Effect of Vitamin B_{12} on the Activity of Inhibited AChE

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Abstract

Human serum AChE activity is measured in vitro using modified Ellman method and shows a wide range between (4.925-8.755) µmole/3min/ml. The activity in men was higher than in women. The possible medical importance of Vitamin B_{12} as a remover of the effects of poisonous and inhibiting compounds of AChE as possible substitute of Atropine was tested, the results showed that Vitamin B_{12} behaved as a reactivator, and the values of reactivation of both of Vitamin B12 and Atropine on the activity of inhibited AChE by heterocyclic compounds sometimes appear approximately equal, these results lead to the conclusion that, it is possible to use Vitamin B_{12} instead of Atropine in treatment of poisoning caused by these types of inhibitors. Mechanism of action of Vitamin B_{12} as reactivator was proposed.

الخلاصة

تم في هذا البحث قياس فعالية انزيم الاسيتيل كولين استريز في مصل دم الانسان باستخدام طريقة المان المحورة و قد تروحت الفعالية بين 4.925- 8.755 مايكرومول/3 دقائق/مل.

و ظهر ان الفعالية عند الرجال اعلى مما عند النساء. كذلك تم دراسة تأثير فيتامين ب 12 على فعالية الانزيم المثبط بواسطة بعض المركبات الحلقية غير المتجانسة حيث اظهرت النتائج ان الفيتامين يسلك كمعيد تنشيط لفعالية الانزيم المثبط ولقد وجد ان تاثير فيتامين ب 12 و بالمقارنة مع الاتروبين كمعيدات تنشيط لفعالية الانزيم المثبط يعطي قيم متساوية تقريبا لذا يمكن الاستنتاج انه بالامكان استخدام فيتامين ب 12 بدلا من الاتروبين في معالجة التسمم الحاصل بسبب هذا النوع من المثبطات. اخيرا تم في هذه الدراسة اقتراح الية عمل لفيتامين ب12 كمعيد تنشيط للانزيم المثبط.

Introduction

Cholinesterases from different species were found to differ in their substrate specificity and susceptibility to inhibitors. They are now considered to constitute a family of enzymes which fall broadly into two types depending on their substrate preference [1].

This division is not absolute and holds true more in mammalian than non- mammalian species.

Those, enzyme which preferentially hydrolyse acetyl esters such as ACh are called acetylcholinesterase (AChE) or acetylcholine acetyl hydrolase (EC 3.1.1.7), and those which prefer other types of esters such as butyryl choline are termed butyrylcholinesterase (BChE) or acyi choline acyl hydrolase (EC 3.1.1.8).

BChE is also known as pseudo cholinesterase, non- specific cholinesterase. The main function of AChE is the rapid hydrolysis of neurotransmitter ACh at cholinergic synapses [2].

The hydrolysis reaction proceeds by nucleophilic attack of the carbonyl carbon, acylating the enzyme and liberating choline.

This is followed by a rapid hydrolysis of the acylated enzyme yielding acetic acid, and restoration of the esteratic site [3].

AChE is widely distributed enzyme found in neural and non- nural tissues [4, 5]. It catalyzes the hydrolysis of acetyl choline and certain other choline; thiocholine esters and many non- choline esters [9, 7].

AChE has been identified . in different tissues of variety of vertebrates [8-11] and invertebrates [12,13]. AChE included in the cholinergic transmission is found highly concentrate in each cholinergic fibers [14-17], neuromuscular junctions [18-20], electric organ of electrophorus electrics [21] and Torpedo marmorata [22]. Also it has been detected in a non- neuronal cells like erythrocyte [23-25]. Stomach and intestine [26, 27]; certain snake venous [28] in dopamine containing cell [7] and blood serum [29-34].

