ISSN

Journal of Basic and Environmental Sciences 4 (2017) 178-189 Online: 2356-6388

Print: 2536-9202

**Research Article** **Open Access**

# Spectroscopic Characterization and Biological Activity of Mixed Ligand Complexes of Transition Metals with Levofloxacin and Phenyl Azo compounds

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**Abstract**

Mixed ligand complexes of the type [Levo.- M- ($L_1$ - $L_4$)], where M = Co (II), Ni(II), Cu(II) and Zr (IV), Levo. is levofloxacin drug as a primary ligand and $L_1$-$L_4$ are four azo compounds as secondary ligands, were prepared and characterized by elemental analysis, molar conductivity, FT-IR, UV-Vis spectroscopy, magnetic moments, and thermogravimetric (TG&DTA) analysis. Spectral and elemental analysis data showed that both levofloxacin drug and the secondary ligands azo compounds act as monovalent monodentate coordinating to the metal ion via OO fashion in levofloxacin and through ON fashion in the secondary ligands with octahedral geometry in all cases. The crystal field splitting energy ($\Delta_o$), Racah parameter (B'), nephelauxetic ratio (β), Sinha parameter (δ %) (metal – ligand covalency percent) and the covalency angular overlap parameter (η) have been calculated using the Tanabe Sugano diagram. The antimicrobial activity of the mixed ligand complexes has been studied by screening against some gram positive and gram negative bacteria, where it was found that they have enhanced activity. The antitumor activities of some selected complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug.

Keyword: mixed ligand complexes, levofloxacin, azo compounds, anti-microbial, antitumor activity

Received; 25 Feb. 2017, In Revised form; 10 Mar. 2017, Accepted; 10 Mar. 2017, Available online 1 April, 2017

## Introduction

One of the major applications of the transition metal complexes is their medical testing as antibacterial and antitumor agents aiming to discover an effective and safe therapeutic regimen for the treatment of bacterial infections and cancers [1]. In addition, many mixed ligand metal complexes have also provoked wide interest because they possess a diverse spectrum of biological and pharmaceutical activities, including antitumor, anti-HIV, antioxidative, antifungal, and antibacterial activities [2–15]. In view of the wide biological activities exhibited by drug-M-ligand complexes, it is meaningful to extend these studies on mixed ligands metal complexes containing levofloxacin- azo dyes. So, this paper reports the synthesis, characterization, and antibacterial studies of mixed ligand transition metal complexes prepared from levofloxacin as a primary ligand and four azo compounds as secondary ligands. The spectral parameters were determined after elucidation of the molecular structure and the antibacterial and antitumor activities of some complexes were screened.

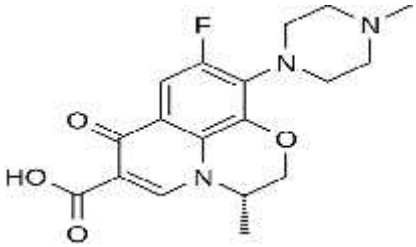
## 1. Experimental

Materials

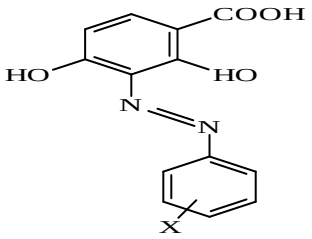
All reagents used in the present study were of the highest quality (Merck, Aldrich and Sigma chemicals) and were used without further purification. Levofloxacin was obtained from Egyptian Company for Chemicals and Pharmaceuticals. Freshly bidistilled water was used whenever water is necessary.

### Preparation of the mixed ligands solid complexes

Mixed ligand Co(II), Ni(II), Cu(II) and Zr(IV) complexes were prepared from hydrated metal chloride (hydrated zirconyloxychloride for Zr complexes) , levofloxacine as a primary ligand, and four azo compounds as secondary ligands. Levofloxacin is an antibiotic, having the following structural formula:



The azo compounds were prepared as described [16] by coupling of 2,4-dihydroxybenzoic acid (in NaOH) with the diazonium salts of m-nitroaniline, p-aminobenzoic acid, p-aminophenol and p-anisidine ($L_1$-$L_4$), respectively. They have the following formulae:



X = m-$NO_2$($L_1$), p-COOH($L_2$), p-OH($L_3$) and p-$CH_3$($L_4$)

Aqueous solution of 1.00 mmol of hydrated metal chlorides (oxychloride in case of Zr) was added with stirring to an ethanolic solution of 1.00 mmol of each of the secondary ligand ($L_1$– $L_4$) and the mixture was refluxed for ≈ 3 hours. To the above mixture, an ethanolic solution of levofloxacin (1.00 mmol) was added and the mixture (1 : 1 : 1 molar proportion) was again refluxed in water bath for 3 hours. The complexes were precipitated by adding few drops of triethylamine solution. The mixture was cooled, and the solid complexes obtained were filtered off and washed with water followed by ethanol and dried under vacuum.

**Physical measurements**

Elemental analysis; (C, H and N) were carried out in the microanalytical centre, Cairo University, Giza, Egypt. Metal ion content (Co, Ni and Cu) was determined by EDTA titration under the appropriate conditions [17]. Infrared spectra were recorded as KBr disc technique using FT–IR spectrometer Model Nicolet is10-thermo-scientific within the wavenumber range 4000 – 400 $cm^{-1}$, at Faculty of Science, Benha University. Thermogravimetric analysis was carried out using Shimadzu TGA – 50H thermal analyzer within the range 25ºC–800ºC. All measurements were done under nitrogen atmosphere at heating rate of 10ºC per minute. The electronic absorption spectra in solution (DMF as a solvent) and in solid state (Nujol mull technique) were recorded on Jasco V-530 (UV-Vis) double beam spectrophotometer (Japan) with scanning speed 400 nm/min and band width 2.0 nm using 10 mm matched quartz cell at room temperature in the range 800 – 200 nm. The magnetic moment values were measured at room temperature using a Sherwood scientific magnetic susceptibility balance. Molar conductivities of the complexes ($10^{-3}$ M) in DMF were obtained using a conductivity bridge YSI model 32.

