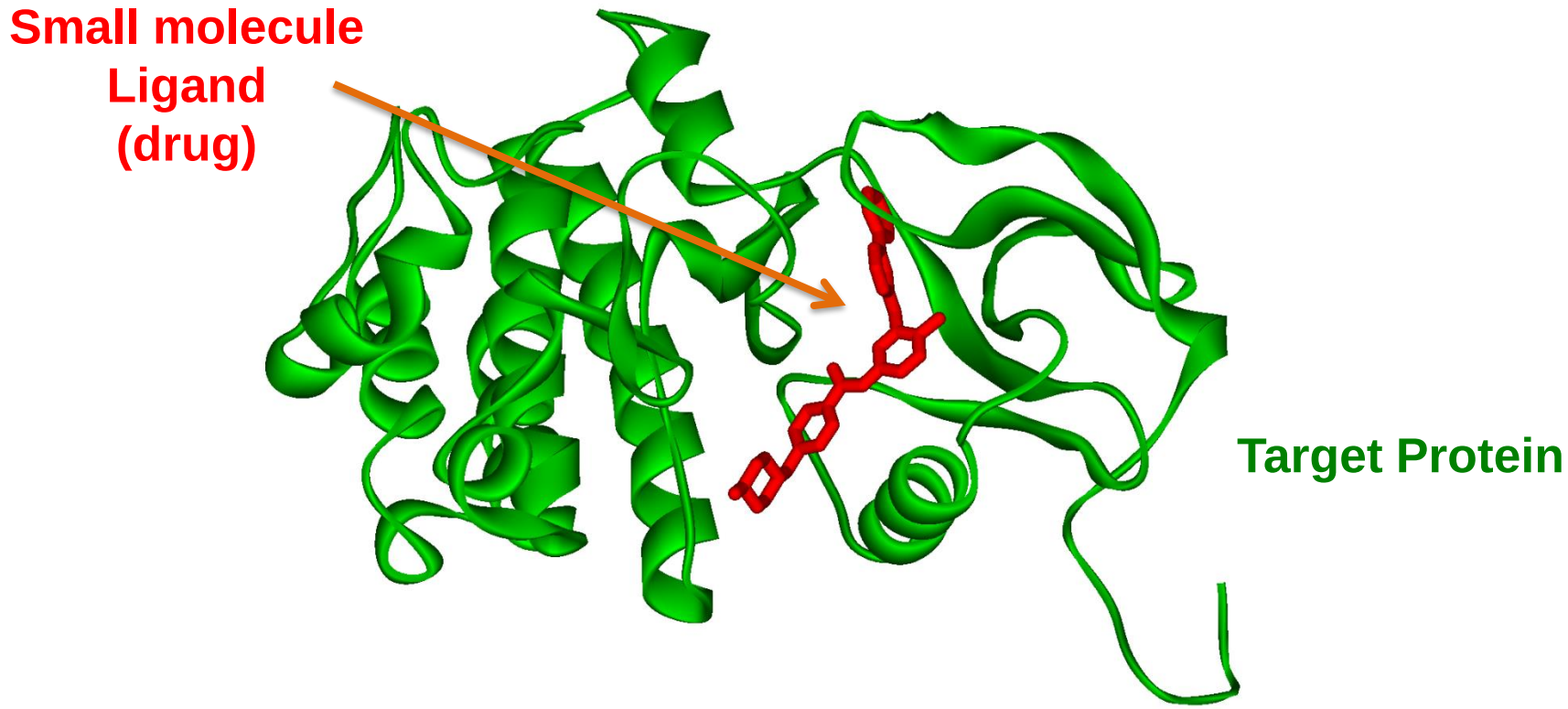
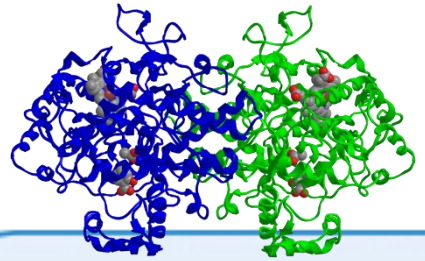
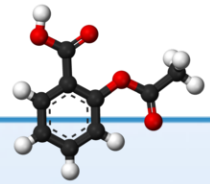


- There are two major types of drug design. The first is referred to as **ligand-based drug design** and the second, **structure-based drug design**.



Target identification



CADD

Structure-based CADD

Target structure

Ligand docking

De novo design

MD

Pharmacophore modeling

Ligand-based CADD

Ligand structure information

QSAR

Pharmacophore modeling

Ligand based virtual screening

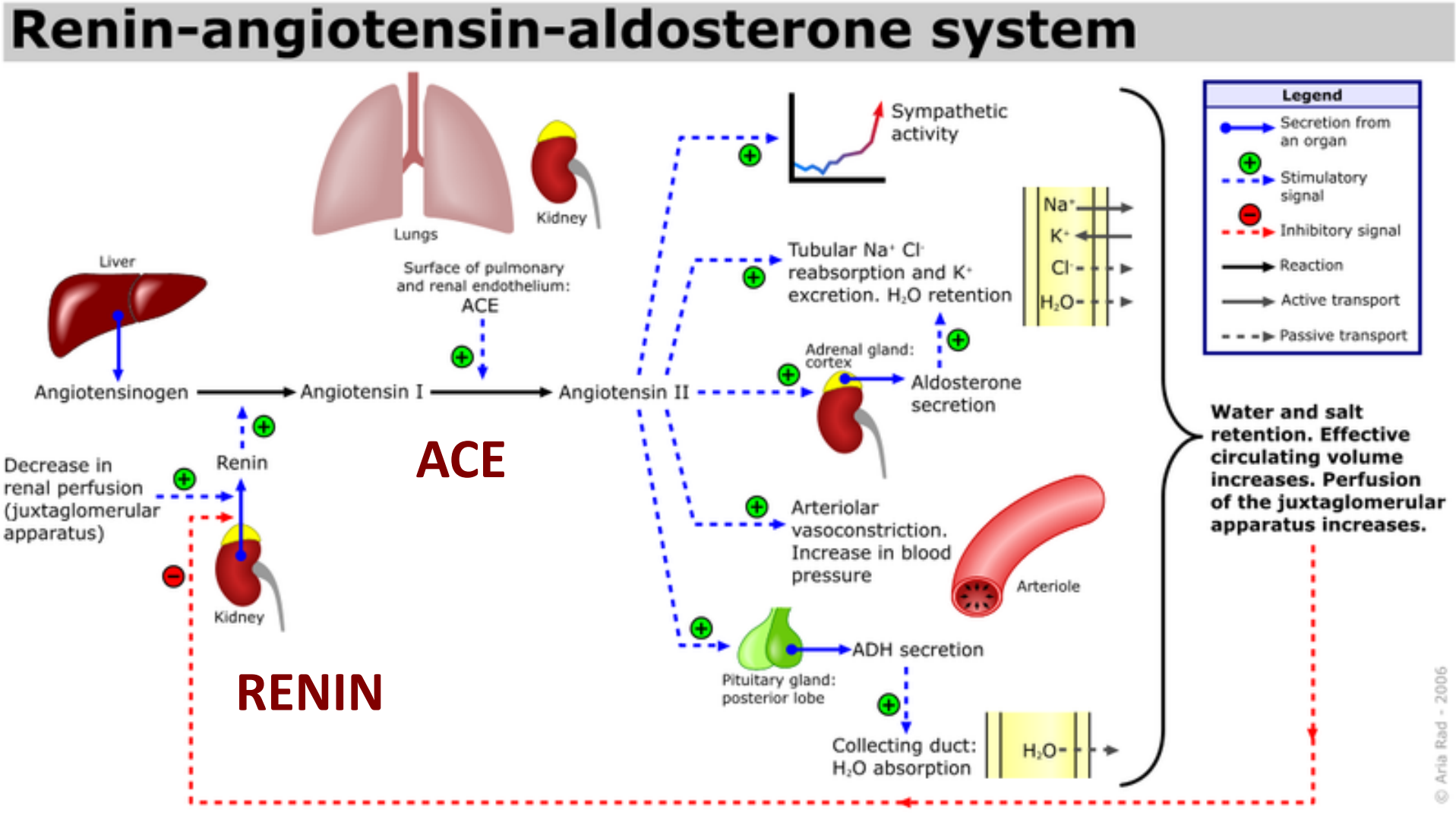
Lead optimization

Drug candidate

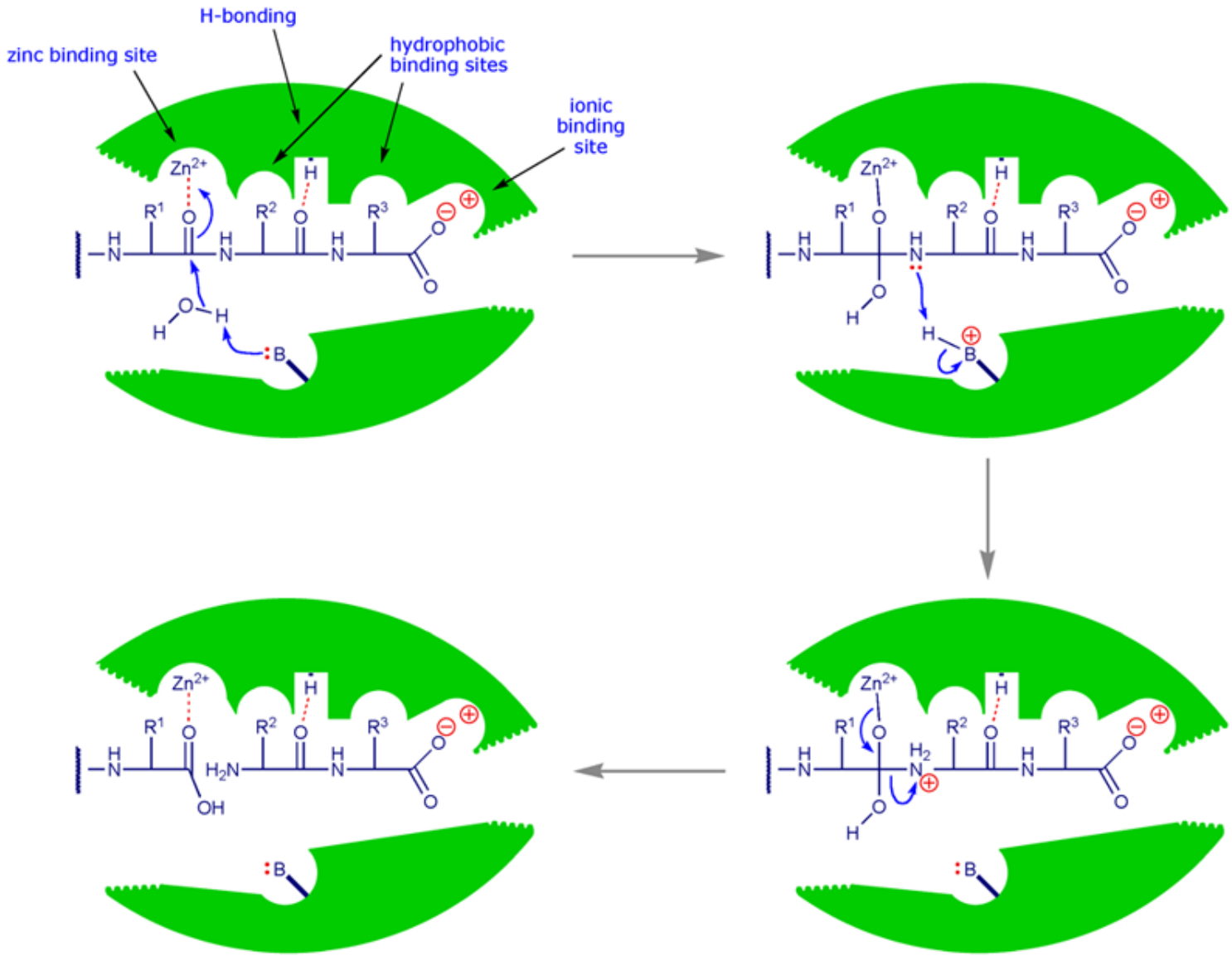
Ligand-based (indirect) DD

- Ligand-based drug design (or indirect drug design) relies on knowledge of molecules that bind to the biological target of interest.
- These molecules may be used to derive a pharmacophore model which defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.
- In other words, a **model of the biological target** may be built based on the knowledge of **what binds to it** and this model in turn may be used to design new molecular entities that interact with the target.

Inhibitors of the Angiotensin Converting Enzyme (ACE): THE RENIN-ANGIOTENSIN SYSTEM (RAS)



- Angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE), which removes a further two residues, and is found in the pulmonary circulation, as well as in the endothelium of many blood vessels.
- Angiotensin II increases blood pressure by increasing the amount of salt and water the body retains, although angiotensin is also very good at causing the blood vessels to tighten (a potent vasoconstrictor).
- Molecules inhibiting ACE are therefore anti-hypertensive drugs


H-bonding
zinc binding site
hydrophobic binding sites
ionic binding site
Zn^{2+}
R^1
R^2
R^3
H
O
N
B
H_2N
OH

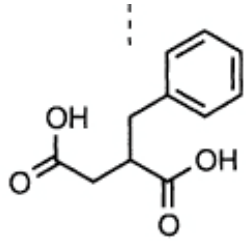
- **ACE (Angiotensin Converting Enzyme) inhibitors**
- **ACE structure was not known when captopril was developed: the structure of peptide teprotide from Brazilian viper's venom that blocked ACE was known.**

Between 1970 and 1973, Squibb scientists randomly tested about 2,000 chemical structures for ACE inhibitor activity4 but could not find what they wanted.

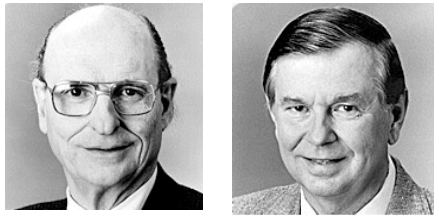
Their luck changed on Wednesday, 13 March 1974, when they decided to follow up some newly published research on an inhibitor of carboxypeptidase A — an exopeptidase thought to have a similar active site to ACE.

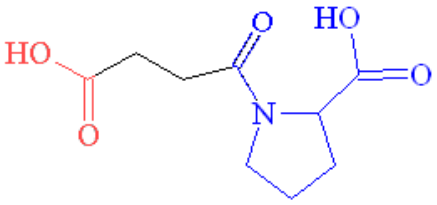
Then, 60 compounds and 18 months later, they ad captopril, and early clinical studies confirmed its antihypertensive effects.

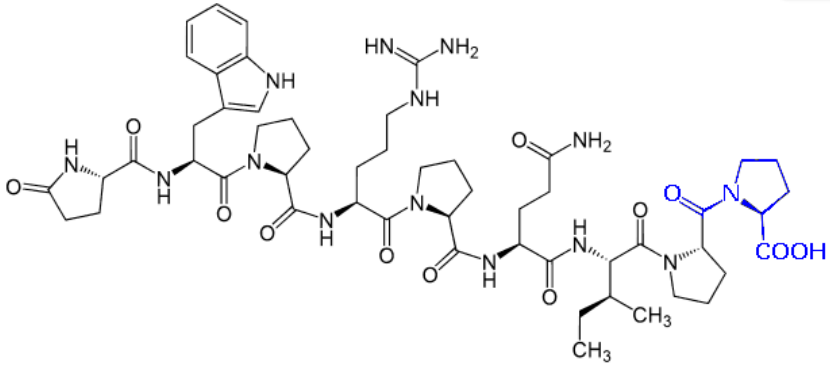
M. Ondetti and D. Cushman

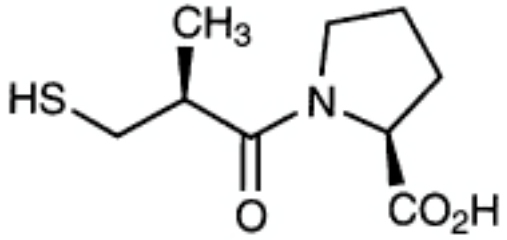


Acido L-benzilsuccinico









Captopril

Due to the critical role of ACE in cardiovascular and renal diseases, it has been an attractive target for drug design.

The development of captopril, the first marketed orally active ACE inhibitor approved for treatment of human hypertension, was accomplished in 1981 by Cushman and Ondetti and coworkers based on three key features of the enzyme.

1) First, ACE was thought to be a carboxypeptidase that

catalyzed the hydrolysis of dipeptides from the C-terminus

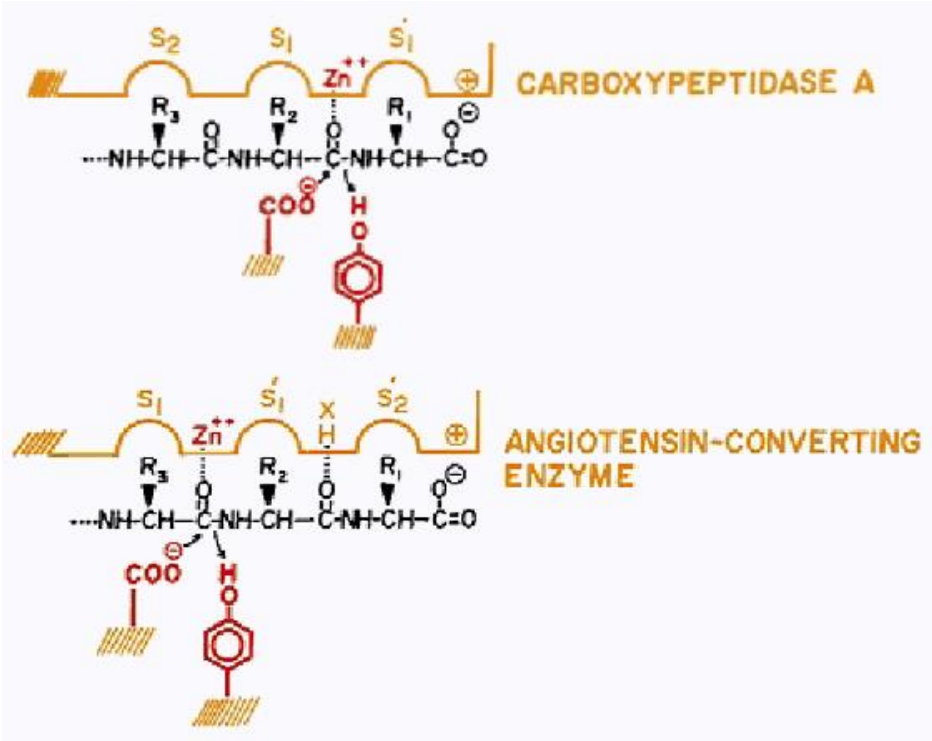
of oligopeptides.

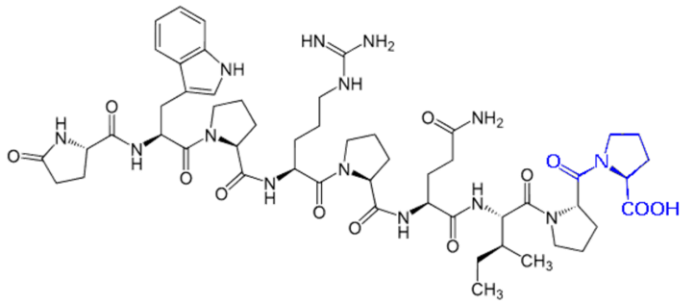
2) In addition, it was inhibited by chelating agents and could be reactivated by zinc and other divalent cations.

In this regard it resembled the much better characterized enzyme carboxypeptidase A from bovine pancreas (an enzyme found in the digestive system of all mammals), whose sequence and 3D structure were elucidated in 1967. Cushman and Ondetti and coworkers (10-12) speculated that these two metallopeptidases shared a similar catalytic mechanism.

3) Third, the sequences of the bradykinin-potentiating peptides pointed to acyl prolines as likely drug candidates.

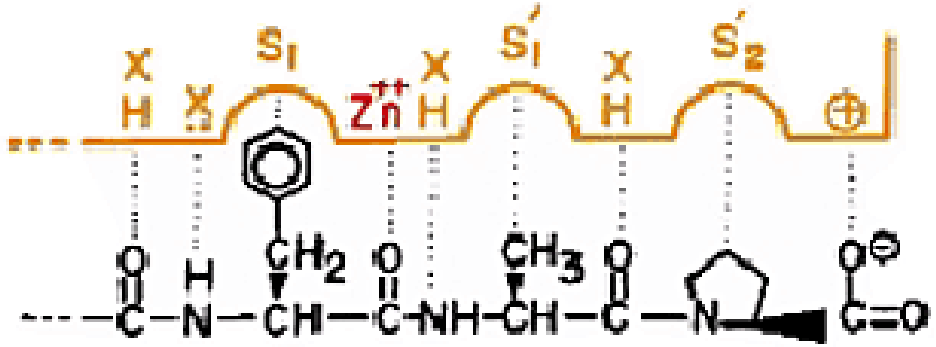
Succinyl proline as first hit compound from LBDD based on teprotide (Pro-rich peptide) and L-benzylsuccinic acid



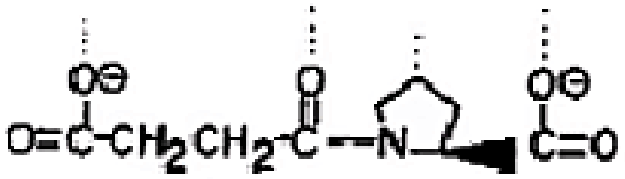


teprotide

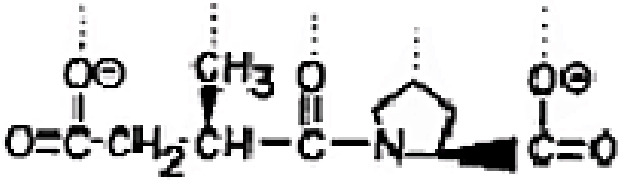
- teprotide (SQ 20,881), which showed the greatest ACE inhibition potency and hypotensive effect in vivo.
- Teprotide had limited clinical value due to its peptide nature and lack of activity when given orally.



