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New Tetra-dentate Schiff Base Ligand N₂O₂ and Its Complexes with Some of Metal Ions: Preparation, Identification, and Studying Their Enzymatic and Biological Activities

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Abstract

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In present work, new tetra-dentate ligand, titled 3,5-bis ((E)-5-Bromo-2-hydroxy benzylidene amino) benzoic acid (H₃L), was prepared via an acid-catalyzed condensation process. New four metallic ligand complexes with Co(II), Ni(II), Cu(II) and Zn(II) ions, were also prepared from the refluxing of equivalent moles. Ligand's structure and its complexes; were confirmed by numerous characterization methods, including Ultraviolet-Visible, Infrared, Mass Spectrometer, ¹H and ¹³C Nuclear Magnetic Resonance spectra, atomic absorption, magnetic moments, and molar conductivity measurements. The results of the spectroscopic analyzes proved that the prepared ligand acts as tetradentate bi-ionic ligand and it was bonded to the metal ions by two nitrogen atoms of the two azomethine groups and by two oxygen atoms of the two phenolic hydroxyl groups after losing their two protons. Octahedral structure proposed to all prepared complexes. The (anti-bacterial) and (anti-fungal) activities of these compound were screened against (E. coli, S. aureus, Klebsiella spp., S. epidermidis,), and (Candida albicans). The results indicated that these compounds have moderated inhibition behavior. The activity of the prepared compounds against Acetyl Choline Esterase Enzyme (AChE) have also studied and the obtained data indicated the presence of different inhibition behavior.

Key words: Acetylcholinesterase (AChE) activity, Anti-bacterial and antifungal, Tetradentate (N₂O₂) Schiff base complexes.

Introduction:

Generally, the ease of preparation of organic Schiff bases compounds (from the condensation among an aldehydes and a primary amines), and its ability to form stable coordination complexes (via chelation by azomethine group) with a different and huge number of metal ions in diverse oxidation states and coordination numbers, motivated the researchers in different scientific fields to prepare and use these privileged organic compounds ¹⁻⁶. The poly or tetra-dentate Schiff base ligands with N_2O_2 coordination system and their metallic complexes have been also gained the attention of scientific researchers due to excellent complexation ability, so it is used in analytical field to remove of some heavy pollutant metals (such as

Hg(II) and Ag(I)) from liquid media, in selective separation and purification of Cu(II) ions from mixtures, used as optical sensors for the determination of copper ion, and used as modified electrodes sensor of the aliphatic alcohols ⁷⁻¹¹. In biological fields, the complexes of N₂O₂ Schiff base ligands were used as anti-bacterial against gram negative and gram-positive types, antifungal, anticancer, antioxidant, as enzyme inhibitors, antiinflammatory, and DNA-cleavage in presence of hydrogen peroxide ¹²⁻²⁰. In catalytic reactions the tetra-dentate Schiff base ligands have been used for controlling the radical polymerization, as catalysts for oxidation of phenol, a good catalytic for increasing the chain length of the imine compartment, used as active catalyst for Suzuki and Heck reactions of Iodobenzene, used to catalyze the aerobic oxidation of catechol derivative, and used as catalyst for conversing of Naphthalene derivative to derivative of Naphthoquinone (K₃ vitamin), in green synthesis process ²¹⁻²⁶. Also some chelated complexes N₂O₂ Schiff base ligands reveals its coefficients as photoactive materials due to their high intensity of fluorescence ²⁷.

