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DNA-binding and Kinetics AChE Inhibition studies of Cobalt(II) and Copper(II) Complexes Derived from 5-[4-(dimethylamino) phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbothioamide

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Abstract

A series of transition metal complexes of the general formula [MLCl(H2O)2] ,M=Co(II) and Cu(II) with new ligand, L=3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamides. The new ligand was synthesized by ring closure of 3-((2E)-[4 -(dimethylamino) phenyl] prop-2-enoyl)-2-H-chromen-2-one with thiosemicarbazide. The new ligand and its cobalt(II) and copper(II) complexes were fully characterized by (C.H.N.S) elemental analyses, FT-IR, NMR and UV-Visible spectra. The observed spectral data and magnetic moments measurements confirmed the octahedral environment around the metal ions. The application part of the recent paper was the screening of the new poly dentate-based pyrazoline ligand and its CoL and CuL complexes with thymus DNA act as powerful sequence-specific gene modulators, by exerting their effect from transcription regulation to gene modification. The DNA binding abilities of the 5-[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and it Cu, Co complexes was evaluated by UV–Vis titration experiments, which was a useful method to calculate the DNA binding constants in vitro, all of the solutions exhibited a slight hypochromic without any significant spectral shift. The binding constants of the compounds were (7.8 * 104, 8 * 108, 6.1* 103) M Close, Cu and Co respectively. In a third part of study, a series of chromene and its complexes with Co and Cu were synthesized and their acetyl cholinesterase enzyme (AChE) inhibitory effects were investigated. The ligand and its complexes were determined to be very good inhibitors against for AChE with different type of inhibition (mix and non-competitive) inhibition.

Keywords: ligands 3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamides, cobalt(II) and copper(II) complexes, Thymus DNA, AChE .

1. Introduction

During the last few decades, considerable progress has been made in the field of anticancer chemistry, although tumor resistance to drugs and other side effects of drug administration are crucial problems in this field [1, 2]. To address these problems, researchers are designing new chemotherapeutic agents based on transition metal ions. Many metal complexes exhibit their effect as drugs by binding to DNA or proteins using different binding methods, and is the basic mode of action of many well-known drugs. [3-5]. The DNA interaction phenomenon may be useful in the design of selective and effective new pharmacological compounds. Metal complexes interact with DNA via covalent binding and most aromatic moieties bind DNA via non-covalent binding such as intercalation or groove binding [6, 7]. Transition metal complexes can act as artificial metallonuclease [8] because of the diversity in them structural geometry and the adjustable oxidation state of the coordinated metal ion. Numerous biological experiments have suggested that DNA is one of the primary cellular targets for many anticancer agents. Particularly, in cancer cells, DNA can be preferentially damaged, due to the interactions with anticancer agents, therefore inhibition/blockage of cell division causes cell death [9]. The DNA interacting molecules are usually bound to DNA non-covalently by three modes: intercalation, groove binding and static electronic interactions .Acetyl

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cholinesterase (AChE, EC. 3.1.1.7) is a crucial enzyme used to control transmission between neurons when the process is either mediated or modulated by the neurotransmitter acetylcho-line (ACh) [10]. ACh is released by the axon terminal or varicosities of the transmitter neuron into the extracellular space to interact with the receptors of the other neuron. To maintain control of neurotransmission, it is necessary for AChE, after Ach executes its function, to catalyze ACh hydrolysis, converting Ach to choline (Ch) and acetate. After ACh hydrolysis, Ch is reabsorbed by the axon terminal to produce more Ach [11, 12]. ACh acts as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system (NS) and as a preganglionic and a postganglionic transmitter in the parasympathetic NS of verte-brates and invertebrates [11, 13]. The mentioned interests in literature survey about applications of pyrazoline ligands and their complexes encouraged us to prepared new complexes and study their biological significance.

2. Experimental

All reagents and chemicals were commercially were used as received from suppliers. The starting materials; 3-acetyl-4a,8a-dihydro-2H-chromen-2-one, 4-(dimethylamino)benzaldehyde, thiosemicarbazide (Merck), acetone 99 %, P ,CuCl2.2H2O and CoCl2.6H2O were supplied from Sigma-Aldrich, chemistry department, college of Science, Mustansiriyah university. The melting points were determined in open capillaries and are uncorrected. Electrical conductivity measurements of the complexes were recorded at (25 °C) for (10–3) M solutions of the test in (DMSO) using Eutech Instruments Philips digital conductivity meter with dipping-type conductivity cell conductivity meter. Elemental microanalysis was executed recorded via microanalysis (C.H.N.) analyzer, Eurovector EA 3000. The tests were recorded at Central Laboratory/University of Teheran, Islamic Republic of Iran. ^{1}H and ^{13}C- NMR spectra for the precursors[M], ligands [H2L1 and H2L2] and complexes were recorded in (DMSO-d6) using Brucker, ideal: Ultra-Shield 500 MHz, origin: varian and reported in ppm (δ), at University of Teheran, Islamic Republic of Iran. The chloride contents for complexes were resolute via potentiometric titration method on (686-titro processor-665, Dosinatmetrom Swiss) in Ibn Siena Enterprise / Baghdad, Iraqi Ministry of Industry. The magnetic susceptibility of complexes at room temperature with Sherwood Scientific Apparatus. Samples were recorded at College of Sciences, University of Al- Mustansiriyah, Iraq.Metal content of complexes were specified using a Shimadzu (A.A 7000) atomic absorption spectrophotometer. The sample were recorded in Ibn Siena Enterprise / Baghdad, Iraqi Ministry of Industry. Mass spectra of ligands and some complexes were found via technique using electrospray technique on the SciexEsi mass analysis. The spectra were recorded at University of Teheran, Islamic Republic of Iran. The vibration absorptions spectra were recorded on Shimadzu FT-IR spectrometer at Mustansiriyah University, College of Science. The UV-Visible spectra of the ligand and its complexes were measured in the region (200–800) nm on Varian Cary 100 Conc. UV–Visible spectrometer, Table 1.

2.1. Synthesis of chalcone

(1.65 g, 10 mmoles) of 3-acetyl-4a,8a-dihydro-2H-chromen-2-one dissolved in (20 ml) ethanol was added gradually to (10 mmoles) of (dimethylamino)benzaldehyde followed by 5% NaOH solution. The reaction mixture was stirred at room temperature for four hours. orange precipitate was formed, collected by filtration and were re crystallization using chloroform and ethanol to give orange crystals of the chalcone after keeping the precipitate overnight for 24 hours, Scheme (1).

