**Preparation, Structural Identification and Biomedical Evaluation of Some New Complexes**

**Alyaa Khider Abbas\* , Asmaa Edrees Fadhil**

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

College of Pharmacy, Al-Turath University, Baghdad, Iraq

**Abstract**

A new (AH) nano mono azo ligand [ 2-amino-6-oxo-6, 7- dihydro-1H-purin-8-yl)diazenyl)nitrobenzene with its metal ions complexes [Ag(I),Cu(II), Zn(II)] were successfully synthesized and identified depending on elemental analyses, magnetic susceptibility, molar conductance, spectroscopic techniques (FT-IR,UV-Vis, HNMR) and thermal analysis (TGA). The FT-IR spectra showed that ligand acts as a neutral N, N-bidentate. Molar ratio results were deducted that the metal to ligand [M:L] ratio was [1:1] for [Ag(I) and Zn(II)] complexes, while it was [1:2] for [Cu(II)]complex. The stability constant and Gibbs free energy were researched spectrophotometry for all metal ions complexes, since all the complexes have high stability determined the crystallography advantage of the ligand (AH) with silver and copper complexes were performed by SEM and X-ray diffraction they were found that they have nanoscopic properties. the data was shown that the studied compound has nano properties. Microbiological investigation for the ligand (AH) and its complexes with assurance on its application as a novel antibacterial and antifungal agent. In addition, the anti-oxidant and anti-inflammatory properties were examined. They showed high effectiveness

**Keywords**

spectroscopic techniques; anti-microbial; anti-oxidant; burn healing.

1. **Introduction**

Azo dyes, which represent approximately or more than half of all commercial dyes or colorants, are by far the most significant dye class. They have been widely applied in numerous scientific and technological fields. [1,2] More studies has been done on azo dyes than any other dye class because of how easily diazotization and azo coupling operations can be carried out[3]. Azo dyes containing heterocyclic rings have also been researched due to their excellent qualities. They serve as efficient colouring agents in a variety of textile-related applications. It has been possible to create excellent colorants with strong chromophores, gorgeous colours, and high-level dyeing and fastness properties by using heterocyclic rings as coupling components[4]. The synthetic azo compounds displayed antifungal, anticancer, and antibacterial action, all without being hazardous to healthy cells. Due to their low toxicity which includes allergic and hyperactive reactions—azo dyes are frequently utilized in food, medicine, paint, polymer chains, and other typical products. Recent years have seen a large number of investigations on azo compound production and conformational characteristics [5-7].

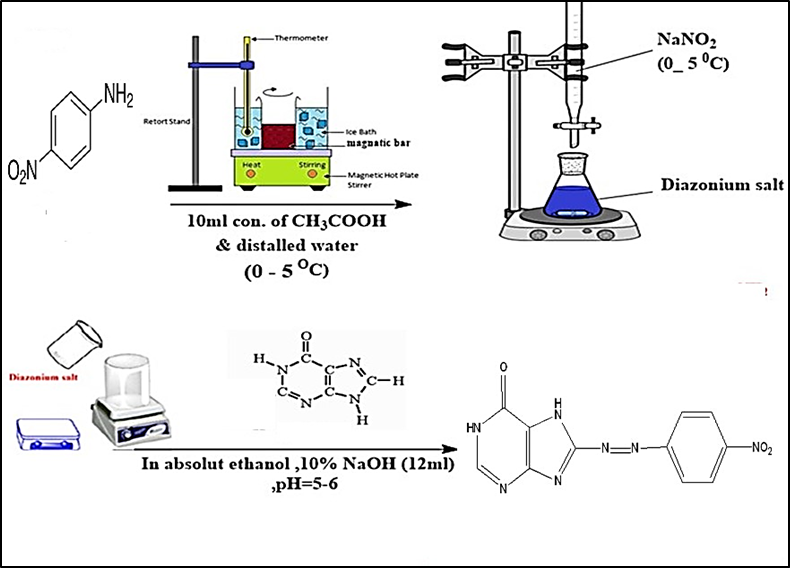
Hypoxanthine, a purine derivative, has long been recognized as a pivotal molecule in various cellular processes[8,9]. Despite its relatively low abundance compared to other purine metabolites, emerging research has shed light on its multifaceted roles in cellular metabolism, signalling pathways, and disease pathogenesis. This review comprehensively explores the metabolism of hypoxanthine, its physiological functions, and its implications in health and disease. Additionally, it discusses the potential therapeutic strategies targeting hypoxanthine metabolism for the treatment of various disorders. Purine metabolism is a highly regulated biochemical pathway crucial for cellular function and homeostasis. Hypoxanthine, an intermediate metabolite in this pathway, plays pivotal roles in nucleotide synthesis, energy metabolism, and signaling cascades. While historically overshadowed by its counterparts adenine and guanine, recent advancements have highlighted the significance of hypoxanthine in diverse physiological processes and pathological conditions[10].In this work, the main goal is to prepare a new complexes of [Ag(I),Cu(II), and Zn(II)]from the ligand (AH)and identified by different physicochemical methods based on various spectral methods, thermal analyses and analytical techniques. The antimicrobial, antioxidant, and anti-inflammatory activities were screened.