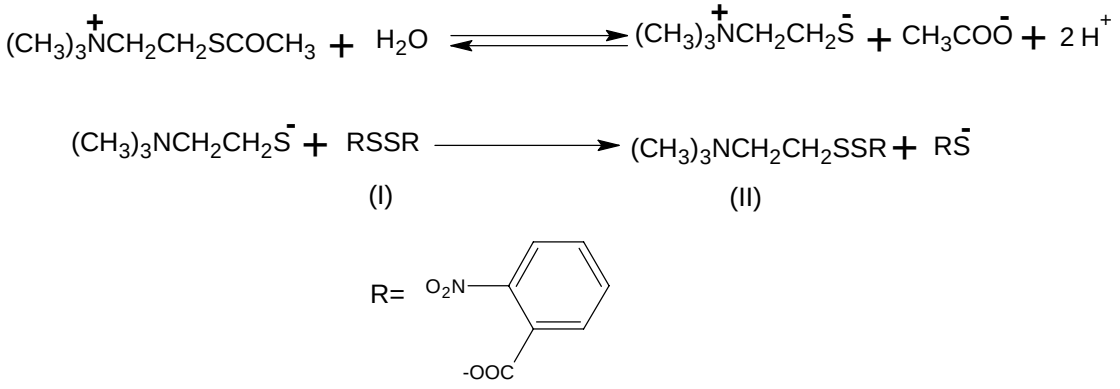
Certain analyses have suggested that the enzyme molecule is a dimer, each protomer contains two non- identical chain, the a- chain, which contains the active site, as the function of (3- chain remains unknown for the time being probably acting as a receptor protomer [35].

The active site of AChE was considered to consist of two subsities; a negatively charged or "anionic" site, to which the positively charged quaternary nitrogen moiety binds, and an esteratic site containing the actual catalytic residues, probably both an electophilic and a nucleophilic group [36].

Experimental

Acetylcholinesterase activity was assayed by Ellman method [37]. The principle of the method is the measurement of the rate of production of thiocholine as acerylthiocholine hydrolyzed.

This was accomplished by the continuous reaction of the thiol with 5,5- Dithio bis-nitrobenzoic acid (I) to produce the yellow color of 5- thio-2-nitrobenzoic acid (II). The rate of color production is measured at 430 nrn in a photometer.



Vitamin B_{12} and Atropine were selected to test their reactivation effects on the activity of inhibited AChE by heterocyclic compounds. The solubility of these compounds was tested in sodium phosphate buffer (0.2 M, pH= 7.3) which was used in enzyme activity determination.

1. AChE activity was measured in human serum using the modified Ellman method as follows:

(50 µl) of DTNB solution (0.001M) was added to 2.25 ml of phosphate buffer solution (pH= 7.3, 0.2M), then (l0 µl) of serum was added, mixed well and (2 ml) of the mixture was transferred to a measuring cell (3 mm), then (34 µl) of ASChI was added, the change in absorbency was measured before and after adding the substrate at (430 nm) for (3 min). The enzyme activity was calculated as the concentration in µmole of the substrate hydrolyzed to each (ml) of sample in (3 minute) and expressed as (µmole/ 3 min/ml).

2. (a) A stock solution (0.6 mg/ml) concentration of Vitamin B_{12} was prepared and then the following concentrations (0.6, 1.4, 2.7, 4, 5.4, 6.8) µgm/ ml were prepared.

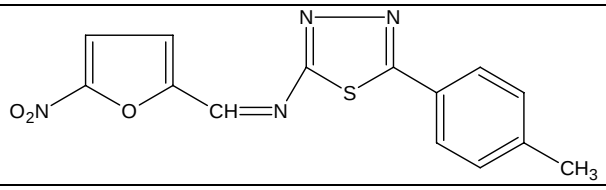
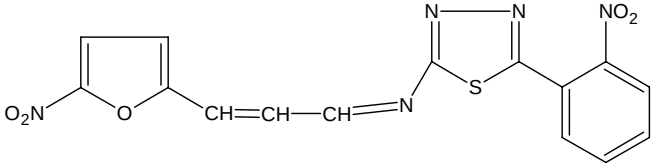
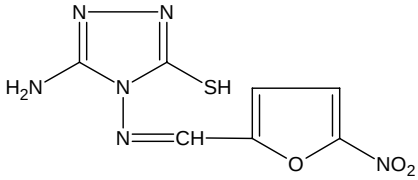
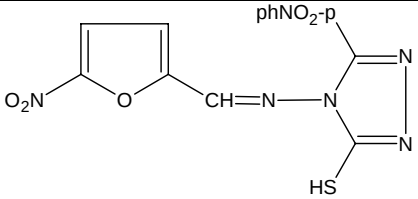
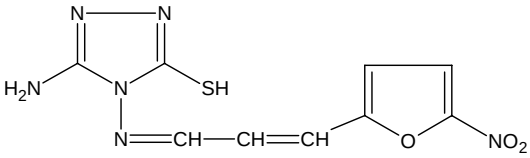
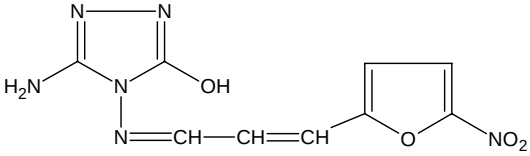
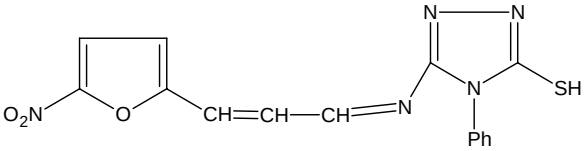
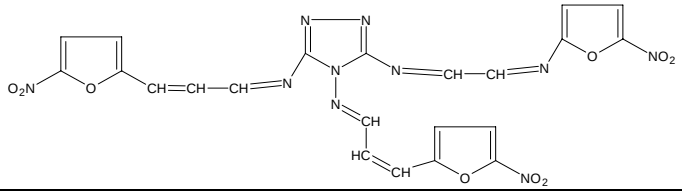
(b) A stock solution ($0.6X10^{-1}$ mg/ml) concentration of Atropine was prepared and then the following concentrations (0.6, 1.4, 2.7, 4, 5.4. 6.8) µgm/ ml were prepared.