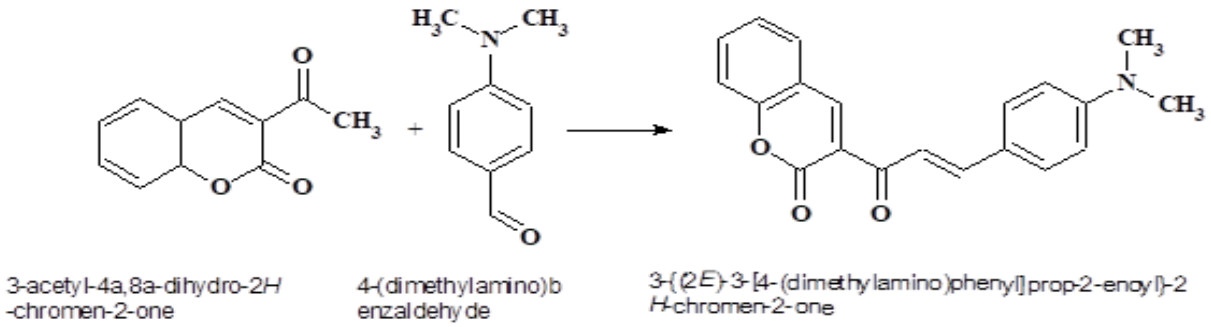
2.2. Synthesis of Ligand [5-[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbothioamide]

[(0.286 g, 1mmole) of chalcone[3-{(2E)-3-[4-(dimethylamino) phenyl] prop-2-enoyl}- 2H-chromen-2-one] and thiosemicarbazide (1.35 g,2.2 m moles) in 1:2 molar ratio were mixed with 100ml of hot ethanol. The reaction mixture were refluxed for 30 minutes in a water bath before the pH of the solutions we adjusted to slightly alkaline by using

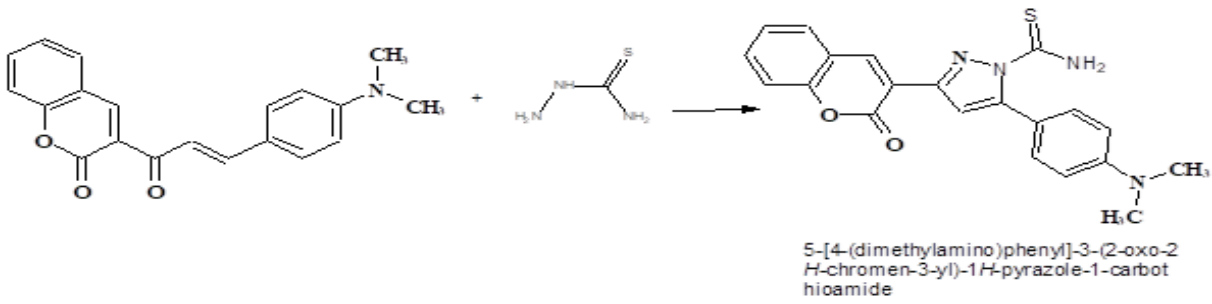
10% NaOH solution (pH=7.5) Before Again, the reaction mixture was reflux for about 14 hours. The deep yellowish precipitate was filtered off, dried in oven then re crystallization from hot ethanol give yellow crystals of ligand, Scheme (2).

2.3. Synthesis of metal complexes

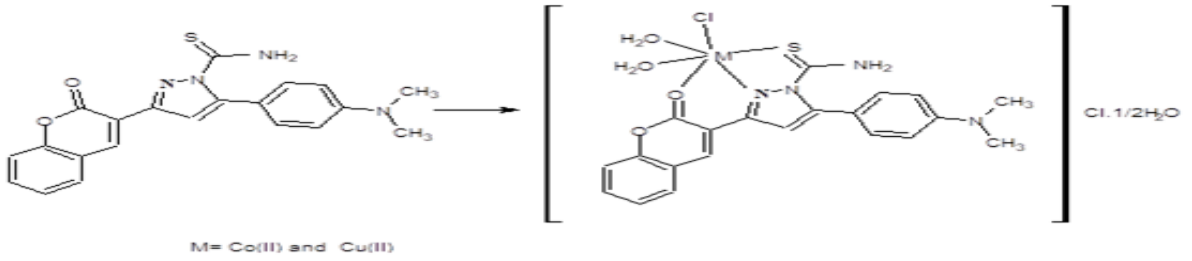
A mixtures (1.282 m moles) of ethonolic solution of ligand and metal chlorides (1.282 m moles (0.217) CuCl2.2H2O),(0.349 g, CoCl2.6H2O),and (0.36 gm of ZnCl2) with (1 mmole, 0.433g gm) of ligand dissolved in (15 ml) of hot ethanol were refluxed for (three) hrs, then add drops of 5% NH4OH solution to maintain the pH to≈6-7). The precipitate was filtered off, dried in vacuum desiccator, Table (1).



Scheme 1: Synthesis of chalcone A.



Scheme 2: Synthesis of Ligand L



Scheme 3: Synthesis of [MLCl(H2O)2]Cl complexes,M=Co(II) and Cu(II).

2.4. Biochemical studies

A quantity of 20 L stock solution of each 5-[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide compound (5 mM) and chelating with Co and Cu metals complexes was diluted with 3 mL phosphate buffer (100 mM, pH = 7.4). An increasing volume of CT-DNA solution was added into the solution. Then, the solution was stirred and incubated at 25 C for 10 min. The UV–Vis spectra in the absence and presence of DNA were recorded using a Shimadzu UV-2501

spectrophotometer. The binding constant Kb was calculated from a D/ΔƐapp vs. D plot according to the following equation [14]: D/ΔƐapp = D/ΔƐ + 1/[(ΔƐ) Kb] where D is the concentration of DNA, ΔƐapp = [ƐA - ƐF], ƐA=Aobs/[compound], ΔƐ = [ƐB - ƐF], and ƐB and ƐF correspond to the extinction coefficients of the DNA–compound adduct and unbound compound, respectively. In the third part of this study, the inhibitory effects of prepared compounds on AChE activities were determined accord- ing to the Ellman test [15].

