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ORIGINAL PAPER

Novel Schiff base (E)-2-((4-chloro-3-nitrophenylimino)(phenyl) methyl)-5-methoxyphenol and Mixed Ligand Complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II): synthesis, structure elucidation and potency study as antibacterial, antimalarial, antioxidant, antidibetic and anticancer agents

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Abstract

Novel Schiff Base (E)-2-((4-chloro-3-nitrophenylimino)(phenyl)methyl)-5-methoxyphenol (S_1) synthesized by condensing 2-hydroxy-4-methoxy benzophenone and 4-chloro-3-nitroaniline in ethanol and used for synthesis of five new mixed ligand complexes of Mn(II) Fe(III), Co(II), Ni(II), and Cu(II). The synthesized Schiff base ligand (S_1) has been characterized by IR, UV–Visible, ^{1}H-NMR, ^{13}C-NMR spectra and all the synthesized complexes were characterized by elemental analysis, IR, electronic, thermal methods (TGA-DTA), Powder XRD analysis, magnetic susceptibility and molar conductivity measurements. All the complexes were proposed to have octahedral geometry. All the synthesized compounds were screened for their antimicrobial, antidibetic, antioxidant, antimalarial and anticancer activity. The obtained results indicated towards potential of these complexes as antimalarial and antioxidant agents.

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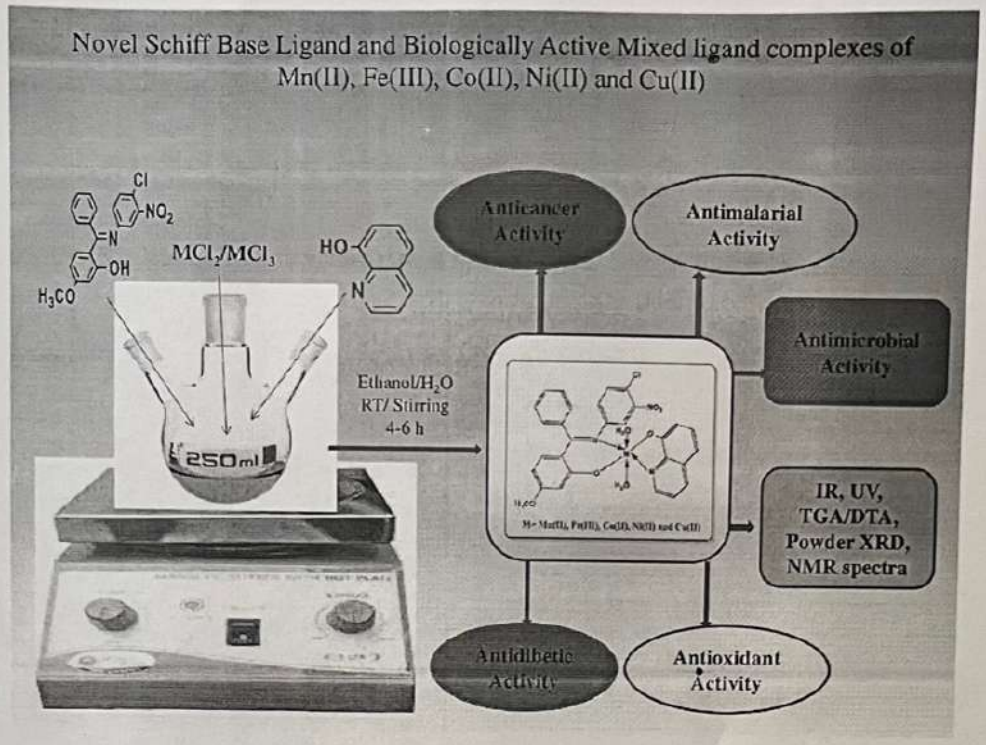
Graphical Abstract



Keywords Anticancer · Antidibetic · Antimalarial · Antioxidant · 8-hydroxyquinoline · Powder XRD

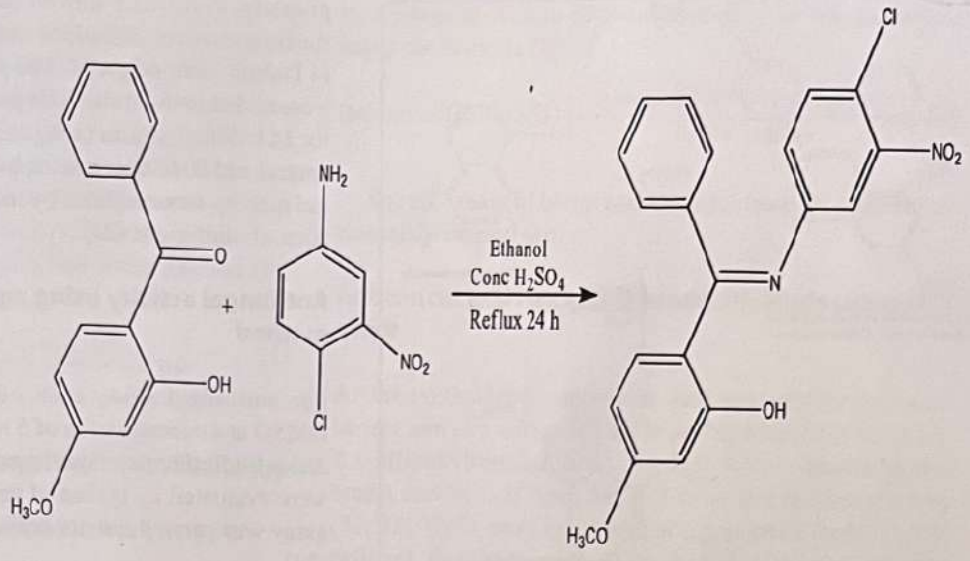
Introduction

Mixed ligand complexes has attracted great attention these days owing to their ease of formation, simple work up procedure, high yield and various applications [1, 2]. The therapeutic and diagnostic properties of transition metal complexes have attracted attention leading to their applications in many areas of modern medicine [3]. Schiff base complexes have suitable biomimetic properties that can mimic the structural features of active sites, and they have been widely used in various fields such as biochemical reactions and biological regulation [4]. The antimicrobial [5–12] and anticancer activities [13] of Schiff base ligands and their metal complexes have been extensively studied.

