

## Note

## DNA Interaction and Cytotoxicity of Cyclometalated Ruthenium(II) Complexes as Potential Anticancer Drugs

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To evaluate the anticancer activity of the cyclometalated ruthenium(II) complexes  $[\text{Ru}(\text{bpy})_2(\text{C}^{\wedge}\text{N})]\text{Cl}$ , we have studied the interaction of these complexes using calf thymus DNA (CT-DNA) and cytotoxicity assays with two tumor (L1210 and HeLa) and a non-tumor (BALB/3T3 clone A31) cell lines. It is suggested that the complexes act as intercalators and/or DNA minor groove binders. Moreover, the complexes display favorable cytotoxicity activities with L1210 and HeLa, which in all cases were significantly more favorable than cisplatin. In contrast, the complexes exhibit appreciably lower cytotoxicity toward BALB/3T3 clone A31.

**Key words** cyclometalated ruthenium(II) complex; anticancer drug; cytotoxicity; oxidative DNA cleavage; lipophilicity

The discovery of cisplatin (*cis*-diamminedichloridoplatinum(II)) by Rosenberg was one of the most significant events for cancer chemotherapy.<sup>1)</sup> However, the use of cisplatin is restricted by its high toxicity, which leads to undesirable side effects and incidents of drug resistance. With the aim of overcoming these limitations, new platinum-based and non-platinum anticancer drugs are under development. A relatively new line of investigation focuses on ruthenium chemistry in an alternative metallopharmaceutical approach to platinum.<sup>2,3)</sup> The higher coordination number of ruthenium compared with platinum provides additional coordination sites, which can potentially be used to fine-tune the properties of the complex, for example, by influencing the way the complex interacts with DNA.<sup>4)</sup> It is well accepted that ruthenium complexes can display anticancer effects with a fairly high selectivity, the lethality of the tumor cells being higher than that of the normal cells.<sup>5–10)</sup> Several ruthenium complexes that display an activity comparable to that of cisplatin have been described, and in some cases activities are even better.<sup>11–16)</sup> Pfeffer and colleagues<sup>17,18)</sup> and Chao and colleagues<sup>19,20)</sup> have found that some cyclometalated

ruthenium(II) complexes are good candidates for becoming anticancer drugs. A cyclometalated ruthenium fragment with a metal–carbon  $\sigma$  bond is a crucial element for a potential anticancer drug. The ruthenium–carbon bond is known to lower the redox potential of ruthenium(III/II) couple dramatically, so that cyclometalated ruthenium(II) complexes may serve as good catalysts for the generation of hydroxyl radicals by virtue of the Fenton-like reaction. In addition, they have good lipophilicity that allows their entries into the cells.<sup>18,20)</sup> From two features these cyclometalated ruthenium(II) complexes may lead to excellent anticancer drugs because many cancer cells possess low levels of catalase activity.<sup>21)</sup>

In the present study, we have synthesized the cyclometalated ruthenium(II) complexes as shown in Fig. 1,  $[\text{Ru}(\text{bpy})_2(\text{C}^{\wedge}\text{N})]\text{Cl}$ , where bpy is 2,2'-bipyridine and  $\text{C}^{\wedge}\text{N}$  is the deprotonated cyclometalating ligand (2-phenylpyridine (phpy), 2-(4-methylphenyl)pyridine (mphpy), or diphenyldiazene (dphdaz)). To evaluate the anticancer activity of these complexes physicochemical properties such as the redox potentials of Ru(III/II) couples ( $E_{1/2}$ ), the cytotoxicity ( $\text{IC}_{50}$ ) obtained by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay,<sup>22)</sup> and lipophilicity ( $\log P_{o/w}$ ) have been determined. There is evidence that DNA is the target for these complexes, which is similar to that of the well-established platinum drugs.<sup>23–26)</sup> Many studies on their mode of action and on structure–activity relationship have been performed. However, many aspects of the tumor-inhibiting action displayed by ruthenium complexes are still unknown.

