Synthesis, characterization and cytotoxic activity study of Cu (II), Co (II), Mn (II), Ni (II) and Cr (III) Metal Complexes with new guanidine Schiff base against the hepatocellular Carcinoma (HCAM) cancer cell

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Abstract

In this research, a new guanidine ligand was synthesized from the condensation reaction of 1,2-hydrazinedicarboximidamide and indol-3-carboxaldehyde and its derived metal transition complexes of Co (II), Ni (II), Mn (II), Cu (II) and Cr (III) have been synthesized by reaction of metal chlorides with guanidine ligand in the molar ratio 2:1 (M: L). The guanidine ligand and its metal complexes were characterized by different spectroscopic and analytical techniques, these studies result suggests that the metal complexes have tetrahedral geometry. The cytotoxic activity of the guanidine Schiff base and its metal complexes were studied on hepatoma cellular carcinoma (HCAM) cell line.

Keywords: Guanidine-indole; Thioureas; Complexes; MTT assay

Introduction

Guanidine and its derivatives can be seen in many natural compounds that have a significant area of biological activities such as anti-inflammatory, anti-diabetic, anti-clotting agents, exhibit cytotoxic, antiviral, antibacterial, and anti-parasitic [1, 2]. Guanidine is an important class of compounds in organic and biochemistry that possesses the formula HN=C(NH$_2$)$_2$, where the carbon atom is bonded to three nitrogen atoms. Which is among the strongest known organic bases and it has very weak pKa that are difficult to accurately measure in water. There are several approaches for the synthesis of guanidine from different materials and reagents, one of these approaches is the diversion of thioureas to guanidine in the presence of a coupling reagent. Their conversion to guanidine regularly needs initial activation [3]. A number of reviewed articles had been reported on Schiff base compounds derived from guanidine are of prominence in organic synthesis, as they are used as intermediates to prepare a number of organic compounds [4-7]. Some Schiff base derivatives were prepared by the interaction of aminoguanidine with the different substituted benzaldehyde [8, 9], as these compounds proved to possess anti-bacterial and anti-cancer activities [10]. Also, various aminoguanidine derivatives exhibit anti-tumor activity by forming metal ion complexes [11, 12]. Three complexes of copper (II) were synthesized from 2-aminobenzimidazole and o-vanillin as primary ligand and N, N-donor heterocyclic bases (1,10-phenanthroline and 2,2'-bipyridyl) as co-ligand are the examples containing guanidine Schiff base ligand, and these complexes considered to have a first vision on their potential anti-cancer activity against MCF-7 (human breast cancer)cell lines as well as anti-inflammatory, antipyretic and analgesic activities [13].

In this paper, five new guanidine-indole complexes were synthesized from the Schiff base reaction of...
1,2-hydrazone dicarboximidamide with indol-3-carboxaldehyde. All complexes have a tetra-coordinated metal centre, binding two donors of the corresponding Schiff base guanidine ligand via the imine nitrogen atom and two nitrogen atoms of the indole unit. This motivates us to study the biological activity of guanidine ligand and its complexes against hepatoma cellular carcinoma (HACM) cell line.

Experimental

Materials and Methods

Aldrich and Merck obtained all the reagents and solvents and used them without further purification. Infrared spectra were collected using KBr pellets on FTIR-84005-SHIMADZU. Melting points were obtained using Thermo-scientific apparatus. Ligand spectrum 1H and 13C-NMR was obtained on Bruker-400HZ and recorded at room temperature in DMSO. UV-Visible spectra on uv-win5 were reported using 10mm quartz cells. Mass spectra were run at 70 eV using glass tubes with a diameter of 0.324 cm. Thin layer chromatography (TLC) has tracked the completion of reactions.

Synthesis of the Guanidine ligand and its metal complexes

General synthetic route of the guanidine ligand, (L)

