B. Antiacetylcholine esterase agents

These drugs inhibit acetylcholinesterase and cause acetylcholine accumulation in all cholinergic junctions and synapses.

They are effective on both nicotinic and muscarinic receptors. These compounds bind competitively to the enzyme prior to acetylcholine and prevent hydrolysis.

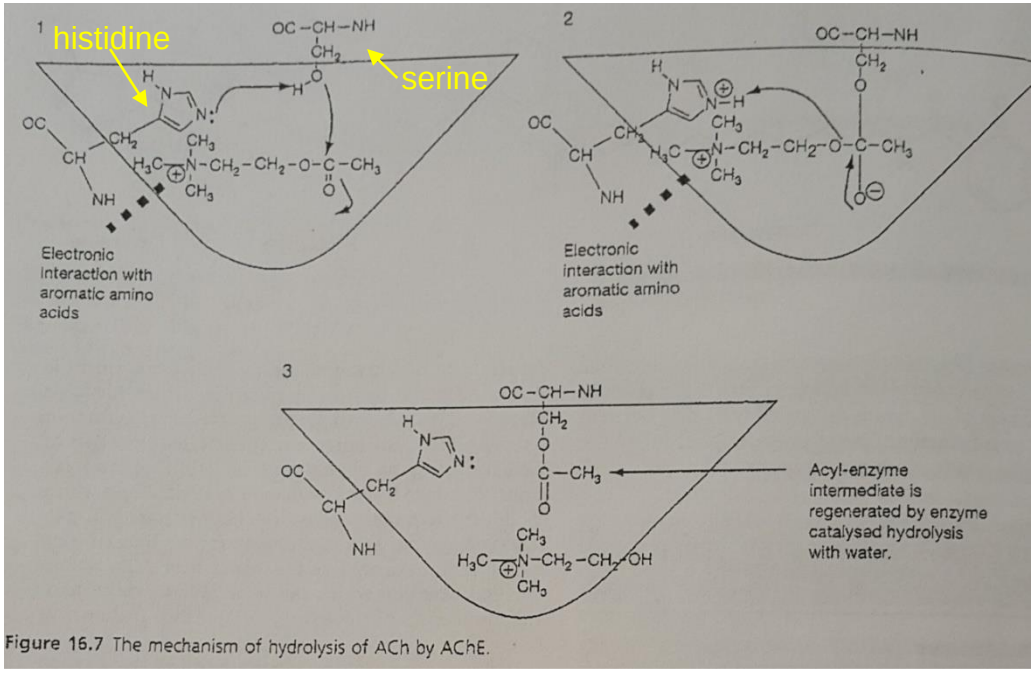


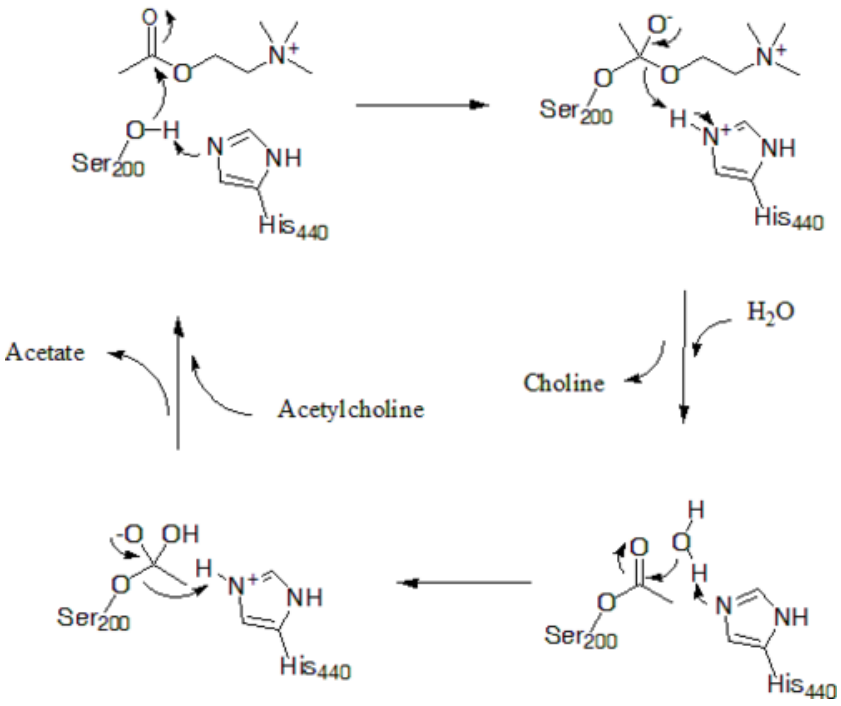
Figure 16.7 The mechanism of hydrolysis of ACh by AChE.

