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Full Paper

Microwave-Assisted Synthesis of Arene Ruthenium(II) Complex as Apoptosis Inducer of A549 Cells

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An arene ruthenium(II) complex coordinated with 2-(2-chlorophenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline, [(η^6-C_6H_6)Ru(*o*-ClPIP)Cl]Cl (**1**), has been prepared by using microwave-assisted synthesis technology. The anti-tumour activity of this complex against various tumour cells has been evaluated by MTT assay and the results show that complex **1** exhibits selective inhibitory activity against the growth of human lung adenocarcinoma A549 cells with $IC_{50} = 31.58$ μM. Further studies by flow cytometric analysis showed that apoptosis of A549 cells was observed when dealt with complex **1**. Furthermore, complex **1** exhibits excellent binding affinity with DNA molecules which was confirmed by spectroscopy methods, as well viscosity and melting point experiments. As a result, the conformation of DNA molecules was disturbed by complex **1**.

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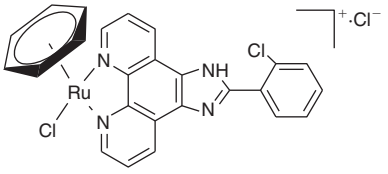
Introduction

Arene Ru^{II} complexes have long been studied as promising anti-tumour candidates, because of their low toxicity to human normal cells but high inhibitory activity against various tumour cells.[1–5] In recent years, several arene ruthenium(II) complexes have been designed,[6–8] and their in vivo and in vitro anti-tumour activity,[9–11] as well as their mechanism to inhibit the growth of tumour cells has been investigated. Dyson and co-workers reported that (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*p*-pta)Cl_2 (RAPTA-C), a classic arene Ru^{II} complex, exhibited prominent anti-tumour activity by inhibiting cell cycle progression at the G2/M phase and inducing apoptosis of tumour cells.[12,13] Sadler et al. also indicated that arene Ru^{II} complexes coordinated by phenanthroline lighands could suppress the growth of various tumour cells at low initial concentration by intercalating into the base-pair of duplex DNA,[14–16] and the presence of the arene ligand played a key role for binding affinity of these Ru^{II} complexes with DNA molecules.[17,18]

Furthermore, a large body of evidence suggests that Ru complexes coordinated with phenanthroimidazole derivatives, a large aromatic planar ligand, usually exhibit high anti-tumour activity by inducing apoptosis of tumour cells.[19–22] Ji and co-workers showed that Ru^{II} polypyridyl complexes bearing 1*H*-imidazo[4,5-*f*][1,10]phenanthroline analogues, such as $[Ru(bpy)_2(bfipH)]^{2+}$ and $[Ru(phen)_2(bfipH)]^{2+}$ (bpy = 2,2′-bipyridine; phen = 1,10-phenanthroline; bfipH = 2-(benzofuran-2-yl)imidazo[4,5-*f*][1,10]phenanthroline), showed high binding affintiy to DNA, which was attributed to the ideal planarity of the intercalating ligand.[23,24] More recently, our research group found that imidazole phenanthrolines themselves displayed excellent inhibitory activity by induced apoptosis of lung cancer A549 cells via the NF-κB signalling pathway.[25] Studies in our research group suggest that an arene Ru^{II} complex coordinated with imidazole phenanthroline shows promising anti-tumour activity by inducing cell cycle arrest at the S-phase.[26] However, the anti-tumour mechanism and the DNA-binding behaviour of these kinds of arene complexes is still not clear, and it is still very important to design novel candidates with high activity and selectivity.

In this paper, we report the microwave synthesis of an arene Ru^{II} complex coordinated by 2-(2-chlorophenyl)-1*H*-imidazo [4,5-*f*][1,10]phenanthroline, [(η^6-C_6H_6)Ru(*o*-ClPIP)Cl]Cl (**1**), as shown in Scheme 1. We found that complex **1** could selectively inhibit the growth of A549 cells with similar levels to cisplatin. Apoptosis of A549 cells induced by complex **1** was observed by flow cytometric analysis. The DNA-binding properties of complex **1** with calf thymus deoxyribonucleic acid (CT-DNA) was investigated by spectroscopic methods and the DNA cleavage ability of complex **1** was evaluated through gel



Scheme 1. The molecular structure of arene Ru^{II} complex **1**.

electrophoresis experiments. Results show that complex **1** intercalates to CT-DNA, inducing DNA damage resulting in apoptosis of A549 cells.

Experimental

Chemicals

Ruthenium(III) chloride hydrate was obtained from Mitsuwa Chemicals. 1,10-Phenanthroline monohydrate, 1,3-cyclohexadiene, and 2-chlorobenzaldehyde were purchased from Aldrich. All chemicals including solvents were obtained from commercial vendors and used as received. Calf thymus DNA (CT-DNA) and pBR-322 DNA was purchased from Guangzhou Ruizhen Biotechnology Co. 1,10-Phenanthroline-5,6-dione was prepared by a similar method reported in the literature.[27] $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ was prepared according to literature.[6] All aqueous solutions were prepared with doubly distilled water. The Tris-HCl buffer was made from tris(hydroxymethyl)aminomethane, Tris (617 mg) and NaCl (292.5 mg), adjusted to pH 7.2 by HCl (0.1 M); this buffer was used for ultraviolet (UV) titration, fluorescence quenching, thermal denaturation, circular dichroism (CD) spectra, and viscosity measurements.