The ligand L (C38H28N10) was prepared from the reaction of thiourea (24.57 g, 0.3228 mol) and hydrazine hydrate (8 g, 0.0689 mol) in dry ethanol (100 ml), before the mixture was subjected to heating for a period 3 hours with stirring. Cool the reaction mixture at room temperature and the resulting precipitate was then filtered and cleansed through recrystallization from ethanol to isolated the 1,2-hydrazone dicarboximidamide product [14]. Indol-3-carboxaldehyde (5.8 g, 0.039 mol) in dry ethanol (25ml) and glacial acetic acid (2-3) drops was added. This combination was stirred about ten minutes, the solution of the 1,2-hydrazone dicarboximidamide (1.12 g, 0.0096 mol) in dry ethanol (15ml) was then added gradually and the reaction mixture was refluxed with stirring for six hours, the yellow precipitate was retrieved through filtration, rinsed by ethanol several times and then allowed to dry. Ultimately, the target product was acquired through there crystallization from ethanol (Scheme 1). Yellow colour; yield: 75 %; M.P 255-258 °C; FTIR (ν cm⁻¹): 3107 (ν N-H), 3059 (ν CH aromatic), 1672 (ν C=N), 1516-1440 (ν C=C), 1365 (ν C-N), 1132(ν N-N); 1H NMR (DMSO, 400 MHz; δ ppm): δ: 11.5 (s, 2H), 8.92 (s, 2H, 2CH =N), 7.14-8.40 (m, 10H, Ar-H); 13C NMR (DMSO, 400 MHz; δ ppm): 111.7, 111.9, 112.1, 112.4, 120.3, 120.5, 122.1, 122.4, 122.6, 122.8, 124.2, 124.7, 129.5, 131.8, 137.0, 137.2 (aromatic carbons), 155.11 (C=N-N), 185.01 (CH=N); MS: m/z: 624[M⁺], 118[M⁺], 146[M⁺], 341[M⁺]; UV-vis. in DMSO, cm⁻¹ (transition): 279 (π→π*) and 357 (n→π*).

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H

\text{Scheme 1. Synthesis of guanidine ligand (L).}
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Synthesis of transition metal complexes (1–5)

The guanidine L(C38H28N10) (0.156g,1mmol) in (2:4:4 ml) of (DMF: EtOH: MeOH) respectively was mixed with metal chlorides (2mmol) of CuCl₂,2H₂O, CoCl₂,6H₂O, MnCl₂.4H₂O, NiCl₂.6H₂O and CrCl₃.6H₂O. This reaction mixture was carried out by heating under reflux with stirring for three hours, through filtration the precipitate was separated from the reaction. The precipitate was washed with diethyl ether and dried in a desiccator over activated silica gel [15] (Fig.1).

Complex 1.

Pale brown colour; yield: 59 %; M.P>300 °C; FTIR (ν cm⁻¹):1637 (ν C=N), 1521 (ν C=C), 1444 (ν C-N), 1122(ν N-N), 640(νM-N) and 486 (νM-NH); UV-vis. in DMSO, cm⁻¹ (transition):262, 296 (π→π*), 336 (n→π*) and 342 (d-d); MS: m/z: 750[M⁺]λₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑ euler1.75.

Complex 2.
Green colour; yield: 66 %; M.P>300 °C; FTIR (v cm⁻¹): 1635 (v C=N), 1562 (v C=C), 1446 (v C-N), 1131 (v N-N), 532(v-M-N) and 430 (v-M-NH); UV-vis. in DMF, cm⁻¹ (transition): 261, 298 (π→π*), 338 (n→π*), 497 (d-d), 383 (L-M); MS: m/z: 741[M+H]⁺; A∞(ohm⁻¹ cm² mol⁻¹) 70.3; μeff (BM) 2.34.

Complex 3.

Bright yellow colour; yield: 57 %; M.P>300 °C; FTIR (v cm⁻¹): 1635 (v C=N), 1577 (v C=C), 1395 (v C-N), 1125 (v N-N), 788(v-M-N) and 530 (v-M-NH); UV-vis. in DMF, cm⁻¹ (transition): 262, 298 (π→π*), 336 (n→π*) and 515, 733 (d-d); MS: m/z: 734[M+H]⁺; A∞(ohm⁻¹ cm² mol⁻¹) 88.9; μeff (BM) 5.46.

Complex 4.

Yellow colour; yield: 45 %; M.P>300 °C; FTIR (v cm⁻¹): 1633 (v C=N), 1577 (v C=C), 1395 (v C-N), 1130 (v N-N), 500 (v-M-N) and 493 (v-M-NH); UV-vis. in DMF, cm⁻¹ (transition): 265, 299 (π→π*), 332 (n→π*) and 473 (d-d); MS: m/z: 742[M+2H]⁺; A∞(ohm⁻¹ cm² mol⁻¹) 87.4; μeff (BM) 3.56.

Complex 5.

