Journal of Molecular Structure 1075 (2014) 17–25

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

# Synthesis, characterization and *in vitro* anticancer activity of 18-membered octaazamacrocyclic complexes of Co(II), Ni(II), Cd(II) and Sn(II)

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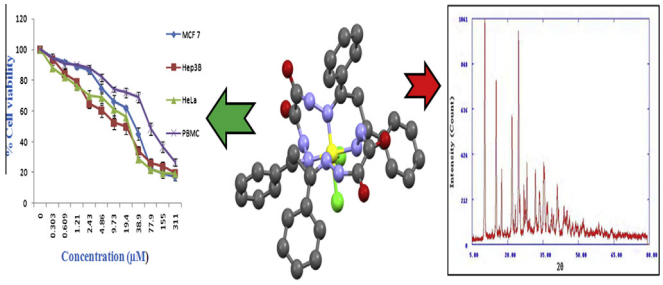
## HIGHLIGHTS

- 18-Membered Schiff base polyaza macrocyclic complexes of the type [$MLX_2$] have been synthesized.
- Synthesis by template condensation reaction of oxalyl dihydrazide with dibenzoylmethane and metal salt.
- The complexes have been characterized by different techniques.
- Anticancer activity by using MTT assay, against different human cancer cell lines.
- These complexes showed good *in vitro* anticancer activity.

## GRAPHICAL ABSTRACT




*Dose response Curve, XRD pattern and energy minimized cylindrical bonded 3–dimensional molecular structures of complex* $[C_{34}H_{28}N_8O_4SnCl_2]$. *Color scheme: Nitrogen (blue), Oxygen (red), Chlorine (green), Carbon (grey), Sn(II) (yellow). Hydrogen atoms have been omitted for clarity*

## ARTICLE INFO

*Article history:*
Received 13 April 2014
Received in revised form 24 June 2014
Accepted 24 June 2014
Available online 30 June 2014

*Keywords:*
Octaazamacrocyclic complexes
Dibenzoylmethane
Oxalyl dihydrazide
MTT assay
Anticancer activity

## ABSTRACT

An effective series of 18 membered octaazamacrocyclic complexes of the type [$MLX_2$], where X = Cl or $NO_3$ have been synthesized by template condensation reaction of oxalyl dihydrazide with dibenzoylmethane and metal salt in 2:2:1 molar ratio. The formation of macrocyclic framework, stereochemistry and their overall geometry have been characterized by various physico-chemical studies viz., elemental analysis, electron spray ionization–mass spectrometry (ESI–MS), I.R, UV–Vis, $^1$H NMR, $^{13}$C NMR spectroscopy, X-ray diffraction (XRD) and TGA/DTA studies. These studies suggest formation of octahedral macrocyclic complexes of Co(II), Ni(II), Cd(II) and Sn(II). The molar conductance values suggest nonelectrolytic nature for all the complexes. Thermogravimatric analysis shows that all the complexes are stable up to 600 °C. All these complexes have been tested against different human cancer cell lines i.e. human hepatocellular carcinoma (Hep3B), human cervical carcinoma (HeLa), human breast adenocarcinoma (MCF7) and normal cells (PBMC). The newly synthesized 18-membered octaazamacrocyclic complexes during *in vitro* anticancer evaluation, displayed moderate to good cytotoxicity on liver (Hep3B), cervical (HeLa) and breast (MCF7) cancer cell lines, respectively. The most effective anticancer cadmium complex ($C_{34}H_{28}N_{10}CdO_{10}$) was found to be active with $IC_{50}$ values, 2.44 ± 1.500, 3.55 ± 1.600 and 4.82 ± 1.400 in micro-molar on liver, cervical and breast cancer cell lines, respectively.



## Introduction

The family of complexes with aza macrocyclic ligands has remained a focus of scientific attention for many decades [1]. schiff

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http://dx.doi.org/10.1016/j.molstruc.2014.06.073
0022-2860/

base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications [2]. A number of macrocyclic complexes with tetra aza macrocyclic ligands, such as cyclen, cyclam and bicyclam have been reported to exhibit antitumor activity [3]. The design and synthesis of the macrocyclic complexes of oxalyl dihydrazide is a field of interest because of their applications as anticancer, antiviral, antibacterial and antifungal agents [4–6]. Extensive research work has been carried out on platinum-based chemotherapeutic compounds [7,8]. Despite of their remarkable success with high efficiency against human testicular, ovarian, bladder, head and neck carcinomas, several side effect such as limited water solubility and the dose-dependent toxicities, mainly nephrotoxicity, cytotoxicity and emetogenesis are the major drawbacks associated with these drugs [8–10]. Moreover, the importance of aza-macrocyclic transition metal complexes is due to the role they play as models for protein metal binding sites in biological systems, as synthetic ionophores [11], electrocatalyst in fuel cells [12], M.R.I contrast agents [13,14], luminescent sensors [15], anticancer drugs [16] and radioimmunotherapeutic agents [17]. These extensive applications have been worth investigating for the design of new macrocyclic ligands and their metal complexes for biological and industrial applications [18]. Coordination compounds containing macrocyclic ligands have been studies in recent decades owing to their wide applications in biological and sensor field. The first non-platinum complex tested in clinical trials was *cis*-[$(CH_3CH_2O)_2$ $(bzac)_2Ti(VI)$] used against a wide variety of ascites and solid tumors [19,20]. One of the potential approaches in anticancer chemistry is focused on the design of new macrocyclic metal complexes with different substituent and labile sites which may increase their cytotoxicity, especially to cancer cells. In this context, a wide range of macrocyclic metal complexes have been synthesized and their *in vitro* anticancer activity has been tested against different cancerous cell lines along with normal cells. The Pd(II) complexes have been derived from a salen ligand and tested against a human hepatoma cancer cell line [21]. Tin based complexes exhibit a broad spectrum of biological activity which includes organotin derivatives having bactericidal, fungicidal, antitumor and acricidal activity [22]. Recently, a number of metal complexes have been synthesized and some of these complexes were also tested for their cytotoxicities on various cancer cell lines and their results showed moderate to good anticancer activity [23]. In this present work we have reported the synthesis and characterization of octaazamacrocyclic complexes of Co(II), Ni(II), Cd(II) and Sn(II) derived from oxalyl dihydrazide and dibenzoylmethane which were also studied for their *in vitro* anticancer activity. The evaluation of *in vitro* anticancer activity of these complexes was carried out against different human cancer cell lines (Hep3B, HeLa and MCF7) by using MTT assay [24].

