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Original article

Transition metal complexes of a new hexadentate macroacyclic N_2O_4-donor Schiff base: Inhibitory activity against bacteria and fungi

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ABSTRACT

A new hexadentate macroacyclic N_2O_4 – donor ligand, glyoxal bis(*N*-nitroso phenylglycine) (gbnp), has been designed and structurally characterized. Manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) complexes of gbnp have been prepared and characterized by elemental analyses, molar conductance measurements, magnetic moment, spectral (IR, ^{1}H NMR, electronic and FAB mass) and thermal studies. The molecular dynamics of the ligand was studied by the variable temperature NMR studies, which suggest the presence of two isomeric forms. The antimicrobial activity of all the compounds was studied against *Escherichia coli, Bacillus cirroflagellosus, Aspergillus niger* and *Candida albicans*. Among the new complexes, copper(II) complex has the highest potential against all the microorganisms tested.

1. Introduction

The chemistry of nitroso compounds has been intensively studied in the past decades, especially in the field of asymmetric synthesis [1,2], nitroso Diels–Alder reaction [3,4], *N*-nitroso aldol reaction [5] and nitroso ene reaction [6]. In 1992, nitric oxide (NO) was selected by *Science* magazine as the "Molecule of the Year" [7] due to its participation in several biological functions [8]. In the same year, a book dealing exclusively with metal nitrosyls (metal–NO compounds) was published [9]. NO binds to the metal centers of hemecontaining biomolecules such as the enzyme guanylyl cyclase forming Fe–NO bonds [10,11]. These observations helped to make the study of metal–NO compounds interdisciplinary in nature, bridging traditional inorganic chemistry with biochemistry and pharmacology.

Aim of the present work was to insert NO group in amino acid Schiff bases. As we know amino acid Schiff bases readily form complexes with metal ions which play an important role as the basic compounds for modeling more complicated amino acid Schiff bases [12–16]. They are key intermediates in a variety of metabolic reactions involving amino acids [17]. The preparative accessibility, diversity and structural variability make Schiff bases very attractive and have achieved recognition for their role in biological systems such as mutagenesis and carcinogenesis [18–27]. In addition to the biochemical research, Schiff bases and their metal complexes are being used as analytical reagents also [28].

In continuation of our study on coordination chemistry of amino acid Schiff bases [29,30], we thought it would be useful to synthetic coordination compounds of a Schiff base containing prudent *N*-nitroso moieties and to evaluate them for their antimicrobial and anticancer activities. Here we report the synthesis and spectroscopic characterization of manganese(II), cobalt(II), nickel(II) copper(II) and zinc(II) complexes of gbnp (Fig. 1). A comparative antibacterial and antifungal activity of these compounds is evaluated.

2. Experimental

2.1. Methods

2.1.1. Synthesis of the ligand [gbnp]

The preparation of glyoxal bis(*N*-nitroso phenylglycine) (gbnp) involves the following steps as outlined in Scheme 1.

2.1.1.1. Synthesis of N-phenylglycine ester [pge]. It was prepared by following the procedure as described in the literature [31].

2.1.1.2. Synthesis of N-nitroso phenylglycine ester [npge]. A suspension of pge (1.79 g, 0.01 mol) in 20 ml of (1:1) aqueous acetic acid

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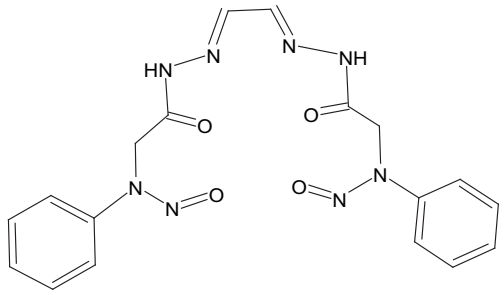


Fig. 1. Structure of the ligand.

was taken and cooled to 0 °C. A solution of sodium nitrite (0.79 g, 0.01 mol) in 8 ml water was added dropwise with stirring for 30 min, maintaining the temperature between 0–5 °C. Further, the reaction mixture was stirred for 2 h. Thick oil separated was extracted with ether. Ether layer was washed with distilled water several times. Ether was evaporated to get back the oil and dried over anhydrous calcium chloride. The purity was confirmed by TLC and ^{1}H NMR analysis. Yield: 80%.

