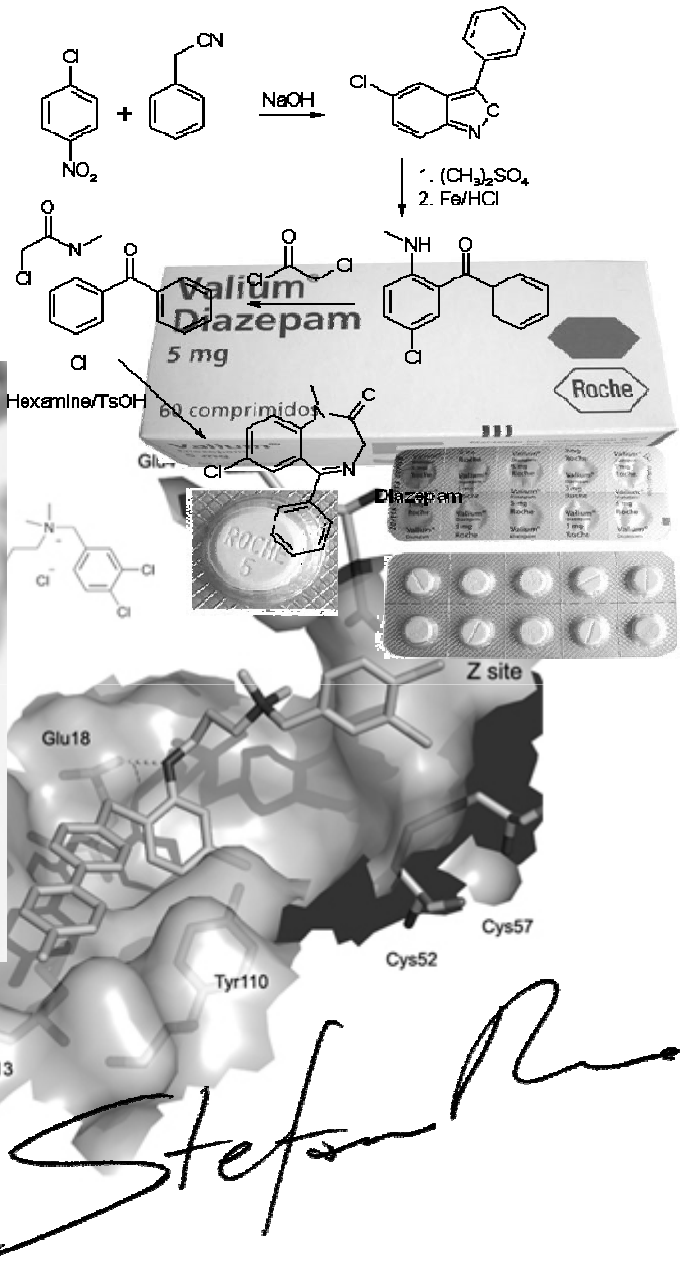
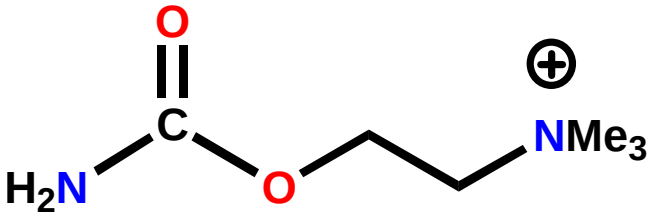
Chimica Farmaceutica e Tossicologica – Parte II



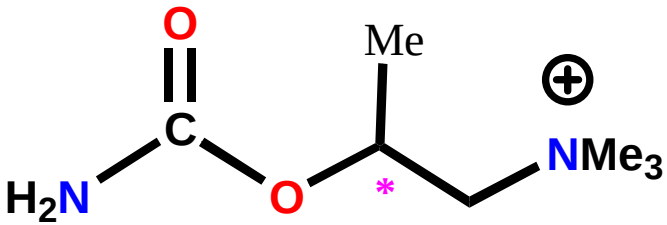
COLINERGICI, ANTICOLINERGICI & ANTICOLINESTERASICI

Parte III

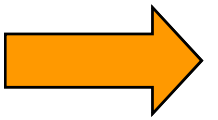
An alternative to cholinergic agonists?



Carbachol

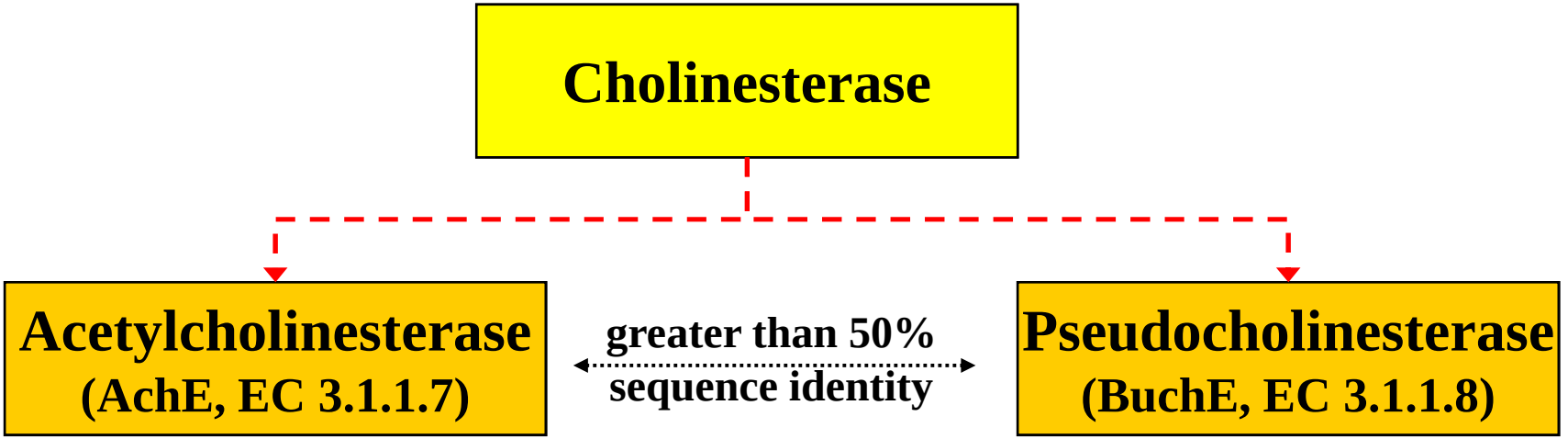


Bethanechol



14. Acetylcholinesterase

14.1 Cholinesterase classification



It is mainly found at:

- ***neuromuscular junctions;***
- ***cholinergic nervous system;***
- ***red blood cell membranes.***

It is mainly found at:

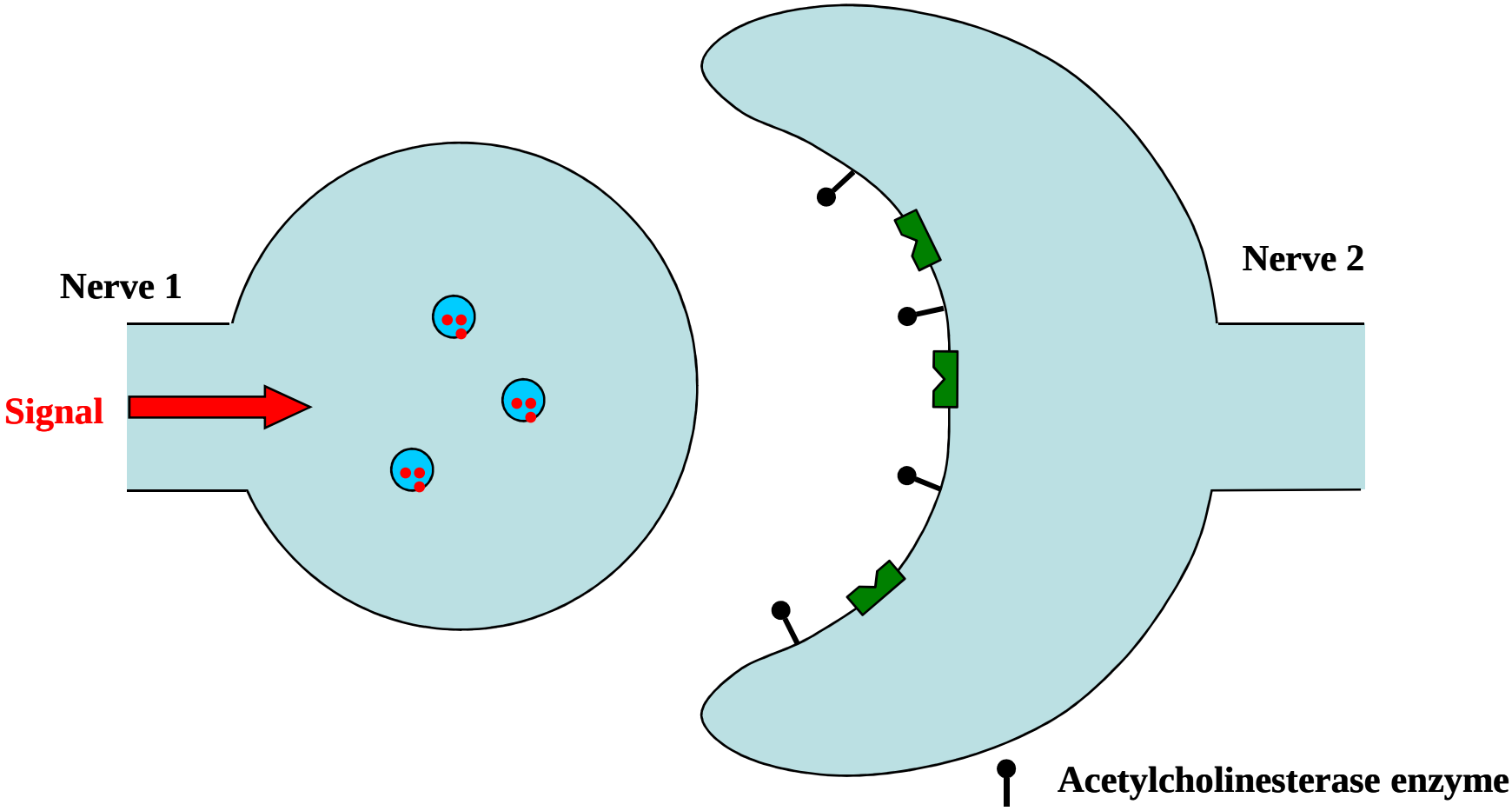
- ***plasma;***
- ***liver (where it is synthesized);***
- ***pancreas.***

Pseudocholinesterase is also called *butyrylcholinesterase*. Butyrylcholine is a synthetic compound and does not occur in the body naturally. It is involved in the metabolism of a few drugs, including the following: *succinylcholine, mivacurium, procaine, cocaine, heroin (diacetylmorphine) and aspirin.*

14. Acetylcholinesterase

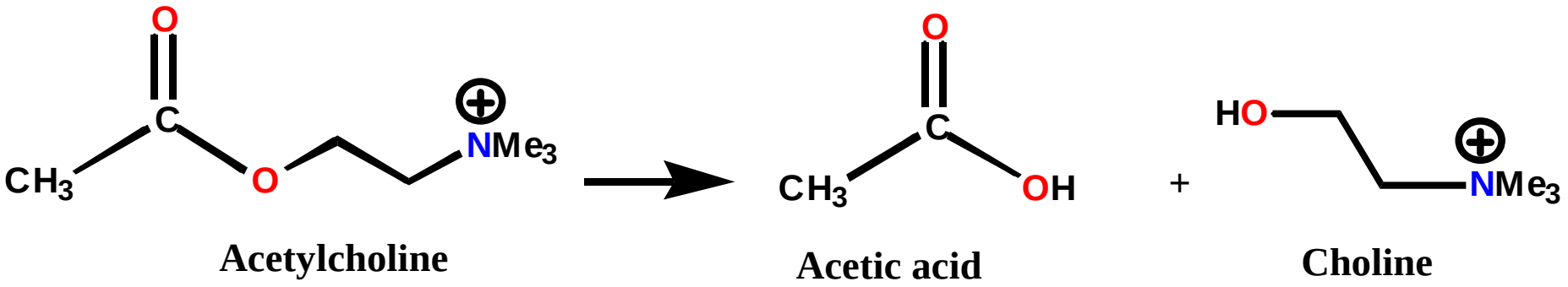
14.1 Role

- **Hydrolysis and deactivation of acetylcholine**
- **Prevents acetylcholine reactivating receptor**



14. Acetylcholinesterase

14.2 Hydrolysis reaction catalysed

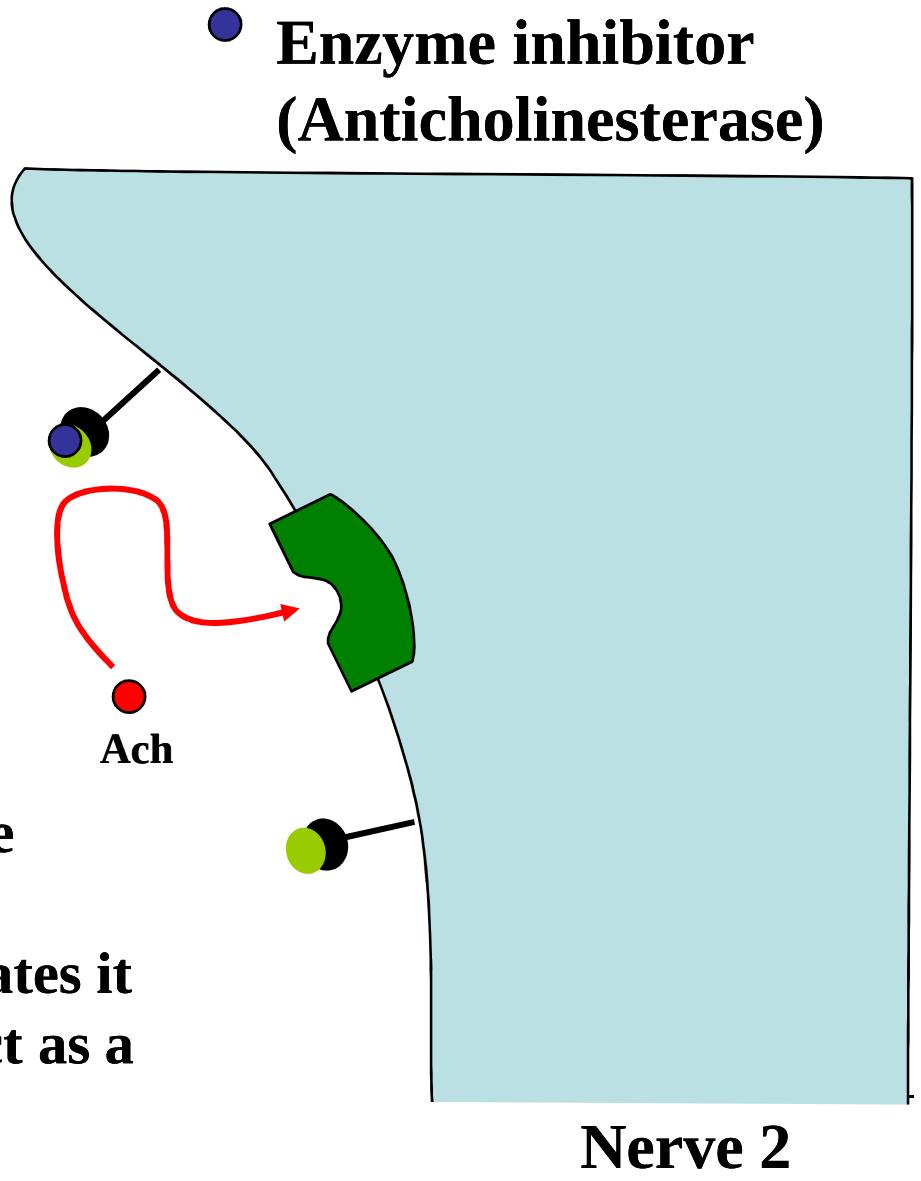


active **inactive**

Acetylcholinesterase has one of the fastest reaction rates of any of our enzymes, breaking up each molecule in about 80 microseconds.

14. Acetylcholinesterase

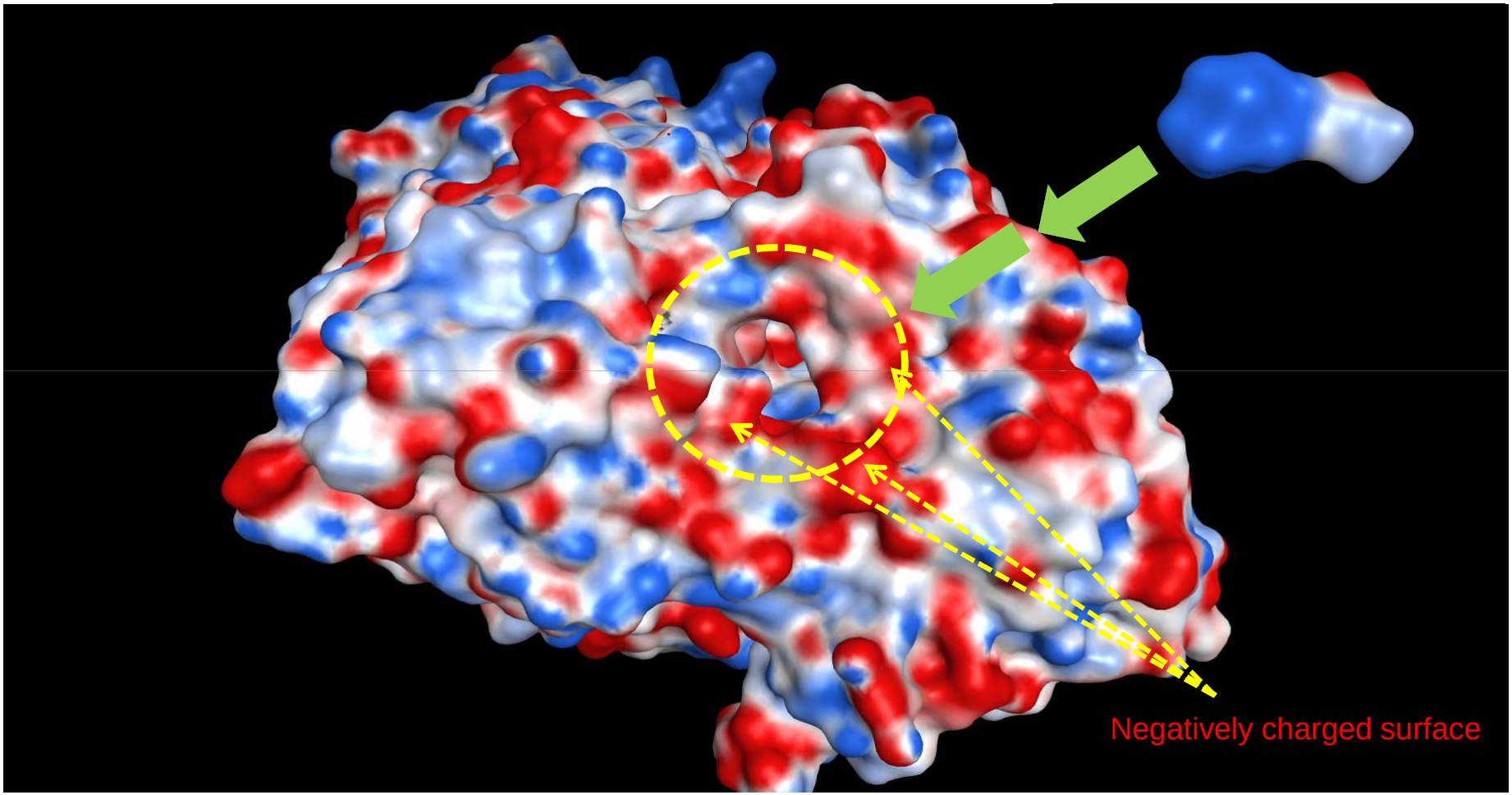
14.3 Effect of inhibition



- Inhibitor blocks acetylcholinesterase
- Ach is unable to bind
- Ach returns to receptor and reactivates it
- Enzyme inhibitor has the same effect as a cholinergic agonist

14. Acetylcholinesterase

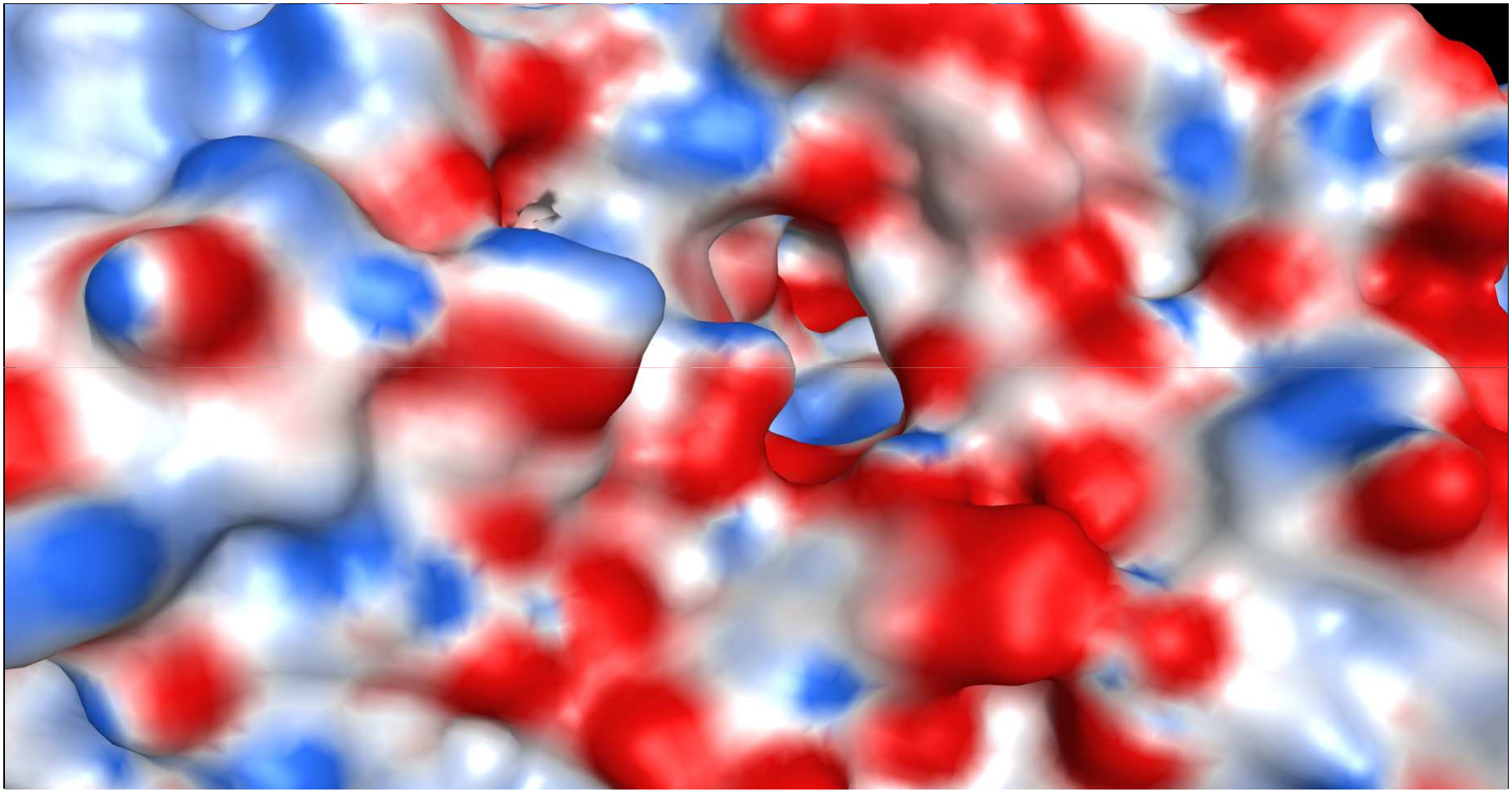
14.4 Structure of enzyme complex



Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil (PDB code: 4EY7); J.Med.Chem. 55: 10282-10286, 2012