1. **Experimental**
2. Materials and instruments

The materials were utilized of analytical grade and without further purification. (C.H.N) is used to define the elemental analyses of the (AH) mono azo ligand and its complexes by (Eure EA 3000 Elemental analyzer), while the metal content was calculated by atomic absorbtion (A.A.) and using ("Nova350.0 Spectrophotometer") . The SHMADZU 8400s spectrophotometer was utilized to enrolment infrared spectra in the range (400- 4000) cm-1 using KBr. The UV-Vis spectra of all the compounds under investigation were measured with (SHMADZU1800–UV-Vis spectrophotometer). The measuring of 1HNMR spectra were done with a (BRUKR AV 400. [Avance-III]) (400 MHz. and 100 MHz.). Thermal analyses (T.G.A.) was performed on (SDT Q600 V20.9 Build). The melting points of each chemical were calculated using the (Gallnkamps) melting point device. The Mohr method was used to find the cloride concentration in the complexes, wether its in the form of counter ion or coordinated .Using a Sherwod Scientific Auto Magnetic Susceptibility Balance Model, to assessed the magnetic susceptibilities of the investigated complexes at surrounding temperature. Finally the molar condicutence of the synthesised complexes were estimated by HI9811-5 HANNA instruments.

1. Synthesis of hetero azo ligand (AH)

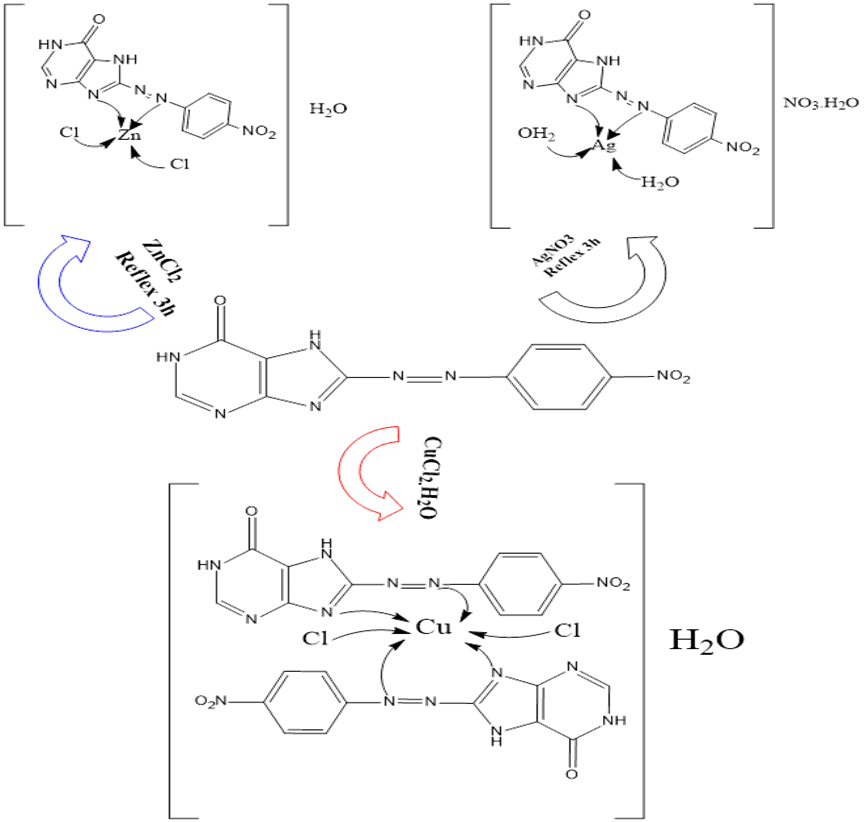
The new mono azo ligand (AH) was prepared by utilizing general procedure as previously notified [3]. P-nitro aniline. (1.38gm; 0.01mol) where deployed as primary amine to prepare diazoniume salt, while (1.36gm; 0.01mol) of hypoxanthine as Coupling. Scheme (1) represents the new (AH) ligand Preparation process.



Scheme 1: Proposed route for Ligand (AH) Synthesis

1. Preparation of new metal ions Complexes

(Cu-AH) complex was synthesized in molar ratio [1:2; M:AH], while (Ag-AH and Zn-AH) complexes were synthesize in mole ratio [l:l; M:AH]. The synthesized path was depicted in scheme 2 and follow the reaction with TLC technology[5] by utilizing a mixture solvents [1.5m/ ammonia, 1m/ butanol and 1m/ methanol]. Table 1 was shown some physical and chemical features.



Scheme 2: Proposed route for Ligand AH- Synthesis

Table 1: Physical Features And Elemental Analyses Properties

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Comp. (M.wt) (gm/mol) | Color  λmax  (nm) | M:L | Ʌm  (S.mol-1.cm2) | % Experimental  % (Theoretical) | | | | |
| C | H | N | M | Cl |
| 1 | AH(C11 H 7N 7O3)  285 | yellow  420 | --- | --- | 46.82  46.31 | 3.000  2.450 | 34.74  34.38 | --- | --- |
| 2 | [Ag(AH)(H2O)2]NO3.2H2O  526.86 | orange  480 | 1:1 | 80.1 | 25.88  25.05 | 2.921  2.84 | 21.72  21.25 | 20.900  20.47 | --- |
| 3 | [Cu(AH)2 Cl2].H2O 722.08 | green  620 | 1:2 | 37.3 | 36.87  36.53 | 2.44  2.21 | 27.94  27.12 | 8.450  8.79 | 9.408  9.82 |
| 4 | [Zn(AH) Cl2].H2O  439.65 | pink  510.00 | 1:1 | 26.7 | 30.70  30.04 | 2.23  2.04 | 22.61  2.30 | 15.15  14.88 | 16.75  16.15 |