**Antimicrobial Screening**

The antibacterial activity of some selected mixed ligands complexes toward four bacterial strains (two gram positive: *Streptococcus pyogenes* and *Staphylococcus epidermidis* and two gram negative bacteria; *Proteus vulgaris* and *Klebsiella pneumonia*) was evaluated by agar well diffusion method [18]. The tested compounds were dissolved in DMF which have no inhibition activity to get concentration of 100 mg/ml. A hot nutrient agar solution (20 ml) was poured into the sterilized petri dishes and allowed to attain room temperature. The seed layer medium was melted and cooled to ≈ 45ºC with gentle shaking. The previously grown subculture was added to the seed layer medium aseptically and mixed well. It was immediately raked into the petri dishes and allowed to attain room temperature. Then wells were made with the sterile cork porer and to these wells, 50 µl in concentration of 100 mgl/ml of the tested compound was added and the plates were allowed to cool for one hour to facilitate the diffusion. The plates were incubated at 37ºC for 24 hours. Antibacterial activity of the complexes was evaluated by measuring the diameter of zone of inhibition in mm. The medium with DMF as solvent was used as a negative control whereas media with Ampicillin were screened separately for its standard antibacterial activity.

**Antitumor activity assay**

Human hepatocellular carcinoma (HepG2) cell line was obtained from the American Type Culture Collection (ATTC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% $CO_2$ and were subcultured two to three times a week.

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning R 96-well tissue culture plates, then incubated for 24 hrs. The tested compounds were then added into 96-

well plates (six triplicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubation for 24 hr, the number of viable cells was determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI1640 medium without phenol red then 10 µl of the 12mM MTT stock solution (5 mg of MTT in 1.0 ml of PBS) to each well including the untreated control.

The 96 well plates were then incubated at 37ºC and 5%$CO_2$ for 4 hrs. An 85 µl aliquot of the media was removed from the wells, 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37ºC for 10 min. then the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc. USA) to determine the number of viable cells and the percentage of viability was calculated as: $(1 - \frac{\text{ODt}}{\text{ODc}}) \times 100$ , where ODt is the mean optical density of the wells treated with the tested samples and ODc is the mean optical density of the untreated cells.

The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration ($IC_{50}$), the concentration required to cause toxic effect in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration [19,20]. All measurements were carried out at the Regional Center for Mycology and Biotecnology, Al-Azhar University, Cairo, Egypt.

## 2. Results and discussion

### Physical properties

All the complexes are colored, stable in air and have high melting points. They are freely soluble in DMSO and DMF but sparingly soluble in other common organic solvents. Results of elemental analysis (Table 1) are in good agreement with the calculated values of the proposed formulae. The values reveal that the levofloxacin : metal : secondary ligand ratio is 1 : 1 : 1. The molar conductance values ($\Lambda_m$; $ohm^{-1}cm^2mol^{-1}$) are in the range 7.33 – 14.6 $ohm^{-1}cm^2mol^{-1}$ indicating the nonionic nature of the complexes.

Table (1): Elemental analysis and molar conductivity of some selected mixed Levovloxacin – ($L_1$-$L_4$) complexes.

| Complex | Tentative formula | M.Wt. | Elemental analysis* | | | | $\Lambda_m$** |
|---|---|---|---|---|---|---|---|
| | | | %C | %H | %N | %M | |
| Levo-L1-Co | $[C_{31}H_{29}N_6O_{11}FCoCl]$ | 774.99 | 48.04 (48.10) | 3.77 (4.11) | 10.84 (10.04) | 7.60 (8.11) | 9.04 |
| Levo-L1-Ni | $[C_{31}H_{29}N_6O_{11}FNiCl]$ | 774.74 | 48.06 (47.31) | 3.77 (3.88) | 10.85 (11.05) | 7.58 (7.15) | 7.33 |
| Levo-L1-Cu | $[C_{31}H_{29}N_6O_{11}FCuCl]$ | 779.61 | 47.76 (46.23) | 3.75 (3.62) | 10.78 (11.56) | 8.15 (8.44) | 7.86 |
| Levo-L2-Zr | $[C_{32}H_{29}N_5O_{12}F\text{-}ZrCl]$ | 806.29 | 47.67 (47.03) | 3.63 (4.11) | 8.69 (8.66) | | 8.86 |
| Levo-L2-Co | $[C_{32}H_{30}N_5O_{12}FCoCl]$ | 790.00 | 48.65 (47.93) | 3.83 (4.32) | 8.87 (9.06) | 7.46 (8.04) | 5.44 |
| Levo-L2-Ni | $[C_{32}H_{30}N_5O_{12}F\text{-}NiCl]$ | 789.76 | 48.67 (48.31) | 3.83 (4.31) | 8.87 (9.34) | 7.43 (7.21) | 11.5 |
| Levo-L2-Cu | $[C_{32}H_{30}N_5O_{12}FCuCl]$ | 794.62 | 48.37 (48.33) | 3.81 (3.89) | 8.81 (9.11) | 8.00 (8.42) | 10.3 |
| Levo-L3-Zr | $[C_{31}H_{28}N_5O_{10}F\text{-}ZrCl]$ | 776.27 | 47.97 (46.82) | 3.64 (3.66) | 9.02 (8.43) | | 9.44 |
| Levo-L3-Co | $[C_{31}H_{29}N_5O_{10}FCoCl]$ | 745.74 | 49.93 (48.31) | 3.92 (4,25) | 9.39 (9.05) | 7.90 (8.21) | 7.33 |
| Levo-L3-Ni | $[C_{31}H_{29}N_5O_{10}F\text{-}NiCl]$ | 744.74 | 50.00 (49.22) | 3.93 (4.44) | 9.40 (9.33) | 7.88 (7.32) | 11.5 |
| Levo-L3-Cu | $[C_{31}H_{29}N_5O_{10}FCuCl]$ | 749.60 | 49.67 (48.82) | 3.90 (4.41) | 9.34 (8.88) | 8.48 (8.04) | 14.6 |
| Levo-L4-Co | $[C_{32}H_{30}N_5O_9FCoCl]$ | 742.00 | 51.80 (50.36) | 4.08 (4.38) | 9.44 (9.31) | 7.96 (7.18) | 12.7 |
| Levo-L4-Ni | $[C_{32}H_{30}N_5O_9FNiCl]$ | 741.76 | 51.82 (50.66) | 4.08 (3.88) | 9.44 (9.03) | 7.91 (8.33) | 9.88 |
| Levo-L4-Cu | $[C_{32}H_{30}N_5O_9FCuCl]$ | 746.62 | 51.48 (51.24) | 4.05 (3.79) | 9.38 (10.22) | 8.51 (8.19) | 10.6 |