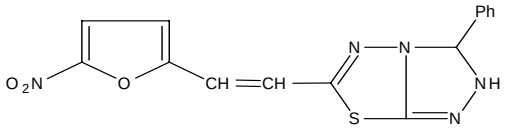
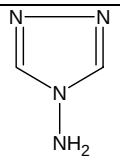
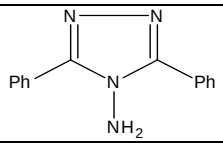
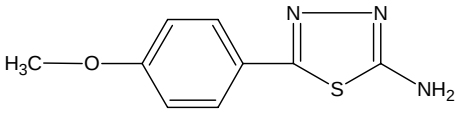
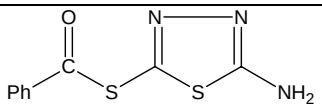
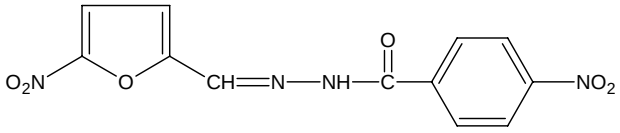
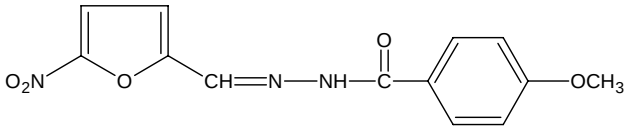
($1x\ 10^{-4}$ M) concentration of all inhibitors was selected to determine the enzyme activity with and without using the inhibitor and under the same conditions by following the method described in the section (1).

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 section (1) 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)methylene 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

Vitamin B_{12} contains multifunctional groups (nucleophilic centres); its effect as reactivator was examined and compared with the effect of Atropine on the activity of inhibited AChE by heterocyclic compounds. Different concentrations of vitamin B_{12} and Atropine (0.6- 6.8) µg/ ml, with fixed concentration of inhibitors ($5x\ 10^{-4}M$) were used. The two compounds shows an increase in percentage of reactivation with increasing concentration.

The behavior of AChE enzyme in the presence of inhibitors and reactivators are shown in fig (1 and 2). The effect of vitamin $B_{]2}$ as reactivator on the activity of inhibited AChE by some inhibitors are approximately equal to Atropine fig (3).

From the results obtained and in the absence of any side effects of vitamin B_{12}, it can recommend to use vitamin B_{12} instead of atropine in the treatment of systemic poisoning caused by heterocyclic compounds.

In general Atropine, as antimuscarinic agents have only a moderate effect on the action of acetyl choline at nicotinic receptor sites, thus at autonomic ganglia, where transmission primarily involves an action of acetylcholine on nicotinic receptors, atropine produces only partial block, so when it is used as premeditation for anesthesia, atropine decreases bronchial and

salivary secretion and there is usually an increase in heart rate and some times a tachycardia as well as inhibition of secretions (causing dry mouth) and relaxation of smooth muscle in t muscle in the gut, urinary tract and biliary tree [39].

