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Synthesis and characterization of transition metal complexes of hydrochloride salt of 3-chlorobenzaldehyde hydralazine hydrazone: a new class of possible anti-cariogenic agents

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A novel hydrazone, formed by the condensation of the hydrochloride salt of hydralazine with 3-chlorobenzaldehyde, and its Co (II), Ni(II), Cu(II) and Zn(II) complexes have been synthesized. Their structures have been elucidated on the basis of elemental analyses, conductance measurements, magnetic moments, and spectral (infrared, ^{1}H NMR, UV–visible, electrospray ionization (ESI) mass) and thermal studies. The bidentate behaviour of the ligand is proposed on the basis of spectral studies. Interestingly, all four complexes exhibit different geometry around the metal centre. The conductance data of the complexes suggest them to be non-electrolytes. The ESI mass spectra of the complexes support their monomeric nature. The compounds were tested against two Gram-positive and three Gram-negative bacterial strains and three fungal strains. Excellent inhibitory activity is observed against the Gram-positive bacteria *Streptococcus mutans* and *Enterococcus faecalis* which play major roles in tooth decay. Among the fungal strains used, *Candida albicans* is inhibited predominantly.

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Keywords: hydrazone; hydralazine hydrochloride; 3-chlorobenzaldehyde; cariogenic properties; antimicrobial activity; Supporting Information

Introduction

Dental caries is one of the most common infectious microbial diseases. Caries results from the interplay of three main factors: dietary carbohydrates, cariogenic bacteria within dental plaque and susceptible hard tooth surfaces.[1] Dental decay is mainly due to demineralization which is caused by acids produced by bacteria, particularly *Streptococcus mutans* and others that ferment dietary carbohydrates. *Streptococcus mutans* synthesizes an adherent, water-insoluble glucan from sucrose and other appropriate carbohydrate substrates at low pH values, which causes the organisms to adhere firmly to the tooth surface[2] leading to the formation of a dental plaque. The formation of dental plaque plays an important role in the development of caries and periodontal diseases in humans.[3,4] Theoretically, the inhibition of each step in the process of caries formation contributes to the prevention of dental caries.[5]

Various steps have been taken to prevent dental caries. Traditionally, control of dental plaque relied on non-specific removal of plaque by mechanical means. It was followed by the use of fluoridated toothpastes[6] and other topically applied fluorides[7] because of the well-proven anti-caries efficacy of fluorides. Since fluorides have limited effects on pit and fissure caries, various non-fluoride agents like arginine,[8] probiotics,[9] novamin,[10] dentrifrices,[11] plant extracts like neem,[12] tulsi,[13] tea,[14] hop plant[15] etc. have been used extensively in the prevention of such dental caries. However, these exhibit a more general disruptive effect on the oral microbiota affecting the overall biological equilibrium within the oral cavity, which is inhabited by thousands of different species of bacteria.[16] As our knowledge of oral diseases and their mechanisms widens, treatments need to be much more specific, targeting a particular set of organisms. As it is well known that *S. mutans* and *Enterococcus faecalis* play important roles in dental caries, it is important to develop agents which predominantly inhibit these bacteria while maintaining overall biological equilibrium within the oral cavity.

Hydrazones are a class of compounds which are well known for their antimicrobial activity.[17–19] Hydralazine-based hydrazones[20] and 3-chlorobenzaldehyde-based hydrazones[21] have been evaluated separately for their antibacterial and antifungal activity and they are found to exhibit excellent activity. But as hydrazones are prone to undergo degradation and bacteria can develop resistance to them, complexation of hydrazones with biocompatible metal ions is useful in this regard for long-term effectiveness.[22] Even the most potent bacterial resistance is

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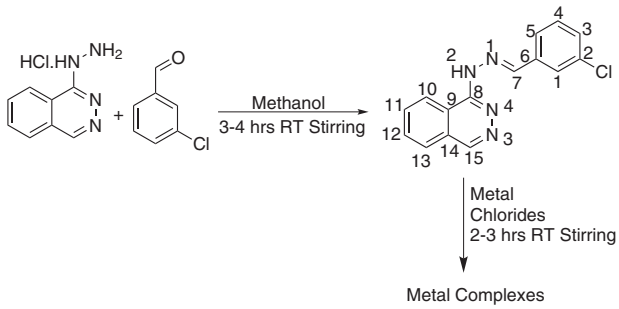
unlikely in this case because of the unique binding, coordination and redox properties of these metal complexes, making them promising novel antimicrobial agents.

In view of the above observations, the hydrochloride salt of 3-chlorobenzaldehyde hydralazine hydrazone and its complexes with late transition metals have been synthesized and evaluated for their antimicrobial activity.



