

Spectroscopic and Characterization of Medical and Physical Applications of Metal Complexes of Schiff bases in nano-sized Shape: Mg(II), Ca(II) and Ba(II) Schiff base Complexes

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Abstract: Mg(II), Ca(II) and Ba(II) metal chelates with formulas, $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$, $[\text{Ca}(\text{HL})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ that HL is the Schiff base moiety formed from condensation of 2-thiophenecarboxaldehyde with 2-chlorophenyl hydrazine have been prepared and characterized by elemental analysis and spectroscopic measurements (infrared and ¹H-NMR). Elemental analysis of the complexes suggests the stoichiometry is 1:2 (metal-ligand). Infrared spectra of the complexes agree with the coordination to the central metal atom through the nitrogen of the 2-chlorophenyl hydrazine(-Ph-NH-) group and the sulfur atom of the thiophene ring. Biological properties of prepared compounds were studied included antimicrobial activity and cytotoxicity. A potent antimicrobial activity evaluated by disc inhibition zone and variety degree of activity recorded against gram-positive; gram-negative and fungal strains. Cytotoxicity against two cell lines; MCF-7 and HePG-2 were evaluated and IC₅₀ values were calculated. The $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ and $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$ had the highest antitumor effect with IC₅₀ values; 32.6 and 30.4 μg/ml for MCF-7 cell line and 37.5 ; 39.5 μg/ml for HePG-2 cell line.

Keywords: Spectroscopic, alkaline earth metal complexes, Schiff base.

I. INTRODUCTION

Schiff base ligands are considered “privileged ligands” because they are easily prepared by the condensation between aldehydes and imines. Stereogenic centers or other elements of chirality (planes, axes) can be introduced in the synthetic design. Schiff base ligands are able to coordinate with many different metals [1], and to stabilize them in various oxidation states. The Schiff base complexes have been used in catalytic reactions [2] and as models for biological systems [3]. Chiral Schiff bases derived from the condensation of salicylaldehydes with 2-amino alcohols have found widespread use as ligands in asymmetric synthesis. These compounds act as tridentate ONO ligands, and a great number of metallic complexes derived from them have been described in the literature [4]. Depending upon the nature of the metal centre, these chiral complexes are able to promote a variety of enantioselective transformations. It has been reported that the structure of the substituent bonded to the imino nitrogen affects the coordination geometry of the complex [5]. During the past two decades, considerable attention has been paid to the chemistry of the metal complexes of Schiff bases containing nitrogen and other donors [6]. This may be attributed to their stability, biological activity [7] and potential applications in many fields such as oxidation catalysis, electrochemistry [8]. The complexes make these compounds effective and stereospecific catalysts for oxidation, reduction and hydrolysis and they show biological activity, and other transformations of organic and inorganic chemistry. It is well known that some drugs have

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higher activity when administered as metal complexes than as free ligands [9]. Nickel(II) Schiff base complexes containing sulfur donors have received considerable attention due to the identification of a sulfur rich coordination environment in biological nickel centers, such as the active sites of certain ureases, methyl-S-coenzyme-M-methyl reductase and hydrogenases [10]. The zinc(II) ion is known to have a high affinity towards nitrogen and sulfur donor ligands. Dowling and Perkin investigated Zn(II) complexes with mixed N, O and S coordination to understand the reactivity of the pseudo-tetrahedral zinc center in proteins [11]. The transition metals zinc and copper are some of the most frequently occurring elements integrated into essential biochemical pathways. There are a number of biologically important molecules showing the catalytic activity [12] or molecules involved in transfer processes, like oxygen transfer, and incorporating transition metals into their active sites. In contrast to zinc(II), the copper(II) ions are redox active and play a crucial role in catalytic sites of oxidoreductases. The cyclic redox process enables these ions to act as pro- or anti-oxidants. The published opinions on the structure and pro- or anti-oxidant activity relationships are, however, quite inconsistent [13]. We report herein the results of our studies on the metal complexes of a Schiff base derived from 2-thiophenecarboxaldehyde and 2-chlorophenyl hydrazine. Tentative structures have been proposed on the basis of analytical, spectral and conductance data. The antimicrobial activity of prepared compounds were assayed in vitro by the disk diffusion method against seven microorganism strains and compared with that of free Schiff bases. Also, cytotoxicity effect against two cell lines; MCF-7 and HePG-2 were evaluated and IC50 values were calculated.

II. MATHEMATICAL MODEL

Tires and suspensions are considered in two degrees of freedom quarter car model as shown in Figure. This model is based on the bouncing motion of the vehicle due to random road surfaces. In this model only 1/4th of the vehicle is taken in to consideration for the vibration analysis. The model is two dimensional. Basically this model is consisting of

2-1- Chemicals

Reagent grade 2-thiophenecarboxaldehyde, 2-chlorophenyl hydrazine, and alkaline earth metal salts (magnesium(II)chloridehexahydrate, calcium chloride anhydrous and barium(II) chloride dihydrate) were received from Merck and Aldrich Companies, and other chemicals and solvents were purchased and used as received.

2-2- Synthesis of Schiff base (HL)

The Schiff base (HL, Fig. 1) was been prepared according to the previous procedure [14, 15]: An ethanolic solution of 2-thiophenecarboxaldehyde (1 mmol, 25 mL) was added to an ethanolic solution of 2-chlorophenyl hydrazine (1 mmol, 25 mL) and refluxed for 2 hour in a water bath. After concentration of the solution, the precipitate was separated, filtered, washed with ethanol, and dried over anhydrous calcium chloride under vacuum. Anal.:Calcd. For HL: C, 55.81; H, 3.83; N, 11.83; S, 13.55. Found: C, 55.24; H, 3.90; N, 11.50; S, 13.24. The calculated mass spectrum: m/e: 236.72 (100.0%), 240 (1.5%).

2-3- Synthesis of the Mg(II), Ca(II) and Ba(II) Schiff base complexes

A mixture of HL (2 mmol, 50 mL) in ethanol was added to an aqueous solution of magnesium(II)chloridehexahydrate, calcium chloride anhydrous and barium(II) chloride dihydrate (1mmol, 10 mL). The mixture of reaction was refluxed for 2 hours and then excess solvent was distilled. The precipitated compounds that separated were filtered, washed with ethanol, and dried over CaCl₂ in vacuum. Anal.:Calcd. For [Mg(HL)₂(Cl)₂].6H₂O: C, 39.05; H, 4.47; N, 8.28; S, 9.48; Mg, 3.59. Found: C, 38.94; H, 4.41; N, 8.12; S, 9.43; Mg, 3.48. Anal.:Calcd. For [Ca(HL)₂(Cl)₂].2H₂O: C, 42.59; H, 3.57; N, 9.03; S, 10.34; Ca, 6.46. Found: C, 42.37; H, 3.52; N, 8.93; S, 10.27; Ca, 6.36. Anal.:Calcd. For [Ba(HL)₂(H₂O)₂].Cl₂.2H₂O: C, 35.06; H, 3.48; N, 7.43; S, 8.51; Ba, 18.01. Found: C, 34.88; H, 3.41; N, 7.39; S, 8.43; Ba, 17.93.