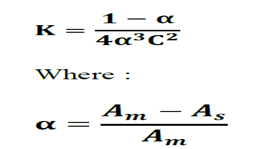
1. **Results And Discussion**

Scheme (1) outlines the synthetic pathways leading to the synthesis of novel azo Hypoxanthine azo ligand [AH]. The synthesis of the ligand (AH) utilized starting materials containing primary aromatic amine moiety [p-nitro anline]. That was subjected to diazotization reactions at (0-5) °C which yielded diazonium salt as inter- mediates, so the diazonium salt will be decomposed and explosive at a temperature high than (0-5) °C; therefore, it is synthesized in this range of temperatures and is used immediately [5, 6]. Then diazonium salt was coupled with Hypoxanthine as a nucleophile in an ethanolic alkaline solution. So, the alkaline means increases nucleophilicity and avoids dissociation [11], while the diazonium salt acts as an electrophile and the major interest of the diazo component for the synthesized ligand [AH] are that the short time interaction, very high yield and the method involve only one easy step, Additionally, the nitrogen atoms include an azo moiety with a pair of electrons, which acts as a binding site for coordination with a metal ion. The presence of donor atoms, such as the hetro nitrogen atom (N7) in Hypoxanthine, which is situated in ortho the site relative to the azo moiety, has also offered additional coordination site [2,7]. So the ligand [AH] was acted as a nonionic N, N-bidentate ligand when interaction with selected metal ions [Ag(I), Cu(II), Zn(II)]. The synthesized ligand [AH] and their selected metal ions complexes are stable, solids non hydroscopic, owned good keeping qualities, As well as, all complexes nonelectrolyte excpet (Ag-AH) complex is (1:1) electrolyte with nitrate ion as counter ionand soluble in water. All synthetic substances' analytical data are proportional to their respective ones. The formulation of all synthesized compounds was made depending on elemental analysis, (FT-IR, UV-Vis,1H-NMR spectra, Thermal Analysis (TGA), melting points, and magnetic susceptibility.

1. Stoichiometric, stability constant, and Gibbs free energy

The mole ratio approach, which is the widely used method for determining the composition of a complex in solution, was used [12]. Figure (S1) was shown this procedure and it was used to achieve the results. The final result illustrates the evolution of a [1:1] [M: L] mole ratio for all synthesized complexes except [Cu -AH]complex has [1:2][M:L]

The stability constant can be defined spectrophotometrically [4] by the following equations were employed to estimate the stability constant for Cu (II) complexes with (1:2) (M:L) is



(α) is the degree of dissociation..

(As) the absorption of a solution with a stoichiometric (1:1) ratio (M: L).

(Am) The absorptions of a solution including an excess of ligands.

(C) The synthetic solution's concentrations in a molar

For Ag(I) and Zn (II) [1:1] ,[M:L] complexes, The stability constant can be computed

using the following

Formula.

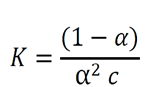


Table 3: K, Ln K, and ΔG of AH-complexes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Complex** | **As** | **Am** | **α** | **K** | **Ln K** | **ΔG(KJ/mole)** |
| [Ag(AH)(H2O)2]NO3.2H2O | 0.280 | 0.291 | 0.037 | 7.03 X 107 | 13.46 | -30544.21 |
| [Cu(AH)2 Cl2].H2O | 0.090 | 0.169 | 0.467 | 1.30 x 107 | 14.08 | -31945.33 |
| [Zn(AH) Cl2].H2O | 0.163 | 0.178 | 0.084 | 1.29 x105 | 11.77 | -26713.08 |

The results were shown in Table (3) the stability increases in the following order: [Cu(AH)2Cl2].H2O< [Ag(AH)(H2O)2]NO3.2H2O< [Zn(AH) Cl2].H2O. From the following equation, the thermodynamic coefficient of ΔG (Gibbs free energy) was found [13]:

ΔG = - RT ln K Where: R is the gas constant, which is 8.31 J/mole-1. K

T = Room temperature (Kelvin). we discovered through ΔG data we indicated that all complexes synthesize spontaneously

1. Thermogravimetric Analysis (TGA)

Survey the data of the thermal analysis of the ligand (AH) and its metal complexes can expect a decomposition of the ligand and metal oxide as well as several water molecules [11,12]. This survey was measured at room temperature to 800 c0 in argon gas [Figure (S2)(a-d)] and the data was summarized in Table (4). The TGA curve of the ligand (AH) was depicted mainly four decomposition steps, within the temperature ranges (25-48) C0,(48-300) C0,(300-575) C0 (575-800) C0 with a mass loss of 2.11% (2.10 %calc.), 26% (26.66% calc.),25.61% (24.91%) and 9.96% (9.82%) respectively [Figure (S2)a]. The thermogravimetric analyses of the complexes were displayed in five sequential Steps. The first step in all complexes includes the loss of lattice water in the range (25-80) C0 However, the rest of the steps.[14-16] was shown to release the ligand molecule and chloride ion or nitrate ion, while decomposition process ended with the formation of metal oxide as final residue.

