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To Determine the Relation of Chirality on the Biological Activity in Case of Organophosphate Poisoning.

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ABSTRACT

Objectives

The aim of project is to determine the relation of chirality on the biological activity in case of organophosphate poisoning.

Materials and Methods

Obidoxime and Trimedoxime, a bis 4, 4'bis pyridinum dioxime are promising compounds as antidote for organophosphate poisoning. Litrature reveal that both bis pyridinum oxime having propelene to heptylene bridge. Yet there is no report found chain with a chiral centre. So it is worth to synthesize and test the titled compounds, having pharmacophore moieties of the three important series of compounds and screen them for *in-vitro* potency and derive the relationship between the chirality of the molecule verses its activity.

Results

Bispyridinum aldoxime was clearly more potent than pralidoxime in reactivating AChE in organophosphate poisoning patient.

Keywords-: Organophosphate, Chiral, Pharmacophore, Bispyridium aldoxime, AChE.

I. INTRODUCTION

Antidotes are the drugs which are used to recover the damage done by the poisons and in this work antidote specially refer to the compounds which are reactivators of organophosphates.

Poison is defined as any chemical, biological, or any other material or thing which is causing harm to the body and may lead to death.

Atropine seemed to be the only antidote available for the treatment of poisoning with organophosphorus compounds (OPC) until 1951, when Jandorf made the first step towards the solution of this problem by showing that hydroxylamine could destroy OPCs in vitro. These results inspired Wilson to try adding the oximes forever – the enzyme-inhibitor complexhydroxylamine to tetraethyl pyrophosphate (TEPP) inhibited acetyl cholinesterase (AChE) in vitro, obtaining thus the first known successful reactivation of the previously irreversibly phosphorylated AChE.

The state of Andhra Pradesh, southern India, is an area of intensive agricultural production. Pesticide use is high, and the state has one of the highest reported rates of pesticide poisoning in India.

Pesticides are currently classified by the WHO on the basis of theirtoxicity in untreated animals from Class Ia (extremely hazardous) to Class III (slightly hazardous), and compounds unlikely to cause ill health.

A. Organophosphate poisoning

Pesticides are toxic chemicals and are easily available and often stored in an improper manner due to lack of awareness of their hazards.

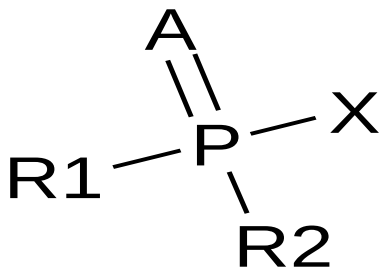
Since the removal of organochlorine insecticides from use, organophosphate insecticides have become the most widely used insecticides available today. More than forty of them are currently registered for use and all run the risk of acute and sub acute toxicity. Organophosphates are used in agriculture, in the home, in gardens, and in veterinary practice. Organophosphorus compound given by multiple route and can cause similar symptom but lead to serious additive toxicity. It is important to understand, however, that there is a wide range of toxicity in these agents and wide variation in cutaneous absorption, making specific identification and management quite important.

OP pesticides inhibit acetyl cholinesterase (AChE) at the muscarinic and nicotinic synapses by depositing a phosphoryl group at the enzyme's active site, this results in an

accumulation of acetylcholine and uncontrolled activation of cholinergic synapses. The 'ageing' of inhibited AChE is particularly important since aged enzyme cannot be reactivated by oximes. The therapeutic window for oximes is, therefore, very much determined by the rate of ageing.

a). *General Chemical Structure*

The insecticides with double bonded sulfur are organothiophosphates, but are converted to organophosphates in the liver. Phosphonate contains an alkyl (R-) in place of one alkoxy group (RO-). "X" is called the "leaving group" and is the principal metabolite for a specific identification.



where

A = oxygen or sulfur

R1 = alkoxy

R2 = alkyl, alkoxy, or tertiary amine

X = a good leaving group

(Eg. F, CN, Thiomalate, P-Nitrophenyl Etc)

A is usually oxygen or sulfur but may also be selenium. When A is other than oxygen, biological activation is required before the compound becomes effective as inhibitor of cholinesterase. Phosphothionates [R1 R2 P(S)X] have much poorer electrophonic character than their oxygen analogs and much weaker hydrogen bond-forming molecules because of the sulfur atom. Their antagonistic capacity is a million time weaker than oxygen analogue. The R group imparts lipophilicity to the molecule and contributes to its absorption through the skin.*[1] Wilson and gisvold pp 568

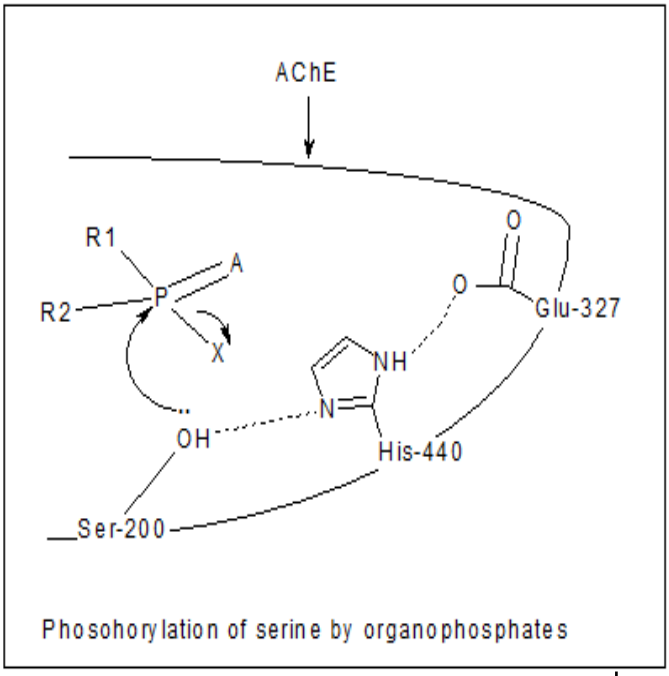
b). Mechanism of Poisoning

Inhibition of AChE by organophosphate compounds takes place in two steps

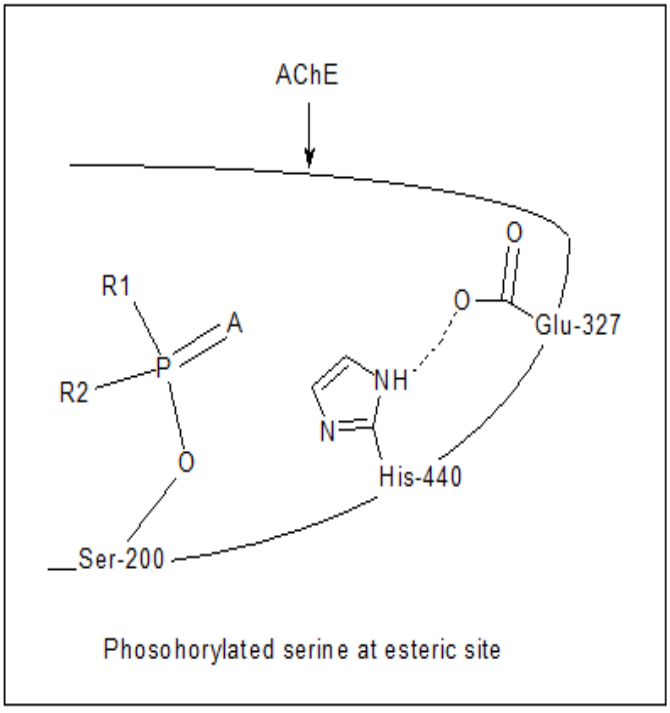
(i) Association of enzyme and inhibitor and

(ii) Phosphorylation step

The serine residue at the esoteric site forms a stable phosphoric ester with the organ phosphorous inhibitor.



Phosohorylation of serine by organophosphates



Phosohorylated serine at esteric site

c). Chemical Classes

To some degree, the occurrence of poisoning depends on the rate at which the pesticide is absorbed. Breakdown occurs chiefly by hydrolysis in the liver; rates of hydrolysis vary widely from one compound to another. In the case of certain organophosphates whose breakdown is relatively slow, significant temporary storage in body fat may occur. Some organophosphates such as diazinon and methyl parathion have significant lipid solubility, allowing fat storage with delayed toxicity due to late release.[43] Delayed toxicity may also occur atypically with other organophosphates, specifically dichlorofenthion and demeton-methyl.

Many organothiophosphates readily undergo conversion from thions (P=S) to oxons (P=O). Conversion occurs in the environment under the influence of oxygen and light, and in the body, chiefly by the action of liver microsomes. Oxons are much more toxic than thions, but oxons break down more readily. Ultimately, both thions and oxons are hydrolyzed at the ester linkage, yielding alkyl phosphates and leaving groups, both of which are of relatively low toxicity. They are either excreted or further transformed in the body before excretion.

The distinction between the different chemical classes becomes important when the physician interprets tests from reference laboratories. This can be especially important when the lab analyzes for the parent compound (i.e., chlorpyrifos in its thiophosphate form) instead of the metabolite form (chlorpyrifos will be completely metabolized to the oxon after the first pass through the liver).[5]

Within one or two days of initial organophosphate binding to AChE, some phosphorylated acetylcholinesterase enzyme can be de-phosphorylated (reactivated) by the oxime antidote pralidoxime. As time progresses, the enzymephosphoryl bond is strengthened by loss of one alkyl group from the phosphoryl adduct, a process called aging. Pralidoxime reactivation is therefore no longer possible after a couple of days, [6] although in some cases, improvement has still been seen with pralidoxime administration days after exposure.

d). Organophosphate-Induced Delayed Neuropathy (OPIDN)

Rarely, certain organophosphates damage to the afferent fibers of peripheral and central nerves because of neurotoxicity and associated with inhibition of "neuropathy target esterase" (NTE). This delayed syndrome has been termed organophosphate-induced delayed neuropathy (OPIDN), and is manifested chiefly by weakness or paralysis and paresthesia of the extremities persist for weeks to years. These rare occurrences have been found shortly after an acute and often massive exposure, but in some cases, symptoms have persisted for months to years. Only a few of the many organophosphates used as pesticides have been implicated as causes of delayed neuropathy in humans. EPA guidelines require that organophosphate and carbamate compounds which are candidate pesticides be tested in susceptible animal species for this neurotoxic properties[8-10]

Three epidemiologic studies with an exposed group and a control group also suggest that a proportion of patients acutely poisoned from any organophosphate can experience some long-term neuropsychiatric sequelae. The findings show significantly worse performance on a battery of neurobehavioral tests, including memory, concentration, and mood, and compound-specific peripheral neuropathy in some cases. These findings are subtle and may sometimes be picked up only on neuropsychologic testing rather than on a neurologic exam.[11-15]Follow-ups of case series have occasionally found some individuals reporting persistent headaches, blurred vision, muscle weakness, depression, memory and concentration problems, irritability, and/or development of intolerance to selected chemical odors[16, 17]

e). Intermediate Syndrome

It is characterized by acute respiratory paresis and muscular weakness, primarily in the facial, neck, and proximal limb muscles. In addition, it is often accompanied by cranial nerve palsies and depressed tendon reflexes. Like OPIDN, this syndrome lacks muscarinic symptomatology, and appears to result from a combined pre- and post-synaptic dysfunction of neuromuscular transmission. Symptoms do not respond well to atropine and oximes; therefore treatment is mainly supportive.[17]The most common compounds involved in this syndrome are methyl parathion, fenthion, and dimethoate, although one case with ethyl parathion was also observed.[1]

f). Signs And Symptoms of Poisoning

Symptoms of acute organophosphate poisoning develop during or after exposure, within minutes to hours, depending on the method of contact. Ii is given by the gastrointestinal route and dermal route to produce toxic effect. All signs and symptoms are cholinergic in nature and affect muscarinic, nicotinic, and central nervous system receptors.

The critical symptoms in management are the respiratory symptoms. Like bronchospasm and bronchorrhea can occur, producing tightness in the chest, wheezing, productive cough, and pulmonary edema. A life threatening severity of poisoning is signified by loss of consciousness, incontinence, convulsions, and respiratory depression. The causes of respiration are death and affect the CVS sign is bradycardia which can progress to sinus arrest. Toxic myocardiopathy has been a prominent feature of some severe organophosphate poisonings.[19]

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Some of the most commonly reported early symptoms include headache, nausea, dizziness, and hypersecretion, sweating, salivation, lacrimation, and rhinorrhea, muscle twitching, weakness, tremor, incoordination, vomiting, abdominal cramps, and diarrhea all signal worsening of the poisoned state. Diagnostic sign is miosis , blurred and/or dark vision, anxiety and restlessness. Psychiatric symptoms including depression, memory loss, and confusion have been reported.

Children have different sign than adult like bradycardia, muscular fasciculations, lacrimation, and sweating. Seizures (22%-25%) and mental status changes including lethargy and coma (54%-96%) were common. In comparison, only 2-3% of adults present with seizures. Other common presenting signs in children include flaccid muscle weakness, miosis, and excessive salivation

g). Treatment For Organophosphate Poisoning

- Airway protection. Administer oxygen by mechanically assisted pulmonary ventilation if respiration is depressed before administering atropine, to minimize the risk of ventricular fibrillation**.**
- Atropine sulfate. Administer atropine sulfate intravenously, or intramuscularly dose 300 mg per day may be required, or even continuous infusion.[24,25,23] The objective of atropine antidot therapy is to antagonize the effects of excessive concentrations of acetylcholine. Atropine does not reactivate the cholinesterase enzyme or accelerate disposition of organophosphate.
- Recrudescence of poisoning may occur if tissue concentrations of organophosphate remain high when the effect of atropine wears off. Atropine is often a life-saving agent in organophosphate poisonings. Favorable response to a test dose of atropine (1 mg in adults, 0.01 mg/kg in children under 12 years) can help differentiate poisoning by anticholinesterase agents from other conditions.
- Glycopyrolate has been studied as an alternative to atropine. Ampules of 7.5 mg of glycopyrolate were added to 200 mL of saline and this infusion was titrated to the desired effects of dry mucous membranes and heart rate above 60 beats/min. During this study, atropine was used as a bolus for a heart rate less than 60 beats/ min.
- Pralidoxime**.** Before administration of pralidoxime, draw a blood sample (heparinized) for cholinesterase analysis (since pralidoxime tends to reverse the cholinesterase depression) in cases of severe poisoning by organophosphate pesticides in which respiratory depression, muscle weakness. Pralidoxime works by reactivating the cholinesterase and also by slowing the "aging" process of phosphorylated cholinesterase to a non-reactivatableform.