*values between parentheses are found values ** $ohm^{-1}cm^2mol^{-1}$

**Thermal analysis**

Thermal methods of analysis open up a new possibility for the investigation of metal complexes. It is taken, in addition to elemental analysis, as a tool to confirm the chemical structure of the complexes. The degradation steps of the samples can be followed up and the different degradation fragments can be estimated in the light of molecular structure of the complexes as given in Table (2). Representative examples of thermograms are given in Fig.'s (1 and 2) from which it is shown that the complexes under study are thermally degraded in, more or less, three main steps.

1- The first step, maximally ended at 189ºC, is due to the removal of either physically adsorbed or coordinated water molecules attached to the solid complexes in addition to some gases produced from the pre-decomposition of the complexes such as HCl ,HF or $CO_2$.

2- The second degradation step, within the temperature range 225ºC - 329ºC is due to the thermal decomposition of the azo molecules acting as secondary ligands. The experimental weight loss of this step agrees well with the calculated values of the degraded fragment (*c.f*. Table 2).

3- The final step, represented by strong endothermic peaks centered at ≈ 575ºC, is due to the decomposition of the levofloxacin moiety of the complexes leading to the formation of metal oxides as final products from which the % of metal ion is calculated as given in Table (2).

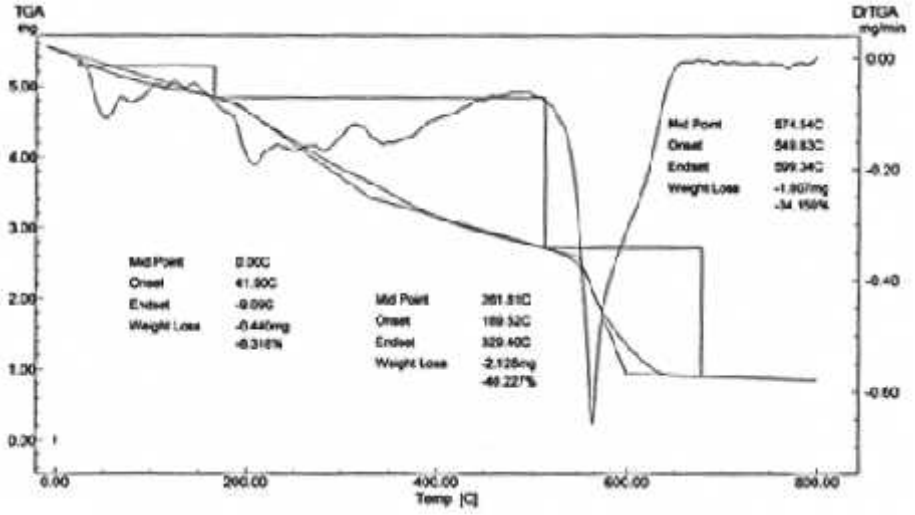



Fig (1): TGA and DTA curves of the complex levovolxacin – Ni – $L_1$

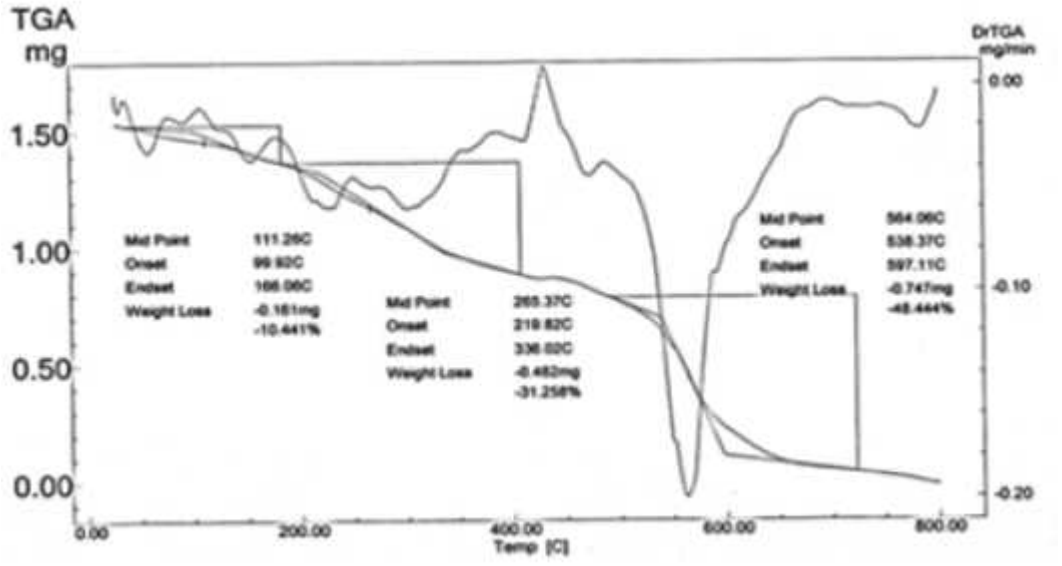



Fig (2): TGA and DTA curves of the complex levovolxacin – Cu – $L_3$

Table (2): Thermogravimetric data for levofloxacin – M – $L_1$-$L_4$ complexes

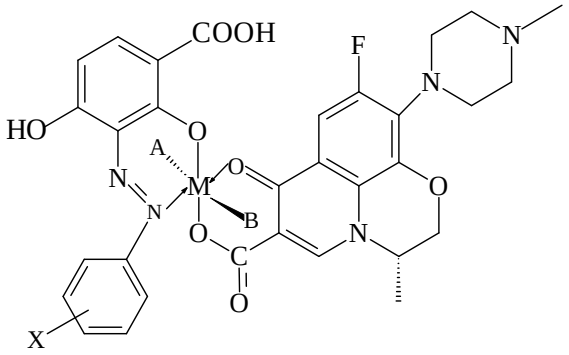
| Complex | Temp.(ºC) | Weight loss % | Assignment |
|---|---|---|---|
| Lev-Ni-L1 | 189.5<br>261.8<br>574.54 | 8.31 (9.57)<br>40.23(39.99)<br>34.16(47.55) | Removal of coordinated $H_2O$ molecule, HCl, HF<br>Beginning of decomposition of L1<br>Decomposition of Levofloxacin Moiety leading to $NiO_2$; 17.30 %. Ni% = 13.59 (15.22) |
| Lev-Cu-L1 | 66.55<br>225.7<br>574.83 | 7.80<br>33.33(39.74)<br>42.39(44.76) | Removal of hydrated $H_2O$ molecule.<br>Beginning of decomposition of L1, HCl gas<br>Decomposition of Levofloxacin Moiety leading to $Cu_2O$; 149 %. Cu% = 13.17 (14.44) |
