Sensitivity of Serum Acetylcholine Esterase Toward Derivatives of Oxadiazole

Dr. **Fatin F. Al-Kazzaz*, Dr. Rehab A. M. Al-Hasani* & Zyzaffon N.***

Received on: 8 /9/2010
Accepted on: 3/2/2011

Abstract

This work addresses the kinetic analysis of the interaction of some oxadiazoles (L_1 , L_2, L_3 & L_4) in ethanol with serum acetylcholinesterase. It was found that ethanol have inhibitory effect (25.18%) on AChE enzyme for this reason niglicable it as solvent and instead of it use dimethyl sulfoxide which had no effect. The % inhibition of L_1 , L_2, L_3 & L_4 at 10^{-7} M was 45.42,71.51,54.67&74.27 respectively and it elevated with increasing the concentration till at 10^{-1} M it reached 53.62,99.08,56.22&99.43 respectively. The effect of both L_2 & L_4 was reversible in nature. Michaelis – Menten constant and maximum velocity for the hydrolysis of acetyl thiocholine iodide by AChE were determined in control and treated systems. Line weaver – Burk plot and their secondary replots indicated that the nature of inhibition in both compounds was noncompetitive inhibition. The value of Ki was estimated also. The mechanism of action of these types of compounds acting as inhibitors to the AChE is suggested.

حساسية انزيم استايل كولين استريز تجاه بعض المشتقات الجديدة للاوكسادايزول

الخلاصة

يهدف هذا العمل الى دراسة حركية التاثر مابين بعض المركبات الحلقية الغير متجانسة الجديدة لمشتقات الاميدازول (L_1 , L_2, L_3& L_4) و الايثانول وبين انزيم الاستيل كولين استريز في المصل (AChE) . لقد وجد ان للايثانول تاثير تثبيط (25.18%) على فعالية الانزيم لذلك استخدم DMSO كمذيب بدلاً عنه و بلغت % للتثبيط للمركبات L_1 & L_2 , L_3 , L_4 عند تركيز 10^{-7} مولاري 74.27,54.67,71.51,45.42 على التوالي وازداد التثبيط بزيادة التركيز حيث عند 10^{-1} مولاري بلغ 99.43,56.22,99.08,53.62 على التوالي . اظهر المركبين L_6, L_2 تأثير تثبيط معكوس. تم قياس وحساب ثابت ميكائيلس – منتن (Km) والسرعة العظمى V_{max} لتحلل الاستيل ثايوكولين ايوديد بأنزيم (AChE) في مصل لاشخاص طبيعيين بوجود وغياب المركبات اعلاه. اشيرت نتائج رسومات لينوويفر – برك ورسوماتهم الثانوية ان طبيعة التثبيط لكلا المركبين هي غير تنافسية وعليه تم حساب ثابت التثبيط Ki .لقد تم اقتراح ميكانيكية للتأثير التثبيطي لهذا النوع من المركبات على الانزيم AChE.

***College of Science, University of Al – Mustansiryah/ Baghdad**

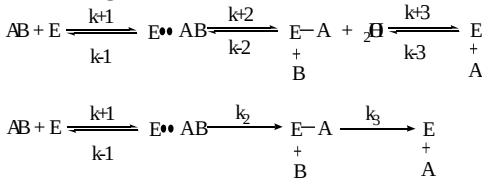
https://doi.org/10.30684/ etj.29.4.2
University of Technology-Iraq, Baghdad, Iraq/2412-0758

Introduction

In the central nerves system (CNS), acetylcholinesterase (AChE) fulfills a vital role at cholinergic synapses by rapidly hydrolyzing a neurotrans mitter acetylcholine (ACh)(1). This enzyme is known to rapidly eliminate ACh after its release at cholinergic synapse, thus allowing precise temporal control of muscle contraction(1). There are two types of the present enzyme acetylcholinesterase (E.C.3.1.1.7) (AChE), also known as RBCs cholinesterase, or (most formally) acetylcholine acetyl hydrolase , found primary in the blood and neural synapses(2) .

Pesudocholinestrase (E.C.3.1.1.8) (BChE or BuChE), also known as plasma cholinestrase, butyryl cholinestrase, acylcholine acylhydrolase, found primarily in the liver[3] .

The difference between the two types of cholinesterase has to do with their respective preferences for substrate : the former hydrolyses acetylcholine more quickly ; the latter hydrolyses butyrylcholine more quickly[3]. Both two enzymes are thought to hydrolyze substrate such as acetyl choline and butyrylcholine by a Ping-Pong Bi-Bi kinetic mechanism that can be viewed as an ordered uni-Bi reaction since water, the second substrate is present in excess as shown in the following scheme.



