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Short communication

Synthesis and structure–activity relationships of tetrazolato-bridged dinuclear platinum(II) complexes: A small modification at tetrazole C5 markedly influences the *in vivo* antitumor efficacy

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ABSTRACT

We synthesized and characterized 15 new derivatives of the highly anticancer-active platinum(II) complex [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-tetrazolato-*N*2,*N*3)]$^{2+}$ (**5-H-Y**) by making substitutions at tetrazole C5. We then evaluated the comprehensive structure–cytotoxicity relationships of a total of 23 derivatives in two murine lymphocytic leukaemia cell lines, sensitive and resistant to cisplatin. We also report the *in vivo* antitumor efficacy of three ester derivatives, two of which exhibited much higher efficacy than oxaliplatin against mouse homografted Colon-26 colorectal tumor.

Platinum-based drugs such as cisplatin [1,2] are currently used to treat a variety of cancers [3–6]. Tetrazolato-bridged dinuclear platinum (II) complexes with the general formula [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-5-R-tetrazolato-*N*2,*N*3)]$^{n+}$ (n = 1 or 2) are currently being developed as next-generation anticancer drug candidates [7,8], and many are reported to be effective against cancers with intrinsic [9] or acquired [10,11] resistance to platinum-based drugs, the target molecule of which is believed to be cellular DNA [12–17]. We previously reported that tetrazolato-bridged complexes have a unique DNA binding mode [10,18–21] and a different cellular uptake pathway [11] than currently available platinum-based drugs. In the present study, we synthesized and characterized 15 new derivatives of the lead compound [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-tetrazolato-*N*2,*N*3)]$^{2+}$ (**5-H-Y**) by making substitutions at tetrazole C5 [22]. The structure–cytotoxicity relationships of a total of 23 derivatives of **5-H-Y** were then investigated in two murine lymphocytic leukaemia cell lines, one sensitive (L1210) and one resistant (L1210R) to cisplatin. We also report the *in vivo* antitumor efficacy of three derivatives against mouse homografted Colon-26 colorectal tumor.

First, we synthesized and characterized 15 new tetrazolato-bridged derivatives of **5-H-Y** (complexes **1**–**15**; Fig. 1) *via* the process shown in Scheme 1. As reported previously [9,23], [*cis*-Pt$(NH_3)_2$(μ-OH)]$_2(NO_3)_2$ was subjected to ligand substitution reactions with 1.1 equivalents of different tetrazole derivatives at 40 °C; substitution of tetrazolate for one of the OH bridges produced the desired products, which were then purified by recrystallization. Complexes **3**, **9** and **10** were obtained by saponification of the corresponding complex containing ester A or B. The complexes were characterized by means of ^{1}H, ^{13}C, ^{19}F (for **11**–**13**), or ^{195}Pt NMR spectroscopy and electrospray-ionization mass spectrometry (see SI). The synthesis of complexes **16**–**23** was reported previously [24]. Complexes **1**–**23** were grouped depending on the type of substituent introduced at tetrazole C5 (Fig. 1 and Table 1).

Next, we evaluated the *in vitro* cytotoxicity of **1**–**23** in L1210 and the subline L1210R, which is resistant to cisplatin due to its reduced internalization of cisplatin as compared with the parenteral line [11]. Cytotoxicity was evaluated by using an MTT assay to determine the half maximal inhibitory concentrations (IC_{50}) of the complexes (Table 1, which includes data for **5-H-Y**, [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-ethyl tetrazolato-*N*2,*N*3-5-acetate)]$^{2+}$ (**EtAc**), [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-tetrazolato-*N*2,*N*3-5-acetate)]$^{+}$ (**Ac**), and [{*cis*-Pt$(NH_3)_2$}$_2$(μ-OH)(μ-5-methyltetrazolato-*N*2,*N*3)]$^{2+}$ (**Me**) for comparison; see Fig. 1). Nineteen of the 23 complexes were more cytotoxic than cisplatin in L1210 cells. We calculated the resistance factor for 22 of the platinum(II) complexes (except **3**), which was defined as the relative ratio of the IC_{50} values of each complex in the two cell lines (RF = $IC_{50\ (L1210R)}/IC_{50\ (L1210)}$; Table 1). The RF of complexes **1**, **2** and **4**–**23** ranged from 0.4 to 1.7 (mean RF, 0.80), indicating that many of these complexes circumvented cross-resistance to cisplatin. Overall, 19 of the 23 complexes had comparable or higher cytotoxicity in L1210R cells than in L1210 cells. The series of tetrazolato-bridged complexes were predicted to inhibit cell growth by means of a mechanism of action different from those of clinical Pt drugs [9], though **5-H-Y** inhibits DNA replication and also