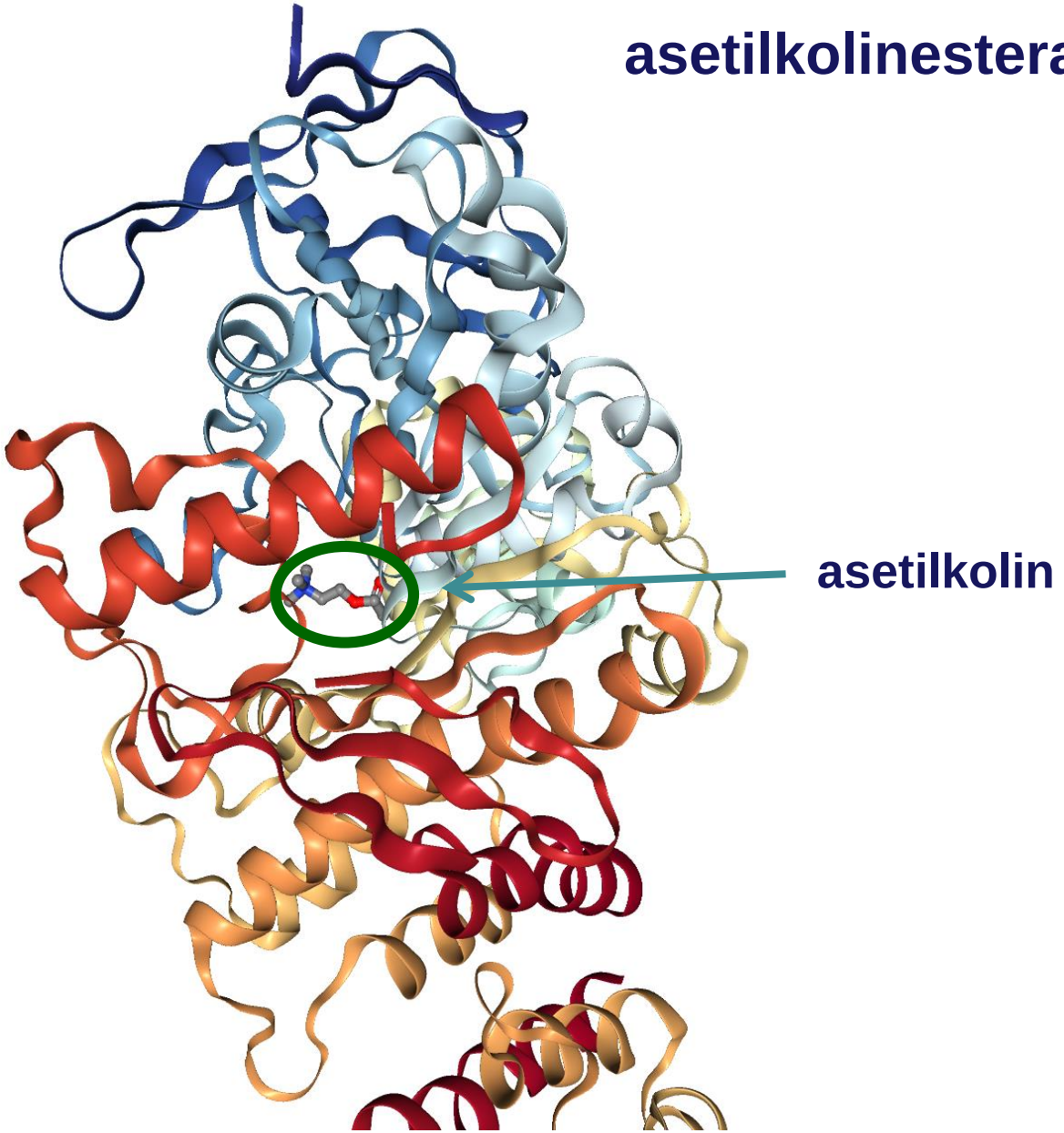
B. Antiacetylcholine esterase agents

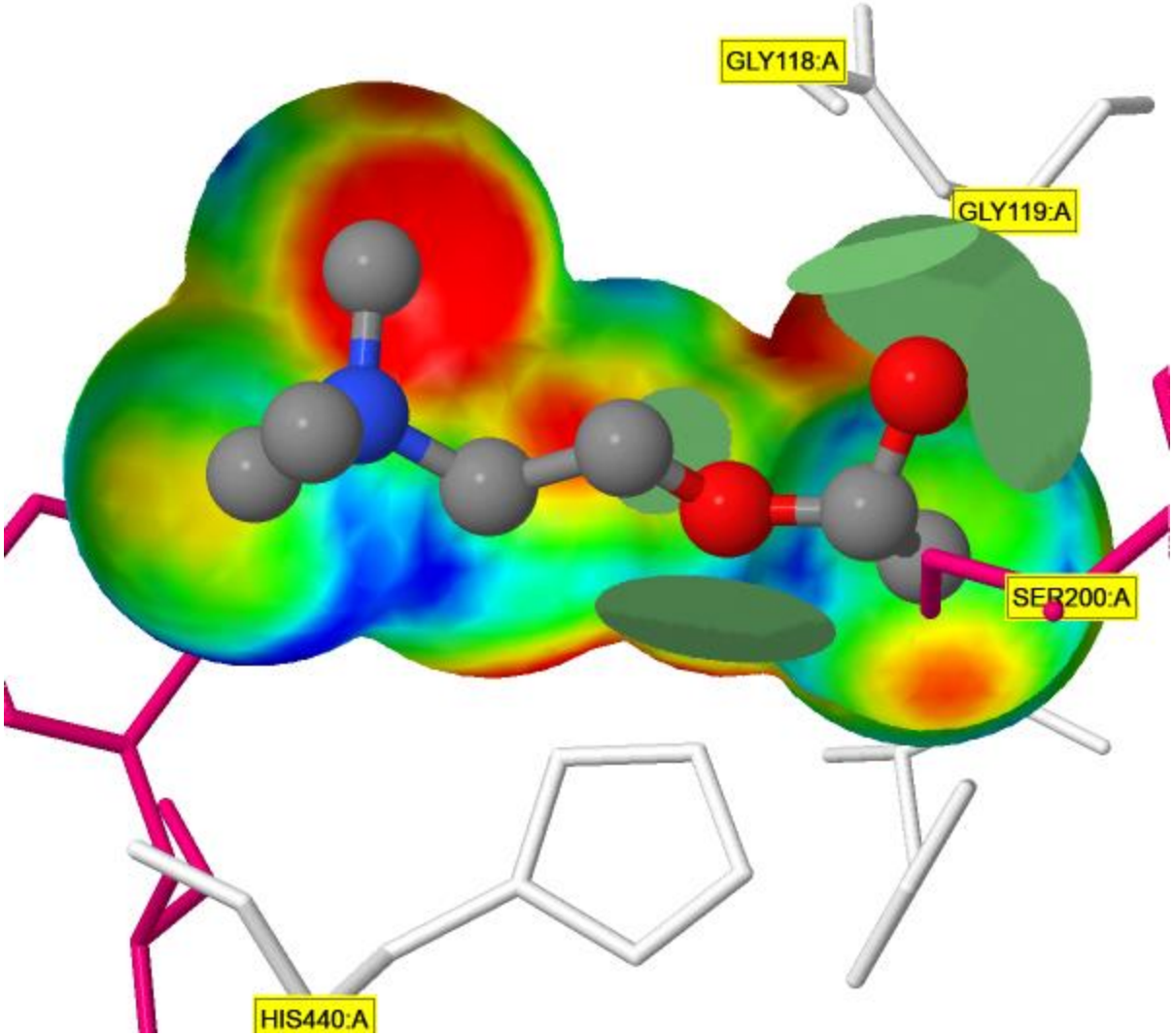
- Drugs that prevent the degradation of acetylcholine (ACh) by acetylcholinesterase
- Viewed as indirect-acting cholinergic agonists
- Lack selectivity (muscarinic, ganglionic, and neuromuscular)

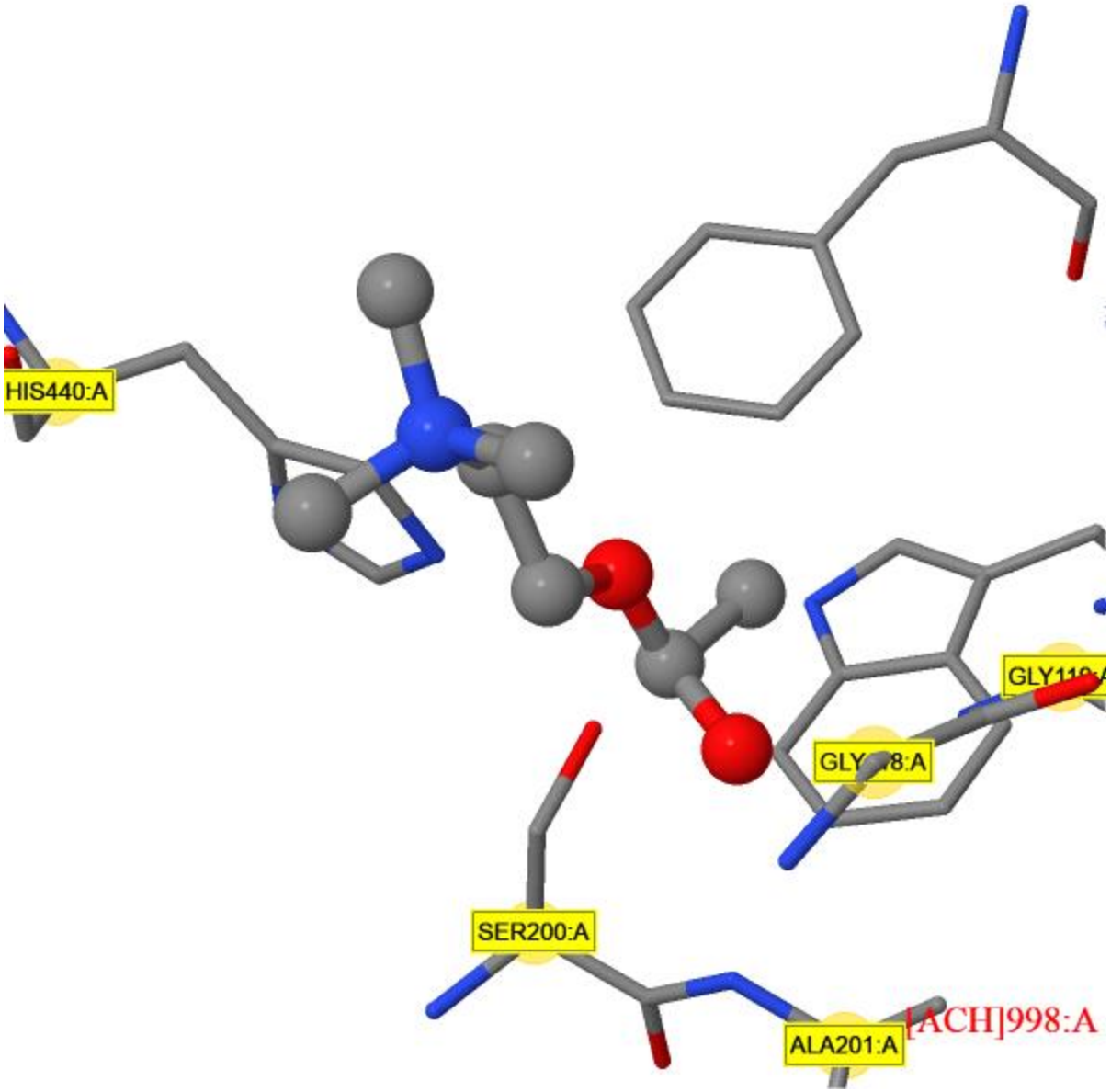
B. Antiacetylcholine esterase agents

Anticholinesterases; myasthenia gravis, atonias in the gastrointestinal tract and glaucoma. These compounds are also used as nerve gases and insecticides. Compounds except competitive antagonists are in two groups as carbamic acid and phosphoric acid derivatives. The mechanism of action of both group is similar. The esteratic region of the acetylcholine esterase reacts with phosphoric acid or carbamic acid esters and inhibits the hydrolysis of acetylcholine.




asetilkolinesteraz
asetilkolin


GLY118:A
GLY119:A
SER200:A
HIS440:A


HIS440:A
GLY118:A
SER200:A
ALA201:A
[ACH]998:A

Anticholinesterases;

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

•B. Antiacetylcholine esterase agents

Anticholinesterases are divided into 3 groups according to the drugs mechanism.