2.5. Determination of biological activity

A stock concentration solution (0.01M) of each target derivatives (5,6) has been prepared and then different concentrations (10-2, 10-3, 10-5, 10-7, 10-9and 10-11M)of each compound were prepared by diluting it with (DMSO) as solvent. AChE activity is measured in human serum as follows:

(50 µL) of DTNB solution (0.001M) was added to(2.25 ml) of sodium phosphate buffer solution(pH= 7.3,0.2M), 0.25ml of inhibitor was mixed with 2 ml of the same buffer, then (10 µL) of serum was added, mixed well and (2 ml) of the mixture was transferred to a measuring cell (1cm),then (34 µl) of (AChI 0.06M) was added, the changes in absorbency was measured after adding the substrate at 430 nm for 3 min. The inhibition percentage was calculated by comparing the activity between with and without inhibitor under the same conditions according to the equation:

$$\%Inhibition = (100 - (Vi\ /\ Vo)) \times 100$$

Vi is the activity in the presence of inhibitor and Vo is the activity in the absence of inhibitor.

2.6. Determination the type of inhibition

Constant concentrations of inhibitors (second higher inhibition) were being used with different concentrations of substrate (0.02, 0.04, 0.06 and 0.08M) to study the type of inhibition. These concentrations were prepared using the stock solution (0.1M) of AChI. The enzyme activity was determined with and without the inhibitors using the linewer-burk equation by ploting 1/V vs. 1/[s]following values were then calculated as follows: 1) Ki, 2) Apparent Vmax (Vmapp), 3) Apparent Km (Kmapp), 4) type of inhibition [14].

3. Results and Discussion

The new 2-pyrazoline-based ligand was prepared by Claisen-Schmidt condensation of 3-acetyl coumarine with N,N-dimethyl amino benzaldehyde followed by second step of ring closure the chalcone product ,A with the alkaline solution of thiosemicarbazide. The progress of reactions completion was indicated by TLC technique after determination the purification in ethyl acetate: hexane eluent chromatogram. The table (1) represents the main characterization of the chalcone, ligand and its complexes through measurements of elemental analyses, melting points, colors and their percent yields. However the elemental analysis data for the chalcone, and the free pyrazoline ligand are consistent with their chemical formula $C_{21}H_{18}N_4O_2S$. The metal complexes of cobalt(II) and copper(II) were determined their molecular formulas on the basis of (C.H.N.S) elemental analyses and flame atomic absorption spectroscopy (FAAS) and the observed results are in well-agreement with their theoretical calculations. Almost metal complexes derived from L ligand are insoluble in methanol, ethanol and DMSO due to their bi nuclear structures compared with the complexes formed with ligand which are sparingly soluble in DMSO, ethanol and methanol.

3.1. NMR Study

The H NMR spectrum of derivative chalcone in d6-DMSO solvent, Figure (1) showed singlet peak at 3.77 ppm assigning to the nuclear spin of up-field aliphatic –CH3 protons attached to N-atom to 6H protons. The de shielded protons of –CH=CH- were observed as singlet and doublet peaks around (8.80-9.80) ppm respectively. The spin-coupling constants (J) for the olefenic moiety confirmed the Claisen-Schmidt. The absorptions around (6.90-7.20) ppm are mainly ascribed to the aromatic Ar-H protons. The ring closure of chalcone with the thiosemicarbazide afforded 2-pyrazoline ligand which exhibited new peaks at around 9.02, (8.39-8.14) ppm which are resulted from the nuclear spin of –SH and –NH2 directly bonded to –N1 of pyrazole ring then supported the thiol and thione tautomer. As well as the appearance of doublet-doublet peaks at around (7.74-8.035) ppm and 4.35 ppm confirmed the vicinal and germinal –CH-CH2- in the pyrazoline ring [15, 16].

3.2. LC-MS spectra

The liquid-chromatography mass spectrum, Figure (1) of the free ligand showed peak m/z=390.90 assigning to the molecular ion of C21H18N4O2S+ and confirms the suggested structure. As well as the base peak with relative intensity 10% at m/z=290.05 would have given strong evidence for cleavage of weak points of –C6H4-N(Me)2 and C4H4 respectively [16].

3.3. FT-IR Spectra

The 2-pyrazoline ligands showed in its IR spectrum strong bands (1627-1635) and (1108-1329) cm-1 which are attributed to vibration modes of υ-C=N of pyrazoline ring, thioamide moiety H2N–C=S respectively [15,16].The disappearance of υ-C=O absorption in the IR spectra of ligand would have given strong evidence for ring closure to afford 2-pyrazoline ring , as well as, strong absorption at 1722 cm-1 never change that is belonged to carbonyl of coumarine derivative [9]. The IR spectra of cobalt(II) and copper(II) complexes showed broad absorption at around (3300-3420) cm-1 assigning to the hydrogen bonded –NH2 and –OH of coordinated water molecules in the inner-sphere of complexes structures. As well as the new shoulder bands in the regions (1590-1545) cm-1 beside the medium bands at (1265-1277) cm-1 could be associated with υ-C=N of pyrazoline ring . However the lowering in the wave numbers of υ(C=S) and υ(C=N) in regions (1090-1085) and (1545-1590) cm-1 respectively, confirms the participation of sulfur of thioamide moiety and nitrogen of 2-pyrazoline rings in the binding with metal ions to form stable five-member chelate rings. Furthermore the weak bands observed around (570-495) and (435-410) cm-1 indicating the appearance of new coordination bonds of M-N, M-O and M-S respectively [17].

3.4. Electronic Spectra and Magnetic Susceptibility measurements

The electronic spectra were recorded in the range (10,000-25,000) cm^{-1}. The cobalt(II) complex display three bands in the regions (1500, 750, 1030, 12100, 11000) cm^{-1} assigning to $^4T_1g(F) \rightarrow {}^4T_2g(F)$, $^4T_1g(F) \rightarrow {}^4A_2g(F)$ and $^4T_1g(F) \rightarrow {}^4T_1g(P)$ and confirms the octahedral geometry [17]. The geometry confirmed by high magnetic moment value in the range 4.75 B.M. due to the spin-orbital coupling [17, 18]. Furthermore, the green solution of copper(II) complex in DMSO showed two electronic spectral bands in the regions 13.800 and 28.600 cm^{-1} which may be assigned to the $^2Eg \rightarrow {}^2T_2g$ and LMCT transitions respectively confirming the distorted octahedral geometry which is further confirmed by the μ_{eff} in the range 1.75 B.M.

