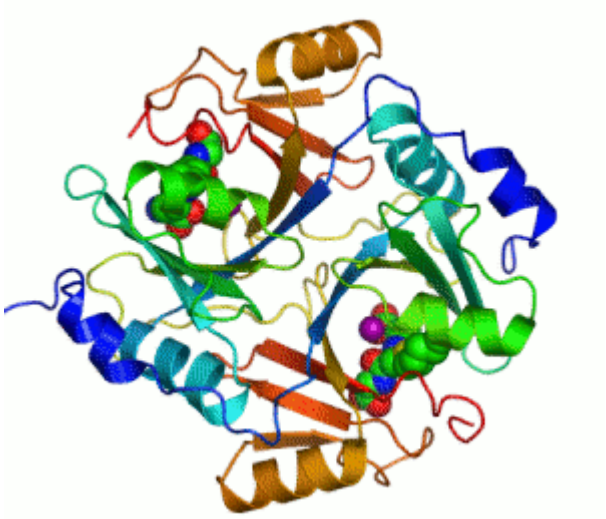
Structure Based Drug Desig (SBDD)

- Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy.
- If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.
- Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

Structure-based drug design and development

Targeting proteins: the enzymes

- Inhibitors of enzymes of invading pathogens: antiviral and antibacterial drugs. Chemoterapic agents, antitumoral agents.
- Inhibitors of endogenous enzymes: anticholestorolemic, anti-hypertensive drugs, etc.....



Target identification

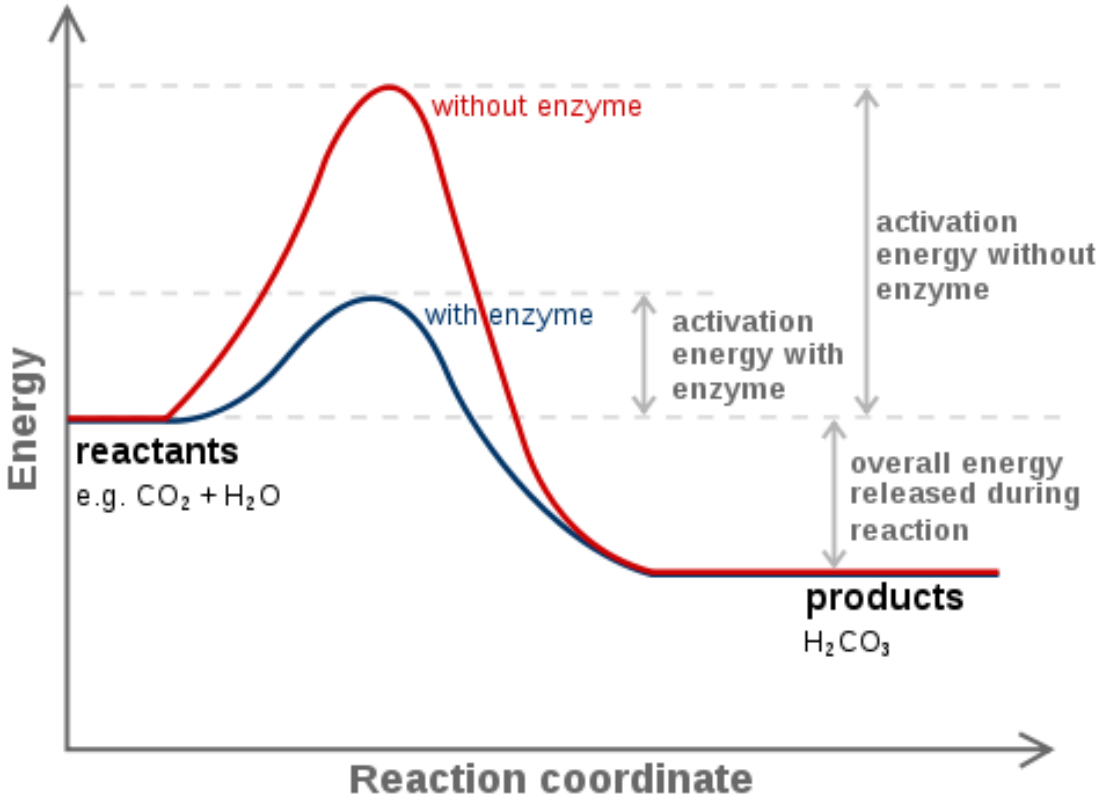
- The structural information for all targets is generally obtained **by X-ray crystallography or NMR**.
- However, in the case of targets with no experimentally determined structures, several computational approaches such as **ab initio modeling**, threading and comparative modeling can be used to predict 3D structures.
- **Homology modeling** or comparative modeling builds 3D structures for unknown proteins based on the known homologous protein structures

Drugs targeting enzymes:

Drug name	Target Enzyme	Therapeutic application
Captopril	ACE (protease)	Anti-hypertensive
Enalapril	ACE	Anti-hypertensive
Aliskiren	Renin (protease)	Anti-hypertensive
Zanamavir, Oseltamivir	Neuraminidases glycosidase	anti-influenza
N-butyl-deoxynojirimicin	glycosidases	disorders of lisosomial accumulation
imatininib	Abl-kinase	antitumoral
omeprazol	proton/K pump	antiacid, gastric ulcera
indinavir	HIV protease	anti-HIV

Targeting enzymes…

- Enzymes are catalyst...

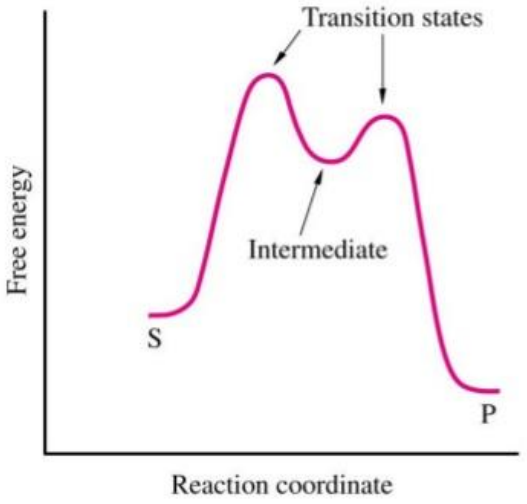


Enzyme inhibitors

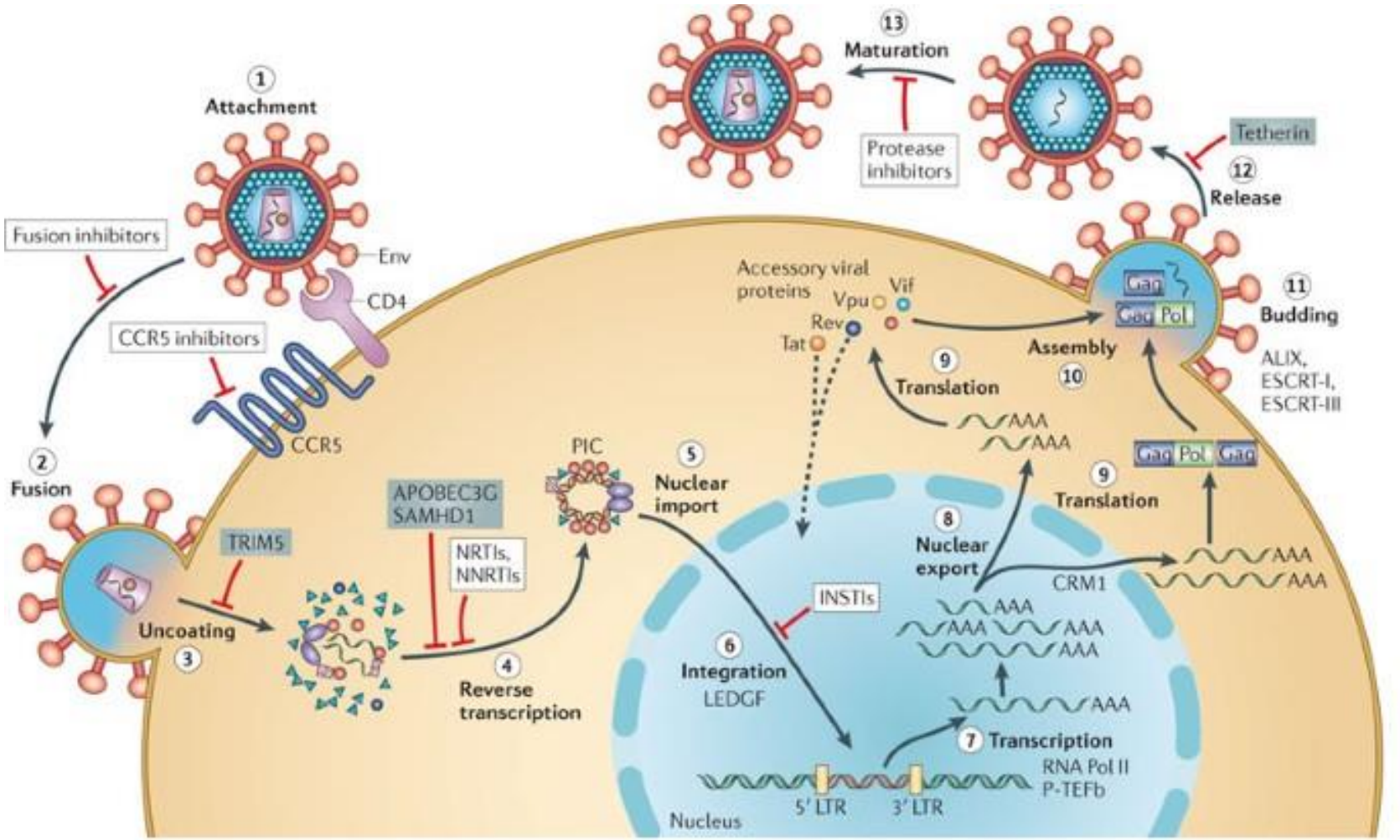
- Molecules that resemble (mimick) the **enzyme substrate** but cannot be transformed by the enzyme
- Inhibitors can also resemble to the **reaction intermediate** of the enzyme-catalyzed reaction

Intermediates

- Intermediates are stable.
- In reactions with intermediates, 2 TS's are involved.
- The slowest step (rate determining) has the highest activation energy barrier.
- Formation of intermediate is the slowest step.



HIV life cycle: the role of HIV aspartyl protease and antiviral drugs based on its inhibition (antiretroviral drugs)



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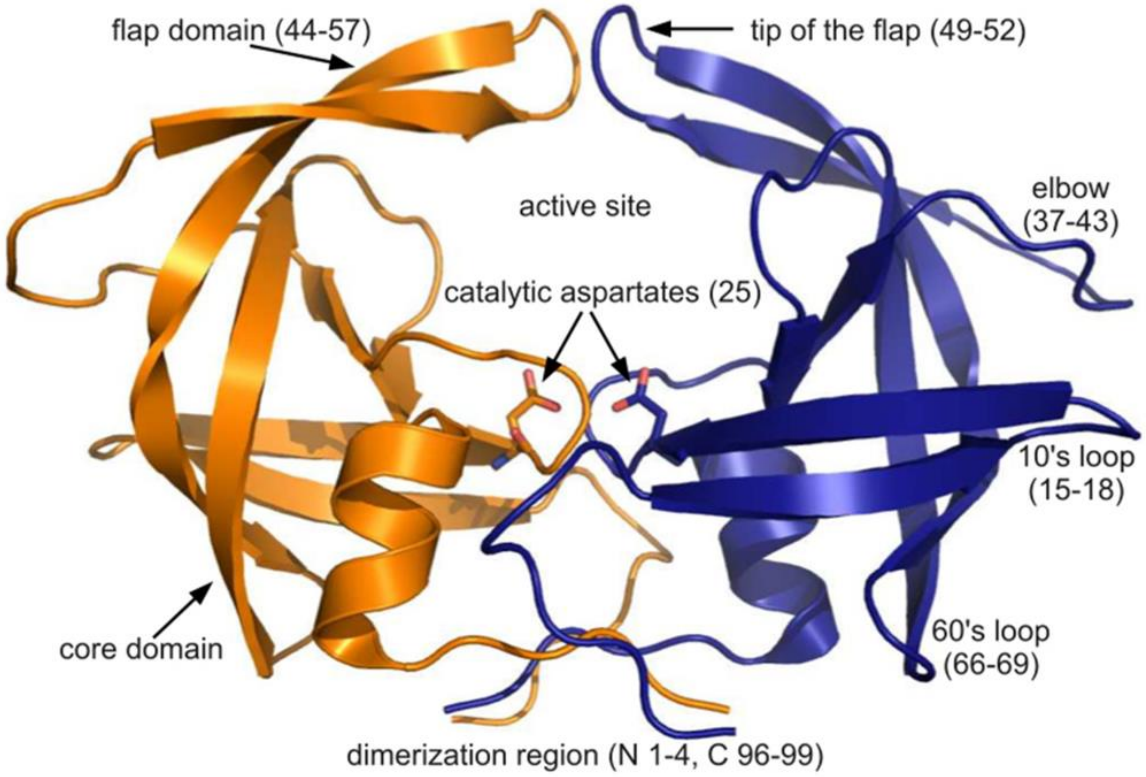
HIV protease inhibitors (antivirals)



- HIV aspartyl protease is necessary to generate important HIV proteins by hydrolysis of larger precursors before budding from host cell.
- HIV protease is a small protein (99 aa), dimeric, with a C2 rotational symmetry, the active site is at the interface of the two subunits

Schema della disposizione del peptide consenso nel sito attivo della aspartil proteasi

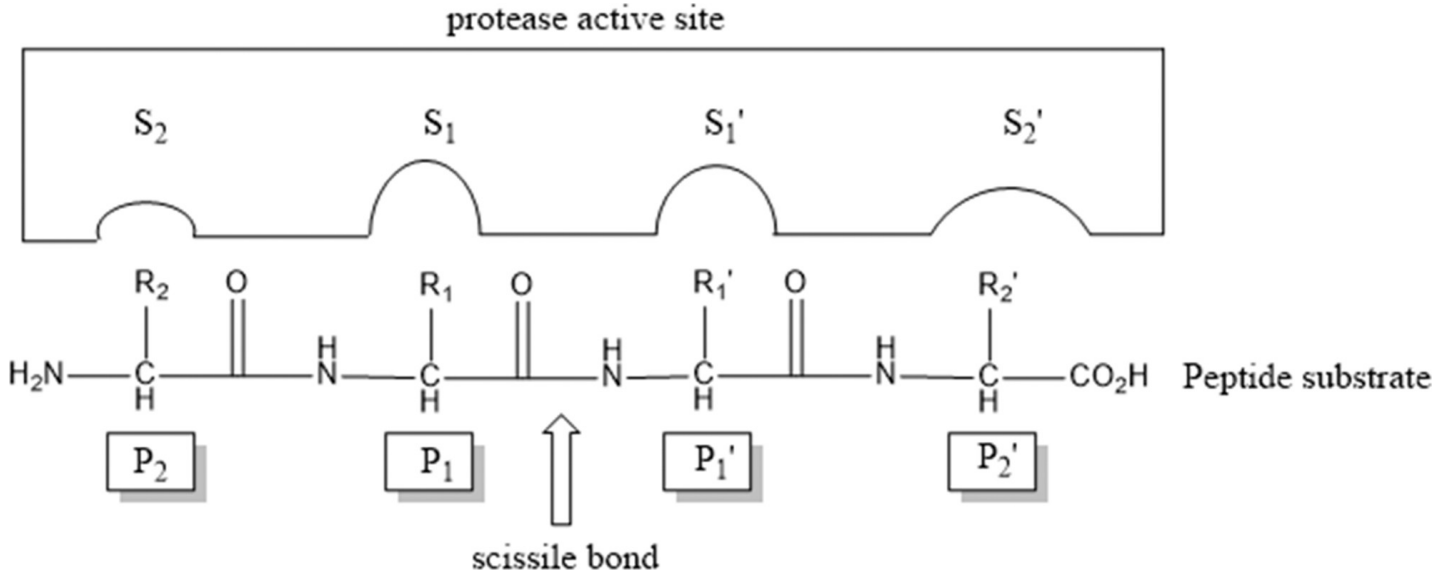
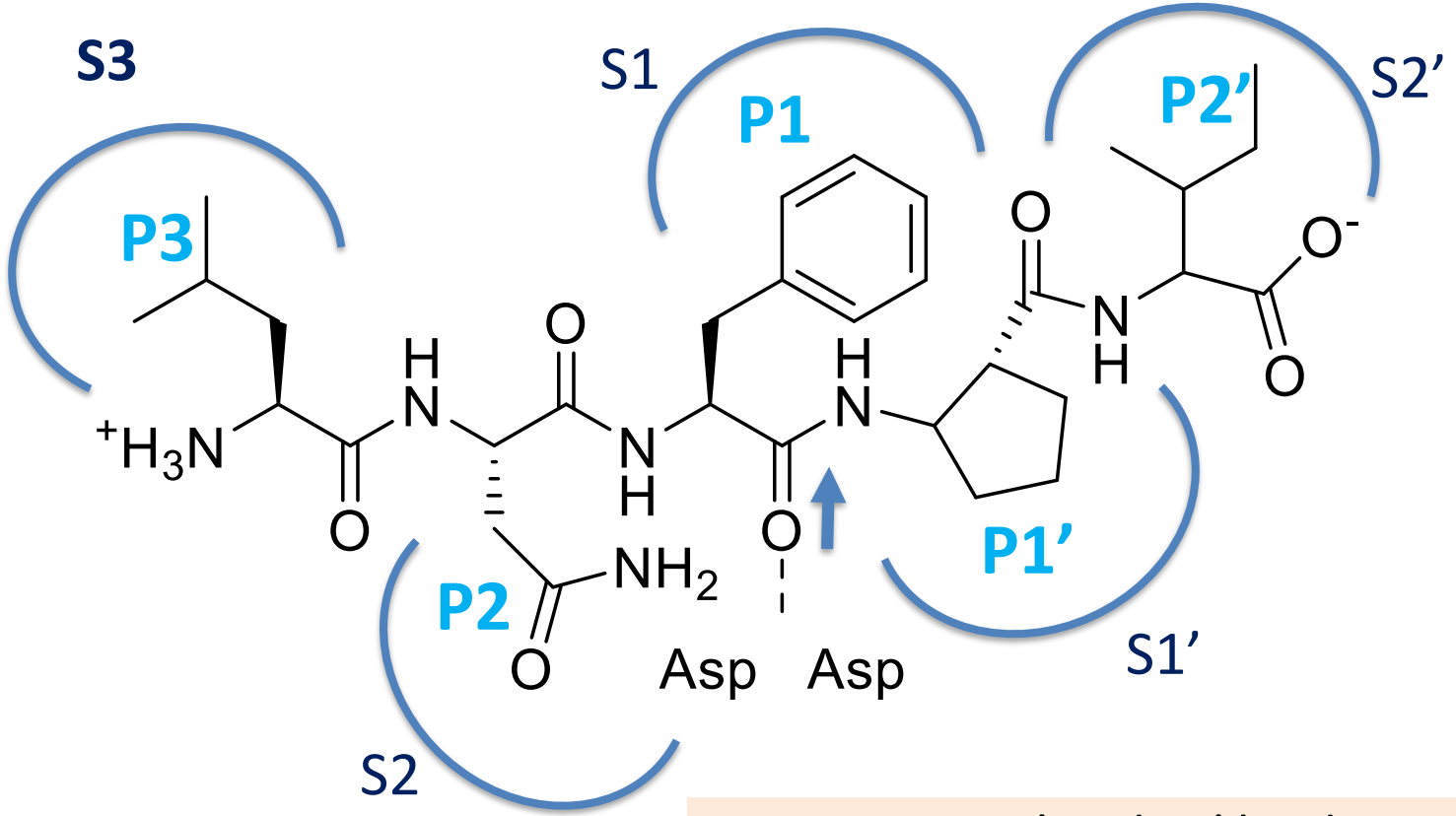


Figure 1. Subsite nomenclature for proteolytic enzymes is shown.
Amino acid residues to the left of the polypeptide scissile amide bond are numbered sequentially, beginning with P1 and increasing toward the N-terminus. Amino acid residues to the right of the scissile bond are numbered sequentially, beginning with P1' and increasing toward the C-terminus. Complimentary regions of the protease active site employ the corresponding S numbering

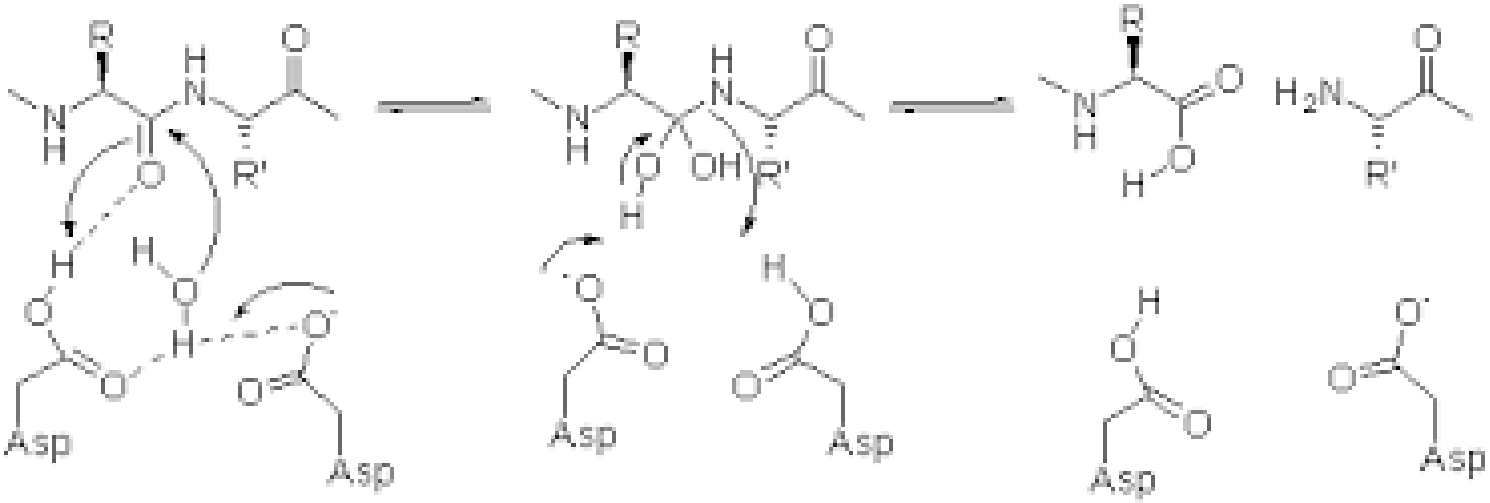
The natural substrate of HIV protease is the pentapeptide sequence

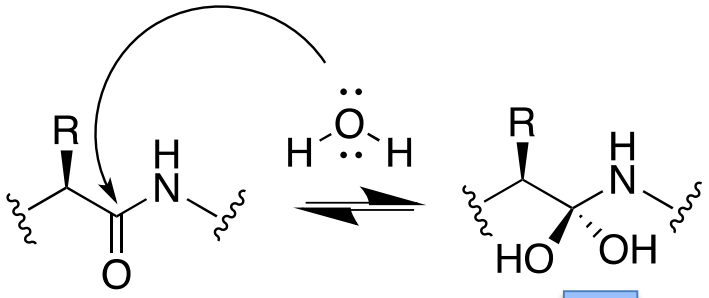
Leu-Asn-Phe-Pro-Ile



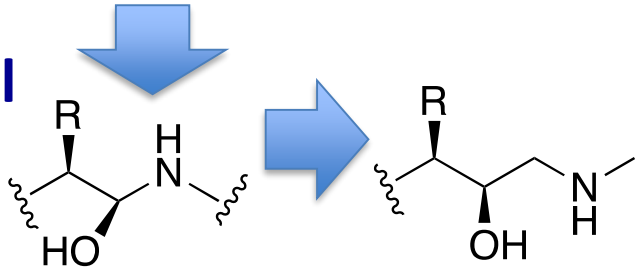
- S3, S2, S1, S1' and S2' binding pockets in the protease active site

Mimetics of sp^3 tetrahedric intermediate of aspartyl protease hydrolytic reaction