Cancer, diabetes and malaria are serious health concerns in countries like India and hence the primary targets of medicinal chemistry research. Cancer is a group of diseases involving abnormal cell growth which can spread to other body parts. The platinum-based complex 'Cisplatin', owing to its anticancer properties, has attracted researchers to work in this area. Still the research is ongoing in order to find different metal complexes with less side effects and similar or better cytotoxicity [14]. Diabetes resulting from insulin deficiency or insulin resistance is a serious chronic disorder around the world [15–17]. Two types of situations are identified regarding this disease viz. type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes. Although various drugs are used to treat non-insulin dependent diabetes, the complications involved with this disease such as kidney failure, micro-and macrovascular disease, retinopathy, neuropathy and atherosclerosis has created an urgent need for the search of orally active drugs [15–17]. Malaria is one of the most infectious diseases affecting to health and developmental growth of developing countries [18]. 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. The antimalarial potential of Schiff base ligands and their metal complexes is well known [19]. Transition metal Schiff base complexes are attractive catalysts in the oxidation of a variety of organic compounds because of their low cost, easy preparation, and chemical and thermal stability [20, 21].

In present investigation we report synthesis of novel Schiff base (S_1) using condensation reaction of 2-hydroxy-4-methoxybenzophenone with 4-chloro-3-nitroaniline. The Schiff base was further used to synthesis five mixed ligand complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) using 8-hydroxyquinoline (HQ) as secondary ligand. All the

Scheme 1 Synthesis of Schiff base ligand (S_1)



2-hydroxy-4-methoxy benzophenone

(*E*)-2-(((4-chloro-3-nitrophenyl)imino) (phenyl)methyl)-5-methoxyphenol

synthesized compounds were characterized using elemental analysis, spectral, thermal methods, Powder XRD analysis, molar conductance and magnetic susceptibility measurements. All the compounds were screened for their biological activities such as anticancer, antidibetic, antimalarial, antimicrobial and antioxidant activities.

Experimental

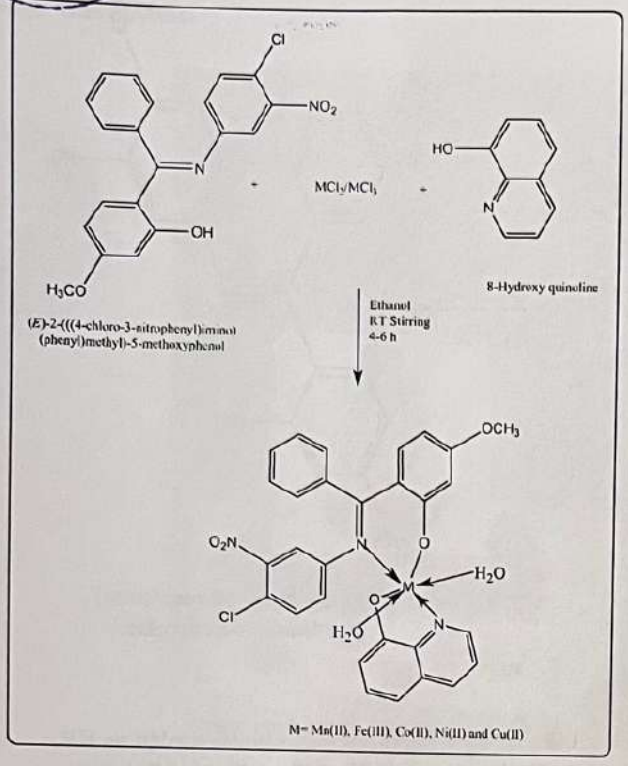
Materials and methods

Metal salts; ($MnCl_2.2H_2O$, $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$,) and 8-hydroxyquinoline were purchased from S.D. Fine Chemicals Private Limited. 2-hydroxy-4-methoxybenzophenone and 4-chloro-3-nitroaniline 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 [22].

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) sulphate as a standard. IR spectra of complexes were recorded as KBr pellets in the region of 4000–400 cm^{-1} on a Shimadzu and Bruker spectrophotometers. Electronic spectra were recorded by preparing 10^{-3} M solutions of complexes in DMSO using Shimadzu UV-1800 UV/Visible Scanning spectrophotometer (double beam). ^{1}H-NMR spectrum of Schiff Base ligand was recorded using Bruker 400 MHz spectrometer. The thermal analysis of complexes were done using a DTG-60H detector with (TGA 1 SF/1100/358, 04.02.2016 17:33:31) module with heating rate 10.00 k/min. The Powder XRD spectra were recorded on an Ultima IV instrument with X-Ray 40 kV/20 mA.

Synthesis of Schiff's base ligand (S1)

A hot ethanolic solution (20 mL) of 2-hydroxy-4-methoxybenzophenone (2.28 g, 10 mmol) was added drop wise into an ethanolic solution (20 mL) of 4-chloro-3-nitroaniline (0.93 g, 10 mmol). Catalytic amount of conc. H_2SO_4 was added to the resultant mixture and then refluxed for 24 h under stirring. The progress of reaction was monitored by TLC at regular interval of time. The resultant hot solution was reduced to 1/3 of its original volume and kept aside at RT for cooling and then the reaction mixture was poured on crushed ice. A yellow crystalline solid product obtained was separated, filtered with Whatmann paper under suction, washed with cold water and ethanol and then recrystallized from the same solvent. (M.p. = 48–50 °C, Yield: 83%) (Scheme 1).



