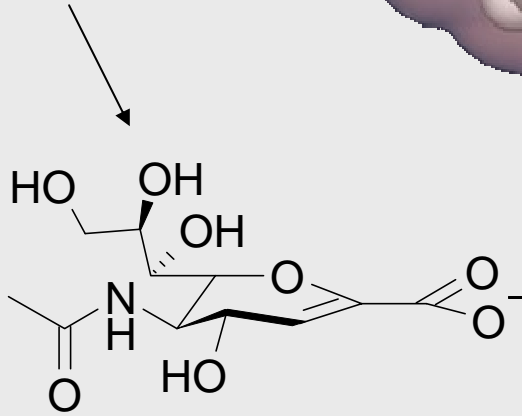
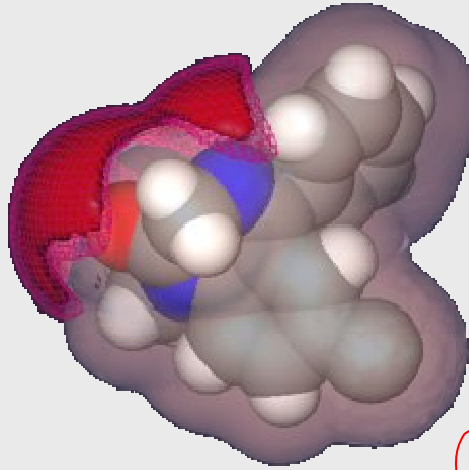
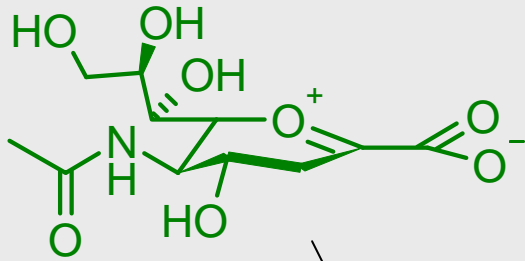


Relenza



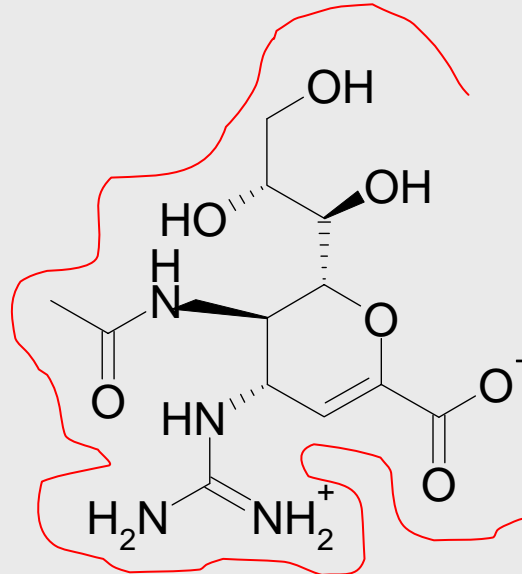
Neuraminidasa

GRID (www.moldiscovery.com)



