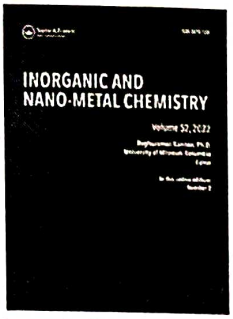

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Biologically active mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) as potential antimalarial, antidibetic and anticancer agents

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ABSTRACT

Mixed ligand complexes of transition metal ions Co(II), Ni(II), Cu(II) and Zn(II) were synthesized using bidentate Schiff's base (E)-2-((benzo[d]thiazol-2-yliminomethyl)-6-methoxyphenol (L1), derived from condensation reaction of 2-aminobenzothiazole and 2-hydroxy-3-methoxybenzaldehyde, and 5-Chloro-8-hydroxyquinoline (5CHQ) (L2) as ligands. All the synthesized compounds were characterized using elemental analysis, IR, electronic, ^{1}H and ^{13}C NMR spectra, TGA-DTA and Powder XRD analysis, molar conductivity and magnetic susceptibility measurements. Based on the results obtained all the synthesized complexes are proposed to have tetrahedral geometry. All the synthesized compounds were screened for their antioxidant, antimicrobial, antimalarial, antidibetic, anticancer activities and cytotoxicity study using MTT assay. The complexes are proposed to have potential antimalarial, antidibetic and anticancer activities.

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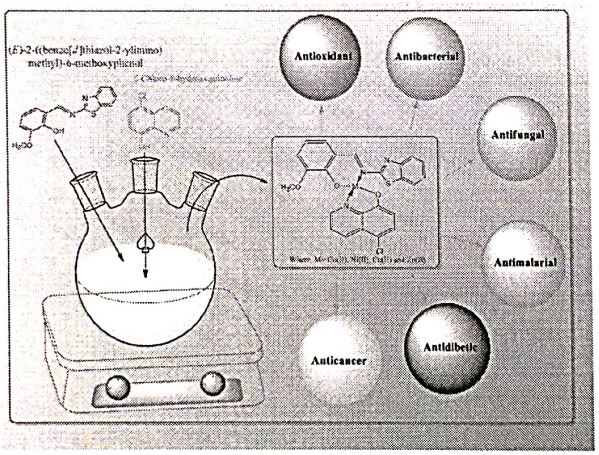
Schiff base mixed complexes; anticancer; antidibetic; antimalarial; antioxidant

GRAPHICAL ABSTRACT



Introduction

Cancer, diabetes and malaria are serious health concerns in countries like India and hence the primary targets of chemistry research. Cancer is a group of diseases involving abnormal cell growth which can spread to other body parts and caused deaths of millions of people throughout the world. The platinum based complex 'Cisplatin' owing to its anticancer activities has motivated worldwide researchers to work in this area which has produced large number of molecules. Still the research is ongoing in order to find different metal complexes with less side effects and similar or better cytotoxicity.[1] Diabetes resulting from insulin deficiency or insulin resistance is a serious chronic disorder around the world.[2–4] Two main types disease viz. type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes are known. Although various drugs are available in the market at present, the complications involved such as kidney failure, micro-and macrovascular disease, retinopathy, neuropathy and atherosclerosis has created an urgent need for the search of orally active drugs.[2–4] Malaria is one of the most infectious diseases affecting to health and developmental growth of developing countries. It is present in 91 countries, mostly in tropical and subtropical regions and the incidence of malaria becoming serious owing to globalization in the world.[5,6] The antimalarial potential of coordination

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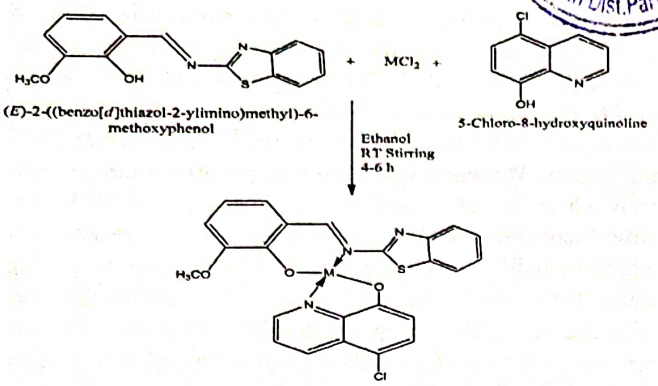

Figure 1. Synthesis of mixed ligand complexes.

compounds is well established and the research is still continued.

Mixed ligand complexes have gained considerable attention recently owing to their importance in biological systems. Such complexes play vital roles in the chemistry of living matter.[7,8] Schiff's bases are one of the most important ligands due to their versatility and exhibit a broad range of biological activities such as antiviral, antibacterial, anti-inflammatory, antimalarial, antifungal, anti-proliferative and antipyretic properties.[9–15] 8-hydroxyquinoline is a bidentate organic ligand which has attracted attention of chemists, medicinal chemists and professionals in health sciences owing to wide range of biological activities, including antimicrobial, anticancer and antifungal effects.[16]

This has prompted us to undertake synthesis of mixed ligand complexes of transition metal ions Co(II), Ni(II), Cu(II) and Zn(II) using Schiff base (E)-2-((benzo[d]thiazol-2-yliminomethyl)-6-methoxyphenol (L1), derived from condensation reaction of 2-aminobenzothiazole with 2-hydroxy-3-methoxybenzaldehyde, and 5-Chloro-8-hydroxyquinoline (L2) as ligands and their structure elucidation using various techniques. All the synthesized complexes are proposed to have tetrahedral geometry and were screened for their antioxidant, antimicrobial, antimalarial, antidibetic, anticancer activities and cytotoxicity study using MTT assay.