Scheme 2 Synthesis of mixed ligand complexes

General procedure for synthesis of mixed ligand complexes

To the aqueous ethanolic solution of the metal chloride salts (10 mmol) [Mn(II) (0.195 g), Fe(III) (0.326 g), Co(II) (0.286 g), Ni(II) (0.285 g) and Cu(II) (0.204 g)] was added drop wise a mixture of an ethanolic solution of Schiff's base ligand (S_1) [0.382 g, 10 mmol (20 mL)] and 8-hydroxyquinoline (HQ) [0.175 g, 10 mmol (20 ml)] in 1:1:1 molar ratio. The reaction mixture was stirred continuously at RT for 4-6 h. The complexes precipitated were washed with ethanol, filtered and dried. All the complexes were recrystallized from ethanol. Scheme 2 represents the synthesis of the mixed ligand complexes.

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 [23]. 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 in diameter were bored using a sterile cork borer. Solutions of all the compounds 1 mg/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).

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 [24].

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 min. 25 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 (neubar 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 of 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 [25].

Anti-malarial activity

In vitro semi-quantitative test for screening of antimalarial 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 was 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 was read at 405 nm [26, 27]. Lastly percentage inhibition of hematin by compounds was calculated using following formula (1),

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

Antidibetic activity using α-amylase inhibition assay

Importance alpha-amylase enzyme in the body

In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic alpha amylases into maltose, maltotriose and small maltooligosaccharides. The digestive enzyme (alpha-amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post-prandial hyperglycemia in diabetic condition [28, 29]. Treatment of diabetes include: improvement of the activity of insulin at the objective tissues, with the utilization of sensitizers (biguanides, thiozolidinediones); incitement of endogenous insulin discharge with the utilization of sulfonylureas (glibenclamide, glimepiride), and decrease of the interest for insulin utilizing particular enzyme inhibitors (**acarbose**, miglitol).

Assay of amylase inhibition

In vitro amylase inhibition was studied using the method reported by Bernfeld [30]. 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 (fungal 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).

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

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-diphenyltetrazolium bromide] assay against human breast cancer cell lines MCF-7 to assess the cytotoxicity [31, 32]. 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 microplates, 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 coloured 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 aluminium foil). Triplicate samples were analyzed by measuring the absorbance of each sample by microplate reader at a wavelength of 550 nm.

Antioxidant property 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 [33] with some modifications and compared with ascorbic acid as standard antioxidant compound. 100 µL of 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 calculated [34] using the formula (3),

Table 1 Elemental analysis, molar conductance and magnetic moments

Complex	Colour	Elemental analysis Found (Calculated) (%)				Molar conductivity (Ohm^{-1} cm^2 mol^{-1})	Magnetic susceptibility μ_{eff} (B.M.)
		M	*C*	*H*	*N*		
C_1 $C_{29}H_{24}Cl\,N_3O_7Mn$	Amber yellow	8.91 (8.90)	56.46 (56.48)	3.92 (3.90)	6.81 (6.82)	12.5	5.38
C_2 $C_{29}H_{24}Cl_2\,N_3O_7Fe$	Dark brown	9.04 (9.06)	56.38 (56.40)	3.92 (3.91)	6.80 (6.78)	76	5.32
C_3 $C_{29}H_{24}Cl\,N_3O_7Co$	Charcoal grey	9.49 (9.47)	56.10 (56.13)	3.90 (3.89)	6.77 (6.78)	9.54	4.39
C_4 $C_{29}H_{24}Cl\,N_3O_7Ni$	Seafoam green	9.46 (9.44)	56.12 (56.13)	3.90 (3.88)	6.77 (6.76)	13.6	3.10
C_5 $C_{29}H_{24}Cl\,N_3O_7Cu$	Olive green	10.16 (10.14)	55.68 (55.70)	3.87 (3.89)	6.72 (6.73)	11.8	1.85

$$\text{Percent inhibition}(\%) = \frac{[A(\text{control}) - A(\text{sample})]}{A(\text{control})} \times 100 \quad (3)$$

where A(control) is absorbance of DPPH and A(sample) is absorbance of reaction mixture (DPPH with sample).

Results and discussion

All the complexes were obtained in 68–72% yield, were thermally stable and found to be decomposing above 295 °C. The complexes were partially soluble in methanol and completely soluble in DMF, DMSO.

Magnetic susceptibility

The Mn(II) and Fe(III) complexes exhibited μ_{eff} values of 5.38 and 5.32 B.M. indicating presence of five unpaired electrons in these complexes. The Co(II) and Ni(II) complexes exhibited μ_{eff} values of 4.39 and 3.10 B.M. The higher than expected value in these complexes indicated presence of orbital contribution in addition to spin contribution in these complexes [35]. The Cu(II) complex exhibited expected value of 1.85 B.M. indicating presence of one unpaired electron.

Molar conductance

The observed low molar conductance values (9.54–13.60 Ohm^{-1} cm^2 mol^{-1}) for Mn(II), Co(II), Ni(II) and Cu(II) complexes indicated their non-electrolytic nature [36]. However, the high value (76 Ohm^{-1} cm^2 mol^{-1}) recorded for Fe(III) complex indicated 1:1 electrolytic nature [37]. The elemental analysis data along with colour, molar conductance and magnetic susceptibility values recorded for all the synthesized complexes are represented in Table 1.

