Effect of Nioxim on the Activity of Inhibited AChE

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Abstract

This study is designed to show the effects of Nioxim on the activity of inhibited AChE by heterocyclic compounds in sera. The results obtained showed that both Oxime causes decrease in percentage of inhibit ion. The mechanism of action of the Oxime as reactivators to inhibited AChE activity were suggested which attributed to the ability of this compound to attack the thion group of the inhibited enzyme (enzyme-inhibitor complex) by the nucleophilic oxygen atom and then liberate the free enzyme.

الخلاصة

تم في هذا البحث دراسة تأثير النيو اوكسيم على فعالية أنزيم الاسيتيل كولين استريز المثبط بواسطة بعـض المركبات الحلقية غير المتجانسة في مصل دم الانسان حيث أظهرت النتائج ان المركب يسبب اعادة تنشـيط لفعاليـة الانزيم كذلك تم في هذه الدراسة اقتراح الية عمل لمركبات الاوكسيم كمعيد تنشيط لفعالية الانزيم المثبط تظهر قابليـة هذا المركب على مهاجمة معقد الانزيم-المثبط (عن طريق ذرة الاوكسجين النيوكليوفيلية) و تحرير الانزيم الحر.

Introduction

Cholinesterase is one of many important enzymes needed for the proper functioning of the nervous systems of humans, other vertebrates, and insects [1].

This enzyme works on the "synapses" which is an electrical switching centers found throughout the nervous system of humans, other vertebrates, and insects. Muscles, glands, and nerve fibres called "neruons" are stimulated or inhibited by constant firing of signals across these synapses [2].

Stimulating signals are usually carried by a chemical called "acetyl choline". Stimulating signals are discontinued by a specific type of cholinesterase enzyme, acetylcholinesterase, which breaks down the acetylcholine. These important chemical reactions are usually going on all the time at a very fast rate, with acetylcholine causing stimulation and acetyl cholinesterase ending the signal. If cholinesterase-affecting insecticides are present in the synapses, however, this situation is thrown out of balance. Acetylcholine can then build up, causing a "jam" in the nervous system. Thus, when a person receives to great an exposure to cholinesterase inhibiting compounds, the body is unable to break down the acetylcholine [3].

Organ phosphorous (OP's) compounds inhibit AChE irreversibly by forming phosphorylated serine (phosphorylated enzyme) in the esteratic site of AChE; regeneration of this site by spontaneous hydrolysis of the complex is very slow.

The enzyme- phosphate ester complexes formed from the action of OP's on AChE are hydrolyzed only slowly, producing prolonged inhibition[4].

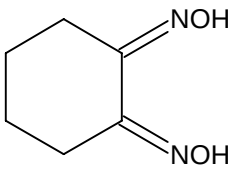
It is possible to reactivate the enzyme by several compounds given soon after the AChE phosphorylation due to poisoning by these substances[5].

Experimental

One Oxime was selected to test its reactivation effets on the activity of inhibited AChE by heterocyclic compounds. The solubility this compound was tested in sodium phosphate buffer (0.2 M, pH= 7.3) which was used in enzyme activity determination.

The compound was chosen namely Nioxim(1,2-cyclohexanediondioxime.