Brown colour; yield: 60 %; M.P>300 °C; FTIR (v cm⁻¹): 1635 (v C=N), 1577 (v C=C), 1398 (v C-N), 1122 (v N-N), 520 (v-M-N) and 420(v-M-NH); UV-vis. in DMF, cm⁻¹ (transition): 260, 295 (π→π*), 343 (n→π*), 495, 520 (d-d) and 420 (L-M); MS: m/z: 727[M⁺]⁺; A∞(ohm⁻¹ cm² mol⁻¹) 89.2; μeff (BM) 3.56.

Figure 1. Proposed structure of the guanidine ligand metal complexes 1-5.

Biological activity

Maintenance of cell cultures

HCAM cell line was got from the Iraqi centre of cancer and continued in RPMI-1640 supplemented with 10% Fatal bovine, 100 units/mL penicillin, and 100 μg/mL streptomycin. cells were passaged by Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37 °C and 5% CO₂. was applied to humidify the atmosphere.

Combination Cytotoxicity Assays

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbrom ide) cell viability assay was performed on 96-well plates, to determine the cytotoxic effect. The HCAM cell line was seeded at approximately 1 × 10⁴ cells/well. After 24h or a confluent monolayer was performed, cells were treated with the prepared compounds using different concentrations (50, 250, 500, 750, 1000) μg/mL. Cell viability was recorded after 72 hours of treatment by removing the medium, adding 28 μL of 2 mg/mL solution of MTT and incubating the cells for 2 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100 μL of dimethyl sulfoxide followed by 37 °C incubation for 15 min with shaking [16]. Absorbency was run on a microplate reader at 620 nm (test wavelength); the assay was reported in triplicate. The rate of inhibition for cell growth (the percentage of cytotoxicity) was considered as the following equation: Proliferation rate as (PR)=B/A*100 where A is the mean optical density of untreated wells and B is the optical density of treated wells and IR= 100- PR [17]. In addition to the concentration causing half the maximum inhibitory (IC50) was determined.

Result and discussion

The analytical and physical data (Table 1), spectral data (Tables 2 and 3) reveal that, the guanidine ligand (L) (C₆H₆N₁₀) was synthesized by condensation reaction of 1,2-hydrazinedicarboximidamide with Indol-3-carboxaldehyde in (1:4) molar ratio as shown in Scheme.1. The complexes 1-5 are designed in (1:2) (L: M) stoichiometric ratio (Fig.1). Based on the results of all the analytical studies, metal complexes 1-5 agreed to the proposed general formula of [M₂L₂] Cl₂.

Conductivity

The high molar conductance values of the complexes (1-5) in DMF (10⁻¹ M) invention in the 70.3-89.2 ohm⁻¹ cm² mol⁻¹ range as in Table 1,
representing that, all the complexes are an electrolyte [18]. This confirms that, the anion is not coordinated to the metal ion.

**FT-IR spectra**

The manners of bonding between the ligand and the metal ion can be exposed by comparing the IR spectra of the solid complexes (1-5) with that of the guanidine ligand (L). The IR spectral data of the ligand (Fig. 2a) and its metal complexes (Figs. 2b-f) are offered in Table 2. The IR spectrum of the ligand shows absorption band at 1672 cm⁻¹ due to azomethine ν(CH=N) [19], a broad medium band appears at 3107 cm⁻¹ assigned to the stretching vibration of the ν(N-H). The comparatively strong bands located at 1516, 1365 and 1132 cm⁻¹ are assigned to the ν(C=C), ν(CN) and ν(N-N) respectively. In all the metal complexes (1-5) indicated that the absorption band of azomethine group ν(CH=N) was shifted to lower frequencies in the range 1633-1637 cm⁻¹ upon complexation with central metal ions, suggesting that the coordination happens via azomethine nitrogen [20]. Its packages weaken the ν(N-H) group, underscoring their association which appears in the ligand region 3107cm⁻¹ Disappears in the complexes. New vibrations at 500-788 cm⁻¹ and 420-530 cm⁻¹ which are not present in the free guanidine L are attributed to the existence of ν(M-N) and ν(M-NH).

**Magnetic moments**

The magnetic moments values of the complexes (1-5) lie in the 1.75-5.46 B.M as shown in Table (3). This strongly favor a distorted tetrahedral geometry around the metal ion and has para-magnetic properties [21].