IR Spectra

IR spectra for complexes were compared with those of ligands considering few prominent peaks. IR spectrum of primary ligand, i.e. free Schiff base (S_1) exhibited strong band around at 1660 cm^{-1} and a broad band in the range 3445–3489 cm^{-1} which indicated the presence of ketimine ν(C=N) and phenolic-OH groups, respectively. A peak for aromatic asymmetric C–H at 2995 cm^{-1} was also observed [13]. In secondary ligand 8-hydroxyquinoline, two characteristic peaks at 1605 and 3450 cm^{-1} were observed which can be assigned to presence of ν(C=N) and ν(OH) vibrations, respectively [38].

In the IR spectra of complexes, the peaks for ketimine ν(C=N) group is shifted towards lower frequency at 1627–1635 cm^{-1} and the phenolic –OH broad band at 3445–3489 cm^{-1} was disappeared indicating N atom of ketimine and deprotonated phenolic-O atoms both are involved in coordination with the metal atom with reference to Schiff's base primary ligand. Similar observations were also noted for 8-hydroxyquinoline ligand as the band for ν(C=N) was shifted to lower frequency 1591–1598 cm^{-1} and the peak observed for phenolic –OH was disappeared in IR spectra of all the complexes indicating the ligand coordinated to metal(II) ion in a bidentate manner via imine-N and deprotonated phenolic-O atoms, respectively [38].

The C–O stretching bands, in case of the Schiff base ligands (S_1) at 1310 cm^{-1} and 8-hydroxyquinoline at 1284 cm^{-1} was shifted to higher wave numbers at 1322 cm^{-1} to at 1382 cm-1 after complexation [39–41].

The appearance of weak to medium intensity bands in far-IR region in the ranges 409–460 and 510–607 cm^{-1} which correspond to ν(M–O) and ν(M–N) modes, respectively, [42] further confirmed the metal–ligand binding. All the mixed ligand complexes exhibited an additional broad peak in the range 3200–3351 cm^{-1} due to ν(OH) stretching of coordinated water molecules [3]. The strong evidence for the

presence of coordinated water molecules was also provided from TGA/DTA analysis of the complexes.

^{1}H-NMR

^{1}H-NMR spectrum of synthesized Schiff base ligand (S_1) was recorded in $CDCl_3$ solvent by using TMS as internal standard. In NMR spectrum of ligand first signal appeared as sharp singlet at δ 3.87 ppm indicating methoxy proton present in ligand. The multiplet signal for aromatic protons of substituted aniline ring in Schiff base was observed at δ 7.63–7.55 ppm while the another multiplet signal for aromatic protons of substituted benzophenone phenyl moiety in Schiff base was observed around δ 6.40–7.59 ppm. The last peak observed at δ 12.71 ppm was due to phenolic -OH proton of substituted benzophenone moiety in ligand. These NMR data helps to confirm the structure of synthesized Schiff base ligand (S_1) [42].

^{13}C-NMR

The ^{13}C-NMR spectrum of synthesized Schiff base ligand (S_1) was recorded in $CDCl_3$ solvent using TMS as an internal standard. The chemical shifts observed at 200, 166.23 and 166.33 ppm indicates to carbon atoms of (C=N), (C–OH) and(C–OCH_3) groups, respectively. The chemical shift at 145.92 ppm indicates the chemical shits due to carbon atom of (C–N). Signals of aromatic carbon were displayed in region 101.06 to 138.24 ppm and chemical shift at 55.65 ppm referred to carbon atom of –OCH_3 (methoxy carbon).

Electronic spectra

The electronic spectra of synthesized Schiff base ligand (S_1) and mixed ligand complexes were recorded in chloroform solvent at room temperature. The electronic spectrum of free Schiff base ligand shows two absorption bands at 265 and 383 nm which can be due to ($\pi \rightarrow \pi^*$) transition of aromatic benzene ring and ($n \rightarrow \pi^*$) transition due to C=N group.

The electronic spectrum of Mn(II) complex exhibited two absorption bands at wavelength 260 and 388 nm is attributed to ligand field transition. Another peak observed at 399 nm was due to LMCT transition. The next peak at 644 nm indicated d-d electronic transition in metal complex. The Fe(III) complex exhibited absorption bands at low intensity at 661, 615 and 380 nm. The Co(II) complex displayed absorption bands at 241, 315 nm indicating ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$) transitions due to ligand field. The band at 398 nm was assigned to LMCT transition. Two bands observed at 630 and 971 nm were assigned due to $^4T_{1g} \rightarrow {}^4A_{2g}$ and $^4T_{1g} \rightarrow {}^4T_{2g}$ d-d transitions, respectively. The Ni(II) complex exhibited four absorption bands at wavelength 795, 691, 487 and 264 nm, respectively. These transitions can be attributed due to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ and LMCT, respectively. The Cu(II) complex exhibited broad absorption band at wavelength 382–364 nm indicating $^2E_g \rightarrow {}^2T_{2g}$ transition. Based on the results obtained all the complexes are proposed to have octahedral geometry [42–44].

Thermal analysis

Thermal analysis of synthesized complexes was carried out using TGA and DTA curves. The TGA curves of all the complexes exhibited weight loss in two to three different steps. In first step loss of coordinated water molecules is observed at 150–180 °C temperature. The second major weight loss observed in the temperature rage 200–400 °C indicated loss of coordinated Schiff base ligand in the complexes. In last step 30–40% weight loss was observed in complexes beyond 400 °C temperature due to loss of coordinated 8-hydroxyquinoline ligand. Finally all the metal complexes were converted into their respective metal oxide beyond 750 °C temperature indicating all the complexes are thermally stable compounds.

In the DTA curves of all the complexes peaks were observed beyond 400 °C up to 750 °C temperature. This was sufficient to indicate that all these complexes undergoes decomposition in the temperature range 400–750 °C.

