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RESEARCH ARTICLE

Study the Effect of Some Heterocyclic Compounds on the Cholinesterase Enzyme in Human Blood Serum

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Abstract

This research involve synthesis of three type of heterocyclic derivatives (thiadiazole, thiadiazole-shiff base, thiadiazole-azo) by three step, the first step react p-hydroxybenzoic acid with thiosemicarbazide to get compound (1), the second step include react (1) with (p-chloro benzaldehyde, p-hydroxy benzaldehyde) to get thiadiazole-shiff base derivatives (2,3) respectively, in other side the third step involve react (1) with 1-naphthol and salicylaldehyde to get, thiadiazole-azo derivatives (4,5) respectively, all these derivatives were characterize by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectra .After that the effect of these compounds was studied on the activity of the enzyme acetyl cholinesterase in the human serum. Compounds that have the donated groups in the ring showed a high inhibition ratio, either the compounds containing the withdrawing groups in the ring showed a low inhibition ratio because the effect of resonance.

Keywords: *Thiadiazole, thiadiazole-shiff base, thiadiazole-azo, cholinesterase*

Introduction

Thiadiazoles derivatives are have biocidal activities such as anticancer, anti inflammatory, anti-tubercular, and pesticide agents [1, 2].For that we prepare to synthesis derivatives of these compound such as, thiadiazole-shiff base, thiadiazole-azo. Shiff base or imine (-C=N) unit have a wide of applications in different areas such as, organic and inorganic chemistry, biological chemistry and medicinal uses [3].

Azo compounds Azo derivatives are very important of organic colorants because it is have a conjugated chromophore azo (-N=N-) group also uses as biological-medical studies and advanced applications in organic synthesis. [4,5]. Acetylcholine (Ach) is accountable for cholinergic transmission and it's the most plentifully neurotransmitter in to the body and the fundamental neurotransmitter in the brain [6].

Acetylcholine esterase (AchE 3.1.1.7) is kindred of enzymes that catalytic action is hydrolysis of a neurotransmitter acetylcholine (Ach) in two compounds is choline and acetic acid [7].

There are two main forms of Cholinesterase namely in mammalian brain. Acetylcholine esterase (AchE) and Butyl cholin esteras (BuchE),While (AchE) is located in excitable tissue such as nerve or muscle, in placental tissue and in most erythrocytes .as well as (BuchE) is the sitting more generally in the body inclusive within the central and peripheral nervous suit, plasma and liver [8]. This enzyme is a complex molecule composed of four identical units. Each unit contains a single peptide chain containing (57) amino acid and (9) carbohydrate chains and molecular weight per unit is (85000) Dalton [9]

Materials and Methods

The chemicals and solvent compound were from BDH, sigma and Aldrich chemical companies.^{1}H-NMR and ^{13}C-NMR spectra were obtained with a model Bruker AM(400MHz)spectrometer for using DMSO-d6 solution in an appropriate deuterated solvent. Elemental analyses (C.H.N.S) were carried out using a C.H.N EA-99 mth instrument.

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FT-IR spectro were registered as KBr discs in range (4000-400) cm^{-1} on a shimadzu 8400FT-IR spectrophotometer.

Preparation of 4-(5-amino-1, 3, 4-thiadiazol-2-yl) phenol compound (1) [10]

In a round bottom flask (100 ml) was placed a mixture of (0.01m) thiosemacarbazide in (8) ml of $POCl_3$ and (0.01 m) of 4-hydroxybenzoic acid, the reaction mixture was (3hr), then the solution coolded and add (30ml) distill water, then refluxed (4hr). The reaction mixture was cooled and protected at (24hr).the solvent was then removed and the resulting solid was recrystallized from ethanol to give compound (1).