Materials and Methods:

All chemicals were acquired from suppliers companies and used as received. The IR spectra of all prepared compounds have been obtained (as a discs of KBr) in the range of 400-4000 cm⁻¹ by using Shimadzu spectrophotometer (FTIR) model (4800S). The electronic spectra registered by using (Cary 100 con.) spectrophotometer. A (digital) SMP30 Stuart apparatus utilized for detecting melting points. Mass analysis of ligand has been done with the (Shimadzu) GC-MS QP-2010. NMR spectra related to (¹H and ¹³C) recorded with Bruker DMX-500 spectrophotometer (300 MHz) by using (DMSO-d₆) as a solvent. The contents of metal ions determined with novAA350Analytic Jena spectrophotometer. The magnetic moment of complexes obtained by (Sherwood Scientific) Magnetic Susceptibility Balance. The molar Conductivity obtained for complex's solutions in $(10^{-3} \text{ M}, \text{DMSO} \text{ solution})$, using WTW 82362 Weilhiem-Germany, Inolab Multi 740.

Methods

Preparation of Ligand

A (25 mL) ethanolic solution contains (0.01 mol, 2.01 g) of 5-Bromo-2-hydroxy benzaldehyde, was acidified with glacial acetic acid (few drops), then added gradually to stirred ethanolic solution (15 mL), contains 3,5-diaminobenzoic acid (0.005 mol, 0.760 g). The product formed after (5 min.) as light yellow precipitate, then the final reaction mixture was refluxed for (1 hr.). The formed precipitate was filtered off, and then washed by ethanol, diethyl ether, and chloroform and distilled water (Scheme 1).



Scheme 1. Synthesis rout of ligand

Preparation of Complexes

We mixed (0.001 mol, 5 mL ethanol) from each metallic salt, with (0.001 mol, 25 mL ethanol) from ligand. Few drops of Triethylamine were added to ligand solution before mixing to liberate the phenolic oxygen atom. The reaction mixtures were refluxed for 3 hrs., and then the obtained colored products, were filtered, washed with diethyl ether, and chloroform (Scheme 2).

$$[M (H_2O)_{\mathbf{X}} (Cl_2)_{\mathbf{Y}} (CH_3COO)_{\mathbf{Z}}] + H_3L \xrightarrow{drops of (Ethyl)_3N} [M(HL)(H_2O)_2]$$

$$M = Co(II), Ni(II); X=6, Y=2, Z=0; M = Cu(II), X=2, Y=2, Z=0; M = Zn(II), X=2, Y=0, Z=2$$

Scheme 2. Preparation rout of complexes

Results:

Characterization of prepared compounds:

Some of physical properties, metal content and yield percentages of ligand and its complexes, are listed in Table 1.

| | | 0 | | | / |
|--|--|--------------|-----------------|--------|-----------------|
| Comp. | Chem. formula(M.Wt g.mol ⁻¹) | Color | M.P (°C) | Yield% | M% Calc.(found) |
| $[H_3L]$ | $C_{21}H_{13}Br_2N_2O_4$ (517.14) | Light yellow | 331-333 | 87 | |
| $[Co(HL)(H_2O)_2]$ | $C_{27}H_{31}Br_2N_3CoO_6$ (712.29) | Light brown | > 390 | 67 | 8.27 (8.12) |
| [Ni HL)(H ₂ O) ₂] | C ₂₇ H ₃₁ Br ₂ N ₃ NiO ₆ (712.05) | Dark brown | > 390 | 61 | 8.24 (7.15) |
| [Cu HL)(H ₂ O) ₂] | C ₂₇ H ₃₁ Br ₂ N ₃ CuO ₆ (716.90) | Olive | 254-258 | 72 | 8.86 (8.95) |
| [Zn HL)(H ₂ O) ₂] | C ₂₇ H ₃₁ Br ₂ N ₃ ZnO ₆ (718.76) | Lemon | 385-388 dec. | 42 | 9.10 (13.00) |

Mass Spectrum of Ligand

The Fig. 1, represents mass spectrum of ligand, which showed a mother ion peak at (m/z=518), corresponding to [M+H]. The suggested

pathways of ligand fragmentation and the structural assignments of each observed fragment are described in (Scheme 3).