Table 4: The thermogravimetric analyses of the (AH) ligand and its complexes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Comp. & Molecular  formula | Stage | Extent of Thermal Decomposition () | Proposed Part | % Mass loss | |
| Calculate  % | Found  % |
| AH  C11H7N7O3 | 1 | (25-48) | H3C0.25 | 2.10 | 2.117 |
| 2 | (50-300) | H2C6 | 26.66 | 26.79 |
| 3 | (300-575) | C4.75N | 24.91 | 25.619 |
| 4 | (575-800) | N2 | 9.82 | 9.967 |
| Residue | >800 | N4O3 | 36.49 | 35.52 |
| [Ag(AH)(H2O)2]NO3.2H2O  AgC11H15N8O10  526.86 | 1 | 25-80 | H2O | 3.41 | 2.867 |
| 2 | 80-400 | H2O.C3H6 | 11.38 | 11.78 |
| 3 | 400-500 | C5H3 | 11.95 | 12.80 |
| 4 | 500-675 | C3 H3 ­N7.5 | 27.14 | 27.39 |
| 5 | 675-800 | N0.7O3 | 10.43 | 10.04 |
| Residue | >800 | AgO5 | 35.49 | 35.09 |
| [Cu(AH)2 Cl2].H2O  CuC22H16N14O7Cl2  722.54 | 1 | 25-50 | H2O0.25 | 0.83 | 0.95 |
| 2 | 40-275 | Cl1.35 O0.75 | 8.29 | 8.44 |
| 3 | 275-490 | C6.5 H14 Cl 0.65 | 15.91 | 16.41 |
| 4 | 490-698 | C15.5N3 | 31.55 | 31.39 |
| 5 | 698-800 | N7.5 | 14.53 | 14.79 |
| Residue | >800 | Cu N3.5O6 | 28.86 | 28.05 |
| [Zn(AH) Cl2].H2O  ZnC12H9N7O4Cl2 | 1 | 25-55 | H2O0.5 | 2.27 | 1.434 |
| 2 | 55-84 | Cl2 O0.5 | 17.97 | 17.63 |
| 3 | 84-560 | C6.5H7 | 19.34 | 18.84 |
| 4 | 560-698 | C4.5N1.3 | 16.4 | 16.73 |
| 5 | 698-800 | N4.2 | 13.38 | 13.83 |
| Residue | >800 | ZnN1.5O3 | 30.58 | 31.54 |

1. Infrared Spectroscopy

The FTIR spectrum of the ligand (AH) was compared with the spectra of the synthesized metal ion complexes in order to determine its identity. Table(5) provides a summary of the significant bands for the ligand (AH) and its metal ions complexes, while [Figure(S3)] appeared to the most moieties vibration ware carried out in the range (400-4000)cm-1 using KBr disk. In the spectrum of the ligand (AH) a band at (1573) cm-1 which related to ʋ(C=N) in the imidazole ring for Hypoxanthine, was altered in position and shape in the spectra of all complexes due to coordination with metal ion as was shown in the [Figures (S3) (b-d)] and [Table(5)][18-20]. The bands ʋ (N-H) and ʋ (C=O), in the free ligand (AH) spectrum was unaltered in the spctra of all complexes, suggesting that these moieties did not within the chelating ring[21] , Table 5 and Figure[S3(a-d)] but, small changes in location or form occasionally associated with a drop or increase in resonance was observed due to chelating [7], The special bands for the azo compound ʋ(N=N) and ʋ (C-N=N-C) , These bands were appeared at (1473,1419 and 1373)cm-1 and (1259) cm-1 in the spectrum of [AH] respectively, The position and intensity of these bands were diminished in the complexes spectra, an indication that nitrogen of the azo moiety is part of the coordination,[Figure(S3)(a&b)] [22-24]. Several new bands appear in the range (503-514)cm-1,(603-605) cm-1,659, and(318-320) cm-1, These bands noticed in this region may belong to ʋ(M-Nazo), ʋ(M-Nimd), ʋ(M-O)H2O and ʋ(M-Cl) respectively. This will support our result as regards the chelation sites of the ligand with metal ions and from the above, we conclude that the ligand (AH) acts as a neutral N,N bidentate ligand forming Penta chelating ring[25-27].

Table 5: FT-IR spectral bonds(cm-1) of the free ligand (AH) and its complexes

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Com.** | **ʋ(OH)** | **ʋ(NH2)** | **ʋ (C=O)py** | **ʋ (C=N)imd** | **ʋ (N=N)** | **ʋ**  **(-CN=N-C-)** | **ʋ**  **(M-N)imd** | **ʋ**  **(M-N)**  **azo** | **ʋ**  **(M-Cl)** | **ν**  **(MO)**  **H2O** |
| AH | 3344  b | [3174  3112]  d | [1691  1668 ]  d | 1573  Sh. | [1473  1419  1373] t | 1259  W | --- | --- | --- | --- |
| [Ag(AH)(H2O)2]NO3.2H2O | 3342  m | [3166  3114]  d | [1693  1672]  d | 1560  w | [1415  1375]d | 1261  W | 603  w | 503  w | --- | 459  vw |
| [Cu(AH)2 Cl2].H2O | [3423  3442]  d | [3186  3145]  d | [1706  1664]  d | 1544  w | 1382  Sh. | 1255  w | 605  m | 514  w | 420  vw | --- |
| [Zn(AH) Cl2].H2O | 3342  m | [3172  3114]  d | [1693  1672]  d sh. | 1564  w | [1415  1375]  d | 1263  w | 605  w | 503  vw | 418  vw | --- |

w: weak Sh : sharp, br: broad, s: strong , t: triple, py: pyrimidine , m: medium , d:double , imd: imidazole

1. Electronic Spectra and magnetic muserment data

The electronic spectrum of the ligand (AH) in Absolut ethanol [10-4 M] with the rang (280-1100)nm was shown in Figure (S4-A) its displayed two band, the first band was noticed at (295 nm,33898 cm-1 )which was related to (π→π\*) intramolecular transition of heterocyclic and aromatic moieties [28]. However the second band appeared at (420 nm , 23809 cm-1), this band was attributed to conjected system (n→ π\*) transition of the intramolecular charge transfer taking place via carbonyl ,nitro and azo moieties [29-30].The electronic spectra for the dia magnetic (d10) Ag(I)and Zn(II) complexes were shown a sharp absorption band [Figures 4 (b and c)] and Table (6), which was assigned to the charge transfer CT [26,31].

The electronic spectrum of the [Cu(AH)2Cl2] H2O complex Figure(S4-d) was displayed two band at( 925 nm,10810 cm-1) and (780 nm, 12820 cm-1),assignable to (2B1g→2A1g) (υ1) and (2B1g→2B2g) (υ2) transition respectively .The (υ3) transition (2B1g→2Eg ) was obscured with the charge transfer band at (620 nm, 16129 cm-1)[32-33], which is characteristic for distorted octahedral within Jahn-Teller deformation (D4h). The magnetic moment (µeff=1.33 B.M).