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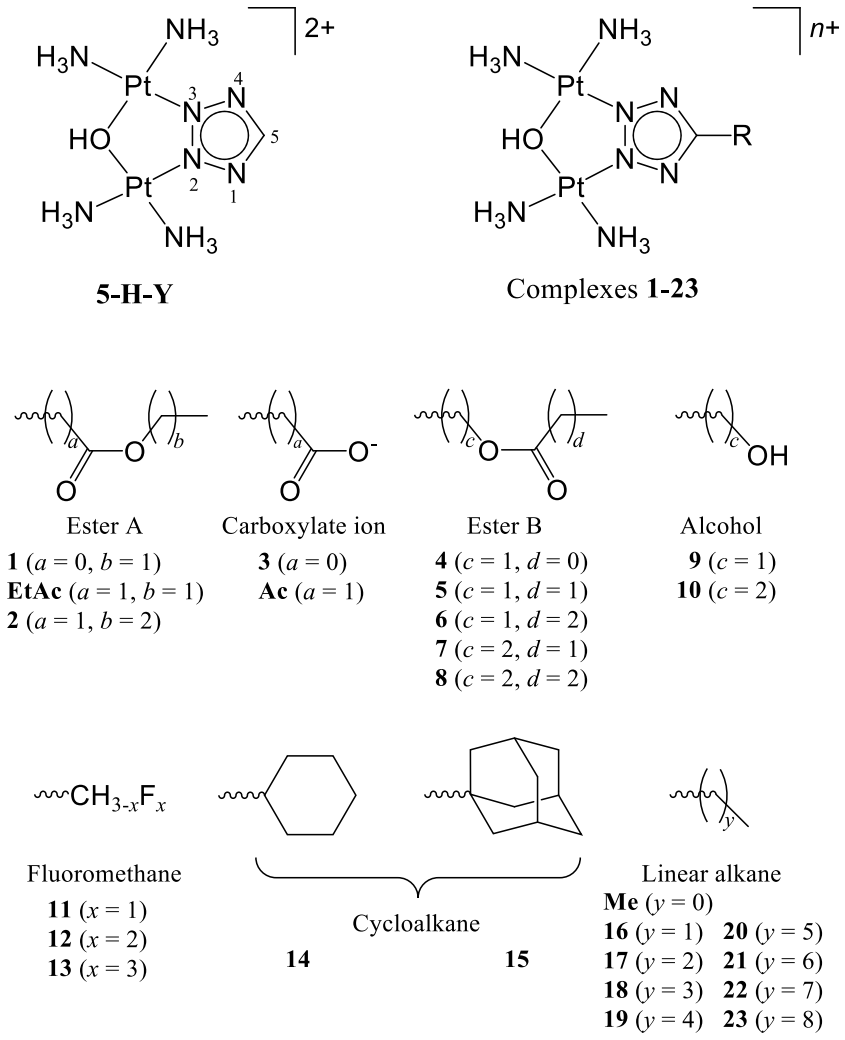
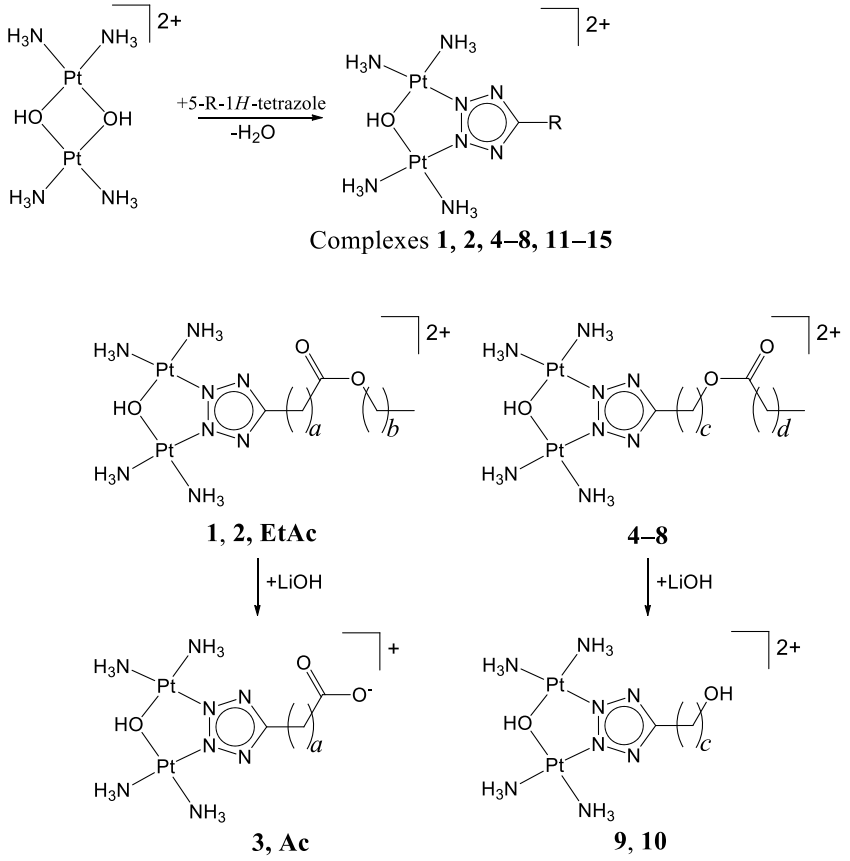