Experimental

Materials and methods

Metal salts ($CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, and $ZnCl_2.H_2O$) and 5-Chloro-8-hydroxyquinoline were purchased from S. D. Fine Chemicals Private Limited. 2-aminobenzothiole and 2-hydroxy-3-methoxybenzaldehyde were obtained from Merck Chemicals Limited. All the chemicals used were of AR grade. Solvents used were double distilled and dried using molecular sieves before use.[17]

Melting points or decomposition temperatures of all the synthesized compounds were measured using a simple capillary tube method and are uncorrected. Molar conductance values of all the synthesized complexes were measured by preparing 10^{-3} M solutions in DMF solvent using Equiptronics conductivity meter with an inbuilt magnetic stirrer (Model:Eq-664) at room temperature. Magnetic susceptibilities were determined on the SES Instrument's magnetic susceptibility Gouy's balance (Model: EMU-50) at room temperature using copper (II) sulfate as a standard. IR spectra of complexes were recorded as KBr pellets in the region of 4000–400 cm^{-1} using Shimadzu Spectrophotometer. Electronic spectra were recorded by preparing 10^{-3} M solutions of complexes in DMSO using Shimadzu UV-1800 UV/Visible Scanning spectrophotometer (double beam). The ^1H and ^{13}C NMR spectra were recorded using Bruker 400 MHz spectrometer. The thermal analysis of complexes was done using a Mettler instrument (DTG-60 H detector) with heating rate 10.00 °C/min. The Powder XRD spectra were recorded on an Ultima IV instrument with X-Ray 40 kV/20 mA.

Preparation of Schiff's base ligand (L1)

The Schiff's base ligand (L1) was prepared using the procedure reported earlier[18] with 85% yield.

General procedure for the synthesis of mixed ligand complexes

To the 10 mL aqueous solution of respective metal salts (10 mmol) was added 10 mL hot ethanolic KOH solution of primary Schiff's base ligand (L_1) [0.284 g, 10 mmol] with continuous stirring. This was followed by 10 mL hot ethanolic KOH solution of secondary ligand 5-CHQ (L_2) [0.175 g, 10 mmol]. The reaction mixture was stirred continuously at RT for 4–6 h. The precipitates obtained were filtered off, washed with (1:1) ethanol:water solution, recrystallized from ethanol and dried. Figure 1 depicts the synthesis scheme.

Antimicrobial activity

Antibacterial activity using well plate method

All the synthesized compounds were screened for their antibacterial activities against pathogenic organisms like *E. coli* and *B. subtilis* and the results obtained were compared with standard drug Streptomycin.[19] The inoculums of the microorganism were prepared from the bacterial cultures. 15 mL of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify. 100 μL of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Wells of 6 mm diameter were bored using a sterile cork borer. Solutions of all the compounds (100, 200, 300 μg/mL) in DMSO were prepared. 100 μL of plant extract solutions were added to the wells. The petri plates incubated at 37 °C for 24 h. Streptomycin (1 mg/mL) was prepared as a positive control and DMSO was taken as negative control. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI). All the determinations were performed in triplicate.

Antifungal activity using agar well plate diffusion method

For antifungal study each compound was dissolved in DMSO at a concentration of 5 mg/mL and stored in a refrigerator till further use. Antifungal activities of the compounds were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of Hufford.[20] Sabouraud dextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121 °C for 15 minutes. Twenty 5 mL of molten (45 °C) SDA medium was aseptically transferred into each 100 mm × 15 mm sterile Petri dish. For counting of spore (fungi) were suspended in normal saline to make volume up to 1 mL and then counted with help of heamocytometer (nebular chamber). Once the agar was hardened, 8 mm wells were bored using a sterile cork borer. Then 0.1 mL (100 μL) from each stock solution of the compounds having final concentration of 5 mg/mL was placed in each the well and the plates were incubated for 24 h at 29 °C. Two wells in each petri dish were supplemented with DMSO and reference antifungal drug Clotrimazole (1 mg/mL) dissolved in DMSO serve as negative and positive control respectively. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition.[21]

In vitro semi-quantitative test for screening of anti-malarial activity

A mixture containing 50 μL of 0.5 mg/mL hematin chloride freshly dissolved in 0.1 M NaOH, 100 μL of 0.5 M sodium acetate buffer (pH 4.4), and 50 μL of the synthesized compound potential anti-malarial drug solution and positive control used was chloroquine diphosphate, whereas the negative control distilled water, was put in microtube and incubated at 37 °C for 18 h. The tube was then centrifuged for 8 min at 4000 rpm. The supernatant was removed and the pH of reaction was measured. The final pH of the mixture should be between (5.0–5.2). It is important that the solutions be added to the plate in this order. The solution mixture in the wells were washed with 200 μL DMSO per well to remove free hematin chloride. The plate was centrifuged again, discharging the supernatant afterwards. The β–hematin remaining was then dissolved in 200 μL of 0.1 M NaOH to form an alkaline hematin that can be measured spectrophotometrically. Finally, the absorbance read at 405 nm.[22,23] Lastly percentage inhibition of hematin by compounds was calculated by using following formula (1),

$$\textit{Percent inhibition}\ (\%) = \frac{\textit{Reading of control} - \textit{Reading of treated cells}}{\textit{Readig of control}} \times 100 \quad (1)$$

Antidibetic activity using α-amylase inhibition assay

In vitro amylase inhibition was studied using the method reported by Bernfeld.[24] In brief, 500 μL of the test compound (1 mg/mL) was allowed to react with 500 μL of 0.1 M phosphate buffer pH 6.9 containing α-amylase enzyme [diastage (0.5%)]. After 10 min incubation at 25 °C, 500 μL of 1% starch solution in 0.1 M phosphate buffer (pH 6.8) was added. Then the solution was again incubated at 25 °C for 10 min. The same operation was performed for the controls where 500 μL of the enzyme was replaced by buffer. After incubation, 1000 μL of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 10 min and cooled. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula (2).

$$\textit{Percent inhibition}\ (\%) = \frac{\textit{Abs540 (Control)} - \textit{Abs 540 (Extract)}}{\textit{Abs 540 (Control)}} \times 100 \quad (2)$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

Anticancer activity and cytotoxicity study using MTT assay

All the synthesized compounds were screened for their anticancer activity using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay against human breast cancer cell lines MCF-7 to assess the cytotoxicity.[25,26]

Cells were incubated at a concentration of 1×10^4 cells/mL in culture medium for 24 h at 37 °C and 5% CO_2. Cells were seeded at a concentration (70 μL) 10^4 cells/well in 100 μL culture medium and 100 μL herbal extracts into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37 °C and 5% CO_2 in CO_2 incubator. After incubation the medium was completely removed and added 20 μL of MTT reagent (5 mg/min PBS). After addition of MTT, cells incubated for 4 h at 37 °C in CO_2 incubator. The wells were observed for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only. After removing the medium completely added 200 μL of DMSO (kept for 10 min) and incubated at 37 °C (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by microplate reader at a wavelength of 550 nm.

