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Binuclear Co(II), Ni(II) and Cu(II) complexes of a new bis(tridentate) hydrazone ligand: Synthesis, thermal, spectroscopic, biological, molecular docking and theoretical studies

Fatma Samy; Ph. D. (fatmasame@edu.asu.edu.eg; <https://orcid.org/0000-0001-8677-8777>) *corresponding author*

(Tel.: 00201096418414; Fax: 0222581243)

Magdy Shebl, Full professor (magdyshebl@edu.asu.edu.eg; <https://orcid.org/0000-0003-4377-2273>)

Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, Cairo 11341, Egypt

Binuclear Co(II), Ni(II) and Cu(II) complexes of a new bis(tridentate) hydrazone ligand: Synthesis, thermal, spectroscopic, biological, molecular docking and theoretical studies

Fatma Samy and Magdy Shebl

Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, Cairo 11341, Egypt

Email: fatmasame@edu.asu.edu.eg (00201096418414), magdyshebl@edu.asu.edu.eg (00201221534851)

Fax: 0222581243

Abstract

A new hydrazone ligand; 4,6-bis(2-hydroxynaphthalen-1-yl)methylenehydrazonoethyl)benzene-1,3-diol (H_4L ; DHNAPH) was synthesized by the reaction of 4,6-bis(1-hydrazonoethyl)benzene-1,3-diol (NH_2DAR) with 2-hydroxy 1-naphthaldehyde. Co(II), Ni(II) and Cu(II)- complexes ($[M_2L(H_2O)_6].nH_2O.mEtOH$; $M = Co, Ni \text{ or } Cu$, $n = 0.5 \text{ or } 1$ and $m = 0.5 \text{ or } 0$) of DHNAPH have been successfully synthesized. Characterization of DHNAPH and its complexes was performed by analytical, spectral (IR, mass, UV-Vis, 1H NMR and ESR), magnetic susceptibility, molar conductivity and thermal gravimetric analysis (TGA) techniques. The scanning electron microscopy (SEM) was used for detection of the morphology of DHNAPH and its Co- and Ni-complexes, which refers that the DHNAPH complexes are in the nano scale. The analytical data, magnetic moments and spectral studies recognized octahedral geometries for DHNAPH complexes. DHNAPH acts as a bis(dibasic triadentate) *via* ($C=N_{azomethine}$ and 2 OH) with metal in complexes. The optimized structure of DHNAPH and its complexes has been done theoretically by Hyperchem program and structural parameters were linked with the IR experimental data. The activity of DHNAPH and its complexes against Hepatocellular carcinoma, fungi and bacteria has been tested. The new complexes are more active than DHNAPH and the highest antitumor activity was given by copper(II) complex. The DNA-binding of DHNAPH and Cu- DHNAPH has been investigated. Molecular docking studies showed that all the tested compounds show good binding score revealing good fitting in between the DNA strands. There is agreement between docking data and DNA binding results.

Keywords: Hydrazones; 4,6-Diacetylresorcionl; Theoretical study; Antitumor and antimicrobial activity; DNA-binding and molecular docking.

Introduction

Hydrazones have attracted a great and continuous interest, due to their simple synthetic methods, easy complexation with different metal ions as well as their diverse applications. Hydrazones and their complexes have many biological, catalytic and analytical applications [1-3]. The biological activity of these compounds includes antifungal, anticonvulsant, antibacterial, antimalarial, analgesic, antimicrobial, anticancer, anti-inflammatory, antiviral and antituberculosis activities [1,4-10].

The coordinating behavior of the di-carbonyl compound; 4,6-diacetylresorcinol (2,4-dihydroxy-5-acetylacetophenone) (DAR) has been studied [11-16]. In addition, some symmetrical and asymmetrical Schiff bases, hydrazones and other ligands [17-32] have been constructed from DAR. Complexation of these ligands with different metal ions gave polynuclear (mono-, bi- and trinuclear) complexes, which showed variable biological applications. However, antitumor [20,24,26,28] and molecular docking [25] studies of these compounds are limited.

Naphthaldehyde-based compounds have numerous presentations such as electrochemical, catalytic, photoluminescence, analytical, cytotoxic, antimicrobial and *in vitro* anticancer, antiproliferative, antioxidant, catechol's studies [33-43]

Transition metal compounds play important role in bio-inorganic chemistry and redox enzyme systems [44]. Copper is one of the most important elements in the human body. It catalyzes many reactions [45]. The Co, Ni and Cu- complexes have an interest role in bioinorganic chemistry such as antibacterial, antifungal, anticancer, DNA cleavage, antioxidant and DNA binding activities [46-55].

Based on the previously mentioned facts and as an expansion of our interest in hydrazones derived from DAR [27,28], the current study aims to synthesize a new *bis*(tridentate) hydrazone ligand; 4,6-bis(2-hydroxynaphthalen-1-yl)methylenehydrazono)ethyl)-benzene-1,3-diol (DHNAPH) (Scheme 1) and investigate its coordinating ability towards cobalt(II), nickel(II) and copper(II) ions. DHNAPH and its complexes were characterized by various analytical and spectroscopic techniques. The antitumor and antimicrobial activities were examined. The optimized structures of the compounds were carried out and the theoretical data were linked with the experimental data. The activity of DHNAPH and its complexes in contrast to Hepatocellular carcinoma, fungi and bacteria was investigated. The DNA-binding of DHNAPH and Cu- DHNAPH was explored and molecular docking studies using MOE (MOE, 2019.0102) software (PDB ID: **1BNA**) were investigated.

2. Experimental

2.1. Materials

Metal salts, $\text{LiOH} \cdot \text{H}_2\text{O}$, hydrazine hydrate (100%), resorcinol, acetic anhydride, zinc(II) chloride and 2-hydroxy-1-naphthaldehyde were either Aldrich, BDH or Merck products. Organic solvents were reagent grade chemicals and used without purification.

