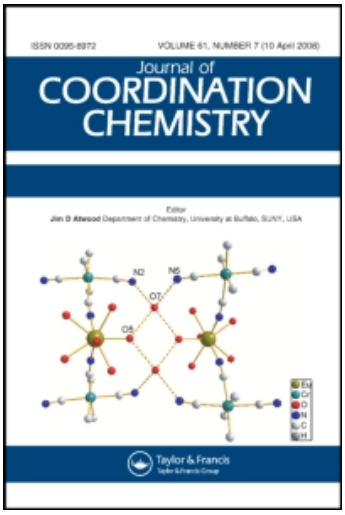

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SYNTHESIS, CHARACTERIZATION AND ANTITUMOR ACTIVITY OF TERNARY COMPLEXES OF *ALL-TRANS* RETINOIC ACID WITH RARE EARTH METALS

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SYNTHESIS, CHARACTERIZATION AND ANTITUMOR ACTIVITY OF TERNARY COMPLEXES OF ALL-*TRANS* RETINOIC ACID WITH RARE EARTH METALS

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Eight new ternary complexes of all-*trans* retinoic acid (HRA) with rare earth metals have been synthesized and characterized by elemental analysis, molar conductance, IR, TG-DTA, UV and ^{1}HNMR. The general formula of the complexes is $M(RA)_2 \cdot Ac \cdot nH_2O$ where M = Y, La, Nb, Sm, Eu, Gd, Er, Tm, $n = 4–8$. Antitumour activity of $Sm(RA)_2 \cdot Ac \cdot 4H_2O$ and $Y(RA)_2 \cdot Ac \cdot 8H_2O$ was tested with human bladder cancer line EJ cells. The results obtained indicated that the inhibitory rates of the two complexes were significantly greater than that of HRA itself ($p < 0.05$). Especially, $Y(RA)_2 \cdot Ac \cdot 8H_2O$ effects on inducing differentiation, modulating the expressions of P_{21} and mutation of P_{53} genes *in vitro*.

Keywords: Antitumor activity; all-*trans* retinoic acid; rare earth complexes; human bladder cancer; induce differentiation; P_{21} and mutation P_{53} gene

INTRODUCTION

The demonstrated therapeutic effectiveness of HRA on acute promyelocytic leukemia (APL) prompted a great deal of interest in studies of HRA and its derivatives in medicine and chemistry. It has been found that HRA possesses certain effects on liver cancer, stomach cancer, and cutis cancer.[1–6]

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However, studies of the antitumor effects of complexes of HRA with rare earth metals on human bladder cancer and the mechanisms have not been reported.

In order to develop highly effective, low poisonous new antitumor medicines, eight rare earth complexes of HRA have been synthesized in our laboratory. The structures and properties of the complexes have been studied and the antitumor activity has been investigated. The studies indicate that the Sm(III) and Y(III) complexes have greater effects against human bladder cancer line EJ cells than HRA.

EXPERIMENTAL

Apparatus and Reagents

Microanalyses for carbon and hydrogen were determined on a Varian EL (Germany) elemental analyzer and rare earth metals were titrated by EDTA volumetric methods using xylenol orange tetraodium salt as indicator. A DDS-11A digital conductometer (Zhejiang Xiaoshan Instrument Standard Spares Plants, China), a 170SX FT-IR spectrophotometer (Nicolet, USA), a UV-240 spectrophotometer (Shimadzu, Japan), an FT-80A nuclear magnetic resonance instrument (Varian, USA). The reagents used included: rare earth oxides (99.99% Yulong Chemical Works Shanghai, China) which were transformed into $M(Ac)_3 \cdot nH_2O$; EJ cells (Beijing Medical Science College, China); RPMI1640 (Gibco, USA); ConA, NAD, PMS (Sigma, USA); NBT (Promega, USA); P_{21}, P_{53} genes (DAKO, Denmark).

Synthesis of the Complexes

The ligand HRA (3.0 mmol) in absolute EtOH (80 mL) was stirred at room temperature for 30 min. After the pH was adjusted to about 6.2, a rare earth(III) acetate (1.0 mmol) was added to the solution, and the solution was stirred continuously for 5 h under a N_2 atmosphere in the dark. The products were isolated by filtration, washed several times with absolute EtOH and dried *in vacuo*.