B. Antiacetylcholine esterase agents

- B1. Competitive antagonists
- B2. Short-acting inhibitors (Carbamates) (Reversibl)
- B3. Long-acting inhibitors (Organophosphorus) (Irreversib)

https://www.youtube.com/watch?v=-gIqZ8IxctE

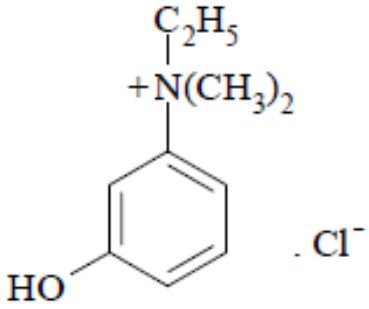
B. Antiacetylcholine esterase agents

B1. Competitive antagonists

B. Antiacetylcholine esterase agents

B1. Competitive antagonists

Edrophonium ENLON, EDROPONIUM CHLORIDE

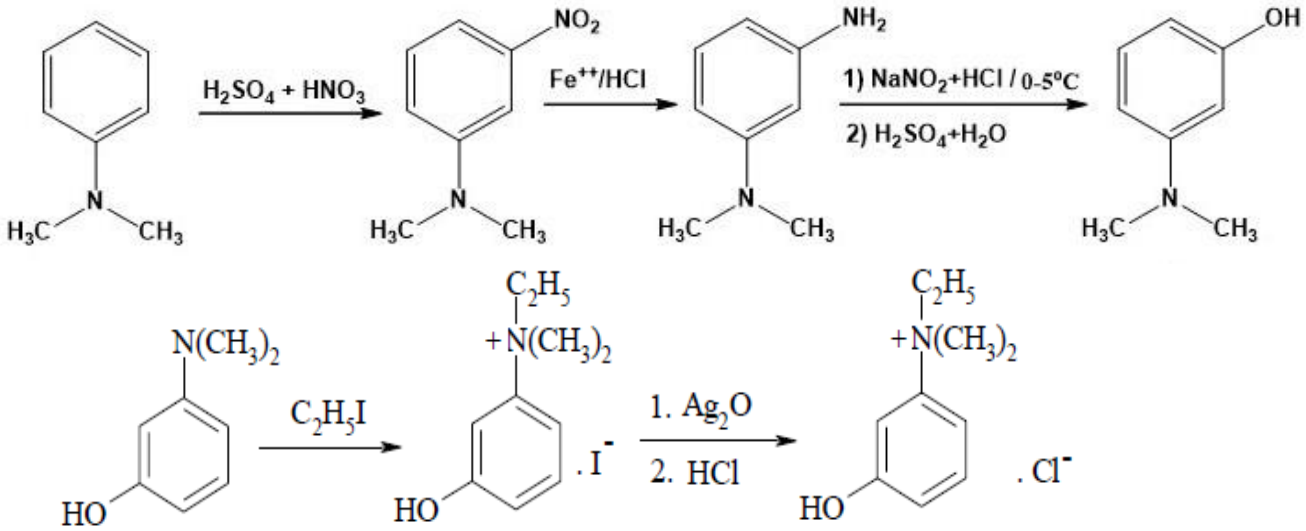


Quaterner alcohol such as Edrophonium act as competitive antagonists with ACh to AChE.

It is used for treating myasthenia gravis*. It's effect is very short like 5-10 minutes.

N,N-Dimethyl-N-ethyl-N-(3-hydroxyphenyl)ammonium chloride

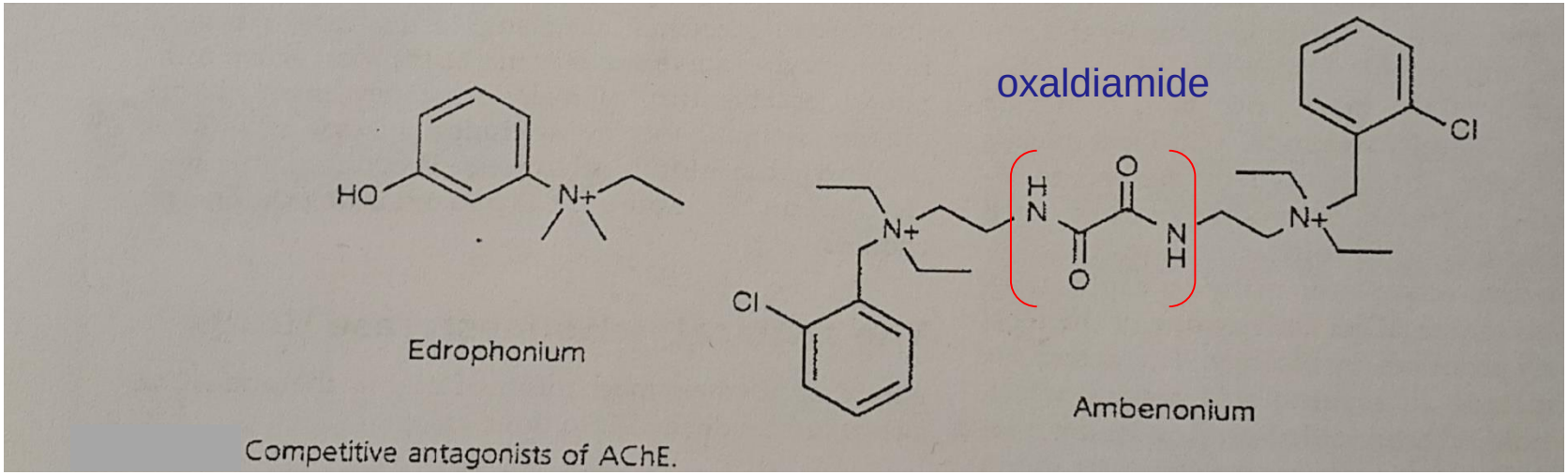
Synthesis:



*An autoimmune disease in which the body produces antibodies to its cholinergic receptors. Severe muscle weakness and fatigue occurs.

B. Antiacetylcholine esterase agents

B1. Competitive antagonists



Competitive antagonists of AChE.

Ambenonium Mytelase R

Ambenonium is another competitive inhibitor of AChE. It is composed of two moieties structurally related to edrophonium connected by an oxaldiamide bridge. This is thought to provide a competitive shield of the two active sites in the AChE.

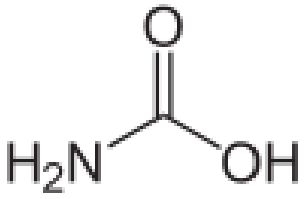
It is used as bromide salt for treating myasthenia gravis

B. Antiacetylcholine esterase agents

- B2. Short-acting inhibitors (Carbamates) (Reversibl)

- A significant proportion of the compounds in this group are esters (carbamate derivatives) of carbamic acid with bases containing substituted phenyl or pyridine groups.

- They compete for binding to the enzyme with acetylcholine. This bond is reversible.



B. Antiacetylcholine esterase agents

- B2. Short-acting inhibitors (Carbamates) (Reversibl)

The remaining groups of anti-AChE drugs all cause formation of a covalent bond with the serine residue in AChE, which is more resistant to nucleophilic attack by water than the normal acetyl group and thus slows down recycling of the enzyme.

A good anticholinesterase compound should be:

- Contain a leaving group which is equal in efficiency of dissociation to the acyl group in ACh.

- The leaving group should produce a residue bound to serine, which is less susceptible to hydrolysis than acetate

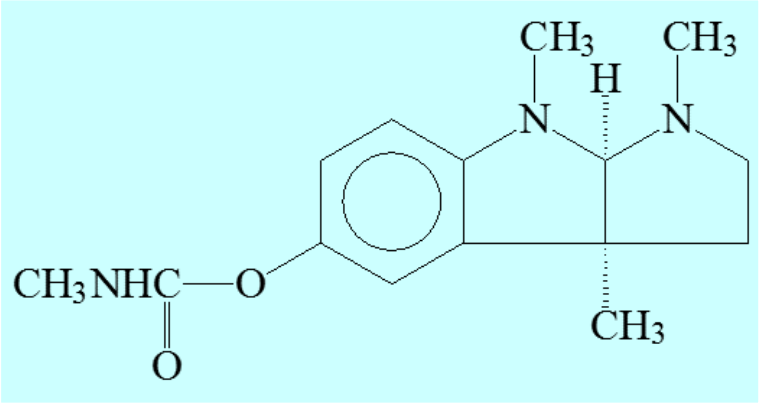
- Contain a positively charged moiety to fix the molecule in the right position/orientation in the enzyme's active site.

Physostigmine ESERİN

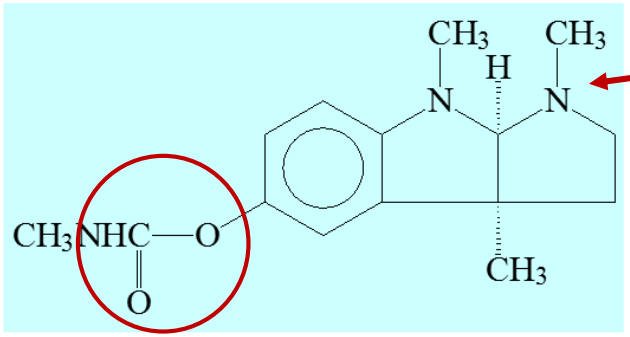
(AChE = Acetylcholinesterase)

Physostigmine was discovered in 1864 and its structure was established in 1925.

It is extracted from the plant *Physostigma venenosum* and it was the first compound as an anti-AChE inhibitor.