Scheme (1): kinetic mechanisms for interaction of a substrate (AB) with acetylcholinestrase (E)

The scheme represents a Ping-Pong Bi-Bi kinetic mechanisms. Reactivation of E occurs as a result of addition of water as a second substrate. The center panel represents a simplification of the Ping-Pong Bi-Bi mechanisms to an ordered Uni-Bi reaction since water is always present in excess[4] .

A cholinesterase inhibitor (or anticholinestrase) suppresses the action of the enzyme because of essential function chemicals that interfere with the action of AChE are potent neurotoxins, causing excessive salivation and eye – watering in low doses, followed by muscle spasms and ultimately death (examples are snake venoms and the nerve gases sarin (isopropyl methyl fluoro phosphorate) and ethyl S-diisopropyl amino ethyl methylthiophospharate[5]. One counteracting medication is pralidoxime[5]. Among the most common AChE inhibitors are phosphorus-based compound, which are designed to bind to the active site of enzyme[6].

Outside of biochemical warfare, anti ChE are also used for reversing medication induced paralysis during anesthesia, as well as in the treatment of myasthenia gravis, glaucoma, and Alzhymer's disease[7] . Such compounds are used for killing insects in a range of products including sheep dip, organophosphate pesticides and carbamate pesticides[8]. In addition to acute poisoning, a semi acute poisoning characterized by strong mental disturbances can occur. Also, prolonged exposure can cause birth defects[9].

Oxadiazoles are becoming at great interest their wide range applications.The oxadiazole ring associated with antifungal hypoglycemic, analgesic, herbicidal and antimycobacterial properties[10]. The -SH group attached to a heterocyclic nucleus may induce fungicidal activity[11]. Compounds containing –N=C-S linkage are reported as antiirradiation agents, anthelmintics, fungicides and pesticides[12]. The oxoanalogs of 5-substituted -1,3,4-oxadiazoles are reported to exert adverse effect against several pathogenic fungi[13]. Kubo et al[14] investigated the herbicidal activity of a large number of 1,3,4-oxadiazoles and conclude that the presences a halophenoxymethyl group at 5-position influences the activity of the compound and oxadiazole ring may has no special effect. Caldwell and Burkhalter [15] have reported that 3-substituted amino - methyl-5-substituted -1,3,4-oxadiazole-2-thiones are tuberculostatic and fungicidal. Also reported in the literature that some Mannich bases of 5-substituted phenyl-1,3,4-oxadiazole-2-thion possess central nervous system depressant activity[16,17,18,19]. Keeping this in view the title compounds have been prepared.

The present work describes the preparation of new Mannich bases derived from 2-thion-1,3,4-oxadiazole and different aliphatic amines, in an attempt to introduce the methyl amino moiety in the structure of mercapto oxadiazole ring which is known to possess a therapeutical applications. The kinetics of ChE with inhibitors occur in two stages as indicated in scheme (2) which involves the formation of a reversible enzyme – inhibitor complex (EH.IX) followed by formation of the enzyme inhibitor bond with displacement of leaving group[20].

$$EH + IX \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} EH\bullet IX \xrightarrow{k_2} EI + HX$$

Where EH the enzyme and IX the inhibitor

Scheme(2) : The general mechanism of ChE's inhibition

Materials and Methods

1- Materials

All reagents were of analytical grade. Acetyl thiocholine (used as substrate, ASCh), 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) were purchased from the Sigma chemical Co., St. Louis, MO, U.S.A.

The instruments used are:

-Spectrophotometer double beam(Shimadzu UV-210A,Japan)

-P^H meter model 1470 Universal digital

-Incubator model 854- Schuabach Memmert.

-Centrifuge Janetzk T30, Germany

-Blance sartorius, Germany

-Melting points were recorded on a Gallenkamp MF B600 010F melting point apparatus.

-Elemental analyses (C.H.N.S) were obtained using EA-034 .mth.

-Infrared spectra were recorded using FT-IR-8300 Shimadzu in the range of (4000-250) cm^{-1}.

The title compound (L) (5-phenyl-1,3,4-oxadiazole-2-thione) was prepared through the reaction of benzoic hydrazide with carbon disulfide in the presence of potassium hydroxide in 95% ethanol[21]. The structure of this compound was confirmed on the basis of its melting point and FT-IR spectra, Table (1).