Since atropine crosses the blood brain barrier CNS effects in the elderly mav include amnesia, confusion and excitation [40].

It is interesting to note that Vitamin B_{12} in the present study having multi- nucleophilic groups in its structure, therefore, several possible proposed mechanisms of action of Vitamin B_{12} as reactivator is shown in schemes (1-4).

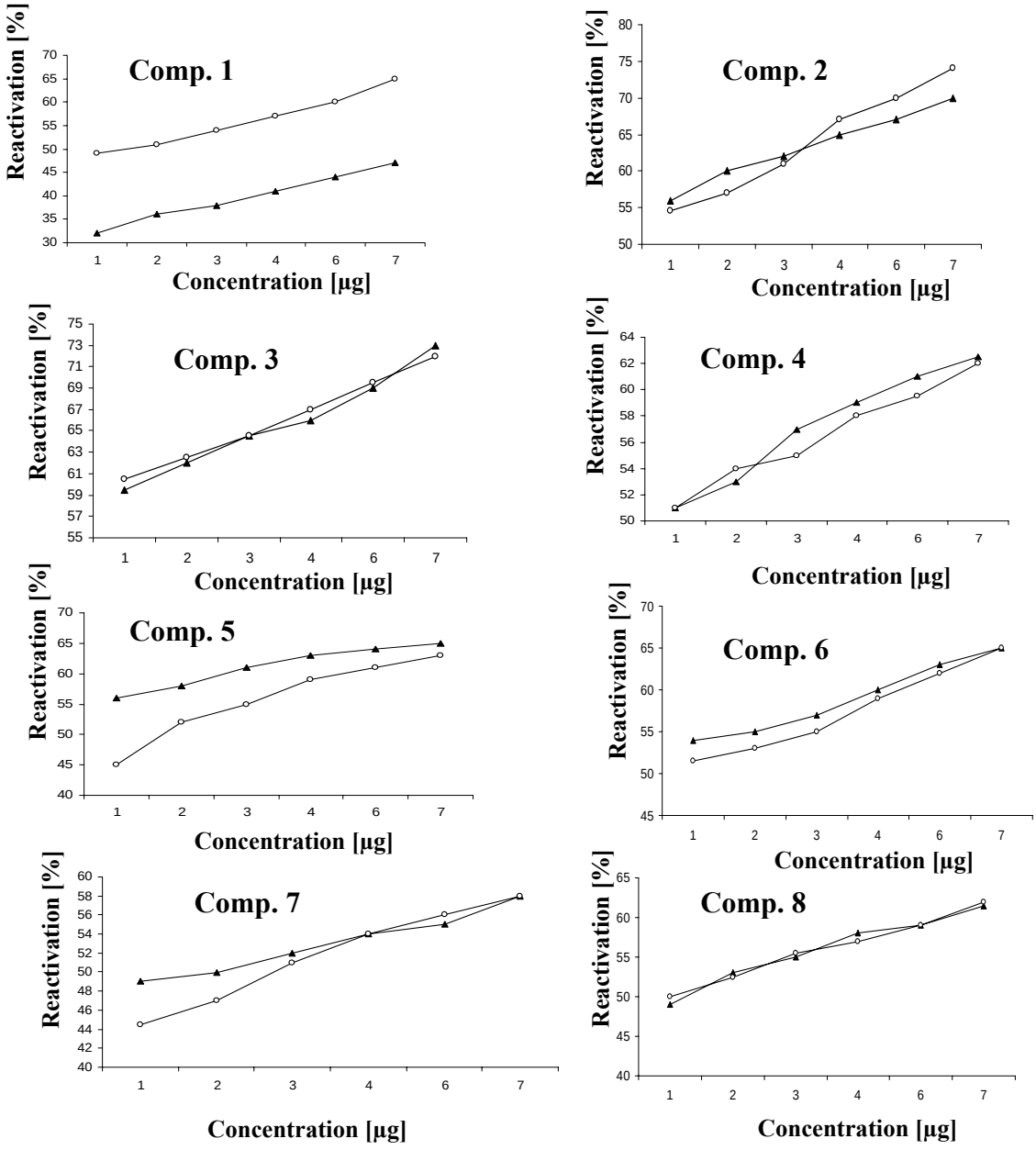


Fig. 1-: Effect of different concentrations of (▲) B_{12} and (○) Atropine one the activity of inhibited AChE by compounds 1 to 8