2.2. Synthesis of DHNAPH, ligand

The methods in the literature were used to prepare 4,6-diacetylresorcinol (DAR) [56] and 4,6-bis(1-hydrazonoethyl)benzene-1,3-diol (NH_2DAR) [12]. The ligand, 4,6-bis(2-hydroxynaphthalen-1-yl)methylene)hydrazono)ethyl)benzene-1,3-diol (DHNAPH) was synthesized (Scheme 1) by dropwise addition of NH_2DAR (10 mmol/ethanol 30 mL) suspended to ethanolic solution of 2-hydroxy-1-naphthaldehyde (20 mmol). The mixture was heated under reflux for 2 h, and the ligand (DHNAPH) was filtered off, washed several times by hot ethanol and dried to give pure yellow product with m.p. $>300^\circ\text{C}$; yield 73 %.

(Scheme 1)

2.3. Synthesis of DHNAPH complexes

An ethanolic solution of the ligand (DHNAPH) was heated to reflux with an aqueous solution of LiOH for $\frac{1}{2}$ hour then an ethanolic solution of metal acetate was added, in molar ratio 1:4:2 (DHNAPH : LiOH : M) and heated to reflux for 6-8 h. The obtained complexes were precipitated, filtered off then washed with distilled water then with EtOH , lastly washed with diethyl ether and complexes were dried in desiccators.

2.4. Measurements

Microanalyses (C, H and N) of DHNAPH and its complexes were determined in Central laboratory at faculty of science ASU, Egypt. Co(II) , Ni(II) and Cu(II) contents were estimated by EDTA complexometrically after decomposition of the chelates by using conc. HNO_3 . Melting point of DHNAPH and decomposition temperatures of the prepared complexes were determined by a digital Stuart SMP3 melting point apparatus. A Bruker WP 200 SY spectrometer was used to record ^1H NMR spectrum of DHNAPH, using dimethylsulfoxide, $\text{DMSO}-d_6$ as a solvent and TMS (tetramethylsilane) as an internal reference. Infra-red (IR) spectra of the DHNAPH compounds were recorded on a Nicolet 6700 FT IR spectrometer. UV-Vis. spectra of DHNAPH and its complexes were determined as

solutions in DMF (dimethylformamide) and/or Nujoll mulls on Jasco UV-Vis. spectrophotometer model V-550. The electron spin resonance spectrum of Cu-DHNAPH complex was measured on an Elexsys (E500), Bruker company instrument. The calibration of the magnetic field was done using 2,2'-Diphenyl-1-picrylhydrazyl (DPPH). At room temperature, magnetic susceptibility measurements for DHNAPH-complexes were performed by using Johnson Matthey magnetic susceptibility balance (Alfa product) Model No. (MKI). Pascal's constants for the diamagnetism of atoms existing in the chelates [57] were utilized to correct the calculated effective magnetic moment values. Molar conductivities of DHNAPH complexes (1×10^{-3} M) solutions were recorded by using the corning conductivity meter NY 12631 model 441. Mass spectra of DHNAPH and its complexes were recorded on Shimadzu apparatus (a Gas chromatographic GCMSqp 1000 ex). A Shimadzu-50 instrument is used to record TG (heating rate = 10 °C/min). The SEM apparatus, FEI company, Netherlands, Model Quanta 250 FEG was utilized to determine the morphology for DHNAPH and its complexes.

2.5. Molecular orbital calculations

Hyperchem (7.52) program was used to find the optimized structures of complexes, in semi-empirical (PM3 level) [58].

2.6. Biological studies

The disc diffusion technique was followed to study the antimicrobial activity of DHNAPH and its binuclear complexes against a pathogenic fungus, Gram-negative and Gram-positive bacteria [59]. The antitumor activity of DHNAPH and its complexes was investigated in contrast to Hepatocellular carcinoma cells at Al-Azhar University in the regional center for (mycology & biotechnology) by determining the effect of the test samples on cell morphology and cell viability following literature procedure [60].

2.7. DNA Binding studies

DHNAPH and its Cu- complex were dissolved in DMSO and then added to the Calf Thymus DNA (CT-DNA) and the mixtures were then incubated at 37 °C for one h. The electrophoresis process was carried out according to the subsequent technique; ~ 0.25 g of agarose were dissolved in TAE buffer (25 mL) and boiled. When the gel reaches about 55 °C, it was poured into the gel cassette fitted with comb and left to solidify. Then, the gel is employed in the electrophoresis chamber flooded with

buffer (TAE). The DNA sample is full cautiously with bromophenol blue into the wells, along with standard DNA marker and electricity (100 V) is passed till the dye front reaches the end of gel. After that, the gel is removed and cautiously stained with ETBR solution (10 µg/mL) for ~10-15 min and then the gel is destained and the bands are observed under UV transilluminator of a gel documentation system (BIO-RAD, Gel Doc 2000) [61,62].

2.8. Molecular docking studies

The MOE (MOE, 2019.0102) software (PDB ID: **1BNA**) were used in molecular docking studies for DHNAPH and its complexes (**1-3**) [63].

3. Results and discussion

This work interest to develop the method to isolate novel DHNAPH compounds, the scheme 1 showed the syntheses of DHNAPH complexes. The new complexes of DHNAPH, are powdery solids, colored and stable at normal laboratory temperature. The physical and spectroscopic techniques are utilized to characterize the new complexes (**Tables 1–6**). All new complexes (**Scheme 1**) have octahedral geometries. The decomposition temperatures of all DHNAPH complexes are higher than 300 °C (**Table 1**). The formation of binuclear complexes, with Co(II), Ni(II) and Cu(II) ions is maintenance by elemental analysis. All new complexes are sparingly-soluble in most organic solvents, but they are soluble in DMF and DMSO.

