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Research Article

Biological evaluation, synthesis and characterization of transition metal complexes of *N*-Methyl *o* and *p*-substituted benzohydroxamic acids

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Abstract

The four new transition metal complexes as Copper (II), Nickel (II), Cobalt (II) and Cadmium (II) of *N*-Methyl benzohydroxamate compounds have been prepared by refluxing 2:1 molar ratio of the *o*- and *p*-substituted hydroxamic acid ligands along with transition metal salts in hot toluene, using a Dean Stark water separator. The elemental analysis and FT-IR Spectroscopic technique were used to characterizing the hydroxamic acid ligands and its transition metal complexes. Spectroscopic analysis exhibits that the hydroxamic acid ligand behaves as a bidentate chelator and coordinating with metal atom through carbonyl oxygen and deprotonated hydroxyl group. The antibacterial and antifungal activity of hydroxamic acid ligands and their transition metal complexes were evaluated. Transition metal complexes display stronger antibacterial activity than their ligands because of the chelating property of ligands to metal ions and as a result chelation increases the lipophilicity of the complexes. However, ligands and their transition metals complexes did not display any antifungal activity against the *Aspergillus Niger* (fungus). This study shows that several transition metal complexes are successfully synthesized and these complexes exhibit high antibacterial activity with less toxicity. This research encourages further synthesis of transition metal complexes and the invention of future metal based drugs.

Keywords: Agar well method; Antibacterial activity; Antifungal activity; Coordination; FT-IR; Hydroxamic acids; Metal complexes

Introduction

Hydroxamic acids having a general formula RCONHOH derived from oxoacids by substituting the hydroxyl (OH) by NHOH. These compounds are the derivates of both hydroxylamines and carboxylic acids **[1]**. Hydroxamic acids play an essential role in many biochemicals, industrial, analytical

pharmaceutical fields. The antioxidant and antiradical properties of hydroxamic acids have also been reported [2]. It has also been observed that the hydroxamic acids are act as effective corrosion inhibitors for copper [3]. The hydroxamic acids are utilized in the production of therapeutics targeting cancer, Alzheimer's, cardiovascular diseases, allergic diseases and malaria [4, 5]. Furthermore, hydroxamate compounds have been employed as insecticides and antimicrobials [6]. Hydroxamic acid moieties has recently been imparted for their potential use as, tumor growth, inhibitors of hypertension, asthma, inflammation and other pharmaceutical application [7, 8]. These bidentate hydroxamic acid ligands are behave as a chelated ligand toward metals atoms as copper, nickel, cadmium, iron [1].

The transition metal complexes with various ligands play a major role in the form of numerous chemical compounds [9]. These diverse transition metals as, zinc copper, nickel and iron display important role in many biological systems [10] and show strong antibacterial activity against the different pathogenic microorganisms. The strong interaction of transition metal ion along with antibiotic drugs has shown the antifungal, antioxidant, antimalarial activities [10, 11]. Transition metal complexes display variable oxidation states and interact with various negatively charged molecules. Hence, the transition metal complexes have started the production of new metal-based drugs having pharmaceutical application and offers therapeutic opportunities [12, 13]. Transition metal compounds also used as anti-inflammatory and antidiabetic and anti-infective agents. These complexes have many side effects but most widely used anticancer agent due to its great efficacy [12]. Some of the transition metals such as cupper, vanadium, titanium and gold metal complexes show significant anticancer activity [14].

In this analysis, four new transition metal complexes as Copper (II), Nickel (II), Cobalt (II) and Cadmium (II) of *N*-Methyl benzohydroxamate were synthesized and characterized by elemental analysis and FT-IR Spectroscopic technique. Antibacterial and antifungal activity of these compounds has been determined.

