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Research Article

Synthesis Characterization and Antimicrobial Activity of Mixed Ligand Complexes of L-ascorbic Acid and its Derivatives with Uracil Base

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ABSTRACT

Some Organometallic Complexes of La (III), Ce (III), Nd (III), and Gd (III) with mixed Ligand of Uracil and L-ascorbic acid and its isopropylidine derivative were synthesized. Uracil complexes were prepared with some of the inner transition metals in a stoichiometric ratio of (1:1 v/v) (M^{n+}: L), where M^{3+} = La (III), Ce (III), Nd (III) and Gd (III) ions. The Complexes were characterized by the physicochemical and spectroscopic techniques as electric conductivity, metal contents, IR, UV–Visible, ^{1}HNMR C^{13}NMR, Mass Spectroscopy and molar conductance techniques. The stoichiometric ratios of the synthesized complexes were confirmed by using molar ratio method. The dissociation constant of Uracil ligand was determined spectro-photometrically. Solvent effect on the electronic spectra of the Uracil ligand was examined using solvents with different polarities. In addition, biological activity of the synthesised metal complexes against *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* bacteria and *Aspergillus niger, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride* fungi respectively were examined *in-vitro*.

Introduction

Coordination chemistry is undoubtedly the most active research area in inorganic chemistry. Several thousands of coordination complexes have been synthesized and investigated during the past few decades. Ever since the importance of the coordination phenomenon in biological processes was realized, much metal containing macromolecules have been synthesized and studied to understand the role of these ligands in biological systems. They also contribute to the development of new metal-based chemotherapeutic agents. These have resulted in the emergence of an important branch of inorganic chemistry, *viz*. bioinorganic chemistry because in several cases, the metal chelates are more antimicrobial than the chelating agents themselves.[1] The importance of pyrimidine derivatives arises from their biological, medicinal and agricultural applications.[2-4] Metal complexes of pyrimidine have been extensively studied in recent years owing to their great variety of biological activity ranging from antimalarial, antibacterial, antitumoral, antiviral activities, etc.[5,6] Uracil is a naturally occurring pyrimidine derivative found in nucleic acids.[7] It is a pyrimidine base that consists of four different binding sites and belongs to a group of essential pyrimidines that play a fundamental role in the structure and function of enzymes and drugs. Uracil was used to synthesis antibacterial[8] and antitumor agents.[9] Some of the derivatives of uracil exhibit significant pharmacological activity and have been used as antitumor, antibacterial, and antiviral drugs.[10] The interaction of metal ions with nucleobases is of great interest because of their relevance to metal's essential, medical or toxic bioactivity, where nucleobase molecules can coordinate as exogenous ligands in metalloproteins, function as cofactors in the enzymatic systems, and

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construct important cell structures, e.g. RNA. Therefore, we reviewed the interaction of uracil and its derivatives with metal ions and the applications of some complexes.

Ascorbic acid is a six-carbon compound related to glucose. It is found naturally in citrus fruits and many vegetables. (Drug bank) L-Ascorbic acid is a white to very pale yellow crystalline powder with a pleasant sharp, acidic taste, almost odorless. Ascorbic acid is a potent reducing, and antioxidant agent that fights bacterial infections, detoxifies reactions, and forms collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries.[11] It is available in synthetic form and found naturally in many medicinal plants, including Indian gooseberry, amla citrus fruits like limes, oranges, lemons, and green leafy vegetables,[12] which are potent for potentiation antioxidant activity. Amla is one of the major sources of Ascorbic acid. A literature survey has been done for the content estimation of ascorbic acid in amla. Analytical methods such as UPLC (Klimczak I. *et al.,* 2015), and[13] HPLC (Saikh M. *et al.,* 2013)[14] were reported for estimation of Ascorbic acid in bulk.

Investigations about how inner transition metal cations interact with biological molecules are essential for a better assessment of the role and effects of metal ions in biological systems. Uracil is a base that can coordinate to the metal ion through any of the two nitrogen atoms and the two carbonyl oxygens of the pyrimidine ring. This paper describes the coordination tendency of uracil, its derivatives, and the biological uses of some complexes of uracil and its derivatives. The structural reviews of metal complexes of uracil and its derivatives as single ligand and mixed ligand showed that in most complexes of uracil and its derivatives, the ligands act as bidentate where O(4) and N(3) are favorable sites for cation binding. The involvement of O(4) as unidentate is observed in an M(III) complex of uracil derivative, and involvement of N(1), N(3) as unidentate was observed in organometallic complexes of uracil derivatives.

