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Synthesis and biological evaluation of platinum(II) complexes containing (1*R*,2*R*)-N^1-alkyl-1,2-diaminocyclohexane and D-(+)-camphorate ligands

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ABSTRACT

Five platinum(II) complexes with N-monoalkyl derivatives of 1*R*,2*R*-diaminocyclohexane as ligands and D-(+)-camphorate anion as leaving group have been synthesized and characterized by elemental analysis, IR, ^{1}H NMR, ESI-MS, and HRMS spectra. All complexes were evaluated for their *in vitro* cytotoxicity against four human tumor cell lines and most of them showed promising cytotoxic activity, especially compounds **3** and **4** with branched alkyl substituent at one of nitrogen atoms. Preliminary mechanism study by flow cytometry and agarose gel electrophoresis was also carried out in comparison with cisplatin and oxaliplatin.

1. Introduction

Most of platinum-based antitumor drugs available in clinic are adhered to the classical structure–activity relationship (SAR) summarized by Cleare and Hoeschele in 1973 [1]. These cisplatin analogues possess similar properties including narrow antitumor spectrum, severe toxicity, intrinsic and acquired cross-resistance [2–4]. Much effort has been made to overcome these drawbacks by designing novel platinum complexes based on the non-classical SAR. And many studies during the last two decades demonstrated that cross-resistance between cisplatin and its analogues could be overcome by introducing sterically hindered platinum(II) complexes [5–8]. *cis*-Amminedichloro(2-methylpyridine)platinum(II) (ZD0473) is a sterically hindered platinum(II) complex with a bulky methylpyridine ligand which is responsible for its ability to overcome platinum resistance [9]. Besides, other strategies used in designing novel platinum-based anticancer agents have focused on integrating biologically active ligands with platinum moieties, such as doxorubicin [10], amino acids [11], and sugars [12].

It has been proved that traditional Chinese medicines (TCMs) play an important role in cancer chemotherapy. And continuous attempts have been made to exploit the benefits of TCM in designing a new chemical entity with special biological activity [13–15]. Norcantharidin, a demethylated form of cantharidin, has recently been introduced as leaving group to prepare TCM-based platinum complexes which showed remarkable antitumor activity [16].

Camphor, a traditional Chinese medicine, has long been used to relieve pain, stop tickle, eliminate inflammation, and kill worm and acariasis as early as in the Ming Dynasty. We have recently reported a novel series of platinum complexes with various diamines as carrier ligands and camphorate anion as leaving group, which showed good antitumor activity. Among them, complex **1** containing 1*R*,2*R*-diaminocyclohexane (1*R*,2*R*-DACH) ligand showed *in vitro* and *in vivo* antitumor activities that were superior to those of carboplatin [17]. In this study, our strategy is to integrate the camphorate anion with Pt-moieties containing N-monoalkyl derivatives of 1*R*,2*R*-diaminocyclohexane as carrier ligands. The resulting compounds were shown in Scheme 1. And their biological activity on four human tumor cell lines and preliminary mechanism of action were also discussed.

2. Experimental

2.1. Materials and instruments

Potassium tetrachloroplatinate(II), 1*R*,2*R*-diaminocyclohexane and disodium D-(+)-camphorate were obtained from a local chemical company. All reagents were of high purity and used without any further purification. Elemental analysis for C, H, and N was performed with a Perkin–Elmer 1400C instrument. Elemental analysis

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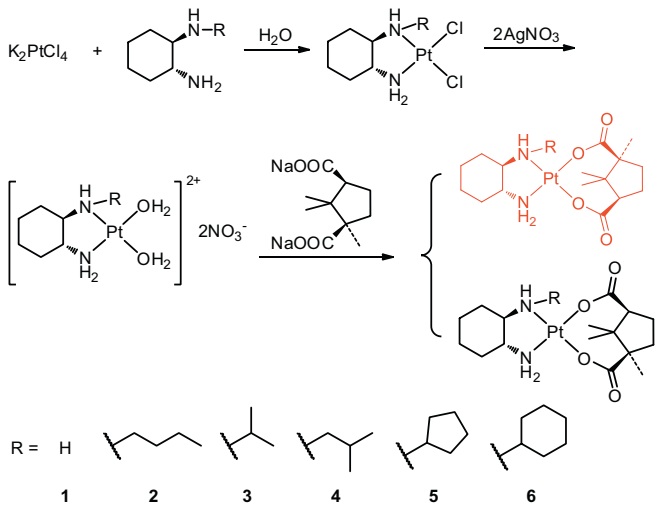

Scheme 1. The synthetic route to obtain platinum(II) complexes **1**–**6**.

for Pt was carried out on a J-A1100 inductively coupled plasma (ICP) spectrometer. ESI-MS and HRMS spectra were carried out in an Agilent 6224 TOF LC/MS instrument. IR spectra were scanned by a Nicolet IR200 spectrophotometer in the range of 4000–400 cm^{-1} in KBr pellet. ^1H NMR spectra were recorded on a Bruker DRX-500 spectrometer at 500 MHz in d_6-DMSO using TMS as an internal reference. UV spectra were tested in a Shimadzu UV-1700 instrument.