Powder XRD analysis

The powder XRD analysis of all synthesized mixed ligand complexes were used to determine the particle size of complexes and hence to determine their nature, viz. crystalline or amorphous. The particle size of mixed ligand complexes was calculated by using Scherer's formula, Eq. (4)

$$D = \frac{K\lambda}{\beta \mathrm{Cos}\theta} \tag{4}$$

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 [45]. The inter planner spacing (d) is calculated by using Brag's Eq. (5),

$$n\lambda = 2d \sin\theta \tag{5}$$

The mean particle size of complexes is the range of 03.51–35.45 nm. All the XRD patterns of complexes exhibited sharp crystalline peaks indicating semi-crystalline

Table 2 Powder XRD data

Complex	Reflexes	2θ	Miller indices	Inter planar spacing (*d*) (A°)	Crystal size (*D*) (nm)	FWHM
C_1 $C_{29}H_{24}Cl\,N_3O_7Mn$	Peak1	09.94	111	8.89	00.28	48.331
	Peak2	15.50	220	5.71	28.23	0.4954
	Peak3	23.28	400	3.81	05.98	2.3658
	Peak4	26.06	500	3.41	03.44	4.1262
	Peak5	35.36	610	2.53	00.68	21.142
Average Crystal size					07.72 nm	
C_2 $C_{29}H_{24}Cl_2\,N_3O_7Fe$	Peak1	09.40	111	9.40	65.34	0.2129
	Peak2	16.02	300	5.52	26.69	0.5249
	Peak3	26.68	500	3.33	01.01	14.070
	Peak4	27.76	510	3.21	02.64	5.3910
Average Crystal size					23.92 nm	
C_3 $C_{29}H_{24}Cl\,N_3O_7Co$	Peak1	09.94	111	8.89	00.20	68.503
	Peak2	16.16	220	5.47	09.36	1.4951
	Peak3	25.84	420	3.44	00.97	14.548
Average Crystal size					03.51 nm	
C_4 $C_{29}H_{24}Cl\,N_3O_7Ni$	Peak1	06.56	111	13.50	23.61	0.588
	Peak2	09.88	211	08.96	37.00	0.376
	Peak3	16.12	411	05.50	33.01	0.424
	Peak4	18.40	422	04.82	37.34	0.376
	Peak5	25.92	631	03.43	35.55	0.400
Average Crystal size					33.30 nm	
C_5 $C_{29}H_{24}Cl\,N_3O_7Cu$	Peak1	06.68	111	13.22	42.20	0.329
	Peak2	09.84	211	08.99	24.62	0.565
	Peak3	13.14	311	06.72	32.90	0.424
	Peak4	16.10	411	05.50	39.65	0.353
	Peak5	26.70	631	03.33	37.88	0.376
Average Crystal size					35.45 nm	

nature of the complexes. The obtained results are represented in Table 2.

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 obtained results were compared with standard drug Streptomycin. It was observed that the complexes of Mn(II), Fe(III), Ni(II) and Cu(II) exhibited better antibacterial activities compared to the free Schiff base ligand (S_1). The Co(II) complex exhibited poor antibacterial activity against both pathogenic organism. Figure 1a–f represents the results obtained.

The increased activity of complexes can be explained on the basis of Overtone's concept and Tweedy's chelation theory [46]. According to chelation theory, on chelation the polarity of metal ion will be reduced and lipophilicity of the complexes will be increased. Due to this, these complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organisms.

Antifungal activity using agar well plate diffusion Method

All the synthesized compounds were screened for their antifungal activity against fungal pathogens *C. albicans* and *A. niger* and the obtained results were compared with standard Clotrimazole. The Schiff base (S_1), Mn(II), Co(II) and Ni(II) complexes exhibited moderate to good antifungal activity against both these pathogens. The Cu(II) complex exhibited moderate activity against *A. niger* but was inactive against *C. albicans*. The Fe(III) complex was inactive against both the pathogens. Table 3 represents results obtained from antimicrobial activity screening for all the synthesized compounds.

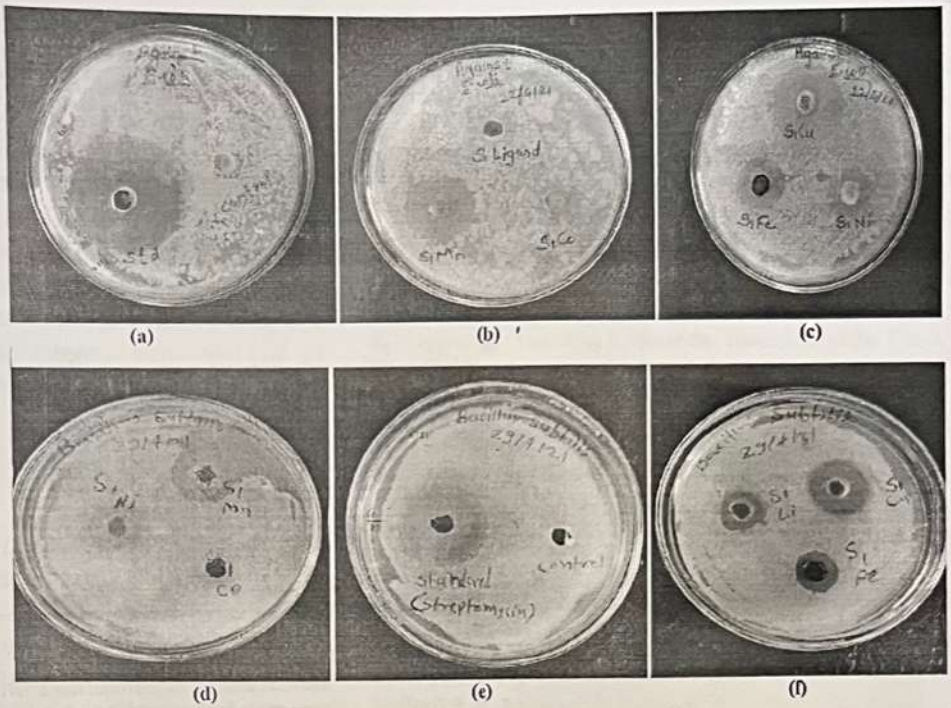


Fig. 1 Antibacterial activity results against *E. coli* [(**a–c**)] and against *B. Subtilis* [(**d–f**)]

