Synthesis, Characterization and Studying the Enzyme Activity of New Benzothiazole Schiff Base Ligand (HL) and Its Complexes with Some of Metal Ions

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Abstract

A new heterocyclic Schiff bases ligand (HL) derived from condensation of 2-Amino-4-methylbenzothiazole with 4-Diethylaminosalicylaldehyde have been synthesized and characterized by (FTIR & UV.Vis) spectroscopies, (^{1}H & ^{13}C)NMR spectra, mass spectrum, elemental microanalysis (C,H,N,S). Metal complexes with Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions have been also synthesized and characterized by (FTIR & UV.Vis) spectroscopies, flame atomic absorption, molar conductivity measurements and magnetic susceptibility. These studies indicate that the mole ratio (L:M) is (2:1) for Co(II) complex and (1:1) for other complexes. The spectral results indicate that the ligand coordinates with metal ions as monobasic bidentate, via azomethine nitrogen and phenolic oxygen atoms. The study suggested octahedral geometry for Co(II), Ni(II) and Zn(II) complexes and square planar and tetrahedral geometries for Cu(II) and Cd(II) complexes, respectively. The enzyme activity of the ligand and its metal complexes with acetylcholine esterase1 **(AChE**) have also been studied. The study of enzyme activity indicates that the ligand and its metal complexes revealed different inhibition behaviors.

Key words: Benzothiazol, Schiff bases Complexes, Enzyme Activity

Introduction

Benzothiazoles are bicyclic ring system with multiple applications which have been the subject of great interest because of their biological activities like antibacterial[1], antifungal[2], antiviral[3], anticancer[4], antitubercular[5], anthelmintic[6], anticonvulsant[7], cytotoxic[8], anti-inflammatory[9] and enzymatic[10] activities. Heterocycles bearing nitrogen, sulphur and thiazole moieties constitute the core structure of a number of biologically interesting compounds[11]. Schiff base complexes derived from heterocyclic compounds have found increased interest in the context of bioinorganic chemistry[12]. Although non-participation of nitrogen atom of benzothiazole moiety in coordination with metal ions[13], Schiff base compounds derived from 2-aminobenzothiazole and salicylaldehyde or its derivatives can be attached to central metal ion via nitrogen atom of azomethine group (Schiff base) and hydroxyl group to form six member chelate ring[13 and 14].

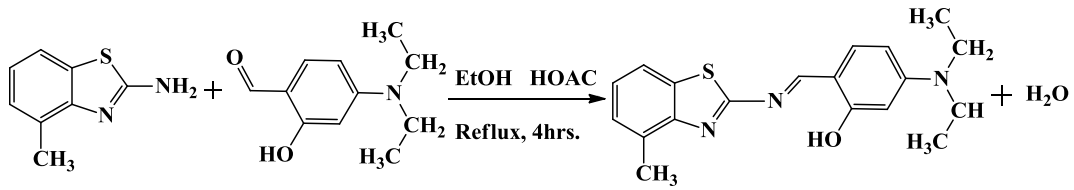
Experimental

Reagents and Physical Measurements:

All reagents and solvents were obtained from commercial sources and used as received without further purification. Melting points were recorded by a Stuart melting point (digital) SMP30 apparatus. FTIR spectra were recorded by a Shimadzu (FT-IR) model 4800S spectrophotometer in the range (4000-400) cm^{-1}. The U.V.-visible spectra of compounds were recorded by using a (U.V-Visible) spectrophotometer type Cary 100con. in the range (800–200) nm. Mass analysis of prepared ligand was performed on GC-MS QP-2010 (Shimadzu). NMR spectra of ligand was recorded at a Bruker DMX-500 spectrophotometer (300 MHz), by using DMSO-d_6 and $(CD_3)_2CO$ for ^1H and ^{13}C spectra, respectively. Elemental analyses (C,H,N,S) of complexes were performed on an EA: Company: Euro Vector, Model: EA 3000A. Magnetic susceptibility of prepared complexes were determined at (R.T) °C by Auto Magnetic Susceptibility Balance (Sherwood Scientific). The metal percentages were determined by using a Phoenix-986 AA spectrophotometer. Conductivity measurements were recorded at (R.T) °C for solutions of samples in DMSO solvent using an Inolab Multi 740, WTW 82362 Weilhiem-Germany. All physical measurements have been done at Al-Mustansiriyah University, Collage of Science, Chemistry Department, except the NMR spectra have been down at Al al-Bayt University, Jordan.

Preparation of ligand (HL)

A solution of 2-Amino-4-methylbenzothiazol (1.64 g, 10 mmol) in absolute ethanol (20 mL) was added gradually to acidified solution of 4-diethylaminosalicylaldehyde (1.93 g, 10 mmol) in (15 ml) from same solvent. The final reaction mixture refluxed for 4 hrs., The formed bright yellow microcrystal precipitate filtered off, washed with distilled water and cold absolute ethanol, dried at room temperature, and finally recrystallized from hot absolute ethanol. The synthesized ligand dissolved in the following solvent Ethanol, Acetone, Ethyl ether, DMSO and DMF. Purity of ligand (HL) was detected by (TLC) using silica gel as stationary phase and (Hexane/ Ethylacetate) as eluent, in ratio (60:40)%. Scheme (1), represents the preparation reaction of ligand (HL).



2-Amino-4-methyl benzothiazol | 4-Diethylamino salicylaldehyde | 5-(diethylamino)-2-(((4-methylbenzo[*d*] thiazol-2-yl) imino)methyl)phenol (HL)

Scheme (1)

Preparation of Metal Complexes

The metallic complexes have been prepared by using the same method, where the salts of [$CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2.H_2O$ and $CdCl_2.2H_2O$] were dissolved in (7 ml) ethanol and added to (15 ml) ethanolic solution of (HL) in (1:1) (metal:ligand) mole ratio respectively with stirring. The pH of resultant solutions were justified by adding few drops of (KOH) solution. Color change has been noticed after mixing both solutions. The reaction mixture then heated under reflux for 4hrs. The complexes precipitates were obtained after reducing the volume of reaction mixture by slow evaporation at room temperature. Finally all precipitates filtered off, washed with distilled water and ethanol, respectively, then dried at room temperature. Table (1) represents some physical properties and metal percentage of ligand and its metal complexes.