Table 6: The data electronic spectra of the (AH) ligand and its complexes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **ℷ(𝑛m)** | **Wavenumber**  **(Cm)** | **Assignment** | **hybridization** | **Geometry** |
| **AH** | 295  420 | 33898  23809 | n →π\* | --- | --- |
| **[Ag(AH)(H2O)2]NO3**  **.2H2O** | 480 | 20833 | CT | Sp3 | Tetrahedral |
| **[Cu(AH)2 Cl2].H2O** | 925  780  620 | 10810  12820  16129 | 2B1g→ 2A1g  B1g →2B2g  CT | sp3d2 | Distorted  octahedral |
| **[Zn(AH) Cl2].H2O** | 510 | 19607 | CT | Sp3 | Tetrahedral |

1. The 1H-NMR Spectrum of ligand [AH]

The 1H-NMR analyses corroborate to the FT-IR spectrum results. The chemical shifts (𝛿) in ppm for numerous types of protons for the ligand (AH) were noticed for NH Pyrmidine at (11.24,1H ppm) , Aromatic at (7.58-8.15ppm, 4H), NH2 at (6.72ppm,2H) [34,35], The 1H-NMR spectrum was obtained in DMSO-d6 solution at 𝛿 (2.5)ppm. [36].

1. Assess X-Ray diffraction

Figure S5 (A and B) was depicted the (AH) and [Zn (AH) Cl2].H2O x-ray diffraction patterns in the range (5 ≤ 2ϴ ≤ 80 ). The degree of crystallinity of these compounds was investigated using an X-RAY source crystal (CuKα). Three of the strongest reflection peaks for (AH ligand) and [Zn (AH) Cl2].H2O [Figure 6] between (27.7839-11.2906) and (29.9534-17.1921), respectively. The primary reflection peaks for (AH) are located at (29.9534). The location of this band is (11.2906) of [Zn(AH) Cl2].H2O. The semi-crystalline nature and particle size of (AH ligand and [Zn(AH) Cl2].H2O are derived from these data were estimated for (X-RD patterns) on the highest intensity value compared with the other bands employing the renowned Dedye-Scherrer equation: D = K λ / 𝛽 cos ϴ [28,37] Where D: The particle size (nm), 𝛽 =Full width at half maximum (FWHM) of x-ray diffraction peak, ϴ = Bragg angle λ = 0.15406 n is Xray wavelength nm, K = 0.9 (Constant). The D of (AH ligand) and [Zn(AH) Cl2].H2O is [4.15 and 1.844]nm respectively . These values[Table 7] emphasize that particle size is located within the nano scales range [38]. the interplanar spacing (d)was calculated from the status of the intense peak according to the Braggs equation [n λ=2d sin e] [28].where [λ=1.5406AO;n=integer number. the (d) data calculated and observed for the AH ligand is [2.9810-2.9807] A while for [Zn(AH) Cl2].H2O is [3.2095-3.2083]A.[39-41].

Table 7: Particle size of ligand AH and [Zn(AH) Cl2].H2O complex

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compounds** | **2ϴ** | **FWHM** | **D (nm)** | **d**  **calculate (A)** | **d**  **found (A)** |
| AH | 29.9534 | 0.28430 | 4.915 | 2.9810 | 2.9807 |
| [Zn(AH) Cl2].H2O | 27.7839 | 0.75500 | 1.844 | 3.2095 | 3.2083 |

1. Scanning Electron Microscopy (SEM) analysis

An electron microscope called an SEM produces a beam of electrons that interact with the atoms in the sample to produce a wide variety of signals, some of which provide details about the sample's surface topography and composition. The surface homogeneities and crystalline structures of the ligand (AH ) with its complexes was performed using the SEM technique on a cross sectional area of 100 nm and an enlarging power of (60.00 KX). The SEM pictures demonstrated heterogeneous surfaces with various forms that vary with various compounds and change in particle volume (Table 8) Through the SEM technique, it can be concluded that the ligand and its complexes have nanoscale properties the particle size is in the nanometer range [19,25,43].

Table 8: SEM Technique for AH Ligand and its Complexes:

|  |  |  |
| --- | --- | --- |
| **Compound** | **Average volume (nm)** | **shape** |
| AH | 45.75 | coral |
| [Ag(AH)(H2O)2]NO3.2H2O | 52.67 | coral |
| [Cu(AH)2 Cl2].H2O | 53.83 | coral |

1. Study Antibacterial and anti-fungi activity

Microbes are the primary cause of the majority of diseases, therefore it's important to find a cure and stop their growth. The remedies for these come from two different sources, one of which is being medication separated from living things like penicillin and penicillium fungus. The chemical compounds that are created by the chemical are the other source. Azo chemicals and Hypoxanthine are widely known for their extremely potent bacterial inhibition properties. as well as antifungal action in many cases, the metal complex's activity is superior to the ligand alone [44-46]. Hypoxanthine has shown outstanding pharmacological characteristics. According to the findings of the current investigation, they had varied deactivation capacities against the two types of chosen bacteria and Candida albincans [Table 9], as will be stated below:-

**For Staphylococcus aureus:-**

[Ag(AH)(H2O)2]NO3.2H2O >[Cu(AH)2Cl2].H2O > [Zn(AH) Cl2].H2O > AH

**For klebsiella pneumonia**

[Cu(AH)2Cl2].H2O > [Zn(AH)Cl2].H2O > AH > [Ag(AH)(H2O)2]NO3.2H2O

**For Candida albincans**

[Ag(AH)(H2O)2]NO3.2H2O>[Cu(AH)2Cl2].H2O > [Zn(AH)Cl2].H2O > AH

The metal complexes were shown highly activity and sensitivity to against bacteria and fungi were used than free ligand. The mechanism of action of metal chelate could be discussed according to chelation theory and overtones concept [2] by permeability the cell wall that contains lipid, which passage only lipid soluble metals. On chelation, the polarity of metal ion is reduced due to overlap with ligand orbital and increase in the lipophilicity boost permeation of the complex into lipid membrane and inhibition of protein synthesis and nucleic acid [4,45,46].