Experimental

Analytical and Physical Measurements

C, H, and N microanalyses were conducted at SAIF, CDRI, Lucknow, Uttar Pradesh, India. Standard EDTA methods determined the metal contents. Electronic spectra (DMF) were recorded on a Gary 14 spectro-photometer. The magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee, Uttarakhand, India. The IR spectra were recorded on a Infrared spectrophotometer from 4000–400 cm^{-1} using KBr at SAIF, Punjab University, Chandigarh. The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz). ESR spectra were recorded using an X-band (9.4 GHz) EMX Bruker spectrometer equipped with an Oxford Helium continuous flux cryostat, allowing us to perform the experiments from 4K to room temperature (RT). The microwave power was varied from 2 mW to 350 mW to obtain the saturation curves. A field calibration was performed using an ESR standard, establishing that 0 dB (197 mW) corresponds to 0.11 mT. The ESR simulation spectra were made using the software Easy Spin. The conductivity was measured on a digital conductivity meter (HPG System, G-3001).

Metal complexes were tested for in vitro antibacterial activity against some bacterial strains using a spot on the lawn on Muller Hinton Agar (MHA). Four test pathogenic bacterial strains, viz., *Bacillus cereus* (MTCC 1272), where Microbial type culture collections (MTCC), *S. typhi* (MTCC 733), *E. coli* (MTCC 739), and *S. aureus* (MTCC 1144), *K. pneumonia* (MTCC 1377) bacteria and *A. niger, A. flavus, F. oxisporium,* and *T. viride* fungi were considered for determination of minimum inhibitory concentration (MIC) of selected complexes. The test pathogens were sub-cultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C (24 hours). Working cultures were stored at 4°C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at -70°C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). The organism was grown overnight in 10 mL of BHI broth, centrifuged at 5.000 g for 10 minutes, and the pellet was suspended in 10 mL of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD_545) was adjusted to obtain 108 cfu/mL followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

Determination of MIC

Minimum Inhabitation Concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. The antimicrobial activity of the compounds was evaluated using spot on lawn on MHA (HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 108 Cfu/mL of the test pathogen, and 10 mL were overlaid on MHA. From 1000X solution of compound (1 mg/mL of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (Streptomycin and Griseofulvin) were also prepared in the same manner: 5 µL of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24 hours. Zones of inhibition of compounds were considered after subtraction of the inhibition zone of DMSO. Negative control (with no compound) was also observed.

Antimicrobial Studies

The antibacterial tests were prepared and characterized according to the standard method. All strains were isolated from the laboratory of microbiology. The identity of all the strains was confirmed. A bacterial suspension was prepared and added to the Nutrient Agar, the fungi

were added to surrounded Agar. All this before medium solidification and under aseptic condition. Then the different concentrations of complexes was placed on the surface of the culture. The bacteria were incubated at 37°C for 24 hours, The fungi were incubated at 28°C for 72 hours.

Bacterial & Fungi Cultures

Plate cultures of nutrient agar medium were used for the culture of bacteria. The medium was prepared by dissolving 14 g, and culture of fungi's 32.5 g of powder in 500 mL of sterile distilled water, Then the medium was sterilized by autoclaving at 121°C for 15 minutes.

Synthesis of Binary Complex

The general procedure for synthesizing M (III) Ligand complexes: La, Ce, Nd, Gd binary metal complexes with Uracil. The binary metal complexes were synthesized by mixing 10 mL solution of metal salts (0.01 mol) with 10 mL of Uracil (0.01 mol) in hot ethanol by keeping the Metal-Ligand ratio (1:1 v/v). The mixture was refluxed for about 4 to 6 hours on a water bath with continuous stirring. The pH of the solution was adjusting about 5 to 6 by adding an acidic buffer solution in ethanol. The volume of the solution was reduced to half. The solid-colored products thus obtained were filtered, washed with distilled water and cold ethanol, and then dried in a vacuum over anhydrous calcium chloride a dedicator.

Metal Complexes 2(a-d) was synthesized similarly using compound 1 and various selected Metal Chlorides. Characterization data are presented in Table 1.

Synthesis of Mixed Ligand Complexes

Mixed Ligand complexes of the type $[M(URA)(AA)(H_2O)_2]^+$ and $[M(URA)(IPAA)(H_2O)_2]^+$ (Fig. 1) were subjected to syntheses by adding 10 mL of binary complexes solution (0.01 mol) and 10 mL of AA/IPAA in hot ethanol by keeping the metal complexes- Ligand (1:1 v/v). The reaction mixture was refluxed for 6 to 8 hours on a steam bath with continuous stirring, and the solution's volume was reduced to half of its original volume. The solid-colored compounds obtained were filtered off, washed with water, cold ethanol diethyl ether, and dried in a vacuum over anhydrous calcium chloride in desiccators. Metal Complexes 3(a-d) and 4(a-d) were synthesized in a similar manner using compound 2(a-d) and selected Ligand L- Ascorbic acid (L-AA) and 5, 6-O-Isopropylidine-L-Ascorbic Acid (IPAA), respectively. Characterization data are presented in Table 2 and Table 3.