Synthesis of 2-(2-Chlorophenyl)-1H-imidazo[4,5f][1,10] phenanthroline (o-ClPIP)

The ligand 2-(2-chlorophenyl)-1*H*-imidazo[4,5*f*][1,10] phenanthroline (*o*-ClPIP) was prepared by a similar method as the literatures with some modifications.[28] 1,10-Phenanthroline-5,6-dione (347 mg, 1.6 mmol), 2-chlorobenzaldehyde (224 mg, 1.6 mmol), and ammonium acetate (2.53 g) was dissolved in 20 mL acetic acid and the resulting solution was heated at reflux at 110°C for 4 h. Then 20 mL of water was added and the pH value was adjusted to 7.0 at room temperature. A yellow precipitate was obtained after filtration, before drying undervaccum. The products were purified silica gel chromatography using ethanol as eluent to give the title compound (348 mg, 65.9 %).

Synthesis of [(C_6H_6)Ru(o-ClPIP)Cl]Cl (**1**)

The arene ruthenium(II) complex **1** was prepared according to a literature procedure[29] but with some modifications. A mixture of $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ (75 mg, 0.15 mmol) and *o*-ClPIP(99.3 mg, 0.3 mmol) in dichloromethane (20 mL) was heated at reflux at 60°C in a sealed reaction vessel in a microwave reactor (Anton Paar Monowave 3000, Graz, Austria) for 30 min. A yellow precipitate was obtained after rotary evaporation, which was purified by recrystallisation from distilled water to give the title compound (105 mg, 90.7 %). ^{1}H NMR (500 MHz, [D_6]DMSO) δ 10.00 (d, $J = 4.6$ Hz, 2H), 9.35 (s, 2H), 8.20 (dd, $J = 8.0$, 5.4 Hz, 2H), 7.99 (dd, $J = 7.4$, 1.6 Hz, 1H), 7.72 (d, 1H), 7.58 (dt, $J = 16.1$, 7.3 Hz, 2H), 6.35 (s, 6H). ^{13}C NMR (126 MHz, [D_6]DMSO) δ 154.67, 150.99, 143.90, 133.13, 132.72, 132.52, 132.15, 130.93, 129.68, 127.98, 126.63, 87.32. *m/z* (ESI) 547.1 $[M\text{-}Cl]^+$.

Cell Lines and Cell Culture

Human cancer cell lines, including human lung adenocarcinoma A549, human hepatocellular carcinoma SMMC-7721, colorectal adenocarcinoma SW620, and human immortalised keratinocyte HaCaT cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). All cell lines were maintained in either Roswell Park Memorial Institute 1640 (RPMI-1640) or Dulbecco's Modified Eagle Medium (DMEM) media supplemented with fetal bovine serum (10 %), penicillin (100 units/mL), and streptomycin (50 units/mL) at 37°C in a CO_2 incubator (95 % relative humidity, 5 % CO_2).

MTT Assay

Cell viability was determined by measuring the ability of cells to transform 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple formazan dye.[30] Cells were seeded in 96-well tissue culture plates for 24 h. The cells were then incubated with the tested compounds at different concentrations for different periods of time. After incubation, 20 μL of MTT solution (5 mg mL^{-1} in phosphate buffered saline, PBS) was added to each well, followed by incubation for a further 5 h. The medium was aspirated and replaced with 150 μL/well of DMSO to dissolve the formazan salt formed. The colour intensity of the formazan solution, which reflects the cell growth condition, was measured at 570 nm using a microplate spectrophotometer (SpectroAmax^{TM} 250).

Flow Cytometric Analysis

The apoptosis rate was analysed by flow cytometry as previously described.[31] Treated or untreated cells were trypsinised, washed with PBS, and fixed with 75 % ethanol overnight at −20°C. The fixed cells were washed with PBS and stained with propidium iodide (PI) for 4 h in darkness. The stained cells were analysed with an Epics XL-MCL flow cytometer (Beckman Coulter, Miami, FL).

Results and Discussion

Synthesis and Characterisation

The use of microwave reactors are becoming a rapidly expanding area in synthetic chemistry in both organic and inorganic synthesis.[32] The technique potentially reduces overall processing time, while enhancing yield and product purity over traditional synthesis methods, especially in the synthesis of Ru complexes which typically require many hours of heating at reflux in high-boiling solvents to affect a reaction. Arene Ru^{II} complex **1** was prepared from $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ and *o*-ClPIP at 60°C under microwave irradiation for 30 min in a Pyrex vessel, in a yield of 90.7 %, which is markedly higher than the methof from conventional synthesis.[33]

The electrospray ionisation mass spectrum (ESI-MS) of complex **1** exhibited a peak at *m/e* 547.1 (100 %) ascribed to $[M\text{-}Cl]^+$, which was in agreement with the theoretical value. The peak at δ 6.33 ppm (s, 6H) in the ^{1}H NMR spectrum was assigned to resonance of the corresponding protons **H-1** in the C_6H_6 ring; the peaks at δ 10.00 (d, $J = 5.0$ Hz, 2H), 9.35 (s, 2H), and 8.20 (dd, $J = 8.0$, 5.0 Hz, 2H) ppm were ascribed to protons in the phenanthroline ring *o*-ClPIP of **H-2**, **H-4**, and **H-3,** respectively (Fig. 1a). This was confirmed by the ^{1}H-^{1}H COSY spectrum of complex **1** (Fig. 1b) which shows **H-3** coupled with **H-2** and **H-4**, and **H-6**, **H-7**, **H-8**, and **H-9** in the benzene ring of phenanthroline coupled with each other.