## Experimental

### *Materials and methods*

The metal salts $CoX_2{\cdot}6H_2O$, $NiX_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$ and $SnCl_2{\cdot}2H_2O$ (X = Cl or $NO_3$), dibenzoylmethane (*all E. Merck chemicals*) and oxalyl dihydrazide (*Acros organics*) were used as received. All the reactions were carried out under anhydrous condition.

### *Synthesis of the complexes*

#### *Synthesis of cobalt complexes*

The synthesis of dichloro/dinitrato (7,9,16,18-tetraphenyl-3,4,12,13-tetraoxa-1,2,5,6,10,11,14,15-octaazacyclooctadecane-6, 9,15,18-tetraene) cobalt (II) complexes, $[CoLX_2]$, has been carried out by template condensation method. The condensation of oxalyl dihydrazide and dibenzoylmethane in the presence of the $CoX_2{\cdot}6H_2O$, (X = Cl or $NO_3$), in methanol takes place easily. It was carried out by stirring a hot methanolic solution (50 mL) of oxalyl dihydrazide (1.181 gm, 10 mmol) with the divalent metal salt of cobalt (5 mmol) dissolved in minimum quantity of methanol (~20 mL). The content of reaction mixture was boiled under reflux for 0.5 h. after that dibenzoylmethane (2.25 g, 10 mmol) in 20 mL methanol was added in the refluxing mixture and refluxing was continued for 8–10 h. Now the mixture was concentrated to half of its volume and kept in desiccator for ~16 h. The solid product thus produced was filtered washed several times with methanol and then with acetone and dried in vacuo.

#### *Synthesis of nickel complexes*

In the synthesis of dichloro/dinitrato (7,9,16,18-tetraphenyl-3,4,12,13-tetraoxa-1,2,5,6,10,11,14,15-octaazacyclooctadecane-6, 9,15,18-tetraene) nickel (II) complexes, $[NiLX_2]$, a similar procedure was adopted as above except that in place of the cobalt salts now nickel salts $NiX_2{\cdot}6H_2O$ (X = Cl or $NO_3$) were used.

#### *Synthesis of cadmium complex*

A similar procedure was adopted for the synthesis of cadmium complex, $[CdL(NO_3)_2]$, as above except that in place of the nickel salts now cadmium salt $Cd(NO_3)_2{\cdot}4H_2O$ was used.

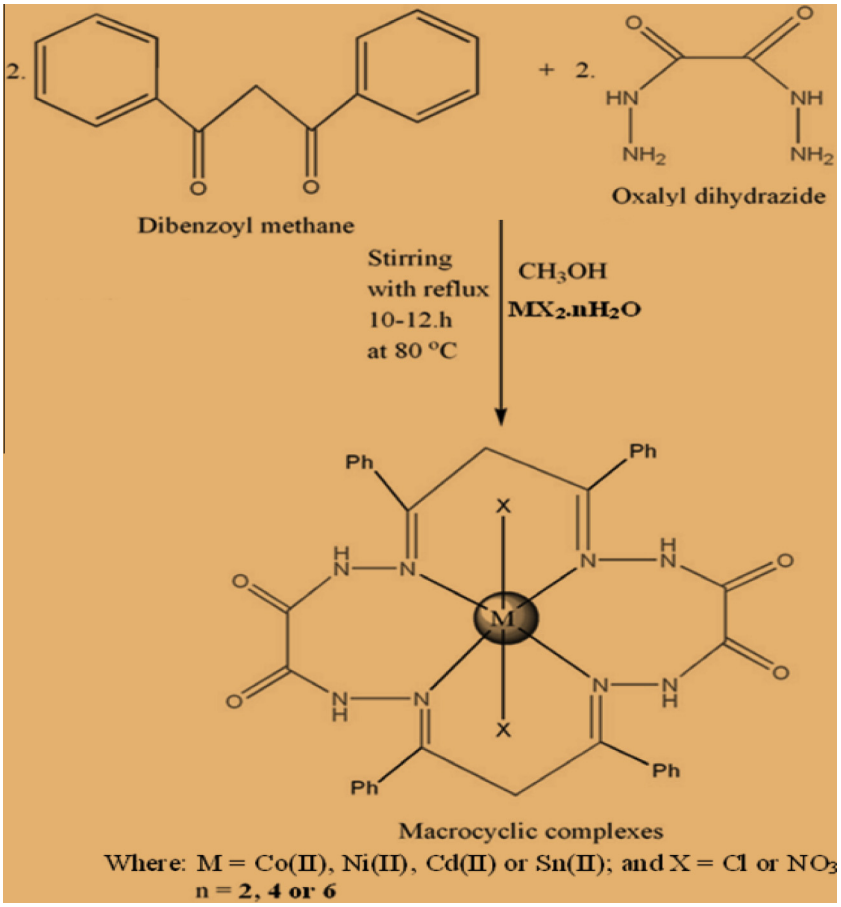
#### *Synthesis of tin complex*

In the synthesis of tin macrocyclic complex, $[SnLCl_2]$, here also the similar procedure was adopted as above except that in place of the cadmium salt now tin salt $SnCl_2{\cdot}6H_2O$ was used.

### *MTT assay*

The MTT assay is a colorimetric assay for measuring the cellular growth that reduces the tetrazolium yellow dye, MTT, to its insoluble formazan (purple color) by mitochondrial dehydrogenases of living cells. MTT is used to determine the cytoxicity of potential drugs and other toxic compounds [24,25]. The insoluble purple formazan product is dissolved into a colored solution by the addition of a suitable solvent. At certain wavelength, the absorbance of this colored solution can be measured. The potency of the drug in causing cell death can be concluded through the production of dose–response curves when the amount of purple formazan produced by untreated control cells. The Hep3B cell line was maintained in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum. The cells were plated at a density of $5 \times 10^3$ cells per well in a 96-well plate and cultured for 24 h at 37 °C. Stock solutions of the synthesized steroids were prepared in a 1:1 mixture of DMSO and THF. The cells were subsequently exposed to drugs. The plates were incubated for 48 h, and cell proliferation was measured by adding 20 μL of MTT dye (5 mg/mL in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37 °C in a humidified chamber containing 5% $CO_2$. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 150 μL dimethyl sulfoxide and absorbance was read at 570 nm. The absorption values were expressed as the percent cell viability, according to the control group as 100%.