Figure 1. Mass spectrum of ligand



NMR Spectra of Ligand:

The ¹H NMR spectrum of ligand, shows signals at (δ =12.70 ppm, s, 2H, phenolic OH), and (δ = 9.03 ppm, s, 2H, azomethinic protons) ². The aromatic protons are noticed as singlet (five protons) and doublet (four protons). The acidic proton of carboxylic group (COOH) did not appear as separated signal due to exchange with solvent ⁷. ²⁸. Where it appeared as broad and low intensity

signal at (δ =13.1 ppm) overlapped with the signal of phenolic hydroxyl group (O-H), as shown in Fig.2. ¹³C NMR spectrum of (H₃L), Fig.3, showed significant signals at (δ =166.4) ppm, (δ =163.0 ppm, and (δ = 159.2) ppm, assigned to carboxylic (C=O), phenolic (C-O), and azomethinic (C=N) carbons ⁶. Signals of aromatic carbons appeared at the range of (δ =110.8) ppm to (δ =135.8) ppm ²⁹. Baghdad Science Journal 2022, 19(1): 155-167



Figure 2. ¹HNMR spectrum of ligand



Figure 3. ¹³C NMR spectrum of ligand

NMR Spectra of Zn(II) Complex:

In ¹HNMR spectrum of Zn(II) complex, (Fig.4), the two phenolic protons disappeared due to coordination. Signals of two azomethinic protons shifted to downfield region (appeared at δ = 8.51 ppm), this confirmed the involvement of this characteristic group in coordination ¹. The signals of triethylamine molecule appeared at (δ =0.842 ppm, t, 3H) and (δ =2.53 ppm, q, 2H), respectively ²⁴. The ligand spectrum also revealed another new signal at (δ =5.16 ppm, s, 2H), related to coordinated H₂O ³⁰. The ¹³C signals of Zn(II) complex did not appear clearly due to poor solubility.

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Figure 4. ¹HNMR spectrum of Zn(II) complex

FTIR Spectra:

The infrared bands assignments of ligand and its metallic complexes have been listed in Table 2. The FTIR spectrum of ligand, displayed the stretching frequency of carboxylic (O-H) group as medium intensity broadband at the frequency of (2500-3100) cm⁻¹ ³¹. Stretching frequency of the carboxylic (C=O) group appeared as strong band at (1697) cm⁻¹ for free ligand, this group disappeared in spectra of all complexes due to formation of carboxylate ion since we added triethylamine (as deprotonating agent) to their solutions ^{31,32}. The stretching frequency of the (CH=N) group appeared as a strong band in (1620) cm⁻¹ for ligand, this band has been shifted to lower frequencies in the spectra of prepared complexes, this shifting proofs the

bonded of it with metal ions ²⁹. The ligand phenolic (C-O) bonds revealed stretching band at (1279) cm⁻ , which blue shifted in spectra of complexes, because it coordinated with metal ions. The additional and new bands noticed in complexes spectra at the frequency range (532-523 and 463-449) cm⁻¹, were attributed to v(M-O) and v(M-N), respectively ³³. The spectra of all prepared complexes also revealed new bands at (3438-3388) cm⁻¹ assigned to stretching vibrational of coordinated water molecules ³³. The new bands appeared at (879-877) cm⁻¹ in complexes spectra, also proofs the presence of coordinated (H₂O) molecules ³³. The Fig. 5 represents the FTIR spectra of ligand and Cu(II) complex.



