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Antimicrobial activity of newly synthesized hydroxamic acid of pyrimidine-5-carboxylic acid and its complexes with Cu(II), Ni(II), Co(II) and Zn(II) metal ions

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ABSTRACT

*Four metal complexes of new hydroxamic acid, 2,4,6-trioxo-1,3-di-p-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) with Cu(II), Ni(II), Co(II) and Zn(II) metal ions have been synthesized. The hydroxamic acid and its metal complexes were characterized by simple analytical techniques such as repeated melting point (M.P.) determination, elemental analysis, running their thin layer chromatography for single spot, and spectroscopic techniques such as I.R., H^1-NMR and UV-Vis. (only for metal chelates) spectroscopy. The antimicrobial activity of the hydroxamic acid and their metal complexes were screened against two species of bacteria and two species of fungi by Serial Dilution Method. Metal complexes were found more active against both bacteria as well as fungi in the antimicrobial screening test.*

Keywords: Hydroxamic acids, antimicrobial activity, metal complexes

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INTRODUCTION

Hydroxamic acids show wide spectrum of biological activities and generally have low toxicities [1-2]. Hydroxamic acids are very well known for their antibacterial [3-5], antifungal [6-7], antitumor [8-10], antituberculous [11] and antimalarial [12] properties. Hydroxamic acids are inhibitors of enzymes such as prostaglandin H_2 synthatase [13], peroxidase [14], urease [15] and matrix metalloproteinase [16]. Cinnamohydroxamic acids have been used for treatment of the symptoms of asthma and other obstructive airway diseases which inhibit 5-lipoxygenase [17]. A number of hydroxamic acid analogues have been shown to inhibit DNA (dinucleic acid) synthesis by inactivating the enzyme ribonucleotide reductase (RNR) [18]. Naturally occurring hydroxamic acid, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) is a powerful antibiotic present in maize [19]. Antiradical and antioxidant properties of hydroxamic acids have also been observed [20]. Hydroxamic acids play important role in many chemical, biochemical, pharmaceutical, analytical and industrial fields [21]-[25]. These diverse biological activities of hydroxamic acids are due to their complexation properties towards transition metal ions [26-27]. Siderophores are Fe(III) complexes of naturally occurring hydroxamic acids, involved in the processes of iron transport from the environment to the living organisms [28-29]. Hydroxamic acids after deprotonation acts as bidentate ligands and octahedral complexes are formed through the co-ordination of two oxygen atom of the –CONHO- group. This type

of coordination has been studied with Cr(III), Fe(III), Ni(II), Co(II) and Zn(II) ions in the solid state as well as in solutions, indicating the formation of octahedral complexes [30].

We report herein the synthesis, structural features and antimicrobial activity of new hydroxamic acid, 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) as well as its metal complexes **4a-d** with Cu(II), Ni(II), Co(II) and Zn(II) metal salts.

EXPERIMENTAL SECTION

Reagents and methods

All chemicals used in the present investigation were of analytical reagent grade. 1,3- Di-*p*-tolylbarbituric acid was synthesized by previously known method in the laboratory. Copper acetate monohydrate, nickel acetate tetrahydrate, cobalt acetate tetrahydrate and zinc acetate dihydrate were purchased from E-Merck. Triethyl amine and ethyl chloroformate were purchased from Spectrochem. Hydroxylamine hydrochloride potassium hydroxide and diethyl ether were obtained from S.D. Fine chemicals limited, India. All the synthesized compounds were analyzed for C, H and N by elemental analyses, model 1108 (EL-III). H^1-NMR spectra (400MHz) were recorded on JNM ECX- 400P (Joel, USA) spectrometer using TMS as an internal standard. IR absorption spectra were recorded in the 400-4000 cm^{-1} range on a Perkin-Elmer FT-IR spectrometer model 2000 using KBr pellets. UV-Visible spectra of metal complexes were recorded in DMSO solvent at room temperature on Simadzu Spectro Photometer (model no. 1601). Melting points were determined using Buchi M-560 and are uncorrected. These reactions were monitored by thin layer chromatography (TLC) on aluminium plates coated with silica gel 60 F_{254} (Merck). UV radiation and iodine were used as the visualizing agents.