2.2. Preparation of target compounds

The general synthetic route to obtain the complexes containing D-(+)-camphorate anion is shown in Scheme 1. The preparation of (1*R*,2*R*)-N^1-alkyl-1,2-diaminocyclohexane has been reported previously [18–20]. To a solution of K_2PtCl_4 (0.42 g, 1 mmol) in distilled water (10 mL) was added (1*R*,2*R*)-N^1-alkyl-1,2-diaminocyclohexane (1 mmol) in distilled water (10 mL). The reaction mixture was then stirred at 25 °C for 12 h in the dark to give yellow precipitate as the intermediate *cis*-[$Pt(Am)_2Cl_2$] ($(Am)_2$ = (1*R*,2*R*)-N^1-alkyl-1,2-diaminocyclohexane). *cis*-[$Pt(Am)_2Cl_2$] was then treated with two equiv $AgNO_3$ in distilled water (80 mL) at 40 °C for 18 h to form an pellucid solution containing the aqua-diamine-dinitratoplatinum intermediate by filtering off AgCl deposits. The solution was then mixed with equimolar disodium D-(+)-camphorate at 50 °C for 12 h stirring and the solution was concentrated to 20 mL by rotary evaporation, and kept at 4 °C for several hours. The resulting pale yellow solid was filtered off and white solid products were obtained by recrystallization from water with active charcoal. Then they were dried in vacuo to obtain the final product.*cis*-[(1*R*,2*R*)-N^1-butyl-1,2-diaminocyclohexane-*N,N'*][(+)-camphorato-*O,O'*]platinum(II) (**2**). White solid. Yield: 0.22 g (39%). ^1H NMR (d_6-DMSO/TMS): δ 0.85 (t, 3H, CH_2CH_3), 0.98 (s, 6H, $C(CH_3)_2$), 1.20 (s, 3H, CCH_3), 1.10–1.89 (m, 16H, $NHCH_2CH_2CH_2CH_3$ and CH_2 of DACH and camphorato), 2.25 (t, 1H, COC*H*), 2.43–2.88 (m, 4H, $NHCH_2$ and NHC*H* and NH_2CH), 4.80–6.80 ppm (m, 3H, CHN*H* and $CHNH_2$); IR (KBr): 3429 (br), 3187, 3117, 2957, 1603, 1457, 1382, 1348, 1169, 1125, 909, 801 cm^{-1}; ESI-MS: *m/z* (%): 563 (87), 564 (100), 565 (90) $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd. for $C_{20}H_{37}N_2O_4Pt$: 564.24011, found: 564.24202. *Anal.* Calc. for $C_{20}H_{36}N_2O_4Pt$: C, 42.62; H, 6.44; N, 4.97; Pt, 34.61. Found: C, 42.70; H, 6.40; N, 4.99; Pt, 34.35.*cis*-[(1*R*,2*R*)-N^1-isopropyl-1,2-diaminocyclohexane-*N,N'*][(+)-camphorato-*O,O'*]platinum(II) (**3**). White solid. Yield: 0.24 g (43%). ^1H NMR (d_6-DMSO/TMS): δ 1.02 (s, 6H, $C(CH_3)_2$), 1.22 (s, 3H, CCH_3), 1.09–1.85 (m, 18H, $NHCH(CH_3)_2$ and CH_2 of DACH and camphorato), 2.27 (t, 1H, COC*H*), 2.45–2.70 (m, 3H, 2NHC*H* and NH_2CH), 4.65–6.44 (m, 3H, CHN*H* and $CHNH_2$); IR (KBr): 3420, 3194, 3121, 2939, 1596, 1457, 1384, 1350, 1173, 1126, 801 cm^{-1}; ESI-MS: *m/z* (%): 549 (90), 550 (100), 551 (83) $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd. for $C_{19}H_{35}N_2O_4Pt$: 550.22446, found: 550.22303. *Anal.