## Experimental

**Chemicals** Ruthenium(III) chloride hydrate (assay: 38.45% for Ru) was obtained from Mitsuwa Chemicals. 2,2'-Bipyridine (bpy) was obtained from Wako Pure Chemical Industries, Ltd. 2-Phenylpyridine (phpy), 2-(4-methylphenyl)pyridine (mphpy), and diphenyldiazene (dphdaz) were purchased from Tokyo Chemical Industry. Calf thymus DNA (CT-DNA) and  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$  were purchased from Sigma-Aldrich, and the concentration of CT-DNA was deter-

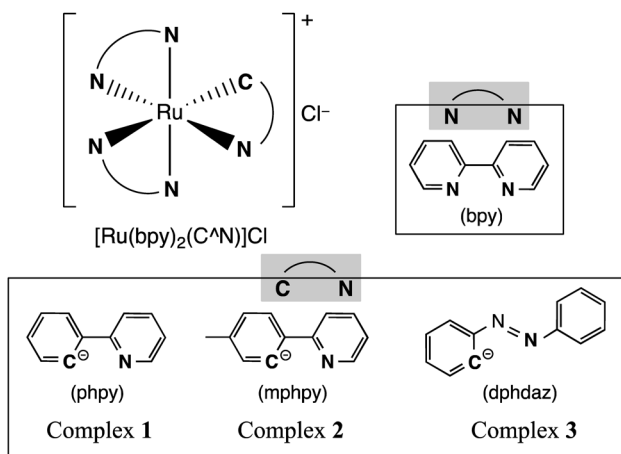


Fig. 1. Cyclometalated Ruthenium(II) Complexes  $[\text{Ru}(\text{bpy})_2(\text{C}^{\wedge}\text{N})]\text{Cl}$  Used in This Study

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mined spectrophotometrically using the molar extinction coefficient  $\epsilon_{257} = 6.6 \times 10^3 \text{ M (base)}^{-1} \text{ cm}^{-1}$ .<sup>27)</sup> The plasmid pBR322 DNA (4361 bp) was obtained from the Nippon gene, and these DNAs were used without further purification. All other reagents and solvents were of guaranteed grades and were used as received. All aqueous solutions were prepared with Millipore "Milli Q" grade water, and experiments were carried out in a buffer consisting of 5 mM Tris-HCl/50 mM NaCl at pH 7.5 unless otherwise noted.