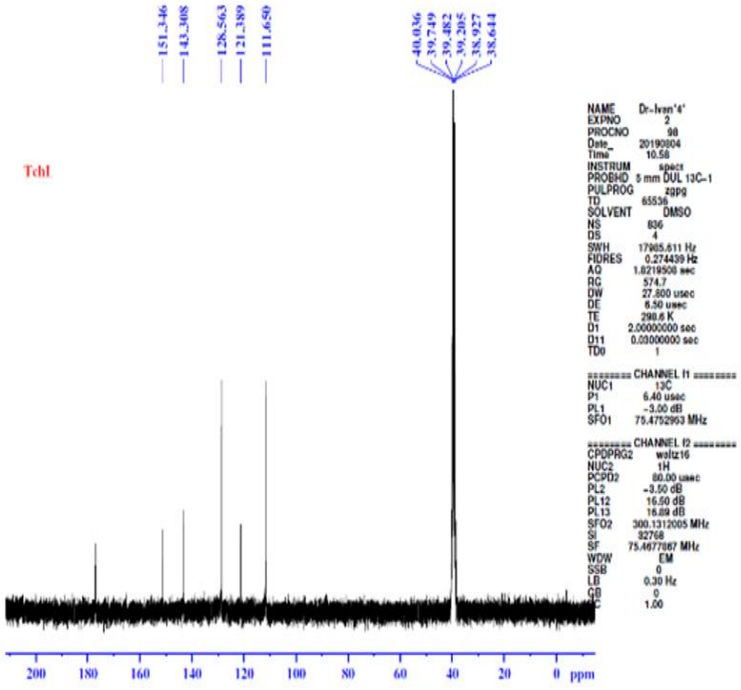


Fig.1: 1H NMR spectrum of (L) in DMSO-d6 solvent.