3.1. Characterization of the ligand; DHNAPH

The characteristic physical and analytical data of DHNAPH and its complexes are tabulated in Table 1. Table 2 summarizes the ¹H NMR spectral data of DHNAPH relative to TMS in DMSO-*d*₆ and **Fig. 1** represents the spectrum. The signals detected at 13.84 and 12.82 ppm (**Table 2**) are characteristic for -OH protons of 4,6-diacetylresorcinol and 2-hydroxy-1-naphthaldehyde, respectively [28,64,65]. These signals disappeared upon addition of D₂O. Signals detected at 9.82 and in the range, 6.37-8.72 ppm may be ascribed to -CH and aromatic protons, respectively. Lastly, signals detected at 2.47 ppm may be related to -CH₃ protons. The infra-red spectrum of DHNAPH (**Table 3**) showed three characteristic bands at 2998, 1577 and 1088 cm⁻¹, which may be attributed to ν(OH...N), ν(C=N)azomethine and ν(C-O)phenolic, respectively [28]. In addition, the two bands observed at 3049

and 2925 cm^{-1} , may be attributed to $\nu(\text{C-H})_{\text{aromatic}}$ and $\nu(\text{C-H})_{\text{aliphatic}}$, respectively. The uv-vis. spectrum of DHNAPH in dimethylformamide (Table 4) displayed three bands; the first one occurs at 279, which may be ascribed to $\pi-\pi^*$ transitions within the aromatic rings. The second band occurs at 340, which may be ascribed to $n-\pi^*$ transitions. The last band occurring at 398 nm, may be related to charge transfer within the entire molecule [53,54].

The mass spectrum of DHNAPH (Fig. 2) exhibited the molecular ion peak at $m/z = 530.54$ amu, which is in a complete agreement with the formula weight calculated with the aid of elemental analyses (F.W. 530.58). In addition, fragmentation pattern of DHNAPH [Scheme S1 (Supplementary material)] confirms the structure of the ligand.

Table 1

Table 2

Fig.1

Fig.2

3.2. Characterization of DHNAPH complexes

The obtained colored complexes are stable at room temperature and exhibited high decomposition temperatures (above $300\text{ }^{\circ}\text{C}$), indicating their thermal stability. The different physical and spectroscopic techniques were employed to characterize them. Elemental analyses (Table 1) showed formation of complexes with molar ratios 2:1; M: DHNAPH (M = Co, Ni or Cu). The molar conductivity data (Table 4) of DHNAPH-complexes (**1-3**) were measured at room temperature in dimethylformamide. The values lie in the range $5.94\text{--}7.74\text{ }\Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$, presenting the non-electrolytic characters of the complexes [66].

3.2.1. IR spectra

The IR spectral data (Table 3) of the isolated complexes were compared with that of the uncomplexed DHNAPH to decide the coordination sites of chelation. The spectra of all complexes displayed a strong broad band in the range $3399\text{--}3421\text{ cm}^{-1}$, which may be due to $\nu(\text{OH})$ of the coordinated or non-coordinated water and/or ethanol molecules combined with the complexes. This is confirmed by the appearance of new non-ligand bands at $855\text{--}856\text{ cm}^{-1}$, which may be ascribed to the rocking mode of the coordinated H_2O molecules [67]. The IR spectra of the complexes showed a blue shift for $\nu(\text{C=N})_{\text{azomethine}}$ (from 1577 cm^{-1} for DHNAPH to $1534\text{--}1540\text{ cm}^{-1}$ for complexes) and $\nu(\text{C-}$

O) (from 1088 cm^{-1} to 1052-1059 cm^{-1}). This indicated that DHNAPH bonded to metal ions through N-azomethine and O-C groups [67-71]. This is supported by the appearance of new bands in the regions 529-552 cm^{-1} and 452-471, which are related to stretching vibrations of metal—oxygen and metal—nitrogen, respectively [72-76].

Table 3

3.2.2. Electronic spectra and magnetic moment measurements

The electronic spectral data of DHNAPH complexes (**1-3**) in DMF solution and as Nujol mulls are collected in Table 4. The electronic spectrum of the dark red Co(II) complex (**1**) exhibited an absorption band at 533 nm, which may be ascribed to the ${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^4\text{A}_{2\text{g}}(\text{F})$ transition in an octahedral geometry [77]. The effective magnetic moment of Co-DHNAPH complex is 3.52 B.M. which is lower than expected for octahedral Co(II) complexes suggesting an antiferromagnetic interaction between Co(II) ions [78-80].

The electronic spectrum of the olive green Ni(II) complex (**2**) exhibited an absorption band at 445 nm, which may be ascribed to the ${}^3\text{A}_{2\text{g}} \rightarrow {}^3\text{T}_{1\text{g}}(\text{P})$ transition in an octahedral geometry [23]. As Nujol mulls, another band was observed at 694 nm, which may be ascribed to the ${}^3\text{A}_{2\text{g}} \rightarrow {}^3\text{T}_{1\text{g}}(\text{F})$ electronic transition in an octahedral geometry [81]. The effective magnetic moment value of Ni-DHNAPH complex is 3.25 B.M., confirming the octahedral geometry around Ni(II) in the complex [82].

The electronic spectrum of the brown Cu(II) complex (**3**) -as Nujol mulls- displayed an absorption band at 699 nm, which may be ascribed to the ${}^2\text{E}_{\text{g}} \rightarrow {}^2\text{T}_{2\text{g}}$ transition in a distorted octahedral geometry [83]. The effective magnetic moment value of Cu-DHNAPH complex is 1.58 B.M., which refers to one unpaired electron (d^9) [84].

Consequently, the electronic spectral data and magnetic moment values indicate that all complexes have octahedral geometry.

Table 4

3.2.3. ESR spectra

A powder ESR spectrum (Fig. 3) of $[\text{CuL}(\text{H}_2\text{O})_6] \cdot \frac{1}{2}\text{H}_2\text{O}$ at room temperature is characteristic for a distorted octahedral geometry [85]. The spectrum of Cu-DHNAPH complex exhibits two signals ($g_{\parallel} = 2.163$ and $g_{\perp} = 2.115$). Based on $g_{\parallel}$ value, which is an important function for signifying covalent character of metal–ligand bonds [86] ($g_{\parallel} > 2.3$ for ionic character and $g_{\parallel} < 2.3$ for covalent character), a covalent character for the Cu-DHNAPH bond was indicated. Also, the exchange interaction parameter term “ G ” was calculated by the equation; $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$ [87]. The calculated G value is 1.42 (lower than four), suggesting a copper–copper exchange interaction.

