Synthesis, Characterization, and Antioxidative Activity of Ternary Rare-Earth Complexes of 5-Fluorouracil-1-acetic Acid and 2,2′-Bipyridine

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Seven new solid ternary complexes of 5-fluorouracil-1-acetic acid (HFAA) and 2,2′-bipyridine (Bipy) with rare-earth metals have been synthesized. Elemental analysis, molar conductivity, IR, TG-DTA, UV, and ^{1}H NMR spectra have been used to characterize these complexes. The general formula for the complexes is $M(FAA)_3Bipy \cdot nH_2O$, where M = Y, La, Ce, Sm, Gd, Dy or Er, n = 0, 2 or 5. The antioxidative activity of these complexes was tested. The results obtained showed that the suppression ratios of these complexes for $O_2^{\bullet -}$ free radical are mostly higher than those of HFAA and Bipy.

The cycle-specific schedule-dependent antimetabolite 5-fluorouracil (5-Fu) has been in clinical use for 40 years and has evolved as an important agent in the treatment of a large spectrum of tumours, including all gastrointestinal cancers and breast cancer. In the meantime, it may appear a little harmful to liver, kidney, and digestive system. For these reasons, many researchers including us have synthesized many 5-fluorouracil derivatives and their metal complexes in order to develop new antitumour drugs with higher antitumour activity and relatively low toxic side effects [1—11]. Among these compounds, ternary metal complexes of 5-fluorouracil derivatives, however, have rarely been reported, the only report concerned the ternary complexes of pyruvic acid isonicotinoyl hydrazone and 5-fluorouracil-1-acetic acid (HFAA) with rare earths [2].

In this paper, the synthesis, characterization, and antioxidative activity of seven ternary rare-earth complexes of 5-fluorouracil-1-acetic acid and 2,2′-bipyridine (see Formula 1) has been reported for the first time.

EXPERIMENTAL

The starting compounds included rare-earth carbonates which were transformed from respective oxides (99.99%, Yuelong Chemical Works, Shanghai, China), 2,2′-bipyridine (Shanghai Factory of Chemical Reagents, China), nitro blue tetrazolium (NBT, Sigma, USA), *N*-methylphenazine methosulfate (PMS, Sigma, USA), nicotinamide adenine dinucleotide (NADH, Sigma, USA). HFAA was synthesized by nucleophilic substitution reaction of 5-fluorouracil with α-chloroacetic acid in aqueous medium according to the literature method [11]. Solvents and reagents used were of anal. grade.

Carbon, hydrogen, and nitrogen were determined using a Varian EL elemental analyzer. The amounts of metals were determined by titration with EDTA. Cerium content was obtained by weighing the CeO_2 residue after calcinating the cerium complex at 800 °C. IR spectra were recorded on a Nicolet 170SX FTIR spectrophotometer, using KBr discs in the range $\tilde{\nu}$ = 200—4000 cm^{-1}. TG-DTA analyses were carried out with a Dupont 1090-B thermal analyzer in a nitrogen

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Table 1. Characterization of the Complexes

Compound	Formula	w_i(calc.)/% w_i(found)/%				Molar conductivity	Yield
		Metal	C	H	N	S cm^2 mol^{-1}	%
I	$Y(FAA)_3 \cdot Bipy$	11.02	41.70	2.50	13.90	20.21	42
		11.18	41.53	2.83	14.04		
II	$La(FAA)_3 \cdot Bipy \cdot 5H_2O$	14.68	35.53	3.19	11.84	18.92	45
		14.35	35.06	3.17	11.71		
III	$Ce(FAA)_3 \cdot Bipy \cdot 5H_2O$	14.79	35.49	3.19	11.82	20.52	44
		15.23	34.99	3.10	11.87		
IV	$Sm(FAA)_3 \cdot Bipy \cdot 5H_2O$	15.70	35.11	3.16	11.70	20.02	45
		16.03	34.99	2.72	11.64		
V	$Gd(FAA)_3 \cdot Bipy \cdot 2H_2O$	17.27	36.92	2.66	12.30	19.23	46
		16.87	37.28	2.74	11.96		
VI	$Dy(FAA)_3 \cdot Bipy \cdot 2H_2O$	17.74	36.71	2.64	12.23	21.35	48
		17.84	36.70	2.52	11.84		
VII	$Er(FAA)_3 \cdot Bipy$	18.90	38.01	2.28	12.66	19.63	46
		18.60	38.47	2.53	12.66		

