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Study on the cytotoxic activity of platinum(II) complexes of (1*R*,2*R*)-N^1-cyclopentyl-1,2-cyclohexanediamine with substituted malonate derivatives

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ABSTRACT

Three platinum(II) complexes of (1*R*,2*R*)-N^1-cyclopentyl-1,2-cyclohexanediamine with malonate derivatives were designed, synthesized and spectrally characterized. MTT assay showed that the complexes possessed positive cytotoxic effect on the four human solid tumor cell lines. Among the complexes, complex **2** demonstrated the strongest cytotoxic activity compared to cisplatin and oxaliplatin against HepG2 cell line (IC_{50} = 3.04 μM). Furthermore, the results of gel electrophoresis revealed that complex **2** interacted with DNA in a different mode from that of cisplatin. Mechanism studies of cell proliferation inhibition and cellular uptake indicated that complex **2** entered HepG2 cell more efficiently than cisplatin, exhibited massive G2 accumulation and then induced apoptosis.

Application and research of platinum-based anticancer drugs have been getting rapid development since the discovery of the first-generation platinum anticancer cisplatin in 1960s.[1] The achievement of cisplatin enormously inspired scholars to make continuous efforts on the platinum-centered structural changes,[2–5] aiming at exploring more effective drugs. However, it is difficult to acquire impressive effects without any serious problems that occurred in cisplatin chemotherapy (e.g., myelosuppression and neurotoxic effects).[6,7] Carboplatin and oxaliplatin following cisplatin approved by the FDA have been well known and universally acknowledged,[8–10] suggesting the important role of 1*R*,2*R*-diaminocyclohexane (DACH) as carrier ligand to influence the formation of platinum-DNA adducts, together with the function of cyclobutane-1,1-dicarboxylate (CBDA) as leaving group to improve physical properties of the platinum complexes.[9,11,12]

Using various derivatives of CBDA and/or DACH, a series of related platinum analogs have been designed, prepared and tested in clinic (e.g., cycloplatin, miboplatin, tetraplatin, L-NDDP and TRK-710).[3,13–15] It is worth mentioning that a successful application case in gastric cancer in South Korea, heptaplatin (cis-malonato-[4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]platinum(II)), is reported to have prominent water-solubility and stability. Moreover, heptaplatin even has better clinical effect and less toxicity (especially less nephrotoxicity) than cisplatin,[16,17] indicating that a malonato moiety as leaving group exhibits good efficacy. It is noted that proper leaving groups may dexterously improve biological properties of the platinum complexes by regulating the lipophilic and hydrophilic property to a perfect balance, in which good water-solubility and less side effects could be obtained.[18]

Apart from classical platinum-based compounds, sterically hindered platinum(II) complexes have been paid much attention, such as picoplatin (cis-amminedichlorido(2-methylpyridine) platinum (II)).[19–21] The methyl group located axially above the plane of pyridine-platinum brings about steric hindrance, which can reduce the hydrolysis rate of platinum complexes (approximately half of cisplatin) and the sensitivity in interacting with other biological nucleophiles like glutathione.[22] In this way, the properties of targeting and anti-tumor activity are both enhanced. Instead of taking cisplatin as the leading structure, our group previously designed a class of *N*-monoalkyl 1*R*,2*R*-DACH derivatives owning different cycloalkyl substituents (e.g., complex **1**, Fig. 1) and the cytotoxic results showed that platinum(II) complexes bearing a cyclopentyl group at the N^1 position of 1*R*,2*R*-DACH have high cytotoxicity against A549 and MCF-7 cell lines.[23,24] Encouraged by the previous successes, we tried to combine N^1-cyclopentyl-1*R*,2*R*-DACH as the carrier ligand with 2-substituted propane-1,1-dicarboxylates as the leaving ligands, resulting in a series of sterically hindered platinum(II) complexes (Fig. 1). Herein, methyl, ethyl and benzyl group