Fig. 3

3.2.4. Thermal analysis

The thermal stability of the Co(II) & Cu(II) complexes (**1&3**) and the nature of solvent (water or ethanol) molecules were examined by using TGA [88] and Table 5 lists TGA data of the Co(II) & Cu(II) complexes. The results of thermal analyses (Table 5) showed a good agreement with the suggested formulae of the Co(II) & Cu(II) complexes (Table 1).

The first decomposition step of Co-DHNAPH complex (**1**) (Scheme 2) shows elimination of $\frac{1}{2}$ non-coordinated EtOH and $\frac{1}{2}$ non-coordinated H_2O molecules (46-138 °C) with weight loss: 3.72% & calc.: 4.08%. The second step (139-260 °C) with weight loss: 4.08% & calc.: 4.59%, is due to loss of two coordinated H_2O molecules. The third step (261-412 °C) with weight loss: 72.23% & calc.: 72.22%, is due to elimination of 4 H_2O ; 2 $\text{C}_{11}\text{H}_7\text{N}$; 2 CH_3CN ; 2 C_2H_2 ; 2 CO . The final Residue; 2 CoO was observed above 412 °C (Found: 20.12% & Calc.: 19.10%).

The first decomposition step of Cu-DHNAPH complex (**3**) (Fig. 4) exhibits elimination of $\frac{1}{2}$ non-coordinated H_2O molecule (44-121 °C) with weight loss: 1.36% & calc.: 1.17%. The second step (122-250 °C) with weight loss: 4.07% & calc.: 4.67%, is due to loss of two coordinated H_2O molecules. The third step (251-438 °C) with weight loss: 67.25% & calc.: 67.28%, is due to loss of 4 H_2O ; 2 $\text{C}_{11}\text{H}_7\text{N}$; 2 CH_4 ; 2 HCN ; 2 CO . The final Residue (2 CuO with carbon) was observed above 438 °C (Found: 27.32 & Calc.: 26.87).

Table 5

Fig. 4

Scheme 2

Kinetic data

The Coats–Redfern model is used to calculate the kinetic and thermodynamic parameters (Table 6) of the complexes [89]. The Eyring equation is used to calculate other thermodynamic parameters of activation. The next remarks are concluded:

[a] ΔH^* values ($2328.1-1*10^{11}$ kJmol⁻¹) are positive for all steps, this raises that decomposition of these steps are endothermic [90].

[b] The positive ΔS^* values (41.63-172.1 Jmol⁻¹) denote the activated complex is less ordered than the reactants and/or the reactions are fast. On the other hand, the negative value (-13.20 Jmol⁻¹) indicates that the reactants are less ordered than the activated complex and/or the reaction is slow.

[c] The positive values of ΔG^* (40.87-95.18 kJmol⁻¹) illustrate the autocatalytic action of metal ion on thermal decomposition of the chelates and non-spontaneous processes [91].

Table 6

3.2.5. Mass spectra

Mass spectra have supported the proposed molecular formulae of Ni(II) and Cu(II) complexes (2&3) (Fig. 5). The molecular ion peaks (m/z) of these compounds are [752.3 (M) & 753.3 (M+1)] and [761.65 (M)], respectively. This data agree with the F.Wt. deduced from elemental analysis (Table 1) for anhydrous complexes (2&3) 752.06 and 761.72, respectively. Scheme 3 showed fragmentation pattern of Cu(II) complex.

Fig. 5

Scheme 3

3.2.6. Morphological study

In order to determine the particle size and the morphology of DHNAPH and its complexes, scanning electron microscope (SEM) has been utilized. Fig. 6 shows SEM images of DHNAPH, Co(II) 1 and Ni(II) 2 complexes. DHNAPH has sheet like morphology (average diameter 3.02 μ m), which is change by complexation with Co(II) and Ni(II) ions to be aggregate spherical shape and cluster with average diameter 67.85 and 113.17 nm, respectively. The complexes are in/or close to nano range.

Fig. 6

3.3 Molecular orbital calculations

The *Hyperchem 7.52* program with (*PM3* level) semi-empirical was used to give optimized structures of DHNAPH and its complexes. Structural parameters of DHNAPH and its complexes are collected in **Table 7**. The heat of formation of the complexes (-455.03 to -746.86 kcal/mol) are more negative than that of DHNAPH (28.79 kcal/mol), this leads to DHNAPH ligand's stability is less than that of its complexes. Dipole moment (μ) of DHNAPH (5.284 D) is less than that of its complexes (5.484 -15.87 D), which indicates that the DHNAPH complexes have higher reactivity than DHNAPH [92]. E_{HOMO} and E_{LUMO} have negative values (-8.3537 to -8.9027 & -0.3133 to -3.4114 eV), which refers the stability of compounds [93]. E_{HOMO} of DHNAPH is lower than that of its complexes but E_{gap} and E_{LUMO} of DHNAPH are higher than that of its complexes. This means that the DHNAPH complexes are more active than DHNAPH [16]. The global softness (S) (0.116-0.199 eV⁻¹), *softness* (σ) (0.233- 0.398 eV) and global hardness (η) (2.515-4.295 eV) values affect on the reactivity and molecular stability. The electronegativity (χ) is in the range 4.608-5.926 eV⁻¹, which refers to capacity of the compounds to attract electrons toward themselves [93]. The electrophilicity index (ω) is in the range 2.472-6.983 eV, which refers electrophilicity behavior [16].