atmosphere at room temperature and 800 ℃. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. ^{1}H NMR spectra were measured with an FT-80A nuclear magnetic resonance instrument using DMSO-d_6 as solvent and TMS as internal reference. Electrolytic conductances measurements were made with a DDS-11A digital conductometer with DMSO as solvent (solution of $c \approx 10^{-3}$ mol dm^{-3}) at 25 ℃. Absorbances were determined on a 751 spectrophotometer at $\lambda = 560$ nm.

Complexes *I*—*VII*

Rare-earth carbonate (1.5 mmol) was added to 40 cm^3 of an aqueous solution containing HFAA (6 mmol). The mixture was warmed and stirred continuously for 15 h keeping the temperature below 80 ℃. The pH of the solution was finally about 6—7. Unreacted rare-earth carbonate was filtered off. A solution of Bipy (2 mmol) in 10 cm^3 of ethanol was added dropwise to the filtrate with stirring. The product precipitated immediately and stirring was continued for 5—6 h, keeping the temperature below 80 ℃. Then the precipitate was collected by filtration, washed with EtOH—H_2O (φ_r = 2:1) several times and dried in a vacuum desiccator to constant mass.

Antioxidative Activity Tests

The superoxide radicals ($O_2^{\bullet -}$) were produced by a system of NADH/PMS/NBT and measured by the amount of NBT reduced by $O_2^{\bullet -}$ [12]. The final concentrations (in 5 cm^3) of the reagents used were c(NADH) = 73 μmol dm^{-3}, c(PMS) = 15 μmol dm^{-3}, c(NBT) = 50 μmol dm^{-3}, c(Tris—HCl buffer, pH = 8) = 0.016 mol dm^{-3}, and c(tested compound) = 30 μmol dm^{-3}. The reaction was left for 5 min at room temperature. The amount of reduced NBT was detected by the absorbance at 560 nm, since the reduced product, blue formazan, absorbs at this wavelength. The suppression ratios for $O_2^{\bullet -}$ were calculated as follows

$$\text{Suppression ratio} = 100 \times \frac{A_0 - A}{A_0}$$

where A is the absorbance in the presence of the ligand or the complexes and A_0 is the absorbance in the absence of the ligand or the complexes.

RESULTS AND DISCUSSION

Complexes *I*—*VII*, their elemental composition and molar conductivity data are listed in Table 1. The complexes are air-stable and soluble in water, easily soluble in DMSO and THF, insoluble in other common organic solvents. The molar conductivity of these complexes in DMSO solution at 25 ℃ varies from 18.92 to 21.35 S cm^2 mol^{-1}, indicating that they are nonelectrolytes in DMSO [13]. Elemental analyses show that the complexes have the general formula $M(FAA)_3Bipy \cdot nH_2O$, where M = Y, La, Ce, Sm, Gd, Dy or Er, n = 0, 2 or 5.

The thermal behaviour of all seven complexes was similar. Data of thermal analysis are given in Table 2. The endothermic peaks appear in the DTA curve of complexes *II*, *III*, and *IV* around 125 ℃ suggesting that the water molecules are either coordinated water or crystal water. The percent of mass loss, in the TGA curve, corresponds to five water molecules. For the complexes *V* and *VI*, the endothermic peaks appear around 110 ℃ corresponding to two water molecules and the water molecules are also either coordinated water or crystal water. The rest complexes do not show mass loss in the TGA curve under

Table 2. Thermal Data of HFAA and the Complexes

Compound	Water loss		Dec. temp.	Residue	
	Temp./℃	$-\Delta w$/%	℃	$-\Delta w$/%	Formula
HFAA			300		
I			342	13.75	Y_2O_3
II	125	9.63	335	17.11	La_2O_3
III	130	9.72	358	18.02	CeO_2
IV	123	9.68	374	18.02	Sm_2O_3
V	110	4.11	364	19.65	Gd_2O_3
VI	112	4.05	366	20.12	Dy_2O_3
VII			373	21.50	Er_2O_3