14. Acetylcholinesterase

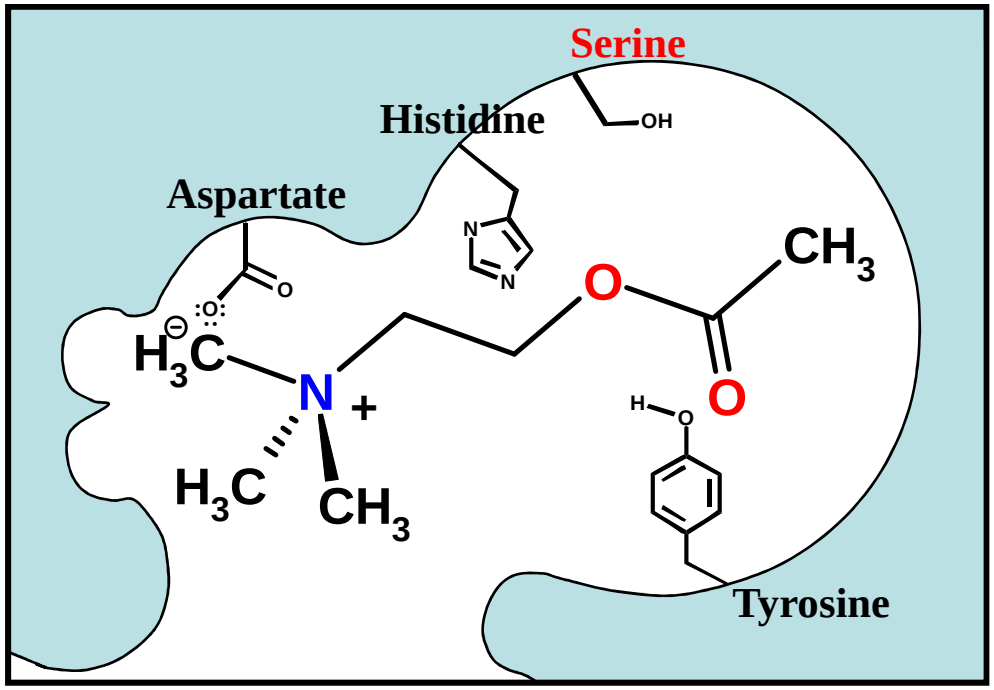
14.4 Structure of enzyme complex



Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil (PDB code: 4EY7); J.Med.Chem. 55: 10282-10286, 2012

14. Acetylcholinesterase

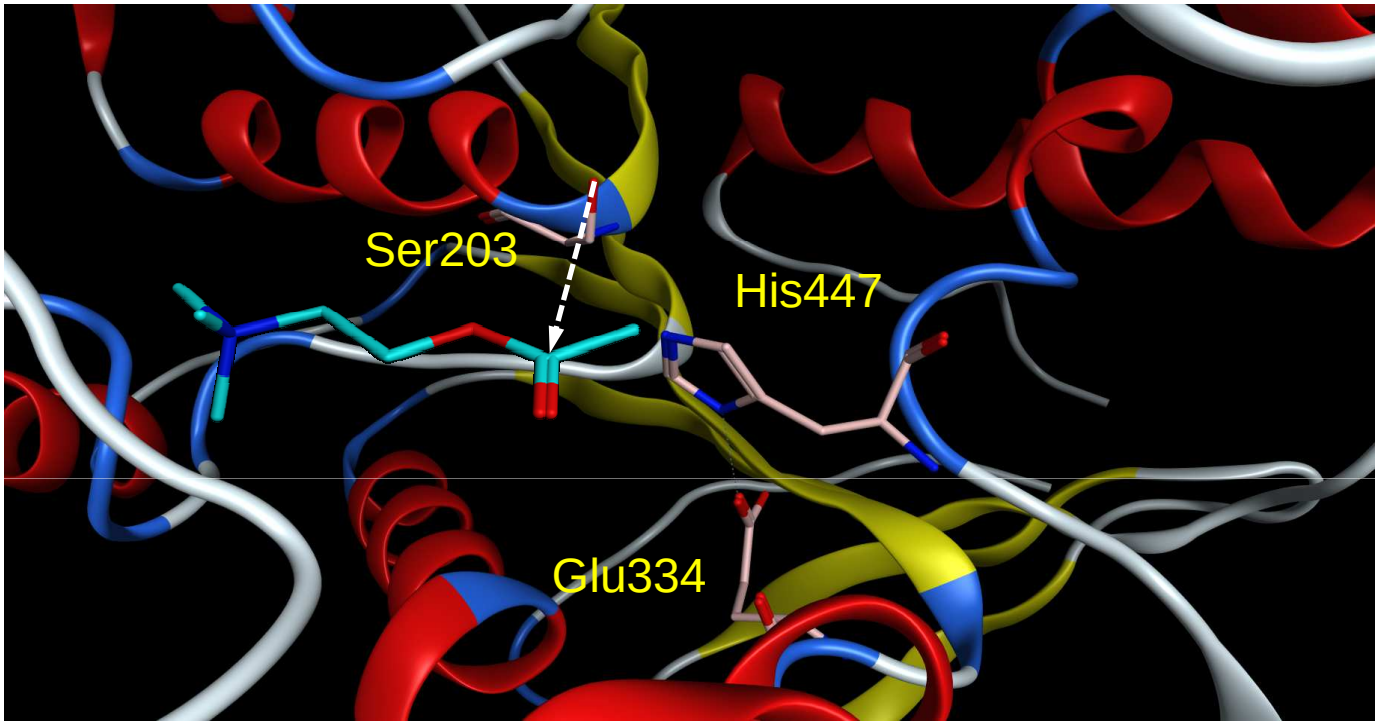
14.5 Active site - binding interactions



- **Anionic binding region similar to cholinergic receptor site**
- **Binding and induced fit strains Ach and weakens bonds**
- **Molecule positioned for reaction with His and Ser**

14. Acetylcholinesterase

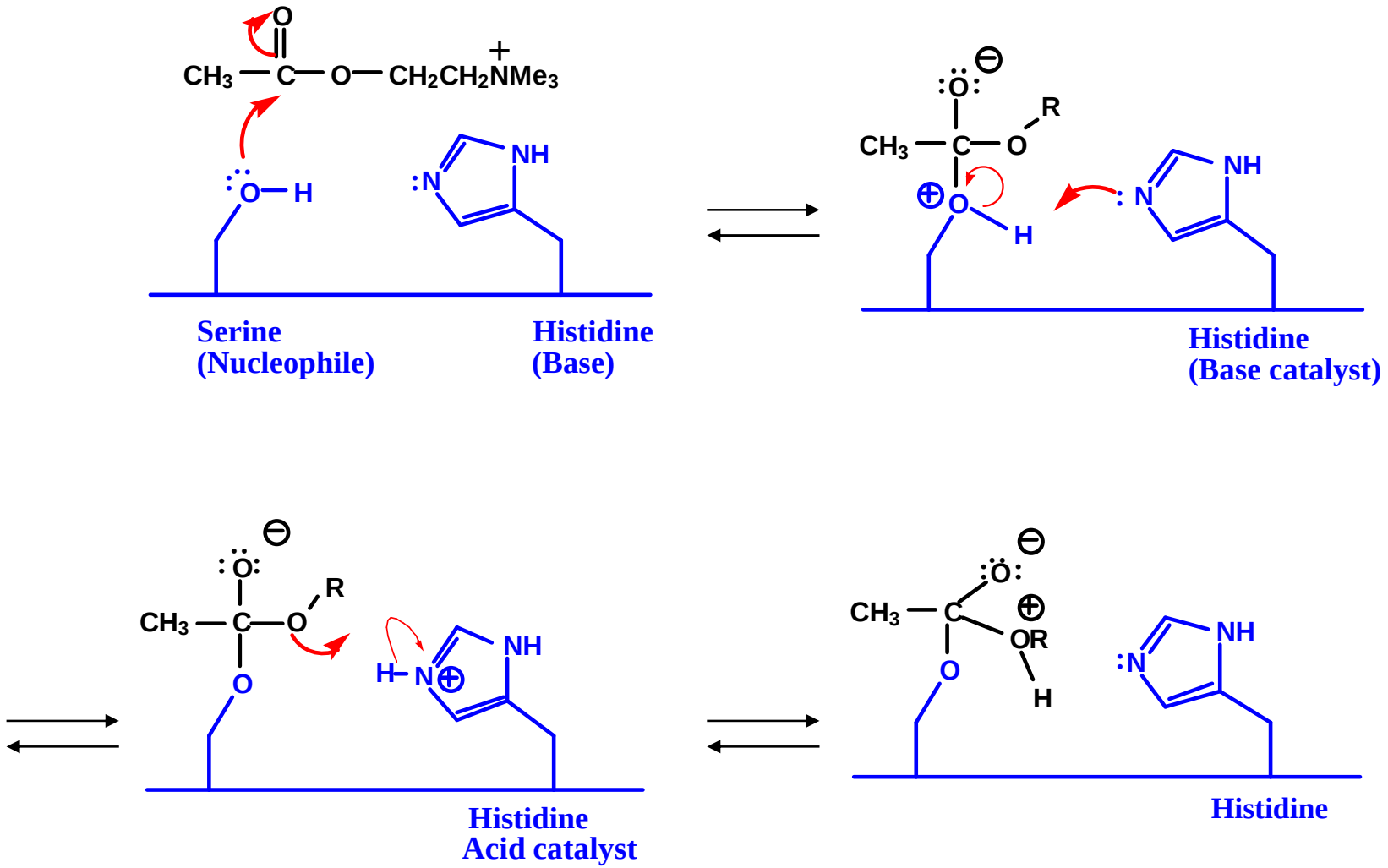
14.5 Active site - binding interactions



The initial structural study of AChE revealed a 20 Å deep active site gorge. The catalytic site is located at the base of the gorge and contains the catalytic triad (**His447**, **Glu334**, and **Ser203** in human AChE). A second or peripheral site extends beyond **Tyr337** (human AChE) at the catalytic/peripheral site interface to the entrance of the gorge and contains numerous aromatic side chains. Kinetic and thermodynamic studies have shown that inhibitors can interact with either or both of the two binding sites in AChE. The peripheral site contributes to catalytic efficiency by ensuring that most substrate molecules that collide with and transiently bind to the peripheral site proceed on to the catalytic site. It also provides a modest allosteric activation of the acylation step of catalysis with certain bound cationic substrates.

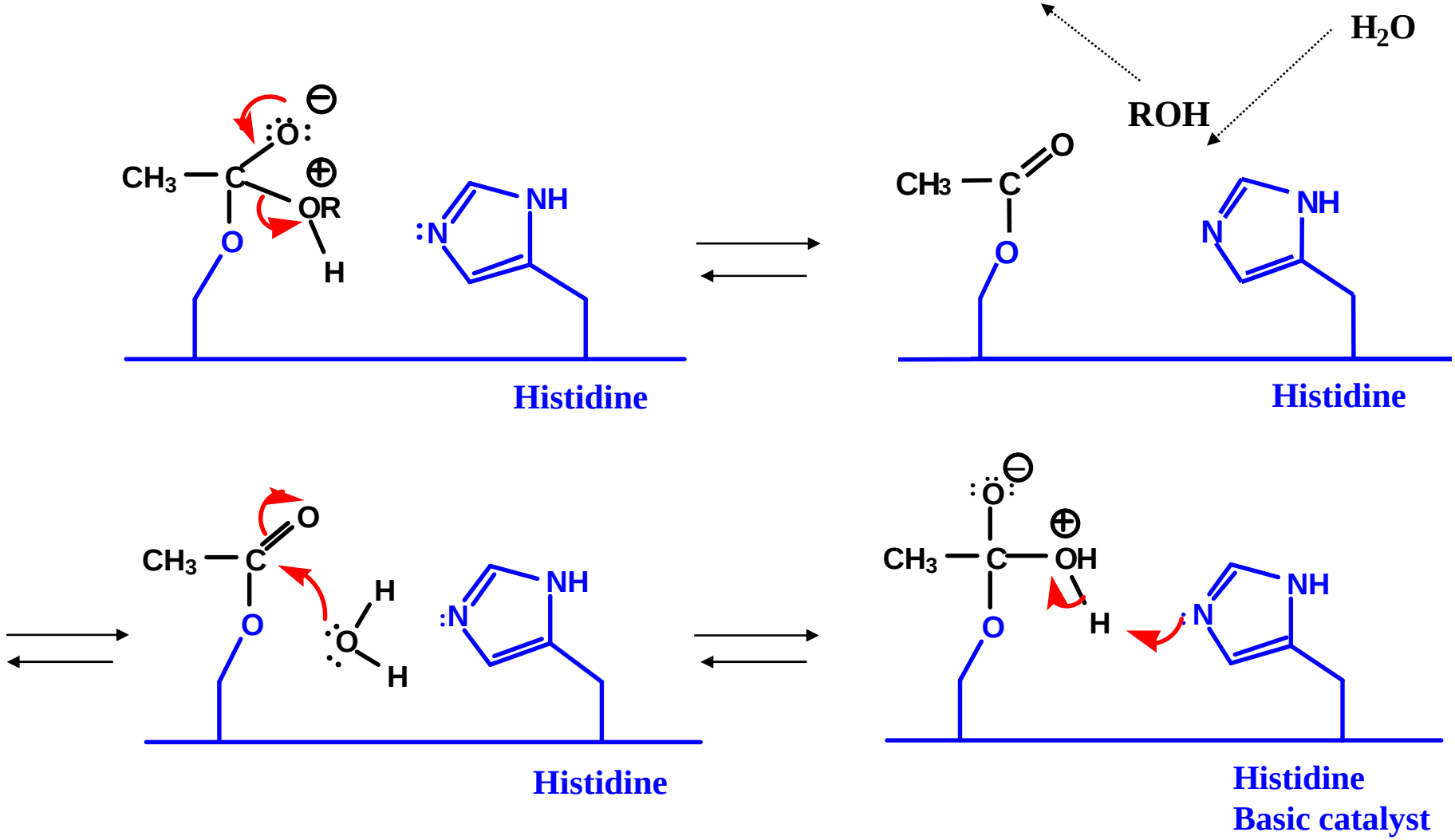
14. Acetylcholinesterase

14.6 Active site - Mechanism of catalysis



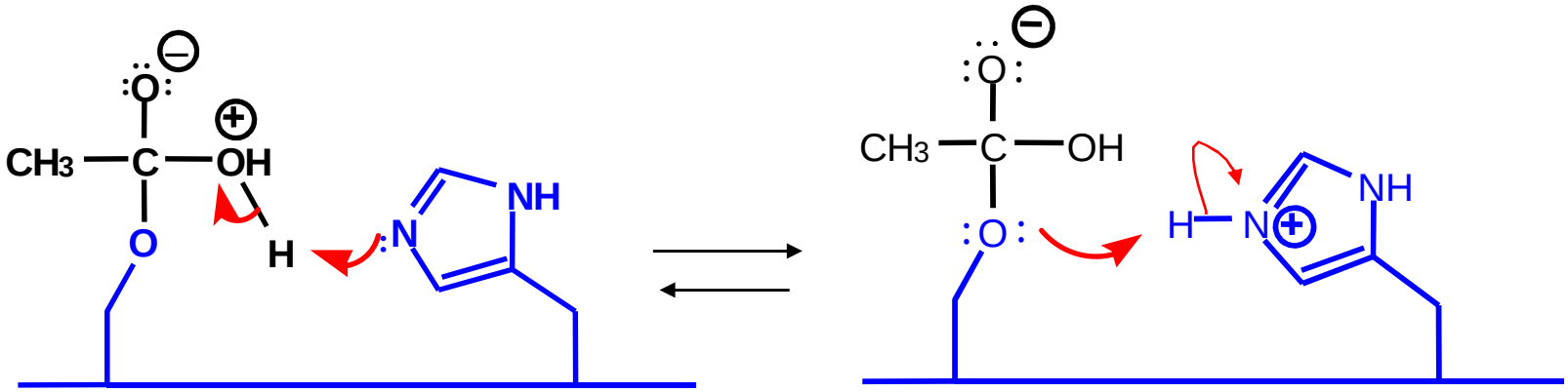
14. Acetylcholinesterase

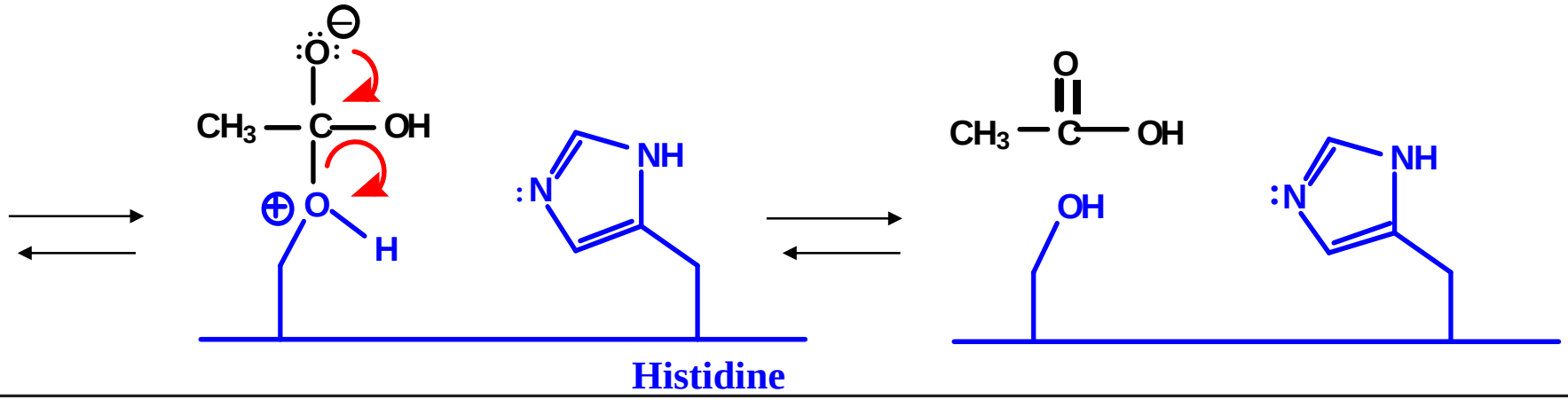
14.6 Active site - Mechanism of catalysis



14. Acetylcholinesterase

14.6 Active site - Mechanism of catalysis





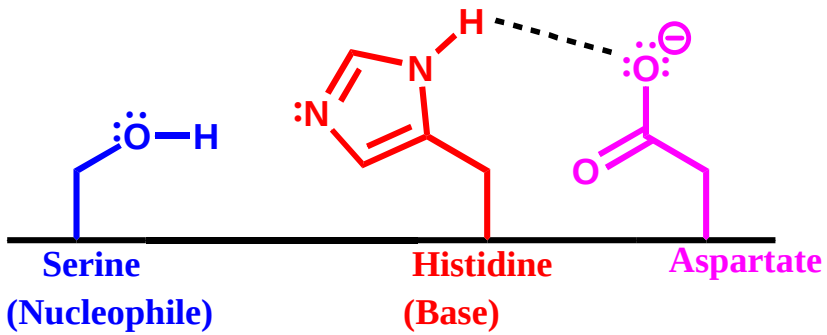
14. Acetylcholinesterase

- **Serine and water are poor nucleophiles**
- **Mechanism is aided by histidine acting as a basic catalyst**
- **Choline and serine are poor leaving groups**
- **Leaving groups are aided by histidine acting as an acid catalyst**
- **Very efficient - 100 x 10^6 faster than uncatalysed hydrolysis**
- **Acetylcholine hydrolysed within 100 μsecs of reaching active site**
- **An aspartate residue is also involved in the mechanism**

14. Acetylcholinesterase

The catalytic triad

- **An aspartate residue interacts with the imidazole ring of histidine to orientate and activate it**

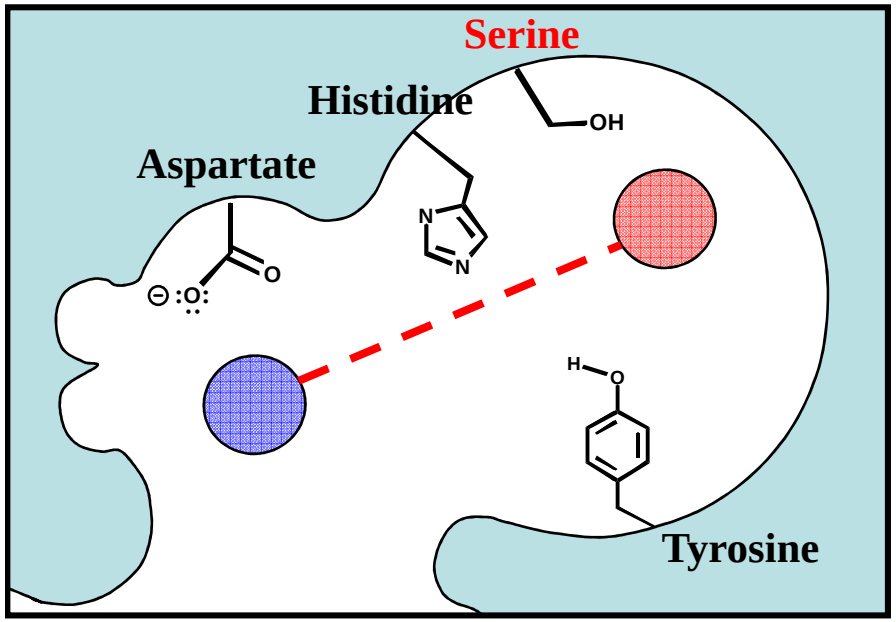


15. Anticholinesterases

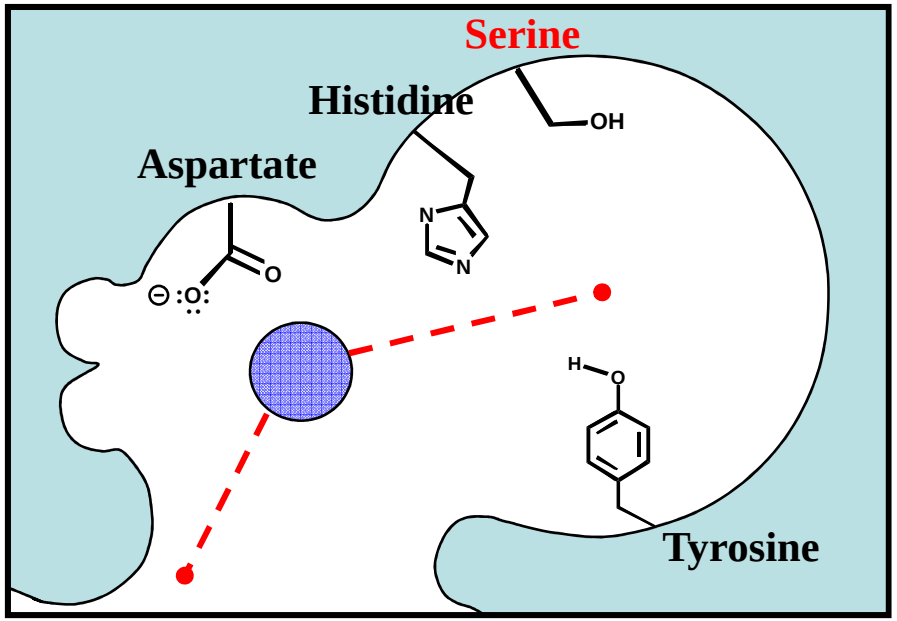
- **Inhibitors of acetylcholinesterase enzyme**
- **Block hydrolysis of acetylcholine**
- **Acetylcholine is able to reactivate cholinergic receptor**
- **Same effect as a cholinergic agonist**

In principle, we have at least two possible strategies:

Design competitive substrates



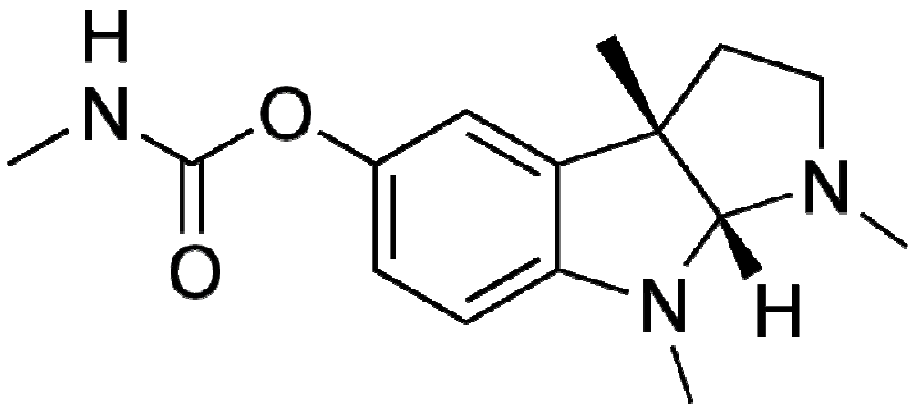
Design competitive inhibitors



… looks at mother nature!