Fig. 1. Previously reported structures of **5-H-Y**, **EtAc**, **Ac**, and **Me**, and of 23 derivatives of **5-H-Y** with the general formula [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-5-R-tetrazolato-*N*2,*N*3)]$^{n+}$ (n = 1 or 2). Synthetic methods for complexes **1–15** are newly reported in this study; those for complexes **16–23** we reported previously [24].

RNA transcription, arresting cells in the S/G2 phase [21], just like cisplatin [25,26]. In our previous study, cisplatin uptake into L1210R cells was decreased compared with that into L1210, whereas most tetrazolato-bridged complexes were more efficiently taken up into L1210R [11]. Therefore, the circumvention of cross-resistance to cisplatin may result from a different cellular uptake pathway from cisplatin, as well as formation of the unique Pt-DNA adduct, which are poor substrates for DNA repair [27–29]. The average cytotoxicity exhibited by the 26 complexes (including **EtAc**, **Ac**, and **Me**) in L1210 cells was in the substituent order of alcohol ≥ fluoromethane ≥ linear alkane > ester B > ester A > cycloalkane ≫ carboxylate ion; and that in L1210R cells was fluoromethane > alcohol ≥ linear alkane ≥ ester B > ester A ≫ cycloalkane ≫ carboxylate ion. Complexes **1**, **2**, and **EtAc** (ester A derivatives) were more cytotoxic with increasing number of methylene groups. Complex **3** (carboxylate ion derivative), which was a hydrolysed form of ester A, had almost no cytotoxicity, which is consistent with a previous report that **Ac** is not internalized by L1210 or L1210R cells [11]. This lack of cellular uptake, and therefore, cytotoxicity may be a result of complex **3** and **Ac** having an overall charge of +1, whereas all the other derivatives examined had an overall charge of +2 [11,30]. Complexes **4**, **5**, **6**, and **8**, but not **7** (ester B derivatives), and complexes **9** and **10** (alcohol derivatives) all had high cytotoxicity. Thus, derivatives containing ester A or ester B substituents may be useful for designing improved pro-drugs for the treatment of cancers. Currently, the fluorination of drug candidates is a common means of altering the physicochemical properties of lead compounds [31]. In the present study, the cytotoxicity of the fluoromethane derivatives (**11**−**13**) decreased with increasing number of fluorine atoms. The two cycloalkane derivatives had different cytotoxicities, with the derivative with the bulkier substituent (adamantane, **15**) having a much lower cytotoxicity than that with the less bulky substituent (cyclohexane, **14**). In addition, the cytotoxicities of the