Antitumor Activity

EJ cells were cultured in RPMI1640 media (control group also containing 0.0001% DMSO) supplemented with various concentrations of HRA or

complexes of HRA with rare earth metals, determined by MTT assay from 24 to 96 h.[7,8] The DMSO group was tested to understand the effect of DMSO. The induced differentiation experiments were carried out by Concanavalin A (ConA) agglutination reaction, cloning efficiency of double deck soft agar culture and lactic dehydrogenase isoenzyme (LDH) spectra. The expressions of P_{21} and mutation P_{53} were determined by the immunohistochemistry ABC staining method.[9]

RESULTS AND DISCUSSION

Compositions and Properties of the Complexes

Elemental compositions, molar conductances and molecular formulae of the complexes are listed in Table I. The complexes are unstable in air. They are soluble in DMSO and DMF, slightly soluble in absolute ethanol and acetone, and insoluble in water. The molar conductances of these complexes in DMF solution vary from 6.18 to 12.59 $S\,cm^2\,mol^{-1}$, indicating that they are nonelectrolytes.[10]

IR Spectra

The IR data of HRA and its complexes are given in Table II. The bands observed in the spectra of the free ligand at 1288 and 919 cm^{-1}, assigned to the δ_{OH} and ρ_{rOH} vibrations of the carboxyl group,[11] disappeared upon complexation, indicating that the carboxyl group of HRA coordinated through the OH oxygen. Subsequently, the complexes displayed both symmetric and asymmetric stretching vibrations of COO^- at 1562–1568

TABLE I Elemental analyses and molar conductances for the complexes $M(RA)_2 \cdot Ac \cdot nH_2O$

$M(RA)_2 \cdot Ac \cdot nH_2O$	C%		H%		M%		Λ ($S \cdot cm^2 \cdot mol^{-1}$)
	Found	*Calc.*	*Found*	*Calc.*	*Found*	*Calc.*	
$Y(RA)_2 \cdot Ac \cdot 8H_2O$	56.05	56.60	7.68	8.20	9.88	10.00	12.59
$La(RA)_2 \cdot Ac \cdot 8H_2O$	53.41	53.62	7.33	7.77	15.21	14.79	8.43
$Nd(RA)_2 \cdot Ac \cdot 4H_2O$	58.25	57.73	6.95	7.45	16.50	16.49	8.11
$Sm(RA)_2 \cdot Ac \cdot 4H_2O$	56.83	57.34	6.69	7.39	16.82	17.06	6.21
$Eu(RA)_2 \cdot Ac \cdot 8H_2O$	52.26	52.78	7.24	7.66	15.80	15.95	6.18
$Gd(RA)_2 \cdot Ac \cdot 4H_2O$	57.54	56.88	6.86	7.36	17.51	17.72	6.20
$Er(RA)_2 \cdot Ac \cdot 8H_2O$	51.65	52.07	7.05	7.54	17.18	17.25	7.06
$Tm(RA)_2 \cdot Ac \cdot 6H_2O$	53.64	53.96	6.81	7.39	18.41	18.01	6.36

TABLE II Data of the important IR data of HRA and its rare earth complexes (cm^{-1})

	HRA	*Y*	*La*	*Nd*	*Sm*	*Eu*	*Gd*	*Er*	*Tm*
ν_{OH} (H_2O)		3366	3402	3364	3402	3396	3427	3382	3362
$\nu_{C=O}$	1686								
$\nu_{C=O}$ ($CH_3CO_2^-$)		1713	1711	1710	1714	1712	1715	1713	1712
$\nu_{C=C}$	1601	1601	1604	1601	1601	1601	1601	1601	1601
δ_{C-H}*	961	973	975	972	974	973	974	973	972
ν_{as}		1562	1583	1586	1570	1570	1581	1585	1567
ν_s		1377	1376	1373	1375	1375	1376	1376	1377
$\Delta\nu$		185	207	213	195	195	205	199	190
δ_{OH}	1288								
ρ_{rOH}	919								
$\rho_{rC-H}(CH_3CO_2^-)$		1067	1072	1071	1068	1071	1069	1068	1069
δ_{C-H} (methyl)	1438	1422	1415	1414	1420	1418	1423	1424	1424
ρ_r (H_2O)		782	781	776	778	779	778	780	782
ρ_ω (H_2O)		538	538	534	538	538	540	538	536
ν_{M-O}		510	513	509	513	514	513	513	512
		409	411	426	416	414	414	415	414

* CH, *trans* double bond.

and 1373–1377 cm^{-1}, respectively. The $\Delta\nu = \nu_{as} - \nu_s = 190–213\,cm^{-1}$ is larger than for sodium mandelate ($\Delta\nu = 154\,cm^{-1}$), which strongly suggests monodentate coordination of the ligand carboxyl group with rare earth ions.[12] The bands at about 1713 and 1069 cm^{-1} may be assigned to the $\nu_{C=O}$ and ρ_{rC-H} of CH_3COO^-, respectively[13] suggesting that the acetoxy also coordinates to the rare earth metals since HRA did not have similar bands. This is confirmed by our ^{1}HNMR studies.