2.1.1.3. Synthesis of 2-[nitroso (phenyl) amino] acetohydrazide (npah). The mixture of npge (2.08 g, 0.01 mol) and hydrazine hydrate (0.49 ml, 0.01 mol) in 20 ml absolute ethanol was refluxed at water bath temperature for 4–5 h. The solid separated was filtered and washed with ethanol. The crude product was recrystallized from hot ethanol and dried *in vacuo*. Yield: 82%. M.P.: 115 °C.

2.1.1.4. Synthesis of glyoxal bis(N-nitroso phenylglycine) (gbnp). To npah (1.94 g, 0.01 mol) in 20 ml absolute ethanol, glyoxal (0.58 g, 0.01 mol) was added and refluxed on a water bath for 4–5 h. The reaction mixture was cooled. The solid separated was filtered, washed with ethanol and recrystallized from hot ethanol. Yield: 87%. M.P.: 155 °C.

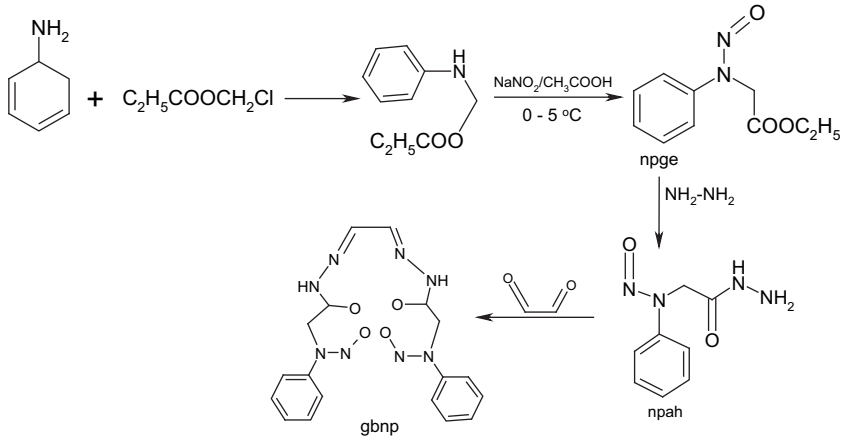
*2.1.1.5. Preparation of the complexes (**1**–**5**).* The respective hydrated metal chlorides [0.01 mol, M = manganese(II), cobalt(II), nickel(II), copper(II), zinc(II)] dissolved in 10 ml of ethanol was added to ethanolic (20 ml) solution of gbnp (0.41 g, 0.01 mol) and refluxed for 4–5 h. The colored complexes so obtained were filtered, washed with ethanol and dried under vacuum over anhydrous $CaCl_2$. Yield: 80–85%.

3. Pharmacology

The *in vitro* antimicrobial activity of all the compounds was studied against Gram negative (*Escherichia coli*) and Gram positive (*Bacillus cirroflagellosus*) bacteria and fungi, *Aspergillus niger* and *Candida albicans* by agar well diffusion method [32,33]. The nutrient broth was prepared by dissolving peptone (0.5%), yeast extract (0.15%), beef extract (0.15%), sodium chloride (0.36%) and monopotassium phosphate (0.13%) in distilled water (100 ml). The pH of the solution was adjusted to 7.2 by adding sodium hydroxide solution (4%) and the resulting solution was autoclaved for 20 min at 15 psi. Each test sample (5 mg) was dissolved in DMSO (1 ml) and 0.1 ml of this solution (50 μg) was used for testing.

4. Results and discussions

The analytical data of the ligand and its metal complexes is summarized in Table 1 and are in good agreement with those required by the general formula $[M(gbnp)]Cl_2 \cdot nH_2O$ [M = manganese(II), cobalt(II), nickel(II), copper(II), zinc(II), $n = 2, 3$]. Attempts to crystallize the ligand and its complexes, suitable for X-ray diffraction studies were unsuccessful. The obtained



Scheme 1.