Scheme 1. Synthesis of 15 new tetrazolato-bridged dinuclear Pt(II) complexes.

linear alkyl chain derivatives followed a U-shaped pattern, which is consistent with those reported in a different cancer cell line [24].

Next, we evaluated the *in vivo* antitumor efficacy of the three ester A-type (**1**, **EtAc**, and **2**) derivatives against homografted Colon-26 colorectal tumors in BALB/c mice, since **EtAc** is one of the lead compounds among tetrazolato-bridged complexes [9]. Oxaliplatin, an anticancer drug currently used to treat colorectal cancer, was used as the positive control. Each of the platinum(II) complexes was dissolved in 5% glucose and administered to the mice as a single dose at 10 mg/kg *via* the tail vein on day 0, which was 7 days after inoculation with Colon-26 colorectal cancer cells. No mice in any groups died and none showed marked body weight loss (Fig. 2, top panel), although there was a weak body weight loss signal in mice treated with **2** at day 7 after administration. The body weight loss was not significant in comparison with the other groups and so we conclude that it was transient. Furthermore, the abnormalities of internal organs by the macroscopic findings were not observed. Tumor growth in the mice treated with **1** or **2** was much slower than that in mice treated with oxaliplatin, as well as that in untreated control mice (Fig. 2, bottom panel). Mean terminal tumor volume in the mice treated with **1** or **2** was 35% and 26% that in the control group; the corresponding value for mice treated with **EtAc** and oxaliplatin was 74% and 71%, respectively. Interestingly, the structural difference between **EtAc** and these two highly active derivatives is a single CH_2 moiety, indicating that a small modification at tetrazole C5 had a marked influence on the compound's *in vivo* antitumor efficacy.

In this study, we found that a variety of substitutions at tetrazole C5 increased the cytotoxicity of the tetrazolato-bridged complex framework. Also, the *in vivo* study of ester A-type derivatives showed that even small structural modifications can result in improvements in antitumor efficacies, and these ester A-type derivatives were found to be much more active than oxaliplatin. This suggests that tetrazolato-bridged complexes are a potentially important platform for developing highly active anticancer drugs for use in patients with advanced cancer.

Table 1
Cytotoxicity and resistance factor (RF) of cisplatin, **5-H-Y**, and 23 derivatives of **5-H-Y** with substitutions at tetrazole C5 in two murine leukaemia cell lines, one sensitive (L1210) and one resistant to cisplatin (L1210R).