The broad features at about 3362–3402 and 610 cm^{-1} in the spectra of the complexes are attributed to crystal water. Two absorptions at about 780 and 538 cm^{-1} suggest that some of the water molecules coordinate to the metal ions as well.[13] By comparison of the far-IR spectra of the complexes with that of HRA, new peaks appear at 509–514 and 409–426 cm^{-1}, indicating the formation of the M–O bond.[14]

Thermal Analysis

The thermal behavior of all eight complexes was similar. Data are given in Table III. Two endothermic peaks corresponding to water loss in the DTA curve (60°C and 190°C) suggested that the water molecules are either coordinated to the rare earth ions or present as crystal water. This conclusion is consistent with the IR studies. The exothermic peak indicating the beginning of decomposition appeared at higher temperature for the complexes (around 280–350°C) than for the ligand (190°C), indicating that the former are more stable than the latter. There were two other exothermic

TABLE III Thermal data of HRA and its rare earth complexes

Sample	*Water loss*		*Loss rate* (%)	*Dec. temp.*		*Residue*	
	T_1 (°C)	T_2 (°C)		T_1 (°C)	T_2 (°C)	*Rate* (%)	*Formula*
HRA		165		190			
Y	80	161	16.98	348	495	24.44	Y_2O_3
La	64	170	15.51	340	475	34.50	La_2O_3
Nd	85	187	8.80	315	400	38.76	Nd_2O_3
Sm	80	161	10.01	325	420	38.48	Sm_2O_3
Eu	60	185	16.05	285	460	36.30	Eu_2O_3
Gd	70	183	8.01	280	467	38.95	Gd_2O_3
Tm	65	190	12.00	360	435	41.50	Tm_2O_3

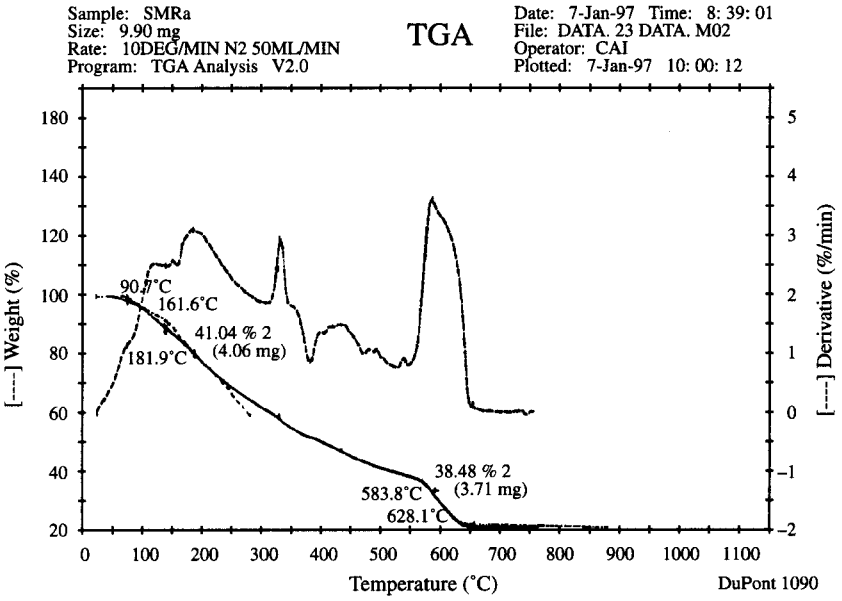

FIGURE 1 TG curve of $Sm(RA)_2 \cdot Ac \cdot 4H_2O$.

peaks at higher temperatures. The complexes decomposed completely at *ca* 700°C and the residues were rare earth oxides. A TG curve of the Sm(III) complex is shown in Figure 1.