2-4- Measurements

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The elemental analyses of carbon, hydrogen, nitrogen and sulfur contents were performed using a Perkin Elmer CHNS 2400 (USA). The molar conductivities of freshly prepared $1.0 \times 10^{-3} \text{ mol/cm}^3$ dimethylsulfoxide (DMSO) solutions were measured for the dissolved HL Schiff base Mg(II), Ca(II) and Ba(II) complexes using Jenway 4010 conductivity meter. The electronic absorption spectra of HL Schiff base complexes were recorded in DMSO solvent within 900-200 nm range using a UV2 Unicam UV/Vis Spectrophotometer fitted with a quartz cell of 1.0 cm path length. The infrared spectra with KBr discs were recorded on a Bruker FT-IR Spectrophotometer ($4000-400 \text{ cm}^{-1}$). The $^1\text{H-NMR}$ spectra were recorded on Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz spectra in deuterated dimethylsulphoxide (DMSO- d_6). Chemical shifts are quoted in δ and were related to that of the solvents. Scanning electron microscopy (SEM) images were taken in Joel JSM-6390LA equipment, with an accelerating voltage of 20 KV.

III. RESULT AND DISCUSSION

3-1- Physical measurements data

Mg(II), Ca(II) and Ba(II) Schiff base HL complexes were synthesized in powder form with high melting points (Table 1). They are not soluble in ethanol, diethyl ester, or chloroform, but are partially soluble in DMSO and DMF. Elemental analysis suggests that the complexes have 1:2 (metal-ligand) molar ratios. The molar conductance of the Mg(II), Ca(II) and Ba(II) Schiff base complexes in DMSO are 20, 52 and $410 \mu\text{s}$ indicating there is electrolytic nature only for $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ complex, these meaning that anions occurred out of the coordination sphere. Based on the conductivity measurements as well as elementary chemical analysis the suggested formulae are $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$, $[\text{Ca}(\text{HL})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$.

3-2- Infrared spectra

Main characteristic infrared absorption bands of HL and its Mg(II), Ca(II) and Ba(II) complexes, along with their assignments, are presented in Table 2.

N-H, C=N and C=C vibration motions

The infrared spectrum of the HL ligand (Fig. 2) exhibits a band at 1595 cm^{-1} assignable to $\nu(\text{C}=\text{N})$ of the azomethine group and an intense bands at (1492, 1452 and 1407 cm^{-1}) corresponding to the C=C stretching of the benzene and thiophene rings [16, 17]. The comparison of the positions of these bands with those observed in the infrared spectra of its Mg(II), Ca(II) and Ba(II) complexes indicated that the band at 1595 cm^{-1} did not show a marked shift, this discussed that azomethine group unshared in the complexation toward Mg(II), Ca(II) and Ba(II) ions, while that bands at 3328 and 3306 cm^{-1} which assigned to stretching vibration motions $-\text{NH}$ of phenylhydrazine moiety is a mild decreasing in an intensity or absence. This fact suggests the coordination of HL through the nitrogen Ph-NH of phenylhydrazine. Proof of coordination to the N atom is provided by the occurrence of the new absorption bands within $412-443 \text{ cm}^{-1}$ in the IR spectra of all HL complexes [16,17].

C-S-C and C-S vibration motions

The observed medium intensity band at 912 cm^{-1} in the free HL ligand, which is ascribed to $\delta(\text{CSC})$ of thiophene ring vibration [15], shifted to lower values for the three HL complexes, suggesting the involvement of the sulfur atom in the bonding with the metal's ions. The band assigned to the stretching of $\nu(\text{C}-\text{S})$ is similarly shifted to lower frequencies. This also confirms that the sulfur atom is taking part in the complex formation [15].

3-3- Electronic spectra

Upon the electronic spectrum of the HL Schiff base ligand, the two essential absorption bands were observed at 310 nm, (360 and 550) nm and assigned to the transitions $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, respectively. These transitions were

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existed also in the spectra of the complexes, but they shifted to different lower intensities, confirming the coordination of the ligand to the metal ions (Fig. 3).

3-4- ^1H NMR Spectra

The ^1H -NMR spectra of the HL Schiff base ligand and magnesium(II) complex shows multiplet signals at 6.752-7.527 ppm, corresponding to the aromatic ring protons of the phenyl and thiophenemoieties [18]. The singlet at 9.894 ppm was attributed to the proton of the azomethine group [19]. The signal of the $-\text{NH}$ proton in the phenylhydrazinemoiety was observed at 8.479 ppm. In comparison between the ^1H -NMR data of HL and its $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$ complex the mode of coordination in between the ligand and metal ions was clarified. Through the discussion of ^1H -NMR spectrum of Mg(II) complex, we find that the signal specialized to $-\text{NH}$ proton of phenylhydrazinemoiety shifted to upfield. The appearance of new strong intense peak at 3.381 ppm can be assigned to the H-proton of H_2O , which attached to the Mg(II) metal to complete the six coordination sphere (Fig. 4).

3-5- SEM images

Scanning electron microscopy images (Fig. 5) is a simple tool used to give an impression about the microscopic aspects of the physical behavior of Schiff base as a complexing agent and of the complex formation. Although this tool is not a qualified method to confirm complex formation but it can be a reference to the presence of a single component in the synthetic products. The pictures of the Mg(II), Ca(II) and Ba(II) complexes show a small particle size with a nano feature products.

3-6- Biological experiments

3-6-1- Screening of antimicrobial activities

The antimicrobial activity of the Schiff base moiety (HL) and Mg(II), Ca(II) and Ba(II) metal chelates was in vitro screened by the standard disc diffusion technique [20, 21].

3-6-1-1- Microbial strains and growth conditions

Seven microorganisms were used to assess the antimicrobial properties, including the Gram-positive *Bacillus subtilisa*, *Staphylococcus aureus* and *Streptococcus pneumonia*, the Gram-negative *Escherichia coli*, *Pseudomonas solanarium* and the fungal *Aspergillus nigaer* and *Penicillium*. All strains were obtained from Microanalysis unit, Alexandria university and maintained on standard suitable cultivation condition of each organism. Active cultures were prepared by transferring a loop of cells from the agar slant to a test tube containing 5 ml of Nutrient Broth for bacteria and YPD Broth for the mold. They were then incubated overnight to the logarithmic phase of growth at 37 °C for bacteria (6–10 h) and 30 °C for the yeast (12–16 h). Culture purity was examined by streaking each culture on plates of Nutrient Agar for bacteria [20]. The turbidity of the cell suspensions was measured at 600 nm and adjusted to the required adjusted to the required concentration of 10^5 - 10^6 CFU/ml using the McFarland standard [22].