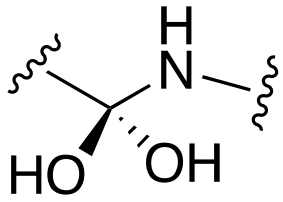


remove a hydroxyl



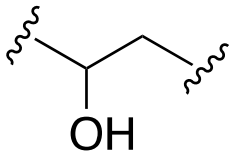
add a carbon: ethanolamine stable transition state analogue

Other intermediate analogues

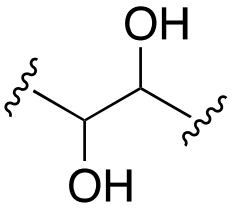


transition state

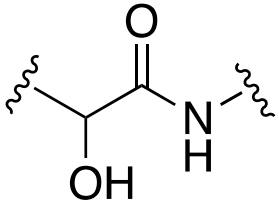
ANALOGUES



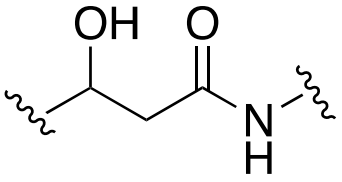
hydroxyethylene



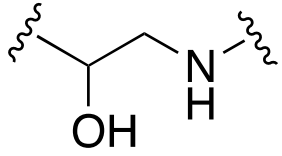
dihydroxyethylene



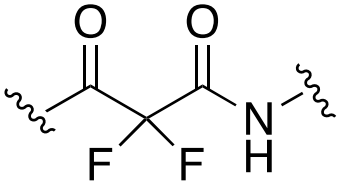
norstatin



statin

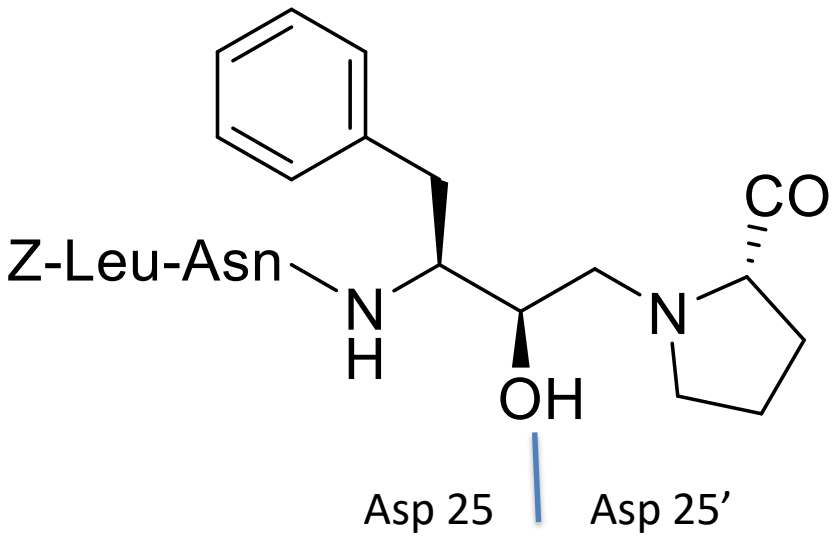


ydroxyethylamine



staton

Hit compound:



IC_{50} = 750 nM

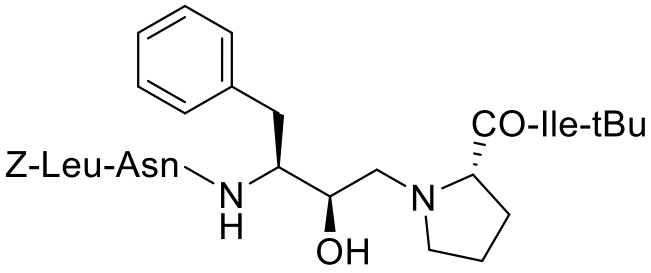
Structure-based drug design

- Hydroxyethylamino **intermediate isoster** of the Leu-Asn-Phe-Pro-Gln-Ile peptide, inhibit the protease with IC_{50} 750 nM
- Insert the quinoline group (S3), tBu group (S2'), to increase affinity:

compound 3, IC_{50} = 23 nM

- To further increase target affinity introduce Boc amide instead of tBu ester and the bicyclic structure (saquinavir, IC_{50} = 0.4 nM)

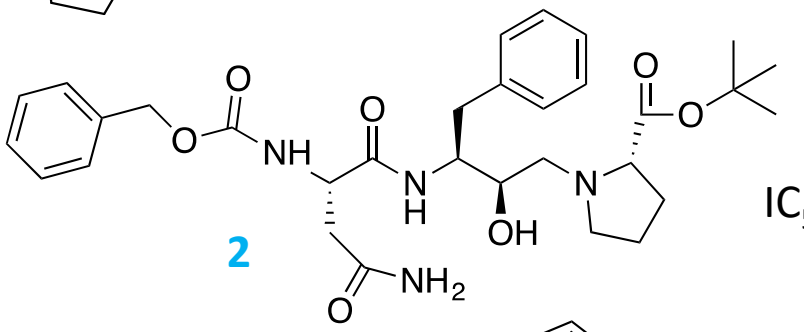
Enzyme affinity (pK_A)



IC_{50} = 750 nM

HIT 1

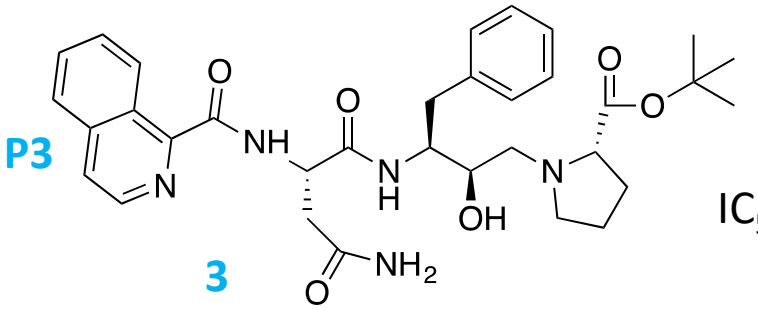
P2'



IC_{50} 140 nM

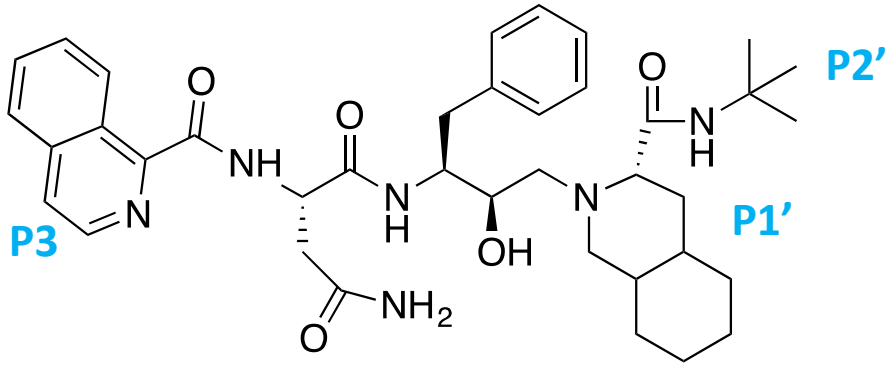
2

Add Asn



IC_{50} = 23 nM

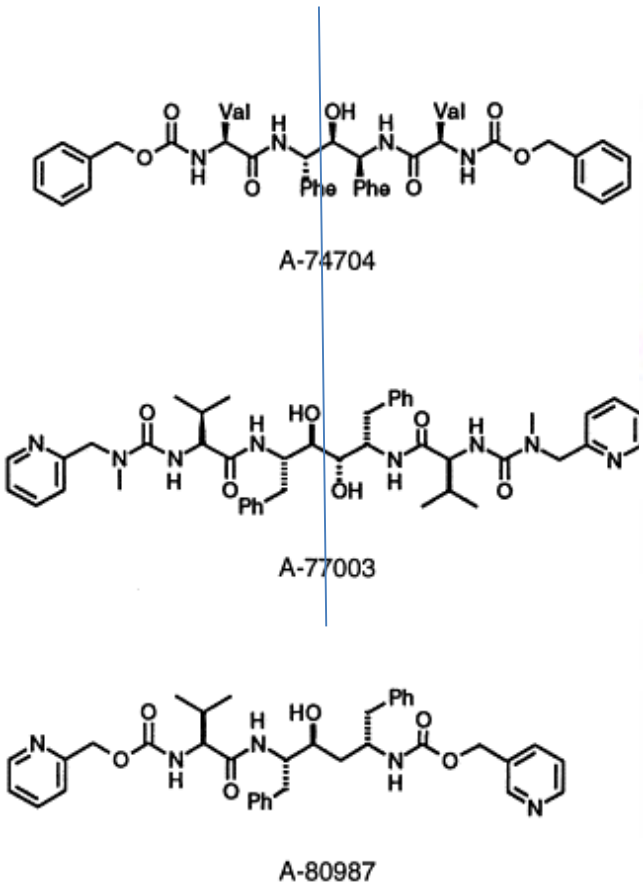
3

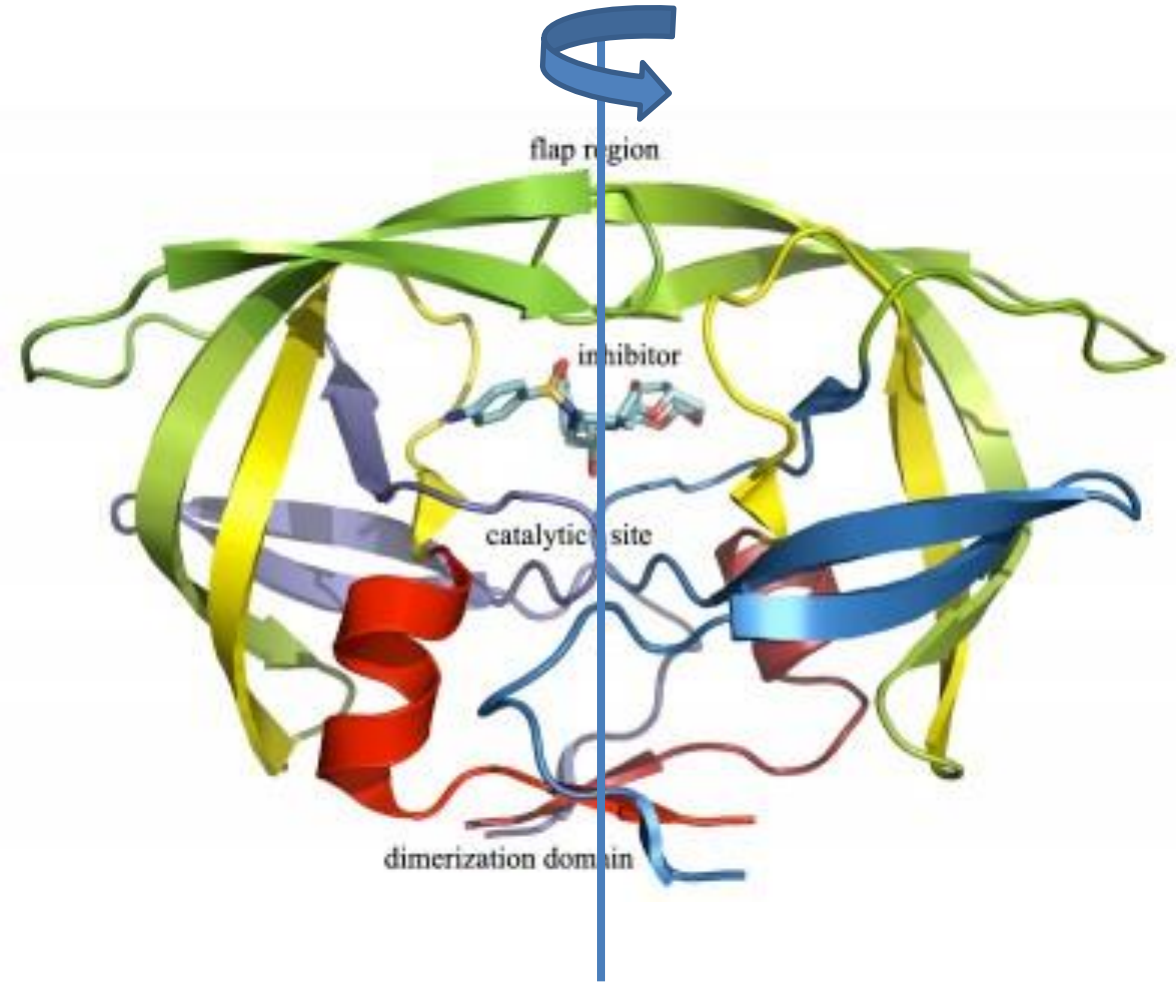


Saquinavir (Roche)

IC_{50} = 0.4 nM

The symmetry (C2) of the target inspired to project symmetric leads





Ritonavir and Lopinavir (Abbott Pharmaceuticals)

1) Symmetry for selectivity HIV proteases vs human proteases

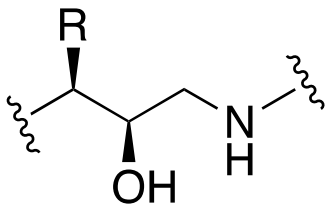
2) More resistant to peptidases (metabolism)

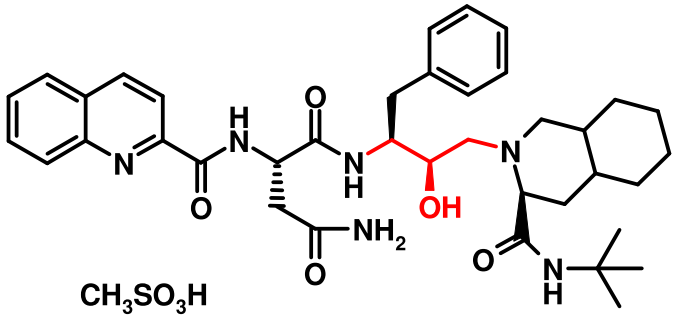
3) The **symmetric** compound A74704 (Abbott) is very active (IC_{50} = 3 nM)

4) The diol equivalent A77003 is ten-fold more active (IC_{50} = 0,22 nM) the amide NH are in optimal positions to form hydrogen bonds with Gly 27 and 27' of protease

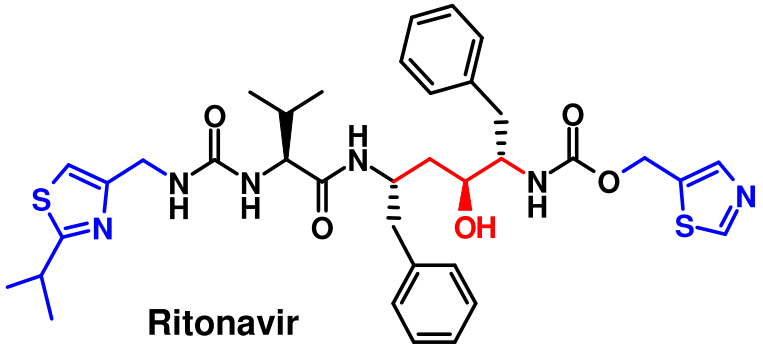
5) Only the (R) hydroxyl forms 2 hydrogen bonds with Asp, the removal of the (S) hydroxyl leads to an increase of the activity (A 80987)

6) Pharmacokinetic optimization: Ritonavir and Lopinavir (IC_{50} 30 and 17 nM)

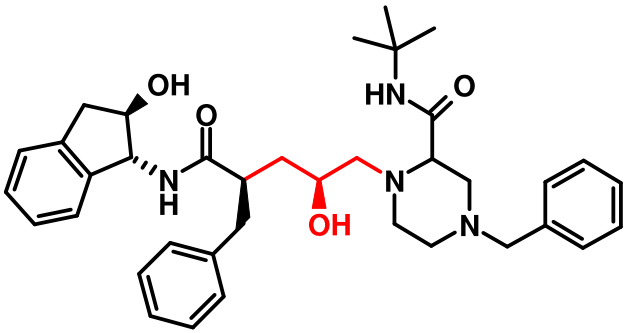




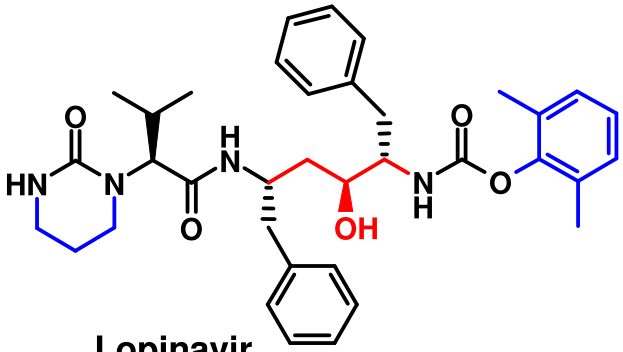
Saquinavir



Ritonavir



Indinavir



Lopinavir

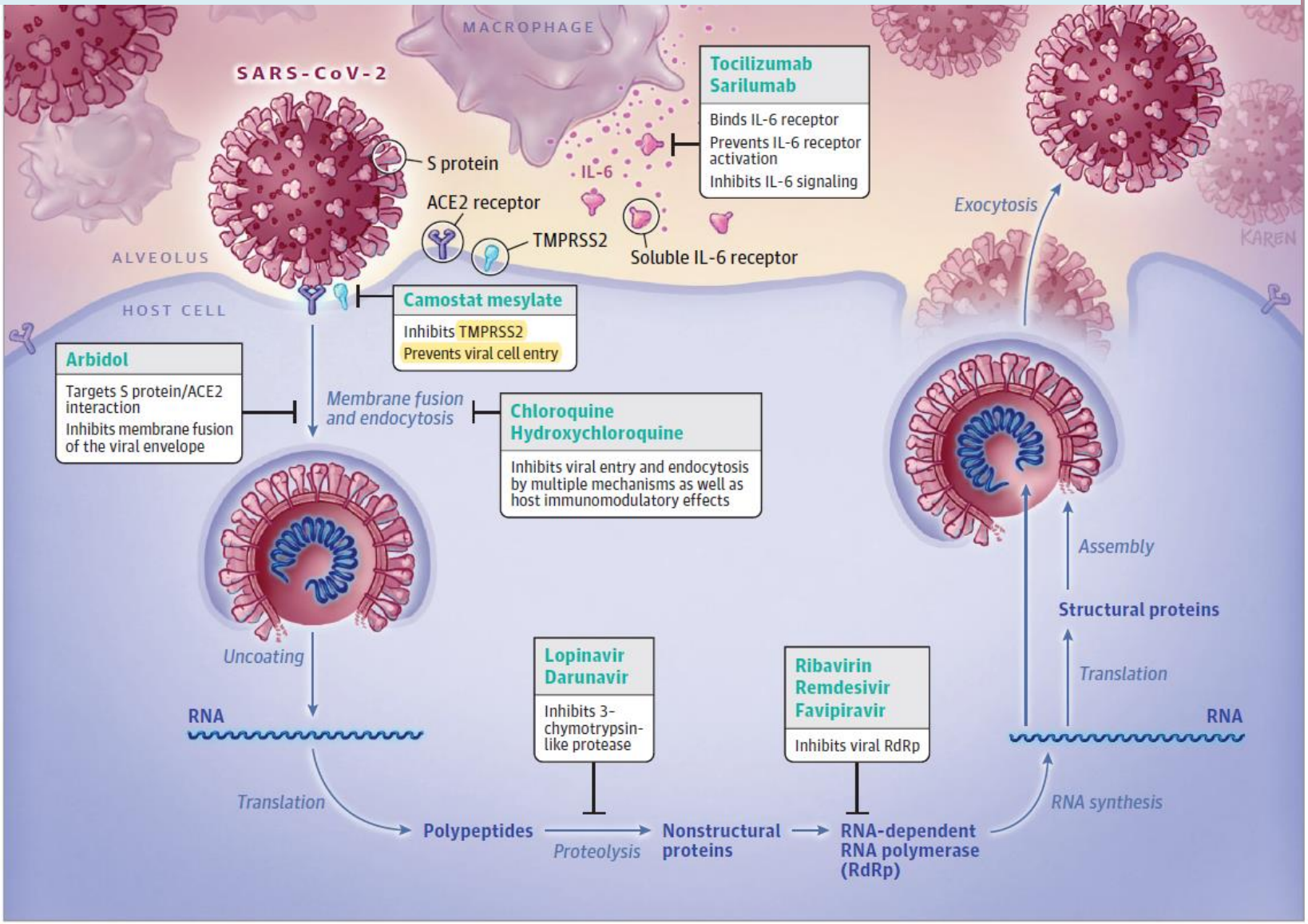
Farmaci basati su piccole molecole autorizzati da AIFA contro il covid-19

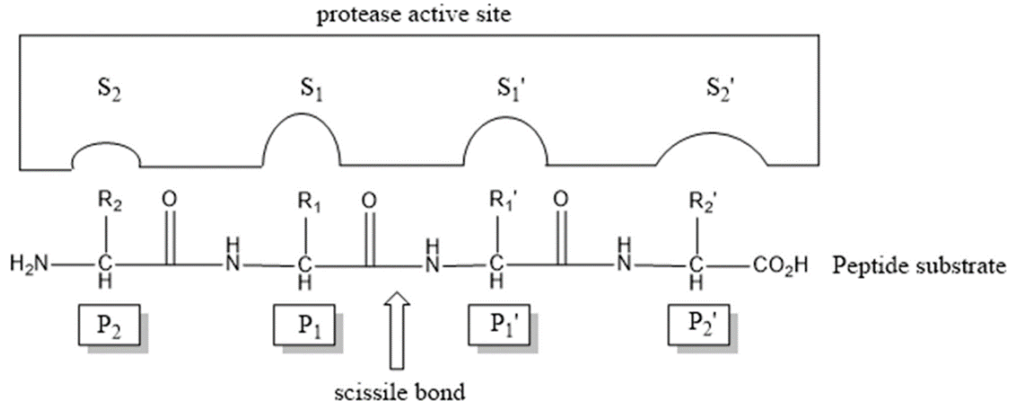
- In Italia sono stati finora autorizzati due antivirali orali per il trattamento della malattia da coronavirus 2019 (COVID-19) negli adulti che non necessitano di ossigenoterapia supplementare e che presentano un elevato rischio di sviluppare una forma severa di COVID-19:

- **Paxlovid (PF-07321332/ritonavir)** dell'Azienda Pfizer Europe MA EEIG
- Inibitore della **cisteina-proteasi Mpro o 3CLpro**
- **Lagevrio (molnupiravir)** dell'Azienda Merck Sharp & Dohme

- In più è stato autorizzato il **Veklury (remdesivir)** che deve essere iniettato
- Entrami inibitori della **RNA polimerasi RNA dipendente virale (RdRp)**

Inhibiting proteases: looking for SARS-CoV2 therapeutics





TARGET – substrato

Substrate preferences for 3CL proteases

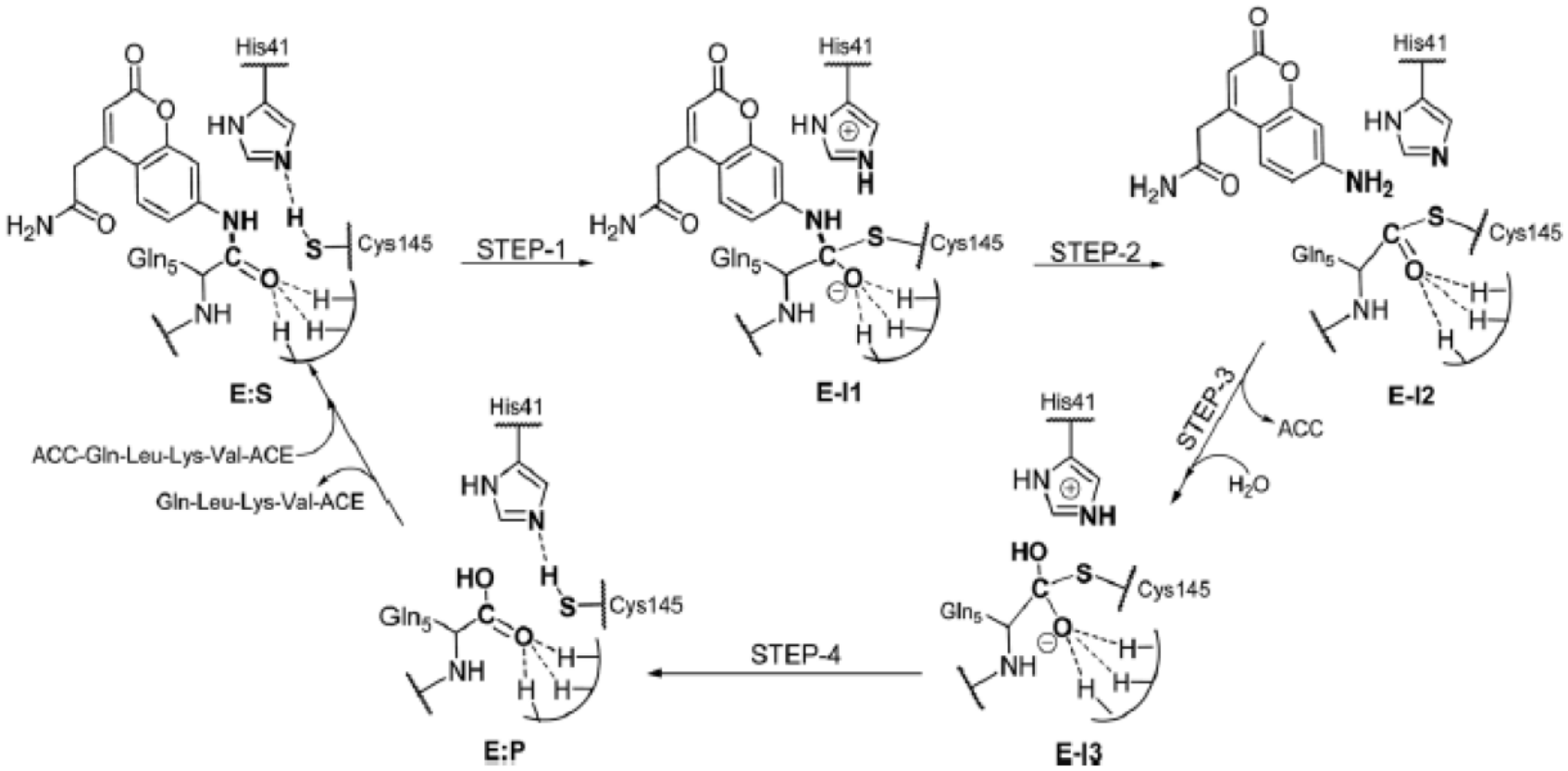
Position	Substrate preference
P5	No strong preference
P4	Small hydrophobic residues
P3	Positively charged residue
P2	High hydrophobicity and absence of beta-branch
P1	Glutamine
P1'	Small residues
P2'	Small residues
P3'	No strong preference

La proteasi $3CL^{pro}$ catalizza il taglio in 11 siti conservati della proteina virale il quale processamento porta a pp1a, proteina non strutturale necessaria al ciclo replicativo.