Physostigmine



Pyrrolidine nitrogen can be ionised at blood pH thus assisting active site binding

Carbamate residue forms covalent bond with serine

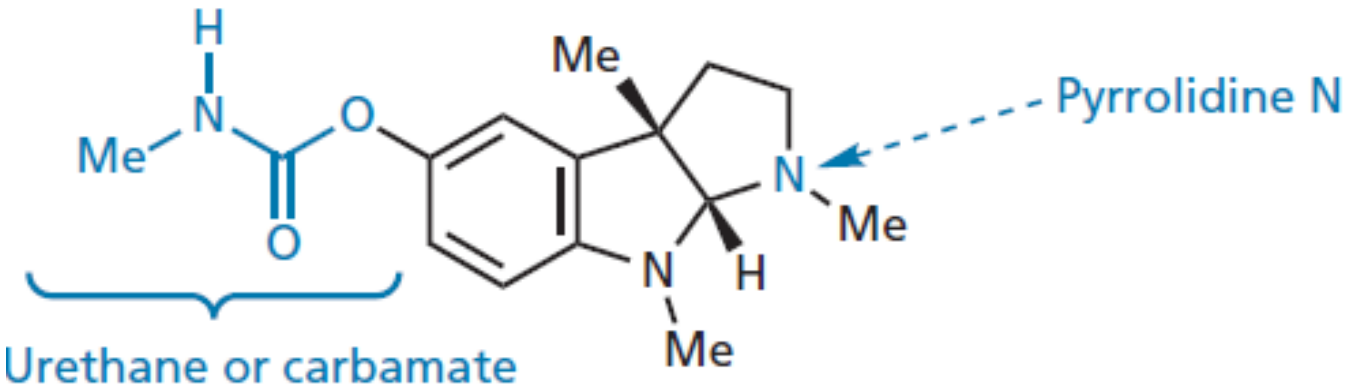
Physostigmine has a tertiary amine group, but its effective form is thought to be the quaternary amine formed by ionization in aqueous media. Because the effect decrases whenever the pH of the media increases.

Increases the motility of intestines and bladder. It used in the treatment of overdoses of Atropine (anticholinergic), phenothiazines and tricyclic antidepressant drugs.

Phyostigmine has serious side effects which limit its use, but it provided a prototype which medicinal chemists could use as a lead compound (prototype).

B. Antiacetylcholine esterase agents

- B2. Short-acting inhibitors (Carbamates) (Reversibl)



Physostigmine

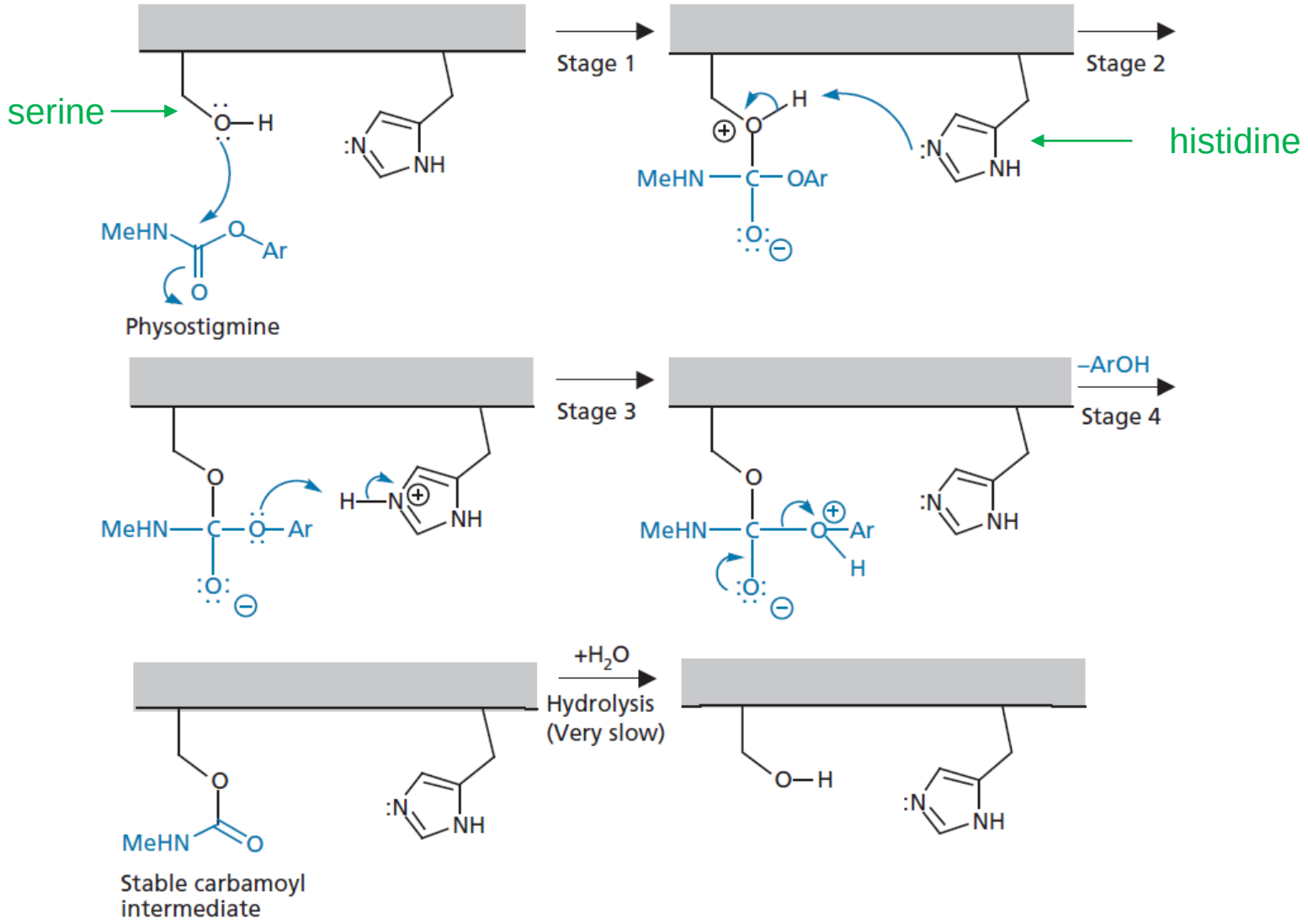
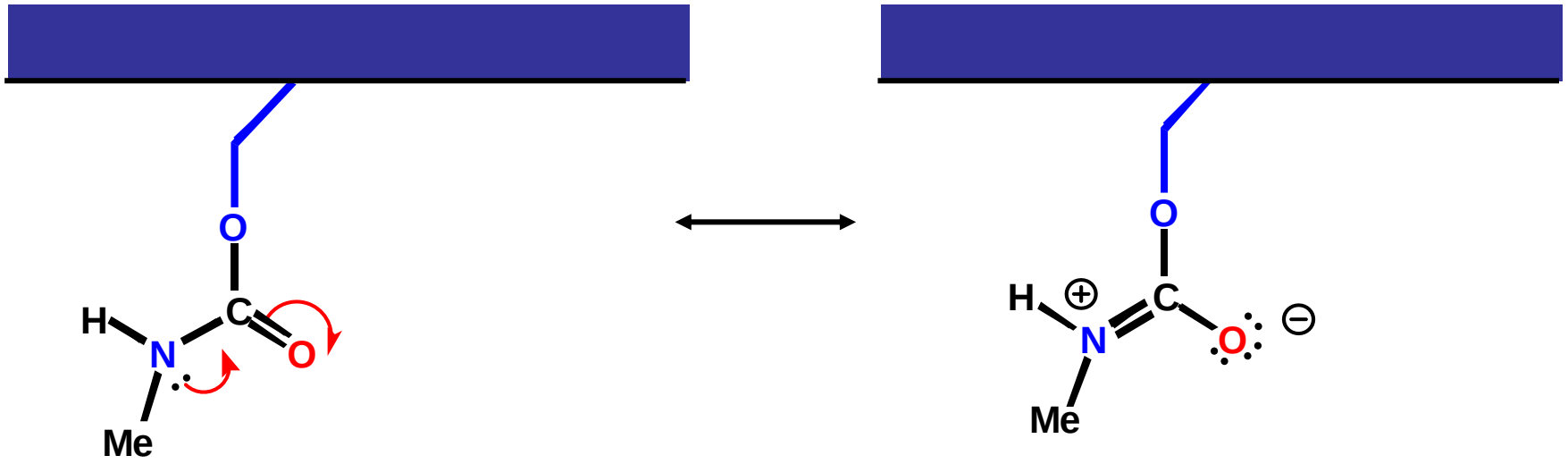


FIGURE 22.45 Mechanism of inhibition by physostigmine (Ar represents the tricyclic system of physostigmine).

Mechanism of action

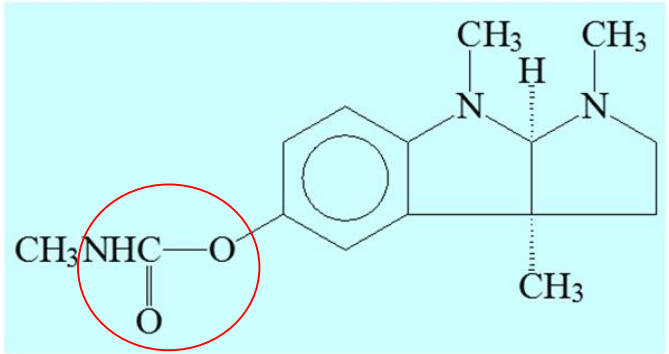


Carbonyl group 'deactivated'

Development of anticholinesterase active compounds from physostigmine

The three structural elements mentioned above with regard of the structure of physostigmine are as follows;

1. A **phenol carbamate** is required since its easily hydrolysed thus it readily transfers its carbamate group to serine. Addiditonally, the hydrophobic interaction produced by the benzene is important.



B. Antiacetylcholine esterase agents

- B2. Short-acting inhibitors (Carbamates) (Reversibl)

2. The carbamate group works exactly as the nitrogen in carbachol which stabilites the linkage by providing its lone pair to the positive carbonyl carbon, making it less attractive to weak nucleophiles (water in this case).