Table 1
Analytical data of gbnp and its complexes.

Compound code	Compound	Empirical formula	Formula weight	Found (calcd.) %					Melt/dec. pt. (°C)	$\Lambda_M(\Omega^{-1}$ cm^2 mol$^{-1})$
				C	H	N	M	Cl		
–	gbnp	$C_{18}H_{18}N_8O_4$	410.10	52.40 (52.70)	4.10 (4.42)	27.15 (27.30)	–	–	155	–
1	$[Mn(gbnp)]Cl_2 \cdot 3H_2O$	$C_{18}H_{24}Cl_2MnN_8O_7$	590.00	36.22 (36.61)	4.00 (4.06)	18.18 (18.90)	9.00 (9.30)	11.95 (12.03)	220	76.21
2	$[Co(gbnp)]Cl_2 \cdot 2H_2O$	$C_{18}H_{22}Cl_2CoN_8O_6$	576.00	36.30 (36.80)	3.24 (3.81)	19.26 (19.44)	10.00 (10.22)	12.14 (12.32)	230	78.20
3	$[Ni(gbnp)]Cl_2 \cdot 2H_2O$	$C_{18}H_{22}Cl_2NiN_8O_6$	575.70	37.22 (37.51)	3.35 (3.82)	19.12 (19.45)	10.10 (10.17)	12.05 (12.33)	205	74.50
4	$[Cu(gbnp)]Cl_2 \cdot 2H_2O$	$C_{18}H_{22}Cl_2Cu\ N_8O_6$	580.60	37.12 (37.20)	3.28 (3.78)	19.10 (19.29)	10.55 (10.93)	12.15 (12.22)	210	79.67
5	$[Zn(gbnp)]Cl_2 \cdot 2H_2O$	$C_{18}H_{22}Cl_2ZnN_8O_6$	582.40	37.00 (37.08)	3.64 (3.77)	19.04 (19.23)	11.12 (11.21)	12.00 (12.10)	208	73.48

Table 2
Thermogravimetric characteristics of the complexes under study.

Compound	Process	Temp °C	Product (No. of moles)	Mass %		Residue %	
				Calcd.	Exptl.	Calcd.	Exptl.
1	Decomposition of coordination sphere	80–100	H_2O (3)	9.15	9.00	12.00	11.50
		100–450	L (1) + Chloride (2)	81.35	81.00		
2	Decomposition of coordination sphere	90–120	H_2O (2)	6.25	6.00	13.00	12.68
		120–500	L (1) + Chloride (2)	83.33	83.20		
3	Decomposition of coordination sphere	100–120	H_2O (2)	6.25	6.12	12.97	12.45
		120–550	L (1) + Chloride (2)	83.38	83.10		
4	Decomposition of coordination sphere	80–120	H_2O (2)	6.20	6.00	13.69	13.22
		120–500	L (1) + Chloride (2)	82.67	82.40		
5	Decomposition of coordination sphere	90–110	H_2O (2)	6.18	6.10	13.97	13.80
		110–450	L (1) + Chloride (2)	82.42	82.25		

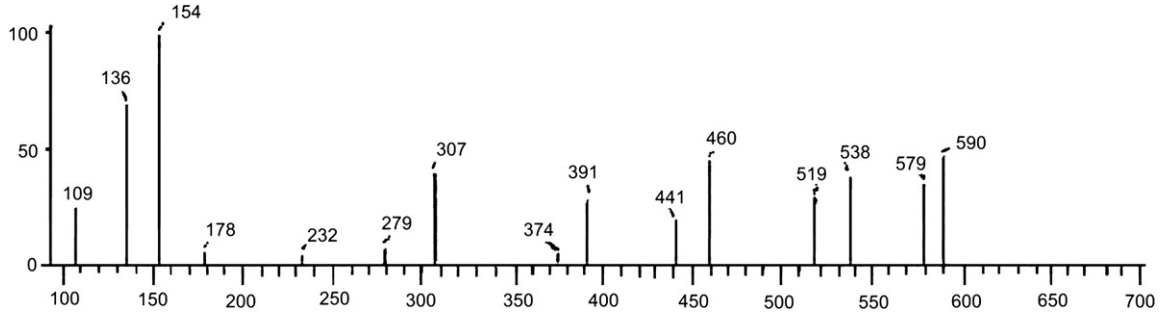


Fig. 2. FAB mass spectrum of $[Mn(gbnp)]Cl_2 \cdot 3H_2O$.