Table 9 :Rate of AH ligand inhibition against and their complexes

|  |  |  |  |
| --- | --- | --- | --- |
| **Compounds** | **Gram(-) Negative** | **Gram(+) Positive** | **Candida** |
| ***Staphylococcus aureus*** | ***klebsiella pneumonia*** |
| Amoxicillin | 20 | 10 | --- |
| Fluconazole | --- | --- | 20 |
| AH | 12 | 13 | 13 |
| [Ag(AH)(H2O)2]NO3.2H2O | 26 | 12 | 22 |
| [Cu(AH)2 Cl2].H2O | 23 | 17 | 21 |
| [Zn(AH) Cl2].H2O | 19 | 14 | 17 |

1. Burns Healing Effect of [Zn(AH) Cl2].H2O

Burns are one of the leading causes of death in children and one of the most frequent issues in health organizations, according to global epidemiological studies. Through the destabilization of the cell membrane, protein coagulation, exhaustion of energy supplies, and cellular hypoxia, burns destroy the tissues and produce necrosis. Additionally, when subjected to traumatic events, antigen challenges, and infectious agents, burns pose serious risks to other body sections. vital biological reaction to the repair of harmed connective and epithelial tissues is wound healing [2,4,19]. Burns’ healing impact was tested to see if [Zn(AH)Cl2].H2O has anti-inflammatory activity. The ability of [Zn(AH) Cl2].H2O at (1.5mM), by calculating the number of days needed to recover the findings, the ability of silver sulfadiazine (positive control), and a negative control, to cure burns, was evaluated [Table 10] was displayed the results. The complex was able to heal burns in [14] days as opposed to silver sulfadiazine (positive control), which was needed [16] days and [18] days without any treatment (negative control)days to recover. Healing was also seen to occur without any side effects, bleeding, fever, adhesion to the wound, or germ contamination. The main reason for the increase in the anti-inflammatory activity in selected complexes due to the presence of zinc ions and Hypoxanthine, which contain heterocyclic rings with highly effective anti-inflammatory [47-50].

Table 10: Following various treatments, the recovery of burn healing in mice

|  |  |  |
| --- | --- | --- |
| **No. of comp.** | **Treatment** | **Period of recovery** |
| 1 | [Zn(AH) Cl2].H2O | 14 Days |
| 2 | Without any treatment | 18 Days |
| 3 | Silver sulfadiazine | 16 Days |

1. Realization Radical Scavenging Performance

By using a radical scavenging technique DPPH (1,1-diphenyl-2-picrylhydrazyl), the in vitro antioxidant activity of the (AH) ligand was assessed. The positive control used was ascorbic acid [2]. it was reducing capacity. At the four concentrations that were looked at (25, 50, 100, and 200 mg/mL) [31,32], the AH ligand is more effective as antioxidant than vitamin C. It was (36±1.530) at 25 mg/mL AH ligand concentrations. At 200 mg/mL, DPPH radical scavenging activity increased dramatically (64.93±2.79). Table 11 and Figure S6 show the results[25-31].

Table 11: DPPH radical scavenging performance of AH ligand and vitamin C

|  |  |  |
| --- | --- | --- |
| **Concentration**  **(mg/ml)** | **DPPH Radical Scavenging Activity (Mean ± SD; %)** | |
| **AH** | **Vitamin C** |
| **12.5** | 34±1.075 | 42.20±1.408 |
| **25** | 36±1.530 | 57.91±3.423 |
| **50** | 39±1.917 | 65.20±2.567 |
| **100** | 63.20±2.067 | 78.67±1.850 |
| **200** | 64.93±2.79 | 85.03±0.598 |

1. **Conclusion:**

In our work, we prepared three newly Nano complexes derived from monoazo-hypoxanthine, which they analyzed employing a variety of spectroscopic, thermal and physicochemical techniques. The (AH) ligand act as nutral N,N bidentate with [1:1; M:L] when attached to silver ion and zinc ion with tetrahedral geometry, however when attached cooper ion had [1:2;M:L] with distorted octahedral. The synthesized ligand (AH) and its complexes were shown high effectiveness against two different types of bacteria, as well as against fungi, in addition to they are effectiveness as an effective antioxidant when compared with ascorbic acid. Zn-AH Complex was highly effective against burns and infections when compared with silver sulfadiazine by Calculating the number of healing days.