Ki = 1 000 nM

Relenza

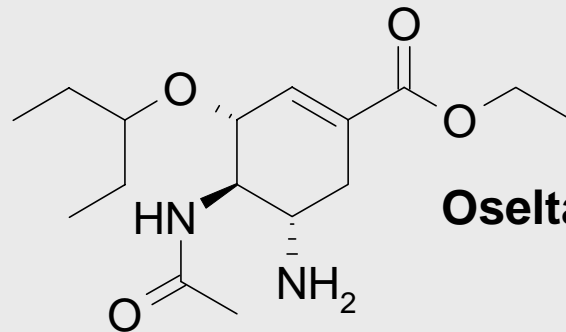
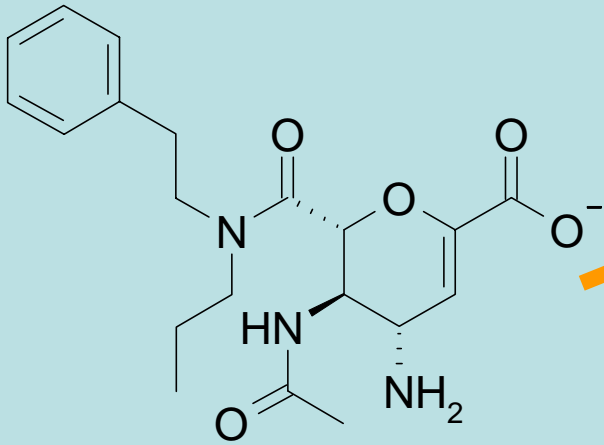
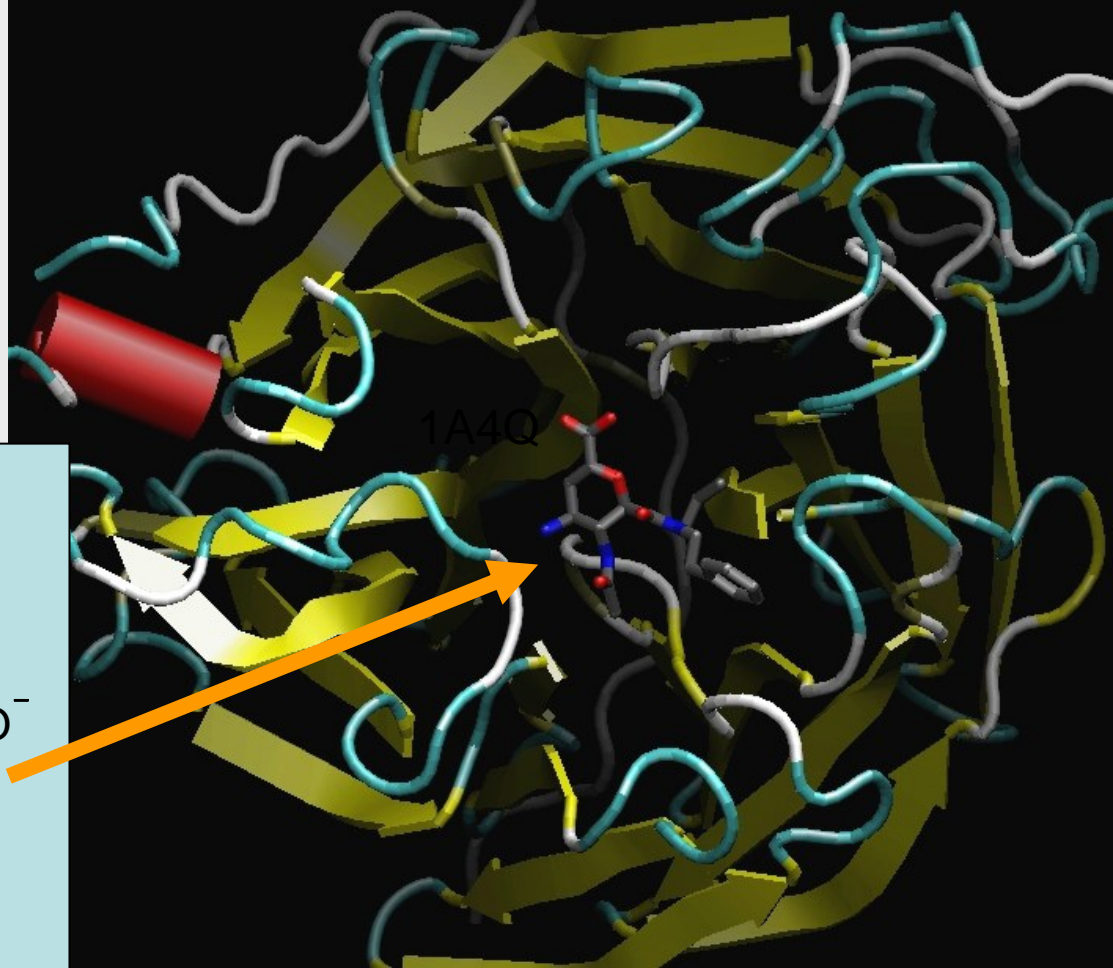


Mark von Itzstein *et al.* (1993)
Nature **363**, 418 – 423.

Zanamivir

Ki = 0.1 – 0.2 nM

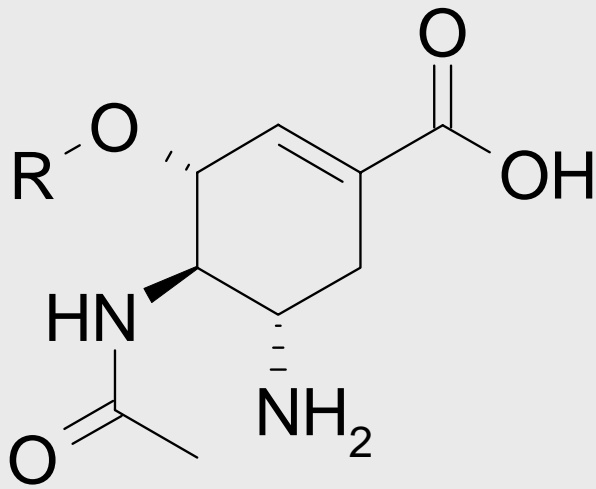
Neuraminidasa



Oseltamivir



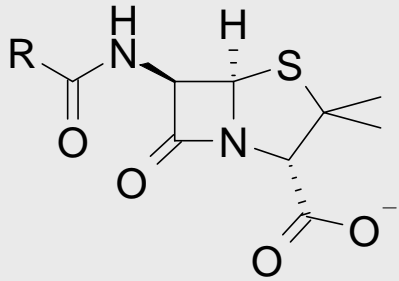
Neuraminidasa



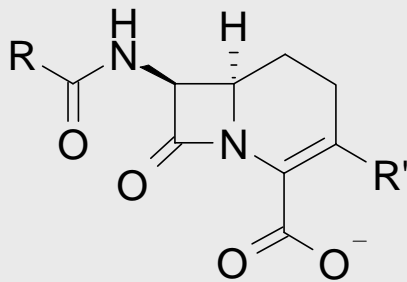
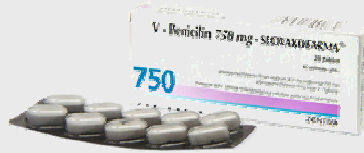
Oseltamivir