Biological Studies

Complex **1** was evaluated in a comparative in vitro MTT cell viability assay with three tumorigenic cell lines, human lung adenocarcinoma A549, human hepatocellular carcinoma SMMC-7721, and colorectal adenocarcinoma SW620, versus normal cells (human immortalised keratinocyte HaCaT cells). The IC_{50} values for complex **1** and the positive control cisplatin are depicted in Table 1. These data indicate that **1** selectively inhibits A549 cells at similar levels to cisplatin, whereas the activity is much lower against SMMC-7721 and SW620 cells.

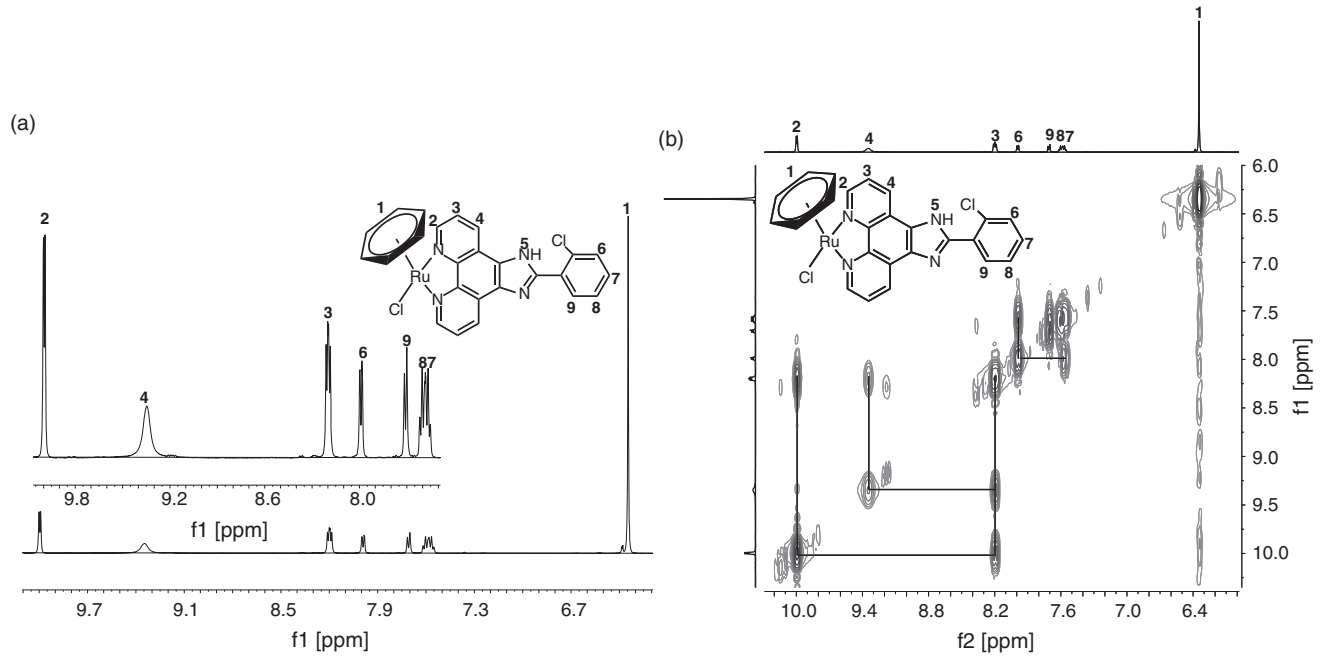


Fig. 1. (a) ^{1}H NMR spectra of complex **1**. (b) ^{1}H-^{1}H COSY of complex **1**.

Table 1. IC_{50} values of complex 1 on tumorigenic cell lines after 72 h of incubation, determined using the MTT assay

Complexes	IC_{50} [μM]			
	A549	SMMC7721	SW620	HaCaT
1	31.58	>100	>100	>100
Cisplatin	16.54	2.92	5.74	7.48

Arene ruthenium complex **1** exhibited low toxicity towards normal human HaCaT cells compared to cisplatin, which shows that **1** is a selective inhibitor for lung adenocarcinoma with low toxicity against normal human cells.

At this point it was unclear what the anti-tumour mechanism of arene Ru^{II} complexes was. To investigate the anti-tumour mechanism of complex **1** further, A549 cells were exposed to different concentrations of **1** (0, 50, 75, and 100 μM) for 24 h and examined by flow cytometric (FCM) analysis. Results show a prominent increase in cell apoptosis at 24 h in Fig. 2. Upon increasing the concentration of complex **1**, the level of apoptosis rises markedly, reaching 39.88 % for the 100 μM experiment, approximately four-fold higher than that of the control (10.86 %), indicating that the arene Ru^{II} complex might be inhibiting the growth of A549 cells by induced apoptosis.