Note: Pralidoxime is of limited value and may actually be hazardous in poisonings by the cholinesterase-inhibiting carbamate compounds

- Dosage of Pralidoxime**:** *Adults and children over 12 years:* 1.0-2.0 g by intravenous. *Children under 12 years:* 20-50 mg/kg body weight (depending on severity) intravenously.
- Skin decontamination**.** In patients who have been poisoned by organophosphate contamination of skin, clothing, hair, and/or eyes. For decontamination antidote must be used. If there are any indications of weakness, ataxia, or other neurologic impairment, clothing should be removed and a complete bath and shampoo given while the victim are recumbent, using copious amounts of soap and water. Attendants should wear rubber gloves and Surgical green soap used. Contaminated clothing, leather shoes should be promptly removed, bagged, and laundered before returning.
- Pulmonary ventilation. In poisonings to give large doses of organophosphate, monitor pulmonary ventilation carefully, even after recovery from muscarinic symptomatology.
- Hydrocarbon aspiration may complicate poisonings that involve ingestion of liquid concentrates of organophosphate pesticides. Pulmonary edema and poor oxygenation in these cases will not respond to atropine and should be treated as a case of acute respiratory distress syndrome.
- Cardiopulmonarymonitoring**.**Some organophosphates have significant cardiac toxicity.
- Seizure control. Rarely, in severe organophosphate poisonings, convulsions occur despite therapy with atropine and pralidoxime.
- Contraindications. Morphine, succinylcholine, theophylline, phenothiazines, and reserpine are contraindicated in organophosphate poisoning.

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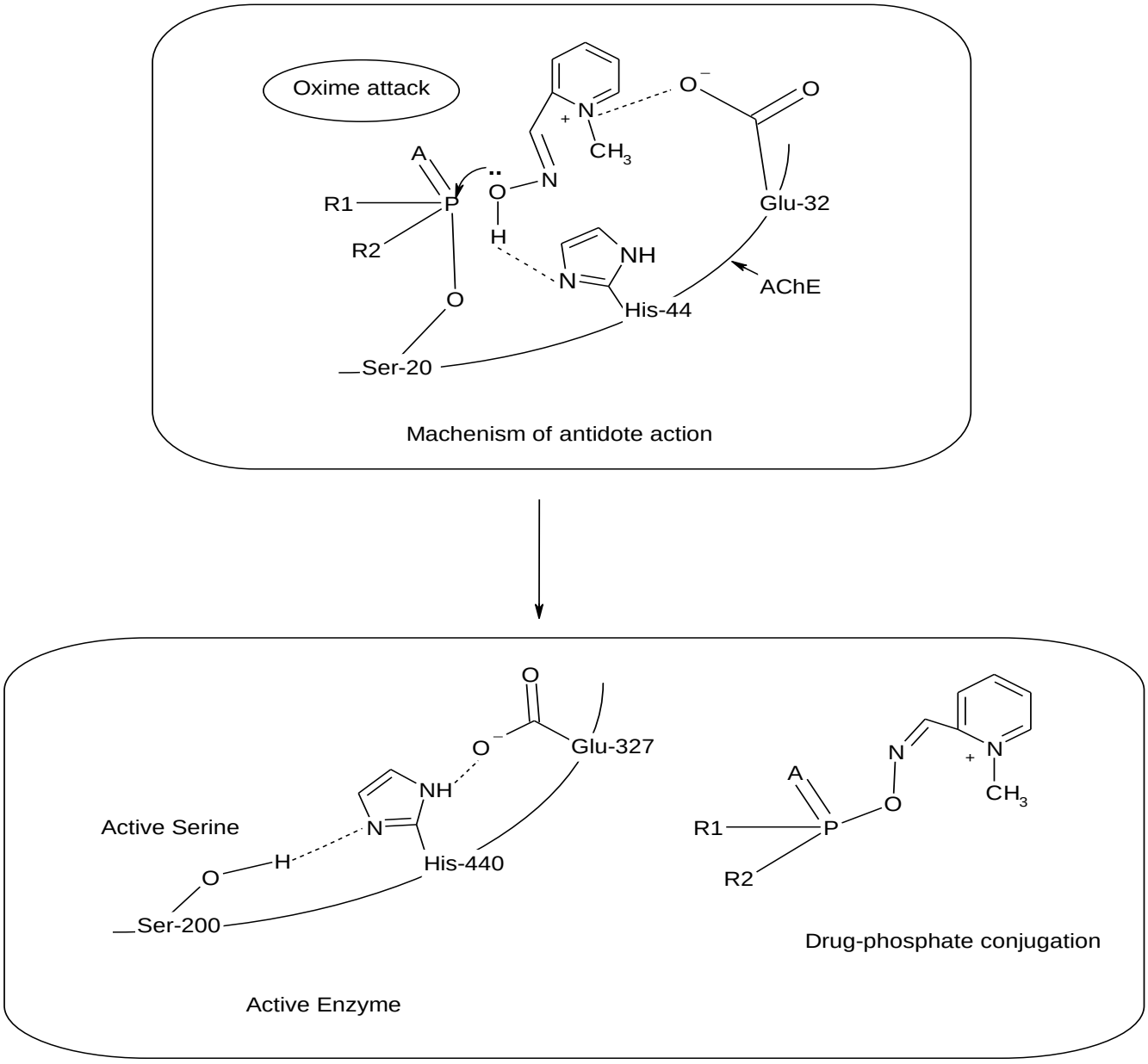
B. Mechanism of Action of Antidote To the organophosphates



Oximes can bind reversibly to the AChE molecule (active centre), allosteric (peripheral) site or at both sites of the enzyme. Every oxime has a strong positive charge in its molecule that navigates it to the negatively charged anionic site at the active centre of AChE, attracting thus the oxime molecule closer to the molecule of the OP residuum.

Thereafter, the oxime is also called as **nucleophilic attack** at the phosphorus atom of the OP residuum, and form unstable enzyme inhibitor-oxime complex. The ultimate result is the splitting of the complex into a phosphorylated oxime and reactivated enzyme

Birlane	ethoprop	Folex	Curamil
chlormephos+	Mocap	methamidophos+	pyridaphenthion
Dotan	ethyl parathion+	Monitor	Ofunack
chlorphoxim	E605	methidathion+	quinalphos
Baythion-C	Parathion	Supracide	Bayrusil
chlorpyrifos	thiophos	Ultracide	ronnel
Brodan	etrimfos	methyl parathion+	Fenchlorphos
Dursban	Ekamet	E 601	Korlan
Lorsban	famphur+	Penncap-M	schradan+
chlorthiophos+	Bash	methyl trithion	OMPA
Celathion	Bo-Ana	mevinphos+	sulfotep+
coumaphos+	Famfos	Duraphos	Bladafum
Asuntol	fenamiphos+	Phosdrin	Dithione
Co-Ral	Nemacur	mipafox+	Thiotepp
crotoxyphos	fenitrothion	Isopestox	sulprofos
Ciodrin	Accothion	Pestox XV	Bolstar
Cypona	Agrothion	monocrotophos+	Helothion
crufomate	Sumithion	Azodrin	temephos
Ruelene	fenophosphon+	naled	Abate

II. OBJECTIVE OF WORK AND DELIVERABLES

A. Objectives

The aim of project is to determine the relation of hilarity on the biological activity in case of organophosphate poisoning.

The project has been designed meticulously based on following points

- Therapeutic: the compounds formed would be showing their effect after consumption of poison.
- Prophylactic: some compounds may be capable of showing the efficiency if they are taken before consumption of poison

Along with these points the decisive intention is to become well versed with the techniques and practice of Medicinal Chemistry.

B. Motivation

- Pesticide poisoning kills hundreds of thousands of people in the southern India each year. The majority are from deliberate self-poisoning with organophosphorus pesticides (OP). The current response from a public health, medical and research perspective is inadequate.
- Number of antidote available to study the clinical practice.

C. Selection Criteria

- Organophosphorus pesticides reactivate AChE and inhibited by nerve agents in vitro and in vivo.
- Organophosphorus pesticides given with atropine to survive experimental animals but poisoned with multiple lethal doses.
- When given with atropine, ability to pass the blood brain barrier and reach the CNS and therapeutic use.
- Ability to express some “direct pharmacological effects” such as influence on the liberation of acetylcholine from pre-synaptic nerve endings (58),
- Oxime’s produce own toxicity itself,
- Chemical and pharmaceutical stability of the oxime.

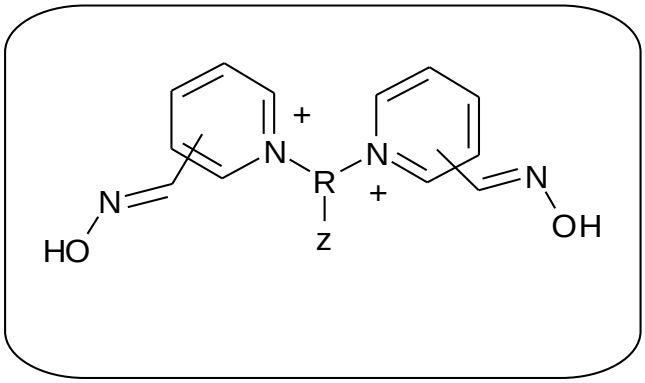
D. *Deliverables on the Basis of Common Features of Antidotes*

- Quaternary Nitrogen:- It is very much essential because positive charge of nitrogen will form ionic

bond with the glutamate residue present in the esteric site of the AChE

- Bivalent pyridine compound:- It is proved that bivalent pyridine antidotes have more reactivation capacity than monovalent one.
- Oxime:- It is a prerequisite for nucleophilic attack on the phosphorous atom, present in the organophosphate and oxime should be present on the pyridine ring because the compounds with oxime anion that is bound on the pyridinium ring are considered as the compounds able to reactivate OPC-inhibited AChE by dephosphorylating the enzyme molecule and restore its activity.

So rational design based on the common features of antidotes is



Where R = chain with 3-4 carbons

Z = any group to introduce chirality in the molecule References

Medical Aspects of Chemical and Biological Terrorism Chemical Terrorism and Traumatism Alexander Monov and Christophor Dishovsky Editors Sofia 2005 Publishing House of the Union of Scientists in Bulgari

III. MATERIALS AND METHODS

A. Present Work

It is evident from literature that Obidoxime and trimedoxime, a bis 4, 4'bis pyridinum dioxime are promising compounds as antidote for organophosphate poisoning. Litrature reveal that both bis pyridinum oxime having propelene to heptylene bridge. Yet there is no report found chain with a chiral centre. So it is worth to synthesize and test the titled compounds, having pharmacophore moieties of the three important series of compounds and screen them for *in-vitro* potency and derive the relationship between the chirality of the molecule verses its activity

B. Materials:-

a). Part 1 Synthesis

The material required are l-aspartic acid, D-aspartic acid, 2-pyridine carboxaldehyde, 3-pyridine carboxaldehyde, 4-pyridine carboxaldehyde, sodium borohydride, acetic anhydride, pyridine, DMAP, copper sulfate, benzylchloroformate, tetrabromo methane, hydroxylamine hydrochloride, triphenyl phosphine are purchased from sigma-aldrich chemicals

The solvents like Tetrahydrofuran, Methanol, and Dichloromethane are purchased from renchem fine chemicals ltd.

b). Part 2 Evaluation

Acetylthiocholine iodide and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich.

3-monocrotophos (dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) was synthesized in IICT laboratory work aimed to transfer newer technologies for the bulk production of these potential insecticides.

C. Experimental

a). Part 1 Synthesis

- General procedure

Methyl ester of L and D aspartic acid were prepared in usual manner by stirring amino acid with H2SO4 in methanol, further amino group is protected with Cbz then ester is reduced to alcohol form using Sodium borohydride. This rssulting alcohol is treated with Tetrabromo methane and triphenylphosphine in Acetonitrile to get dibromo derivative.

Pyridine 2, 3 and 4 aldehydes were converted to respective pyridine 2, 3 and 4 aldoxime by classical method by stirring aldehydes with hydroxyl amine hydrochloride.

Pyrine aldoximes were stirred at a define temperature with dibromo derivatives of aspartic acids in either acetonitrile or n-butanol to form the desired product.

The synthesis of title compounds have been planned and achieved as per Scheme 1 and Scheme 2, Scheme 3, Scheme 4 and Scheme 5 and also tried Scheme 6

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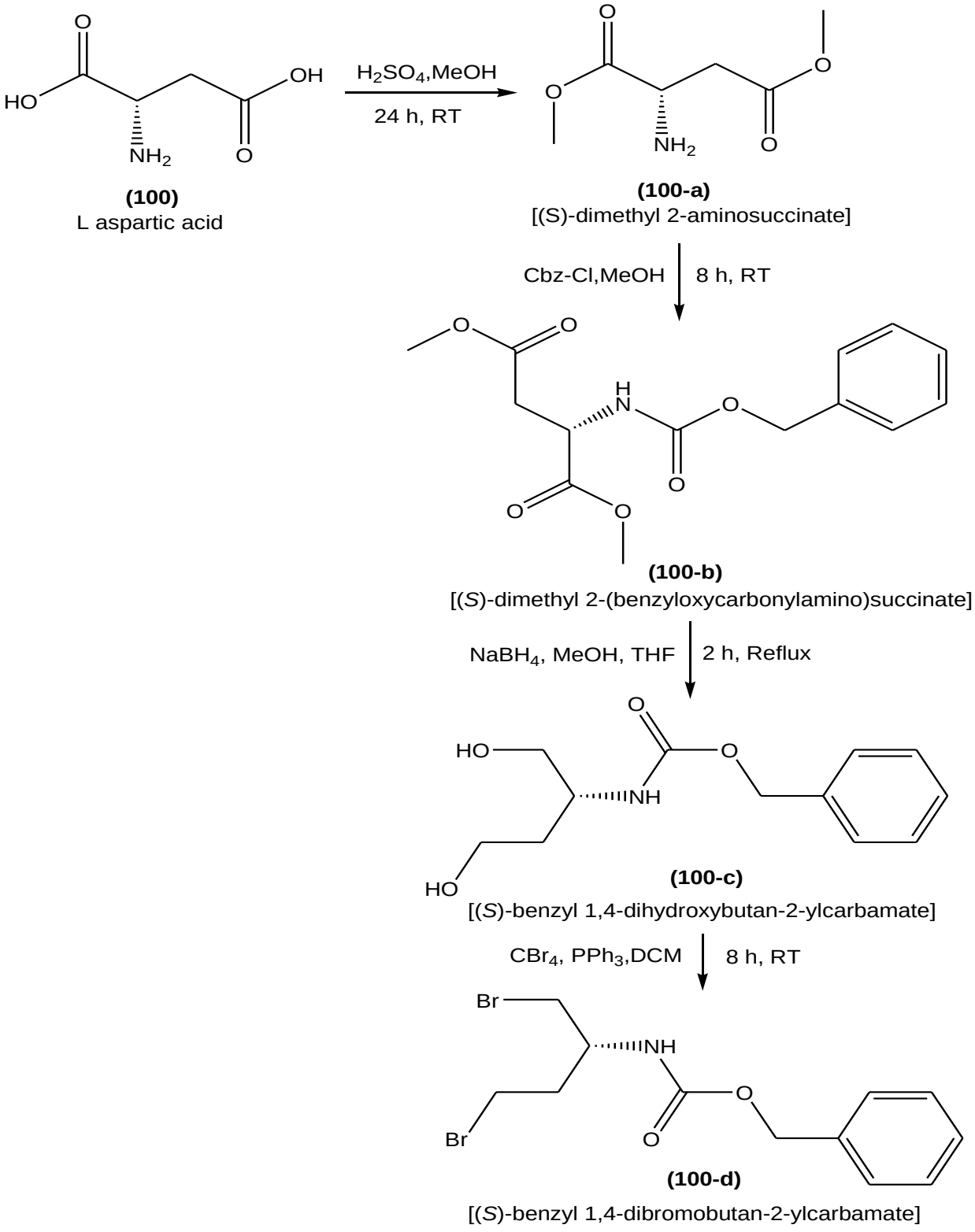
Scheme 1