R:	Ki (nM)
-H	6 300
-CH ₃	3 700
-CH ₂ CH ₃	2 000
-CH ₂ CH ₂ CH ₃	180
-CH ₂ CH ₂ CF ₃	225
-CH ₂ OCH ₃	2 000
-CH ₂ CH=CH ₂	2 200
-CH ₂ CH ₂ CH ₂ CH ₃	300
-CH ₂ CH(CH ₃) ₂	200
-CH(CH ₃)CH ₂ CH ₃	10
-CH(CH ₂ CH ₂ CH ₃) ₂	16
Cyclopentyl	22
Cyclohexyl	60
Phenyl	530
-CH(CH₂CH₃)₂	1

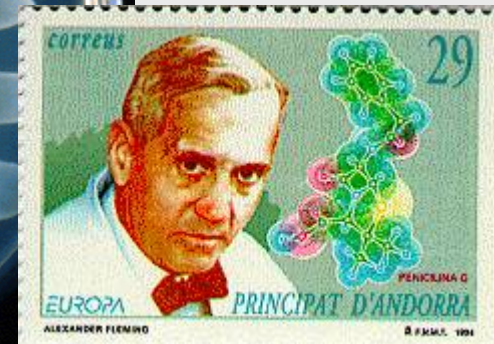
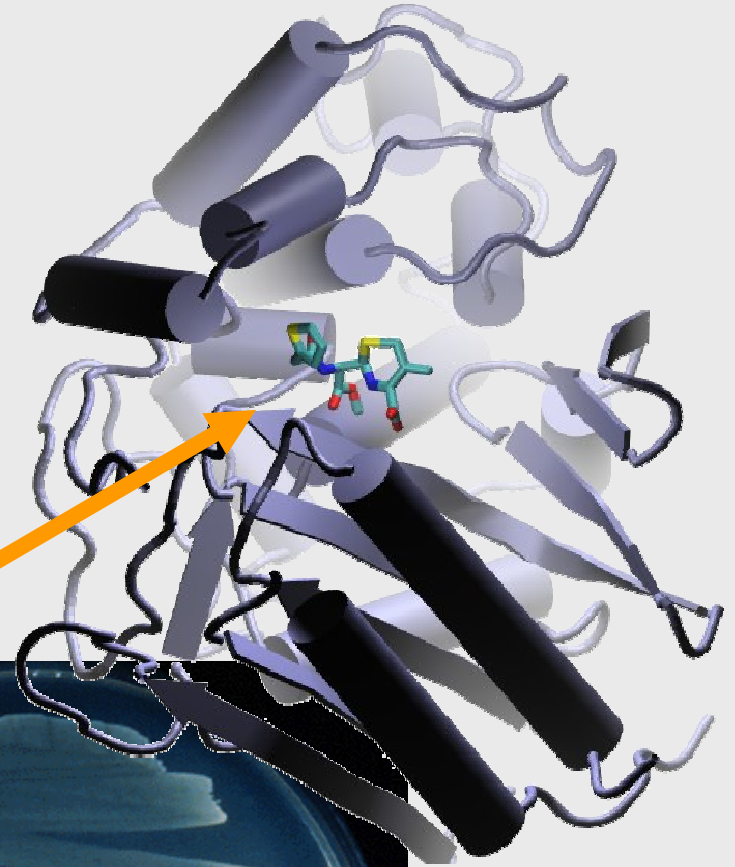
D-alanyl-D-alanin karboxypeptidasy EC 3.4.16.4, transpeptidasa



peniciliny

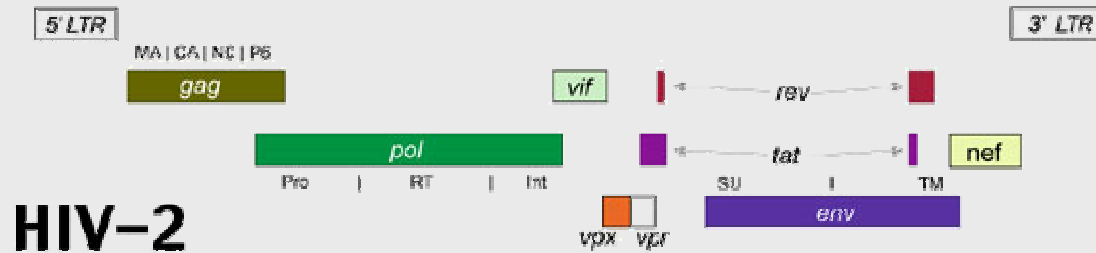
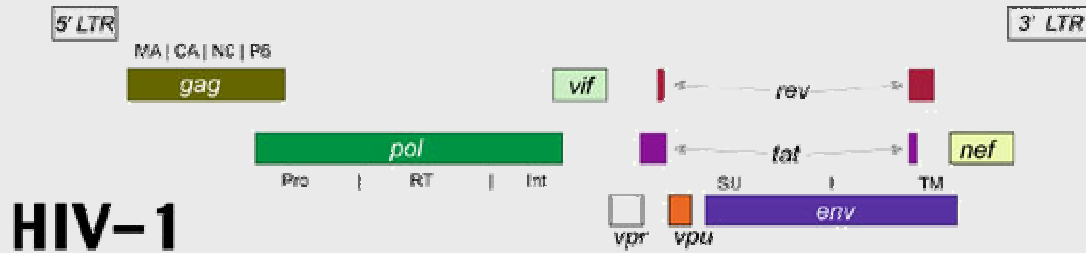