^{1}HNMR Spectrum of Ligand (HL)

^{1}H-NMR spectrum of the ligand, Figures (1) and (2), shows a signal at (δ=12.38 ppm, 1H) assigned to phenolic (O-H) group[15], while the singlet signal appeared at (δ=9.01 ppm, 1H) refers to azomethine proton[16]. The spectrum reveals signals related to protons of aromatic rings at (δ=7.83 ppm, d, 1H, benzo.), (δ=7.60 ppm, d, 1H, phen.), (δ=7.23-7.26 ppm, d,t 2H, benzo.), (δ=6.45 ppm, d, 1H, phen.) and (δ=6.16 ppm, s, 1H, phen.)[16]. The quartet signal of methylene group appeared at (δ=3.42-3.49 ppm, 4H)[16] beside the signal of DMSO-d_6 water molecules[17]. The triplet signal of methyl group appeared at (δ=1.12-1.17 ppm, 6H)[16]. The singlet signal of benzothiazole methyl group appeared also beside the signal of DMSO-d_6 at (δ=2.61 ppm, 3H)[16].

^{13}C-NMR spectrum of Ligand (HL)

The ^{13}C-NMR spectrum of ligand, Figure (3) in $(CD_3)_2CO$ exhibited chemical shift, at (δ=164.4 ppm) assigned to benzothiazole azomethine carbon atom (C_7)[16]. The chemical shift of Schiff base azomethine carbon atom (C_8) appeared at (δ=153.5 ppm). The chemical shifts appeared at (135.6, 135.5), (132.0), (126.29), (123.8), (118.5), (104.9) and (96.4) ppm were assigned to the carbon atoms of aromatic rings (C_{10}, C_{12}), (C_5), (C_4, C_6), (C_3, C_{14}), (C_2, C_9), (C_1, C_{13}) and (C_{11}) respectively[16]. The chemical shifts appeared at (δ=43.9) and (δ=11.5) ppm were assigned to the carbon atoms (C_{15} , C_{17}) and (C_{16} , C_{18}) of ethyl group, respectively[18]. The methyl carbon atom of benzothiazole moiety appeared at (δ=17.0 ppm)[19].

Mass Spectrum of Ligand (HL)

The mass spectrum of ligand, Figure (4), showed the mother ion peak at (*m/z*=339), as a base peak, which corresponds to (M^+). Suggested fragmentation pathways and structural assignments of fragments are described in scheme (2).

FTIR-Spectra of Ligand (HL) and its Metallic Complexes

The main stretching frequencies of characteristic bands related to the free ligand and its metal complexes and their assignments are presented in Table (2). The υ(O-H) vibration of phenolic hydroxyl group which appeared at (3387 cm^{-1}) in free ligand spectrum, disappeared[20] at all spectra of its metal complexes which indicate clearly the participation of oxygen atom of this group in coordination with metal ions after losing its proton. The phenolic υ(C–O) stretching vibration in the free Schiff base is observed at 1253 cm^{-1}, which is shifted by 11–14 cm^{-1} towards lower wave numbers in the complexes, thus indicating coordination of the phenolic oxygen to the metal ions[21]. The υ(C=N) vibrational frequency of the Schiff base which appeared at (1633 cm^{-1}) shifted to lower frequencies[13] in all complexes except Cd(II) complex appeared at (1635 cm^{-1}). It is thought that the lone pairs of ring sulfur and nitrogen have very less probability to take part in coordination, because of the lone pair of electrons of the sulfur is involved in aromaticity of the ring and hence comparatively less available. Also the presence of azomethine group attached at 2-position sterically hinders the attack of a Lewis acid on ring nitrogen and sulfur, thus making these donor sites less favorable for coordination[12,22]. In addition, the presence of phenolic hydroxyl group at ortho position makes the nitrogen atom of Schiff base azomethine group more favorable for coordination by forming six chelation ring with central metal ions. Although non-participation of nitrogen atom of benzothiazole moiety in coordination with metal ions[13], this group suffered from changing in position as a result of changing the electronic environment around it[13]. The υ(M–O) and υ(M–N) vibrations were observed at (563-540) cm^{-1} and (476- 457) cm^{-1}, respectively[20]. The IR spectra of complexes reveal a considerable peak observed in the 3200–3580 cm^{-1} range assigned to the νa(OH) and νs(OH) vibration modes from coordinated water molecules [23]. The presence of coordinated water was also confirmed by the medium strength bands at 825–827 cm^{-1}, characteristic of ρr(H_2O) frequencies[20].

Electronic Spectra and Magnetic Properties of the Prepared Complexes

The magnetic susceptibility measurements were used in combination with electronic spectral data to establish the structure of complexes. The effective magnetic moment (μeff) values were observed at room temperature (300 K) for the complexes have been listed in Table(3) with electronic spectra of ligand and its metal complexes. The electronic spectra of ligand and its metal complexes were recorded in DMSO solutions at wave lengths range (200-800) nm.

The UV-Vis spectrum of yellow solution of the prepared ligand reveals two peaks at (275 nm, 36363 cm^{-1}) and (440 nm, 22727 cm^{-1}), the first absorption peak may be assigned to $\pi \rightarrow \pi^*$ transition of -CH=N- groups and phenyl rings. The second high intensity absorption peak may be assigned to intra-ligand charge transfer transitions (ILCT)[24].

The absorption peaks observed in spectrum of Co(II) complex at (265 nm, 37735 cm^{-1}) and (275 nm, 36363 cm^{-1}), were assigned to ligand field transitions. The absorption peak at (360 nm, 27777 cm^{-1}), was assigned to the (MLCT) transition[25,26]. The spectrum also reveals new high intensity absorption peak at (445 nm, 22471 cm^{-1}), assigned to the higher energy, third spin allowed ($^4T_1g(F) \rightarrow {}^4T_1g(p)$) ($\upsilon_3$) transition[27]. At a high concentration, the spectrum revealed another new peak in visible region at (560 nm, 17857 cm^{-1}), assigned to the second spin allowed transition, $^4T_1g(F) \rightarrow {}^4A_2g(F)$ (υ_2)[14]. The first low energy, spin allowed transition ($^4T_1g(F) \rightarrow {}^4T_2g(F)$) ($\upsilon_1$) has not been observed, but calculated theoretically,(914 nm, 10934 cm^{-1}). The value of 10Dq is equal to energy difference between $^4T_1g(P)(\upsilon_3)$ and $^4A_2g(F)(\upsilon_2)$ transitions[28]. The other ligand field parameters ($B^{\backslash}$) and (β) have been calculated and their values were (497 cm^{-1}) and (0.51), respectively. The value of (β), indicates moderate covalence character of the bonds between ligand and metal. The calculated value of effective magnetic moment was seen at (4.48) B.M within the expected range of octahedral geometry[26].