Figure 5. FT-IR spectra of [H₃L] ligand and Cu(II) complex

| Table 2. Assignment of FT-IR (v ⁻ , cm ⁻¹), of prepared compounds | | | | | | |
|--|-------------------------|--------|--------|---|------------------|------------------|
| Compound | v(OH, H ₂ O) | v(C=O) | v(C=N) | vas(COO ⁻) vs(COO ⁻) | v(C-O) v(C-N) | v(M-O) v(M-N) |
| [H ₃ L] | 2500-300 | 1697 | 1620 | | 1279 1192 | |
| $[\mathbf{C}_{\mathbf{O}}(\mathbf{H})(\mathbf{H},\mathbf{O})]$ | 2288 | | 1616 | 1562 | 1304 | 526 |
| $[CO(\Pi L)(\Pi_2 O)_2]$ | 2200 | | 1010 | 1379 | 1173 | 451 |
| | 3375 | | 1618 | 1566 | 1306 | 528 |
| | | | | 1381 | 1174 | 449 |
| $[C_{11} HI] (H O)$ | 3431 | | 1612 | 1570 | 1311 | 532 |
| $[Cu \Pi L)(\Pi_2 O)_2]$ | | | | 1377 | 1178 | 463 |
| [7n HI)(H O) | 3438 | | 1616 | 1566 | 1308 | 523 |
| $[LII \Pi L)(\Pi_2 U)_2]$ | | | | 1383 | 1157 | 459 |

Molar Conductance, Magnetic Properties and Electronic Spectra of Complexes:

The conductivity values of complexes were recorded at temperature of laboratory, for DMSO solutions (10^3 M) , and they confirmed a nonelectrolyte nature of complexes. Values of effective magnetic moment were (Co(II)= 4.88 B.M), (Ni(II)= 3.15 B.M) (Cu(II)= 1.8 B.M), and (Zn(II)= 0.00 B.M), and these values were in agreement with suggested geometries. The electronic spectra of all prepared compounds were recorded for (DMSO solution, 10^{-4} M). The discolored solution of (H₃L) ligand, shows two peaks the first at (258 nm, 38759 cm⁻¹), assigned to

 $\pi \rightarrow \pi^*$ of benzene rings ³⁴. The second absorption peak was noticed at (337 nm, 29673 cm⁻¹), assigned to $n \rightarrow \pi^*$ transitions of nonbonding electrons related to (HC=N) groups ²⁹. In spectra of all complexes the second peak shifted to higher wavelength (red shift), where appeared at the range of (396-403 nm), this confirms the involvement of two (CH=N) groups in coordination with metallic ions. Spectrum of Cu(II) complex, showed new peak at (690 nm, 14492 cm⁻¹) assigned to ²Eg \rightarrow ²T₂g transition of octahedral geometry ^{29,35}. The Fig. 6 represents the electronic spectra of ligand and Co(II) complex Table 3.



Figure 6. Electronic spectra of ligand and Co(II) complex

| Table 3. Molar Conductance, Magnetic Properties, Electronic Spectra, and Suggested Geometry of | of |
|--|----|
| Prepared Compounds | |

| Compound | $\lambda \max nm,$ (υ cm ⁻¹) | € Max M ⁻¹ . C ⁻¹ | Assignment | µeff (B.M) | Molar conductance (µs. cm ⁻¹) | Geometry |
|--|---|--|---|---------------|---|----------|
| $[H_3L]$ | 258, (38759) 337, (0.43) | 7100 4300 | $\pi \rightarrow \pi^* n \rightarrow \pi^*$ | | | |
| $[Co(HL)(H_2O)_2]$ | 260, (1.77) 398, (0.7) | 17700 7000 | $\pi \rightarrow \pi^*$ CT | 4.88 | (7.3) | Oh |
| [Ni(HL)(H ₂ O) ₂] | 258, (0.58) 396, (0.17) | 5800 1700 | $\pi \rightarrow \pi^*$ CT | 3.15 | (3.6) | Oh |
| [Cu(HL)(H ₂ O) ₂] | 259, (1.15) 403, (0.381) 690, (0.0725) | 11500 3810 725 | $\pi \rightarrow \pi^* CT$ $^2Eg \rightarrow ^2T_2g$ | 1.80 | (2.9) | Oh |
| [Zn(HL)(H ₂ O) ₂] | 258, (1.25) 284, (0.72) 403, (0.66) | 12500 7200 6600 | $\begin{array}{c} \pi \rightarrow \pi^{*} \\ \pi \rightarrow \pi^{*} CT \end{array}$ | (0.00) | (2.5) | Oh |