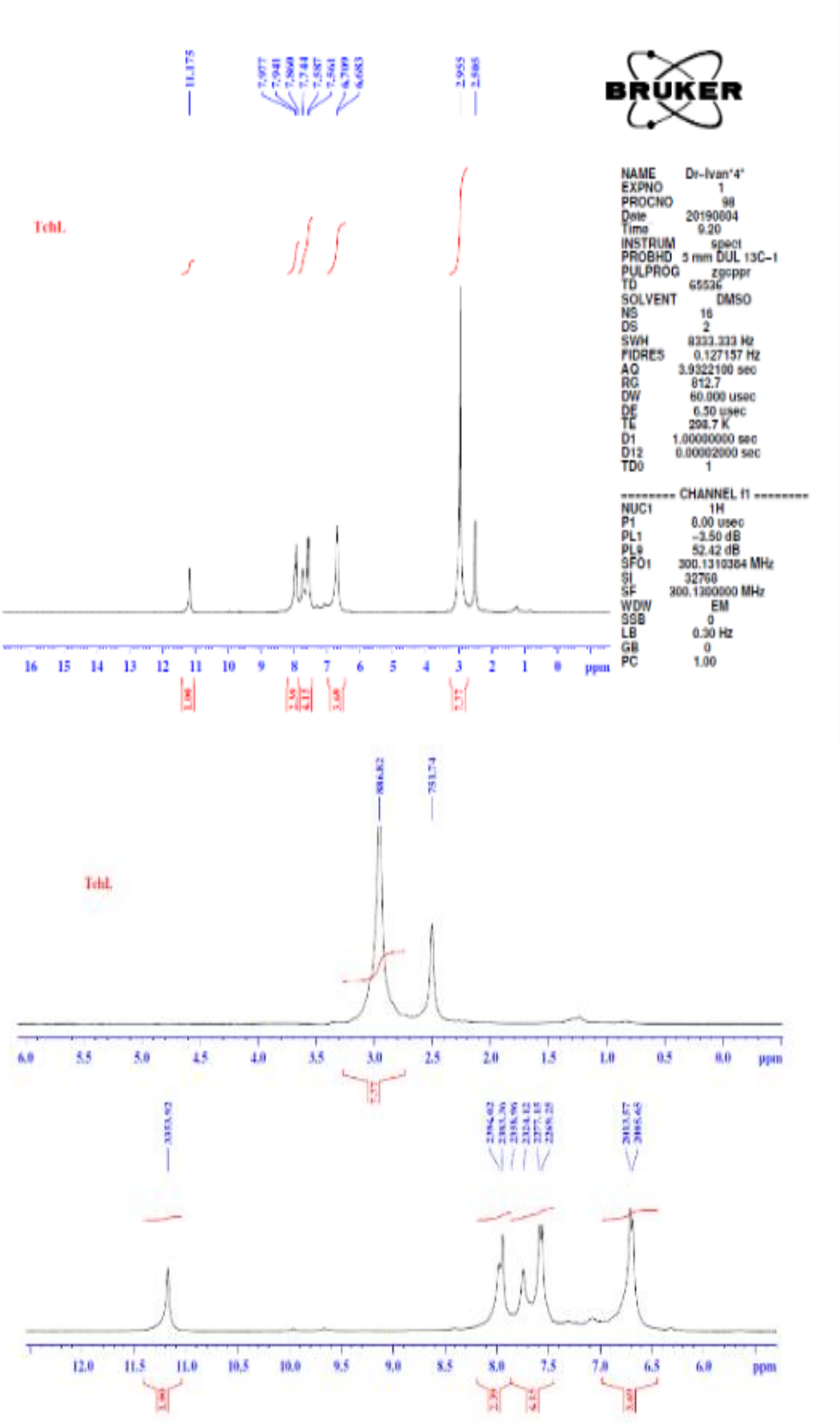


Fig. 2: 13C NMR spectrum of (L) in DMSO-d6 solvent

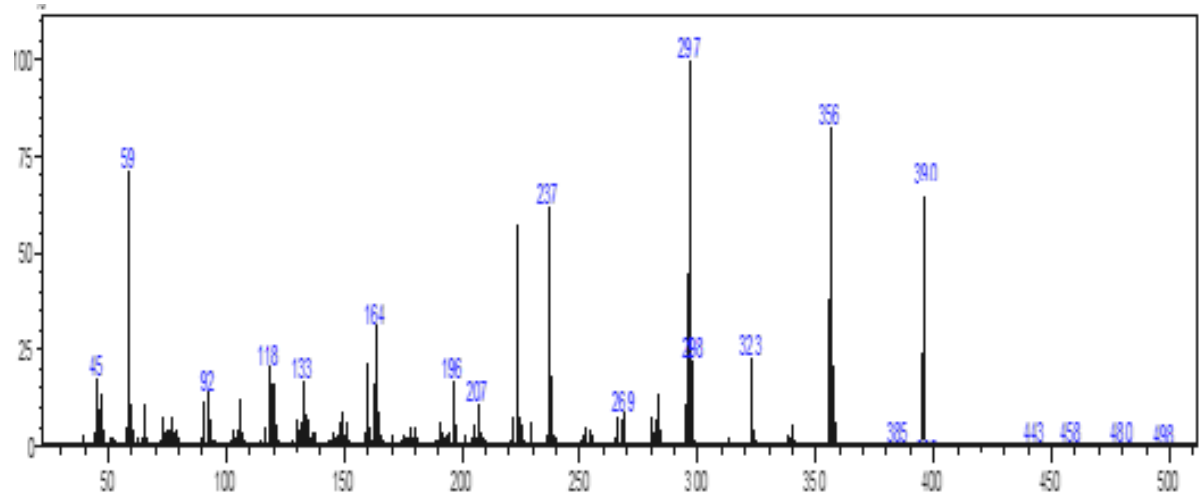


Fig. 3: Mass spectrum of L.

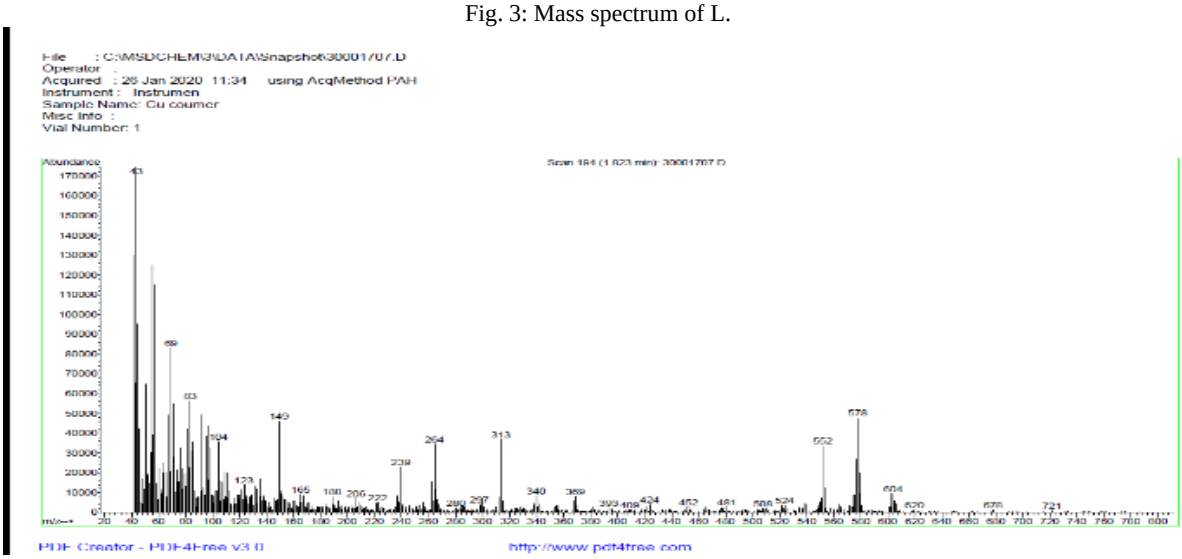


Fig. 4: LC-MS spectrum of CuL complex at 10 eV

3.5. Biological Study

The DNA binding abilities of the 5-[4-(dimethylamino)phenyl]-3-(2-oxo- 2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and with Cu(II), Co(II) complexes was evaluated by UV–Vis titration experiments, which was a useful method to calculate the DNA binding constants in vitro. After Calf Thymus DNA (CT DNA) was added to the phosphate buffer solution containing constant ligand and complexes concentrations, all of the solutions exhibited a slight hypochromic without any significant spectral shift. The binding constants of the compounds were calculated by using a plot of D/ΔƐapp versus D, as shown in Figure 5 and Table 1. Bis-naphthalimide derivative 4a with the shortest linker showed the largest binding constant (7.8 * 104, 8 * 108, 6.1* 103) M for close, Cu and Co binding with DNA respectively, Figure (5).

The change in ligand and complexes UV absorption spectra with increasing concentration of CT-DNA is shown in Figure (6). Specifically, both compounds showed hypochromicity upon increasing DNA concentration, indicating that both compounds interacted with the DNA helix. However, a weak hypochromic effect is indeed observed, and no red shift is apparent.