15. Anticholinesterases

15.1 Physostigmine (eserine)



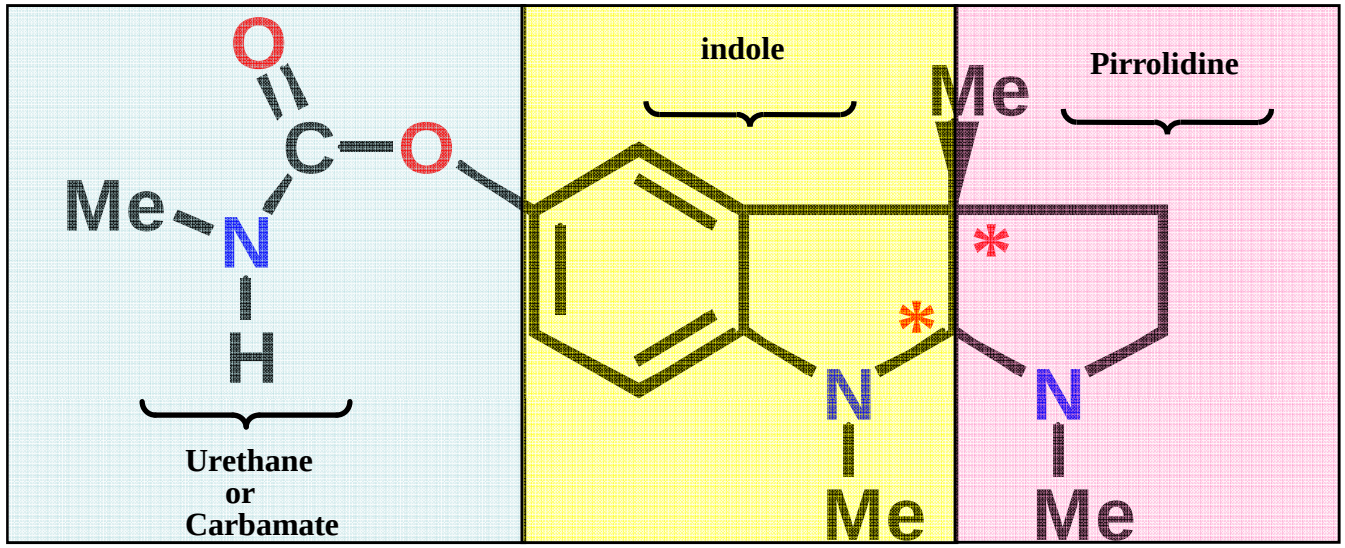
Physostigma venenosum

- **Natural product from the Nigerian calabar bean**

In 1934, Dr. Mary Walker of London conducted a successful trial of physostigmine in a patient with *myasthenia gravis*. Dr. Walker discovered that a subcutaneous injection of physostigmine temporarily restored muscle function in a patient with myasthenia gravis. At that time, physostigmine was used as an antidote for curare poisoning.

15. Anticholinesterases

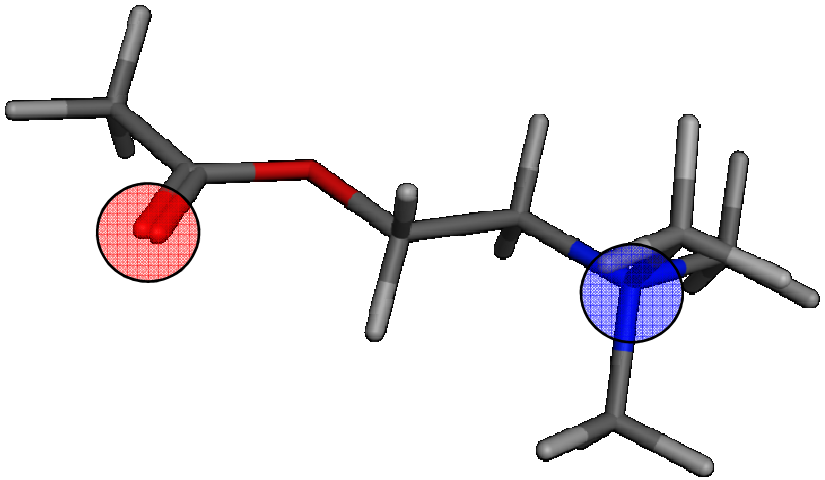
15.1 Physostigmine (eserine)



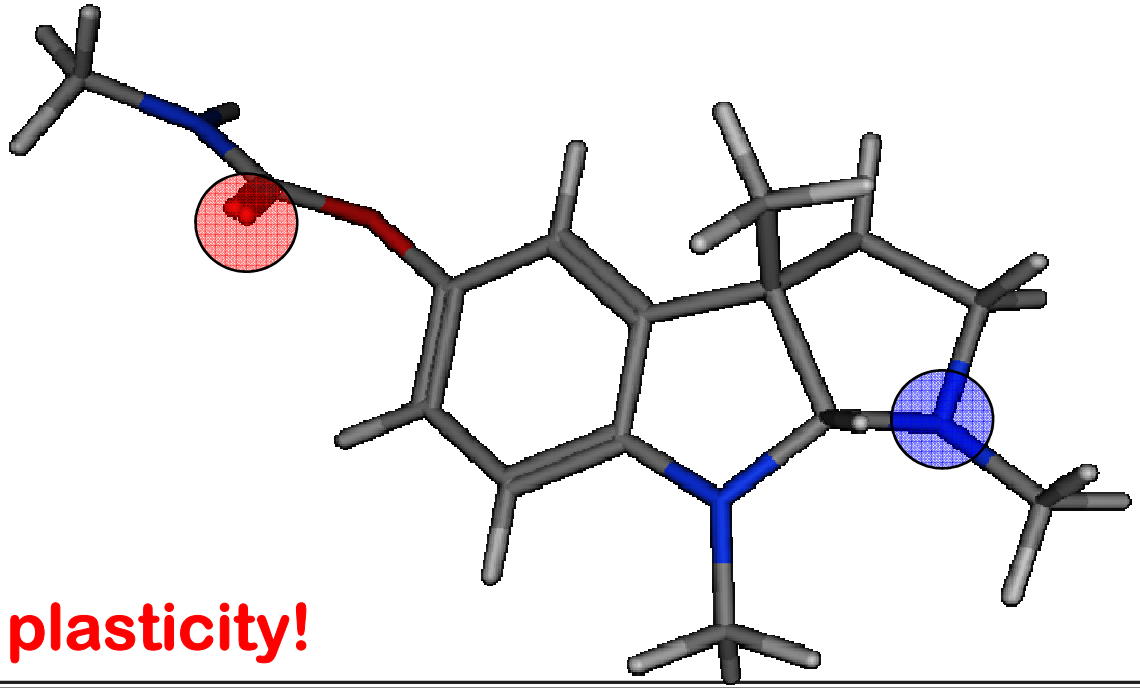
[(3aR,8bS)-3,4,8b-trimethyl-2,3a-dihydro-1H-pyrrolo[2,3-b]indol-7-yl] N-methylcarbamate

- **Carbamate is essential (equivalent to ester of Ach)**
- **Aromatic ring is important**
- **Pyrrolidine N is important (ionised at blood pH)**
- **Pyrrolidine N is equivalent to the quaternary nitrogen of Ach**

Where are the analogies with Ach?

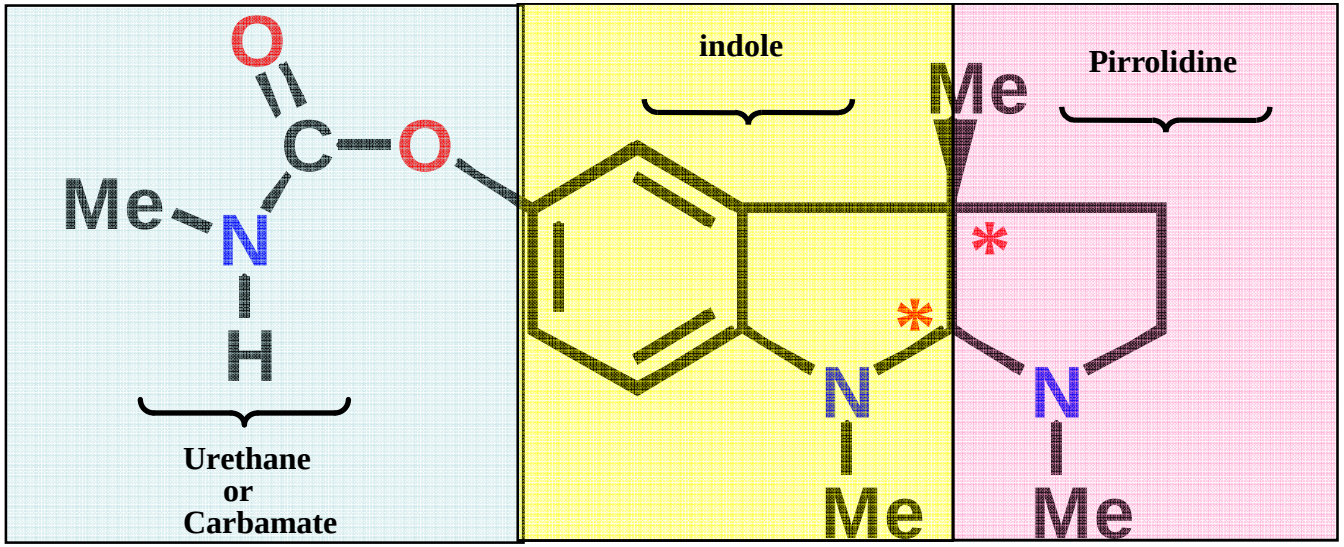


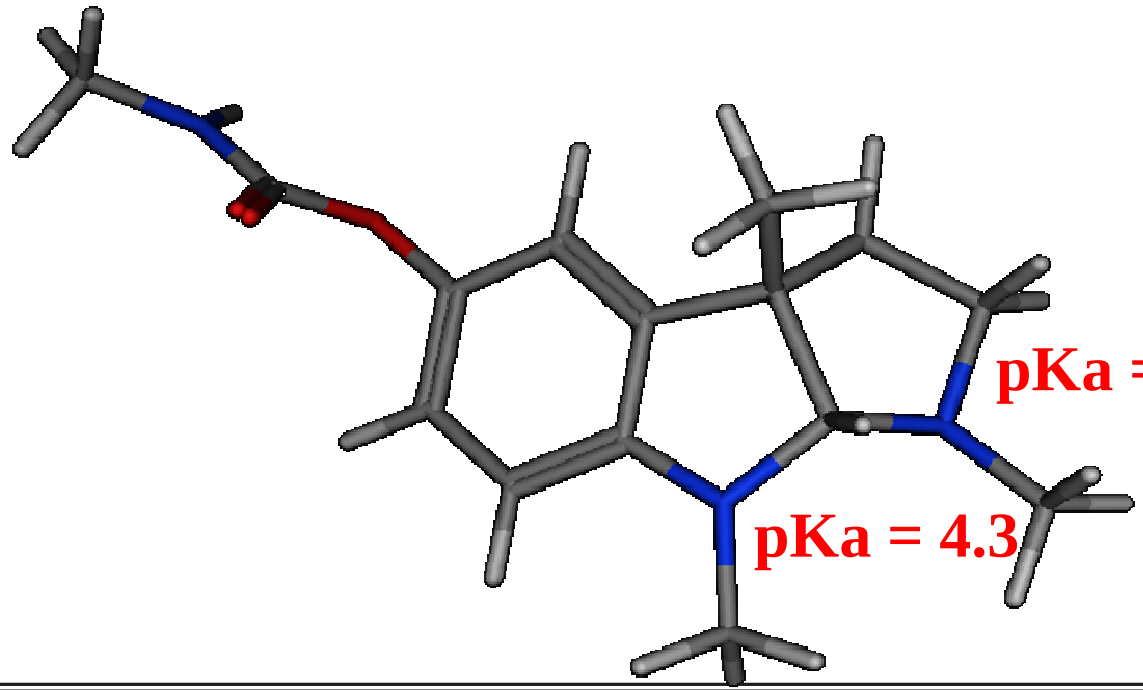
5.2 Å



7.8 Å

Enzymatic plasticity!

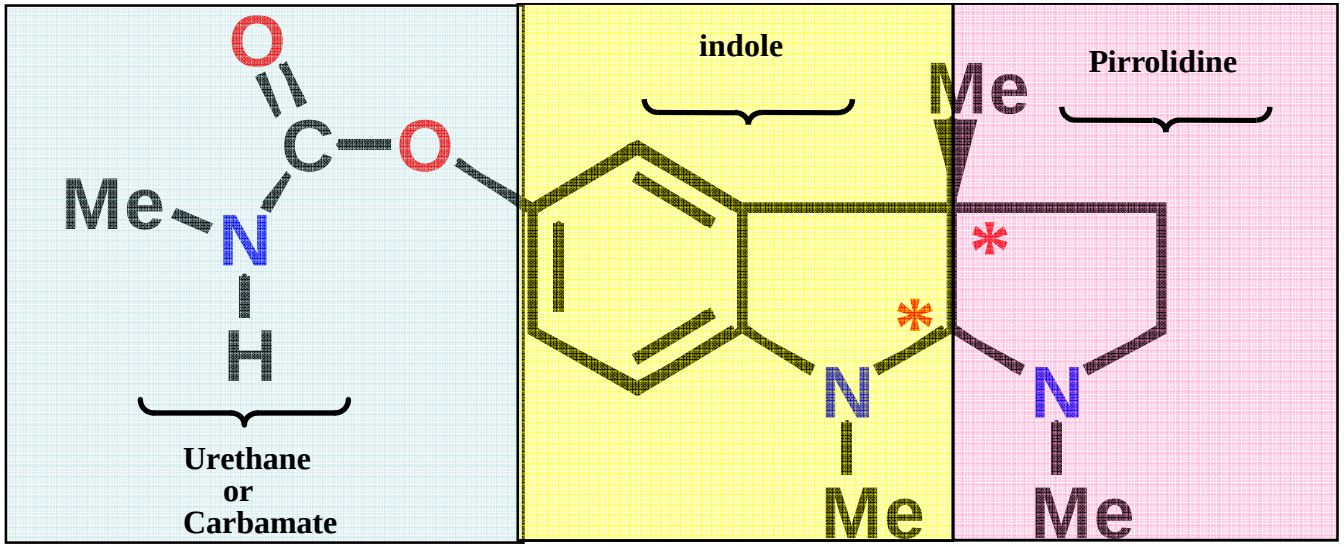




logP = 1.65

pKa = 8.3

pKa = 4.3

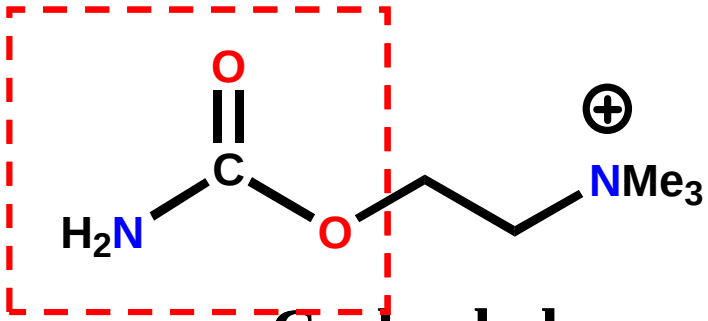


•**Physostigmine is used to treat *myasthenia gravis*, *glaucoma*, *Alzheimer's disease* and *delayed gastric emptying*.**

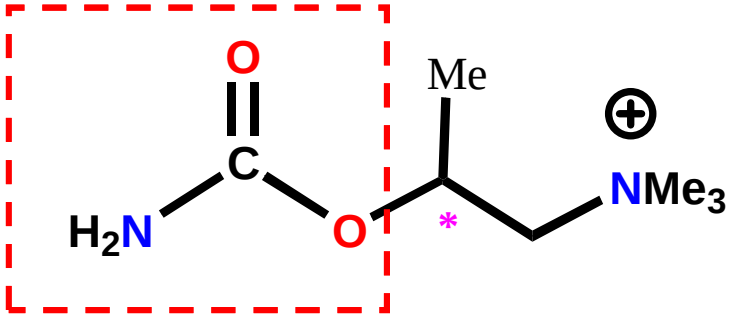
•**It can cross the blood-brain barrier, and physostigmine salicylate is used to treat the central nervous system effects of *atropine*, *scopolamine* and other *anticholinergic drug overdoses*.**

•**Other side effects may include nausea, vomiting, diarrhea, anorexia, dizziness, headache, stomach pain and sweating.**

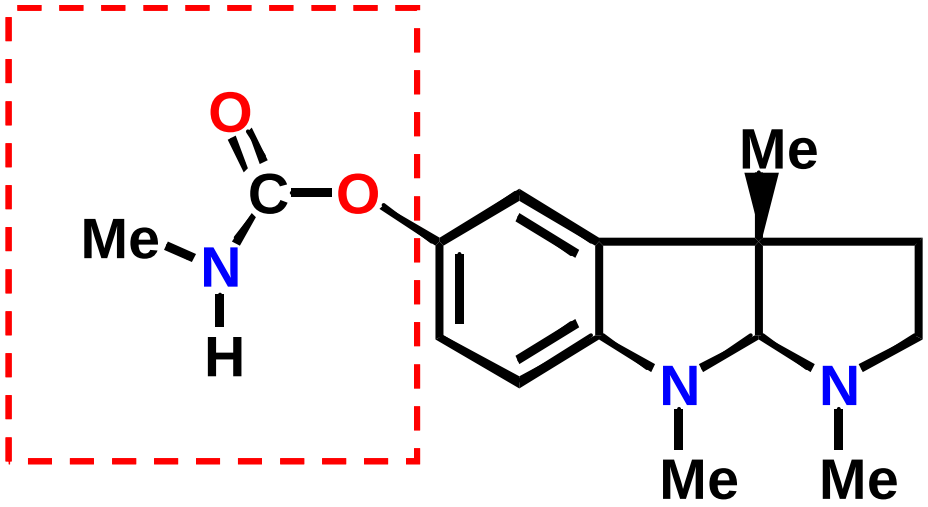
Do you remember? Nice copy&paste!!!



