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Research paper

Design, synthesis and biological evaluation of demethylcantharidate-linked platinum(II) complexes of *N*-monoalkyl-1*R*,2*R*-diaminocyclohexane derivatives

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ABSTRACT

Nine platinum(II) complexes with *N*-monoalkyl 1*R*,2*R*-DACH derivatives as carrier ligands and demethylcantharidate as a leaving group were synthesized and spectrally characterized. All the complexes showed considerable cytotoxicity against tested human cancer cell lines: A549, HCT116 HepG-2 and MCF7 cell lines. Especially, complex **2** exhibited potent cytotoxicity against A549 (1.01 μM) and HCT116 (0.83 μM) cell lines, and showed no cross-resistance to cisplatin against SGC7901/CDDP cell line (RF = 1.44). In addition, the typical compounds were further studied by flow cytometric analysis and western blot method. The results indicated that they induced apoptosis by a mitochondrial-dependent pathway, which were similar to cisplatin.

1. Introduction

Cisplatin has been widely used in the treatment of testicular, ovarian, bladder and head/neck tumors for decades since its first approval by FDA in 1978 [1]. After the success of cisplatin, a large number of anticancer platinum complexes have been studied. Among them, carboplatin and oxaliplatin have been accepted worldwide, along with five other platinum(II) complexes gained approval regionally in different nations (nedaplatin and miriplatin in Japan, lobaplatin and dicycloplatin in China, heptaplatin in Korea) [2]. However, these clinical used platinum drugs possess similar drawbacks, including severe side effects and intrinsic and/or acquired resistance [3–5]. Several strategies have been employed in order to overcome these drawbacks, among which application of bioactive ligands is a promising one in the design of novel platinum complexes. Such resulting complexes may have dual mechanisms of action, i.e., one from the proper biologically active ligand and the other from Pt pharmacophore [6–12].

Traditional Asian medicine, especially traditional Chinese medicine (TCM), is a treasure yet to be efficiently explored [13,14]. Artemisinin [15] as antimalarial agent and arsenic trioxide [16] for the use in leukaemia is one of modern drugs successfully mined from traditional Chinese medicine. The platinum-TCM conjugates may generate a novel strategy to combine the two kinds of old drugs together synergistically [17]. The platinum(II) complexes containing camphorato, derivative of long used TCM camphor, showed promising *in vitro* and *in vivo* anticancer activity [18,19]. Cantharidin is another TCM used for the treatment of liver, lung and intestinal cancers for a long time. Demethylcantharidin is a synthetic analogue with similar biological activity and much less toxic than cantharidin. Combination platinum(II) moieties with demethylcantharidate as a leaving group have resulted in a series of novel complexes. The relative platinum complexes showed more potent antitumor activity against some cancer cell lines than cisplatin/oxaliplatin and had no cross-resistance with cisplatin. Moreover, they possessed a dual antitumor mechanism, namely, inhibition of PP2A (demethylcantharidin) and platination of DNA (platinum moiety) [20–22].

So far, numerous platinum complexes containing 1*R*,2*R*-diaminocyclohexane (1*R*,2*R*-DACH) and its derivatives as carrier ligands have been reported after oxaliplatin was approved worldwide. In our previous research [23–28], a number of platinum(II) complexes of *N*-monoalkyl 1*R*,2*R*-DACH as carrier ligands have been investigated and some of these compounds showed better anticancer activity towards some cancer cell lines than their counterparts with 1*R*,2*R*-DACH as ligand. To continue our research, a series of platinum(II) complexes were obtained in this study with the above mentioned ligands and demethylcantharidate as a leaving ligand. The *in vitro* cytotoxicity of the synthesized complexes

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against six human solid cancer lines was studied. Additionally, the mechanism of cellular death of typical complexes was also investigated in this paper.

2. Results and discussion

2.1. Synthesis and characterization

Complexes **1**–**9** were synthesized *via* Scheme 1. The intermediates **M1–M9** were obtained by a routine method described by our previous study [23–25]. Demethylcantharidate was prepared by a Diels-Alder reaction between furan and maleic anhydride, followed by hydrogenation using Pd/C as catalyst [20]. All intermediates were characterized by ^{1}H NMR, FT-IR, and ESI-MS spectra together with microanalysis. Platinum(II) complexes **1**–**9** were spectrally characterized by ^{1}H NMR, ^{13}C NMR (complexes **2** and **7**), FT-IR and ESI-MS spectra as well as element analyses.