* Calc. for $C_{19}H_{34}N_2O_4Pt$: C, 41.52; H, 6.24; N, 5.10; Pt, 35.50. Found: C, 41.60; H, 6.18; N, 5.15; Pt, 35.29.*cis*-[(1*R*,2*R*)-N^1-(2-methylpropyl)-1,2-diaminocyclohexane-*N,N'*][(+)-camphorato-*O,O'*]platinum(II) (**4**). White solid. Yield: 0.20 g (36%). ^1H NMR (d_6-DMSO/TMS): δ 0.89 (d, 6H, $CH(CH_3)_2$), 0.99 (s, 6H, $C(CH_3)_2$), 1.22 (s, 3H, CCH_3), 1.05–1.89 (m, 13H, $NHCH_2CH(CH_3)_2$ and CH_2 of DACH and camphorato), 2.25 (t, 1H, COC*H*), 2.40–2.85 (m, 4H, $NHCH_2$ and NHC*H* and NH_2CH), 4.69–6.70 (m, 3H, CHN*H* and $CHNH_2$); IR (KBr): 3434, 3186, 3117, 2938, 1608, 1383, 1347, 1169, 1125, 801 cm^{-1}; ESI-MS: *m/z* (%): 563 (79), 564 (100), 565 (87) $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd. for $C_{20}H_{37}N_2O_4Pt$: 564.24011, found: 564.24143. *Anal.* Calc. for $C_{20}H_{36}N_2O_4Pt$: C, 42.62; H, 6.44; N, 4.97; Pt, 34.61. Found: C, 42.74; H, 6.36; N, 5.01; Pt, 34.41.*cis*-[(1*R*,2*R*)-N^1-cyclopentyl-1,2-diaminocyclohexane-*N,N'*][(+)-camphorato-*O,O'*]platinum(II) (**5**). White solid. Yield: 0.24 g (42%). ^1H NMR (d_6-DMSO/TMS): δ 0.95 (s, 6H, $C(CH_3)_2$), 1.25 (s, 3H, CCH_3), 1.12–1.98 (m, 20H, CH_2 of DACH and cyclopentyl and camphorate), 2.22 (t, 1H, COC*H*), 2.55–2.78 (m, 3H, 2NHC*H* and NH_2CH), 4.76–6.55 (m, 3H, CHN*H* and $CHNH_2$); IR (KBr): 3434 (br), 3178, 3113, 2944, 1602, 1457, 1382, 1348, 1126, 801 cm^{-1}; ESI-MS: *m/z* (%): 575 (85), 576 (100), 577 (80) $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd. for $C_{21}H_{37}N_2O_4Pt$: 576.24011, found: 576.24175. *Anal.* Calc. for $C_{21}H_{36}N_2O_4Pt$: C, 43.82; H, 6.30; N, 4.87; Pt, 33.89. Found: C, 43.90; H, 6.24; N, 4.92; Pt, 34.10.*cis*-[(1*R*,2*R*)-N^1-cyclohexyl-1,2-diaminocyclohexane-*N,N'*][(+)-camphorato-*O,O'*]platinum(II) (**6**). White solid. Yield: 0.23 g (39%). ^1H NMR (d_6-DMSO/TMS): δ 0.97 (s, 6H, $C(CH_3)_2$), 1.24 (s, 3H, CCH_3), 1.15–1.95 (m, 22H, CH_2 of DACH and cyclohexyl and camphorate), 2.24 (t, 1H, COC*H*), 2.57–2.83 (m, 3H, 2NHC*H* and NH_2CH), 4.80–6.67 (m, 3H, CHN*H* and $CHNH_2$); IR (KBr): 3431 (br), 3187, 3109, 2934, 2859, 1602, 1456, 1383, 1349, 1126, 800 cm^{-1}; ESI-MS: *m/z* (%): 589 (76), 590 (100), 591 (90) $[M+H]^+$; HRMS: *m/z* $[M+H]^+$ calcd. for $C_{22}H_{39}N_2O_4Pt$: 590.25576, found: 590.25425. *Anal.* Calc. for $C_{22}H_{38}N_2O_4Pt$: C, 44.81; H, 6.50; N, 4.755; Pt, 33.09. Found: C, 44.89; H, 6.47; N, 4.805; Pt, 33.28.