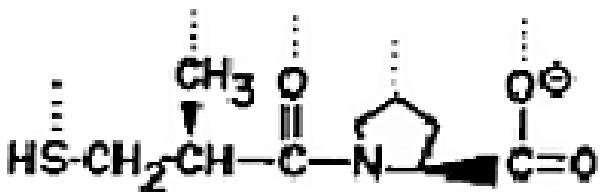
VENOM PEPTIDE ANALOG



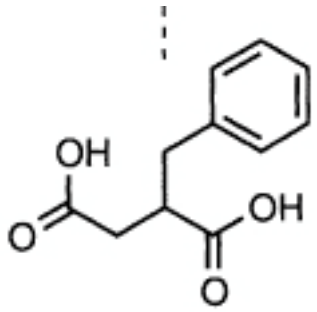
SUCCINYL-Pro



D-2-METHYLSUCCINYL-Pro



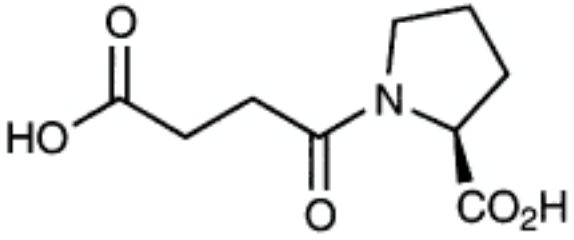
CAPTOPRIL



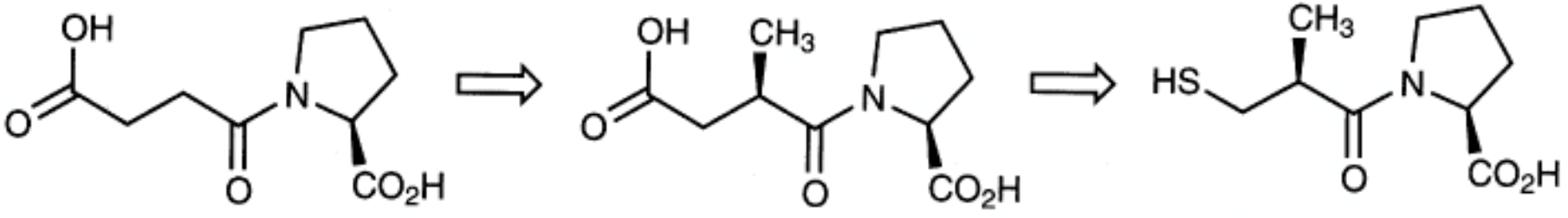
Acido L-benzilsuccinico

Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro

Teprotide

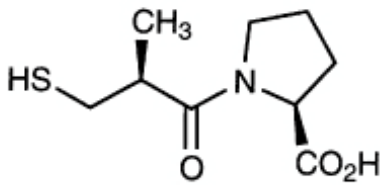


Succinil prolina

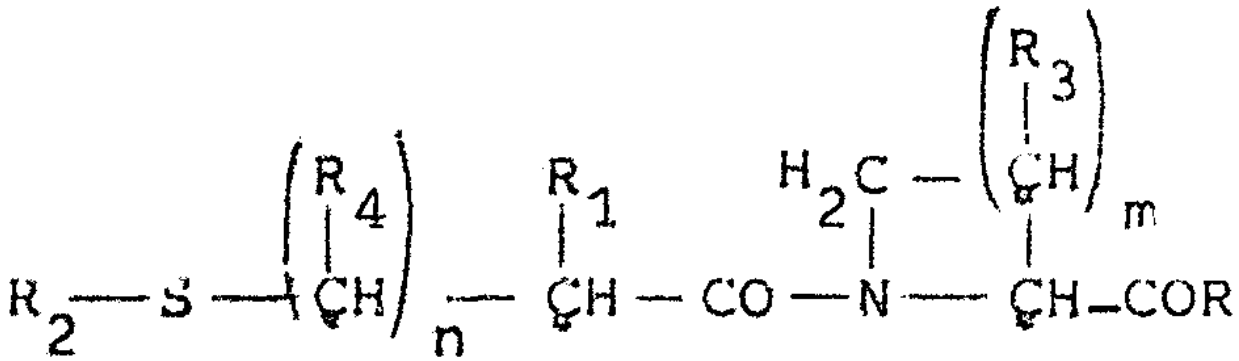


Captopril

Patent captopril (1977) inventors: Ondetti and Cushman



This invention relates to new proline derivatives and related compounds which have the general formula



Captopril structure extension allowed higher target affinity/specificity

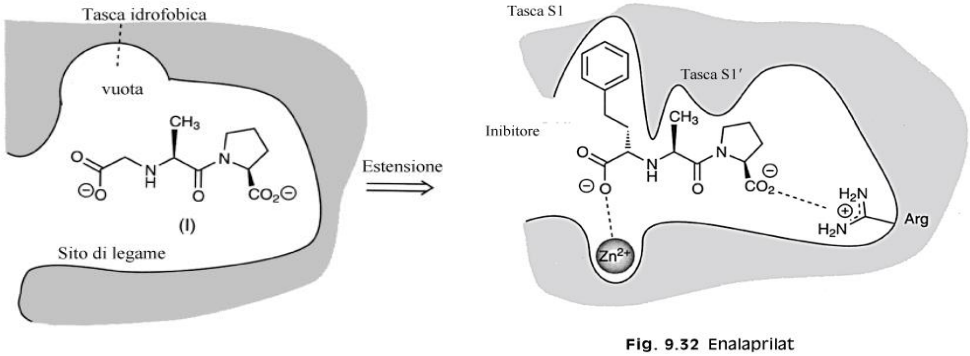
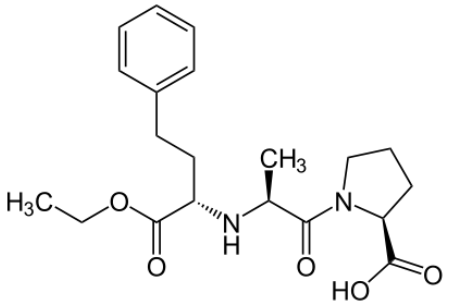
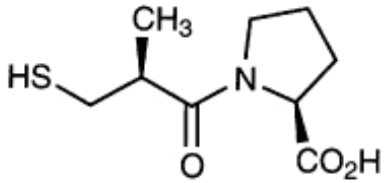


Fig. 9.32 Enalaprilat

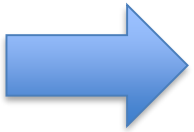


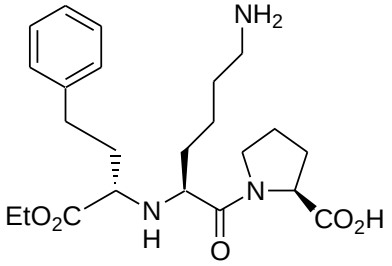
enalapril

me too
me better strategy

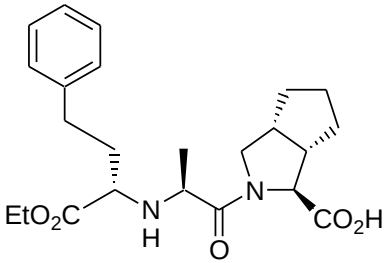


Captopril

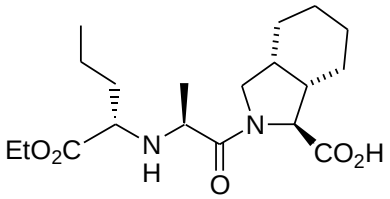




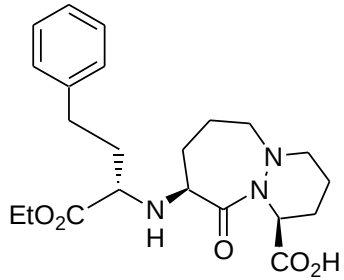
Lisinopril (Merck)



Ramipril (Hoechst)-D)



Peridopril (Servier-FR)



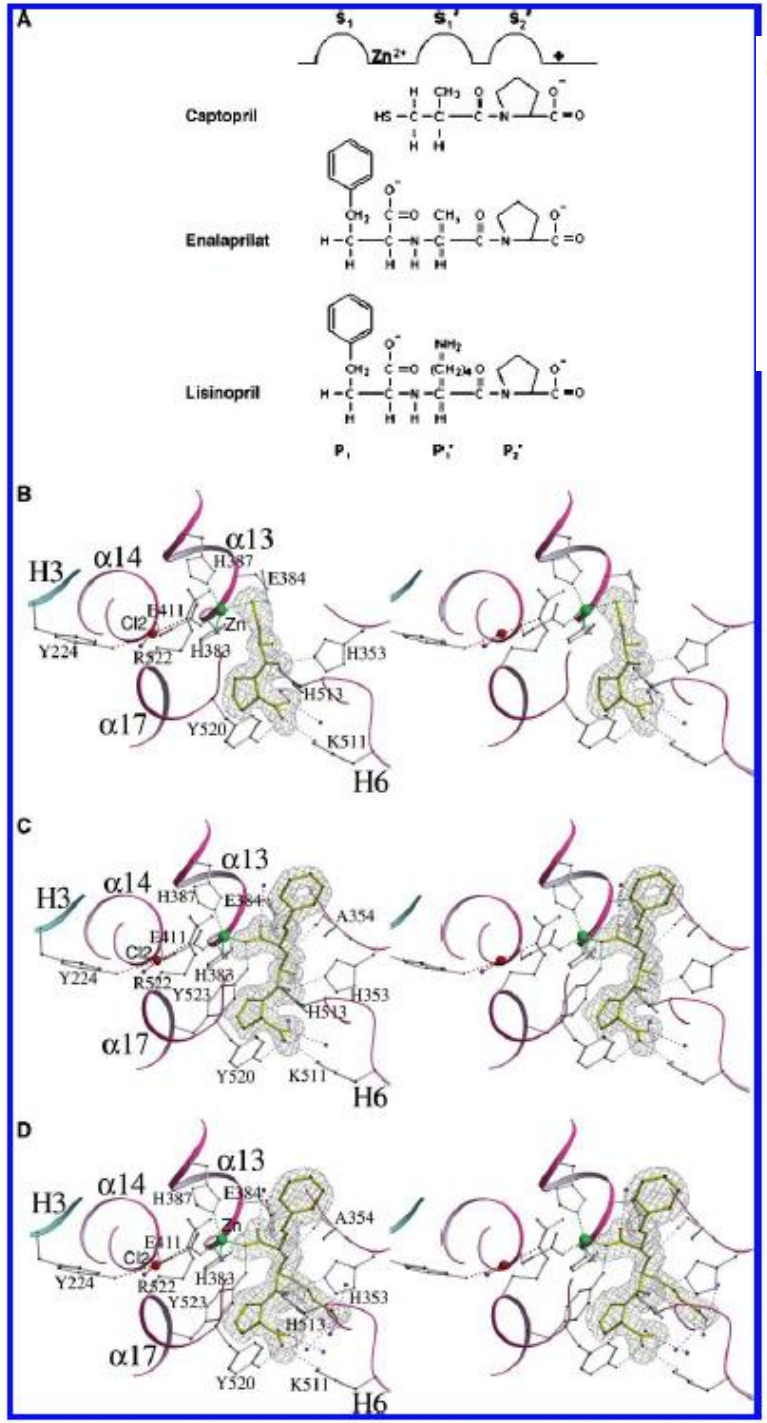
cilazipril (Hoffmann-La Roche-UK)

Other ACE inhibitors

In parallel, another approach to ACE inhibitors used rational drug design based on the inhibition of a different zinc proteinase, thermolysin.

Two N-carboxyalkyl dipeptides emerged from this search, enalapril and lisinopril, which were approved for marketing in 1985 and 1987, respectively.

Over the years many more active ACE inhibitors were developed by the pharmaceutical industry, more or less empirically, and 17 are approved for use. Collectively, they serve as the first line of approach to the treatment of hypertension.



emical structure of ACE inhibitors captopril, enalaprilat, and lisinopril. (B–D) Stereo representations o

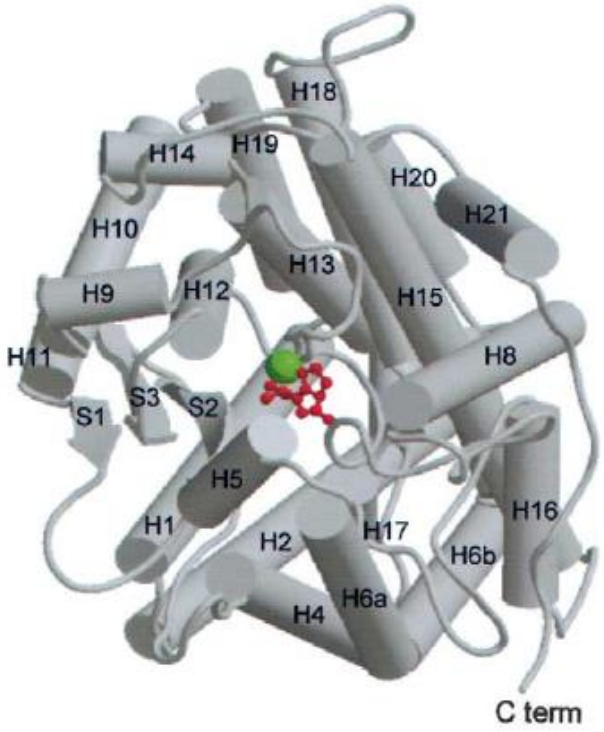
Structural Details on the Binding of Antihypertensive Drugs Captopril and Enalaprilat to Human Testicular Angiotensin I-Converting Enzyme†,‡

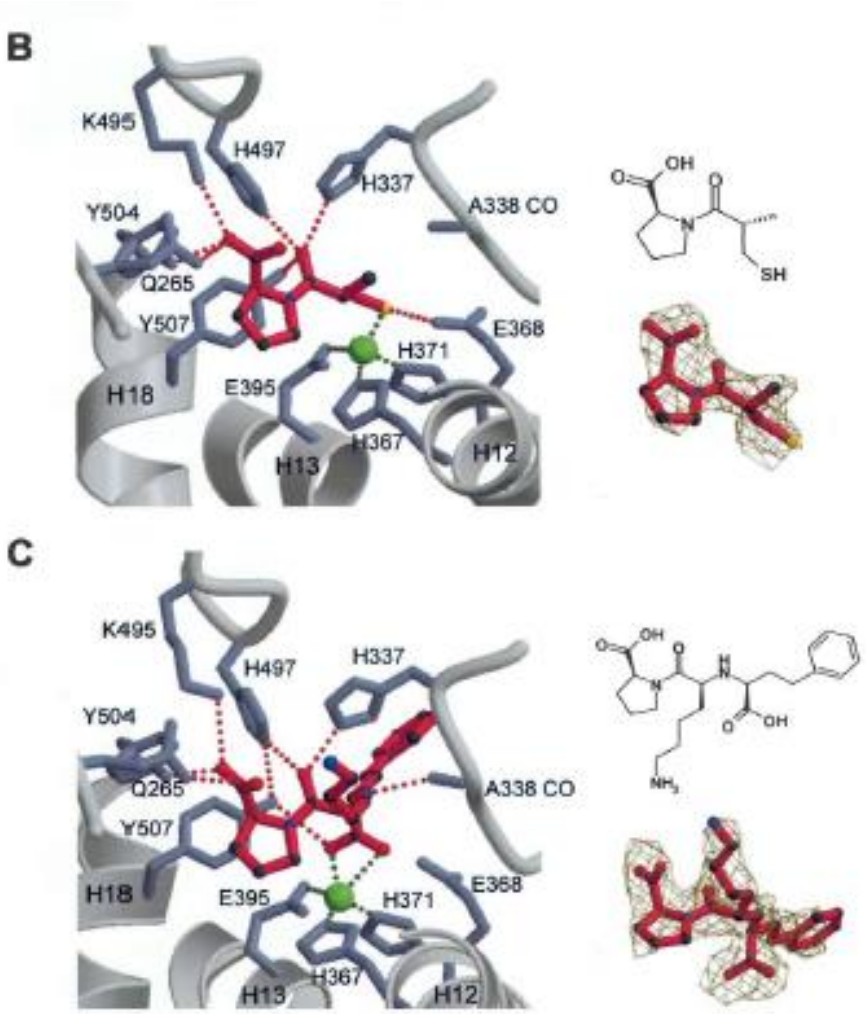
Ramanathan Natesh,§,|| Sylva L. U. Schwager,⊥ Hazel R. Evans,§ Edward D. Sturrock,*,⊥ and K. Ravi Acharya*,§

Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom, and Division of Medical Biochemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory 7925, South Africa

Received March 16, 2004; Revised Manuscript Received May 10, 2004

captopril and lysinopril into ACE active site



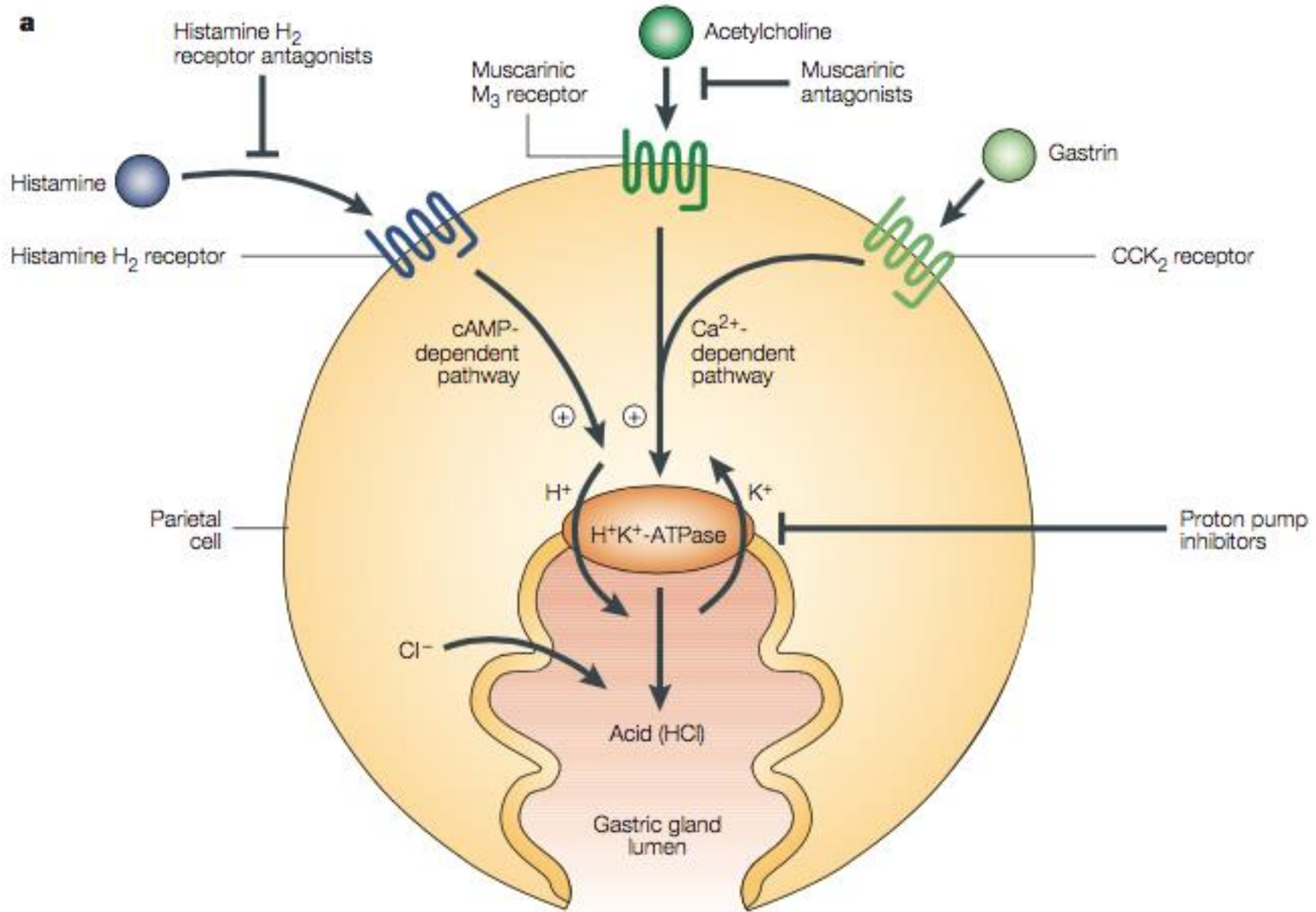


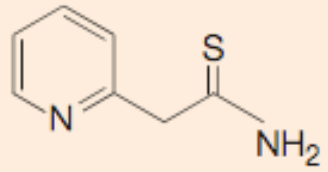
A PROTON-PUMP INHIBITOR EXPEDITION: THE CASE HISTORIES OF OMEPRAZOLE AND ESOMEPRAZOLE

Lars Olbe, Enar Carlsson and Per Lindberg

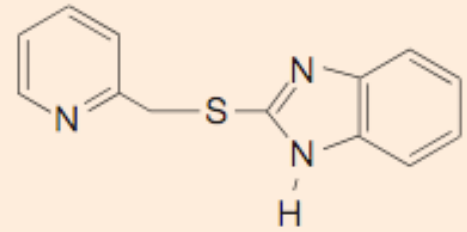
Thirty years ago, disorders associated with inappropriate levels of gastric acid were a major problem for which treatment options were limited, and approaches to the control of gastric acid secretion were thus the focus of considerable drug discovery efforts. Here, we summarize how one such programme led to the development of the proton-pump inhibitor omeprazole (Losec, Prilosec), a conceptually new drug that proved clinically superior to previous antisecretory drugs in the treatment of acid-related disorders, and which became the world's best-selling drug in the late 1990s. We then describe how the antisecretory and clinical effects were further improved by the development of esomeprazole (Nexium), a single enantiomer of omeprazole, which was launched in 2000.

Gli inibitori della pompa protonica (H^+-K^+ -ATPase): farmaci anti-ulcera gastrica

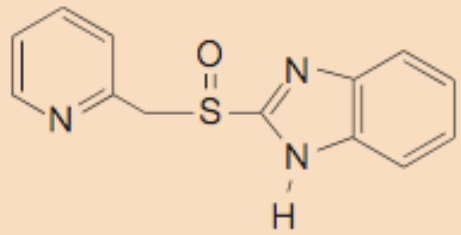




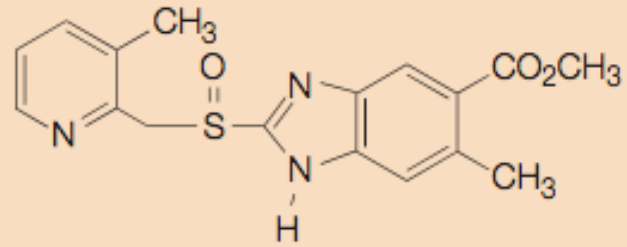
[CMN 131]



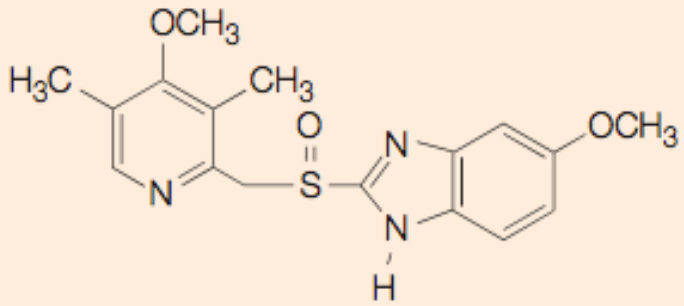
[H 124/26] (1973)



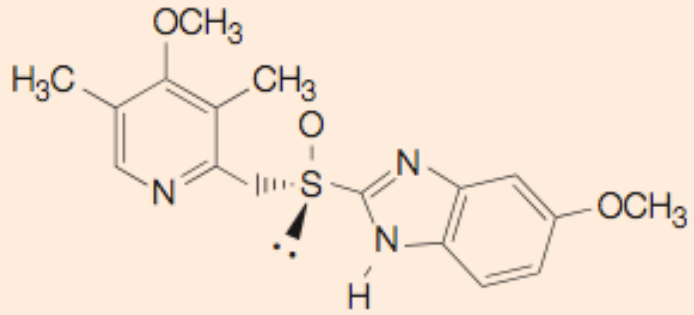
Timoprazole
[H 83/69] (1974)



Picoprazole
[H 149/94] (1976)



Omeprazole
[H 168/68] (1979)



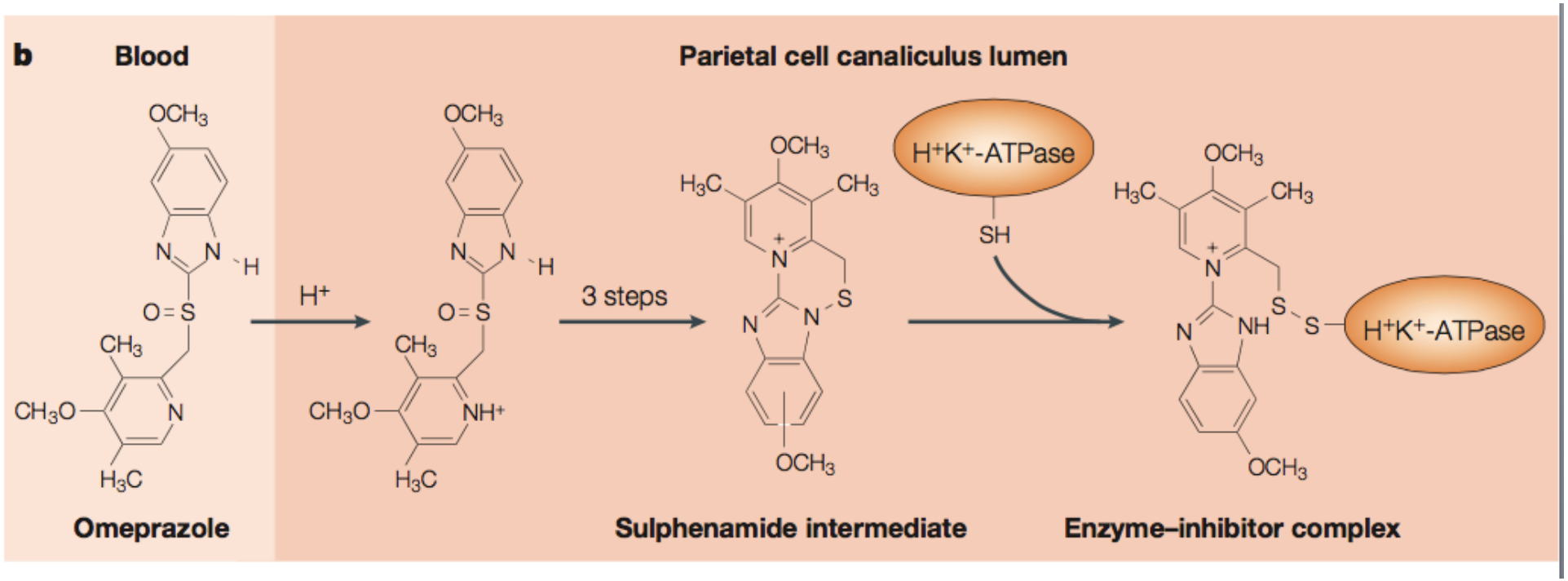
Esomeprazole
(1983)