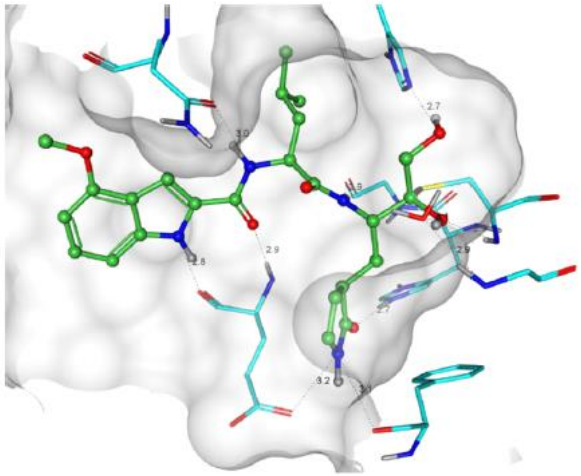
legame peptidico scisso (P1-P1'):

Gln–(Ser/Ala/Gly)

TARGET – meccanismo d'azione



MOA: irreversible covalent bond with Sars-CoV2Mpro



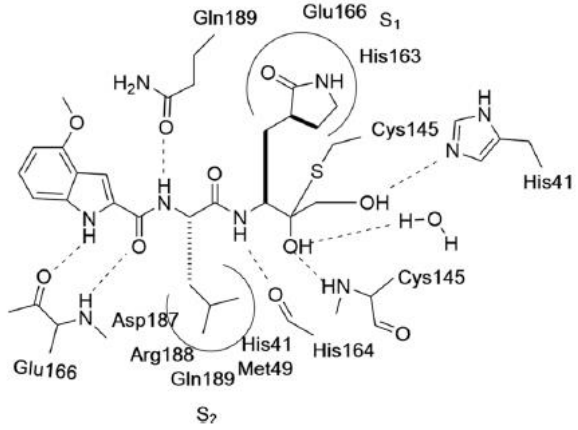
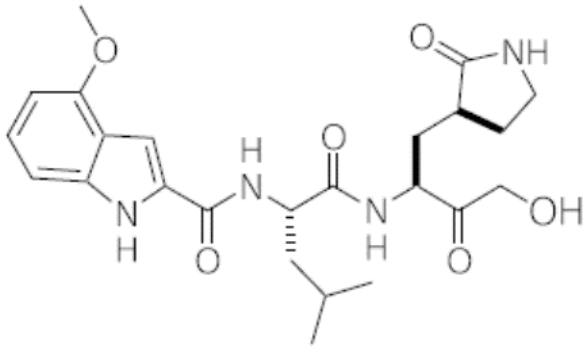
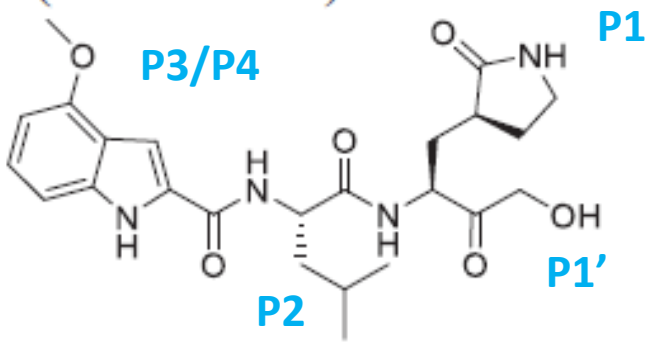


Figure 5. Cocrystal structure of the covalent adduct of 4 bound to SARS CoV-2 3CL^{pro} (6XHM). The Connolly surface for the inhibitor

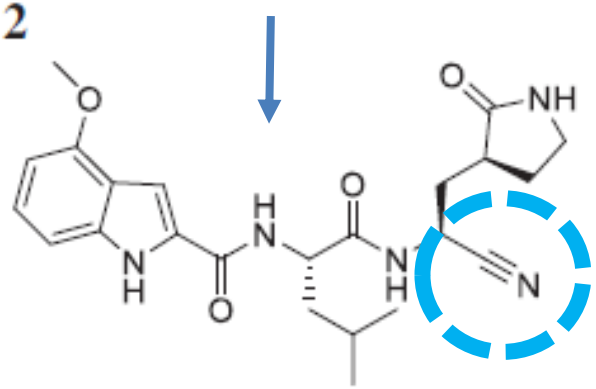


(PF-00835231)

1 (PF-00835231)



2



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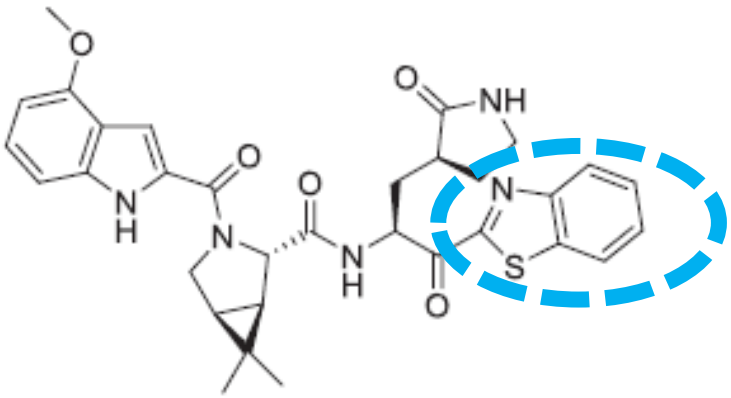


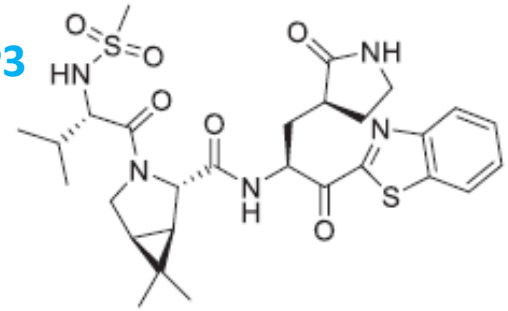
To improve upon the low passive absorptive permeability (Papp < 0.207 × 10−6 cm/s) (21) and poor oral absorption of **1** in animals, we aimed to remove the hydrogen bond donor (HBD) of the P1′ a-hydroxymethyl ketone moiety in 1 because increased HBD count has been shown to be correlated with poor oral bioavailability **(Lipinsky rule of 5)**.

To this end, we pursued two functional groups precedented as **covalent warheads** for cysteine proteases in parallel**: nitriles and benzothiazol-2-yl ketones-**

Introduction of a 6,6-dimethyl-3-azabicyclo[3.1.0]hexane as a cyclic leucine mimetic at P2 removed the HBD from the P2/P3 amide linkage.

4

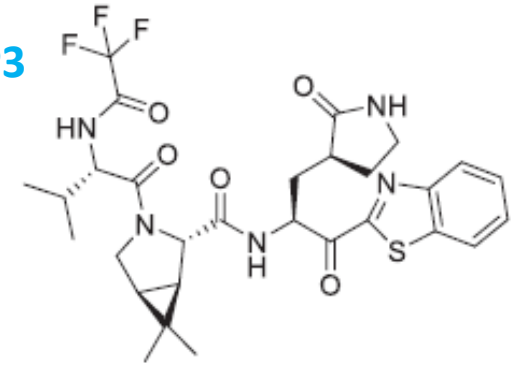
P3



The methanesulfonamide in compound 4 extends underneath Gln189, productively engaging P3 pocket residues and achieving improved hydrogen-bonding interactions with the Glu166

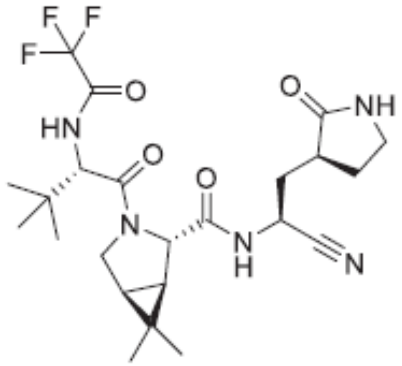
5

P3



An effort to identify alternate P3 capping groups to sulfonamide led to trifluoroacetamide. Compound 5 exhibited comparable biochemical potency (Ki = 12.1 nM) to 4 but with greatly improved SARS-CoV-2 Vero E6 antiviral activity.

6 (PF-07321332)

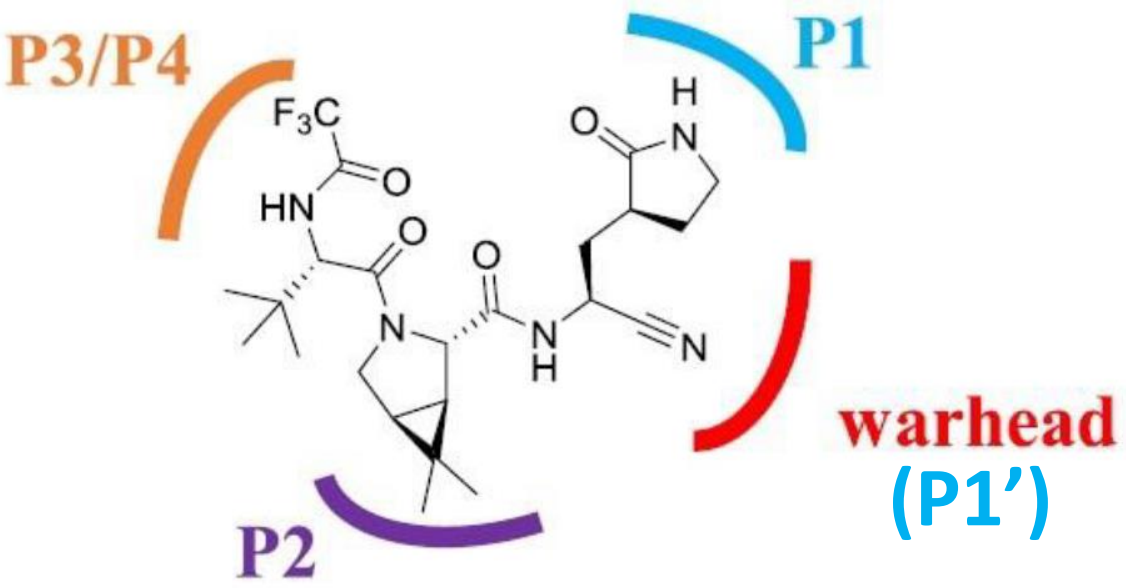


The P1’ nitrile of 6 forms
a reversible covalent thioimidate adduct with the catalytic Cys145

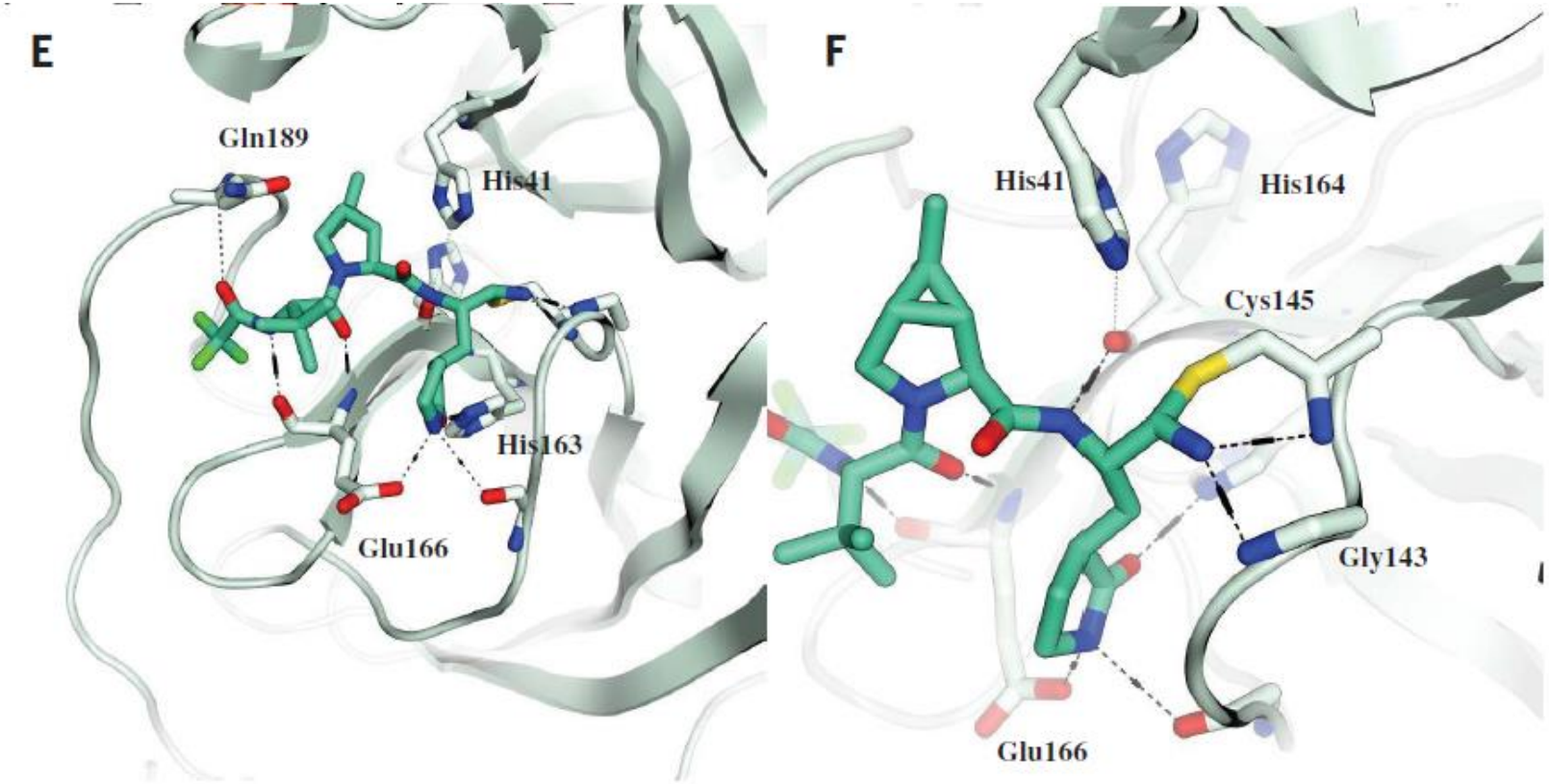
Il farmaco PF-07321332

FARMACO – struttura

Interazione di PF-07321332 con le tasche idrofobiche di SARS-CoV-2 M^{pro}



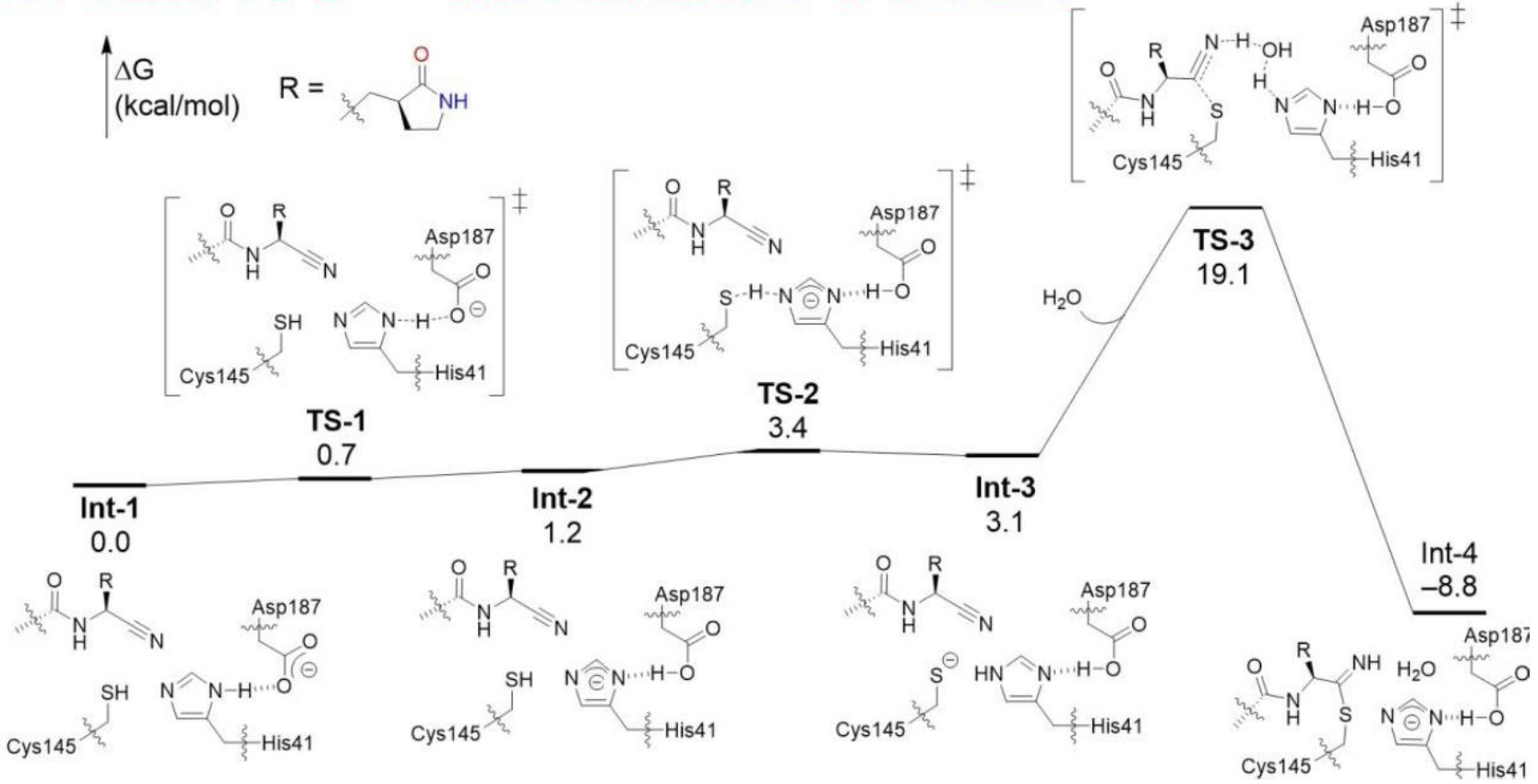
Co-crystal structures



(E) SARS-CoV-2 Mpro–bound crystal structure of clinical candidate PF-07321332 (6).
(F) A reversible covalent Cys145 adduct is formed with the nitrile substituent in compound 6.

Mechanism of action

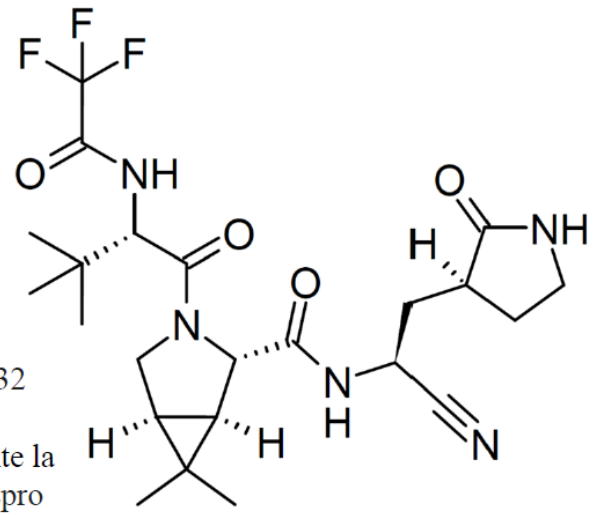
FARMACO – meccanismo d'azione



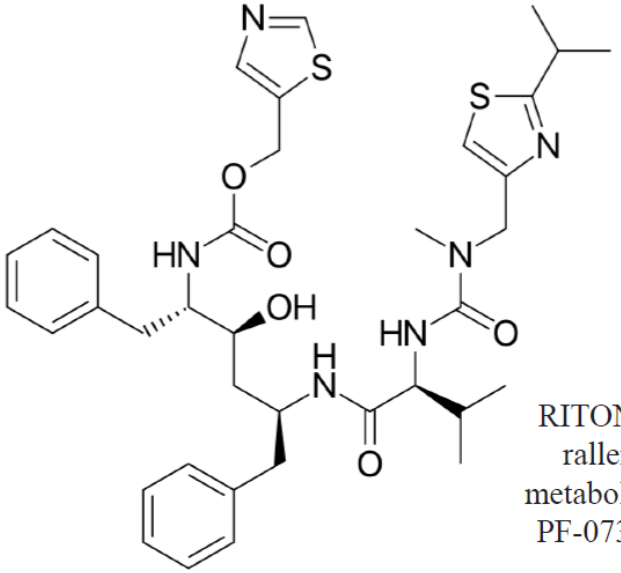
Paxlovid

FARMACO – composizione

PAXLOVID è il farmaco orale che prevede la combinazione di PF-07321332 e ritonavir.