(100)
L aspartic acid

(100-a)
[(S)-dimethyl 2-aminosuccinate]

(100-b)
[(*S*)-dimethyl 2-(benzyloxycarbonylamino)succinate]

(100-c)
[(*S*)-benzyl 1,4-dihydroxybutan-2-ylcarbamate]

(100-d)
[(*S*)-benzyl 1,4-dibromobutan-2-ylcarbamate]

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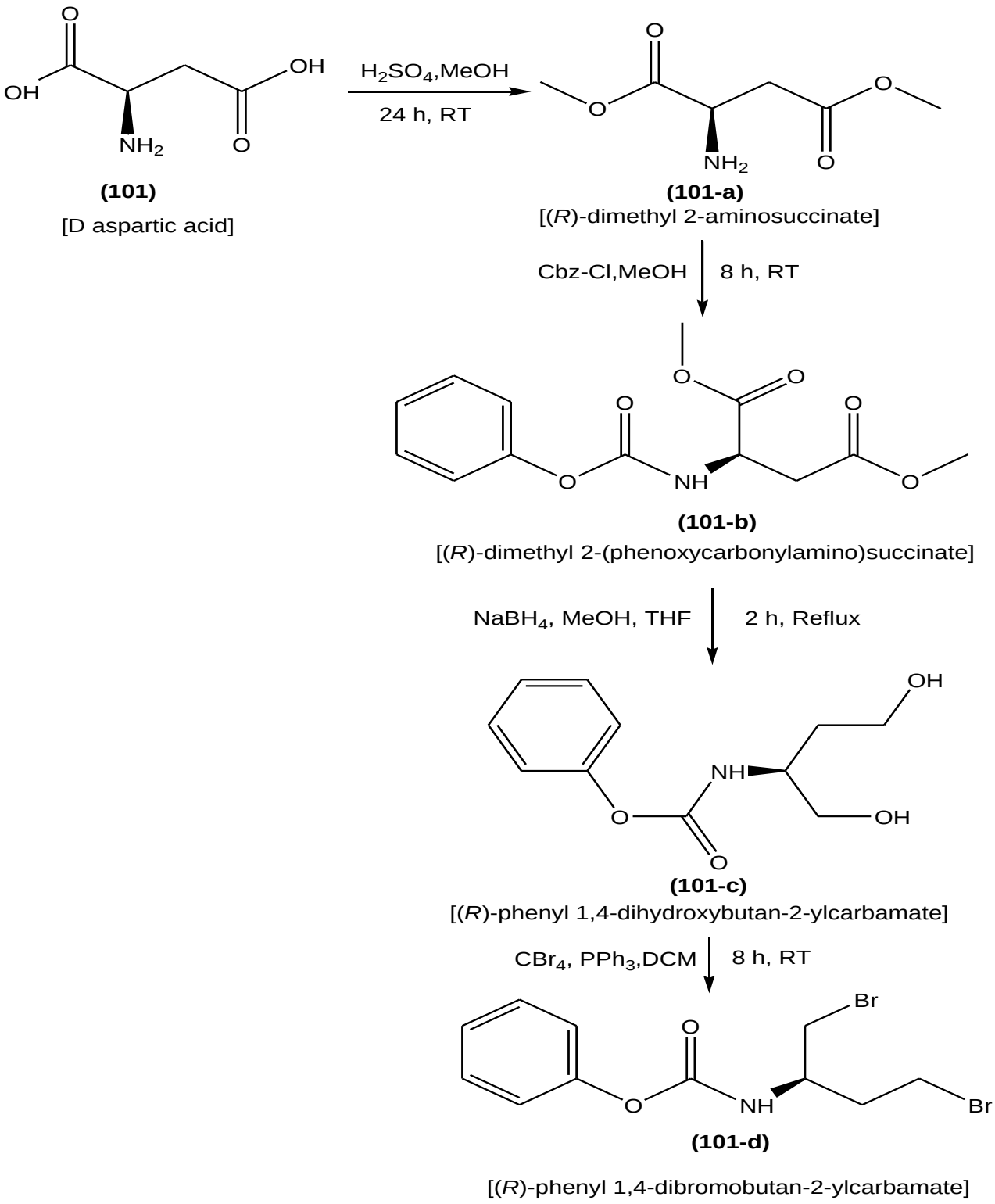
Scheme 2



(101)

[D aspartic acid]

(101-a)

[(*R*)-dimethyl 2-aminosuccinate]

(101-b)

[(*R*)-dimethyl 2-(phenoxycarbonylamino)succinate]

(101-c)

[(*R*)-phenyl 1,4-dihydroxybutan-2-ylcarbamate]

(101-d)

[(*R*)-phenyl 1,4-dibromobutan-2-ylcarbamate]

b). Step-1 Esterification

L aspartic acid was mixed with H2SO4 (0.3 eq) taken in methanol and stirred for 24 hours at room temperature. Excess Methanol were distilled out and remaining acid is neutralized with concentrated sodium bicarbonate aqueous solution. Resulting mixture was extracted with chloroform. The methyl ester was obtained as yellow colored dense liquid which slowly converts to crystalline solid. On recrystallization we got (100-a) {(S)-diethyl (2-aminosuccinate)} which comes as white solid.

c). Step-2 Protection of Amino Group

Methyl ester of aspartic acid {(S)-dimethyl (2-aminosuccinate)} (100-a) (1 eq) was taken in methanol; to this sodium bicarbonate (1.5 eq) was added. In this mixture Benzylchloroformate (1.2 eq) was added and stirred for 8 hours at room temperature. The resulting mixture was filtered and remaining methanol was removed and product (100-b) {(S)-dimethyl 2-(benzyloxycarbonylamino) succinate} was obtained as yellow liquid which get crystallized on standing overnight.

d). Step-3 Reduction of Ester group to Alcohol

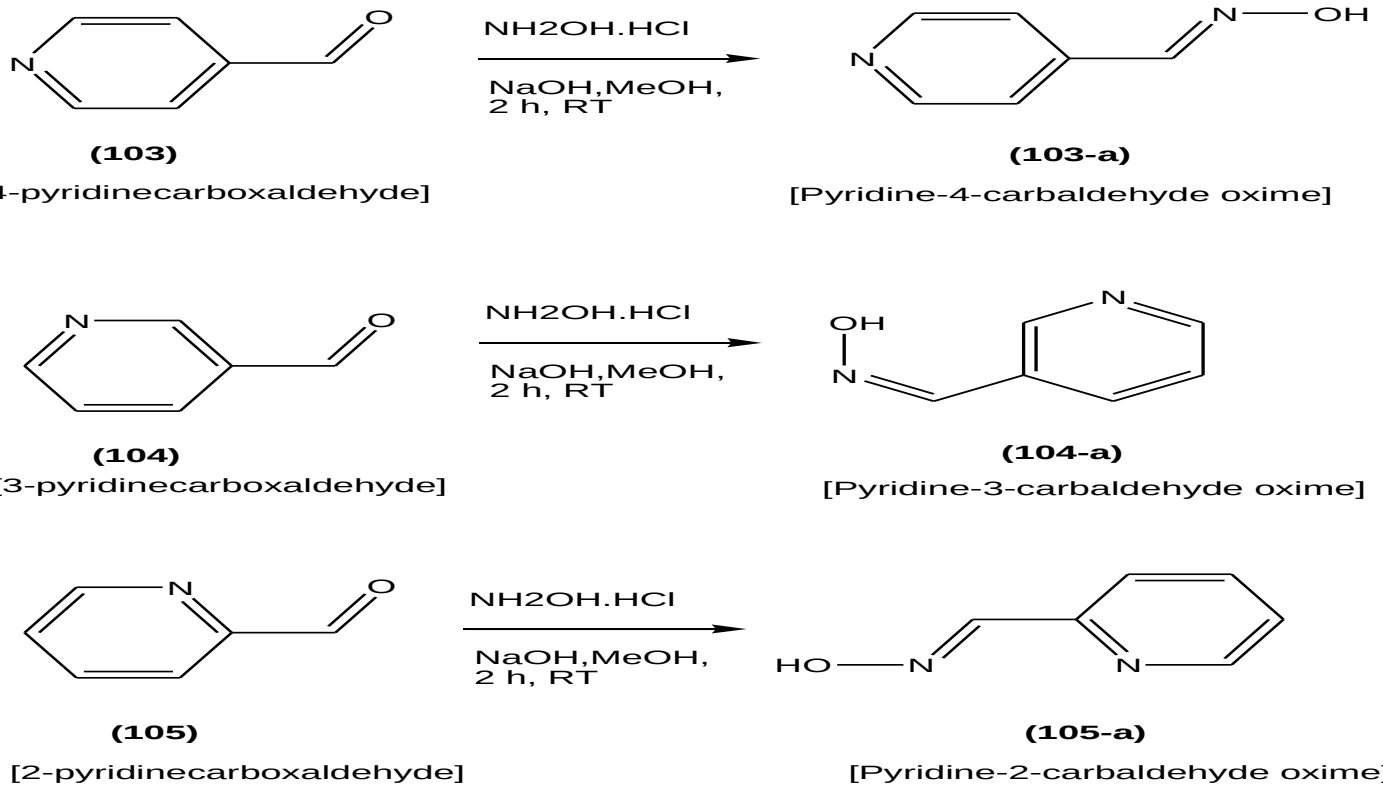
Sodium borohydride(2.2 eq) was taken in THF : Methanol (2:1) system and Amino protected ester (100-b) {(S)-dimethyl 2-(benzyloxycarbonylamino) succinate} (1 eq) dissolved in methanol and slowly added to previous system under nitrogen atmosphere and refluxes for 4 hours and left at room temperature for further 2 hours. The resulting mixture was neutralized and product 100-(c) {(S)-benzyl 1, 4-dihydroxybutan-2-ylcarbamate} was obtained as colorless liquid.

e). Step-4 Alcohol To Dibromo Derivative

The alcohol (110-c) {(S)-benzyl 1, 4-dihydroxybutan-2-ylcarbamate} (1 eq) was taken in acetonitrile and triphenylphosphine (2.2 eq) was added to this solution and stirred for 15 minutes at room temperature. The tetrabromomethane (2.2 eq) was dissolved in acetonitrile and added very slowly to the previous mixture under nitrogen atmosphere and stirred for 8 hours at room temperature.

The product (100-d) {(S)-benzyl 1, 4-dibromobutan-2-ylcarbamate} ware obtained in 5-10% Ethyl acetate: Hexane solution by column chromatography as white solid.

Scheme 3



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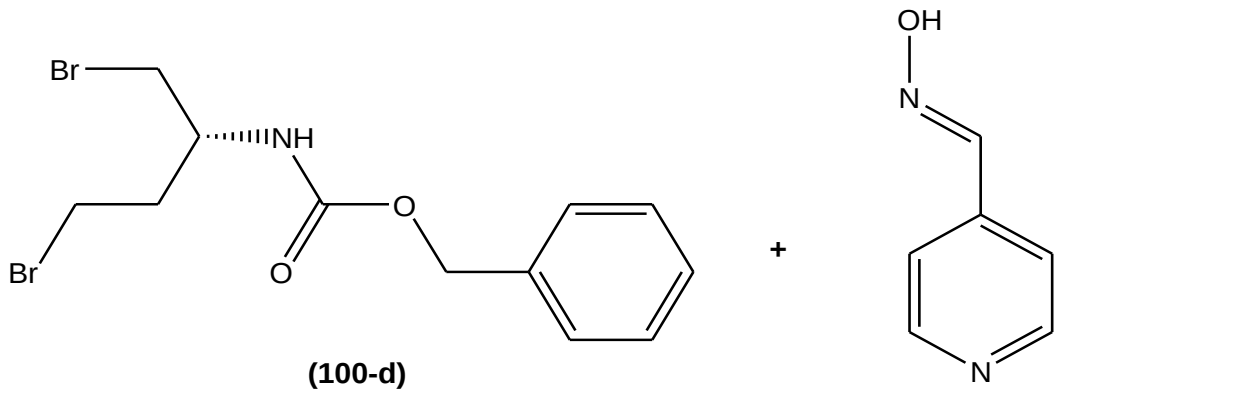
f). Step-5 Pyridine Aldehyde To Pyridine Aldoxime

4-Pyridinecarboxaldehyde (105) (1 eq) was taken in methanol to this hydroxyl amine hydrochloride (1.5 eq) was added and instantly sodium hydroxide (1.5 eq) was added to the solution and stirred for 4 hours. After that mixture were neutralized with 0.1 N HCl. And resulting mixture was extracted with chloroform to get desired product (105-a). Product was obtained as slightly pink solid

Scheme 4

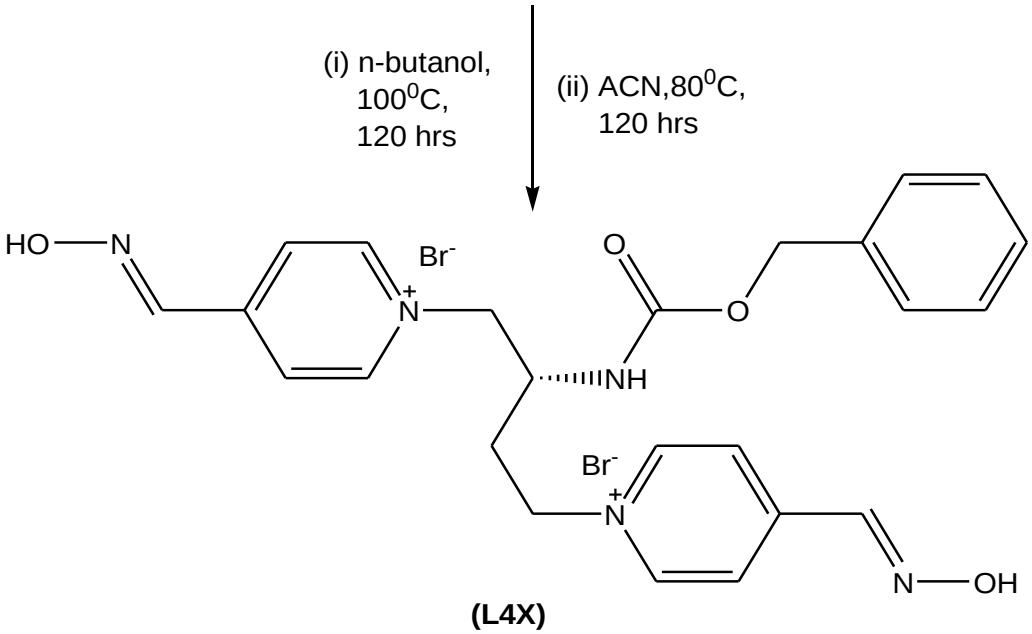


(100-d)