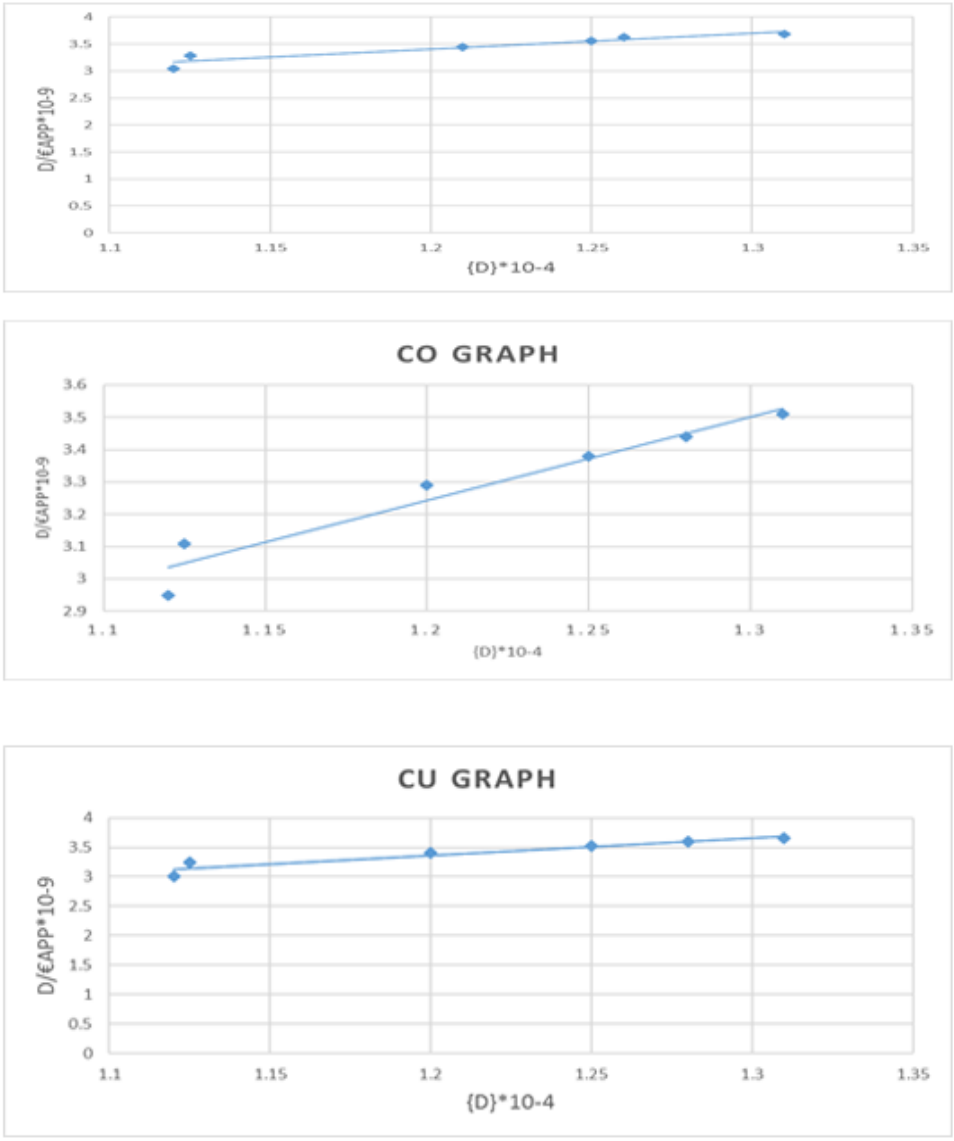


Fig. 5: D/Δ£app vs. D plot -[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and with Cu , Co complexes (50 µM) with CT DNA in PBS buffer (50.0 mM, pH = 7.4).

Table 1: Kb values of-[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and with Cu , Co complexes with CT DNA in PBS.

Compound	Kb(M^{-1})
a	$7.8 * 10^3$
b	$8 * 10^8$
c	$6.1 * 10^3$

The double-helical DNA consists of two complementary, anti-parallel, sugar–phosphate poly-deoxyribonucleotide strands which are associated with specific hydrogen bonding between nucleotide bases [9]. The backbone of these paired strands defines the helical grooves, within which the edges of the heterocyclic bases are exposed. The biologically relevant B-form of the DNA double helix is characterized by a shallow–wide major groove and a deep–narrow minor groove [9, 12]. Compounds most commonly bind to DNA by intercalating between the DNA base pairs or by binding in the DNA minor groove. Natural products such as netropsin and distamycin A are crescent-shaped molecules that contain an oligo (pyrrole carboxamide) chain and cationic end side chains that result in high affinity for the AT-rich sequences of DNA (30, 31). In principle, hybrid molecules that contain both intercalating and

minor-groove binding functionalities should be able to interact more strongly with DNA than those having either of the individual functionality, and thus should have a prolonged residence time on DNA allowing them to interfere with DNA processing enzymes. Transition metal complexes that are suitable for binding and cleaving nucleic acids are of significant current interest due to their various applications in nucleic acid chemistry, like foot-printing studies and sequence-specific binding agents and also as a putative anticancer drugs (33).The discovery of cis-platin in 1965 has given an impetus to study transition metal complexes as anticancer agents (34). Transition metal complexes with their varied coordination geometry, and versatile redox and spectral properties are often suitable for designing metal-based anticancer drugs (35). Since the last few decades, cis-platin and its analogues have been clinically used as effective chemotherapeutic drugs. Iron-bleomycins (Fe-BLMs) are natural antitumor chemotherapeutic agents that oxidatively cleave cellular DNA by targeting the deoxyribose sugar moiety [19, 20].

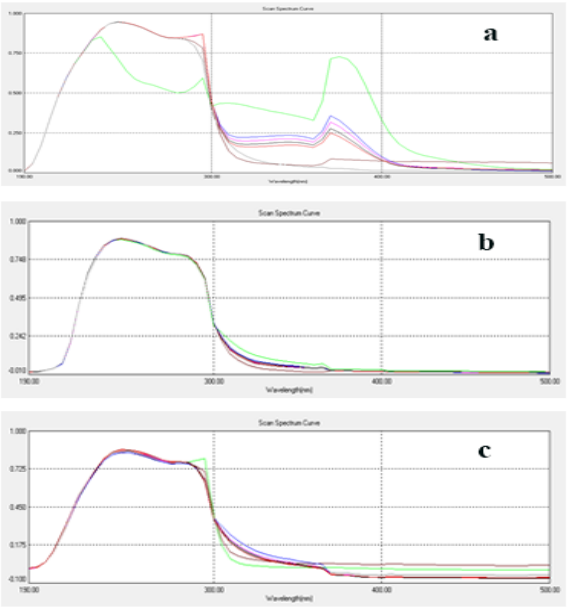


Fig. 6: Absorption spectra of compound -[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and with Cu , Co complexes, (2×10−5mol/L) in the presence of calf thymus DNA (CT-DNA) in tris buffer containing. [DNA]

In man acetylcholine can be hydrolyzed by choline esterase1 and acetylcholine esterase1). Measurements of choline esterase activity are clinically important in cases of suspicion of toxicity with organophosphate pesticides and heritable deficiency of the enzyme with risk of prolonged apnea following an aesthesia with the muscle relaxant succinylb is choline (suxamethonium). In this regard synthesizing and performing microbiological tests and evaluating the toxicity of compounds have been popular in many nations. Present work determined the activity of human AChE in the absence and presence of chromene and pyrimidine under different substrate concentrations and designed to investigate the biological activity and effects of prepared compounds. First experiment tried to study the effect of solvent DMSO which did not show any inhibitory effect as found and as Jaffer et al[41] found too. Then examine the chromene and its complexes with Co and Cu in the mixture at different concentrations (10^{-3}, 10^{-4},10^{-6},10^{-8},10^{-10},10^{-12}) M. Before each set of inhibition experiments were conducted, the AChE activity was measured by using four different concentrations of acetylthiocholineiodide (substrate) (0.02, 0.04, 0.06 and 0.08) M as in Figure (7).

The effect of different concentrations of each inhibitor at acetylcholine concentrations on AChE activity is illustrated in Figure 8.