Thus carbamate agents such as physostigmine undergo the hydrolysis via the action of AChE, but form a carbamoyl serine (or methyl carbamoyl serine), which is more stable than acetate against hydrolysis required for the restoration of the active size.

Regenaration takes 30-60 minutes compared with 150 microseconds in the case of the acetylated enzyme. This is forty million times slower than the hydrolysis of serine acetate.

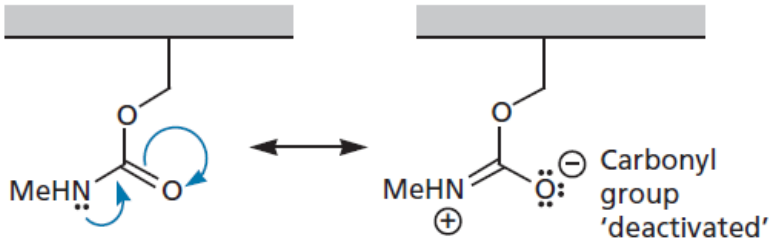
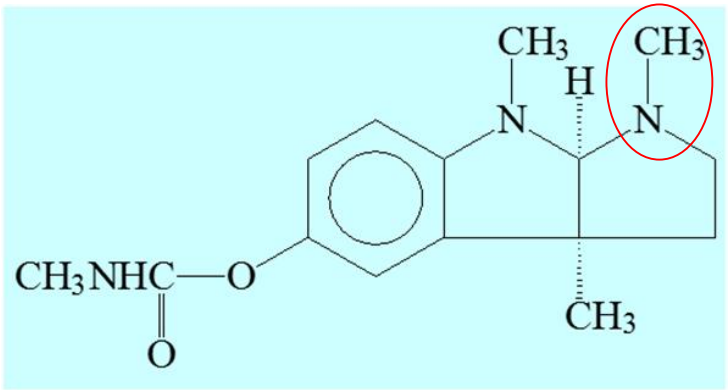
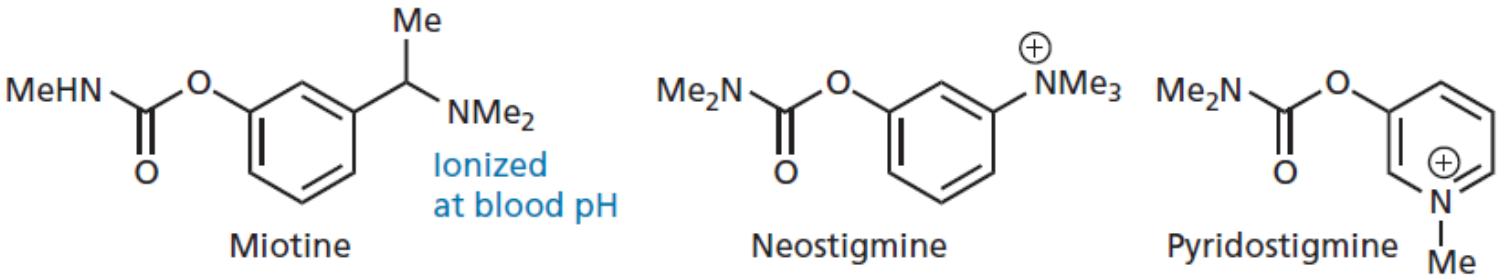


FIGURE 22.46 Stabilization of the carbamoyl–enzyme intermediate.

3. In physostigmine the methyl pyrrolidine is not a quaternary ammonium, yet it is protonated at physiological pH, hence satisfying the third rule which requires a **positively charged** group for binding to the active site.



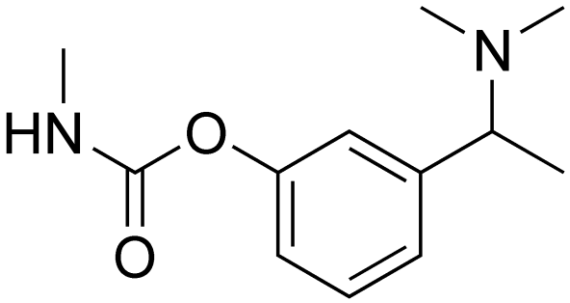
Analogues of Physostigmine

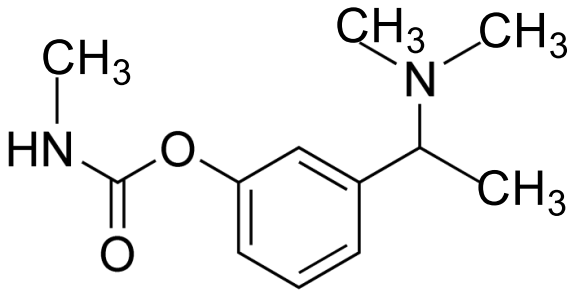


Analogues of physostigmine. Miotine is a chiral molecule that has been studied as a racemate.

B2. Short-acting inhibitors (Carbamates) (Reversibl Inhibitors)

Miotine



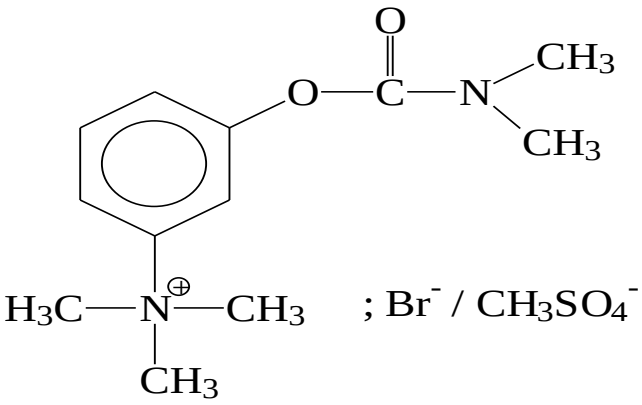


[3-[1-(Dimethylamino)ethyl]phenyl] *N*-methylcarbamate

Miotine followed physostigmine as a first synthetic AChE inhibitor. It is still prone to chemical hydrolysis and its non-permanently charged tertiary amine group grants it access to the CNS, leading to undesired side effects there.

B2. Short-acting inhibitors (Carbamates) (Reversibl Inhibitors)

Neostigmine PROSTİGMİNE, PLANTİGMİN, NEOSTİGMİNE®



3-[[(Dimethylamino)carbonyl]oxy]-N,N,N-trimethylbenzenammonium bromide

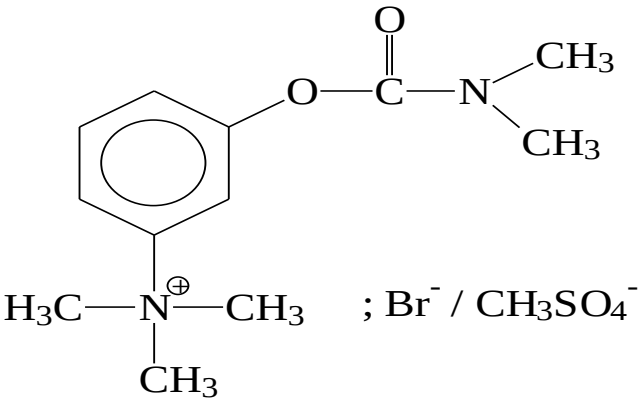
It is obtained the modification of Miotine

Neostigmine is a further modification on miotine where the methylcarbamate group is replaced with dimethylcarbamate, making the compound more resistant to chemical hydrolysis due to the added inductive effect of the second methyl group.

This permanently charged quaternary nitrogen in neostigmine prevents it from crossing the BBB, making it a safe drug from a CNS perspective, and encourages binding to the enzyme active site. It is more effect than physostigmine. It is used as Br and methylsulphate salt.

B2. Short-acting inhibitors (Carbamates) (Reversibl Inhibitors)