**Syntheses of Ruthenium(II) Complexes** *cis*-[Ru(bpy)<sub>2</sub>Cl<sub>2</sub>] $\cdot$ 2H<sub>2</sub>O was prepared in agreement with a previous report<sup>28)</sup> and used directly in subsequent reactions without further purification. A mixture of *cis*-[Ru(bpy)<sub>2</sub>Cl<sub>2</sub>] $\cdot$ 2H<sub>2</sub>O (0.26 g, 0.50 mmol), C<sup>^</sup>N ligand (0.52 mmol), NH<sub>4</sub>PF<sub>6</sub> (0.26 g, 1.6 mmol), and *N*-ethylmorpholine (1.3 mL, 10 mmol) in H<sub>2</sub>O-methanol (1:5, v/v, 15 mL) was heated under an argon atmosphere at reflux for 4 h. The reaction mixture was then cooled to room temperature and the solvent was removed under vacuum. The crude product was dissolved in acetonitrile and purified by column chromatography (neutral alumina, acetonitrile-toluene, 1:1, v/v). The major purple band was collected and the solvent was removed under vacuum. The resulting solid was dissolved in acetonitrile and added to vigorously stirred diethyl ether to precipitate the product [Ru(bpy)<sub>2</sub>(C<sup>^</sup>N)]PF<sub>6</sub>, which was filtered and washed with diethyl ether. The complex [Ru(bpy)<sub>2</sub>(C<sup>^</sup>N)]PF<sub>6</sub> was metathesized to the corresponding chloride salt by column chromatography (QAE Sephadex A-25, 0.2 M KCl), [Ru(bpy)<sub>2</sub>(C<sup>^</sup>N)]Cl ([Ru(bpy)<sub>2</sub>(phpy)]Cl (**1**), [Ru(bpy)<sub>2</sub>(mphpy)]Cl (**2**), [Ru(bpy)<sub>2</sub>(dphdaz)]Cl (**3**)). [Ru(bpy)<sub>2</sub>(phpy)]PF<sub>6</sub>: *Anal.* Calcd for C<sub>31</sub>H<sub>24</sub>N<sub>5</sub>PF<sub>6</sub>Ru: C, 52.25; H, 3.39; N, 9.83%. Found: C, 52.11; H, 3.69; N, 9.89%. <sup>1</sup>H-NMR in DMSO-*d*<sub>6</sub>,  $\delta_{\text{H}}$  (400 MHz): 8.74 (1H, d, *J*=8.2 Hz), 8.66 (1H, d, *J*=8.2 Hz), 8.59 (2H, t, *J*=7.6 Hz), 8.12 (1H, d, *J*=8.4 Hz), 8.06 (1H, t, *J*=7.9 Hz), 7.92–7.84 (5H, m), 7.76–7.67 (3H, m), 7.62 (1H, d, *J*=5.2 Hz), 7.52 (1H, t, *J*=6.6 Hz), 7.46 (1H, d, *J*=5.5 Hz), 7.36–7.31 (3H, m), 7.01 (1H, t, *J*=6.0 Hz), 6.81 (1H, t, *J*=7.7 Hz), 6.75 (1H, t, *J*=6.7 Hz), 6.29 (1H, d, *J*=6.9 Hz). [Ru(bpy)<sub>2</sub>(mphpy)]PF<sub>6</sub> $\cdot$ H<sub>2</sub>O: *Anal.* Calcd for C<sub>32</sub>H<sub>28</sub>N<sub>5</sub>OPF<sub>6</sub>Ru: C, 51.62; H, 3.79; N, 9.41%. Found: C, 51.96; H, 4.15; N, 9.54%. <sup>1</sup>H-NMR in DMSO-*d*<sub>6</sub>,  $\delta_{\text{H}}$  (400 MHz): 8.73 (1H, d, *J*=8.4 Hz), 8.66 (1H, d, *J*=8.2 Hz), 8.60 (2H, t, *J*=6.5 Hz), 8.05 (2H, d, *J*=8.0 Hz), 7.94–7.84 (4H, m), 7.76 (1H, d, *J*=8.0 Hz), 7.72–7.63 (4H, m), 7.52 (1H, d, *J*=6.6 Hz), 7.42 (1H, d, *J*=5.0 Hz), 7.38–7.32 (3H, m), 6.97 (1H, t, *J*=6.0 Hz), 6.62 (1H, d, *J*=7.8 Hz), 6.11 (1H, d, *J*=7.7 Hz), 1.96 (3H, s). [Ru(bpy)<sub>2</sub>(dphdaz)]PF<sub>6</sub> $\cdot$ H<sub>2</sub>O: *Anal.* Calcd for C<sub>32</sub>H<sub>27</sub>N<sub>6</sub>OPF<sub>6</sub>Ru: C, 50.73; H, 3.59; N, 11.09%. Found: C, 51.36; H, 3.85; N, 11.41%. <sup>1</sup>H-NMR in DMSO-*d*<sub>6</sub>,  $\delta_{\text{H}}$  (400 MHz): 8.74 (1H, d, *J*=8.4 Hz), 8.69 (1H, d, *J*=8.1 Hz), 8.51 (1H, d, *J*=7.6 Hz), 8.45 (1H, d, *J*=8.4 Hz), 8.27 (1H, d, *J*=7.8 Hz), 8.09–7.93 (5H, m), 7.69 (1H, d, *J*=5.1 Hz), 7.54 (1H, d, *J*=4.8 Hz), 7.49–7.44 (4H, m), 7.36 (1H, d, *J*=4.8 Hz), 7.15 (1H, t, *J*=7.3 Hz), 7.08–7.03 (3H, m), 6.96–6.90 (3H, m), 6.80 (1H, d, *J*=7.5 Hz).