The biochemical tests indicated that chromene and its complexes with Co and Cu have caused noticed inhibitory effects on enzyme activity compared with the measured normal values of enzyme activity 1.025 μ_{mol}/2min/ml, Table (2).

Table (2) showed that the greater inhibition percent was found at concentrations (10^{-3}) M for each compounds, these can be attributed to the presence of more than one nucleophile sides in compounds, chromene derivative compound having NH_2, carbonyl group in carbothioamide ring linked to pyrazole ring attached to chromene ring showed good AChE inhibitions, whereas Co and Cu complexes to chromene derivative still keep inhibition for AChE at same level, these groups may compete with substrate led to good orient to active site gorge of enzyme [19, 20].

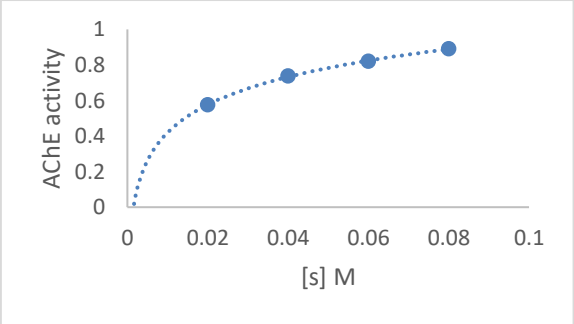


Fig. 7: The Michaelis-Menten plot of AChE at different concentrations of substrate and without inhibitor.

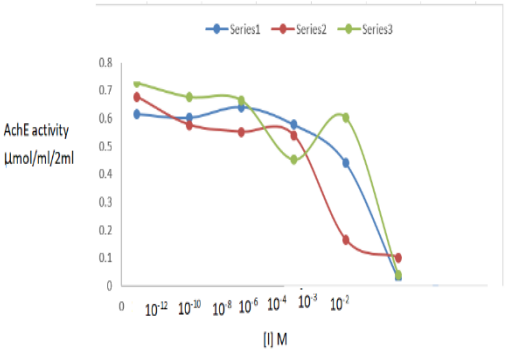


Fig. 8: Effect of different chromene and its complexes with Co and Cu concentrations (10^{-3}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}, 10^{-12}) M on AChE activity.

Huiet al, disclosed a rational design of novel series of potent 3-substituted 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives as AChE inhibitors, binding to the active site of human AChE substrate domain. Molecular modeling studies led to the identification of 3-substituted 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives, and the biological data were in full agreement with the proposed binding mode. 3-Substituted 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives may represent a leading structure to generate enzyme inhibitors as novel therapeutically entities for severe neurodegenerative diseases. The pharmacological study of 3-substituted 5*H*-thiazolo[3,2-*a*] pyrimidine derivatives targeting Alzheimer's disease will be reported in due course.

Hayrettin et al [19, 20] designed, synthesized, and evaluated the biological activities as potential acetylcholine esterase and butyrylcholin esterase inhibitors of a series of 6H-benzo[c]chromen-6-one, and 7,8,9,10-tetrahydro-benzo[c]chromen-6-one derivatives has been benzo [c] chromen-6-one moiety is present in various plant-derived nutrition, however, it has negligible inhibitory potential to inhibit cholinesterase enzymes, and found that the generation of benzo[c]chromen-6-one derived compounds for the inhibition of cholinesterase enzymes.

Table 2: The effect of different concentrations of chromene and its complexes with Co and Cu on the human serum AChE activity.

	Inhibition con. (M)	**AChE activity µmol/2min/ml**	**%Inhibition**
control	Zero	1.025	-
Close	10^{-3}	0.025	97.56
	10^{-4}	0.587	42.73
	10^{-6}	0.437	57.36
	10^{-8}	0.65	36.58
	10^{-10}	0.662	35.41
	10^{-12}	0.712	*30.53
Cu	10^{-3}	0.0875	91.51
	10^{-4}	0.15	85.71
	10^{-6}	0.525	48.78
	10^{-8}	0.537	47.60
	10^{-10}	0.562	45.17
	10^{-12}	0.662	35.41*
Co	10^{-3}	0.0125	98.78
	10^{-4}	0.425	58.53
	10^{-6}	0.562	45.17
	10^{-8}	0.625	39.02
	10^{-10}	0.587	42.73
	10^{-12}	0.6	41.46*

* Maximum inhibition concentration in each compound.

3.6. 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 (9) and Table (3).

From this presentation the study indicated that K_m was varied from higher or the same in the presence of chromene and its complexes with Co and Cu compared with non-inhibiting system. A high Km means the lesser affinity of substrate toward enzyme and the higher inhibitor affinity to fits very well into the active-site cleft of the enzyme which presence in 10^{-6}, 10^{-4} concentrations of Close and Co respectively (mix inhibition), in contrast Cu does not compute with substrate on the active site of enzyme (noncompetitive inhibition) .The chromene and pyrazole and carbothioamide group contributed toward acetyl cholinesterase inhibitor activity, and Cu, Co complexation it decrease the potency of the compounds when compare with the uncomplexation compounds (less Km and less Vmax). Coplexated atom must be the strong electron withdrawing nature of potent activity because it decreases electron density in the rings due to inductive effect.

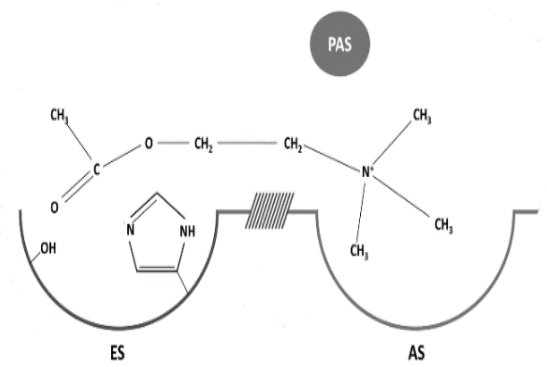


Fig. 9: Schematic representation of AChE binding sites: ES – esteratic site, AS – anionic substrate binding site, PAS – peripheral anionic binding site (46).