3-6-1-2- Agar disk diffusion assay

The antimicrobial activity of the Schiff base moiety (HL) and Mg(II), Ca(II) and Ba(II) metal chelates was screened using the agar disk diffusion method [23]. Target organisms were inoculated in melted (at 50°C) trypticase soy agar + 0.6% yeast extract medium. Mold strains were inoculated in melted (at 50 °C) potato dextrose agar medium with heavy inoculum. Then the inoculated medium were poured over a solid layer of uninoculated agar medium in sterilized Petri-dishes and left to solidify at 4°C (surface layer should be constant in volume and horizontally homogenous). Discs of Whatman No. 1 filter paper (6.0 mm in diameter) were sterilized by autoclaving at 121°C for 15 min. An accurate weight of each crystal compound was dissolved in DMSO (200 µg /ml) and aseptically added to each disc and left to dry. Each disc was aseptically placed on the middle of agar surface (in triplicate) and left at 4°C for 1 hr then plates

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were incubated. The antimicrobial activity of target compounds was recorded as three degree of inhibition effect, mild ; moderate and marked degree.

3-6-2-Cytotoxicity assay

Evaluation of Cytotoxic Effects of certain chemical compound operated in the Regional Center for Mycology & Biotechnology, Al-Azhar University, Egypt. Mammalian Cell lines: MCF-7 cells (human breast cancer cell line were obtained from VACSERA Tissue Culture Unit. Chemicals Used; Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St.Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza. Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH₂O and filtered through a Whatmann No. 1 filter paper. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/ml gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂ and were sub cultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability. For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10⁴ cells per well in 100 µl of growth medium Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in humidified incubator with 5% CO₂ for a period of 48 h. three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37 °C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated [24, 25].

3-7- Antimicrobial activity screening

The antimicrobial activity of the Schiff base ligand (HL) and Mg(II), Ca (II) and Ba(II) metal chelates was evaluated against seven microorganisms , including the Gram-positive *Bacillus subtilisa*; *Staphylococcus aureus* and *Strepto- coccus pneumonia* , the Gram-negative *Escherichia coli* and *Pseudomonds solanarium* and the mold *Aspergillusnigaerand Penicillium* . The inhibition zones of the Schiff base moiety (HL) and Mg(II), Ca(II) and Ba(II) metal chelates are shown in Table 3 and Figure 6 a; b and c. Results indicated that prepared compounds exhibited variety degree of antimicrobial activity. Prepared compounds [Ca(HL)₂(Cl)₂].2H₂O and [Mg(HL)₂(Cl)₂.6H₂O had marked antimicrobial effect against *Bacillus subtilisa*; *Strepto- coccus pneumonia* and *Pseudomonds solanarium* bacterial strains and *Aspergillusnigaer* fungal strain. On the other hand, prepared compounds exhibited moderate to mild antimicrobial activity with other microorganisms strains. The possible mechanisms of action of the test compounds based on their structures, which can bind the microorganism cell walls. The different element binding with Schiff base ligand (HL) in different prepared compounds has important role for increases its ability to bind the cell wall. This binding inhibits the biosynthesis of the peptidoglycan layer of bacterial cell walls, which affects penetration through the cell wall [26,27]. The variety of antimicrobial activity and type of bacteria affected may be due to differences in the ability to bind the cell wall, which is based on the structure of the prepared compound.

3-8- Cytotoxicity evaluation

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Cytotoxic and antitumor activities of the Schiff base ligand (HL) and Mg(II), Ca (II) and Ba(II) metal chelates were evaluated against cell lines MCF-7 and HePG-2 and drug vinblastine as standard and result are recorded as viability percentage in Table 4 and 5 and presented in Figure 7 and 8. The results indicated that the prepared compound $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ had the highest cytotoxicity effects against cell line MCF-7 followed by compounds $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$ and $[\text{Ca}(\text{HL})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ in comparison with Schiff base moiety (HL). On the other hand the cytotoxicity effects of prepared compounds against HePG-2 cell line were $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ followed by $[\text{Ca}(\text{HL})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$. Also, the result indicated that no proportional relation between the concentration of prepared compounds and cytotoxic activity. The calculated IC_{50} values, that is, the concentration ($\mu\text{g}/\text{mL}$) of a compound able to cause 50% of cell death with respect to the control culture, are presented in Fig. 9. vinblastine is used as a reference compound. The HepG2 cells line are sensitive to Schiff base (HL) with the IC_{50} value of $22.5 \mu\text{g}/\text{ml}$ and MCF-7 cell line also sensitive to Schiff base (HL) with the IC_{50} value of $32.1 \mu\text{g}/\text{ml}$. The $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ and $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$ had the highest cytotoxic effect against the two cell lines tested with IC_{50} values; 32.6 and $30.4 \mu\text{g}/\text{ml}$ for MCF-7 cell line and 37.5 ; $39.5 \mu\text{g}/\text{ml}$ for HePG-2 cell line. The $[\text{Ca}(\text{HL})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ had the lower cytotoxicity effect (IC_{50} value; $75.6 \mu\text{g}/\text{ml}$) for MCF-7 cell line and had no inhibition effect for HePG-2 cell line. The observed cytotoxicity behavior of $[\text{Ba}(\text{HL})_2(\text{H}_2\text{O})_2] \cdot \text{Cl}_2 \cdot 2\text{H}_2\text{O}$ and $[\text{Mg}(\text{HL})_2(\text{Cl})_2] \cdot 6\text{H}_2\text{O}$ might be attributed to its ability to inhibit topoisomerase I, since topoisomerase I inhibitors are known to be efficient in reducing cell proliferation of ovarian and liver cancer cells. [28, 29 and 30].

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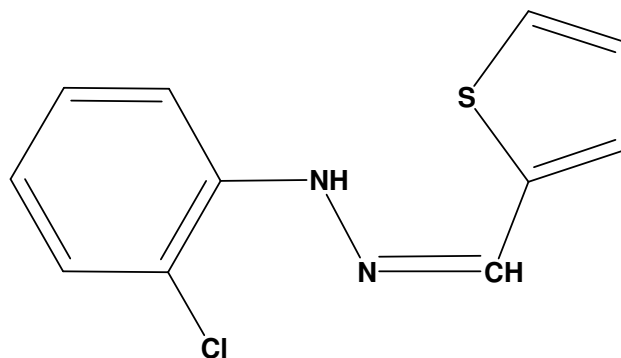


Fig. 1: Schiff base N-(2-chloro-phenyl)-N'-thiophen-2-ylmethylene-hydrazine (HL).