2.3. Cell culture

The *in vitro* antitumor activity of resulting complexes were tested on A549 (human non-small cell lung cancer), MCF-7 (human breast carcinoma), HepG2 (hepatocellular carcinoma), and HCT-116 (human colorectal cancer) cell lines, respectively. They were all cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg mL^{-1} of streptomycin, and 100 μg mL^{-1} of penicillin in an atmosphere of 5% CO_2 at 37 °C.

2.4. Cytotoxicity assay (IC_{50} values)

The *in vitro* cytotoxicity of all complexes (IC_{50} values) were determined by MTT assay (MTT = 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The suspension of 1000 cells $well^{-1}$ was plated in 96-well culture plates with culture medium and was incubated for 24 h at 37 °C in a 5% CO_2 incubator. The complexes were dissolved in ultrapure water firstly and diluted to the required concentration with culture medium. Then the diluted solution of complexes was added to the wells, and the cells were incubated at 37 °C in a 5% CO_2 incubator for 48 h. After that, the cells were treated with 10 μL MTT dye solution (5 mg mL^{-1}) for 4 h cultivation. The media with MTT solution were removed with 100 μL of DMSO solution. The absorbance of formazane solution was measured at 540 nm with an automatic microplate ELISA reader. The IC_{50} value was determined from the chart of cell viability (%) against dose of complex added (μM).

2.5. Cell cycle analysis

A Cell Cycle and Apoptosis Analysis Kit (Beyotime Institute of Biotechnology, China) was used in the cell cycle progression study. Firstly, 1×10^6 HCT-116 cells were cultured in a 6-well plate and incubated at 37 °C in 5% CO_2 overnight. Then cells were treated with the selected complexes for 12 h at the concentration of 30 μM. Successively, cells were harvested and washed twice with PBS solution. After that, cells were fixed in cold 70% ethanol and stored at 4 °C. On the day of analysis, ethanol was removed by centrifugation and cells were washed twice with PBS, and then treated with mixed solution of RNase and propidium iodide (PI) for 30 min at 37 °C. The samples were processed by a flow cytometer (FAC Scan, Becton Dickenson, USA). The results were analyzed by using FCSEXPRESS software.

2.6. Gel electrophoresis experiment

DNA cleavage induced by complexes **1**, **3**, **6**, and cisplatin was investigated by agarose gel electrophoresis. pET22b plasmid DNA (50 ng μL^{-1}) was used as the target in this experiment. Based on the previous reports by Lippard and co-workers [23,24], we made some modifications of experiment conditions in consideration of the specificity of our compounds. Appropriate dilutions of compounds were made before dissolving them in ultrapure water, and different volumes of solutions were added to the tubes to achieve a set of concentrations in the range of 1–100 μM. pET22b DNA (5 μL) was added to each tube, and then the mixtures of tested complexes and pET22b plasmid DNA were incubated at 37 °C for 24 h. After that, the agarose gel (1% w/v) containing ethidium bromide (0.01%) was prepared using TA buffer (50 mM Tris–acetate, pH 7.5). The mixtures with loading buffer (1 μL) underwent electrophoresis in agarose gel in TA buffer (50 mM Tris–acetate, pH 7.5) at 90 V for 60 min. The bands were photographed using a Gel Image System (Tanon 2500, China).

3. Results and discussion

3.1. Synthesis and characterization

The synthesis of complexes **2**–**6** is shown in Scheme 1. The ligands (1*R*,2*R*)-N^1-alkyl-1,2-diaminocyclohexane were prepared in the previous studies [18–20]. The resulting complexes were characterized by ^{1}H NMR, MS, and IR spectra together with elemental analysis. For IR spectra in the range of 3109–3194 cm^{-1}, the N–H stretching vibrations of complexes **2**–**6** are red shifted compared to the single amino group of the corresponding ligands due to amino group coordination with Pt(II) ion. Also it is notable that the asymmetric stretching vibrations of (C–O) signals appear between 1596 and 1608 cm^{-1}, characteristic of coordinated dicarboxylate ligands, whereas the symmetric stretching vibrations of (C–O) show in the range of 1382–1384 cm^{-1} [21]. The $\Delta\nu$ [ν_{as}(C–O) – ν_s (C–O)] values of these complexes are in the range of 212–225 cm^{-1}, greater than 200 cm^{-1}, in line with the structures of the resulting complexes with bidentate-coordinated carboxylate ligands [22]. ^{1}H NMR spectroscopy of the platinum(II) complexes was performed with d_6-DMSO as the solvent, and the expected signals were accordingly observed. The broad signals of hydrogen atoms belonging to amino groups appear in the range of δ 4.65–6.80 ppm due to amine coordination with platinum(II), shifting high-field relative to the metal-free ligands in the range of δ 8.85–9.42 ppm. Besides, the structures of all the complexes were further determined by HRMS spectra and elemental analysis for C, H, N, and Pt.

The alkyl/cycloalkyl substituent at N^1 position of target complexes with an optically active ligand D-(+)-camphorate may produce diastereoisomeric mixtures for complexes **2**–**6** as shown in Scheme 1. In order to clarify the structures of target complexes, we have made theoretic calculations for complex **2**–**6** to investigate which diastereoisomer is energically preferred by using HyperChem 7.5 [25]. After building the molecule and constraining bond angle for PtN_2O_2 plane, we chose AMBER as the molecular mechanics force field which assigns atom types and parameters to the molecule. Then amber2 was selected as the parameter set for the AMBER force field. At last, Polak–Ribiere was applied as the minimization algorithm to optimize the structure of the molecule and calculate the minimal energy of the molecule, as for complex **3**, which are illustrated in Fig. 1.