Table 7

Table 8 showed that the bond length of C=N(azomethine) for DHNAPH (1.3008 Å) increased by complexation (1.3522 - 1.3821 Å), indicating that stability of complexes increases, where C=N(azomethine) frequencies (1577 cm⁻¹) decrease to 1534-1540 cm⁻¹ [2,92]. The positive slope of relations between (M-O) bond lengths and (C-O) frequencies refers to the bond lengths for M-O bonds decrease with decreasing of C-O frequencies [2]. This due to as stability of complex increases when the M-O became stronger and its length decrease, as well as the C-O became weaker and C-O frequencies decrease.

$$\text{O(R)-M} = -11.64 (1.23) + 0.01282 (0.00117) \nu\text{C-O}, R = 99.18\%$$

$$\text{O(N)-M} = -3.598 (0.678) + 0.005178 (0.000642) \nu\text{C-O}, R = 98.48\%$$

Table 8

Table 9 showed that QSAR properties (surface area, volume, hydration energy, Log p, refractivity, polarizability) of complexes increased about that of DHNAPH. QSAR properties values of the DHNAPH complexes are similar due to similarity between the complexes. The relationships of the linear free energy of bioactivity *versus* the QSAR data (**Table 9**) calculated using *Hyperchem 7.52*,

showed that the negative slopes refer to a decrease in the bioactivity with the increase of both volume and hydration energy. This observation is supported by the positive slope of polarizability with bio data, because the polarizability is inversely proportional with the hydration energy.

$$B = -0.0575 \text{ volume} + 92.827 \quad R^2 = 0.9977$$

$$B = -1.6598 \text{ hydration energy} - 76.214 \quad R^2 = 0.9617$$

$$B = 17.857 \text{ polarizability} - 1146.1 \quad R^2 = 0.9231$$

B= Values of *Candida albicans* (ATCC 10231) 0.5 mg/mL/ Control #

Table 9

3.4. Biological activity

3.4.1. Antimicrobial activity

The antimicrobial activity (Table 10 & Fig. 7) of the new ligand (DHNAPH) and its complexes has been examined against some bacteria and fungi including Gram +ve bacteria; *S. aureus* & *B. subtilis*, Gram -ve bacteria; *S. typhimurium* & *E. coli* and *C. albicans* & *A. fumigatus*. DHNAPH was biologically inactive towards all organisms at the current concentration. Some complexes were active towards the Gram +ve bacteria; *B. subtilis* and *C. albicans*. Towards *B. subtilis*, nickel(II) **2** and copper(II) **3** complex showed low activity. Towards *C. albicans*, all complexes are active and activity varies from low (Co(II) complex) to intermediate (Ni(II) complex) and high (Cu(II) complex). The noticed antimicrobial activity of some complexes may be ascribed to their ability to destruct the cell walls, leading to a change in the cell permeability properties and thus -in turn- causes the death of the cell [94].

Table 10

Fig. 7

3.4.2. Antitumor activity

The efficiency of DHNAPH and its Co(II), Ni(II) and Cu(II)-complexes (Table 11 & Fig. 8) as antitumor agents was explored *in vitro* in contrast to human hepatocellular carcinoma cell line. With $IC_{50} = 200 \mu\text{g/mL}$, DHNAPH showed the lowest activity of the synthesized compounds. On complex-

formation, the activity of DHNAPH remarkably increases ($IC_{50} = 7.29-121 \mu\text{g/mL}$). The higher activity of the complexes than DHNAPH may be owing to the increased conjugation in the DHNAPH skeleton as a result of complex-formation [95]. The sequence of activity of the DHNAPH complexes is : copper(II) > nickel(II) > cobalt(II)-complexes i.e. the highest activity of the complexes is given by Cu(II)- DHNAPH with $IC_{50} = 7.29 \mu\text{g/mL}$, which is lower than that of cisplatin; $IC_{50} = 15.9 \mu\text{g/mL}$. The noteworthy activity of the Cu-DHNAPH complex may be resulted from the significant action of copper in numerous enzymes that catalyze a large number of reactions [96,97]

Table 11

Fig.8

3.5. DNA- binding

Agarose gel electrophoresis was applied in the CT-DNA binding study of DHNAPH and Cu-DHNAPH complex (Fig. 9). The gel after electrophoresis obviously showed that the intensity of all the DNA samples has been detracted partially, which may be related to the interaction with CT-DNA.

Fig. 9a showed that in case of fixed concentration of DNA and different concentrations of DHNAPH; the ligand has the partial ability to destroy DNA at concentration of 2 mg/mL with 400 ng of DNA, otherwise at fixed concentration (Fig. 9b) of DHNAPH (1 mg/mL) with different concentrations of DNA, DHNAPH has the ability to destroy DNA at 200 ng, but at high concentration of DNA more than 400 ng DHNAPH has not the ability to cleave DNA. In addition, it was noted that in case of fixed concentration of DNA and different concentrations of Cu- DHNAPH complex; the complex has (Fig. 9c) the ability to destroy DNA at concentration of 0.25 mg/mL with 400 ng of DNA, otherwise at fixed concentration of complex (Fig. 9d) (0.5 mg) with different concentrations of DNA, the complex has the ability to destroy DNA at 200 and 400 ng, but at high concentration of DNA (800 ng), the complex has not the ability to cleave DNA.

By comparing the ligand with its Cu(II) complex, it was noted that at fixed concentration of DNA (400 ng/mL), DNA is destroyed at concentration of 0.25 mg/mL for Cu- DHNAPH complex and partial cleavage at 2 mg/mL for DHNAPH. On the other hand, at different concentration of DNA, DNA is destroyed at concentration of (200 ng DNA + 1 mg/mL ligand) and (200-400 ng DNA + 0.5 mg/mL complex), respectively. This means that Cu- DHNAPH complex is more active than its ligand, where destruction of DNA occurred at low concentration of Cu- DHNAPH complex. The cleavage ability of the copper(II) complex may be ascribed to the presence of Cu^{2+} ions and aromatic DHNAPH ligand

which promotes the possibility of double strand scission directly after the DNA has suffered a single strand break [98].

3.6. Molecular Docking study

All the molecular docking studies were recorded using Molecular Operating Environment (MOE, 2019.0102) software. All minimizations were performed with MOE until an RMSD gradient of 0.05 kcal·mol⁻¹Å⁻¹ with MMFF94x force field and the partial charges were automatically calculated.