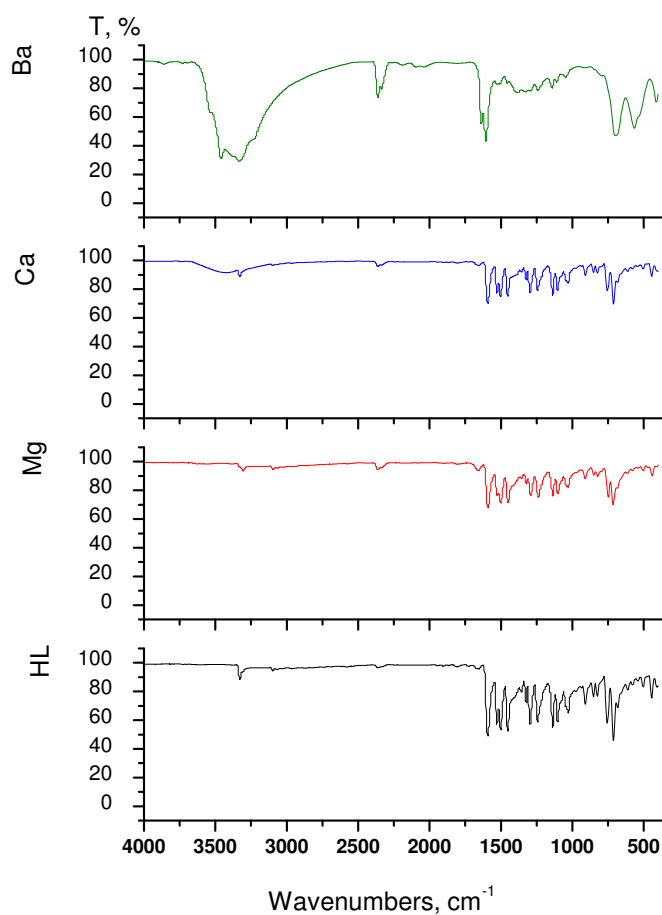


Fig.2: Infrared spectra of HL, $[Mg(HL)_2(Cl)_2] \cdot 6H_2O$, $[Ca(HL)_2(Cl)_2] \cdot 2H_2O$ and $[Ba(HL)_2(H_2O)_2] \cdot Cl_2 \cdot 2H_2O$ complexes

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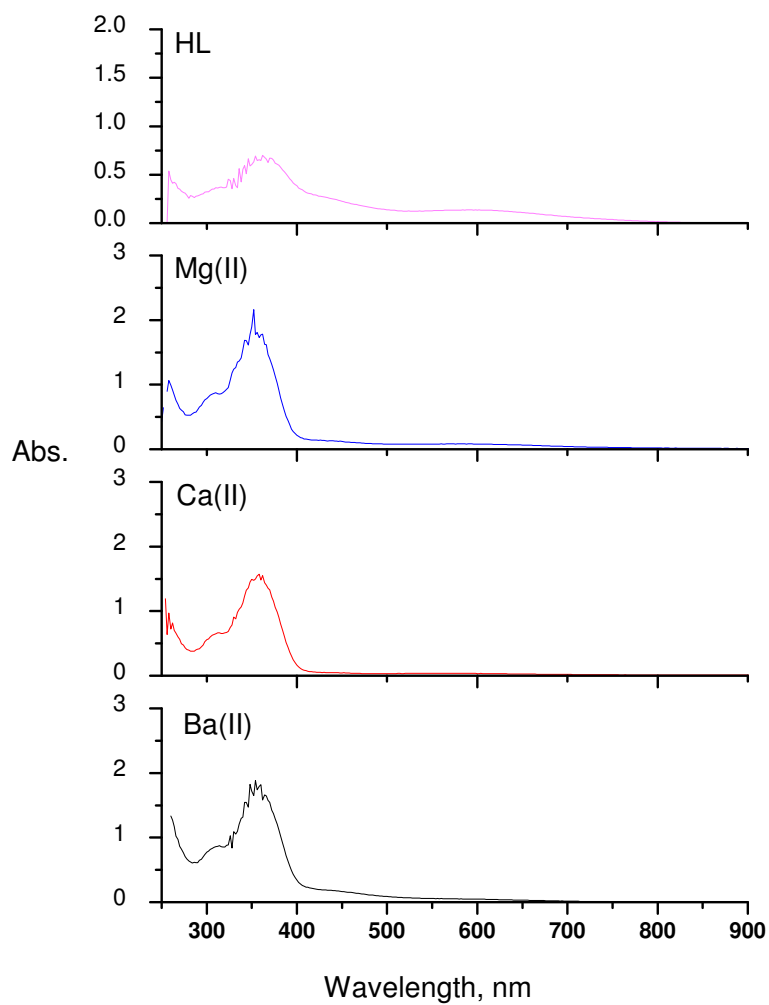


Fig. 3: Electronic spectra of HL, $[Mg(HL)_2(Cl)_2] \cdot 6H_2O$, $[Ca(HL)_2(Cl)_2] \cdot 2H_2O$ and $[Ba(HL)_2(H_2O)_2] \cdot Cl_2 \cdot 2H_2O$ Schiff base complex

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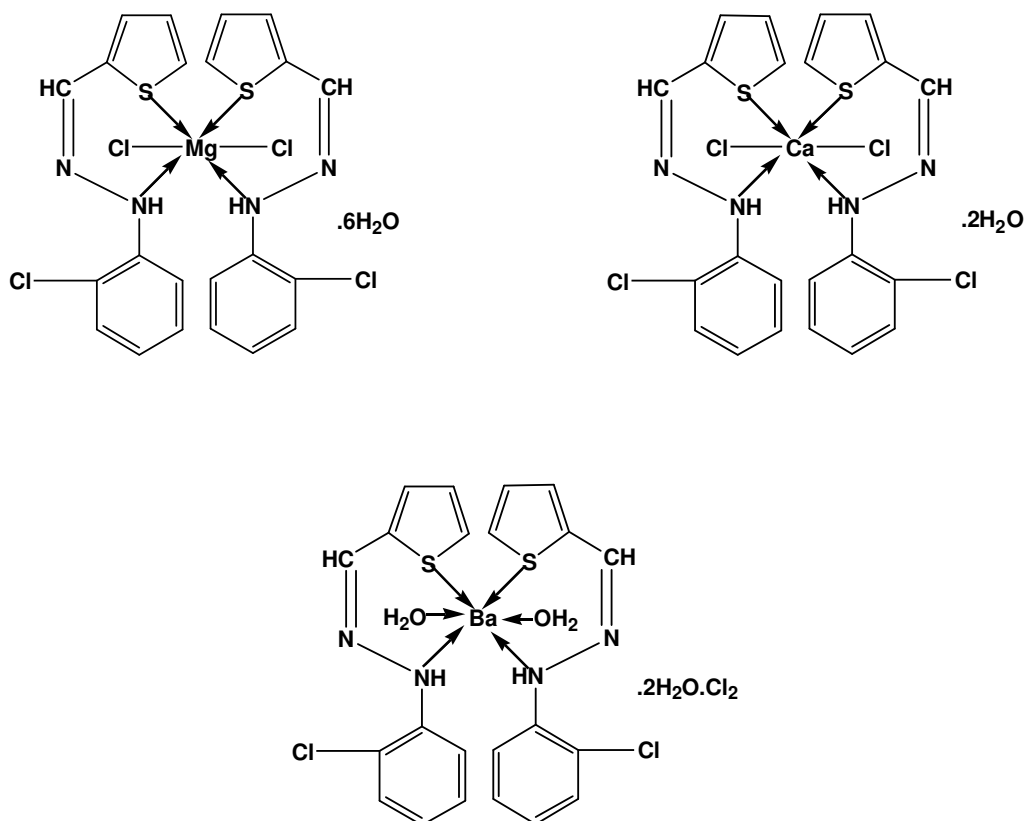