The spectrum of Ni(II) complex exhibited two peaks in UV. region at (265 nm, 37735 cm^{-1}) and (350 nm, 28571 cm^{-1}), were assigned to ligand field spectra[25]. At a high concentration the spectrum showed two new peaks in Visible region at (683 nm, 14641 cm^{-1}) and (624 nm, 16025 cm^{-1}), were assigned to $^3A_2g(F) \rightarrow {}^3T_2g(F)$ (υ_1) and $^3A_2g(F) \rightarrow {}^3T_1g(F)(\upsilon_2)$,respectively[14]. The third high energy, spin allowed transition $^3A_2g(F) \rightarrow {}^3T_1g(P)(\nu_3)$ has been not observed practically, but calculated theoretically,(413nm, 24156 cm^{-1}) by applying the bands ratios of (υ_2/υ_1)[25] on (d^8) diagram. The 10Dq value is equal to the lowest energy transition (υ_1)[28]. The others ligand field parameters ($B^{\backslash}$) and (β) have been calculated and their values were (366 cm^{-1}) and (0.35), respectively. The small value of (β), confirmed the presence of strong covalence bond between ligand and metal. The magnetic moment value was (3.4 B.M) and the ligand field parameters confirmed an octahedral configuration around Ni(II) ion[29].

The spectrum of Cu(II) complex revealed two peaks in UV. region, exactly at (265 nm, 37735 cm^{-1}) and (350 nm, 28571 cm^{-1}), were assigned to ligand field spectra[25]. The spectrum also showed third peak at (420 nm, 23809 cm^{-1}) assigned to $^2B_1g \rightarrow {}^2B_2g + {}^2Eg$ transition[28]. The position of this peak is in a good agreement with that reported for highly distorted octahedral geometry. The magnetic moment value (1.78 B.M) and the ligand field parameters confirmed the square-planar configuration around Cu(II) ion[20].

The spectra of Zn(II) and Cd(II) complexes exhibited three absorption peaks , at (275 nm, 36363 cm^{-1}),(280 nm, 35714 cm^{-1}), (365 nm, 27397 cm^{-1}),(350 nm, 28571 cm^{-1}) and (440 nm, 22727 cm^{-1}),(440 nm, 22727 cm^{-1}). The first two peaks in both complexes were assigned to ligand field spectra[25]. The third peak in two complexes were assigned to metal to ligand charge transfer (MLCT)[24]. The magnetic moments of the Zn(II) and Cd(II) complexes were found to be diamagnetic. According to these results in addition to the results of elemental analyses and flame atomic absorption, we can suggest octahedral and tetrahedral geometries for both complexes, respectively.

Molar Conductance of Prepared Complexes

The values of molar conductance of Ni(II) and Zn(II) complexes in DMSO were (41.6 & 33.5 $S.cm^2 .mole^{-1}$), indicated the (1:1) ratio electrolyte nature[29]. The values of the other complexes Co(II), Cu(II) and Cd(II) in DMSO were within the range (11.4-16.7 $S.cm^2 .mole^{-1}$), indicated the non-electrolyte nature[30] of these complexes. According to all previously mentioned analyses, we proposed the following structures of prepared complexes as shown in Figure (5).

Studying of Enzyme Activity

Methods

Determination of AChE Activity

Human serum AChE activity was determined using Ellman et al. method[31] .

Determination of Biological Activity of ligand and its Metal Complexes and Type of Inhibition[32].

The inhibition percentage was calculated by comparing the activity between with and without inhibitor under the same conditions according to the following equation[32]:

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

Results and Discussion

Present work determined the activity of human AChE in the absence and presence of ligand and its metal complexes under different substrate concentrations and designed to investigate the biological activity and effects of a series of compounds listed in Table (4).

First experiment tried to study the effect of solvent DMSO which did not show any inhibitory effect. Then examine the ligand and complexes in the mixture at different concentrations (10^{-1}, 10^{-3}, 10^{-5},10^{-7}) M. Before each set of inhibition experiments were conducted, the AChE activity was measured by using four different concentrations of acetyl thiocholineiodide (substrate) (0.02, 0.04, 0.06, 0.08) M as shown in Figure(6). The effect of maximum concentration of each inhibitor at different acetylcholine concentrations on AChE activity is illustrated in Figure(7).

The biochemical tests indicated that all compounds have caused noticed inhibitory effects on enzyme activity compared with the measured normal values of enzyme activity, Table (4).

Table (4) showed that the greater inhibition percent was found at concentrations (10^{-3}) M, for Cu(II) and Cd(II) complexes and (10^{-7}) M, for ligand and other complexes. These can be attributed to the presence of more than one nucleophile sides in mother thiazole compound which may led to good orient to active site gorge beside the new nucleophile center that produce after complexion with metals. The greatest significant inhibition was found at concentration (10^{-3} M) in compound Cu(II) and Cd(II). It has been observed that the nature of these metals to chelate with ligand make a less steric hinders compared to other complexes which gave it more freedom to compute with substrate.

Study Type of Inhibition

The second part of this study is to determine the type of inhibition and kinetic parameters (Km,Vmax, and Ki) at different concentrations of substrate and under the same conditions by using Linweaver-Burk equation as shown in Figure (8) and Table(5).

From this presentation the study indicated that K_m was varied from higher, lesser or the same in the presence of ligand and its metal complexes compared with non-inhibition system. A high Km means that the inhibitor fits very well into the active-site cleft of the enzyme which is present in ligand, Cu(II), and Co(II), on other hand, Zn(II) complex does not compute with substrate on the active site of enzyme (noncompetitive inhibition), the affinity of ASCh (substrate) increases in the presence of Ni(II) and Cd(II) complexes, which appeared uncompetitive inhibition with K_m value lesser than control.