Antioxidant activity using DPPH radical scavenging activity

All the synthesized compounds were characterized for their antioxidant activity using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity as per the method described[27] with some modifications and compared with ascorbic acid as standard antioxidant compound. 100 μL of

Table 1. Elemental analysis, molar conductance and magnetic moments.

Compound	Elemental analysis Found (Calculated) (%) M	C	H	N	S	Molar conductance ($Ohm^{-1}.cm^2.mol^{-1}$)	Magnetic moment μ_{eff} (B.M.)
$[Co(L_1)(L_2)]$	11.31 (11.32)	55.34 (55.36)	3.15 (3.16)	8.07 (8.08)	6.16 (6.18)	16.05	3.89
$[Ni(L_1)(L_2)]$	11.27 (11.29)	55.37 (55.39)	3.10 (3.11)	8.07 (8.09)	6.16 (6.17)	13.04	3.12
$[Cu(L_1)(L_2)]$	12.09 (12.1)	54.86 (54.87)	3.07 (3.08)	8.00 (8.02)	6.10 (6.11)	14.36	1.91
$[Zn(L_1)(L_2)]$	12.41 (12.45)	54.66 (54.67)	3.06 (3.07)	7.97 (7.98)	6.08 (6.09)	17.86	Diamagnetic

Synthetic compounds with the concentration of 1000 μg/mL was mixed with 100 μL DPPH (0.2 mmol/L in methanol) in 96 well plate, in control only methanol and for standard Ascorbic acid (1000 μg/mL in 100 μL) used. The resultant absorbance was recorded at 515 nm after 30 min. incubation at 37 °C. The percentage of scavenging activity was derived using the following formula (3).[28]

$$Percentage\ inhibition\ (\%) = \frac{A(Control) - A(Sample)}{A(Control)} \times 100 \quad (3)$$

where A(Control) is absorbance of DPPH and A(Sample) is absorbance of reaction mixture (DPPH with Sample).

Results and discussion

The complexes can be represented by general formula [M(L1)(L2)] where M = Co(II), Ni(II), Cu(II), Zn(II), L1 = Schiff's base ligand (E)-2-((benzo[d]thiazol-2-ylimino-methyl)-6-methoxyphenol and L2 = 5-chloro-8-hydroxyquinoline (5-CHQ). All the complexes were obtained in 72–76% yield, were having light yellow to green color, thermally stable and were found to decomposed above 300 °C. All the complexes were soluble in DMF, DMSO and $CHCl_3$. The elemental analysis data along with yield, molar conductance and magnetic susceptibility values recorded for all the synthesized complexes are represented in Table 1.

Molar conductance and magnetic properties

Molar conductance measurements for all the synthesized complexes were carried out by preparing 10^{-3} M solutions in DMF solvent. The low values obtained for all the synthesized complexes indicated non-electrolytic nature of complexes.

The Co(II), Ni(II) and Cu(II) complexes were paramagnetic, while Zn(II) complex was diamagnetic. The magnetic moment value of 3.89 B. M. recorded for Co(II) complex indicated the presence of three unpaired electrons in this complex and is attributed to spin contribution only. Somewhat greater magnetic moment value (3.12 B.M.) than expected (2.89 B.M.) was recorded for Ni(II) complex which can be devoted to the presence of orbital contribution in addition to spin contribution in this complex.[29] The magnetic moment value of 1.91 B.M. observed for Cu(II) complex indicated presence of one unpaired electron while Zn(II) complex was diamagnetic.

IR spectra

IR spectra were recorded in the range 4000–400 cm^{-1} and interpreted using few main peaks. The spectrum of Schiff base ligand (L1) exhibited a weak band at 3520 cm^{-1} due to phenolic (–OH) group. The other band at 3053 cm^{-1} could be attributed to (C-H) of aromatic ring. The main strong band found at 1583 cm^{-1} indicates (C = N) in Schiff base ligand. In the spectrum of 5-chloro-8-hydroxyquinoline (5CHQ) (L2), a broad peak was observed at 3400–3450 cm^{-1} which is attributed to symmetric stretching of (O-H) group. The strong band due to (C = N) at 1607 cm^{-1} was also observed.[9]

The broad peak observed in the range of 3200–3450 cm^{-1} due to phenolic ν(-OH) in ligands was absent in complexes which indicated M-L bonding through oxygen atom of phenolic –OH groups of both these ligands. The band observed in the range 1580–1610 cm^{-1} due to ν(-C = N) in the spectra of ligands was shifted at lower frequency in complexes. This indicated that both these ligands are coordinated with the respective metals through nitrogen of ν(-C = N) group. The peaks observed in the range of 414–453 cm^{-1} and 540–552 cm^{-1} in case of complexes, can be attributed to the stretching vibrations of ν(M-N) and ν(M-O) respectively.[9,18] Two peaks observed at 1638 and 672 cm^{-1} due to ν(C = N) in the thiazole ring of the Schiff Base ligand were unchanged in the spectra of complexes which confirms that the nitrogen and sulfur atoms present in thiazole ring remains uncoordinated.[16]

Electronic spectra

Nujol mull electronic spectra of all the synthesized compounds were recorded. The absorption bands at 221and 261 nm in the spectrum of Schiff base ligand (L1) can be attributed to ($\pi \rightarrow \pi^*$) transition of benzenoid system of benzothiazole moiety. The next bands observed at 271 and 276 nm correspond to ($n \rightarrow \pi^*$) transition in Schiff base ligand (L1).[30] The electronic spectrum of secondary ligand 5-CHQ (L2) exhibited presence of absorption bands at (294, 307 nm) respectively which are attributed to ($\pi \rightarrow \pi^*$) transition and those at λ_{max} (354, 372, 391 nm) can be assigned due to ($n \rightarrow \pi^*$) transition.[18]

Table 2. Powder XRD analysis of complexes.