complexes are insoluble in water, methanol and ethanol but soluble in DMF and DMSO. The molar conductance values fall in the range of 74.50–79.67 Ω^{-1} cm^2 mol^{-1}, suggesting 1:2 electrolytic nature for the complexes [34]. All the complexes lost lattice held water molecules in the 80–120 °C temperature range (Table 2). The FAB mass spectrum (Fig. 2) of $[Mn(gbnp)]Cl_2 \cdot 3H_2O$ shows the molecular ion peak at 590, which supplements the proposed composition for the complex.

IR spectral data of the ligand in comparison with those of the complexes coordination of the ligand through carbonyl oxygens, azomethine nitrogens and oxygens of nitroso groups. Table 3 lists such selected vibration bands and the respective assignments.

The presence of new bands at 530–570 cm^{-1} and 410–430 cm^{-1} in the spectra of the complexes correspond to ν(M–N) and ν(M–O) vibration bands [35,36].

The electronic spectral measurements were used for assigning the stereochemistries of complexes based on the positions and number of the d–d transition peaks. The electronic spectrum of the ligand shows three peaks at 27,855, 32,362 and 39,062 cm^{-1}, assigned to $\pi^* \leftarrow n$ of C=N, N=O and C=O chromophores respectively [37]. On complexation, these peaks have shifted to higher wavelengths suggesting coordination of azomethine nitrogen, nitroso oxygen and carbonyl oxygen. The cobalt(II) complex exhibit two d–d bands at 15,200 cm^{-1} and 19,200 cm^{-1} assigned to $^4T_{1g} \rightarrow {}^4A_{2g}$ and $^4T_{1g} \rightarrow {}^4T_{2g}$, respectively, in an octahedral geometry [32,38]. The spectrum of nickel(II) complex exhibit three bands at 11,148 cm^{-1}, 17,667 cm^{-1} and 26,737 cm^{-1}, which are due to $^3A_{2g} \rightarrow {}^3T_{1g}$ (ν_1), $^3A_{2g} \rightarrow {}^3T_{1g}$ (ν_2) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (ν_3) transitions respectively and suggest an octahedral geometry [38]. The ν_2/ν_1 values of 2.1 and 1.58 for cobalt(II) and nickel(II) complexes also suggest octahedral geometry around the metal ions.

Ligand field parameters (Table 4) such as Racah interelectronic-repulsion parameter (B'), ligand field splitting energy (Dq), covalency factor (β) and ligand field stabilization energy (LFSE) have been calculated for cobalt(II) and nickel(II) complexes [38]. The B' values for the complexes are lower than the free ion value, which is an indication of orbital overlap and delocalisation of d-orbitals. The β values obtained are less than unity suggesting a considerable amount of covalent character of the metal–ligand bonds. The β value for the nickel(II) complex is less than the cobalt(II) complex, indicating the greater covalent nature of the former.

The electronic spectrum of copper(II) complex exhibits a band at 16,778 cm^{-1} which is assigned to the transition $^2E_g \rightarrow {}^2T_{2g}$ in an octahedral geometry [38]. The manganese(II) complex in DMSO solution show one band in the 14,700–18,200 cm^{-1} region probably due to metal-ligand charge transfer transition [39].

Magnetic moment values (Table 4) along with electronic spectral data were used to determine the stereochemistry of the isolated complexes. The room temperature magnetic moments of all the complexes are in agreement with the spin free values. The magnetic moment values for manganese(II), cobalt(II) and nickel(II) indicate the high spin octahedral nature of the complexes.

The ^1H NMR spectra of gbnp at different temperatures are depicted in Fig. 3. The spectrum at room temperature (30 °C) exhibited two signals for methylene groups (4.82, 5.15 ppm), -NH protons (11.88, 12.01 ppm) and azomethine protons (7.72, 7.77 ppm) indicating the existence of *cis* and *trans* isomeric forms [40]. It is proposed that, in this compound, restricted rotation about the >C=N linkage as well as the partial double bond character of the amide C–N

Table 3
Diagnostic IR bands of gbnp and its complexes.