cefalosporiny



Human immunodeficiency virus (HIV)

genom



proteasa
reverzní transkriptasa
integrasa

Three arrows point from the enzyme names to the corresponding domains in the HIV-2 diagram: 'proteasa' points to the Pro domain of the pol gene, 'reverzní transkriptasa' points to the RT domain of the pol gene, and 'integrasa' points to the Int domain of the pol gene.

Human immunodeficiency virus (HIV)

léčiva

Nucleoside Analogs

Combivir (AZT plus 3TC)	Epivir (lamivudine or 3TC)	Hivid (zalcitabine or ddC)	Retrovir (zidovudine ZDV or AZT)	Videx (didanosine or ddI)	Zerit (stavudine or d4T)	Ziagen (abacavir)
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Non-Nucleosides

Rescriptor (delavirdine or DLV)	Sustiva (efavirenz or DMP)	Viramune (nevirapine or NVP)
---	--------------------------------------	--

Adjunctive Anti-Retrovirals

Hydrex (hydroxyurea or HU)	Droxia (hydroxyurea or HU)
--------------------------------------	--------------------------------------

Nucleotide

Proveon (adefovir or PMEA)

Protease Inhibitors

Agenerase (amprenavir or 141)	Fortovase (saquinavir soft gels FTV)	Crixivan (indinavir or IDV)	Invirase (saquinavir capsules SQV)	Norvir (ritonavir or RTV)	Viracept (nelfinavir or NFV)
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Produced in part with support from the Massachusetts Dept. of Public Health HIV/AIDS Bureau and Dupont Pharmaceuticals. Pills shown actual size. © 1999

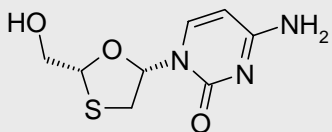
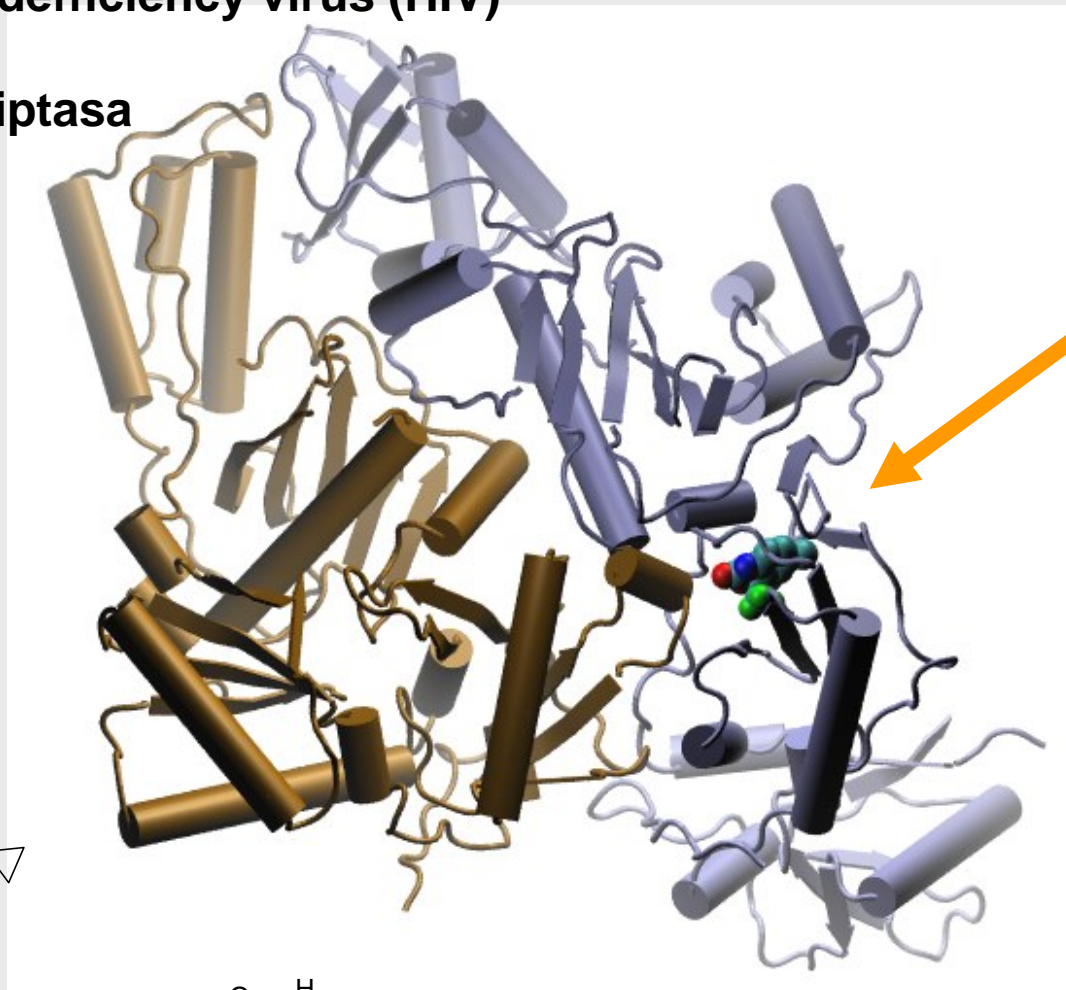