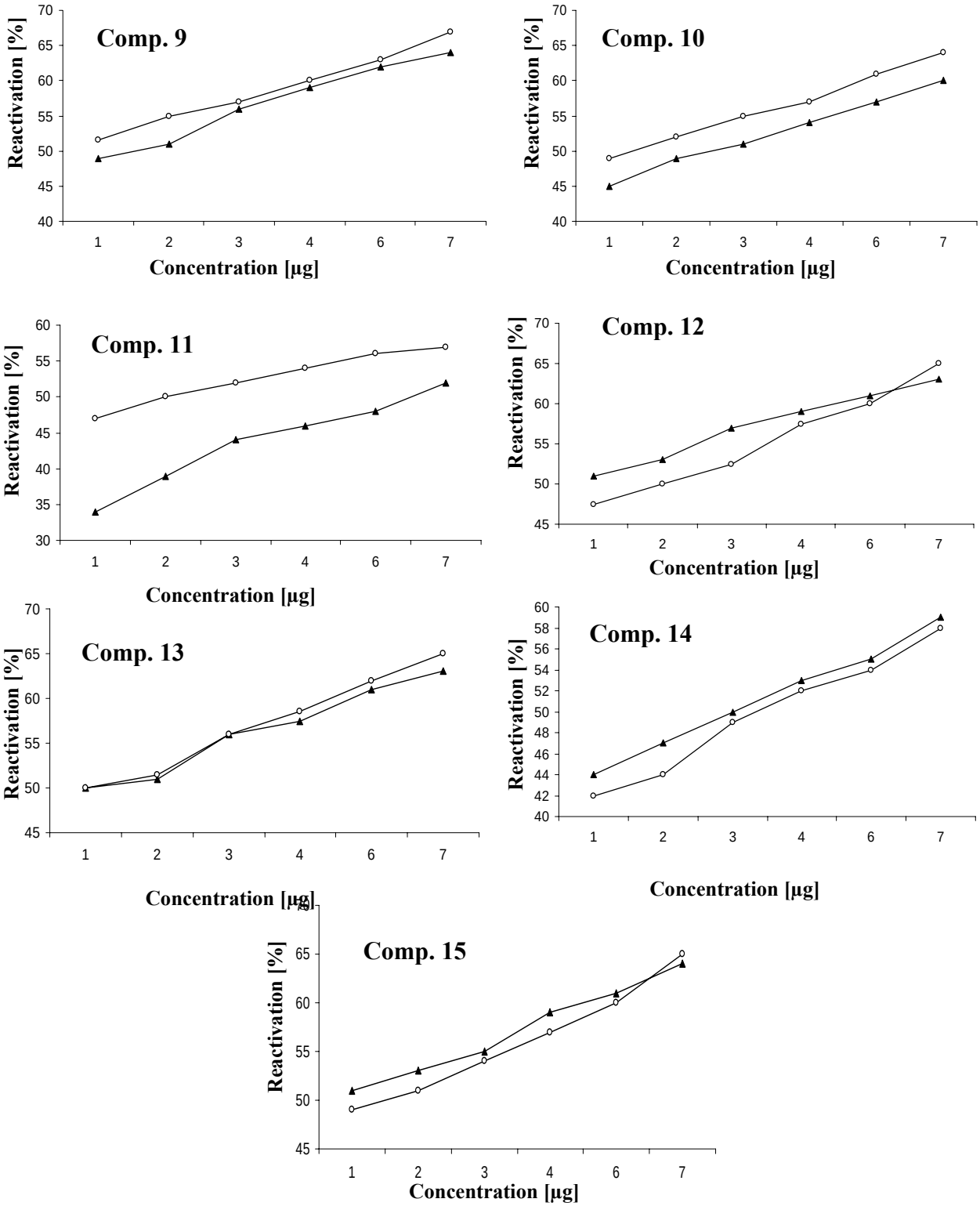


Fig. 2-: Effect of different concentrations of (▲) B_{12} and (○) Atropine one the activity of inhibited AChE by compounds 9 to 15