M.wt = 142.-16

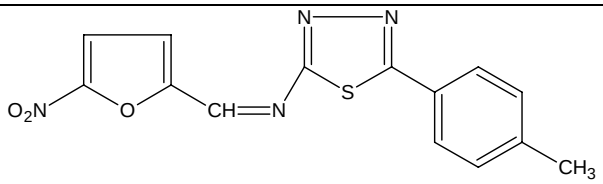
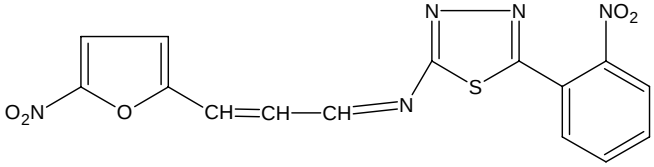
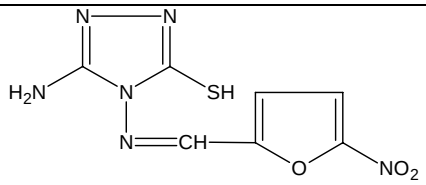
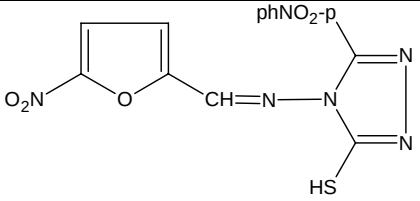
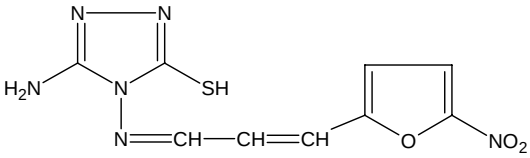
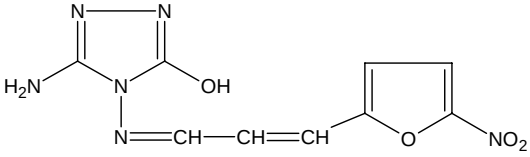
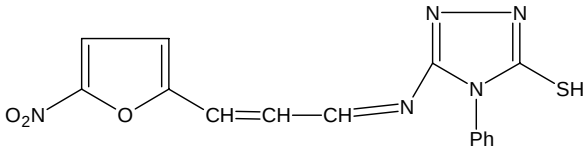


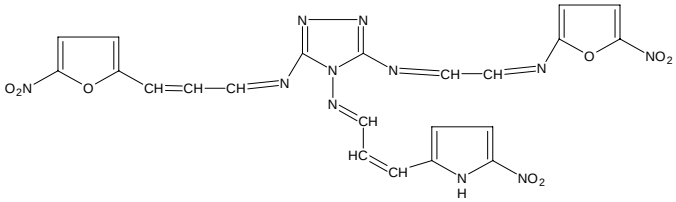
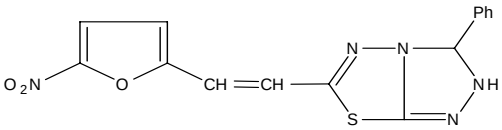
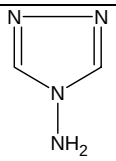
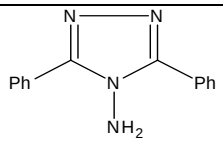
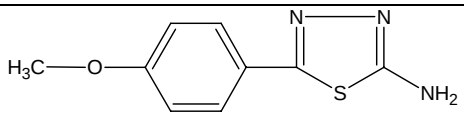
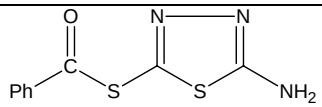
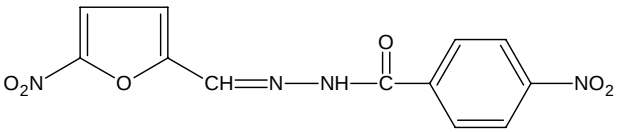
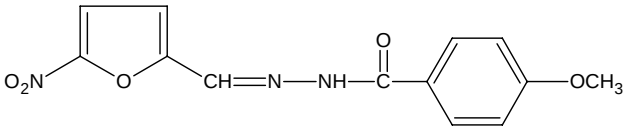
Procedure:

A stock solution (0.1 M) concentration of Nioxim was prepared and then the following concentrations (0.5,1,2,3,4, 5) X 10^{-3} M were prepared. ($1X10^{-4}$M) concentration of all inhibitors was selected lo determine the enzyme activity with and without the inhibitor and under the same conditions as follows:

(50 µl) of DTNB solution (0.001 M) was added to 2.25 ml of phosphate buffer solution (l ml of inhibitor mixed with 1.25 ml of buffer (pH=7.3, 0.2M)), then (10 µl) of serum was added, mixed well and (2 ml) of the mixture was transferred lo a measuring cell (3 mm), finally (34 µl) of ASChI (0.06M) was added, the change in absorbency was measured before and after adding the substrate at (430 inn) for (3 min). After that the enzyme activity was determined in the presence of inhibitor and possible reactivator (1.25 ml buffer mixed with 0.75 ml of possible reactivator). The method described in above was followed for the measurement of the activity of enzyme.