PF-07321332
inibisce
covalentemente la
proteasi 3CLpro



RITONAVIR
rallenta il
metabolismo di
PF-07321332

FARMACO – efficacia e tossicità

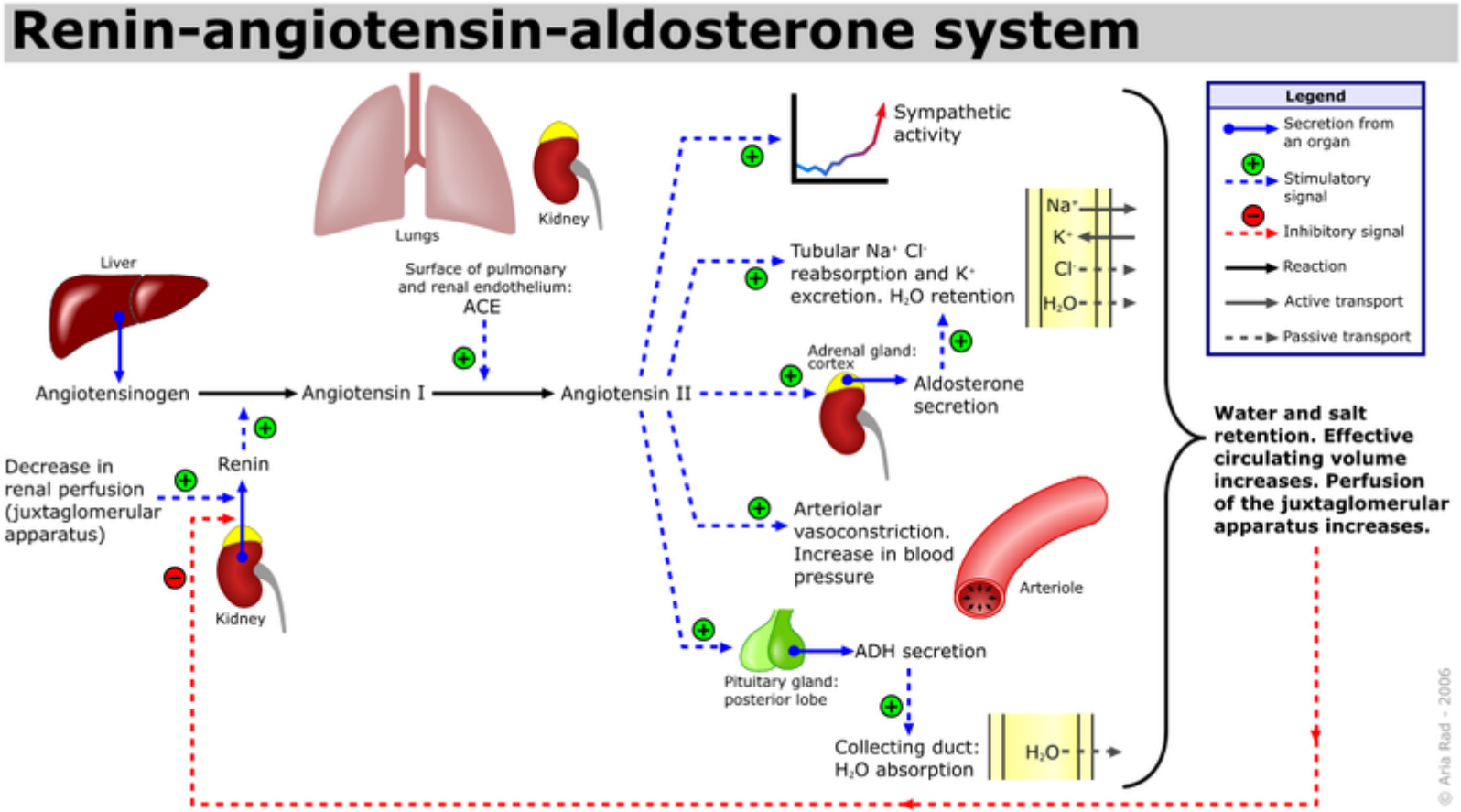
- PAXLOVID (PF-07321332; ritonavir) riduce il rischio di ospedalizzazione o di morte dell'89%, comparato al placebo in adulti ad alto rischio non ospedalizzati affetti da Covid-19. Questi dati provvisori derivano da uno studio di fase 1/2 EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) randomizzato e doppio cieco. Durante lo studio non ci sono stati morti tra i pazienti a cui è stato somministrato PAXLOVID.

- Pazienti trattati entro 3 giorni dall'insorgenza dei sintomi, entro il 28° giorno di studio:
  Il 0.8% dei pazienti trattati con PAXLOVID sono stati ospedalizzati (3/389 ospedalizzati, nessun morto);
  Il 7.0% dei pazienti trattati col placebo sono stati ospedalizzati o morti (27/385 ospedalizzati, di cui 7 morti).

- Pazienti trattati entro 5 giorni dall'insorgenza dei sintomi, entro il 28° giorno di studio:
  L'1.0% dei pazienti trattati con PAXLOVID sono stati ospedalizzati (6/607 ospedalizzati, nessun morto);
  Il 6.7% dei pazienti trattati con placebo sono stati ospedalizzati o morti (41/612 ospedalizzati, di cui 10 morti).

- Gli eventi avversi blandi sono confrontabili tra i pazienti trattati con PAXLOVID (19%) e quelli col placebo (21%), mentre eventi avversi gravi (1.7% e 6.6%) ed eventi avversi gravi con interruzione dello studio (2.1% e 4.1%) sono stati riscontrati nei pazienti trattati con PAXLOVID e placebo, rispettivamente. Inoltre, il farmaco è selettivo per SARS-CoV-2 M^{pro}, la quale presenta un meccanismo che non è presente in nessuna proteasi umana, pertanto si correla una bassa tossicità.

Renin inhibitors

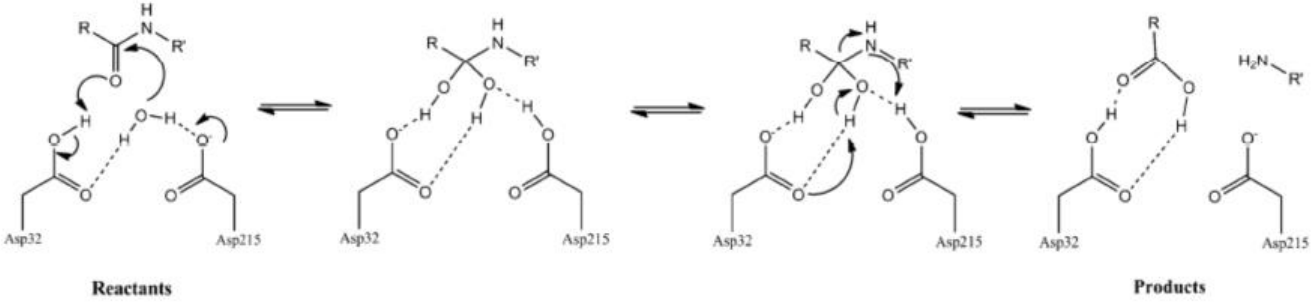


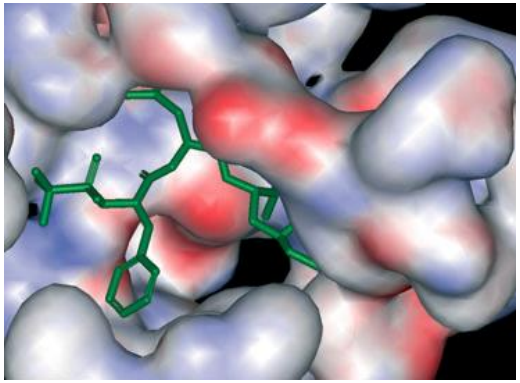
Enzyme inhibitors

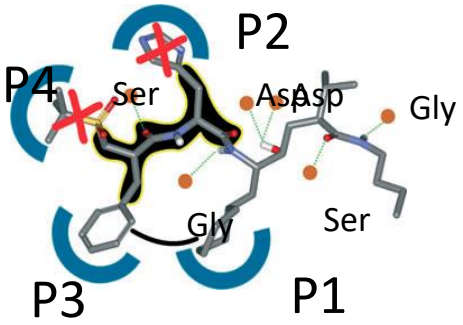
- Molecules that resemble (mimick) the enzyme substrate but cannot be transformed by the enzyme
- Inhibitors can also resemble to the transition state (or intermediate) of the enzyme-catalyzed reaction

...and stabilizes the transition state of a reaction....

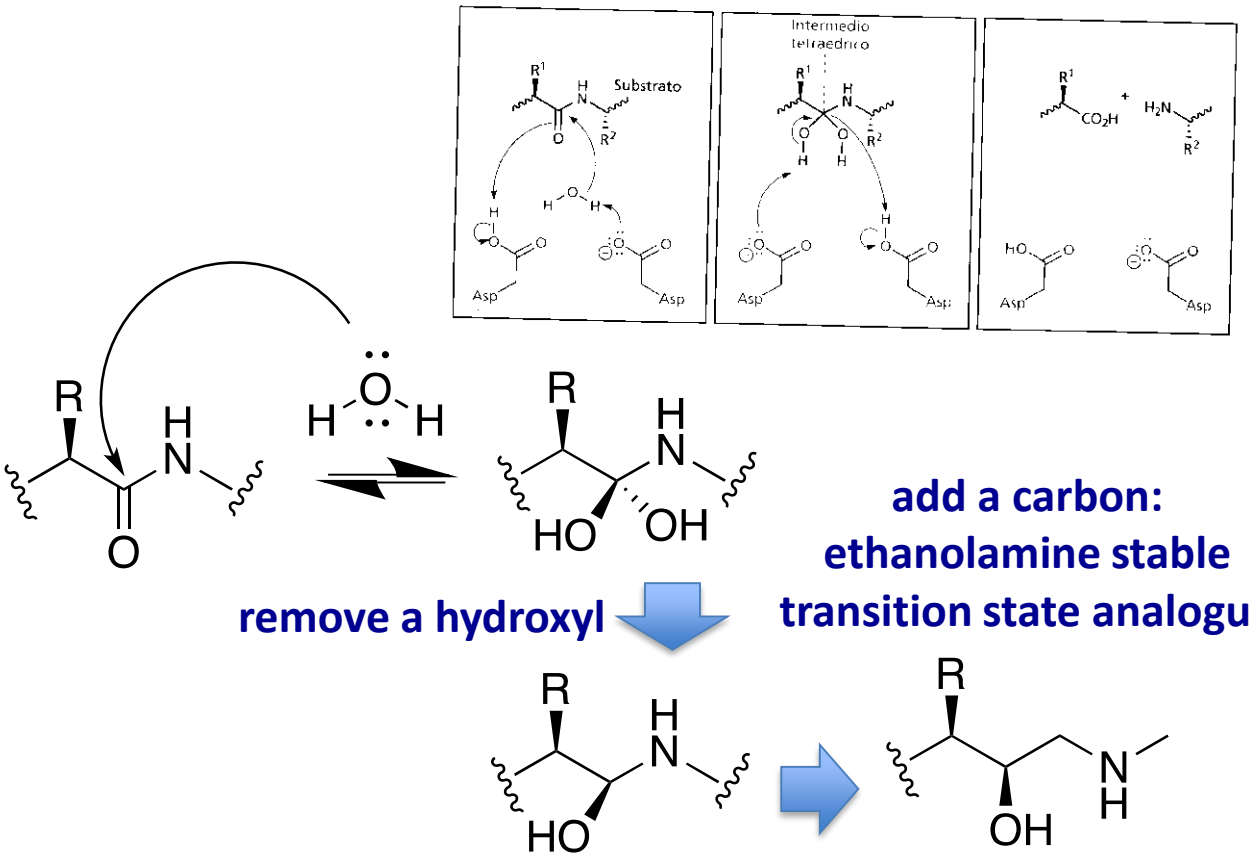
proteases mechanism of action



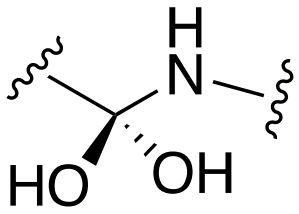




Mimetics of sp^3 reaction intermediate of enzymatic hydrolytic reaction

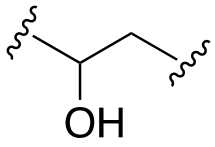


Other reaction intermediate analogues

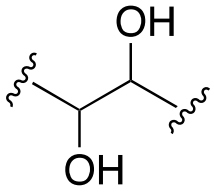


transition state

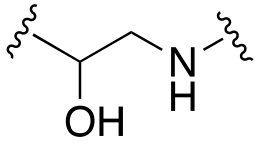
ANALOGUES



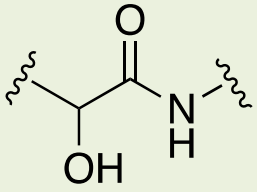
hydroxyethylene



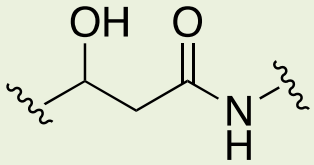
dihydroxyethylene



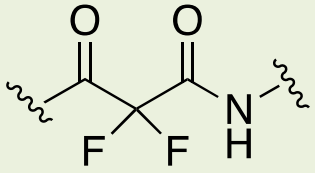
ydroxyethylamine



norstatin

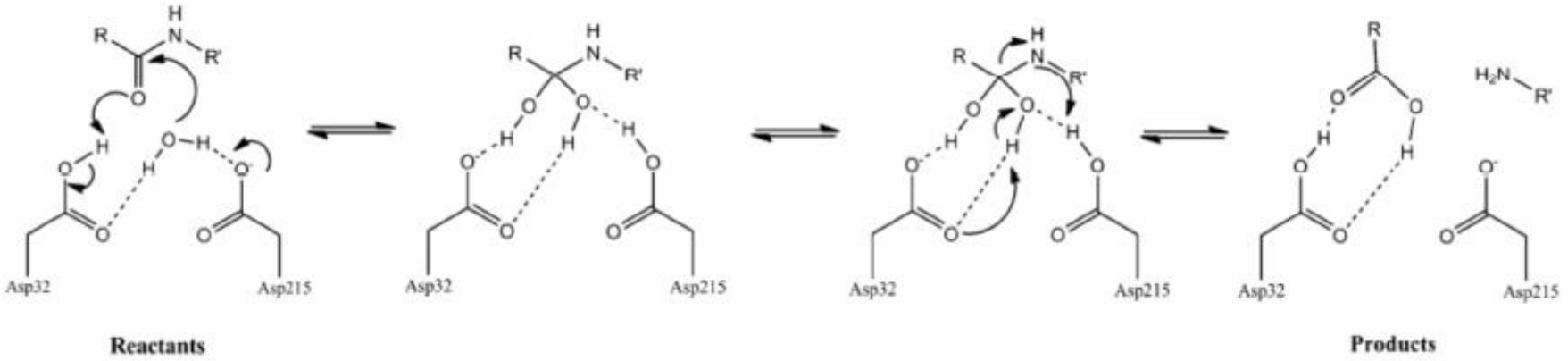


statin

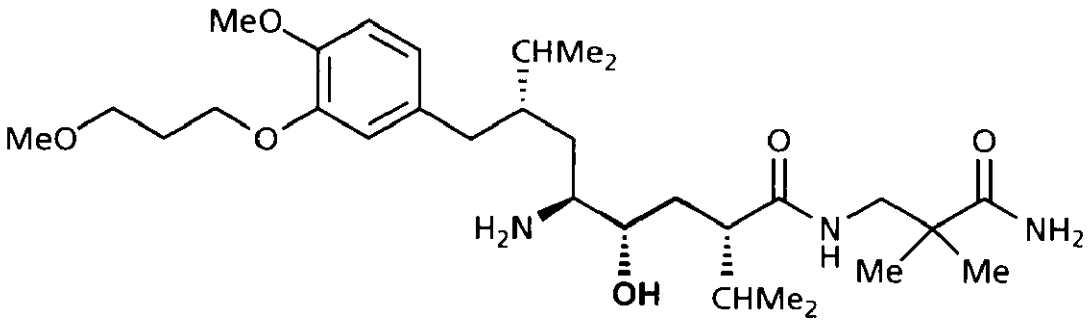


staton

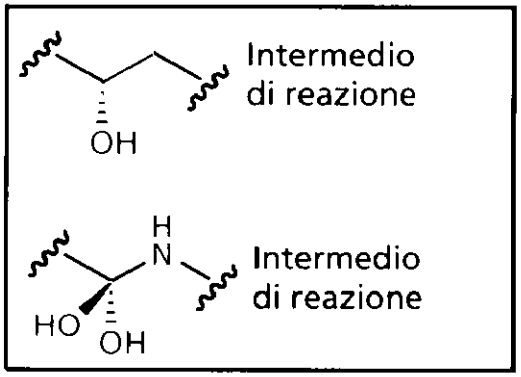
Renin inhibitors: mimetics of the sp^3 transition state (tetrahedric) of the amide hydrolysis reaction catalyzed by proteases



ALISKIREN: a rationally designed renin inhibitor



Mimetico dello
stato di transizione
idrossietilenico



The story of Aliskiren discovery....

- Structure-Based Drug Design and the Discovery of Aliskiren (Tekturna): Perseverance and Creativity to Overcome a R&D Pipeline Challenge.

Nissim Claude Cohen

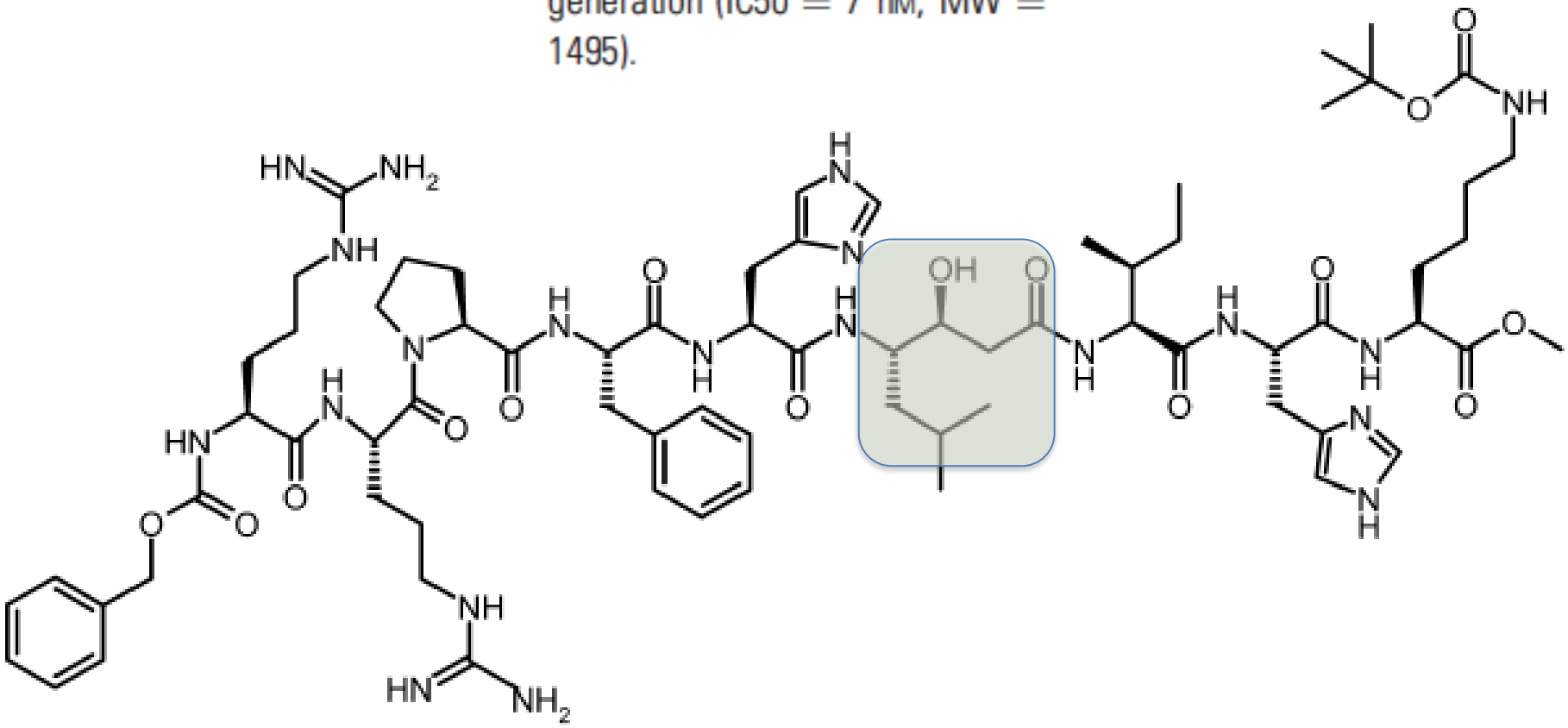
Chem Biol Drug Des 2007; 70: 557–565

Peptide and Peptidomimetic inhibitors

- The first generation of inhibitors was based on the structure of the natural peptide substrate of renin:
- Asp-Arg-Val- Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His –Asp
- The amide bond between Leu and Val is chemically modified to be a TRANSITION STATE ANALOGUE

Peptide-based inhibitors

Figure 3: CGP29287: example of peptide inhibitor of the first generation (IC50 = 7 nM; MW = 1495).



The peptidomimetic approach was unsuccessful

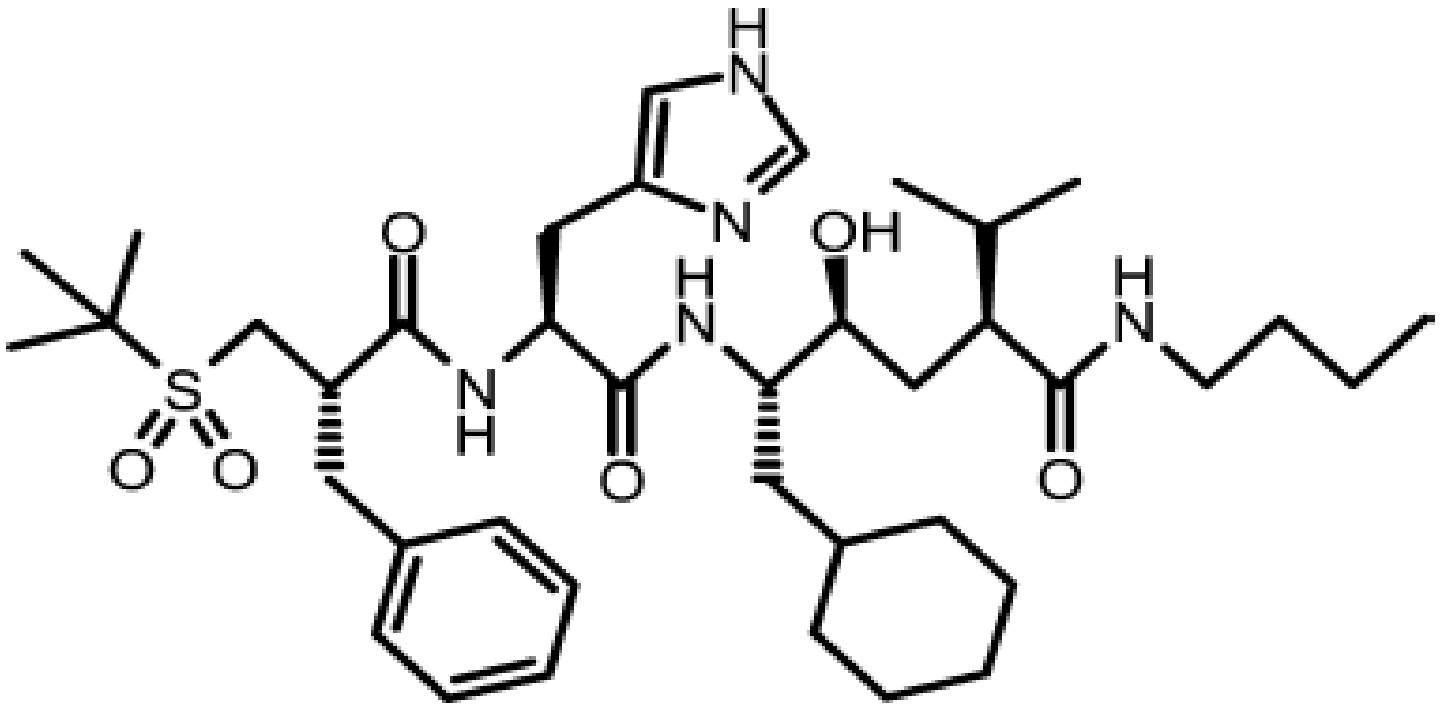
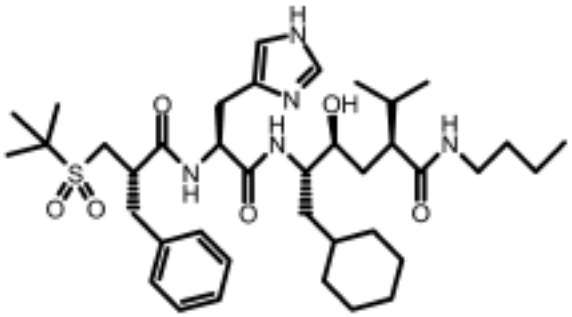
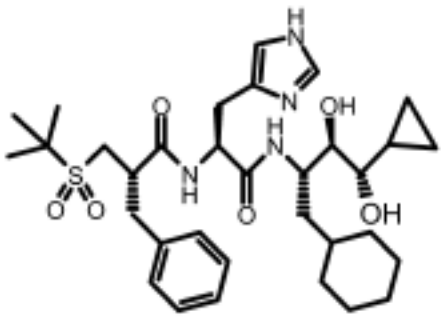


Figure 4: CGP38560 was withdrawn from clinical development for insufficient pharmacokinetics: poor oral absorption (< 1%); rapid biliary excretion (half-life of 7 min).