DNA-Binding Properties of Arene Ruthenium(II) Complexes

Electronic Spectra

Electronic absorption spectroscopy is one of the most common methods to study the interaction of ruthenium complexes with DNA. In general, ruthenium complexes have characteristic spectroscopic properties, which undergo hypochromism and red shift in the presence of DNA; the degree of change is usually proportional to the binding strength. Electronic spectra were recorded to clarify the interaction of complexes **1** with CT-DNA, as shown in Fig. 3.

As shown in Fig. 3, there was a characteristic absorption at 271 nm in the electronic spectra of complex **1** which was attributed to the IL (intraligand charge transfer) absorption, and an absorption appeared at 295 nm which was attributed to LMCT (ligand to metal charge transfer) absorption. Upon the addition of CT-DNA, the absorption of IL and electronic spectra undergo apparent hypochromism, and the hypochromism calculated for IL absorption and LMCT absorption was ~16 and 12 %, respectively.

The intrinsic binding constant (K) was calculated according to the decay of the IL absorption using the Stern–Volmer equation:[34]

$$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = [b - (b^2 - 2K^2C_t[\mathrm{DNA}]/S)]^{1/2}/2KC_t$$

$$b = 1 + KC_t + K[\mathrm{DNA}]/2S$$

The intrinsic binding constant K calculated for complex **1** is $\sim 2.47 \times 10^5\ \mathrm{M}^{-1}$, which shows that complex **1** exhibits a certain DNA-binding affinity.

Fluorescence Quenching

Owing to the low fluorescence of complex **1** in Tris-HCl buffer (pH = 7.2), fluorescence quenching of ethidium bromide (EB) and DNA was carried out to further study the DNA-binding behaviour of complex **1**. Fluorescence quenching can be used to study the affinity of a complex to DNA, not just its binding mode,[35] and the results are illustrated in Fig. 4.

A strong fluorescence was observed in the range of 550–690 nm at room temperature, the maxima was at ~600 nm when excited at 530 nm. Upon increasing the concentration of complex **1**, the emission intensity of the EB-DNA system deceased dramatically, resulting from the replacement of EB which is a classical DNA intercalator.[36,37] According to these results we conclude that complex **1** competitively binds with DNA over EB, which is in agreement with the electronic spectra, indicating that complex **1** interacts with DNA in an intercalative mode.

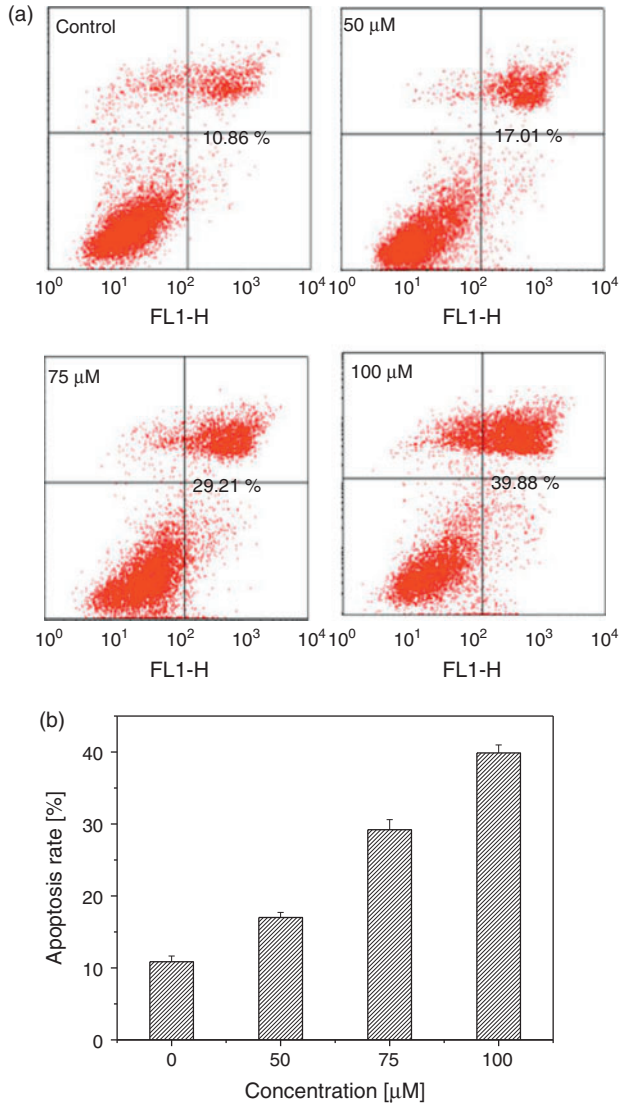


Fig. 2. (a) FCM analysis of the apoptosis of A549 cells treated with complex **1** after 24 h. The effect of complex **1** (50, 75, and 100 μM) on A549 cell apoptosis was determined by flow cytometry. Untreated (control) cells or cells treated for 24 h were harvested, fixed, stained with Annexin V/PI, and assessed for cell apoptosis distribution by flow cytometric analysis. (b) Percentages of apoptotic cells were measured by the annexin V/PI flow cytometry analysis, as described in the Experimental section. The *y*-axis plots the sum of early and late apoptotic cells as the mean ± standard deviation of three independent experiments.