It can be observed from Fig. 1 that the configuration B (E = 87.907 kcal/mol) is more thermodynamically stable than configuration A (E = 149.228 kcal/mol). Analogous calculation results were also obtained for other complexes (data not shown). So it is proposed that complexes **2**–**6** prefer to adopt configuration B shown in Fig. 1 and Scheme 1 (red one).

Besides, considering that there is an 8-membered ring in all compounds due to Pt coordinating with D-(+)-camphorate, we tested the stability of all compounds in aqueous solution by UV–Vis spectral analysis at the concentration of 0.5 mg/mL. The UV absorption spectra in the range of 200–600 nm for compound **3** was shown in Fig. 2 and no obvious changes of absorption peak and UV absorbance were observed within 12 h, suggesting that compound **3** is relatively stable in aqueous solution. The similar results were obtained for other compounds in the same experimental condition (data not shown).

3.2. In vitro cytotoxicity

The *in vitro* cytotoxicities of complexes **2**–**6** were tested by MTT assay against four human tumor cell lines including HepG2 (human hepatocellular carcinoma), MCF-7 (human breast carcinoma), A549 (human non-small-cell lung cancer), and HCT-116 (human colorectal cancer) after 48 h incubation, which were compared with those of cisplatin and oxaliplatin (Table 1). The cytotoxicity of parent compound **1** on HL-60 (leukemia), 3AO (ovarian), BEL-7402 (hepatocarcinoma), and A549 cell lines has been tested in our previous report [17], suggesting that the cytotoxicity of complex **1** was very close to that of cisplatin against HL-60 and slightly better than cisplatin against 3AO, however, it was far less than cisplatin on A549 and BEL-7402 cell lines. Table 1 showed that the complexes with D-(+)-camphorate anion, which were modified in the substituent of nitrogen atom, generally displayed good cytotoxicity against selected cell lines. Based on the IC_{50} values, it was noted that the compounds with a chain alkyl substituent at one of nitrogen atoms of carrier ligands (**2**–**4**) were more potent *in vitro* than those with a cycloalkyl substituent (**5**–**6**). Generally, the alkyl substituent at the nitrogen atom increased the cytotoxicity of complexes on non-small-cell lung cancer cell line (A549) and hepatocarcinoma cell line relative to that of parent compound **1**.

As for MCF-7 cell line, compounds **3** (R = isopropyl, IC_{50} = 6.0 - μM) and **4** (R = 2-methylpropyl, IC_{50} = 8.0 μM) showed comparable antitumor effect to those of cisplatin and oxaliplatin, especially compound **3**, 2.3-fold less potent than oxaliplatin. With respect to A549 cell line, the most active agent was compound **4**, which revealed similar cytotoxicity with positive control, about 2.0-fold less potent than cisplatin and oxaliplatin. Besides, compound **3** had desirable antitumor effect in both HCT-116 and HepG2 cell lines, and the IC_{50} values were 5.1-fold less than that of oxaliplatin for HCT-116 and 7.7-fold less than that of cisplatin in HepG2. Generally, compounds **3** and **4** with branched alkyl substituent at one