Astra program start in 1969, the first SPECIFIC anti-acid drug omeprazole was marketed in 1988 (Losec in Europe, Prilosec in USA)

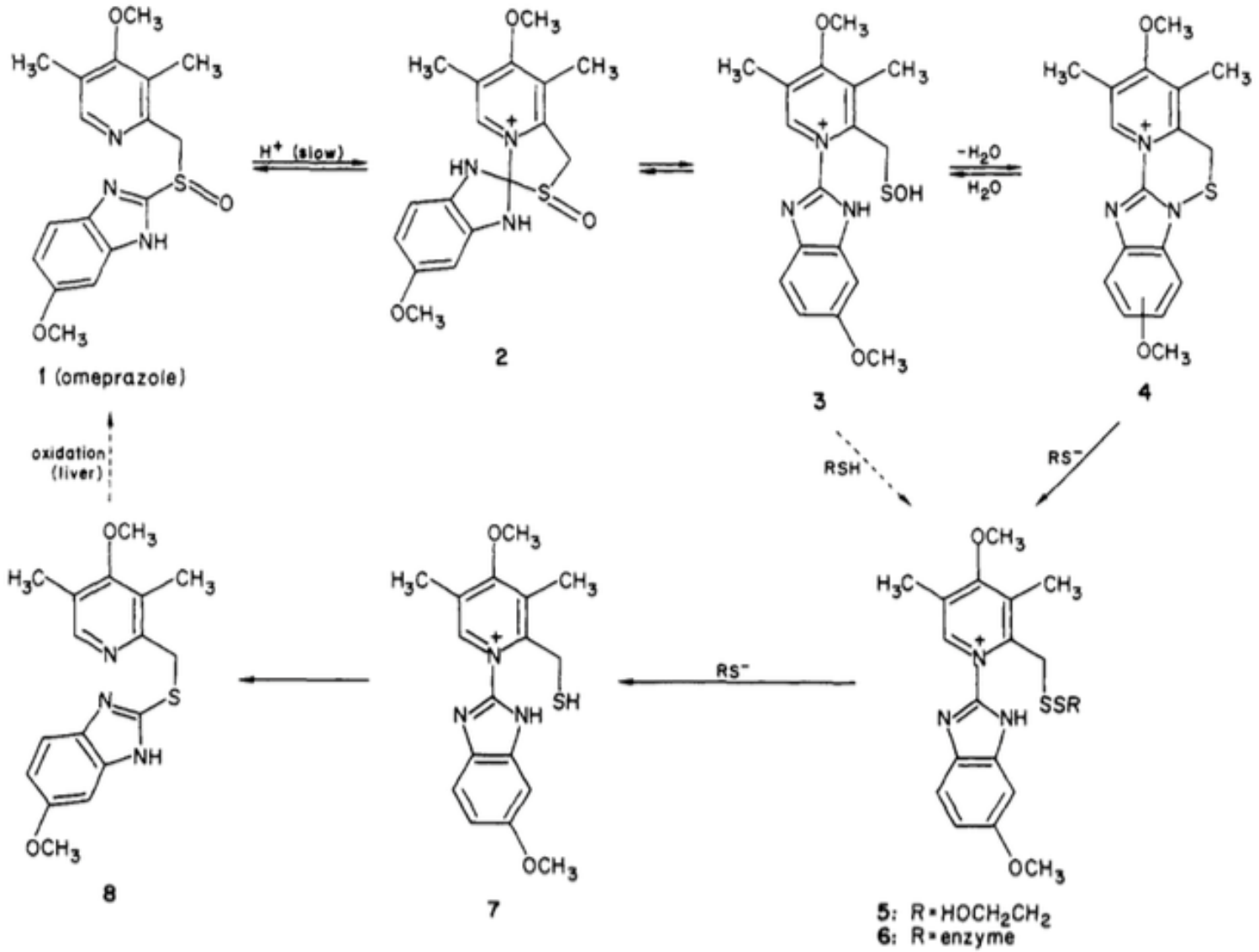
From Hit to Lead :

- CMN 131 active but toxic (toxicity problem)
- Isosteric replacement of thiourea with indole: H124/126
- This molecule was yet patented by an hungarian company as anti-tubercolosis (patent problem)
- A metabolite was more active: tiomoprazole (sulfoxide instead of sulfide)
- Hit to lead optimization: increase the basicity of pyridine nitrogen by introducing EDG on the aromatic ring, picoprazole and omeprazole (H168/68) (pharmacokinetic!)
- 1982: started clinical trials on omeprazole
- Study of the mechanism: omeprazole is a PRODRUG and acid-promoted conversion to the real active drug: the sulphenamide

mechanism of action: omeprazole (prodrug!) is activated by acids to the active sulphenamide



J Med Chem 1986



The S-enantiomer (esomeprazole) is the most active one

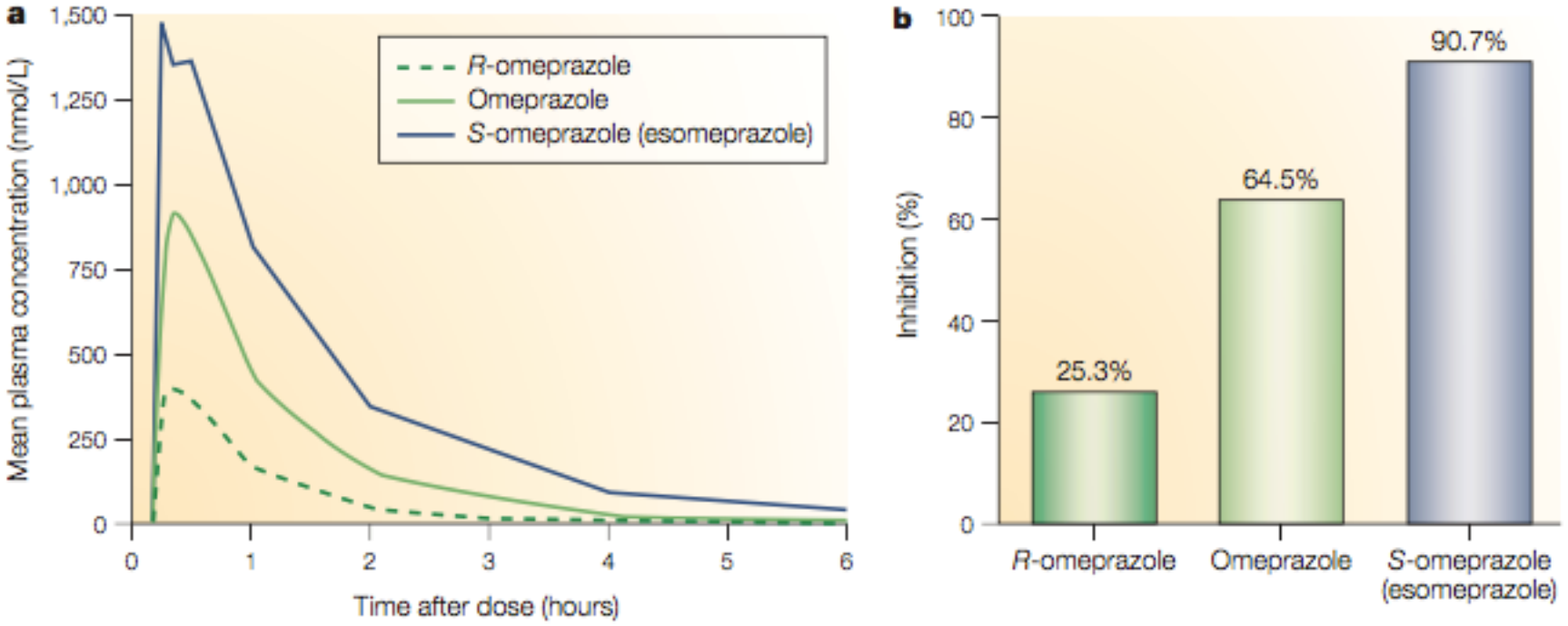


Figure 4 | **Effects of racemic omeprazole and its enantiomers. a** | Drug plasma concentrations and **b** | inhibition of pentagastrin-stimulated gastric acid secretion in healthy subjects ($n = 4$) after oral administration of 15 mg of *R*-omeprazole, omeprazole and esomeprazole at time 0 (REF. 42).

The synthesis

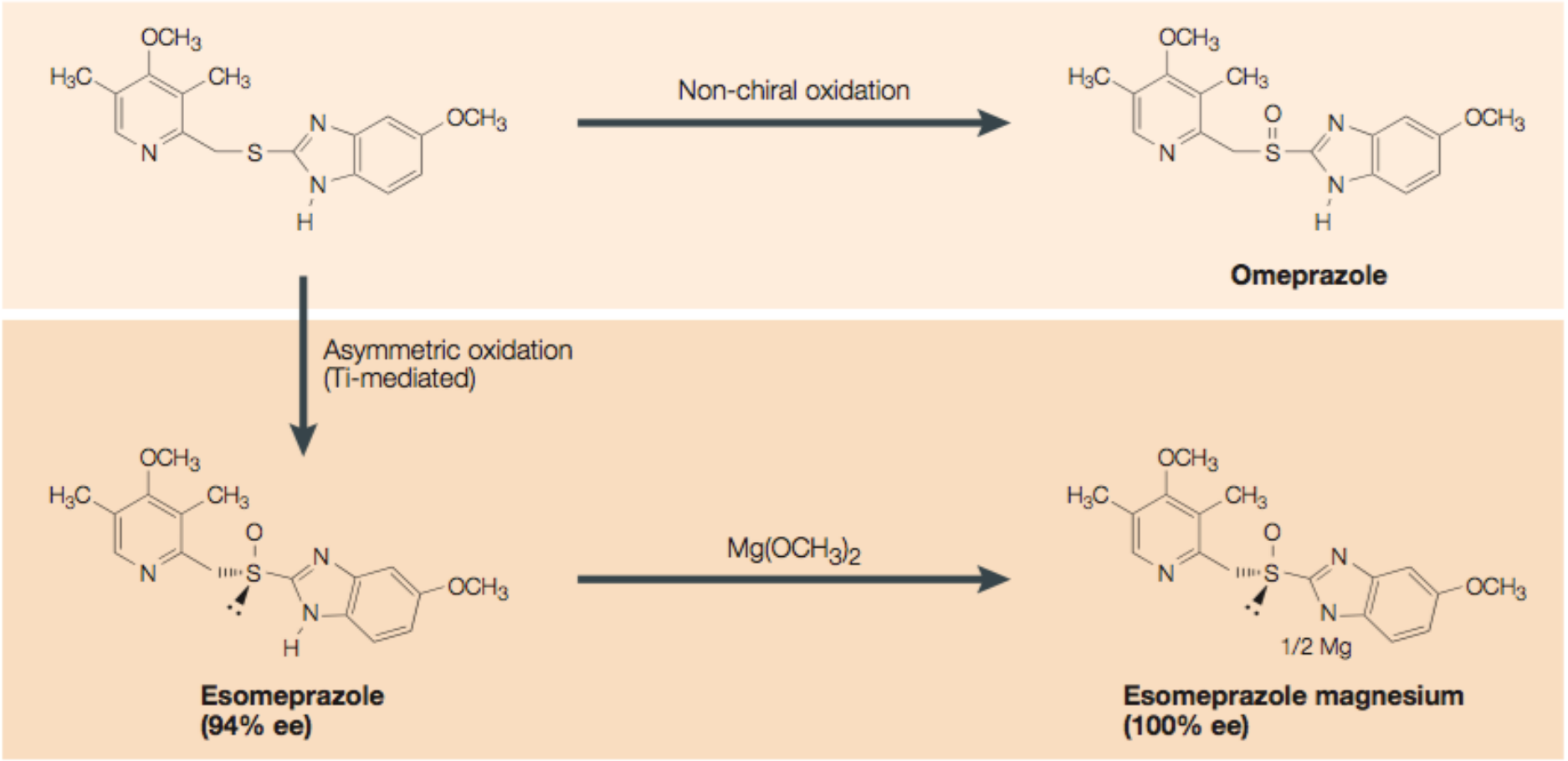
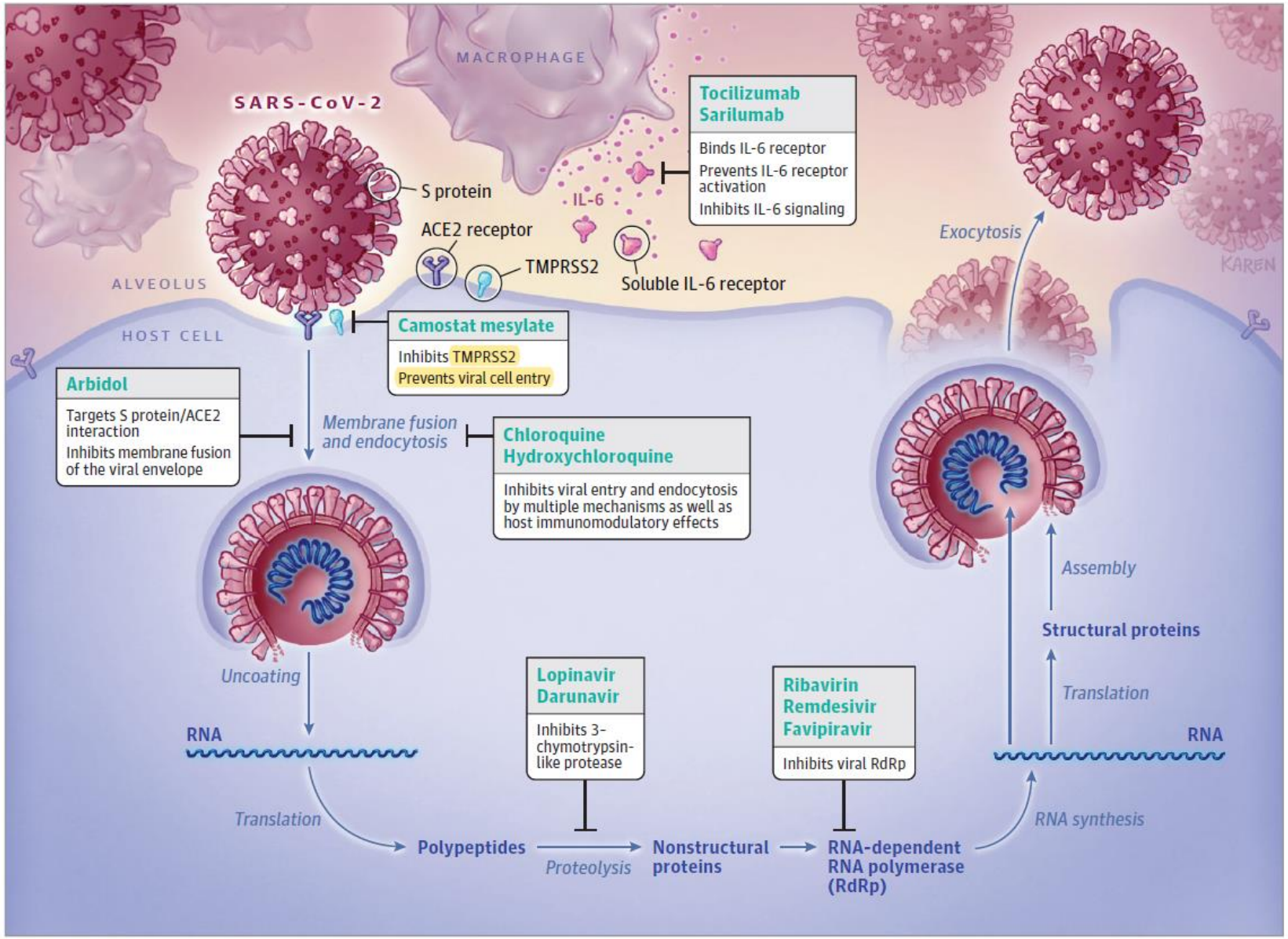


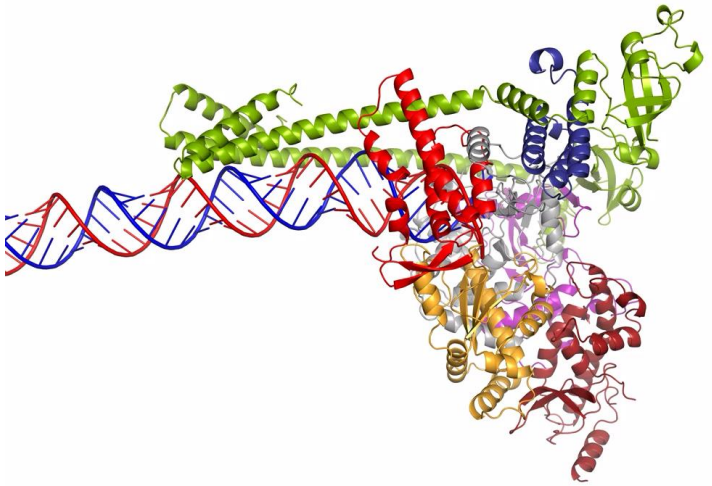
Figure 3 | **Synthesis of omeprazole and esomeprazole.** The large-scale production of esomeprazole is achieved by asymmetric oxidation of the same sulphide intermediate as is used in the production of omeprazole, which gives a 94% enantiomeric excess (ee). This is increased to 100% by preparing a magnesium salt of of esomeprazole and then performing a crystallization.

The SARS-CoV2 lifecycle: how to block it



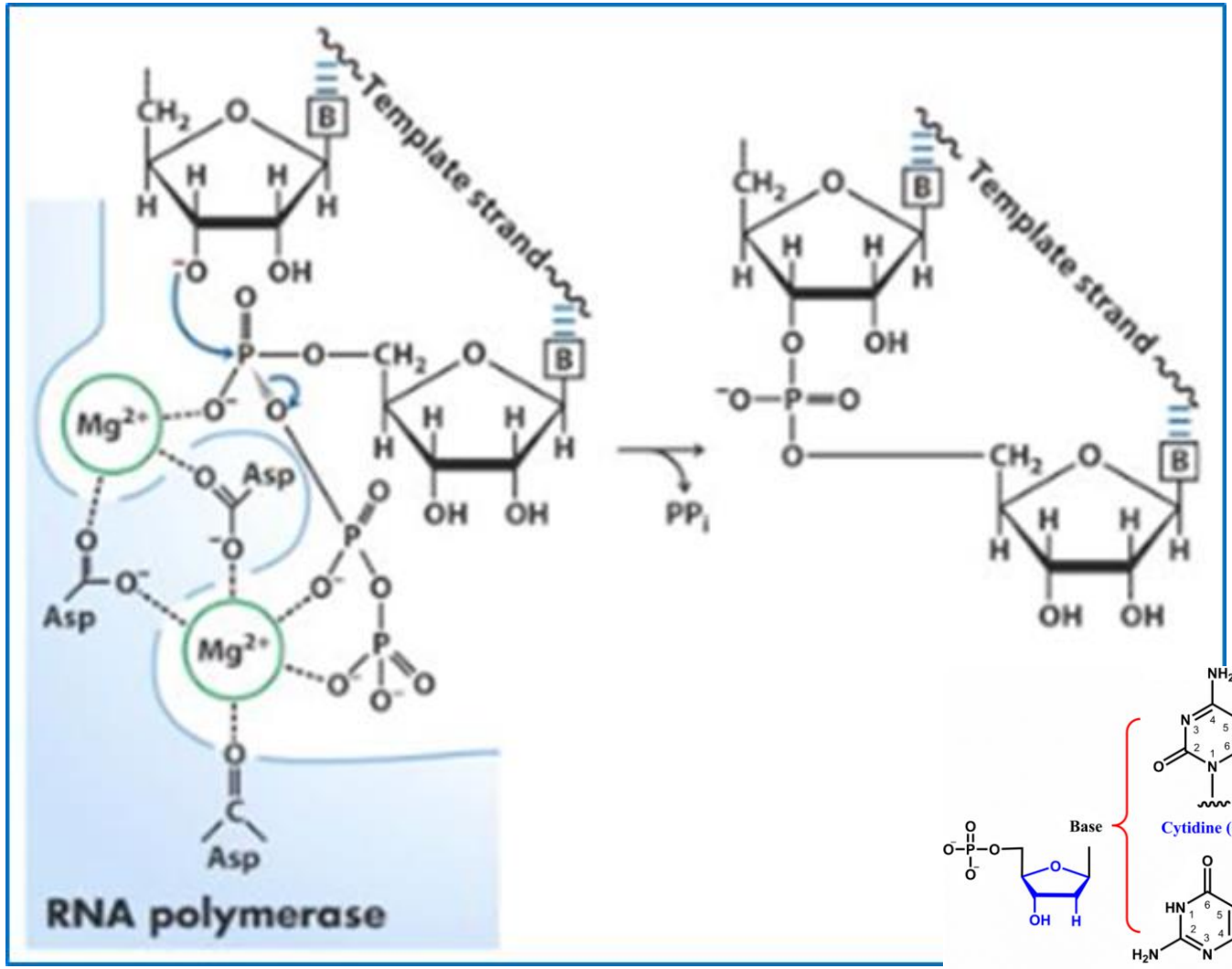
The target: the RNA-dependent RNA polymerase (RdRp)

- This enzyme catalyze the formation of phopshodiester bonds among nucleotides in the presence of metal cations, elongation of nucleotidic chain in direction5’ → 3’
- The structure of this enzyme is very conserved among RNA viruses