**Physical Measurements** UV-visible (UV-Vis) spectra were obtained with a Shimadzu UV-2500PC spectrophotometer equipped with a temperature controller. Emission spectra were recorded on a JASCO FP-6500 fluorescence spectrophotometer. Cyclic voltammograms were obtained by using an ALS/CHI electrochemical analyzer model 630C with a 20-mL one-compartment three-electrode electrochemical cell. A three-electrode system was used with a 3-mm diameter

glassy carbon working electrode (BAS), an Ag/AgCl reference electrode (BAS RE-1B), and a platinum wire counter electrode (BAS). Cyclic voltammetric measurements were carried out in anaerated 5 mM Tris-HCl/50 mM NaCl (pH 7.5) at 25.0 $\pm$ 0.1 $^{\circ}$ C. All redox potentials ( $E_{1/2}$ ) were calculated using  $(E_{\text{pa}} + E_{\text{pc}})/2$  at a scan rate of 0.1 V s<sup>-1</sup>.

**Competitive DNA-Binding Experiments** Using the emission spectral method, the relative bindings of complexes **1–3** and [Ru(bpy)<sub>3</sub>]Cl<sub>2</sub> to CT-DNA were examined with an ethidium bromide (EtBr)-bound CT-DNA solution in 5 mM Tris-HCl/50 mM NaCl at pH 7.5. The emission intensities at 604 nm (470 nm excitation) were measured at various complex concentrations. The experiments were carried out by titrating ruthenium(II) complexes (0–105  $\mu$ M) into EtBr-DNA solution containing 10  $\mu$ M EtBr and 100  $\mu$ M CT-DNA.

#### Agarose Gel Electrophoresis of Plasmid pBR322 DNA

The oxidative DNA cleavage reactions catalyzed by complexes **1–3** were examined by gel electrophoresis experiment. Supercoiled pBR322 DNA (50  $\mu$ M in DNA bp) in the absence and presence of 5 mM H<sub>2</sub>O<sub>2</sub> was treated with 25  $\mu$ M ruthenium(II) complexes in 5 mM Tris-HCl/50 mM NaCl at pH 7.5, and the solutions were incubated at 37 $^{\circ}$ C for 5 h. 4',6-Diamidino-2-phenylindole (DAPI) and methyl green (MG) (25  $\mu$ M) were used as groove binders. Subsequently, the samples were loaded on 1% (w/v) agarose gel containing 0.5  $\mu$ g mL<sup>-1</sup> ethidium bromide (EtBr) in TAE buffer (40 mM Tris-acetate/1 mM ethylenediaminetetraacetic acid (EDTA)) and after running the gel at 100 V for 1 h. The bands were visualized by photographing the fluorescence of intercalated ethidium bromide under a UV (302 nm) illuminator.