Fig. 4: Proposed structures of $[Mg(HL)_2(Cl)_2] \cdot 6H_2O$, $[Ca(HL)_2(Cl)_2] \cdot 2H_2O$ and $[Ba(HL)_2(H_2O)_2] \cdot Cl_2 \cdot 2H_2O$ Schiff base complex

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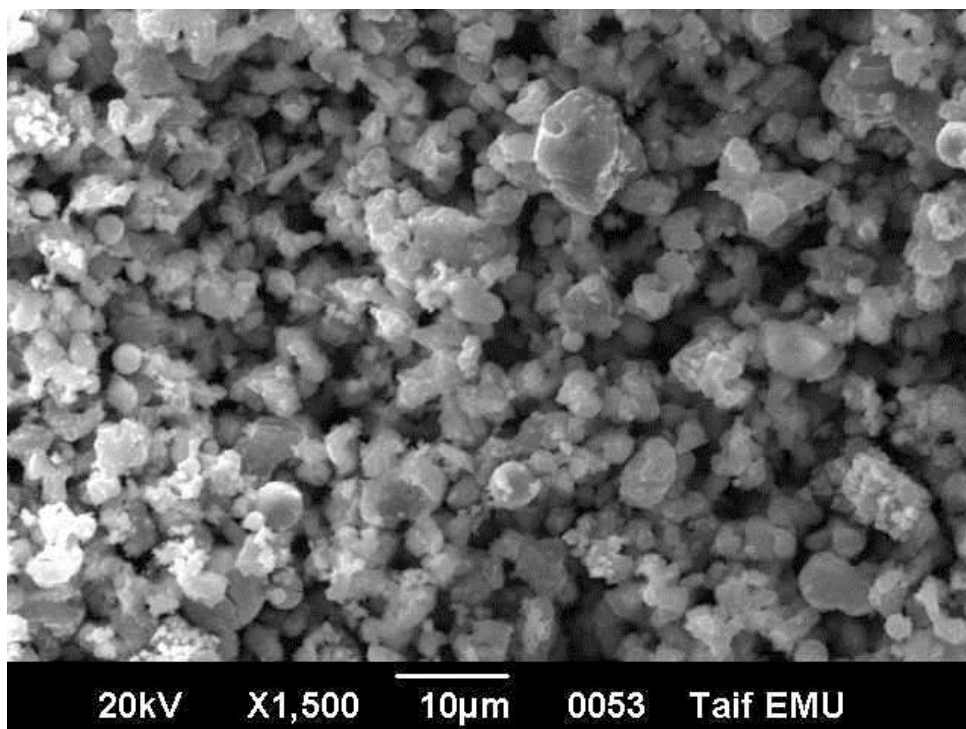


Fig. 5a: SEM image of [Mg(HL)₂(Cl)₂].6H₂O Schiff base complex

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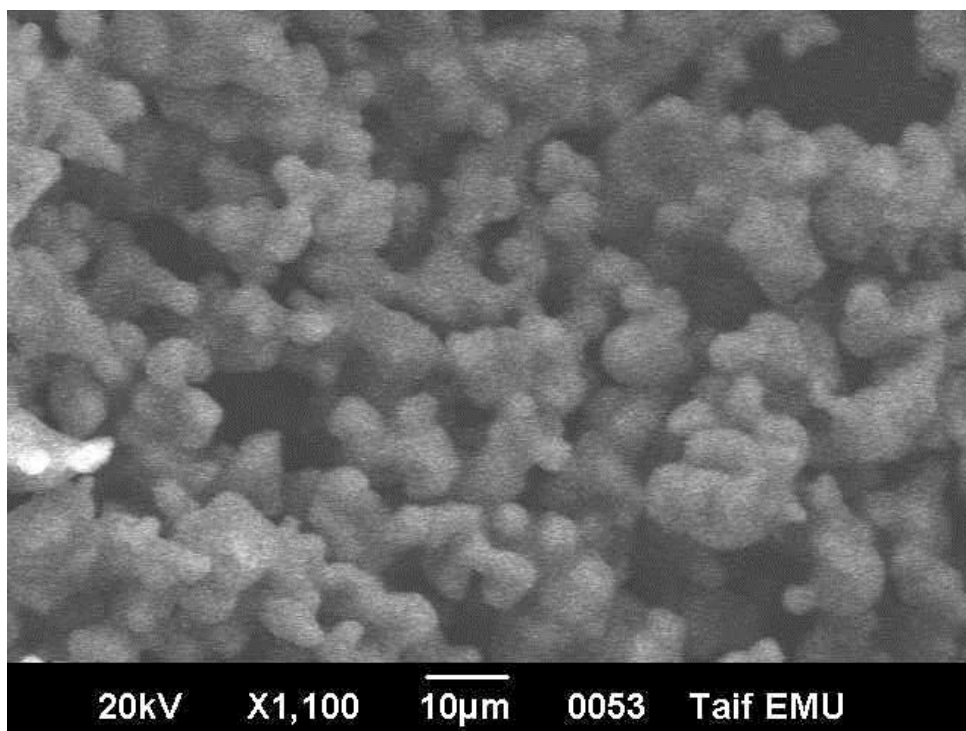
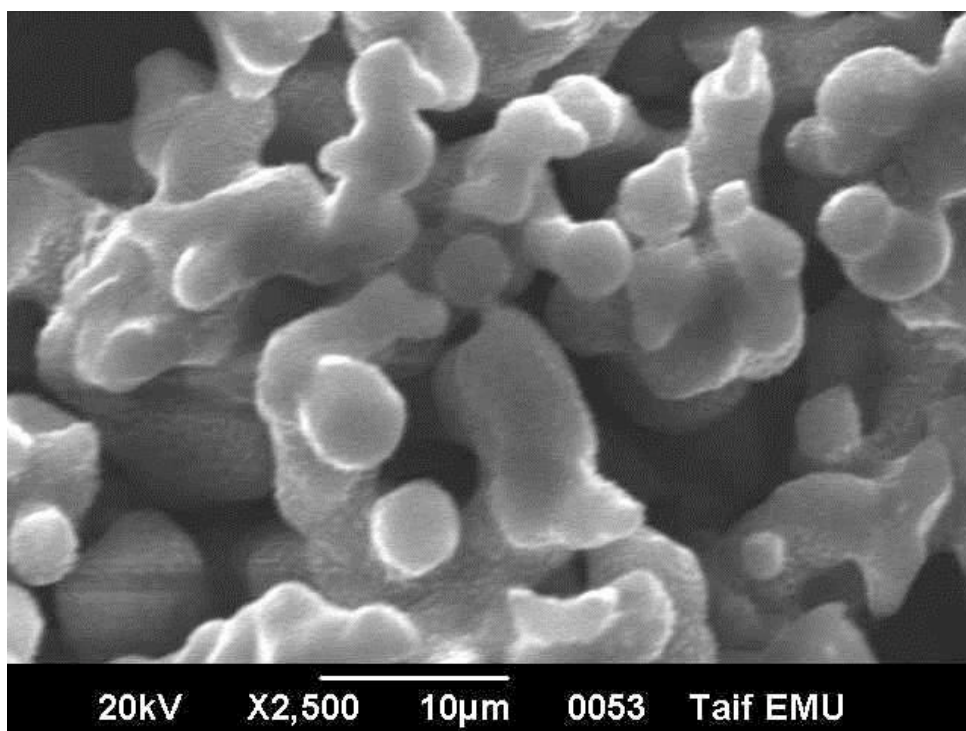