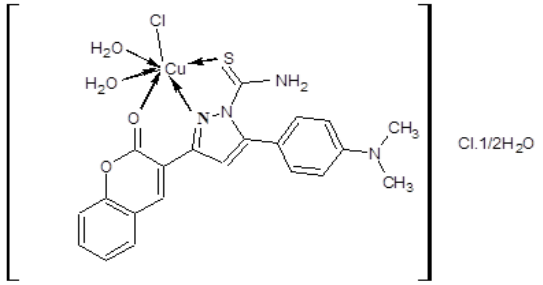
The affinity is obviously influenced by several factors, for example size, three-dimensional structure, presence of groups which easily bind non covalently to groups in or close to the active site etc. A consequence of such a good fit could be attributed to the orientation in space such that the covalent or hydrogen bonding to the serine residue. The Vmax value for control sample (1 **μ_{mol}/ml/min)** was higher than in inhibited samples, so it is clear that the amount of active enzyme Vmax present in non-inhibiting system. The biochemical tests revealed that Ki for Co and Cu complexes are higher (1.11×10^{-5}, 1.76×10^{-5}) than Close (3.7×10^{-7}) which mean that lesser Ki value have higher affinity to binding with enzyme at as clear in Table (2).

The 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 chromene and its complexes with Co and Cu exhibit different and same types of inhibition. The mixed inhibition by Close and Co can explain 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-competitive inhibition of Cu can be explain according to the classical models described that the inhibitor bind to another site that cause conformational change lock the enzyme and prevent the substrate binding or decreasing substrate affinity to AChE. In the case of reversible inhibitors being commonly applied in neurodegenerative disorders treatment, special attention is paid to currently approved drugs (donepezil, rivastigmine and galantamine) in the pharmacotherapy of Alzheimer's disease, and toxic carbamates used as pesticides. "Anionic" subsite is the binding site for some quaternary ligands that acts as the inhibitors of AChE (43, 44). In addition, some quaternary oximes, which act as deactivator's of AChE after inhibition by organophosphates, bind to "anionic" site (46).t22 20.

Table 3: Kinetic properties of AChE with and without hormone and its complexes with Co and Cu

Sample	Inhibitor Conc.(M)	K_m (M)	V_{max}(μ_{mol}/ml/min)	K_i (M)	Inhibition type
Control	Zero	0.02	1	-	-
Close	10^{-6}	0.066	0.729	3.7×10^{-7}	Mix
Cu	10^{-4}	0.02	0.1	1.11×10^{-5}	Non
Co	10^{-4}	0.043	0.299	1.76×10^{-5}	Mix

A new series of some novel pyrazinamide condensed 1,2,3,4-tetrahydropyrimidines was prepared and synthesized compounds were evaluated for acetyl and butyl cholinesterase (AChE and BuChE) inhibitor activity. The titled compounds exhibited weak, moderate or high AChE and BuChE inhibitor activity with an IC50 value of 0.11 μM and 3.4 μM [19].The researchers recently [20] reported herein the straightforward two-step synthesis and biological assessment of novel racemic benzo chromeno pyrimidinones as non-hepatotoxic, acetylcholine esterase inhibitors with antioxidative properties and found significantly lower inhibition for *h*AChE in comparison with *Ee*AChE, the IC50 values ranging from 1279 to 3657 nM. Compound 3Bbwas the most potent inhibitor with an IC50 value of 1279 nM. Recently the researchers [19] have focused on the synthesis a series of novel 1,2,3,4-tetrahydropyrimidines of biological interest and analyzed for their structures. The acetyl and butyl cholinesterase inhibitor activity data revealed that the all synthesized compounds proved to be active against acetyl and butyl cholinesterase enzymes. The novel studies of cytoxicity of pyrazoline complexes have docked and [17, 18] in *vitro* studied of dihydrobenzimidazo pyrimidine derivatives against acetylcholinesterase (AChE) and found that the compounds exhibit potent inhibitory activities with an IC50 of 46.8 nM and 42.5 nM respectively [19].



Scheme 3: Octahedral geometry of the prepared [CuLCl. $(H_2O)_2$]Cl.1/2(H_2O)]

Table 4: Elemental analyses and some physical properties of (L) and its complexes.

Compound Color	Δ S.cm^2/mole	M.P ^{0}C	%Found % (Calculated)				
			C	H	N	S	M[a]
L	12	187-189	64.60 (64.00)	4.65 (4.37)	8.21 (7.98)		
[CoL] Olive	75	300 dec	45.34 (59.58)	4.75 (3.84)	10.88 (11.01)		10.60 (9.22)
[Cu L] Olive	80	298 dec	44.96 (43.90)	3.99 (3.22)	9.33 (9.66)		11.33 (10.80)

Table.5 The electronic spectra in DMSO and magnetic moment for prepared complexes.

Compounds	**nmλ**	**$\sum_o$ L.mol^{-1} cm^{-1}**	**Assignment**	**μ_{eff}**
ligand				
[CoLCl.2H2O]	740	1500,750,1030,12100,11000	$^4T_1g_{(F)} \rightarrow {}^4T_2g_{(F)}$	4.75
	600	730	$^4T_1g_{(F)} \rightarrow {}^4A_2g_{(F)}$	
	500	1030	$^4T_1g_{(F)} \rightarrow {}^4T_1g_{(P)}$	
	395	12100	LMCT	
	230	11000	€→€*	
[CuLCl.2H2O]	625	150	$^2B_1g \rightarrow {}^2B_2g$	1.85
	510	90	$^2B_1g \rightarrow {}^2Eg$	
	345	2600	LMCT	
	276	12100	€→€*	

4. Conclusions

According to the data obtained from elemental analyses, FT-IR spectra and magnetic moments, the octahedral environment was concluded around cobalt(II) and copper(II) ions Furthermore, the results obtained from IR spectra confirmed that the ligand coordinated to the metal ions through nitrogen atom of pyrazoline ring –N2 and sulfur atom of thioamide respectively forming stable five-member chelate ring, Scheme (4).

The DNA binding abilities of the 5-[4-(dimethylamino)phenyl]-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-1-carbithioamide and it Cu , Co complexes appeared that all of the solutions exhibited a slight hypochromic without any significant spectral shift [23]. The binding constants of the compounds were calculated by using a plot of D/ΔƐapp versus D. Close ,Cu and Co showed the largest binding constant ($7.8 * 10^4$, $8 * 10^8$, $6.1* 10^3$) M for binding with DNA respectively . Specifically, both

compounds showed hypochromicity upon increasing DNA concentration, indicating that both compounds interacted with the DNA helix. In other hand the chromene and its complexes with Co and Cu were determined to be very good inhibitors against for AChE with different type of inhibition (mix and non-competitive) inhibition.

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