Biological Studies:

Both of (*in-vitro*) antimicrobial studies of prepared compounds were accomplished by following disc diffusion method. We prepared two concentrations $[10^{-3} \& 10^{-4}]$ M, from each prepared compounds to evaluate the susceptibilities of bacteria and fungi, by measuring the diameter (mm) of inhibition zone (IZ), which surrounded the holes, after incubating the plate for 24 hrs., at (37 °C). (DMSO) solvent did not reveal any growth of inhibition, so it was used as negative control ²⁹. For concentration of $[10^{-4} M]$, all prepared compounds

did not reveal any inhibitor effect against the growth of bacteria and fungi, except (H₃L) ligand, showed moderate inhibitor effect against the growth of *S*. epidermidis. The solution of Ni(II) complex at $[10^{-3} \text{ M}]$ exhibited inhibitor effect against the growth of E. coli only. The solutions of (H₃L), Co(II), and Zn(II) At $[10^{-3} \text{ M}]$, only exhibited inhibitor effects against the growth of Candida fungi. The obtained results of antimicrobial studies are listed in Table 4. The results of both studies graphically are represented in Fig.7.

| Table 4. Data of Zone Inhibition (mm), of prepared compounds. | | | | | | |
|--|--------------------------------|----------------|---------|----------------|---------|--|
| Comp. (Symbol) | G ⁺ bacteria | | Gra | Fungal | | |
| [Cons.] | | | | | | |
| $A-E = 10^{-3} M,$ | S. aureus | S. epidermidis | E. Coli | Klebsiella sp. | Candida | |
| $F-J=10^{-4}M$ | | | | | | |
| (DMSO) | 0 | 0 | 0 | 0 | 0 | |
| $\left[\mathrm{H}_{3}\mathrm{L}\right]^{\mathrm{a}}(\mathrm{A})$ | 0 | 11 | 11 | 10 | 11 | |
| $[Co(HL)(H_2O)_2]^a(B)$ | 13 | 11 | 11 | 10 | 12 | |
| $[Ni(HL)(H_2O)_2]_2^a(C)$ | 0 | 0 | 11 | 0 | 0 | |
| $\left[Cu(HL)(H_2O)_2\right]^a(D)$ | 10 | 10 | 0 | 10 | 0 | |
| $[Zn(HL)(H_2O)_2]^a(E)$ | 0 | 14 | 12 | 14 | 19 | |
| $[H_3L]^b$ (F) | 0 | 10 | 0 | 0 | 0 | |
| $\left[\operatorname{Co}(\operatorname{HL})(\operatorname{H}_2\operatorname{O})_2\right]^{\mathrm{b}}(\operatorname{G})$ | 0 | 0 | 0 | 0 | 0 | |
| $[Ni(HL)(H_2O)_2]_2^{b}(H)$ | 0 | 0 | 0 | 0 | 0 | |
| $\left[\operatorname{Cu}(\operatorname{HL})(\operatorname{H}_2\operatorname{O})_2\right]^{\mathrm{b}}(\operatorname{I})$ | 0 | 0 | 0 | 0 | 0 | |
| $\left[\text{Zn}(\text{HL})(\text{H}_2\text{O})_2\right]^{\text{b}}(\text{J})$ | 0 | 0 | 0 | 0 | 0 | |





Figure 7. Zone diameter (mm) of inhibition for synthsized compounds agaist picted pathogenes

Determination the Activity of AChE:

Ellman et al. ³⁶ procedure was followed for evaluating the AChE activity in human serum, by mixing (50 μ L, 0.001 M) of DTNB solution with (2.25 mL, 0.2 M) of sodium phosphate buffer solution (pH=7.3), the final solution mixed well with (10 μ L) of serum. 2 mL were added from the later solution and mixed with (34 μ L, 0.06 M) of acetyl thiocholineiodide (ASChI). We recorded the absorbance change for (3 min) at (λ = 430 nm) before and after adding of the substrate .