For the other cell lines i.e. HeLa and MCF7, all the procedure are the same as described above for Hep3B cell lines. Doxorubicin (Doxo) and 5-Fluorouracil (5-Fu) were used as cytotoxic drugs of reference.




**Scheme 1.**

## Materials and physical measurements

The elemental analyses for carbon, hydrogen and nitrogen were obtained from the Central Drug Research Institute (Lucknow, India). The $^1$H NMR was recorded on a JEOL GSX 300 MHz FX-1000 spectrometer using DMSO-$d_6$ as a solvent and tetramethylsilane ($Me_4Si$) as an internal standard. The IR spectra (4000–400 $cm^{-1}$) were recorded as KBr discs on the Perkin Elmer – 2400 spectrometer. Metal and chloride ions were determined volumetrically [26] and gravimetrically [27], respectively. The electronic spectra of the compounds in DMSO were recorded on a Pye-Unicam 8800 spectrophotometer at room temperature. The electrical conductivities were obtained with a systronics type 302 conductivity bridge equilibrated at 25 ± 0.01 °C using $10^{-3}$ M DMSO solution at room temperature. The TGA and DTA were performed on a Schimadzu Thermal Analyzer under nitrogen atmosphere using alumina powder as reference. The XRD pattern and different parameters were collected with a Rigaku, Mini Flex II powder diffractometer using X-ray radiation. Peripheral Blood Mononuclear Cell (PBMC) isolation was done by fresh blood (10–15 mL) which was kindly

**Table 1**
Elemental analyses, *m*/*z* values, colors, yields, molar conductance and melting points of the metal complexes.

| Compounds | *m*/*z* (Calc.) | Yield (%) | Anal. found (calc.) % | | | | | Molar conductivity ($ohm^{-1}$ $cm^2$ $mol^{-1}$) | Color (M.p. °C) |
|---|---|---|---|---|---|---|---|---|---|
| | | | M | Cl | C | H | N | | |
| $[CoLCl_2]$ $C_{34}H_{28}N_8O_4$ $CoCl_2$ | 742.40 (742.49) | 65 | 7.80 (7.94) | 9.60 (9.55) | 55.50 (55.99) | 3.74 (3.80) | 15.10 (15.09) | 18 | Light pink (240 °C) |
| $[CoL(NO_3)_2]$ $C_{34}H_{28}N_{10}CoO_{10}$ | 795.31 (795.59) | 67 | 7.50 (7.41) | – | 51.40 (51.33) | 3.60 (3.55) | 17.50 (17.61) | 19 | Light salmon (235 °C) |
| $[NiLCl_2]$ $C_{34}H_{28}N_8O_4$ $NiCl_2$ | 741.95 (742.25) | 66 | 7.5 (7.90) | 9.41 (9.56) | 55.20 (55.01) | 3.50 (3.80) | 14.98 (15.09) | 20 | Powder blue (252 °C) |
| $[NiL(NO_3)_2]$ $C_{34}H_{28}N_{10}NiO_{10}$ | 795.10 (795.35) | 74 | 7.6 (7.38) | – | 51.00 (51.35) | 3.41 (3.55) | 17.63 (17.61) | 21 | Sky blue (250 °C) |
| $[CdL(NO_3)_2]$ $C_{34}H_{28}N_{10}CdO_{10}$ | 849.10 (849.07) | 69 | 13.10 (13.24) | – | 47.90 (48.10) | 3.40 (3.33) | 16.30 (16.40) | 22 | Creamy white (254 °C) |
| $[SnLCl_2]$ $C_{34}H_{28}N_8O_4$ $SnCl_2$ | 802.30 (802.25) | 70 | 14.51 (14.79) | 8.75 (8.84) | 51.10 (50.90) | 3.50 (3.52) | 13.80 (13.96) | 21 | Light yellow (260 °C) |

**Table 2**
IR spectral data of the complexes ($cm^{-1}$).

| S. no. | Compounds | ν(N—H) | ν(C=N) | ν(C—H) | δ(C—N) | δ(C—H) | ν C=O(free) [CO—NH] moiety | ν(M—N) |
|---|---|---|---|---|---|---|---|---|
| 1 | $C_{34}H_{28}N_8O_4\ CoCl_2$ | 3260 (s) | 1607 | 3260 | 1200 | 1503 | 1667 | 418 |
| 2 | $C_{34}H_{28}N_{10}CoO_{10}$ | 3182 (s) | 1642 | 3182 | 1381 | 1511 | 1666 | 460 |
| 3 | $C_{34}H_{28}N_8O_4\ NiCl_2$ | 3281 (s) | 1615 | 3281 | 1269 | 1527 | 1670 | 420 |
| 4 | $C_{34}H_{28}N_{10}NiO_{10}$ | 3285 (s) | 1511 | 3285 | 1385 | 1520 | 1674 | 424 |
| 5 | $C_{34}H_{28}N_{10}CdO_{10}$ | 3284 (s) | 1612 | 3284 | 1388 | 1535 | 1686 | 421 |
| 6 | $C_{34}H_{28}N_8O_4\ SnCl_2$ | 3286 (s) | 1591 | 3285 | 1353 | 1527 | 1682 | 425 |

provided by Blood bank of Jawaharlal Nehru Medical College, A.M.U, Aligarh (India). The blood sample was diluted with the same volume of phosphate buffered saline (PBS). The diluted blood sample was carefully layered on Ficoll-Histopaque (Sigma Aldrich, USA). The mixture was centrifuged under at 900*g* for 10 min at 20–22 °C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with three volumes of PBS for twice and resuspended RPMI-1640 media