**Electronic spectra**

The electronic spectral data for the guanidine ligand (L) and its metal complexes (1-5) in DMSO solution are summarized in Table (3). The ligand (Fig. 3a) in DMSO solution shows two bands at 357 nm (ε = 12880 mol⁻¹cm⁻¹) and 279 nm (ε = 2580 mol⁻¹cm⁻¹), which may be assigned to the n→π* and π→π* transitions respectively [22]. The bands appeared in the UV-vis spectra (Figs. 3b-f) of the complexes (1-5) in the 332-343 and 260-299 nm ranges were assigned to the ligand field (L.F). Thus, the band appeared in the 342-733 nm region indicated d →d transition in the complexes. The bands at 383 and 420 nm in the spectra of complexes 2 and 5 was due to the metal to ligand charge transfer transition (C.T) [23].

**1H, 13C-NMR spectra for the guanidine ligand (L)**

The ¹H-NMR spectrum for the ligand guanidine (L) in Fig.4 showed the following characteristic chemical shift (DMSO-d₆ as a solvent): the spectrum showed singlet signal at 11.51 ppm due to proton of NH group, and singlet signal at 8.92 ppm attributed to the azomethine proton (CH=N). As well as signals at 8.40-7.14 ppm range detected as multiple due to aromatic protons. The ¹³CNMR spectrum of a ligand (L), Fig.5 in DMSO-d₆ solvent displayed that the chemical shift at 185.01ppm due to carbon of (CH=N) group, and the chemical shift at 155.11 ppm assigned to the carbon atom of (C=N-N) group. While the chemical shift at 111.7-137.2 ppm range attributed to the aromatic carbons [24].

**Mass Spectra for the guanidine ligand (L) and its complexes**

The electrospray (ESI⁺) mass spectra of ligand (L) and its metal complexes (1-5) exhibited successive fragments related to the structures. The parent ion peak for the ligand (Fig. 6a) observed at m/z 624 corresponding to M⁺ for C₅H₁₀N₁₀; requires 624.25. The other peak fragments were shown to give at m/z 118 (M⁺) for C₆H₁₄N₁₀, m/z 146 (M⁺) for C₈H₁₆N₂ and m/z 341 (M⁺) for C₂₈H₁₇N₈. Complex 1 (Fig. 6b) gave a molecular ion peak at m/z 750 which corresponds to M⁺ for [Cu₂(C₃₉H₃₈N₁₀)] Cl₂; requires 749.77. As well as the complex 2 (Fig. 6c) reveal a molecular ion peak at m/z 741 corresponding to [M⁺H]⁺ for [Cu₂(C₃₉H₃₈N₁₀)] Cl₂; requires 740.55. While the complex 3 (Fig. 6d) gave ion peak at m/z 734 which corresponds to [M⁺H]⁺ for [Mn₂(C₃₉H₃₈N₁₀)] Cl₂; requires 732.56. The complex 4 (Fig. 6e) exhibited a molecular ion peak at m/z 742 corresponding to [M+2H]⁺ for [Ni₂(C₃₉H₃₈N₁₀)] Cl₂; requires 740.07, and the complex 5 (Fig. 6f) gave ion peak at m/z 727 which corresponds to M⁺ for [Cr₂(C₃₉H₃₈N₁₀)] Cl₂; requires 726.67 [25].

**Anticancer Activity**

To examine the cytotoxic activity of the guanidine ligand (L) and its metal complexes (1-5), they were
tested for effects on the viability of the HCAM cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and cisplatin is one of the most usually used anticancer drugs [26]. All of the complexes were tested from their low concentration (50 µg/ml) to higher concentration (1000 µg/ml). In vitro cytotoxic activity investigation revealed that complexes 1, 2 and 3 exhibited high activity with concentration of 50, 750 and 1000 µg/ml, and IC50 values of 87.73, 180 and 432 µg/ml, respectively. The results revealed that the cytotoxic activity of the Cu (II), Mn (II) and Co (II) complexes [27, 28] could be correlated with the donor atoms as (N8-type (Figs. 7-9). It is also important to note that the guanidine ligand (L) and complexes (4, and 5) did not show marked inhibition against the HCAM cancer cell line as shown in Table 4.

