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The effect of some Thiosemicarbazide derivatives and their complexes on Human Serum Cholinestrase Activity

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Abstract: The inhibitory effect of some 1-phenyl-3-methyl-5-pyrazolone thiosemicarbazone and 1-phenyl-3-methyl-5-pyrazolone-4-phenyl thiosemicarbazone and their complexes with Cr(III),Mo(V) and W(VI) on the activity of human serum cholinesterase have been studied in vitro. Some of these compounds showed a remarkable activity even at low concentration . From the degree of inhibition obtained, the time of incubation and inhibitor concentration for the highest inhibition have been determined. The results obtained from (Linweaver –Burk) plot indicates that the inhibition is Non-Competitive. The inhibition percentages obtained confirmed that the complexes has a higher inhibition than ligands.

Keywords: Thiosemicarbazide derivatives , complexes , Human , Cholinestrase.

Introduction :

Acetyle choline esterase (AChE) (E.C.3.1.1.7) is an enzyme which occurs at high specific activity in the brain and in nervous tissue and it is readily detected in the membranes of muscles and erethrocytes(1).

AChE are a group of enzyme of most important toxicological significance to all animals. They have been repeatedly implicated to be readily inhibited by phosphate and carbamate organoesters that are commonly used as insecticides (2). However, little is known about the effect of metal complexes on these enzyme , both in the direct vicinity of the active site and at neighboring regions that may influence the active site, is important for the rational design of inhibitors, deactivators affinity reagents and other ligands for AChE.

A large number of compounds have been synthesized and tested as a cholinesterase inhibitors. They belong to different types of organic organometalic classes, such as alkaloids, organophosphorus compounds, ketones(3-5). Schiff base derivatives demonstrated a wide biological activity and spectrum , such as antifungal(6), hypoglacemic (7), and other therapeutic values (8).

In this study, thiosemicarbazaid derevatives and their complexes were syntheside and screened to determine their anti-cholinesterase activity aiming to find new cholinesterase inhibitors in addition to their known wide biological activity.

Materials and methods

1- INSTRUMENTATION:

A pye – Unicom sp3-100 infrared spectrophotometer was used to recorded the ir spectra as KBr disc , UV/VIS spectra were measured by a HITACHI U-2000 spectrophotometer, Elemental Analysis were done by atomic absorption AA-680G (Shimadzu). Electrical conductance was measured on conductivity CDC304 (Jenway4070) Melting points determined by an electric heated block apparatus (Gallen Kamp), and were uncorrected. PH-meter model 720 Orion, Spectrophotometer ur-120-02 shimadzu , water Deionized B114 Elgastat.

2-MATERIALS

The hydrated metal chlorides [CrCl3.6H2O] , [MoCl5.6H2O] , [WCl6.6H2O] were supplied by BDH chemicals, ethanol absolute , diethyl ether, di methyl sulfoxide , 4-phenylthiosemicarbazide, 1-phenyl-3-methyl-5-pyrazolone and thiosemicarbazide, DTNB , ASCHI, Phosphate buffer. All of their materials supplied by Aldrich, BDH and Fluka AG Buchs companies .

3-ENZYME ASSAY

The ligands(L1 and L2) and their complexes were prepared as in literature(9), the physical properties are listed in Table (1).

The ligands and their complexes were dissolved in dimethyl sulphoxide(DMSO) and stock solutions were made for each compounds. Different volumes from these

stocks were added to the assay mixture and the enzyme activity was determined according to the WHO method(10) with minor modification. Phosphate buffer (2.25 mL) was used in the assay medium containing 50 µL of 5,5-Dithiobis(2-nitrobenzoic acid)(DTNB) solution and 10 µL serum. Two mL of this medium was transferred into 3mm cuvatte and 34 µL of the substrate was added . the absorbance was read at 430 nm. DMSO was used as a vehicle solution (control) and showed no inhibitory effect on the activity of the enzyme. Lineweaver-Burk plot was employed in order to know the kind of enzyme inhibition and calculate the values of Km, Ki and Vmapp.