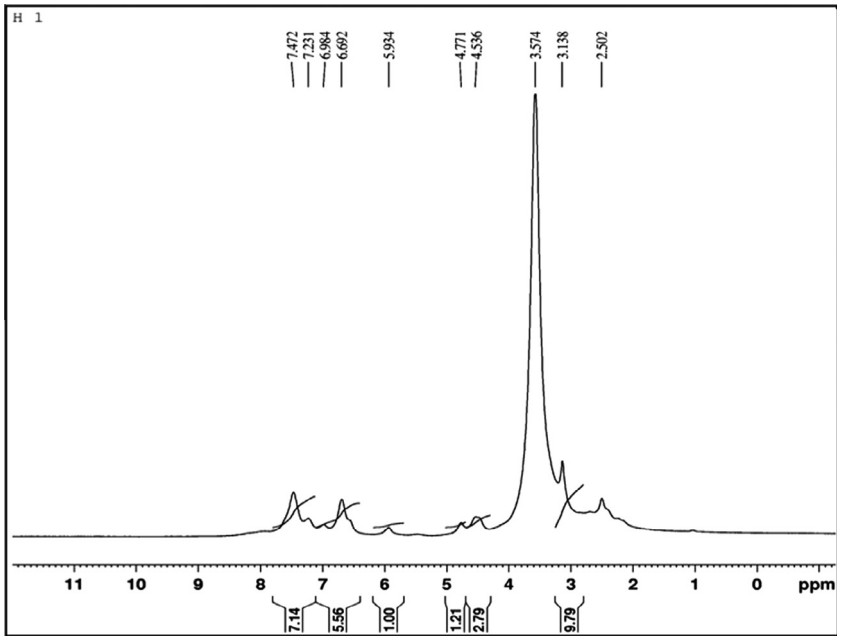



**Fig. 1.** $^1H$ NMR spectra of macrocyclic complex, [$SnLCl_2$].

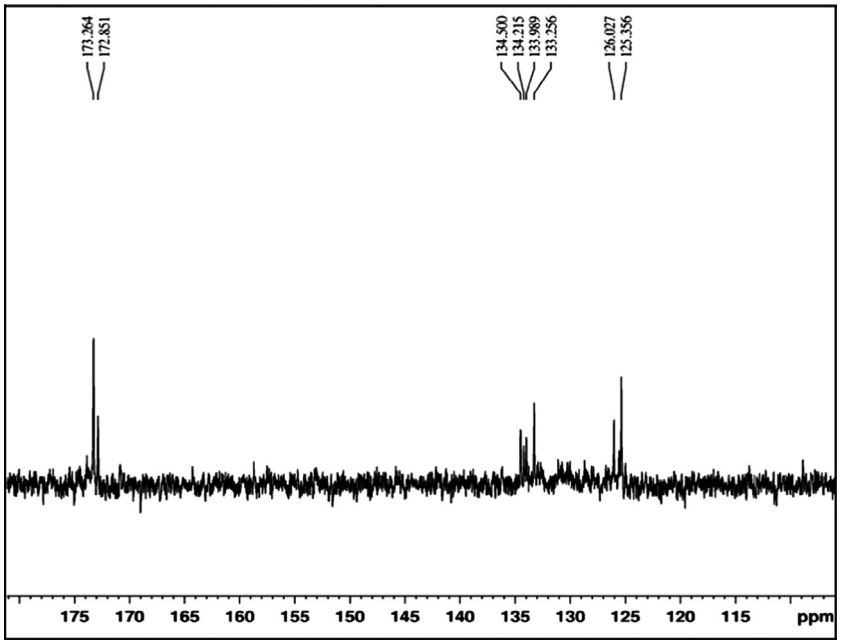



**Fig. 2.** $^{13}C$ NMR spectra of macrocyclic complex, [$SnLCl_2$].

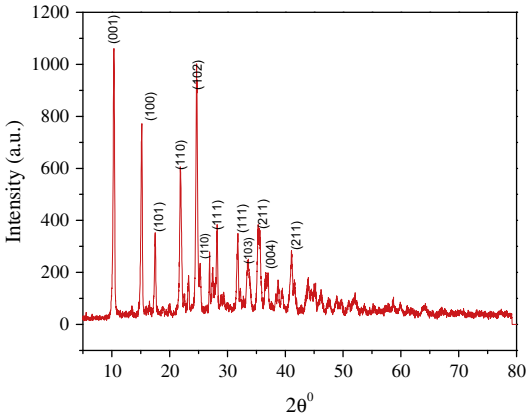



**Fig. 3.** X-ray diffraction (XRD) pattern of macrocyclic complex, $[SnLCl_2]$.

(Sigma Aldrich, USA) with 10% antibiotic and antimycotic solution (Sigma Aldrich, USA) and v/v fetal calf serum (FCS) (Sigma Aldrich, USA). Cell counting was performed to determine the PBMC cell number with equal volume of trypan blue [28]. The Hep3B (human hepatocellular carcinoma), MCF7 (human breast adenocarcinoma) and Hela (human cervical carcinoma) cell lines are procured from Cell Repository–National Centre for Cell Science, Pune (India).

## Results and discussion

A new series of octaazamacrocyclic Schiff base complexes $[MLX_2]$ (M = Co(II), Ni(II), Cd(II) and Sn(II); X = Cl or $NO_3$) have been prepared by the template reaction of respective metal ions, with oxalyl dihydrazide and dibenzoylmethane in a 1:2:2 molar ratio as shown in Scheme 1. All the complexes were stable in atmosphere and soluble in DMF (dimethylformamide) and DMSO (dimethyl sulfoxide). Their color and melting points are given in Table 1. Their thermal stabilities (TGA/DTA) were also studied which are discussed in Section 'Thermal analyses'. The molar conductivities of the complexes were recorded in DMSO at room temperature. As reported by Sears et al. the molar conductance values for 1:1 electrolyte in this solvent ranges from ~23 to 42 $ohm^{-1}\ cm^2\ mol^{-1}$. However, Greenwood et al. have suggested 50–70 $ohm^{-1}\ cm^2\ mol^{-1}$ as the range for 1:1 electrolyte in this solvent [29]. For newly synthesized complexes the recorded molar conductivity values (18–22 $ohm^{-1}\ cm^2\ mol^{-1}$) are quite low which indicate that $Cl^-$ or $NO_3^-$ groups are not ionized remaining intact in the coordination sphere being attached with central metal atom. The metal to ligand stoichiometry of the complexes was ascertained on the basis of elemental analyses and position of molecular ion peaks in the mass spectra (Table 1). The analytical data for elemental analyses suggest that the proposed macrocyclic complexes have 1:1 metal to ligand stoichiometry.