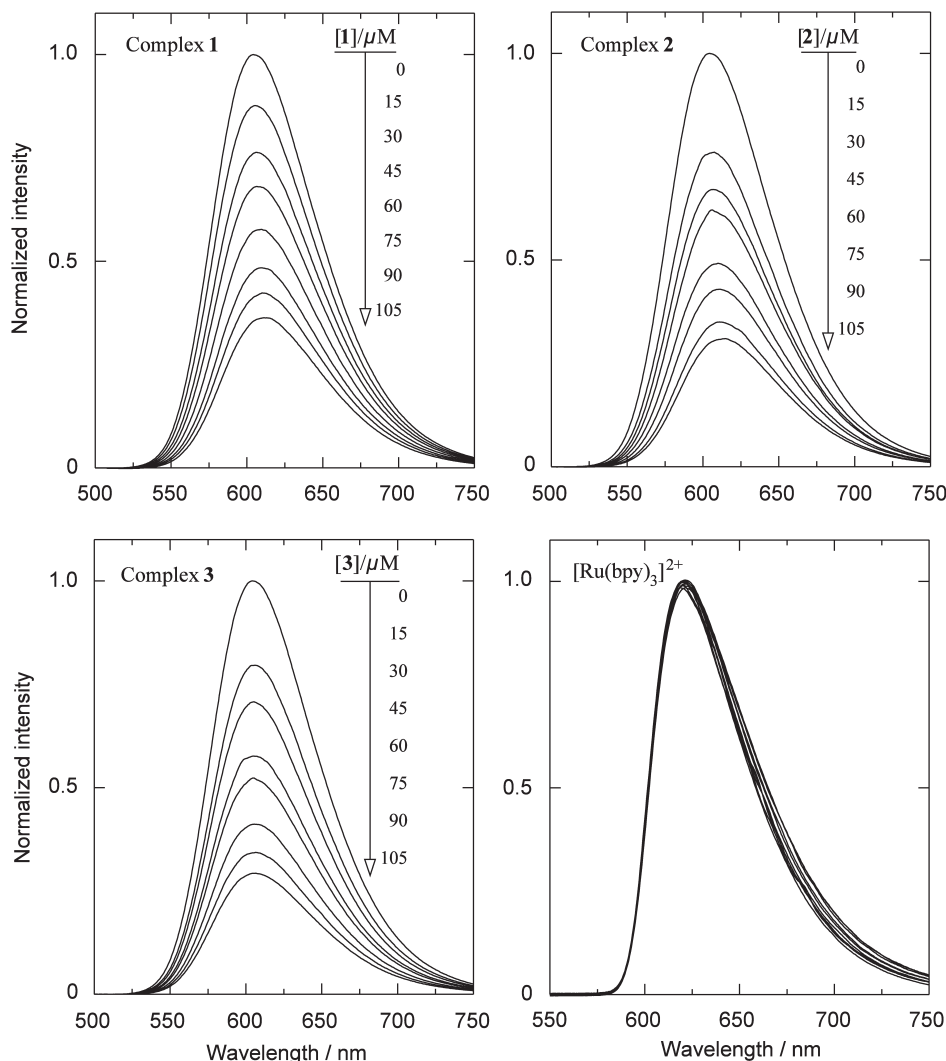
**In Vitro Cytotoxicity Assay** The L1210 murine leukemia (JCRB 9026), the HeLa human cervical cancer (JCRB 9004), and the BALB/3T3 clone A31 murine (JCRB 9005) cell lines were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). These cell lines were cultured as a suspension in RPMI 1640 media (Sigma) supplemented with 10% heat-inactivated fetal bovine serum and no antibiotics. The cells were grown at 37 $^{\circ}$ C in a 5% CO<sub>2</sub> humidified atmosphere. Cytotoxicity was determined by MTT assay.<sup>22)</sup> In brief, 200  $\mu$ L aliquots of a cell suspension containing 10<sup>5</sup> cells mL<sup>-1</sup> were pipetted into each well of the 96-well microtiter plate. The cells were treated for 48 h in the presence of various concentrations of ruthenium(II) complexes. Following exposure to the complexes, 10  $\mu$ L of a 50 mg mL<sup>-1</sup> MTT solution was added to each well and the plate was left at 37 $^{\circ}$ C for 4 h. The culture media were removed and the plate was washed with 0.01 M phosphate buffered saline. The precipitated dye was solubilized by adding 200  $\mu$ L of 2-propanol. The absorption was measured by using a microplate reader at 570 nm. The IC<sub>50</sub> value was defined as the complex concentration that reduced the absorbance by 50% of the absorbance of complex-free control.