Molecular docking process was performed to assess the binding mode of the DHNAPH compounds on DNA that is related to their activity. The X-ray crystallographic structure of DNA was downloaded from the protein data bank (PDB ID: **1BNA**) [63]. All the tested compounds show good binding score revealing good fitting in between the DNA strands. DHNAPH shows only interactions with strand B with the minimal number of interactions. The metal complexes show higher number of interactions than DHNAPH with both strands confirming their good biological activity. The results are summarized in **Table 12 & Fig. 10**, where; DG: DNA Guanine base; DC: DNA Cysteine base; DA: DNA Adenine base; A: DNA one strand; B: DNA second strand.

The free ligand (DHNAPH) formed four bonds with the amino acid residues, one of them is H-donor bond between the N of the azomethine on the resorcinol moiety (Nazomethine of DHNAPH) and DG-B14 (distance = 3.72 Å). There are three bonds between DG-B16 and (N, =CH & OH) on the naphthayl moiety (distance = 3.45, 3.39 and 3.75 Å, respectively). It is notable that free ligand revealed a moderate binding energy score (S = -6.9913 kcal/mol.) (**Fig. 10a**).

While, Co- DHNAPH complex (**1**) (**Fig. 10b**) formed seven bonds with the amino acid residues, three of them between O of water molecule and (DG-A10, DC-A11 & DG-B14) (3.01, 2.95 & 2.97 Å, respectively), and two of them between O of another water molecule and (DG-B16 & DA-B17) (2.99 & 3.83 Å, respectively), as well as two bond between DA-B18 and O of naphthayl and water molecule (2.92 & 3.39 Å, respectively) but Ni- DHNAPH complex (**2**) (**Fig. 10c**) formed eight bonds, one H-donor bond between DC-A11 and O of water molecule (3.06 Å), and two of them between DG-B14 and two O water molecules (3.56 & 3.20 Å), and two (acceptor H- bonds) of them between another water molecule with (DG-B16 & DA-B17) (3.33 & 3.63 Å), as well as two of them between DA-B18 and (OH & =N) (3.33 & 2.93 Å) and donor H- bond between DC-B15 and O of water molecule (2.88 Å).

On the other hand, Cu- DHNAPH complex (**3**) (**Fig. 10d**) formed seven bonds with the amino acid residues in the active site of protein. Four hydrogen bonds are between DC-A11 and O of water

molecules (3.70, 2.85, 3.28 & 3.09 Å). Two bonds are H-acceptor between O of water molecules and DG-A12 (3.25 & 4.12 Å). The seventh bond is H-acceptor bond between O of water molecule and DA-B17 (3.27 Å).

There is an agreement between docking data and DNA binding results, where Cu- DHNAPH complex is more active than its ligand, due to Cu- DHNAPH complex destroyed DNA at low concentration than that of DHNAPH and this confirmed by docking data, Cu- DHNAPH complex formed seven bonds with **1BNA**, while DHNAPH formed four bonds with **1BNA**.

Table 12

Fig. 10

Conclusion

New binuclear complexes of Co(II), Ni(II), Cu(II) with 4,6-bis(2-hydroxynaphthalen-1-yl)methylene)hydrazono)ethyl)benzene-1,3-diol (H₄L) have been synthesized. The characterization of complexes was performed by analytical, spectral (IR, mass, UV-Vis and ESR), magnetic susceptibility, molar conductivity measurements and TGA techniques. The scanning electron microscopy is used for detection of the morphology of DHNAPH and Co(II) and Ni(II) complexes. The analytical data, magnetic moments and spectral studies recognized octahedral geometries for DHNAPH complexes. DHNAPH acts as bis(dibasic triadentate) *via* (C=N_{azomethine} and 2 OH) with metal in complexes. The optimized structure of DHNAPH and its complexes has been done theoretically by Hyperchem program. The structural parameters were linked with the IR experimental data. The activity of DHNAPH and its complexes in contrast to Hepatocellular carcinoma, fungi and bacteria has been tested. The new complexes are more active than DHNAPH and the highest antitumor activity is given by copper(II) complex. The DNA-binding of DHNAPH and Cu- DHNAPH has been investigated. There is agreement between docking data and DNA binding results, where Cu- DHNAPH complex destroyed DNA at low concentration than that of the ligand and this confirmed by docking data.

Conflict of interest

The authors of the article do not have any conflict of interest.

Data availability statement

The data that support the findings of this study are openly available from the corresponding authors

+ upon reasonable request. Additional supporting information section at the end of this article.

ORCID

Fatma Samy <https://orcid.org/0000-0001-8677-8777>.

Magdy Shebl <https://orcid.org/0000-0003-4377-2273>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Table 1. Analytical and physical data of the ligand (DHNAPH) and its complexes.

No.	Reaction	Complex [F. Wt]	Color	Yield (%)	Elemental analysis, % Found/(Calc.)			
					C	H	N	M
		Ligand [530.58]	Orange	73	72.35 (72.44)	4.96 (4.94)	10.94 (10.56)	-----
1	H ₄ L + Co(OAc) ₂	[Co ₂ L(H ₂ O) ₆].½H ₂ O.½EtOH [784.54]	Dark red	87	50.65 (50.52)	4.48 (4.88)	7.01 (7.14)	15.00 (15.02)
2	H ₄ L + Ni(OAc) ₂	[Ni ₂ L(H ₂ O) ₆].H ₂ O [770.08]	Olive green	85	49.66 (49.91)	4.60 (4.71)	6.97 (7.28)	15.02 (15.25)
3	H ₄ L + Cu(OAc) ₂	[Cu ₂ L(H ₂ O) ₆].½H ₂ O [770.73]	Brown	89	49.60 (49.87)	4.20 (4.58)	6.92 (7.27)	16.21 (16.49)

Table 2. ¹H NMR spectral data of DHNAPH.

Ligand	a CH ₃ (6H)	b CH (2H)	c H-aromatic (12H)	d (1H)	e (1H)	g (2H)	f (2H)
H ₄ L	2.47	9.82	6.49-8.72	6.37	8.4	12.82 (exchangeable with D ₂ O)	13.84 (exchangeable with D ₂ O)

Table 3. Characteristic IR spectral data of DHNAPH and its complexes.