In the IR spectra of all platinum complexes, the N—H stretching vibrations appeared between 3202 and 3440 cm^{-1}, shifting to lower frequencies than those of the free alkyl amine. The ν_{as}(C=O) signal of the complexes appears in the range from 1650 to 1622 cm^{-1}, characteristic signals of coordinated dicarboxylates, and the C—O signal appeared in the range of 1311–1325 cm^{-1}. All ^{1}H NMR and ^{13}C NMR data are compatible to the molecular structures proposed in Scheme 1. In the ^{1}H NMR spectra of complexes **1**–**9**, the broad signals of hydrogen atoms belonging to amino groups appear in the range of δ 6.17–6.50 ppm due to amine coordination with metal ions, shifting to high-field compared with the metal-free ligands in the range of δ 8.80–9.40 ppm (appeared only when d_6-DMSO was used as NMR solvent, complexes **2** and **7**). Moreover, the signals of C—H protons connected to the amino groups were observed between 2.48 and 2.94 ppm, clearly shifting high-field relative to the corresponding signals ($\delta \approx 3.5$ ppm) in the free ligands. Besides, all the complexes showed 100% of $[M+H]^+$ or $[M+Na]^+$ peaks in the ESI-MS spectroscopy, which have three main ion peaks due to the existence of ^{194}Pt (33%), ^{195}Pt (34%) and ^{196}Pt (25%) isotopes. Elemental analysis data of C, H and N for each complex were in good fit with the empirical formula proposed.

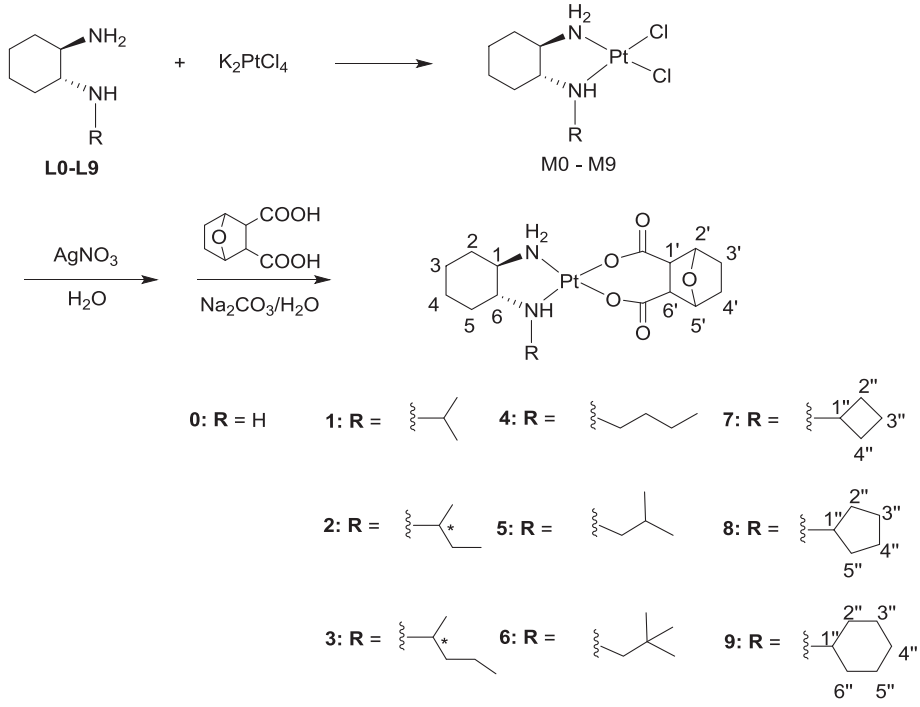
Notably, complexes **2** and **3** had an extra stereogenic carbon center along with other three stereogenic centers [24]; however, no effort was devoted to isolating the pure enantiomers in this study. Therefore, both *R* and *S* configurations at the α-carbon atom are present in the final compounds.

2.2. In vitro cytotoxicity studies

The cytotoxicity of complexes **0**–**9** was determined by MTT assays against A549, HCT116, HepG-2 and MCF7 human cancer cell lines. The *in vitro* and *in vivo* suppression of growth of cancer cells (especially hepatocellular carcinoma cells) by complex **0** were reported before by Yee-Ping Ho's group [20], and the *in vitro* cytotoxicity of complex **0** was measured in this paper as another positive control, along with cisplatin and oxaliplatin. The result of cytotoxicity against four cancer cell lines are expressed as IC_{50} values listed in Table 1.

According to the IC_{50} values, most of the synthesized complexes showed comparable or better cytotoxicity to cisplatin and oxaliplatin against A549, HCT116 and MCF7 cancer cell lines. However, only complex **2** possessed comparable cytotoxicity against HepG-2 cell line with positive controls.

It is noted that the antiproliferative properties of the synthesized platinum complexes with *N*-monoalkylsubstituted 1*R*,2*R*-DACH as carrier ligands against A549 and HCT116 cancer cells were enhanced compared with complex **0**. Significantly, complex **2** were 3–4 times more potent than cisplatin/oxaliplatin against A549 cell line and 2–3 folds more potent than cisplatin/oxaliplatin against HCT116 cell line. Furthermore, complexes **5** and **7** had better cytotoxicity against MCF7 cell line than complex 0, and complex 5 was slightly more potent than cisplatin against MCF7 cell line. However, the HepG-2 cell line was much less responsive to our complexes than complex **0**, which indicated that the steric hinderance in the carrier ligands might reduce the cytotoxicity of the platinum complex against HepG-2 cell line.



Scheme 1. The synthetic method and related chemical complexes studied in this work.