## Results and Discussion

**Properties of the Complexes** All of the synthesized complexes gave satisfactory elemental analyses. The X-ray crystal structures of **1** and **2** have been reported previously.<sup>29–31)</sup> Indisputable structural assignment of **3** would arise from the crystallographic analysis of single-crystal; however, it was not possible to obtain single-crystals suitable for X-ray crystallographic analysis of any of the newly synthesized com-

Table 1. UV-Vis Absorption Spectral and Electrochemical Data of  $[\text{Ru}(\text{bpy})_2(\text{C}^{\wedge}\text{N})]\text{Cl}$  and  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  in 5 mM Tris-HCl/50 mM NaCl (pH 7.5)

Complex	$\lambda_{\text{max}}/\text{nm}$ ( $10^{-4} \epsilon/\text{M}^{-1} \text{cm}^{-1}$ )	$E_{1/2}/\text{V}$ vs. Ag/AgCl ( $\Delta E_p/\text{mV}$ )
<b>1</b>	362 (0.90), 410sh (0.72), 489 (0.64), 539 (0.68)	0.28 (71)
<b>2</b>	364 (0.90), 414sh (0.74), 486 (0.66), 539 (0.72)	0.25 (60)
<b>3</b>	333 (0.97), 493 (1.04)	0.61 (73)
$[\text{Ru}(\text{bpy})_3]^{2+}$	453 (1.37)	>1.0

Fig. 2. Emission Spectral Changes of the Binding of  $10 \mu\text{M}$  EtBr to  $100 \mu\text{M}$  CT-DNA upon Addition of Increasing Amounts (0– $105 \mu\text{M}$ ) of **1–3** and  $[\text{Ru}(\text{bpy})_3]^{2+}$  $\lambda_{\text{ex}}=470 \text{ nm}$ .

plexes. To conclude, although the structures of **1–3** were not proven beyond any doubt, the structures are consistent with  $^1\text{H-NMR}$  data described. The UV-Vis absorption spectral and electrochemical data of complexes **1–3** and  $[\text{Ru}(\text{bpy})_3]^{2+}$  in 5 mM Tris-HCl/50 mM NaCl (pH 7.5) are summarized in Table 1. The metal-to-ligand charge-transfer (MLCT) band maxima of **1–3** at the lowest energy are red-shifted by *ca.* 40–90 nm relative to the MLCT band maximum of  $[\text{Ru}(\text{bpy})_3]^{2+}$ . Moreover, complexes **1–3** exhibit much less positive redox potentials for Ru(III/II) couples (0.28, 0.25, and 0.61 V) with respect to  $[\text{Ru}(\text{bpy})_3]^{2+}$  (>1.0 V), indicating that **1–3** can be expected to serve as effective catalysts for Fenton-like reaction. It is evident that these dramatic cathodic shifts may be associated

with decreased charges on the ruthenium centers following coordination by the  $\sigma$ -donor  $\text{C}^{\wedge}\text{N}$  ligands.

**DNA Binding Studies** To investigate the binding mode between the respective ruthenium(II) complex and CT-DNA, fluorescence competition experiments with EtBr were employed. EtBr is a planar cationic dye that can intercalate into the DNA helix. Although EtBr is only weakly emissive, the EtBr-DNA adduct is a strong emitter. Quenching of the fluorescence may be performed to determine the extent of the binding between the respective ruthenium(II) complex and CT-DNA. One reason for the quenching is the reduction in the number of binding sites on the DNA that is available to the EtBr presumably because of competition with complexes