Fig. 5b: SEM image of $[Ca(HL)_2(Cl)_2].2H_2O$ Schiff base complex



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Fig. 5c: SEMImage of $[Ba(HL)_2(H_2O)_2].Cl_2.2H_2O$ Schiff base complex

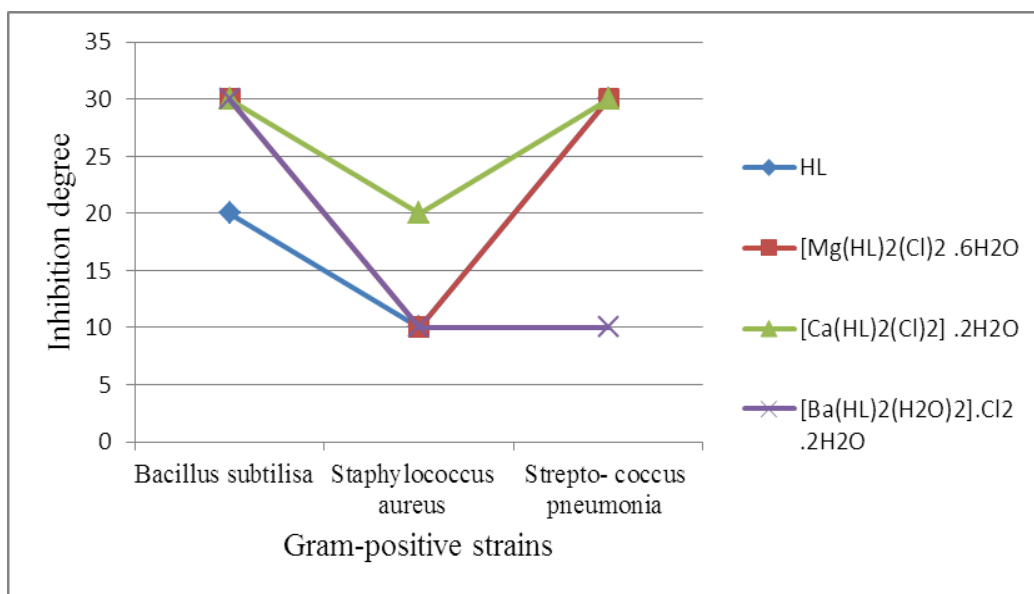


Fig. 6a: Antimicrobial activity of prepared compound against tested Gram-positive strains.

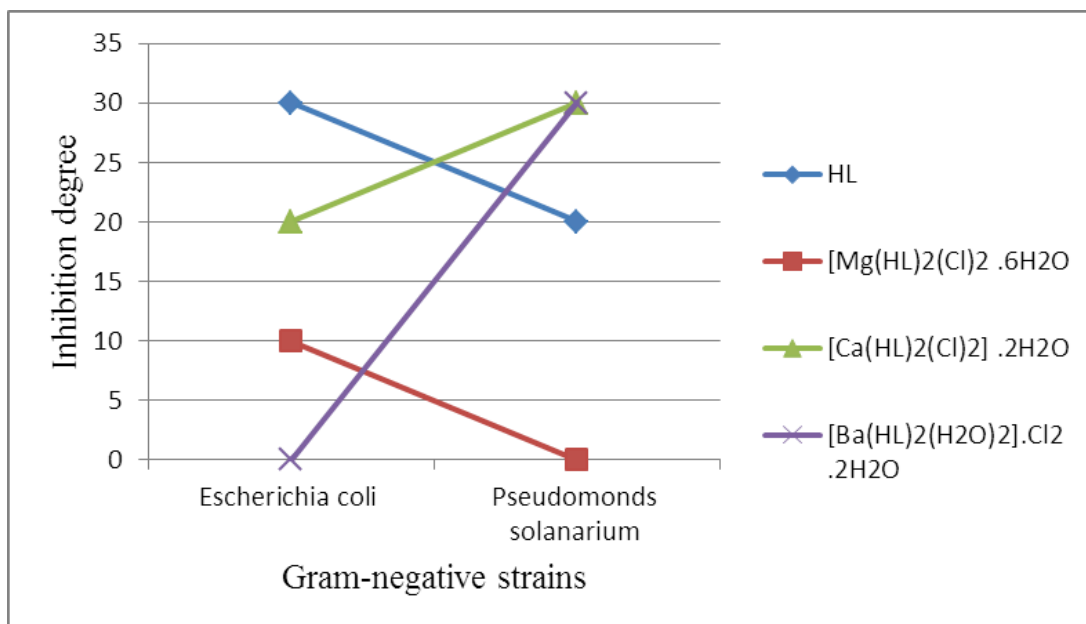


Fig. 6b: Antimicrobial activity of prepared compound against tested Gram-negative strains.