Electronic Spectra

The electronic spectra of the Nd(III) and Er(III) ternary solid complexes are summarized in Table IV and shown in Figure 2. By comparison with their

TABLE IV Electronic spectra parameters of the Nd(III) and Er(III) complexes

Complex	ν (cm^{-1})	*Assignment*	*Covalant parameters*
$Nd(RA)_2 \cdot Ac \cdot 4H_2O$	12487	$^4I_{9/2}-{}^4F_{5/2}$	$\beta = 0.9894$
	13426	$^4F_{7/2}, {}^4S_{3/2}$	$\delta = 1.071$
	15913	$^4F_{9/2}$	$b^{1/2} = 0.0728$
	17129	$^4G_{5/2}, {}^2G_{7/2}$	
	19066	$^4G_{7/2}$	
$Er(RA)_2 \cdot Ac \cdot 8H_2O$	12580	$^4I_{15/2}-{}^4I_{9/2}$	$\beta = 0.9944$
	15328	$^4F_{9/2}$	$\delta = 0.5620$
	18382	$^4S_{3/2}$	$b^{1/2} = 0.0529$
	19290	$(^2H,{}^4G)_{11/2}$	
	21810	$^4F_{5/2}$	

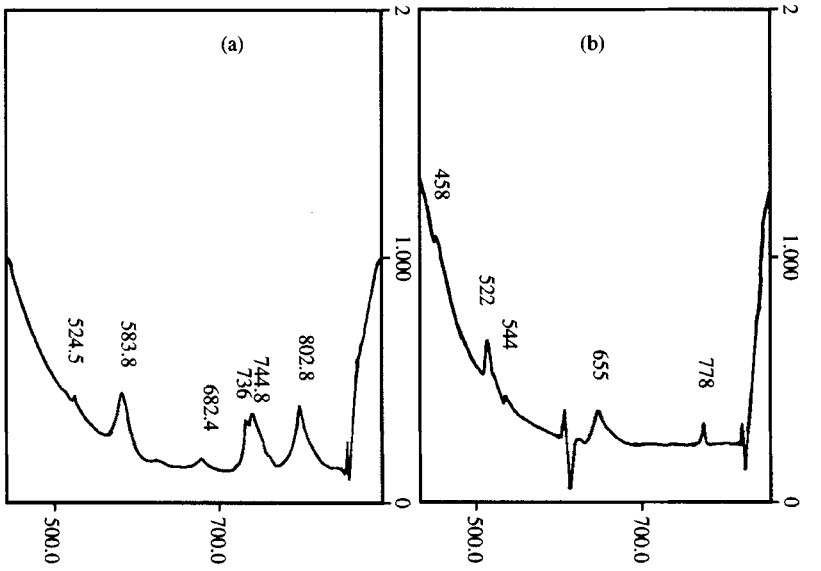

FIGURE 2 Electronic spectra of (a) $Nd(RA)_2 \cdot Ac \cdot 4H_2O$ and (b) $Er(RA)_2 \cdot Ac \cdot 8H_2O$.

hydrated cations, the absorption peaks of the rare earth complexes varied to some degree. The measured spectral parameters, including Shiha's parameter (δ), the naphelauxetic ratio (β) and the bonding parameter ($b^{1/2}$), were used to indicate the nature of the bonding between the metal and the ligand.[15,16] Positive values of β, δ, $b^{1/2}$ and δ less than 1.5 indicated that the coordinate bond of the metal ion with the ligand is poor covalent. The $b^{1/2}$ of the heavy rare earth complex is less than that of the light rare earth complex, indicating that the affinity of heavy rare earth ions for water is greater than that of light rare earth ions.

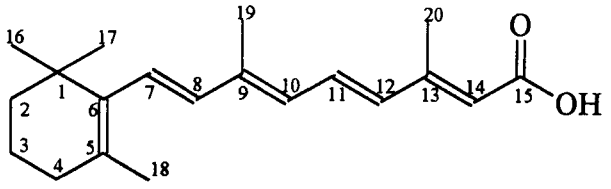

FIGURE 3 Structure of HRA.

^{1}HNMR Spectra

^{1}HNMR spectra of HRA and $La(RA)_2 \cdot Ac \cdot 8H_2O$ were studied using d_6-DMSO as solvent and Ref. 17. The structural formula of HRA and the numbering of the H atoms are shown in Figure 3.