| Lev-Zr-L1 | 80.12<br>220.08<br><br>510.45 | ----<br>Composite 3 steps<br>--- | Removal of hydrated $H_2O$ molecules.<br>Beginning of decomposition of L1, HCl, HF and $CO_2$ gases<br>Decomposition of Levofloxacin Moiety leading to $Zr_2O_3$; 20.73 %. Zr% = 17.64 (16.41) |
| Lev-Ni-L2 | 71.56<br>329.60<br>573.11 | ----<br>45.30(47.22) | Removal of hydrated $H_2O$ molecules.<br>Beginning of decomposition of L2, HCl, $CO_2$ gases<br>Decomposition of Levofloxacin Moiety leading to NiO; 10.64 %. Ni% = 8.36 (7.73) |
| Lev-Zr-L2 | 69.59<br>123.61 &<br>265.37<br>494.73 | ----<br>31.25 (31.39)<br>--- | Removal of hydrated $H_2O$ molecules.<br>Beginning of decomposition of L2, HCl, HF and $CO_2$ gases<br>Decomposition of Levofloxacin Moiety leading to $Zr_2O_3$; 18.27 %. Zr% = 15.55 (11.55) |
| Lev-Cu-L3 | 111.26<br>265.37<br>564.06 | ----<br>31.25(31.29)<br>48.44(49.10) | Removal of hydrated HCl, $CO_2$, $H_2O$.<br>Beginning of decomposition of L3.<br>Decomposition of Levofloxacin Moiety leading to CuO; 9.86 %. Cu% = 7.87 (8.64) |

Values between parentheses are theoretical values.

**FT-IR**

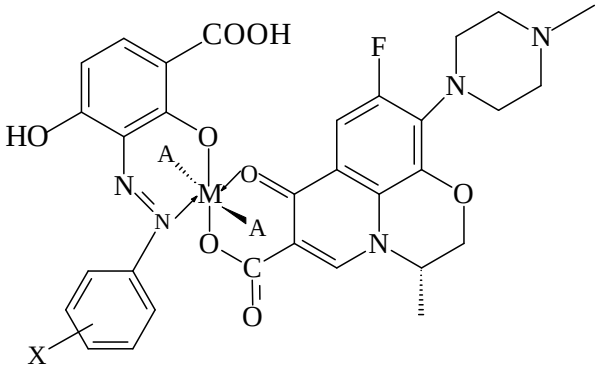
Fundamental IR spectral bands of levofloxacin and azo compounds were examined and compared to those of mixed ligands complexes. The IR spectrum of free levofloxacin is characterized mainly by the strong bands at 3262, 1725, 1453, 1164 and 982 $cm^{-1}$ due to stretching vibration of $V_{OH,}$ $V_{CO.}$ $V_{CN,}$ $V_{COO^-}$ and $\delta_{OH}$, respectively. On the other hand, the spectra of the free azo compounds acting as secondary ligands are characterized by the bands within the ranges 3424-3376 $cm^{-1}$, 1671-1625 $cm^{-1}$, 1446-1427 $cm^{-1}$, 1158-1155 $cm^{-1}$ and 1092-975 $cm^{-1}$ due to $V_{OH,}$ $V_{C=O.}$ $V_{N=N,}$ $V_{COO-}$ and $\delta_{OH}$, respectively. On complexation, the stretching vibration bands due to N=N and OH groups of the azo compounds are shifted to lower wavenumber indicating that the azo compounds coordinate to metal ion via their OH group in o-position to the azo group along with the N=N center in ON fashion acting as monovalent monodentate ligands. At the same time, the stretching vibrations of OH and $COO^-$ groups of the levofloxacin moiety also suffer a shift to lower wavenumber indicating the contribution of these groups in complex formation via OO fashion and shows that levofloxacin acts as monovalent monodentate ligand. The far infrared region of the spectra of the metal chelates shows new sets of bands within the region 600 – 400 $cm^{-1}$ which are due to the stretching vibrations of M – O, M – N and M – Cl bonds.