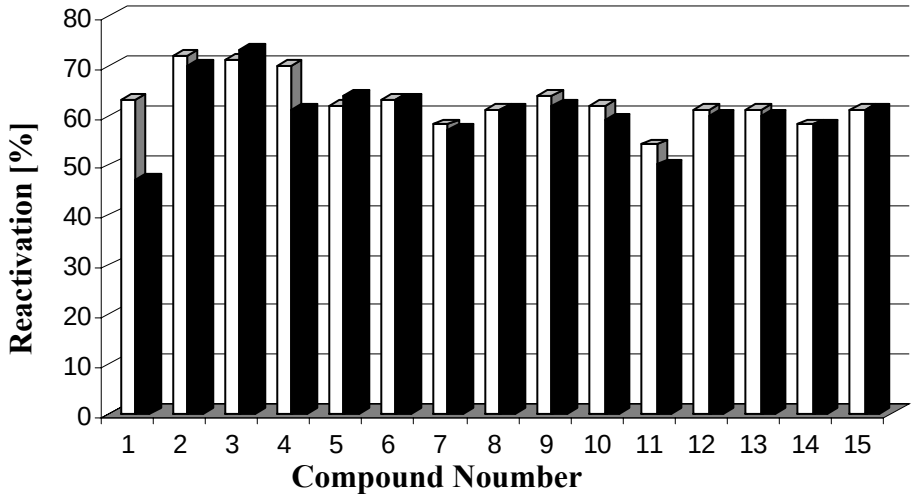
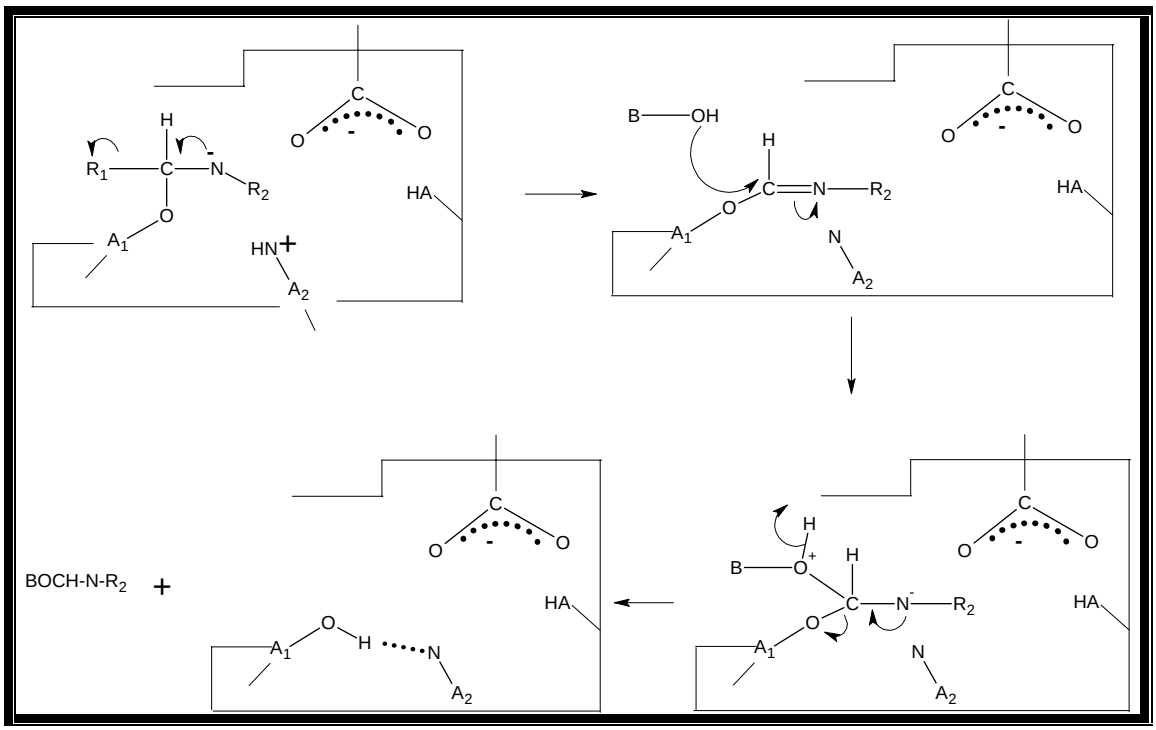
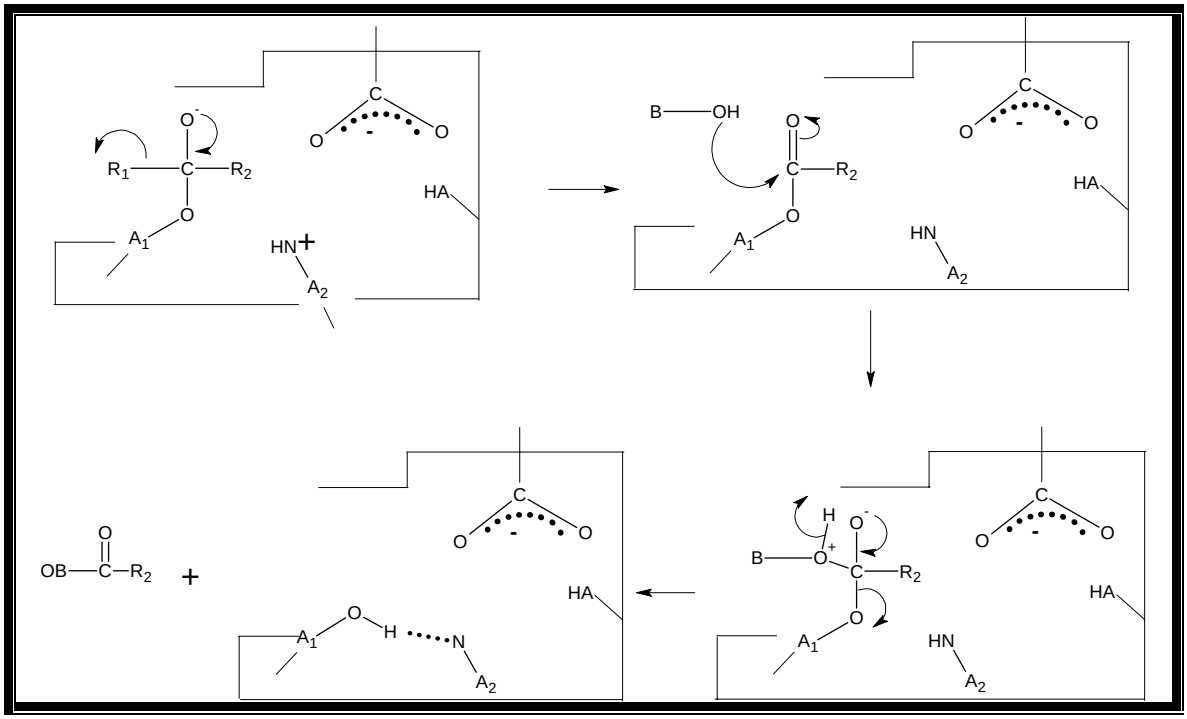