The biochemical tests revealed that Ki (the binding affinity of the inhibitor) for compounds Cu(II) and Cd(II) complexes is higher than the rest of compounds as well as Table (4) has showed previously the inhibition percent of Cu(II) and Cd(II) complexes were the greater (83 and 87) % respectively. This difference in Ki values enables to conclude that not all of the assumptions underlying classic Michaelis–Menten equations are being obeyed and that the data are consistent with the kinetics of a tight-binding inhibitor. Also, the results demonstrated that, the ligand and its metal complexes exhibited different types of inhibition at maximum own concentration. The mixed inhibition by ligand and Cu(II) and Co(II) can be explained in order to inhibitors structure that make a conformational changes after binding to –SH,-COOH, imidazole group of Ser, His, Glu in AChE, which are either localized in the active center or are important in determining the active conformation of enzyme molecule. On the other hand, non and uncompetitive inhibition can be explained according to the classical models described that the inhibitor bind to another site that caused conformational changing lock of the enzyme and preventing the substrate binding or decreasing substrate affinity to AChE.

In comparison, inhibition by metal ions is related to the binding affinity towards the amino acid side chain.

Proteins containing the histidine residue are the most vulnerable to the metal binding such as by zinc and copper [33 and34]. The imidazole group of histidine provides the strongest cation-π attraction that may interact with nitrogenous cations of substrates or free metal ions [35,36]. Copper, cadmium, and zinc have been reported to display noncompetitive inhibition behavior towards ChE activity, while mercury has been reported to act as an irreversible inhibitor [37].

Conclusions

Condensation of 2-amino-4-methylbenzothiazole and 4-diethylamino salicylaldehyde produces a new Schiff bases ligand having potential binding sites towards metal ions to form six member chelate ring. Heterocyclic Schiff base ligand acts as monobasic bidentate ligand by coordinating through azomethine nitrogen and phenolic oxygen atoms. Different geometries have been obtained from coordination of the prepared ligand with selected bivalent metal ions. DMSO has been used in preparation of solution in studying of enzyme activity which did not reveal any inhibitory effect. The inhibition concentration was (10^{-3})M for Cu(II) and Cd(II) complexes and was (10^{-7})M for ligand and other complexes. Ligand and Co(II), Cu(II) complexes showed competitive inhibition. Complexes of Ni(I) and Cd(II) revealed Un-Competitive inhibition. Zn(II) complex exhibited Non-Competition behavior.

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Table (1): Physical Properties, Yield Percentage and Elemental Analysis of Ligand and Its Metal Complexes

Compound Symbol	Chemical formula (M.Wt) g.mole^{-1}	Color	M.p ^{0}C	Yield%	Elemental Analysis Found (Calculated)				
					C%	H%	N%	S%	M%
(HL)	$C_{19}H_{21}N_3OS$ (339.45)	Bright yellow	140-142	83	67.58 (67.23)	6.33 (6.24)	12.69 (12.38)	9.44 (9.45)	-----
$[Co(L)_2(H_2O)_2]$	$C_{38}H_{44}N_6O_4S_2Co$ (771.86)	Orange	317-319	67	60.42 (59.13)	5.57 (5.75)	11.61 (10.89)	7.75 (8.31)	8.10 (7.64)
$[Ni(L)(H_2O)_4]Cl$	$C_{19}H_{28}ClN_3O_5Ni$ 504.65	Brown	204-206	52	44.57 (45.22)	5.11 (5.59)	7.67 (8.33)	6.68 (6.35)	12.40 (11.63)
$[Cu(L)(H_2O)Cl]$	$C_{19}H_{22}ClN_3O_2SCu$ 455.46	Brown	128-130	60	48.88 (50.10)	4.78 (4.87)	9.38 (9.23)	6.23 (7.04)	14.70 (13.95)
$[Zn(L)(H_2O)_4]Cl$	$C_{19}H_{28}ClN_3O_5SZn$ (511.34)	Yellow	298-300	58	43.83 (44.63)	4.90 (5.52)	8.49 (8.22)	5.38 (6.27)	13.31 (12.79)
$[Cd(L)(H_2O)Cl]$	$C_{19}H_{22}ClN_3O_2SCd$ (504.33)	Yellow	89-93	59	44.85 (45.25)	4.90 (4.40)	7.70 (8.33)	5.96 (6.36)	21.89 (22.29)

Table (2): FTIR Spectral Data (cm^{-1}) for Ligand (HL) and Its Metal Complexes

Compound Symbol	*υ(O-H)* Coord.(H_2O) ρr(H_2O)	*υ(C–H) Arom. Aliph.*	*υ(C=N) Schiff. (benzo.)*	*υ(C=C) Benzen*	*υ(C-O) υ(C-S-C)*	υ(M–O) υ(M–N)
(HL)	**3387(br) --- ---**	**3099(w) (2924-2866)(w)**	**1633(m) (1583)(m)**	**1570(m) 1510(m)**	**1253(m) 740(s)**	**---- ----**
$[Co(L)_2(H_2O)_2]$	**--- (3500-3580)(br) (825)m**	**(3082-3065)(w) (2974-2862)(w)**	**1612(m) (1554)(m)**	**1508(m) 1479(s)**	**1244(m) 732(w)**	**563(w) 468(w)**
$[Ni(L)(H_2O)_4]Cl$	**--- (3225-3430)(br) (827)w**	**3088(w) (2968-2937)(w)**	**1607(sho) (1579)(m)**	**1510(w) 1485(w)**	**1246(m) 744(w)**	**555(w) 464(w)**
$[Cu(L)(H_2O)Cl]$	**--- (3380-3440)(br) (825)w**	**3054(w) (2964- 2866)(w)**	**1610(m) (1572)(s)**	**(1519-1460)(m)**	**1244(m) 727(w)**	**543(w) 460(w)**
$[Zn(L)(H_2O)_4]Cl$	**--- (3450-3530)w (825)m**	**3065(w) (2968-2902)(w)**	**1610(m) (1558)(s)**	**1508(m) 1479(s)**	**1244(s) 748(w)**	**550(w) 476(w)**
$[Cd(L)(H_2O)Cl]$	**--- (3468-3552) (825)m**	**3075(w) (2982-2866)(w)**	**1635(m) (1589)(m)**	**(1512, 1491)(s)**	**1240(m) 742(s)**	**540(w) 457(w)**

s = strong w = weak m = medium sho=shoulder

Table (3): Electronic Spectral Data, Magnetic Moments, Molar Conductance and Proposed Geometries of Ligand and its Metal Complexes