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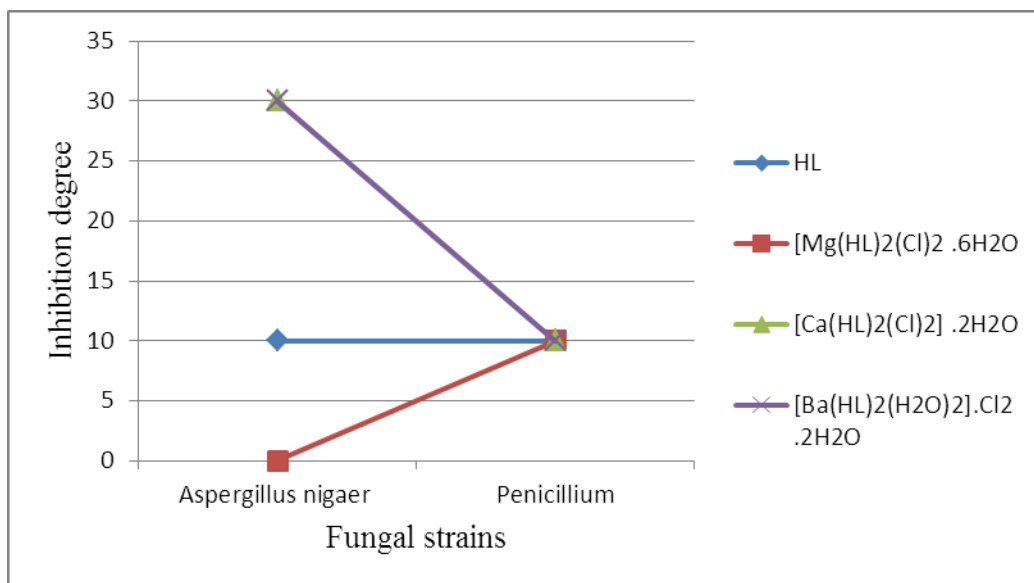


Fig. 6c: Antimicrobial activity of prepared compound against tested fungal strains.

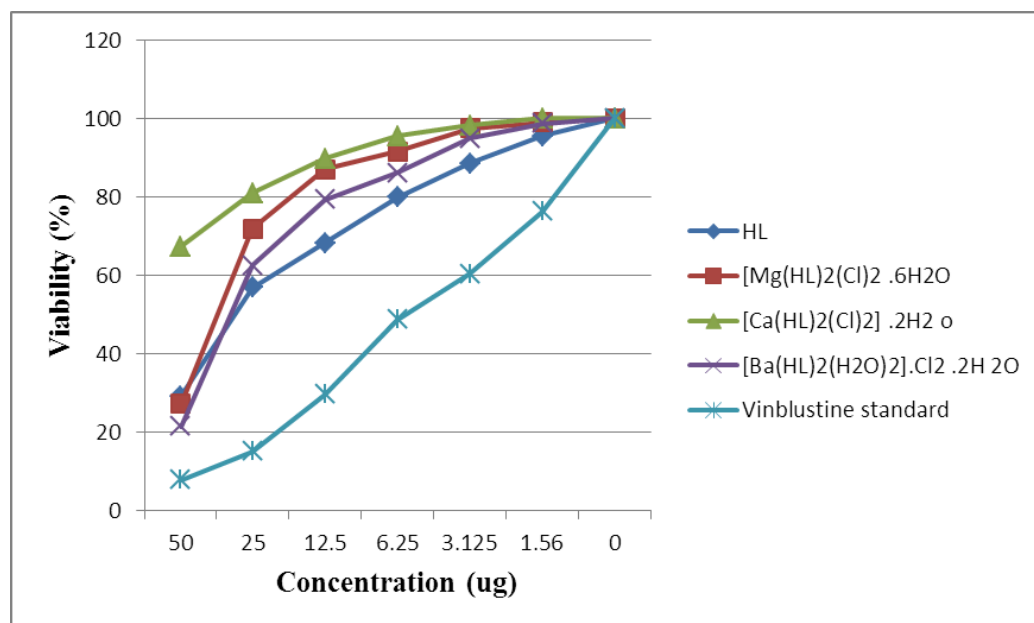


Fig.7: Cytotoxicity of prepared compounds against cell line MCF-7

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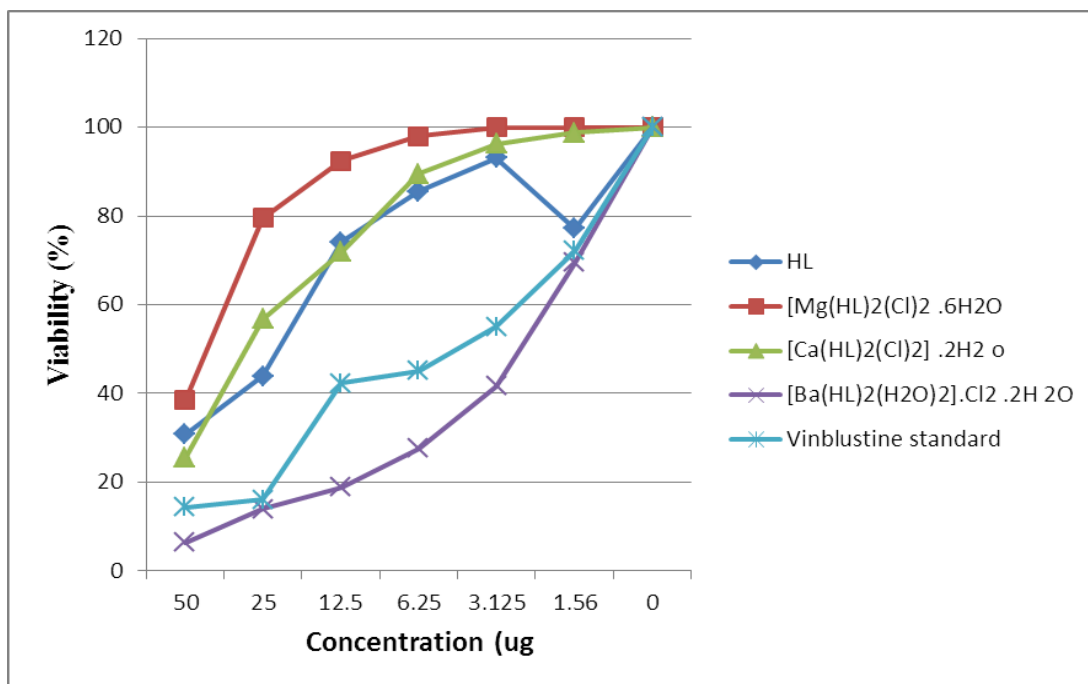