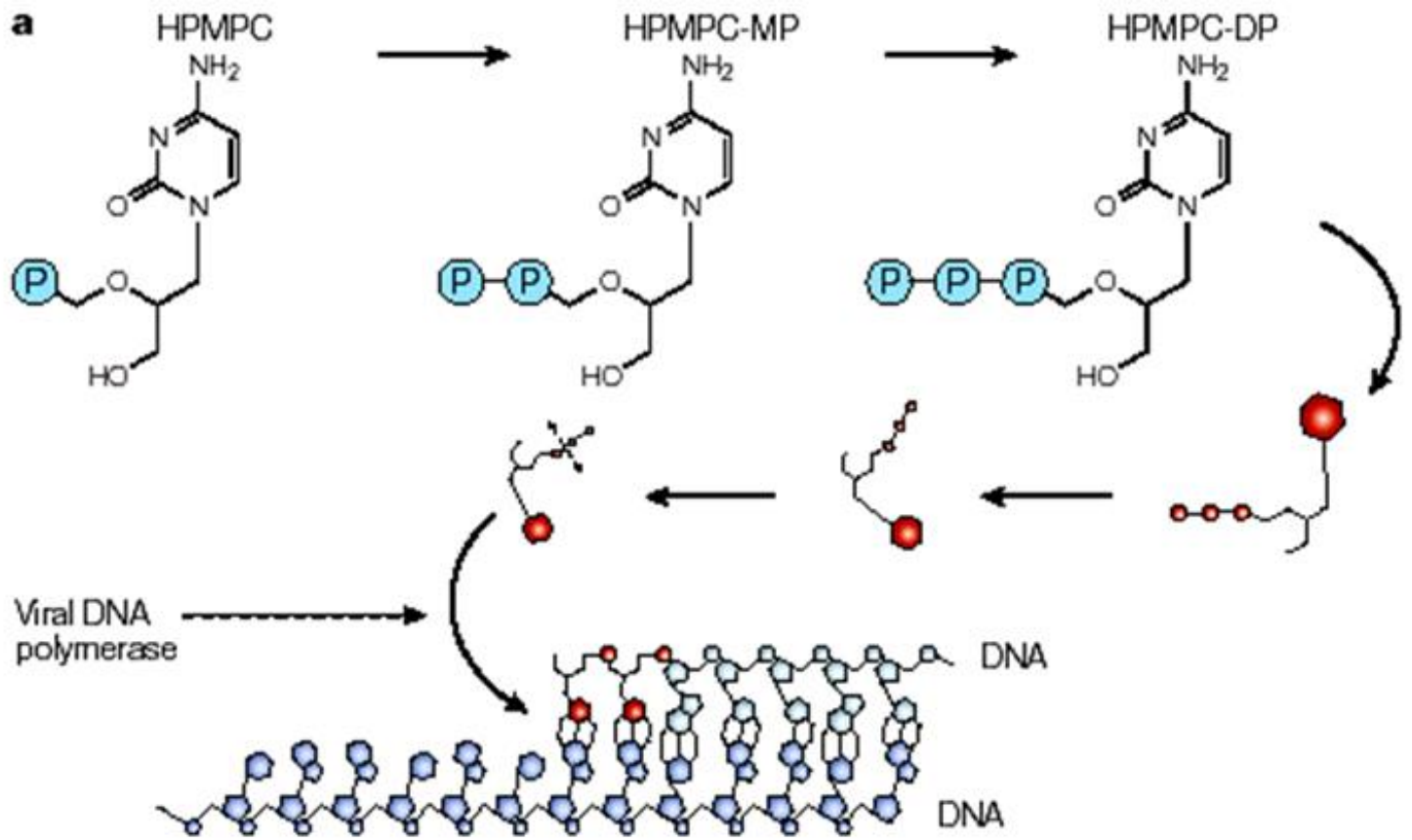


Hillen, H.S., Kokic, G., Farnung, L. *et al.* *Nature* 584, 154–156 (2020). DOI: 10.1038/s41586-020-2368-8

mechanism of action of chain terminators: substrate analogues of viral DNA/RNA polymerase

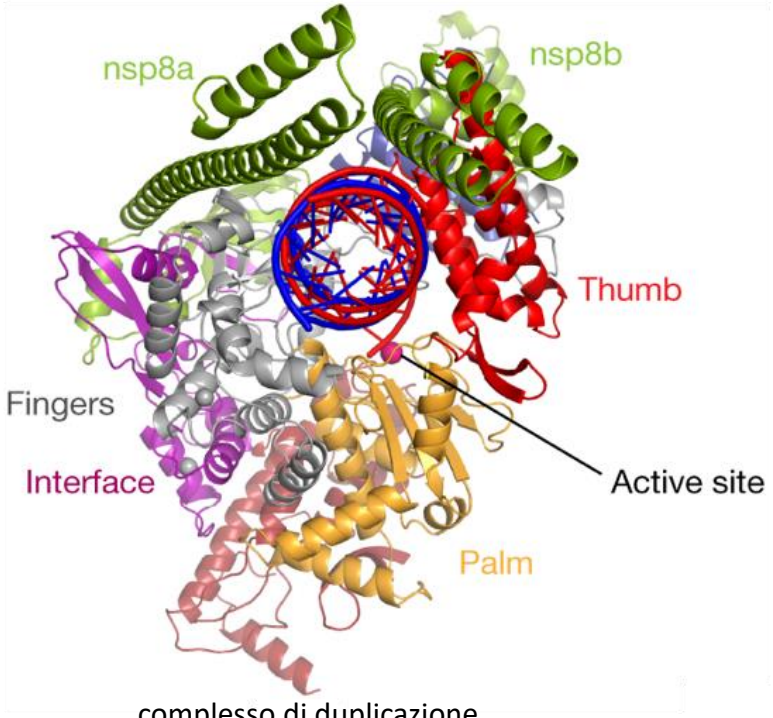


mechanism of action of chain terminators: substrate analogues of viral DNA/RNA polymerase



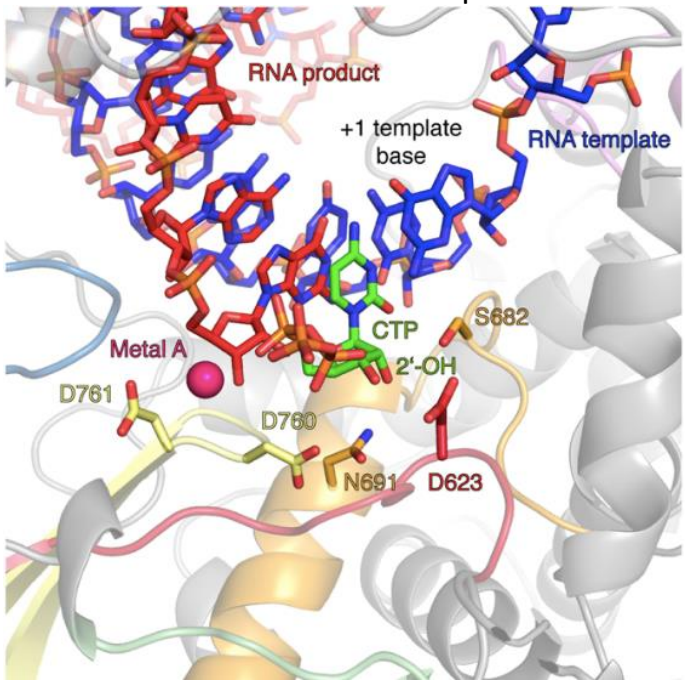
RdRp of SARS-CoV-2 (nsp12)

Cryo-EM structure



complesso di duplicazione

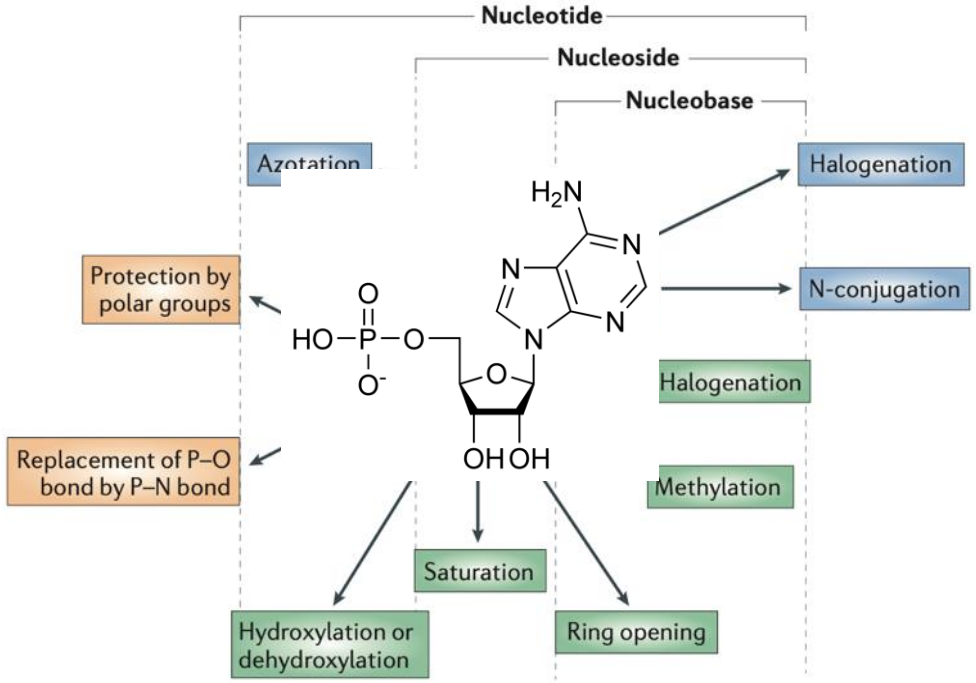
Sito catalitico: residui polari



Hillen, H.S., Kokic, G., Farnung, L. *et al. Nature* 584, 154–156 (2020). DOI: 10.1038/s41586-020-2368-8

The strategy: analogues of nucleotides

***Ligand Based Drug Design* (LBDD)**



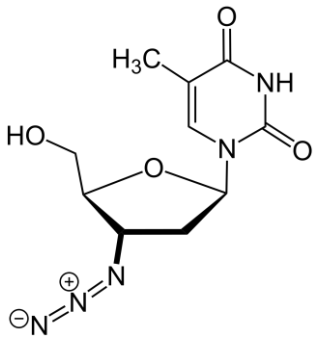
Thre categories of antiviral drugs based on nucleoside or nucleotide analogues:

- The mutagenic nucleosides
- Chain terminators *obligate*
- Chain terminators *delayed*

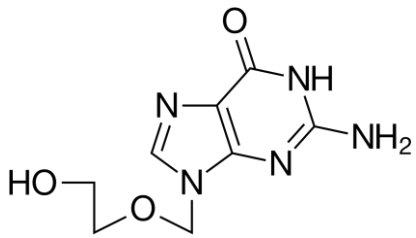
➢ Used in clinic from around 50 years

Jordheim, L., Durantel, D., Zoulim, F. *et al.* *Nat Rev Drug Discov* 12, 447–464 (2013). DOI: 10.1038/nrd4010

Nucleoside analogues

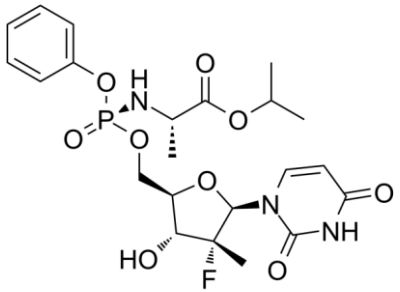


Azidotimidine (AZT)

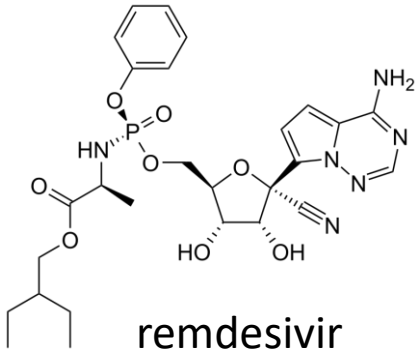


Acyclovir

Obliged chain truncators (no 3’ OH)



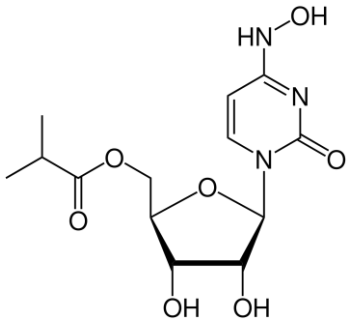
sofosbuvir



remdesivir

Delayed chain truncators (with 3’ OH)

molnupravir

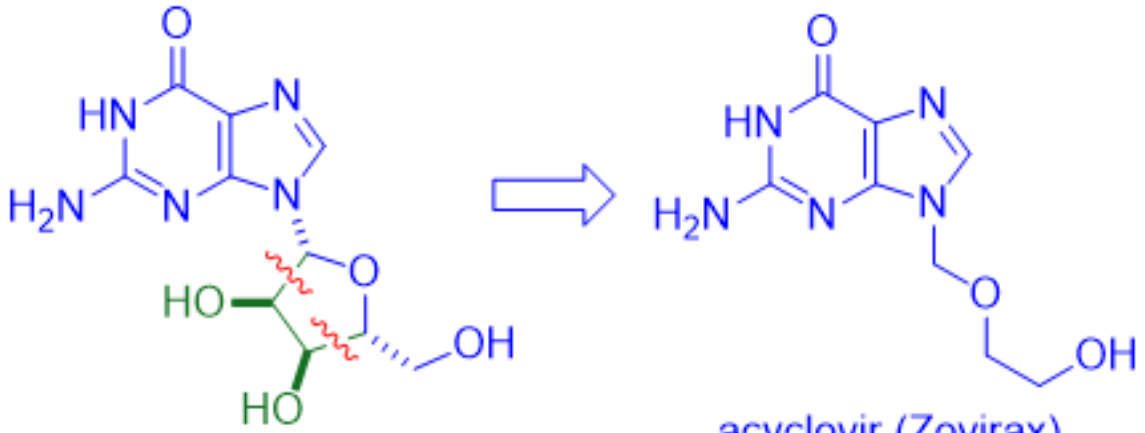


Mutagenic (with 3’ OH)

Cellular effects induced by antiviral and cytotoxic nucleoside and nucleotide analogues

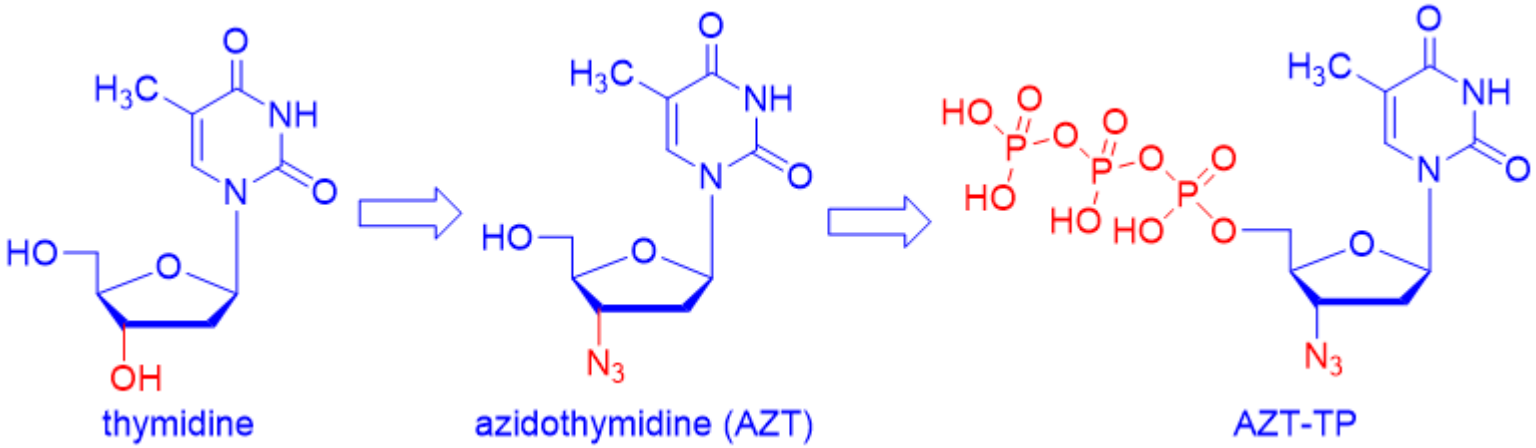
- **Termination of chain elongation**
- Nucleotide analogues are in competition with physiological nucleotides during the initiation and extension of DNA or RNA chains by cellular or viral polymerases
- **Obligate or delayed**
- **Accumulation of mutations in viral progeny**
- These analogues have a 3‘ hydroxyl group on their carbohydrate group, which enables sustained chain elongation after their incorporation into DNA or RNA. Subsequent mismatching may lead to mutagenesis, resulting in reduced infectivity; this concept has been validated for many riboviruses with the notable exception of hepatitis C virus1
- **Induction of apoptosis**
- The exact mechanism by which nucleoside analogues induce apoptosis in cancer cells is still not clearly understood

Acyclovir: inhibitor of DNA polymerase in herpes simplex virus (HSV)



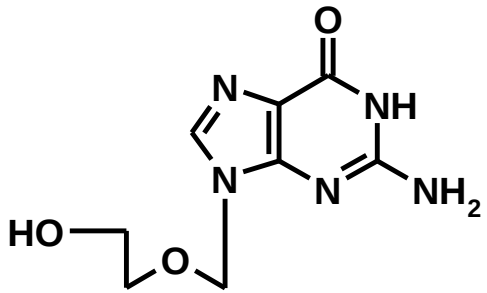
- Gertrude “Trudy” Elion, who shared the 1988 Nobel Prize with George Hitchings, led a team at Wellcome Research Institute that discovered acyclovir (Zovirax) for the treatment of herpes simplex virus (HSV). One of the Wellcome chemists, Howard Schaeffer, established that the intact sugar ring of compounds (such as guanosine) was not essential for binding to enzymes needed for DNA synthesis. Cutting off the diol fragment of the sugar fragment led to the discovery of acyclovir, which became one of the most successful antiviral drugs at the time.

Azidothymidine (AZT, zidovuline): anti-HIV, obligate CT

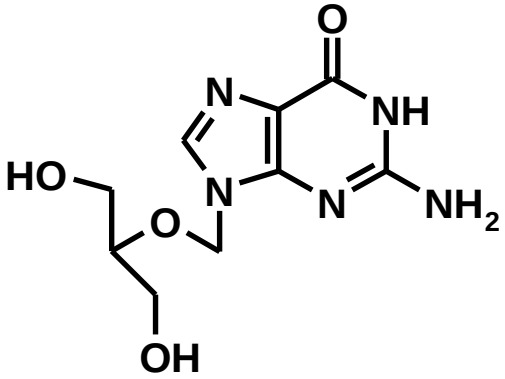


- AZT is an **obligate a chain truncator**
- AZT is also reasonably selective. AZT-TP inhibits the synthesis of DNA by reverse transcriptase about 100-times better than it inhibits the synthesis of DNA by the host-cell DNA polymerase in the cell nucleus.
- More impressively, AZT inhibits HIV replication at concentrations about 1,000-fold less than it takes to inhibit the replication of the host-cell lymphocytes. In other words, AZT kills the virus faster than normal healthy cells thus providing a therapeutic window.

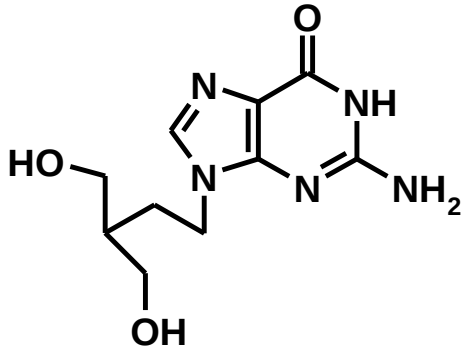
Me-too drugs and prodrugs



acyclovir

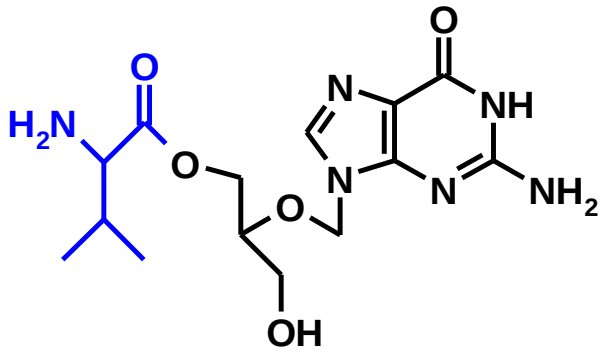


gancyclovir



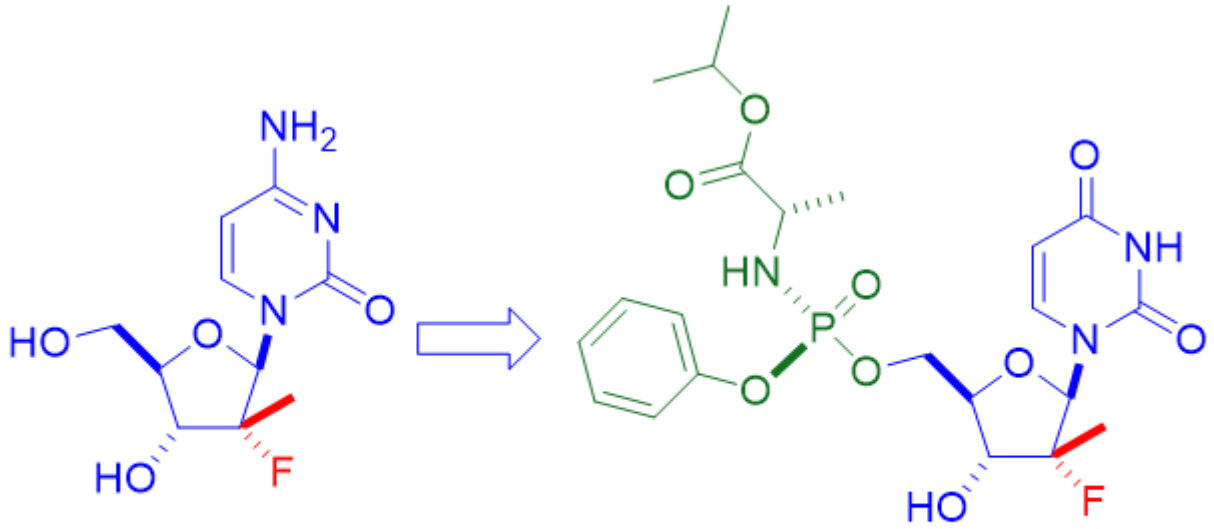
pencyclovir

- Following the discovery of acyclovir, its “me-too” drugs, ganciclovir (Cytovene, 1988) and penciclovir (Denavir, 1996), followed.



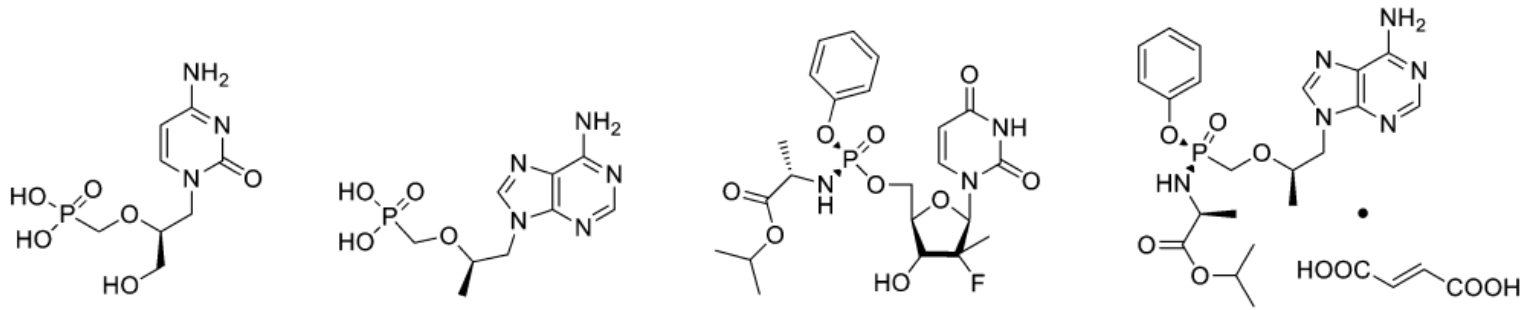
Valganciclovir (oral prodrug)

Sofosbuvir (delayed CT)



- Sofosbuvir is a prodrug of a unique nucleoside PSI-6130, which is fascinating on its own right because it has a rare fluorine-containing tertiary carbon at the 2’-position of its ribose ring. This is a testimony of how important that the fluorine atom has become in modern drug discovery.
- While no fluorine-containing drugs existed before fludrocortisone was approved in 1955, nowadays, more than 20% of all drugs contain one or more fluorine atoms.

Prodrug strategies to reduce polarity of phosphates and phosphonates



1, cidofovir **2**, tenofovir **3**, sofosbuvir **4**, tenofovir alafenamide

Figure 2. Chemical structures of key phosphonates and mono(phosphate/phosphonate) prodrugs approved for clinical use.