1. **References:**
2. Crespi, S., Simeth, N.A. & König, B. (2019) Heteroaryl azo dyes as molecular photoswitches. Nat Rev Chem 3, 133–146.
3. Hussein, N. A., & Abbas, A. K. (2021) Synthesis spectroscopic characterization and thermal study of some transition metal complexes derived from caffeine azo ligand with some of their applications. Eurasian Chem. (4) 66-92.
4. Abbas, A. K. (2016). Preparation, Characterization, and Biological Evaluation of some Lanthanide (ΙΙΙ) ions Complexes with 3-(1-methyl-2-benzimidazolylazo)-Tyrosine. Baghdad Science Journal, 13(2)128-142.
5. Jasim, D. J., & Abbas, A. K. (2022) Synthesis, identification, antibacterial, medical and dying performance studies for azo-sulfamethoxazole metal complexes. Eurasian Chem. 4 16-40
6. Abbas, N. F., & Abbas, A. K. (2020) Novel complexes of thiobarbituric acid–azo dye: structural, spectroscopic, biological activity and dying. Biochem. Cell. Arch, 20(1), 2419-2433.
7. Merino, E. (2011). Synthesis of azobenzenes: the coloured pieces of molecular materials. Chemical Society Reviews, 40(7), 3835-3853.
8. Fadhil, A. E., & Abbas, A. K. (2023). Synthesis and Structural Views on New Azo Ligand and Its Metal Complexes with Some of their Application. Iraqi Journal of Science,64(12) 6119-6134.
9. Toledo-Ibelles, P., Gutiérrez-Vidal, R., Calixto-Tlacomulco, S., Delgado-Coello, B., & Mas-Oliva, J. (2021). Hepatic accumulation of hypoxanthine: a link between hyperuricemia and nonalcoholic fatty liver disease. Archives of medical research, 52(7), 692-702.
10. Furuhashi, M., Koyama, M., Higashiura, Y., Murase, T., Nakamura, T., Matsumoto, M., ... & Miura, T. (2020). Differential regulation of hypoxanthine and xanthine by obesity in a general population. Journal of diabetes investigation, 11(4), 878-887.
11. Ohe, T. and Watanabe, Y. (1980). Purification and Properties of Hypoxanthine phosphoriboribosyltrans ferase from Streptomyces cyanogenus, Agrc.Biol.Chem, 44(9) 1999-2006.
12. Benkhaya, S., M'rabet, S. and El Harfi, A., 2020. Classifications, properties, recent synthesis and applications of azo dyes. Heliyon, 6(1) e03271.
13. Skoog, D.A., Holler, F.J. and Crouch S.R.(2017). Principles of Instrumental Analysis, 7th edition , Cengage Learning
14. Greine, W., Neise, L. and Stocker,H. (1995). Thermodynamics and statistical Mechanics, Springer-Verlag.
15. Kha;eel, A.M.N. and Jaafer, M.I.(2017). Synthesis and Characterization of boron and 2-aminophenol Schiff base ligands with their Cu(Ⅱ) and Pt(Ⅱ) Complexes and evaluation as antimicrobial agents, Oriental Journal of Chemistry, 33(05), 2394-2404.
16. Al-Daffay, R.K. and Al-Hamdani, A.A.(2022). Synthesis, Characterization and Thermal Analysis of a New Acidic azo Ligand’s Metal Complexes, Baghdad Sci. J., 19(3), 121-133.
17. Abbas, H. and Al-Hassani, R(2018). Spectroscopic Characterization and Bioactivity studies of new Co(Ⅱ), Ni(Ⅱ), Cu(Ⅱ), Au(Ⅲ) and Pt(Ⅳ) Complexes with didentate (NO) donor azo dye ligand, International Journal of Medical Science and Dental Research, 1(01)38-51.
18. Patel, K. D., & Patel, H. S. (2017). Synthesis, spectroscopic characterization and thermal studies of some divalent transition metal complexes of 8-hydroxyquinoline. Arabian Journal of Chemistry, 10, S1328-S1335.
19. Abu-Khadra, A. S., Farag, R. S., & Abdel-Hady, A. E. D. M. (2016). Synthesis, characterization and antimicrobial activity of Schiff base (E)-N-(4-(2-hydroxybenzylideneamino) phenylsulfonyl) acetamide metal complexes. American Journal of Analytical Chemistry, 7(03), 233.
20. Abd, Z. and Abbas A.K. (2024). Metal Complexes of Adenine Azo Ligand: Synthesis, Identification and study some of their Applications, Iraqi Journal of Science, 65(3) 1212-1229.
21. Mahmoud, W. A., Ali, A. A. M., & Kareem, T. A. (2015). Preparation and spectral characterization of new azo imidazole ligand 2-[(2-cyano phenyl) Azo]-4, 5-Diphenyl imidazole and its complexes with Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg (II) ions. Baghdad Science Journal, 12(1).
22. Dabish, R.A. and Abbas, A.K. (2021). Synthesis and Spectral Studies of Some New Complexes Containing Azo Ligand with Anticancer, Antibacterial and Dyeing Performance. Annals of the Romanian Society for Cell Biology,25(4) 7968-8006.
23. Abbas, A., & Kadhim, R. S. (2017). Metal complexes of proline-azo dyes, synthesis, characterization, dying performance and antibacterial activity studies. Orient J Chem, 33(1) 402-417.
24. Husham, M., Witwit, I.N., and Ali, A.M.(2020).Synthesis of new azo imidazole ligand and fabricating its chelate complexes with metallic ions, Journal of Physics, Conference Series, 1660, 1-9
25. Al-Majidi, S. M., & Al-Khuzaie, M. G. (2019). Synthesis and Characterization of New Azo Compounds Linked to 1, 8-Naphthalimide and Studying Their Ability as Acid-Base Indicators. Iraqi Journal of science, 60(11) 2341-2352.
26. Abbas, A.K., Al-Qaysi, W.W. (2023). Synthesis, Characterization of Novel Nano Azo Compounds as a New pH Sensor, 48(1), 399-415.