Neostigmine PROSTİGMİNE, PLANTİGMİN, NEOSTİGMİNE®

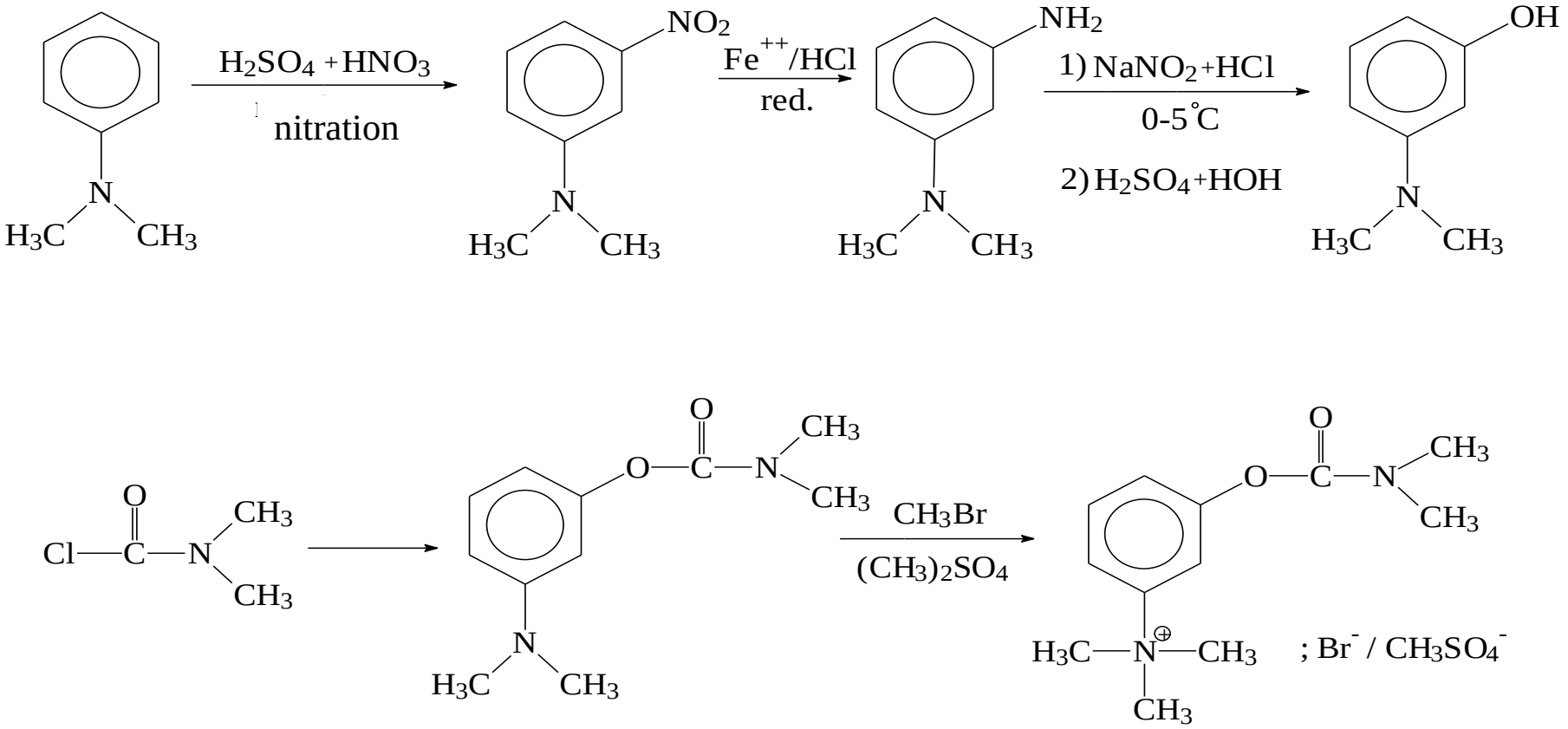


It is used as muscle, intestine and bladder atony, Myestania Gravis* and an antagonist of curar.

Pharmacokinetics: Absorbs very little from gastrointestinal tract. Following parenteral administration, it is rapidly eliminated as methyl sulfate and excreted in the urine as unchanged and its metabolites. It is partially metabolized by hydrolysis of the ester.

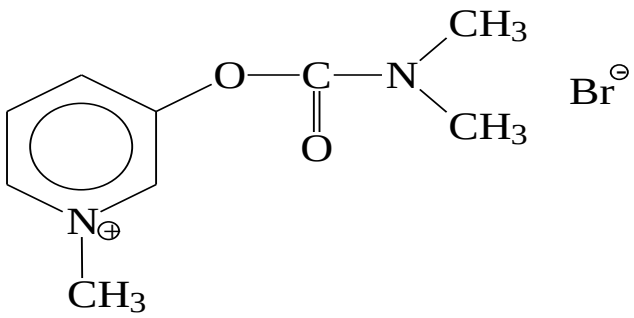
* It is a an autoimmune disease where antibodie to the nicotinic recoptors are produced which decrase the number of functional nicotinic receptor. The syndrome is associated with general weakness in all muscle, specially the small ones such as those int he head, neck, and extremities.

Synthesis of Neostigmine:



B2. Short-acting inhibitors (Carbamates) (Reversibl Inhibitors)

Pyridostigmine MESTİNON®



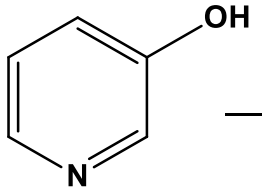
3-(Dimethylaminocarbonyloxy)-methyl-pyridinium bromide

Pyridostigmine is close in structure to both neostigmine and miotine. The aromatic ring (leaving group) is preserved but attached to a methylpyridine ring rather than a benzene ring. A permanent charge is provided by the aromatic ring as well.

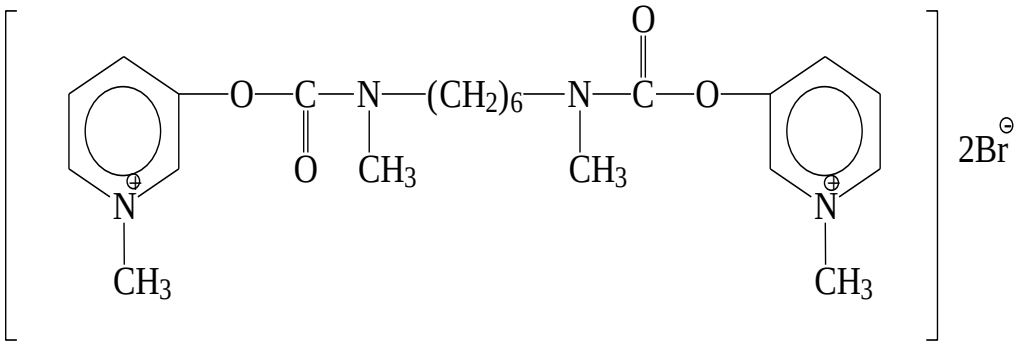
It is used for myesthenia gravis and it leads to better patient complicance than neostigmine as the dose frequency is less (every 3-6 hours). Pyridostigmine is used to reverse anaesthesia-produced paralysis in the same fashion as neostigmine.

Side effects; Difficulty in breathing, broncho spasm, dyspnea, bradycardia, myosis in the eyes, contraction and cramping of the muscular muscles, frequency of urination, excessive sweating, tear, saliva, nausea, vomiting and diarrhea.

Synthesis:????



Distigmine Br UBRETID tb



1,6-Bis-[N-methyl-N-(1-methyl-3-pyridyloxycarbamoyl]hexan dibromide

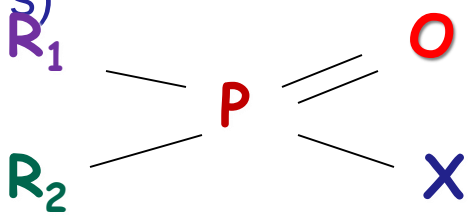
.

Used in atony after surgery

B. Antiacetylcholine esterase agents

B3. Long-acting inhibitors (Organophosphorus)
(Irreversible Inhibitors)

Organophosphate agents (Irreversible Inhibitors)

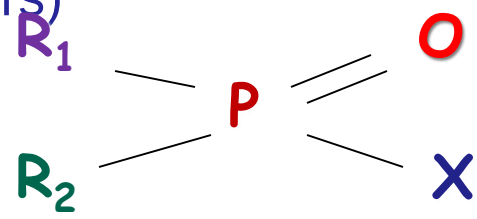


Irreversible inhibitors of AChE act in a similar way as the carbamates, but the phosphate bond is stable for hundreds of hours.

R_1 = Alkoxy
R_2 = Alkoxy, alkyl, tertiary amine
X = Halogen (F), nitrile, p-nitro-phenoxy, organic acide

It is further strengthened by the ageing phenomenon, where the phosphorylated serine on the active site loses one of its oxygens, leading to a phosphine-serine bond, which is even more resistant to hydrolysis.

Organophosphate agents (Irreversible Inhibitors)



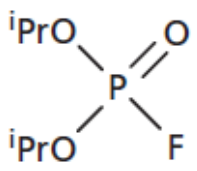
These compounds were first intended to be (but fortunately never used) as chemical warfare agents. The prototype compounds were dyflos and sarin (nerve gases). These compounds irreversibly phosphorylate the serine hydroxyl motif in AChE.

R_1 = Alkoxy
R_2 = Alkoxy, alkyl, tertiary amine
X = Halogen (F), nitrile, p-nitro-phenoxy, organic acide

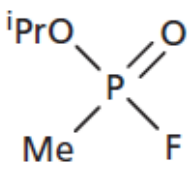
The reaction product is very stable and can not be hydrolysed by weak nucleophiles, even with histidine catalysis. The increases the availability of endogenous ACh in the synaptic gap, leading to a constant signalling at the smooth and skeletal muscles, paralysis and death.

The synthesis of the enzyme again takes weeks or even months. Most of these drugs have toxic effects and have been developed as chemical warfare agents.