Compound Symbol	λ_{max} nm (υ cm^{-1})	Assignment	Ligand field parameters			Molar Conductance $S.cm^2.mole^{-1}$	μ_{eff} (B.M) Suggested Geometry
			10Dq cm^{-1}	($B^{`}$) cm^{-1}	(β)%		
HL	275 (36363)	$\pi \rightarrow \pi^*$	---			---	---
	440 (22727)	ILCT					
$[Co(L)_2(H_2O)_2]$	265 (37735) 275 (36363) 360 (27777)	ligand field	4614	497	0.51	11.4	(4.48) O.h
	445 (22471)	(MLCT) & $^4T_1g(F) \rightarrow {}^4T_1g(p)$ (υ_3)					
	560 (17857) 914(10934)*	$^4T_1g(F) \rightarrow {}^4A_2g(F)$ (υ_2) $^4T_1g(F) \rightarrow {}^4T_2g(F)$($\upsilon_1$)*					
$[Ni(L)(H_2O)_4]Cl$	265 (37735) 350 (28571)	ligand field	14641	366	o.35	38.6	(3.4) O.h
	683 (14641) 624 (16025) 413(24156)*	$^3A_2g(F) \rightarrow {}^3T_2g(F)(\upsilon_1)$ $^3A_2g(F) \rightarrow {}^3T_1g(F)(\upsilon_1)$ $^3A_2g(F) \rightarrow {}^3T_1g(P)(\upsilon_3)$*					
$[Cu(L)(H_2O)Cl]$	265 (37735) 350 (28571)	Ligand field	---			16.7	(1.78) S.P
	420 (23809)	$^2B_1g \rightarrow {}^2B_2g + {}^2Eg$					
$[Zn(L)(H_2O)_4]Cl$	275 (36363) 365 (27397)	Ligand field	---			33.5	(0.0) O.h
	440 (22727)	(MLCT)					
$[Cd(L)(H_2O)Cl]$	280 (35714) 350 (28571)	Ligand field	---			13.8	(0.0) T.h
	440 (22727)	(MLCT)					

Table (4): The Effect of Different Concentrations of Ligand and Its Metal Complexes on the Human Serum AChE Activity.

Compound	Inhibition Conc. (M)	AChE activity μ_{mol}/3min/ml	%Inhibition
control	zero	1.25	-
HL	10^{-1}	0.4	**68**
	10^{-3}	0.5	**60**
	10^{-5}	0.675	**46**
	10^{-7}	0.375	**70***
$[Co(L)_2(H_2O)_2]$	10^{-1}	0.48	61
	10^{-3}	0.537	57.04
	10^{-5}	0.45	64
	10^{-7}	0.42	**66.4***
$[Ni(L)(H_2O)_4]Cl$	10^{-1}	0.38	69.6
	10^{-3}	0.4	68
	10^{-5}	0.47	62
	10^{-7}	0.375	**70***
$[Cu(L)(H_2O)Cl]$	10^{-1}	0.25	80
	10^{-3}	0.212	**83.04***
	10^{-5}	0.425	66
	10^{-7}	0.287	77
$[Zn(L)(H_2O)_4]Cl$	10^{-1}	0.537	57.04
	10^{-3}	0.687	45.04
	10^{-5}	0.4	68
	10^{-7}	0.375	**70***
$[Cd(L)(H_2O)Cl]$	10^{-1}	0.2	84
	10^{-3}	0.1625	**87.04***
	10^{-5}	0.637	49.04
	10^{-7}	0.35	72

*** Maximum Inhibition Concentration of Each Compound**

Table (5): The Kinetic Properties of AChE with and without Ligand and Its Metal Complexes

Sample	Inhibitor Concentration (M)	K_m (M)	V_{max} (μ_{mol}/ml/min)	K_i (M)	Inhibition type
Control	Zero	0.046	1.1835	-	-
HL	10^{-7}	0.0658	0.1057	9.75×10^{-9}	Mix
$[Co(L)_2(H_2O)_2]$	10^{-7}	0.051	0.0684	6.106×10^{-9}	Mix
$[Ni(L)(H_2O)_4]Cl$	10^{-7}	0.0303	0.0624	5.48×10^{-9}	Uncomp.
$[Cu(L)(H_2O)Cl]$	10^{-7}	0.0303	0.0624	5.48×10^{-9}	Uncomp.
$[Zn(L)(H_2O)_4]Cl$	10^{-7}	0.046	0.0767	6.89×10^{-9}	Non comp.
$[Cd(L)(H_2O)Cl]$	10^{-3}	0.0302	0.1018	9.411×10^{-5}	Uncomp.