Circular Dichroism

The interaction of complex **1** with CT-DNA was further studied by circular dichroism.[8] By observing the change in CD signal of CT-DNA upon addition of complex **1**, conformational changes of DNA caused by the binding of complex **1** can be elucidated. These results are shown in Fig. 5.

There is a positive CD signal apparent in the range of 230–290 nm for CT-DNA, with the maxima at 280 nm. When complex **1** was added to the solutions, the positive CD signal of CT-DNA decreased distinctly, which indicated the conformation of double-stranded DNA was disturbed by complex **1**. On the basis of the above analysis, complex **1** disturbs the DNA conformation after binding with DNA, which might lead to a series of changes in the biological process.

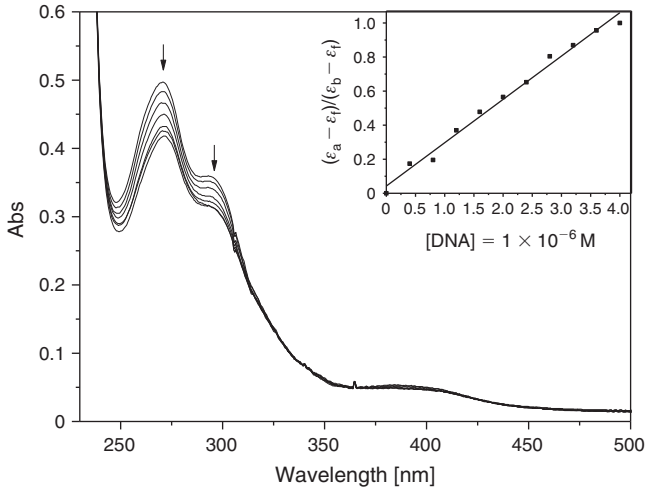


Fig. 3. Electronic spectra of **1** in Tris-HCl buffer (pH = 7.2) in the absence and presence of calf thymus DNA. [Ru] = 10.0 μM.

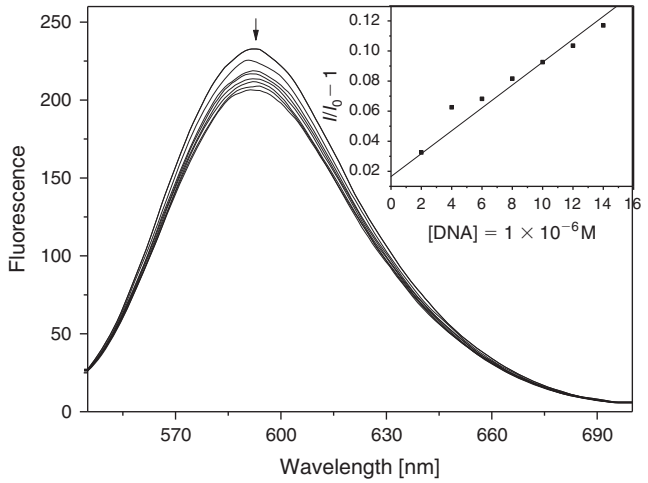


Fig. 4. Emission spectra of EB-DNA in Tris-HCl buffer (pH = 7.2) in the absence and presence of complex **1**. [EB] = 16 μM, [DNA] = 100 μM. Arrow shows the emission intensity changes upon increasing concentration of **1**.

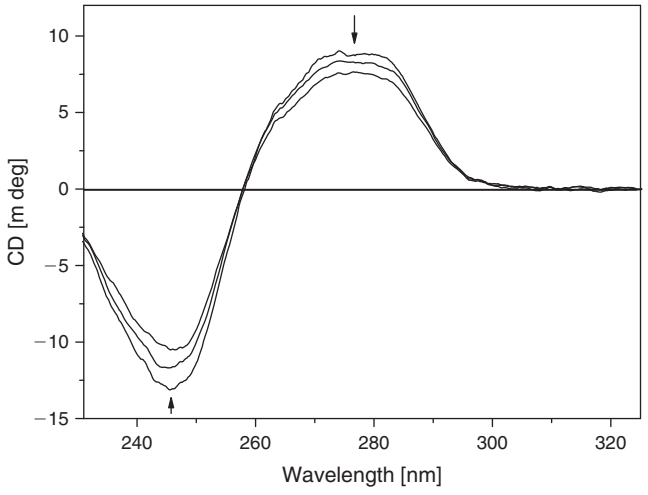


Fig. 5. The change of CD spectra of CT-DNA with the increasing concentration of complex **1**. [DNA] = 100 μM, [Ru] = 0, 2, 4 μM.

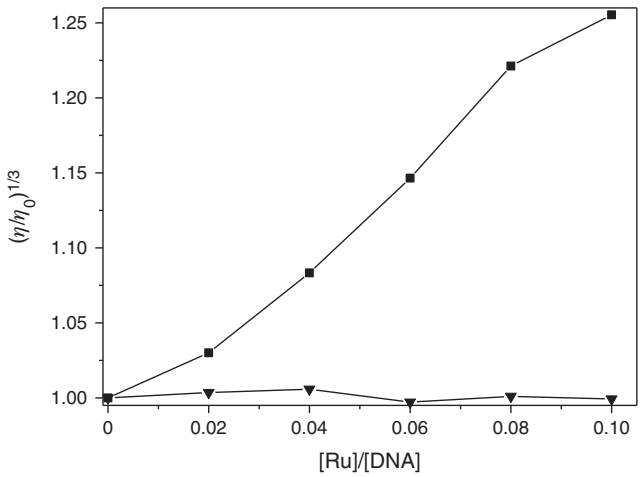


Fig. 6. Effects of complex **1** (■) and $[Ru(bpy)_3]^+$ (▼) on the relative viscosity of CT-DNA.