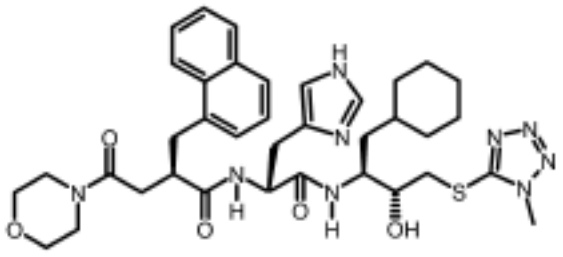
CGP38560 (0.7 nM)

Ciba Geigy



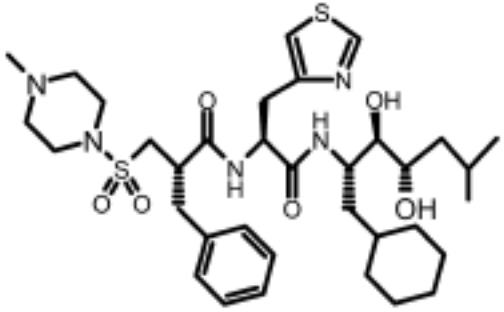
Remikiren (1.0 nM)

Roche



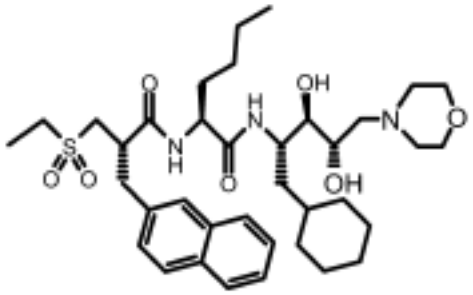
YM-21095 (0.5 nM)

Yamanouchi



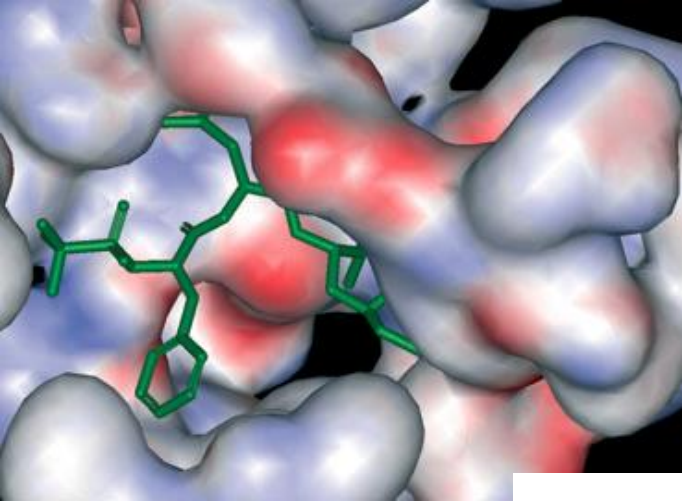
Zankiren A-72517 (1.1 nM)

Abbott

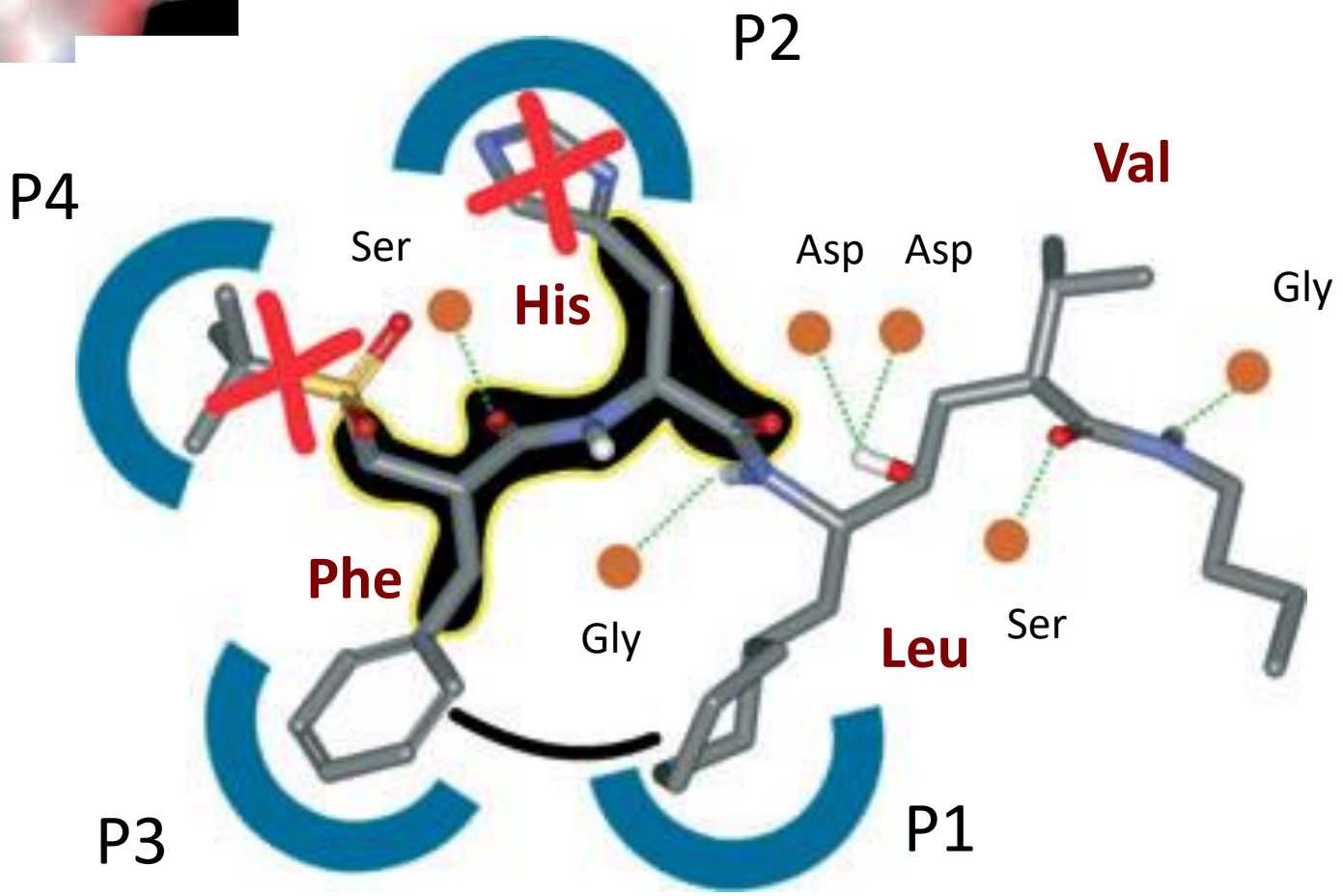


BW-175 (3.3 nM)

Glaxo-Wellcome



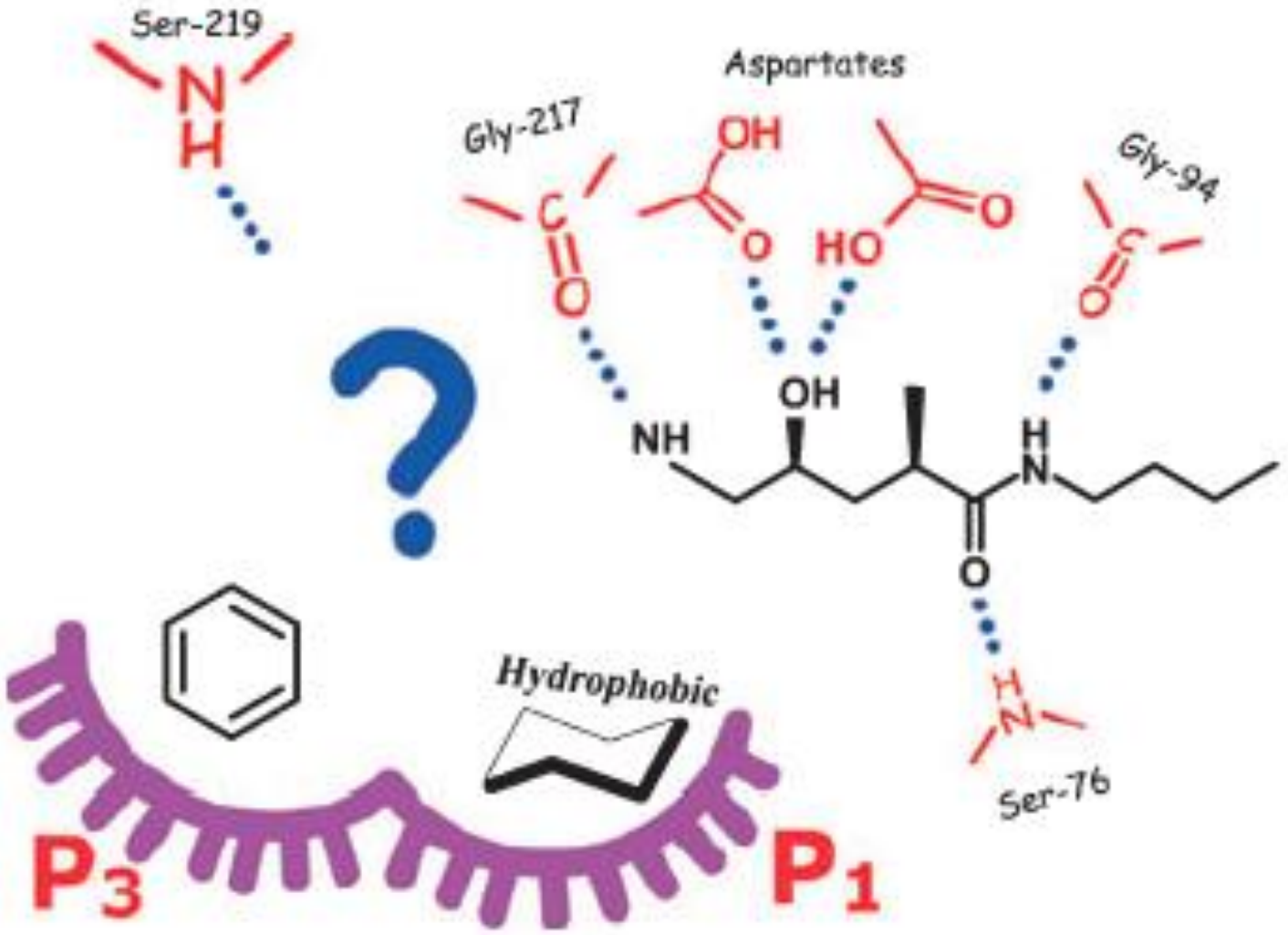
6 intra-molecular interactions
and 4 binding sites
P1 and P3 more important than P2 and P4
CGP 38560

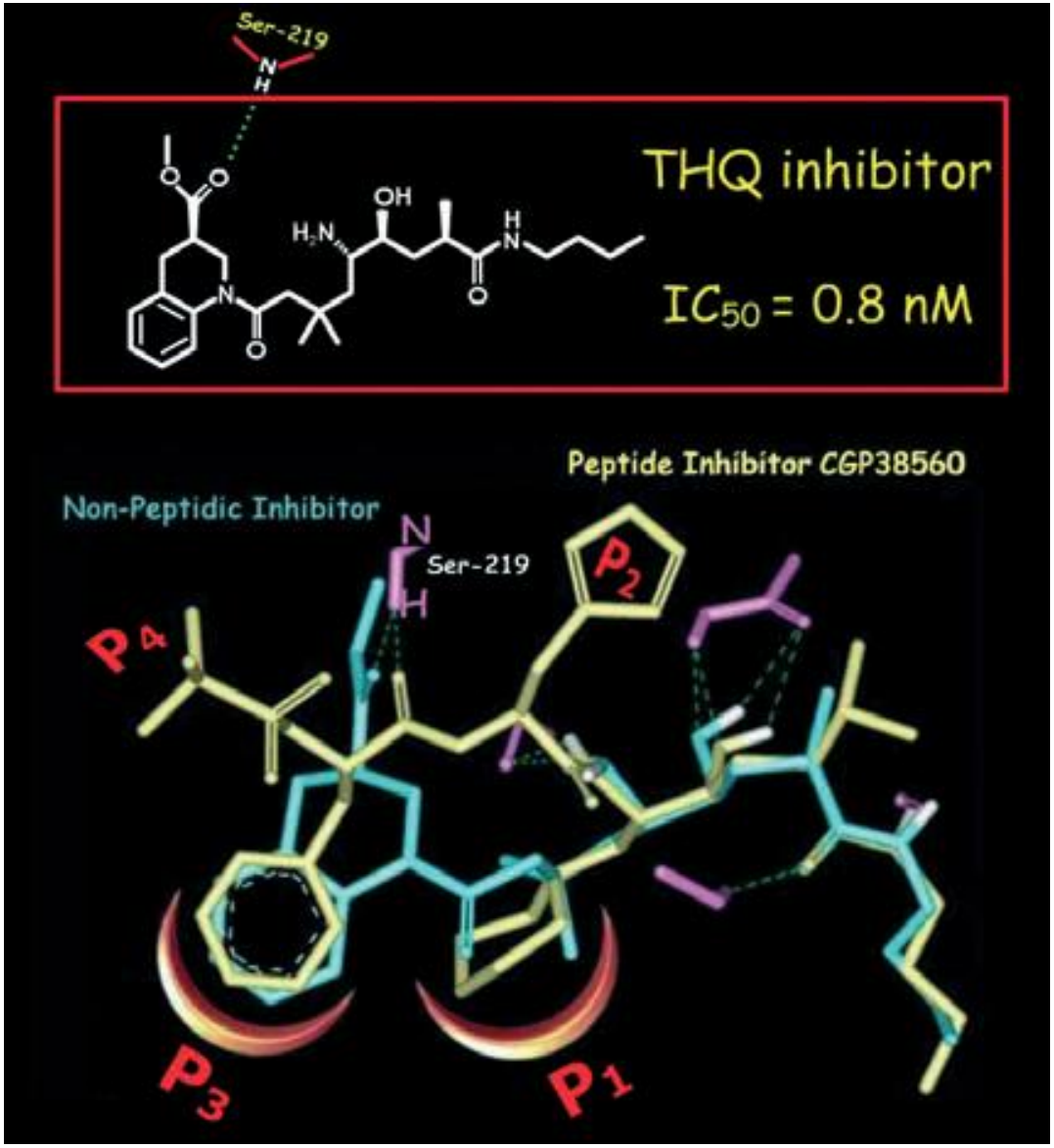


From peptide to non-peptide

- We can remove the peptidic backbone; we however maintained the possibility of a hydrogen-bond with the serine residue. The two side-chains in P1 and P3 are not connected together any more by the peptidic backbone but we can, because of their close proximity, connect them by a direct chemical link. P2 and P4 were sacrificed, but we kept in mind that they could be used if necessary.

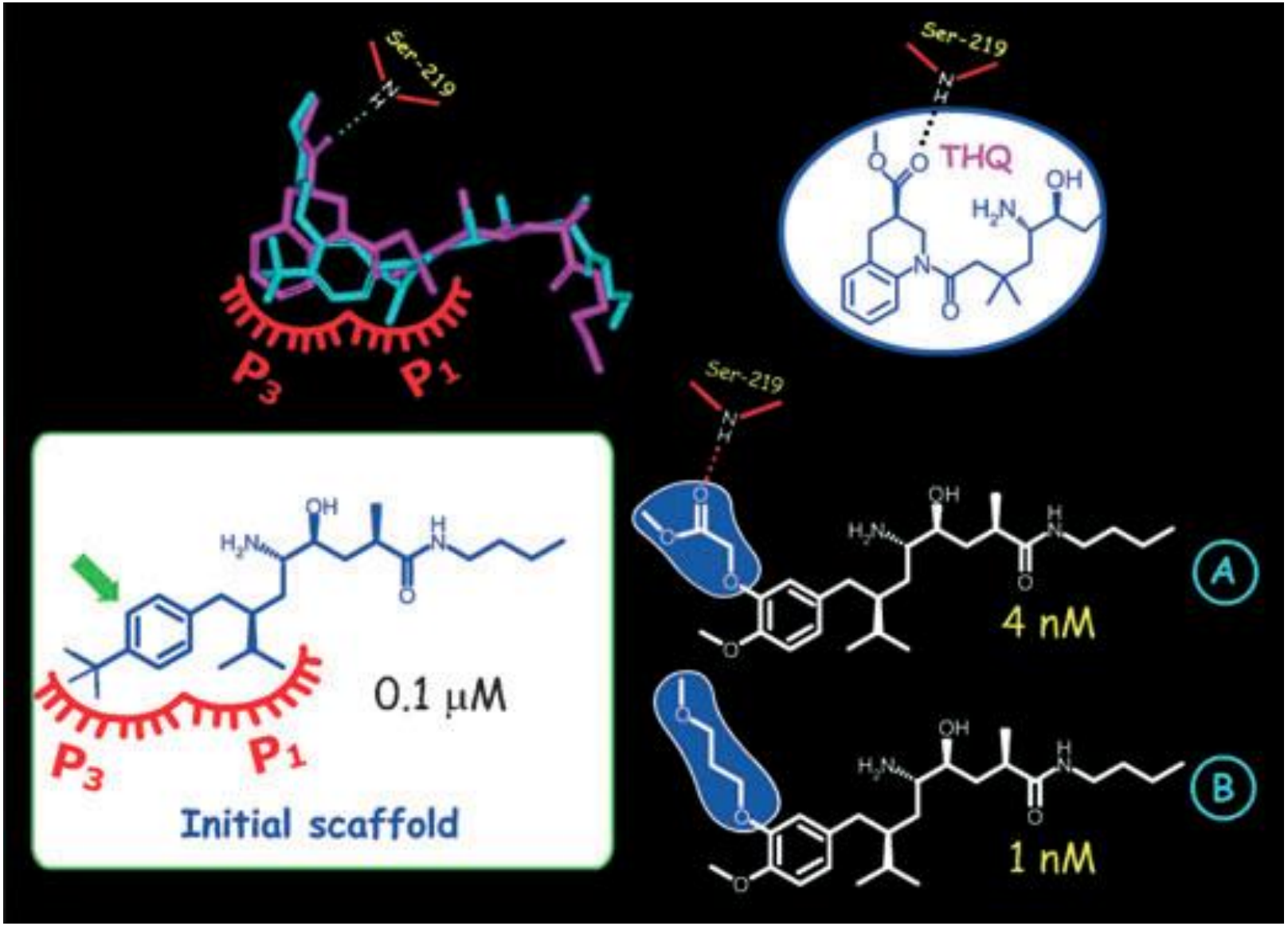
The operational strategy



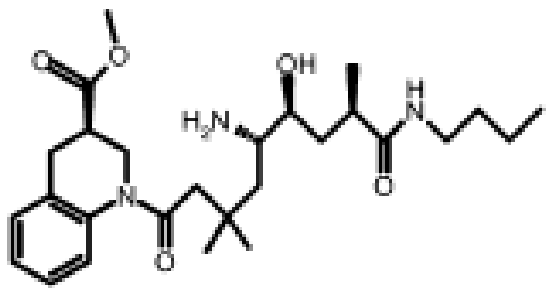


The first potent non-peptide renin inhibitor discovered: a tetrahydroquinoline (THQ) lead.

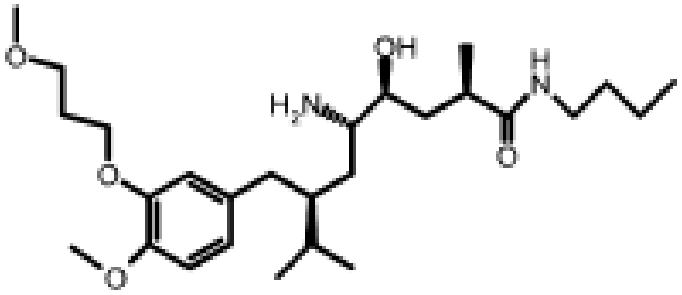
From micromolar to nanomolar affinity in the phenoxy series



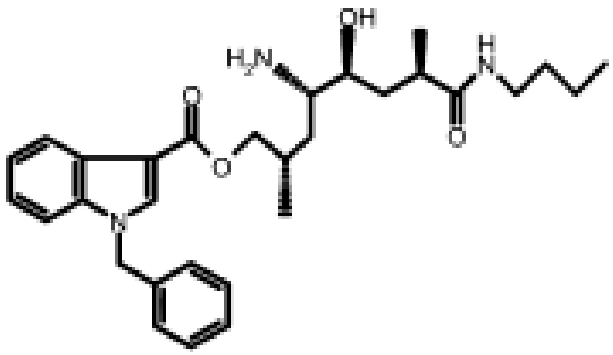
third generation of renin inhibitors



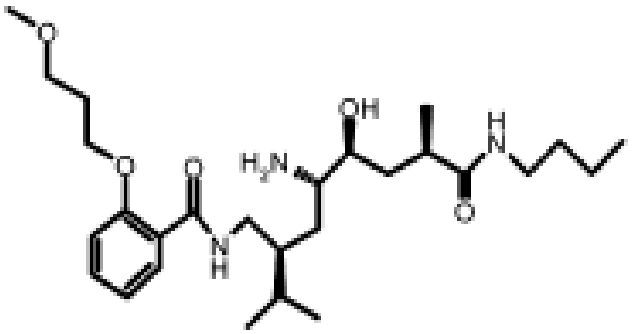
THQ (0.8 nM)



Phenoxy (1 nM)

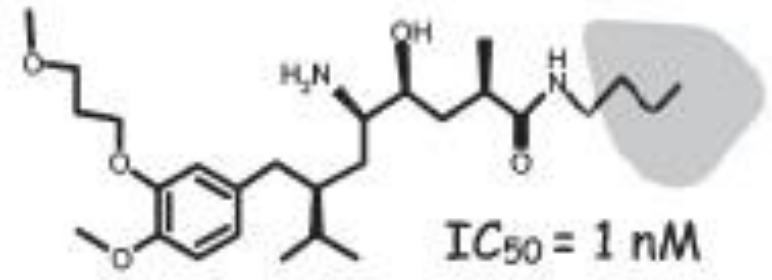


Indole (3 nM)



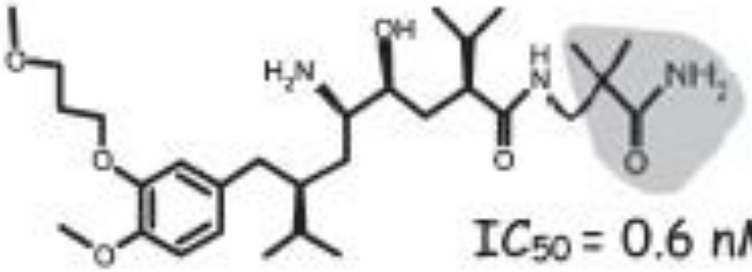
Salicylamide (0.9 nM)