Lékařství:

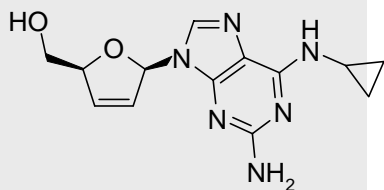
inhibitory

Human immunodeficiency virus (HIV)

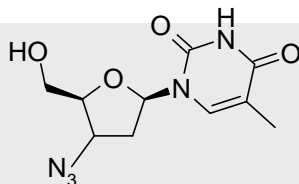
reverzní transkriptasa



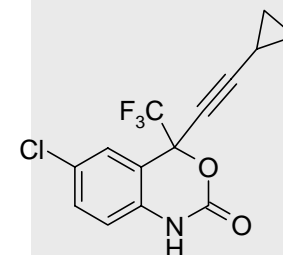
lamivudine



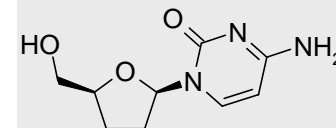
abacavir



AZT



efavirenz

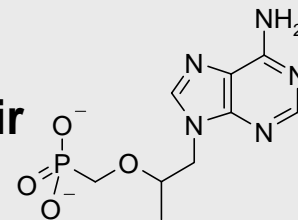


ddC

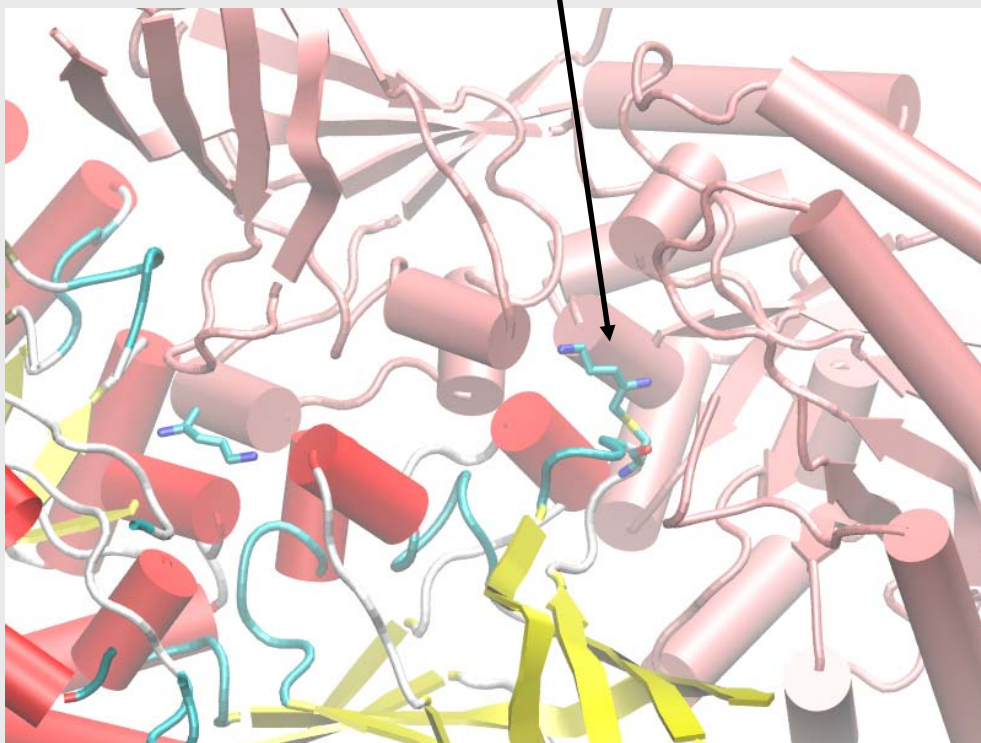
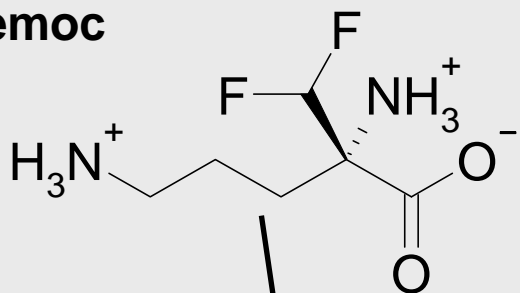
tenofovir