Elemental analysis confirmed the formation of Levo: M:$L_1$-$L_4$ complexes with stoichiometric ratio 1:1:1, accordingly the mode of bonding of such complexes can be represented as follows:




X = m-$NO_2$ ($L_1$), p-COOH ($L_2$), p-OH ($L_3$) and p-$CH_3$ ($L_4$)

A = $H_2O$ and B = Cl




A = $H_2O$ (O for $Zr^{4+}$)

**Magnetic measurements and electronic spectra**

**i. Magnetic susceptibility**

Magnetic susceptibility measurements at room temperature exhibit paramagnetism for Co(II), Ni(II) and Cu(II) complexes and diamagnetism for Zr(IV) complexes. The $\mu_{eff}$ values, term symbols and ground state symbols are listed in Table (3). Cobalt(II) complexes in high spin octahedral field with electronic configuration $t_{2g}^5e_g^2$ are orbitally degenerate which causes an orbital angular momentum contribution to the magnetic moment and the experimental magnetic moment values lie between spin-only, $\mu_{SO} = [4S (S + 1)]^{1/2} = 3.88$ BM and $\mu_{SL} = [4S (S + 1) + L (L + 1)]^{1/2} = 5.2$ BM values. The obtained magnetic moment ($\mu_{eff}$) values for the complex ranged between 4.08 and 4.11 BM lying in the range between $\mu_{SO}$ and $\mu_{SL}$ indicating an octahedral geometry around Cobalt(II) with orbital contribution to the spin – only magnetic moment values[21, 22]. The Ni(II) complexes show magnetic moment values of 2.87 – 2.9 BM indicating an octahedral environment around Ni(II) ion [23,24]. The observed magnetic moments for the Cu (II) complexes lie within the range 1.75 – 1.80 sBM suggesting a distorted octahedral geometry for Cu(II) complex [25]. The complexes of $ZrO^{2+}$ are diamagnetic as expected from its $4d^0$ electronic configuration.

**ii. Electronic spectra**

Electronic absorption spectra of the mixed ligands complexes were scanned in solution using DMF as solvent and in solid state using Nujol mull technique. The electronic absorption spectral data in Nujol mull (wavenumber; $cm^{-1}$) are given in Table (3) and representative examples for Ni(II) complexes in DMF and Nujol mull are shown in Fig.'s (3 and 4). The difference in spectral bands on going from Nujol mull to DMF is due to the destruction of the crystalline state hence, the environment differs in the solid state than that in solution. Inspection of the data obtained shows that:

1- Cobalt (II) complexes show three bands at 16666-16806 $cm^{-1}$,14749-14925 $cm^{-1}$ and 13679-13698 $cm^{-1}$ which are due to $d \rightarrow d$ electronic transition of the types $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$ ($V_1$), $^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$ ($V_2$) and $^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$ ($V_3$), respectively [26]. The use of Tanabe Sugano diagram for the spin allowed electronic transition in $d^7$ configuration was used to calculate the crystal field splitting energy ($\Delta_o$), Racah parameter (B'), nephelauxetic ratio ($\beta$) and crystal field stabilization energy of the complexes.

2- The electronic spectra of Ni (II) complexes show the three spin allowed bands at (31055-31250 $cm^{-1}$), (22222-22573 $cm^{-1}$) and (18155-18181 $cm^{-1}$) which are assigned to electronic transition type $^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$, $^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$ and $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}$ respectively. The spectral data are calculated using Tanabe Sugano diagram for $d^8$ configuration.

3- Copper (II) complexes show two spin allowed transition bands within the range 22321– 22727 $cm^{-1}$ and 18797– 19230 $cm^{-1}$ due to the $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$ and $^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ transitions, respectively. It was reported that Cu (II) complexes showed a broad asymmetric band in the region 20576 $cm^{-1}$ expected for a $d \rightarrow d$ transition in an octahedral Cu(II) complex [27]. The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a $d^9$ ion. [28]

4- Finally, the electronic configuration of Zr (IV) complexes ($d^0$) confirms the absence of any $d \rightarrow d$ transitions. The absorption bands in their spectra are due to $n \rightarrow \pi^*$ or CT interaction (Zr → L).

The spectral data are fully assigned in Table (3).