Table 3 Antimicrobial activity results

Complex	Concentration	Zone of Inhibition (mm)			
		Antibacterial activity		Antifungal activity	
		E. coli	*B. subtilis*	*C. albicans*	*A. niger*
S_1 $C_{20}H_{15}Cl\,N_2O_4$	5 mg/mL	00	16	09	10
C_1 $C_{29}H_{24}Cl\,N_3O_7Mn$	5 mg/mL	17	24	06	09
C_2 $C_{29}H_{24}Cl_2\,N_3O_7Fe$	5 mg/mL	13	16	00	00
C_3 $C_{29}H_{24}Cl\,N_3O_7Co$	5 mg/mL	00	12	08	09
C_4 $C_{29}H_{24}Cl\,N_3O_7Ni$	5 mg/mL	11	18	07	08
C_5 $C_{29}H_{24}Cl\,N_3O_7Cu$	5 mg/mL	22	23	00	08
Standard (streptomycin)	1 mg/mL	31	32	–	–
Standard (Clotrimazole)	1 mg/mL	–	–	14	13

Antidibetic activity using α-amylase inhibition Assay

The antidibetic activity of all the synthesized compounds was recorded with the help of α-amylase inhibition assay and the obtained results were compared with standard acarbose (80.80% inhibition). All the synthesized compounds exhibited moderate to good antidibetic activity in terms of α-amylase inhibition. Surprisingly the Schiff base ligand (S_1) exhibited highest value of 59.09% amongst all the synthesized compounds whereas all the complexes exhibited values in the range 53.03–55.55%. Figure 2 represents results obtained.

Anticancer activity and cytotoxicity study using MTT assay

All the synthesized complexes were screened for their anticancer activity and cytotoxicity study against human breast cancer cell line MCF-7 using MTT assay. Figure 3 represents graphical representation of the results obtained from anticancer activity.

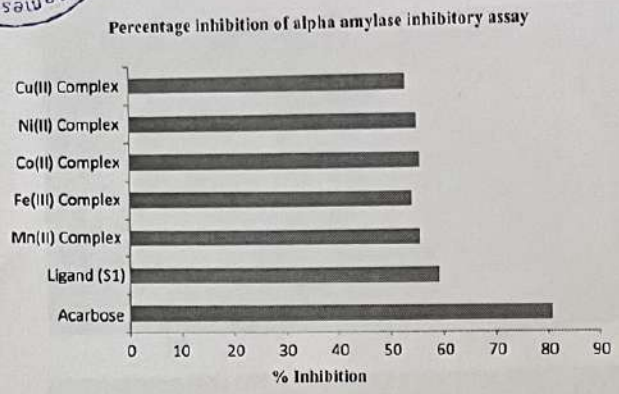


Fig. 2 Graphical representation of antidibetic activity

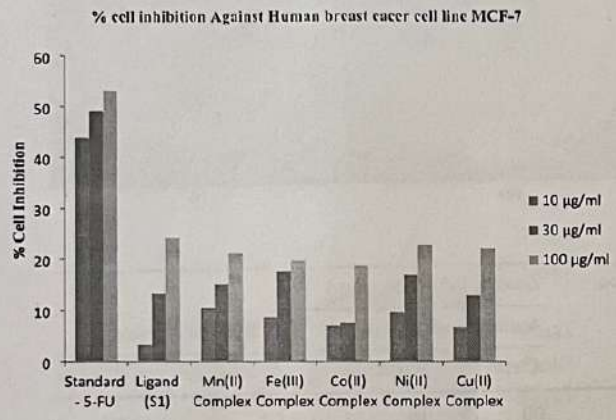


Fig. 3 Graphical representation of results obtained for anticancer activity

The IC_{50} values above 100 µM are noted for all the synthesized compounds and were compared with that of the standard drug 5-Flourouracil (FU) which exhibited IC_{50} value of 60.76 µM. All the compounds exhibited moderate to good anticancer activity against human breast cancer cell line MCF-7. The cell viability values decreased and percent inhibition values increased with increasing dose concentration which 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 using DPPH radical scavenging assay

All the synthesized compounds were screened for their antioxidant activities and obtained results were compared with standard ascorbic acid (Vitamin C) on the basis of free radical scavenging effect of stable DPPH free radical activity. The results of complexes were interpreted on the basis of percent inhibition at 1000 µg/mL concentration. The DPPH radical scavenging activity of mixed ligand complexes was

Table 4 Anti-oxidant activity (DPPH radical scavenging activity)

Sample Code	Concentration (µg/mL)	ABS at 515 (nm)	Percent inhibition (%)
Control	–	0.454	–
S_1 $C_{20}H_{15}Cl\,N_2O_4$	1000	0.357	21.36
C_1 $C_{29}H_{24}Cl\,N_3O_7Mn$	1000	0.093	79.51
C_2 $C_{29}H_{24}Cl_2\,N_3O_7Fe$	1000	0.026	94.27
C_3 $C_{29}H_{24}Cl\,N_3O_7Co$	1000	0.091	79.95
C_4 $C_{29}H_{24}Cl\,N_3O_7Ni$	1000	0.123	72.90
C_5 $C_{29}H_{24}Cl\,N_3O_7Cu$	1000	0.229	49.55
Standard–Acarbose	1000	0.010	97.79