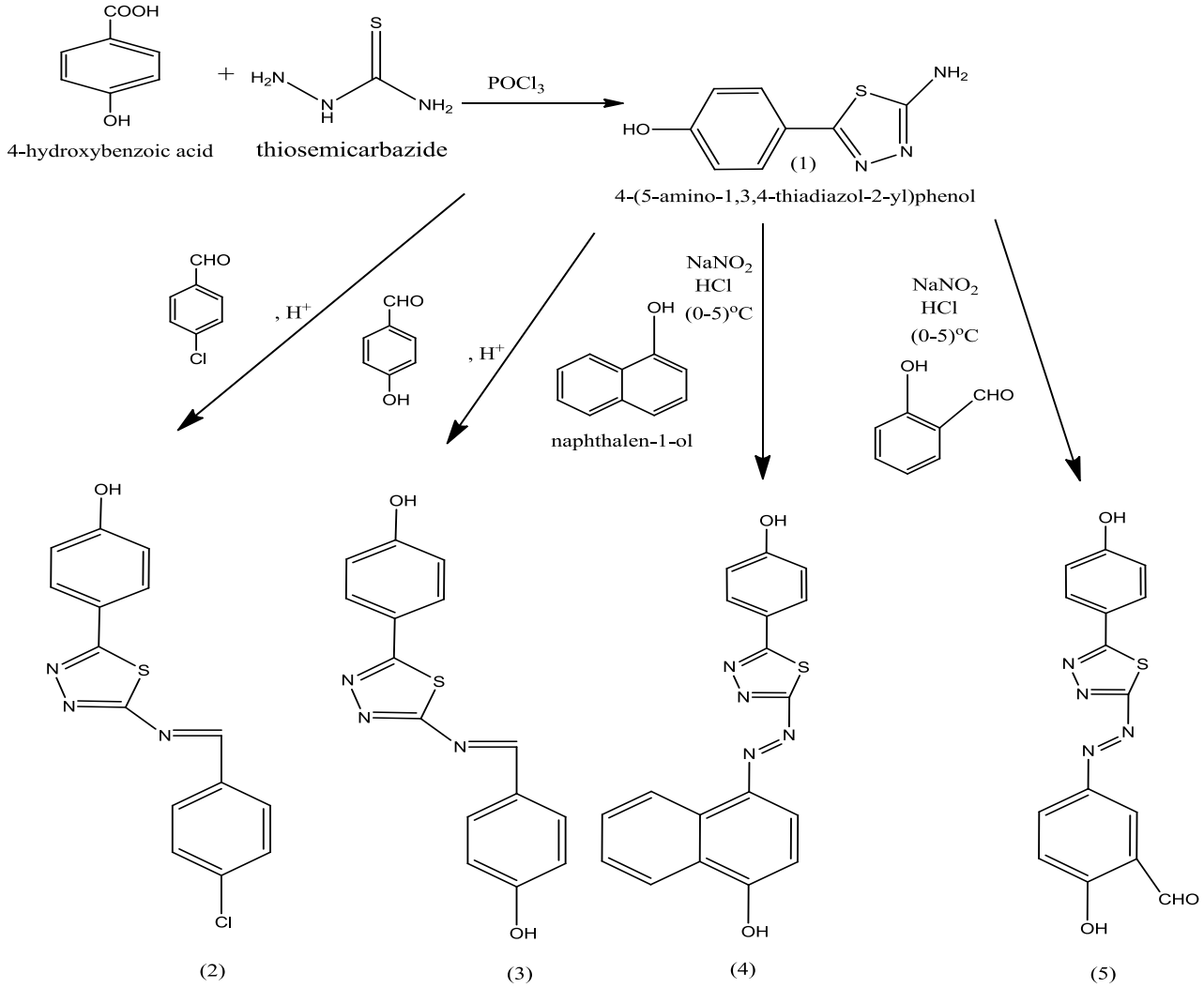
Synthesis Shiff base Derivatives (2, 3) [11]

A mixture of (0.01mol) of (p-chloro benzaldehyde, p-hydroxy benzaldehyde) and (0.01mol) (1) was refluxed for 3hr in (20 mL) of ethanol and Add (3) drops of acetic acid. The reaction was cooled and kept for about (24 hs). The crystals form was filtered to give compound (2, 3) respectively.

Synthesis azo dye Derivatives (4, 5) [12]

Azo derivatives (4, 5) were synthesized by dissolved (1) compound (0.01mol) in (50 ml) of distilled water and (4 ml HCl). That solution was diazotized with (0.01 mol in 25 ml distilled water ($NaNO_2$) was cooled and add up to drop-wise to solution of (1) .The resulting reaction mixture was stirred of 20 minutes.

The resulting diazonium chloride solution was added drop-wise with cooled condition and stirring continuously at (0-5) ^oC to solution of (1-naphthole, salicylaldehyde) (0.01 mol) dissolved in 100 ml ethanol. The reaction mixture was stirred for another 2 hr at (0-5)^oC in ice-bath. After completion of reaction, the mixture was added to the ice cold water (200ml) with stirring. Crude the product was isolated by filtration, washed with distilled water and dried to get (4, 5) azo derivatives.



Scheme 1: Synthesis of heterocyclic derivatives

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Determination of Serum Acetylcholine Esterase (AchE) Activity .Using (MHO) Modifier Method [13]

- Put (2,25ml) from phosphate buffer solution (Ph=7.3) (0.2M) in test tub and add for it (50µL) from detector solution (5, 5-Dithio-2, 2-bisnitro benzoic acid) (BTNB) (0.001M) and (10 µL) of serum .then mixttne components by using vortex mixer.
- Taken (2ml) from mix and placed in the measuring cell, then added to it (34 µL) from base material Acetyl thio choline iodide (Ac.s.ch.I) (0.06M).

Absorbance was read at wave length at (412 nm) before and after the base material was added for each 3minte of enzyme reacted with base material

Calculation

The Acetylcholine esterase (AchE) activity is express in international Units (U/ml=µmoles/min/ml) at 37 °C and it's calculated from: [14]

$$U/ml = \frac{x \,.y\,.\,\triangle A_{unknown}}{13.6\,.\,z} = 14.71\,.\,\triangle A_{unknown}$$

x= the volume in milliliters at when the absorbance is read. y= the serum dilution, 100 for normal serum

$\triangle A_{unknown}$ = its increase in absorbance corrected for the blank.

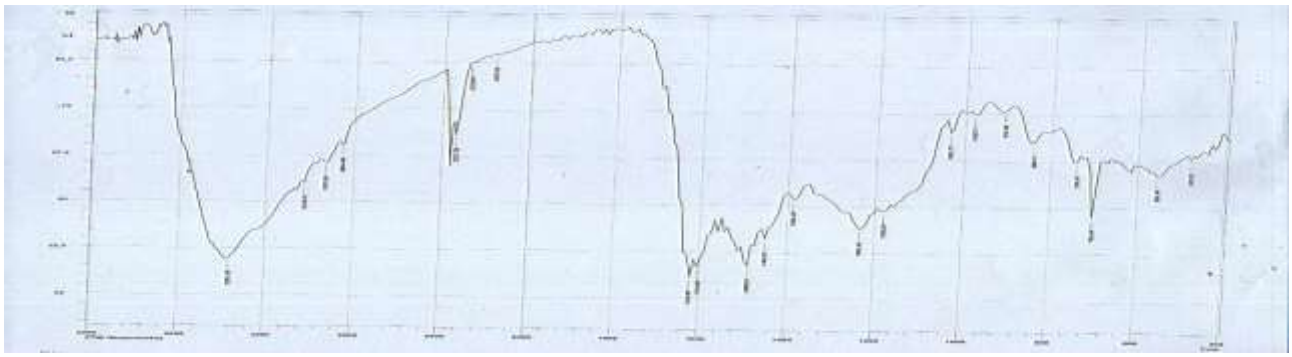
13.6=the millimolar absorptivity of the 5-thio-2-nitrobenzoate for a 1-cm cuvette