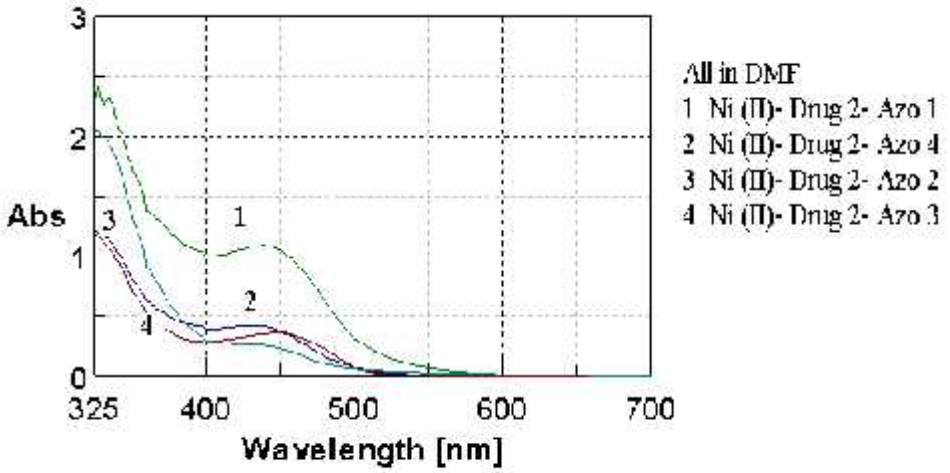



Fig (3): Electronic absorption spectra of Ni(II) complexes in DMF

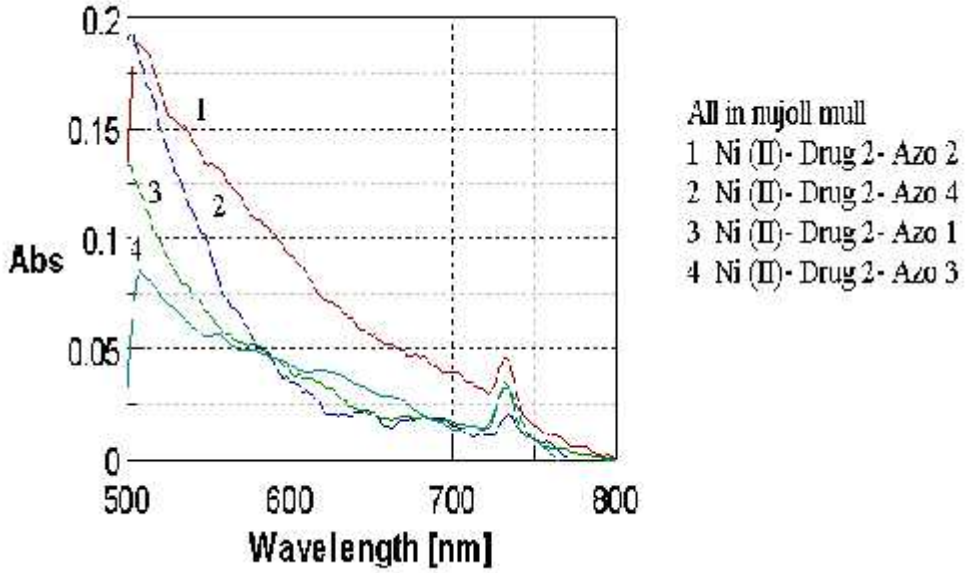



Fig (4): Electronic absorption spectra of Ni(II) complexes in Nujol mull

Table (3): electronic absorption spectra and magnetic moment data of the mixed ligands complexes in DMF and Nujol mull

| Complex | Nojol mull | | Assignment | Magnetic data | | |
|---|---|---|---|---|---|---|
| | $\lambda_{(nm)}$ | W.No. ($cm^{-1}$) | | Term Symbol | Ground state | $\mu_{eff}$ (BM) |
| Levo-L1-Co | 360<br>402<br>730 | 27777.8<br>24875.6<br>13698.6 | $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^4T_{1g(P)}(\nu_2)$<br>$\rightarrow {}^4A_{2g(F)}(\nu_3)$ | $^4F$ | $^4T_{1g}$ | 4.09 |
| Levo-L1-Ni | 493<br>625<br>745 | 20284.0<br>16000.0<br>13422.8 | $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^3T_{1g(F)}(\nu_2)$<br>$\rightarrow {}^3T_{1g(P)}(\nu_3)$ | $^3F$ | $^3A_{2g}$ | 2.87 |
| Levo-L1-Cu | 593<br>700<br>762 | 16863.4<br>14285.7<br>13123.4 | $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$<br>$^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ | $^2D$ | $^2E_g$ | 1.79 |
| Levo-L1-Zr | 335<br>425 | 29850.75<br>23529.41 | M→L<br>charge transfere | | | Dia. |
| Levo-L2-Ni | 505<br>600sh<br>743 | 19802.0<br>16666.7<br>13459.0 | $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^3T_{1g(F)}(\nu_2)$<br>$\rightarrow {}^3T_{1g(P)}(\nu_3)$ | $^3F$ | $^3A_{2g}$ | 2.828 |
| Levo-L2-Zr | 420<br>488 | 23809.5<br>20491.8 | M→L<br>charge transfere | | | Dia |
| Levo-L3-Co | 355<br>400<br>735 | 28169.0<br>25000.0<br>13605.4 | $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^4T_{1g(P)}(\nu_2)$<br>$\rightarrow {}^4A_{2g(F)}(\nu_3)$ | $^4F$ | $^4T_{1g}$ | 3.872 |
| Levo-L3-Ni | 507<br>595<br>741 | 19723.9<br>16806.7<br>13495.3 | $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^3T_{1g(F)}(\nu_2)$<br>$\rightarrow {}^3T_{1g(P)}(\nu_3)$ | $^3F$ | $^3A_{2g}$ | 2.828 |
| Levo-L3-Cu | 552<br>645<br>725 | 18115.9<br>15503.9<br>13793.1 | $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$<br>$^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ | $^2D$ | $^2E_g$ | 1.732 |
| Levo-L3-Zr | 442 | 22624.4 | M→L<br>charge transfere | | | Dia |
| Levo-L4-Co | 362<br>404<br>732 | 27624.3<br>24752.5<br>13661.2 | $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^4T_{1g(P)}(\nu_2)$<br>$\rightarrow {}^4A_{2g(F)}(\nu_3)$ | $^4F$ | $^4T_{1g}$ | 3.872 |
| Levo-L4-Ni | 322<br>435<br>550 | 31055.90<br>22988.51<br>18181.82 | $^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\nu_1)$<br>$\rightarrow {}^3T_{1g(F)}(\nu_2)$<br>$\rightarrow {}^3T_{1g(P)}(\nu_3)$ | $^3F$ | $^3A_{2g}$ | 2.828 |
| Levo-L4-Cu | 600<br>720 | 16666.7<br>13888.9 | $^2a_{1g(D)} \rightarrow {}^2b_{1g(D)}$<br>$^2e_{g(D)} \rightarrow {}^2b_{1g(D)}$ | $^2D$ | $^2E_g$ | 1.732 |

**Calculation of the spectral data**

From the electronic absorption spectra and by the use of Tanabe-Sugano diagrams, the values of crystal field splitting energy ($\Delta_o$), Racah parameter (B'), nephelauxetic ratio (β) and the crystal field stabilization energy can be determined. For Co (II) complexes, the configuration is $d^7$ ion and from the high spin Tanabe Sugano diagram, the ground state is $^4T_{1(F)}$ and the spin multiplicity is a quartet. The diagram shows that there are three quartet excited states; $^4T_2$, $^4A_2$ and $^4T_{1(P)}$. From diagram one can predict that there are three spin-allowed transitions ($V_1$, $V_2$ and $V_3$).

- The position on the horizontal axis (Δ/B) where the ratio between the lines representing $V_2/V_1$ is determined, then a vertical line is drawn at this position.
- The value on the vertical axis (E/B) that corresponding to the spin-allowed transitions is found so as to determine $V_1$/B, $V_2$/B and $V_3$/B.
- Knowing the values of $V_1$, $V_2$ and $V_3$ the values of B and Δ can be calculated.