Table 1-: Heterocyclic Compounds used for interaction with AChE

Comp. No.	Structure	Name	Mwt.
1		5-(p-methyl phenyl)-3-[5'-nitro-2'-furyl] methylamino]-l,3,4-thiodiozole	314
2		5-(o-nitro phenyl)-3-[3(5'-nilro-2'-furyl)Prop-2-enylidene amino)]-1 ,2J,4-thiodizole	326
3		5-Amino-2-mercapto -4-[5'-nilro-2'-furyl)methylcne amino)]-4H-1,2,4-triazole	254
4		3-Mercapto -5-(p-$N0_2$ phenyl)-4H-1,2,4-triazole	238
5		**5-Amino-3-mercapto -4- [3-(5'-nitro-2-furyl)Prop-2-methylene amino)]-4H-l,2,4-triazole**	219
6		**5-Amino-3-hydroxy -4-[3'-(5'-nitro-2-furyl)Prop-2-enylidene amino)]-4H-1,2,4-triazole**	264
7		**3-Mercapto-4-phenyl -5-[3-(5-nitro-2'-furyl)Prop-2-enylidene) amino]-4H-l,2,4-triazole**	341

8		**Tri[3(5-nitro-2'-furyl) Prop-2-enylidene) amino]-4H-l,2,4-triazole**	574
9		**3-phenyl -6-[5'-nitro-2'-furyl) ethyl]-l,2,4-triazolc[3,4,6],[1,3,4] thiodiazole**	339
10		4-Amino-**1**,2,4-Triazole	84
11		3,5-diphenyl-4-amino-l,2,4-triazolc	236
12		2-(amino-5-(p-mcthoxy phenyl)1,3,4-thiadiazole	206
13		2-(amino-5-thio benzoyloxy-1,3,4-thiadiazole	237
14		5-Nitrofurylidene -N-p-N0$_2$-benzoic acid hydrizde	304
15		5-Nilrofurylidcne-N-p-methoxy-benzoic acid hydrizde	289

Results and Discussion

Acetylcholinesterase can be reactivated by many substances which have the ability to reactivate it and some are used as organophosphorous antidotes, like obidoxime chloride and pralidoxime, others are also available like diacetylmonoxime and trimedoxime bromide. In the present study, the compounds tested to reactivate the enzyme is Nioxim contain nucleophilic groups more than other oximes, to reactivate the inhibited AChE by heterocyclic compounds in human serum.

Various concentrations of Nioxim (0.5-5)x 10^{-3} M were used with a fixed concentration (1x 10^{-4}M) of inhibitors as shown in tables (2-4)

All concentrations which used causes decrease in the inhibition percentage of the inhibited AChE activity, the degree of reactivation increased with the increased concentration of Nioxim until maximum reactivation was reached. The behavior of AChE activity in the presence of inhibitors and Nioxim of various concentrations are shown in figures (1 and 2).

The influence of Nioxim as reactivater is mainly attributed to the ability of Nioxim to attack the active groups of the inhibited enzyme (enzyme- inhibitor complex) by the nucleophilic oxygen atom and then liberate the free enzyme according to the proposed mechanism shown in scheme (1 and 2).

Table (2) Effect of different concentrations of *Nioxime* on the activity of inhibited *AChE* by compounds (1) to (5).