Where,
Metal Chlorides=$CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, Anhydrous $ZnCl_2$

Scheme 1. Synthetic route to the ligand (with atom numbering scheme) and its transition metal complexes.

Experimental

Reagents and Instrumentation

All the solvents used in the present investigation were of analytical grade and were used without further purification. The starting materials 1-hydrazinophthalazine hydrochloride and 3-chlorobenzaldehyde were obtained from Sigma Aldrich. Hydrated metal chlorides and anhydrous zinc chloride were obtained from S. D. Fine Chemicals, India.

The metal content of Co(II), Ni(II) and Cu(II) complexes was determined gravimetrically and that of Zn(II) complex was determined volumetrically after wet ashing with a mixture of HCl and $HClO_4$. The chloride content of the complexes was determined as AgCl gravimetrically. Carbon, hydrogen and nitrogen were determined with a Thermoquest CHN analyser. Infrared (IR) spectra were recorded in the region 400–4000 cm^{-1} with a Nicolet 170 SX FT-IR spectrometer using KBr discs. NMR spectra were recorded using a Bruker Avance 500 MHz spectrometer in DMSO-d_6 with tetramethylsilane as an internal standard. UV–visible spectra were recorded with a Varian Cary Bio spectrophotometer in the range 200–1100 nm using DMF as the solvent. Electrospray ionization (ESI) mass spectra were obtained with a Shimadzu-2010A. Magnetic susceptibility measurements were made using a Johnson Matthey magnetic susceptibility balance at room temperature with $Hg[Co(SCN)_4]$ as the calibrant. Conductance measurements were made in DMF solution (10^{-3} M) using an ELICO-M-82 conductivity bridge with a type CC-01 cell and cell constant of 0.5. Thermal studies were carried out with a TGA7 analyser (PerkinElmer, USA) in the temperature range 25–1000°C at a heating rate of 10°C min^{-1}.

Synthesis of 3-Chlorobenzaldehyde Hydralazine Hydrazone (LH.HCl)

To a hot solution of hydralazine hydrochloride (1 g, 0.005 mol) in dry methanol (30 ml) was added a hot methanolic solution (10 ml) of 3-chlorobenzaldehyde (0.7 g, 0.005 mol) followed by refluxing for 6 h[23] (Scheme 1). The progress of the reaction was monitored using thin-layer chromatography. The reaction mixture was cooled to room temperature and the off-white precipitate formed was filtered under suction, washed several times with dry methanol and dried under vacuum.

LH.HCl: yield 75%; m.p. 285°C. ^{1}H NMR (500 MHz, DMSO-d_6, ppm); 14.57 (s, 1H, N2H), 9.07 (s, 1H, C7H), 9.18 (br, 1H, C10H), 9.07 (s, 1H, C15H), 8.48 (s, 1H, C1H), 8.22–8.26 (m, 1H, C13H), 8.22–8.26 (m, 1H, C11H), 8.15–8.18 (m, 1H, C12H), 7.53–7.60 (m, 1H, C5H), 7.92 (d, J = 8 Hz, 1H, C3H), 7.53–7.60 (m, 1H, C4H). ^{13}C NMR (DMSO-d_6, ppm): 151.10 (C8), 148.20 (C7), 144.78 (C15), 135.88 (C2), 133.87 (C6), 135.18 (C11), 133.67 (C12), 130.92 (C3), 130.63 (C4), 128.13 (C13), 128.08 (C14), 127.90 (C5), 126.84 (C1), 125.35 (C10).

General Procedure for Synthesis of Co(II), Ni(II), Cu(II) and Zn(II) Complexes

A suspension of LH.HCl (0.3 g, 0.001 mol) in methanol (30 ml) was added to a methanolic solution of transition metal chloride and stirred for 2–3 h. The precipitate obtained was filtered under vacuum, washed with methanol and recrystallized from a mixture of water (20 ml) and ethanol (5 ml). All complexes were obtained in moderate to good yields (65–70%). The complexes were freely soluble in DMF and DMSO and found to be electrically non-conducting in DMF solution. The analytical data (Table 1) are satisfactory as regards the molecular formulae given. The melting range of all complexes is above 300°C.