The Mannich bases (L_1, L_2, L_3 and L_4) were prepared as described in our previous work[21,22,23] . A mixture of ethanolic solution of (L) (5-phenyl-1,3,4-oxadiazole-2-thione) (0.01 mole) and formaldehyde (1.5 ml, 35%) was treated with ethanolic solution of suitable amine (dibutyl, dipropyl, dicyclopentyl and dicyclohexyl) (0.01 mole) with stirring by slow addition. The solution was stirred for an hour and left over night in a refrigerator. The precipitate was filtered, washed with cold ethanol and dried. The various 3-substituted amino methyl-5-phenyl-1,3,4-oxadiazole-2-thione are reported in table (1) and characterized by elemental analysis and FT-IR spectra.

Blood sampling

Five ml of blood was drawned from the same subject by vein directly after the clotting, in centrifuge at 3000rpm for 10 min, the serum sample was separated and used immediately as a source of enzyme.

2- Method

a- Determination of acetylcholine esterase activity

Cholinesterase activity was measured in human serum using the modified Ellman method[24] as follows :

(50 µl) of DTNB solution (0.001 M) is added to (2.25 ml) of sodium phosphate buffer solution (pH = 7.3, 0.2 M) , then (10 µl) of serum is added, mixed well and (2 ml) of the mixture is transferred to a measuring cell (3 mm), then (34 µl) of acetyl thiocholine iodide (ASChI 0.06 M) is added, then change in absorbency is measured after adding the substrate at 430 nm for 3 min. the enzyme activity is calculated as the concentration in µ mole of the substrate hydrolyzed to each (ml) of sample in (3minute) and expressed as (µ mole / 3min. / ml) .

b- Determination the biological activity of oxadiazole derivatives

A stock solution (0.05 M) concentration of each compound in table (1)

is prepared and then different concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}) M of each compounds are prepared by diluting it with dimethyl sulfoxide (DMSO) as solvent. ChE activity is measured in human serum as described in previous section (a) in presence 0.25 ml of each compound dissolved in 2 ml of the same buffer.

The inhibition percentage is calculated by comparing the activity with and without the inhibitor and under the same conditions according to the equation :

$$\% \text{ Inhibition} = 100 - \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}} * 100$$

c- Determination the type of inhibition

A constant (10^{-1}, 10^{-5}M) of inhibitor is used with different concentrations of substrate (0.02, 0.04, 0.06, 0.08 M) to study the type of inhibition. These different concentrations are prepared from the stock solution (0.1M) ASChI. The enzyme activity is determined with and without the inhibitor – using the Lineweaver – Burk equation by ploting 1/v vs. 1/[s][25] . The following values were calculated

1) Ki , 2) Apparent ν_{max} (ν_{mapp}) , 3) Apparent K_m (K_{mapp}), 4) Type of inhibition .

Results and discussion

The activity of human AChE in the absence and presence of oxadiazole derivatives under different substrate

concentrations was determined. The present work is designed to investigate the biological activity and effects of a series of compounds in table (1). First experiment tried to study the effect of solvent (Ethanol & DMSO) then examine the compound (L) (oxadiazole – 2 – thione) in the mixture at different concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4},10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M) it abvious (L) had inhibitor action, so the determine the present of inhibition is calculated. The following findings are observed in table (2).

The results suggested that ethanol acts as inhibitor of enzyme which is agreed with Baker[26] and showed a non understandable data with the compound (L), for this reason it regarding to use dimethyl sulfoxide (DMSO) as solvent because it did not show any inhibitory effect as found and as Jaffer et al found too[27].

From figure (1) The Michaelis – Menten constant Km and maximum velocity (V_{max}) for the hydrolysis of acetylcholine iodide (ASCh) by AChE were calculated as shown in table (3).

The affinity of substrate to the enzyme (AChE) in the absence of inhibitor was higher than in presence of each inhibitors for both concentration 10^{-1}, 10^{-5} except in (10^{-1} M L_4) shows same affinity to enzyme. The biochemical tests revealed that all compounds cause good inhibitory effects on enzyme activity, Table (4). The normal value of the enzyme activity ranges between (1.5-2.7) µmol/3min/ml .

The relationships between compounds concentration versus the activity of enzyme are shown in figures (2).

From figures it notice that the change of AChE activity with [I] is very wide in L_2, L_4 but very narrow with L_1, L_3 , this is may be due to long chain of the resubsituted in L_1, L_3 compounds .From these results it is observe that any increase in compounds concentrations causes increases in percentage of enzyme inhibition. The greater inhibition of each compound is demonstrated at concentration (10^{-1} M) as shown in figure (3) .