Z= the number of minutes of incubation

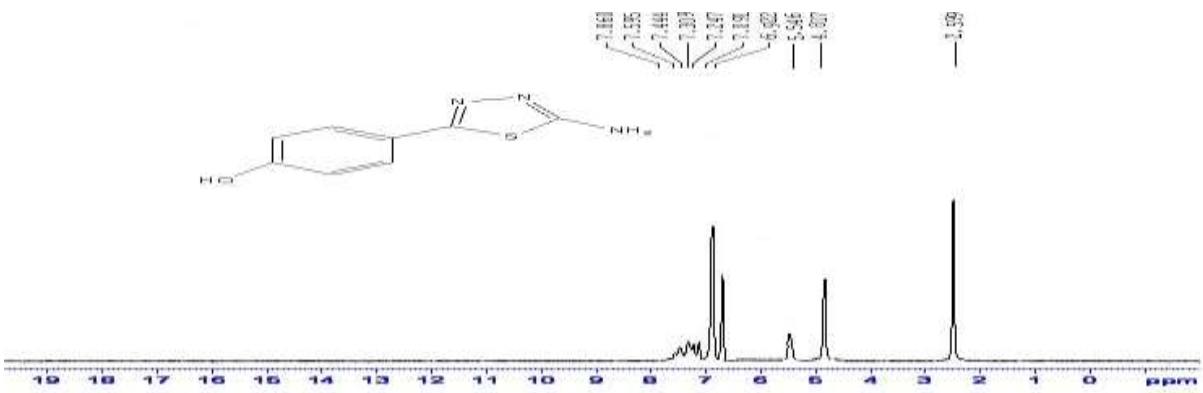
Results and Discussion

Compound (1)

Compound was obtained as solid brown yield 78%,Rf =0.4, Melting Point (205-207)^{O}C. FT-IR data of compound(1) shows absorption at (3348) cm^{-1} for U (O-H) phenol which Overlapped with absorption pack of (NH_2) group, The band at (1635) cm^{-1}, which due to (C=N) thiadiazole ,also shows band at (1600) cm^{-1} for (C=C)aromatic ring, and band for (C-S) at (1157)cm^{-1}. The^{1}H-NMR (DMSO-d_6) spectrum data of compound show δ: 6.8-7.5(m, 4H, Ar-H), 5.52(m, 2H, NH_2), 4.8(s, 1H.OH).



FT-IR for compound (1)



^{1}H-NMR for compound (1)

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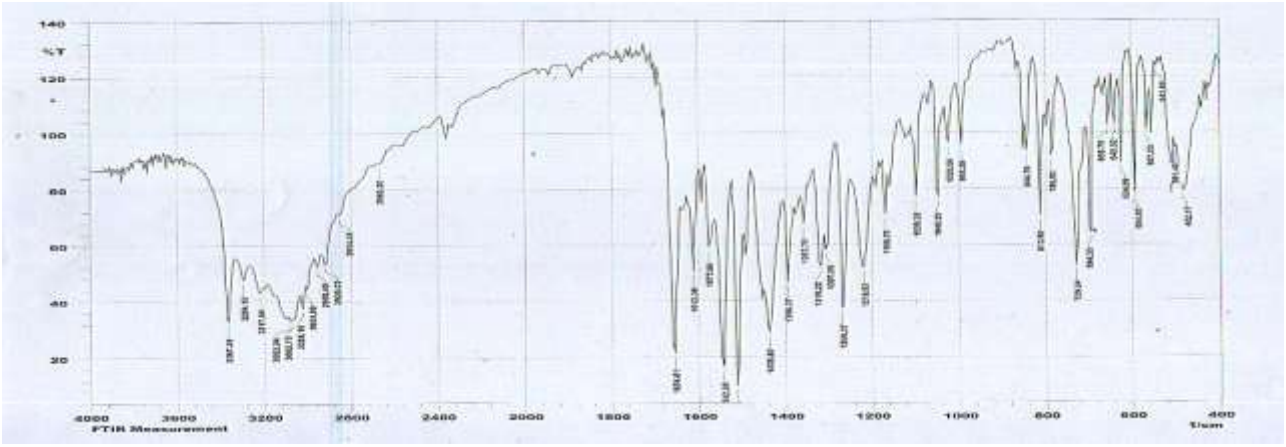
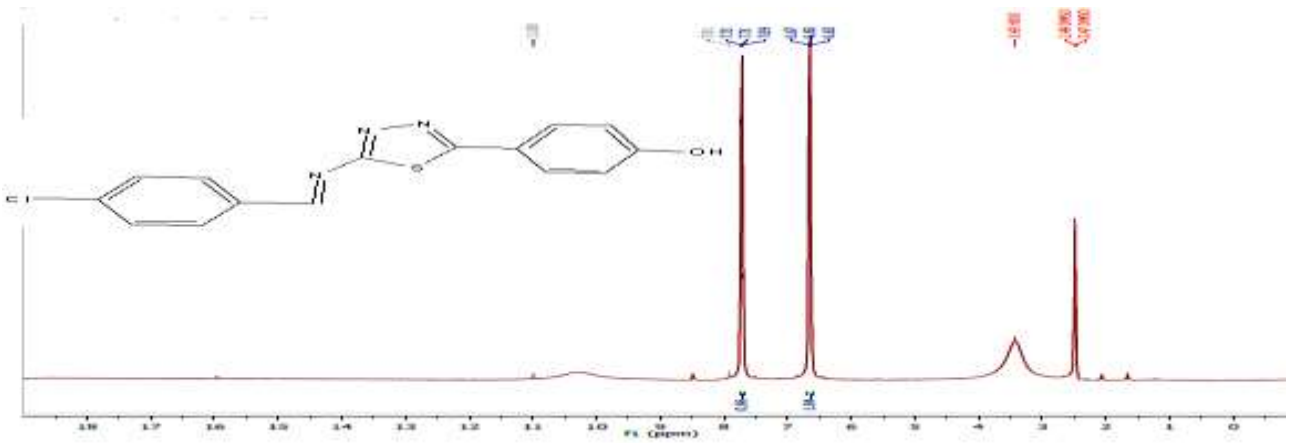
Compound (2)