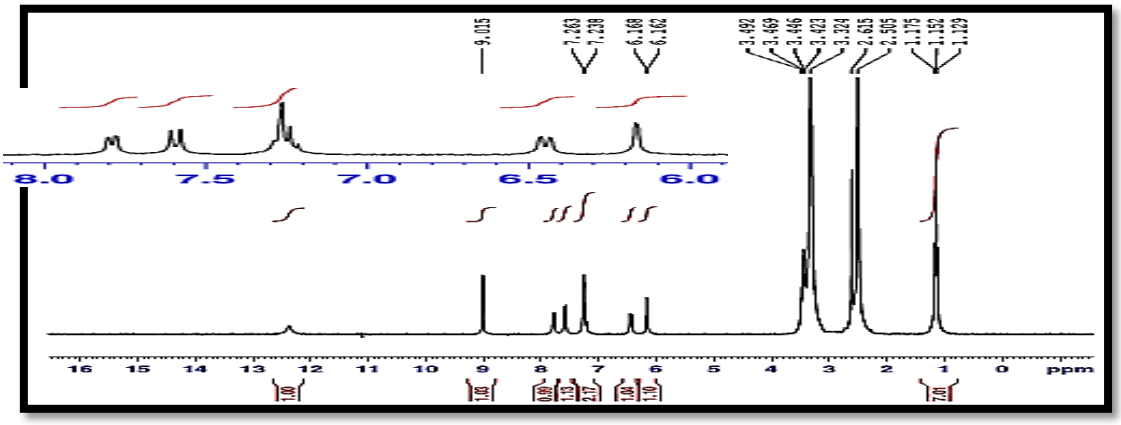


Figure (1): ^{1}HNMR Spectrum of Ligand (HL) in DMSO-d_6

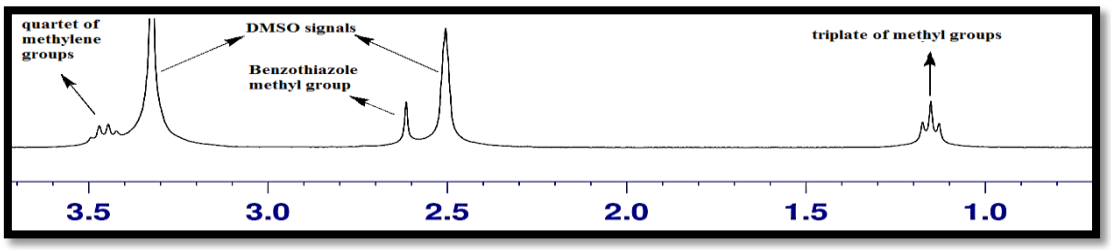


Figure (2): Expansion ^{1}HNMR Spectrum of Ligand (HL) in DMSO-d_6

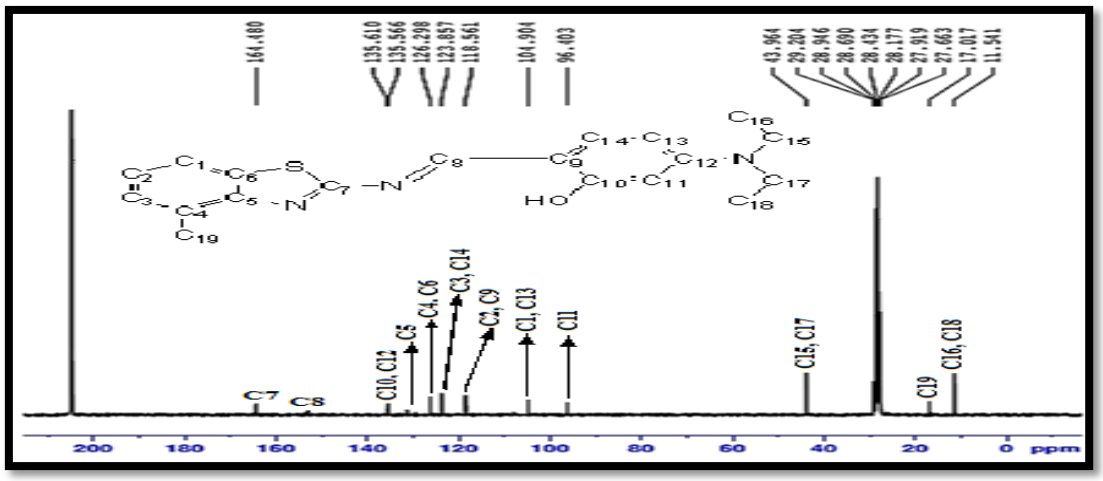


Figure (3): ^{13}CNMR Spectrum of Ligand in $(CD_3)_2CS$

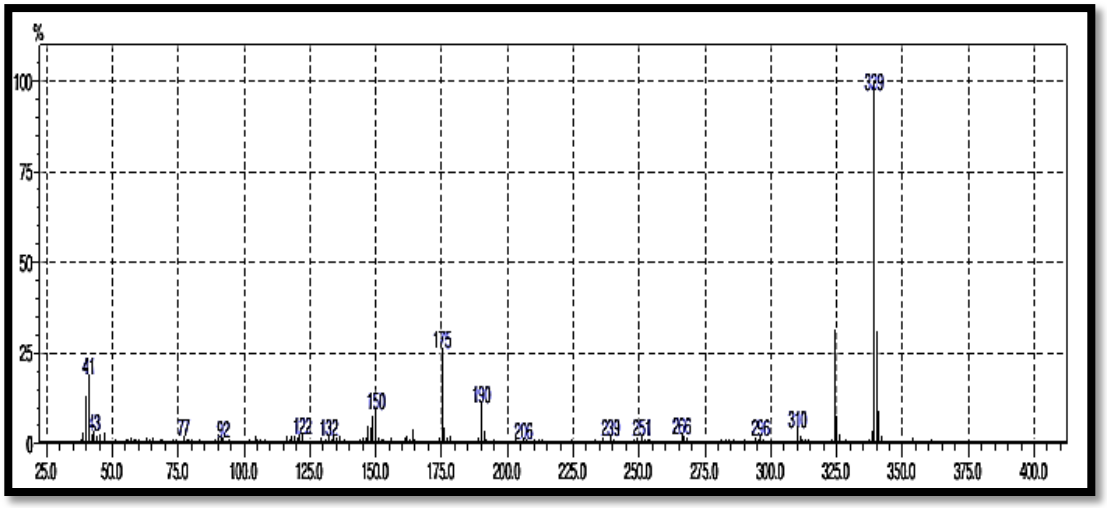


Figure (4): Mass Spectrum of Ligand (HL)