[(*S*)-benzyl 1,4-dibromobutan-2-ylcarbamate]

(103-a)

[Pyridine-4-carbaldehyde oxime]



(L4X)

{(*E*)-1,1'-((*S*)-2-(benzyloxycarbonylamino)butane-1,4-diyl)bis(4-((*E*)-(hydroxyimino)methyl)pyridinium) bromide}

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g). Step-6 Coupling

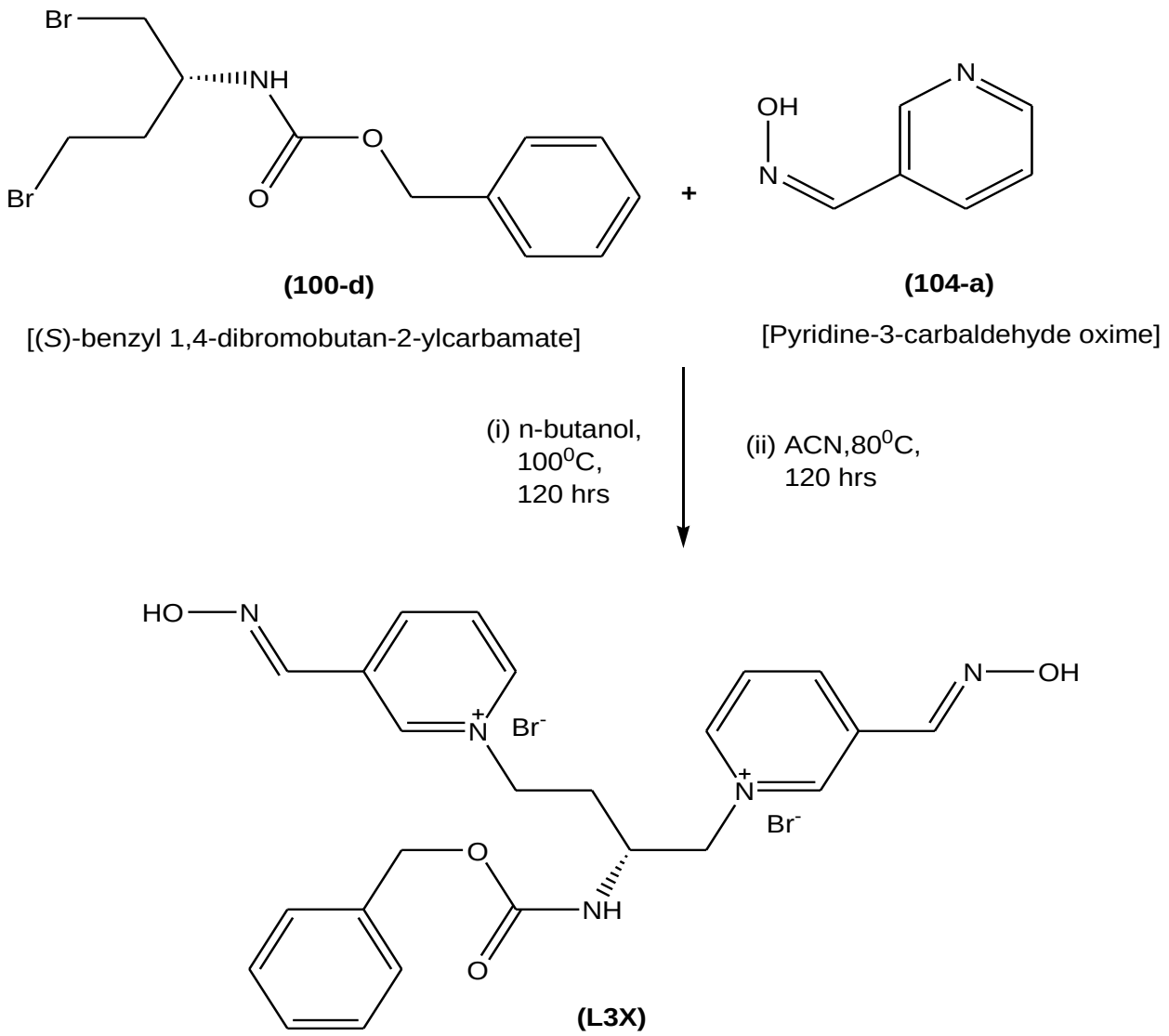
Method 1

Pyridine aldoxime 3(a) (2.2 eq) was dissolved in acetonitrile in seal tube. In this mixture Dibromo derivative of aspartic acid 1(d) (1 eq) was added and heated for 120 hours at 80^0 C. Final product final 1 was obtained by column chromatography in 40% Methanol : Chloroform system as brown colored semisolid.

Method 2

Pyridine aldoxime 3(a) (2.2 eq) was taken in n-butanol in seal tube. In this mixture Dibromo derivative of aspartic acid 1(d) (1 eq) was added and heated for 120 hours at 80^0 C. Final product final 1 was obtained by column chromatography in 40% Methanol : Chloroform system as brown colored semisolid.

Scheme 5



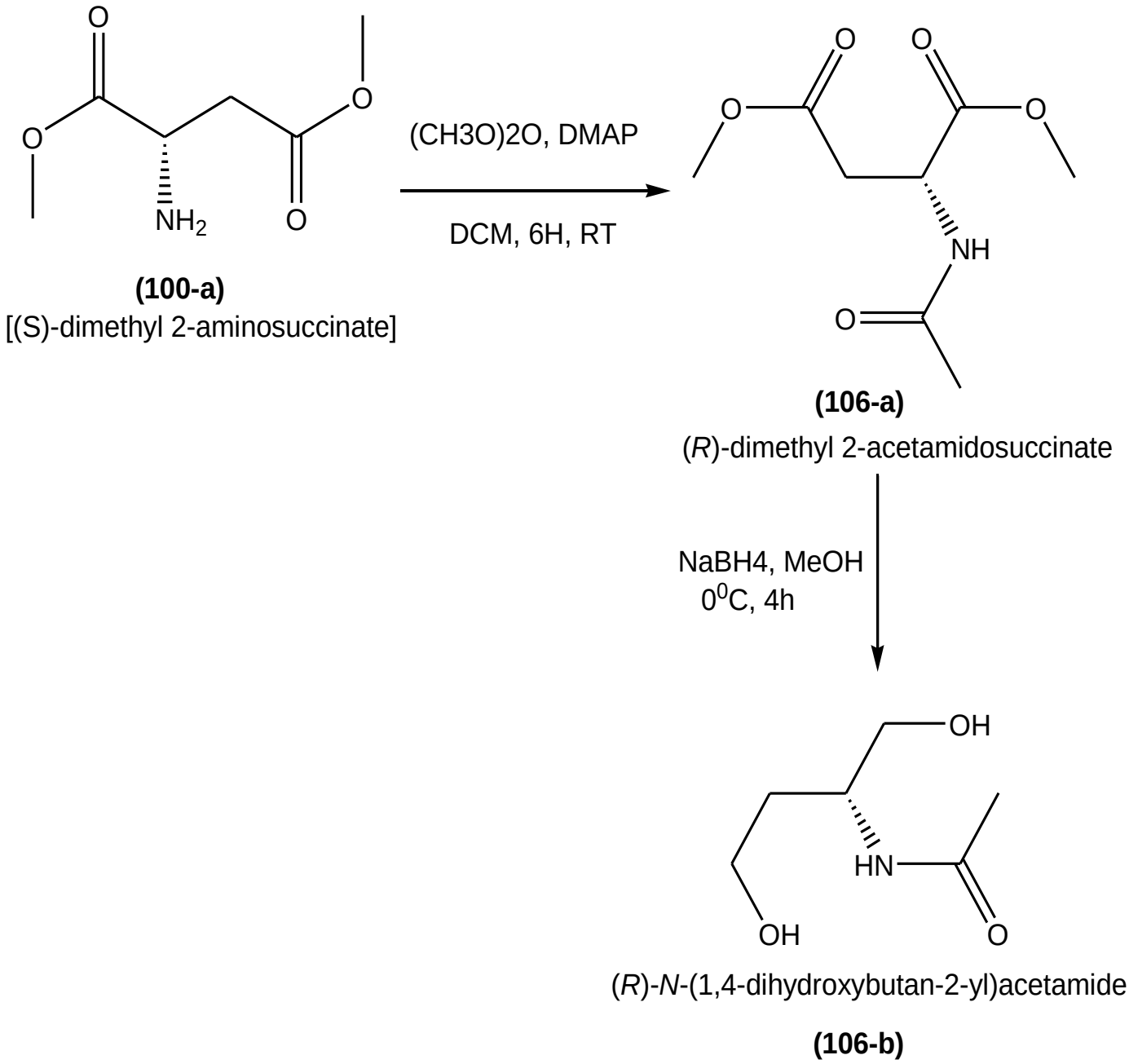
(100-d) [(*S*)-benzyl 1,4-dibromobutan-2-ylcarbamate]

(104-a) [Pyridine-3-carbaldehyde oxime]

(L3X)

{(*E*)-1,1'-((*S*)-2-(benzyloxycarbonylamino)butane-1,4-diyl)bis(3-((*E*)-(hydroxyimino)methyl)pyridinium) bromide}

Scheme 6



(100-a)
[(S)-dimethyl 2-aminosuccinate]

(106-a)
(*R*)-dimethyl 2-acetamidosuccinate

(*R*)-*N*-(1,4-dihydroxybutan-2-yl)acetamide

(106-b)

h). *Acyl Protection of Amino Group*

Methyl ester of aspartic acid {(S)-dimethyl (2-aminosuccinate)} (100-a) (1eq) was dissolved in DCM and pyridine (1.5eq) was added to it. After stirring the previous mixture for 10 minutes DMAP (catalytic amount) was added. Then acetic anhydride (1.2eq) was added at 0^0 C temperature and kept at RT for 6 hours to get good yield. Desired product 106-b {(R)-dimethyl 2 acetamidosuccinate} will appear as white colored solid.

I). Reduction of Acyl-Ester

Sodium borohydride(2.2 eq) was taken in MeOH, N acyl ester 106-b {(R)-dimethyl 2 acetamidosuccinate} (1eq) was dissolved in small amount of methanol and added to the

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previous mixture very slowly at 0^0C, and kept at 0-RT for further 4 hours.Reaction mixture

quenched with concentrated ammonium chloride solution and sodium sulfate was added to this. Methanol was removed and remaining mixture was extracted with ethyl acetate to get 106-b { (R)-N-(1,4dihroxybutane-2-yl)acetamide)}

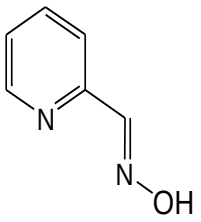
C. Biological Evaluation

The AChE activity was estimated in brain tissue at different intervals by the method described by Ellman et al. (1961), and the amount of protein was estimated by the method of Lowry et al. (1951). Brain tissue was homogenized. 10% w/v) in 0.1 M Tris-HCl buffer (pH 8) using a Potter-Elvehjam homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 5000 × g for 10 min and the supernatant was further centrifuged at 5000 × g for 10 min. The resultant supernatant was used as the enzyme source for the estimation of AChE. A typical run in 96-well plates consisted of **75 µ L** of 0.1 M phosphate buffer, pH 7.5, **25 µ L** DTNB (dithiobis-2-nitrobenzoic acid) (0.16 mM), and **25 µ L** of homogenate for each well. The reaction was initiated by adding **25 µ L** of acetylthiocholine iodide (0.4 mM) as substrate at 27 ± 1°C. The developed color was read at 412 nm in a spectrophotometer (Molecular devices, Supported with software soft maxpro-3; Sunnyvale, CA, USA).

IV. REVIEW OF LITERATURE

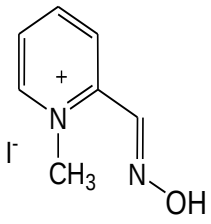
A. Past Work

The first triumphant pioneering experiment was done by Wilson and Ginsberg in 1955 for the reactivation of Acetylcholine esterase (AChE). They have synthesized and tested molecules 2-Pyridinealdoxime (2 PAM) methiodide



2-Pyridinealdoxime

(1)



2-Pyridinealdoxime methiodide

(2)

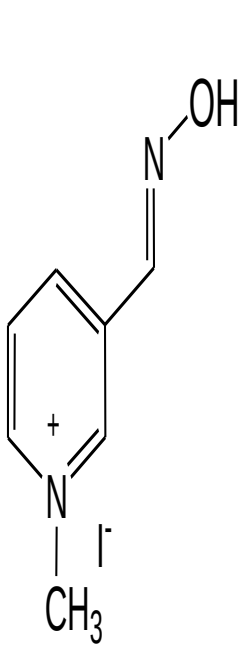
The quaternary oxime (2) was found a million times better than non methylated compound (1).

(Ref Ginsburg S, Wilson IB, A Powerful Reactivator of Alkylphosphonate-inhibited Acetylcholine Esterase. Biochim. Biophys. Acta.1955; 18: 168)

These people also synthesized tertiary O-acetyloxime, quaternary O-acetyloxime, and quaternary pyridine ketoximes and compared with (2).

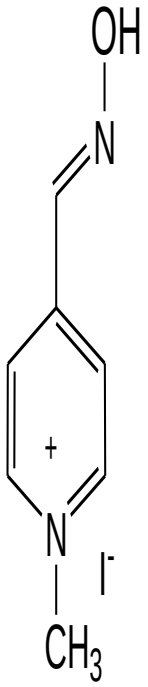
(Ref Ginsburg S, Wilson IB, Oxime of Pyridine Series. JACS 1957Jan 20; 79:481-5)

In 1966 Hobbiger et al. reported new potent reactivators of AChE inhibited by tetraethylpyrophosphate (TEPP)



3-PAM

(3)



4-PAM

(4)

Earlier a lot of compounds have been studied by Hobbiger and coworkers related to pyridine, indole, indoline, quinoline and isoquinoline (hobbiger 1958). The most successful reactivators have so far emerged are structurally related to pyridine. The order of activities found 2-PAM>4-PAM>3-PAM

This study suggested that quaternary nitrogen and an oxime or hydroximic acid is an essential feature of high activity.