Compound was obtained as brown and solid, yield 80%,Rf =0.3, **M.P (205-207)^{O}C**. FT-IR spectrum data of compound(2)show absorption at (3082) cm^{-1} for(Ar-H), (3367) cm^{-1} (O-H) ,(3294) cm^{-1} (OH),and show new band at (1654) for (C=N) thiadiazol and new band at (1612) for (C=N) shiff base and band at (1269)cm^{-1} for (C-S),also band at (813) for (C-Cl). The^{1}H-NMR (DMSO-d_6) spectrum data of compound appear δ: 6.6-7.7(m, 8H, Ar-H), 11.00(m, 2H, OH), 7.9(s, 1H.C=H).



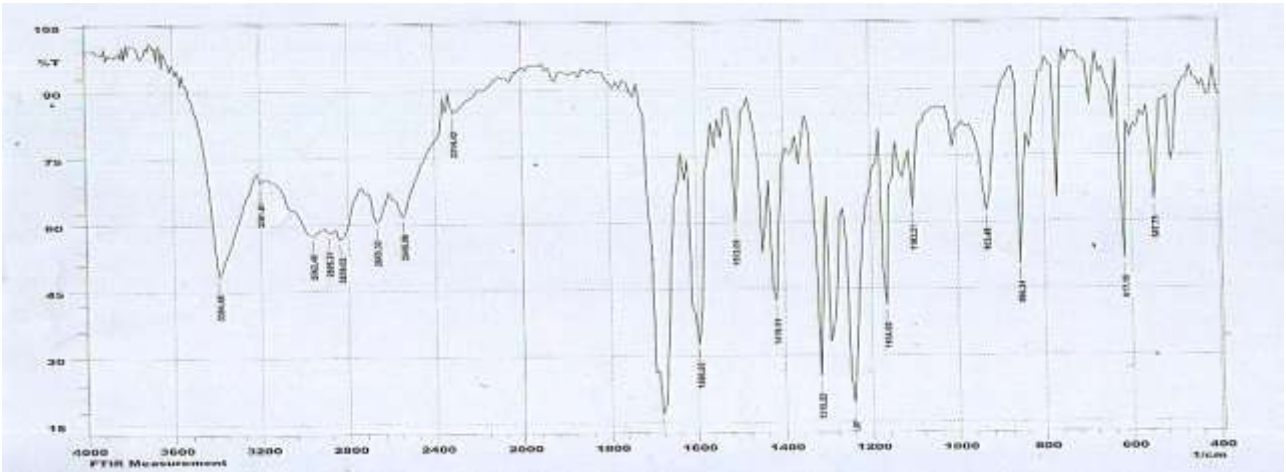
FT-IR for compound (2)



^{1}H-NMR for compound (2)

Compound (3)

Solid yield 77%,Rf =0.3, **M.P (205-207)^{O}C** The FT-IR spectrum da(3)appear absorption at (3082) cm^{-1} for(Ar-H),(3394) cm^{-1} (O-H), (3201) cm^{-1} (OH),and show new band at (1666) for (C=N) thiadiazol and new band at (1569) for (C=N) shiff base and band at (1232)cm^{-1} for (C-S). The^{13}C-NMR (DMSO-d_6) spectrum data of compound show δ: 191(C8), 145(C7), 125(C9), 140(C4, C13), 114-138(Carom.).



FT-IR for compound (3)