Evaluation the Enzyme Activity of Prepared Compounds:

From stock (DMSO solution, 0.1 M) of each compounds, we prepared series of concentrations $(10^{-3}, 10^{-5}, 10^{-7}, 10^{-9} 10^{-11} \text{ and } 10^{-13} \text{ M})$. The percentage of enzyme inhibition calculated under the same conditions, where we compared the

activity of enzyme (without and with) inhibitor, according to the following equation:

%Inhibition

= 100

The activity in the presence of inhibitor The activity in the absence of inhibitor

*** 100**

Determination the Inhibition Type:

Four concentrations [0.02, 0.04, 0.06 and 0.08] M, of acetylthiocholine iodide (AChTI, substrate), were used to study the inhibition type, were being used with constant concentrations of inhibitors (lower and higher) by using (0.1M) of AChI as stock solution. The activity of enzyme was assayed using

the lineweaver-burk equation in presence and absence of tested compounds by ploting 1/[V]versus 1/[S], then the type of inhibition constant Ki, maximum velocity Vmax and michalis-menton constant Km were detwrmined. At first, we examined the effect of solvent (DMSO), which no longer exhibited any inhibitory effect as observed and as Z. Nabeel observed too ³⁷. The tested complexes in the mixture at exceptional concentrations [10⁻³, 10⁻⁵, 10⁻⁷, 10⁻⁹, 10⁻¹¹ and 10⁻¹³ M] (Fig.8). Figure 9 illustrates the characteristic effects of inhibitors concentrations [M] acetylcholine (AChE) concentrations.



Figure 8. Michaelis-Menten plot for activity of AChE towered substrate



Figure 9. Effect of concentrations [M] of prepared complexes on AChE activity

As illustrated above, benzylidene amino benzoic acid (ligand) complexes caused noticeable inhibition effects on enzyme activity, if we make a comparison with the normal value of it (1.27 umol/2min/mL). The second section of this study included an understanding of the type of inhibition and calculate the some kinetic parameters, such as;

Km, Vmax, and Ki, at extraordinary of substrate concentrations and beneath of the same stipulations. The graph of Lineweaver-Burk showed that the complexes inhibited AChE by two types of inhibition (Mix and Non), and gave various values of Ki (Table 5), and (Figs.-10-12), illustrate all results of these two studies.

| Table 5. Kinetic parameters and the type of inhibition | | | | | | |
|--|--------------------|---------------------------------------|-------------------------------|-----------------------|-----------------|--|
| Comp. | Inhibitor Conc.(M) | $\mathbf{K}_{\mathbf{m}}(\mathbf{M})$ | $V_{max} (\mu_{mol}/mL/2min)$ | $K_{i}(M)$ | Inhibition type | |
| Control | 0.00 | 0.016 | 2 | - | - | |
| Со | 10 ⁻³ | 0.05 | 1.25 | 1.6×10^{-5} | Mix | |
| Cu | 10 ⁻³ | 0.016 | 0.833 | 7.14×10^{-4} | Non | |
| Zn | 10 ⁻⁵ | 0.016 | 1.11 | 1.25×10^{-5} | Non | |



Figure 10. The Lineweaver-Burk Plot of AChE activity of Co(II) complex at maximum inhibitory concentration (10⁻³ M)



Figure 11. The Lineweaver-Burk Plot of AChE activity of Cu(II) complex, at maximum inhibitory concentration (10⁻³ M)



Figure 12. The Lineweaver-Burk Plot of AChE activity of Zn(II) complex at maximum inhibitory concentration (10⁻⁵ M)