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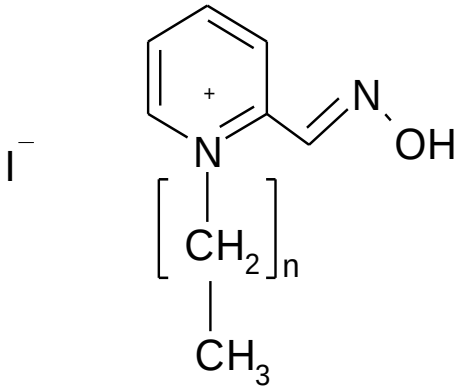
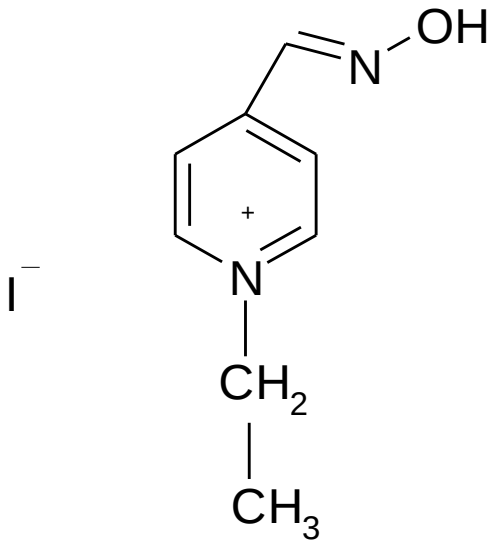
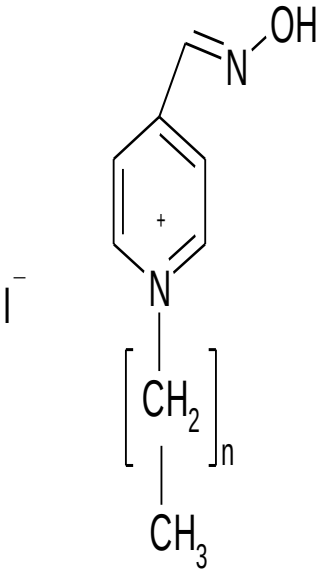
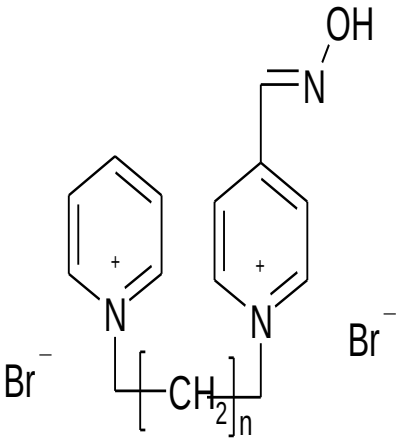


5 (n = 1), 6 (n = 2),
7 (n = 3)

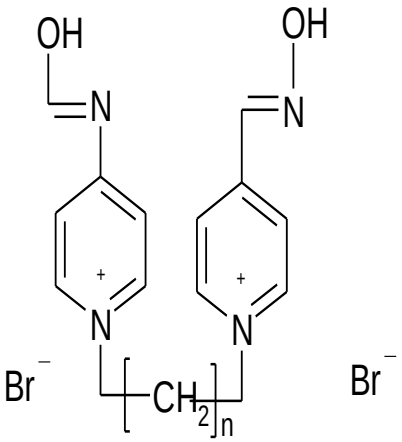


8





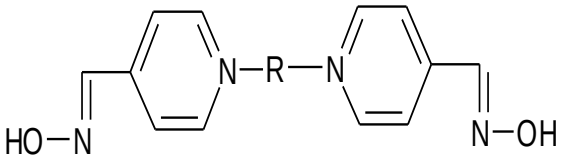
13(n =3), 14(n = 4), 15(n = 5)



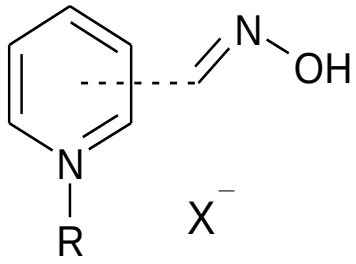
16 (n =2), 17(n =3),
18(n = 4), 19(n = 5)

All the compounds have been subjected to the preliminary screening for antidotal activity. Under the experimental condition choosen bispyridinium ion were found markedly superior to the 2-PAM iodide as antidote to TEPP *in-vivo.*

Poziomek et al. synthesized and tested a number of 1,1'-polymethelenebis(4-formylpyridinium bromide) dioximes and N-substituted 2- and 4- formyl pyridinium halide oxime molecules.

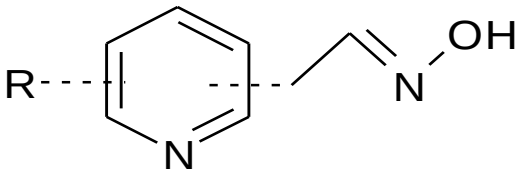


21 [R = -$(CH_3)_2$-], 22 [R = -$(CH_3)_3$-]
23 [R = -$(CH3)_4$-], 24 [R = -$(CH3)_5$-],
25 [R = -$(CH3)_{10}$-]



N substituted 2- and 4- formyl pyridinium halide oxime (26 -31)

These compounds are even more rapid reactivators of the AChE than PAM although they are structurally related to the less active 4-PAM. Under the test condition compound 24 was found most active with an exception to the compound 25 which is too toxic for chemotherapeutic use. All the bisquaternary compounds are active when used with atropine. (Pzimek et al.1958) In 1959 Ginsberg and Wilson synthesized pyridine (tertiary) and quaternary pyridine nucleous for the reactivation of alkylophosphonate inhibited AChE



Tertiary pyridinealdoxime

No	Oximino-formyl	R	X
26 27 28 29 20 31	2 2 4 2 4 4	Ethyl Allyl Allyi 2-hydroxyethyl 2-hydroxyethyl butyl	I Br Br Br Br Br

(32)

CH=NOH	2	2	2	2	2	3	4	4
R	-	6CH_3	3CH_3	3OH	3OCH_3	-	-	5,6 C_6H_4

CH=NOAC	2	2	3	4
R	-	6CH_3	-	-

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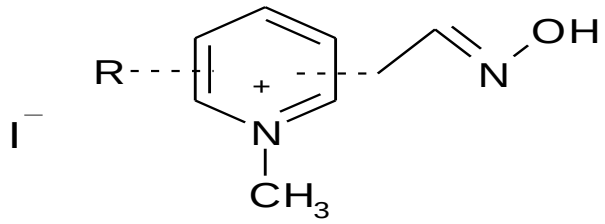
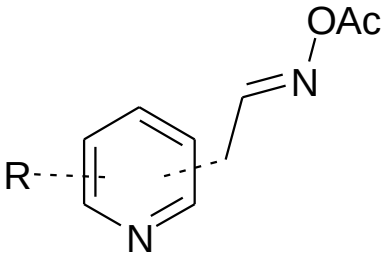


Quaternary pyridinealdoxime

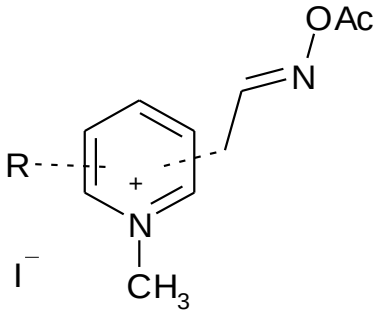
(33)

CH=NOH	2	2	2	2	2	3	4	4



Tertiary pyridine O-acetylaldoxime

(34)

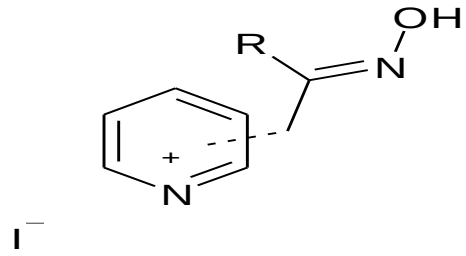


Quaternary pyridine O-acetylaldoxime

(35)

R	-	Cl	$3CH_3$	3OH	$3OCH_3$	-	-	5,6 C_6H_4

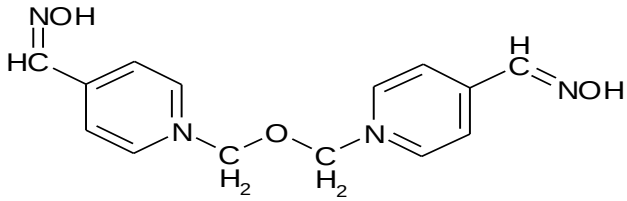
CH=NOH	2	2	3	4	4
R	CH_3	C_6H_5	CH_3	CH_3	C_6H_5



Quaternary Pyridine Ketoxime

(36)

Obidoxime was synthesized by Luttrringhaus and Hagedorn in 1964. The first clinical trial showed that this bispyridinum aldoxime was clearly more potent than pralidoxime in reactivating AChE in organophosphate poisoning patient (erdmen and von Clarmman 1963)



Obidoxime

(37)

HI-6(1, 2-hydroxy imino methyl-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxopropane dichloride) was synthesized in 1960s by Hagedorn and colleges, which has been extensively Investigated for the treatment of soman poisoning. HLo7

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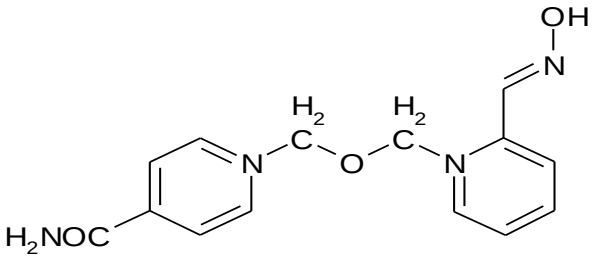
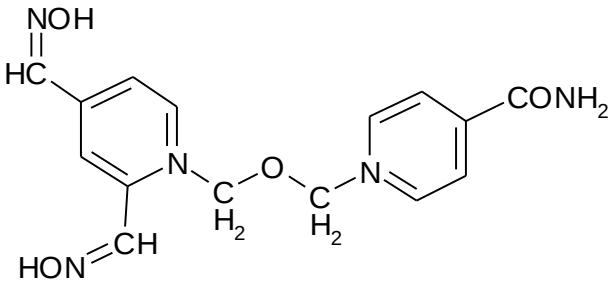
proposed reactivator of choice against nerve gas poisoning. However its synthesis is bit difficult and its development is fewer advances than that of HI-6(Kusic et al. 1985). Both the oximes HI-6 and HLo-7 were less effective against pesticide poisoning in comparison with obidoxime and 2-PAM



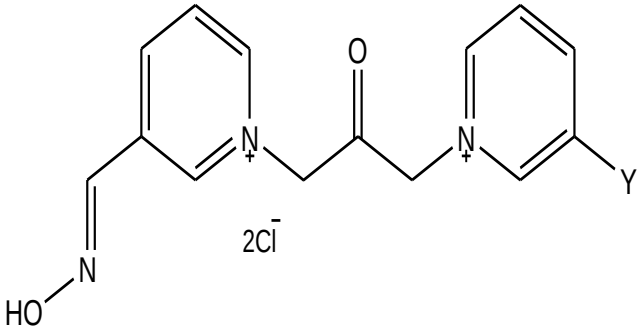
HI-6

(38)

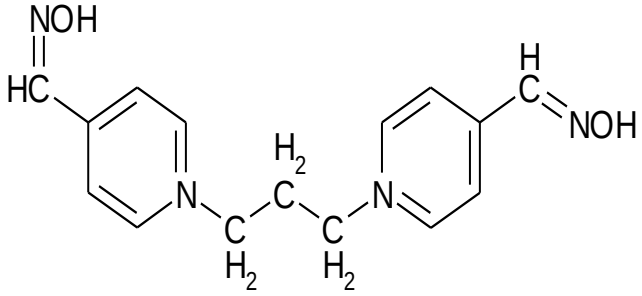


HLo-7

(39)



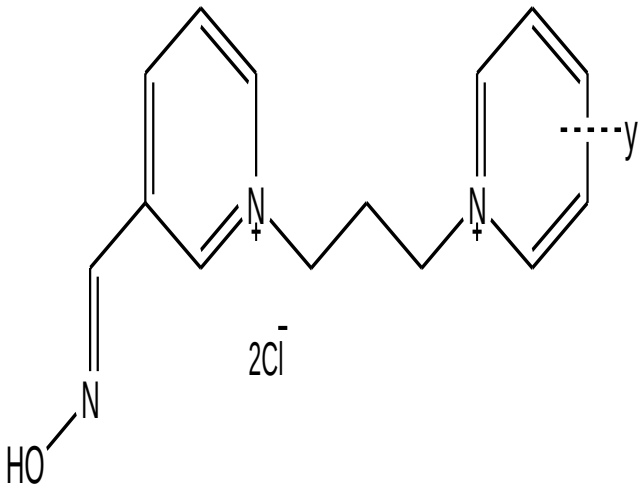
(40)



Trimedoxime

(41)

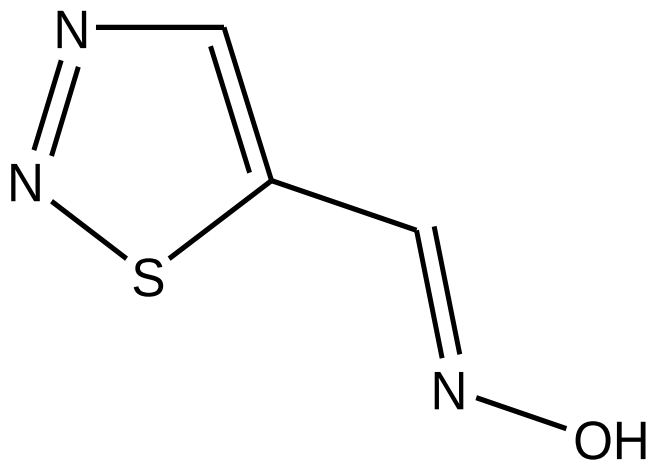
Arun et al. reported on quaternary salt of 3,3'-bis pyridine monooximes: synthesis and biological activity. Two new series of asymmetrically substituted 3,3'-bis pyridinium monoxides bridged by ox propane and propane groups were synthesized and tested.