From this figure it is observed that two substituted with linear chain or cyclic substituted derivatives of imidizol compound exhibits approximately a highly percentage of inhibition (more than 95%) .

The present work study the effect of L_2 and L_4 compounds on the activity of cholinesterase enzyme. In same line there are other studies that refer to the inhibitory effect of some classes of compounds on enzyme activity such as phenindione[20], organophosphatase and carbamates[9], organophosphate paraoxon[8], tacrine[28], 2-pyridinealdoxime methyl halide[29], porphin compounds[30].

It has also been found that some alkaloids such as cathinone, cathine, ephedrine and diethyl phosphoryl cause rise in enzyme activity and are classified as reactivator to the cholinesterase enzyme[8,31,32].

Study type of inhibition

The second part of this study include determine the type of inhibition and kinetic parameters (K_{mapp} , V_{mapp} and Ki) at different concentrations of substrate and under the same conditions by using linweaver Burk equation and one plotted as shown in figures (4) , table (5) .

The results demonstrated that (L_2, L_4) exhibit in the samety of inhibition (non competitive).Unlike the situation of irreversible inhibition, however, the binding is week and the enzyme activity is restored when the inhibitor dissociates from the enzyme inhibitor complex.The hydrolysis of acetylcholine by the acetylcholinesterase involves initial attack of hydroxyl group of the amino acid serine residue to form covalent bond with the carbonyl carbon of acetylcholine and attraction of the acetylcholine cationic heads to the enzyme anionic site, which leads to the formation of acetylcholinesterase-acetylcholine complex (ES) as in figure (4), afterwards the acetyl group is catalytically transferred to a serine residue present at the esteratic site while choline molecule is lost and later the hydrolysis takes place to produce acetic acid and regenerated enzyme[33].

In order to understand the action of L_2, L_4 as inhibitors to cholinesterase enzyme, the following proposed mechanism was studied, that other studies reported inhibition effect of the oxadiazole compounds on the activity enzymes could be explained due to the facts bellow :

a- Molecular interactions between the atoms N,O & S of the oxadiazole moieties with the active site.

b- The oxadiazole compounds containing moieties showed both types of liquid crystals lyotropic & thermotropic[34,35,36] .

Froede et al, suggested theory of non competitive inhibition based on the binding of inhibitor to the acetyl enzyme and the free enzyme was proven correct by demonstrating that inhibition ion increase the steady – state concentration of acetyl enzyme, as predicted by the theory by contrast. The traditional theory that the inhibitor bind to the enzyme – substrate complex and free enzyme predicts that the amount of acetyl enzyme will be drastically reduced when the inhibition is high. A third theory involving all three types of binding remains possible[37] .

The conclusion from present study that aliphatic oxadiazole derivatives showed an inhibitory effect on AChE. The type of this inhibitory is non – competitive, also ethanol had inhibitory effect while DMSO had non effect upon AChE activity

References

[1]Quinn,D.M, Acetylcholineestrase : enzyme structure, reaction dynamics and virtual transition states", Chem. Rev., (1987), 87, 955-979 .

[2]Mnchatterjea & Rana S., "Text book of medical biochemistry",(2005), 6th Ed., 565 .

[3]Hung Y.J., Hung Y. and Baldassarre H., "Recombinanat human butyryl cholinesterase from milk of transgenic animals to protect against organophosphate poisoning", Proc Nati Acad Sci., USA, (2007), 34, 13603-13608 .

[4]Froed H.C. and Wilson I.B., "Direct determination of acetyl-enzyme intermediate in the acetylcholinesterase – catalyzed hydrolysis of acetylcholine and acetylthio choline", J. Biol. Chem., (1984), 259, 11010-11013 .

[5]Holmes J.H., Kanfer I. and Zwarenstein H., "Effect of benzodiazepine derivatives on human blood cholinesterase in vitro", Res. Commun. Chem. Pathol. Pharmacol., (1978), 21 (2), 367-370 .

[6]Gentinetta R. and Brodbeck U., " Acetylcholinesterase is not inhibited

by pyridoxal 5 – phosphate", Biochem. Biophys. Acta , (1986), 10 ; 884 (3), 603-605 .

[7]Masterman D., " Cholinesterase inhibitors in the treatment of Alzheimer's disease and related dementias", Clin. Geriatr. Med., (2004), 20, 59-68 .

[8]Clint R., Ahmed K. and Lester G., "Interactions of rate brain AChE with the defergent triton X-100 and the organo phosphate paraoxon", Toxi. Sci., (2001), 63, 208-213 .