From this analysis, the study showed that Km ranged from higher, same in the presence of complexes relative to the non-inhibiting model. A high value of (km) indicates a lower affinity of substrate [s] toward the enzyme and a higher affinity of inhibitors to attach with the (active-site) cleft of the enzyme, that is present in Co(II) complex (mix inhibition); while, the Cu(II) and Zn(II) does not quantify the substrate at the (activesite) of the enzyme, (non-competitive inhibition). AChE showed inhibition constant (Ki) in the range $(10^{-4}-10^{-5} \text{ M})$ in presence of maximum inhibitors concentrations, which is probably due to variant type of inhibition from non and mix. (Table 5), clearly revealed that the (Vmax) value of control sample (2 µmol/mL/2min) in normal sample was larger than that of inhibited samples, so it is evident, that the amount of active enzyme (Vmax) is present in non-inhibited system.

Mesut et al. ³⁸ found that the synthesized derivatives of sulfonamides show potential inhibitor properties for AChE with Ki constants in the range of $2.54 \pm 0.22 - 299.60 \pm 8.73 \mu$ M. The derivatives of sulfonamides exhibited different inhibition type. We determined that the derivatives (S1, S1i, S3, and S3i) showed a competitive inhibition effect, whereas others (S2, S2i, S4, and S4i) showed mixed-type inhibition. As a result, the sulfonamide derivatives can used as an alternative acetylcholinesterase inhibitor due to this effect. Inhibitors with fewer side effects are thought to be important in the treatment of AD.

39 al. showed Ahmed et antiacetylcholinesterase activity of 4-(1.3-Dioxoisoindolin-2-yl)-N-Phenyl Benzamide Derivatives was assessed by Ellman's test. Compound 4g in this series exhibited the highest inhibitory potency (IC50 = $1.1 \pm 0.25 \mu$ M) compared to donepezil (IC50 = $0.41 \pm 0.12 \mu$ M) as reference drug. Catarina et al. ⁴⁰ concluded in general, tert-butyl derivatives effectively inhibited AChE, being compound 33 the most potent $(IC50 = 8.3 \pm 0.3 \,\mu\text{M}; \text{Ki } 5.2 \,\mu\text{M})$. The data pointed to a non-competitive inhibition mechanism of action, which was also observed for the standard donepezil.

Conclusions:

The newly synthesized Schiff bases ligand and its complexes with Co(II), Ni(II) Cu(II), and Zn(II), were characterized via various and different physical and analytical studies. The collected data proved that the ligand behaves as a dibasic N_2O_2 tetra-dentate ligand; with forming thermally stable mononuclear metallic complexes. According to the results of different techniques, we suggest an octahedral environment around each metal ions. The synthesized compounds revealed moderate inhibition behavior against some of the chosen pathogens (gram-positive, gram-negative bacteria and Candida fungi) for solution of $(10^{-3}M)$, while the lowest concentration of prepared compounds (10^{-4} M) , did not exhibit any noticeable inhibition behaviors. The enzyme activity of complexes against Acetyl Choline Esterase Enzyme (AChE) were also studied and the obtained data indicated the presence of two different inhibition behaviors (mixed and non-competitive).

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mustansiriyah University.

Authors' contributions statement:

Basim H. Al-Zaidi and Zaizafoone N. Nasif contributed in analysis, interpretation, and drafting the MS, Marry contributes in revision & proofreading of the MS, Amer S. Mahdi contributes in conception and design of the MS, and Ahmad H. Ismail contributes in acquisition of MS data.

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ليكاند قاعدة شف جديد رباعي المخلب ذو الصيغة N2O2 ومعقداته مع بعض الايونات الفلزية: التحضير، التكاند قاعدة شف جديد رباعي التشخيص ودراسة فعالياتها الانزيمية والبايولوجية

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الخلاصة:

الكلمات المفتاحية: فعالية الأستيل كولين استريز (AChE) ، مضادات البكتيريا والفطريات معقدات قواعد شف رباعية المخلب. (N2O2)