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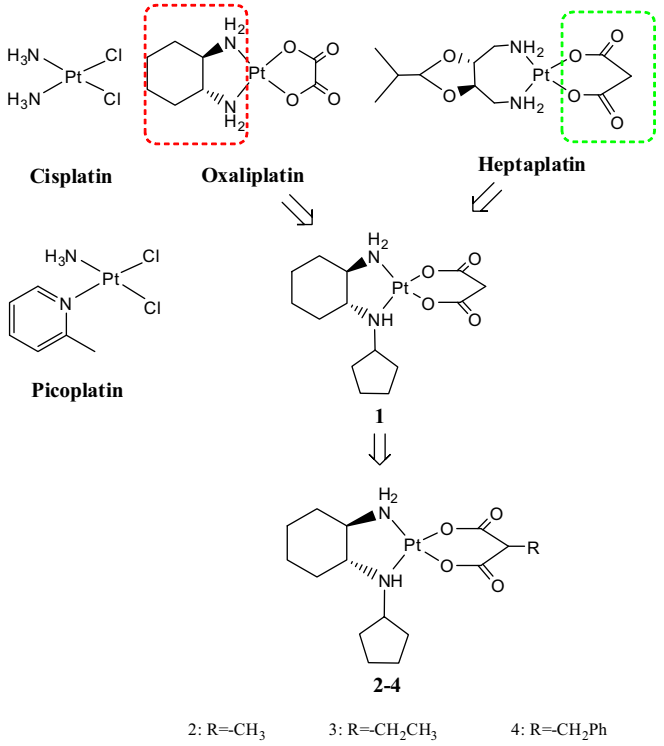
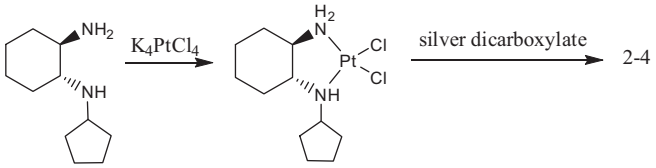


Figure 1. Chemical structures of cisplatin, oxaliplatin, heptaplatin, picoplatin, precursor complex **1** and the target complexes **2**–**4**.

were chosen as the substituents introduced to the C-2 position of malonate. We hope the introduction of the substituents could regulate the compounds' lipophilicity, so that, they may promote transporting complexes to the possible target DNA or proteins inside the tested cell lines and result in good anti-proliferation effects.[25] Specific structures of the novel platinum(II) complexes are presented in Figure 1. The synthetic route of the target complexes **2**–**4** is as shown in Scheme 1.[26] Complexes **2**–**4** were synthesized by a mixture of cis-dichloro[(1*R*,2*R*)-N^1-cyclopentyl-1,2-diaminocycloh-exane-*N*,*N*′] platinum(II) and the corresponding silver dicarboxylate in distilled water. (1*R*,2*R*)-N^1-cyclopentyl-1,2-cyclohexanediamine (L) as ligand was prepared according to our previous Letters.[24,27] 2-Substitued malonate derivatives were prepared as introduced in the literature methods.[28–30] All the synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR spectra as well as ESI-MS spectroscopy. In addition, complex **2** was also characterized by ^{195}Pt NMR spectroscopy and single crystal X-ray structural analysis.[31] Aqueous solubility of complex **2** was measured as about 5.8 mg/mL which was similar to oxaliplatin (6 mg/mL). Single crystals of complex **2** were grown from its aqueous solution. The compound crystallized in a triclinic system with space group $P\bar{1}$, a = 11.282(4) Å, b = 13.503(5) Å, c = 15.137(5) Å, α = 73.710(4)°, β = 69.756(4)°, γ = 69.411(4)°, Z = 4 and V = 1992.0 (12) Å^3. Molecular structure of complex **2** was presented in Figure 2 and the corresponding crystallographic data were shown in Table 1. It is noted that there are four independent molecules in the asymmetric unit. Again, the stereo configuration of the chiral nitrogen atom derived from coordination is *S* as reported previously.[24,25,27]

The in vitro cytotoxicity of all the targeted complexes (**2**–**4**) were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[32] against four human solid tumor cell lines including A549, HCT-116, HepG2 and MCF-7. The cytotoxic effects of these complexes are presented in Table 2, in terms of IC_{50} values (50% of cell growth inhibition). Cisplatin



Scheme 1. Synthetic route of the target complexes.