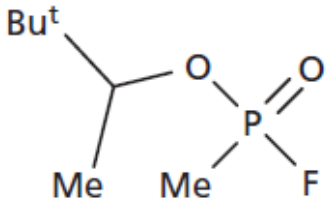
Organophosphate agents (Irreversible Inhibitors)



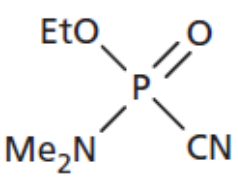
Dyflos
(Diisopropyl
fluorophosphonate)



Sarin



Soman



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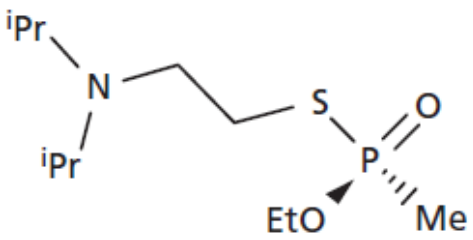


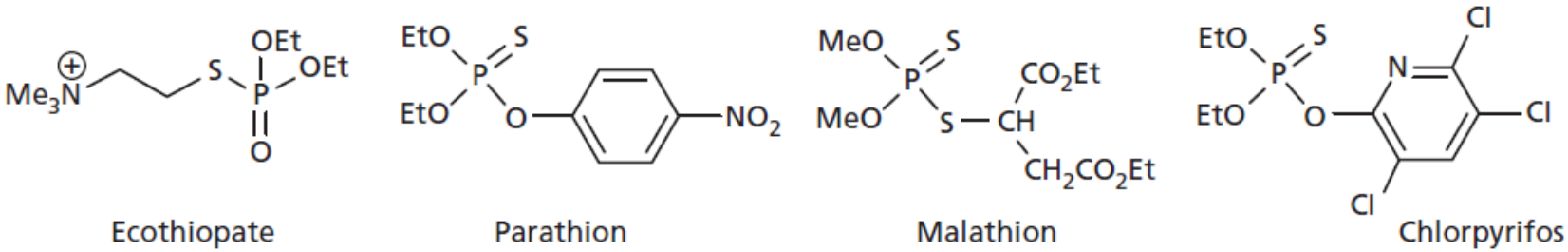
VX

FIGURE 22.48 Examples of nerve agents.

Dyflos and sarin are extremely active and have serious side effects, thus limiting their use to local ophthalmic administration for the treatment of glaucoma.

The binding of these agents is irreversible and a new enzyme is needed in order for the cells to restore ACh hydrolysing capacity.

Organophosphate agents (Irreversible Inhibitors)



Ecothiopate Parathion Malathion Chlorpyrifos

FIGURE 22.50 Organophosphates used as medicines and insecticides.

The number of organophosphates synthesised as insecticides is estimated to be 50 000. Organophospahtes are generally very lipid soluble (ecothiopate is an exception, and is soluble and stable in aqueous solutions), and many of them are chemically unstable.

Organophosphate agents (Irreversible Inhibitors)

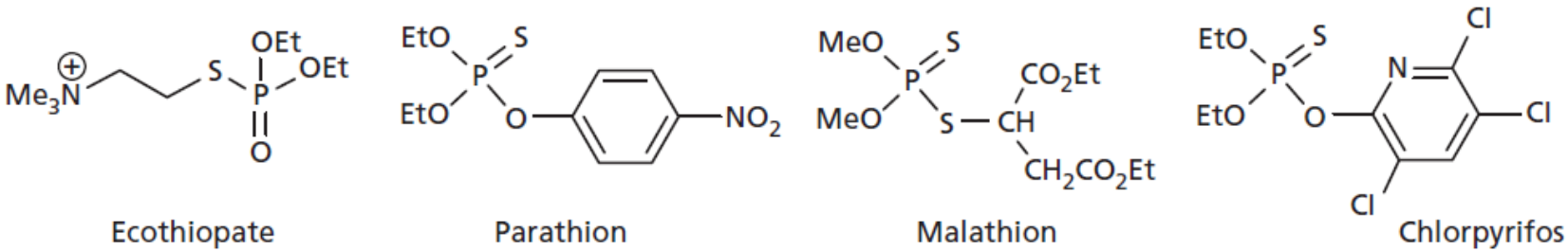


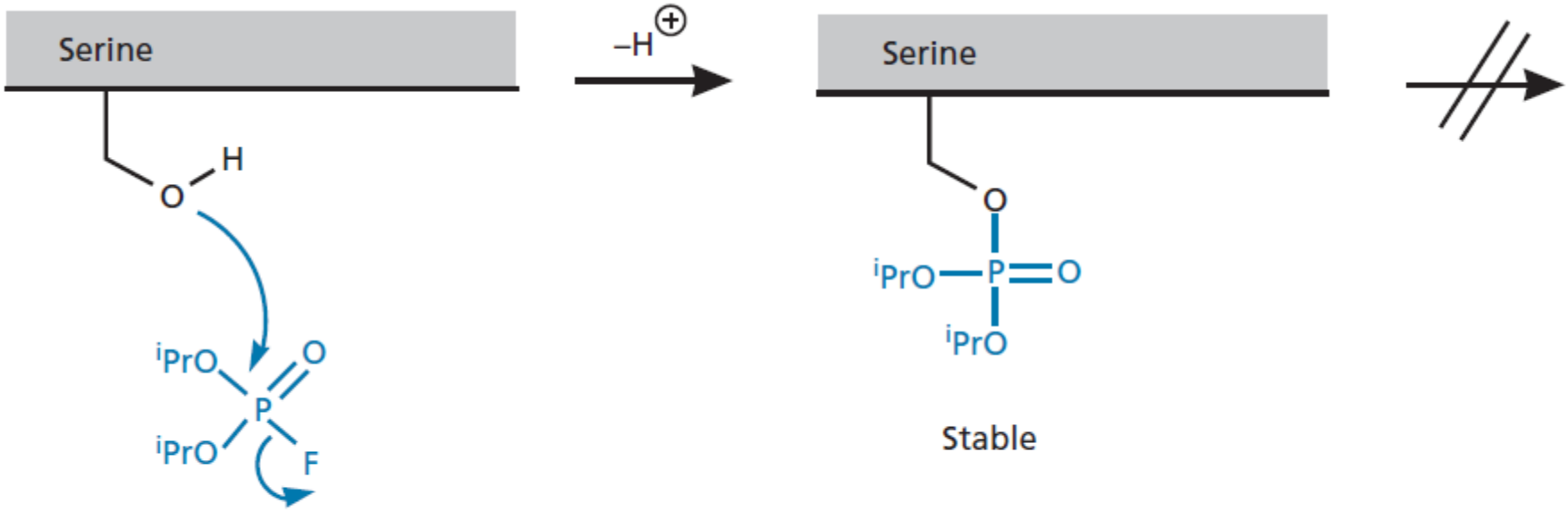
FIGURE 22.50 Organophosphates used as medicines and insecticides.

Ecothiopate, malathion, and parathion have satisfactory stability.

Ecothiopate was developed to selectivity bind to AChE. This was achieved by introducing a quaternary ammonium group at a suitable distance (the two carbon bridge rule) from the phosphorylating head group.

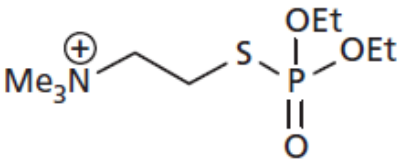
Ecothiopate is more potent than dyflos, and its stability in aqueous solutions (up to several weeks) enables its use as eyedrops for the tratment of glaucoma. It dissociates from AChE over a few days.

Nerve gases



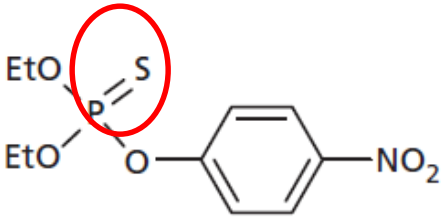
Simplified mechanism of action of dyflos at the active site of acetylcholinesterase.