Table 3. IR and UV Data for Ligands and their Complexes

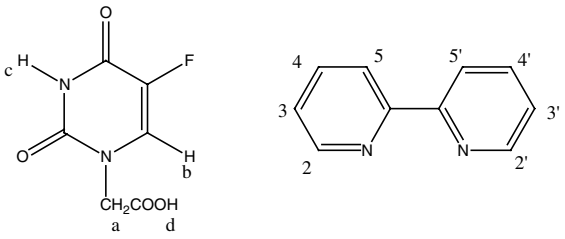
Compound	$\tilde{\nu}$/cm^{-1}											λ_{max}
	ν(OH)^a	ν(OH)^b	ν(C$_2$═O)	ν(C$_4$═O)	ν_{as}(COO$^-$)^c	ν_s(COO$^-$)^c	$\{\Delta\tilde{\nu}\}$	ν(C—F)	π(C—H)^d	ν(M—O)	ν(M—N)	/nm
HFAA		3191	1705	1668				1242				272
NaFAA	3333		1696	1673	1605	1440	165	1235				
Bipy					1578	1452			770			280
I			1705	1668	1568	1438	130	1241	762	425	276	281
II	3396		1707	1671	1569	1444	125	1244	765	440	280	278
III	3422		1698	1668	1570	1442	128	1240	758	432	278	283
IV	3428		1696	1665	1567	1439	128	1238	766	437	279	282
V	3447		1694	1675	1565	1435	130	1242	766	418	272	284
VI	3406		1705	1671	1568	1448	120	1244	760	436	280	282
VII			1715	1663	1575	1459	116	1246	752	423	282	280

a) ν(OH) of H_2O; *b*) ν(OH) of COOH; *c*) plus ν(C═C) and ν(C═N) of Bipy; *d*) π(C—H) of Bipy.

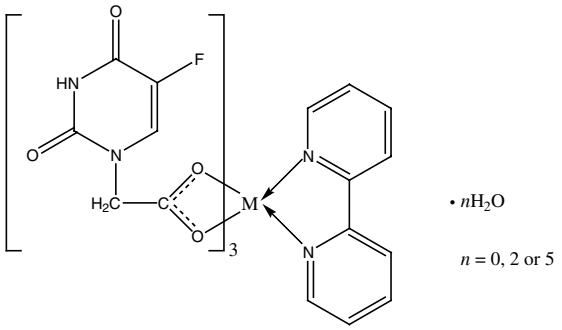
300 ℃, suggesting that they do not contain any water molecules. These results agree with the composition of the complexes determined by elemental analysis. The exothermic peak indicating the beginning of decomposition appears at higher temperature for the complexes (around 340—370 ℃) than for the ligand HFAA (300 ℃), indicating that the former are more stable than the latter. There are two other exothermic peaks at higher temperature for the complexes. On heating to about 700 ℃, the complexes are decomposed completely and the residues are rare-earth oxides.

The principal IR data of the ligands and their complexes are given in Table 3. The bands observed at 3191 cm^{-1} in the spectra of HFAA assigned to ν(OH)(COOH) disappear on complexation. Subsequently, the complexes displayed both symmetric and asymmetric vibrations of COO$^-$ at 1435—1459 cm^{-1} and 1565—1575 cm^{-1}, respectively. These indicate the coordination of the —COOH group of the ligand HFAA after deprotonation to form the M—O bond in the complexes. $\Delta\tilde{\nu}$ ($\Delta\tilde{\nu} = \tilde{\nu}(\nu_{as}(COO^-)) - \tilde{\nu}(\nu_s(COO^-))) = 116$—$130$ cm^{-1} is smaller than that of NaFAA (sodium salt of HFAA) ($\Delta\tilde{\nu} = 1605 - 1440 = 165$ cm^{-1}), which strongly suggests the coordination of carboxyl group of the ligand HFAA with rare-earth ion in bidentate mode [14]. The characteristic vibration of ν(C—F) varies less for the complexes, suggesting that the fluorine atom does not coordinate to metal ion. The strength of the π(C—H) of the ligand Bipy varied stronger in the complexes, and the band also shifted to higher field by about 4—18 cm^{-1}. The characteristic bands of pyridine ring were overlapped with the stretching vibration of HFAA in the complexes, so it is difficult to observe the variety. By comparison of the far-IR spectra of the complexes with those of the ligands, new peaks assigned to ν(M—O) and ν(M—N) appear at 418—440 cm^{-1} and 272—282 cm^{-1} [15], indicating the complexation of the ligands with rare-earth metals. A broad band appears at about 3400 cm^{-1} assigned to ν(OH)(H_2O) in the IR spectra of some complexes, indicating that these complexes contained water molecules. No characteristic band due to coordinated water is observed in the far-IR spectra of these complexes, which proves that all the water molecules are crystal water. This is consistent with the results of elemental analysis. The complexes *VII* and *I* did not show the band at this region, suggesting they do not contain water.