Fig. 8: Cytotoxicity of prepared compounds against cell line HePG-2

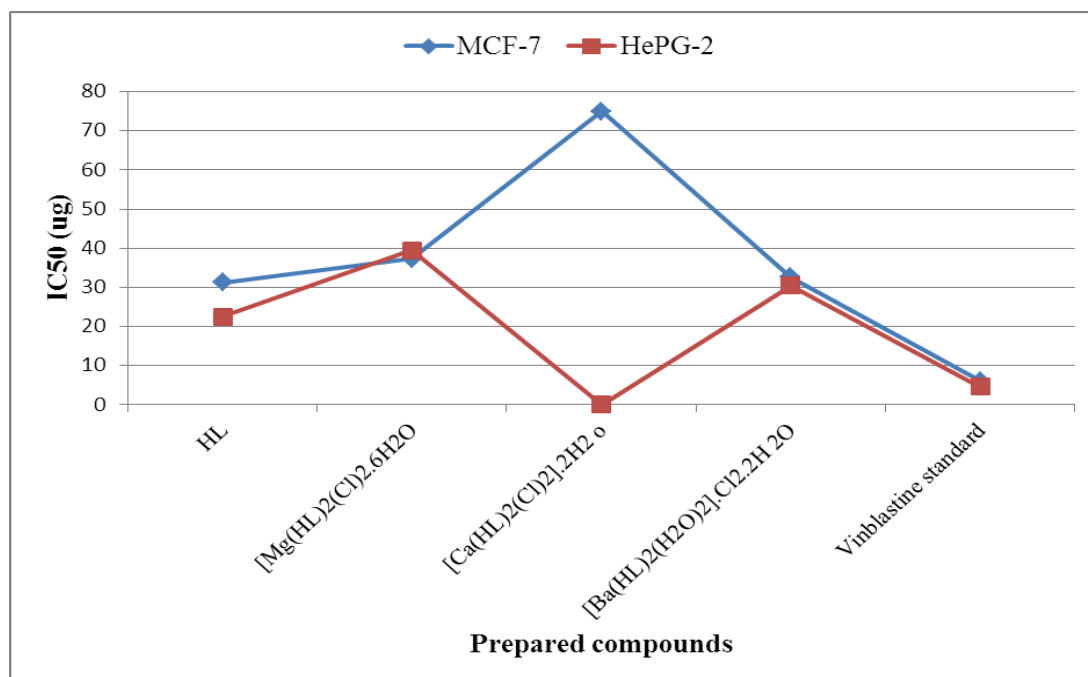


Fig. 9: IC₅₀ values of prepared compounds with MCF-7 and HePG-2 cell lines.

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Table 1: Analytical and physical data for HL Schiff base complexes

Compounds	Mp (°C)	Color	$\Lambda_M (\Omega^{-1} \text{cm}^2 \text{mol}^{-1})$
[Mg(HL) ₂ (Cl) ₂].6H ₂ O	>300	brown	20
[Ca(HL) ₂ (Cl) ₂].2H ₂ O	>300	brown	52
[Ba(HL) ₂ (H ₂ O) ₂].Cl ₂ .2H ₂ O	>300	brown	410

Table 2: Infrared characteristic bands frequencies (cm⁻¹) of the HL ligand complexes

Compounds	$\delta(\text{H}_2\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{C})$ phenyl + thiophene	$\delta(\text{CSC})$	$\nu(\text{C}-\text{S})$	$\nu(\text{M}-\text{N})$
HL	--	1595	1492, 1452, 1407	912	710	---
[Mg(HL) ₂ (Cl) ₂].6H ₂ O	1659	1591	1525, 1502, 1450	910	685	441
[Ca(HL) ₂ (Cl) ₂].2H ₂ O	1654	1593	1527, 1503, 1452	908	683	443
[Ba(HL) ₂ (H ₂ O) ₂].Cl ₂ .2H ₂ O	--	1606	1527, 1455, 1381	909	699	412

Table 3: Antimicrobial activity of prepared compounds

Compound name				
Organism strain	HL	[Mg(HL) ₂ (Cl) ₂].6H ₂ O	[Ca(HL) ₂ (Cl) ₂].2H ₂ O	[Ba(HL) ₂ (H ₂ O) ₂].Cl ₂ .2H ₂ O
Gram-positive				
<i>Bacillus subtilis</i>	++	+++	+++	+++
<i>Strepto-coccus pneumonia</i>	+	+	++	+
<i>Staphylococcus aureas</i>	+++	+++	+++	+
Gram-negative				
<i>Eshrecia Coli</i>	+++	+	++	-
<i>Pseudomondssolanarium</i>	++	-	+++	+++
Mold				
<i>Aspergillusnigaer</i>	+	-	+++	+++
<i>Penicillium</i>	+	+	+	+

- : No antimicrobial activity + : Mild activity ++ Moderate activity
+++ Marked activity

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Table 4: Evaluation of cytotoxicity of prepared compounds against cell line MCF-7

Concentration (µg)	Viability%				
	HL	[Mg(HL) ₂ (Cl) ₂ .6H ₂ O]	[Ca(HL) ₂ (Cl) ₂ .2H ₂ O]	[Ba(HL) ₂ (H ₂ O) ₂ .Cl ₂ .2H ₂ O]	Vinblastine standard
50	29.17	27.32	67.40	21.51	7.82
25	56.85	71.85	80.93	62.43	15.18
12.5	68.18	86.93	89.74	79.28	29.60
6.25	79.86	91.54	95.42	86.15	48.75
3.125	88.57	97.38	98.13	94.82	60.35
1.56	95.46	98.96	100	98.67	76.24
0	100.00	100.00	100.00	100.00	100.000

Table 5: Evaluation of cytotoxicity of prepared compounds against cell line HePG-2

Concentration (µg)	Viability (%)				
	HL	[Mg(HL) ₂ (Cl) ₂ .6H ₂ O]	[Ca(HL) ₂ (Cl) ₂ .2H ₂ O]	[Ba(HL) ₂ (H ₂ O) ₂ .Cl ₂ .2H ₂ O]	Vinblastine standard
50	30.75	38.42	25.42	6.42	14.38
25	43.92	79.56	56.73	13.97	16.13
12.5	74.14	92.37	71.86	18.86	42.25
6.25	85.57	97.94	89.54	27.59	45.13
3.125	93.04	100	96.28	41.62	55.00
1.56	77.16	100	98.71	69.46	72.13
00	100.00	100.00	100.00	100.00	100.00

Table 6: IC₅₀ (µg) values of prepared compounds after 72 h continuous exposure of tumor cell lines

Compound	Tumor type / cell line	
	MCF-7	HePG-2
HL	31.20	22.50
[Mg(HL) ₂ (Cl) ₂ .6H ₂ O]	37.30	39.50
[Ca(HL) ₂ (Cl) ₂ .2H ₂ O]	75.0	-
[Ba(HL) ₂ (H ₂ O) ₂ .Cl ₂ .2H ₂ O]	32.60	30.40
Vinblastine standard	6.10	4.60