[Zn(LH)(L)Cl]: ^{1}H NMR (500 MHz, DMSO-d_6, d ppm): 12.38 (s, 1H, N2H), 8.30–8.33 (m, 1H, C7H), 8.16 (s, 1H, C10H), 8.30–8.33 (m, 1H, C15H), 8.32 (s, 1H, C1H), 7.73–7.82 (m, 1H, C13H), 7.73–7.82 (m, 1H, C11H), 7.73–7.82 (m, 1H, C12H), 7.44–7.45 (m, 1H, C5H), 7.84–7.86 (m, 1H, C3H), 7.44–7.45 (m, 1H, C4H). ^{13}C NMR (DMSO-d_6, ppm): 149.06 (C7), 151.49 (C8), 137.99 (C15), 131.85 (C11), 132.50 (C6), 133.61 (C12), 130.27 (C3), 127.17 (C1), 126.78 (C5), 137.84 (C2), 126.51 (C13), 127.13 (C14), 129.14 (C4), 125.93 (C10), 123.80 (C9).

Results and Discussion

Physical Properties

Analytical data are in good agreement with the formulae suggested (Table 1).

Molar Conductivity

The molar conductivity of the complexes measured in DMF at a concentration of 10^{-3} M falls in the range 0.16–0.56 Ω^{-1} cm^2 mol^{-1} (Table 1). These values are much less than those expected for 1:1 electrolytes. Hence the complexes are non-electrolytic in nature.

IR Spectral Studies

The diagnostic IR frequencies of the ligand (LH.HCl) and the corresponding metal complexes are listed in Table 2. The IR spectrum of the ligand exhibits very strong bands at 1617 and 1590 cm^{-1} which are assigned to ν(C = N) (azomethine nitrogen) and $\nu(C=N)_{ring}$ (phthalazine ring) modes, respectively. In the spectra of the complexes, these two bands shift to lower wavenumber and appear in the ranges 1562–1600 and 1525–1535 cm^{-1},

Table 1. Analytical, electronic, conductance and magnetic moment data of the ligand and its complexes

Compound	Empirical formula	Elemental analysis (%)[a]					λ_{max} (nm)	μ_{eff} (BM)[b]	Λ_m (Ω^{-1} cm^2 mol^{-1})
		C	H	N	M	Cl			
Ligand (LH)	$C_{15}H_{11}ClN_4$	63.45 (63.72)	3.83 (3.92)	19.56 (19.82)	—	—	330	—	—
$[Co(LH)_2Cl_2].2MeOH$	$C_{32}H_{30}N_8O_2Cl_4Co$	50.49 (50.61)	3.78 (3.98)	14.82 (14.76)	7.59 (7.76)	9.11 (9.33)	555, 472	4.1	0.56
$[Ni(L)_2].MeOH$	$C_{31}H_{24}N_8OCl_2Ni$	56.72 (56.92)	3.54 (3.70)	17.07 (17.13)	8.73 (8.97)	—	411, 820	Dia	0.32
$[Cu(L)_2]$	$C_{30}H_{20}N_8Cl_2Cu$	58.50 (57.47)	4.32 (3.22)	8.75 (17.87)	13.85 (10.14)	—	604	1.7	0.16
[Zn(LH)(L)Cl]	$C_{30}H_{21}N_8Cl_3Zn$	54.28 (54.16)	3.02 (3.18)	16.56 (16.84)	9.71 (9.83)	5.22 (5.33)	—	Dia	0.29

[a]Calculated values in parentheses.
[b]Dia = diamagnetic.

Table 2. Diagnostic IR bands (cm^{-1}) of the ligand and its transition metal complexes[a]

Compound	ν(NH)	ν(C = N)	ν(C = N) ring
Ligand (LH)	3346b	1617s	1590s
$[Co(LH)_2Cl_2].2MeOH$	3423m	1600	1535s
$[Ni(L)_2].MeOH$	n.o.	1562s	1535s
$[Cu(L)_2]$	n.o.	1597s	1526s
[Zn(LH)(L)Cl]	3446	1590s	1525s

[a]b = broad; s = strong; m = medium; n.o. = not observed.

respectively. These shifts suggest the coordination of the ligand through the azomethine nitrogen and phthalazine ring nitrogen. The LH.HCl ligand exhibits tautomerism: it can associate with the metal ion in either phthalazine tautomeric form or hydrazine tautomeric form.[24] In the case of LH.HCl and its Co(II) and Zn(II) complexes, the ν(NH) band is observed in the range 3346–3446 cm^{-1}. The absence of the ν(NH) band in the case of Ni(II) and Cu(II) complexes suggests coordination of ligand in phthalazone tautomeric form.

