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Synthesis, Characterization and Antimicrobial Activity of Novel Hydroxamic Acids of Pyrimidine-5-Carboxylic Acid and Their Complexes

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Abstract- A series of metal complexes of Cu(II), Ni(II) and Co(II) have been synthesized with new hydroxamic acids, 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (5) and 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (6). During the course of present investigations, simple analytical and spectroscopic techniques such as repeated melting point (M.P.) determination, elemental analysis, running their thin layer chromatography for single spot, I.R., H^1-NMR and UV-Vis. (only for metal chelates) spectral studies were employed to indentify the purity and structure of hydroxamic acids and their metal chelates. Free ligands and their metal complexes have been screened for their antimicrobial activity against various species of fungi and bacteria.

Keywords: Hydroxamic acids, antimicrobial activity, metal complexes.

I. INTRODUCTION

Hydroxamic acids play important role in many chemical, biochemical, analytical, pharmaceutical and industrial fields [1]-[6]. Hydroxamic acids have a wide spectrum of biological activities and generally have low toxicities [7]. Antibacterial [8]-[10], antifungal [11], antitumor [12] and anti-inflammatory activities of hydroxamic acids are due to their ability to inhibit various enzymes, such as matrix metalloproteinases [13], urease [14] and proxidase [15]. Anticancer properties of some aliphatic and aromatic hydroxamic acids have also been studied [16]-[17]. Suberoylanilide hydroxamic acid (SAHA) has been proved as potent drug for cancer therapy [18]. Recent studies show that hydroxamic acid derivatives strongly inhibit melanin synthesis via deactivation of tyrosinase[19]. Antiradical and antioxidant properties of hydroxamic acids have also been observed [20]. Biological activities of hydroxamic acids are due to their complexing properties towards transition metal ions [21]-[22].

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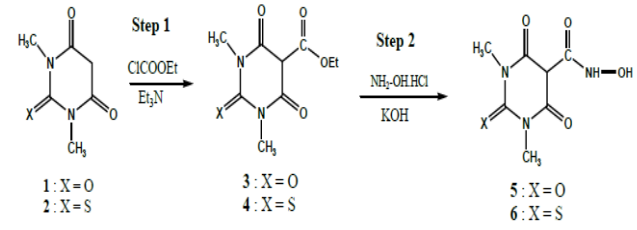
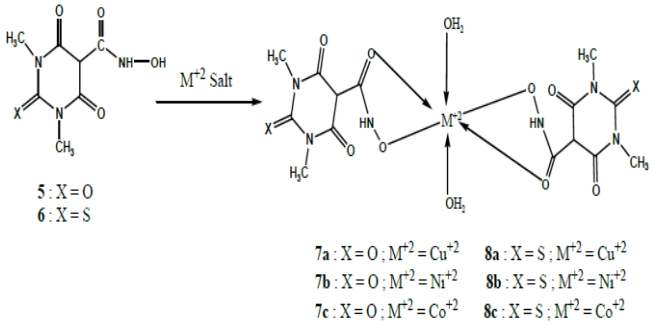
In view of the above applications, we herein report the synthesis, structural features and antimicrobial activities of some new hydroxamic acids viz. 1,3-dimethyl-2,4,6-trioxo-1, 2, 3, 4, 5, 6-hexahydropyrimidine-5- carboxylic acid hydroxamide (**5**) and 1, 3- dimethyl- 4, 6-dioxo- 2-thioxo-1,2,3,4,5,6-hexahydropyrimidine- 5-carboxylic acid hydroxamide (**6**) as well as their metal complexes **7a-c** & **8a-c** with Cu(II), Ni(II) and Co(II) metal salts.