[9]ziga j., Robert r. and marija s., Cell lab. Links, (2007), 48(8), 1465-1468

[10]Garrod L.P. and Garady F.D., "Antibiotic and chemotherapy", (1972),3rd Ed., John Wiley and Sons, New York, NY, USA,.

[11]Huheey I.E., "Inorganic chemistry – principles of structure and reactivity", (1983), Harpar and Row, New York, 421,425, 119 .

[12]Martyneto L.I. and Spitynes V.I., "Methodological aspects of the course in inorganic chemistry", (1986), Mir publishers Moscow.

[13]Ram V.J. and Pandey N.H., Agric Bio. Chem., (1973), 37

[14]Kubo H. and Sato R., Chem. Abs., (1970), 73, 108594t .

[15]Caldwell H.C. and Burkhalter J.H., J. Am. Pharm. Assoc. Sci., Edn., (1958), 47 .

[16]Vishnu I.L. and Pandey H.N., "1,3,4-Oxadiazoles: part III. Mannich bases derived from 5-(o-) hydroxyl phenyl-1,3,4-oxadiazole-2-thione", J. Ind. Chem. Soc., LI, (1974),634-635 .

[17]Suman S.P. and Bahel S.C., "5-Substituted-1,3,4-oxadiazoles and related compounds as possible fungicides",J. Ind. Chem. Soc., LVI, (1979), 712-715 .

[18]Goswan B.N. and Nath S.C., "Synthesis of 3,5-disubstituted 1,3,4-oxadiazole-2-thiones as potential fungicidal agents", J. Heter. Chem., (1984), 21,205-208 .

[19]Zuhar M.E., Suha K.Z. and Nadir A,B., "Synthesis and anti-microbial evaluation of substituted 1,3,4-oxadiazole-2-thiones", Eur. J. Med. Chem., (1988), 23, 133-137 .

[20]Falah S.D., "Inhibitional AChE activity by new derivatives of phenindione", Al-Taqani J., (2005), 18(1), 44-57 .

[21]Al – Hasani R.A.M., M.Sc. Thesis, (1999), Al-Mustansirya University , Iraq, Baghdad, (1999) .

[22]Al – Hasani R.A.M., PhD. Thesis, (2004) , Al-Nahrain University , Iraq, Baghdad, (2004) .

[23]Al – Rubaie Z.A.J., M.Sc. Thesis, (2007), Baghdad University , Iraq, Baghdad, (2007) .

[24]Ellman G.L., Courtney D. and Andres V., "A new and rapid colorimetric determination of AChE activity", Biochem. Pharmacol., (1961), 7, 88-95 .

[25]Linweaver H. and Burke D., J. Am. Chem. Soc., (1934), 56, 658.Baker G.M. and Chen C.H., "The effect of ethanol on the structural stability of acetycholine receptor forms of acetycholinesterase", Biochem. Biophys. Acta

[26](1989), 15, 99263, 333-340 .

[27]Jaffer H.J., Mahond M.J. and Al-Azzawi M.J., J. Biol. Sci. Res., (1988), 19, 793 .

[28]Abdulaziz A., Mohammed A.and Abdullah S., "Sensitivity of bovin refinal AChE toward tacrine : kinetic characterization", J. Biochem. Mol. Toxi., (1998), 12 (4), 245-251 .

[29]Mounter L.A and Ellin R.I., "The inhibition of AChE by2-pyridinealdoxime methyl halide", Molecular Pharma., (1968), 4, 425-456 .

[30]Lee B., Park M. and Yu B., "Inhibition of electric eel AChE by porphin compounds", Bioorg. Med. Chem. Lett., (1998), 16, 8 .
[31]Mansour M. M., M.Sc Thesis, (2000), Al-Mustansirya University, Iraq, Baghdad, (2000) .
[32]Wei L., Kang Y. and Chen L., "Reactivation kinetic of diethyl phosphoryl AChE", Euro. J. Biochem., (2005), 151 (3), 525-529
[33]Smith E., Hill R. and Lehman I., "Principles of biochemistry",(1983) , 7th Ed., John Wiley and Sons, New York, NY, USA,.
[34]Oh, Molecular Cells, (2000), 10 (3), 275-280 .
[35]Viviane T. and Mark T., Natural Medicine, (2008), 10, 379-381 .
[36]Janiefz S. and Wedel A., Issues of Nature Medicine, (2002), 6, 184,476.
[37]Froed H.C., Wilson I.B. and Kaufman H., "Acetylcholinesterase, theory of noncompetitive inhibition ", Arch. Biochem. Biophys., (1986), 247 (2), 420-423 .