NMR Spectral Studies

The numbering scheme used for the assignments of the protons in the ligand and its Zn(II) complex is shown in Scheme 1. The ^1H NMR spectrum of the ligand exhibits a singlet at 14.57 ppm due to N2H. The singlet at 9.07 ppm, corresponding to two protons, is due to C7H and C15H protons. In the ^1H NMR spectrum of the Zn(II) complex, though there is no possibility for N2H proton to coordinate to the metal ion, its signal is shifted downfield to 12.38 ppm, and also there is a slight downfield shift of all the ring protons which indicates that the Zn(II) complex is present in free form (free from HCl salt). Downfield shift of C7H by 0.75 ppm in the Zn(II) complex compared to the ligand indicates involvement of azomethine nitrogen in coordination. All the other peaks are observed in the expected region.

A sharp peak at 2.17 ppm and a broad peak at 3.61 ppm in ^1H NMR spectrum and a signal at 48.47 ppm in the ^{13}C NMR spectrum of the ligand suggest the presence of lattice-held methanol in the ligand.

Mass Spectral Studies

The peak at $m/z = 283$ corresponds to the molecular ion peak $(M)^+$ of the ligand. The ESI mass spectra of Co(II), Ni(II), Cu(II) and Zn(II) complexes show the molecular ion peaks at $m/z = 693$ (M^+), 621 $(M+1)^+$, 626 $(M-1)^+$ and 662 (M^+), respectively, supporting the monomeric nature of the complexes.

Electronic Spectral Studies and Magnetic Behaviour

The electronic spectral study was carried out in order to assign the geometry around the central metal ions. The absorption wavelengths are listed in Table 1. The appearance of two d–d bands at 555 and 472 nm in the case of the Co(II) complex is attributed to $^4T_{1g} \rightarrow {}^4A_{2g}$ (ν_2) and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3) transitions, respectively, indicating an octahedral geometry around the Co(II) ion.[25] The electronic spectrum of the Ni(II) complex exhibits two bands at 411 and 820 nm, assignable to transitions $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1A_{2g}$, respectively, expected for square-planar

geometry.[26] In the case of the Cu(II) complex, only one band appears at 604 nm which can be assigned to $^2T_2 \rightarrow {}^2E$ transition expected for an tetrahedral geometry around the metal ion.[27] The magnetic moments of 4.1 BM for the Co(II) complex and 1.7 BM for the Cu(II) complex suggest high-spin octahedral and tetrahedral arrangements around these metal ions, respectively. The Ni(II) complex is diamagnetic indicating a square-planar arrangement.

Thermal Studies

Thermal study of the Co(II), Ni(II), Cu(II) and Zn(II) complexes was carried out in the temperature range 25–1000°C, with a heating rate of 10°C min^{-1} in nitrogen atmosphere.

The Co(II) complex decomposes in three stages. The first mass loss of 8.42% (calcd 8.44%) in the temperature range 40–50°C corresponds to the loss of two lattice-held methanol molecules. This temperature range rules out the possibility of lattice-held water molecules. In the second step, two chlorine atoms are lost in the range 200–320°C with a weight loss of 9.11% (calcd 9.33%). In the third step, the ligand (hydrazine tautomeric form) is lost in the range 320–800°C with a mass loss of 74.5% (calcd 74.46%).

The Ni(II) complex exhibits a two-step decomposition. In the first step, the weight loss is 4.9% (calcd 4.98%) in the range 40–50°C corresponding to the loss of one lattice-held methanol molecule. The second step corresponds to loss of the ligand (phthalazone tautomeric form) in the range 300–800°C with a weight loss of 86.08% (calcd 86.13%).

The Cu(II) complex decomposes in a single step in the range 300–600°C with a weight loss of 89.02% (calcd 89.86%) corresponding to loss of two ligand molecules in phthalazone tautomeric form.

A two-step decomposition is observable for the Zn(II) complex. In the first step, a chlorine atom is lost in the range 260–310°C with a weight loss of 5.22% (calcd 5.33%). In the second step, a weight loss of 84.7% (calcd 84.84%) is observed due to the loss of two isomeric ligand molecules, one in phthalazone tautomeric form and the other in hydrazine tautomeric form.

A plateau is obtained after heating the metal complexes above 800 °C, which corresponds to the formation of stable metal oxide. Tentatively assigned structures of the complexes are shown in Fig. 1.