Materials and methods

Reagents

All the chemicals were obtained from sigma Aldrich (99%) and utilized without any purification. All the chemicals and transition metal salts were of analytical grade. In this research work the chemicals which are utilized; *N*-Methylhydroxylamine Hydrochloride, *o*-Mithoxybenzoyl chloride *p*-Nitrobenzoyl chloride, Sodium Hydrogen carbonate ($NaHCO_3$), Potassium hydroxide (KOH), Toluene, Methanol, Ethyl ether, Ethyl alcohol, Dimethyl sulfoxide (DMSO), cobalt chloride, nickel chloride, copper sulphate and cadmium acetate.

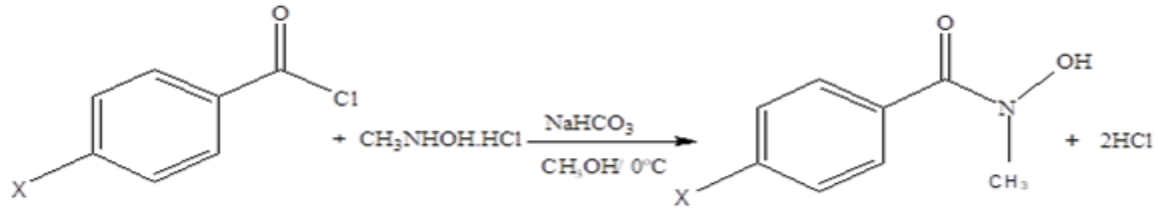
Synthesis of *o*- and *p*-substituted benzohydroxamic acid

The *o*- and *p*-substituted *N*-methyl benzohydroxamic acids, *N*-methyl *o*-methoxybenzohydroxamic acid (L_1) and *N*-methyl *p*-nitrobenzohydroxamic acid (L_2) have been prepared by in a similar fashion according to the method described earlier by Ulrich and Sayigh [15]. The *N*-methylhydroxylamine hydrochloride (25 mmol) was added to the dissolving mixture of sodium hydrogen carbonate (50mmol) and was poured *o*- and *p*-substituted benzoyl chloride (25mmol) dropwise. The ratio between these compounds was 2:1 molar ratio respectively. Sodium hydrogen carbonate ($NaHCO_3$) act as a catalyst. The resulting mixture was further stirred for 35 minutes. After the filtration, the solvent was evaporated. The solid mass was obtained on removing solvent at low pressure are

dissolved in hot acetic ether and again filtered. On cooling the hydroxamic acids have started to precipitate and the filtrate were placed in refrigerator overnight to obtain the desire crystals. The general synthetic method is carried out shown in (Scheme 1).

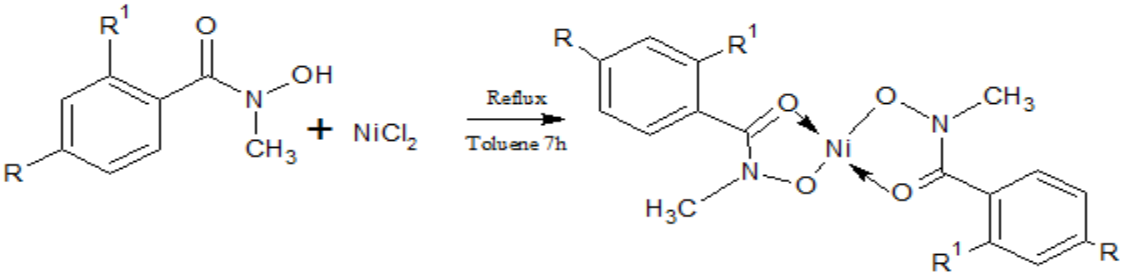
Synthesis of Metal (II) *N*-Methyl benzohydroxamate complex

The Metal (II) *N*-methyl benzohydroxamate compounds have been prepared by refluxing 2:1 molar ratio of the ligand *N*-methylbenzohydroxamic acid with transition metal (II) salts in hot toluene about 6-7h with refluxing and Dean-Stark apparatus was used to remove the water. After cooling and filtration, the solution was evaporated at low pressure. Solid residue was precipitated by boiling acetic ether and recrystallized in ethyl alcohol (Scheme 2).