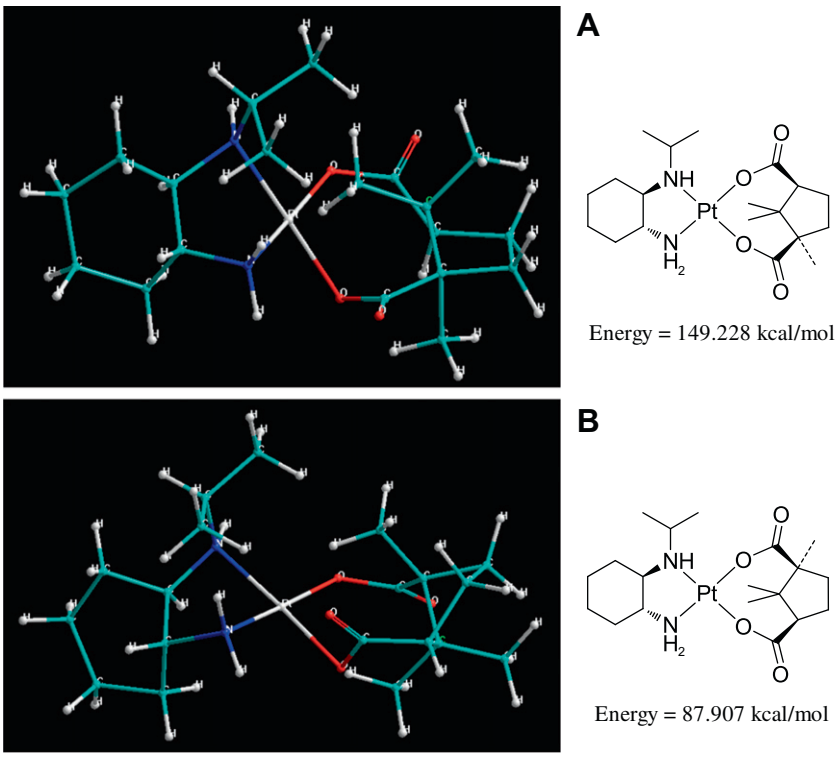

Fig. 1. Theoretically optimized structure of complex 3 (A isomer: E = 149.228 kcal/mol; B isomer: E = 87.907 kcal/mol).

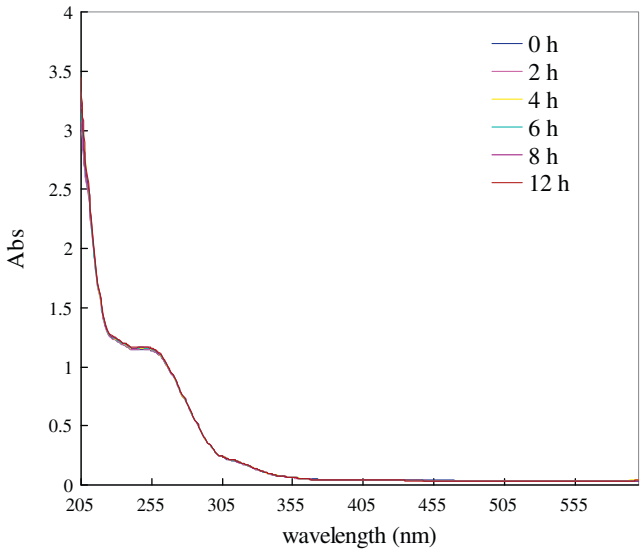

Fig. 2. UV absorption spectra of complex **3** in aqueous solution within 12 h.

of nitrogen atoms of carrier ligands were the most effective agents on the selected tumor cell lines.

3.3. Cell cycle block analysis

We analyzed the DNA content of cells stained with propidium iodide by flow cytometry to examine the effect of complexes on HCT-116 cell cycle progression. The DNA histograms were shown in Fig. 3, which were obtained after cells were treated with the selected compounds **3** and **4** for 12 h incubation at the concentration of 30 μM.

Table 1
In vitro cytotoxicity of complexes **1**–**6** against selected human tumor cell lines after 48 h incubation.

Compd.	R	IC_{50} (μM)[a]			
		A549[b]	HCT-116[c]	HepG2[d]	MCF-7[e]
1	H	19.7 ± 1.8[f]	n.d.[g]	n.d.	n.d.
2	Butyl	32.0 ± 2.2	41.8 ± 3.6	36.7 ± 1.9	31.3 ± 2.0
3	Isopropyl	12.5 ± 1.4	11.1 ± 0.7	9.2 ± 1.0	6.0 ± 0.5
4	2-Methylpropyl	7.8 ± 0.9	15.7 ± 1.8	13.5 ± 1.5	8.0 ± 1.1
5	Cyclopentyl	>100	>100	>100	41.0 ± 3.5
6	Cyclohexyl	40.0 ± 3.0	33.9 ± 4.8	47.7 ± 3.8	29.3 ± 3.8
Cisplatin		3.6 ± 0.2	1.7 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Oxaliplatin		3.9 ± 0.4	2.2 ± 0.2	1.4 ± 0.1	2.6 ± 0.3

[a] IC_{50} value represents the drug concentration suppressing 50% tumor cell proliferation; values are mean ± SD from three independent experiments.
[b] Human non-small-cell lung cancer cell line.
[c] Human colorectal cancer cell line.
[d] Human hepatocellular carcinoma cell line.
[e] Human breast cancer cell line.
[f] Data from L. Wang, S. Gou et al. [17].
[g] n.d. = Not determined.

It was observed that HCT-116 tumor cells exposed to cisplatin and oxaliplatin for 12 h were blocked in the S (44.23%) and G2/M (37.57%) phase, respectively. It was also noted that compounds **3** and **4** produced cell accumulation in the S phase, with the population of 38.31% and 38.39%, respectively, after 12 h exposure at a concentration equal to that of cisplatin. Based on the investigation we can conclude that compounds **3** and **4** inhibit tumor cell proliferation mainly by inhibiting DNA synthesis similar to cisplatin, different from oxaliplatin.