Carbachol

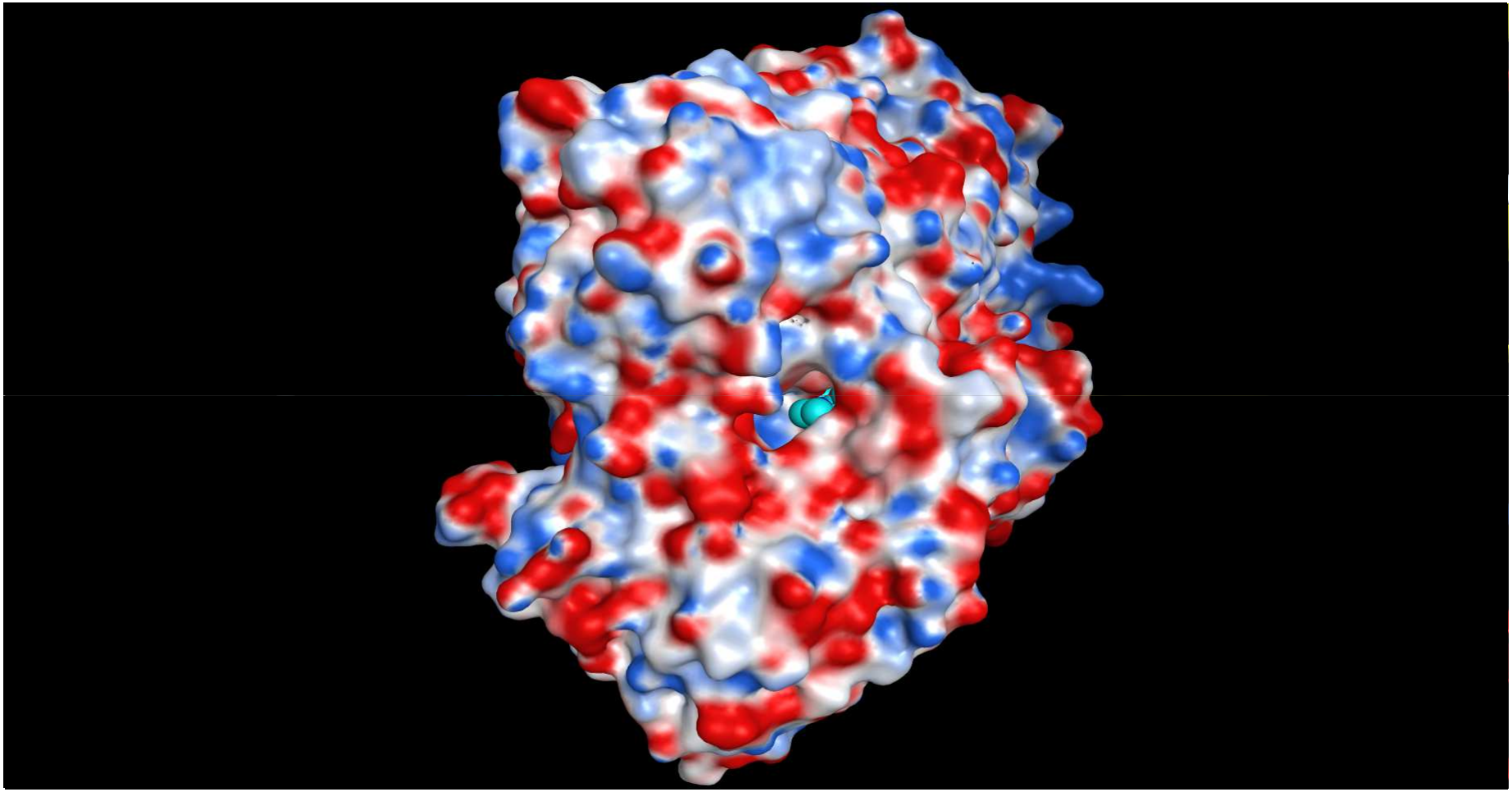


Bethanechol

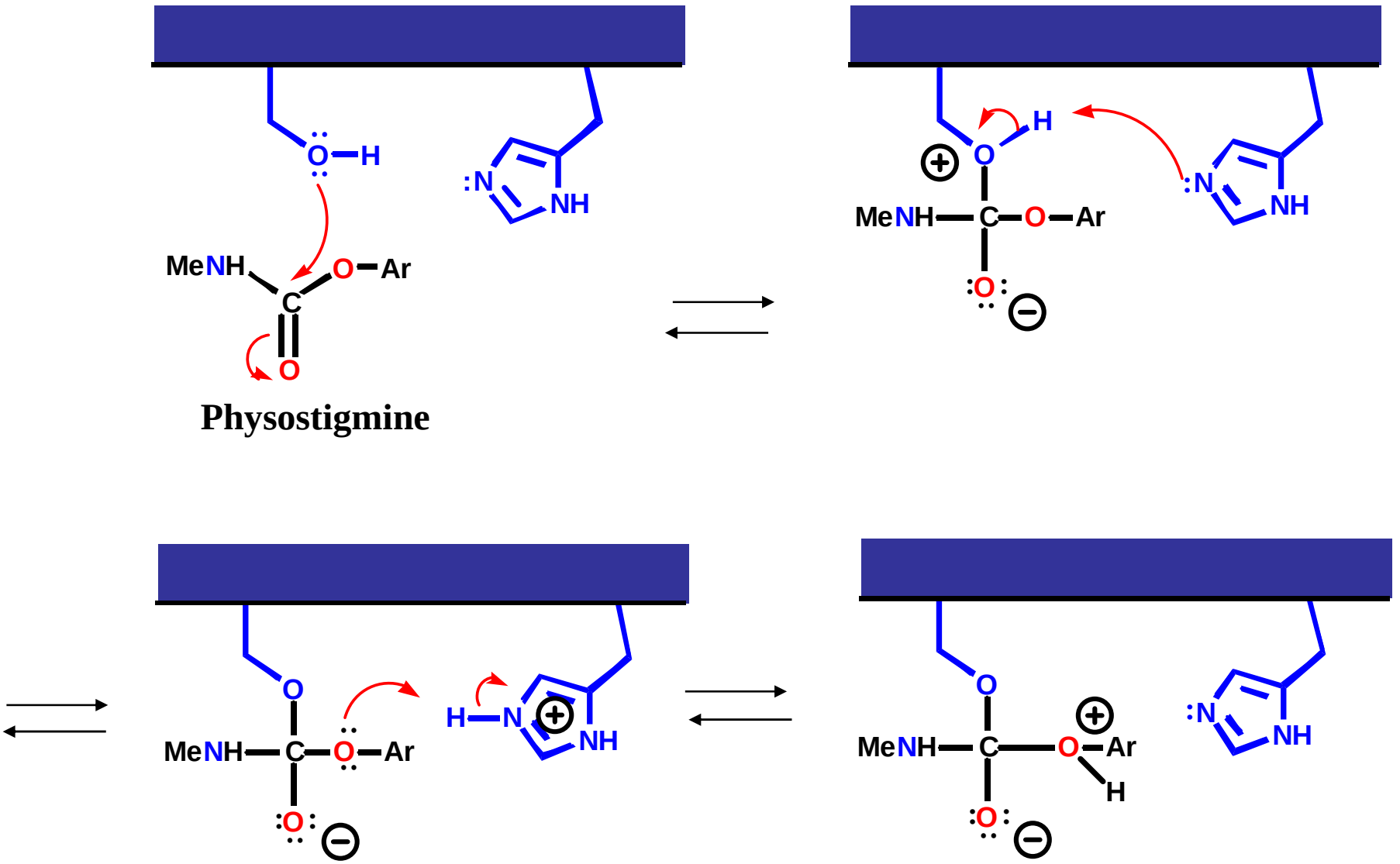


Physostigmine

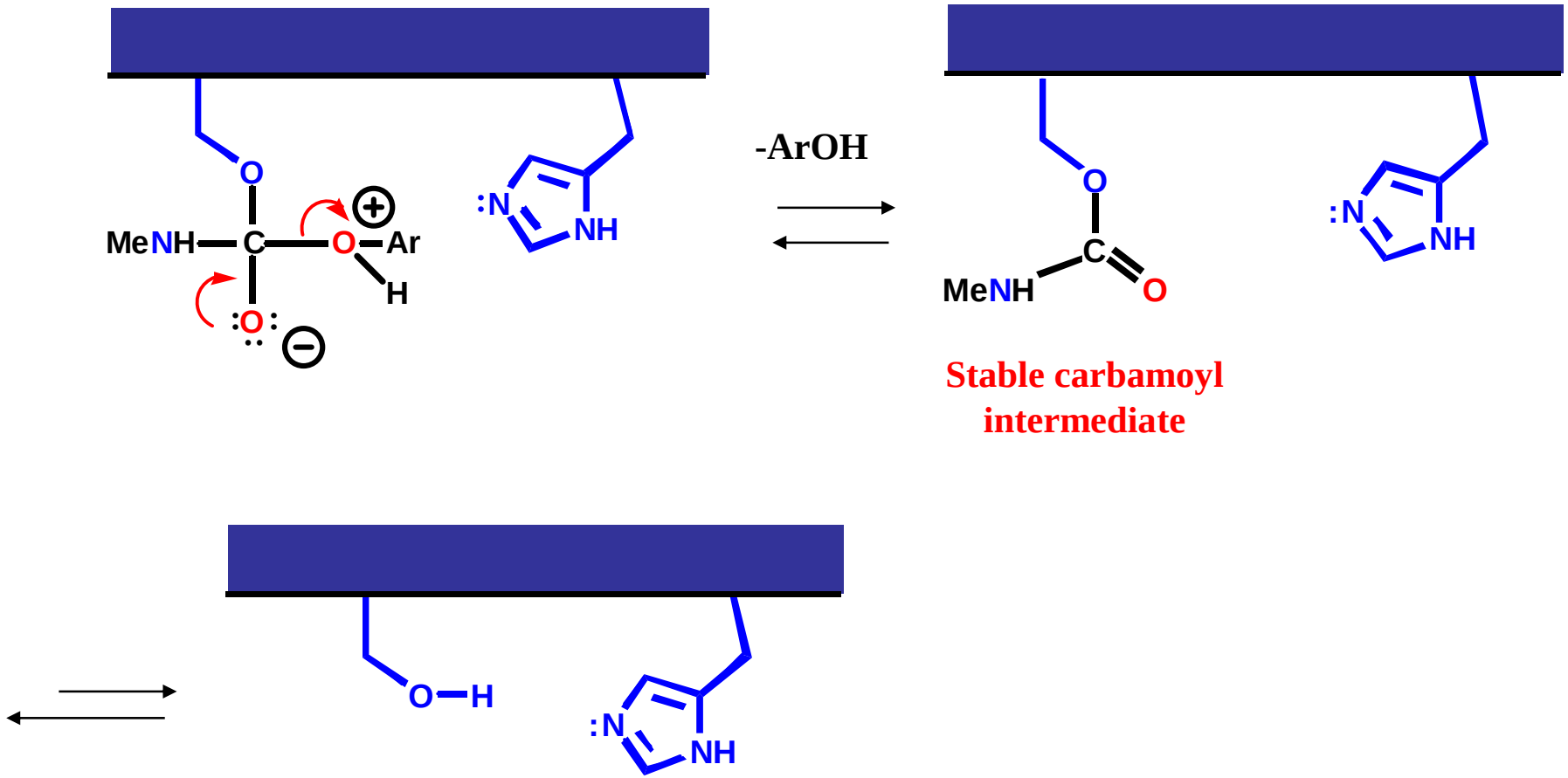
15.2 Mechanism of action



15.2 Mechanism of action

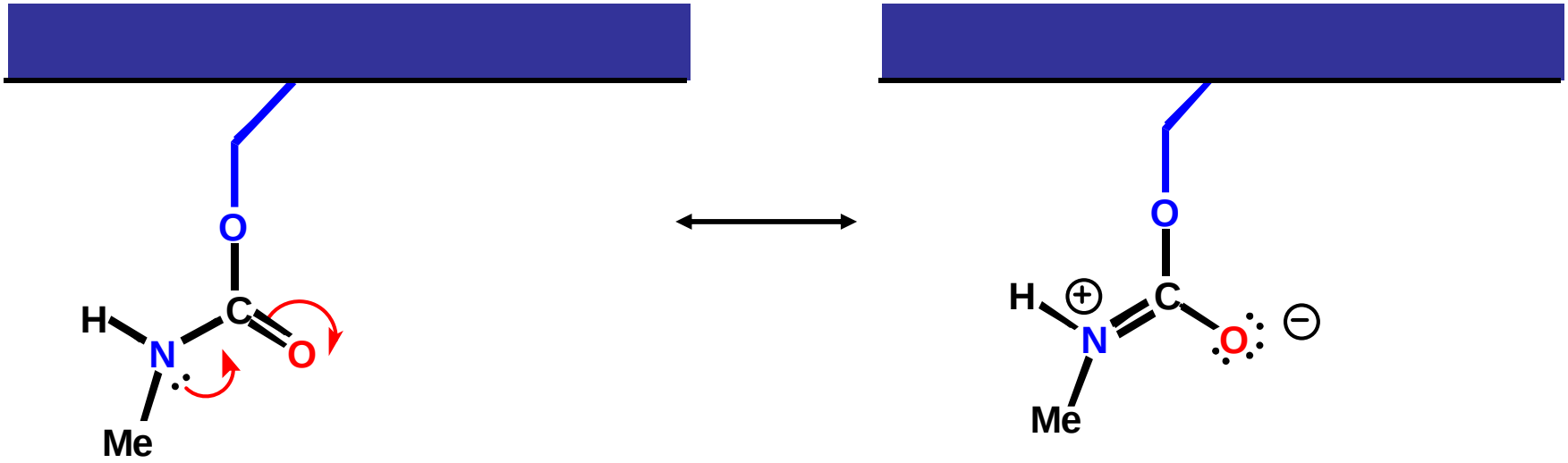


15.2 Mechanism of action



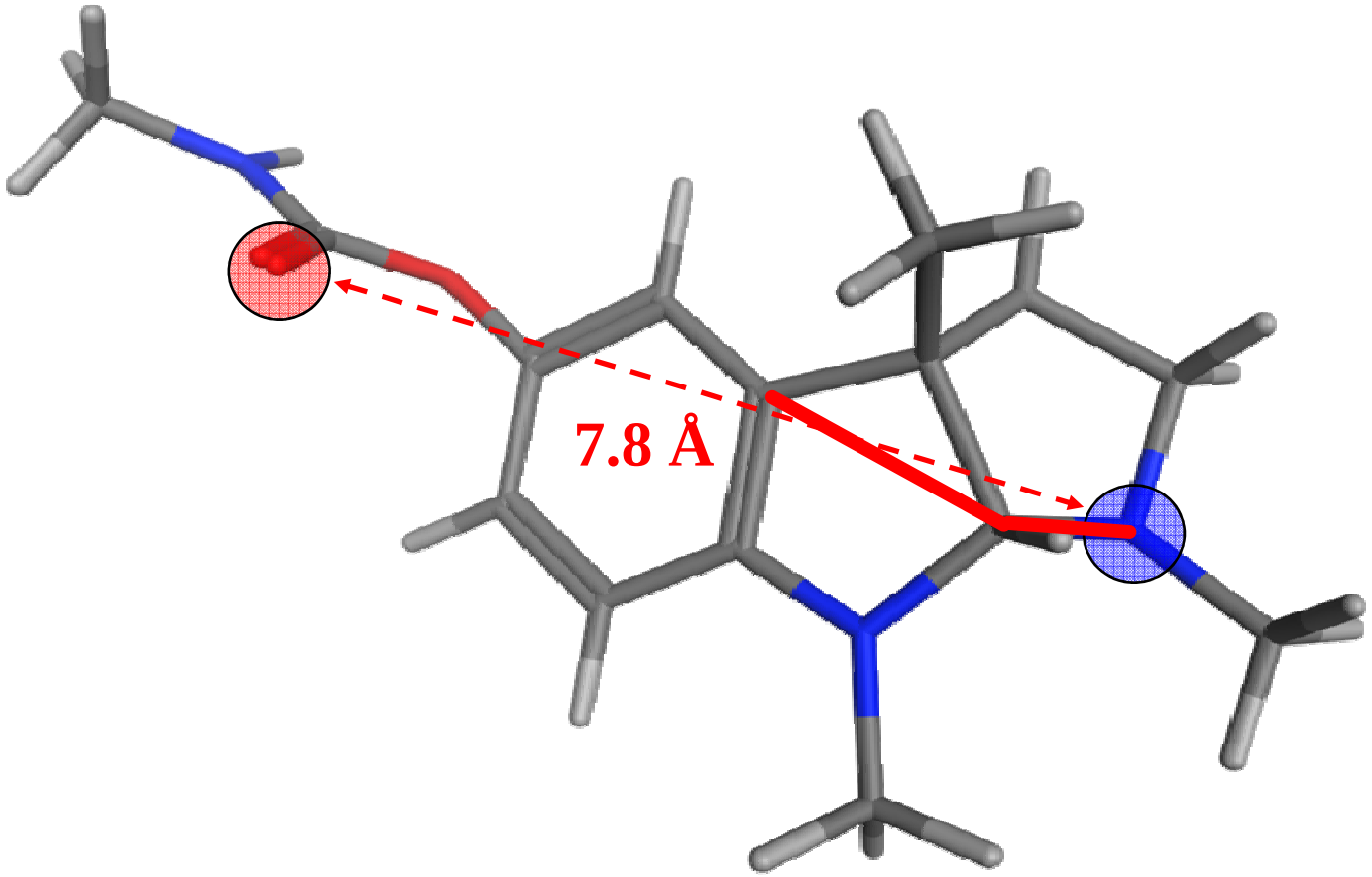
Rate of hydrolysis slower by 40 x 10^6

15.2 Mechanism of action

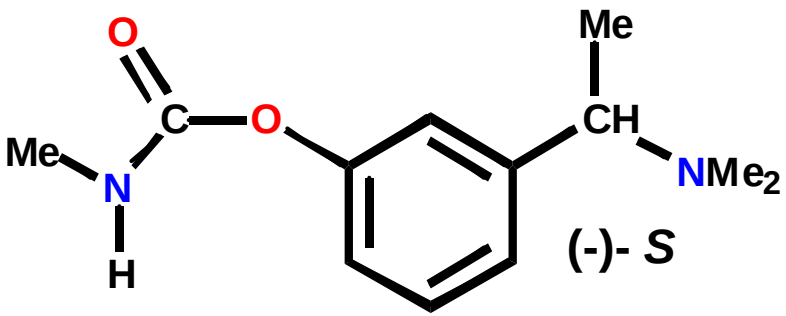


Carbonyl group 'deactivated'

15.3 As usual we need a good idea: Physostigmine analogues.

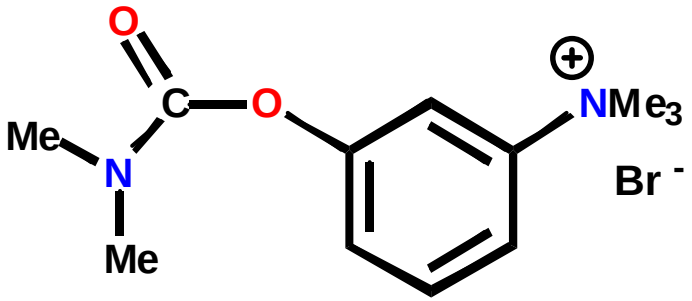


15.3 Physostigmine analogues



Miotine **(log P = 1.6 pKa = 8.6)**

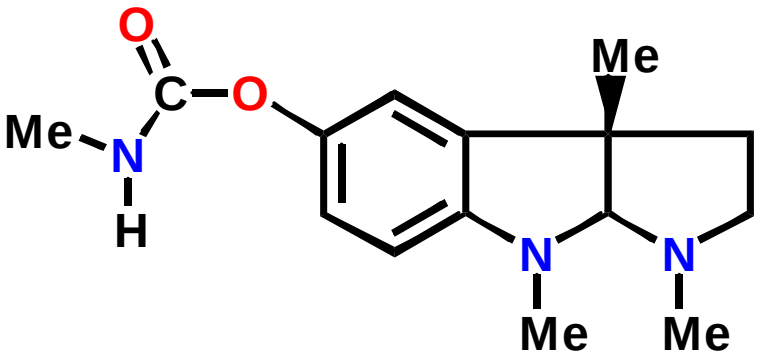
- **Simplified analogue**
- **Miotine was the first *synthetic carbamate* that was used clinically**
- **Susceptible to hydrolysis**
- **Crosses BBB as free base**
- **CNS side effects**



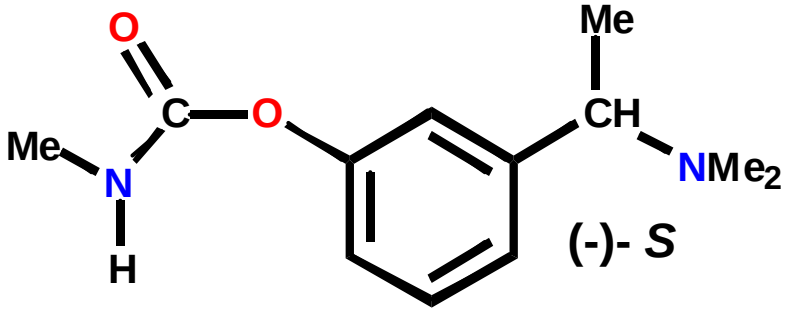
Neostigmine

- **Fully ionised**
- **Cannot cross BBB**
- **No CNS side effects**
- **More stable to hydrolysis**
- **Extra *N*-methyl group increases stability**

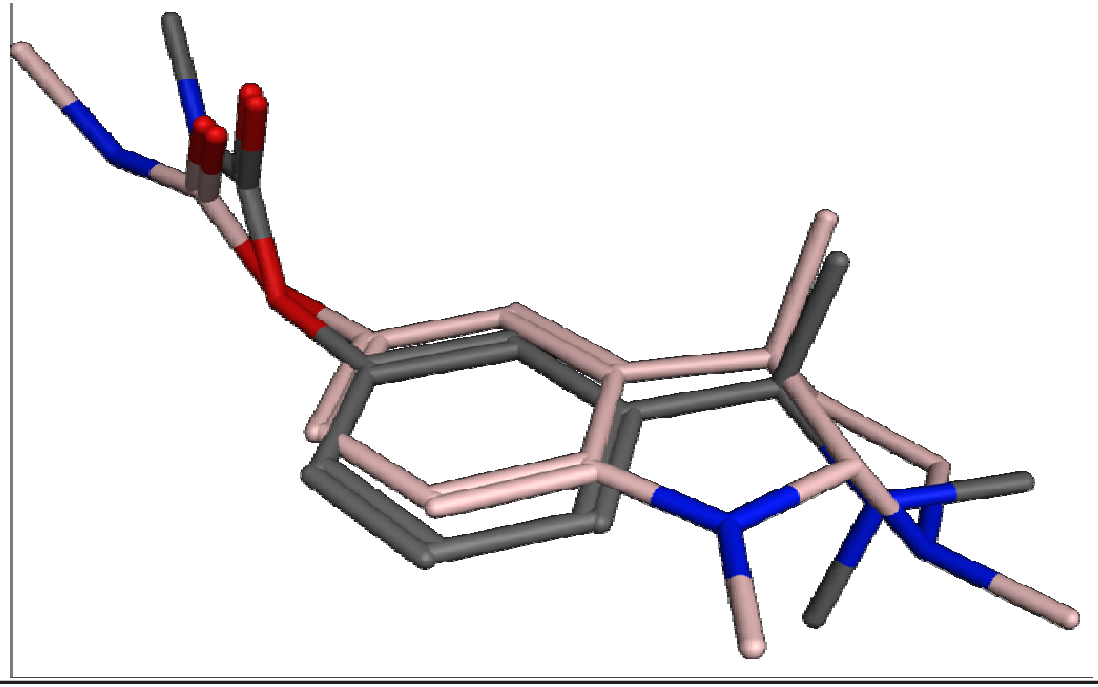
15.3 Physostigmine analogues



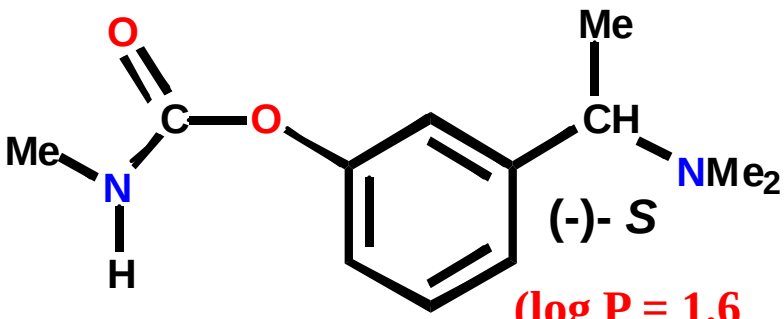
Physostigmine



Miotine

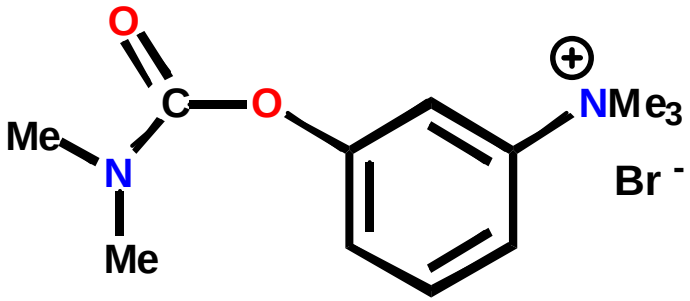


15.3 Physostigmine analogues



(log P = 1.6
pKa = 8.6)