Lead optimization



IC_{50} = 1 nM

initial lead: *CGP54061A*



IC_{50} = 0.6 nM

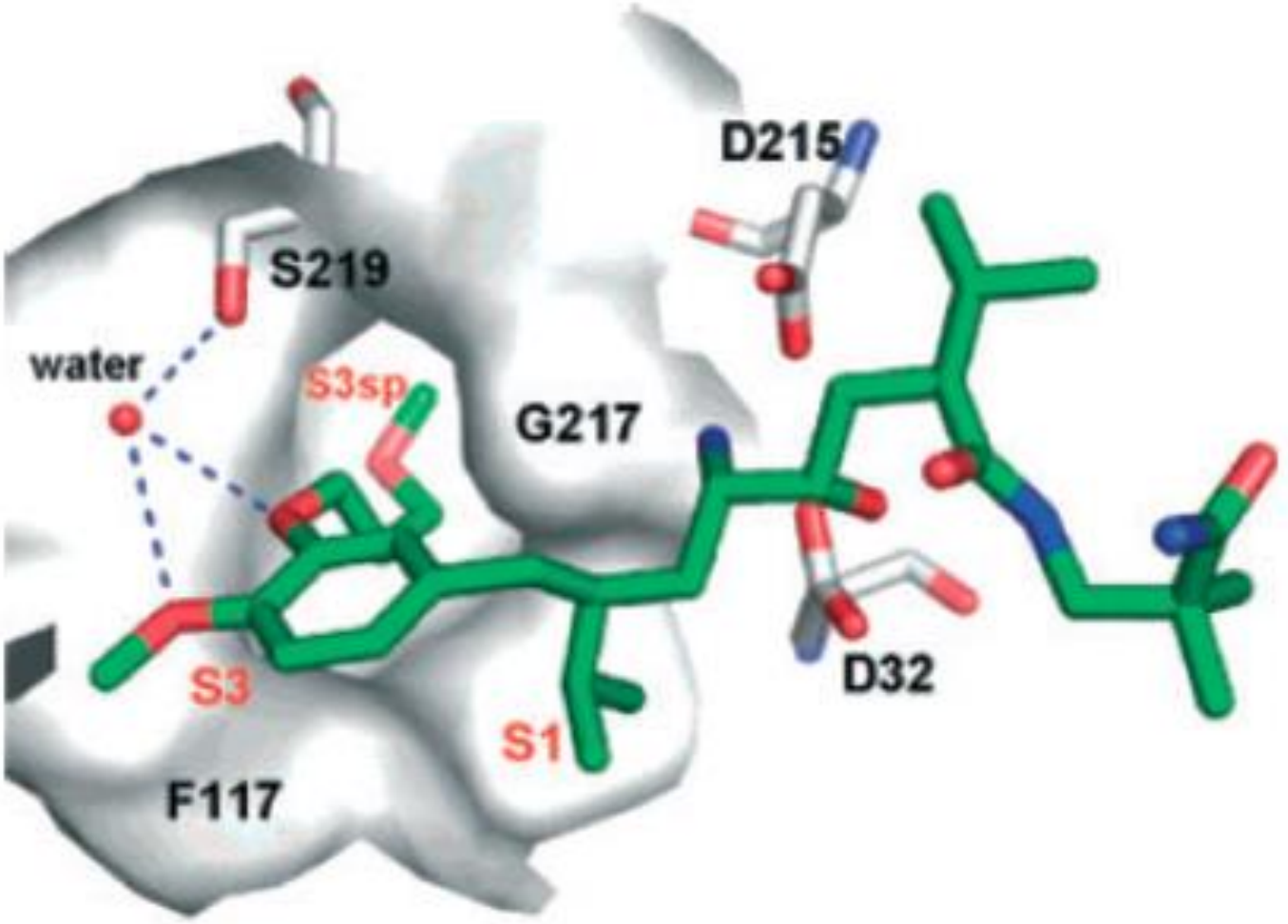
CGP60536B - SPP100 - Aliskiren

Tekturna - Rasilez

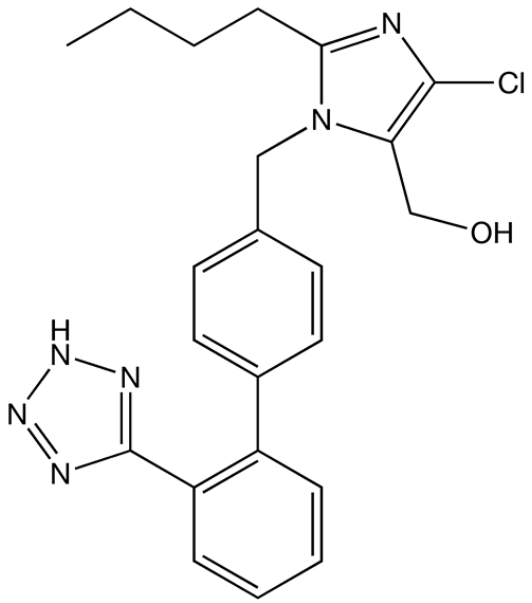
Optimization

- Potency
- Aqueous solubility
- logP
- Stability
- Specificity (enzymes)
- Specificity (species)
- Toxicity

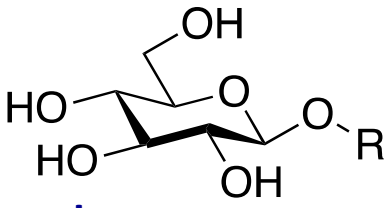
Complex between Aliskiren (Tekturna) and renin



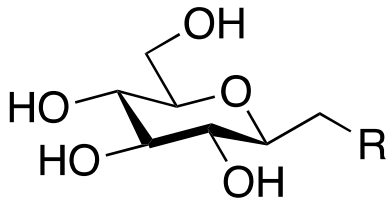
Losartan, an Angiotensin II receptor inhibitor was similarly developed by ligand-based optimization



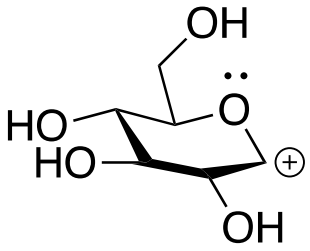
Two types of glycosidase inhibitors



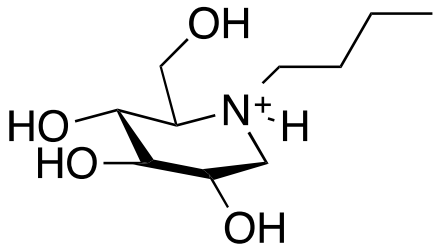
substrate



C-glycoside



transition state



N-butyl deoxynojirimicin: antitumoral, Gaucher disease

- Substrate analogues: C-glycosides
- Transition state analogues: azasugars or iminoglycosides

Chemical Science

EDGE ARTICLE

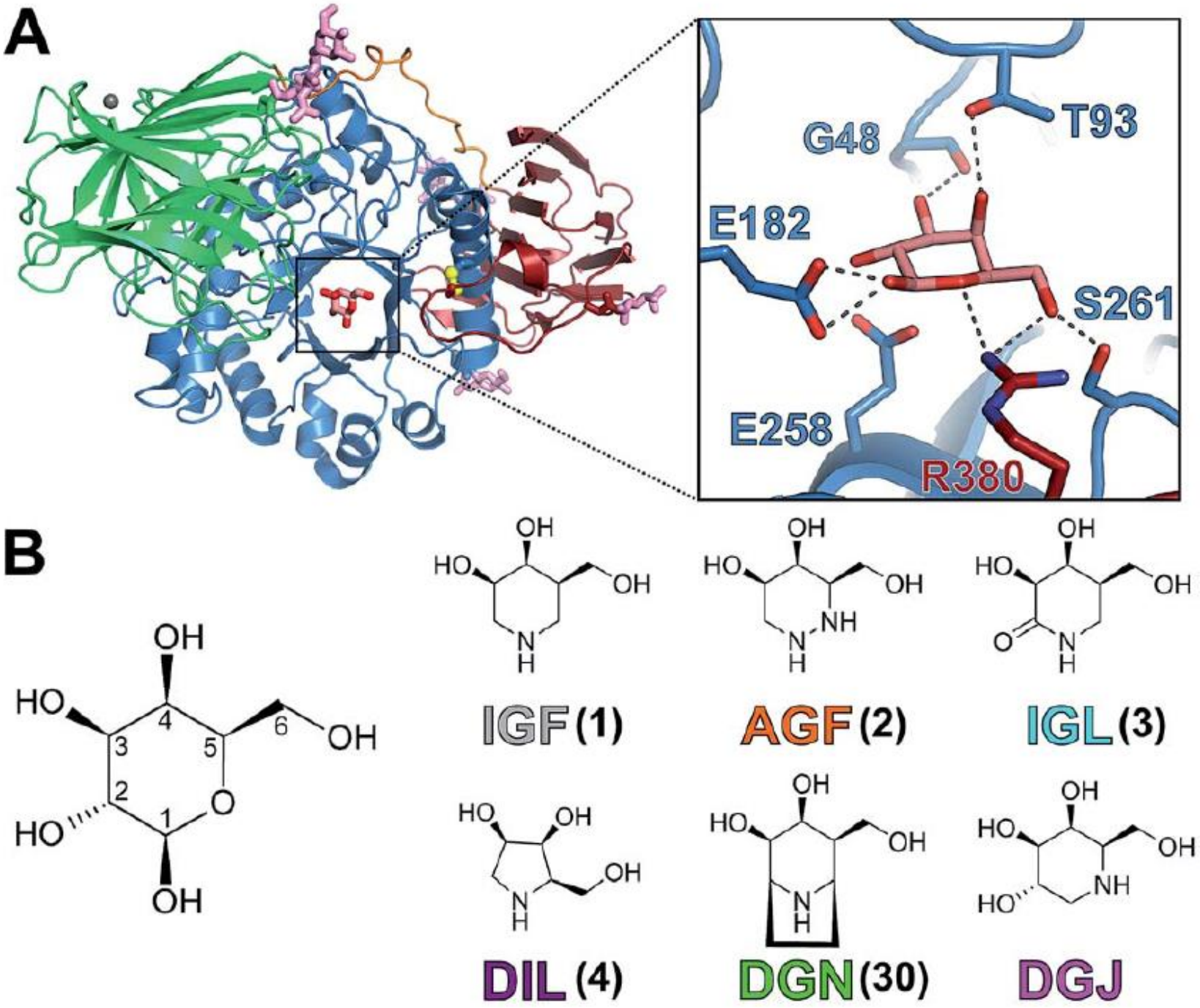
Cite this: *Chem. Sci.*, 2015, **6**, 3075

Azasugar inhibitors as pharmacological chaperones for Krabbe disease†‡

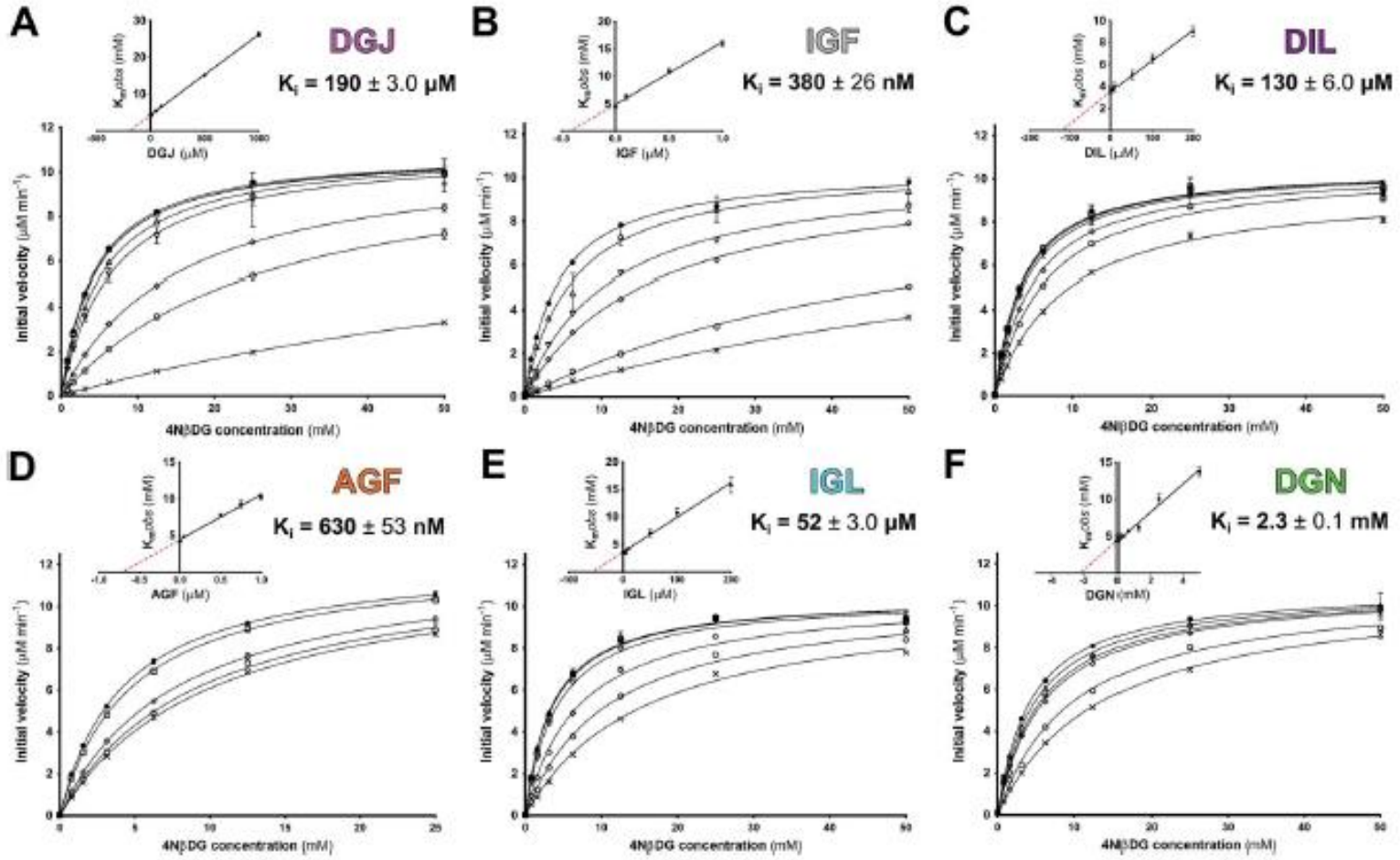
Chris H. Hill,§[a] Agnete H. Viuff,§[b] Samantha J. Spratley,[a] Stéphane Salamone,[b] Stig H. Christensen,[b] Randy J. Read,[a] Nigel W. Moriarty,[c] Henrik H. Jensen*[b] and Janet E. Deane*[a]

Not only enzyme inhibitors.....azasugars as molecular chaperones

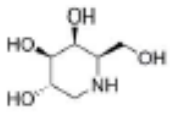
- Pharmacological chaperone therapy (PCT) has recently emerged as an alternative strategy for treating diseases caused by partially defective proteins. In cases where mutant enzyme is trapped in the ER due to instability or misfolding, specific binding of a small molecule chaperone is hypothesized to stabilize the correctly-folded enzyme, allowing functional material to leave the ER, and decreasing removal of the protein by ER-associated degradation.
- Although not completely understood, several biochemical mechanisms for pharmacological chaperones (PCs) have been proposed including the acceleration of folding, slowing of unfolding, template-based induction of correct folding, and thermodynamic stabilization. To attain selectivity, PCs are often active-site-specific competitive inhibitors; hence the ideal PC would bind the enzyme in the ER, stabilize the protein, restore correct trafficking, then dissociate in lysosomes where the PC would be outcompeted by an excess of substrate. Restoration of just 10–15% of activity is sufficient to prevent disease


A
G48
T93
E182
S261
E258
R380
B
IGF (1)
AGF (2)
IGL (3)
DIL (4)
DGN (30)
DGJ

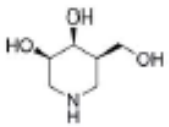
Azasugar derivatives are competitive inhibitors of GALC



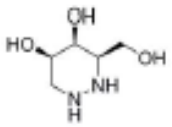
Azasugars stabilize GALC to thermal denaturation



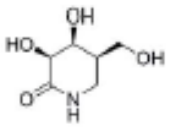
DGJ



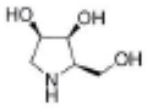
IGF



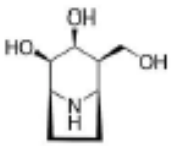
AGF



IGL

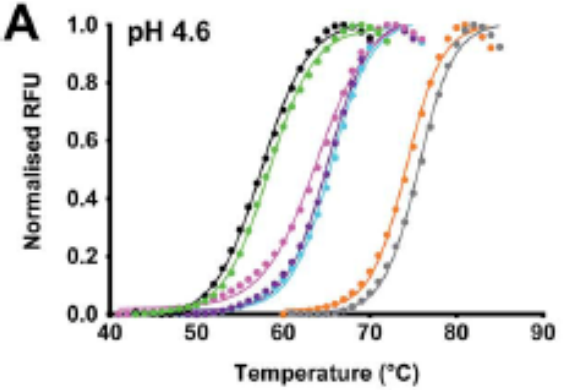


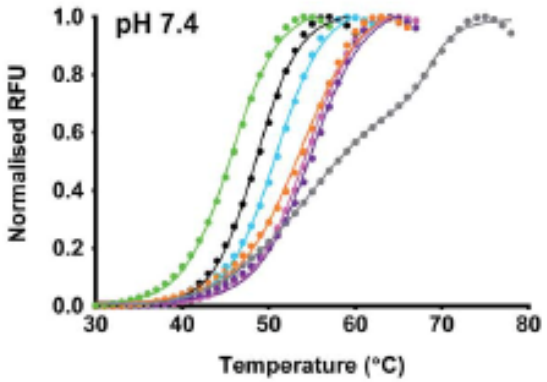
DIL



DGN

A

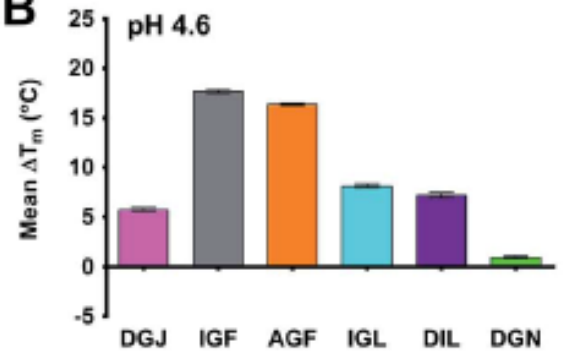


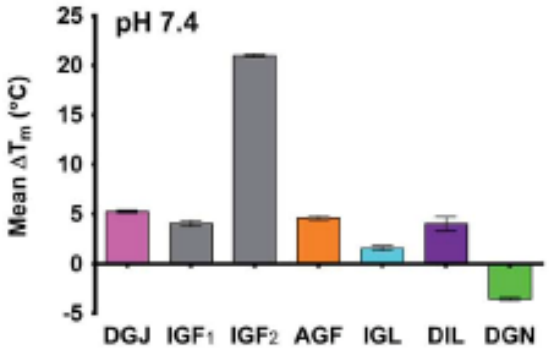


C

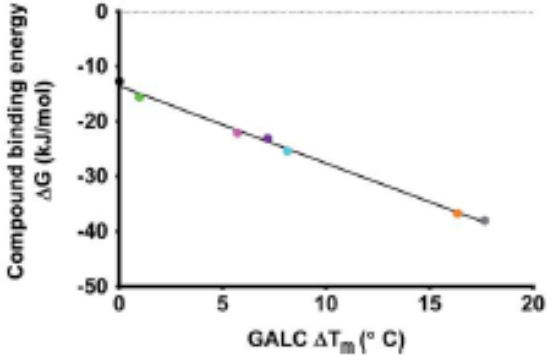
Compound	ΔT_m (°C) pH 4.6	ΔT_m (°C) pH 7.4
DGJ	**+5.7** ± 0.2	**+5.2** ± 0.1
IGF_1	**+17.7** ± 0.1	**+4.0** ± 0.3
IGF_2	n/a	**+21.0** ± 0.2
AGF	**+16.4** ± 0.1	**+4.6** ± 0.1
IGL	**+8.1** ± 0.2	**+1.6** ± 0.2
DIL	**+7.2** ± 0.3	**+4.0** ± 0.7
DGN	**+1.0** ± 0.1	**-3.6** ± 0.1

B

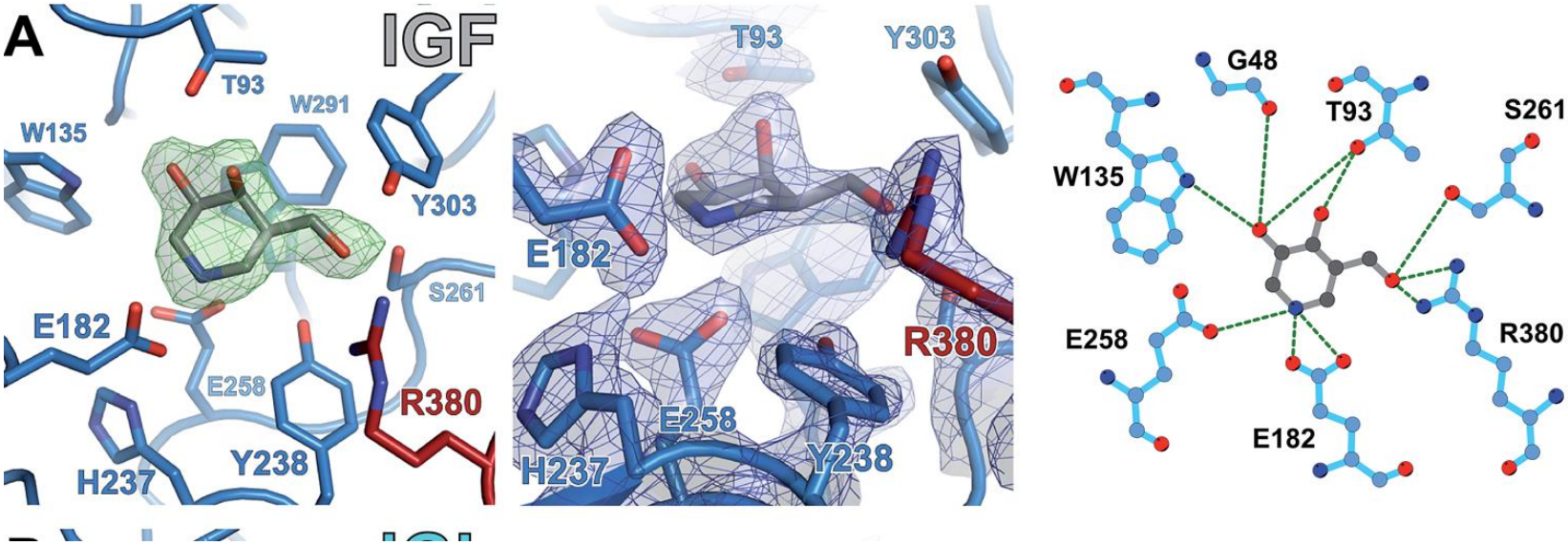




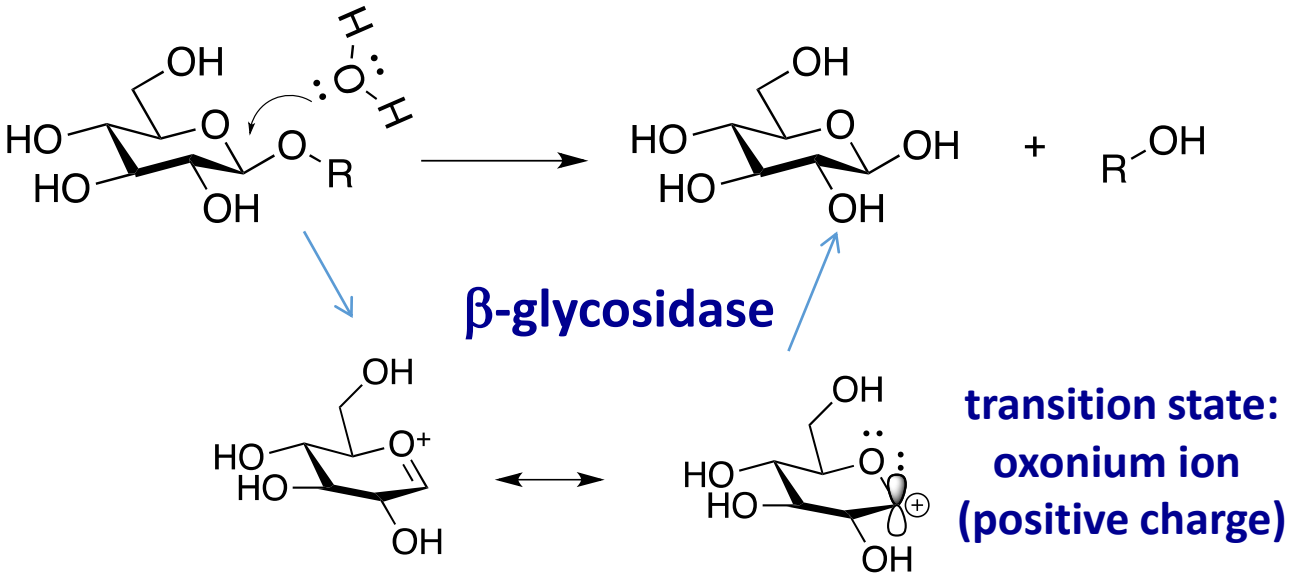
D



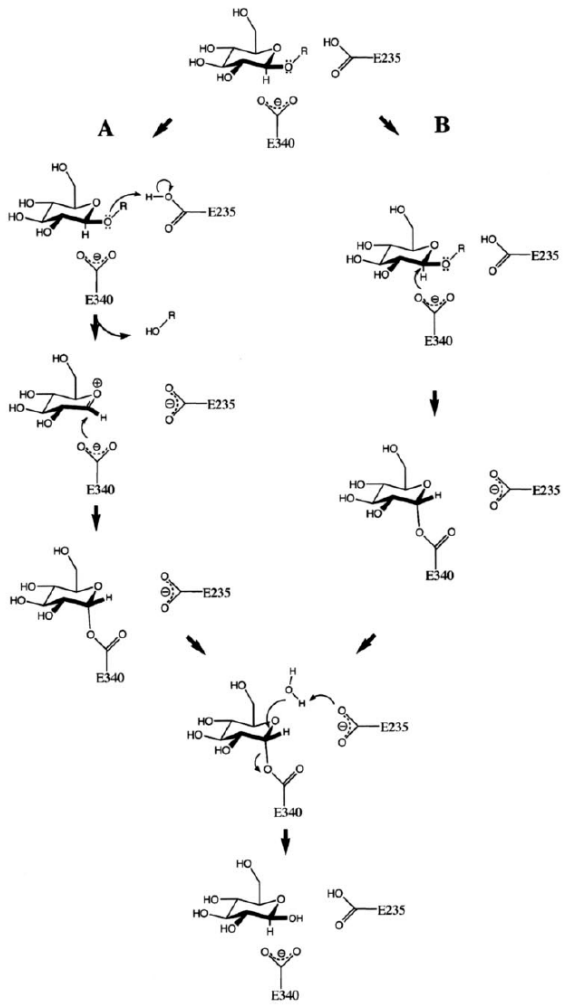
Azasugars bind specifically to the active site of GALC