Nephelauxetic ratio (β) is given by β = B'/B, where B' represents Racah value in complex which is always smaller than the B value of free ion (1100 $cm^{-1}$ and 1040 $cm^{-1}$ for Co(II) and Ni(II), respectively) due to the nephelauxetic effect. From the β values, the covalence

factor ($b^{1/2}$), Sinha parameter (δ %) (metal – ligand covalency percent) and the covalency angular overlap parameter (η) have been calculated using the relations [29,30]:

$b^{1/2} = 1/2[(1 – \beta)^{1/2}]$, $\delta\ \% = [(1 – \beta)/\beta]x100$ and $\eta = (1 – \beta)^{1/2} / \beta^{1/2}$.

The spectral parameters for Co(II) and Ni(II) complexes are given in Table (4)

Table (4): Spectral parameters of Co(II) and Ni(II) mixed ligands complexes.

| Complex | Spectral parameters | | | | | |
|---|---|---|---|---|---|---|
| | $\Delta_o$ ($cm^{-1}$) | B' ($cm^{-1}$) | B | %δ | η | CFST ($cm^{-1}$) |
| Co(II)complexes | 17448 | 759 | 0.69 | 44.93 | 0.670 | -13958 |
| Ni(II) complexes | 18787 | 606 | 0.58 | 71.53 | 0.846 | -22544 |

**Biological activity**

The antimicrobial activity of the mixed ligands – drug complexes is tested against representatives of gram positive bacteria (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and gram negative bacteria (*Proteus vulgaris* and *Klebsiella pneumonia)*. Standard drug; Ampicillin and DMF solvent control were screened separately for their antibacterial activity. The antibacterial results, expressed as inhibition zone diameter (mm) and % activity index (relative to ampicillin), are given in Table (5) from which it is clear that the mixed ligands complexes show high activity against the tested organisms. Also, percent Activity Index data show that Cu (II) complexes have the highest activity followed by Ni (II), Co (II) and finally Zr (IV) complexes.

Increased activity of metal chelates had been explained by Overtone's concept [32] and the Tweedy's theory [33], according to which chelation reduces the polarity of the ligand due to partial sharing of its negative charge with the metal, favoring transportation of the complexes across the lipid layer of the cell membrane. The positive results suggested the very diffusion of the complexes into the bacterial cells and are able to kill the bacterium as indicated by the zones of inhibition of bacterial growth.

Table (5): Antibacterial activities of some mixed ligands complexes in terms of inhibition zone diameter (mm) and % activity index.

| Complex | *Strept. Pyog.* | | *Staph. Epid.* | | *Prot. Vulgaris* | | *Kleb. Pne.* | |
|---|---|---|---|---|---|---|---|---|
| | Inh. zone | % Ac.Ind | Inh. zone | % Ac.Ind | Inh. zone | % Ac.Ind | Inh. Zone | % Ac.Ind |
| Ampicillin | 25 | 100 | 25 | 100 | 22 | 100 | 23 | 100 |
| Levo-L1-Co | 22 | 88 | 21 | 84 | 18 | 82 | 20 | 87 |
| Levo-L1-Ni | 21 | 84 | 22 | 88 | 21 | 96 | 21 | 91 |
| Levo-L1-Cu | 22 | 88 | 21 | 84 | 18 | 82 | 19 | 83 |
| Levo-L1-Zr | 21 | 84 | 22 | 88 | 17 | 77 | 14 | 61 |
| Levo-L2-Ni | 23 | 92 | 22 | 88 | 20 | 90 | 21 | 91 |
| Levo-L2-Zr | 19 | 76 | 23 | 92 | 19 | 86 | 20 | 87 |
| Levo-L3-Co | 21 | 84 | 19 | 84 | 19 | 86 | 22 | 74 |
| Levo-L3-Ni | 21 | 84 | 20 | 80 | 21 | 96 | 19 | 83 |
| Levo-L3-Cu | 22 | 88 | 23 | 92 | 20 | 90 | 21 | 91 |
| Levo-L3-Zr | 19 | 76 | 20 | 80 | 17 | 77 | 20 | 87 |
| Levo-L4-Co | 21 | 84 | 19 | 76 | 20 | 91 | 21 | 83 |
| Levo-L4-Ni | 20 | 80 | 21 | 84 | 20 | 90 | 22 | 96 |
| Levo-L4-Cu | 22 | 88 | 20 | 80 | 21 | 96 | 20 | 87 |

**Antitumor activity**

The cytotoxic activities of some selected mixed ligands complexes viz; levofloxacin–L1 and levofloxacin–L3 complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The relation between surviving cells and drug concentration is plotted to get the survival curve from which the 50% inhibitory concentration ($IC_{50}$) was estimated. The results are represented graphically in Fig.'s (3 and 4) and the lethal concentrations ($IC_{50}$) values are listed in Table (6).

Table (6): Lethal concentration ($IC_{50}$) of the mixed Levo. - Co (II), Ni (II), Cu (II) and Zr (IV) complexes with ligands $L_1$ and $L_3$ on HEPG2.

| Complex | $IC_{50}$ (µg/ml) | |
|---|---|---|
| | Levo-$L_1$ complex | Levo-$L_3$ complex |
| Vinblastine | 4.6 | |
| Co (II) | 6.15 | 6.25 |
| Ni (II) | 8.24 | 6.31 |
| Cu (II) | 5.53 | 6.52 |
| Zr (IV) | 13.33 | 12.52 |