3.4. DNA cleavage pattern

In the study on the interaction with DNA, we introduced agarose gel electrophoresis and used pET22b plasmid DNA as the

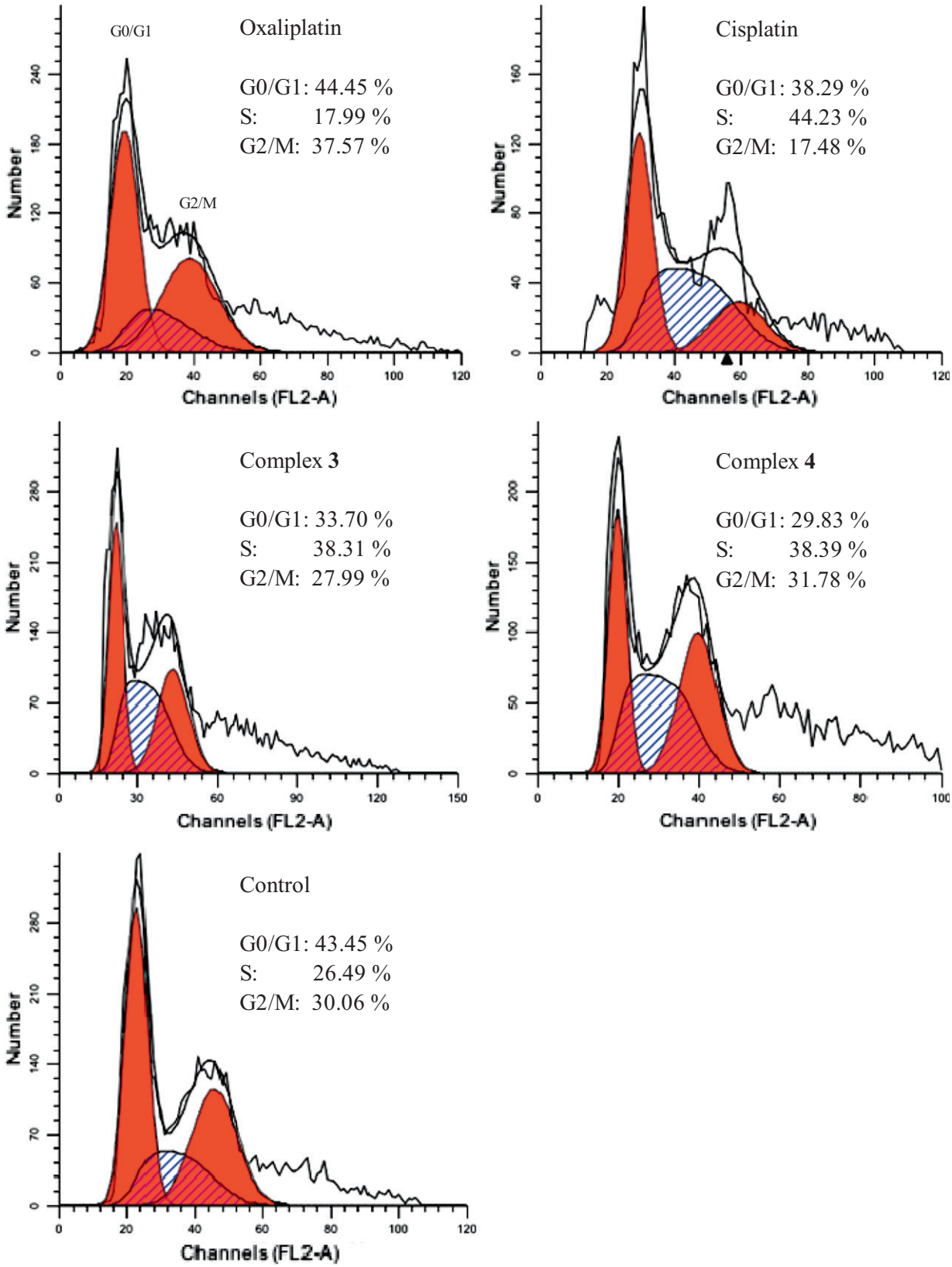

Fig. 3. Cell cycle distribution of HCT-116 cells by FACS analysis exposed for 12 h to complexes **3** and **4**, cisplatin, and oxaliplatin.

target. In order to clarify the different DNA cleavage pattern of compounds with different substituents at one of nitrogen atoms, complexes **1**, **3**, and **6** were chosen to test their ability to distort plasmid DNA.