2a. $[La(URA)(H_2O)_4]^{3+}$: UV-Visible (λ_{Max} in nm) 281 (in DMSO) IR (in cm^{-1}): 3422-3381, 855-835(O-H in Coordinated water), 3178, 3110, 1532, 1511, 1418 (–NH), 2823, 2808 (-C-H str.), 1739, 1683 (-C=O), 1260(-C-N); 457-426 (M-N Bond), 408-422 (M-O Bond); ^{1}HNMR: δ 8.42, 7.41(s, -NH), δ 8.26, 8.86 ppm (d, -CH=CH-), δ 4.63 ppm (s, H_2O) ^{13}CNMR: δ 170.22, 153.93, 146.20, 103.23 (C1 to C4 Uracil); Mass (FAB): 323(M+), 307, 276, 275, 161, 116, 112, 96, 94, 85, 71(BP), 70, 67, 54, 43.

2b. $[Ce(URA)(H_2O)_4]^{3+}$: UV-visble (λ_{Max} in nm) 279 (in DMSO) IR (in cm^{-1}): 3424-3383, 857-837(O-H in Coordinated water), 3180, 3122, 1534, 1513, 1420 (–NH), 2825, 2810 (-C-H str.), 1740, 1685 (-C=O), 1263(-C-N); 460-428 (M-N Bond), 423-410 (M-O Bond); ^{1}HNMR: δ 8.43, 7.44(s, -NH), δ 8.29, 8.88 ppm (d, -CH=CH-), δ 4.66 ppm (s, H_2O) ^{13}CNMR: δ 170.26, 153.93, 146.22, 103.36 (C1 to C4 Uracil); Mass (FAB): 324(M+), 308, 277, 275, 161, 116, 96, 94, 85, 71(BP), 70, 67, 54, 43.

2c. $[Nd(URA)(H_2O)_4]^{3+}$: UV-visible (λ_{Max} in nm) 286 (in DMSO) IR (in cm^{-1}): 3426-3385 (O-H in Coordinated water), 3182, 3125 (–NH), 2823, 2807 (-C-H str. in Uracil ring), 1743, 1687 (-C=O), 1535, 1516 (-NH bend) 1266(-C-N); 462-429 (M-N Bond), 425-412 (M-O Bond); ^{1}HNMR: δ 8.45, 7.46 (s, -NH), δ 8.32, 8.90 ppm (d, -CH=CH-), δ 4.67 ppm (s, H_2O) ^{13}CNMR: δ 170.26, 153.93, 146.23, 103.32 (C1 to C4 Uracil); Mass (FAB): 328(M+), 256, 184, 112, 96, 70(BP), 67, 67, 61, 55, 43.

2d. $[Gd(URA)(H_2O)_4]^{3+}$: UV-visible (λ_{Max} in nm) 280 (in DMSO) IR (in cm^{-1}): 3429-3387, 862-839(O-H in Coordinated water), 3185, 3127 (–NH), 2829, 28185 (-C-H str.), 1745, 1689 (-C=O), 1536, 1517 (-NH bend) 1267(-C-N); 463-430 (M-N Bond), 427-413 (M-O Bond); ^{1}HNMR: δ 8.47, 7.49 (s, -NH), δ 8.35, 8.93 ppm (d, -CH=CH-), δ 4.68 ppm (s, H_2O); ^{13}CNMR: δ 170.26, 153.93, 146.25, 103.30 (C1 to C4 Uracil); Mass (FAB): 341(M+), 305, 269, 195, 123, 107, 89, 71, 65, 54, 43.

3a. $[La(URA)(AA)(H_2O)_2]^{3+}$: UV-visible ($\lambda_{Max}$ in nm) 258 (in DMSO) IR (in cm^{-1}): 3418-3389, (O-H in coordinated water), 3316, 3226 (O-H str. in CH-OH), 3183, 3122 (-NH), 2828, 2803 (-C-H str. in Uracil ring), 1736, 1685 (-C=O), 1538, 1512 (-NH bend), 1669, 1506, 1448 (-C=C-), 1448 (-C-H), 1382 (C-O); 1254, 916 (-C-N); 457-426 (M-N Bond), 408–422 (M-O Bond); ^{1}HNMR: δ δ 4.76, 3.76, 3.48 ppm (m,

Table 1: Physical parameters and UV-Visible, IR, NMR and Mass data of $[M(URA)(H_2O)_4]^{3+}$

			Elemental Analysis Calculated (Found)		
Complex	*Colour*	*M. P. in °C*	*C%*	*H%*	*N%*
$[La(URA)(H_2O)_4]^{3+}$	White	338–340	14.86 (14.81)	3.71 (3.69)	8.67 (8.65)
$[Ce(URA)(H_2O)_4]^{3+}$	Red	342–344	14.80 (14.76)	3.70 (3.67)	8.63 (8.64)
$[Nd(URA)(H_2O)_4]^{3+}$	Brown	346–348	14.62 (14.59)	3.65 (3.62)	8.52 (8.49)
$[Gd(URA)(H_2O)_4]^{3+}$	Pink	350–352	14.06 (13.99)	3.51 (3.49)	8.20 (8.17)