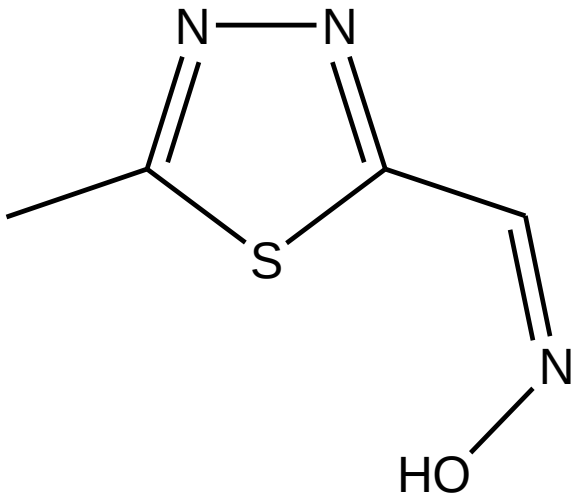
(42)

Compound	Substituents(Y)
41 a	CONH2
41 b	CONHCH3
41 c	CONHBu
41 d	CONHC5H9
41 e	CONHC6H11
41 f	CONHC6H5
41 g	COOC6H11
41h	CH=NOH

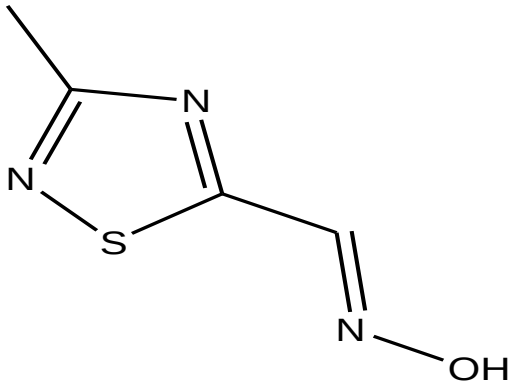
HP Benschop et al. synthesized thiadiazole-5-caroaldoxime and tested against organophosphate poisoning in rabbits, mice, rats and guinea pig (Benschop et al. 1979)



(43)

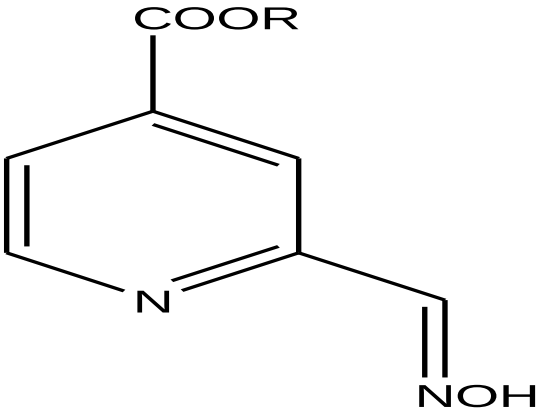


(44)

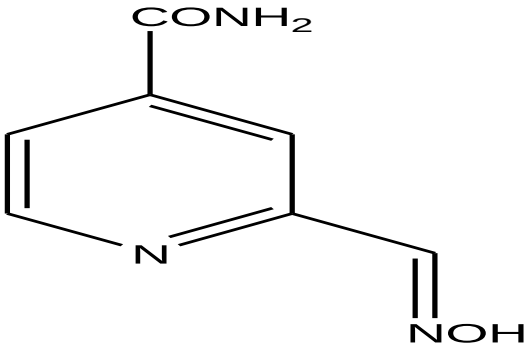


(45)

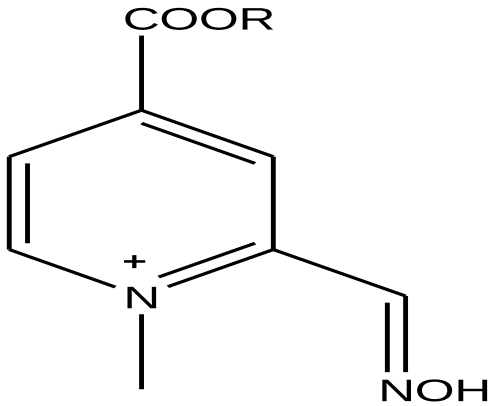
Bielavesky et al. reported the choline esterase reactivators derived from pyridine-2-carbaldoxime (Bielavesky et al.1998)



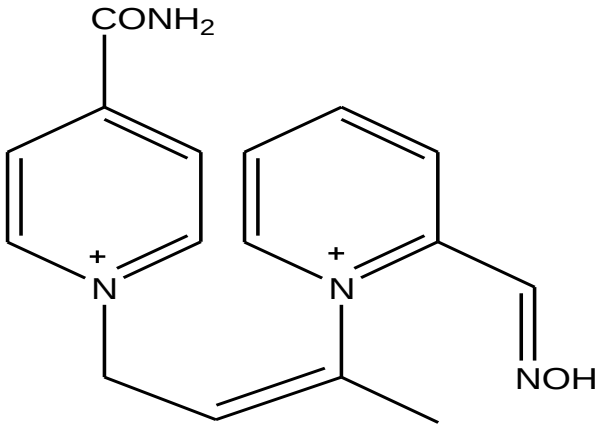
(46a, R=CH_3); (46b, R=C_2H_5)



(47) (48a, R=CH_3)



(48a,R=CH_3) (48b,R=C_2H_5)



(49a, X=Br) (49b, R=Cl)

In comparison with analogous compounds containing the –CH2-O-CH2- Bridge, the Butelenediyl Bridge gives compounds which are somewhat more stable but more toxic (Patoka et al. 1970). The compounds 45 a, 45 c, and 46a were tested in-vitro for their therapeutic effect against the organophosphate insecticide fosdrin (Mevinphos).

Dane AG et al reported synthesis of quaternary salts of 2-[(hydroxylimino) methyl] imidazole (dane et al 1991)

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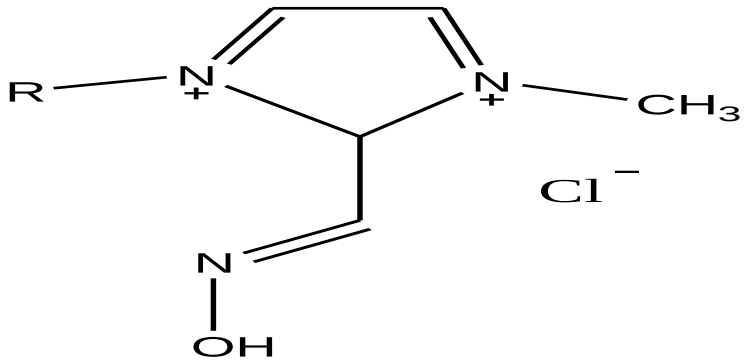

(50)

SNo.	R		
50a	CH_2OCH_2 CH_2OCH_3		
50b	$CH_2OCH_2CH_2CH(OCH_3)CH_3$		
50c	$CH_2OCH_2CH_2Si(CH_3)$		
50d	$CH_2OCH_2CH_2CH=Si(CH_3)$		
50e	CH_2CH_2CN		
50f	$CH_2CH_2CH_2CN$		
50g	$CH_2CH_2CH_2CH_2CN$		
50h	$CH_2CH_2CH_2CH_2$ CH_2COOCH_3	50r	$CH_2OCH_2CH_2NO_2$
50i	CH_2CH_2F	50s	$CH_2OCH_2C(CH_3)_2NO_2$
50j	$CH_2CH_2OCH_2CH_2F$	50t	$CH_2CH_2SO_2CH_3$
50k	$CH_2OCH_2CH_2CH_2CH_2Cl$	50u	$CH_2OCH_2CH_2SO_2CH_3$
50 l	$CH_2CH_2OCH_2Cl$	50v	$CH_2CH_2N^+(CH_3)_2Cl$
50m	CH_2CH_2Br	50w	$CH_2CH_2C_4H_5N^+$ Cl
50n	$CH_2CH_2CH_2Br$	50x	$CH_2CH_2N(CH_2)SO_2CH_3$
50o	$CH_2OCH_2CH_2CH_2Br$	50y	$CH_2CH_2N(CH_2)SO_2CF_3$
50p	$CH_2OCH_2C(CH_3)_2CH_2Br$	50z	$CH_2CH_2N(CH_2)SO_2$ Ph
50q	$CH_2CH_2NO_2$		

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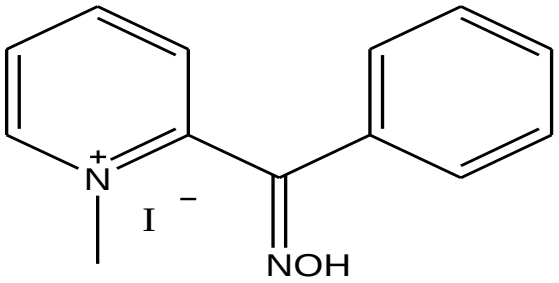
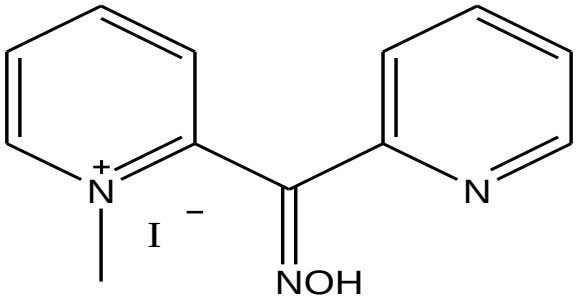
Kuca et al. synthesized monopyridinium oximes and tested against nerve agent poisoning. All the three monoquaternary ketoximes were against nerve agents like Sarin, Cyclosarin, VX and Tuban. All ketoximes were able to reactivate VX-inhibite AChE only. The reason for this too low reactivation is probably due to pressure of ketoximes group instead of aldoxime group. The aldoxime group is currently most preffered functional group of AChE reactivators (kassa et al. 2002)



(51)



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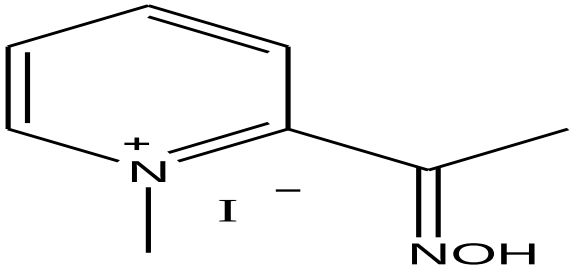
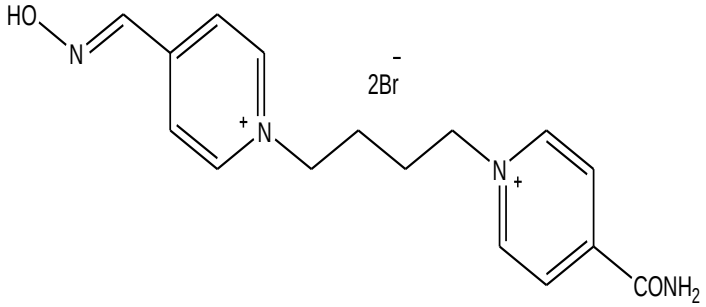
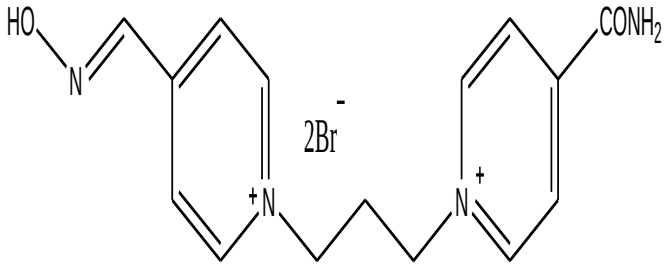


Kuca et al. reported the synthesis and reactivation of efficacy of a new asymmetric bisquaternary reactivate 1-(4-hydoxyiminomethylpyridinium)-4-(4-cabamoyl pyridinium) butane bromide(kuca et al. 2003)



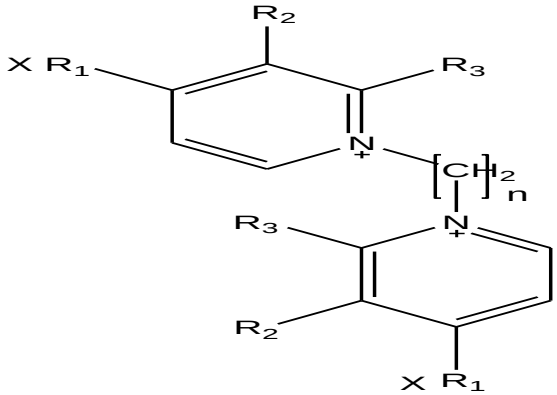
(54)

Kamil Kuca et al have reported in the same year, synthesi and reactivation efficacy of serin inhibited AChE by 1-(4-hydroxyiminomethylpyridinium)-3-(carbamoylpyridinium)-propane dibromide(Kuca et al, 2003)



(55)

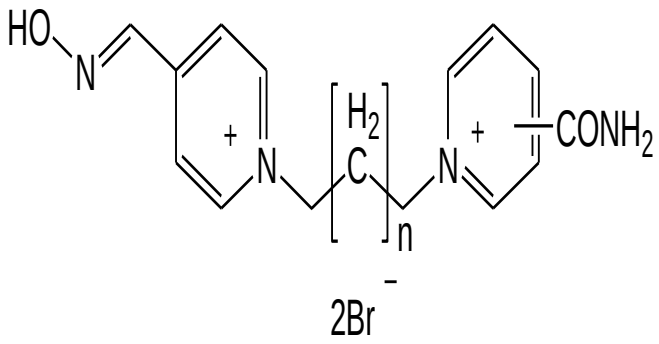
Pang et al. reported on regional designed alkaline-linked bispyridiniumal doxime as improved Ache deactivators. To improve the potency of 2-PAM for treating OP poisoning they demonized 2-PAM and analogues of it



(56).R_1 = -CH=NOH, R_2 = H, R_3 = H, X = Br^-
(57). R_1 = H, R_2 = -CH=NOH, R_3 = H, X = Br^-
(58). R_1 = H, R_2 = H, R_3 = -CH=NOH, X = Br^-

a: (n) =2, b: (n) = 3, c: (n) = 4, d: (n) = 5, e: (n) = 6, f: (n) = 7, g: (n) = 8, h: (n) = 9

In 2005 Chennamaneni SR et al. synthesized alkylene linked bis pyridinium oximes and tested for their reactivation potency for TEPP inhibited AChE .



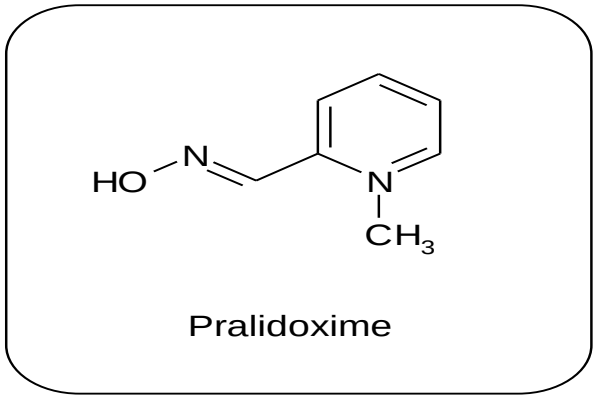
(59)

	n	$CONH_2$
59 a	1	4
59 b	1	3
59 c	2	4
59 d	2	3
59 e	3	4
59 f	3	3
59 g	4	4

	n	$CONH_2$
59 h	4	3
59 i	5	4
59 j	5	3
59 k	6	4
59 l	6	3
59 m	7	4
59 m	7	3

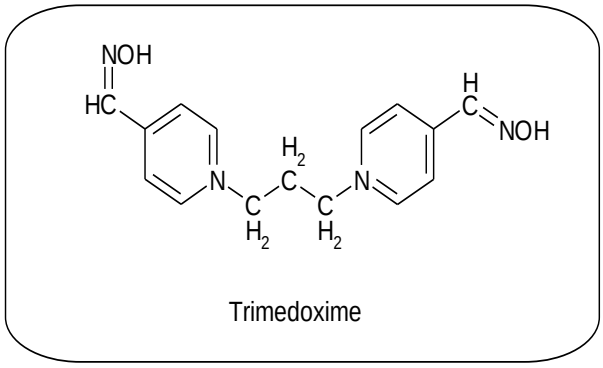
B. All Available Antidotes in Market

a). Pralidoxime



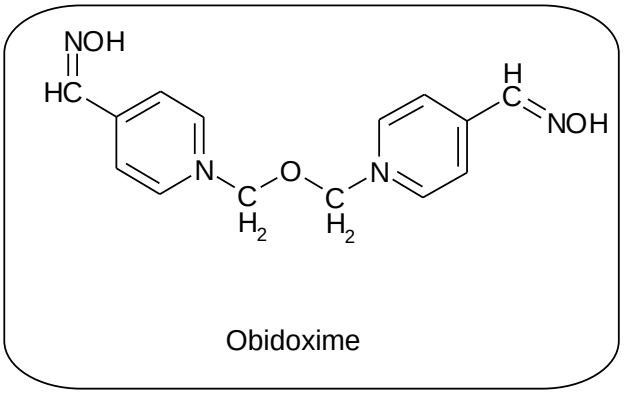
Pralidoxime (2-pyridine aldoxime methyl chloride) or 2-PAM, usually as the chloride or iodide salts, belongs to a family of compounds called oximes that bind to organophosphate-inactivated acetylcholinesterase. It is used to combat poisoningby organophosphates or acetylcholinesterase inhibitors (nerve agents) in conjunction with atropine and diazepam. It is a white solid.