### *ESI-mass spectra*

Mass spectra of all the synthesized macrocyclic complexes of Co(II), Ni(II), Cd(II) and Sn(II) showed *m/z* peaks at 742.40, 795.31, 741.95, 795.10, 849.10 and 802.30 that corresponded to $C_{34}H_{28}N_8O_4CoCl_2$, $C_{34}H_{28}N_{10}CoO_{10}$, $C_{34}H_{28}N_8O_4NiCl_2$, $C_{34}H_{28}N_{10}NiO_{10}$, $C_{34}H_{28}N_{10}CdO_{10}$ and $C_{34}H_{28}N_8O_4SnCl_2$ moieties, respectively. The proposed molecular formulae of synthesized complexes was confirmed by comparing their molecular formula weights with respective *m/z* values (Table 1) that are in good agreement for aforementioned complexes.

### *Infrared spectra*

In all the complexes the presence of a single band in the region 3185–3285 $cm^{-1}$ may be assigned to ν(N—H) stretching [30–33]. It was observed that a pair of bands corresponding to $\nu(NH_2)$ at 3300 and 3310 $cm^{-1}$ which were present in the i.r. spectrum of oxalyl dihydrazide were absent in the infrared spectra of all the complexes. The disappearance of these bands and appearance of absorption bands near 1605–1642 $cm^{-1}$ which are assignable to imine ν(C=N) stretching vibration is consistent [34] with the macrocyclic structure given in Scheme 1. The value of ν(C=N) is lower than that usually found for azomethine linkage which may be explained on the basis of drift of lone pair density of azomethine nitrogen towards the metal atom [35,36] indicating the involvement of the π electrons throughout the macrocyclic framework. This fact confirm that coordination takes place through nitrogen of C=N groups. The bands present in the region ~3000 to 3183 $cm^{-1}$ may be assigned to ν(C—H) stretching [30]. The strong band present in the region 1660–1685 $cm^{-1}$ may be assigned to the C=O group of the CO—NH moiety [35] in these complexes. The bands present in the range of 1100–1270 $cm^{-1}$ an all complexes are assigned to ν(C—N) stretching [37]. The far IR spectra show bands in the region ~410 to 460 $cm^{-1}$ corresponding to ν(M—N) vibrations [38,39]. The presence of bands in all the complexes in the region 418–460 $cm^{-1}$ originate from ν(M—N) vibrational modes and gives idea about coordination of azomethine nitrogen [40]. However, no bands characteristics free amines were observed which strongly supports the proposed structure of all these complexes (Table 2).

### *$^1H$ and $^{13}C$ NMR spectroscopic analyses*

The $^1H$ NMR as well as $^{13}C$ NMR spectra of tin complex $[SnLCl_2]$ fully suggest the structure of complexes as given in Scheme 1. The $^1H$ NMR spectrum (Fig. 1) in DMSO-$d^6$ shows broad signal at 7.231–7.472 ppm range due to amide (—CONH) protons [37,41,42]. The multiplet in the region 6.692–6.984 ppm may be assigned to aromatic protons [43,44]. A singlet observed at 3.5745 ppm is assigned to methylene protons of [N=C(Ph)—$CH_2$—(Ph)C=N] moiety. The $^{13}C$ NMR spectrum of tin complex was also recorded in DMSO-$d^6$. The signals observed in the region 124.38–136.60 ppm have been assigned to aromatic carbon attached to nitrogen atoms. Carbon atoms which have a distance from nitrogen atoms show upfield shift. A signal observed at 173.264 ppm may be assigned to carbonyl (>C=O) carbon. Similarly a band appeared in the region 125.356–126.027 ppm due to carbon of two methylene group in the complex as shown in Fig. 2.

### *UV–Vis studies*

The UV–Vis absorption spectra of Co(II) and Ni(II) complexes were recorded in the 8300–30,000 $cm^{-1}$ range in dimethyl sulfoxide solution ($10^{-3}$ M). The UV–Vis spectra of cobalt complexes shows absorption in the region ca. 8300–9200 $cm^{-1}$ ($\nu_1$), 12,600–15,800 $cm^{-1}$ ($\nu_2$) and 18,800–20,650 $cm^{-1}$ ($\nu_3$), respectively. These values resemble to the reported octahedral complexes [45,46]. The various bands are assignable to: $^4T_{2g}(F) \leftarrow {}^4T_{1g}$, ($\nu_1$), $^4A_{2g}(F) \leftarrow {}^4T_{1g}$, ($\nu_2$) and $^4T_{1g}(P) \leftarrow {}^4T_{1g}$, ($\nu_3$) transitions, respectively. These transitions suggest that the symmetry of these complexes is not ideal octahedral i.e. $D_{4h}$. The electronic spectra of Ni(II) complexes exhibit a well discernable band on the low energy side and the other bands are observed in the region ca. 16,600–17,300 $cm^{-1}$ ($\nu_2$) and 26,900–28,000 $cm^{-1}$ ($\nu_3$), which are assigned to $^3T_{1g}(F) \leftarrow {}^3A_{2g}$ ($\nu_2$), and $^3T_{1g}(P) \leftarrow {}^3A_{2g}$, ($\nu_3$), respectively. The low energy band ($\nu_1$), splits into two bands in the range ~9500 to 10,000 and 11,500–12,500 $cm^{-1}$, which can be assigned to $^3E_g \leftarrow {}^3B_{1g}$ and $^3B_{2g} \leftarrow {}^3B_{1g}$, assuming the effective symmetry to be $D_{4h}$. The

**Table 3**
The XRD parameters of the value of Sn(II) complex.

| Identification of the complex | $[SnLCl_2]$ |
|---|---|
| Empirical formula | $C_{34}H_{28}N_8O_4\ SnCl_2$ |
| Formula weight | 802.30 (g $mol^{-1}$) |
| Wave length | 1.540598 Å |
| Crystal system (powder) | Hexagonal |
| Lattice parameter | $a$ = 6.987 Å, $b$ = 6.987 Å and $c$ = 8.876 Å |
| Lattice parameter | Alpha = 90°, Beta = 90° and Gamma = 120° |
| 2$\theta$ Min–max | 5.00–80.00 |
| Lattice type | P |

spectral results are consistent with distorted octahedral nature of these complexes [46,47].