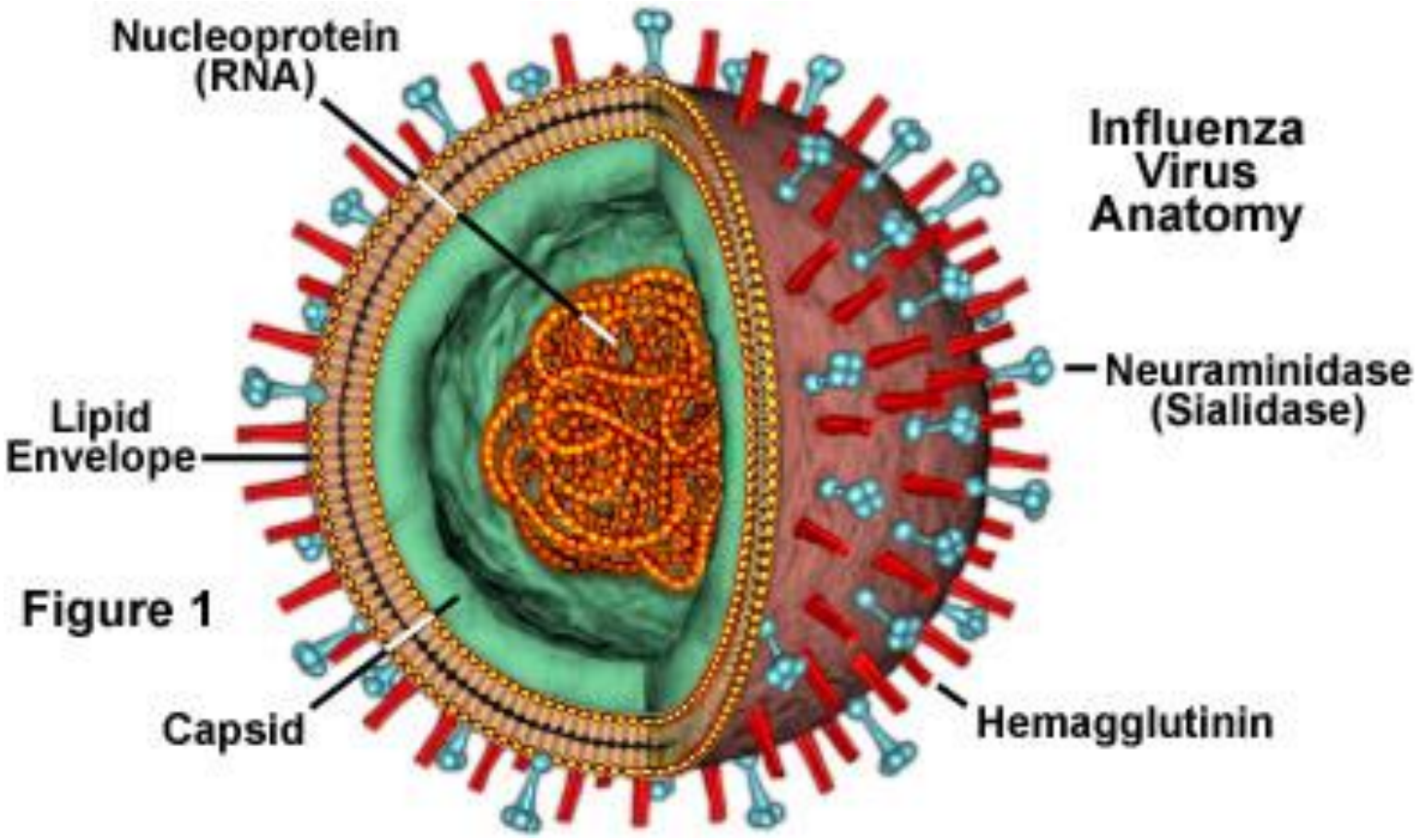
glycosidases inhibitors or molecular chaperones



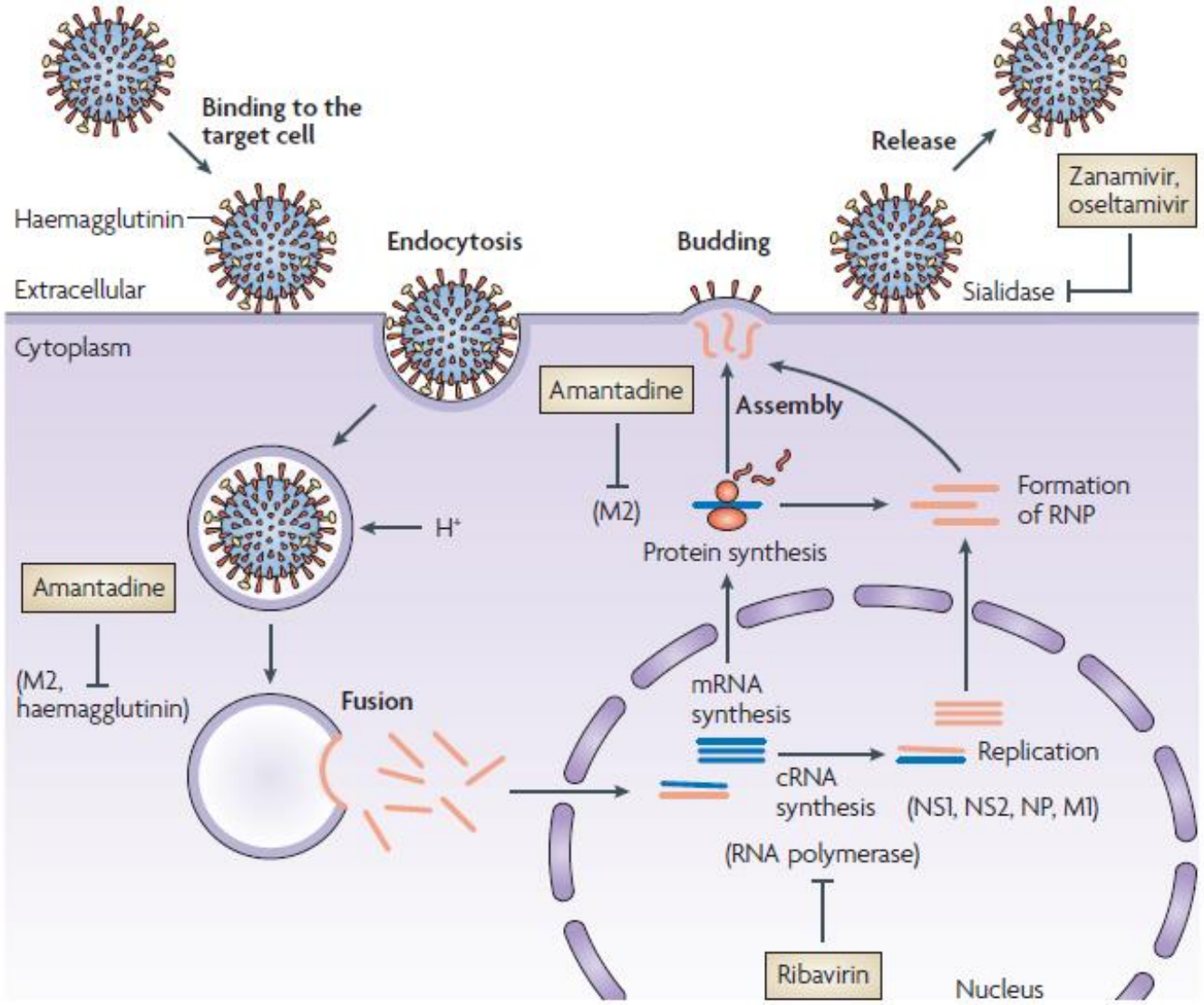
- The beta-glycosidase stabilizes the transition state positive charge by interaction with negatively charged amino acid residues (Asp, Glu)


A
B
E235
E340

SBDD: Sialidase or neuraminidase inhibitors: antiviral (anti-influenza) drugs



Sialic acid is an important anchor point on host cells for viruses adhesion, invasion, and budding



SBDD: Sialidase or neuraminidase inhibitors: antiviral (anti-influenza) drugs

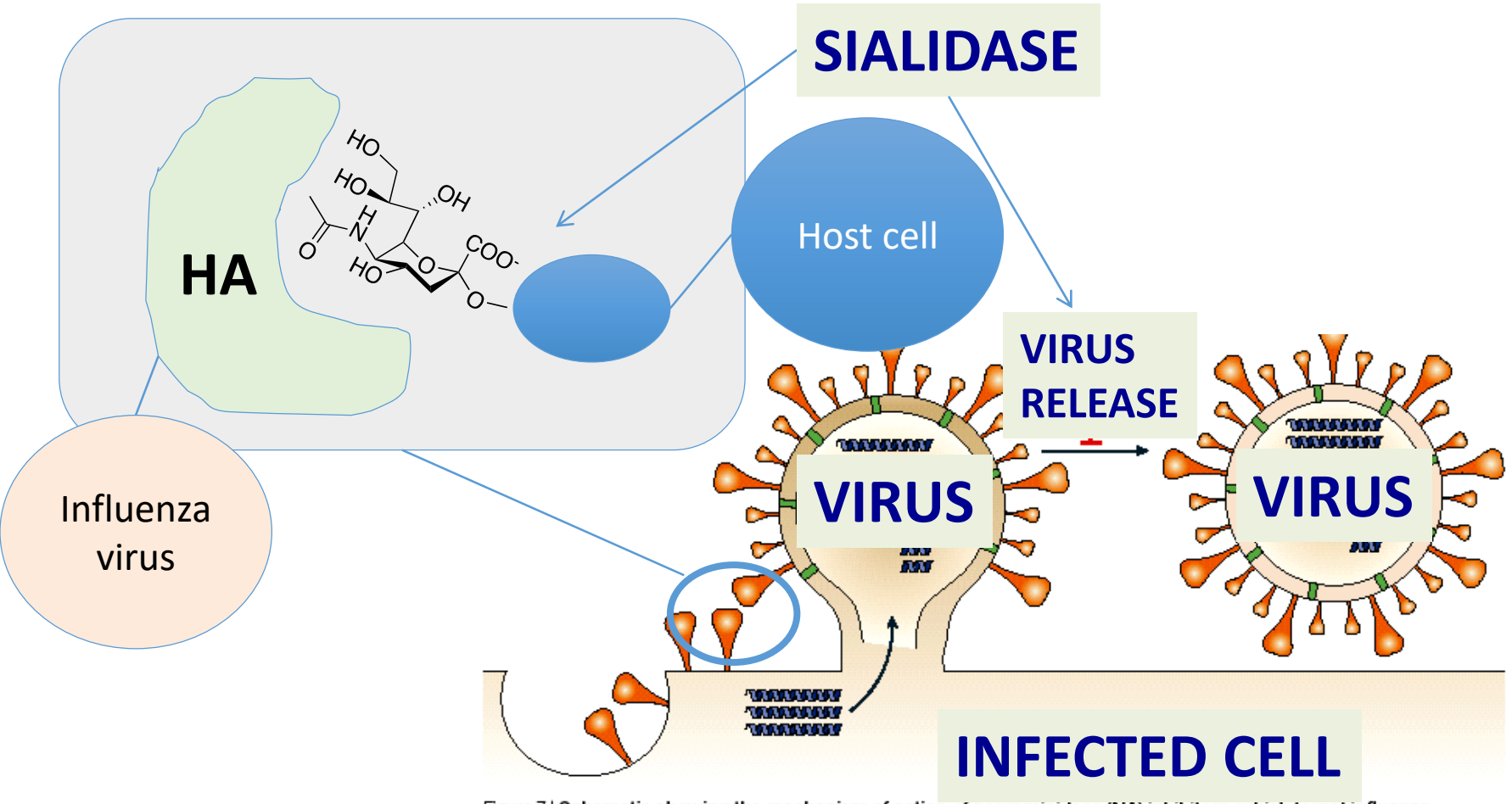
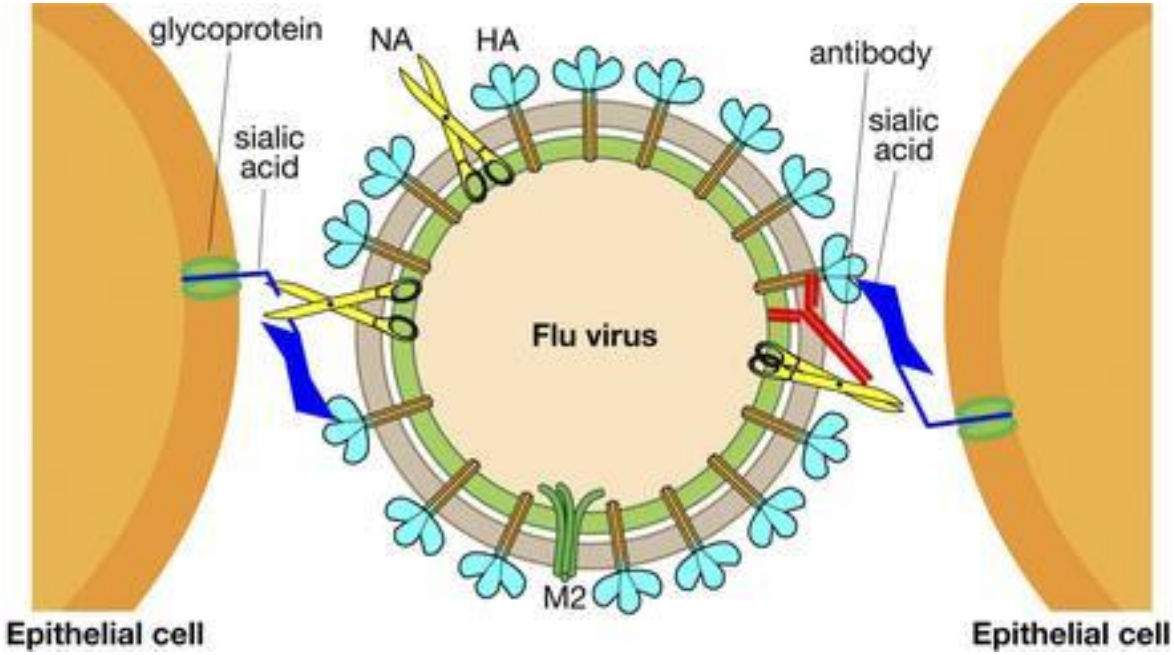
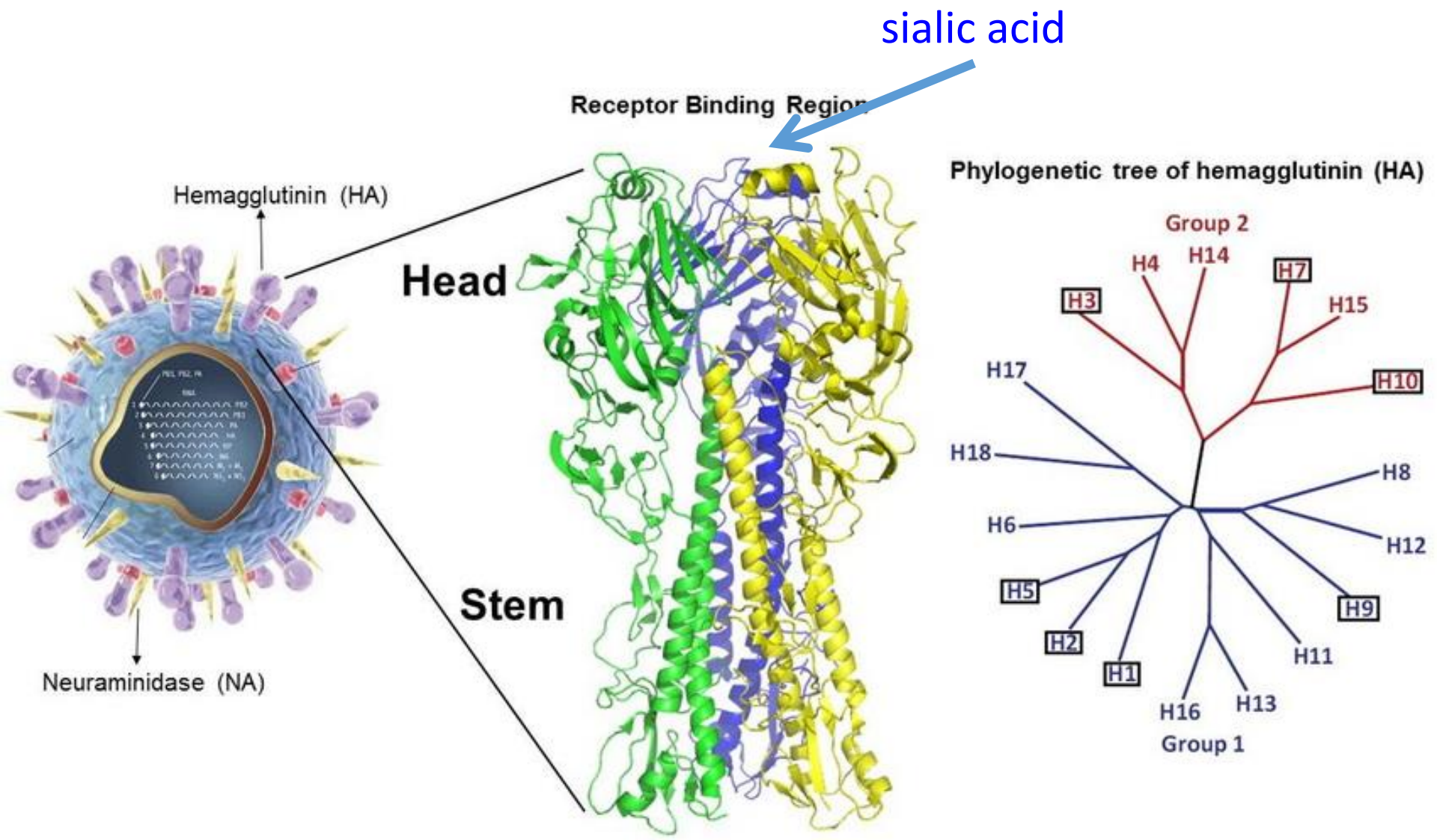


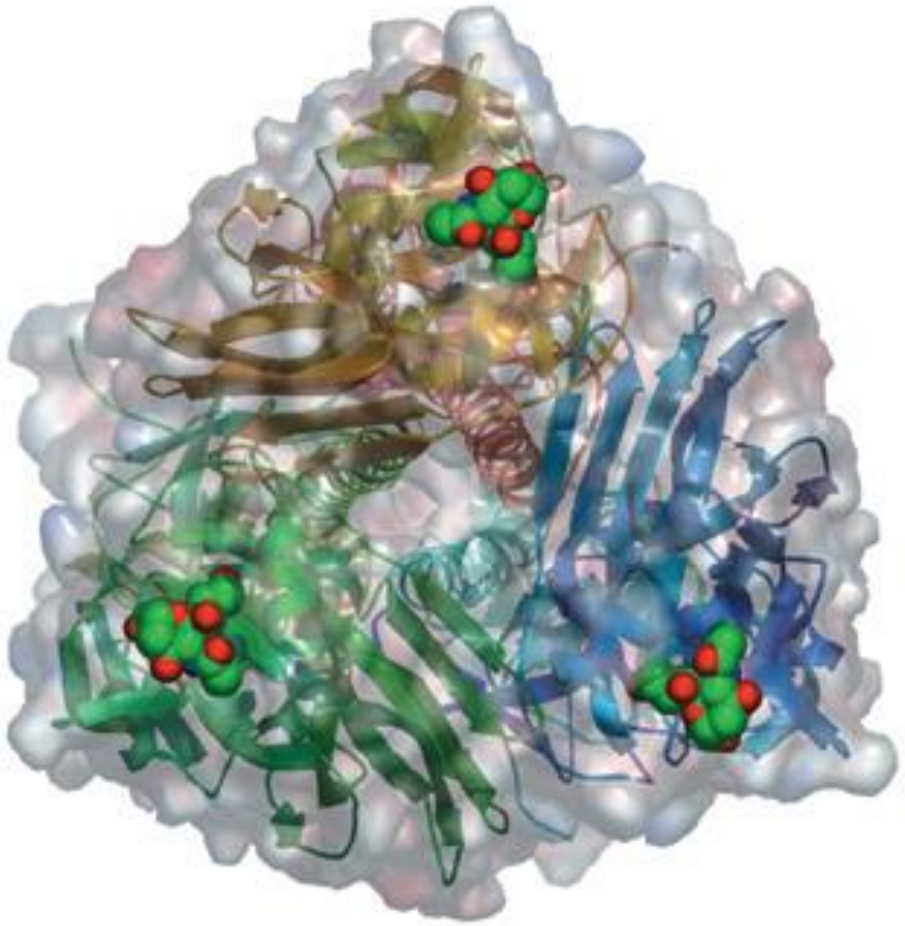
Figure 7 | **Schematic showing the mechanism of action of neuraminidase (NA) inhibitors, which target influenza viruses.** NA facilitates the release of virus particles from infected cells by cleaving a sialic acid residue from the cell-surface glycoprotein. By blocking this reaction, NA inhibitors prevent the release of virus.


glycoprotein
NA
HA
antibody
sialic acid
sialic acid
Flu virus
M2
Epithelial cell
Epithelial cell

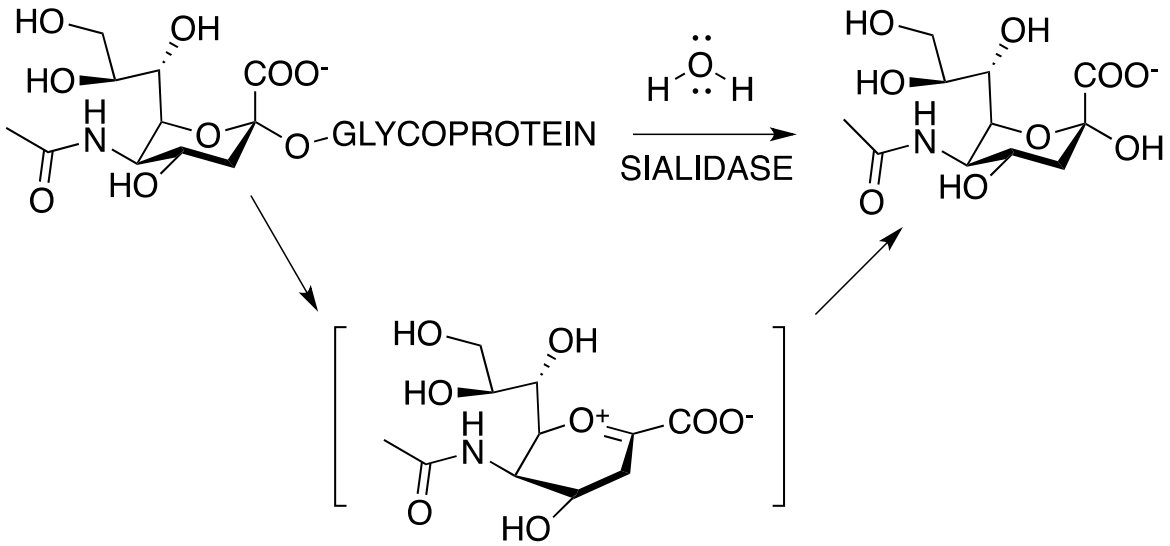
Neuraminidase (NA) and hemagglutinin (HA) on influenza virus capsid



A view of the influenza virus haemagglutinin trimer complexed with N-acetylneuraminic acid (Neu5Ac; in CPK form).