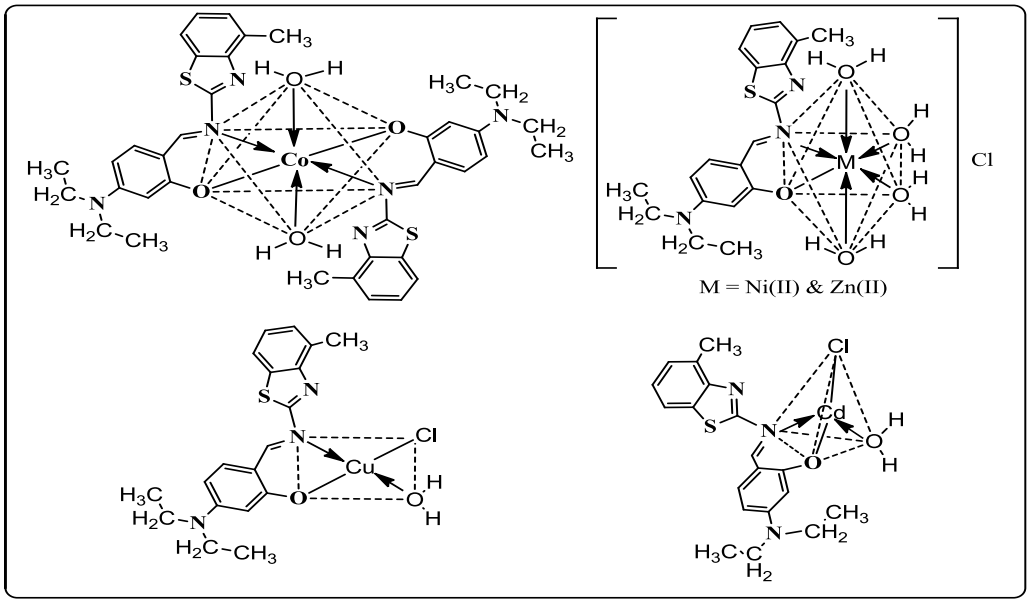


Figure (5): Proposed Structures of the Prepared Complexes

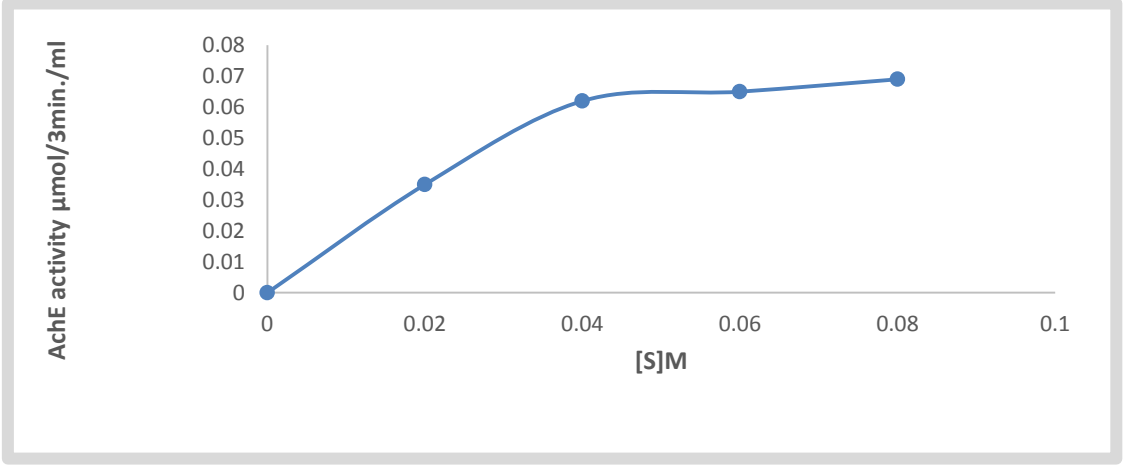


Figure (6): Michaelis-Menten Plot of AChE with Different Concentrations of Substrate without Inhibitor.

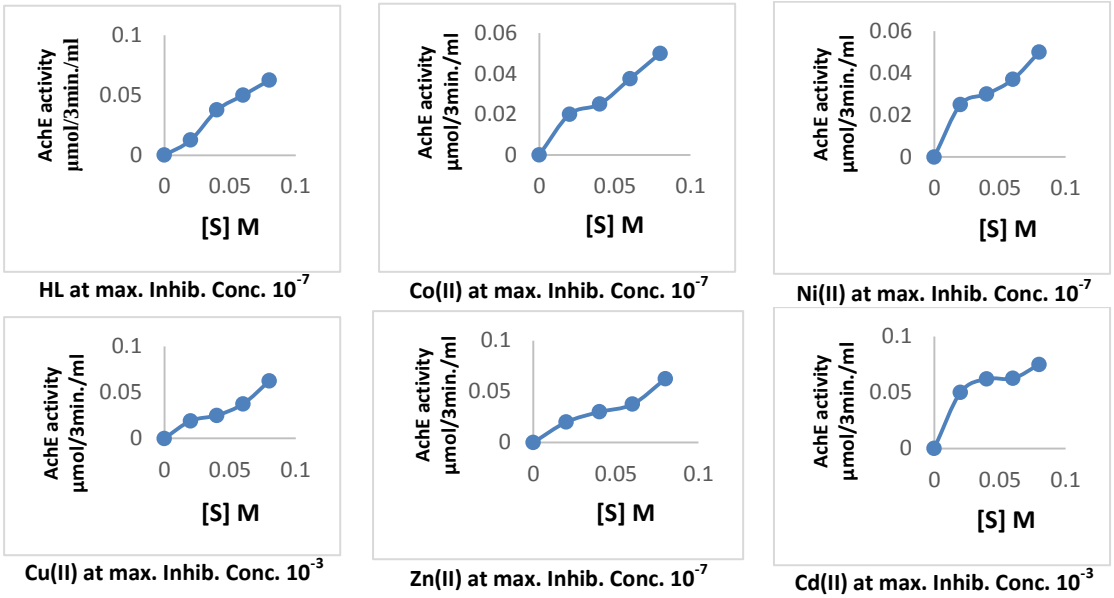


Figure (7): Michaelis-Menten Plots of AChE with Different Concentrations of Substrate in Presence of Inhibitors.