II. RESULTS AND DISCUSSION

In this present work synthesis of 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydro-pyrimidine-5-carboxylic acid hydroxamide (**5**) and 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide (**6**) were carried out by adding an aqueous solution of hydroxylamine hydrochloride and potassium hydroxide drop-wise to a methanolic solution of ethyl 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydro-pyrimidine-5-carboxylate (**3**) and ethyl 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**4**) respectively. The solution was continuously stirred for 72 hours at room temperature, which on acidification give crude solid. **(Scheme 1).** Compounds **5** and **6** on stirring with different metal salts, gave corresponding metal complexes **7a-c** and **8a-c (Scheme 2).**



Scheme 1



Scheme 2

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All the metal chelates obtained were colored solid and stable at room temperature and insoluble in most of the common organic solvents. The analytical and spectroscopic data are in good agreement with theoretical values for the ligands and metal complexes **(Table 1)**

Table1. Analytical data and physical properties of the hydroxamic acids 5, 6 and metal complexes 7a-c, 8a-c.

S.No.	Compds.	Molecular Formula	Color	Percentage Elemental Analysis Calc./ (Found)			M.P. /D.T. (ºC)	Yield (%)
				C	H	N		
1	**5**	$C_7H_9N_3O_5$	Creamish White	39.07 (41.50)	4.18 (4.10)	19.53 (19.22)	121^{0}C	85%
2	**7a**	$[Cu(C_7H_8N_3O_5)_2.2H_20]$	Green	31.87 (31.60)	3.03 (2.90)	15.93 (15.45)	343^{0}C	84%
3	**7b**	$[Ni(C_7H_8N_3O_5)_2.2H_20]$	Light Blue	32.18 (32.60)	3.06 (2.78)	16.10 (15.85)	393^{0}C	76%
4	**7c**	$[Co(C_7H_8N_3O_5)_2.2H_20]$	Violet	32.18 (32.45)	3.06 (2.92)	16.09 (15.87)	346^{0}C	79%
5	**6**	$C_7H_9N_3O_4S$	Light Yellow	36.36 (37.20)	3.89 (3.62)	18.18 (17.80)	94^{0}C	94%
6	**8a**	$[Cu(C_7H_8N_3O_4S)_2.2H_20]$	Bluish Green	30.05 (29.80)	2.86 (2.72)	15.02 (14.84)	270^{0}C	71%
7	**8b**	$[Ni(C_7H_8N_3O_4S)_2.2H_20]$	Bluish Green	30.28 (30.15)	2.89 (2.80)	15.14 (14.94)	320^{0}C	70%
8	**8c**	$[Co(C_7H_8N_3O_4S)_2.2H_20]$	Purple	30.27 (30.30)	2.88 (2.58)	15.13 (14.83)	360^{0}C	75%

Antimicrobial Activity

All the newly synthesized hydroxamic acids **5** and **6** and their metal chelates **7a-c** and **8a-c** were tested for their antimicrobial activity against two bacteria *Staphylococcus aureus* and *Escherichia Coli* and two fungi *Aspergillus niger* and *Aspergillus flavus* .

III. EXPERIMENTAL

Reagents and methods

All chemical used in the present investigation were of analytical reagent grade. 1,3- Dimethyl barbituric acid and 1,3-dimethyl-2-thiobarbituric acid were purchased from Alfa aesar Grate Britain. Copper acetate monohydrate, nickle acetate tetrahydrate and cobalt acetate tetrahydrate 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 analysed for C, H and N by elemental analyser, model 1108 (EL-III). H^1-NMR spectra (400MHz) were recorded on JNM ECX- 400P (Jeol, 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 pallets. UV-Vis. 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 acids

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

Synthesis of ligand **5** was carried out in two steps as follows:

Step 1: Synthesis of ethyl 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate **(3).**

Ethyl 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate (**3**) was synthesized by the reported method of **Kuhne *et al*** [23]. 1,3-Dimethyl barbituric acid (**1**) [5g, 0.032 mol.] and triethyl amine [3.69ml, 0.034 mol.] and dimethyl aminopyridine (DMAP) [0.15g] were dissolved in 20 ml of dichloromethane (DCM) and the solution was cooled to 0^0 C. Then ethyl chloroformate [3.33ml, 0.033 mol.] was added drop-wise over half an hour. The mixture was subsequently stirred for 12 hours at 0^0C, 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 crude product was recrystallised from ethyl alcohol to yield pure **3**.