Miotine



Neostigmine

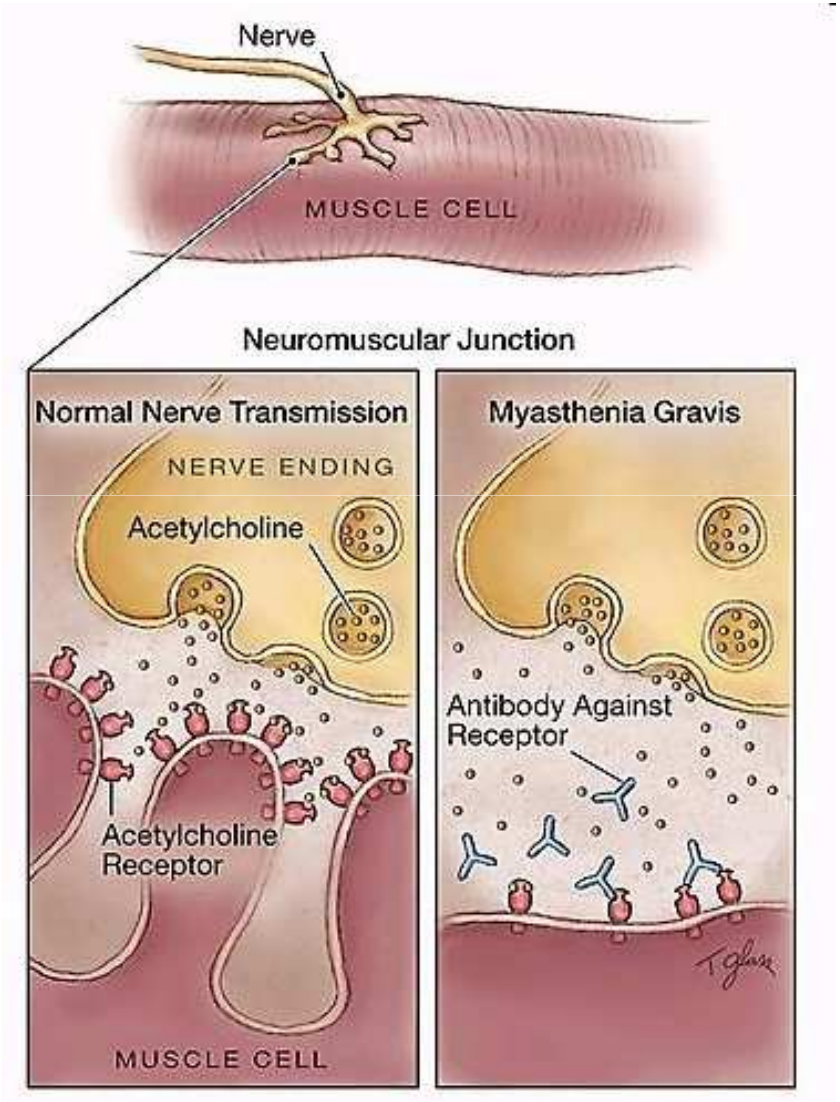
- **Crosses BBB as free base**

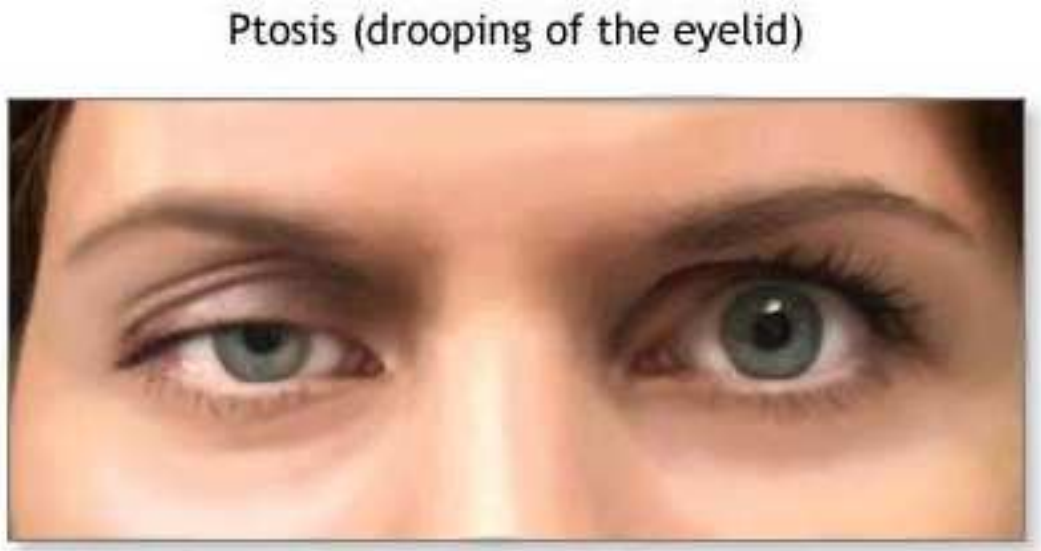
Alzheimer's Disease (AD)

- **Cannot cross BBB**

Myasthenia gravis

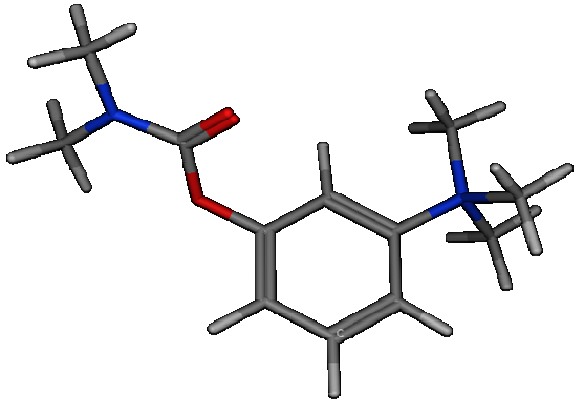
15.4 Myasthenia gravis



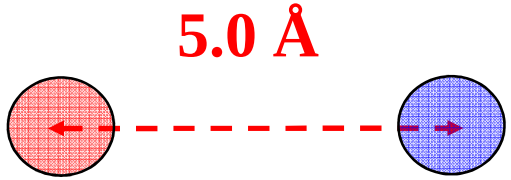


Myasthenia gravis (sometimes abbreviated MG; from the Greek *myastheneia,* lit. 'muscle disease', and Latin *gravis*, 'serious') is a neuromuscular disease leading to fluctuating muscle weakness and fatiguability. At about 14 cases per 100,000 (in the U.S.), it is one of the lesser known autoimmune disorders. Weakness is typically caused by circulating antibodies that block acetylcholine receptors at the post-synaptic neuromuscular junction, inhibiting the stimulative effect of the neurotransmitter acetylcholine.

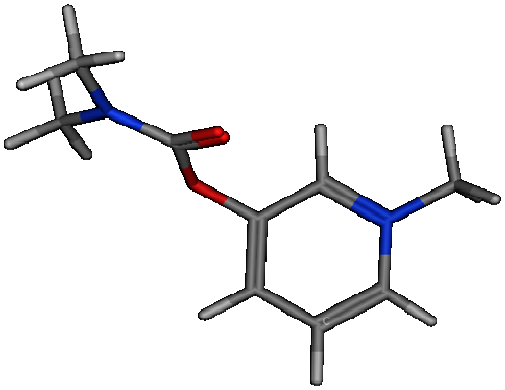
Therapeutically Active Physostigmine analogues



Neostigmine



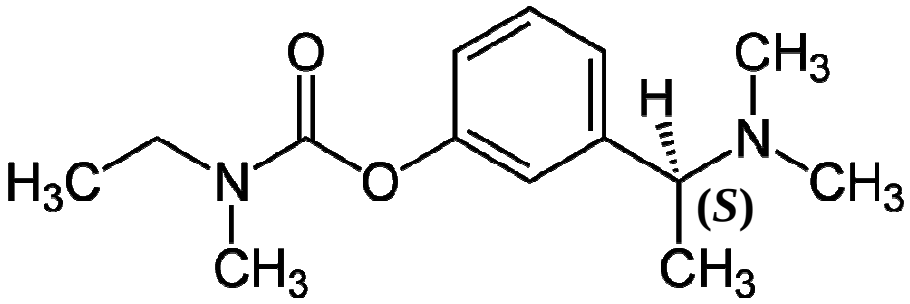
Prostigmin ®



Pyridostigmine

Mestinol ®

Competitive Substrate analogues of Ach:



Rivastigmine

log P = 2.4
pKa = 8.6
PSA = 34 Å^2

Rivastigmine, an acetylcholinesterase suicide substrate, *inhibits both butyrylcholinesterase and acetylcholinesterase* (unlike donepezil, which selectively inhibits acetylcholinesterase), for the treatment of mild to moderate dementia of the Alzheimer's type and dementia due to Parkinson's disease. The drug can be administered orally or via a transdermal patch; the latter form reduces the prevalence of side effects,[which typically include nausea and vomiting.

15.4 Myasthenia gravis

Therapeutic treatment

Treatment is by medication and/or surgery. Medication consists mainly of ***cholinesterase inhibitors*** to directly improve muscle function and ***immunosuppressant drugs*** to reduce the autoimmune process. ***Thymectomy*** is a surgical method to treat MG.

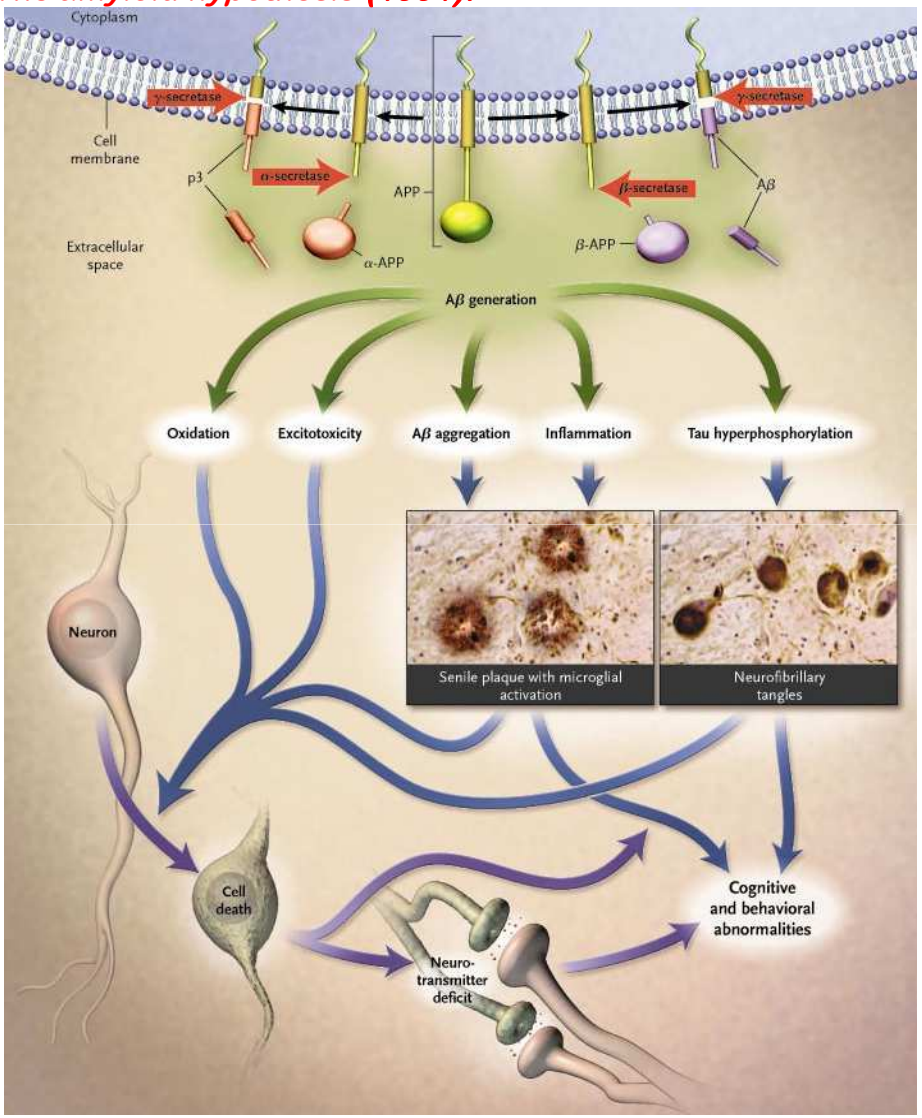
Acetylcholinesterase inhibitors: ***neostigmine*** and ***pyridostigmine*** can improve muscle function by slowing the natural enzyme cholinesterase that degrades acetylcholine in the motor end plate; the neurotransmitter is therefore around longer to stimulate its receptor.

Immunosuppressive drugs: ***prednisone***, ***cyclosporine*** and ***azathioprine*** may be used. Patients are commonly treated with a combination of these drugs with a cholinesterase inhibitor. Treatments with some immunosuppressives take weeks to months before effects are noticed.

Thymectomy: is the surgical removal of the ***thymus***. It is mainly carried out in an adult because the thymus loses most of its functional capacity after adolescence. The role of the thymus prior to adolescence is to educate T-lymphocytes (T-cell) to a specific response where they populate the lymphoid organs, for storage until needed. However, the procedure is more controversial in patients who do not show thymus abnormalities.

15.5 Alzheimer's Disease (AD)

The *amyloid hypothesis (1991):*



Alzheimer's disease (AD), also known simply as Alzheimer's, is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. It is the most common type of dementia.

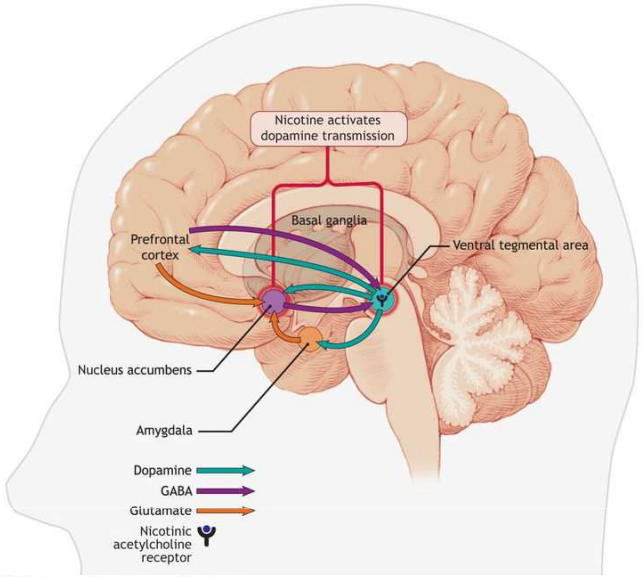
The most striking early symptom is loss of short term memory (amnesia), which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, cognitive (intellectual) impairment extends to the domains of language (aphasia), skilled movements (apraxia), recognition (agnosia), and those functions (such as decision-making and planning) closely related to the frontal and temporal lobes of the brain as they become disconnected from the limbic system, reflecting extension of the underlying pathological process. These changes make up the essential human qualities, and thus AD is sometimes described as a disease where the victims suffer the loss of qualities that define human existence.

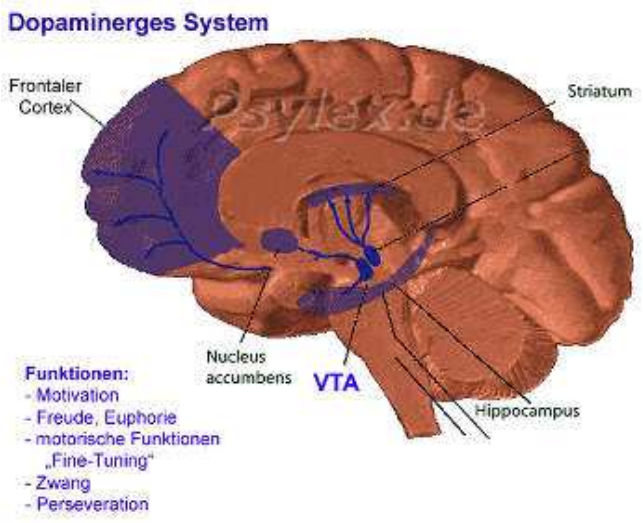
This pathological process consists principally of neuronal loss or atrophy, principally in the temporoparietal cortex, but also in the frontal cortex, together with an inflammatory response to the deposition of **amyloid plaques** and **neurofibrillary tangles.**

Legenda: amyloid beta precursor protein (APP); amyloid beta (Aβ)

15.5 Alzheimer's Disease (AD)

The *dopaminergic hypothesis (2017):*





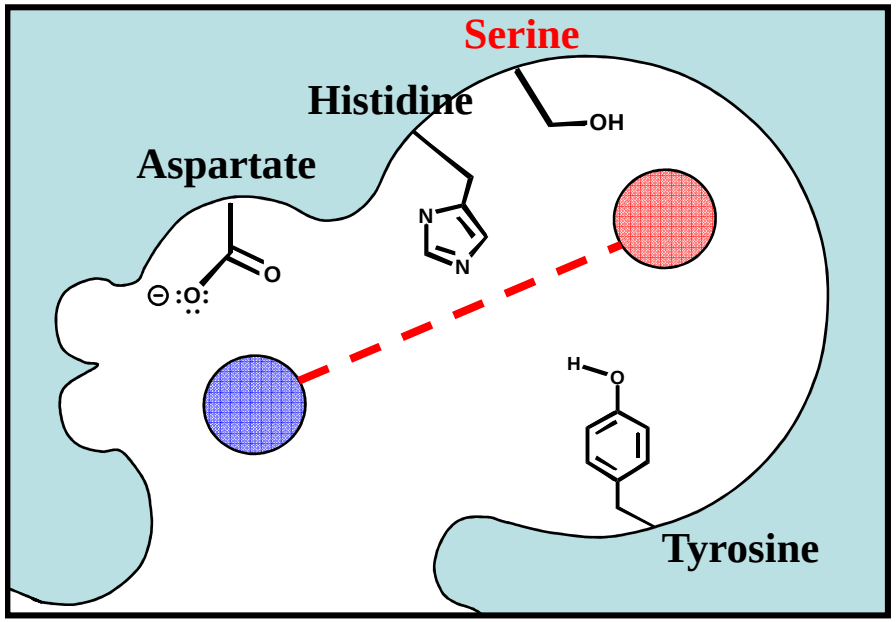
The most striking early symptom is loss of short term memory (amnesia), which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories.

Alzheimer, scoperto il meccanismo all'origine della malattia. E' nell'area che regola l'umore

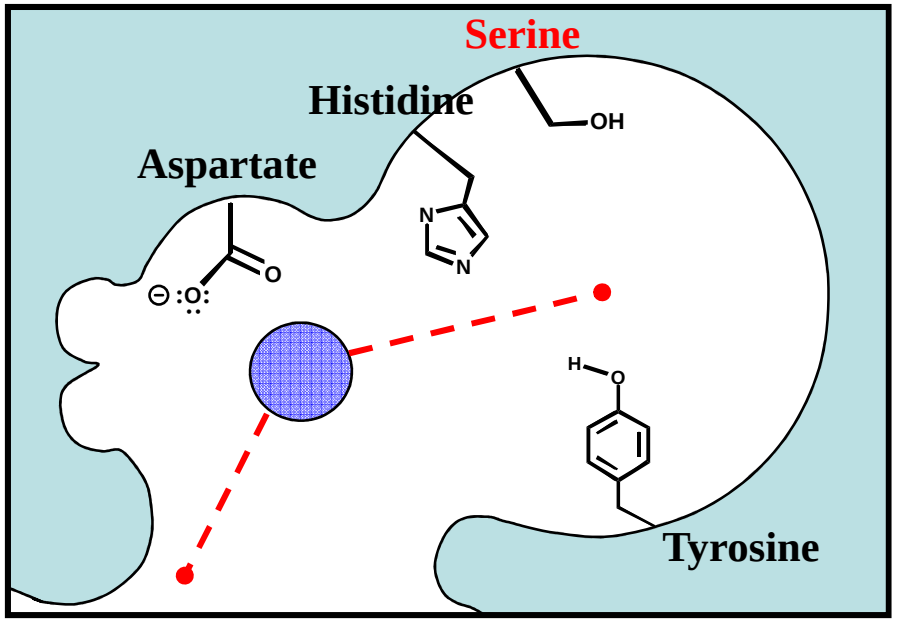
La scoperta italiana, appena pubblicata su Nature Communications. Aggiunge un importante tassello nella comprensione di una malattia che colpisce circa mezzo milione di italiani over 60

In principle, we have at least two possible strategies:

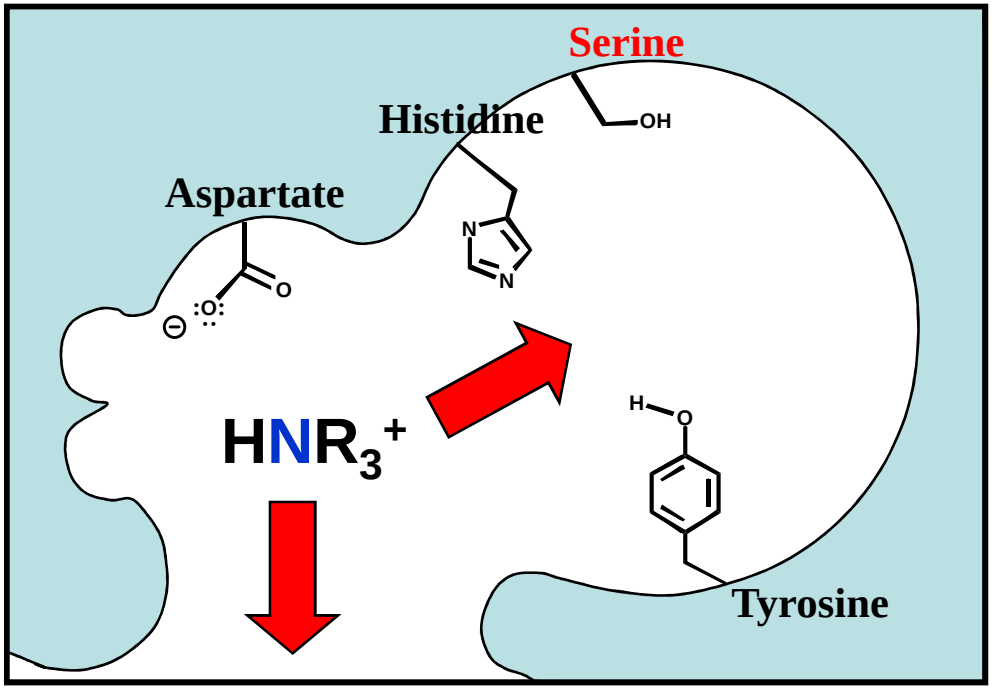
Design competitive substrates



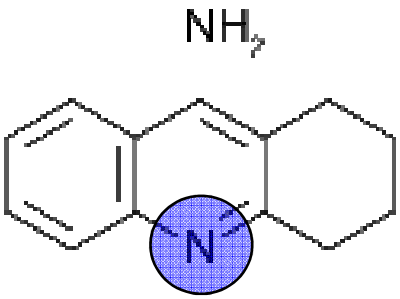
Design competitive inhibitors



Simple…



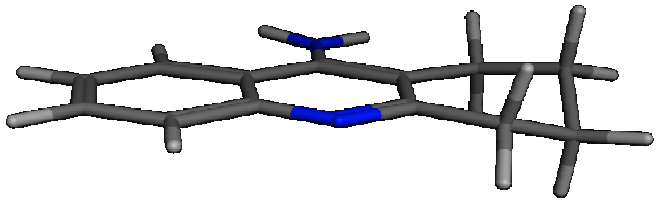
Competitive Inhibitor analogues of Ach:



Tacrine

1,2,3,4-tetrahydroacridin-9-amine

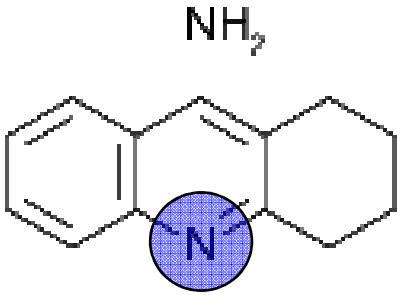
log P = 2.7
pKa = 9.9
PSA = 39 Å^2



In 1993, Tacrine (Cognex®) became the first FDA approved treatment of the memory problems of Alzheimer's disease, but this was not without controversy, and many practitioners believed the drug was ineffective and hepatotoxic.