No.	IR Spectra (cm) ⁻¹						
	$\nu(\text{OH})$ phenolic / H ₂ O / EtOH	$\nu(\text{C-H})$ aromatic	$\nu(\text{C-H})$ aliphatic	$\nu(\text{C=N})$ azomethine	$\nu(\text{C-O})$ phenolic	$\nu(\text{M-O})$	$\nu(\text{M-N})$
H₄L	2998	3049	2925	1577	1088		
1	3415	3034	2925	1534	1059	552	452
2	3399	3057	2926	1540	1052	529	471
3	3421	3050	2923	1535	1053	540	467

Table 4. Electronic spectra, magnetic moments and molar conductivity data of DHNAPH and its complexes.

No.	M_{complex}	μ_{eff}	Conductance ^a ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)	Electronic spectral bands λ_{max} (nm)	
	B.M.	B.M.		DMF ^b (ϵ_{max})	Nujol mull
H₄L	---	---	---	279 (0.325), 340 (0.316), 398 (0.345)	---
1	4.98	3.52	6.75	454, 494, 533	495
2	4.59	3.25	7.74	445	433, 694
3	2.23	1.58	5.94	411	431, 699

^a Solutions in DMF (10⁻³ M). ^b Concentrated solutions.Values of ϵ_{max} are in parentheses and multiplied by 10⁻⁴ (L cm⁻¹ mol⁻¹).

Table 5. Thermal analyses data (TG) of DHNAPH-complexes (**1&3**).

No.	Complex	Temperature range (°C)	%Loss in Wt.		Assignment
			Found	Calc.	
1	[Co ₂ L(H ₂ O) ₆].½EtOH.½H ₂ O	46-138	3.72	4.08	Lattice (½ EtOH & ½ H ₂ O)
		139-260	4.08	4.59	2 H ₂ O
		261-412	72.23	72.22	4 H ₂ O; 2 C ₁₁ H ₇ N; 2 CH ₃ CN; 2 C ₂ H ₂ ; 2 CO
		Above 412	20.12	19.10	Residue 2 CoO
3	[Cu ₂ L(H ₂ O) ₆].½H ₂ O	44-121	1.36	1.17	Lattice ½ H ₂ O
		122-250	4.07	4.67	2 H ₂ O
		251-438	67.25	67.28	4 H ₂ O; 2 C ₁₁ H ₇ N; 2 CH ₄ ; 2 HCN; 2 CO
		Above 438	27.32	26.87	Residue 2 CuO + 4 C

Table 6. Temperature of decomposition and activation parameters (E*, ΔH*, ΔS* and ΔG*) determined from DTG results for the decomposition of DHNAPH- complexes.

No.	Complex	Stage	DTG peak (°C)	E* (KJ/mol)	A (S ⁻¹)	ΔH* (KJ/mol)	ΔS* (KJ/mol.k)	ΔG* (KJ/mol)
1	[Co ₂ L(H ₂ O) ₆].½EtOH.½H ₂ O	1 st	86	45.27	44.55	832560	41.63	40.97
		3 rd	345	135.8	132.9	9*10 ¹⁰	126.5	89.31
3	[Cu ₂ L(H ₂ O) ₆].½H ₂ O	1 st	85	91.34	90.63	5*10 ¹²	172.1	76.00
		2 nd	177	40.00	38.53	2328.1	-13.20	40.87
		3 rd	302	136.7	134.2	1*10 ¹¹	129.3	95.18

E* and A are the activation energy and the Arrhenius pre-exponential factor, respectively.

Table 7. Structural parameters of DHNAPH and its metal complexes.

No.	Heat of Formation, kcal/mol	Dipole moment [μ /D]	HOMO Energy, [eV]	LUMO Energy, [eV]	ΔE_{gap} [eV]	ω [eV]	χ [eV ⁻¹]	S [eV ⁻¹]	σ [eV]	η [eV]
H4L	28.79	5.284	-8.9027	-0.3133	8.5894	2.472	4.608	0.116	0.233	4.295
1	-743.12	6.844	-8.4409	-3.4114	5.0295	6.983	5.926	0.199	0.398	2.515
2	-746.86	15.87	-8.3537	-2.2088	6.1449	4.539	5.281	0.163	0.325	3.072
3	-455.03	5.484	-8.6557	-1.4873	7.1684	3.588	5.072	0.140	0.279	3.584

Table 8. The selected bond lengths of optimized structures of DHNAPH and its metal complexes.

Compound	C=N(azo)	C-O(R)	C-O(N)	N(azo)-M	O(R)-M	O(N)-M
H4L	1.3008	1.3647	1.3683	----	----	----
1	1.3821	1.2444	1.3446	1.9138	1.9403	1.8848
2	1.3723	1.2516	1.3289	1.8762	1.8539	1.8467
3	1.3522	1.3518	1.3548	1.9159	1.85796	1.8567

M = Co (**1**); Ni (**2**); Cu (**3**), (R) = resorcinol, (N) = naphthaldehyde, (M-N) & (M-O) in Å and azo = azomethine.

Table 9. QSAR properties of DHNAPH and its complexes.

No.	Surface area (approx)	Surface area (Grid)	Volume	Hydration Energy	Log p	Refractivity	polarizability
1	579.55	761.04	1375.89	-23.28	0.64	169.96	59.41
2	711.00	877.12	1610.87	-46.11	1.65	179.52	64.19
3	717.89	886.55	1607.17	-46.13	1.65	179.52	64.21
3	703.08	859.94	1598.30	-46.50	1.65	179.52	64.23

Table 10. Antimicrobial activity of DHNAPH₂ and its complexes.

, nearest whole mm. Mean* of zone diameter												
<i>Organisms</i> <i>Sample</i> Conc/No.	Gram - positive bacteria				Gram - negative bacteria				Yeasts and Fungi**			
	<i>Staphylococcus aureus</i> (ATCC 25923)		<i>Bacillus subtilis</i> (ATCC 6635)		<i>Salmonella typhimurium</i> (ATCC 14028)		<i>Escherichia coli</i> (ATCC 25922)		<i>Candida albicans</i> (ATCC 10231)		<i>Aspergillus fumigatus</i>	
	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml
H4L	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	9 L	7 L	-	-
2	-	-	10 L	8 L	-	-	-	-	16 I	12 I	-	-
3	-	-	9 L	7 L	-	-	-	-	32 H	27 H	-	-
Control #	35	26	35	25	36	28	38	27	35	28	37	26

* = Calculated from 3 values.