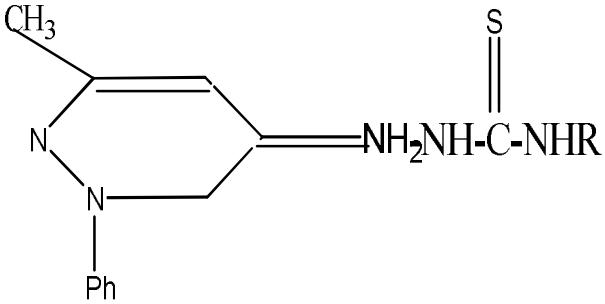


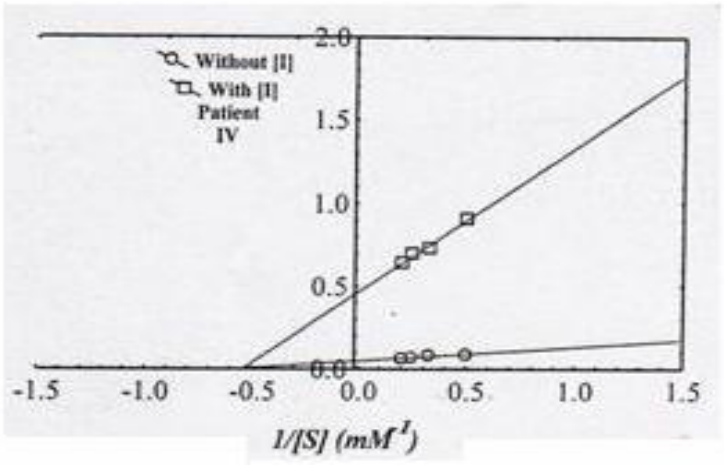
Figure (1): structure of ligands (L1; R=H) and (L2; R=Ph).

Results and Discussion

The effects of the synthesized compounds on serum cholinesterase activity in vitro, were carried out. Some of these compounds showed an encouraging inhibitory action as compared to a known inhibitors such as dibucaine and NaF(11). Enzyme activity for AChE in serum has been measured in vitro according to Ellman's modified method for different samples of man and women , the range of enzyme activity (12) is between (4.41±0.6 - 8.0±1.14) µmol/3min./ml.

The amount of activity is estimated for each sample. At the optimum time of incubation each enzyme sample was incubated with various concentration of inhibitors. Therefore, concentration compounds , which exhibited highest degree of inhibition also the inhibitor effect of DMSO solvent on the activity of AChE has been studied in the work, and not give any effect on the activity of enzyme (13).

Type of inhibition ,Vmapp. and Ki were estimated by measuring the enzyme activity in the absence and presence of inhibitor at different concentration of substrate under the optimum conditions by using Lineweaver-Burk equation and plots as shown in table (2,3) and Fig.(2). The results suggested that compounds (I-VI) for normal persons acted as non- competitive inhibitions Km remained constant while Vmax changed dramatically.



Figure(2): Lineweaver-Burk pilot of AchE in serum

Ache mechanism of inhibition of the enzyme is predictable for studied compounds as follows ; in the mechanism of the enzyme AChE, the hydroxyle group of the amino acid seiren acid attacks carbonyl group of choline ester and at the same time, hydrogen from the acidic group H-A protonates the oxygen of the carbonyl function of the ChE. The inhibition will track theacidic hydrogen of tyrosine and prohibit its attachment to the carbonyl Carbone

enzyme toward nucleophilic attack of carbonyl group of seiren . this results information of quarter nary compounds which will be attached electrostatcally to the anionic side . this will lead to connection via hydrogen bonding between (N-H) group of complexes and the receptor with nitrogen of imidazole of histiden at the esteratic site to form inhibitor complex for enzyme which lead to inhibited AChE fig.(3).