Because of the flat configuration like a frisbee and the high pKa closed to 10, tacrine has the capacity to slice through cell membranes almost as easily as ethyl alcohol.

Competitive Inhibitor analogues of Ach:

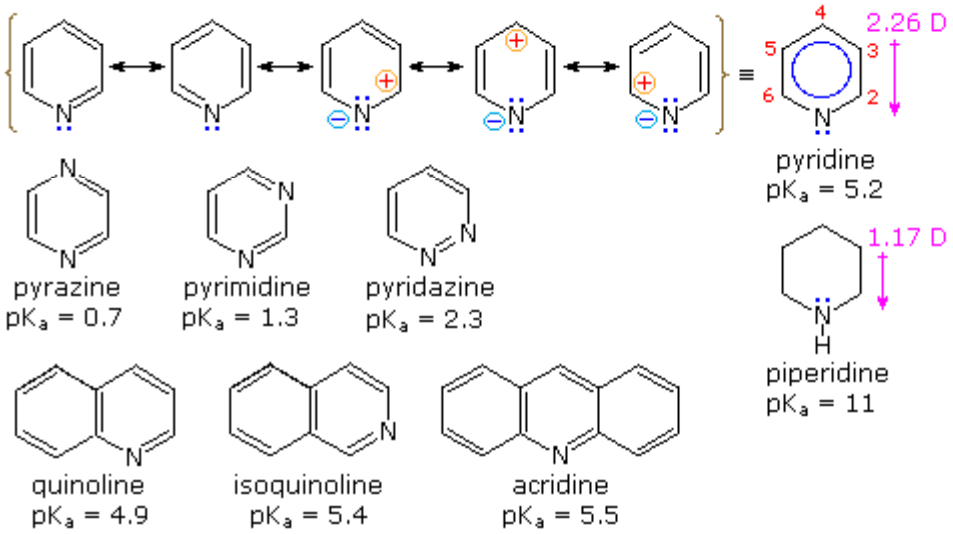


Tacrine

log P = 2.7

pKa = 9.9

PSA = 39 Å^2

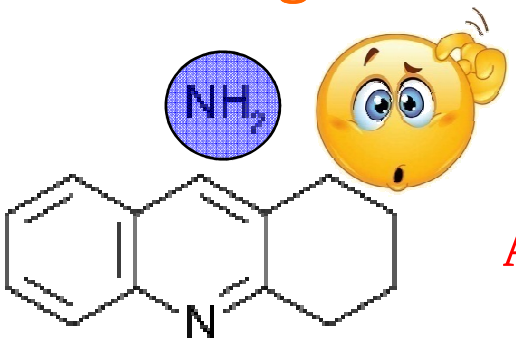


Pyridine Basicities

Substituent(s)	pK$_a$
4-methyl	6.0
3-methyl	5.7
2,6-dimethyl	6.7
2,4,6-trimethyl	7.5
2,6-di-*t*-butyl	3.6
3-methoxy	4.9
4-methoxy	6.5
4-dimethylamino	9.7
2-chloro	0.7
4-chloro	3.8
2,6-dichloro	−2.9

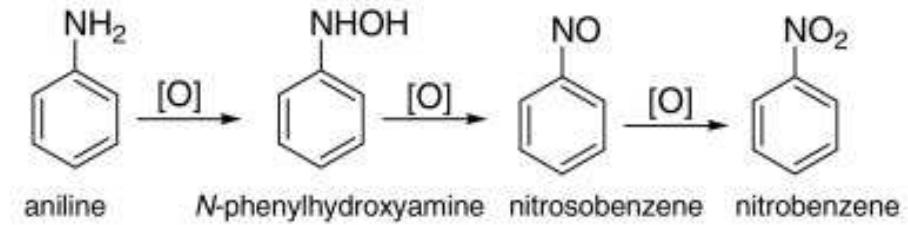
Thanks, Zorro96!!

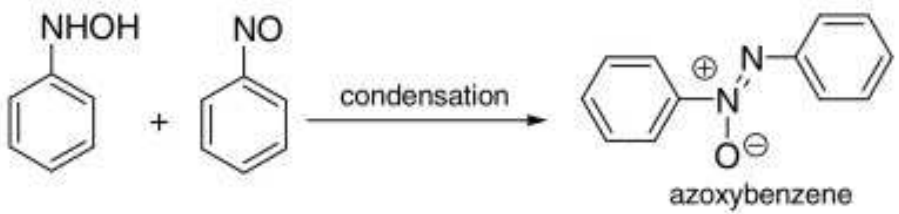
Competitive Inhibitor analogues of Ach:

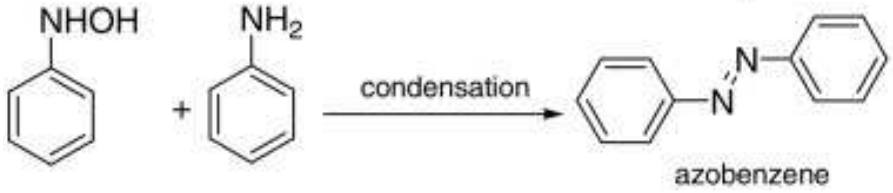


Tacrine

Aniline oxidation:

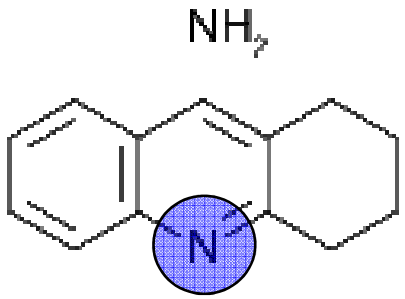






Thanks, Zorro96!!

Competitive Inhibitor analogues of Ach:



Tacrine

1,2,3,4-tetrahydroacridin-9-amine

log P = 2.7
pKa = 9.9
PSA = 39 Å²

In 1993, Tacrine (Cognex®) became the first FDA approved treatment of the memory problems of Alzheimer's disease, but this was not without controversy, and many practitioners believed the drug was ineffective and hepatotoxic.

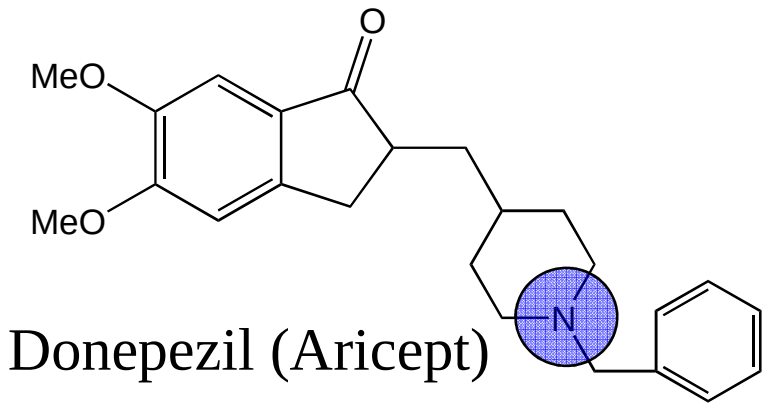
Because of the flat configuration like a frisbee and the high pKa closed to 10, tacrine has the capacity to slice through cell membranes almost as easily as ethyl alcohol.

Multi-targeting pharmacology of Tacrine:

Tacrine (Cognex®) has numerous mechanisms of action. The putative principle mechanism of action for Alzheimer's disease *is as a non-competitive reversible acetylcholinesterase inhibitor* somewhat selective for action in the central nervous system. But the lesions of Alzheimer's exceed the boundaries of the cholinergic system to include noradrenaline neurotransmitter deficits, serotonin neurotransmitter deficits, decreased vascular perfusion. It may be fortunate that tacrine has a wide variety of actions. Tacrine blocks sodium and potassium channels. It has direct post synaptic muscarinic activity, alters monoamine (serotonin and noradrenaline) uptake, increases the release of 5-HT, noradrenaline, and dopamine; inhibits monoamine oxidase A and B, stimulates cholinergic firing, interacts with N-methyl-d-aspartate-phencyclidine (NMDA) receptors.

Two other curious actions of tacrine are not shared by pure anticholinesterase inhibitors such as physostigmine and presumably donepezil (Aricept®). First, Alzheimer's disease is associated with decreased cerebral blood flow. Tacrine significantly increases cerebral blood flow in patients who have Alzheimer's disease. Second, much has been made of the role of amyloid deposition in the pathology of Alzheimer's disease. Tacrine actually blocks the secretion of β-amyloid precursor protein. It appears that tacrine is uniquely suited to treat Alzheimer's disease.

Competitive Inhibitor analogues of Ach:



Donepezil (Aricept)

log P = 4.2
pKa = 8.2
PSA = 39 Å^2

(*RS*)-2-[(1-benzyl-4-piperidyl)methyl]- 5,6-dimethoxy-2,3-dihydroinden-1-one

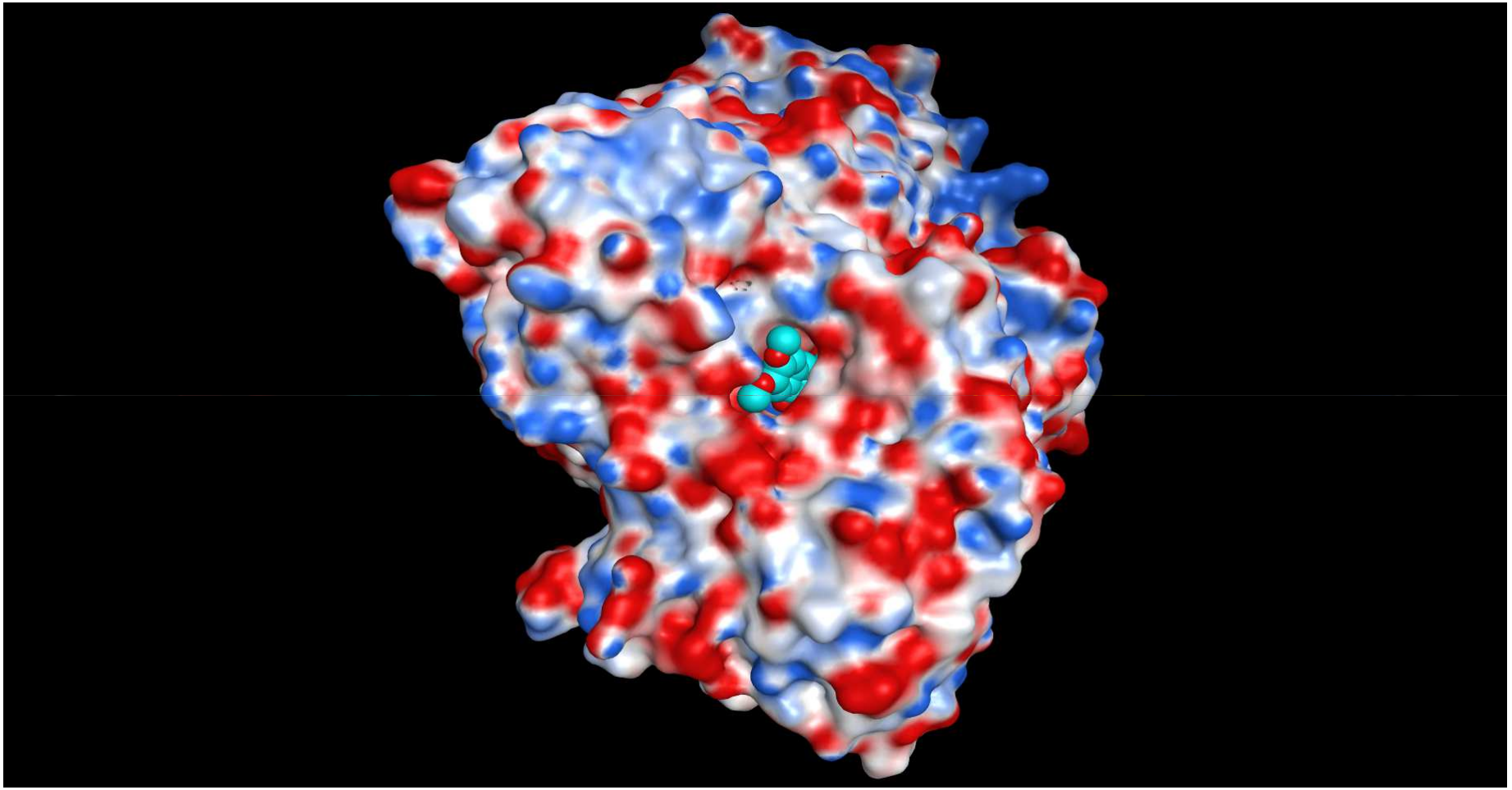
In 1996, Donepezil hydrochloride was the second drug approved by the U.S. FDA for the *palliative treatment* of mild to moderate AD. It is a new class of ChE inhibitor having an N-benzylpiperidine and an indanone moiety that shows longer and more selective action.

In fact, clinical studies show that physostigmine has not optimal oral activity, brain penetration and pharmacokinetic parameters, while tacrine has hepatotoxic liability.

Competitive Inhibitor analogues of Ach:

Donepezil (Aricept), is a centrally acting reversible acetyl cholinesterase inhibitor. Its main therapeutic use is in the treatment of Alzheimer's disease where it is used to increase cortical acetylcholine. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase. If this proposed mechanism of action is correct, donepezil's effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact. Donepezil has been tested in other cognitive disorders including Lewy body dementia and Vascular dementia, but it is not currently approved for these indications.
Donepezil has also been studied in patients with Mild Cognitive Impairment, schizophrenia, attention deficit disorder, post-coronary bypass cognitive impairment, cognitive impairment associated with multiple sclerosis, and Down syndrome.

Mechanism of action



A flavor of Donepezil discovery:

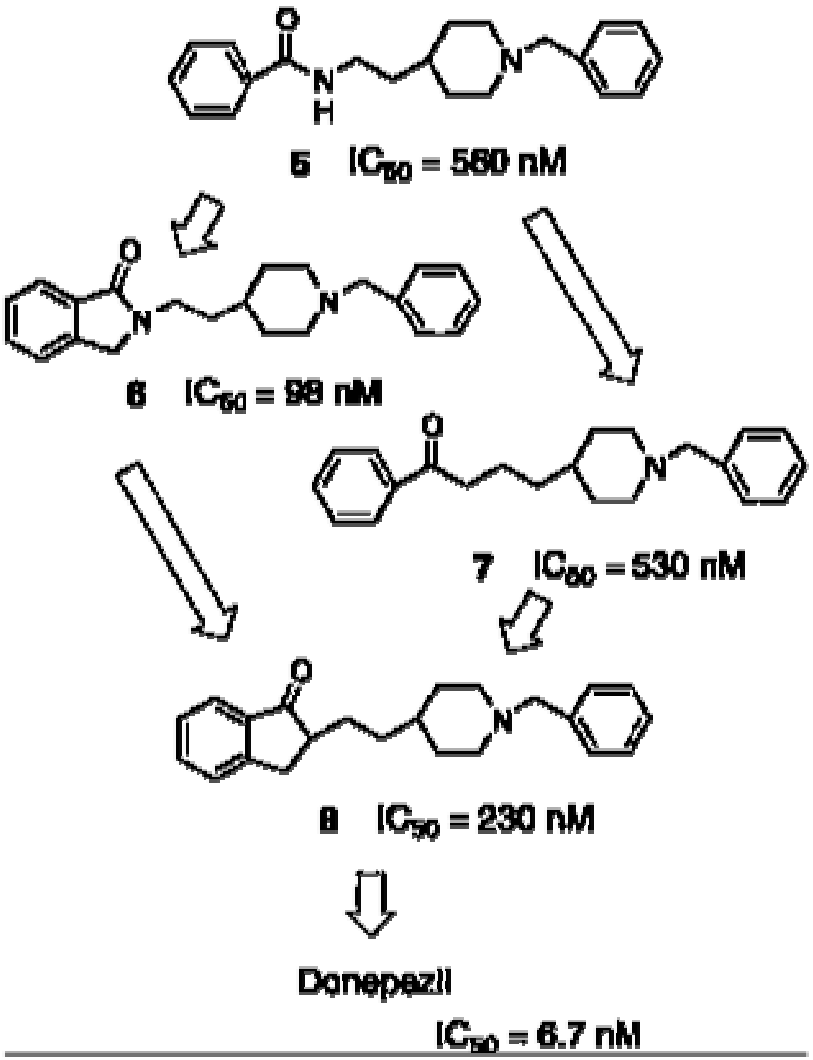
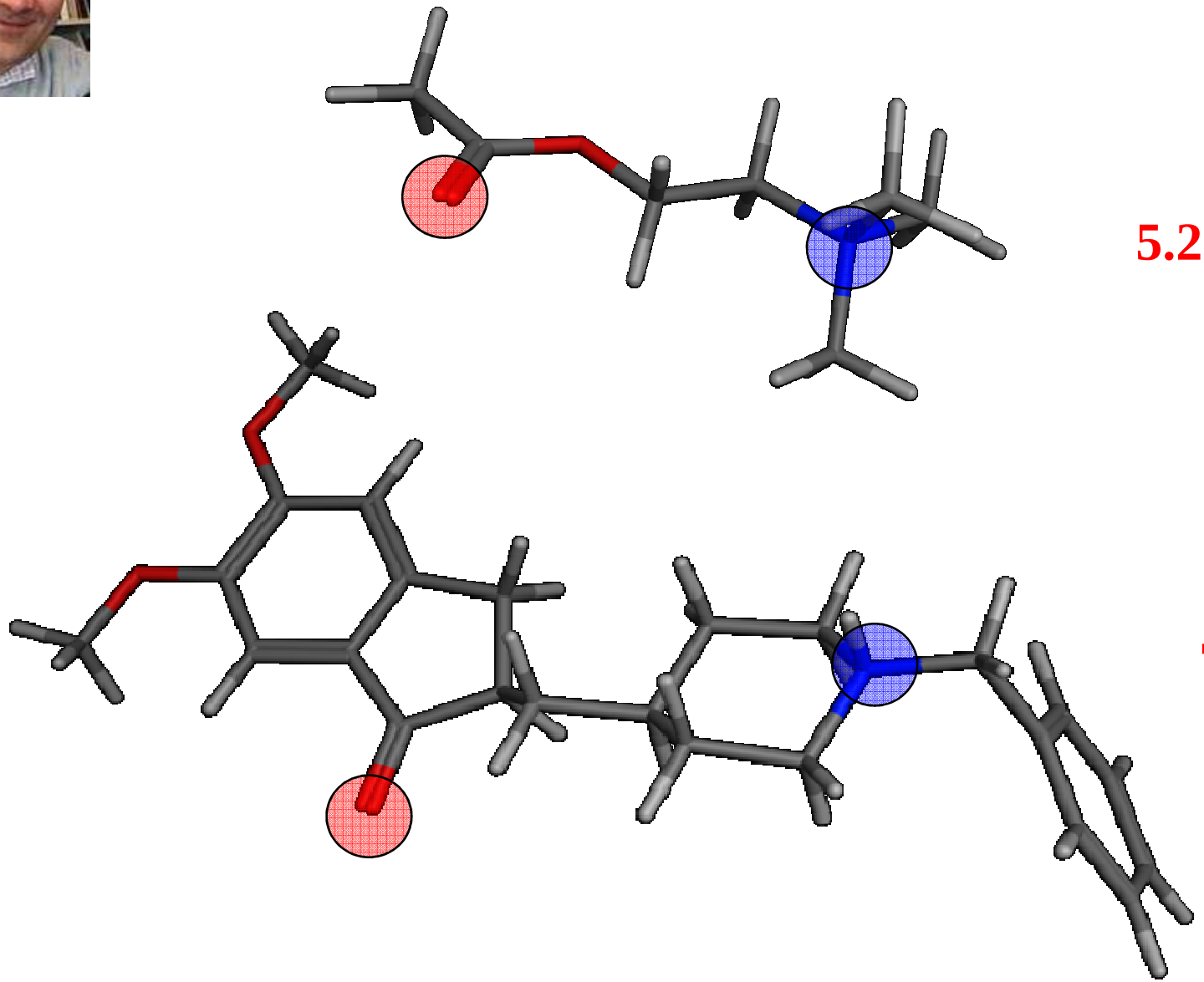
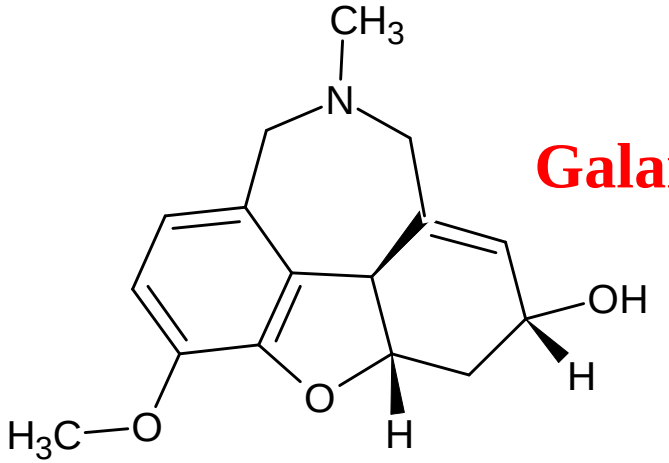


Fig. 5. Second stage of drug design. Reproduced from H. sugimoto, J. Syn. Org. Chem. Jpn. **56**, 320 – 327 (1998), with permission.