Compound (1)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	**5.500**	**0**	**100**
0	**1.125**	**79.545**	**20.455**
$5x10^{-4}$	**5.075**	**39.091**	**60.909**
$1x10^{-3}$	**4.675**	**35.909**	**64.091**
$2x10^{-3}$	**4.325**	**27.723**	**72.273**
$3x10^{-3}$	**3.975**	**21.364**	**78.636**
$4x10^{-3}$	**3.525**	**15.000**	**85.000**
$5x10^{-3}$	**3.350**	**7.723**	**92.273**
Compound (2)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	5.175	0	100
0	1.350	73.913	26.087
$5x10^{-4}$	3.350	35.266	64.734
$1x10^{-3}$	3.600	30.435	69.565
$2x10^{-3}$	3.875	25.121	74.879
$3x10^{-3}$	4.175	19.324	80.676
$4x10^{-3}$	4.325	16.425	83.575
$5x10^{-3}$	4.500	13.043	86.957
Compound (3)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	5.925	0	100
0	1.800	69.620	30.380
$5x10^{-4}$	16.878	37.131	62.869
$1x10^{-3}$	24.473	31.646	68.354
$2x10^{-3}$	27.004	29.536	70.464
$3x10^{-3}$	29.536	27.004	72.996
$4x10^{-3}$	31.646	24.473	75.527
$5x10^{-3}$	37.131	16.878	83.122
Compound (4)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	5.200	0	100
0	-1.075	79.327	20.673
$5x10^{-4}$	3.500	32.692	67.308
$1x10^{-3}$	3.700	28.846	71.154
$2x10^{-3}$	3.925	24.519	75.841
$3x10^{-3}$	4.245	18.365	81.634
$4x10^{-3}$	4.475	13.942	86.058
$5x10^{-3}$	4.600	11.538	88.462
Compound (5)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	7.275	0	100
0	1.275	82.474	17.526
$5x10^{-4}$	3.500	51.890	48.110
$1x10^{-3}$	3.800	47.766	52.234
$2x10^{-3}$	4.175	42.612	57.388
$3x10^{-3}$	4.550	37.457	62.543
$4x10^{-3}$	4.825	33.677	66.323
$5x10^{-3}$	5.250	27.835	72.165

Table (3) Effect of different concentrations of *Nioxime* on the activity of inhibited *AChE* by compounds (6) to (10).

Compound (6)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	5.525	0	100
0	1.650	70.136	29.964
$5x10^{-4}$	3.375	38.914	61.086
$1x10^{-3}$	3.550	35.747	64.253
$2x10^{-3}$	4.175	24.434	75.566
$3x10^{-3}$	4.525	18.100	81.900
$4x10^{-3}$	4.825	12.670	87.330
$5x10^{-3}$	5.25	4.977	95.023
Compound (7)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**Activity Recovered[%]**
Nil	6.500	0	100
0	1.425	78.077	21.923
$5x10^{-4}$	3.575	45.000	55.000
$1x10^{-3}$	3.775	41.923	58.077
$2x10^{-3}$	4.175	35.769	64.231
$3x10^{-3}$	4.675	28.077	71.923
$4x10^{-3}$	5.325	18.077	81.823
$5x10^{-3}$	6.150	5.385	94.615
Compound (8)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**Activity Recovered[%]**
Nil	6.150	0	100
0	1.350	78.049	21.951
$5x10^{-4}$	4.100		***66.667***
$1x10^{-3}$	4.325	29.675	70.325
$2x10^{-3}$	4.550	26.016	73.984
$3x10^{-3}$	4.725	23.171	76.829
$4x10^{-3}$	4.950	19.512	80.488
$5x10^{-3}$	5.425	11.789	88.211
Compound **(9)**			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**Activity Recovered[%]**
Nil	**7.275**	0	100
0	**1.450**	80.069	19.931
$5x10^{-4}$	**$5x10^{-4}$**	36.082	63.918
$1x10^{-3}$	**$1x10^{-3}$**	32.646	67.354
$2x10^{-3}$	**$2x10^{-3}$**	29.553	70.447
$3x10^{-3}$	**$3x10^{-3}$**	22.337	77.663
$4x10^{-3}$	**$4x10^{-3}$**	15.464	84.536
$5x10^{-3}$	**$5x10^{-3}$**	9.622	90.378
Compound (10)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**Activity Recovered[%]**
Nil	6.750	0	100
0	1.425	78.889	21.111
$5x10^{-4}$	4.550	32.593	67.407
$1x10^{-3}$	4.825	28.519	71.481
$2x10^{-3}$	5.100	24.444	75.556
$3x10^{-3}$	5.300	21.481	78.519
$4x10^{-3}$	5.925	12.222	87.778
$5x10^{-3}$	6.150	8.889	91.111

Table (4) Effect of different concentrations of *Nioxime* on the activity of inhibited *AChE* by compounds (11) to (15).