Organophosphate drugs

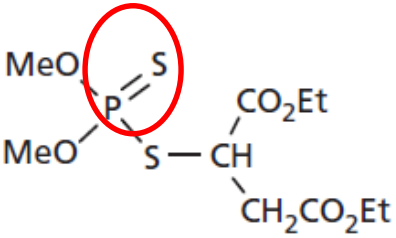


Ecothiopate

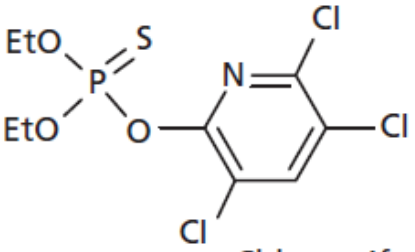
Phospholine Iodide ®



Parathion



Malathion

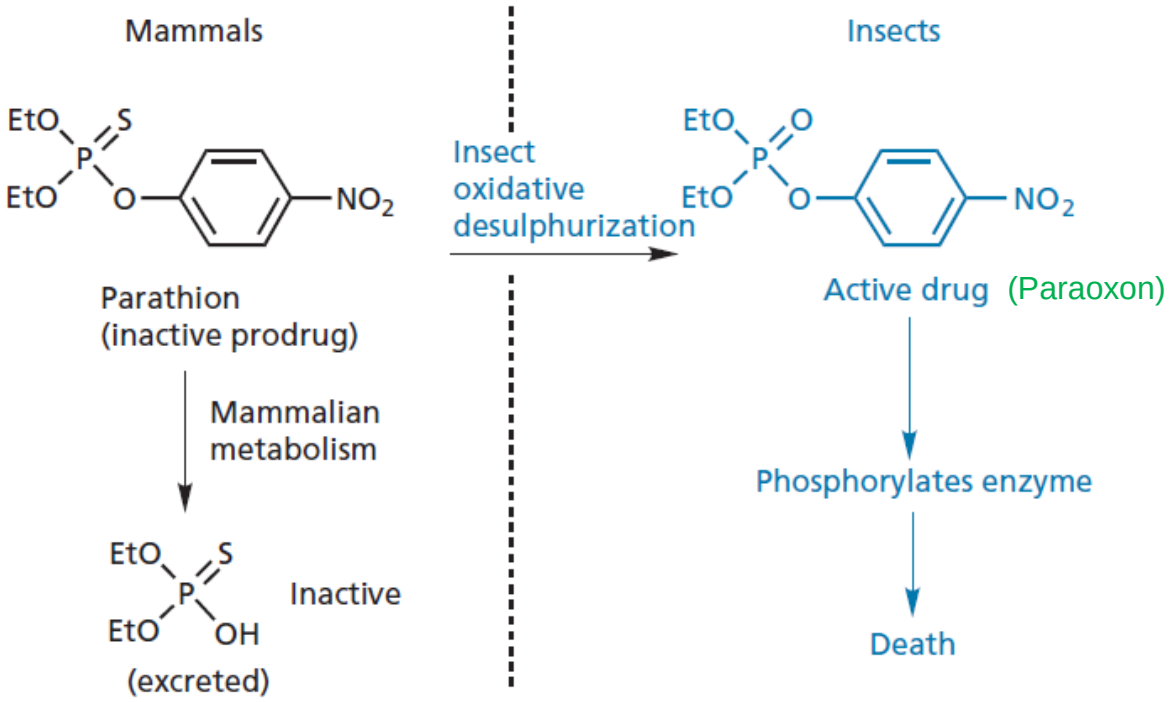


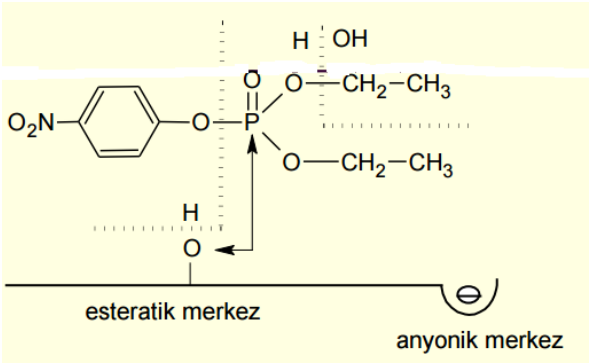
Chlorpyrifos

Organophosphates used as medicines and insecticides.

It is used glaukom

Chlorpyrifos is used for plant protection. However its licence was cancelled on 08 April 2016 due to damage to human health by the Ministry of Food, Agriculture and Livestock.



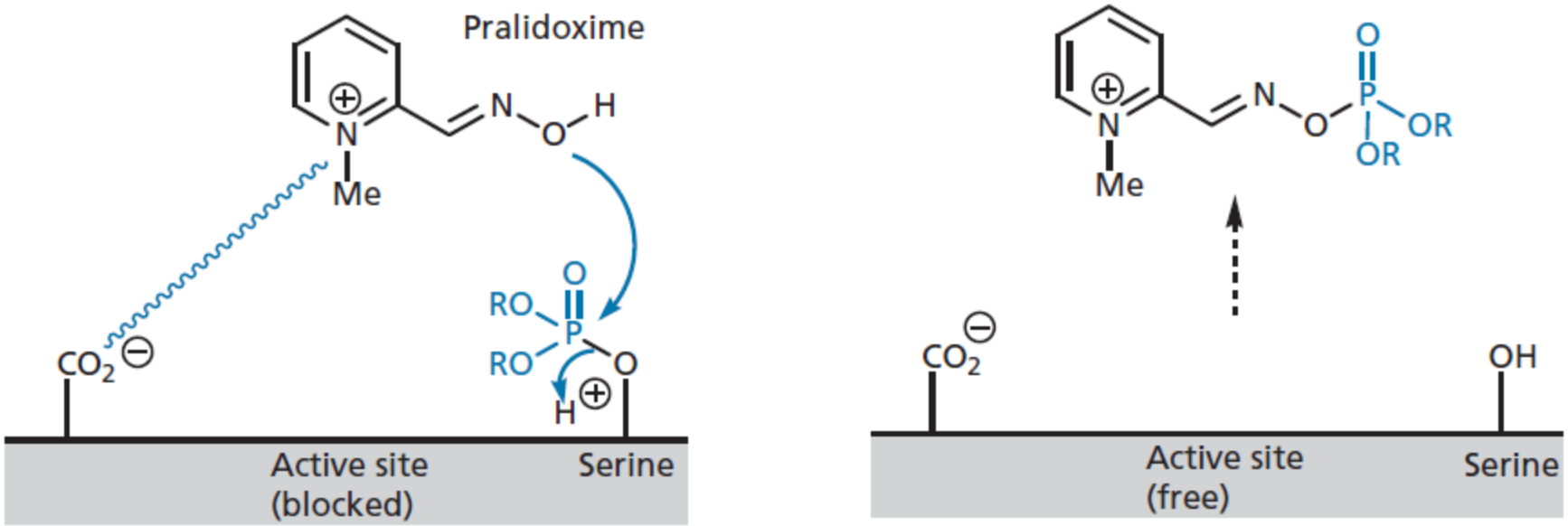


Metabolism of insecticides in mammals and insects.

One of the ester groups of the paraoxone enzyme-linked organophosphate undergoes enzymatic reaction over time, an alcohol unit breaks down and the acidic P-OH group is formed. This change in phosphated enzyme is called “aging or aging Fos. Nucleophilic binding of the oxime molecule to the aged organophosphate group is not possible, and

therefore no enzyme regeneration can occur

Pralidoxime (PAM): It is antidote to irreversible AChE inhibitors and removes the inhibitor that inhibits the enzyme (It is a cholinesterase reactivator. Used in combination with atropine for the treatment of organophosphorous anticholinesterases.)

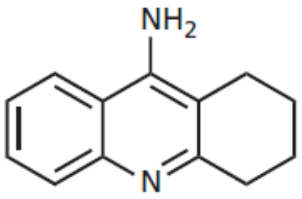


Pralidoxime as an antidote for organophosphate poisoning.

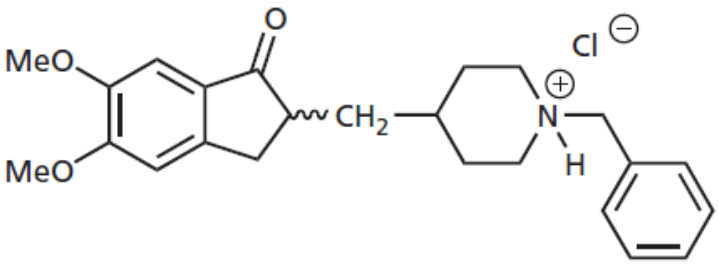
Hydrolysis of phosphates.

Centrally acting cholinergic drugs

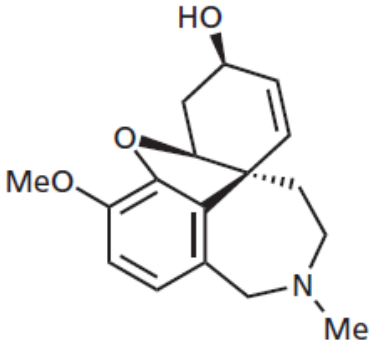
In Alzheimer's disease, cholinergic neurons in the CNS have decreased. Therefore, reversible anticholinesterase compounds (acetylcholinesterase inhibitor) have been developed to improve the loss of cognitive functions. They have an effect on **muscarinic receptors**. Takrin is found as the first drug but not used due to its hepatotoxic effect



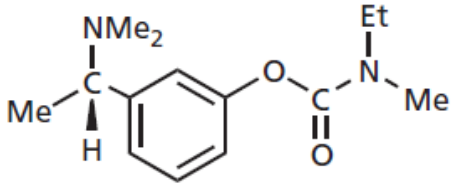
Tacrine (Cognex, Parke-Davis)



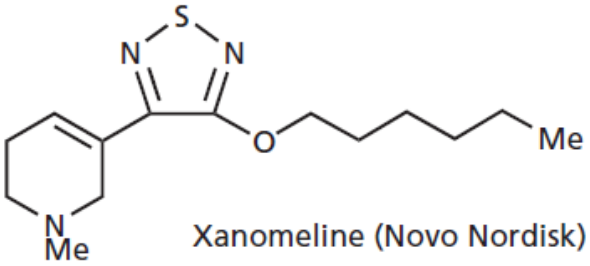
Donepezil (Aricept, Eisai)



Galantamine (Reminyl, Shire)



Rivastigmine (Exelon, Novartis)



Xanomeline (Novo Nordisk)

References:

- *Pharmaceutical Chemistry Edited by: David G Watson, Churchill Livingston. Elseiver, 2011.*
- *Medicinal Chemistry : A Molecular and Biochemical Approach, Third Edition, Deited by: Thomas Nogrady, Donald F. Weaver, Oxford University Press, 2005.*

Questions:

1.Describe the structure-effect relationships in the development of short-acting anticholinesterase (anti-acetylcholinesterase) drugs.

2.What is the first short-acting anticholinesterase compound and what are the important points to consider in the derivatives developed through this compound.

3.Please, write the possible synthesis reaction of Pyridostigmine compound from 3-hydroxy-pyridine as a starting material.

4.Demonstrate how the pyridostigmine compound inhibits the AChE enzyme.

5.Compare the compounds of physostigmine and neostigmine chemically and write down which compound should be preferred for treating.