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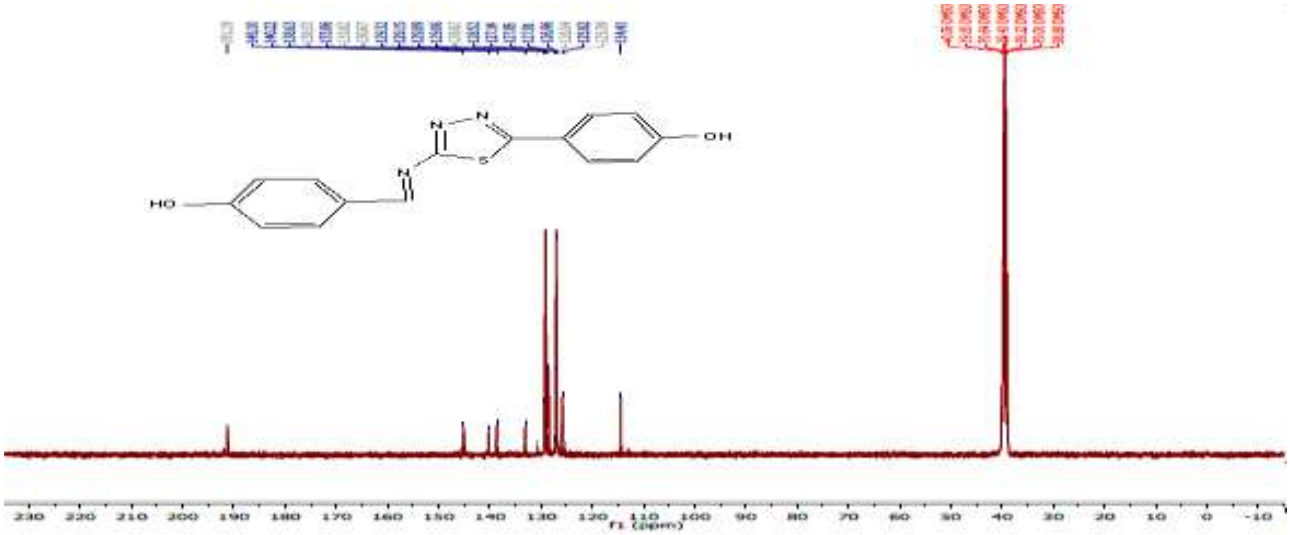
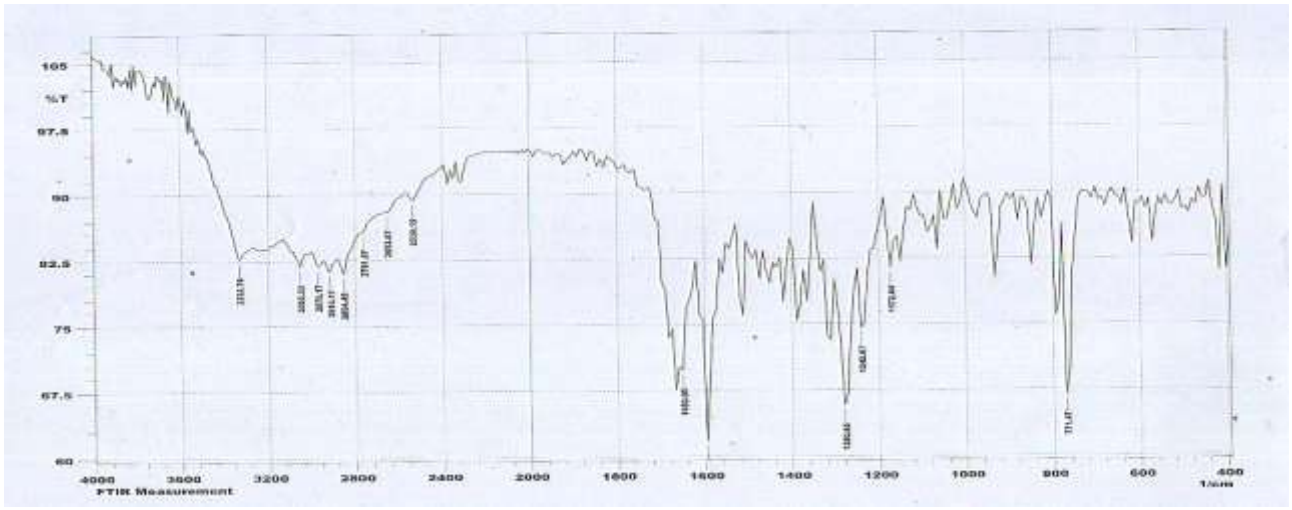


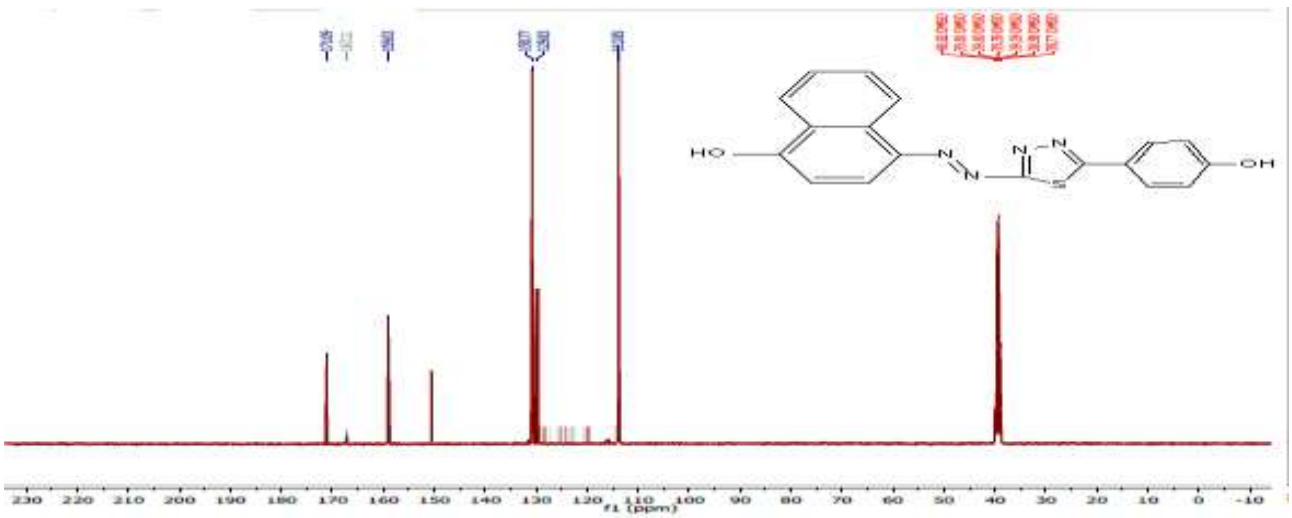
^{13}C-NMR for compound (3)