Table 1
Physical data of the ligand (L) and its metal complexes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Empirical formula</th>
<th>FW</th>
<th>Color</th>
<th>Yield (%)</th>
<th>M.P (°C)</th>
<th>Molar conductance Am (Ω cm² mol⁻¹)</th>
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<tr>
<td>1</td>
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<td>Pale brown</td>
<td>59</td>
<td>&gt;300</td>
<td>77.6</td>
</tr>
<tr>
<td>2</td>
<td>[Co₂(C₃H₂₂N₁₀)₂] Cl₂</td>
<td>811.5</td>
<td>Green</td>
<td>66</td>
<td>&gt;300</td>
<td>70.3</td>
</tr>
<tr>
<td>3</td>
<td>[Mn₂(C₃H₂₂N₁₀)₂] Cl₂</td>
<td>803.5</td>
<td>Bright yellow</td>
<td>57</td>
<td>&gt;300</td>
<td>88.9</td>
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<tr>
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<td>[Ni₂(C₃H₂₂N₁₀)₂] Cl₂</td>
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<tr>
<td>5</td>
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<td>Brown</td>
<td>60</td>
<td>&gt;300</td>
<td>89.2</td>
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Table 2
IR frequencies of the bands (cm⁻¹) of ligand (L) and its metal complexes.

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<th>ν (NH)</th>
<th>ν (CH=N)</th>
<th>ν (C=C)ₚₜ</th>
<th>ν (N-N)</th>
<th>ν (C-N)</th>
<th>ν (M-N)</th>
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<td>1132</td>
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<td>1521</td>
<td>1122</td>
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<td>486</td>
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<td>1446</td>
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<td>1577</td>
<td>1122</td>
<td>1398</td>
<td>520</td>
<td>420</td>
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Table 3
The electronic absorption spectral bands (nm) and magnetic moment (B.M) for the ligand (L) and its complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ max (nm)</th>
<th>Eₘₐₓ mol⁻¹ cm⁻¹</th>
<th>Assignment</th>
<th>Suggested structure</th>
<th>µeff (B.M)</th>
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Table 4
IC50 values for the ligand and its complexes against HCAM cell

<table>
<thead>
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<th>Line</th>
<th>Compound</th>
<th>Concentration µg/ml</th>
<th>IC50 µg/ml</th>
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<tbody>
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<td>1</td>
<td>L</td>
<td>50</td>
<td>87.73</td>
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<tr>
<td>2</td>
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<td></td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td></td>
<td>432</td>
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<tr>
<td>4</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
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</tr>
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Fig. 2c. FT-IR spectrum of complex (2).

Fig. 2d. FT-IR spectrum of complex (3).

Fig. 2e. FT-IR spectrum of complex (4).

Fig. 2f. FT-IR spectrum of complex (5).

Fig. 3a. UV-Vis. spectrum of guanidine (L).

Fig. 3b. UV-Vis. spectrum of complex (1).
Fig. 3c. UV-Vis. spectrum of complex (2).

Fig. 3d. UV-Vis. spectrum of complex (3).

Fig. 3e. UV-Vis. spectrum of complex (4).

Fig. 3f. UV-Vis. spectrum of complex (5).

Fig. 4. $^1$H NMR spectrum of guanidine (L).

Fig. 5. $^{13}$C NMR spectrum of guanidine (L).
Fig. 6a. ESI-Mass spectrum of guanidine (L).

Fig. 6b. ESI-Mass spectrum of complex (1).

Fig. 6c. ESI-Mass spectrum of complex (2).

Fig. 6d. ESI-Mass spectrum of complex (3).
Fig. 6e. ESI-Mass spectrum of complex (4).

Fig. 6f. ESI-Mass spectrum of complex (5).

Fig. 7. Rate viability of HCAM cell line treated with five replicates of concentrations(µg/ml) from Cu (II) complex with IC50 in µg/ml.

Fig. 8. Rate viability of HCAM cell line treated with five replicates of concentrations(µg/ml) from Co (II) complex with IC50 in µg/ml.

Fig. 9. Rate viability of HCAM cell line treated with five replicates of concentrations(µg/ml) from Mn (II) complex with IC50 in µg/ml.
Conclusions

Five new metal complexes have been synthesized and described from binucleation ligand and well characterized through conventional analytical approaches and by various spectral physicochemical methods. The results of FTIR, electronic transition, $^1$H and $^{13}$C-NMR and mass spectrometry. In addition to the measurements of magnetic sensitivity and electric conductivity led to the conclusion that the metal complexes 1-5 assumed a distorted tetrahedral geometry. Among the five complexes, only Co (II) and Mn (II) complexes showed a good result against liver cancer cells, and they need further investigations to improve and identify the most active complex.

Conflicts of interest

There are no conflicts to declare

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REFERENCES