Substituent type	Complex	IC_{50} (μM)[a]		RF[b]
		L1210	L1210R	
–	Cisplatin	17.8 ± 2.1	65.0 ± 8.5	3.7
H	**5-H-Y**[c]	1.5 ± 0.1	1.4 ± 0.3	1.0
Ester A	**1**	24.8 ± 1.9	23.3 ± 3.4	0.9
	EtAc[c]	8.7 ± 0.3	4.6 ± 0.5	0.5
	2	4.0 ± 0.8	2.3 ± 0.4	0.6
Carboxylate ion	**3**	> 160	> 160	–
	Ac[c]	> 160	> 160	–
Ester B	**4**	2.8 ± 0.3	3.1 ± 0.4	1.1
	5	2.2 ± 0.1	1.7 ± 0.2	0.8
	6	2.5 ± 0.2	1.7 ± 0.9	0.7
	7	42.5 ± 8.5	15.0 ± 2.2	0.4
	8	2.8 ± 0.3	2.8 ± 0.4	1.0
Alcohol	**9**	3.1 ± 0.3	3.0 ± 0.2	1.0
	10	3.4 ± 0.3	3.1 ± 0.2	0.9
Fluoromethane	**11**	2.2 ± 0.1	1.4 ± 0.0	0.6
	12	3.3 ± 0.2	1.5 ± 0.0	0.5
	13	5.5 ± 0.7	2.9 ± 0.3	0.5
Cycloalkane	**14**	8.8 ± 1.8	4.0 ± 0.2	0.5
	15	63.8 ± 2.5	79.5 ± 14.1	1.3
Linear alkane	**Me**[c]	1.2 ± 0.1	1.4 ± 0.4	1.2
	16	1.3 ± 0.1	0.7 ± 0.1	0.5
	17	1.3 ± 0.1	1.0 ± 0.1	0.7
	18	1.8 ± 0.0	1.0 ± 0.2	0.5
	19	2.2 ± 0.3	1.3 ± 0.4	0.6
	20	5.4 ± 0.4	3.8 ± 0.9	0.7
	21	10.2 ± 0.2	9.1 ± 1.1	0.9
	22	9.7 ± 1.0	10.2 ± 0.1	1.1
	23	4.1 ± 0.4	6.9 ± 0.7	1.7

[a] Data are presented as the mean ± standard deviation of four experiments.
[b] RF is the relative ratio of the IC_{50} value for each cell line (RF = IC_{50} (L1210R)/IC_{50} (L1210)).
[c] Data reported in reference [11] are included for comparison.

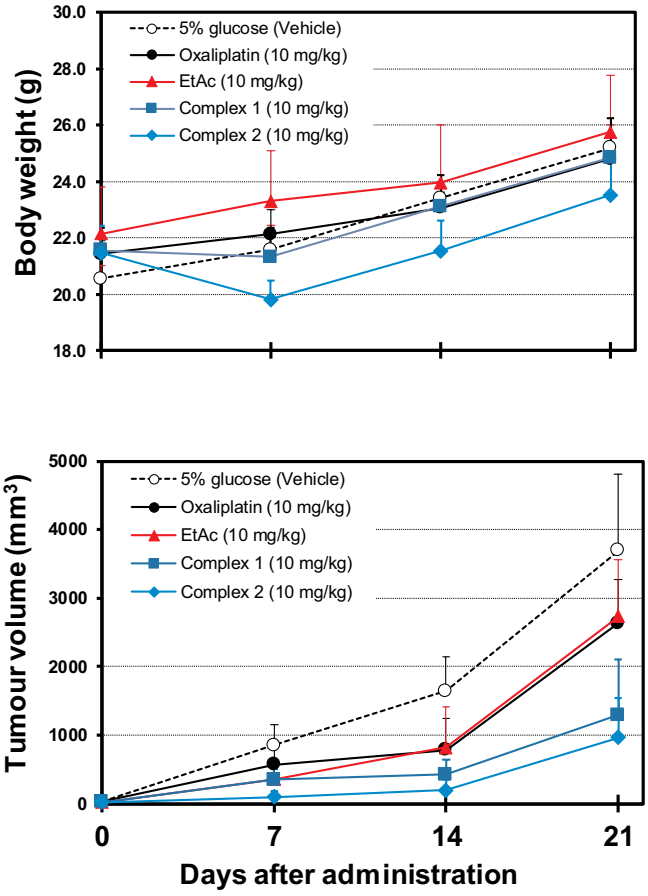


Fig. 2. Body weight and tumor volume in BALB/c mice laterally homografted with Colon-26 murine colorectal cancer cells after treatment with 10 mg/ kg of oxaliplatin, **EtAc**, **1**, or **2**. Data for untreated control mice are indicated by open circles. Mice were treated with a single dose of the test compounds only on day 0. Body weight and tumor volume were measured weekly starting on day 0. Each data point represents the mean of six body weights or tumor volumes, and the error bars indicate standard deviations of the mean.

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