Step 2: Synthesis of 1,3-dimethyl- 2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide **(5)** from ethyl 1,3- dimethy l-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylate **(3).**

Synthesis of 1,3-dimethy l-2,4,6-trioxo- 1,2,3,4,5, 6-hexahydopyrimidine- 5-carboxylic acid hydroxamide (**5**) was carried out by adopting a method similar to that described by **Griffith *et al*** [24]. The mixture of hydroxylamine hydrochloride [3.08g, 0.044 mol] and aqueous potassium hydroxide [3.70g, 0.066 mol] was added drop-wise to a methanolic solution of ethyl 1,3-dimethy l-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylat e **(3)** [5g, 0.022 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 *in vacuo* to yield a solid. The crude product was recrystallised from hot water to yield pure compound **5**.

Synthesis of 1,3-dimethyl- 4,6-dioxo-2- thioxo-1,2,3,4,5 ,6-hexahydropyrimidine-5-carboxylic acid hydroxamide **(6)**

The synthesis of **6** was carried out in two steps starting from 1,3-dimethyl-2-thiobarbituric acid (**2**) by a similar method as described for the synthesis of **5**.

Synthesis of metal chelates

Synthesis of Cu(II), Ni(II) and Co(II) chelates of

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1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide **(5).**

Copper acetate monohydrate [0.232g, 0.00115 mol.] in cold water was added with stirring to 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide **(5)** [0.50 g, 0.0023 mol.] in EtOH (20 ml) in a round bottom flask. The contents were stirred for about 4 hours and then 1ml glacial acetic acid was added and further stirred for 2 hours until a green precipitate of **7a** appeared. The precipitate was filtered, washed with small amounts of Et_2O and dried over $CaCl_2$ in a vacuum desiccator.

Similarly, complexes **7b** of Ni(II) and **7c** of Co(II) with 1,3-dimethyl-2,4,6-trioxo-1,2,3,4,5,6-hexahydropyrimidine-5-carboxylic acid hydroxamide **(5)** were synthesized by taking nickle acetate tetrahydrate and cobalt acetate tetrahydrate, respectively.

Synthesis of Cu(II), Ni(II) and Co(II) chelates of 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyri midine-5-carboxylic acid hydroxamide **(6).**

Copper acetate monohydrate [0.216g, 0.00108 mol.] in cold water was added with stirring to 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyrimidi ne-5-carboxylic acid hydroxamide (**6**) [0.50 g, 0.00216 mol.] in EtOH (20 ml) in a round bottom flask. The content was stirred for about 4 hours and then 1ml glacial acetic acid was added and further stirred for 2 hours until a bluish green precipitate of **8a** appeared. The precipitate was filtered, washed with small amounts of Et_2O and dried over $CaCl_2$ in a vacuum desiccator.

Similarly, complexes **8b** of Ni(II) and **8c** of Co(II) with 1,3-dimethyl-4,6-dioxo-2-thioxo-1,2,3,4,5,6-hexahydropyri midine-5-carboxylic acid hydroxamide (**6**) were synthesized by taking nickle acetate tetrahydrate and cobalt acetate tetrahydrate, respectively.

Infrared Spectra

In the IR spectra (**Table 2**), carbonyl stretching vibrations of hydoxamic acids exhibit a medium sharp intensity band in the region 1685-1660 cm^{-1} [25]. This band has shifted towards negative region 1660-1601 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 vibrations, obtained in the region 1120-1100 cm^{-1} in the IR spectra of ligands, have shifted to lower side by 45-30 cm^{-1} in the IR spectra of their metal complexes suggesting the coordination of ligand to the metal ion through oxygen of the N-O moiety [26]. The presence of water molecules within coordination sphere of all chelates were supported by broad bands in the region 3550-3290 cm^{-1} and 850-802 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 550-520 cm^{-1} is probable due to formation of M-O bonds [27].