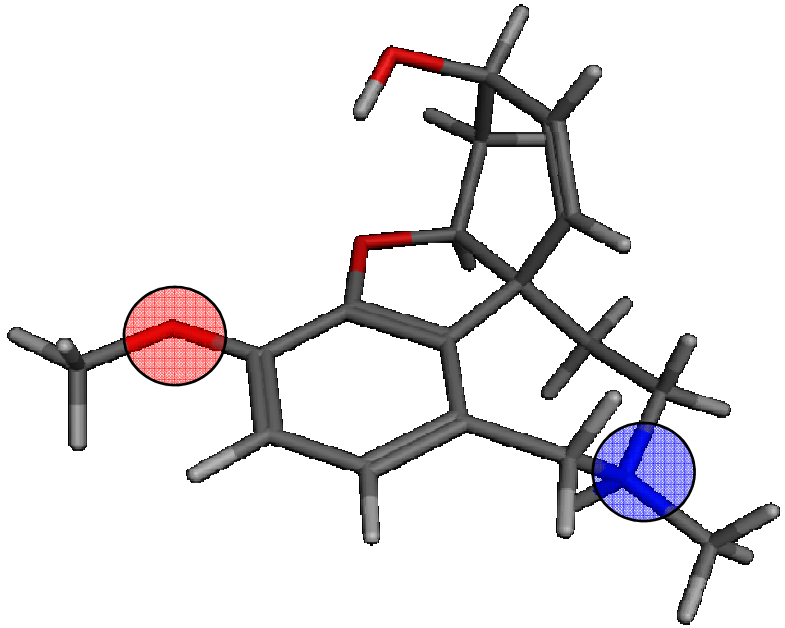
Where are the analogies with Ach?



Mother nature…

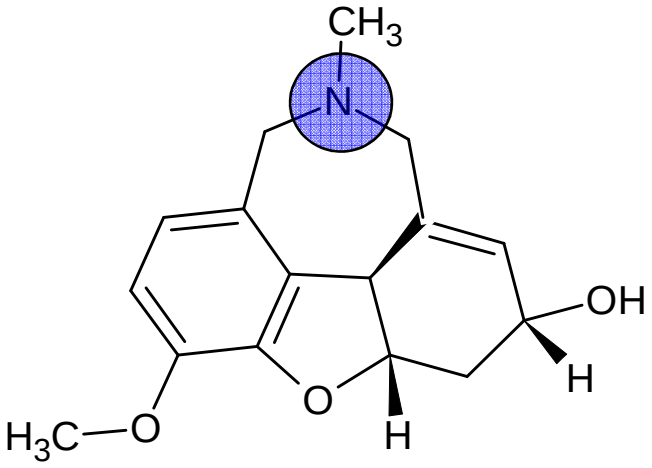


Galantamine



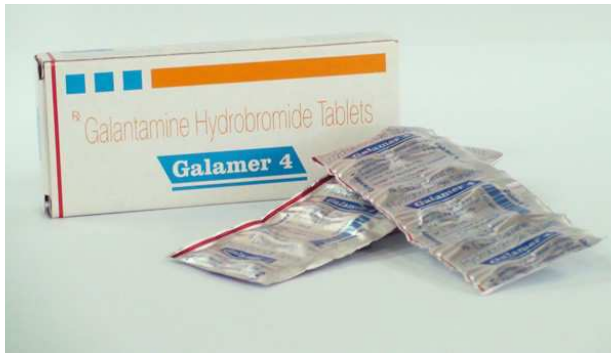
6.5 Å

Competitive Inhibitor analogues of Ach:



Galantamine
(Reminyl)

log P = 1.3
pKa = 8.9
PSA = 42 Å^2



(4a*S*,6*R*,8a*S*)- 5,6,9,10,11,12- hexahydro- 3-methoxy- 11-methyl- 4a*H*- [1]benzofuro[3a,3,2-*ef*][2]benzazepin- 6-ol

Galantamine (also called galanthamine in some studies) hydrobromide was approved in 2001 by the FDA.

Galantamine was discovered accidentally in the 1950s by a Bulgarian pharmacologist in the bulbs and flowers of wild Caucasian snowdrops, *Galanthus woronowii* (Sramek et al 2000). Initially, it was used as an agent to reverse the effect of curare in anesthetic practice.

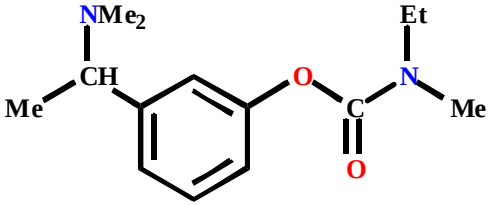
Some curiosities…

Donepezil and **Rivastigmine** are active centrally, in contrast to **Galantamine**, which is more active peripherally. Furthermore, Rivastigmine preferentially inhibits the G1 isoform of cholinesterase, predominantly located in the cortex, hippocampus and in neuritic plaques, while Donepezil and Galantamine are not selective for any cholinesterase isoforms and have wide cholinergic activity both centrally and peripherally. The cholinergic activity of Rivastigmine, in contrast to Donepezil and Galantamine, is apparently more targeted at clinically relevant brain sites. The pharmacological profile of Rivastigmine results in it having a low potential to interact with other drugs and it may be used with a high margin of safety in patients having a wide variety of concomitant diseases.

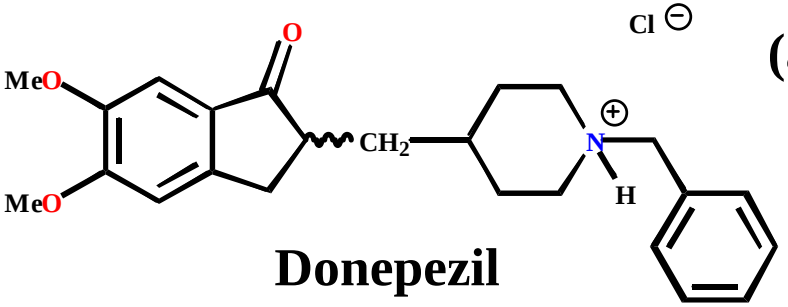
15.5 Anticholinesterases as ‘Smart Drugs’

- **Act in CNS**
- **Must cross blood brain barrier**
- **Used to treat memory loss in Alzheimers disease**
- **Alzheimers causes deterioration of cholinergic receptors in brain**
- **Smart drugs inhibit Ach hydrolysis to increase activity at remaining receptors**

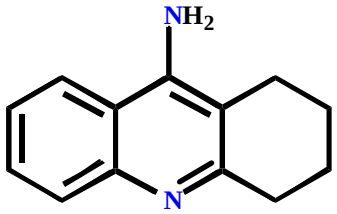
15.5 Anticholinesterases as 'Smart Drugs'



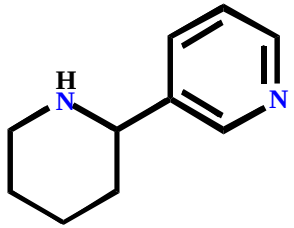
Rivastigmine (Exelon)
(analogue of physostigmine)



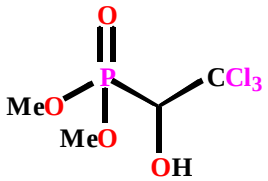
Donepezil



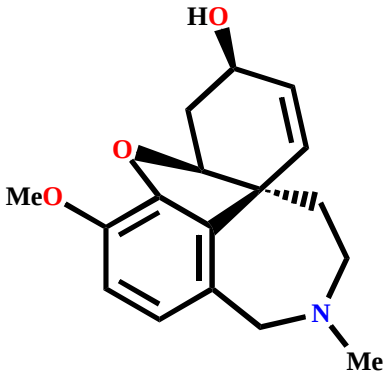
Tacrine (Cognex)
Toxic side effects



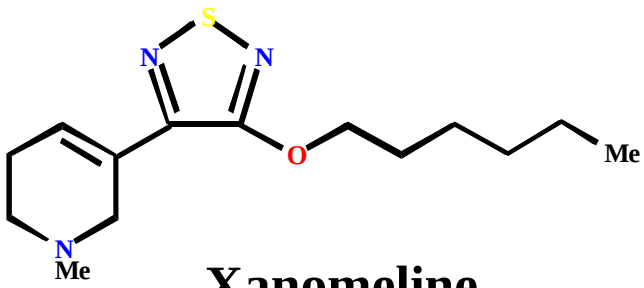
Anabaseine
(ants and marine worms)



Metrifonate
(organophosphate)



Galanthamine
(daffodil and snowdrop bulbs



Xanomeline

Nootropics

Nootropics (from Greek, 'acting on the mind'), popularly referred to as "smart drugs", are substances which boost human cognitive abilities (the functions and capacities of the brain). The word nootropic is derived from the Greek words *noos* or "mind" and *tropein* meaning "to ward." Typically, nootropics are alleged to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth.

Most alleged nootropic substances are nutrients or plant components (herbs, roots, beans, bark, etc.), available over the counter at health food and grocery stores, and are used as nutritional supplements. Some nootropics are drugs, used to treat people with cognitive learning difficulties, neural degradation (Alzheimer's and Parkinson's), and for cases of oxygen deficit to prevent hypoxia. These drugs have a variety of human enhancement applications as well, are marketed heavily on the World Wide Web, and are used by many people in personal cognitive enhancement regimens.

With some nootropics the effects are subtle and gradual, such as with most nerve growth inducers, and may take weeks or even months before any cognitive improvement is noticed. At the other end of the spectrum are nootropics which have effects that are immediate, profound, and obvious. While scientific studies support some of the claimed benefits, it is worth noting that many of the claims attributed to a variety of nootropics have not been formally tested.

15.5 Alzheimer's Disease (AD)

Therapeutic treatment

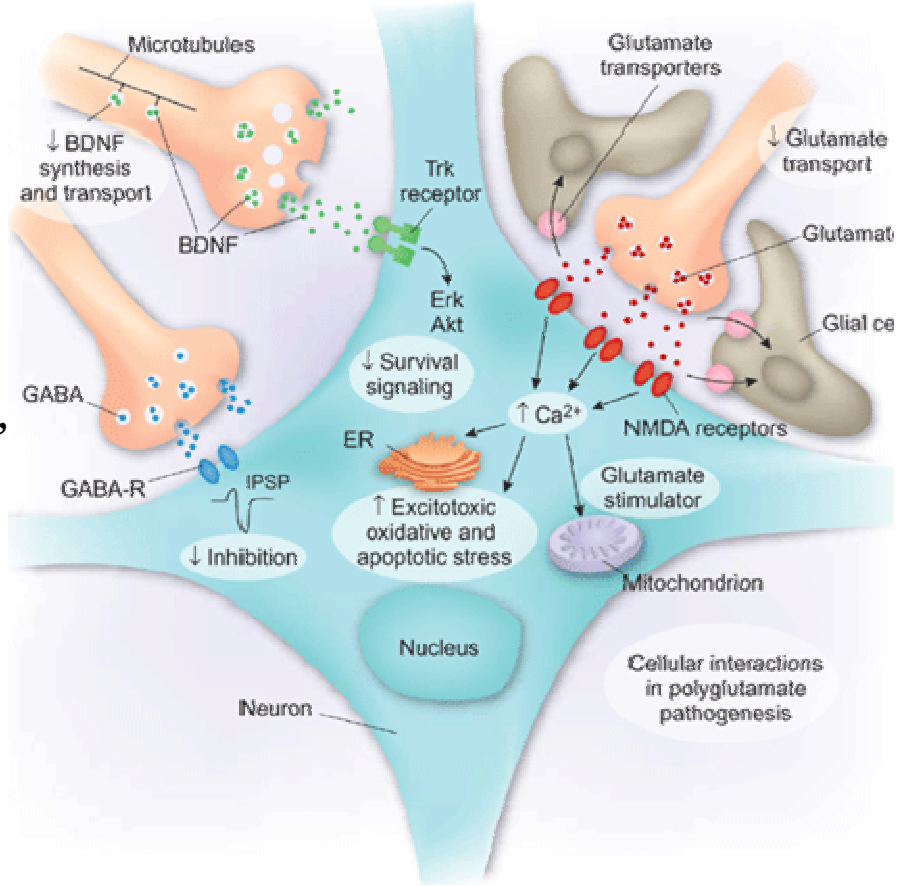
Four medications are currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to treat the cognitive manifestations of AD: three are ***acetylcholinesterase inhibitors*** and the other is ***memantine***, an NMDA receptor antagonist. No drug has an indication for delaying or halting the progression of the disease.

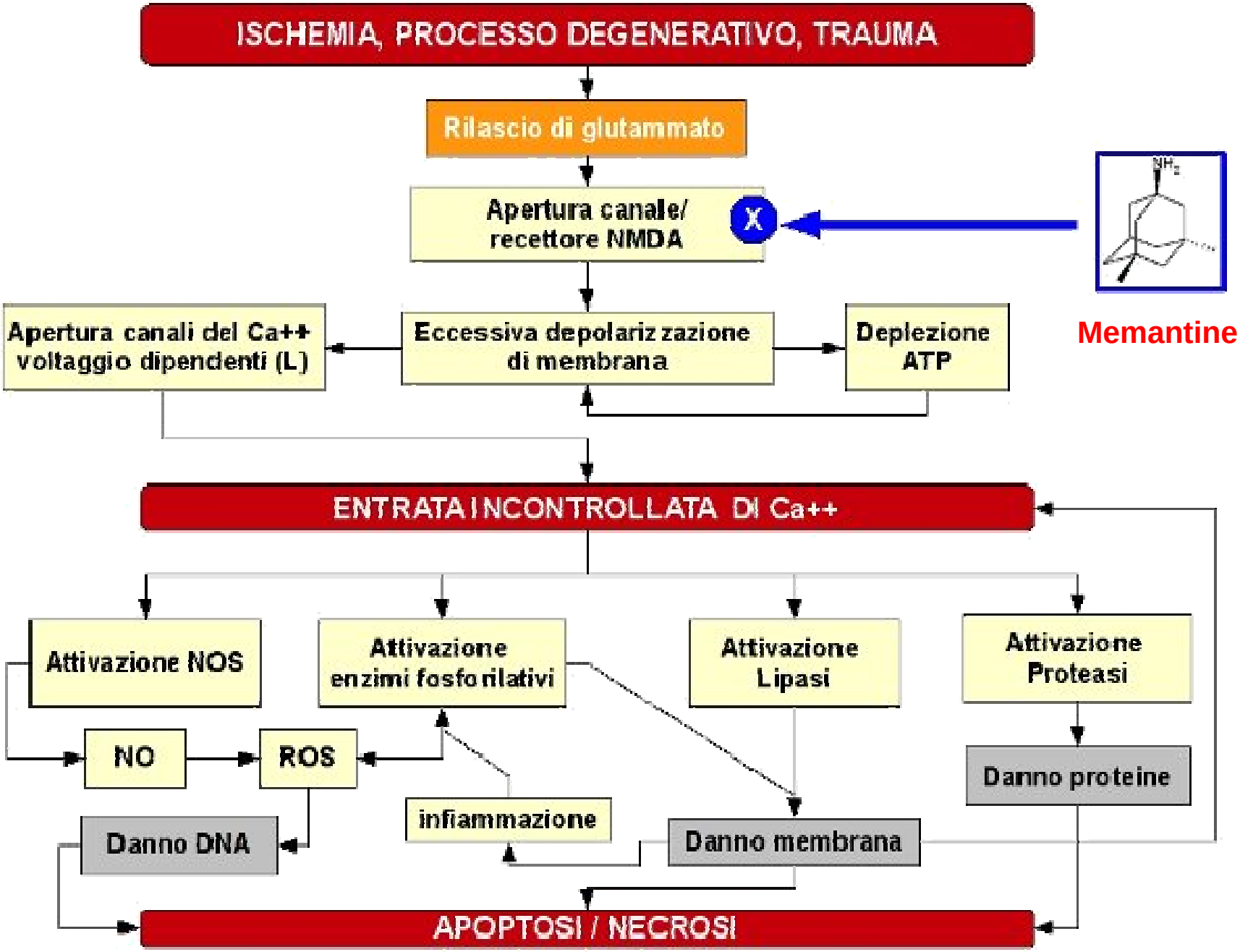
Glutamate is a important excitatory neurotransmitter of the nervous system, although excessive amounts in the brain can lead to cell death through a process called ***excitotoxicity*** which consists of the overstimulation of glutamate receptors. Excitotoxicity occurs not only in Alzheimer's disease, but also in other neurological diseases such as Parkinson's disease and multiple sclerosis. ***Memantine***, is a noncompetitive ***NMDA receptor antagonist*** first used as an anti-influenza agent. It acts on the glutamatergic system by blocking NMDA receptors and inhibiting their overstimulation by glutamate. Memantine has been shown to be moderately efficacious in the treatment of moderate to severe Alzheimer's disease. Its effects in the initial stages of AD are unknown. Reported adverse events with memantine are infrequent and mild, including hallucinations, confusion, dizziness, headache and fatigue. The combination of memantine and donepezil has been shown to be "of statistically significant but clinically marginal effectiveness".

Excitotoxicity…

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters such as ***glutamate*** and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (***glutamate receptors***) such as the **NMDA receptors** and **AMPA receptors** are overactivated. Excitotoxins like NMDA (*N*-Methyl-D-aspartate) and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate (**50-100 μM**), can cause excitotoxicity by allowing high levels of calcium ions (Ca^{2+}) to enter the cell.

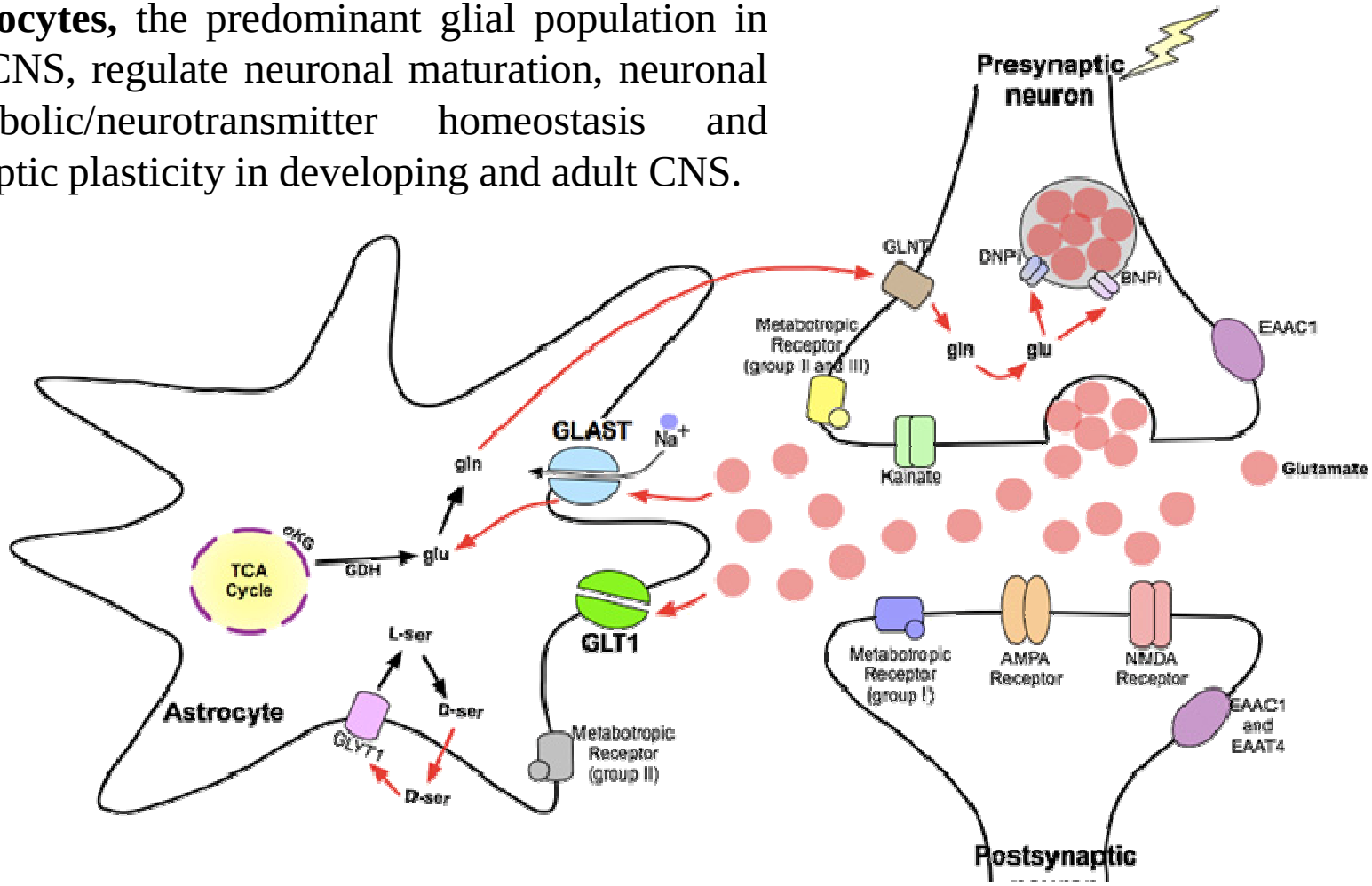
Ca^{2+} influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA.




ISCHEMIA, PROCESSO DEGENERATIVO, TRAUMA
Rilascio di glutammato
Apertura canale/ recettore NMDA
X
Memantine
Apertura canali del Ca++ voltaggio dipendenti (L)
Eccessiva depolarizzazione di membrana
Deplezione ATP
ENTRATA INCONTROLLATA DI Ca++
Attivazione NOS
Attivazione enzimi fosforilativi
Attivazione Lipasi
Attivazione Proteasi
NO
ROS
Danno proteine
infiammazione
Danno DNA
Danno membrana
APOPTOSI / NECROSI

Excitotoxicity…

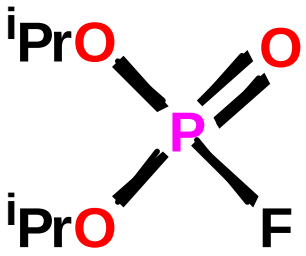
Astrocytes, the predominant glial population in the CNS, regulate neuronal maturation, neuronal metabolic/neurotransmitter homeostasis and synaptic plasticity in developing and adult CNS.