Synthesis of the hydroxamic acid

Synthesis of 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**)

Synthesis of ligand **3** was carried out in two steps as follows:

Step 1: Synthesis of ethyl 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**2**)**.**
Ethyl 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**2**) was synthesized by the reported method of Kuhne *et al* [31]. 1,3- Di-*p*-tolylbarbituric acid [5g, 0.016 mol.], triethyl amine [2.30ml, 0.0168 mol.] and dimethyl aminopyridine (DMAP) [0.10g] were dissolved in 20 ml of dichloromethane (DCM) and the solution was cooled to 0^0C. Then ethyl chloroformate [1.60ml, 0.0165 mol] was added drop-wise over half an hour. The mixture was subsequently stirred for 12 hours at 0^0C and then, allowed to warm to the room temperature for 7 hours. The product is extracted in chloroform and dried over Na_2SO_4. Further, chloroform was evaporated to dryness and the crude product was recrystallised from ethyl alcohol to yield pure compound **2**.

Step 2: 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) from ethyl 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**2**)**.**

Synthesis of 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) was carried out by adopting a method similar to that described by Griffith *et al* [32]. The mixture of hydroxylamine hydrochloride [1.87g, 0.026 mol] and aqueous potassium hydroxide [2.19g, 0.039 mol] was added drop-wise to a methanolic solution of ethyl 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**2**) [5g, 0.013 mol]. The solution was stirred at room temperature for 72 hours and then acidified to pH 5.5 using 5% HCl solution. After filtration the solvent was removed under vacuum to yield a crude solid. The crude product was recrystallised from hot water to yield pure compound **3**.

Synthesis of metal complexes

Synthesis of Cu(II), Ni(II), Co(II) and Zn(II) complexes of 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**)**.**

Copper acetate monohydrate [0.136g, 0.00068 mol.] in cold water was added with stirring to 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) [0.50 g, 0.00136 mol.] in EtOH (20 ml) in a round bottom flask. The contents were stirred for about 6 hours and then reduce to half volume under vacuum.

Yellowish brown precipitate of **4a** was appeared after adding petroleum ether. The precipitate was filtered, washed with small amounts of Et_2O and dried over $CaCl_2$ in a vacuum desiccator.

Similarly, complexes **4b** of Ni(II) , **4c** of Co(II) and **4d** of Zn(II) with 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) were synthesized by taking nickel acetate tetrahydrate, cobalt acetate tetrahydrate and zinc acetate dihydrate respectively.

Infrared Spectra

In the IR spectra (**Table 1**), carbonyl stretching vibrations of hydoxamic acid exhibit a medium sharp, intense band in the region 1660 cm^{-1} [33]. This band has shifted towards negative region 1626-1609 cm^{-1} in the metal complexes indicating the coordination of the ligand with the metal ion through oxygen of the carbonyl group. The symmetric N-O stretching vibration which obtained in the region 1120 cm^{-1} in the IR spectra of ligand, have shifted to lower side in the IR spectra of their metal complexes suggesting the coordination of ligand to the metal ion through oxygen of the N-O moiety [34]. The presence of water molecules within the coordination sphere of all metal chelates were supported by broad bands in the region 3450-3280 cm^{-1} and 850-800 cm^{-1} due to stretching and deformation modes of coordinated water molecules, respectively. The appearance of new band in the IR spectra of metal chelates in the region 551-519 cm^{-1} is probable due to formation of M-O bonds [35].

Table1. IR spectral data of hydroxamic acid, 3 and its metal complexes, 4a-d

Compound	ν(C=O)cm^{-1}	ν(C-N) cm^{-1}	ν(N-O) cm^{-1}	ν(M-O) cm^{-1}
3	1660	1349	1120	-
4a	1609	1327	1036	551
4b	1624	1355	1023	519
4c	1626	1384	1023	540
4d	1629	1350	1025	541

H^1-NMR Spectra

The hydroxamic acid **3** shows a one proton singlet at δ 1.14 due to –N**H**-O proton, probably due to the magnetic anisotropy of the neighboring carbonyl group, electronegativity of nitrogen and H- bonding [36]. One proton singlet in hydroxamic acid **3** appeared at δ 2.49 due to –N-O**H** proton [37]. Due to proton exchange in D_2O this signal disappeared in the spectra indicating the possibility of –OH proton. In the hydroxamic acid **3,** a one proton singlet due to $–C_5$-**H** proton was obtained at δ 5.26. A multiplet due to six protons of two –C$\mathbf{H_3}$ groups of hydroxamic acid **3** appeared at δ 2.01 – 2.09 and another multiplet due to eight protons of two aromatic rings, Ar-**H** was observed at δ 7.17 - 7.20 in the hydroxamic acid **3**. H^1-NMR spectra of metal complexes **4a-d** were not taken due to very less solubility in suitable organic solvents.