Compounds	$\nu(OH)_{water}$	ν(C=O)	ν(N–H)	ν(C=N)	ν(N=O)	ν(M–N)	ν(M–O)
gbnp	–	1678s	3197m	1585m	1478m	–	–
1	3438b	1603s	3051m	1511m	1404w	535w	412w
2	3369b	1603s	3092m	1517m	1401w	534w	421w
3	3375b	1605s	3110m	1519m	1452m	537w	428w
4	3430b	1604s	3105m	1512m	1410w	570w	422w
5	3497b	1612s	3085m	1512w	1437m	542w	419w

b = broad, m = medium, w = weak.

Table 4
Magnetic susceptibility and electronic spectral data of compounds.

Compounds	μ_{eff} (B.M.)	d–d bands (λ_{max} in cm^{-1})	Dq (cm^{-1})	B' (cm^{-1})	β	ν_2/ν_1	LFSE (K cal mol^{-1})
1	5.98	–	–	–	–	–	–
2	4.00	7,150[a], 15,200, 19,200	805	863	0.89	2.1	27.60
3	2.67	11,148, 17,667, 26,737	1114.8	730	0.70	1.58	38.22
4	1.80	16,778	–	–	–	–	–
5	Diamagnetic.	–	–	–	–	–	–

[a] Calcd. value.

bond led to the formation of isomers. To overcome the rotational restriction and to find the coalescence temperature, the ligand, gbnp was studied by variable temperature ^{1}H NMR technique.

The ligand exist as *Z*/*E* geometrical isomers about the >C=N bond of Schiff base moiety as well as *cis*/*trans* amide conformers (Fig. 4). As the temperature was lowered below 30 °C (at 20 °C), the two signals of methylene (Fig. 5a), azomethine and –NH group became prominent indicating the presence of two isomers (ratio 1:3). When the temperature was raised to 41 °C, one of the two signals of each group started diminishing in the isomer ratio 1:4. At still higher temperature (at 58 °C), only one signal predominates in the ratio 1:8 indicating a single amide conformer at and above 58 °C. In this investigation it is observed that, during the conversion of *cis*/*trans* amide conformers, the –NH proton (Fig. 5b) of predominant form has shifted to the upfield region at elevated temperatures whereas the position of methylene and azomethine protons has remained the same.

The ^{1}H NMR spectrum of zinc(II) complex recorded in DMSO-d_6 solution state shows only one set of signals indicating the existence of the molecule in only one isomeric form. On complexation, the –NH proton has shifted downfield to 11.86 ppm due to the involvement of adjacent amide >C=O group in coordination. The signal due to azomethine proton observed at 7.71 ppm exhibited a downfield shift (0.10 ppm) suggesting the involvement of azomethine nitrogen in coordination. The methylene protons and the aromatic protons have appeared at 5.04 and 7.44–7.68 ppm respectively. Thus ^{1}H NMR spectral observations supplement infrared spectral assignments, for the ligating mode of the ligand.

The ESR spectra of the copper(II) complex at both 300 K and 77 K show one intense absorption band at high field, which is isotropic due to the tumbling motion of the molecules. The g_{iso} at 300 and 77 K are 2.1132 and 2.1182 respectively. Mononuclear nature of the complex was also evident from the absence of a half field signal, due to $m_s \pm 2$ transitions, ruling out any Cu–Cu interaction [41].

From the *in vitro* antimicrobial screening results (Table 5 and Fig. 6), it is observed that the ligand is moderately active against bacteria, *E. coli*, *B. cirroflagellosus* and fungi, *A. niger*, *C. albicans*. All the complexes have shown higher activity in comparison with the ligand and metal salts against both bacteria and fungi used. The activity of the ligand has enhanced on complexation. Among the complexes, copper(II) complex has the highest potential against all the microorganisms even more than the standard drugs used. Such an enhanced activity of the complexes can be explained on the basis of Overtone's concept and Tweedy's chelation theory [42]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalisation of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism. The variation in the effectiveness of the different compounds against different organisms depends on the impermeability of the cells of microbes or difference in ribosome of the microbial cells [43].