Compound (4) (E)-4-((5-(4-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl) diazenyl) naphthalen-1-ol

This compound was product as Brown solid yield 83%, Rf =0.43, **M.P (205-207)^{O}C** spectrum data of compound (4) show absorption at (3055) cm^{-1} for(Ar-H),(3332) cm^{-1} (O-H), and show new band at (1650) for (C=N) thiadiazol and new band at (1600) for (C=N) shiff base and band at (1280)cm^{-1} for (C-S). The^{13}C-NMR (DMSO-d$_6$) spectrum data of compound show δ: 1711(C8), 167(C7), 124(C4, C5), 150, (C4, C12), 113-130(Carom.). The^{13}C-NMR (DMSO-d$_6$) spectrum data of compound show δ: 171 (C8), 167(C7), 150(C4, C5), 113-130(Carom.)



FT-IR for compound (4)



^{13}C-NMR for compound (4)

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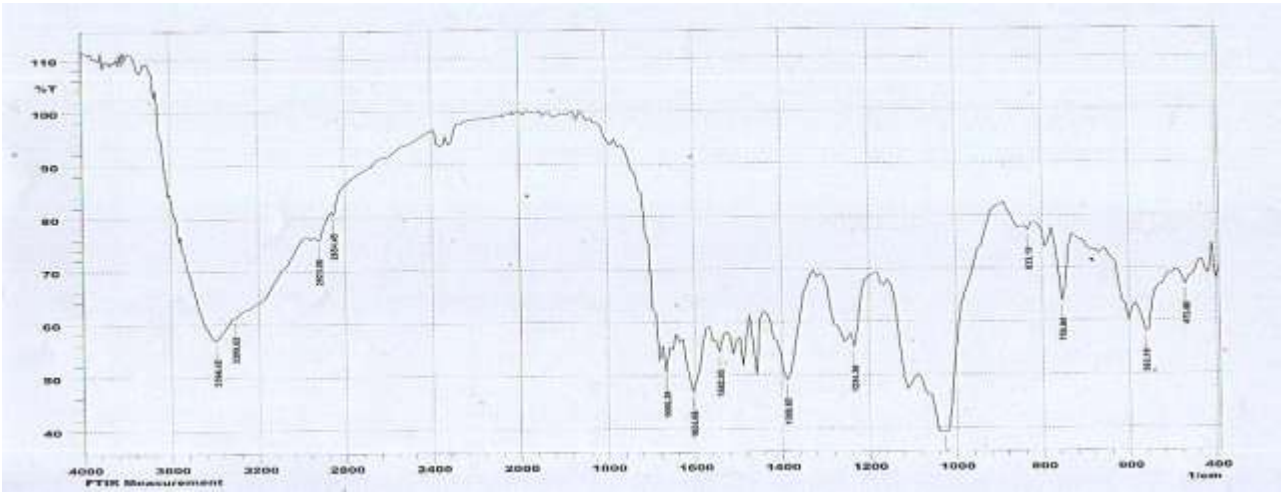
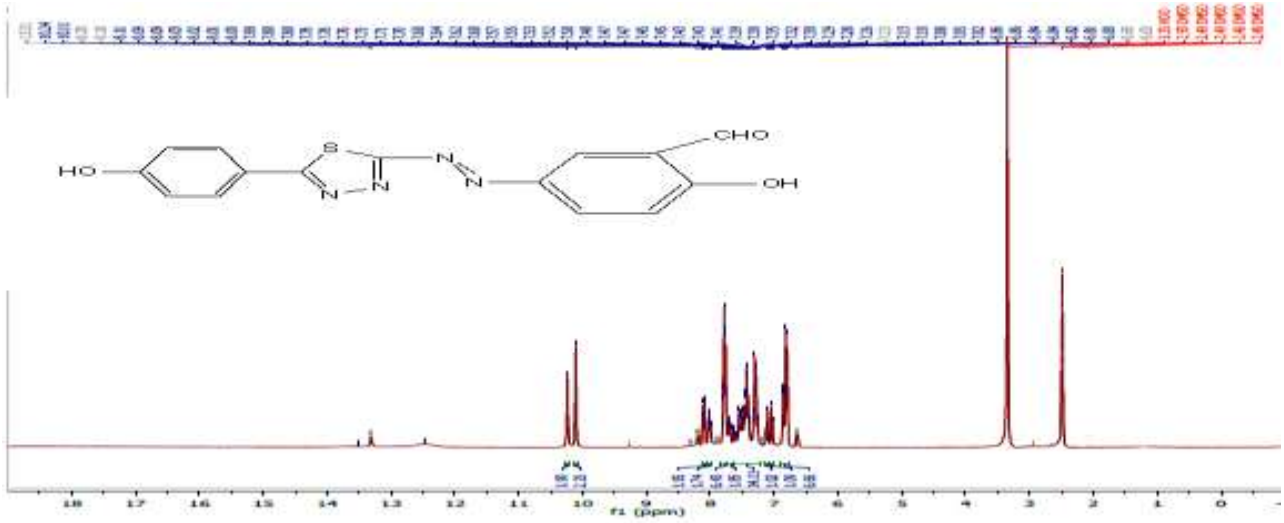
Compound (5): (E)-2-hydroxy-5-((5-(4-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl) diazenyl) benzaldehyde

This compound was product as solid brown yield 89%,Rf =0.29, **M.P (205-207)^{o}C** The FT-IR spectrum data of compound(5)show absorption at (3080) cm^{-1} for(Ar-H),(3394) cm^{-1} (O-H) ,(3309) cm^{-1} (OH),and show new band at (1666) for (C=N) thiadiazol and new band at (1604) for (C=N) shiff base and band at (1232)cm^{-1} for (C-S), other band show at (2845)cm^{-1} for(C-H) aldehyde, (1700)cm^{-1} for (C=O). The^{1}H-NMR (DMSO-d_6) spectrum data of compound appear δ: 6.6-8.2(m, 7H, Ar-H), 13.3(s, 1 H, CHO), 10.2, 10, 1(s, 2H.OH).