The protide technology

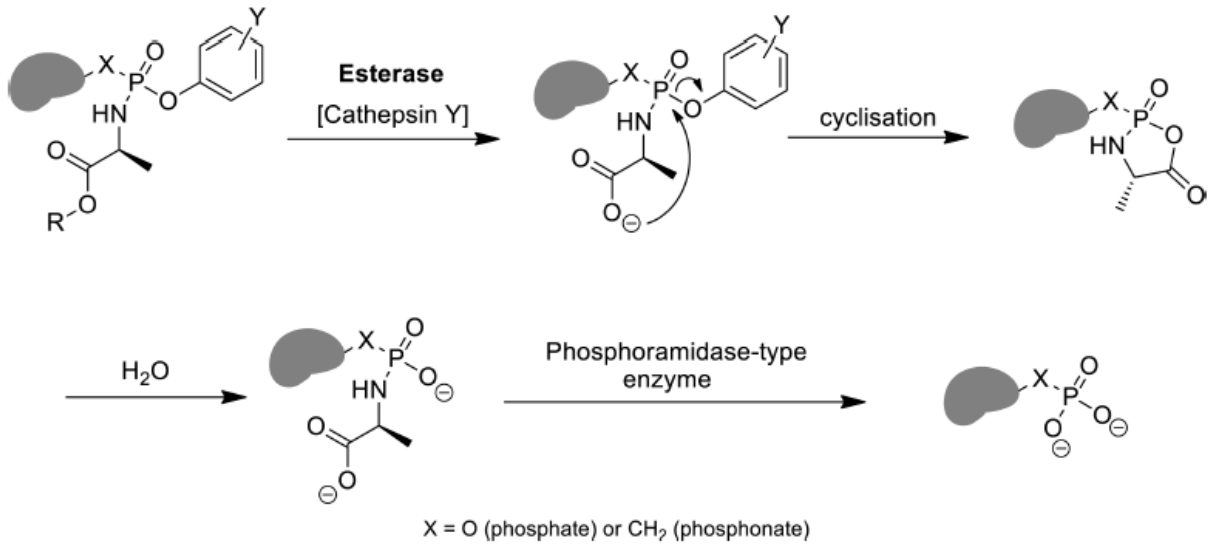
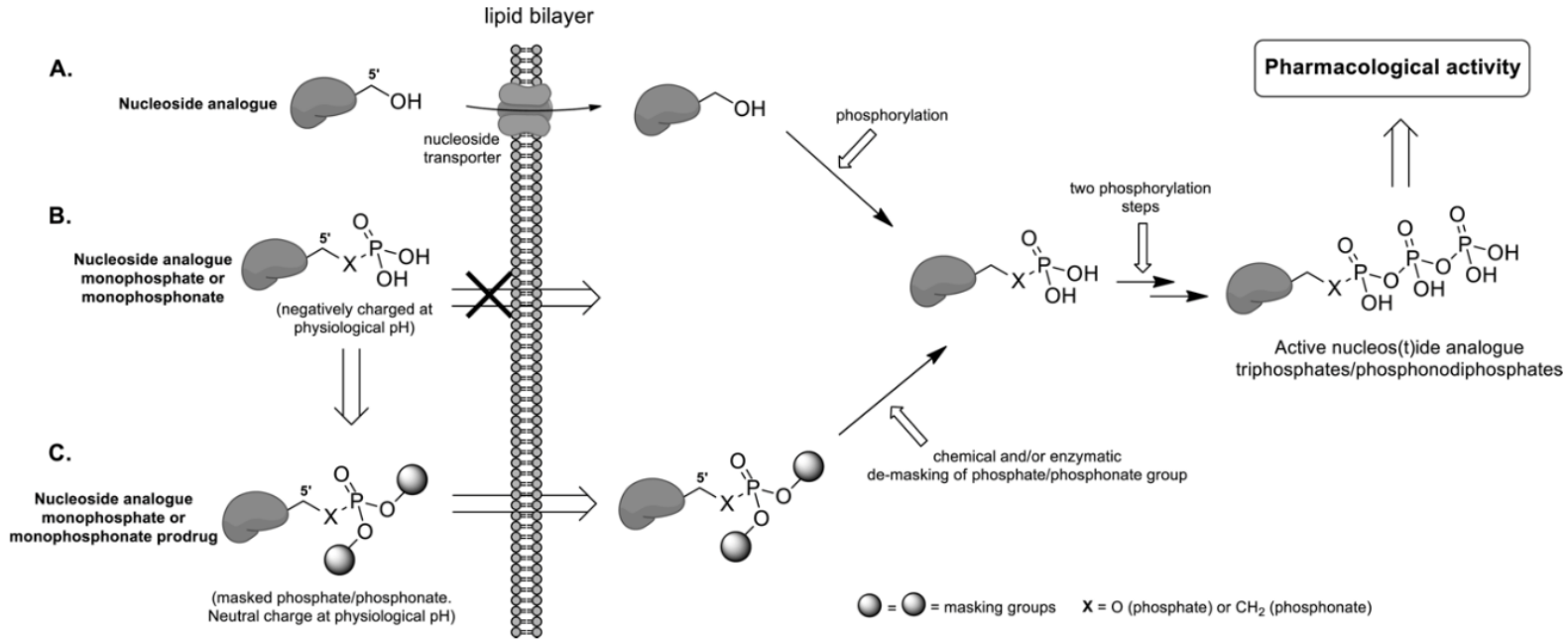


Figure 3. Postulated mechanism of ProTides in vivo breakdown to release the nucleoside analogue monophosphate or monophosphonate.

The pharmacokinetic problem of nucleoside analogues

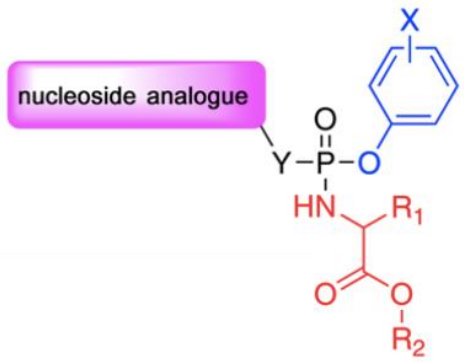
1) Low membrane penetration
2) The first phosphrylation is inefficient (RDS)



Thornton P. J., Kadri H., Miccoli A., Mehellou Y., *J. Med. Chem.* 59, 10400−10410 (2016) DOI: 10.1021/acs.jmedchem.6b00523

The ProTide technology

Prodrugs of monophosph(on)ate nucleoside analogues developed by Chris McGuigan of Univeristy of Cardiff from ‘80, phosphate group is masqued by an aryloxy-phosphoramidate

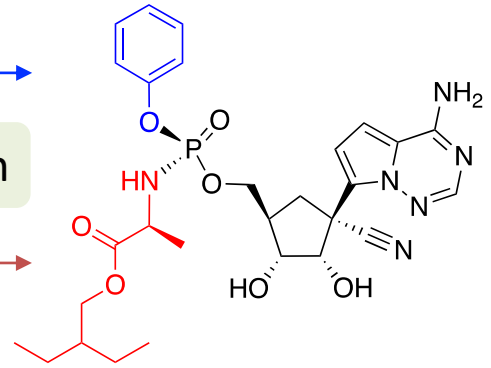


Y=O o CH_2
X = substituent
R_1= H, Me, CH_2iPr, -CH_2Ph, …
R_2 = Me, Et, iPr, tBu, Bn, …

Substituted aryl

PK optimization

Aminoacid ester



remdesivir (Veklury®)
GS-5734

Mehellou Y., Rattan H. S. and Balzarini J. *J. Med. Chem.*, 61, 2211−2226 (2018) DOI: 10.1021/acs.jmedchem.7b00734

Other prodrug strategies
HepDirect (vedi ciclofosfamide)

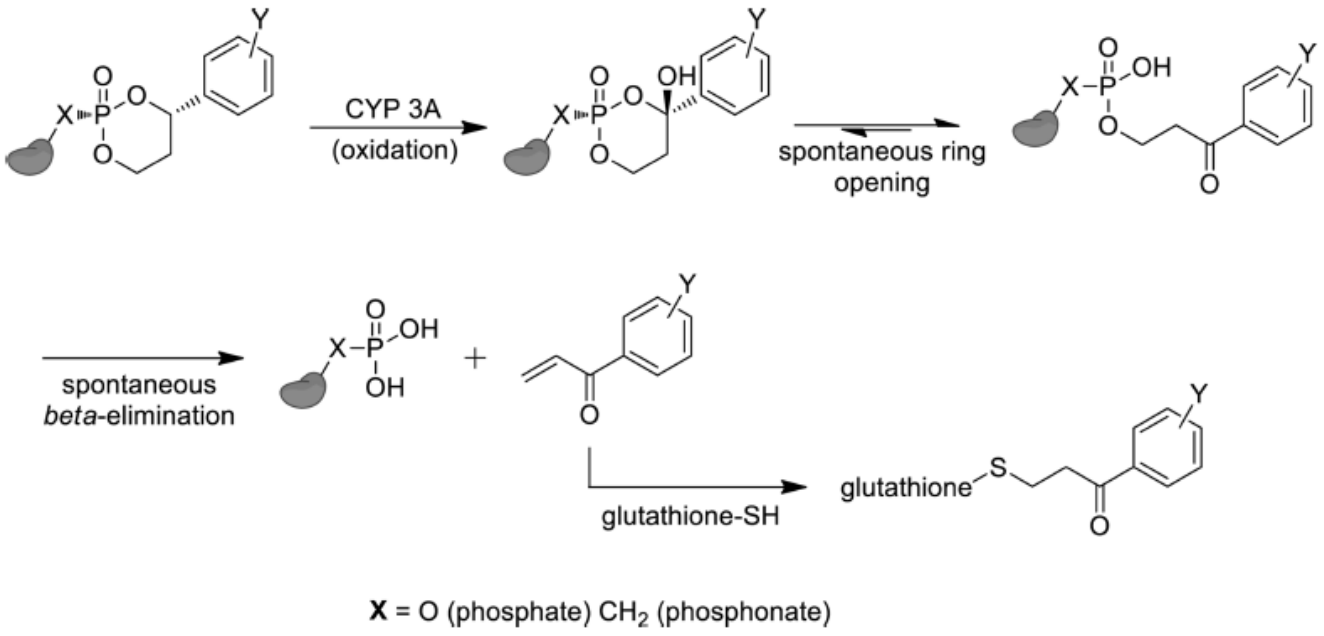


Figure 5. Mechanism of CYP450-mediated metabolism of HepDirect prodrugs in the liver.

HepDirect prodrugs

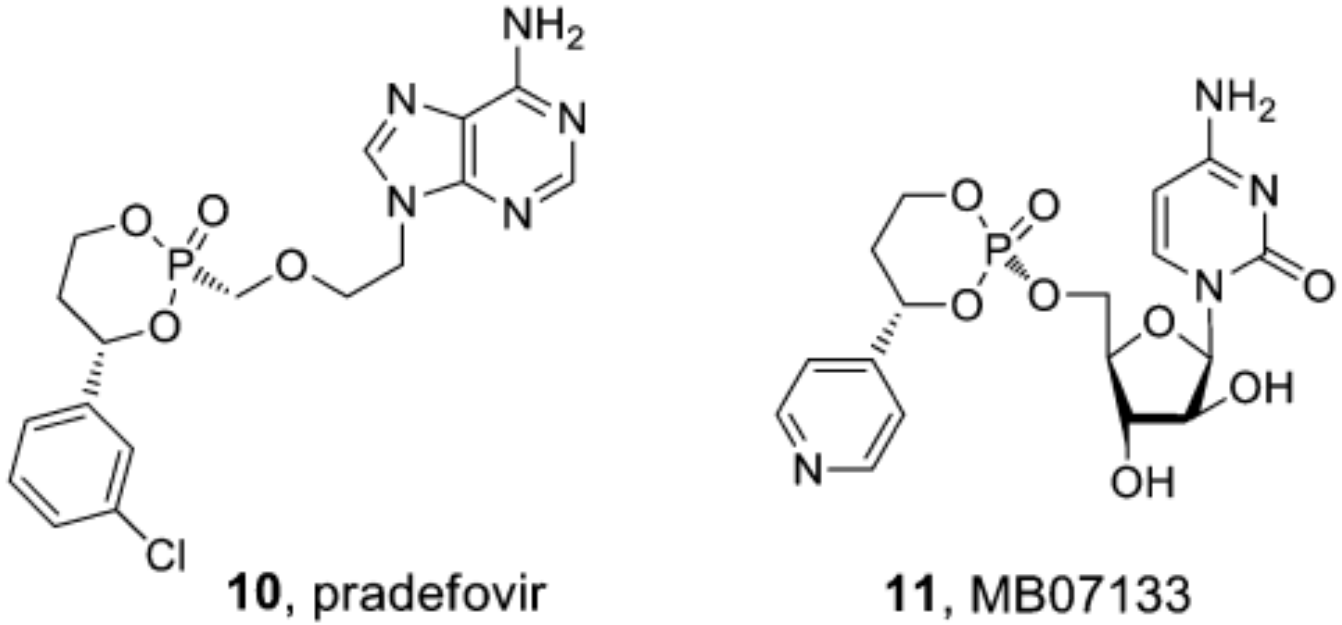


Figure 6. Chemical structures of two nucleotide HepDirect clinical candidates.

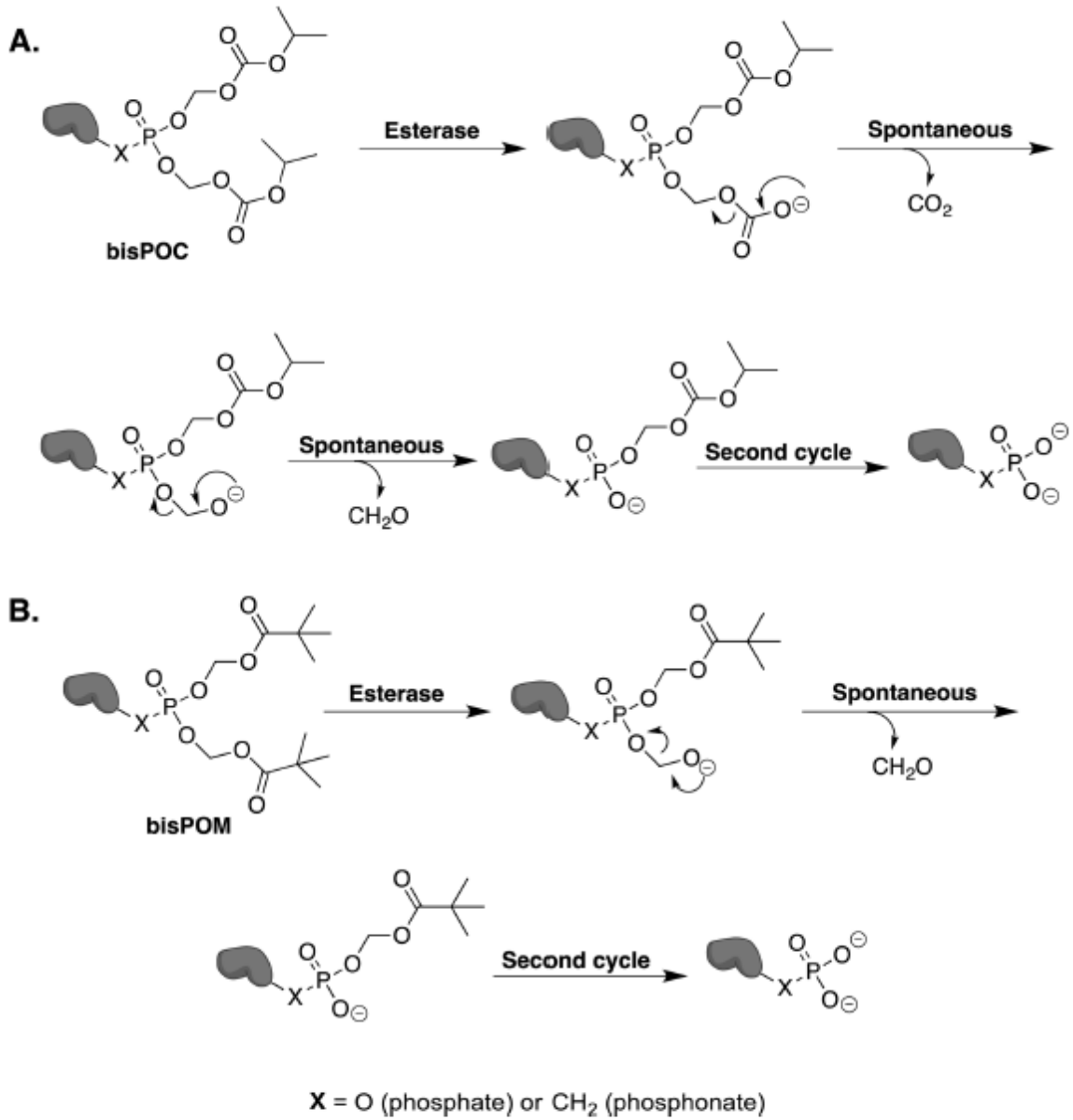
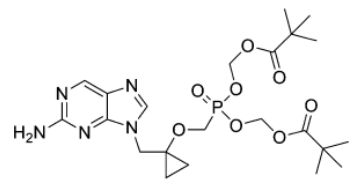
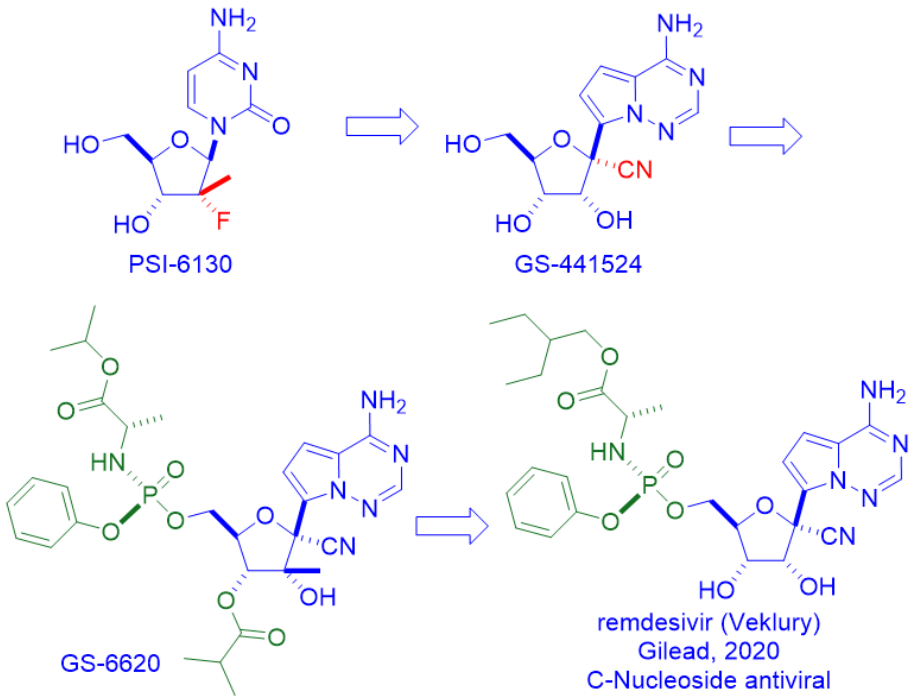


Figure 8. Metabolism of (A) bisPOC and (B) bisPOM prodrugs.



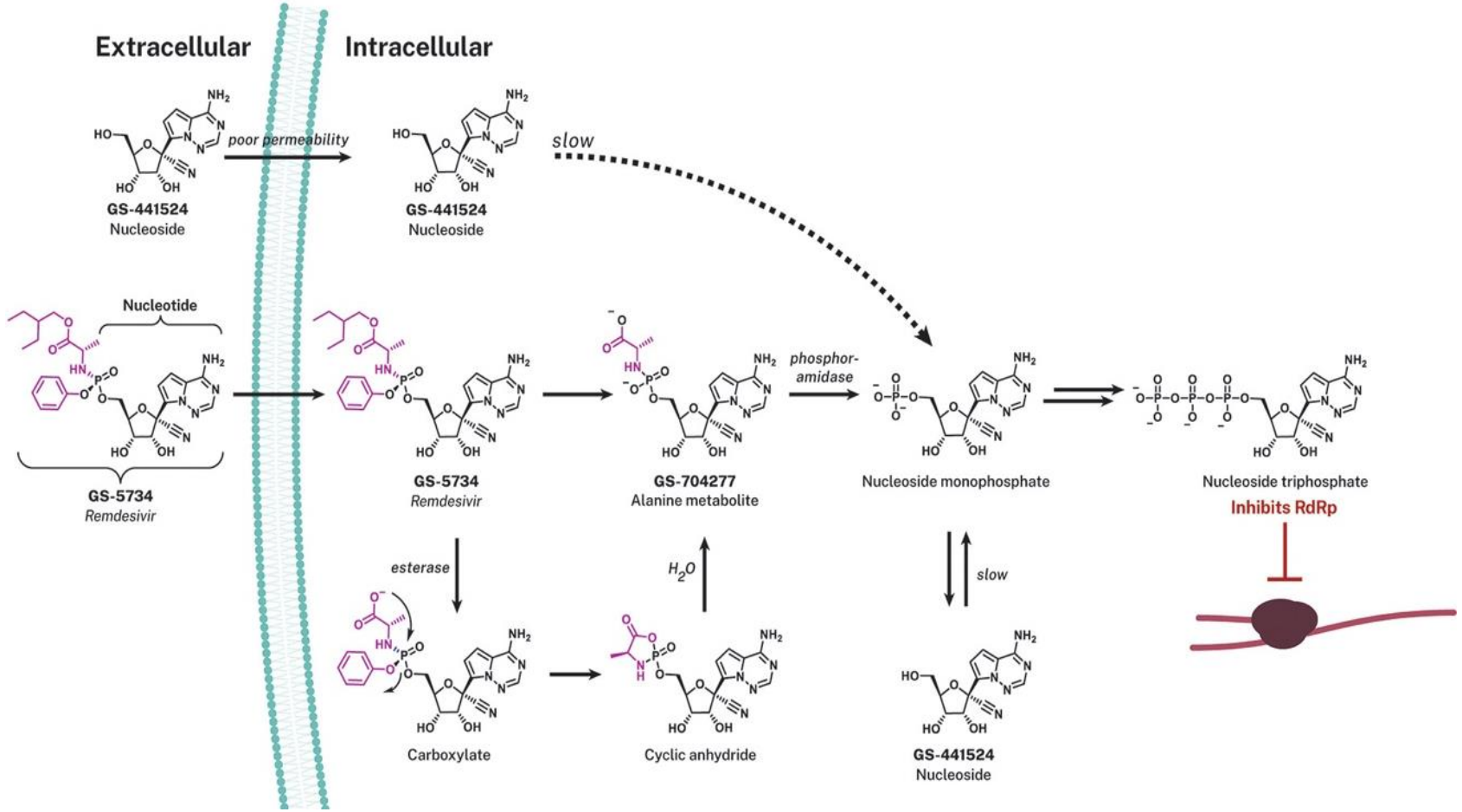
14, besifovir

Figure 9. Chemical structure of besifovir, an anti-HBV bisPOM prodrug clinical candidate.



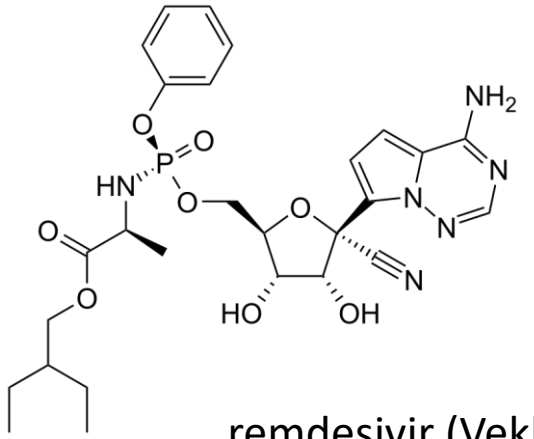
- Then came along the Ebola virus (EBOV), a member of the Filoviridae family. EBOV is a RNA virus. Screened an assembly of approximately 1000 compounds, largely nucleosides and nucleotides from Gilead's collection.
- They heavily focused on ribose analogs that could target RNA viruses since this would encompass many emerging viral infections ranging from respiratory pathogens belonging to the Coronaviridae family such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), to mosquito-borne viruses of the Filoviridae family such as Dengue and Zika.
- Remdesivir has to be given via injection, whereas sofosbuvir is orally bioavailable.