Where X is NO_2

Scheme 1. Reaction between hydroxylamine and *p*-substituted benzoyl chloride



Where R is NO_2 and R^1 is OCH_3

Scheme 2. Reaction between the *o*- and *p*-substituted hydroxamic acids and transition metal ions

Anti-bacterial assay

Agar well method was utilized to determine the antibacterial activity of the synthesized compounds against different types of bacteria including *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Staphylococcus aureus*. The Mueller Hinton agar plates were made by following instructions. The wells of 6 milimeters (mm) width were made in to the petri dishes seeded with bacterial culture by the help of sterilized cork borers. Transition metal hydroxamate complexes were added in 2mL of DMSO. The 100 mL of stock solution (2mg/mL in dimethyl sulfoxide) of complexes were poured in each well. The petri dishes were then incubated overnight at 37°C. Pure DMSO and Amoxicillin (30 µg/mL) were utilized as positive and negative controls. The antibacterial activity of the complexes was investigated by measuring the diameters of inhibition zone in millimeters **[16, 17]**.

Ant-fungal assay

Anti-fungal assay of synthesized compound has been determined by the agar well diffusion method. *Aspergillus Niger* (fungi) was utilized for antifungal activity of synthesized compound and this fungus was isolated from environment. The solution of hydroxamic acids and their TMCs (0.02mg)

used for antifungal activity were made in 1mL of DMSO assayed against *Aspergillus niger* (fungus). After this, the solution of potato dextrose agar (PDA) was prepared and autoclaved then this potato dextrose agar (PDA) solution was poured in petri dishes under. The walls (6mm) were prepared in petri dishes by sterilized borers. The antifungal activity of the compounds was noted after 3, 7, and 14 days. Moreover, the diameter of inhibition zone was determined at the end of the incubation period [13, 18].

Results and discussion

Synthesis of *o*- and *p*-substituted benzohydroxamic acid and their complexes

These ligands, *N*-methyl *o*-methoxybenzohydroxamic acid (L_1) and *N*-methyl *p*-nitrobenzohydroxamic acid (L_2) were prepared by reacting *N*-methylhydroxylamine hydrochloride with acid chloride. Sodium hydrogen carbonate ($NaHCO_3$) was utilized as catalyst. Transition metal hydroxamate complexes were obtained by the reaction between some transition metal ions such as, Copper (II), Nickel (II), Cobalt (II) and Cadmium (II) and the relevant hydroxamic acids ligands (L_1 and L_2) in 1:2 molar ratios in hot toluene. The calculated values of ligands and complexes are in good yield with according to the observed (Table 1 & 2).

Table 1. Analytical data along with physical properties of the hydroxamic acid ligands

Compound	Colour	Yield (%)	M.P. (°C)	Percentage found (Calculated)		
				C	H	N
L_1	Colourless crystals	70%	138-139	59.19 (59.85)	5.84 (6.08)	6.54 (7.69)
L_2	Orange crystals	85%	112-113	48.49 (48.98)	3.85 (4.10)	13.54 (14.28)

Table 2. Analytical data along with physical properties of the transition metal complexes

Compounds	Colour	Yield (%)	M.P.(°C)	Percentage found (Calc.)		
				C	H	N
$L_1Cu(II)$	Sharp green solid	64%	155-156	50.86 (51.00)	4.49 (4.75)	6.29 (6.60)
$L_2Cu(II)$	Light green solid	68%	259-260	41.19 (42.34)	3.08 (3.10)	12.28 (12.34)
$L_1Ni(II)$	Light green solid	65%	185-186	51.48 (51.59)	4.70 (4.81)	5.47 (6.68)
$L_2Ni(II)$	Lemon green solid	73%	124-125	42.05 (42.80)	3.10 (3.13)	11.28 (12.47)
$L_1Co(II)$	Dark pink solid	60%	181-182	51.23 (51.56)	4.72 (4.80)	6.45 (6.68)
$L_2Co(II)$	Dark brown Solid	65%	247-248	42.65 (42.77)	3.08 (3.13)	12.28 (12.47)
$L_1Cd(II)$	White solid	67%	292-293	45.47 (45.72)	4.11 (4.26)	5.68 (5.92)
$L_2Cd(II)$	Sharp yellow solid	70%	272-273	37.11 (38.22)	2.35 (2.80)	11.01 (11.14)