FT-IR for compound (5)



^{1}H-NMR for compound (5)

Table 1: Physical and analytical data of heterocyclic compounds (1-5)

Comp.	color	m.p ^{o}C	Yield%	Molecular formula (Mol.wt)	Found (calc.)%		
					C	H	N
1	white	190	78	$C_8H_7N_3OS$ (193.23)	(49.73) 49.69	(3.65) 3.88	(21.75) 21.46
2	yellow	249	80	$C_{15}H_{10}ClN_3OS$ (315.78)	(57.05) 56.98	(3.19) 3.29	(13.31) 13.39
3	yellow	195	77	$C_{15}H_{11}N_3O_2S$ (297.33)	(60.59) 60.42	(3.73) 3.75	(14.13) 14.48
4	brouwn	83	83	$C_{18}H_{12}N_4O_2S$ (348.38)	(62.06) 62.45	(3.47) 3.16	(16.08) 16.23
5	yellow	231	89	$C_{15}H_{10}N_4O_3S$ (326.33)	(55.21) 55.29	(3.09) 3.11	(17.17) 17.69

Table 2 shows the organic compounds as inhibitors with their inhibitors

Comp.	Molecular formula	inhibition ratio%
1	$C_8H_7N_3OS$	75.4
2	$C_{15}H_{10}ClN_3OS$	54.4
3	$C_{15}H_{11}N_3O_2S$	74.2
4	$C_{18}H_{12}N_4O_2S$	47.3
5	$C_{15}H_{10}N_4O_3S$	24.6

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Table (2) shows the effect of these compounds on the acetyl cholinesterase, which has been studied *(In Vitro*) in human serum. The effectiveness was determined once without using the inhibitory substance and other time using the inhibitory substance by adding (1ml) of the inhibitory material and mixed with (1.25ml) of the buffer solution and then followed the method to determine the effective enzyme shown in the practical part. After that, the rate of inhibition was determined according to the following law:

$$\text{percent inhibition} = 100 - \frac{A_{\text{with inhibitor}} \times 100}{A_{\text{without inhibitor}}}$$

The effect of the solvent (DMSO) on the enzyme was studied and showed no inhibitory effect. It does not affect in the enzyme activity [15]

Table 3: shows the effect of the compound (1)

Inhibitor Conc.(M)	Enzyme Activity (µmoles/3min/ml)	Inhibition %
None	8.38	0.0
0.045	2.05	75.4%
0.0045	2.20	73.7%
0.00045	2.3	71.9%
0.000045	2.5	70.1%

Table 4: shows the effect of the compound (2)

Inhibitor Conc.(M)	Enzyme Activity (µmoles/3min/ml)	Inhibition %
None	8.38	0.0
0.045	3.82	54.4%
0.0045	3.97	52.61%
0.00045	4.26	49.1%
0.000045	4.41	47.4%

Table 5: shows the effect of the compound (3)

Inhibitor Conc.(M)	Enzyme Activity (µmoles/3min/ml)	Inhibition %
None	8.38	0.0
0.045	2.05	74.2%
0.0045	2.35	71%
0.00045	2.79	66.6%
0.000045	2.94	64%

Table 6: shows the effect of the compound (4)

Inhibitor Conc.(M)	Enzyme Activity (µmoles/3min/ml)	Inhibition %
None	8.38	0.0
0.045	4.46	47.3%
0.0045	4.56	45.6%
0.00045	4.85	42.1%
0.000045	5.14	38.6%

Table 7: shows the effect of the compound (5)

Inhibitor Conc.(M)	Enzyme Activity (µmoles/3min/ml)	Inhibition %
None	8.38	0.0
0.045	6.32	24.6%
0.0045	6.47	22.8%
0.00045	6.76	19.3%
0.000045	7.3	12.3 %

It can be seen from the Tables (3, 4, 5, 6, 7) that the compound No. (1) and compound (3) have caused significant and clear inhibiting in the effective enzyme when compared with compounds no. (2, 4, 5,) as these compounds have caused less inhibition, this is due to the fact that the compound (1) contains (-NH2) and the compound (3) contains (-OH) group which donated groups of electron by resonance to wards the nucleophilic nitrogen atom, facilitating the attack at the active site of the enzyme. The compound No. (2) Contains (Cl) and the compound no. (4) contains of (**naphthalen**) and compound number (5) contains of (**benzaldehyde**) which withdrawing groups of electron and thus reducing the nucleophilic attack at the active site of the enzyme. In mechanical decomposition of the acetylcholine esterase Hydrogen bonding occurs between the