Complex	Reflexes	2-Theta	Miller indices	(d) Value (A°)	Crystal size (D) (nm)	FWHM
$[Co(L_1)(L_2)$	Peak1	18.36	111	4.82	19.84	0.7077
	Peak2	23.10	210	3.84	05.24	2.6969
	Peak3	29.62	220	3.01	03.52	4.0661
Average crystal size					09.53 nm	
$[Ni(L_1)(L_2)]$	Peak1	16.34	111	5.41	28.87	0.4850
	Peak2	23.20	211	3.82	10.98	1.2877
	Peak3	29.48	310	3.02	11.75	1.2191
Average crystal size					17.20 nm	
$[Cu(L_1)(L_2)]$	Peak1	06.68	111	13.2	20.37	0.6815
	Peak2	11.32	300	7.80	04.87	2.8554
	Peak3	23.02	442	3.85	02.00	7.0507
Average crystal size					09.08 nm	
$[Zn(L_1)(L_2)]$	Peak1	05.20	111	16.98	14.69	0.541
	Peak2	09.06	300	9.75	15.38	0.518
	Peak3	13.94	421	6.34	15.45	0.518
	Peak4	26.06	831	3.41	19.23	0.424
Average crystal size					16.19 nm	

The electronic spectrum of Co(II) complex exhibited three different absorption bands. The first band at 346 nm is attributed to ligand to metal charge transfer (LMCT). The second and third absorption bands observed at 377 and 399 nm respectively can be assigned to $^4A_1 \rightarrow {}^4E_D$ and $^4A_1 \rightarrow {}^4T_{2D}$ transitions respectively. The electronic spectrum of Ni(II) complex exhibited two absorption bands. The one observed at 292 nm can assigned to $^3T_1(F) \rightarrow {}^3T_1(P)$ transition while the second band at 347 nm is attributed to $^3T_1(F) \rightarrow {}^3T_2(F)$ transition respectively. The electronic spectra of Cu(II) complex shows absorption band at λ max 404 nm can be attributed to $^2E_2 \rightarrow T_2$ (d-d) transition this corresponds to tetrahedral geometry of complex. The electronic absorption spectrum of Zn(II) complex do not exhibit d-d transition due to completely filled d orbital and shows absorption band at 423 nm attributed to LMCT transition. The observed electronic data indicated that all the complexes show tetrahedral geometry.[31]

NMR spectra

^{1}H-NMR spectrum

The ^{1}H-NMR spectrum of synthesized Schiff base ligand (L1) was recorded in $CDCl_3$ and Zn (II) complex in dimethylsulfoxide (DMSO) solvent using TMS as internal standard. In the spectrum sharp singlet at $\delta = 3.93$ ppm indicates presence of methoxy proton in the ligand. The signals of protons of aromatic substituted aldehyde ring are observed at $\delta = 6.91$–7.35 ppm while the signals for the aromatic protons of benzothiazole ring was observed at $\delta = 7.39$–7.98 ppm. The sharp signal at $\delta = 9.27$ ppm was due to azomethine proton in Schiff base ligand (L1). The last peak in spectrum at $\delta = 12.46$ ppm due to phenolic –O-H proton of substituted aldehyde molecule.

The ^{1}H-NMR spectrum of Zn(II) complex was compared with parent Schiff base ligand (L1). The –O-H signal at 12.46 ppm in the spectrum of ligand (L1) is completely disappeared in that Zn(II) complex, indicating the involvement of phenolic –OH in the chelation through the displacement of –OH proton. The peak for azomethine proton observed at $\delta = 9.27$ ppm in the spectrum of ligand (L1) is shifted to lower value at $\delta = 8.49$ ppm in that of Zn(II) complex which may be due resonance and nitrogen atom bonded to metal.[32] The singlet observed at $\delta = 3.81$ ppm is due to methoxy proton in Schiff base ligand (L1). The peaks observed in the range $\delta = 6.90$ to 8.09 ppm can be attributed to aromatic protons of the ligand moiety.

^{13}C-NMR spectrum

The ^{13}C-NMR spectrum of synthesized Schiff base ligand (L1) was recorded in $CDCl_3$ solvent using TMS as internal standard. The peak at $\delta = 167.49$ ppm represents the azomethine carbon. The signals at $\delta = 116.69$, 118.38, 119.38, 121.76, 151.48 and 152.11 ppm represents carbon atoms of benzene ring of substituted benzaldehyde while those at $\delta = 123.05$, 125.28, 125.30, 126.72, 134.73 and 148.53 ppm represents carbon atoms from benzene ring of benzothiazole moiety. The signal at $\delta = 168.93$ ppm was due to carbon of (N = C-S) of thiazole ring and finally the signal at 56.29 ppm was due to methoxy carbon (-OCH_3) of substituted benzaldehyde. Thus both these NMR spectra provide sufficient evidences to confirm the proposed structure of synthesized Schiff base ligand (L1) molecule.

Powder XRD analysis

The nature of synthesized mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) was studied by powder x-ray diffraction method. The obtained results indicate microcrystalline nature in these complexes. The inter planner spacing (d) is calculated by using Brag's equation (4),

$$n\lambda = 2d \sin\theta \tag{4}$$

The mean particle size of complexes was calculated using Scherer's formula (5),

$$D = \frac{K\lambda}{\beta \cos\theta} \tag{5}$$

where K is constant and usually taken as 0.9, D is particle size, λ is wavelength of x-ray radiation, β is Full Width half maximum and θ is diffraction angle.[33] The obtained results are represented in Table 2.

Thermal analysis

The TGA/DTA curves of synthesized mixed ligand complexes were recorded. The experiments were carried out in a nitrogen atmosphere with heating rate of 10.00 K/min and in temperature range 20–800 °C using alumina crucible.