*X-ray diffraction analysis*

The $[SnLCl_2]$ complex was also characterized by X-ray diffraction technique. The X-ray powder diffraction (XRD) was used to determine the type of structure ordering of 18-membered octaazamacrocyclic complex (Fig. 3). The X-ray diffraction of the representative $[SnLCl_2]$ complex was scanned in the range 5–80° at wavelength 1.540 Å. The diffractogram and associated data depict the 2$\theta$ value for each height, relative strength and interplanar spacing ($d$-values). The diffractogram of $[SnLCl_2]$ complex had 12 reflections with maxima at 2$\theta$ = 10.356° and its intensity is 1057 a.u. corresponding to $d$ value 8.53495 Å. The above indexing method also yields Miller indices (hkl), unit cell parameters. The unit cell of $[SnLCl_2]$ complex yielded values of lattice constants, $a$ = 6.987 Å, $b$ = 6.987 Å and $c$ = 8.876 Å. In conjunction with these cell parameters, the conditions such as $a = b \neq c$ and $\alpha = \beta = 90$, $\gamma = 120$ required for samples to be hexagonal were tested and base to be acceptable (Table 3). Hence, it can be reasoned that $[SnLCl_2]$ complex has a hexagonal crystal system. The average crystallite size ($D$) of $[SnLCl_2]$ complex was calculated by following the Debye–Scherrer formula:

$$D = 0.9\lambda/\beta \cdot \cos\theta$$

where constant 0.9 is the shape factor, $\lambda$ is the X-ray wavelength of Cu K$\alpha$ radiation (1.54 Å), $\theta$ is the Bragg diffraction angle and $\beta$ is the full-width at half-maximum (FWHM) of the (0 0 1) plane diffraction peak. The calculated average particle size was found to be ~27.6 nm.