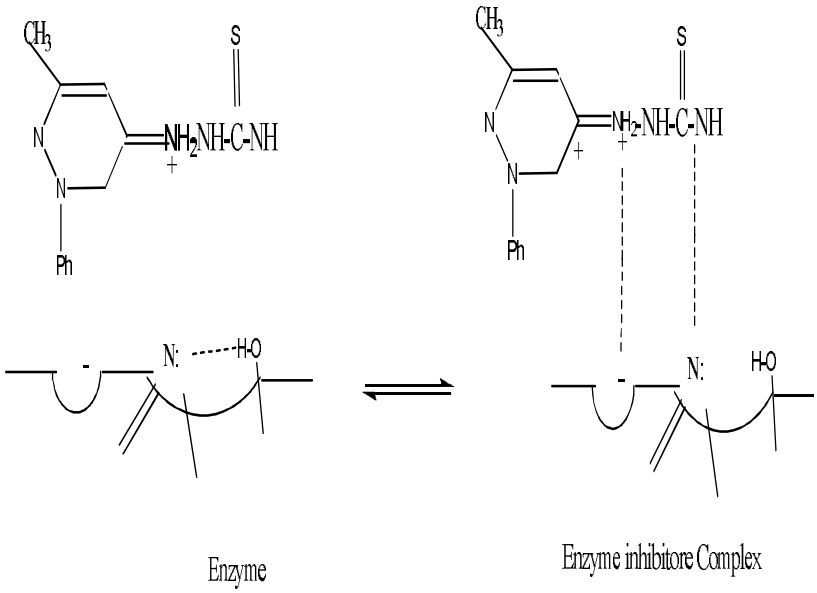


Figure (3): Interaction of the inhibitor with AChE

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Table (1): physical properties of the ligands and their complexes

No.	Comp.	Physical and spectral data
I	L_1	m.p. 174-176 C° , Found: C, 52.98,H,5.37,N,28.24 Cal: C,(53.42),H(5.3),N(28.32), I.R.υ C=N sy. 1640 cm^{-1}vs., υ C=s cm^{-1}sy. 825 vs. UV:294nm,308nm,yield 85%
II	L_2	m.p. 134-136 C° , Found: C, 62.87,H,5.4,N,21.45 Cal: C,(63.14),H(5.3),N(21.65), I.R.υ C=N sy. 1625 cm^{-1}vs., υ C=s cm^{-1}sy. 895m. UV:246nm,310nm,yield 85%
III	$[Cr(L_1)_2 Cl_2]Cl$	m.p.185-187 C°, I.R. υ C=N sy. 1620 cm^{-1}bs., υ C=s cm^{-1}sy. 800 bm. UV:294nm,310nm,595nm.
IV	$[Mo(L_1)_2 Cl_2]Cl_3$	m.p.205-207 C°, I.R. υ C=N sy. 1615 cm^{-1}bs., υ C=s cm^{-1}sy. 795 bm. UV:252nm,310nm,495nm.
V	$[W(L_1)_2 Cl_2]Cl_4$	m.p.211-213 C°, I.R. υ C=N sy. 1625 cm^{-1}bs., υ C=s cm^{-1}sy. 790 bm. UV:284nm,308nm,530nm.
VI	$[Cr(L_2)_2 Cl_2]Cl$	m.p.190-193 C°, I.R. υ C=N sy. 1610m^{-1}bs., υ C=s cm^{-1}sy. 870 bm. UV:284nm,314nm,605nm.
VII	$[Mo(L_2)_2 Cl_2]Cl_3$	m.p.208-210 C°, I.R. υ C=N sy. 1600 cm^{-1}bs., υ C=s cm^{-1}sy. 850 bm. UV:250nm,315nm,501nm.
VIII	$[W(L_2)_2 Cl_2]Cl_4$	m.p.210-212 C°, I.R. υ C=N sy. 1605 cm^{-1}bs., υ C=s cm^{-1}sy. 860 bm. UV:249nm,313nm,545nm.

Table (2): the effect of different concentrations of ligands and complexes on the activity of ACHE.