Table (1) : The derivatives of (5-phenyl-1,3,4-oxadiazole-2-thione) used for inhibited AChE with their physic

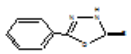
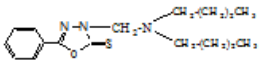
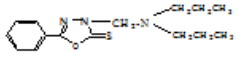
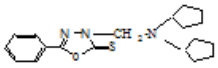
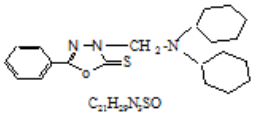
Comp. No.	Symbols	Structure of compounds	Compounds name	Melting point °C	Color	FT-IR	Elemental analysis Found(Calc.)
1-	L	 $C_8H_6N_2SO$ (mother compound)	5-phenyl-1,3,4-oxadiazole-2-thione	278	White	28)12υC=S(υN-H (3100)	C=53.42(52.59) H=2.89(3.05) N=16.28(16.15) S=17.64(16.83)
2-	L_1	 $C_{17}H_{25}N_3SO$	[N(3-(5-phenyl-2-thione-1,3,4-oxadiazole)methyl)dibutyl amine]	78-80	White	)1242υC=S(υN-CH_2 (2869)	C=61.64(60.92) H=6.89(6.54) N=14.01(13.89) S=10.49(11.06)
3-	L_2	 $C_{15}H_{21}N_3SO$	[N(3-(5-phenyl-2-thione-1,3,4-oxadiazole)methyl)dipropyl amine]	54 -56	White	49)12υC=S(υN-CH_2 (2854)	C=63.68(64.32) H=7.18(7.08) N=14.88(14.25) S=10.12(11.04)
4-	L_3	 $C_{19}H_{23}N_3SO$	[N(3-(5-phenyl-2-thione-1,3,4-oxadiazole)methyl)dicyclopentyl amine]	92-95	White	55)12υC=S(υN-CH_2 (2866)	C=66.66(65.94) H=6.89(6.72) N=11.75(12.13)S=9.22(9.40)
5-	L_4	 $C_{21}H_{25}N_3SO$	[N(3-(5-phenyl-2-thione-1,3,4-oxadiazole)methyl)dicyclohexyl amine]	132-136	White	62)12υC=S(υN-CH_2 (2864)	C=67.18(67.09) H=6.96(7.02) N=10.75(9.89) S=8.56(8.62)

Table (2): The effect of ethanol as solvent and mother compound in ethanol at different concentrations in (AChE) activity

Concentration (M)	Activity (µ mol / 3 min / ml)	% Inhibition
Control	1.39	-
Effect of DMSO	1.39	-
Effect of ethanol	1.04	92.23
10^{-1}	0.108	27.48
10^{-2}	0.658	52.67
10^{-3}	1.008	27.48
10^{-4}	0.633	54.46
10^{-5}	0.991	28.70
10^{-6}	1.35	2.87
10^{-7}	0.1	92.80
10^{-8}	0.408	70.64
10^{-9}	0.916	34.10
10^{-10}	0.841	39.49

Table (3): The kinetic properties of AChE without and with compound using Michaelis – Menten plots (a,b,c,d) (figure 1)

Compounds	K_M (M)	V_{max} (µ mol / min / ml)
Control	0.028	2.200
L_2		
10^{-1} M	0.048	1.900
10^{-5} M	0.040	0.550
L_4		
10^{-1} M	0.027	0.016
10^{-5} M	0.045	0.683

Table (4): The effect of different concentration of compounds on the activity of AChE in human serum

Inhibition conc. (M)	Enzyme activity (µmol/3min/ml)	% Inhibition
L_1		
10^{-1}	0.800	53.63
10^{-3}	0.842	51.22
10^{-5}	0.916	46.87
10^{-7}	0.941	45.42
L_2		
10^{-1}	0.025	99.08
10^{-3}	0.050	98.17
10^{-5}	0.710	71.53
10^{-7}	0.775	71.51
L_3		
10^{-1}	1.191	56.22
10^{-3}	1.200	55.89
10^{-5}	1.210	55.52
10^{-7}	1.233	54.67
L_4		
10^{-1}	0.016	99.42
10^{-3}	0.025	99.09
10^{-5}	0.533	80.41
10^{-7}	0.700	74.27