In Fig. 4a, as for cisplatin (lanes 2–5), the electrophoretic mobility of supercoiled DNA (form I) and open circular DNA (form II) increased with the concentration of cisplatin and a little increased density of form II was observed at high concentration (100 μM),

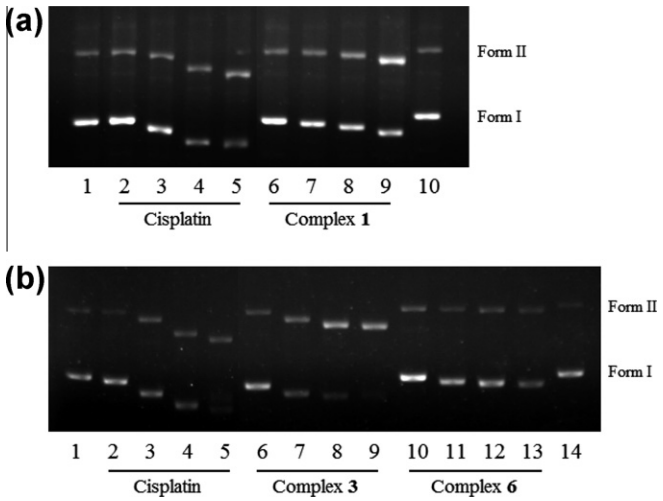

Fig. 4. Gel electrophoretic mobility pattern of pET22b plasmid DNA incubated at 37 °C with various concentrations of tested compounds for 24 h. (a): untreated plasmid DNA (lanes 1 and 10), concentrations of 1, 10, 50, and 100 μM of cisplatin (lanes 2–5), concentrations of 1, 10, 50, and 100 μM of complex **1** (lanes 6–9); (b): untreated plasmid DNA (lanes 1 and 14), concentrations of 1, 10, 50, and 100 μM of cisplatin (lanes 2–5), concentrations of 1, 10, 50, and 100 μM of complex **3** (lanes 6–9), concentrations of 1, 10, 50, and 100 μM of complex **6** (lanes 10–13). Form I and Form II indicate supercoil and unwinding (open circle) forms of pET22b, respectively.

suggesting that cisplatin produced open circle DNA partly, and supercoiled DNA was cleaved to produce short DNA strand. Moreover, it is noted that the total amount of forms I and II had a significant decrease in the range of 50–100 μM, indicating that plasmid DNA was partly degraded by cisplatin.

With respect to complex **1** shown in Fig. 4a (lanes 6–9), the electrophoretic mobility of form I and form II increased slightly with the drug concentration and the density of open circle DNA (form II) had a significant increase at high concentrations (50–100 μM). Then it can be found that complex **1** mainly produced open circle DNA and there was no obvious degradation of DNA, which was different from cisplatin.

Fig. 4a and b were obtained by two parallel experiments with different drugs on the same condition. The same behavior of plasmid DNA interacting with cisplatin was observed in Fig. 4b (lanes 2–5). In Fig. 4b (lanes 6–9), complex **3** showed a cleavage ability to produce a large amount of open circle DNA, which was similar to complex **1**, different from cisplatin. Interestingly, from lanes 10–13, complex **6** did not give rise to open circle DNA, but caused degradation of plasmid DNA at a certain degree, different from complex **3**.

Based on the discussion above, it can be concluded that complexes **1** and **3** show a similar distorting pattern (mainly nicking effect) toward plasmid DNA, indicating that the compound with an alkyl substituent at one of the nitrogen atoms may not change the mode of action toward DNA compared with the parent compound **1**. However, complex **6**, with a cycloalkyl substituent, demonstrates a different effect (mainly degrading effect) on DNA relative to its analogous complexes **1** and **3**, but similar to cisplatin, interestingly.

4. Conclusion

In conclusion, we designed and synthesized five novel platinum(II) complexes with N-monoalkyl 1*R*,2*R*-diaminocyclohexane derivatives as carrier ligands and D-(+)-camphorate anion as the leaving group. The *in vitro* cytotoxicity results showed that most of complexes had good antitumor activity against selected cell lines. Compounds **3** and **4**, with a branched alkyl substituent at one of nitrogen atoms, displayed not only better antitumor activity but also broader spectrum. Besides, the branched alkyl substituent at the nitrogen atom increased the cytotoxicity of complexes on non-small-cell lung cancer cell line (A549) and hepatocarcinoma cell line in comparison to that of parent compound **1**. Flow cytometry demonstrated that DNA synthesis phase in the cell cycle was one of the targets for typical complexes **3**, **4**, and cisplatin. Besides, representative complexes (**1**, **3**, and **6**) and positive drug in this study possessed DNA cleaving ability in the agarose gel experiment, especially complex **6**, with a cycloalkyl substituent, demonstrating a different effect (mainly degrading effect) on DNA relative to its analogous complexes (**1** and **3**), but similar to cisplatin. As such, we can conclude that alkyl and cycloalkyl substituents at N^1 position have different influences in DNA-drug interaction pattern. However, the specific mechanism needs to be further clarified.

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