Compound (11)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**Activity Recovered[%]**
Nil	6.525	0	100
0	1.400	78.544	21.456
$5x10^{-4}$	3.350	48.659	51.341
$1x10^{-3}$	3.575	45.211	54.789
$2x10^{-3}$	4.175	36.015	63.985
$3x10^{-3}$	4.400	32.567	67.433
$4x10^{-3}$	4.725	27.586	72.414
$5x10^{-3}$	5.200	20.307	79.693
Compound **(12)**			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	7.000	0	100
0	2.700	61.429	38.571
$5x10^{-4}$	3.750	46.429	53.571
$1x10^{-3}$	4.025	42.500	57.500
$2x10^{-3}$	4.325	38.214	61.786
$3x10^{-3}$	4.900	30.000	70.000
$4x10^{-3}$	5.050	27.857	72.143
$5x10^{-3}$	5.300	24.286	75.714
Compound (13)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	6.350	0	100
0	2.375	62.598	37.402
$5x10^{-4}$	4.050	36.220	63.780
$1x10^{-3}$	4.400	30.709	69.291
$2x10^{-3}$	4.675	26.378	73.622
$3x10^{-3}$	4.825	24.016	75.984
$4x10^{-3}$	5.250	17.323	82.677
$5x10^{-3}$	5.450	14.173	85.827
Compound (14)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	6.050	0	100
0	0.900	85.124	14.786
$5x10^{-4}$	2.775	18.595	45.868
$1x10^{-3}$	3.000	25.620	49.587
$2x10^{-3}$	3.650	34.298	60.331
$3x10^{-3}$	3.975	39.669	65.702
$4x10^{-3}$	4.500	50.413	74.380
$5x10^{-3}$	4.925	54.132	81.405
Compound (15)			
Oxime Cone.[M]	**Enzyme activity**	**Inhibition[%]**	**ActivityRecovered[%]**
Nil	7.425	0	100
0	1.325	82.155	17.845
$5x10^{-4}$	5.125	30.976	69.024
$1x10^{-3}$	5.500	25.926	74.074
$2x10^{-3}$	5.825	21.549	78.451
$3x10^{-3}$	6.025	18.855	81.145
$4x10^{-3}$	6.225	16.162	83.838
$5x10^{-3}$	6.450	13.131	86.869