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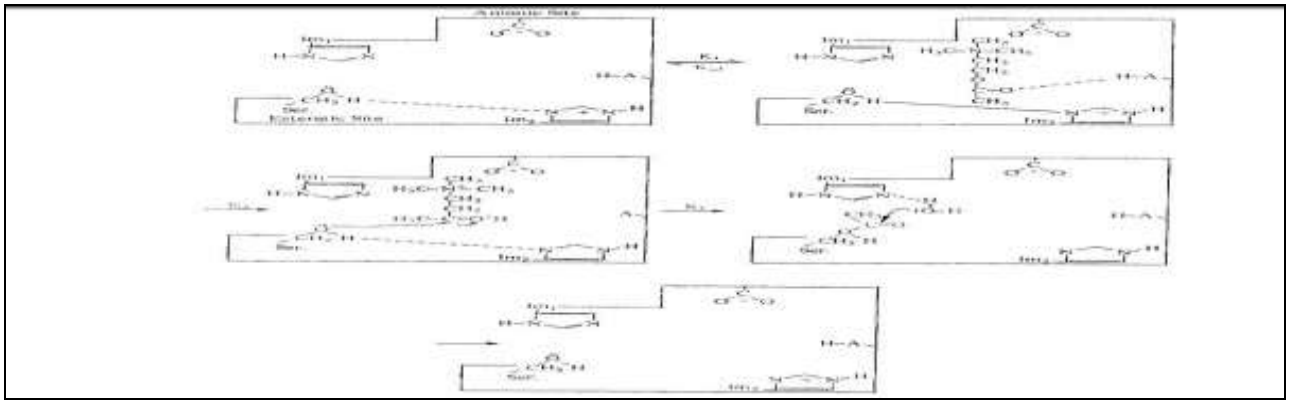
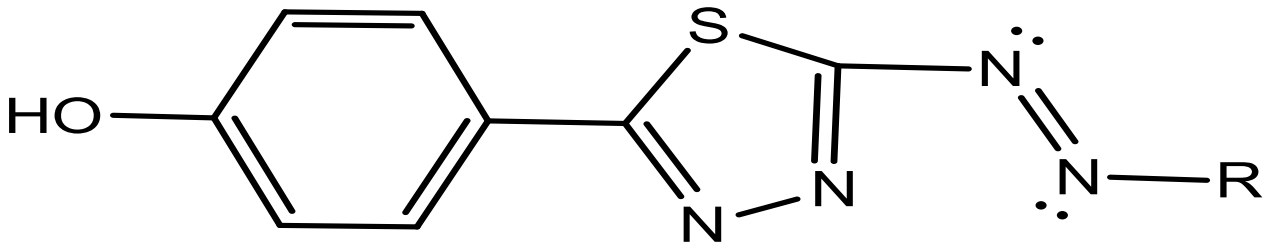
hydroxyl group of serine acid (ser-CH2-OH) and amidazole (Im2) Located in the active site of the enzyme. This link leads to an increase in nucleophilic hydroxyl group of serine acid, There is a link between the hydrogen of acid group in the acid of the tyrosine with the oxygen atom of the Carbonyl group(C=O) for aster cholin, This leads to an increase in the electrolyte of carbon group of the Carbonyl group(C=O) for aster cholin as shown in the following diagram:

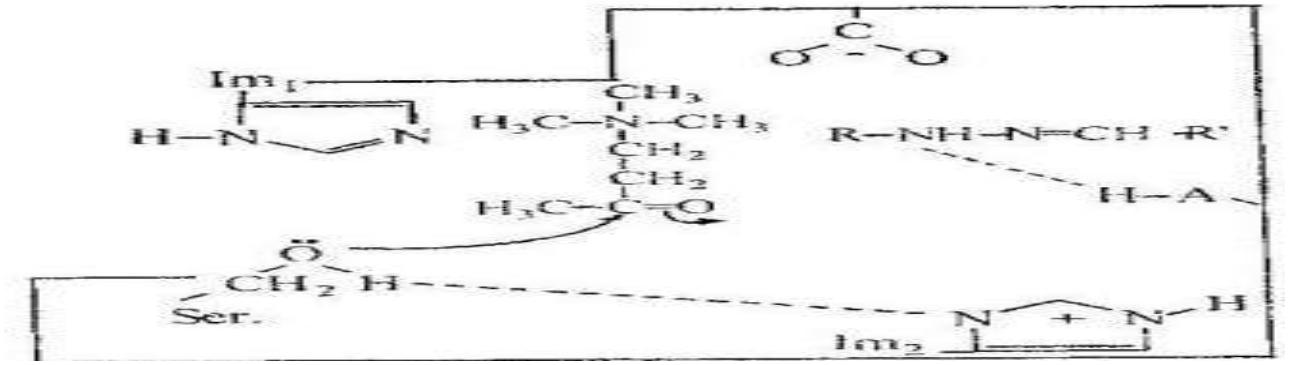


While the use of thiadiazol compounds as inhibitors:



These compounds possess electronic doublets on the nitrogen atoms, which are two active sites, and can act as a base and acquire the hydrogen of acid group of the tyrosine acid and link with him.

Thus preventing its association with the oxygen atom of the carbonyl group(C=O) to the aster cholin against the nucleophilic attack of the hydroxyl group of serine acid, thereby inhibiting the action of choline acetase, as shown in the following figure:



On the other hand, the presence of the group (-NH2) and (-OH phenolic) in the compounds (No.1,3), respectively, leads to the Protonatioon process of the amidazole ring(Im2) located in the active site of the enzyme, which leads to the lack of the occurrence of hydrogen between the Amidazol ring(Im2) and the hydroxyl group of Searin. Thus, the nucleophilic attack on the carbonyl group (C=O) will be reduced. Thus, the enzyme will be ineffective against the nucleophilic attack of choline acetate. We conclude from the above that these organic compounds under study can be used as inhibitors of cholinesterase and particularly compounds containing strong donated group. The results of this study were compared with other recent studies using the semicarbazone [16] and vitamin B12 [17] compounds, which showed the effect of these compounds on the active cholinesterase enzyme.

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