Table 5 Results of antimalarial activity screening

Compound	Concentration (mg/mL)	Absorbance (nm)	Percentage Inhibition (%)
Control	–	1.19	–
S_1 $C_{20}H_{15}Cl\,N_2O_4$	1000	0.608	48.90
C_1 $C_{29}H_{24}Cl\,N_3O_7Mn$	1000	0.473	60.25
C_2 $C_{29}H_{24}Cl_2\,N_3O_7Fe$	1000	0.427	65.54
C_3 $C_{29}H_{24}Cl\,N_3O_7Co$	1000	0.410	64.11
C_4 $C_{29}H_{24}Cl\,N_3O_7Ni$	1000	0.489	58.90
C_5 $C_{29}H_{24}Cl\,N_3O_7Cu$	1000	0.517	56.55
Standard	1000	0.370	68.90

significantly better than the free Schiff base ligand (S_1). The Fe(III) complex exhibited highest inhibition value which is very close to that of standard ascorbic acid indicating excellent antioxidant activity. The Mn(II), Co(II) and Ni(II) complexes exhibited good activity whereas the Cu(II) complex exhibited moderate antioxidant activity. The result indicated that the complexes are much better free radical scavenger than the free Schiff base ligand (S_1) molecule [47]. The obtained results are represented in Table 4.

Antimalarial activity

All the synthesized compounds were screened for their antimalarial activity and the obtained results are represented

in Table 5. All the synthesized compounds exhibited better antimalarial activities close to the standard used. The complexes Fe(III), Co(II) and Mn(II) exhibited 65.54, 64.11 and 60.25% inhibition values which are close to the standard value 68.90%. Thus these complexes exhibited excellent antimalarial activity and can act as potential antimalarial agents. The Ni(II) and Cu(II) complex exhibited 58.90 and 56.55% inhibition value which is more than the Schiff base ligand (S_1) (48.90%). Still it can be clearly observed that all the synthesized complexes exhibited good to excellent antimalarial activity than parent Schiff base ligand and can be considered as potential antimalarial agents.

Conclusions

Room temperature synthesis of novel Schiff base ligand (S_1) and mixed ligand complexes of Mn(II), Fe(III), Co(II), Ni(II), Cu(II) is reported here. All the synthesised compounds were characterized using various techniques. The magnetic susceptibility data indicated that all the complexes are paramagnetic in nature. IR and electronic spectral data indicated towards binding of metal atoms with nitrogen and oxygen atoms in ligands. Powder XRD data indicated semi-crystalline nature of the complexes. Thermal analysis indicated involvement of water molecules in the coordination sphere and thermal stability of complexes. Thus based on the obtained results, all the complexes are proposed to have octahedral geometry.

All the synthesized complexes were screened for their biological activities such as antibacterial, antifungal and antioxidant activities. The antibacterial results indicated that all the complexes exhibited better activities as compared to Schiff base ligand (S_1) which can be explained based on Tweedy's chelation theory. The Schiff base ligand (S_1), Mn(II), Co(II) and Ni(II) complexes exhibited moderate antifungal activity against both *C. albicans* and *A. niger* whereas remaining complexes exhibited varying activities.

The results of antidibetic activity measurements using α-amylase inhibition assay indicated that all the compounds exhibited moderate to good activity in comparison with the standard used. All the complexes exhibited good anticancer activity against human breast cancer cell line MCF-7 as compared to the standard drug 5-Fuorouracil. In case of antioxidant activity measurements the Fe(III) complex exhibited highest inhibition thus showing excellent activity whereas remaining complexes exhibited moderate to good activity. The Mn(II), Fe(III) and Co(II) complexes exhibited excellent antimalarial activity whereas remaining complexes viz. Ni(II), Cu(II) and Schiff base ligand (S_1) exhibited moderate activity as compared to standard used. Overall all the complexes exhibited various biological activities and hence are potential candidates for future studies as drug molecules.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13738-021-02431-5.