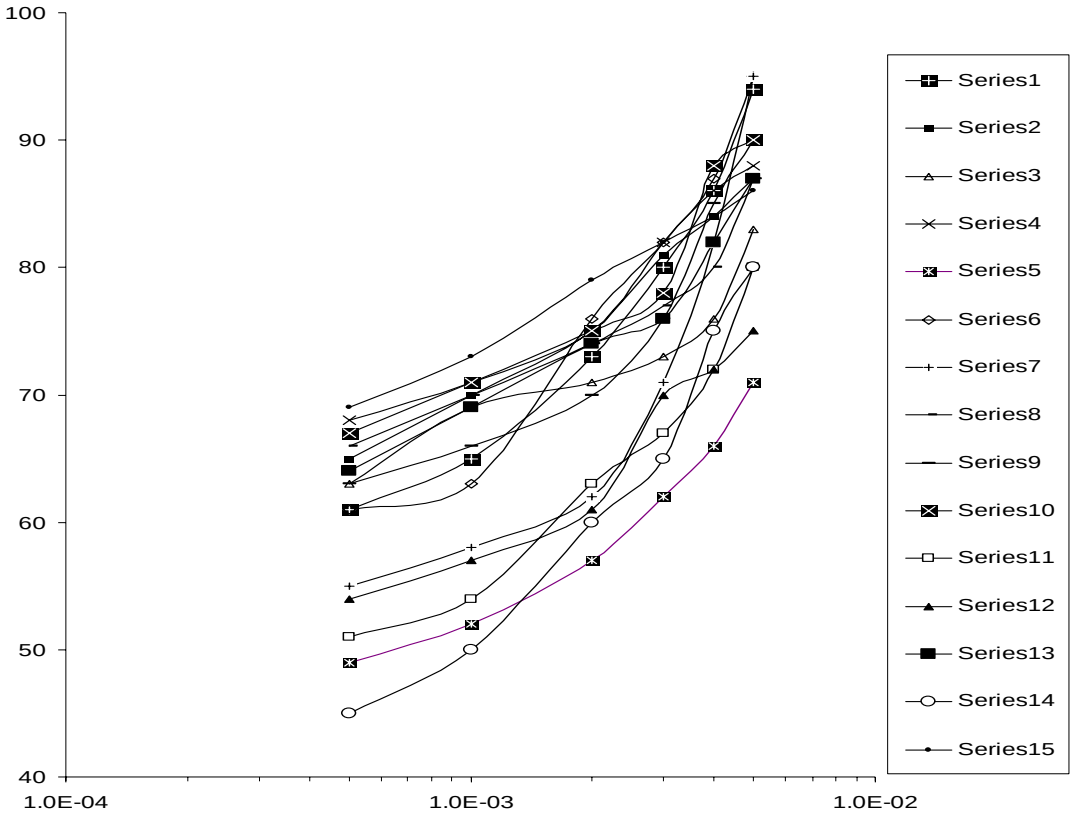


Fig.1 -: Effect of different concentrations of Nioxim on the activity of inhibited AChE by compounds (1-15)