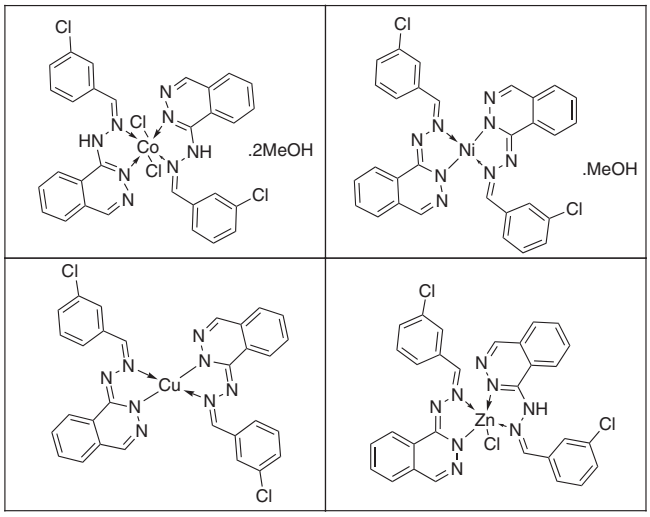


Figure 1. Tentatively assigned structures of the complexes.

Antibacterial and Antifungal Activity

The ligand and metal complexes were evaluated for antimicrobial activity against clinical isolates, using the serial dilution method in duplicate.[28] The antibacterial activity was tested against Gram-positive strains *Enterococcus faecalis* and *Streptococcus mutans* and Gram-negative strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using ciprofloxacin as reference standard. Antifungal activity was tested against *Candida albicans*, *Aspergillus fumigatus* and *A. niger* using ketoconazole as reference standard. The data obtained are summarized in Table 3.

The antibacterial activity is related to the cell wall structure of the bacteria. The thick cell wall of Gram-positive bacteria is made up of many layers of peptidoglycan and teichoic acids, whereas the thin cell wall of Gram-negative bacteria is made up of a few layers of peptidoglycan which are surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility, meaning that some antibiotics kill only Gram-positive bacteria leaving them ineffective against Gram-negative pathogens.[29] From the behaviour of the tested complexes which show more activity against Gram-positive than against Gram-negative bacteria, it could be expected that the observed activity is due to inhibition of cell wall biosynthesis.

The activity of the ligand is not significant against *S. mutans* whereas the metal complexes show excellent activity, also better

Table 3. Antibacterial and antifungal activity, measured as minimum inhibitory concentration (MIC), of the ligand and its transition metal complexes

Sample	Antibacterial MIC (μg ml^{-1})					Antifungal MIC (μg ml^{-1})		
	Gram positive		Gram negative					
	E. faecalis	*S. mutans*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*A. fumigatus*	*A. niger*
Ligand (LH)	3.12	25	50	—	25	0.19	3.12	6.25
$[Co(LH)_2Cl_2].2MeOH$	3.12	0.78	100	50	100	0.19	3.12	3.12
$[Ni(L)_2].MeOH$	6.25	0.78	25	—	25	3.12	—	25
$[Cu(L)_2]$	3.12	0.78	25	50	50	0.19	6.25	6.25
[Zn(LH)(L)Cl]	12.50	0.78	25	—	50	0.19	—	12.50
Ciprofloxacin	3.12	1.56	1.56	0.92	1.56	—	—	—
Ketoconazole	—	—	—	—	—	0.19	0.78	0.39

than that of the standard. In the case of *E. faecalis*, activity of Ni(II) and Zn(II) complexes is moderate, whereas that of the ligand and Co(II) and Cu(II) complexes is equivalent to the standard used. Such increased antibacterial activity of Cu(II) chelates compared to Ni(II) chelates has also been demonstrated for the Cu(II) derivatives of 2,6-diacetylpyridine bis(2-thenoylhydrazone).[30] Among the fungal strains used, *C. albicans* is inhibited predominantly by the ligand and its complexes except for the Ni(II) complex. The enhanced activity of the complexes compared to the ligand can be attributed to the enhanced lipophilic nature of the former.

Conclusions

3-Chlorobenzaldehyde hydralazine hydazone and its Co(II), Ni(II), Cu(II) and Zn(II) complexes have been synthesized, and characterized on the basis of elemental analyses, conductance measurements, magnetic moment, and spectral (IR, UV–visible, NMR (^{1}H, ^{13}C, 135-DEPT, CH-COSY), ESI mass) and thermal studies. In the complexes, the ligand acts as a neutral bidentate ligand coordinating through azomethine nitrogen and phthalazine ring nitrogen in either hydrazine (in the case of the Co(II) complex) or phthalazone (in the case of the Ni(II) and Cu(II) complexes) tautomeric form; in the case of the Zn(II) complex there is a mixture of hydrazine form and phthalazone form. Thermal studies support the presence of lattice-held methanol molecules in case of the Co(II) and Ni(II) complexes. The synthesized compounds exhibit excellent activity against the Gram-positive bacteria *E. faecalis* and *S. mutans*, and against the diploid fungus *C. albicans*. The synthesized complexes have the potential to act as anti-cariogenic agents.

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