Table (5): The kinetic properties of AChE with L_6 and L_2 compounds

Comp. No.	K_{mapp} (M)	V_{mapp} (µmol/ml/min)	Ki (M)	Inhibition
L_2				Non competitive
10^{-1}	0.33	0.500	1×10^{-1}	
10^{-5}	-	0.660	2×10^{-5}	
L_6				Non competitive
10^{-1}	0.038	0.027	2.8×10^{-3}	
10^{-5}	-	0.285	4×10^{-6}	

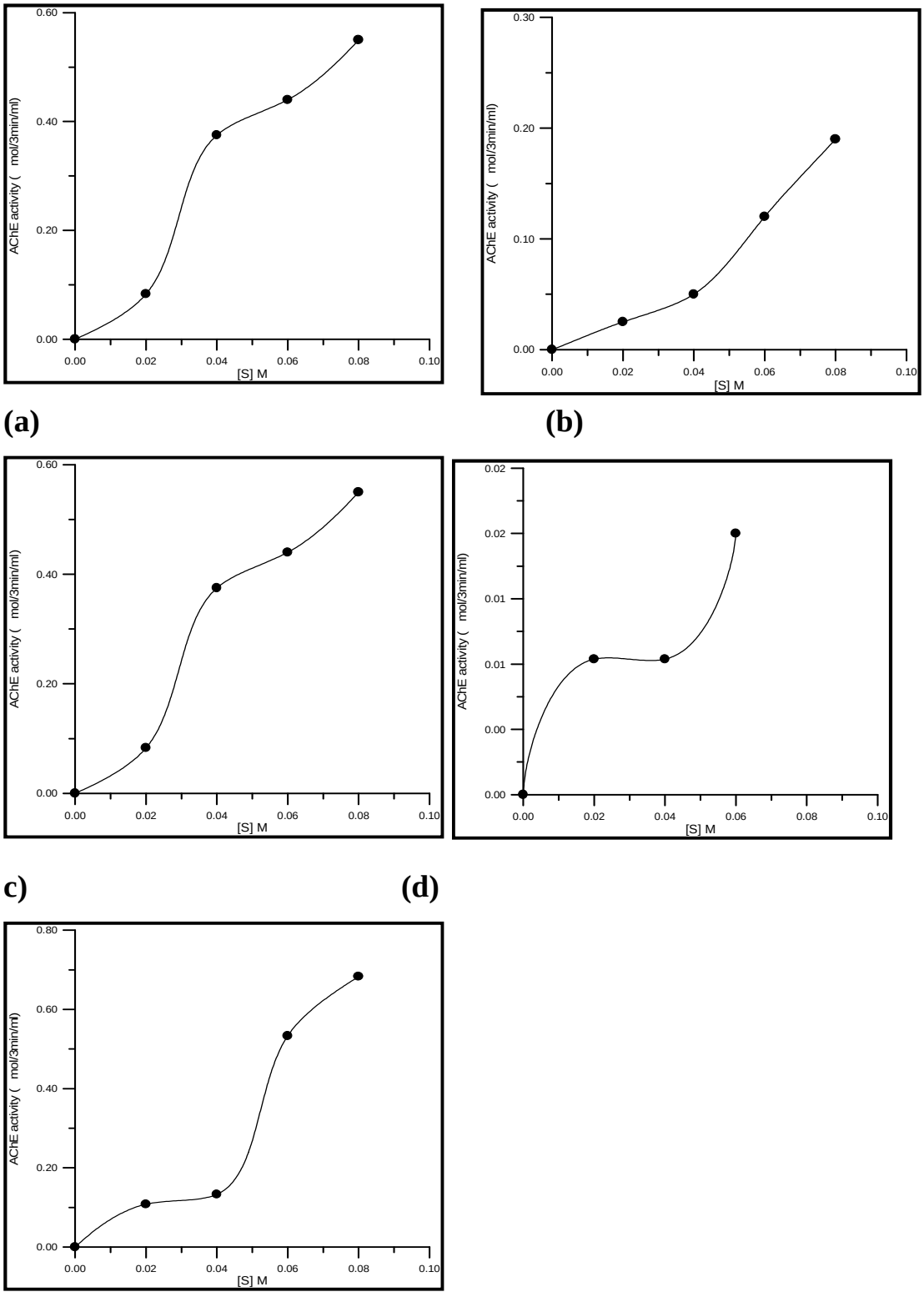
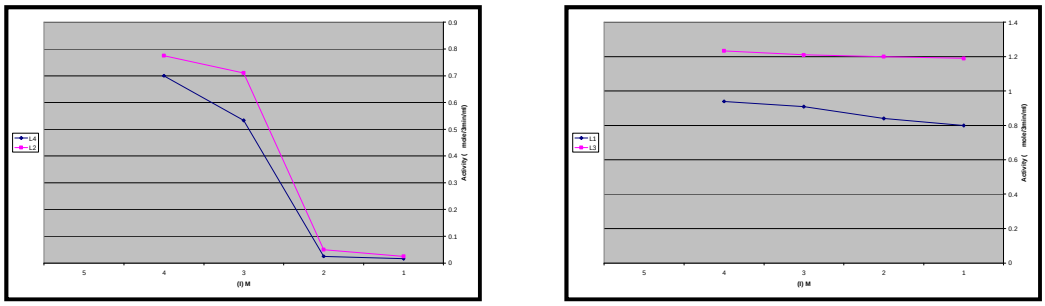