1. Trimedoxime (TMB-4)



Chemically, TMB-4 Cl is a 1,3-bis(4-hydroxyimminomethyl-1-pyridinio)propane-dichloride and was synthesised in the USA in 1957 (24) and patent-protected in 1967 (25). Trimedoxime bromide (INN), also known as dipyroxime or TMB-4, is an oxime used in the treatment of organophosphate poisoning. Experiments have shown that TMB-4 is more potent reactivators of the DFP-inhibited AChE than PAM-2 (26) and by 15 % to 40 % better reactivators than LüH-6 in case of tabun inhibition (27).

2. Obidoxime (LüH-6, Toxogonin)

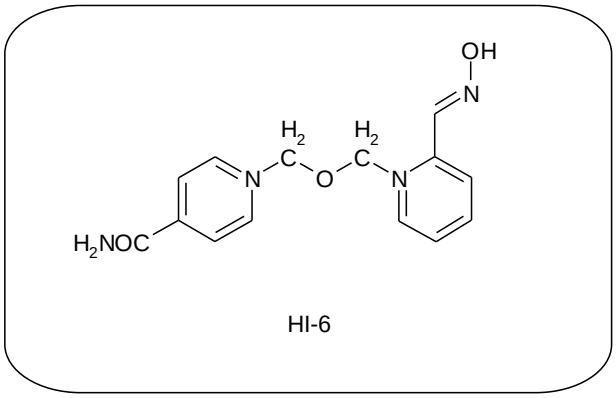


Obidoxime

Obidoxime (LüH-6, Toxogonin) Chemically, LüH-6 Cl is a [1,3-bis(4hydroxyimminomethyl-1-pyridinio)-2-oxapropane] dichloride. Obidoxime is a member of the oxime family used to treat nerve gas poisoning. Oximes are drugs known for their ability to reverse the binding of organophosphorus compounds to the enzyme acetylcholinesterase (AChE).

AChE is an enzyme that removes acetylcholine from the synapse after it creates the required stimulation on the next nerve cell. If it gets inhibited, acetylcholine is not removed after the stimulation and multiple stimulations are made, resulting in muscle contractions and paralysis.Organophosphates (such as nerve gases) are well-known inhibitors of AChE. They bind to a specific place on the enzyme and prevent it from functioning normally by changing the OH group on the serine residue and by protonating(quaternary nitrogen, R_4N^+) the nearby nitrogen atom located in the histidineresidue.

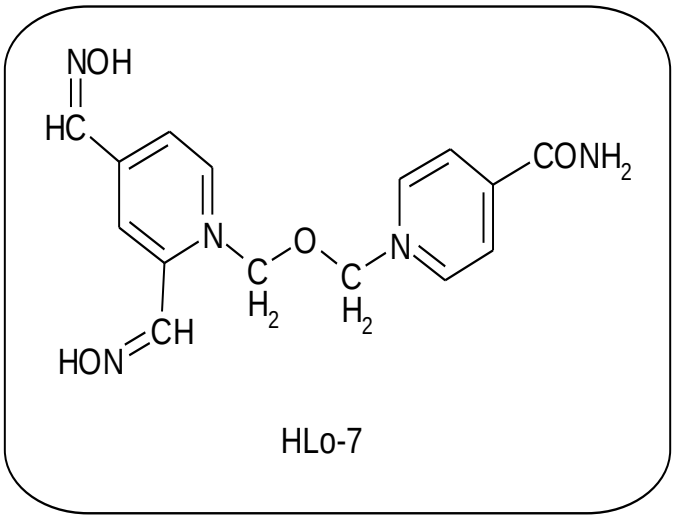
3. HI-6



HI-6

It was synthesised in 1966 and given the code name HS-6, after the last name initials of Ilse Hagedorn and Klaus Schoene (39). Current treatment of organophosphorus poisoning, resulting in overstimulation and desensitization of muscarinic and nicotinic receptors by acetylcholine (ACh), consists of the administration of atropine and oxime reactivators. However, no versatile oxime reactivator has been developed yet and some mortality still remains after application of standard atropine treatment, probably due to its lack of antinicotinic action.

4. HLo-7

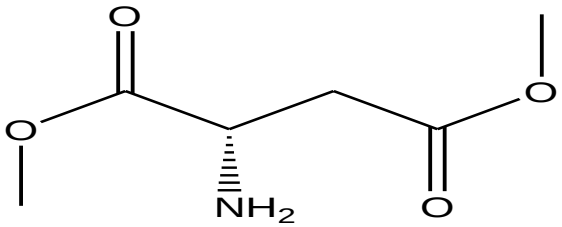


HLo-7

HLö-7 The fourth and the last important “Hagedorn oxime” (after LüH-6, HS-6 and HI-6) is HLö-7, named after Ilse Hagedorn and Marianne Löffler and also synthesised in Freiburg, Germany in 1986 (46). Chemically, this oxime is a 1-[[[4-(aminocarbonyl)pyri dinio]methoxy]methyl]-2,4-bis[(hydroxyimino)methyl] pyridinium-diiodide. The new oxime reactivates AChE inhibited by any of the four major nerve agents (4750), as well as the enzyme inhibited by cyclosarin (51).

V. RESULTS

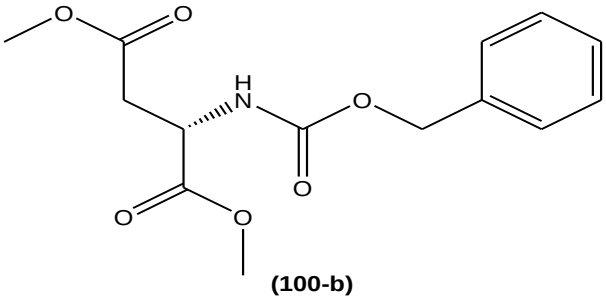
1. *100a*



(100-a)
[(S)-dimethyl 2-aminosuccinate]

Physical Properties: white colored crystalline solid Mass: 162 (M^++H = 161+1)

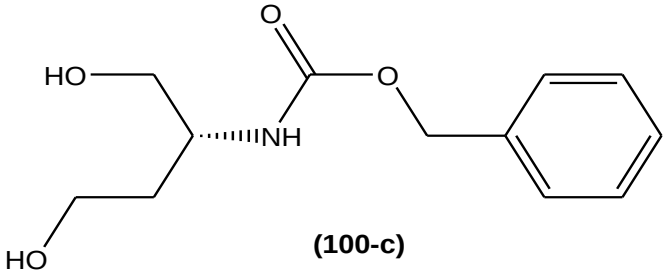
2. *100b*



(100-b)
[(*S*)-dimethyl 2-(benzyloxycarbonylamino)succinate]

Physical Properties: Slightly yellow colored pleasant smelling liquid, slowly solidifies to white colored mass Mass: 296 (M^++H = 295+1)

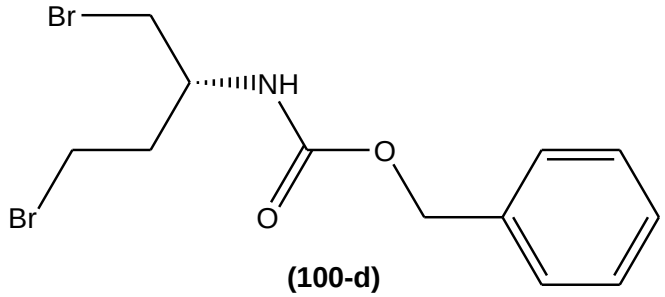
3. *100c*



(100-c)
[(*S*)-benzyl 1,4-dihydroxybutan-2-ylcarbamate]

Physical Properties: Slightly yellow colored liquid Mass: 240 (M^++H = 239+1)

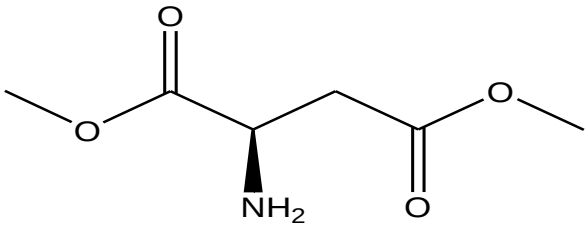
4. *100d*



(100-d)
[(*S*)-benzyl 1,4-dibromobutan-2-ylcarbamate]

Physical Properties: White colored solid, irritating to eyes Mass: 388 (M^++Na = 365+23)

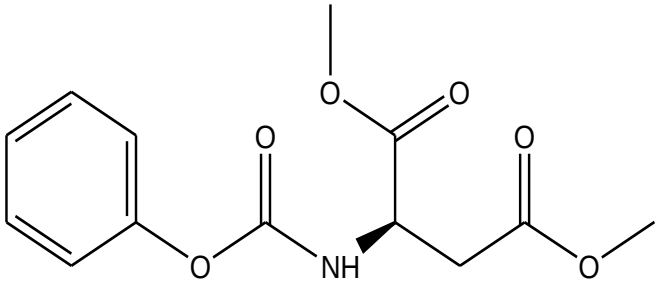
5. *101a*



(101-a)
[(*R*)-dimethyl 2-aminosuccinate]

Physical Properties: white colored crystalline solid Mass: 162 (M^++H = 161+1)

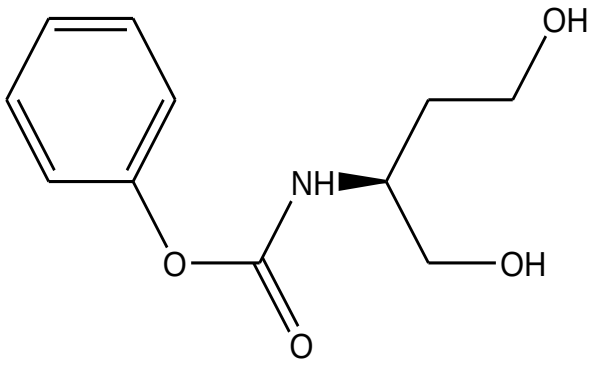
6. *101b*



(101-b)
[(*R*)-dimethyl 2-(phenoxycarbonylamino)succinate]

Physical Properties: Slightly yellow colored pleasant smelling liquid, slowly solidifies to white colored mass Mass: 296 (M^++H = 295+1)

7. 101c

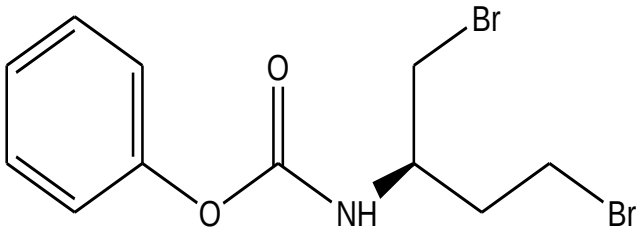


(101-c)

[(*R*)-phenyl 1,4-dihydroxybutan-2-ylcarbamate]

Physical Properties: Slightly yellow colored liquid Mass: 240 (M^++H = 239+1)

8. 101d

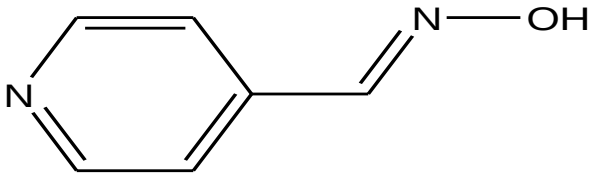


(101-d)

[(*R*)-phenyl 1,4-dibromobutan-2-ylcarbamate]

Physical Properties: White colored solid, irritating to eyes Mass: 388 (M^++Na = 365+23)

9. 103a

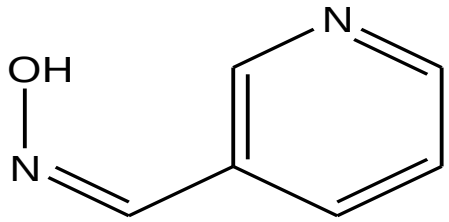


(103-a)

[Pyridine-2-carbaldehyde oxime]

Physical Properties: Mass: 123 (M^++H = 122+1)

10. 104a

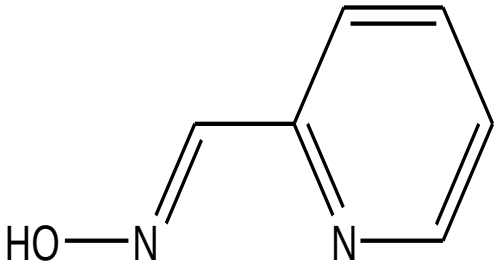


(104-a)

[Pyridine-3-carbaldehyde oxime]

Mass: 123 (M^++H = 122+1)

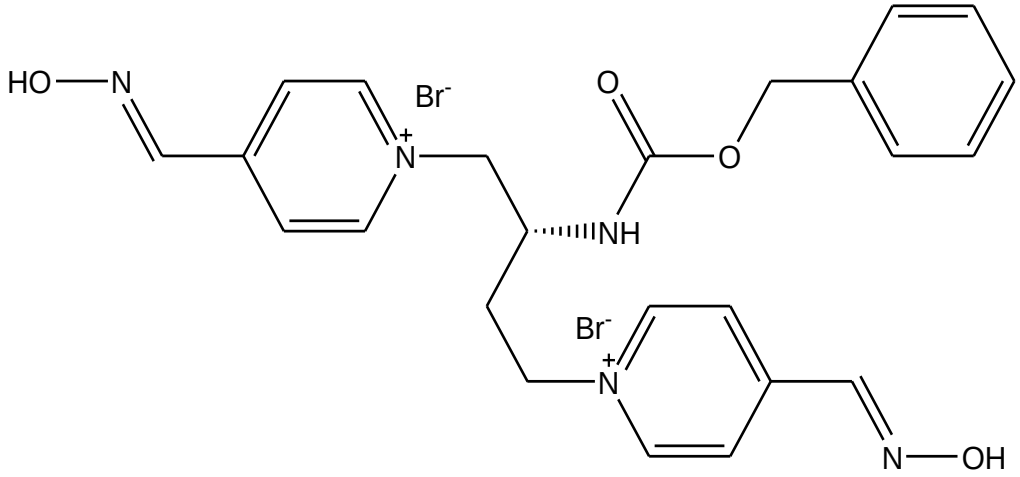
11. 105a



(105-a)