The ProTide remdesivir

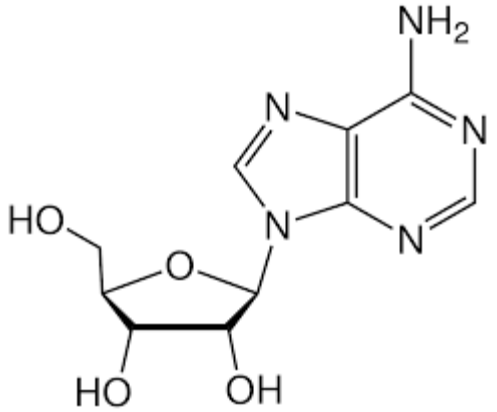


Eastman R.T., Roth J.S., Brimacombe K.R. *et al. ACS Cent. Sci.* 2020, 6, 672−683 DOI: 10.1021/acscentsci.0c00489

Remdesivir (Veklury®)



remdesivir (Veklury®)
GS-5734

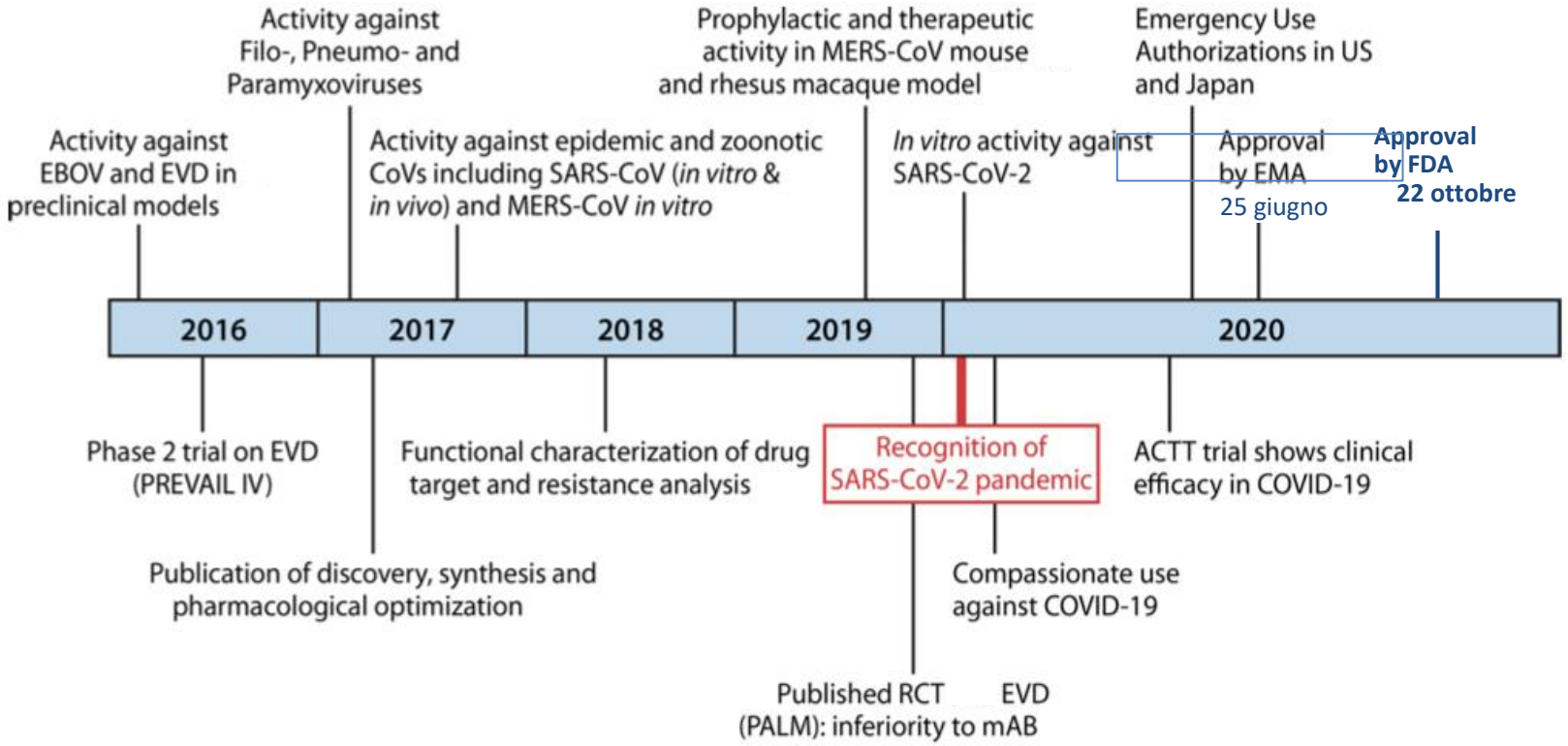


adenosine

- Remdesivir (GS-5734) is the phosphoramidate prodrug of the **1'-cyano-C-nucleoside analogue** of the adenosine
- Wide spectrum antiviral action (*Paramyxovirus*, *Pneumovirus*, *Filoviridae* e *Coronavirus*) it was initially developed against the virus of Ebola

Malin JJ, Suárez I, Priesner V, Fätkenheuer G, Rybniker J. *Clin Microbiol Rev* 34, e00162-20 (2021) DOI: 10 .1128/CMR.00162-20

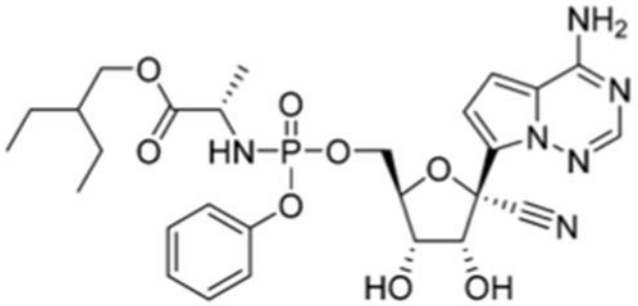
The remdesivir (Veklury®)



Malin JJ, Suárez I, Priesner V, Fätkenheuer G, Rybniker J. *Clin Microbiol Rev* 34, e00162-20 (2021) DOI: 10 .1128/CMR.00162-20

Il ProTide remdesivir: absorption

La biodisponibilità orale del remdesivir è bassa quindi necessita di somministrazione per **via endovenosa**

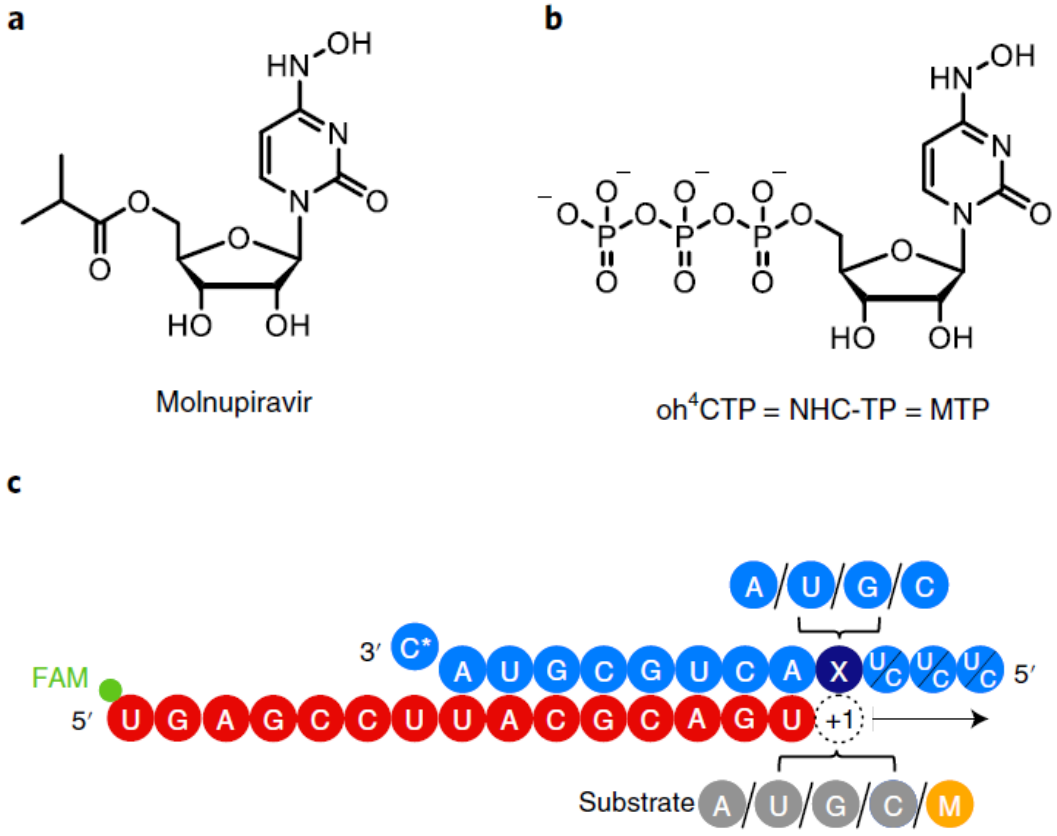


4a *Sp* and *Rp* isomers (~1:1)

remdesivir (Veklury®)
GS-5734

Siegel D., Hui H. C., Doerffler E., *et al. J. Med. Chem.* 60, 5, 1648–1661 (2017) DOI: 10.1021/acs.jmedchem.6b01594

Mutagenic nucleoside: the molnupravir



Molnupiravir is an isopropylester prodrug of the nucleoside analog β-d-N4-hydroxycytidine (NHC or EIDD-1931)

The fluoroquinolone antibiotics

Journal of Medicinal Chemistry

ACS AuthorChoice

Perspective

pubs.acs.org/jmc

Origins of the Quinolone Class of Antibacterials: An Expanded "Discovery Story"

Miniperspective

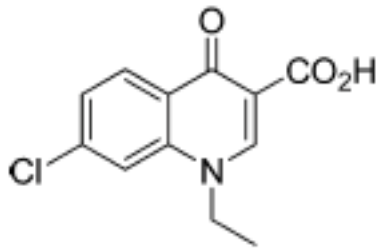
Gregory S. Bisacchi*

AstraZeneca, 35 Gatehouse Drive, Waltham, Massachusetts 02451, United States

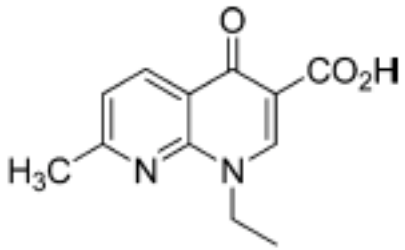
The following excerpt from a 2005 review of the field of antibacterial quinolones is representative of descriptions in the scientific literature concerning the origin of the class:

“The first antimicrobial quinolone was discovered about 50 years ago as an impurity in the chemical manufacture of a batch of the antimalarial agent chloroquine (Figure 2). It demonstrated anti Gram-negative antibacterial activity, but its potency and antimicrobial spectrum were not significant enough to be useful in therapy. Building on this lead, however, subsequently nalidixic acid was commercialized.”

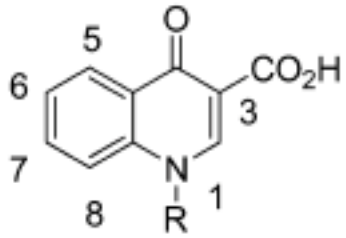
the origin of nalidixic acid published by its discoverers at Sterling Drug (now part of Sanofi) and moreover may even be making inferences beyond the data available from Sterling’s published literature (i.e., “... potency and antimicrobial spectrum [of compound 1] were not significant enough to be useful in therapy”



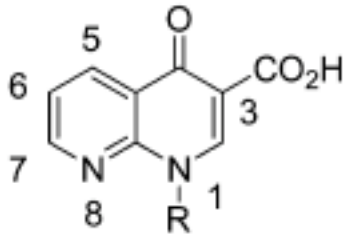
1
"active impurity/ by-product" (lead compound)



2
nalidixic acid

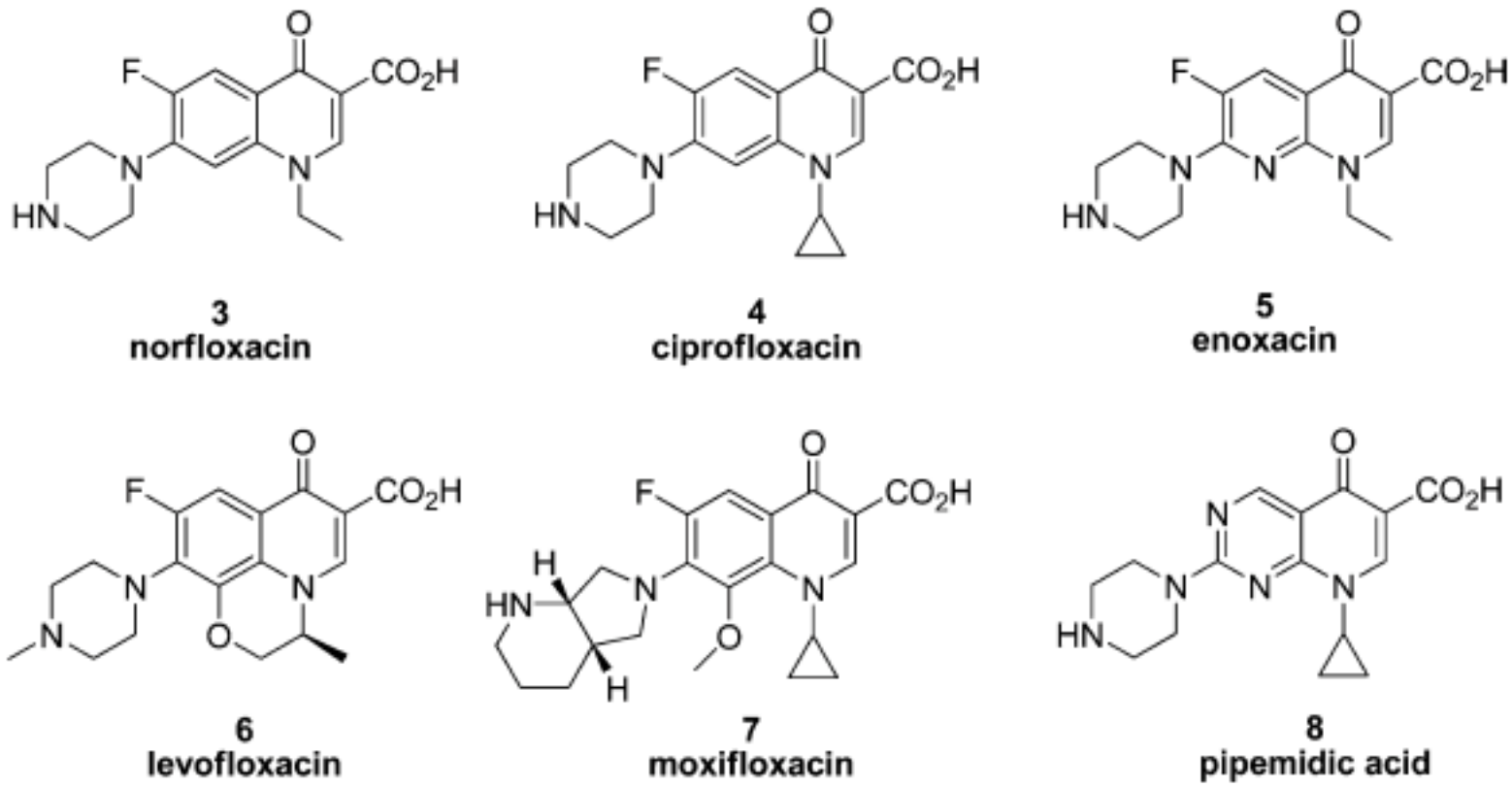


quinolone core



1,8-naphthyridone core

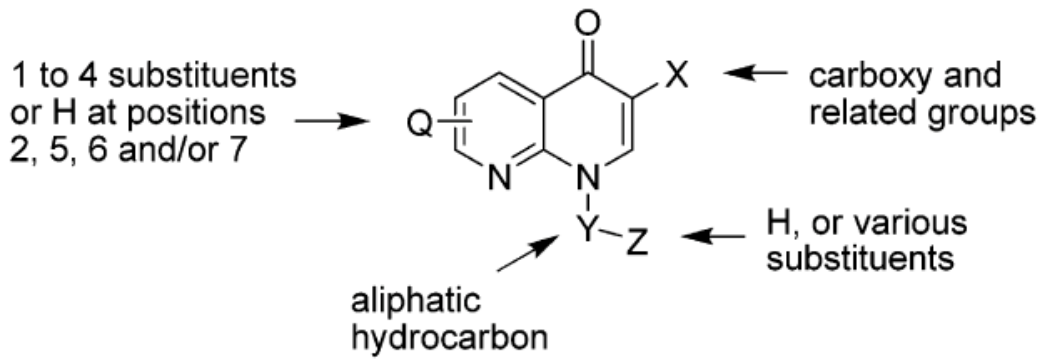
Quinolone and naphtyridone-derived antibiotics



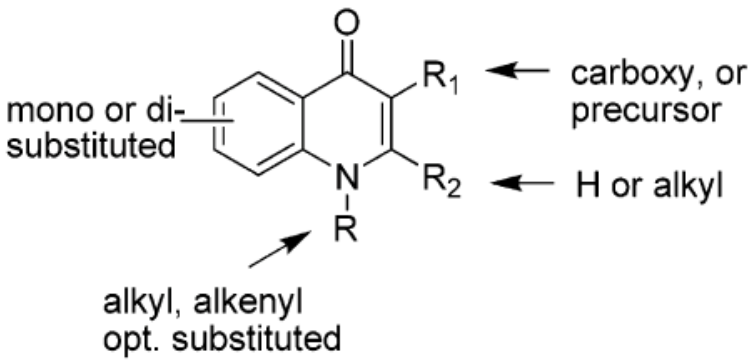
During the past 2 decades or so, launches of new agents having the quinolone core have surpassed launches of new 1,8- naphthyridone-based drugs. Over the years, the clinically useful microbiological spectrum of the quinolone class has expanded further to include many Gram positive pathogens, such as Staphylococcus aureus

patents

Sterling BE612258
Filed Jan 1961
Published July 1962



ICI GB830832
Filed Feb 1957
Published Mar 1960



mechanism of action and selectivity

The dual action against DNA gyrase and topoisomerase IV has subsequently proved to be the same mechanism for all the antibacterial quinolones.

These remarkable enzymes are involved in maintaining the integrity of the supercoiled DNA helix during replication and transcription. If their action is impeded the bacterial chromosome remains unwound and is too long and large to fit into the two progeny cells.
The comparable mammalian enzyme is not susceptible to the actions of the quinolones at concentrations used in clinical practice.

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 289, NO. 18, pp. 12300–12312, May 2, 2014
© 2014 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.

Fluoroquinolone-Gyrase-DNA Complexes

*TWO MODES OF DRUG BINDING**

Received for publication, October 21, 2013, and in revised form, January 30, 2014 Published, JBC Papers in Press, February 4, 2014, DOI 10.1074/jbc.M113.529164

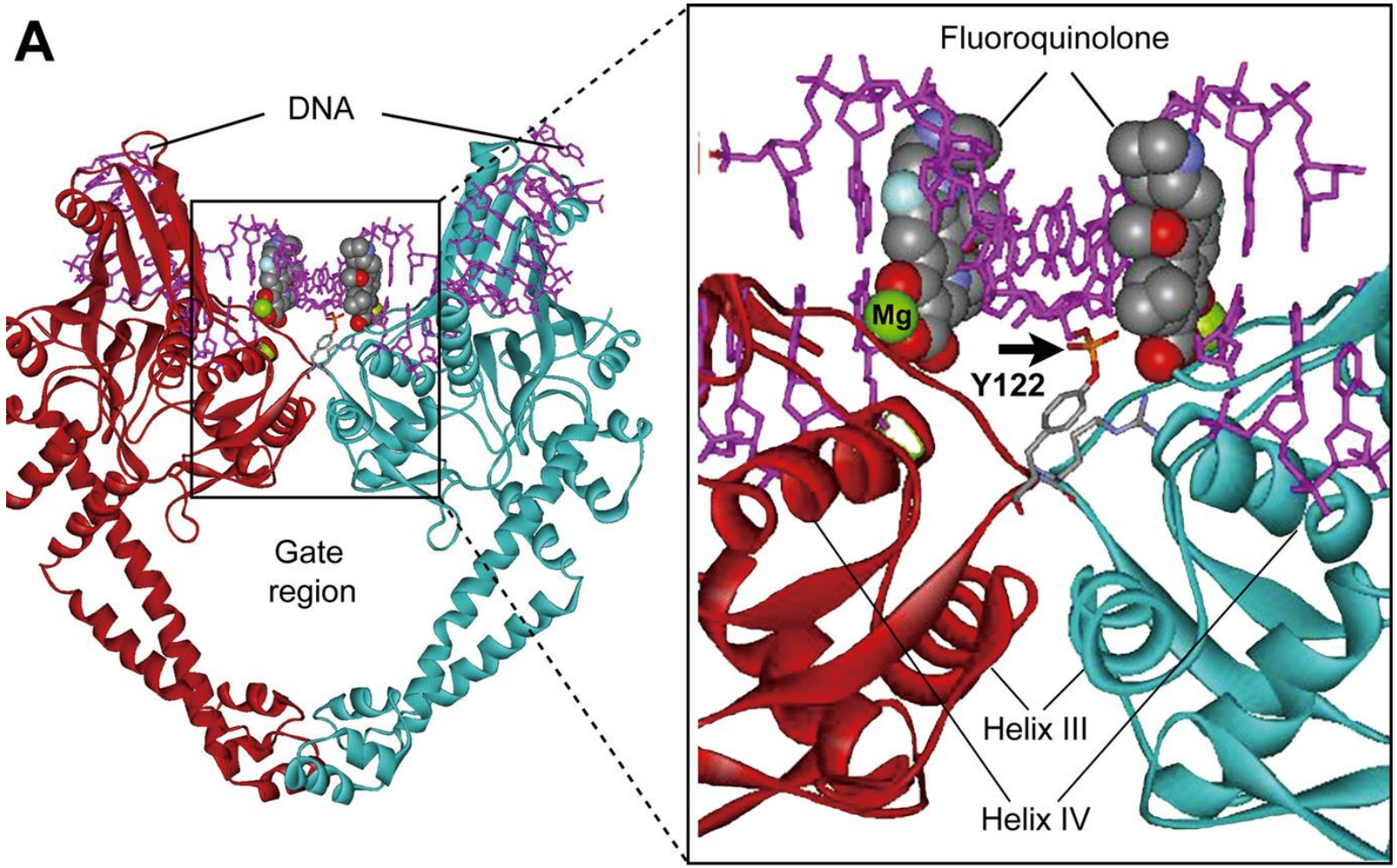
Arkady Mustaev[‡], Muhammad Malik[‡], Xilin Zhao[‡], Natalia Kurepina[‡], Gan Luan[‡], Lisa M. Oppegard[§], Hiroshi Hiasa[§], Kevin R. Marks[¶], Robert J. Kerns[¶], James M. Berger[‖1], and Karl Drlica[‡2]

From the [‡]Public Health Research Institute and Department of Microbiology and Molecular Genetics, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Newark, New Jersey 07103, [§]Department of Pharmacology, University of Minnesota Medical School, Minneapolis, Minnesota 55455, [¶]Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, Iowa 52242, and [‖]Department of Molecular and Cell Biology, California Institute for Quantitative Biosciences, University of California, Berkeley, California 94720

DNA gyrase and topoisomerase IV control bacterial DNA topology by breaking DNA, passing duplex DNA through the break, and then resealing the break.