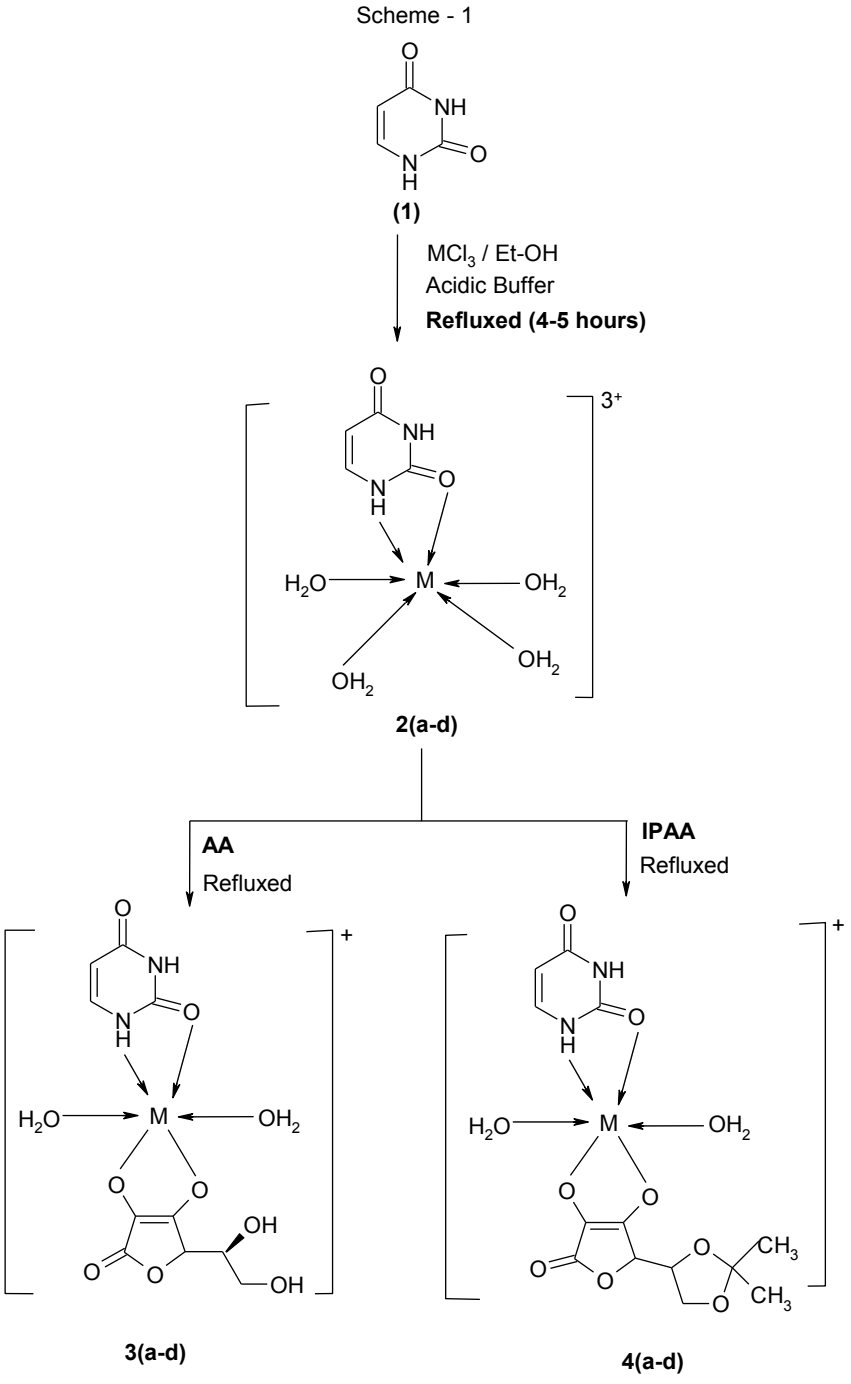

Fig. 1: The figure showed that of the studied complexes ML and ML2 Geometric isomerism.