** = identified on the basis of routine cultural, morphological and microscopical characteristics.

– = No effect.

L: Low activity = Mean of zone diameter $\leq 1/3$ of mean zone diameter of control.I: Intermediate activity = Mean of zone diameter $\leq 2/3$ of mean zone diameter of control.H: High activity = Mean of zone diameter $> 2/3$ of mean zone diameter of control.

#: Chloramphenicol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

Table 11. Antitumor activity of DHNAPH and its metal- complexes against HepG-2.

No.	Compound	Inhibition concentration 50%. (IC ₅₀) (μg/mL)
	Ligand	200
1	[Co ₂ L(H ₂ O) ₆].½H ₂ O.½EtOH	121
2	[Ni ₂ L(H ₂ O) ₆].H ₂ O	50.5
3	[Cu ₂ L(H ₂ O) ₆].½H ₂ O	7.29
	cisplatin	15.9

IC₅₀ = inhibition concentration 50%.

Table 12. Docking results of DHNAPH and its metal- complexes.

Compound	S mol)/(kcal	DNA Base	Interacting groups	Type of interaction	Length (Å°)
H₄L	-6.9913	DG-B14	N	H-bond (acceptor)	3.72
		DG-B16	N	H-bond (acceptor)	3.45
		DG-B16	=CH	H-bond (donor)	3.39
		DG-B16	OH	H-bond (acceptor)	3.75
1	-6.3758	DG-A10	OH	H-bond (acceptor)	3.01
		DC-A11	OH	H-bond (donor)	2.95
		DG-B14	OH	H-bond (acceptor)	2.97
		DG-B16	OH	H-bond (donor)	2.99
		DA-B17	OH	H-bond (acceptor)	3.83
		DA-B18	O	H-bond (acceptor)	2.92
		DA-B18	OH	H-bond (donor)	3.39
2	-6.3465	DC-A11	OH	H-bond (donor)	3.06
		DG-B14	OH	H-bond (acceptor)	3.56
		DG-B14	OH	H-bond (acceptor)	3.20
		DC-B15	OH	H-bond (donor)	2.88
		DG-B16	OH	H-bond (acceptor)	3.33
		DA-B17	OH	H-bond (acceptor)	3.63
		DA-B18	OH	H-bond (acceptor)	3.33
		DA-B18	=N	H-bond (donor)	2.93
3	-6.4247	DC-A11	OH	H-bond (donor)	3.70
		DC-A11	OH	H-bond (acceptor)	2.85
		DC-A11	OH	H-bond (acceptor)	3.28
		DC-A11	OH	H-bond (acceptor)	3.09
		DG-A12	OH	H-bond (acceptor)	3.25
		DG-A12	OH	H-bond (acceptor)	4.12
		DA-B17	OH	H-bond (acceptor)	3.27

Fig. 1. ^1H NMR spectrum of DHNAPH ligand

Fig. 2. Mass spectrum of DHNAPH ligand.

Fig. 3. ESR spectrum of Cu(II)- complex (**3**) at room temperature.

Fig. 4. TGA/DrTGA diagrams of the Cu(II)- complex (**3**).

Fig. 5. The mass spectrum of Cu(II)- complex (3).

Ligand

Co(II)- complex

Ni(II)- complex

Fig. 6. SEM of the ligand, Co(II)- and Ni(II)- complexes (1&2).

Fig. 7. Graph of antiyeastal and antifungal activity of the ligand (H_4L), $Co(II)$ -, $Ni(II)$ - and $Cu(II)$ -complexes (**1-3**).

Fig. 8. Graph of (IC_{50}) of antitumor activity for the ligand (H_4L), $Co(II)$ -, $Ni(II)$ - and $Cu(II)$ -complexes (**1-3**) and Cisplatin against HepG-2.

Fig. 9a. The pattern of DNA binding of the agarose gel electrophoresis diagram showing lane L- marker 1kb DNA Ladder, lane 1; DNA control, lane 2; DNA+DMSO, lanes (3, 4 & 5); (400 ng) DNA+ (0.5, 1 & 2 mg/mL) of DHNAPH.

Fig. 9b. The pattern of DNA binding of the agarose gel electrophoresis diagram showing lane L- marker 1kb DNA Ladder, lane 1; DNA control, lane 2; DNA+DMSO, lanes (3, 4 & 5); (200, 400 & 800 ng) DNA+ (1 mg/mL) of DHNAPH.

Fig. 9c. The pattern of DNA binding of the agarose gel electrophoresis diagram showing lane L- marker 1kb DNA Ladder, lane 1; DNA control, lane 2; DNA+DMSO, lanes (3, 4 & 5); (400 ng) DNA+ (1, 0.5 & 0.25 mg/mL) of Cu- complex.

Fig. 9d. The pattern of DNA binding of the agarose gel electrophoresis diagram showing lane L- marker 1kb DNA Ladder, lane 1; DNA control, lane 2; DNA+DMSO, lanes (3, 4 & 5); (200, 400 & 800 ng) DNA+ (0.5 mg/mL) of Cu- complex.

Fig. 10a. 2D & 3D diagram of DHNAPH showing its interaction with the DNA binding site.

Fig. 10b. 2D & 3D diagram of Co(II)- complex (1) showing its interaction with the DNA binding.

Fig. 10c. 2D & 3D diagram of Ni(II)- complex (2) showing its interaction with the DNA binding

Fig. 10d. 2D & 3D diagram of compound Cu(II)- complex (3) showing its interaction with the DNA binding.

Scheme 1. Synthesis of ligand (DHNAPH) and its metal complexes (1-3).

Scheme S1. Mass fragmentation of DHNAPH.

Scheme 2. TGA fragmentation of Co(II) complex (1).

Scheme 3. Mass fragmentation pattern of Cu(II) complex (3).