H^1-NMR Spectra

The hydroxamic acids **5** and **6** show a one proton singlet at δ 1.26 and 1.74 respectively due to –N**H**-O proton, probably due to magnetic anisotropy of the neighboring carbonyl group, electronegativity of nitrogen and H- bonding [28].One proton singlet in hydroxamic acids **5** and **6** at δ 2.18 and 2.48 are due to –N-O**H** protons [29]. Due to proton exchange in D_2O this signal disappeared in the spectra indicating the possibility of –OH proton. Six proton multiplet for two –C**H**$_3$ group protons of hydroxamic acids **5** and **6** appear at δ 3.09 - 3.20 and 3.32-3.43, respectively. The hydroxamic acids **5** and **6** show a one proton singlet due to –C_5-**H** proton at δ 4.27 and 5.25, respectively. H^1-NMR of metal complexes **7a-c** and **8a-c** were not taken due to very less solubility in common organic solvents.

Table 2. IR spectral data of hydroxamic acids 5, 6 and their metal complexes 7a-c and 8a-c.

Comp-ound	ν(C=O)cm^{-1}	ν(C-N) cm^{-1}	ν(N-O) cm^{-1}	ν(N-C=S) cm^{-1}	ν(M-O) cm^{-1}
5	1685	1332	1103	-	-
7a	1647	1325	1043	-	520
7b	1660	1316	1049	-	535
7c	1630	1313	1042	-	522
6	1664	1360	1117	1387, 1280	-
8a	1629	1350	1025	1287	541
8b	1602	1379	1061	1273	523
8c	1627	1379	1060	1263	514

UV- vis. Spectra

In the electronic spectra of Cu(II) complexes **7a** and **8a**, three absorption bands in the region. 13888-15338, 15625-18867 and 20408-24096 cm^{-1} have been observed [30] 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 [31]-[33]. The electronic spectra of Ni(II) complexes **7b** and **8b** exhibit three bonds in the region 12987, 15151-16129 and 21739 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 [34]-[35]. In the electronic spectra of Co(II) complexes **7c** and **8c** three absorption bands in the region 12987-13157, 14814-15334 and 17543-18518 cm^{-1} were seen [36] 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.

Antimicrobial activity

Synthesized ligands **5**,**6** and metal chelates **7a-c**, **8a-c** were tested for their antimicrobial activity against two bacteria ***Staphylococcus aureus*** and ***Escherichia coli*** and two fungi ***Aspergillus niger*** and ***Aspregillus flavus*** by adopting Serial Dilution Method [37-38]. The micro-organisms were cultured in nutrient agar medium [38] 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.

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Measured quantities of the test compounds were dissolved in propylene glycol. First set was prepared for primary screening by taking 1ml (1500µg/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°C for 24 hours for bacteria and at 28°C for 96 hours for fungi. The Minimum Inhibitory Concentration (**MIC**) values were determined at the end of incubation period. Active synthesized compounds, found in the primary screening were further tested for secondary screening by taking 1ml (1000µg/ml) of seeded broth against all microorganisms.

The experimental results of **MIC** values show moderate activity of all the compounds against both bacteria and fungi **(Table 3)**. Further, it has been found that the metal complexes were more active than hydroxamic acids. Among the metal complexes **8a** was found most active against both bacteria and fungi.

Table 3. The minimum inhibitory concentration (µg/ml) MIC values of hydroxamic acids 5, 6 and their metal complexes 7a-c, 8a-c.

S.N	Compound	Bacteria		Fungi	
		Staphylococcus aureus	Escherichia coli	Aspergillus niger	Aspergillus flavus
1	5	325	500	500	500
2	7a	250	162	162	125
3	7b	250	325	325	325
4	7c	500	250	325	325
5	6	325	250	250	325
6	8a	162	125	125	125
7	8b	250	250	250	250
8	8c	325	325	325	250

IV. CONCLUSION

Six new metal chelates, **7a-c** and **8a-c** with ligands **5** and **6** have been synthesized and characterized. All the synthesized hydoxamic acids and their metal chelates were screened for antimicrobial activity. A comparative study of the MIC values of the ligands and the complexes show that complexes exhibit higher antimicrobial activity than free ligands.

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