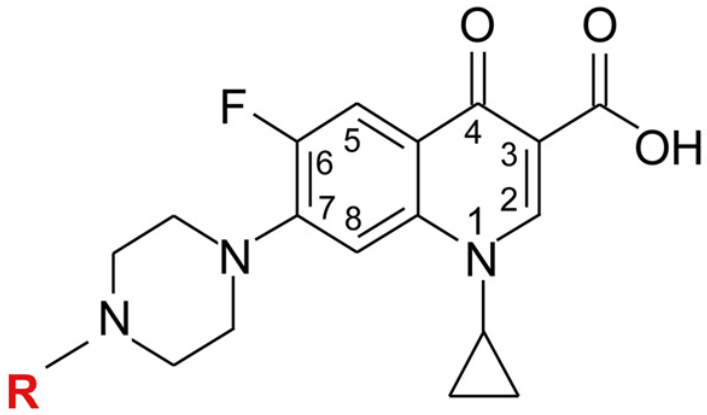
This process is subject to reversible corruption by fluoroquinolones, antibacterials that form drug-enzyme-DNA complexes in which the DNA is broken.
The complexes, called cleaved complexes because of the presence of DNA breaks, have been crystallized and found to have the fluoroquinolone C-7 ring system facing the GyrB/ParE subunits.

The fluoroquinolones and some antitumor drugs reversibly trap type II topoisomerases on DNA as ternary complexes in which DNA is broken.
Knowledge of these “cleaved complexes,” which block DNA replication and transcription, is central to establishing how quinolones and related antitopoisomerase agents act.



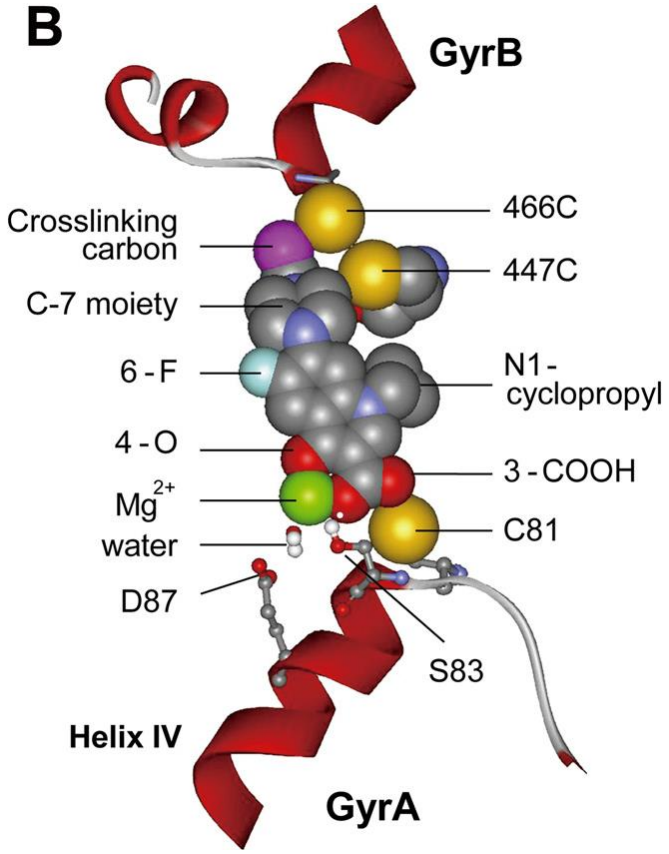
- Quinolone antibiotics, first developed in the 1960s, kill bacteria by blocking enzymes called class II topoisomerases, which normally untangle DNA during cell replication. These enzymes usually cut DNA's double helix, pass another part of the strand through the gap, and then mend the cut. But quinolones bind to the enzymes, preventing them from mending their cuts.

- In the 1980s, researchers added fluorine atoms to the quinolones' structures. This allowed the antibiotics to penetrate tissues throughout the body, including the central nervous system, and boosted their effectiveness against a broad range of bacterial infections.



R-group	Compound
-H	Ciprofloxacin
$-C(O)-CH_3$	Cip-Ac
$-C(O)-CH_2-Cl$	Cip-AcCl
$-C(O)-CH_2-Br$	Cip-AcBr

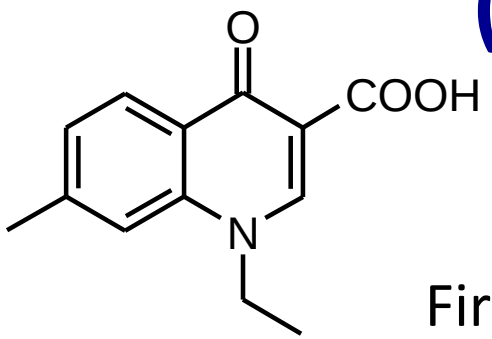
The present report describes a biochemical approach for mapping quinolone-protein contacts. For this work, we prepared derivatives of the fluoroquinolone ciprofloxacin (Cip)3 that contained a haloacetyl group located at the distal nitrogen of the C-7 piperazinyl ring (Cip-AcCl and Cip-AcBr; see Fig. 1C for fluoroquinolone structures). These derivatives were expected to react with cysteines in cleaved complexes if located within cross-linking distance of the bound drug.



A strong interaction was observed between Cip-AcCl and GyrB-E466C gyrase, as predicted by crystal structures.

Surprisingly, a strong interaction also occurred with GyrA-G81C gyrase, indicating the existence of a second binding mode. The GyrA-G81C variation is naturally occurring, which made intracellular tests straightforward: when living cells were treated with haloacetylated ciprofloxacin, a GyrA-G81C E. coli variant and the equivalent M. smegmatis variant exhibited effects consistent with GyrA-based cross-linking.

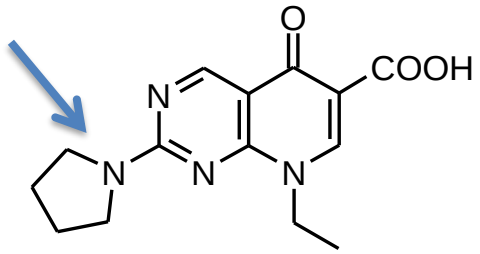
Fluoroquinolone antibiotics by LBDD (QSAR)



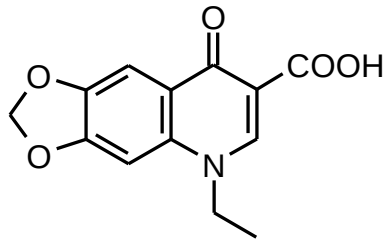
Nalidixic acid, quinolone,
discovered in 1963,

First generation of quinolone antibiotics
Enlarged spectrum of action

Nitrogenated ring

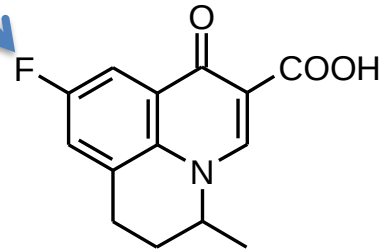


Piromidic acid

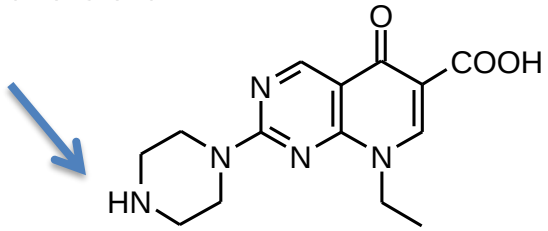


Oxolinic acid

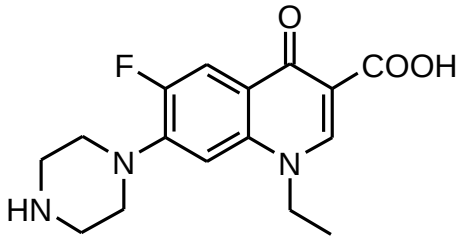
fluorine



flumechin

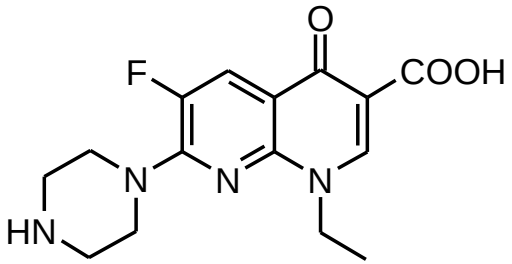


Pipemidic acid

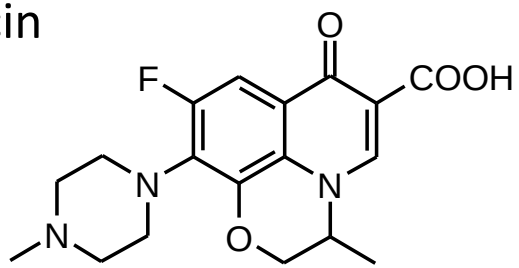


Norfloxacin (1980)

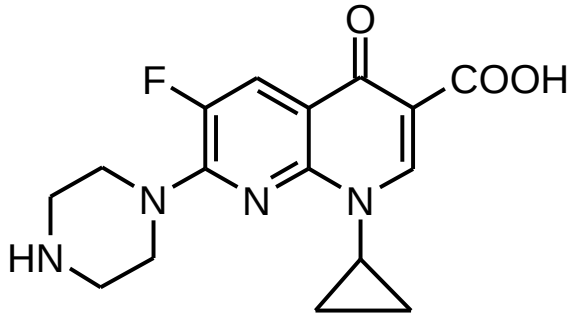
- Improved activity (200-1000 fold)
- Improved pharmacokinetic:
- Good oral activity
- High hematic concentration
- Fast tissue distribution
- Gram- and Gram+ bacteria: wider spectrum of action



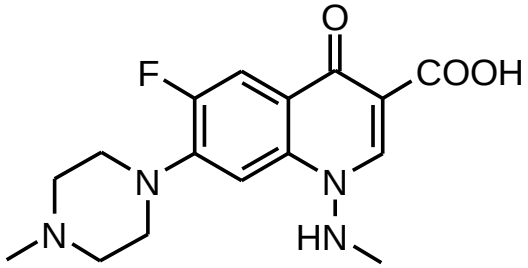
Enoxacin



Ofloxacin

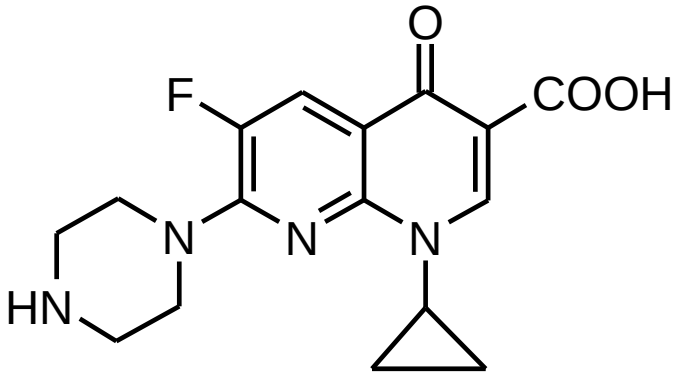


Ciprofloxacin



Amifloxacin

Ciprofloxacin



Overall, ciprofloxacin is the most potent of the currently available fluoroquinolones against Gram-negative bacteria, although some of the new fluoroquinolones currently under investigation may challenge this.

Ciprofloxacin is also active against P. aeruginosa and Acinetobacter spp. It is very active against Haemophilus influenzae, Moraxella catarrhalis and Neisseria spp., including β-lactamase-producing strains of Neisseria gonorrhoeae.

Following oral absorption the drug is widely distributed in body water, with concentrations in most tissues and in phagocytic cells approximating to those in plasma. Most of the absorbed dose can be recovered from faeces and urine.

Significant untoward reactions are uncommon; the most frequent being gastrointestinal tract disturbances (approximately 3.4%) and rashes (<1%). CNS disturbances, typical of quinolones, have been reported in 1.1% of patients.15

- Aug. 27, 2013 -- The FDA is strengthening its warning that a popular class of antibiotics, called fluoroquinolones, may cause sudden, serious, and potentially permanent nerve damage called peripheral neuropathy.
- Fluoroquinolones are antibiotics that are commonly used to treat a variety of illnesses such as respiratory and urinary tract infections. These medicines include ciprofloxacin (Cipro), gemifloxacin (Factive), levofloxacin (Levaquin), moxifloxacin (Avelox), norfloxacin (Noroxin), and ofloxacin (Floxin). More than 23 million patients received a prescription for one of them in 2011.
- Peripheral neuropathy is damage to the nerves that send information to and from the brain and spinal cord and the rest of the body. Damage interrupts this connection, and the symptoms depend on which nerves are affected. In general, the symptoms are in the arms and legs and include numbness, tingling, burning, or shooting pain.
- Peripheral neuropathy has been listed as a side effect of fluoroquinolones since 2004. There have been reports of long-lasting nerve damage and disability in patients taking this type of medication.
- A recent FDA review revealed that the existing warnings for fluoroquinolones were inadequate. The FDA's newest alert requires that all drug labels and medication guides for fluoroquinolones be updated to better emphasize the risk for serious and potentially irreversible peripheral neuropathy.

When antibiotics turn toxic

Commonly prescribed drugs called fluoroquinolones cause rare, disabling side effects. Researchers are struggling to work out why.

Nature 2018

- THE DARK SIDE OF FLUOROQUINOLONES
  Some FDA-approved fluoroquinolones were swiftly withdrawn from the market after severe adverse reactions and several deaths — trovafloxacin, withdrawn in 1999, damaged livers, for instance. But others became the drug of choice both for serious infections and for routine complaints, despite rare side effects.

LBDD and quantitative structure-activity relationship (QSAR): from HIT to LEAD

- In LBDD context, quantitative structure-activity relationship (QSAR) in which a correlation between calculated properties of molecules and their experimentally determined biological activity may be derived.
- These QSAR relationships in turn may be used to predict the activity of new analogs.
- The QSAR equation (Hansch) are generally used in lead development (from hit to lead) not in hit discovery
- We will see the general form of a QSAR equation later in the course (Hansch equation).

the quantitative structure-activity relationship (QSAR)

Multilinear regression analysis (MLRA) relating a set of independent variables (descriptors) x_{ij} for a molecule *i* with a property y_i (i.e., the pharmacological activity) of this molecule:

$$y_i = c_0 + c_1 xi_1 + c_2 xi_2 + \ldots c_n x_{in}$$

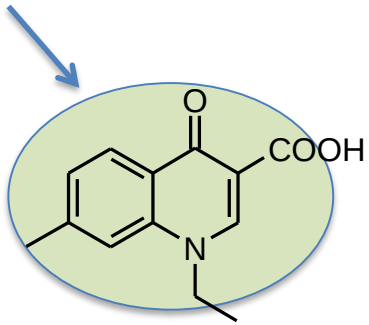
In this case, it has to be decided from the very beginning how many descriptors, *n*, will be used to represent each molecule..

Congeneric set of molecules

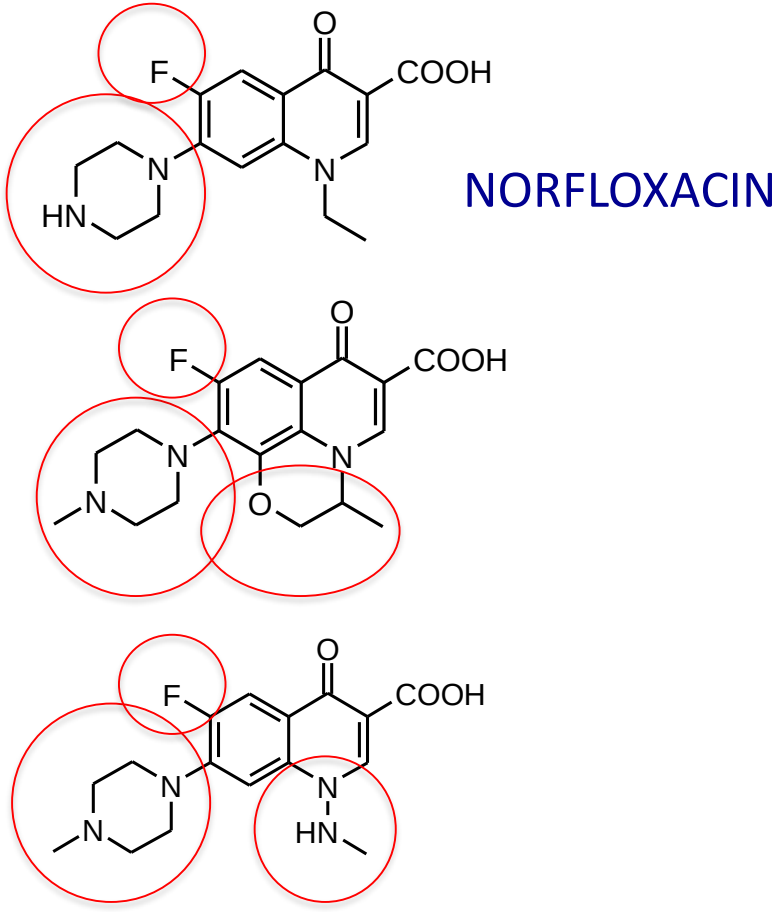
- For a congeneric set of molecules having the same skeleton/ scaffold, this might be an easy task by choosing descriptors for the various substituents on the skeleton as was used in linear free energy models such as the Hammett or Taft type of approaches.

The QSAR equation applies to a congeneric group of molecules.

Fluoroquinolone antibiotics (NEW GENERATION)



Nalidixic acid,
COMMON SCAFFOLD



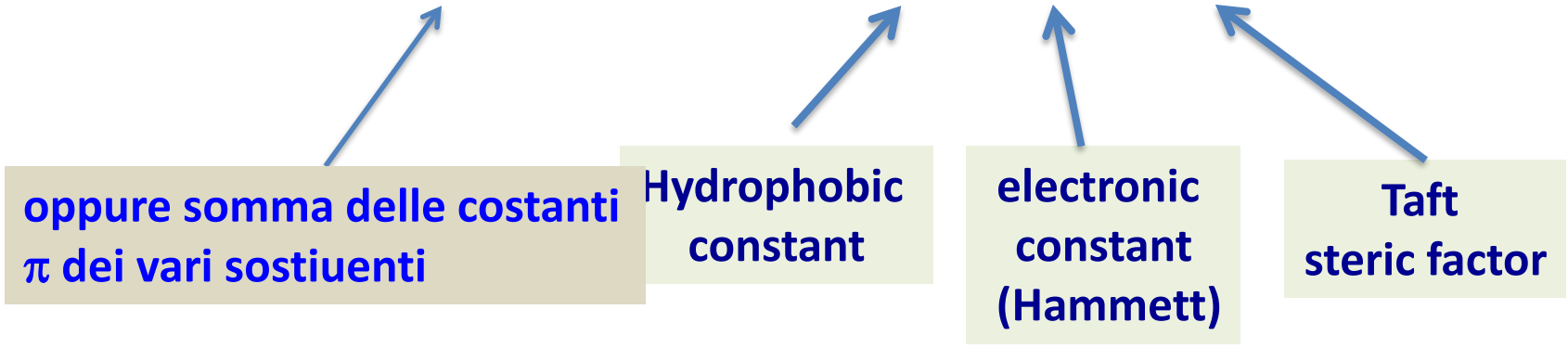
NORFLOXACIN

- Scaffold in common, substituents can vary; predicting biological activity of derivatives differing by substituents

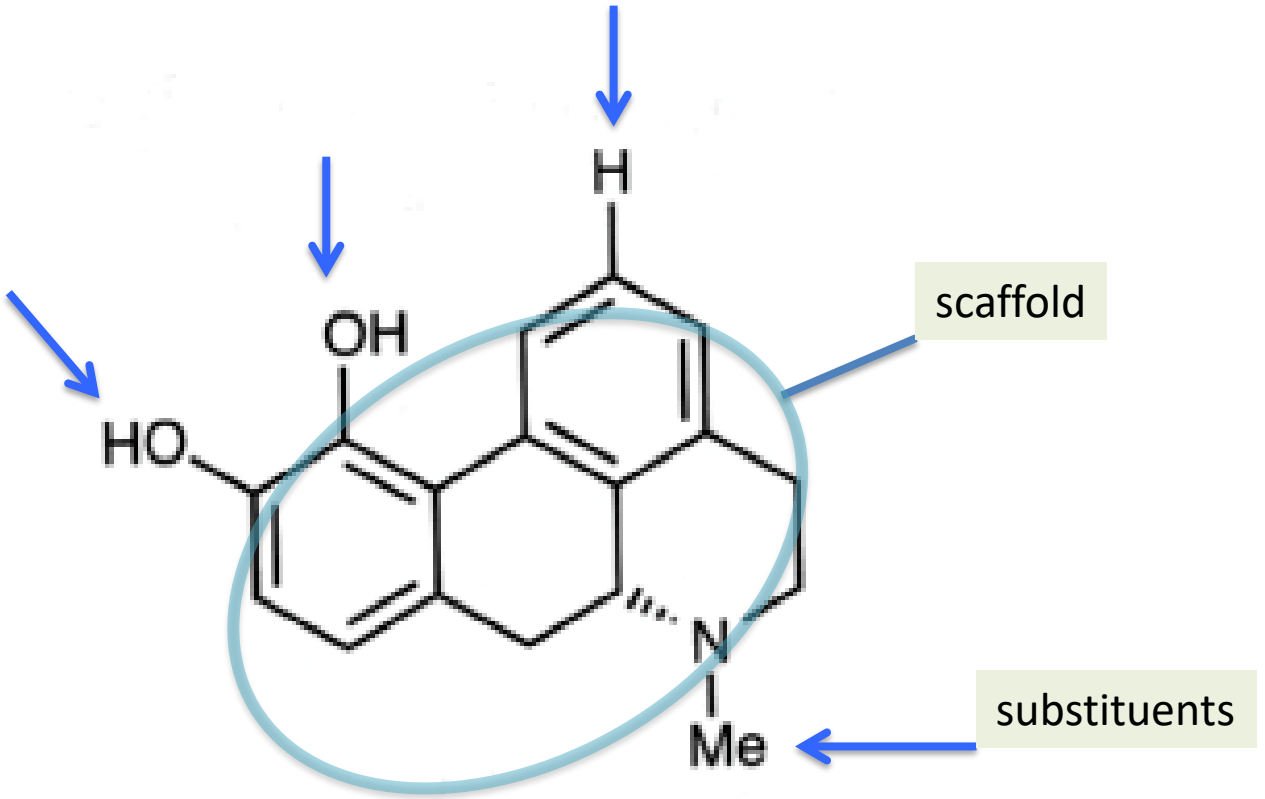
The effect of substituent on biological activity in a family of congeneric drugs: the Hansch equation, the base of QSAR.