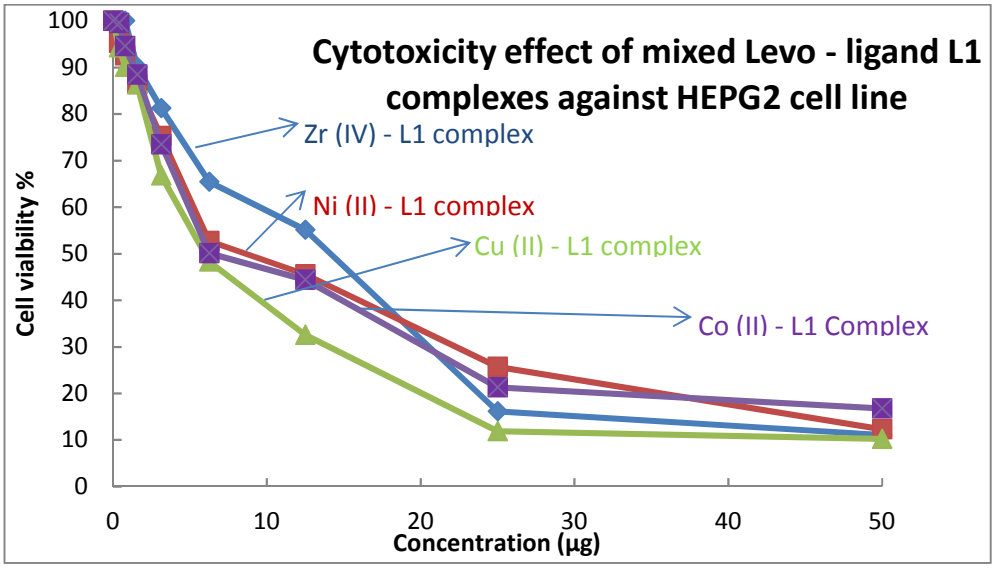



Fig (3): Cytotoxicity effect of mixed Levo-ligand $L_1$ complexes against HEPG2 cell line

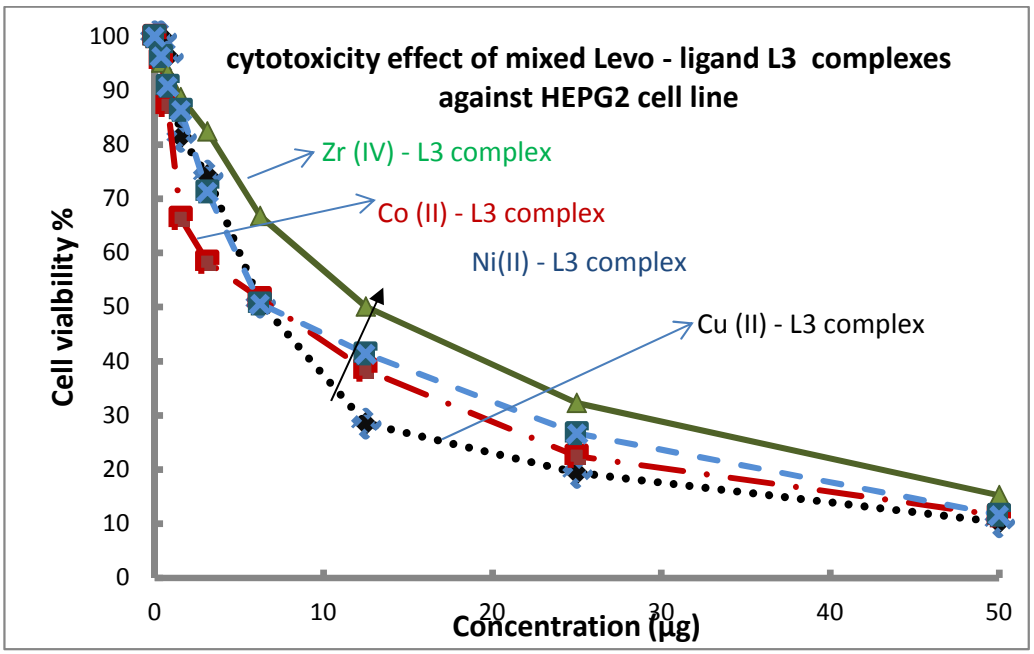



Fig (4): cytotoxicity effect of mixed Levo - ligand L3 complexes against HEPG2 cell line

Inspection of the cytotoxic data shows that mixed complexes of levofloxacine with $L_1$ are, in general, more effective than those with $L_3$ complexes and the metal ions are arranged according to their cytotoxicity as Cu (II) > Co (II) > Ni (II) > Zr (IV) for mixed ligand complexes with $L_1$ while Co (II) > Ni (II) > Cu (II) > Zr (IV) for complexes with $L_3$. The enhanced activity of metal complexes may be attributed to the increase in conjugation in the ligand moiety takes place in complexation process [33]. Shier [34] suggested that compounds having $IC_{50}$ values 10 – 25 µg/ml are considered to have weak cytotoxic activities, while those having intermediate values (ranging from 5 – 10 µg/ml) are classified as moderately active. On the other hand, compounds with $IC_{50}$ values less than 5µg/ml are considered to be very active. Consequently, the mixed ligands complexes under study are considered to be ranged from very active to moderately active except for ZrO complexes which was found to have weak activity with $IC_{50}$ values equal 13.3 and 12.5 µg/ml for complexes with $L_1$ and $L_3$, respectively. In general, these complexes seem to be promising as an anticanser agent due to their high cytotoxic activity.

## 3. Conclusions

This work presents spectroscopic characterization and biological activity of mixed ligand complexes of Co (II), Ni (II), Cu (II) and Zr (IV) with levofloxacin drug as primary ligand and four phenyl azo compounds as secondary ligands. The analytical and physicochemical data show satisfactory agreement with the proposed formulae confirming the structure of the prepared complexes. In brief, the results obtained can be summarized as follows:

1- The stiociometric ratio of the complexes is (1:1:1) (Levofloxacin:M:Azo) and the complexes are fairly stable in air and have non electrolytic nature.

2- Levofloxacin acts as monobasic monodentate ligand coordinated to metal ion through OO fashion whereas the azo compounds coordinate via ON fashion acting also as monobasic monodentate ligands.

3- The complexes are of octahedral structure around metal ions. The spectral data for each complex was determined by the aid of Tanabe Sugano diagrams.

4- The complexes show high antibacterial activity toward gram positive (*Streptococcus pyogenes* and *Staphylococcus epidermidis*) and gram negative (*Proteus vulgaris* and *Klebsiella pneumonia)* bacteria.

5- The cytotoxic activities of some selected mixed ligands complexes were tested against HEPG2 cell line and compared to that of Vinblastine as a standard drug. The study reveals that the mixed ligands complexes are considered to be ranged from very active to moderately active against HEPG2 Cell line except for ZrO complexes which was found to have weak activity with slightly high $IC_{50}$.

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