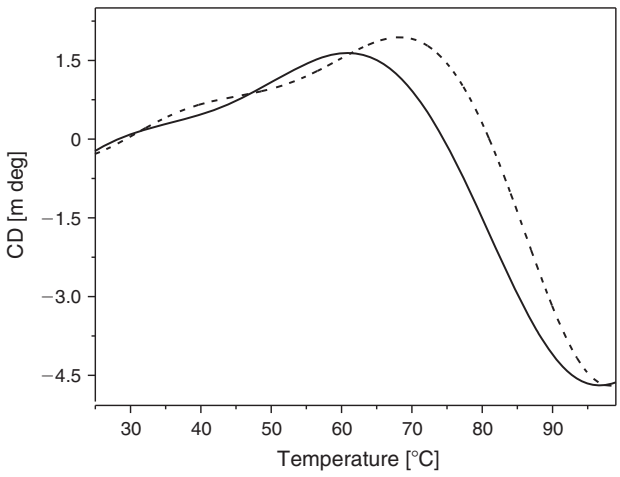


Fig. 7. Typical CD melting curves of CT-DNA without (—) or with **1** (- - -) in Tris-HCl buffer (pH = 7.2). The CD signal was recorded with a positive band at 280 nm. [DNA] = 100 μM; [Ru] = 10 μM.

Viscosity Experiments

The relative viscosity of DNA increases with the addition of metal complexes that bind by intercalation. Viscosity measurements have proven to be an important method to study the mode of binding of compounds to DNA. In general, intercalation of a compound into DNA is known to cause an observable increase in the viscosity of a DNA solution due to an increase in the distance of base pairs at the intercalation site.[38] Viscosity changes were measured using CT-DNA with increasing concentrations of the complex **1**, as shown in Fig. 6.

The effects of complex **1** versus $[Ru(bpy)_3]^+$ on the relative viscosity of rod-like DNA are shown in Fig. 6. It is well known that $[Ru(bpy)_3]^+$ interacts with DNA in a classical electronic effect.[39] On increasing the concentration of complex **1**, the relative viscosity of the DNA increased steadily compared to $[Ru(bpy)_3]^+$. Thissuggests that complex **1** inserts into the base-pairs of CT-DNA via an intercalative mode, which is in agreement with the spectroscopic studies.

Melting Point Experiments

Melting point (T_m) studies were undertaken to further examine the conformation of CT-DNA after binding with complex **1**. In general, double-stranded DNA will gradually dissociate to single strands following an increase in temperature, which can be followed by examining the CD signal decrease at 280 nm.[40] A large change in the T_m of DNA is an indication of a strong interaction between a complex and DNA. The interaction of complex **1** with CT-DNA was characterised by measuring the effects on melting temperature of DNA, and the results are shown in Fig. 7.

The melting point (T_m) for CT-DNA in the absence and presence of complex **1** was determined by CD spectra to be ~80.1 ± 0.12 and 84.9 ± 0.15°C (ΔT_m = 4.8°C), respectively. These data are comparable to that observed for $[Ru(bpy)_2(dpoq)](ClO_4)_2{\cdot}H_2O$ (ΔT_m = 4.0°C) and $[Ru(phen)_2(dpoq)](ClO_4)_2{\cdot}H_2O$ (ΔT_m = 5.8°C) (dpoq = dipyrido[1,2,5]oxadiazolo [3,4-b]quinoxaline),[41] indicating that complex **1** stabilises the conformation of CT-DNA by intercalating into the base pairs of the double helix of DNA.[42]

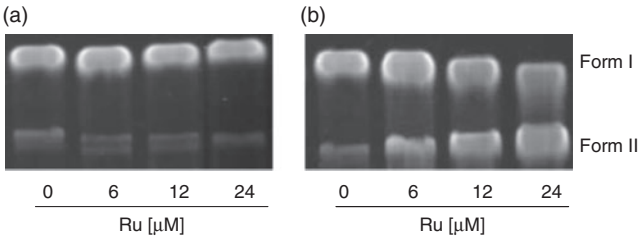


Fig. 8. Photocleavage of plasmid pBR-322 DNA in the absence and presence of complex **1** without light irradiation (a) and with light irradiation at 365 nm for 30 min (b).

*Photocleavage of pBR-322 DNA by Arene Ruthenium(II) Complex **1***

The cleavage activity on plasmid DNA induced by ruthenium(II) complexes was tested by agarose gel electrophoresis. Circular plasmid DNA in the intact supercoiled form moves at the front in electrophoresis; if breakage occurs on one strand (nicked), the supercoiled DNA migrates after the circular form.[43] The photocleavage of plasmid pBR-322 DNA by complex **1** was evaluated by gel electrophoresis, as shown in Fig. 8.