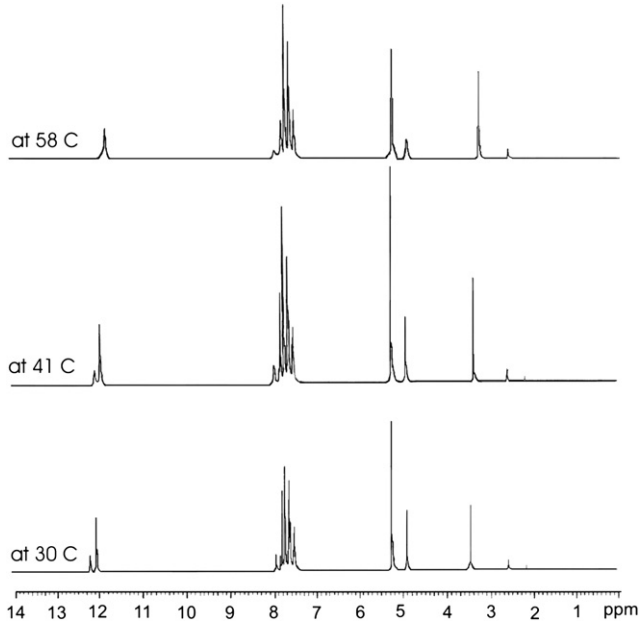


Fig. 3. Variable-temperature ^{1}H NMR spectrum of gbnp.

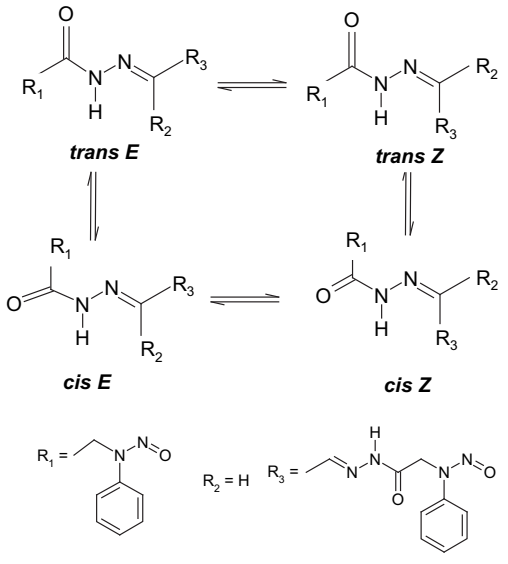


Fig. 4. *cis*/*trans* conformers.

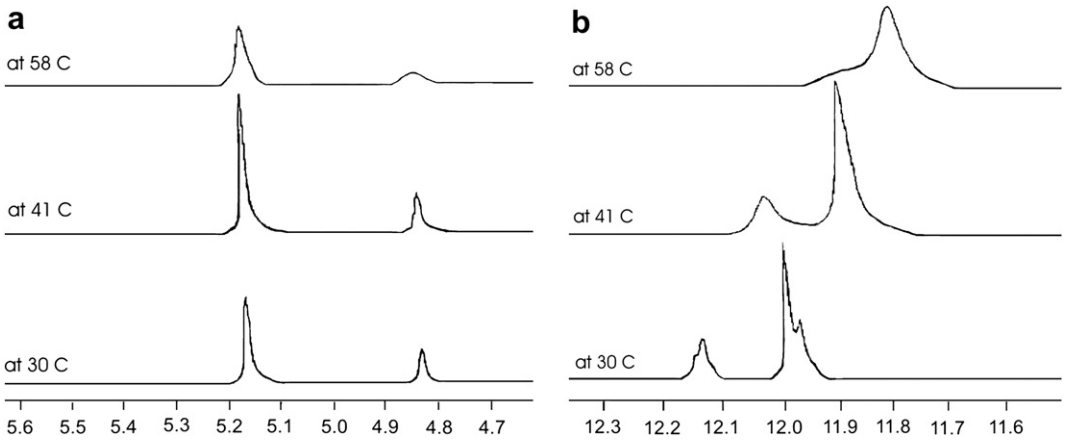


Fig. 5. Competition between isomers for stereointerconversion resulting in the formation of plateau and peak coalescence, **a** (−CH_2 group) and **b** (−NH group).