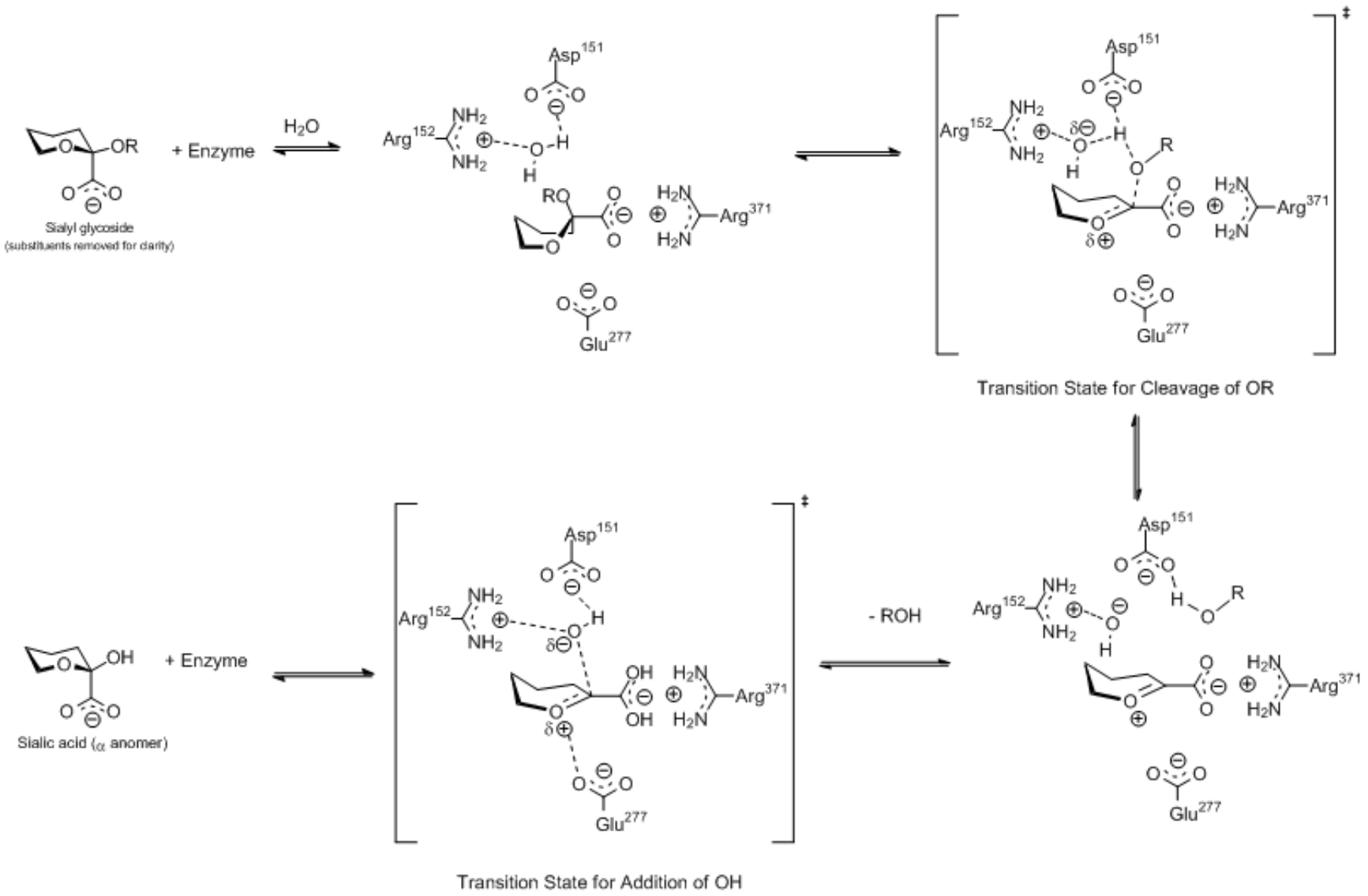


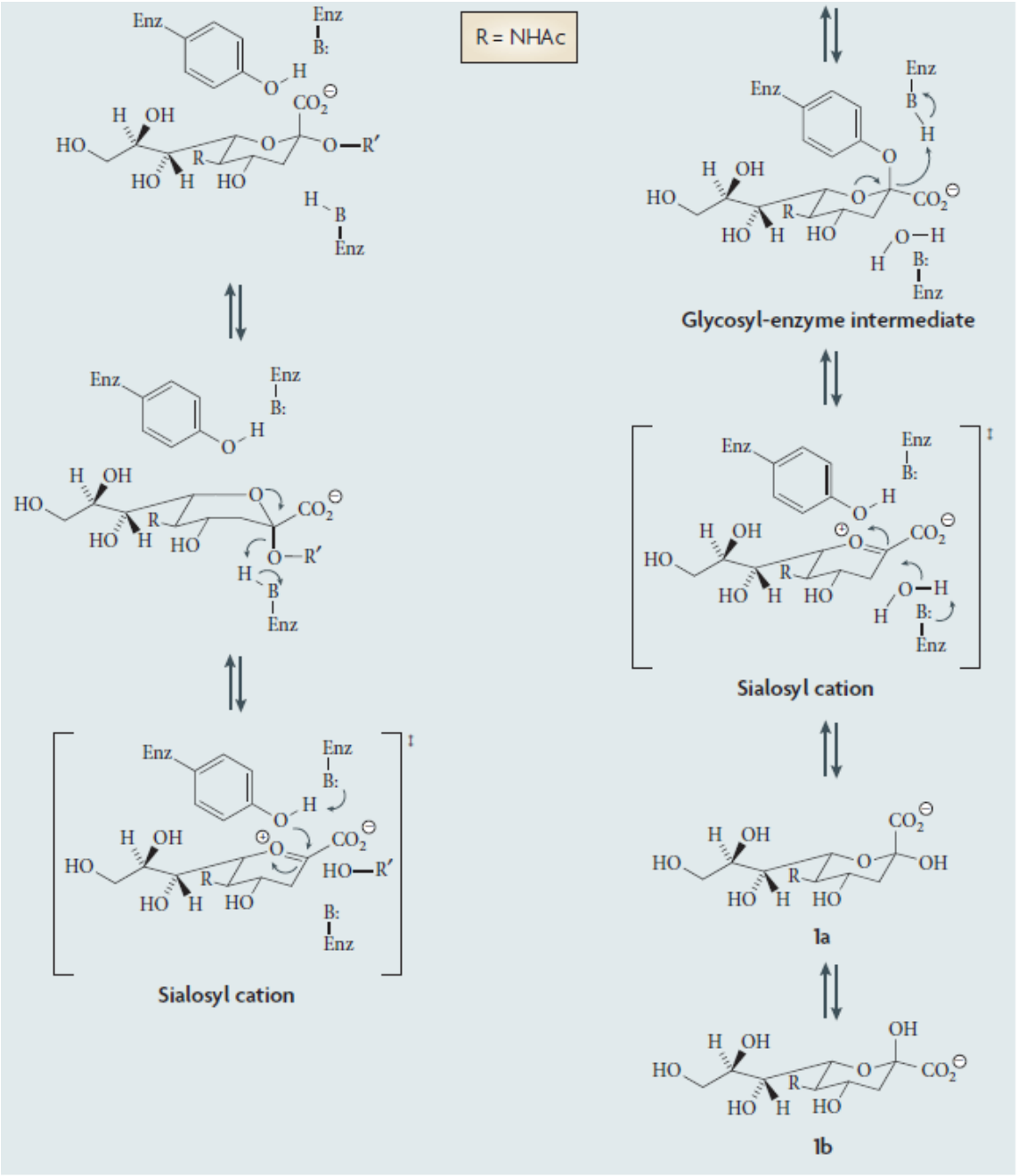
Mechanism of sialidase action



- NA facilitates the release of virus particles from infected cells by cleaving sialic acid (neuraminic acid) from cell- surface glycoproteins.
- Blocking this reaction, NA inhibitors prevent virus release

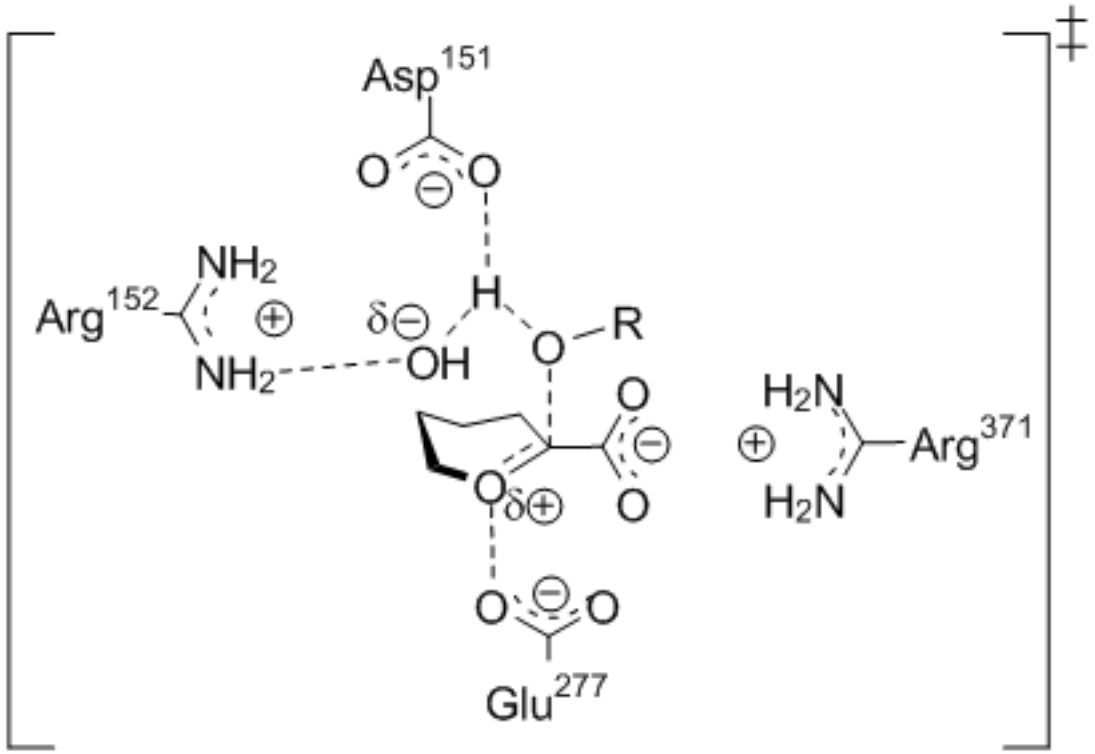
Mechanism of neuraminidase-catalyzed cleavage of sialic acid



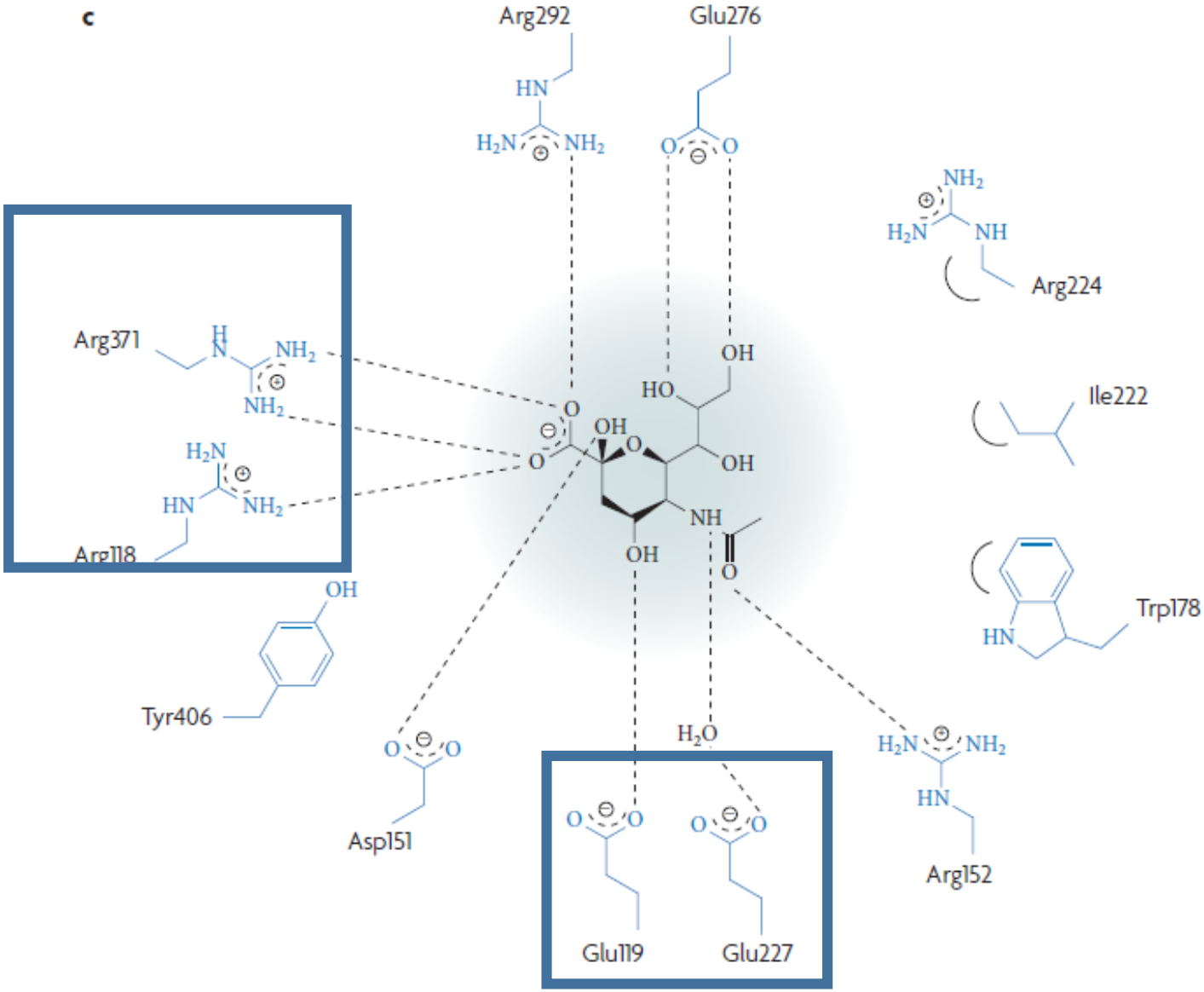


Participation of vicinal Tyr

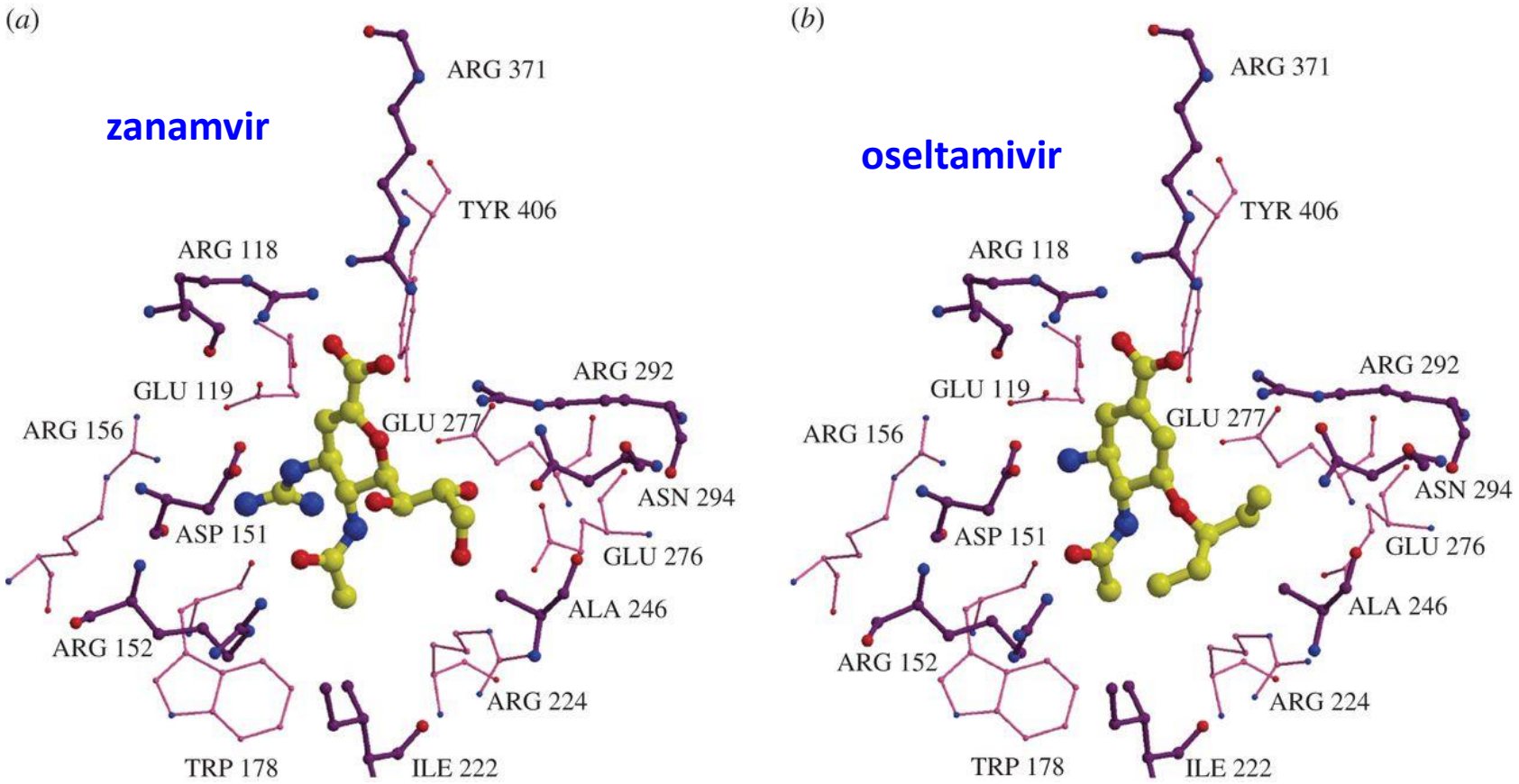
Transition state



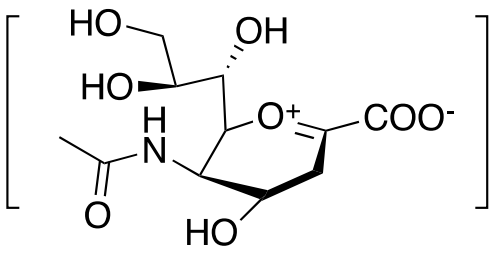
Proposed transition state for hydrolysis of glycosidic bond to sialic acid catalyzed by neuraminidase. The sugar has been simplified for clarity.


c
Arg292
Glu276
Arg224
Arg371
Ile222
Arg118
Trp178
Tyr406
Asp151
Arg152
Glu119
Glu227

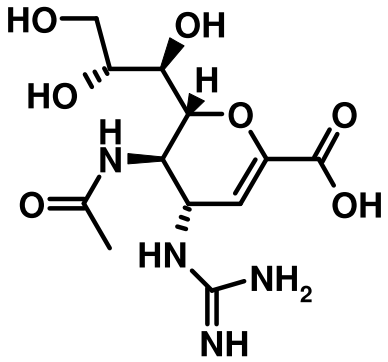
zanamvir and oseltamivir into Neuraminidase active site



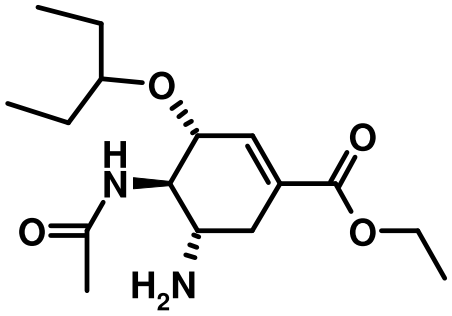
sialidase is a glycosidase: the transition state is an oxonium ion that is mimicked by antiviral drugs zanamavir (Relenza) and Oseltamivir



oxonium ion TT



Zanamavir

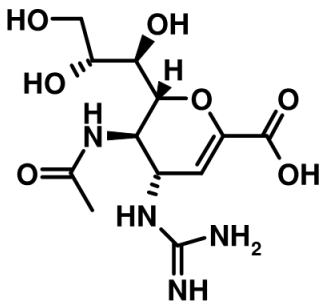


Oseltamivir

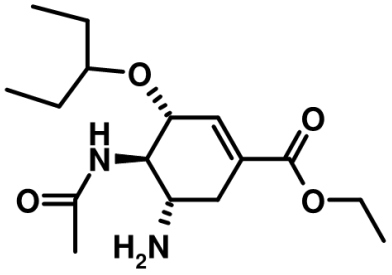
Transition state analogues

Zanamavir: too polar, spray
Oseltamivir (Tamiflu) orally active

- Hydrophobic, apolar, logP > 0
- Hydrophilic, polar, logP < 0
- Intermediate polarity, logP about 0



Zanamavir

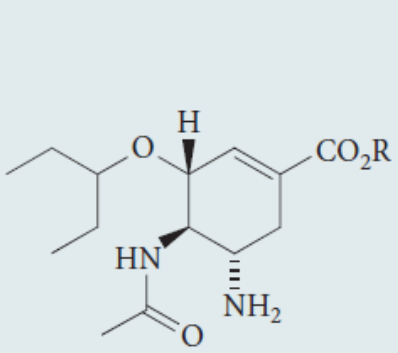


Oseltamivir

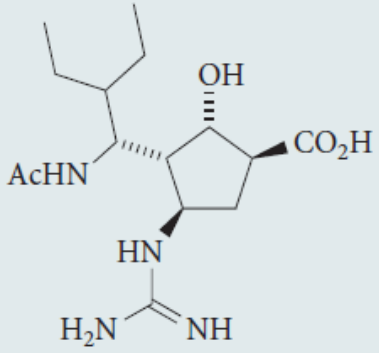
Log P = -3

Log P = 1.16

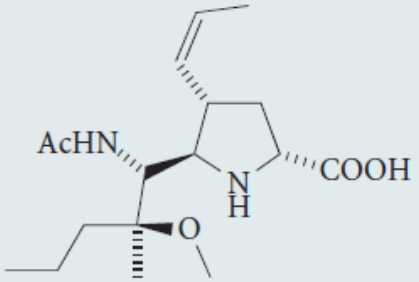
Replacing the carbohydrate core with other cycles



8 R = H GS4071
11 R = Et GS4104, Oseltamivir



9 Peramivir, BCX-1812



10

A range of core templates was used, including cyclohexenes such as oseltamivir carboxylate (compound 8, FIG. 3; originally known as GS 4071)[31]; cyclopentanes such as peramivir[53] (compound 9, FIG. 3); and pyrrolidines such as A-315675 (compound
10, FIG. 3