TGA: From TGA curves it was observed that all the complexes were thermally stable up to 140 °C. No weight loss occurs in this region indicating absence of any water molecule bonded to the metal atoms.[34] The TGA curves of Co(II), Ni(II) and Zn(II) complexes exhibited single weight loss step while that of Cu(II) complex exhibited two weight loss steps.

The major weight loss of 79.12% for Co(II) complex in the temperature range of 351–785 °C, of 76.08% for Ni(II) complex in the temperature range of 334–690 °C, and of 85.30% for Zn(II) complex in the temperature range of

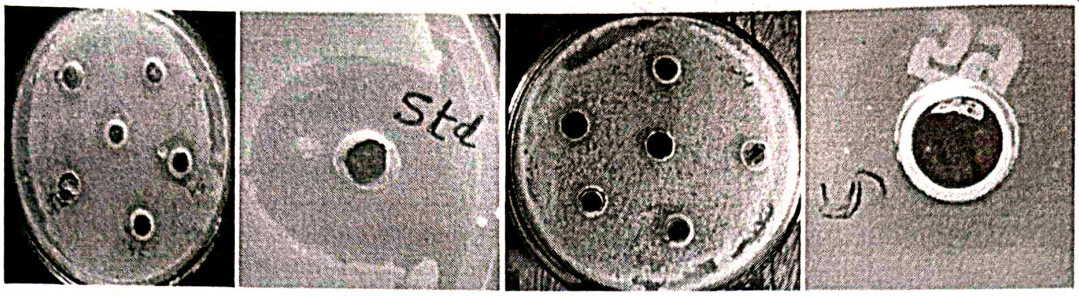

Figure 2. Antibacterial activity (a, b) against *E. coli* and (c, d) against *B. Subtilis*.

Table 3. Antimicrobial activity.

		Zone of inhibition (mm)			
		Antibacterial activity		Antifungal activity	
Compound	Concentration (mg/mL)	*E. coli*	*B. Subtilis*	*C. albicans*	*A. niger*
SB (L_1)	5	10	15	08	09
$[Co(L_1)(L_2)]$	5	25	20	07	07
$[Ni(L_1)(L_2)]$	5	14	13	00	00
$[Cu(L_1)(L_2)]$	5	12	10	00	00
$[Zn(L_1)(L_2)]$	5	20	16	05	07
Standard (streptomycin)	1	31	32	–	–
Standard (Clotrimazole)	1	–	–	14	13

372–777 °C was observed. This major weight loss in all three complexes can be attributed to the decomposition of both the organic ligands. In case of Cu(II) complex, the first weight loss of 25.77% is observed in the temperature range 210–403 °C which can be attributed to decomposition of heterocyclic ligand (5-Cl-8-HQ). The second weight loss of 71.66% is observed in the temperature range 403–763 °C which can attributed to decomposition of Schiff base organic ligand (L1). The remaining weight of final residues corresponds to the percentage of metal oxides remained.[35]

DTA: The DTA curves of Co(II), Ni(II) and Zn(II) complexes exhibited two peaks. The first endothermic peak at 160 °C for Co(II) and 145 °C for Ni(II) complexes owing to crystalline rearrangement process occurring. The second sharp exothermic peaks observed in the range 400–600 °C for Co(II) and Ni(II) complexes were due to decomposition of organic ligands.

In case of Zn(II) complex two exothermic peaks are observed in the range 500–700 °C which indicated complete decomposition of organic ligands. The DTA curve of Cu(II) complex exhibited only one broad exothermic peak in the range 380–570 °C owing to complete decomposition of organic ligands.

Antimicrobial activity

Antibacterial activity using well plate method

All the synthesized compounds were screened for their antibacterial activities against pathogenic organism like *E. coli* and *B. subtilis* and the results were compared with standard drug Streptomycin. The results are represented in Figure 2(a)–(d).

All the four complexes were found to be more potent as compared to the Schiff base ligand (L1). The enhanced activity in complexes as compared to ligand (L1) can be explained based on Overtone's concept and chelation theory.[36] As per chelation theory the polarity of metal ion decreases on complexation owing to sharing of its positive charge with donor groups and possible π-electron delocalization over the whole chelate ring. This results in increased lipophilic character the central metal atom which favors the cell permeation and increase the activity.[36]

The Co(II) and Zn(II) complexes shown good results but Ni(II) and Cu(II) complexes exhibited moderate activity for both the species. The complexes were found to be more potent than Schiff base ligand (L1).

Antifungal activity using agar well plate diffusion method

All the synthesized compounds were screened for their antifungal activity against *C. albicans* and *A. niger* fungal pathogens and the results were compared with the standard Clotrimazole. The results obtained were not much encouraging in cases of antifungal activity measurements. The Schiff base ligand (L1) and Co(II) complex exhibited moderate antifungal activity whereas Zn(II) complex exhibited poor to moderate activity. The Ni(II) and Cu(II) complexes were inactive. Table 3 represents the results obtained from antimicrobial screening.

Antimalarial activity

Figure 3 represents graphical representation of results obtained from antimalarial activity for all the synthesized mixed ligand complexes.

All the synthesized compounds exhibited antimalarial activities close to the standard used. The Co(II), Ni(II) and Zn(II) complexes exhibited 65.71, 65.96, and 64.62% inhibition values respectively which are very close to the standard value 68.90% and thus exhibited excellent. Thus these can act as potential antimalarial agents. The Cu(II) complex exhibited 56.89% inhibition value which is less than the Schiff base ligand (L1) (58.99%). Overall all the synthesized compounds exhibited good to excellent antimalarial activity and can be considered as potential antimalarial agents.

Antidibetic activity using α-amylase inhibition assay

Table 4 represents the results obtained from antidibetic activity evaluation. The Schiff base ligand (L1) exhibited percent inhibition value of 60.60% which is greater than Co(II), Cu(II) and Zn(II) complexes (53.53, 56.06 and 54.54% inhibition respectively). The Ni(II) complex was able to show greater inhibition as compared to Schiff base ligand (L1) i.e., 62.12%. The Standard acarbose exhibited percent inhibition value of 80.80%. All the synthesized compounds were able to show good α-amylase inhibition activity in comparison to the standard used.