Figure (1): The Michaelis – Menten plots of AChE with different concentration of substrate without inhibitor (a),With inhibitor 10^{-1} L_2 (b), 10^{-5} L_2 (c), 10^{-1} L_4 (d), 10^{-5} L_4 (e)



(a) **(b)**

Figure (2): between concentration of The relationships compounds, L_2, L_4 and AChE activity (a) , L_1, L_3 and AChE activity (b)

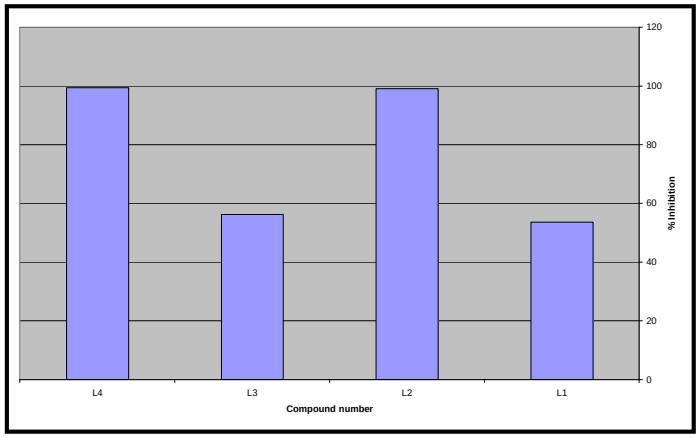
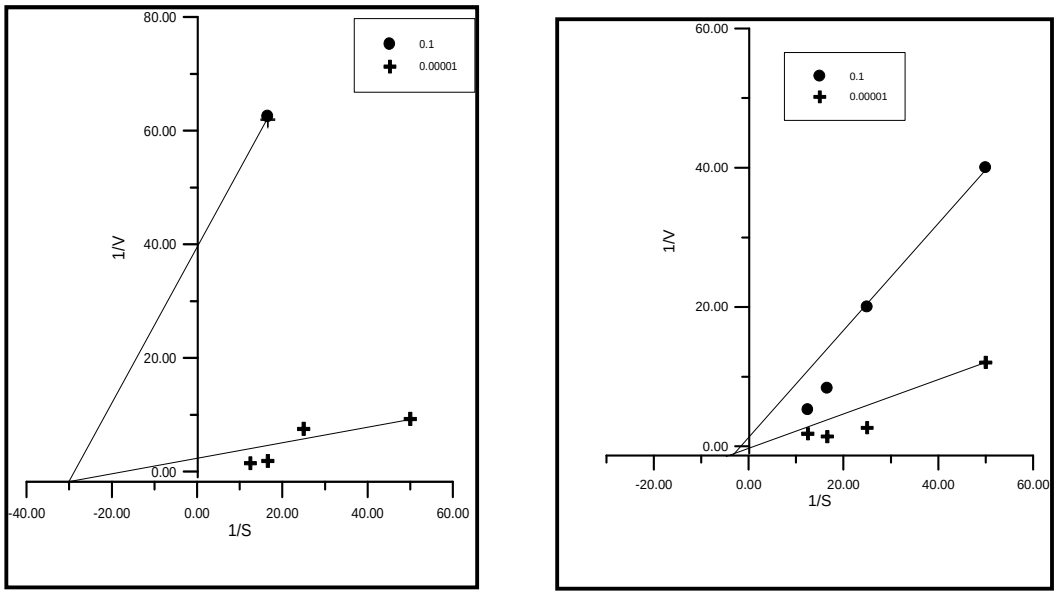


Figure (3): The percentage of inhibition of AChE by (10^{-1} M) of compound L_1, L_2, L_3, L_4



(a) **(b)**

Figure (4): Lineweaver – Burk plots of AChE with compound L_4 (a) and L_2 (b) at concentration (10^{-1} and 10^{-5} M)