Table 2: Physical parameters and UV-Visible, IR, NMR and Mass data of $[M(URA)(AA)(H_2O)_2]^+$

			Elemental Analysis Calculated (Found)		
Complex	*Colour*	*M. P. in °C*	*C%*	*H%*	*N%*
$[La(URA)(AA)(H_2O)_2]^+$	White	354–356	26.03 (25.98)	3.03 (3.01)	6.07 (5.98)
$[Ce(URA)(AA)(H_2O)_2]^+$	Red	358–360	26.96 (25.94)	3.02 (3.00)	6.05 (5.97)
$[Nd(URA)(AA)(H_2O)_2]^+$	Brown	362–364	25-73 (25.69)	3.00 (2.96)	6.00 (5.96)
$[Gd(URA)(AA)(H_2O)_2]^+$	Pink	354–356	25.04 (25.00)	2.92 (2.89)	5.84 (5.78)

Table 3: Physical parameters and UV-Visible, IR, NMR, and Mass data of $[M(URA)(IPAA)(H_2O)_2]^{+.}$

			Elemental Analysis Calculated (Found)		
Complex	*Colour*	*M. P. in °C*	*C%*	*H%*	*N%*
$[La(URA)(IPAA)(H_2O)_2]^+$	White	354–356	26.03 (25.98)	3.03 (3.01)	6.07 (5.98)
$[Ce(URA)(IPAA)(H_2O)_2]^+$	Red	380–382	26.03 (25.98)	3.03 (3.01)	6.07 (5.98)
$[Nd(URA)(IPAA)(H_2O)_2]^+$	Brown	384–386	31.74 (31.71)	3.58 (3.56)	13.22 (13.19)
$[Gd(URA)(IPAA)(H_2O)_2]^+$	Pink	376–378	30.98 (30.94)	3.50 (3.47)	12.91 (12.89)

H_4, H_5, H_6), δ 4.88 ppm (s, C5 & C6-OH), 4.71 (s, for H_2O), δ 8.41, 7.42 (s, -NH), δ 8.26, 8.86 ppm (d, -CH=CH-) ^{13}CNMR: δ 174.09, 118.62, 156.26, 77.06, 69.72, 62.91 ppm (C1 to C6 of AA) and δ 170.20, 153.91, 146.26, 103.32 ppm (C1 to C4 Ura); Mass (FAB): 461(M+), 424, 393, 362, 300, 315, 251, 167, 112, 94, 85, 71, 67, 43.

3b. $[Ce(URA)(AA)(H_2O)_2]^{3+}$: UV-visible ($\lambda_{Max}$ in nm) 268 (in DMSO) IR (in cm^{-1}): 3420-3391, (O-H in coordinated water), 3318, 3228 (O-H str. in CH-OH), 3186, 3126 (–NH), 2838, 2805 (-C-H str. in Uracil ring), 1738, 1687 (-C=O), 1538, 1516 (-NH bend), 1661, 1508, 1450 (-C=C-), 1450 (-C-H), 1384 (C-O); 1257, 918 (-C-N); 459-429 (M-N Bond), 410-425 (M-O Bond); ^{1}HNMR: δ δ 4.78, 3.77, 3.50 ppm (m, H_4, H_5, H_6), δ 4.91 ppm (s, C5 & C6-OH), 4.73 (s, for H_2O), δ 8.43, 7.45 (s, -NH), δ 8.28, 8.88 ppm (d, -CH=CH-) ^{13}CNMR: δ 174.11, 118.66, 156.25, 77.03, 69.78, 62.96 ppm (C1 to C6 of AA) and δ 170.23, 153.95, 146.29, 103.31 ppm (C1 to C4 Ura); Mass (FAB): 462(M+), 425, 394, 363, 302, 317, 252, 168, 113, 95, 86, 71, 68, 42.

3c. $[Nd(URA)(AA)(H_2O)_2]^{3+}$: UV-visible ($\lambda_{Max}$ in nm) 278 (in DMSO) IR (in cm^{-1}): 3423-3389, (O-H in coordinated water), 3320, 3231 (O-H str. in CH-OH), 3179, 3129 (-NH), 2839, 2811 (-C-H str. in Uracil ring), 1741, 1689 (-C=O), 1541, 1518 (-NH bend), 1673, 1506, 1452(-C=C-), 1449 (-C-H), 1386 (C-O); 1259, 920 (-C-N); 462-431 (M-N Bond), 426-412 (M-O Bond); ^{1}HNMR: δ δ 4.80, 3.78, 3.53 ppm (m, H_4, H_5, H_6), δ 4.92 ppm (s, C5 & C6-OH), 4.76 (s, for H_2O), δ 8.45, 7.47 (s, -NH), δ 8.30, 8.89 ppm (d, -CH=CH-) ^{13}CNMR: δ 174.09, 118.65, 156.27, 77.06, 69.75, 62.96 ppm (C1 to C6 of AA) and δ 170.25, 153.96, 146.24, 103.31 ppm (C1 to C4 Ura); Mass (FAB): 465(M+), 429, 397, 366, 304, 316, 255, 171, 116, 98, 89, 71, 67, 43.