[Pyridine-4-carbaldehyde oxime]

Mass: 123 (M^++H = 122+1)

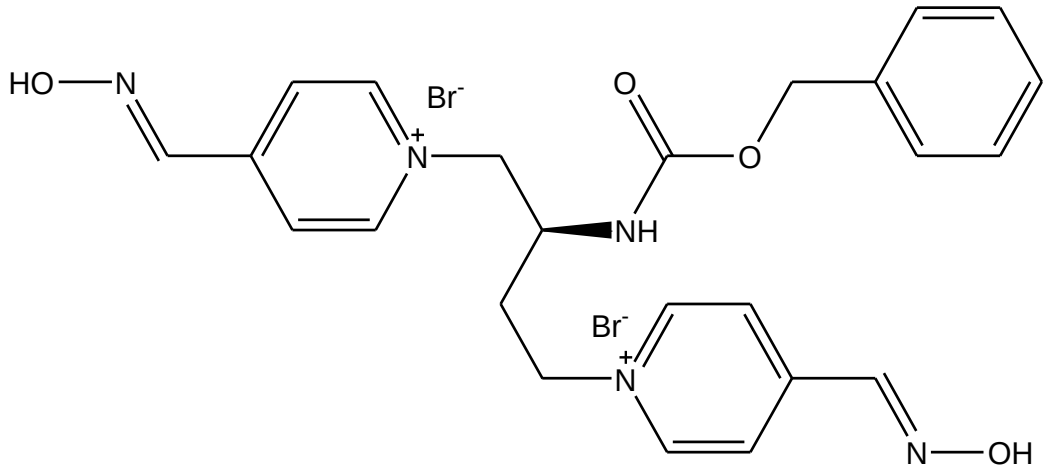


(L4X)

{(*E*)-1,1'-((*S*)-2-(benzyloxycarbonylamino)butane-1,4-diyl)bis(4-((*E*)-(hydroxyimino)methyl)pyridinium) bromide}

Mass: 449

12. D4X

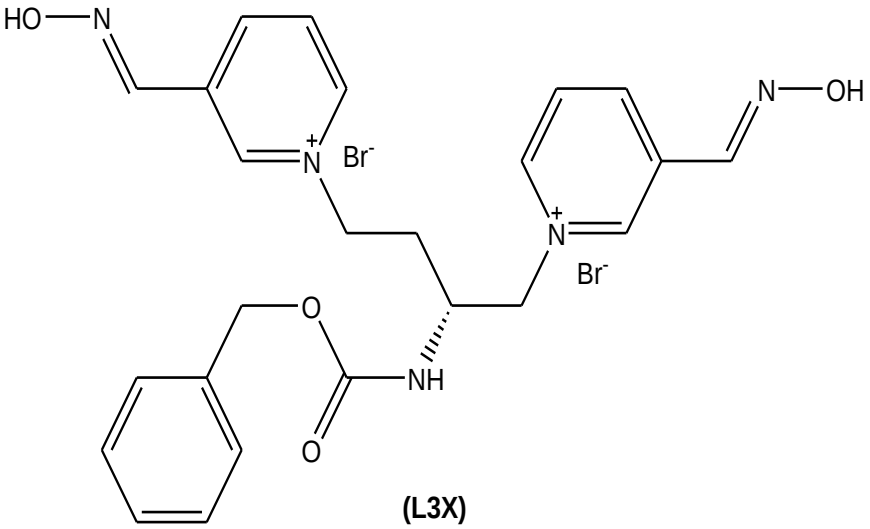


(D4X)

(*E*)-1,1'-((*R*)-2-(benzyloxycarbonylamino)butane-1,4-diyl)bis(4-((*E*)-(hydroxyimino)methyl)pyridinium) bromide

Mass: 449

13. L3X

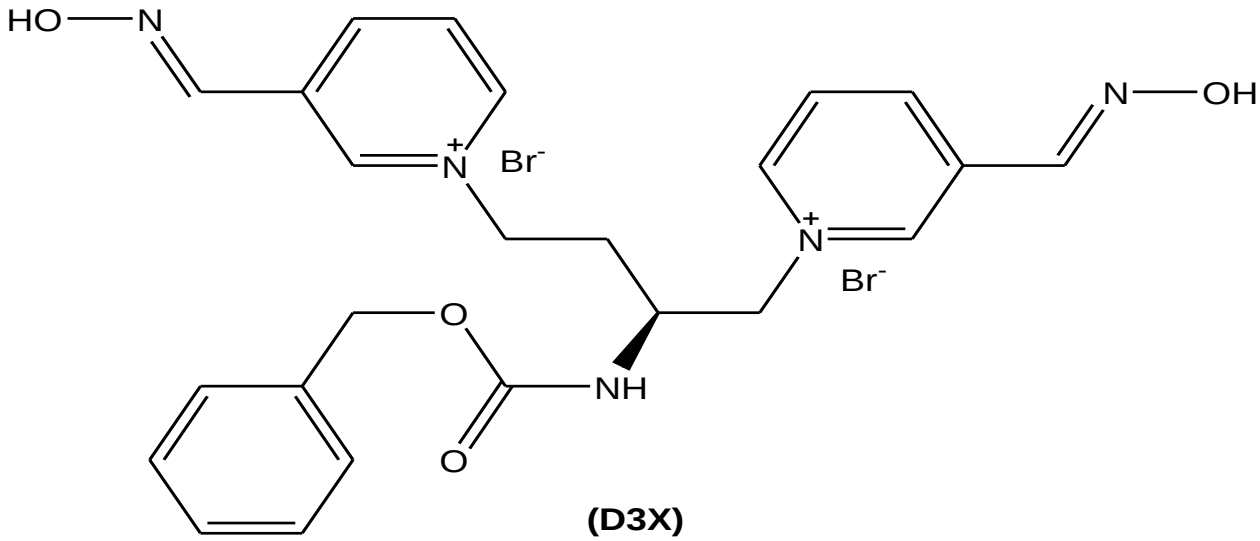


(L3X)

{(*E*)-1,1'-((*S*)-2-(benzyloxycarbonylamino)butane-1,4-diyl)bis(3-((*E*)-(hydroxyimino)methyl)pyridinium) bromide}

Mass: 449

14. D3X

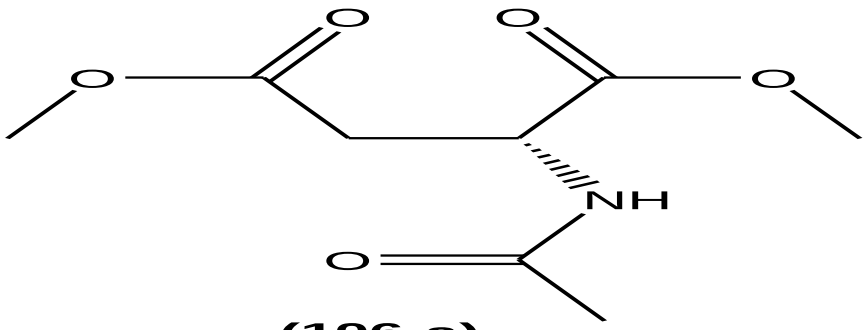


(D3X)

{(*E*)-1,1'-((*R*)-2-(benzyloxycarbonylamino)butane-1,4-diyl) bis(3-((*E*)-(hydroxyimino)methyl)pyridinium) bromide}

Mass: 44z

15. N-Ac protection

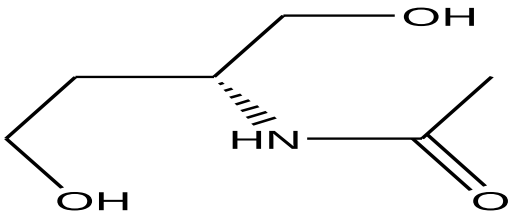


(106-a)

(*R*)-dimethyl 2-acetamidosuccinate

Mass: 204 (M^++H = 203+1)

16. 106-b



(*R*)-*N*-(1,4-dihydroxybutan-2-yl)acetamide

(106-b)

Mass: 186 (M^++K = 147+39)

REFERENCE

[1]. Eddleston M[1]. Patterns and problems of deliberate self-poisoning in the developing world. QJM. 2000 Nov; 93(11):715-31.

[2]. Agarwal SB. A clinical, biochemical, neurobehavioral and sociopsychological study of 190 patients admitted to hospital as a result of acute organophosphorus poisoning. Environ Res 1993 Jul; 62(1):63-70. 10. Taylor

[3]. P.Anticholinesterase agents. In: Gilman AG and Goodman LS (eds), The Pharmacological Basis of Therapeutics. New York: Macmillan Publishing Co. Inc.; 1985, pp.110-28.

[4]. de Kort WL[1], Kiestra SH, and Sangster B. The use of atropine and oximes in organophosphate intoxications: A modified approach. J Toxicol Clin Toxicol. 1988;26(3-4):199-208.

[5]. Jamal JA. Neurological syndromes of organophosphorus compounds. *Adverse Drug React Toxicol Rev* 1997;16(3):133-70

[6]. Steenland K, Jenkins B, Ames RG, et al. Chronic neurological sequelae to organophosphate pesticide poisoning. Am J Public Health. 1994 May; 84(5):731-736

[7]. Savage EP, Keefe TJ, Mounce LM, et al. Chronic Neurological Sequelae of Acute Organophosphate Pesticide Poisoning. Archives of Environmental Health 1988;43:38-45.

[8]. Rosenstock L, Keifer M, Daniell W, et al. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *Lancet* 1991;338:223-7.

[9]. Gershon S. PSYCHIATRIC SEQUELÆ OF CHRONIC EXPOSURE TO ORGANOPHOSPHORUS INSECTICIDES. Lancet 1961 June; 277(24):1371-1374.

[10]. Metcalf DR and Holmes JH. EEG, psychological, and neurological alterations in humans with organophosphorus exposure. *Ann NY Acad Sci* 1969;160:357-65.
[11]. HOLMES JH, GAON MD. Observations on acute and multiple exposure to anticholinesterase agents. Trans Am Clin Climatol Assoc.1956- 1957; 68:86-100.
[12]. Hirshberg A and Lerman Y. Clinical problems in organophosphate insecticide poisoning: The use of a computerized information system. *Fundam Appl Toxicol* 1984; 4:S209-14.
[13]. Agarwal SB. A clinical, biochemical, neurobehavioral and sociopsychological study of 190 patients admitted to hospital as a result of acute organophosphorus poisoning. Environ Res 1993 Jul; 62(1):63-70.
[14]. Miller CS and Mitzel HC. Chemical sensitivity attributed to pesticide exposure versus remodeling. *Arch Environ Health* 1995; 50:119-29. 2010 Aug; 43(3) :38-45.
[15]. De Bleecker J, Willems J, Van Den Neucker K, et al. Prolonged Toxicity with Intermediate Syndrome After Combined Parathion and Methyl Parathion Poisoning. J Toxicol Clin Toxicol. 2008;30:333-345.
[16]. DeBleecker J, Van Den Neucker K, and Colardyn F. Intermediate syndrome in organophosphorous poisoning: A prospective study. *Crit Care Med* 1993;21:1706-11.
[17]. Aldridge WN and Nemery B. Toxicology of trialkylphosphorothioates with particular reference to lung toxicity. Fundamental and Applied Toxicology 1984; 4:S215-S223.
[18]. Bardin PG, Van Eeden SF, Moolman JA, et al. Organophosphate and carbamate poisoning. *Arch Intern Med* 1994;154:1433-41.
[19]. Zwiener RJ, Ginsburg CM. Organophosphate and Carbamate Poisoning in Infants and Children. Pediatrics 1988;81:121-683 .
[20]. Sofer S, Tal A, and Shahak E. Carbamate and organophosphate poisoning in early childhood. Pediatric Emergency Care. 1989;5(4):222-225
[21]. S llivan JB and Blose J. Organophosphate and carbamate insecticides. In: Sullivan JB and Krieger GR (eds), Hazardous Materials Toxicology. Baltimore, MD: Williams and Wilkins, 1992, pp. 1015-26.
[22]. Goswamy R, Chaudhuri A, and Mahashur AA. Study of respiratory failure in organophosphate and carbamate poisoning. Heart Lung. 1994;23(6):466-72.
[23]. Holmes JH and Gaon MD. Observations on acute and multiple exposure to anticholinesterase agents. *Trans Am Clin Climatol Assoc* 1957; 68:86-103.
[24]. Fernando R. Pesticide poisoning in the Asia-Pacific region and the role of a regional information network. J Toxicol Clin Toxicol 1995; 33(6):677-82.
[25]. Hirshberg A and Lerman Y. Clinical problems in organophosphate insecticide poisoning: The use of a computerized information system. *Fundam Appl Toxicol* 1984; 4:S209-14.
[26]. Miller CS and Mitzel HC. Chemical sensitivity attributed to pesticide exposure versus remodeling. Arch Environ Health. 1995; 50(2):119-29.
[27]. DeBleeker J, Willems J, Van Den Neucker K, et al. Prolonged toxicity with intermediate syndrome after combined parathion and methyl parathion poisoning. *Clin Toxicol* 1992;30:333-45.
[28]. DeBleecker J, Van Den Neucker K, and Colardyn F. Intermediate syndrome in organophosphorous poisoning: A prospective study. *Crit Care Med* 1993;21:1706-11.
[29]. Aldridge WN and Nemery B. Toxicology of trialkylphosphorothioates with particular reference to lung toxicity. *Fundam Appl Toxicol* 1984; 4:S215-23.
[30]. Bardin PG, Van Eeden SF, Moolman JA, et al. Organophosphate and carbamate poisoning. *Arch Intern Med* 1994;154:1433-41.
[31]. Malik GM[1], Mubarik M, Romshoo GJ. Organophosphorus Poisoning in the Kashmir Valley, 1994 to 1997. N Engl J Med 1998 Apl; 338:1078-1079.
[32]. Nagami H[1], Nishigaki Y, Matsushima S, Matsushita T, Asanuma S, Yajima N, et al. Hospital-based survey of pesticide poisoning in Japan, 1998-2002. Int J Occup Environ Health 2005; Apr-Jun;11(2):180-4.
[33]. DuBois KP. The toxicity of organophosphorous compounds to mammals. Bull World Health Organ 1971; 44(1-2-3): 231-240.
[34]. Pasquet J, Mazuret A, Fournel J, et al. Acute oral and percutaneous toxicity of phosalone in the rat, in comparison with azinphosmethyl and parathion. Toxicol Appl Pharmacol 1976;37:85-92.
[35]. Garcia-Repetto R, Martinez D, and Repetto M. Coefficient of distribution of some organophosphorus pesticides in rat tissue. *Vet Hum Toxicol* 1995;37:226-9.
[36]. Gallo MA and Lawryk NJ. Organic phosphorus pesticides. In: Haves WJ and Laws ER (eds), Handbook of Pesticide Toxicology, vol 2, Classes of Pesticides. San Diego, CA: Academic Press Inc., 1991.