- For a group of molecules with related structure (congeneric group), the most complete QSAR equation is the Hansch equation

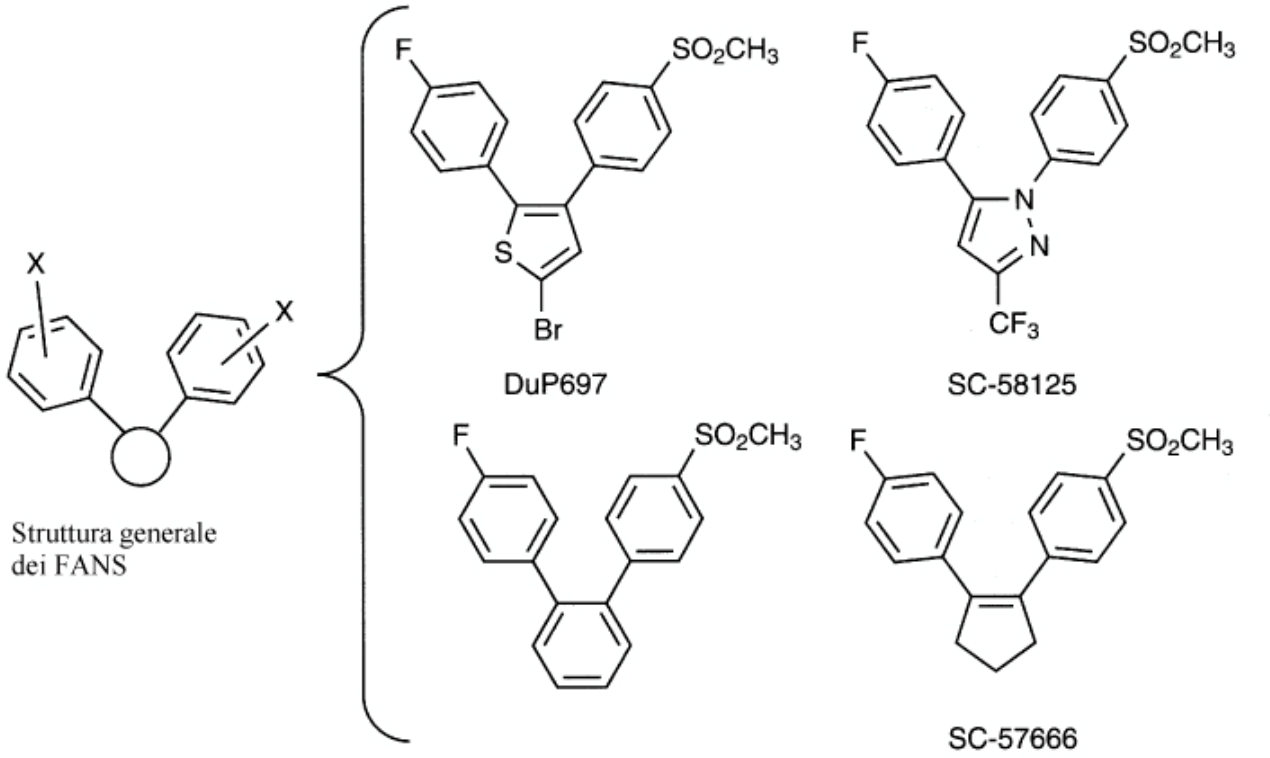
$$\text{Log } (1/IC_{50}) = -k_1 (\log P)^2 + k_2 \log P + k_3 \Sigma\pi + k_4 \Sigma\sigma + k_5 Es + k_6$$



Molecules belonging to a congeneric group have the same scaffold an do differ by the substituents



Applicability of Hansch equation



Congeneric groups of NSAID
non steroidal anti-inflammatory drugs

- **⇒ INTRODUCTION**

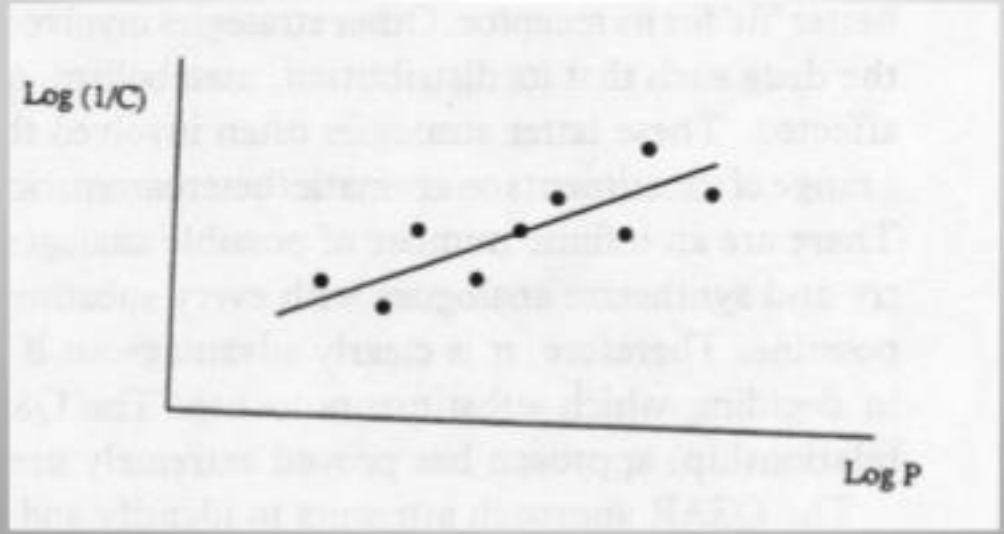
The identification of a new drug molecule requires a lot of synthesis, time and money. It was identified that out of billion molecules synthesized, around one or two molecules reach the clinical trials. This produces hurdle in the discovery new chemical entities (NCEs) for the treatment of various diseases.

The quantitative structure activity relationship approach has proved extremely useful in tackling this problem.

QSAR approach attempts to identify and quantify the physicochemical properties of a drug and to see whether any of these properties has an effect on the drug's biological activity.

Graphs and equations

A range of compounds is synthesized in order to vary one physicochemical property and to test how this affects the biological activity. A graph is then plot the biological activity 0n the y-axis versus the physicochemical feature on x-axis.



⇒PHYSICOCHEMICAL PROPERTIES

—

a) Hydrophobicity Log P (partition coefficient)

LogP = [drug] in octanol / [drug] in water

- Vary log P & see how this affects the biological activity.
- Biological activity normally expressed as 1/C, where C = [drug] required to achieve a defined level of biological activity. The more active drugs require lower concentration.

- Plot log 1/C vs. log P
- Typically over a small range of log P, *e.g*. 1-4, a straight line is obtained

$$\log 1/C = k1 \log P + k2$$

If graph is extended to very high log P values then get parabolic curve. Reasons:

- poorly soluble in aqueous phase
- trapped in fat depots
- more susceptible to metabolism

For parabolic curve

$$\text{Log } 1/C = -k (\log p)2 + k2 \log p + k3$$

- The substituent hydrophobicity constant(π)

$$\pi x = \log px - \log ph$$

Ph= partition coefficient of std compound

Px= partition coefficient for std with substituent

b) Steric Effect

The bulk, size and shape of drug will influence how easily it can approach and interact with binding site. A bulky substituent may help to orientate a drug properly for maximum binding n increase activity.

The hydrophobicity constant of substituents (π)

- The hydrophobicity constant of a given substituent indicates how much the substituent x is more hydrophobic than an hydrogen atom
- Its value is obtained by measuring the P of a standard compound with or without the substituent x:

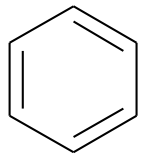
$$\pi_x = \log P_x/P_H = \log P_x - \log P_H$$

- If π_x>0 then the substituent x is more hydrophobic than an hydrogen, if π_x<0 is more hydrophilic than an hydrogen
- π values of some common substituents:

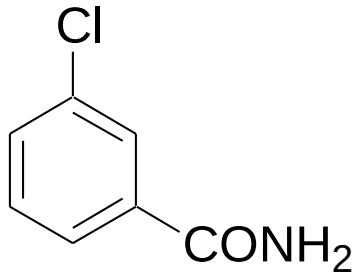
Group	CH_3	t-Bu	OH	OCH_3	CF_3	Cl	Br
π (aliphatic)	**0.50**	**1.68**	**-1.16**	**0.47**	**1.07**	**0.39**	**0.60**
π (aromatic)	**0.52**	**1.68**	**-0.67**	**-0.02**	**1.16**	**0.71**	**0.86**

Additivity of π values

$$\text{Log } P_{(\text{chlorobenzamide})} = \log P_{(\text{benzene})} + \pi_{Cl} + \pi_{CONH2}$$



BENZENE



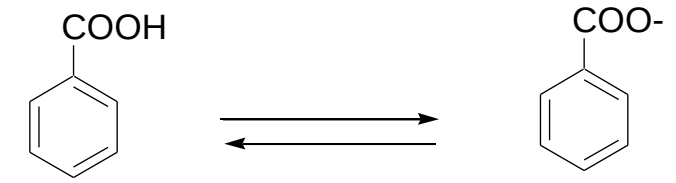
META-CLOROACETAMMIDE

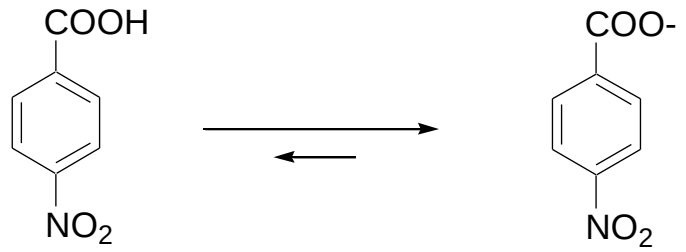
The electronic constant σ (Hammett constant)

- The electronic properties of the substituents have effect on the ionization (pKa) and polarity of the drug, and, as a consequence on pharmacokinetic properties.
- The electronic constant of Hammett (σ) is a measure of the electron-withdrawing (EW) and electron-donating (ED) properties of a substituent and is calculated measuring the dissociation constant of para-substituted benzoic acids in relation to unsubstituted benzoic acid.

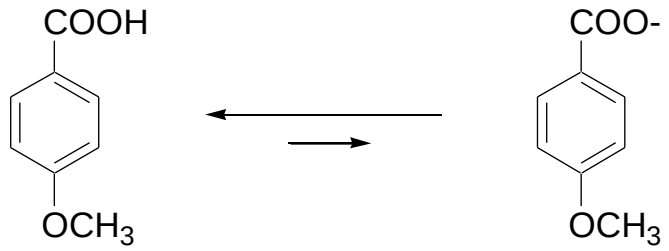
$$K_H = [PhCOO^-]/[PhCOOH]$$

The EWG groups stabilize the dissociated form (conjugate base), making Ka to increase (pKa decreases), while ED destabilize the dissociated form (Ka decreases, pKa increases).



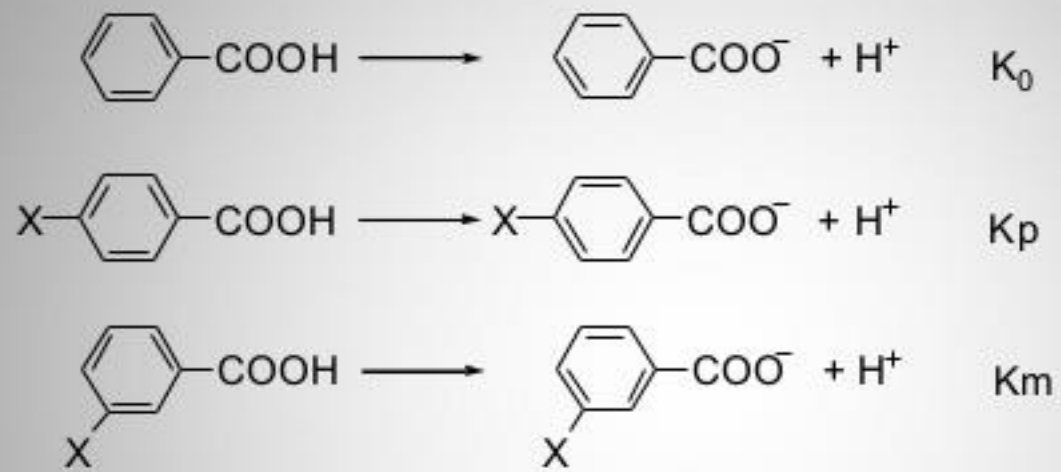


NO_2: EWG



OCH_3: EDG

Examples

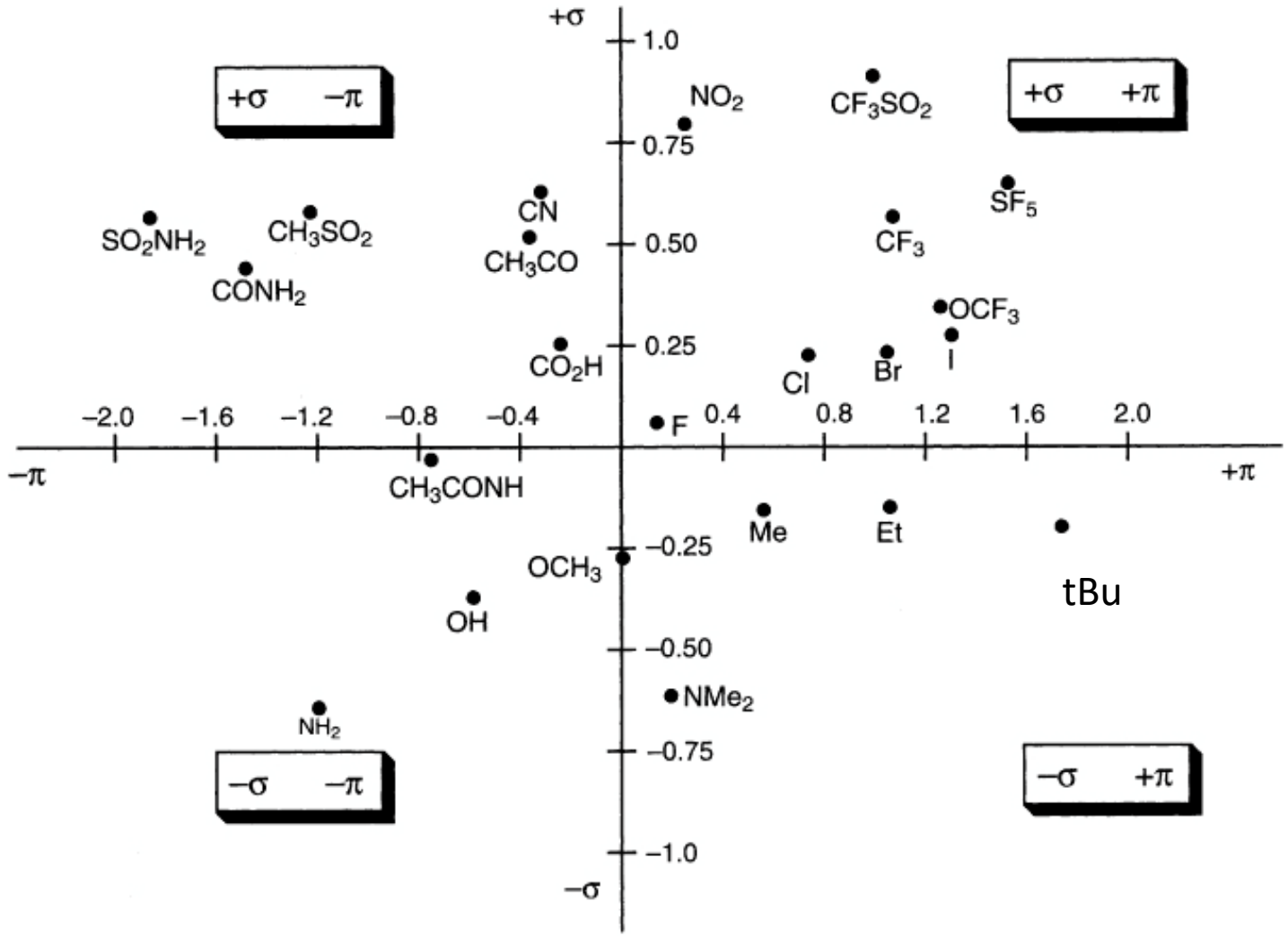


$$\sigma_{para} = \log_{10} \frac{Kp}{K_0}$$

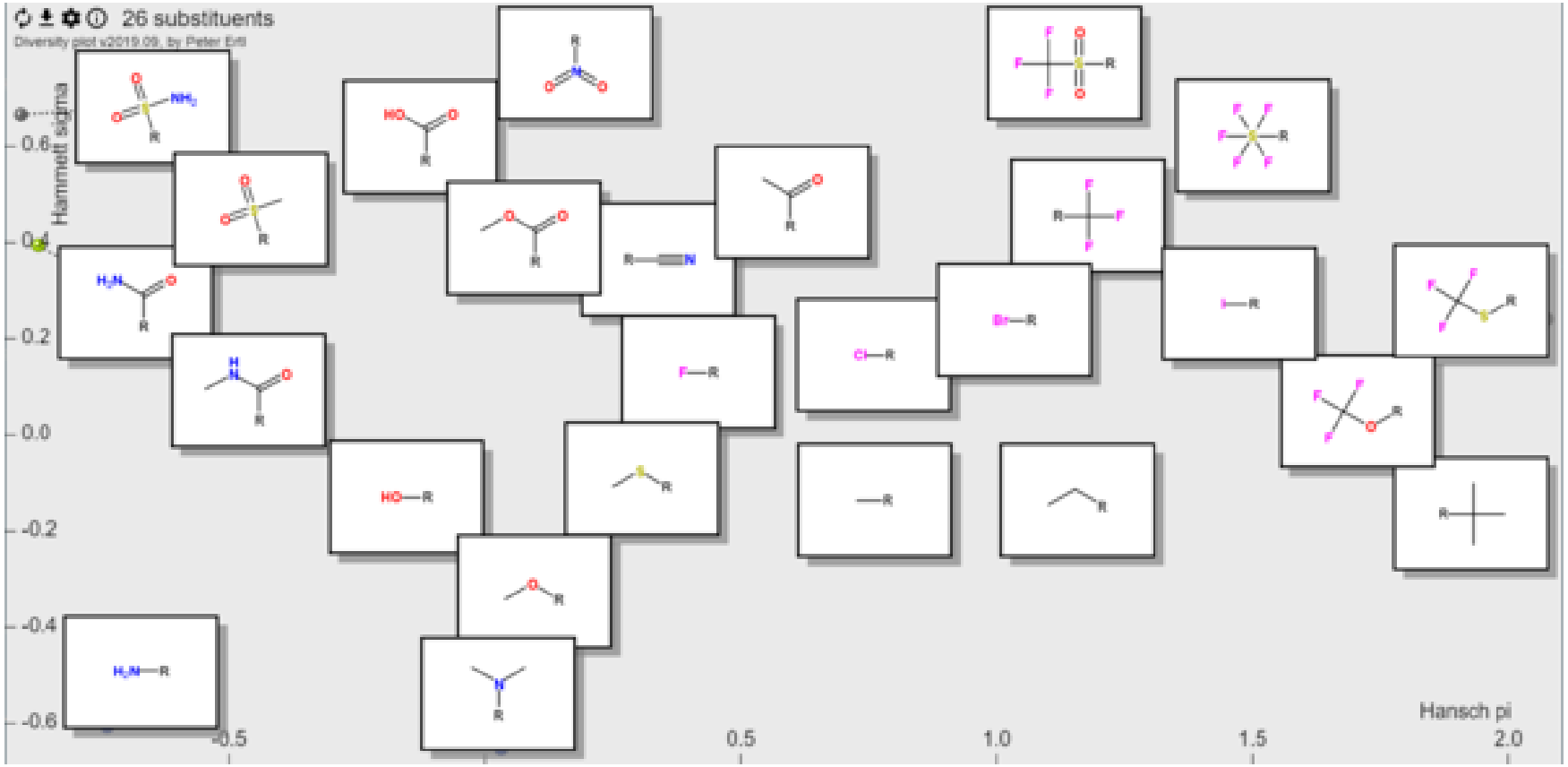
$$\sigma_{meta} = \log_{10} \frac{Km}{K_0}$$

- $\sigma_x = \log Kx/K_H = \log Kx - \log K_H$
- $\sigma_x > 0$ if x is a EWG
- $\sigma_x < 0$ if x is a EDG
- The Hammet constant accounts for inductive and resonance effects, its value will depend on the position of the substituent on the aromatic ring (m or p).
- For instance the NO_2 group has $\sigma_p = 0.78$, $\sigma_m = 0.71$.
- The OH group has $\sigma_p = -0.37$, $\sigma_m = 0.12$

The Craig diagram



Craig diagram



Examples are:

Taft's steric factor (Es) (~1956), the value for Es can be obtained by comparing the rates of hydrolysis of substituted aliphatic esters against a std ester under acidic conditions.

$$Es = \log kx - \log k0$$

Kx represents rate of hydrolysis of an aliphatic ester having substituent X

K0 represent rate of hydrolysis of reference ester.

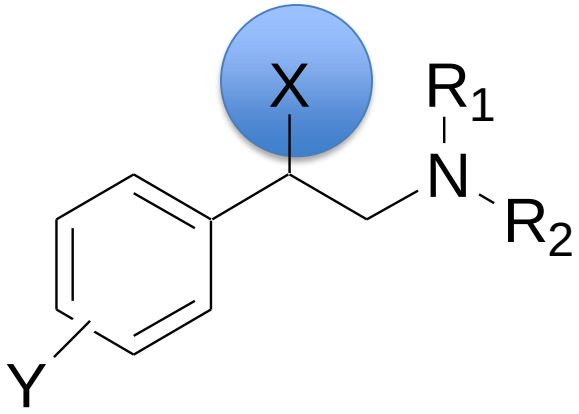
Exemples of Hansch equations

Congeneric group of β-haloarylamines as beta-blockers (anti-aritmic drugs): the activity depends on π e σ of substituent x:

$$\log (1/C) = 1.22\pi - 1.59\sigma + 7.89$$

This equation suggests that the biological activity increases with π and decreases with σ.

Optimal substituents: π positive, σ negative

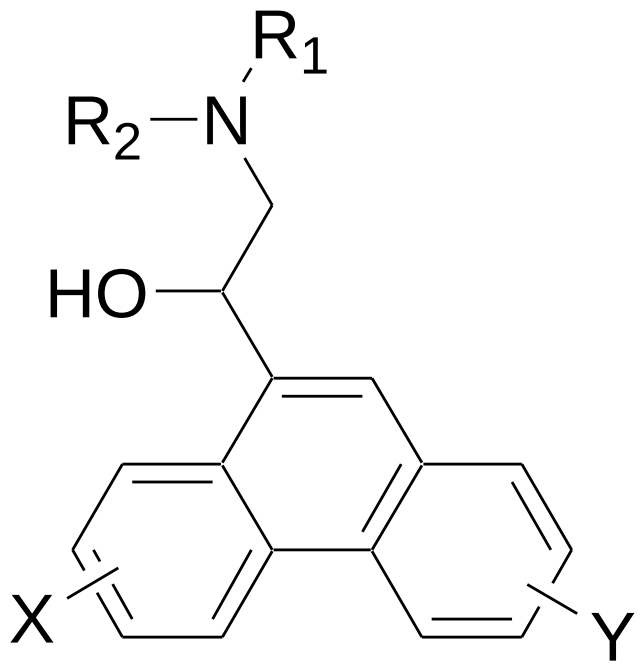


Family of fenantren-aminocarbinols (antimalaric drugs) has the following equation.

Different substituent X and Y into the aromatic rings have an additive effect on biological activity:

$$\text{Log } (1/C) = -0.015\ (\pi_{sum})^2 + 0.14\ \pi_{sum} + 0.27\ \Sigma\pi_x + 0.40\ \Sigma\pi_Y + 0.65$$

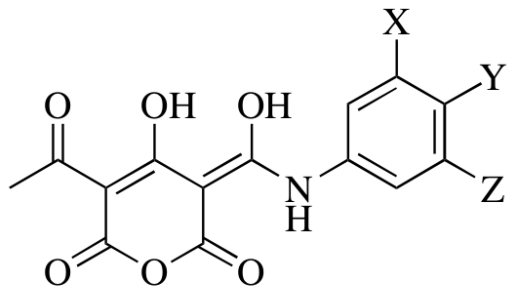
$$\Sigma\sigma_x + 0.88\ \Sigma\sigma_Y + 2.34$$



π_{sum} is equivalent to logp

Exercise

1) Pour la famille de molécules avec la formule suivante :



on a calculé expérimentalement la suivante équation de Hansch :

$\log 1/C = -0{,}14\ \Sigma\pi + 1{,}35\ \Sigma\sigma - 0{,}72$

Suggérer des substituants qui maximisent l'activité biologique dans ce type de composés

Application of QSAR

- Diagnosis of MOA of drug.
- Prediction of activity.
- Prediction of toxicity.
- Lead compound optimization.
- Environmental chemistry.

Advantages

- Quantifying the relationship between structure and activity provides an understanding of the effect of structure on activity.
- It is also possible to make predictions leading to synthesis of novel analogues.
- The results can be used to help understand interaction between functional groups in the molecules of greatest activity with those of their target

Disadvantages

- False correlations because biological data that are considerable experimental error.
- If training dataset is not large enough , the data collected may not reflect the complete property

- Features may not be reliable. This is particularly serious for 3D features because 3D structures of ligands binding to receptor may not available

Limitations of Hansch analysis

- 1. There must be parameter values available for the substituent's in the data set
- 2. A large number of compounds is required.
- 3. Depends on biological results (Chance of error)
- 4. Interrelationship of parameters
- 5. Groups should be selected in such a way that it should contain at least one representative from each cluster.

6. Lead optimization technique, not a lead discovery technique.
7. Risk of failure in “too far outside” predictions