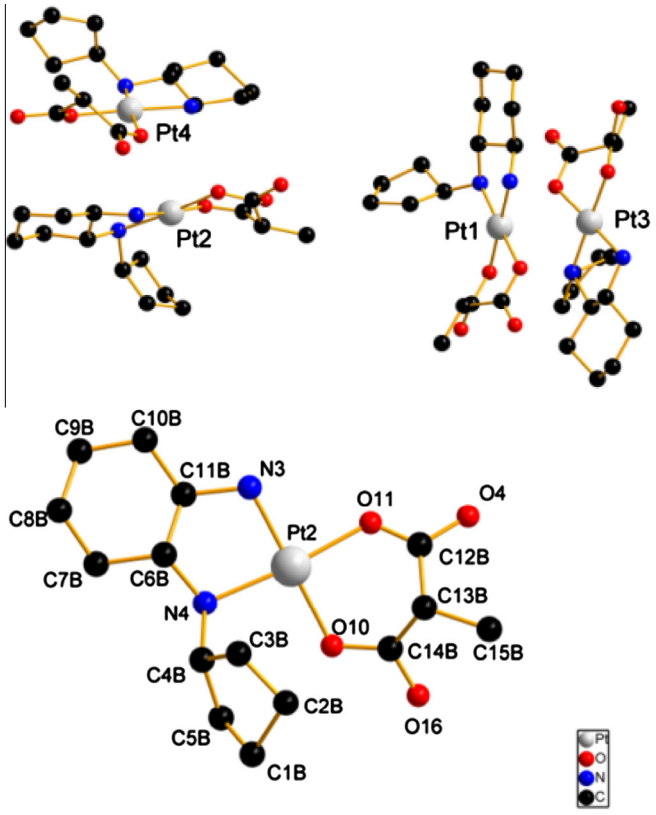


Figure 2. Crystal structure of complex **2**. The asymmetric unit consists of four independent molecules. One of them was selected as representative to display selected bond lengths (Å) and angles (°) for **2**: Pt(2)–N(3) 2.02(3), Pt(2)–N(4) 2.07 (3), Pt(2)–O(11) 2.03(2), Pt(2)–O(10) 2.06(3), N(3)–Pt(2)–O(11) 91.5(10), N(3)–Pt (2)–O(10) 175.7(11), O(11)–Pt(2)–O(10) 88.2(11), N(3)–Pt(2)–N(4) 83.8(11), C (12B)–C(13B)–C(15B) 116(4), C(6B)–N(4)–C(4B) 107(4), C(6B)–N(4)–Pt(2) 109(2), C(4B)–N(4)–Pt(2) 116(3), C(5B)–C(4B)–N(4) 110(5), C(3B)–C(4B)–N(4) 116(5), C (3B)–C(4B)–C(5B) 108(7). Hydrogen atoms and free water molecules were omitted for clarity.

and oxaliplatin were used as positive controls. Furthermore, the cytotoxicity of the precursor complex **1** is demonstrated in Table 3 for comparison.

Notably, according to the IC_{50} values summarized in Table 2, complex **2** was the most effective agent among the synthesized complexes against four human solid tumor cell lines, where the IC_{50} values ranged from 3.04 to 21.50 μM. Complex **2** had the strongest cytotoxic activity against HepG2 (IC_{50} = 3.04 μM) compared to cisplatin (IC_{50} = 4.00 μM) and oxaliplatin (IC_{50} = 3.57 μM). What's more, the cytotoxic activity of complex **2** was quite close to cisplatin and oxaliplatin in HCT-116 and MCF-7 cell lines. However, complex **2** showed less cytotoxic activity against A549 (IC_{50} = 21.50 μM vs IC_{50} = 12.16 μM (cisplatin) and 10.02 μM (oxaliplatin), respectively). Complex **4** exhibited good cytotoxic activity against HCT-116 (IC_{50} = 7.43 μM) and MCF-7 (IC_{50} = 5.79 μM), especially 0.88-fold more potent against MCF-7 than oxaliplatin. However, the cytotoxic activity of complex **4** against A549 and HepG2 was