3d. $[Gd(URA)(AA)(H_2O)_2]^{3+}$: UV-visible ($\lambda_{Max}$ in nm) 272 (in DMSO) IR (in cm^{-1}): 3426-3395, (O-H in coordinated water), 3323, 3232 (O-H str. in CH-OH), 3187, 3129 (-NH), 2831, 2816 (-C-H str. in Uracil ring), 1743, 1690 (-C=O), 1539, 1519 (-NH bend), 1674, 1511, 1454 (-C=C-), 1449 (-C-H), 1389 (C-O); 1262, 922 (-C-N); 463–434 (M-N Bond), 428–413 (M-O Bond); ^{1}HNMR: δ δ 4.82, 3.80, 3.55 ppm (m, H_4, H_5, H_6), δ 4.94 ppm (s, C5 & C6-OH), 4.79 (s, for H_2O), δ 8.47, 7.50 (s, -NH), δ 8.32, 8.90 ppm (d, -CH=CH-) ^{13}CNMR: δ 174.05, 118.66, 156.25, 77.04, 69.74, 62.93 ppm (C1 to C6 of AA) and δ 170.22, 153.95, 146.24, 103.33 ppm (C1 to C4 Ura); Mass (FAB): 479(M+), 443, 412, 381, 320, 236, 161, 116, 96, 84, 71(BP), 70, 54, 43.

4a. $[La(URA)(IPAA)(H_2O)_2]^+$: UV-visible ($\lambda_{Max}$ in nm) 277 (in DMSO) IR (in cm^{-1}): 3428-3393, 864-846 (O-H in coordinated water), 2910 (-C-H), 1771, 1741, 1677 (-C=O), 1662, 1509, 1445, (-C=C-) 1429 (-C-H), 1389 (C-O), 3188, 3126 (–NH), 2823, 2809 (-C-H str. in uracil), 1530, 1516, 1411 (-NH bend) 1256, 1169, 912(-C-N); 452-432 (M-N Bond), 420-410 (M-O Bond); ^{1}HNMR: 1.69 ppm (s, for the isopropylidene methyl proton) δ 4.68, 3.76, 3.44 ppm (m, H_4, H_5, H_6), δ 4.64 ppm (s, for the H_2O), δ 8.39, 7.44 (s, -NH), δ 8.26, 8.84 ppm (d, -CH=CH-) ^{13}CNMR: 170.09, 150.22, 118.12, 108.83, 74.14, 73.29, 64.69, 25.70, 25.30 (C1 to C9 IPAA) and 170.20, 153.91, 146.26, 103.32 (C1 to C4 Ura); Mass (FAB): 501(M^+), 465, 437, 377, 316, 231, 161, 97, 86, 71, 70, 63, 54, 43, 28.

4b. $[Ce(URA)(IPAA)(H_2O)_2]^+$: UV-visible ($\lambda_{Max}$ in nm) 274 (in DMSO) IR (in cm^{-1}): 3430-3395, 866-847 (O-H in coordinated water), 2916 (-C-H), 1774, 1743, 1678 (-C=O), 1664, 1511, 1446, (-C=C-) 1422 (-C-H), 1392 (C-O), 3190, 3129 (–NH), 2823, 2809 (-C-H str. in uracil), 1533, 1518, 1418 (-NH bend) 1258, 1171, 915(-C-N); 452-432 (M-N Bond), 420-410 (M-O Bond); ^{1}HNMR: 1.72 ppm (s, for the isopropylidene methyl proton) δ 4.71, 3.78, 3.46 ppm (m, H_4, H_5, H_6), δ 4.67 ppm (s, for the H_2O), δ 8.41, 7.45(s, -NH), δ 8.29, 8.86 ppm (d, -CH=CH-) ^{13}CNMR: 170.06, 150.23, 118.15, 108.82, 74.13, 73.25, 64.65, 25.76, 25.32 (C1 to C9 IPAA) and 170.26, 153.88, 146.23, 103.36 ppm (C1 to C4 Ura); Mass (FAB): 502(M^+), 466, 438, 378, 317, 232, 162, 98, 87, 72, 71, 63, 54, 43, 28.

4c. $[Nd(URA)(IPAA)(H_2O)_2]^+$: UV-visible ($\lambda_{Max}$ in nm) 278 (in DMSO) IR (in cm^{-1}): 3433-3397, 867-849 (O-H in coordinated water), 2910, 1569 8C-H), 1776, 1745, 1680 (-C=O), 1667, 1513, 1447, (-C=C-) 1429 (-C-H), 1395 (C-O), 3191, 3132 (–NH), 2835, 2813 (-C-H str. in uracil), 1530, 1516, 1411 (-NH bend) 1260, 1173, 917(-C-N); 456-435 (M-N Bond), 425-414 (M-O Bond); ^{1}HNMR: 1.75 ppm (s, for the isopropylidene methyl proton) δ 4.72, 3.8, 3.49 ppm (m, H_4, H_5, H_6), δ 4.69 ppm (s, for the H_2O), δ 8.43, 7.46 (s, -NH), δ 8.30, 8.88 ppm (d, -CH=CH-) ^{13}CNMR: 170.13, 150.26, 118.18, 108.79, 74.16, 73.22, 64.66, 25.76, 25.36 δ ppm (C1 to C9 IPAA) and 170.21, 153.94, 146.22, 103.36 δ ppm (C1 to C4 Ura); Mass (FAB): 506(M^+), 469, 441, 381, 320, 235, 166, 101, 90, 75, 71, 70, 63, 54, 43, 28.