Anticancer activities and cytotoxicity study using MTT assay

Anticancer activity measurements shed light on ability of compound to control abnormal growth of cells. MTT assay is widely accepted and most reliable method for measuring cell proliferation, cell viability or cytotoxicity.[37] The results obtained from antibacterial measurements indicated complexes are more active than the Schiff base ligand (L1) which prompted us to carry out anticancer activity of complexes against human breast cancer cell line MCF-7 using MTT assay. Figure 4 represents graphical representation of results obtained from anticancer activity and cytotoxicity study using MTT assay.

The IC_{50} values above 100 μM are noted for all the synthesized compounds while the standard drug 5-Fluorouracil (5-FU) exhibited IC_{50} value of 60.76 μM. Thus all the compounds exhibited moderate to good anticancer activity against human breast cancer cell line MCF-7. The cell viability values indicated that these complexes are more toxic to cancer cells than normal cells. Considering all these observed facts all these complexes could be considered as potential anticancer agents.

Antioxidant activity

All the synthesized compounds were screened for their antioxidant activity using DPPH method and results compared with standard ascorbic acid. The Zn(II) complex shown good antioxidant activity with 63.21% inhibition as compared to the standard used (97.79%). The Schiff base ligand (L1) recorded moderate activity with 51.76% inhibition. Remaining compounds were inactive.

Conclusions

All the mixed ligand complexes were proposed to have tetrahedral geometry. All the synthesized compounds were screened for their antimicrobial, antimalarial, antidibetic, anticancer and antioxidant activities. Based on the results

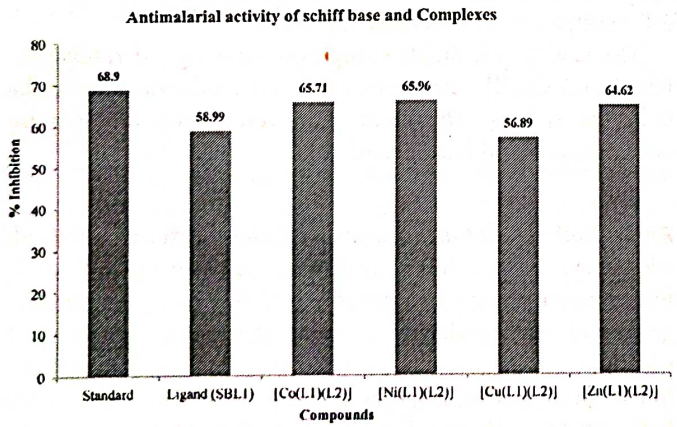

Figure 3. Graphical representation of antimalarial activity.

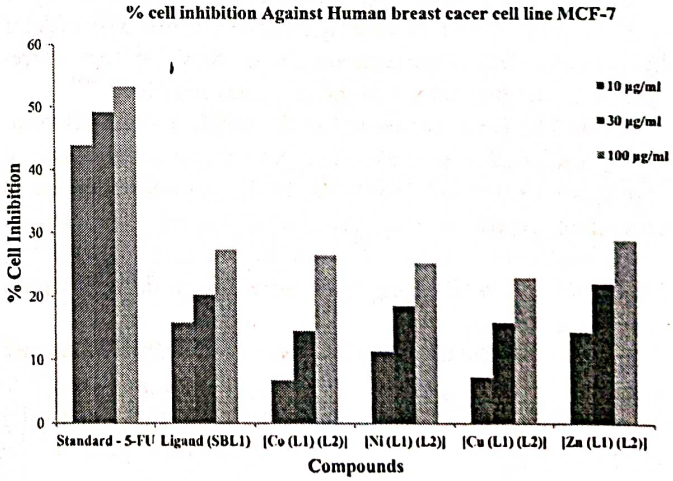

Figure 4. Graphical representation of anticancer activity.

Table 4. Antidibetic activity i.e., percent inhibition of α-amylase.

Compound	Concentration (μg/mL)	ABS at 540 nm	Percentage inhibition (%)
Control	–	1.98	–
SB (L_1)	1000	0.78	60.60
[Co(L_1)(L_2)]	1000	0.92	53.53
[Ni(L_1)(L_2)]	1000	0.75	62.12
[Cu(L_1)(L_2)]	1000	0.87	56.06
[Zn(L_1)(L_2)]	1000	0.90	54.54
Standard-Acarbose	1000	0.38	80.80

obtained all the complexes can be proposed to have potential antimalarial, antidibetic and anticancer activities and are good candidates for future drugs.