that are non-emissive under the experimental conditions.<sup>32,33</sup> Whereas  $[\text{Ru}(\text{bpy})_3]^{2+}$  caused no decrease of the emission intensity as the amounts of complex were increased, complexes **1–3** caused appreciable decreases in the emission at 604 nm as shown in Fig. 2. These results indicate that **1–3** compete with EtBr in binding to DNA. The apparent DNA binding constants  $K_{\text{app}}$  for the complexes were estimated by using the method described previously.<sup>34</sup> The  $K_{\text{app}}$  values of **1** to **3** were calculated as  $1.3 \times 10^6$ ,  $1.6 \times 10^6$  and  $1.6 \times 10^6 \text{ M}^{-1}$ , respectively. Because EtBr intercalates into DNA through interactions with the minor groove,<sup>35</sup> an intercalative and/or minor groove binding interaction of **1–3** is suggested. It may be due to the complexes interacting with DNA with approximately equal degrees of intercalation or minor groove binding, although  $[\text{Ru}(\text{bpy})_3]^{2+}$  does not intercalate.<sup>36</sup>

**Chemical Nuclease Activity** Chemical nuclease activity of complexes **1–3** was studied in the presence of oxidizing agent  $\text{H}_2\text{O}_2$ . When circular plasmid DNA is subjected to agarose gel electrophoresis, a relatively fast migration is observed in the intact, supercoiled, covalently closed circular form (form I). If a break occurs on one strand (nicking), the supercoiled structure of DNA will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) will be generated and will oscillate between form I and form II. Complexes **1–3** induced cleavage of plasmid pBR322 DNA in 5 mM Tris-HCl/50 mM NaCl (pH 7.5) in the presence of 5 mM  $\text{H}_2\text{O}_2$  (Fig. 3, lanes 4, 8, 12), whereas  $[\text{Ru}(\text{bpy})_3]^{2+}$  was no chemical nuclease active (data not shown). In addition, DNA cleavage experiments performed in the presence of dimethyl sulfoxide (DMSO) or KI led to inhibition of DNA cleavage. This clearly indicates that the observed DNA cleavage by **1–3** in the presence of  $\text{H}_2\text{O}_2$  is due to hydroxyl radicals ( $\cdot\text{OH}$ ) produced by the Fenton-like reaction (Eq. (1)).

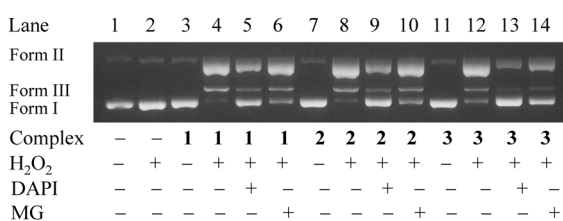
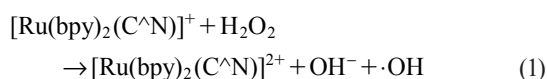


Fig. 3. Agarose Gel Electrophoresis Diagram Showing Oxidative Cleavage of pBR322 DNA by the Reaction of 5 mM  $\text{H}_2\text{O}_2$  with  $25 \mu\text{M}$  **1**, **2**, or **3** in the Presence of  $25 \mu\text{M}$  DAPI or MG in 5 mM Tris-HCl/50 mM NaCl (pH 7.5, 37°C) for 5 h

The chemical nuclease activity follows the order  $\mathbf{1} \approx \mathbf{2} > \mathbf{3}$ . The complexes **1** and **2** possessed lower redox potentials (Table 1) and exhibited more efficient cleavage activities. These cyclometalated ruthenium(II) complexes thus can be expected to enhance tumor selectivity because many cancer cells possess low levels of catalase.<sup>21</sup> As mentioned above, the complexes reported herein may function as minor and/or major groove binders. Therefore, the groove binding preferences of the complexes using the minor groove binder 4',6-diamidino-2-phenylindole (DAPI) and major groove binder methyl green (MG) have been determined (Fig. 3, lanes 5 and 6 for **1**, 9 and 10 for **2**, 13 and 14 for **3**). The observed DNA-binding constants of both MG<sup>37</sup> and DAPI<sup>38</sup> are in the order of  $10^{-6} \text{ M}^{-1}$ . The addition of DAPI effectively inhibited DNA cleavage by complexes **1–3**, suggesting that **1–3** primarily interact with DNA through the minor groove.