Table 5
In vitro antimicrobial activity of gbnp and its metal complexes through agar well diffusion method.

Compound	Zone of inhibition (mm)[a] (standard deviation)			
	Antibacterial		Antifungal	
	B. C.	E. C.	A. N.	C. A.
$MnCl_2 \cdot 6H_2O$	14.3(2.1)	20.3(1.2)	22. 7(2.3)	21.3(1.5)
$CoCl_2 \cdot 4H_2O$	26.7(1.5)	27.3(1.2)	20. 7(1.1)	19.7(2.1)
$NiCl_2 \cdot 6H_2O$	20.7(0.6)	20.0(1.7)	20. 7(1.1)	19.7(2.1)
$CuCl_2 \cdot 2H_2O$	19.7(0.6)	19.0(1.0)	20. 3(1.1)	20.3(0.6)
$ZnCl_2 \cdot 2H_2O$	21.3(1.1)	24.0(1.0)	19.0(1.0)	19.7(0.6)
1	21.7(1.1)	21.3(1.5)	27. 3(1.5)	27.7(1.5)
2	31.3(1.1)	28.3(0.6)	21. 3(1.5)	21.3(1.5)
3	21.7(2.1)	22.0(1.7)	23. 7(1.1)	21.3(1.5)
4	34.7(0.6)	34.0(2.0)	32. 7(1.1)	31.3(1.5)
5	23.3(0.6)	21.3(0.6)	22. 7(2.1)	23.3(1.5)
gbnp	16.0(1.0)	16.3(1.5)	16. 7(0.6)	19.3(0.6)
Norfloxacin	28.3(0.6)	28.3(1.2)	–	–
Griseofulvin	–	–	29(1.0)	29.3(0.6)

E. C. = *Escherichia coli*, B. C. = *Bacillus cirroflagellosus*, A. N. = *Aspergillus niger*, C. A. = *Candida albicans*.
Key to interpretation: less than 10 mm – inactive; 10–15 mm – weakly active; 15–20 mm – moderately active; More than 20 mm – highly active.
[a] Average values of triplicates.

5. Conclusion

A newly synthesized macroacyclic ligand, glyoxal bis(*N*-nitroso phenylglycine) (gbnp) has potential binding sites towards metal ions. It acts as a hexadentate ligand by coordinating through two azomethine nitrogens, two amide carbonyl oxygens and two nitroso oxygens in N_2O_4 fashion. It forms octahedral complexes with manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) ions. FAB mass spectral analysis reveals the existence of monomeric complex and thermal studies support the presence of lattice held water molecules. The antibacterial and antifungal screening of the ligand and the complexes shows that the ligand is less active compared to the complexes. Among the complexes, [Cu(gbnp)] $Cl_2 \cdot 2H_2O$ has the highest potential against all the microorganisms. The proposed structure of the complex is given in Fig. 7.

6. Experimental protocols

6.1. Chemistry

The carbon, hydrogen and nitrogen contents were determined using Heraus C H N rapid analyzer. The chloride content of the complexes was determined as AgCl gravimetrically [44]. Infrared