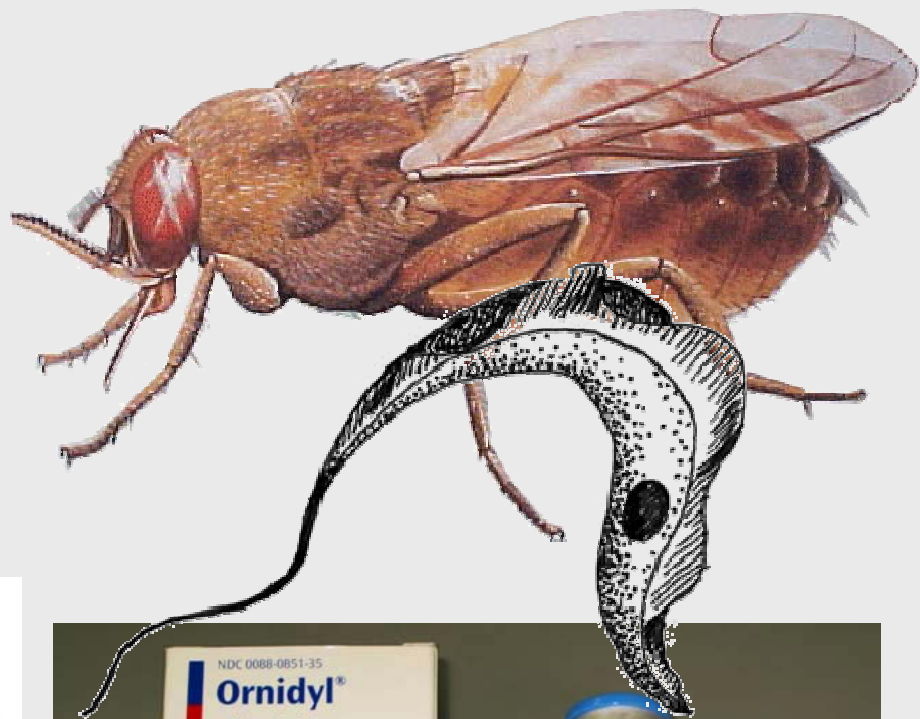
ÚOCHB AV ČR



Lékařství:
ornitindekarboxylasa (EC 4.1.1.17)
Spavá nemoc



2TOD



α -difluoromethylornithin – eflornithine
produkce zastavena 1995

Deficience enzymů

Fenylketonurie **fenylalaninhydroxylasa**
poškození mozku, mentální retardace

Lysosomové nemoci:

Gaucherova nemoc **β -glukosidasa**
defektní vývoj sleziny, kostí, jater, poruchy krve tvorby

Fabryho nemoc **α -galaktosidasa**
defektní vývoj srdce a ledvin

Pompeho nemoc **α -glukosidasa**
defektní vývoj svalů a srdce

Morquiova nemoc **β -galaktosidasy** (různé isoenzymy)
defektní vývoj kostí

Deficiencie enzymů

Fenylketonurie

fenylalaninhydroxylasa

Phenoptin – tetrahydrobiopterin – ukončená III. fáze

Lysosomové nemoci:

Gaucherova nemoc

β -glukosidasa

Cerazyme – rekomb. β -glukosidasa

Genz 112638 – „chemický chaperon“ – II. fáze

Fabryho nemoc

α -galaktosidasa

Fabrazyme – rekomb. α -galaktosidasa

Pompeho nemoc

α -glukosidasa

Myozyme – rekomb. α -glukosidasa – schváleno 2006

Morquiova nemoc

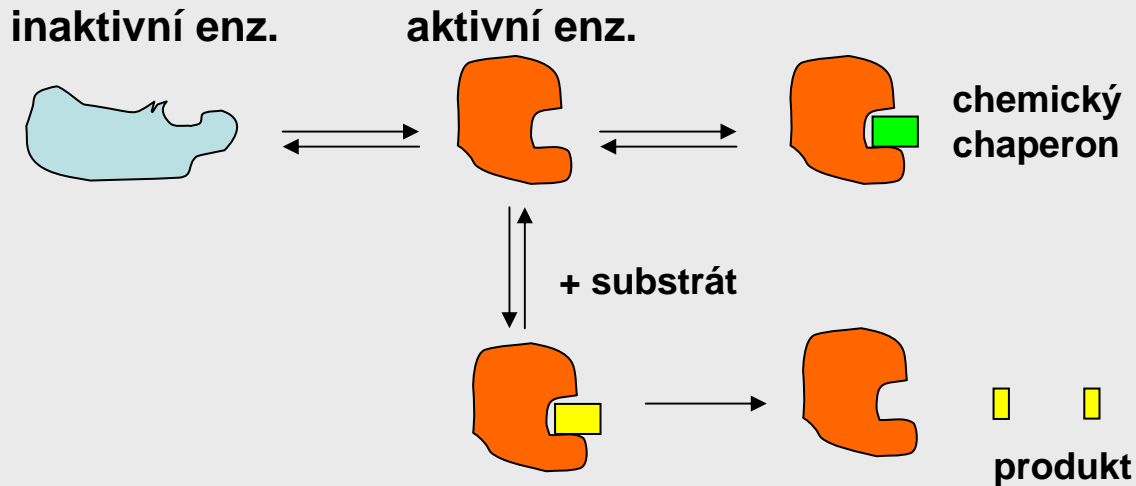
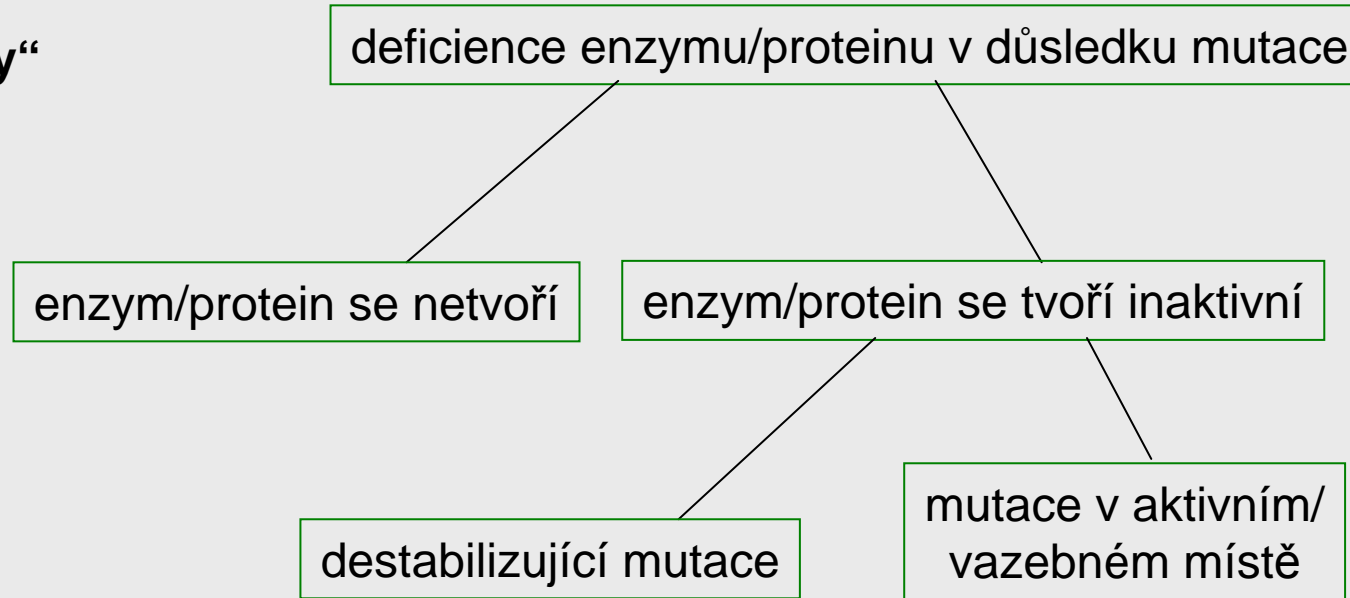
β -galaktosidasy (různé isoenzymy)

„chemický chaperon“ – *in vitro* a *in vivo*



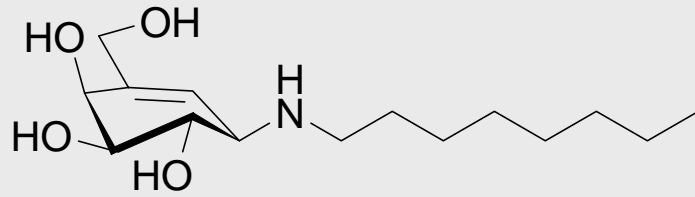
Deficience enzymů

„chemické chaperony“



Deficience enzymů

„chemické chaperony“



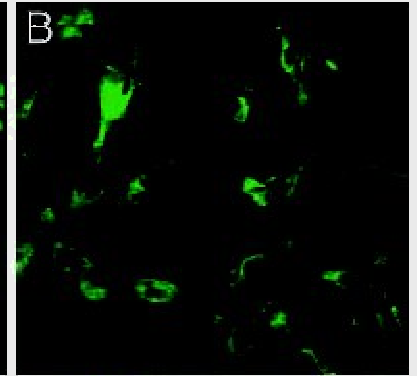
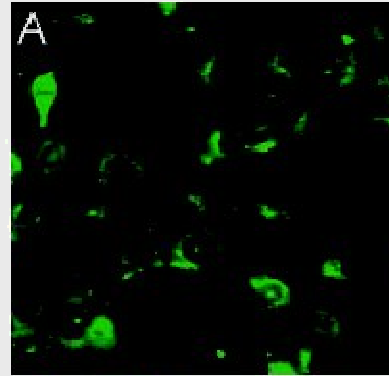
chemický chaperon