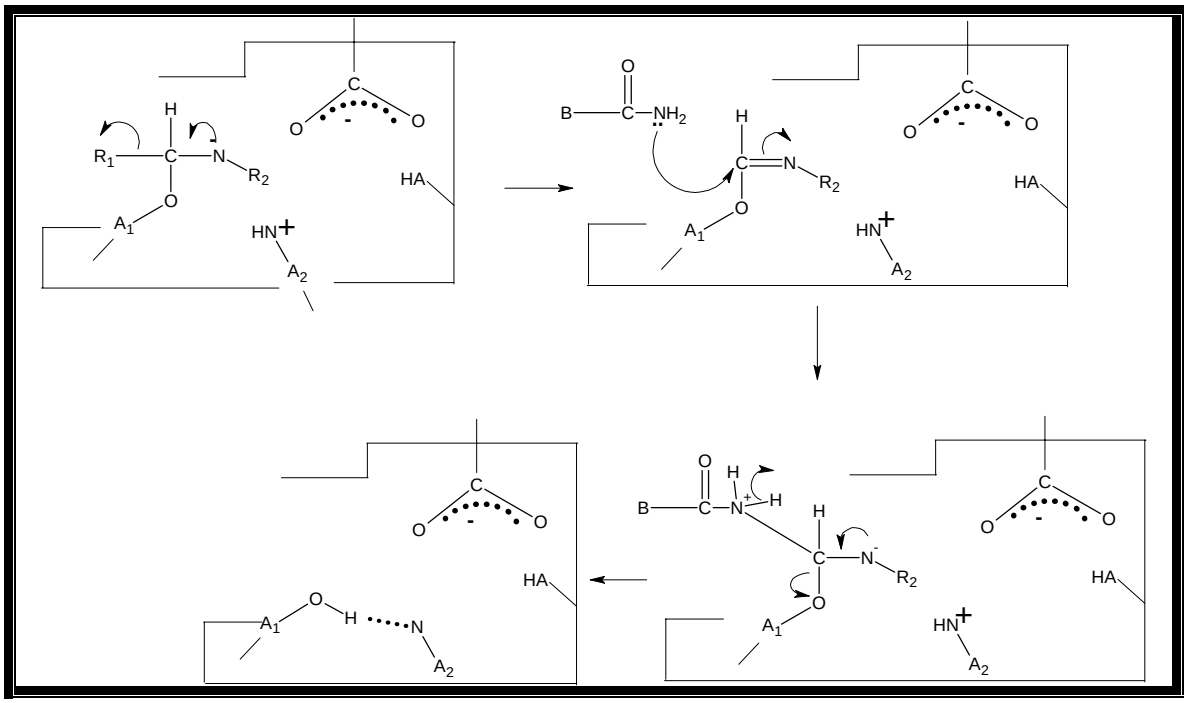
Fig. 3:- Diagram shows the effect of (6.8 µg/ml) of Vitamine B_{12} and Atropine on the activities of inhibited AChE by compounds (1-15).