The chemical shifts for HRA: δ12.3 ppm (1H, s, H_{15}), 6.97–6.00 (5H, m, $H_{12,11,10,8,7}$), 5.70 (1H, s, H_{14}), 2.24 (3H, s, H_{20}); and for $La(RA)_2 \cdot Ac \cdot 8H_2O$: δ6.82–6.00 ppm (5H, m, $H_{12,11,10,8,7}$), 5.58 (1H, s, H_{14}), 2.16 (3H, s, H_{20}), 1.69 (3H, s, $CH_3CO_2^-$), 3.35 (br, H_2O). The broad single peak due to the carboxyl proton of the free ligand disappeared in the La(III) complex. The single peak at 1.69 ppm may be attributed to the Me proton of $CH_3CO_2^-$. These changes indicate that the carboxyl groups of the ligand and the $CH_3CO_2^-$ are coordinated to the La^{3+} ion. A broad peak at 3.35 ppm in the complexes may be attributed to the coordinated water. ^{1}HNMR spectra of HRA and the La(III) complex are in Figure 4.

Antitumor Studies

Data on inhibitory effects of HRA, $Y(RA)_2 \cdot Ac \cdot 8H_2O$ and $Sm(RA)_2 \cdot Ac \cdot 4H_20$ against human bladder cancer line EJ cells (tested by Lanzhou Medicine College) are given in Table V. First, by comparison of the experimental groups including DMSO, HRA, and M(III) complexes with the control group, we found that the experimental groups inhibited the proliferation of EJ cells ($p < 0.01$); Subsequently, we also compared HRA and M(III) complexes with the DMSO group, in order to get rid of the effects of DMSO. As shown in Table V, the data suggested that the antitumor effects of HRA and M(III) complexes were not induced by DMSO, since their biochemical activities were great than the latter. Lastly, by comparison of the M(III) complexes with HRA, we found that EJ cells were obviously inhibited after they had been treated with Sm(III) and Y(III), indicating that the antitumor effects of Sm(III) and Y(III)

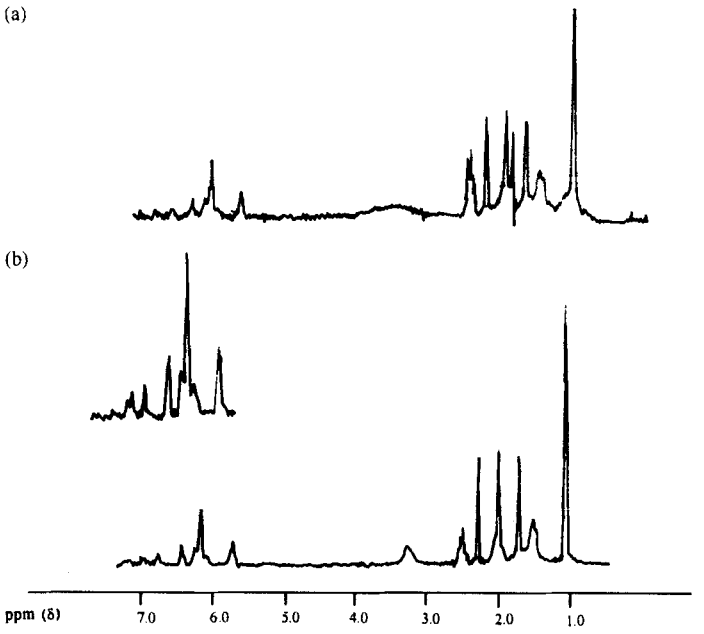

FIGURE 4 ^{1}HNMR spectra of (a) $La(RA)_2 \cdot Ac \cdot 8H_2O$ and (b) HRA.

TABLE V Inhibitory effects of HRA, $Y(RA)_2 \cdot Ac \cdot 8H_2O$ and $Sm(RA)_2 \cdot Ac \cdot 4H_2O$ against EJ cells