**In Vitro Cytotoxicity Assay** It is well known that lipophilicity of the metal complexes is critical for their cellular selective uptake. Several lipophilicity descriptors can be used to characterize the equilibria between two immiscible liquid phases. Among these, the most commonly used descriptor in medicinal chemistry is the 1-octanol-water partition coefficient,  $\log P_{o/w}$ .<sup>39</sup> The  $\log P_{o/w}$  values determined for **1–3** were greater than 2, indicating that **1–3** may facilitate their cell uptake efficiency and enhance anticancer activity. Hence, complexes **1–3** have been screened against tumor (murine leukemia (L1210), human cervical carcinoma (HeLa)) and non-tumor (murine (BALB/3T3 clone A31, abbreviated hereafter as BALB)) cell lines. The cytotoxicity of the complexes has been investigated in comparison with cisplatin under identical conditions by using an MTT assay. The  $\text{IC}_{50}$  values of **1–3** and cisplatin are summarized in Table 2. As shown in Table 2, complexes **1–3** display favorable cytotoxicity activities against the two tumor cell lines (L1210 and HeLa), which were in all cases significantly higher than cisplatin. The higher cytotoxicity activities of complexes **1–3** are probably due to the effective generation of hydroxyl radicals by virtue of the Fenton-like reaction because of the low levels of catalase activity for tumor cells.<sup>21</sup> The higher cytotoxicity activities of **1** and **2** may be due to the chemical nuclease activity ( $\mathbf{1} \approx \mathbf{2} > \mathbf{3}$ ). Interestingly, complexes **1–3** exhibit appreciably lower cytotoxicity toward the non-tumor cell line (BALB) relative to cisplatin, indicating that complexes **1–3** could be served as promising potential anticancer drugs.

## Conclusion

We have synthesized the cyclometalated ruthenium(II) complexes  $[\text{Ru}(\text{bpy})_2(\text{C}^{\wedge}\text{N})]\text{Cl}$  and investigated the interaction of their complexes with DNA and cytotoxicity assays with two

Table 2. Cytotoxicity ( $\text{IC}_{50}$ ) of Complexes **1–3** and Cisplatin against Two Tumor (L1210, HeLa) and a Non-tumor (BALB) Cell Lines after 48 h of Incubation

Complex	$\text{IC}_{50}$ (a)/mM			$r_{\text{L1210}}$ (b)	$r_{\text{HeLa}}$ (c)
	L1210	HeLa	BALB		
<b>1</b>	$0.50 \pm 0.13$	$1.7 \pm 0.2$	$12 \pm 2$	24	7.1
<b>2</b>	$0.20 \pm 0.03$	$1.5 \pm 0.5$	$11 \pm 1$	55	7.3
<b>3</b>	$2.6 \pm 0.2$	$8.6 \pm 2.0$	$27 \pm 2$	10	3.1
Cisplatin	$4.8 \pm 0.3$	$7.5 \pm 2.6$	$0.86 \pm 0.03$	0.18	0.11

a) Data of  $\text{IC}_{50}$  show the mean  $\pm$  S.D. of three independent experiments. b)  $r_{\text{L1210}} = \text{IC}_{50}(\text{BALB})/\text{IC}_{50}(\text{L1210})$ . c)  $r_{\text{HeLa}} = \text{IC}_{50}(\text{BALB})/\text{IC}_{50}(\text{HeLa})$ .

tumor (L1210 and HeLa) and a non-tumor (BALB) cell lines. It is suggested that complexes **1–3** act as intercalators and/or minor groove binders. Moreover, complexes **1–3** display favorable cytotoxicities against L1210 and HeLa, which were in all cases significantly higher than cisplatin. In contrast, the complexes exhibit appreciably lower cytotoxicity toward BALB. Overall, our study provides new insight into the design of anticancer drugs that can enhance tumor selectivity.

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**Conflict of Interest** The authors declare no conflict of interest.

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