References

1. M. Kiruthika, R. Elayaperumal, T. Vennila, Der Chem. Sin. **3**(5), 1175 (2012)
2. S.I. Mirza, M. Saleem, Z.A. Mirza, Chem. Sci. Trans. **8**(1), 7 (2019)
3. J. Devi, N. Batra, Spectrochim. Acta Part A **135**, 710 (2015)
4. S. Maddela, A. Makulaa, R. Maddela, Toxicol. Environ. Chem. **96**, 1 (2014)
5. P. Mishra, H. Rajak, A. Mehta, J. Gen. Appl. Microbiol. **51**, 133 (2005)
6. M.S. Karthikeyan, D.J. Prasad, B. Poojary, K.S. Bhat, B.S. Holla, N.S. Kumari, Med. Chem. **14**(22), 7482 (2006)
7. J. Patole, D. Shingnapurkar, S. Padhyea, C. Ratledgeb, Bioorg. Med. Chem. Lett. **16**, 1514 (2006)
8. A.A. Ardakani, H. Kargar, N. Feizi, M.N. Tahir, J. Iran. Chem. Soc. **15**, 1495 (2018)
9. H. Kargar, A.A. Ardakani, K.S. Munawar, M. Ashfaq, M.N. Tahir, J. Iran. Chem. Soc. **18**, 2493 (2021)
10. A. Sahraei, H. Kargar, M. Hakimi, M.N. Tahir, J. Mol. Struct. **1149**, 576 (2017)
11. H. Kargar, A. Ardakani, M.N. Tahir, M. Ashfaq, K.S. Munawar, J. Mol. Struct. **1229**, 129842 (2021)
12. H. Kargar, F.A. Meybodi, R.B. Ardakani, M.R. Elahifard, V. Torabi, M.F. Mehrjardi, M.N. Tahir, M. Ashfaq, K.S. Munawar, J. Mol. Struct. **1230**, 129908 (2021)
13. H.F.A. El Halim, G.G. Mohamed, M.N. Anwar, Appl. Organomet. Chem. **32**(1), 3899 (2018)
14. V. Jevtovic, S. Ivkovic, S. Kaisarevic, R. Kovacevic, Contempo. Mater. **1**(2), 133 (2010)
15. H. Zhang, Y. Yuetao, F. Dawei, W. Yipeng, Q. Song, Hindawi Publish. Corpo. Evid. Based Complement. Alternat. Med. (2011)
16. G. Mariappan, B.P. Saha, S. Datta, D. Kumar, P.K. Haldar, J. Chem. Sci. **123**(3), 335 (2011)
17. S.N. Shukla, P. Gaur, S. Jhariya, B. Chaurasia, P. Vaidya, D. Dehariya, M. Azam, Chem. Sci. Trans. **7**(3), 424 (2018)
18. V. Singh, Pharma Innov. J. **8**(5), 403 (2019)
19. L.C.S. Pinheiro, L.M. Feitosa, F.F. Da Silveira, N. Boechat, Annal. Braz. Acad. Sci. **90**(1 Suppl. 2), 1251 (2018)
20. H. Kargar, Trans. Met. Chem. **39**, 811 (2014)
21. H. Kargar, V. Torabi, A. Akbari, R.B. Ardakani, M.N. Tahir, J. Iran. Chem. Soc. **16**, 1081 (2019)
22. B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, 5th edn. (Longman group, London, 1989)
23. D. Moonmun, R. Majumder, A. Lopamudra, Ind. J. Pharm. Sci. **79**(1), 79 (2017)
24. C.D. Hufford, M.J. Funderburk, J.M. Morgan, L.W. Robertson, J. Pharm. Sci. **64**(5), 789 (1975)
25. S. Umadevi, G.P. Mohanta, V. Chelladurai, P.K. Manna, R. Manavalan, J. Nat. Rem. **3**, 185 (2003)
26. S.A. Amolegbe, S. Adewuyi, C.A. Akinremi, J.F. Adediji, A. Lawal, A.O. Atayese, J.A. Obaleye, Arab. J. Chem. **8**(5), 742 (2015)
27. S. Jaber, A.L. Saleh, P. Lutgen, M. Qutob, Q. Abu-Remeleh, M. Akkawi, J. Pharma. Pharmacol. **3**, 63 (2015)
28. M.J. Roux, R. Martinez-Maza, A. Le Goff, B. Lopez-Corcuera, C. Aragon, S. Supplisson, J. Biol. Chem. **276**(21), 17699 (2001)
29. M. Lankisch, P. Layer, R.A. Rizza, E.P. DiMagno, Pancreas **17**(2), 176 (1998)
30. P. Bernfeld, Enzymology **1**, 149 (1955)

31. N. Horiuchi, K. Nakagawa, Y. Sasaki, K. Minato, Y. Fujiwara, K. Nezu, Y. Ohe, N. Saijo, Cancer Chemother. Phramocol. **22**(3), 246 (1988)
32. P. Senthilraja, K. Kathiresan, J. Appl. Pharma. Sci. 5(03), 080 (2015)
33. H. Kumar, S.A. Javed, S.A. Khan, A. Mohammad, Eur. J. Med. Chem. **43**, 2688 (2008)
34. X.J. Duan, W.W. Zhang, X.M. Li, B.G. Wang, Food Chem. **95**, 37 (2006)
35. S.O. Podunavac-Kuzmanovic, S.L. Markov, L.S. Vojinovic, Acta Period. Technol. 35(1), 280 (2004)
36. W.J. Geary, Coord. Chem. Rev. **7**, 81 (1971)
37. S.I. Mirzal, M. Saleem, Z.A. Mirza, Chem. Sci. Trans. **8**(1), 7 (2019)
38. K. Nakamoto, Y. Morimoto, A.E. Martell, J. Am. Chem. Soc. **83**, 4528 (1961)
39. H. Kargar, R.B. Ardakani, V. Torabi, A. Sarvian, Z. Kazemi, Z.C. Natanzi, V. Mirkhani, A. Sahraei, M.N. Tahir, M. Ashfaq, Inorg. Chim. Acta **514**, 120004 (2021)
40. H. Kargar, R.B. Ardakani, V. Torabi, M. Kashani, Z.C. Natanzi, Z. Kazemi, V. Mirkhani, A. Sahraei, M.N. Tahir, M. Ashfaq, K.S. Munawar, Polyhedron **195**, 114988 (2021)
41. H. Kargar, V. Torabi, A. Akbari, R.B. Ardakani, A. Sahraei, M.N. Tahir, Struct. Chem. **30**, 2289 (2019)
42. P. Subbaraj, A. Ramu, N. Raman, J. Dharmaraja, Int. J. Emer. Sci. Eng. (IJESE) **1**, 7 (2013)
43. A.T. Numan, E.M. Atiyah, R.K. Al-shemary, S.S. Abd_Ulrazzaq, J. Phys. **1003**, 012016 (2018)
44. S.E.H. Etaiwa, M. Abd El-A Dina, H. Abd El-Z Eman, A.A. Elham, Spectrochim. Acta Part A **79**, 1331 (2011)
45. S.A.S. Alazawi, A.A.S. Alhamadani, Um-Salama Sci. J. **4**(1), 102 (2007)
46. B.G. Tweedy, Phytopathol. **55**, 910 (1964)
47. P.E. Ikechukwu, A.A. Peter, Bioinorg. Chem. Appli. 890734 (2015)