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References

1. Jevtovic, V.; Ivkovic, S.; Kaisarevic, S.; Kovacevic, R. Anticancer Activity of New Copper (II) Complexes Incorporating a Pyridoxal-Semicarbazone Ligand. *Contemp. Mater.* **2010**, *1*, 133–137. DOI: 10.5767/anurs.cmat.100102.en.133J.
2. Zhang, H.; Yuetao, Y.; Dawei, F.; Yipeng, W.; Song, Q. Hypoglycemic Properties of Oxovanadium (IV) Coordination Compounds with Carboxymethyl-Carrageenan and Carboxymethyl-Chitosan in Alloxan-Induced Diabetic Mice. *Evid.-Based Complement. Alternat. Med.* **2011**, *2011*, 1–7. DOI: 10.1155/2011/691067.
3. Mariappan, G.; Saha, B. P.; Datta, S.; Kumar, D.; Haldar, P. K. Design, Synthesis and Antidiabetic Evaluation of Oxazolone Derivatives. *J. Chem. Sci.* **2011**, *123*, 335–341. DOI: 10.1007/s12039-011-0079-2.
4. Shukla, S. N.; Gaur, P.; Jhariya, S.; Chaurasia, B.; Vaidya, P.; Dehariya, D.; Azam, M.; Synthesis, C. In Vitro Anti-Diabetic, Antibacterial and Anticorrosive Activity of Some Cr(III) Complexes of Schiff Bases Derived from Isoniazid. *Chem. Sci. Trans.* **2018**, *7*, 424–444. DOI: 10.7598/cst2018.1509.
5. Singh, V. Metal Complexes as Antimalarial Potential: A Review. *Pharm. Innov. J.* **2019**, *8*, 403–406.
6. Pinheiro, L. C. S.; Feitosa, L. M.; Silveira, F. F. D.; Boechat, N. Current Antimalarial Therapies and Advances in the Development of Semi-Synthetic Artemisinin Derivatives. *An. Acad. Bras. Cienc.* **2018**, *90*, 1251–1271. DOI: 10.1590/0001-3765201820170830.
7. Numan, A. T.; Atiyah, E. M.; Al-Shemary, R. K.; Abd_Ulrazzaq, S. S. Composition, Characterization and Antibacterial Activity of Mn(II), Co(II), Ni(II), Cu(II) Zn(II) and Cd(II) Mixed Ligand Complexes Schiff Base Derived from Trimethoprim with 8-Hydroxy Quinoline. *J. Phys.: Conf. Ser.* **2018**, *1003*, 012016. DOI: 10.1088/1742-6596/1003/1/012016.
8. Matangi, S.; Pragathi, J.; Bathini, U.; Gyana Kumari, C. Synthesis, Characterization and Antimicrobial Activity of Transition Metal Complexes of Schiff Base Ligand Derived from 3-Ethoxy Salicylaldehyde and 2-(2-Aminophenyl) 1-H-Benzimidazole. *E-J. Chem.* **2012**, *9*, 2516–2523. DOI: 10.1155/2012/287909.
9. Rasheed, R. T. Synthesis of New Metal Complexes Derived from 5-Nitroso-8-Hydroxy Quinoline and Salicylidene P-Imino Acetophenone with Fe (II), Co (II), Ni (II) and Cu (II) Ions. *JNUS.* **2012**, *15*, 68–73. DOI: 10.22401/JNUS.15.4.08.
10. Vinusha, H. M.; Kollur, S. P.; Revanasiddappa, H. D.; Ramu, R.; Shirahatti, P. S.; Nagendra Prasad, M. N.; Chandrashekar, S.; Begum, M. Preparation, Spectral Characterization and Biological Applications of Schiff Base Ligand and Its Transition Metal Complexes. *Results Chem.* **2019**, *1*, 100012. DOI: 10.1016/j.rechem.2019.100012.
11. Mohapatra, R. K.; Saikishore, V. P.; Azam, M.; Biswal, S. K. Synthesis and Physicochemical Studies of a Series of Mixed Ligand Transition Metal Complexes and Their Molecular Docking Investigations against Coronavirus Main Protease. *Open Chem.* **2020**, *18*, 1495–1506. DOI: 10.1515/chem-2020-0190.
12. Abou Melha, K. S.; Al-Hazmi, G. A.; Althagafi, I.; Alharbi, A.; Keshk, A. A.; Shaaban, F.; El-Metwaly, N. Spectral, Molecular Modeling, and Biological Activity Studies on New Schiff's Base of Acenaphthaquinone Transition Metal Complexes. *Bioinorg. Chem. Appl.* **2021**, *2021*, 6674394. DOI: 10.1155/2021/6674394.
13. Mahmoud, W. H.; Mohamed, G. G.; El Sayed, O. Y. Coordination Compounds of Some Transition Metal Ions with New Schiff Base Ligand Derived from Dibenzoyl Methane. Structural Characterization, Thermal Behavior, Molecular Structure, Antimicrobial, Anticancer Activity and Molecular Docking Studies. *Appl. Organomet. Chem.* **2017**, *32*, 4051. DOI: 10.1002/aoc.4051.
14. Devi, J.; Batra, N. Synthesis, Characterization and Antimicrobial Activities of Mixed Ligand Transition Metal Complexes with Isatin Monohydrazone Schiff Base Ligands and Heterocyclic Nitrogen Base. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.* **2015**, *135*, 710–719. DOI: 10.1016/j.saa.2014.07.041.
15. Mumtaz, A.; Mahmud, T.; Elsegood, M. R.; Weaver, G. W. Synthesis and Characterization of New Schiff Base Transition Metal Complexes Derived from Drug Together with Biological Potential Study. *J. Nucl. Med. Rad. Ther.* **2016**, *7*, 1000310. DOI: 10.4172/2155-9619.1000310.
16. Saadeh, H. A.; Sweidan, K. A.; Mubarak, M. S. Recent Advances in the Synthesis and Biological Activity of 8-Hydroxyquinolines. *Molecules.* **2020**, *25*, 4321. DOI: 10.3390/molecules25184321.
17. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Group: England, **1989**.
18. Devi, J.; Kumari, S.; Malhotra, R. Spectroscopic Studies, and Biological Activity of Organosilicon(iv) Complexes of Ligands Derived from 2-Aminobenzothiazole Derivatives and 2-Hydroxy-3-Methoxy BenzaldehydePhosphorus. *Sulfur Silicon Relat. Elem.* **2012**, *187*, 587–597. DOI: 10.1080/10426507.2011.634465.
19. Moonmun, D.; Majumder, R.; Lopamudra, A. Quantitative Phytochemical Estimation and Evaluation of Antioxidant and Antibacterial Activity of Methanol and Ethanol Extracts of Heliconia Rostrata. *Ind. J. Pharma. Sci.* **2017**, *79*, 79–90. DOI: 10.4172/pharmaceutical-sciences.1000204.
20. Hufford, C. D.; Funderburk, M. J.; Morgan, J. M.; Robertson, L. W. Two Antimicrobial Alkaloids from Heartwood of Liriodendron Tulipifera L. *J. Pharm. Sci.* **1975**, *64*, 789–792. DOI: 10.1002/jps.2600640512.
21. Umadevi, S.; Mohanta, G. P.; Chelladurai, V.; Manna, P. K.; Manavalan, R. Antibacterial and Antifungal Activity of Andrographis Echiodes. *J. Nat. Remedies.* **2003**, *3*, 185–188.
22. Amolegbe, S. A.; Adewuyi, S.; Akinremi, C. A.; Adediji, J. F.; Lawal, A.; Atayese, A. O.; Obaleye, J. A. Iron(III) and Copper(II) Complexes Bearing 8-Quinolinol with Amino-Acids Mixed Ligands: Synthesis, Characterization and Antibacterial Investigation. *Arabian J. Chem.* **2015**, *8*, 742–747. DOI: 10.1016/j.arabjc.2014.11.040.
23. Jaber, S.; Saleh, A. L.; Lutgen, P.; Quto, B. M.; Abu-Remeleh, Q.; Akkawi, M. Bicarbonate In-Vitro Effect on Beta-Hematin Inhibition by Artemisia Sieberi Aqueous Infusion. *J. Pharma. Pharmacol.* **2015**, *3*, 63–72. DOI: 10.17265/2328-2150/2015.02.003.
24. Bernfeld, P. Amylase Alpha and Beta. *Enzymology.* **1955**, *1*, 149–158. DOI: 10.1016/0076-6879(55)01021-5.
25. Horiuchi, N.; Nakagawa, K.; Sasaki, Y.; Minato, K.; Fujiwara, Y.; Nezu, K.; Ohe, Y.; Saijo, N. In Vitro Antitumor Activity of Mitomycin C Derivative (RM-49) and New Anticancer Antibiotics (FK973) against Lung Cancer Cell Lines Determined by Tetrazolium Dye (MTT) Assay. *Cancer Chemother. Pharmacol.* **1988**, *22*, 246–250. DOI: 10.1007/BF00273419.
26. Senthilraja, P.; Kathiresan, K. In Vitro Cytotoxicity MTT Assay in Vero, HepG2 and MCF-7 Cell Lines Study of Marine Yeast. *J.*