*Sample**	*Time* (h)	$OD = \bar{X} \pm OD$ (10^{-6} M)	$OD = \bar{X} \pm OD$ (10^{-7} M)	$OD = \bar{X} \pm OD$ (10^{-8} M)	$OD = \bar{X} \pm OD$ (10^{-9} M)
Control	72	1.462 ± 0.012	1.462 ± 0.012	1.462 ± 0.012	1.462 ± 0.012
HRA		$1.370 \pm 0.066^{\dagger}$	1.468 ± 0.038	$1.468 \pm 0.060^{\dagger}$	1.460 ± 0.033
DMSO		1.340 ± 0.045	1.462 ± 0.075	1.272 ± 0.072	1.380 ± 0.062
Y(III)		$1.330 \pm 0.054^{\dagger}$	1.422 ± 0.072	$1.350 \pm 0.043^{\dagger}$	$1.312 \pm 0.013^{\ddagger,\P}$
Sm(III)		$1.245 \pm 0.043^{\ddagger}$	$1.393 \pm 0.021^{\dagger}$	$1.283 \pm 0.066^{\ddagger}$	1.370 ± 0.068
Control	96	1.494 ± 0.048	1.494 ± 0.048	1.494 ± 0.048	1.494 ± 0.048
HRA		$1.422 \pm 0.036^{\dagger}$	1.508 ± 0.062	$1.434 \pm 0.008^{\dagger}$	1.468 ± 0.067
DMSO		1.380 ± 0.024	1.452 ± 0.012	1.342 ± 0.053	1.442 ± 0.024
Y(III)		$1.360 \pm 0.080^{\dagger}$	1.462 ± 0.009	$1.408 \pm 0.039^{\dagger}$	$1.448 \pm 0.030^{\ddagger}$
Sm(III)		$1.334 \pm 0.026^{\ddagger,\P}$	1.431 ± 0.028	$1.309 \pm 0.050^{\ddagger,\P}$	1.417 ± 0.048

* Each concentration contained five wells and repeated for six independent experiments ($n = 5$).
† Represents $p < 0.05$.
‡ Represents $p < 0.01$ compared with control.
¶ Represents $p < 0.05$ compared with HRA. "*t*" text.

complexes were great than that of HRA ($p < 0.05$) and transition metal(II) complexes.[18]

The effects of induced differentiation of $Y(RA)_2 \cdot Ac \cdot 8H_2O$ in EJ cells were tested on the minimum ConA concentration of agglutination, colony

efficiency in double deck soft agar culture for 14 days, and assays of LDH isoenzyme. The results are summarized in Tables VI–VIII, respectively. As shown in these tables, the malignancy of human bladder cancer line EJ cells were reduced after treatment with 10^{-9} M Y(III) complexes and the EJ cells were induced differentiation to more mature.

TABLE VI Data of the minimum ConA concentration of agglutination

Sample	*Minimum ConA concentration* (μg/ml)
Control	3.125
10^{-6} M HRA	6.25
10^{-9} M HRA	6.25
10^{-6} M $Y(RA)_2 \cdot Ac \cdot 8H_2O$	6.25
10^{-9} M $Y(RA)_2 \cdot Ac \cdot 8H_2O$	12.5

TABLE VII Data of colony efficiency in double deck soft agar culture for 14 days

*Sample**	*Number of colony*	*Colony efficiency* (%)
Control	28	28
10^{-6} M HRA	14	14**
10^{-9} M HRA	22	22**
10^{-6} M $Y(RA)_2 \cdot Ac \cdot 8H_2O$	23	23**
10^{-9} M $Y(RA)_2 \cdot Ac \cdot 8H_2O$	10	10**

* Each concentration group contained 100 cells.
** Represents $p < 0.01$ compared with control. "χ^2" text.

TABLE VIII Data of LDH isoenzyme electrophoresis OD (%)

Sample	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Control	22.4	32.2	26.6	5.6	8.8
10^{-9} M HRA	24.1	27.0	25.9	10.8	14.3
10^{-9} M $Y(RA)_2 \cdot Ac \cdot 8H_2O$	19.4	29.4	23.1	10.3	12.5

TABLE IX Data of the positive expression of P_{21} and mutation P_{53}

*Sample**	*Concentration* (M)	P_{21}		P_{53}	
		Number	*Rate* (%)	*Number*	*Rate* (%)
Control		666	66.6	580	58.0
HRA	10^{-6}	460	46.0**	437	43.7**
	10^{-9}	630	63.0**	460	46.0**
$Y(RA)_2 \cdot Ac \cdot 8H_2O$	10^{-6}	590	59.0**	440	44.0**
	10^{-9}	530	53.0**	400	40.0**

* Each group contained 1000 cells.
** Represents $p < 0.01$ compared with control. "χ^2" text.

Immunohistochemistry was used to detect the expression of P_{21} and mutation P_{53}. The results are listed in Table IX. The positive rate of P_{21} and mutation P_{53} in EJ cells treated with the Y(III) complex are lower than those treated with HRA, indicating that $Y(RA)_2 \cdot Ac \cdot 8H_2O$ induced the anti-tumour effect through down-regulating the expression of P_{21} or mutation P_{53} gene.

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