Gel electrophoresis was carried out for **1** on pBR-322 DNA after incubation, with and without light irradiation at 365 nm for 30 min. As shown in Fig. 8a, little DNA cleavage was observed for the control (0) and for the sample containing complex **1** (6, 12, and 24 μM) without light irradiation. While under light irradiation, the amount of supercoiled conformation (Form I) of plasmid pBR-322 DNA diminishes gradually with increasing concentrations of complex **1**, whereas the nicked formation (Form II) of DNA increases. These results indicate that complex **1** inhibits the growth of tumour cells by disturbing the conformation of DNA molecules but not by direct DNA damage, which may be the reason for low toxicity to human normal cells.

Conclusion

In summary, arene Ru^{II} complex **1** was synthesised using microwave-assisted technology, in a high yield of 90.7%.

According to MTT results, complex **1** exhibited selective inhibition to A549 cells at similar levels to cisplatin. Flow cytometry indicated that arene Ru^{II} complex **1** could induce apoptosis of A549 cells. The interaction of complex **1** with CT-DNA was studied by spectroscopy, viscosity, and melting point experiments and the results suggest that complex **1** interacts with CT-DNA by intercalation, with an intrinsic binding constant of $2.47 \times 10^5\ M^{-1}$. Furthermore, studies by gel electrophoresis indicate that complex **1** cleaves plasmid pBR-322 DNA from the supercoiled form to the nicked form effectively under the irradiation of light, which shows potential utility in chemotherapy. Taken together, these results suggest that arene Ru^{II} complex interacts with DNA causing induction of A549 cell apoptosis.