UV- Visible Spectra

Cu (II) complex

In the electronic spectra of Cu (II) complex, **4a**, three absorption bands in the region. 13157, 16949 and 23809 cm^{-1} have been observed, which correspond to the transitions $^2B_{1g} \rightarrow {}^2A_1g$, $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_1g \rightarrow {}^2E_1g$ suggesting distorted octahedral geometry [38-40].

Ni (II) complex

The electronic spectra of Ni (II) complex, **4b,** exhibit three bonds in the region 13333, 16129 and 20833 corresponding to the transitions $^3A_{2g} \rightarrow {}^2T_{2g}(F)$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$, $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ respectively which show an octahedral geometry for these complexes [41-42].

Co (II) complex

In the electronic spectra of Co (II) complex, **4c** three absorption bands in the region 12903, 14925 and 20200 cm^{-1} were seen, which may correspond to the transition $^4T_{1g} \rightarrow {}^4T_{2g}(F)$, $^4T_{1g} \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$, respectively, indicating an octahedral geometry [43-44].

Zn (II) Complex

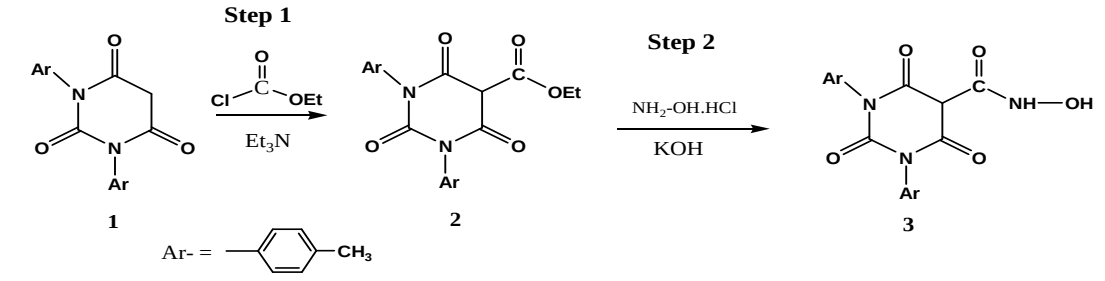
No significant absorption was noticed in Zn (II) complex, **4d**, above 400nm probably due to diamagnetic nature and completely filled d- orbitals. In the Zn (II) complex only transitions due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ were seen.

Antimicrobial activity

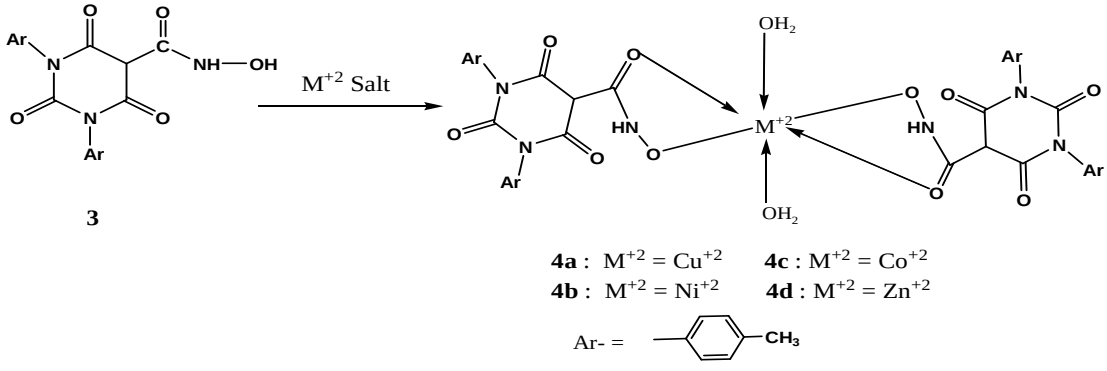
Synthesized ligand **3** and metal chelates **4a-d** were tested for their antimicrobial activity against two bacteria *Staphylococcus aureus* and *Escherichia coli* and two fungi *Aspregillus flavus* and *Aspergillus niger* by adopting Serial Dilution Method [45-46]. The micro-organisms were cultured in nutrient agar medium [46] which was prepared by taking 6.0 gm peptone, 1.50 gm beef extract, 1.0 gm dextrose, 3.0 g yeast extract, 1.50 g agar (for slant) in 1 liter distilled water for bacteria and 10.0g peptone, 20.0g dextrose, 20.50g agar (for slant) in 1 liter distilled water for fungi. Measured quantities of the test compounds were dissolved in propylene glycol. First set was prepared for primary screening by taking 1ml (2000µg/ml) of seeded broth (obtained by 1:100 dilution of the incubated micro-organism broth culture) in 10 well cleaned sterilized test tubes and gradual dilution process was continued for all the ten tubes using a fresh pipette each time. All the above sets of tubes were incubated at 37^{o}C for 24 hours for bacteria and at 28^{o}C for 96 hours for fungi. The Minimum Inhibitory Concentration (MIC) values were determined at the end of the incubation period. Active synthesized compounds, found in the primary screening were further tested for secondary screening by taking 1ml (1500µg/ml) of seeded broth against all microorganisms.