4d. $[Gd (URA)(IPAA)(H_2O)_2]^+$: UV-visible ($\lambda_{Max}$ in nm) 278 (in DMSO) IR (in cm^{-1}): 3439-3399, 868-850(O-H in coordinated water), 2916 (-C-H), 1778, 1747, 1662 (-C=O),

1669, 1514, 1449, (-C=C-) 1429 (-C-H), 1397 (C-O), 3194, 3132 (–NH), 2838, 2812 (-C-H str. in uracil), 1532, 1519, 1412 (-NH bend) 1262, 1174, 919(-C-N); 458-437 (M-N Bond), 426-416 (M-O Bond); ^{1}HNMR: 1.78 ppm (s, for the isopropylidene methyl proton) δ 4.74, 3.83, 3.50 ppm (m, H_4, H_5, H_6), δ 4.71 ppm (s, for the H_2O), δ 8.45, 7.49 (s, -NH), δ 8.33, 8.89 ppm (d, -CH=CH-) ^{13}CNMR: 170.13, 150.26, 118.16, 108.81, 74.19, 73.26, 64.67, 25.73, 25.32 (C1 to C9 IPAA) and 170.23, 153.94, 146.28, 103.35 (C1 to C4 Ura); Mass (FAB): 519(M+), 483, 455, 395, 334, 250, 179, 136, 116, 91, 71, 64, 43.

Antimicrobial Activity

The antimicrobial activity of all prepared complexes was tested selected strain of bacteria and fungi as in Table 4 and Table 5. The products (1g) were dissolved in sterile distilled water at a concentration of 10 mL, then take (1 mL) of the tube (1) and transported into the tube (2) and continued

Table 4: Anti-bacterial Activity of Synthesised Compounds [SM = streptomycin inhibition diameter in mm, highly active = +++ (inhibition zone>15), moderatively active = ++ (inhibition zone 10-15), slightly active = + (inhibition zone 10), inactive inhibition zone -6) for bacteria]

Compound / *Code*	*Salmonella typhi*		*BucillusSubsniss*		*Staphylococcusaureus*		*Klebsiella pneumonia*	
	50 ppm	*100 ppm*	*50 ppm*	*100 ppm*	*50 ppm*	*100 ppm*	*50 ppm*	*100 ppm*
2a	++	+++	++	+++	++	+++	++	+++
2b	++	+++	++	++	++	++	++	+++
2c	+	++	+	++	++	++	++	++
2d	++	+	++	++	+	++	+	++
3a	++	+++	++	+++	++	+++	++	+++
3b	++	+++	++	++	++	++	++	+++
3c	+	++	+	++	++	++	++	++
3d	+	+	++	++	+	++	+	++
4a	++	+++	++	+++	++	+++	++	+++
4b	++	+++	++	++	++	++	++	+++
4c	+	++	+	++	++	++	++	++
4d	+	++	+	++	+	+	+	+
SM	+++	++++	+++	++++	+++	++++	+++	++++

Table 5: Antifungal activity of the synthesized compound derivatives [Std- Griseofulvin inhibition diameter in mm, Highly active = ++++ (inhibition zone > 20-25), More Active = +++ (inhibition zone > 12-20), Moderatively active = ++ (inhibition zone 6-12), slightly active = + (inhibition Zone less than 6), Inactive inhibition zone - for Fungi].

Compound code	*Aspergillus niger*		*Aspergillus flavus*		*Fusarium oxisporium*		*Trichoderma viride*	
	50 ppm	*100 ppm*	*50 ppm*	*100 ppm*	*50 ppm*	*100 ppm*	*50 ppm*	*100 ppm*
2a	++	++	++	+++	++	+++	++	+++
2b	+	++	+	++	+	++	++	+++
2c	++	+++	+	++	+	++	++	+++
2d	++	++	+	++	+	++	+	+++
3a	+	++	+	++	+	++	+	+++
3b	+	++	+	++	+	++	+	+++
3c	++	+++	++	+++	++	+++	++	+++
3d	++	+++	++	++	++	++	++	+++
4a	+	++	+	++	++	++	++	++
4b	+	++	+	+	+	++	+	++
4c	++	+++	++	+++	++	+++	++	+++
4d	++	++	+	++	++	+++	++	++
GF	+++	++++	+++	+++	+++	++++	+++	++++

the dilution into reach (5) tubes of concentration for each product. The plates were incubated inverted at 37°C for 24 hours in case of bacteria and at 28°C for 48 hours in fungi. After incubation, the inhibition zones were recorded in mm. A diameter less than 10 mm indicates no effect.