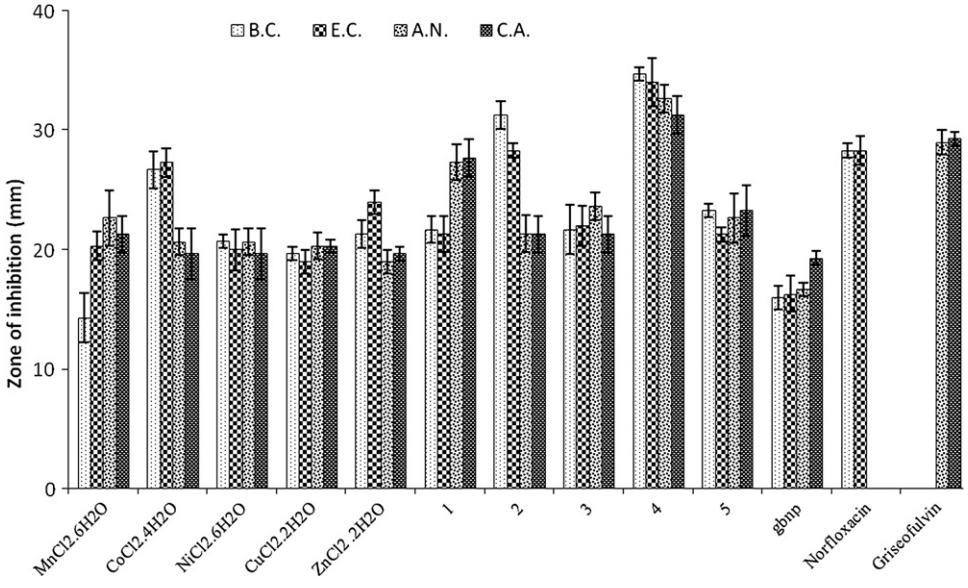


Fig. 6. Antimicrobial activities of metal salts, metal complexes (**1**–**5**), gbnp and the standards.

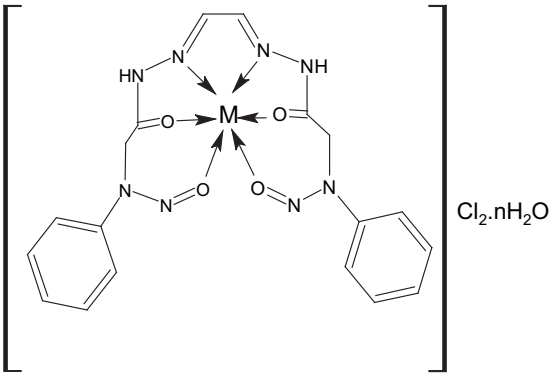


Fig. 7. The proposed structure of the complexes.

spectra (in a KBr matrix) were recorded in the region 4000–400 cm^{-1} on a Thermo Nicolet 320 FT-IR spectrometer. ^1H NMR spectra were recorded in DMSO-d_6 as the solvent at 400 MHz on a BRUKER AMX 400 spectrometer using tetramethylsilane (TMS) as an internal reference. Electronic spectra of the complexes were recorded on a Cary-Bio-50 Varian in DMSO solution (10^{-5} M for UV-region and 10^{-2} M for visible region). The magnetic susceptibility measurements were carried out on a Faraday balance using $Hg[Co(NCS)_4]$ as the calibrant and diamagnetic corrections were made by direct weighing of the ligand for diamagnetic pull. FAB mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10ma) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. Conductance measurements were recorded in DMSO (10^{-3} M) using Elico conductivity bridge type CM-82, provided with a dip type conductivity cell fitted with platinum electrodes. ESR spectra were recorded on Varian E-4 X-band spectrometer using tetracyanoethylene (TCNE) as 'g' ($g = 2.0027$) marker at room temperature and also at liquid nitrogen temperature. The thermal studies of the complexes were made with Mettler Toledo TGA/SDTA851^e with star^e software, under nitrogen atmosphere with a heating rate of 10 °C/min in the temperature range of 50–1000 °C.

6.2. Biological evaluation

One day prior to the experiment, the bacterial and fungal cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37 °C. Inoculation medium containing 24 h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25 ml in each dish) into petri dishes and then allowed to attain room temperature. The cups were made by punching the set of agar with a sterile cork borer and scooping out the punched part. The diameter of each cup was 5 mm. Norfloxacin and Griseofulvin were used as the standards for antibacterial and antifungal tests, respectively. DMSO was used as the solvent control. The entire test samples and the standards were tested at a concentration of 50 μgm. The petri dishes were prepared in triplicate and allowed to stand for an hour in order to facilitate the diffusion of the drug solution and then were incubated at 37 °C for 48 h. The zones of inhibition against all the microorganisms were measured in millimetres and the standard deviations were calculated for each strain used.

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