Inhibitore Concentration M	Activity of inhibited Enz. U/ml	Inh.%	Activity Recovery%
I			
0	0.732	0	100
$1x10^{-4}$	0.201	97.25	2.74
$5x10^{-4}$	0.192	97.37	2.622
$1x10^{-3}$	0.173	98.3	1.7
II			
0	0.56	0	100
$1x10^{-4}$	0.211	96.3	3.7
$5x10^{-4}$	0.182	96.5	3.5
$1x10^{-3}$	0.169	97.3	2.7
III			
0	0.756	0	100
$1x10^{-4}$	0.0156	97.936	2.063
$5x10^{-4}$	0.0105	98.611	1.388
$1x10^{-3}$	0.0097	98-716	1.283
IV			
0	0.755	0	100
$1x10^{-4}$	0.017	97.748	2.251
$5x10^{-4}$	0.010	98.675	1.324
$1x10^{-3}$	0.0095	98.741	1.258
V			
0	0.757	0	100
$1x10^{-4}$	0.0127	98.322	1.677
$5x10^{-4}$	0.0087	98.85	1.149
$1x10^{-3}$	0.0056	99.233	0.766
VI			
0	0.759	0	100
$1x10^{-4}$	0.0108	98.577	1.422
$5x10^{-4}$	0.0061	99.196	0.803
$1x10^{-3}$	0.0051	99.328	0.671
VII			
0	0.775	0	100
$1x10^{-4}$	0.010	98.709	1.290
$5x10^{-4}$	0.006	99.225	0.774
$1x10^{-3}$	0.0048	99.445	0.554
VIII			
0	0.780	0	100
$1x10^{-4}$	0.0078	99.0	1.0
$5x10^{-4}$	0.0072	99.076	0.923
$1x10^{-3}$	0.0060	99.230	0.769

Table(3): Kinetic Parameter of the Inhibited ACHE using Lineweaver-Burk Plot

Type of Inhibition	Vmapp (U/ml)	Ki (M)
L_1	0.463	$2.05x10^{-5}$
L_2	0.512	$3.17x10^{-5}$
$[Cr(L_1)_2 Cl_2]Cl$	0.475	2.01×10^{-5}
$[Mo(L_1)_2 Cl_2]Cl_3$	0.517	3.10×10^{-5}
$[W(L_1)_2 Cl_2]Cl_4$	0.658	4.53×10^{-5}
$[Cr(L_2)_2 Cl_2]Cl$	0.515	5.22×10^{-6}
$[Mo(L_2)_2 Cl_2]Cl_3$	0.417	3.46×10^{-5}
$[W(L_2)_2 Cl_2]Cl_4$	0.367	2.55×10^{-5}

تأثير بعض مشتقات الثايوسيميكاربازايد ومعقداتها على فعالية أنزيم الكولين استريز في مصل دم الإنسان

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الخلاصة:

تم في هذا البحث دراسة تأثير بعض مركبات 1-فنيل-3-مثيل-5-بايروزولون ثايوسميكاربازون و1-فنيل-3-مثيل-5-بايروزولون-4-فنيل ثايوسميكاربازون ومعقداتها مع الكروم(III) , الموليبدينيوم (V) والتنكستنVI) على فعالية أنزيم الكولين استريز في مصل الدم مختبريا (في الزجاج in vitro) حيث درست تأثيرها كمثبطات على فعالية انزيم AChE وتم تعيين زمن حضانة وتركيز المثبط مع الإنزيم لاعطاءه أعلى نسبة مئوية للتثبيط.

وقد أظهرت النتائج المستحصلة من رسم لينويفربرك أن التثبيط يكون غير تنافسي وقد أكدت نتائج حساب النسبة المئوية للتثبيط أن المعقدات لها قوة تثبيطيه أعلى من الليكندات المشتقة منها وقد تم دراسة نوع التثبيط وتحديد قيم الثوابت الفيزياوية لها.