Result and Discussion

Complexes of La (III), Ce (III), Nd (III), and Gd (III) with mixed Ligand of Uracil and L-ascorbic acid and its isopropylidine derivative were synthesized. Uracil complexes were prepared with some of the inner transition metals in a stoichiometric ratio of (1:1 v/v) ($M^{n+:}$ L), where M^{n+} = La (III), Ce(III), Nd(III), and Gd(III) ions. The Complexes were characterized by the Physico-chemical and spectroscopic techniques as elemental analysis, IR, UV–Visible, ^{1}HNMR, ^{13}CNMR, and Mass Spectroscopy. The stoichiometric ratios of the synthesized complexes were confirmed by using molar ratio method. The dissociation constant of Uracil ligand was determined spectro-photometrically. The solvent effect on the electronic spectra of the Uracil ligand was examined using solvents with different polarities. In addition, the biological activity of the synthesized metal complexes against *S. typhi, E. coli, S. aureus* and *K. pneumonia* bacteria and *A. niger, A. flavus, F. oxisporium,* and *T. viride* fungi, respectively, were examined *in-vitro*.

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Reference

1. Claudel M, Justine VS, Katharina MF. New Antimicrobial Strategies Based on Metal Complexes. J Chem. 2017; 2: 849–899.
2. Hung J, Werbel M. Synthesis and antimalarial properties of 1-imino derivatives of 7-chloro-3-substituted-3,4-dihydro-1,9(2H,10H)-acridinediones and related structures. J Hetero. Chem. 1984; 21:74.
3. Oladipo M, Isola KT. Coordination Possibility of Uracil and Applications of Some of Its Complexes: A Review. Res. J. of Pharma., Bio. And Chemical Sci. Oct.-Dec. 2013; 4(4): 386-394.
4. Mahmoud ST, Kamal AM, Ahmed M, Hassanien R, Sayed M, Zaki RM, Mohamed SK, Zawam SA, Shaban AA, Raheem A. Synthesis, reactions, and applications of pyrimidine derivatives, Current Chemistry Letters 2021; 11 (2022): 1-18.
5. Teleb SM, Askar ME, Kalyoubi SA, Gaballa AS. Synthesis Charecterization and antymicrobial activities of some 5-Bromouracil-Metal ion Complexes, 2019; Bull. Chem. Soc. Ethiop. 33(2):255-268.
6. Bhandari SV, Dangre SC, Bothara KG, Patil AA, Sarkate AP, Lokwani DK. Design, synthesis and pharmacological screening of novel nitric oxide donors containing 1,5-diarylpyraz-olin-3-one as nontoxic NSAIDs. Eur. J. Med. Chem. 2009; 44: 4622–4636.
7. 7. Bondock S, Rabie R, Etman HA, Fadda AA. Synthesis and antimicrobial activity of some new heterocycles incorporating antipyrine moiety, Eur. J. Med. Chem.2008; 43: 2122–2129.
8. Wright EG, Gambinu JJ. Quantitative structure-activity relationships of 6-anilinouracils as inhibitors of Bacillus subtilis DNA polymerase III. J. Med. Chem. 1984; 27: 181–185.
9. Watnabe AK, Harada K, Zeidler J, Adamic JM, Takahashi K, Ren WY, Cheng LC, Fox JJ, Chou TC.Synthesis and anti-HIV-1 activity of 2'-"up"-fluoro analogs of active anti-AIDS nucleosides 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxycytidine (DDC) J. Med. Chem. 1990; 33: 2145–2150.
10. Xiong J, Lan YJ, Zhang S F. Lanthanum (III) complex of uracil-1-acetic acid, a new 2D coordination polymer [La (Phen)(UA)3]n·nH2O (Phe =1,10-phenanthroline and UA = uracil-1-acetate). Russian J. Coord. Chem. 2007; 33: 306–311.
11. Sarkar AR, Ghosh P. Interaction of divalent metal ions with Uracil 1 Oxygen coordinating uracil complexes of Mn(II), Fe(II), Co(II), Ni(II) and Cu(II). Inorg. Chim. Acta. 1983; 78:L39–L41.
12. Kufelnicki A, Jaszczak J, Kalinowska-Lis U, Wardak, C Ochocki, J. Complexes of uracil (2,4dihydroxypyrimidine) derivatives. J. Solution Chem. 2006; 35: 739–751.
13. Gaballa AS, Teleb SM, Asker MS, Yalcin E, Seferoglu Z. Synthesis, spectroscopic properties and antimicrobial activity of some new 5-phenylazo-6-aminouracil-vanadyl complexes. J. Coord. Chem. 2011; 64:4225–4243.
14. Averill BA, Principles of General Chemistry 2012; Silberberg; Martin, S. (Eds.), Chemistry: The Molecular Nature of Matter and Change, 4th ed., McGraw Hill Company; New York; 2006; Housecroft, CE Sharpe, A.G. Inorganic Chemistry, 2nd ed., Pearson Education Limited: England; 2005.

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