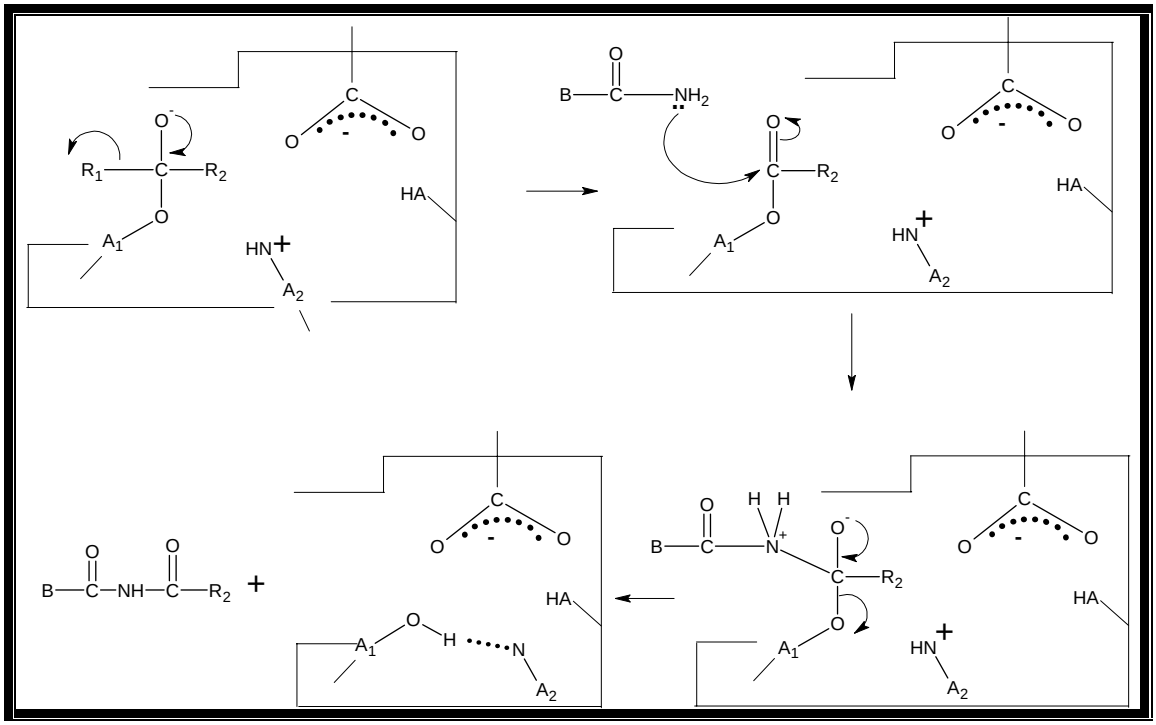
Scheme(1) First suggested mechanism for reactivation AChE by by vitamine B_{12}
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn



Scheme(2) Second suggested mechanism for reactivation AChE by by vitamine B_{12}
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn



Scheme(3) Third suggested mechanism for reactivation AChE by by vitamine B_{12}
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn



Scheme(4) Forth suggested mechanism for reactivation AChE by by vitamine B_{12}
A_1= Ser, Glu, Asp, and Tyr A_2= His, Lys, Arg, Gln, and Asn

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