Acknowledgements

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References

[1] H. K. Liu, P. J. Sadler, *Acc. Chem. Res.* **2011**, *44*, 349. doi:10.1021/AR100140E
[2] G. Süss-Fink, *Dalton Trans.* **2010**, *39*, 1673. doi:10.1039/B916860P
[3] G. S. Smith, B. Therrien, *Dalton Trans.* **2011**, *40*, 10793. doi:10.1039/C1DT11007A
[4] P. Nowak-Sliwinska, J. R. Beijnum, A. Casini, A. A. Nazarov, G. Wagnières, H. Bergh, P. J. Dyson, A. W. Griffioen, *J. Med. Chem.* **2011**, *54*, 3895. doi:10.1021/JM2002074
[5] M. G. Mendoza-Ferri, C. G. Hartinger, A. A. Nazarov, R. E. Eichinger, M. A. Jakupec, K. K. Severin, B. K. Keppler, *Organometallics* **2009**, *28*, 6260. doi:10.1021/OM900715J
[6] B. Therrien, *Coord. Chem. Rev.* **2009**, *253*, 493. doi:10.1016/J.CCR.2008.04.014
[7] J. Canivet, L. Karmazin-Brelot, G. Süss-Fink, *J. Organomet. Chem.* **2005**, *690*, 3202. doi:10.1016/J.JORGANCHEM.2005.02.050
[8] A. Martínez, C. S. K. Rajapakse, R. Sánchez-Delgado, A. Varela-Ramirez, C. Lema, R. J. Aguilera, R. A. Sánchez-Delgado, *J. Inorg. Biochem.* **2010**, *104*, 967. doi:10.1016/J.JINORGBIO.2010.05.002
[9] A. Astarina, M. J. Chow, W. H. Ang, *Aust. J. Chem.* **2012**, *65*, 1271. doi:10.1071/CH12059
[10] T. Ohkuma, N. Utsumi, K. Tsutsumi, K. Murata, C. Sandoval, R. Noyori, *J. Am. Chem. Soc.* **2006**, *128*, 8724. doi:10.1021/JA0620989
[11] H. C. Lo, O. Buriez, J. B. Kerr, R. H. Fish, *Angew. Chem. Int. Ed.* **1999**, *38*, 1429. doi:10.1002/(SICI)1521-3773(19990517)38:10<1429::AID-ANIE1429>3.0.CO;2-Q
[12] C. Scolaro, A. Bergamo, L. Brescacin, R. Delǒno, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava, P. J. Dyson, *J. Med. Chem.* **2005**, *48*, 4161. doi:10.1021/JM050015D
[13] A. K. Renfrew, A. D. Phillips, E. Tapavicza, R. Scopelliti, U. Rothlisberger, P. J. Dyson, *Organometallics* **2009**, *28*, 5061. doi:10.1021/OM900345N
[14] F. Y. Wang, J. J. Xu, A. Habtemariam, J. Bella, P. J. Sadler, *J. Am. Chem. Soc.* **2005**, *127*, 17734. doi:10.1021/JA053387K
[15] A. F. A. Peacock, P. J. Sadler, *Chem. – Asian J.* **2008**, *3*, 1890. doi:10.1002/ASIA.200800149
[16] S. W. Magennis, A. Habtemariam, O. Novakova, J. B. Henry, S. Meier, S. Parsons, I. D. H. Oswald, V. Brabec, P. J. Sadler, *Inorg. Chem.* **2007**, *46*, 5059. doi:10.1021/IC062111Q
[17] M. R. Gill, J. Garcia-Lara, S. J. Foster, C. Smythe, G. Battaglia, J. A. Thomas, *Nat. Chem.* **2009**, *1*, 662. doi:10.1038/NCHEM.406
[18] H. Chen, J. A. Parkinson, R. E. Morris, P. J. Sadler, *J. Am. Chem. Soc.* **2003**, *125*, 173. doi:10.1021/JA027719M
[19] G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3. doi:10.1021/JM100020W
[20] W. J. Mei, X. Y. Wei, Y. J. Liu, B. Wang, *Trans. Met. Chem.* **2008**, *33*, 907. doi:10.1007/S11243-008-9131-6
[21] T. Chen, Y. Liu, W. J. Zheng, J. Liu, Y.-S. Wong, *Inorg. Chem.* **2010**, *49*, 6366. doi:10.1021/IC100277W
[22] S. Shi, T. Xie, T. M. Yao, C. R. Wang, X. T. Geng, D. J. Yang, L. J. Han, L. N. Ji, *Polyhedron* **2009**, *28*, 1355. doi:10.1016/J.POLY.2009.02.035
[23] H. Xu, K. C. Zheng, Y. Chen, Y. Z. Li, L. J. Lin, H. Li, P. X. Zhang, L. N. Ji, *J. Chem. Soc., Dalton Trans.* **2003**, *2260.* doi:10.1039/B300353A
[24] K. J. Du, J. Q. Wang, J. F. Kou, G. Y. Li, L. L. Wang, H. Chao, L. N. Ji, *Eur. J. Med. Chem.* **2011**, *46*, 1056. doi:10.1016/J.EJMECH.2011.01.019
[25] D. D. Sun, W. Z. Wang, J. W. Mao, W. J. Mei, J. Liu, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 102. doi:10.1016/J.BMCL.2011.11.063
[26] Q. Wu, C. D. Fan, T. F. Chen, C. R. Liu, W. J. Mei, S. D. Chen, B. G. Wang, Y. Y. Chen, W. J. Zheng, *Eur. J. Med. Chem.* **2013**, *63*, 57. doi:10.1016/J.EJMECH.2013.01.037
[27] V. Rajendiran, M. Murali, E. Suresh, S. Sinha, K. Somasundaram, M. Palaniandavar, *Dalton Trans.* **2008**, *148.* doi:10.1039/B710578A
[28] Z. Zhang, Q. Wang, Q. Wu, X. Y. Hu, C. X. Wang, W. J. Mei, Y. Y. Tao, W. L. Wu, W. J. Zheng, *Chem. J. Chin. Univer.* **2012**, *33*, 2441 [in Chinese]. doi:10.7503/CJCU20120387
[29] Q. Wu, J. Wu, W. J. Mei, J. H. Yao, W. L. Wu, Y. H. Chen, Y. Y. Tao, *Chin. J. Org. Chem.* **2013**, in press. doi:10.6023/CJOC201303022
[30] T. Chen, Y. S. Wong, *Biomed. Pharmacother.* **2009**, *63*, 105. doi:10.1016/J.BIOPHA.2008.03.009
[31] T. Chen, Y. S. Wong, *J. Agric. Food Chem.* **2008**, *56*, 10574. doi:10.1021/JF802125T
[32] D. Adam, *Nature* **2003**, *421*, 571. doi:10.1038/421571A
[33] C. A. Vock, C. Scolaro, A. D. Phillips, R. Scopelliti, G. Sava, P. J. Dyson, *J. Med. Chem.* **2006**, *49*, 5552. doi:10.1021/JM060495O
[34] W. J. Mei, J. Liu, K. C. Zheng, L. J. Lin, H. Chao, A. X. Li, F. C. Yun, L. N. Ji, *Dalton Trans.* **2003**, *1352.* doi:10.1039/B212443B
[35] W. J. Mei, J. Liu, H. Chao, L. N. Ji, *Trans. Metal Chem.* **2003**, *28*, 852. doi:10.1023/A:1026335221126
[36] B. C. Baguley, M. L. Bret, *Biochemistry* **1984**, *23*, 937. doi:10.1021/BI00300A022
[37] R. F. Pasternack, M. Caccam, B. Keogh, T. A. Stephenson, A. P. Williams, E. J. Gibbs, *J. Am. Chem. Soc.* **1991**, *113*, 6835. doi:10.1021/JA00018A019
[38] P. Zhang, J. Chen, Y. Liang, *Acta Biochim. Biophys. Sin.* **2010**, *42*, 440. doi:10.1093/ABBS/GMQ040
[39] A. Mills, *Analyst* **1998**, *123*, 1135. doi:10.1039/A800030A
[40] C. C. Ju, A. G. Zhang, C. L. Yuan, X. L. Zhao, K. Z. Wang, *J. Inorg. Biochem.* **2011**, *105*, 435. doi:10.1016/J.JINORGBIO.2010.12.004
[41] B. Peng, X. Chen, K. J. Du, B. L. Yu, H. Chao, L. N. Ji, *Spectrochim. Acta A* **2009**, *74*, 896. doi:10.1016/J.SAA.2009.08.031
[42] V. V. Koval, D. V. Pyshnyi, O. S. Fedorova, *J. Biomol. Struct. Dyn.* **2001**, *19*, 515. doi:10.1080/07391102.2001.10506759
[43] J. Sun, S. Wu, Y. Han, J. Liu, L. N. Ji, Z. W. Mao, *Inorg. Chem. Commun.* **2008**, *11*, 1382. doi:10.1016/J.INOCHE.2008.09.015