Infra-red spectroscopy of hydroxamic acid ligands

The IR spectra of hydroxamic acid ligands are reported in solid state in the region 4000-400cm^{-1}. The significant stretching absorptions are given in (Table 3) shows the IR spectra of ligands (L_1 and L_2) and their transition metal complexes. The key infrared absorption bands are *v*(O-H), *v*(C=O), *v*(C-N) and *v*(N-O) stretching vibrations of the hydroxamate moiety. In ligand L_1 and L_2, the *v*(OH) band is found in the range of 3128-3423cm^{-1} as broad band. The absence of *v*(OH) band in the spectra of transition metal complexes indicating the replacement of hydroxyl by transition metal ions and ligand exist as deprotonated form in complexes. The carbonyl group is to be found in the range of 1609-1718cm^{-1} notably, below the general ketonic C-O stretching vibration of 1720cm^{-1}. The existence of C=O peak at lower value in association with broad hydroxyl OH peak can have related with intermolecular hydrogen bonding. The *v*(C-N) and *v*(N-O) appear as sharp bands at 1440-1441cm^{-1} and 917-958cm^{-1} respectively.

Table 3. Infrared spectral data for the ligands (L_1 and L_2) and their transition metal complexes

Compounds	*v*(OH)	*v*(C=O)	*v*(C-N)	*v*(N-O)	*v*(M-O)
L_1	3128	1609	1440	917	---
L_2	3423	1718	1441	958	---
L_2Cu(II)	---	1594	1459	959	511
L_1Ni(II)	---	1603	1474	940	518
L_2Ni(II)	---	1603	1499	952	576
L_1Co(II)	---	1596	1464	938	571
L_2Co(II)	---	1596	1475	939	563
L_1Cd(II)	---	1584	1467	944	498
L_2Cd(II)	----	1584	1475	946	561

Infra-red spectroscopy of transition metal complexes

To compare the vibrational spectra of hydroxamic acid ligands with the derived transition metal complexes. The significant characteristic is the absence of hydroxyl *v*(OH) peak in the IR spectra of complexes, due to the replacement of OH of free ligands by transition metal ions, indicating the existence of ligand as deprotonated form in complexes. The significant peak in the free hydroxamic acid ligands is the carbonyl groups which have stretching frequencies occur in the range of 1609-1718cm^{-1}. The shifting of stretching frequency of C=O group to lower value range 1584-1603cm^{-1}in the IR spectra of transition metal compounds, which confirm the synthesis of metal complexes **[19, 20]**. Thus, suggested on the transition metal ion a five membered chelating ring is formed which furthermore supported the existence of hydroxamic acid as a bidentate chelating ligand in complexes **[21]**.

The C-N bands are reported in the region 1459-1499cm^{-1}on complexation. More interestingly, the carbonyl oxygen acts as a donor atom with electron withdrawal from the carbonyl group enhance the electron density on C-N group. Hence, increase of the C-N frequency and decrease of the carbonyl frequency are expected as outcome in the IR spectra of complexes **[22]**. The strong absorption bands are observed for the *v* (N-O) in the range of 938-959cm^{-1} **[20]**. The weak and short bands are observed in the low energy region are attributed to *v*(M-O) stretching vibration. Several transition

metal complexes show different M-O stretching vibrations such as, for Copper (II) hydroxamate complex, these M-O bands are observed in the region 511cm^{-1}, for Nickel (II) hydroxamate complexes these bands are observed in the region 518-576 cm^{-1}, for Cobalt (II) hydroxamate complexes these bands are observed in the region 563-571cm^{-1} and for Cadmium (II) hydroxamate complexes these bands are observed in the region 498-561cm^{-1}. Presence of these peaks confirm the formation of chelation between transition metal ions as, Copper(II), Nickel(II), Cobalt(II) and Cadmium(II) to carbonyl oxygen(C=O) by *O*, *O* donor sites of synthesized ligands [21, 23].