27. Abbas, A.,& Kadhim, R. S. (2016). Preparation, spectral and biological studies of azo ligand derived from proline with Cu (II), Ag (I) and Au (III) metal ion. J. Appl. Chem, 9(8), 20-31.
28. Jasim, Duaa Jameel, and Alyaakhider Abbas. (2021)"Divalent Metal Complexes Of Azosulfamethaxazole: Synthesis And Characterization With Study Some Of Their Applications."Nat.Volatiles and Essential Oils, 8(1): 8272-8300
29. N.F.Abass and A.K.Abbas “Novel complexes of thiobarbituric acid-azo Dye: structural ,spectroscopic ,biological activity and dying performance,Biochem Cell .Arch.,2011,2419-2433.
30. Mahmoud, S. S., & Khaleel, A. M. N. (2023). Synthesis, Identification and Biological Study of New Pharmaceutical Model Based on Amino Acids with Some of Its Complexes. Iraqi Journal of Science, 5501-5516.
31. Ali, A. A., Al-Hassani, R. M., Hussain, D. H., Rheima, A. M., & Meteab, H. S. (2020). Synthesis, spectroscopic, characterization, pharmacological evaluation, and cytotoxicity assays of novel nano and micro scale of copper (II) complexes against human breast cancer cells. Drug Invention Today, 14(1).
32. Al-Qaysi, W.W. and Abbas, A.K.(2023). Novel Nano Zn+2 – compound from LA Ligand as an Asid-Base Indicator :Synthesis, Characterization, pH Sensor and Fluorescent study, Iraqi Journal of Science, 64(11), 5525-5540.
33. Al-Hamdani, A. A. S., & Abdulridha, M. Q. (2023). Synthesis, Characterization of New Metal Complexes of Co (II), Cu (II), Cd (II) and Ru (III) from azo ligand 5-((2-(1H-indol-2-yl) ethyl) diazinyl)-2-aminophenol, Thermal and Antioxidant Studies. Baghdad Science Journal, 20(5 Suppl.) 1964-1975.
34. Miessler, G.L. and Tarr, D.A. (2011). Inorganic Chemistry, 4th ed., Pearson
35. Abbas, A.K. (2015). Synthesis, Structural and Biological Efficiency Studies of New Azo Ligands and Their Complexes with Zn(Ⅱ), Cd(Ⅱ) and Hg(Ⅱ) Metal Ions, Ibn Al-Haitham Journal for Pure and Applied Science, 28(3)169-186.
36. Emam, S.M., Abou El-Enein S.A. and Monir E.(2017) Spectrochemical and Thermal Studies for Bivalent Metal Complexes of Azo dye Ligand containing Pyrimidine Ring, International Journal of Engineering Research and Technology, 6(9) 1-12.
37. Ali, R. A., Ahamed, L.S., and Alrekabi, Z.G.(2018) Synthesis of new nucleoside analogues from 3,5-disubstituted pyrazoline, Journal of Pharmaceutical Sciences and Research, 10(4) 705-710.
38. S.G.Qazi ,A.R.Rennie, J.K. Cokcroft and M.Vickers (2009),”Use of wide\_angle x\_ray diffraction to measure shape and size off dispersed collide particles”,J.Colloid Interface SCi,105,3380-3451.
39. E.N.Masten,A.G.Fox and M.A.Keef,(2004),International tables for crestallography”,Kluwer Acadimic, Dordrect.
40. Bragg, W.H. and Bragg, W.L. (1913) The Reflaxion of X-rays by Crystals, Proc. R. Soc.Land. A., 88(605)428-438.
41. Humphreys,C.J.(2013). The Significance of Bragg’s law in electron Diffraction and Microscopy and Bragg’s second law. Acta Cryst., A69.45-50.
42. Greenberg B., (1989) Bragg’s Law with Refraction, Acta cryst., A45-238-241.
43. Farhan, R. Z., & Ebrahim, S. E. (2021). Preparing nanosilica particles from rice husk using precipitation method. Baghdad Science Journal, 18(3), 0494-0494.‏
44. Ghomrasni, N. B., Chivas-Joly, C., Devoille, L., Hochepied, J. F., & Feltin, N. (2020). Challenges in sample preparation for measuring nanoparticles size by scanning electron microscopy from suspensions, powder form and complex media. Powder Technology, 359, 226-237.
45. Elsayed, E.H., Wahaib,D., Ali, A., El-Nabey, B. and Elbadawy H.(2023). Synthesis, Characterization, DNA Binding Interactions, DFT Calculations, and Covid-19 Molecular Docking of Novel Bioactive Copper(1) Complexes developed Via unexpected Reduction of Azo-Hydrazo Ligands, BMC Chemistry, 17(159)1-19
46. basil AbdulRazzaq, A., Shami, A. M., & Ghaima, K. K. (2022). Detection of vanA and vanB genes Among Vancomycin Resistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. Iraqi journal of biotechnology, 21(1).‏
47. Al-Hayali, O. Z., Marjani, M. F. A., & Maleki, A. (2023). Evaluation of Rosmarinus officinalis Leaves Essential Oils Activity Against Vancomycin Intermediate Staphylococcus aureus (VISA) Isolated from Baghdad Hospital Patients. Iraqi Journal of Science, 64(5) 2153-2167.‏
48. Ghafil, J. A., Fliyh, M. T., & Al-Mutalib, L. A. (2023). Preparing burn mouse model infected with Pseudomonas aeruginosa: a histopathological study. World Journal of Experimental Biosciences, 11(2) 46-50.‏
49. Tian, J., Wong, K. K., HO, C. M., Lok, C.N., YU, W.Y., Che, C.M., Chiu, J.F. &Tam, P.K. (2007). Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem: Chemistry Enabling Drug Discovery, 2(9), 129-136.
50. Mclaughlin, E.S. and Paterson, A.O.(2012). Burns Prevention Causes and Treatment, Nova Science Publishers, Inc., New York.
51. Nguyen, C.M., Chandler, R., Ratanshi, I. and Logsetty, s. (2020) Handbook of Burns, 1, 529-547