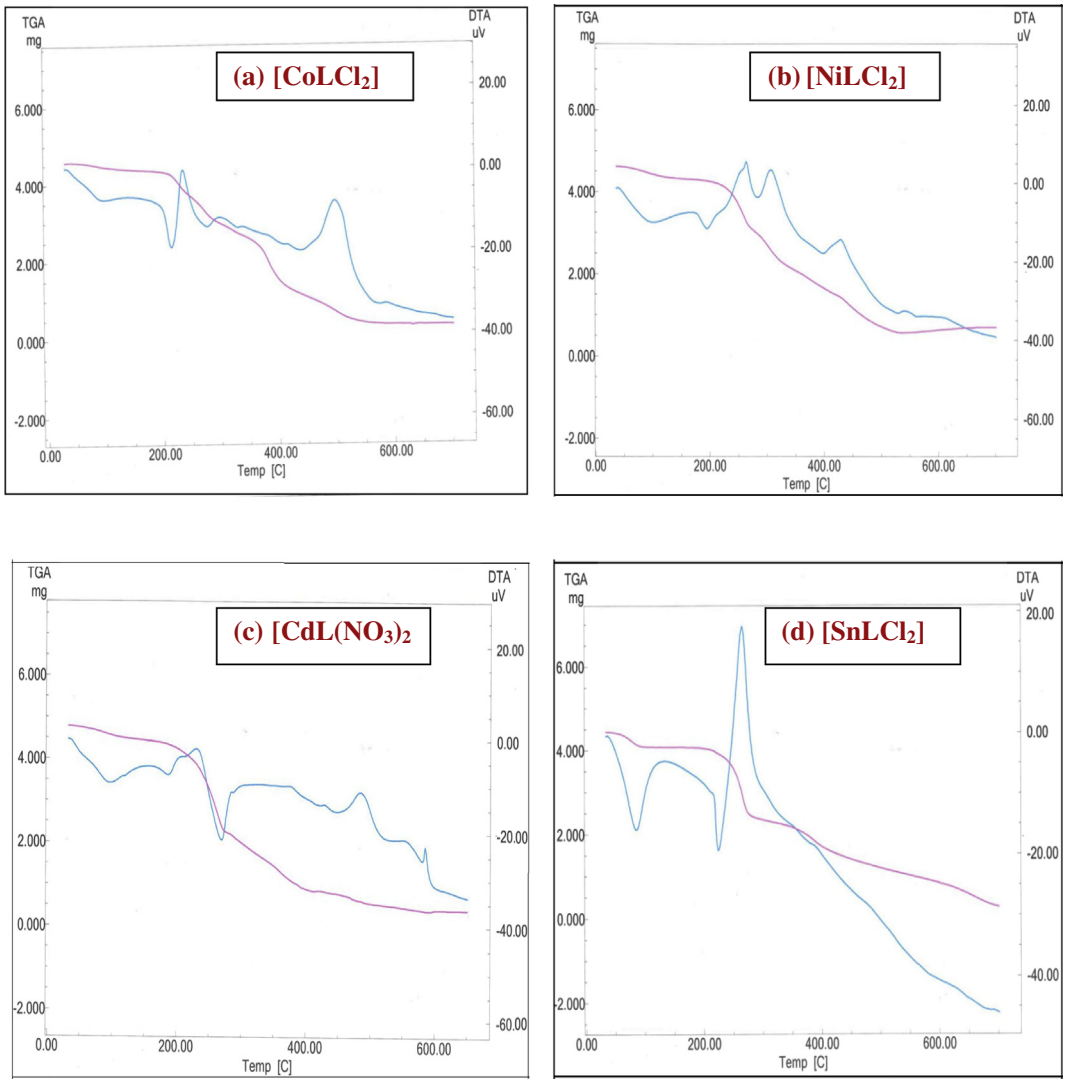



**Fig. 4.** (A–D) TGA/DTA curve of the synthesized macrocyclic complexes.

*Thermal analyses*

Thermal stabilities of all the metal complexes were studied by thermogravimetric analyses (TGA and DTA) in $N_2$ atmosphere at a heating rate of 20 °C $min^{-1}$ in the temperature range 20–700 °C. The thermal analyses show that these compounds undergo three steps of weight loss (Fig. 4). There are two endothermic peaks in the DTA curve of these complexes, the first is the melting point of the complexes and the second corresponds to decomposition of the complexes. The TG studies of $[CoLCl_2]$, $[NiLCl_2]$, $[CdL(NO_3)_2]$ and $[SnLCl_2]$ complexes showed no weight loss upto 140 °C indicating the absence of coordinated water molecules in all these macrocyclic complexes. The weight loss below 140 °C can be attributed to the evaporation of the lattice water molecules. Initial thermal decomposition temperature (IDT) of the complex is found to be ca. 140 °C. The second weight loss about 50% occurs between 140 and 370 °C; the temperature of maximum rate decomposition (Tmax) is between ca. 275 °C and 300 °C in these complexes.

*Cytotoxic potential of inhouse synthesized macrocyclic complexes*

The cytotoxic potential of the inhouse synthesized macrocyclic complexes against Hep3B, HeLa and MCF7 cell lines was assessed by determining the number of viable cells surviving after incubation with the macrocyclic complexes for the stipulated time period using the MTT method as given in Section 'MTT assay'. The cytotoxicity assay suggests a variable cytotoxicity of inhouse synthesized complexes for Hep3B, HeLa as well as MCF7 cell lines which can be attributed to the intrinsic anticancer property of these macrocyclic complexes. Curves of dose-dependent effects of all the complexes on cell viability of different human cancer cell lines (Hep3B, MCF7 and HeLa) and normal cells (PBMC) are displayed in Fig. 5(a–f). Experiments revealed that there was substantial

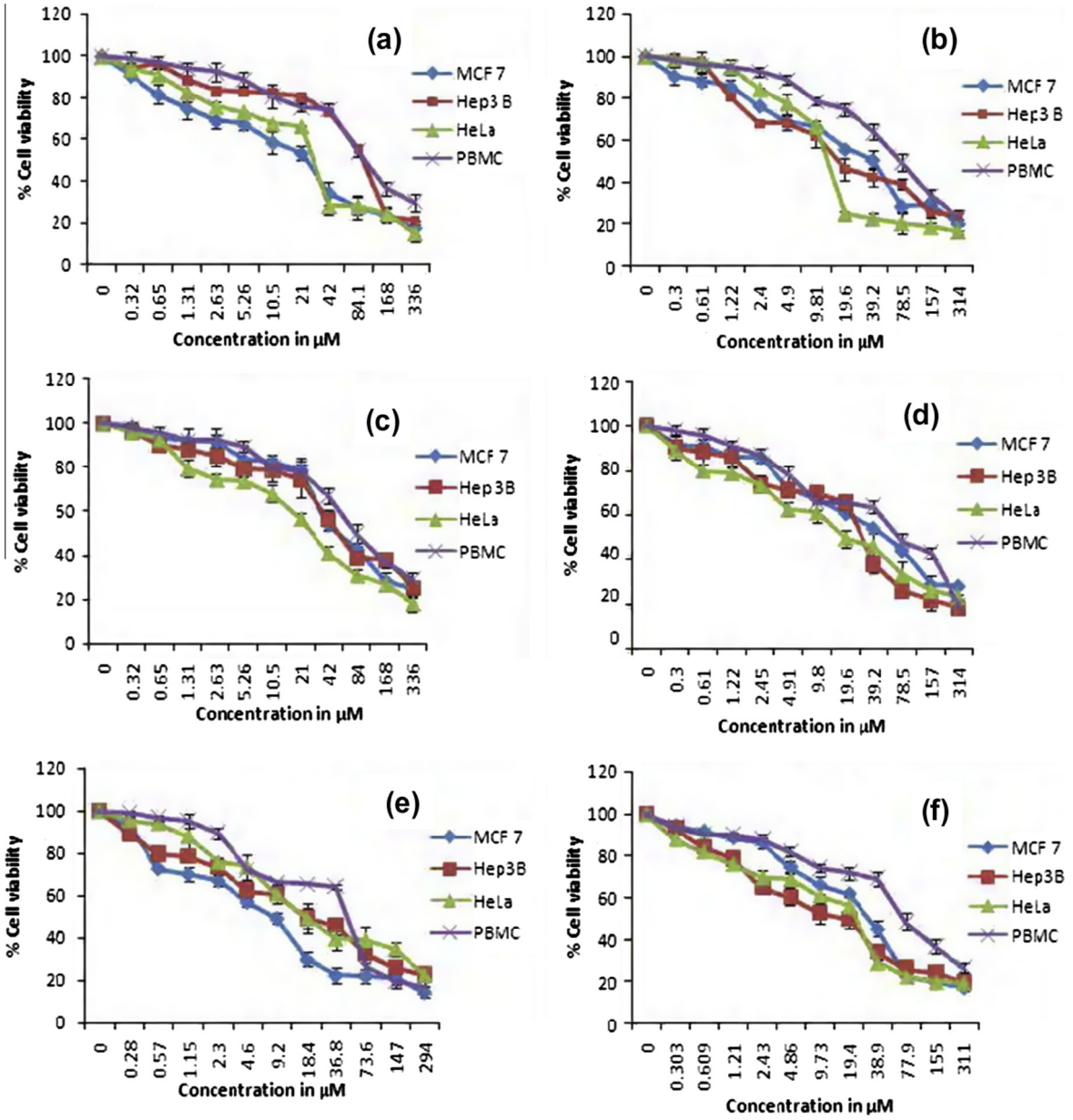



**Fig. 5.** (a–f) Dose-dependent curve of newly prepared Schiff base macrocyclic metal complexes; (a) $C_{34}H_{28}N_8O_4CoCl_2$, (b) $C_{34}H_{28}N_{10}CoO_{10}$, (c) $C_{34}H_{28}N_8O_4NiCl_2$, (d) $C_{34}H_{28}N_{10}NiO_{10}$, (e) $C_{34}H_{28}N_{10}CdO_{10}$ and (f) $C_{34}H_{28}N_8O_4SnCl_2$, against different human cancer cell lines (Hep3B, HeLa and MCF7) and normal cells (PBMC).