Biological activity

Antibacterial activity

The in-vitro antibacterial activities of hydroxamic acid ligands and its metal complexes were evaluated by the agar well assay against different pathogenic bacterial strains as, Gram negative (*Escherichia coli*, *Klebsiella pneumoniae Salmonella typhi*), Gram positive (*Staphylococcus aureus*). The pure dimethyl sulfoxide (DMSO) and Amoxicillin (30µg/mL) were utilized as positive and negative controls. The inhibition zone values are reported in mm given in the (Table 4). When Inhibition zone values are less than 10mm reported as weak, from 10-16mm are moderate and above than 16mm are shown as active [20].

It was reported that all transition metal complexes show inhibition against all tested Gram-negative and Gram-positive bacterial strains at various rates. The newly synthesized transition metal complexes display effective bactericidal activity as compare with its hydroxamic acid ligands, which is due to the chelation property of hydroxamic acid ligand to the metal ions. Thus, chelation will increase the lipophilicity of the complexes and an effective penetration of complexes through the cell wall of bacteria to inhibit the growth of various strains of bacteria [11, 13, 18]. Furthermore, the hydroxamic acids and its transition metal complexes such as, Coppe (II), Nickel (II), Cobalt (II) and Cadmium (II) show moderate action against the four strains of bacteria.

Table 4. Antibacterial activities of hydroxamic acid ligands (L_1 and L_2) and its transition metal complexes

Compounds	Inhibition zone diameter (mm)			
	Escherichia coli	***Klebsiella pneumoniae***	***Salmonella typhi***	***S. aureus***
L_1	10	13	10	8
L_2	11	14	9	13
L_1Co (II)	8	15	7	11
L_2Co(II)	9	11	7	14
L_1Cu(II)	10	12	7	11
L_2Cu(II)	8	12	6	11
L_1Ni(II)	7	14	11	9
L_2Ni(II)	6	13	8	10
L_1Cd(II)	8	15	7	11
L_2Cd(II)	11	12	9	12
Amoxicillin	30	25	30	23

Antifungal activity

The hydroxamic acids and its newly synthesized metal complexes were tested by diffusion method for their antifungal activity against the *Aspergillus Niger* (fungus). The antifungal activity of all compounds was compared with the control. Hydroxamic acids and its transition metal complexes did not

show any antifungal activity against the tested fungus (*Aspergillus Niger)*. The fungal growth is very high in this case therefore; all the ligands and their synthesized complexes are inactive against this fungus. Other reason will be the lesser amount of the ligands and complexes were formed during this study. To show antifungal activity, the higher concentration of test solution of ligands and its metal complexes were required **[11, 13]**.

Conclusion

In this study hydroxamic acids and their transition metal complexes were successfully synthesized and characterized. The biological activities of ligands and their metal complexes were investigated. Characterization of ligands and their metal complexes were carried out by Elemental analysis and IR spectroscopic technique. The spectroscopic studies showed that hydroxamic acid is a bidentate ligand and coordination with metal ion takes place via oxygen atom. Hydroxamic acid ligands and their complexes exhibits strong antibacterial activity but did not display any antifungal activity against the given fungus.

Authors' contributions

Conceived and designed the experiments: N Khan & Samiullah, Performed the experiments: N Khan & Samiullah, Analyzed the data: B Shireen, Contributed materials/ analysis/ tools: I Ali, A Akbar & AU Rehman, Wrote the paper: B Shireen & F Behlil.

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