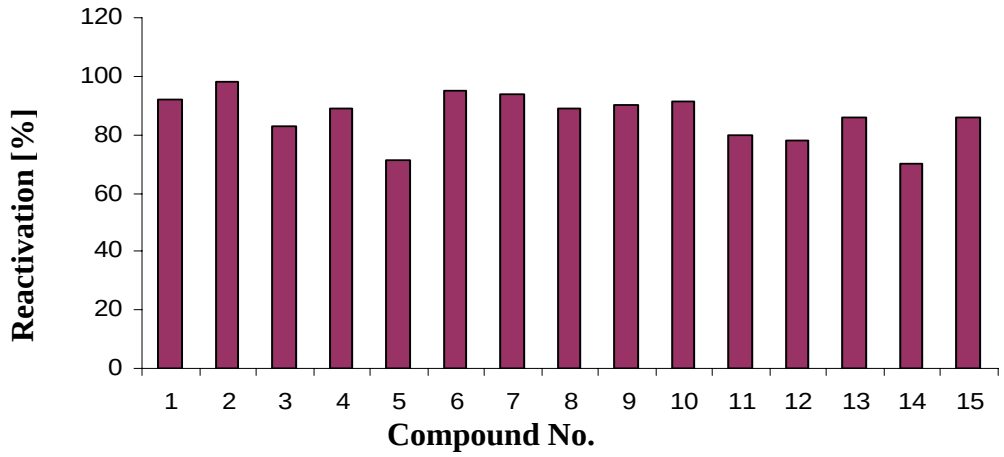
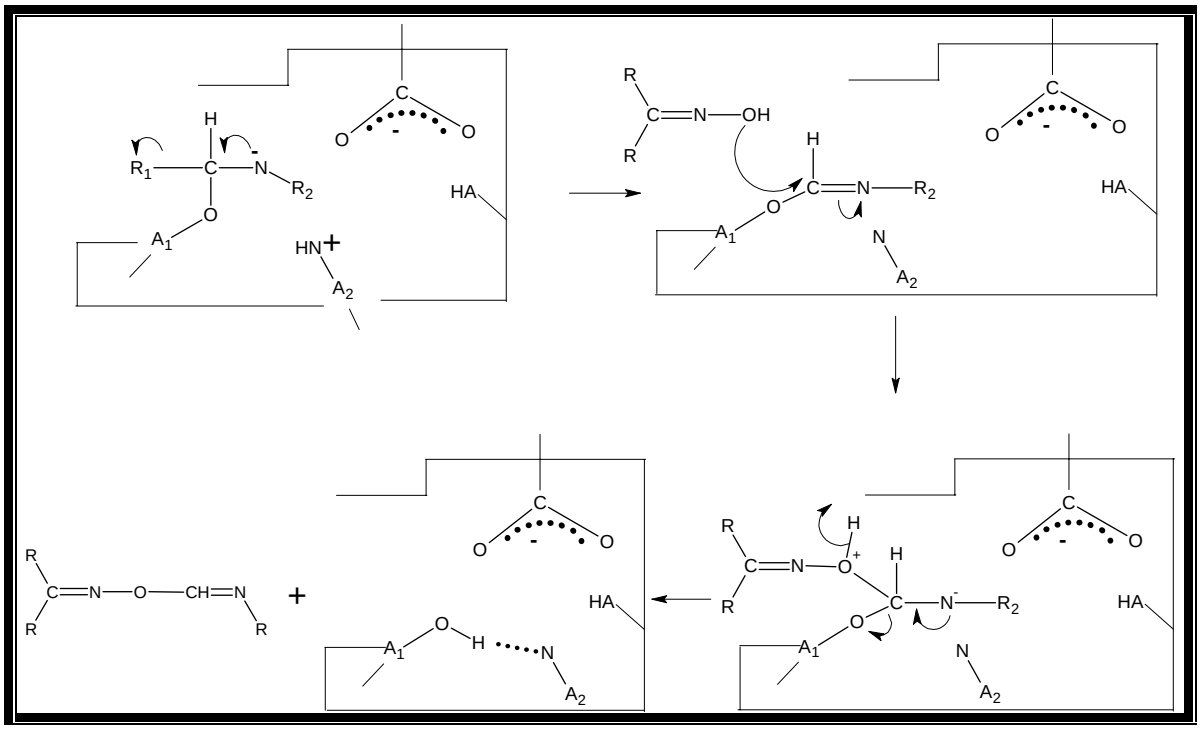
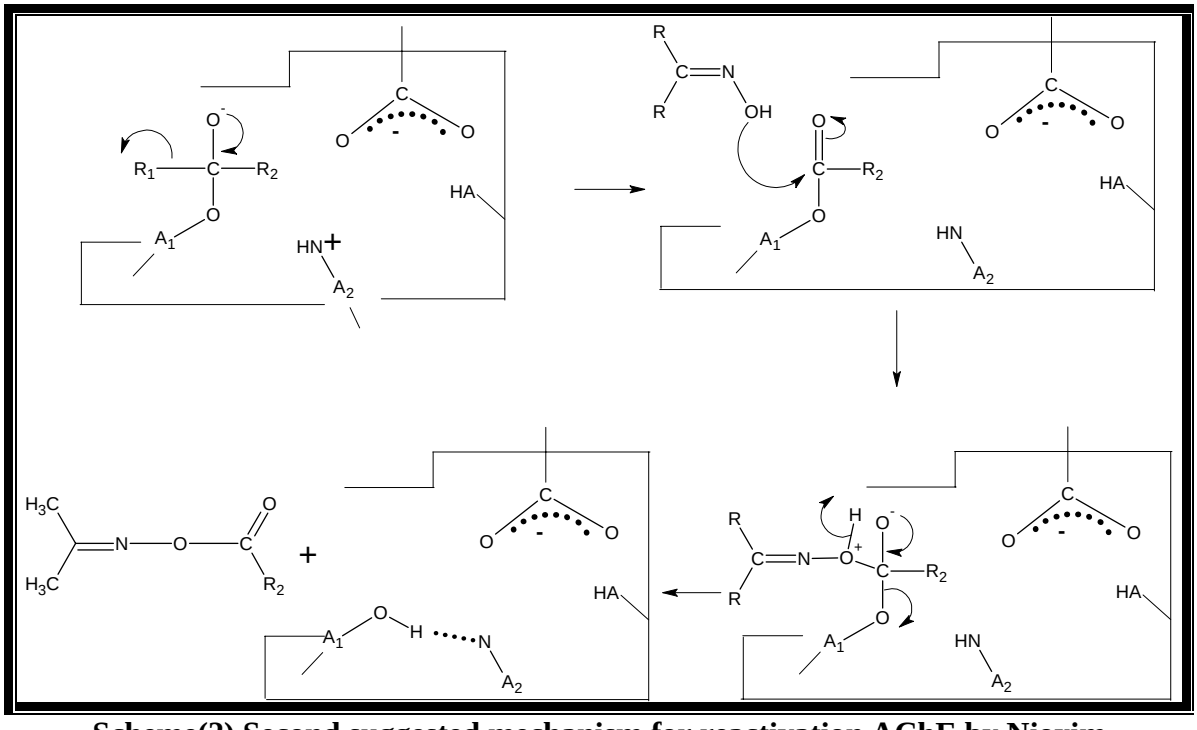


Fig. 2-: Diagram shows the effect of Nioxim on the activity of inhibited AChE by compounds (1-15)



Scheme(1) First suggested mechanism for reactivation AChE by by Nioxim
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn



Scheme(2) Second suggested mechanism for reactivation AChE by Nioxim
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn

References

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