**Table 4**
$IC_{50}$ values in μM of the macrocyclic complexes.

| S. no. | Complexes | Hep3B | HeLa | MCF7 | PBMC |
|---|---|---|---|---|---|
| 1. | $C_{34}H_{28}N_8O_4\ CoCl_2$ | 3.90 ± 0.700 | 7.36 ± 2.060 | 5.12 ± 2.540 | 12.40 ± 0.900 |
| 2. | $C_{34}H_{28}N_{10}CoO_{10}$ | 4.86 ± 0. 639 | 3.95 ± 0.162 | 7.54 ± 0.810 | 14.08 ± 1.500 |
| 3. | $C_{34}H_{28}N_8O_4\ NiCl_2$ | 10.50 ± 1.910 | 7.36 ± 2.988 | 4.46 ± 1.200 | 21.5 ± 2.400 |
| 4. | $C_{34}H_{28}N_{10}NiO_{10}$ | 6.39 ± 0.280 | 4.49 ± 2.190 | 3.56 ± 1.150 | 11.34 ± 1.300 |
| 5. | $C_{34}H_{28}N_{10}CdO_{10}$ | 2.44 ± 1.500 | 3.55 ± 1.600 | 4.82 ± 1.400 | 10.12 ± 2.300 |
| 6. | $C_{34}H_{28}N_8O_4\ SnCl_2$ | 7.85 ± 2.401 | 3.36 ± 2.801 | 3.44 ± 1.702 | 13.41 ± 2.900 |
| 7. | Doxo | 2.15 ± 1.700 | 2.32 ± 1.600 | 1.56 ± 2.700 | 8.87 ± 1.800 |
| 8. | FU | 3.95 ± 1.802 | 3.92 ± 2.800 | 3.18 ± 1.600 | 9.91 ± 2.900 |

As reference drugs – { Doxo = Doxorubicin; FU = 5 – Fluorouracil.

increase in cytoxicity in the cell lines with increasing exposure to drug concentration. The absorption values were expressed as the cell viability in percent, according to the control group as 100%. Assays were performed in triplicate on three independent experiments. The concentration required for 50% inhibition of cell viability ($IC_{50}$) was calculated using the software "Prism 3.0". For each of the tested complexes $IC_{50}$ was calculated and the results are summarized in Table 4. The obtained results were revealed that compounds $C_{34}H_{28}N_8O_4CoCl_2$, $C_{34}H_{28}N_{10}CoO_{10}$, $C_{34}H_{28}N_8O_4NiCl_2$, $C_{34}H_{28}N_{10}NiO_{10}$, $C_{34}H_{28}N_{10}CdO_{10}$ and $C_{34}H_{28}N_8O_4SnCl_2$ showed remarkable inhibitory activities against different human cancer cell lines. It is apparent from the $IC_{50}$ values that all the tested complexes show moderate to good cytoxicity against different human cancer cell lines. The cobalt complex, $[CoLCl_2]$, led to remarkable increase in potency against human hepatocellular carcinoma (Hep3B) cells with $IC_{50}$ values of 3.90 ± 0.700 μM, 5.12 ± 2.540 μM (against MCF7 cell lines) and 7.36 ± 2.060 μM (against HeLa cell lines). The another complex of cobalt, $[CoL(NO_3)_2]$, is more effective against human cervical carcinoma (HeLa) cells with $IC_{50}$ values of 3.95 ± 0.162 μM. The complexes $[NiLCl_2]$ and $[NiL(NO_3)_2]$ are more effective against breast cancer (MCF7) cells with $IC_{50}$ values of 4.46 ± 1.200 μM and 3.56 ± 2.400 μM respectively. Among all these complexes the cadmium complex, $[CdL(NO_3)_2]$, exhibits very good cytotoxicity against all cancerous cells with $IC_{50}$ values of 2.44 ± 1.500 μM against Hep3B cell lines, 3.55 ± 1.600 μM against HeLa cell lines and 4.82 ± 1.400 μM against MCF7 cell lines, respectively. Present study showed that among the three human cancer cell lines tested, HeLa cell lines were found to be sensitive to most of the tested compounds while Hep3B and MCF7 cell lines were found to be sensitive to some selected compounds. Moreover, cadmium complex is relatively more sensitive for all the tested cancer cell lines.

## Conclusion

In the present study an effective series of dichloro/dinitrato (7,9,16,18-tetraphenyl-3,4,12,13-tetraoxa-1,2,5,6,10,11,14,15-octa azacyclooctadecane-6,9,15,18-tetraene) metal (II) complexes of cobalt, nickel, copper, cadmium and tin were derived from oxalyl dihydrazide and dibenzoylmethane and studied for their biological evaluation of cytotoxic activity. The results of cytotoxic study by MTT assay revealed that all the macrocyclic complexes (1–6) showed a considerable broad spectrum of anticancer activity against the three tested human cancer cell lines. In particular the cadmium, ($C_{34}H_{28}N_{10}CdO_{10}$), was the most proficient cytotoxic agent with $IC_{50}$ values below 5.00 μM in all the three tested human cancer cell lines i.e. Hep3B, HeLa and MCF7. On the basis of these observations, it could be the topic of further investigations for searching potential anticancer metal based complexes. Ultimately, it is comprehensible that further development of macrocyclic complexes of different metals can serve as new templates for antitumor chemotherapy and could probably be lead to more active complexes in the area of cancer chemotherapy.

## Acknowledgements

The authors wish to thank the Chairman, Department of Chemistry, Aligarh Muslim University for providing research facilities. We also thank Department of Applied Physics, A.M.U, Aligarh, India for XRD analysis and Cell Repository–National Centre for Cell Science, Pune (India) for providing cancer cell lines. The coauthor Abdul Kareem also thanks UGC, New Delhi for financial assistance.

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