RESULTS AND DISCUSSION

In this present work synthesis of 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**3**) was carried out by adding an aqueous solution of hydroxylamine hydrochloride and potassium hydroxide drop-wise to a methanolic solution of ethyl 2,4,6-trioxo-1,3-di-*p*-tolyl-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**2**). The solution was continuously stirred for 72 hours at room temperature, which on acidification gave crude compound **3 (Scheme I).** Compound **3** on stirring with different metal salts, gave corresponding metal complexes **4a-d (Scheme II).**



Scheme I



Scheme II

All the metal complexes obtained were solid and stable at room temperature and insoluble in most of the common organic solvents. The spectroscopic and analytical data **(Table 2)** are in good agreement with theoretical values for the ligand and metal complexes**.**

Table 2. Analytical data and physical properties of the hydroxamic acid, 3 and metal complexes, 4a-d

S.No.	Compound	Molecular Formula	Color	Percentage Elemental Analysis Calc./ (Found)			M.P. /D.T. (^{0}C)	Yield (%)
				C	H	N		
1	**3**	$C_{19}H_{17}N_3O_5$	Dark Pink	62.12 (61.90)	4.63 (4.52)	11.44 (11.28)	156^{0}C	85%
2	**4a**	$[Cu(C_{19}H_{16}N_3O_5)_2.2H_20]$	Yellowish Brown	54.87 (53.27)	4.33 (4.30)	10.10 (9.90)	248^{0}C	71%
3	**4b**	$[Ni(C_{19}H_{16}N_3O_5)_2.2H_20]$	Light Pink	55.22 (54.70)	4.39 (4.25)	10.13 (10.10)	270^{0}C	70%
4	**4c**	$[Co(C_{19}H_{16}N_3O_5)_2.2H_20]$	Pink	55.20 (54.70)	4.35 (4.25)	10.16 (10.20)	322^{0}C	75%
5	**4d**	$[Zn(C_{19}H_{16}N_3O_5)_2.2H_20]$	Brown	54.67 (53.80)	4.32 (4.30)	10.07 (9.89)	310^{0}C	70%

Antimicrobial Activity

The newly synthesized hydroxamic acid **3** and its metal chelates **4a-d** were tested for their antimicrobial activity against two bacteria *Staphylococcus aureus* and *Escherichia Coli* and two fungi *Aspergillus Niger* and *Aspergillus flavus*. The experimental results of **MIC** values **(Table 3)** show moderate activity of all the compounds against both bacteria and fungi. Further, it has been found that the metal complexes were more active than hydroxamic acid. This increased antimicrobial activity of the complexes as compared to the hydroxamic acid is probably due to the fact that chelation increases the lipophilicity of the complexes, which subsequently enhances the penetration through the lipid layer of the cell membrane and restricts further multiplicity of the microorganism [46]. Among the metal complexes, Cu (II) complex **4a** was found most active against both bacteria and fungi. The higher antimicrobial activity of Cu (II) complex may be due to higher stability constant of copper complexes.

Table 3. The minimum inhibitory concentration (µg/ml) MIC values of hydroxamic acid, 3 and metal complexes, 4a-d

S. No.	Compound	Bacteria		Fungi	
		Staphylococcus aureus	*Escherichia coli*	*Aspergillus niger*	*Aspergillus flavus*
1	**3**	325	325	250	325
2	**4a**	**125**	**125**	**250**	**250**
3	**4b**	325	500	500	325
4	**4c**	500	250	250	325
5	**4d**	500	250	250	250

CONCLUSION

Four new metal chelates, **4a-d** with ligand **3** have been synthesized and characterized. Octahedral geometries have been proposed for the prepared metal complexes. Synthesized hydroxamic acid and its metal chelates were screened for antimicrobial activity against two species of bacteria and two species of fungi. A comparative study of the MIC values of the ligand and its complexes show that complexes exhibit higher antimicrobial activity than free ligand. Among the metal complexes, Cu(II) complex, **4a** was found most active against both bacteria and fungi.

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