-

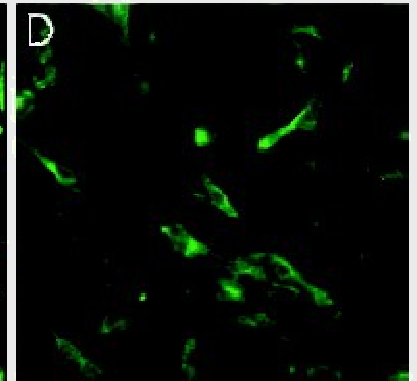
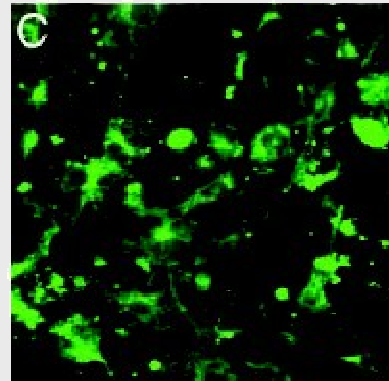
+

GM1-gangliosidosis a
Morquio B disease
(deficience lyzozomální
 β -galaktosidasy)

WT



R201C

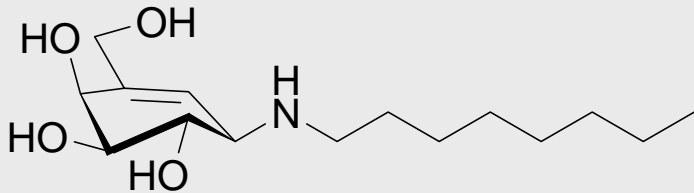


Matsuda *et al.* (2003)
PNAS **100**(26), 15912–15917.

detekce fluorescenčně značeným cholera toxinem

Deficience enzymů

„chemické chaperony“

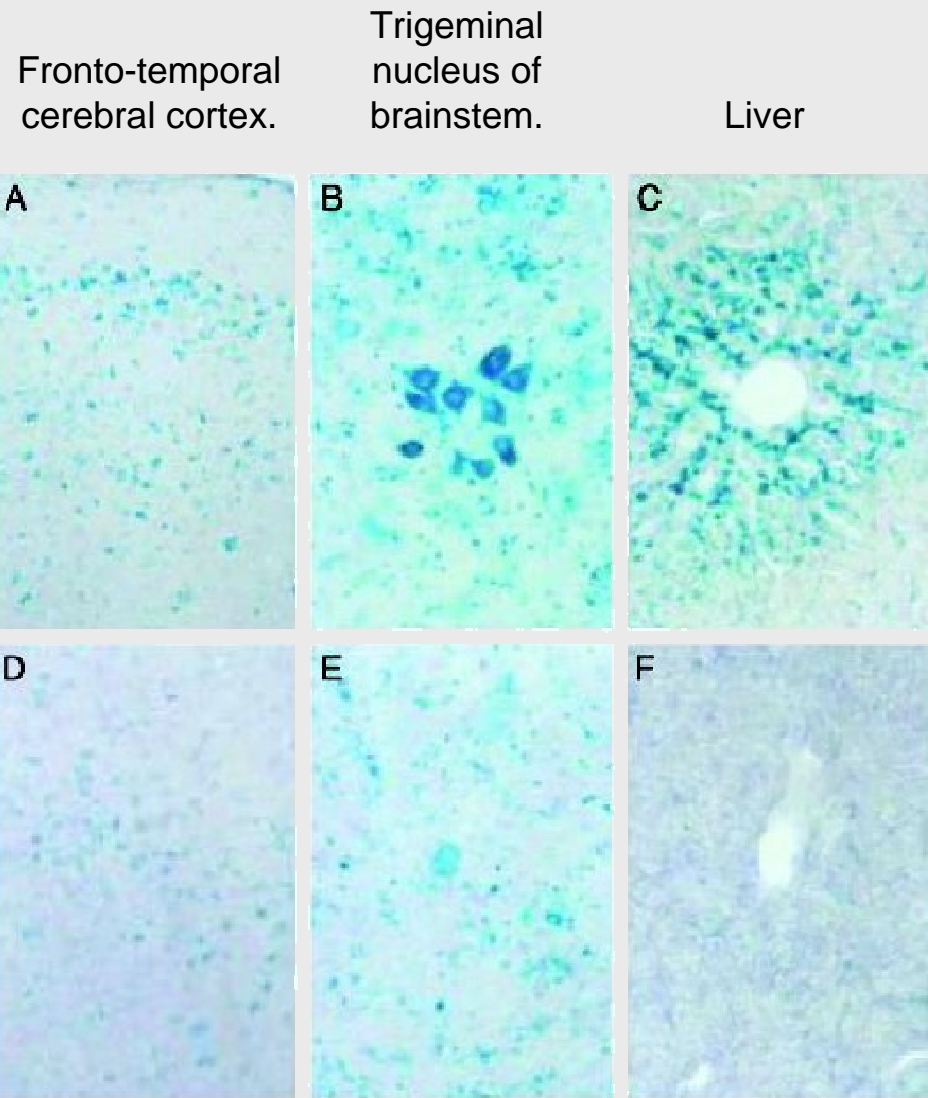


s chem.
chaperonem

GM1-gangliosidosis a
Morquio B disease
(deficience lyzozomální
 β -galaktosidasy)

bez chem.
chaperonu

Matsuda *et al.* (2003)
PNAS **100**(26), 15912–15917.



detekce X-Gal