Appl. Pharm. Sci. **2015**, *5*, 080–084. DOI: 10.7324/JAPS.2015.50313.

27. Kumar, H.; Javed, S. A.; Khan, S. A.; Mohammad, A. 1,3,4-Oxadiazole/Thiadiazole and 1,2,4-Triazole Derivatives of Biphenyl-4-Yloxy Acetic Acid: Synthesis and Preliminary Evaluation of Biological Properties. *Euro. J. Med. Chem.* **2008**, *43*, 2688–2698. DOI: 10.1016/j.ejmech.2008.01.039.
28. Duan, X. J.; Zhang, W. W.; Li, X. M.; Wang, B. G. Evaluation of Antioxidant Property of Extract and Fractions Obtained from a Red Alga, Polysiphonia Urceolata. *Food Chem.* **2006**, *95*, 37–43. DOI: 10.1016/j.foodchem.2004.12.015.
29. Podunavac-Kuzmanovic, S. O.; Markov, S. L.; Vojinovic, L. S. Physico-Chemical Characterization and Anti-Microbial Activity of Copper(II) Complexes with 2-Amino and 2-Methylbenzimidazole Derivatives. *Acta Period. Technol.* **2004**, *35*, 247–254. DOI: 10.2298/APT0435247P.
30. Etaiw, S. E. H.; Abd El-Aziz, D. M.; Abd El-Zaher, E. H.; Ali, E. A. Synthesis, Spectral, Antimicrobial and Antitumor Assessment of Schiff Base Derived from 2-Aminobenzothiazole and Its Transition Metal Complexes. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2011**, *79*, 1331–1337. https://doi.org/10.1016/j.saa2011.04.064. DOI: 10.1016/j.saa.2011.04.064.
31. Mohan, M.; Veena, V.; Kezo, S.; Reddy, K. R. Synthesis, Characterization, Cytotoxic and Antioxidant Studies of Mixed Ligand Schiff Base Complexes Derived from P-Nitroaniline and 2, 4-Dinitroaniline. *Int. J. Pharma. Sci. Res.* **2019**, *10*, 5025–5031. DOI: 10.13040/IJPSR.0975-8232.10(11).5025-31.
32. Kerim, S.; Arzu, O.; Yasemin, U.; Mustafa, E.; Ismail, D.; Kemal, S. Spectroscopic and Theoretical Study of 1,2,4-Triazole-3-One Based Salicylaldimine Complexes and Evaluation of Superoxide-Scavenging Properties. *J. Mol. Struct.* **2009**, *922*, 1–10. DOI: 10.1016/j.molstruc.2009.02.001.
33. Alazawi, S. A. S.; Alhamadani, A. A. S. Synthesis and Characterization of Mixed Ligand Complexes of 8-Hydroxyquinoline and Schiff Base with Some Metal Ions. *Um-Salama Sci. J.* **2007**, *4*, 102–109.
34. Abbas, S. H.; Abbas, H. H.; Musa, H. K. Synthesis, Characterization, Kinetic and Thermodynamic Evaluation from TG-DTA Analysis of New Nickel(II) Mixed Ligand Complexes of Bidentate Salicylaldimine Schiff Bases and 1,10-Phenanthroline. *Bulgarian Chem. Commun.* **2020**, *52*, 68–75. DOI: 10.34049/bcc.52.1.5095.
35. Zhang, Y. H.; He, S. S.; Xie, S. Y.; Wei, P. L.; Wang, S. Z.; Liao, G. H. Two Cu(II) and Co(II) Coordination Polymers: Crystal Structures and Treatment Activity on Human Ovarian Cancer Cells. *Inorg. Nano-Metal Chem.* **2021**, *51*, 188–194. DOI: 10.1080/24701556.2020.1769669.
36. Tweedy, B. G. Plant Extracts with Metal Ions as Potential Antimicrobial Agents. *Phytopathology.* **1964**, *55*, 910–914.
37. Nagaraj, R.; Murugesan, S.; Jegathalaprathaban, R.; Jeyaraj, D. R. Biologically Active Cu(II), Co(II), Ni(II) and Zn(II) Complexes of Pyrimidine Derivative Schiff Base: DNA Binding, Antioxidant, Antibacterial and In Vitro Anticancer Studies, *J. Fluoresc.* **2017**, *27*, 1801–1814. DOI: 10.1007/s10895-017-2118-y.

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