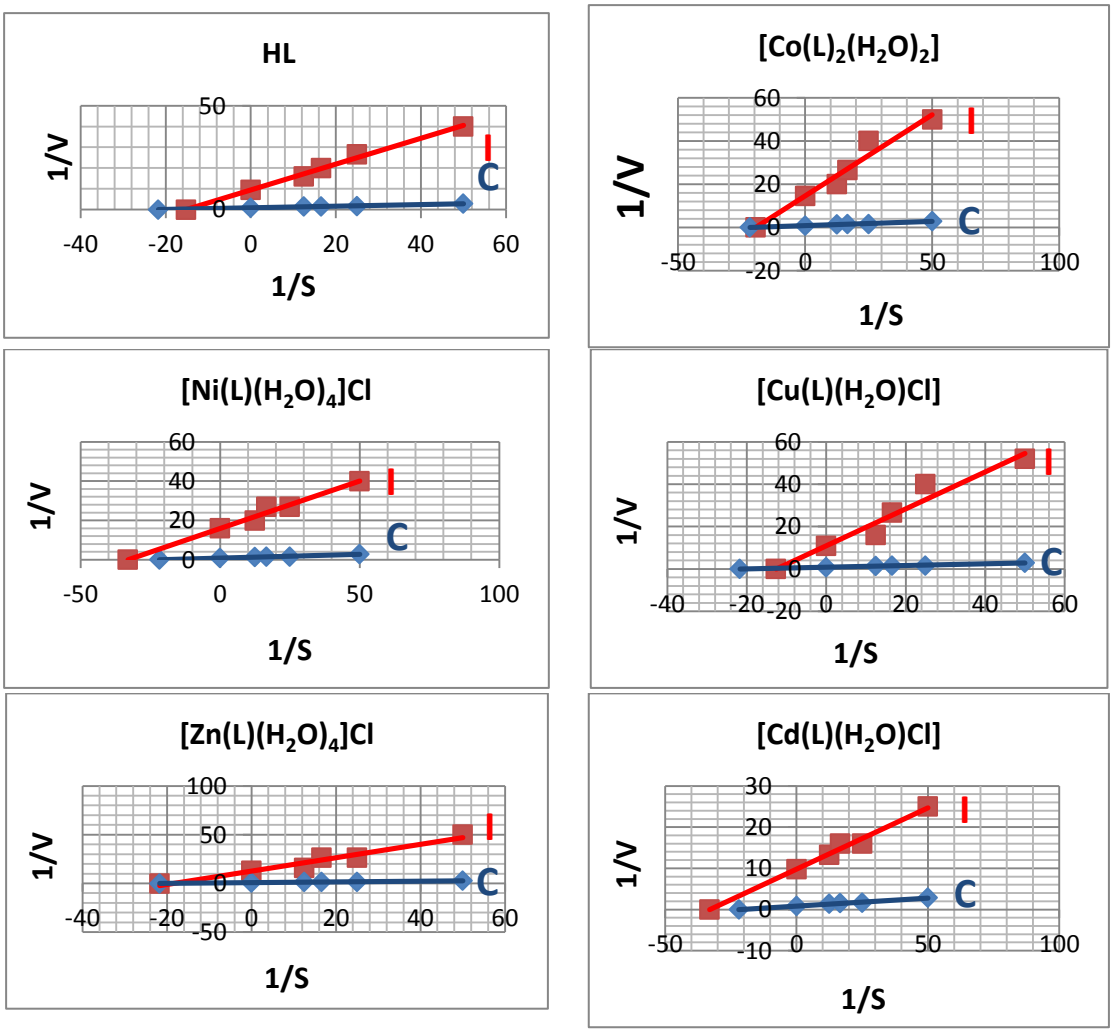
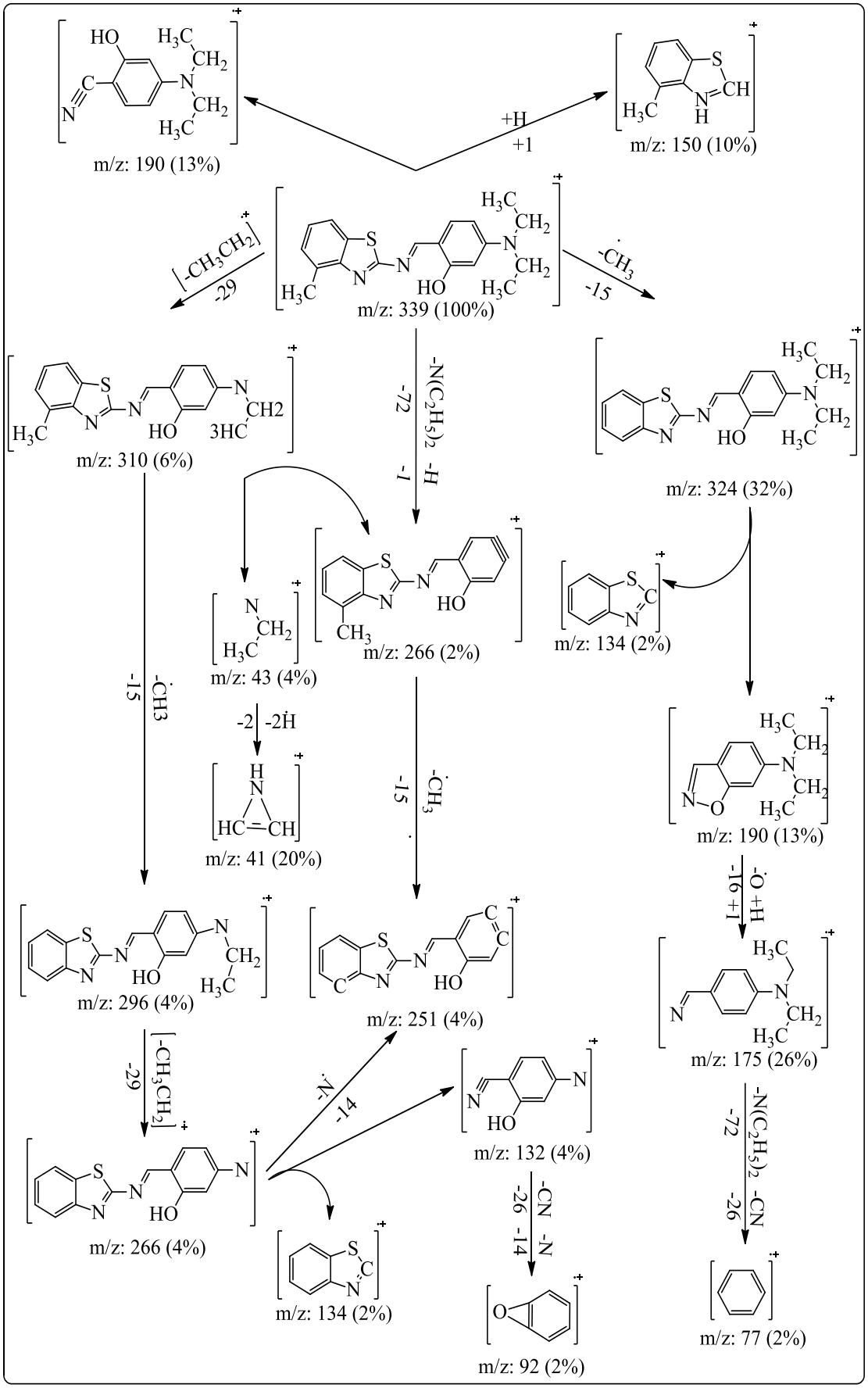


Figure (8): Lineweaver-Burk Plots of AChE of Compound(1-6) all with each Maximum Inhibitory Concentration which Selected.



Scheme (2): Proposed Fragmentation Pathways of Ligand (HL)