The UV absorptions of DMSO solutions of HFAA, Bipy, and the complexes were measured (Table 3). For HFAA and Bipy, λ_{max}/nm are 272 and 280, respectively. They are associated with $\pi \rightarrow \pi^*$. After forming the complex, the λ_{max} exhibits at 278—284 nm. Due to the deprotonation of HFAA, the conjugated effect enhanced and the red shifts were observed obviously. In the complexes, metal ion affects little the



Formula 1. The structure and the spectroscopic denotation of HFAA and Bipy.



Formula 2. The suggested structure of the complexes.

Table 4. Data of Scavenging Effects on $O_2^{\bullet -}$ Radical of the Ligands and Complexes

Compound	Average suppression ratio for $O_2^{\bullet -}$/%
HFAA	12.26
Bipy	2.32
I	53.40
II	33.40
III	63.21
IV	54.78
V	81.70
VI	88.68
VII	46.23

existence of Bipy, so the band of Bipy varies less.

The ^{1}H NMR spectra of the ligands and complex *II* were studied using DMSO-d_6 as solvent. The chemical shifts for HFAA (Formula 1), δ: 11.95 (s, 1H, H_d), 11.80 (s, 1H, H_b), 8.18 (d, 1H, H_c), 4.50 (s, 2H, H_a); for Bipy: δ: 8.67 (d, 2H, H_5, $H_{5'}$), 8.42 (d, 2H, H_2, $H_{2'}$), 7.77 (d, 2H, H_3, $H_{3'}$), 7.25 (d, 2H, H_4, $H_{4'}$); and for complex *II*: δ: 11.71 (s, 1H, H_b), 8.71 (d, 2H, H_5, $H_{5'}$), 8.44 (d, 2H, H_2, $H_{2'}$), 8.03 (d, 1H, H_c), 7.86 (d, 2H, H_3, $H_{3'}$), 7.45 (d, 2H, H_4, $H_{4'}$), 4.09 (s, 2H, H_a), 3.37 (s, 2H, H_2O). When coordinated to the metal ion, the singlet of hydrogen atom of the HFAA COOH at δ = 11.95 disappeared in the complexes, *i.e.* the hydrogen atom of HFAA COOH is replaced by metal ion on complex formation. The δ values of hydrogen atoms of Bipy were shifted to lower field in the complexes, this is due to the decreasing of the electron density of the pyridine ring after the coordination of the nitrogen atom of Bipy [16]. Because of the coordination of HFAA COOH after deprotonation, the electron density of the pyrimidine ring increases. Therefore, the δ values of hydrogen atoms of pyrimidine ring were shifted to higher field [17].

According to the aforementioned data, for the complexes prepared the structure presented in Formula 2 is proposed.

The antioxidative activities of the complexes have been determined. The concentration of the tested compound is 30 μmol dm^{-3}. The suppression ratios of the ligands and the complexes are summarized in Table 4. It can be seen that the ligands and the complexes can scavenge $O_2^{\bullet -}$. The suppression ratios of the complexes for the $O_2^{\bullet -}$ radical are mostly higher than those of the ligands. The scavenging effect of complex *VI* on $O_2^{\bullet -}$ radical is stronger than those of the other complexes.

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