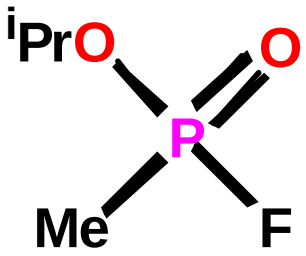
(glutamate aspartate transporter; GLAST) and glutamate transporter-1 (GLT-1)

15.4 Organophosphates

a) Nerve gases

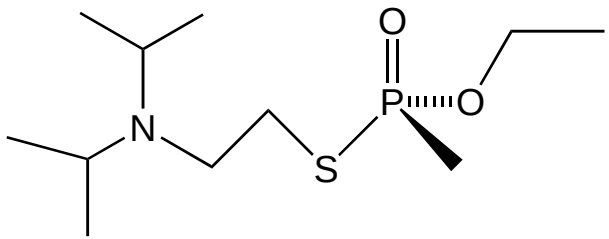


Dyflos
(Diisopropyl fluorophosphonate)



Sarin

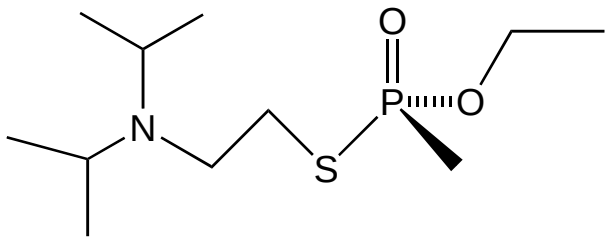
- **Agents developed in World War 2**
- **Agents irreversibly inhibit acetylcholinesterase**
- **Permanent activation of cholinergic receptors by Ach**
- **Results in death**



VX Nerve Agent

The VX nerve agent is the most well-known of the V-series of nerve agents. Its chemical name is O-ethyl-S-[2(diisopropylamino)ethyl] methylphosphonothiolate and its molecular formula is C11H26NO2PS.
The only countries known to possess VX are the United States and Russia. VX agent is considered an area denial weapon due to its physical properties.

With its high viscosity and low volatility VX has the texture and feel of high-grade motor oil. This makes it especially dangerous, as it has a high persistence in the environment. It is odorless and tasteless, and can be distributed as a liquid or, through evaporation, into small amounts of vapor. It works as a nerve agent by blocking the function of the enzyme acetylcholinesterase. Normally, an electric nerve pulse would cause the release of acetylcholine over a synapse that would stimulate muscle contraction. The acetylcholine is then broken down to non-reactive substances (acetic acid and choline) by the acetylcholinesterase enzyme. If more muscle tension is needed the nerve must release more acetylcholine. VX blocks the action of acetylcholinesterase, thus resulting in sustained contractions of all the muscles in the body. Sustained contraction of the diaphragm muscle causes death by asphyxiation.

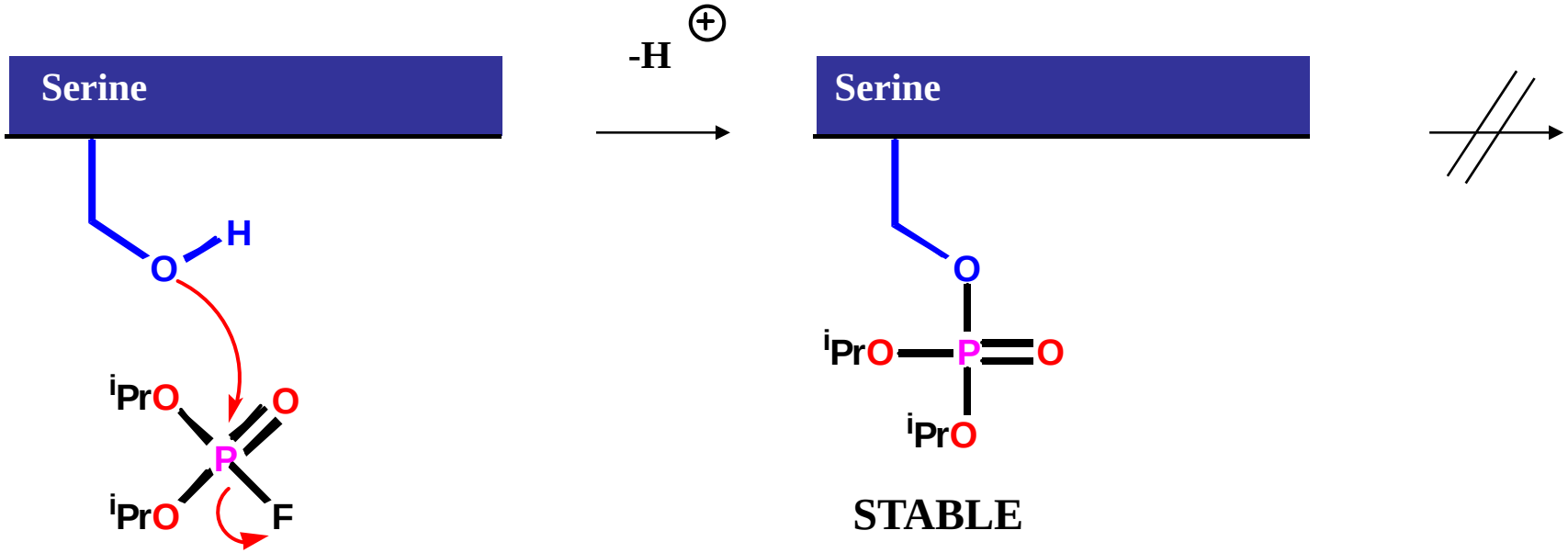


VX Nerve Agent

Often regarded as the deadliest nerve agent created to date, as little as 200 micrograms is enough to kill an average person, depending on method of absorption. Death can be avoided if the appropriate antidote is injected immediately after exposure. The most commonly used antidote is atropine and pralidoxime, which is issued for military personnel in the form of an autoinjector. Standard chemical agent resistance pills are also effective. Atropine works by binding and blocking a subset of acetylcholine receptors (known as muscarinic acetylcholine receptor, mAchR), so that the build up of acetylcholine produced by loss of the acetylcholinesterase function can no longer affect their target. This prevents involuntary muscle actions so that muscles like the diaphragm are not in constant contraction. The injection of pralidoxime regenerates bound acetylcholinesterase.

15.4 Organophosphates

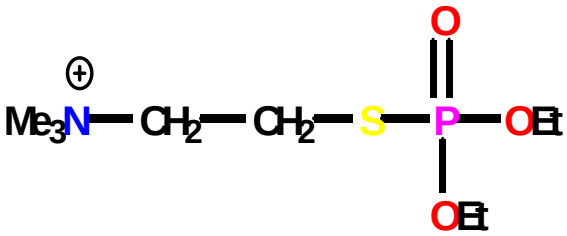
b) Mechanism of action



- **Irreversible phosphorylation**
- **P-O bond very stable**

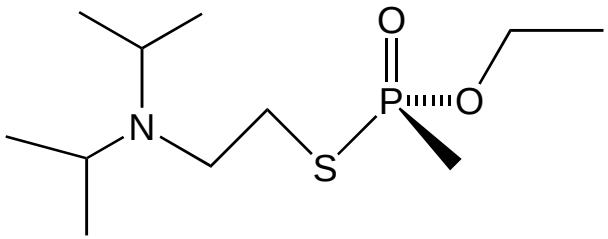
15.4 Organophosphates

c) Medicinal organophosphate



Ecothiopate

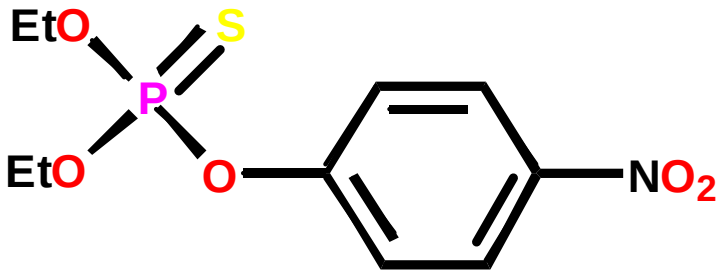
- **Used to treat glaucoma**
- **Topical application**
- **Quaternary N is added to improve binding interactions**
- **Results in better selectivity and lower, safer doses**



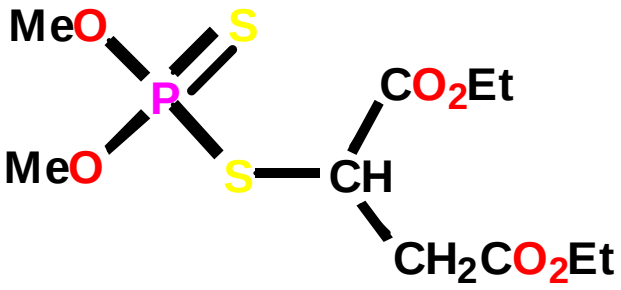
VX Nerve Agent

15.4 Organophosphates

d) Organophosphates as insecticides



Parathion



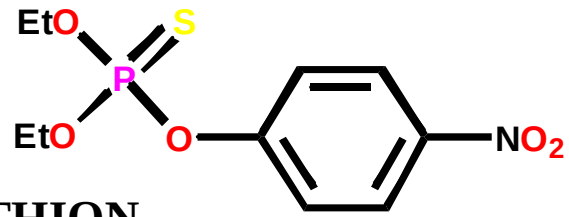
Malathion

- **Relatively harmless to mammals**
- **Agents act as prodrugs in insects**
- **Metabolised by insects to produce a toxic metabolite**

15.4 Organophosphates

d) Organophosphates as insecticides

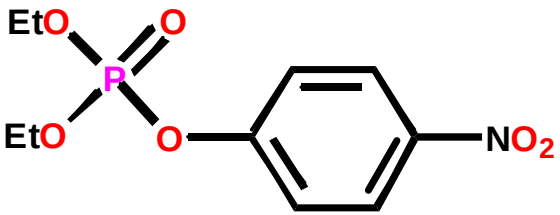
MAMMALS



PARATHION
(Inactive Prodrug)

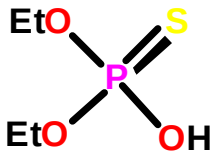
Insect
Oxidative
desulphurisation

INSECTS



Active drug

Mammalian
Metabolism



INACTIVE
& excreted

Phosphorylates enzyme

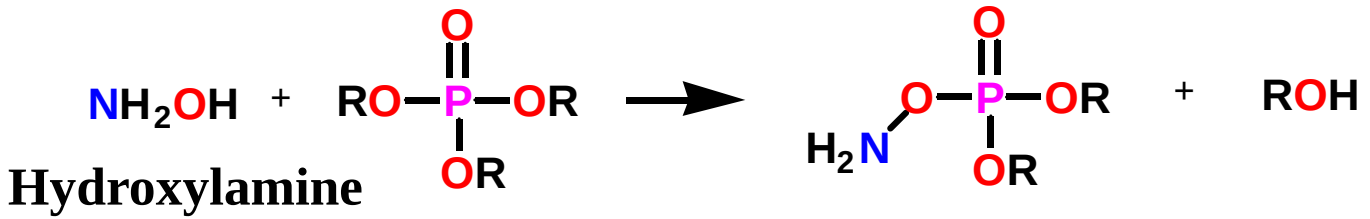
DEATH

15.4 Organophosphates

e) Design of Organophosphate Antidotes

Strategy

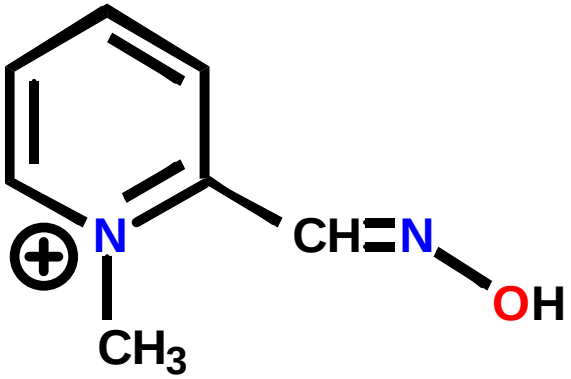
- **Strong nucleophile required to cleave strong P-O bond**
- **Find suitable nucleophile capable of cleaving phosphate esters**
- **Water is too weak as a nucleophile**
- **Hydoxylamine is a stronger nucleophile**



- **Hydroxylamine is too toxic for clinical use**
- **Increase selectivity by increasing binding interactions with active site**

15.4 Organophosphates

e) Design of Organophosphate Antidotes

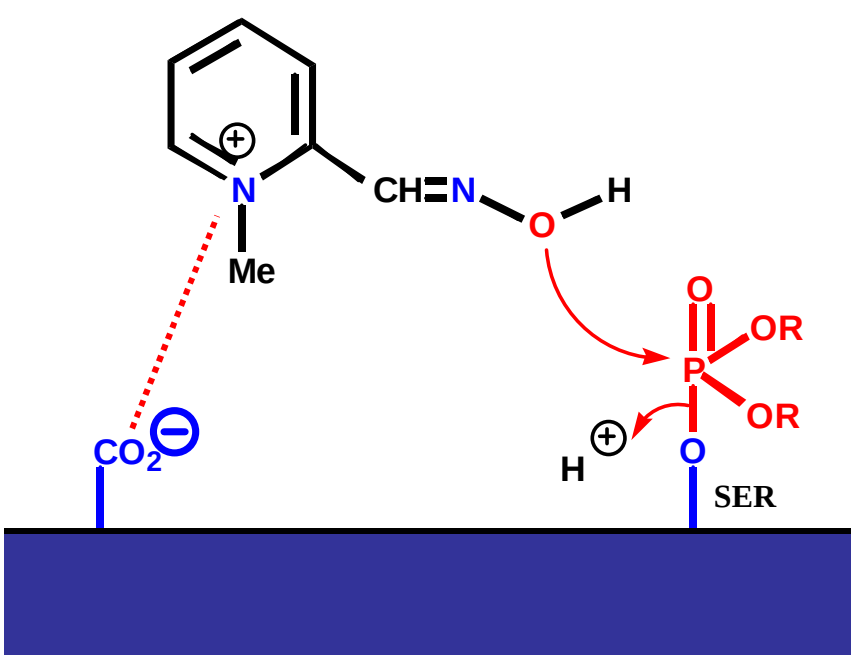


Pralidoxime

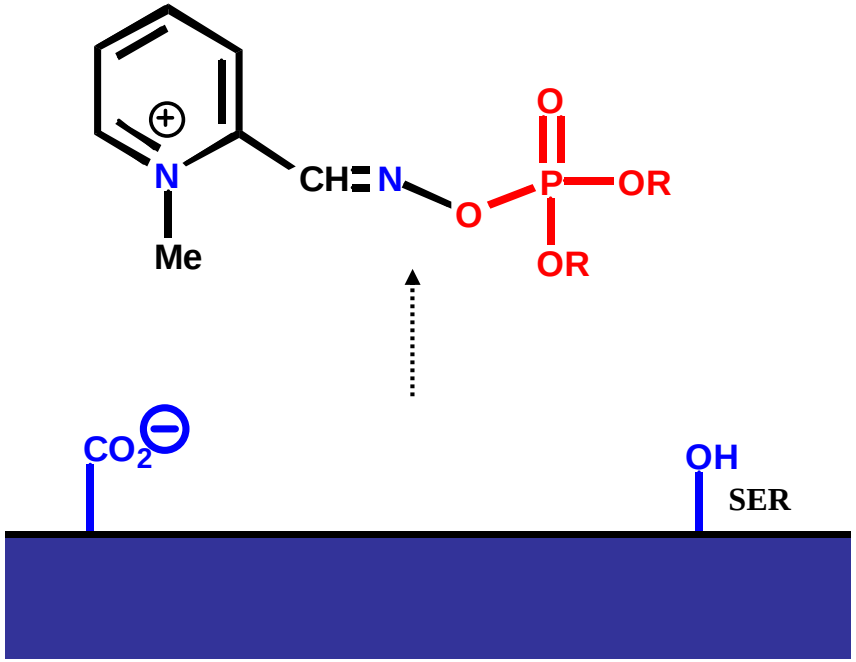
- **Quaternary N is added to bind to the anionic region**
- **Side chain is designed to place the hydroxylamine moiety in the correct position relative to phosphorylated serine**
- **Pralidoxime 1 million times more effective than hydroxylamine**
- **Cannot act in CNS due to charge - cannot cross bbb**

15.4 Organophosphates

e) Design of Organophosphate Antidotes



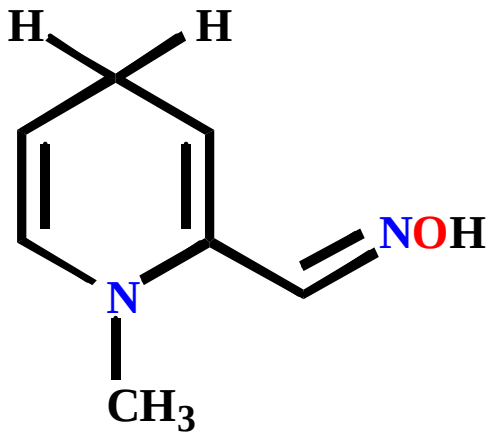
Active Site (Blocked)



Active Site (Free)

15.4 Organophosphates

e) Design of Organophosphate Antidotes



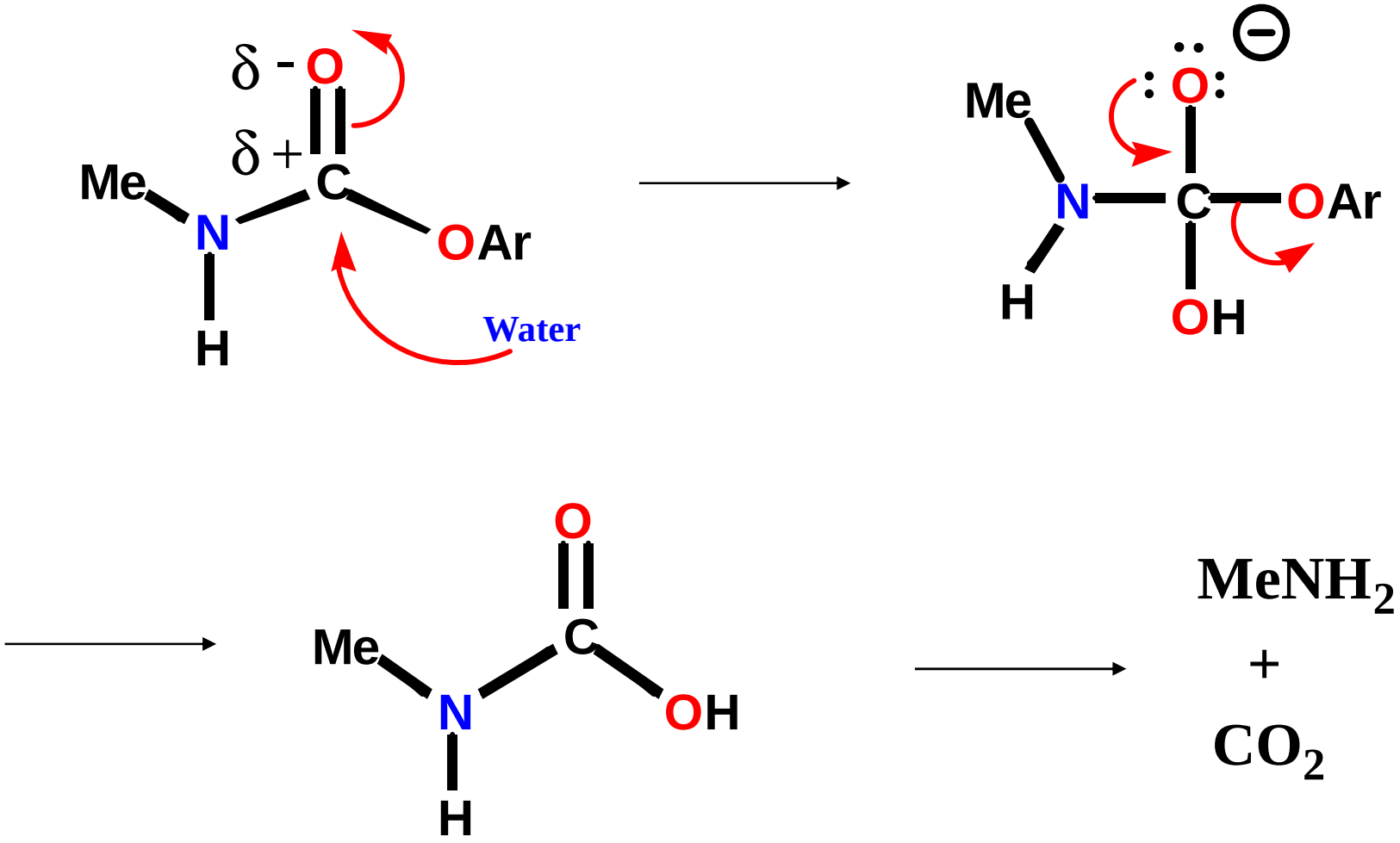
ProPAM

- **Prodrug for pralidoxime**
- **Passes through BBB as free base**
- **Oxidised in CNS to pralidoxime**


TITLE
"CHIMICA FARMACEUTICA E TOSSICOLOGICA II"
DIRECTOR
Stefano Moro
CAMERA
Chimica e Tecnologia Farmaceutiche
DATE
SCENE
TAKE

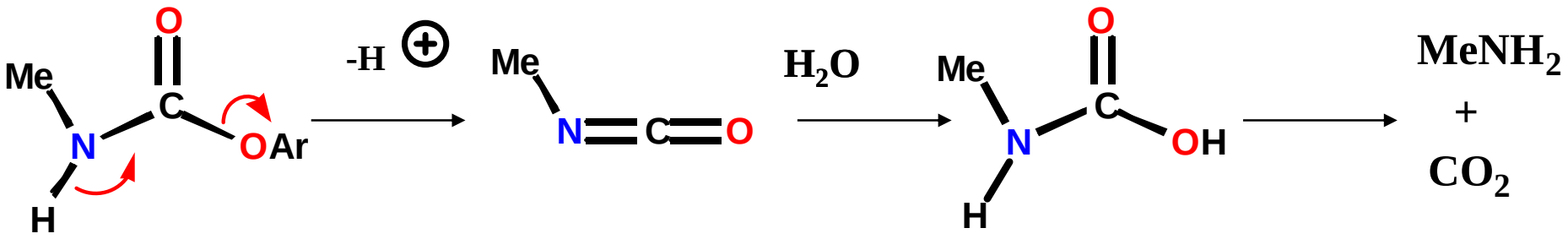
Hydrolysis mechanisms

Possible mechanism 1



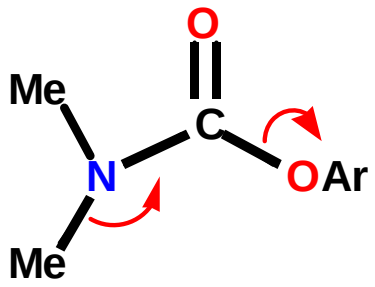
Hydrolysis mechanisms

Possible mechanism 2



Too reactive

Compare:



-Me ⊕

No hydrolysis